



(86) Date de dépôt PCT/PCT Filing Date: 2011/01/06
(87) Date publication PCT/PCT Publication Date: 2011/07/14
(85) Entrée phase nationale/National Entry: 2012/07/03
(86) N° demande PCT/PCT Application No.: US 2011/020418
(87) N° publication PCT/PCT Publication No.: 2011/085129
(30) Priorité/Priority: 2010/01/06 (US61/292,776)

(51) Cl.Int./Int.Cl. *C07D 239/84* (2006.01),
A61K 31/18 (2006.01), *A61K 31/496* (2006.01),
A61K 31/506 (2006.01), *C07C 335/16* (2006.01),
C07C 335/26 (2006.01), *C07D 209/08* (2006.01),
C07D 215/58 (2006.01), *C07D 239/28* (2006.01),
C07D 261/16 (2006.01), *C07D 295/26* (2006.01),
C07D 311/16 (2006.01), ...

(71) Demandeur/Applicant:
ERRICO, JOSEPH P., US

(72) Inventeurs/Inventors:
ERRICO, JOSEPH P., US;
MUGRAGE, BENJAMIN, US;
TURCHI, IGNATIUS, US; ...

(54) Titre : METHODES ET COMPOSITIONS POUR LE DEVELOPPEMENT DE MEDICAMENTS CIBLES
(54) Title: METHODS AND COMPOSITIONS OF TARGETED DRUG DEVELOPMENT

(57) **Abrégé/Abstract:**

Provided herein are compounds having anti-proliferative effect. Also provided are compounds that can modulate the activity of multi-domain proteins comprising a dimerization arm and interdomain tether, such as EGFR, where an untethered, extended conformation is the active state and a tethered conformation is the inactive state, resulting in an autoinhibited configuration. Also provided are methods and pharmacophores for identifying such compounds. Other aspects provide methods or therapeutic treatment for proliferative diseases, disorders, or conditions, such as those associated with EGFR.



(51) Cl.Int./Int.Cl. (suite/continued) *C07D 407/12* (2006.01), *C07D 493/04* (2006.01), *C40B 30/02* (2006.01),
G06F 19/16 (2011.01), *C07K 14/71* (2006.01)

(72) Inventeurs(suite)/Inventors(continued): SILLS, MATTHEW, US; HERRON, JEFFREY D., US;
LODATO, NICHOLAS J., US; ONG, JANE, US; PINE, COREY, US; FEAVER, WILLIAM J., US; ALLOCCO, JOHN, US;
WINES, PAM, US

(74) Agent: SMART & BIGGAR

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
14 July 2011 (14.07.2011)

PCT

(10) International Publication Number
WO 2011/085129 A3(51) International Patent Classification:
A61K 31/535 (2006.01)(21) International Application Number:
PCT/US2011/020418(22) International Filing Date:
6 January 2011 (06.01.2011)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/292,776 6 January 2010 (06.01.2010) US

(72) Inventor; and

(71) Applicant : **ERRICO, Joseph P.** [US/US]; 29 Deer Path Circle, Green Brook, New Jersey 08812 (US).

(72) Inventors; and

(75) Inventors/Applicants (*for US only*): **MUGRAGE, Benjamin** [US/US]; 18 South Main Street, Cranbury, New Jersey 08512 (US). **TURCHI, Ignatius** [US/US]; 475 Robbins Court, Yardley, Pennsylvania 19067 (US). **SILLS, Matthew** [US/US]; 95 Lawrence Drive, Berkeley Heights, New Jersey 07922 (US). **HERRON, Jeffrey D.** [US/US]; 817 Mansfield Road West, Bordentown, New Jersey 08505 (US). **LODATO, Nicholas J.** [US/US]; 851 Dewey Street, Union, New Jersey 07083 (US). **ONG, Jane** [US/US]; 8 Taylor Drive, Franklin Park, New Jersey 08823 (US). **PINE, Corey** [US/US]; 1119 Rowand Court, Voorhees, New Jersey 08043 (US). **FEAVER, William J.** [US/US]; 7 Bearsley Drive, East Brunswick, New Jersey 08816 (US). **ALLOCCO, John** [US/US]; 78 Cloister Place, Staten Island, New York 10306 (US). **WINES, Pam** [US/US]; 14 Lafayette Mills Road, Manalapan, New Jersey 07726 (US).(74) Agent: **BOCK, Joel, N.**; SNR DENTON US LLP, P.O. Box 061080, Wacker Drive Station, Willis Tower, Chicago, Illinois 60606 (US).(81) Designated States (*unless otherwise indicated, for every kind of national protection available*): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.(84) Designated States (*unless otherwise indicated, for every kind of regional protection available*): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- *with international search report (Art. 21(3))*
- *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
- *with sequence listing part of description (Rule 5.2(a))*

(88) Date of publication of the international search report:
1 September 2011

(54) Title: METHODS AND COMPOSITIONS OF TARGETED DRUG DEVELOPMENT

(57) Abstract: Provided herein are compounds having anti-proliferative effect. Also provided are compounds that can modulate the activity of multi-domain proteins comprising a dimerization arm and interdomain tether, such as EGFR, where an untethered, extended conformation is the active state and a tethered conformation is the inactive state, resulting in an autoinhibited configuration. Also provided are methods and pharmacophores for identifying such compounds. Other aspects provide methods or therapeutic treatment for proliferative diseases, disorders, or conditions, such as those associated with EGFR.



WO 2011/085129 A3

TITLE OF INVENTION

METHODS AND COMPOSITIONS OF TARGETED DRUG DEVELOPMENT

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority from U.S. Provisional Application Serial No. 61/292,776, filed on January 6, 2010, which is incorporated herein by reference in its entirety.

INCORPORATION-BY-REFERENCE OF SEQUENCE LISTING

[0002] The Sequence Listing, which is a part of the present disclosure, includes a computer readable form comprising nucleotide and/or amino acid sequences of the present invention. The subject matter of the Sequence Listing is incorporated herein by reference in its entirety.

FIELD OF THE INVENTION

[0003] The present invention generally relates to novel chemical compounds for use in the treatment of disease, methods of manufacture of such compounds and intermediates thereof, and methods of identifying lead molecules for use in quasi-rational drug design.

BACKGROUND OF THE INVENTION

[0004] Rational drug development is a process of developing lead molecules, not by randomly screening thousands of molecules in the blind hope of finding one that shows the desired activity, but rather by deducing the active site of the target and devising a chemical that interacts with that site in the appropriate manner.

[0005] Epidermal Growth Factor Receptor (EGFR) is a member of the ErbB (HER) family receptor tyrosine kinase (RTKs), which regulate cell growth and differentiation and are implicated in many human cancers. EGFR activation and dimerization is discussed in, for example, Burgess et al. (2003) Molecular Cell 12, 541-552 and Ferguson et al. (2003) Molecular Cell 11, 507-517.

[0006] EGF activates its receptor by inducing dimerization of the extracellular region of EGFR. The activation of EGFR has been described through results of disulfide bond mapping as well as X-ray crystal structures. The crystal structures of ligand-bound sEGFR showed that

dimerization is receptor mediated, with two individual ligand molecules present in the dimer. The dimerization interface of activated EGFR is completely occluded by intramolecular interactions, and is an autoinhibited configuration. To activate the receptor, a large domain rearrangement that exposes this occluded interface must accompany EGF binding where EGF does not contribute to the EGFR dimer interface. The EGFR mechanism is in sharp contrast to most other receptor tyrosine kinase activation mechanisms in which the bound ligand contributes directly to the receptor dimerization interface and does not dramatically alter the conformation of the extracellular region of the receptor tyrosine kinase.

[0007] EGFR contains four subdomain I, II, III, and IV. Almost all receptor/receptor contacts observed in the crystal structures of EGFR are mediated by domain II. At the center of the dimer interface is a prominent loop (residues 242–259 of EGFR) that extends from the second C1 module (module 5) of each domain II and reaches across the interface to interact primarily with domain II of its dimerization partner. This domain II loop, which is specific to ErbB receptors, is the “dimerization arm”. The dimerization arm of domain II is completely occluded by intramolecular interactions with domain IV (*i.e.*, an autoinhibited configuration). There are two smaller interaction sites in the dimer that involve side chains from the second and the sixth disulfide-bonded modules of domain II. And the dimer interface may extend into domain IV. While the two receptor molecules approach one another very closely toward the C terminus of domain IV, a well-defined, tight interface is not observed.

[0008] Although EGF and TGF- α clearly do not span the dimer interface, each ligand simultaneously contacts two separate binding surfaces in the same EGFR molecule. The bound EGF or TGF- α molecule resembles a wedge between domains I and III. The relationship between domains I and II is essentially identical to that seen in IGF-1R and in the activated sEGFR dimer, implying that ligand binding does not greatly influence the relative orientation of these two domains. But the relationship between domains II and III differs dramatically in the activated and unactivated structures. A direct intramolecular interaction between cysteine-rich domains II and IV restrains the domain II/III relationship that characterizes the unactivated configuration. This interdomain “tether” is stabilized by essentially identical interactions between the two cysteine rich domains (II and IV) in inactive sErbB3 and sEGFR.

[0009] The intramolecular domain II/IV tether precisely buries the dimerization arm of domain II against domain IV, so that the tethered configurations of sErbB3 and sEGFR cannot dimerize and thus appear to be autoinhibited. Moreover, the two ligand binding surfaces on domain I and III are too far apart in the tethered configuration for a single ligand to bind to both simultaneously. Consequently, the tethered configuration can only form low-affinity interactions with ligand, using just one of its ligand binding surfaces at a time.

[0010] Switching between the unactivated and activated configurations of sEGFR requires domains I and III to be drawn toward one another through a 130° rotation of the rigid domain I/II pair in one plane and a 20° rotation in another. Only this extended configuration of sEGFR is capable of both high-affinity ligand binding and efficient dimerization.

[0011] Based upon energetic calculations, it is currently thought that at any given time, about 95% of sEGFR molecules will be tethered and the remaining 5% will not. The presence of ligand and subsequent binding to domains I and III of the non-tethered form will drive the equilibrium toward the non-tethered form, trapping receptor molecules in the extended state that can dimerize.

[0012] Exposure of the dimerization arm is not sufficient alone to drive EGFR dimerization. Also required is additional contact sites in modules 2 and 6 of domain III. These two additional contact sites and the dimerization arm cooperate at the dimer interface.

[0013] Known strategies of EGFR inhibition are directed to antibody binding of domain III to provide steric hindrance of the required configuration change (e.g., Erbitux). Other conventional strategies are directed to antibody binding of domain II, specifically the dimerization arm, so as to prevent dimerization (e.g., pertuzumab). Still other conventional strategies are directed to antibody binding of domain IV residues that participate in the intramolecular tether (e.g., trastuzumab, Herceptin). But no existing strategies are directed to the tethering mechanism of activation.

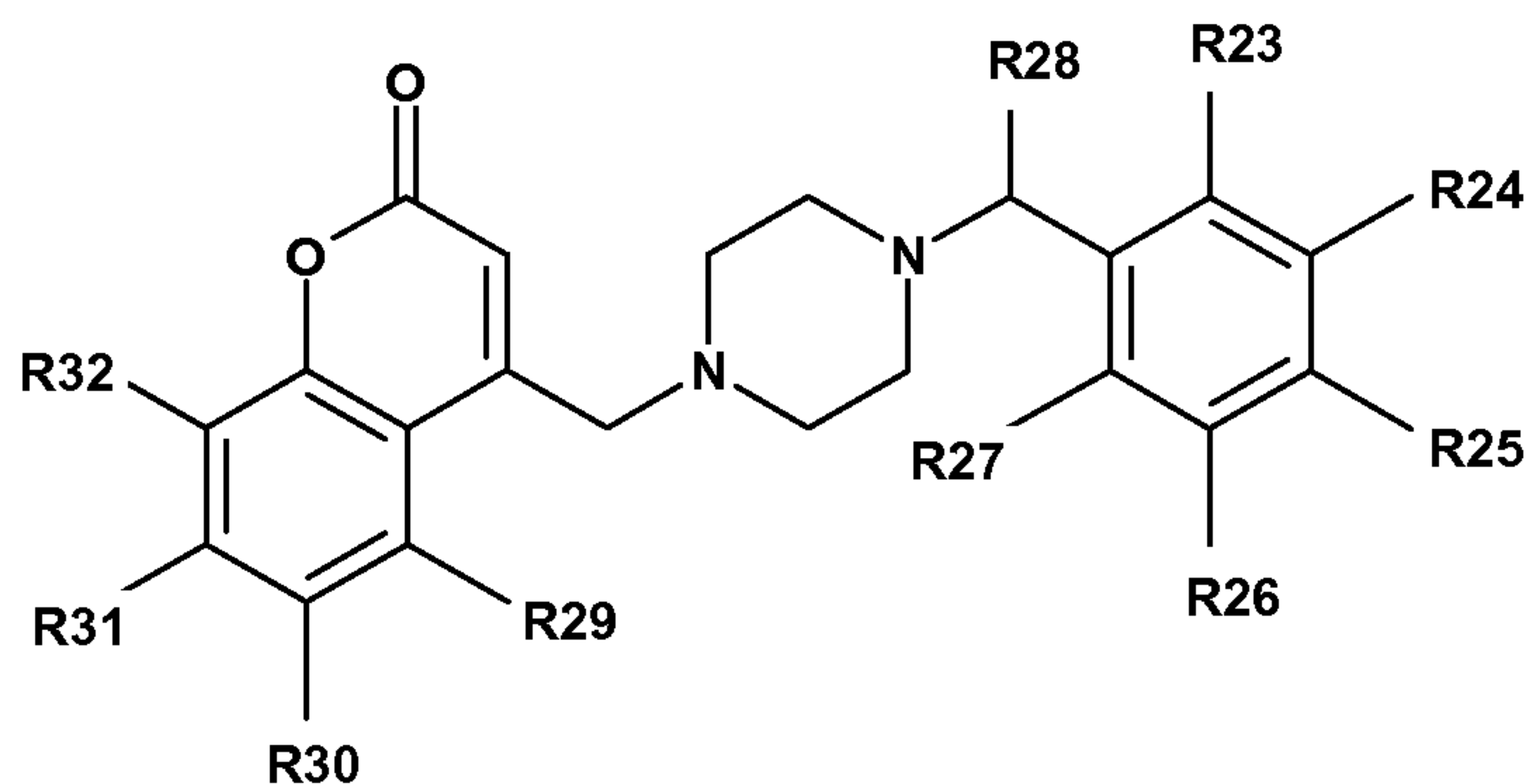
SUMMARY OF THE INVENTION

[0014] Described herein are compounds and compositions having an anti-proliferative effect, along with methods of therapeutic treatment with such compounds. Also provided are methods of discovery of such compounds. An approach described herein identifies modulators of the

activity of multi-domain proteins comprising a dimerization arm and interdomain tether, such as EGFR, where an untethered, extended conformation is the active state and a tethered conformation is the inactive state, resulting in an autoinhibited configuration.

[0015] One aspect of the invention provides small molecule compounds.

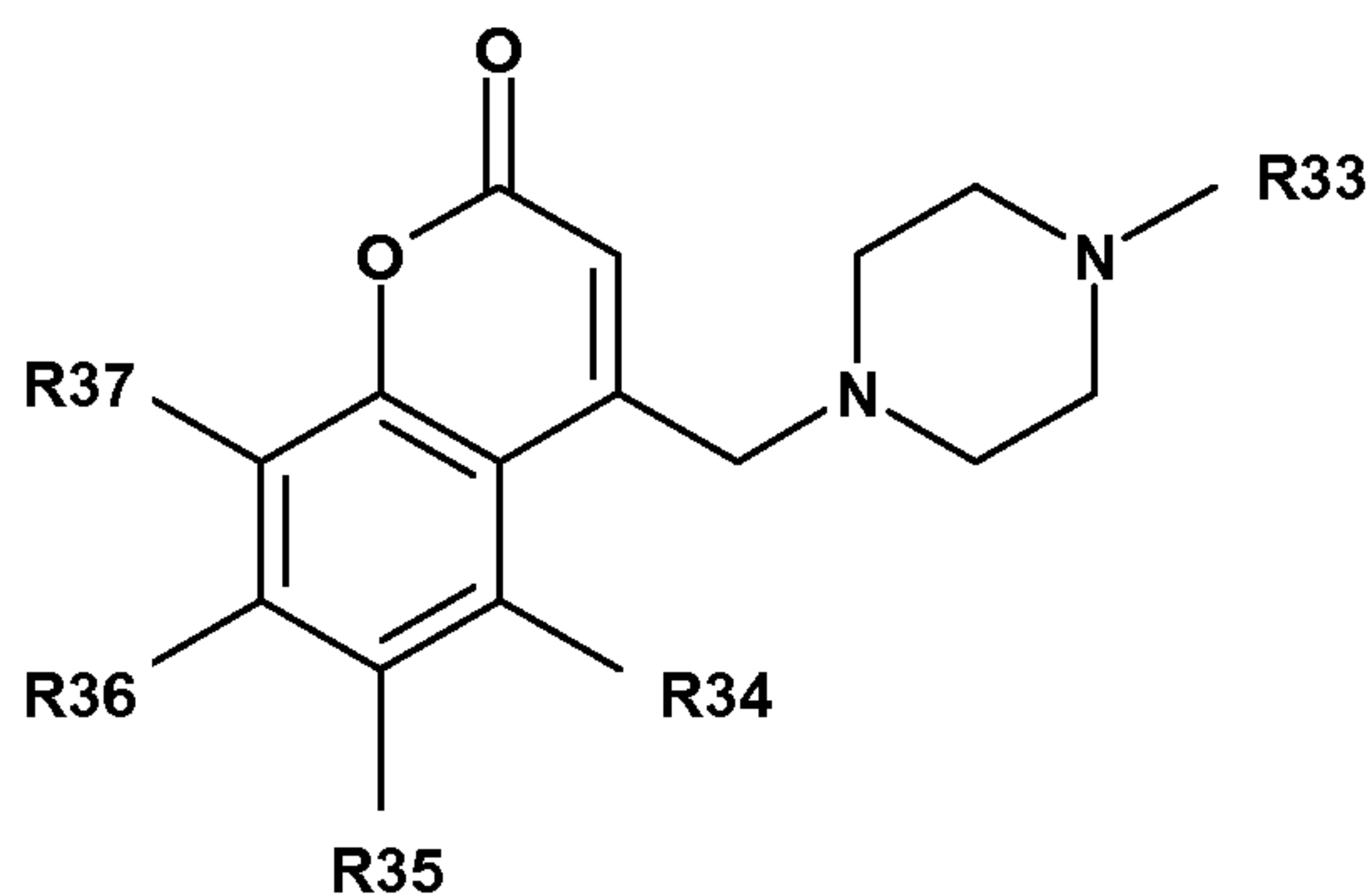
[0016] In one embodiment, compounds have a formula of



Formula (13) (1734-like Type P),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

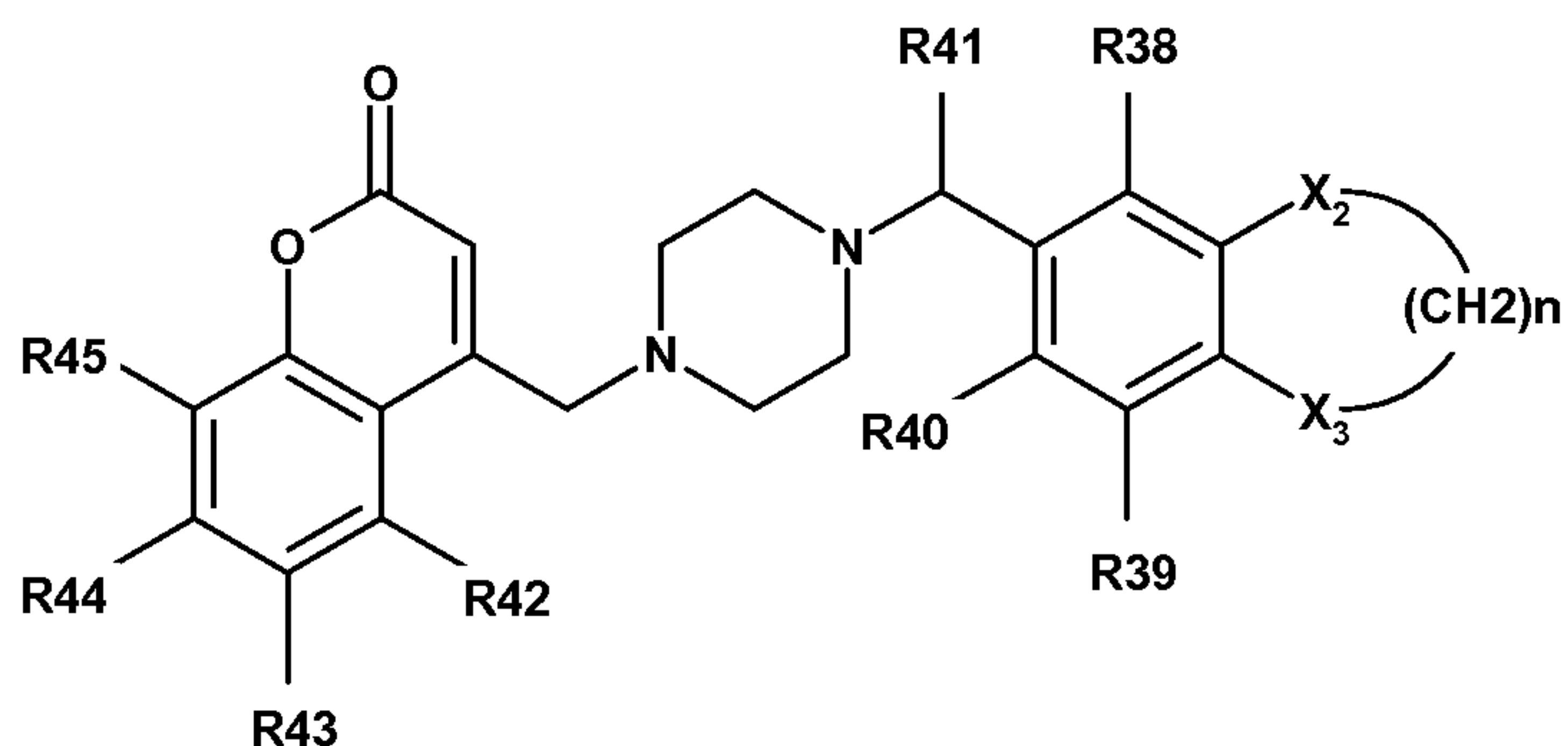
[0017] In one embodiment, compounds have a formula of



Formula (14) (1734-like Type Q),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

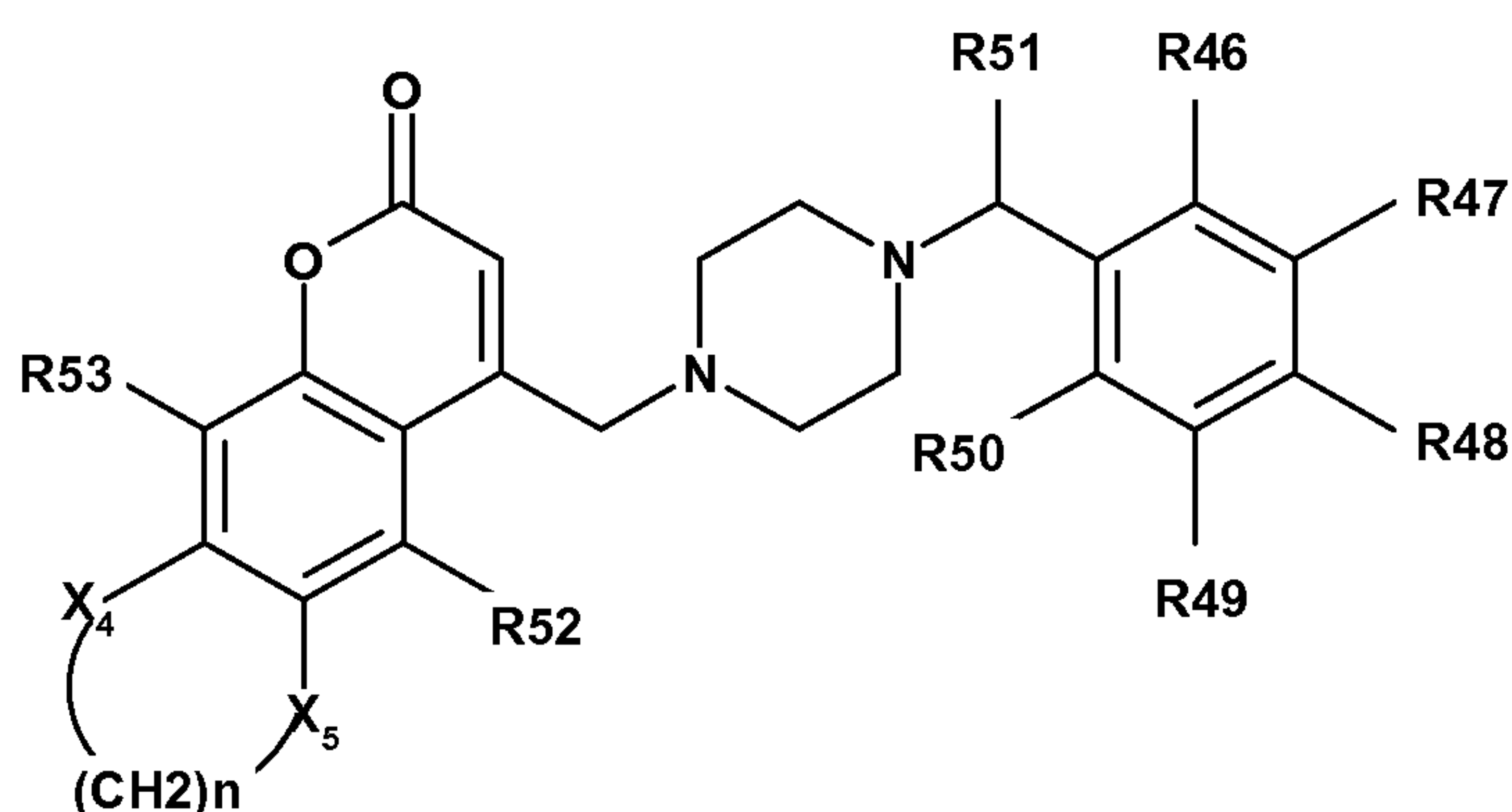
[0018] In one embodiment, compounds have a formula of



Formula (15) (1734-like Type R),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0019] In one embodiment, compounds have a formula of

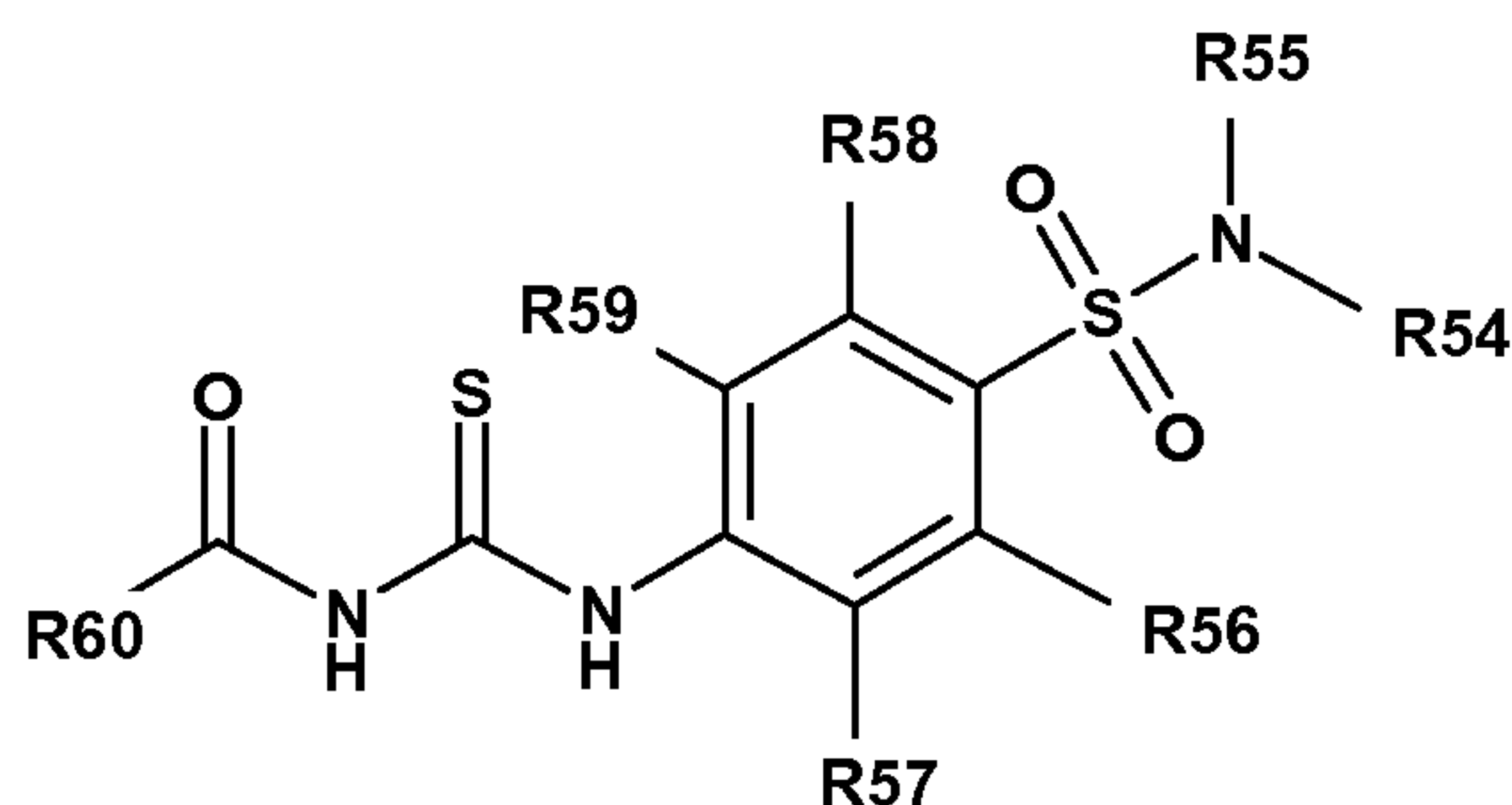


Formula (16) (1734-like Type S),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0020] In one embodiment, a 1734-like compound inhibits EGFR activity and comprises at least five or more of functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9 of a Scheme II 1734-like pharmacophore, with functional groups as defined herein.

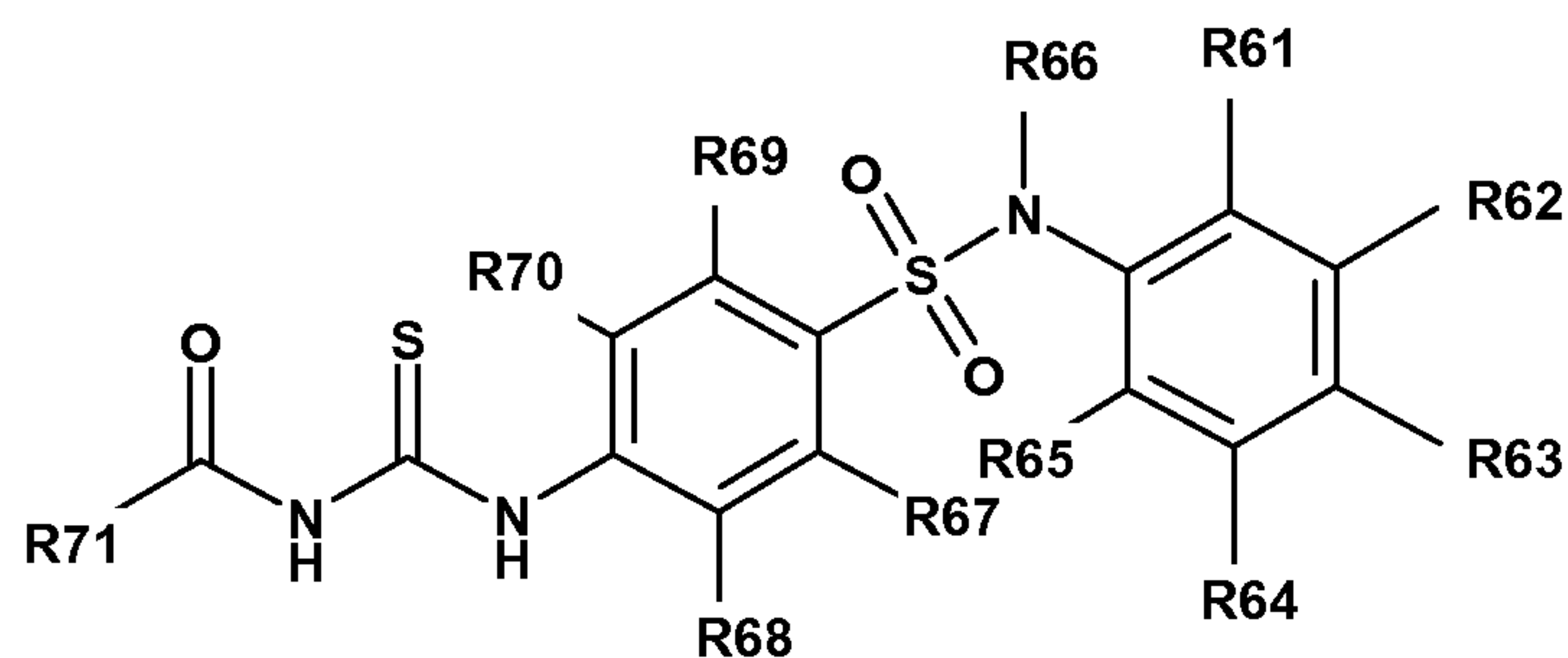
[0021] In one embodiment, compounds have a formula of



Formula (18) (1886-like Type J),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

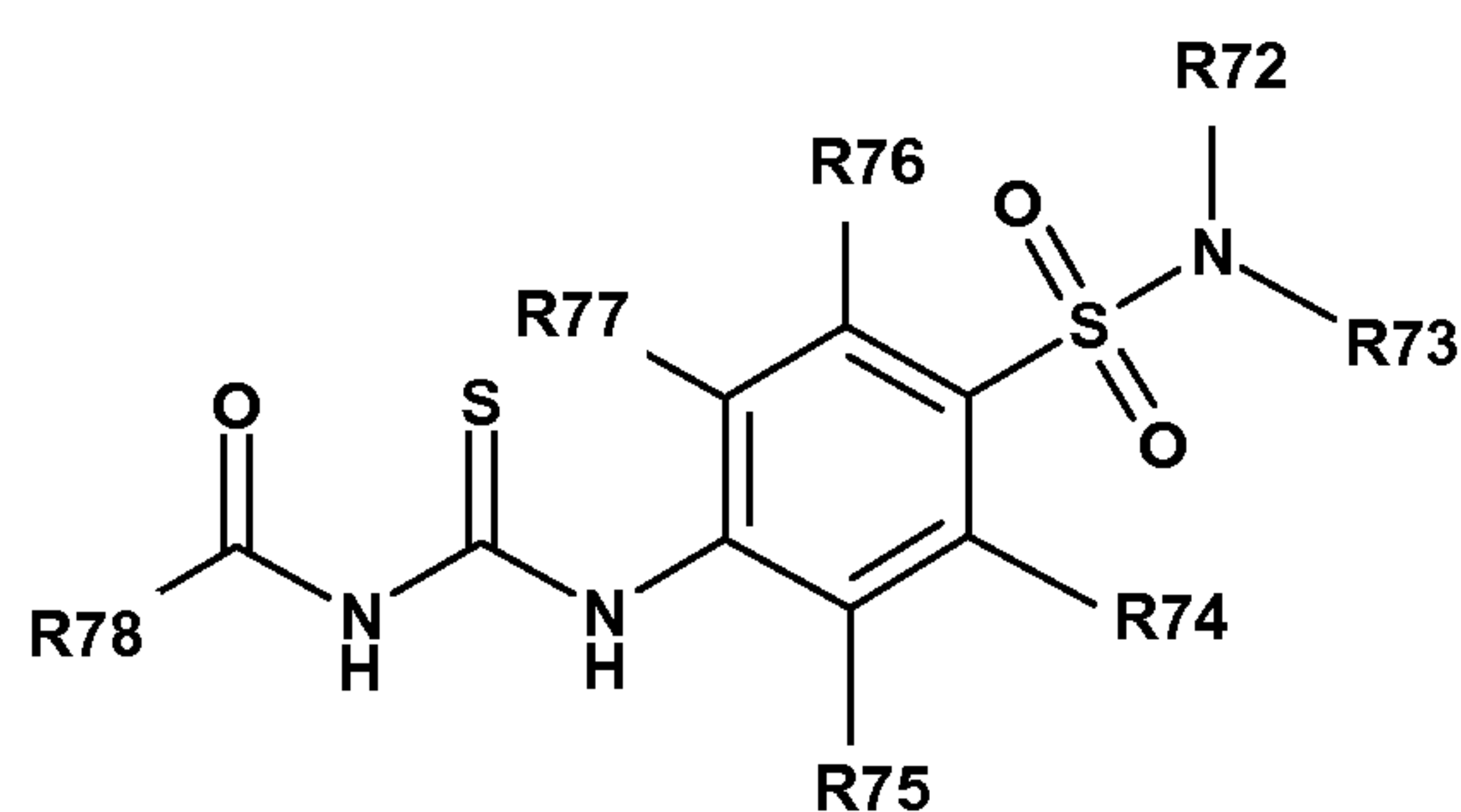
[0022] In one embodiment, compounds have a formula of



Formula (19) (1886-like Type K),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

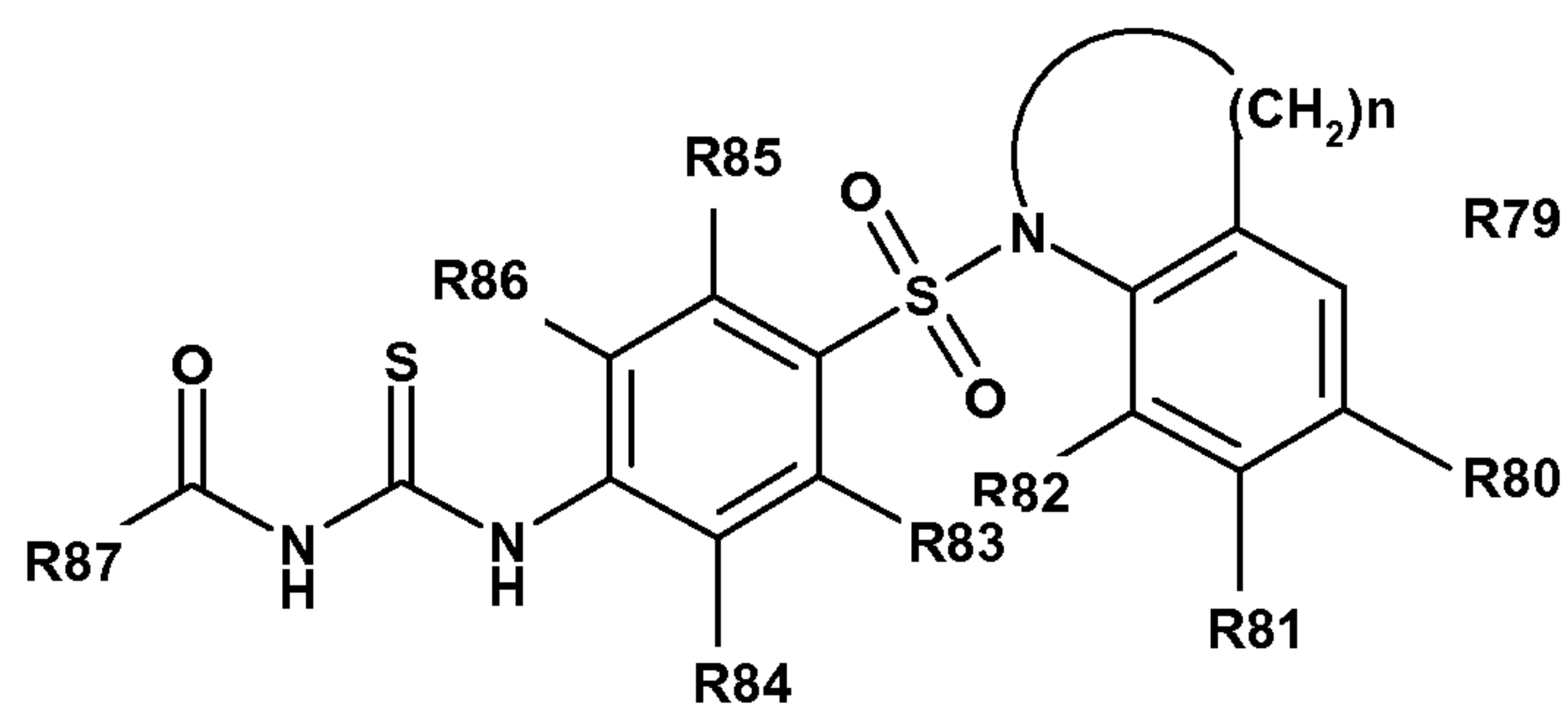
[0023] In one embodiment, compounds have a formula of



Formula (20) (1886-like Type L),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

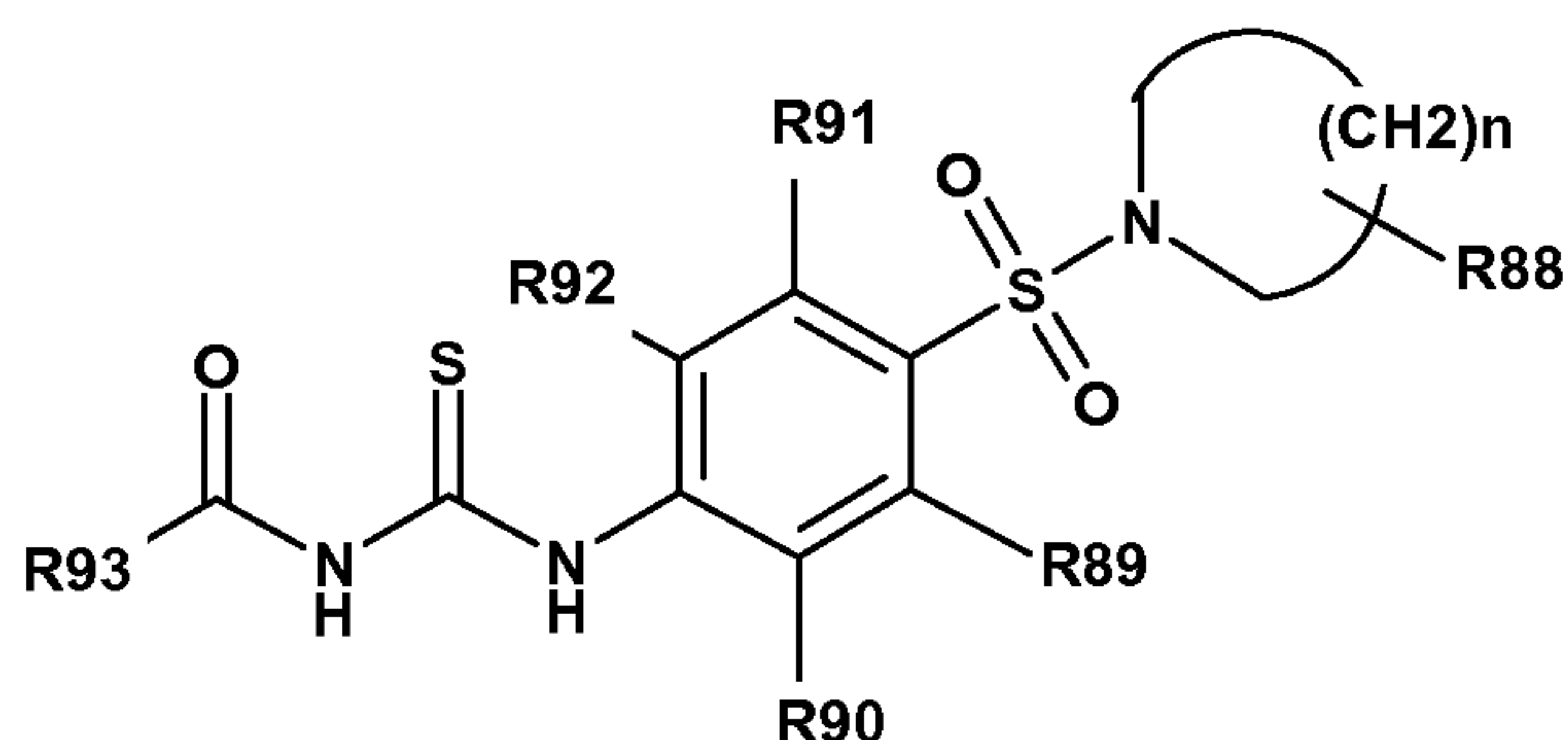
[0024] In one embodiment, compounds have a formula of



Formula (21) (1886-like Type M)

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

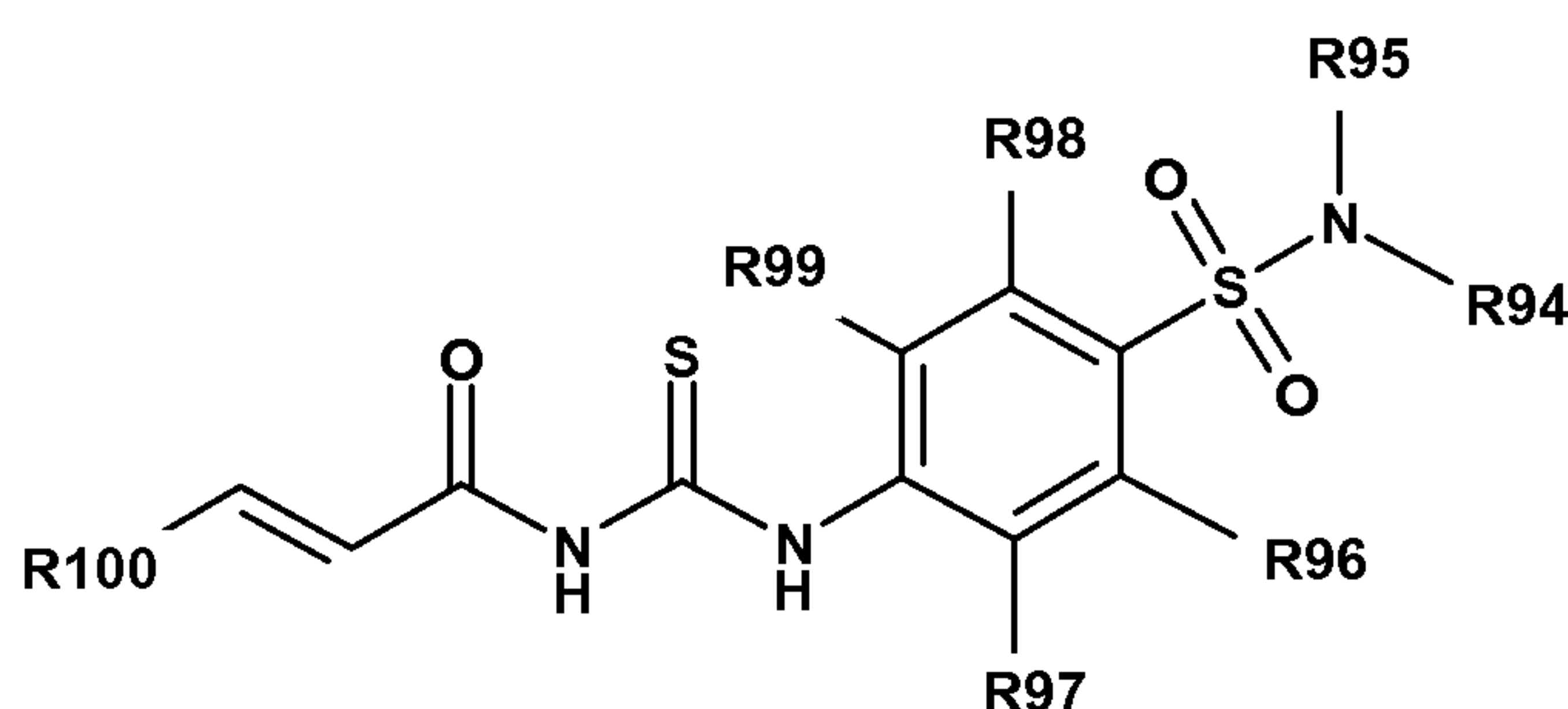
[0025] In one embodiment, compounds have a formula of



Formula (22) (1886-like Type N),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0026] In one embodiment, compounds have a formula of

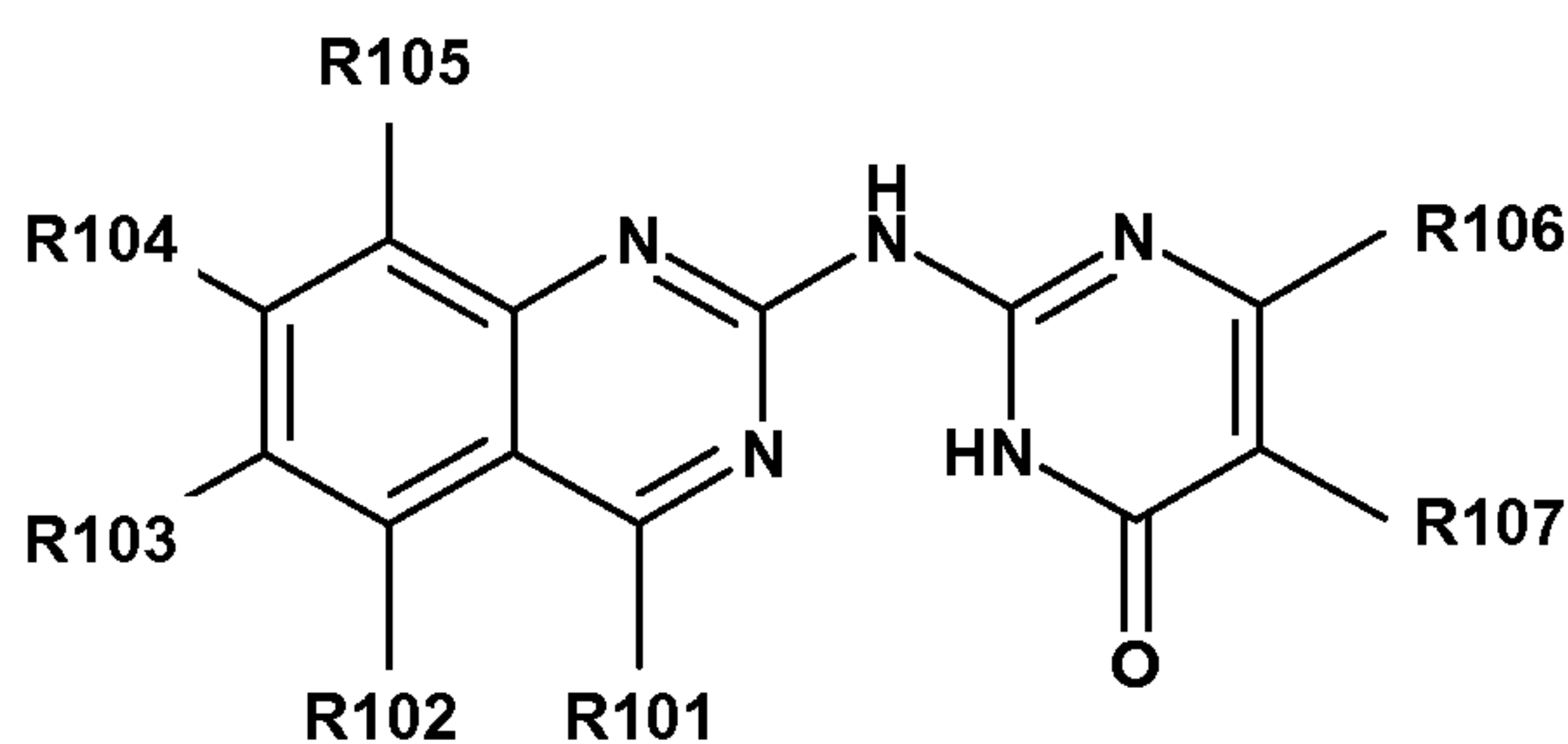


Formula (23) (1886-like Type O),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0027] In one embodiment, a 1886-like compound inhibits EGFR activity and comprises at least seven or more of functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9 of a Scheme III 1886-like pharmacophore, with functional groups as defined herein.

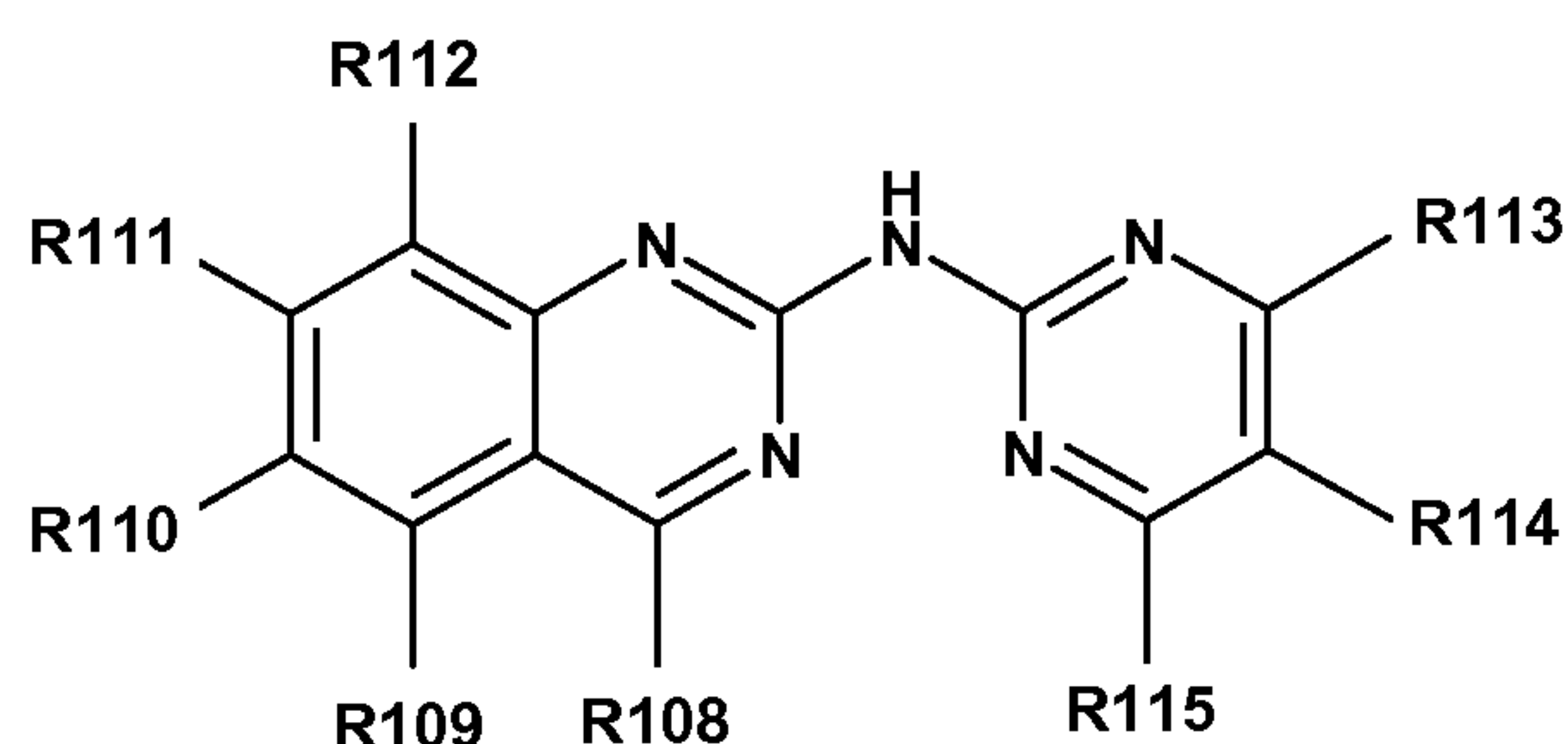
[0028] In one embodiment, compounds have a formula of



Formula (25) (10381-like Type D),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

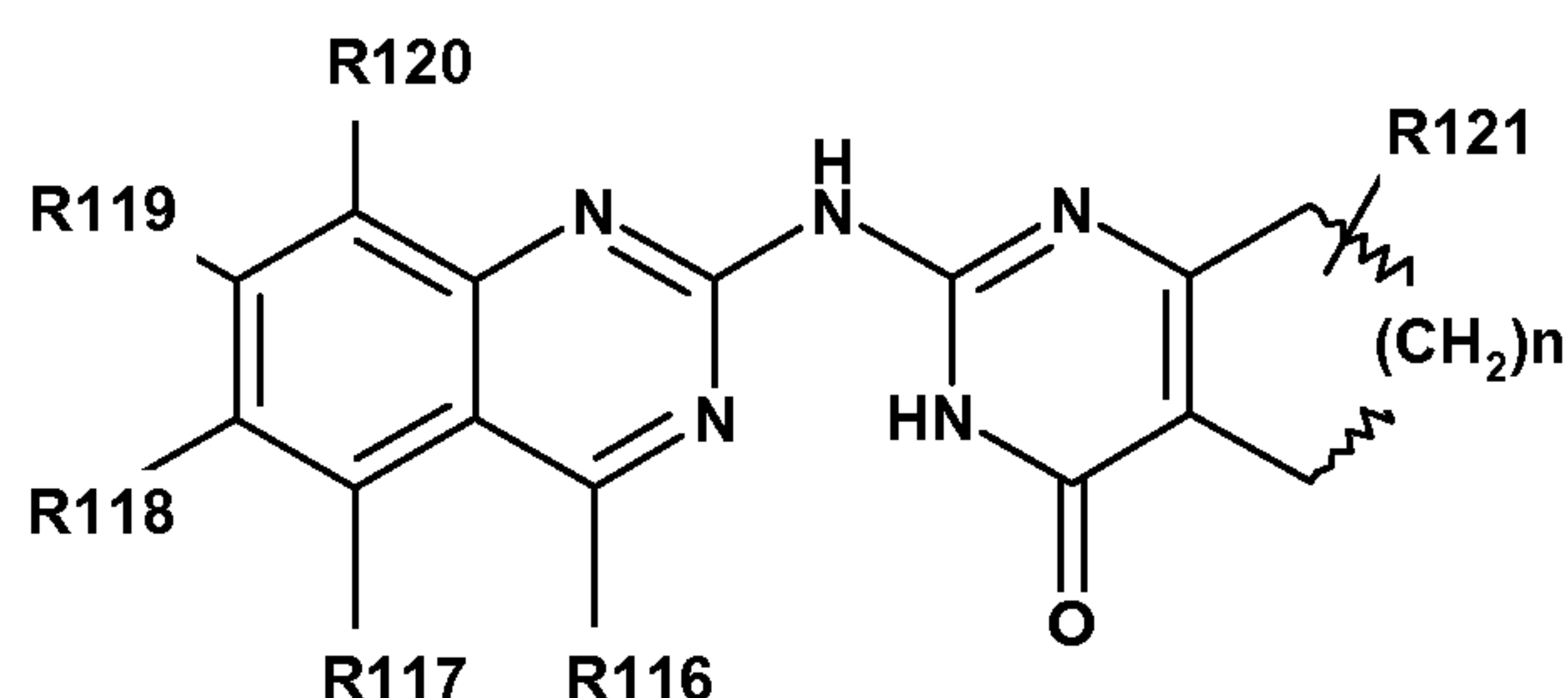
[0029] In one embodiment, compounds have a formula of



Formula (27) (10381-like Type E),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0030] In one embodiment, compounds have a formula of

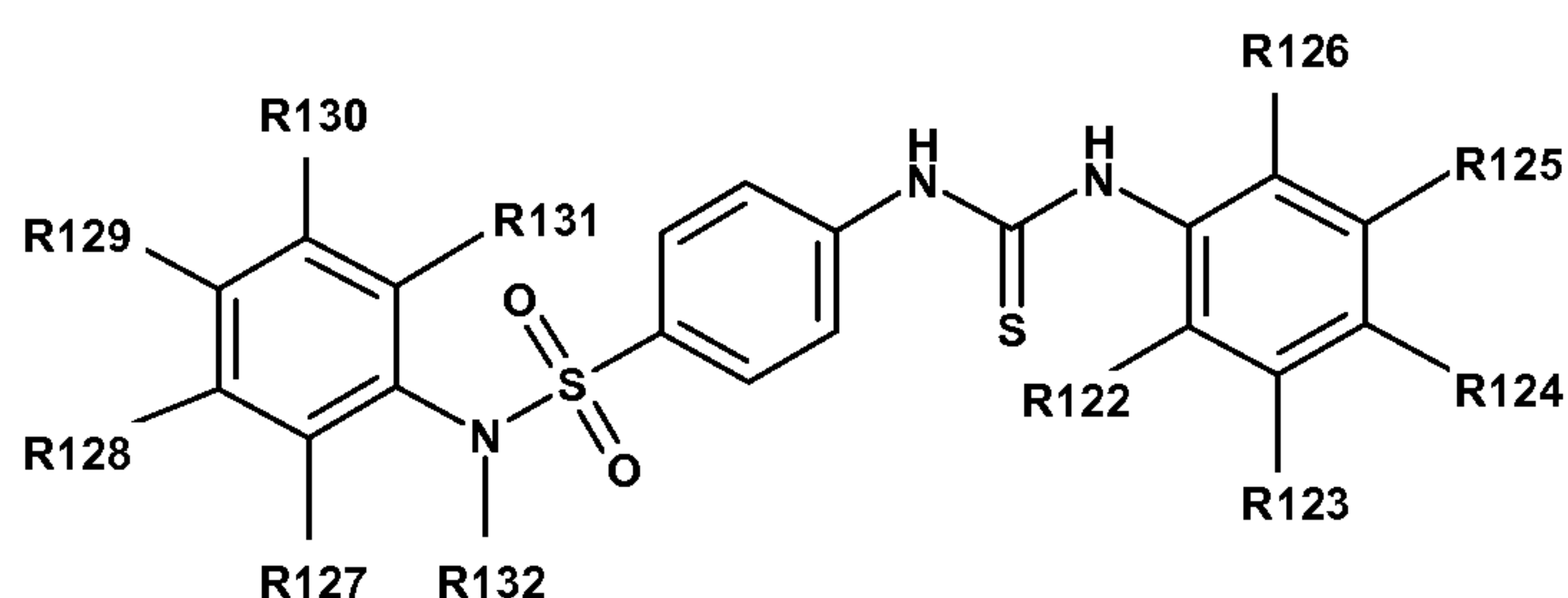


Formula (28) (10381-like Type F),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0031] In one embodiment, a 10381-like compound inhibits EGFR activity and comprises at least eight or more of functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10 of a Scheme IV 10381-like pharmacophore, with functional groups as defined herein.

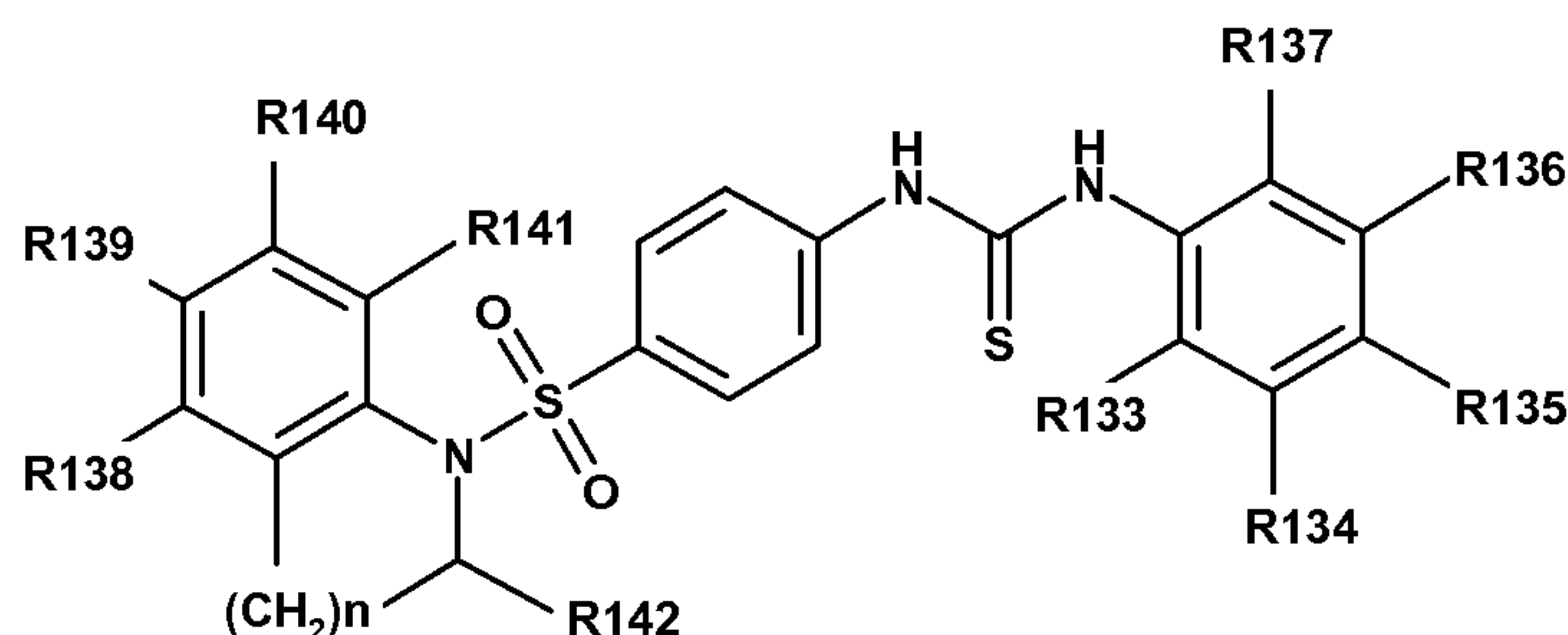
[0032] In one embodiment, compounds have a formula of



Formula (32) (11091-like Type H),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0033] In one embodiment, compounds have a formula of



Formula (33) (11091-like Type I),

with constituents as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

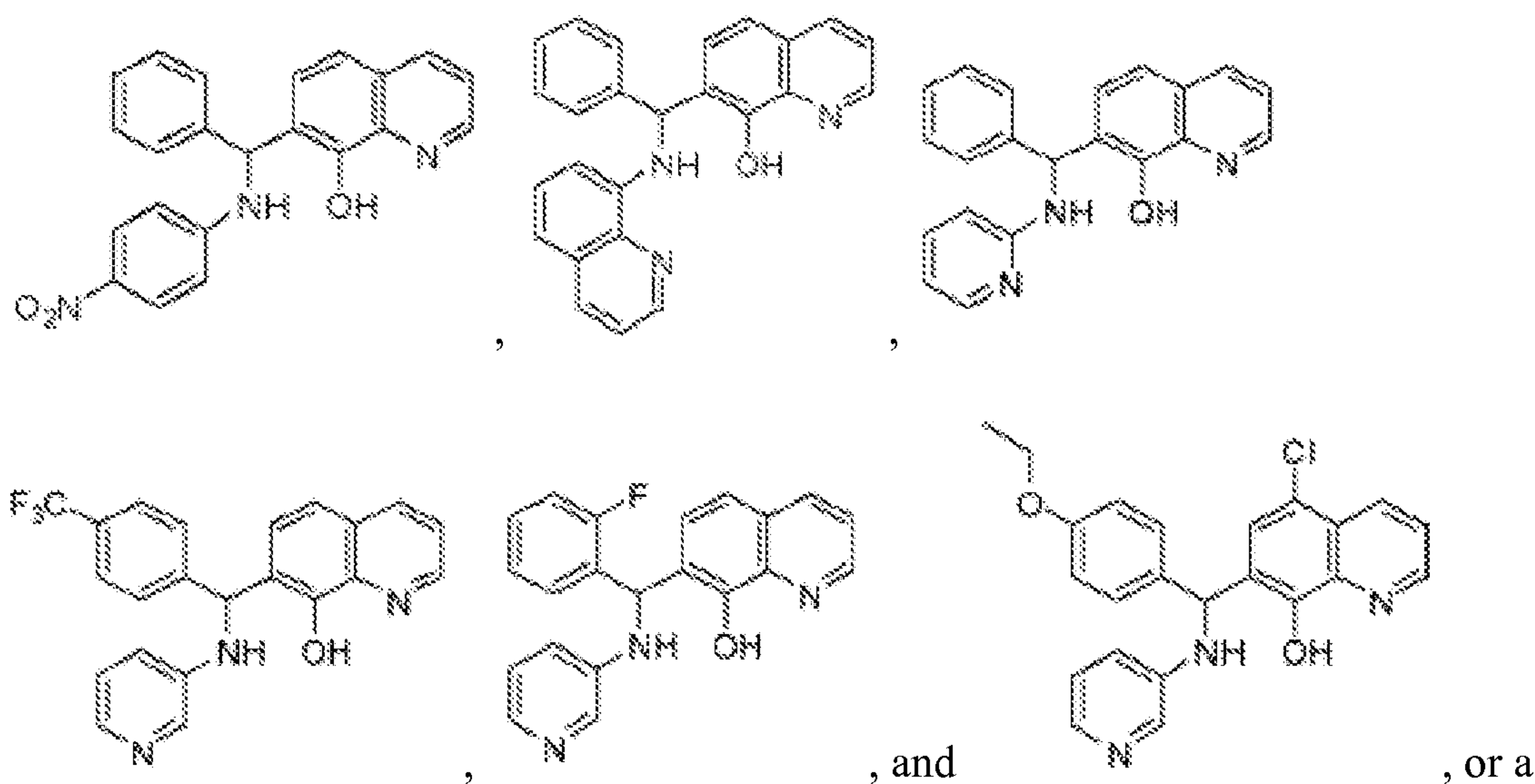
[0034] In one embodiment, a 11091-like compound inhibits EGFR activity and comprises at least nine or more of functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11 of a Scheme V 11091-like pharmacophore, with functional groups as defined herein.

[0035] One aspect of the invention provides a method of treating a proliferative disease, disorder, or condition using a compound described herein.

[0036] In one embodiment, the method of treating a proliferative disease, disorder, or condition comprises administering to a subject in need thereof a composition comprising a therapeutically effective amount of a compound with a formula selected from the group consisting of: Formula (13); Formula (14); Formula (15); Formula (16); Formula (18); Formula (19); Formula (20); Formula (21); Formula (22); Formula (23); Formula (25); Formula (27); Formula (28); Formula (32); and Formula (33), as defined herein, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0037] In one embodiment, the method of treating a proliferative disease, disorder, or condition comprises administering to a subject in need thereof a composition comprising a therapeutically effective amount of a compound with a formula selected from the group consisting of: AD4-1734 (Formula (12)); AD4-1886 (Formula (17)); AD4-10381 (Formula (24)); and AD4-11091 (Formula (31)), or a stereoisomer or pharmaceutically acceptable salt thereof.

[0038] In one embodiment, the method of treating a proliferative disease, disorder, or condition comprises administering to a subject in need thereof a composition comprising a therapeutically effective amount of a compound with a formula selected from the group consisting of:



stereoisomer or pharmaceutically acceptable salt thereof.

[0039] In some embodiments, the composition further comprises a pharmaceutically acceptable carrier or excipient.

[0040] In some embodiments, the treated proliferative disease, disorder, or condition is selected from: cancer; a blood vessel proliferative disorder; a fibrotic disorder; a mesangial cell proliferative disorder; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by a viral infection.

[0041] In some embodiments, the treated proliferative disease, disorder, or condition is associated with EGFR. In some configurations, the proliferative disease, disorder, or condition associated with EGFR is selected from the group consisting of: cancer; a blood vessel proliferative disorder; a fibrotic disorder; a mesangial cell proliferative disorder; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by a viral infection.

[0042] One aspect provides a method for identifying an EGFR inhibitor.

[0043] In one embodiment, the method for identifying an EGFR inhibitor comprises: providing a pharmacophore comprising a scheme selected from the group consisting of Scheme II (as defined herein) as input to a 3-dimensional database; comparing a three dimensional structure of a candidate compound to the three dimensional structure of the pharmacophore; and

selecting a candidate compound with a three dimensional structure that substantially aligns with five or more functional groups of Scheme II (ADS-1734-like).

[0044] In one embodiment, the method for identifying an EGFR inhibitor comprises: providing a pharmacophore comprising a scheme selected from the group consisting of Scheme III (as defined herein) as input to a 3-dimensional database; comparing a three dimensional structure of a candidate compound to the three dimensional structure of the pharmacophore; and selecting a candidate compound with a three dimensional structure that substantially aligns with seven or more functional groups of Scheme III (AD4-1886-like).

[0045] In one embodiment, the method for identifying an EGFR inhibitor comprises: providing a pharmacophore comprising a scheme selected from the group consisting of Scheme IV (as defined herein) as input to a 3-dimensional database; comparing a three dimensional structure of a candidate compound to the three dimensional structure of the pharmacophore; and selecting a candidate compound with a three dimensional structure that substantially aligns with eight or more of functional groups of Scheme IV (AD4-10381-like).

[0046] In one embodiment, the method for identifying an EGFR inhibitor comprises: providing a pharmacophore comprising a scheme selected from the group consisting of Scheme V (as defined herein) as input to a 3-dimensional database; comparing a three dimensional structure of a candidate compound to the three dimensional structure of the pharmacophore; and selecting a candidate compound with a three dimensional structure that substantially aligns with nine or more of functional groups of Scheme V (AD4-11091-like).

[0047] In some embodiments of the method for identifying an EGFR inhibitor, similarity between the three-dimensional structure of the candidate compound and the three-dimensional structure of the pharmacophore is indicative of an ability of the candidate compound to inhibit EGFR by substantially maintaining a tethered inactive configuration of EGFR or substantially preventing stabilization of the untethered active configuration of EGFR.

[0048] Some embodiments of the method for identifying an EGFR inhibitor further comprise determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation; and constructing a pharmacophore, wherein the pharmacophore comprises a plurality of pharmacophoric features that approximates the identity and the spatial orientation of

the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation such that the pharmacophore structural features are complementary to the inactive EGFR configuration. In some configurations, determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation comprises analysis of X-ray crystallographic data derived from a crystalline form of EGFR in an inactive, tethered conformation.

[0049] In some embodiments of the method for identifying an EGFR inhibitor, at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of domain II of EGFR in a tethered inactive conformation. In some embodiments of the method for identifying an EGFR inhibitor, at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of a cleft region between domain II and domain IV of EGFR in a tethered inactive conformation.

[0050] Some embodiments of the method for identifying an EGFR inhibitor further comprise determining a docking affinity of the candidate molecule for the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation. In some configurations, docking affinity is quantified by energy gained upon interaction of the candidate molecule with the target biomolecule, energy required to attain the docked conformation relative to the lowest energy conformation, or a combination thereof.

[0051] Other objects and features will be in part apparent and in part pointed out hereinafter.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] Those of skill in the art will understand that the drawings, described below, are for illustrative purposes only. The drawings are not intended to limit the scope of the present teachings in any way.

[0053] FIG. 1A shows pharmacophore 21_thr100_glu105 (see Example 3; Table 1). FIG. F10A: Pharmacophore 21_thr100_glu105. FIG. F10B: Pharmacophore 21_thr100_glu105 aligned to AD4-1886.

[0054] FIG. 1B shows AD4-1886 aligned to pharmacophore 21_thr100_glu105.

[0055] FIG. 2: AD4-11091 aligned to the pharmacophore model, Pharm1886-6. (see Example 3; Table 3).

[0056] FIG. 3 shows the binding site at the interface of Domain II and Domain IV of the Inactive Form of EGFR (1NQL.pdb) as determined by the site finder in MOE. The Carbon Atoms of the Domain II Residues are Colored Red and those of the Domain IV Residues are Colored Blue.

[0057] FIG. 4 shows Pharm-1nql-glue-6 aligned to the hit AD4-1734.

[0058] FIG. 5 shows DockPharm1505-2 aligned to the hit AD4-10381.

[0059] FIG. 6 is a series of two-dimensional representations of AD4-1886 and AD4-1886-like compounds docked with the EGFR/cetuximab complex. Docking of compound AD4-1886 to EGFR is depicted, for example, in FIG. 6A. Docking of compound AD4-11883 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6B. Docking of compound AD4-11975 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6C. Docking of compound AD4-11409 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6D. Docking of compound AD4-11638 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6E. Docking of compound AD4-11645 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6F.

[0060] FIG. 7 is a series of two-dimensional representations of AD4-11091 and AD4-11091-like compounds docked with the EGFR/cetuximab complex. Docking of compound AD4-11091 to EGFR is depicted, for example, in FIG. 7A. Docking of compound AD4-12509 (an AD4-11091-like compound) to EGFR is depicted, for example, in FIG. 7B. Docking of compound AD4-12423 (an AD4-11091-like compound) to EGFR is depicted, for example, in FIG. 7C. Docking of compound AD4-12528 (an AD4-11091-like compound) to EGFR is depicted, for example, in FIG. 7D. Docking of compound AD4-12522 (an AD4-11091-like compound) to EGFR is depicted, for example, in FIG. 7E. Docking of compound AD4-12504 (an AD4-11091-like compound) to EGFR is depicted, for example, in FIG. 7F.

[0061] FIG. 8 is a series of two-dimensional representations of AD4-1734 and AD4-1734-like compounds docked with inactive EGFR. Docking of compound AD4-1734 to EGFR is depicted, for example, in FIG. 8A. Docking of compound AD4-10631 (an AD4-1734-like

compound) to EGFR is depicted, for example, in FIG. 8B. Docking of compound AD4-10188 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8C. Docking of compound AD4-10186 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8D. Docking of compound AD4-10633 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8E. Docking of compound AD4-10174 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8F.

[0062] FIG. 9 is a series of two-dimensional representations of AD4-10381 and AD4-10381-like compounds docked with inactive EGFR. Docking of compound AD4-10381 to EGFR is depicted, for example, in FIG. 9A. Docking of compound AD4-11340 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9B. Docking of compound AD4-12632 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9C. Docking of compound AD4-12681 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9D. Docking of compound AD4-12732 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9E. Docking of compound AD4-11511 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9F.

[0063] FIG. 10 is a series of line and scatter plots showing % inhibition of EGFR as a function of concentration of Tykerb (FIG. 10A) or Iressa (FIG. 10B) either alone or in combination with AD4-10628. A shift in the dose-response curve to the left indicates a more potent response.

[0064] FIG. 11 is a scatter plot showing Dose Reduction Index (DRI) as a function of F_a for DRI Tykerb and DRI AD4-10628.

[0065] FIG. 12 is a histogram showing a summary of Combination Index (CI) values at 90% inhibition (ED90) for a series of AD4 compounds in combination with Tykerb. Compound 4 is Iressa. Compound 5 is Tarceva. The balance of compounds are AD4 compounds described herein. Response below the dark middle line (i.e., $CI < 0.9$) indicates synergism.

[0066] FIG. 13 is a cartoon depicting conformations of EGFR. FIG. 13A shows EGFR as a tethered monomer. FIG. 13B shows EGFR as an untethered monomer. FIG. 13C shows EGFR in a ligand stabilized extended conformation. FIG. 13D shows EGFR as a ligand induced activated dimer.

[0067] FIG. 14 is a cartoon depicting ligand-induced dimerization and activation of the kinase domain of EGFR.

DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED EMBODIMENTS

[0068] Described herein are compounds and compositions having an anti-proliferative effect, along with methods of therapeutic treatment with such compounds and methods of discovery of such compounds. Various small molecule compounds described herein can hold proteins of multiple domains together in a tethered, inactive state. Also provided are methods to identify the structural requirements of such inhibitors, screen for effective inhibitors, optimize the structure of identified candidates, and utilize identified small molecule compounds in therapeutic treatment regimes.

[0069] One aspect of the invention is directed to small molecule compounds efficacious in treating proliferative diseases or conditions. Various embodiments of compounds described herein can have an anti-proliferative effect. Various embodiments of compounds described herein can hold multiple domain proteins in a tethered, inactive state. Various embodiments of compounds described herein can have an inhibitory effect on EGFR. Compounds described herein have been demonstrated to be empirically effective in treating proliferative diseases and conditions.

[0070] One aspect of the invention is directed to therapeutic treatment of proliferative diseases and disorders using compounds and compositions described herein.

[0071] One aspect of the invention is directed to compounds, methods, and apparatuses for developing one or more drugs for one or more targeted therapies. More specifically, the approach described herein identifies modulators of the activity of multi-domain proteins comprising a dimerization arm and interdomain tether, where an untethered, extended conformation is the active state and a tethered conformation is the inactive state, resulting in an autoinhibited configuration. The pharmacophoric approach described herein is based upon a mechanistic understanding of conformation-dependent protein receptor activation mechanisms, thus avoiding conventional combinatorial chemistry and high throughput screening techniques.

BIOMOLECULE TARGET SELECTION

[0072] Desirable target enzymes include those for which there exists crystallography data sufficient to discern a ligand binding, activation, and/or dimerization mechanism. The various methods of the invention can be used to generate pharmacophore models for a variety of multi-domain protein targets (crystallized with and/or without ligand) having an interdomain tether associated with activation state. Thus is provided compounds that can prevent untethering and stabilization of the extended conformation, and methods for identifying such compounds.

[0073] It shall be understood that the types of biomolecule target for the lead molecules generated by the methods of the present invention can include one or more of EGFR (i.e., ErbB1), HER2/c-neu (ErbB-2), Her 3 (ErbB-3), and Her 4 (ErbB-4).

EGFR

[0074] Described herein is targeting of various portions of the domains of EGFR so as to prevent stabilization of the untethered, extended conformation. In other words, a small molecule inhibitor can be used to hold proteins of domain II and IV together in the tethered, inactive state. This strategy can provide for some retention of the basal levels of EGFR signaling, retention of EGF response, and/or reduce EGF-independent dimerization. Such a therapeutic effect would slow rapid growth of cancer cells (which are more sensitive given increased expression levels of EGFR) but retain at least a portion of basal EGFR activity necessary for healthy tissue function.

[0075] Known strategies of EGFR inhibition are directed to antibody binding of domain III to provide steric hindrance of the required configuration change (e.g., Erbitux). Other conventional strategies are directed to antibody binding of domain II, specifically the dimerization arm, so as to prevent dimerization (e.g., pertuzumab). Still other conventional strategies are directed to antibody binding of domain IV residues that participate in the intramolecular tether (e.g., trastuzumab, Herceptin). But, in contrast to the approach described herein, the above conventional strategies do not prevent untethering or stabilization of the extended conformation.

[0076] As described above, EGFR has an autoinhibited configuration in which the dimerization arm of domain II is completely occluded by intramolecular interactions with

domain IV (see e.g., FIG. 13). EGF activates its receptor by inducing dimerization of the extracellular region of EGFR (see e.g., FIG. 13D). Almost all receptor/receptor contacts observed in the crystal structures of EGFR are mediated by domain II, specifically, a prominent loop (residues 242–259 of EGFR) that extends from the second C1 module (module 5) of each domain II (i.e., the dimerization arm). The unactivated configuration is characterized by a direct intramolecular interaction between cysteine-rich domains II and IV, which restrains the domain II/III relationship (see e.g., FIG. 13A). This interdomain “tether” is stabilized by essentially identical interactions between the two cysteine rich domains (II and IV) in inactive sEGFR. Switching between the unactivated and activated configurations of sEGFR requires domains I and III to be drawn toward one another through a 130° rotation of the rigid domain I/II pair in one plane and a 20° rotation in another (see e.g., FIG. 13B). Only this extended configuration of sEGFR is capable of both high-affinity ligand binding (see e.g., FIG. 13C) and efficient dimerization (see e.g., FIG. 13D). In the activated and dimerized configuration, the dimerization arm of domain II reaches across the interface to interact primarily with the corresponding domain II arm of its dimerization partner (see e.g., FIG. 13D). EGFR dimerization also requires interaction of contact sites in modules 2 and 6 of domain III. The presence of EGF ligand and subsequent binding to domains I and III of the non-tethered form will drive the equilibrium toward the non-tethered form, trapping receptor molecules in the extended state that can dimerize.

[0077] The approach described herein provides for some retention of the basal levels of EGFR signaling. In healthy individuals there exists a baseline signal from EGFR necessary for growth, with enhanced EGF levels promoting accelerated growth in, for example, wound recovery. But cancer cells have been demonstrated to exhibit more EGFR, which increases the probability of the untethered conformation and subsequent EGF binding to the unoccluded domain I/III ligand binding site, thereby activating EGFR. An inhibitor that holds multi-domain proteins of EGFR together in a tethered, inactive state can allow for some basal levels of EGFR signaling.

[0078] Furthermore, small molecule inhibitors that prevent stabilization of the untethered state (see e.g., FIG 14B depicting untethered state, and FIG 14C depicting stabilized untethered state) of EGFR can be used in conjunction with other anti-EGFR therapeutic agents. Use of

small molecule inhibitors described herein in conjunction with other anti-EGFR therapeutic modalities can allow decreased dosage and/or increased maximal inhibition. For example, use of such small molecule inhibitors can be used in conjunction with Erbitux (which binds domain III blocking EGF), which would allow a lower dosage of Erbitux and/or increased maximal inhibition.

[0079] In various embodiments, domain II of EGFR, as existing in the tethered state, is targeted so as to prevent opening (i.e., the configuration change from tethered to open, see e.g., FIG. 13A-B depicting tethered and untethered conformations). In various embodiments, the cleft between domain II and domain IV is targeted so as to prevent opening (i.e., the configuration change from tethered to open). A single small molecule can be used to span the two domains. Alternatively, a series of small molecules (e.g., at least two small molecules) in several compartments can be used in conjunction so as to span the two domains. In various embodiments, domain II of EGFR, as existing in the untethered state, is targeted so as to prevent stabilization of the untethered state (see e.g., FIG. 13B-C, depicting untethered and stabilized conformations). In various embodiments, domain III of EGFR is targeted (e.g., modules 2 and 6 of domain III, which are interacting contact sites required for EGFR dimerization) so as to prevent stabilization of the untethered state.

PHARMACOPHORIC APPROACH

[0080] One aspect is directed to a pharmacophoric approach for developing a drug targeting a multi-domain protein having an interdomain tether associated with activation state. Based upon the activation and dimerization mechanism of a biomolecule of interest, binding targets are identified and characterized. The mechanism and/or binding target can be characterized, for example, via crystallography data. The target binding domains can be expressed as one or more pharmacophore features and/or compiled in a pharmacophore model comprising one or more pharmacophore features.

[0081] Pharmacophore generation can be according to software designed for such a task. Candidate molecules (from, for example, one or more chemical libraries) can be selected from those molecules which align to the pharmacophore models. Preferably, candidate molecules are docked and scored *in silico* for interaction with the target binding domain. Again, docking and

scoring can be according to software designed for such a task. After selection of molecules aligning to one or more pharmacophore models, with optional docking and scoring *in silico*, the selected molecules can be obtained, for example, by chemical synthesis or from a commercial source. The selected molecules can be measured for binding affinity and/or effect on function for the target biomolecule. Such assessment can be according to a biological assay. The tested molecules can be further selected according to desirable measured parameters. The selected molecules and/or the further selected molecules can optionally be further optimized.

Determining Structure Spatial Position

[0082] From the activation and dimerization model of the target biomolecule, target regions can be identified and 3D binding domains can be defined. Definition of the binding domain(s) generally involves the determination of the specific spatial position of the atoms of the portion of the target biomolecule which plays a role in the activation and dimerization mechanism.

[0083] Determination of the spatial position of the binding portion can be achieved by means of various *in silico* techniques. For example, software packages can be used that model the structure of the binding surface and match it to a model of the active surface of the target to assess levels of compatibility. Such software includes CAMAL.

[0084] Determination of the spatial position of the binding portion can be achieved by means of X-ray crystallography. X-ray crystallography can be used to determine the structure of atoms within a structure that is known to play a role in the activation and dimerization mechanism, and to then use this structural information to build a synthetic molecule that binds to one or more of these components and interferes with configuration changes and/or stabilization. Techniques for employing X-ray crystallography for structural determination are known in the art (*see e.g.*, Messerschmidt (2007) X-Ray Crystallography of Biomacromolecules: A Practical Guide, John Wiley & Sons, ISBN-10: 3527313966; Woolfson (2003) An Introduction to X-ray Crystallography, 2d Ed., Cambridge University Press, ISBN-10: 0521423597). Creation of X-ray crystal structures are also known in the art (*see e.g.*, U.S. Patent No. 6,931,325 to Wall and U.S. Patent No. 6,916,455 to Segelke, each incorporated herein by reference). Except as otherwise noted herein, therefore, the process of the present invention can be carried out in accordance with such processes.

[0085] Parameters derived from X-ray crystallography observed diffraction data include, but are not limited to, hydrogen bonders, apolar hydrophobic contacts, salt bridge interactions, polar surface area of the domain, apolar surface area of the domain, shape complementarity score for the antibody-target complex, and explicitly placed water molecules. Also useful is characterization of bonds between atoms. The distance between two atoms that are singly bonded ranges from about 1.45 to about 1.55Å. Atoms that are double bonded together are typically about 1.2 to about 1.25Å apart. Bonds that are resonant between single and double bonds typically have an about 1.30 to about 1.35Å separation.

Construction of Pharmacophores

[0086] A pharmacophore model can be constructed from structural information of biomolecule components playing a role in activation and dimerization, including definition of atom position. Small molecules with complementary features to components of the target biomolecule, such as a component playing a role in activation and dimerization, have the potential to interfere with configuration changes and/or stabilization necessary for activation and dimerization and thus have therapeutic utility.

[0087] In various embodiments, *in silico* approaches can be used for *de novo* structure design with a fragment based approach employing contact statistics, 3D surface models, and docked ligands as templates. From the spatial position information, and/or from other parameters described above, one can derive 3D ligand-receptor models (*e.g.*, interaction pattern, pharmacophore schemes), surface maps (*e.g.*, topography/shape, electrostatic profile, hydrophobicity, protein flexibility), and docking models (*e.g.*, scoring system for ligand binding, minimum energy calculation).

[0088] Techniques for pharmacophore model construction are known in the art and described extensively herein (see *e.g.*, Examples 4-5). Except as otherwise noted herein, therefore, the processes of the present invention can be carried out in accordance with such processes.

[0089] A pharmacophore model or scheme is generally a set of structural features in a ligand that are related, preferably directly related, to the ligand's recognition at a receptor site and its biological activity. Pharmacophore features can be derived from corresponding donor, acceptor, aromatic, hydrophobic, and/or acidic or basic moieties of the corresponding target biomolecule,

especially those features on domains participating in dimerization and activation mechanisms. It shall be understood that additional information about the nature of the atoms in the target biomolecule being used in a pharmacophore scheme, and not simply the spatial location of the atoms, can assist in the modeling process of a new chemical lead. These characteristics include, but are not limited to, the pKa values of the atoms, the rotational rigidity of the bonds holding the atoms in place, the nature of the bonds themselves (single, double, resonant, or otherwise), the projected directionality of hydrogen bond donors and acceptors, *etc.*

[0090] Typical feature components useful in generating a pharmacophore scheme include, but are not limited to, atomic position; atomic radii; hydrogen bond donor features; hydrogen bond acceptor features; aromatic features; donor features; acceptor features; anion features; cation features; acceptor and anion features; donor and cation features; donor and acceptor features; acid and anion features; hydrophobic features, hydrogen bond directionality, and metal ligands (*see e.g.*, Examples 4-5). Such features can be located, for example, at a single atom, centroids of atoms, or at a projected directional position in space.

[0091] It is contemplated that numerous pharmacophore queries can be designed for any given target biomolecule. It is further contemplated that these pharmacophore queries will be useful to identify small molecule ligands which interact with the target biomolecule at a site involved with dimerization and activation, especially towards maintaining a tethered, inactive conformation.

[0092] Exemplary resources for accomplishing such modeling and queries include, but are not limited to MOE (CGG) (providing pharmacophore query and visualization), Glide (Schrodinger) (providing docking and scoring), Accord for Excel (Accelrys) (providing organization of molecular information including chemical structures and formulas), and the ZINC database (UCSF) (providing a library of commercial compounds). One design tool for the generation of pharmacophores from immune system protein - target biomolecule structural binding characterization is MOE, or Molecular Operating Environment (Chemical Computing Group). Model generation uses geometrical and electronic constraints to determine the 3D positions of features corresponding to the immune system protein. The model of these embodiments consists of spherical features in 3D space. The diameter of the spheres can be

adjusted (e.g., about 0.5 to about 3.0 Å). Such models allow matches and/or partial matches of the features.

[0093] Pharmacophoric structural features can be represented by labeled points in space. Each ligand can be assigned an annotation, which is a set of structural features that may contribute to the ligand's pharmacophore (*see e.g.*, Examples 4-5). In various embodiments, a database of annotated ligands can be searched with a query that represents a pharmacophore hypothesis (*see e.g.*, Examples 6-7). The result of such a search is a set of matches that align the pharmacophoric features of the query to the pharmacophoric features present in the ligands of the searched database (*see e.g.*, Examples 6-7, Tables 11-15). The number of hits within the database depends, at least in part, upon the size of the database and the restrictiveness of the pharmacophore query (e.g., partial matches, number of features, *etc.*). Properties and parameters of the molecules present within the search database are used to focus the outcome of the query. For example, compounds with a defined range of molecular weight (MW) or lipophilicity (logP) can be present in the searched section of the library database of compounds.

Candidate Molecules

[0094] The subject methods find use in the screening of a variety of different candidate molecules (*e.g.*, potentially therapeutic candidate molecules). As described above, candidate molecules can be searched using a pharmacophore query. Candidate molecules encompass numerous chemical classes, though typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 50 and less than about 2,500 Daltons. Candidate molecules can comprise functional groups for structural interaction with proteins, particularly hydrogen bonding, and can include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The candidate molecules can comprise cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups.

[0095] In preferred embodiments, the candidate molecules are compounds in a library database of compounds. One of skill in the art will be generally familiar with, for example, numerous databases for commercially available compounds for screening (*see e.g.*, ZINC database, UCSF, with 2.7 million compounds over 12 distinct subsets of molecules; Irwin and Shoichet (2005) *J Chem Inf Model* 45, 177-182). One of skill in the art will also be familiar with

a variety of search engines to identify commercial sources or desirable compounds and classes of compounds for further testing (*see e.g.*, ZINC database; eMolecules.com; and electronic libraries of commercial compounds provided by vendors, for example: ChemBridge, Princeton BioMolecular, Ambinter SARL, Enamine, ASDI, Life Chemicals etc).

[0096] Candidate molecules for screening according to the methods described herein include both lead-like compounds and drug-like compounds. A lead-like compound is generally understood to have a relatively smaller scaffold-like structure (e.g., molecular weight of about 150 to about 350 D) with relatively fewer features (e.g., less than about 3 hydrogen donors and/or less than about 6 hydrogen acceptors; hydrophobicity character xlogP of about -2 to about 4) (*see e.g.*, Angewante (1999) *Chemie Int. ed. Engl.* 24, 3943-3948). In contrast, a drug-like compound is generally understood to have a relatively larger scaffold (e.g., molecular weight of about 150 to about 500 D) with relatively more numerous features (e.g., less than about 10 hydrogen acceptors and/or less than about 8 rotatable bonds; hydrophobicity character xlogP of less than about 5) (*see e.g.*, Lipinski (2000) *J. Pharm. Tox. Methods* 44, 235-249). Preferably, initial screening is performed with lead-like compounds.

[0097] When designing a lead from spatial orientation data, it can be useful to understand that certain molecular structures are characterized as being “drug-like”. Such characterization can be based on a set of empirically recognized qualities derived by comparing similarities across the breadth of known drugs within the pharmacopoeia. While it is not required for drugs to meet all, or even any, of these characterizations, it is far more likely for a drug candidate to meet with clinical success if it is drug-like.

[0098] Several of these “drug-like” characteristics have been summarized into the four rules of Lipinski (generally known as the “rules of fives” because of the prevalence of the number 5 among them). While these rules generally relate to oral absorption and are used to predict bioavailability of compound during lead optimization, they can serve as effective guidelines for constructing a lead molecule during rational drug design efforts such as may be accomplished by using the methods of the present invention.

[0099] The four “rules of five” state that a candidate drug-like compound should have at least three of the following characteristics: (i) a weight less than 500 Daltons; (ii) a log of P less than 5; (iii) no more than 5 hydrogen bond donors (expressed as the sum of OH and NH groups);

and (iv) no more than 10 hydrogen bond acceptors (the sum of N and O atoms). Also, drug-like molecules typically have a span (breadth) of between about 8Å to about 15Å. It will be understood that a candidate molecule, or even a selected molecule, may not meet all, or even any, of these characterizations. Nonetheless, the above guidelines are helpful in drug screening and design.

[0100] As explained above, the number of molecules identified as hits to the pharmacophore depend, at least in part, on the size of the database and the restrictiveness of the pharmacophore query. The number of molecules identified as hits from a pharmacophore query can be reduced by further modeling of fit to the binding site of the target biomolecule. Such modeling can be according to docking and scoring methods, as described below.

Docking and Scoring

[0101] Candidate molecules identified as being complementary to certain features of a target biomolecule as compared to a pharmacophore model (*e.g.*, through a pharmacophore query as described above) can be further selected according to docking affinity for the target biomolecule (*see e.g.*, Examples 6-7). In addition to pharmacophore model generation for database queries, a second sequential and complementary method for compound identification and design can be employed. Pharmacophore queries can filter out compounds quickly and docking and scoring can evaluate ligand-target biomolecule binding more accurately. In the case of protein or enzyme target biomolecules, amino acid residues of different domains in an inactive conformation can be used to define the docking site.

[0102] In various embodiments, selected compounds from the pharmacophore queries are docked to the target binding site using software designed for such analysis (*e.g.*, Glide (Schrodinger, NY). Docking affinity can be calculated as numerical values (*e.g.*, “Glide score”) based upon, for example, energy gained upon interaction of the molecule with the protein (*e.g.*, “g_score”) and/or energy required to attain the docked conformation relative to the lowest energy conformation (*e.g.*, “e_model”) (*see e.g.*, Examples 6-7). For these particular examples, the more negative the score, the better the docking. Preferably, the g_score is less than about -5. Preferably, the e_model score is less than about -30. It is contemplated that the desirable numerical quantification of docking can vary between different target biomolecules.

[0103] In various embodiments, a threshold docking score (e.g., g_score and/or e_model score) can be chosen so as to manage the number of molecules for acquisition and further testing. For example, in some docking studies, a g-score of negative 5.0 (or greater magnitude in a negative direction) is considered a desirable docking score and the cut off is adjusted accordingly. As another example, in some docking studies, a g-score of negative 7.5 (or greater magnitude in a negative direction) is considered a desirable docking score and the cut off is adjusted accordingly. Thus, the magnitude of the g_score can be used to adjust a number of hits to a workable number that can be acquired and tested. As an example, if the total number of compounds identified from a pharmacophore query was about 1,000 to about 3,000, the docking scores can be used to rank such compounds so as to select about 100 to about 200 for further testing. It is contemplated the number of compounds to be selected for further testing could be lower or higher than these estimates. Preferably, magnitude of the g_score is used as a selection criteria, but it is contemplated that e_model score could be similarly used, especially where e_model score is of low magnitude. It is further contemplated that the selection criteria can be based upon both g_score and e_model score, preferably weighted toward g_score.

[0104] Docking and scoring can result in a group of compounds with multiple conformers. Using suitable modeling software (e.g., MOE), 3D structures can be converted to 2D and duplicates thereby removed. The resulting list of preferred chemical structures can be used to search for commercial vendors using, for example, search engines designed for such a task (e.g., eMolecules.com).

Effect on Target Biomolecule

[0105] Candidate molecules selected according to pharmacophore query and/or further selected according to docking analysis can be tested for effect on the target biomolecule. Assessment of effect of a molecule on biomolecule function (e.g., inhibition of enzymatic activity) can be assessed by various methods known in the art (see e.g., Examples 1-3). For example, inhibitory effect of a candidate molecule on the catalytic activity of a target enzyme can be assessed by known activity assays specific for the target enzyme (*see e.g.*, Reymond, ed. (2006) *Enzyme Assays: High-throughput Screening, Genetic Selection and Fingerprinting*, John Wiley & Sons, 386 p., ISBN-10: 3527310959; Eienthall and Danson, Ed. (2002) *Enzyme Assays*, 2d edition, Oxford University Press, 384 p., ISBN-10: 0199638209). As described

herein, an in-cell Western (ICW) screening protocol can be used to evaluate candidate compounds (see e.g., Example 1; Chen et al. (2005) *Analytical Biochemistry* 338, 136-142). Also as described herein, a MTT Cell Proliferation Assay can be used to evaluate candidate compounds (see e.g., Example 2). Also as described herein, an EGF inhibitor assay can be used to evaluate candidate compounds (see e.g., Example 3; Mukku (1984) *J. Biol. Chem.* 259, 6543-6546; Duh et al. (1990) *World J. Surgery* 14, 410-418; Lokeshwar et al. (1989) *J. Biol. Chem.* 264(32), 19318-19326).

Further Refinement

[0106] Further refinement of candidate molecules can be conducted. For example, data from biological assays can be correlated with the docking model so as to further refine lead-like molecules and/or drug-like molecules. Various software packages (e.g., MOE) can be employed to visualize active compound interaction with a target biomolecule to identify sites on the template suitable for modification by *de novo* design. Analogs of active compounds can be identified using similarity and sub-structure searches (*see e.g.*, SciFinder; eModel). Available analogs can be analyzed according to docking and scoring procedures described above. Analogs with desirable docking scores can be acquired and further tested for biological effect on the target biomolecule according to methods described herein. One skilled in the art will understand these, and other, methods of refining and further developing candidate molecules identified by the methods presented herein.

PHARMACOPHORES

[0107] Provided herein are a series of pharmacophores that can be used to identify small molecules that can substantially maintain a non-extended tether inactive configuration of EGFR or substantially prevent stabilization of an extended tether active configuration of EGFR. Pharmacophores include, but are not limited to, a Scheme II pharmacophore (AD4-1734-like), a Scheme III pharmacophore (AD4-1886-like), a Scheme IV pharmacophore (AD4-10381-like), and Scheme V pharmacophore (AD4-11091-like).

[0108] Scheme II pharmacophore (AD4-1734-like)

[0109] A Scheme II pharmacophore (AD4-1734-like) can include functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9.

[0110] Functional group F(II)1 donates an H-bond or forms a salt bridge to a carboxylate side chain of receptor Asp553 of SEQ ID NO: 1 and has coordinates of $r = 56.363$, θ (theta) = 94.368 , and Φ (phi) = -17.752 and a spherical radius of about $1.2\text{\AA}$,

[0111] Functional group F(II)2 is a donor and has coordinates of $r = 53.290$, θ (theta) = 101.494 , and Φ (phi) = -23.244 and a spherical radius of about $1.0\text{\AA}$.

[0112] Functional group F(II)3 forms a hydrophobic contact with a side chain of receptor Val568, imidazolidine side chain of receptor Pro552 and with a side chain of Met253 of SEQ ID NO: 1 and has coordinates of $r = 53.726$, θ (theta) = 97.830 , and Φ (phi) = -18.377 and a spherical radius of about $1.7\text{\AA}$.

[0113] Functional group F(II)4 forms a hydrophobic contact with side chain of receptor Val575, Met253, and with an imidazolidine ring of receptor Pro552 of SEQ ID NO: 1 and has coordinates of $r = 53.647$, θ (theta) = 103.844 , and Φ (phi) = -20.990 and a spherical radius of about $1.4\text{\AA}$.

[0114] Functional group F(II)5 donates an H-bond to a side chain hydroxyl of Thr570 of SEQ ID NO: 1 and has coordinates of $r = 51.093$, θ (theta) = 104.261 , and Φ (phi) = -25.552 and a spherical radius of about $1.2\text{\AA}$.

[0115] Functional group F(II)6 is a donor having directionality of F4 with respect to a backbone carbonyl of receptor Thr570 of SEQ ID NO: 1 and has coordinates of $r = 52.340$, θ (theta) = 103.980 , and Φ (phi) = -27.461 and a spherical radius of about $1.5\text{\AA}$.

[0116] Functional group F(II)7 accepts an H-bond from a receptor backbone NH of Ala573 of SEQ ID NO: 1 and has coordinates of $r = 51.383$, θ (theta) = 106.455 , and Φ (phi) = -24.319 and a spherical radius of about $1.2\text{\AA}$.

[0117] Functional group F(II)8 is an acceptor having directionality of F7 with respect to the backbone NH of receptor Ala573 of SEQ ID NO: 1 and has coordinates of $r = 52.861$, θ (theta) = 107.691 , and Φ (phi) = -25.448 and a spherical radius of about $1.5\text{\AA}$.

[0118] Functional group F(II)9 donates an H-bond to a backbone carbonyl of Asp563 and forms a salt bridge to a side chain carboxylate of receptor Asp563 of SEQ ID NO: 1 and has coordinates of $r = 57.688$, θ (theta) = 99.198, and Φ (phi) = -21.588 and a spherical radius of about 1.2Å.

[0119] A selected candidate compound can substantially align with at least one of functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9. For example, a selected candidate compound can substantially align with at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine of functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9. Preferably, a selected candidate compound can substantially align with at least five of functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9.

[0120] Scheme III pharmacophore (AD4-1886-like)

[0121] A Scheme III pharmacophore (AD4-1886-like) can include functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9.

[0122] Functional group F(III)1 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 and accepts a hydrogen bond from the side chain NH₂ of receptor Gln384 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243, and Φ (phi) = 45.369 and a spherical radius of about 1.2Å.

[0123] Functional group F(III)2 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 87.287$, θ (theta) = 40.739, and Φ (phi) = 51.781 and a spherical radius of about 1.2Å.

[0124] Functional group F(III)3 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 86.320$, θ (theta) = 41.915, and Φ (phi) = 52.323 and a spherical radius of about 1.5Å.

[0125] Functional group F(III)4 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.870$, θ (theta) = 38.463, and Φ (phi) = 41.650 and a spherical radius of about 1.2Å.

[0126] Functional group F(III)5 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 82.241$, θ (theta) = 37.431 , and Φ (phi) = 44.151 and a spherical radius of about $1.5\text{\AA}$.

[0127] Functional group F(III)6 accepts a hydrogen bond from, or forms a salt bridge to, NH^{3+} of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 88.009$, θ (theta) = 37.822 , and Φ (phi) = 54.903 and a spherical radius of about $1.2\text{\AA}$.

[0128] Functional group F(III)7 accepts a hydrogen bond from, or forms a salt bridge to, NH^{3+} of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.513$, θ (theta) = 36.889 , and Φ (phi) = 54.484 and a spherical radius of about $1.5\text{\AA}$.

[0129] Functional group F(III)8 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243 , and Φ (phi) = 45.369 and a spherical radius of about $1.2\text{\AA}$.

[0130] Functional group F(III)9 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 79.652$, θ (theta) = 40.928 , and Φ (phi) = 44.528 and a spherical radius of about $1.6\text{\AA}$.

[0131] A selected candidate compound can substantially align with at least one of functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9. For example, a selected candidate compound can substantially align with at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, or at least nine of functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9. Preferably, a selected candidate compound can substantially align with at least seven of functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9.

[0132] Scheme IV pharmacophore (AD4-10381-like)

[0133] A Scheme IV pharmacophore (AD4-10381-like) can include functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10.

[0134] Functional group F(IV)1 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 49.686$, θ (theta) = 113.993 , and Φ (phi) = -17.014 and a spherical radius of about $1.2\text{\AA}$.

[0135] Functional group F(IV)2 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 48.071$, θ (theta) = 115.388, and Φ (phi) = -16.211 and a spherical radius of about 1.6Å.

[0136] Functional group F(IV)3 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.781$, θ (theta) = 113.121, and Φ (phi) = -17.520 and a spherical radius of about 1.2Å.

[0137] Functional group F(IV)4 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 52.021$, θ (theta) = 114.264, and Φ (phi) = -15.878 and a spherical radius of about 1.5Å.

[0138] Functional group F(IV)5 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.322$, θ (theta) = 111.253, and Φ (phi) = -15.426 and a spherical radius of about 1.2Å.

[0139] Functional group F(IV)6 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 51.575$, θ (theta) = 112.433, and Φ (phi) = -13.827 and a spherical radius of about 1.5Å.

[0140] Functional group F(IV)7 forms a hydrophobic contact with a side chain of receptor Leu243 and a side chain of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 47.767$, θ (theta) = 112.521, and Φ (phi) = -10.196 and a spherical radius of about 1.2Å.

[0141] Functional group F(IV)8 donates a hydrogen bond to a backbone carbonyl of His280 of SEQ ID NO: 1 and has coordinates of $r = 45.184$, θ (theta) = 112.328, and Φ (phi) = -8.875 and a spherical radius of about 1.2Å.

[0142] Functional group F(IV)9 forms a hydrophobic contact with a side chain of receptor Met244 and Leu243 of SEQ ID NO: 1 and has coordinates of $r = 49.512$, θ (theta) = 108.007, and Φ (phi) = -8.504 and a spherical radius of about 1.2Å.

[0143] Functional group F(IV)10 forms a hydrophobic contact with a side chain of receptor Met244 of SEQ ID NO: 1 and has coordinates of $r = 50.431$, θ (theta) = 104.985, and Φ (phi) = -7.420 and a spherical radius of about 1.8Å.

[0144] A selected candidate compound can substantially align with at least one of functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10. For example, a selected candidate compound can substantially align with at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, or at least ten of functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10. Preferably, a selected candidate compound can substantially align with at least eight of functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10.

[0145] Scheme V pharmacophore (AD4-11091-like)

[0146] A Scheme V pharmacophore (AD4-11091-like) can include functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11.

[0147] Functional group F(V)1 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 85.329$, θ (theta) = 34.962 , and Φ (phi) = 53.394 and a spherical radius of about $1.2\text{\AA}$.

[0148] Functional group F(V)2 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 87.180$, θ (theta) = 35.618 , and Φ (phi) = 53.267 and a spherical radius of about $1.5\text{\AA}$.

[0149] Functional group F(V)3 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.349$, θ (theta) = 34.560 , and Φ (phi) = 55.424 and a spherical radius of about $1.5\text{\AA}$.

[0150] Functional group F(V)4 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.958$, θ (theta) = 35.296 , and Φ (phi) = 51.296 and a spherical radius of about $1.2\text{\AA}$.

[0151] Functional group F(V)5 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.060$, θ (theta) = 34.984 , and Φ (phi) = 50.365 and a spherical radius of about $1.5\text{\AA}$.

[0152] Functional group F(V)6 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.884$, θ (theta) = 36.166 , and Φ (phi) = 49.227 and a spherical radius of about $1.2\text{\AA}$.

[0153] Functional group F(V)7 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.006$, θ (theta) = 35.523 , and Φ (phi) = 49.076 and a spherical radius of about $1.5\text{\AA}$.

[0154] Functional group F(V)8 accepts a hydrogen bond to a side chain imidazole of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.100$, θ (theta) = 38.590 , and Φ (phi) = 43.143 and a spherical radius of about $1.2\text{\AA}$.

[0155] Functional group F(V)9 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 84.418$, θ (theta) = 37.489 , and Φ (phi) = 46.968 and a spherical radius of about $1.2\text{\AA}$.

[0156] Functional group F(V)10 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 82.486$, θ (theta) = 38.027 , and Φ (phi) = 47.246 and a spherical radius of about $1.5\text{\AA}$. and

[0157] Functional group F(V)11 forms a favorable hydrophobic interaction with a side chain of receptors Val417 and Ile448 of SEQ ID NO: 1 and has coordinates of $r = 84.438$, θ (theta) = 40.753 , and Φ (phi) = 48.557 and a spherical radius of about $1.2\text{\AA}$.

[0158] Functional group F11 forms a favorable hydrophobic interaction with a side chain of receptors Val417 and Ile448 of SEQ ID NO: 1 and has coordinates of $r = 84.438$, θ (theta) = 40.753 , and Φ (phi) = 48.557 and a spherical radius of about $1.2\text{\AA}$.

[0159] A selected candidate compound can substantially align with at least one of functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11. For example, a selected candidate compound can substantially align with at least two, at least three, at least four, at least five, at least six, at least seven, at least eight, at least nine, at least ten, or at least eleven of functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11. Preferably, a selected candidate compound can substantially align with at least nine of functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11.

[0160] One aspect provides a method for identifying an epidermal growth factor receptor (EGFR) inhibitor comprising: providing a pharmacophore comprising any one of Schemes II, III, IV, or V as input to a 3-dimensional database; comparing a three dimensional structure of a

candidate compound to the three dimensional structure of the pharmacophore; selecting a candidate compound with a three dimensional structure that substantially aligns with six or more functional groups of any one of Schemes II, III, IV, or V; wherein, similarity between the three-dimensional structure of the candidate compound and the three-dimensional structure of the pharmacophore is indicative of an ability of the candidate compound to inhibit EGFR by substantially maintaining a tethered inactive configuration of EGFR or substantially preventing stabilization of the untethered active configuration of EGFR.

[0161] In some embodiments, the method further comprises determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation; and constructing a pharmacophore, wherein the pharmacophore comprises a plurality of pharmacophoric features that approximates the identity and the spatial orientation of the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation such that the pharmacophore structural features are complementary to the inactive EGFR configuration.

[0162] In some embodiments, determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation comprises analysis of X-ray crystallographic data derived from a crystalline form of EGFR in an inactive, tethered conformation.

[0163] In some embodiments, at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of domain II of EGFR in a tethered inactive conformation. In some embodiments, at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of a cleft region between domain II and domain IV of EGFR in a tethered inactive conformation.

[0164] In some embodiments, the method further comprises determining a docking affinity of the candidate molecule for the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation; wherein docking affinity is quantified by energy gained upon interaction of the candidate molecule with the target biomolecule, energy required to attain the docked conformation relative to the lowest energy conformation, or a combination thereof.

COMPOUNDS

[0165] Another aspect of the present invention includes small molecule compounds, identified by the methods described herein. Compounds described herein can have an anti-proliferative effect useful in, for example, treating a proliferative disease, disorder, or condition. Compounds described herein can be useful for the treatment of diseases, disorders, or conditions related to a target biomolecule according to which they were identified from. Various embodiments of compounds described herein can hold multiple domain proteins in a tethered, inactive state. For example, it is well known that inhibition of growth factor proteins has a benefit in treatment of certain conditions in oncology. As another example, inhibition of EGFR has a benefit in treatment of certain conditions associated with EGFR, as discussed further below. Compounds described herein can have an EGFR inhibitory effect useful in, for example, treating a proliferative disease or disorder associated with EGFR. Compounds described herein have been demonstrated to be empirically effective in treating proliferative diseases and conditions.

[0166] Various compounds, including AD4-1734, AD4-1886, AD4-10381, and AD4-10381, were identified as EGFR inhibitors through the pharmacophoric approach described herein (see e.g., Examples 4-5). Such compounds, and derivatives thereof, have utility as therapeutic agents for treatment of proliferative diseases or conditions. For example, compounds described herein can be used as a therapeutic agent for the treatment of an EGFR-associated disease, disorder, or condition. Analogs and derivatives of such compounds are expected to have the same or similar anti-proliferative effects and utility (see e.g., Examples 6-7). Identified compounds and analogs and derivatives thereof are further discussed below.

[0167] While under no obligation to provide an underlying mechanism and in no way limiting the present invention by doing so, it is presently thought that at least a portion of activity of compounds described herein arise from inhibition of EGFR. It is further contemplated that the presently described compounds may have additional modes of action in their effectiveness in treating a proliferative disease, disorder, or condition. Regardless of the underlying mechanism, compounds described herein have been demonstrated to be empirically effective in treating proliferative diseases and conditions.

[0168] The following definitions are provided to better define the present disclosure. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0169] The expression "alkyl", unless specifically limited, denotes a C₁₋₁₂ alkyl group, suitably a C₁₋₆ alkyl group, e.g. C₁₋₄ alkyl group. Alkyl groups may be straight chain or branched. Suitable alkyl groups include, for example, methyl, ethyl, propyl (e.g. n-propyl and isopropyl), butyl (e.g. n-butyl, iso-butyl, sec-butyl and tert-butyl), pentyl (e.g. n-pentyl), hexyl (e.g. n-hexyl), heptyl (e.g. n-heptyl) and octyl (e.g. n-octyl). The expression "alk", for example in the expressions "alkoxy", "haloalkyl" and "thioalkyl" should be interpreted in accordance with the definition of "alkyl". Exemplary alkoxy groups include methoxy, ethoxy, propoxy (e.g. n-propoxy), butoxy (e.g. n-butoxy), pentoxy (e.g. n-pentoxy), hexoxy (e.g. n-hexoxy), heptoxy (e.g. n-heptoxy) and octoxy (e.g. n-octoxy).

[0170] The expression "cycloalkyl", unless specifically limited, denotes a C₃₋₁₀ cycloalkyl group (i.e., 3 to 10 ring carbon atoms), more suitably a C₃₋₈ cycloalkyl group, for example, a C₃₋₆ cycloalkyl group. Exemplary cycloalkyl groups include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl and cyclooctyl. A preferred number of ring carbon atoms is three to six.

[0171] The expression "aryl", unless specifically limited, denotes a C₆₋₁₂ aryl group, suitably a C₆₋₁₀ aryl group, more suitably a C₆₋₈ aryl group. Aryl groups will contain at least one aromatic ring (e.g. one, two or three rings). An example of a typical aryl group with one aromatic ring is phenyl. An example of a typical aryl group with two aromatic rings is naphthyl.

[0172] The expression "heteroaryl", unless specifically limited, denotes an aryl residue, wherein one or more (e.g., 1, 2, 3, or 4, suitably 1, 2 or 3) ring atoms are replaced by heteroatoms selected from N, S and O, or else a 5-membered aromatic ring containing one or more (e.g., 1, 2, 3, or 4, suitably 1, 2 or 3) ring atoms selected from N, S and O. Exemplary monocyclic heteroaryl groups having one heteroatom include: five membered rings (e.g., pyrrole, furan, thiophene); and six membered rings (e.g., pyridine, such as pyridin-2-yl, pyridin-3-yl and pyridin-4-yl). Exemplary monocyclic heteroaryl groups having two heteroatoms include: five membered rings (e.g., pyrazole, oxazole, isoxazole, thiazole, isothiazole, imidazole, such as imidazol-1-yl, imidazol-2-yl imidazol-4-yl); six membered rings (e.g., pyridazine, pyrimidine, pyrazine). Exemplary monocyclic heteroaryl groups having three heteroatoms include: 1,2,3-

triazole and 1,2,4-triazole. Exemplary monocyclic heteroaryl groups having four heteroatoms include tetrazole. Exemplary bicyclic heteroaryl groups include: indole (e.g., indol-6-yl), benzofuran, benzthiophene, quinoline, isoquinoline, indazole, benzimidazole, benzthiazole, quinazoline and purine.

[0173] A saturated group is generally understood as having no double or triple bonds. For example, in a saturated linear hydrocarbon, each carbon atom is attached to two hydrogen atoms, except those at the ends of the chain, which bear three hydrogen atoms. For example, an unsaturated hydrocarbon is generally understood as a carbon structure containing one or more double or triple bonds.

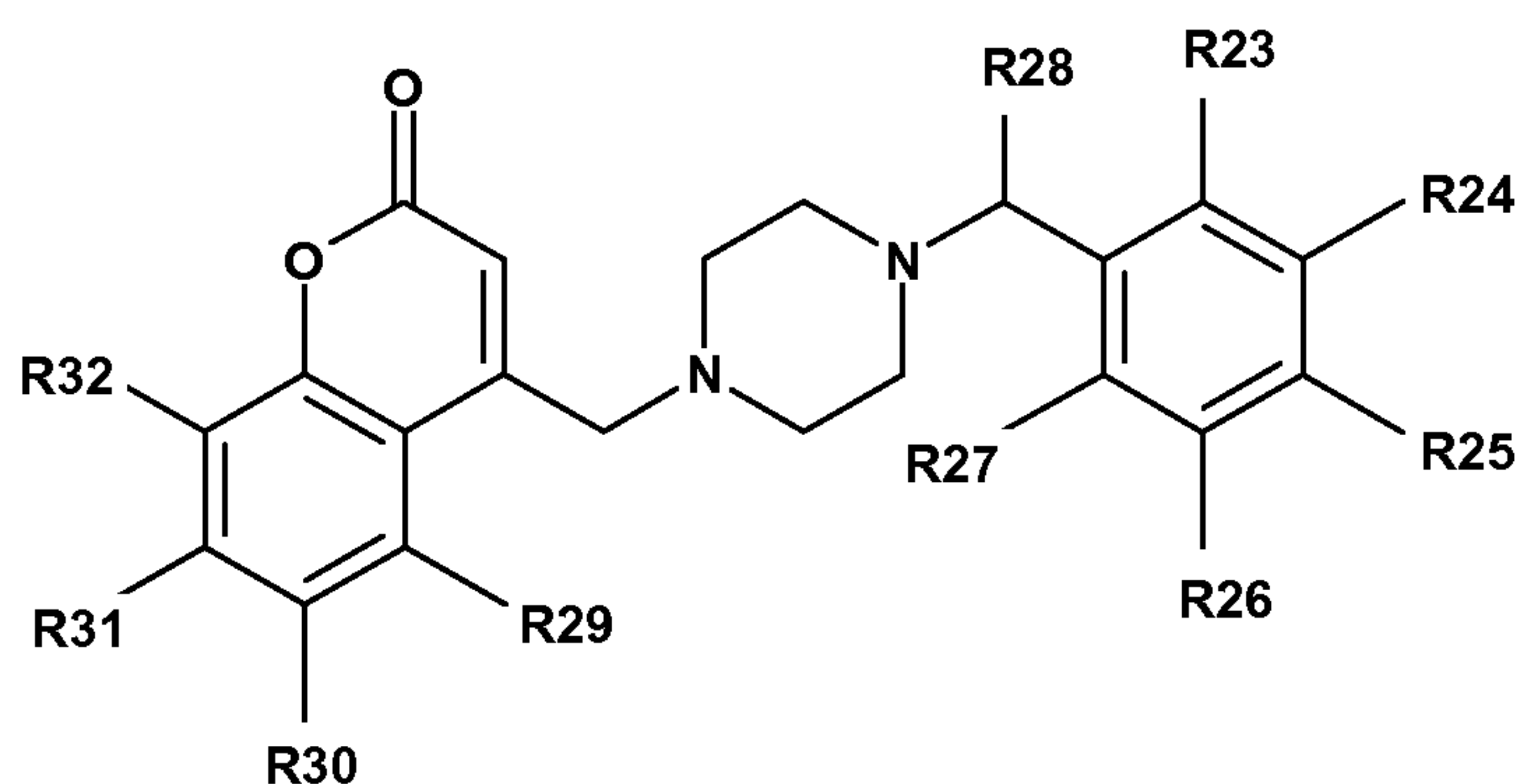
[0174] The term “halogen” or “halo” includes fluorine (F), chlorine (Cl) bromine (Br) or iodine (I).

[0175] The term “amino” refers to the group -NH₂.

[0176] All possible stereoisomers of the claimed compounds are included in the present disclosure. Where a compound described herein has at least one chiral center, it may accordingly exist as enantiomers. Where a compound possess two or more chiral centers it may additionally exist as diastereomers. It is to be understood that all such isomers and mixtures thereof are encompassed within the scope of the present disclosure.

[0177] In view of the close relationship between the free compounds and the compounds in the form of their salts, whenever a compound is referred to in this context, a corresponding salt is also intended, provided such is possible or appropriate under the circumstances. The pharmaceutically acceptable salt can take a form in which a basic side chain is protonated with an inorganic or organic acid. Representative organic or inorganic acids include hydrochloric, hydrobromic, perchloric, sulfuric, nitric, phosphoric, acetic, propionic, glycolic, lactic, succinic, maleic, fumaric, malic, tartaric, citric, benzoic, mandelic, methanesulfonic, hydroxyethanesulfonic, benzenesulfonic, oxalic, pamoic, 2-naphthalenesulfonic, p-toluenesulfonic, cyclohexanesulfamic, salicylic, saccharinic or trifluoroacetic acid. Alternatively it may take the form in which an acidic side chain forms a salt with a metal ion (e.g., sodium, potassium ions and the like) or other positive ion such as ammonium. All pharmaceutically acceptable acid addition salt forms of the compounds described herein are intended to be embraced by the scope of this disclosure.

[0185] One structure derived from the AD4-1734-like pharmacophore is “Type P” as follows:



Formula (13)

[0186] In the above structure, R^{23} and R^{27} of Formula (13) can be hydrogen.

[0187] R^{24} and R^{26} of Formula (13) can be independently selected from represent hydrogen, lower alkyl (one to six carbon, straight chain, branched, or optionally containing unsaturation), alkoxy ($-OR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), or halogen (F, Cl, Br, or I). Preferably, R^{24} and R^{26} of Formula (13) are independently selected from hydrogen or alkoxy.

[0188] R^{25} of Formula (13) can represent hydrogen, lower alkyl, cycloalkyl, substituted alkyl, alkoxy or halogen. Preferably, R^{25} of Formula (13) is selected from hydrogen or alkoxy. Substituted alkyl is defined as a hydrocarbon chain with 1-3 carbons which is substituted with additional functional groups from the following list: carboxyl defined as $-COOR^{10}$, (R^{10} is a lower alkyl group or cycloalkyl group as in the above definition), aryl, aryloxy ($-OAr$). Aryl is defined as an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more of the following groups: lower alkyl defined as C-1 to C-4, straight chain, branched, or optionally containing unsaturation; cycloalkyl, which is defined as C-1 to C-6 optionally containing unsaturation; Aryl including phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; Alkoxy ($-OR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition); Trifluoromethyl, Trifluoromethoxy, Difluoromethoxy, 3, 4-methylenedioxy, 2, 3-methylenedioxy, Nitro or Halogen (F, Cl, Br, I).

[0189] R^{28} of Formula (13) can be selected from hydrogen, lower alkyl or aryl. Preferably, R^{28} of Formula (13) is selected from hydrogen or aryl. More preferably, R^{28} of Formula (13) is hydrogen. Aryl represents an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-,

4-, 5- or 6-position with one or more of the following groups: lower alkyl defined as above, cycloalkyl defined as above, or halogen.

[0190] R^{29} of Formula (13) can be selected from hydrogen, lower alkyl, hydroxy, alkoxy or halogen. Lower alkyl, alkoxy, and halogen are as defined above.

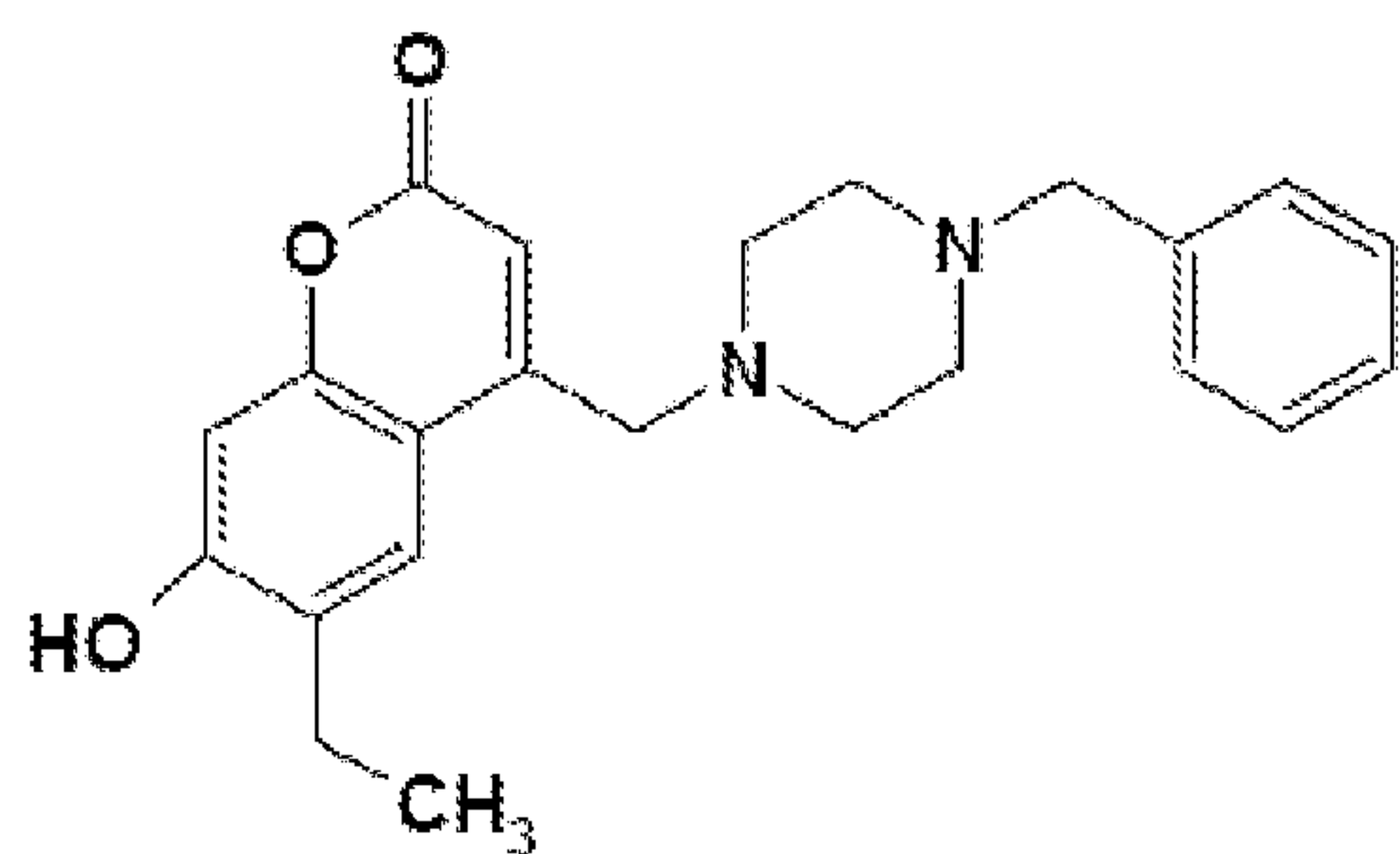
[0191] R^{30} of Formula (13) can be selected from hydrogen, lower alkyl, alkoxy, trifluoromethyl, or halogen. Preferably, R^{30} of Formula (13) is lower alkyl. Lower alkyl, alkoxy, and halogen are as defined above.

[0192] R^{31} of Formula (13) can be selected from hydrogen, hydroxy, alkoxy, lower alkyl, trifluoromethyl or halogen. Preferably, R^{31} of Formula (13) is selected from hydroxy or alkoxy. Lower alkyl, alkoxy, and halogen are as defined above.

[0193] R^{32} of Formula (13) can be selected from hydrogen, lower alkyl, hydroxy, trifluoromethyl or halogen. Preferably, R^{32} of Formula (13) is hydrogen. Lower alkyl and halogen are as defined above.

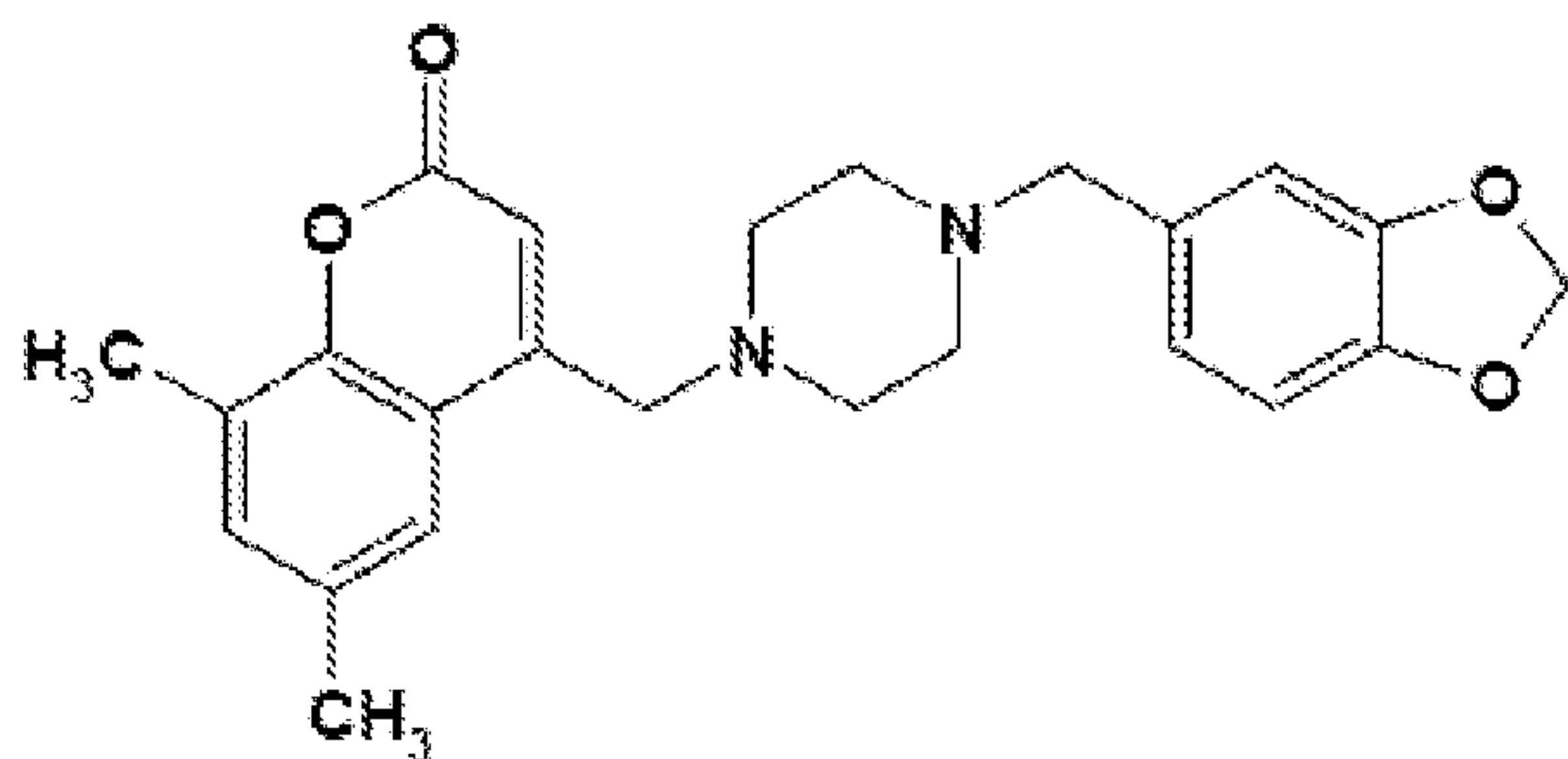
[0194] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (13).

[0195] In some embodiments, the compound of Formula (13) is AD4-10631.



AD4-10631

[0196] In some embodiments, the compound of Formula (13) is AD4-10174.

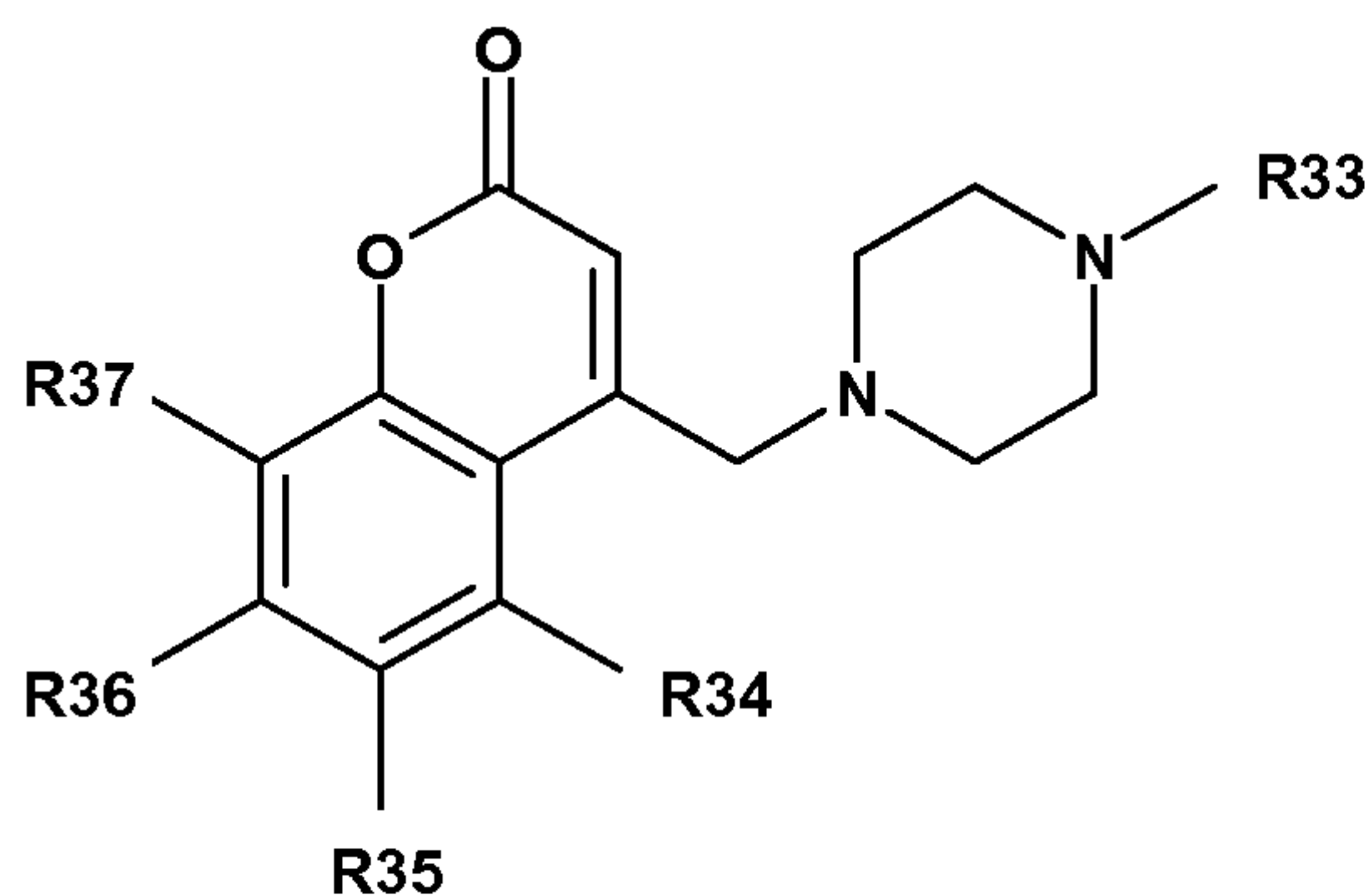


AD4-10174

[0197] In some embodiments, the compound(s) of Formula (13) excludes compound AD4-1734, Formula (12).

[0198] Type Q AD4-1734-like

[0199] One structure derived from the AD4-1734-like pharmacophore is “Type Q” as follows:



Formula (14)

[0200] In the above structure, R³³ of Formula (14) can be selected from lower alkyl, substituted lower alkyl, or aryl. Preferably R³³ of Formula (14) is aryl. Lower alkyl, substituted lower alkyl, and aryl are as defined above.

[0201] R³⁴ of Formula (14) can be selected from hydrogen.

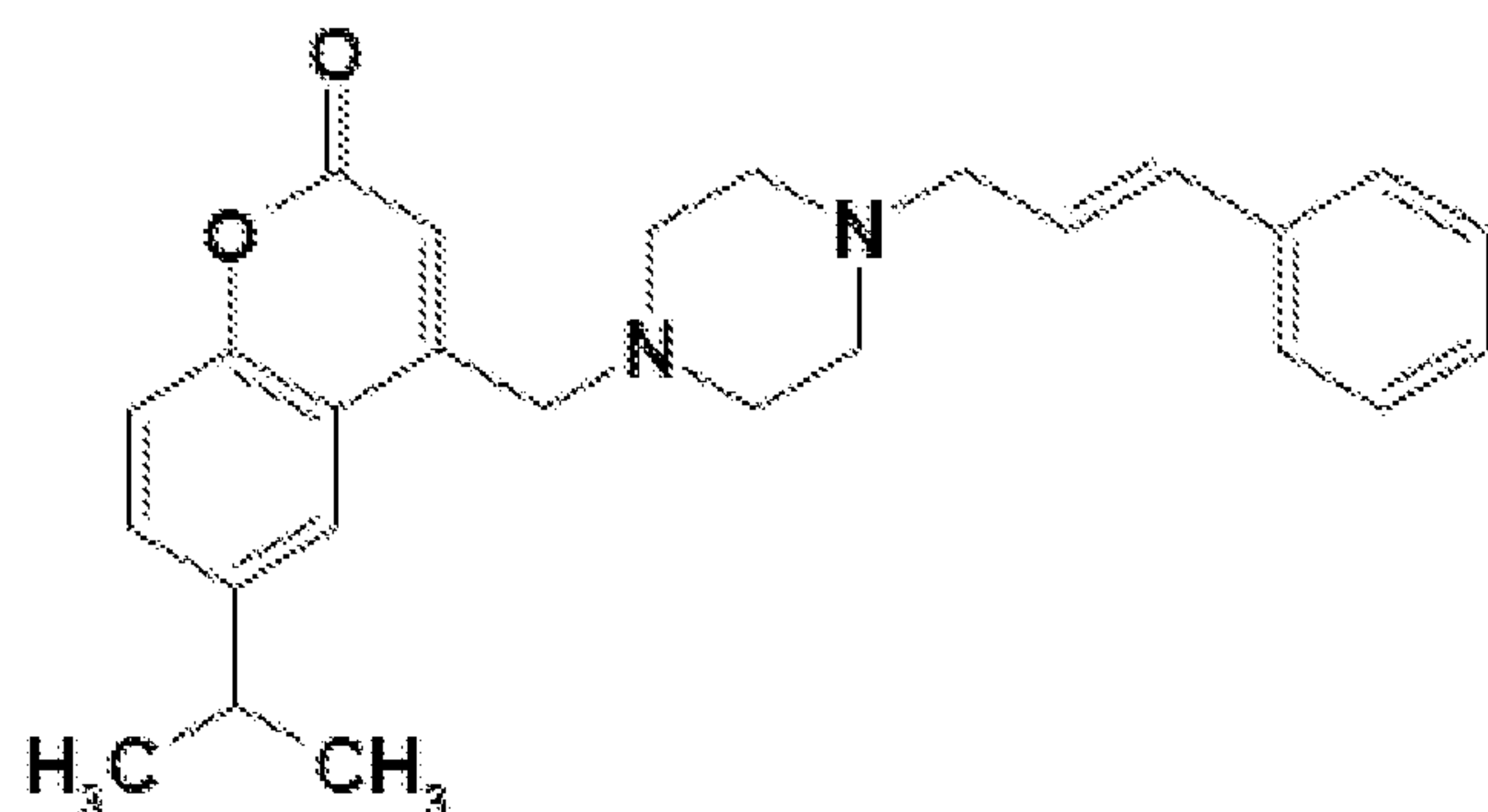
[0202] R³⁵ of Formula (14) can be selected from hydrogen, hydroxy, alkoxy, lower alkyl, trifluoromethyl or halogen. Preferably R³⁵ of Formula (14) is lower alkyl. Lower alkyl, alkoxy, and halogen are as defined above.

[0203] R³⁶ of Formula (14) can be selected from hydrogen, lower alkyl, hydroxy, alkoxy, trifluoromethyl or halogen. Preferably, R³⁶ of Formula (14) is selected from hydroxy or alkoxy. Lower alkyl and halogen are as defined above.

[0204] R³⁷ of Formula (14) can be selected from hydrogen.

[0205] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (14).

[0206] In some embodiments, the compound of Formula (14) is AD4-10188.

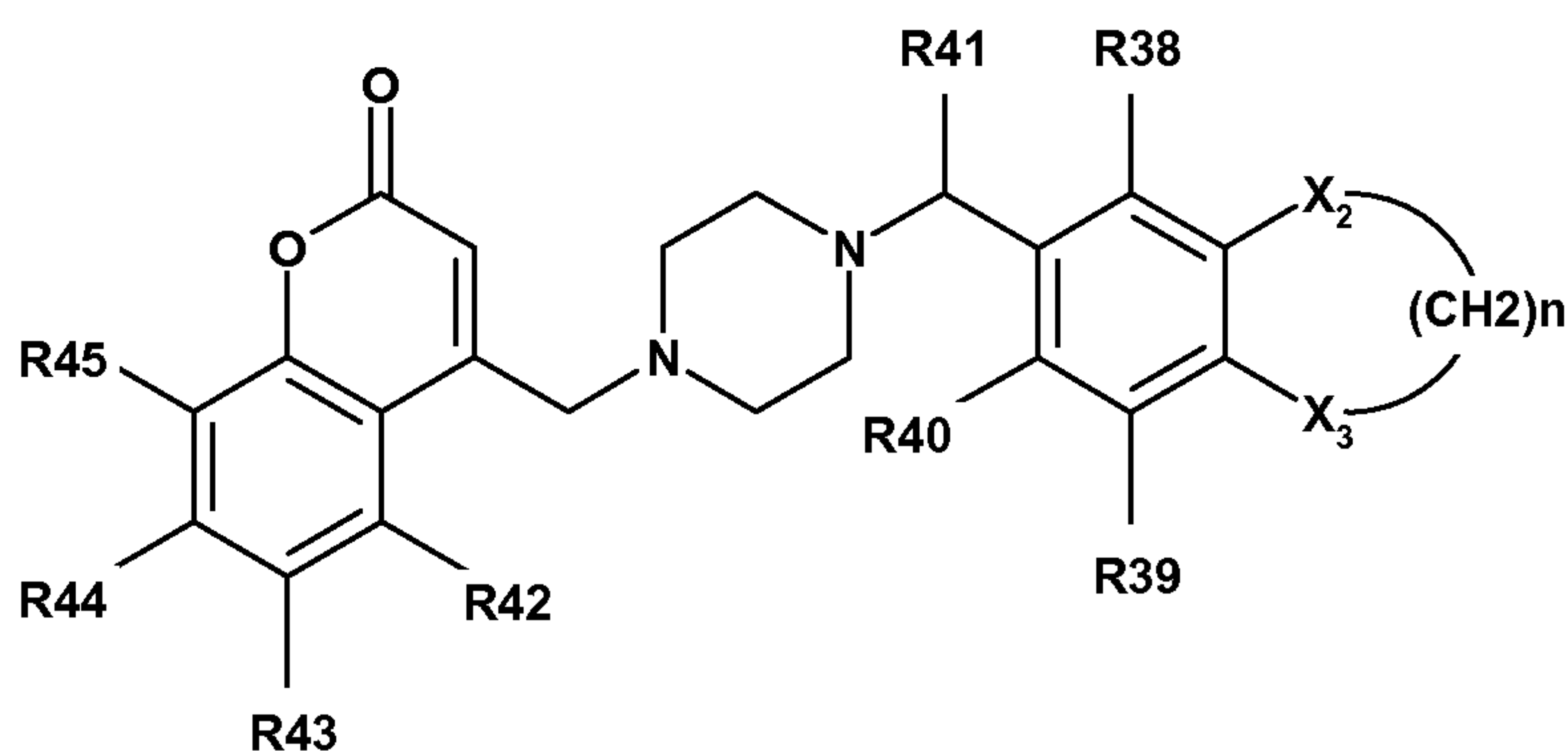


AD4-10188

[0207] In some embodiments, the compound(s) of Formula (14) excludes compound AD4-1734, Formula (12).

[0208] Type R AD4-1734-like

[0209] One structure derived from the AD4-1734-like pharmacophore is “Type R” as follows:



Formula (15)

[0210] In the above structure, X^2 and X^3 of Formula (15) can be independently selected from carbon, oxygen, nitrogen or sulfur atom. Preferably, X^2 and X^3 of Formula (15) are independently selected from oxygen or carbon. In Formula (15), n can be one or two resulting in a 5 or 6 membered ring. Preferably, n of Formula (15) is $n = 1$.

[0211] R^{38} of Formula (15) can be selected from hydrogen.

[0212] R^{39} of Formula (15) can be as defined for R^{24} .

[0213] R^{40} of Formula (15) can be as defined for R^{23} .

[0214] R^{41} of Formula (15) can be selected from hydrogen, lower alkyl or aryl.

[0215] R^{42} of Formula (15) can be selected from hydrogen, lower alkyl, hydroxy, alkoxy, or halogen. Preferably, R^{42} of Formula (15) is hydrogen. Lower alkyl, alkoxy and halogen are as defined above.

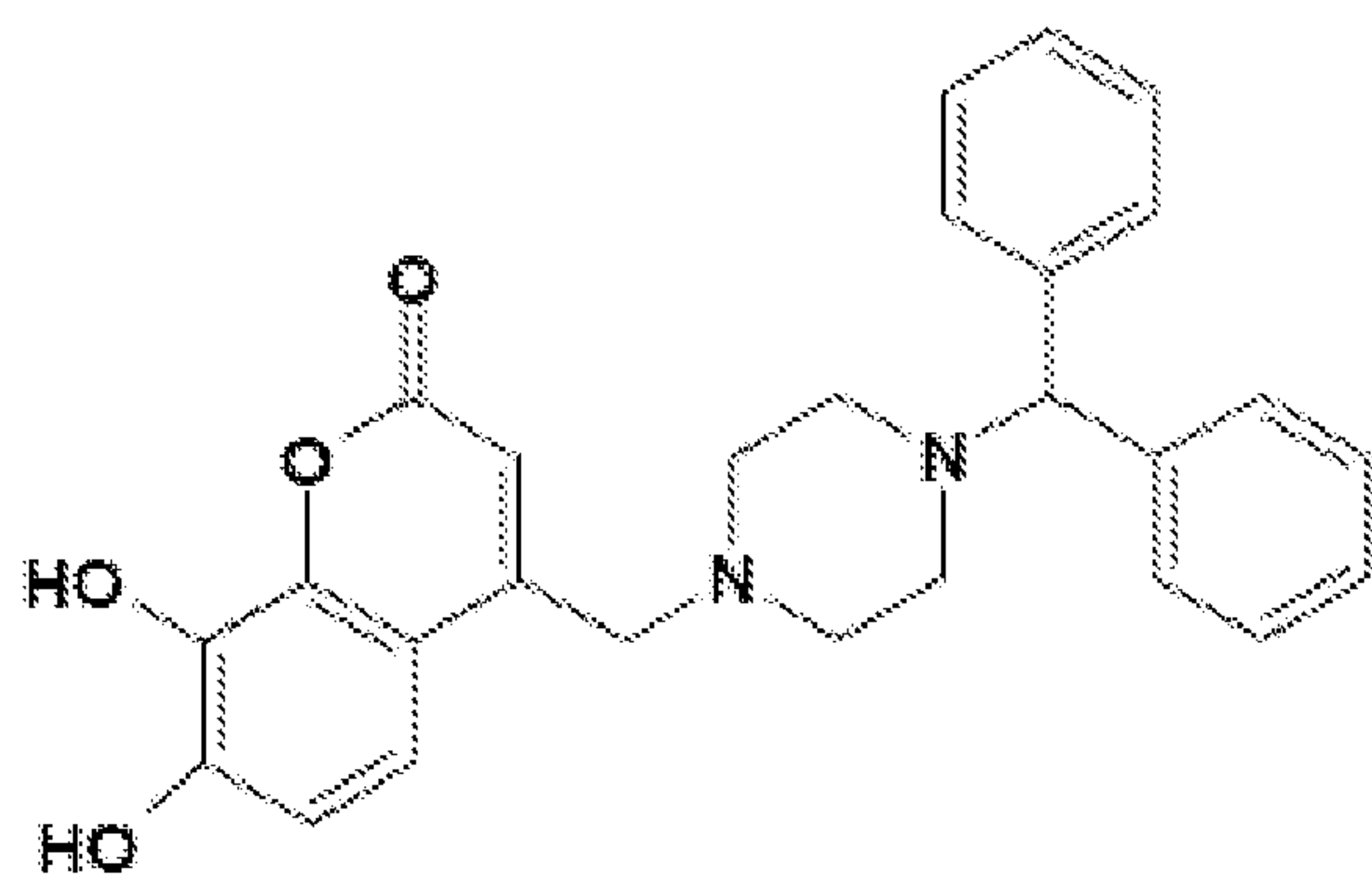
[0216] R^{43} of Formula (15) can be selected from hydrogen, lower alkyl, alkoxy, trifluoromethyl, or halogen. Preferably R^{43} of Formula (15) is lower alkyl. Lower alkyl, alkoxy and halogen are as defined above.

[0217] R^{44} of Formula (15) can be selected from hydrogen, hydroxy, alkoxy, lower alkyl, trifluoromethyl or halogen. Preferably, R^{44} of Formula (15) is selected from hydroxy or alkoxy. Lower alkyl, alkoxy and halogen are as defined above.

[0218] R^{45} of Formula (15) can be selected from hydrogen, lower alkyl, hydroxy, trifluoromethyl, or halogen. Preferably, R^{45} of Formula (15) is hydrogen. Lower alkyl and halogen are as defined above.

[0219] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (15).

[0220] In some embodiments, the compound of Formula (15) is AD4-10633.

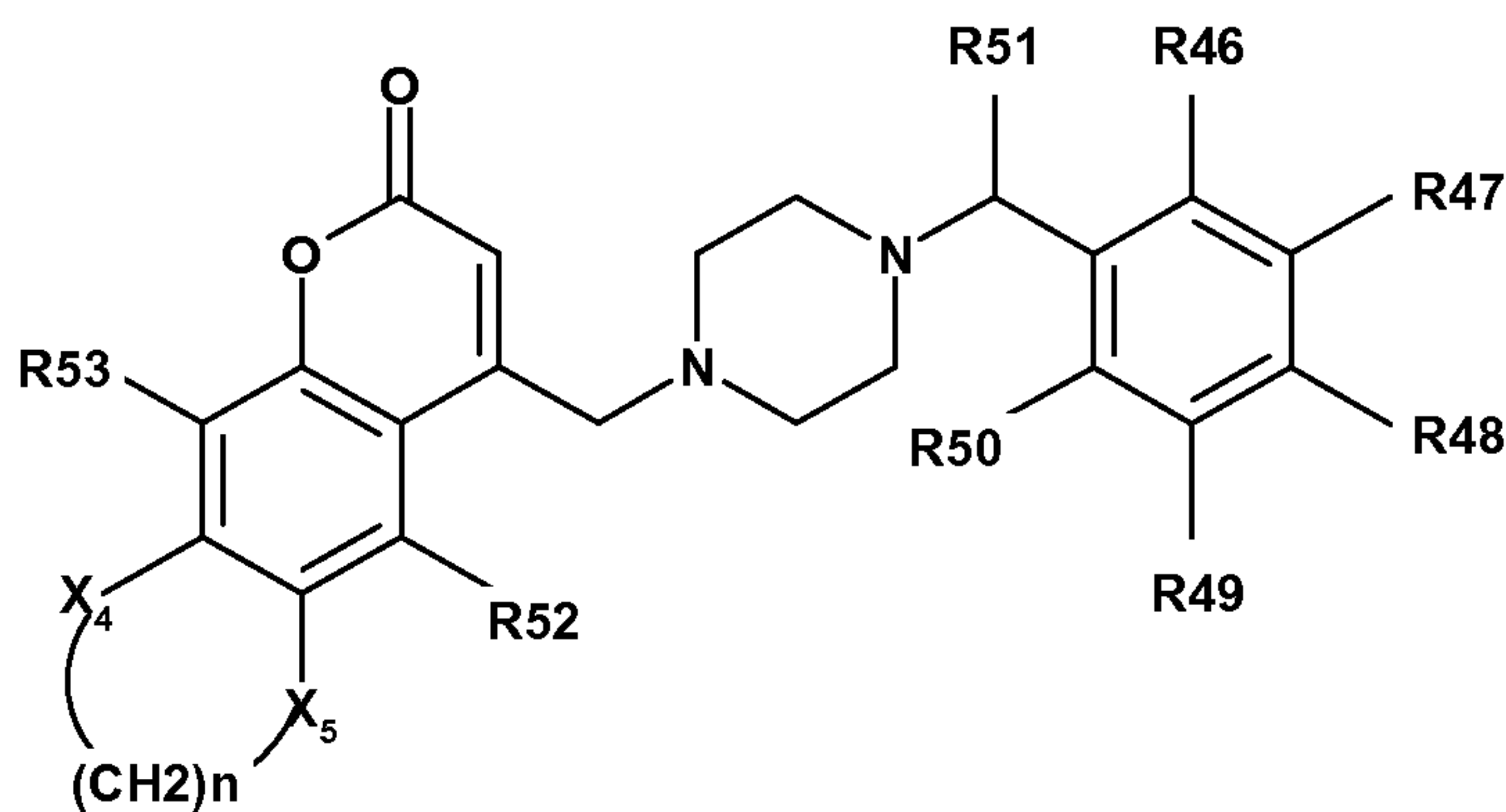


AD4-10633

[0221] In some embodiments, the compound(s) of Formula (15) excludes compound AD4-1734, Formula (12).

[0222] Type S AD4-1734-like

[0223] One structure derived from the AD4-1734-like pharmacophore is “Type S” as follows:



Formula (16)

[0224] In the above structure, X^4 and X^5 of Formula (16) can be independently selected from carbon, oxygen, nitrogen or sulfur atom. Preferably, X^4 and X^5 of Formula (16) are independently selected from oxygen or carbon. In Formula (16), n can be one or two resulting in a 5 or 6 membered ring. Preferably, n of Formula (16) is $n = 1$.

[0225] R^{46} and R^{50} of Formula (16) can be independently selected from hydrogen.

[0226] R^{47} and R^{49} of Formula (16) can be independently selected from hydrogen, lower alkyl, alkoxy or halogen. Lower alkyl, alkoxy and halogen are as defined above.

[0227] R^{48} of Formula (16) can be selected from hydrogen, lower alkyl, cycloalkyl, substituted alkyl, alkoxy, or halogen. Preferably, R^{48} of Formula (16) is selected from hydrogen or lower alkyl. Lower alkyl, cycloalkyl, substituted alkyl, and alkoxy are as defined above. Halogen is F, Cl, Br, or I.

[0228] Alternatively, R^{47} and R^{48} of Formula (16) can be joined to form a ring as described for Formula (15). Preferably, R^{47} and R^{48} of Formula (16) are joined to form a ring a 5-membered ring.

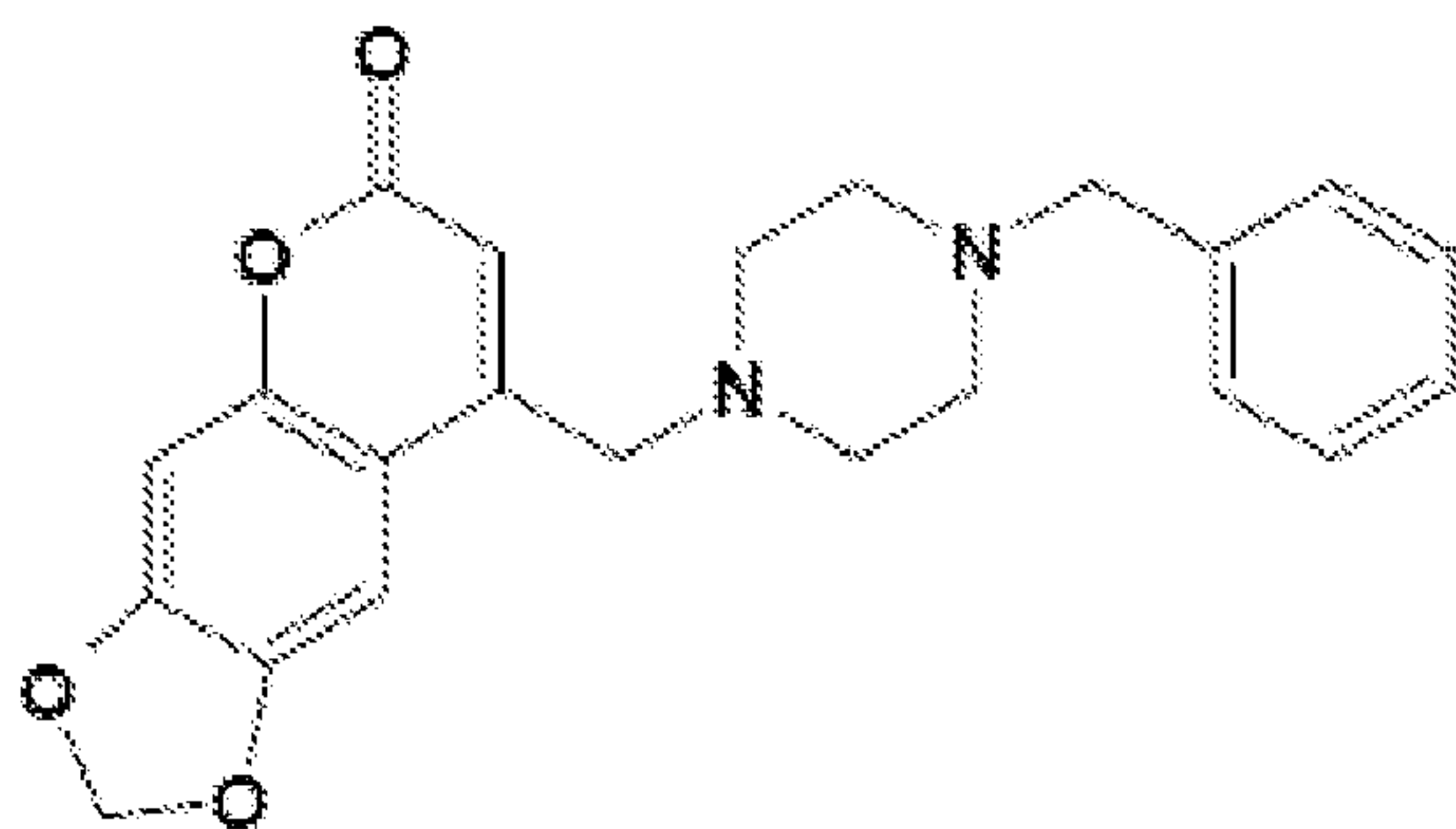
[0229] R^{51} of Formula (16) can be selected from hydrogen, lower alkyl, or aryl. Preferably, R^{51} of Formula (16) is selected from hydrogen or aryl. Lower alkyl or aryl are as defined above.

[0230] R^{52} of Formula (16) can be selected from hydrogen, lower alkyl, hydroxy, alkoxy or halogen. Preferably, R^{52} of Formula (16) is hydrogen. Lower alkyl, alkoxy and halogen are as defined above.

[0231] R^{53} of Formula (16) can be selected from hydrogen, lower alkyl, hydroxy, trifluoromethyl or halogen. Preferably, R^{53} of Formula (16) is hydrogen. Lower alkyl and halogen are as defined above.

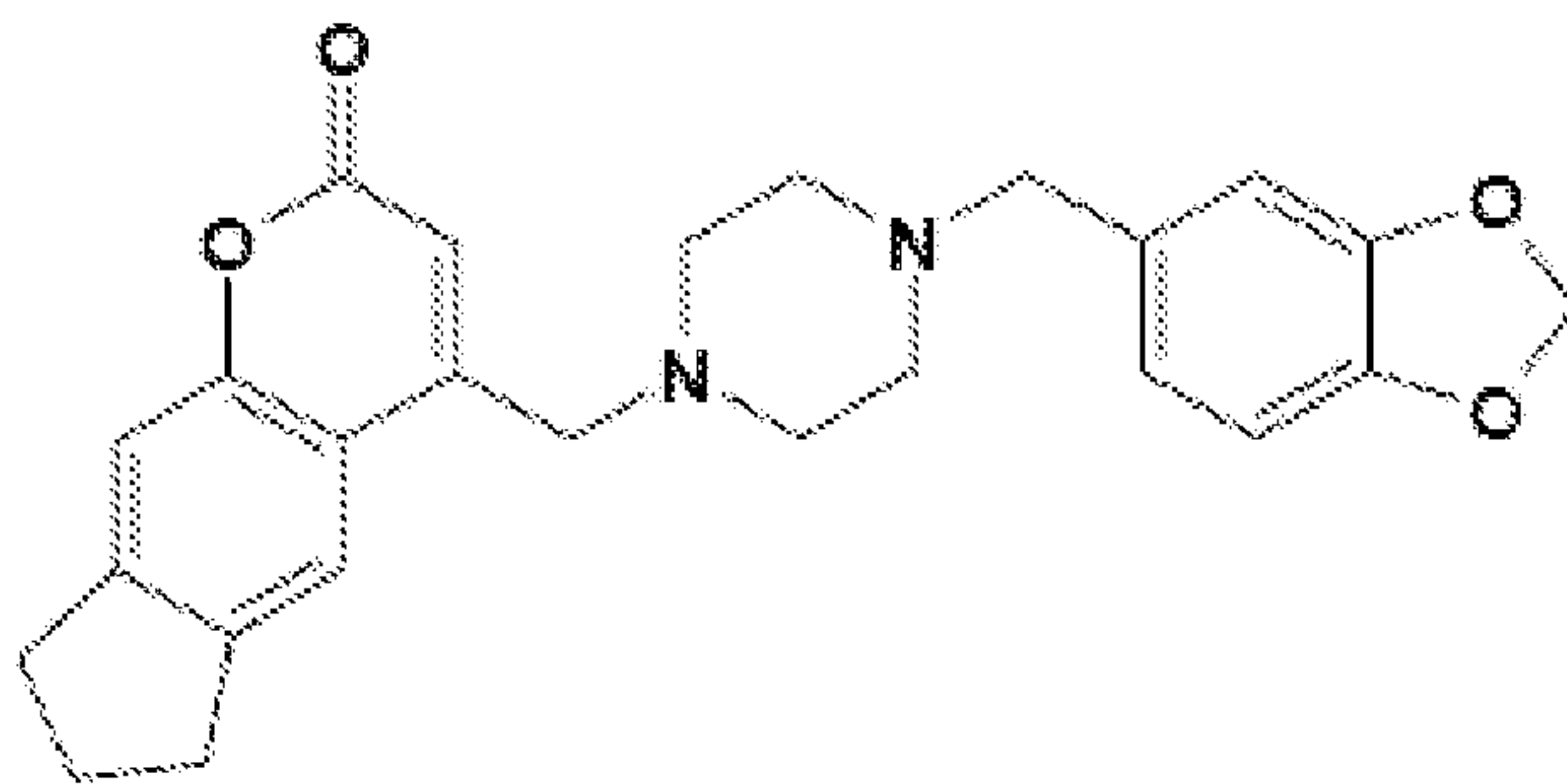
[0232] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (16).

[0233] In some embodiments, the compound of Formula (16) is AD4-10639.



AD4-10639

[0234] In some embodiments, the compound of Formula (16) is AD4-10179.

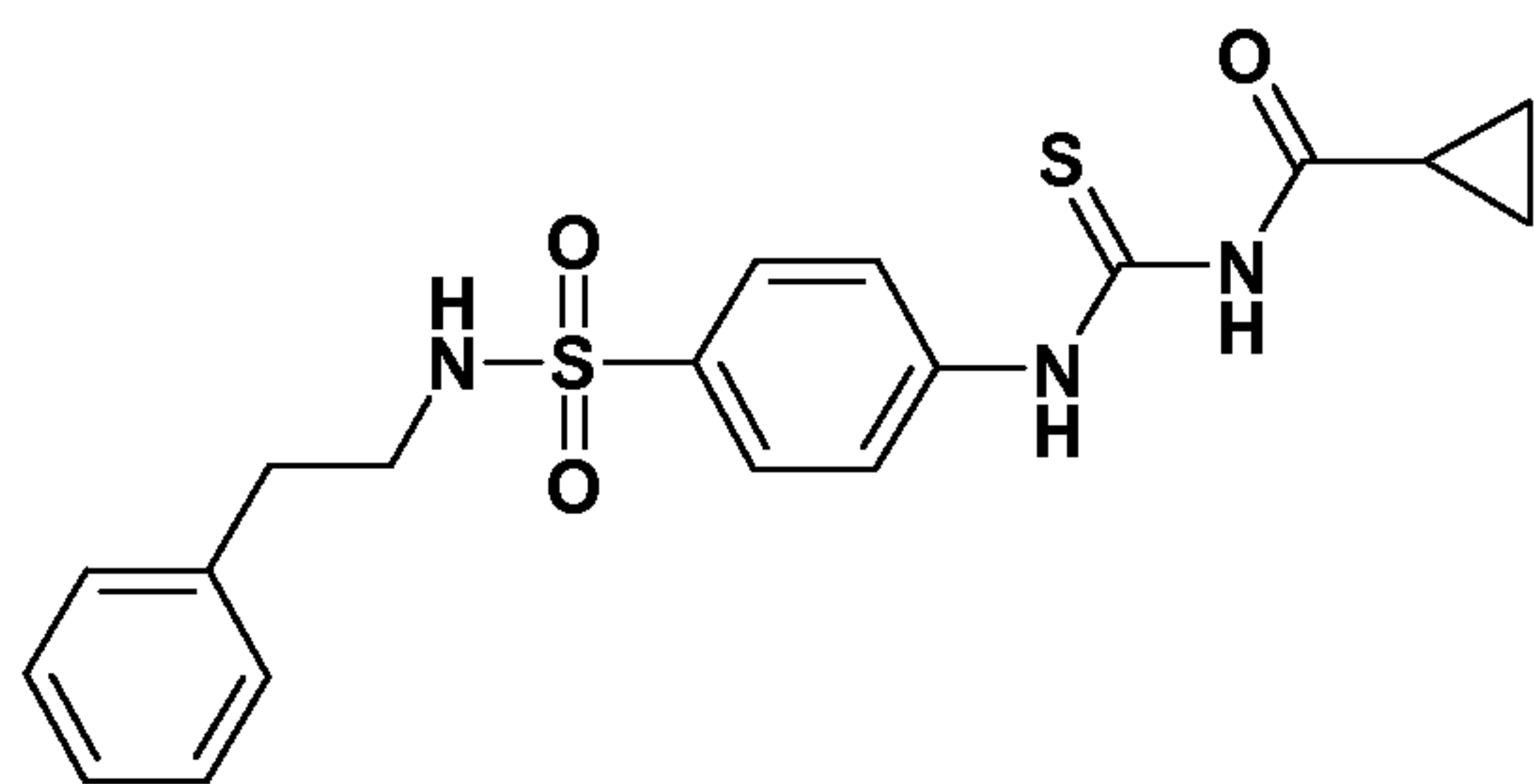


AD4-10179

[0235] In some embodiments, the compound(s) of Formula (16) excludes compound AD4-1734, Formula (12).

[0236] AD4-1886

[0237] AD4-1886 is identified as an inhibitor of epidermal growth factor binding to its receptor (*see e.g.*, Example 4).

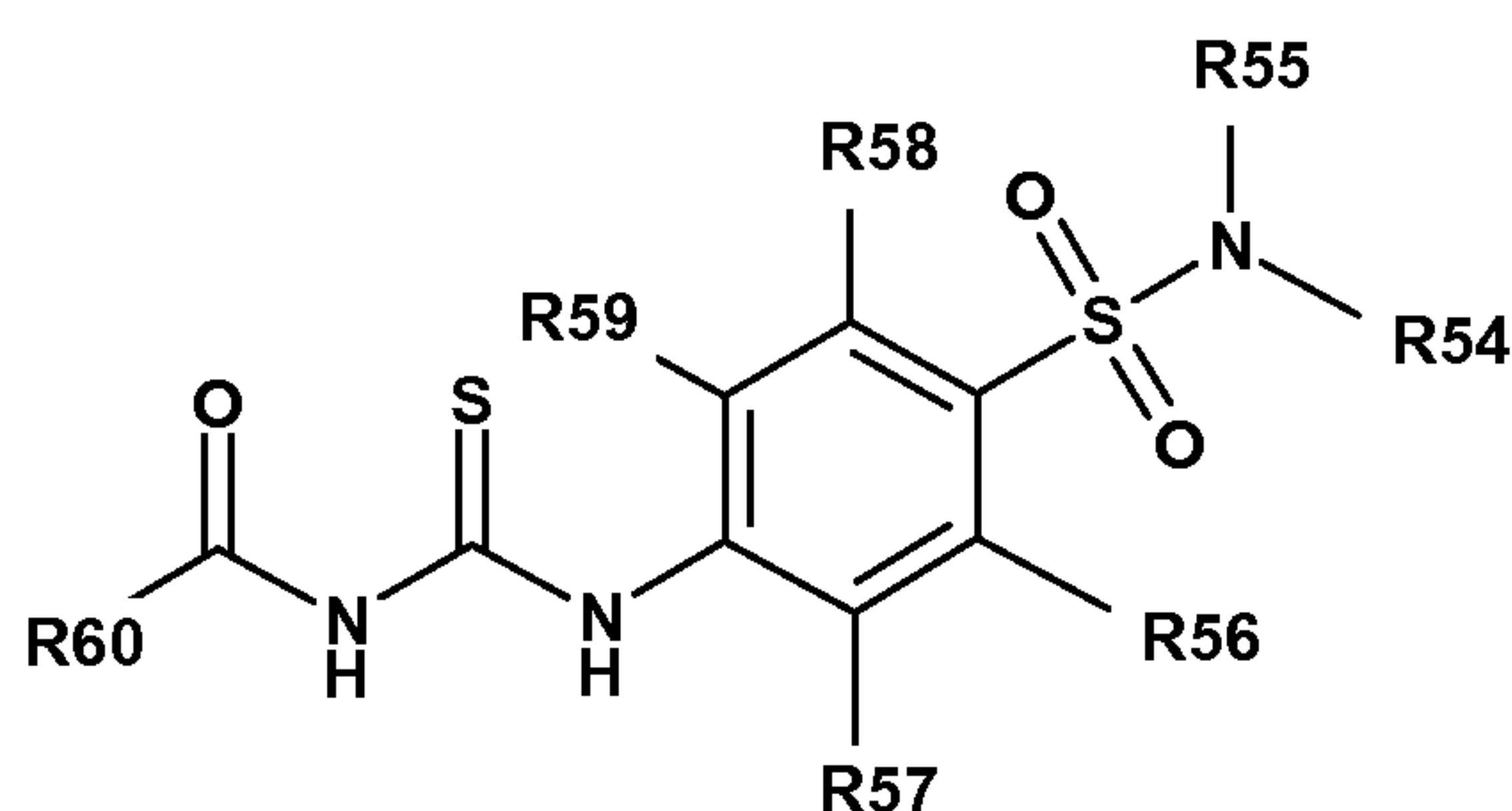


AD4-1886, Formula (17)

[0238] As described herein, a pharmacophore model was utilized to identify small molecules that are AD4-1886-like.

[0239] Type J AD4-1886-like

[0240] One structure derived from the AD4-1886-like pharmacophore is “Type J” as follows:



Formula (18)

[0241] In the above structure, R^{54} of Formula (18) is a 5 or 6 membered heterocyclic containing from 1 to 4 N, O, or S atoms or any combination of those atoms with carbon atoms to form a heterocyclic aromatic ring which is optionally substituted with from 1 to 3 of the following groups: lower alkyl, cycloalkyl, alkoxy ($-OR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), trifluoromethyl, trifluoromethoxy, substituted amino, nitro or halogen (F, Cl, Br, or I). Lower Alkyl is defined as one to six carbon, straight chain, branched, or optionally containing unsaturation. Cycloalkyl is defined as C-1 to C-6 optionally containing unsaturation. As preferred examples, R^{54} of Formula (18) can represent: 4,6-Dimethyl-2-pyrimidine, 2,6-Dimethoxy-4-pyrimidine, 6-Methoxy-4-pyrimidine, 5-Ethyl-2-(1,3,4-Thiadiazole), 5-Methyl-3-Isioxazole, 3-Methoxy-6-pyridazinamine, 2-Thiazole or 2-Methoxy-3-pyrazine.

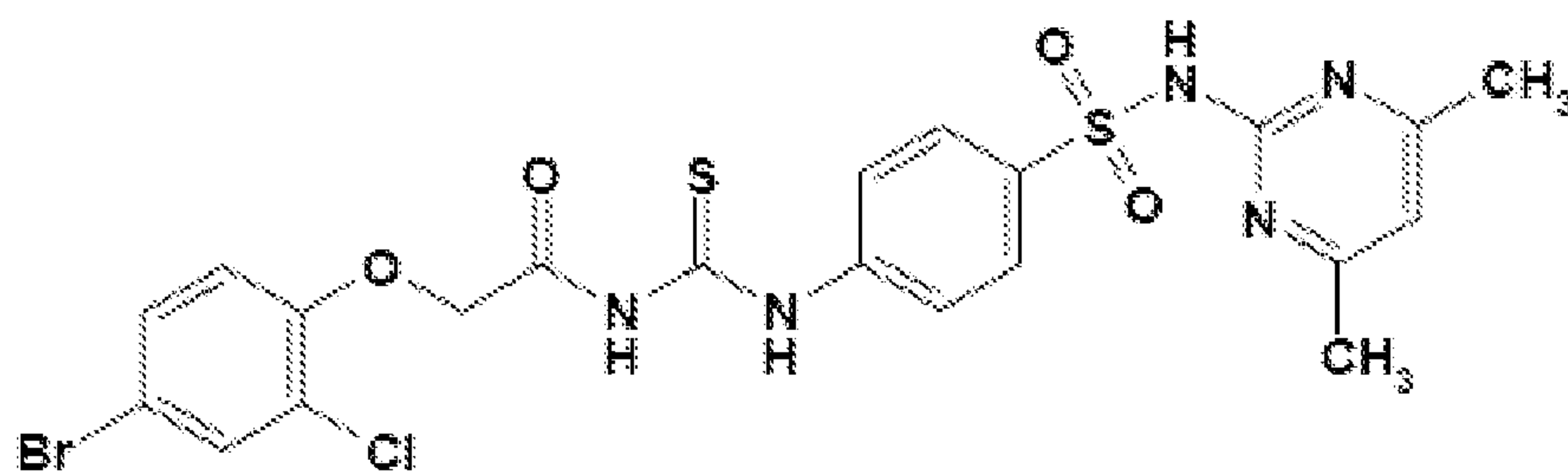
[0242] R^{55} of Formula (18) can be selected from hydrogen or lower alkyl. Preferably, R^{55} of Formula (18) is selected from hydrogen or methyl. Lower alkyl is as defined above.

[0243] R^{56} , R^{57} , R^{58} , and R^{59} of Formula (18) can be independently selected from hydrogen.

[0244] R^{60} of Formula (18) can be selected from lower alkyl, cycloalkyl, or substituted alkyl. Lower alkyl and cycloalkyl are as defined above. Substituted alkyl is defined as a hydrocarbon chain with 1-3 carbons which is substituted with additional functional groups from the following list: carboxyl defined as $-\text{COOR}^{10}$ (R^{10} is a lower alkyl group or cycloalkyl group as in the above definition), aryl, aryloxy ($-\text{OAr}$) where aryl is defined as an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more of the following groups: lower alkyl defined as C-1 to C-4, straight chain, branched, or optionally containing unsaturation, cycloalkyl defined as C-1 to C-6 optionally containing unsaturation, aryl including phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms, alkoxy ($-\text{OR}^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), Trifluoromethyl, Trifluoromethoxy, Difluoromethoxy, 3, 4-methylenedioxy, 2, 3-methylenedioxy, Nitro or Halogen (F, Cl, Br, I).

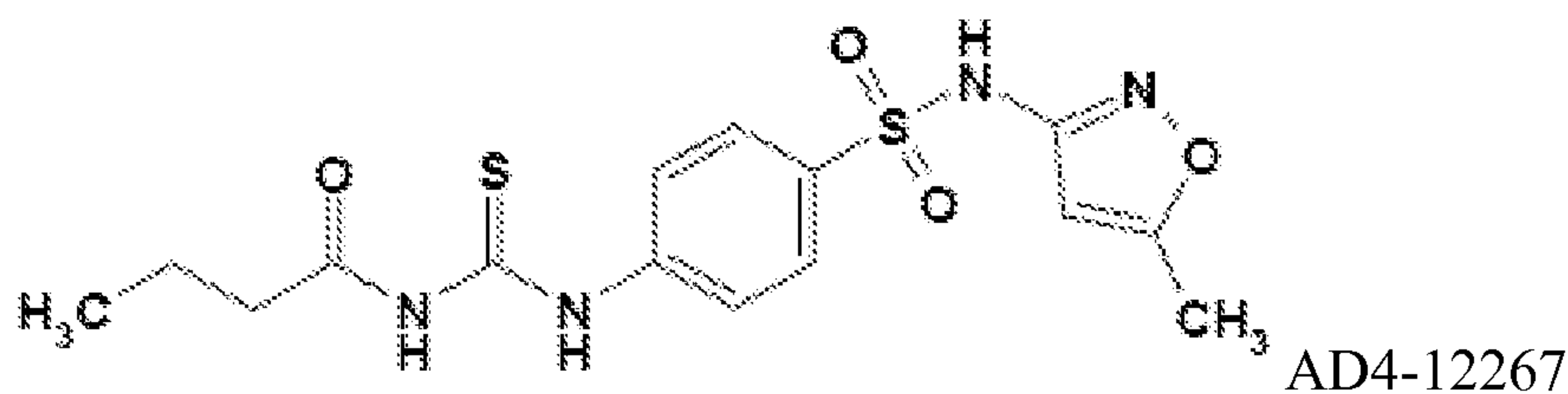
[0245] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (18).

[0246] In some embodiments, the compound of Formula (18) is AD4-12158.



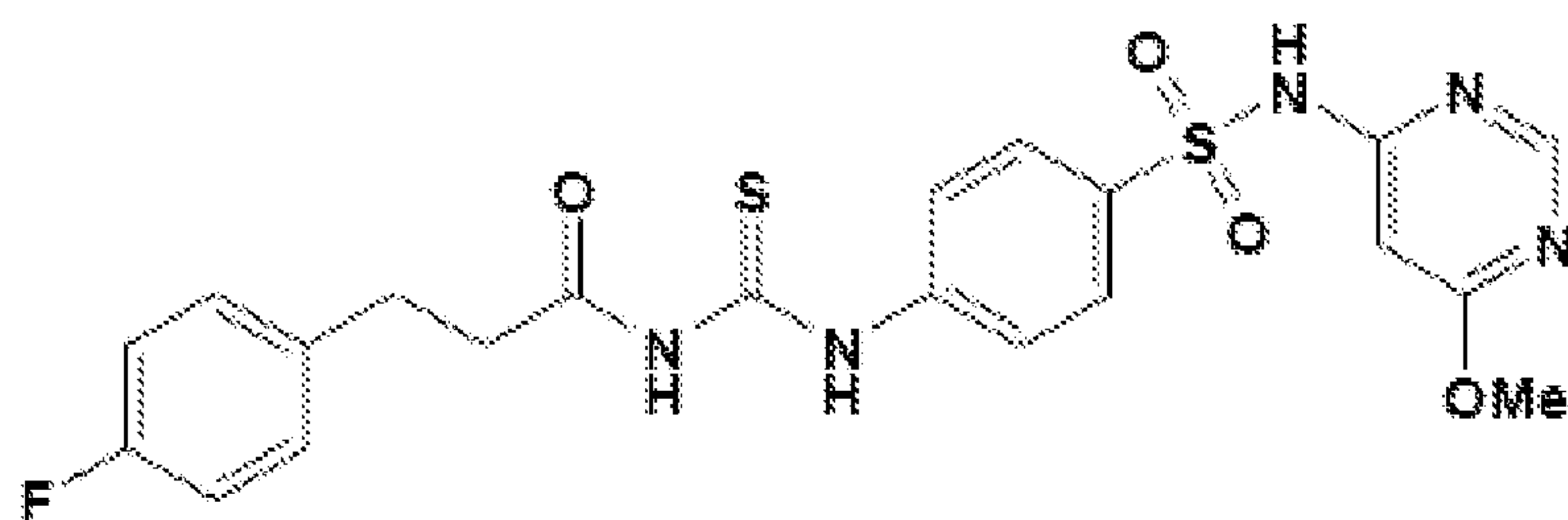
AD4-12158

[0247] In some embodiments, the compound of Formula (18) is AD4-12267.



AD4-12267

[0248] In some embodiments, the compound of Formula (18) is AD4-11384.



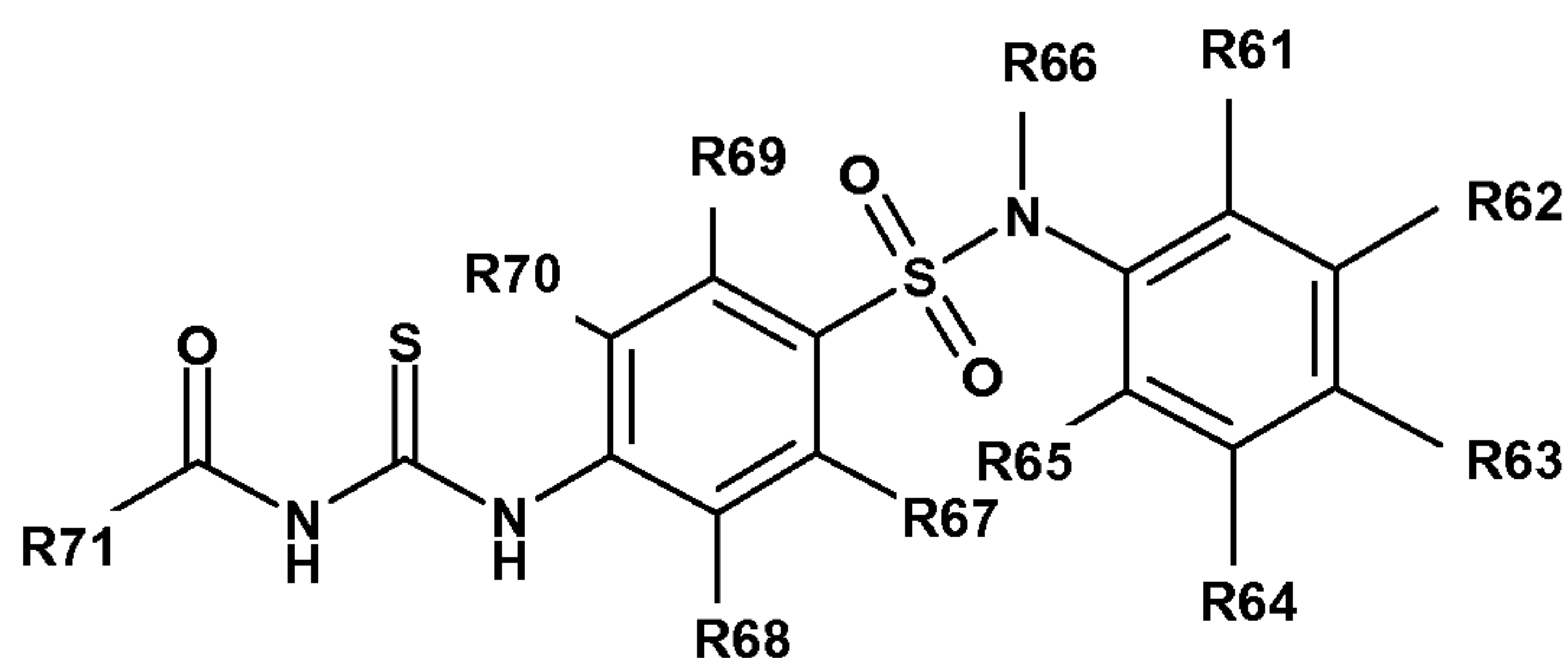
AD4-11384.

[0249]

[0250] In some embodiments, the compound(s) of Formula (18) excludes compound AD4-1886, Formula (17).

[0251] Type K AD4-1886-like

[0252] One structure derived from the AD4-1886-like pharmacophore is “Type K” as follows:



Formula (19)

[0253] In the above structure, R^{61} , R^{62} , and R^{64} of Formula (19) can be independently selected from hydrogen or lower alkyl. Lower alkyl is as defined above.

[0254] R^{63} and R^{65} of Formula (19) can be selected from hydrogen, lower alkyl or alkoxy. Lower alkyl and alkoxy are as defined above.

[0255] R^{66} of Formula (19) can be selected from hydrogen or lower alkyl. Lower alkyl is as defined above.

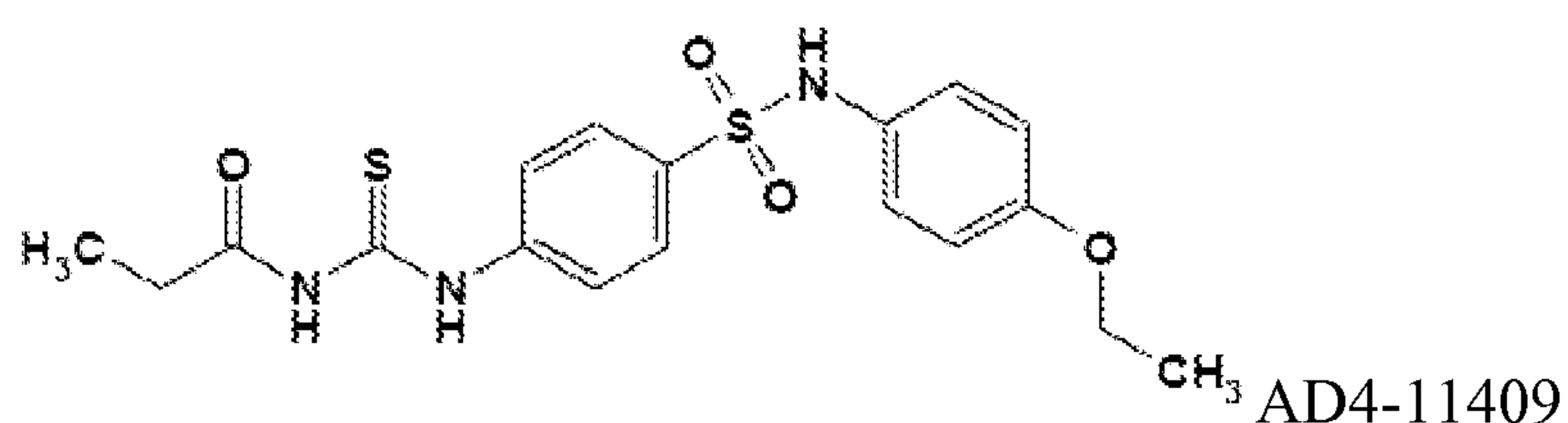
[0256] R^{67} , R^{68} , R^{69} , and R^{70} of Formula (19) can be independently selected from hydrogen.

[0257] R^{71} of Formula (19) can be selected from lower alkyl, cycloalkyl, aryl, substituted alkyl, or heteraryl. Lower alkyl, cycloalkyl, aryl, and substituted alkyl are as defined above. Hereoaryl is defined as a heterocyclic ring containing from 1 to 4 N, O, or S atoms which has optional substitution with one or more of the following functional groups: lower alkyl defined as

C-1 to C-4, straight chain, branched, or optionally containing unsaturation, cycloalkyl defined as C-1 to C-6 optionally containing unsaturation, aryl including phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms, alkoxy ($-\text{OR}^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), trifluoromethyl, trifluoromethoxy, nitro or halogen (F, Cl, Br, I).

[0258] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (19).

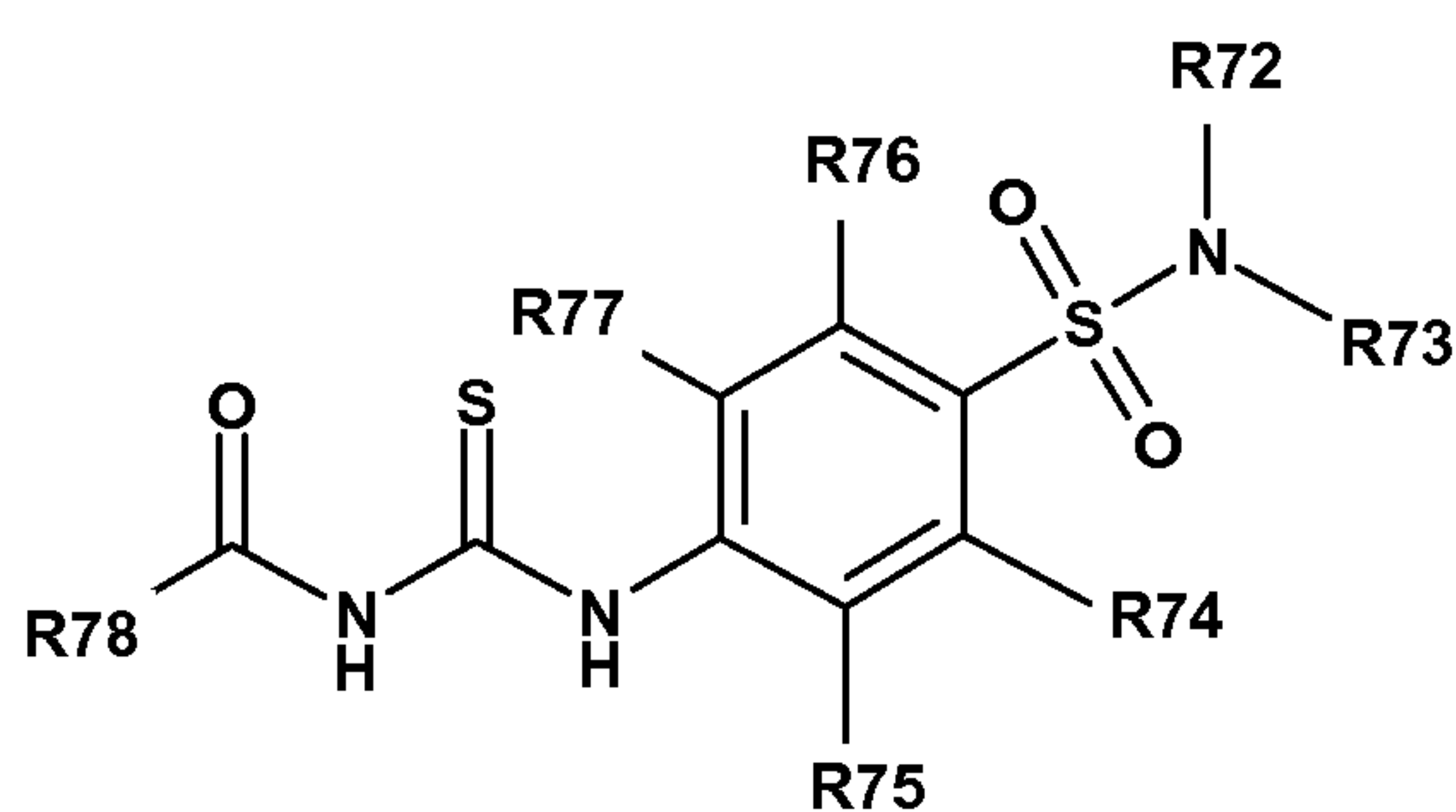
[0259] In some embodiments, the compound of Formula (19) is AD4-11409.



[0260] In some embodiments, the compound(s) of Formula (19) excludes compound AD4-1886, Formula (17).

[0261] Type L AD4-1886-like

[0262] One structure derived from the AD4-1886-like pharmacophore is “Type L” as follows:



Formula (20)

[0263] In the above structure, R^{72} of Formula (20) can be selected from Hydrogen or Lower Alkyl. Lower alkyl is as defined above.

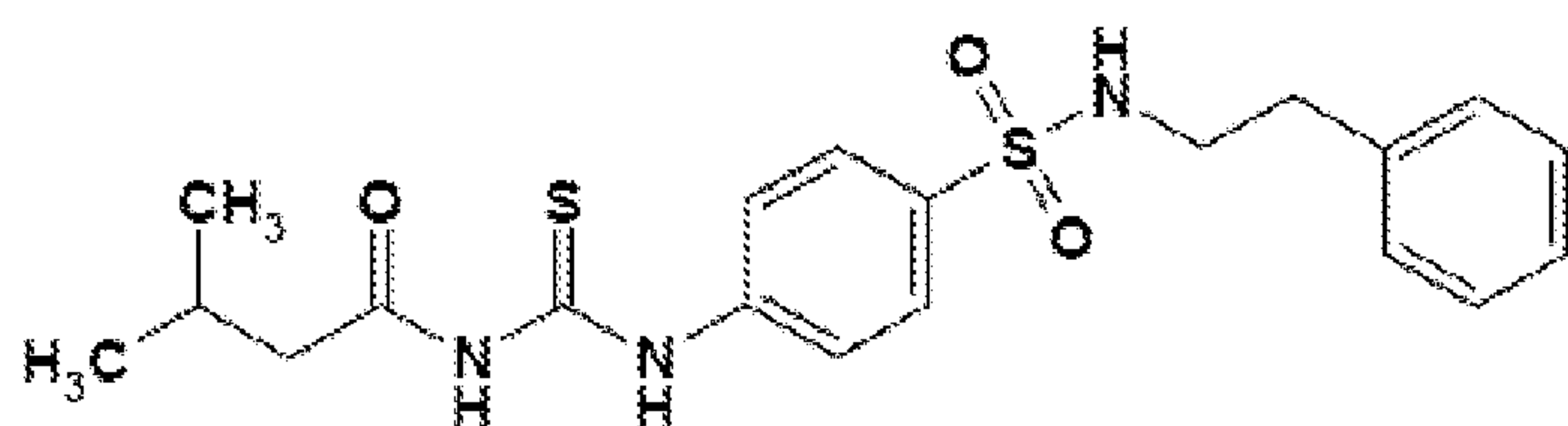
[0264] R^{73} of Formula (20) can be selected from lower alkyl, cycloalkyl, substituted alkyl, or acyl. Lower alkyl, cycloalkyl, and substituted alkyl are as defined above. Acyl represents a substituted ketone $-\text{COR}^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition.

[0265] R^{74} , R^{75} , R^{76} , and R^{77} of Formula (20) can be independently selected from hydrogen.

[0266] R^{78} of Formula (20) can be selected from lower alkyl, cycloalkyl, and substituted alkyl. Lower alkyl, cycloalkyl, and substituted alkyl are as defined above.

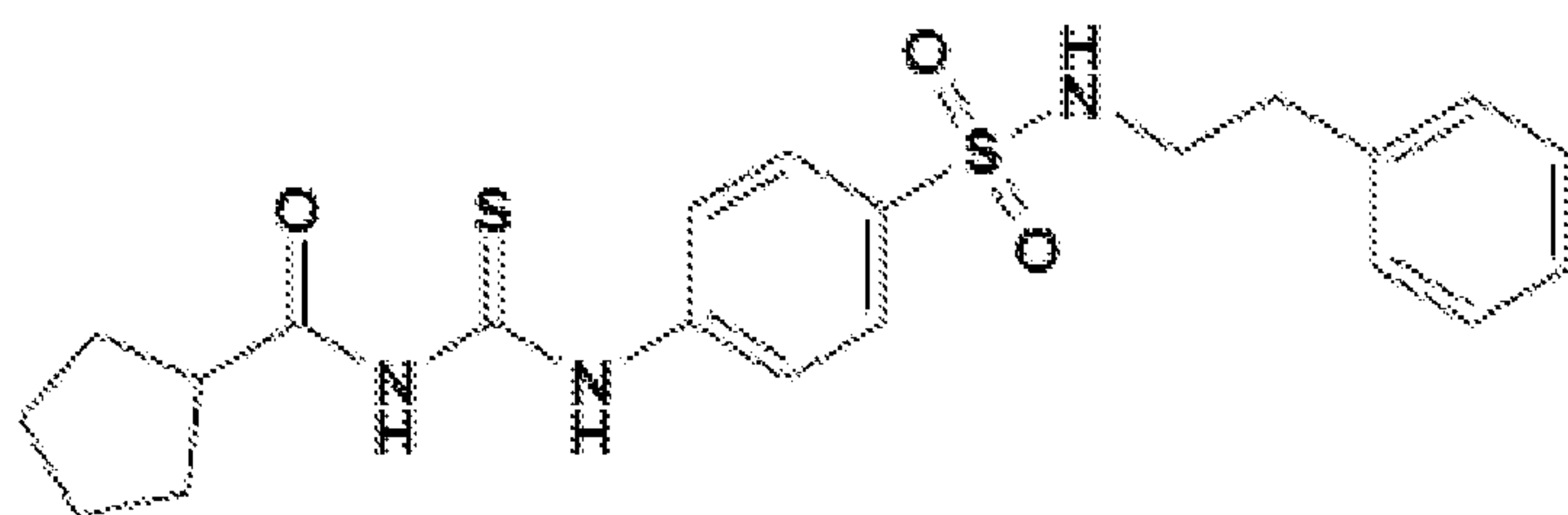
[0267] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (20).

[0268] In some embodiments, the compound of Formula (20) is AD4-10608.



AD4-10608

[0269] In some embodiments, the compound of Formula (20) is AD4-10609.

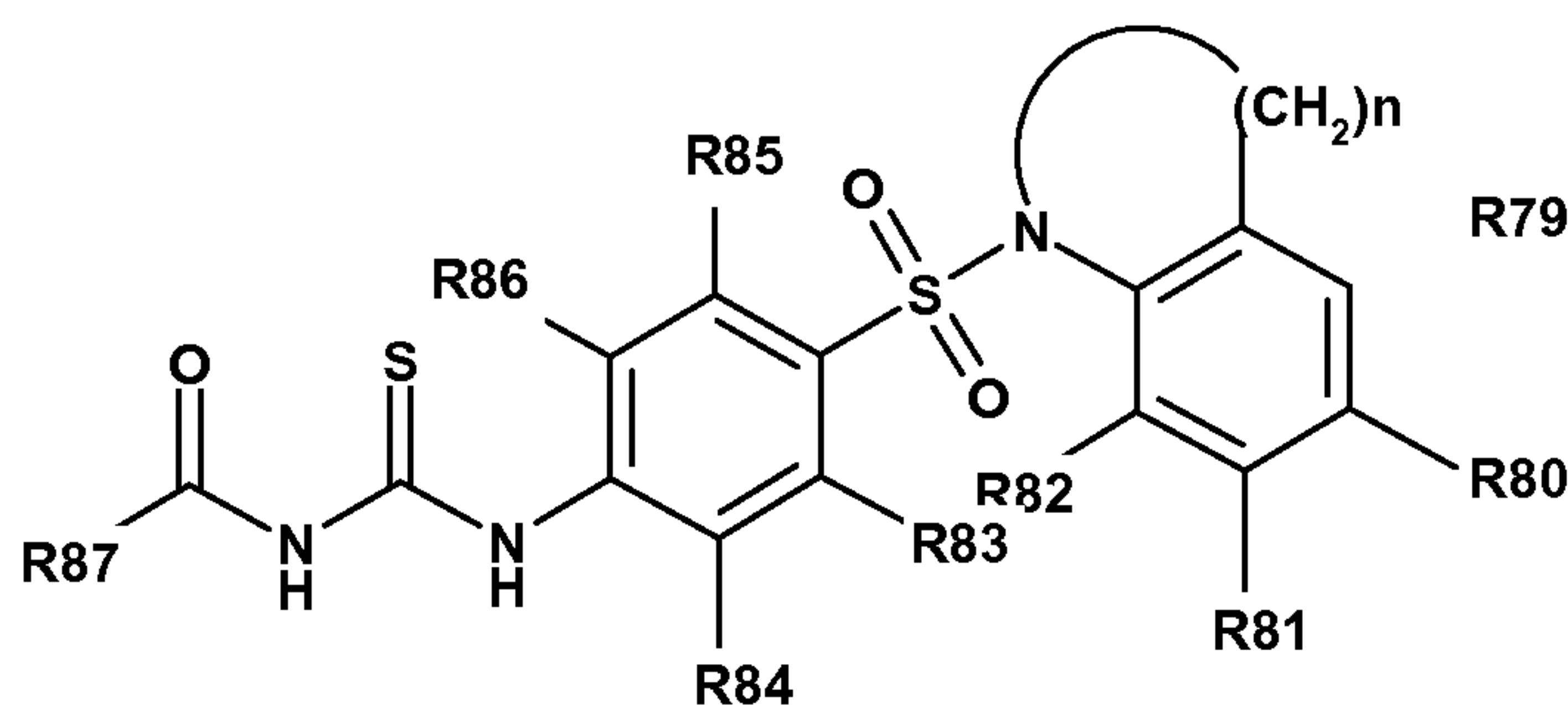


AD4-10609

[0270] In some embodiments, the compound(s) of Formula (20) excludes compound AD4-1886, Formula (17).

[0271] Type M AD4-1886-like

[0272] One structure derived from the AD4-1886-like pharmacophore is “Type M” as follows:



Formula (21)

[0273] In the above structure, n of Formula (21) can be 1 or 2, which represents one or two methylene groups resulting in either a five or six membered fused ring system.

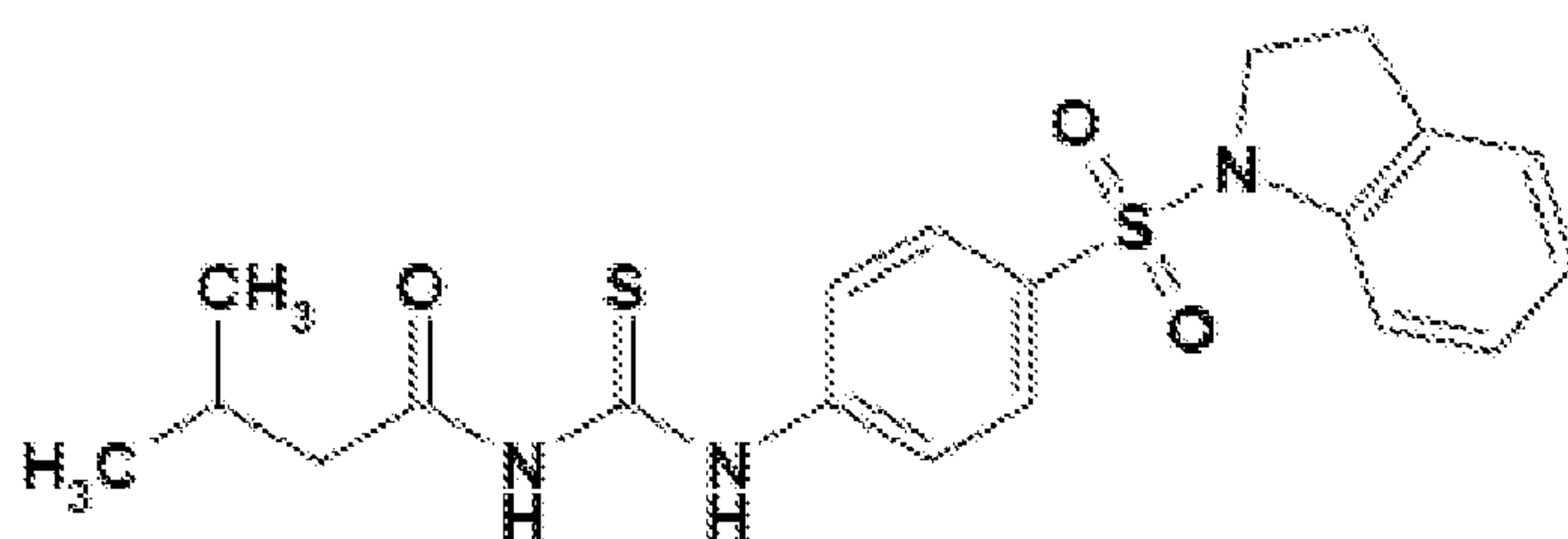
[0274] R^{79} , R^{81} , R^{82} , R^{83} , R^{84} , R^{85} , and R^{86} of Formula (21) can be independently selected from hydrogen.

[0275] R^{80} of Formula (21) can be selected from hydrogen, lower alkyl, alkoxy, halogen, or trifluoromethyl. Lower alkyl and alkoxy are as defined above. Halogen is defined as F, Cl, Br, or I.

[0276] R^{87} of Formula (21) can be selected from lower alkyl, cycloalkyl, substitute alkyl, or aryl. Lower alkyl, cycloalkyl, substitute alkyl, and aryl are as defined above.

[0277] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (21).

[0278] In some embodiments, the compound of Formula (21) is AD4-10626.

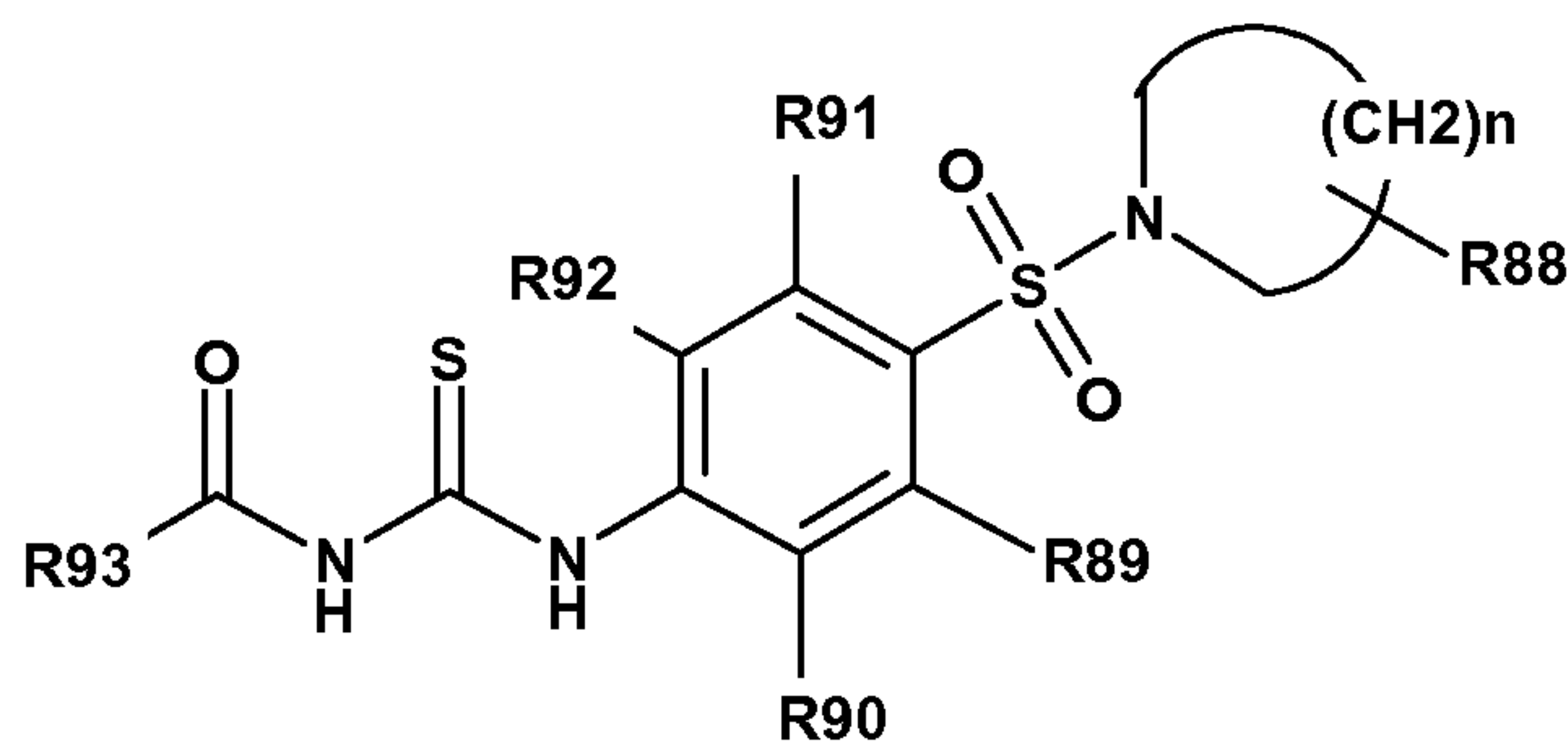


AD4-10626

[0279] In some embodiments, the compound(s) of Formula (21) excludes compound AD4-1886, Formula (17).

[0280] Type N AD4-1886-like

[0281] One structure derived from the AD4-1886-like pharmacophore is “Type N” as follows:



Formula (22)

[0282] In the above structure, n of Formula (22) can be 3, 4 or 5, which represents 3,4,or 5 methylene groups resulting in either a five, six, or seven membered ring (pyrrolidine, piperidine, or hexahydro-1H-azepine).

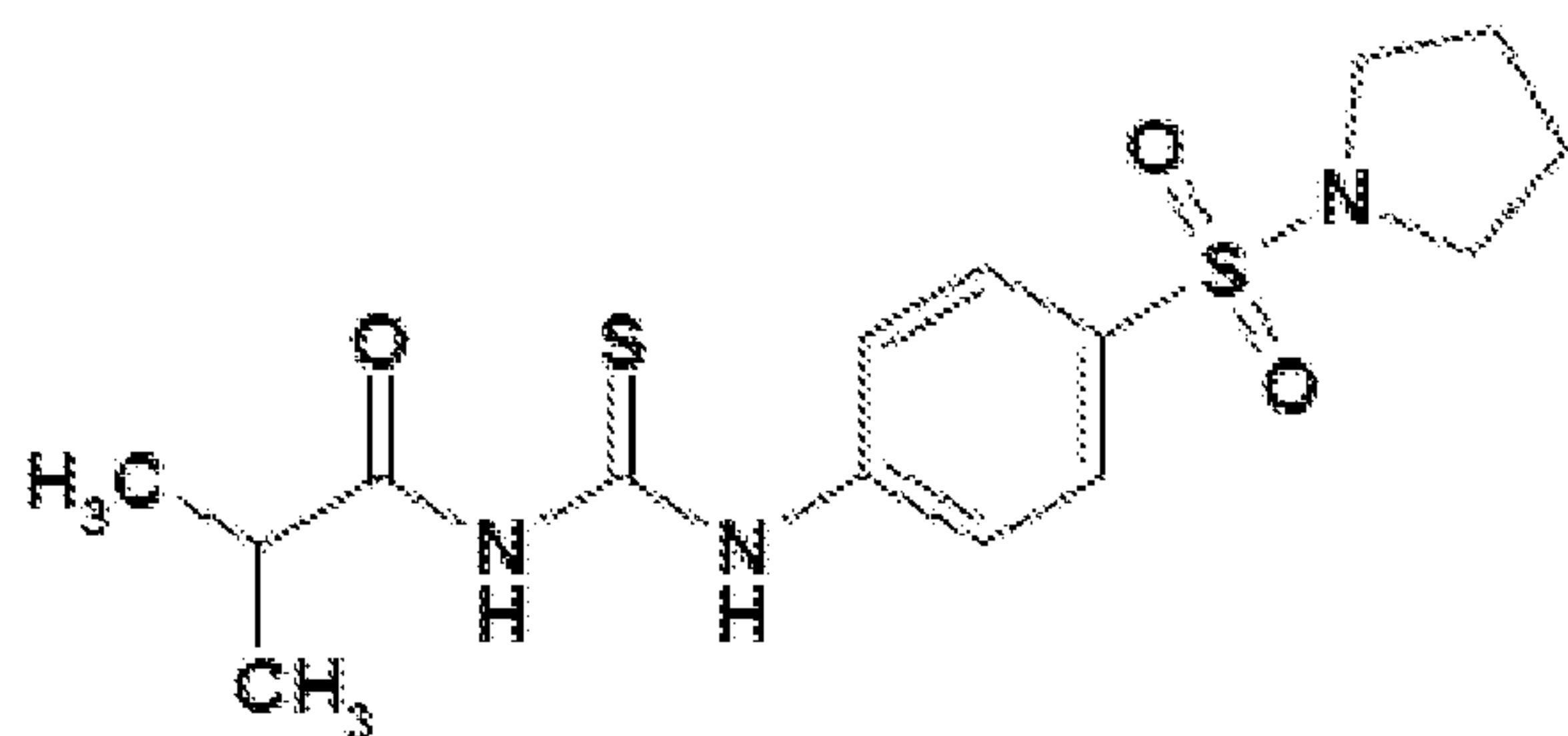
[0283] R^{88} of Formula (22) can be selected from hydrogen or lower alkyl. Lower alkyl is as defined above.

[0284] R^{89} , R^{90} , R^{91} , R^{83} , and R^{92} of Formula (22) can be independently selected from hydrogen.

[0285] R^{93} of Formula (22) can be selected from lower alkyl, cycloalkyl, substituted alkyl, or aryl. Lower alkyl, cycloalkyl, substituted alkyl, and aryl are as defined above.

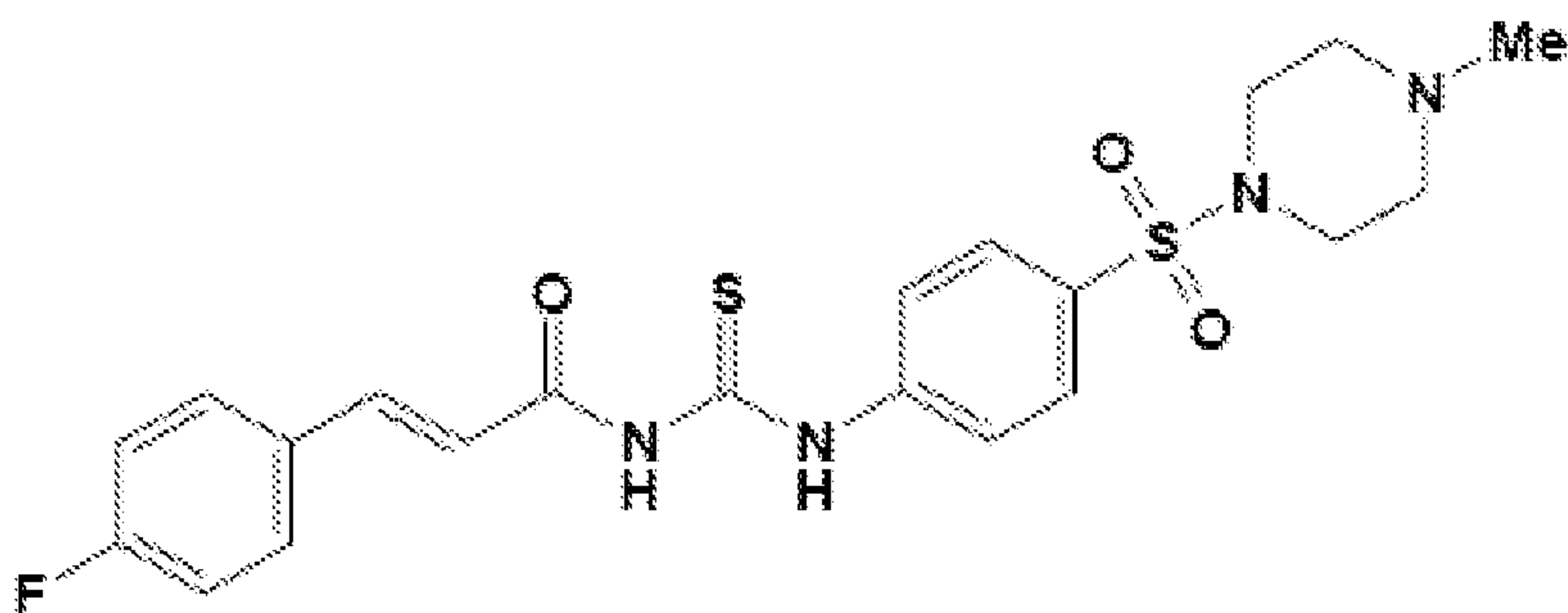
[0286] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (22).

[0287] In some embodiments, the compound of Formula (22) is AD4-11970.



AD4-11970

[0288] In some embodiments, the compound of Formula (22) is AD4-12107.

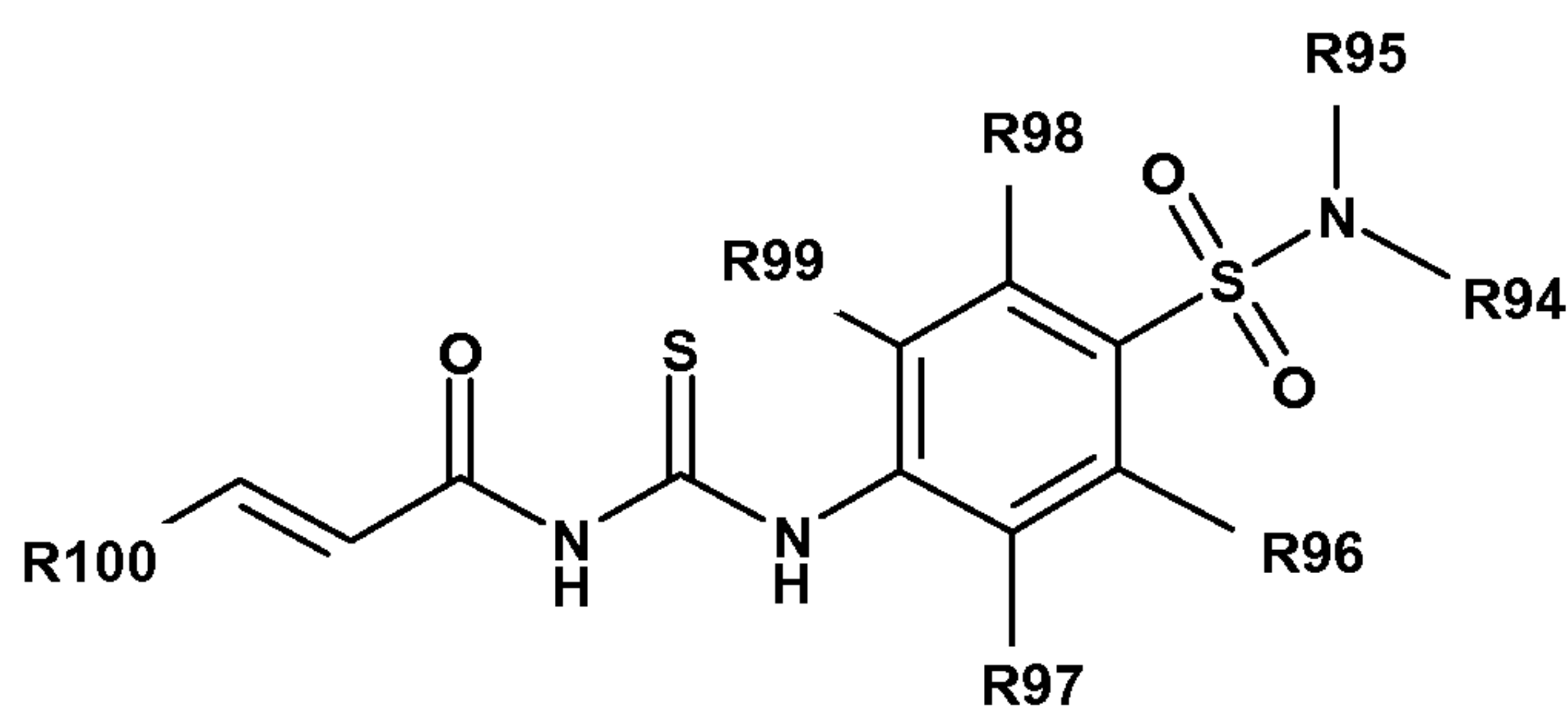


AD4-12107

[0289] In some embodiments, the compound(s) of Formula (22) excludes compound AD4-1886, Formula (17).

[0290] Type O AD4-1886-like

[0291] One structure derived from the AD4-1886-like pharmacophore is "Type O" as follows:



Formula (23)

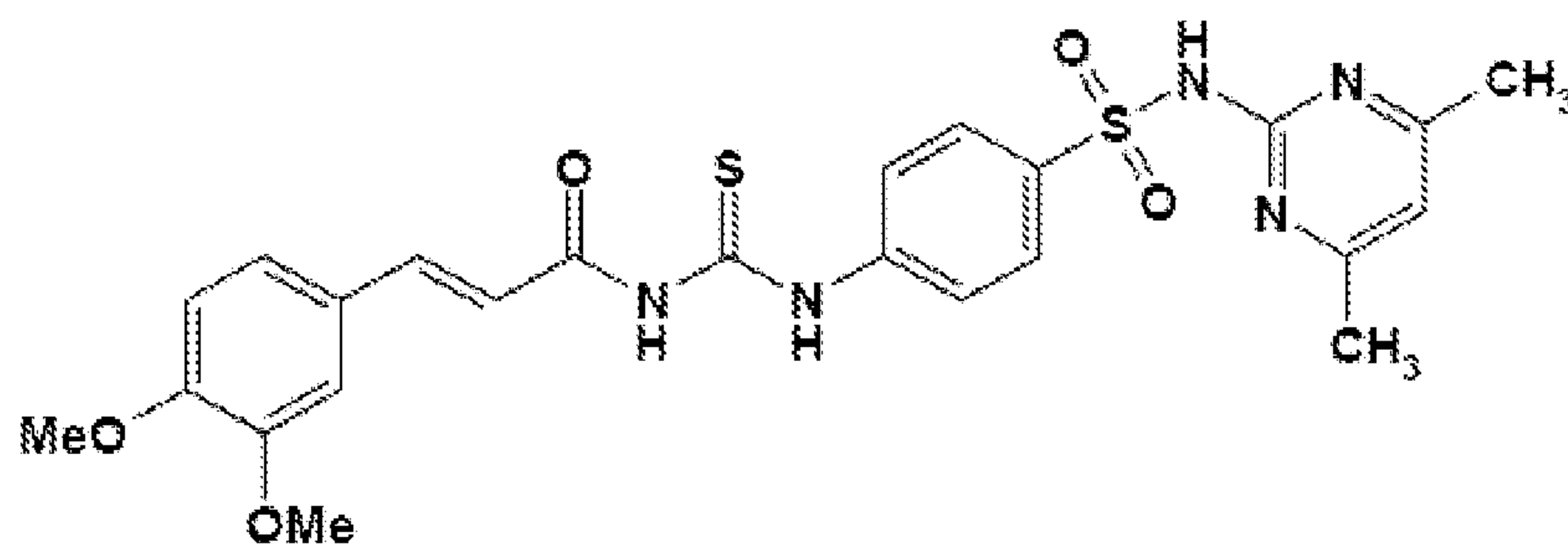
[0292] In the above structure, R^{94} of Formula (23) is a 5 or 6 membered heterocyclic containing from 1 to 4 N, O, or S atoms or any combination of those atoms with carbon atoms to form a heterocyclic aromatic ring which is optionally substituted with from 1 to 3 of the following groups: lower alkyl, cycloalkyl, alkoxy ($-OR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), trifluoromethyl, trifluoromethoxy, substituted amino, nitro or halogen (F, Cl, Br, or I). Lower alkyl is defined as one to six carbon, straight chain, branched, or optionally containing unsaturation. Cycloalkyl is defined as C-1 to C-6 optionally containing unsaturation.

[0293] R^{95} of Formula (23) can be selected from hydrogen or lower alkyl. Lower alkyl is as defined above.

[0294] R^{96} , R^{97} , R^{98} , and R^{99} of Formula (23) can be independently selected from hydrogen.

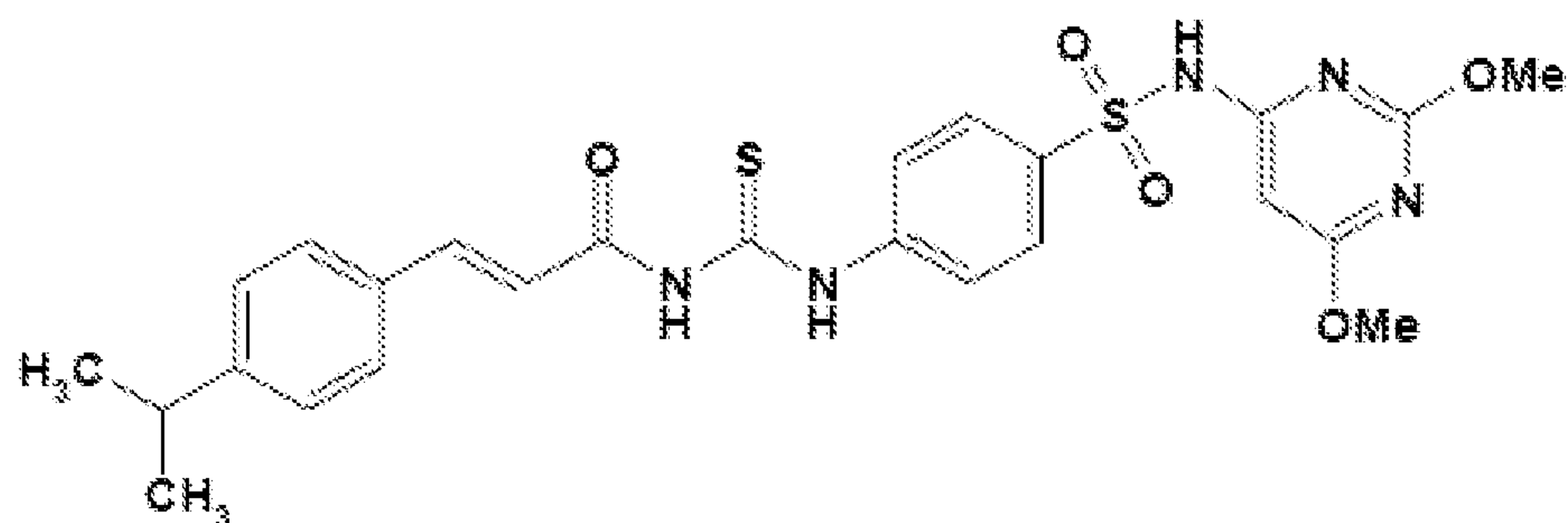
[0295] R^{100} of Formula (23) can be selected from aryl or heteroaryl. Aryl and heteroaryl are as defined above.

[0296] In some embodiments, the compound of Formula (23) is AD4-11883.



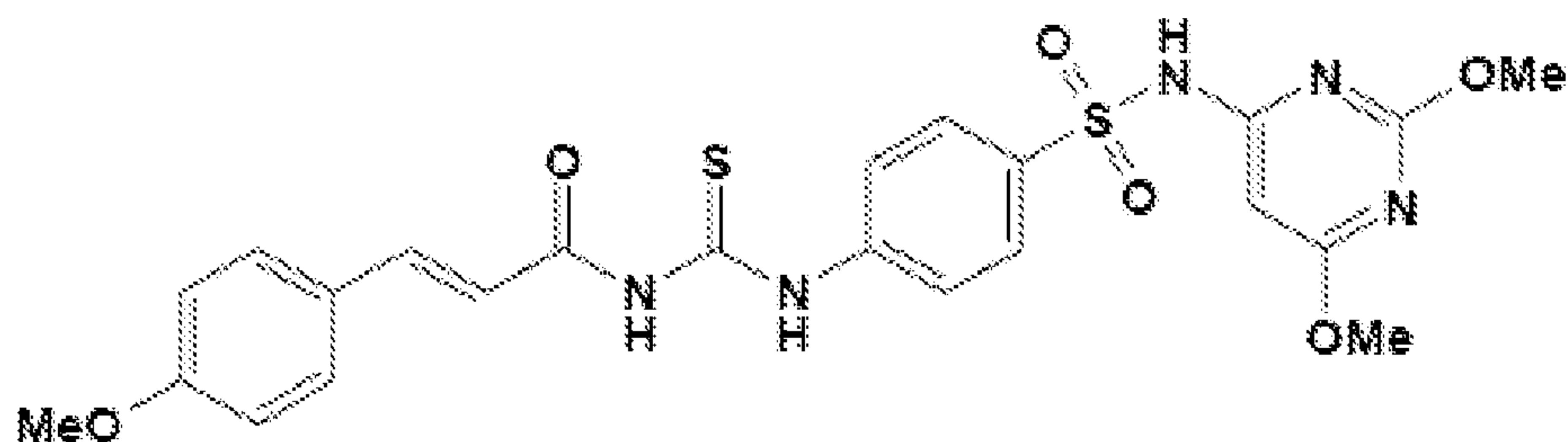
AD4-11883

[0297] In some embodiments, the compound of Formula (23) is AD4-11638.



AD4-11638

[0298] In some embodiments, the compound of Formula (23) is AD4-11645.

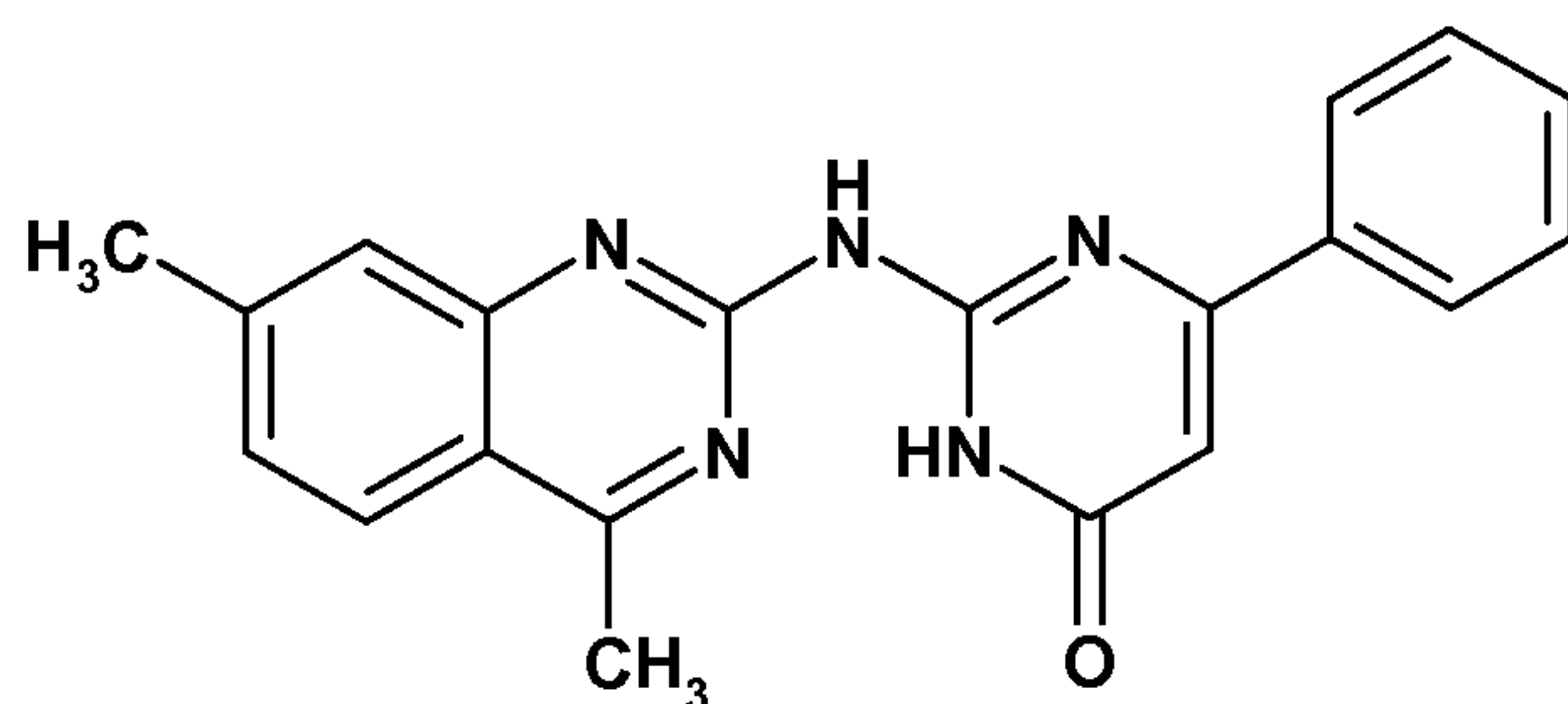


AD4-11645

[0299] In some embodiments, the compound(s) of Formula (23) excludes compound AD4-1886, Formula (17).

[0300] AD4-10381

[0301] AD4-10381 is identified as an inhibitor of epidermal growth factor binding to its receptor (*see e.g.*, Example 5).

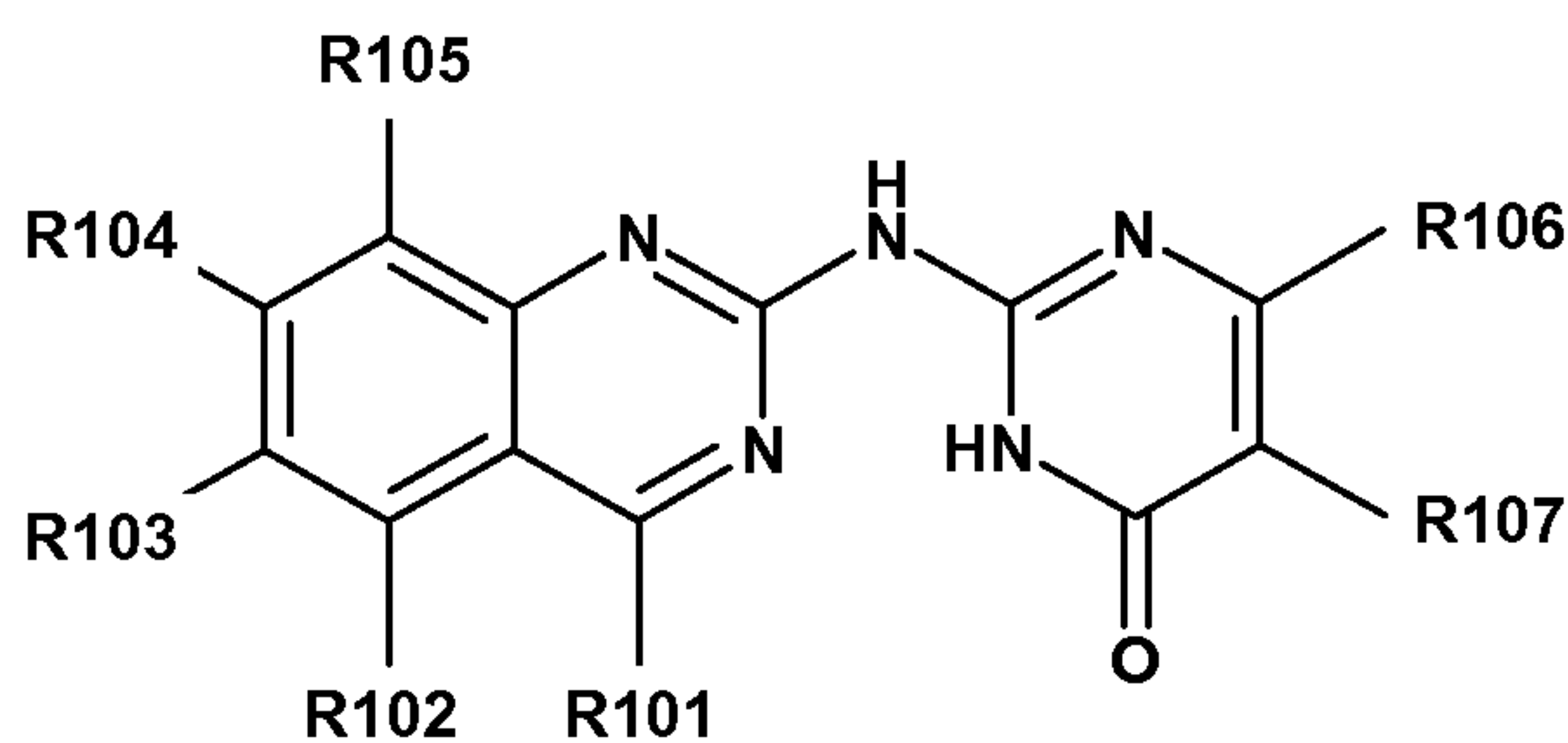


AD4-10381, Formula (24)

[0302] As described herein, a pharmacophore model was utilized to identify small molecules that are AD4-10381-like.

[0303] Type D AD4-10381-like

[0304] One structure derived from the AD4-10381-like pharmacophore is “Type D” as follows:



Formula (25)

[0305] In the above structure, R¹⁰¹ of Formula (25) can be selected from lower alkyl, cycloalkyl or aryl. Preferably, R¹⁰¹ of Formula (25) is selected from methyl or phenyl. Lower alkyl is defined as one to six carbon, straight chain, branched, or optionally containing unsaturation. Cycloalkyl is defined as three to six membered carbocyclic ring optionally containing unsaturation. Aryl represents an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more of the following groups: lower alkyl defined as above, cycloalkyl defined as above, or heteroaryl containing from 1 to 4 N, O, or S atoms.

[0306] R¹⁰² of Formula (25) can be selected from hydrogen.

[0307] R¹⁰³ of Formula (25) can be selected from hydrogen, lower alkyl, cycloalkyl, alkoxy, or halogen. Preferably, R¹⁰³ of Formula (25) is selected from methyl, ethyl, chloro, methoxy, or ethoxy. Lower alkyl is defined as above. Cycloalkyl is defined as above. Alkoxy is defined as -OR¹⁰ where R¹⁰ is defined as a lower alkyl group or cycloalkyl group in the above definition.

[0308] R¹⁰⁴ of Formula (25) can be selected from hydrogen, lower alkyl, cycloalkyl, or alkoxy. Preferably, R¹⁰⁴ of Formula (25) is selected from hydrogen, methyl, or methoxy. Lower alkyl is defined as above. Cycloalkyl is defined as above. Alkoxy is defined as above.

[0309] R¹⁰⁵ of Formula (25) can be selected from hydrogen, lower alkyl, cycloalkyl, or alkoxy. Preferably, R¹⁰⁵ of Formula (25) is selected from hydrogen, methyl, methoxy, or ethoxy. Lower alkyl is defined as above. Cycloalkyl is defined as above. Alkoxy is defined as above.

[0310] R¹⁰⁶ of Formula (25) can be selected from hydrogen, lower alkyl, aryl, or substituted alkyl. Preferably, R¹⁰⁶ of Formula (25) is selected from methyl or phenyl. Lower alkyl is defined as above. Substituted alkyl is defined as lower alkyl group with substitution of additional groups such as an aryl thioether, -S-Ar', where Ar' is defined as phenyl, substituted phenyl or an unsubstituted heteroaryl five or six membered ring containing from 1 to 4 N, O, or

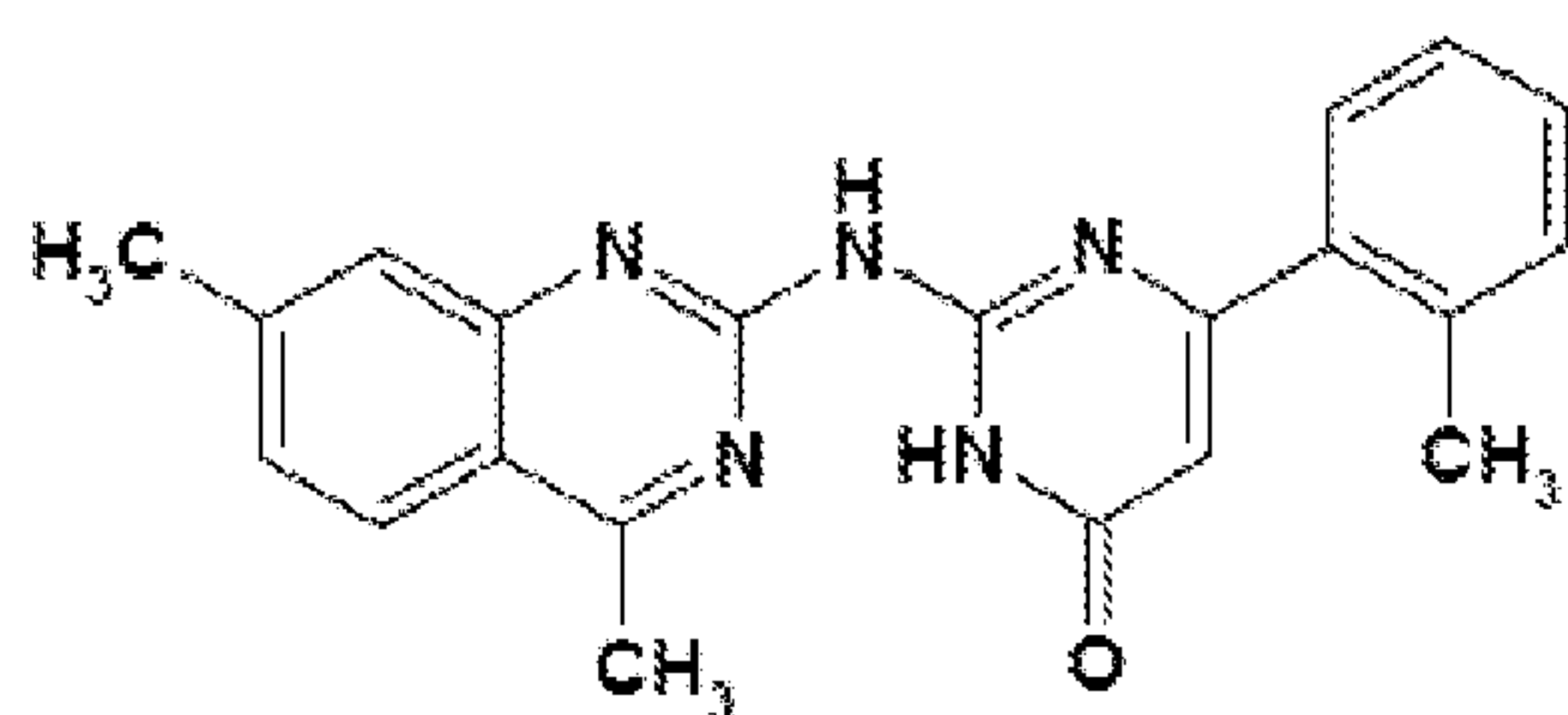
S atoms, or a heteroaryl five or six membered ring containing from 1 to 4 N, O, or S atoms which has one or more optional substitution with the substituent defined as one or more of the following groups: lower alkyl defined as above, cycloalkyl defined as above, unsaturation, bicyclic heterocycles such as benzimidazoles, benzoxazoles, benzthiazoles, or benzopyrazoles. Aryl is defined as an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more of the following groups: lower alkyl defined as C-1 to C-4, straight chain, branched, or optionally containing unsaturation, Cycloalkyl defined as C-1 to C-6 optionally containing unsaturation, aryl including phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms, Alkoxy ($-\text{OR}^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition), trifluoromethyl, trifluoromethoxy, difluoromethoxy, 3, 4-methylenedioxy, 2, 3-methylenedioxy, nitro or halogen (F, Cl, Br, I).

[0311] R^{107} of Formula (25) can be selected from hydrogen, lower alkyl, cycloalkyl, aryl, or substituted alkyl. Preferably, R^{107} of Formula (25) is methyl. Lower alkyl is defined as above. Cycloalkyl is defined as above. Aryl is defined as above. Substituted alkyl is defined as above.

[0312] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (25).

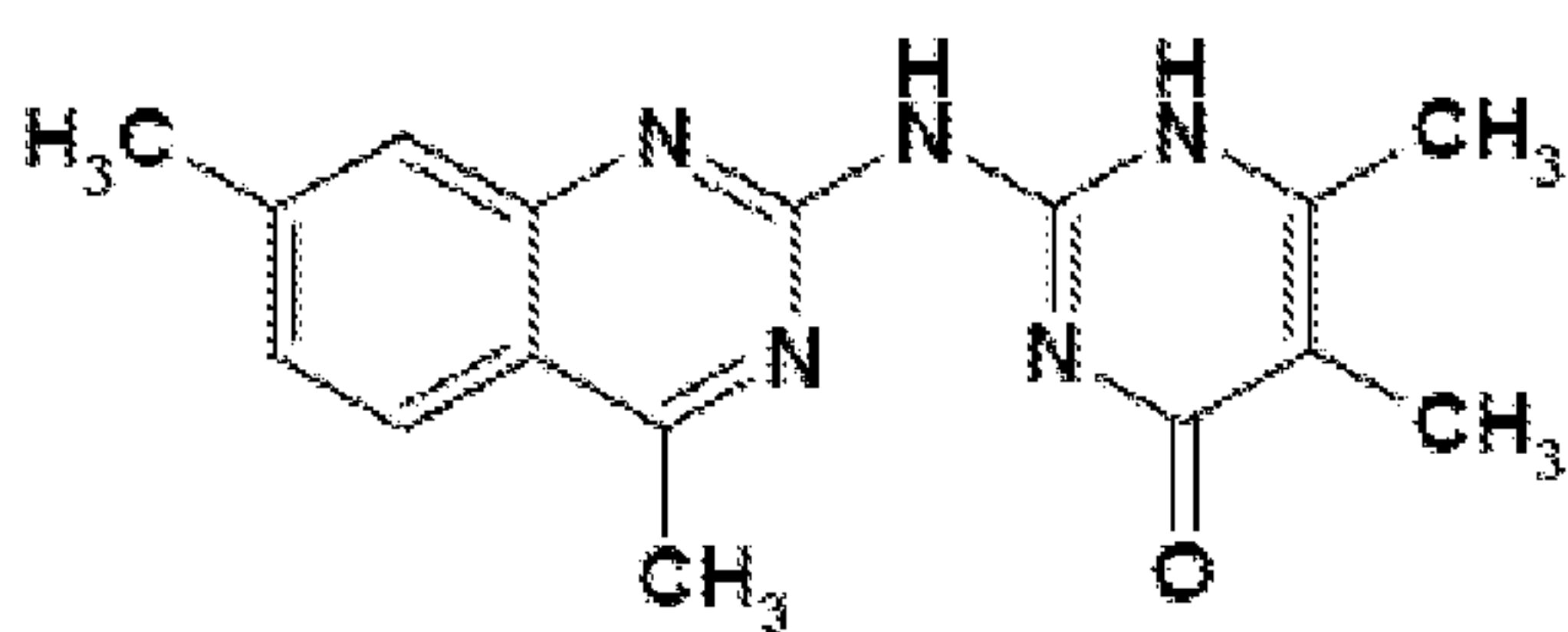
[0313] In some embodiments, the compound(s) of Formula (25) excludes compound AD4-10381, Formula (24).

[0314] In some embodiments, the compound of Formula (25) is AD4-11511.



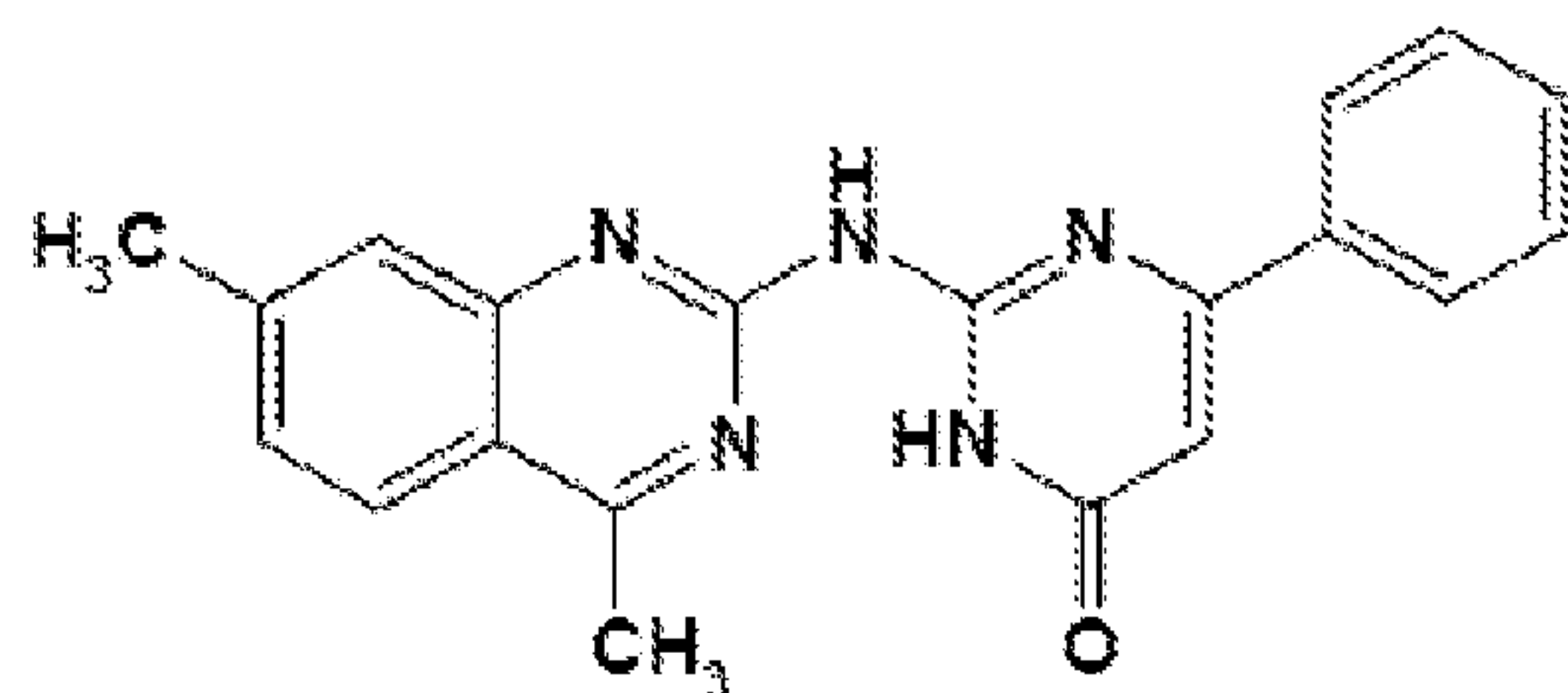
AD4-11511, Formula (26)

[0315] In some embodiments, the compound of Formula (25) is AD4-12632.



AD4-12632

[0316] In some embodiments, the compound of Formula (25) is AD4-10381.

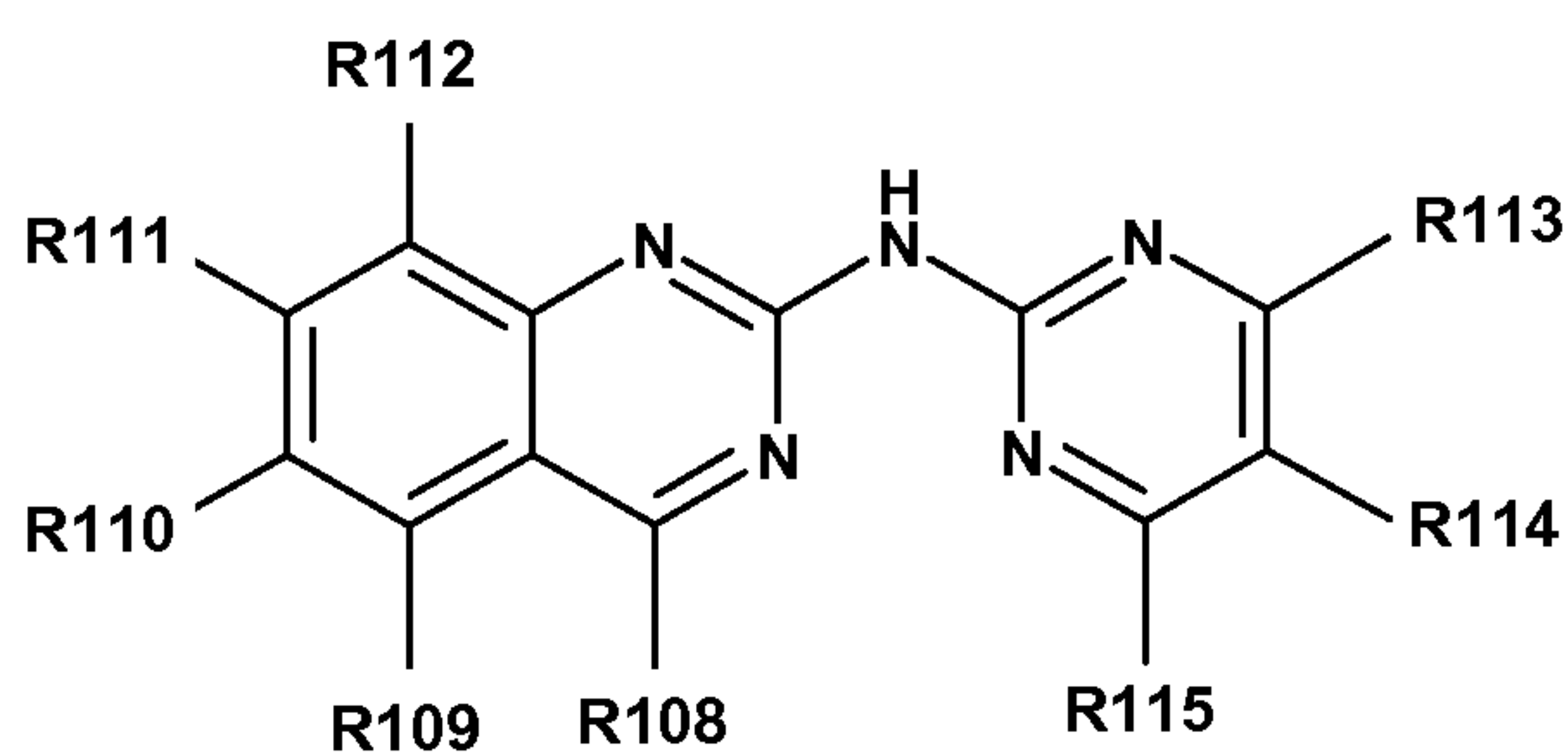


AD4-10381

[0317] In some embodiments, the compound of Formula (25) excludes AD4-10381.

[0318] Type E AD4-10381-like

[0319] One structure derived from the AD4-10381-like pharmacophore is “Type E” as follows:



Formula (27)

[0320] In the above structure, R^{108} of Formula (27) can be selected from lower alkyl, substituted alkyl, cycloalkyl, aryl, amino, or hydroxyl. Preferably, R^{108} of Formula (27) is selected from methyl, phenyl, or hydroxyl. Lower alkyl is defined as above. Substituted alkyl is defined as above. Cycloalkyl is defined as above. Aryl is defined as above.

[0321] R^{109} of Formula (27) can be selected from hydrogen.

[0322] R^{110} of Formula (27) can be selected from hydrogen, lower alkyl, substituted alkyl, cycloalkyl, alkoxy, or halogen. Preferably, R^{110} of Formula (27) is selected from hydrogen, methyl, methoxy, or chloro. Lower alkyl is defined as above. Substituted alkyl is defined as above. Alkoxy is defined as above. Halogen is defined as F, Cl, Br, or I.

[0323] R^{111} of Formula (27) can be selected from hydrogen, lower alkyl, substituted alkyl, cycloalkyl, alkoxy, or halogen. Preferably, R^{111} of Formula (27) is selected from hydrogen, methyl, methoxy, or chloro. Lower alkyl is defined as above. Substituted alkyl is defined as above. Cycloalkyl is defined as above. Alkoxy is defined as above. Halogen is defined as above.

[0324] R^{112} of Formula (27) can be selected from hydrogen or lower alkyl. Preferably, R^{112} of Formula (27) is methyl. Lower alkyl is defined as above.

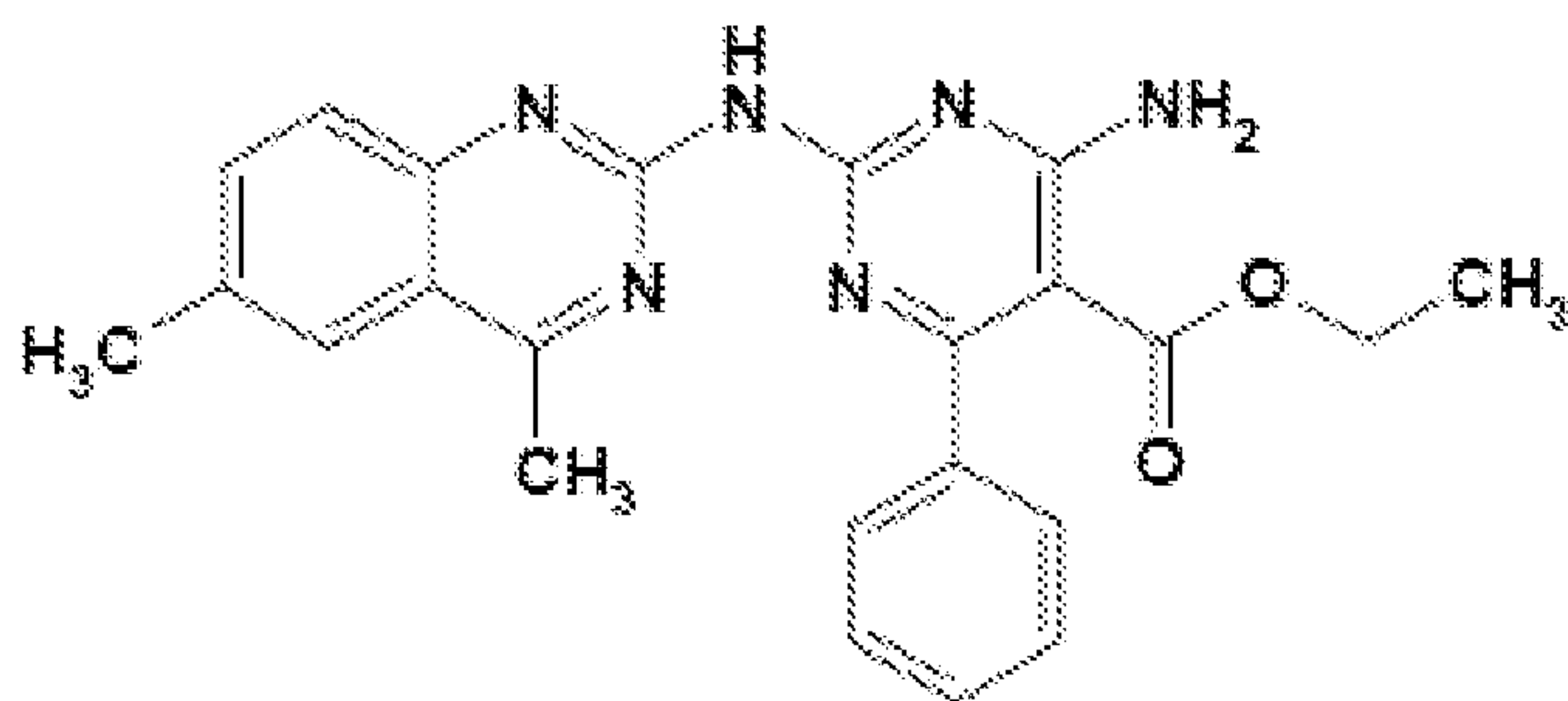
[0325] R^{113} of Formula (27) can be selected from lower alkyl, trifluoromethyl, aryl, or amino. Preferably, R^{113} of Formula (27) is selected from methyl, phenyl, or amino. Lower alkyl is defined as above. Aryl is defined as above.

[0326] R^{114} of Formula (27) can be selected from hydrogen, acyl or carboxyalkyl. Acyl represents a substituted ketone $-COR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition. Carboxyalkyl is defined as a substituted ester group $COOR^{10}$ where R^{10} is defined as a lower alkyl group or cycloalkyl group in the above definition.

[0327] R^{115} of Formula (27) can be selected from lower alkyl, trifluoromethyl, or aryl. Preferably, R^{115} of Formula (27) is methyl, phenyl or trifluoromethyl. Lower alkyl is defined as above. Aryl is defined as above.

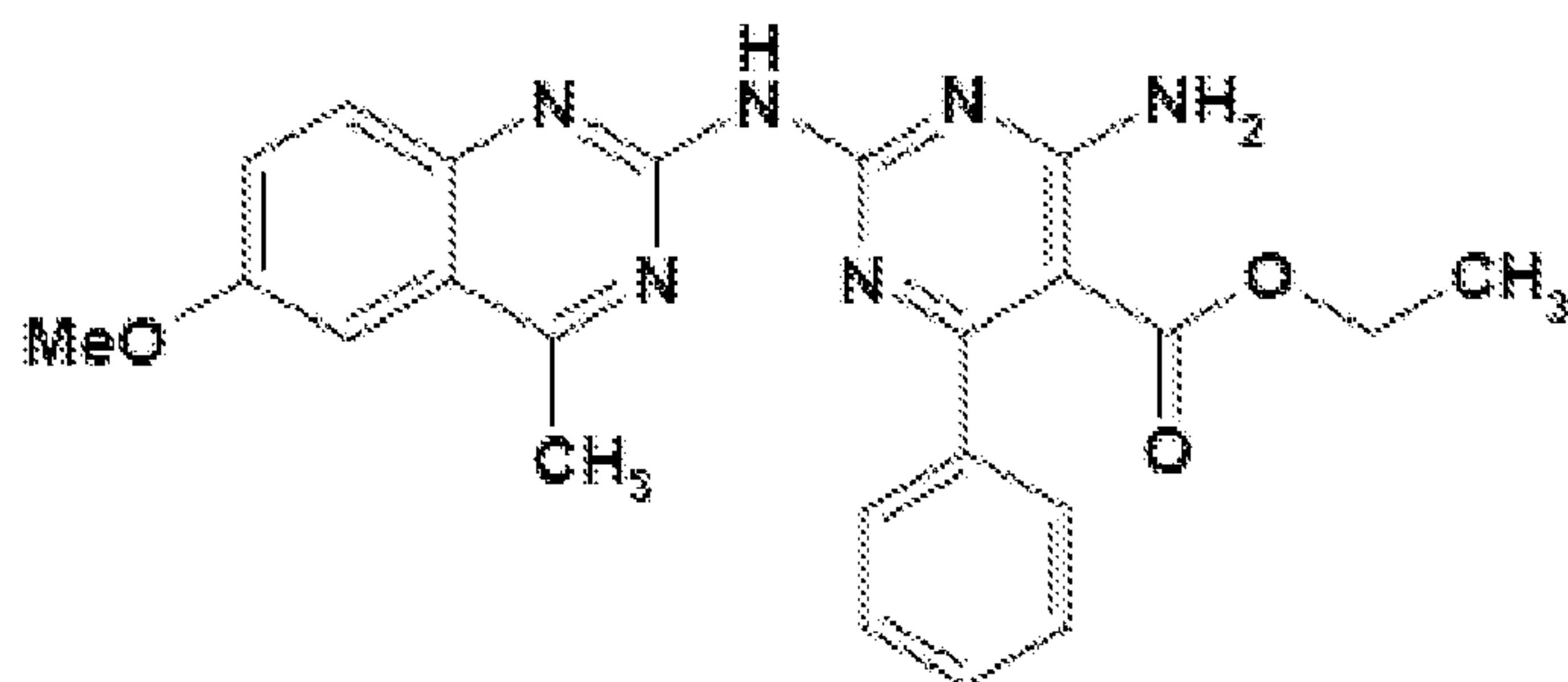
[0328] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (27).

[0329] In some embodiments, the compound of Formula (27) is AD4-12681.



AD4-12681

[0330] In some embodiments, the compound of Formula (27) is AD4-12679.

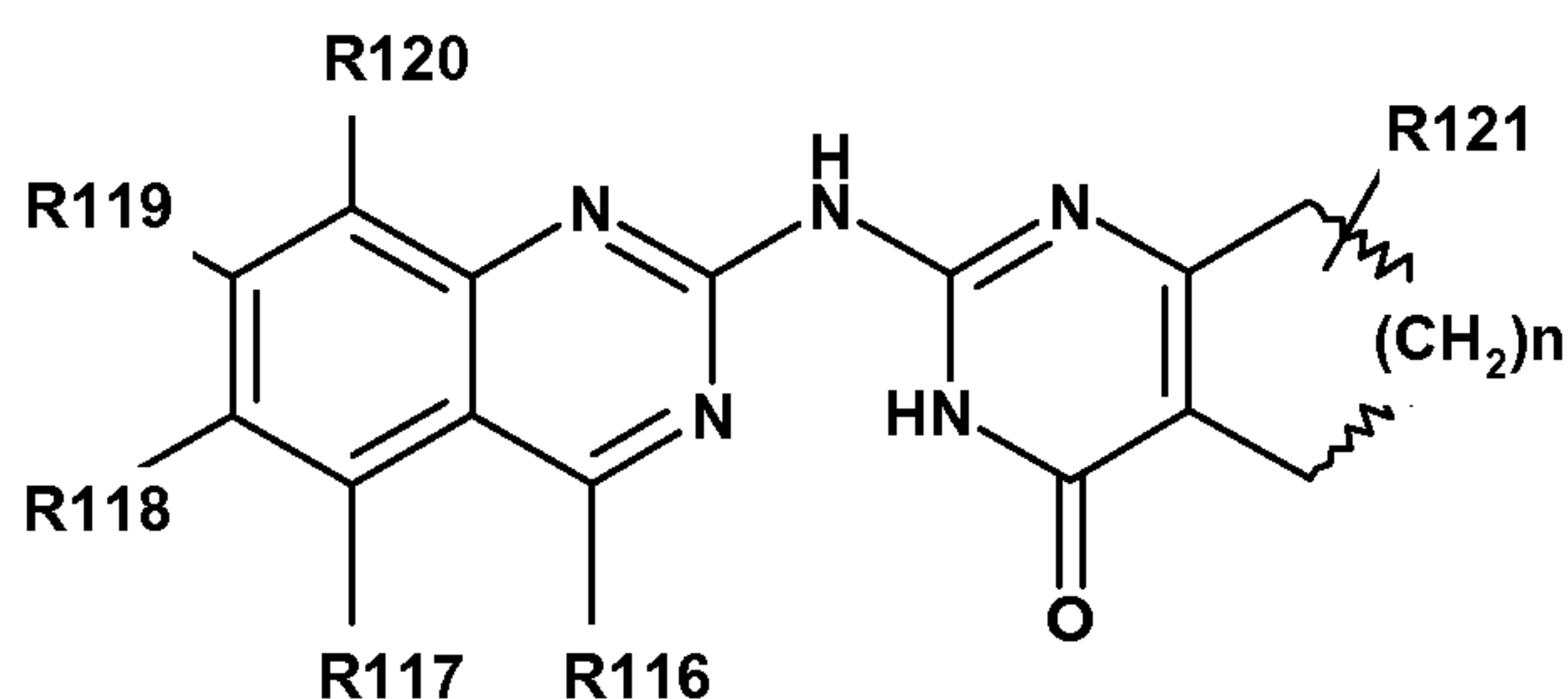


AD4-12679

[0331] In some embodiments, the compound(s) of Formula (27) excludes compound AD4-10381, Formula (24).

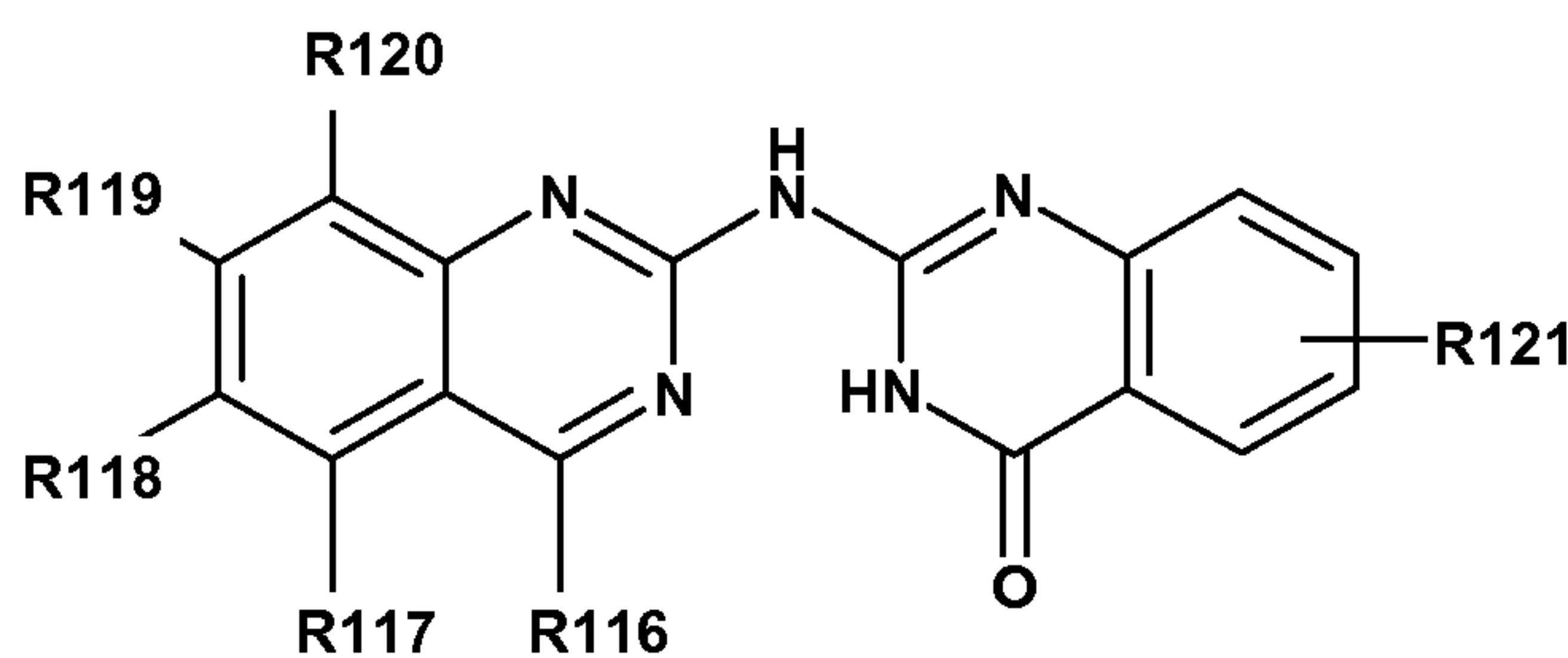
[0332] Type F AD4-10381-like

[0333] One structure derived from the AD4-10381-like pharmacophore is “Type F” as follows:



Formula (28)

[0334] In the above structure, n of Formula (28) can be 1 or 2, which represents one or two methylene groups resulting in either a five or six membered carbocyclic ring. The six membered ring contains optional unsaturation resulting in a fused benzo-ring system as shown below (see Formula (29)).



Formula (29)

[0335] R¹¹⁶ of Formula (28) or Formula (29) can be selected from lower alkyl or aryl. Preferably, R¹¹⁶ of Formula (28) or Formula (29) are independently methyl or phenyl. Lower alkyl is defined as above. Aryl is defined as above.

[0336] R¹¹⁷ of Formula (28) or Formula (29) can be selected from hydrogen.

[0337] R¹¹⁸ of Formula (28) or Formula (29) can be selected from hydrogen, lower alkyl, alkoxy, or halogen. Lower alkyl is defined as above. Alkoxy is defined as above. Halogen is defined as above.

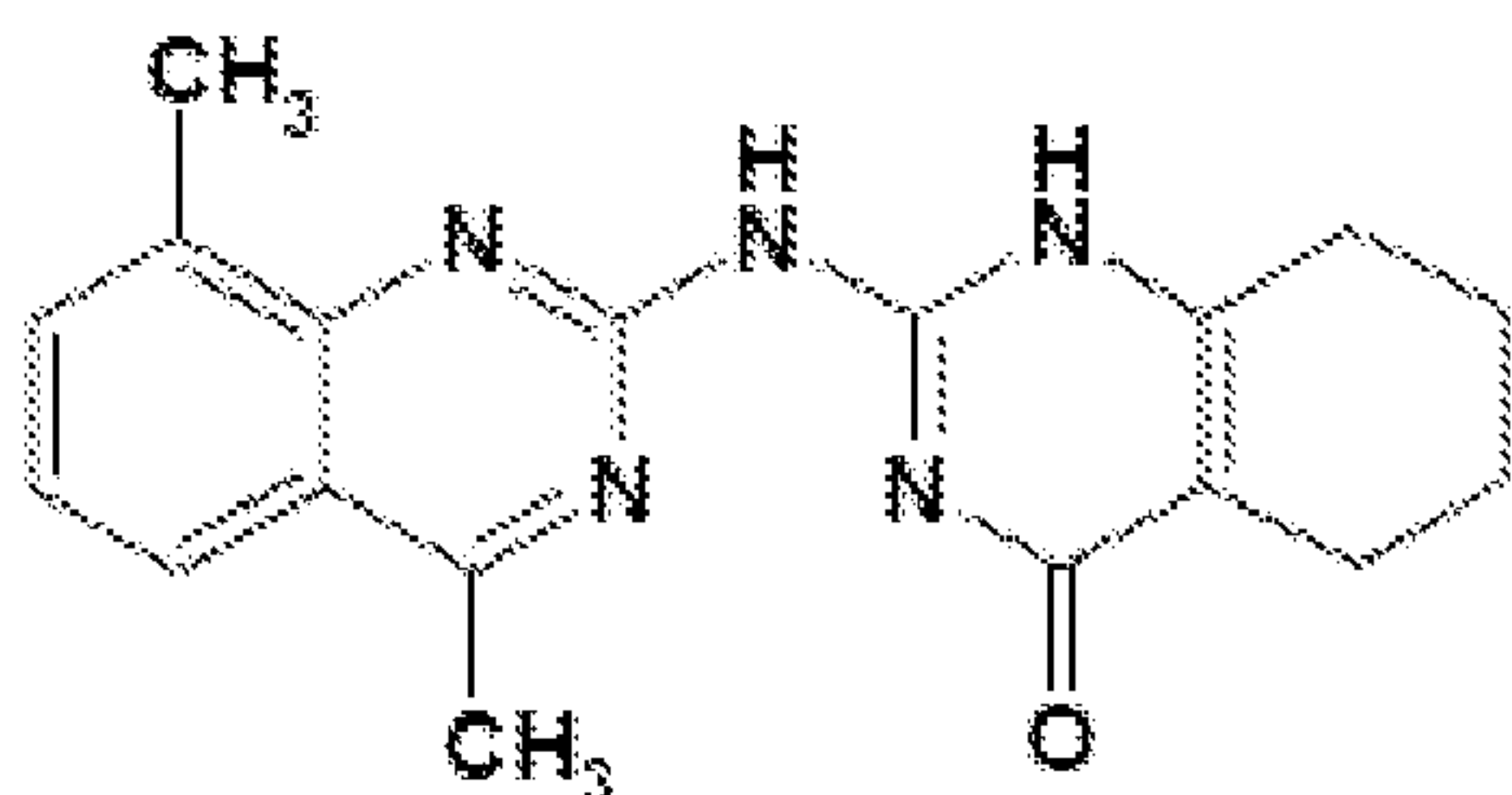
[0338] R¹¹⁹ of Formula (28) or Formula (29) can be selected from hydrogen, lower alkyl, or aryl. Lower alkyl is defined as above. Aryl is defined as above.

[0339] R^{120} of Formula (28) or Formula (29) can be selected from hydrogen, lower alkyl, or alkoxy. Lower alkyl is defined as above. Alkoxy is defined as above.

[0340] R^{121} of Formula (28) or Formula (29) can be selected from hydrogen or aryl. Aryl is defined as above.

[0341] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (28) or Formula (29).

[0342] In some embodiments, the compound of Formula (28) is AD4-11436.

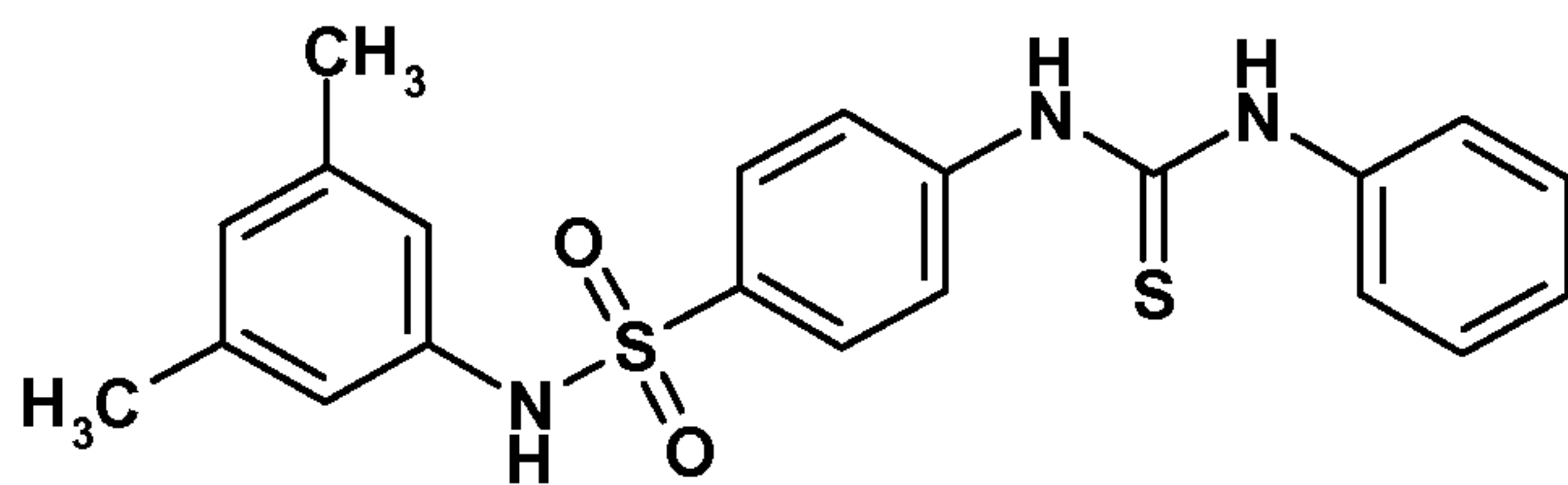


AD4-11436

[0343] In some embodiments, the compound(s) of Formula (28) and Formula (29) excludes compound AD4-10381, Formula (24).

[0344] AD4-11091

[0345] AD4-11091 is identified as an inhibitor of epidermal growth factor binding to its receptor (*see e.g.*, Example 4).

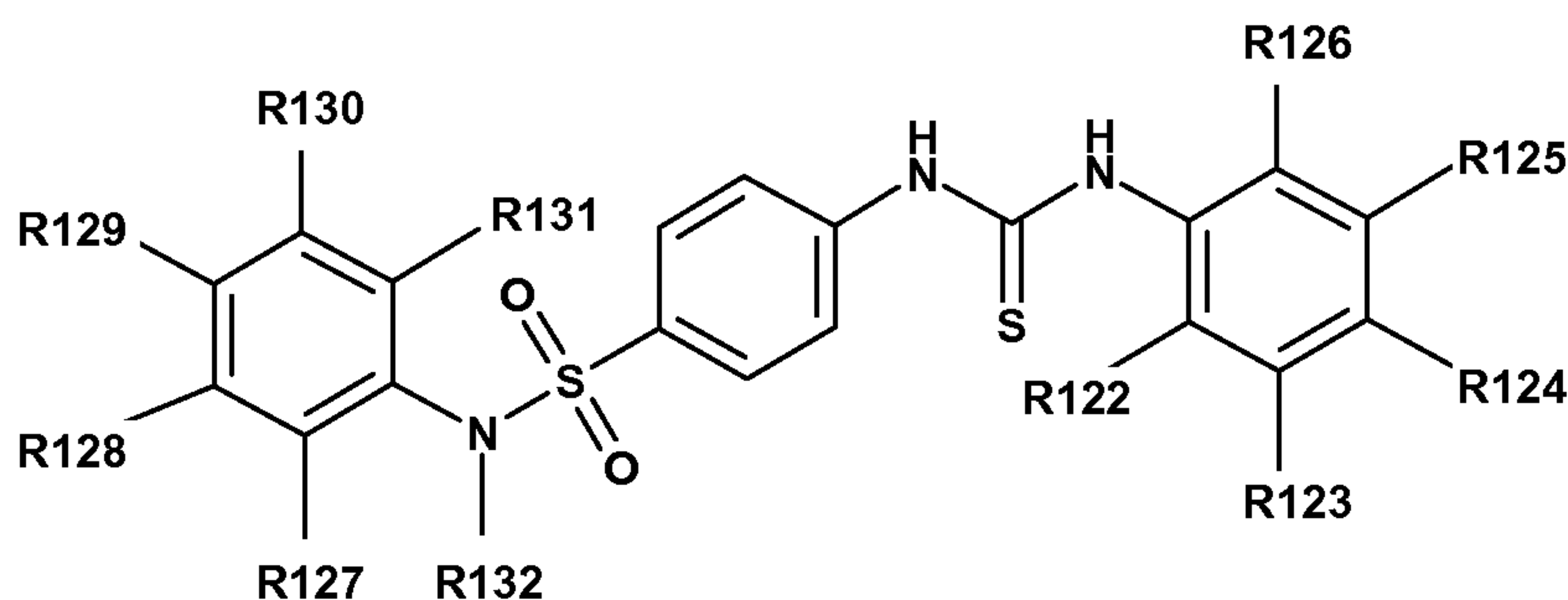


AD4-11091, Formula (31)

[0346] As described herein, a pharmacophore model was utilized to identify small molecules that are AD4-11091-like.

[0347] Type H AD4-11091-like

[0348] One structure derived from the AD4-10381-like pharmacophore is “Type H” as follows:



Formula (32)

[0349] In the above structure, R¹²² and R¹²⁶ of Formula (32) can be independently selected from hydrogen, alkoxy, trifluoromethyl or halogen. Preferably, R¹²² and R¹²⁶ of Formula (32) are independently selected from hydrogen, trifluoromethyl, methoxy, or chloro. More preferably, one or both of R¹²² and R¹²⁶ of Formula (32) is hydrogen. Alkoxy and halogen are as defined above.

[0350] R¹²³ and R¹²⁵ of Formula (32) can be independently selected from hydrogen, trifluoromethyl, halogen, lower alkyl, acyl or alkoxy. Preferably, R¹²³ and R¹²⁵ of Formula (32) are independently selected from halogen or trifluoromethyl. Halogen, lower alkyl, acyl and alkoxy are as defined above.

[0351] R¹²⁴ of Formula (32) can be selected from hydrogen, lower alkyl, alkoxy, acyl or halogen. Preferably, R¹²⁴ of Formula (32) is lower alkyl or halogen. Lower alkyl, alkoxy, acyl and halogen are as defined above.

[0352] R¹²⁷ and R¹³¹ of Formula (32) can be independently selected from hydrogen, lower alkyl, or alkoxy. Preferably, R¹²⁷ and R¹³¹ of Formula (32) are independently selected from hydrogen, methyl, or methoxy. Lower alkyl and alkoxy are as defined above.

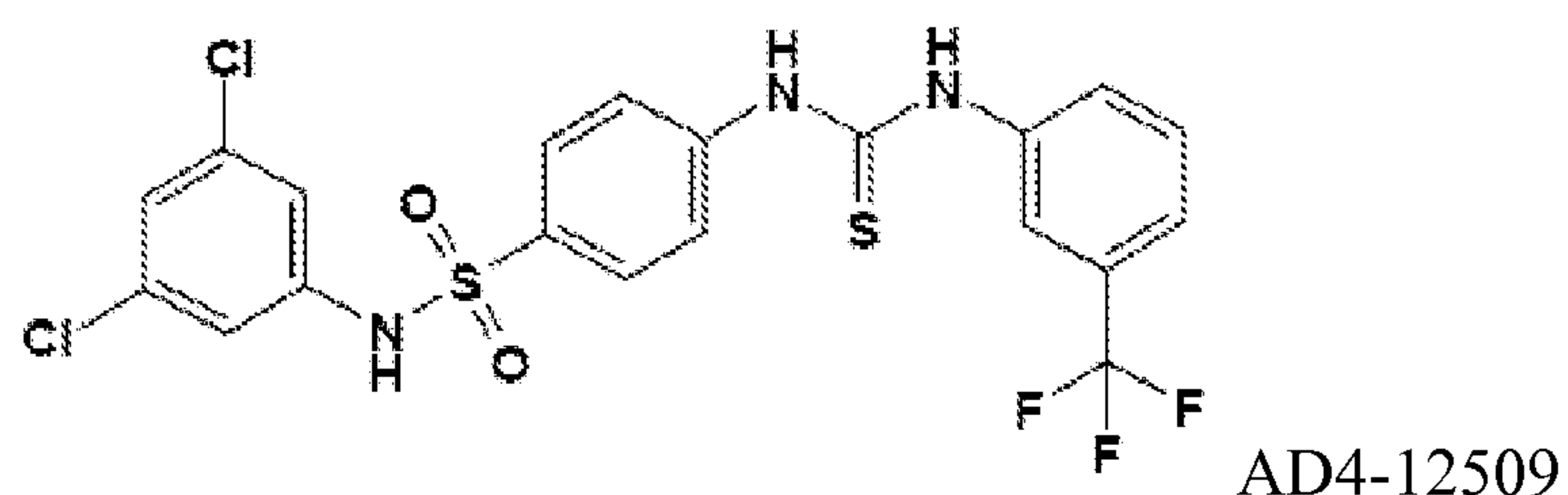
[0353] R¹²⁸ and R¹³⁰ of Formula (32) can be independently selected from hydrogen or halogen. Preferably, R¹²⁸ and R¹³⁰ of Formula (32) are independently selected from hydrogen or chloro. Halogen is as defined above.

[0354] R¹²⁹ of Formula (32) can be selected from hydrogen, halogen, alkoxy, or lower alkyl. Preferably, R¹²⁹ of Formula (32) is selected from hydrogen, chloro, methoxy, or methyl. Halogen, alkoxy, and lower alkyl are as defined above.

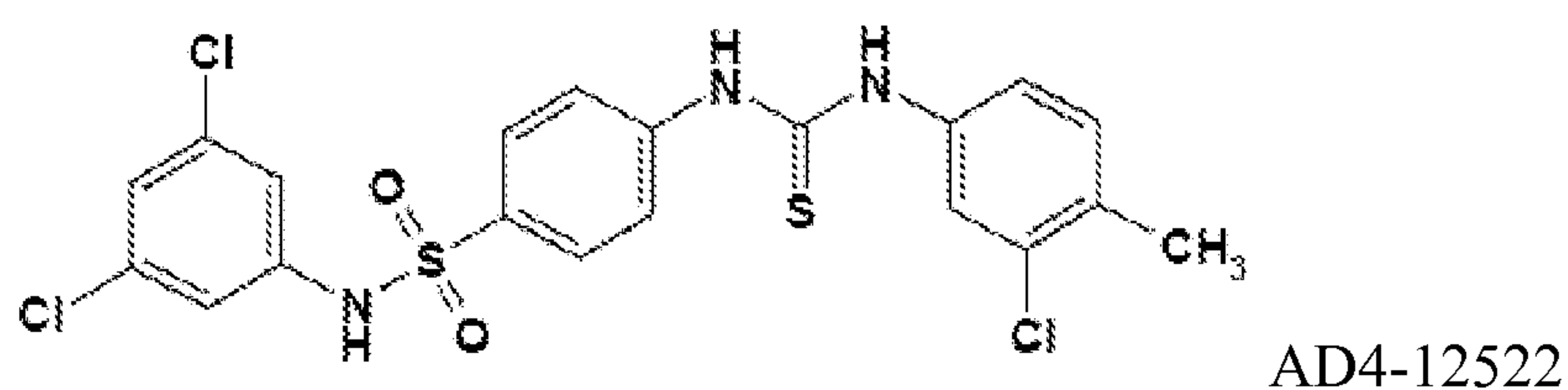
[0355] R¹³² of Formula (32) can be selected from hydrogen or lower alkyl. Preferably, R¹³² of Formula (32) is selected from hydrogen, methyl, or ethyl. Lower alkyl is as defined above.

[0356] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (32).

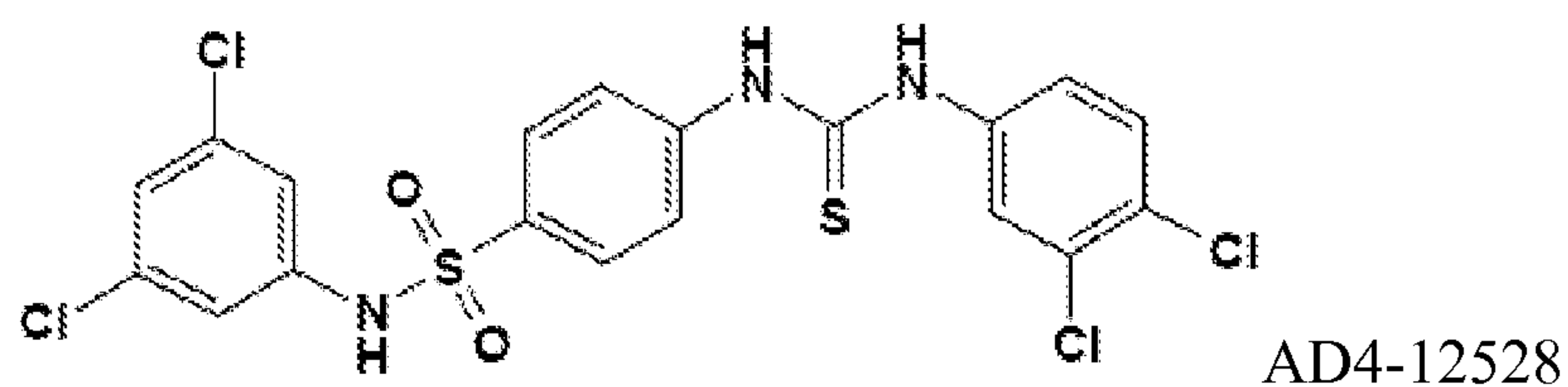
[0357] In some embodiments, the compound of Formula (32) is AD4-12509.



[0358] In some embodiments, the compound of Formula (32) is AD4-12522.



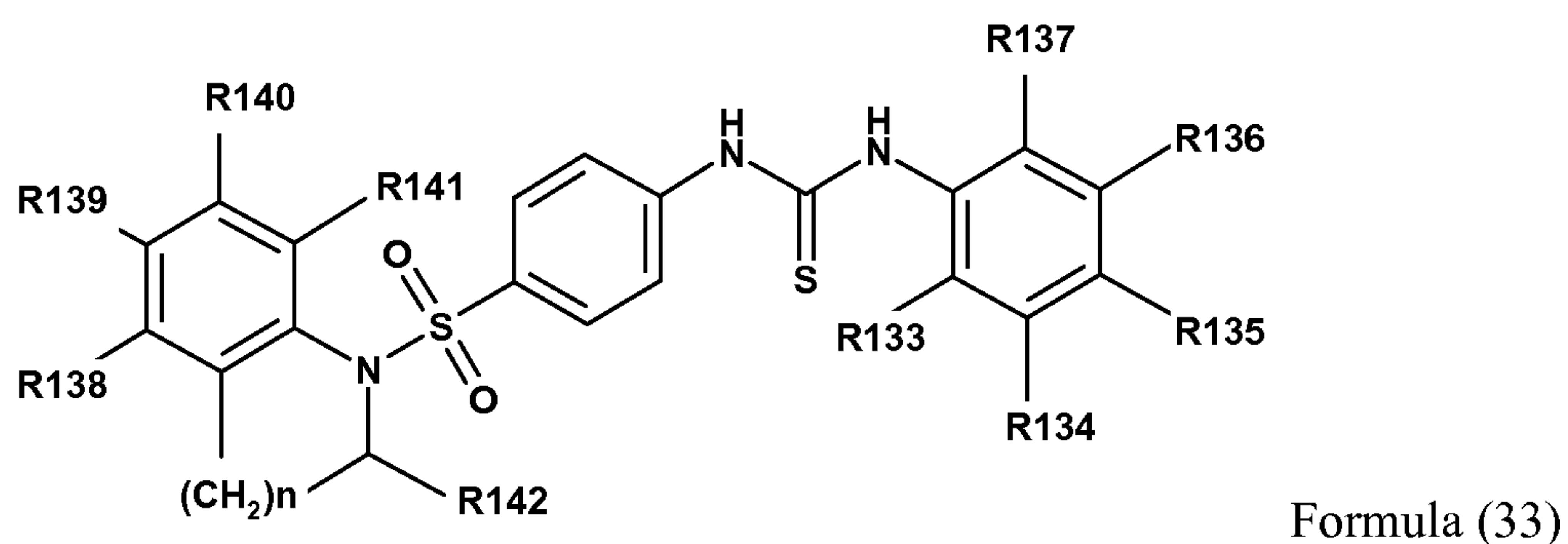
[0359] In some embodiments, the compound of Formula (32) is AD4-12528.



[0360] In some embodiments, the compound(s) of Formula (32) excludes compound AD4-11091, Formula (31).

[0361] Type I AD4-11091-like

[0362] One structure derived from the AD4-10381-like pharmacophore is “Type I” as follows:



[0363] In the above structure, n of Formula (33) can be 1 or 2, which represents one or two methylene groups resulting in either a five or six membered carbocyclic ring.

[0364] R^{133} and R^{137} of Formula (33) can be independently selected from hydrogen, lower alkyl or halogen. Preferably, R^{133} and R^{137} of Formula (33) are independently selected from hydrogen, methyl or chloro. Lower alkyl and halogen are as defined above.

[0365] R^{134} and R^{136} of Formula (33) can be independently selected from hydrogen, trifluoromethyl, or halogen. Preferably, R^{134} and R^{136} of Formula (33) are independently selected hydrogen, trifluoromethyl, or chloro. Halogen is as defined above.

[0366] R^{135} of Formula (33) can be selected from hydrogen or halogen. Preferably, R^{135} of Formula (33) is chloro. Halogen is as defined above.

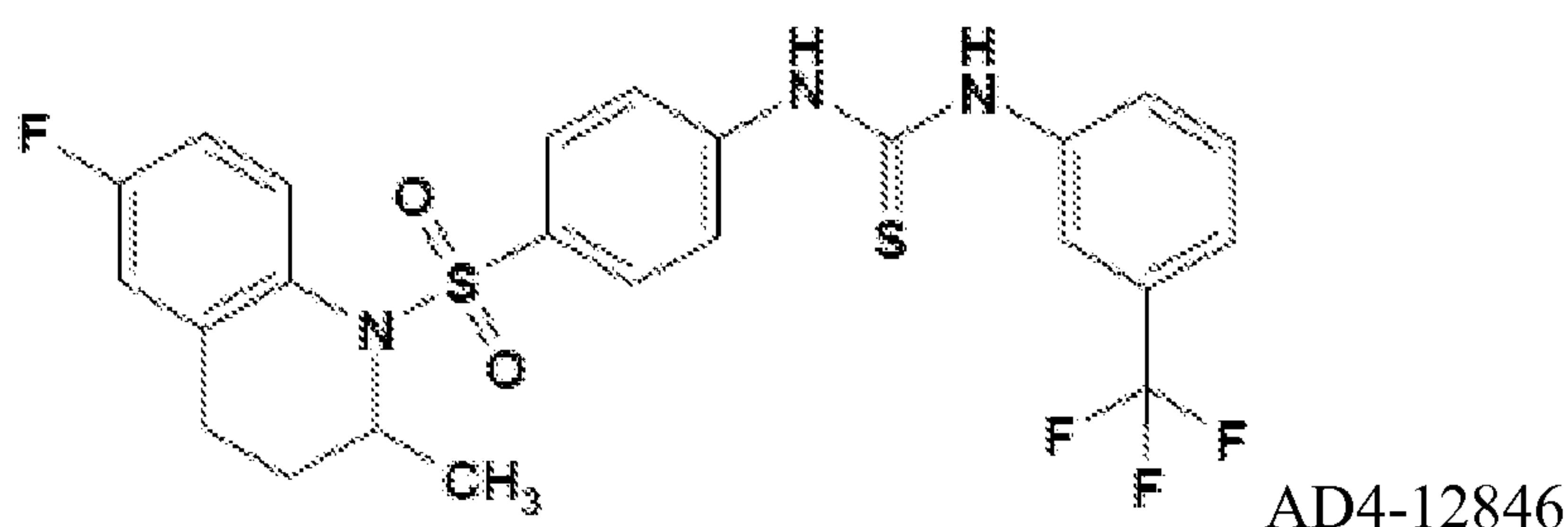
[0367] R^{138} , R^{140} , and R^{141} of Formula (33) can be independently selected from hydrogen.

[0368] R^{139} of Formula (33) can be selected from hydrogen or halogen. Preferably, R^{139} of Formula (33) is selected from fluoro or hydrogen. Halogen is as defined above.

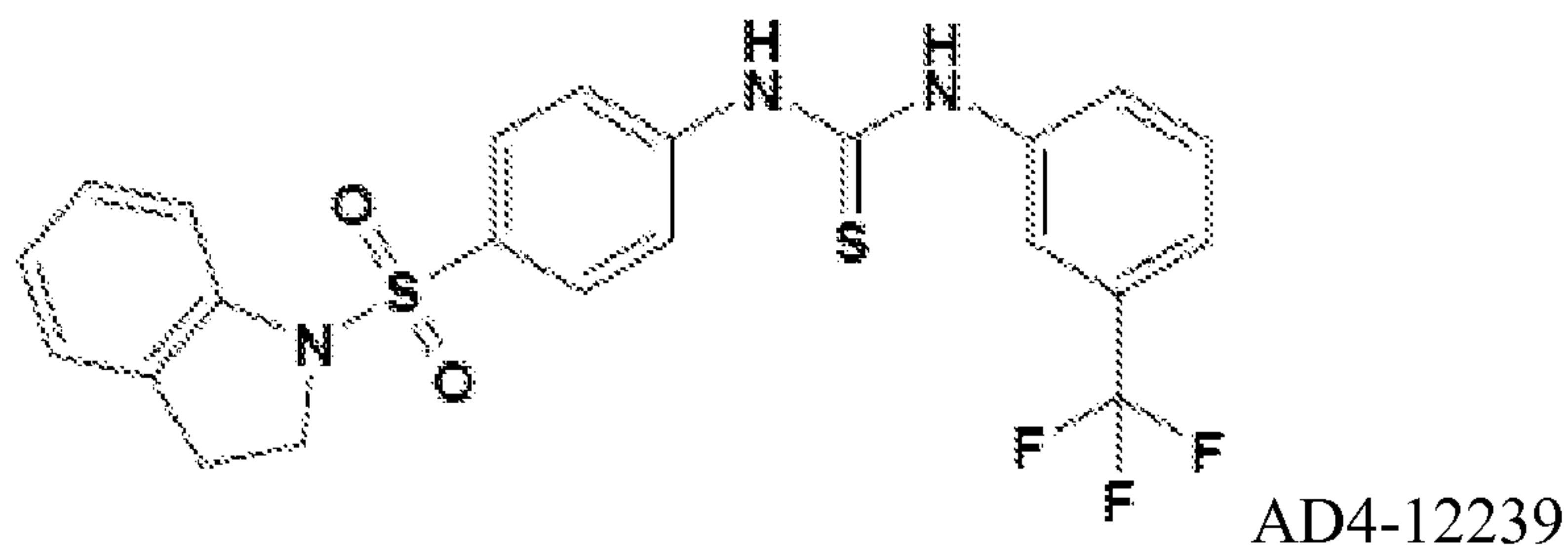
[0369] R^{142} of Formula (33) can be selected from hydrogen or lower alkyl. Preferably, R^{142} of Formula (33) is selected from hydrogen or methyl. Lower alkyl is as defined above.

[0370] In some embodiments, the compound(s) are the enantiomeric isomers of Formula (33).

[0371] In some embodiments, the compound of Formula (33) is AD4-12846.



[0372] In some embodiments, the compound of Formula (33) is AD4-12239.

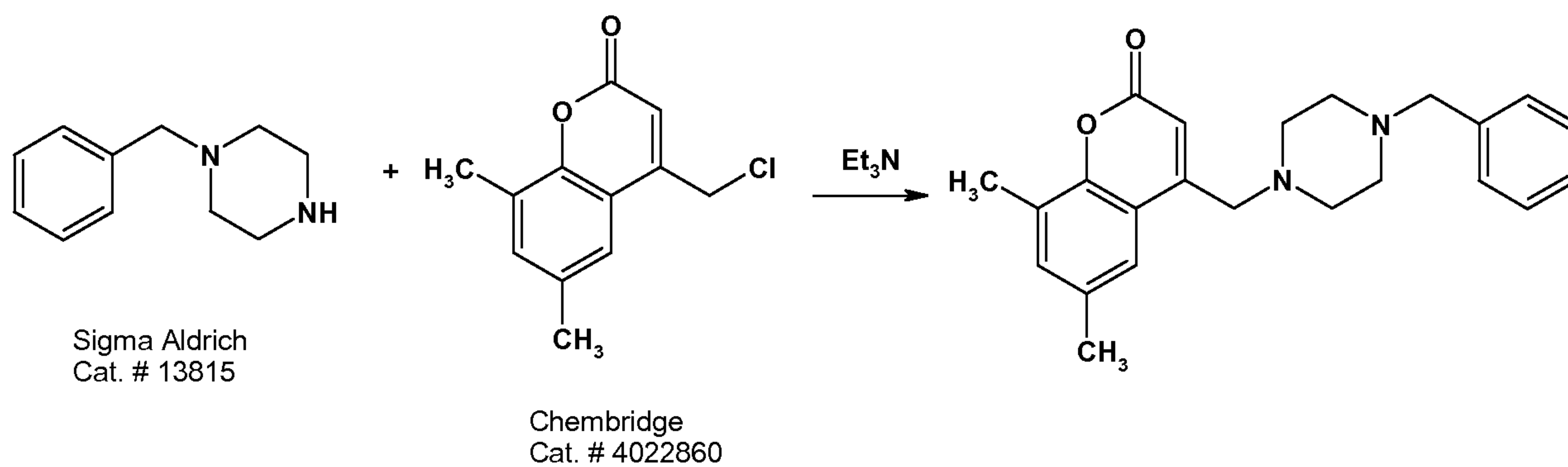


[0373] In some embodiments, the compound(s) of Formula (33) excludes compound AD4-11091, Formula (31).

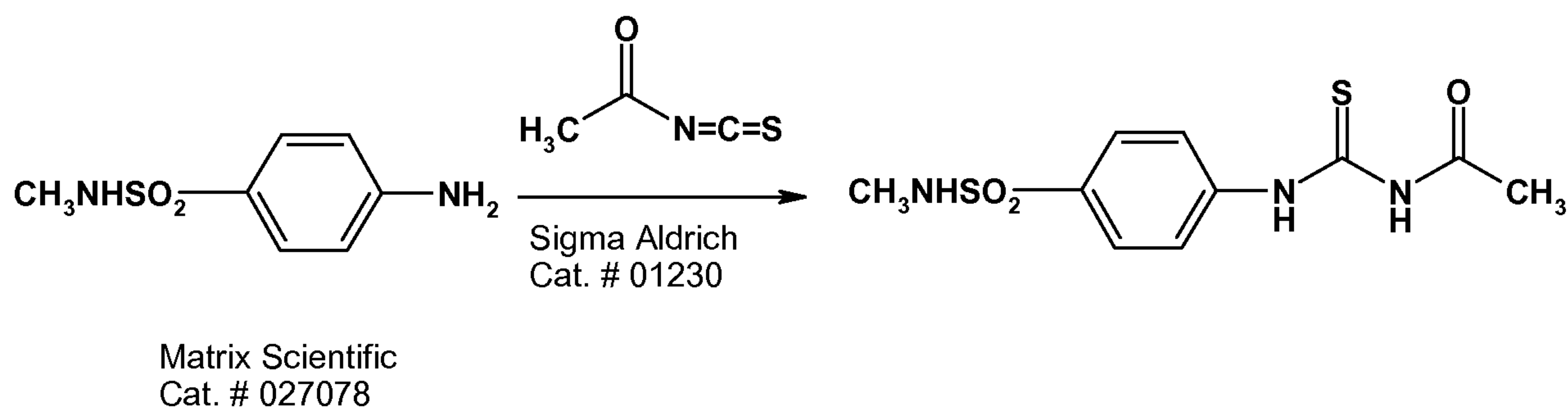
SYNTHESIS

[0374] One aspect provides methods for the synthesis of compounds described herein (see Example 9).

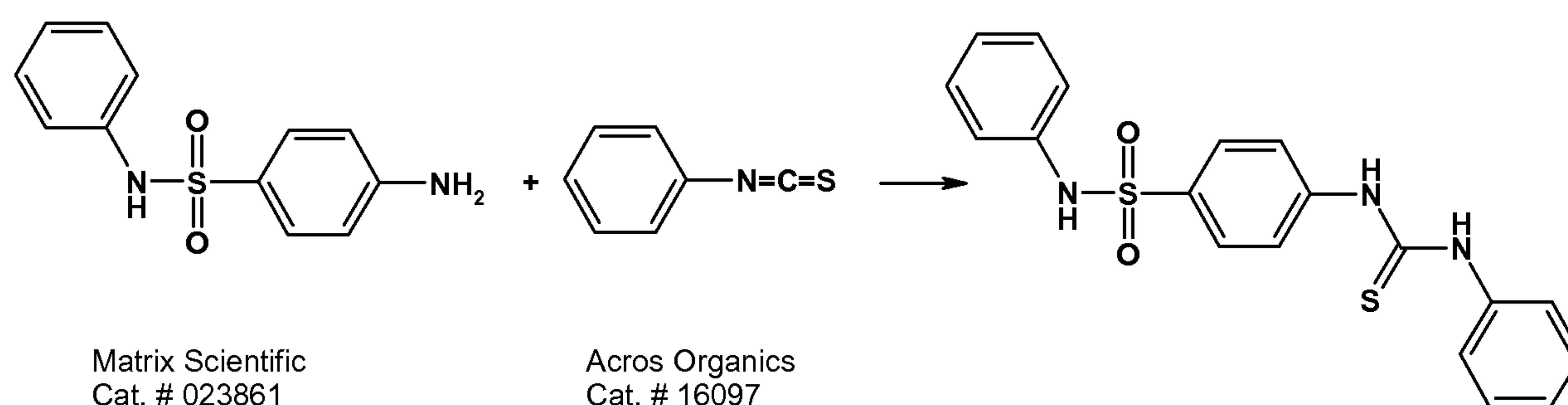
[0375] An AD4-1734-like compound can be synthesized as follows:



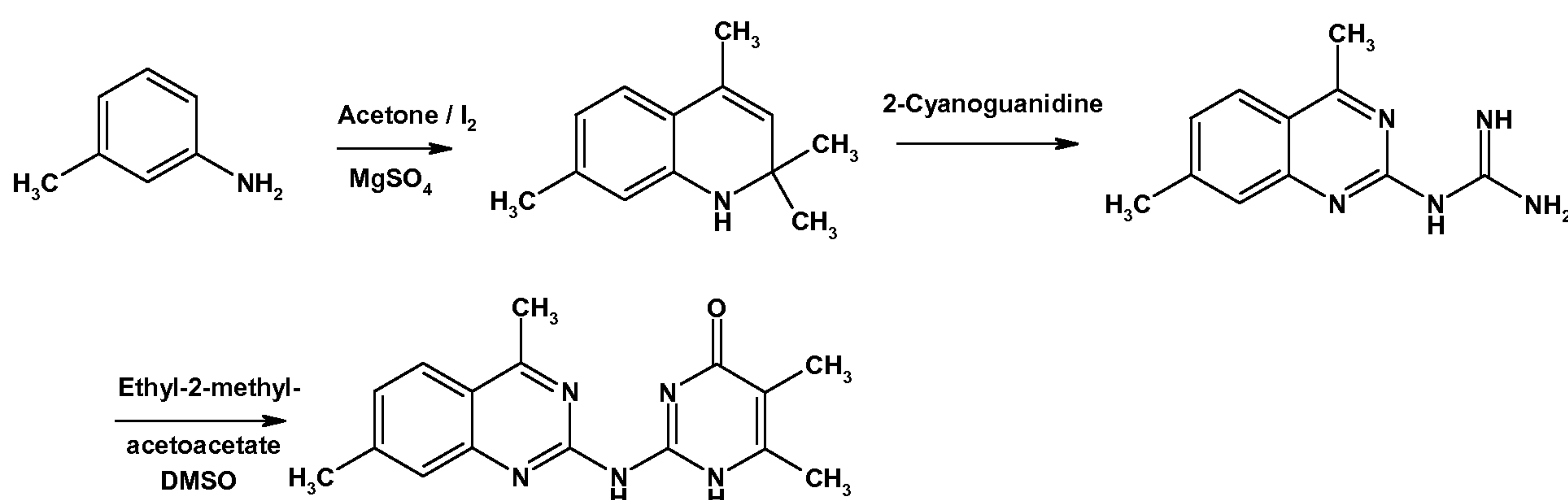
[0376] An AD4-1886-like compound can be synthesized as follows:



[0377] An AD4-11091-like compound can be synthesized as follows:



[0378] An AD4-11511-like compound can be synthesized as follows:



[0379] Further methodology for synthesis of AD4-12632, which is an AD4-11511-like compound, is provided in Example 9.

[0380] The above reactions can include any condition or combination of conditions disclosed in Example 9.

PHARMACEUTICAL FORMULATIONS

[0381] Embodiments of the compositions of the invention include pharmaceutical formulations of the various compounds described herein. A compound described herein can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt form and with or without a pharmaceutically acceptable excipient. The compounds described herein can be formulated by any conventional manner using one or more pharmaceutically acceptable carriers and/or excipients as described in, for example, Remington's Pharmaceutical Sciences (A.R. Gennaro, Ed.), 21st edition, ISBN: 0781746736 (2005). Such formulations will contain a therapeutically effective amount of the agent, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the subject. The

formulation should suit the mode of administration. The agents of use with the current invention can be formulated by known methods for administration to a subject using several routes which include, but are not limited to, parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, and rectal. The individual agents may also be administered in combination with one or more additional agents of the present invention and/or together with other biologically active or biologically inert agents. Such biologically active or inert agents may be in fluid or mechanical communication with the agent(s) or attached to the agent(s) by ionic, covalent, Van der Waals, hydrophobic, hydrophilic or other physical forces.

[0382] Controlled-release (or sustained-release) preparations may be formulated to extend the activity of the agent and reduce dosage frequency. Controlled-release preparations can also be used to effect the time of onset of action or other characteristics, such as blood levels of the agent, and consequently affect the occurrence of side effects.

COMBINATION WITH KINASE INHIBITORS

[0383] Compounds described herein can be used with, or formulated with, known therapeutic compounds. Combination therapy is understood as a therapeutic regimen comprising, e.g., an anti-proliferative compound described herein and a second agent. An anti-proliferative compound and a second agent can be formulated for separate administration or may be formulated for administration together.

[0384] Compounds described herein can be combined with another anti-proliferative compound, such as the EGFR kinase inhibitors, Tykerb, Iressa, and Tarceva, or Erbitux, a humanized monoclonal antibody to the EGF receptor, to produce a greater therapeutic effect than either agent alone. As shown herein, when AD4 compounds were evaluated in a cell proliferation assay with Tykerb, Iressa, Tarceva or Erbitux, the effect of the combination of agents to inhibit cell proliferation was greater than the effect of any of the agents alone (see e.g., Example 8). Specifically, compounds described herein were evaluated with Tykerb, Iressa, Tarceva or Erbitux at a fixed concentration ratio, which was ascertained from the results of dose-response curves of each agent alone.

[0385] An compound described herein, such as an EGFR inhibitor, can be used with, or formulated and used with a second agent that inhibits vascularization of a tumor.

Vascularization of a solid tumor generally refers to formation of blood vessels in a solid tumor. An agent that inhibits the vascularization of a tumor can inhibit vessel initiation, development, or maintenance leading to, for example, the reduction in the number or density of vessels in a tumor.

[0386] A compound described herein can be used with, or formulated and used with a second agent that modifies, for example increasing, permeability of a solid tumor. Permeability of a solid tumor generally refers to the permeability of a solid tumor to a therapeutic. A solid tumor may be said to be permeable to a therapeutic if the therapeutic is able to reach cells at the center of the tumor.

[0387] A compound described herein can be used with, or formulated and used with, a chemotherapeutic second agent. A chemotherapeutic agent refers to a molecule or composition used to treat a malignancy. Such agents can be used in combination with a compound described herein or with a combination therapy described herein. Chemotherapeutic agents include agents that can be conjugated to a compound described herein or can be used in combination with the combination therapy in unconjugated form.

[0388] A compound described herein can be used with, or formulated and used with a second agent that is a biological agent. A biological agent, also called a biologic, are generally understood as a product of a biological system, e.g., an organism, cell, or recombinant system. Examples of such biologic agents include, but are not limited to, nucleic acid molecules (e.g., antisense nucleic acid molecules), interferons, interleukins, colony-stimulating factors, antibodies (e.g., monoclonal antibodies), and cytokines.

[0389] A compound described herein can be used or formulated with an EGFR inhibitor approved for treatment of an EGFR-related condition or disorder. For example, compounds described herein can be used with or formulated with one or more of Tykerb, Iressa, Tarceva, or Erbitux. Tykerb, Iressa, and Tarceva are kinase inhibitors that block EGFR tyrosine kinase activity. Erbitux is a humanized monoclonal antibody that binds to an extracellular epitope on EGFR. Erbitux blocks activation of the receptor by preventing both ligand binding and receptor dimerization. In various embodiments, a compound described herein can lock EGFR into a

dimerization incompetent conformation. Thus, compounds described herein and known EGFR inhibitors, such as those described above, can act in a complementary or synergistic fashion.

[0390] A compound described herein, such as AD4-1734-like compounds, AD4-1886-like compounds, AD4-10381-like compounds, or AD4-11091-like compounds, can be used or formulated with Tykerb. Compounds described herein, such as AD4-1734-like compounds, AD4-1886-like compounds, AD4-10381-like compounds, or AD4-11091-like compounds, can be used or formulated with Iressa. A compound described herein, such as AD4-1734-like compounds, AD4-1886-like compounds, AD4-10381-like compounds, or AD4-11091-like compounds, can be used or formulated with or Tarceva.

THERAPEUTIC USE

[0391] Another aspect is a process of treating a proliferative disease, disorder, or condition with a compound described herein. In various embodiments, a proliferative disease, disorder, or condition is associated with a target biomolecule having an interdomain tether associated with an activation state, such as EGFR. The therapeutic method can include administration of a therapeutically effective amount of a compound of the invention to a subject in need thereof. In some embodiments, the compound is a compound described herein having anti-proliferative effects. In some embodiments, the compound is a compound described herein having EGFR inhibitory activity. In some embodiments, the compound is an EGFR inhibitor that acts to bind one or more domains of EGFR so as to prevent tether extension and maintain an inactive conformation.

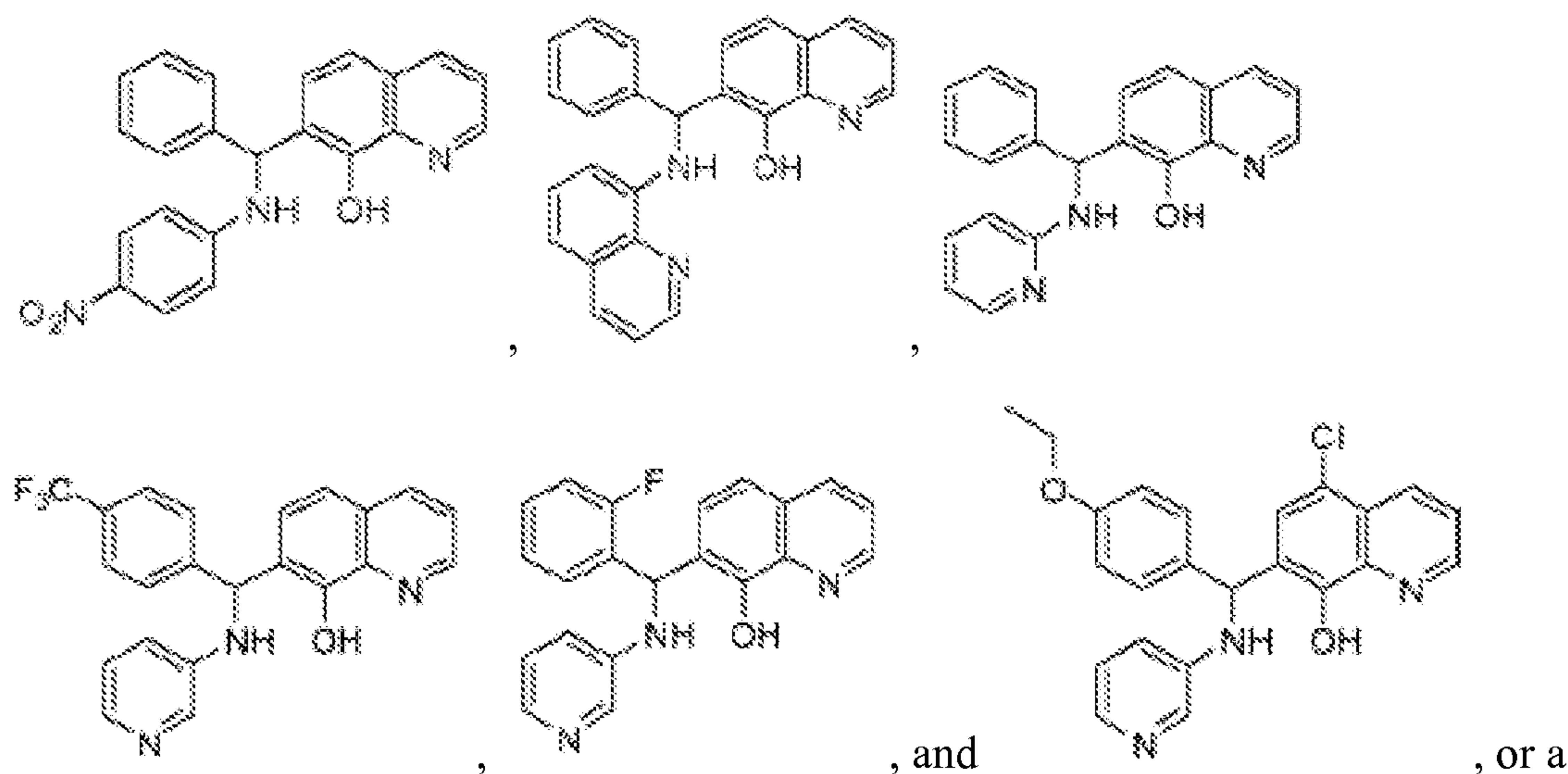
[0392] In various embodiments, the therapeutic method includes administration of one or more compounds described herein.

[0393] For example, the therapeutic method can include administration of one or more compounds according to a formula selected from the following: Formula (13); Formula (14); Formula (15); Formula (16); Formula (18); Formula (19); Formula (20); Formula (21); Formula (22); Formula (23); Formula (25); Formula (27); Formula (28); Formula (32); and Formula (33), as defined above, or a stereoisomer or pharmaceutically acceptable salt thereof.

[0394] As another example, the therapeutic method can include administration of one or more compounds selected from the following: AD4-1734 (Formula (12)); AD4-1886 (Formula

(17)); AD4-10381 (Formula (24)); and AD4-11091 (Formula (31)), or a stereoisomer or pharmaceutically acceptable salt thereof.

[0395] As another example, the therapeutic method can include administration of one or more compounds selected from the following:



stereoisomer or pharmaceutically acceptable salt thereof.

[0396] Methods described herein are generally performed on a subject in need thereof. For example, a subject in need of the therapeutic methods described herein can be diagnosed with a proliferative disease, disorder, or condition, or at risk thereof. As another example, a subject in need of the therapeutic methods described herein can be diagnosed with a disease, disorder, or condition associated with EGFR, or at risk thereof. A determination of the need for treatment can be assessed by a history and physical exam consistent with the disease, disorder, or condition at issue. Diagnosis of the various conditions treatable by the methods described herein is within the skill of the art. The subject can be an animal subject, preferably a mammal, more preferably horses, cows, dogs, cats, sheep, pigs, mice, rats, monkeys, guinea pigs, and chickens, and most preferably a human.

[0397] Examples of proliferative diseases or conditions treatable with compositions described herein include, but are not limited to, cancer; blood vessel proliferative disorders; fibrotic disorders; mesangial cell proliferative disorders; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by virus infections, such as papilloma virus infection.

[0398] While under no obligation to provide an underlying mechanism and in no way limiting the present invention by doing so, it is presently thought that at least a portion of activity of compounds described herein arise from inhibition of EGFR. It is further contemplated that the presently described compounds may have additional modes of action in their effectiveness in treating a proliferative disease, disorder, or condition. Regardless of the underlying mechanism, compounds described herein have been demonstrated to be empirically effective in treating proliferative diseases and conditions.

[0399] Various compounds described herein can be effective for inhibiting EGFR, and thus, effective against diseases or conditions associated with EGFR, such as include, but are not limited to, proliferative diseases. In some embodiments, the proliferative disease treated by a compound described herein is a condition caused by excessive growth of cancer or non-cancer cells that express a member of the EGFR family of receptors. The excess cells generated by a proliferative disease can express EGFR at normal levels or can overexpress EGFR. Particularly suitable diseases or conditions associated with EGFR can be those stimulated by a ligand of EGFR or mutations of such ligands. Examples of such ligands that stimulate EGFR include, but are not limited to, EGF, TGF- α , heparin-binding growth factor (HBGF), β -cellulin, and Cripto-1. Examples of proliferative disease associated with EGFR include, but are not limited to, cancer; blood vessel proliferative disorders; fibrotic disorders; mesangial cell proliferative disorders; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by virus infections, such as papilloma virus infection.

[0400] Cancer, or neoplasia, refers generally to any malignant neoplasm or spontaneous growth or proliferation of cells. A subject having "cancer", for example, may have a leukemia, lymphoma, or other malignancy of blood cells. In certain embodiments, the subject methods are used to treat a solid tumor. Exemplary solid tumors include but are not limited to non small cell lung cancer (NSCLC), testicular cancer, lung cancer, ovarian cancer, uterine cancer, cervical cancer, pancreatic cancer, colorectal cancer (CRC), breast cancer, as well as prostate, gastric, skin, stomach, esophageal, and bladder cancer.

[0401] Treatment of cancer or treating a subject having cancer can include inhibition of replication of cancer cells, inhibition of spread of cancer, reduction in tumor size, lessening or reducing the number of cancerous cells in the body of a subject, or amelioration or alleviation of

symptoms of cancer. A treatment can be considered therapeutic if there is a decrease in mortality or morbidity, and can be performed prophylactically, or therapeutically.

[0402] Methods described herein can be used to treat (e.g., reduce tumor size, decrease the vascularization, and/or increase the permeability of) an established tumor. An established tumor is generally understood as a solid tumor of sufficient size such that nutrients, e.g., oxygen, can no longer permeate to the center of the tumor from the subject's vasculature by osmosis and therefore the tumor requires its own vascular supply to receive nutrients. Methods described herein can be used to treat a solid tumor that is not quiescent and is actively undergoing exponential growth.

[0403] A therapeutic protocol can be modified according to permeability of a solid tumor. Permeability of a solid tumor generally refers to the permeability of a solid tumor to a therapeutic. A solid tumor may be said to be permeable to a therapeutic if the therapeutic is able to reach cells at the center of the tumor. An agent that increases the permeability of a tumor may for example, normalize, e.g., maintain, the vasculature of a solid tumor. Tumor vascularization or tumor permeability can be determined by a variety of methods known in the art, such as, e.g. by immunohistochemical analysis of biopsy specimens, or by imaging techniques, such as sonography of the tumor, computed tomography (CT) or magnetic resonance imaging (MRI) scans.

[0404] Different types of psoriasis display characteristics such as pus-like blisters (pustular psoriasis), severe sloughing of the skin (erythrodermic psoriasis), drop-like dots (guttate psoriasis) and smooth inflamed lesions (inverse psoriasis). The treatment of all types of psoriasis (e.g., psoriasis vulgaris, psoriasis pustulosa, psoriasis erythrodermica, psoriasis arthropathica, parapsoriasis, palmoplantar pustulosis) is contemplated by the invention.

[0405] Blood vessel proliferative disorders refer to angiogenic and vasculogenic disorders generally resulting in abnormal proliferation of blood vessels. The formation and spreading of blood vessels, or vasculogenesis and angiogenesis, respectively, play important roles in a variety of physiological processes such as embryonic development, corpus luteum formation, wound healing, and organ regeneration. They also play a pivotal role in cancer development. Other examples of blood vessel proliferation disorders include, but are not limited to, arthritis, where new capillary blood vessels invade the joint and destroy cartilage, and ocular diseases, like

diabetic retinopathy, where new capillaries in the retina invade the vitreous, bleed and cause blindness. Disorders related to the shrinkage, contraction or closing of blood vessels, such as restenosis, are also implicated.

[0406] Fibrotic disorders refer to the abnormal formation of extracellular matrix. Examples of fibrotic disorders include, but are not limited to, hepatic cirrhosis and mesangial cell proliferative disorders. Hepatic cirrhosis is characterized by the increase in extracellular matrix constituents resulting in the formation of a hepatic scar. Hepatic cirrhosis can cause diseases such as cirrhosis of the liver. An increased extracellular matrix resulting in a hepatic scar can also be caused by viral infection such as hepatitis. Lipocytes appear to play a major role in hepatic cirrhosis. Other fibrotic disorders implicated include atherosclerosis.

[0407] Mesangial cell proliferative disorders refer to disorders brought about by abnormal proliferation of mesangial cells. Mesangial proliferative disorders include, but are not limited to, various human renal diseases, such as glomerulonephritis, diabetic nephropathy, malignant nephrosclerosis, thrombotic microangiopathy syndromes, transplant rejection, and glomerulopathies.

[0408] EGFR (Tuzi et al., 1991, Br. J. Cancer 63:227-233; Torp et al., 1992, APMIS 100:713-719) HER2/neu (Slamon et al., 1989, Science 244:707-712) and the PDGF-R (Kumabe et al., 1992, Oncogene 7:627-633) are known to be overexpressed in many tumors and/or persistently activated by autocrine loops. Overexpression of the receptor and autocrine loops have been demonstrated in most common and severe cancers (see e.g., Akbasak and Suner-Akbasak et al., 1992, J. Neurol. Sci. 111:119-133; Dickson et al., 1992, Cancer Treatment Res. 61:249-273; Korc et al., 1992, J. Clin. Invest. 90:1352-1360; Lee and Donoghue, 1992, J. Cell. Biol. 118:1057-1070). Overexpression of EGFR is known to be associated with cancers of the bladder, brain, head and neck, pancreas, lung, breast, ovary, colon, prostate, and kidney. (see e.g., Atalay et al., 2003, Ann. Oncology 14:1346-1363; Herbst and Shin, 2002, Cancer 94:1593-1611; Modjtahedi et al., 1996, Br. J. Cancer 73:228-235). Overexpression of EGFR can be correlated or associated with poor prognosis of the patients (see e.g., Herbst and Shin, 2002, Cancer 94:1593-1611; Modjtahedi et al., 1996, Br. J. Cancer 73:228-235). HER2 has been associated with breast, ovarian, gastric, lung, pancreas and bladder cancer.

[0409] An inhibitor compound described herein can be used therapeutically either as exogenous materials or as endogenous materials. Exogenous agents are those produced or manufactured outside of the body and administered to the body. Endogenous agents are those produced or manufactured inside the body by some type of device (biologic or other) for delivery to within or to other organs in the body.

[0410] According to the methods described herein, administration can be parenteral, pulmonary, oral, topical, intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, ophthalmic, buccal, or rectal administration.

[0411] An effective amount of a compound described herein is generally that which can exhibit an anti-proliferative effect to an extent such as to ameliorate the treated condition. For example, an effective amount of a compound described herein may inhibit EGFR to an extent such as to ameliorate the treated condition. In some embodiments, an effective amount is that amount of therapy (or combination therapy) that is sufficient to affect a desired result on a cancerous cell or tumor, including, but not limited to, for example, reducing tumor size, reducing tumor volume, decreasing vascularization of a solid tumor, or increasing the permeability of a solid tumor to an agent, either in vitro or in vivo. In certain embodiments, an effective amount of therapy (or combination therapy) is the amount that results in a percent tumor inhibition of more than about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 99%, or about 100%. In certain embodiments, an effective amount of therapy (or combination therapy) is sufficient to achieve a desired clinical result, including but not limited to, for example, ameliorating disease, stabilizing a subject, preventing or delaying the development of, or progression of cancer in a subject. An effective amount of therapy (or combination therapy) can be determined based on one administration or repeated administration. Methods of detection and measurement of the indicators above are known to those of skill in the art. Such methods include, but are not limited to measuring reduction in tumor burden, reduction of tumor size, reduction of tumor volume, reduction in proliferation of secondary tumors, decreased solid tumor vascularization, expression of genes in tumor tissue, presence of biomarkers, lymph node involvement, histologic grade, and nuclear grade.

[0412] In some embodiments, tumor burden can be determined. Tumor burden, also referred to as tumor load, generally refers to a total amount of tumor material distributed throughout the

body of a subject. Tumor burden can refer to a total number of cancer cells or a total size of tumor(s), throughout the body, including lymph nodes and bone marrow. Tumor burden can be determined by a variety of methods known in the art, such as, for example, by measuring the dimensions of tumor(s) upon removal from the subject, e.g., using calipers, or while in the body using imaging techniques, e.g., ultrasound, computed tomography (CT) or magnetic resonance imaging (MRI) scans. Tumor size can be determined, for example, by determining tumor weight or tumor volume.

[0413] When used in the methods of the invention, a therapeutically effective amount of a compound described herein can be employed in pure form or, where such forms exist, in pharmaceutically acceptable salt form and with or without a pharmaceutically acceptable excipient. For example, the agents of the invention can be administered, at a reasonable benefit/risk ratio applicable in a sufficient amount sufficient to inhibit the target biomolecule for which the compound is specific for the treatment or prophylaxis of a disease, disorder, or condition associated with the target biomolecule.

[0414] Toxicity and therapeutic efficacy of such compounds, and pharmaceutical formulations thereof, can be determined by standard pharmaceutical procedures in cell cultures and/or experimental animals for determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀, (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index that can be expressed as the ratio LD₅₀/ED₅₀, where large therapeutic indices are preferred.

[0415] The amount of a compound described herein may be combined with a pharmaceutically acceptable carrier to produce a single dosage form will vary depending upon the host treated and the particular mode of administration. It will be appreciated by those skilled in the art that the unit content of agent contained in an individual dose of each dosage form need not in itself constitute a therapeutically effective amount, as the necessary therapeutically effective amount could be reached by administration of a number of individual doses.

[0416] Administration of a compound described herein can occur as a single event, a periodic event, or over a time course of treatment. For example, modulators can be administered daily, weekly, bi-weekly, or monthly. As another example, a compound can be administered in multiple treatment sessions, such as 2 weeks on, 2 weeks off, and then repeated twice; or every

3rd day for 3 weeks. One of ordinary skill will understand these regimes to be exemplary and could design other suitable periodic regimes. For treatment of acute conditions, the time course of treatment will usually be at least several days. Certain conditions could extend treatment from several days to several weeks. For example, treatment could extend over one week, two weeks, or three weeks. For more chronic conditions, treatment could extend from several weeks to several months or even a year or more.

[0417] The specific therapeutically effective dose level for any particular subject will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration; the route of administration; the rate of excretion of the composition employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts (*see e.g.*, Koda-Kimble et al. (2004) *Applied Therapeutics: The Clinical Use of Drugs*, Lippincott Williams & Wilkins, ISBN 0781748453; Winter (2003) *Basic Clinical Pharmacokinetics*, 4th ed., Lippincott Williams & Wilkins, ISBN 0781741475; Sharqel (2004) *Applied Biopharmaceutics & Pharmacokinetics*, McGraw-Hill/Appleton & Lange, ISBN 0071375503). For example, it is well within the skill of the art to start doses of the composition at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. If desired, the effective daily dose may be divided into multiple doses for purposes of administration. Consequently, single dose compositions may contain such amounts or submultiples thereof to make up the daily dose. It will be understood, however, that the total daily usage of the compounds and compositions of the present invention will be decided by an attending physician within the scope of sound medical judgment.

[0418] Compounds of the invention that inhibit a target biomolecule can also be used in combination with other therapeutic modalities. Thus, in addition to the therapies described herein, one may also provide to the subject other therapies known to be efficacious for particular conditions linked to the target biomolecule. Treatment in accord with the methods described herein can be performed prior to, concurrent with, or after conventional treatment modalities for

a disease, disorder, or condition associated with a target biomolecule for which the compound is specific.

KITS

[0419] Also provided are kits. Such kits can include the compositions of the present invention and, in certain embodiments, instructions for administration. Such kits can facilitate performance of the methods described herein, for example, treatment methodologies or screening methodologies. When supplied as a kit, the different components of the composition can be packaged in separate containers and admixed immediately before use. Components include, but are not limited to one or more compounds described herein, vectors, diagnostic reagents, assay reagents, and/or combinations thereof. Such packaging of the components separately can, if desired, be presented in a pack or dispenser device which may contain one or more unit dosage forms containing the composition. The pack may, for example, comprise metal or plastic foil such as a blister pack. Such packaging of the components separately can also, in certain instances, permit long-term storage without losing activity of the components.

[0420] Kits may also include reagents in separate containers such as, for example, sterile water or saline to be added to a lyophilized active component packaged separately. For example, sealed glass ampules may contain a lyophilized component and in a separate ampule, sterile water, or sterile saline, each of which has been packaged under a neutral non-reacting gas, such as nitrogen. Ampules may consist of any suitable material, such as glass, organic polymers, such as polycarbonate, polystyrene, ceramic, metal or any other material typically employed to hold reagents. Other examples of suitable containers include bottles that may be fabricated from similar substances as ampules, and envelopes that may consist of foil-lined interiors, such as aluminum or an alloy. Other containers include test tubes, vials, flasks, bottles, syringes, and the like. Containers may have a sterile access port, such as a bottle having a stopper that can be pierced by a hypodermic injection needle. Other containers may have two compartments that are separated by a readily removable membrane that upon removal permits the components to mix. Removable membranes may be glass, plastic, rubber, and the like.

[0421] In certain embodiments, kits can be supplied with instructional materials. Instructions may be printed on paper or other substrate, and/or may be supplied as an electronic-

readable medium, such as a floppy disc, mini-CD-ROM, CD-ROM, DVD-ROM, Zip disc, videotape, audio tape, and the like. Detailed instructions may not be physically associated with the kit; instead, a user may be directed to an Internet web site specified by the manufacturer or distributor of the kit.

[0422] Compositions and methods described herein utilizing molecular biology protocols can be according to a variety of standard techniques known to the art (*see, e.g.*, Sambrook and Russel (2006) Condensed Protocols from Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, ISBN-10: 0879697717; Ausubel et al. (2002) Short Protocols in Molecular Biology, 5th ed., Current Protocols, ISBN-10: 0471250929; Sambrook and Russel (2001) Molecular Cloning: A Laboratory Manual, 3d ed., Cold Spring Harbor Laboratory Press, ISBN-10: 0879695773; Elhai, J. and Wolk, C. P. 1988. Methods in Enzymology 167, 747-754; Studier (2005) Protein Expr Purif. 41(1), 207–234; Gellissen, ed. (2005) Production of Recombinant Proteins: Novel Microbial and Eukaryotic Expression Systems, Wiley-VCH, ISBN-10: 3527310363; Baneyx (2004) Protein Expression Technologies, Taylor & Francis, ISBN-10: 0954523253).

[0423] Definitions and methods described herein are provided to better define the present disclosure and to guide those of ordinary skill in the art in the practice of the present disclosure. Unless otherwise noted, terms are to be understood according to conventional usage by those of ordinary skill in the relevant art.

[0424] In some embodiments, numbers expressing quantities of ingredients, properties such as molecular weight, reaction conditions, and so forth, used to describe and claim certain embodiments of the present disclosure are to be understood as being modified in some instances by the term “about.” In some embodiments, the term “about” is used to indicate that a value includes the standard deviation of the mean for the device or method being employed to determine the value. In some embodiments, the numerical parameters set forth in the written description and attached claims are approximations that can vary depending upon the desired properties sought to be obtained by a particular embodiment. In some embodiments, the numerical parameters should be construed in light of the number of reported significant digits and by applying ordinary rounding techniques. Notwithstanding that the numerical ranges and parameters setting forth the broad scope of some embodiments of the present disclosure are

approximations, the numerical values set forth in the specific examples are reported as precisely as practicable. The numerical values presented in some embodiments of the present disclosure may contain certain errors necessarily resulting from the standard deviation found in their respective testing measurements. The recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein.

[0425] In some embodiments, the terms “a” and “an” and “the” and similar references used in the context of describing a particular embodiment (especially in the context of certain of the following claims) can be construed to cover both the singular and the plural, unless specifically noted otherwise. In some embodiments, the term “or” as used herein, including the claims, is used to mean “and/or” unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive.

[0426] The terms “comprise,” “have” and “include” are open-ended linking verbs. Any forms or tenses of one or more of these verbs, such as “comprises,” “comprising,” “has,” “having,” “includes” and “including,” are also open-ended. For example, any method that “comprises,” “has” or “includes” one or more steps is not limited to possessing only those one or more steps and can also cover other unlisted steps. Similarly, any composition or device that “comprises,” “has” or “includes” one or more features is not limited to possessing only those one or more features and can cover other unlisted features.

[0427] All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (*e.g.* “such as”) provided with respect to certain embodiments herein is intended merely to better illuminate the present disclosure and does not pose a limitation on the scope of the present disclosure otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the present disclosure.

[0428] Groupings of alternative elements or embodiments of the present disclosure disclosed herein are not to be construed as limitations. Each group member can be referred to and claimed individually or in any combination with other members of the group or other elements found

herein. One or more members of a group can be included in, or deleted from, a group for reasons of convenience or patentability. When any such inclusion or deletion occurs, the specification is herein deemed to contain the group as modified thus fulfilling the written description of all Markush groups used in the appended claims.

[0429] Citation of a reference herein shall not be construed as an admission that such is prior art to the present disclosure.

[0430] Having described the present disclosure in detail, it will be apparent that modifications, variations, and equivalent embodiments are possible without departing the scope of the present disclosure defined in the appended claims. Furthermore, it should be appreciated that all examples in the present disclosure are provided as non-limiting examples.

EXAMPLES

[0431] The following non-limiting examples are provided to further illustrate the present invention. It should be appreciated by those of skill in the art that the techniques disclosed in the examples that follow represent approaches the inventors have found function well in the practice of the invention, and thus can be considered to constitute examples of modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments that are disclosed and still obtain a like or similar result without departing from the spirit and scope of the invention.

EXAMPLE 1: EGFR INHIBITION ASSAY

[0432] The following example describes general EGFR in-cell Western (ICW) screening protocol. Methods are according to Chen et al. (2005) Analytical Biochemistry 338, 136-142 (incorporated herein by reference), except as otherwise noted.

[0433] Cell Plates: A431 cells (ATCC # CRL-1555) were grown in Dulbecco's Modified Eagle Medium (DMEM; ATCC # 30-2002) supplemented with 10% fetal bovine serum, 105 units/ml pen/strep (Invitrogen #15140155) and 2.1mM L-glutamine (ATCC # 30-2214). Cells were seeded into 96 well tissue culture plates (BD Falcon #353948) at a density of 30,000 cells per well and incubated at 37°C with 5% CO₂ overnight. Two cell plates were prepared for each compound plate.

[0434] Serum Starvation: The cells were serum starved prior to compound addition. The media was removed by aspiration and the cells washed with PBS (200 μ l / well; Invitrogen # 20012-027). The PBS was removed by aspiration and replaced with 200 μ l of DMEM (ATCC # 30-2002) supplemented with 105 units/ml pen/strep (Invitrogen #15140155) and 2.1 mM l-glutamine (ATCC # 30-2214). The cell plates were incubated at 37°C/5% CO₂ for 2 hours.

[0435] Compound Plate Preparation: The test compounds were solvated in 100% DMSO (Sigma #472301-2L) at a concentration of 25 mM. Compounds found not to be completely soluble in 100% DMSO at 25 mM were diluted to 10 mM with 100% DMSO and TFA (Fluka #91699) added to a final concentration of 0.2%. 40 μ l of test compound was added to the appropriate well of a 96 well plate (Falcon #351190). As controls, 6 μ M EGFR kinase inhibitor PD168393 (EMD/Calbiochem # 513033) in 100% DMSO and DMSO alone were added to various wells. Prepared compound plates were stored at RT prior to use and at 4°C long term.

[0436] Compound Dilution Plate Preparation: 250 μ l of DMEM supplemented with 1 mg/ml BSA (Sigma A3059-10G) was added to the appropriate wells of a 96 well plate to prepare the compound dilution plates. Using a multichannel pipettor, 1.25 μ l of compound from the compound plate was transferred into the compound dilution plate. This dilution rate will give a compound concentration in the assay of 125 μ M.

[0437] Compound Addition: The starve media was removed from the cell plates by aspiration. Using a multichannel pipettor, the compound dilution plates were mixed by pipetting up and down three times. 50 μ l of mixed, diluted compound was added to each of two rows/columns on each of two cell plates. The cell plates with compound were incubated at 37°C/5% CO₂ for 4 hours.

[0438] EGF Addition: 20 ng/ml EGF (Upstate #01-107) was prepared in DMEM supplemented with 1 mg/ml BSA. 50 μ l of 20 or 0 ng/ml EGF was added to the appropriate wells without the removal of compound. The compound and EGF were mixed by pipetting up and down three times. The plates were incubated at 37°C/5% CO₂ for 10 min. In some screening assays the final concentration of EGF used for simulation was 6.6, or 12.5 ng/ml rather than 10 ng/ml.

[0439] Fixation and Triton Washing: The EGF+compound was removed by aspiration and 150 μ l of freshly prepared Fixation Solution (1x PBS, Sigma P3813-10PK, and 4%

Formaldehyde, Pierce #28908) was immediately added. The plates were incubated at RT for 20 min without shaking. The Fixation Solution was removed by aspiration and the plates washed four times with 200 μ l each of Triton Wash Solution (1x PBS, Sigma P3813-10PK, and 0.1 % Triton X-100, T8787-50ML) for 5 min with gentle shaking.

[0440] Blocking and Probing: Following the last Triton Wash, the plates were blocked for 1.5h at RT with shaking using 150 μ l of Odyssey Blocking Buffer (LI-COR # 927-40000). The block was removed by aspiration and 50 μ l of diluted primary Ab mix was added. The plates were incubated at 4°C overnight with gentle shaking. The plates were washed five times with 200 μ l of PBST (1x PBS, Sigma P3813-10PK, and 0.1% Tween-20, Sigma P9416-50ML) for 5 min each with shaking. 50 μ l of diluted secondary Ab mix was added and the plates incubated at RT for 1h with shaking. The plates were washed 5x with 200 μ l of PBST for 5 min each with shaking. One final wash with 200 μ l of PBS (Sigma P3813-10PK) for 5 min with shaking was performed prior to scanning.

[0441] Primary Ab Mix contained: 0.1% Tween-20 (Sigma P9416-50ML); 1/500 dilution anti-total EGFR (Invitrogen #AHR5062); 1/800 dilution anti-phospho EGFR (Tyr 1173; Cell Signalling #4407); and Odyssey Blocking Buffer (LI-COR # 927-40000). Some screening assays used a 1/100 dilution of anti-phospho EGFR (Tyr1045; Cell Signalling #2237) rather than the anti-phospho EGFR Ab indicated above.

[0442] Secondary Ab Mix contained: 0.2% 10% Tween-20 (Sigma P9416-50ML); 1/1200 dilution anti-mouse IR680 conjugate (LI-COR 926-32220); 1/1200 dilution anti-rabbit IR800CW conjugate (LI-COR 926-32211); and Odyssey Blocking Buffer (LI-COR # 927-40000). In some screening assays, the dilution of the secondary Ab conjugates was 1/800 rather than 1/1200.

[0443] Plate Scanning: The plates were scanned on an Odyssey Infrared Imaging System from LI-COR Biosciences. The focus offset was set at 3.5 mm and the scanning intensity set at 3 for the 700 channel and 7 for the 800 channel.

[0444] Data Analysis: The % of Maximum and % Inhibition values were calculated as follows. 700 Channel = signal for total EGFR (used to control for variation in cell number). 800 Channel = signal for phosphorylated EGFR. 800_{-EGF} = basal level EGFR phosphorylation + non-specific signal (no compound). $800_{+EGF} = 800_{-EGF} + \text{EGF dependent EGFR receptor phosphorylation (no compound)}$. $700/800_{com} = 700 \text{ or } 800 \text{ channel signals with compound}$. %

Maximum = $\{[(800_{\text{com}}/700_{\text{com}}) - (800_{\text{-EGF}}/700_{\text{-EGF}})] / [(800_{\text{+EGF}}/700_{\text{+EGF}}) - (800_{\text{-EGF}}/700_{\text{-EGF}})]\} \times 100\%$. % Inhibition = 100% - (% Maximum).

EXAMPLE 2: MTT ASSAY

[0445] The following example describes the MTT Cell Proliferation Assay. The MTT Cell Proliferation Assay served as a secondary screen to evaluate active compounds from the primary cell based ICW screening protocol, described above. The MTT assay was used to evaluate toxicity through viability and proliferation effects, and compares the growth of an epithelial carcinoma A431 cell line (American Type Culture Collections (ATCC) cat #CRL-155) and an MDBK line (ATCC cat # CCL-22) derived from a healthy bovine kidney after a three day compound treatment and incubation.

[0446] The MTT Cell Proliferation Assay is a colorimetric assay system that measures the reduction of the tetrazolium salt MTT (3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) into insoluble formazan crystals, produced by the mitochondria of viable cells. After incubation of the cells with the MTT, the formazan crystals formed are solubilized by the addition of a detergent or DMSO (ACS Reagent grade, Sigma cat# 472301). The color can then be quantified by spectrophotometric means. Among the applications for the method are drug sensitivity, cytotoxicity, response to growth factors, and cell activation. The reduction of tetrazolium salts is now recognized as a safe, accurate alternative to radiometric testing.

[0447] Methods are according to manufacturer instructions for MTT Cell Proliferation Assay, ATCC, Cat. No. 30-1010K, except as otherwise noted.

[0448] Each MTT assay tested 11 compounds and 1 standard in a dose response curve with 5 concentrations, and 2.5 fold dilutions. Two replicates on each cell plate and 2 duplicate plates gave 4 individual replicates of each concentration, in each cell line. Concentrations were diluted in 100% DMSO from 25mM stocks. Initial concentrations used and final concentrations tested were as follows: 20 mM to 100 μ M; 8 mM to 40 μ M; 3.2 mM to 16 μ M; 1.3 mM to 6.4 μ M; and 0.51 mM to 2.6 μ M.

[0449] Twelve 96 well plates of each cell line were plated. Plating was done by dislodging cells as in passage protocol, counting on a hemocytometer, re-suspending in standard growth media, and plating using a multichannel pippetor.

[0450] Standards: AD4-10289 was used as the dose response standard, run at one concentration lower than the test compounds; 40, 16, 6.4, 2.6, and 1.0 μ M final concentrations. 10289 was run in place of 1 test compound, with the same number of replicates. 16 μ l of 25 mM stock + 34 μ l DMSO = 8mM starting concentrations, and serial diluted as with test compounds in same plate. AD4-1734 was used at 2 concentrations, 31.125 μ M and 23.5 μ M final concentrations. These concentrations were used on each plate to give 100% , and less than 100 % activity in A431, while also giving less than 50% and 0 activity in DMBK cells. Two replicates of each concentrations was run on each cell plate. All compound addition and cell treatments were done using aseptic technique under a laminar flow aseptic hood.

[0451] Control: 0.5 % DMSO in treatment media was used as the control. The assay was demonstrated to tolerate up to 1.0 % DMSO with no significant growth differences.

[0452] MTT dye (Thiazolyl Blue Tetrazolium Bromide, Sigma cat # M5655) stock was prepared in bulk and stored at 4°C for use: 5 mgs/ml sterile PDS, shielded from light. 4 μ l of this stock per 100 μ ls treatment media per well was used. 130 ml of MTT/treatment was prepared fresh to dye 12 plates; 124.8 ml treatment media + 522 mls of MTT stock .

[0453] Cells (harvested from 95% confluent flasks) were plated at densities of 7500 cells per well. Plates were incubated overnight prior to treatment, with treatment day = Day 0. Compound treatments were prepared prior to washing cells to minimize time cells were dry at room temperature after final aspiration. Compounds were diluted in 100% DMSO in 96 well polypropylene plates: 40 μ l 25 mM stock + 10 μ l DMSO = 20 mM; 20 μ l of 20 mM + 30 μ l DMSO = 8 mM; 20 μ l of 8 mM + 30 μ l DMSO = 3.2 mM; 20 μ l of 3.2 mM + 30 μ l DMSO = 1.3 mM; 20 μ l of 1.3 mM + 30 μ l DMSO = 0.51 mM.

[0454] 5.0 μ l/ml were added to treatment media, in sterile, 2.2 ml deep well plates = 0.5% dmso final. Ten μ l of each dmso concentration was added to 2.0 ml of treatment media and then mixed using a 1200 μ l multichannel pipettor. Cell plates were treated from the deep well plates. Treatment media was standard growth media containing 0.5% FBS and standard additions. This

reduced FBS concentration allowed for a slower growth rate in cells. In plate 1 of each cell line, standard AD4- 10289 was run in lane 3 (see above for rates).

[0455] Media was aspirated from cell plates, and washed with 200 μ l/well sterile PBS. Aspirated PBS wash, and cell plates were treated with 200 μ l of compound/treatment media per well, and incubated at 37°C, 5% CO₂ for 3 days.

[0456] Prior to harvesting, visual observations were made under an inverted microscope. Plates were harvested by: aspirating the growth media; adding back MTT dye/100 μ l treatment media; and incubating plates at 37°C for 1 hour. MTT dye was aspirated. 100 μ l/well dmsol was added, and then shaken on a Bellco plate shaker for 5 minutes, at 4.5 setting.

[0457] Plates were read in a Tecan Sunrise UV plate reader, at 560 nm. Settings: Read mode, Outside Normal 2 sec; Shake settle time 3 sec. Data is reported as % inhibition, calculated for individual plates using control values from that plate.

EXAMPLE 3: TESTING OF IDENTIFIED COMPOUNDS FROM PHARMACOPHORES FOR EGFR INHIBITION

[0458] Identified compounds, representing various pharmacophore models, were tested for ability to inhibit EGFR at 25 μ M.

[0459] AD4-compounds were identified using pharmacophore models (see Example 3, Example 4) and then were docked with the binding site of EGFR (SEQ ID NO: 1) that is recognized by defined CDRs of cetuximab. The inhibition of epidermal growth factor binding by AD4-compounds was then determined (NovaScreen BioSciences, Hanover, MD). Inhibition of EGF binding was determined at 25 μ M concentration.

[0460] For the inhibitor assays, K_D (binding affinity) was 1.04 nM, while B_{max} (receptor number) was 43.0 fmol/mg tissue (wet weight). Receptor source was rat liver membranes. The radioligand was [¹²⁵I]EGF (150-200 Ci/ μ g) at a final ligand concentration of 0.36 nM. A non-specific determinant was used as EGF – [100 nM]. The reference compound and positive control was EGF. Reactions were carried out in 10 mM HEPES (pH 7.4) containing 0.1% BSA at 25°C for 60 minutes. The reaction was terminated by rapid vacuum filtration onto glass fiber filters. Radioactivity trapped onto the filters was determined and compared to control values to ascertain

any interactions of test compounds with the EGF binding site. The EGF inhibitor assays were modified from, for example, Mukku (1984) J. Biol. Chem. 259, 6543-6546; Duh et al. (1990) World J. Surgery 14, 410-418; Lokeshwar et al. (1989) J. Biol. Chem. 264(32), 19318-19326.

EXAMPLE 4: GENERATION OF PHARMACOPHORES FOR TARGET INHIBITION FROM EGFR/CETUXIMAB CRYSTAL STRUCTURE

[0461] The following example describes analysis of the target protein-antibody crystal structure and generation of pharmacophores for EGFR inhibition.

[0462] The protein crystal structure of cetuximab complexed to EGFR has been reported by Ferguson *et al.* (Cancer Cell, 2005, 7, 301-311 and the crystallographic data deposited in the Protein Data Bank as PDB code 1YY9. Structural information which define the position of the atoms of cetuximab was utilized to construct a pharmacophore model used to identify small molecules having similar atoms in similar positions. Small molecules having similar features to the antibody can demonstrate similar biological activity and thus similar therapeutic utility.

[0463] The pharmacophore feature generation and pharmacophore virtual screening module of the Molecular Operating Environment (MOE) software from Chemical Computing Group (CCG) (Montreal, Quebec, Canada) was used in the pharmacophore definitions described below. MOE's pharmacophore applications use a general notion of a pharmacophore being a set of structural features in a ligand that are directly related to the ligand's recognition at a receptor site and its biological activity.

[0464] In MOE, pharmacophoric structural features are represented by labeled points in space. Each ligand is assigned an annotation, which is a set of structural features that may contribute to the ligand's pharmacophore. A database of annotated ligands can be searched with a query that represents a pharmacophore hypothesis. The result of such a search is a set of matches that align the pharmacophoric features of the query to the pharmacophoric features present in the ligands of the searched database. The MOE software suite provides for interactive modifications (positions, radii, as well as other characteristics of the pharmacophoric query can be interactively adjusted); systematic matching (all possible matches of the ligand and the query are systematically examined); partial matching (the search algorithm is capable of finding ligands

that match only a portion of the query); and volume filtering (the query can be focused by adding restrictions on the shape of the matched ligands in the form of a set of volumes).

[0465] The pharmacophore features of this example were generated using the Pharmacophore Query Editor in MOE. All hydrogen bond donor features are spheres of 1.2 Angstroms in radius and are colored purple. All hydrogen bond acceptor features are spheres of 1.2 Angstroms in radius and are colored cyan. All aromatic features are spheres of 1.2 Angstroms in radius and are colored green. All combined acceptor-anion pharmacophore features are spheres of 1.2 Angstroms in radius and are colored grey. All combined donor-acceptor features are spheres of 1.2 Angstroms in radius and are colored pink. All combined donor-cation features are spheres of 1.2 Angstroms and are colored red. All donor, acceptor, aromatic, combined acid-anion, and combined donor-acceptor directionality features are spheres of 1.5 Angstroms in radius and colored dark grey for donors, dark cyan for acceptors, dark green for aromatics, dark cyan for combined acid-anions, and dark grey for combined donor-acceptors. A feature that is marked essential in the pharmacophore query must be contained in the ligand in order for that ligand to be a hit.

[0466] All of the pharmacophore features were derived from the corresponding donor, acceptor, aromatic and acid moieties of the corresponding antibody in complex with its receptor (*e.g.*, cetuximab complexed with EGFr, pdb accession number 1YY9) taken from crystal structures deposited in the protein databank with two exceptions. In some cases two methods provided the MOE software are used to place pharmacophore features. These are explained below.

[0467] The Contact statistics calculated, using the 3D atomic coordinates of a receptor, preferred locations for hydrophobic and hydrophilic ligand atoms using statistical methods. Using this method hydrophobic-aromatic and H-bonding features were placed, as noted in the individual pharmacophore definitions.

[0468] The MultiFragment Search essentially places a relatively large number of copies of a fragment (*e.g.*, 200 copies of ethane) into a receptor's active site. The fragments are placed randomly around the active site atoms and are assumed not to interact with each other; no regard is paid to fragment overlap. Next, a special energy minimization protocol is used to refine the initial placement: the receptor atoms feel the average forces of the fragments, while each

fragment feels the full force of the receptor but not of the other fragments. Using this technique it was possible to place hydrophobic, H-bond donors, acceptors and anions and cations in favorable positions within the receptors for use as MOE pharmacophore features.

[0469] Excluded volumes were generated for the pharmacophores defined below except when indicated. These were derived from the position of the receptor atoms near the antibody binding site. Excluded volumes are positions in space where ligand atoms must be excluded in order to avoid bumping into the receptor. They were generated in MOE by selecting the receptor residues within 5 Angstroms from the antibody and selecting "union" from the pharmacophore query editor in MOE.

[0470] In the Individual Pharmacophore Definitions described below, abbreviations were as follows: F = pharmacophore feature; Donor = Don, Acceptor = Acc, Anion = Ani, Cation = Cat, Acceptor and Anion = Acc&Ani, Donor and Cation = Don&Cat, Donor and Acceptor = Don&Acc, Aromatic = Aro, Hydrophobe = Hyd.

[0471] EGFR complexed with antibody cetuximab (1YY9.pdb)

[0472] The crystal (1YY9.pdb) of protein EGFR (SEQ ID NO: 1) complexed with antibody cetuximab (SEQ ID NO: 5 and SEQ ID NO:6) was analyzed according to the procedures described above. Results showed that two sets of residues of the antibody cetuximab make contact with the receptor. These are Gly54-Asp58 and Thr100-Glu105.

[0473] Hit AD4-1886 from Pharmacophore model 21_thr100_glu105

[0474] Pharmacophore model 21_thr100_glu105 (described below in Table 1 and 2 and depicted in FIG. 1) produced the hit, AD4-1886.

[0475] In the Individual Pharmacophore Definitions described below, abbreviations were as follows: F = pharmacophore feature; Donor = Don, Acceptor = Acc, Anion = Ani, Cation = Cat, Acceptor and Anion = Acc&Ani, Donor and Cation = Don&Cat, Donor and Acceptor = Don&Acc, Aromatic = Aro, Hydrophobe = Hyd.

Table 1

21_thr100_glu10	F(III)1	Derived from the side chain OH of antibody Tyr102. This OH donates an H-bond to the side chain carbonyl of receptor
-----------------	---------	---

5 Partial match, ligand must match at least 7 pharmacophore features. FIG. 1	Don&Acc	Gln408 and accepts an H-bond from the side chain NH2 of receptor Gln384.
	F(III)2 Acc	Derived from the backbone carbonyl of antibody Tyr102 accepting an H-bond from the side chain OH of receptor Ser440.
	F(III)3 Acc2	Directionality of F2 with respect to the OH of Ser440.
	F(III)4 Aro	Derived from side chain phenol ring of the antibody Tyr101 forming a favorable coulombic interaction with the imidazole side chain of the receptor His409.
	F(III)5 Aro2	Directionality of F4 with respect to the imidazole of His409.
	F(III)6 Acc&Ani	Derived from the side chain carboxylate of antibody Asp103. Acceptor accepts an H-bond from or Anion forms a salt bridge to NH3 ⁺ of receptor Lys465.
	F(III)7 Acc2	Directionality of F6 with respect to the side chain of Lys465.
	F(III)8 Don	Derived from the side chain OH of antibody Tyr102. This OH donates an H-bond to the side chain carbonyl of receptor Gln408. This feature is marked essential.
	F(III)9 Don2	Directionality with respect to the side chain carbonyl of receptor Gln408.
	V1	Excluded volume

Table 2: Cartesian and spherical coordinates of features for pharmacophore 21_thr100_glu105.

Feature	X	Y	Z	r	Θ (theta)	Φ (phi)	sphere radius (A)	sphere volume (A ³)	Preferred features
F(III)1 Don&Acc	37.771	38.261	61.320	81.552	41.243	45.369	1.2	7.2	
F(III)2 Acc	35.242	44.754	66.137	87.287	40.739	51.781	1.2	7.2	
F(III)3 Acc2	35.245	45.639	64.234	86.320	41.915	52.323	1.5	14.1	
F(III)4 Aro	39.910	35.496	67.237	85.870	38.463	41.650	1.2	7.2	
F(III)5 Aro2	35.866	34.818	65.306	82.241	37.431	44.151	1.5	14.1	
F(III)6 Acc&Ani	31.030	44.156	69.520	88.009	37.822	54.903	1.2	7.2	
F(III)7 Acc2	30.168	42.269	69.193	86.513	36.889	54.484	1.5	14.1	

F(III)8 Don	37.771	38.261	61.320	81.552	41.243	45.369	1.2	7.2	preferred
F(III)9 Don2	37.200	36.592	60.180	79.652	40.928	44.528	1.6	17.2	

[0476] Hit AD4-11091 from Pharmacophore model Pharm-1886-6

[0477] Pharmacophore model Pharm-1886-6, which was derived from the Glide docked pose of AD4-1886, afforded the hit AD4-11091. This is a partial match model. The ligand must match at least 9 pharmacophore features recited in Table 3 and 4.

Table 3

Pharm-1886-6 Partial match, ligand must match at least 9 pharmacophore features. Figure 2	F(V)1 Acc&Ani	Ligand accepts an H-bond from side chain amino of receptor Lys465
	F(V)2 Acc2	Directionality of F1 with respect to the side chain amino of receptor Lys465
	F(V)3 Acc2	Directionality of F1 with respect to the side chain amino of receptor Lys465
	F(V)4 Don	Ligand donates an H-bond to the side chain carbonyl of receptor Gln411. This feature is marked essential
	F(V)5 Don2	Directionality of F4 with respect to the side chain carbonyl of Gln411
	F(V)6 Don	Ligand donates an H-bond to the side chain nitrogen of receptor Gln411. This feature is marked essential.
	F(V)7 Don2	Directionality of F6 with respect to the side chain nitrogen of Gln411
	F(V)8 Acc	Ligand accepts an H-bond to the side chain imidazole of receptor His409
	F(V)9 Aro	Ligand forms a favorable π - π interaction with the phenyl ring of receptor Phe412
	F(V)10 Aro2	Directionality of F9 with respect to the phenyl ring of receptor Phe412
	F(V)11 Aro	Ligand forms favorable hydrophobic interactions with the side chains of receptor Val417 and Ile448. This feature is marked essential
	V1	Excluded volume

Table 4: Cartesian and spherical coordinates of features for pharmacophore Pharm-1886-6.

Feature	X	Y	Z	r	Θ (theta)	Φ (phi)	sphere radius (A)	sphere volume (A ³)	Preferred features
F(V)1 Acc&Ani	29.158	39.252	69.930	85.329	34.962	53.394	1.2	7.2	
F(V)2 Acc2	30.366	40.690	70.870	87.180	35.618	53.267	1.5	14.1	
F(V)3 Acc2	27.798	40.331	71.112	86.349	34.560	55.424	1.5	14.1	
F(V)4 Don	30.334	37.857	68.525	83.958	35.296	51.296	1.2	7.2	preferred
F(V)5 Don2	30.012	36.233	67.233	82.060	34.984	50.365	1.5	14.1	
F(V)6 Don	32.328	37.488	67.721	83.884	36.166	49.227	1.2	7.2	preferred
F(V)7 Don2	31.212	36.002	66.743	82.006	35.523	49.076	1.5	14.1	
F(V)8 Acc	38.730	36.298	66.517	85.100	38.590	43.143	1.2	7.2	
F(V)9 Aro	35.061	37.556	66.983	84.418	37.489	46.968	1.2	7.2	
F(V)10 Aro2	34.495	37.311	64.976	82.486	38.027	47.246	1.5	14.1	
F(V)11 Aro	36.483	41.320	63.964	84.438	40.753	48.557	1.2	7.2	preferred

[0478] Using the above described methodology, one can generate pharmacophore models for a variety of protein targets (crystalized with ligand).

EXAMPLE 5: GENERATION OF PHARMACOPHORES FOR TARGET INHIBITION FROM INACTIVE EGFR CRYSTAL STRUCTURE

[0479] The following example describes analysis of the target protein crystal structure and generation of pharmacophores for EGFR inhibition. The ligands found by this methodology will interact with residues from Dom II and Dom IV of EGFR and thereby yielding an inactive form of the receptor.

[0480] Methods are according to those described in Example 4, except as noted otherwise. Abbreviations for Individual Pharmacophore Definitions are as described above.

[0481] The protein crystal structure of the inactive conformation of EGFR has been reported by Ferguson et al. (Ferguson, K.M., Berger, M.B., Mendrola, J.M., Cho, H., Leahy, D.J., Lemmon, M.A. (2003) EGF activates its receptor by removing interactions that auto-inhibit ectodomain dimerization *Mol. Cell* 11: 507-517). The binding site was determined using the site finder module in the MOE software. It consists of the interface of residues from Domain II (23 residues, Lys227, Phe228, Lys 235, Asp236, Thr237, Cys238, Pro239, Pro240, Leu241, Met242, Tyr244, Tyr249, Gln250, Met251, Gly257, Lys258, Tyr259, Ser260, Cys265, Val275, His278, Gly279 and Ser280) and Domain IV (16 residues, Arg548, Gly549, Pro550, Asp551, Asn552, Asp561, His564, Val566, Thr568, Cys569, Pro570, Ala571, Gly572, Val573, Met574 and Leu580) (see e.g., FIG. 3).

[0482] Structural information which was derived from contact statistics and MFSS in the program MOE was used to construct pharmacophore models used to identify small molecules having similar atoms in similar positions.

[0483] Two methods provided in the MOE software are used to place pharmacophore features which correspond to hit AD4-1734. These are contact statistics and MFSS, as described above. Both the contact statistics and MFSS algorithms were applied to the Domain II - Domain IV interface binding site of the inactive form of EGFR (1NQL.pdb, see figure above).

[0484] Excluded volumes were generated in MOE by selecting the receptor residues of the binding site at the Domain II and Domain IV interface described above and selecting "union" from the pharmacophore query editor in MOE.

[0485] In the Individual Pharmacophore Definitions described below, abbreviations were as follows: F = pharmacophore feature; Donor = Don, Acceptor = Acc, Anion = Ani, Cation = Cat, Acceptor and Anion = Acc&Ani, Donor and Cation = Don&Cat, Donor and Acceptor = Don&Acc, Aromatic = Aro, Hydrophobe = Hyd.

[0486] Hit AD4-1734 from Pharmacophore model Pharm-1nql-glue-6

[0487] Pharmacophore model Pharm-1nql-glue-6 (see TABLE 5 and 8; FIG. 4) produced the hit AD4-1734. This is a partial match model. The ligand must match at least 5 pharmacophore features.

TABLE 5

Pharm-1nql-glue-6 Partial match, ligand must match at least 5 pharmacophore features. FIG. 4	F(II)1 Don&Cat	Derived from MFSS (see above). Ligand donates an H-bond or forms a salt bridge to the carboxylate side chain of receptor Asp553
	F(II)2 Don	Derived from MFSS. This feature is ignored in this pharmacophore model
	F(II)3 Hyd	Derived from hydrophobic contact statistics. Ligand forms hydrophobic contacts with side chain of receptor Val568, imidazolidine side chain of receptor Pro552 and with the side chain of Met253
	F(II)4 Hyd	Derived from hydrophobic contact statistics. Ligand forms hydrophobic contacts with side chain of receptor Val575, Met253, and with the imidazolidine ring of receptor Pro552. This feature is marked essential
	F(II)5 Don	Derived from MFSS. Ligand donates an H-bond to the side chain hydroxyl of Thr570
	F(II)6 Don2	Directionality of F4 with respect to the backbone carbonyl of receptor Thr570
	F(II)7 Acc	Derived from MFSS. Ligand accepts an H-bond from receptor backbone NH of Ala573
	F(II)8 Acc2	Directionality of F7 with respect to the backbone NH of receptor Ala573
	F(II)9 Don&Cat	Derived from MFSS. Ligand donates an H-bond to the backbone carbonyl of Asp563 and forms a salt bridge to the side chain carboxylate of receptor Asp563. This feature is marked essential
	V1	Excluded volume

TABLE 6: Cartesian and spherical coordinates of features for pharmacophore Pharm-1nql-Glue-6.

Feature	X	Y	Z	r	Θ (theta)	Φ (phi)	sphere radius (Å)	sphere volume (Å ³)	Preferred features
F(II)1 Don&Cat	53.523	-17.135	-4.293	56.363	94.368	-17.752	1.2	7.2	
F(II)2 Don	47.983	-20.609	-10.619	53.290	101.494	-23.244	1	4.2	optional

F(II)3 Hyd	50.511	-16.780	-7.319	53.726	97.830	-18.377	1.7	20.6	
F(II)4 Hyd	48.632	-18.658	-12.837	53.647	103.844	-20.990	1.4	11.5	preferred
F(II)5 Don	44.675	-21.359	-12.586	51.093	104.261	-25.552	1.2	7.2	
F(II)6 Don2	45.067	-23.421	-12.644	52.340	103.980	-27.461	1.5	14.1	
F(II)7 Acc	44.906	-20.294	-14.555	51.383	106.455	-24.319	1.2	7.2	
F(II)8 Acc2	45.475	-21.640	-16.064	52.861	107.691	-25.448	1.5	14.1	
F(II)9 Don&Cat	52.952	-20.952	-9.221	57.688	99.198	-21.588	1.2	7.2	preferred

[0488] Hit AD4-10381 from Pharmacophore model DockPharm1505-2

[0489] Pharmacophore model DockPharm1505-2 (see TABLE 7 and TABLE 8; FIG. 5), afforded the hit AD4-10381. This is a partial match model. The ligand must match at least 8 pharmacophore features.

TABLE 7

DockPharm1505-2 Partial match, ligand must match at least 8 pharmacophore features. FIG. 5	F(IV)1 Acc	Derived from MFSS. Ligand accepts an H-bond from receptor side chain OH of Thr239. This feature is marked essential
	F(IV)2 Acc2	Directionality of F1 with respect to side chain OH of Thr239. This feature is marked essential
	F(IV)3 Aro	Derived from MFSS. F Ligand forms hydrophobic contacts with side chain of receptor Met576 and imidazolidine ring of Pro242
	F(IV)4 Aro2	Directionality of F3 with respect to side chain of receptor Met576 and imidazolidine ring of Pro242
	F(IV)5 Aro	Derived from MFSS. Ligand forms hydrophobic contacts with side chain of receptor Met576 and imidazolidine ring of Pro242
	F(IV)6 Aro2	Directionality of F5 with respect to side chain of receptor Met576 and imidazolidine ring of Pro242
	F(IV)7 Aro	Derived from MFSS. Ligand forms hydrophobic contacts with side chain of receptor Leu243 and side chain of Thr239
	F(IV)8 Don	Derived from MFSS. Ligand donates an H-bond to the backbone carbonyl of His280. This feature is marked essential
	F(IV)9 Aro	Derived from hydrophobic contact statistics. Ligand forms hydrophobic contacts with side chains of receptor Met244 and Leu243. This feature is marked essential

	F(IV)10 Hyd	Derived from MFSS. Ligand forms hydrophobic contacts with side chain of receptor Met244
	V1	Excluded volume

TABLE 8: Cartesian and spherical coordinates of features for pharmacophore DockPharm1505-2.

Feature	X	Y	Z	r	Θ (theta)	Φ (phi)	sphere radius (Å)	sphere volume (Å ³)	Preferred features
F(IV)1 Acc	43.406	-13.282	-20.203	49.686	113.993	-17.014	1.2	7.2	preferred
F(IV)2 Acc2	41.702	-12.124	-20.610	48.071	115.388	-16.211	1.6	17.2	preferred
F(IV)3 Aro	44.536	-14.059	-19.940	50.781	113.121	-17.520	1.2	7.2	
F(IV)4 Aro2	45.616	-12.975	-21.378	52.021	114.264	-15.878	1.5	14.1	
F(IV)5 Aro	45.210	-12.475	-18.241	50.322	111.253	-15.426	1.2	7.2	
F(IV)6 Aro2	46.291	-11.393	-19.681	51.575	112.433	-13.827	1.5	14.1	
F(IV)7 Aro	43.427	-7.811	-18.296	47.767	112.521	-10.196	1.2	7.2	
F(IV)8 Don	41.296	-6.448	-17.166	45.184	112.328	-8.875	1.2	7.2	preferred
F(IV)9 Aro	46.569	-6.963	-15.306	49.512	108.007	-8.504	1.2	7.2	preferred
F(IV)10 Hyd	48.308	-6.291	-13.040	50.431	104.985	-7.420	1.8	24.4	

EXAMPLE 6: LIGAND DOCKING AND SCORING TO EGFR PROTEIN COMPLEXED WITH ANTIBODY CETUXIMAB

[0490] The compounds selected for docking to the target protein, crystal (1YY9.pdb) of protein EGFR (SEQ ID NO: 1) complexed with antibody cetuximab (SEQ ID NO: 5 and SEQ ID NO:6), were those which were found to align to the pharmacophore models generated in the MOE modeling software (*see* Example 3). These compounds were obtained in MOE database format. The 3-dimensional atomic coordinates of these compounds were written to a structure

data format (*.sdf) file using the export command in the MOE database window without adding hydrogens.

[0491] The LigPrep software module of Maestro modeling software (Schrodinger LLC, NY, NY) was next employed to prepare the compounds for docking. The *.sdf file was converted into Maestro format using LigPrep. Hydrogens were then added and any charged groups neutralized. Ionization states were generated for the ligands at 7.0 +/- 1.0 pH units. After this, tautomers were generated when necessary, alternate chiralities were generated and low energy ring conformers were produced. This was followed by removing any problematic structures and energy minimizing the resulting ligands using MacroModel software module. Finally a Maestro file (*.mae) was written of the ligands which were now ready for docking. All of these steps were automated via a python script supplied by Schrodinger, LLC.

[0492] The following describes protein preparation. First the protein structure 1YY9 of EGFR complexed with antibody cetuximab was imported into Maestro in PDB format. Hydrogens were added and any errors such as incomplete residues were repaired. The protein structure was checked for metal ions and cofactors. Charges and atom types were set for metal ions and cofactors as needed. Ligand bond orders and formal charges were adjusted if necessary. The binding site was determined by picking the ligand (for 1YY9 it is either the Thr100-Tyr101-Tyr102-Asp103-Tyr104-Glu105 or Gly54-Gly55-Asn56-Thr57-Asp58 pieces of the antibody) in Maestro (Glide). The program determines the centroid of the picked ligand and draws a 20 Angstrom box which is the default setting with the centroid of the ligand at the center of the box. The box was the binding site for the ligands to be docked. The protein preparation facility, which is automated in Glide, consists of two components, preparation and refinement. The preparation component added hydrogens and neutralized side chains that are not close to the binding site and do not participate in salt bridges. The refinement component performed a restrained minimization of the co-crystallized complex which reoriented side-chain hydroxyl groups and alleviated potential steric clashes.

[0493] The following describes receptor grid generation. Glide searches for favorable interactions between one or more ligand molecules and a receptor molecule, usually a protein. The shape and properties of the receptor are represented on a grid by several different sets of fields including hydrogen bonding, coulombic (i. e., charge-charge) interactions hydrophobic

interactions, and steric clashes of the ligand with the protein. In the first step the receptor must be defined. This was done by picking the ligand. The unpicked part of the structure was the receptor. The ligand was not included in the grid calculation but was used to define the binding site as described above. Scaling of the nonpolar atoms of the receptor was not included in the present docking runs. The grids themselves were calculated within the space of the enclosing box. This is the box described above and all of the ligand atoms must be contained in this box. No pharmacophore constraints were used because the Glide extra precision scoring function performs better without these constraints.

[0494] To use Glide, each ligand must be a single molecule, while the receptor may include more than one molecule, e.g., a protein and a cofactor. Glide can be run in rigid or flexible docking modes; the latter automatically generates conformations for each input ligand. The combination of position and orientation of a ligand relative to the receptor, along with its conformation in flexible docking, is referred to as a ligand pose. All docking runs are done using the flexible docking mode. The ligand poses that Glide generates pass through a series of hierarchical filters that evaluate the ligand's interaction with the receptor. The initial filters test the spatial fit of the ligand to the defined active site, and examine the complementarity of ligand-receptor interactions using a grid-based method. Poses that pass these initial screens enter the final stage of the algorithm, which involves evaluation and minimization of a grid approximation to the OPLS-AA nonbonded ligand-receptor interaction energy. Final scoring is then carried out on the energy-minimized poses. By default, Schrödinger's proprietary GlideScore multi-ligand scoring function is used to score the poses. If GlideScore was selected as the scoring function, a composite Emodel score is then used to rank the poses of each ligand and to select the poses to be reported to the user. Emodel combines GlideScore, the nonbonded interaction energy, and, for flexible docking, the excess internal energy of the generated ligand conformation. Conformational flexibility is handled in Glide by an extensive conformational search, augmented by a heuristic screen that rapidly eliminates unsuitable conformations, such as conformations that have long-range internal hydrogen bonds.

[0495] The settings used in the docking runs of this example were as follows. Grid file was read in. Extra precision (XP) scoring function was used. Docked using conformational flexibility. 5000 poses per ligand for the initial Glide screen were kept (default). Scoring

window for keeping initial poses was 100.0 (default). Best 800 poses per ligand for the energy minimization was kept (default). For the energy minimization, a distance dependent dielectric constant of 2.0 was used and maximum number of conjugate gradient steps was 100 (defaults). The ligand file was then loaded. Molecules with > 120 atoms and/or > 20 rotatable bonds were not docked (default). Van der Waals radii of ligand atoms with partial charges < 0.15 were scaled by 0.80. This was done to mimic receptor flexibility. Constraints and similarity were not used. Poses with Coulomb plus Van der Waals energies > 0.0 were rejected. To ensure that poses for each molecule were conformationally distinct, poses with RMS deviation < 0.5 and/or maximum atomic displacement of 1.3 Angstroms were discarded.

[0496] The following describes Glide Scoring. The choice of best-docked structure for each ligand was made using a model energy score (Emodel) that combines the energy grid score, the binding affinity predicted by GlideScore, and (for flexible docking) the internal strain energy for the model potential used to direct the conformational-search algorithm. Glide also computed a specially constructed Coulomb-van der Waals interaction-energy score (CvdW) that was formulated to avoid overly rewarding charge-charge interactions at the expense of charge-dipole and dipole-dipole interactions. This score was intended to be more suitable for comparing the binding affinities of different ligands than is the “raw” Coulomb-van der Waals interaction energy. In the final data work-up, one can combine the computed GlideScore and “modified” Coulomb-van der Waals score values to give a composite score that can help improve enrichment factors in database screening applications. The mathematical form of the Glide score is:

[0497]
$$\text{GScore} = 0.065 \cdot \text{EvdW} + 0.130 \cdot \text{Coul} + \text{Lipo} + \text{Hbond} + \text{Metal} + \text{BuryP} + \text{RotB} + \text{Site}$$

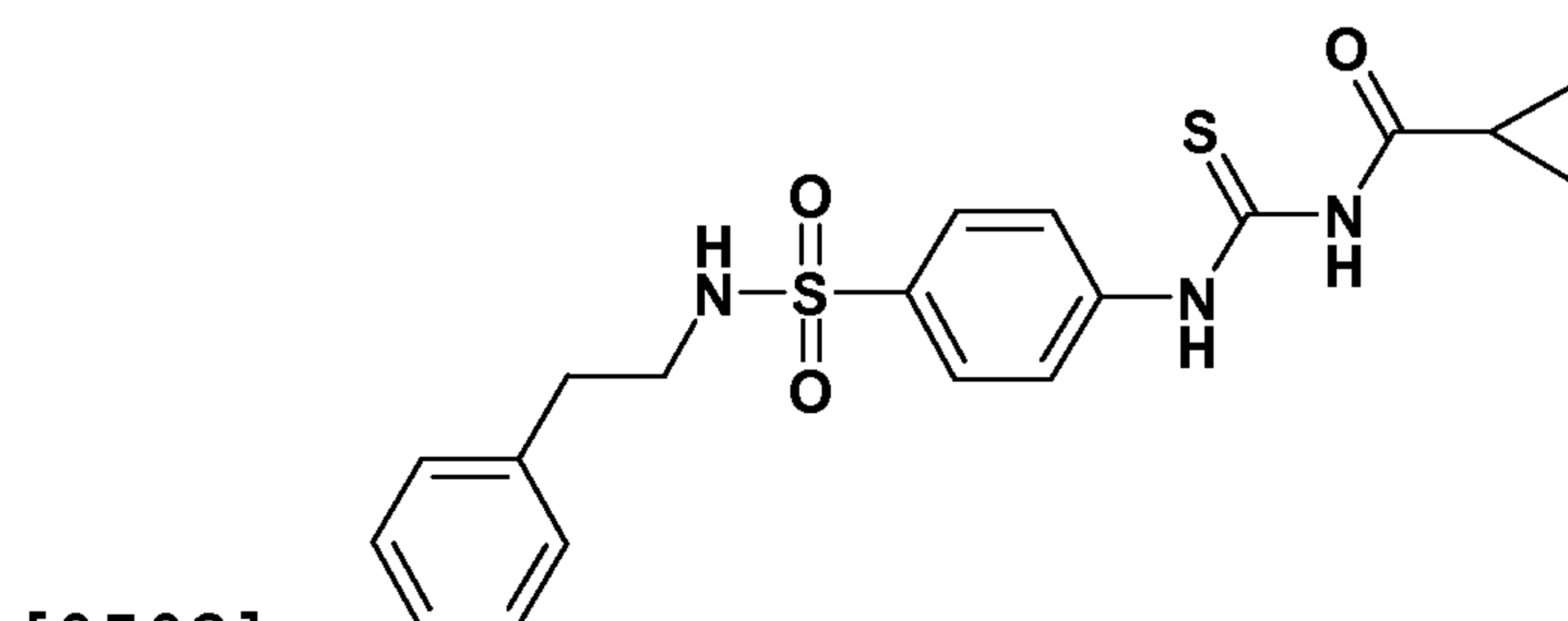
[0498] where EvdW is van der Waals energy (calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums); Coul is the Coulomb energy (calculated with reduced net ionic charges on groups with formal charges, such as metals, carboxylates, and guanidiniums); Lipo is the lipophilic contact term (rewards favorable hydrophobic interactions); HBond is the hydrogen-bonding term (separated into differently weighted components that depend on whether the donor and acceptor are neutral, one is neutral and the other is charged, or both are charged); metal is the metal-binding term (only the

interactions with anionic acceptor atoms are included; if the net metal charge in the apo protein is positive, the preference for anionic ligands is included; if the net charge is zero, the preference is suppressed); BuryP is the penalty for buried polar groups; RotB is the penalty for freezing rotatable bonds; and Site is polar interactions in the active site (polar but non-hydrogen-bonding atoms in a hydrophobic region are rewarded).

[0499] The following describes generation of the virtual compound library that was screened. The lead-like compounds from a free, virtual database of commercially available compounds was downloaded in structure data format (sdf, Molecular Design Limited) from the ZINC database (Irwin and Shoichet (2005) J. Chem. Inf. Model. 45(1), 177-182). The lead-like database is comprised of approximately 890,000 compounds divided into 33 segments. This was used to generate the database of conformers for screening by MOE. Hydrogens were then added. For a pharmacophore search, a database of low energy conformers must be generated. The Conformation Import command was applied to the sdf file above. After the conformers were generated, preprocessing of the conformer database was applied. This step, called feature annotation, determined the types of pharmacophore features in each molecule/conformation and their geometrical relationships. This was then compared with the query and those molecules/conformations that matched the query within the given tolerance were saved as hits.

[0500] AD4-1886-like

[0501] Analysis of compounds from the ZINC database against pharmacophore 21_thr100_glu105 identified from the 1YY9.pdb crystal of protein EGFR (SEQ ID NO: 1) complexed with antibody cetuximab (SEQ ID NO: 5 and SEQ ID NO:6) (*see e.g.*, Example 3) according to the methods described above identified the compound AD4-1886.

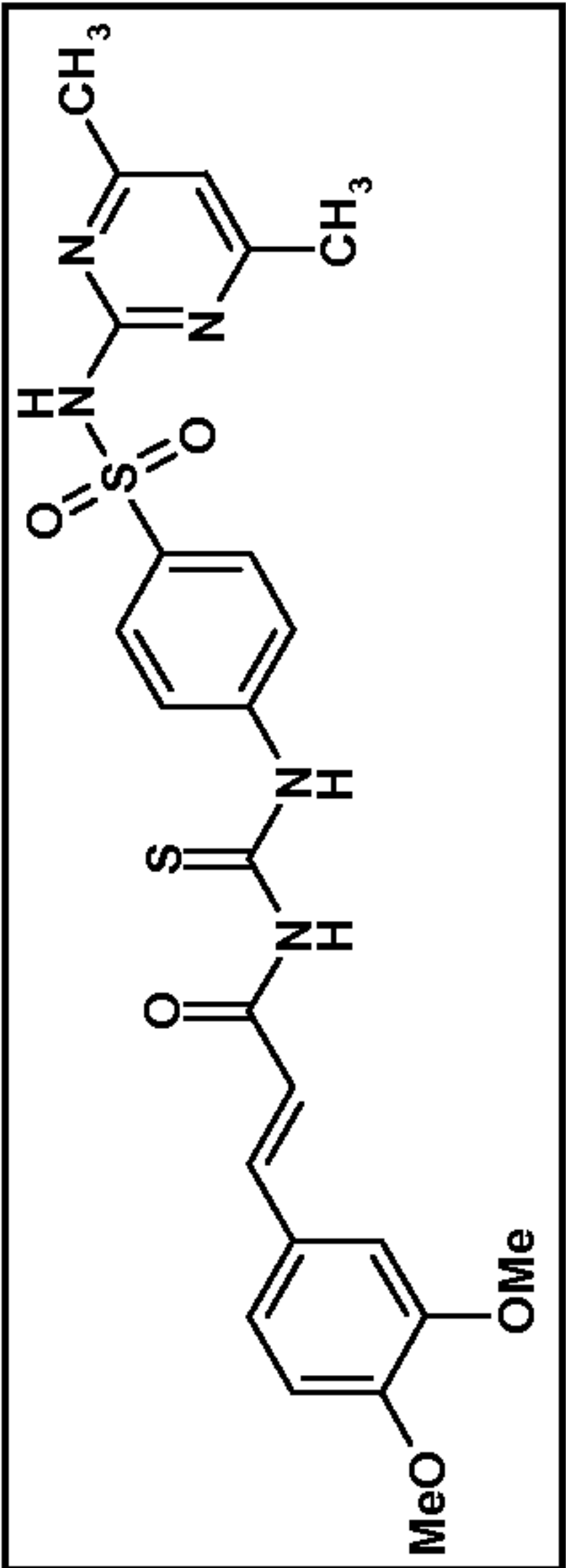
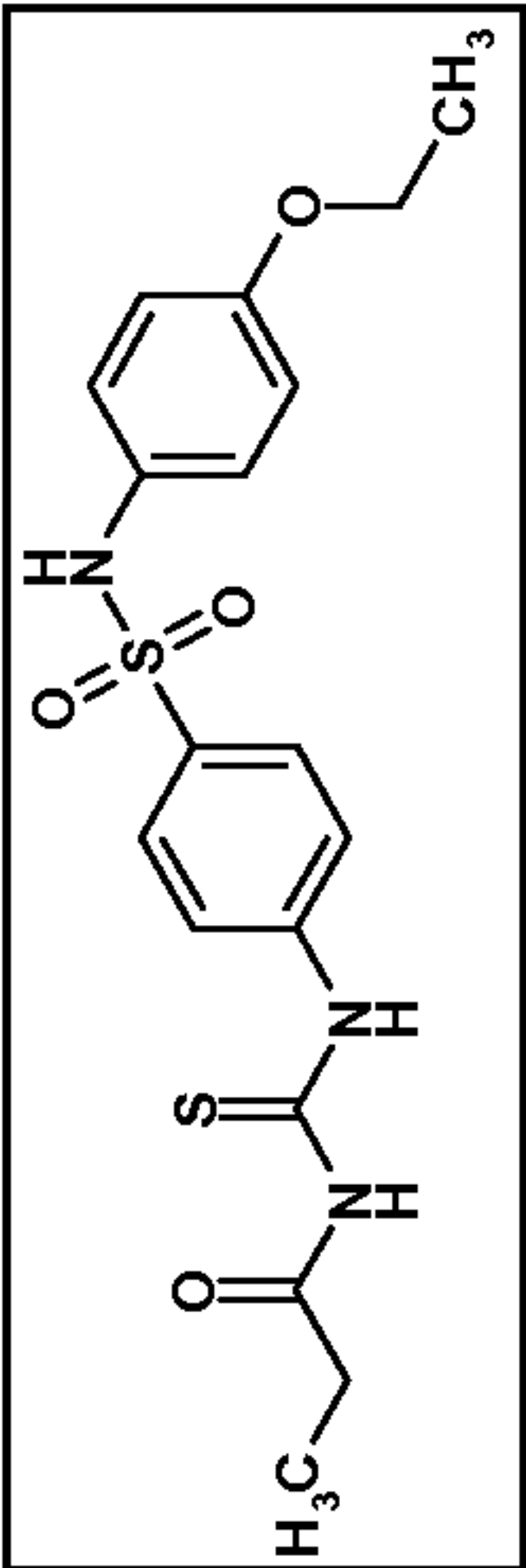


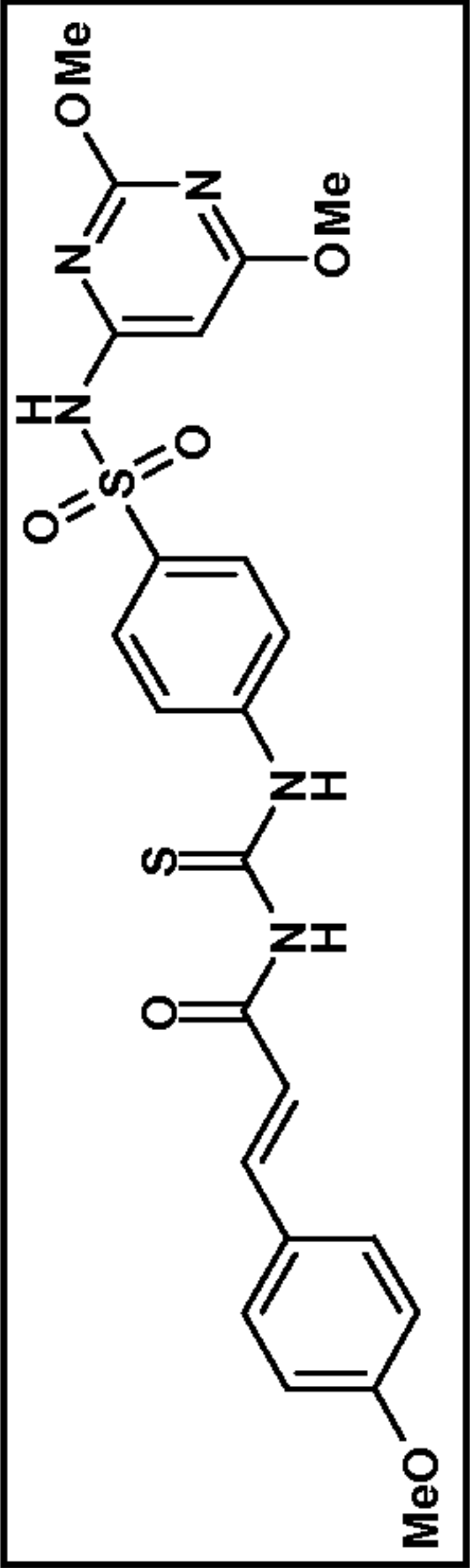
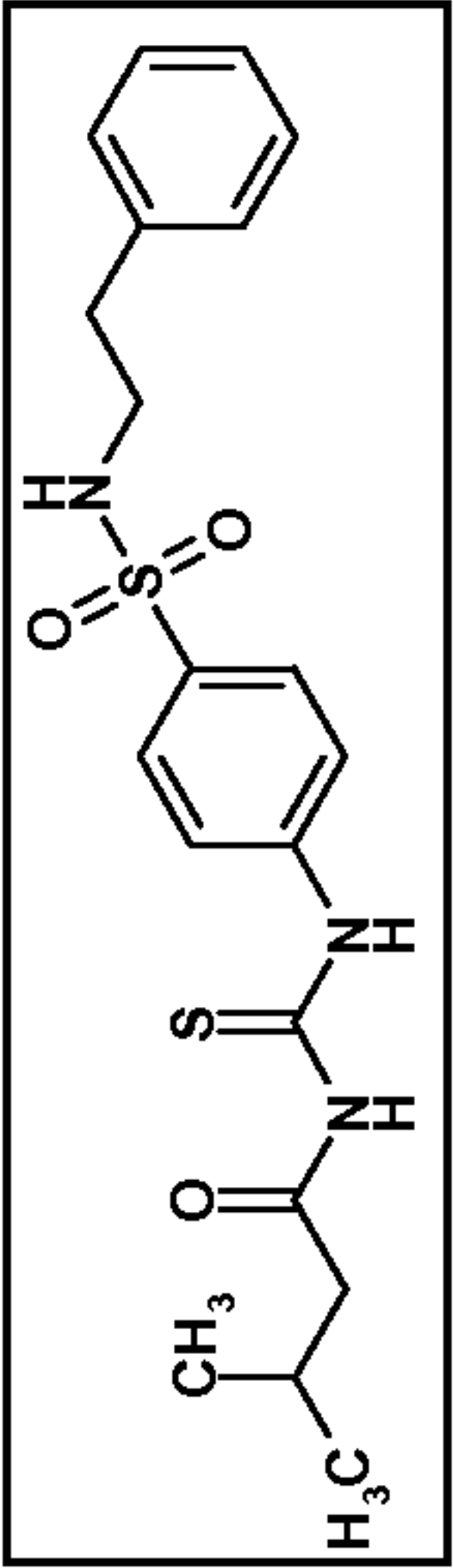
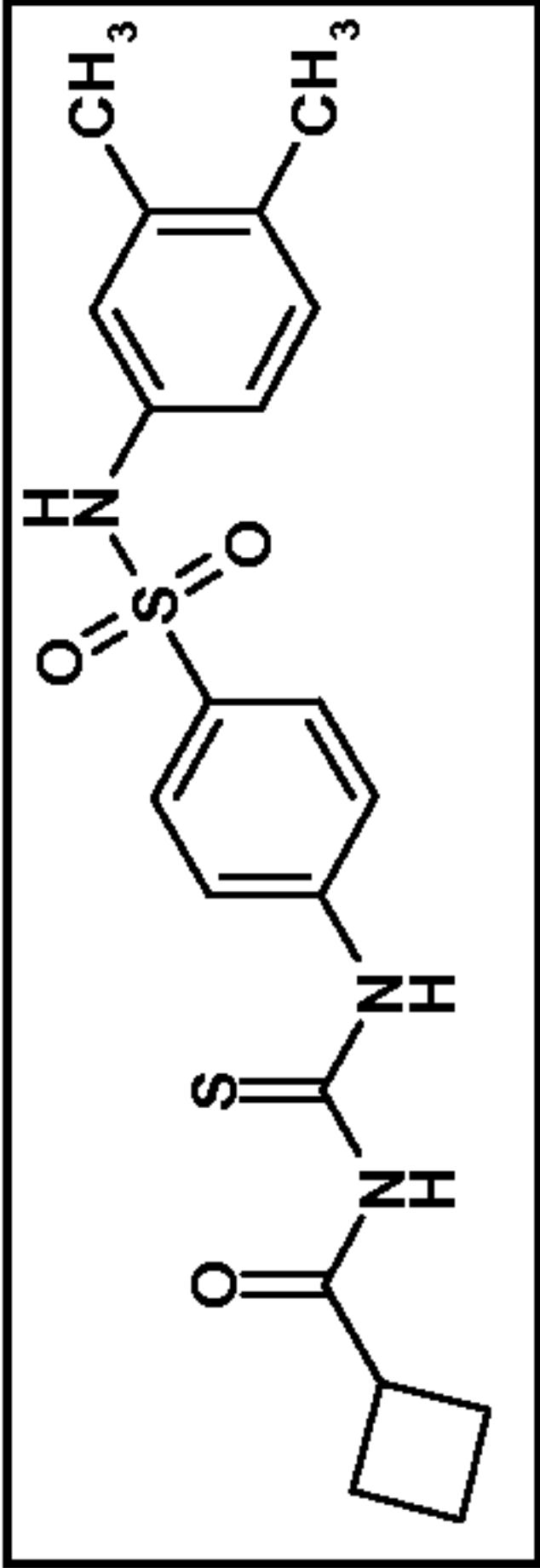
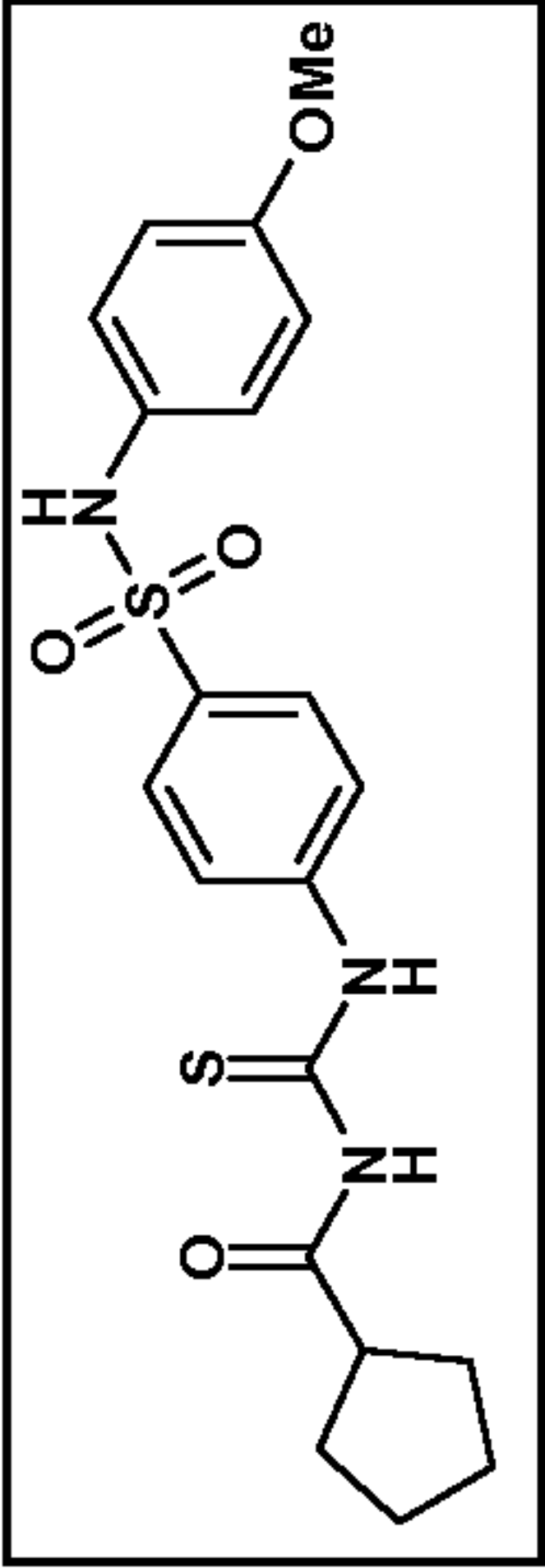
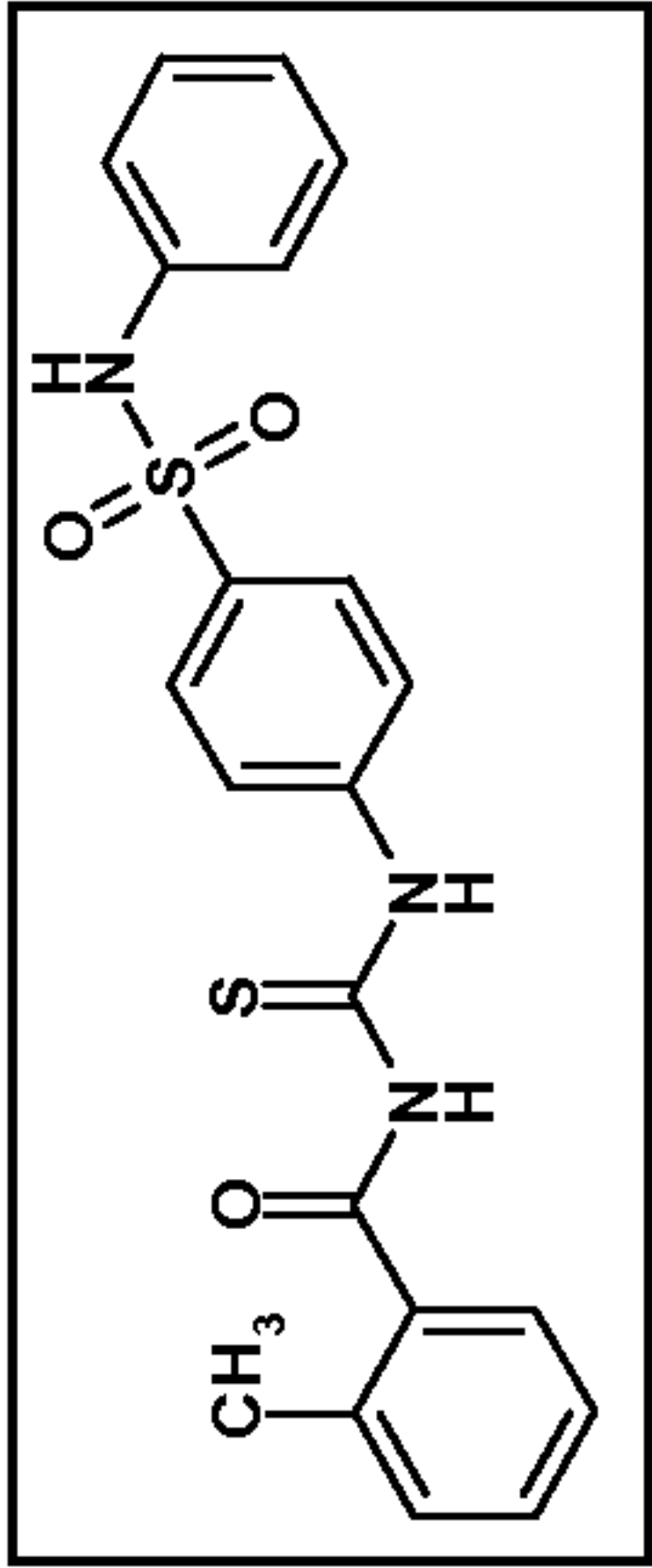
AD4-1886, Formula (17)

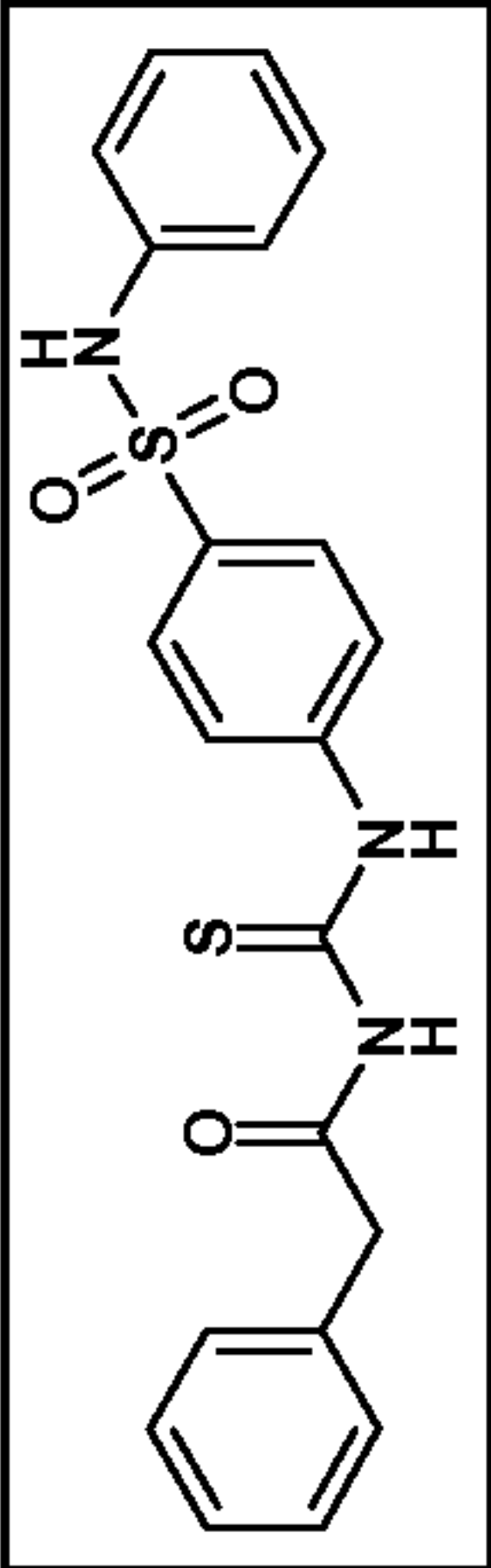
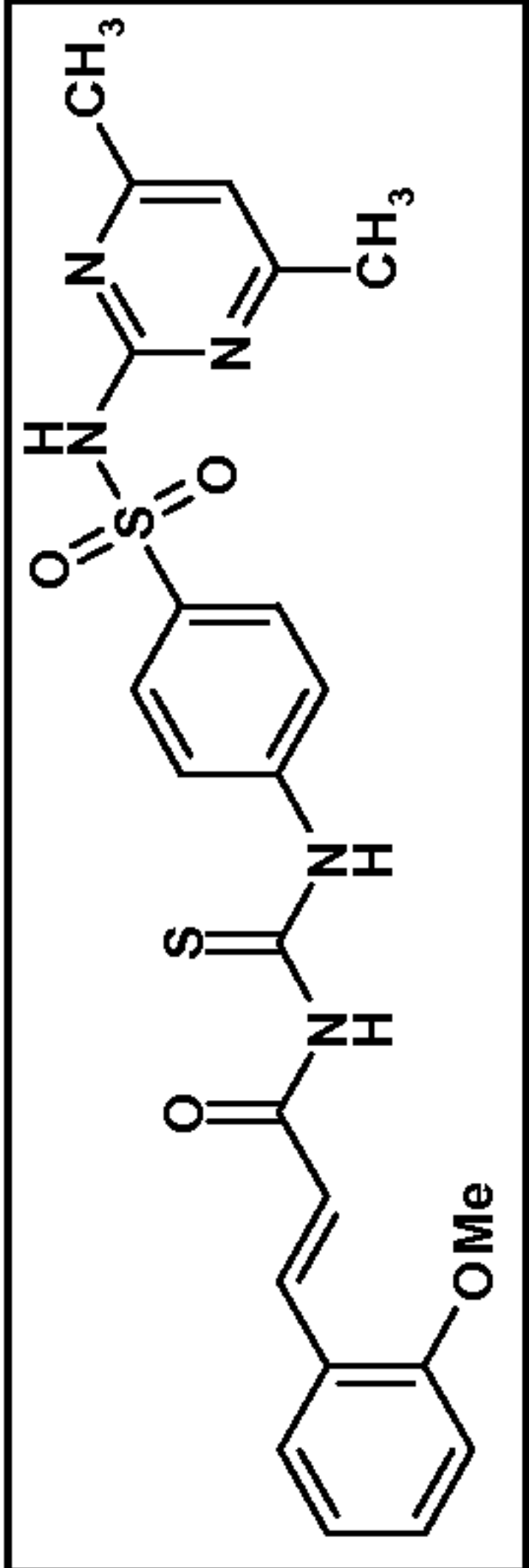
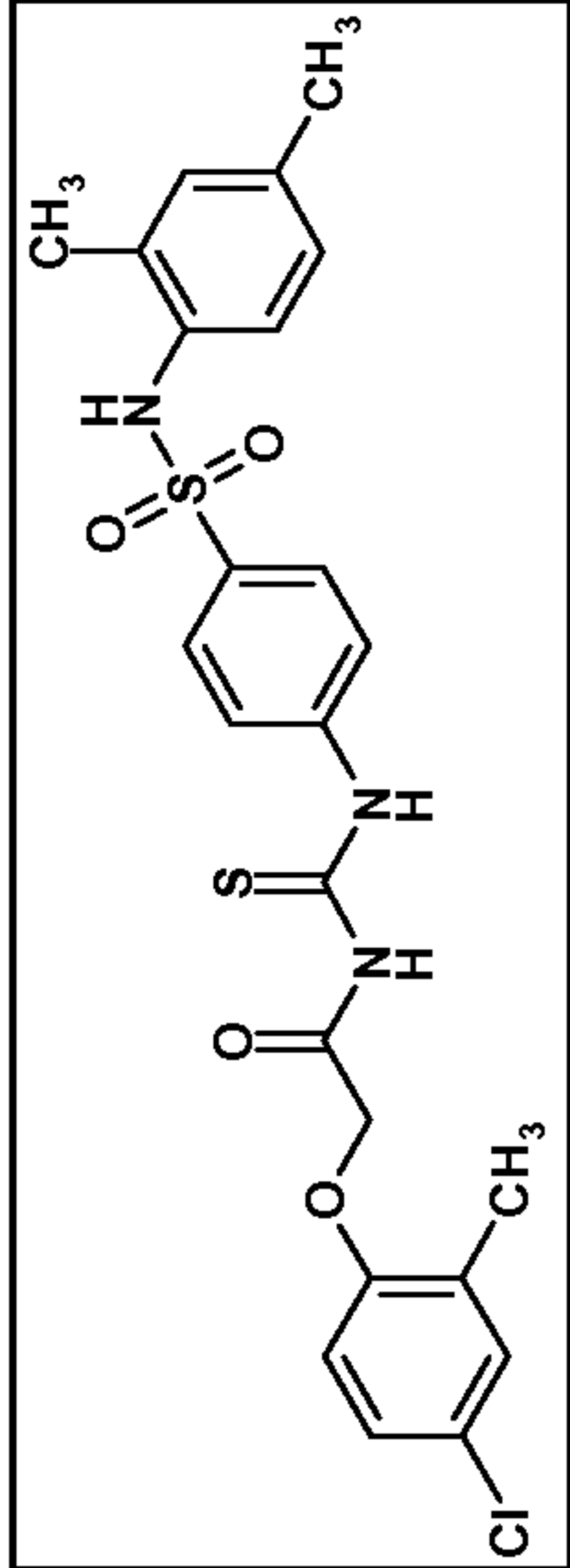
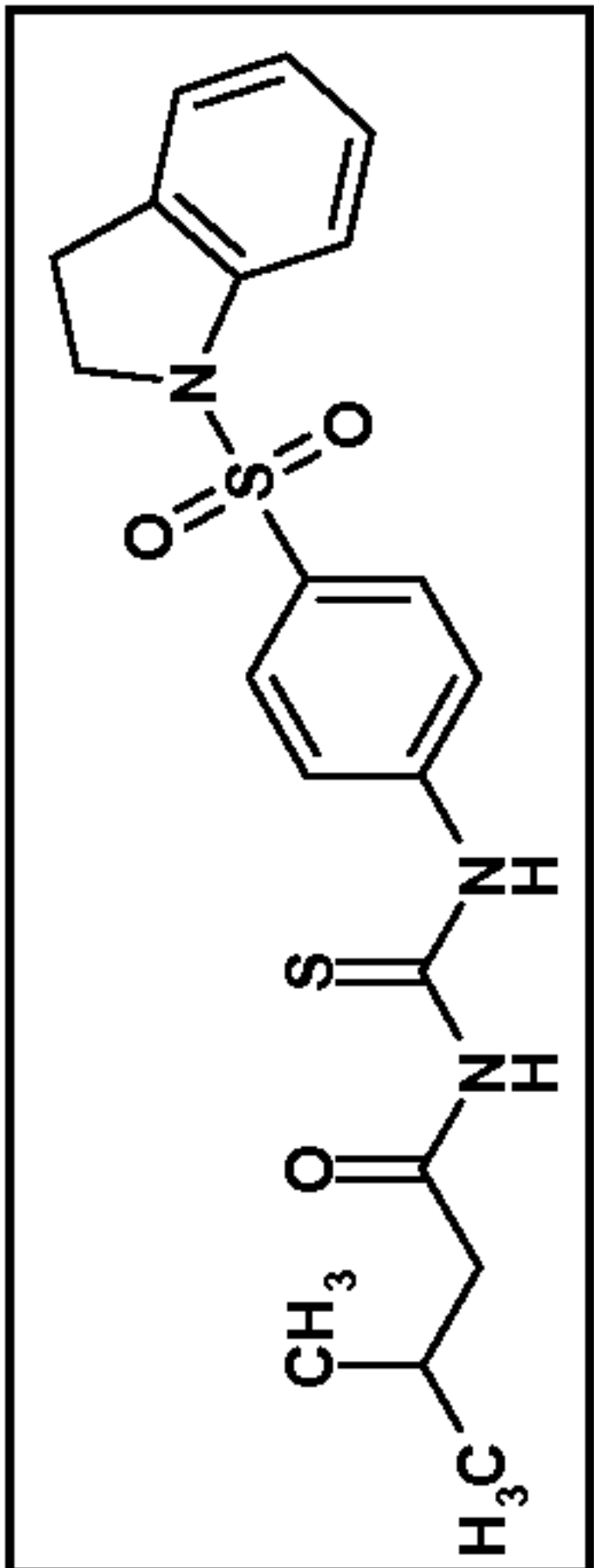
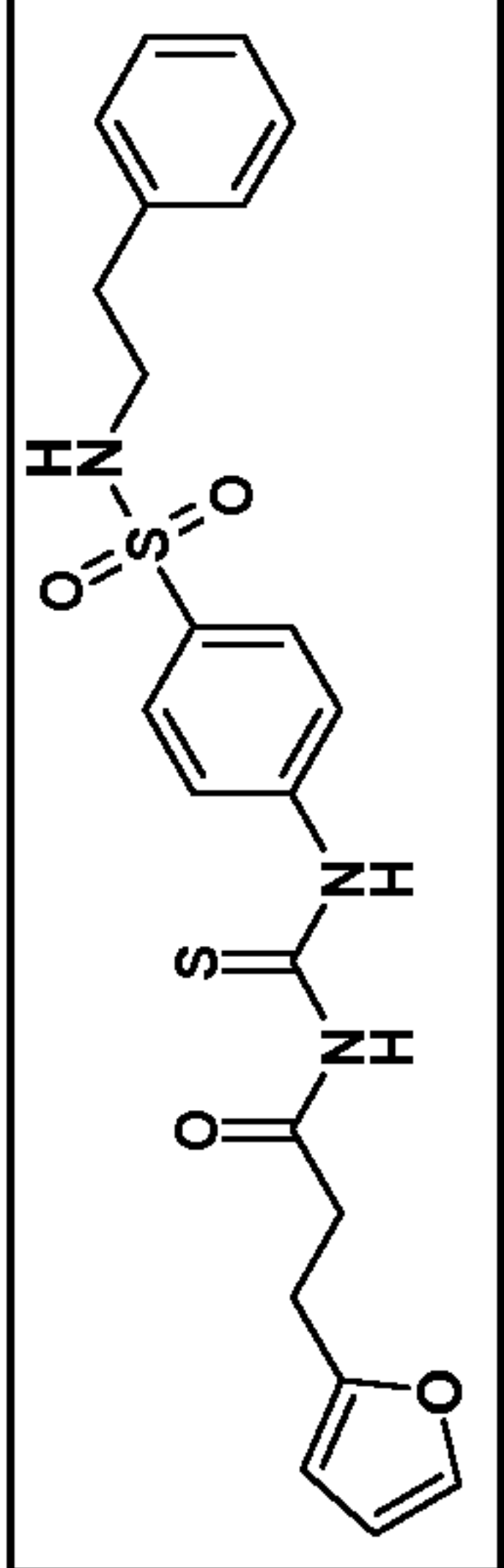
[0503] The compounds in TABLE 9 were identified via AD4-1886 structure similarity searches and were docked to the 1YY9.pdb binding site (erbitux residues thr100_glu105) to

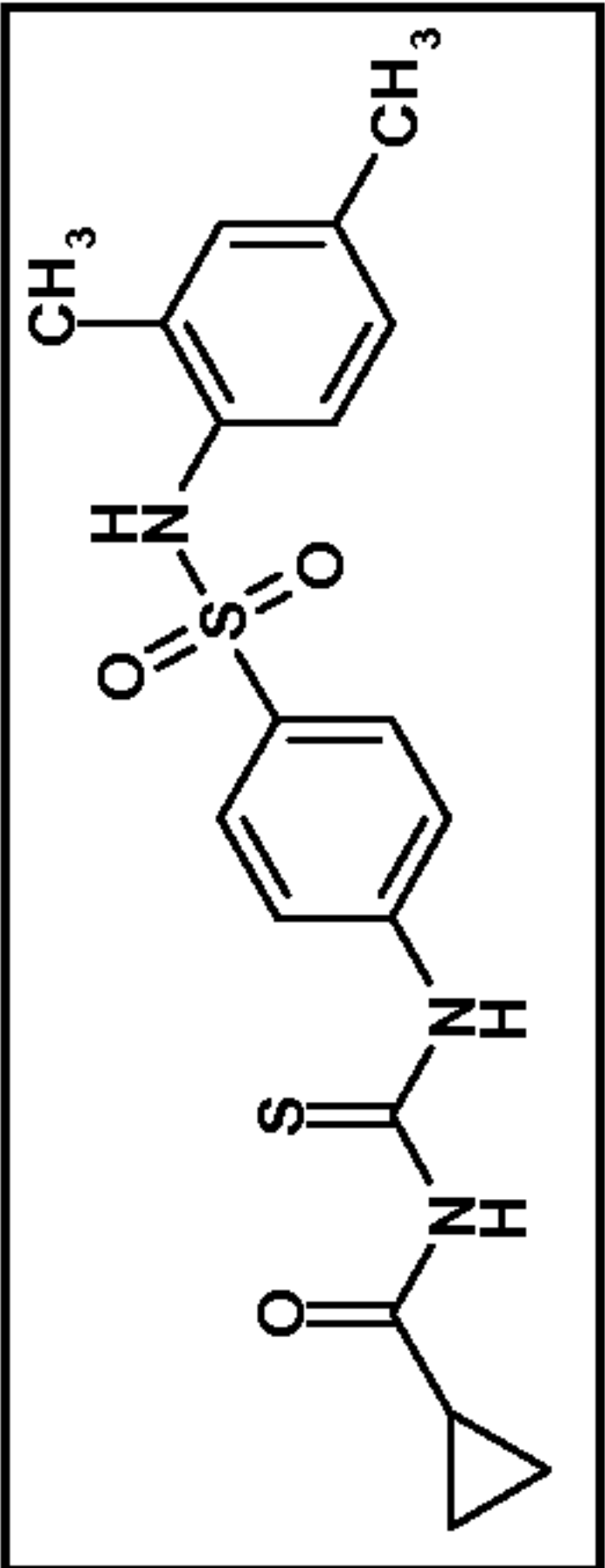
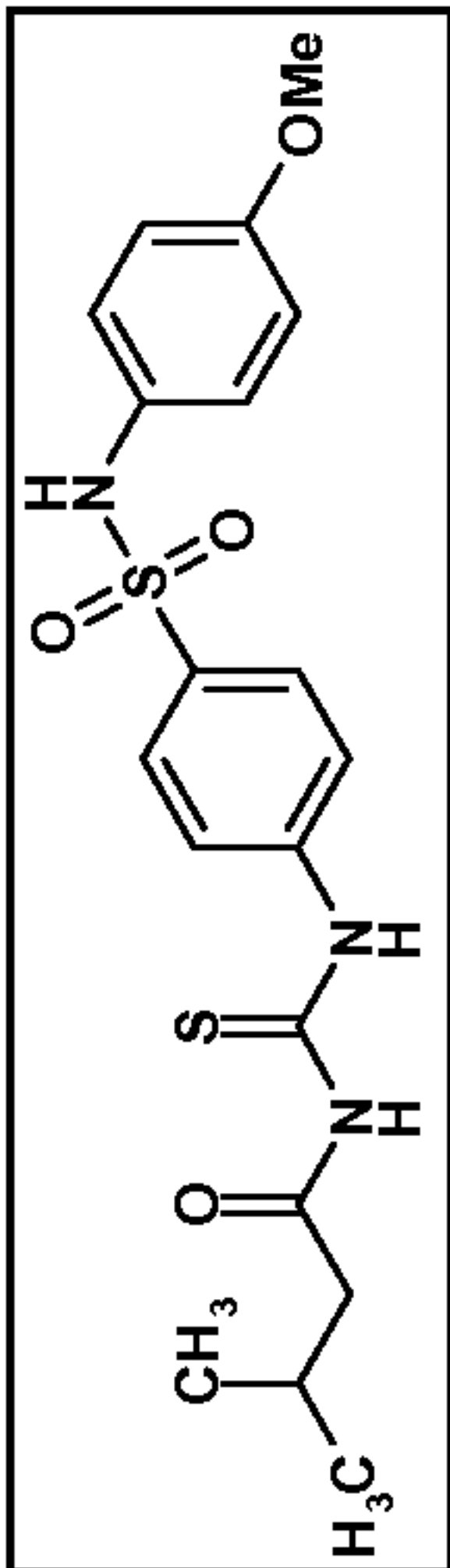
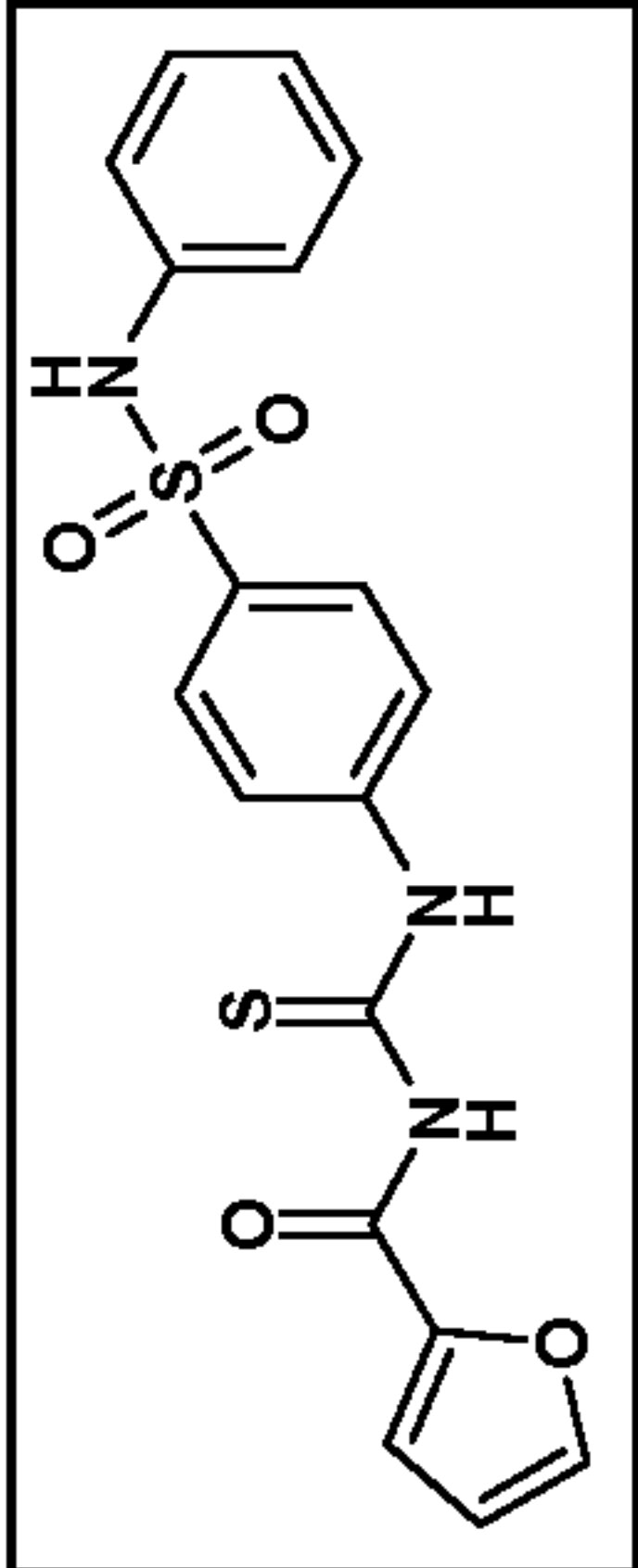
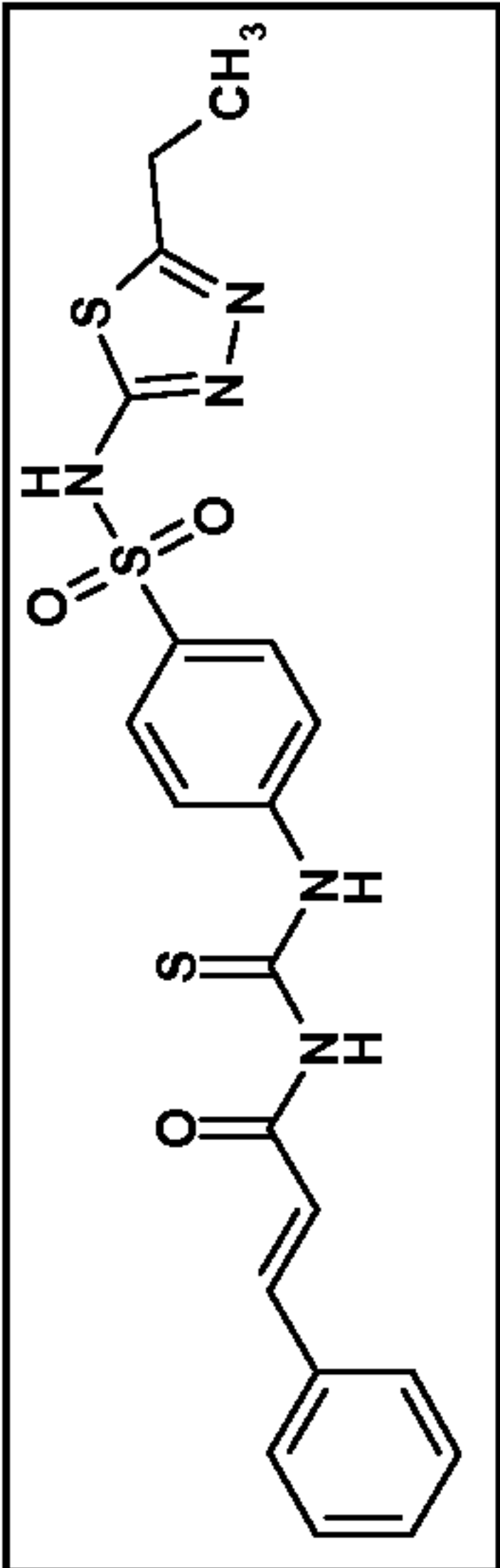
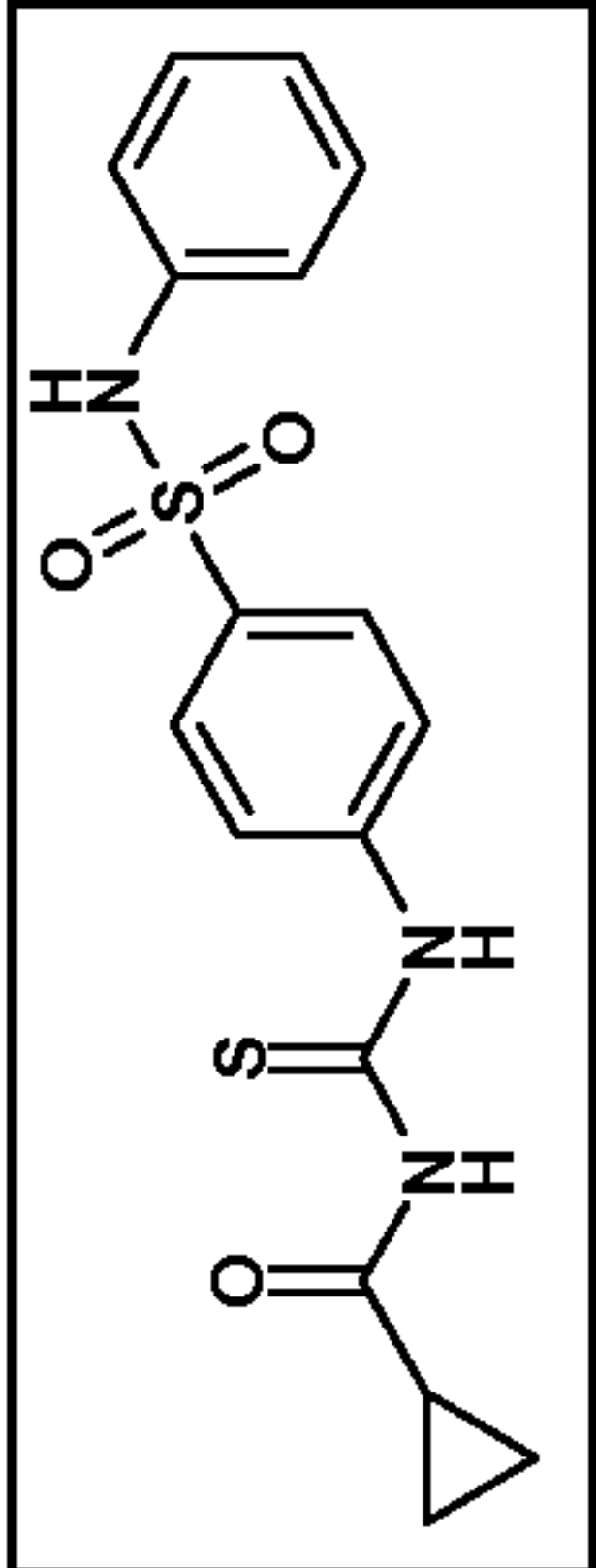
obtain their Glide and Emodel scores. Also depicted in TABLE 9 is ICW assay and MTT assay results (see Example 1, Example 2, Example 7).

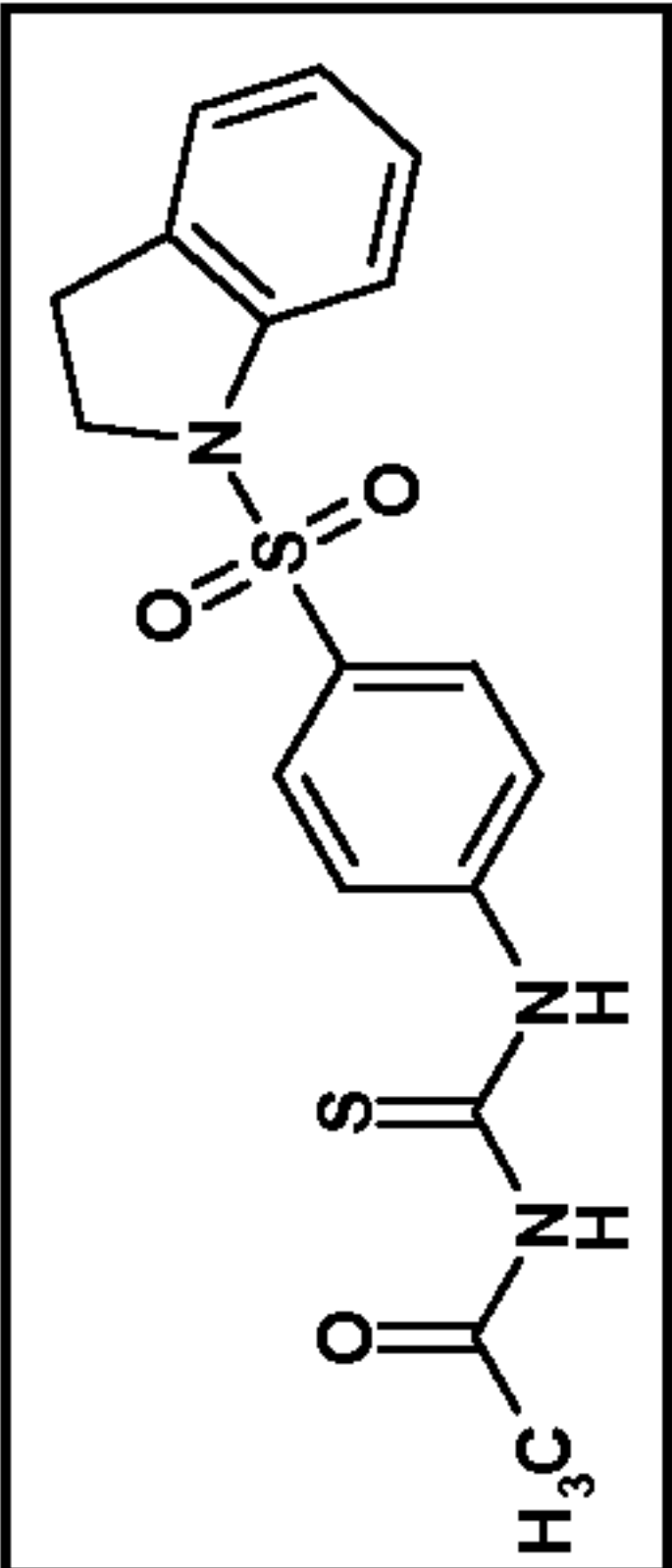
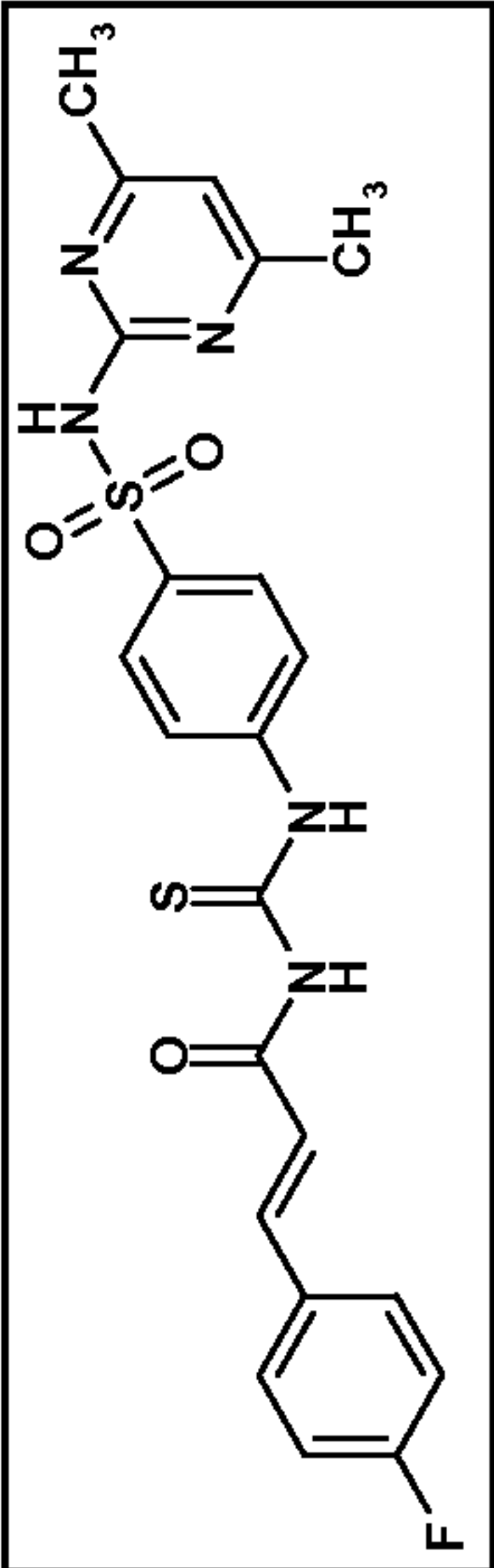
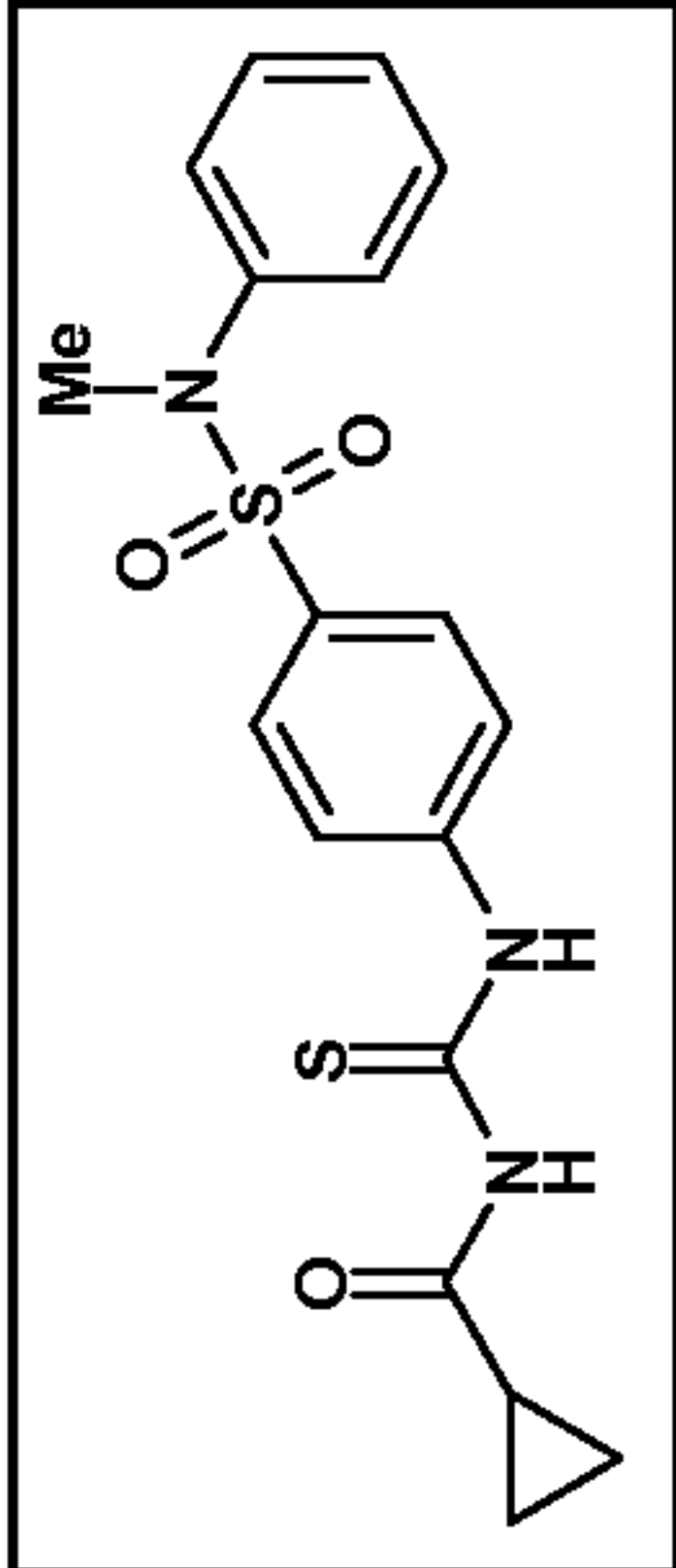
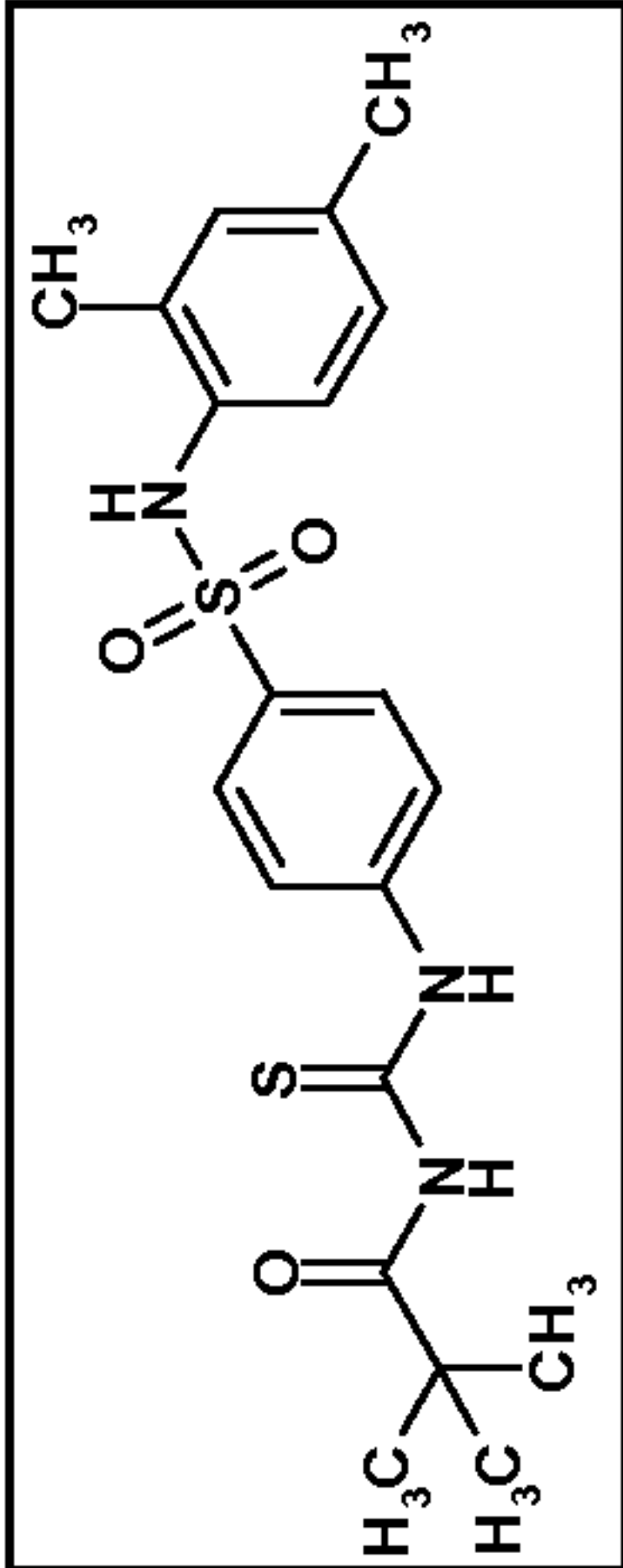
TABLE 9: Glide Score, E-Model score, ICW assay, and MTT Assay Results for AD4-1886-like Compounds.

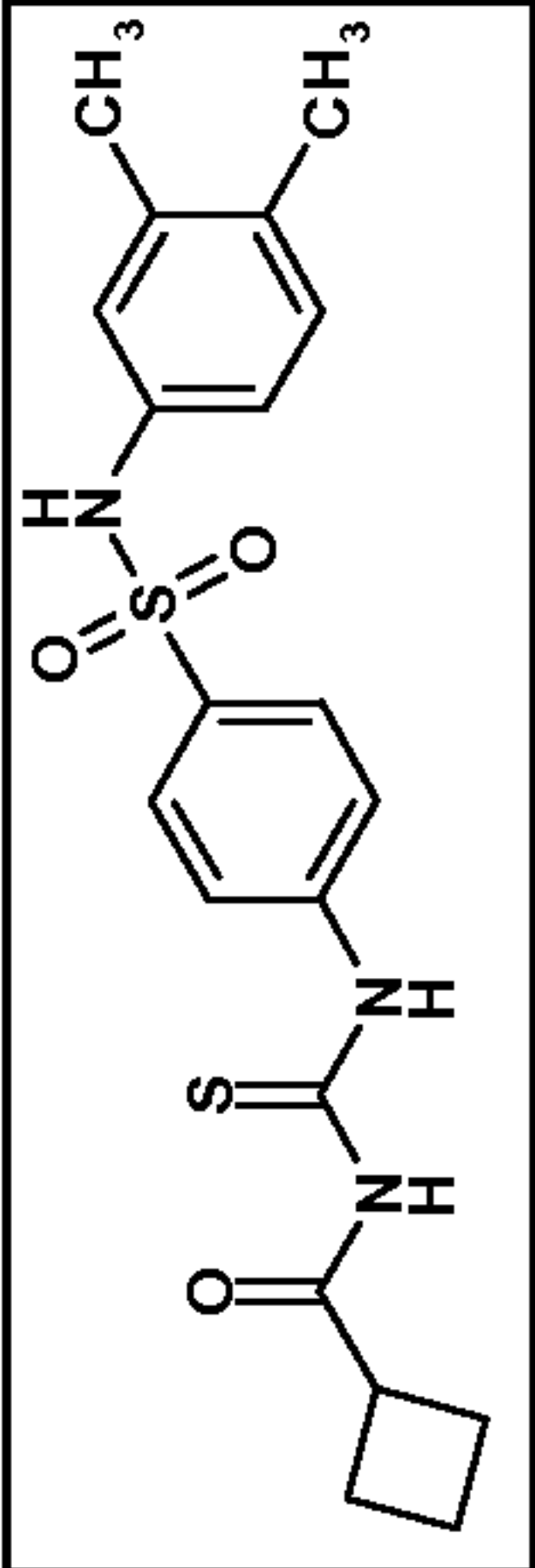
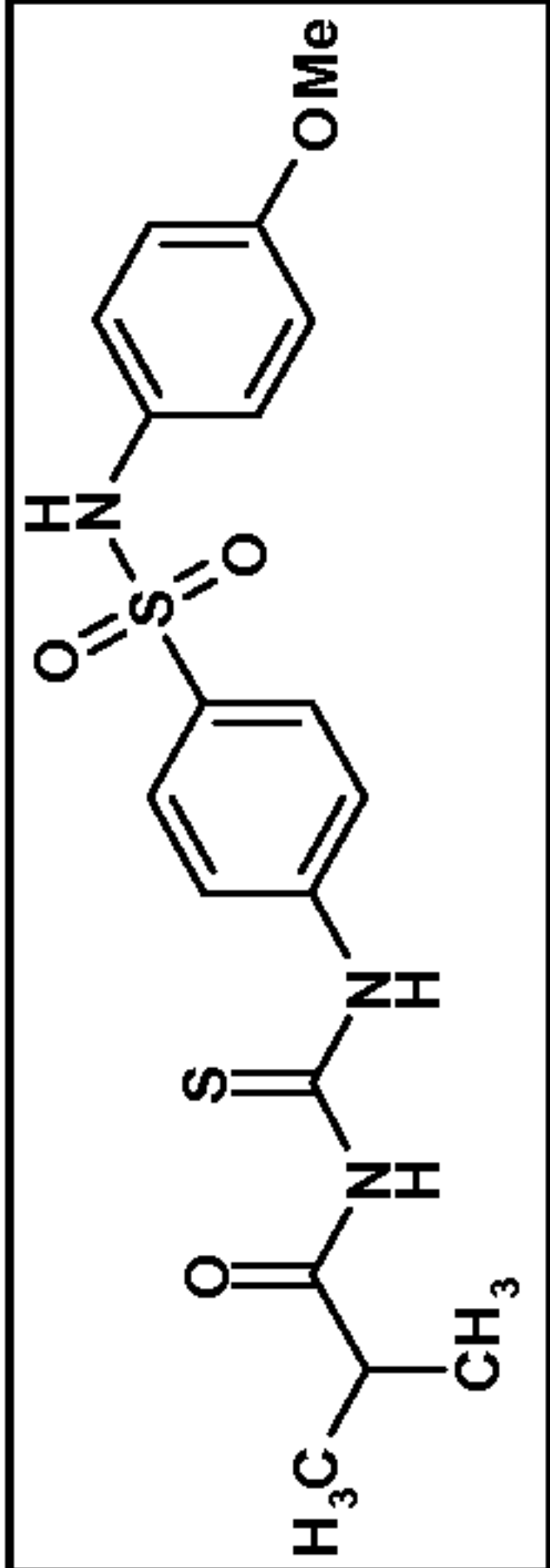
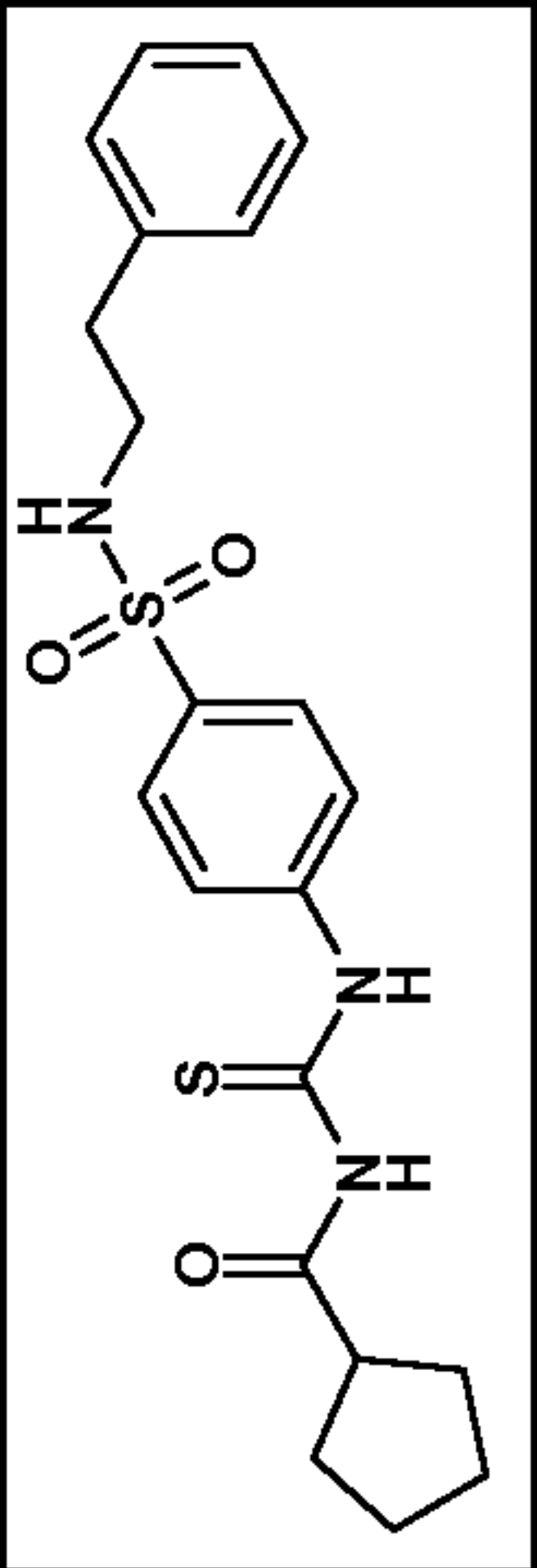
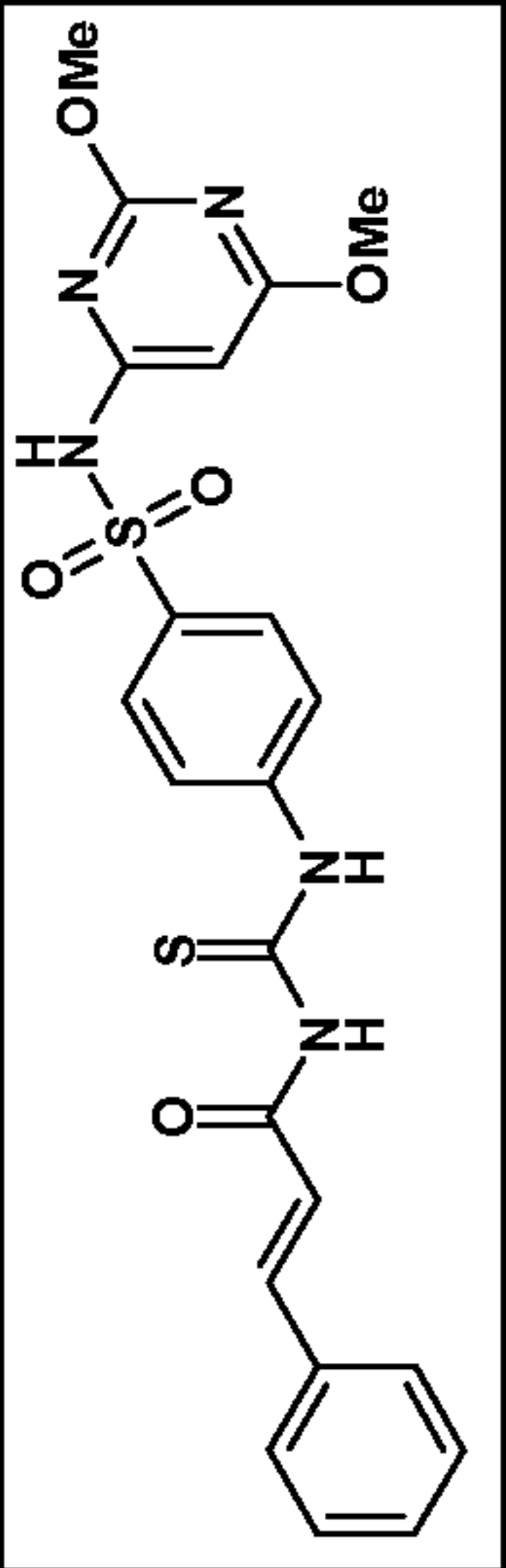
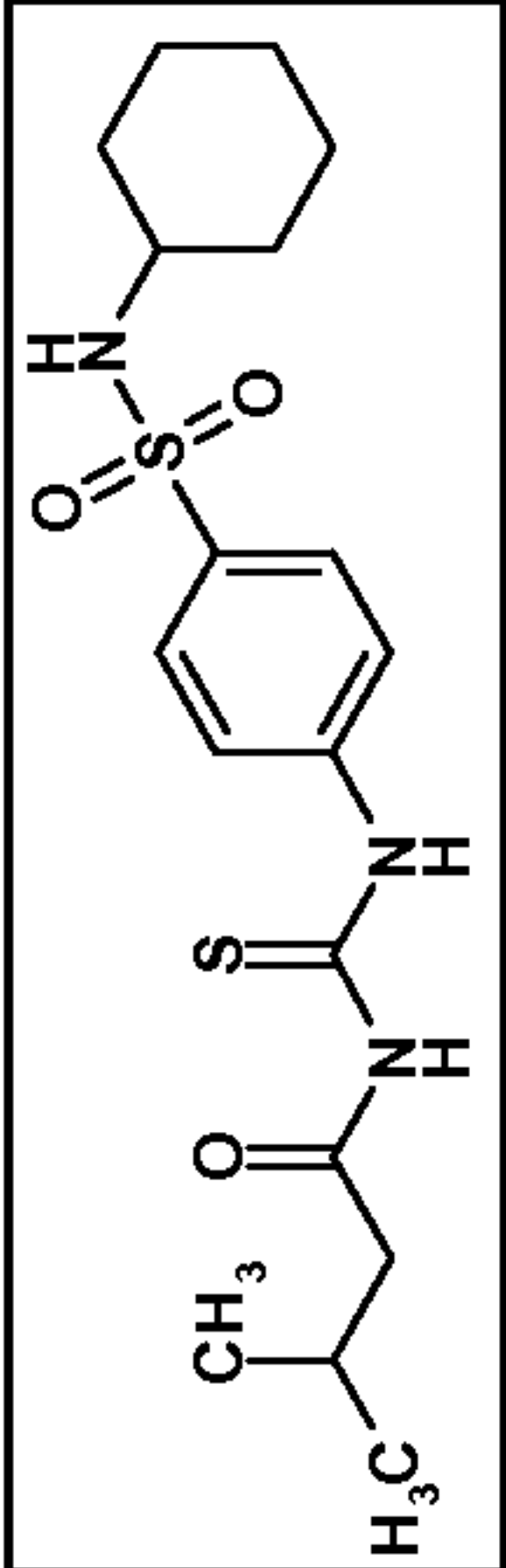
AD4-1886 by ICW Inhibition		Sub-Class	Glide G-Score	Glue-E-Model	ICW Screen	MTT Screen	Erbitux-G-Score	Erbitux-E-Model
	AD4-11883	O	-6.72	-56.32	83.13	19.51/non-convergent	-3.22	-50.29
	AD4-11975	K	-5.19	-59.024	75.826	17.52/17.16(0.98)	-5.78	-36.79
	AD4-11409	K	-5.51	-61.783	74.94	17.18/11.66(0.68)	-2.96	-34.78
	AD4-11638	O	-5.68	-63.037	68.754	11.19/14.90(1.33)	-3.24	-50.39

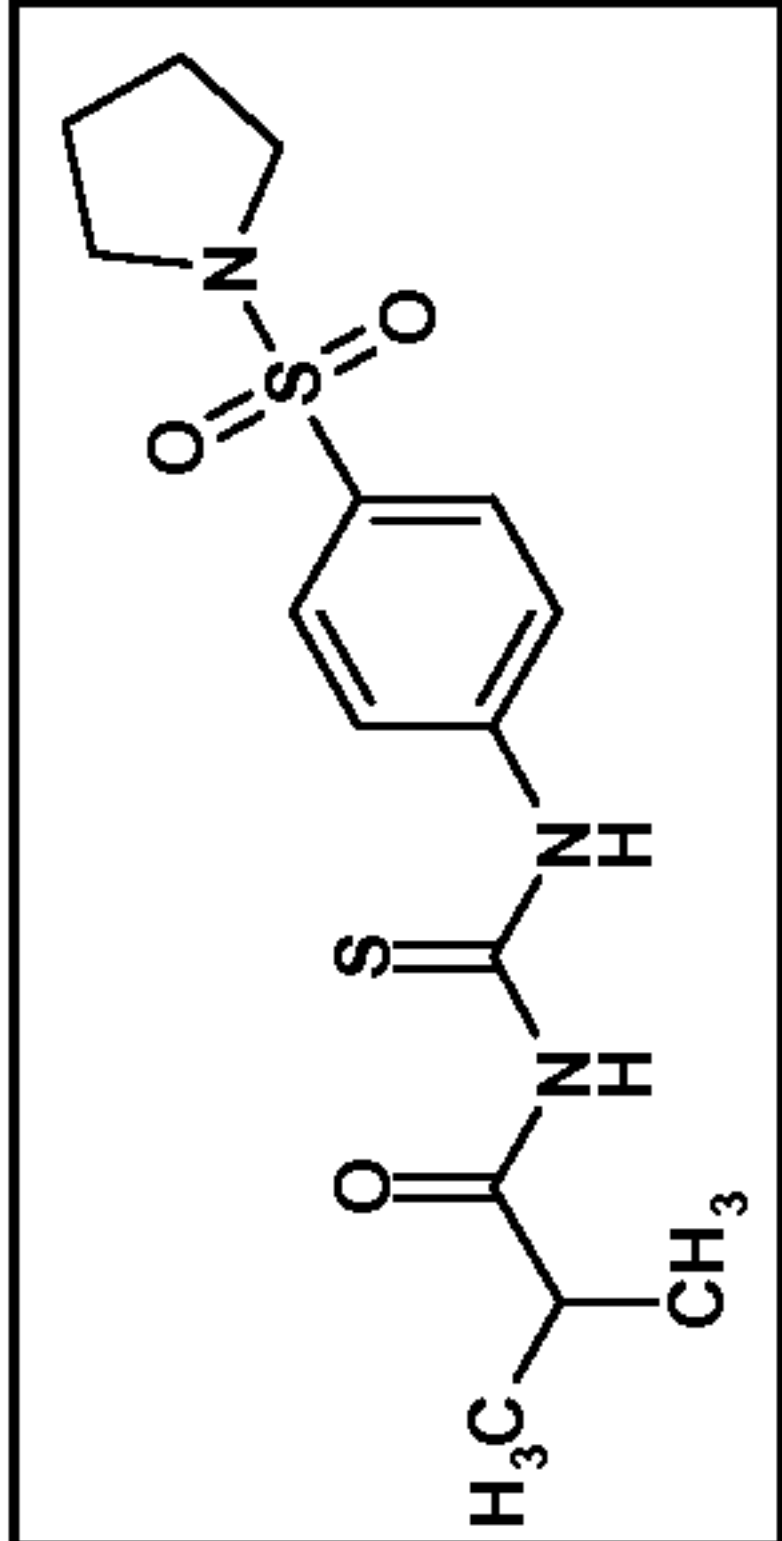
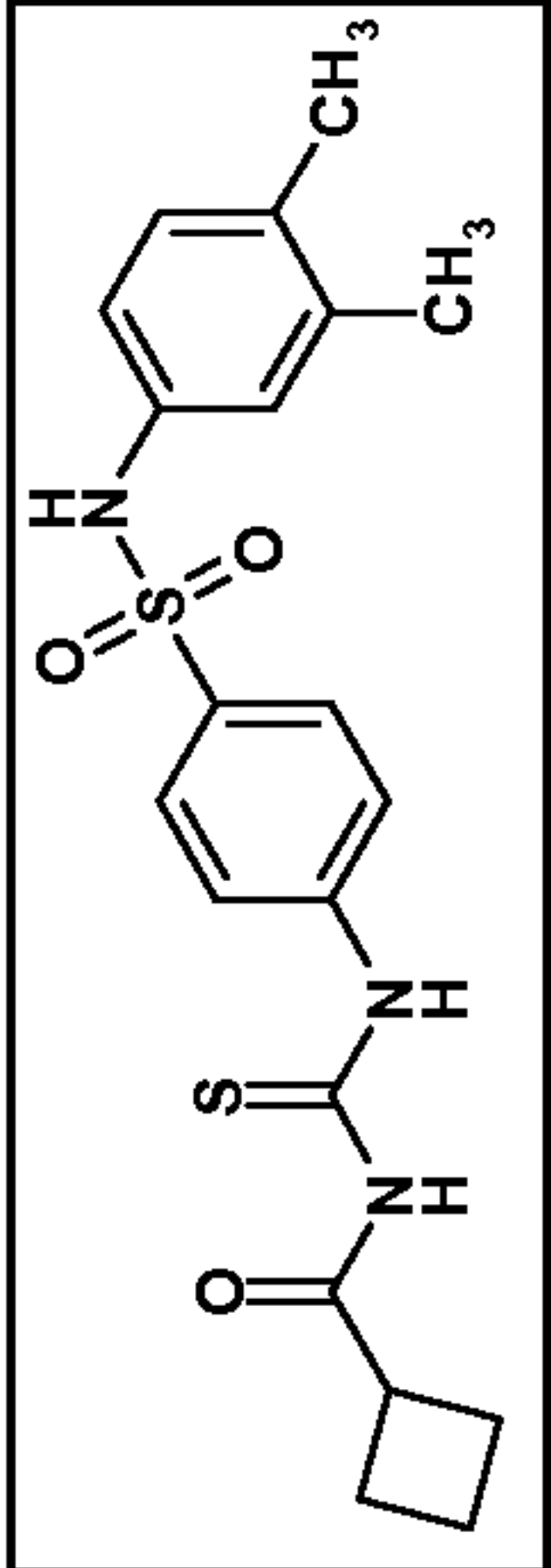
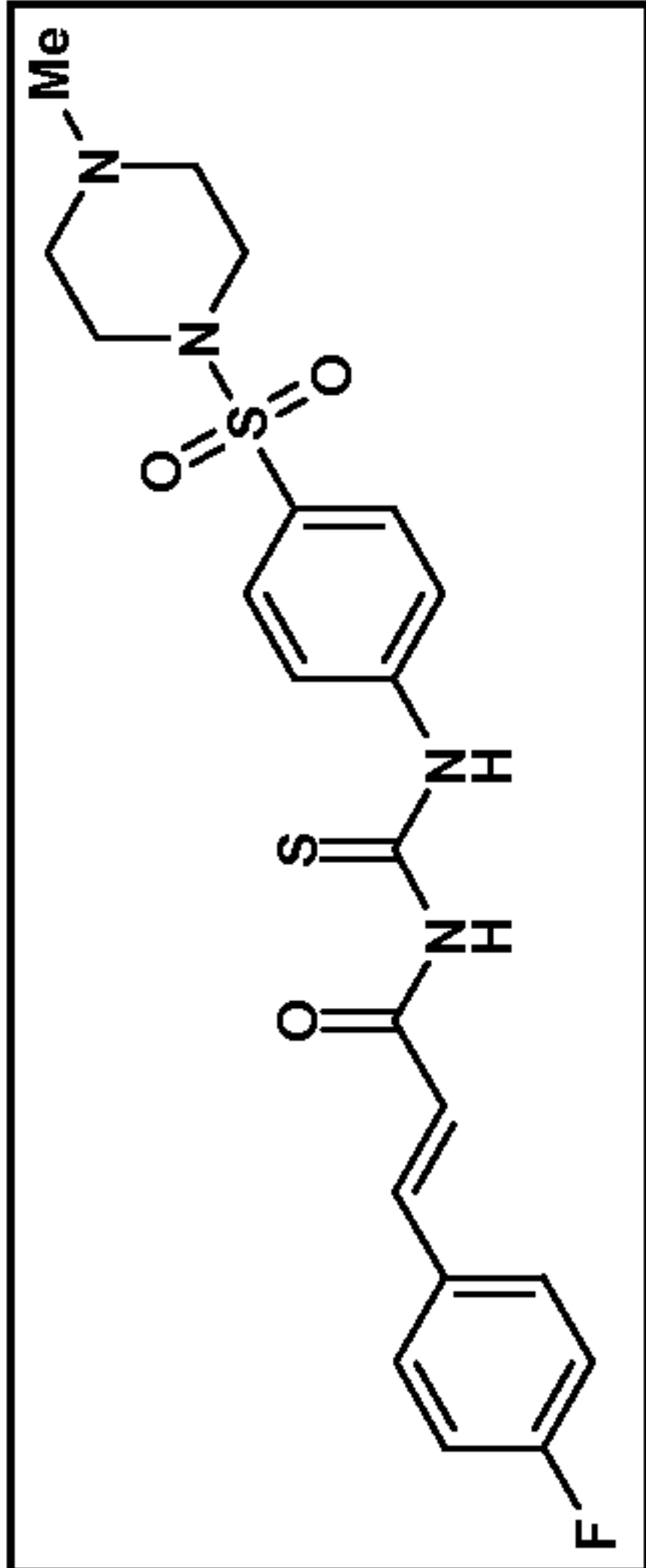
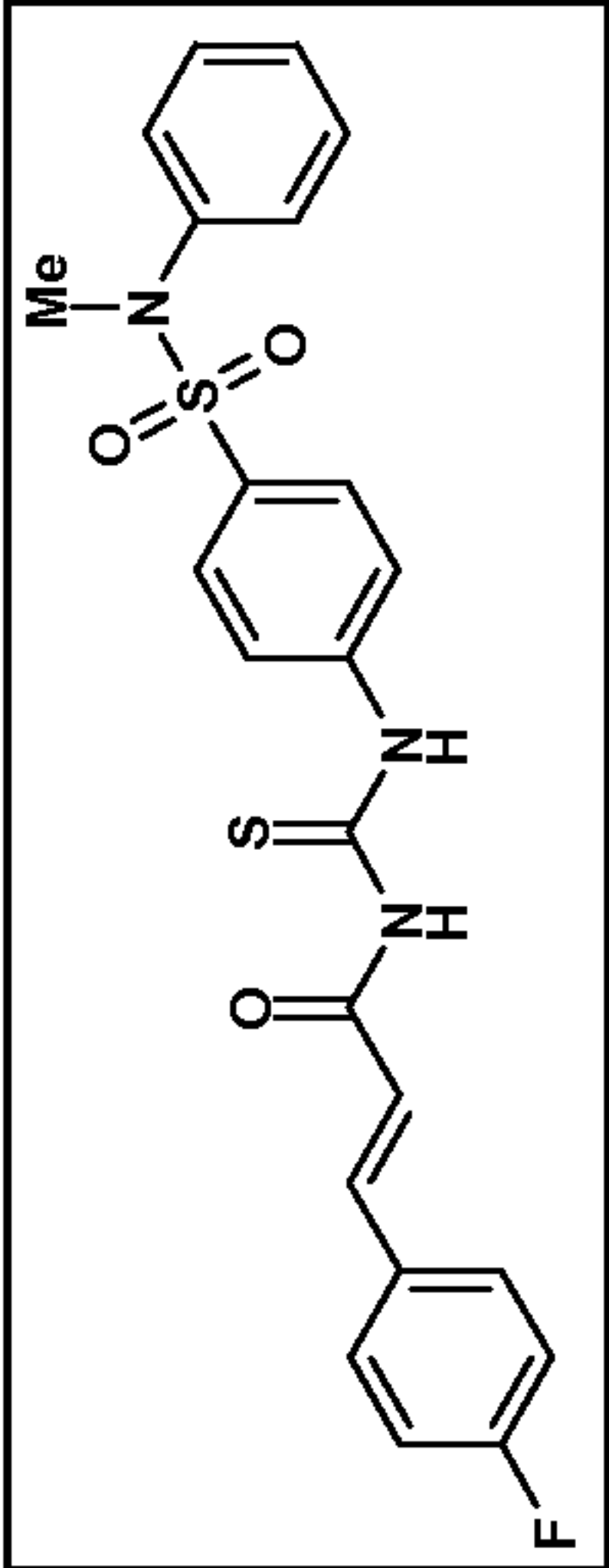
	AD4-11645	O	-7.29	-61.027	66.452	45.60/47.02(1.03)	-3.56	-39.45
	AD4-10608	L	-6.31	-65.526	62.846	6.33/5.35(1.18)	-3.57	-38.88
	AD4-10676	K	-4.99	-35.282	59.637	18.58/8.84(0.48)	-3.48	-39.56
	AD4-12119	K	-4.87	-59.115	55.94	18.64/14.62(0.78)	-1.86	-39.07
	AD4-10706	K	-5.17	-38.332	55.497	15.12/23.11(1.53)	-0.32	-35.65

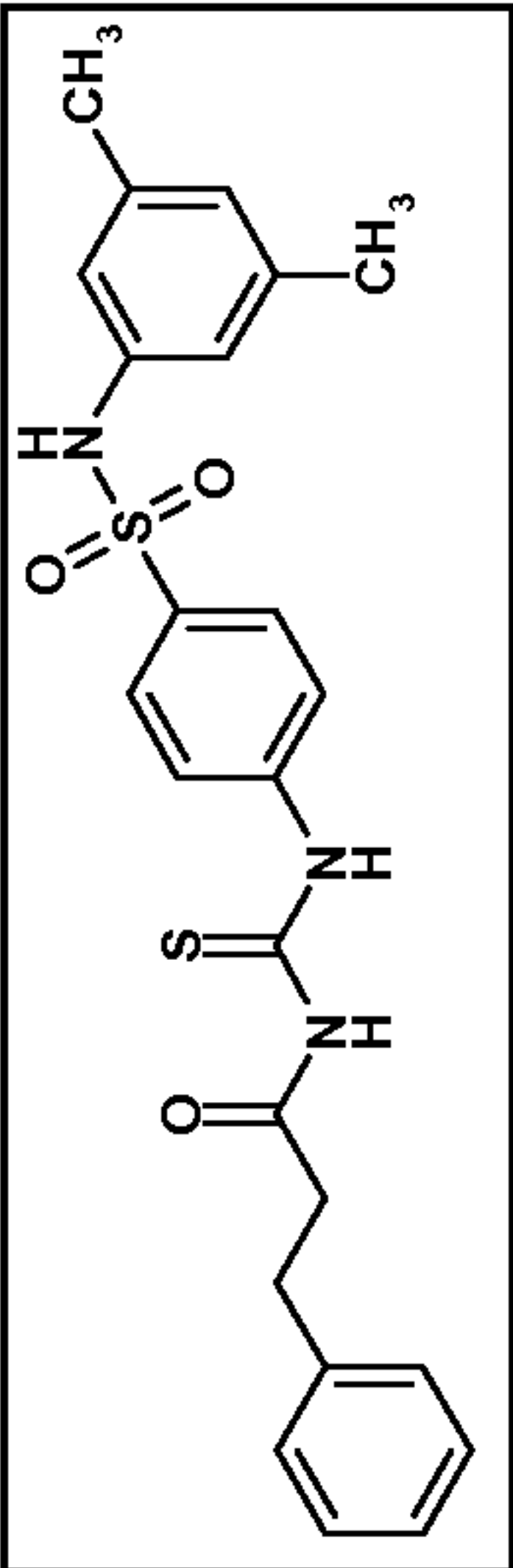
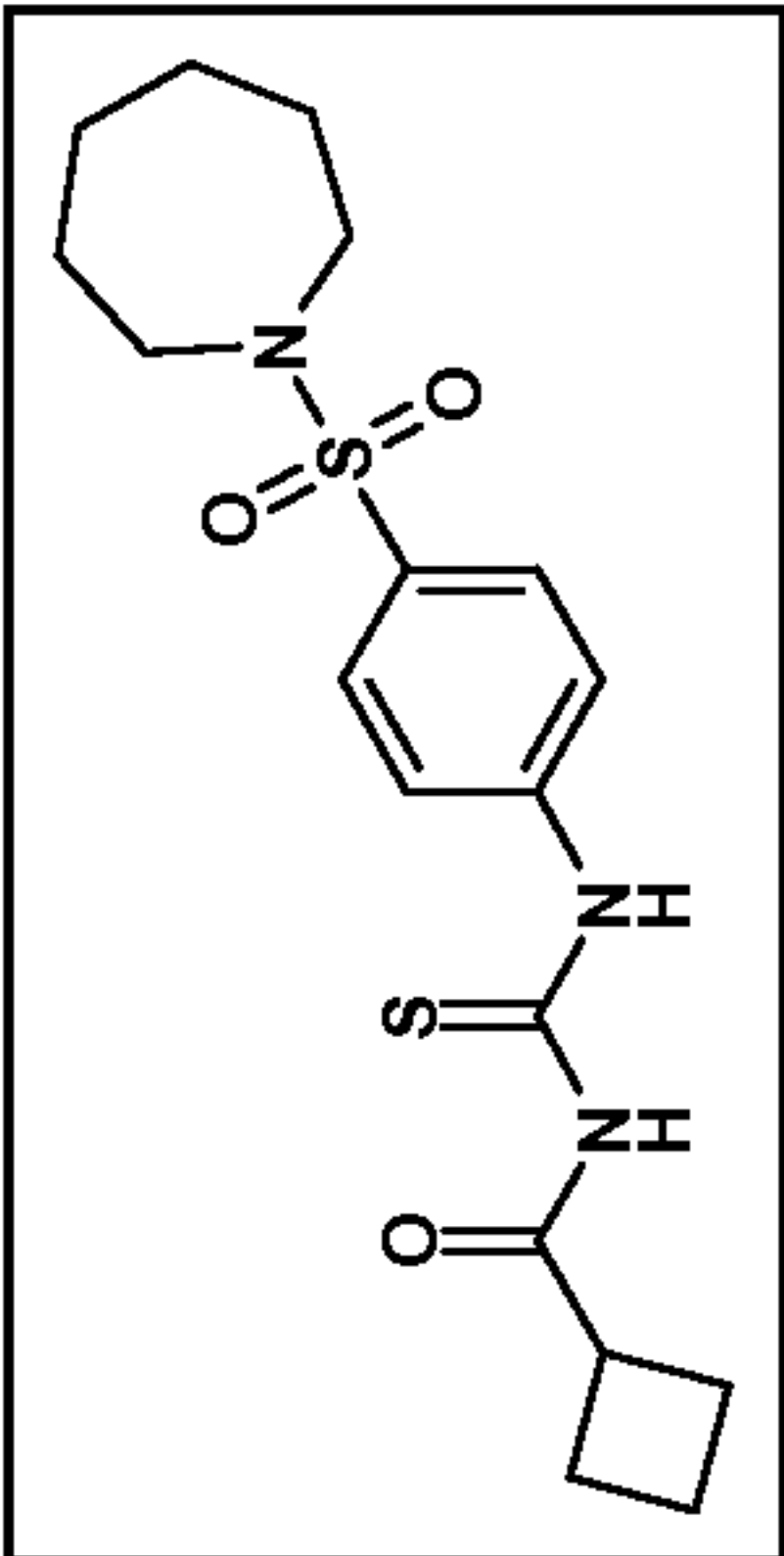
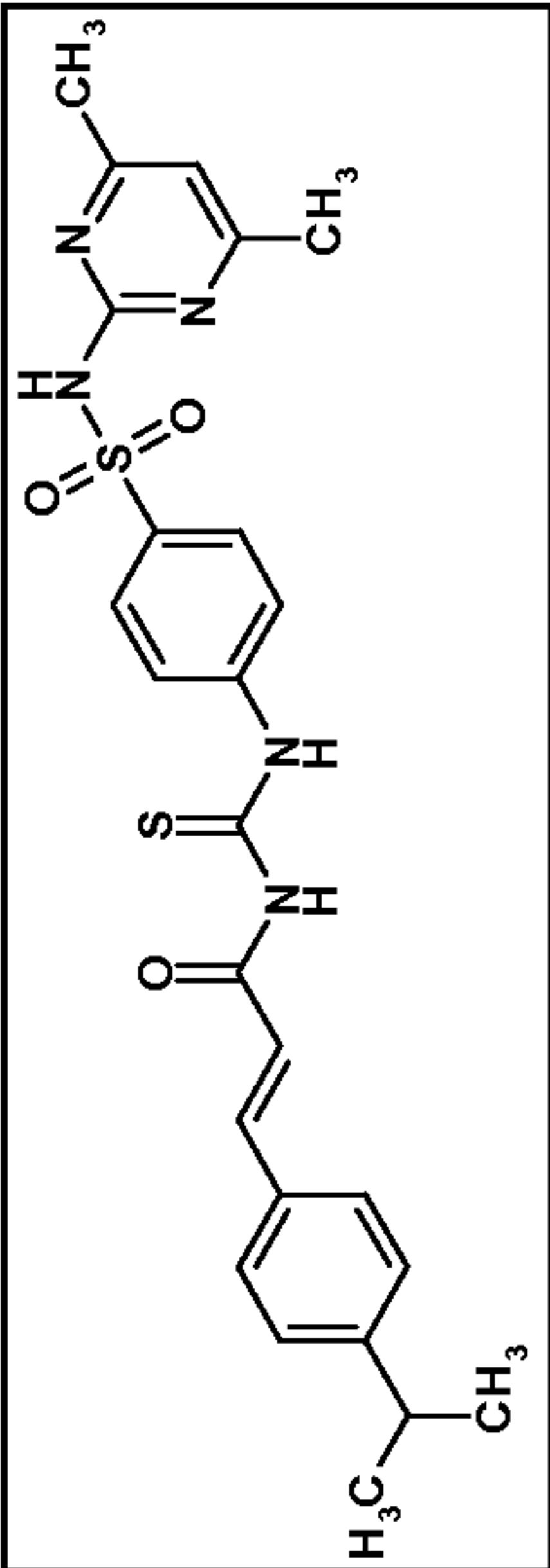
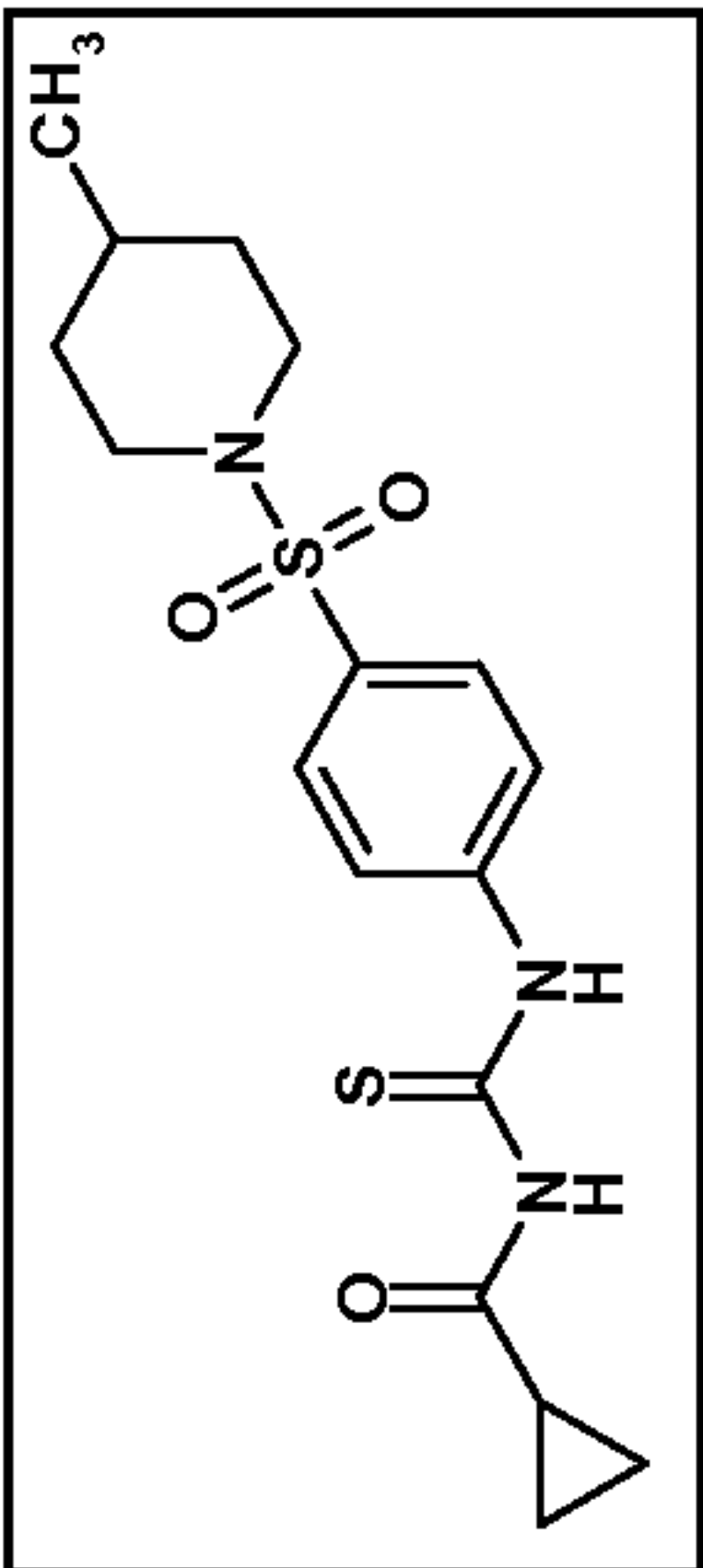
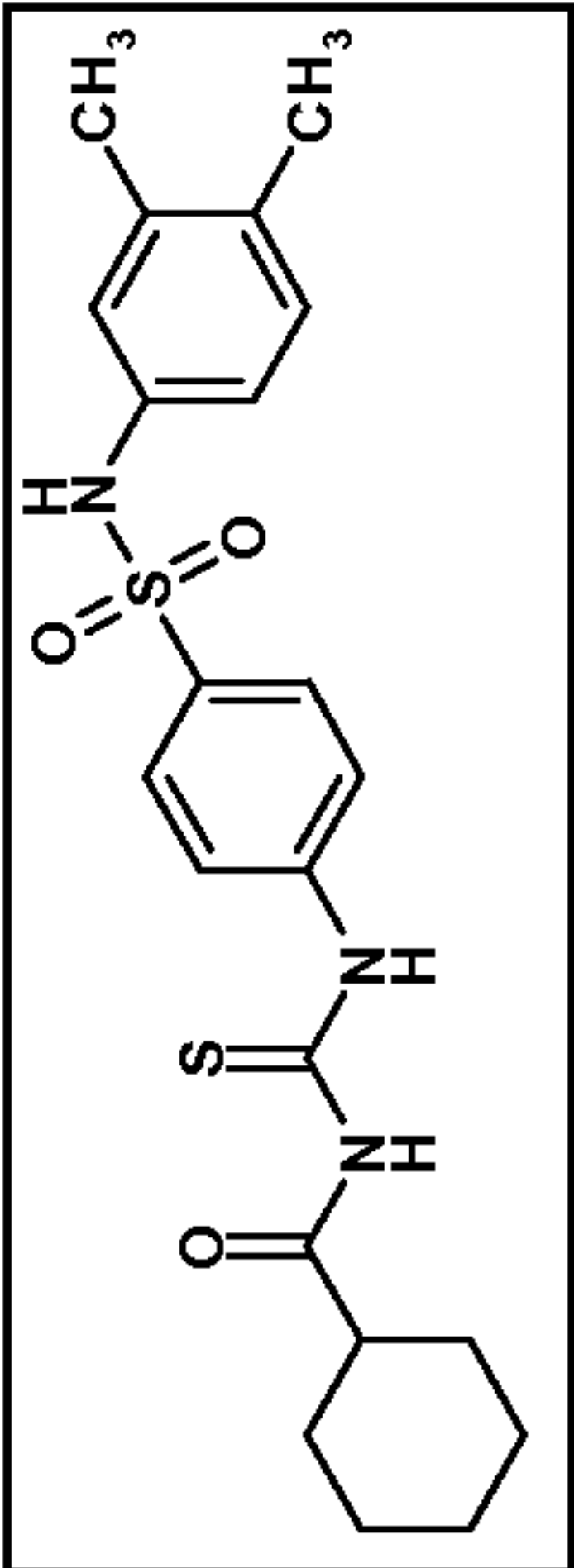
	AD4-11416	K	-5.11	-57.963	53.517	15.37/13.54(0.88)	-5.3	-41.96
	AD4-12148	O	-5.18	-66.098	51.845	42.82/39.24(0.92)	-3.95	-43.35
	AD4-12152	K	-6.63	-69.497	50.528	21.02/16.20(0.77)	-3.38	-44.49
	AD4-10626	M	-7.03	-54.119	50.05	5.31/19.57(3.68)	-4.2	-40.24
	AD4-10624	L	-5.71	-66.733	49.448	25.64/16.76(0.65)	-2.01	-33.65

	AD4-12097	K	-7.24	-55.243	48.543	15.08/9.85(0.65)	-3.72	-36.73
	AD4-12040	K	-5.67	-59.95	47.822	17.13/14.82(0.87)	-4.17	-33.64
	AD4-10705	K	-4.28	-36.375	47.764	23.09/25.20(1.09)	-0.07	-35.1
	AD4-11367	O	-6.03	-60.038	46.872	43.33/43.63(1.01)	-3.56	-32.87
	AD4-11413	K	-5.42	-52.398	46.23	31.40/29.77(0.95)7	-4.38	-41.06

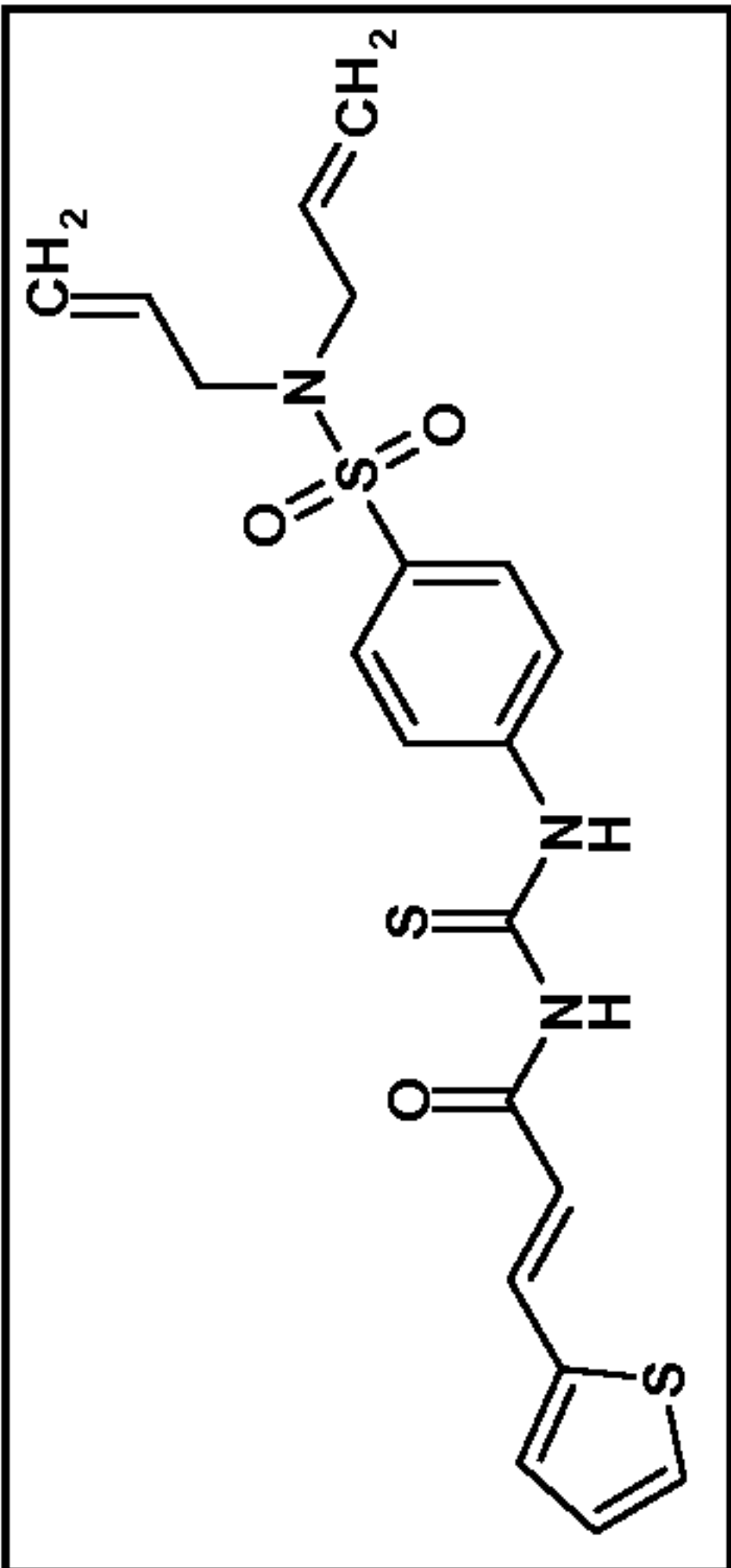
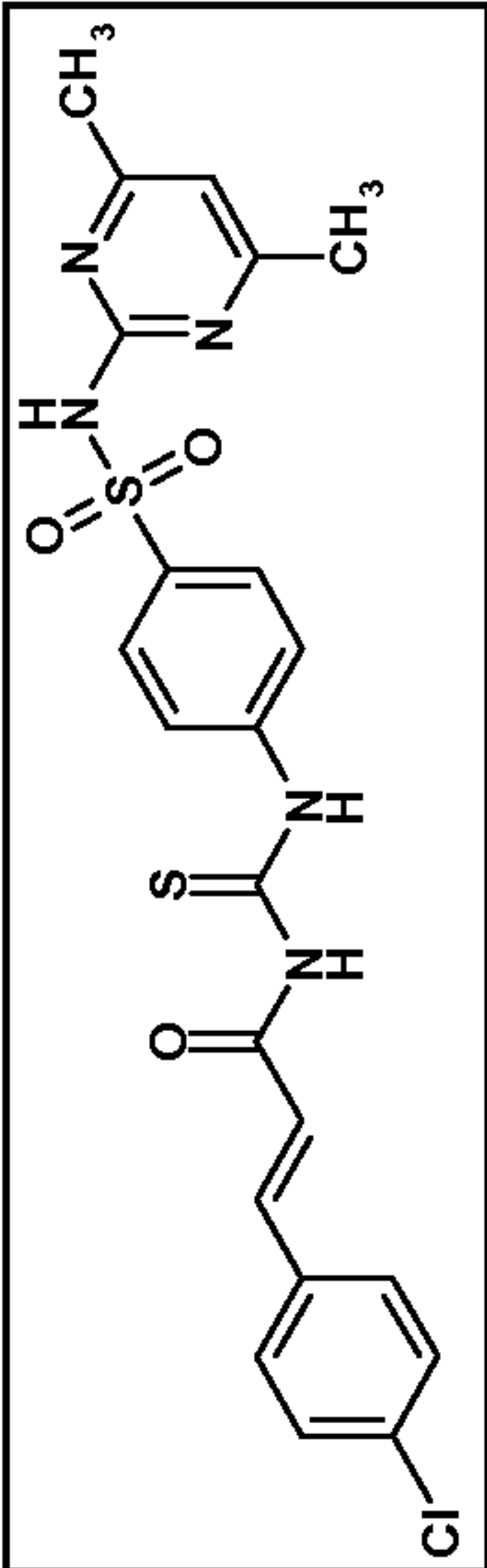
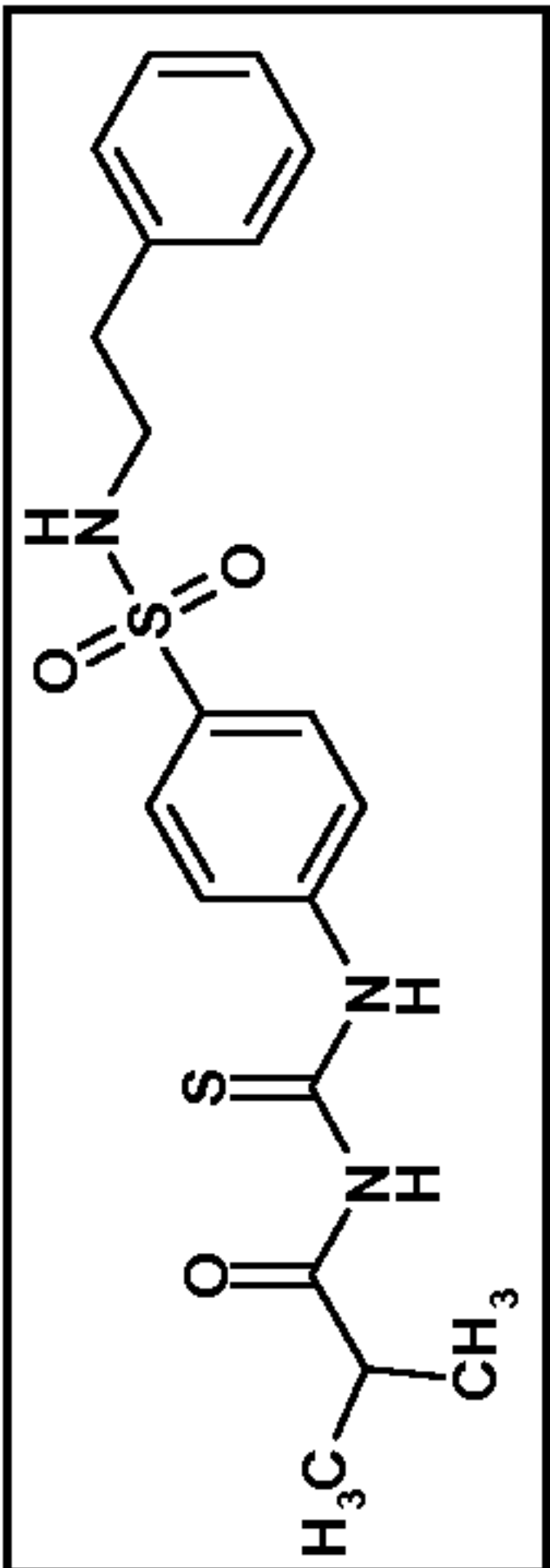
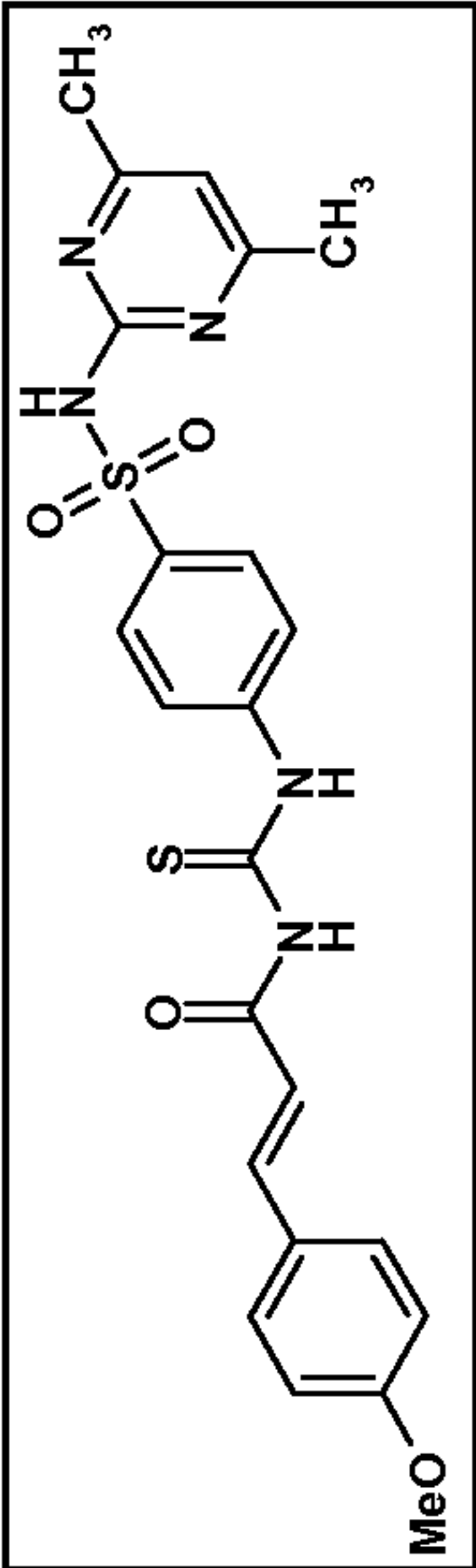
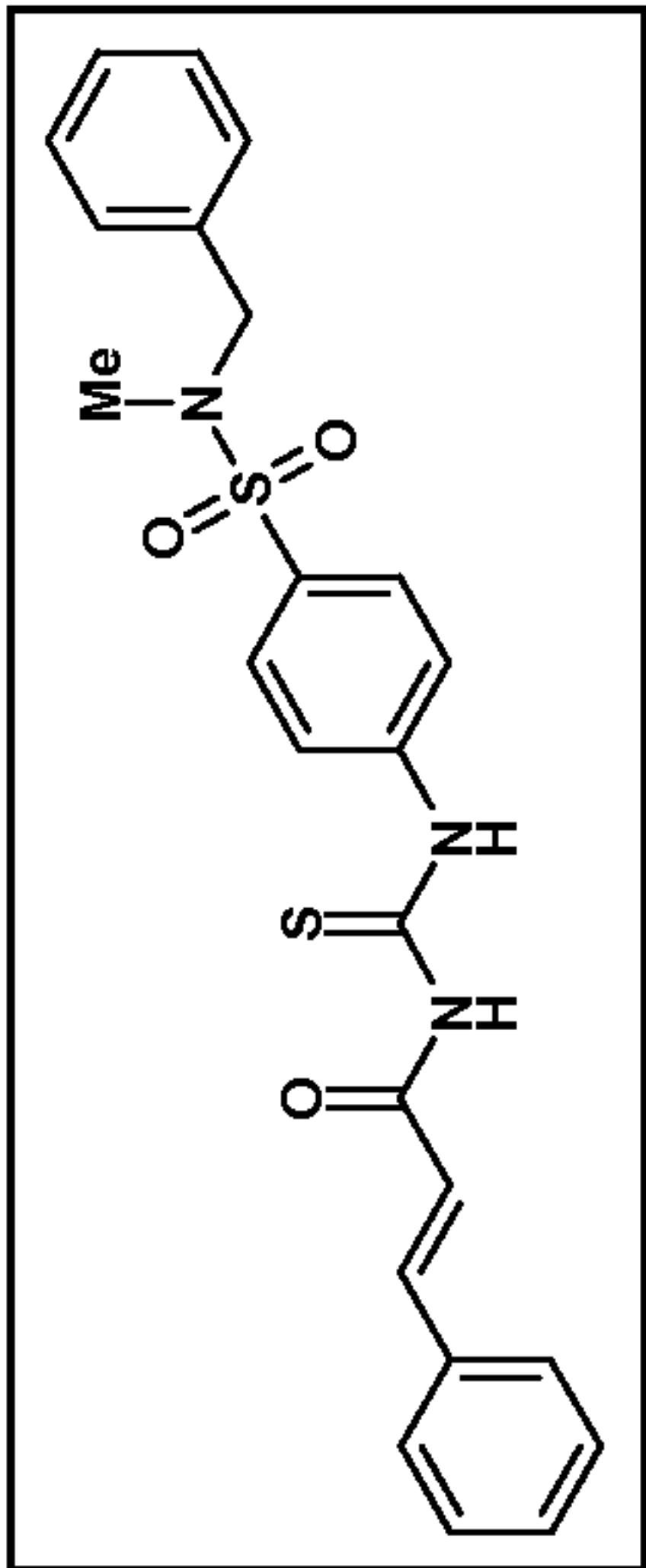
	AD4-10623	M	-6.86	-51.962	46.185	25.78/38.34(1.49)	-4.83	-44.1
	AD4-11871	O	-5.5	-63.42	45.776	49.72/41.84(0.84)	-2.83	-36.58
	AD4-12164	K	-5.78	-50.998	45.64	50.58/non-convergent	-4.3	-45.59
						51.37/549.1(1.07)		
	AD4-11968	K	-7.13	-59.876	45.095	6.49/6.29(0.97)	-3.78	-36.75

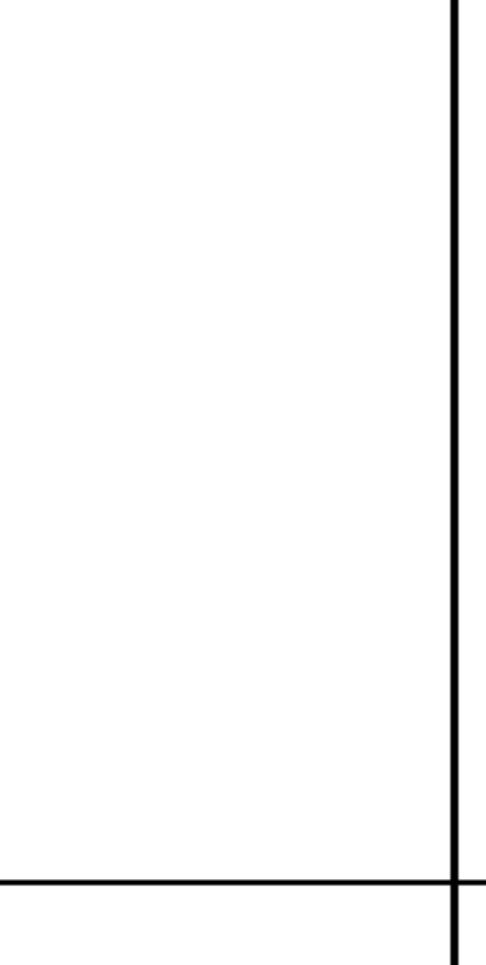
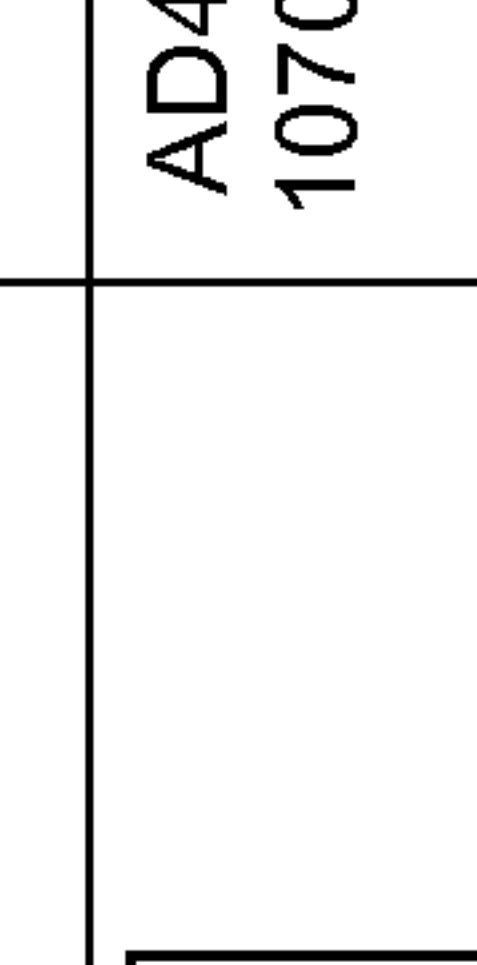
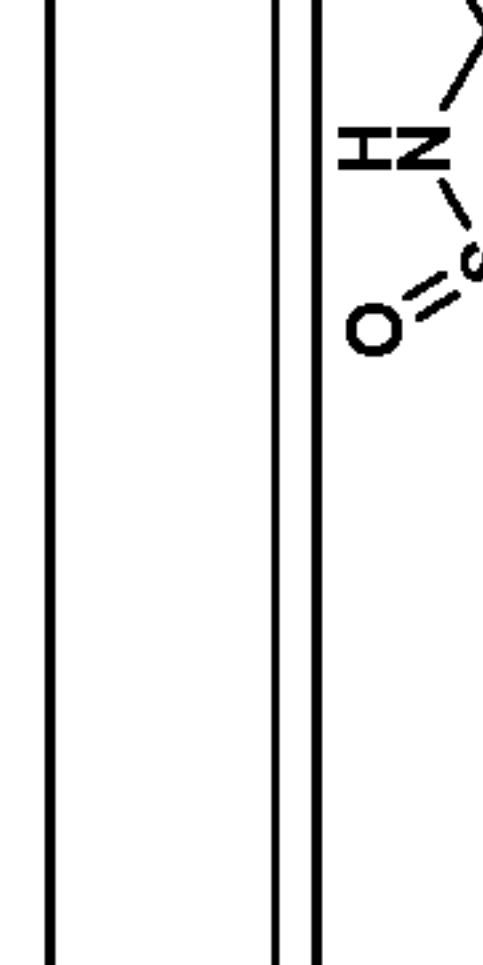
	AD4-11422	K	-5.81	-57.435	44.982	29.46/9.23(0.31)	-3.75	-38.46
	AD4-11971	K	-5.84	-56.864	44.908	41.54/41.10(0.99)	-2.14	-36.33
	AD4-10609	L	-5.54	-63.839	43.828	6.84/6.34(0.93)	-3.09	-40.73
	AD4-11369	O	-5.24	-57.914	43.546	48.64/47.17(0.97)	-3.5	-43.41
	AD4-12041	L	-5.79	-47.552	43.515	6.61/14.29(2.16)	-1.92	-34.81

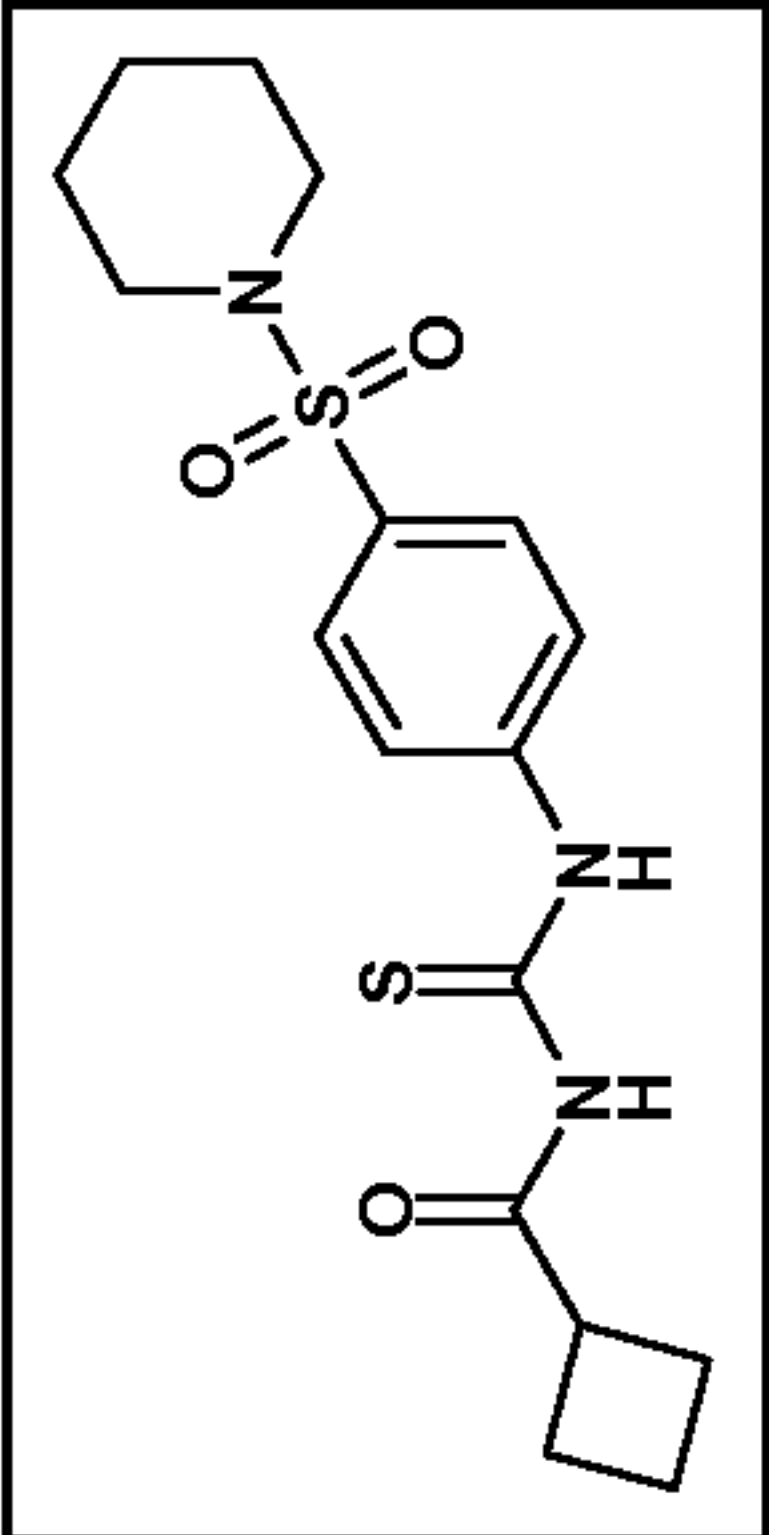
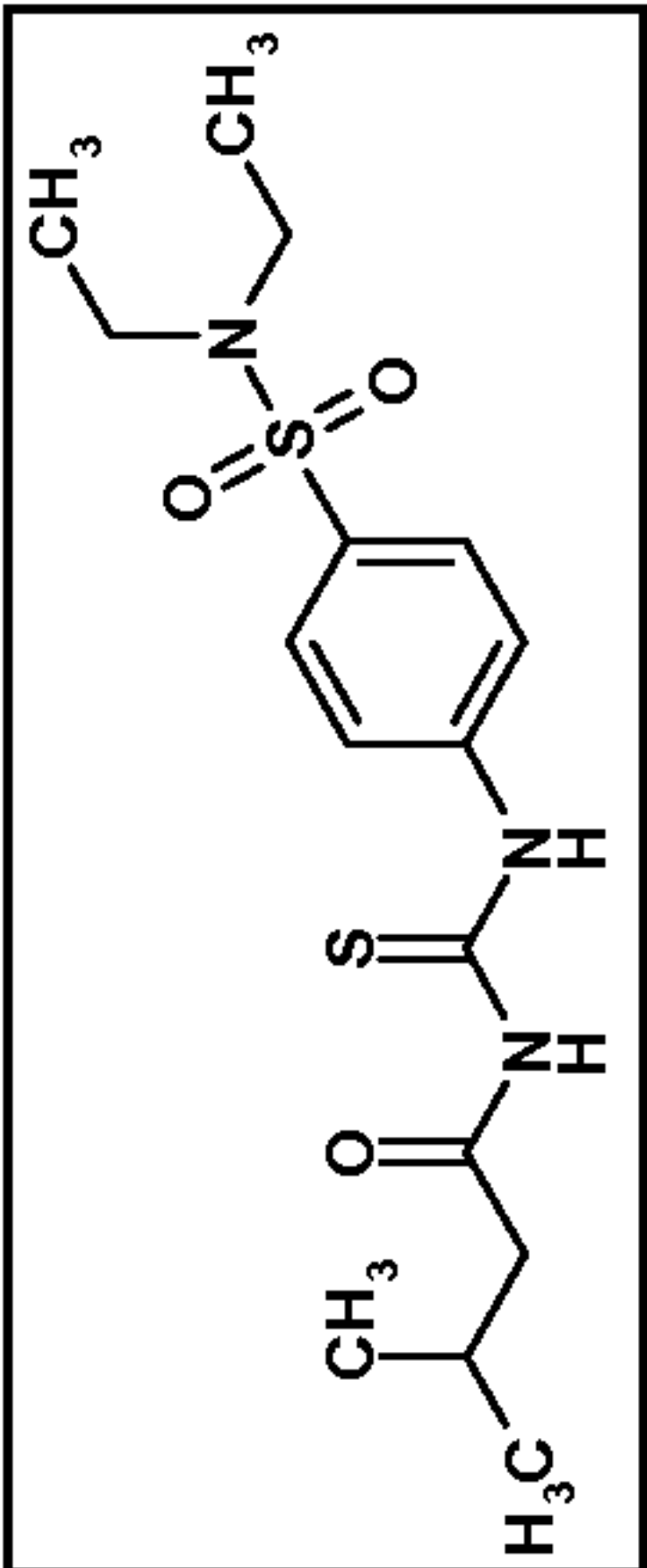
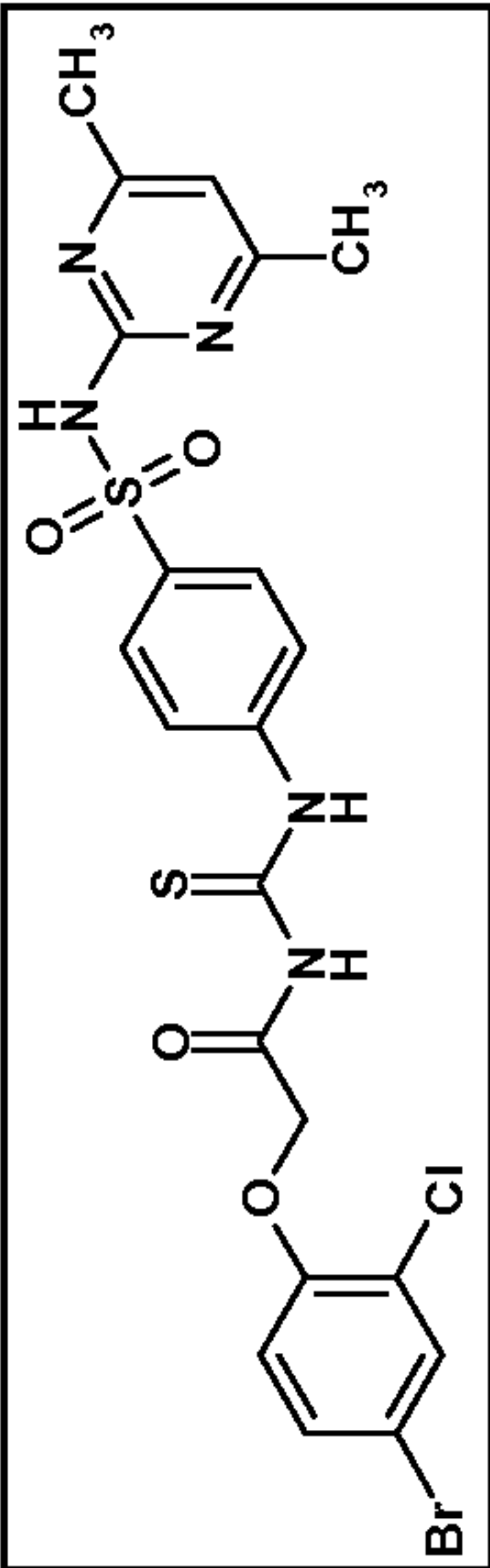
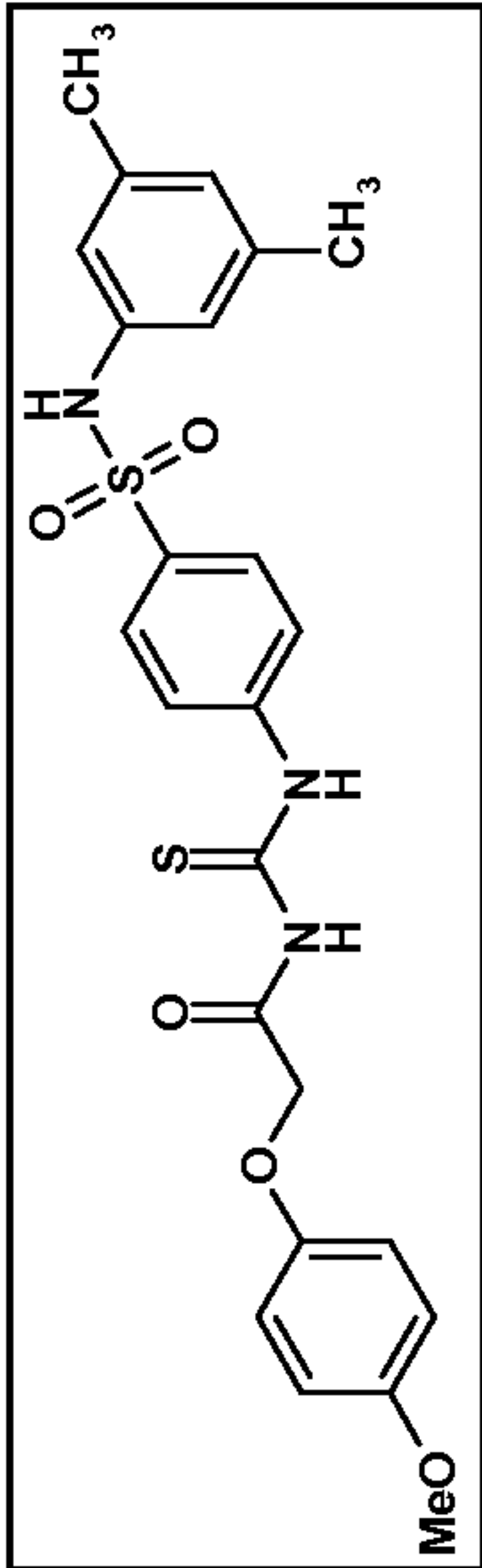
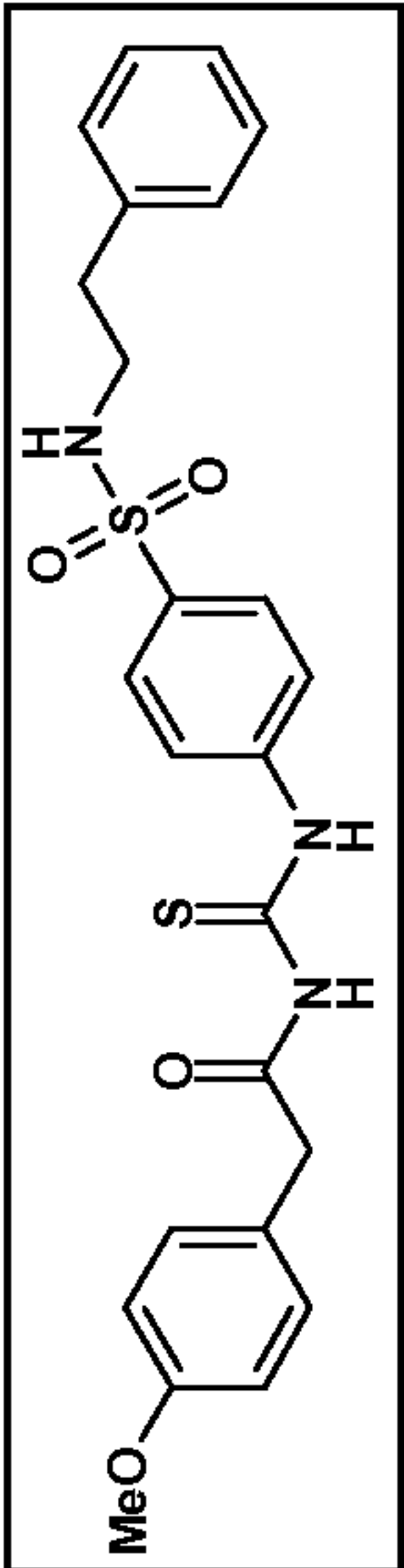
	AD4-11970	N	-5.62	-44.739	43.246	non-convergent	-0.26	-35.75
	AD4-12175	K	-3.1	-60.769	42.36	39.99/9.56(0.24)	-3.36	-29.19
	AD4-12107	N	-6.12	-53.813	41.933	14.08/non-convergent	-3.53	-38.84
	AD4-12280	K	-4.17	-55.921	41.439	10.23/17.68(1.73)	-1.38	-41.29
						5.76/21.24(3.69)		

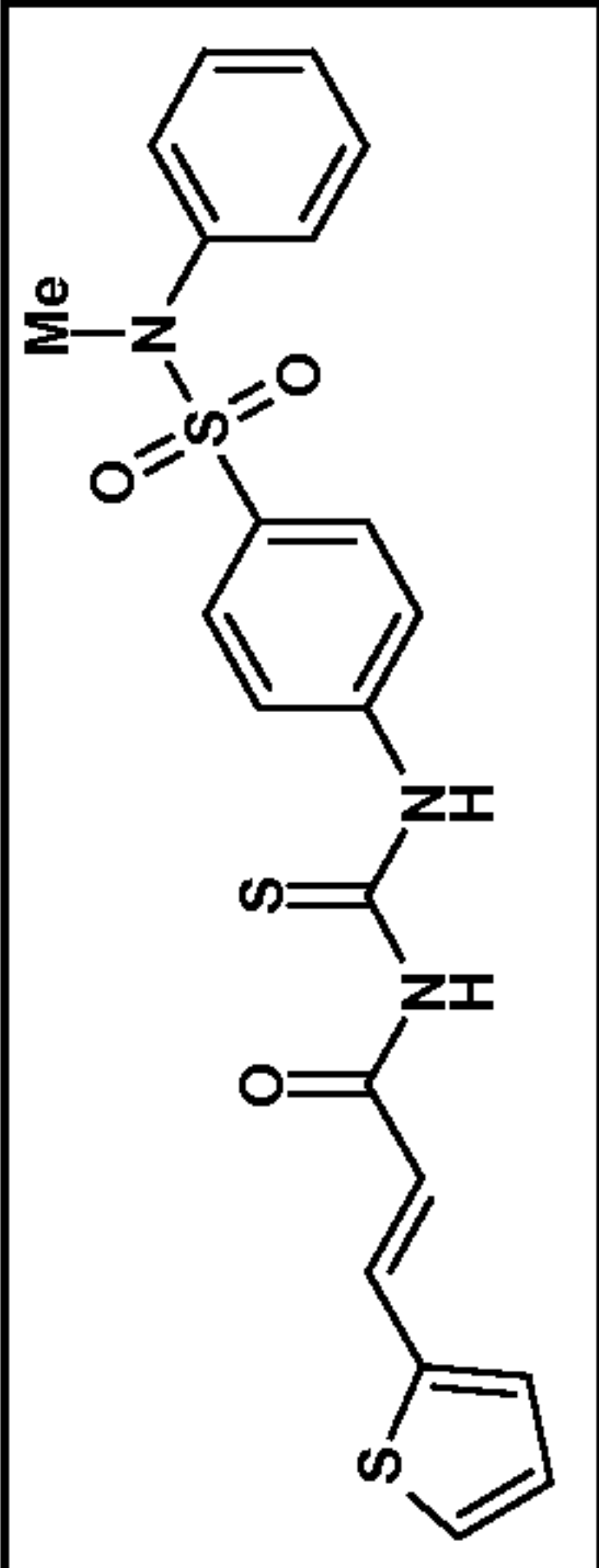
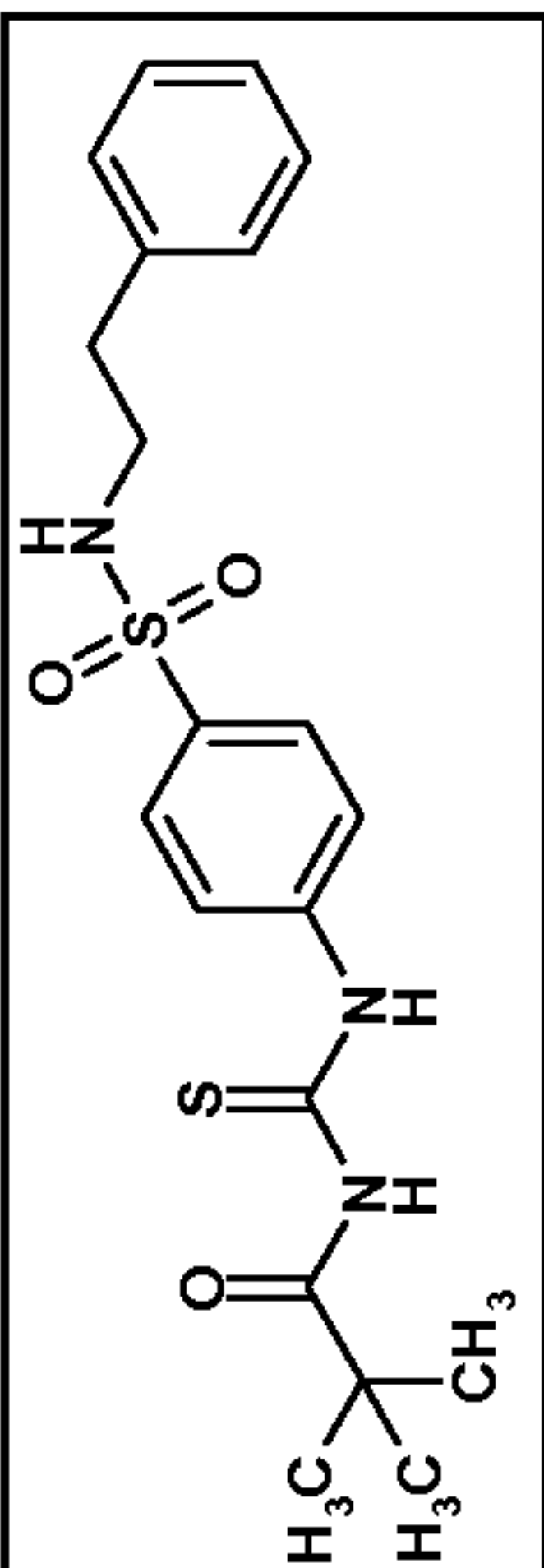
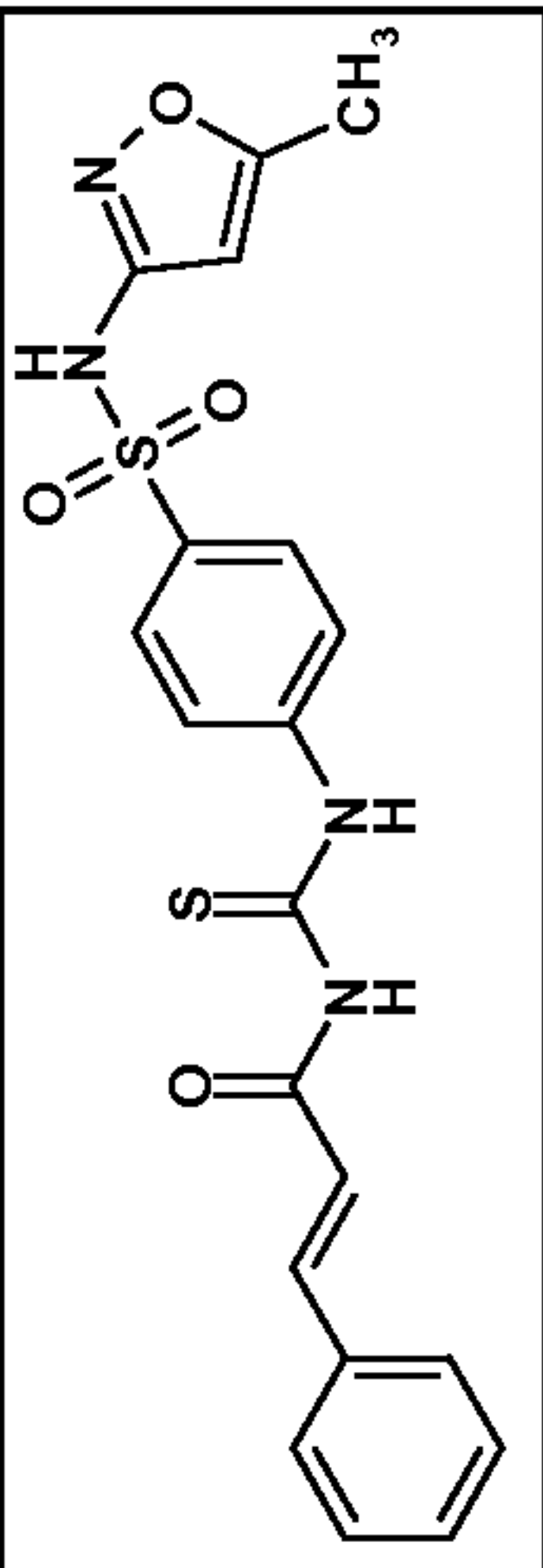
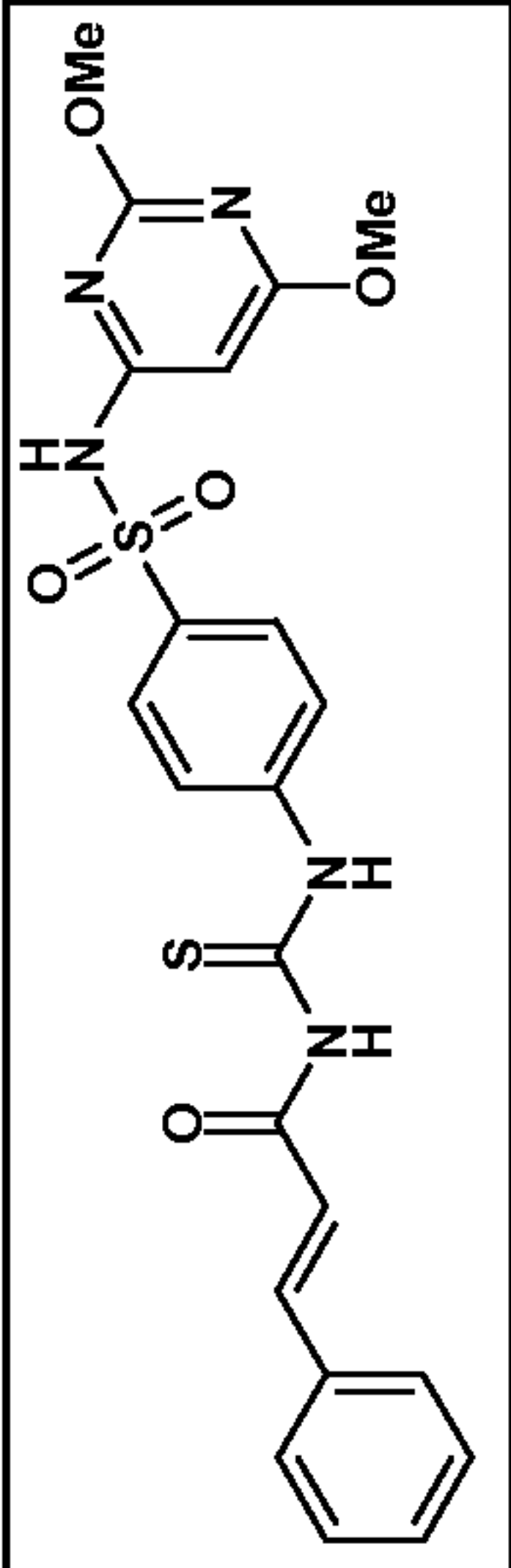
	AD4-12139	K	-4.11	-63.237	41.23	18.56/5.30(0.29)	-5.93	-41.9
	AD4-12104	N	-5.48	-48.445	41.204	38.98/37.55(1.30)	-4.1	-36.8
	AD4-11642	O	-6.28	-65.521	40.922	9.89/9.46(0.96)	-3.16	-44.18
	AD4-12146	N	-2.65	-54.642	40.916	26.57/25.76(0.97)	-3.96	-31.29
	AD4-11417	K	-5.19	-59.747	40.711	25.47/49.29(1.94)	-4.49	-46.82

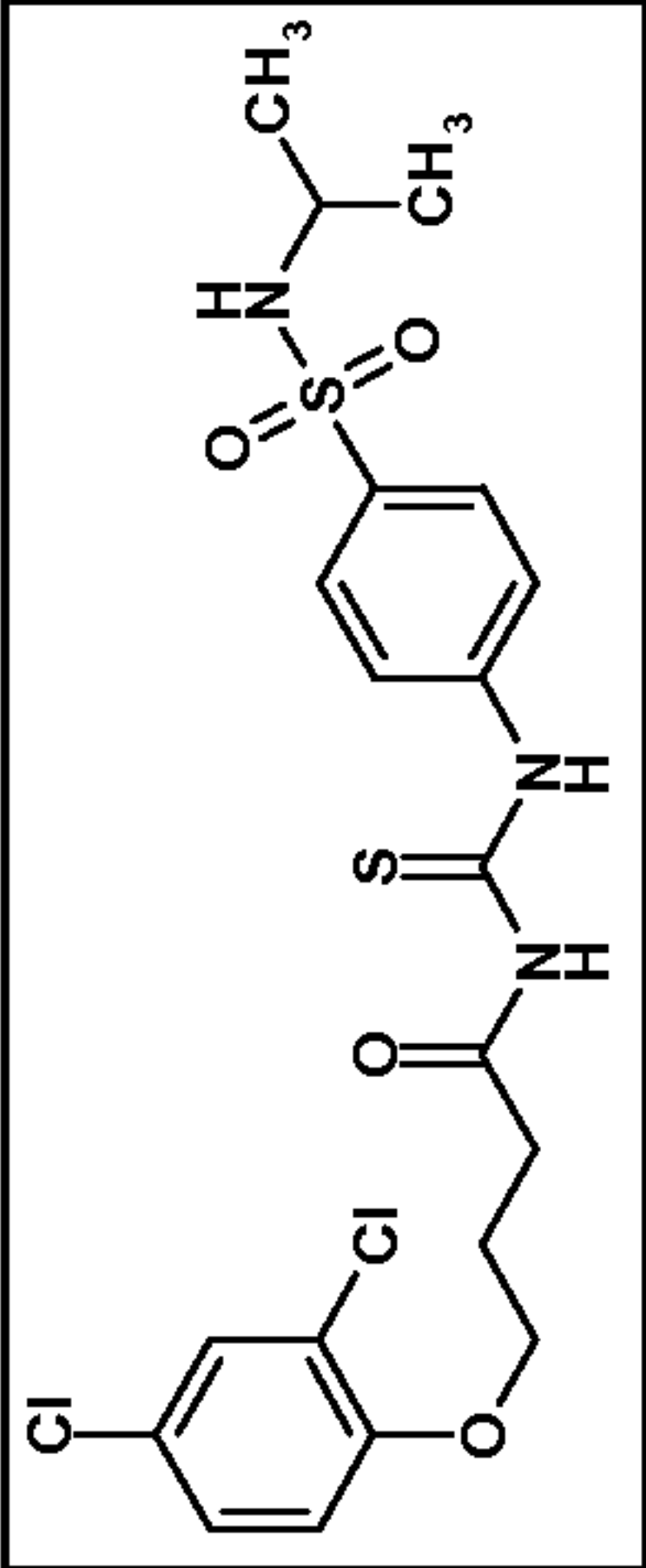
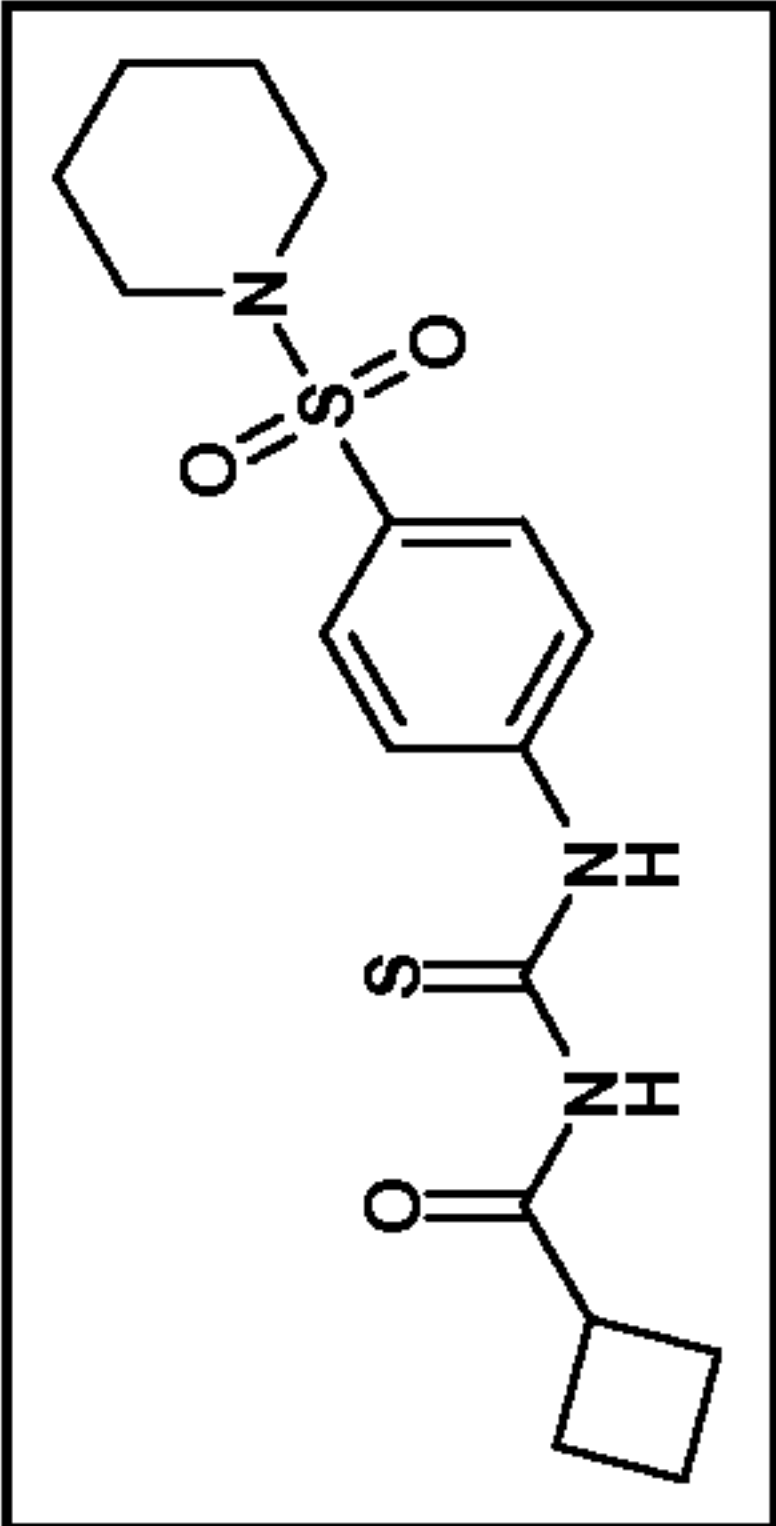
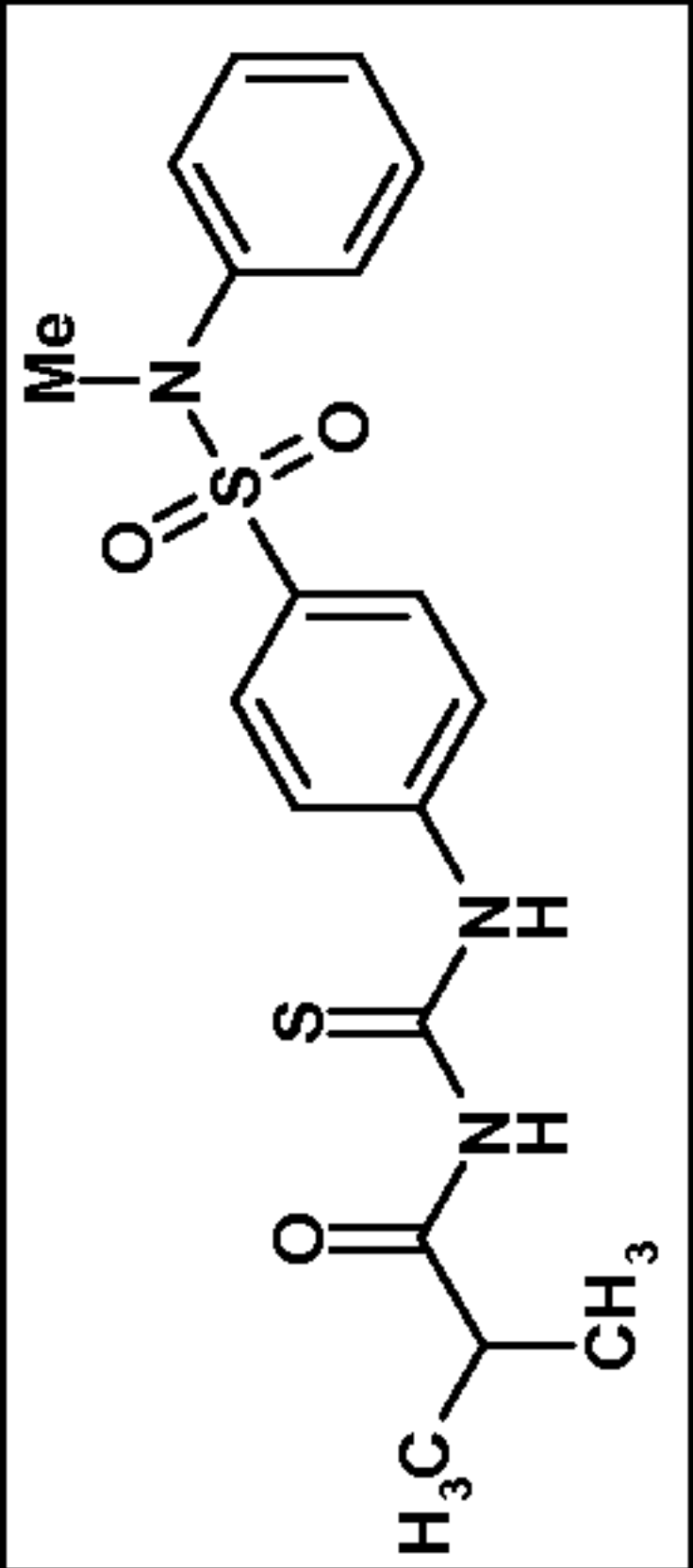
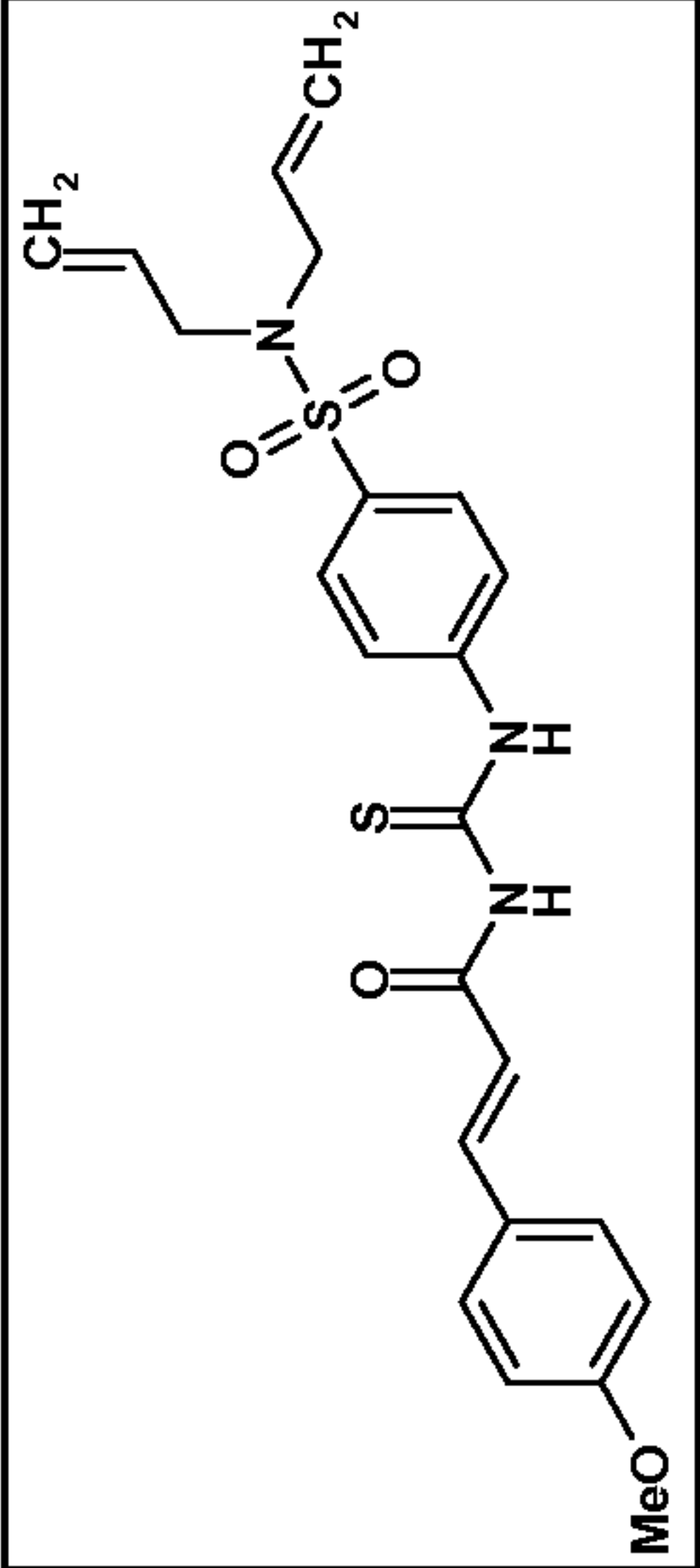
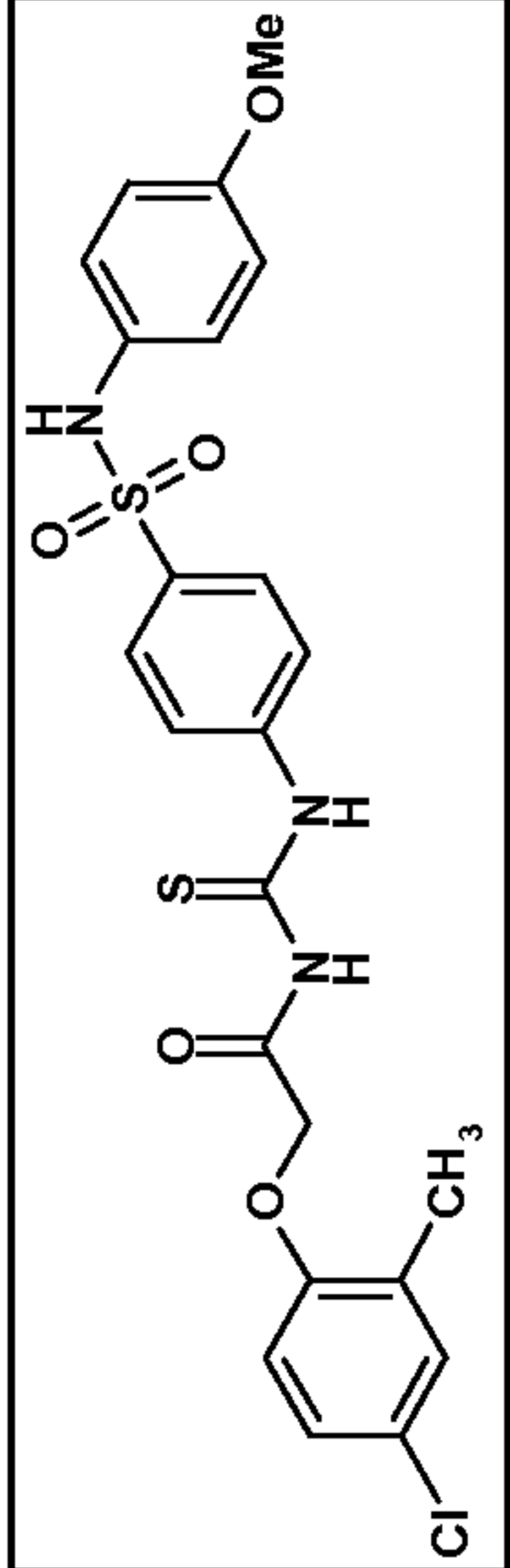
[illegible]

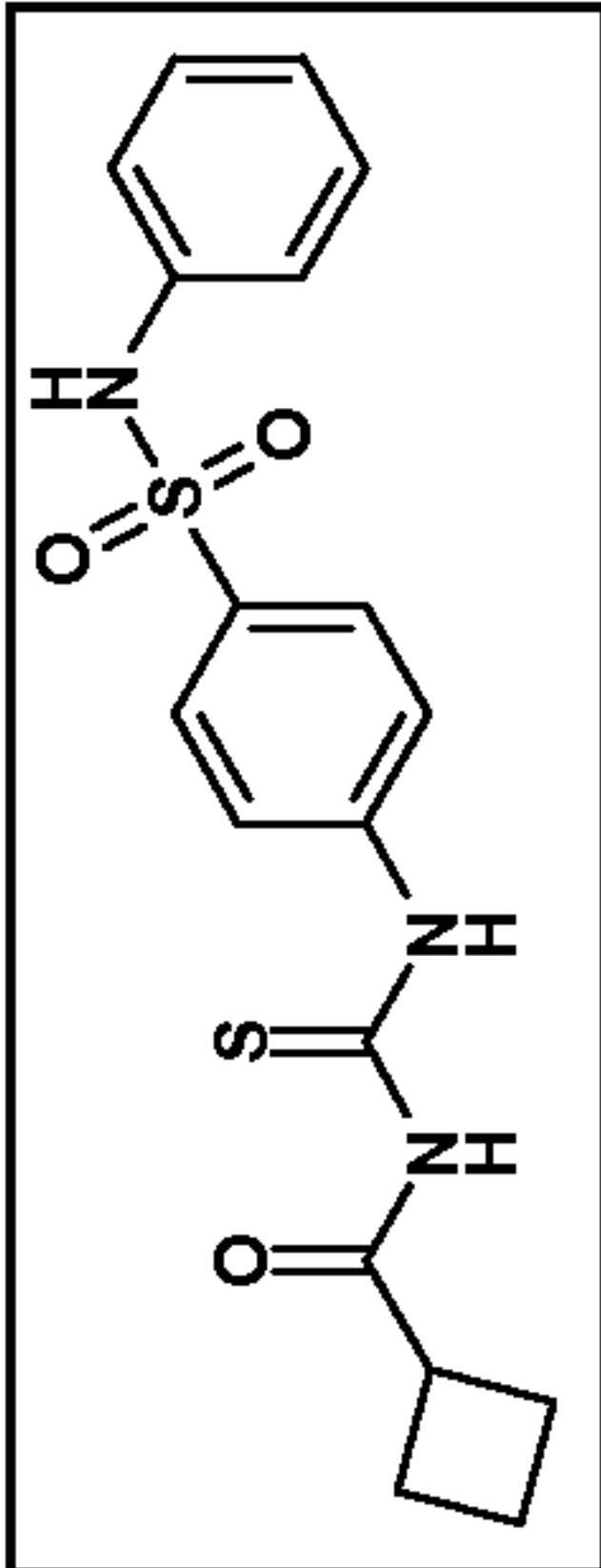
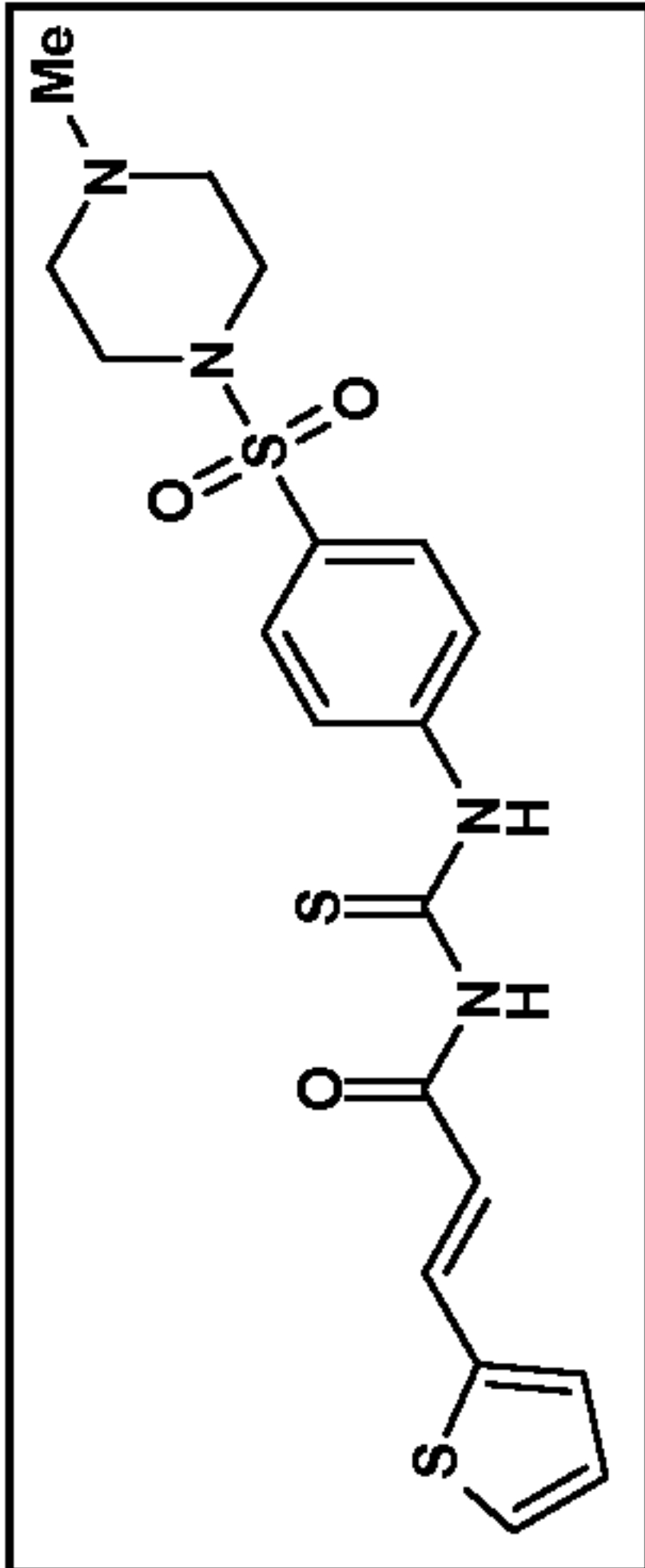
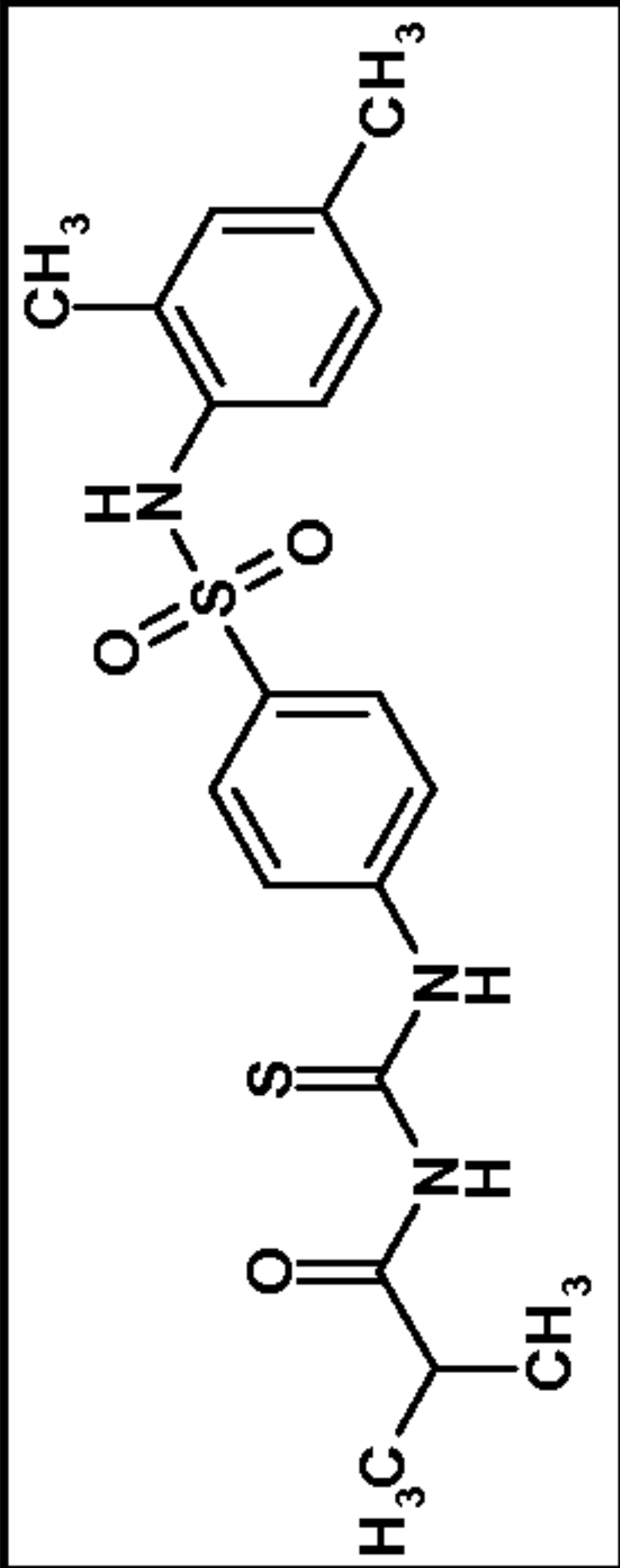
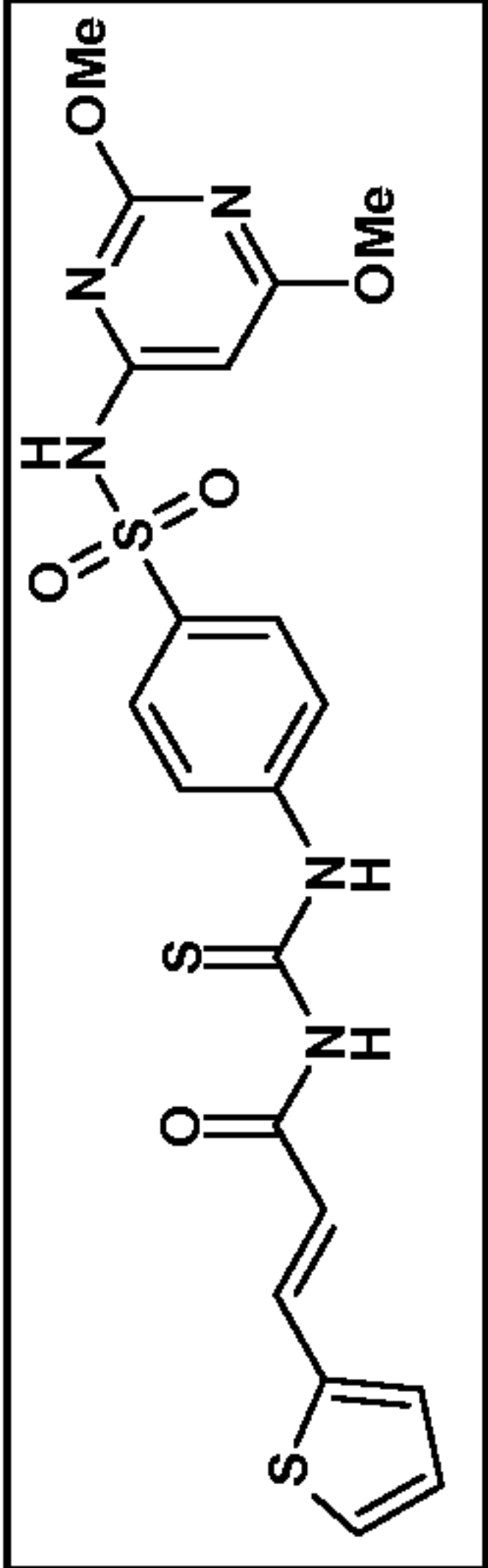
	AD4-11979	L	-4.83	-50.876	39.775	9.31/16.16(1.74)	-2.24	-30.07
	AD4-11648	O	-5.95	-54.648	38.362	46.74/45.07(0.96)	-3.43	-42.24
	AD4-10611	L	-6.72	-63.131	38.285	30.94/20.04(0.65)	-2.77	-42.62
	AD4-11646	O	-5.63	-58.436	37.949	19.43/21.11(1.09)	-3.11	-43.16
	AD4-12126	L	-5.72	-62.255	37.795	14.90/41.85(2.81)	-2.57	-35.52

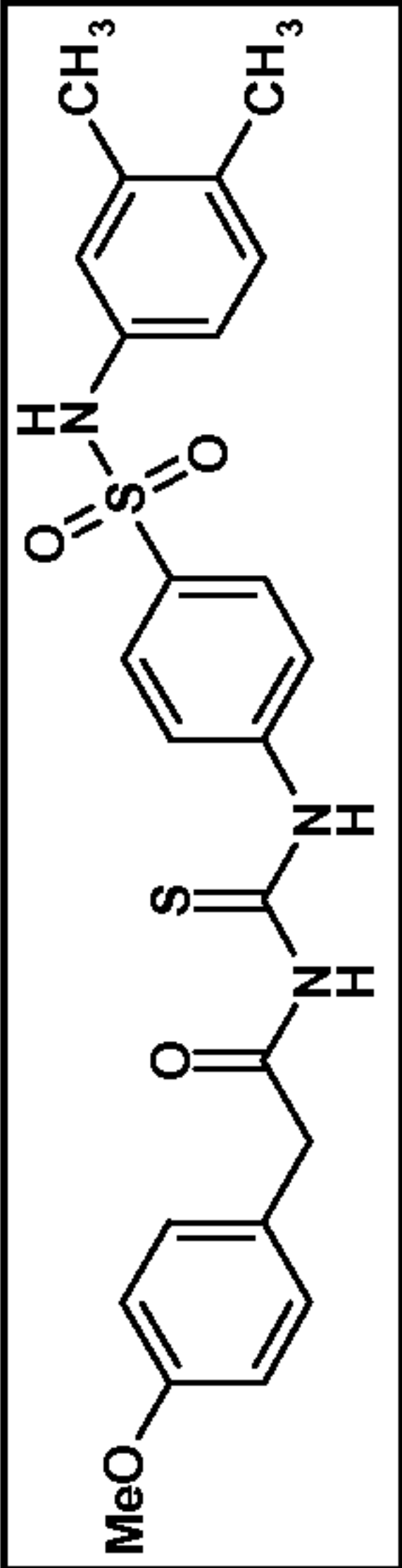
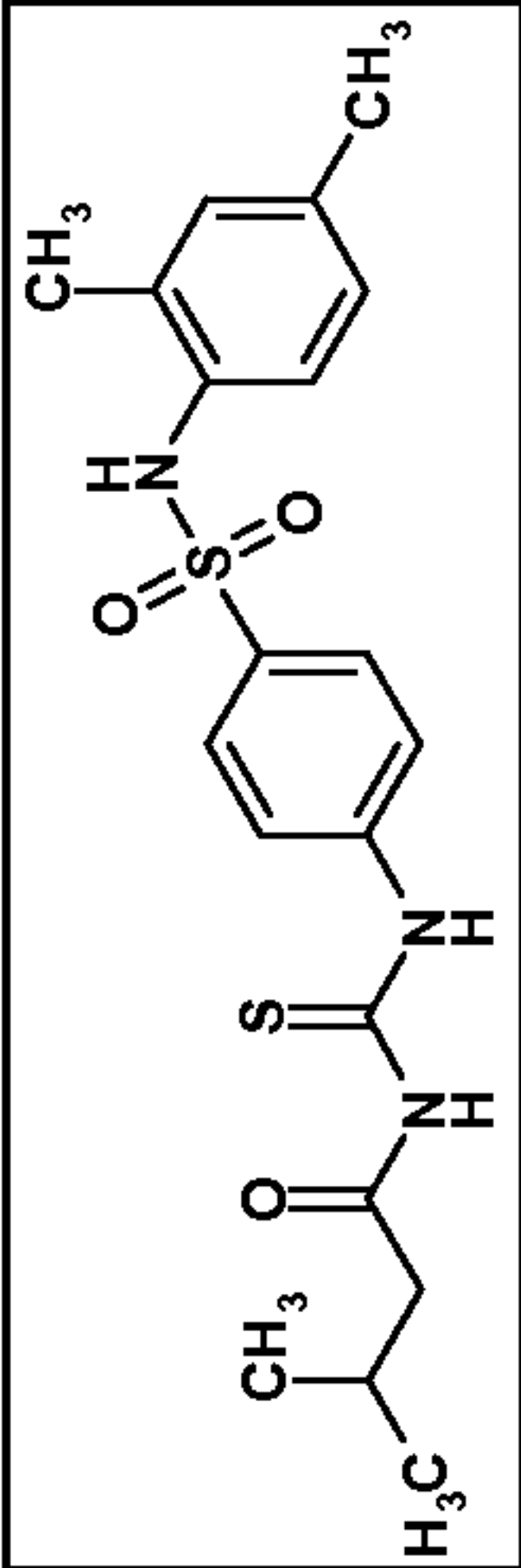
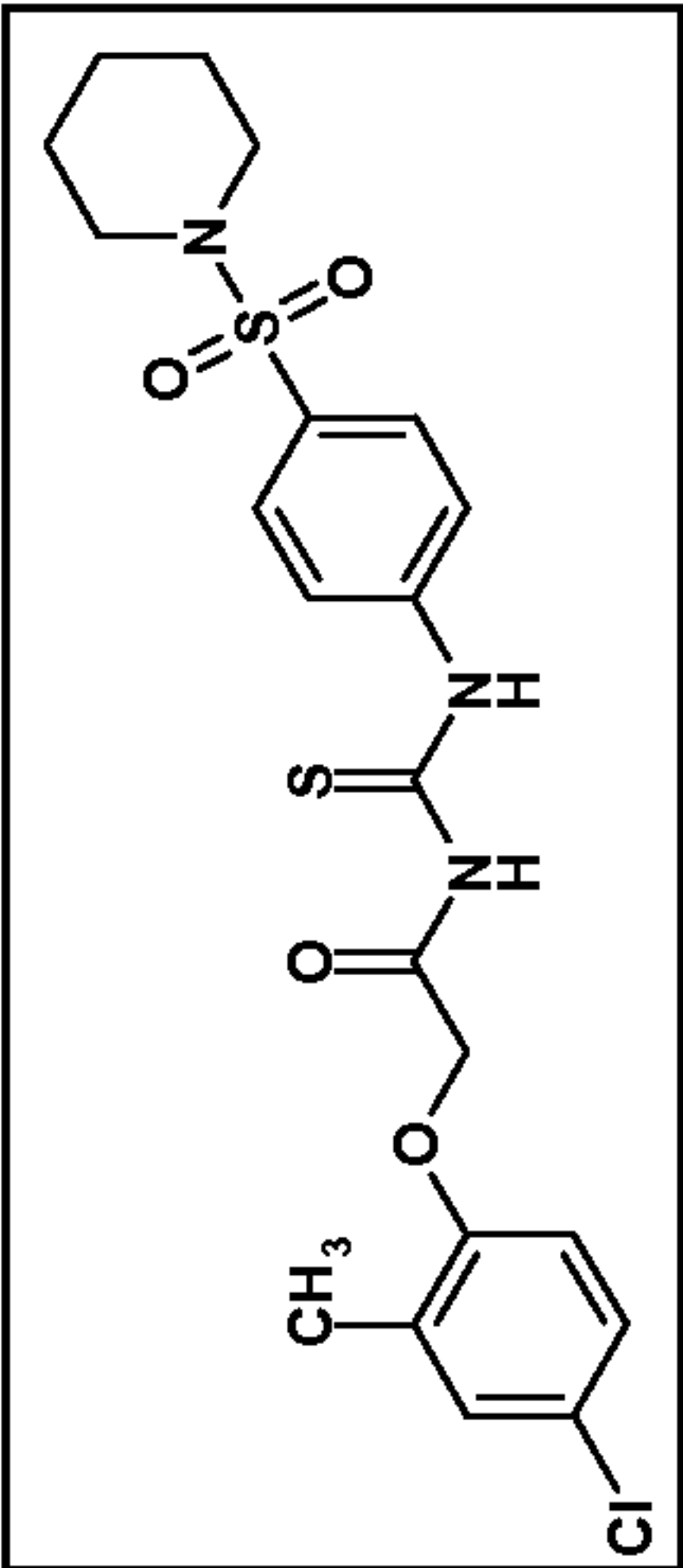
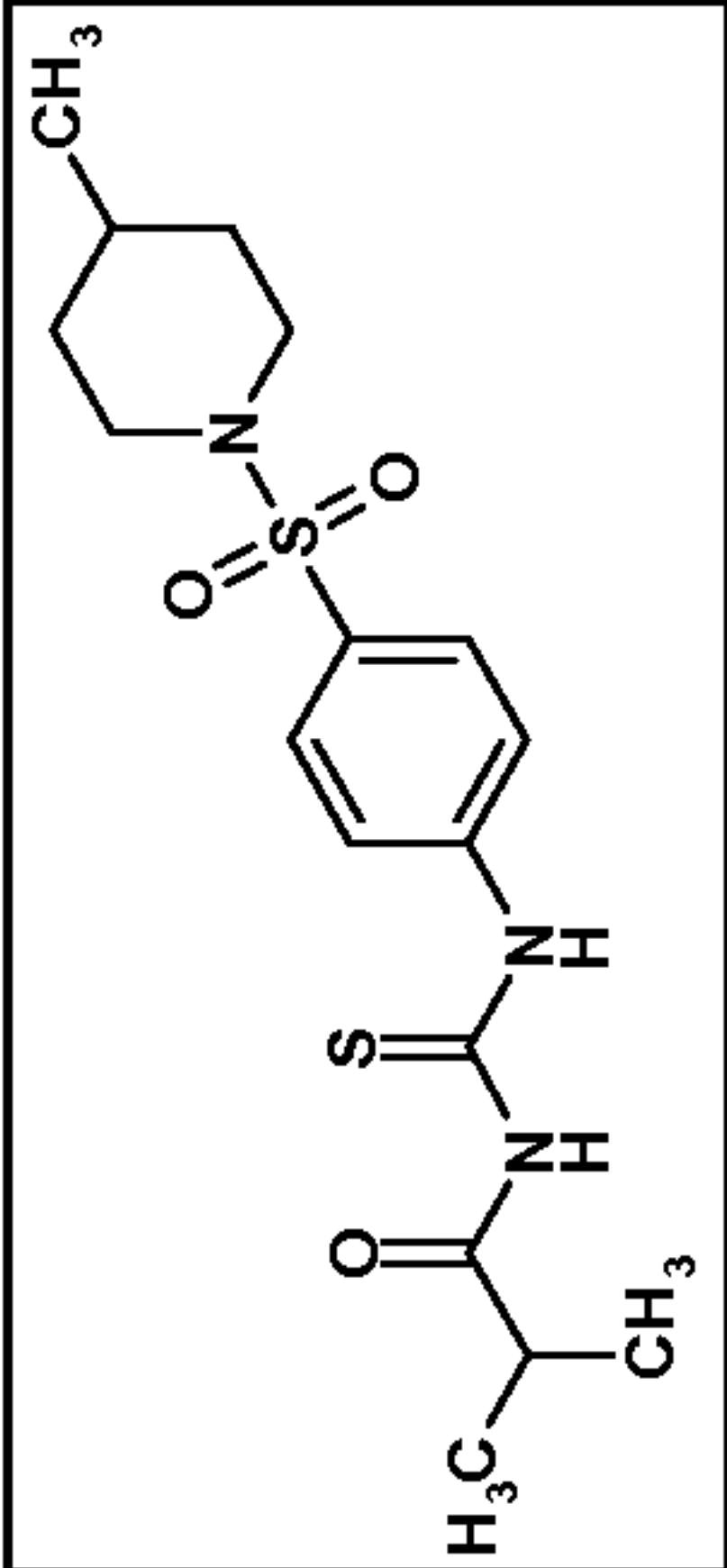
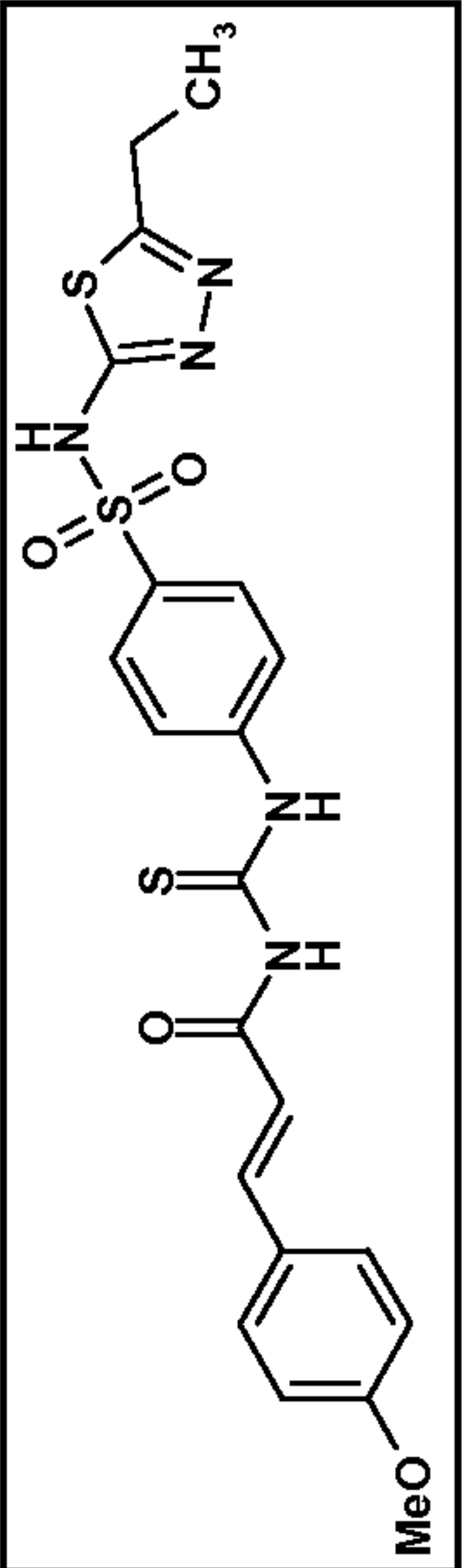
	AD4- 12183	K	-3.58	-64.491	37.695	26.56/17.25(0.46)	-2.96		-34.21	
	AD4- 11880	O	-4.5	-62.253	37.604	47.28/47.33(1.00)	-2.65		-45.91	
	AD4- 12171	L	-5.28	-49.354	37.375	38.92/37.13(0.95)	-4.18		-43.51	
	AD4- 10708	K	-5.35	-35.677	37.233	16.64/35.92(2.16)	-4		-36.76	

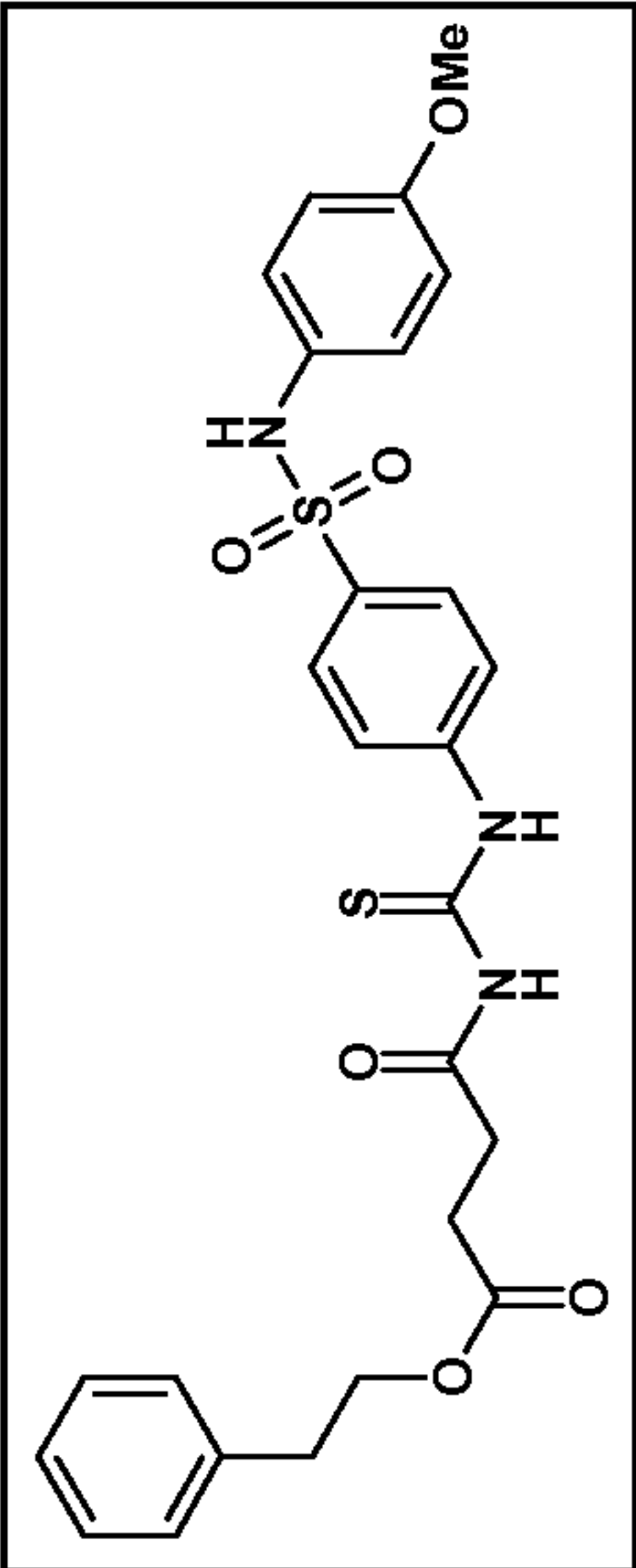
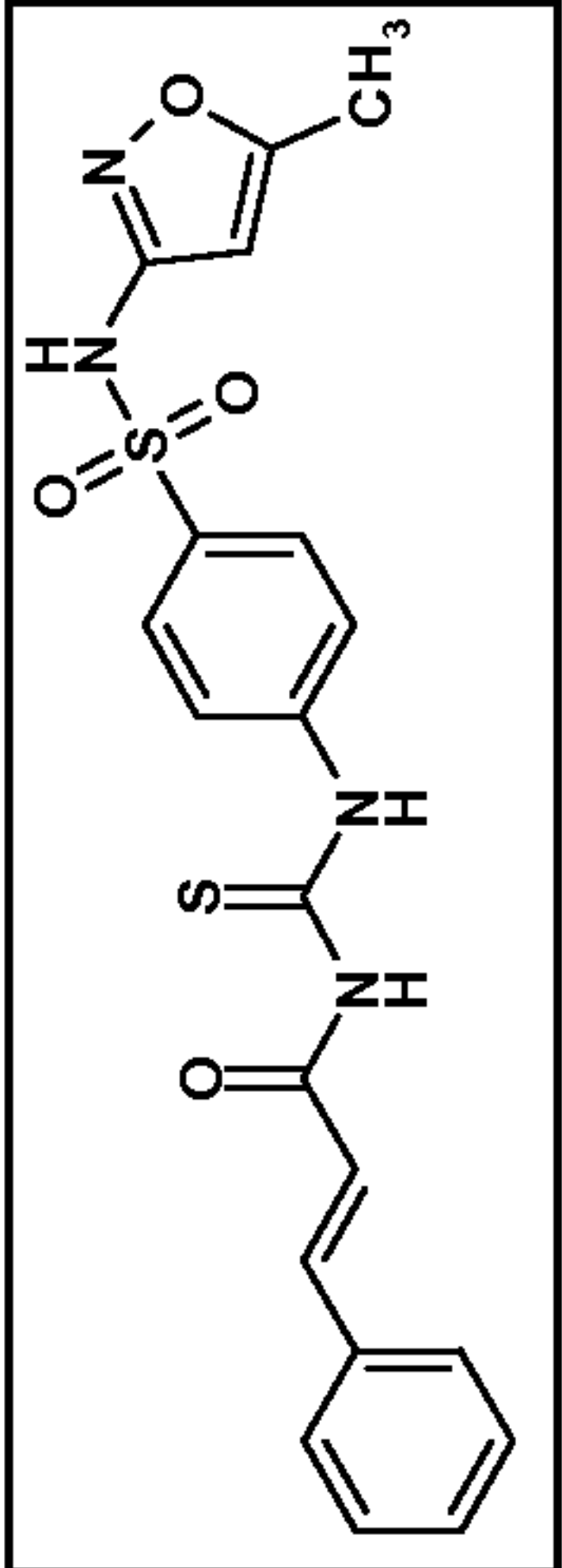
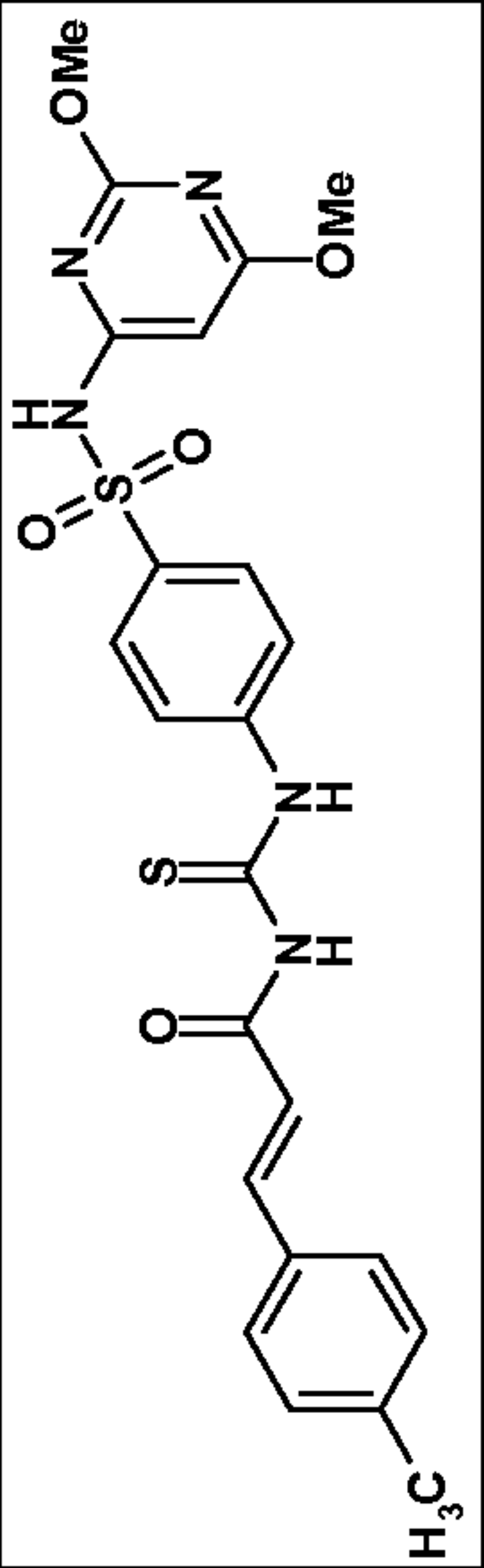
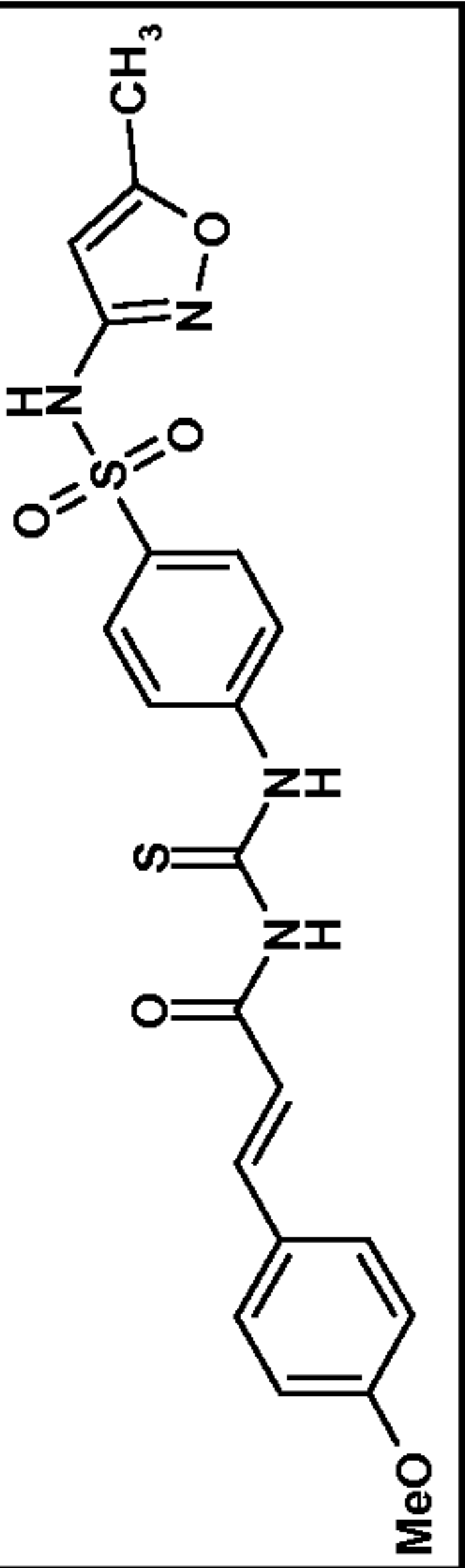
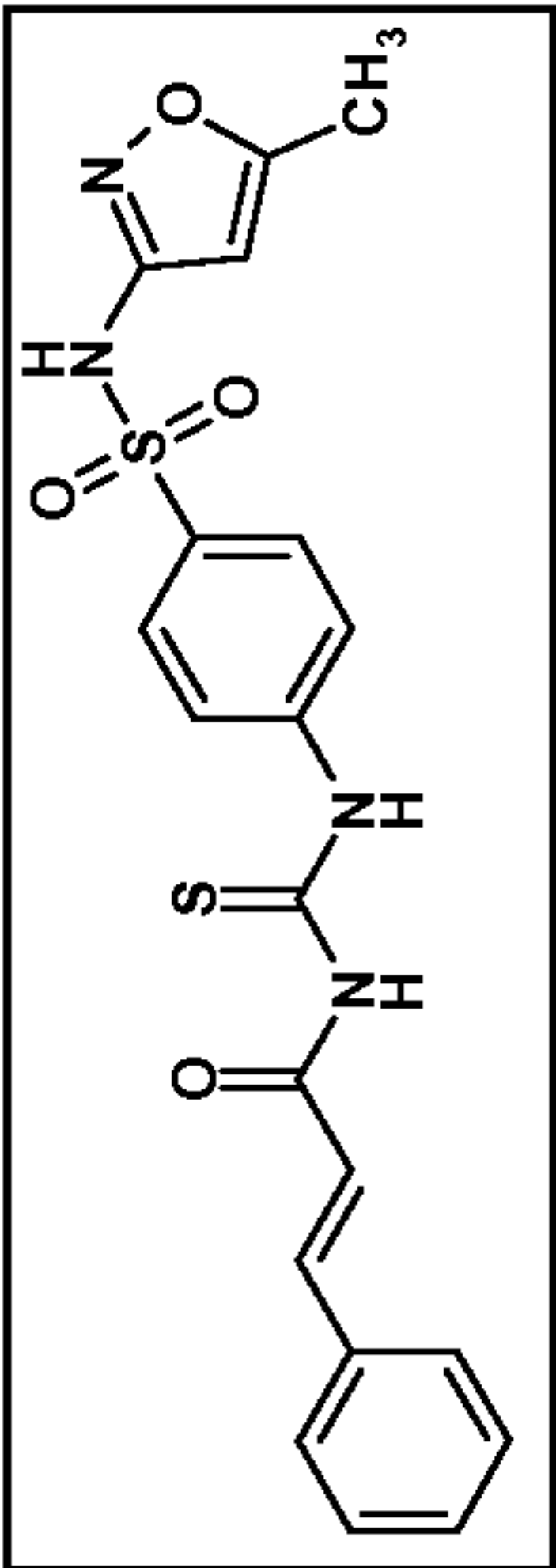
	AD4-11421	N	-5.75	-53.081	37.181	41.46/43.51(1.05)	-3.85	-34.1
	AD4-12049	L	-5.44	-48.47	36.966	32.27/11.96(0.37)	-2.43	-24.87
	AD4-12158	J	-6.58	-71.788	36.727	5.01/4.58(0.91)	-4.16	-49.79
	AD4-12165	K	-6.81	-66.767	36.632	63.62/12.03(0.19)	-3.94	-47.61
	AD4-11415	L	-6.25	-70.703	35.841	15.74/10.52(0.67)	-4.75	-47.34

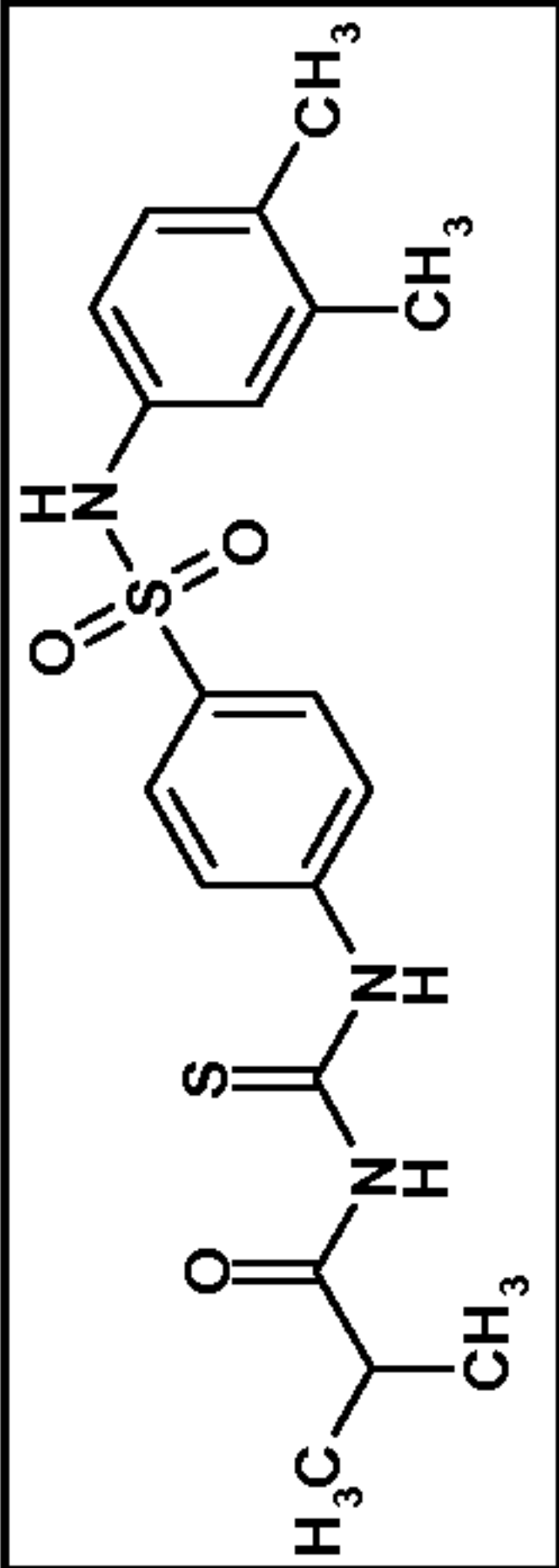
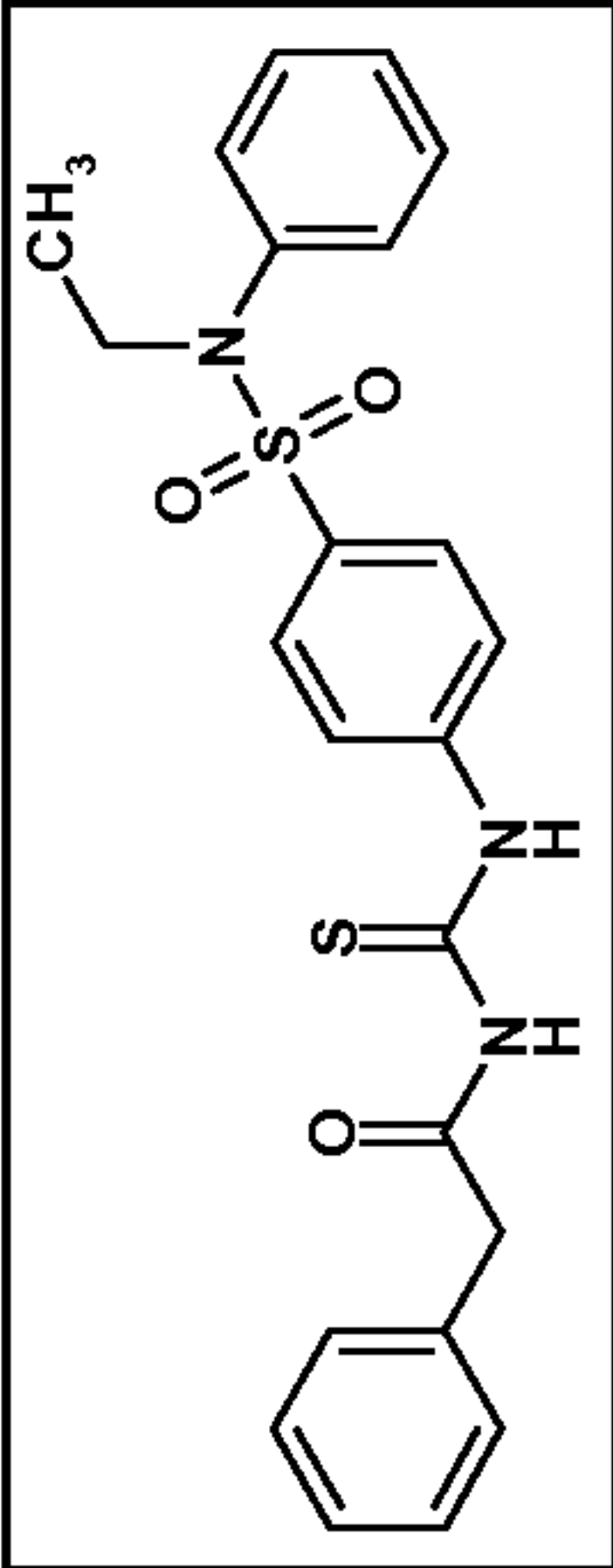
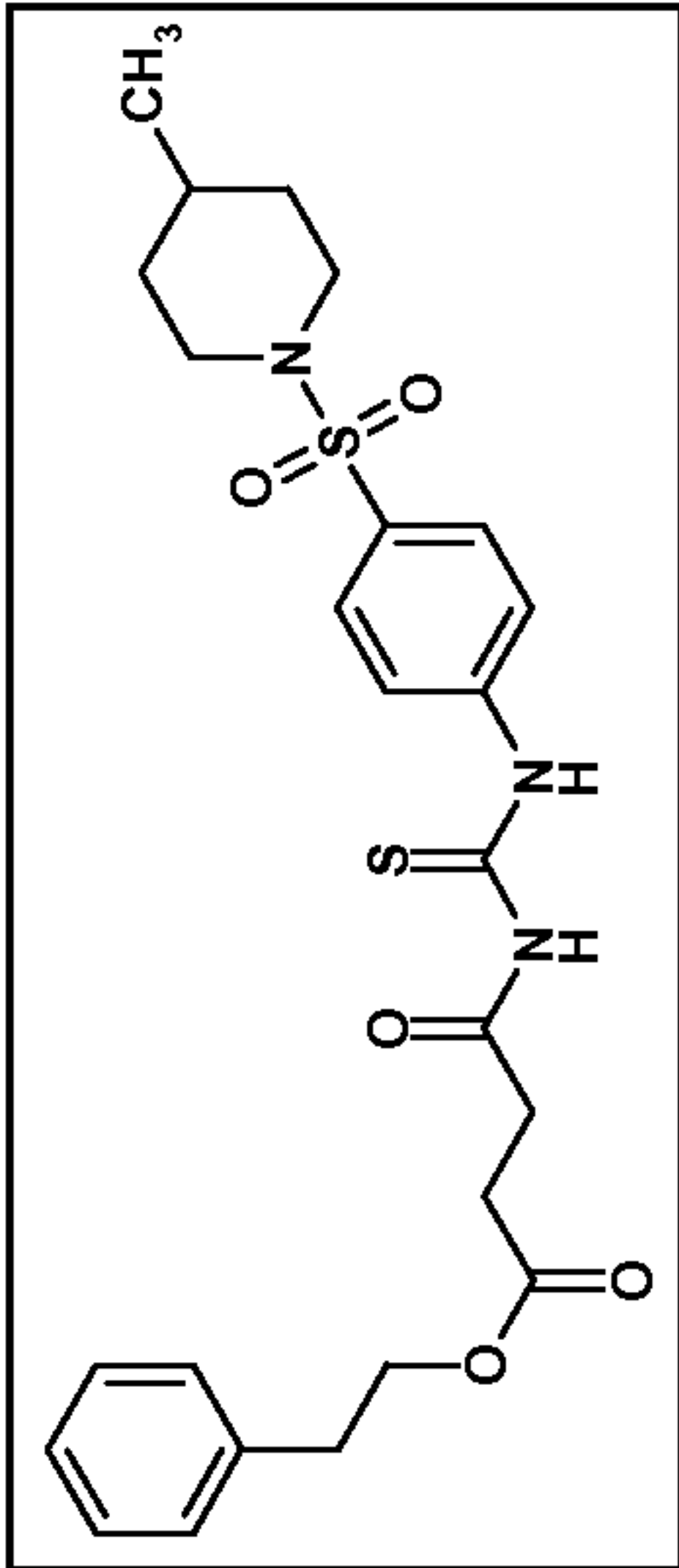
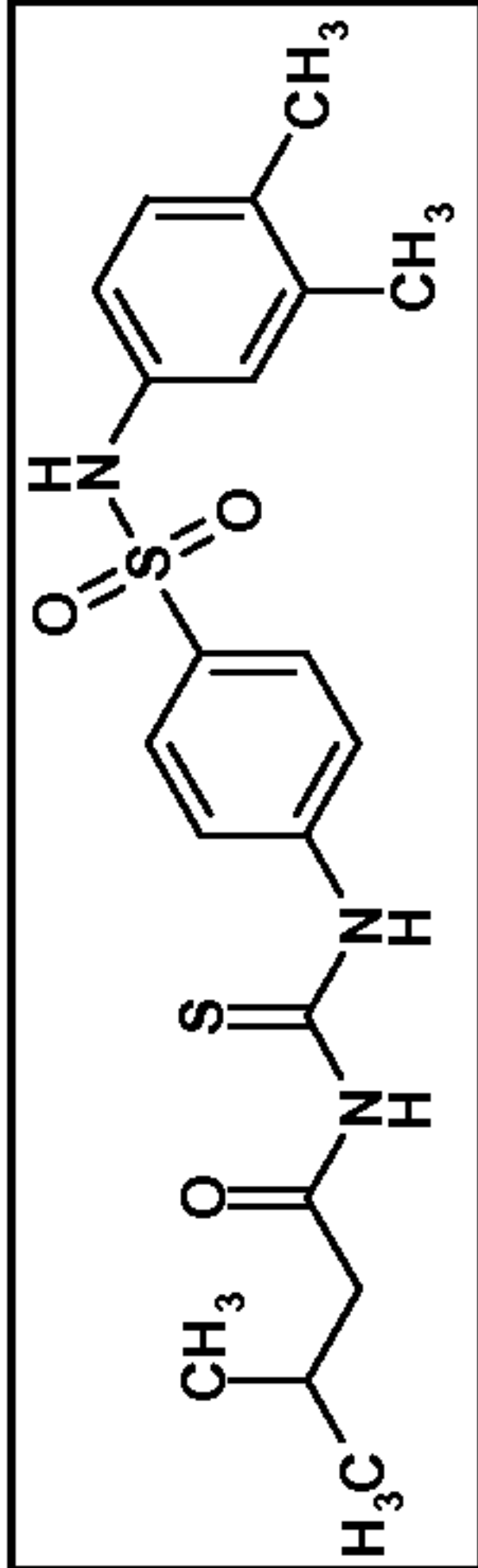
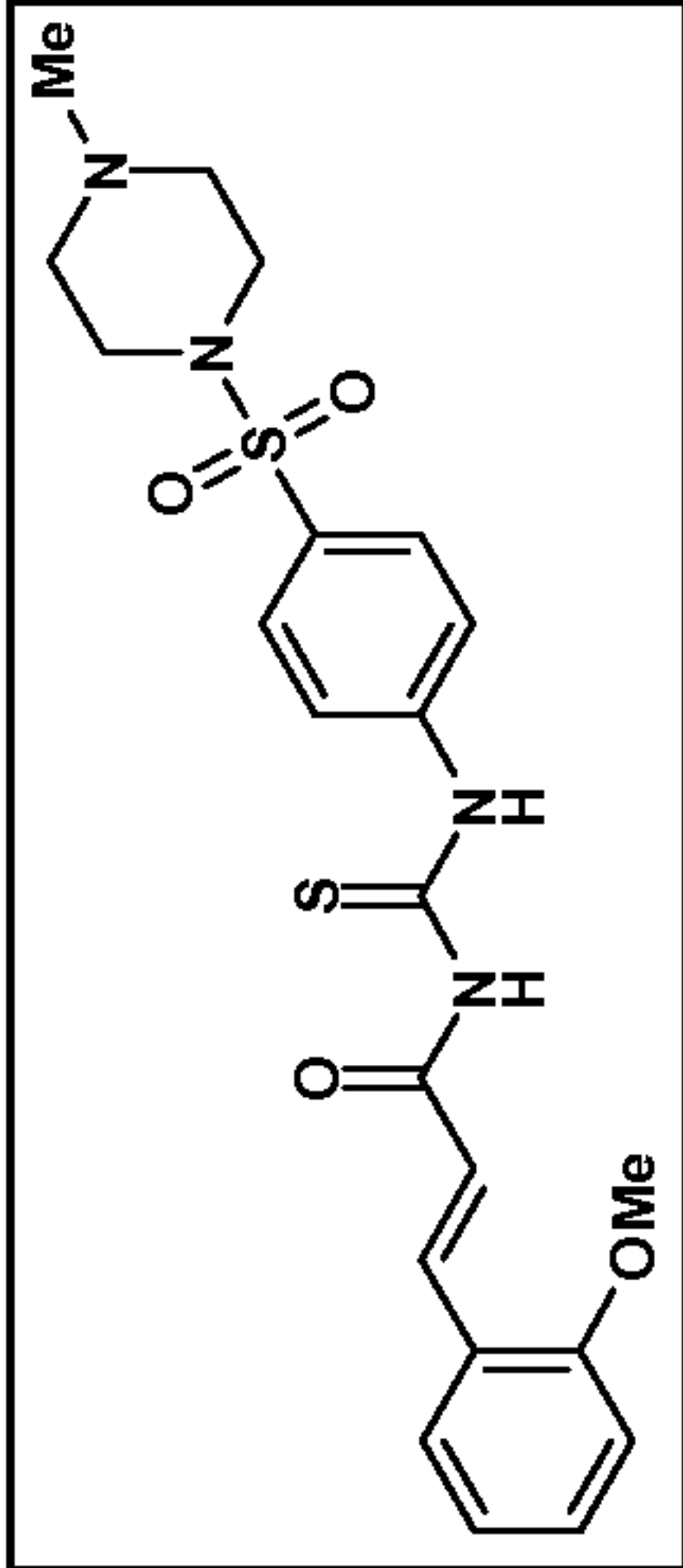
	AD4-12288	K	-5.92	-54.007	35.584	non-convergent	-4.78	-40.58
	AD4-10613	L	-5.8	-59.786	35.514	13.92/11.84(0.85)	-3.36	-37.53
	AD4-12173	O	-4.83	-49.002	35.393	37.26/105.8(2.84)	-2.76	-34.16
						55.76/54.00(0.97)		
	AD4-12122	O	-5.82	-52.564	35.068	non-convergent/40.29	-2.89	-44.52

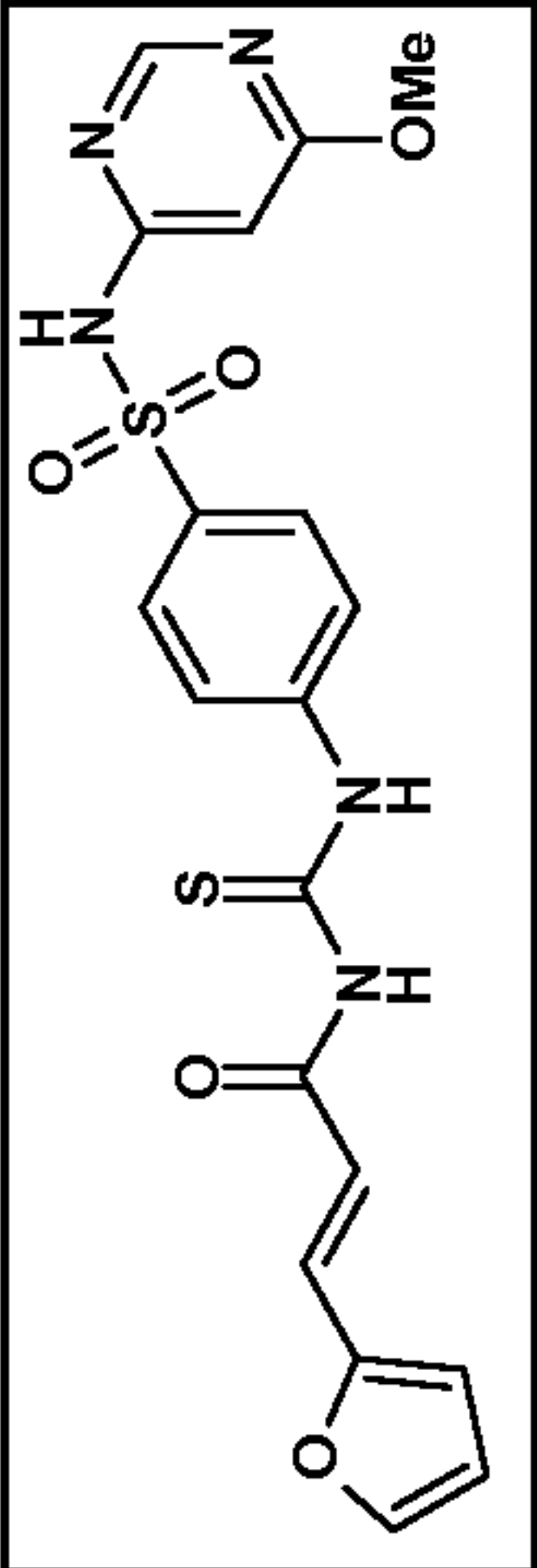
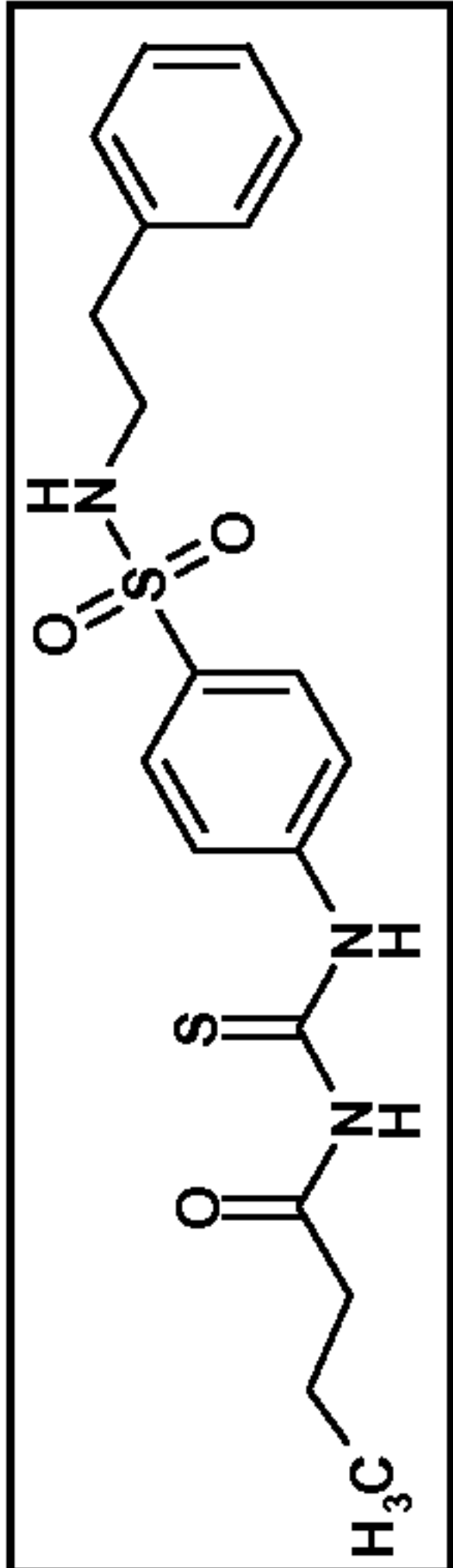
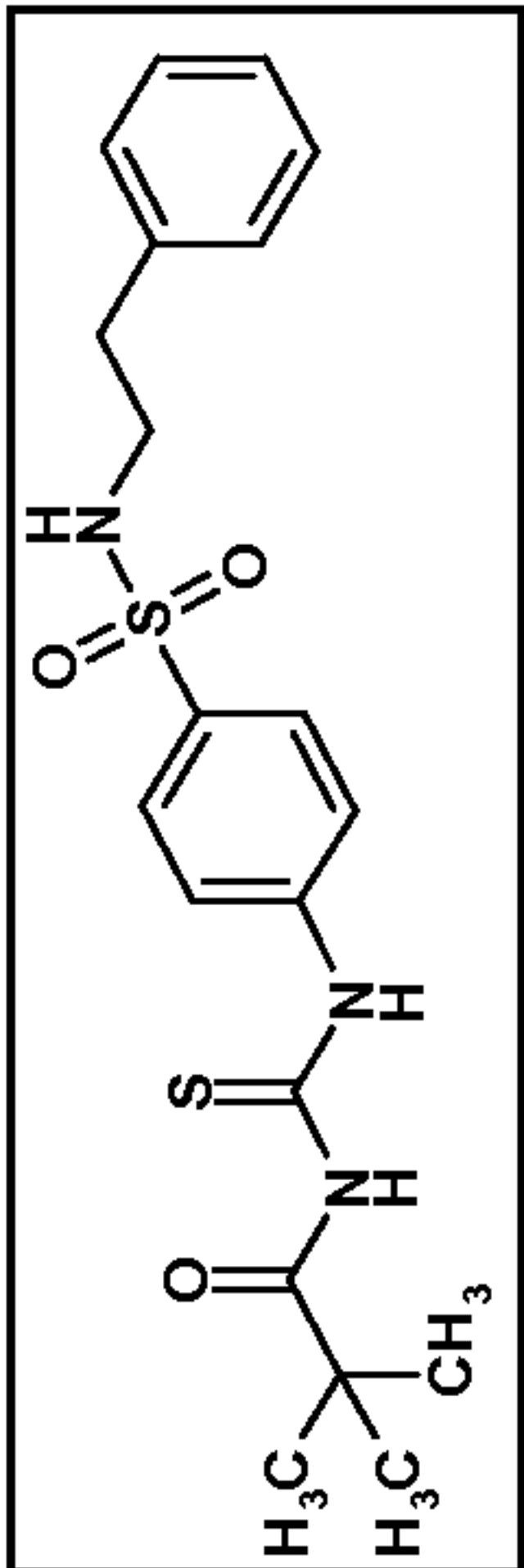
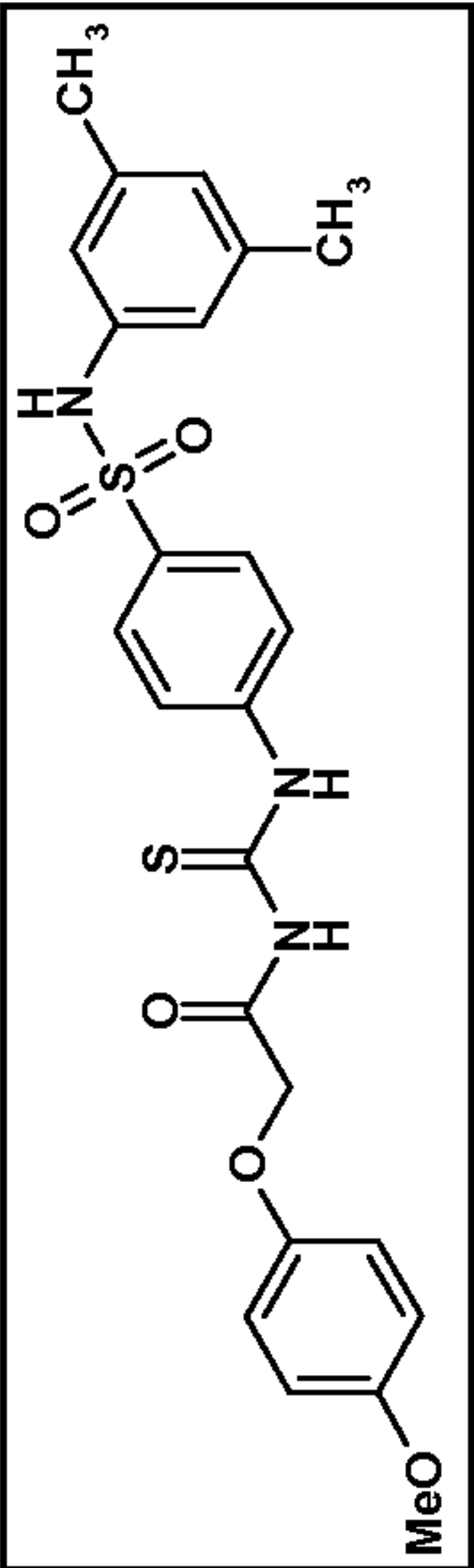
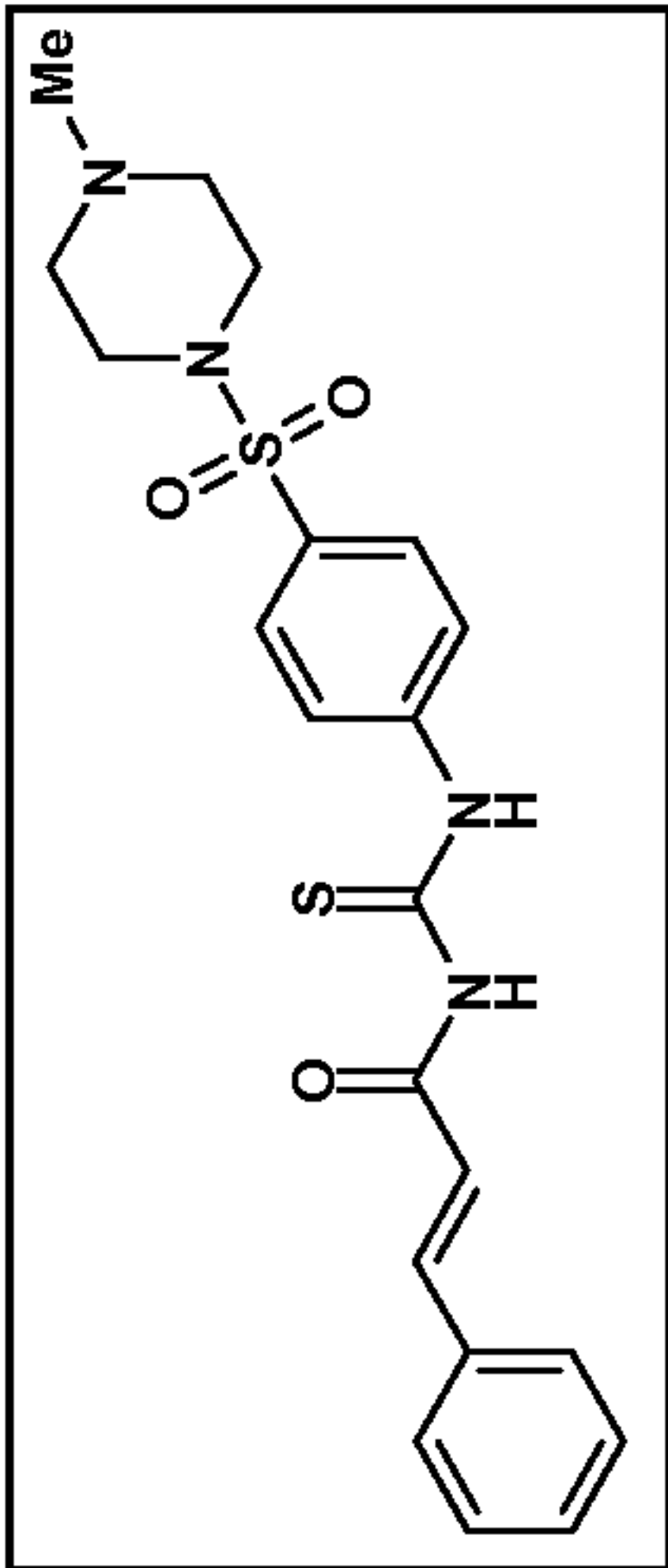
	AD4-11538	L	-6.95	-65.791	34.978	5.48/5.19(0.95)	-3.36	-40.64
	AD4-12064	N	-4.99	-54.071	34.778	47.78/42.73(0.89)	-2.6	-34.85
	AD4-12042	K	-5.83	-56.582	34.592	non-convergent	-2.07	-29.6
	AD4-12016	L	-4.01	-54.12	34.399	15.78/23.53(1.49)	-2.74	-30.33
	AD4-12196	K	-6.07	-70.658	34.379	18.01/36.80(2.04)	-1.74	-49.22

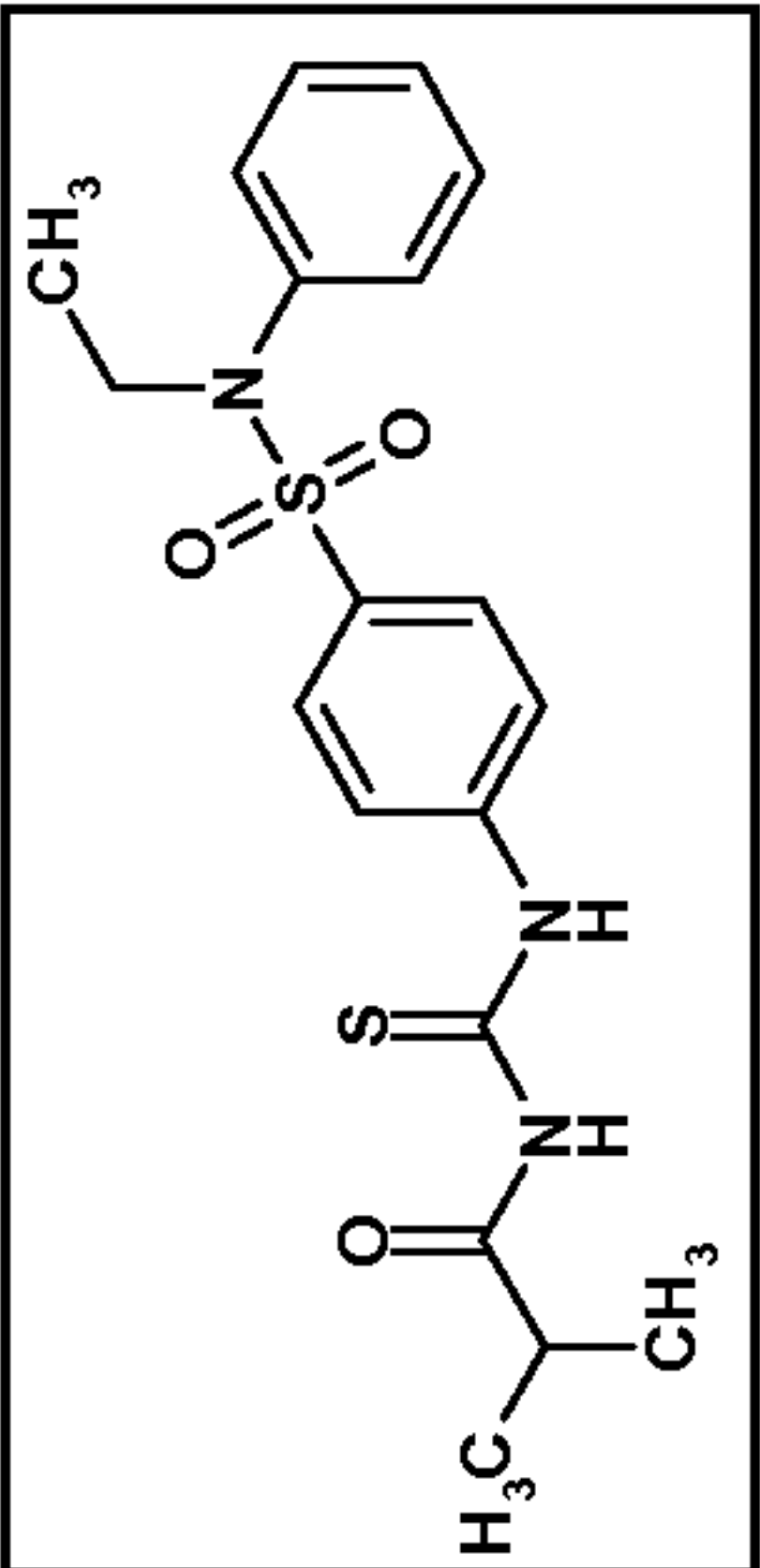
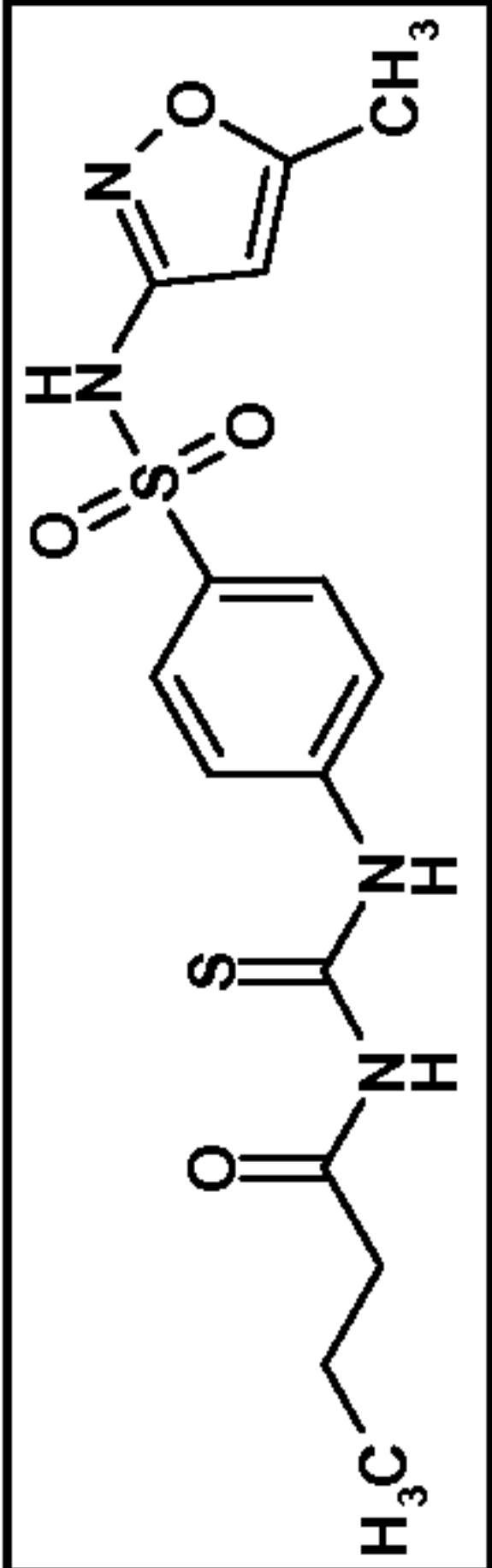
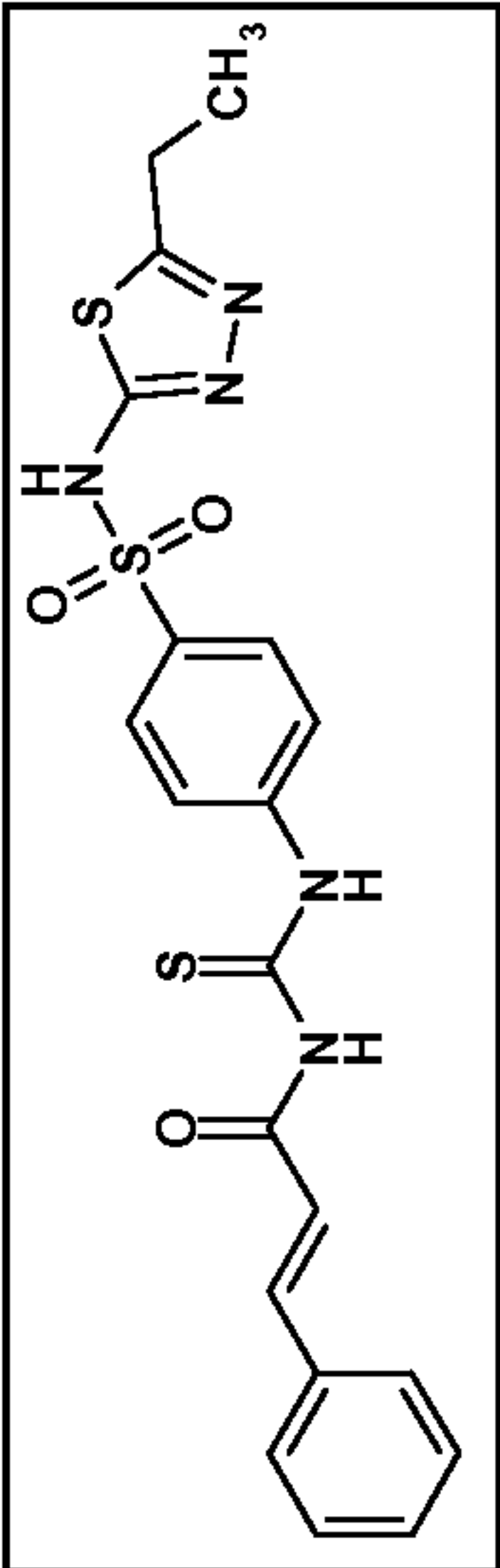
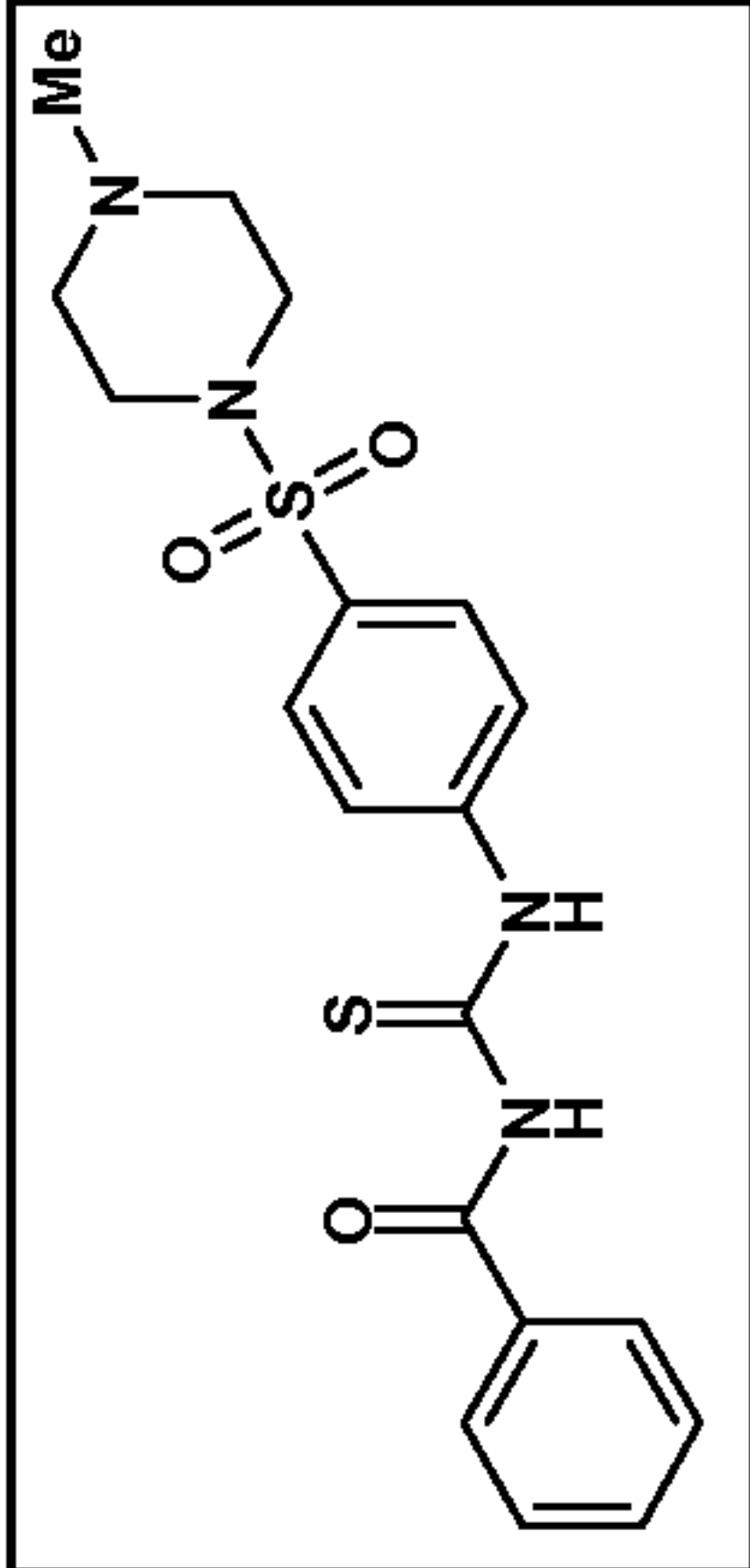
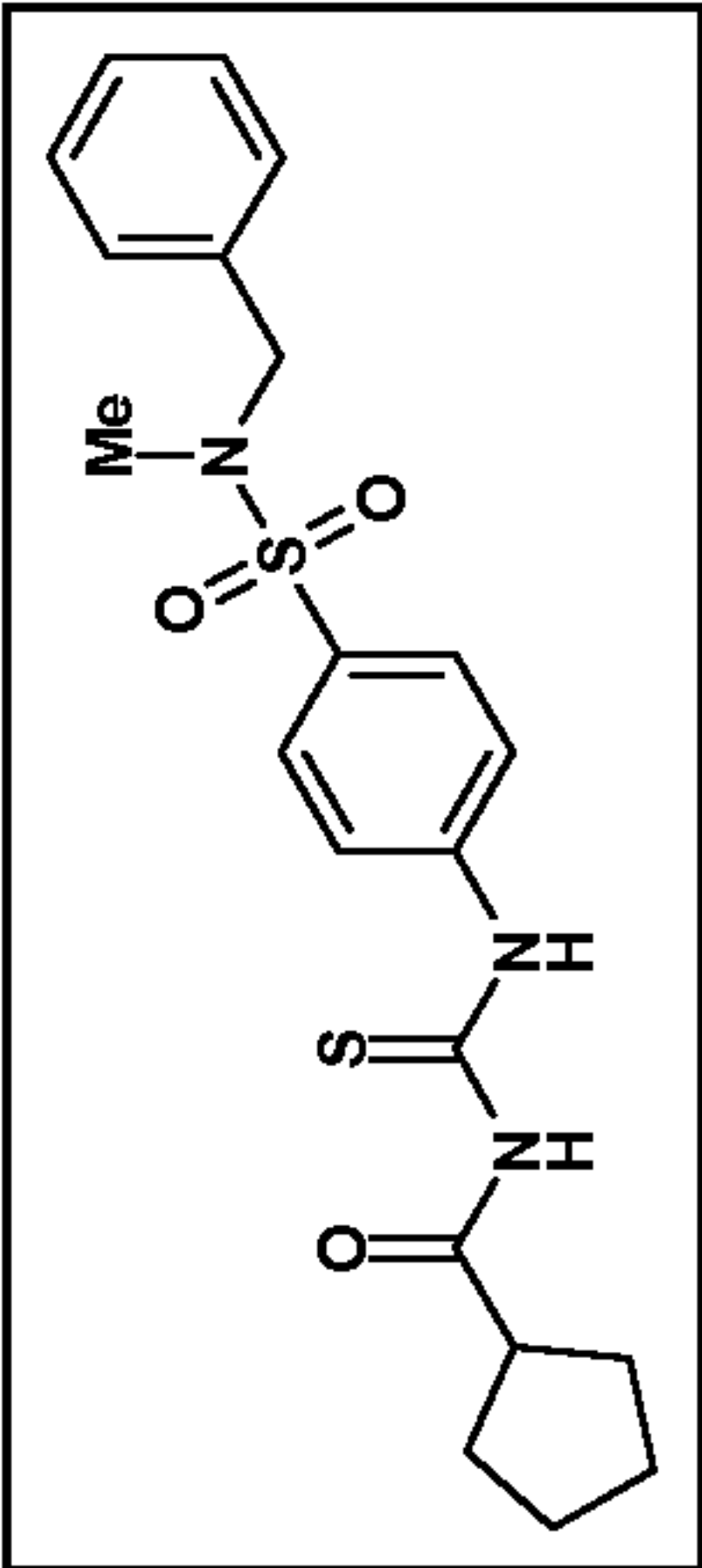
		AD4-12044	K	-5.61	-54.634	34.334	63.18/33.11(0.52)	-2.09	-37.5	
		AD4-12060	N	-5.79	-59.463	34.272	48.53/40.47(0.83)	-3.38	-36.21	
		AD4-12062	K	-6.61	-55.772	34.268	64.51/18.83(0.29)	-3.49	-36.09	
		AD4-11881	O	-6.34	-65.317	34.08	63.31/46.45(0.73)	-3.58	-44.41	

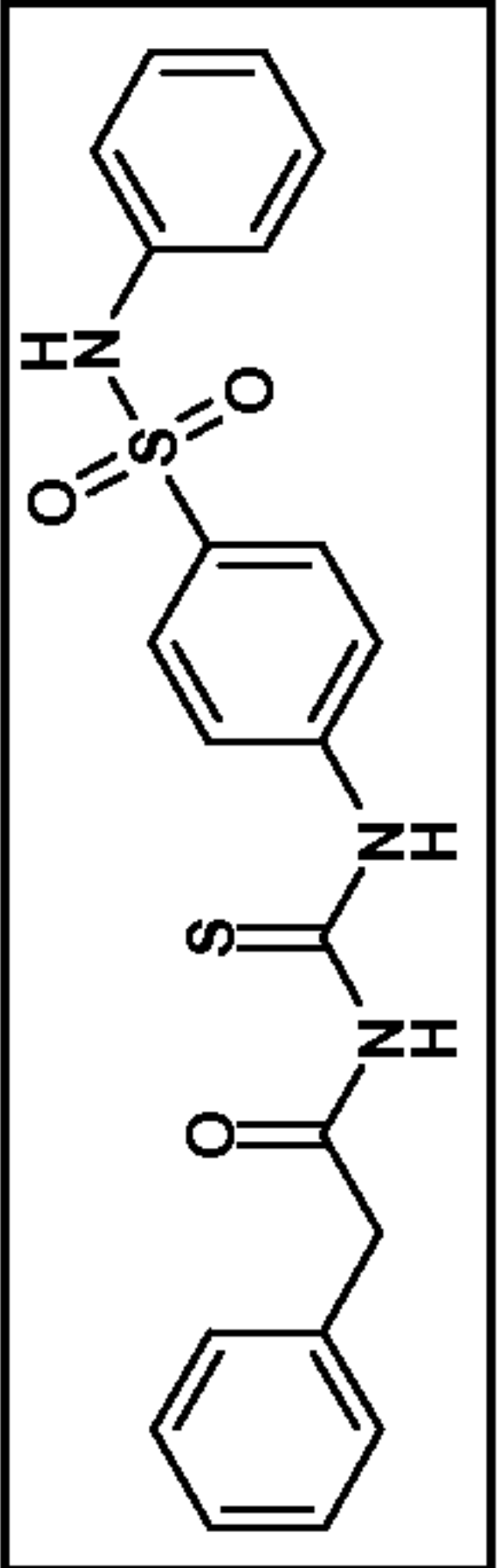
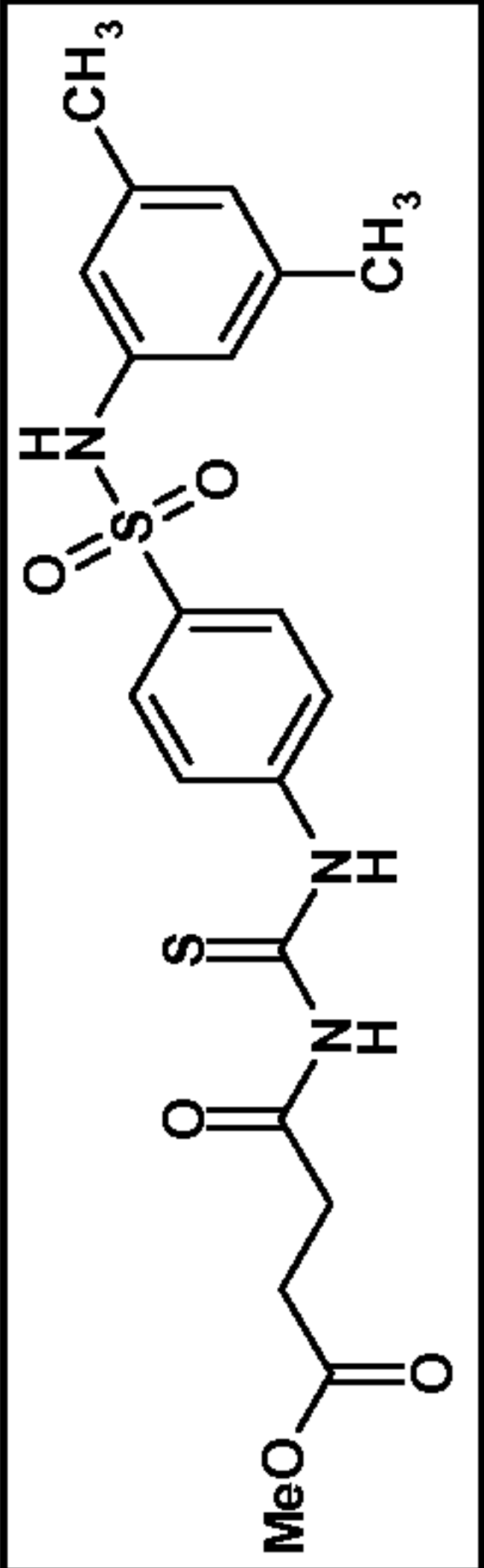
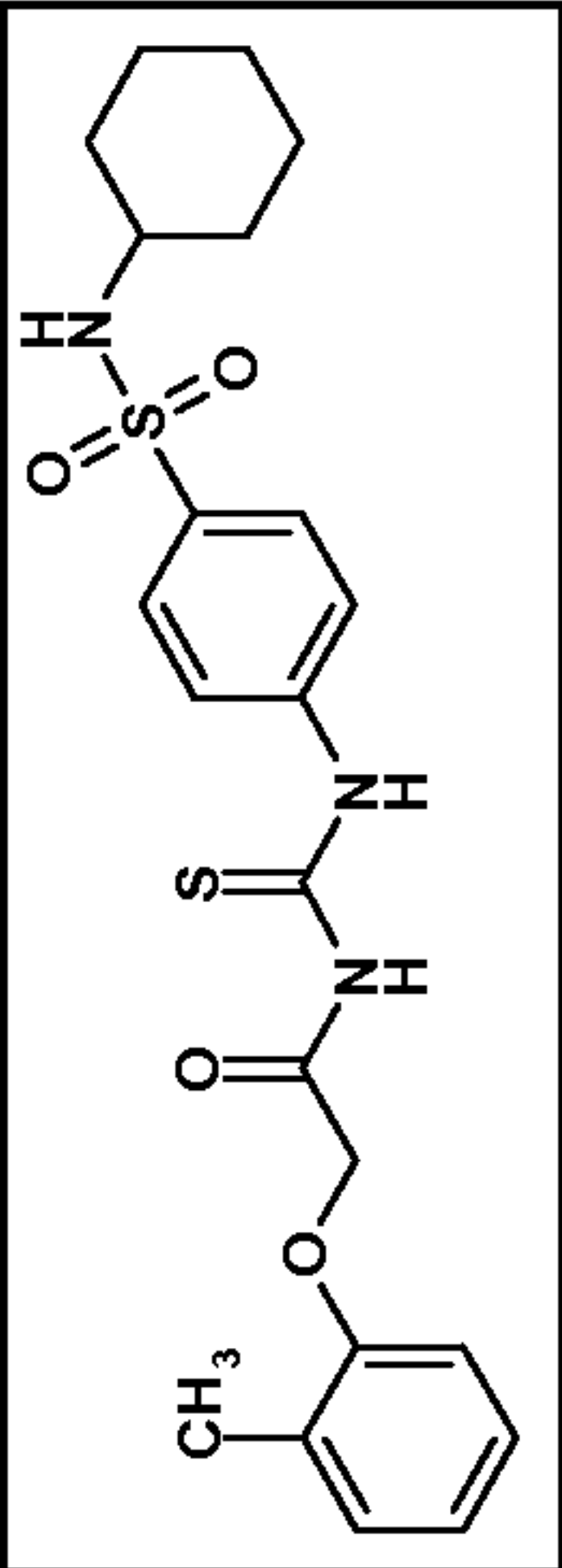
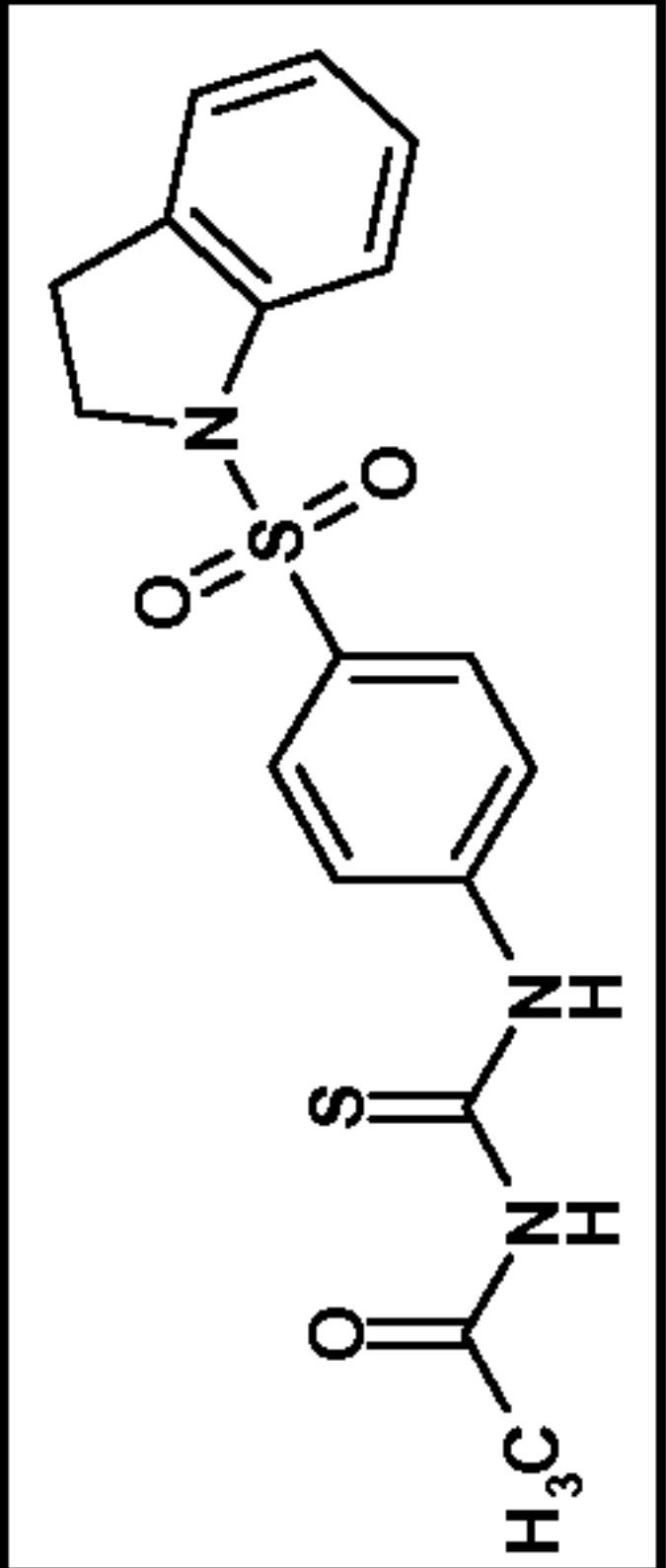
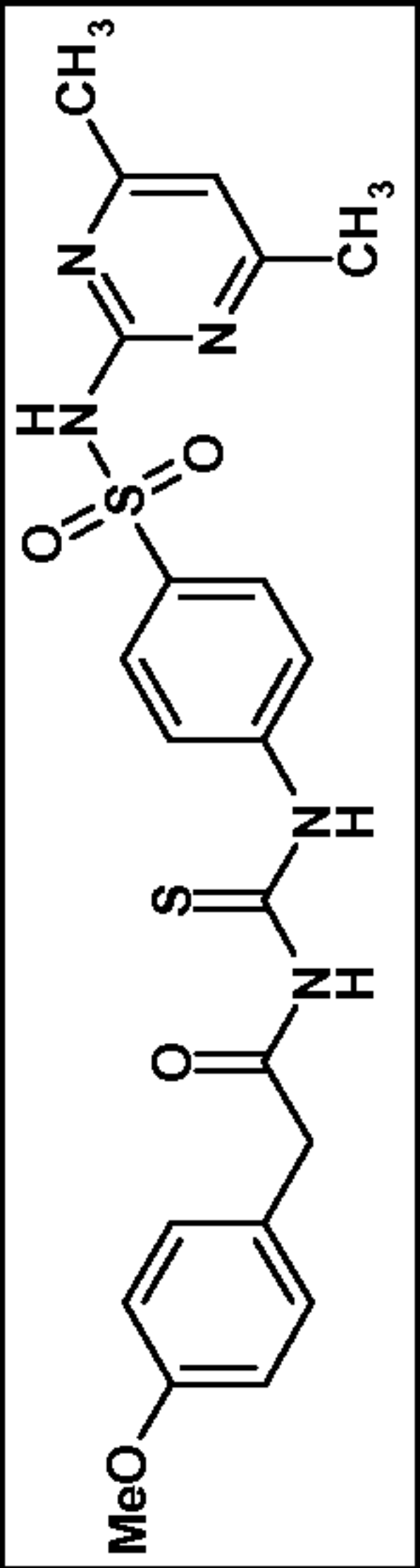
	AD4-11418	K	-4.89	-66.222	34.067	11.91/10.25(0.86)	-3.64	-51.66
	AD4-12010	K	-7.06	-56.321	33.772	6.68/6.16(0.92)	-3.62	-37.4
	AD4-11402	N	-4.4	-60.537	33.635	11.82/17.36(1.47)	-3.56	-43.59
	AD4-11977	N	-4.93	-42.763	33.588	32.51/non-convergent	-3.11	-28.88
	AD4-11637	O	-5.26	-60.687	33.491	non-convergent/17.76	-3.04	-46.33

	AD4-12149	K	-5.38	-67.463	33.331	21.88/36.21(0.60)	-2.96	-49.52
	AD4-12273	O	-4.61	-53.119	33.104	31.74/42.94(1.35)	-2.59	-34.17
	AD4-11874	O	-6.4	-52.192	32.474	34.98/38.97(1.11)	-3.3	-48.79
	AD4-11867	O	-4.91	-55.985	32.432	51.01/48.09(0.94)	-3.11	-43.51
	AD4-11885	O	-5.44	-52.547	32.38	37.83/48.34(1.28)	-2.88	-40.04

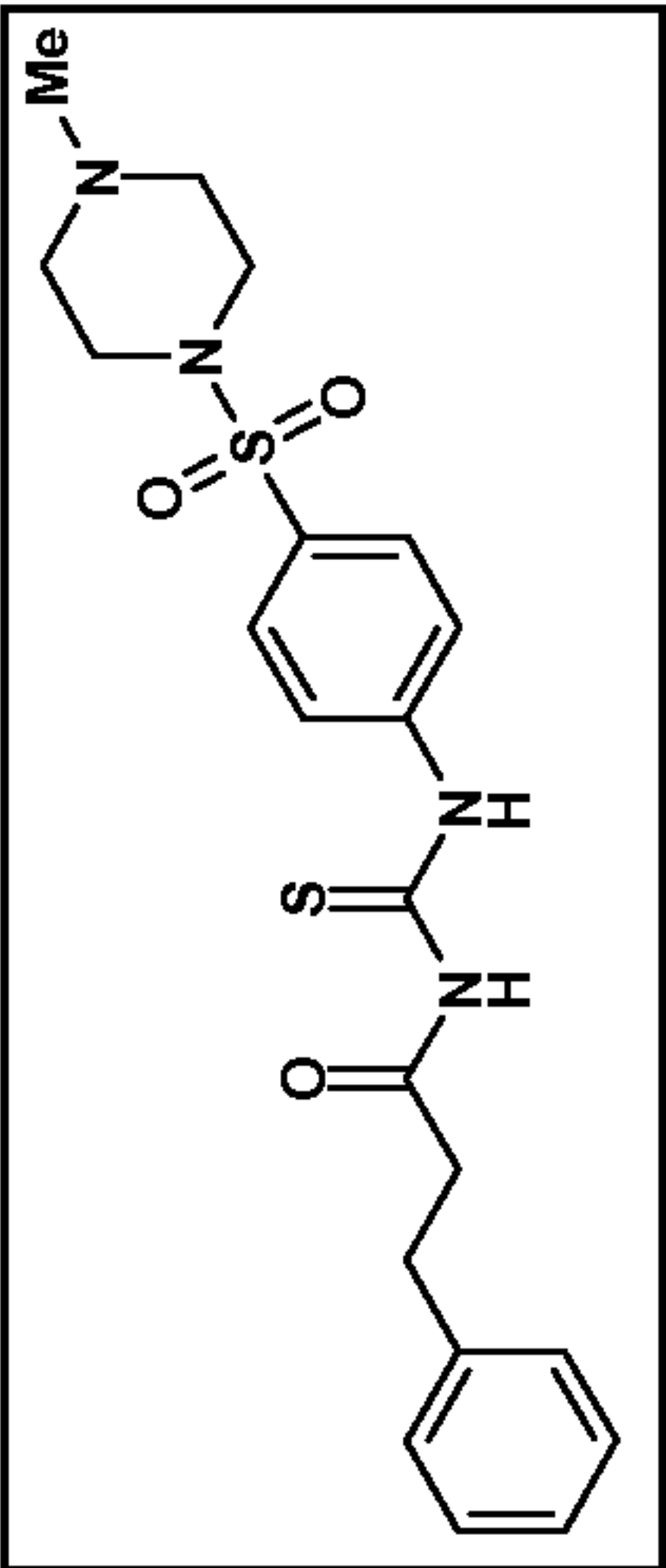
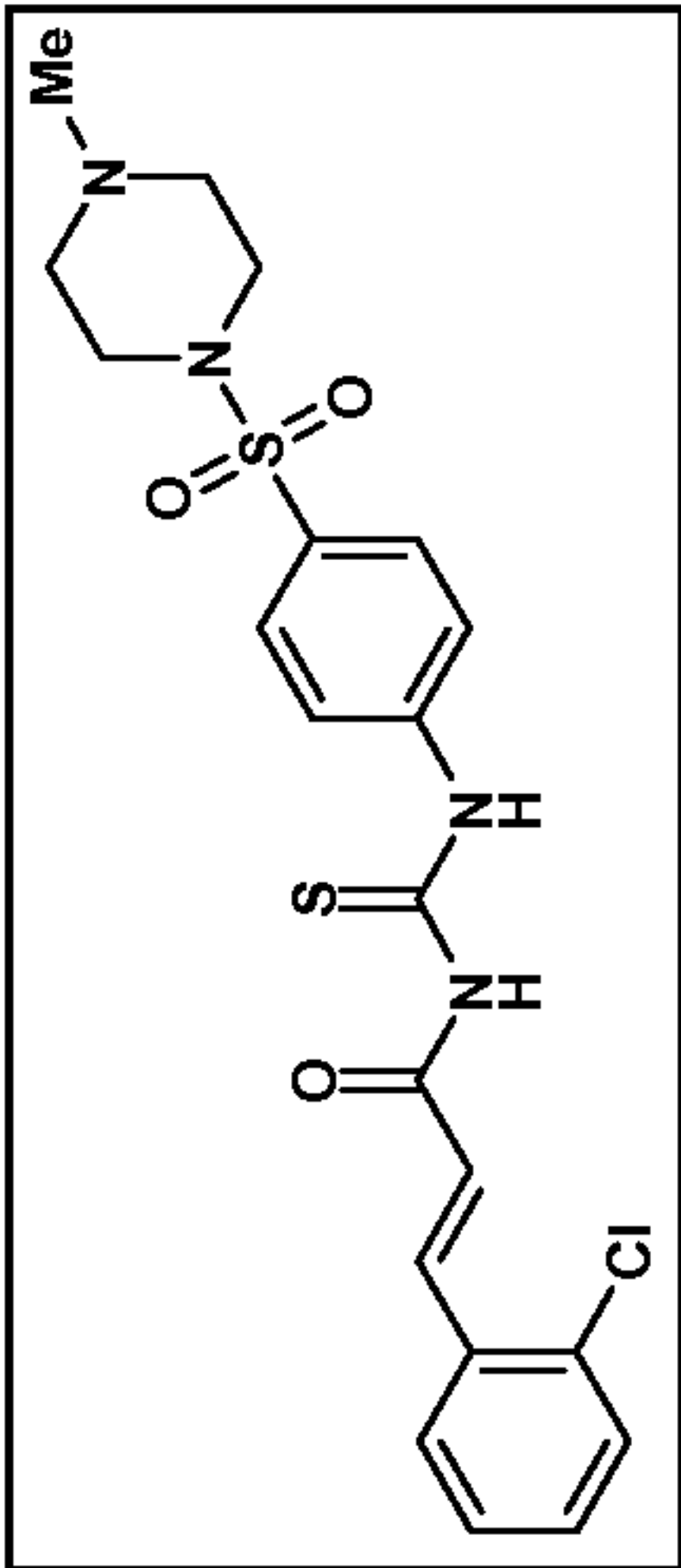
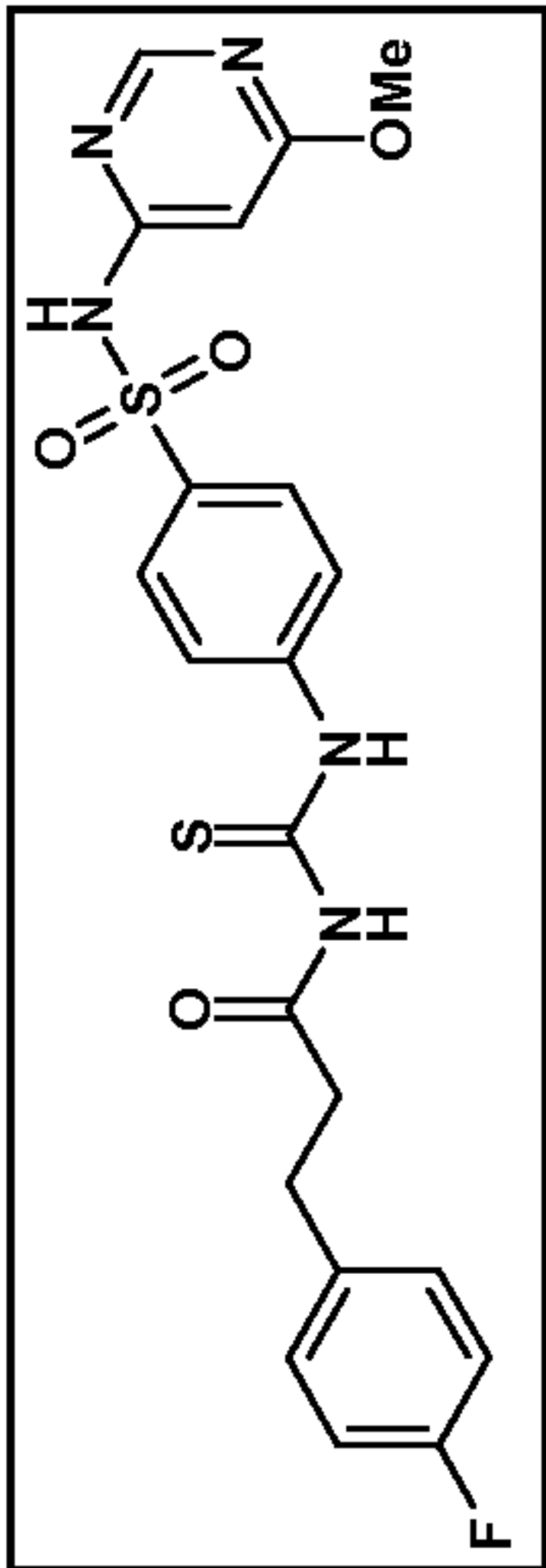
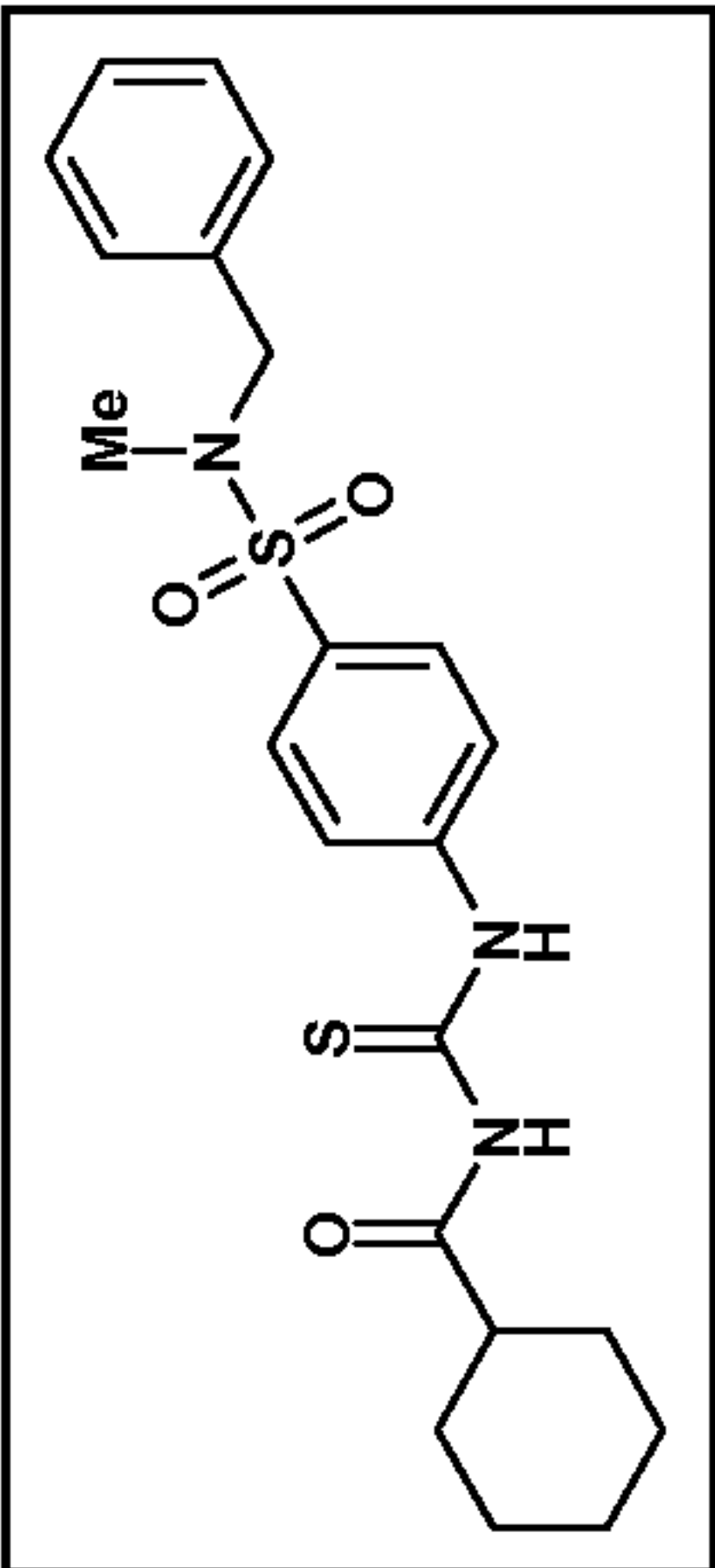
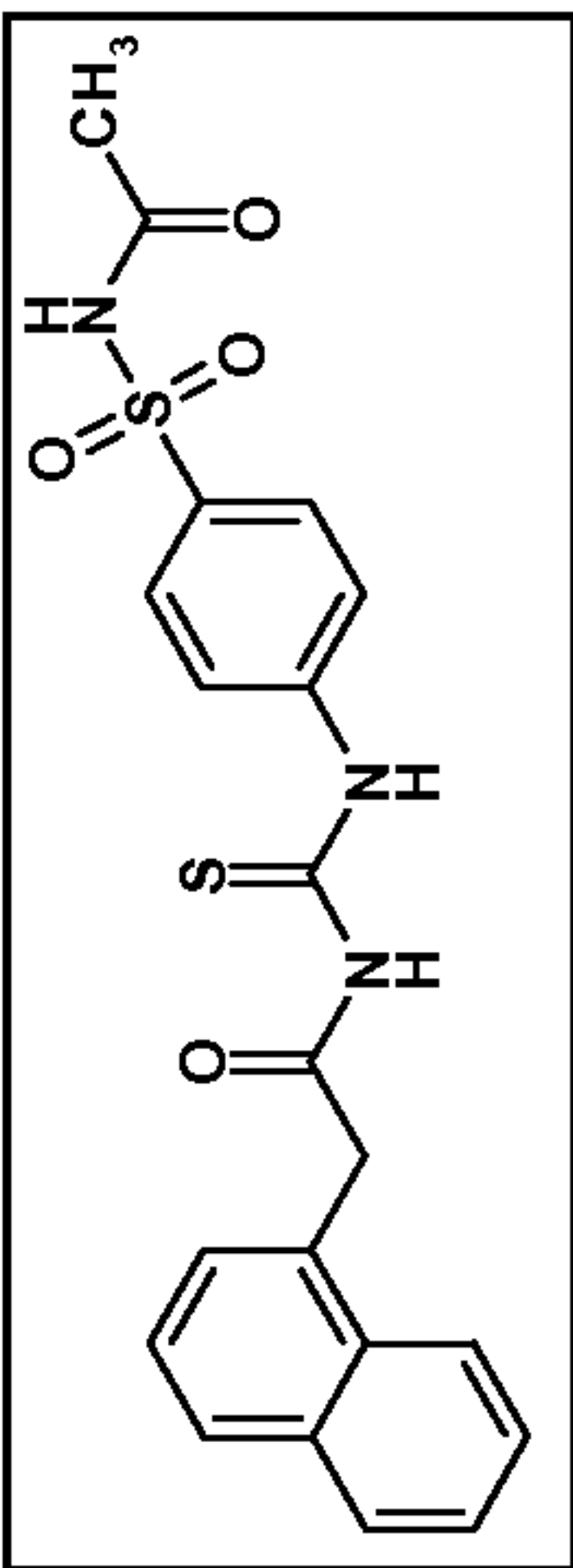
	AD4-12003	K	-5.54	-53.098	32.007	11.53/12.79(1.11)	-4.01	-31.62
	AD4-11412	K	-6.18	-58.012	31.958	11.42/20.02(1.75)	-4.65	-40.44
	AD4-12153	N	-6.74	-58.542	31.36	15.58/21.77(0.72)	-3.2	-49.88
	AD4-12024	K	-5.06	-59.351	31.244	6.77/6.34(0.97)	-3.51	-37.04
	AD4-12265	N	-5.64	-51.979	31.19	38.12/35.06(0.92)	-4.14	-42.09

	AD4-11870	O	-6.19	-57.173	30.849	55.07/47.30(0.86)	-3.16	-44.1
	AD4-10612	L	-6.39	-64.392	30.841	11.68/13.21(1.13)	-1.5	-37.91
	AD4-10313	L	-2.31	-36.062	30.774	20.85/17.62(0.85)	-3.5	-38.52
	AD4-11396	K	-5.9	-61.408	30.745	25.09/21.23(0.85)	-4.36	-46.45
	AD4-12145	N	-6.3	-51.616	30.469	40.50/41.75(1.03)	-1.35	-41.29

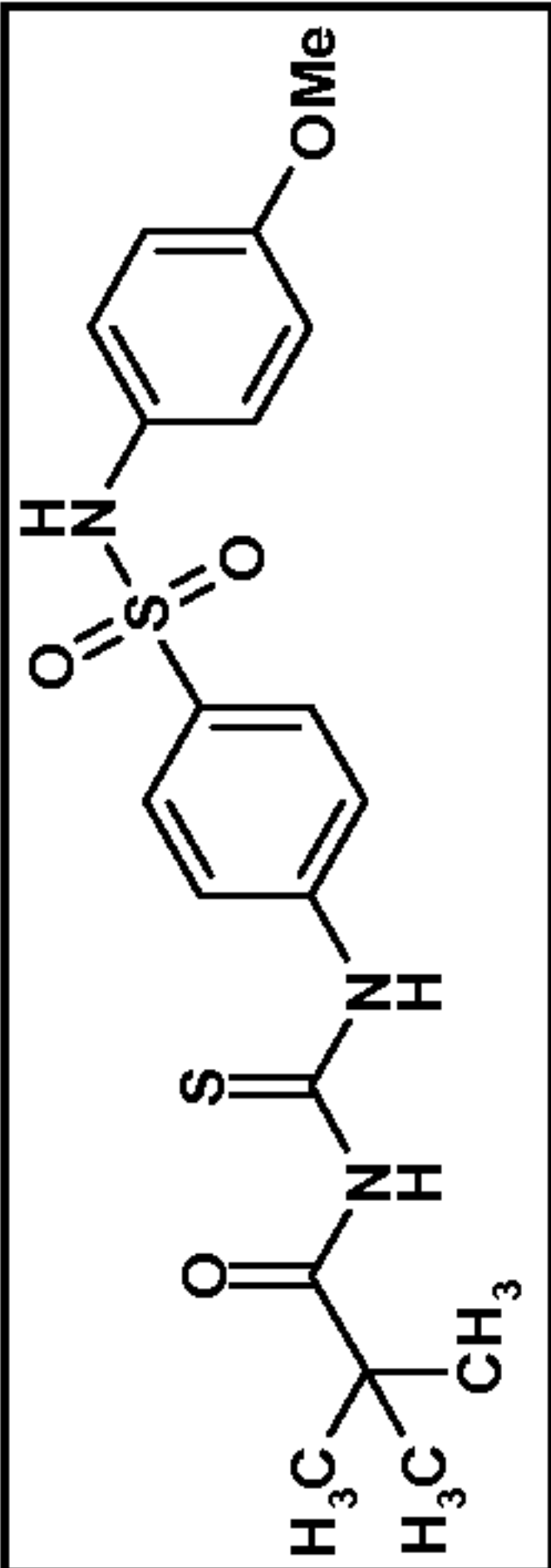
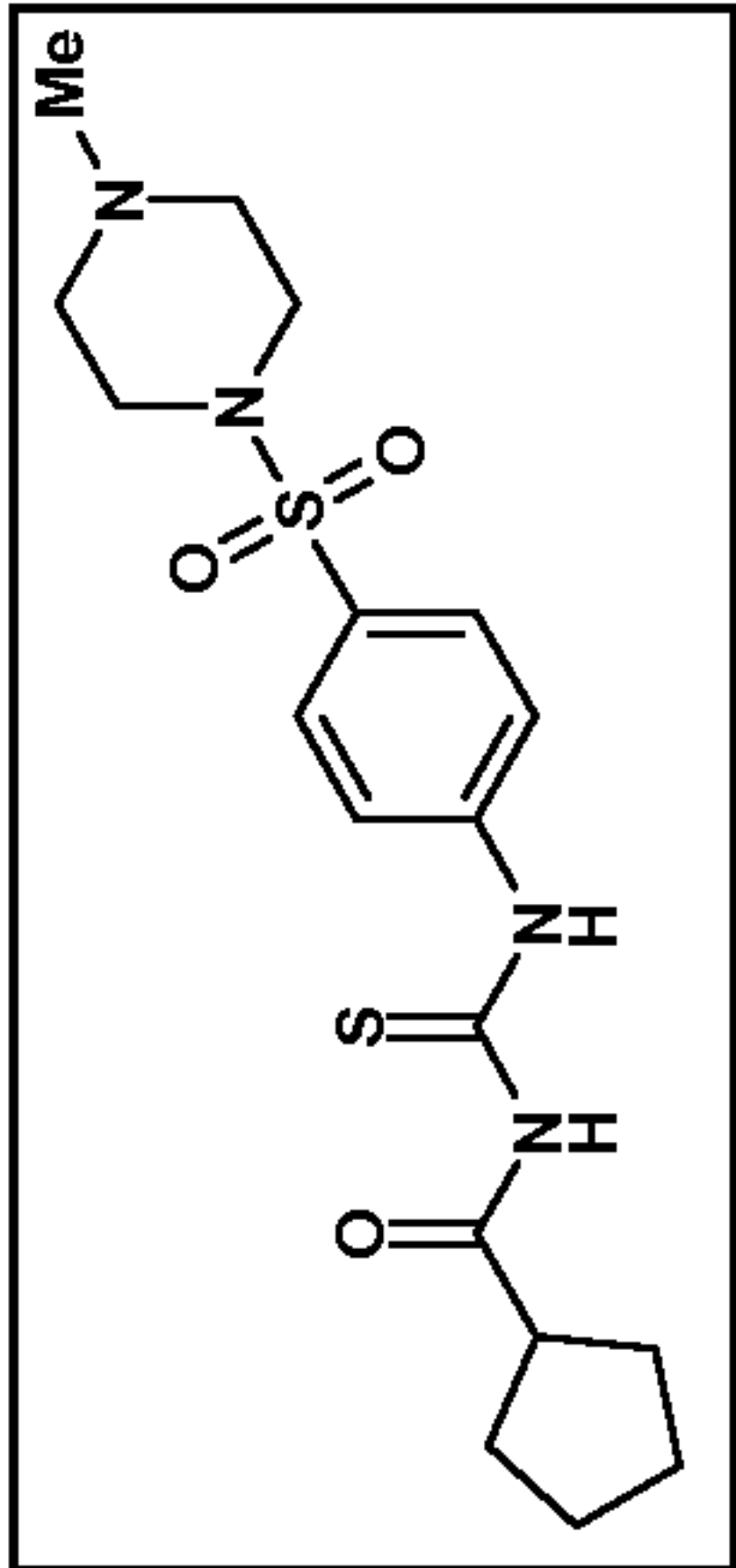
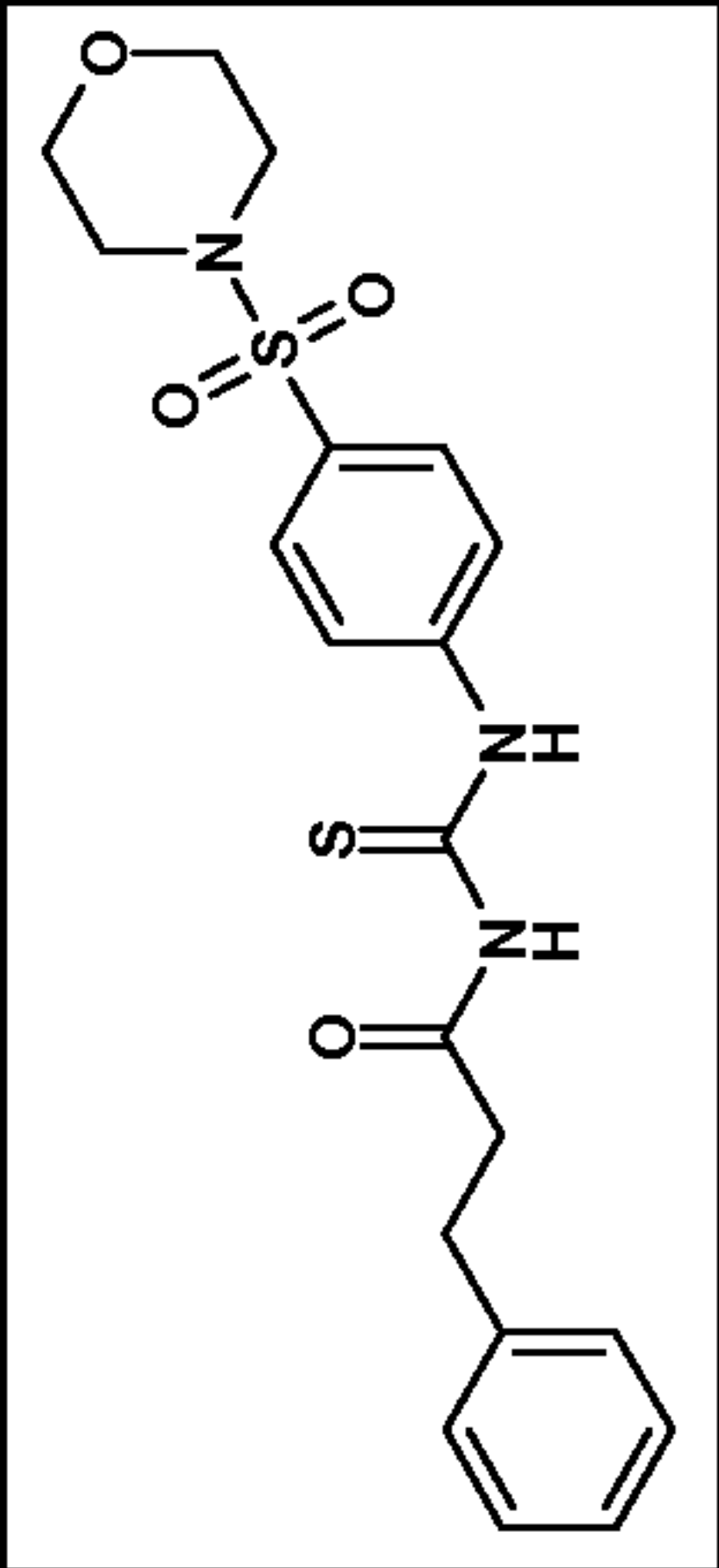
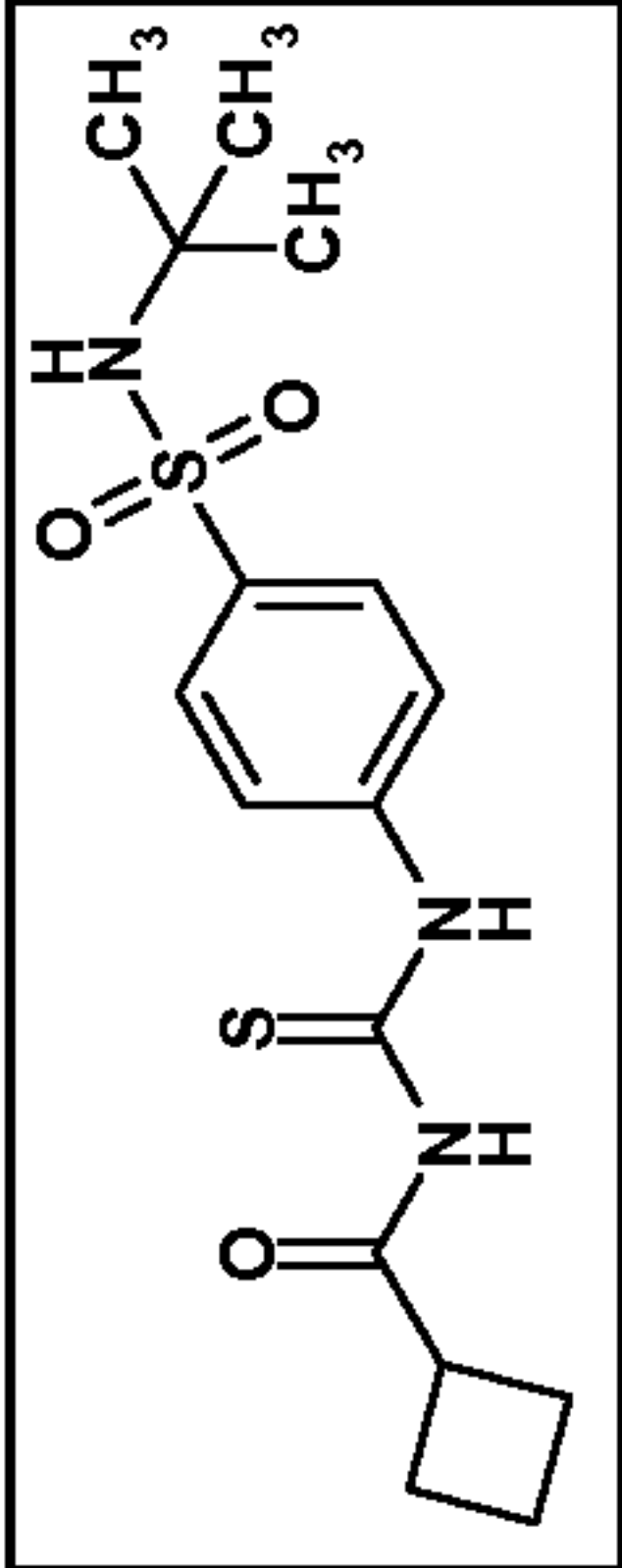
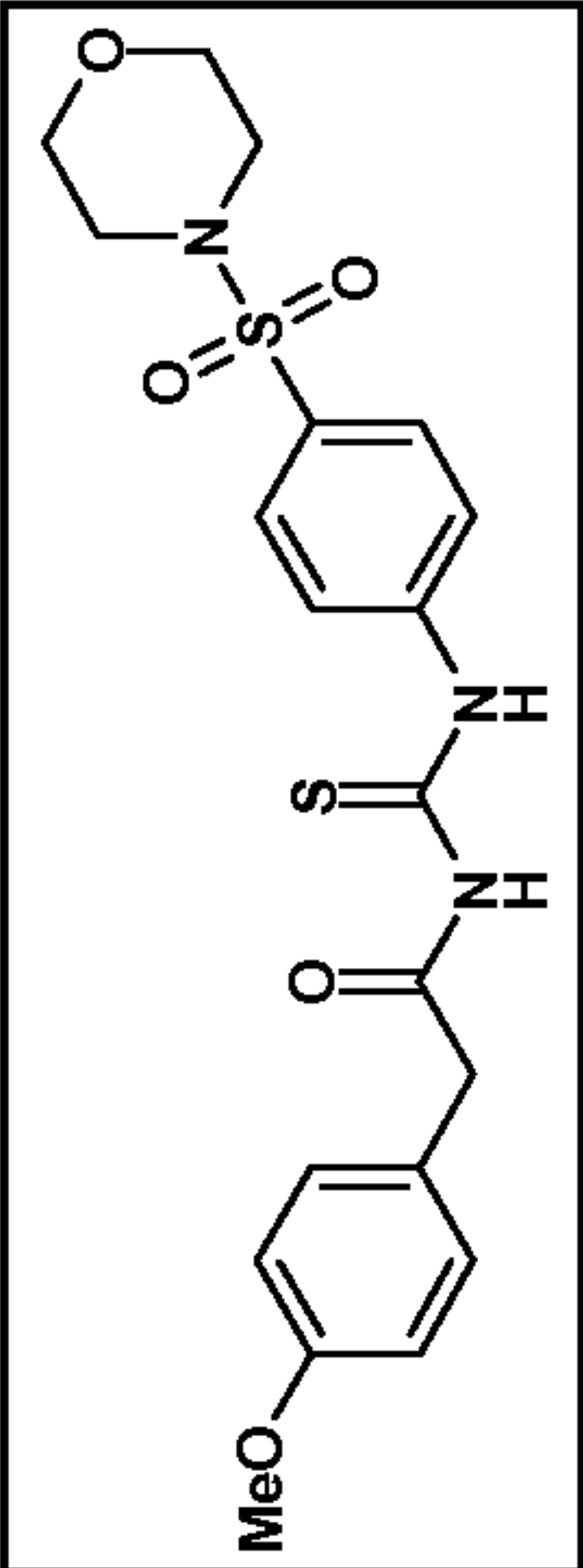
	AD4-12029	K	-5.31	-45.509	30.376	25.32/51.15(2.02)	-3.9	-42.48
	AD4-12267	J	-5.05	-43.093	30.36	non-convergent	-3.71	-30.79
	AD4-11861	O	-6.6	-58.006	30.242		-2.03	-43.96
	AD4-11420	N	-4.8	-54.408	30.052		-3.02	-32.21
	AD4-12039	L	-6.45	-54.185	29.977		-3.16	-35.07

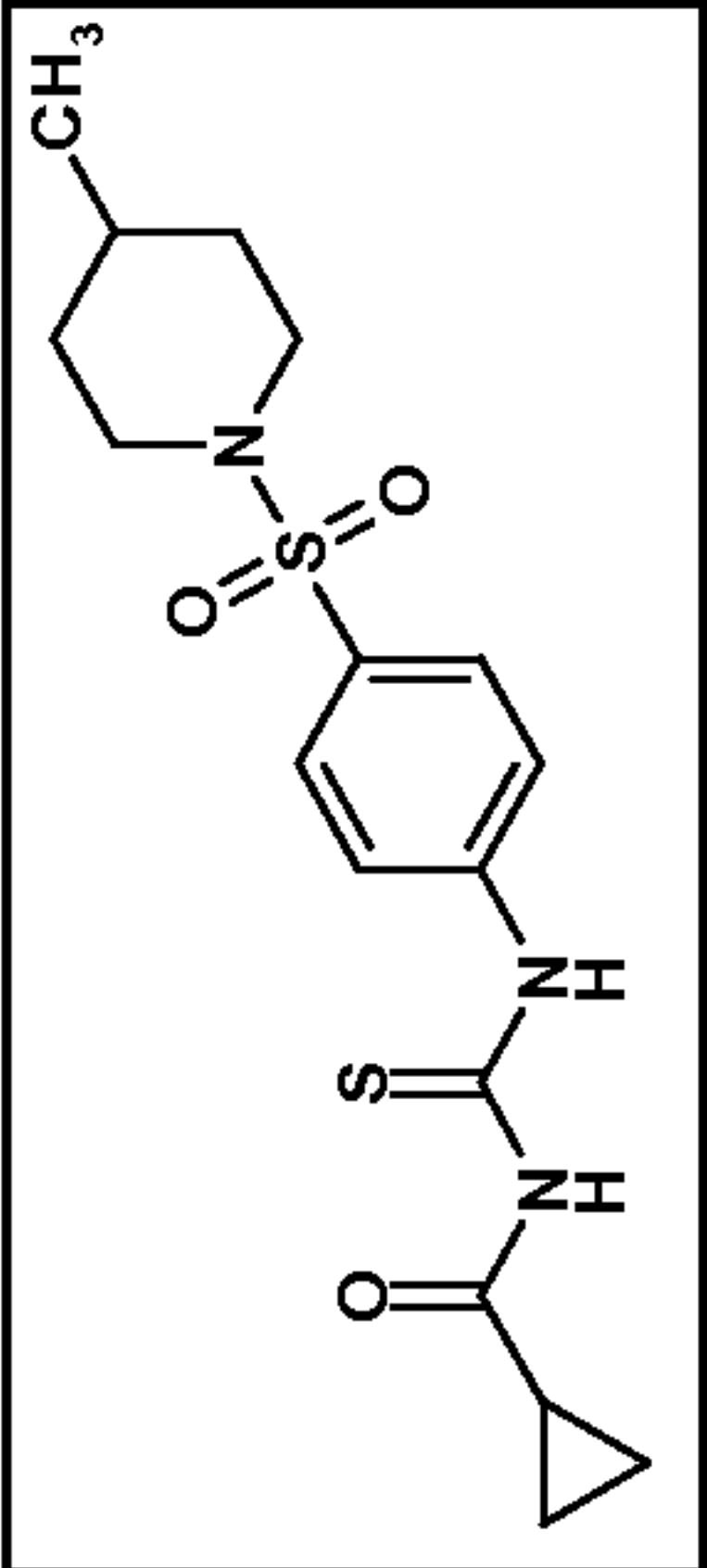
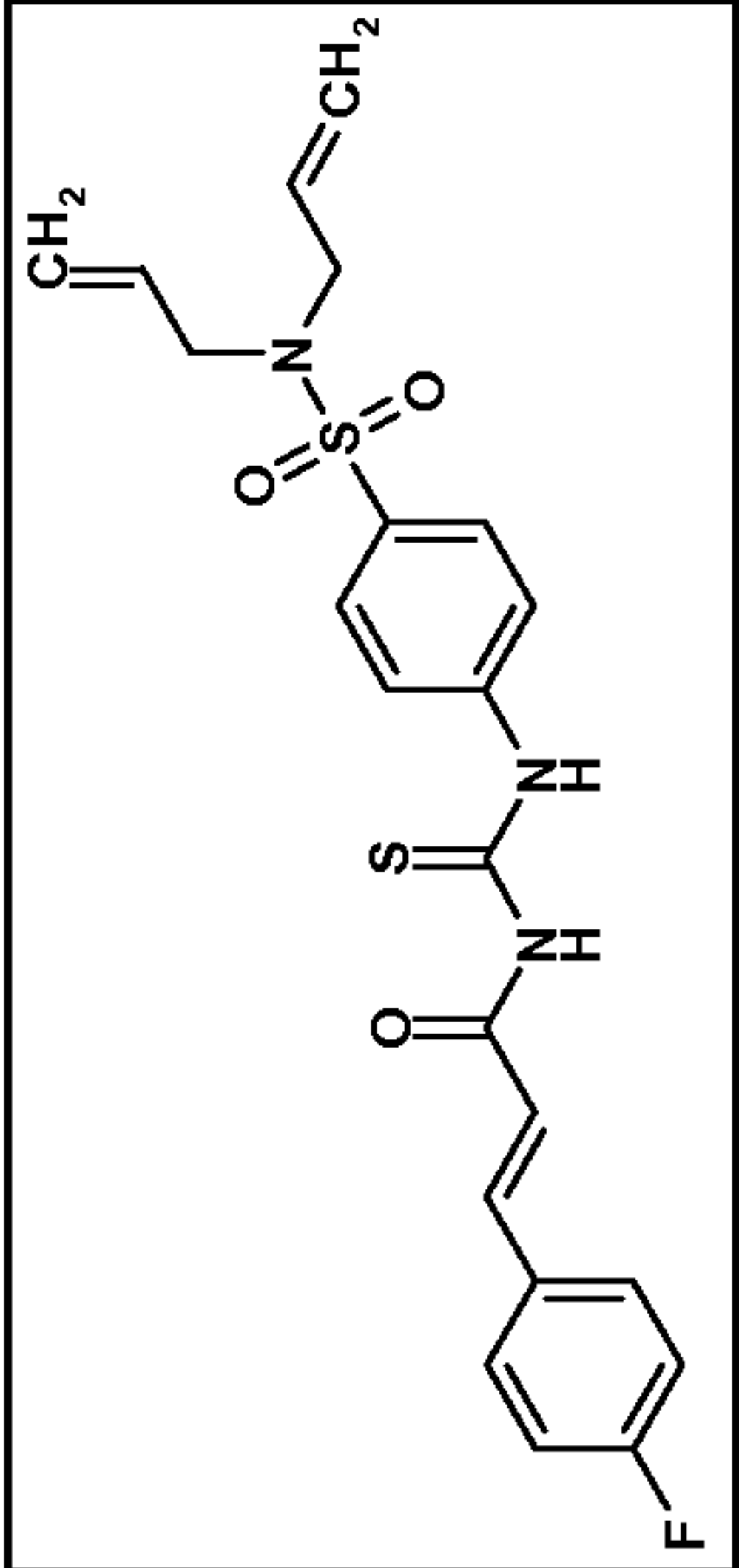
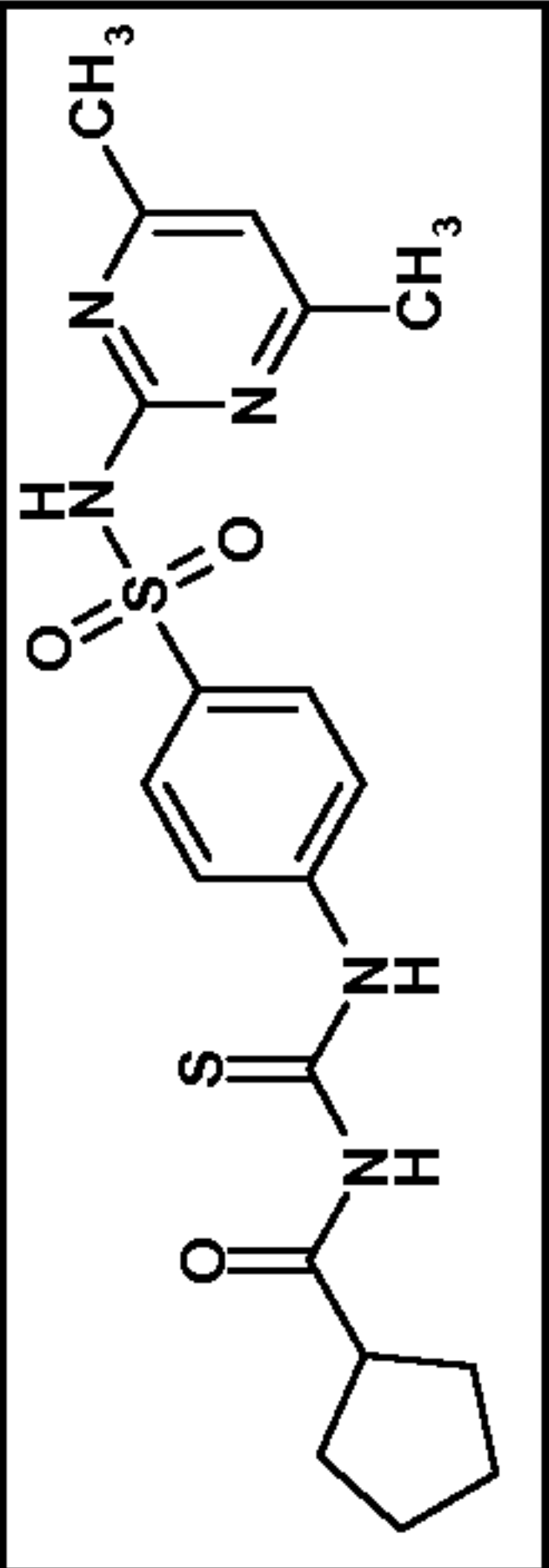
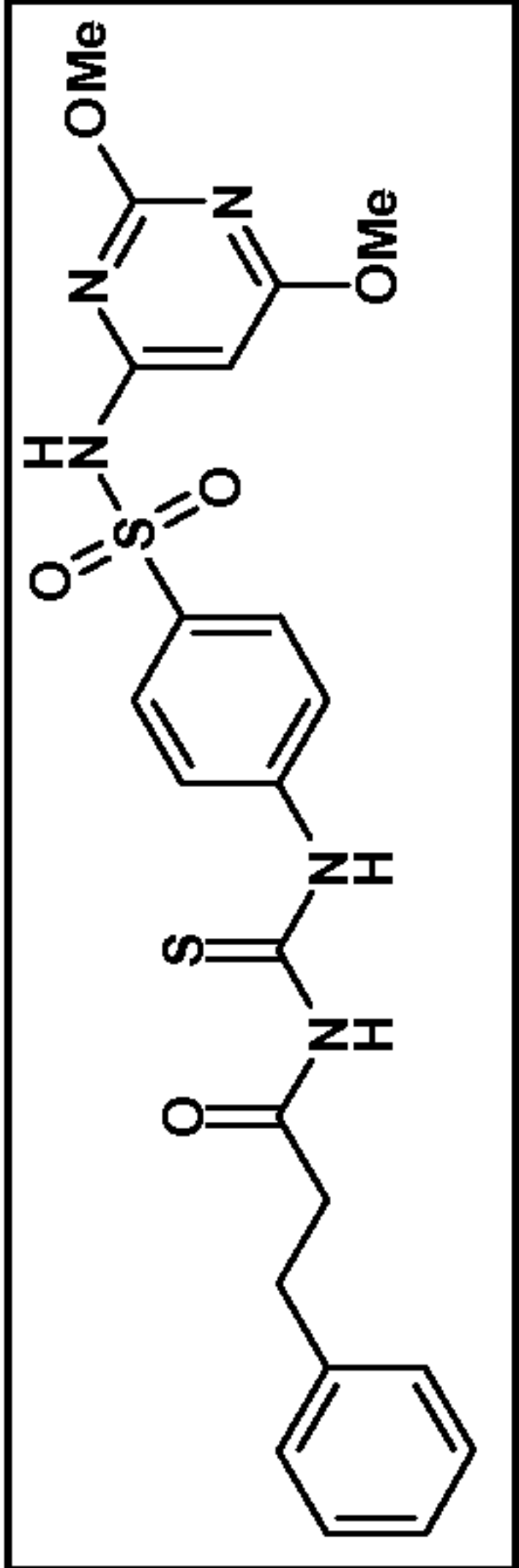
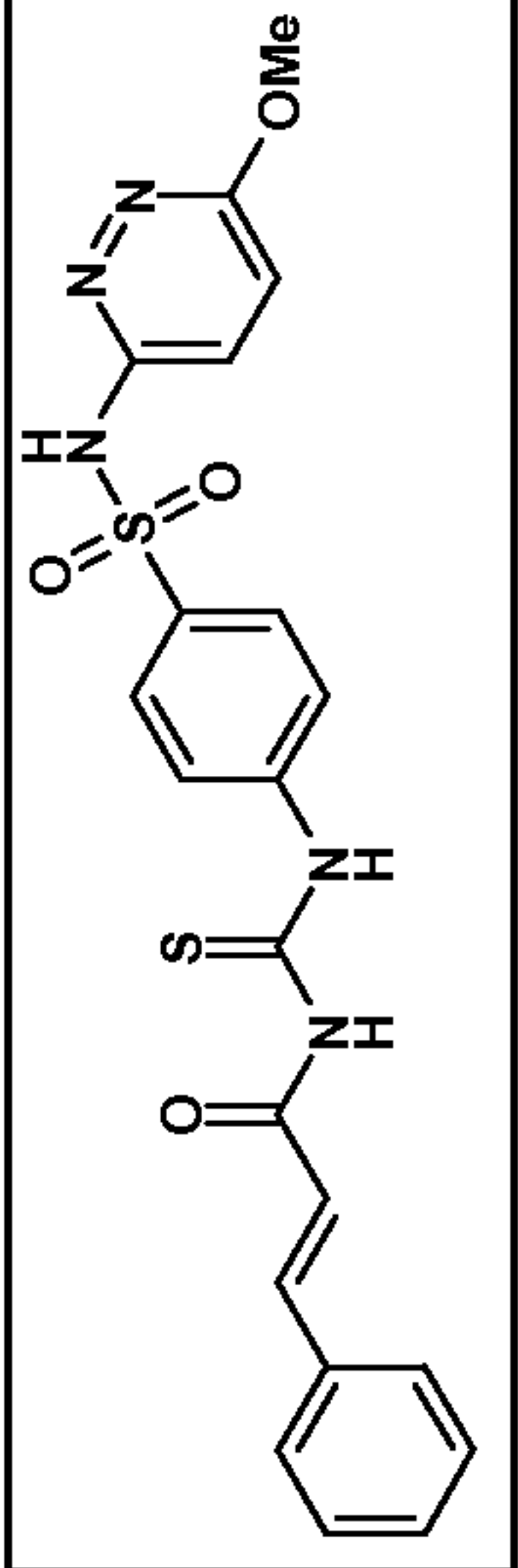
	AD4-12074	K	-5.08	-55.471	29.545	16.24/32.28(1.99)	-2.98	-39.72
	AD4-12093	K	-6.31	-46.102	29.436	non-convergent	-2.96	-39.97
	AD4-12073	L	-5.45	-59.824	29.313	19.54/32.28(1.65)	-5.06	-41.61
						11.59/16.97(1.46)		
	AD4-10103	M	-5.14	-48.394	29.295		-4.54	-44.4
	AD4-12266	J	-5.78	-68.303	29.289	49.64/45.88(0.92)	-4.54	-53.86

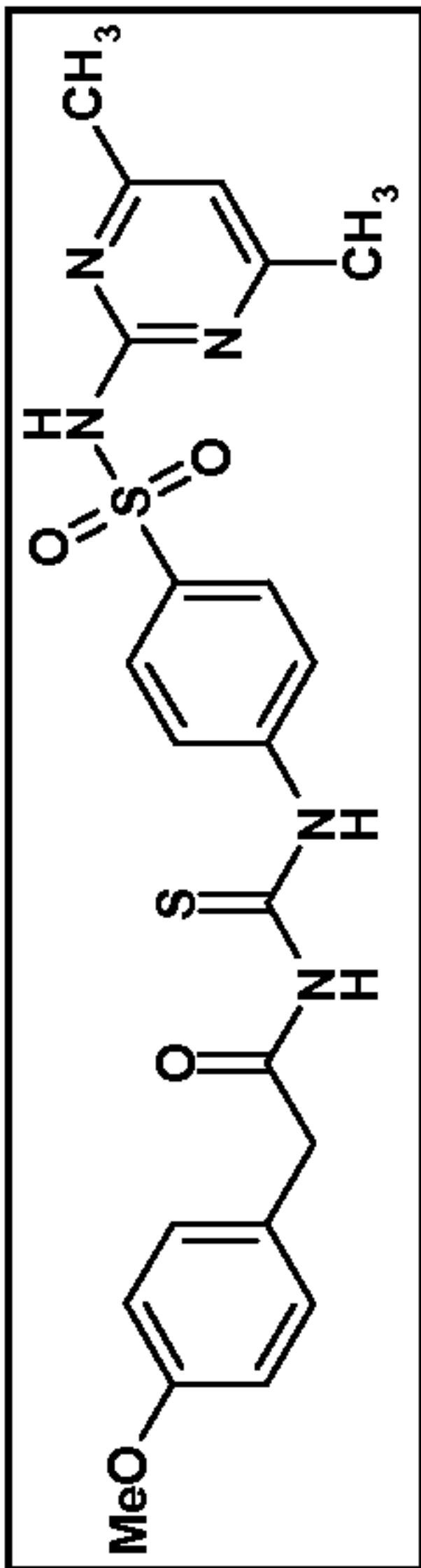
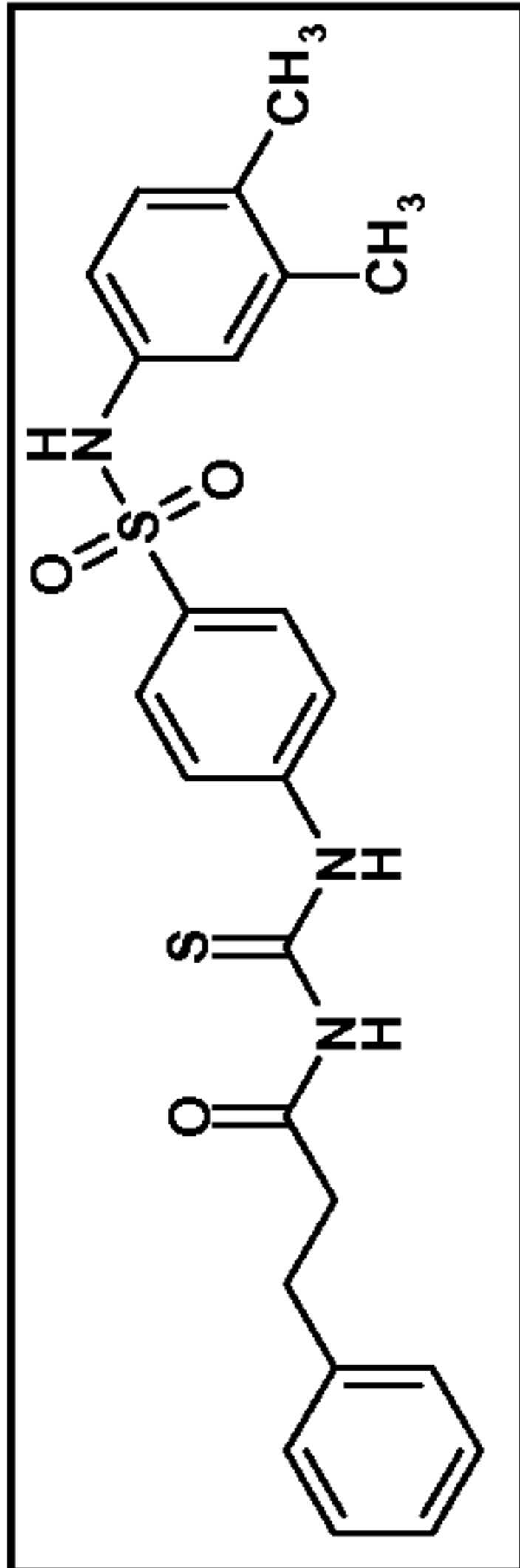
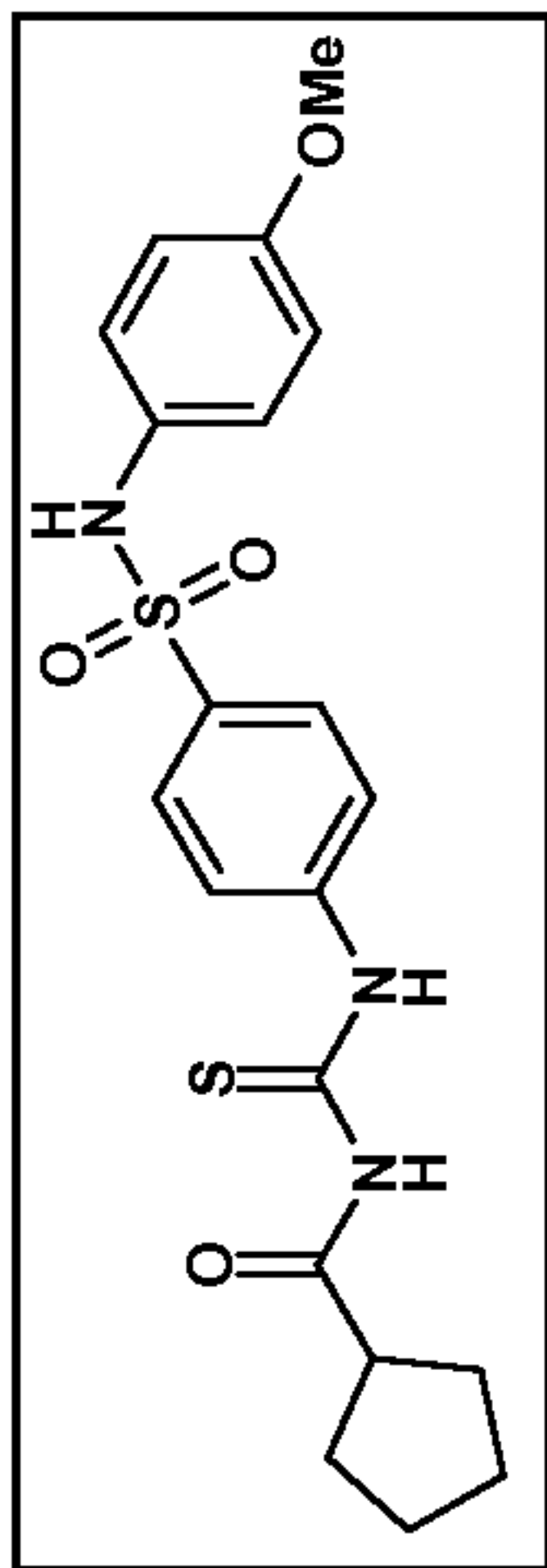
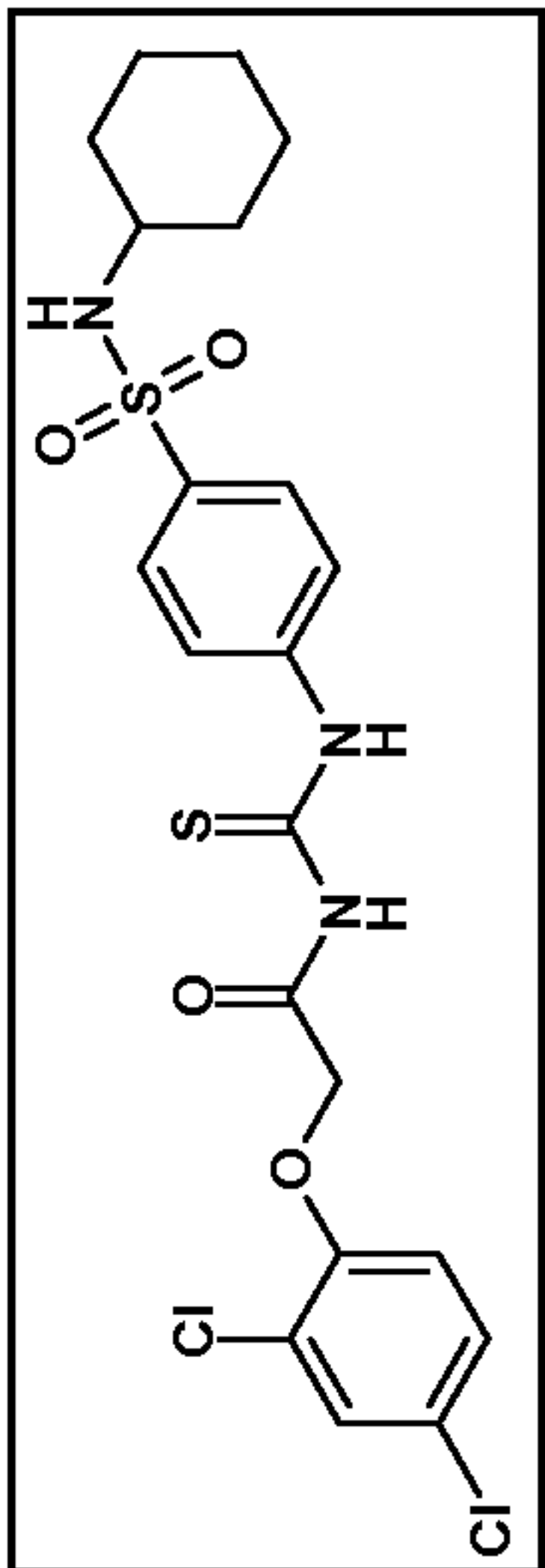
[illegible]

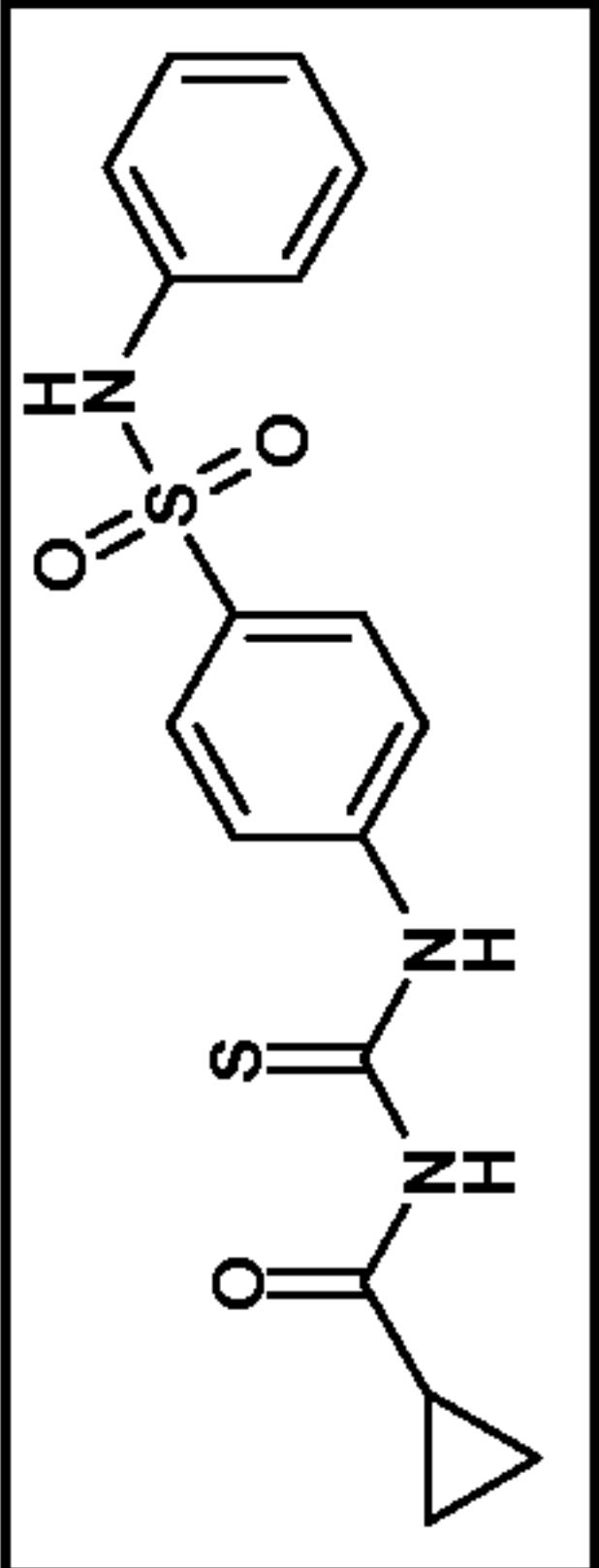
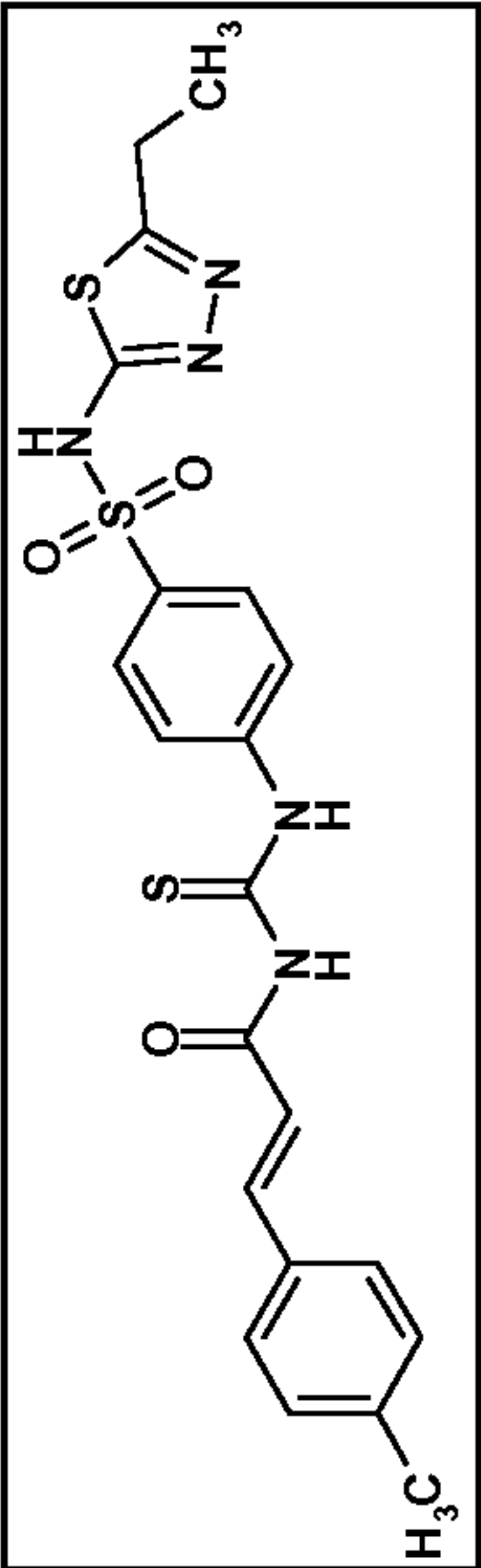
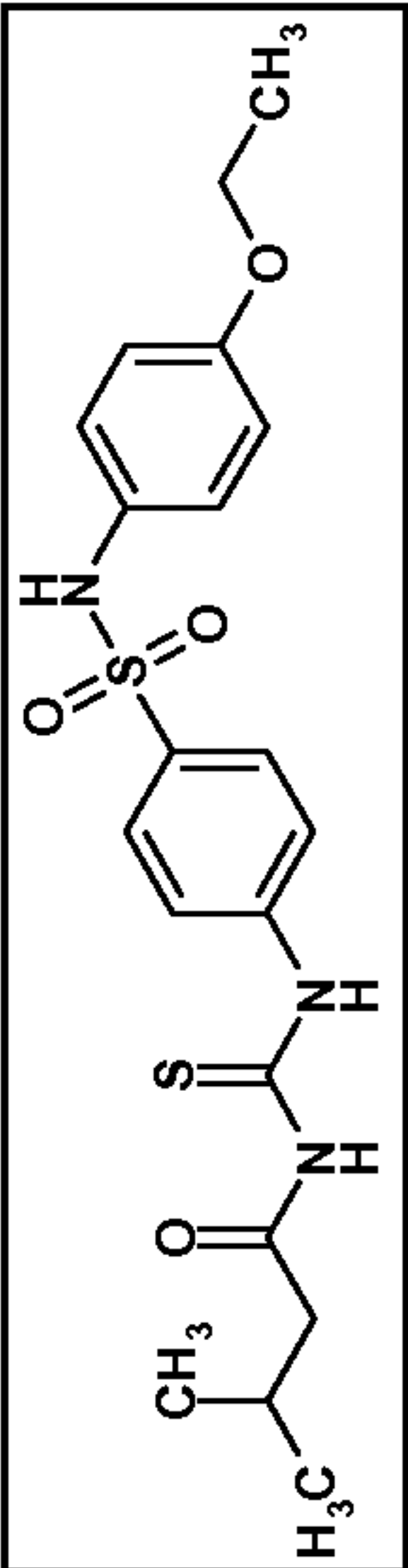
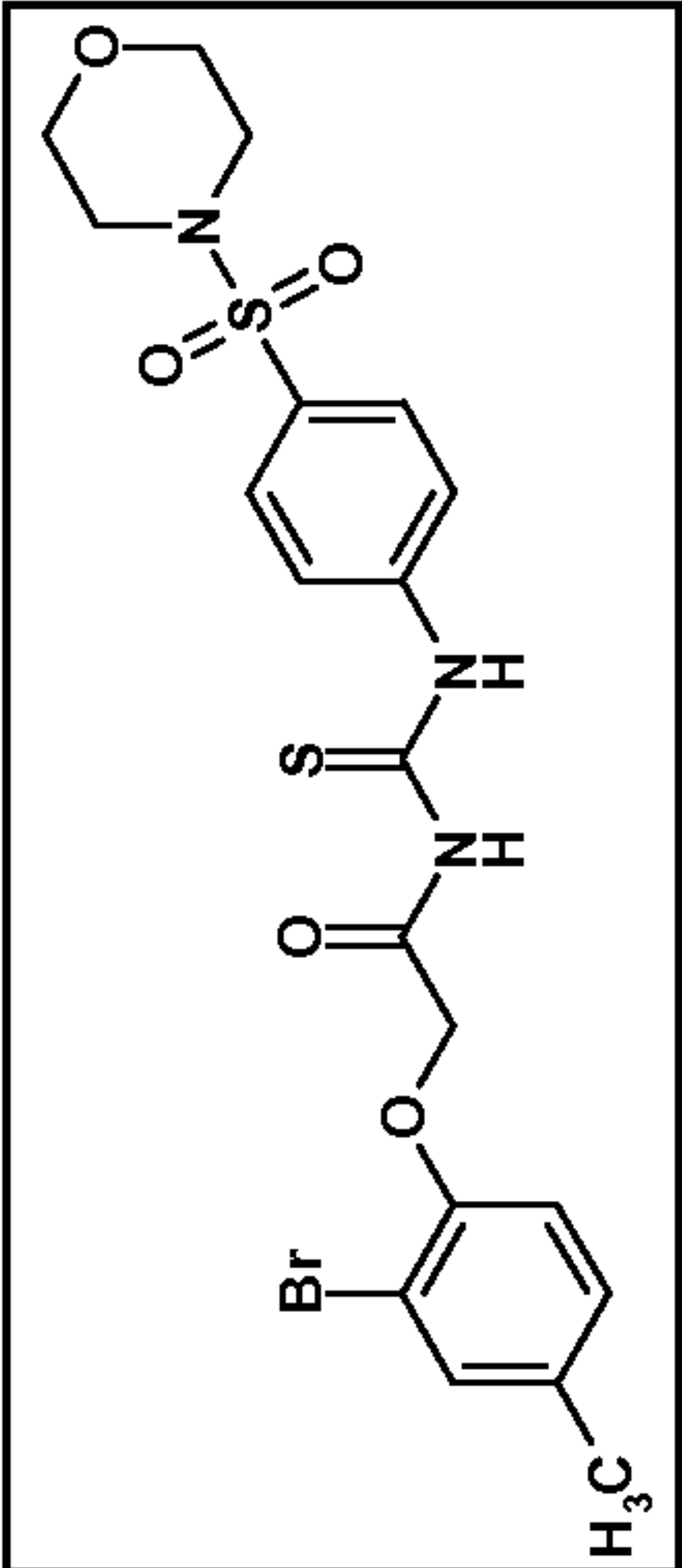
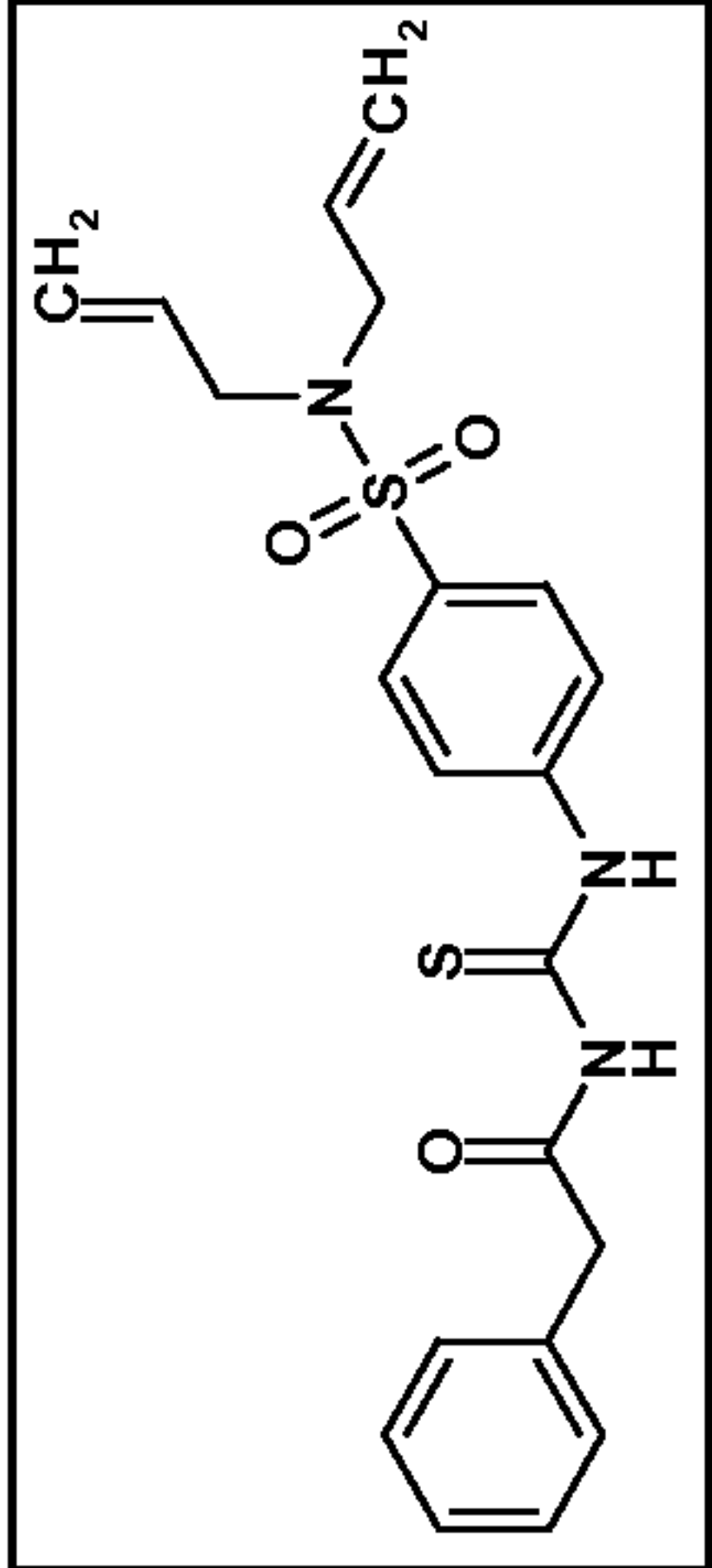
	AD4-11424	N	-5.82	-59.614	28.422			-3.64	-28.82
	AD4-12059	N	-4.54	-58.82	28.33	32.25/37.69(1.17)		-3.42	-40.97
	AD4-11384	J	-5.62	-60.184	28.127			-3.68	-47.04
	AD4-11410	L	-5.68	-61.822	28.101			-3.84	-40.3
	AD4-12071	L	-4.56	-58.755	27.713			-4.19	-41.48

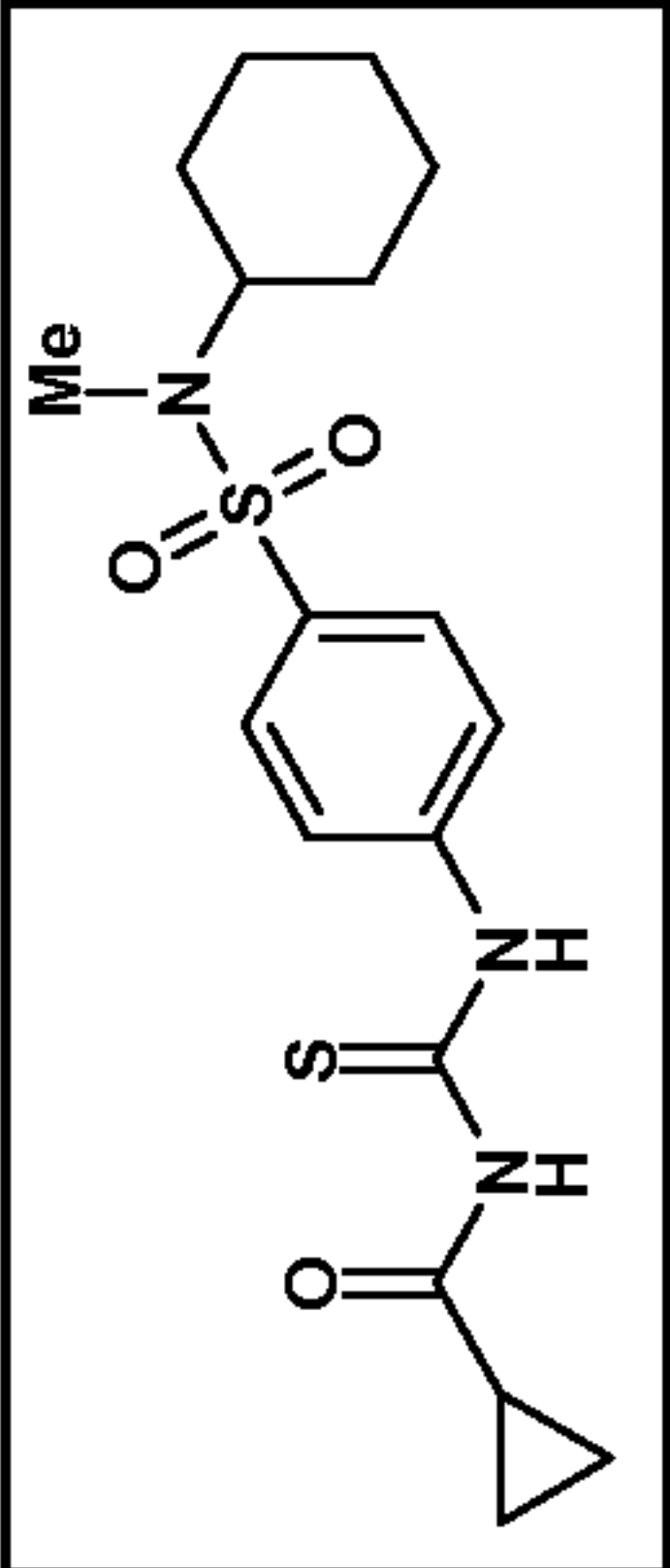
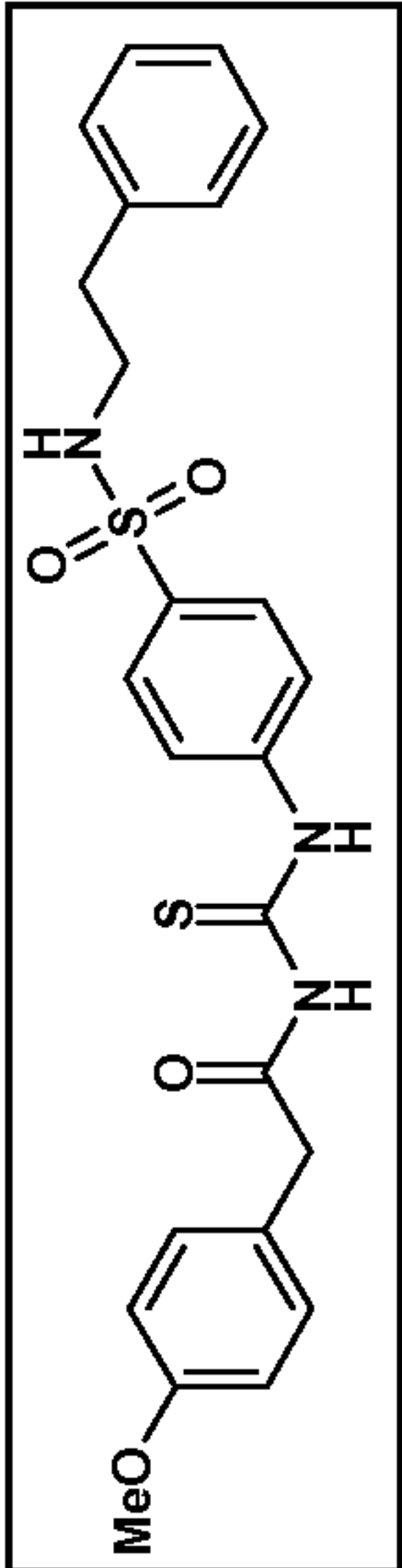
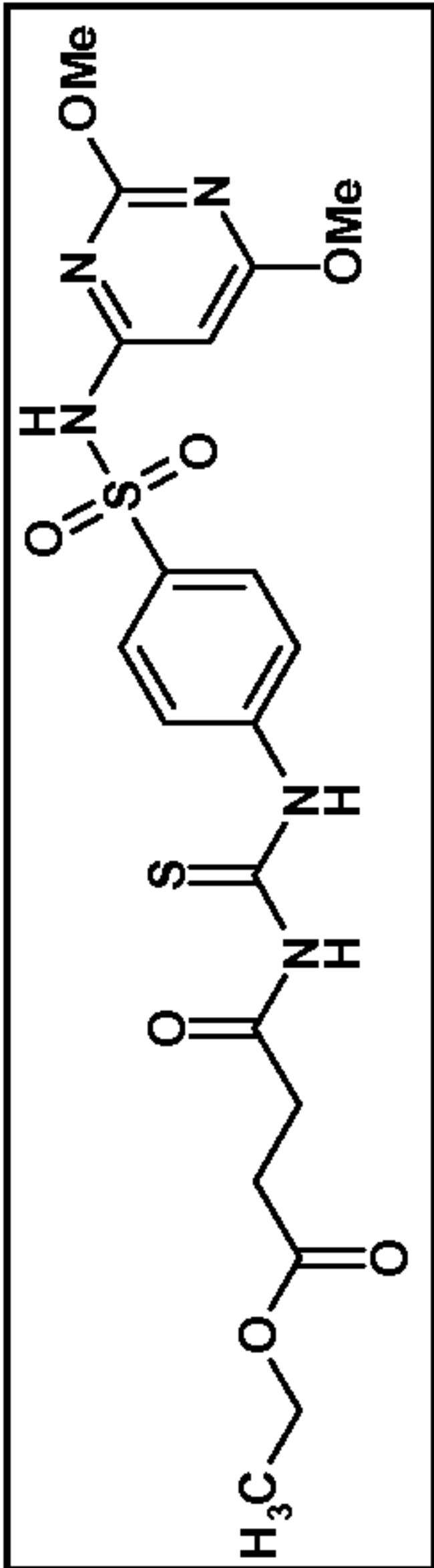
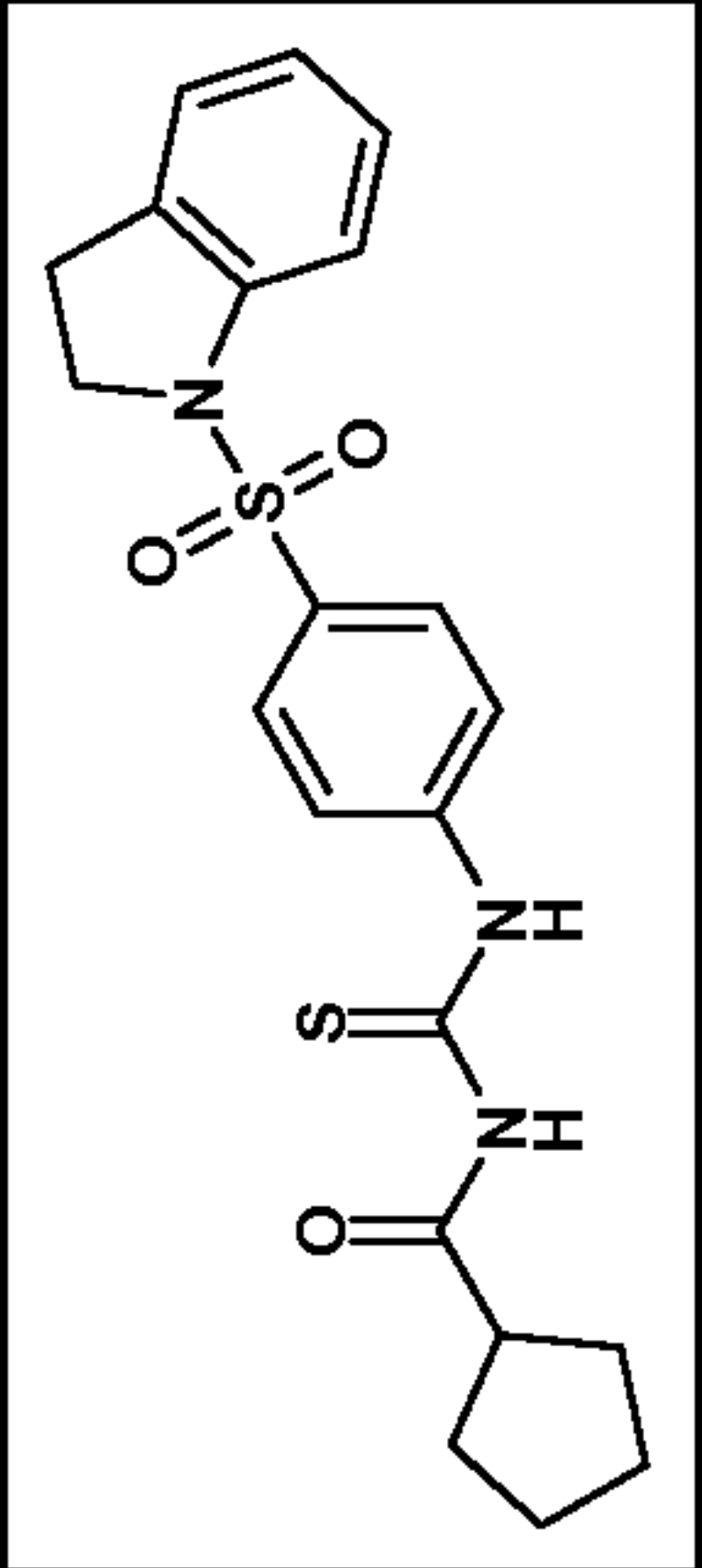
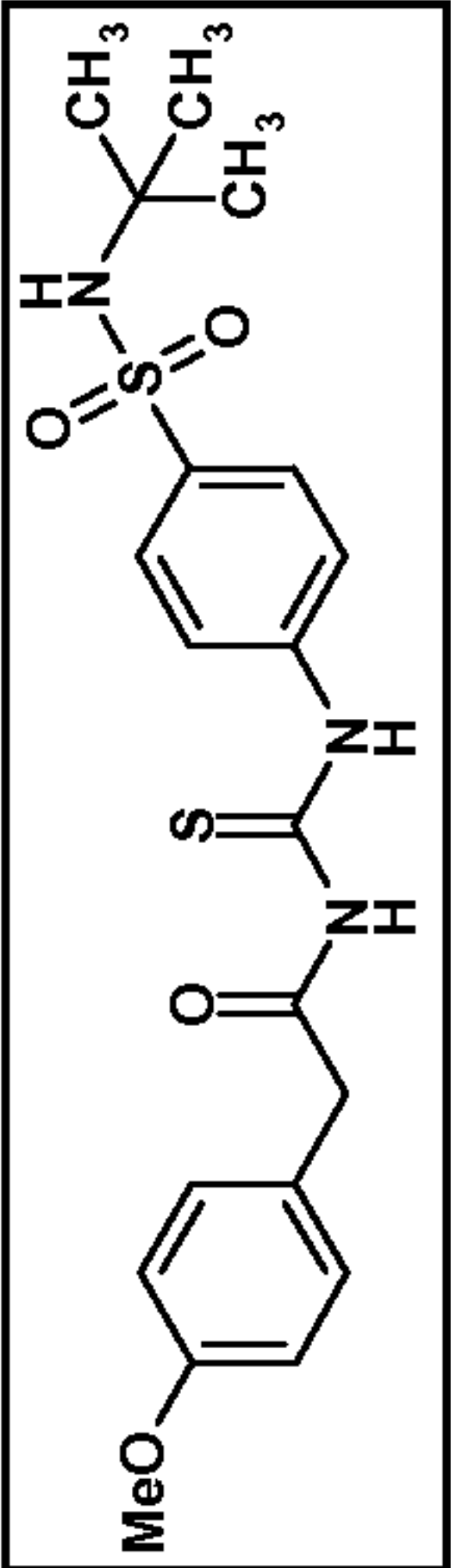
[illegible]

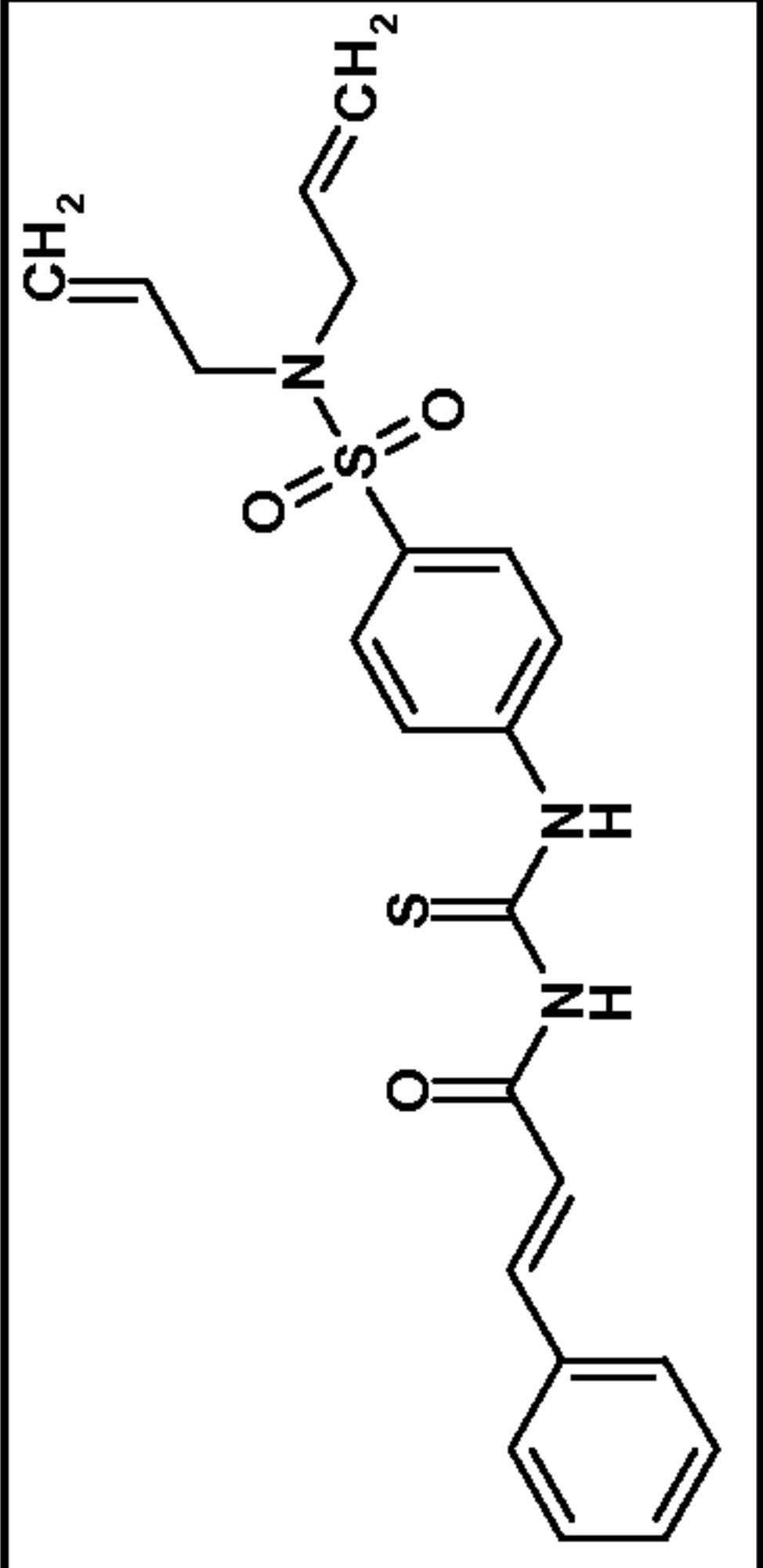
	AD4-11994	K	-6.23	-55.137	27.18		-1.85	-35.39
	AD4-11973	N	-4.87	-45.457	27.176		-3.5	-42.87
	AD4-12147	N	-3	-57.789	26.967		-0.46	-40.6
	AD4-11976	L	-2.46	-54.259	26.918		-2.33	-34.3
	AD4-11406	N	-7.04	-54.126	26.851		-4.09	-39.79

	AD4-12286	N	-4.81	-52.846	26.829	15.46/29.94(1.94)	-4.26	-32.26
	AD4-12157	L	-4.38	-52.787	26.73		-1.28	-39.47
	AD4-12163	J	-6.08	-59.91	26.671		-3.35	-43.82
	AD4-11405	J	-6.94	-58.643	26.497		-4.41	-53.71
	AD4-11888	O	-6.74	-63.413	26.355		-3.37	-41.13

		AD4-11395	J	-6.11	-55.753	26.301				-4.63	-49.78		
		AD4-12103	K	-7.21	-63.637	26.212				-3.1	-44.09		
		AD4-11523	K	-6	-59.162	26.098				-3.83	-33.07		
		AD4-12075	L	-6.25	-65.453	25.956			19.24/40.11(2.08)	-7.04	-41.76		

	AD4-12098	K	-5.89	-53.46	25.946	23.36/16.41(0.70)	0.121	-33.78
	AD4-11879	O	-4.9	-68.161	25.689		-2.48	-47.6
	AD4-12014	K	-5.59	-65.343	25.611		-3.5	-34.96
	AD4-11401	N	-5.59	-56.827	25.557		-3.9	-51.43
	AD4-11978	L	-3.01	-51.532	25.53		-2.15	-31.77

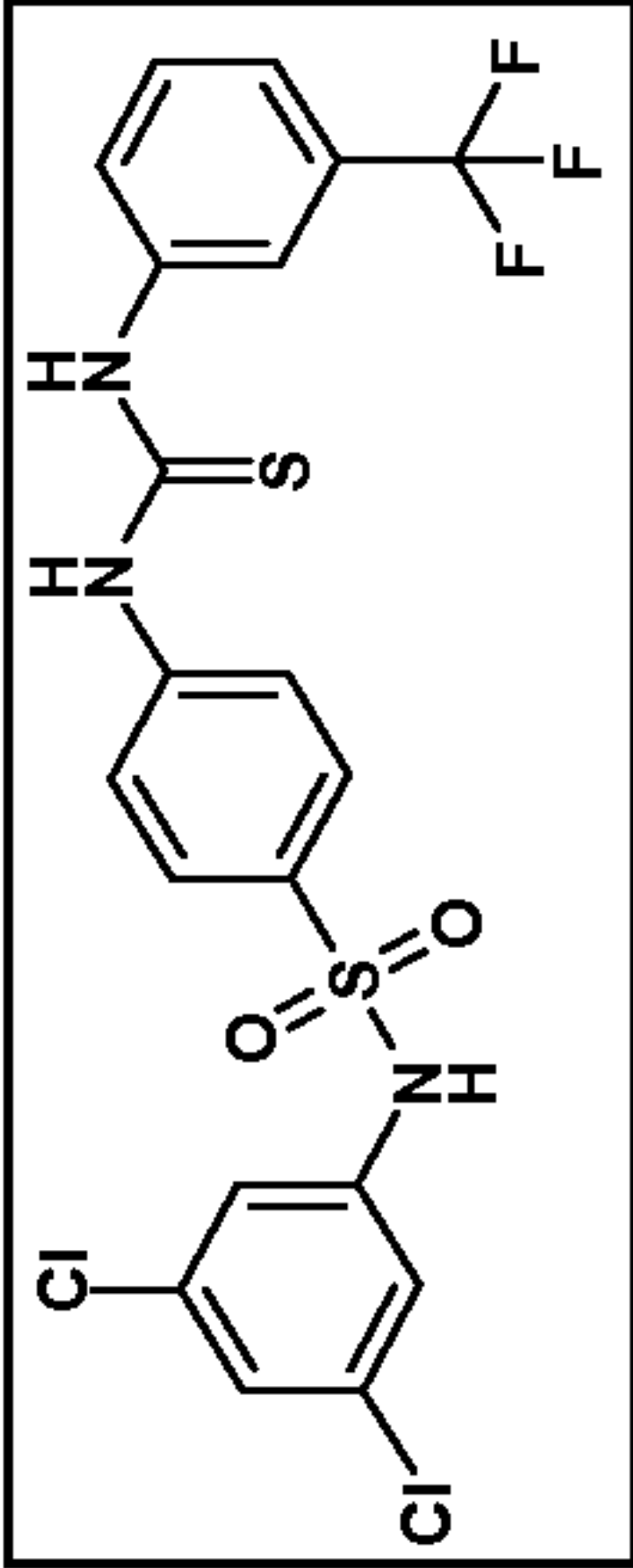
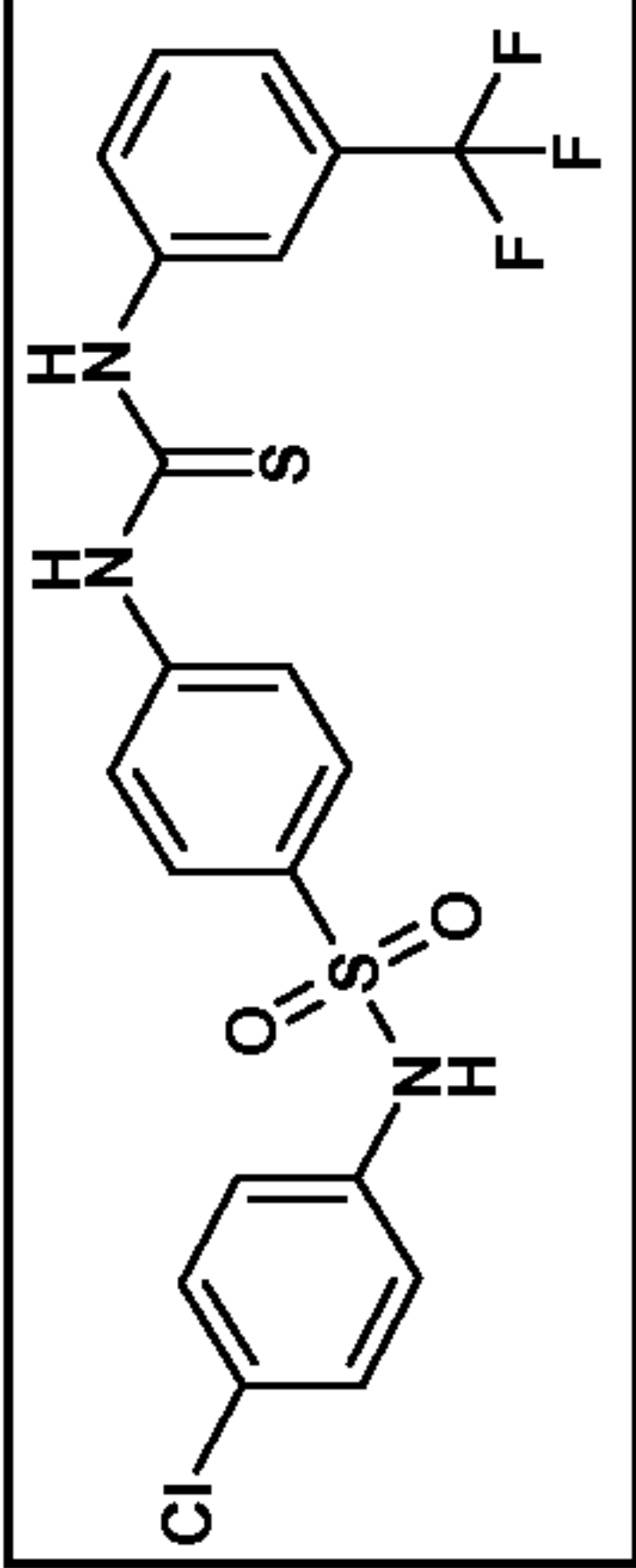
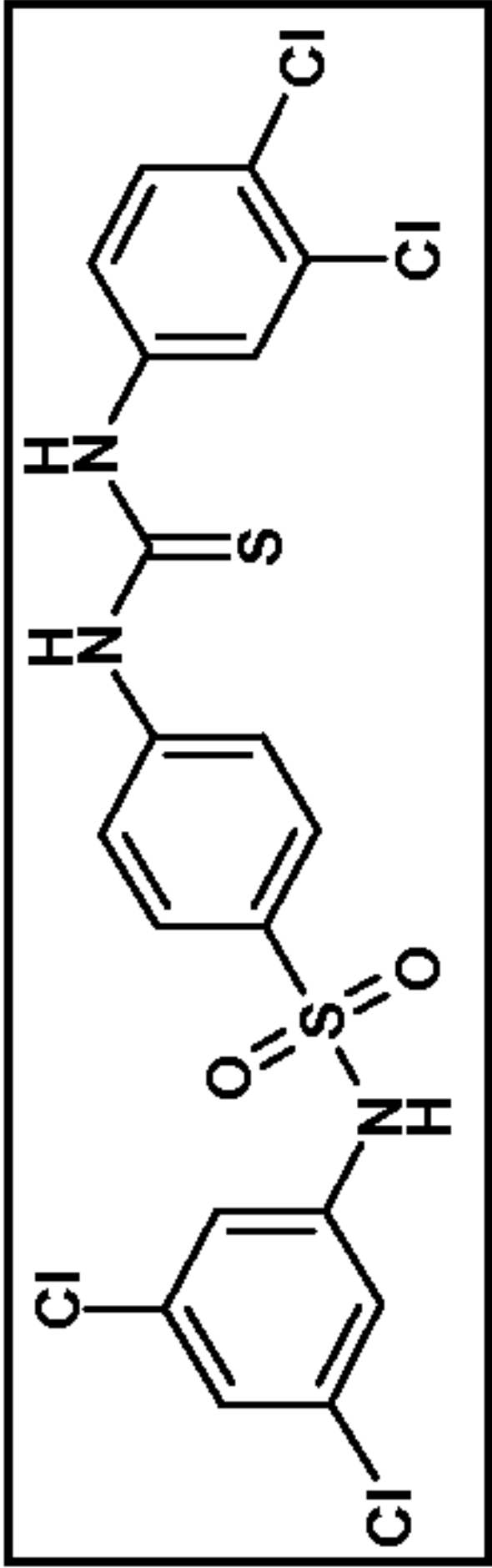
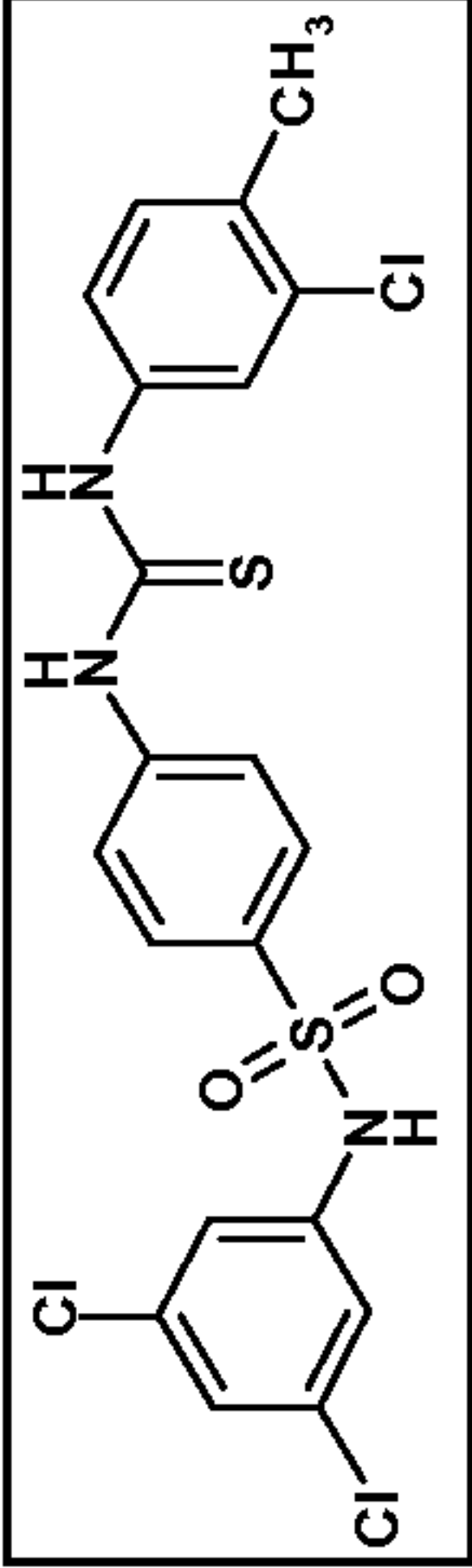
	AD4-11986	L	-5.62	-53.997	25.368		-4.32	-35.72
	AD4-10622	L	-5.32	-69.992	25.347		-5	-49.48
	AD4-11980	J	-4.48	-57.693	25.235	49.82/24.40(0.49)	-2.39	-48.62
	AD4-12291	M	-7.05	-58.86	25.191		-3.47	-34.09
	AD4-12133	L	-5.78	-42.682	25.058		-0.57	-35.65

	AD4-12092	L	-4.77	-52.761	25.019		-1.58	-29.14
--	-----------	---	-------	---------	--------	--	-------	--------

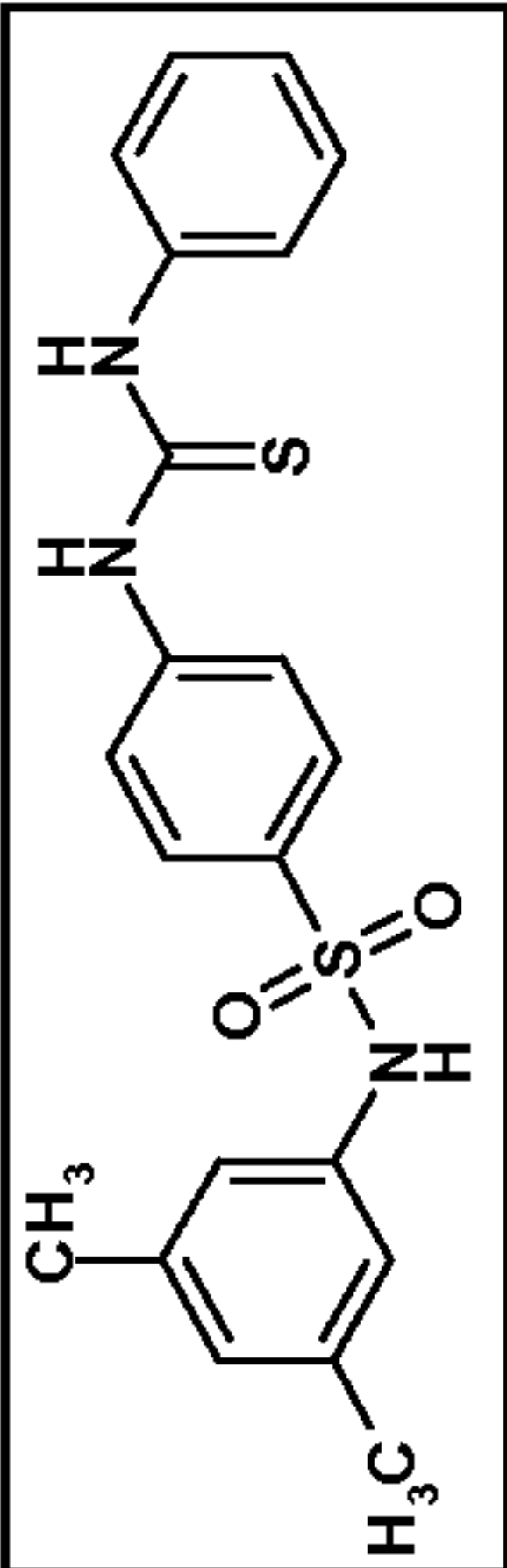
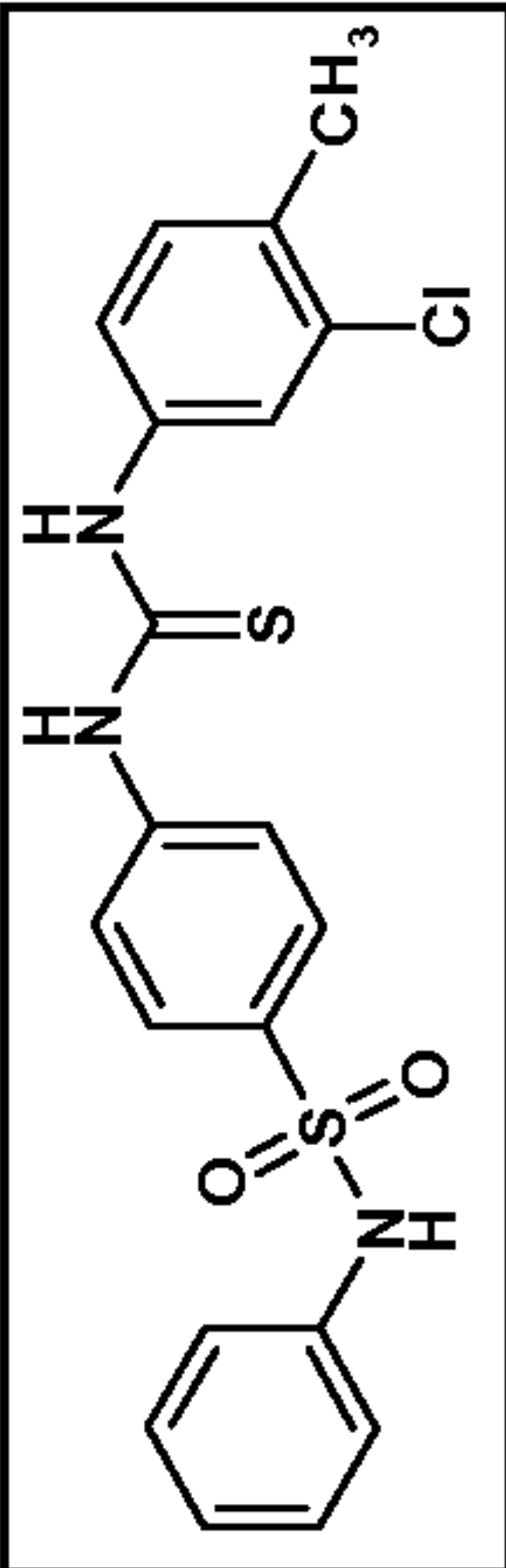
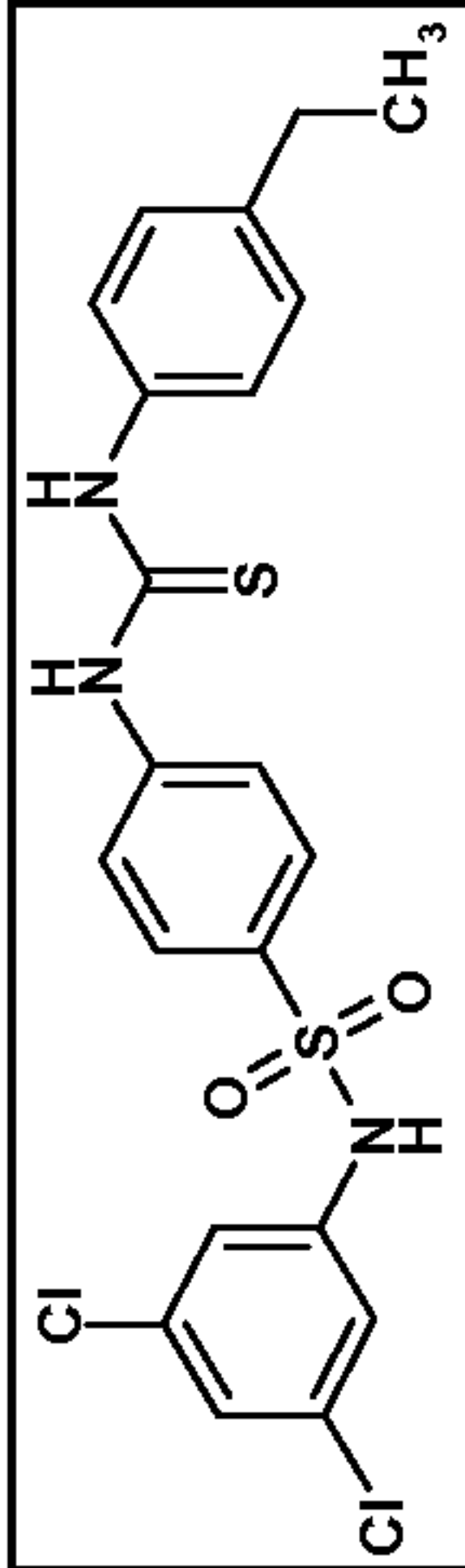
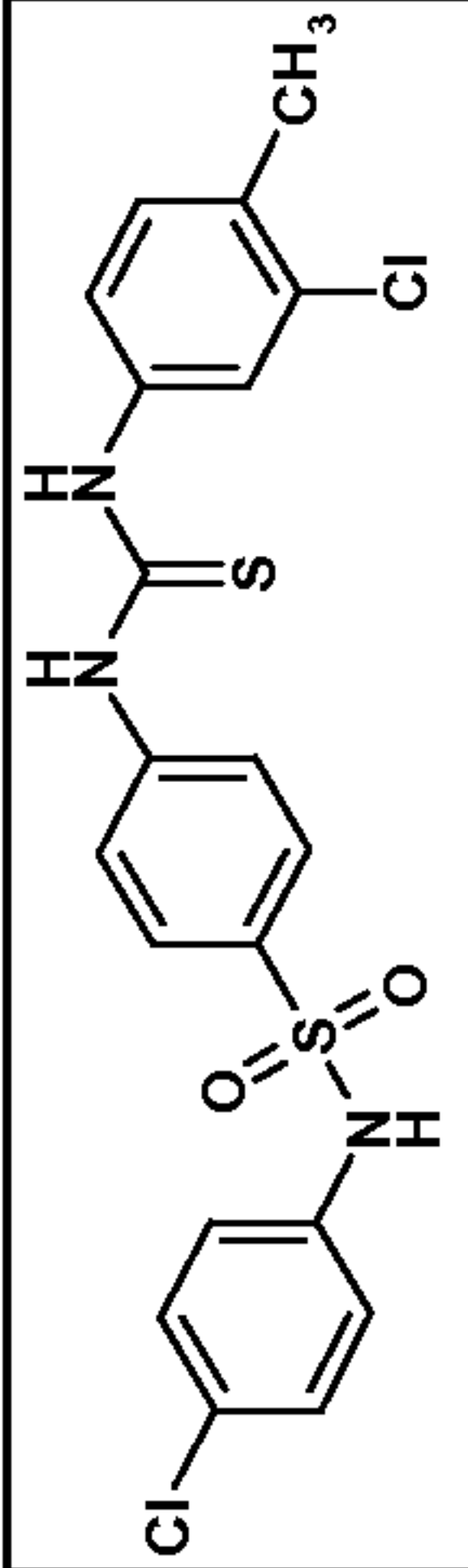
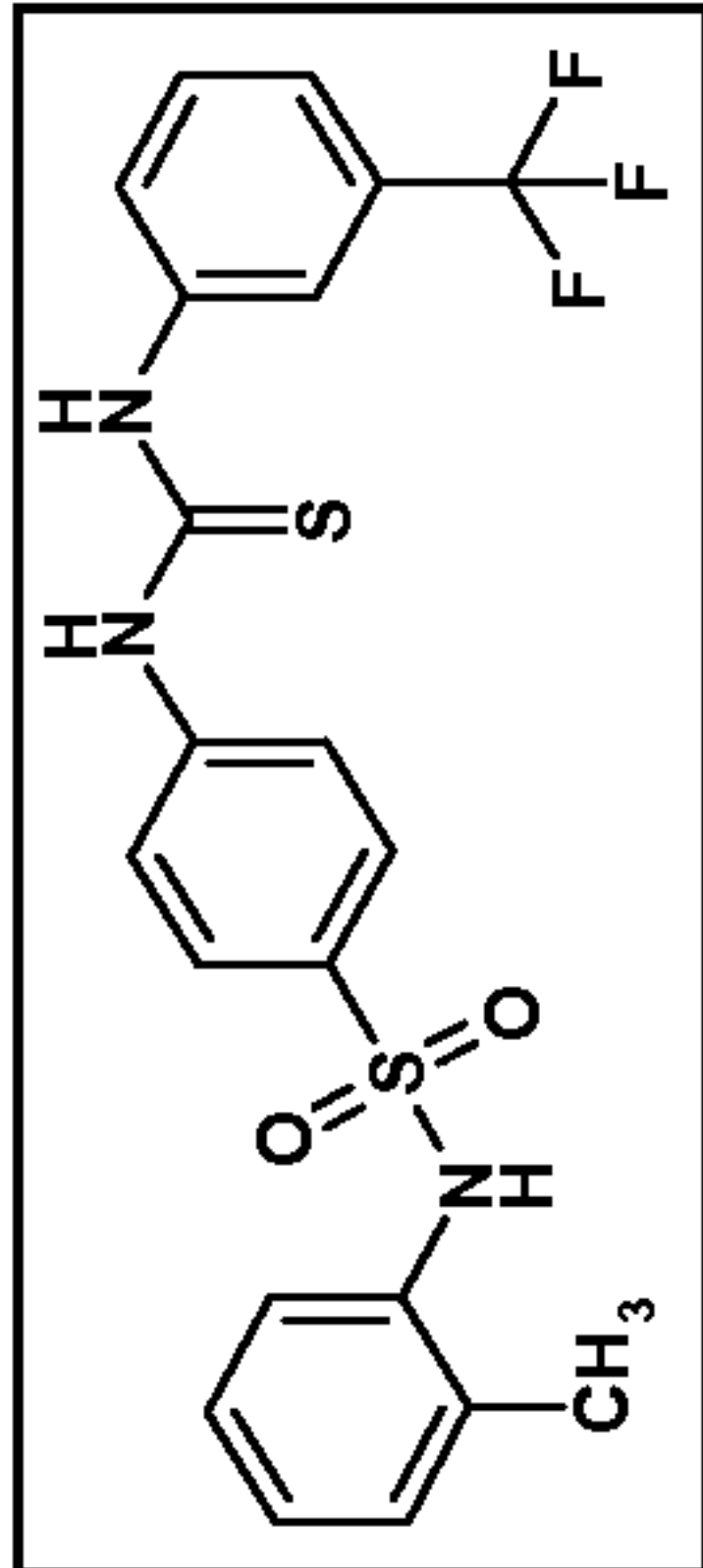
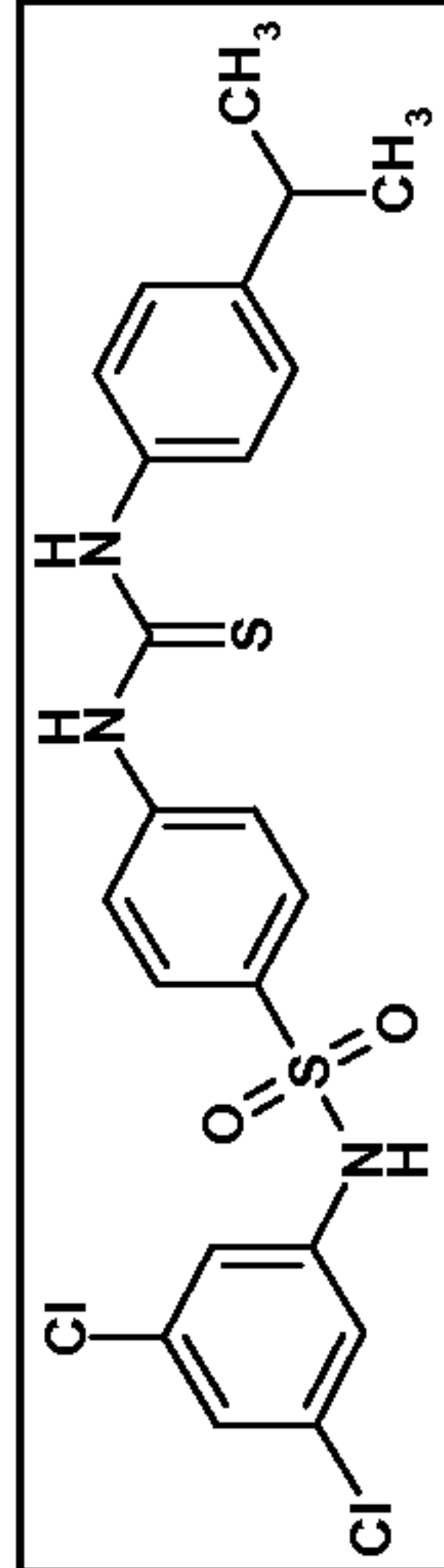
[0504] AD4-11091-like

[0505] The AD4-11091-like compounds in TABLE 10 were identified via Glide docked pose of AD4-1886. Depicted in TABLE 10 is Glide score, E-model score, and ICW assay and MTT assay results (See Examples 1-3).

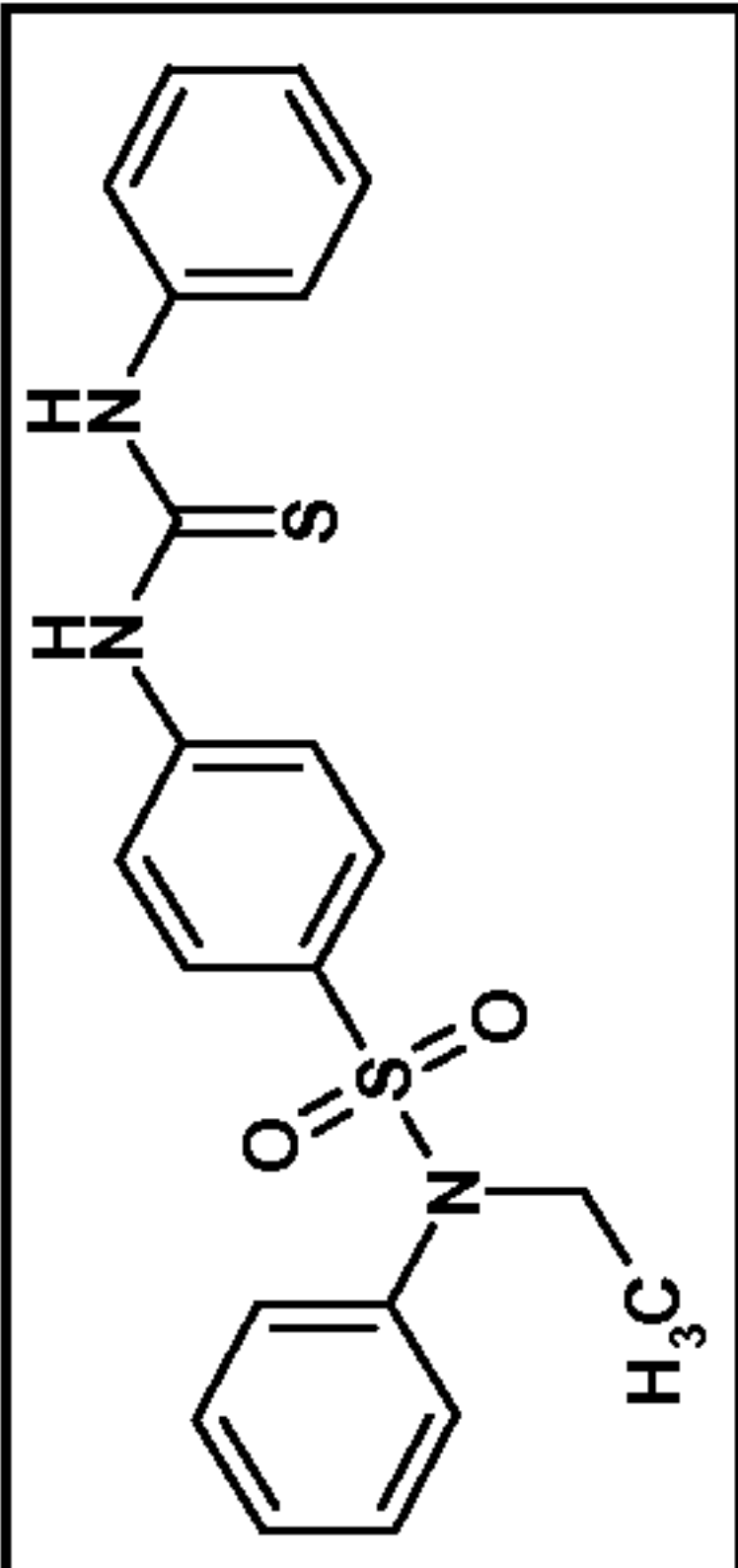
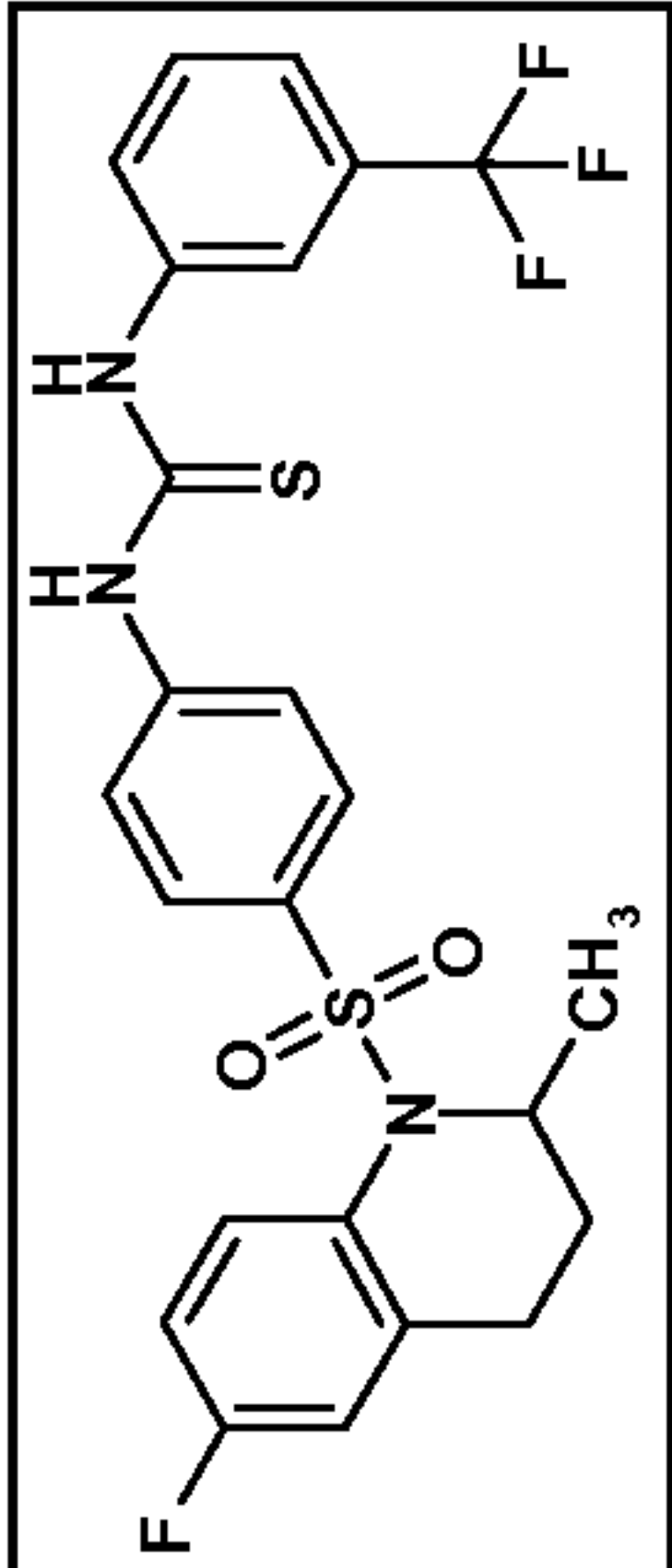
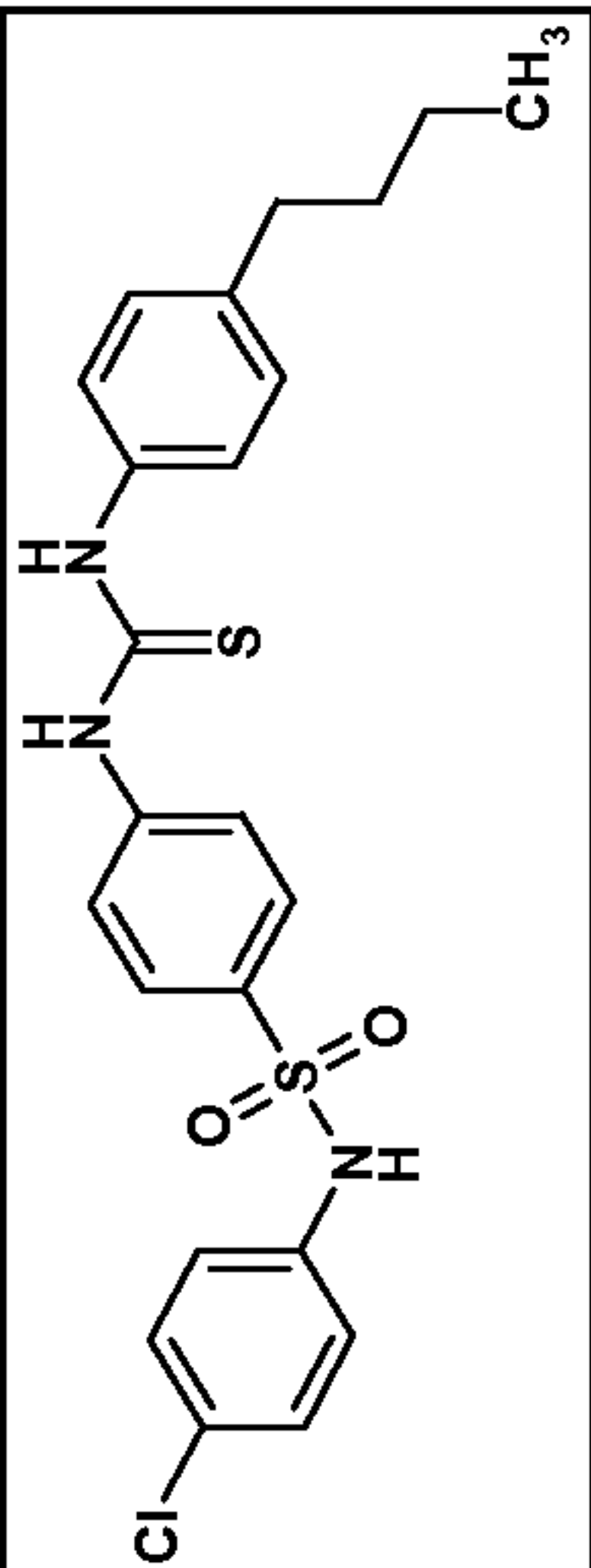
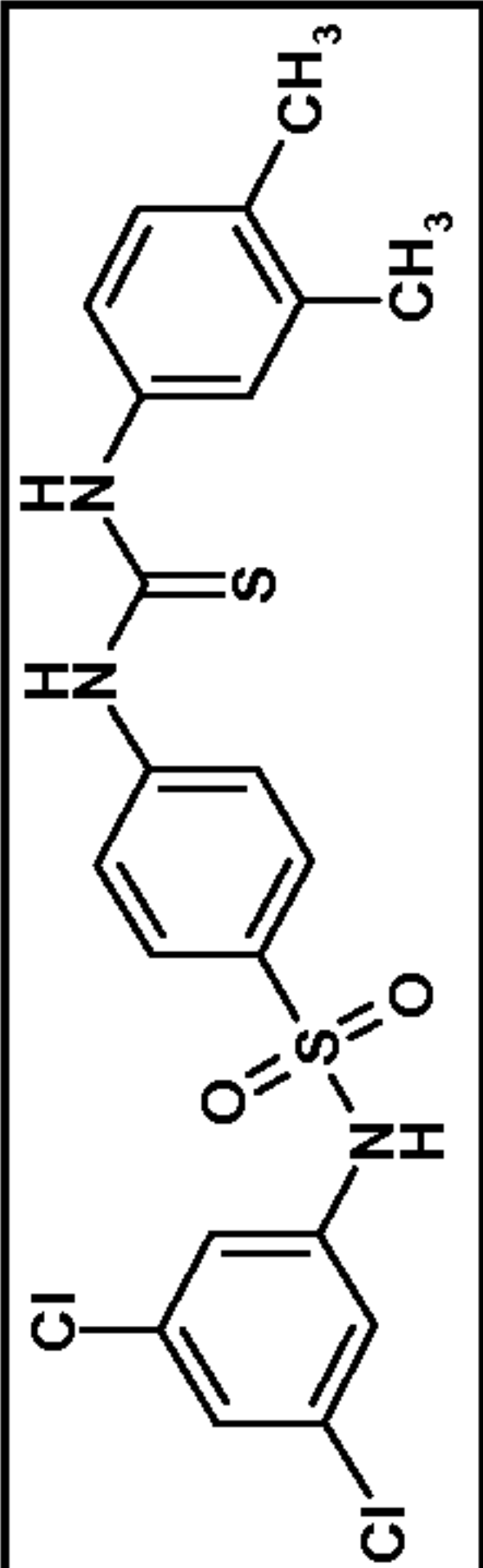
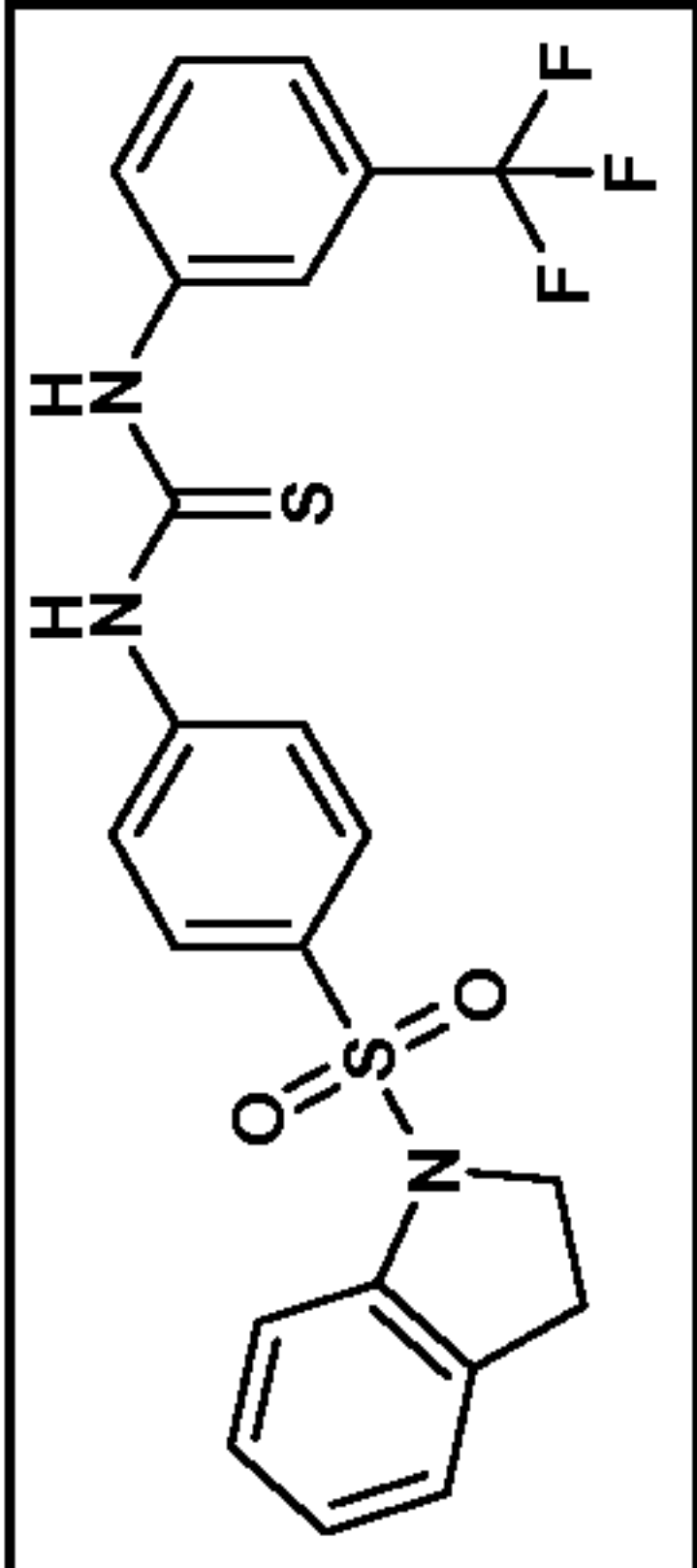
TABLE 10: Glide Score, E-Model score, ICW assay, and MTT Assay Results for AD4-11091-like Compounds.

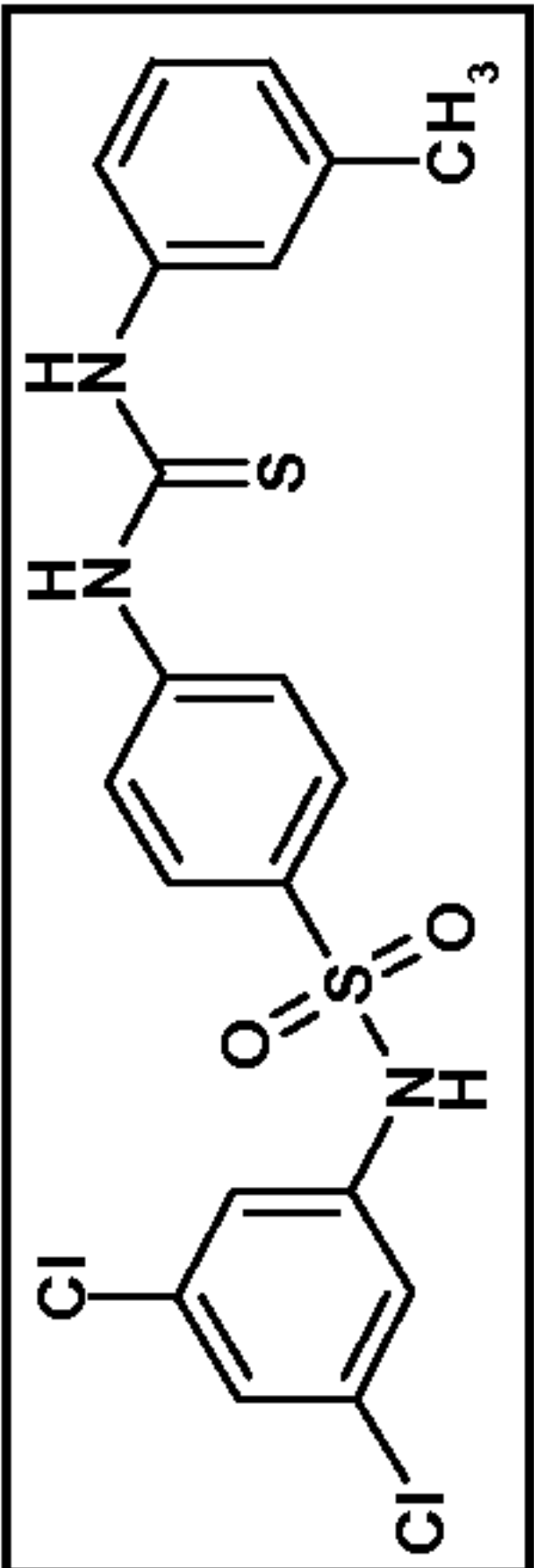
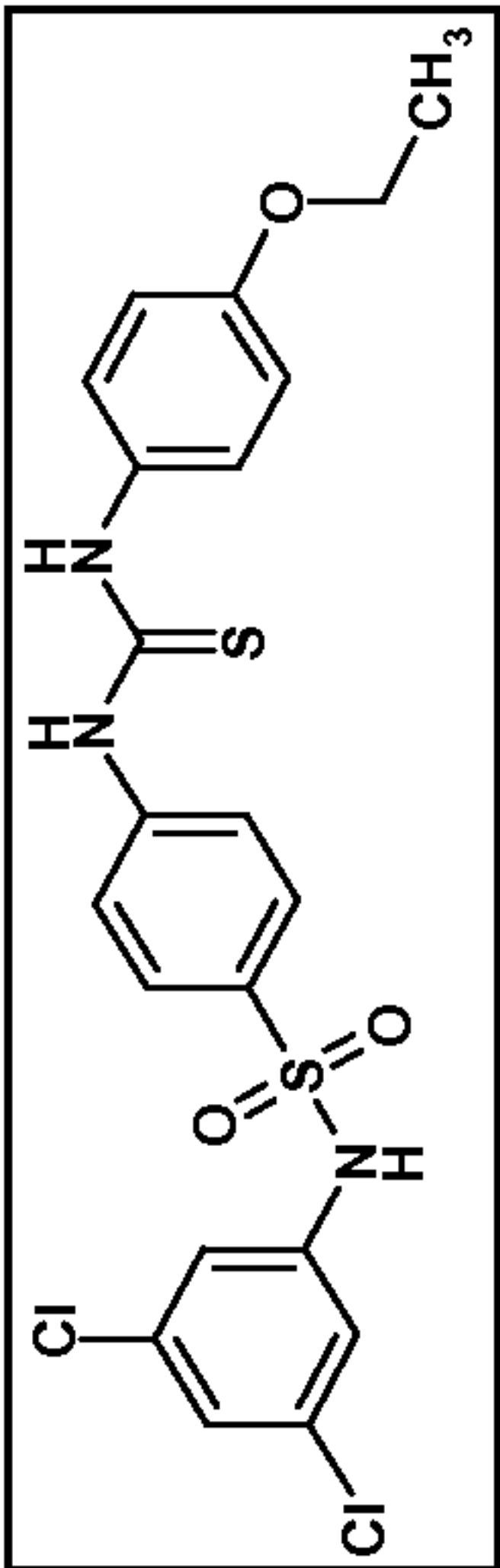
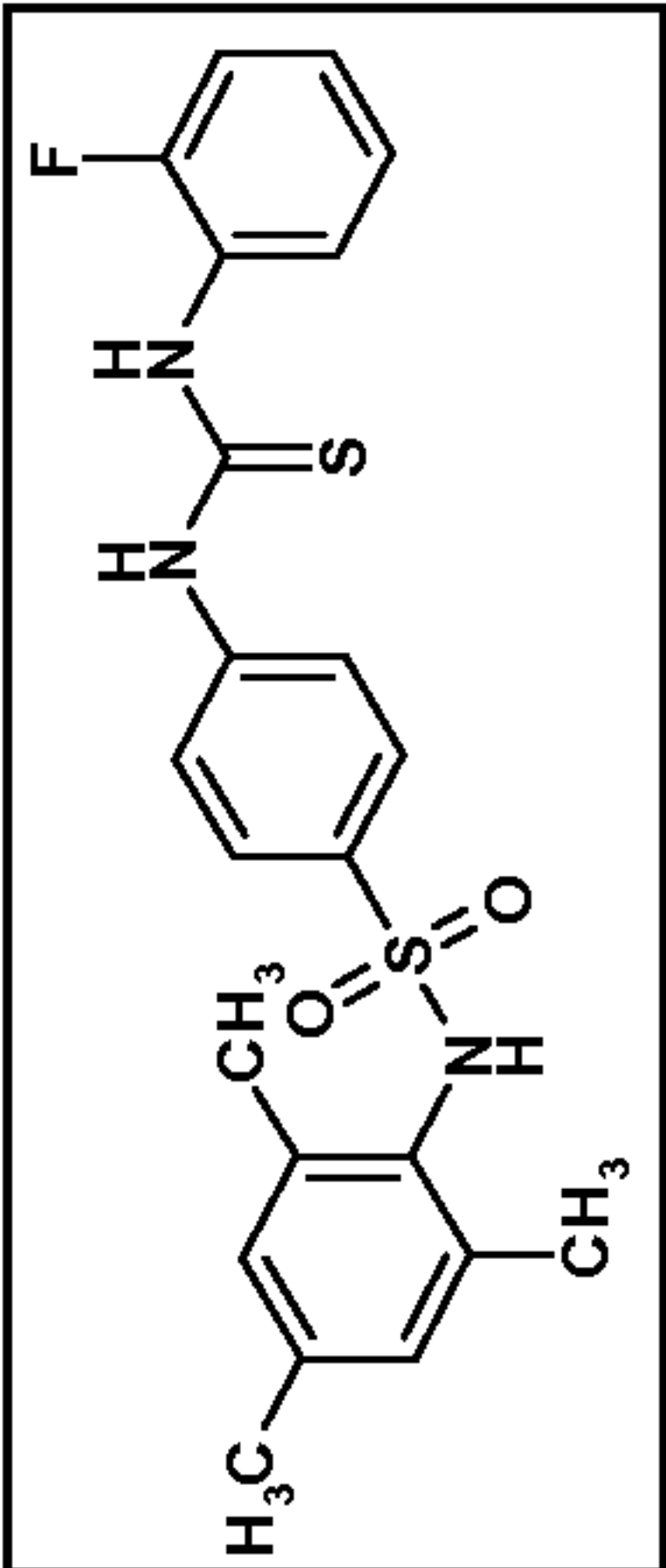
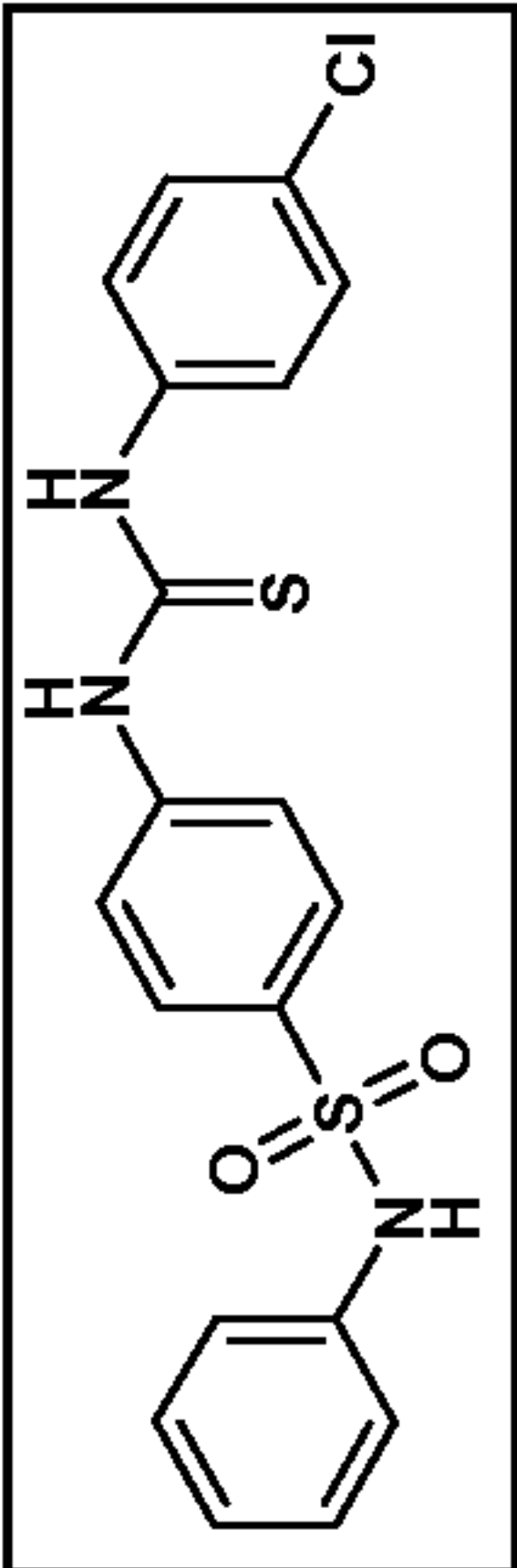
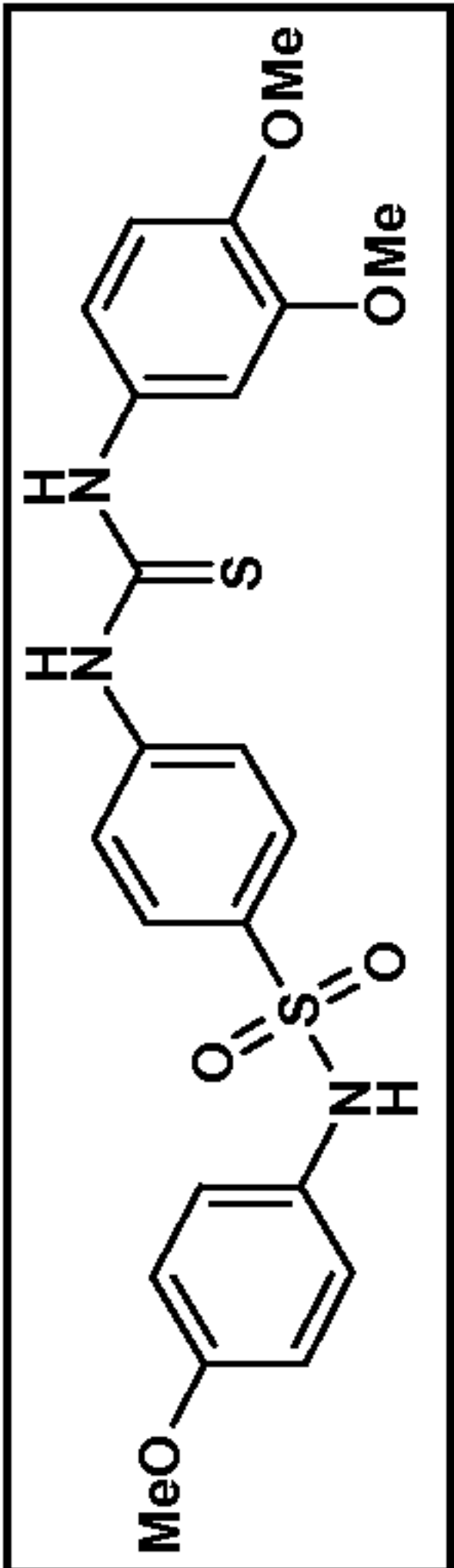
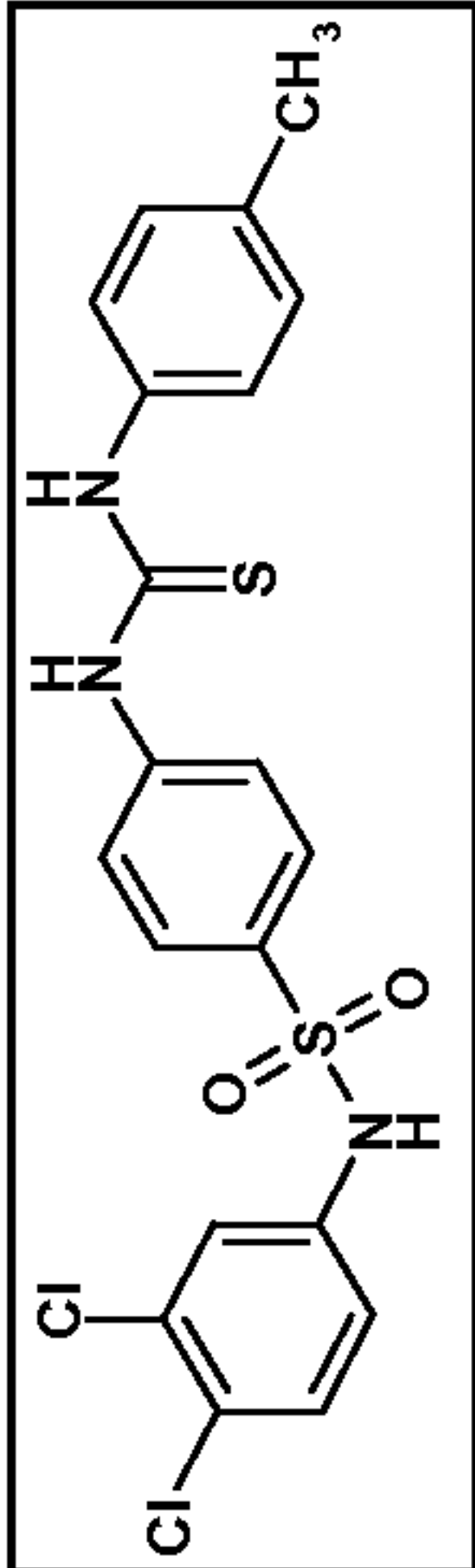
AD4-11091 LIKE by ICW Inhibition	AD4-No.	Glue - G-Score	Glue - E-Model	ICW assay	MTT assay	Erbtux - G-Score	Erbtux - E-Model	Pharmacophore Query
	AD4-12509	-6.15	-54.56	140.975	5.49/5.24(0.95)	-2.169	-37.161	1YY9 - AD4-11091 LIKE
	AD4-12423	-5.16	-62.7	129.155	6.35/5.77(0.91) 4.23/2.92(0.69)	-4.008	-31.651	1YY9 - AD4-11091 LIKE
	AD4-12528	-7.06	-54.89	123.946	5.43/5.30(0.98)	-4.226	-40.6	1YY9 - AD4-11091 LIKE
	AD4-12522	-5.62	-59.43	123.893	6.20/5.33(0.86)	-4.336	-39.519	1YY9 - AD4-11091 LIKE

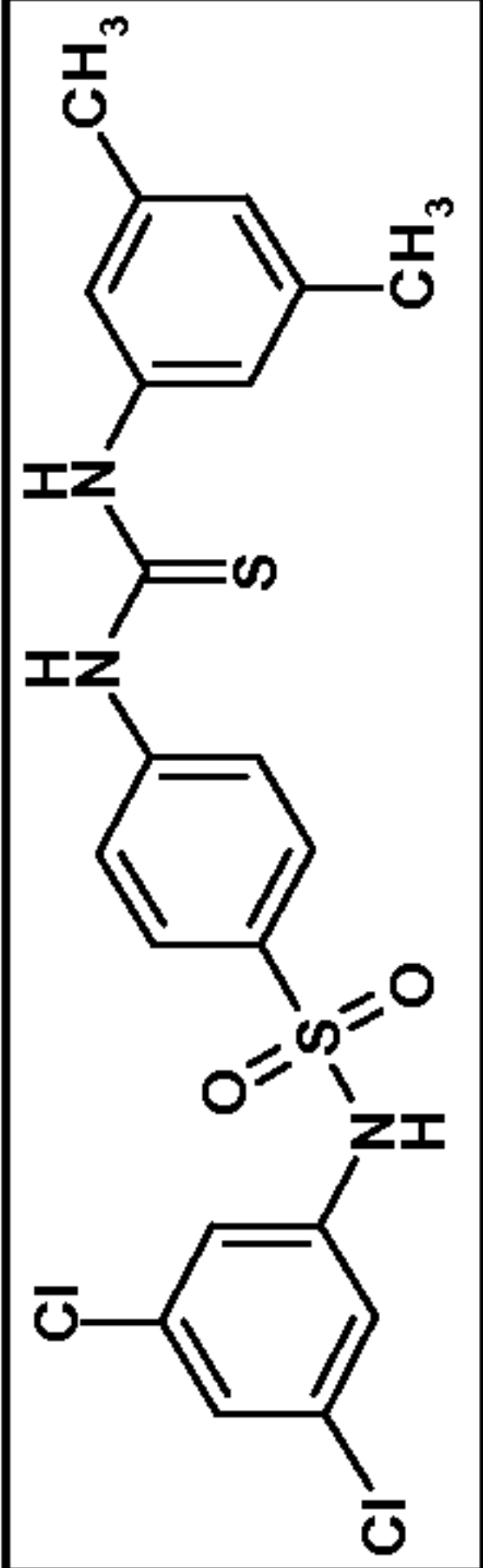
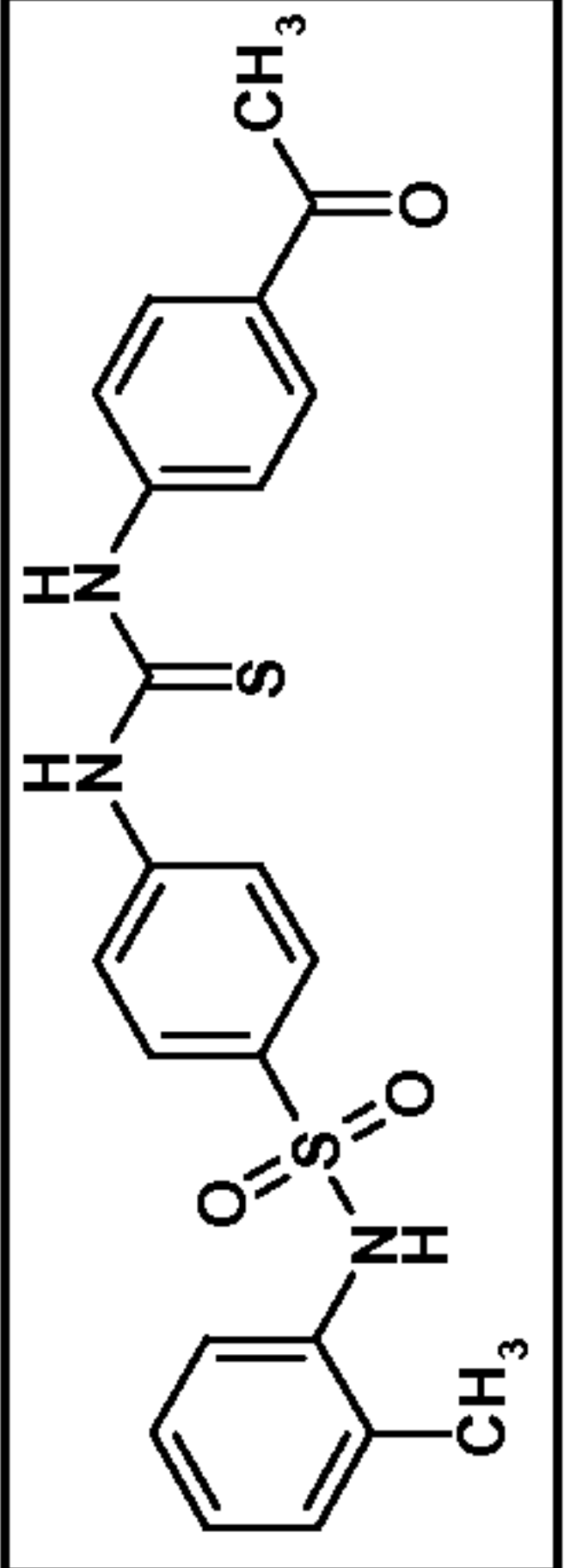
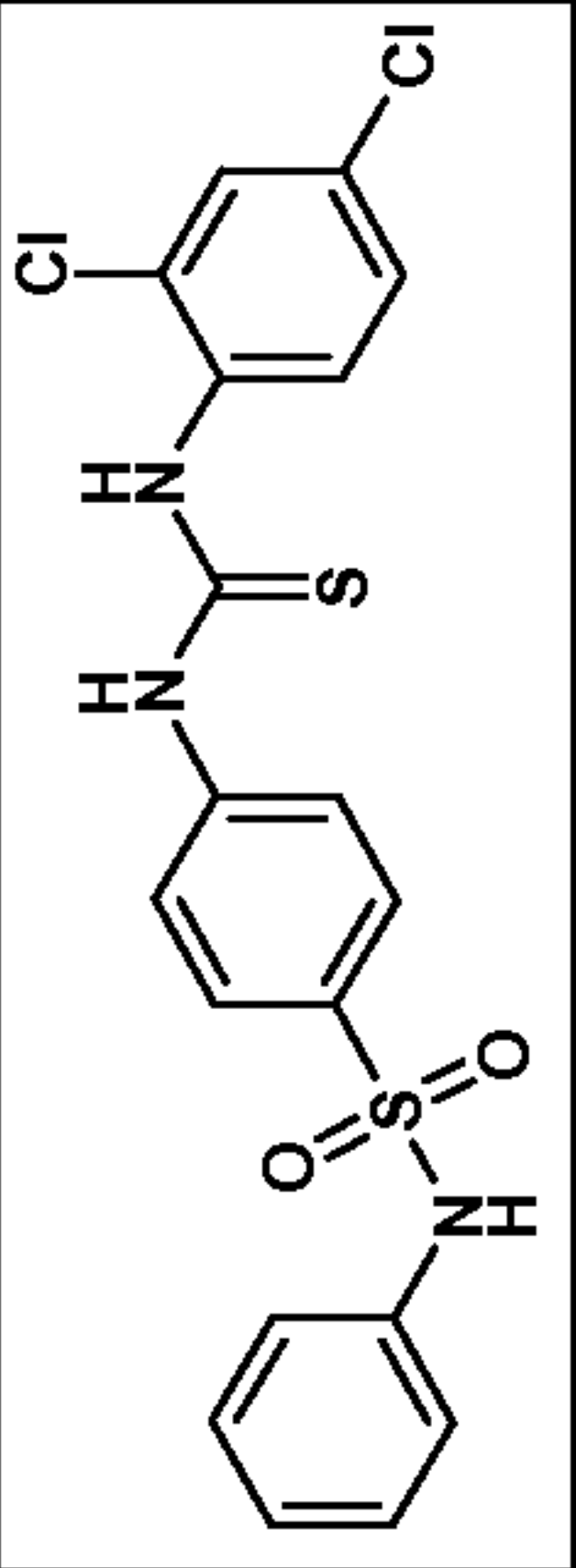
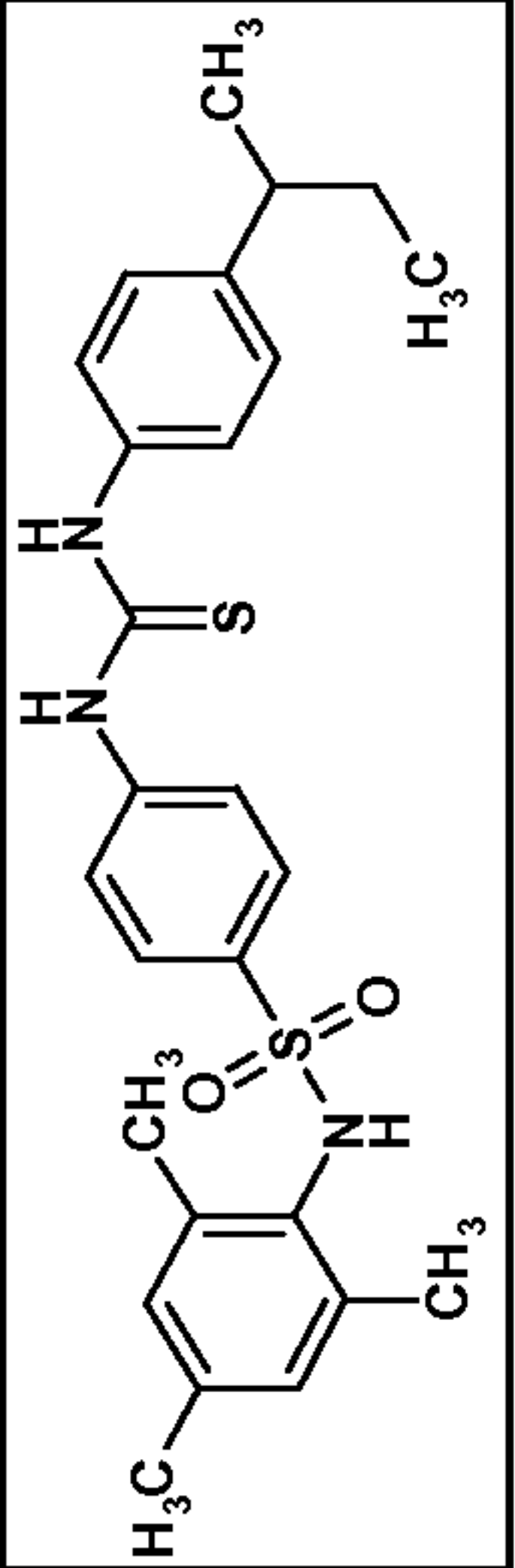
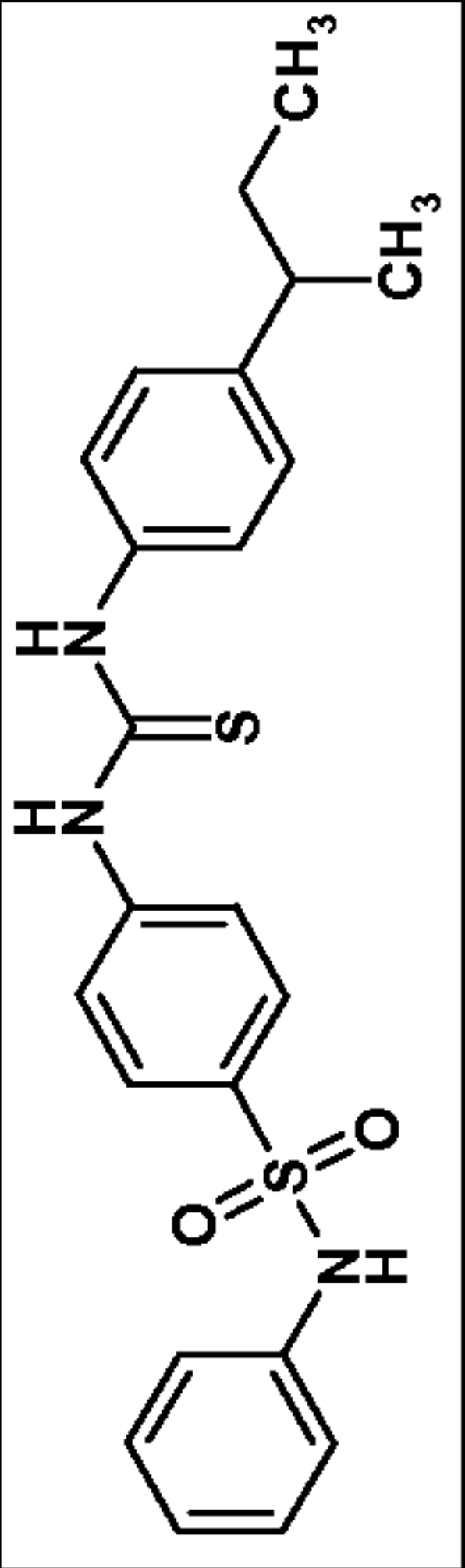
[illegible]

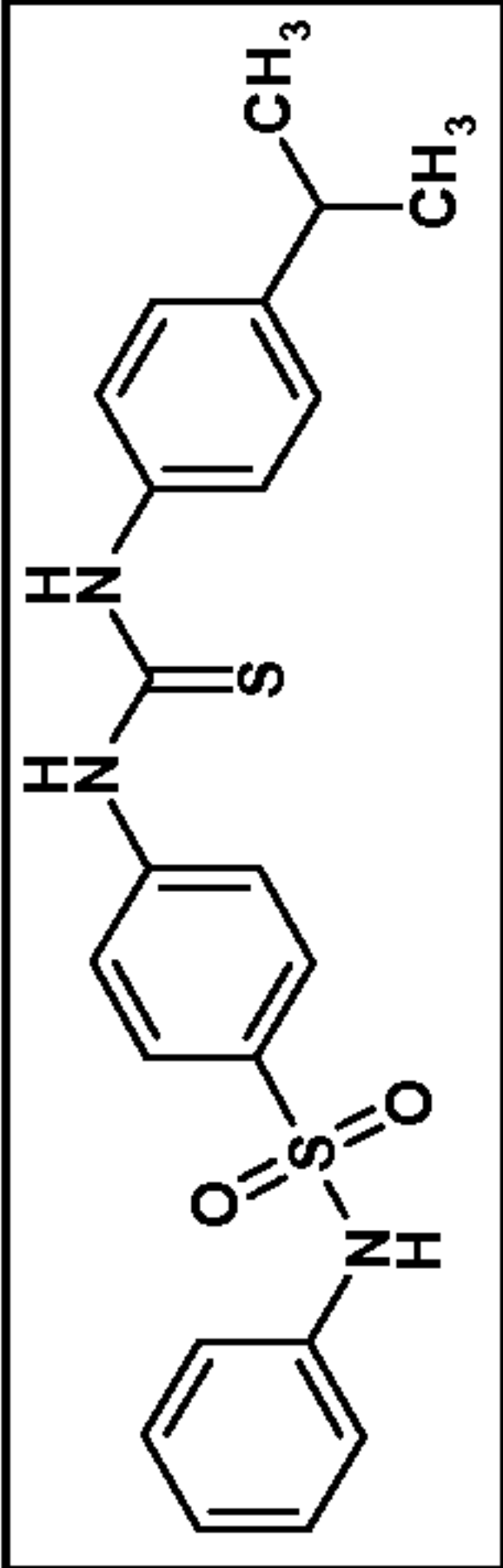
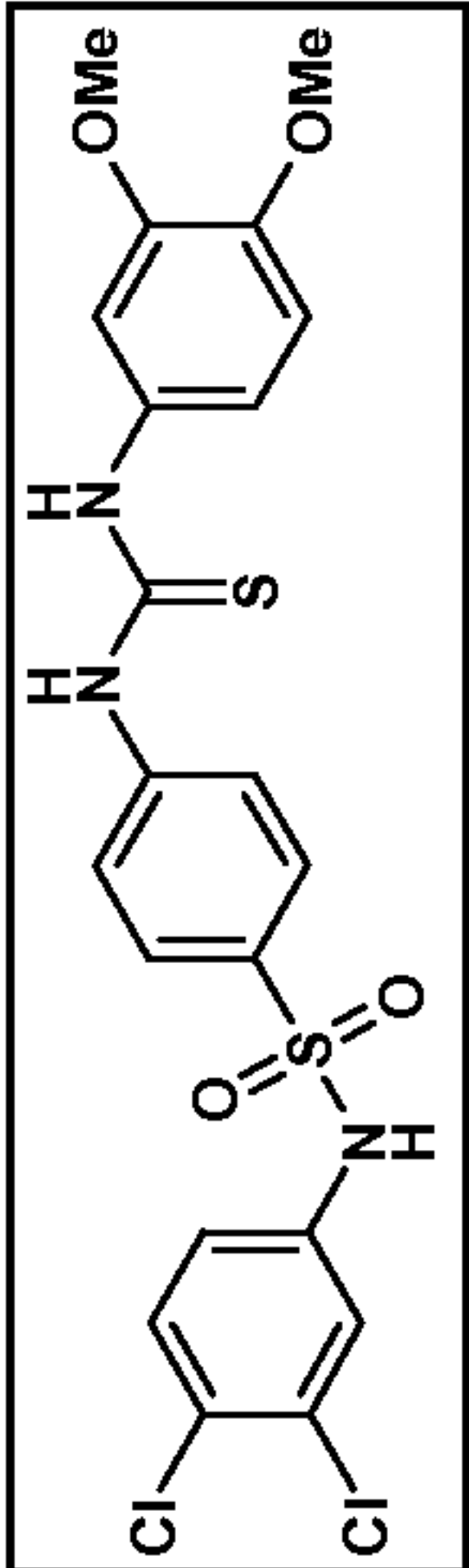
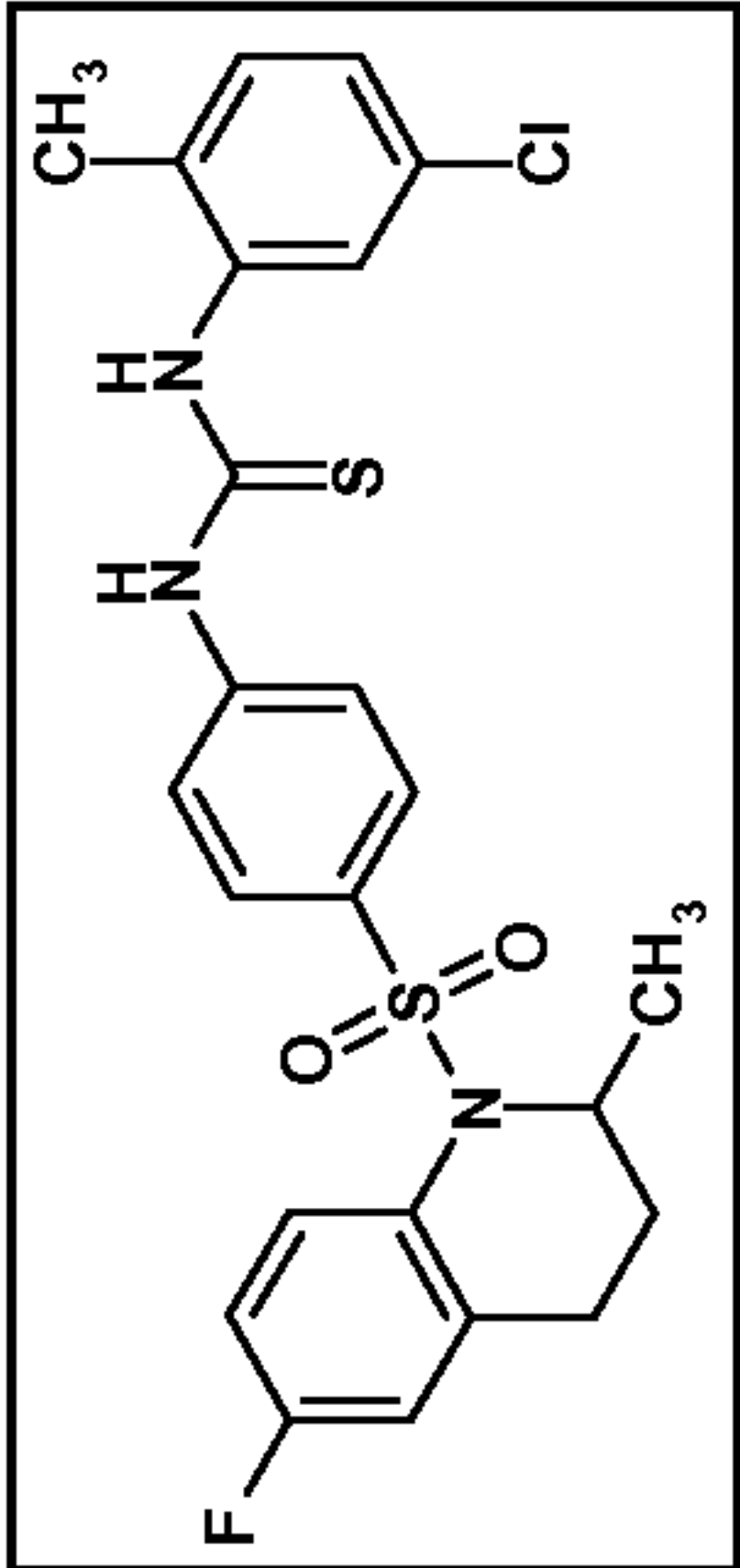
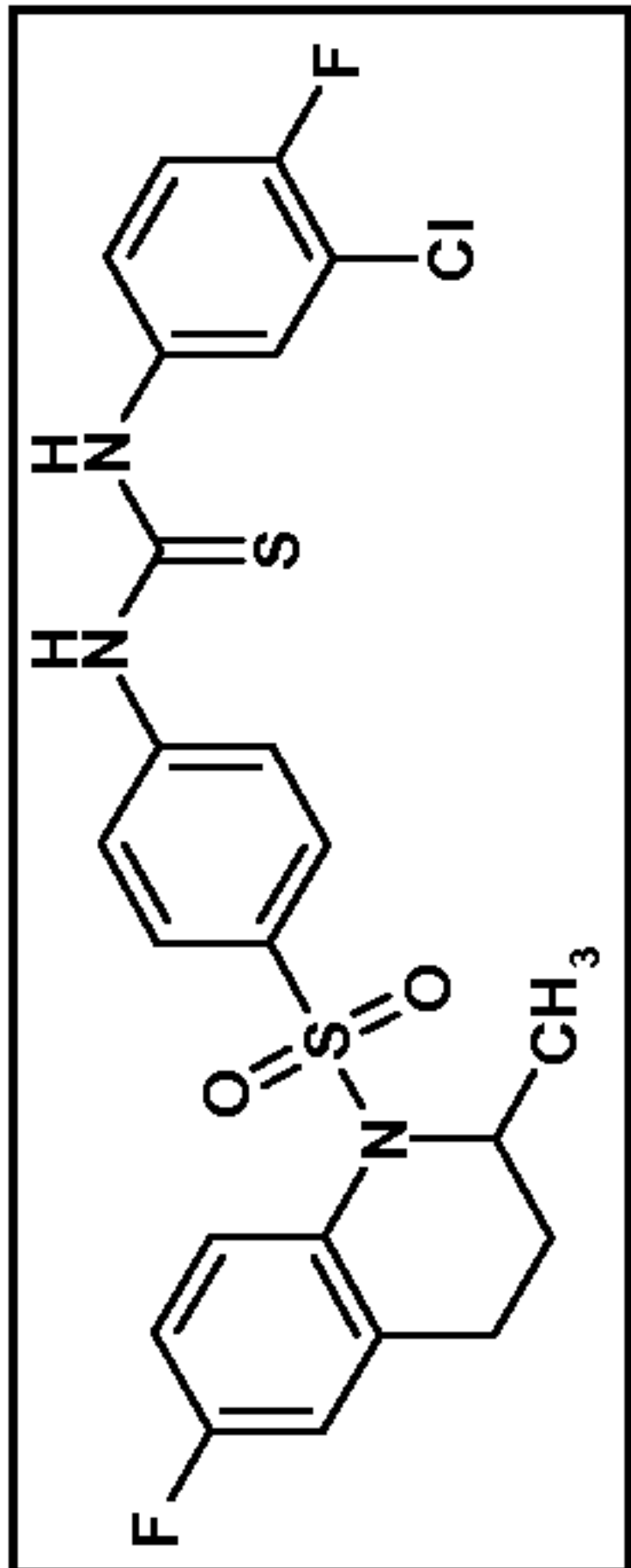
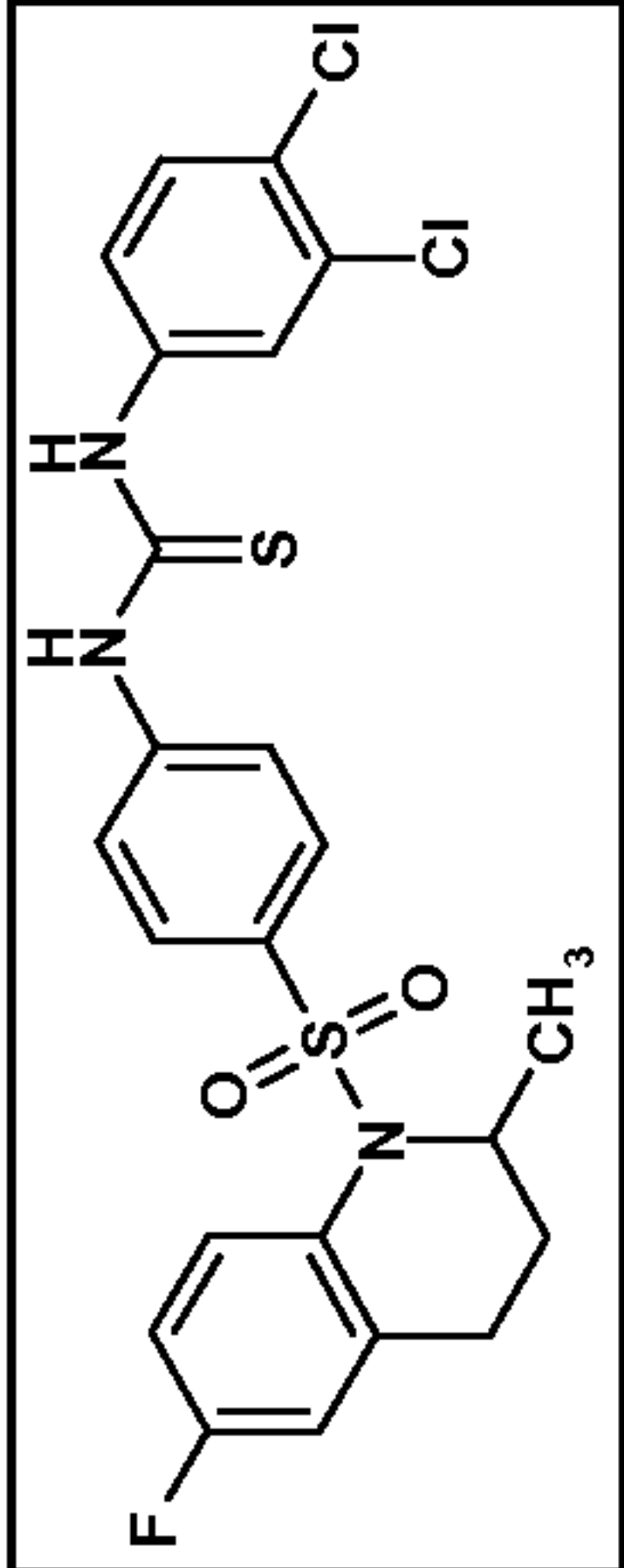
	AD4-11091	-4.2	-43.59	112.858	non-convergent	-3.987	-42.271	1NQL - AD4-1734 top 3
	AD4-12844	-5.23	-51.72	112.768	6.80/6.13(0.90)	-1.121	-36.357	1YY9 - AD4-11091 LIKE
	AD4-12436	-6.93	-61.5	108.826	5.47/5.53(1.01)	-3.738	-34.38	1YY9 - AD4-11091 LIKE
	AD4-12505	-5.92	-59.81	93.3284	5.69/6.02(1.06)	-3.807	-37.664	1YY9 - AD4-11091 LIKE
	AD4-12487	-6.35	-56.12	84.5602	6.45/5.50(0.85)	-3.209	-35.185	1YY9 - AD4-11091 LIKE
	AD4-12424	-6.87	-61.52	82.3761	4.08/4.73(1.16)	-3.929	-44.784	1YY9 - AD4-11091 LIKE

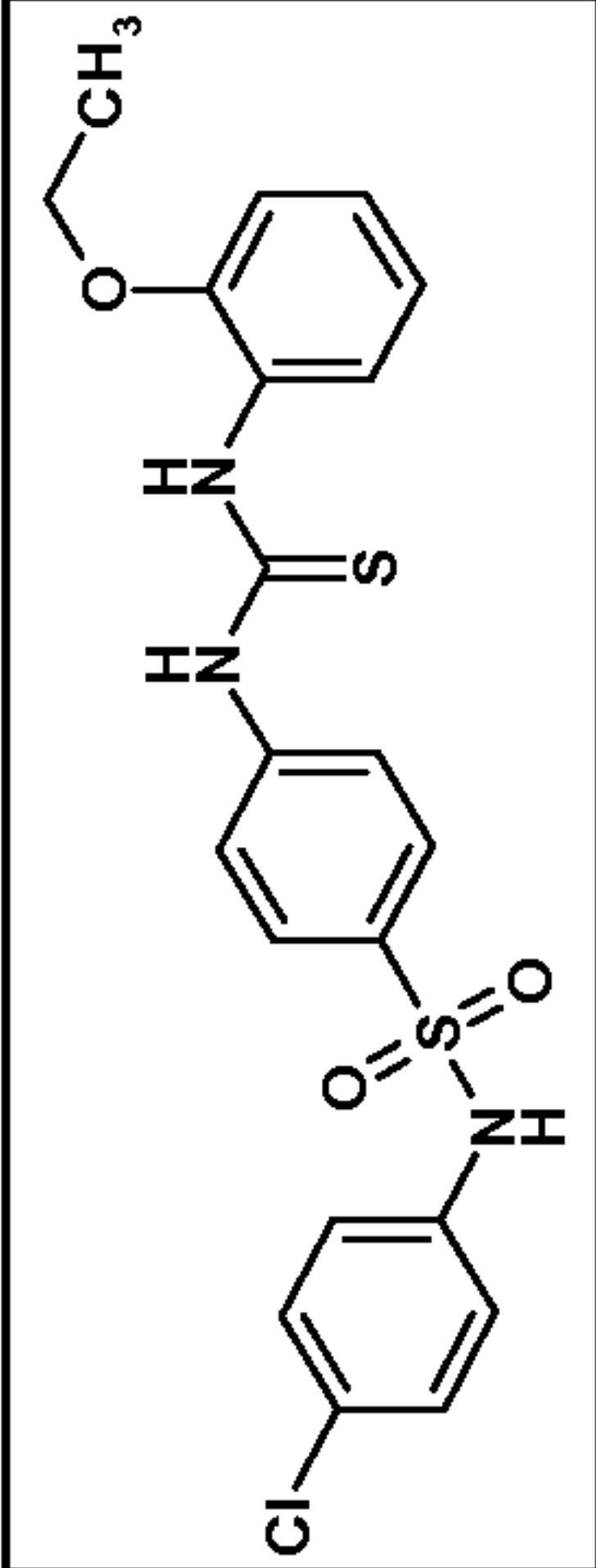
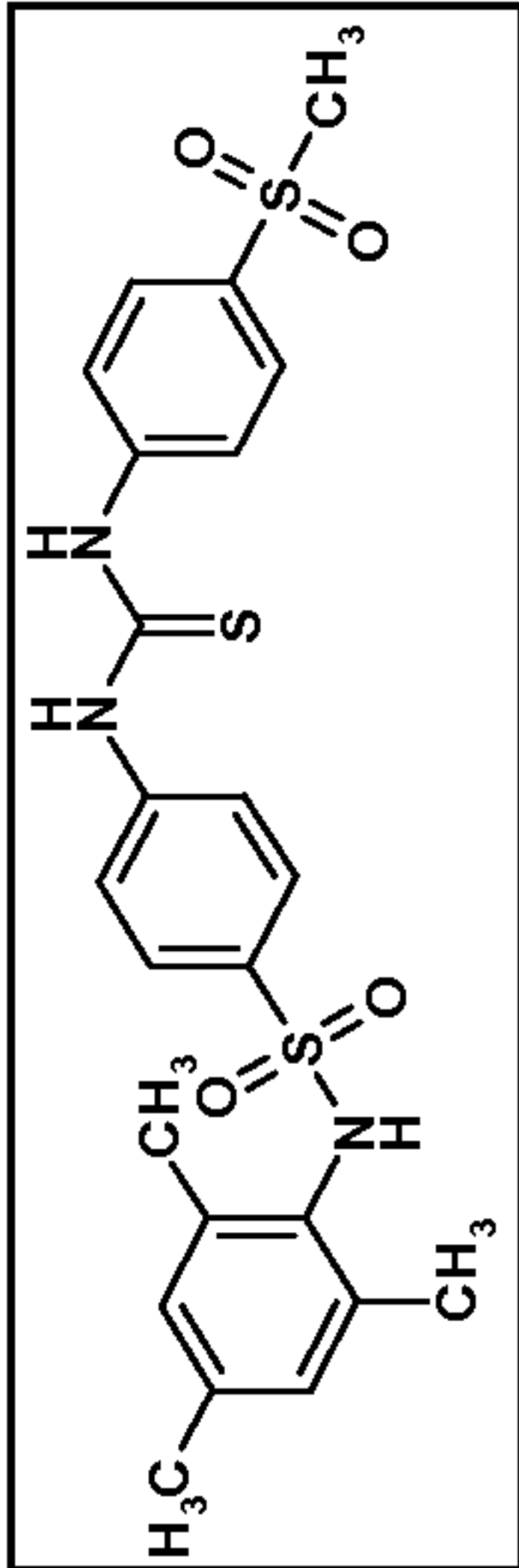
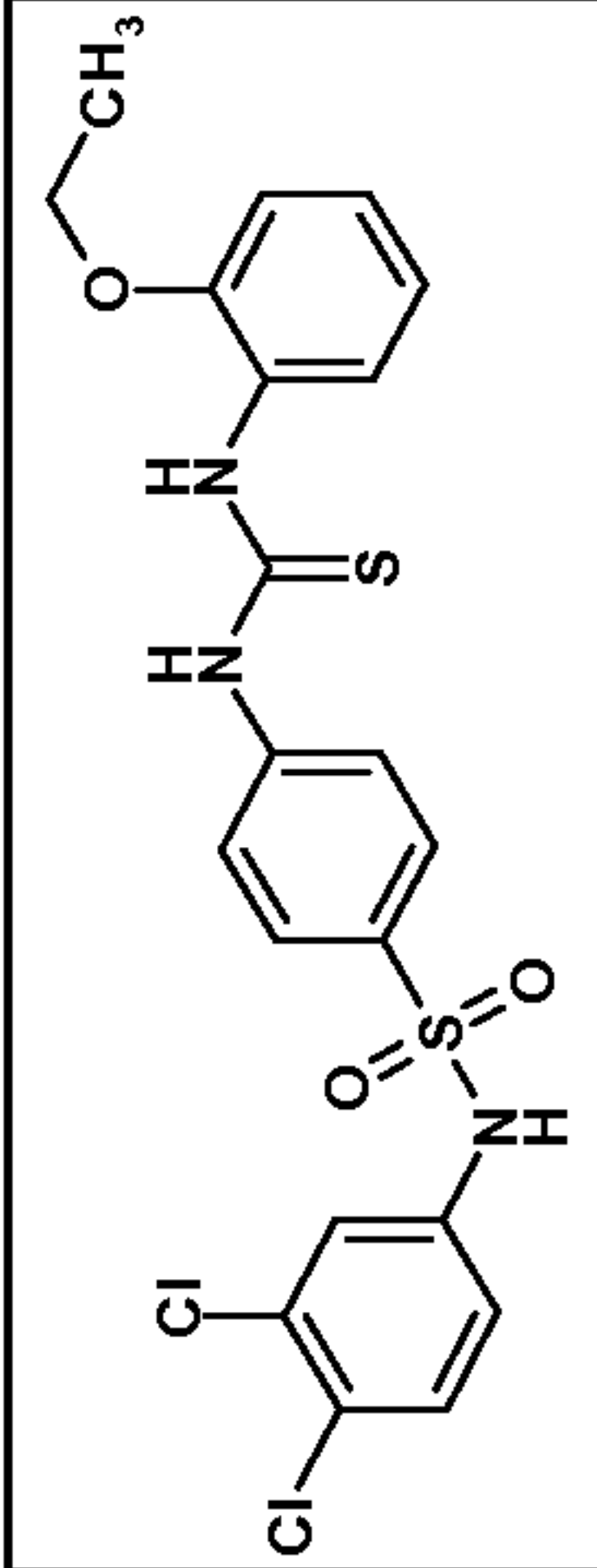
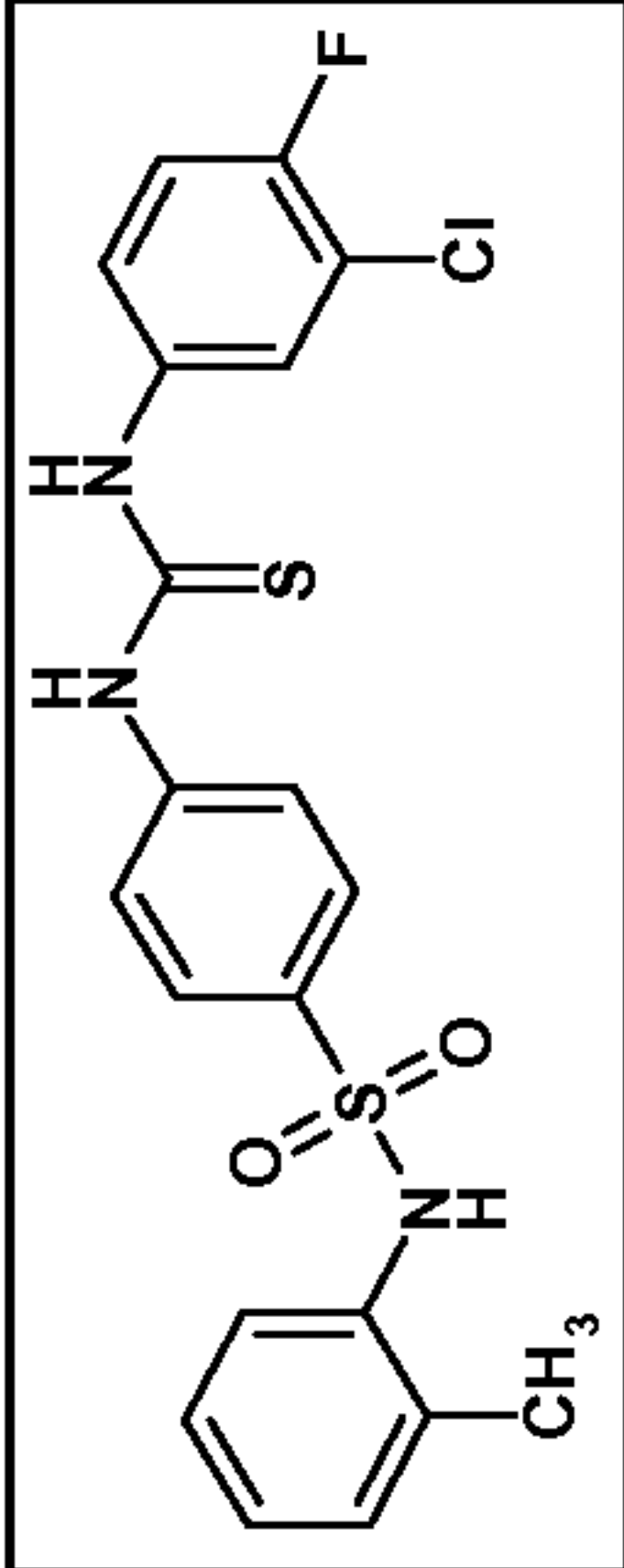
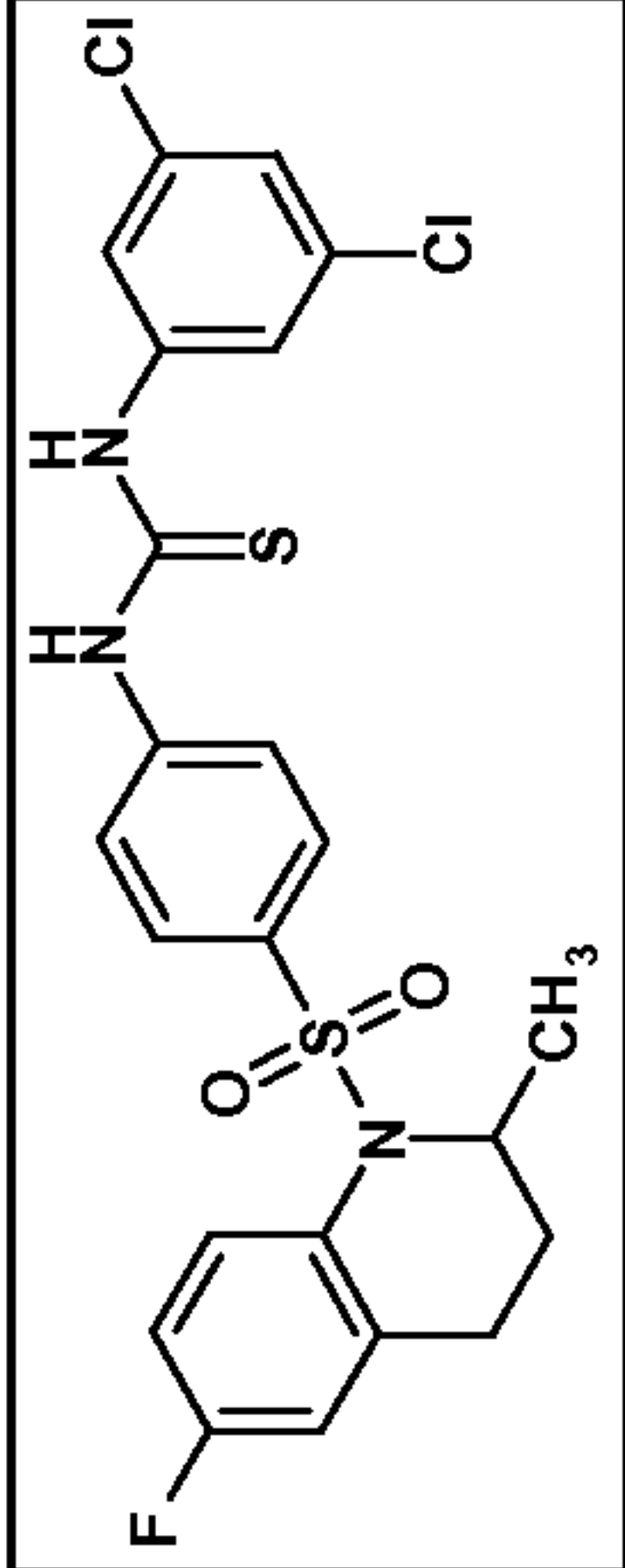
[illegible]

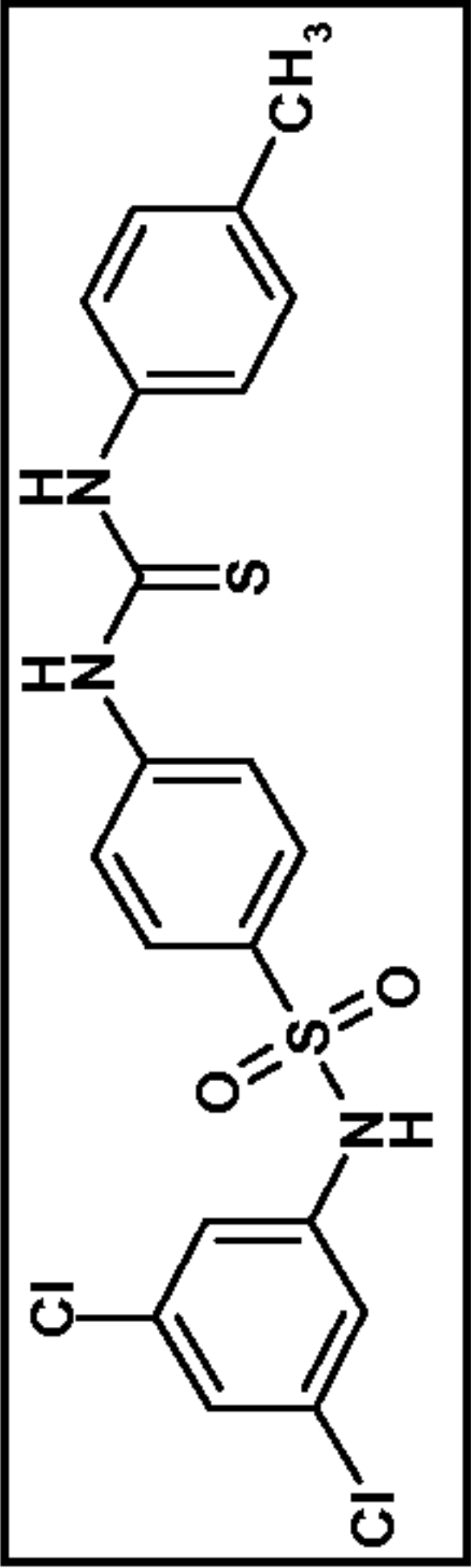
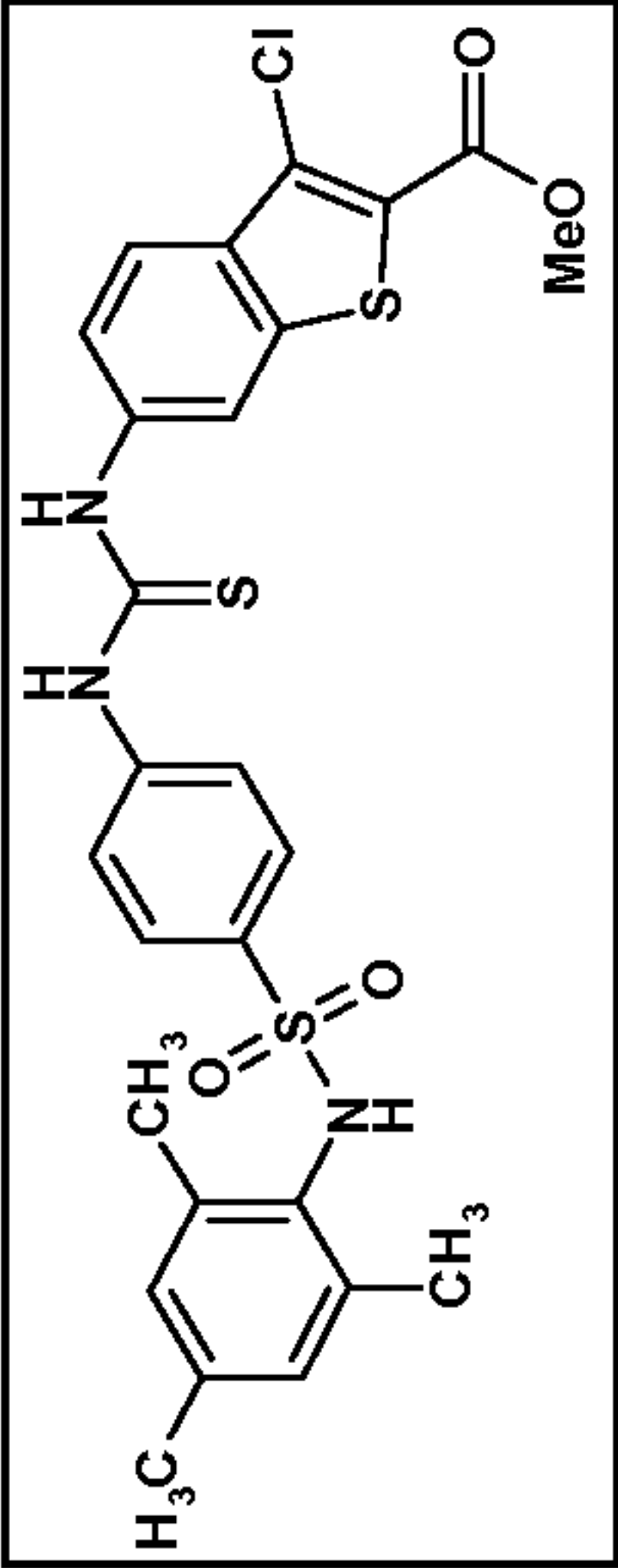
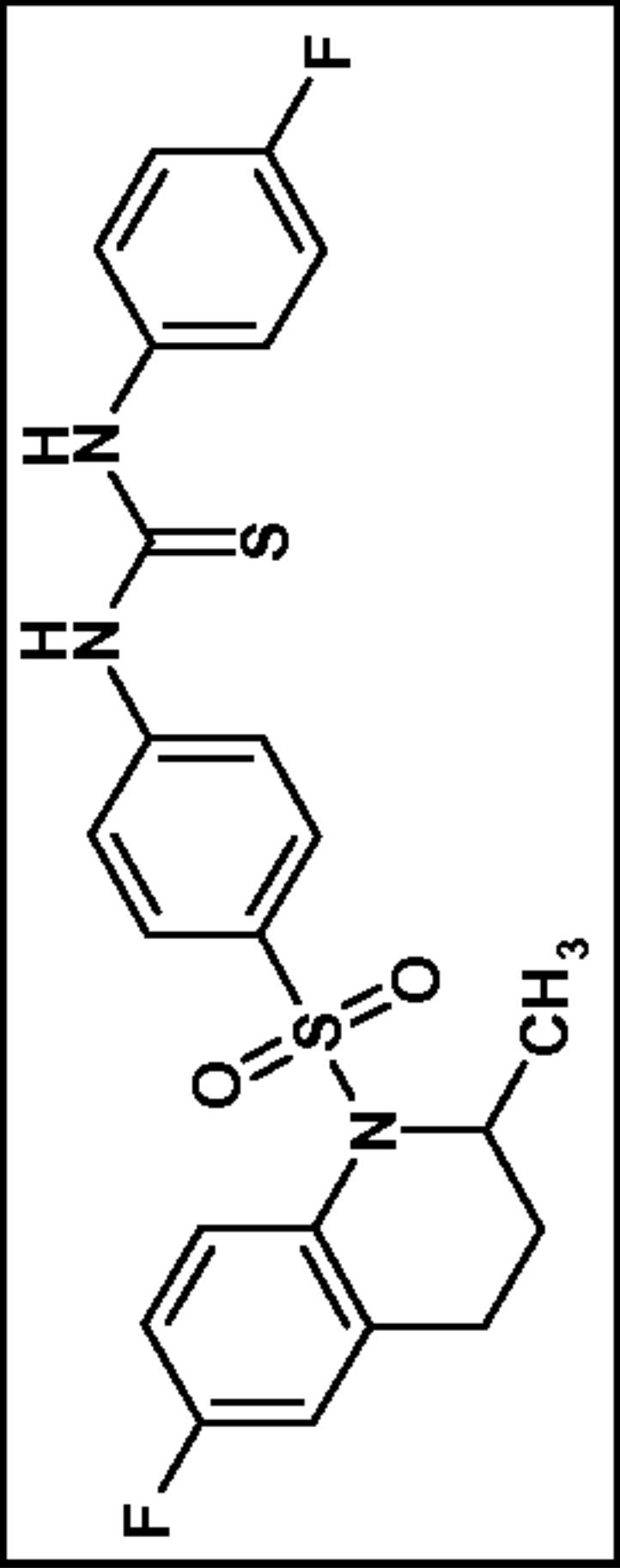
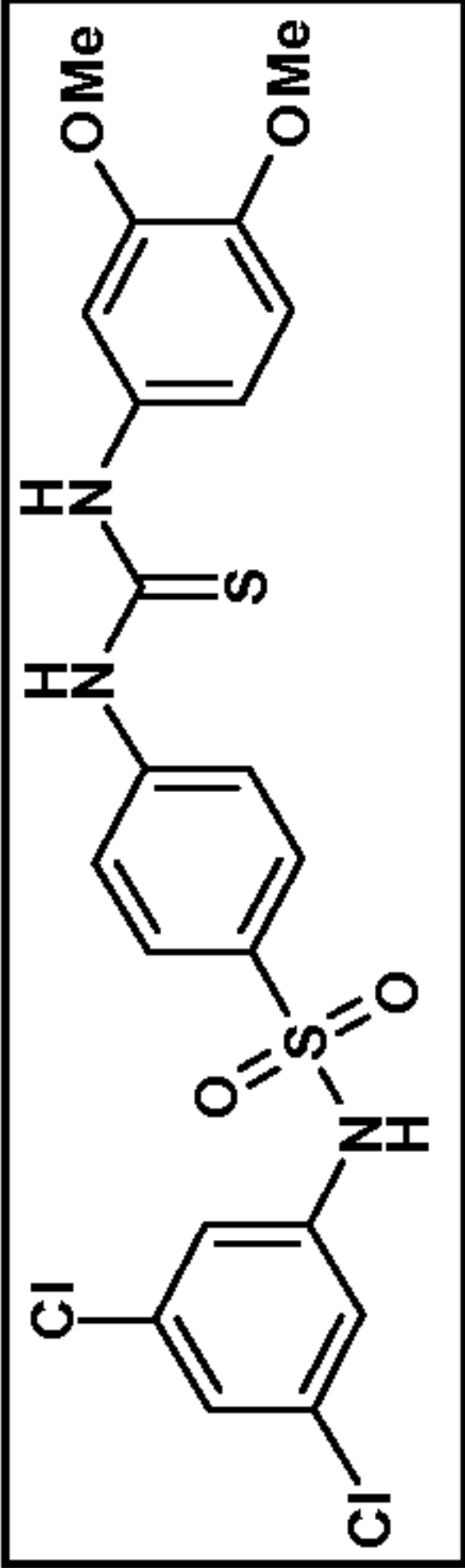
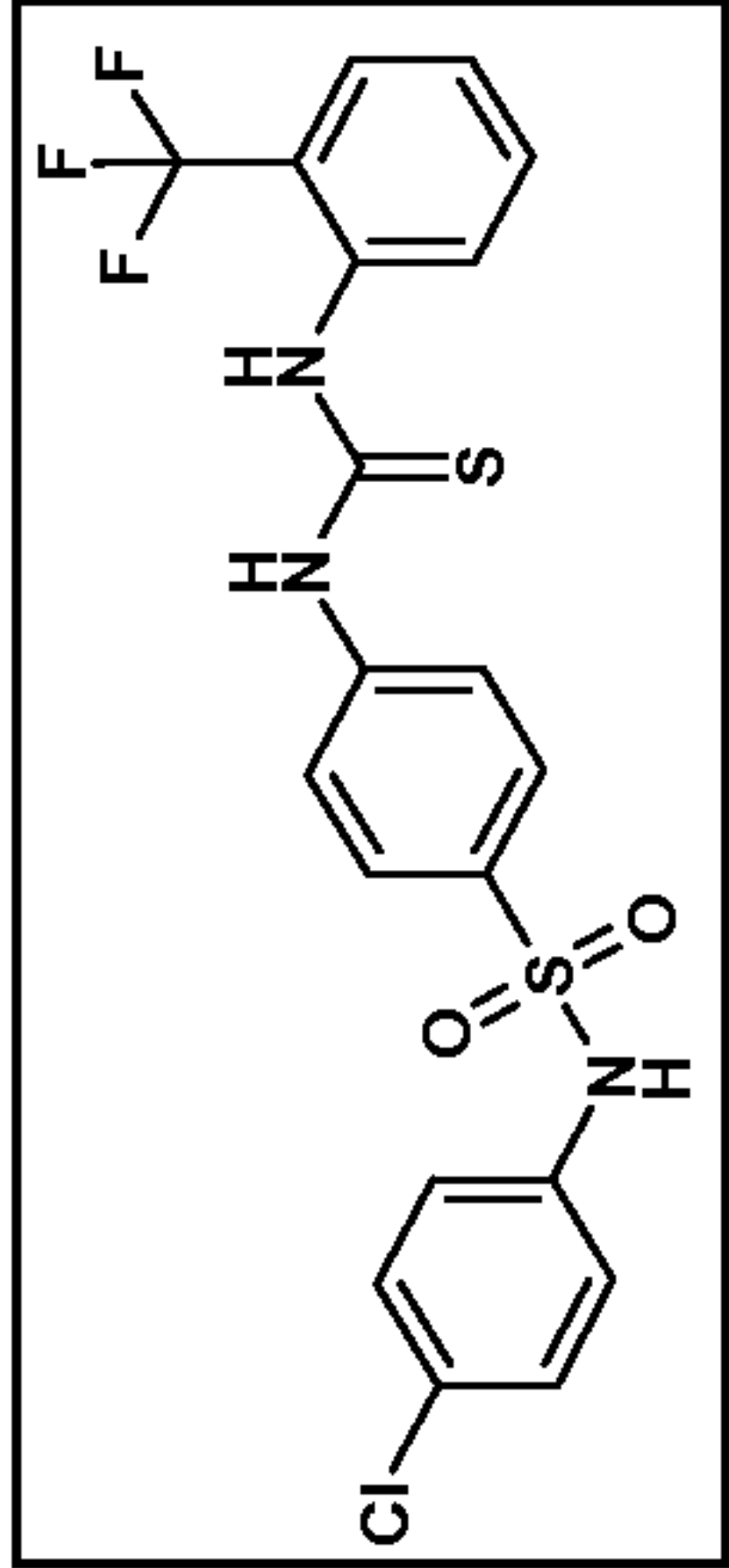
	AD4-12389	-5.83	-54.1	72.5659	12.15/6.07(0.50)	-1.372	-36.929	1YY9 - AD4-11091 LIKE
	AD4-12846	-4.89	-53.74	71.4924	71% at 2.6 microMolar 5.55/5.38(0.97)	-4.09	-40.727	1YY9 - AD4-11091 LIKE
	AD4-12501	-6.53	-53.93	71.3823	4.06/4.06(1.00)	-4.39	-49.596	1YY9 - AD4-11091 LIKE
	AD4-12400	-6.67	-56.3	69.4793	4.05/5.77(1.42)	-4.487	-36.418	1YY9 - AD4-11091 LIKE
	AD4-12239	-4.79	-57.44	67.8291	58% at 2.6 microMolar 16.13/13.77(0.85)	-3.787	-33.019	1YY9 - AD4-11091 LIKE

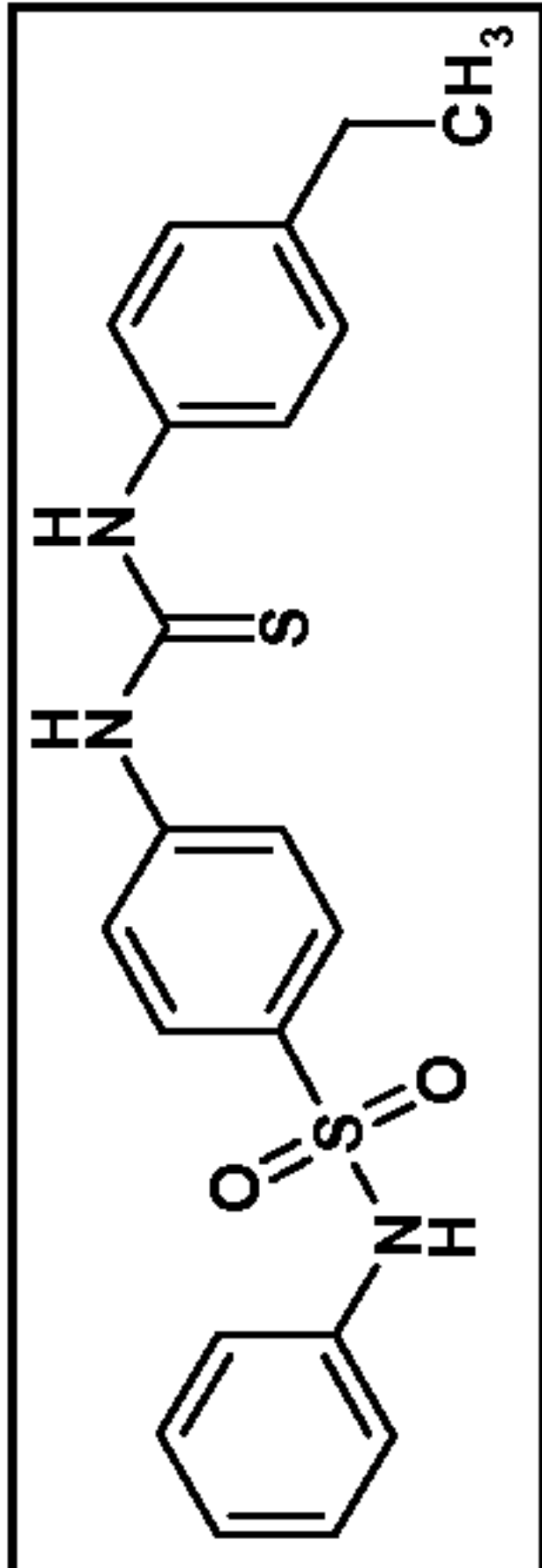
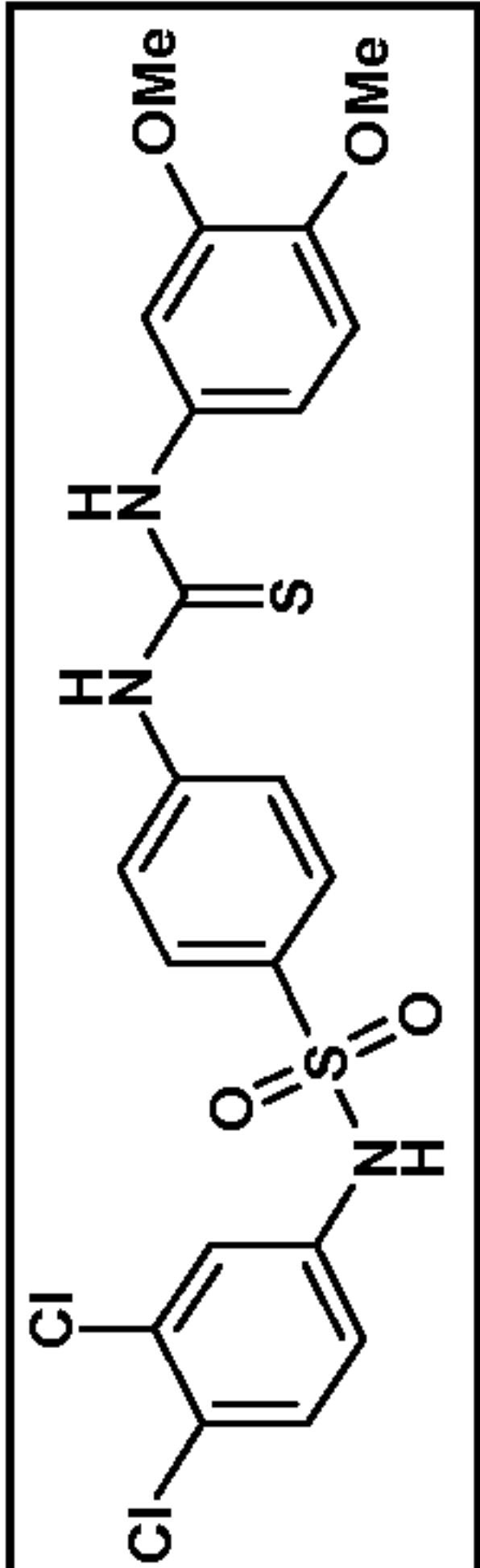
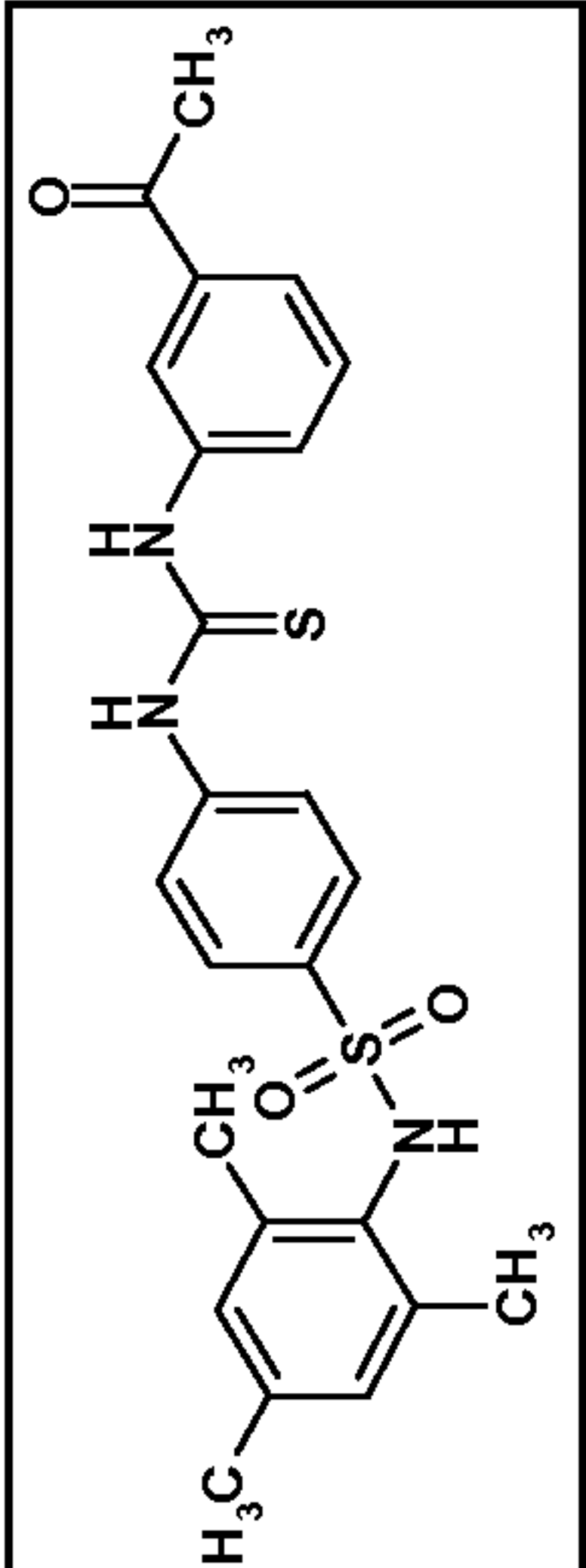
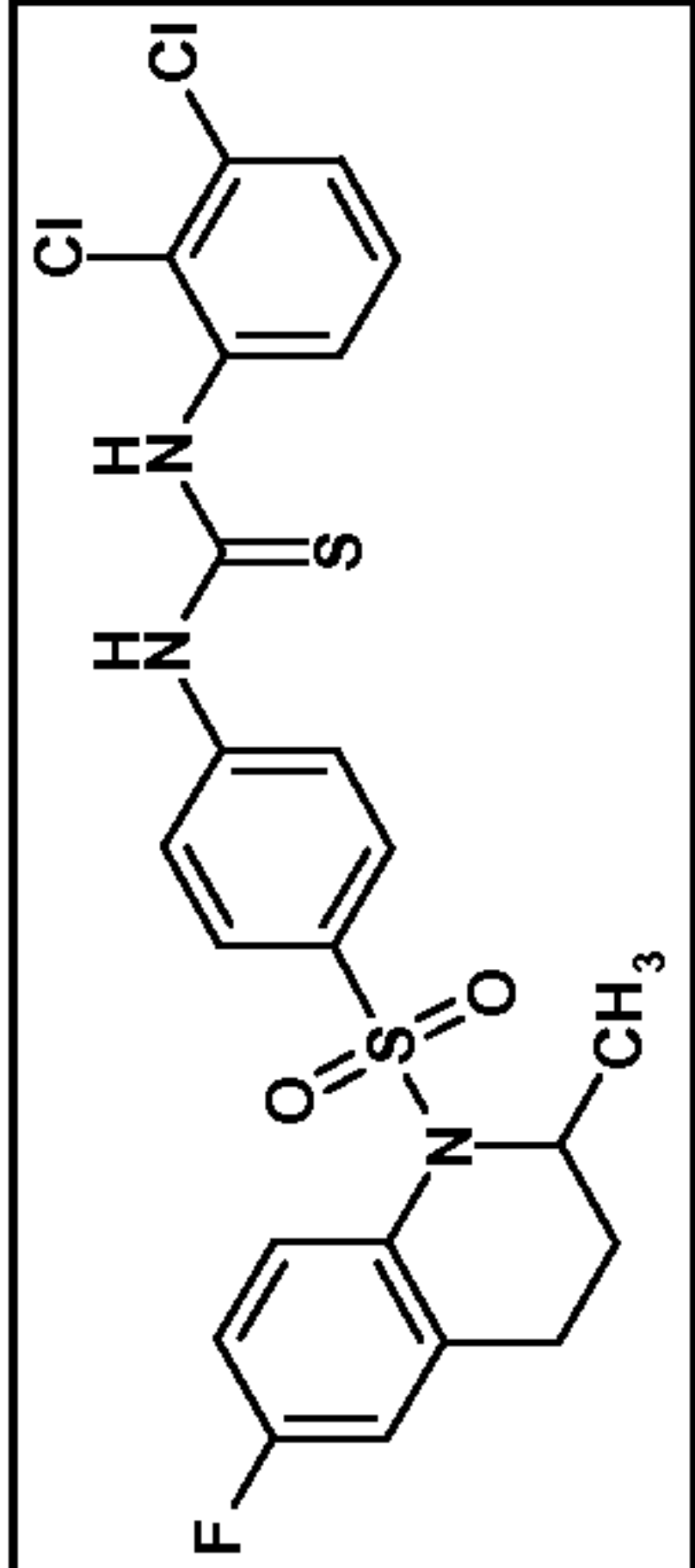
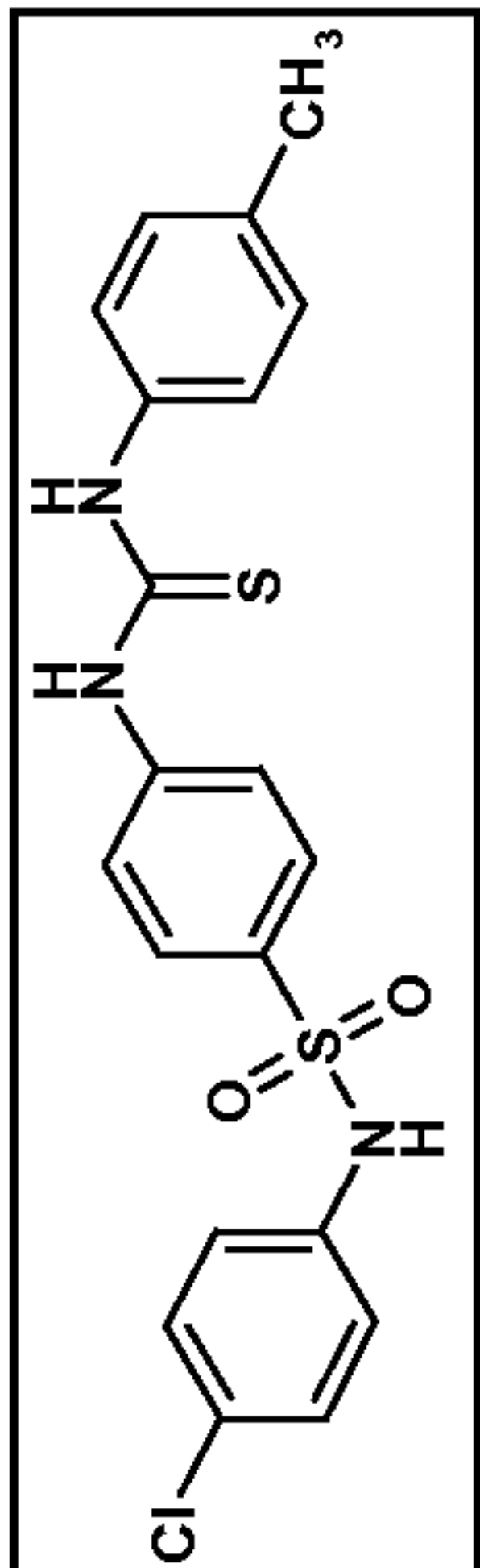
	AD4-12472	-4.86	-50.57	64.2647	6.23/5.42(0.87)	-4.241	-30.519	1YY9 - AD4-11091 LIKE
	AD4-12529	-7.76	-56.15	63.9249	6.35/6.47(1.02)	-4.728	-39.186	1YY9 - AD4-11091 LIKE
	AD4-12395	-4.85	-55.34	57.5206	7.92/6.96(0.88)	-4.241	-44.652	1YY9 - AD4-11091 LIKE
	AD4-12783	-6.55	-55	57.4272	7.00/7.62(1.09)	-4.334	-34.188	1YY9 - AD4-11091 LIKE
	AD4-12433	-5.66	-62.36	54.4095	29.06/38.50(1.32)	-3.35	-37.559	1YY9 - AD4-11091 LIKE
	AD4-12438	-6.13	-61.5	53.245	5.69/5.90(1.04)	-4.367	-31.989	1YY9 - AD4-11091 LIKE

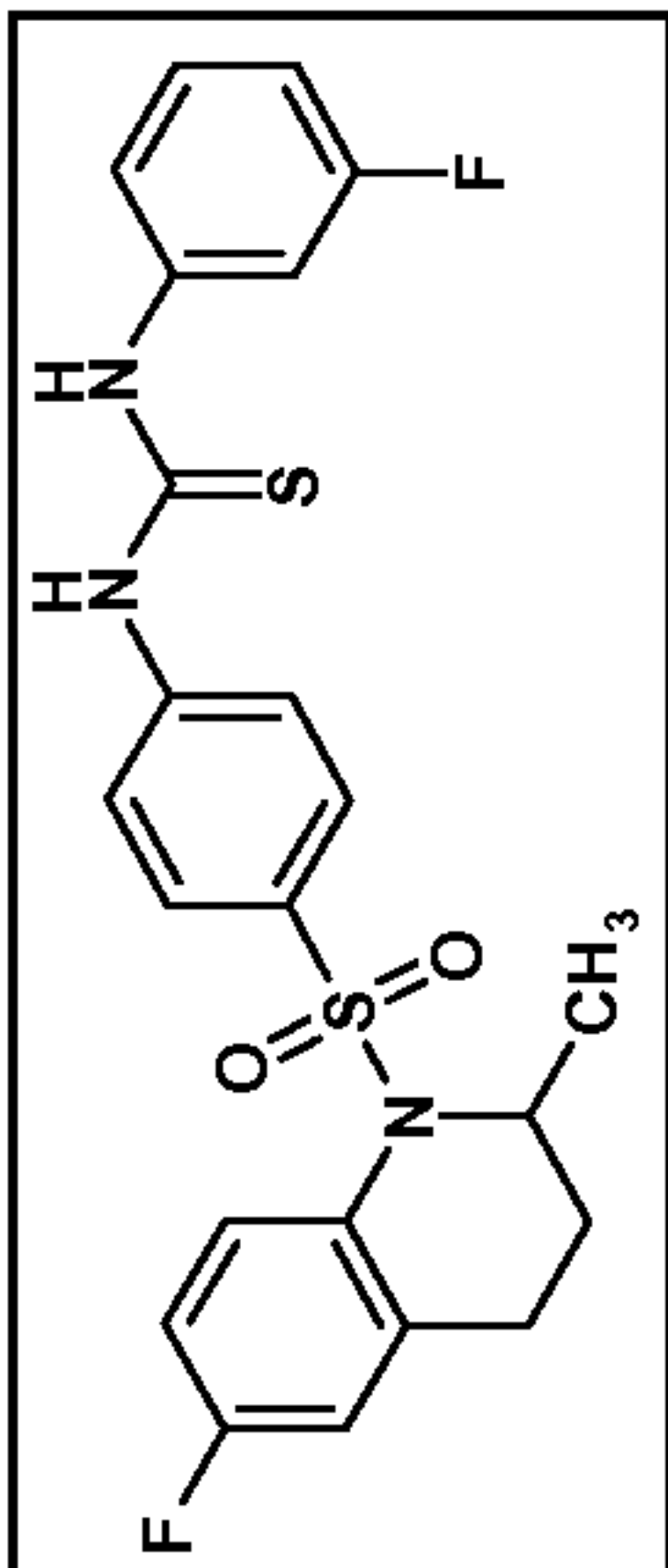
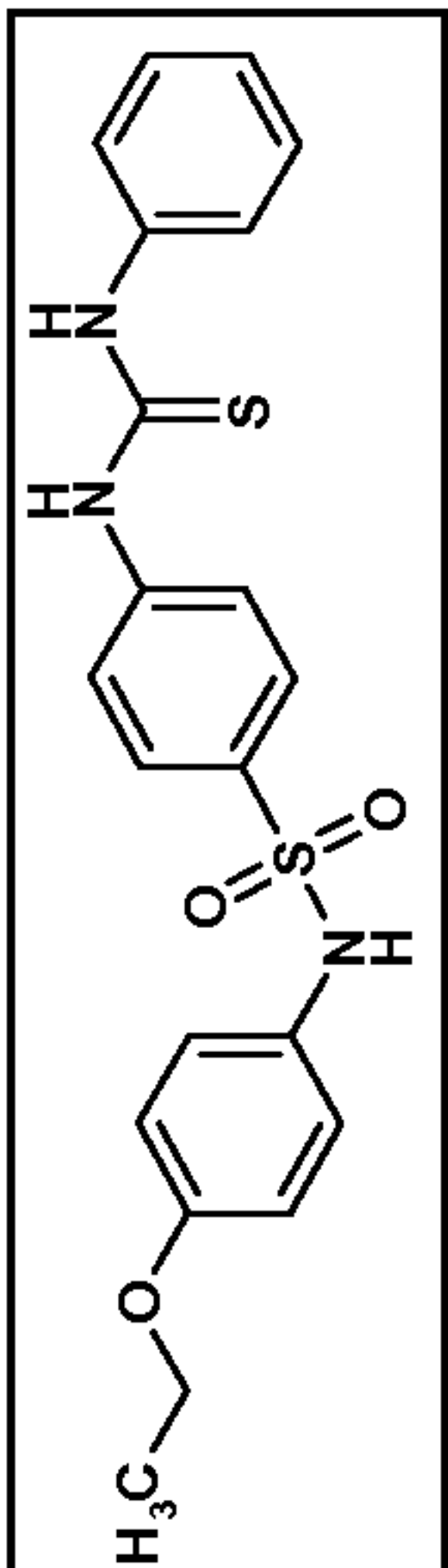
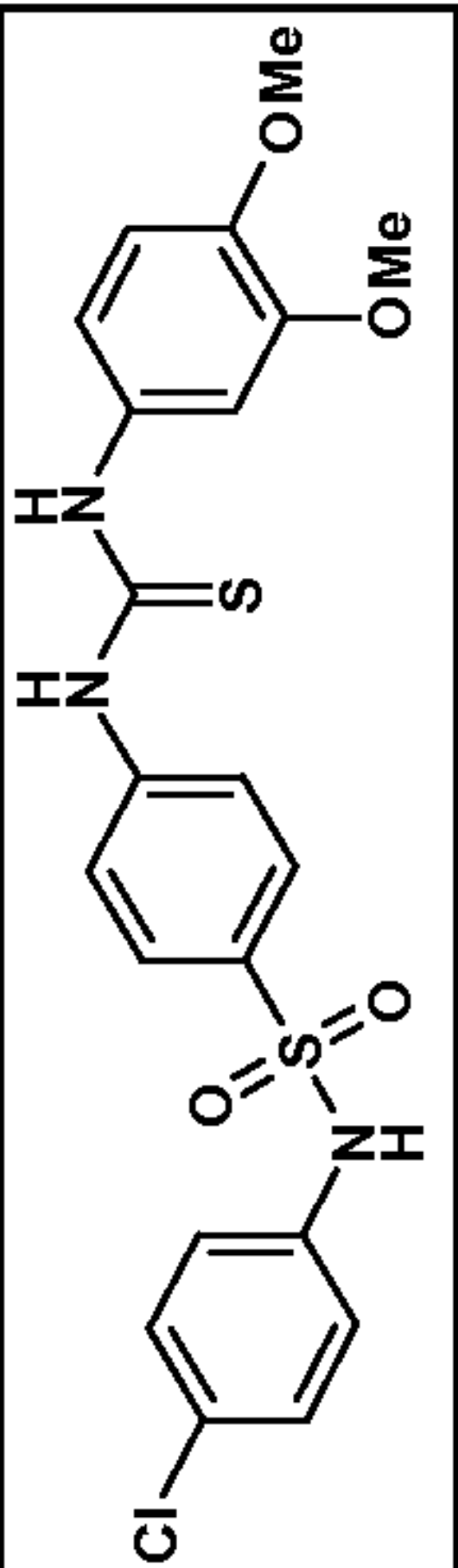
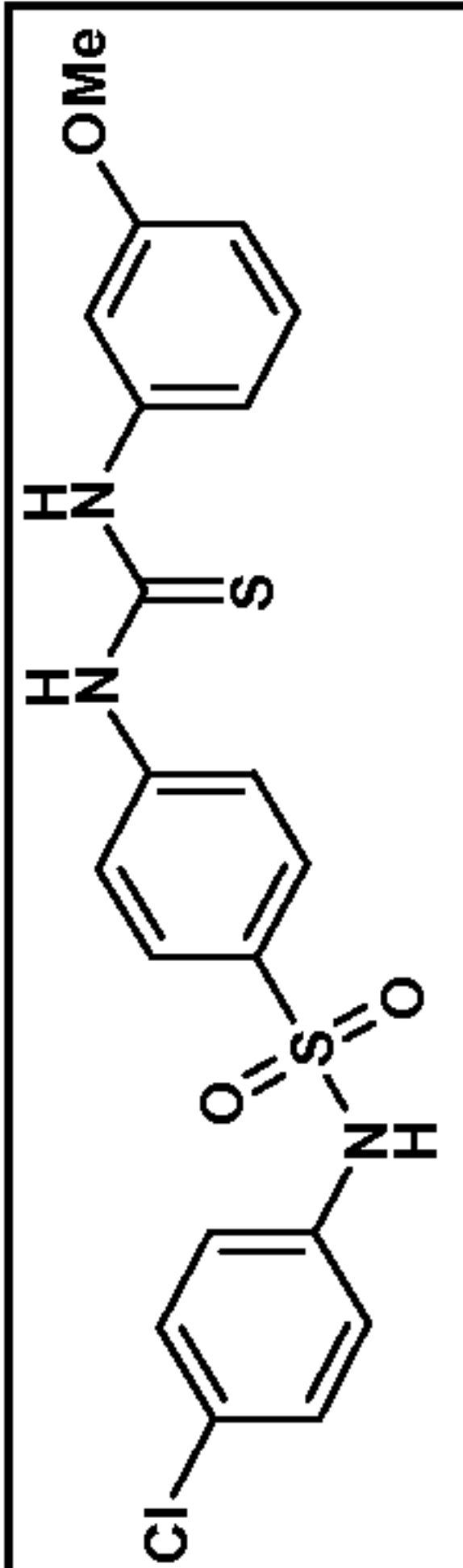
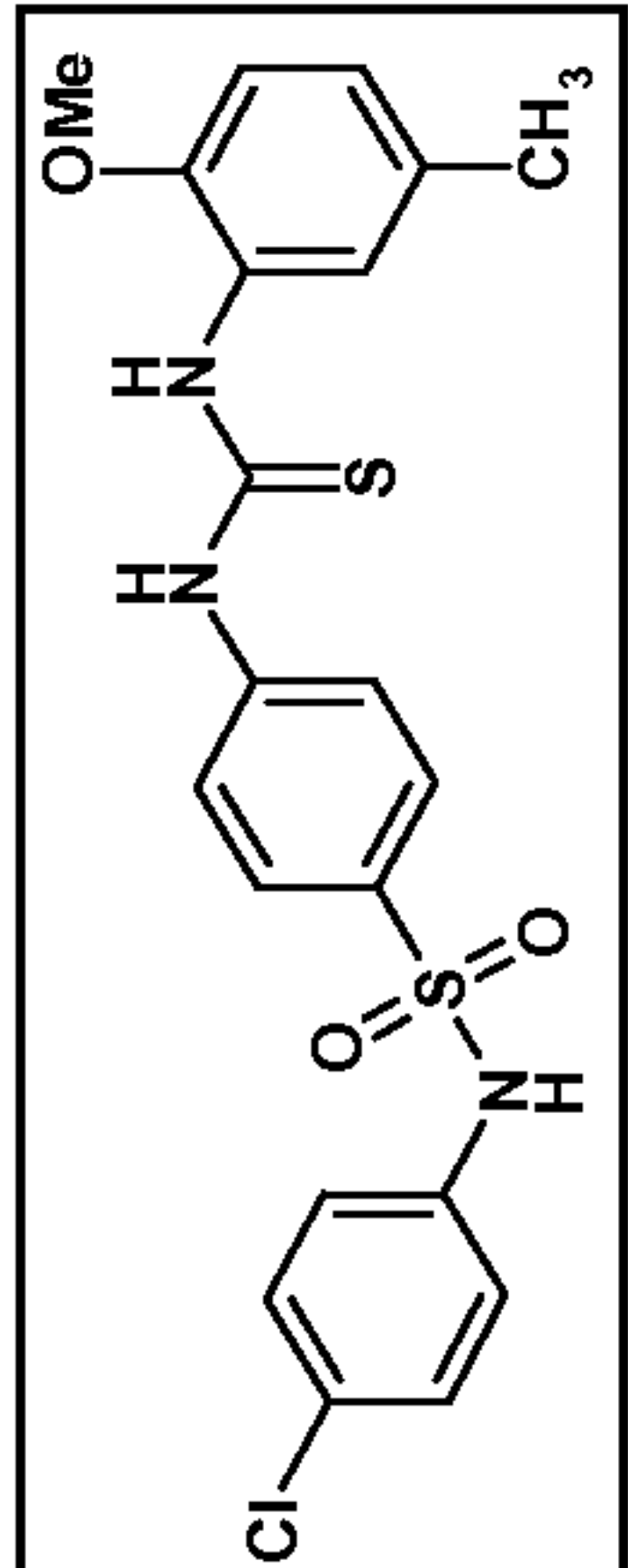
	AD4-12461	-5.04	-52.58	53.1391	4.06/5.91(1.46)	-3.308	-39.144	1YY9 - AD4-11091 LIKE
	AD4-10700	-6.44	-62.8	52.5996	14.69/14.35(0.98)	-1.113	-36.75	1YY9 - AD4-1886 Dockpharm
	AD4-12822	-4.19	-52.61	51.4082	6.92/7.04(1.02)	-3.072	-33.644	1YY9 - AD4-11091 LIKE
	AD4-12403	-5.67	-58.34	50.7196	4.03/5.30(1.32)	-2.864	-39.727	1YY9 - AD4-11091 LIKE
	AD4-12798	-6.21	-53.39	49.531	4.74/5.69(1.20)	-3.7	-41.52	1YY9 - AD4-11091 LIKE

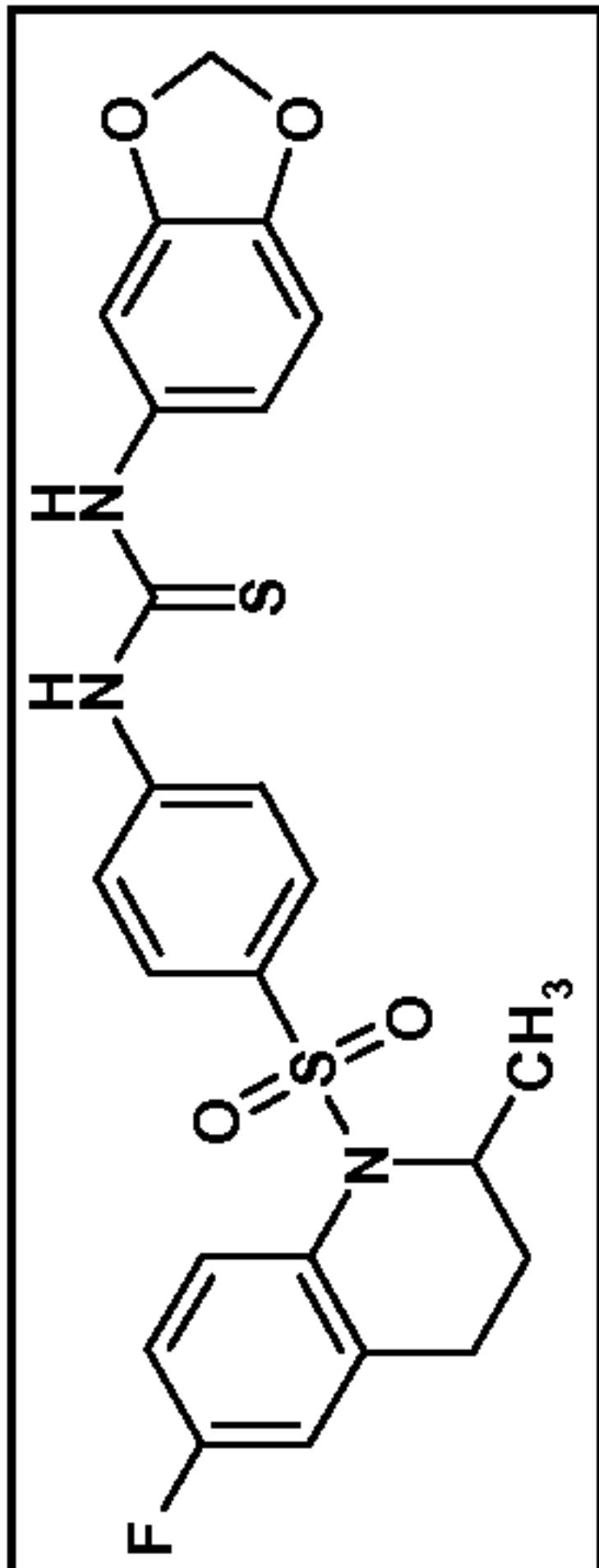
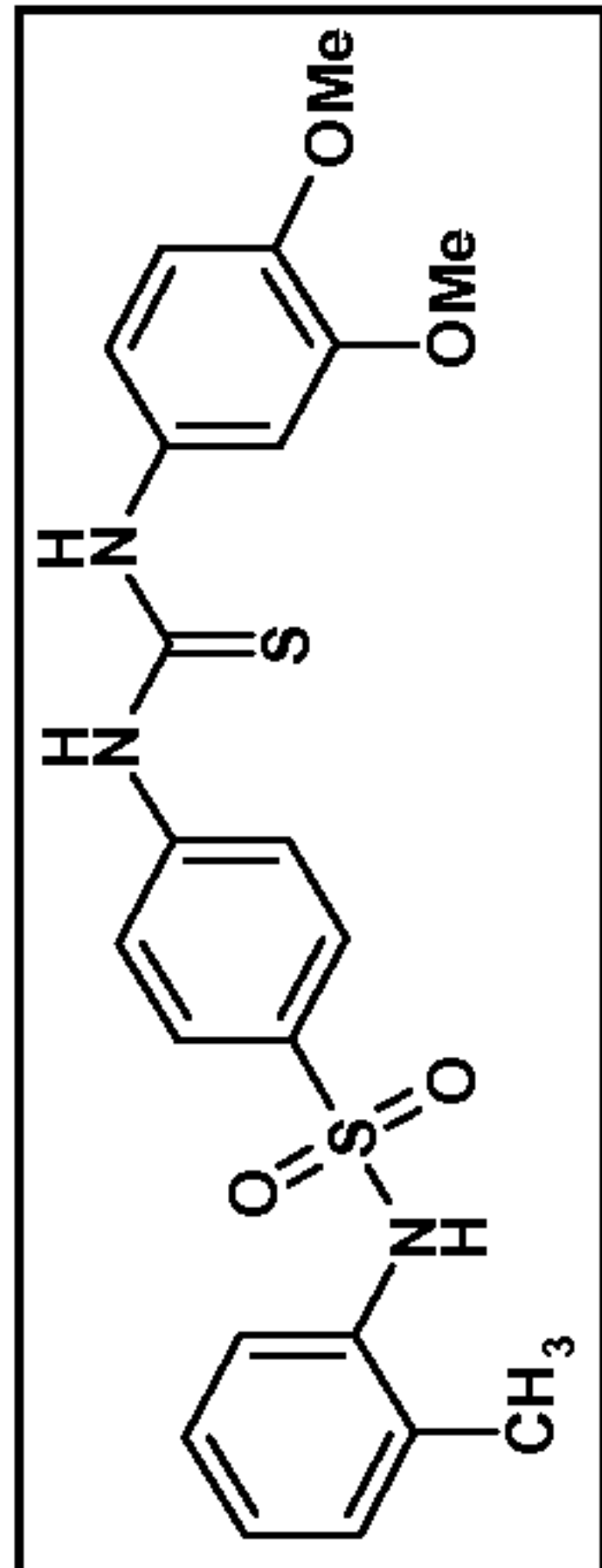
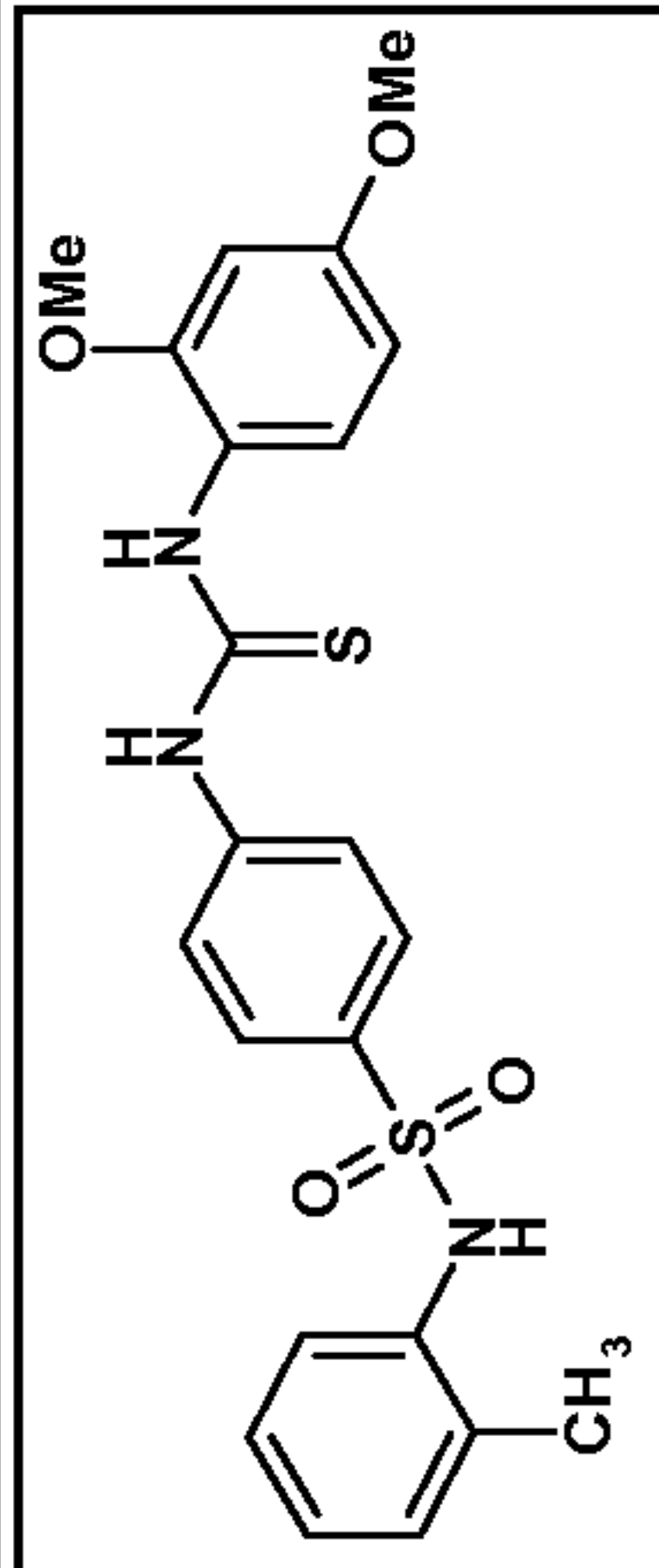
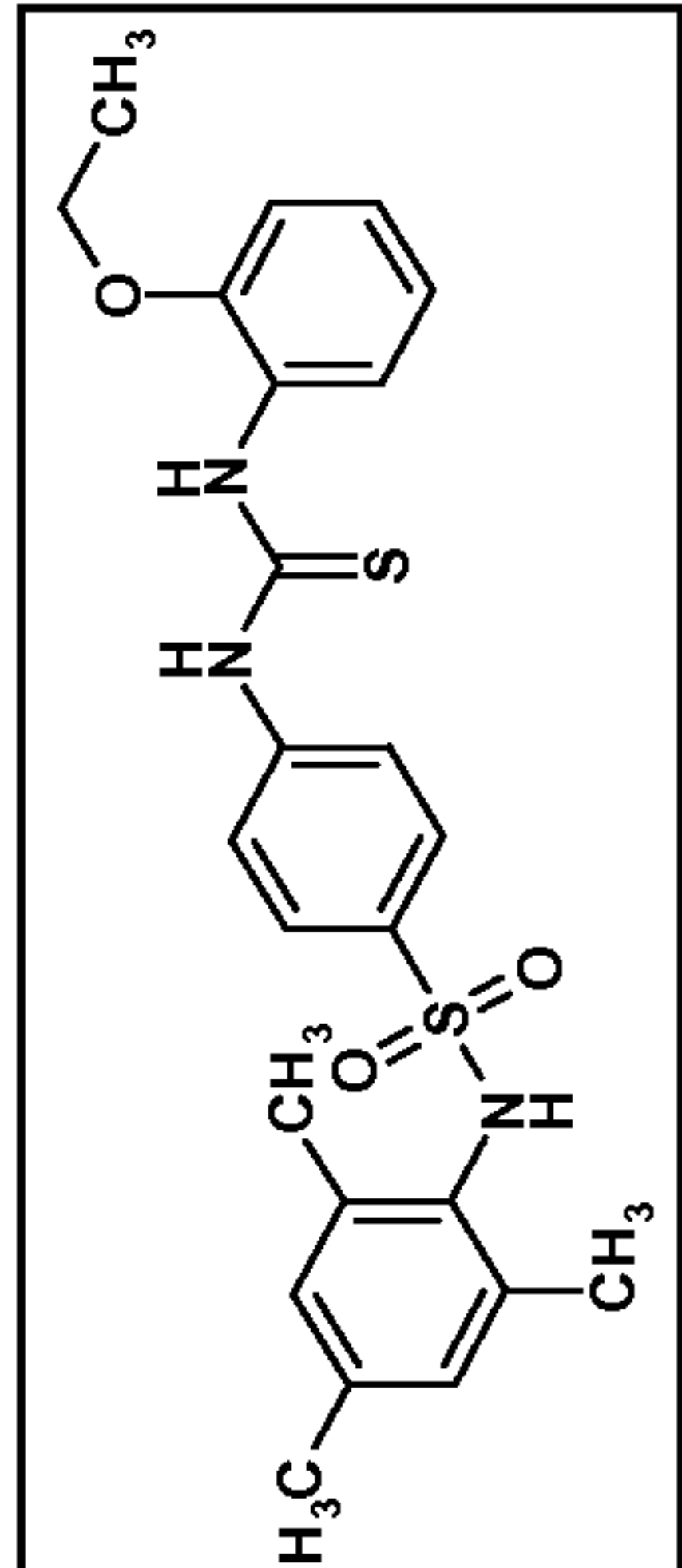
	AD4-12820	-6.64	-59.05	47.471	5.98/5.93(0.99)	-3.737	-43.758	1YY9 - AD4-11091 LIKE
	AD4-11432	-6.34	-60.05	47.0006	7.80/7.82(1.00)	-2.138	-42.697	1YY9 - AD4-11091 LIKE
	AD4-12829	-4.49	-36.92	46.4407	36.97/37.27(1.01)	-4.396	-24.523	1YY9 - AD4-11091 LIKE
	AD4-12836	-5.44	-57.16	46.1658	9.32/8.43(0.90)	-4.633	-35.888	1YY9 - AD4-11091 LIKE
	AD4-12827	-6.59	-56.14	45.9206	6.49/6.40(0.99)	-4.353	-37.272	1YY9 - AD4-11091 LIKE

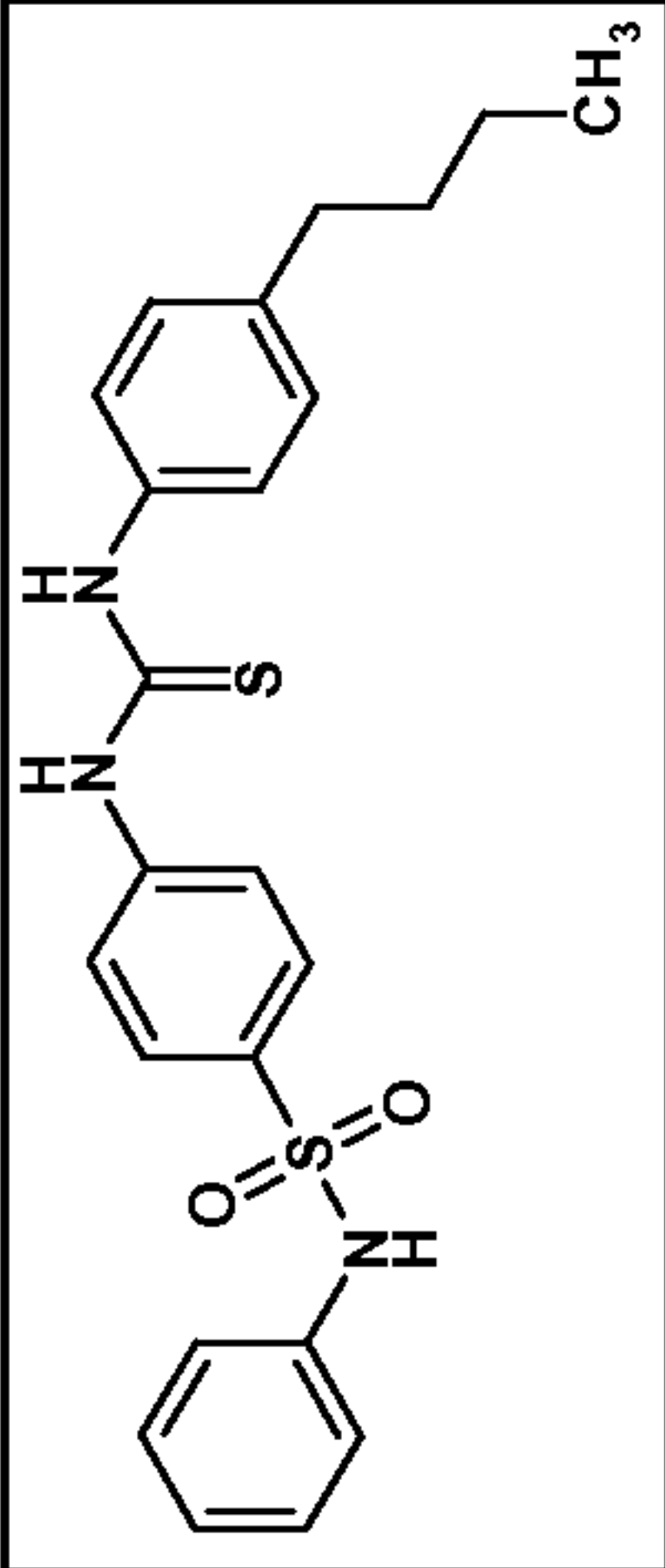
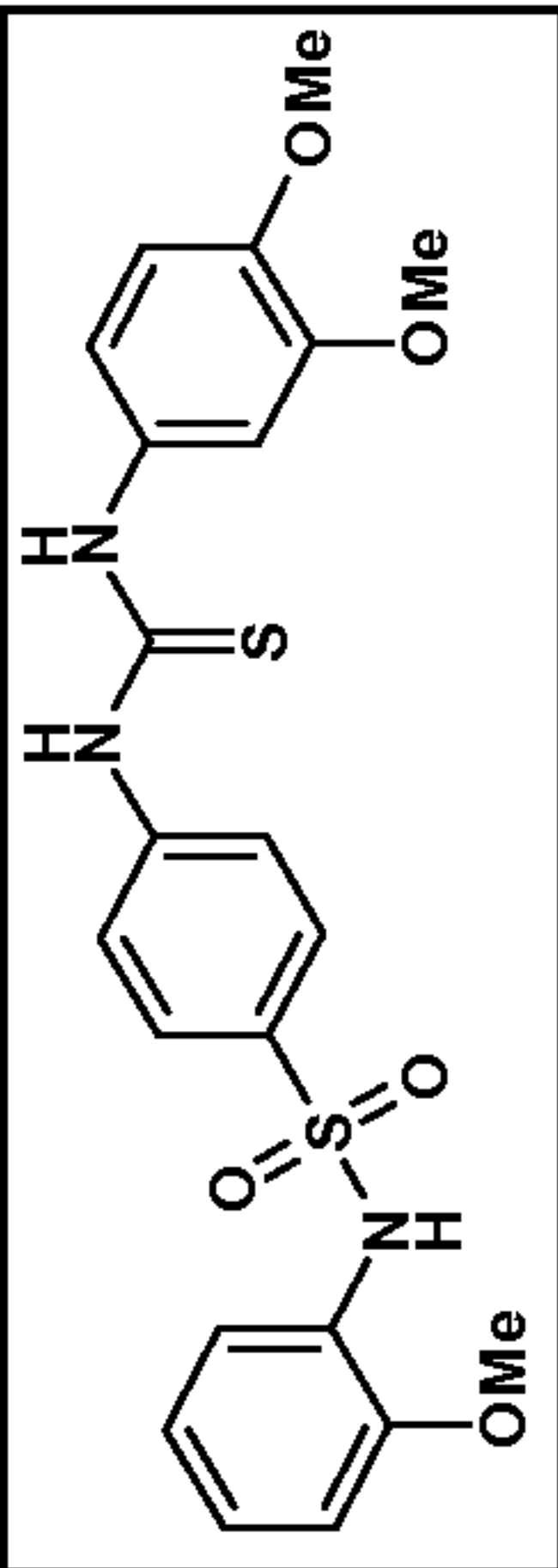
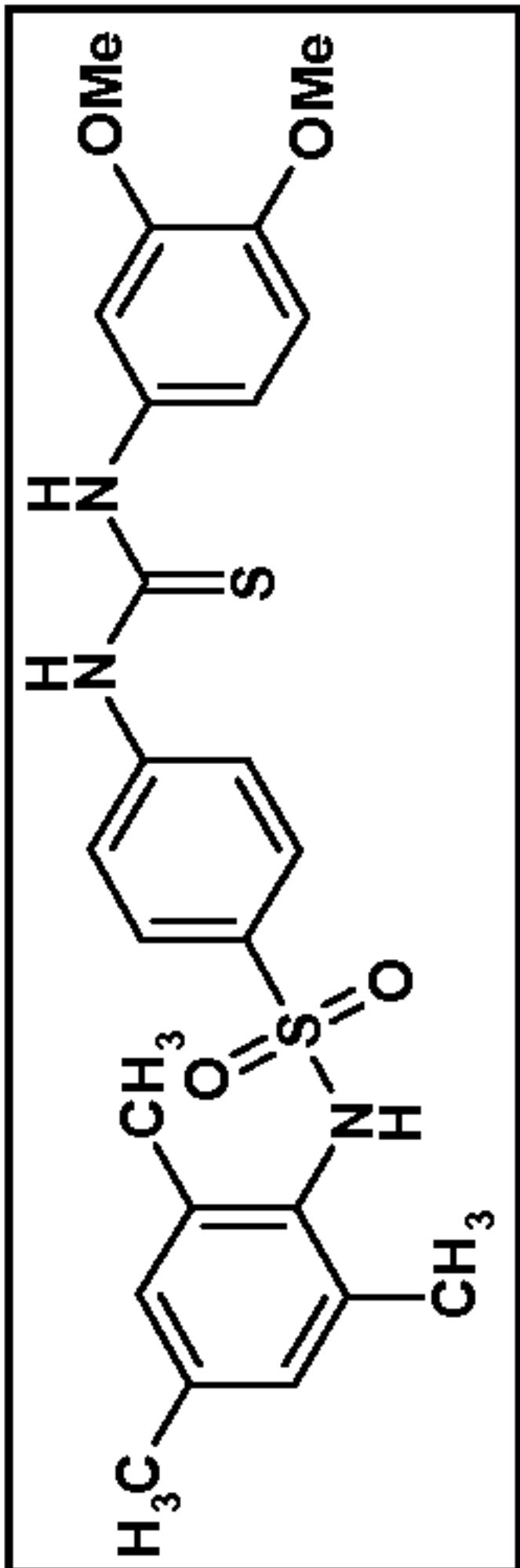
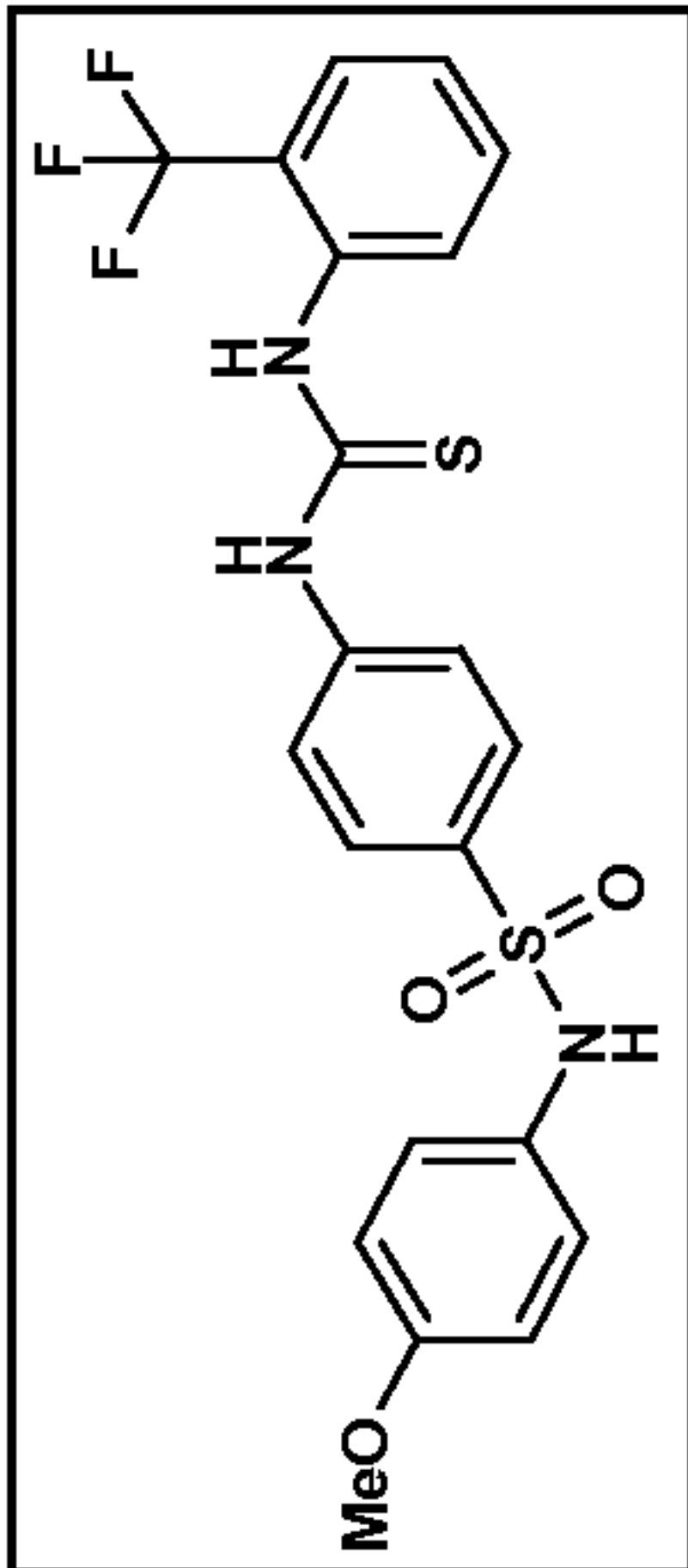
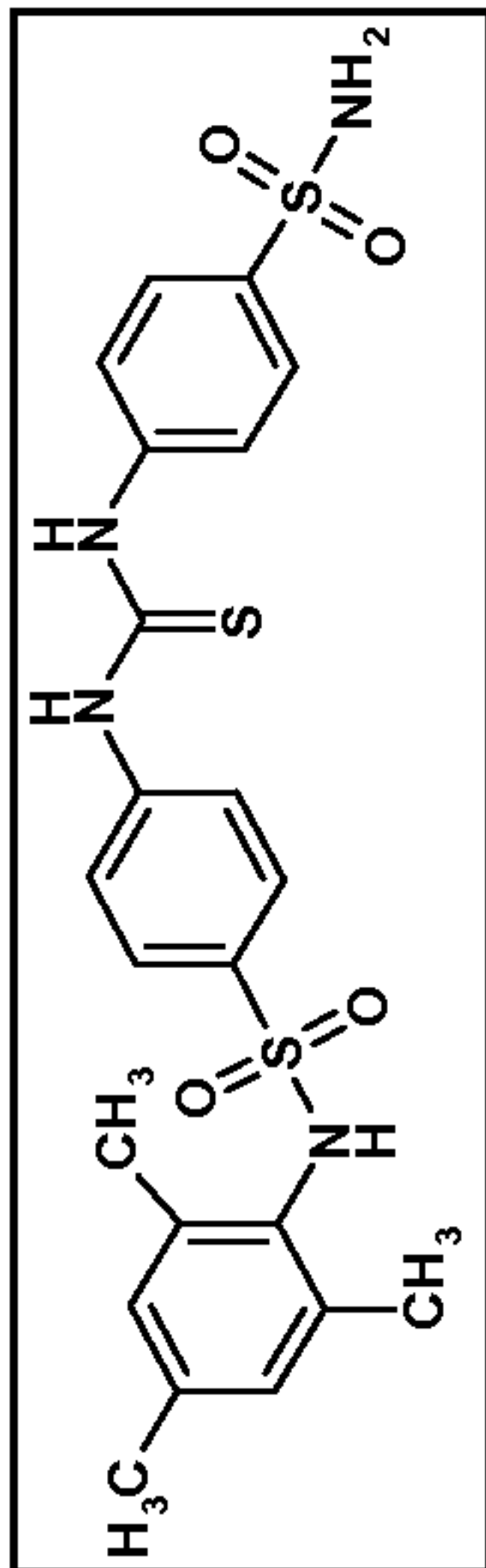
	AD4-12521	-5.59	-54.87	45.7824	6.99/6.54(1.22)	-3.596	-28.511	1YY9 - AD4-11091 LIKE
	AD4-11427	-6.36	-57.89	44.904	11.97/14.48(1.21)	-3.173	-34.903	1YY9 - AD4-11091 LIKE
	AD4-12443	-5.91	-55.75	44.7896	6.71/6.91(1.03)	-3.756	-40.035	1YY9 - AD4-11091 LIKE
	AD4-12477	-5.1	-56.94	44.2167	6.65/5.44(0.82)	-4.704	-45.689	1YY9 - AD4-11091 LIKE
	AD4-12843	-4.42	-54.07	43.3347	74% at 2.6 microMolar 6.26/12.23(1.95)	-3.616	-45.969	1YY9 - AD4-11091 LIKE

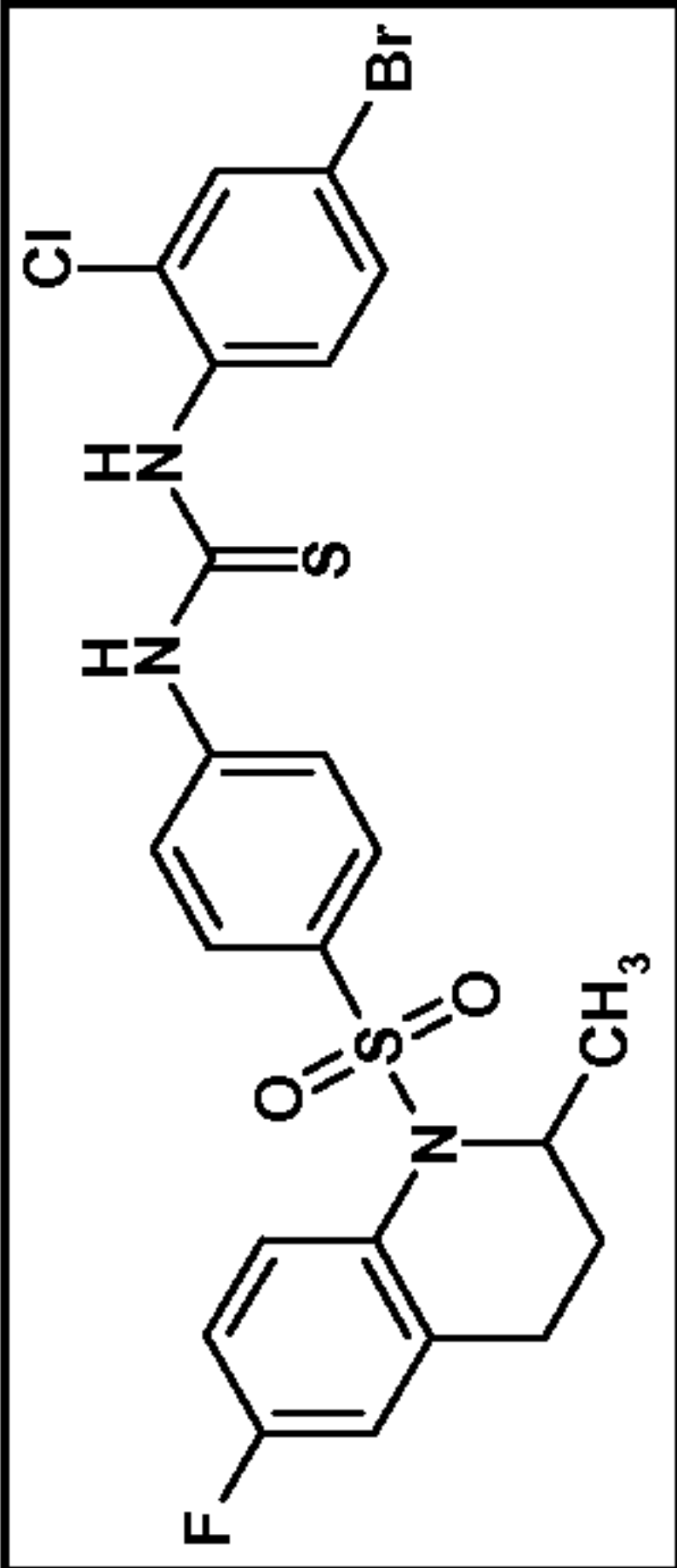
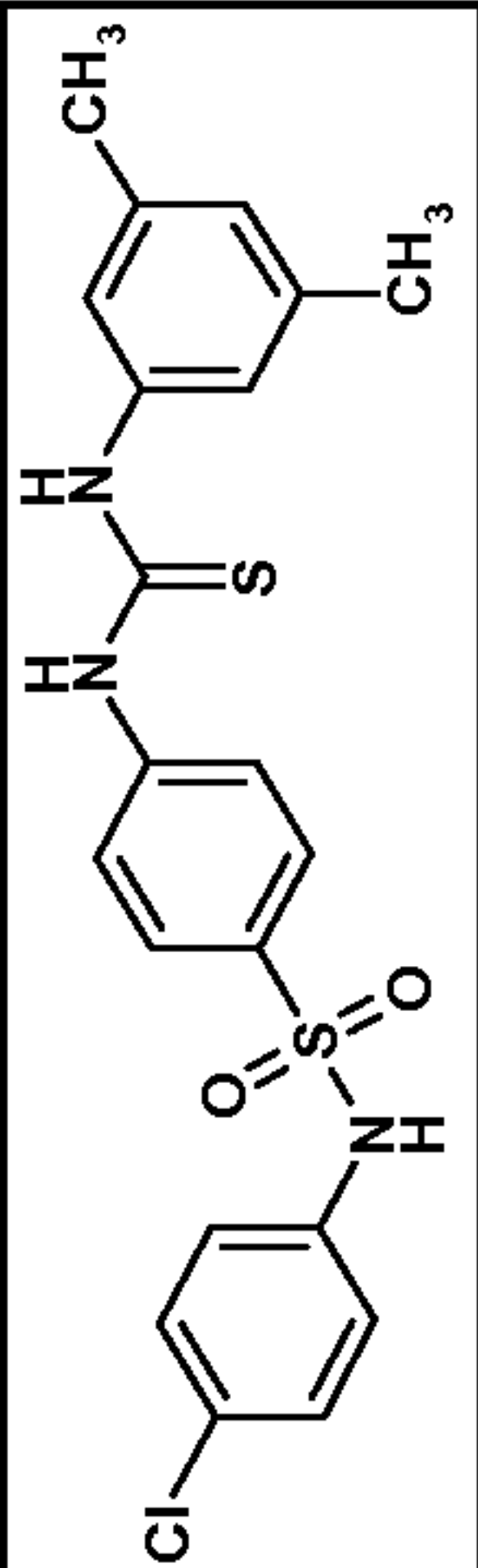
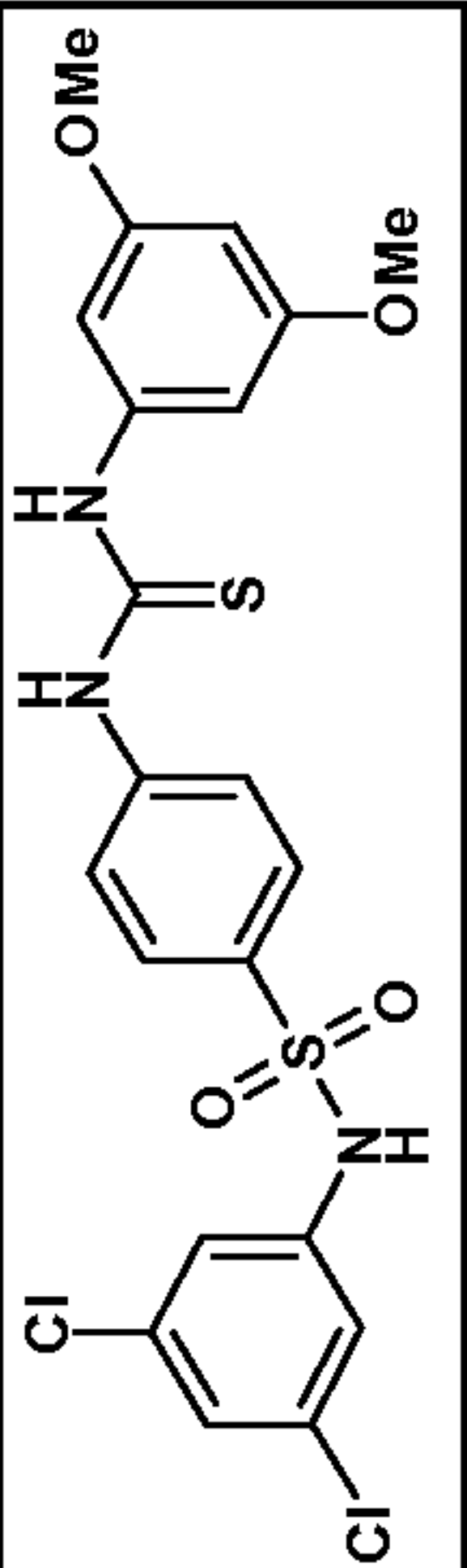
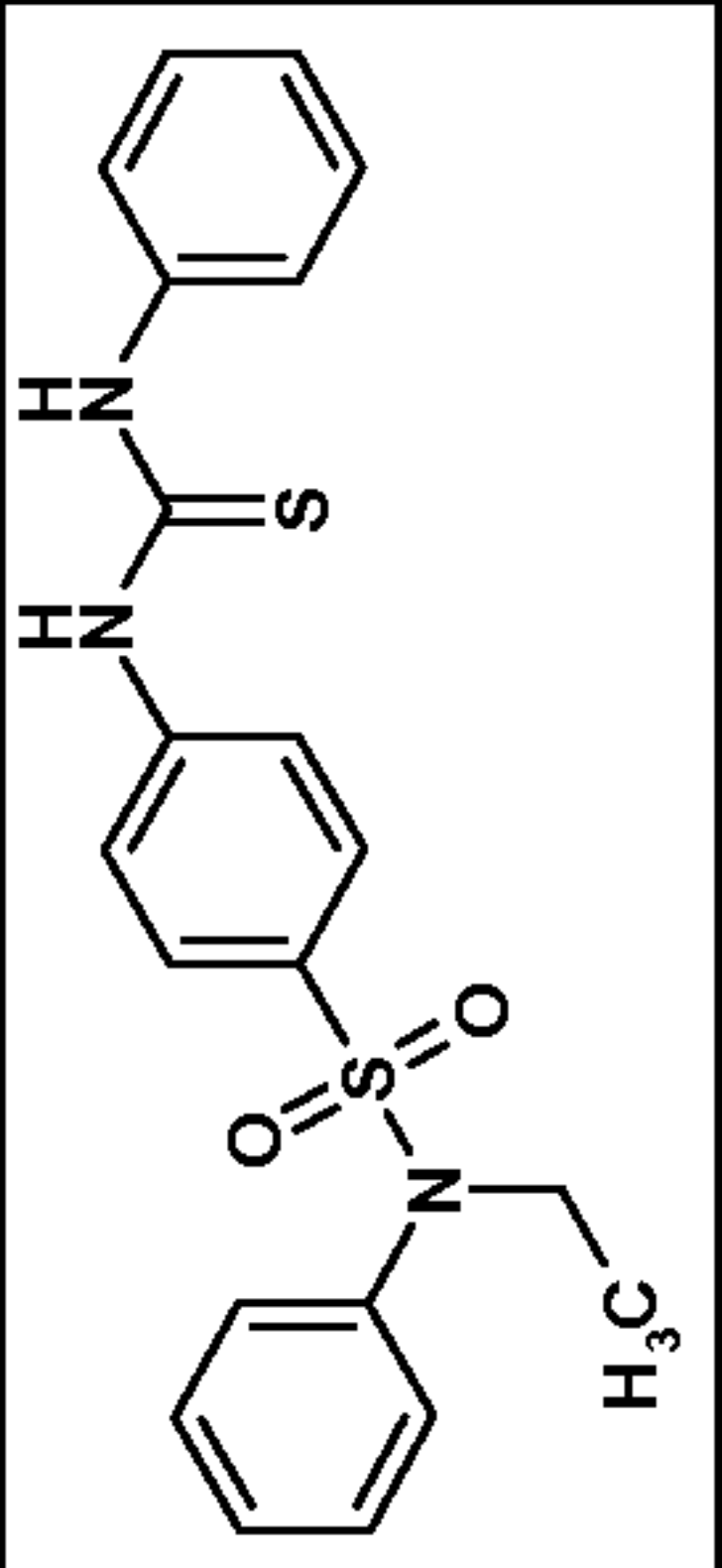
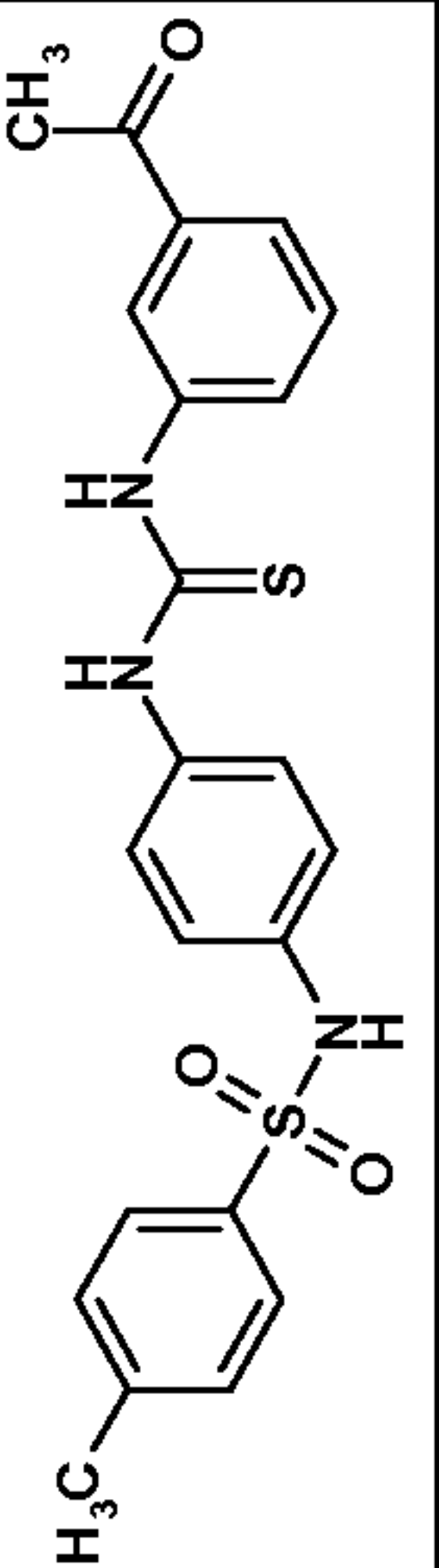
	AD4-12460	-6.63	-60.84	42.5115	6.33/5.61(0.87)	-3.956	-34.204	1YY9 - AD4-11091 LIKE
	AD4-12560	-6.6	-67.91	42.3275	8.88/5.23(0.59)	-2.493	-38.312	1YY9 - AD4-11091 LIKE
	AD4-12850	-4.74	-59.3	42.2628	31.78/35.77(1.13)	-5.454	-36.419	1YY9 - AD4-11091 LIKE
	AD4-12481	-6.81	-56.97	42.006	10.38/11.04(1.06)	-4.203	-42.388	1YY9 - AD4-11091 LIKE
	AD4-12435	-5.71	-52.17	41.4281	6.67/6.15(0.92)	-3.596	-34.513	1YY9 - AD4-11091 LIKE

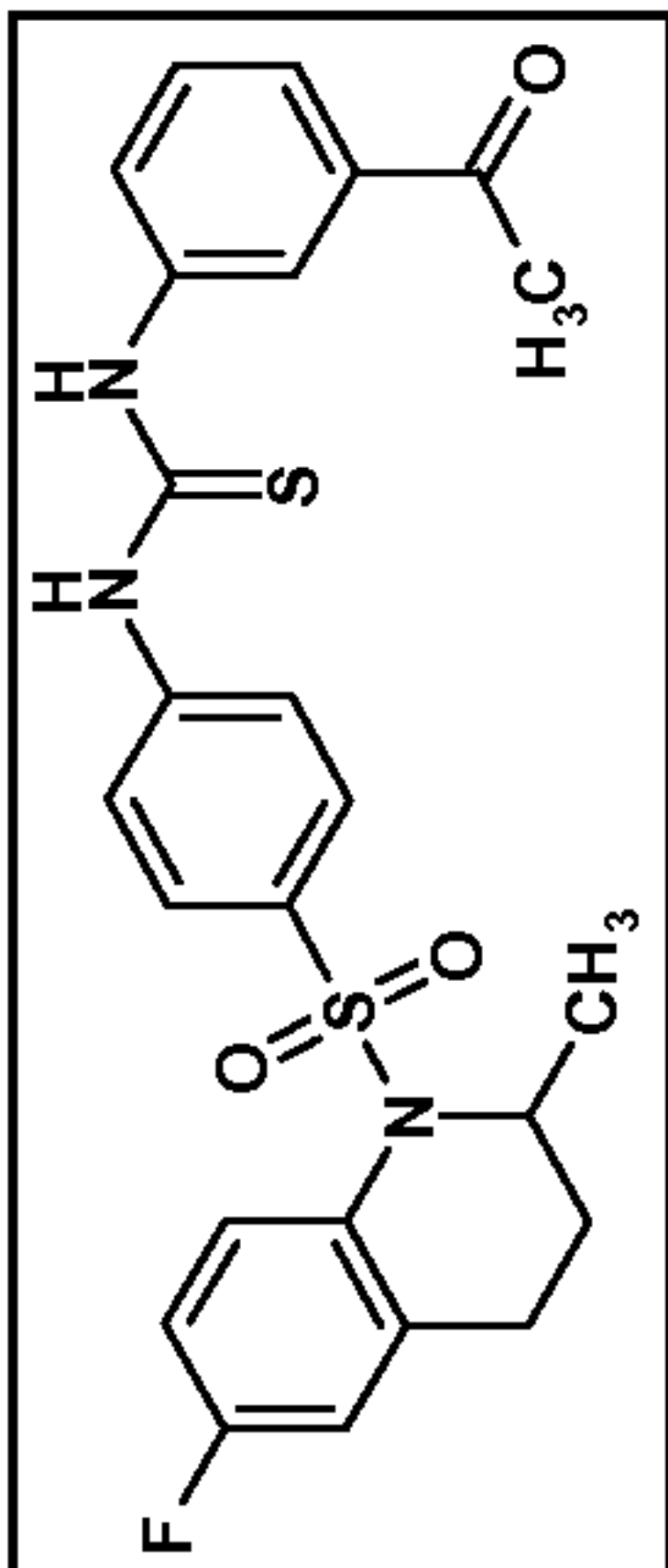
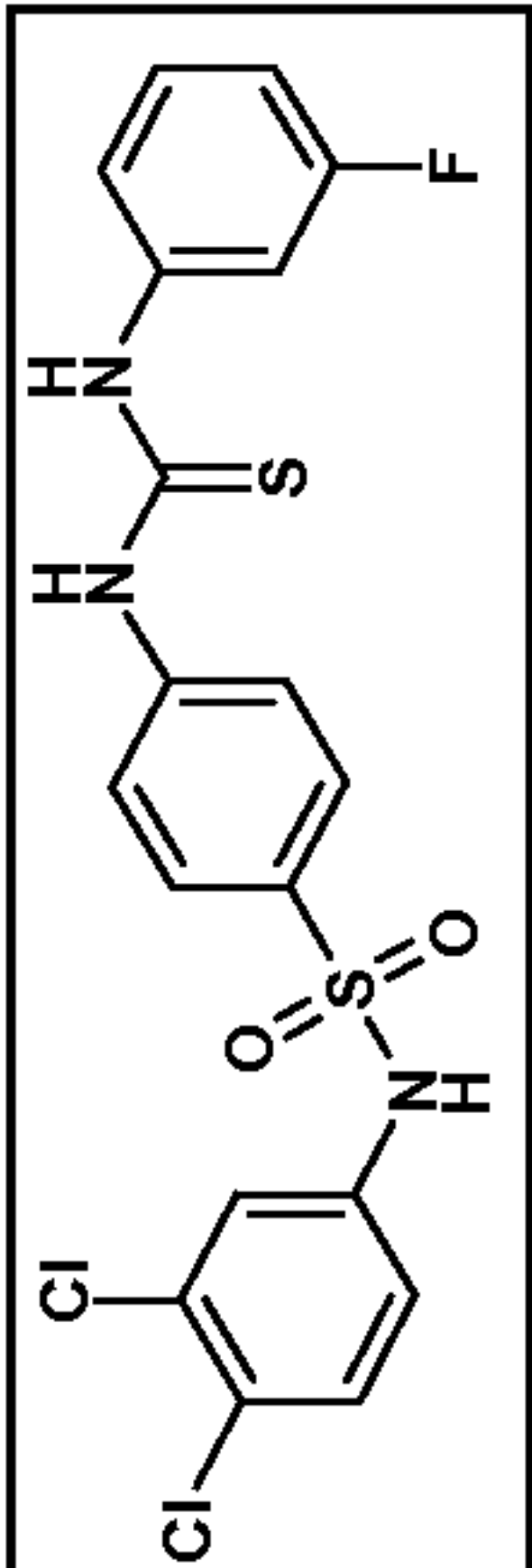
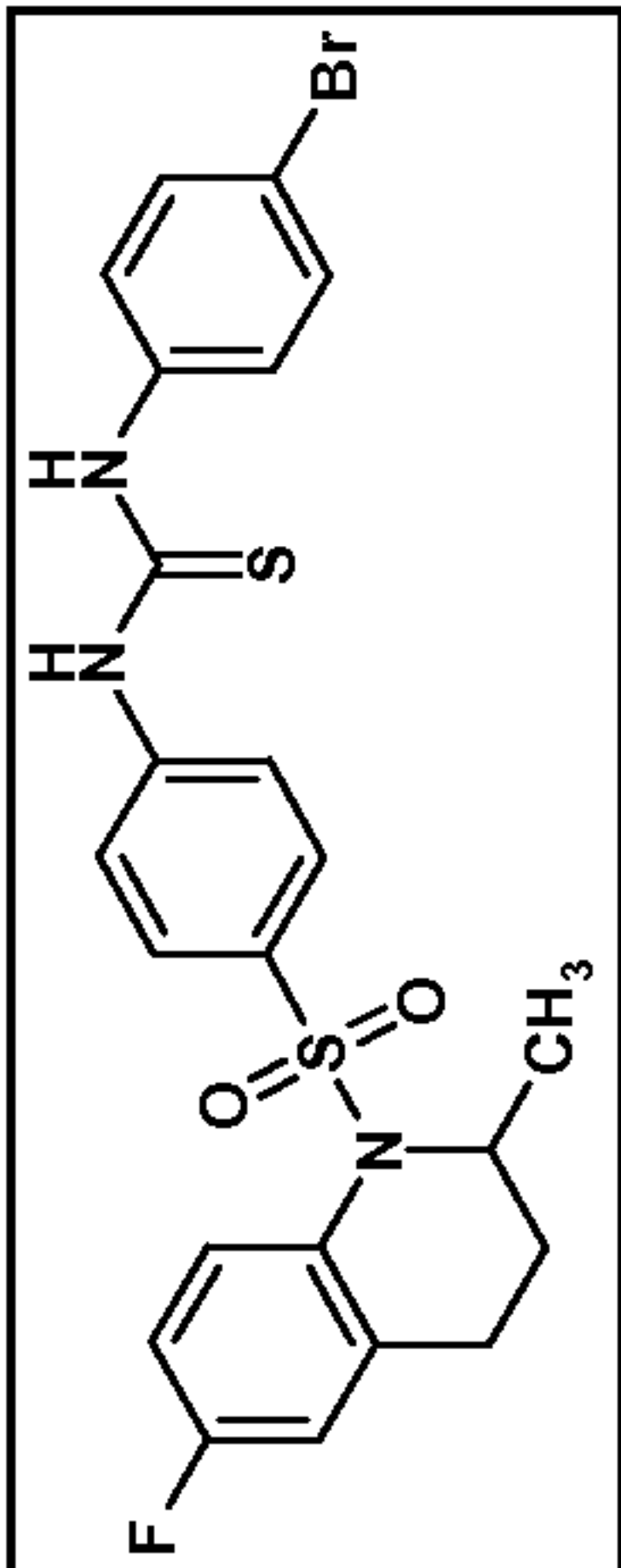
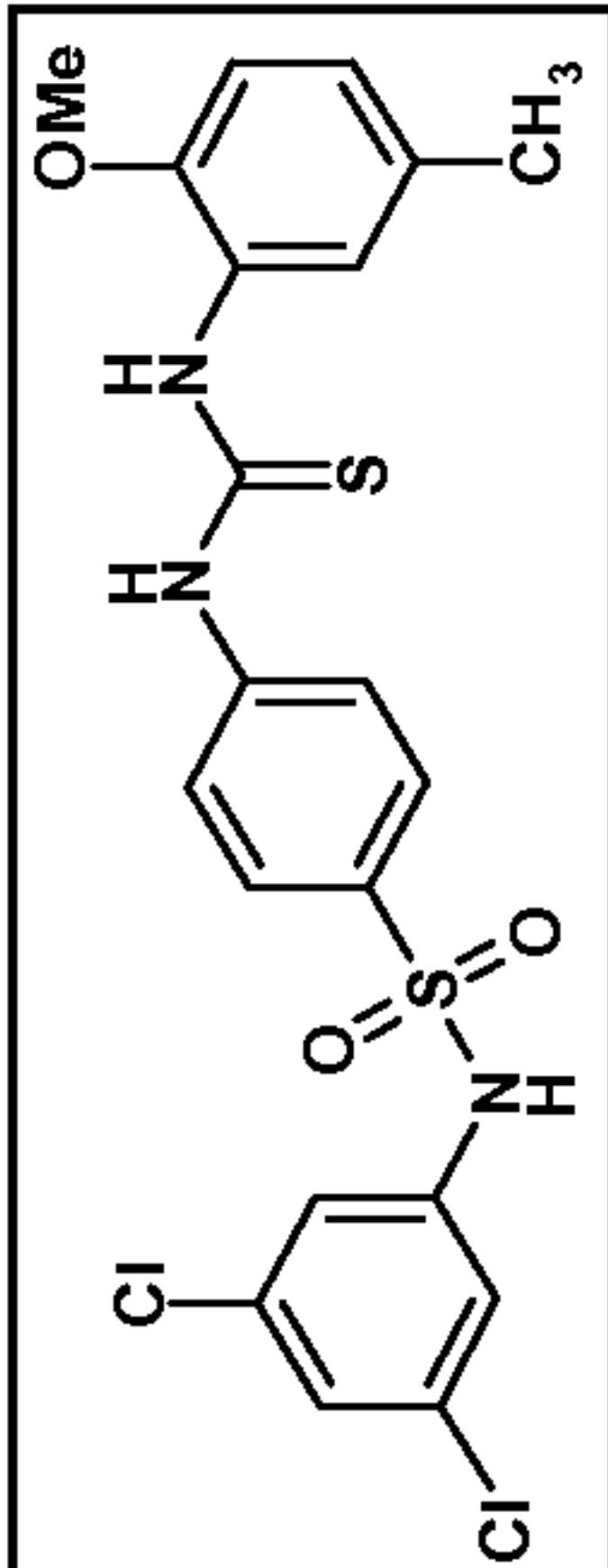
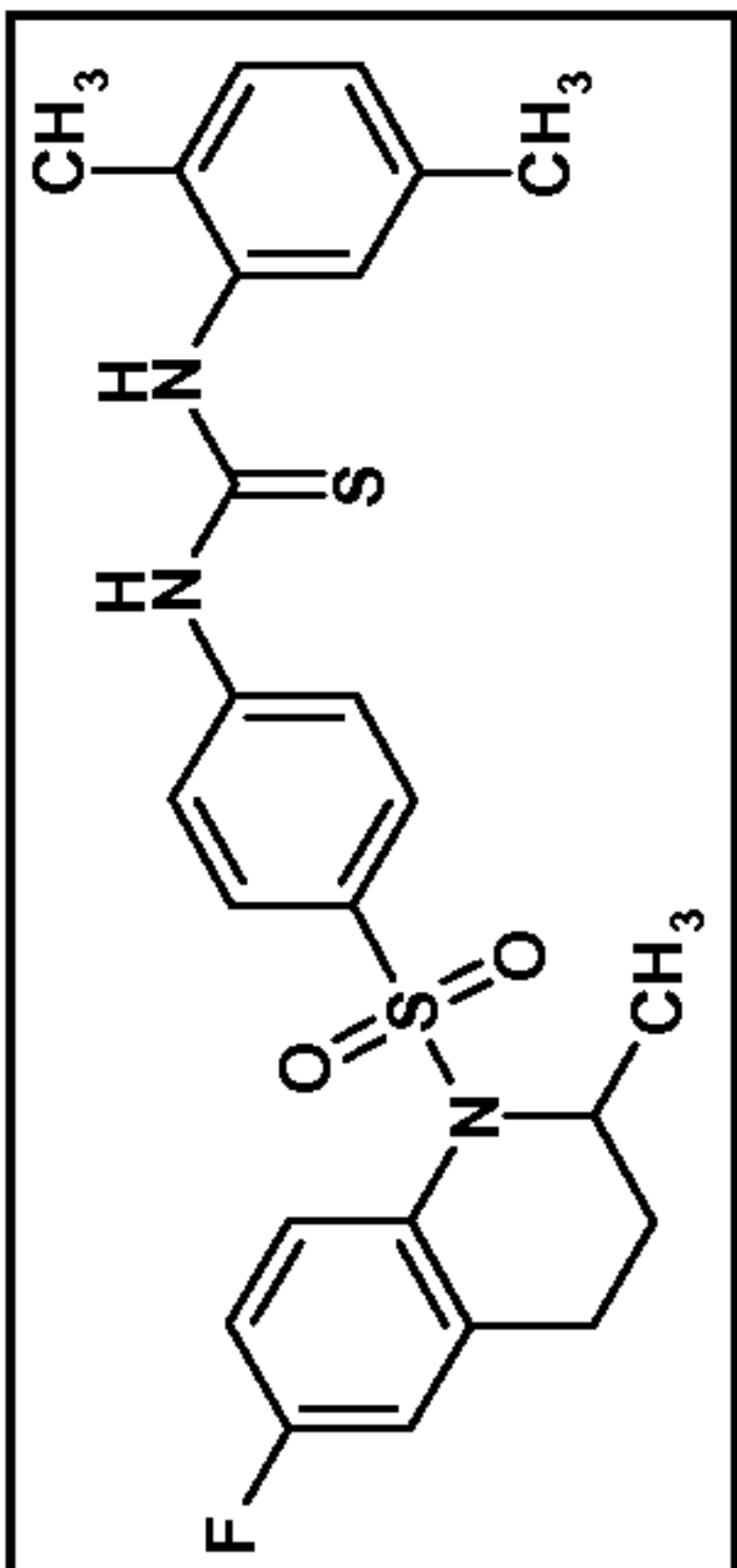
	AD4-12854	-6.57	-55.99	38.7055	6.64/8.19(1.23)	-3.134	-34.386	1YY9 - AD4-11091 LIKE
	AD4-12246	-6.97	-57.98	38.3844	8.36/8.01(0.96)	-3.234	-36.483	1YY9 - AD4-11091 LIKE
	AD4-12391	-6.13	-58.89	37.4191	11.09/9.09(0.82)	-1.131	-37.349	1YY9 - AD4-11091 LIKE
	AD4-12853	-5.17	-52.29	36.92	8.76/7.51(0.86)	-4.265	-38.827	1YY9 - AD4-11091 LIKE
	AD4-12413	-5.58	-54.77	35.1946	6.83/6.35(0.93)	-4.789	-34.803	1YY9 - AD4-11091 LIKE

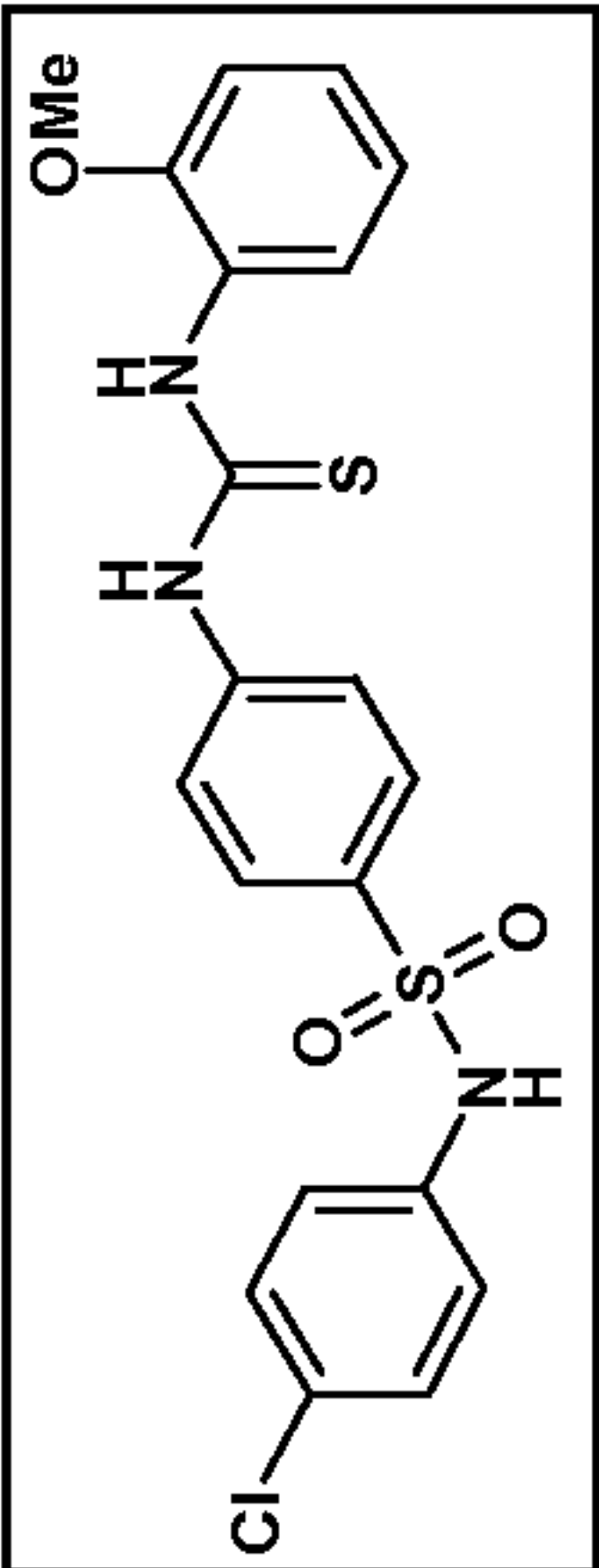
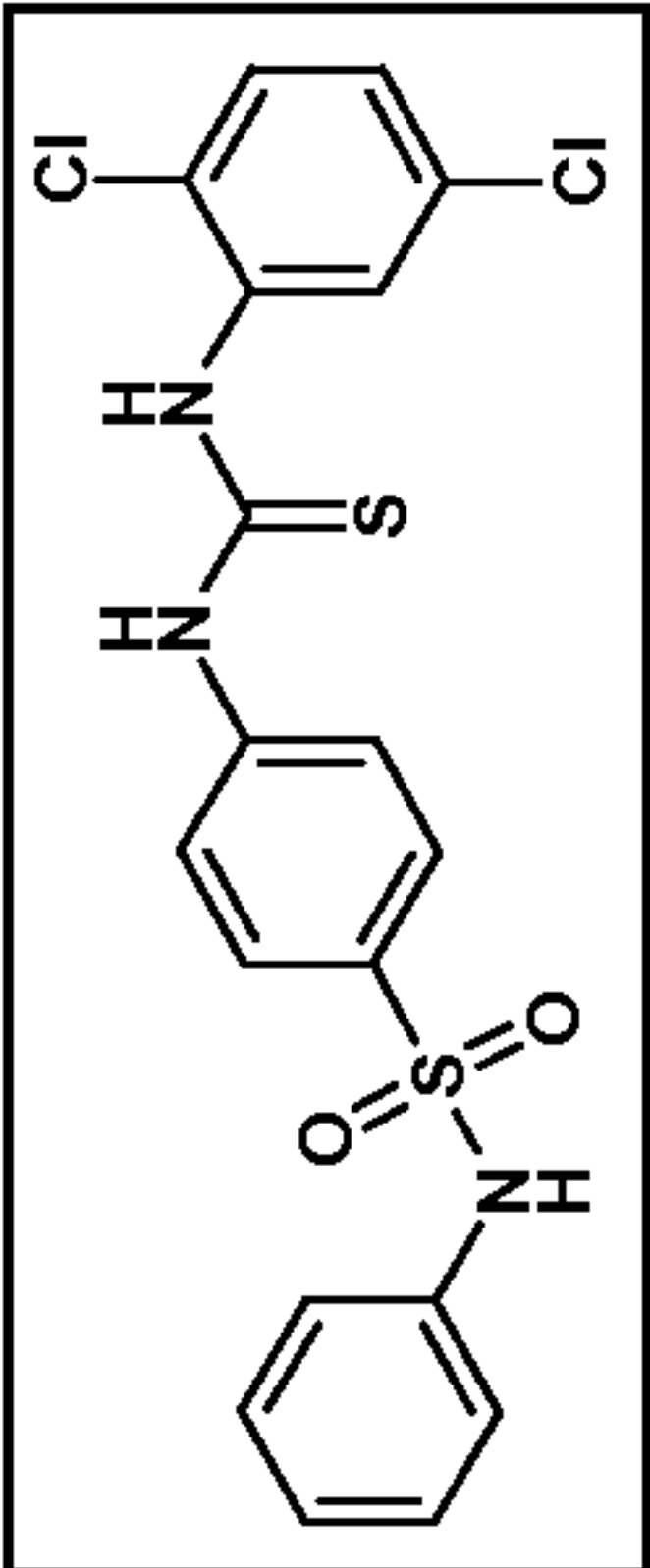
	AD4-12837	-6.48	-49.2	35.1542	6.28/5.68(0.90)	-3.525	-37.042	1YY9 - AD4-11091 LIKE
	AD4-11344	-5.87	-60.29	34.1727	15.06/15.26(1.01)	-2.857	-32.85	1YY9 - AD4-11091 LIKE
	AD4-12525	-6.67	-61.47	34.1311	14.75/12.15(0.82)	-3.692	-39.056	1YY9 - AD4-11091 LIKE
	AD4-12411	-6.38	-56.96	34.0556	6.70/5.18(0.77)	-3.267	-31.845	1YY9 - AD4-11091 LIKE
	AD4-12485	-5.32	-49.68	33.3993	6.98/7.19(1.03)	-3.785	-35.542	1YY9 - AD4-11091 LIKE

	AD4-12828	-4.75	-55.94	32.6456	26.61/63.39(2.38)	-4.427	-40.661	1YY9 - AD4-11091 LIKE
	AD4-12527	-6.11	-56.16	31.9587	26.91/20.01(0.74)	-4.98	-40.481	1YY9 - AD4-11091 LIKE
	AD4-12517	-6.51	-58.67	31.5308	30.39/20.01(0.66)	-3.452	-41.81	1YY9 - AD4-11091 LIKE
	AD4-12465	-5.59	-56.91	31.3476	9.92/6.54(0.66)	-3.435	-35.487	1YY9 - AD4-11091 LIKE

	AD4-12823	-3.94	-58.13	30.8167	4.07/4.06(1.00)	-1.408	-47.23	1YY9 - AD4-11091 LIKE
	AD4-12537	-6.29	-58.32	30.7051	35.37/38.65(1.09)	-3.88	-38.727	1YY9 - AD4-11091 LIKE
	AD4-11428	-6.22	-59.58	29.8673	20.77/15.46(0.74)	-3.692	-42.78	1YY9 - AD4-11091 LIKE
	AD4-12577	-6.85	-61.65	29.4151	6.62/5.92(0.89)	-4.289	-30.521	1YY9 - AD4-11091 LIKE
	AD4-12250	-6.65	-62.41	29.3683	12.68/11.57(0.91)	-3.033	-43.246	1YY9 - AD4-11091 LIKE

	AD4-12832	-3.94	-58.13	29.02	7.71/16.30(2.11)	-1.408	-47.23	1YY9 - AD4-11091 LIKE
	AD4-12512	-5.28	-56.81	28.9126	6.40/5.83(0.91)	-3.555	-34.118	1YY9 - AD4-11091 LIKE
	AD4-12482	-7.87	-59.36	28.3382	6.54/6.05(0.93)	-5.834	-34.551	1YY9 - AD4-11091 LIKE
	AD4-11430	-6.25	-48.49	27.1482	19.83/16.84(0.85)	-3.823	-33.209	1YY9 - AD4-11091 LIKE
	AD4-10693	-6.87	-60.55	26.8206	48.24/35.85(0.74)	-2.528	-44.456	1YY9 - AD4-1886 Dockpharm

	AD4-12825	-5.72	-54.54	26.7866	6.78/5.46(0.81)	-4.103	-37.363	1YY9 - AD4-11091 LIKE
	AD4-12431	-5.95	-50.11	26.7815	6.40/5.57(0.87)	-4.12	-36.705	1YY9 - AD4-11091 LIKE
	AD4-12813	-6.36	-51.7	26.1431	11.76/13.63(1.16)	-5.202	-33.055	1YY9 - AD4-11091 LIKE
	AD4-12499	-5.38	-58.36	26.0767	6.99/6.58(0.94)	-2.372	-38.545	1YY9 - AD4-11091 LIKE
	AD4-12824	-4.66	-56.6	25.9501	Not Active	-2.832	-37.478	1YY9 - AD4-11091 LIKE

	AD4-12502	-5.63	-60.71	25.1182	6.97/5.46(0.78)	-2.992	-38.026	1YY9 - AD4-11091 LIKE
	AD4-12855	-4.57	-57.09	25.0542	6.83/6.25(0.92)	-4.8	-33.063	1YY9 - AD4-11091 LIKE

[0506] Two-dimensional representations of the docked pose of AD4-1886, along with AD4-1886-like compounds were produced. Docking of compound AD4-1886 to EGFR is depicted, for example, in FIG. 6A. Docking of compound AD4-11883 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6B. Docking of compound AD4-11975 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6C. Docking of compound AD4-11409 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6D. Docking of compound AD4-11638 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6E. Docking of compound AD4-11645 (an AD4-1886-like compound) to EGFR is depicted, for example, in FIG. 6F.

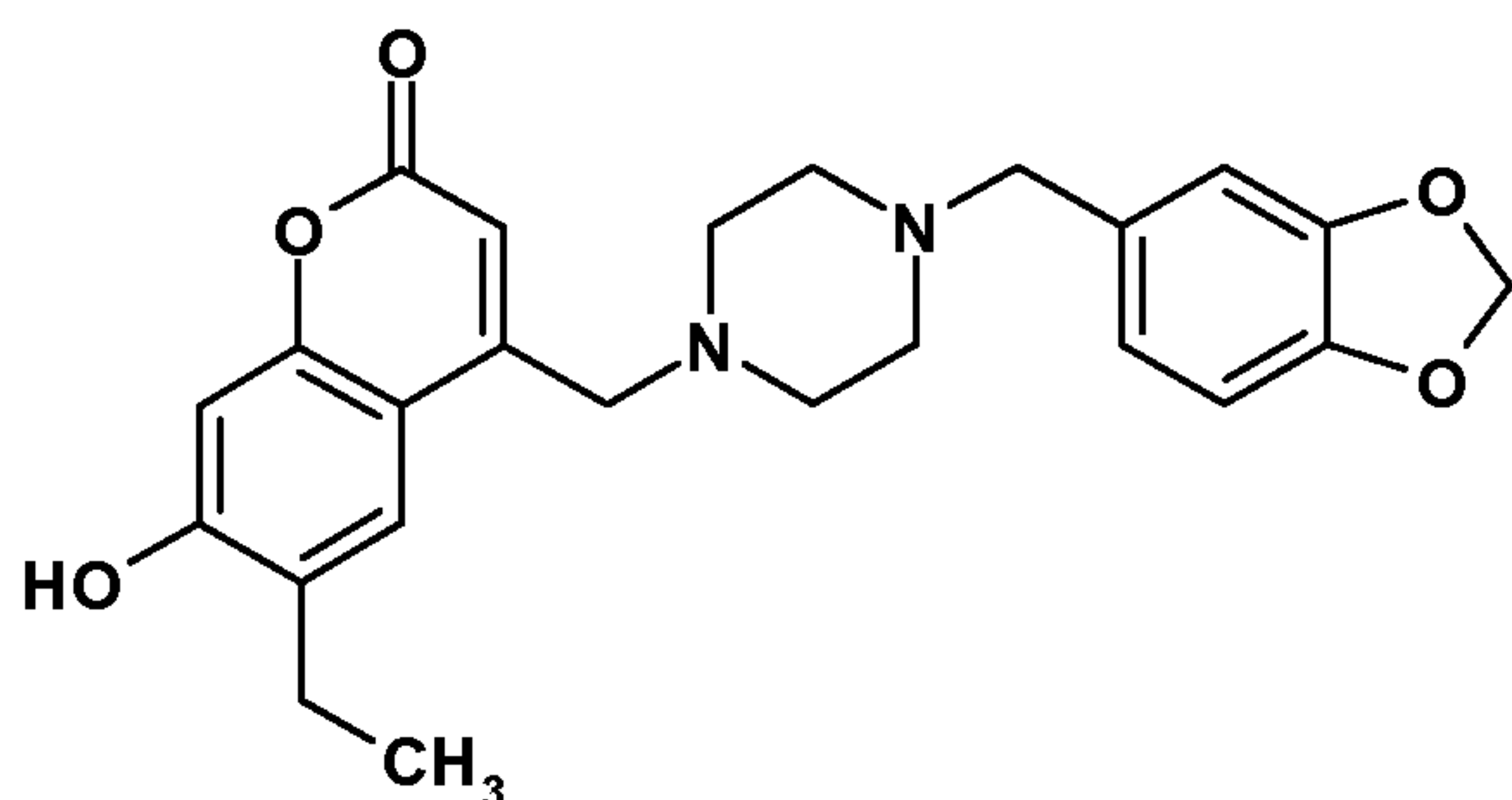
EXAMPLE 7: LIGAND DOCKING AND SCORING TO INACTIVE EGFR PROTEIN

[0507] The compounds selected for docking to the target protein, the inactive folded conformation of EGFR (PDB accession number 1NQL) were those which were found to align to the pharmacophore models generated in the MOE modeling software (*see* Example 4).

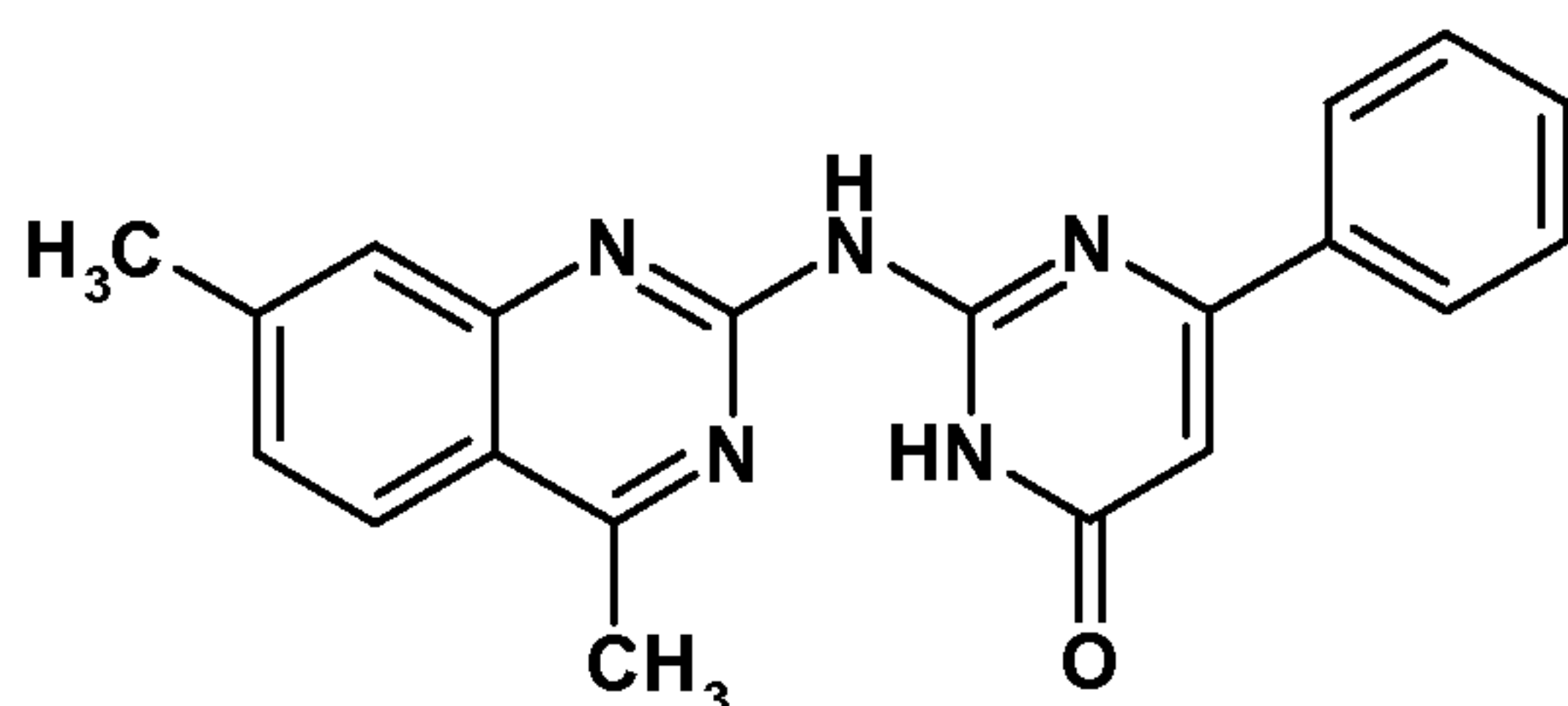
[0508] Methods are according to those described in Example 6, except as otherwise indicated.

[0509] The protein crystal structure of EGFR in its inactive state (1NQL.PDB) was imported into Maestro in PDB format. The binding site was determined by picking a ligand, ZINC3304802 which was one of the pharmacophore hits found by MOE, in Maestro (Glide).

[0510] Analysis of compounds from the ZINC database against the pharmacophores identified from the 1NQL.PDB crystal structure of protein EGFR according to the methods described above identified compounds AD4-1734, and AD4-10381.



AD4-1734, Formula (12)



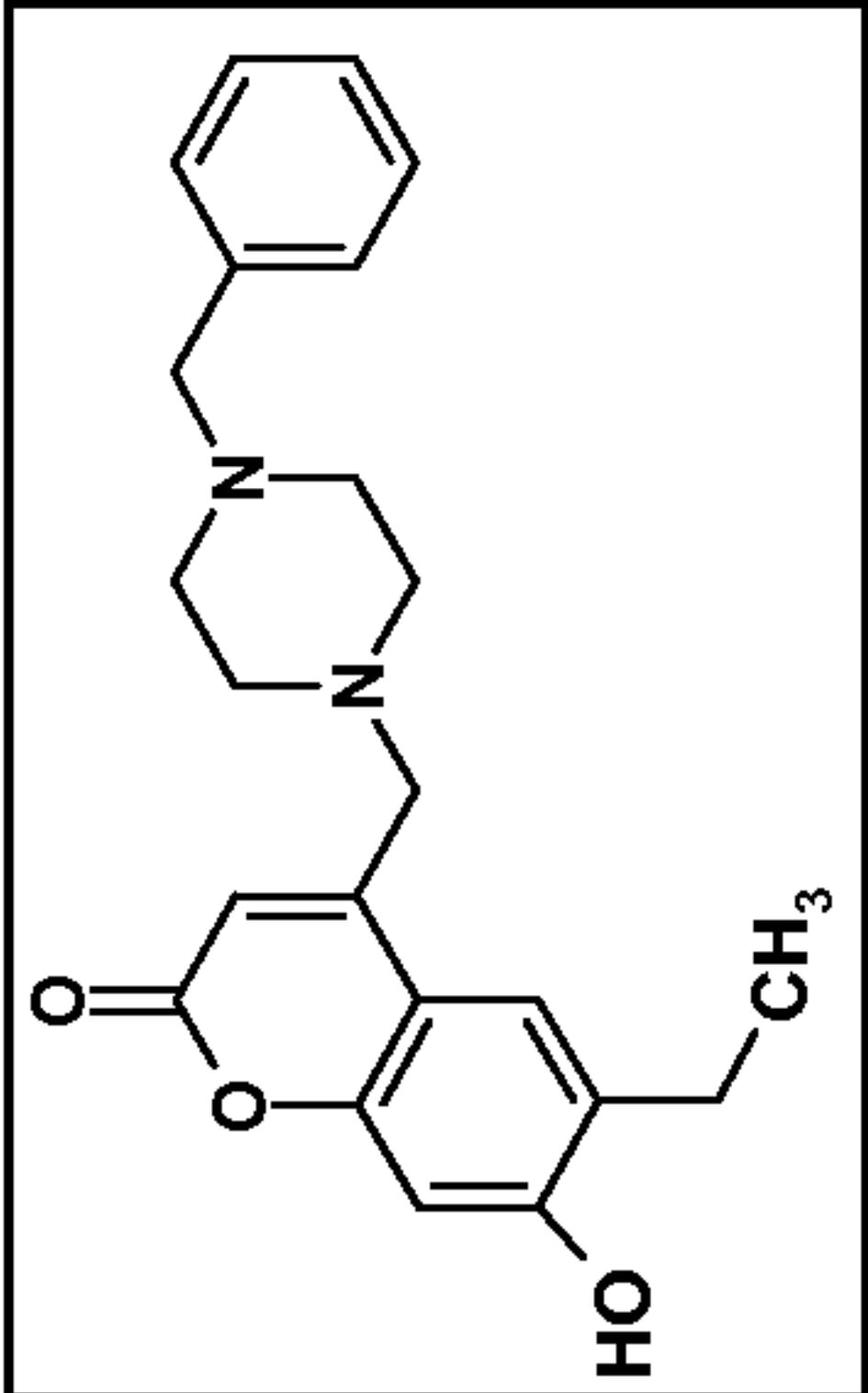
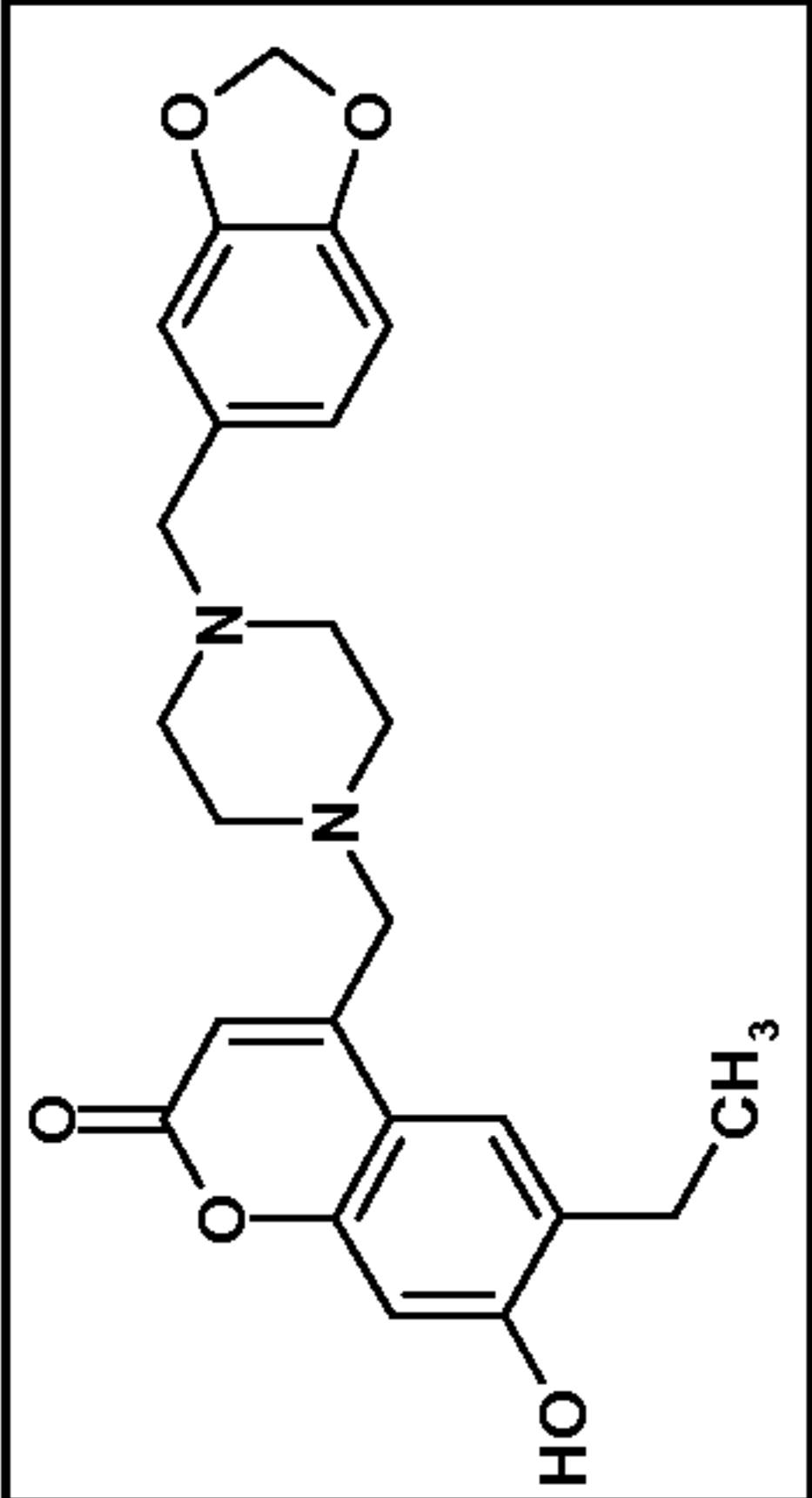
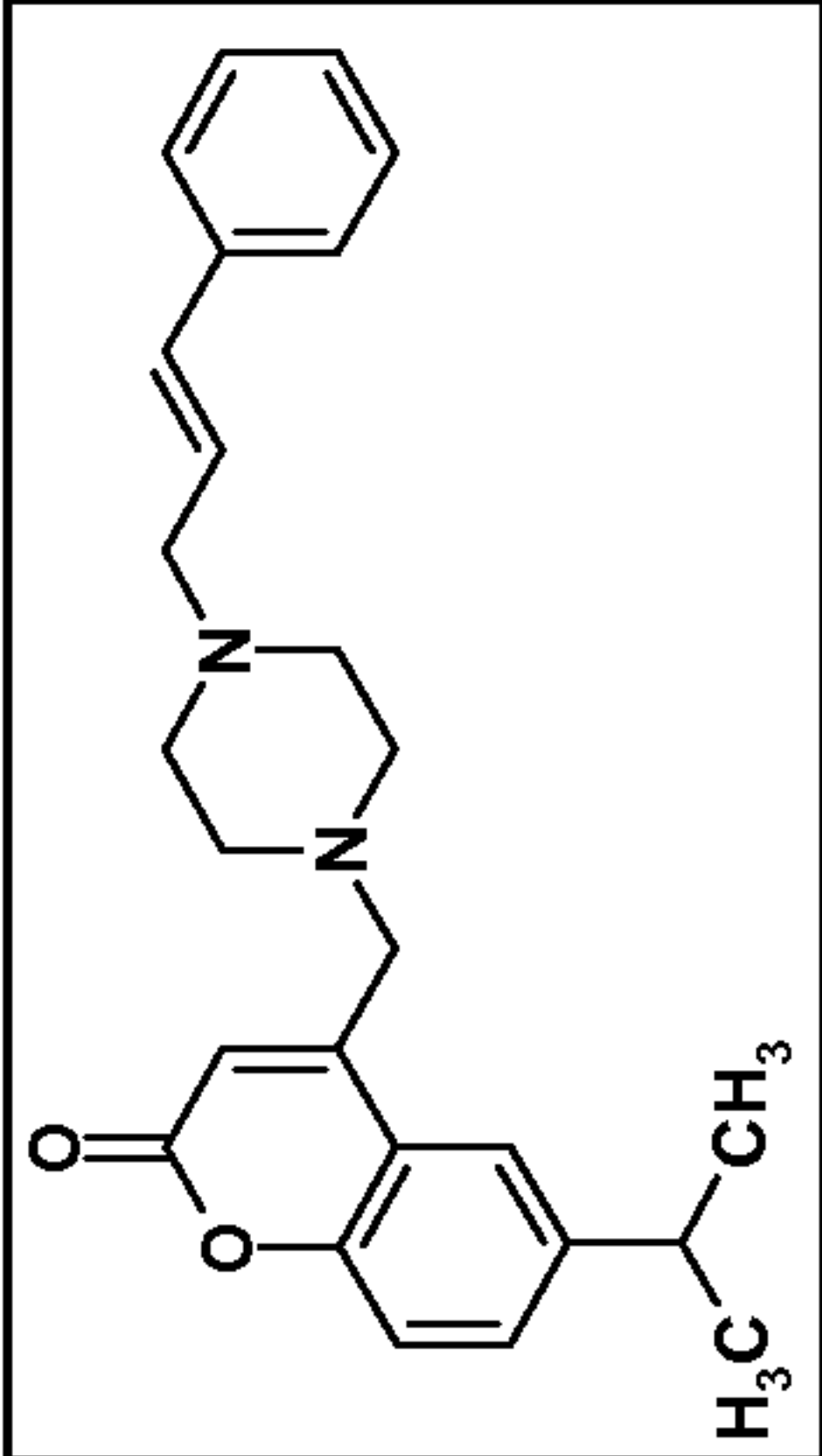
AD4-10381, Formula (24)

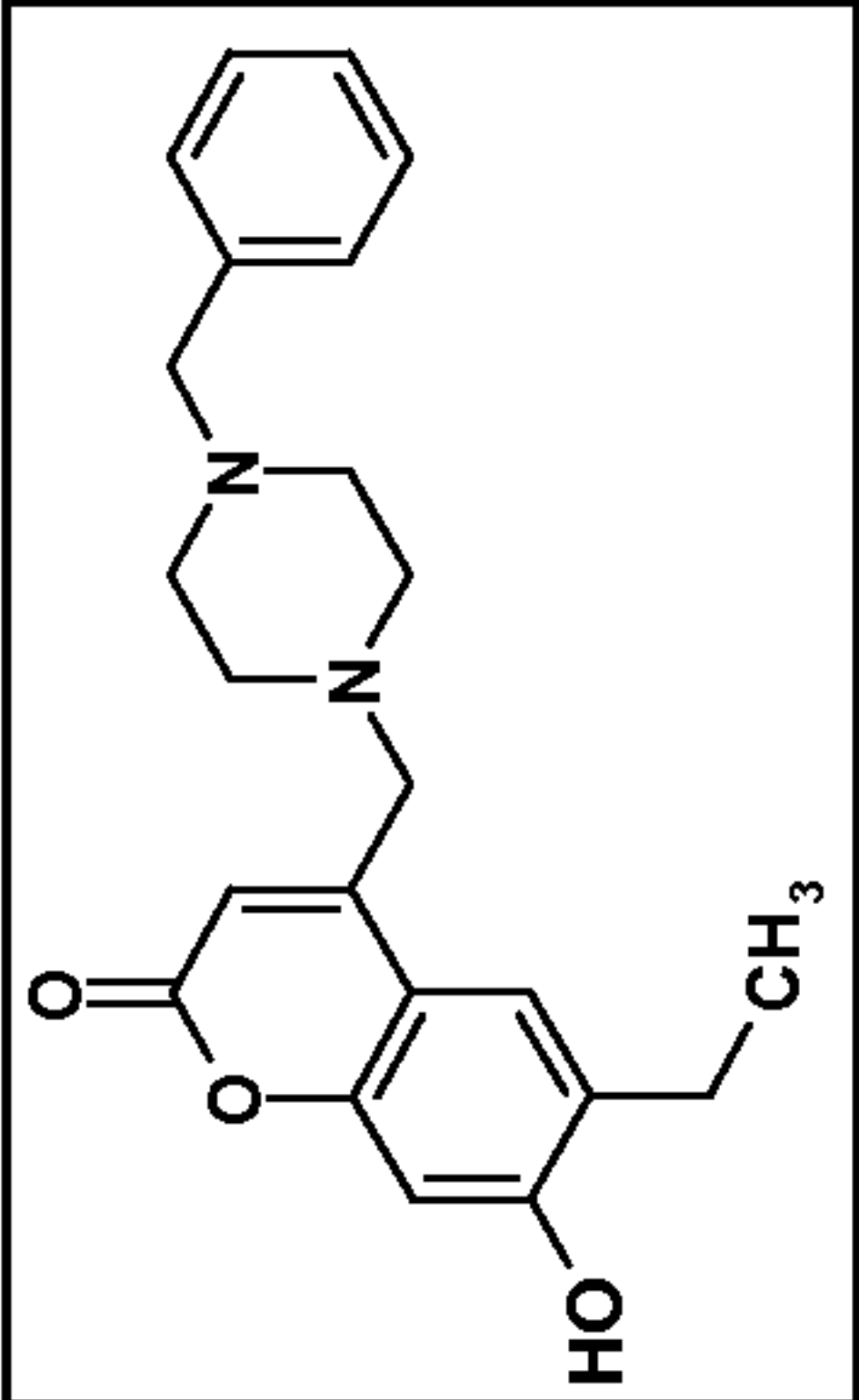
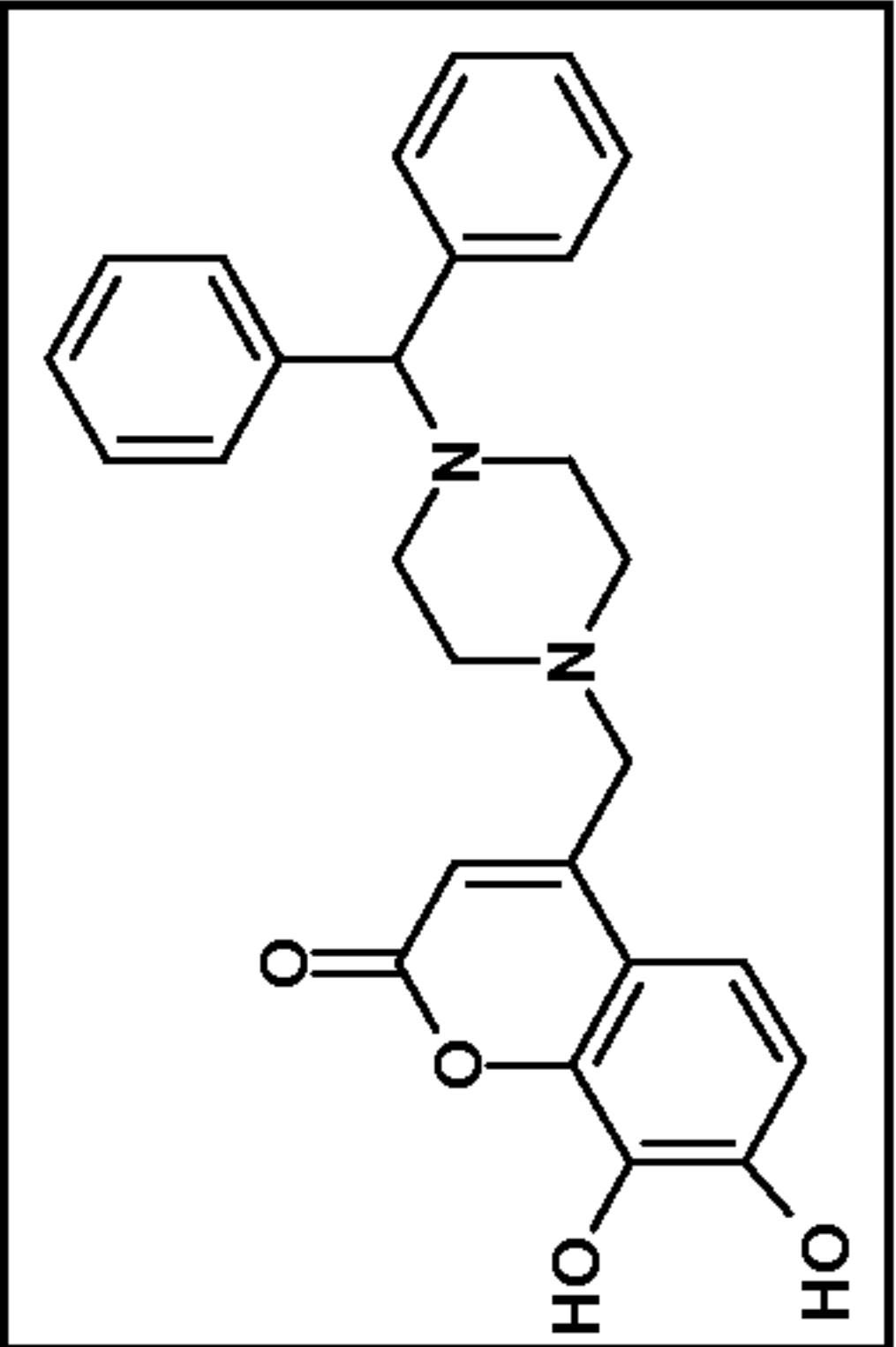
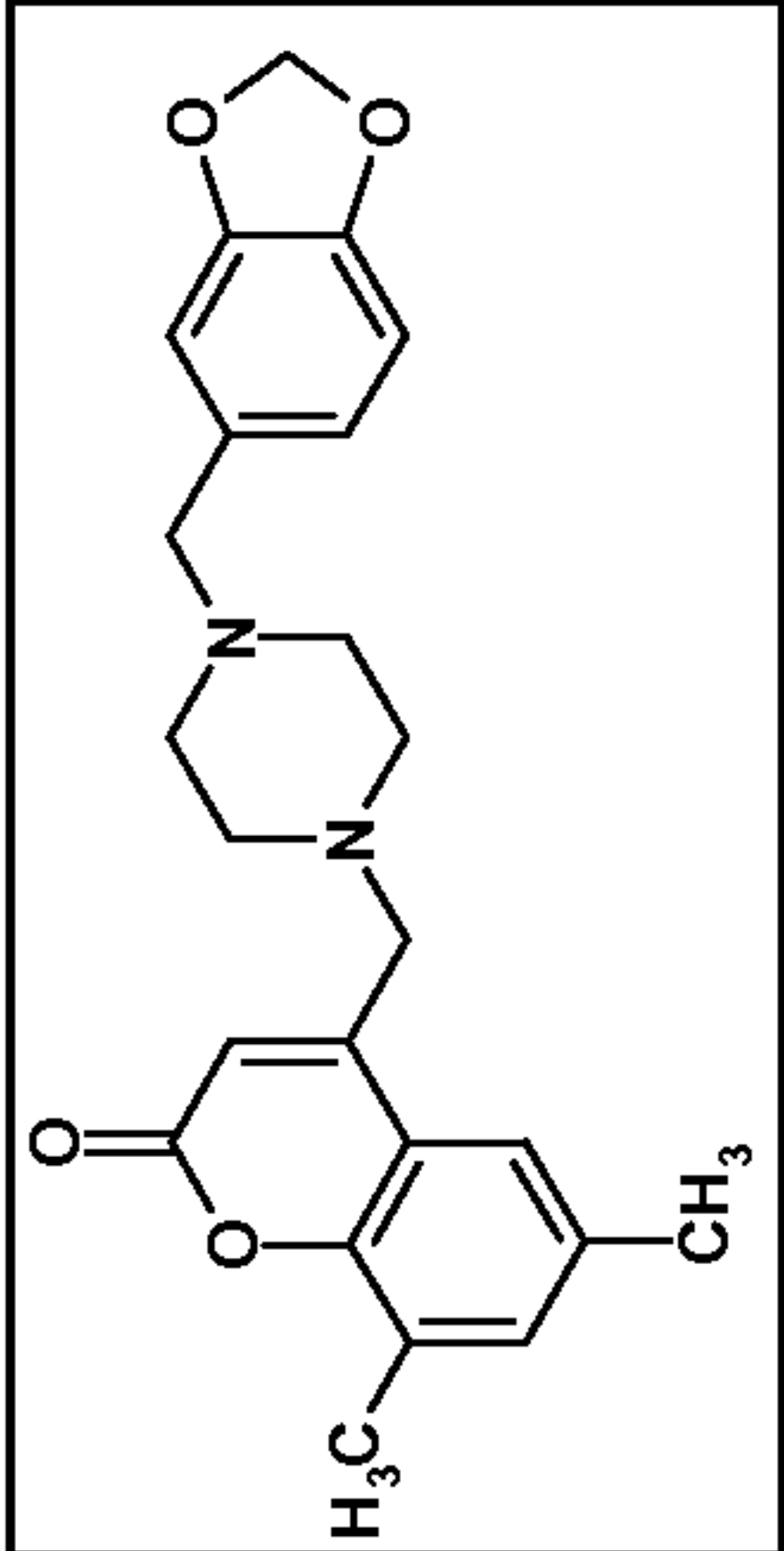
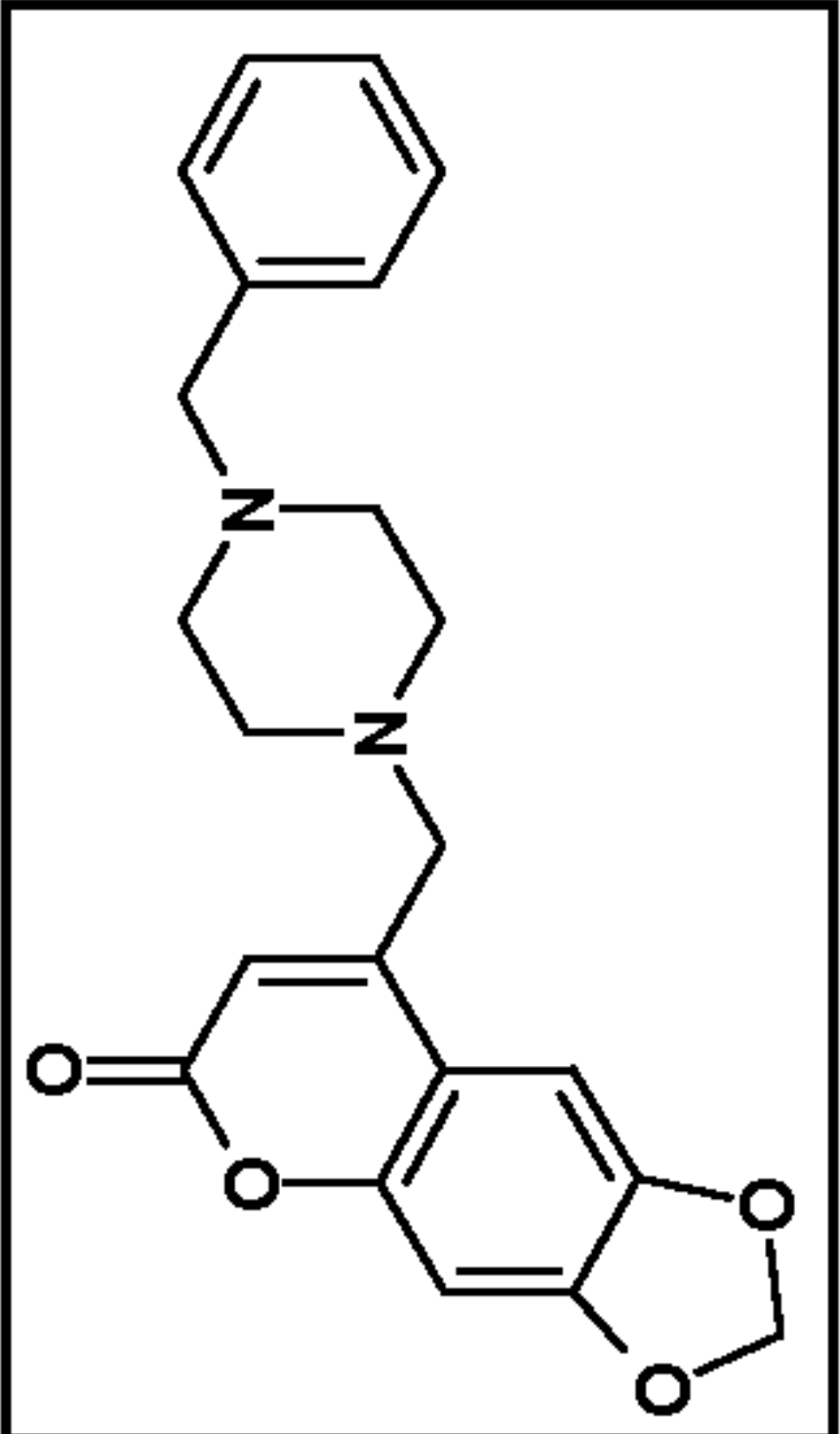
[0511] The compounds in the following tables were identified via structure similarity searches and were docked to the binding site to obtain their Glide and Emodel scores.

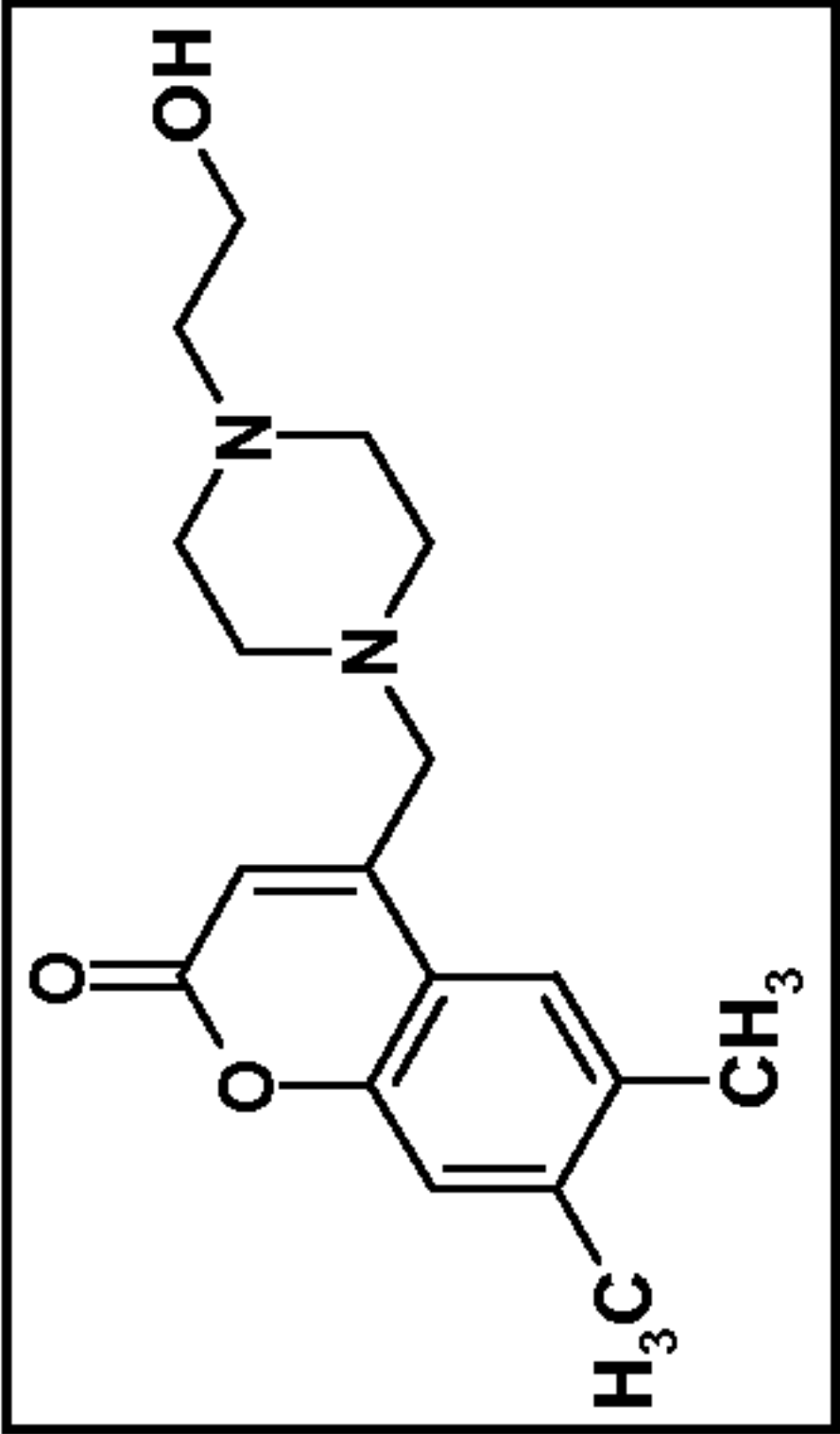
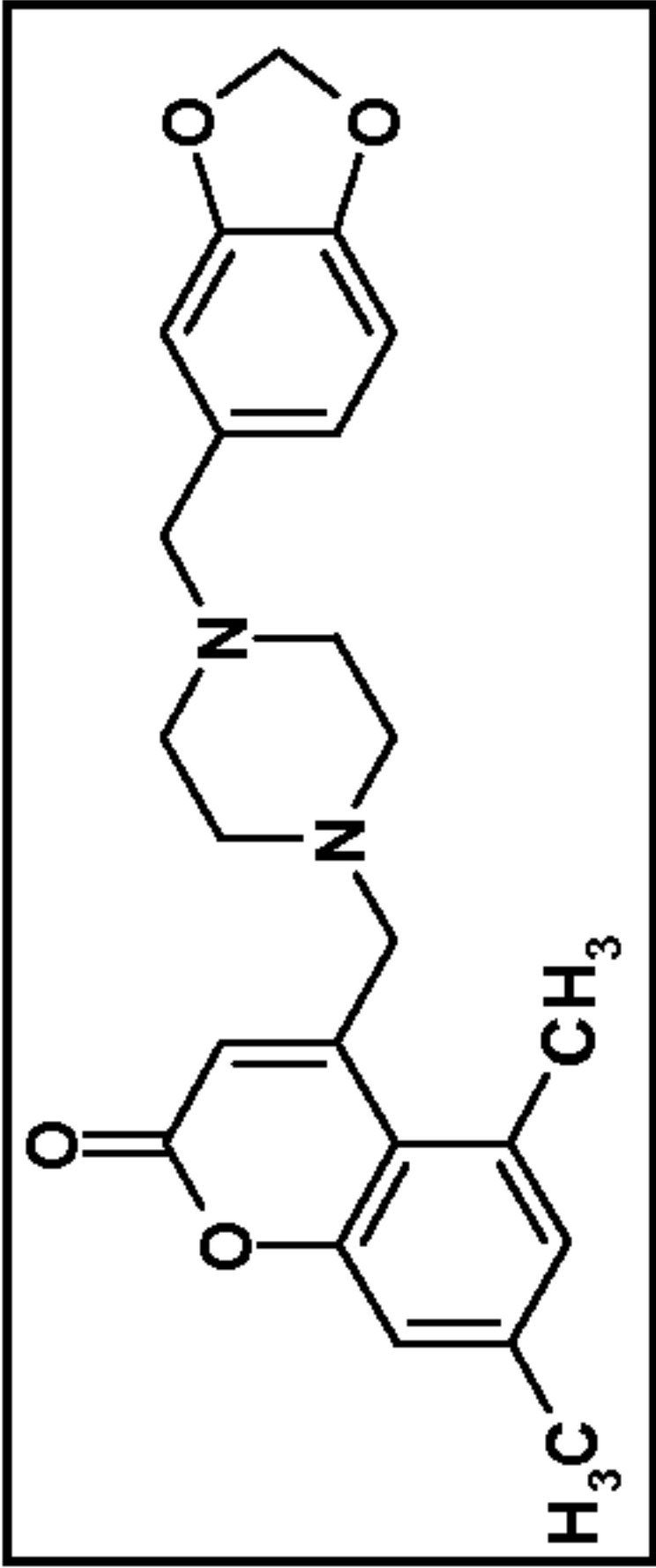
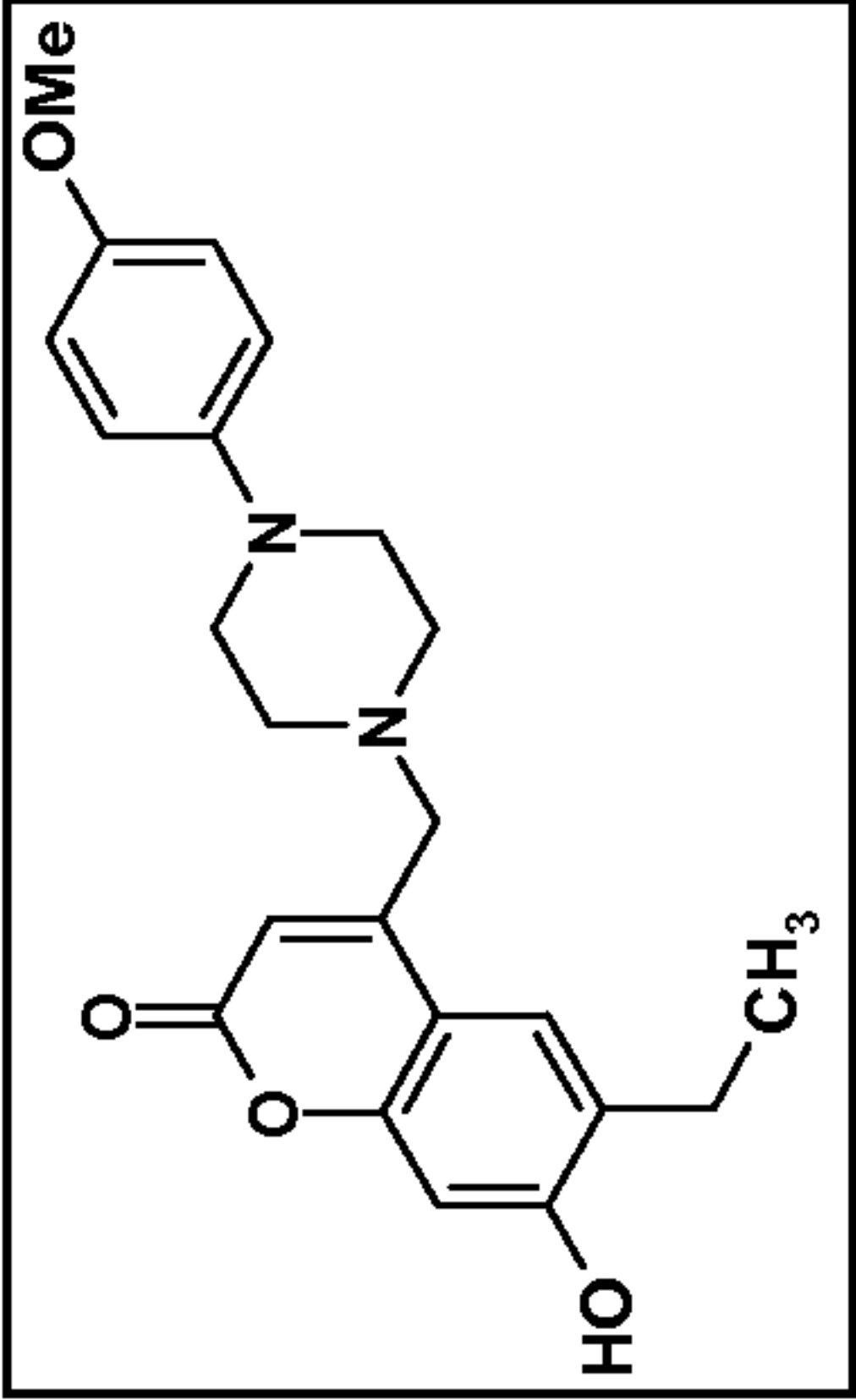
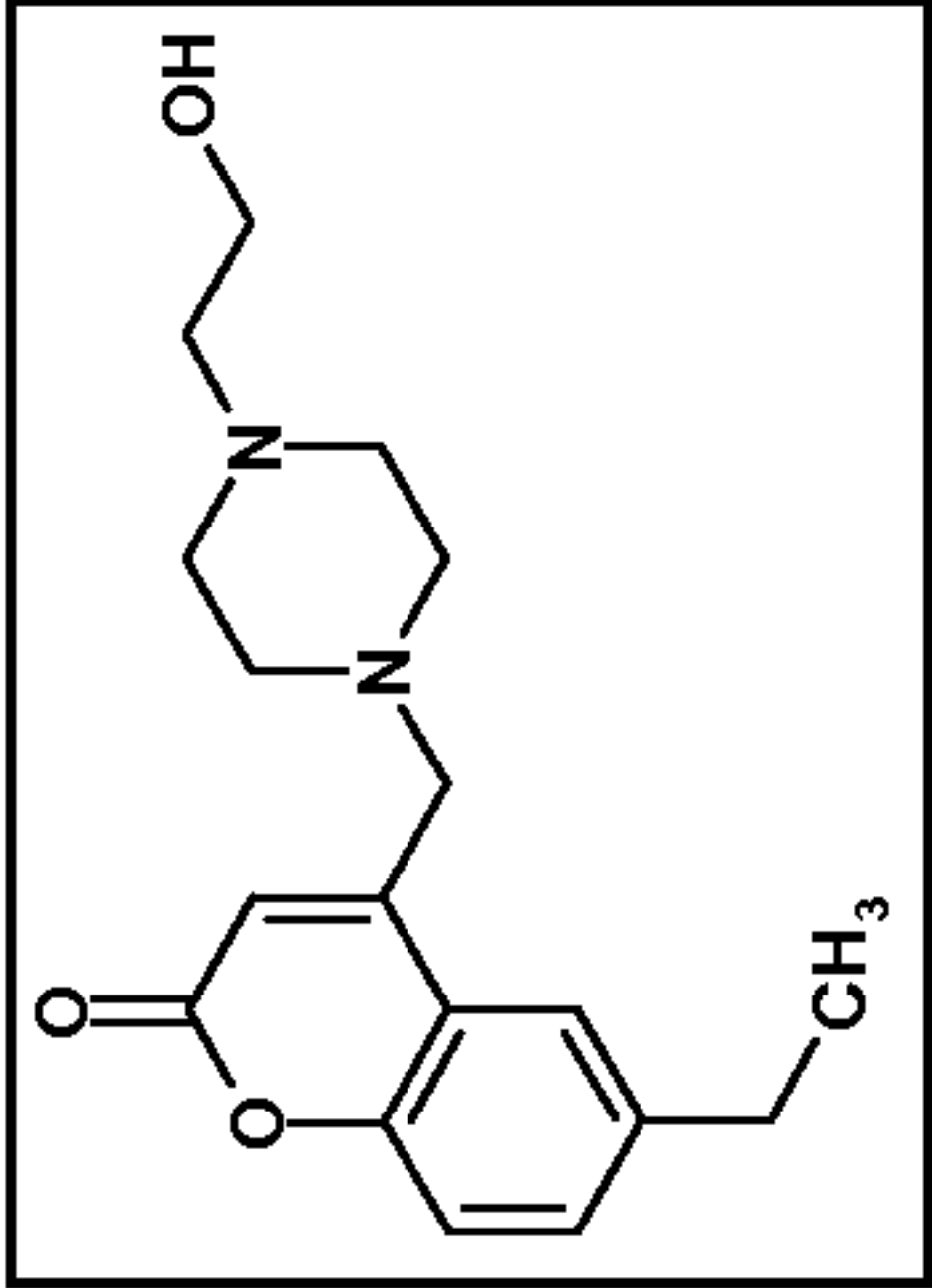
[0512] AD4-1734-like

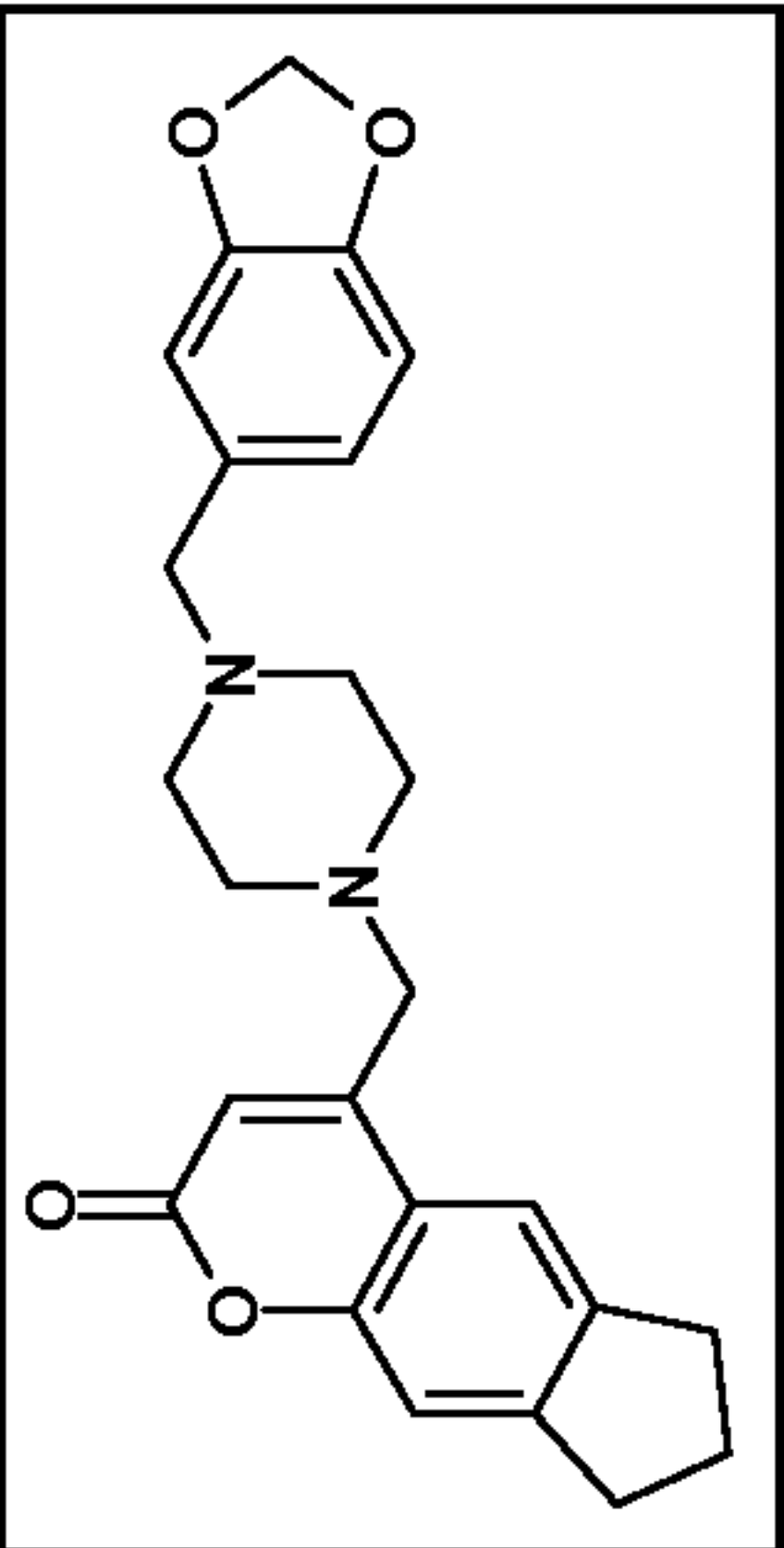
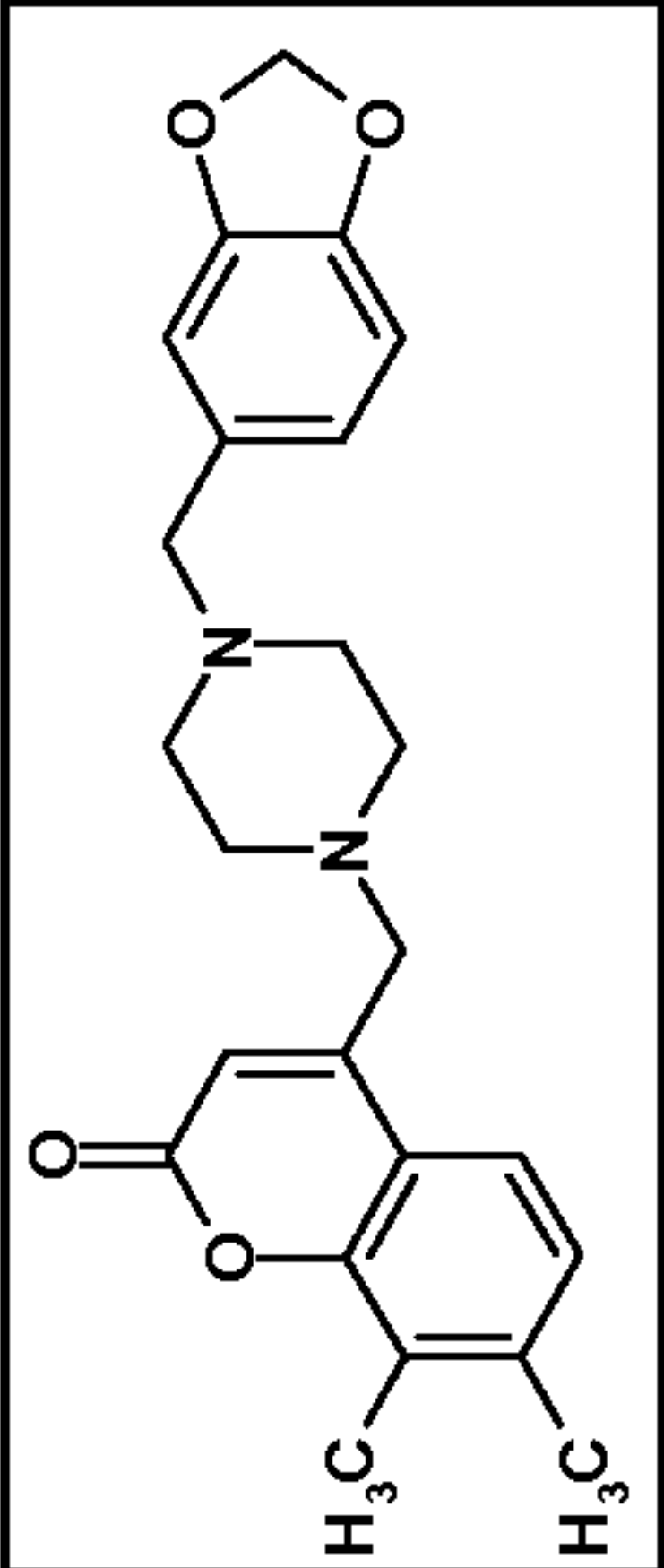
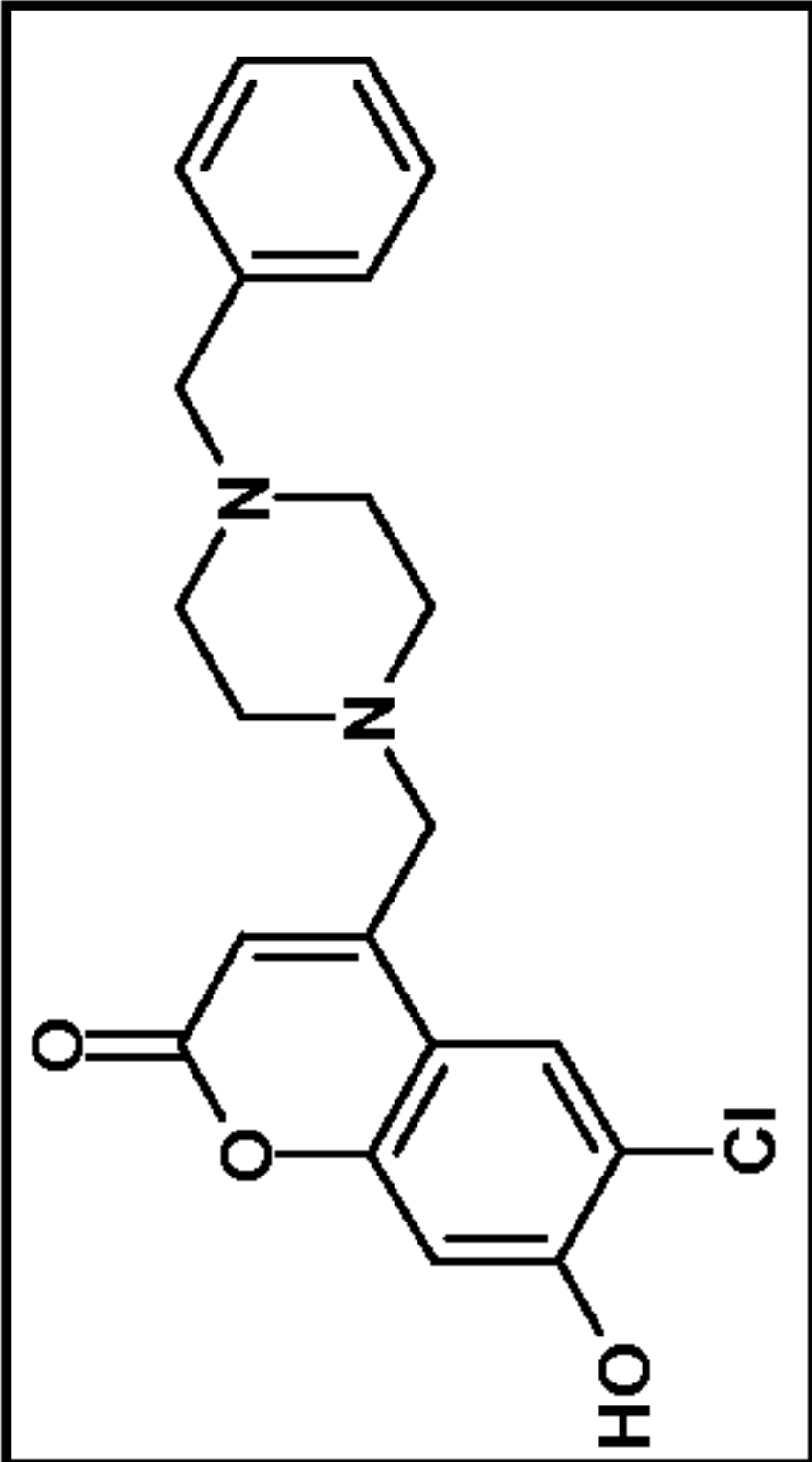
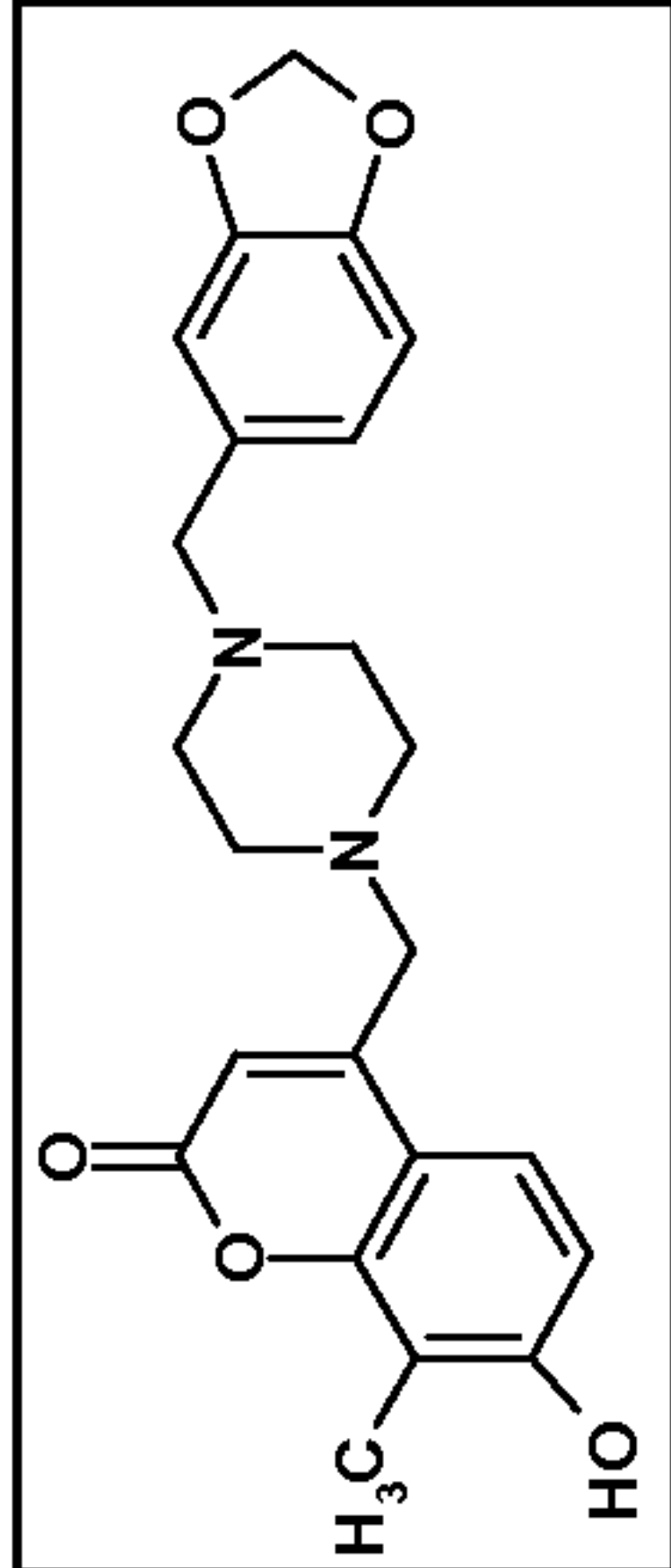
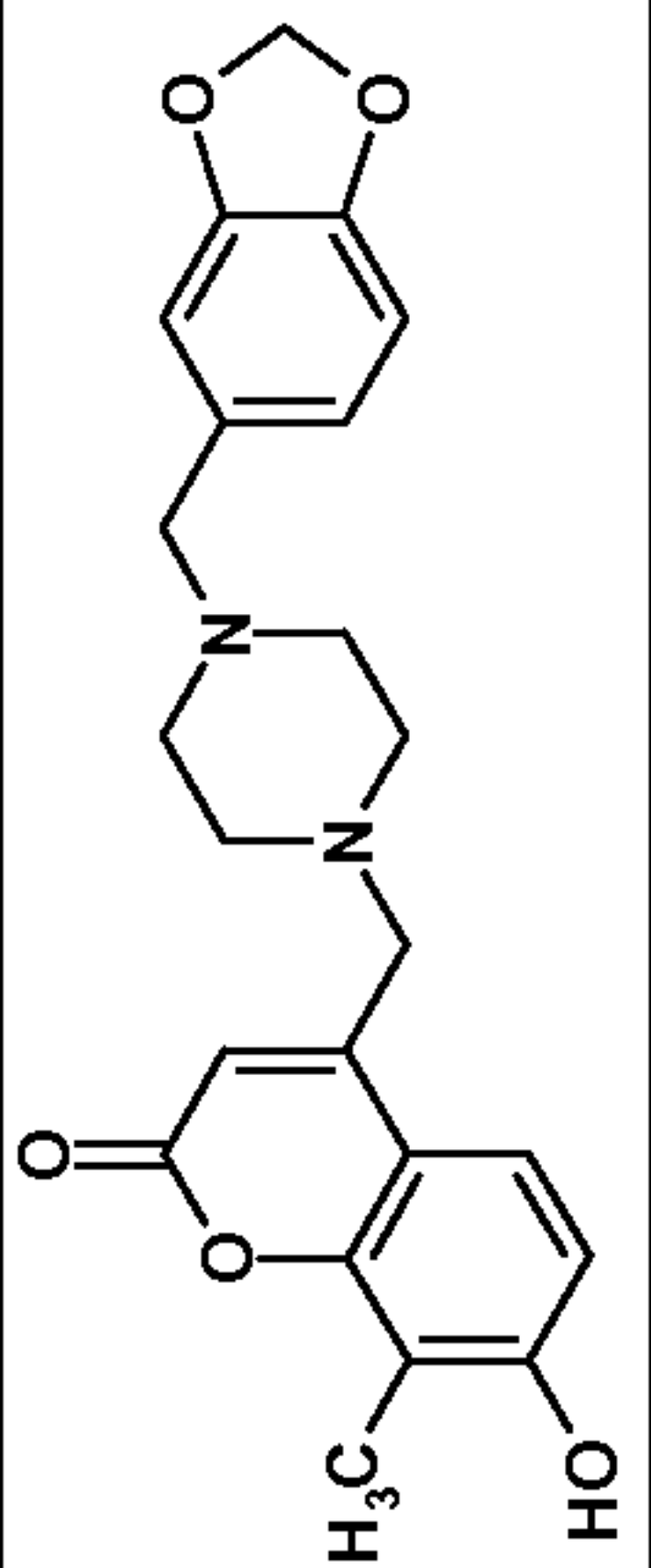
[0513] The compounds in TABLE 11 were identified via AD4-1734 structure similarity searches and were docked to the 1NQL.PDB binding site to obtain their Glide and Emodel scores. Also depicted in TABLE 11 is ICW assay and MTT assay results (See Example 1, Example 2, Example 7).

TABLE 11: Glide Score, E-Model score, ICW assay, and MTT Assay Results for AD4-1734-like Compounds.

	AD4 No.	Sub-Class	E-Model	G-Score	ICW Screen	MTT Screen	MTT Follow-up	Pharmacophore Query
	AD4-10631	P	-4.999	-46.538	104.48	35.64/37.47 (1.05)		1NQL-Glue - AD4-1734 LIKE
	AD4-1734	R	-6.985	-47.431	65.9	22.75/42.50 (1.87)	22.1/40.2(1.82)	1NQL-Glue
	AD4-10188	Q	-6.118	-47.713	53.35	9.05/16.32 (1.80)		1NQL-Glue - AD4-1734 LIKE

	AD4-10186	P	-5.204	-49.183	47.518	40.10/45.74 (1.14)		1NQL-Glue - AD4-1734 LIKE
	AD4-10633	P	-5.019	-48.58	46.398	5.20/non-convergent	Not Convergent	1NQL-Glue - AD4-1734 LIKE
	AD4-10174	R	-7.534	-48.44	45.85	2.26/8.58 (3.80)	4.42/101.2 (22.9)	1NQL-Glue - AD4-1734 LIKE
	AD4-10639	S	-5.635	-48.32	43.695	42.59/non-convergent 27% Max & -12% Max		1NQL-Glue - AD4-1734 LIKE

	AD4-10176	Q	-7.195	-47.579	42.066	50% Max & 11% Max	1NQL-Glue - AD4-1734 LIKE
	AD4-10182	P	-5.62	-40.619	39.51	6.78/43.31 (6.39) 86% Max & 15% Max	1NQL-Glue - AD4-1734 LIKE
	AD4-10171	Q	-6.276	-48.614	39.389	16.52/21.02(1.27)	1NQL-Glue - AD4-1734 LIKE
	AD4-10178	Q	-6.311	-41.019	34.907	22% Max & -19% Max	1NQL-Glue - AD4-1734 LIKE

	AD4-10179	S	-5.484	-53.221	34.742	13.16/13.87(1.05) 38% Max & 20% Max	1NQL-Glue - AD4-1734 LIKE
	AD4-10181	R	-5.427	-45.217	34.604	2.61/15.96 (6.11) 38% Max & 5% Max	1NQL-Glue - AD4-1734 LIKE
	AD4-10180	P	-6.42	-43.904	32.1	16.72/36.31(2.17) 70% Max & 13% Max	1NQL-Glue - AD4-1734 LIKE
	AD4-10637	R	-6.977	-48.57	30.057	20.81/41.33(1.99)	1NQL-Glue - AD4-1734 LIKE
	AD4-10491	R	-5.677	-45.143	29.223	22.22/40.61(1.83)	1NQL-Glue - AD4-1734 LIKE

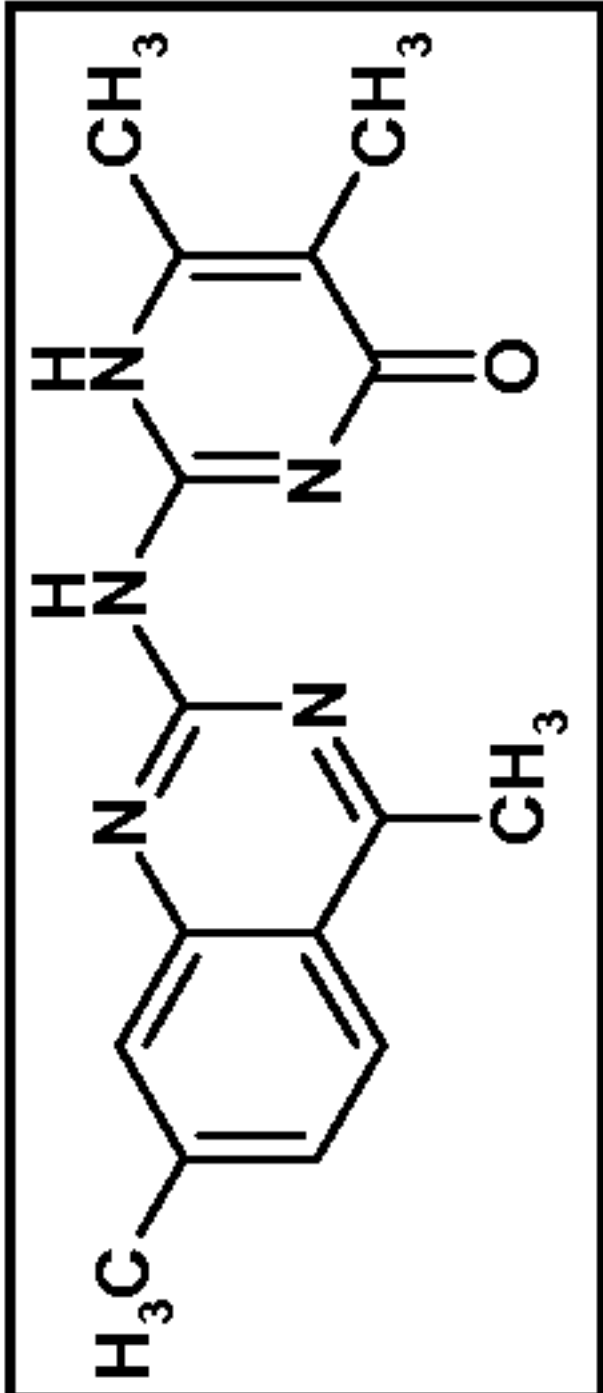
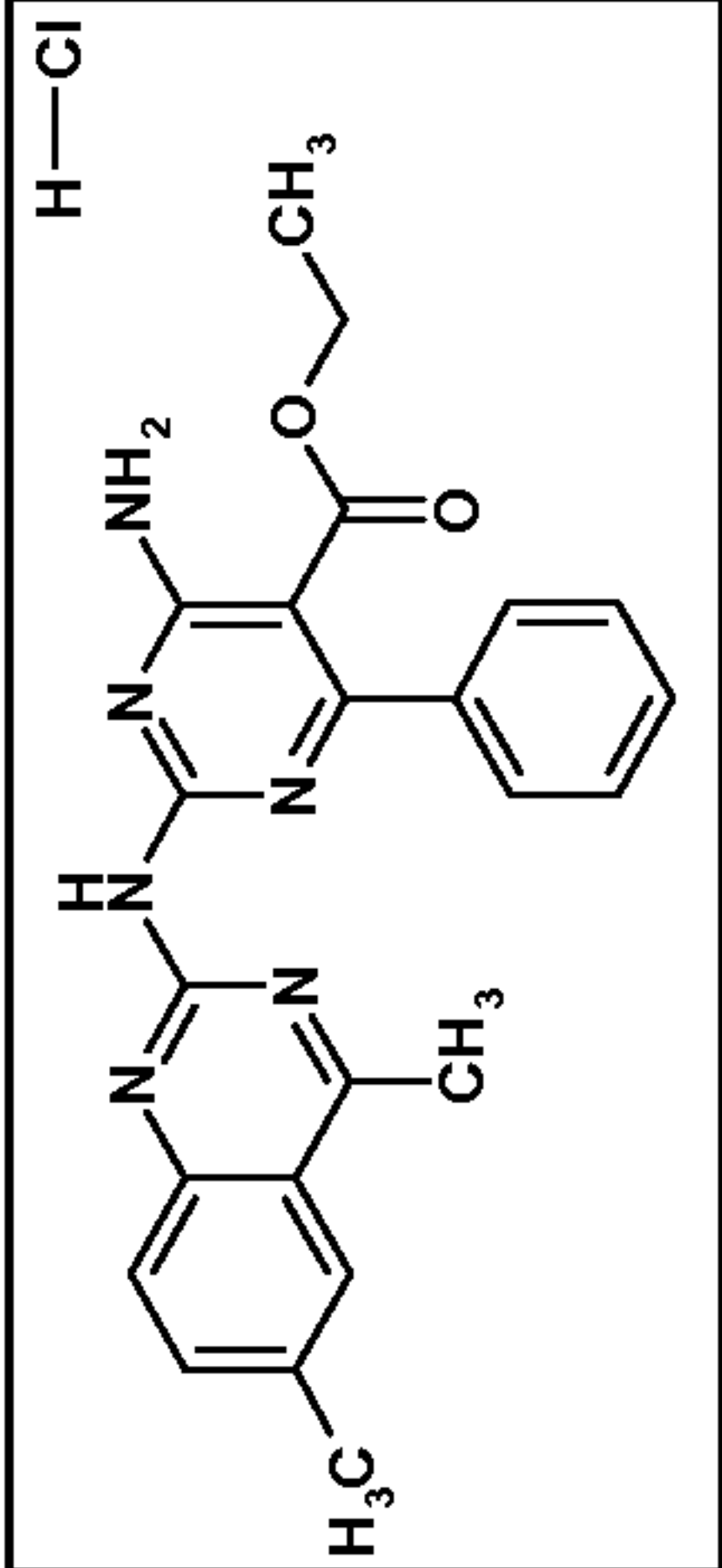
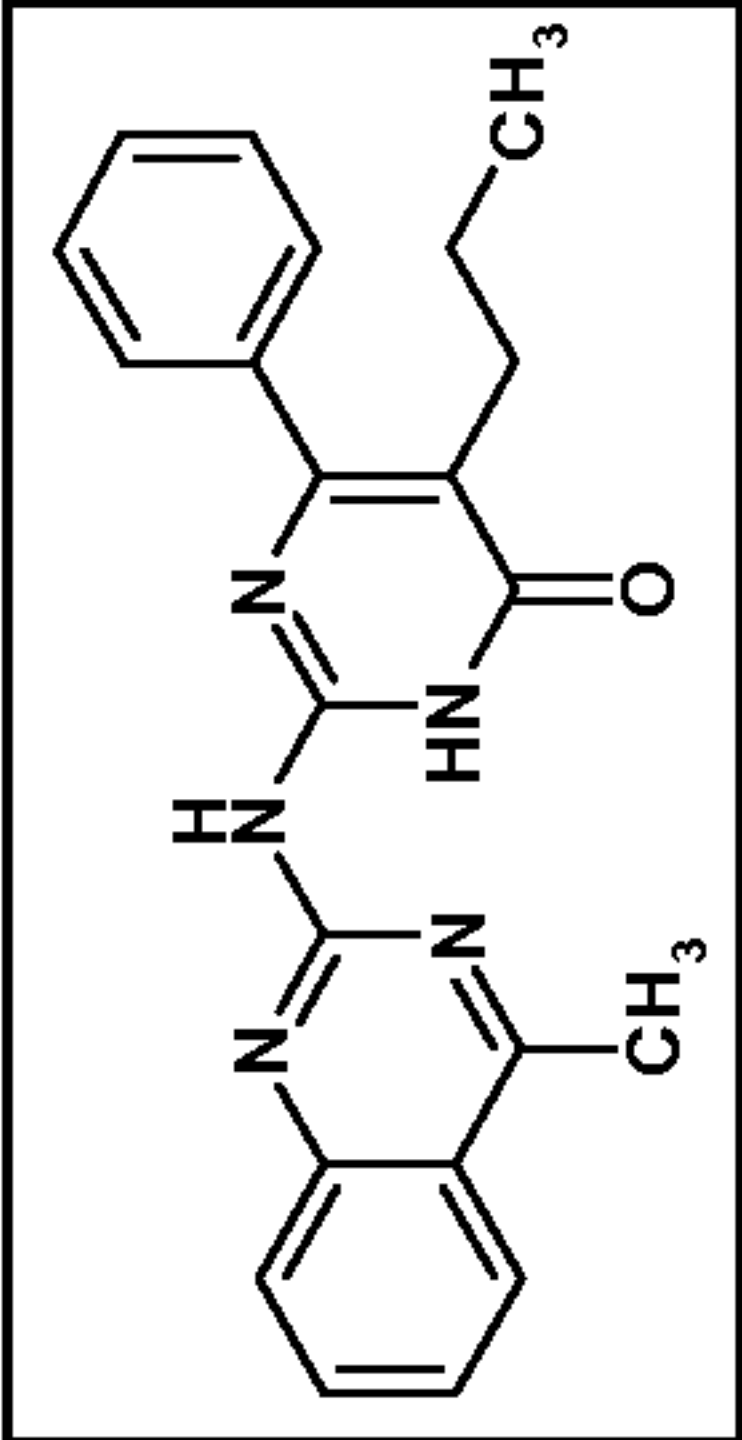
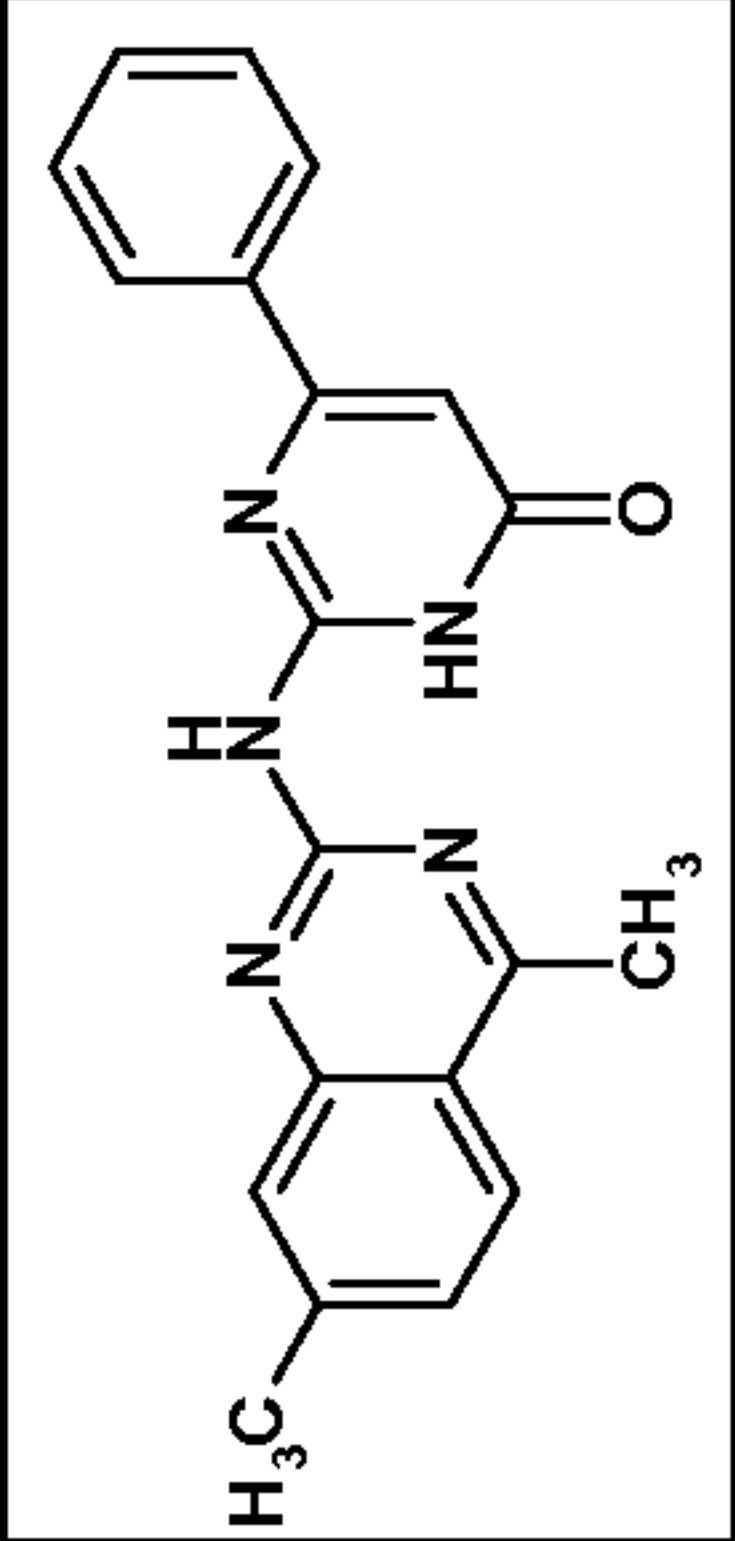
[illegible]

154

[0514] AD4-10381-like

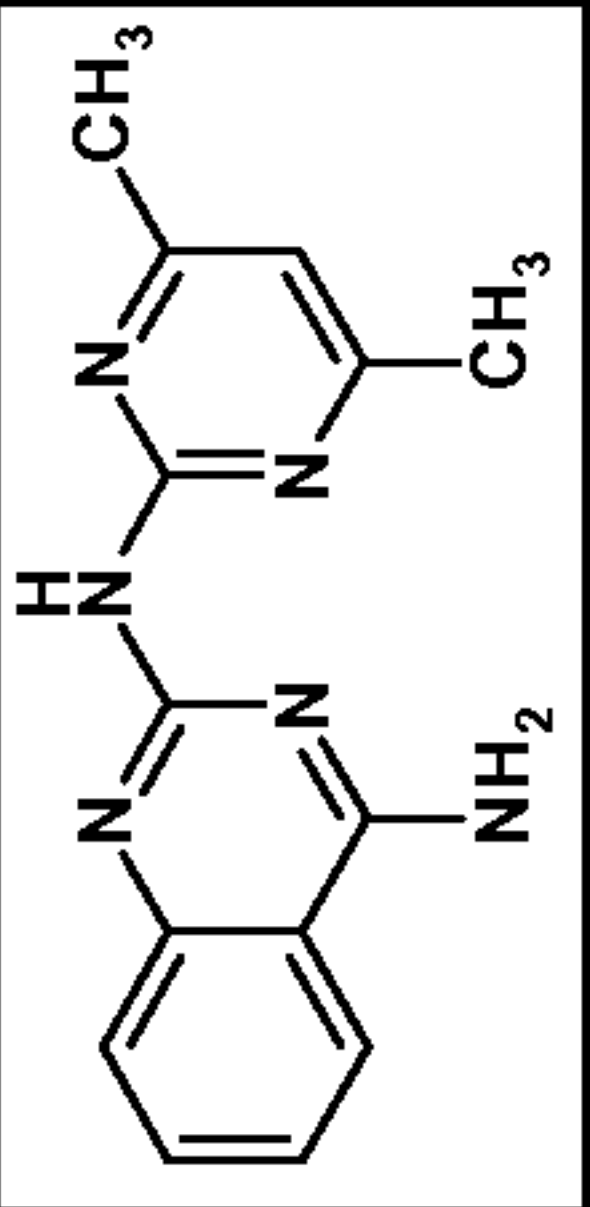
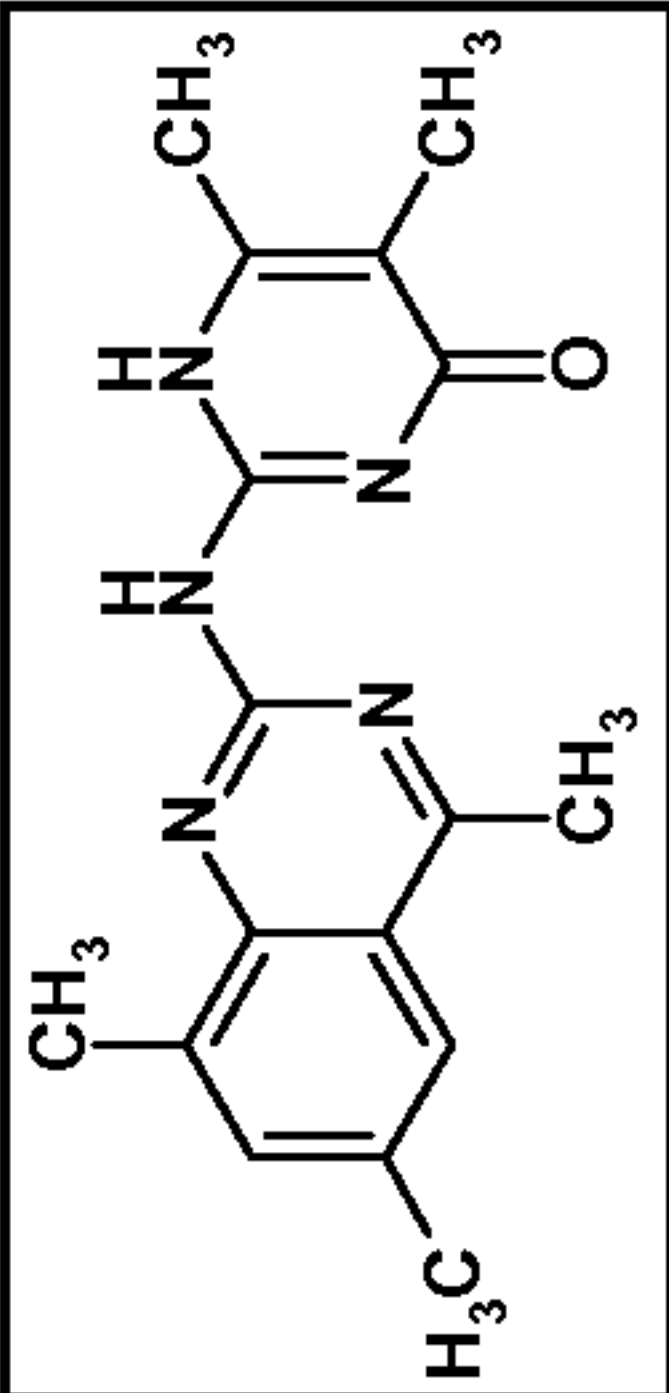
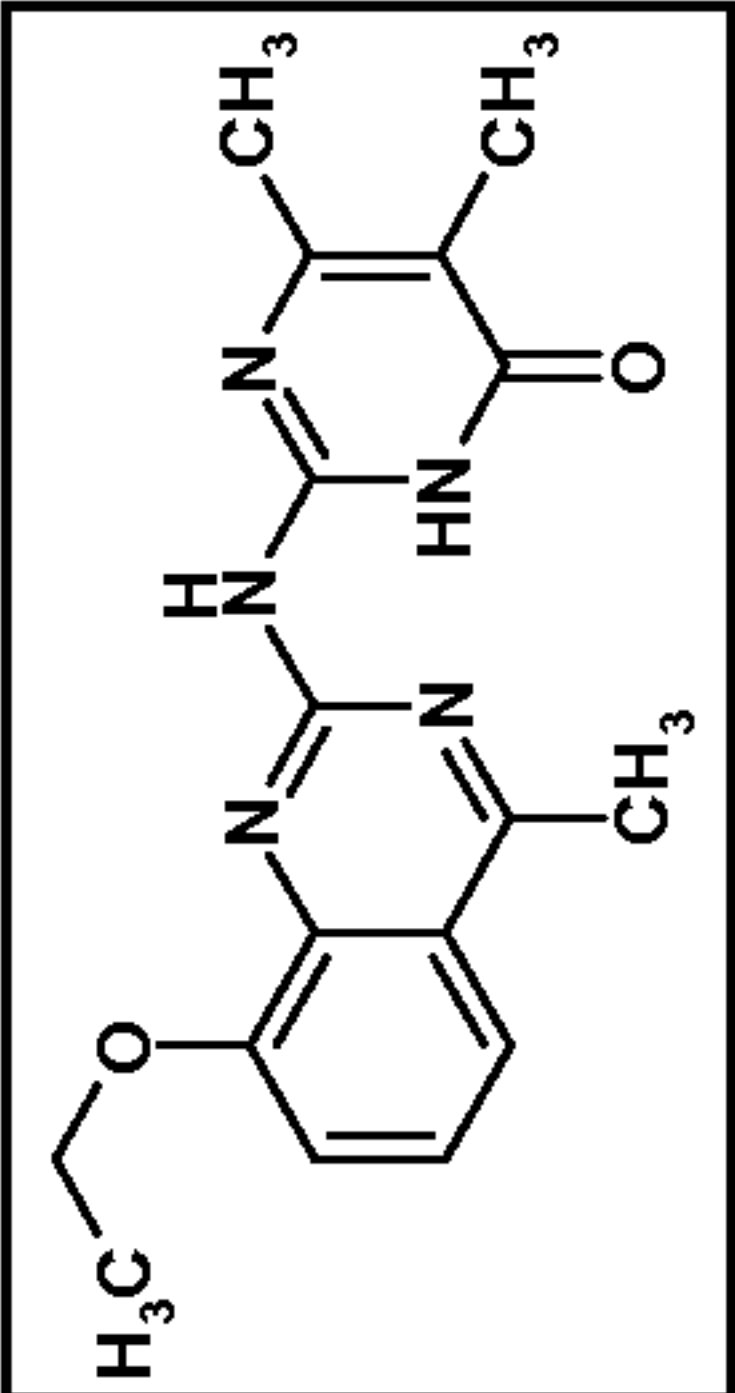
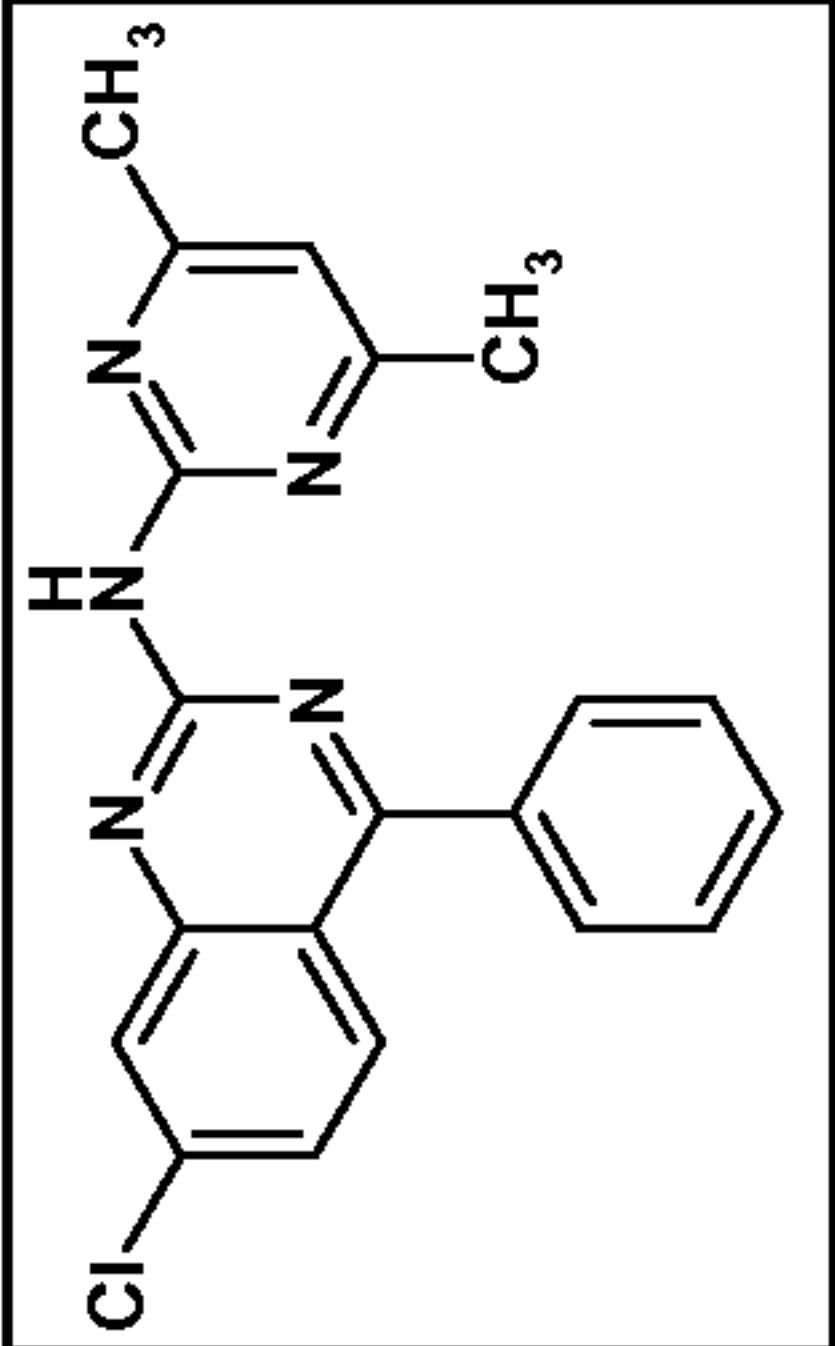
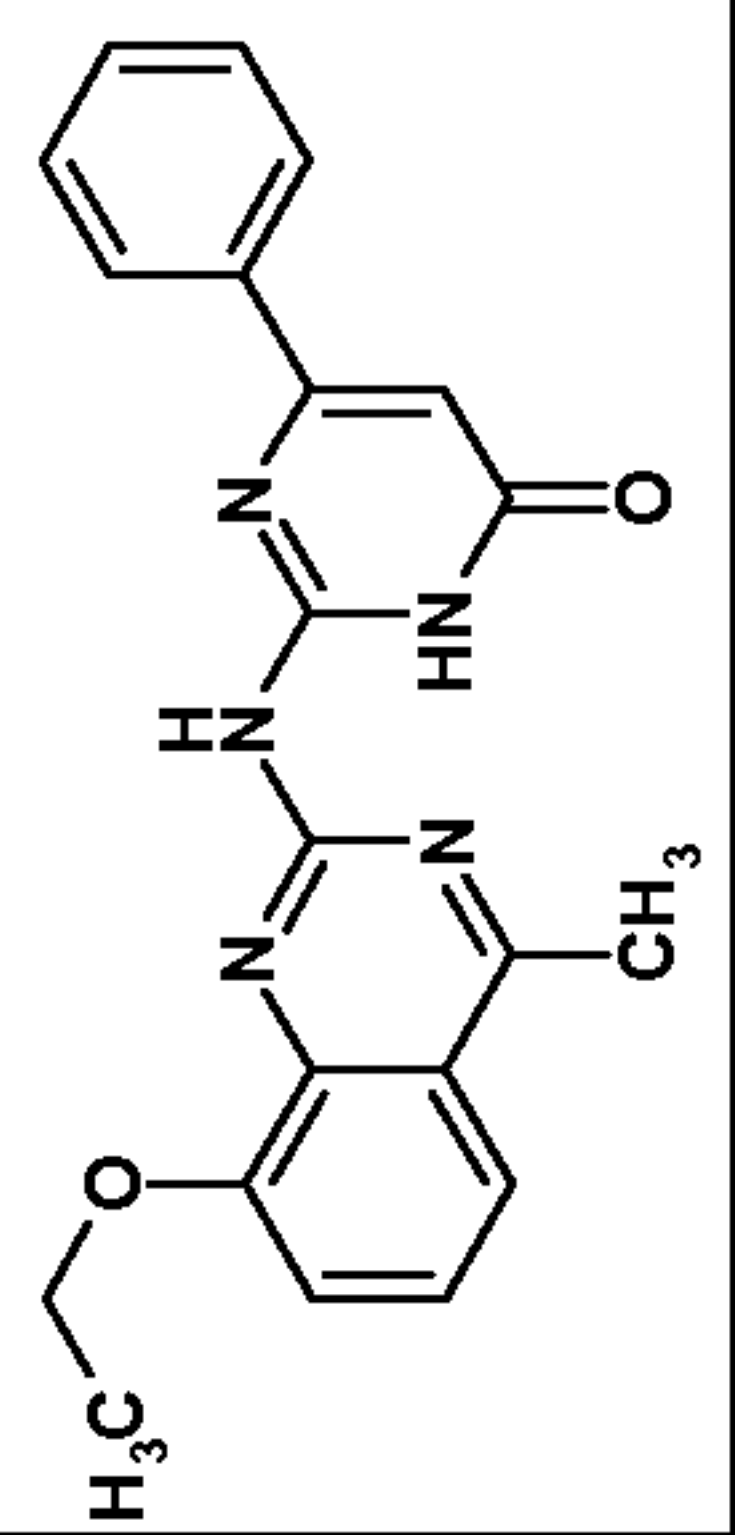
[0515] The compounds in TABLE 12 were identified via AD4-10381 structure similarity searches and were docked to the 1NQL.PDB binding site to obtain their Glide and Emodel scores. Also depicted in TABLE 12 is ICW assay and MTT assay results (See Example 1, Example 2, Example 7).

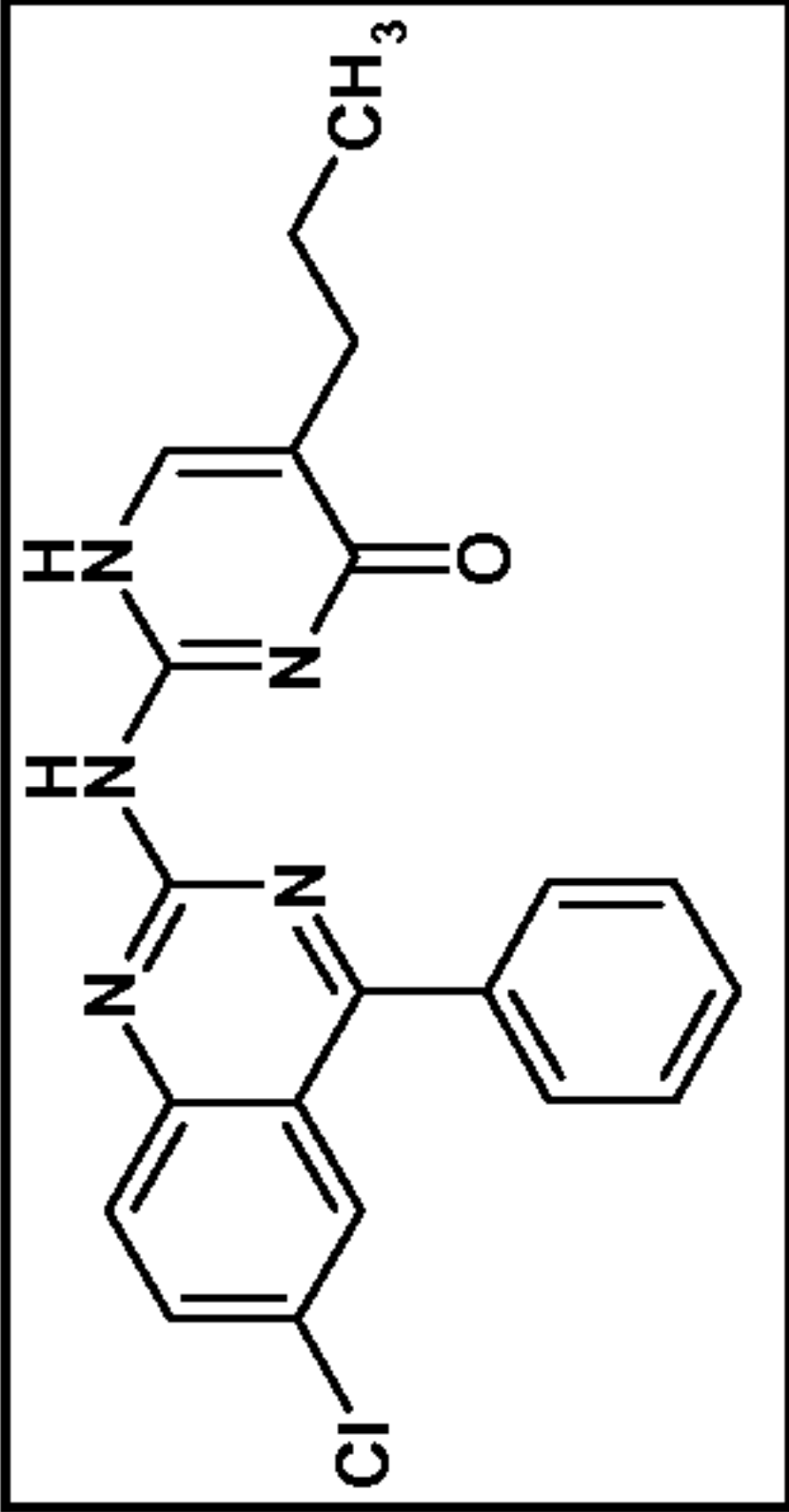
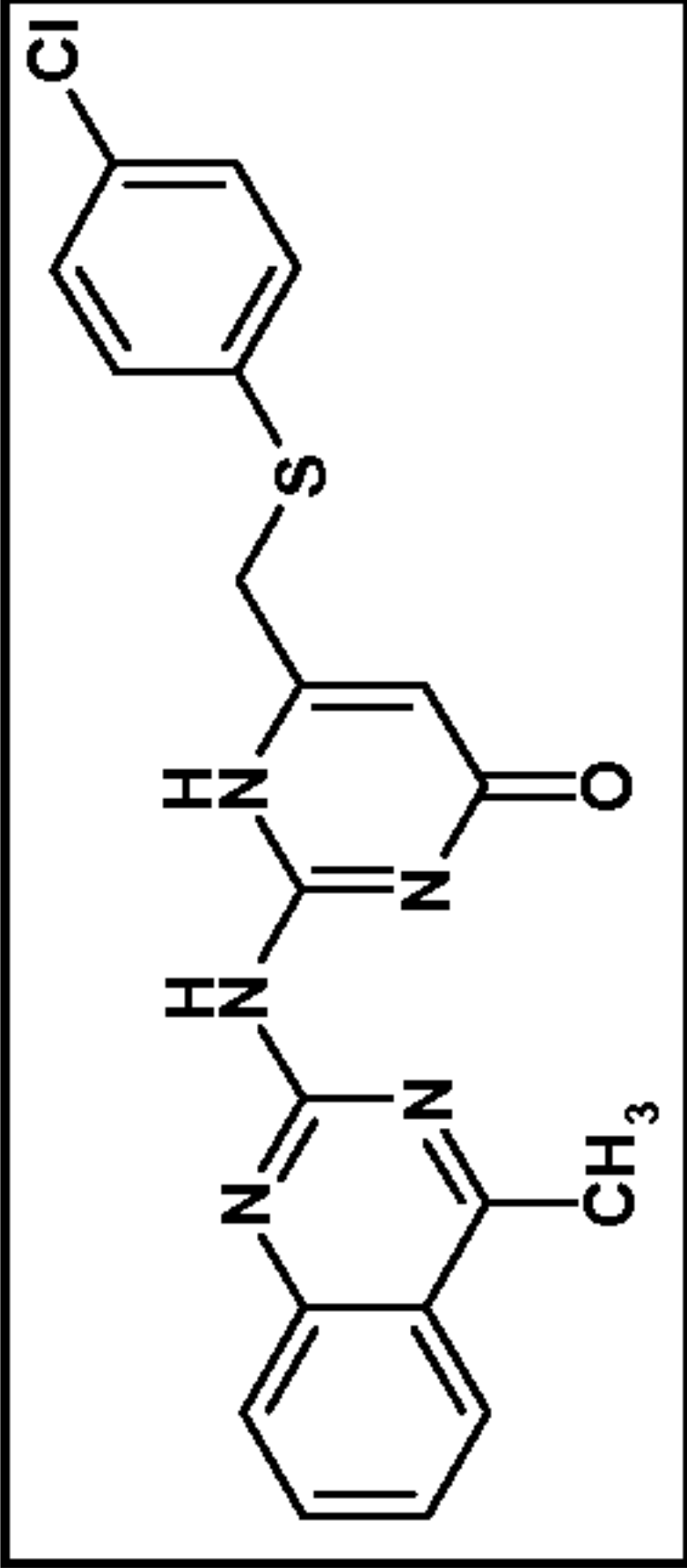
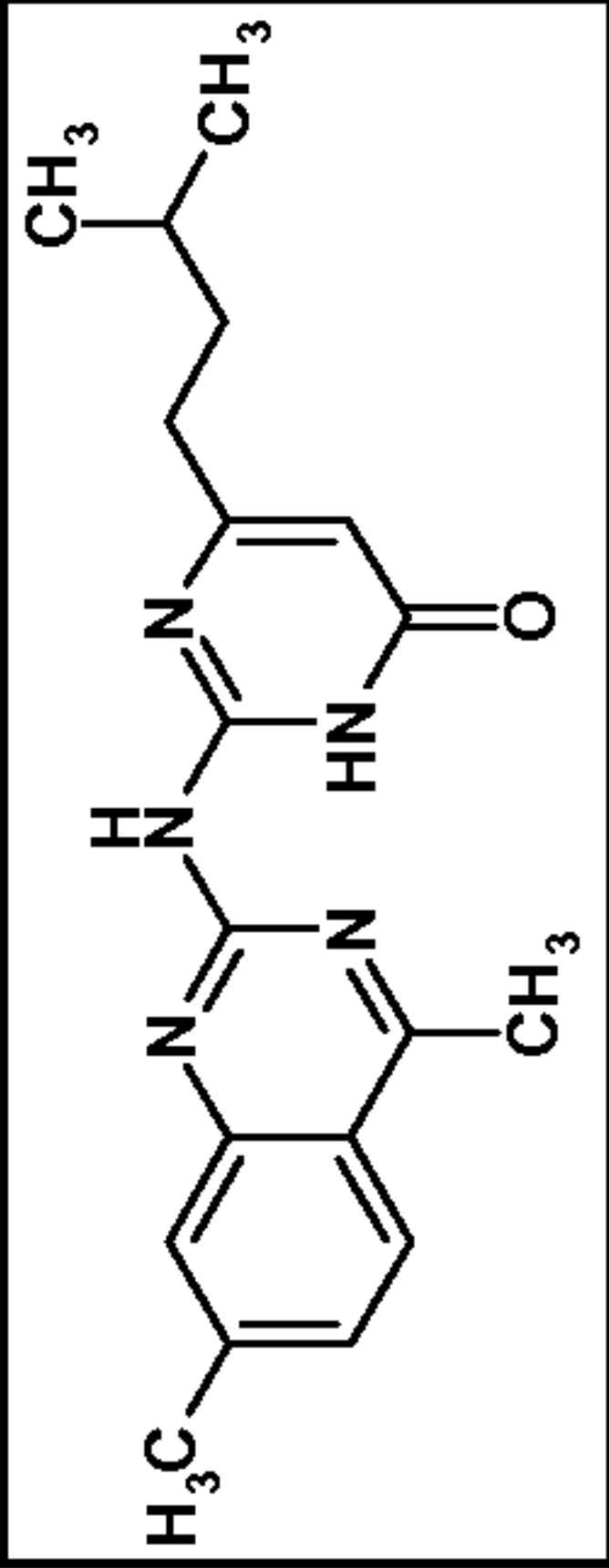
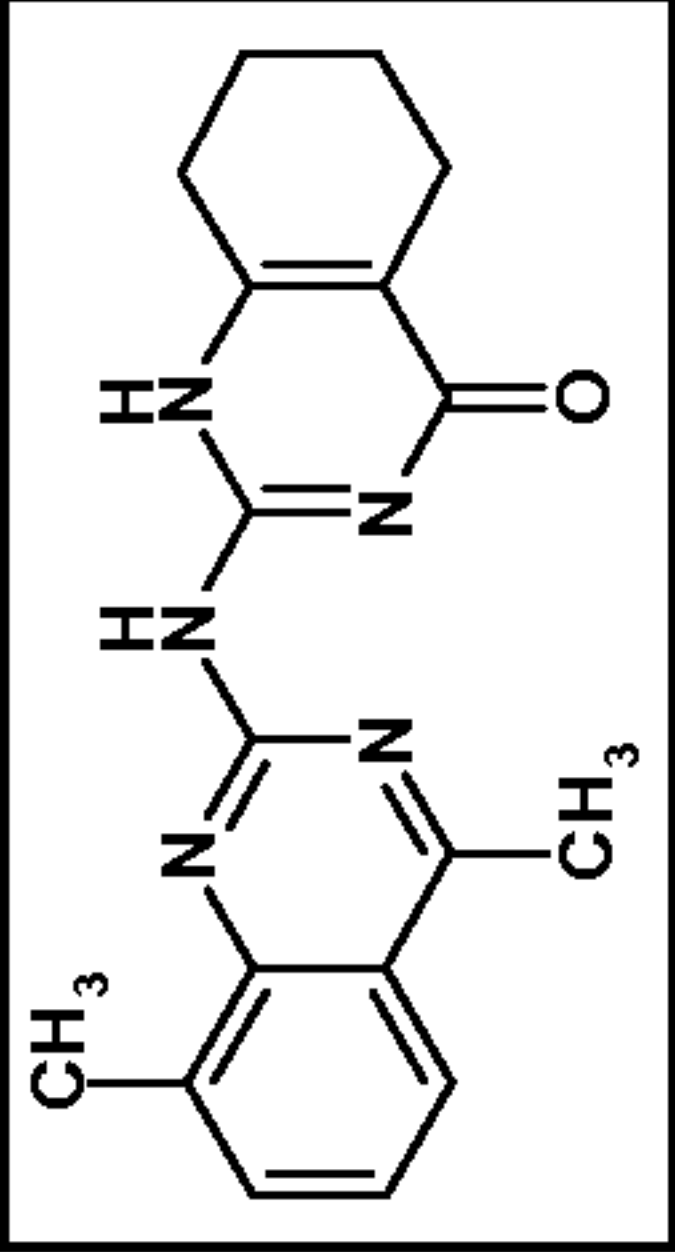
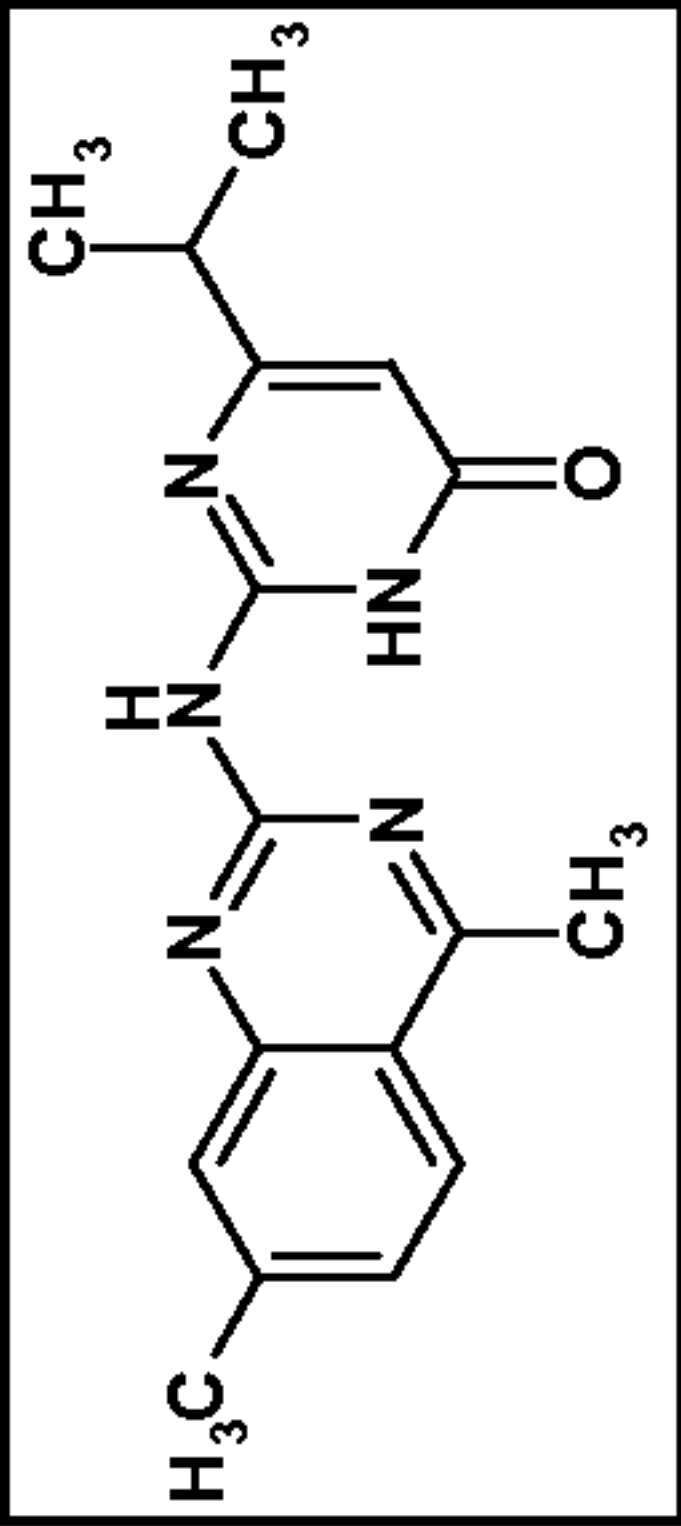
TABLE 12: Glide Score, E-Model score, ICW assay, and MTT Assay Results for AD4-10381-like Compounds.

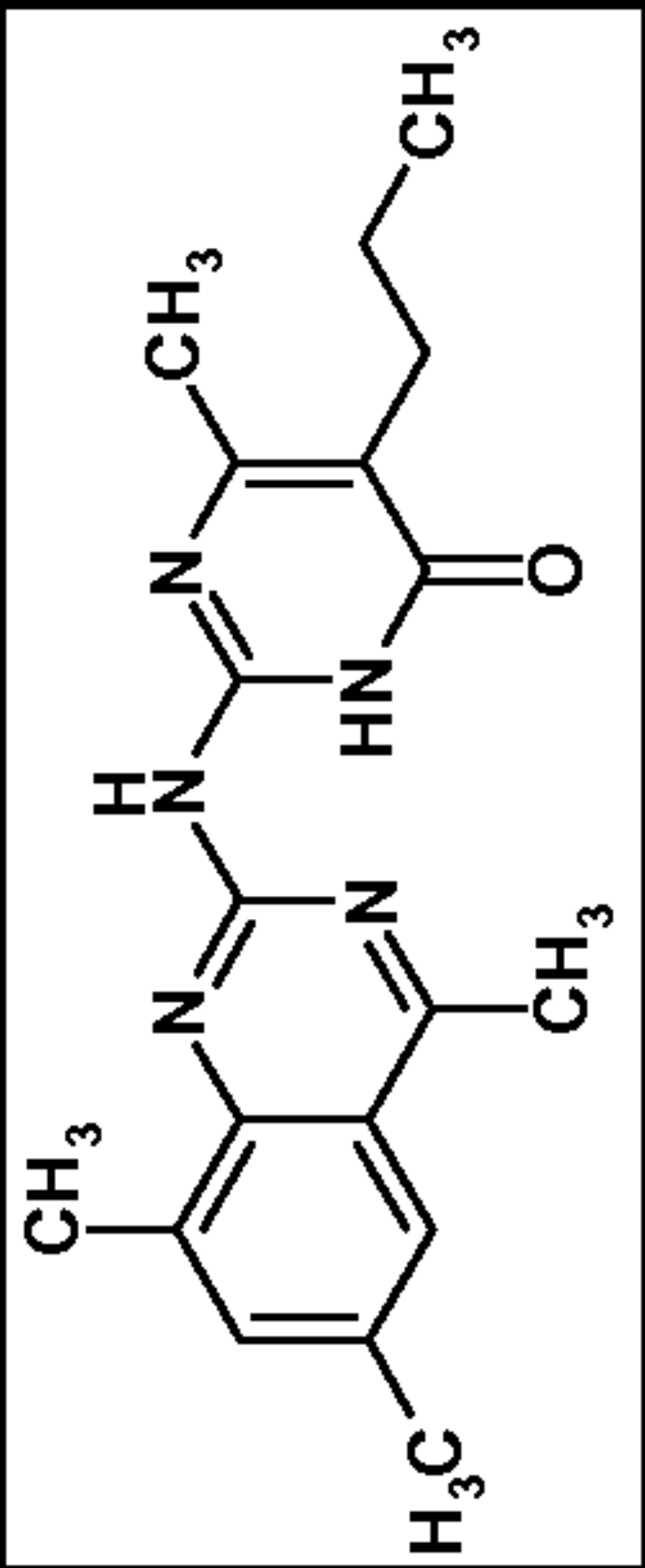
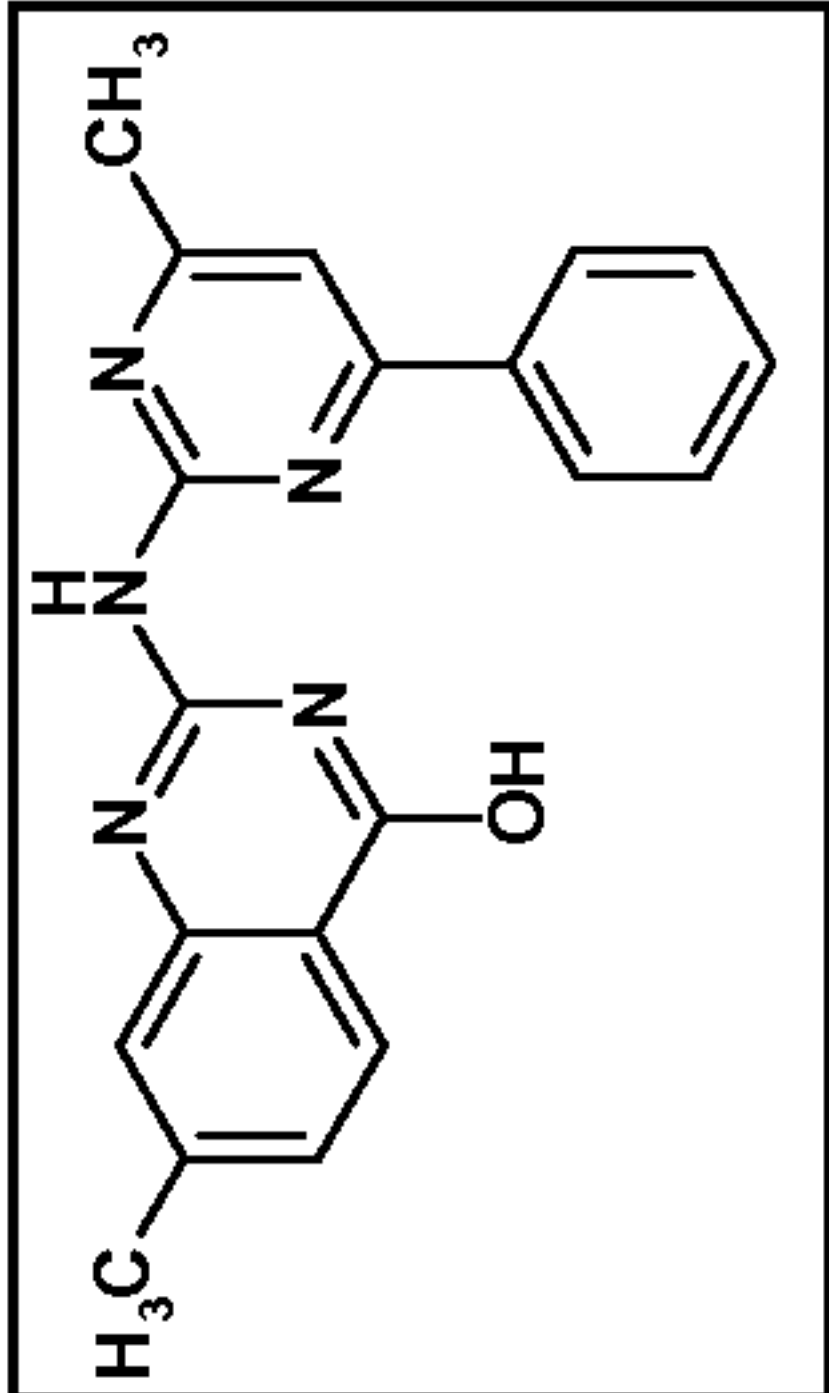
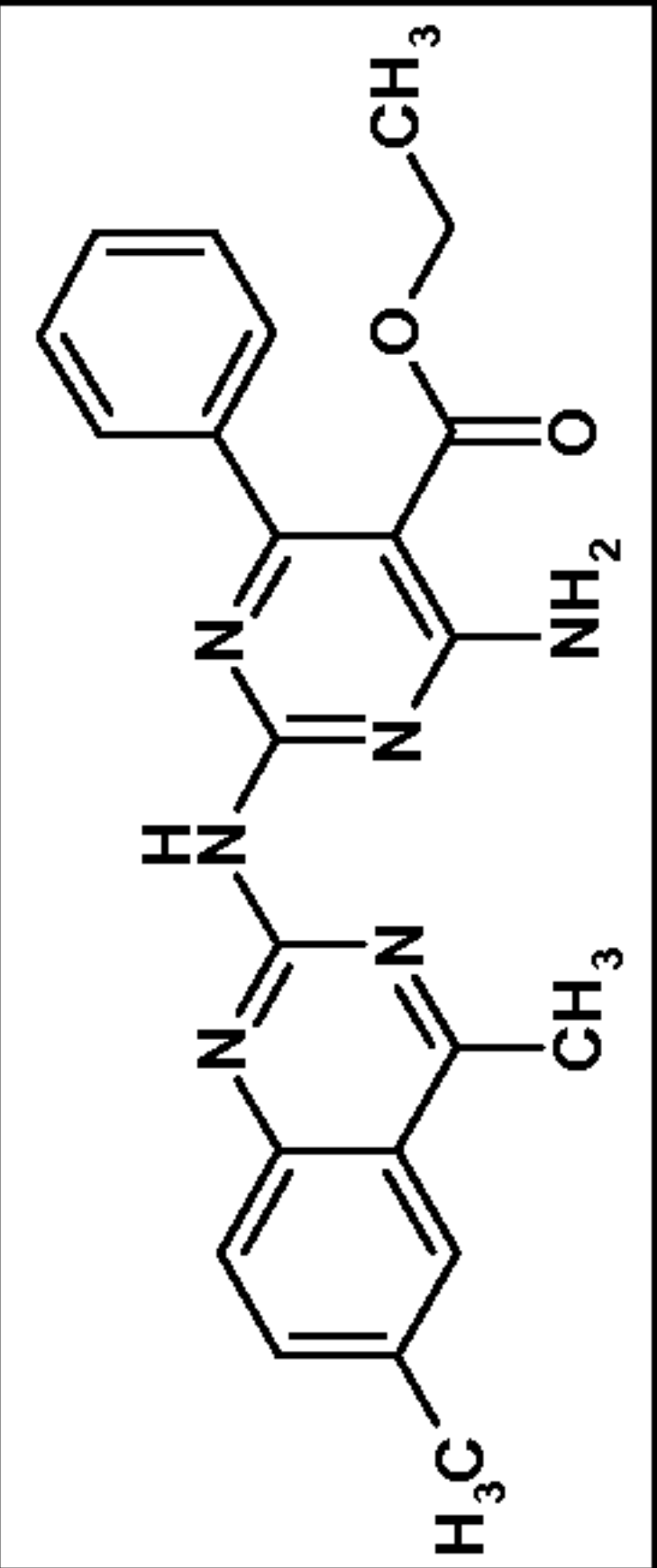
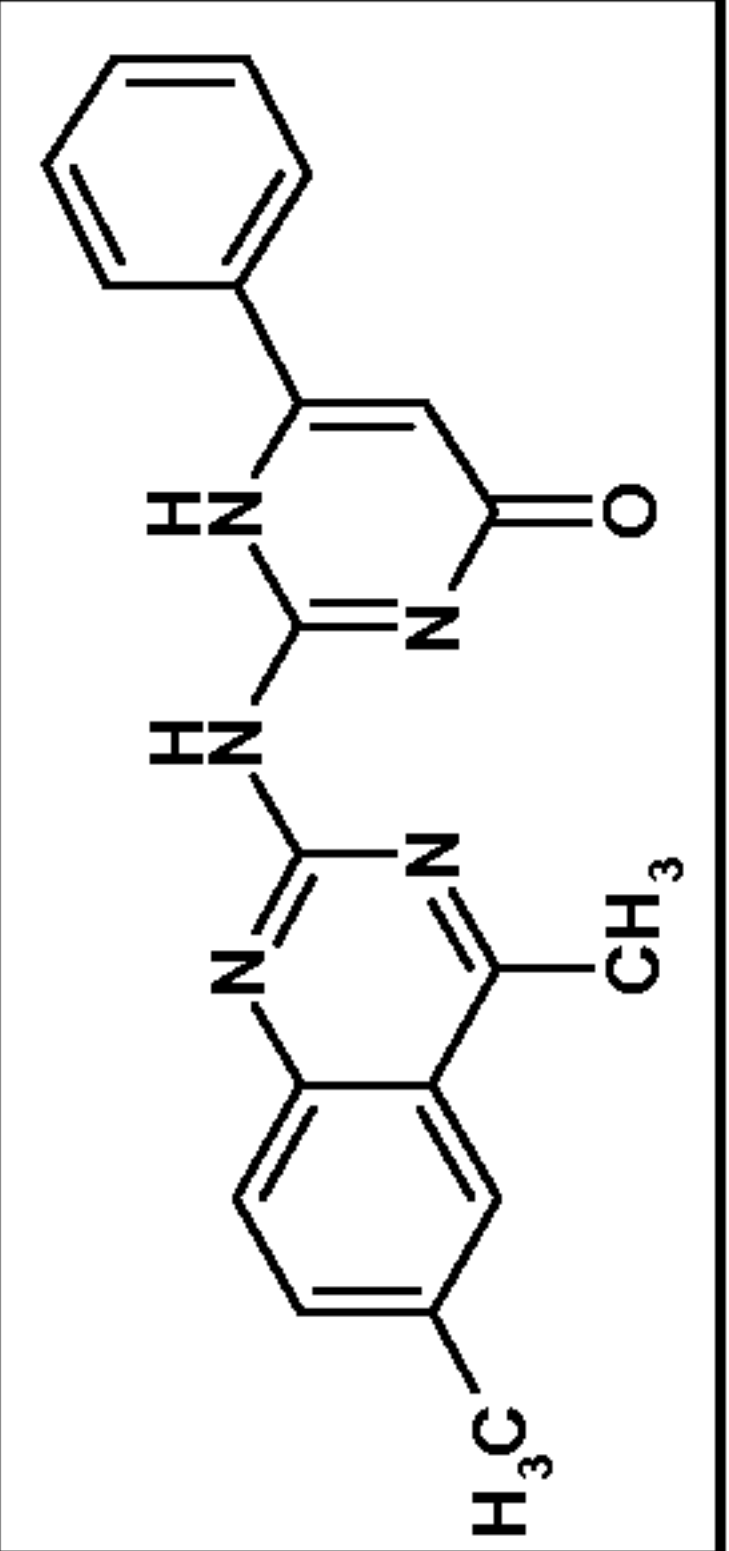
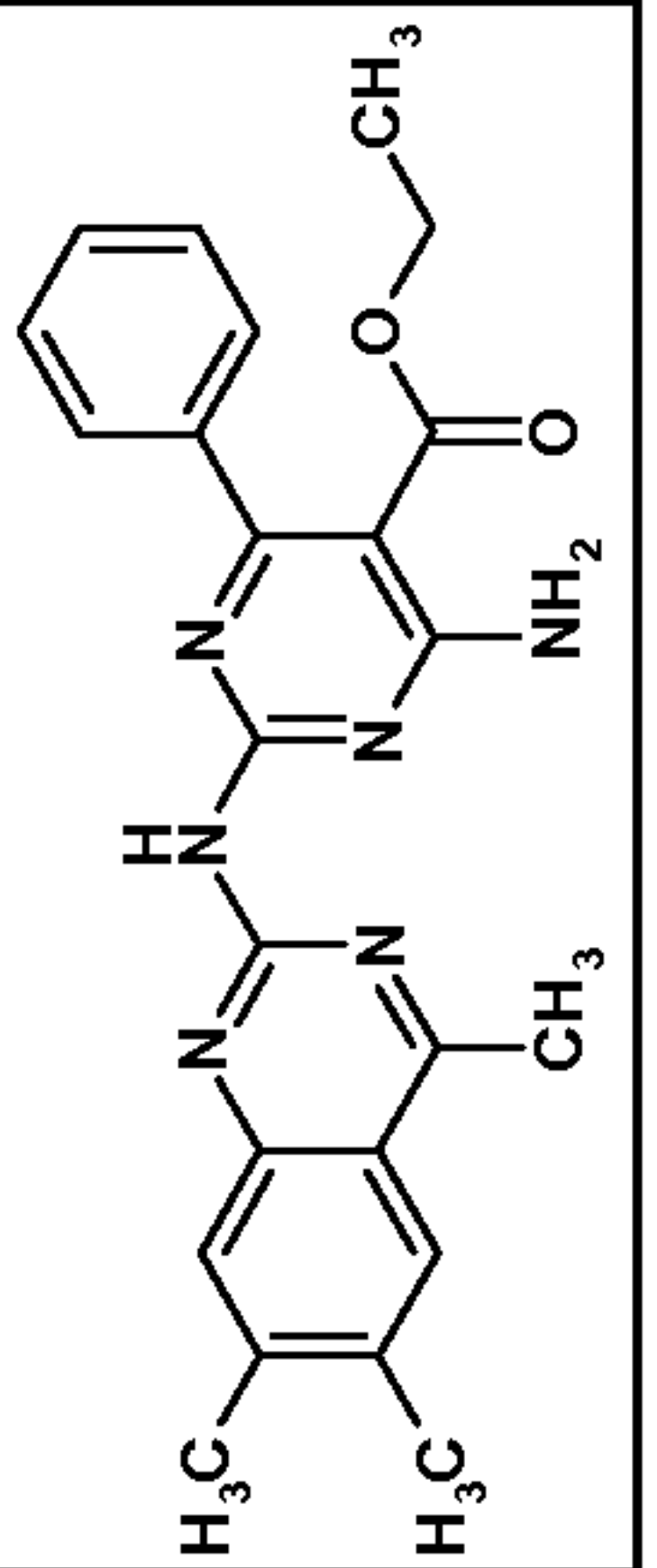
Structure	AD4 No.	Sub-Class	G-Score	E-Model	ICW 1	ICW 2	ICW Avg	MTT Assay IC ₅₀ (A431/MDBK)	MTT Follow-up
	12632	D	-5.83	-34.77	89.66	92.56	91.11	5.67/5.59(0.99)	
	12681	E	-4.03	-45.89	89.93	89.02	89.48	15.63/25.40(1.63)	
	12732	D	-5.1	-47.5	79.12	86.41	82.77	11.07/14.95(1.35)	
	10381 + TFA	D	-6.02	-50.11	87.04	78.48	82.76	2.10/3.01(1.43)	

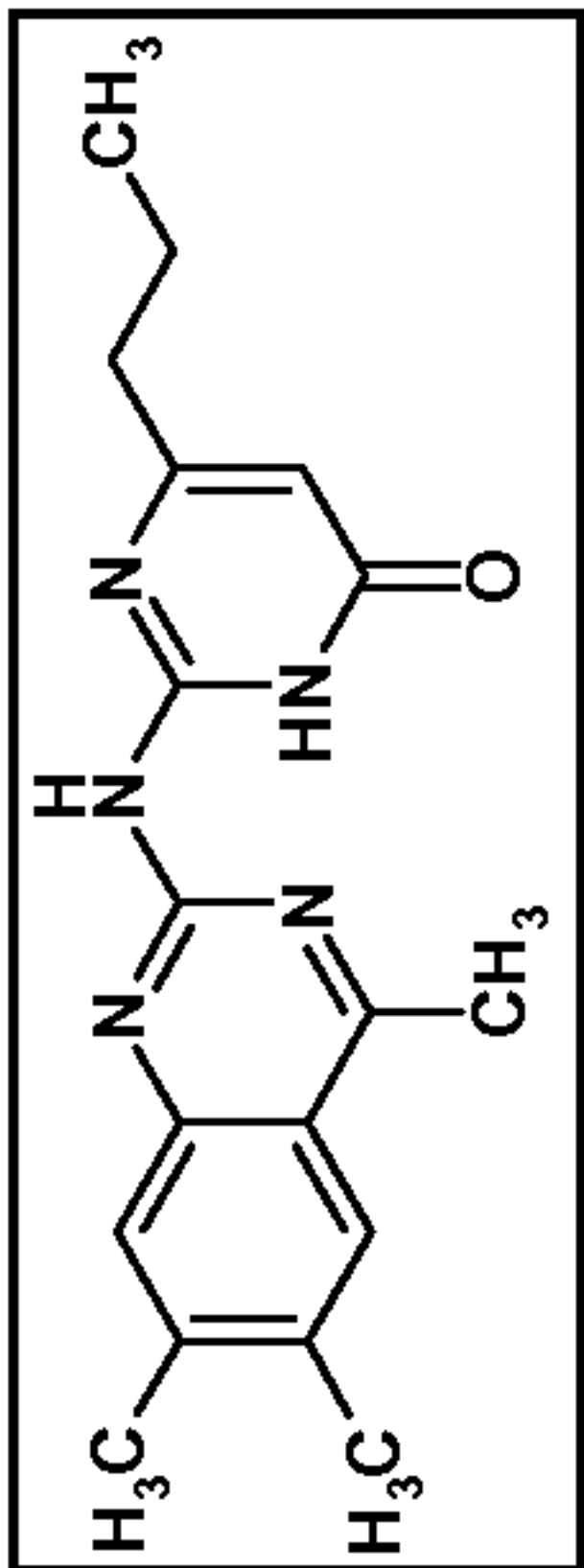
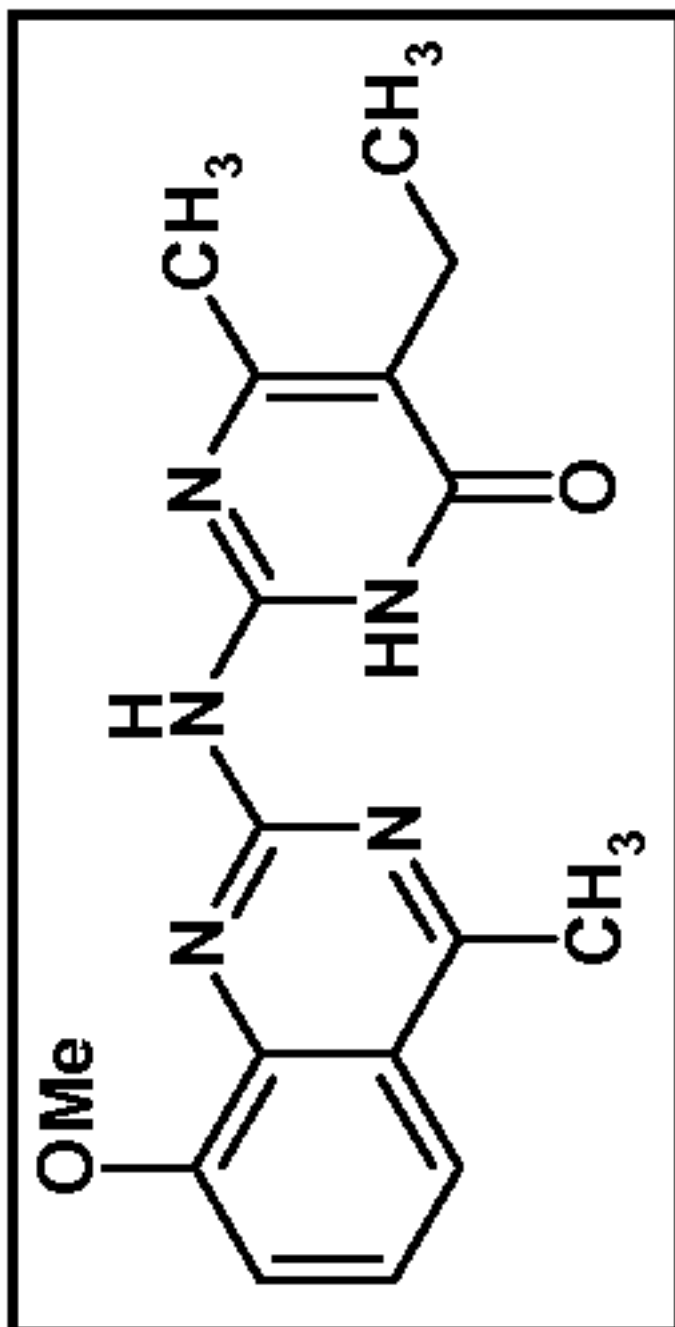
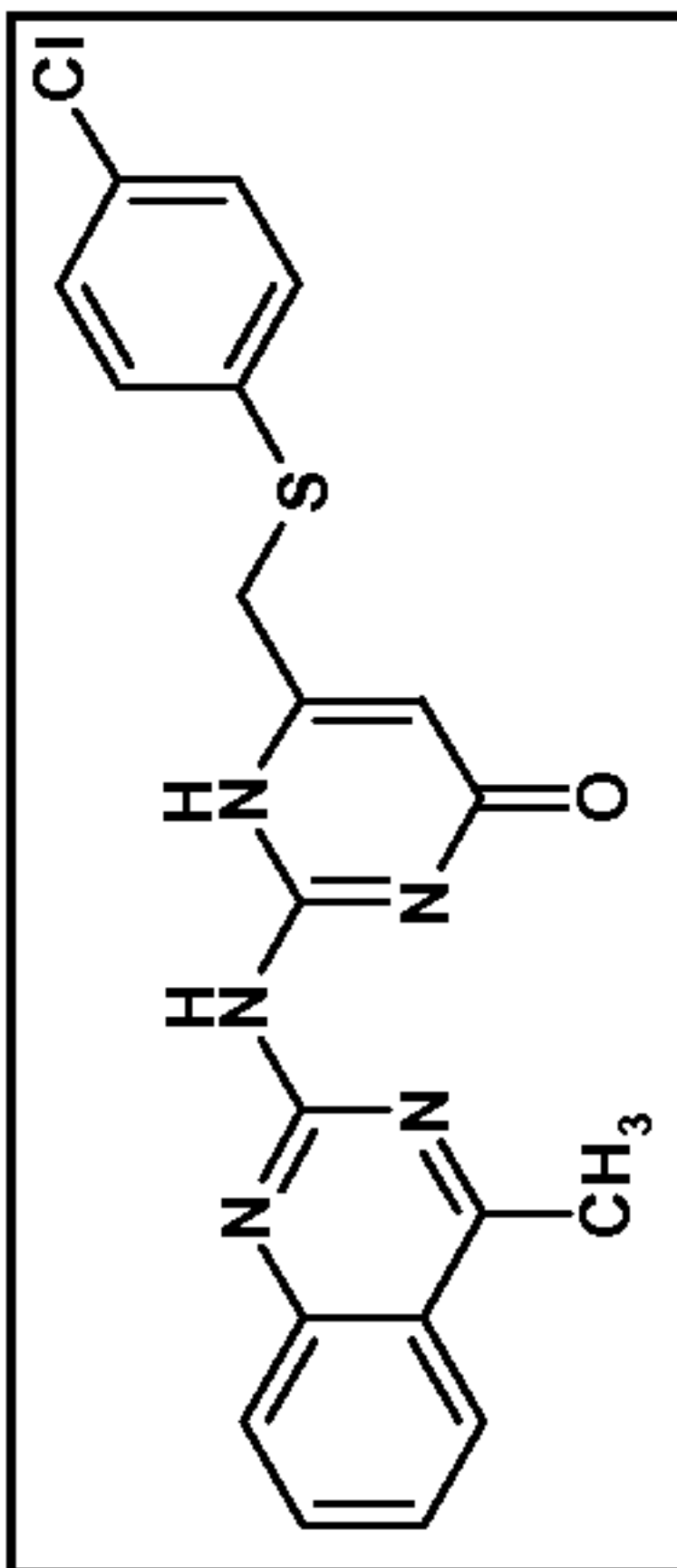
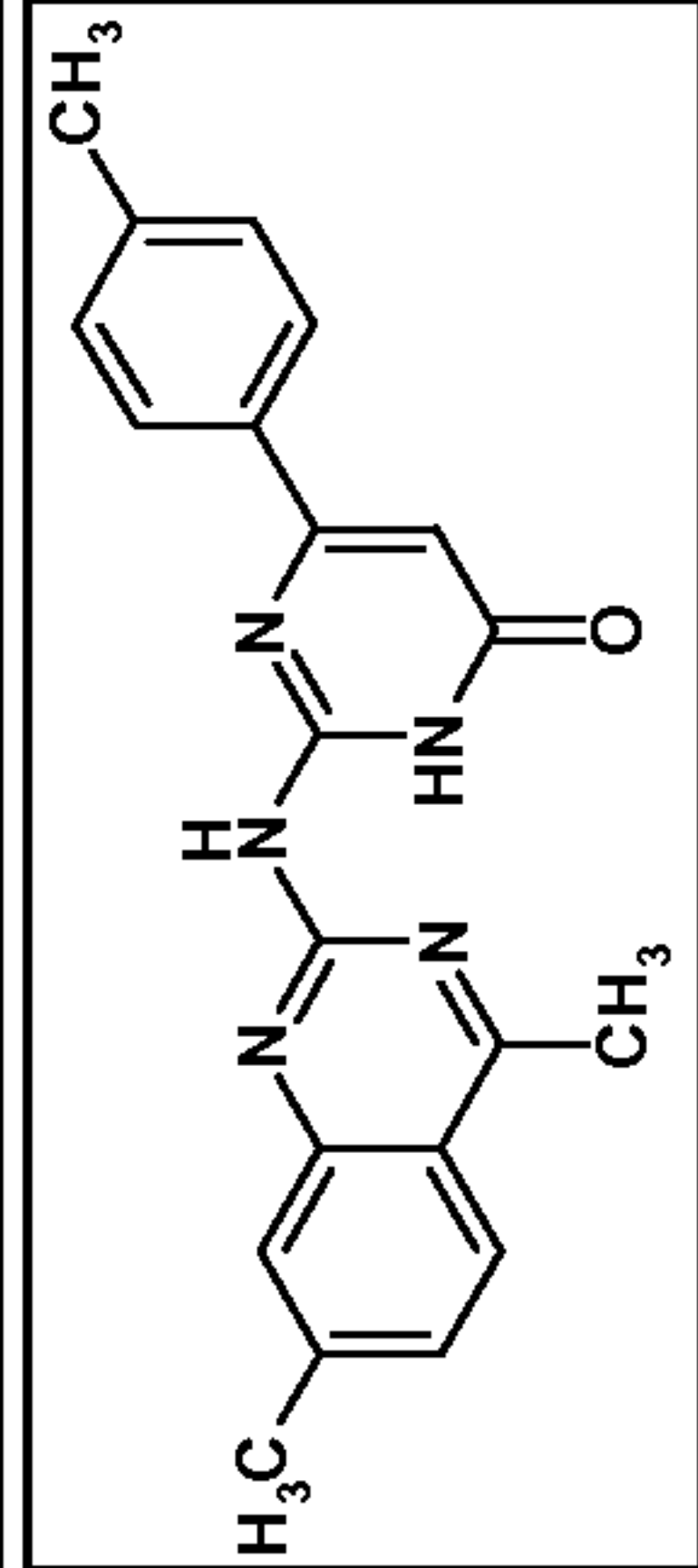
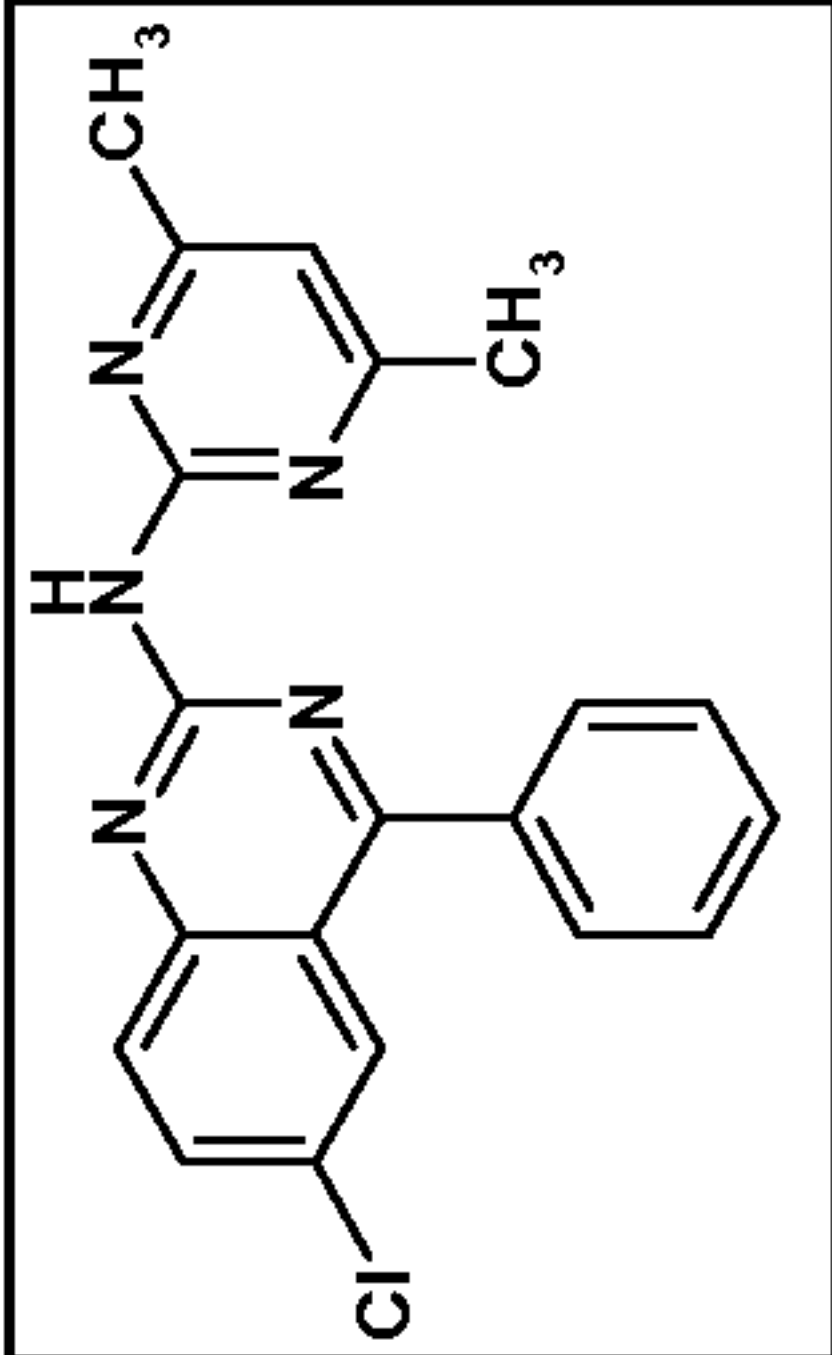
[illegible]

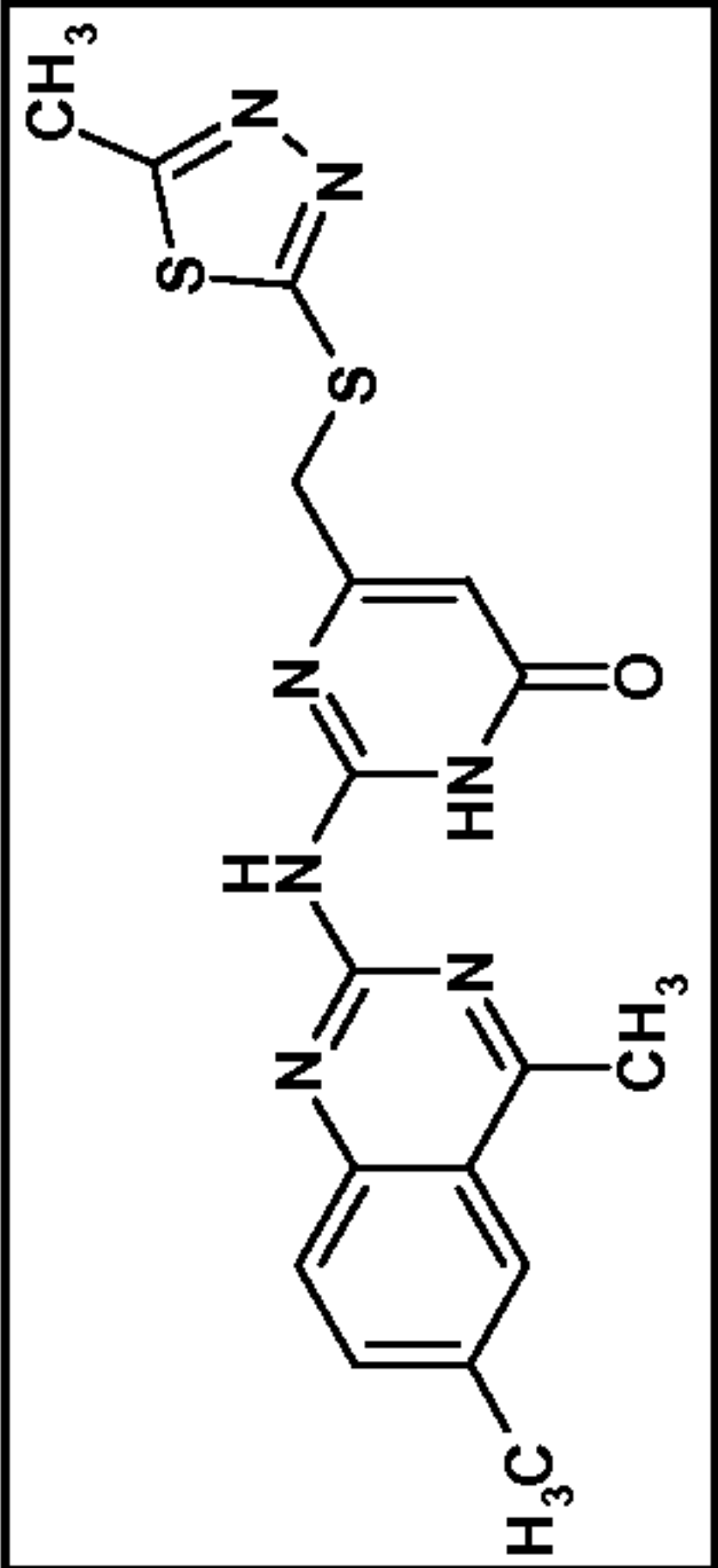
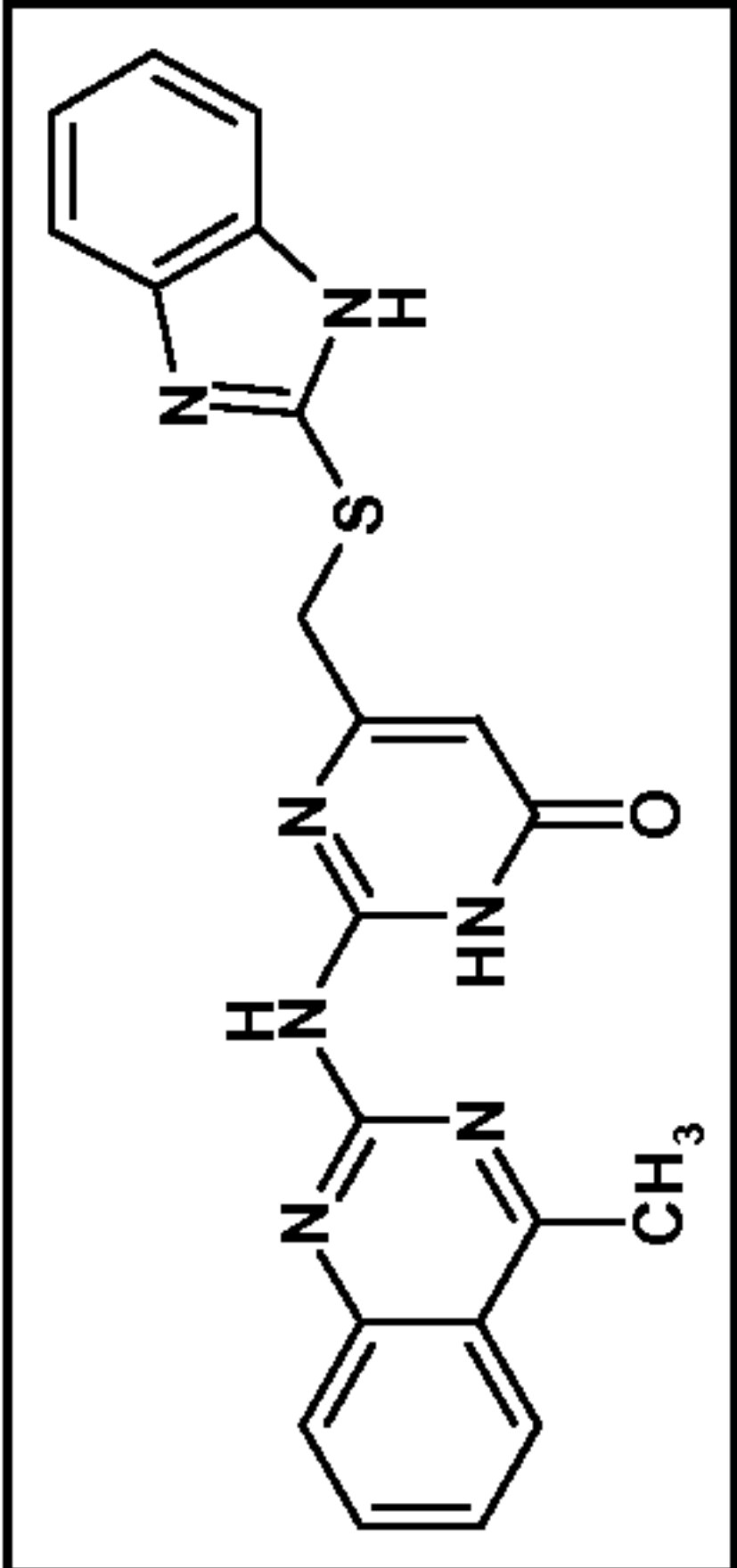
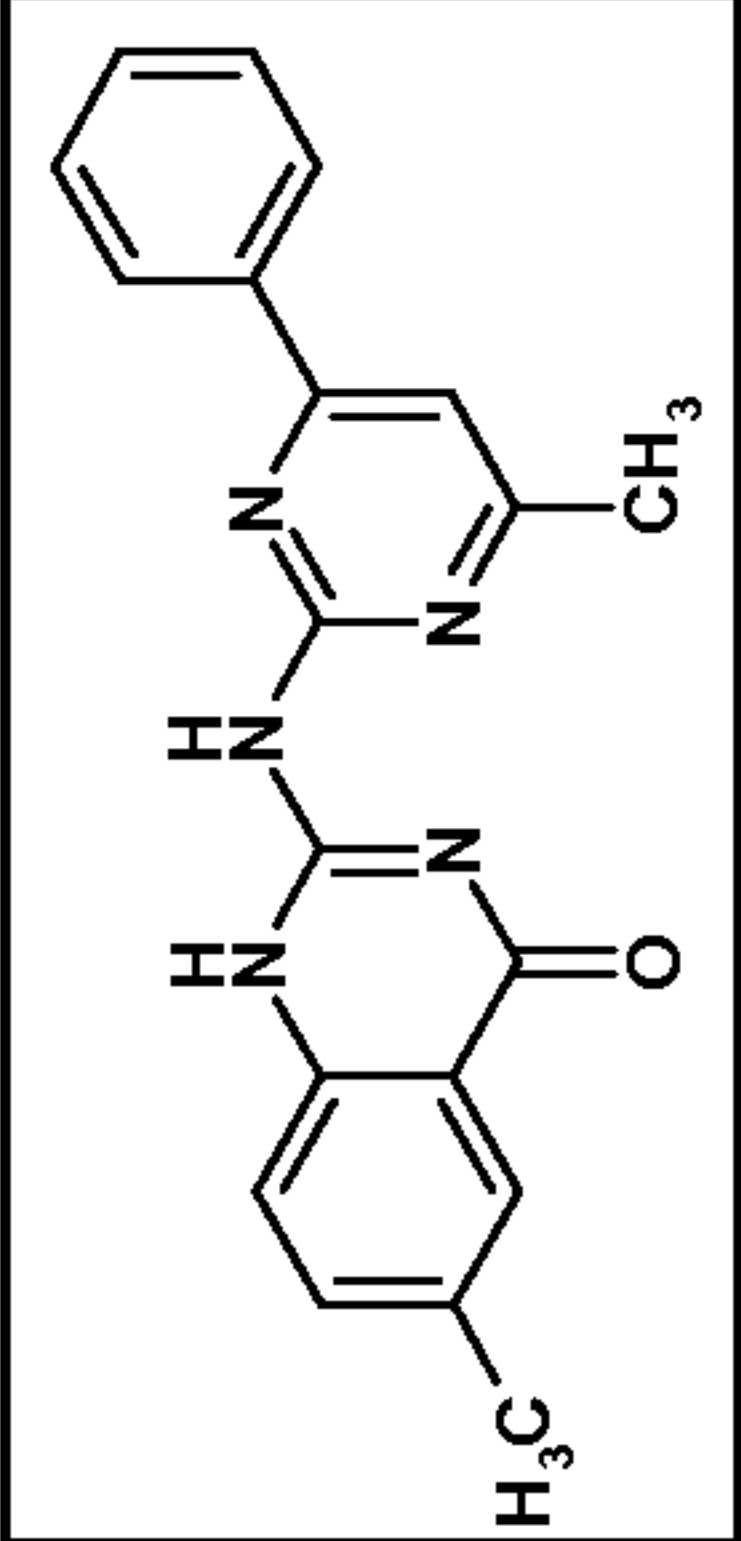
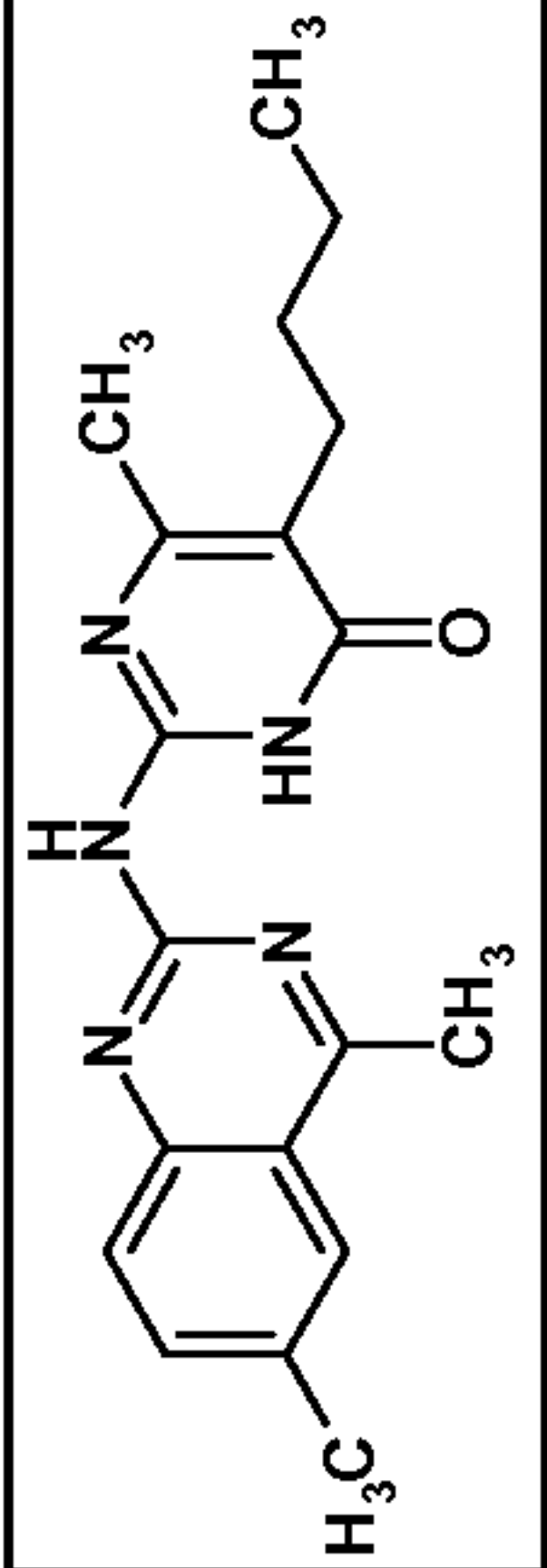
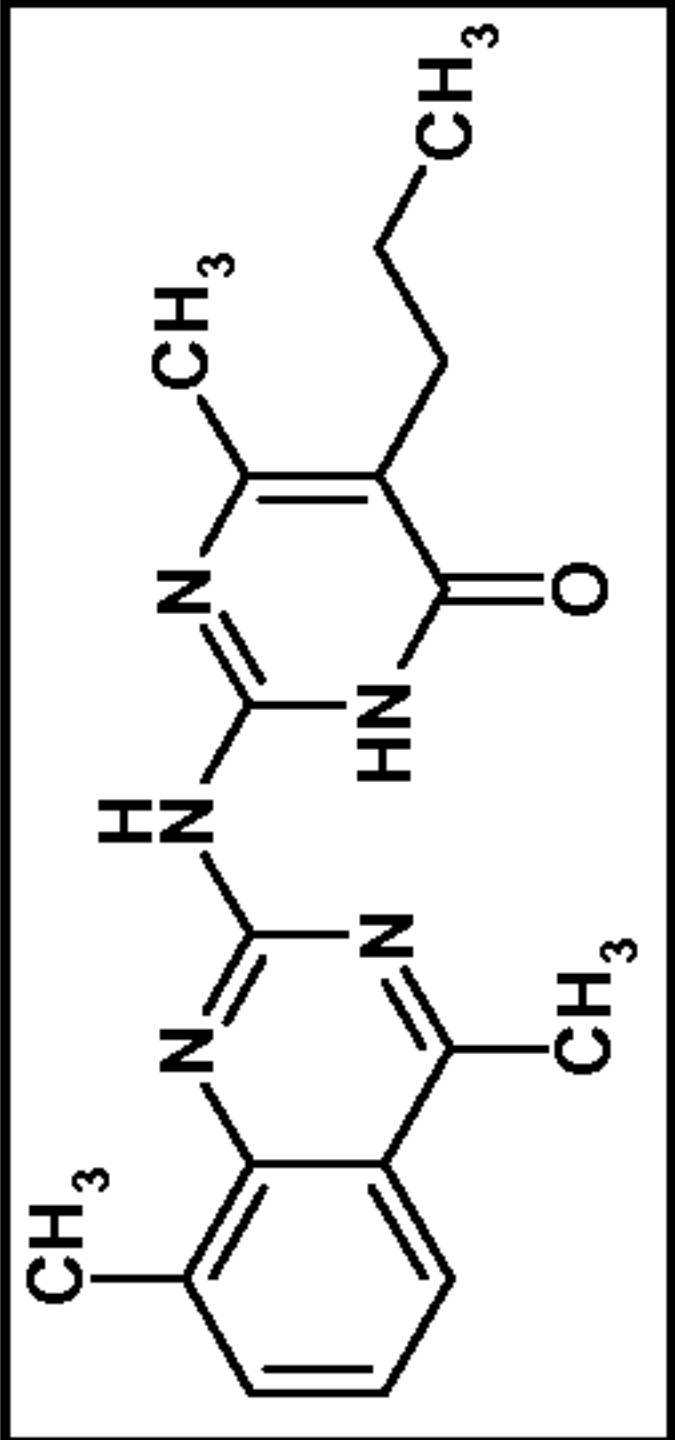
[illegible]

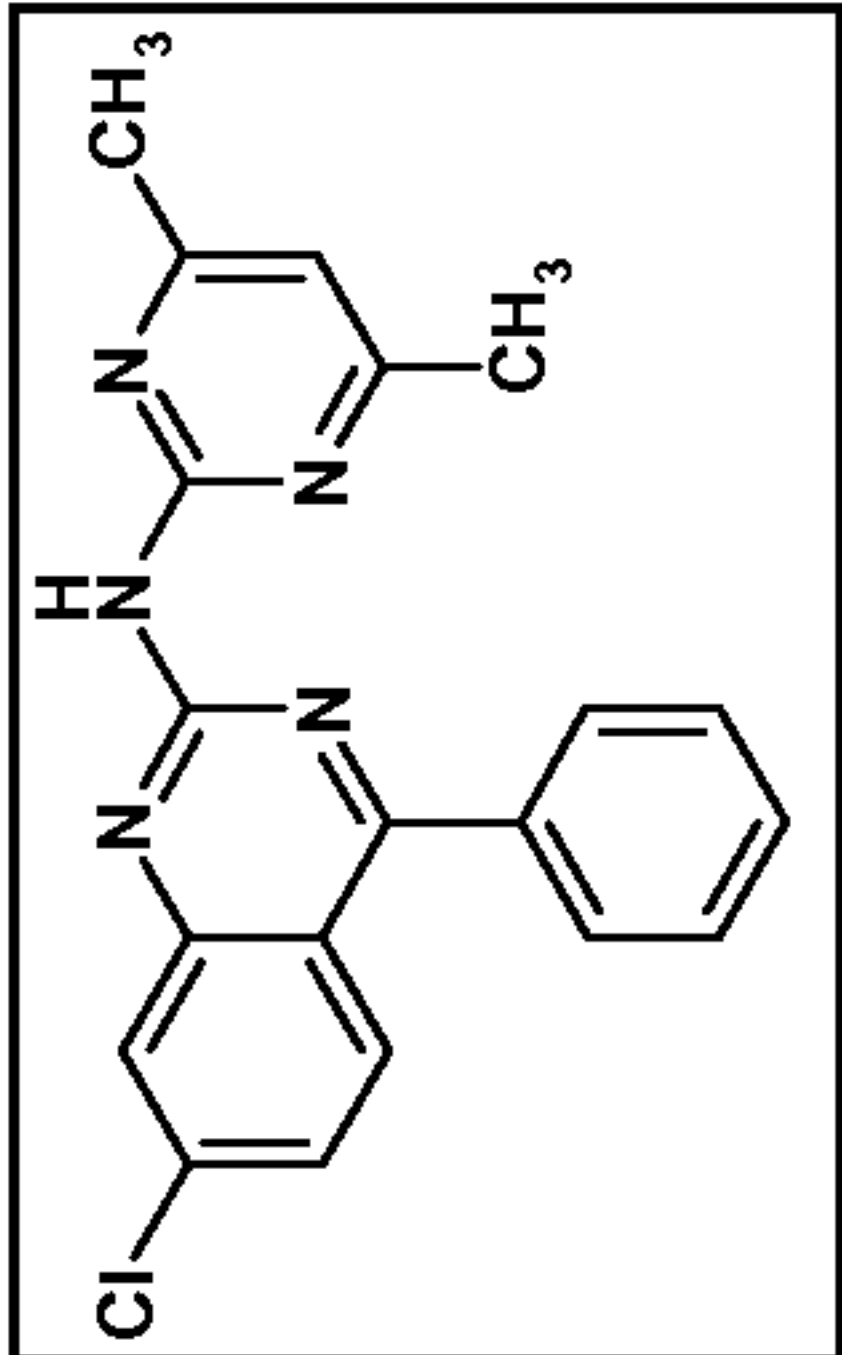
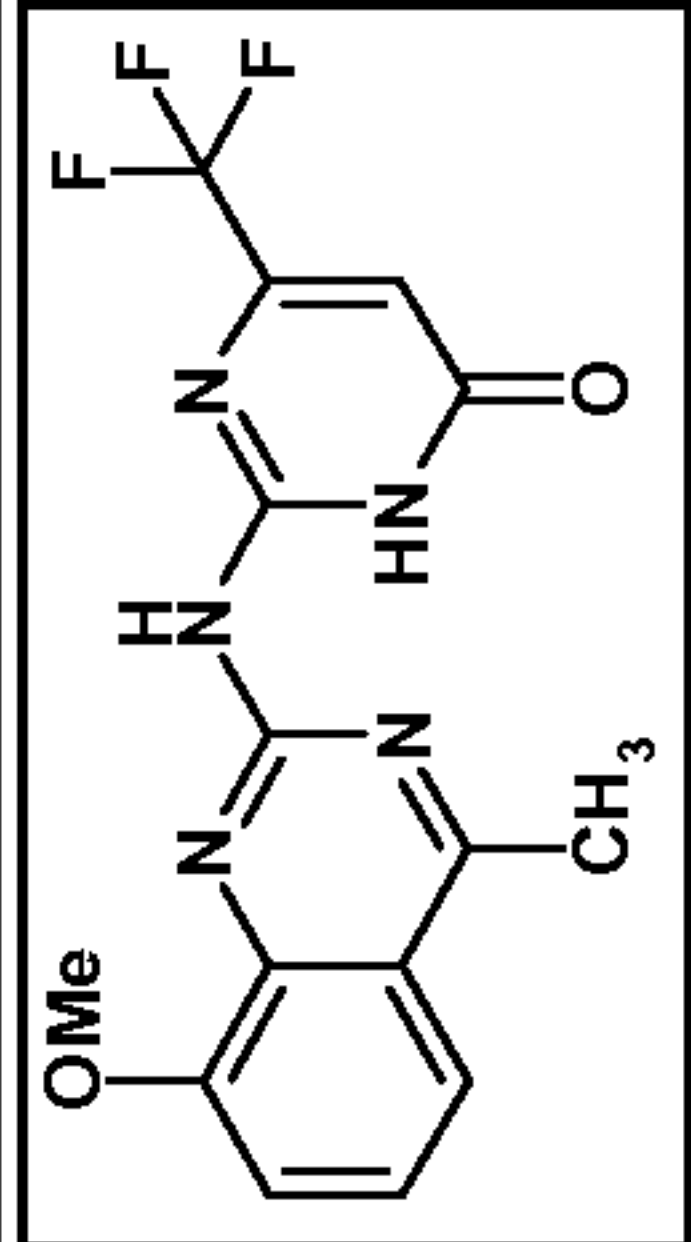
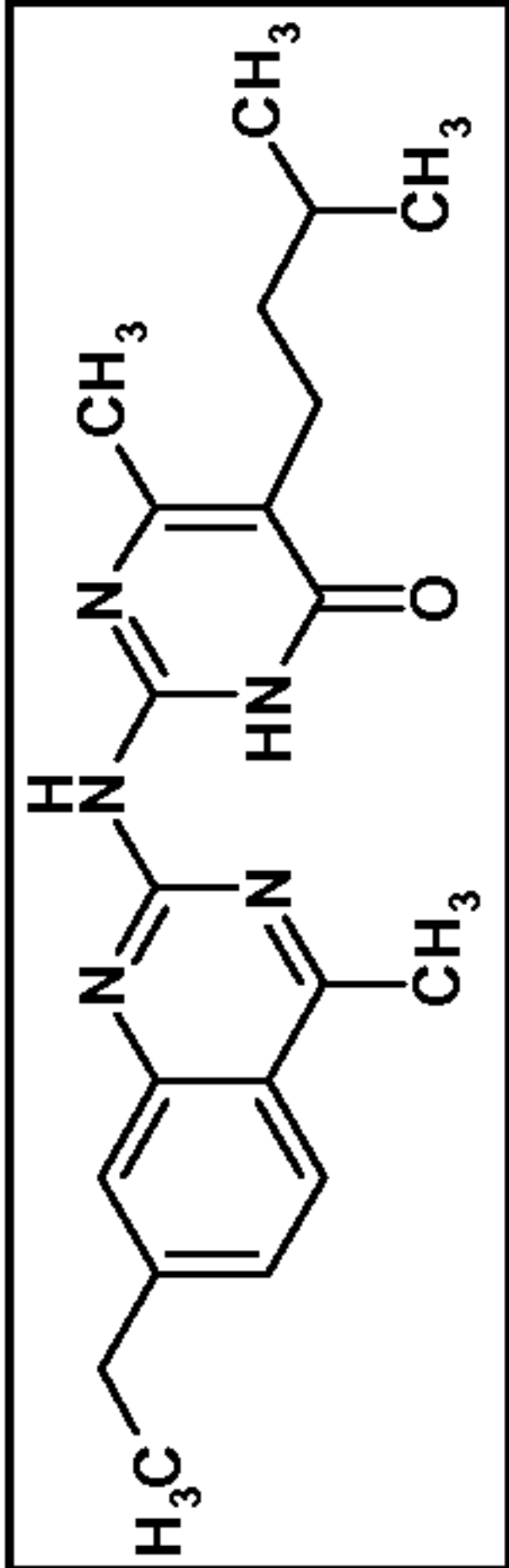
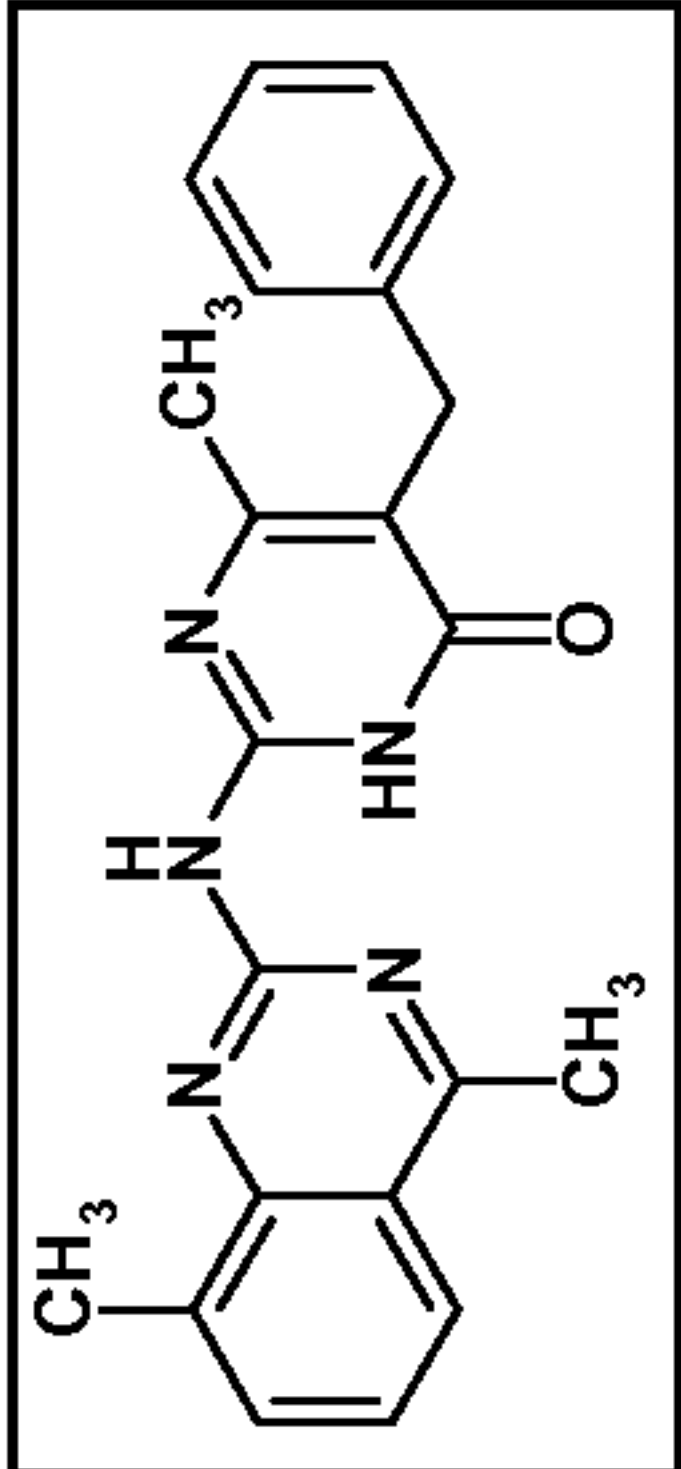
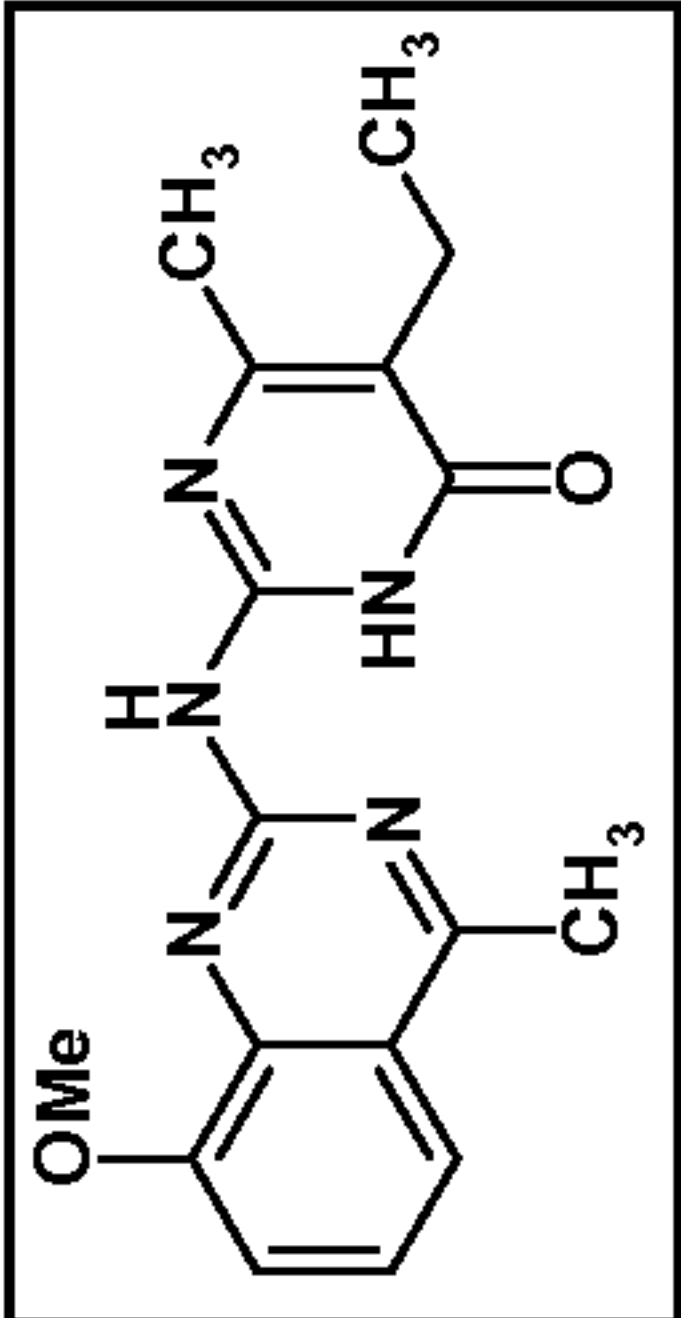
	11829	E	-4.74	-36.8	62.56	68.79	65.68	19.57/16.18	
	12645 + TFA	D	-6.1	-43.6	60.00	69.95	64.97	6.49/6.29(0.97)	
	12735 + TFA	D	-5.8	-44.56	69.46	59.98	64.72	17.92/15.32(0.85)	
	11636	E	-5.64	-48.26	61.28	63.84	62.56	26.75/14.70	
	11492	D	-5.66	-48.59	53.35	71.23	62.29	non-convergent	

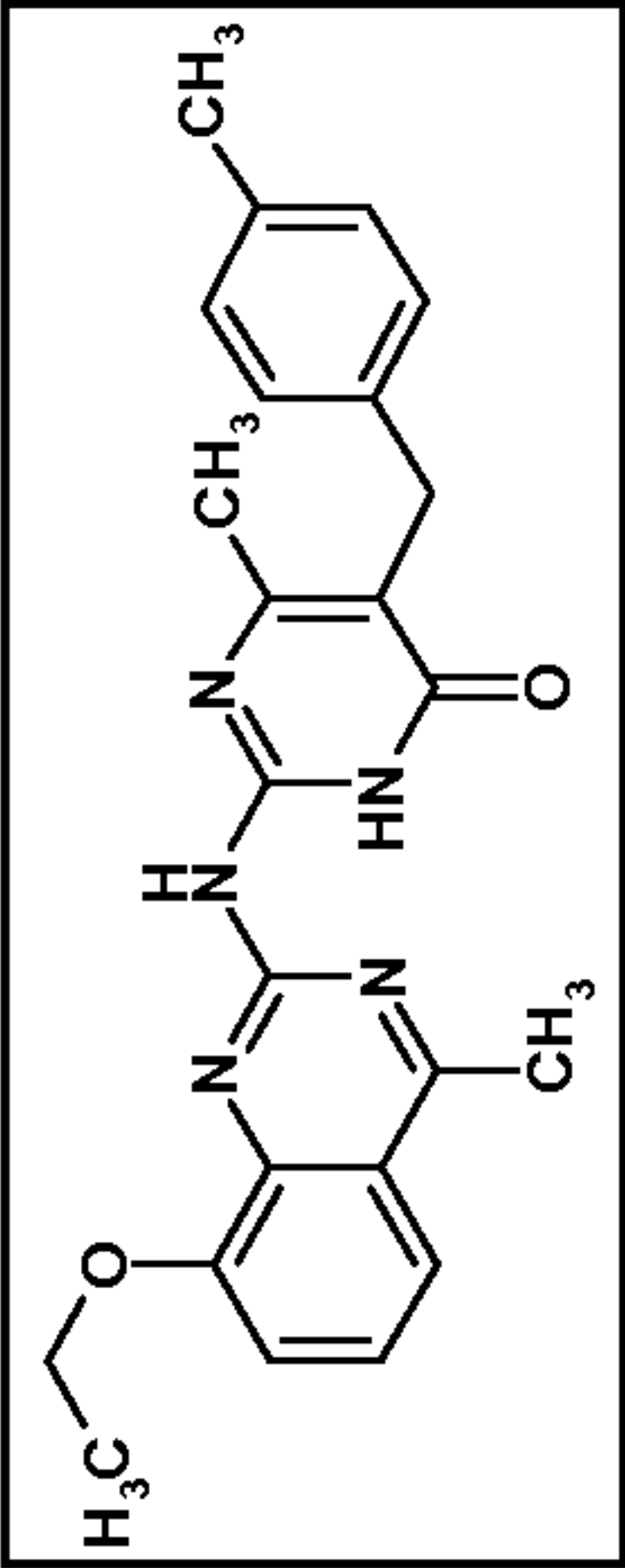
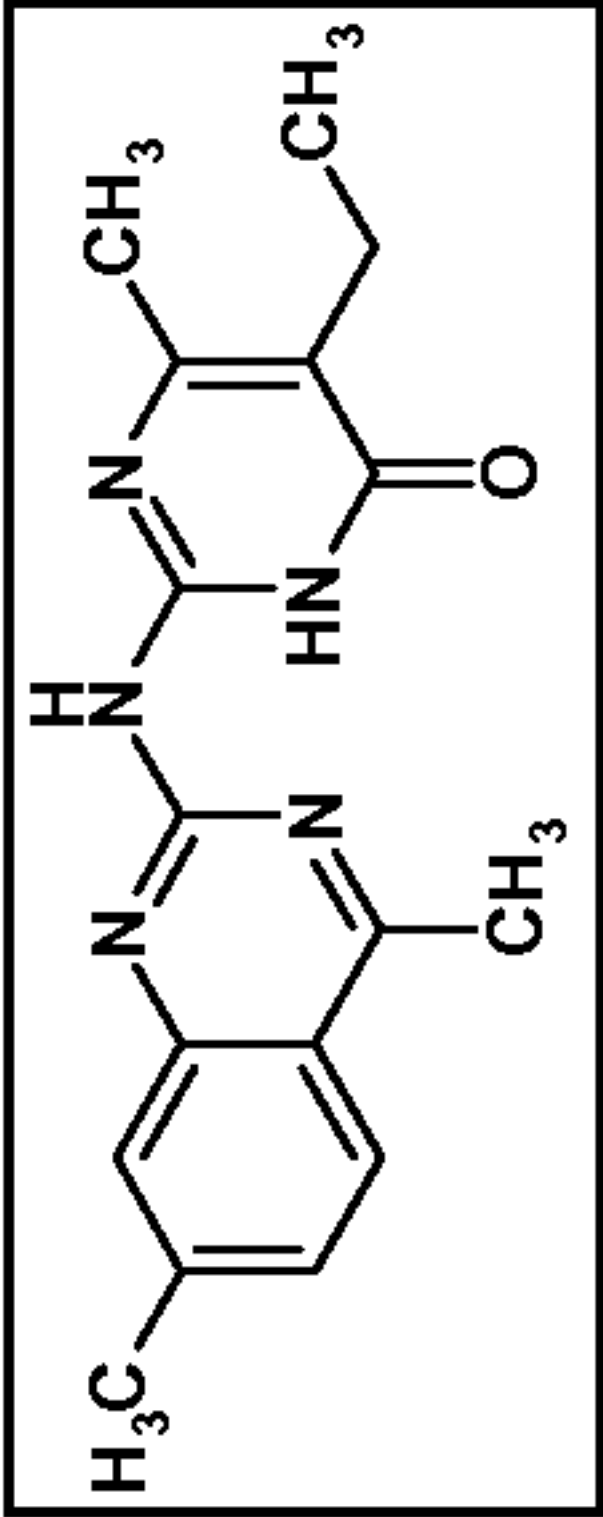
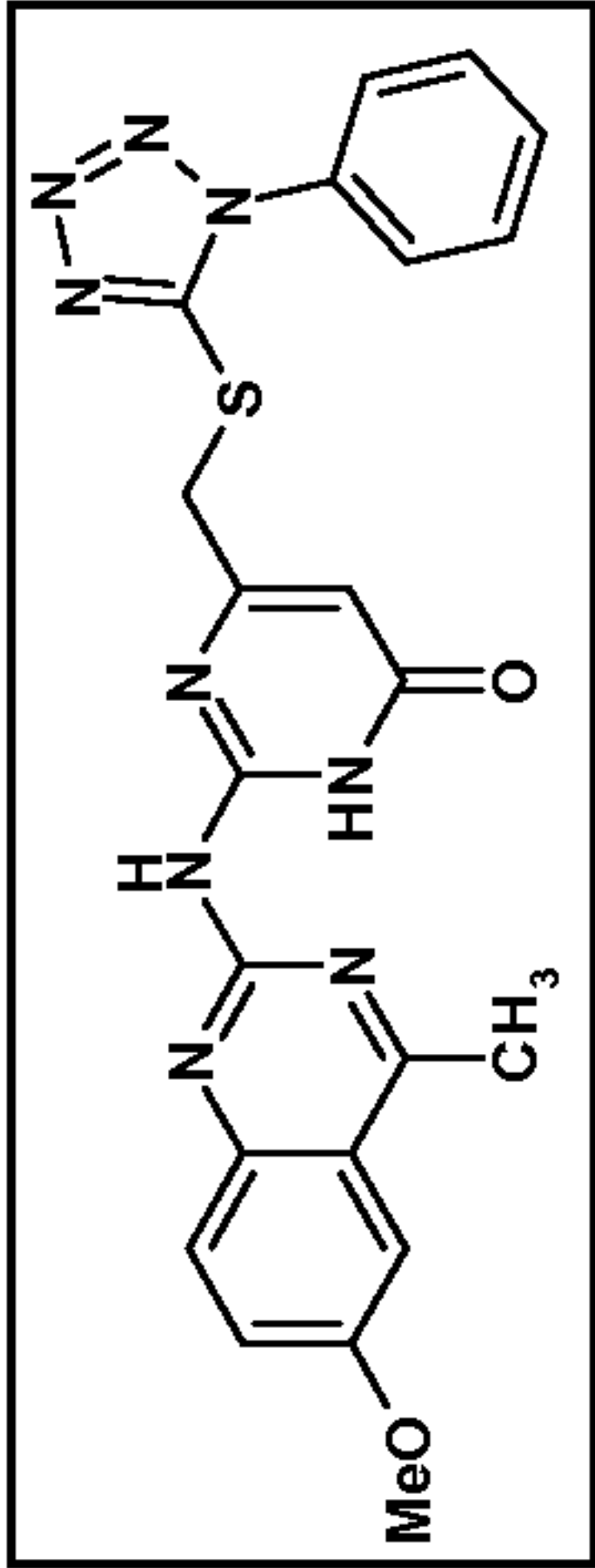
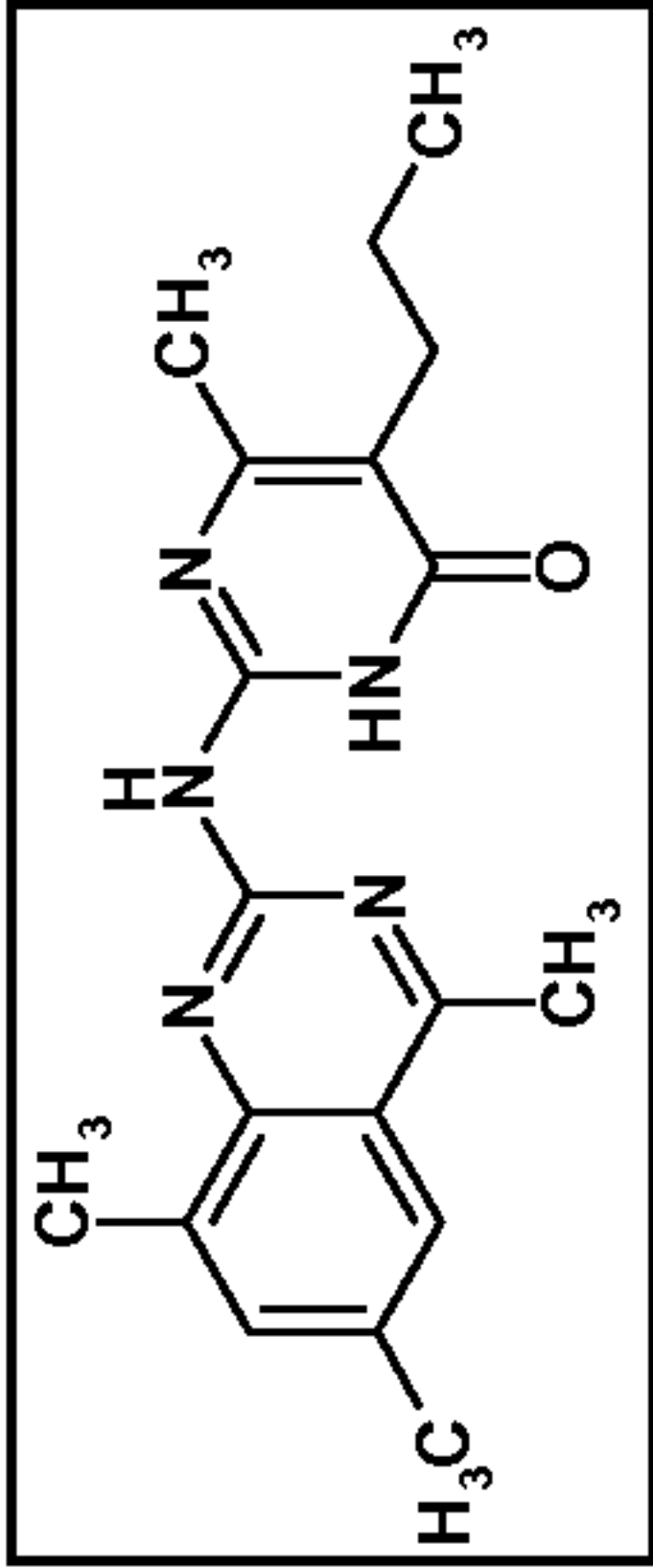
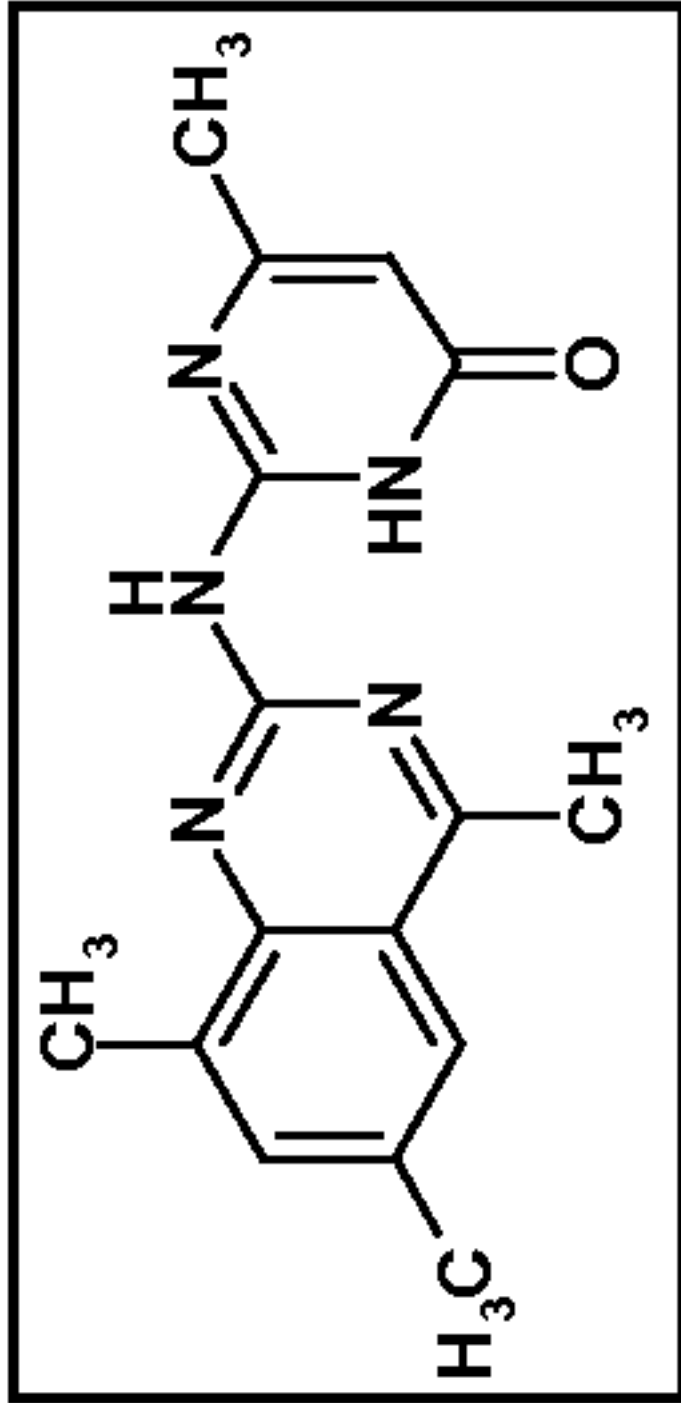
	12660 + TFA	D	-5.55	-47.79	49.87	73.40	61.63	41.54/41.10(0.99)	
	11445 + TFA	D	-4.56	-56.29	59.54	61.02	60.28	9.61/45.14(4.70)	
	11783 + TFA	D	-5.05	-44.32	66.95	53.44	60.19	18.22/14.99(0.82)	
	11436+ TFA	F	-4.46	-43.34	59.16	60.14	59.65	5.86/2.80(0.48)	
	11472	D	-5.57	-43.39	51.74	66.90	59.32	24.68/19.35	

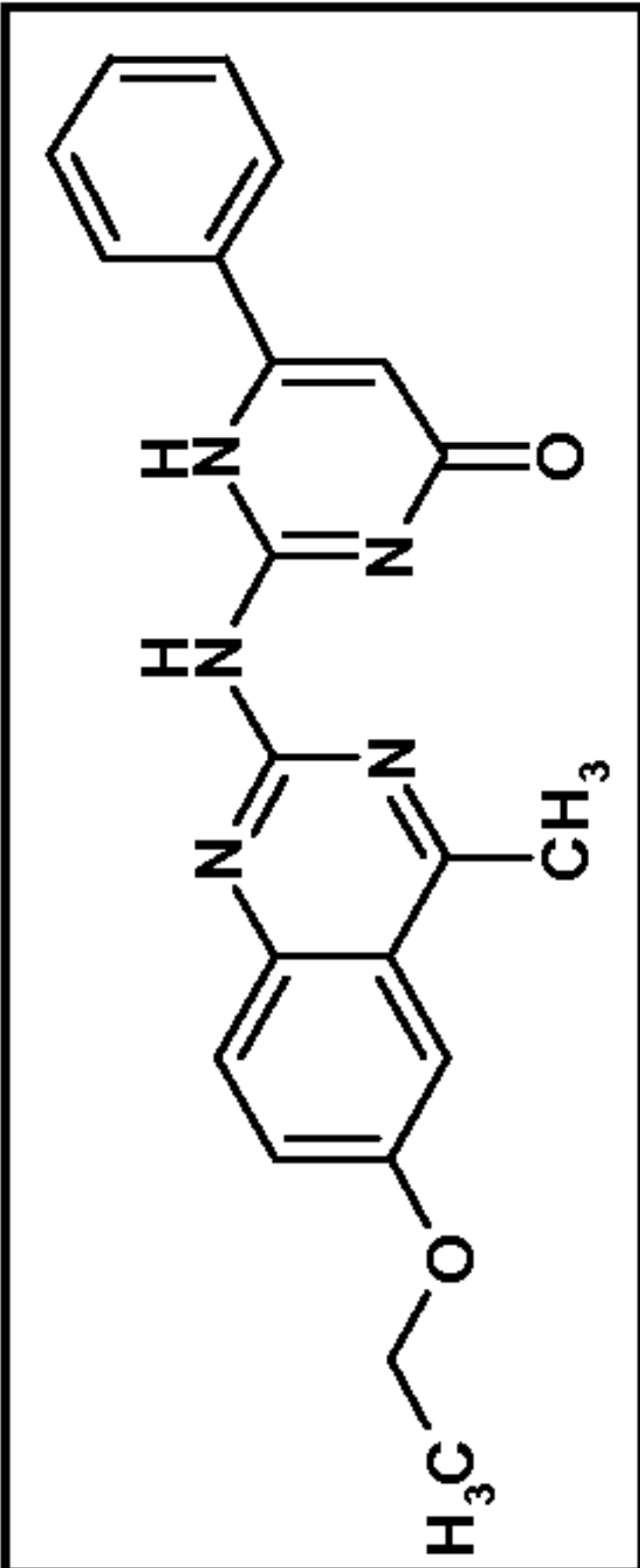
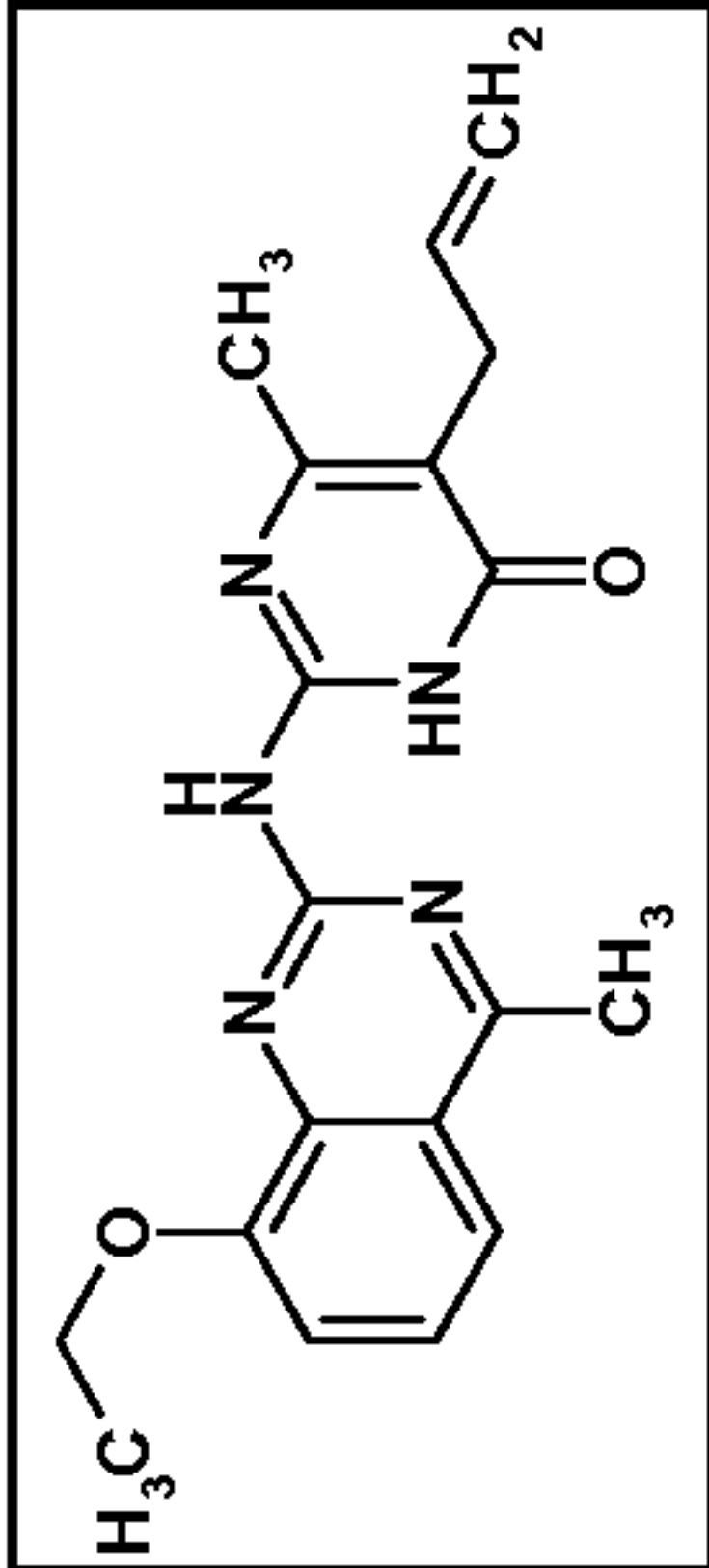
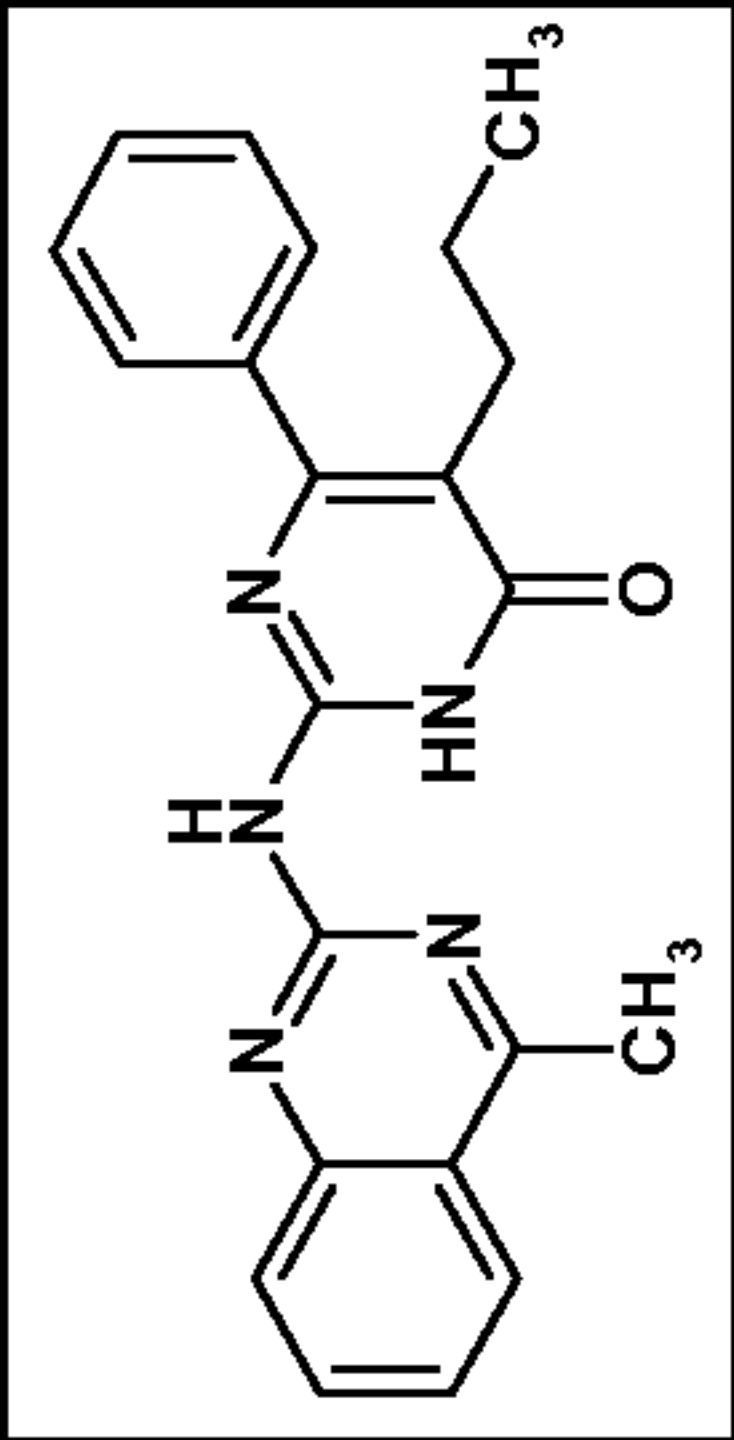
	12612 + TFA	D	-5.87	-46.47	69.21	45.51	57.36	48.51/79.16	
	12693 + TFA	E	-4.92	-42.18	40.36	74.00	57.18	9.01/6.88(0.76)	
	11812	E	-4.52	-47.03	51.90	61.77	56.84	14.65/31.61	
	11601	D	-6.11	-52.7	58.11	54.06	56.08	1.29/2.62 0.738/2.61(normalized)	3.67/48.56(13.23)
	11825	E	-4.03	-50.59	52.17	55.87	54.02	4.06/17.94	

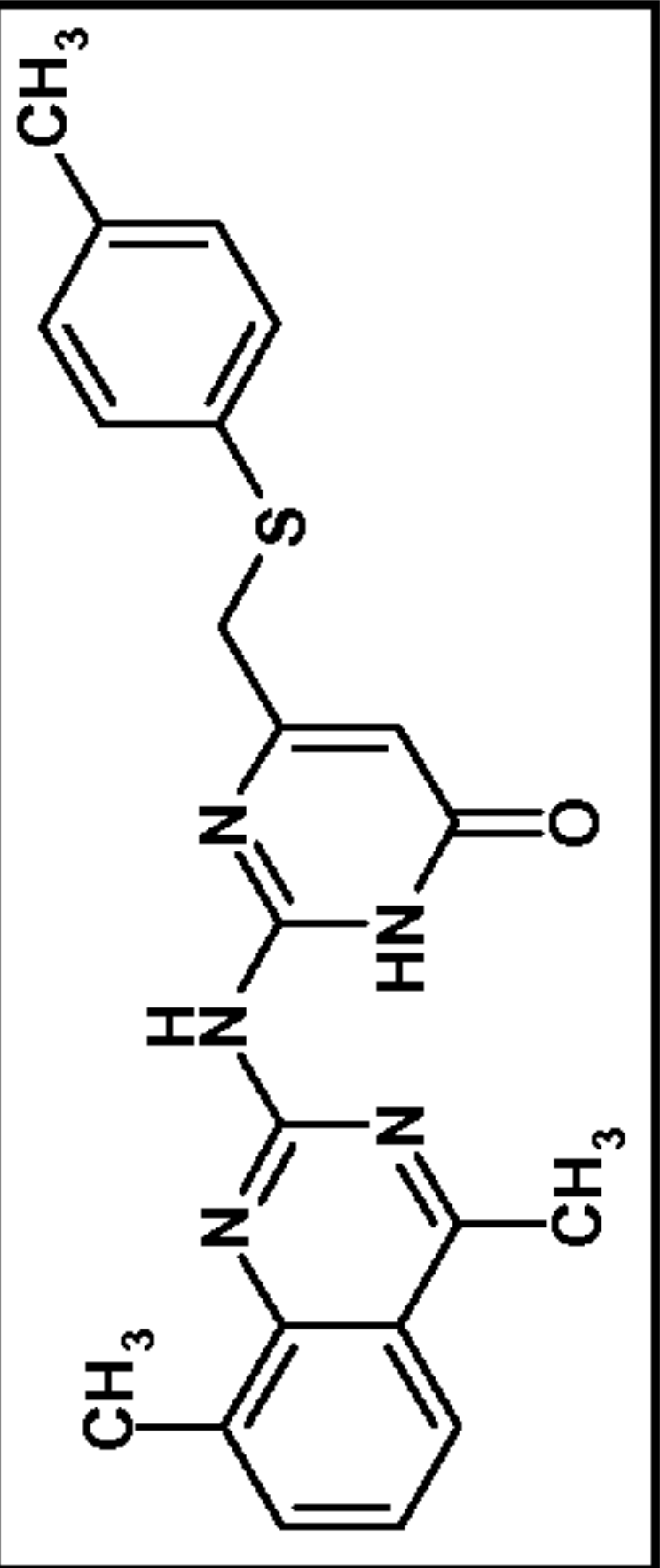
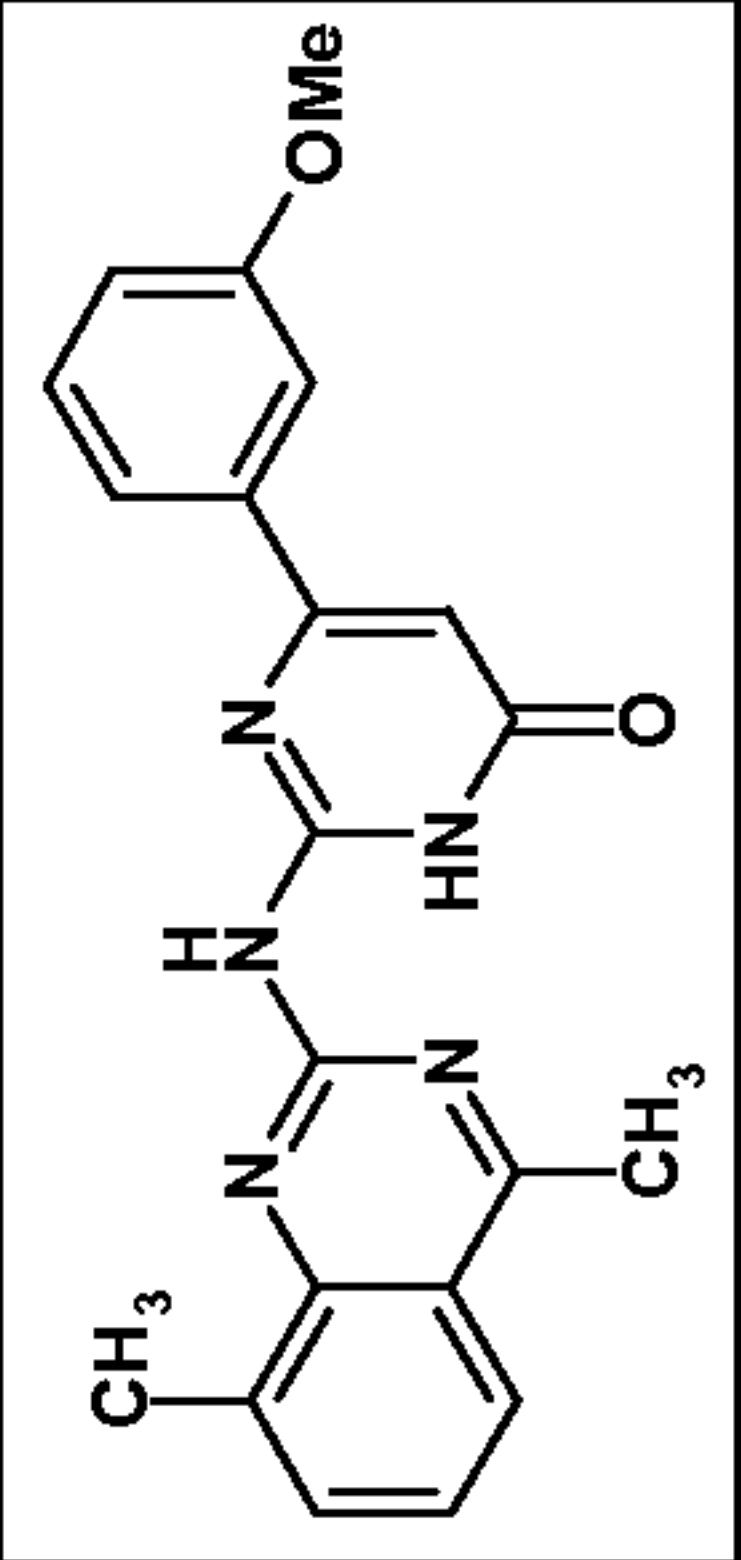
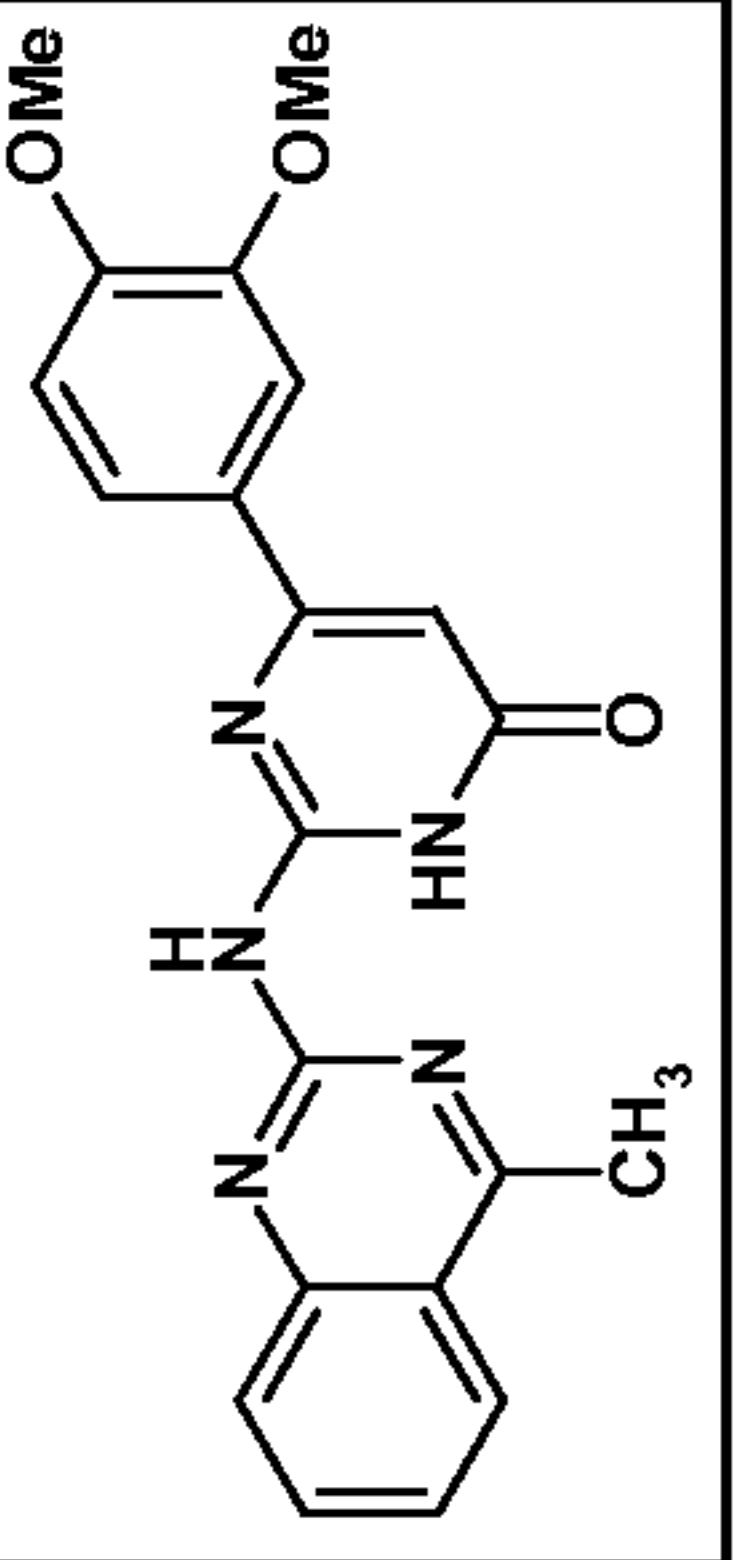
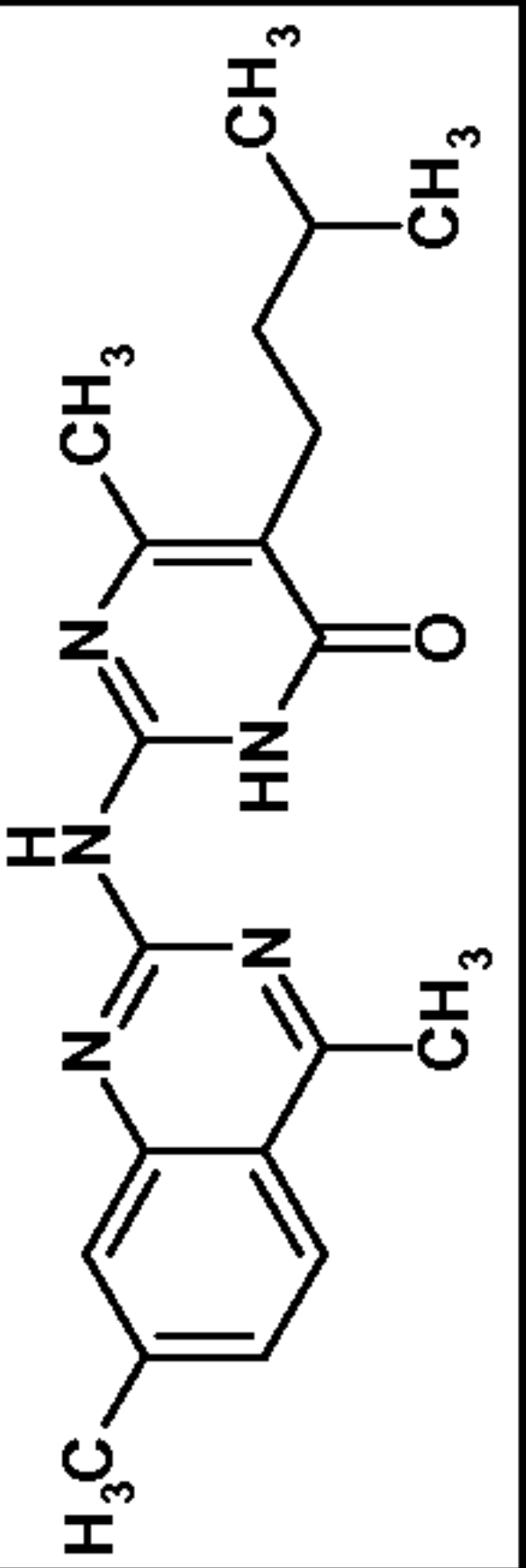
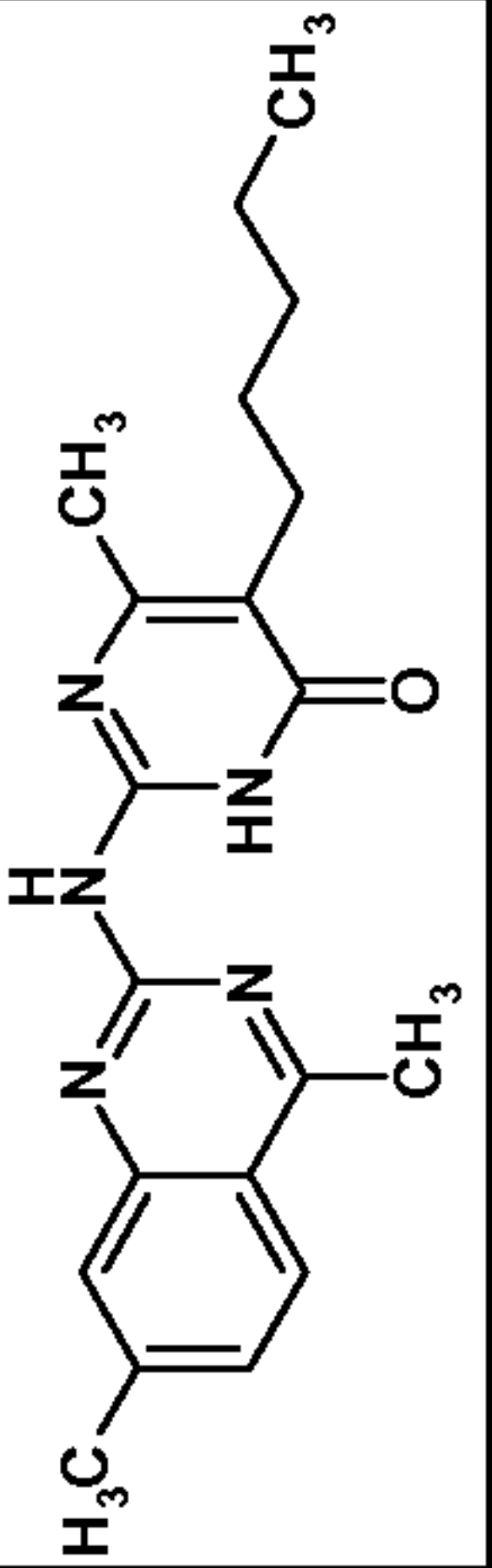
	12673 + TFA	D	-5.89	-44.35	44.70	60.90	52.80	33.08/22.52(0.68)	
	12613	D	-5.37	-45.92	47.72	56.65	52.18	17.58/11.50(0.65)	
	11583 + TFA	D	-5.58	-53.92	47.93	54.26	51.10	47.28/47.33(1.00)	
	12604 + TFA	D	-3.38	-55.15	55.87	46.08	50.98	19.30/29.98(1.55)	
	11316	E	-5.01	-42.35	47.95	53.57	50.76	44.42/19.89(0.45)	

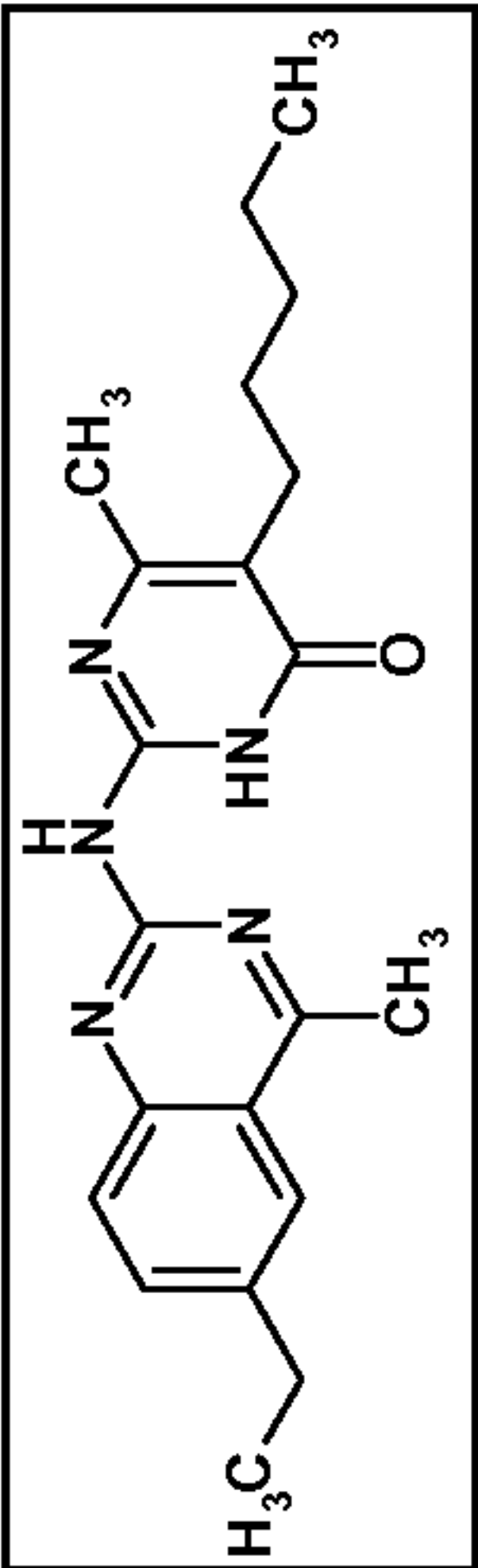
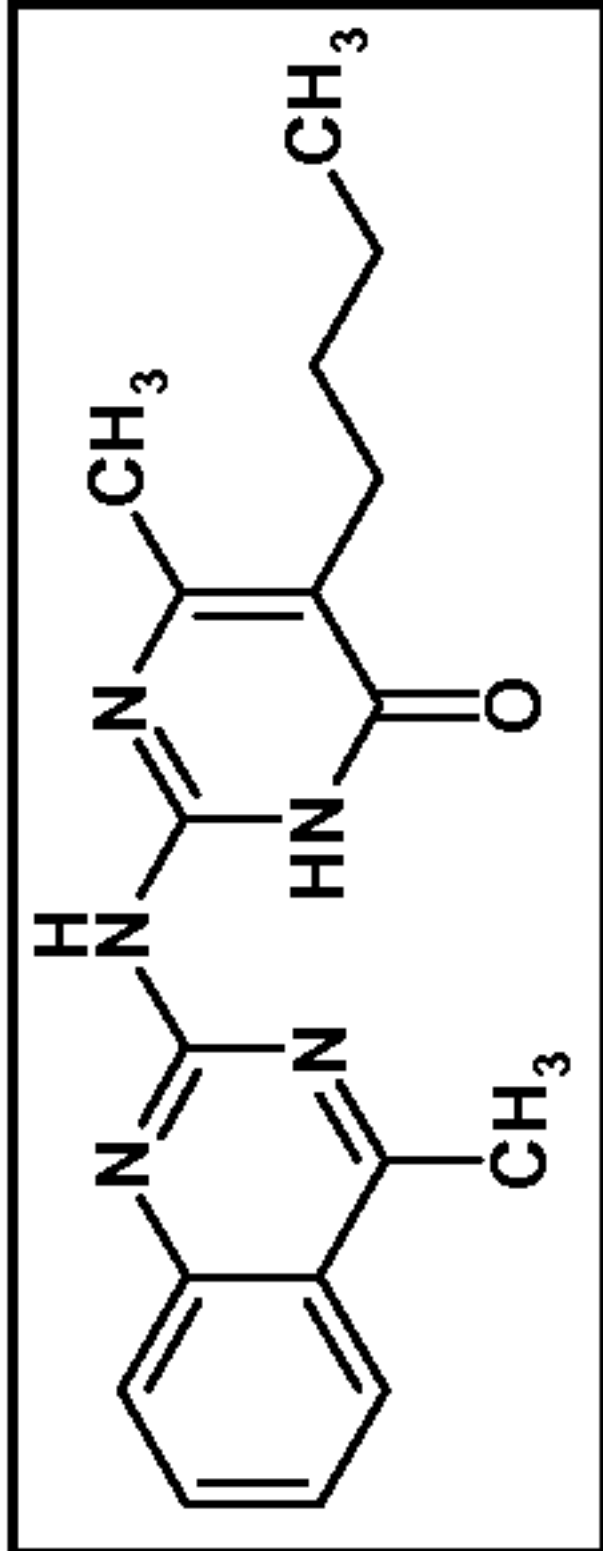
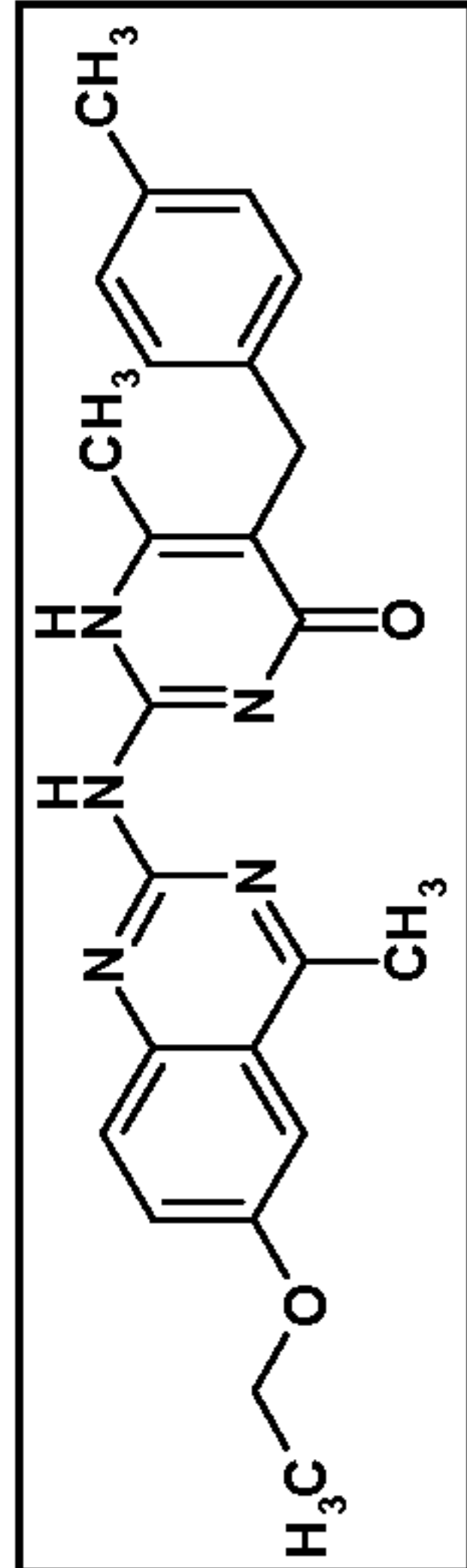
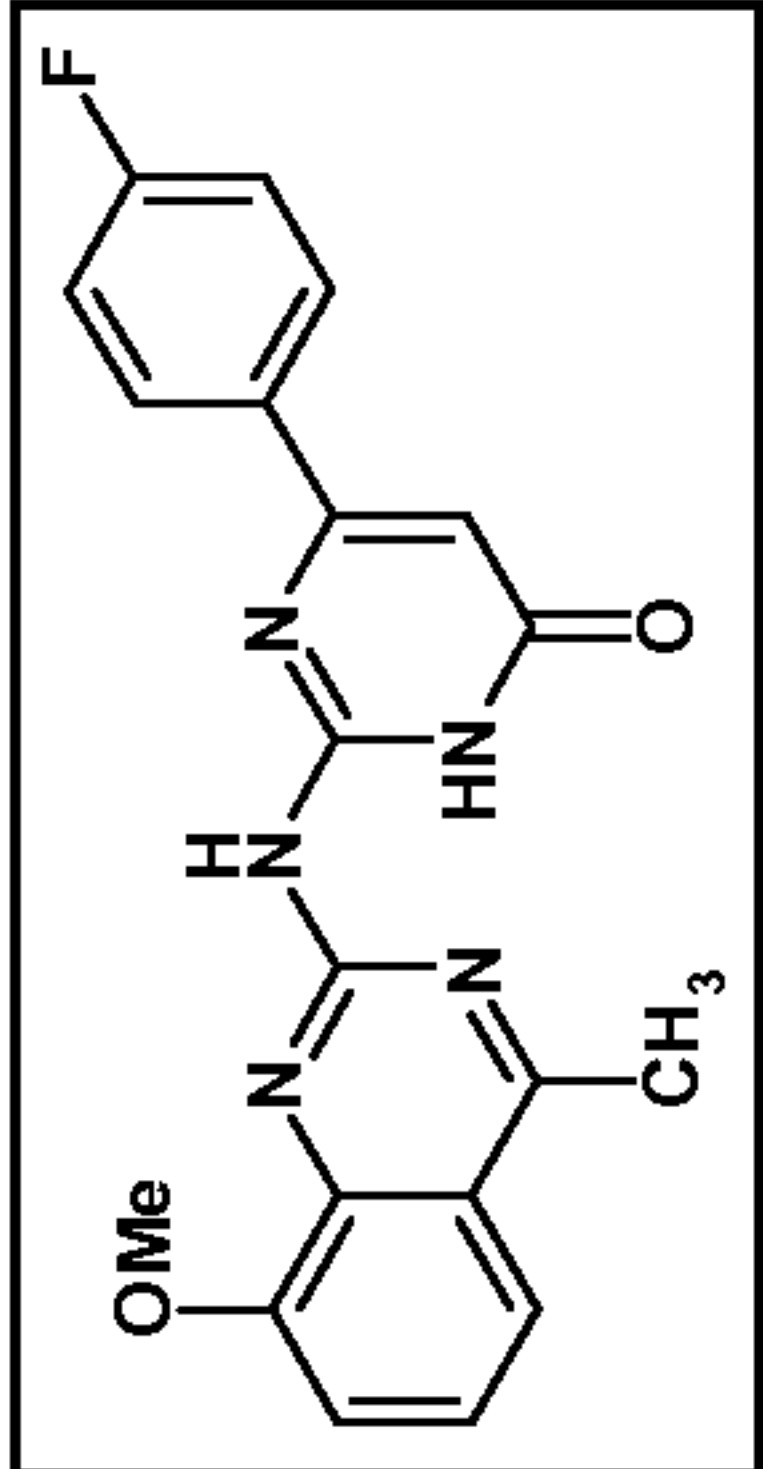
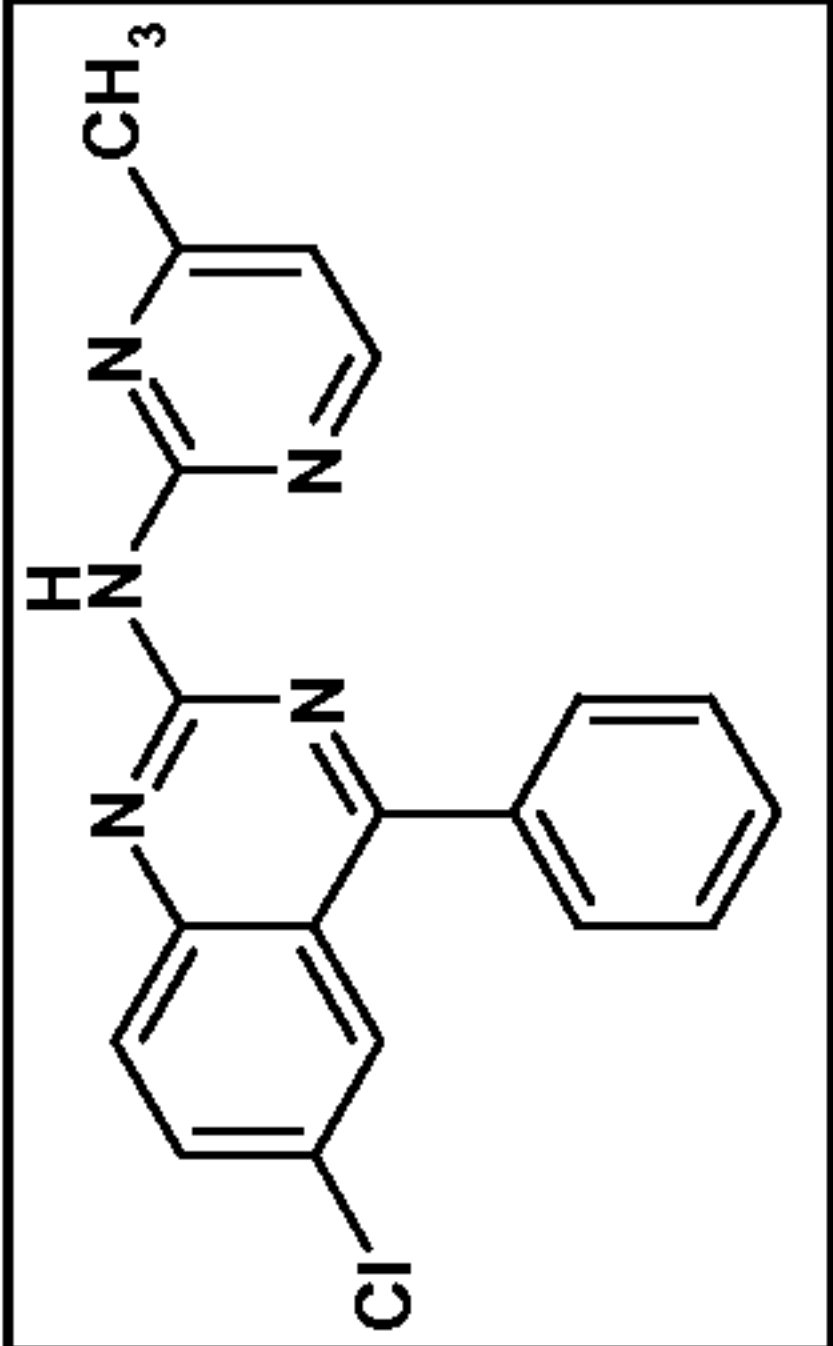
	11450	D	-2.82	-58.11	53.35	48.02	50.68	6.98/6.83	
	11582	D	-6.23	-55.72	59.77	40.01	49.89	2.43/2.44	49.26/55.85(1.13)
	11315	E	-4.77	-43	48.33	50.82	49.58	very selective MDBK 20% at 100 μM	
	11498	D	-5.12	-47.66	42.15	56.48	49.32	5.39/8.77	
	12742	D	-5.3	-45.02	33.49	64.92	49.20	19.60/18.95(0.97)	

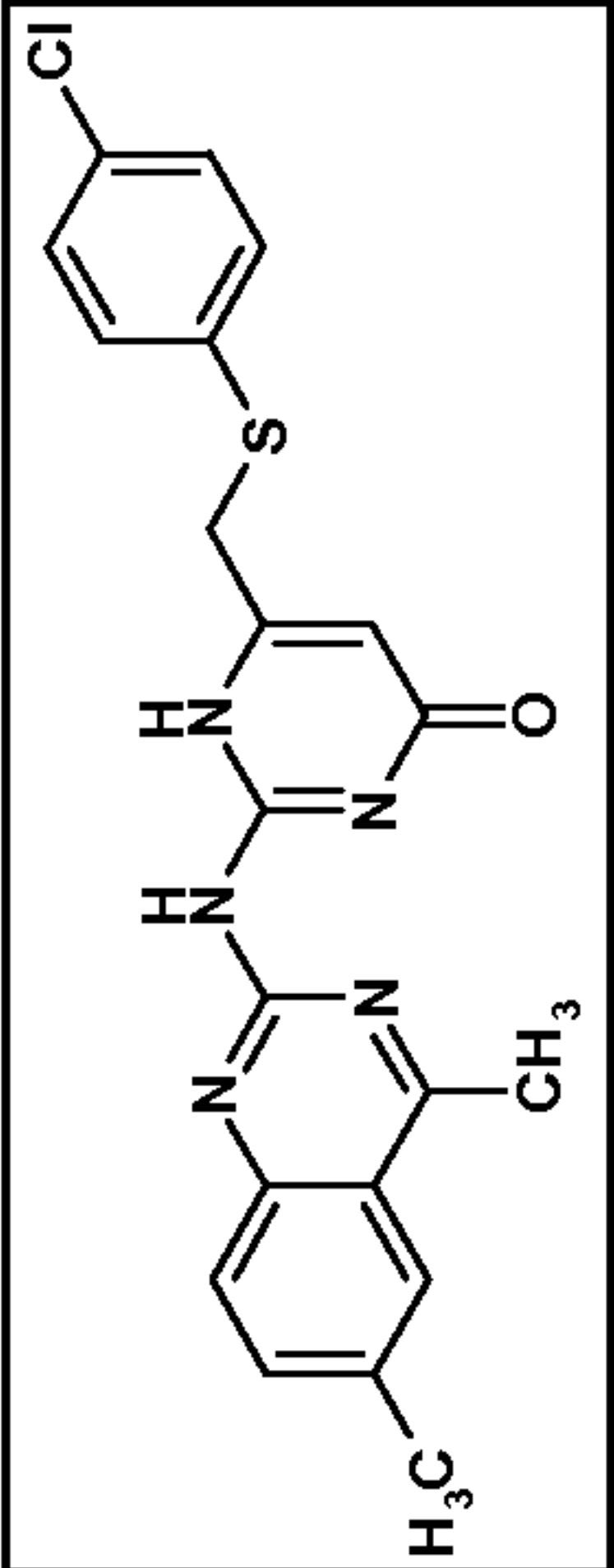
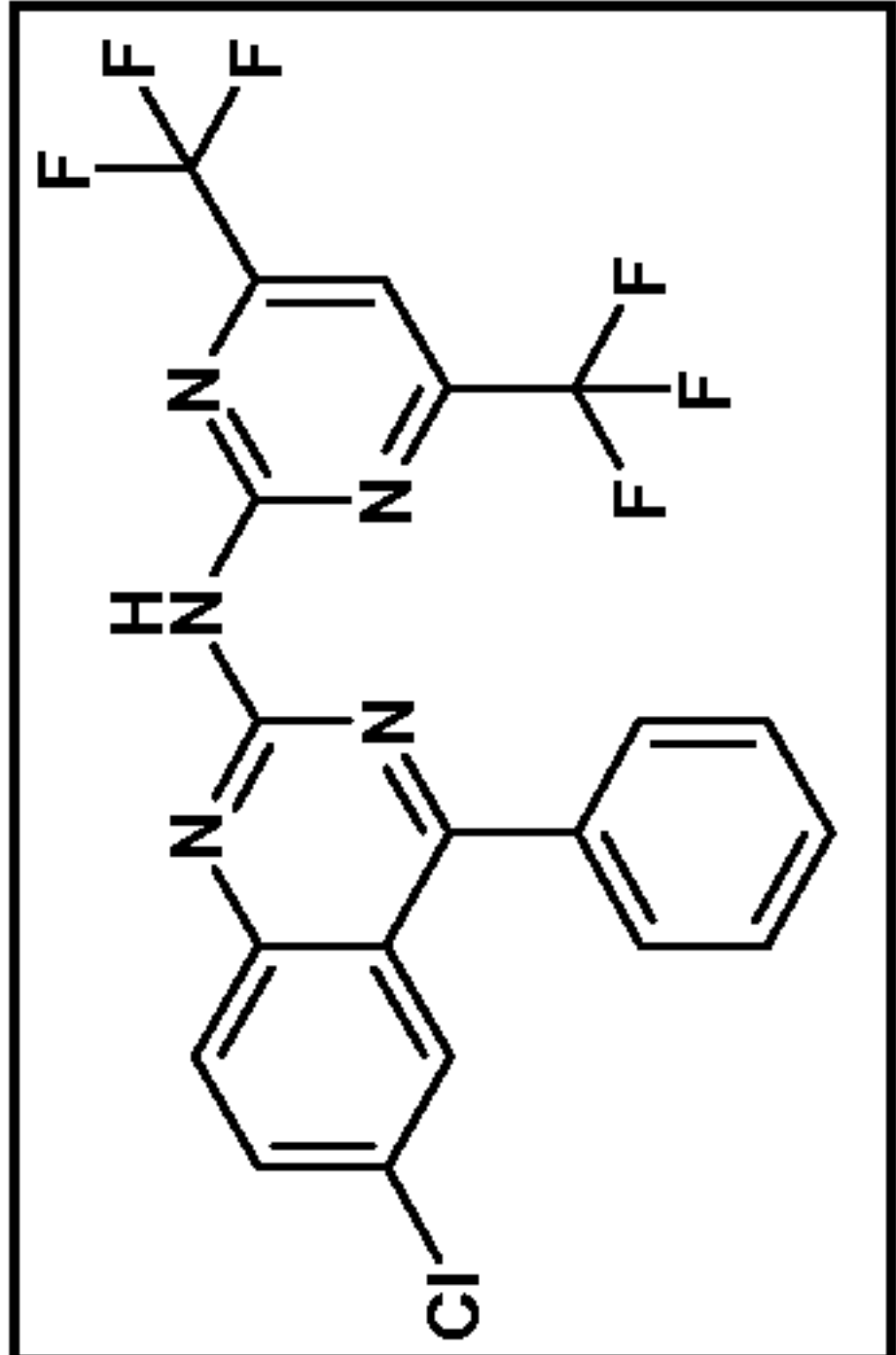
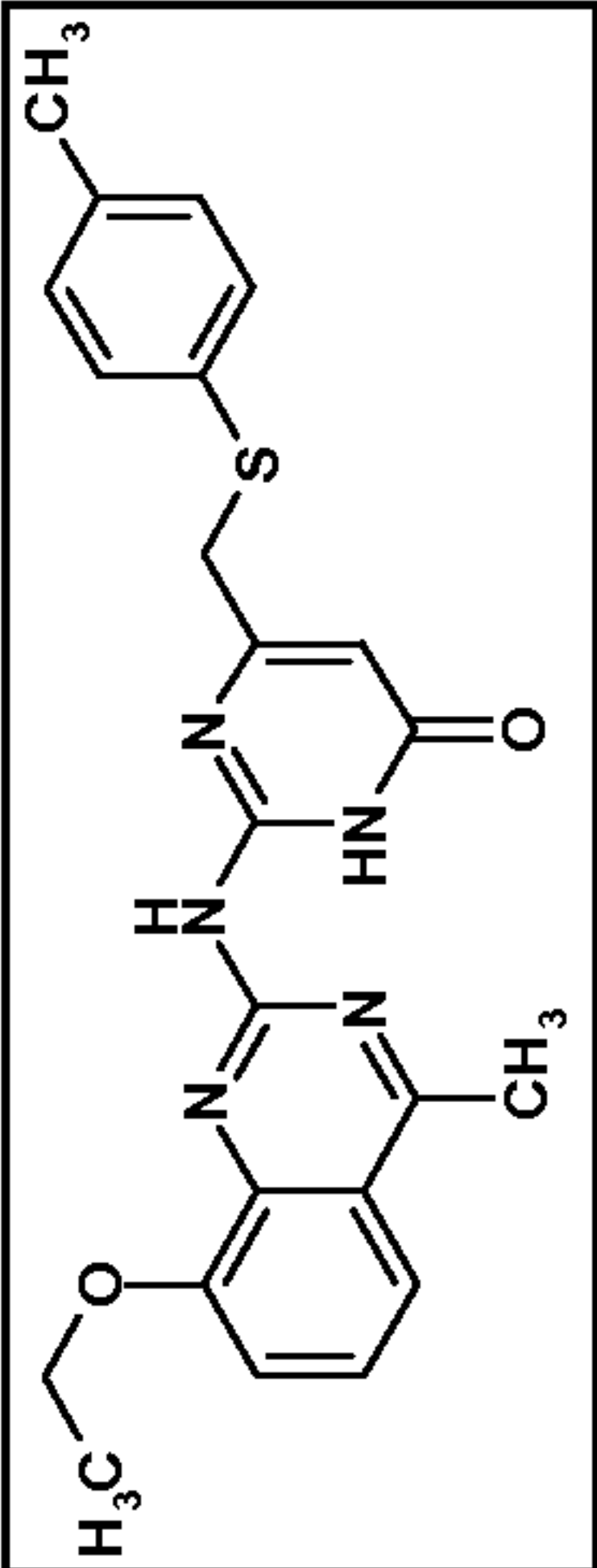
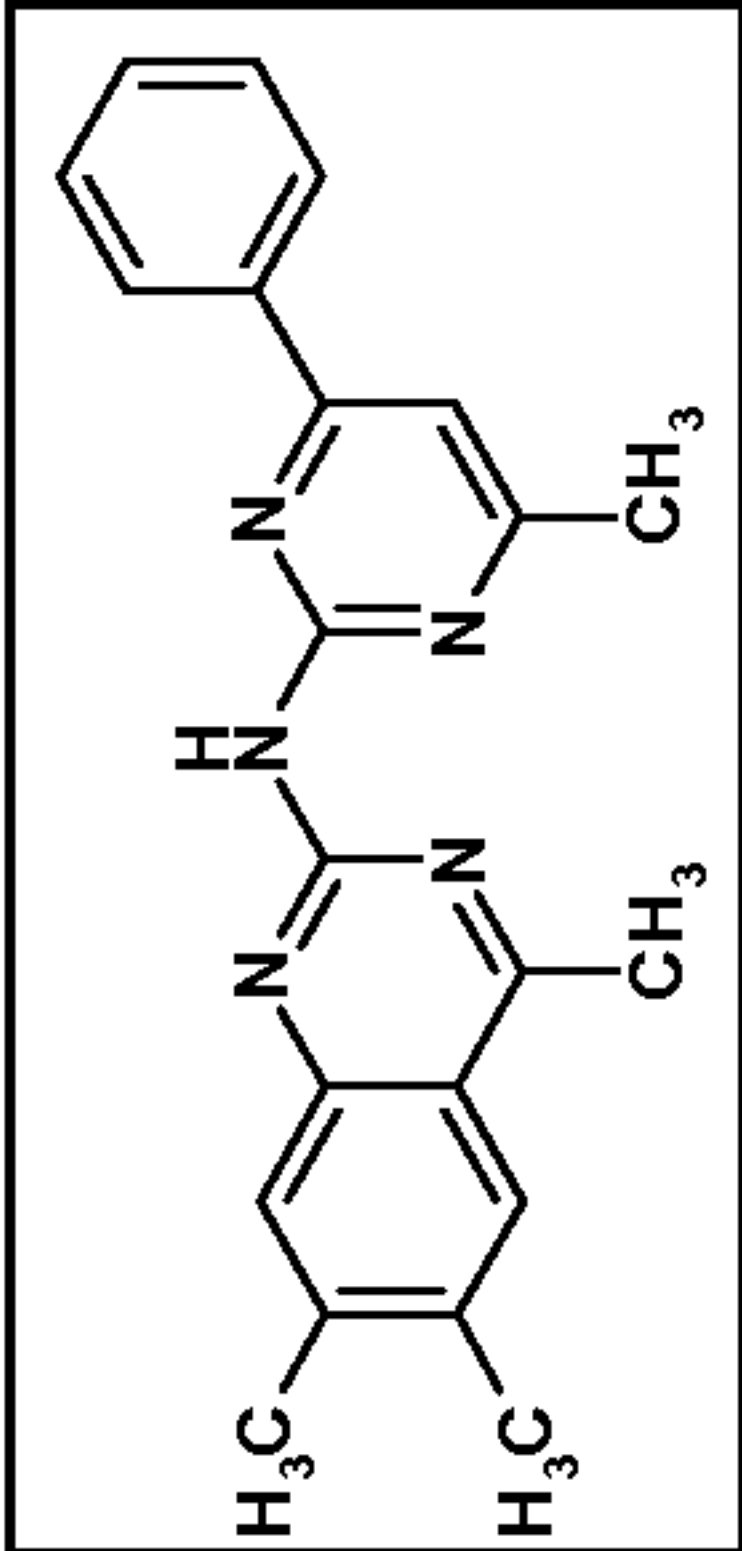
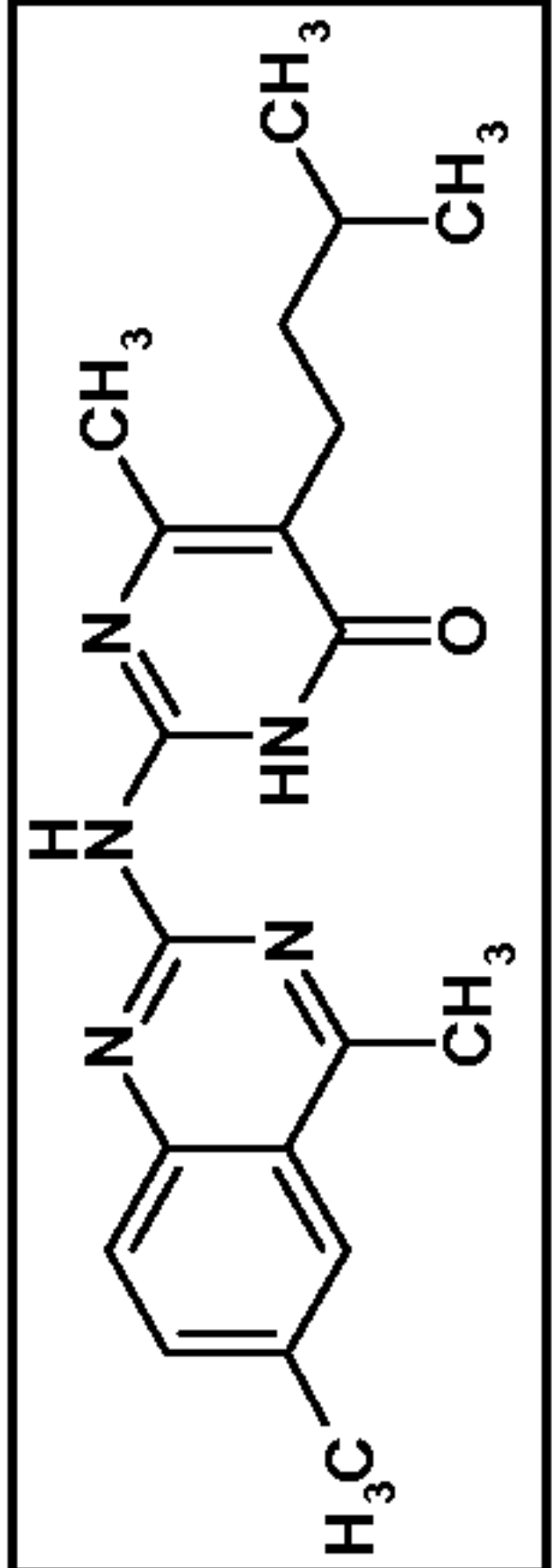
	12636	E	-4.58	-41.6	49.55	48.49	49.02	15.88/34.63(2.18)	
	11758	D	-5.73	-43.23	53.63	41.93	47.78	53.81/576.2	
	11771 + TFA	D	-4.55	-44.55	51.58	42.32	46.95	18.17/42.13(2.32)	
	12611 + TFA	D	-5.46	-53.71	52.69	38.76	45.72	5.32/5.31(1.00)	
	12738 + TFA	D	-5.5	-46.21	53.67	37.02	45.35	45.36/24.62(0.54)	

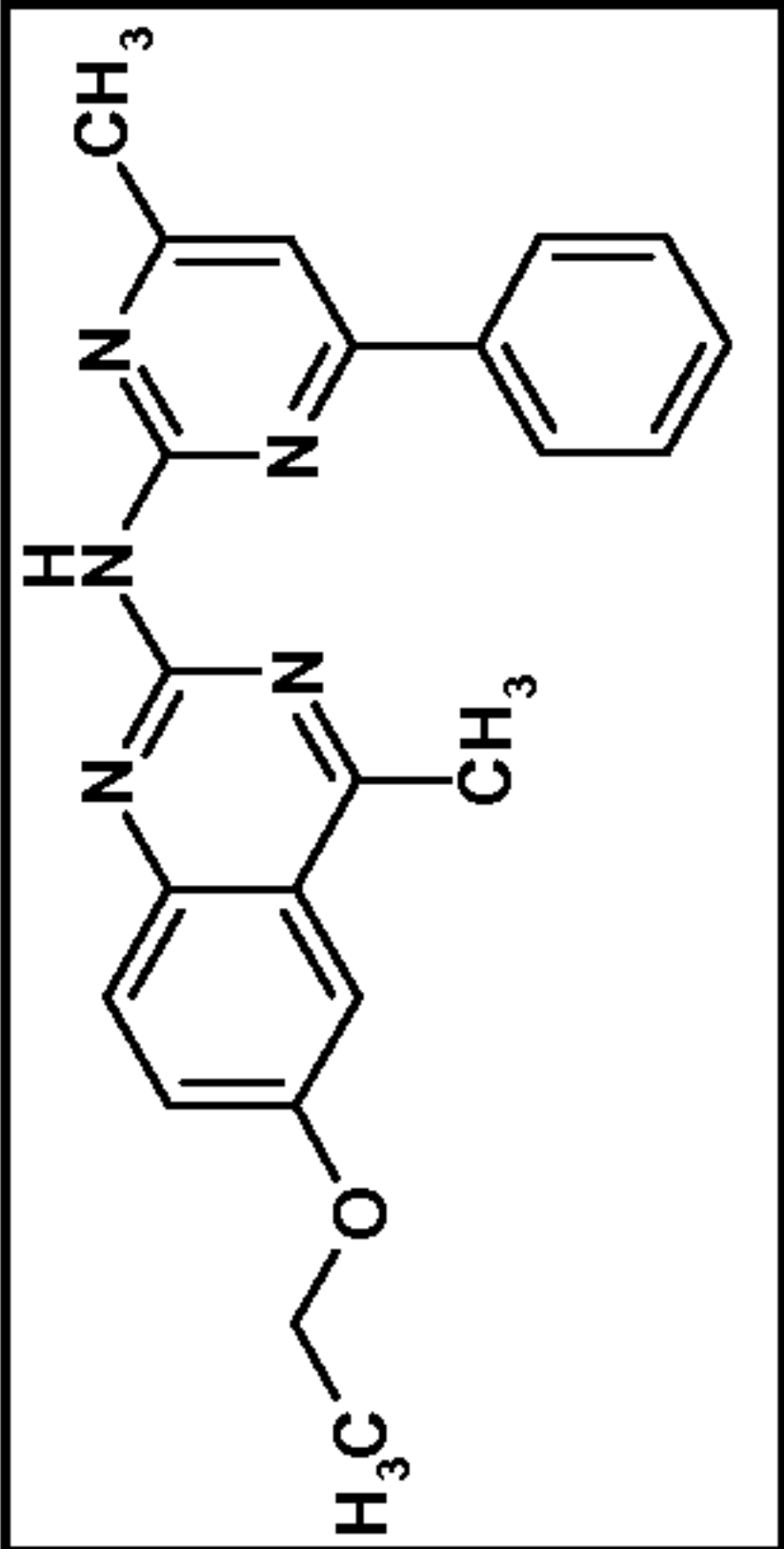
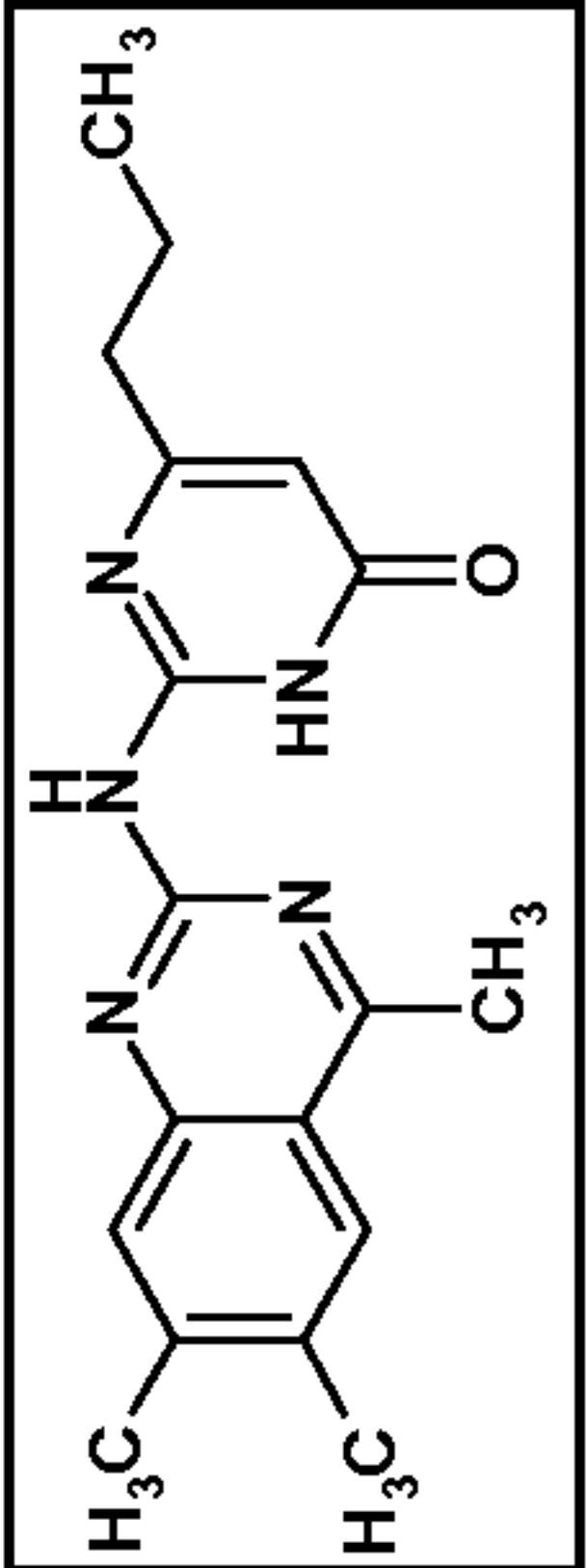
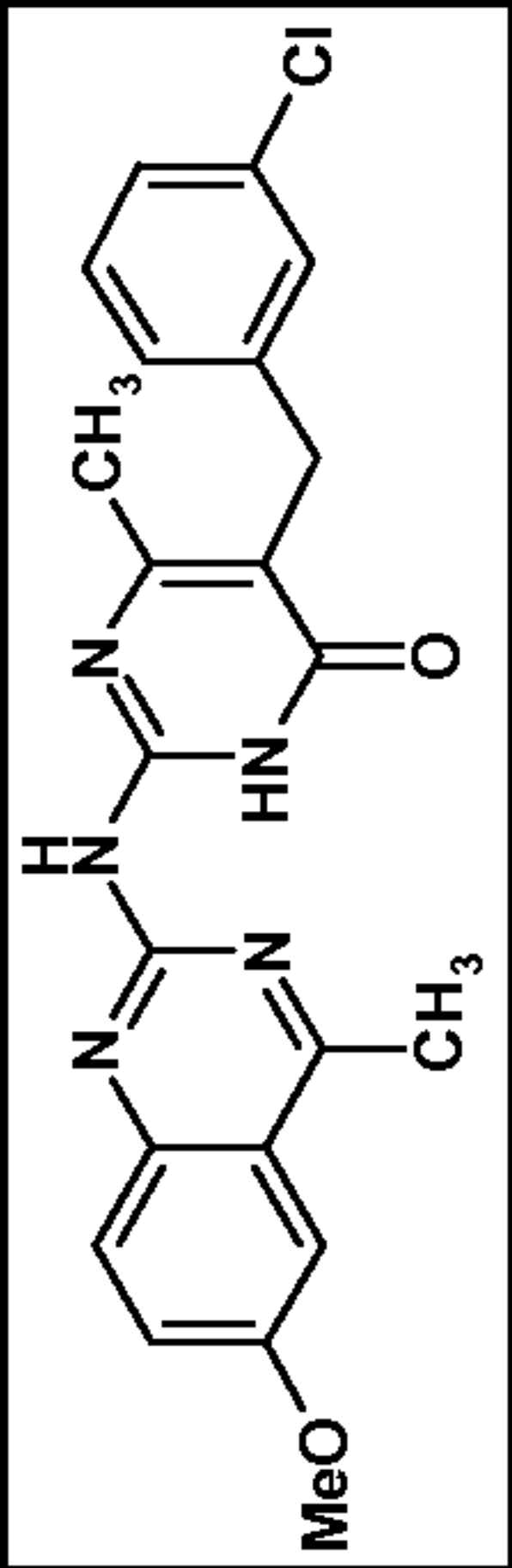
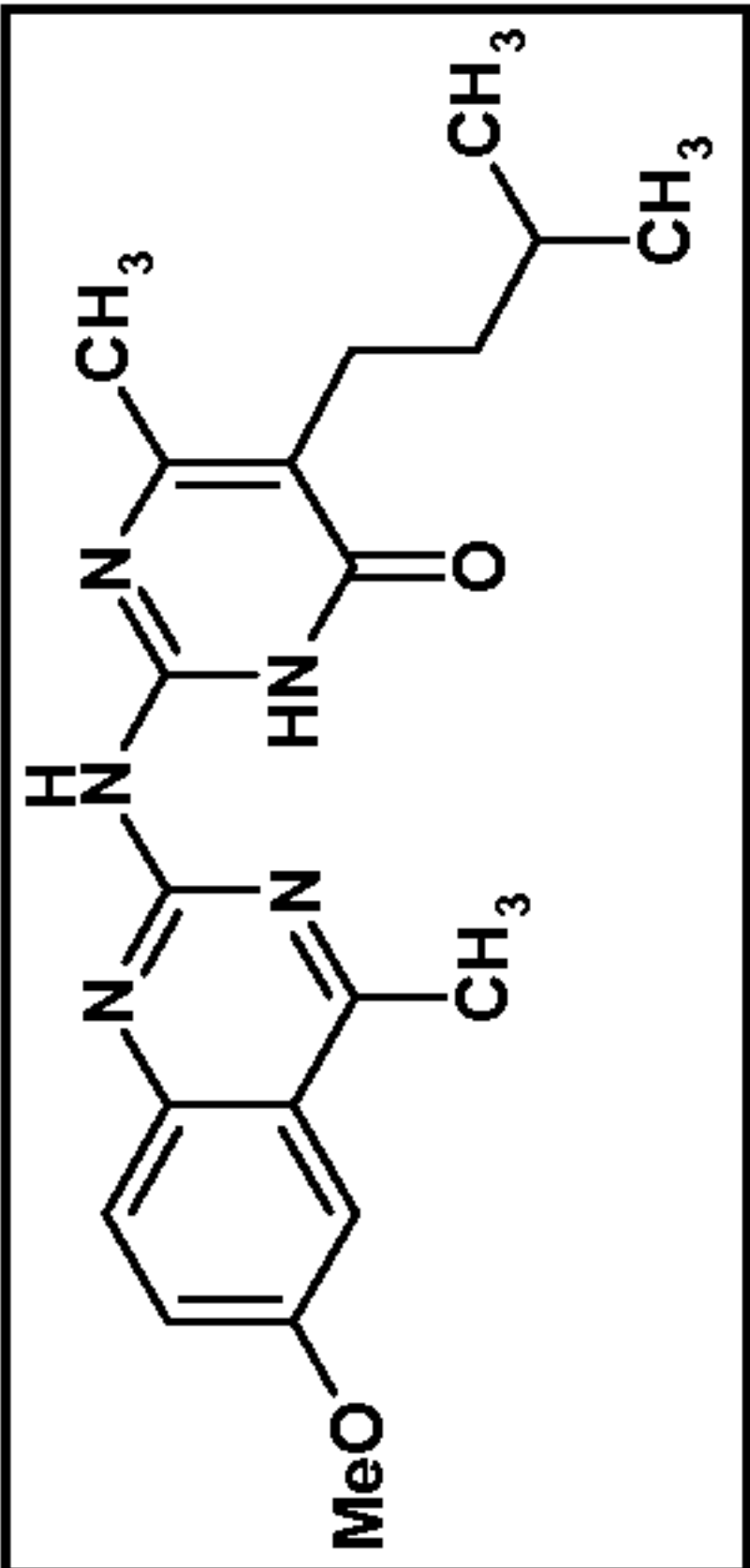
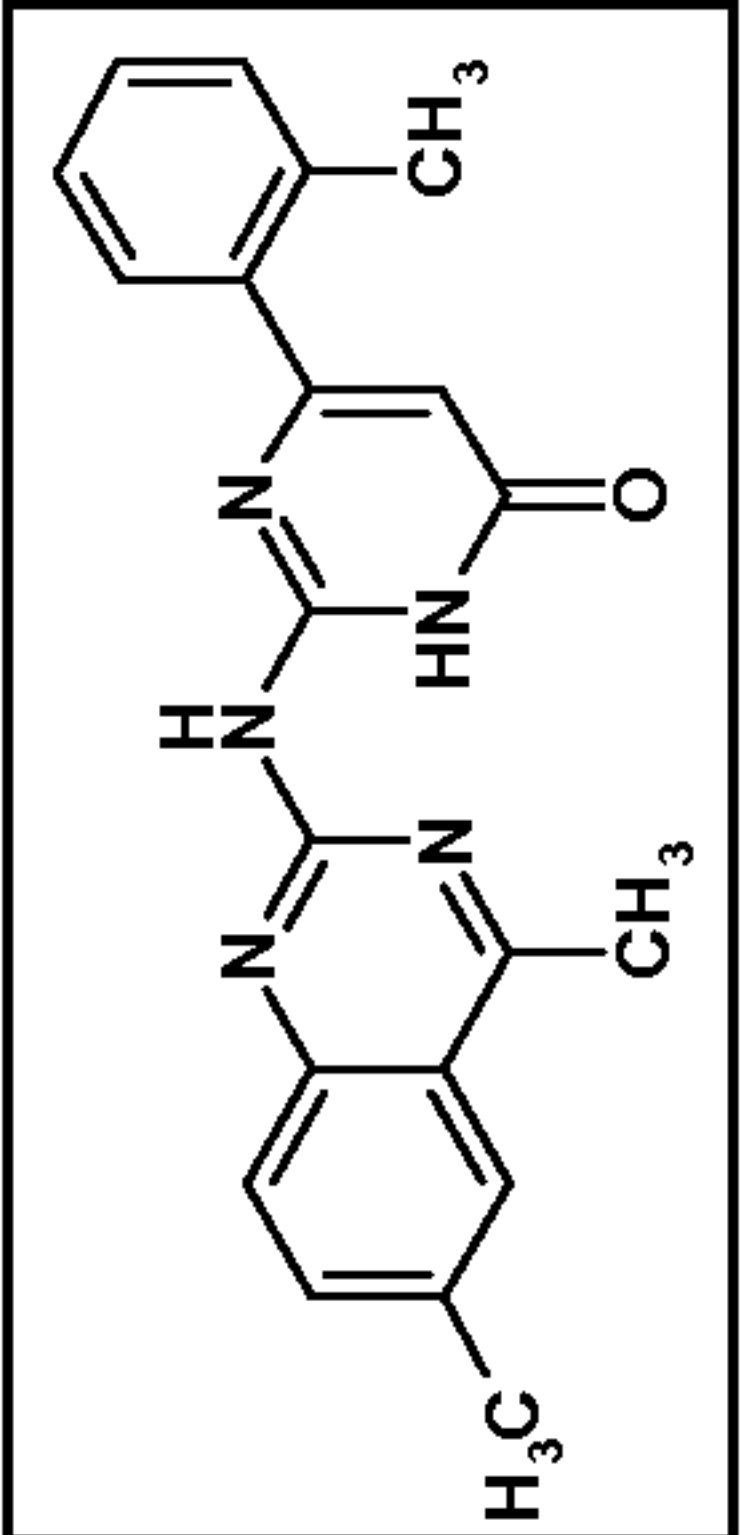
	11753 + TFA	D	-5.13	-57.6	42.44	47.28	44.86	NOT ACTIVE	
	12665 + TFA	D	-5.51	-43.87	51.06	37.29	44.17	17.52/17.16(0.98)	
	11448	D	-4.63	-45.4	50.06	37.38	43.72	3.45/11.8(3.27)	
	12736	D	-5.39	-46.96	32.35	54.51	43.43	7.14/9.82(1.38)	
	11607 + TFA	D	-6.52	-43.34	43.69	41.73	42.71	10.08/8.03(0.80)	

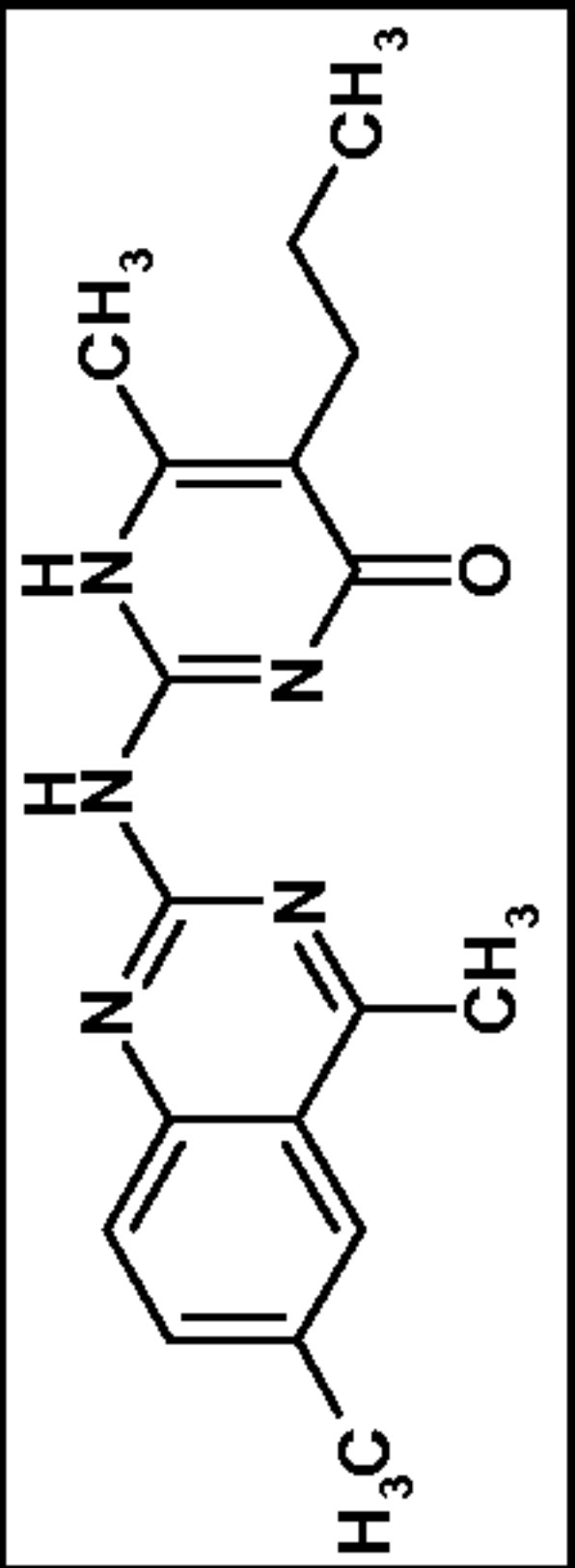
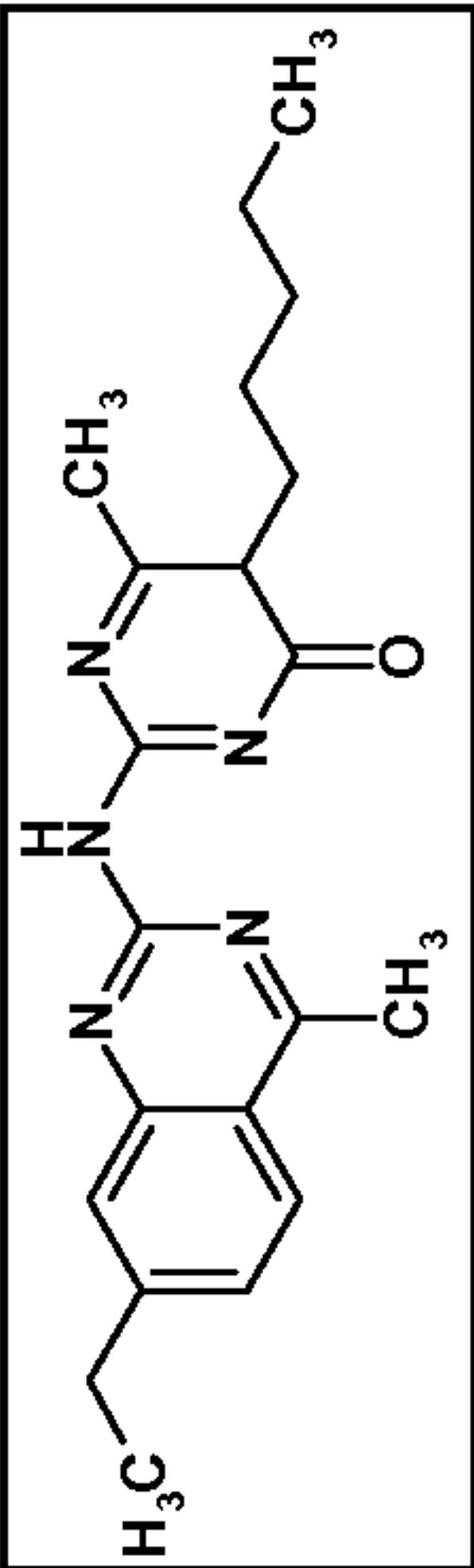
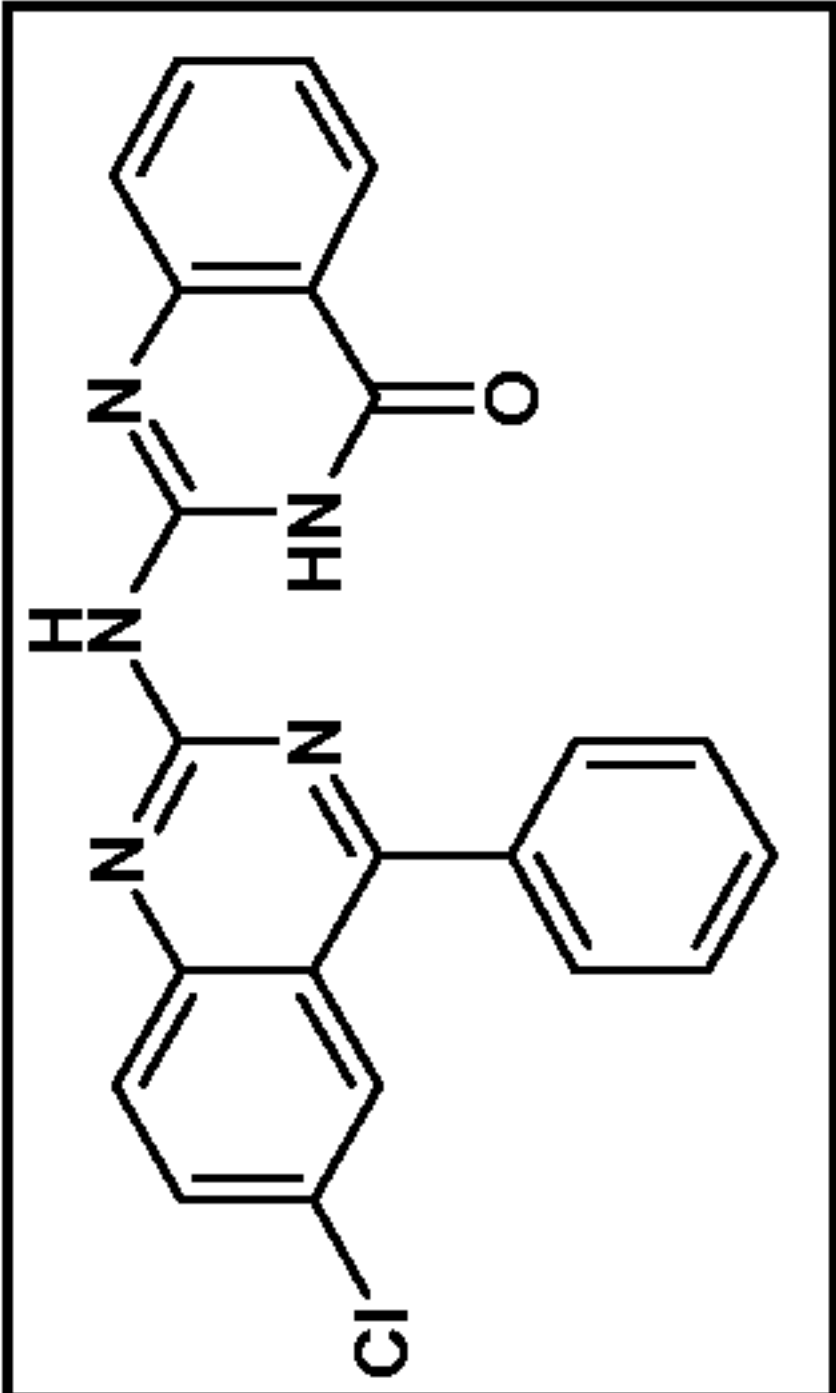
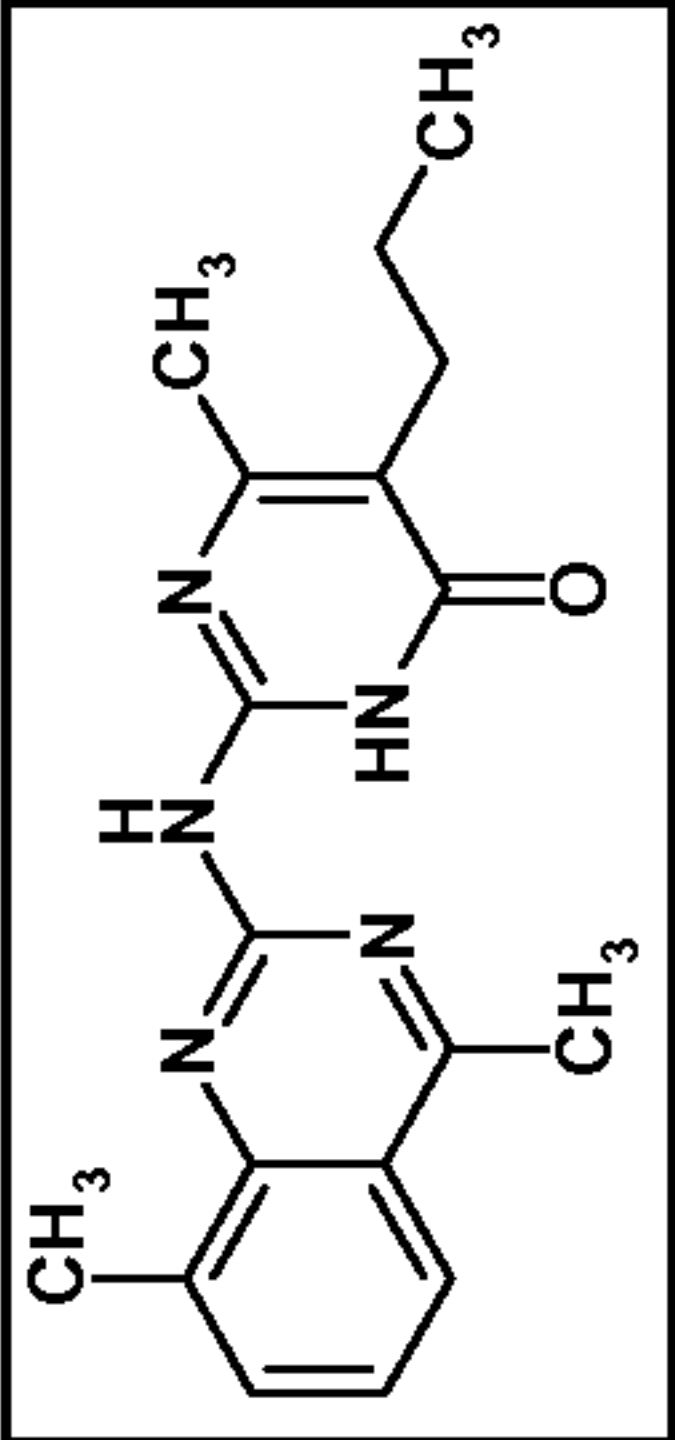
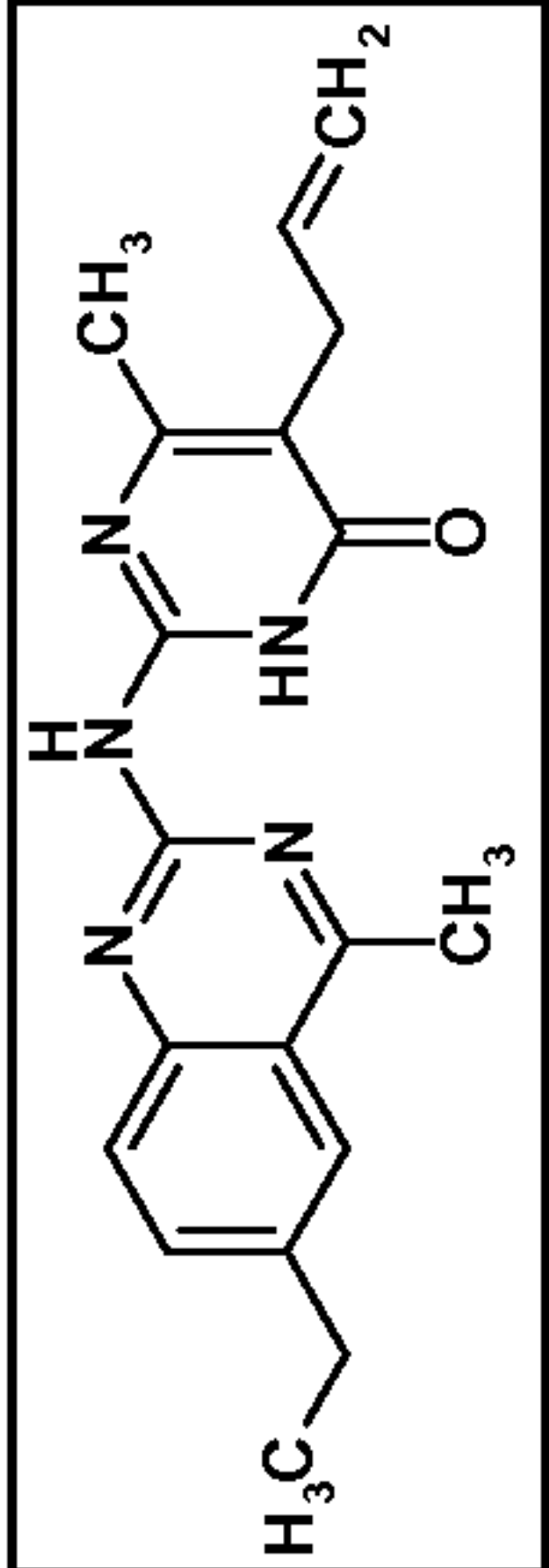
	12608 + TFA	D	-5.19	-51.11	46.64	38.07	42.36	43.13/17.03(0.80)_	
	12737	D	-5.3	-47.17	38.90	45.65	42.27	14.72/6.33(0.43)	
	12609	D	-5.81	-43.86	42.02	40.07	41.04	11.19/23.49(2.10)	
	12683 + TFA	D	-5.91	-44.79	54.37	27.38	40.88	63.42/45.31(0.71)	
	12674	D	-5.8	-34.96	29.69	51.69	40.69	10.67/8.41(0.79)	

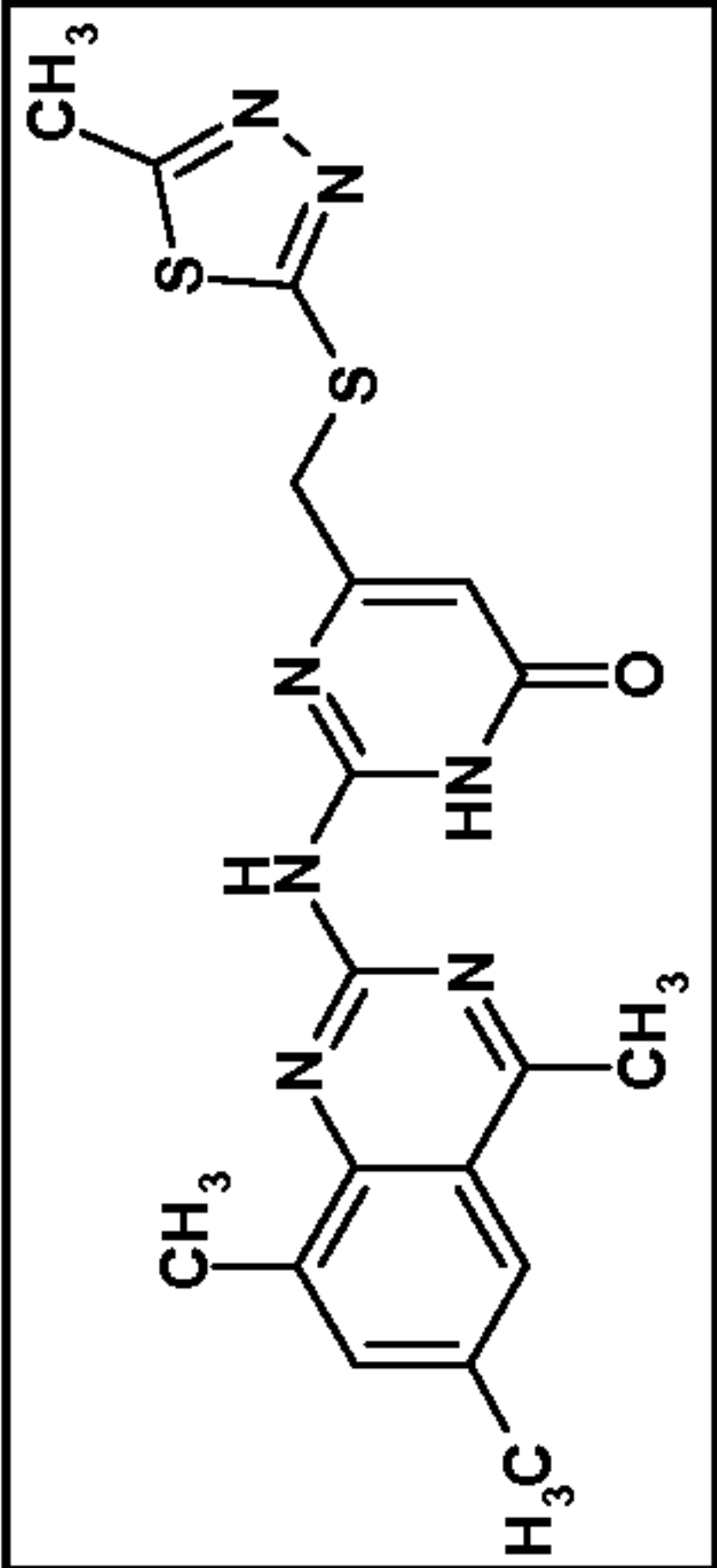
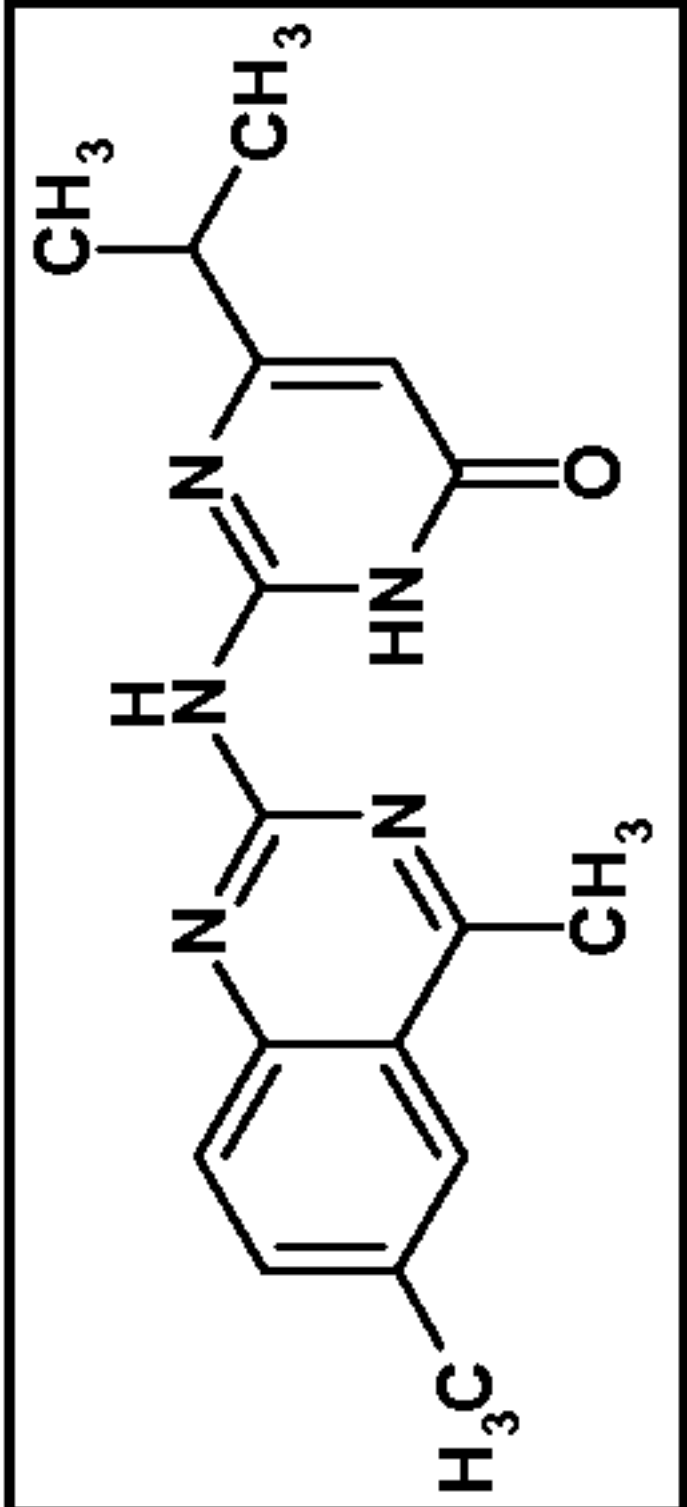
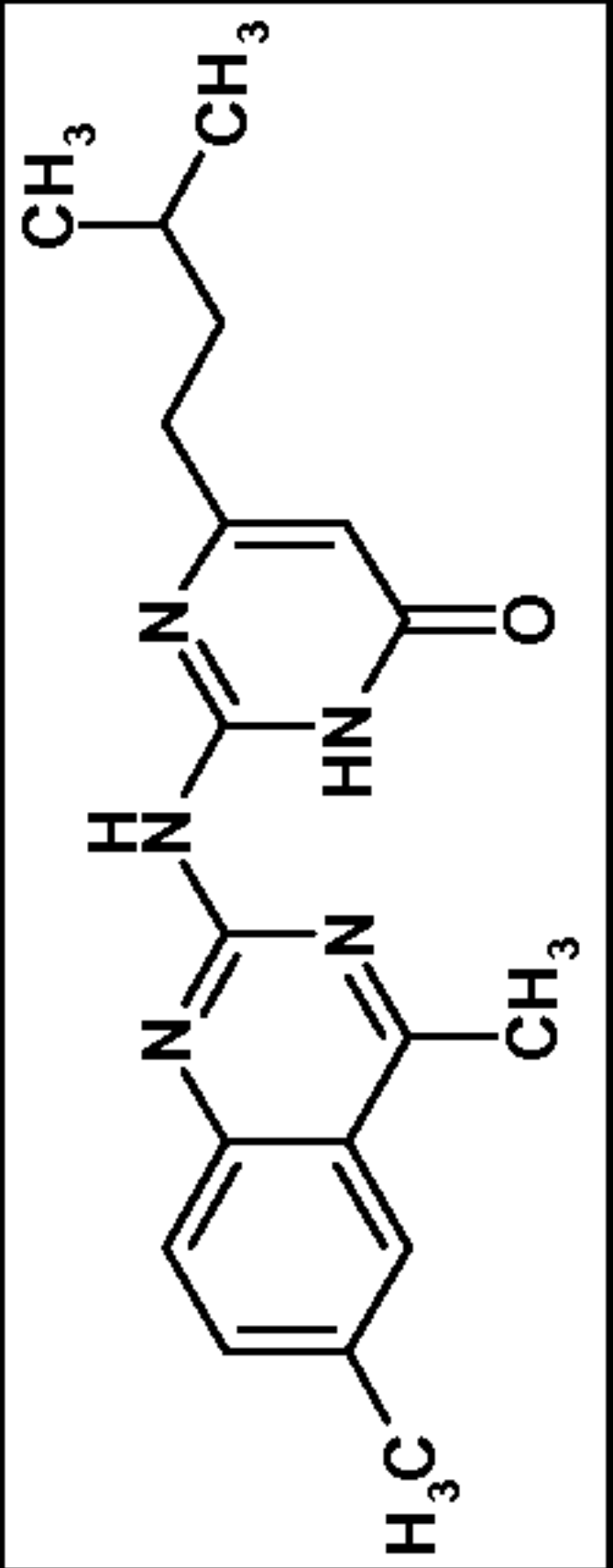
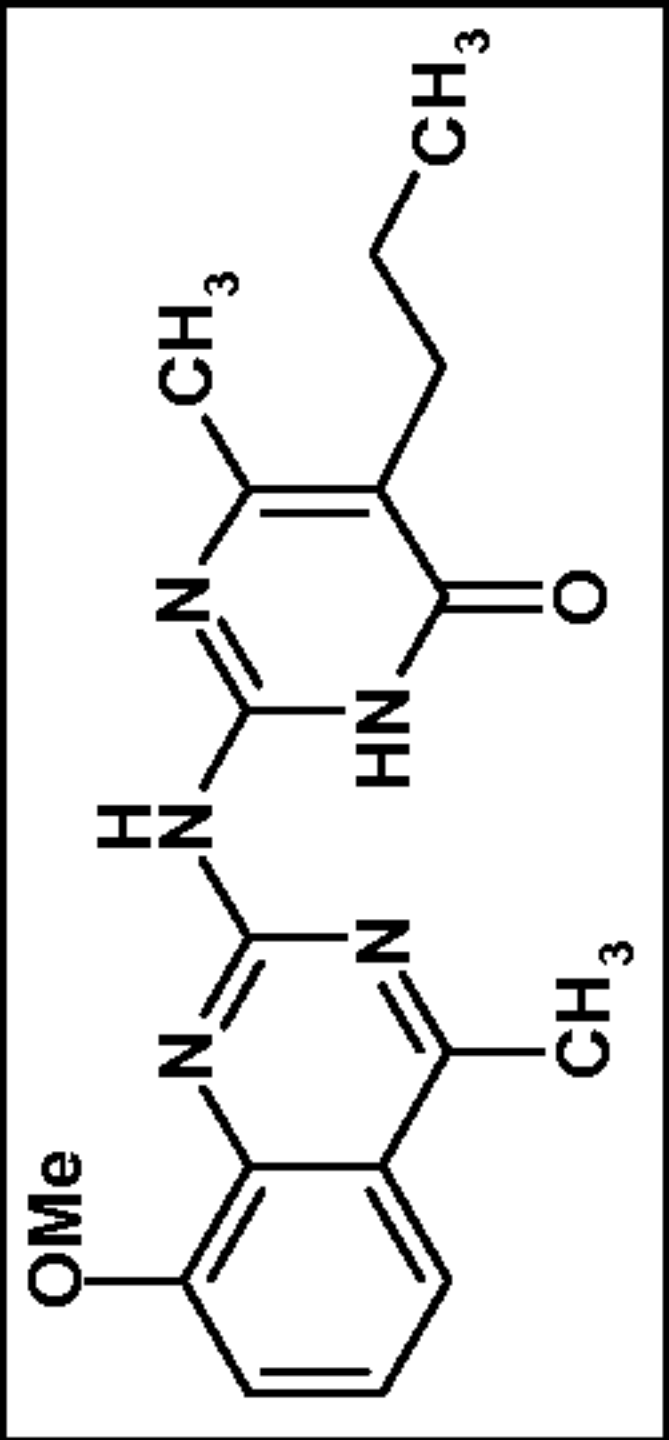
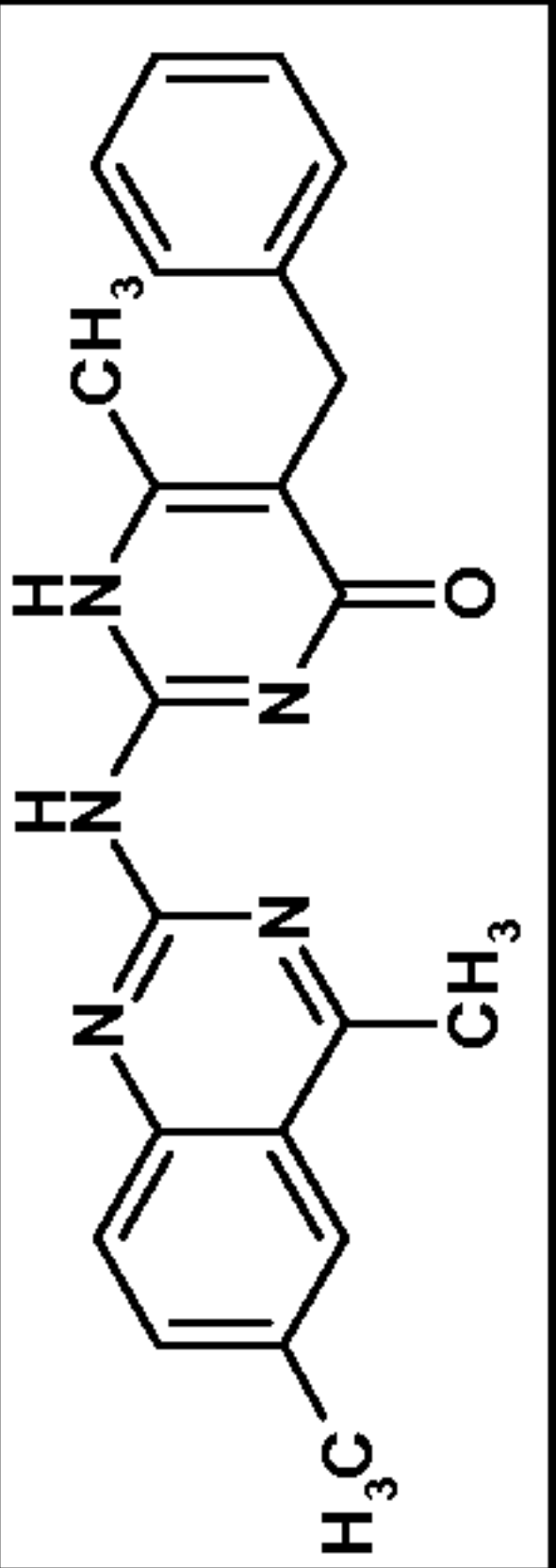
	11707 + TFA	D	-4.94	-55.26	43.70	37.31	40.51	15.70/non-convergent	
	11474	D	-4.35	-53.39	32.19	48.71	40.45	3.57/28.48(7.98)	
	11471 + TFA	D	-4.39	-57.49	37.43	42.40	39.92	36.91/44.14(1.20)	
	11712 + TFA	D	-4.74	-48.22	32.96	46.11	39.53	18.74/17.32(0.92)	
	11730 + TFA	D	-5.24	-46.64	47.15	30.67	38.91	14.48/10.05(0.69)	

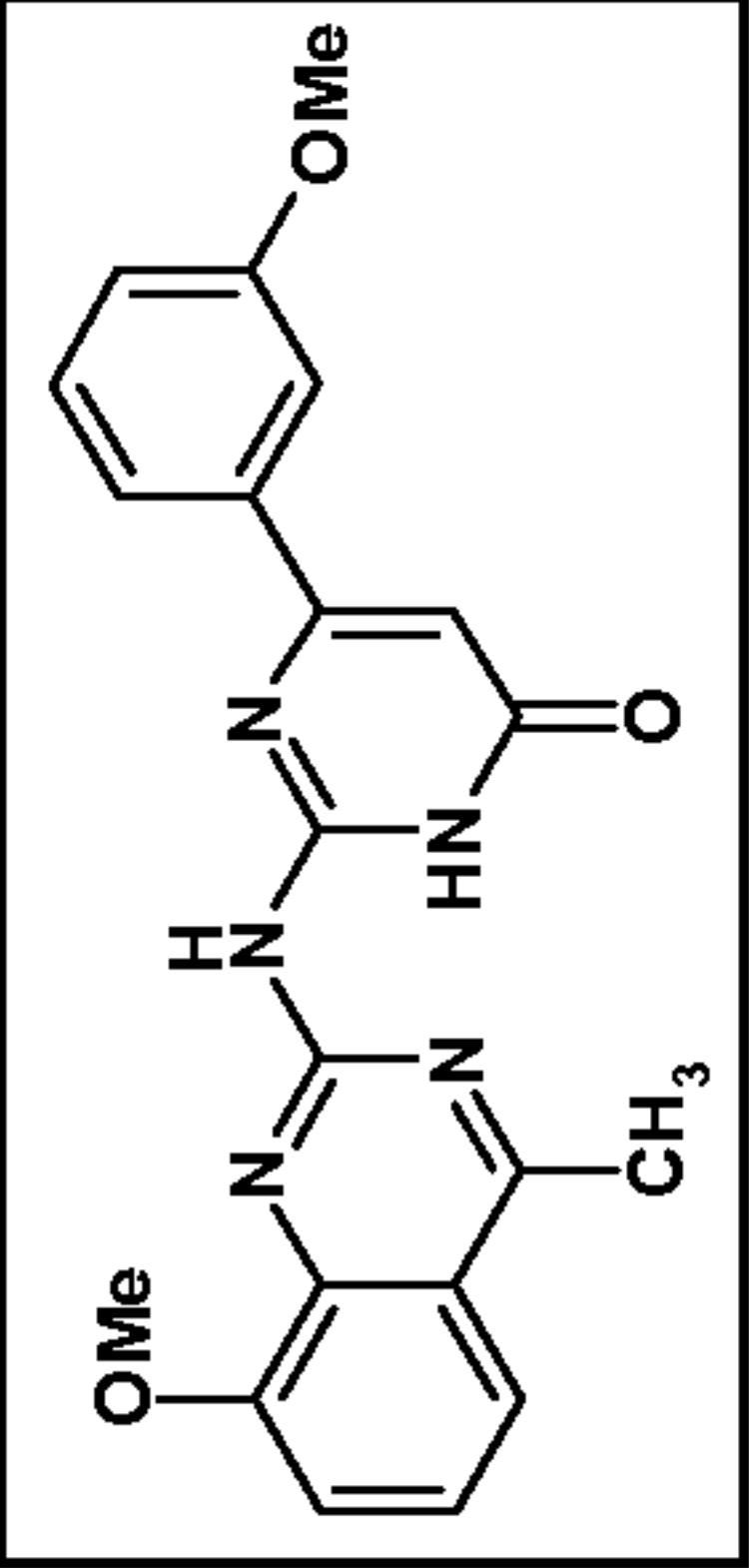
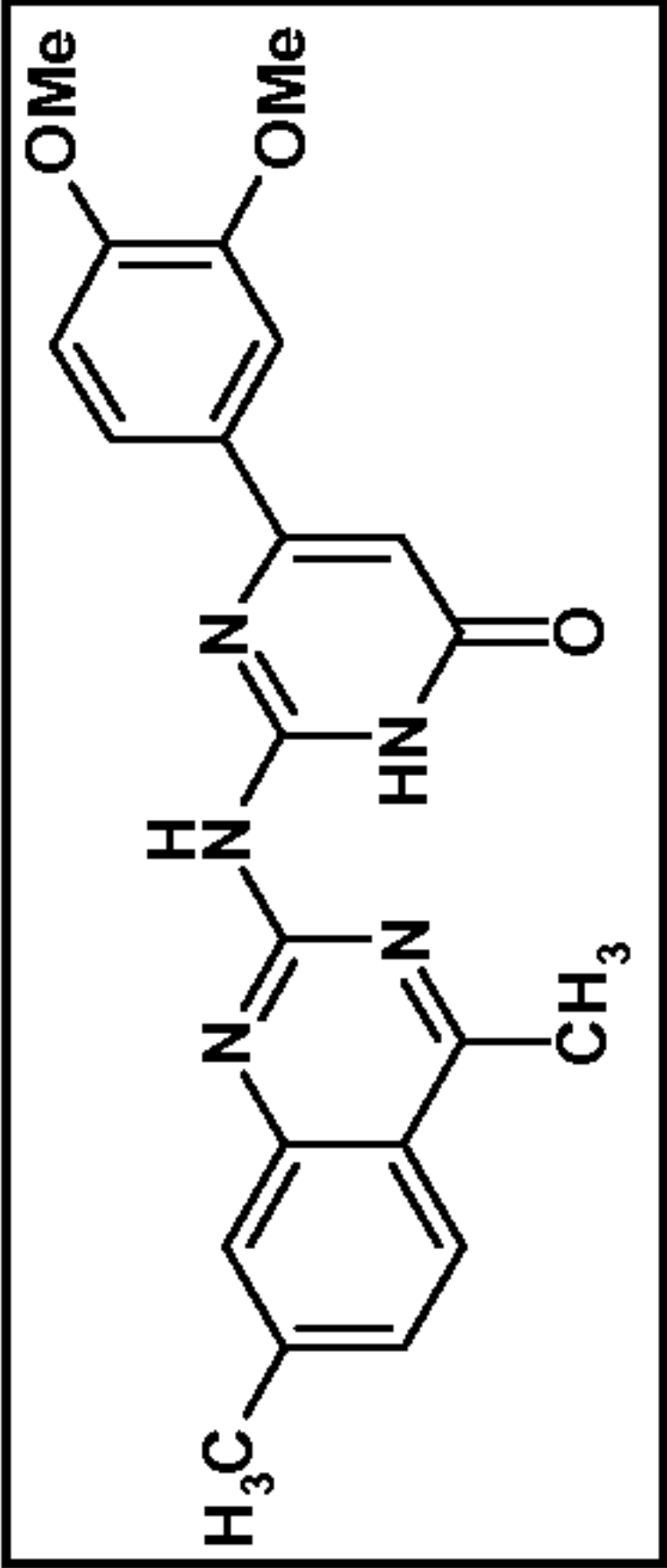
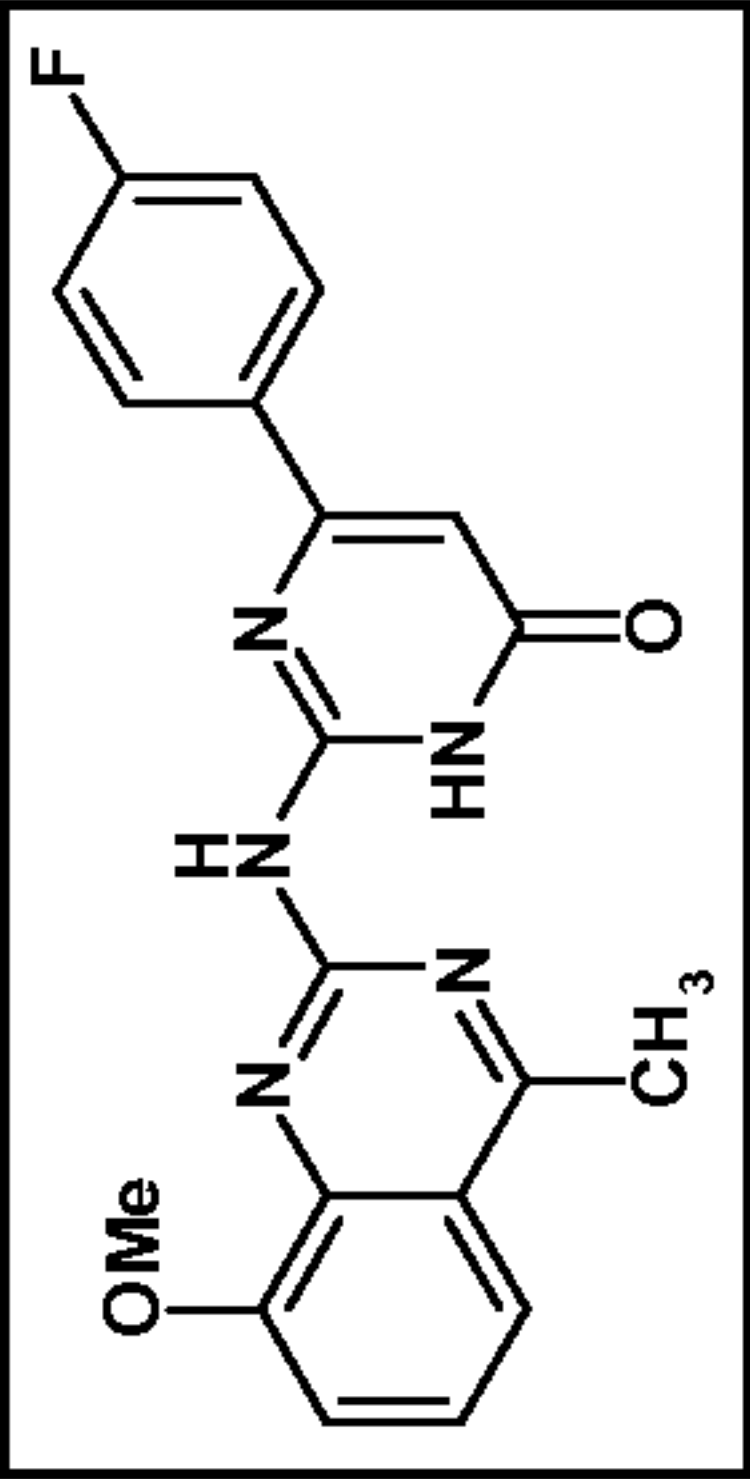
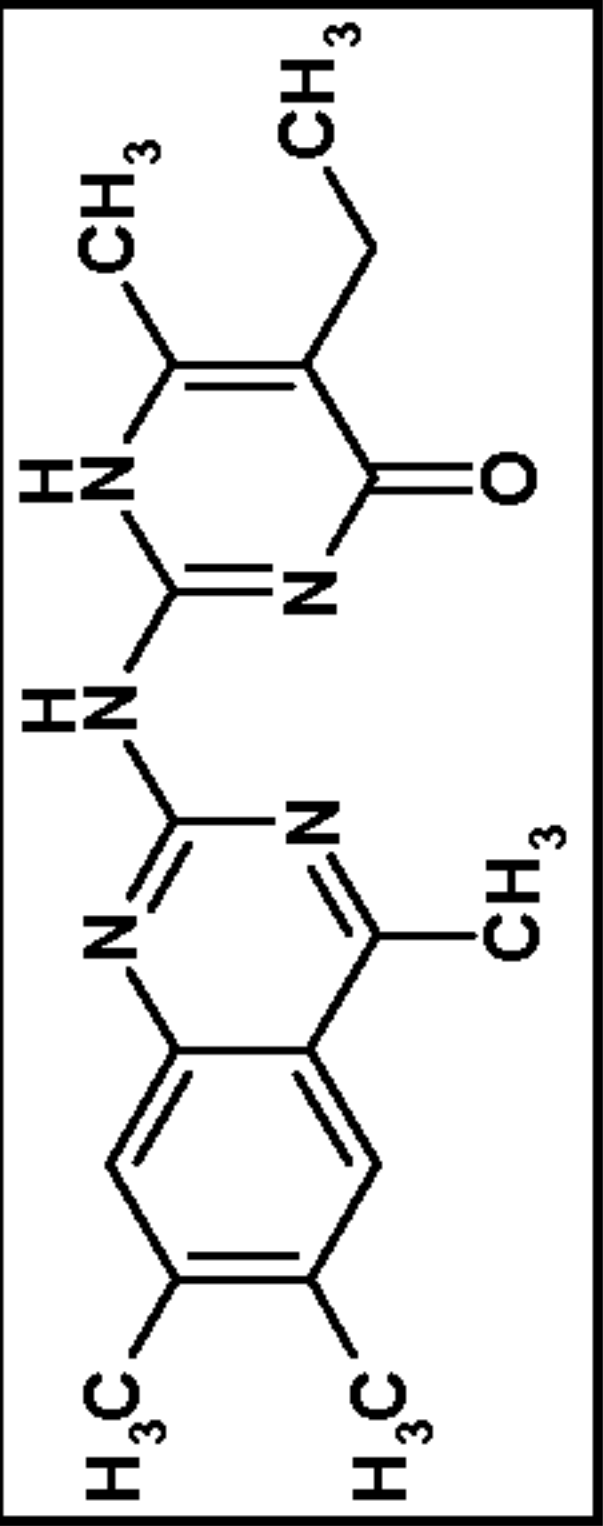
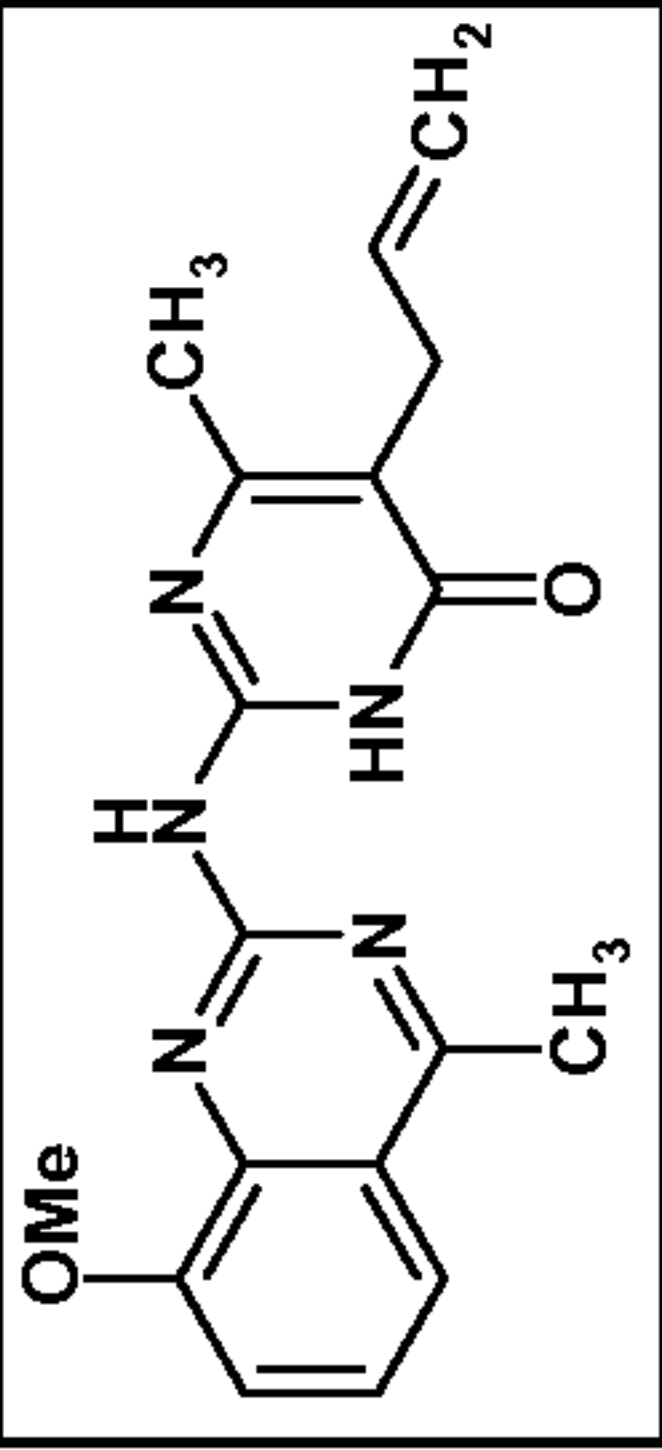
	12226 + TFA	D	-5.67	not docked	-51.38	42.24	35.23	38.74	42.50/49.01(1.15)	
	12603 + TFA	D	not docked	not docked		48.06	29.15	38.61	16.20/10.11(0.62)	
	11442	D	-5.57		-51.14	50.14	25.76	37.95	15.63/118.5	
	12778	D	-3.8		-52.22	31.05	44.08	37.56	4.66/5.34(1.15)	
	11455	E	-5.64		-40.8	48.80	25.90	37.35	10.84/23.41(2.16)	

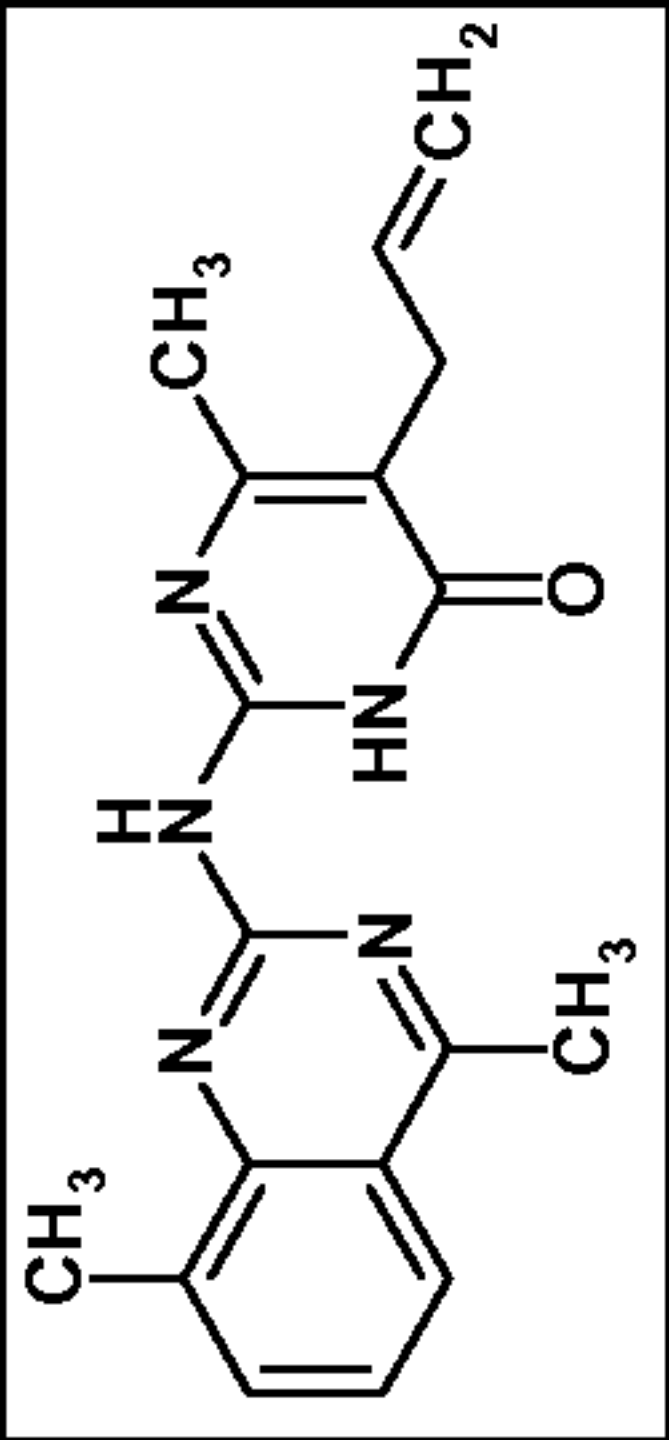
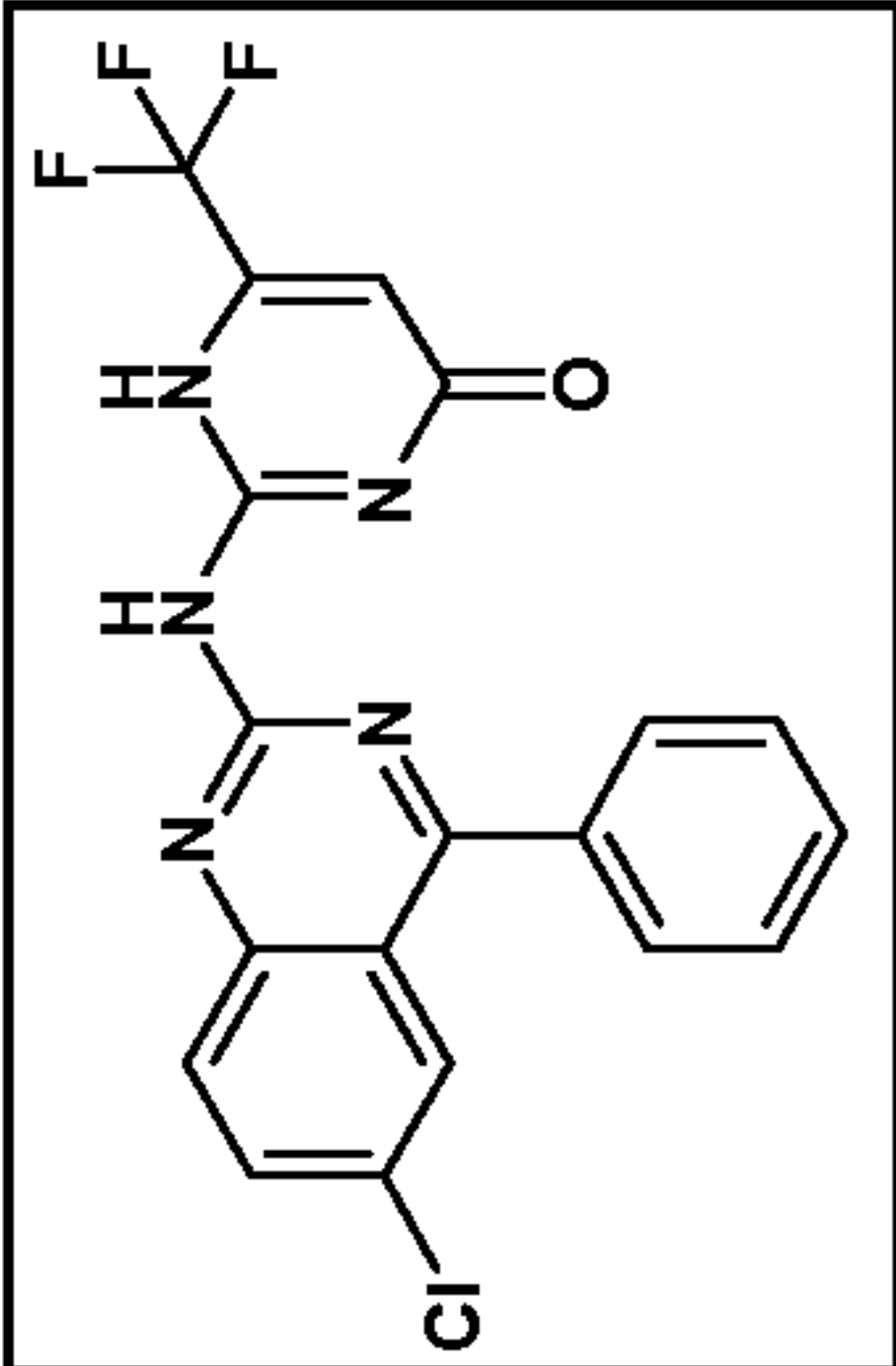
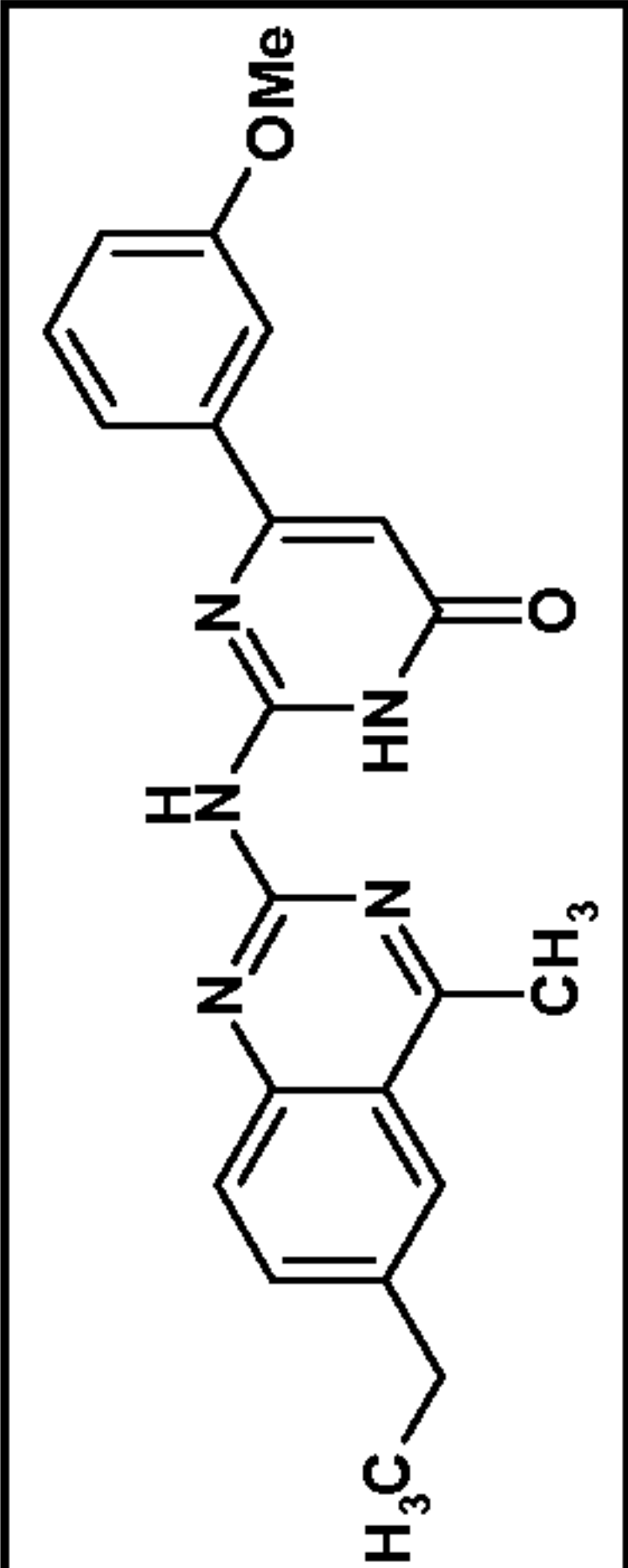
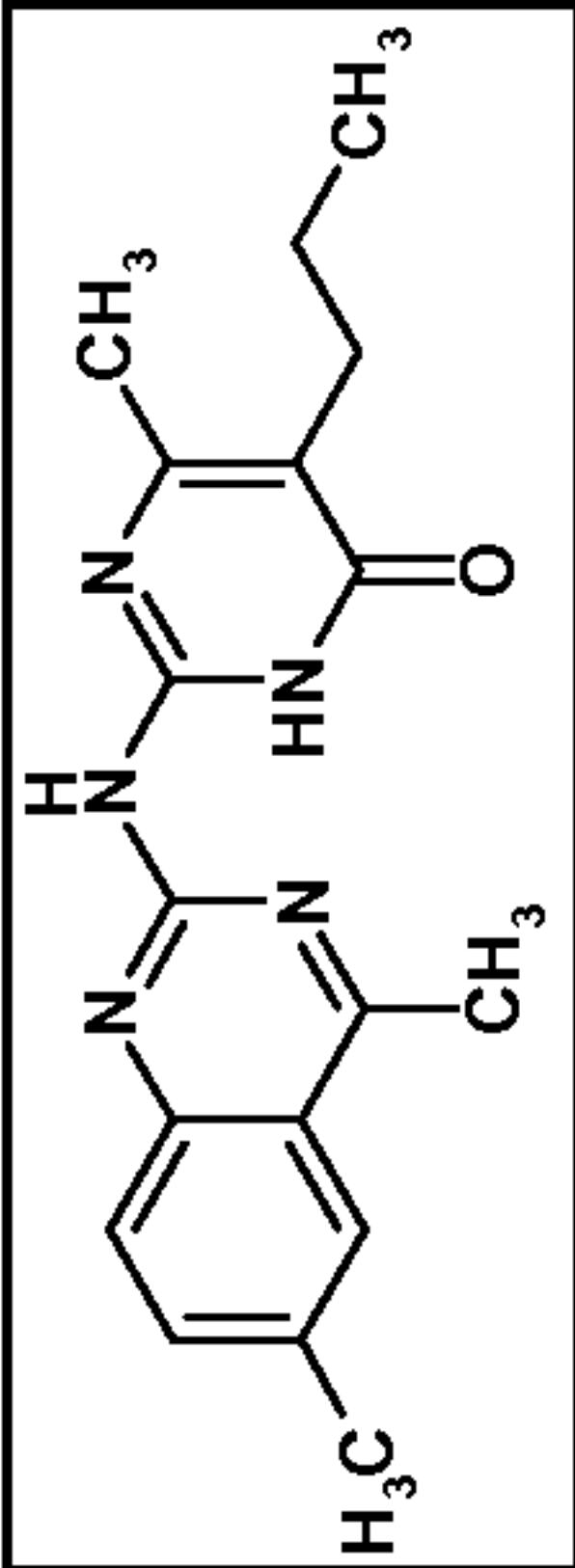
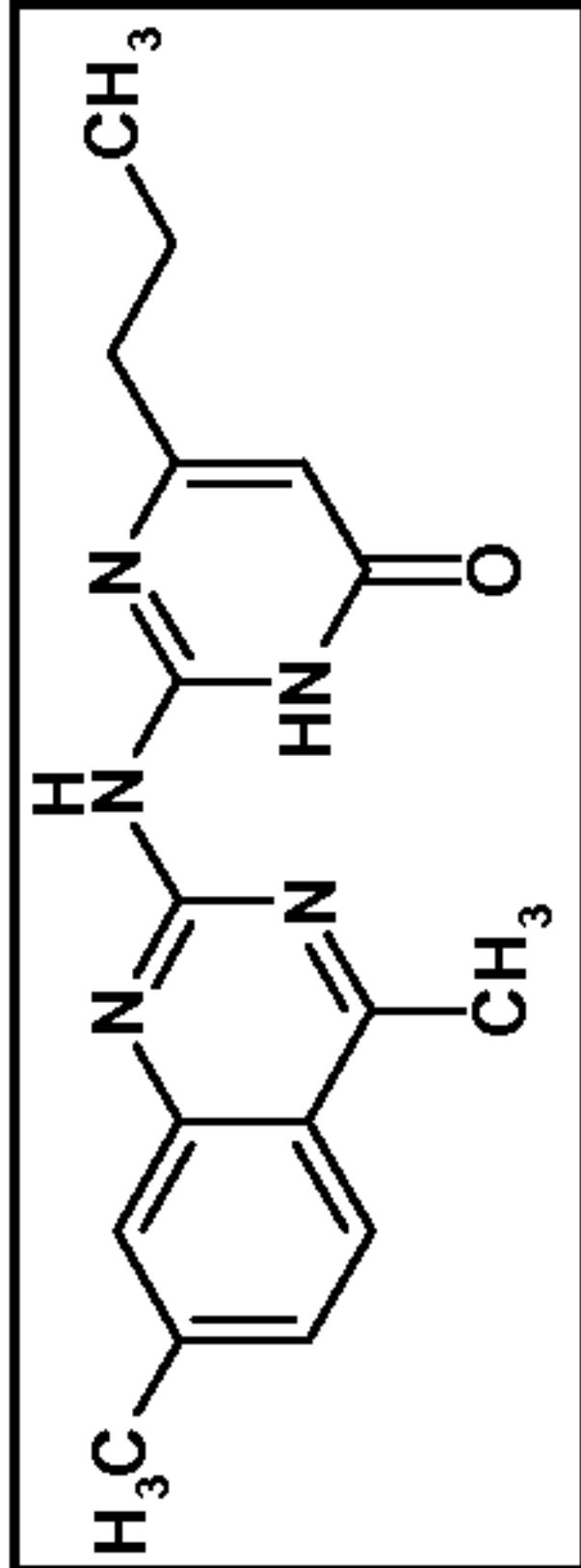
	11826 + TFA	D	-5.44	-59.4	40.52	33.80	37.16	49.43/43.30(0.88)	
	11449	E	-3.52	-42.05	48.00	26.22	37.11	6.41/5.40(0.84)	
	11755	D	-5.33	-64.06	40.96	33.21	37.09	non-convergent	14.14/3.24(0.23)
	11309	E	-5.51	-42.35	33.31	40.70	37.00		
	12666 + TFA	D	-5.29	-49.45	48.16	25.37	36.77	14.48/17.13(1.18)	

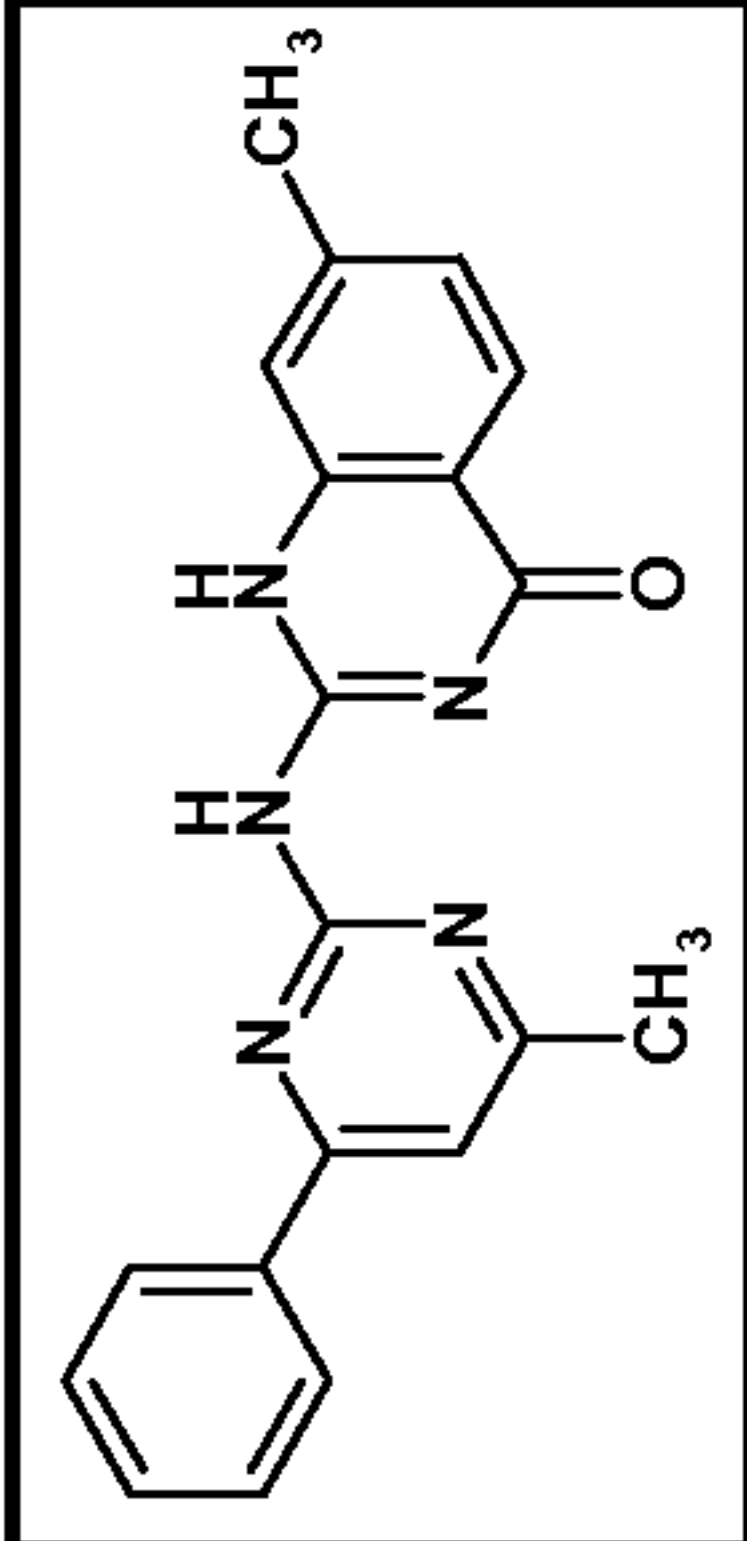
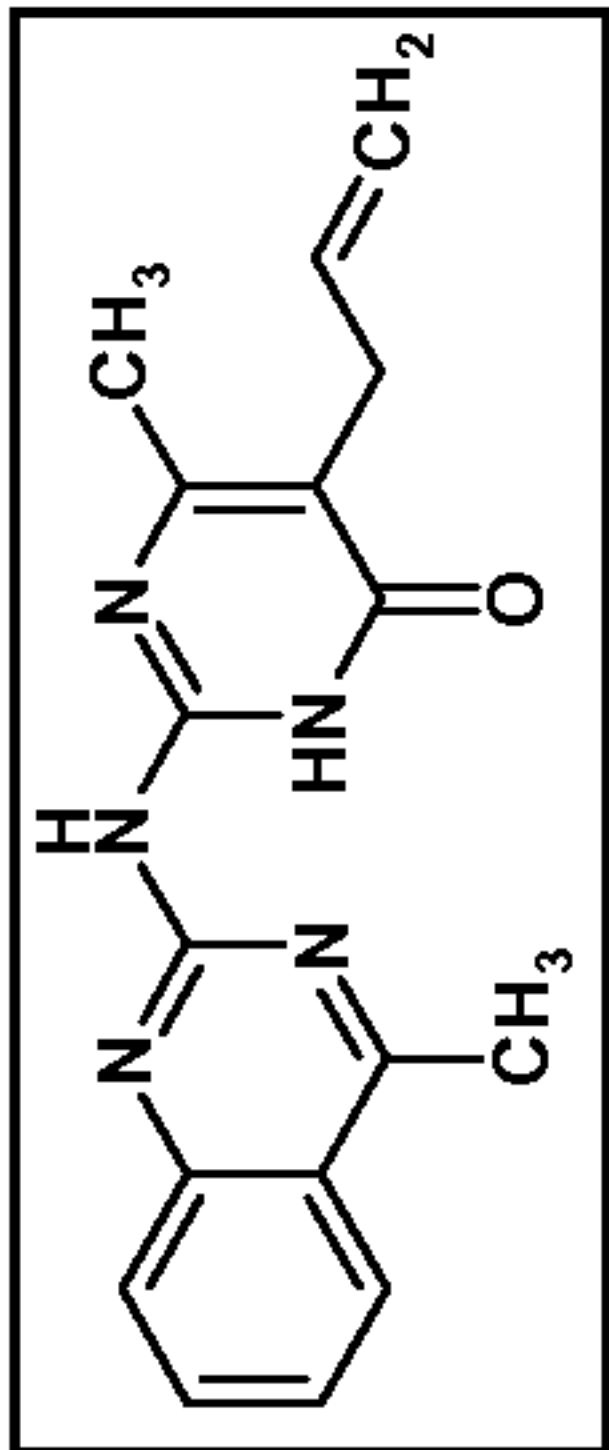
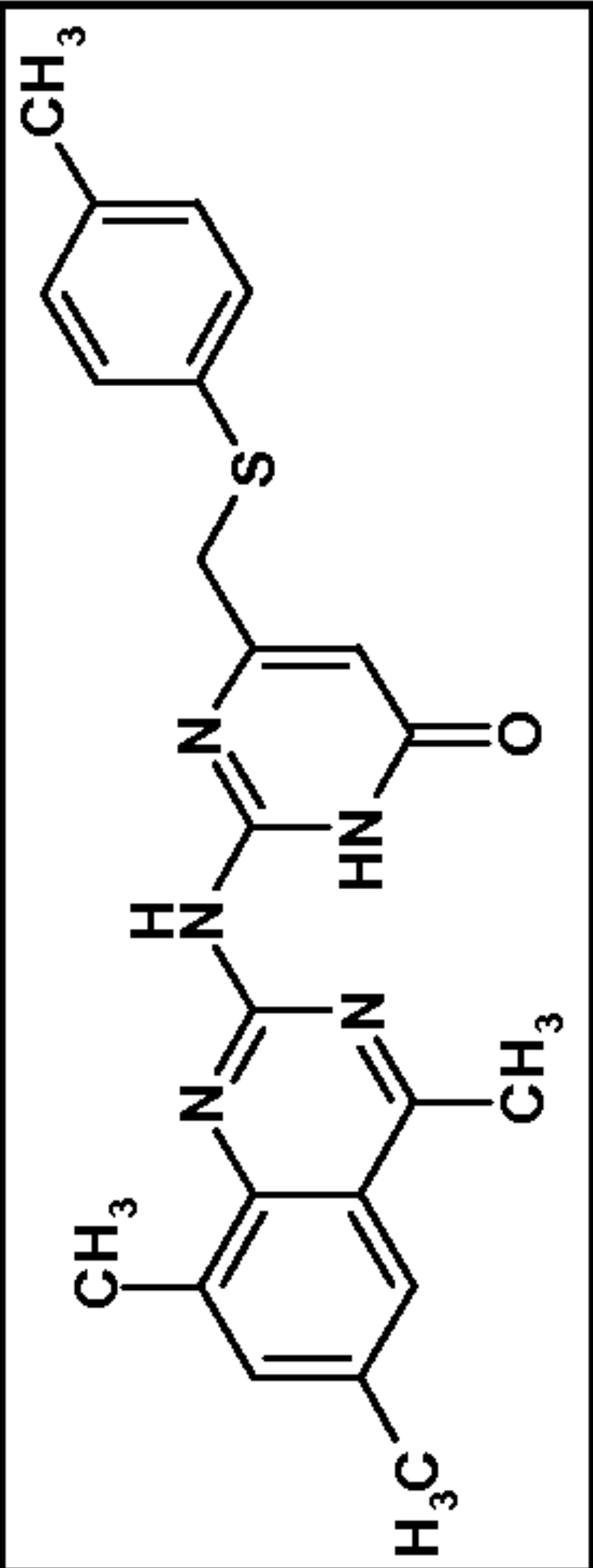
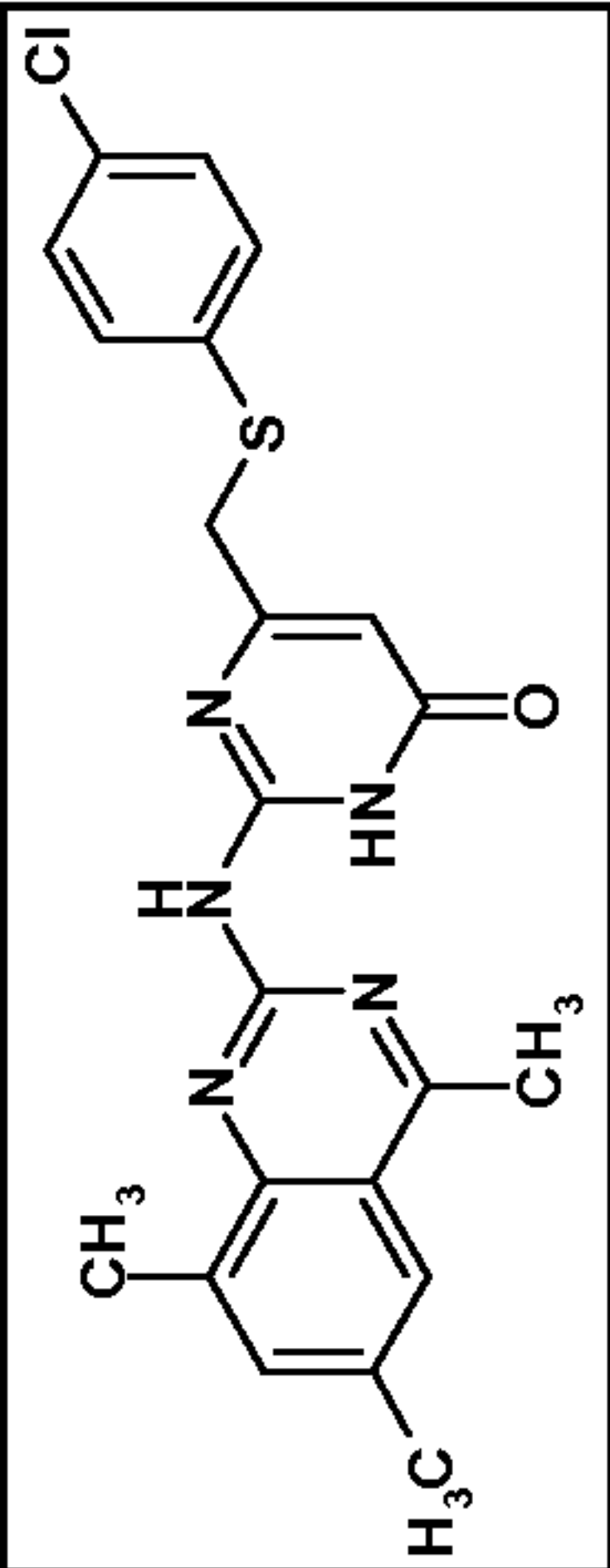
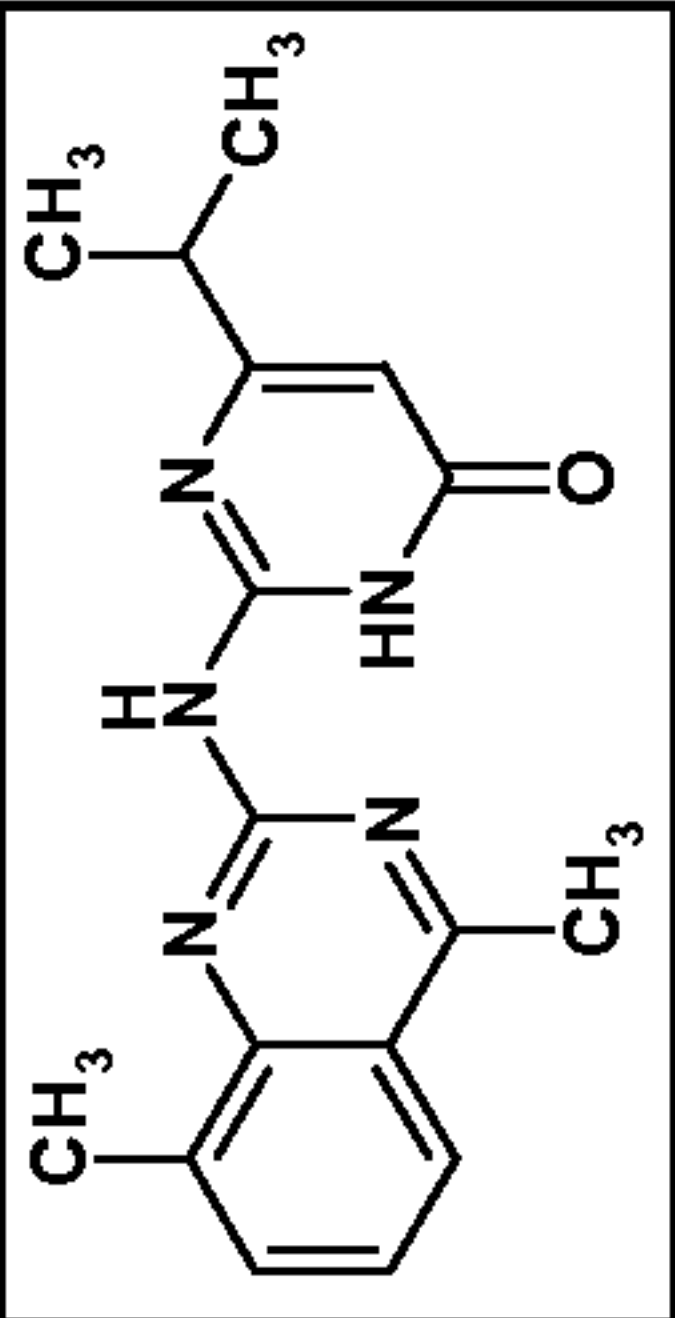
	12688 + TFA	E	-5.44	-42.43	45.86	26.95	36.40	5.04/3.58(0.71)	
	12654 + TFA	D	-5.9	-44.23	37.92	34.22	36.07	15.95/12.62(0.79)	
	11742 + TFA	D	-5.95	-57.7	33.84	37.63	35.74	16.09/non-convergent	
	11456	D	-5.61	-49.17	33.74	37.33	35.54	5.84/5.48(0.94)	
	11785 + TFA	D	-3.71	-54.57	41.06	30.01	35.54	10.35/9.15(0.88)	24.82/25.91(1.04)

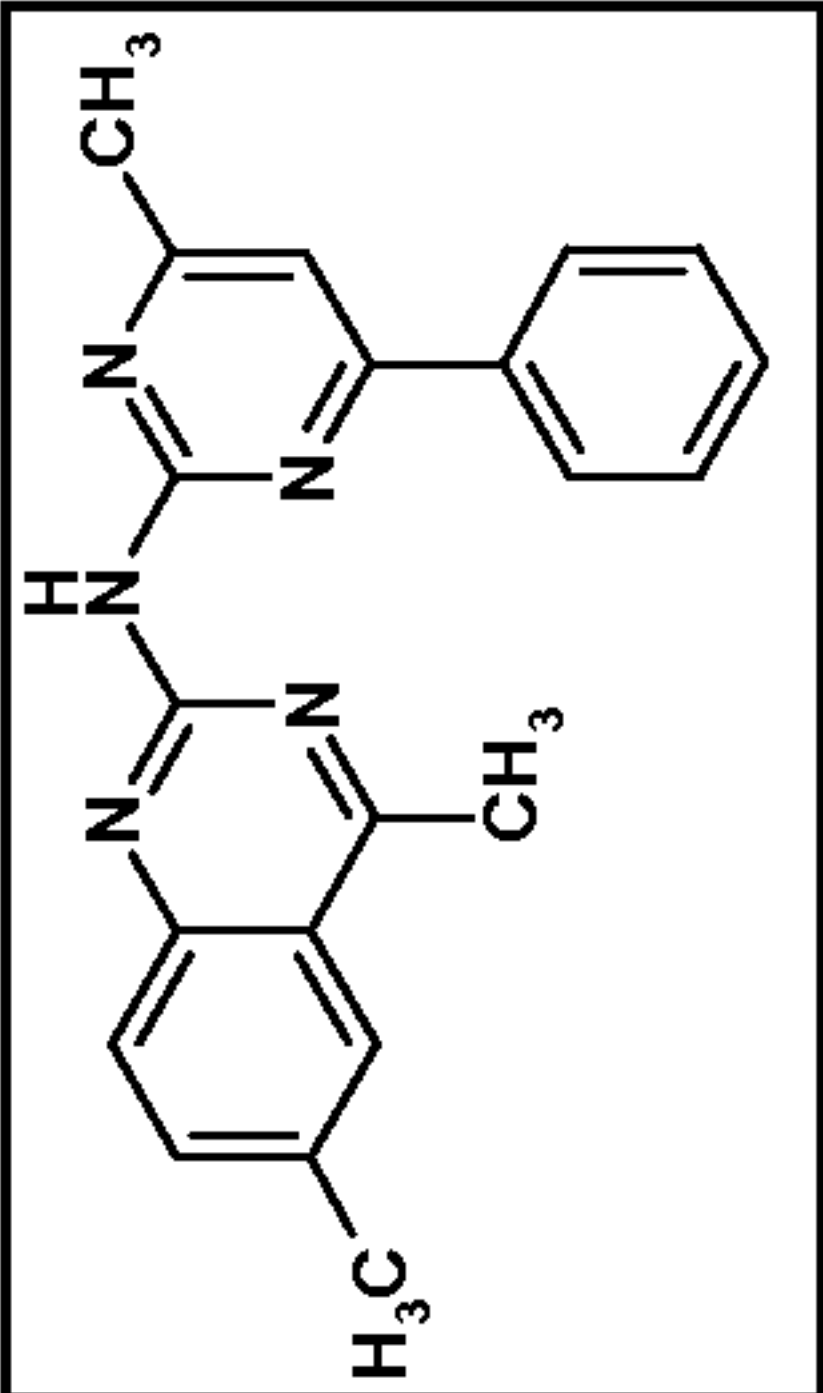
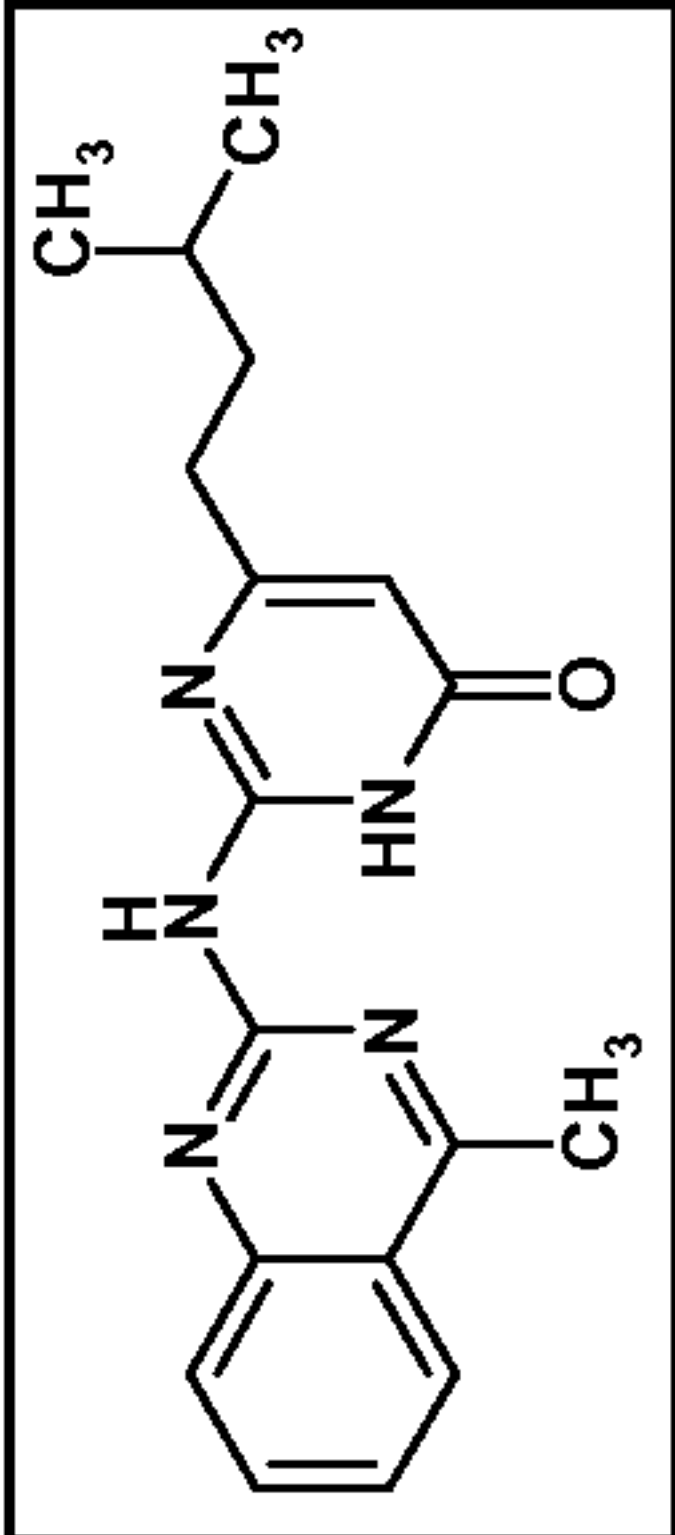
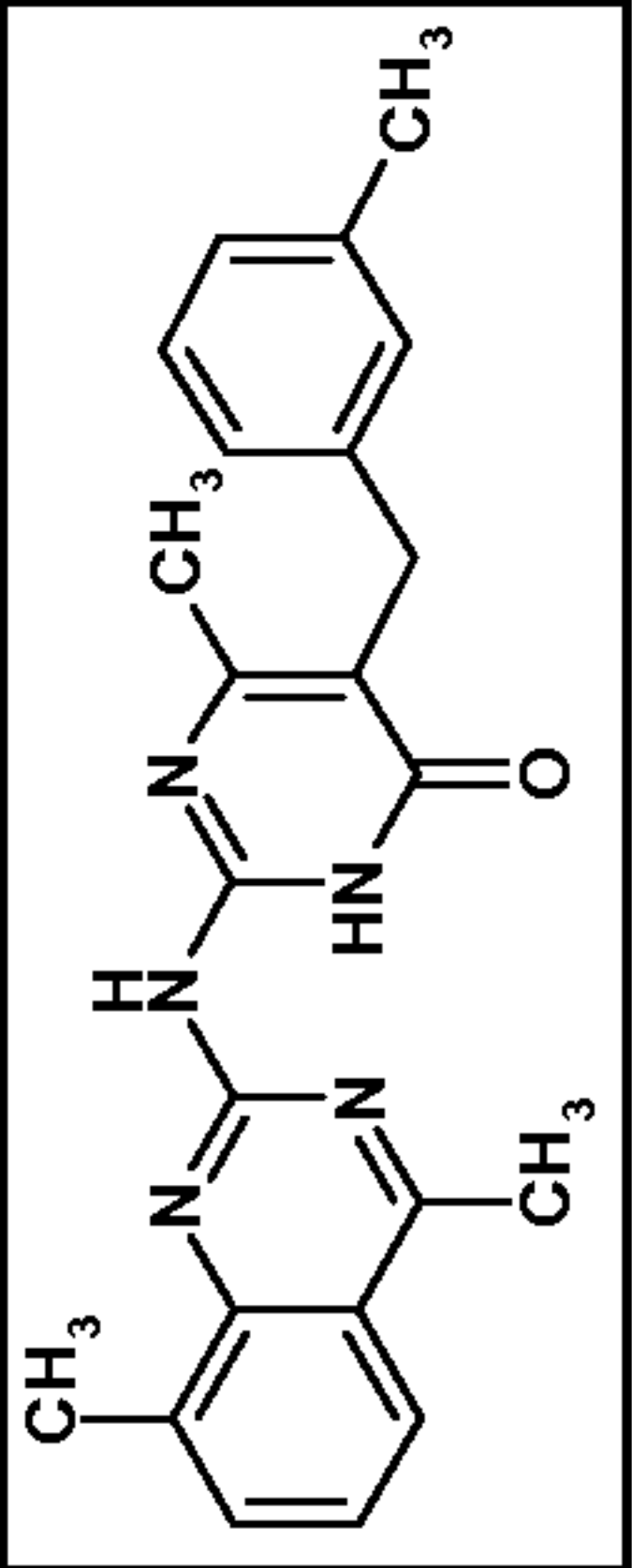
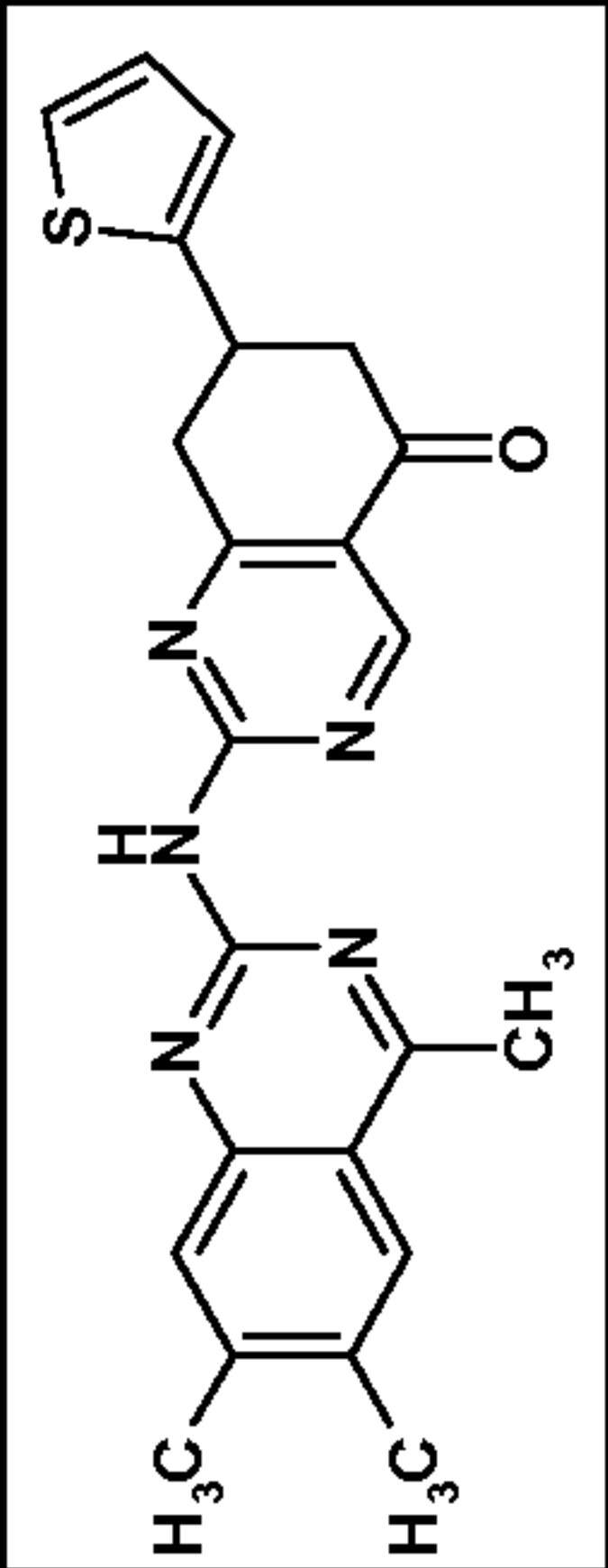
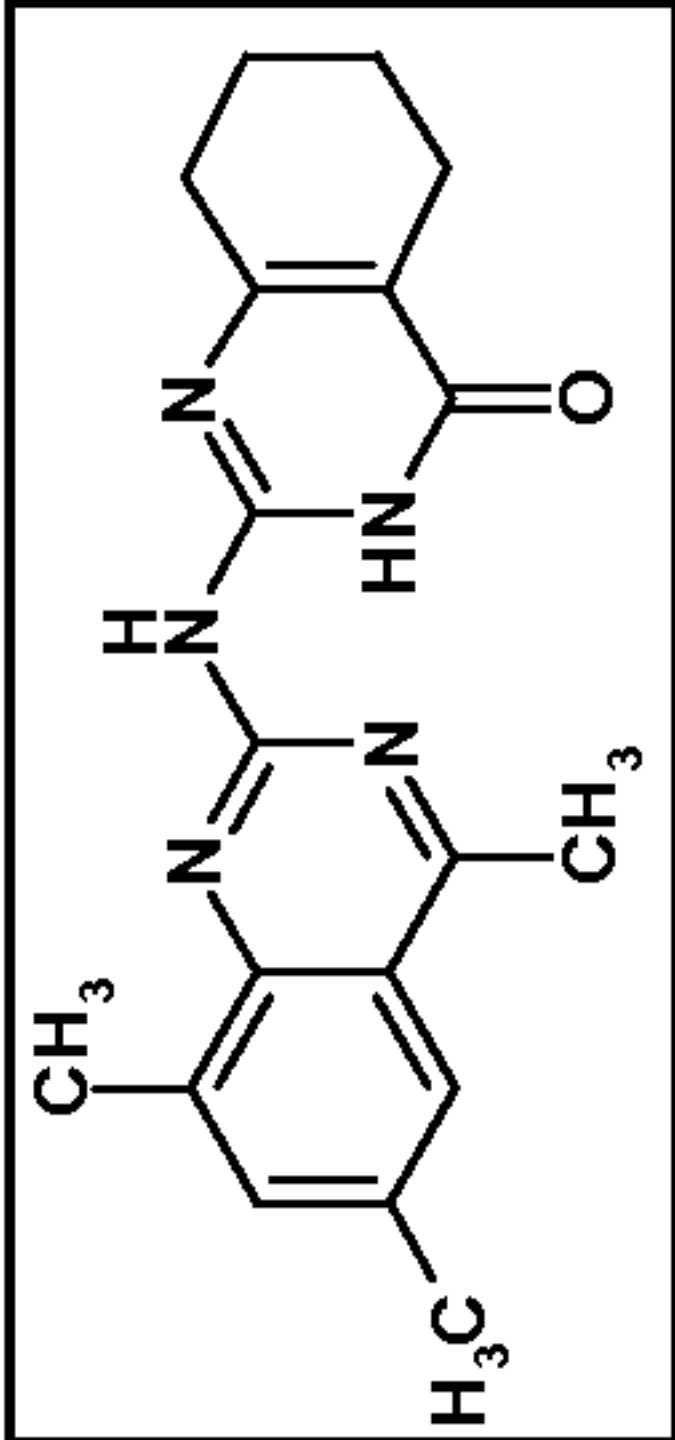
	12661	D	-4.69	-40.42	29.34	41.14	35.24	43.15/38.19(0.89)	
	12214 + TFA	D	-5.35	-48.28	42.29	27.52	34.91	32.43/45.47(1.40)	
	11706 + TFA	F	-1.8	-49.31	37.65	30.99	34.32	17.60/25.45(1.45)	
	12625 + TFA	D	-5.41	-45.41	30.45	38.10	34.27	21.20/17.03(0.80)	
	12739	D	-5.22	-42.52	28.51	39.54	34.03	15.90/9.17(0.58)	

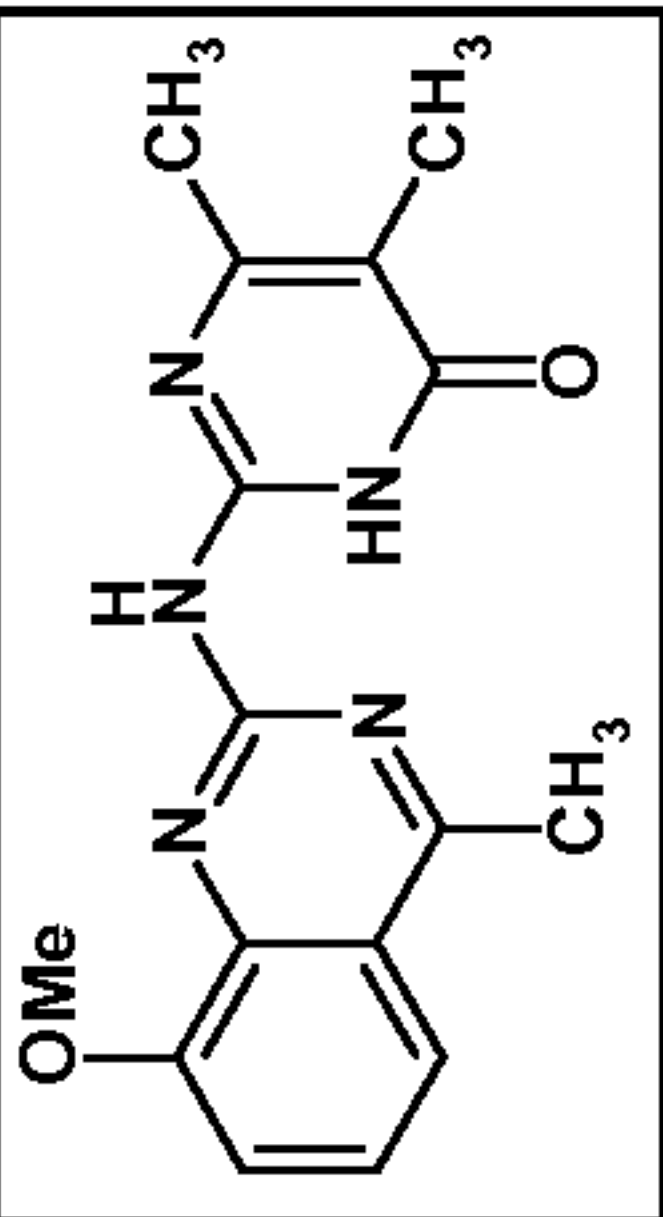
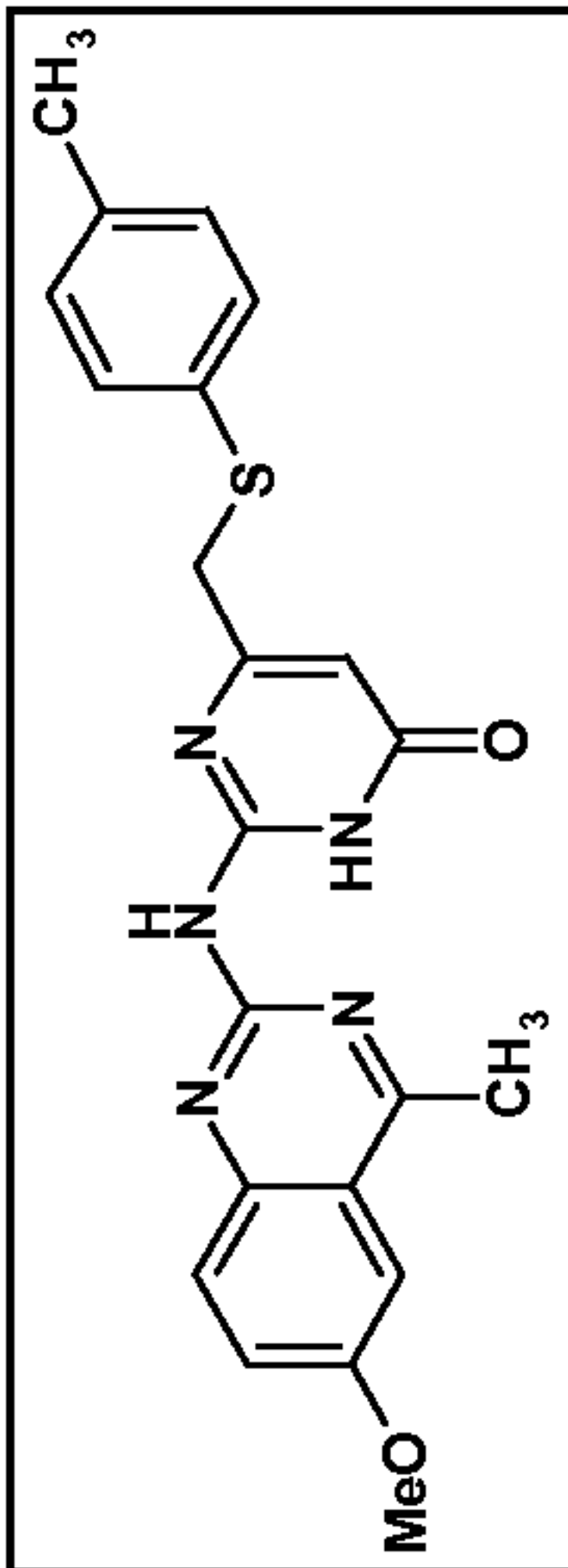
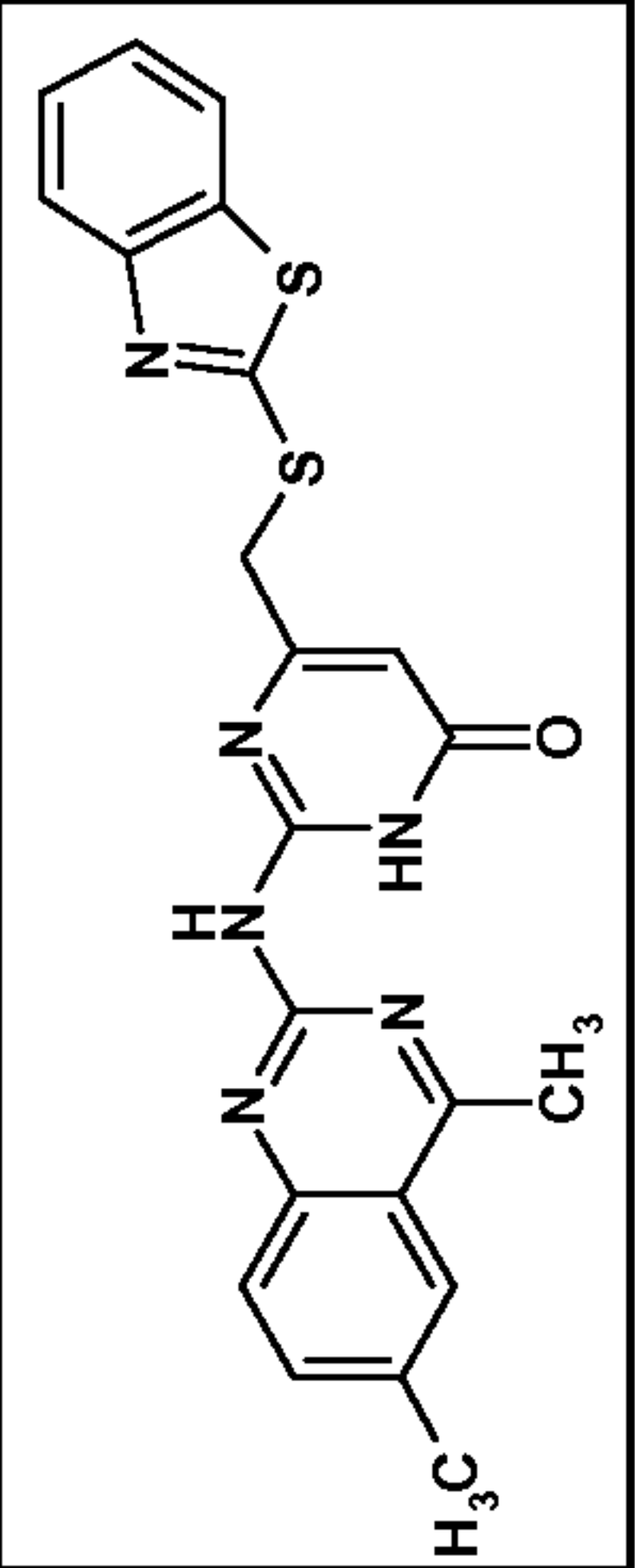
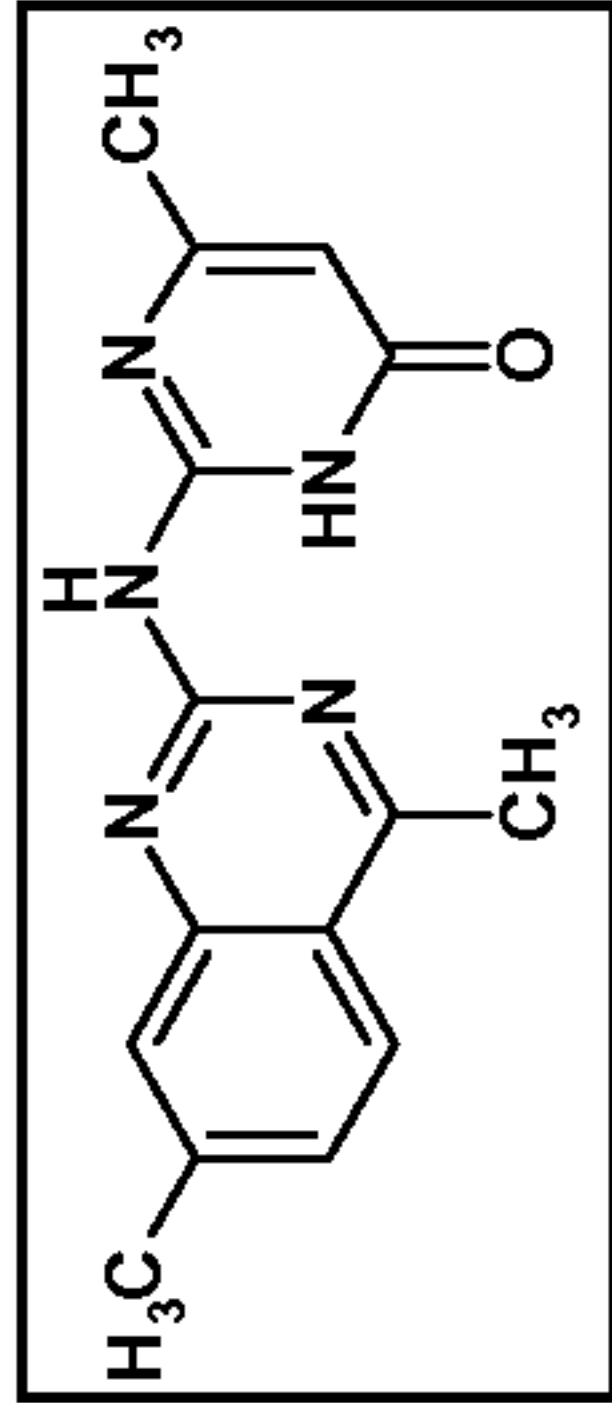
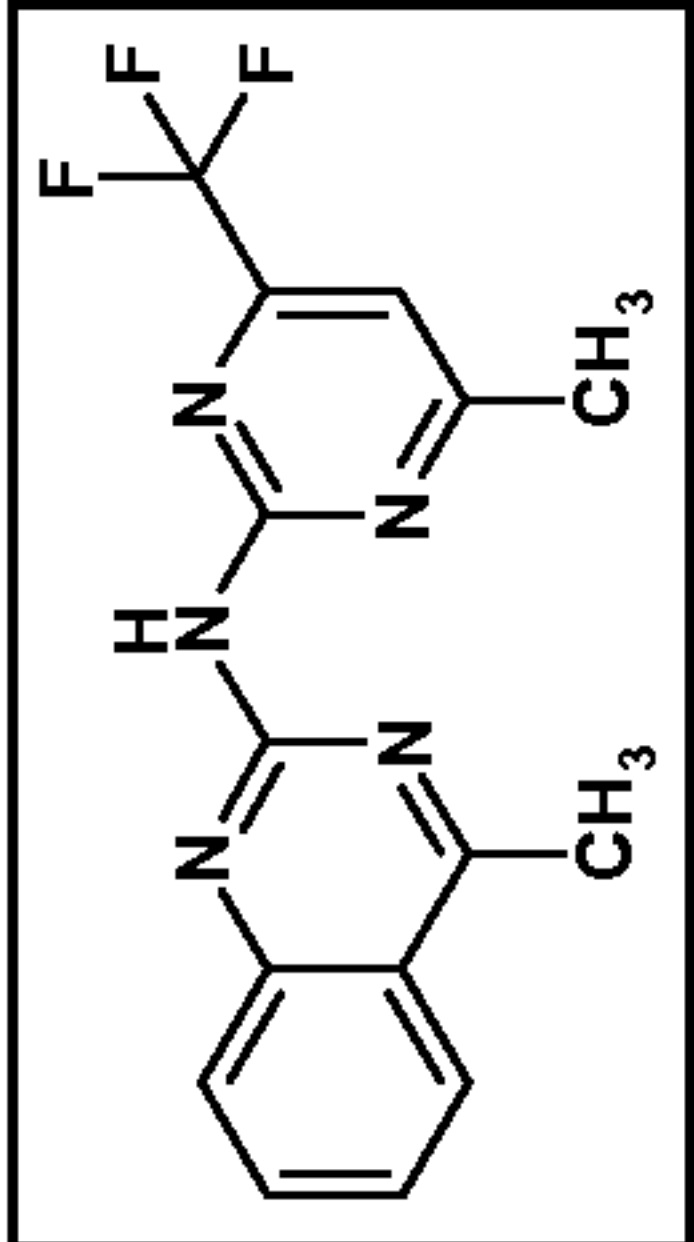
	11476	D	-5.09	-57.08	36.71	31.11	33.91	17.86/13.29(0.74)	
	12741 + TFA	D	-5.68	-44.51	42.10	25.04	33.57	63.20/28.04(0.44)	
	11795 + TFA	D	-4.61	-48.34	35.23	31.81	33.52	26.80/12.87(0.48)	
	11773 + TFA	D	-5.37	-48.14	43.74	23.30	33.52	39.36/20.99(0.53)	
	11294	D	-6.17	-45.01	29.81	37.13	33.47	6.43/19.78(3.08)	

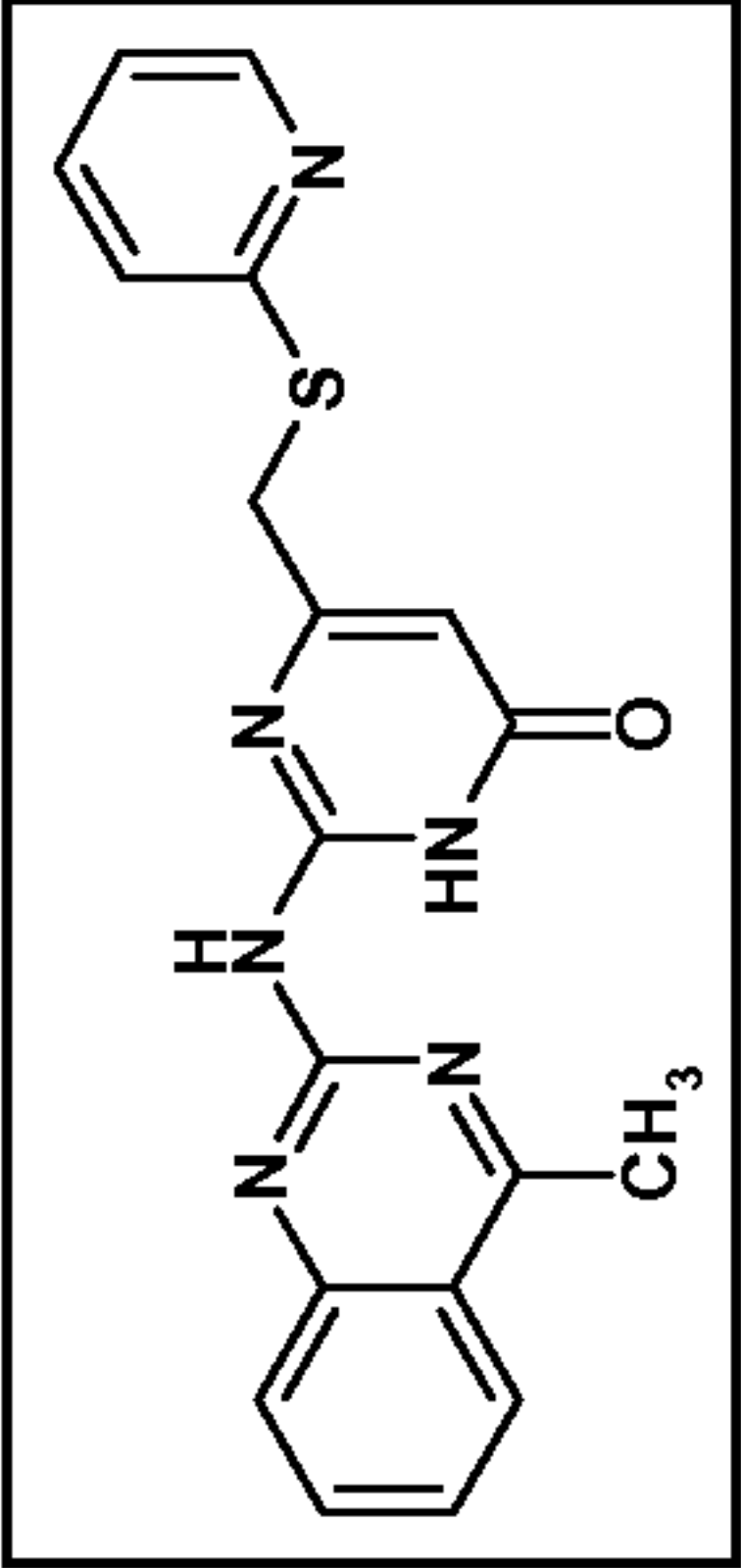
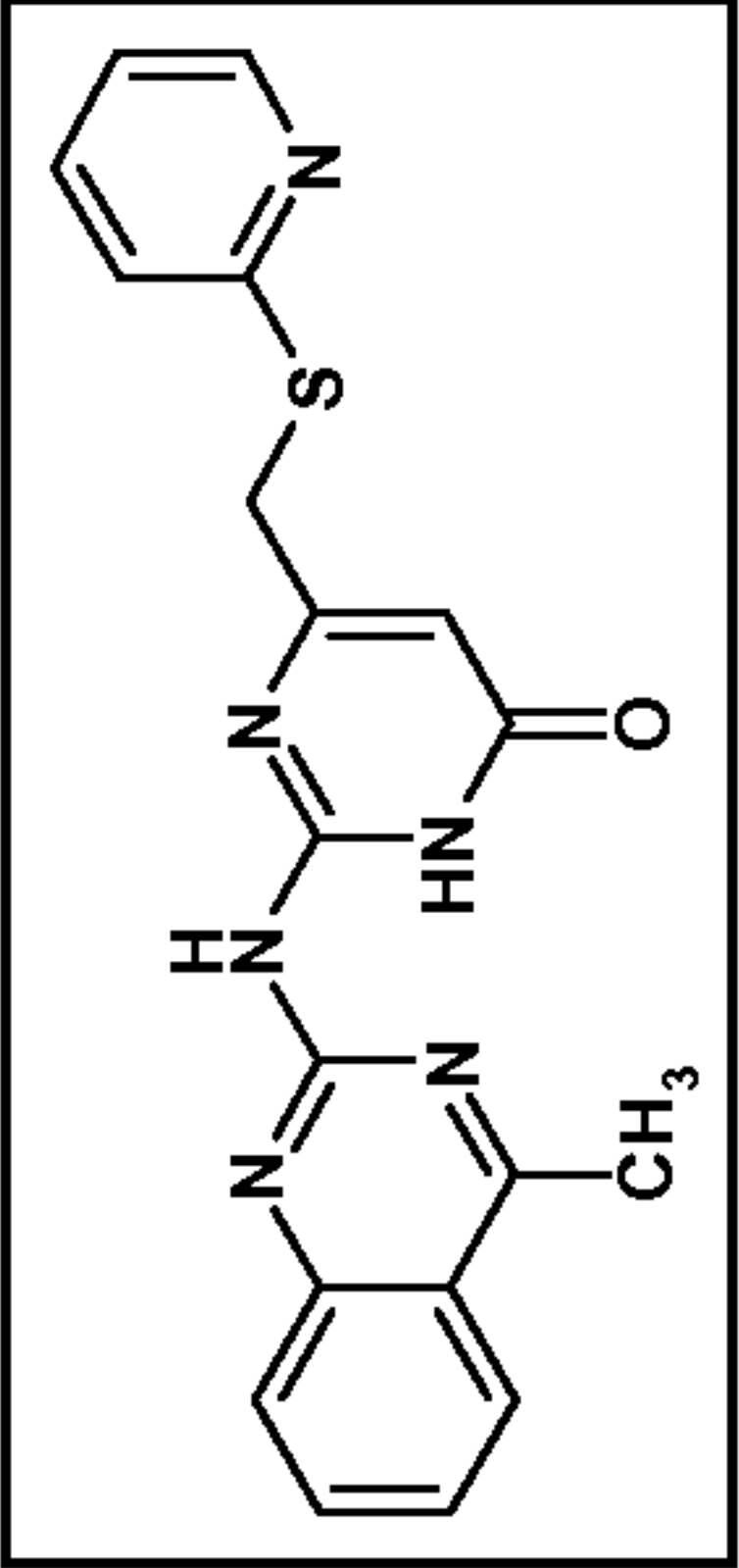
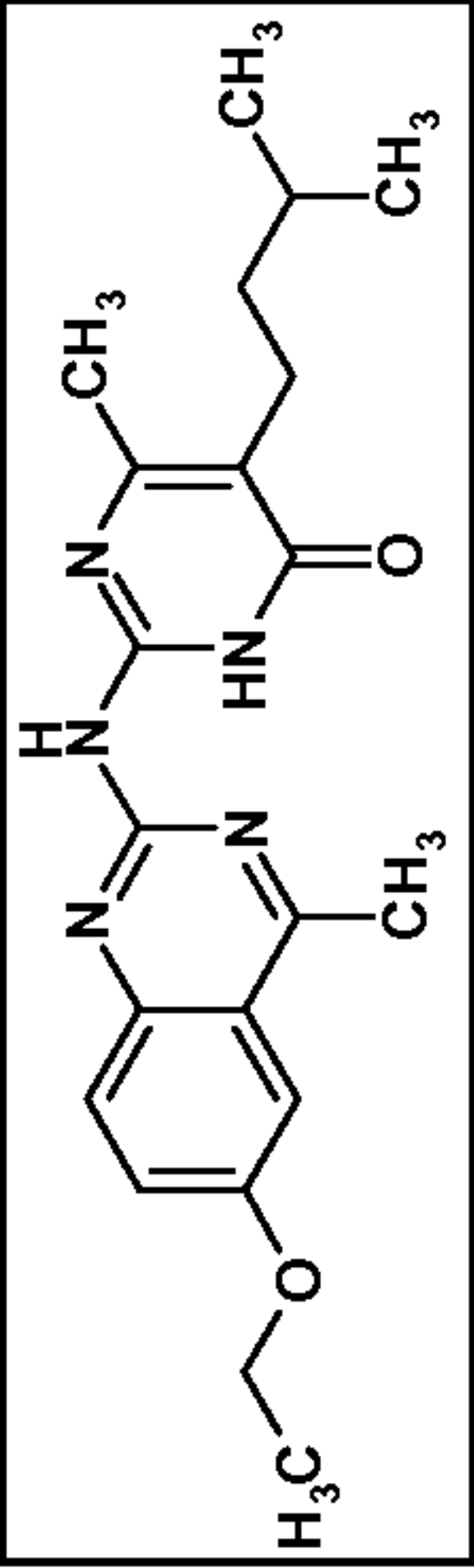
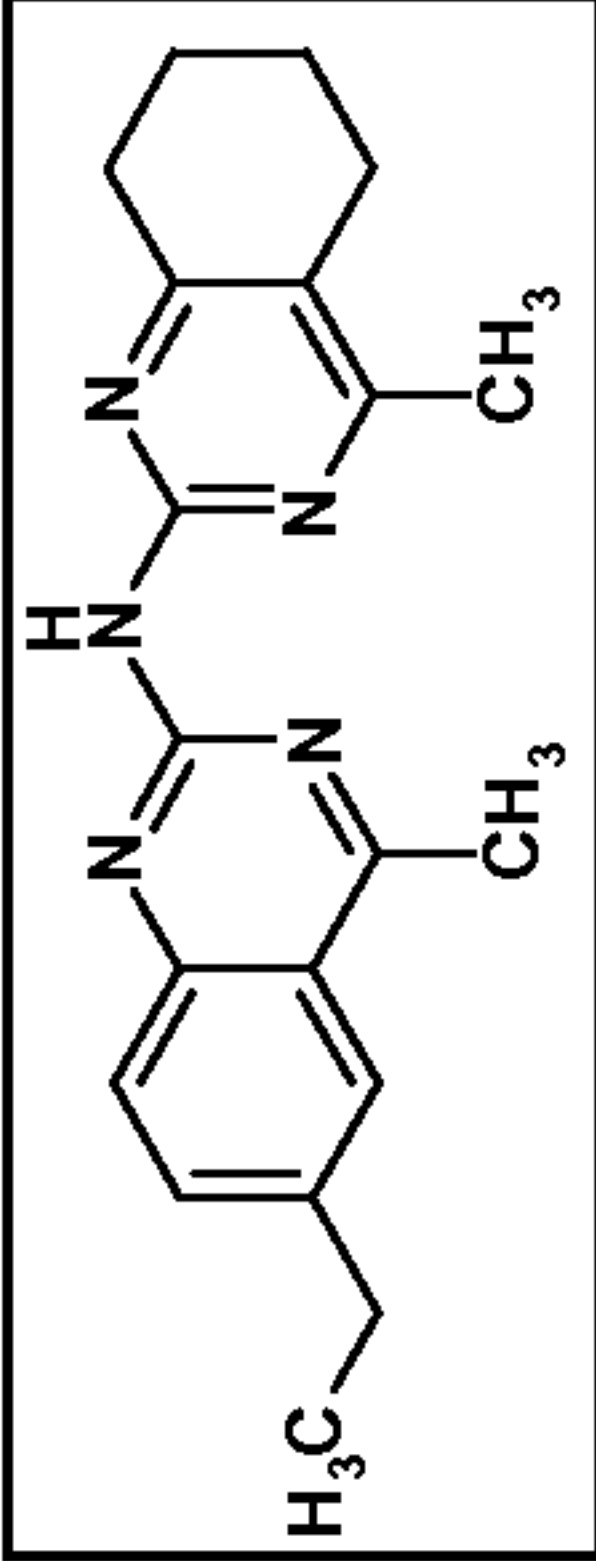
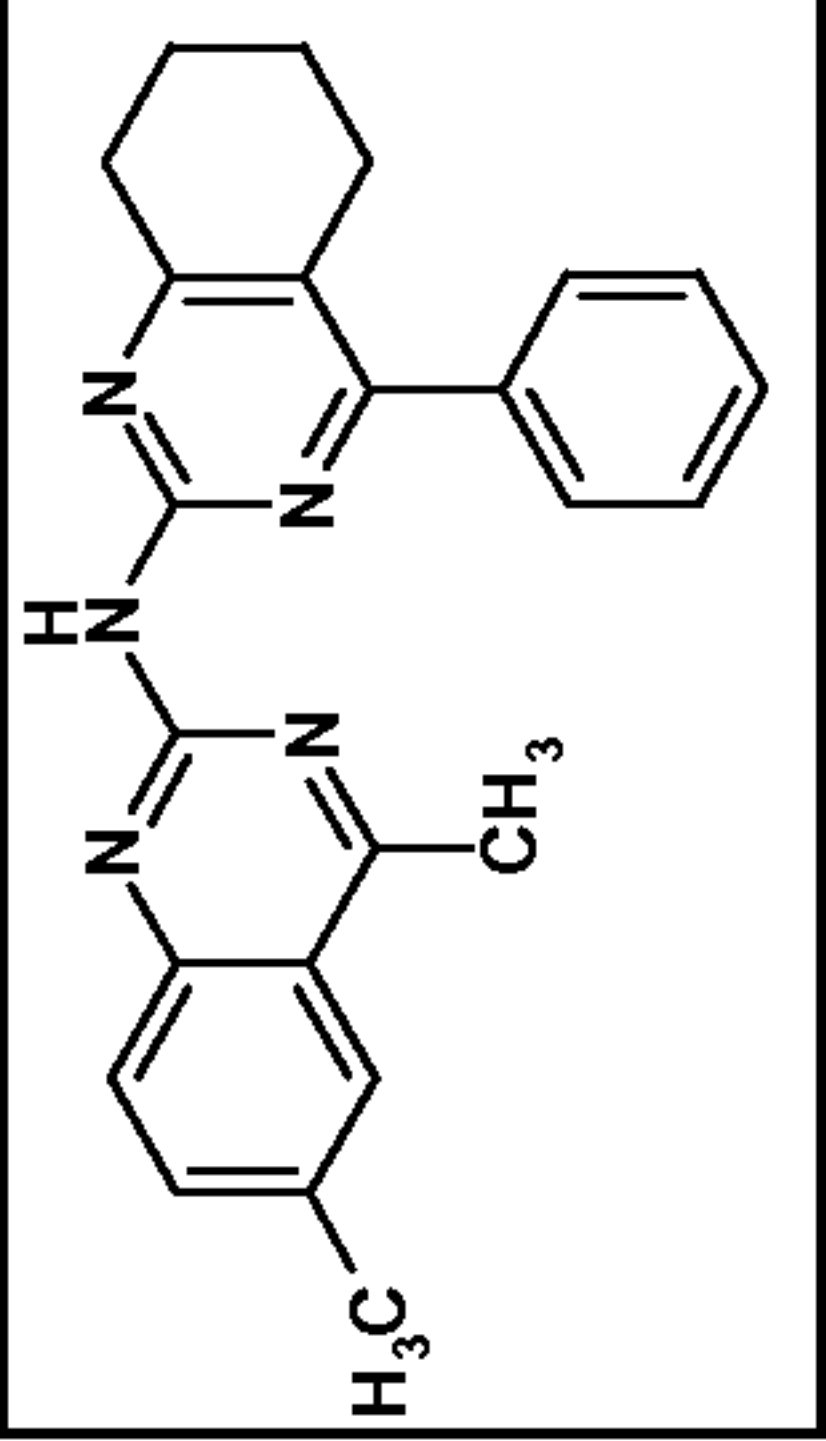
	11513 + TFA	D	-5.96	-56.74	32.64	34.26	33.45	6.73/6.32(0.94)	
	11800 + TFA	D	-4.31	-58.11	34.26	32.02	33.14	6.71/5.86(0.87)	
	12620	D	-4.98	-47.62	32.26	33.77	33.01	5.17/5.30(1.03)	
	11809 + TFA	D	-6.46	-44.94	31.42	34.02	32.72	39.28/10.88(0.28)	
	12746	D	-5.22	-48.45	18.02	47.28	32.65	17.86/11.89(0.67)	

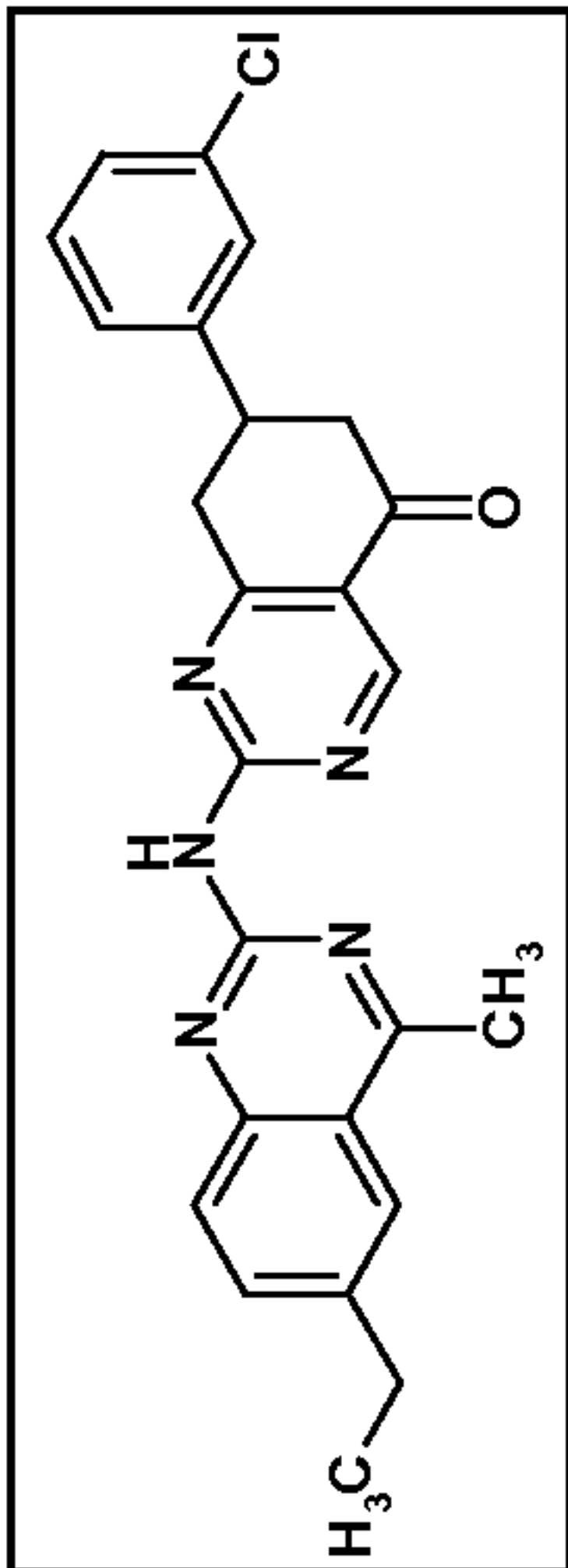
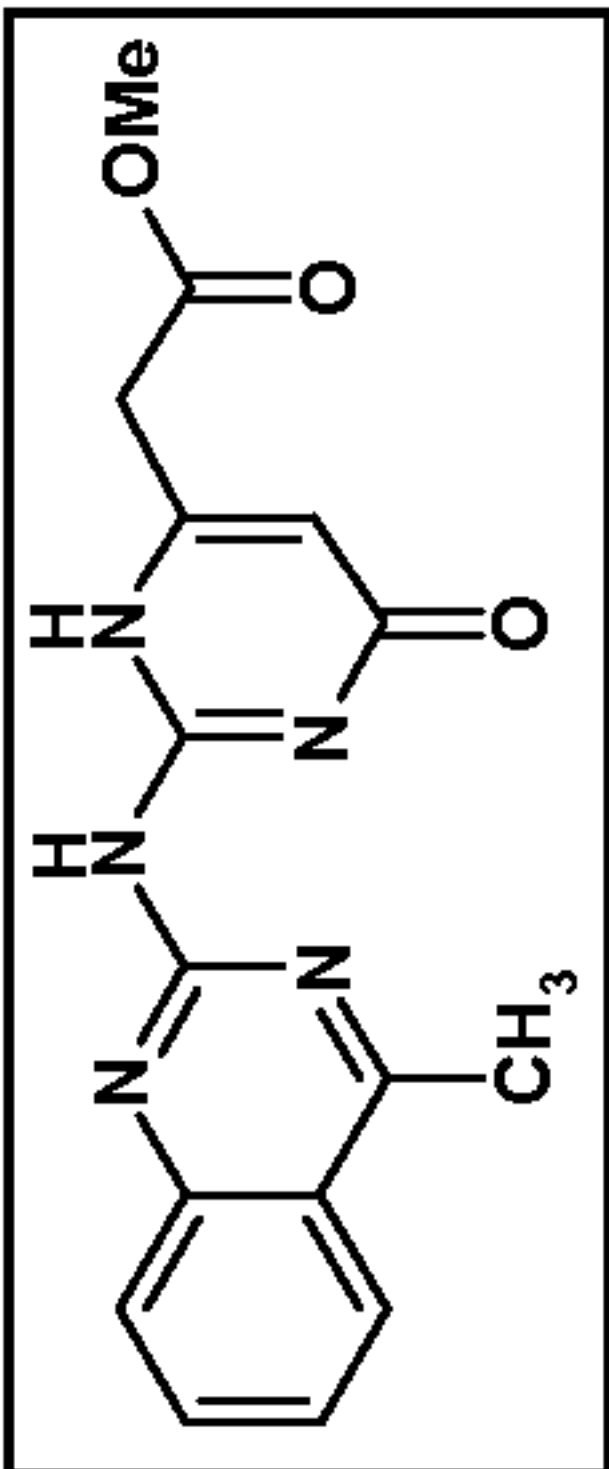
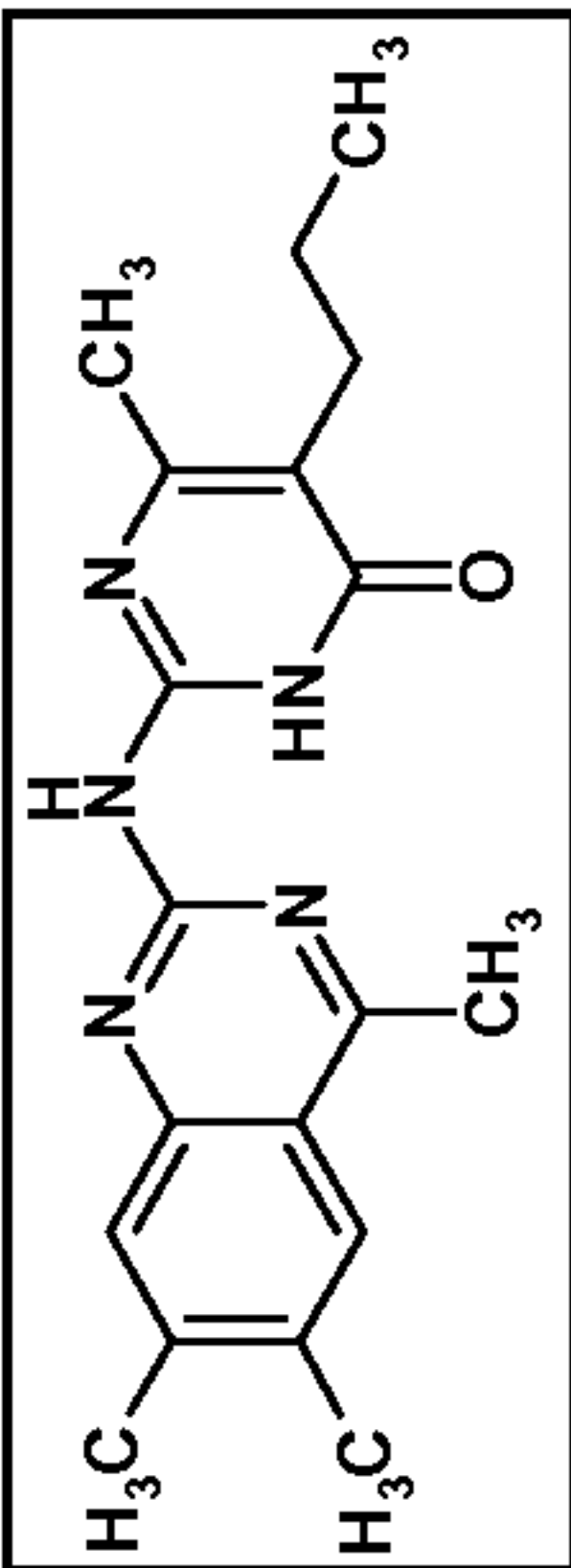
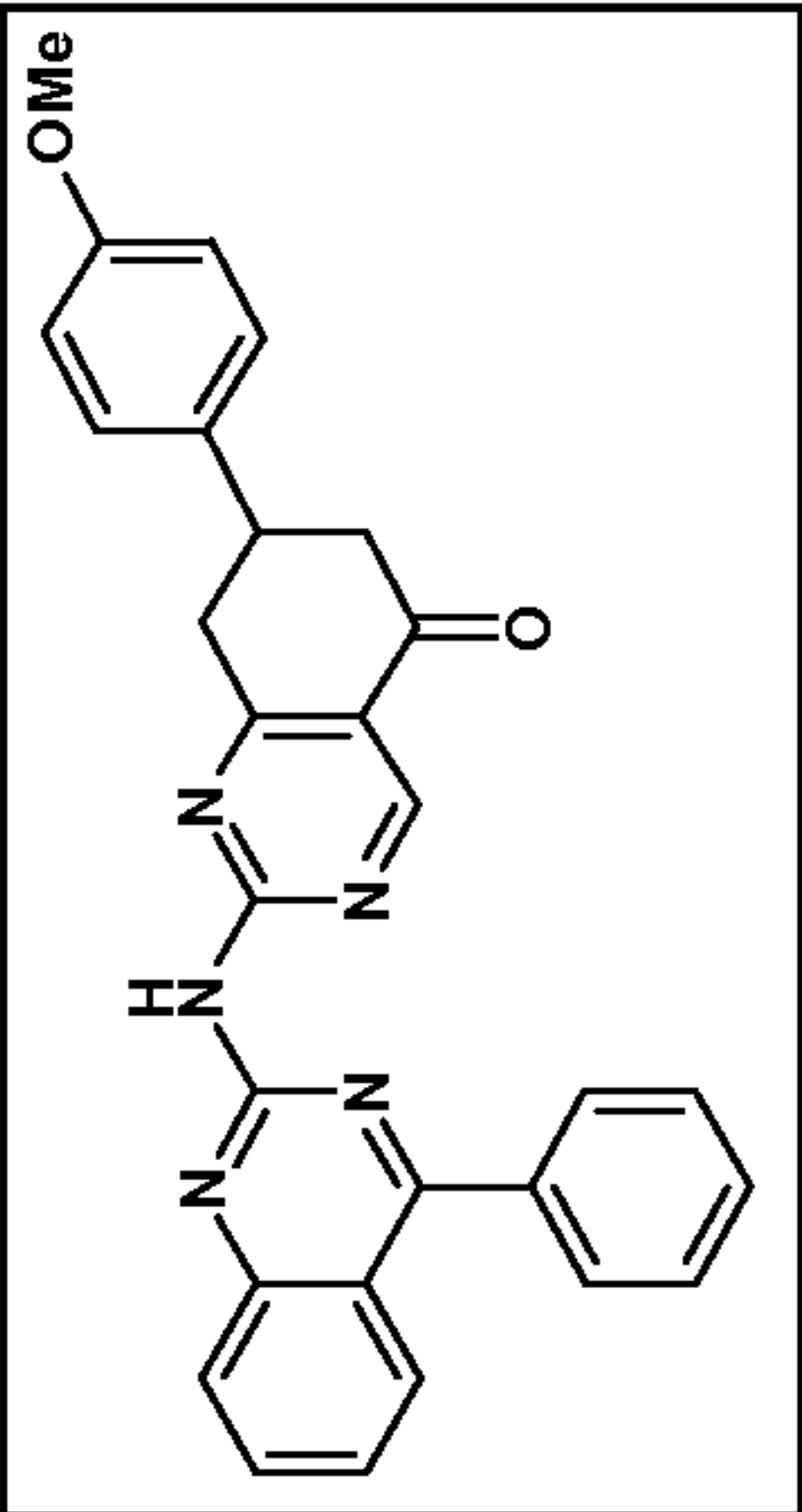
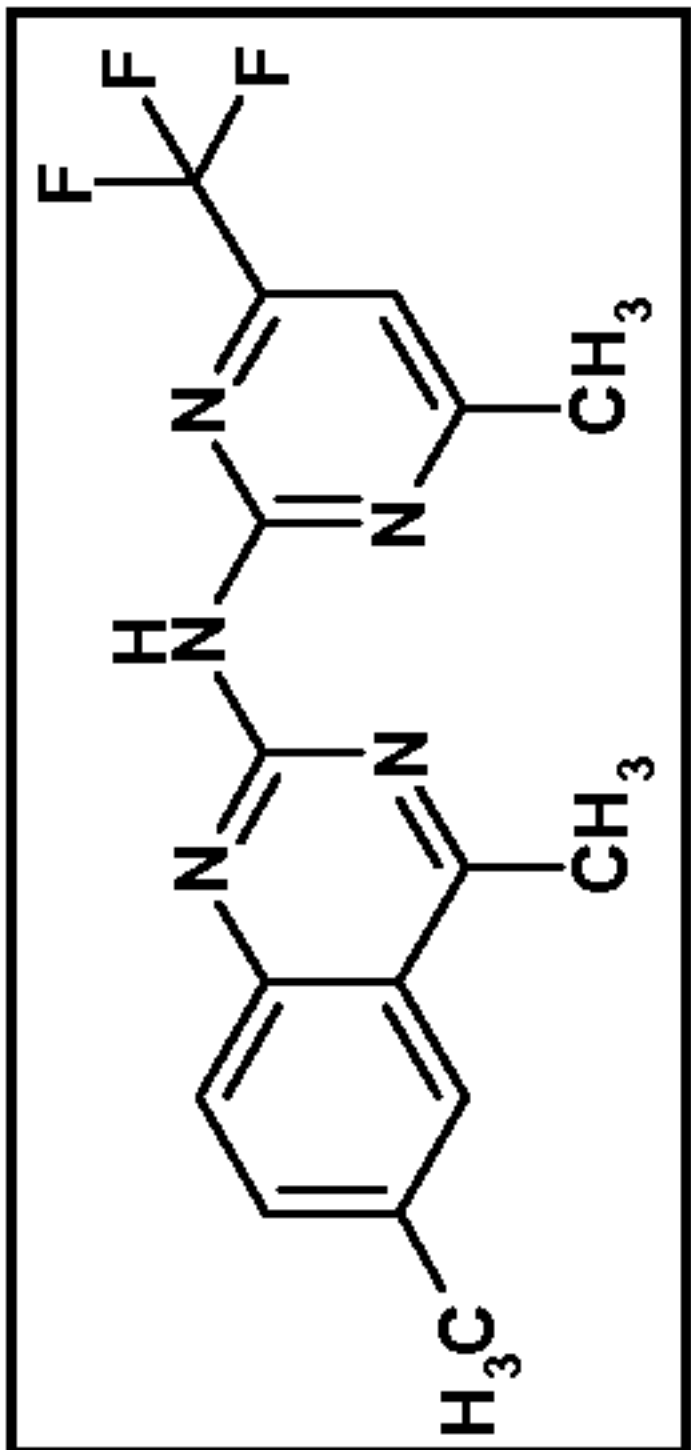
	12607 + TFA	D	-5.11	-44.98	38.40	26.85	32.62	16.57/11.83(0.71)	
	11759+ TFA	D	-5.06	-46.66	38.43	26.39	32.41	14.20/7.36(0.52)	
	11503	D	-5.4	-50.16	25.88	38.53	32.21	6.71/19.02(2.83)	
	12667	D	-5.45	-48.78	29.45	34.27	31.86	36.05/19.55(0.54)	
	12655	D	-5.54	-42.42	30.00	33.36	31.68	11.59/7.89(0.68)	

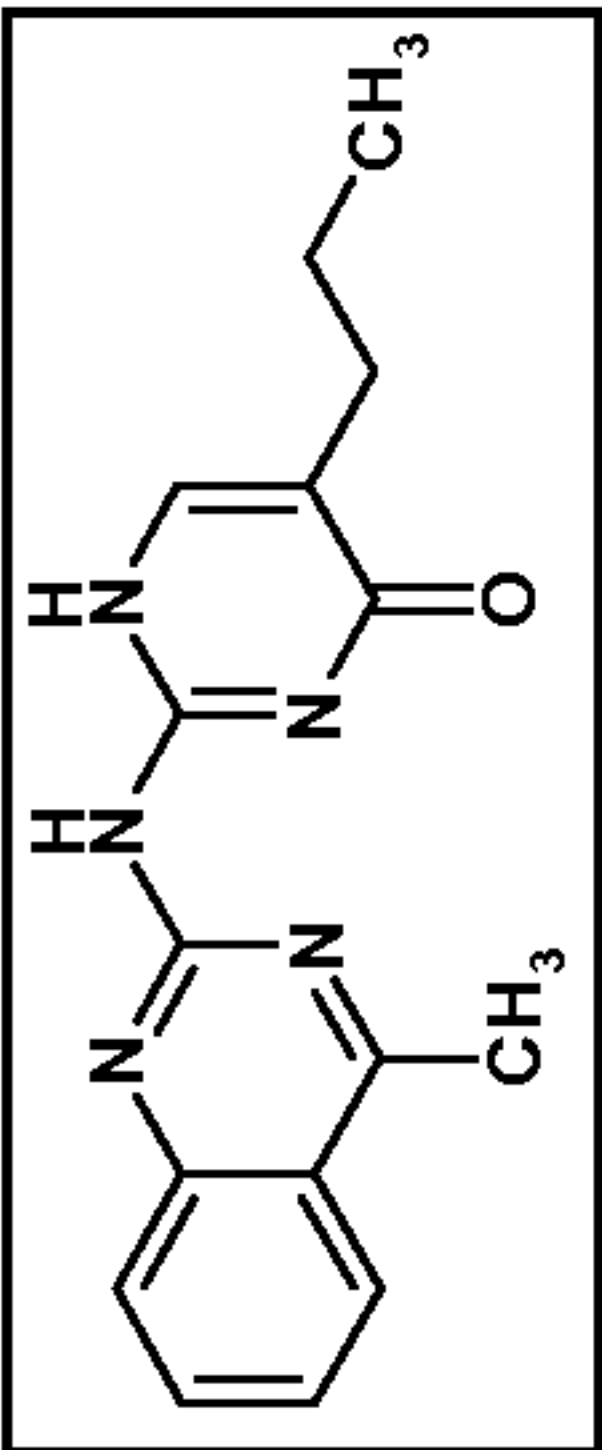
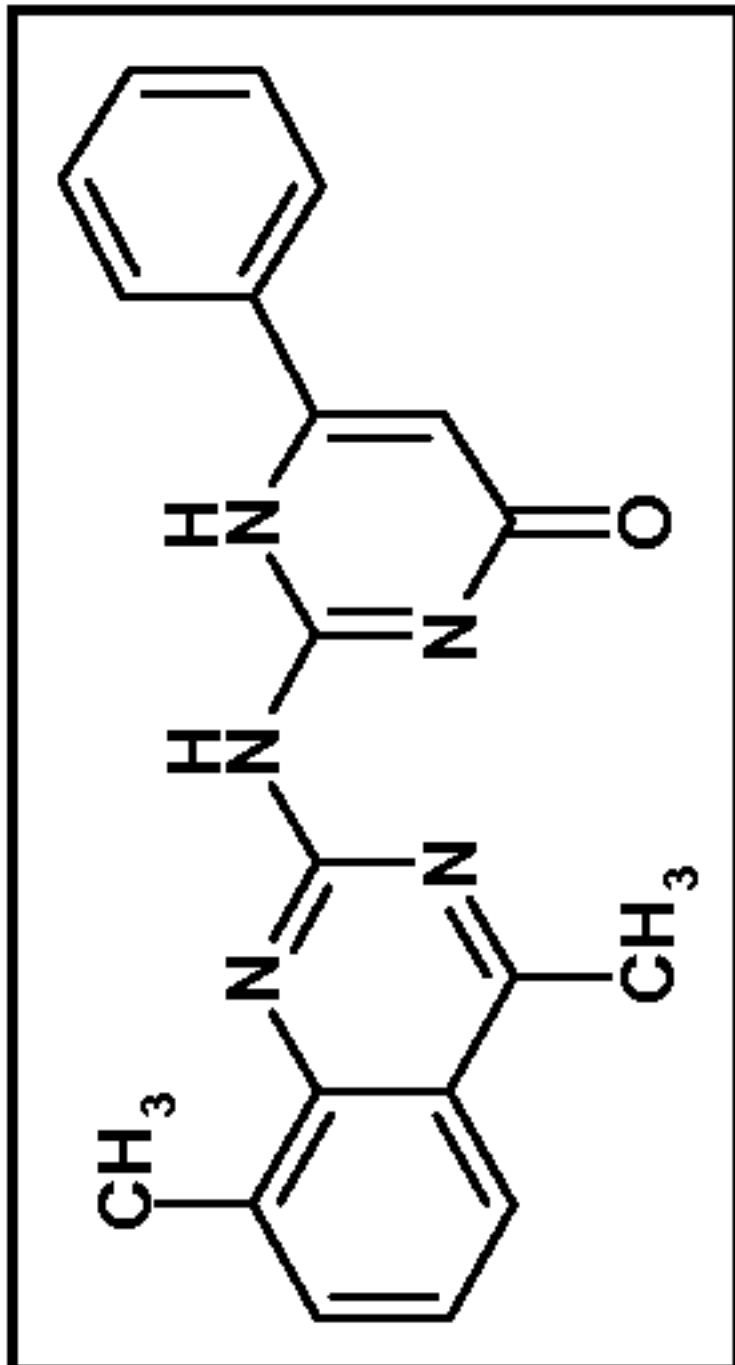
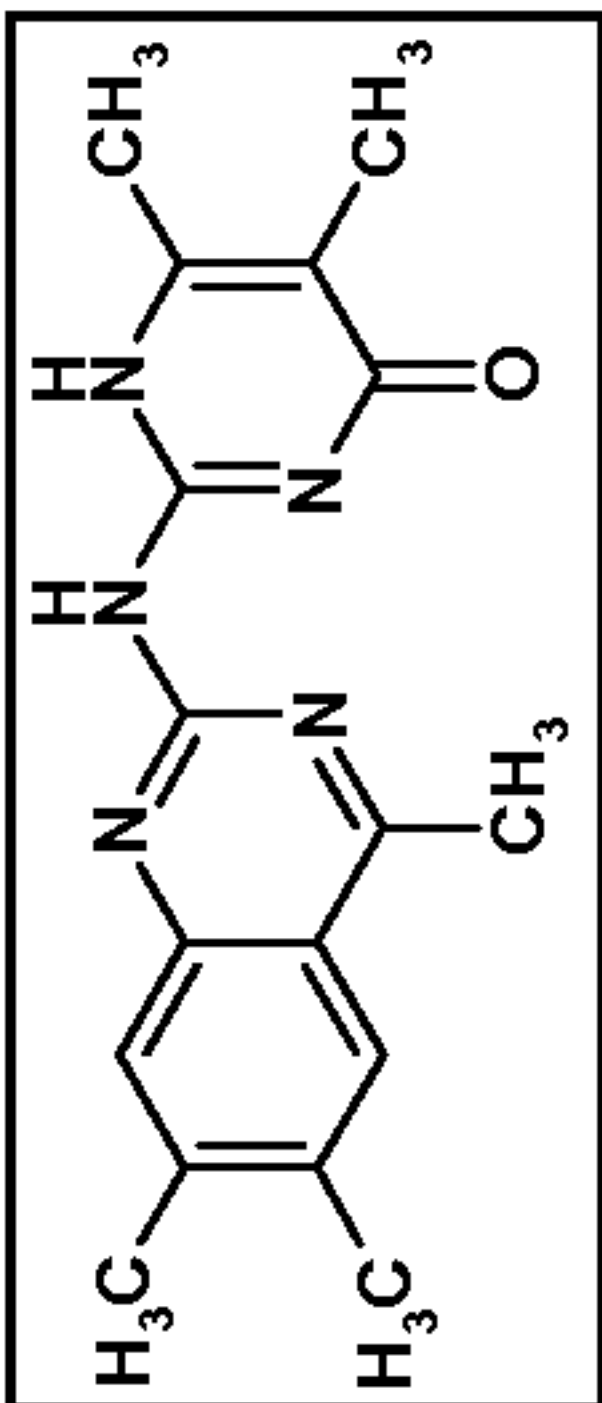
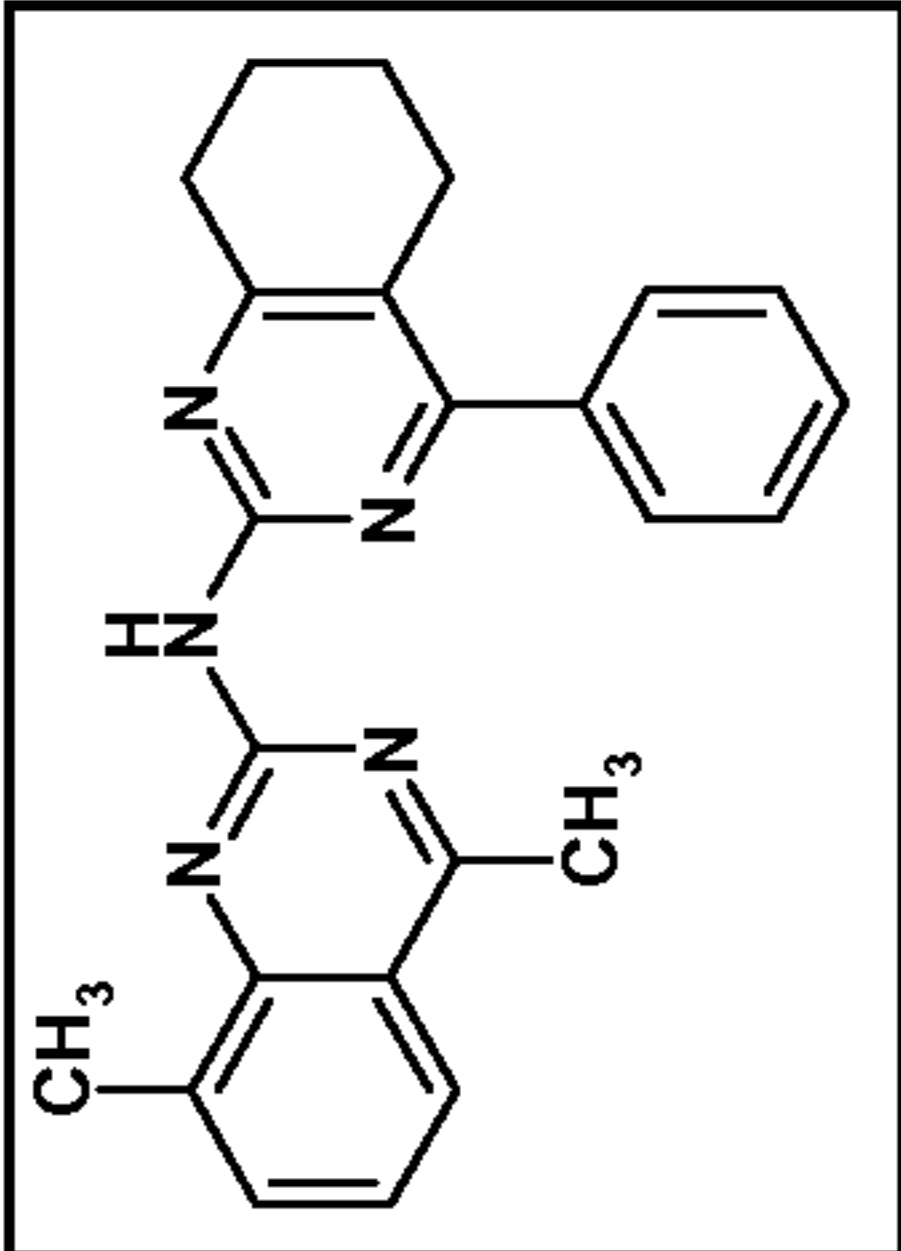
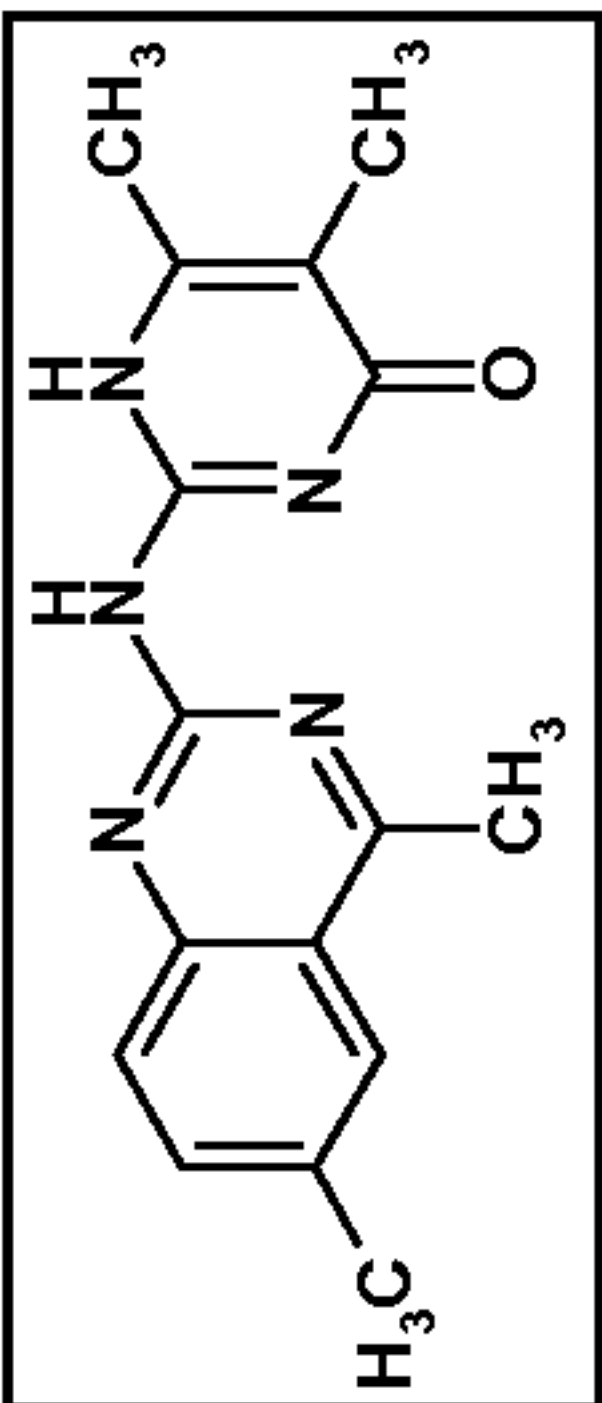
	11610	F	-5.54	-43.39	22.50	40.84	31.67	4.98/17.38	
	12610 + TFA	D	-5.54	-43.39	41.25	21.42	31.33	50.40/19.62(0.39)	
	11727 + TFA	D	-5.45	-54.36	37.00	25.09	31.05	35.11/16.14(0.46)	
	11726 + TFA	D	-5.33	-54.88	35.72	26.02	30.87	NOT ACTIVE	
	12777	D	-5.54	-42.95	24.37	37.17	30.77	12.81/8.96(0.70)	

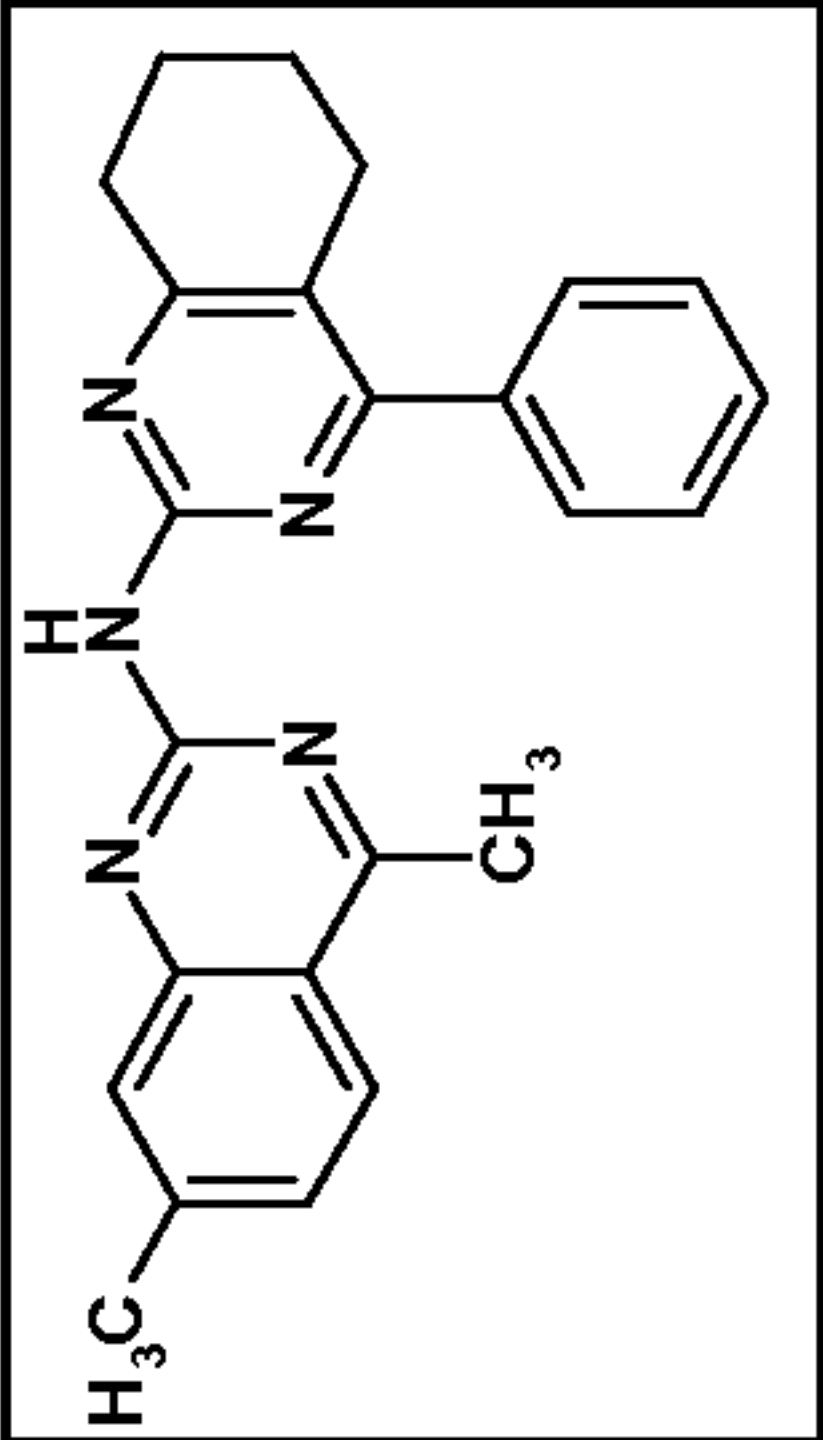
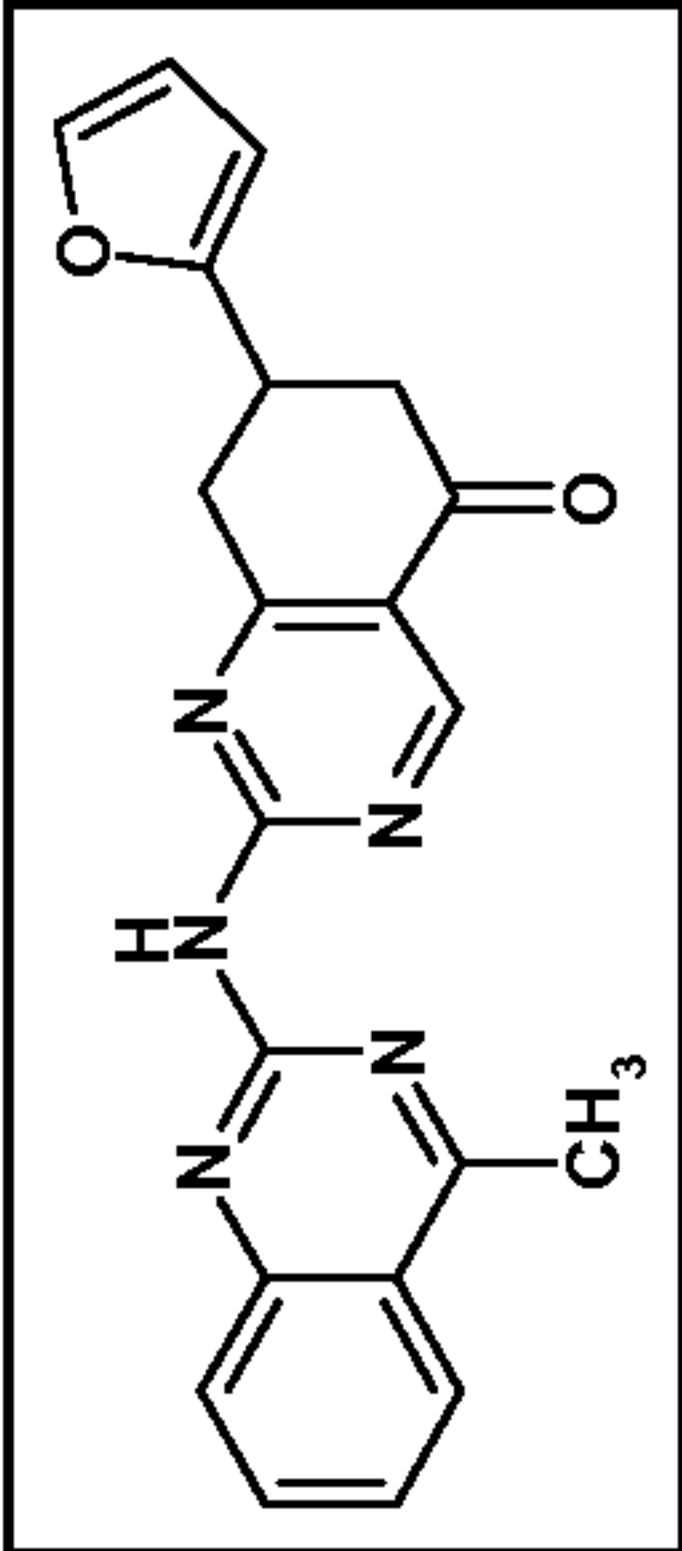
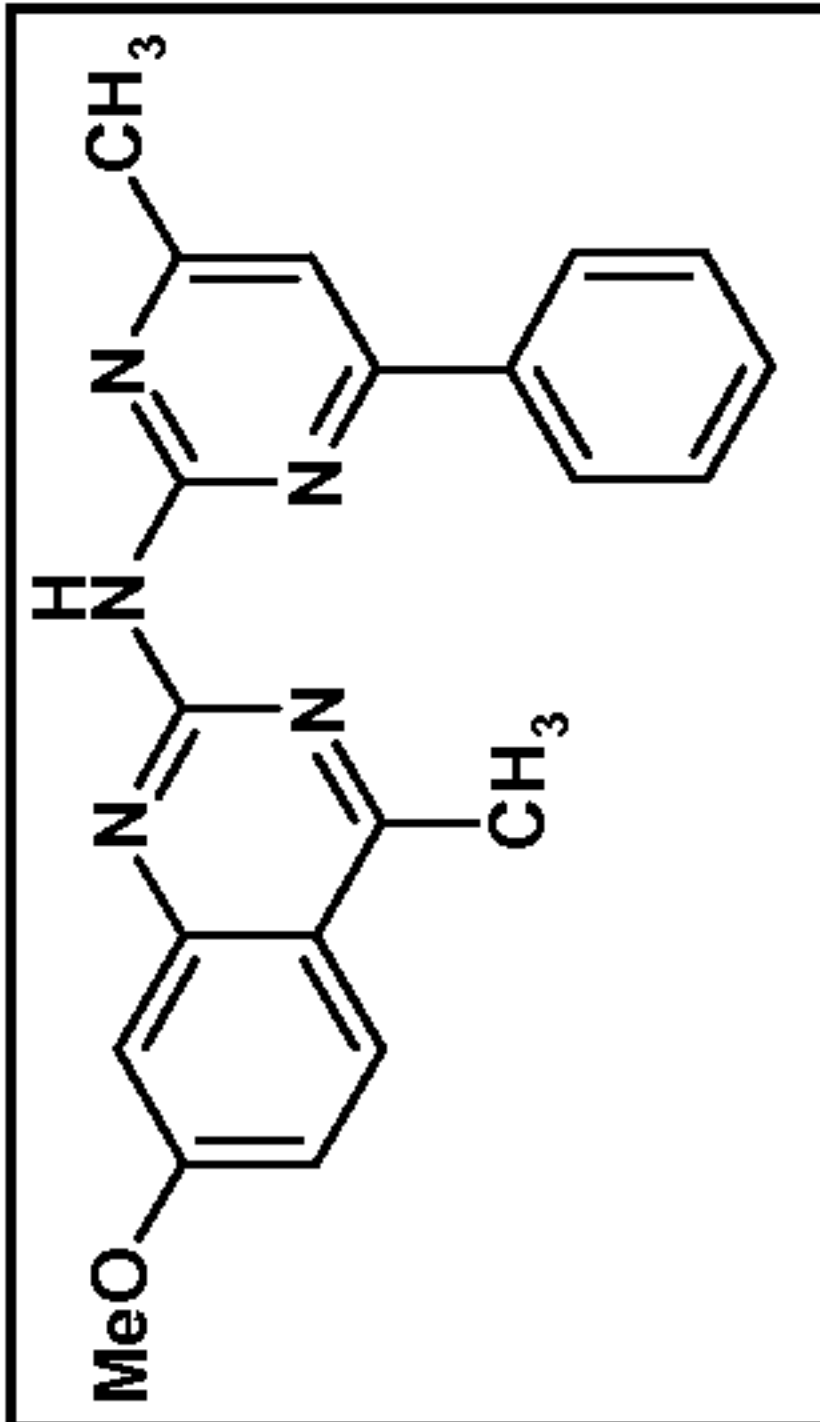
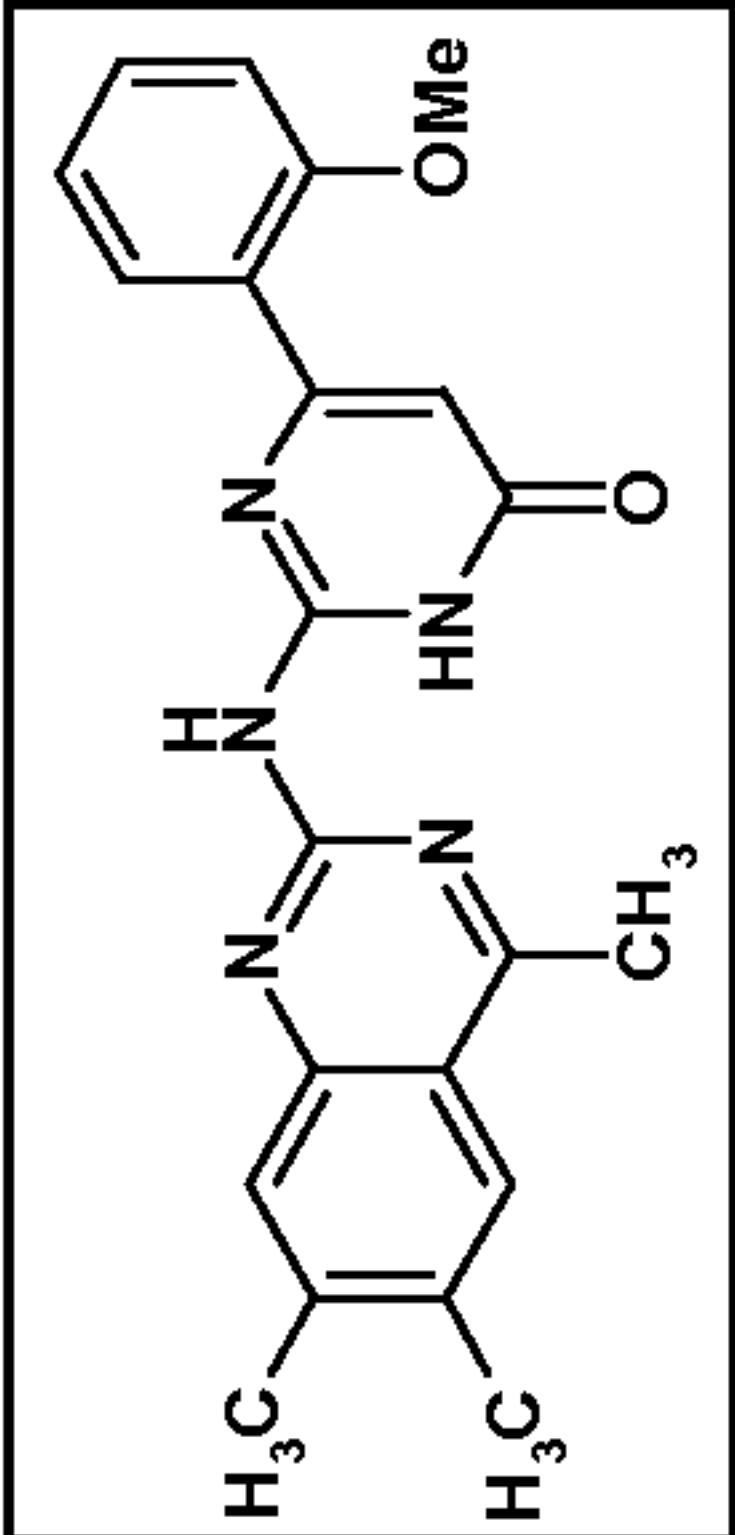
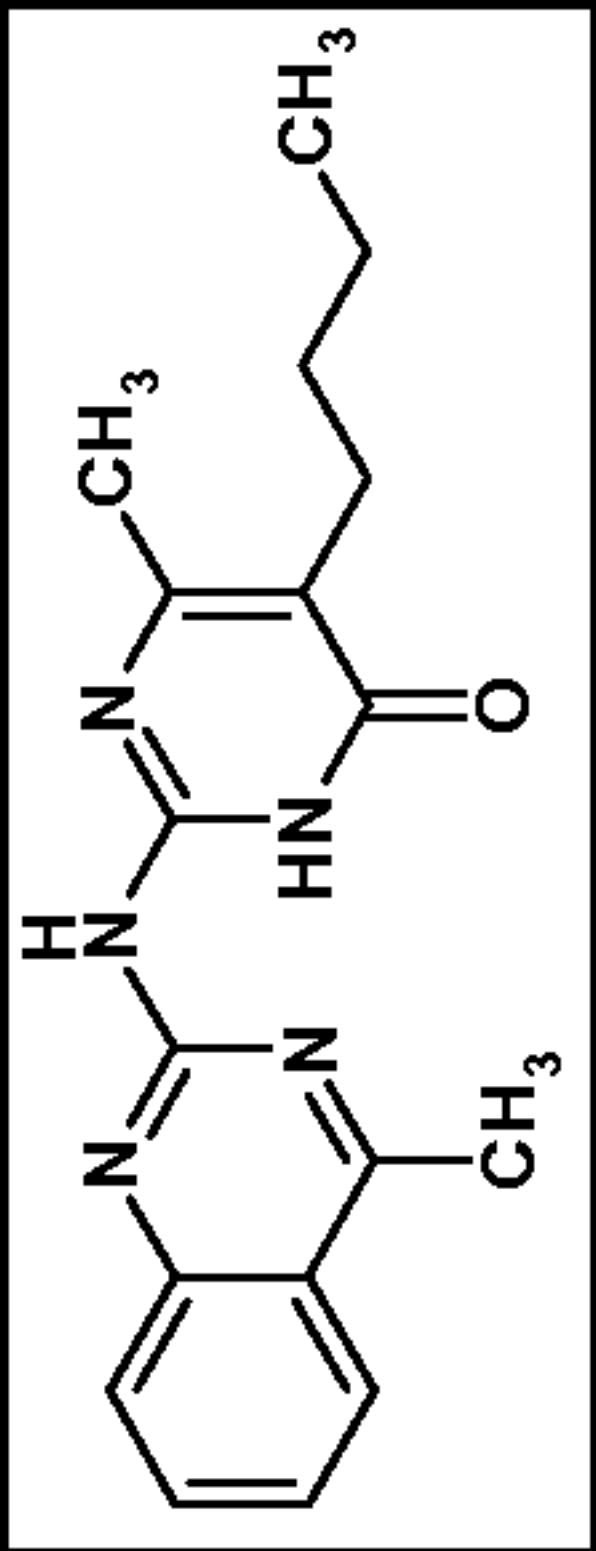
	12689 + TFA	E	-4.12	-45.52	44.07	17.08	30.58	44.11/43.55(0.99)	
	11792	D	-4.91	-45.07	28.62	32.50	30.56	5.36/13.57(2.53)	
	11754 + TFA	D	-5.38	-53.48	34.09	25.85	29.97	5.58/7.55(1.35)	
	11681 + TFA	F	-4.75	-41.75	30.46	29.26	29.86	5.47/4.08(0.75)	
	11483 + TFA	F	-5.7	-45.23	29.55	28.99	29.27		

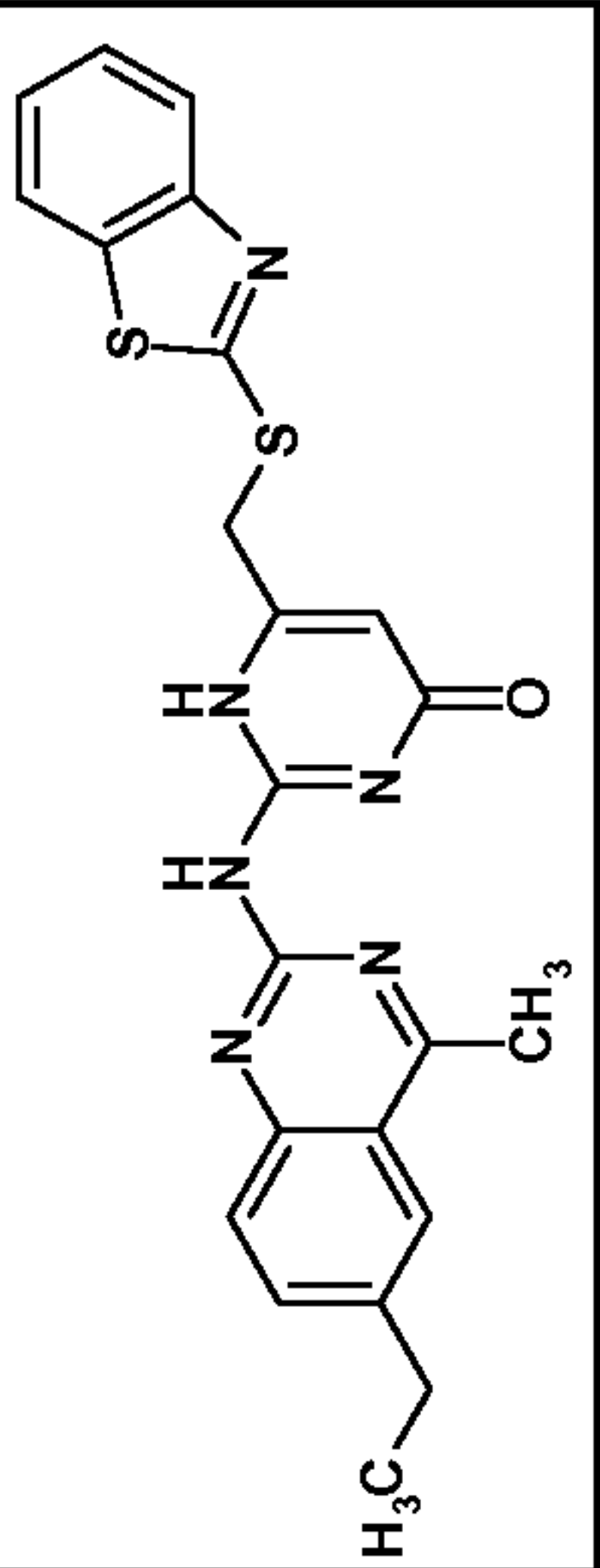
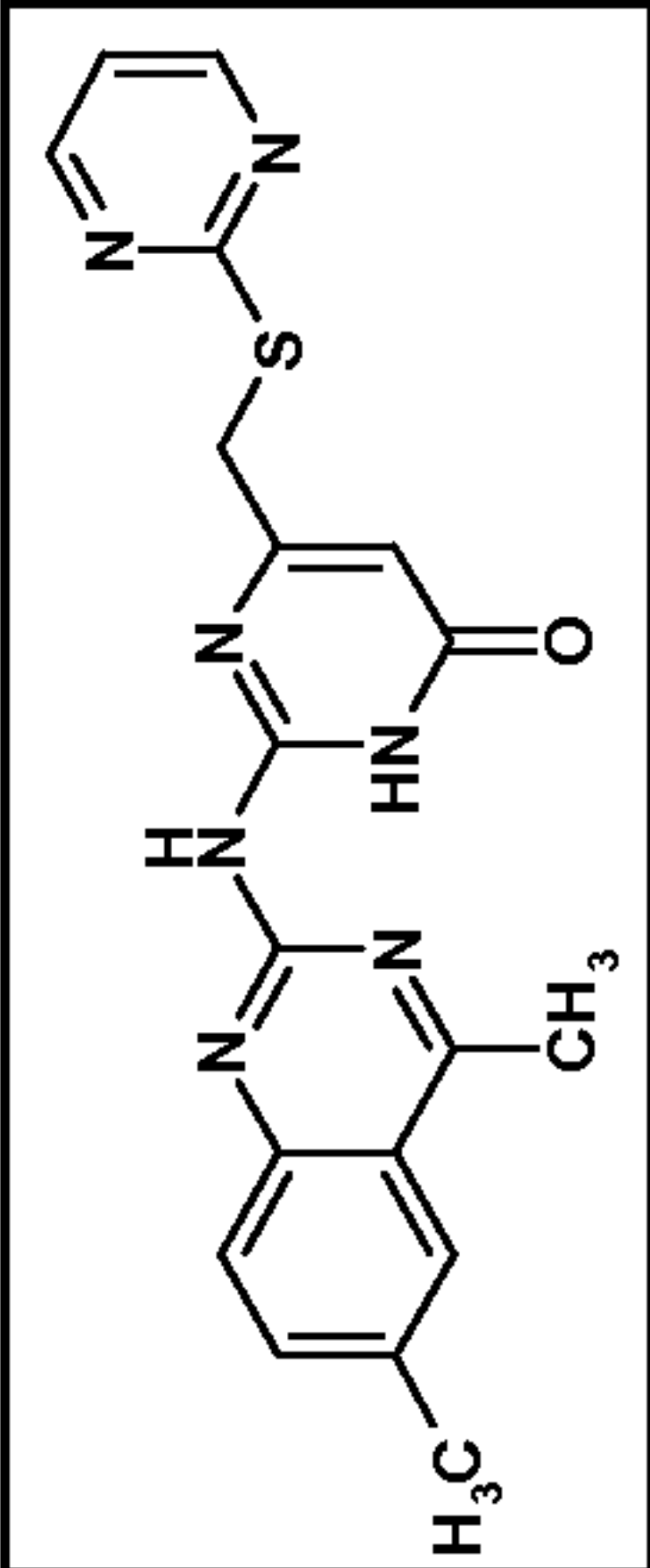
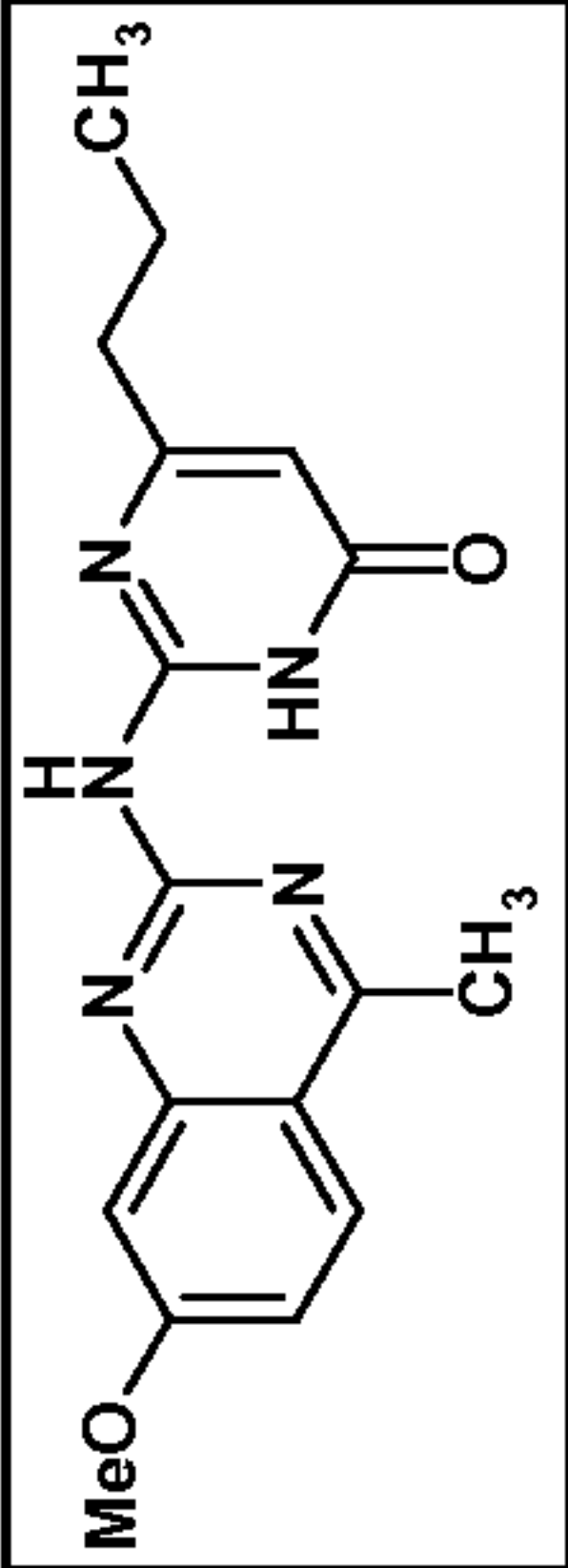
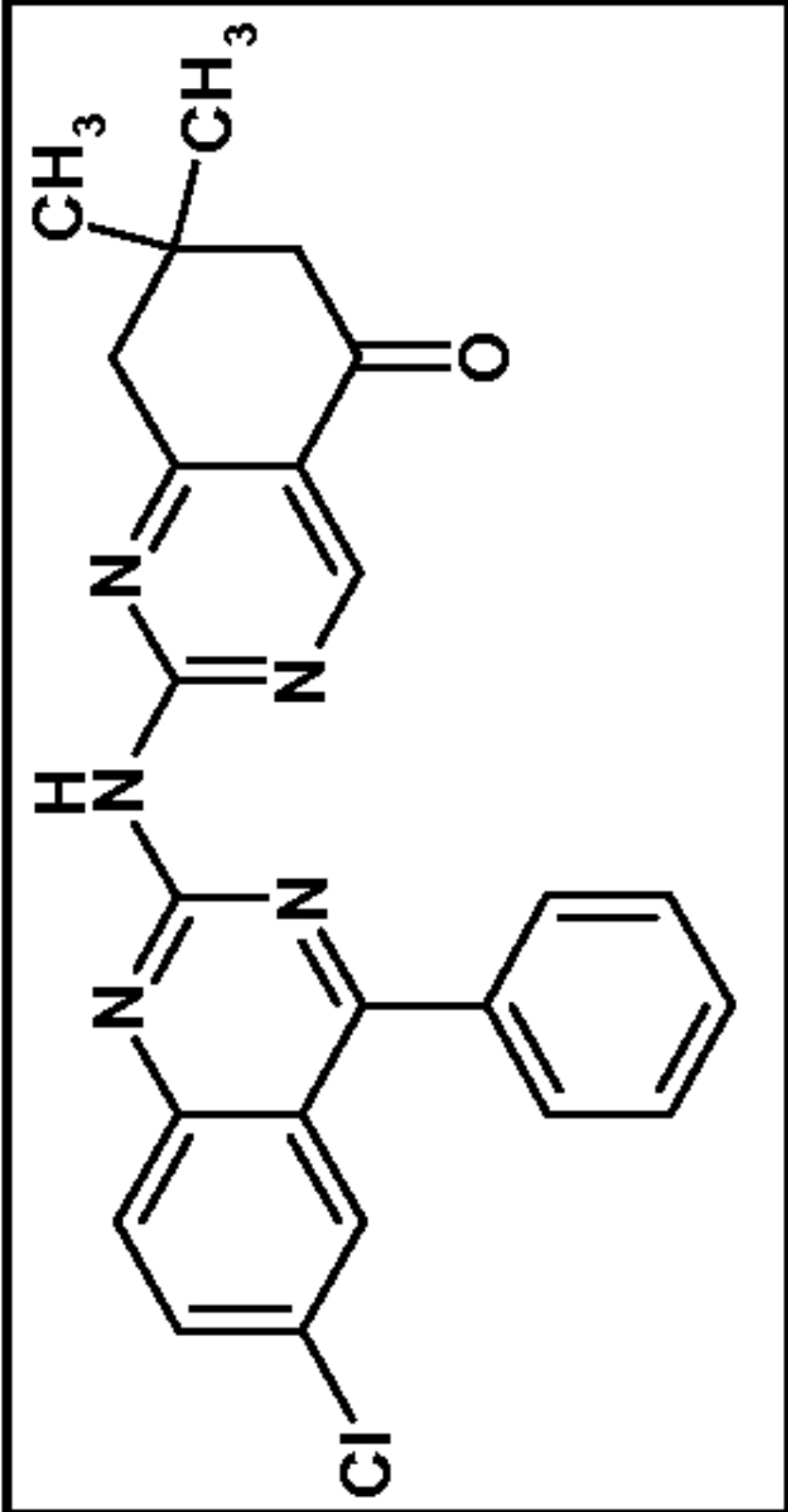
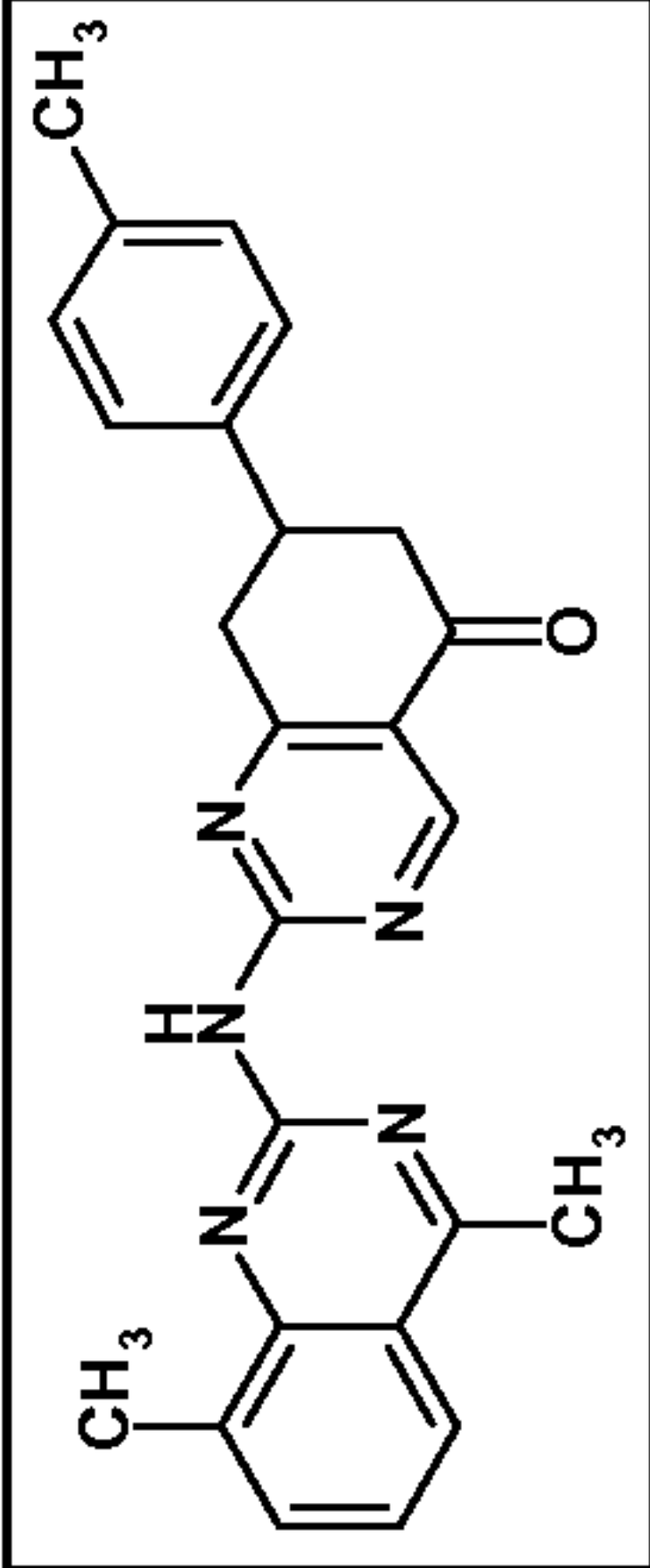
	12615	D	-5.86	-44.34	31.59	26.88	29.24	5.12/5.93(1.16)	
	11617	D	-6.05	-62.56	27.71	30.66	29.18	6.01/19.73(3.28)	
	11592	D	-5.13	-65.33	24.41	33.91	29.16	21.82/18.28(0.84)	
	11725 + TFA	D	-5.93	-41.53	26.65	31.20	28.93	13.00/1.11(0.85)	
	11457	E	-4.91	-34.84	38.67	18.48	28.58	27.40/48.97(1.79)	

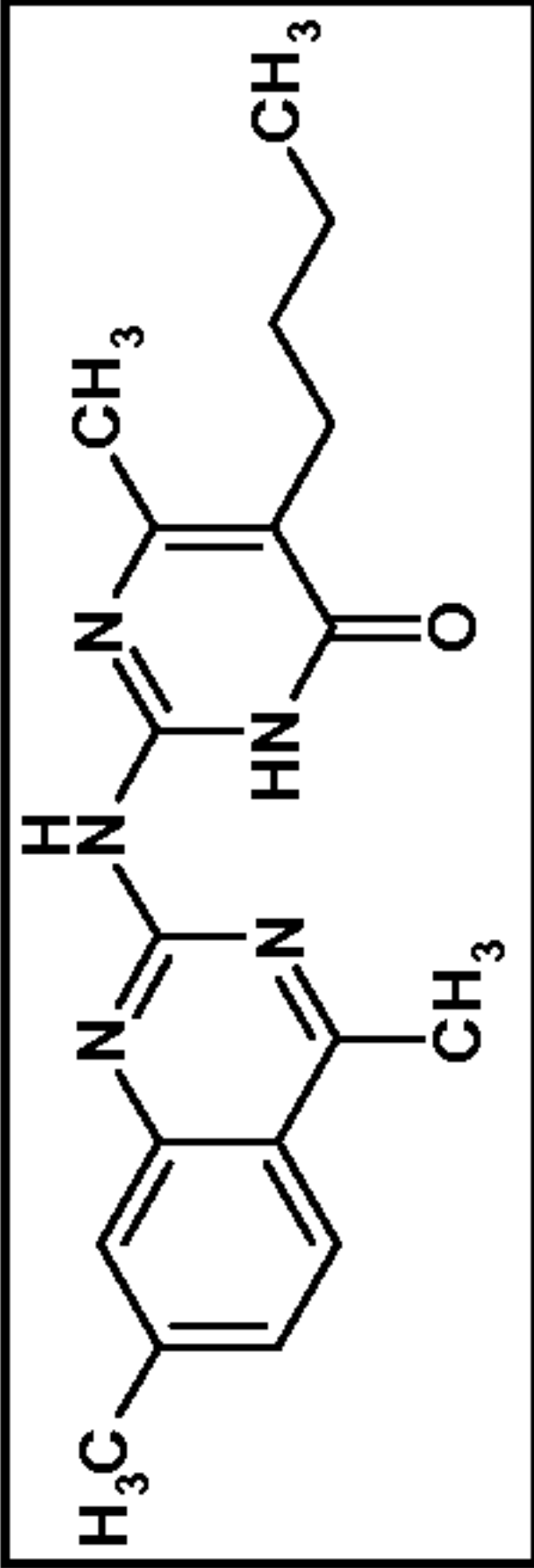
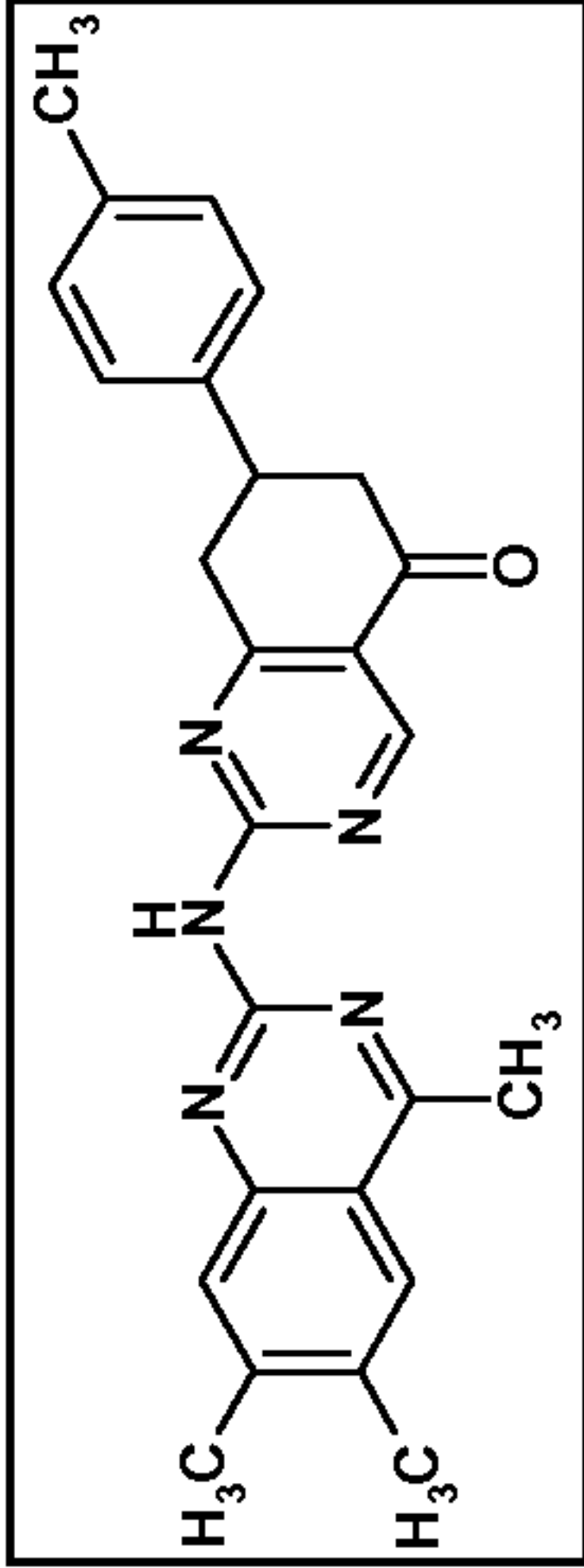
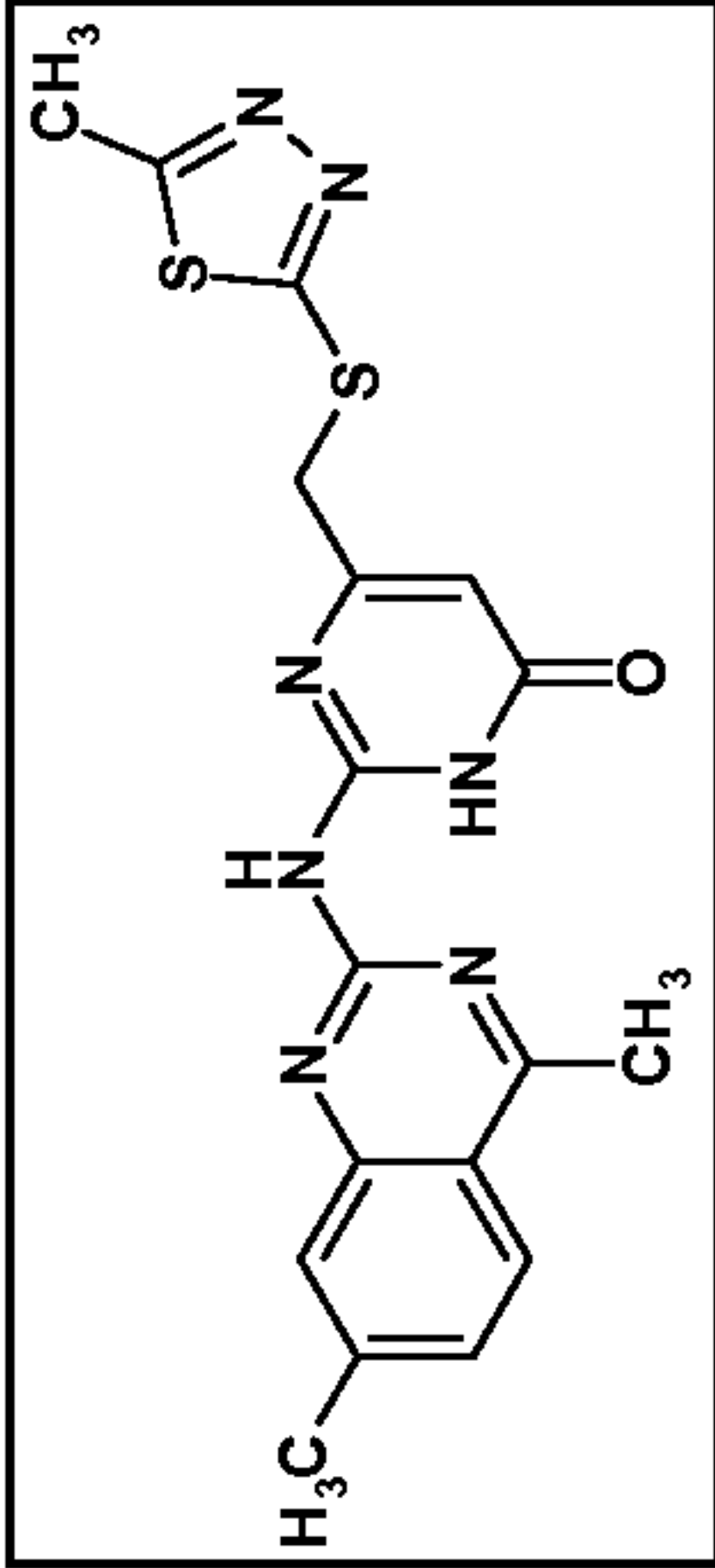
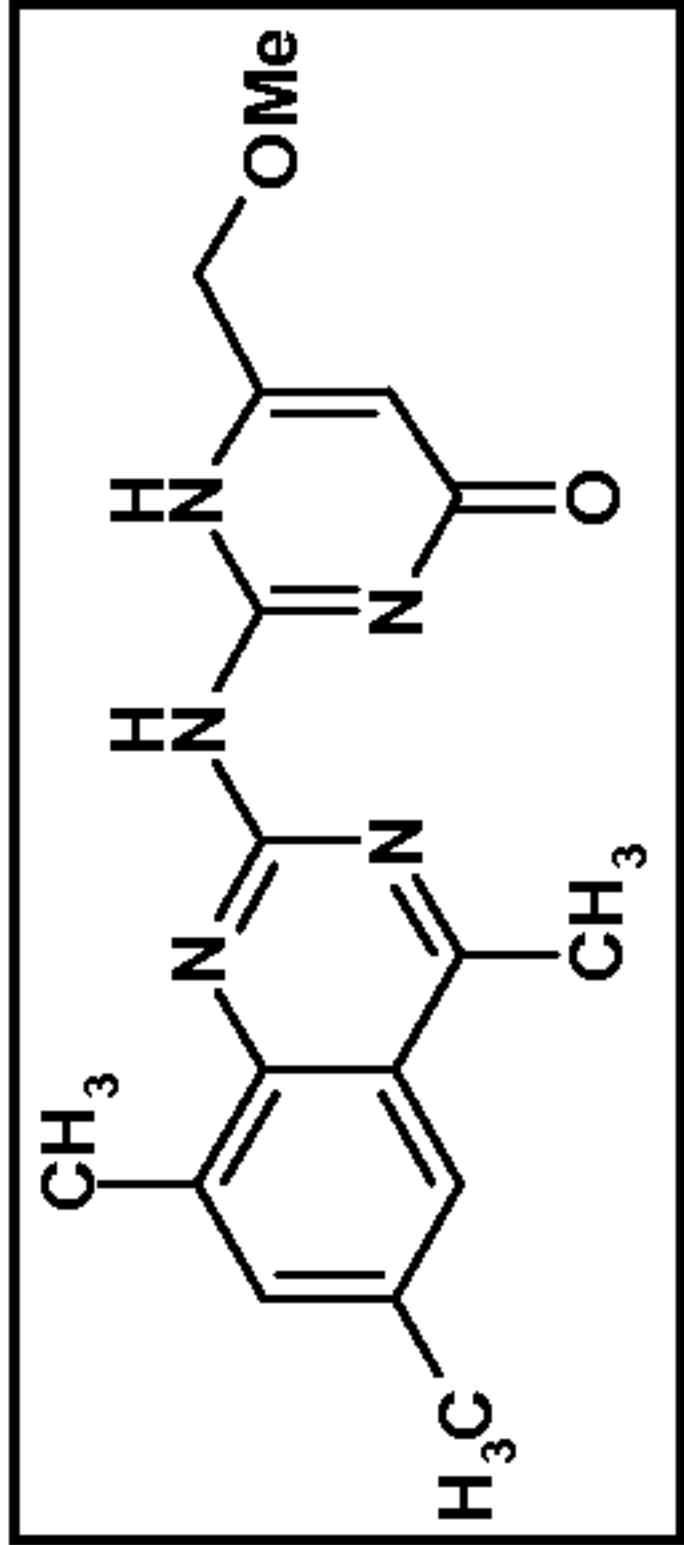
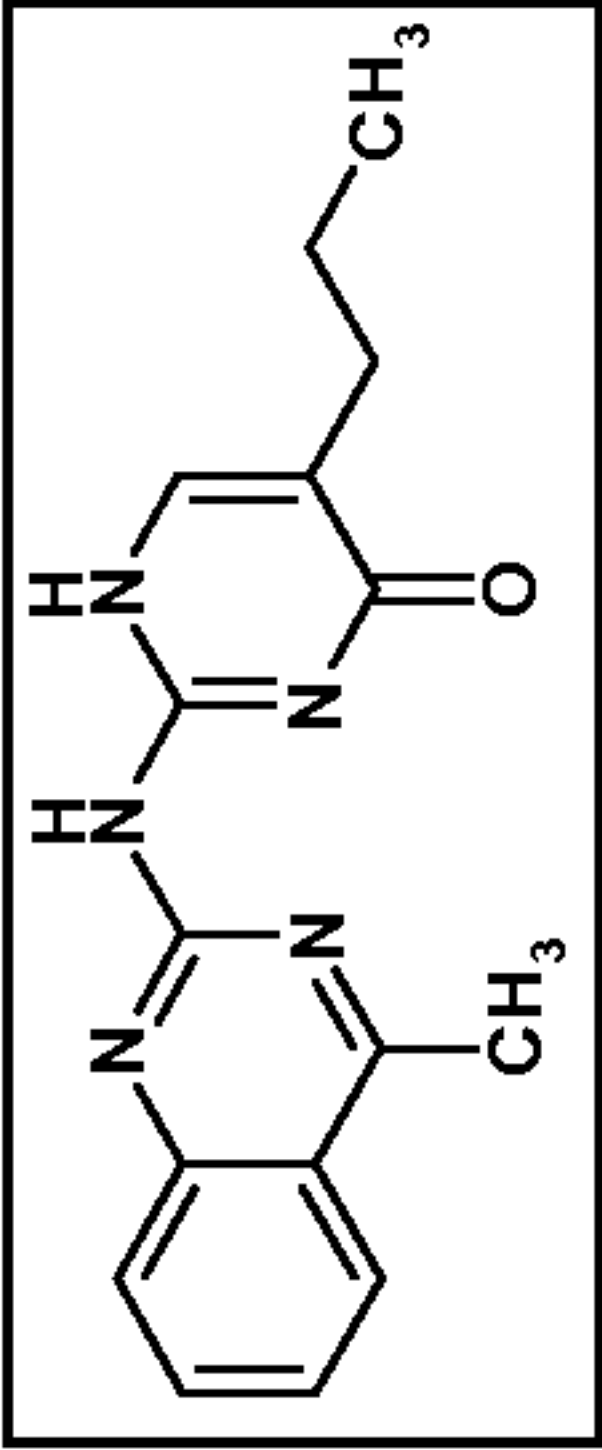
	12623 + TFA	D	-5.2	-54.89	21.11	35.88	28.49	37.25/17.02(0.46)	
	12740 + TFA	D	-5.62	-56.42	28.77	27.91	28.34	49.36/23.63(0.48)	
	12684 + TFA	D	-5.56	-49.03	40.81	15.75	28.28	25.72/19.52(0.76)	
	12213	G	-5.11	-42.88	28.35	27.66	28.01	39.11/38.60(0.99)	
	12227	G	-4.6	-45.64	31.47	23.08	27.27	16.42/17.07(1.04)	

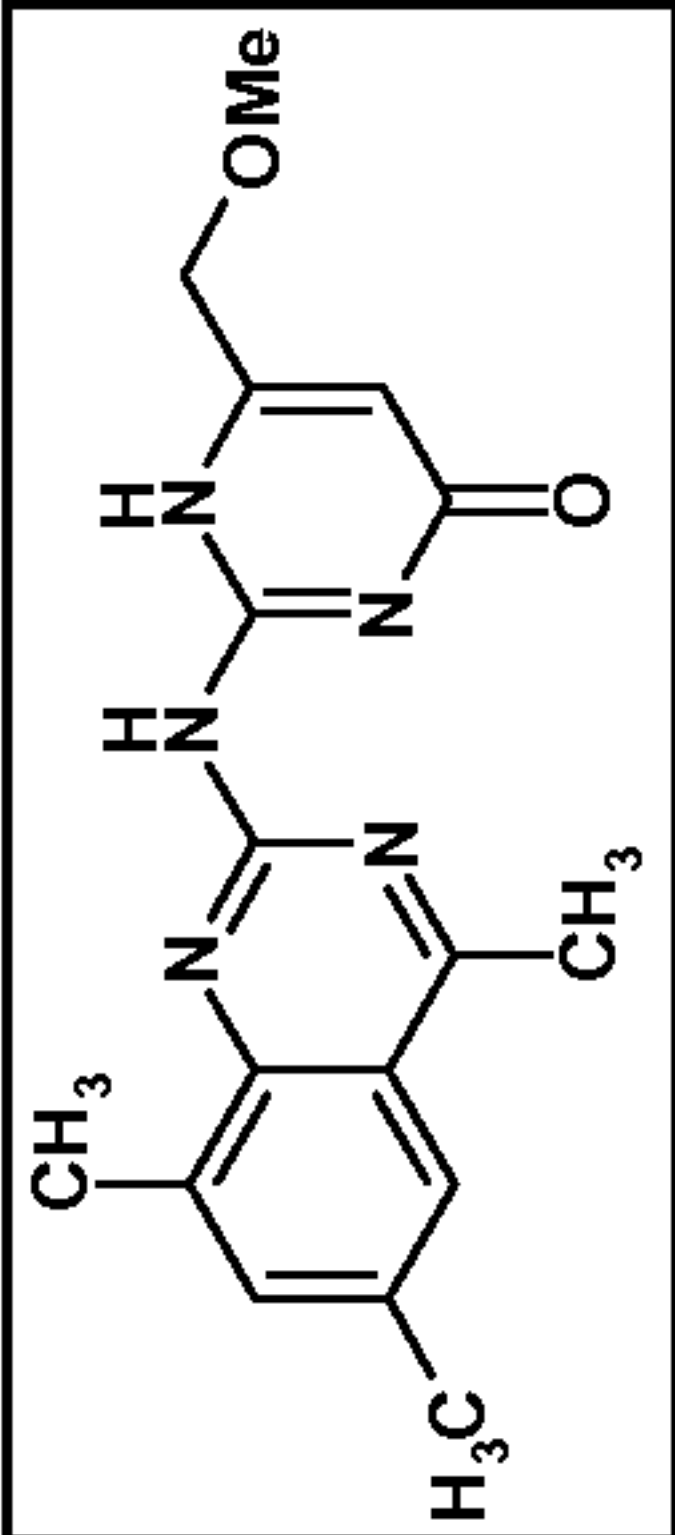
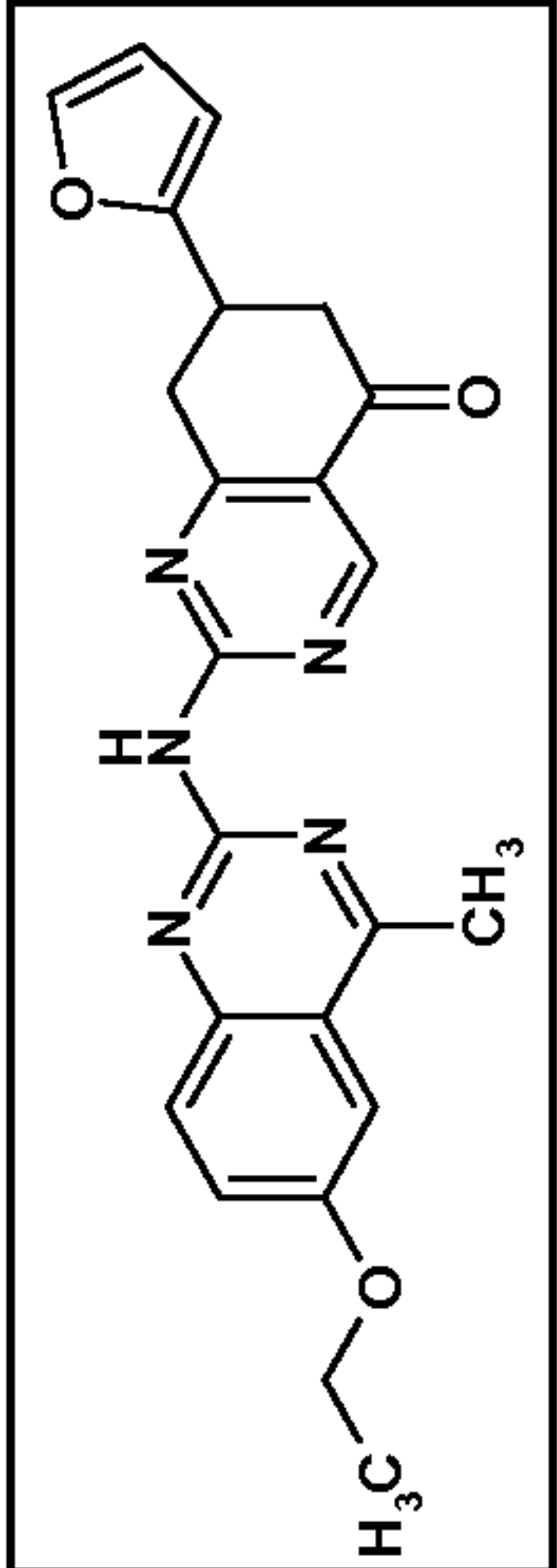
	11680 + TFA	F	-4.78	-38.29	32.59	21.89	27.24	21.86/17.38(0.80)	
	11609	D	-6.98	-54.01	28.83	25.63	27.23	35.98/24.31(0.68)	
	12614 + TFA	D	-5.79	-46.31	20.34	34.09	27.21	19.42/7.56(0.39)	
	11663 + TFA	G	-6.6	-52.02	31.73	21.73	26.73	non-convergent	
	11816 + TFA	E	-4.77	-41.3	39.76	13.58	26.67	14.15/28.12(1.99)	

	12622	D	-5.8	-48.37	21.30	31.34	26.32	20.80/11.52(0.55)	
	11624	D	-5.58	-47.58	22.54	29.61	26.08	14.04/22.37(1.59)	
	12630 + TFA	D	-5.66	-41.51	17.06	34.97	26.02	11.70/8.75(0.75)	
	12229 + TFA	E	-3.71	-32.18	16.57	35.37	25.97	19.23/20.86(1.08)	
	12629 + TFA	D	-5.13	-42.48	19.83	31.95	25.89	12.66/10.71(0.85)	

	11777	G	-3.31	-42.7	37.92	13.81	25.87	6.60/10.66(1.62)	
	11679 + TFA	G	-5.35	-46.98	31.02	20.64	25.83	12.55/8.73(0.70)	
	12676	E	-3.44	-42.61	28.01	23.61	25.81	12.93/18.79(1.45)	
	11746 + TFA	D	-5.48	-45.63	29.51	21.86	25.68	33.53/14.36(0.43)	
	12743 + TFA	D	-4.99	-46.31	9.17	42.12	25.65	6.88/9.86(1.43)	

	11300 + TFA	D	-7.97	-65.69	25.11	26.12	25.61	26.34/7.95(0.30)	
	11751	D	-6.76	-54.92	25.87	25.14	25.51	9.88/6.31(0.64)	
	12669 + TFA	D	-5.44	-42.18	11.73	39.10	25.42	49.32/36.71(0.74)	
	11807 + TFA	G	-5.01	-42.97	30.73	20.02	25.37	5.31/17.78(3.35)	
	11673 + TFA	G	-5.6	-42.41	33.30	17.39	25.35	non-convergent	

	12776 + TFA	D	-5.61	-43.06	21.34	29.30	25.32	29.74/19.03(0.64)	
	11670 + TFA	G	-5.37	-39.67	34.91	15.63	25.27	13.52/24.32(1.80)	
	11469	D	-5.08	-60.97	26.79	23.71	25.25	10.12/11.47(1.13)	
	11625 + TFA	D	-5.97	-44.48	31.51	18.94	25.22	49.03/34.70(0.71)	
	12662	D	-6.33	-40.08	21.64	28.79	25.21		

	12644 + TFA	D	-5.99	-43.95	16.45	33.67	25.06	46.89/30.86(0.66)	
	11674 + TFA	G	-4.7	-38.24	31.91	18.16	25.04	20.20/35.53(1.76)	

[0516] Two-dimensional representations of the docked pose of AD4-1734, and AD4-10381 compounds, along with AD4-1734-like, AD4-10381-like compounds were produced.

[0517] Docking of compound AD4-1734 to EGFR is depicted, for example, in FIG. 8A. Docking of compound AD4-10631 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8B. Docking of compound AD4-10188 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8C. Docking of compound AD4-10186 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8D. Docking of compound AD4-10633 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8E. Docking of compound AD4-10174 (an AD4-1734-like compound) to EGFR is depicted, for example, in FIG. 8F.

[0518] Docking of compound AD4-10381 to EGFR is depicted, for example, in FIG. 9A. Docking of compound AD4-11340 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9B. Docking of compound AD4-12632 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9C. Docking of compound AD4-12681 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9D. Docking of compound AD4-12732 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9E. Docking of compound AD4-11511 (an AD4-10381-like compound) to EGFR is depicted, for example, in FIG. 9F.

EXAMPLE 8: COMBINATORIAL STUDIES

[0519] Studies were initiated to evaluate the ability of various compounds disclosed herein to synergize with several compounds known to inhibit the function of the EGF receptor in a cell proliferation assay (MTT assay). These compounds include Tarceva, Tykerb (non-selective inhibitor of EGFR and HER2 tyrosine kinases), Iressa (selective inhibitor of EGFR kinase), and a mouse antibody homolog of Erbitux (clone 225; inhibits binding of EGF to the EGF receptor). The rationale for this hypothesis is based on the idea that the AD4 compounds interact with a different site than the EGFR antibody, Erbitux, and have a different mechanism of action than the EGFR kinase inhibitors, Tykerb, Iressa or Tarceva.

[0520] Methods are according to Examples 1-7 except as indicated otherwise.

[0521] Known EGFR inhibitors Tykerb (AD4-0003), Iressa (AD4-0004), Tarceva (AD4-0005) and Clone 225 (from which Erbitux was derived) were titrated in the absence or presence of a fixed concentration of AD4 compound. The EGFR kinase inhibitors and AD4 compound were pre-diluted in 100% DMSO (DMSO + 0.2% TFA for AD4 10381) as necessary such that a 1/200 dilution into DMEM+BSA resulted in 2x the final concentration desired. Clone 225 was diluted similarly with the exception that the pre-dilution was made in DMEM+BSA rather than DMSO. The EGFR inhibitor and AD4 compound dilutions were then mixed 1:1 in a 96 well plate. 50 μ l of the mix was then added to the cell plate.

[0522] For the Clone 225 Combination Experiment, Clone 225 (Lab Vision/Thermo Scientific; #MS-269) was tested at concentration of 1, 0.5, 0.25, 0.125, 0.0625 and 0 μ g/ml. The EGF concentration used for stimulation was 10 ng/ml. For the Tarceva Combination Experiment, Tarceva was tested at concentrations of 156, 63, 25, 10, 4 and 0 nM. The EGF concentration used for stimulation was 5 ng/ml. For the Tykerb Combination Experiment, Tykerb was tested at concentrations of 78, 31.25, 12.5, 5, 2 and 0 nM. The EGF concentration used for stimulation was 5 ng/ml. For the Iressa Combination Experiment, Iressa was tested at concentrations of 156, 63, 25, 10, 4 and 0 nM. The EGF concentration used for stimulation was 5 ng/ml.

[0523] The concentration of AD4 compound used in each experiment are provided in the graphs and data tables. A shifting of the inhibitor curve to the left indicates an increase in the effectiveness of the AD4-compound.

[0524] In these studies, the ability of an AD4 compound and the known compound (e.g. Tykerb), either alone or combined in a fixed constant ratio, were evaluated for their ability to inhibit cell proliferation in the MTT assay. From these studies, the following values were calculated: IC₅₀ values for the AD4 compound alone, for Tykerb (or other test compound) alone, and for each compound when combined; the Combination Index (CI), which reflects the degree of antagonism or synergism (see TABLE 13 below); and the Dose Reduction Index (DRI), which is a measure of how many fold the dose of each drug in a synergistic combination may be reduced at a given effect level when compared with the doses of each drug alone.

TABLE 13: Combination Index

KEY:					
Range of C	Description			Graded Symbols	
< 0.1	Very strong synergism			+ + + + +	
0.1-0.3	Strong synergism			+ + + +	
0.3-0.7	Synergism			+ + +	
0.7-0.85	Moderate synergism			+ +	
0.85-0.9	Slight synergism			+	
0.9-1.10	Nearly additive			+/-	
1.10-1.20	Slight antagonism			-	
1.20-1.45	Moderate antagonism			- -	
1.45-3.3	Antagonism			- - -	
3.3-10	Strong antagonism			- - - -	
>10	Very strong antagonism			- - - - -	

[0525] Results showed the following. A series of AD4-Pharma compounds produce a synergistic effect with Tykerb, Iressa and Erbitux to enhance their effect in a cell proliferation assay. These synergistic effects were demonstrated by significant changes in the Dose Reduction Index, the Combination Index and shifts in the dose-response curves.

[0526] The effects of a number of compounds appear to involve positive cooperativity because the effect increases as the concentration of the compound increases. Compounds that demonstrated the greatest degree of positive co-operativity usually demonstrated high DRI values. Some of the compounds that demonstrate the greatest degree of positive co-operativity also show synergistic behavior as evidenced by a low CI value. Iressa and Tarceva, another selective inhibitor of EGFR kinase, did not produce synergistic effects with Tykerb. Compounds acting at the same target (i.e., EGFR kinase) should not be synergistic.

[0527] An example of a shift in the dose-response curve is shown in FIG. 10, where AD4-10628 produced a leftward shift (higher potency) in the dose-response curves for both Tykerb and Iressa. The effect is more evident at higher concentrations of the compound, indicating a positive co-operativity effect may be involved in the compound's action.

[0528] Results for several of the more potent compounds are summarized in TABLE 14. AD4-10628 AD4-11511 produce a leftward shift (i.e., greater potency) in Tykerb's dose-response curve, and demonstrate very high DRI values. Although significant shifts in the dose-response curves for the EGFR kinase inhibitors are produced, these effects are not

translated into significant shifts in the IC₅₀ values (50% inhibition), since the effects of the AD4 compounds are observed to a greater degree at higher concentrations.

TABLE 14: Summary of Median Effect Analysis Studies

Compound	IC ₅₀ Tykerb (nM)	IC ₅₀ Tykerb + Compound (nM)	DRI @ ED97	CI @ ED90
AD4-10628	178	59	126	0.55
AD4-11511	189	118	84	0.87
Iressa	307	160	2	1.15
	IC ₅₀ Iressa (nM)	IC ₅₀ Iressa + Compound (nM)	DRI @ ED97	CI @ ED90
AD4-10628	207	93	105	0.91
AD4-11511	326	245	96	0.93

[0529] DRI values were calculated for the effect of the test compound on Tykerb, as well as for Tykerb's effect on the test compound. In general, most compounds enhanced the effect of Tykerb, as demonstrated by a high DRI, whereas Tykerb usually had a minimal effect on the test compound. As a result, the reported DRI is for the effect of the test compound on Tykerb. This can best be observed by plotting DRI as a function of Fa, or percent effect, ranging from 5% to 97% (see e.g., FIG. 11). AD4-10628 had a significant effect on the activity of Tykerb, which becomes greater at higher activity levels (or doses), whereas Tykerb has little effect on AD4-10628 (see e.g., FIG. 11). As shown in TABLE 14, AD4-10628 and AD4-11511 all produced a fairly high DRI for both Tykerb and Iressa.

[0530] CI values were calculated where the combined effect of the compounds produces 50%, 90%, 95% and 97% (i.e., ED₅₀, ED₉₀, ED₉₅ and ED₉₇) inhibition of cell proliferation. A graph showing the CI values at 90% inhibition is displayed in FIG. 12. Response below the red line (i.e CI < 0.9) indicates synergism. As indicated in FIG. 12, a number of compounds demonstrated synergism. For example, the CI values for AD4-10628 (see TABLE 14) demonstrated significant synergy with Tykerb. In contrast, only AD4-1505 shows synergy with Iressa based on the CI value (data not shown). Since Iressa and Tykerb have similar mechanisms of action, minimal interaction would be expected between these

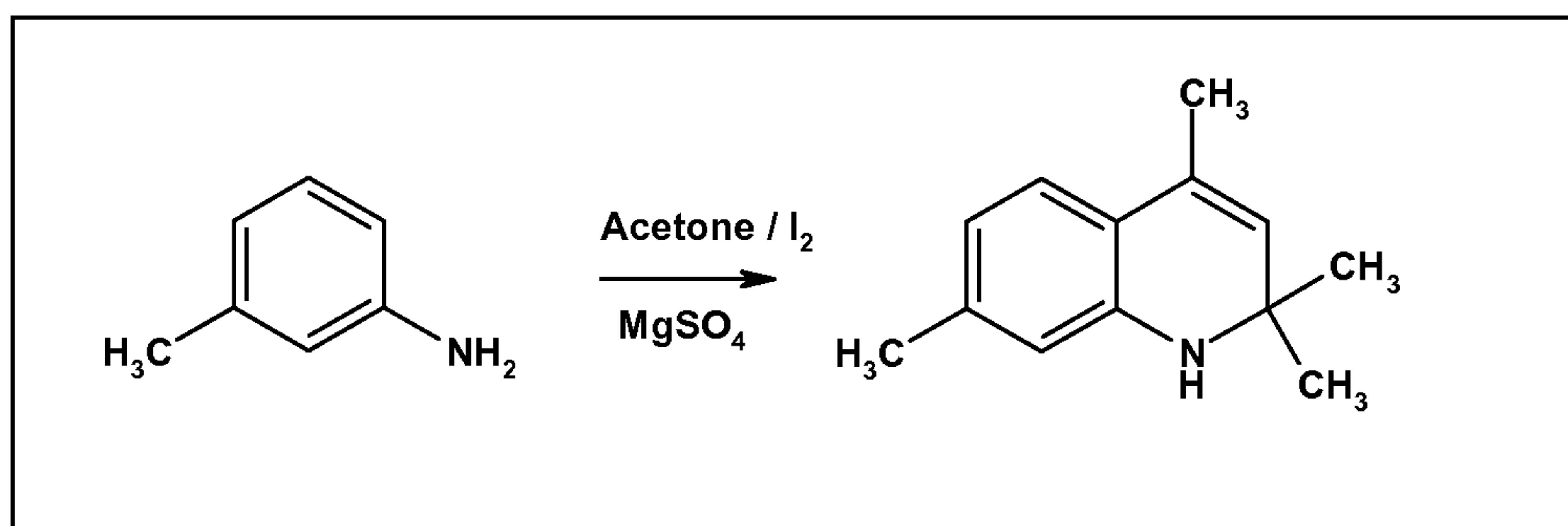
two compounds. As shown in TABLE 14, little interaction is observed based on the DRI and CI values for Iressa.

[0531] These results show that the AD4 compounds produce a significant effect on EGF receptor-mediated cell proliferation through a site that is distinct from either EGF receptor kinase or the EGF receptor. Furthermore, based on their synergistic effect, the compounds may provide a unique method to achieve the same or enhanced therapeutic effect while using a lower therapeutic dose of the marketed compounds, Tykerb, Iressa or Erbitux.

EXAMPLE 9: SYNTHESIS OF COMPOUNDS

[0532] The following example describes synthesis of AD4-12632, which is an AD4-11511-like compound.

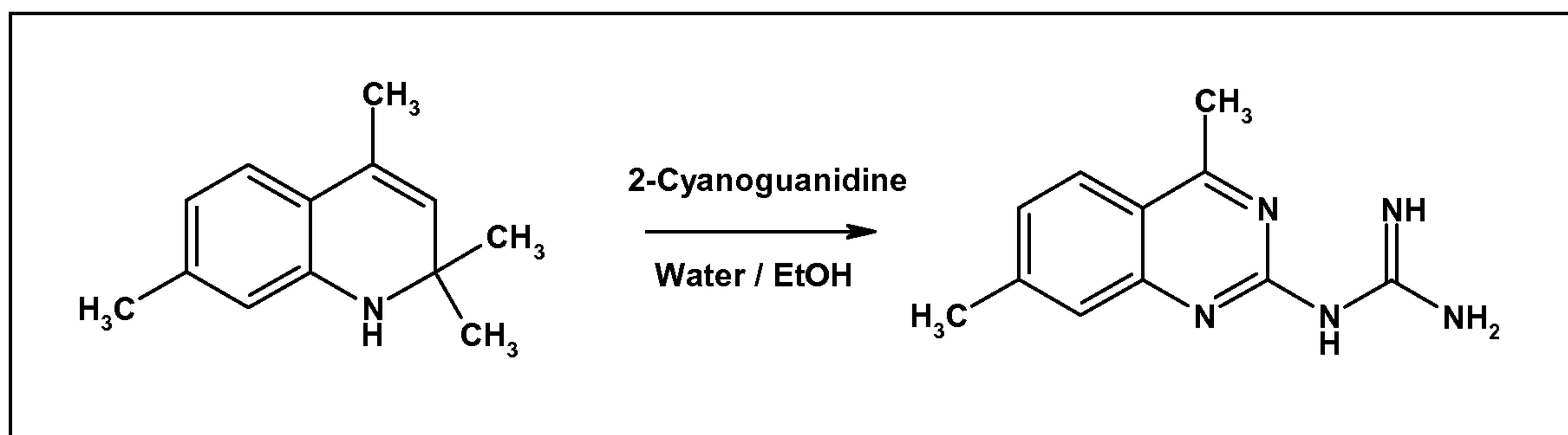
[0533] Step 1 was as follows (see e.g., Hamann 1988 J. Med. Chem. 41(4), 623-639):



[0534] m-Toluidine (across, 32.1 g, 0.3 mol) was dissolved in 250 ml of acetone and treated with 100 g of MgSO₄, iodine (2.6 g, 0.01 mol) and a catalytic amount (200 mg) of t-butyl catechol. The mixture was stirred and heated to reflux temperature overnight. After cooling to room temperature the dark mixture was filtered through Celite which was rinsed with EtOAc. The solution was treated with decolorizing charcoal, filtered, and concentrated using a rotovap. The dark residual oil was purified by fractional distillation. The fraction boiling between 90 - 95 °C @ 0.05 mm/Hg contained the desired product. The light yellow oil solidified after cooling in the refridgerator to give the free base as an off-white waxy solid. The free base was converted to the hydrochloride salt as follows. The freebase was dissolved in Et₂O and cooled in an ice bath. A solution of hydrogen chloride in Et₂O was prepared and a slight molar excess (1.1 equivalents) was added from a dropping funnel. The

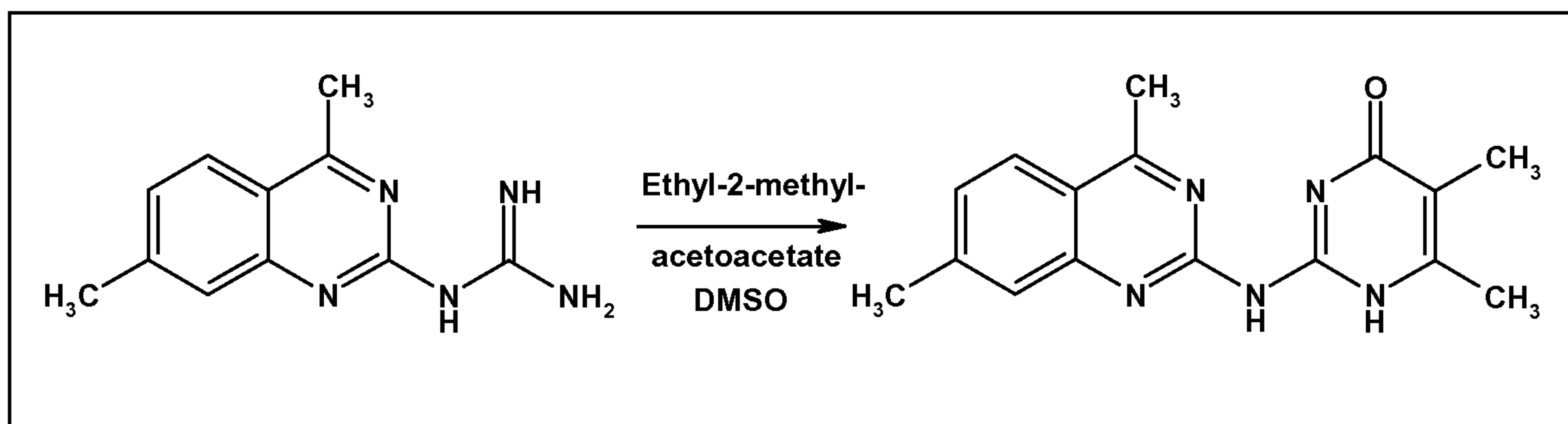
mixture was stirred at 0 °C for 1 h and then filtered to collect 1,2-dihydro-2,2,4,7-tetramethylquinoline hydrochloride as a white solid (MP 213-215 °C).

[0535] Step 2 was as follows (see e.g., Web 2003 Bioorg. Med. Chem. 11, 77-85):



[0536] 1,2-dihydro-2,2,4,7-tetramethylquinoline hydrochloride (9.35 g, 0.004 mol) was suspended with stirring in 30 ml water and 20 ml of EtOH was added followed by 2-cyanoguanidine (Acros, 3.4 g, 0.004 mol). The heterogeneous mixture was heated to 100 °C for 6 h and then cooled to room temperature. The solid was collected by filtration and washed with 500 ml of cold water followed by 100 ml of 2-propanol. The off-white solid was dried and then dissolved by heating to 150 °C in DMSO. Upon cooling to room temperature a white solid formed and was collected by filtration. The solid was rinsed with Et₂O and dried to give 4,7-dimethylquinazoyl-2-guanidine hydrochloride as a white solid (MP 329-331 °C). The hydrochloride salt was suspended in 5N aqueous sodium hydroxide and stirred at room temperature for 5 days. The white solid was collected by filtration and rinsed with 2 x 250 ml water and 100 ml of ice-cold 2-propanol. The solid was dried to give 4,7-dimethylquinazoyl-2-guanidine free base as an off white solid (MP 260-261 °C).

[0537] Step 3 was as follows (see e.g., Shikhaliev 2002 Chem. Het. Compounds 38(11), 1368-1370):

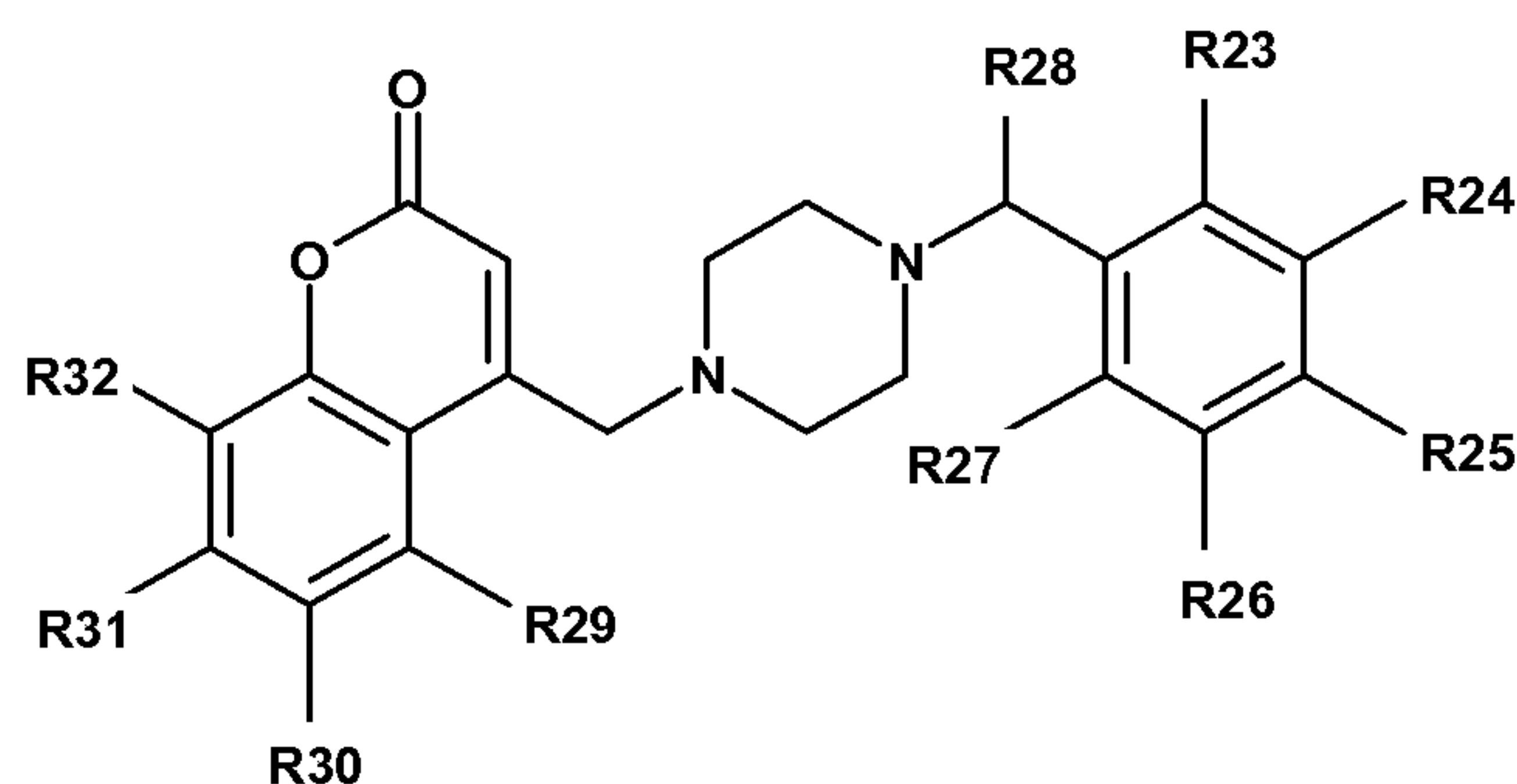


[0538] 4,7-dimethylquinazoyl-2-guanidine (2.15 g, 0.01 mol) and ethyl-2-methyl acetoacetate (1.58 g, 0.011 mol) were stirred in 15 ml DMSO to produce a suspension. The solution was heated to 130 °C overnight to give a clear brown solution and then cooled to room temperature. The solid that formed was collected by filtration. The solid was dissolved in EtOH at room temperature with stirring, treated with decolorizing charcoal and filtered through a bed of Celite. The EtOH solution was cooled to 0 °C to give an off white solid that is collected by filtration (MP 226-228 °C). The resulting product was the AD4-12632 compound.

CLAIMS

What is claimed is:

Claim 1. A compound having a formula of:



Formula (13) (1734-like Type P)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{23} and R^{27} are hydrogen;

R^{24} and R^{26} are independently selected from the group consisting of:

(i) hydrogen;

(ii) C-1 to C-6 lower alkyl;

(iii) alkoxy $-OR^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(iv) halogen;

R^{25} is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of

(a) carboxyl $-COOR^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of: straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; alkoxy $-OR^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; trifluoromethyl; trifluoromethoxy; difluoromethoxy; 3, 4-methylenedioxy; 2, 3-methylenedioxy; nitro; and halogen; and

(c) aryloxy ($-OAr$);

(v) alkoxy $-OR^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(vi) halogen;

R^{28} is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(c) halogen.

R^{29} is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) hydroxy;

(iv) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(v) halogen;

R³⁰ is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) trifluoromethyl; and

(v) halogen;

R³¹ is selected from the group consisting of:

(i) hydrogen;

(ii) hydroxy;

(iii) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(v) trifluoromethyl; and

(vi) halogen; and

R³² is selected from the group consisting of:

(i) hydrogen;

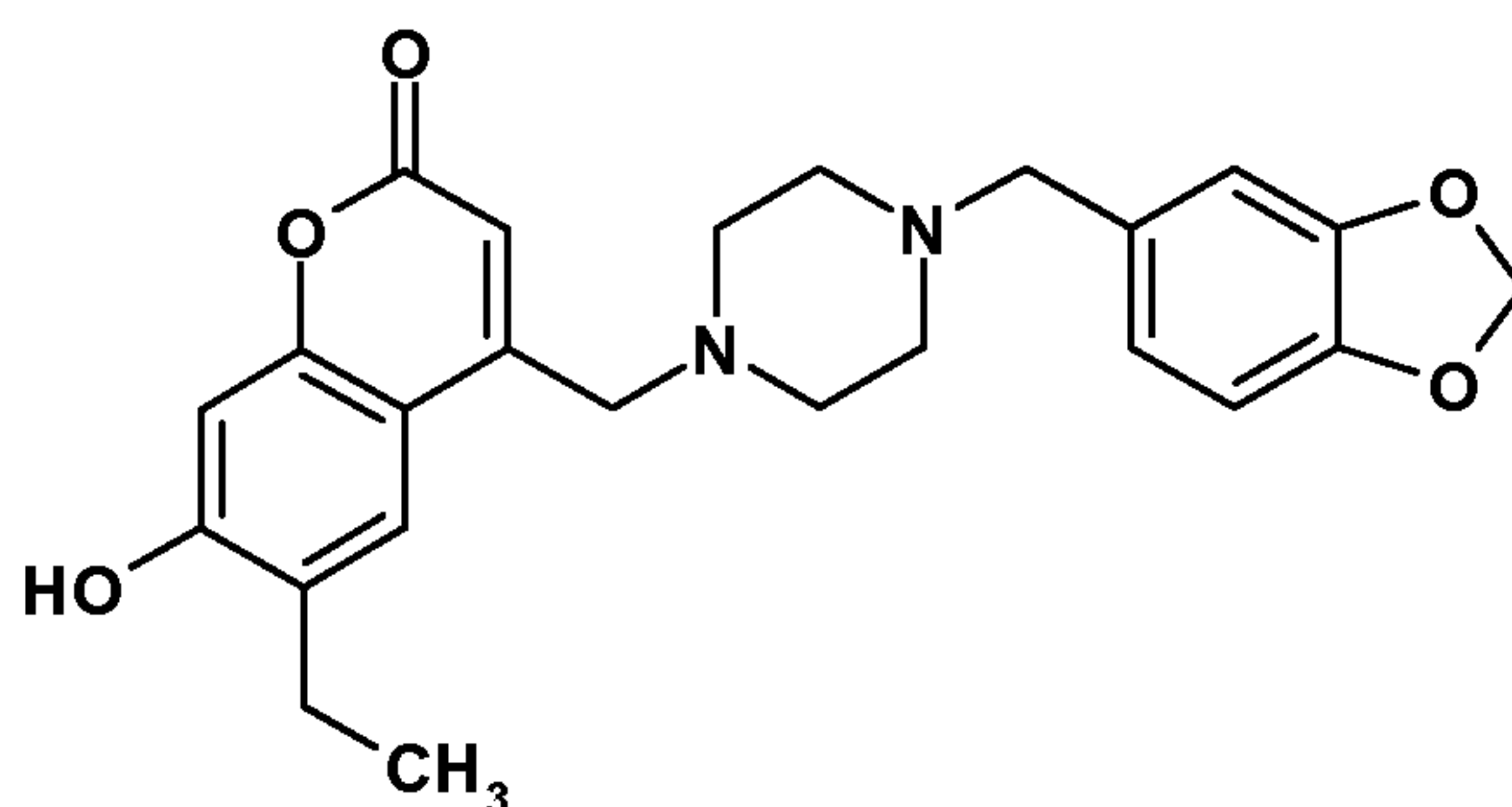
(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) hydroxy;

(iv) trifluoromethyl; and

(v) halogen; and

Formula (13) excludes the compound of Formula (12):



Formula (12)

Claim 2. The compound of claim 1 wherein R^{24} and R^{26} are independently selected from the group consisting of: hydrogen and alkoxy.

Claim 3. The compound of any one of claims 1-2 wherein R^{25} is selected from the group consisting of: hydrogen or alkoxy $-OR^{10}$.

Claim 4. The compound of any one of claims 1-3 wherein R^{28} is selected from the group consisting of: hydrogen or aryl.

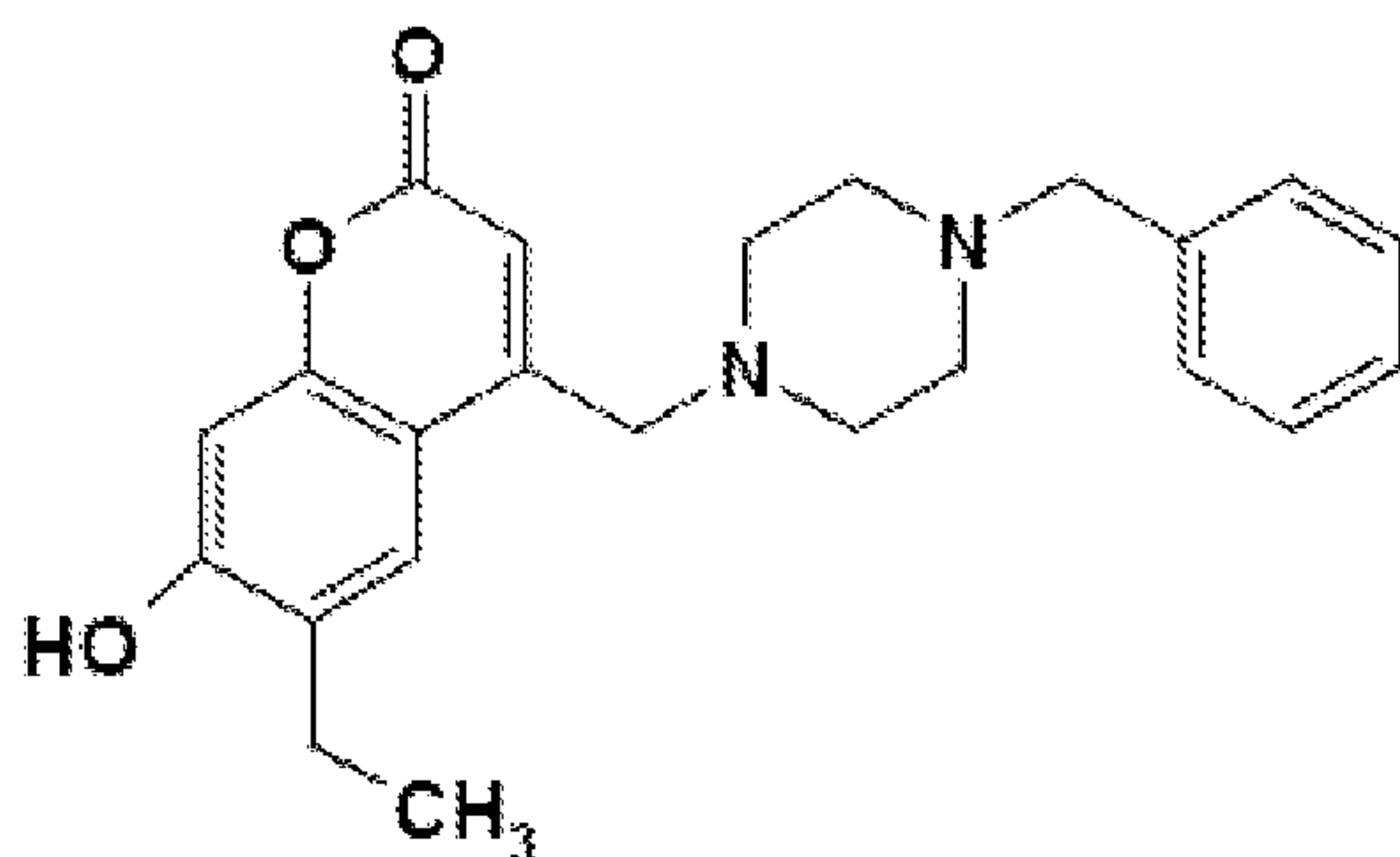
Claim 5. The compound of claim 4 wherein R^{28} is hydrogen.

Claim 6. The compound of any one of claims 1-5 wherein R^{30} is straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation.

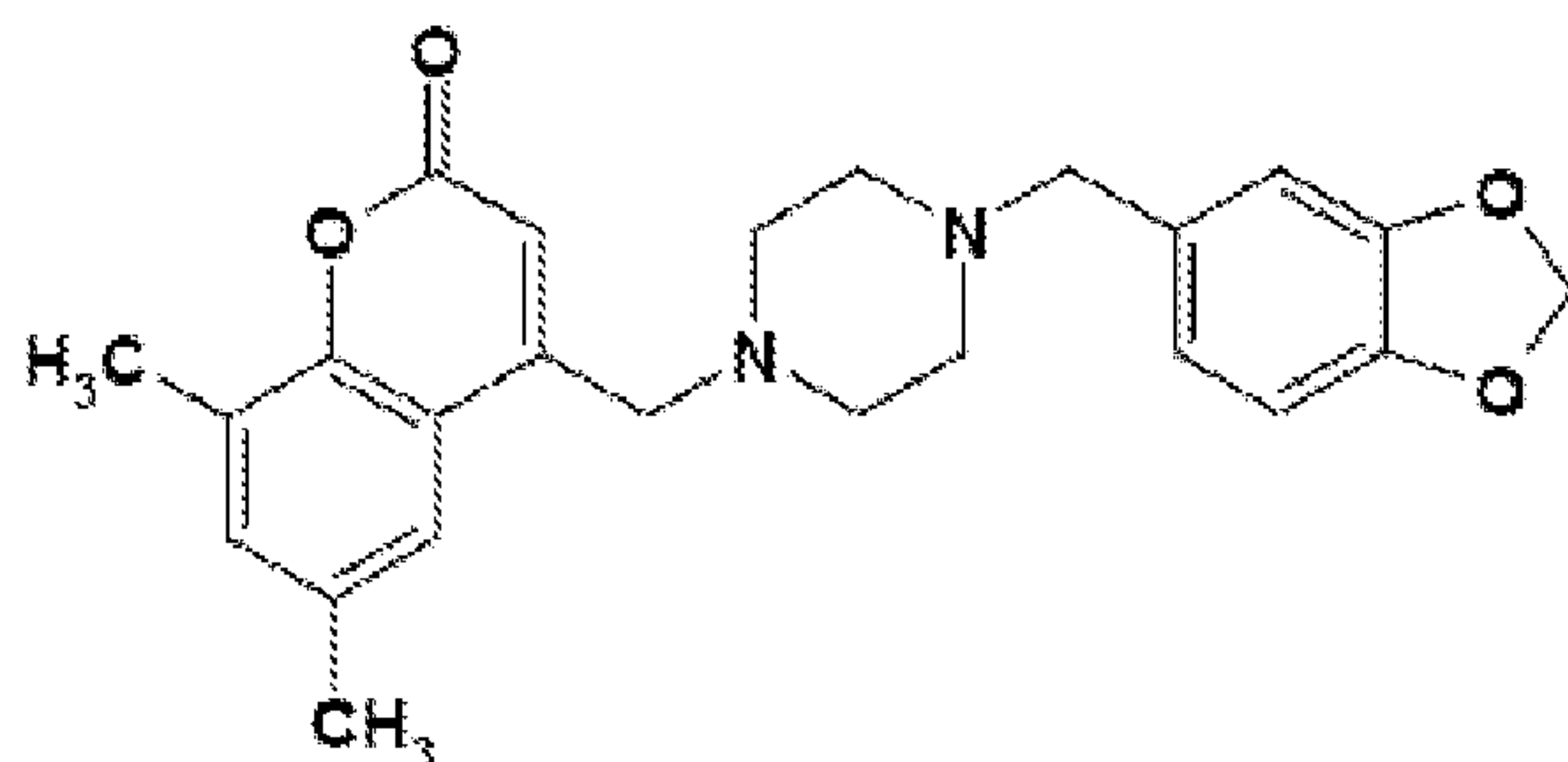
Claim 7. The compound of any one of claims 1-6 wherein R^{31} is selected from the group consisting of: hydroxy or alkoxy $-OR^{10}$.

Claim 8. The compound of any one of claims 1-7 wherein R^{32} is hydrogen.

Claim 9. The compound of claim 1 selected from the group consisting of:

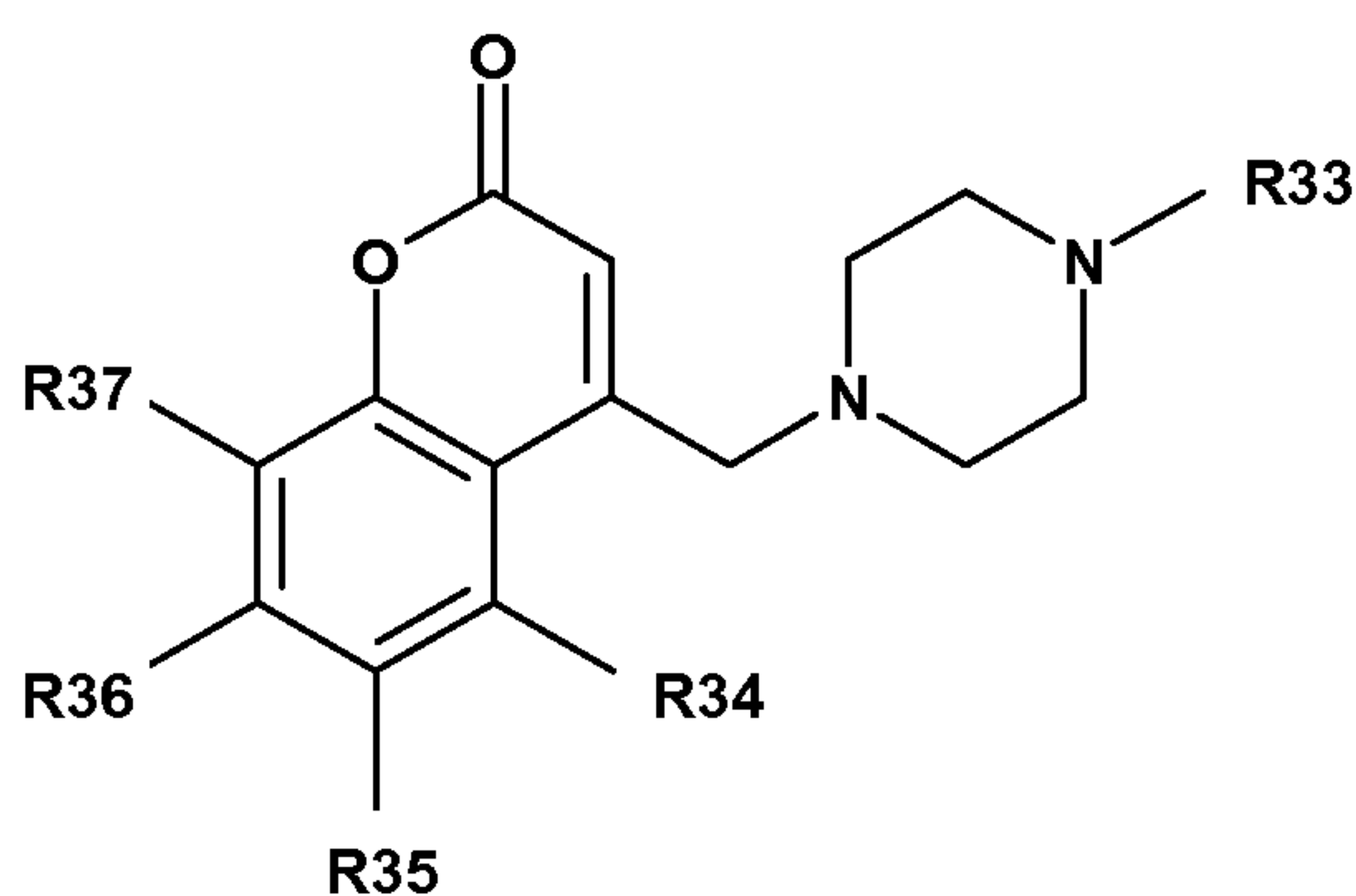


AD4-10631; and



AD4-10174.

Claim 10. A compound having a formula of:



Formula (14) (1734-like Type Q)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R³³ is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl -COOR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of: straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; trifluoromethyl; trifluoromethoxy; difluoromethoxy; 3, 4-methylenedioxy; 2, 3-methylenedioxy; nitro; and halogen; and

(c) aryloxy (-OAr); and

(iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(d) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(e) trifluoromethyl;

(f) trifluoromethoxy;

(g) difluoromethoxy;

(h) 3, 4-methylenedioxy;

(i) 2, 3-methylenedioxy;

(j) nitro; and

(k) halogen;

R³⁴ is hydrogen;

R³⁵ is selected from the group consisting of:

(i) hydrogen;

(ii) hydroxy;

(iii) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(v) trifluoromethyl; and

(vi) halogen;

R³⁶ is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) hydroxy;

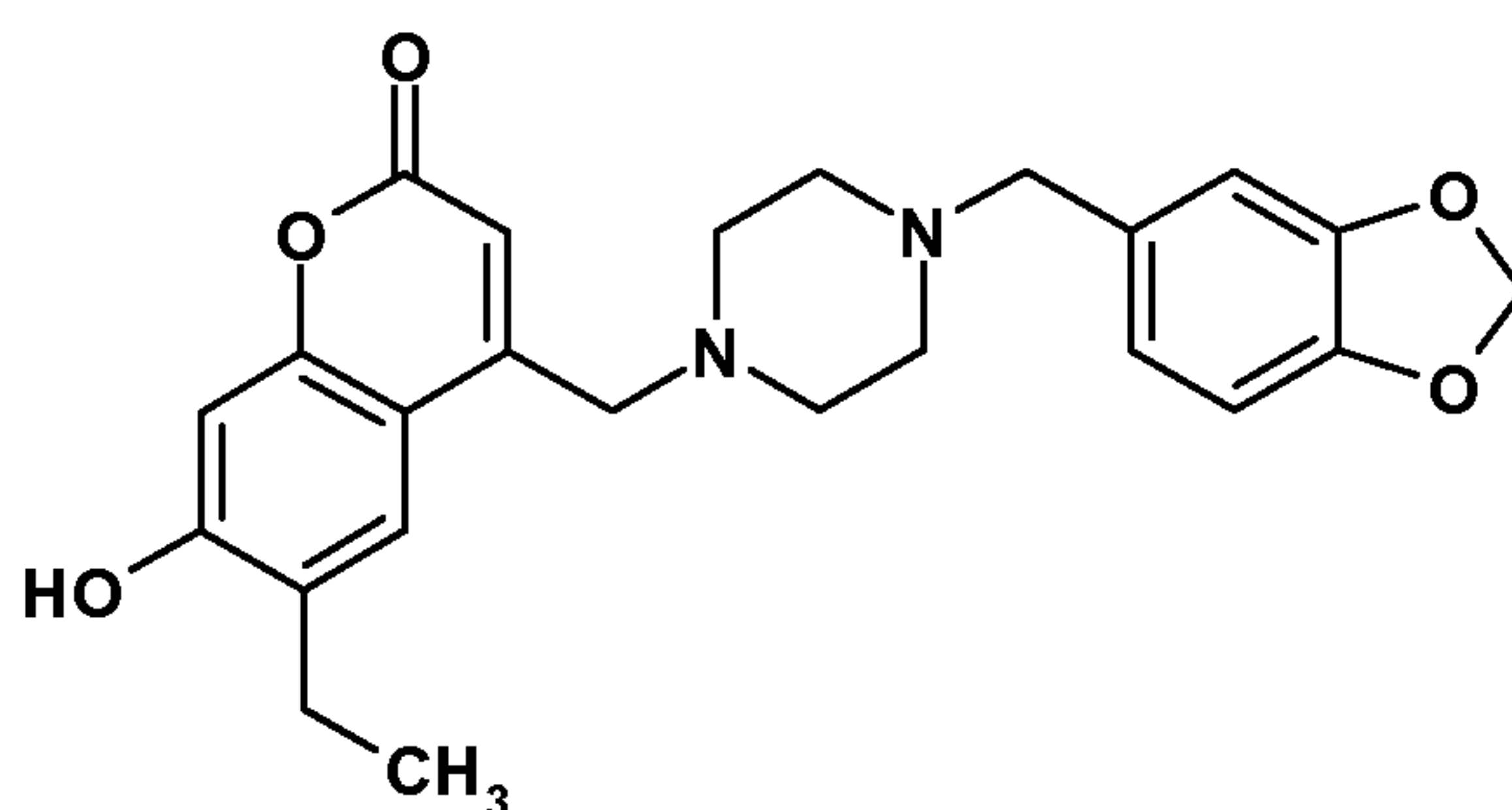
(iv) alkoxy -OR¹⁰ where R¹⁰ is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(v) trifluoromethyl; and

(vi) halogen; and

R³⁷ is hydrogen; and

Formula (14) excludes a compound of Formula (12):



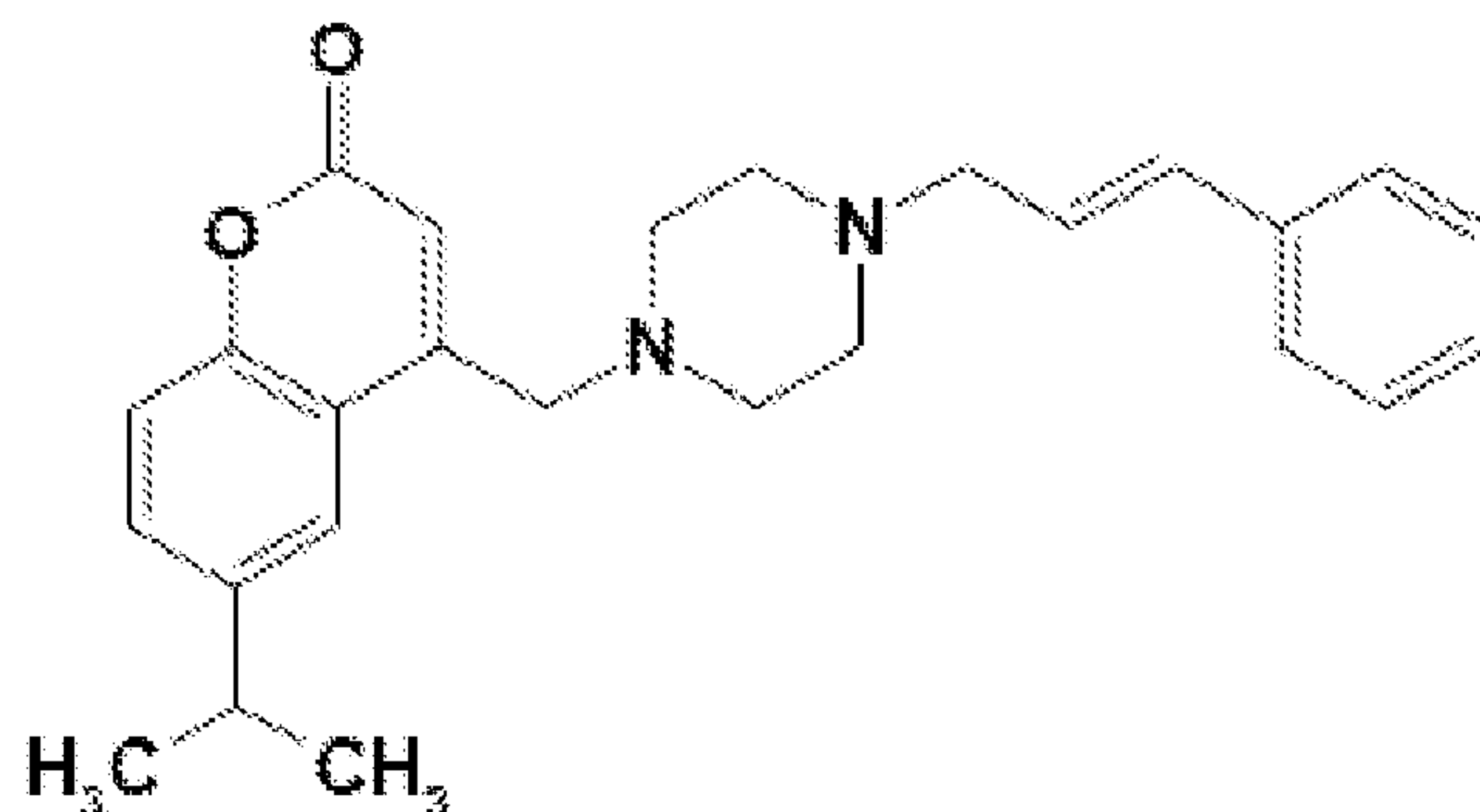
Formula (12).

Claim 11. The compound of claim 10 wherein R³⁵ is straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation.

Claim 12. The compound of any one of claims 10-11 wherein R^{36} is selected from the group consisting of:

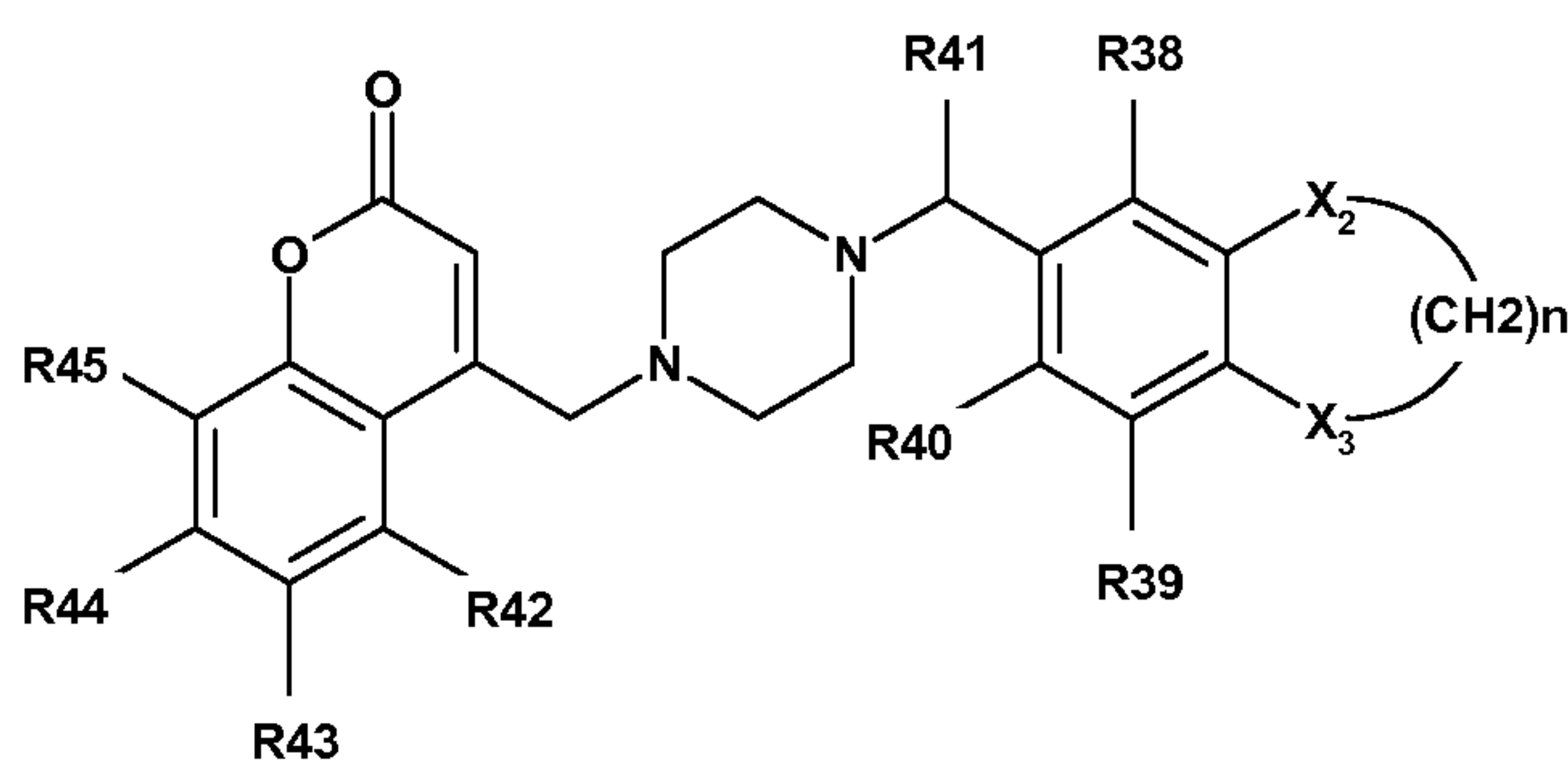
- (i) hydroxy and
- (ii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom.

Claim 13. The compound of claim 10 selected from the group consisting of:



AD4-10188.

Claim 14. A compound having a formula of:



Formula (15) (1734-like Type R)

or a stereoisomer or pharmaceutically acceptable salt thereof;
wherein,

n is 1 or 2;

X^2 and X^3 are independently selected from the group consisting of: carbon, oxygen, nitrogen, and sulfur;

R^{38} is hydrogen;

R^{39} is selected from the group consisting of:

- (i) hydrogen;
- (ii) C-1 to C-6 lower alkyl;
- (iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl

optionally containing unsaturation; and

- (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (iv) halogen;

R^{40} is hydrogen;

R^{41} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally

containing unsaturation; and

- (iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

- (a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

- (d) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

- (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

- (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

- (e) trifluoromethyl;
- (f) trifluoromethoxy;

- (g) difluoromethoxy;
- (h) 3, 4-methylenedioxy;
- (i) 2, 3-methylenedioxy;
- (j) nitro; and
- (k) halogen;

R^{42} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (iii) hydroxy;
- (iv) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (v) halogen;

R^{43} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iv) trifluoromethyl; and
- (v) halogen;

R^{44} is selected from the group consisting of:

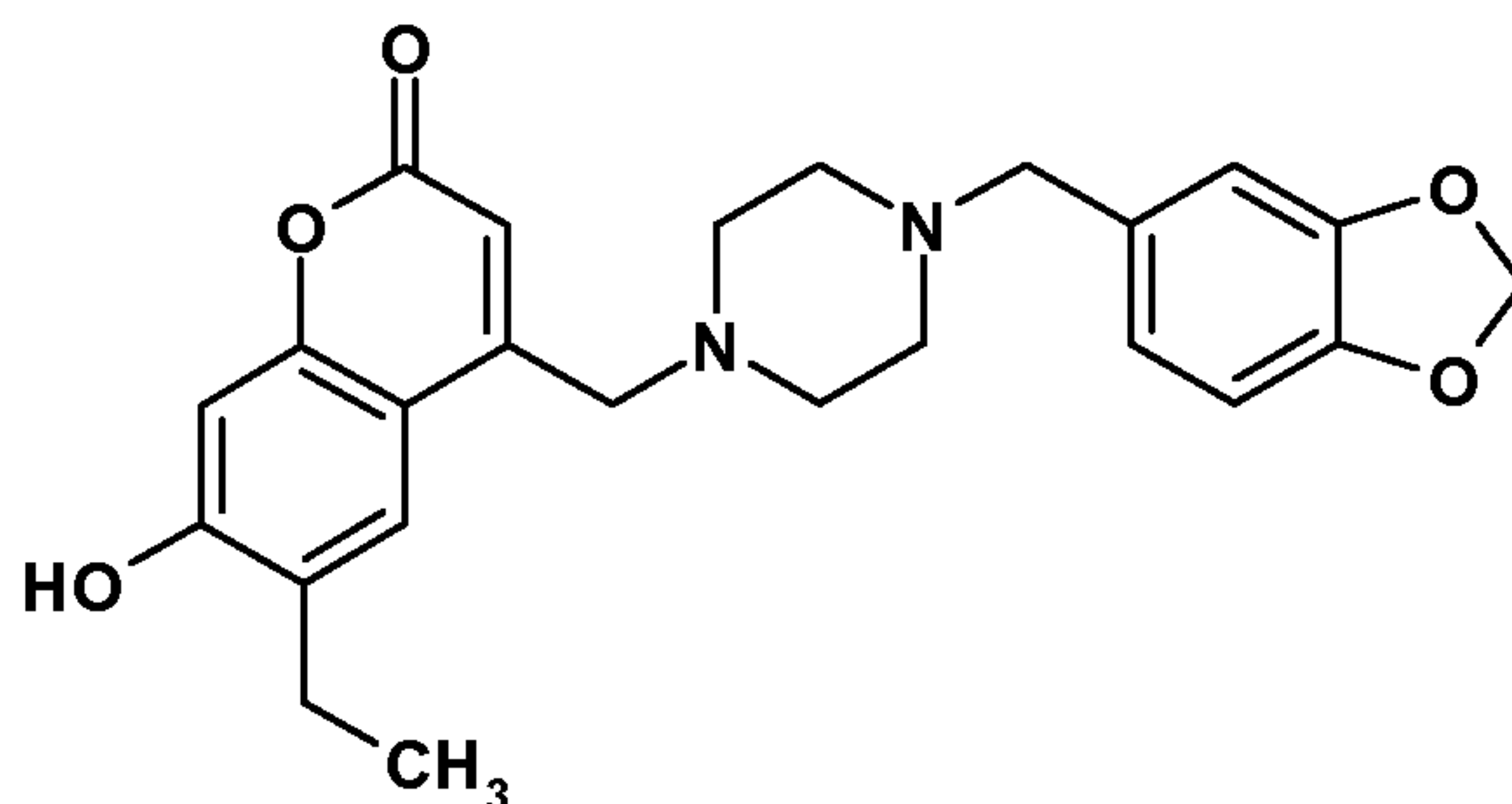
- (i) hydrogen;
- (ii) hydroxy;
- (iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

- (a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
- (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iv) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (v) trifluoromethyl; and
- (vi) halogen;

R^{45} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (iii) hydroxy;
- (iv) trifluoromethyl; and
- (v) halogen; and

Formula (15) excludes the compound of Formula (12):



Formula (12).

Claim 15. The compound of claim 14 wherein n is 1.

Claim 16. The compound of any one of claims 14-15 wherein X^2 and X^3 are independently selected from the group consisting of: oxygen or carbon.

Claim 17. The compound of any one of claims 14-16 wherein R^{42} is hydrogen.

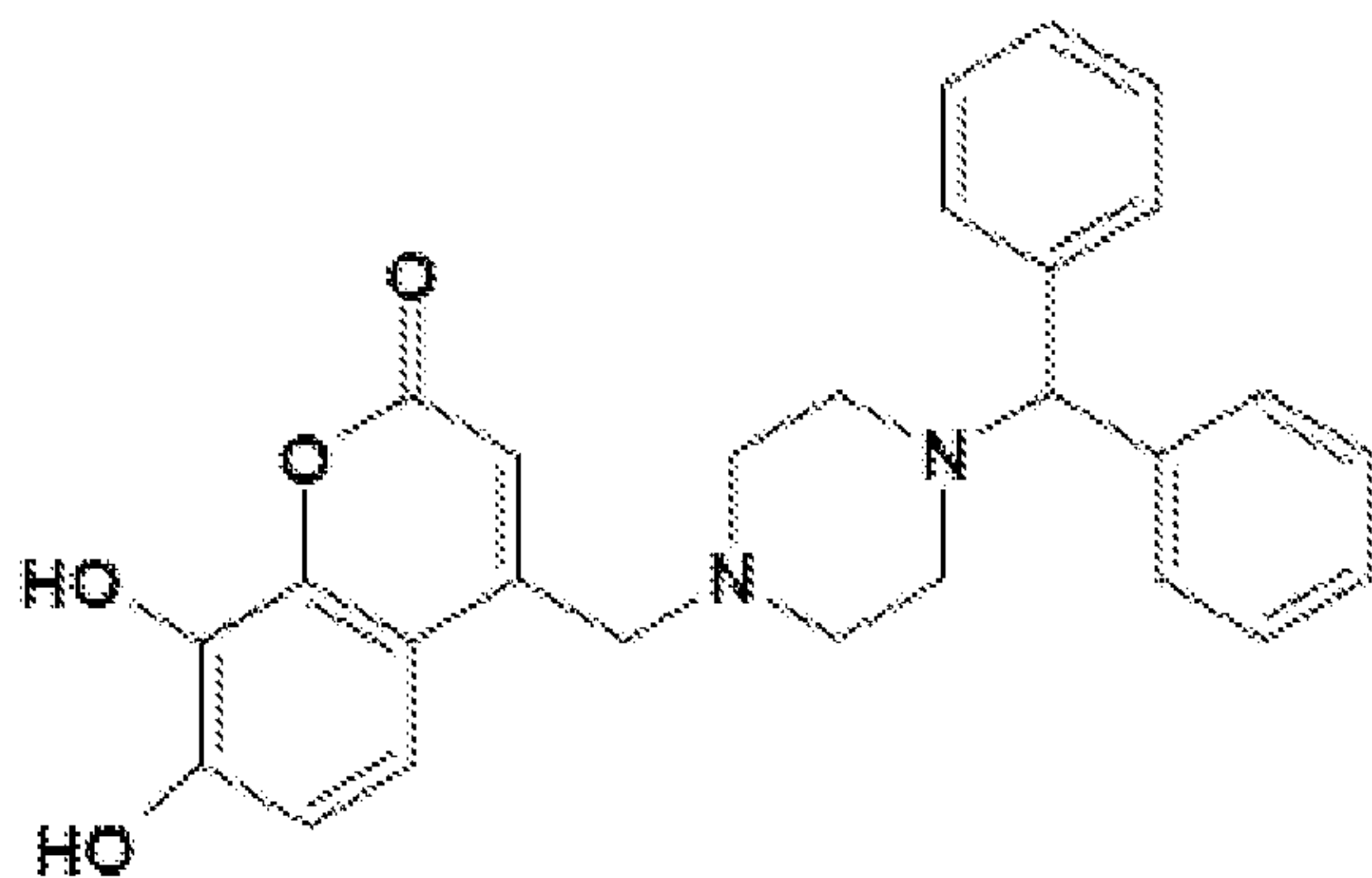
Claim 18. The compound of any one of claims 14-17 wherein R^{43} is straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation.

Claim 19. The compound of any one of claims 14-18 wherein R^{44} is selected from the group consisting of:

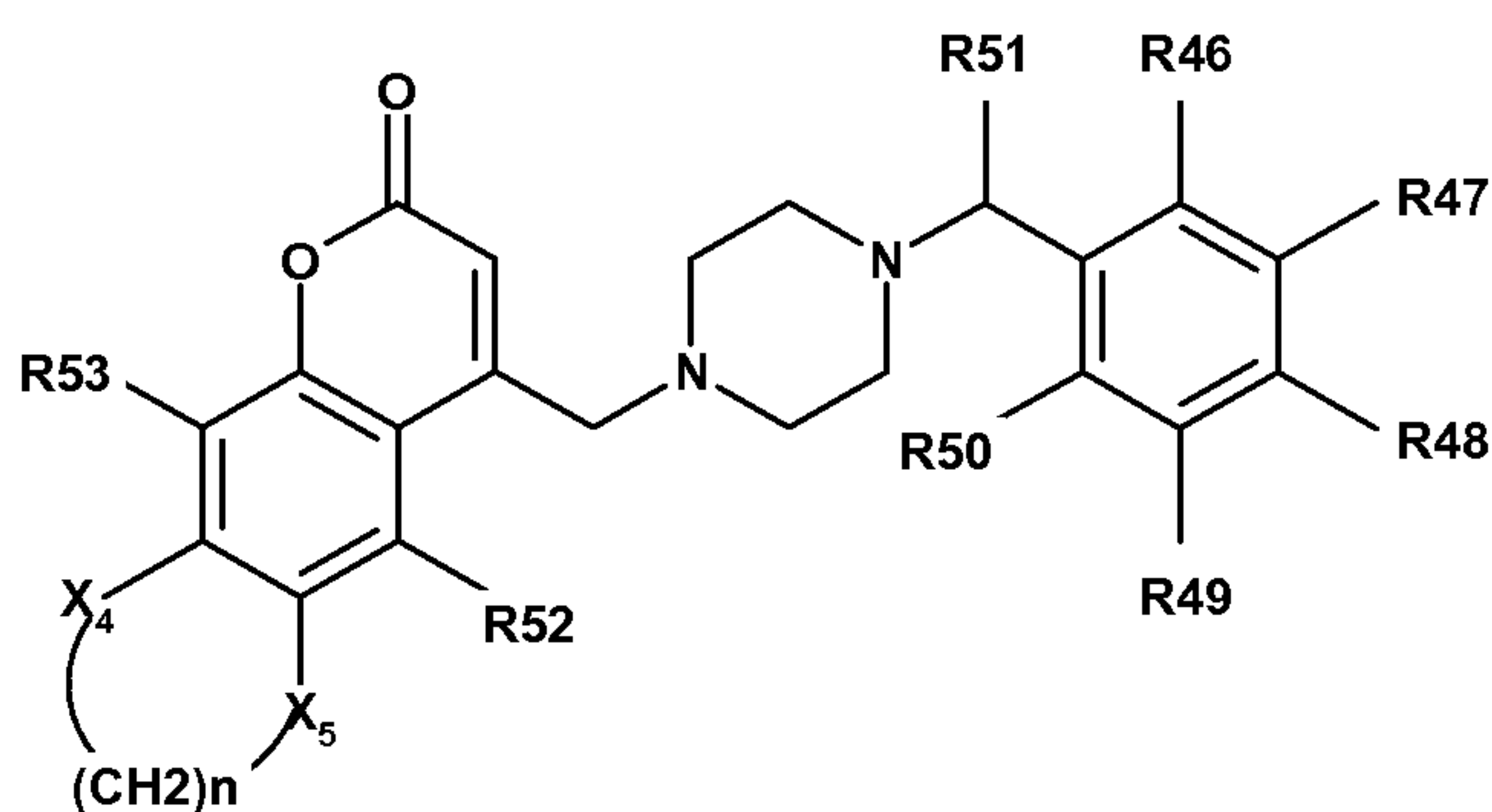
- (i) hydroxy and
- (ii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom.

Claim 20. The compound of any one of claims 14-19 wherein R^{45} is hydrogen.

Claim 21. The compound of claim 14 selected from the group consisting of:



Claim 22. A compound having a formula of:



or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

n is 1 or 2;

X^4 and X^5 are independently selected from the group consisting of: carbon, oxygen, nitrogen and sulfur;

R^{46} and R^{50} are hydrogen;

R^{47} and R^{49} are independently selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) halogen; and

(v) R^{47} and R^{49} form a 5 or 6 membered cycloalkyl ring;

R^{48} is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl $-COOR^{10}$ where R^{10} is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of: (A) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and (B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr);

(v) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(vi) halogen; and

(vii) R⁴⁸ forms a 5 or 6 membered cycloalkyl ring with R⁴⁷;

R⁵¹ is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(d) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(e) trifluoromethyl;

(f) trifluoromethoxy;

(g) difluoromethoxy;

(h) 3, 4-methylenedioxy;

(i) 2, 3-methylenedioxy;

(j) nitro; and

(k) halogen;

R⁵² is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) hydroxy;

(iv) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

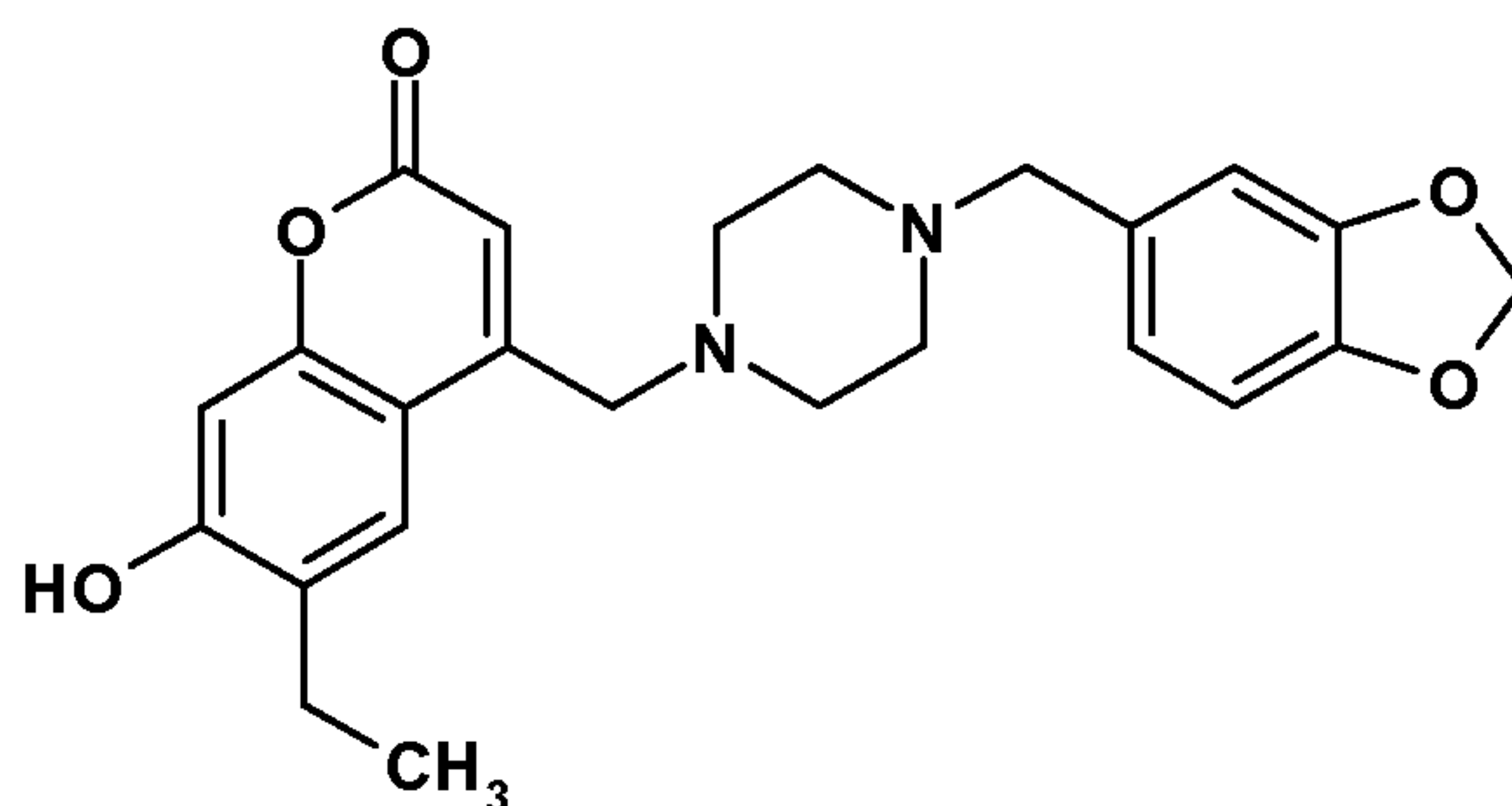
(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(v) halogen;

R^{53} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (iii) hydroxy;
- (iv) trifluoromethyl; and
- (v) halogen; and

Formula (16) excludes the compound of Formula (12):



Formula (12).

Claim 23. The compound of claim 22 wherein n is 1.

Claim 24. The compound of any one of claims 22-23 wherein X^4 and X^5 are independently selected from the group consisting of: oxygen and carbon.

Claim 25. The compound of any one of claims 22-24 wherein R^{48} is selected from the group consisting of: hydrogen or straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation.

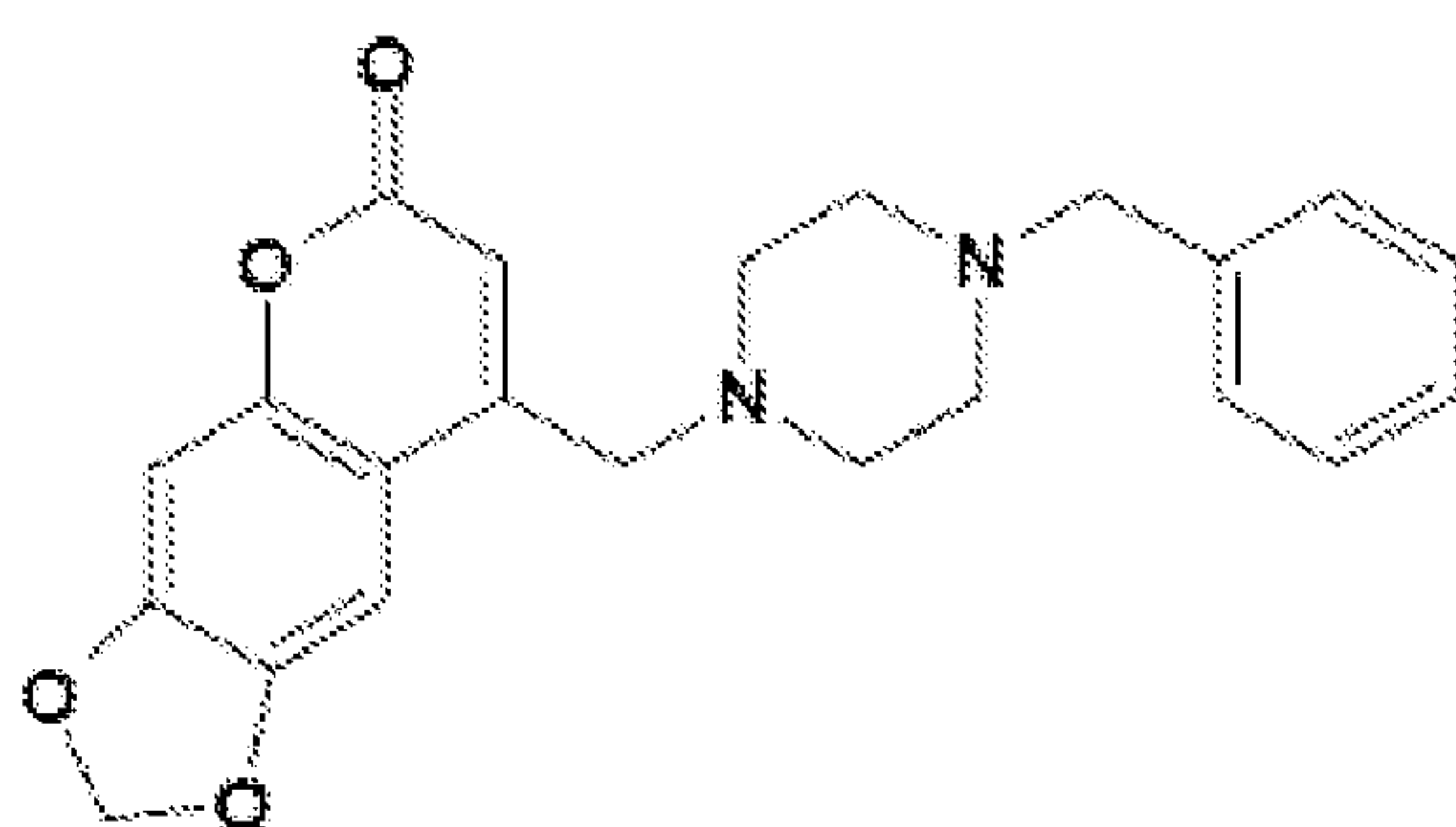
Claim 26. The compound of any one of claims 22-25 wherein R^{47} and R^{48} form a 5 membered cycloalkyl ring.

Claim 27. The compound of any one of claims 22-26 wherein R⁵¹ is selected from the group consisting of:

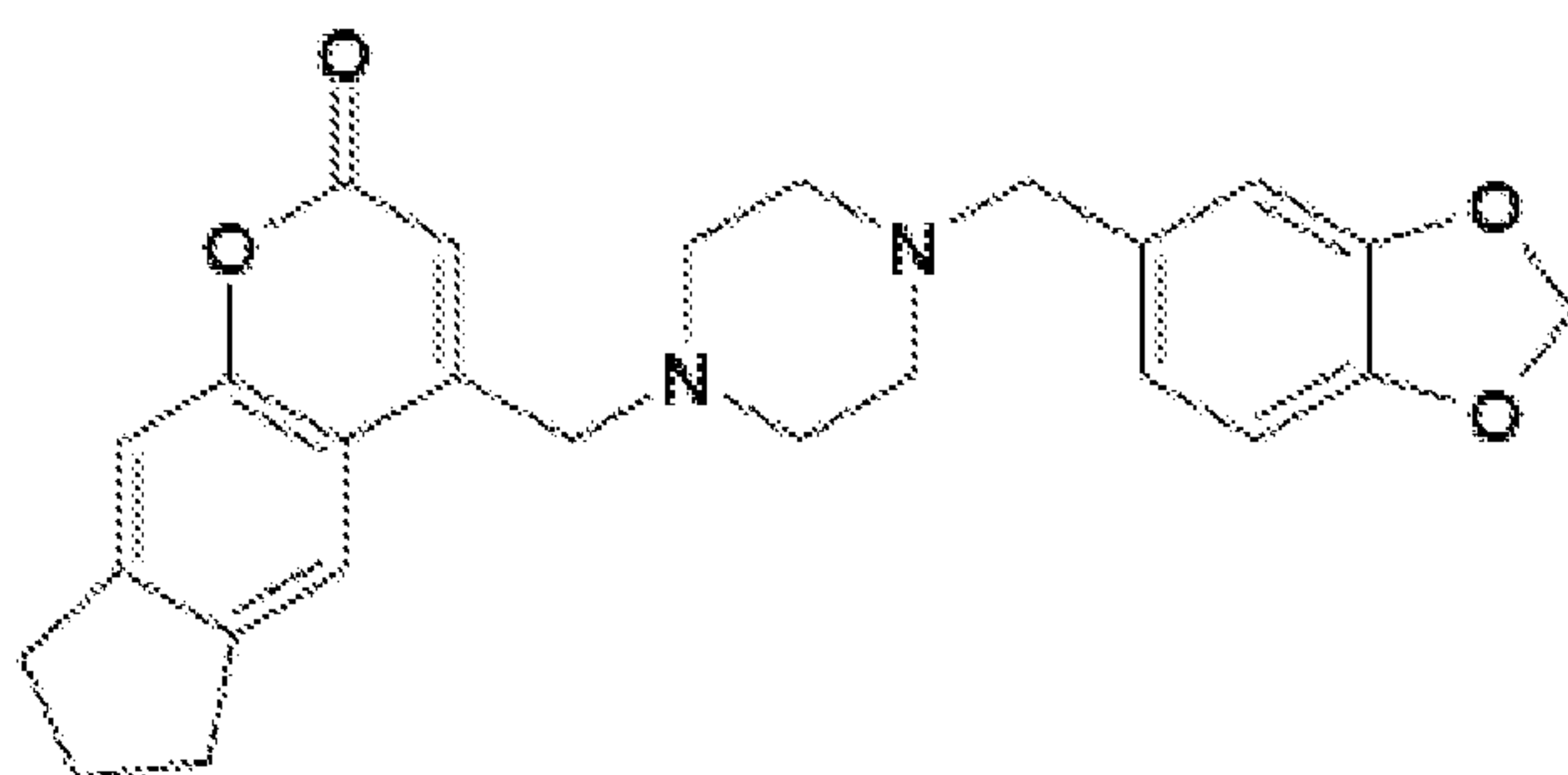
- (i) hydrogen; and
- (ii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:
 - (a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;
 - (d) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:
 - (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (e) trifluoromethyl;
 - (f) trifluoromethoxy;
 - (g) difluoromethoxy;
 - (h) 3, 4-methylenedioxy;
 - (i) 2, 3-methylenedioxy;
 - (j) nitro; and
 - (k) halogen.

Claim 28. The compound of any one of claims 22-27 wherein R⁵² is hydrogen.

Claim 29. The compound of claim 22 selected from the group consisting of:



AD4-10639; and



AD4-10179.

Claim 30. The compound of any one of claims 1-29 that inhibits EGFR activity comprising:

five or more of functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9 of a Scheme II 1734-like pharmacophore;

wherein

functional group F(II)1 donates an H-bond or forms a salt bridge to a carboxylate side chain of receptor Asp553 of SEQ ID NO: 1 and has coordinates of $r = 56.363$, θ (theta) = 94.368 , and Φ (phi) = -17.752 and a spherical radius of about $1.2\text{\AA}$;

functional group F(II)2 is a donor and has coordinates of $r = 53.290$, θ (theta) = 101.494 , and Φ (phi) = -23.244 and a spherical radius of about $1.0\text{\AA}$;

functional group F(II)3 forms a hydrophobic contact with a side chain of receptor Val568, imidazolidine side chain of receptor Pro552 and with a side chain of Met253 of SEQ ID NO: 1 and has coordinates of $r = 53.726$, θ (theta) = 97.830 , and Φ (phi) = -18.377 and a spherical radius of about $1.7\text{\AA}$;

functional group F(II)4 forms a hydrophobic contact with side chain of receptor Val575, Met253, and with an imidazolidine ring of receptor Pro552 of SEQ ID NO: 1 and has coordinates of $r = 53.647$, θ (theta) = 103.844 , and Φ (phi) = -20.990 and a spherical radius of about $1.4\text{\AA}$;

functional group F(II)5 donates an H-bond to a side chain hydroxyl of Thr570 of SEQ ID NO: 1 and has coordinates of $r = 51.093$, θ (theta) = 104.261 , and Φ (phi) = -25.552 and a spherical radius of about $1.2\text{\AA}$;

functional group F(II)6 is a donor having directionality of F4 with respect to a backbone carbonyl of receptor Thr570 of SEQ ID NO: 1 and has coordinates of $r = 52.340$, θ (theta) = 103.980 , and Φ (phi) = -27.461 and a spherical radius of about $1.5\text{\AA}$;

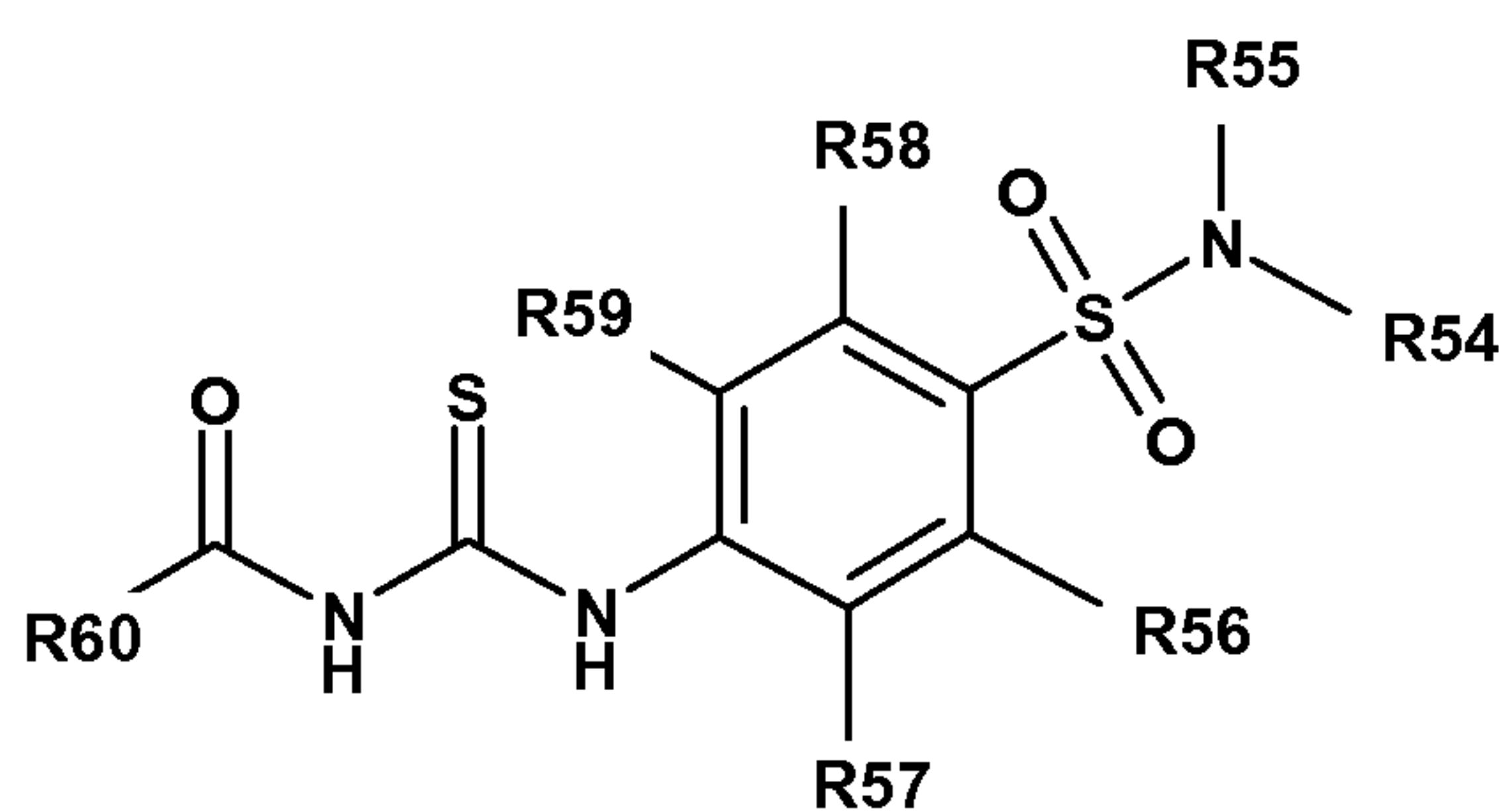
functional group F(II)7 accepts an H-bond from a receptor backbone NH of Ala573 of SEQ ID NO: 1 and has coordinates of $r = 51.383$, θ (theta) = 106.455 , and Φ (phi) = -24.319 and a spherical radius of about $1.2\text{\AA}$;

functional group F(II)8 is an acceptor having directionality of F7 with respect to the backbone NH of receptor Ala573 of SEQ ID NO: 1 and has coordinates of $r = 52.861$, θ (theta) = 107.691 , and Φ (phi) = -25.448 and a spherical radius of about $1.5\text{\AA}$;

functional group F(II)9 donates an H-bond to a backbone carbonyl of Asp563 and forms a salt bridge to a side chain carboxylate of receptor Asp563 of SEQ ID NO: 1 and has coordinates of $r = 57.688$, θ (theta) = 99.198 , and Φ (phi) = -21.588 and a spherical radius of about $1.2\text{\AA}$; and

the compound substantially maintains a non-extended tether inactive configuration of EGFR or substantially prevents stabilization of an extended tether active configuration of EGFR.

Claim 31. A compound having a formula of:



Formula (18) (1886-like Type J)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{54} is selected from the group consisting of:

(i) a 5 or 6 membered heterocyclic containing from 1 to 4 N, O, or S atoms or any combination of those atoms with carbon atoms to form a heterocyclic aromatic ring optionally substituted with from 1 to 3 groups selected from:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(c) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(d) trifluoromethyl,

(e) trifluoromethoxy,

(f) substituted amino,

(g) nitro; and

(h) halogen;

R⁵⁵ is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R⁵⁶, R⁵⁷, R⁵⁸, and R⁵⁹ are hydrogen;

R⁶⁰ is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl -COOR¹⁰ where R¹⁰ is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

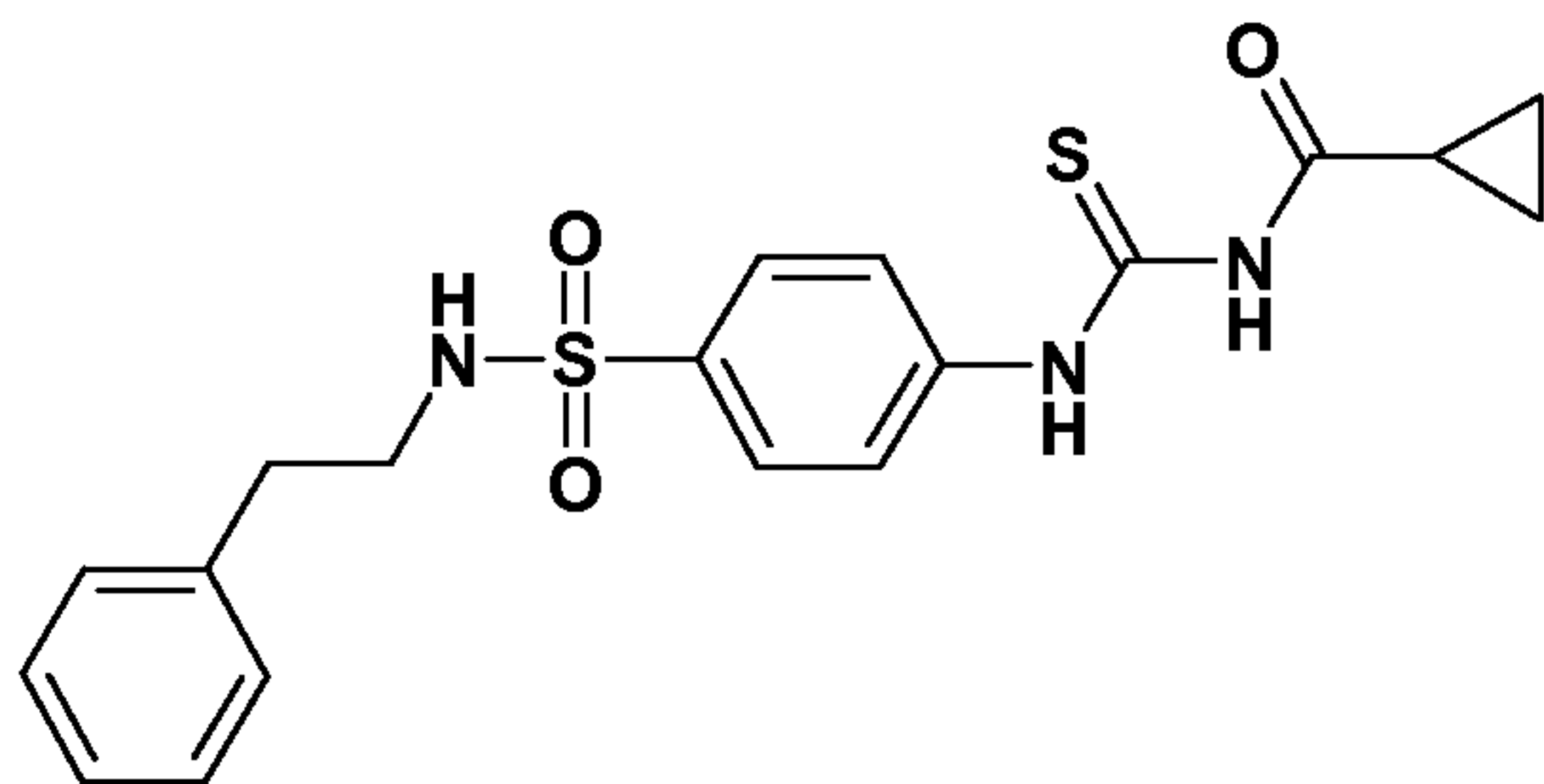
(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr); and

Formula (18) excludes a compound of Formula (17):

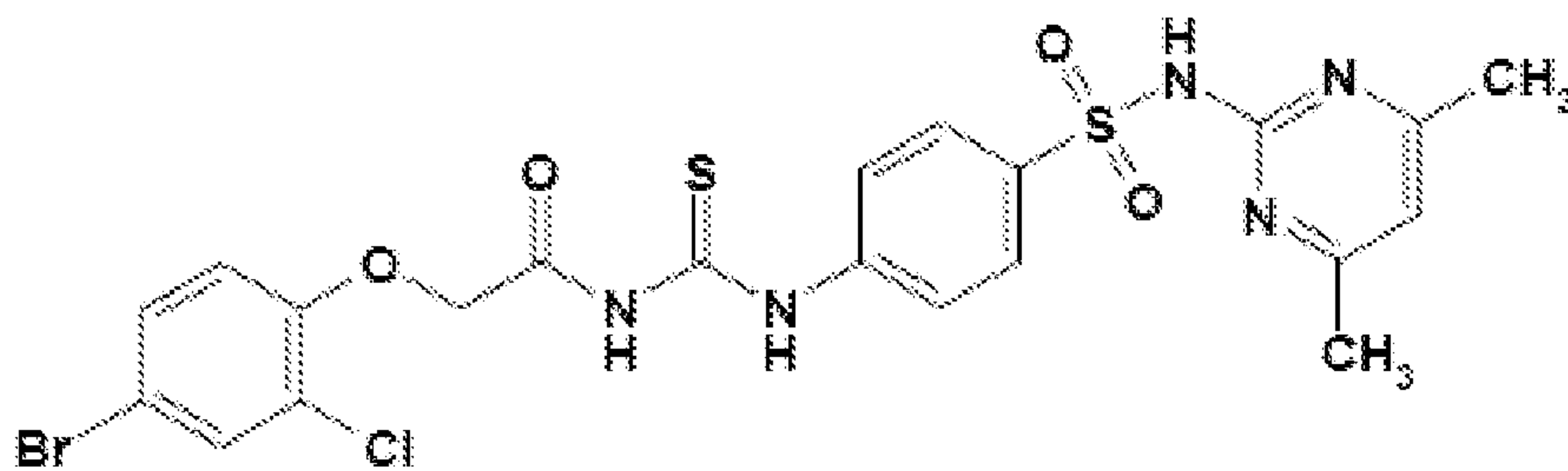


Formula (17).

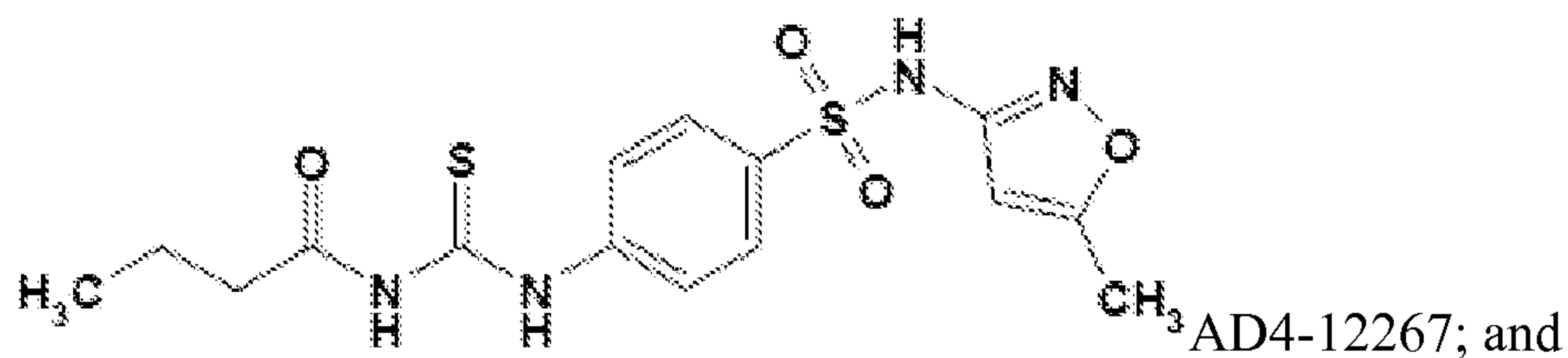
Claim 32. The compound of claim 31 wherein R⁵⁴ is selected from the group consisting of: 4,6-dimethyl-2-pyrimidine; 2,6-dimethoxy-4-pyrimidine; 6-methoxy-4-pyrimidine; 5-ethyl-2-(1,3,4-Thiadiazole); 5-methyl-3-Isoxazole; 3-methoxy-6-pyridazinamine, 2-thiazole; and 2-methoxy-3-pyrazine.

Claim 33. The compound of any one of claims 31-32 wherein R⁵⁵ is selected from the group consisting of: hydrogen and methyl.

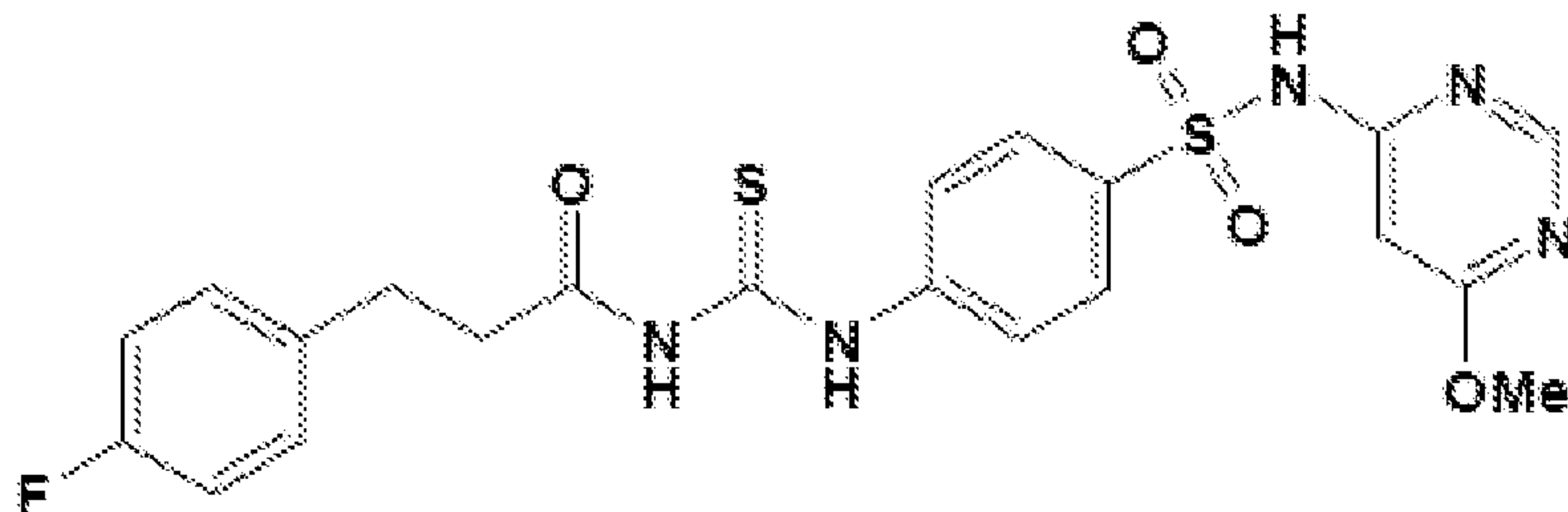
Claim 34. The compound of claim 31 selected from the group consisting of:



AD4-12158;

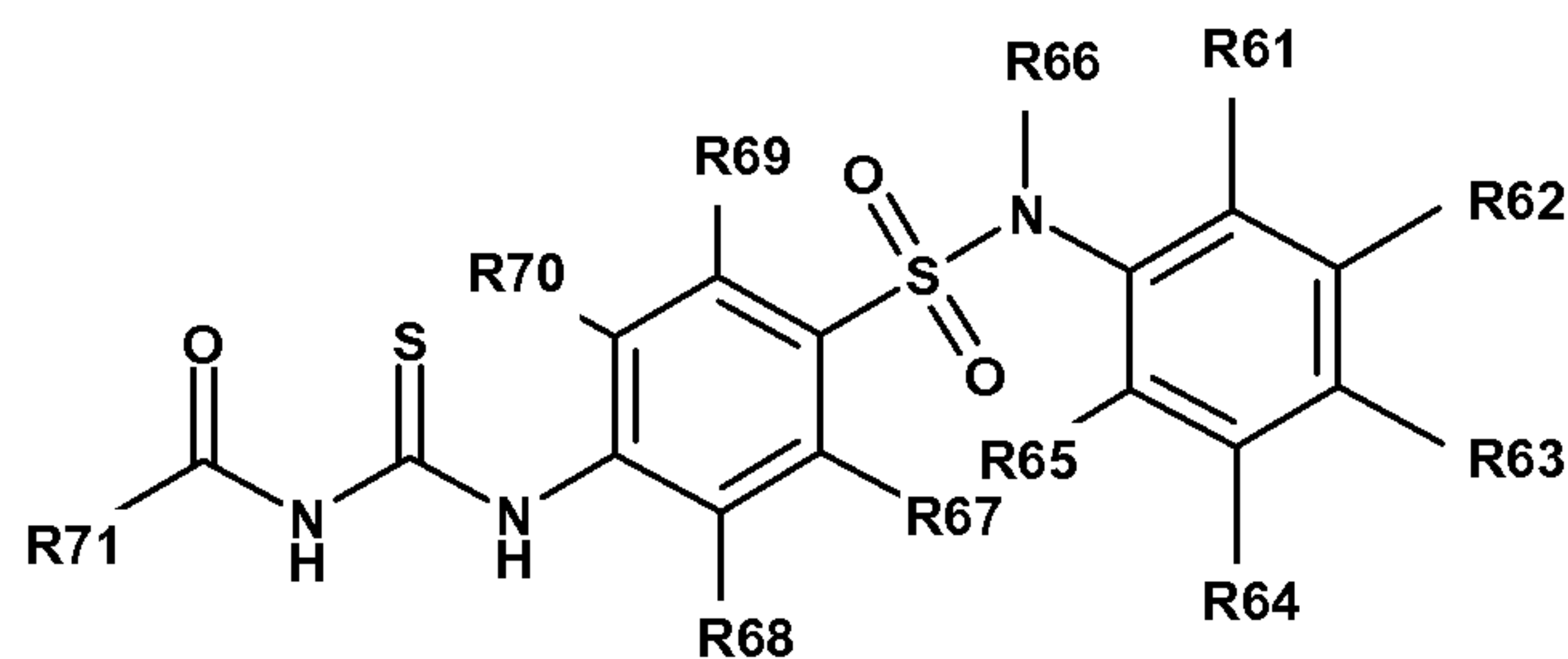


AD4-12267; and



AD4-11384.

Claim 35. A compound having a formula of:



Formula (19) (1886-like Type K)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{61} , R^{62} , and R^{64} are independently selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{63} and R^{65} are independently selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
- (iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

R^{66} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{67} , R^{68} , R^{69} , and R^{70} are hydrogen;

R^{71} is selected from the group consisting of:

- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

- (a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;
- (d) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:
- (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (e) trifluoromethyl;
- (f) trifluoromethoxy;
- (g) difluoromethoxy;
- (h) 3, 4-methylenedioxy;
- (i) 2, 3-methylenedioxy;
- (j) nitro; and
- (k) halogen;
- (iv) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:
- (a) carboxyl -COOR¹⁰ where R¹⁰ is selected from the group consisting of:
- (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr);

(v) heteroaryl containing a heterocyclic ring containing from 1 to 4 N, O, or S atoms optionally substituted with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(c) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(d) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

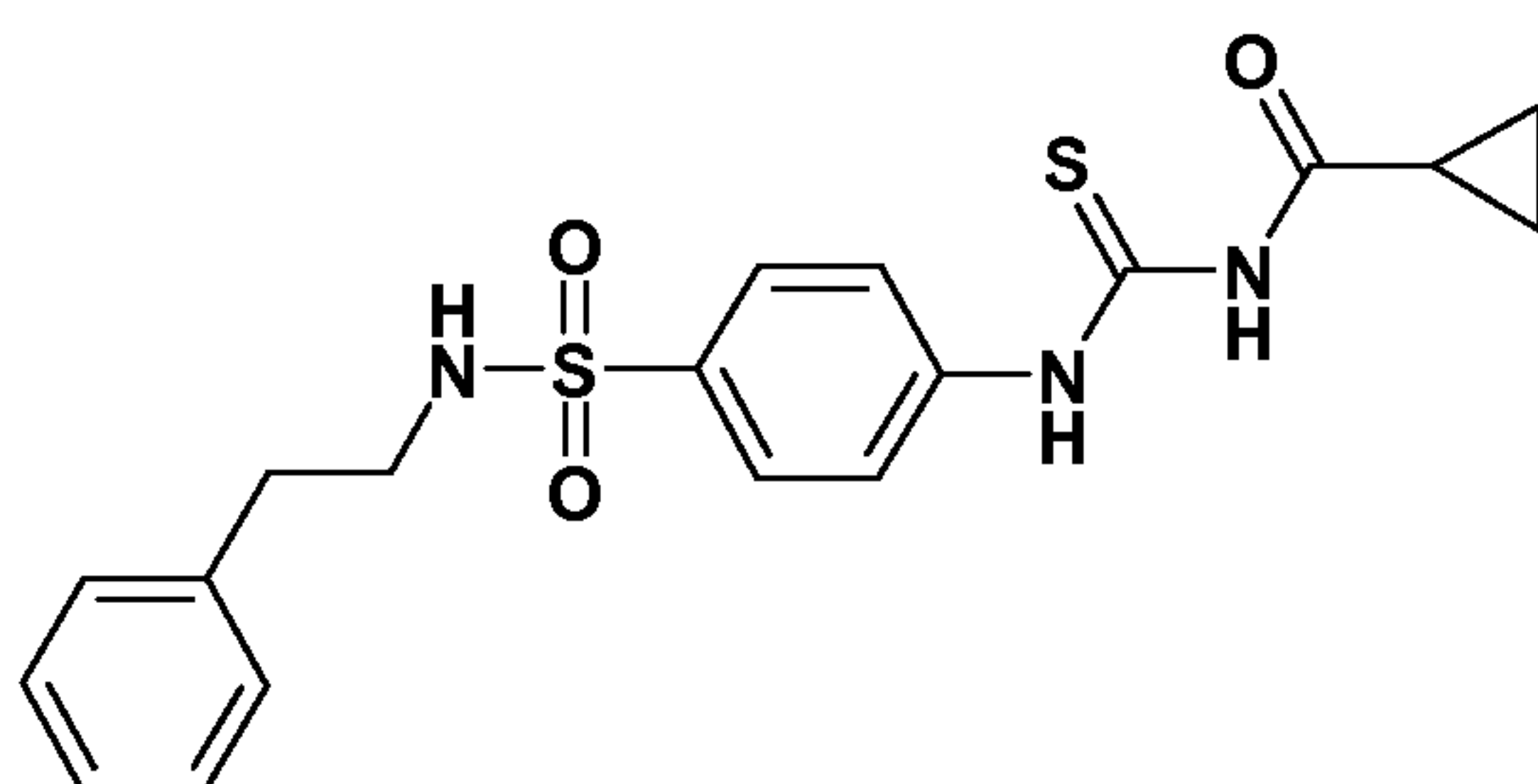
(e) trifluoromethyl;

(f) trifluoromethoxy;

(g) nitro; and

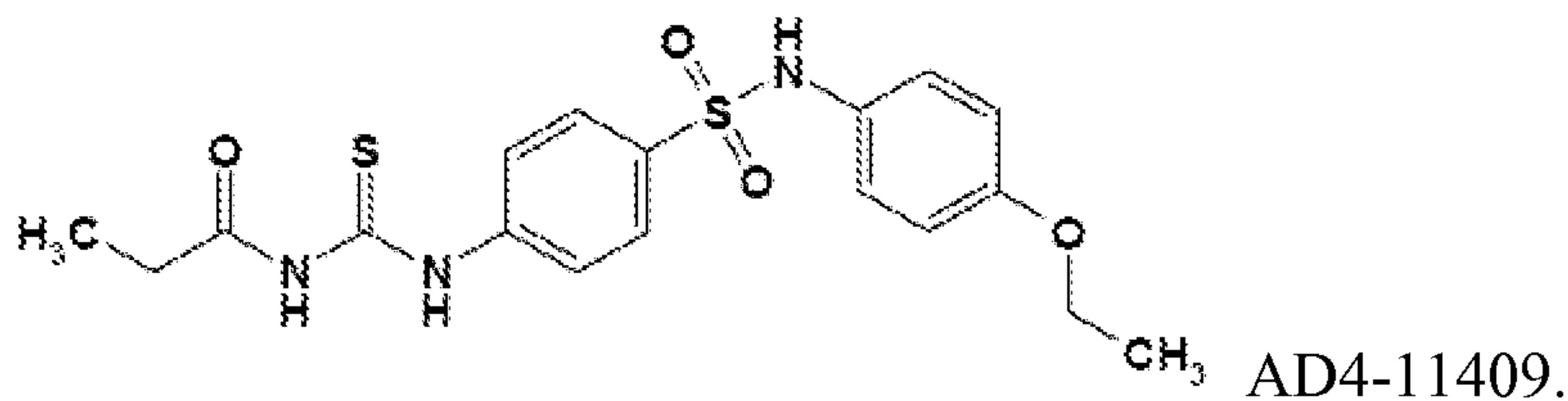
(h) halogen; and

Formula (19) excludes a compound of Formula (17):



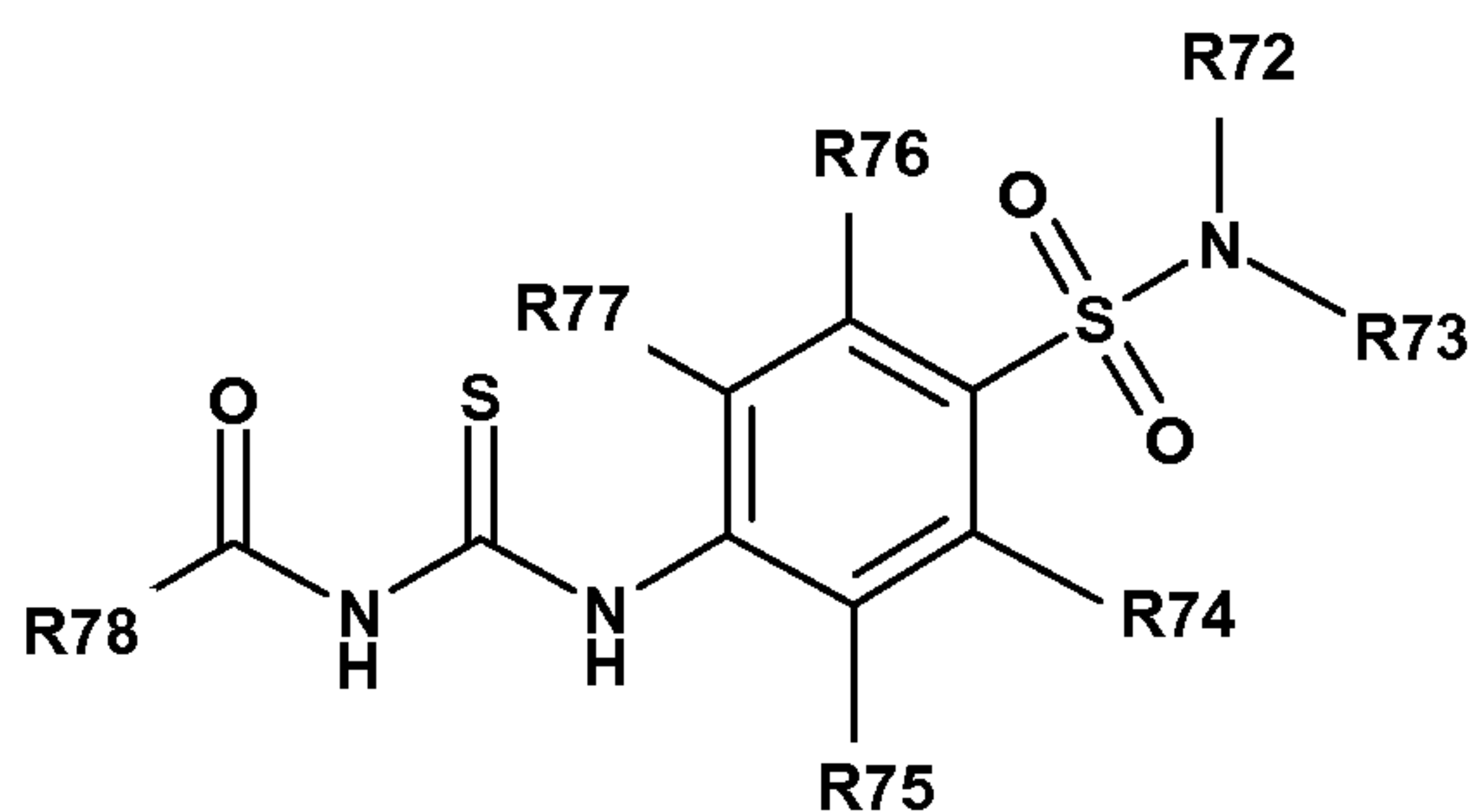
Formula (17).

Claim 36. The compound of claim 35 selected from the group consisting of:



AD4-11409.

Claim 37. A compound having a formula of:



Formula (20) (1886-like Type L)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{72} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{73} is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl $-\text{COOR}^{10}$ where R^{10} is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy $-\text{OR}^{10}$ where R^{10} is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

- (5) trifluoromethyl;
- (6) trifluoromethoxy;
- (7) difluoromethoxy;
- (8) 3, 4-methylenedioxy;
- (9) 2, 3-methylenedioxy;
- (10) nitro; and
- (11) halogen; and
- (c) aryloxy (-OAr); and
- (iv) acyl -COR¹⁰ where R¹⁰ is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- R⁷⁴, R⁷⁵, R⁷⁶, and R⁷⁷ are hydrogen; and
- R⁷⁸ is selected from the group consisting of:
 - (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:
 - (a) carboxyl -COOR¹⁰ where R¹⁰ is selected from the group consisting of:
 - (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

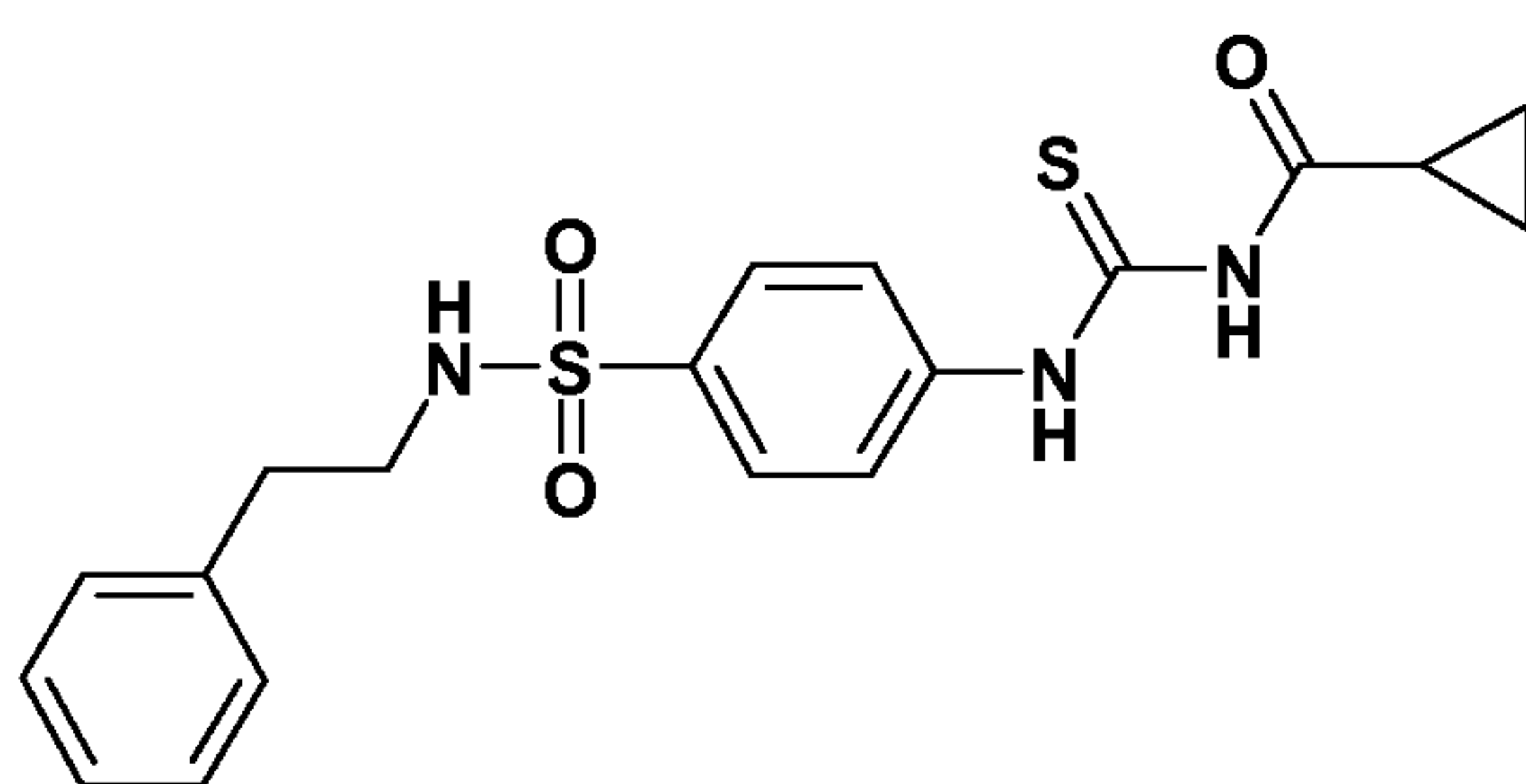
(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

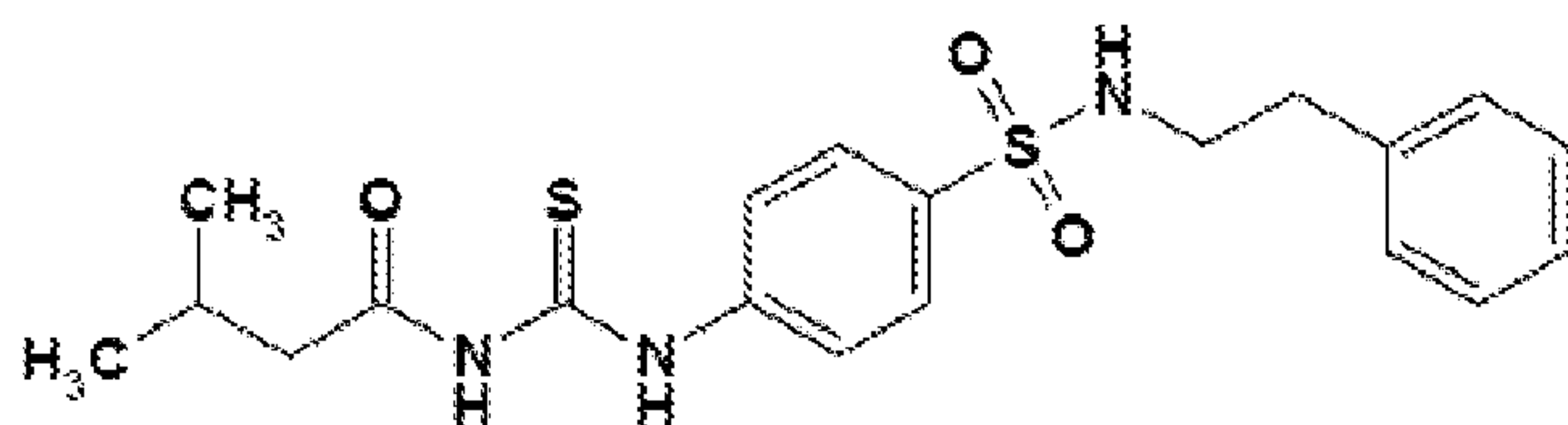
(c) aryloxy (-OAr); and

Formula (20) excludes a compound of Formula (17):

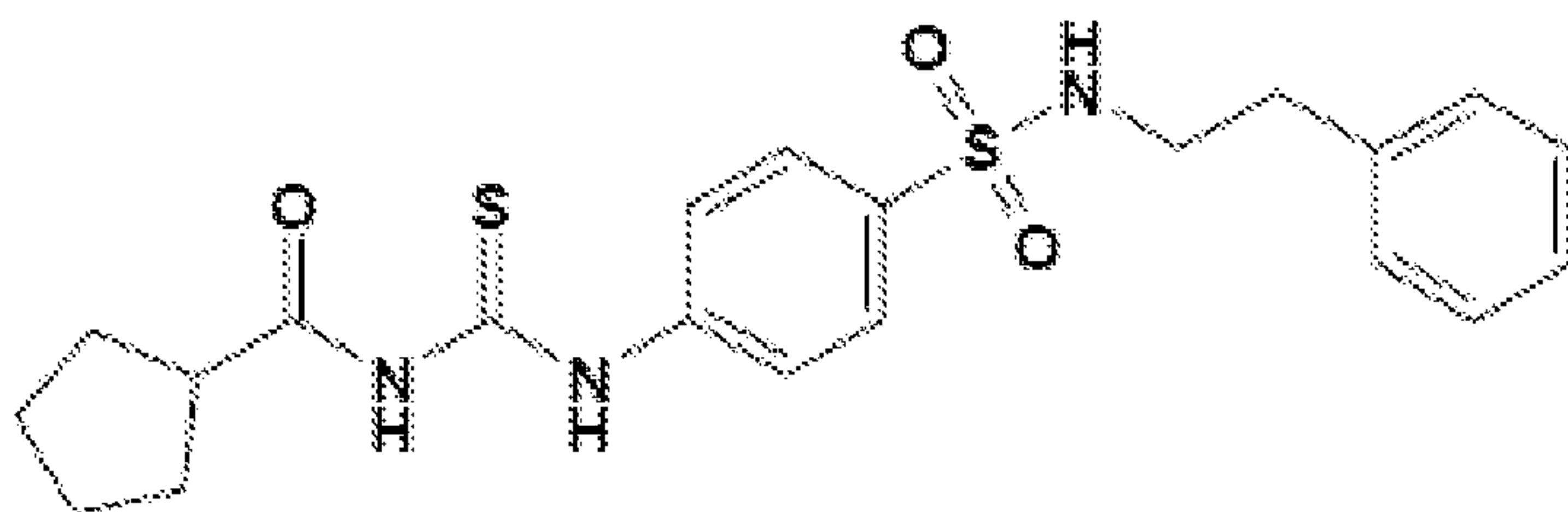


Formula (17).

Claim 38. The compound of claim 37 selected from the group consisting of:

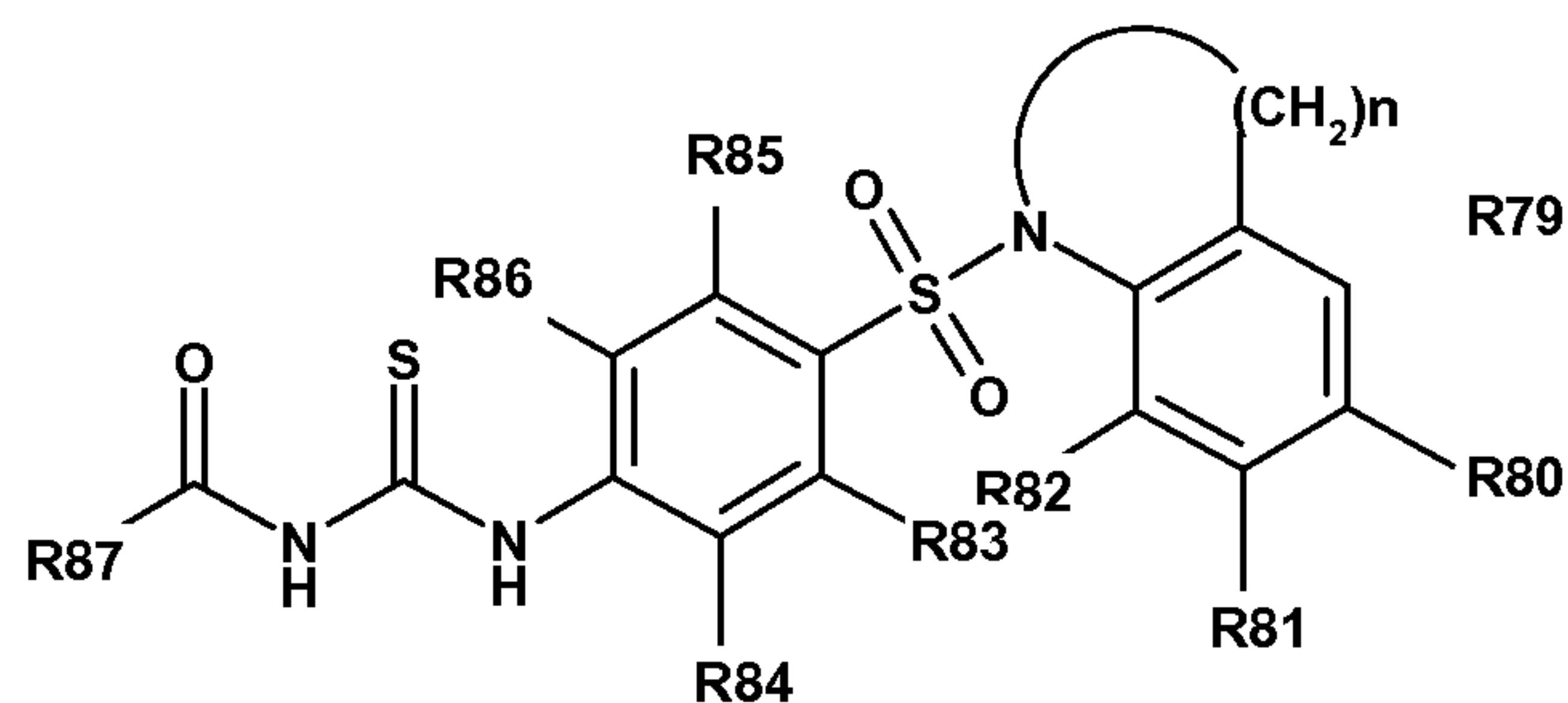


AD4-10608; and



AD4-10609.

Claim 39. A compound having a formula of:



Formula (21) (1886-like Type M)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

n is 1 or 2;

R^{79} , R^{81} , R^{82} , R^{83} , R^{84} , R^{85} , and R^{86} are hydrogen;

R^{80} is selected from the group consisting of:

(i) hydrogen,

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally

containing unsaturation;

(iii) alkoxy $-OR^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) halogen; and

(v) trifluoromethyl;

R^{87} is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl $-\text{COOR}^{10}$ where R^{10} is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; \

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy $-\text{OR}^{10}$ where R^{10} is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

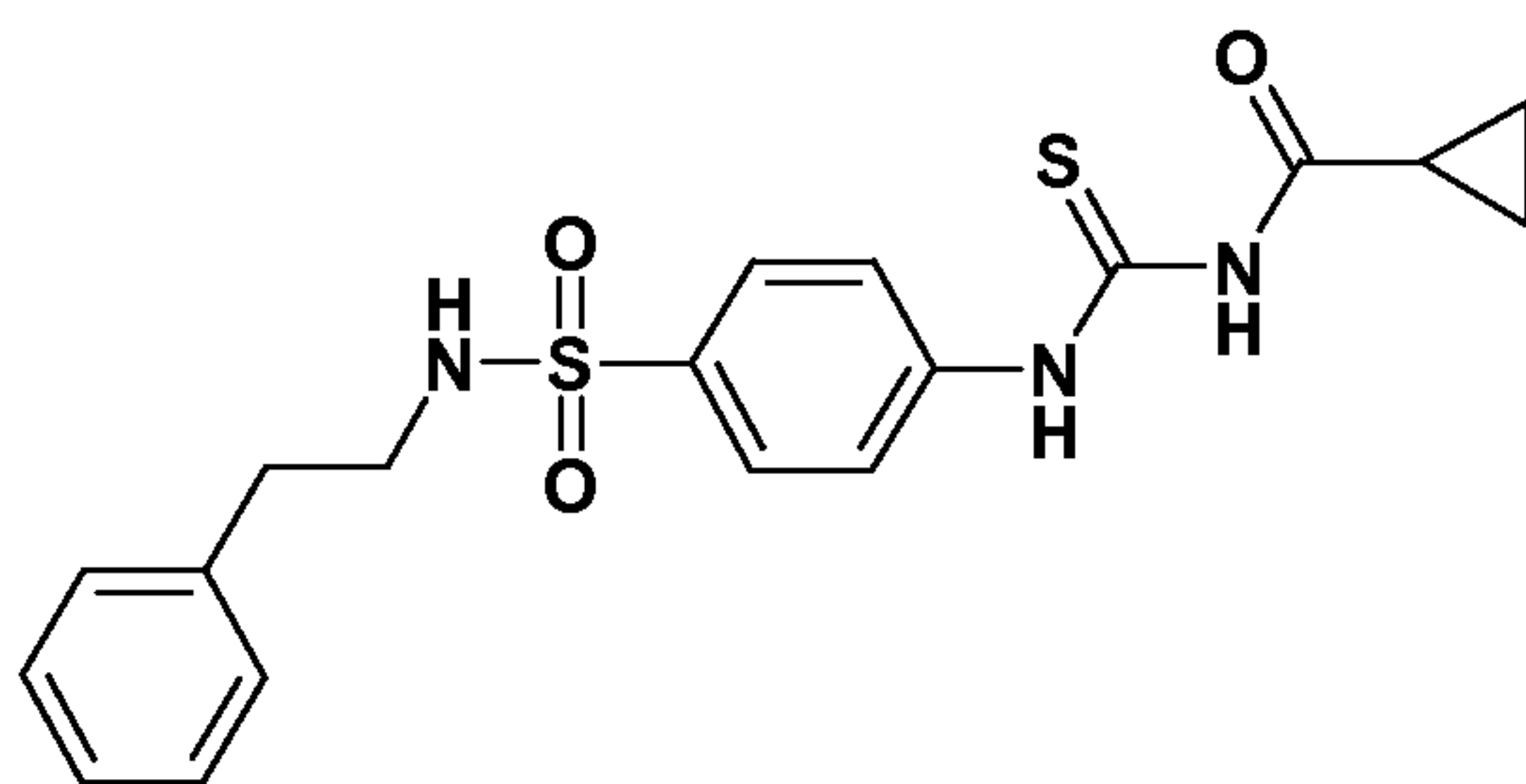
(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

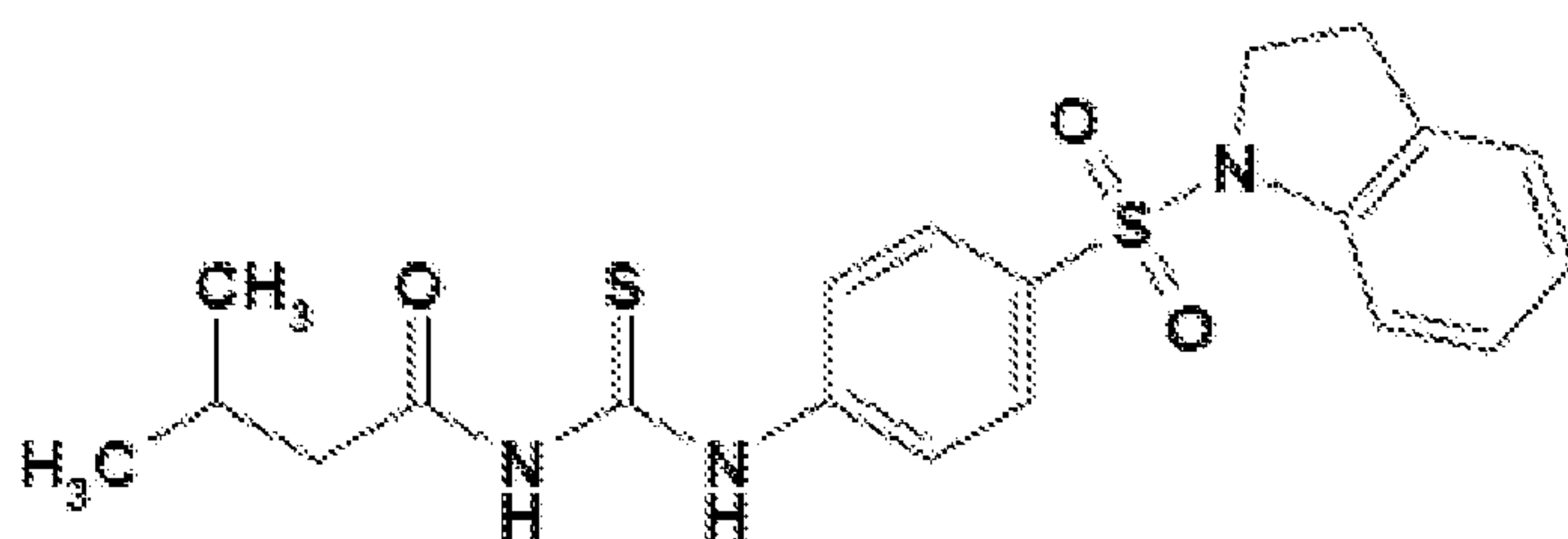
- (9) 2, 3-methylenedioxy;
 (10) nitro; and
 (11) halogen; and
 (c) aryloxy (-OAr); and
 (iii) aryl comprising a phenyl or heteroaryl five or six membered ring containing from 1 to 4 N, O, or S atoms; and

Formula (21) excludes a compound of Formula (17):



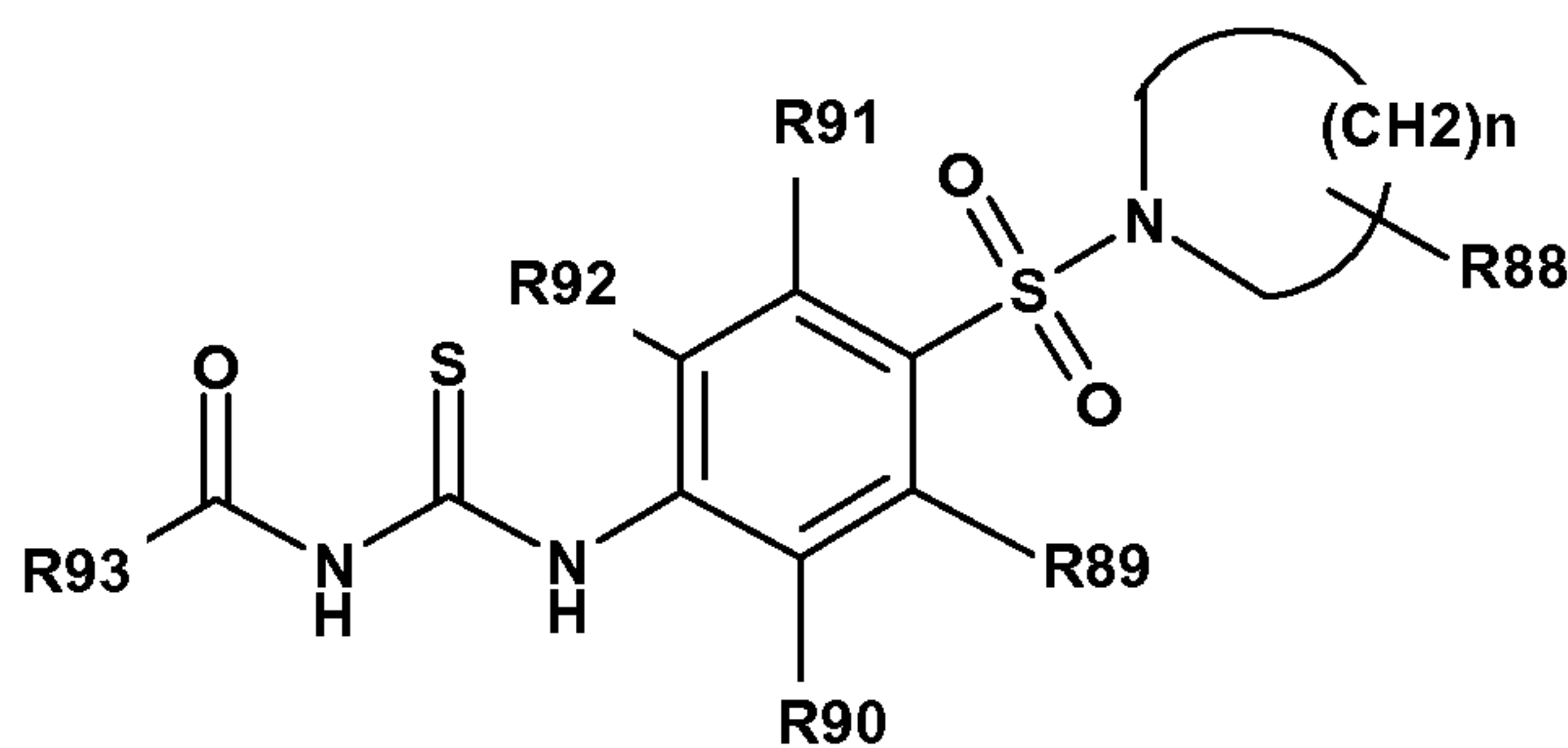
Formula (17).

Claim 40. The compound of claim 39 selected from the group consisting of:



AD4-10626.

Claim 41. A compound having a formula of:



Formula (22) (1886-like Type N)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

n is 3, 4, or 5;

R^{88} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{89} , R^{90} , R^{91} , R^{83} , and R^{92} are hydrogen;

R^{93} is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl $-\text{COOR}^{10}$ where R^{10} is a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation or a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy $-\text{OR}^{10}$ where R^{10} is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

(9) 2, 3-methylenedioxy;

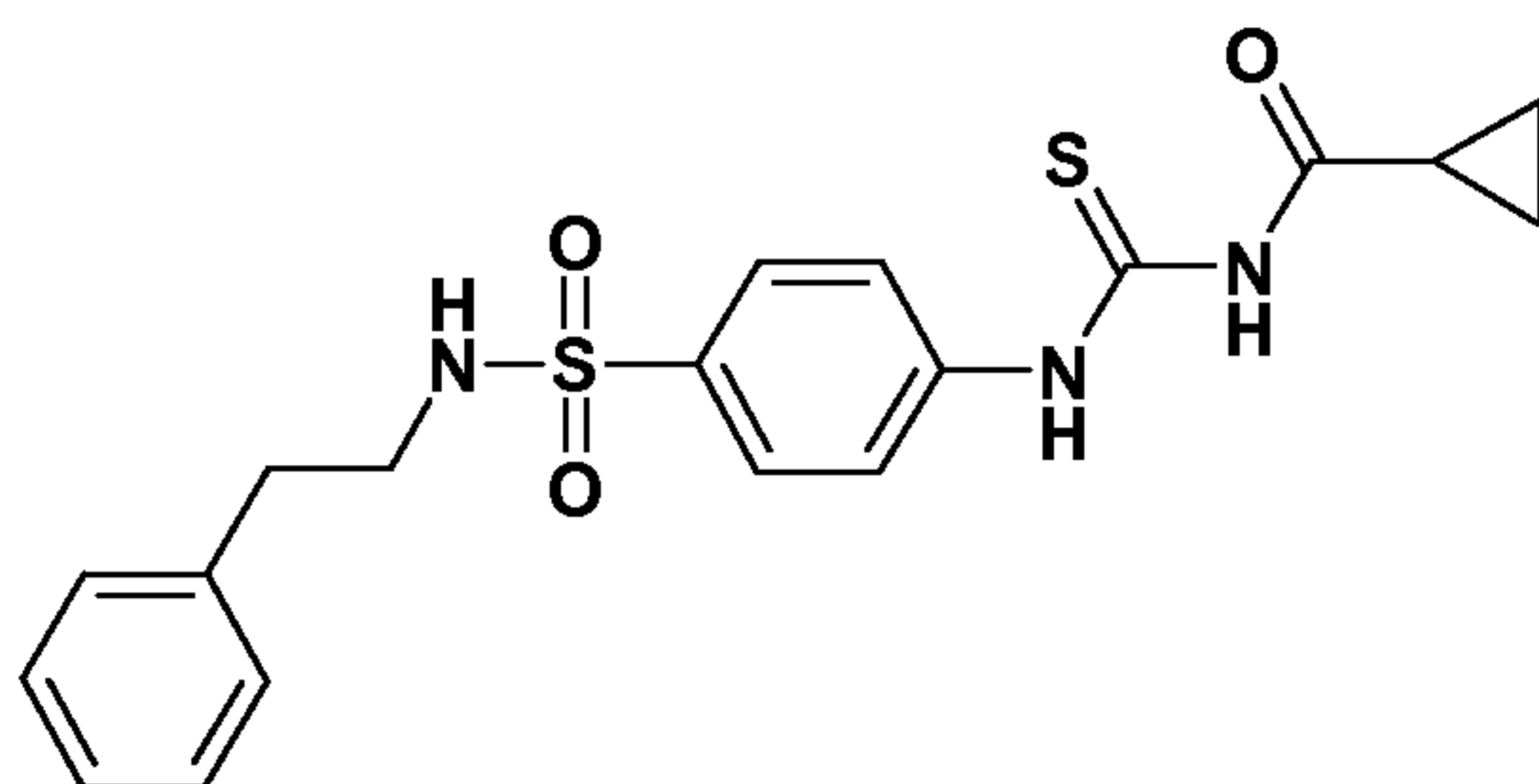
(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr); and

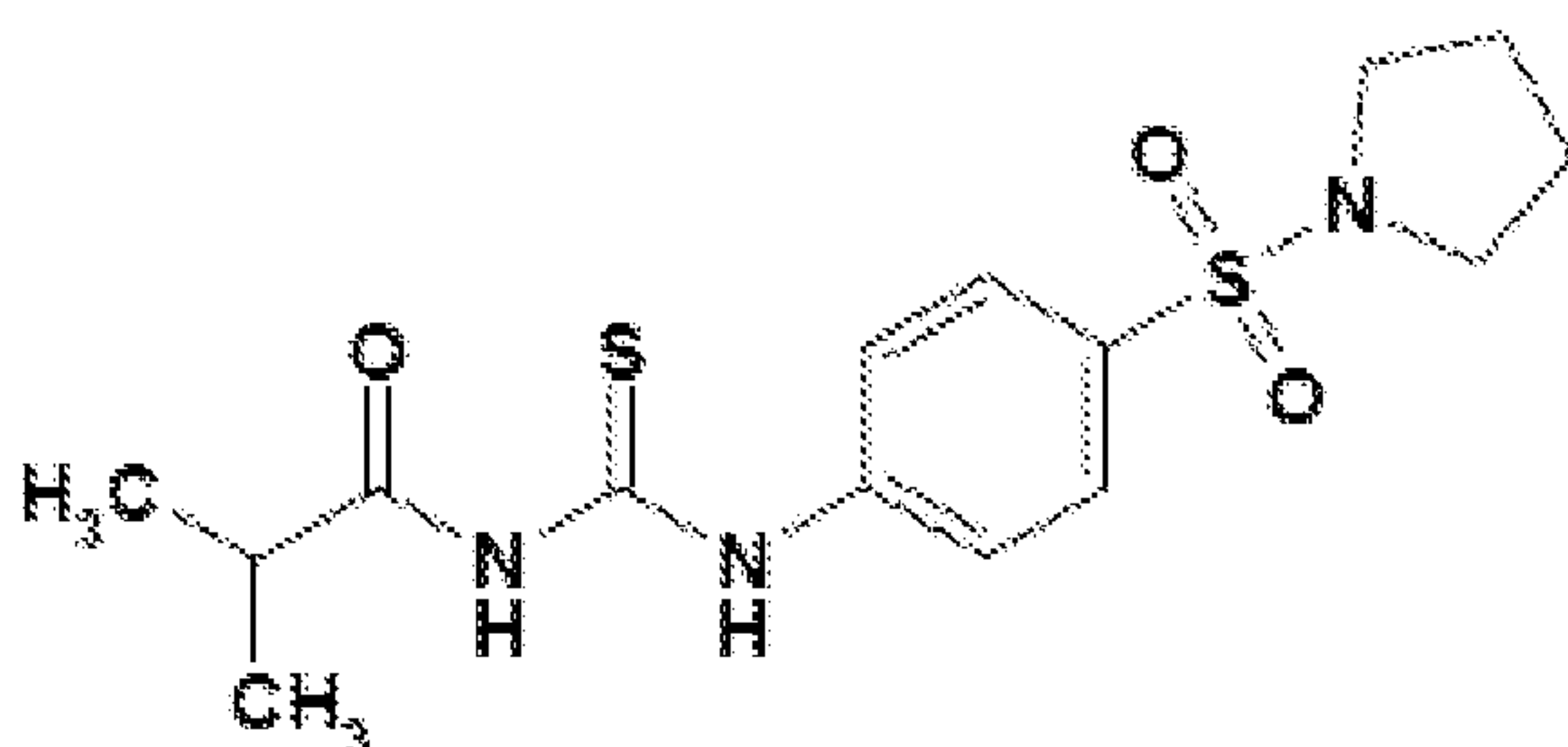
(iv) aryl comprising a phenyl or heteroaryl five or six membered ring containing from 1 to 4 N, O, or S atoms; and

Formula (22) excludes a compound of Formula (17):

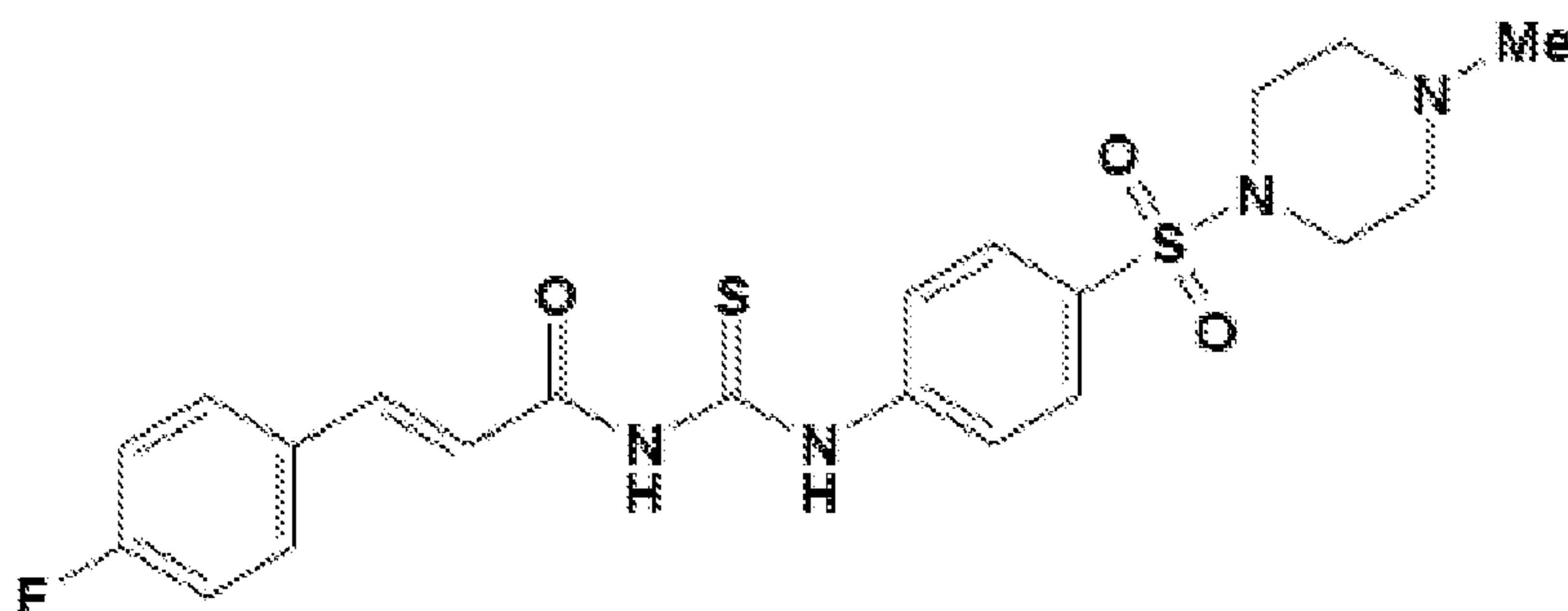


Formula (17).

Claim 42. The compound of claim 41 selected from the group consisting of:

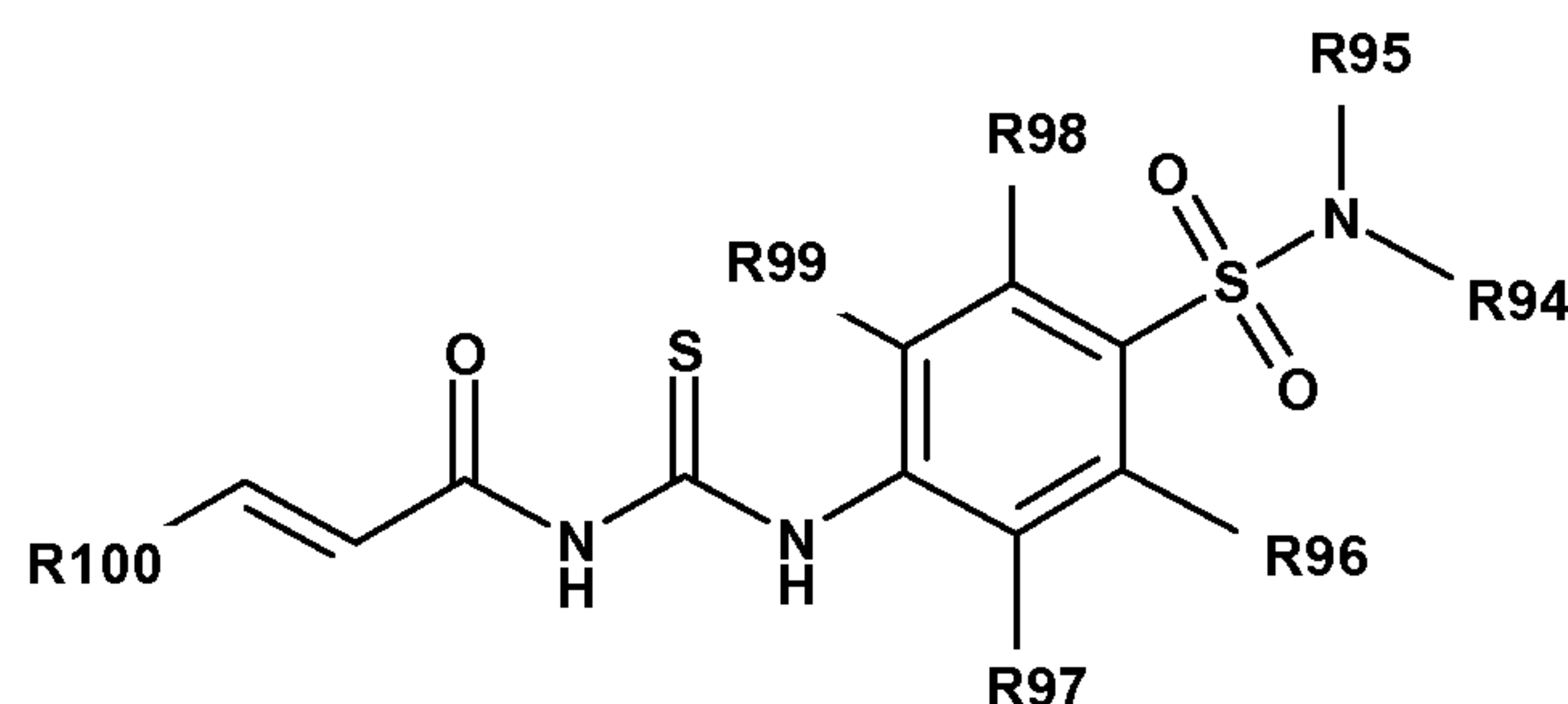


AD4-11970; and



AD4-12107.

Claim 43. A compound having a formula of:



Formula (23) (1886-like Type O)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{94} is a 5 or 6 membered heterocyclic aromatic ring containing from 1 to 4 N, O, or S atoms or any combination of those atoms with carbon atoms optionally substituted with from 1 to 3 of groups selected from:

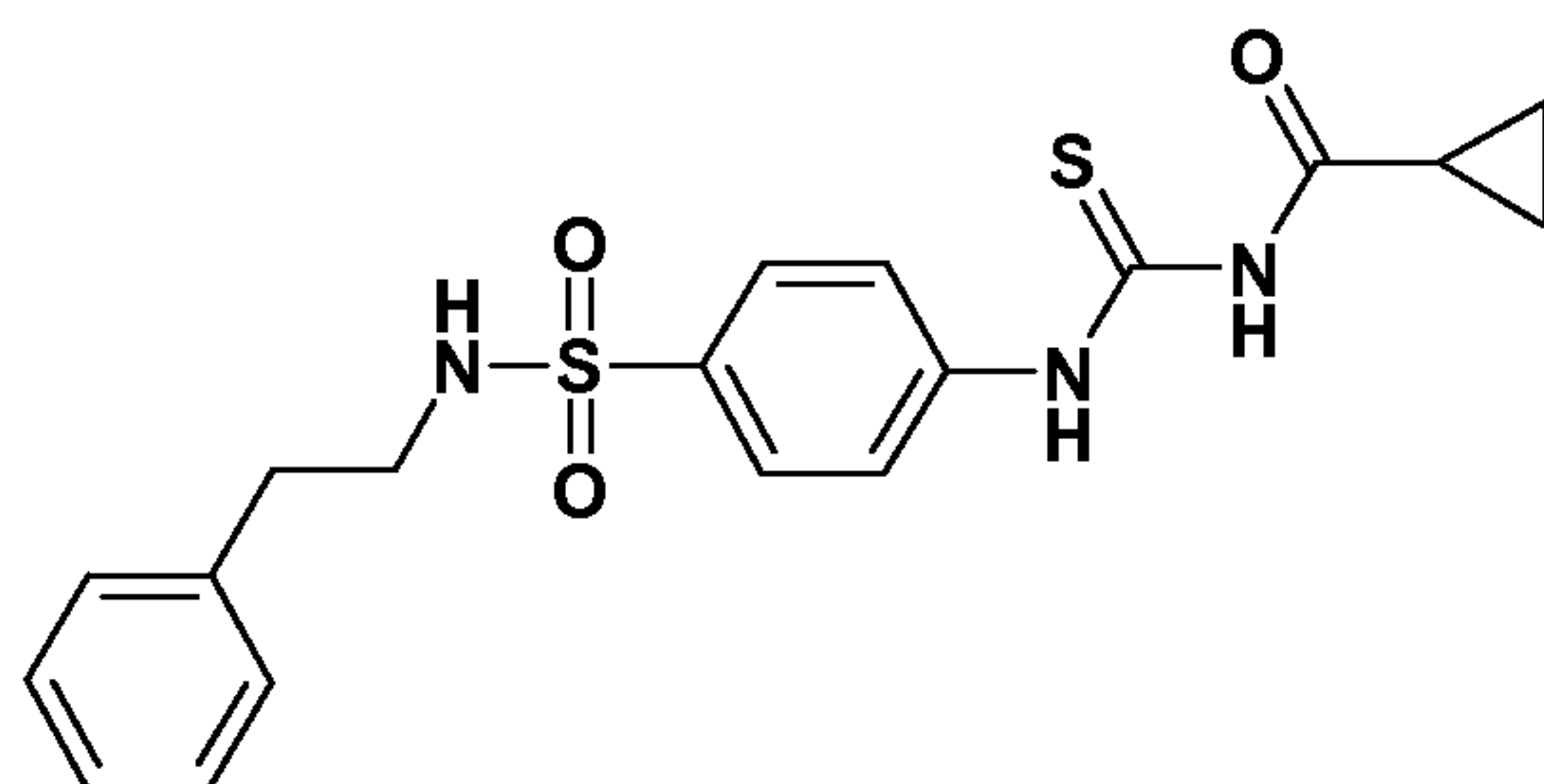
- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iv) trifluoromethyl;
- (v) trifluoromethoxy;
- (vi) substituted amino;
- (vii) nitro; and
- (viii) halogen;

R^{95} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{96} , R^{97} , R^{98} , and R^{99} are hydrogen;

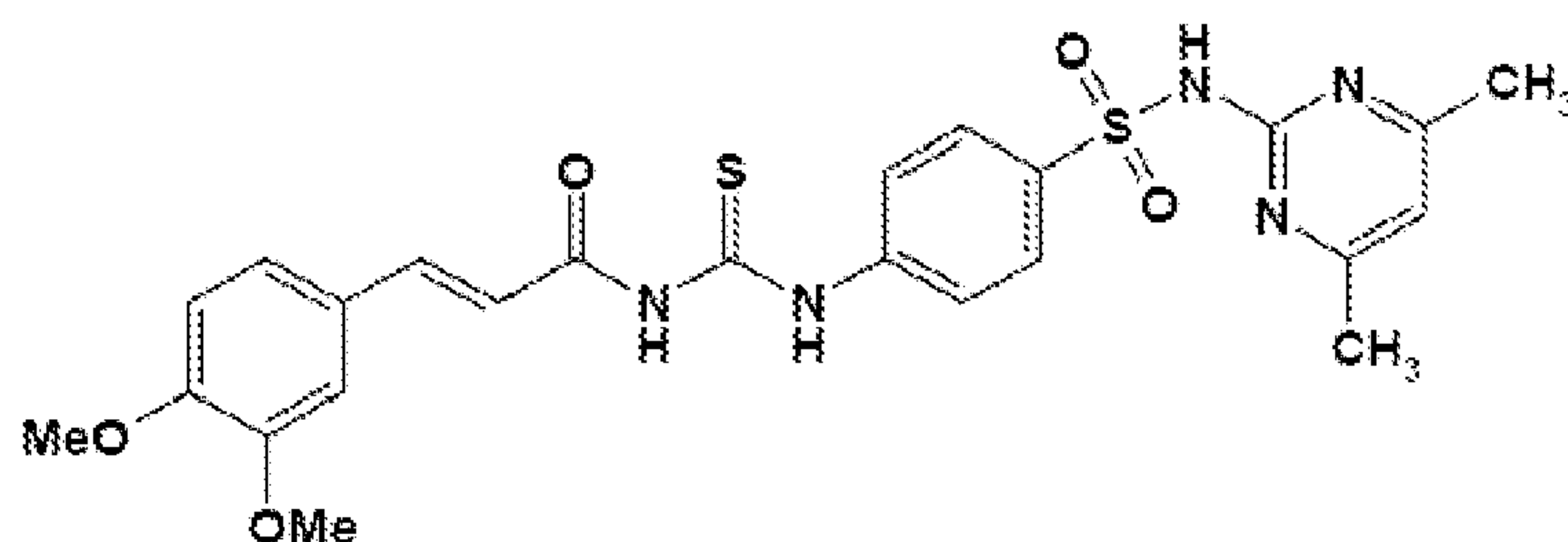
R^{100} is aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; and

Formula (23) excludes a compound of Formula (17):

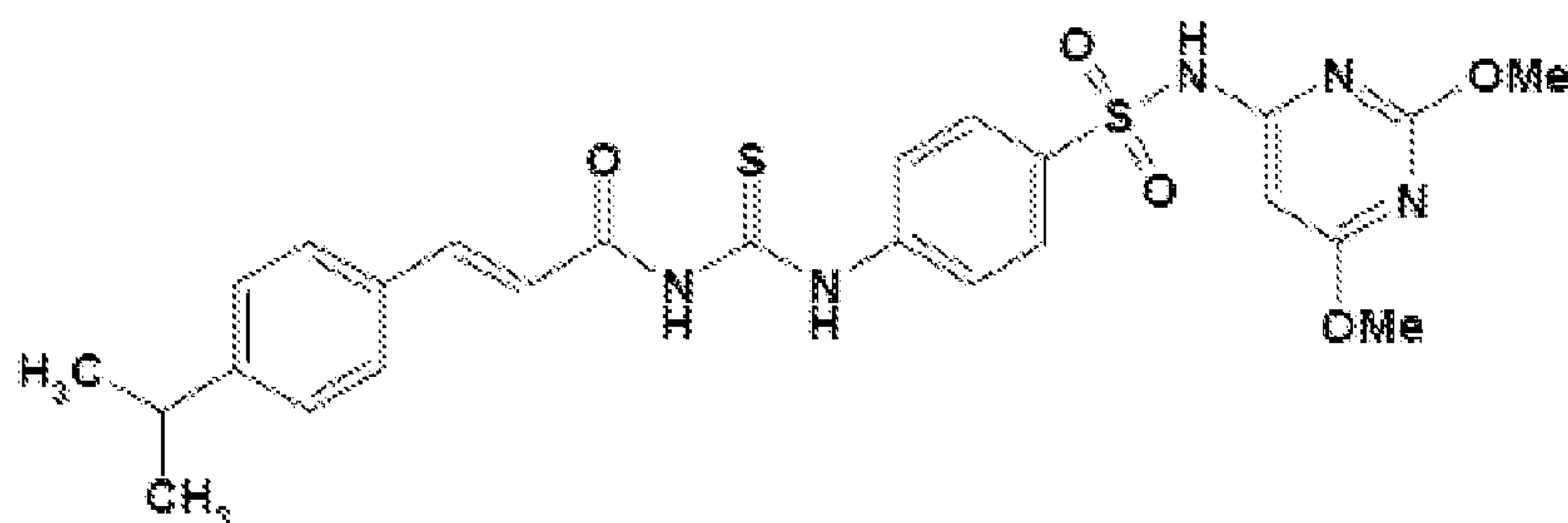


Formula (17).

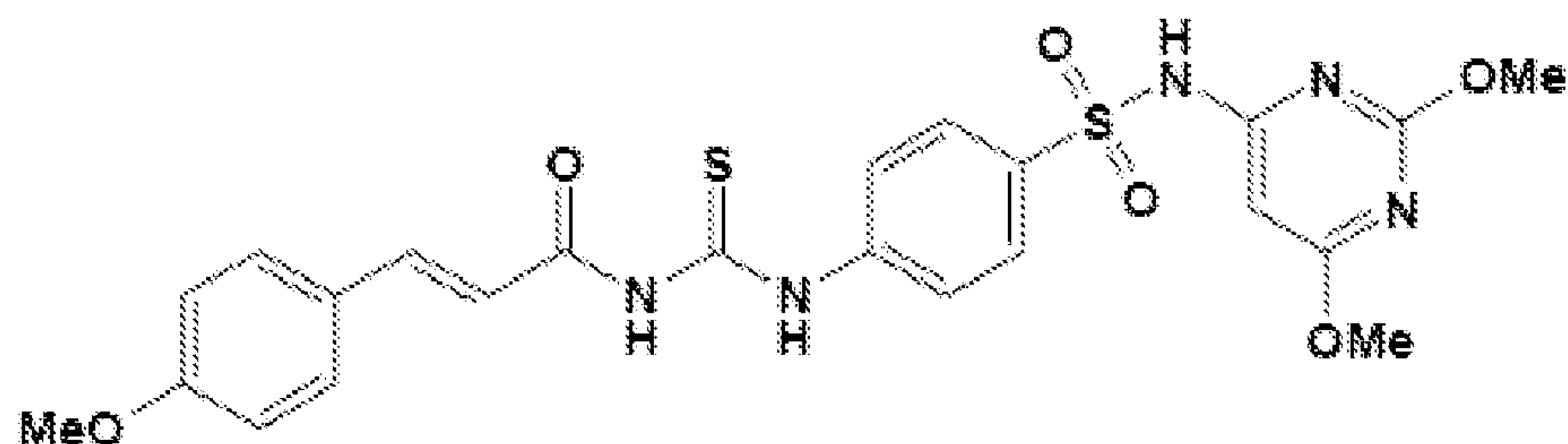
Claim 44. The compound of claim 43 selected from the group consisting of:



AD4-11883;



AD4-11638; and



AD4-11645.

Claim 45. The compound of any one of claims 31-44 that inhibits EGFR activity comprising:

at least seven or more of functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9 of a Scheme III 1886-like pharmacophore;

wherein

functional group F(III)1 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 and accepts a hydrogen bond from the side chain NH_2 of receptor Gln384 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243, and Φ (phi) = 45.369 and a spherical radius of about 1.2Å;

functional group F(III)2 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 87.287$, θ (theta) = 40.739, and Φ (phi) = 51.781 and a spherical radius of about 1.2Å;

functional group F(III)3 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 86.320$, θ (theta) = 41.915, and Φ (phi) = 52.323 and a spherical radius of about 1.5Å;

functional group F(III)4 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.870$, θ (theta) = 38.463, and Φ (phi) = 41.650 and a spherical radius of about 1.2Å;

functional group F(III)5 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 82.241$, θ (theta) = 37.431, and Φ (phi) = 44.151 and a spherical radius of about 1.5Å;

functional group F(III)6 accepts a hydrogen bond from, or forms a salt bridge to, NH^{3+} of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 88.009$, θ (theta) = 37.822, and Φ (phi) = 54.903 and a spherical radius of about 1.2Å;

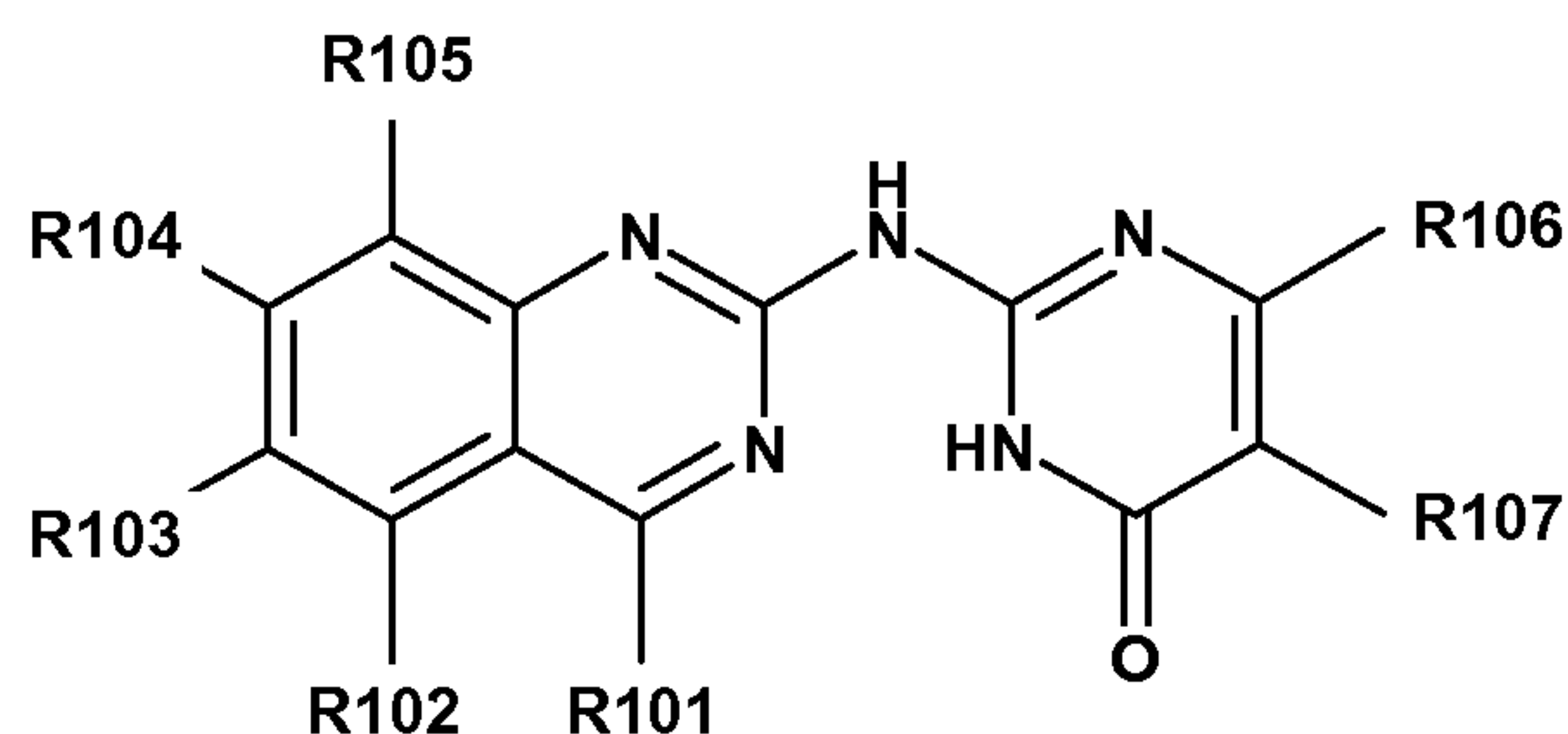
functional group F(III)7 accepts a hydrogen bond from, or forms a salt bridge to, NH^{3+} of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.513$, θ (theta) = 36.889, and Φ (phi) = 54.484 and a spherical radius of about 1.5Å;

functional group F(III)8 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243, and Φ (phi) = 45.369 and a spherical radius of about 1.2Å;

functional group F(III)9 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 79.652$, θ (theta) = 40.928, and Φ (phi) = 44.528 and a spherical radius of about 1.6Å;and

the compound substantially maintains a non-extended tether inactive configuration of EGFR or substantially prevents stabilization of an extended tether active configuration of EGFR.

Claim 46. A compound having a formula of:



Formula (25) (10381-like Type D)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{101} is selected from the group consisting of:

- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (iii) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

- (a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (c) heteroaryl containing from 1 to 4 N, O, or S atoms;

R^{102} is hydrogen;

R^{103} is selected from the group consisting of:

- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl

optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation

or one oxygen or nitrogen atom; and

(v) halogen;

R¹⁰⁴ and R¹⁰⁵ are independently selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally

containing unsaturation;

(iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one

oxygen or nitrogen atom; and

(iv) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl

optionally containing unsaturation;

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation

or one oxygen or nitrogen atom;

R¹⁰⁶ is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally

containing unsaturation;

(iii) aryl having an unsubstituted phenyl ring or a phenyl ring

substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl

optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or

one oxygen or nitrogen atom; and

(c) heteroaryl containing from 1 to 4 N, O, or S atoms;

(d) aryl comprising a phenyl or heteroaryl containing from 1

to 4 N, O, or S atoms;

(e) alkoxy -OR¹⁰ where R¹⁰ is selected from the group

consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(f) trifluoromethyl;

(g) trifluoromethoxy;

(h) difluoromethoxy;

(i) 3, 4-methylenedioxy;

(j) 2, 3-methylenedioxy;

(k) nitro; and

(l) halogen; and

(iv) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl -COOR¹⁰ where R¹⁰ is selected from the group

consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr);

R¹⁰⁷ is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(a) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(c) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(d) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(e) trifluoromethyl;

(f) trifluoromethoxy;

(g) difluoromethoxy;

(h) 3, 4-methylenedioxy;

(i) 2, 3-methylenedioxy;

(j) nitro; and

(k) halogen; and

(v) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:

(a) carboxyl $-\text{COOR}^{10}$ where R^{10} is selected from the group consisting of:

(1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:

(1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy $-\text{OR}^{10}$ where R^{10} is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

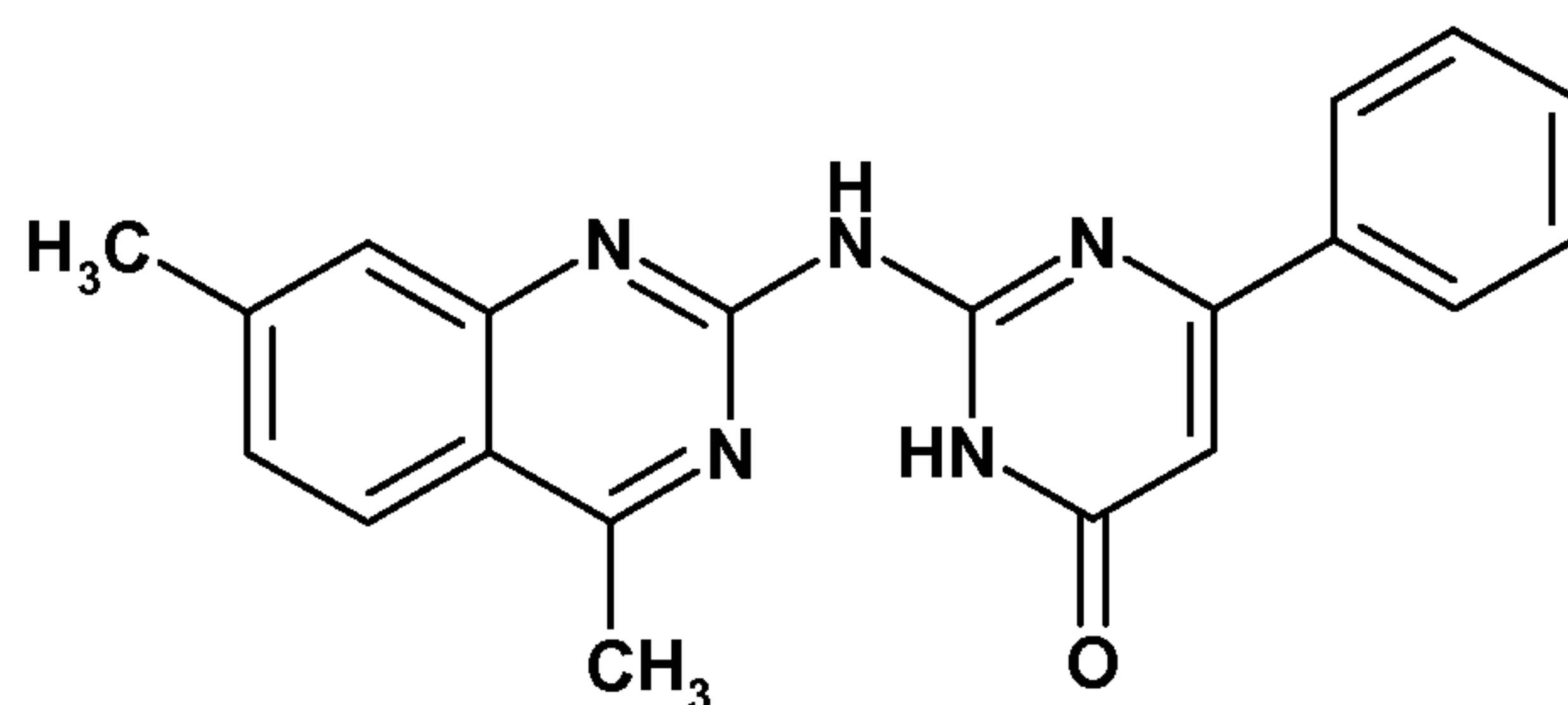
(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr); and

Formula (25) excludes a compound of Formula (24):



Formula (24).

Claim 47. The compound of claim 46 wherein R^{101} is selected from the group consisting of: methyl and phenyl.

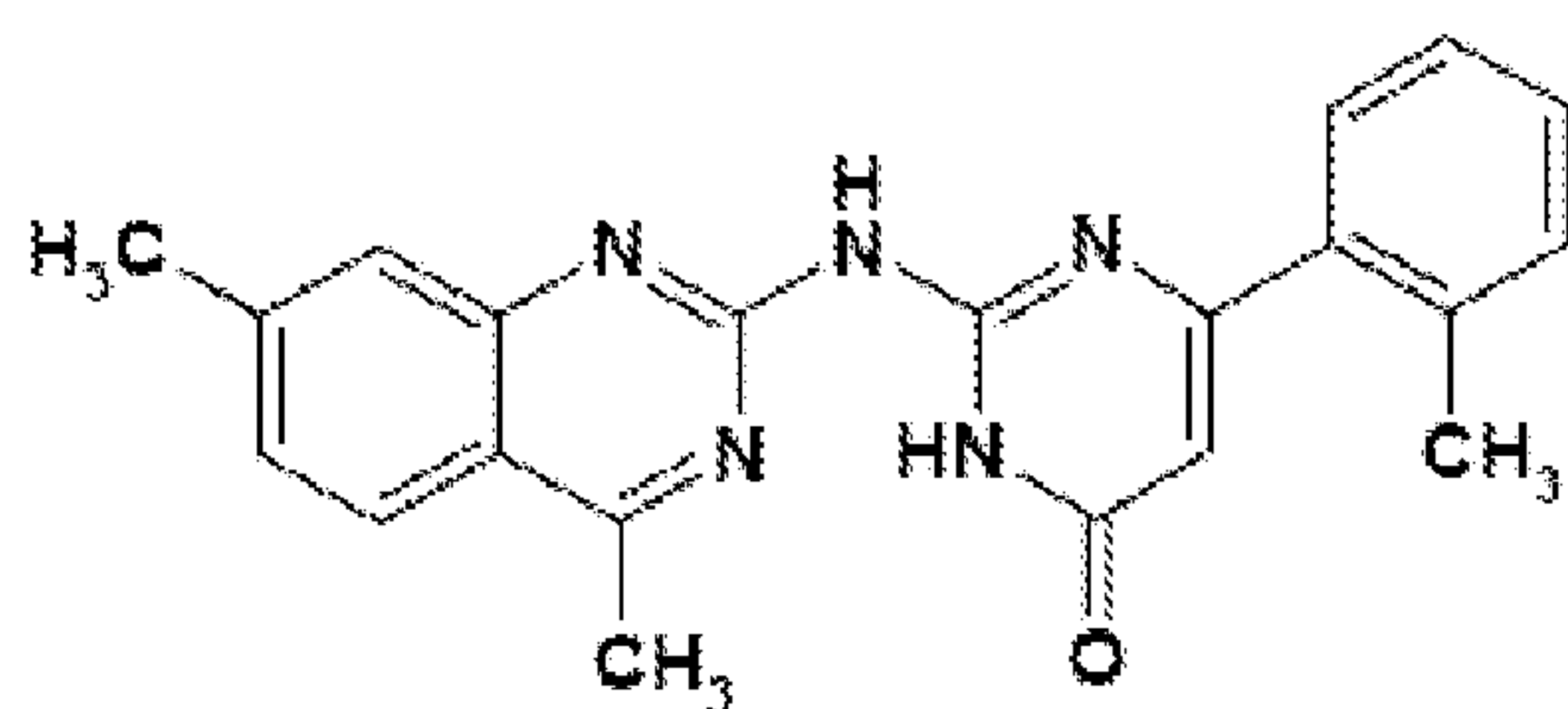
Claim 48. The compound of any one of claims 46-47 wherein R^{103} is selected from the group consisting of: methyl, ethyl, chloro, methoxy, and ethoxy.

Claim 49. The compound of any one of claims 46-48 wherein R^{104} is selected from the group consisting of: hydrogen, methyl, and methoxy.

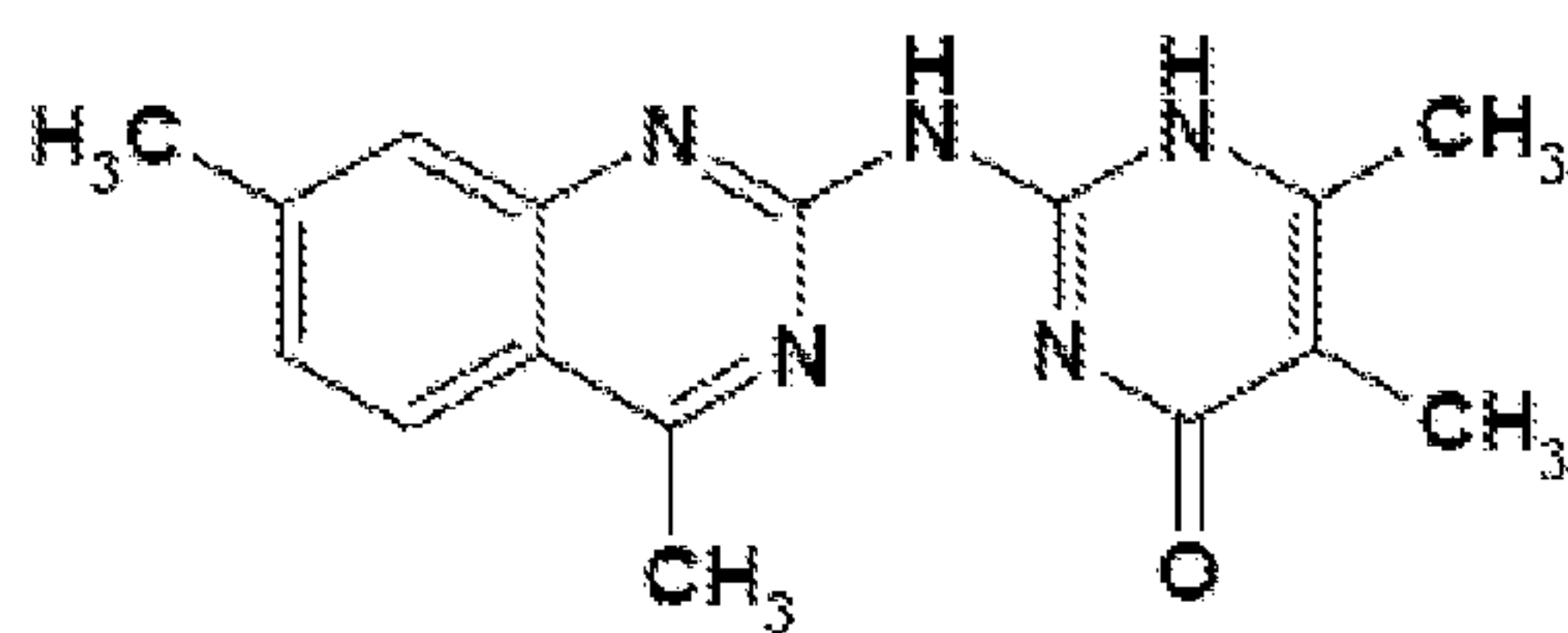
Claim 50. The compound of any one of claims 46-49 wherein R^{105} is selected from the group consisting of: hydrogen, methyl, methoxy, and ethoxy.

Claim 51. The compound of any one of claims 46-50 wherein R^{106} is selected from the group consisting of: methyl and phenyl.

Claim 52. The compound of claim 46 selected from the group consisting of:

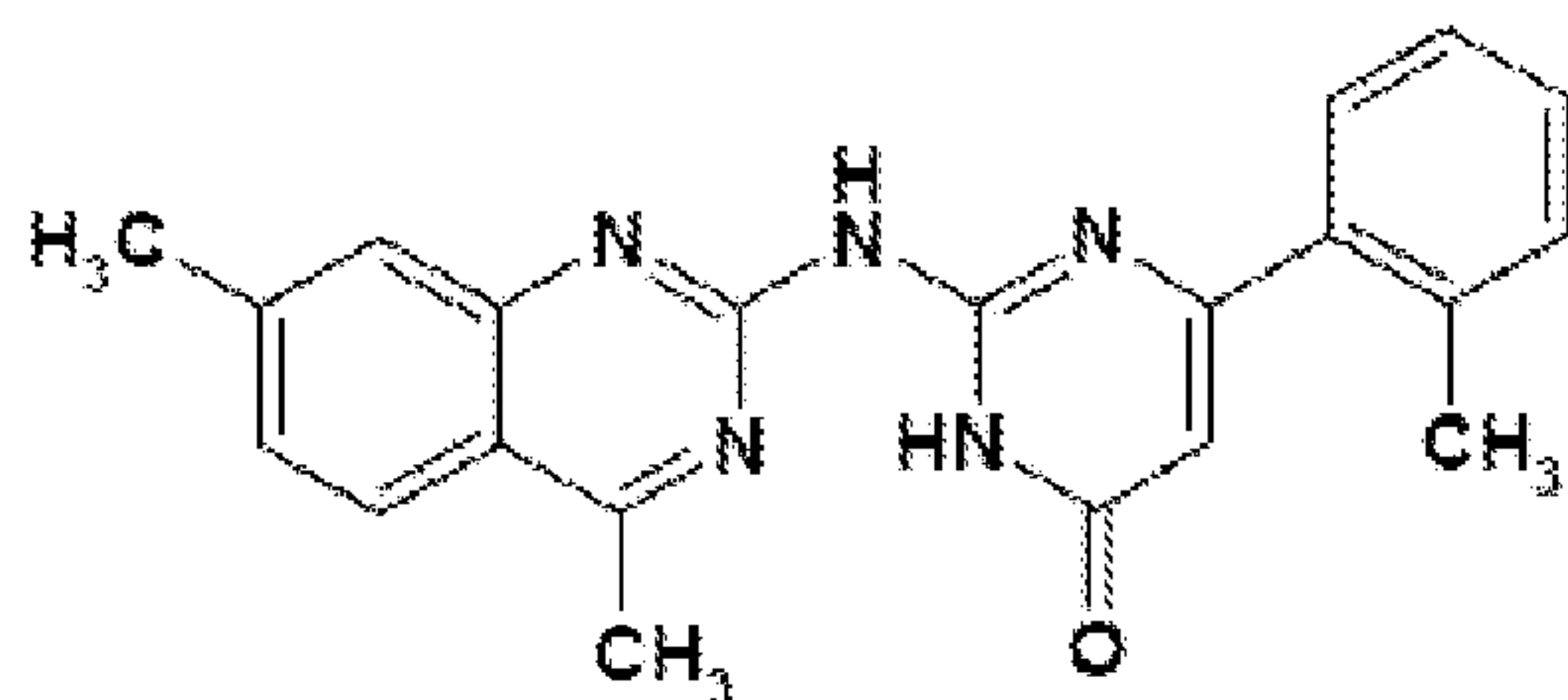


AD4-11511, Formula (26); and



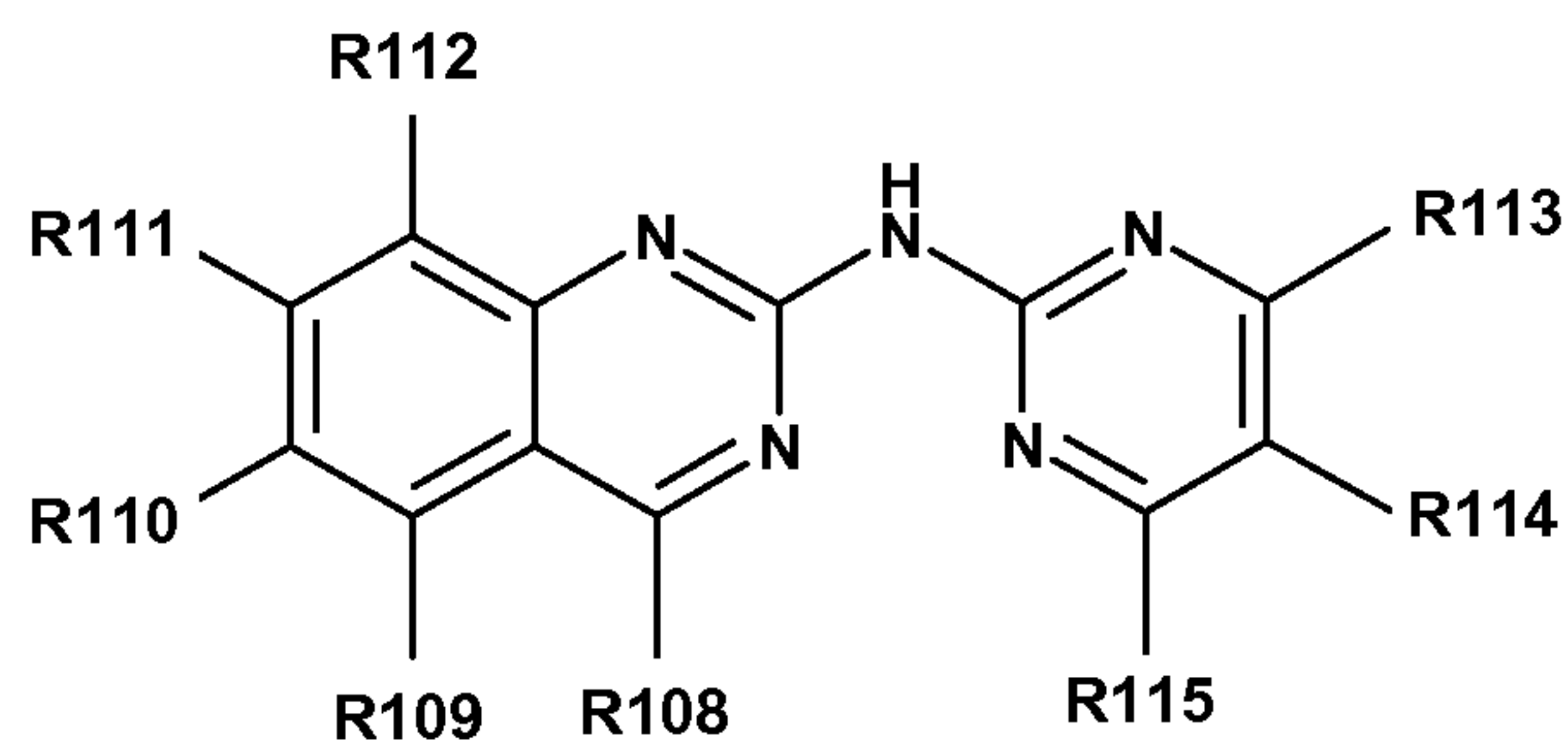
AD4-12632.

Claim 53. The compound of claim 46 selected from the group consisting of:



AD4-11511, Formula (26).

Claim 54. A compound having a formula of:



Formula (27) (10381-like Type E)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{108} is selected from the group consisting of:

- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
- (iii) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:
 - (a) carboxyl $-\text{COOR}^{10}$ where R^{10} is selected from the group consisting of:
 - (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:
 - (1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;
 - (4) alkoxy $-\text{OR}^{10}$ where R^{10} is selected from the group consisting of:
 - (A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (5) trifluoromethyl;
 - (6) trifluoromethoxy;
 - (7) difluoromethoxy;
 - (8) 3, 4-methylenedioxy;

- (9) 2, 3-methylenedioxy;
- (10) nitro; and
- (11) halogen; and
- (c) aryloxy (-OAr);
- (iv) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;
- (v) amino; and
- (vi) hydroxyl;
- R^{109} is hydrogen;
- R^{110} and R^{111} are independently selected from the group consisting of:
 - (i) hydrogen;
 - (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (iii) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (iv) C-1 to C-3 substituted alkyl having at least one substitution group selected from the group consisting of:
 - (a) carboxyl $-\text{COOR}^{10}$ where R^{10} is selected from the group consisting of:
 - (1) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (2) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;
 - (b) aryl having an unsubstituted phenyl ring or a phenyl ring substituted at the 2-, 3-, 4-, 5- or 6-position with one or more groups selected from the group consisting of:
 - (1) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
 - (2) C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(3) aryl having phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

(4) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(A) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(B) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(5) trifluoromethyl;

(6) trifluoromethoxy;

(7) difluoromethoxy;

(8) 3, 4-methylenedioxy;

(9) 2, 3-methylenedioxy;

(10) nitro; and

(11) halogen; and

(c) aryloxy (-OAr);

(v) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(vi) halogen;

R¹¹² is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R¹¹³ is selected from the group consisting of:

(i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(ii) trifluoromethyl;

(iii) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; and

(iv) amino;

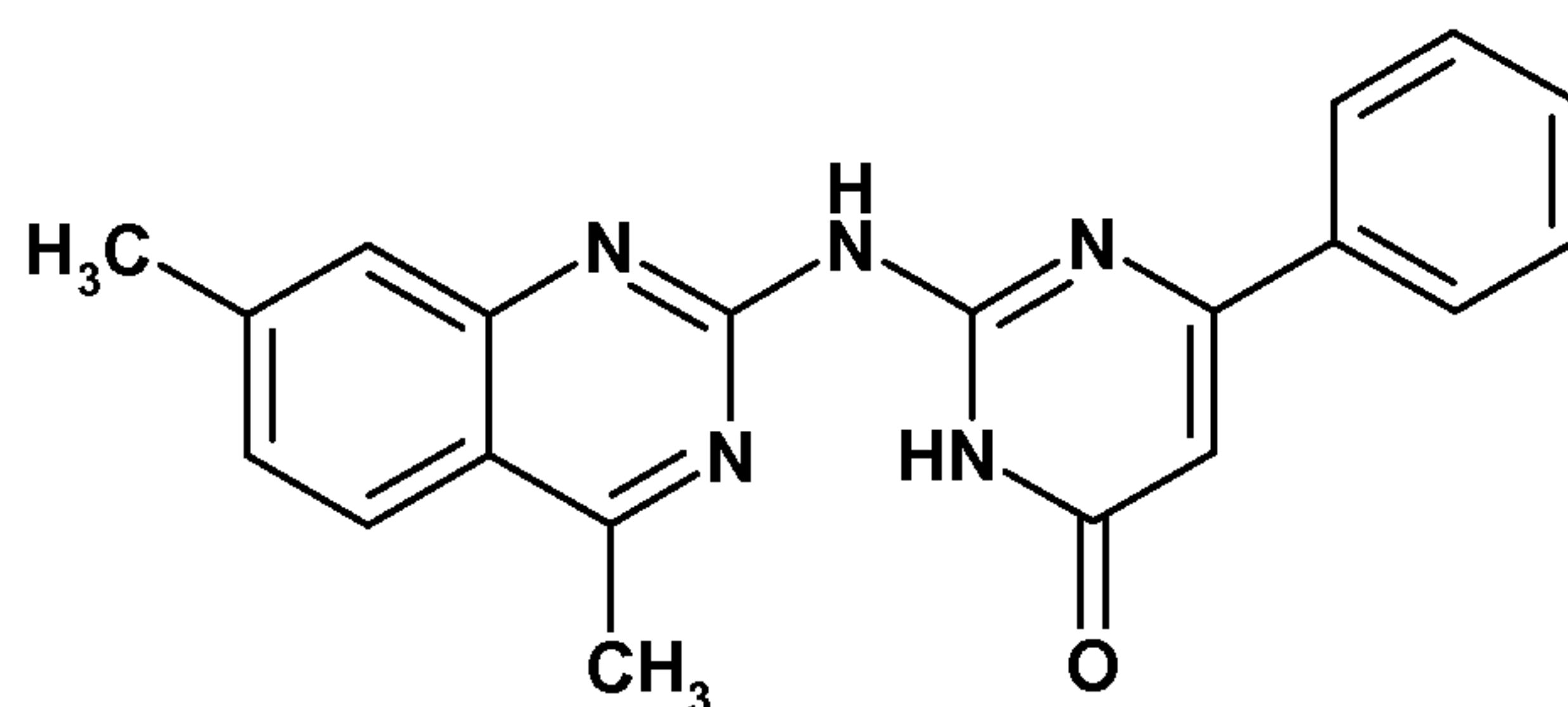
R^{114} is selected from the group consisting of:

- (i) hydrogen;
- (ii) acyl $-COR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (iii) carboxyl $-COOR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

R^{115} is selected from the group consisting of:

- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) trifluoromethyl; and
- (iii) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; and

Formula (27) excludes a compound of Formula (24):



Formula (24).

Claim 55. The compound of claim 54 wherein R^{108} is selected from the group consisting of: methyl, phenyl, and hydroxyl.

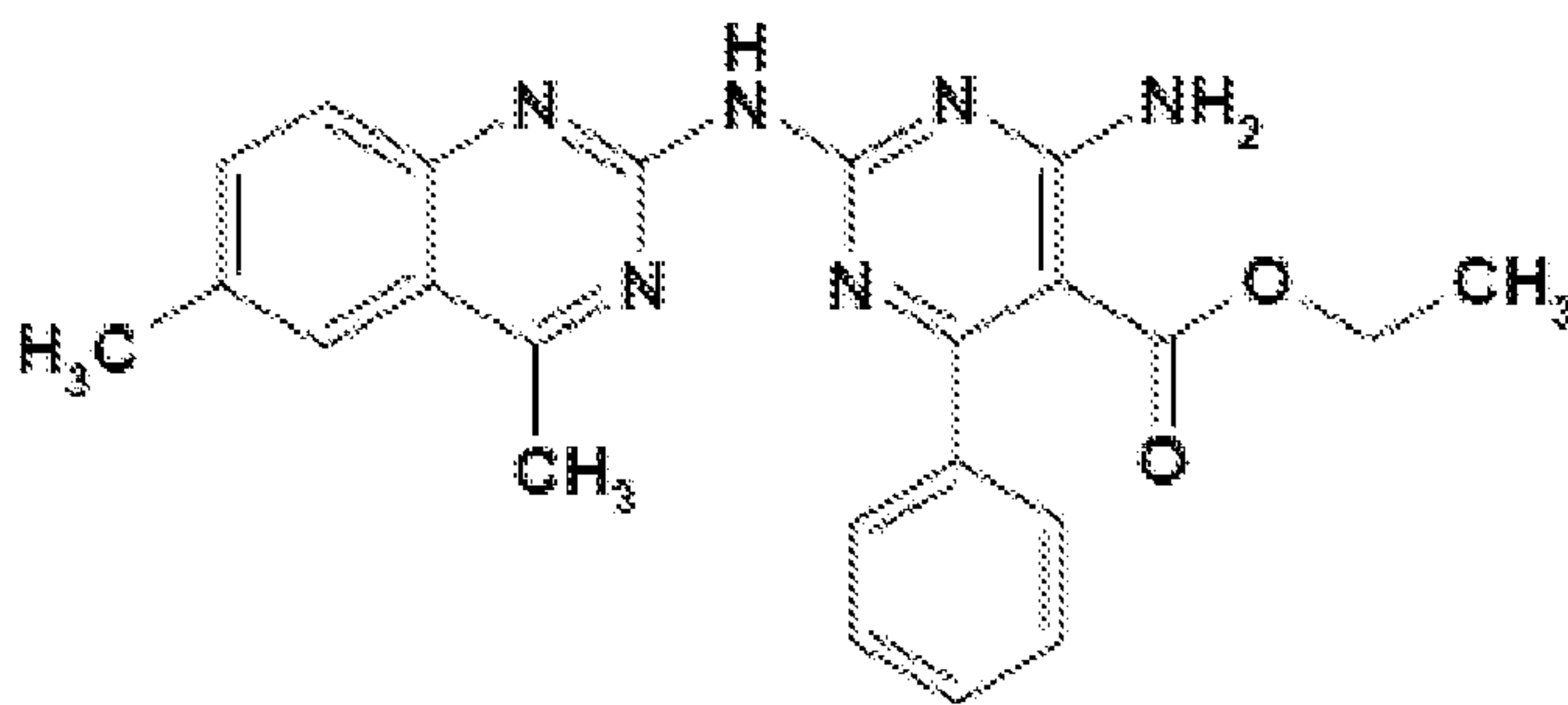
Claim 56. The compound of any one of claims 54-55 wherein R^{110} and R^{111} are independently selected from the group consisting of: hydrogen, methyl, methoxy, and chloro.

Claim 57. The compound of any one of claims 54-56 wherein R^{112} is methyl.

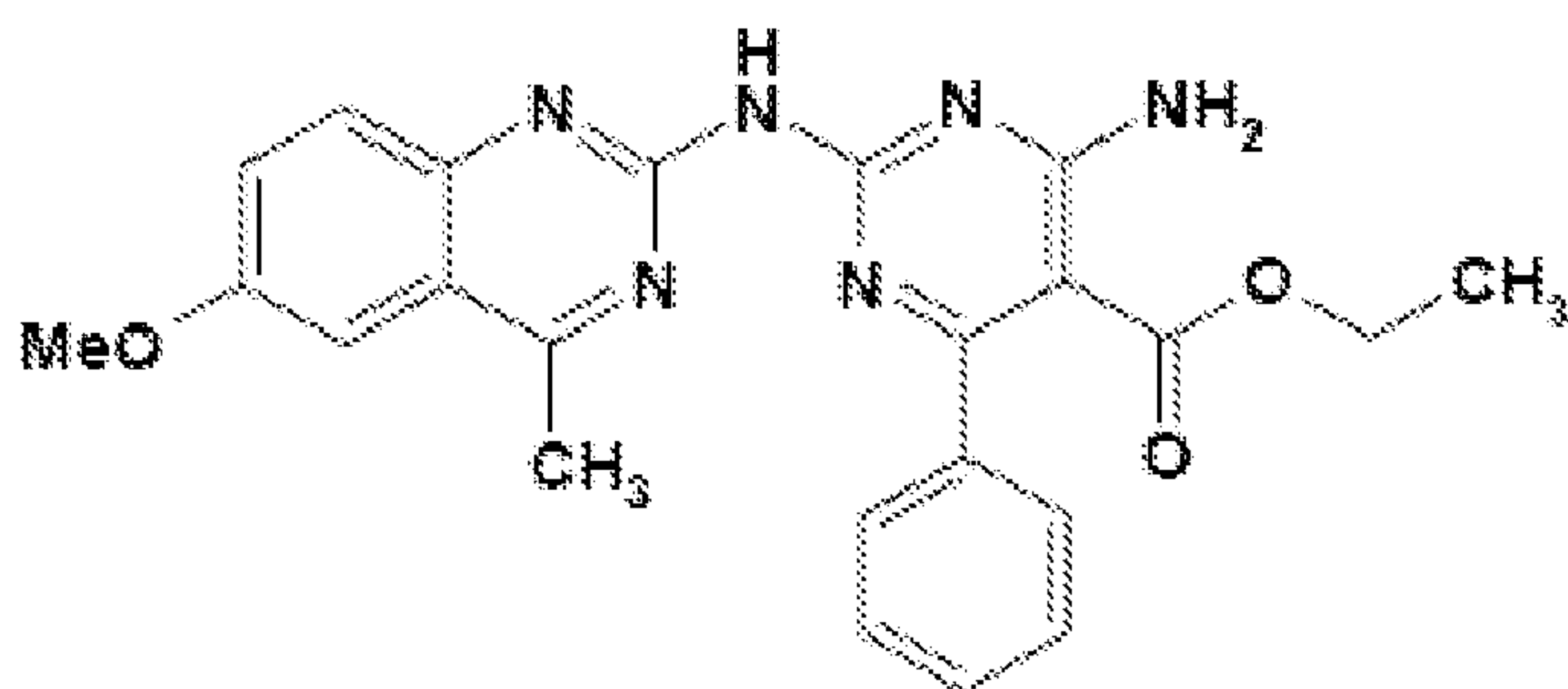
Claim 58. The compound of any one of claims 54-57 wherein R^{113} is selected from the group consisting of: methyl, phenyl, and amino.

Claim 59. The compound of any one of claims 54-58 wherein R^{115} is selected from the group consisting of: methyl, phenyl, and trifluoromethyl.

Claim 60. The compound of claim 54 selected from the group consisting of:

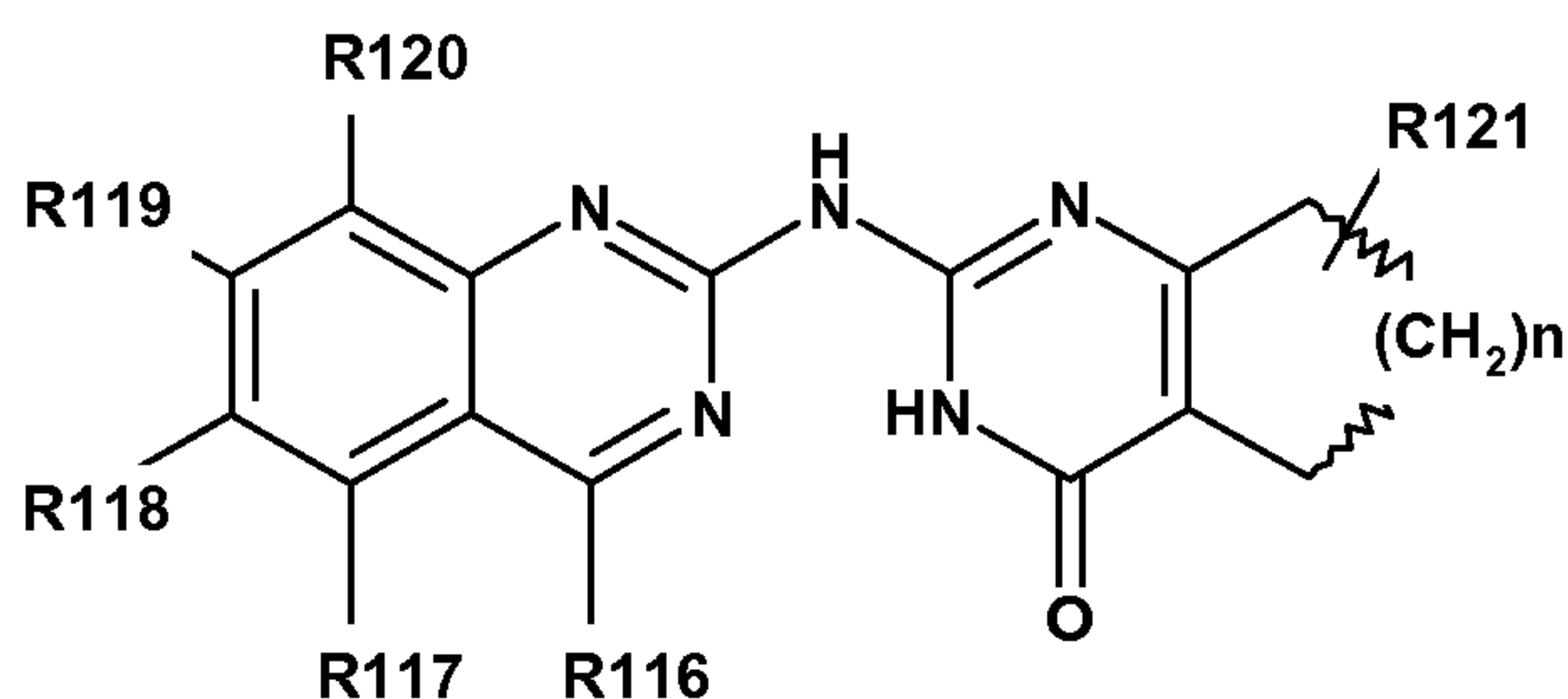


AD4-12681;



AD4-12679.

Claim 61. A compound having a formula of:



Formula (28) (10381-like Type F)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein

n is 1 or 2;

R^{116} is selected from the group consisting of:

- (i) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
- (ii) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

R^{117} of is hydrogen;

R^{118} is selected from the group consisting of:

- (i) hydrogen,
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;
- (ii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and
- (iii) halogen;

R^{119} is selected from the group consisting of:

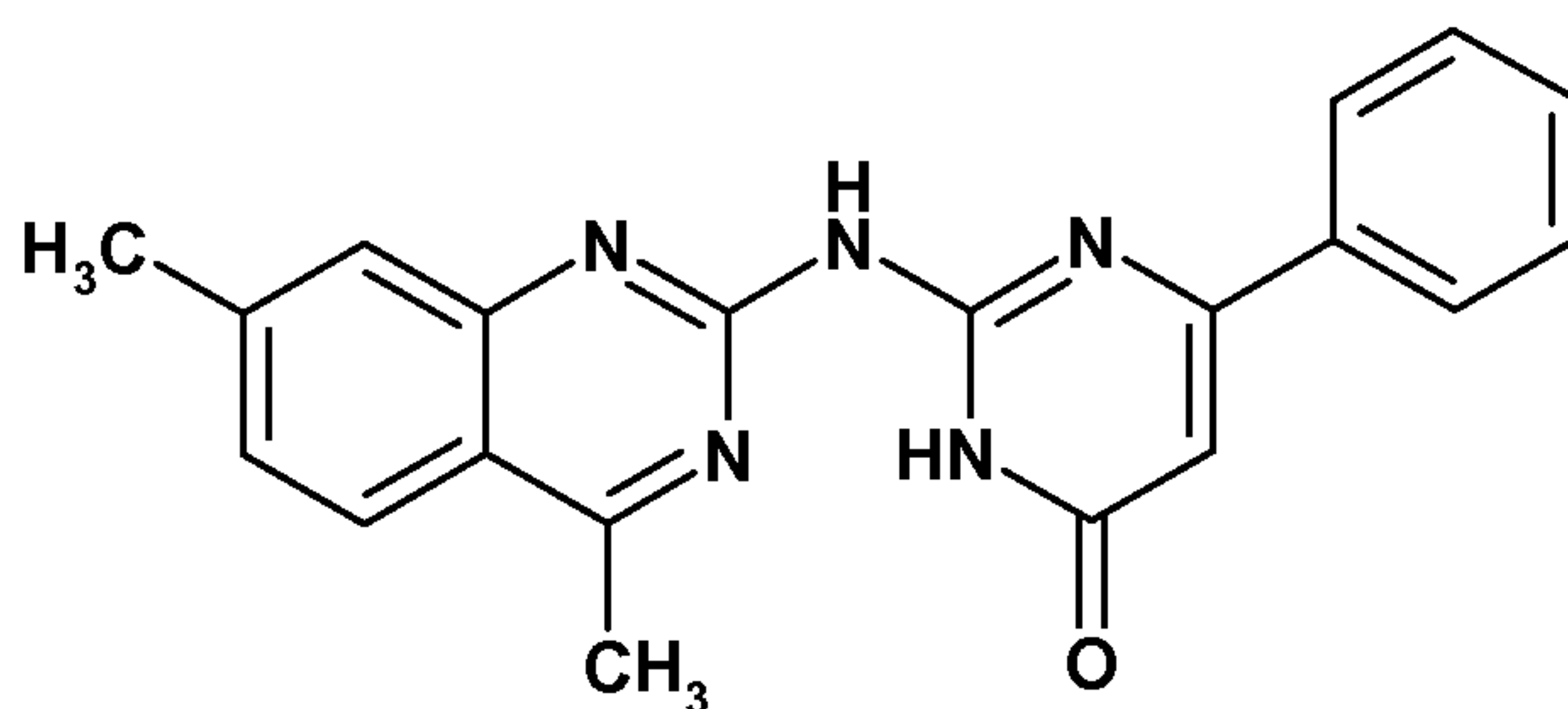
- (i) hydrogen;
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
- (iii) aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms;

R^{120} is selected from the group consisting of:

- (i) hydrogen,
- (ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
- (iii) alkoxy -OR¹⁰ where R¹⁰ is selected from the group consisting of:
 - (a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and
 - (b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

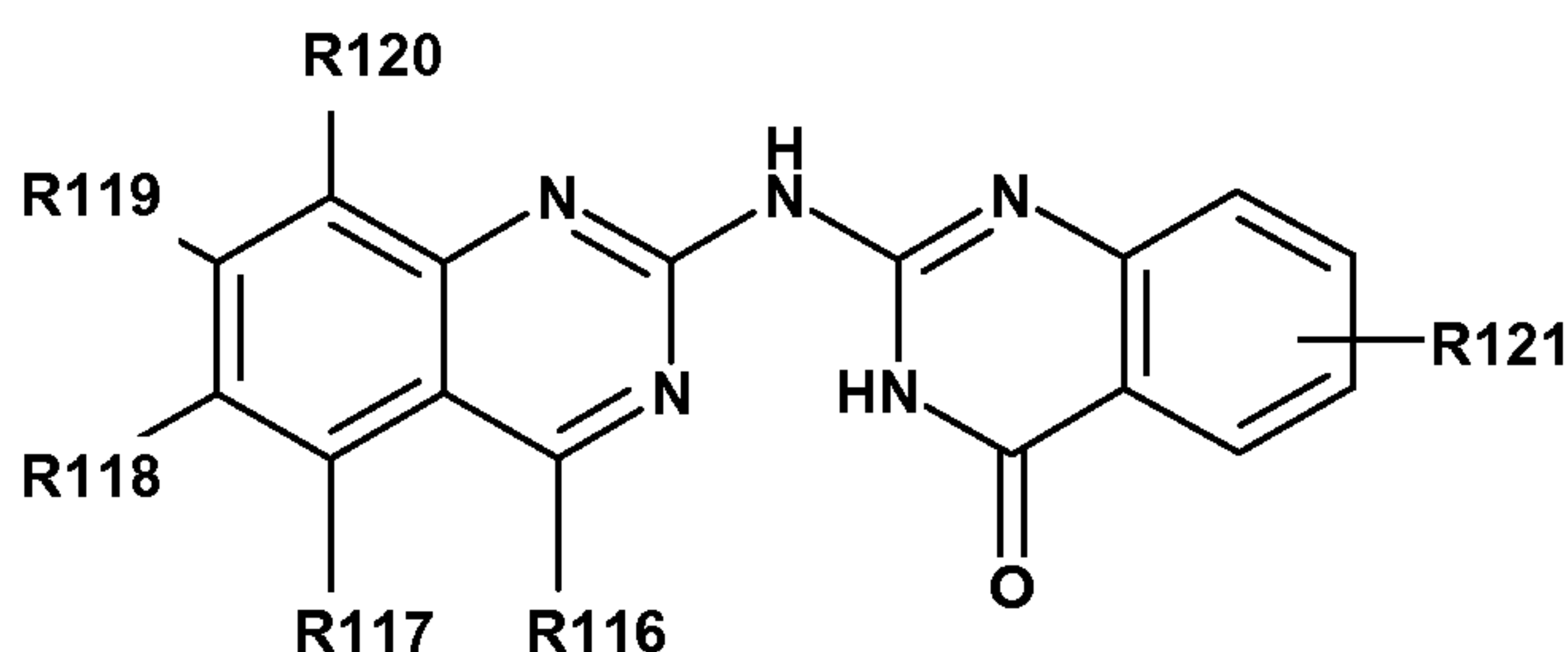
R¹²¹ is selected from the group consisting of: hydrogen and aryl comprising a phenyl or heteroaryl containing from 1 to 4 N, O, or S atoms; and

Formula (28) excludes a compound of Formula (24):



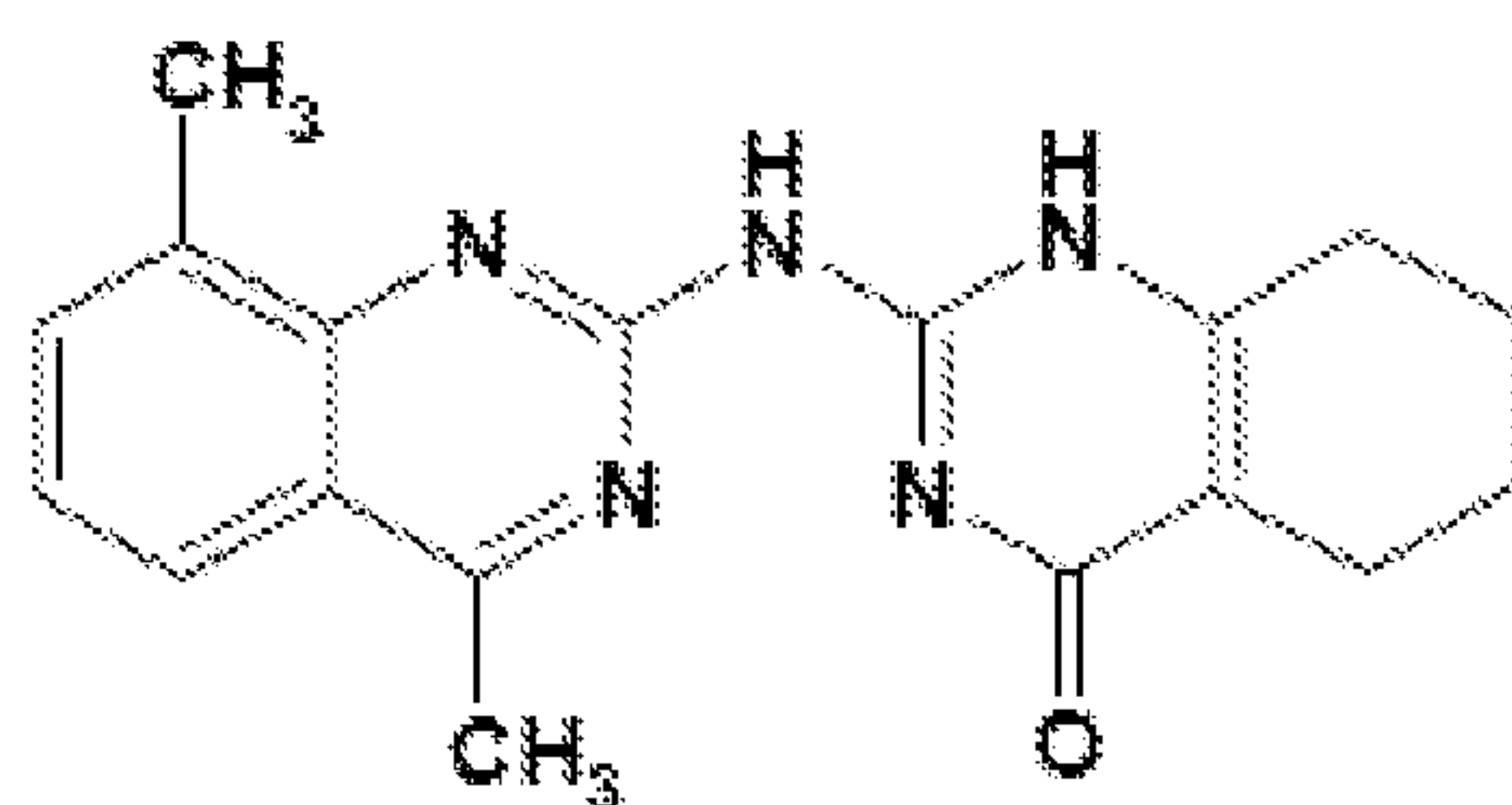
Formula (24).

Claim 62. The compound of claim 61 wherein n is 2 and the compound has a formula of:



Formula (29).

Claim 63. The compound of claim 61 selected from the group consisting of:



AD4-11436.

Claim 64. The compound of any one of claims 46-63 that inhibits EGFR activity comprising:

eight or more of functional groups F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, F(IV)9, and F(IV)10 of a Scheme IV 10381-like pharmacophore;

wherein

functional group F(IV)1 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 49.686$, θ (theta) = 113.993 , and Φ (phi) = -17.014 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)2 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 48.071$, θ (theta) = 115.388 , and Φ (phi) = -16.211 and a spherical radius of about $1.6\text{\AA}$;

functional group F(IV)3 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.781$, θ (theta) = 113.121 , and Φ (phi) = -17.520 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)4 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 52.021$, θ (theta) = 114.264 , and Φ (phi) = -15.878 and a spherical radius of about $1.5\text{\AA}$;

functional group F(IV)5 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.322$, θ (theta) = 111.253 , and Φ (phi) = -15.426 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)6 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 51.575$, θ (theta) = 112.433 , and Φ (phi) = -13.827 and a spherical radius of about $1.5\text{\AA}$;

functional group F(IV)7 forms a hydrophobic contact with a side chain of receptor Leu243 and a side chain of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 47.767$, θ (theta) = 112.521 , and Φ (phi) = -10.196 and a spherical radius of about $1.2\text{\AA}$;

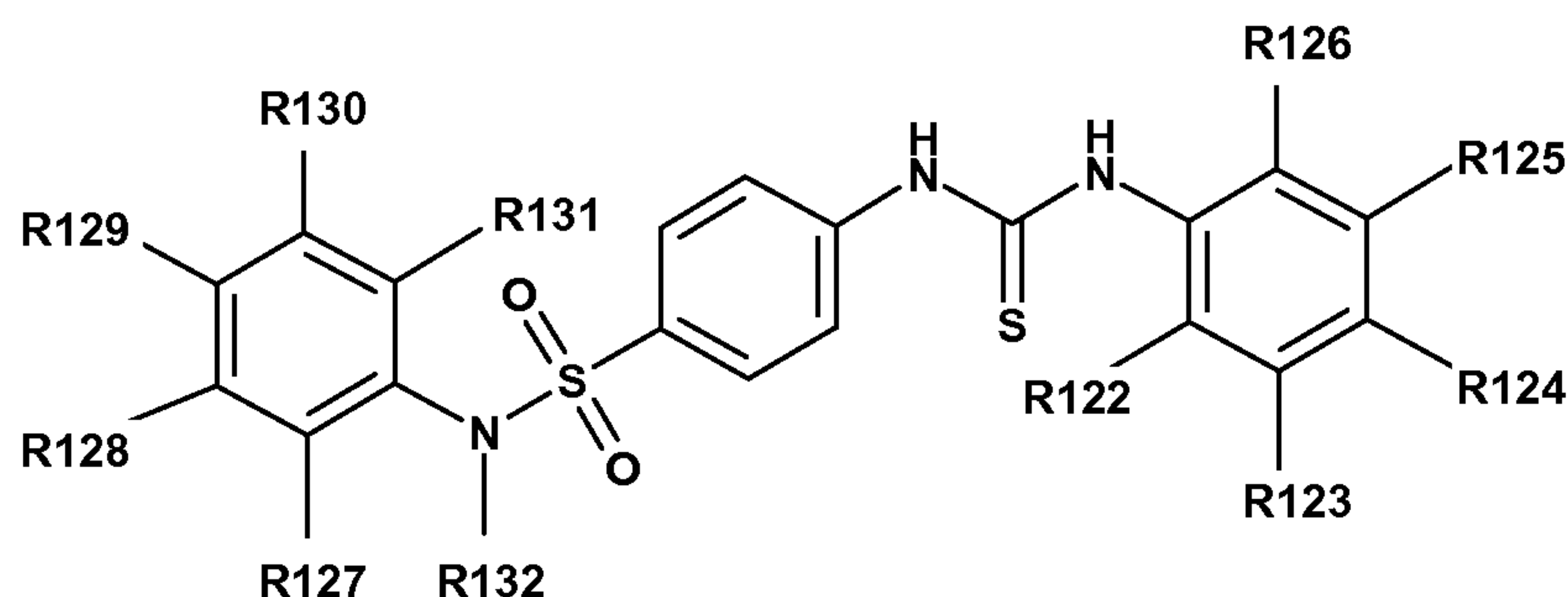
functional group F(IV)8 donates a hydrogen bond to a backbone carbonyl of His280 of SEQ ID NO: 1 and has coordinates of $r = 45.184$, θ (theta) = 112.328 , and Φ (phi) = -8.875 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)9 forms a hydrophobic contact with a side chain of receptor Met244 and Leu243 of SEQ ID NO: 1 and has coordinates of $r = 49.512$, θ (theta) = 108.007 , and Φ (phi) = -8.504 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)10 forms a hydrophobic contact with a side chain of receptor Met244 of SEQ ID NO: 1 and has coordinates of $r = 50.431$, θ (theta) = 104.985 , and Φ (phi) = -7.420 and a spherical radius of about $1.8\text{\AA}$; and

the compound substantially maintains a non-extended tether inactive configuration of EGFR or substantially prevents stabilization of an extended tether active configuration of EGFR.

Claim 65. A compound having a formula of:



Formula (32) (11091-like Type H)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein,

R^{122} and R^{126} are independently selected from the group consisting of:

(i) hydrogen,

(ii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl

optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iii) trifluoromethyl; and

(iv) halogen;

R^{123} and R^{125} are independently selected from the group consisting of:

(i) hydrogen;

(ii) trifluoromethyl;

(iii) halogen;

(iv) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(v) acyl $-COR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(vi) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

R^{124} is selected from the group consisting of:

(i) hydrogen;

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

(iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

(iv) acyl $-COR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(v) halogen;

R^{127} and R^{131} are independently selected from the group consisting of:

(i) hydrogen,

(ii) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom;

R^{128} and R^{130} are independently selected from the group consisting of: hydrogen and halogen;

R^{129} is selected from the group consisting of:

(i) hydrogen;

(ii) halogen;

(iii) alkoxy $-OR^{10}$ where R^{10} is selected from the group consisting of:

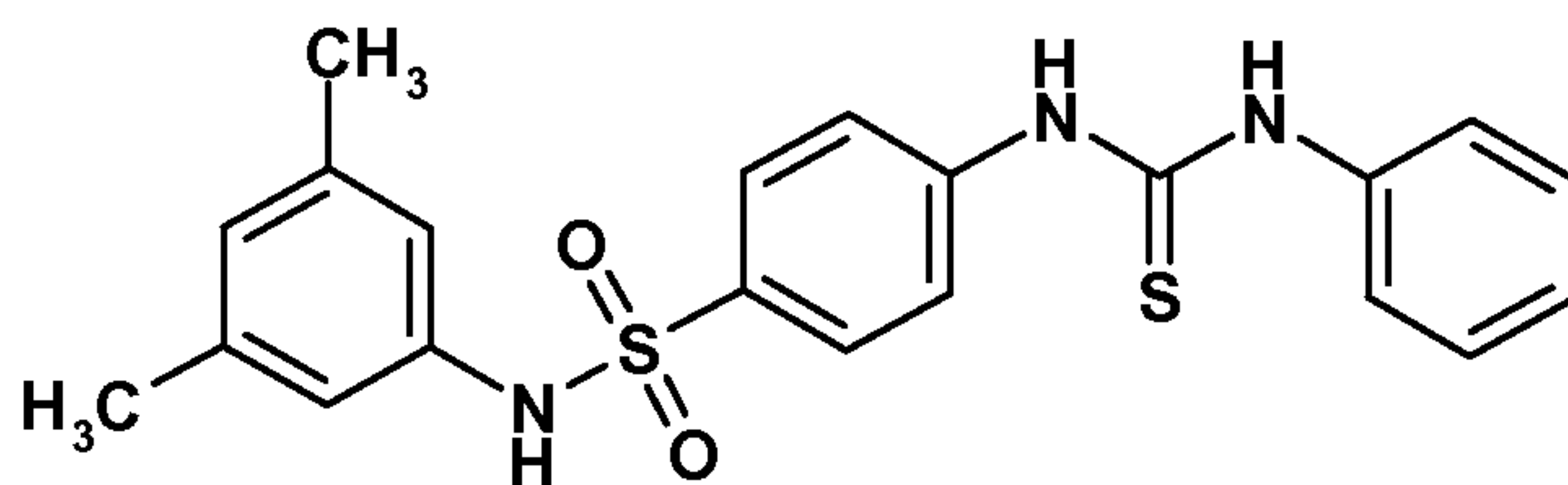
(a) a straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

(b) a C-1 to C-6 cycloalkyl optionally containing unsaturation or one oxygen or nitrogen atom; and

(iv) straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation;

R^{132} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

Formula (32) excludes a compound of Formula (31):



Formula (31).

Claim 66. The compound of claim 65 wherein R^{122} and R^{126} are independently selected from the group consisting of: hydrogen; trifluoromethyl; methoxy; and chloro.

Claim 67. The compound of claim 65 wherein R^{122} or R^{126} is hydrogen.

Claim 68. The compound of any one of claims 65-67 wherein R^{123} and R^{125} are independently selected from the group consisting of: halogen and trifluoromethyl.

Claim 69. The compound of any one of claims 65-68 wherein R^{124} selected from the group consisting of: halogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation.

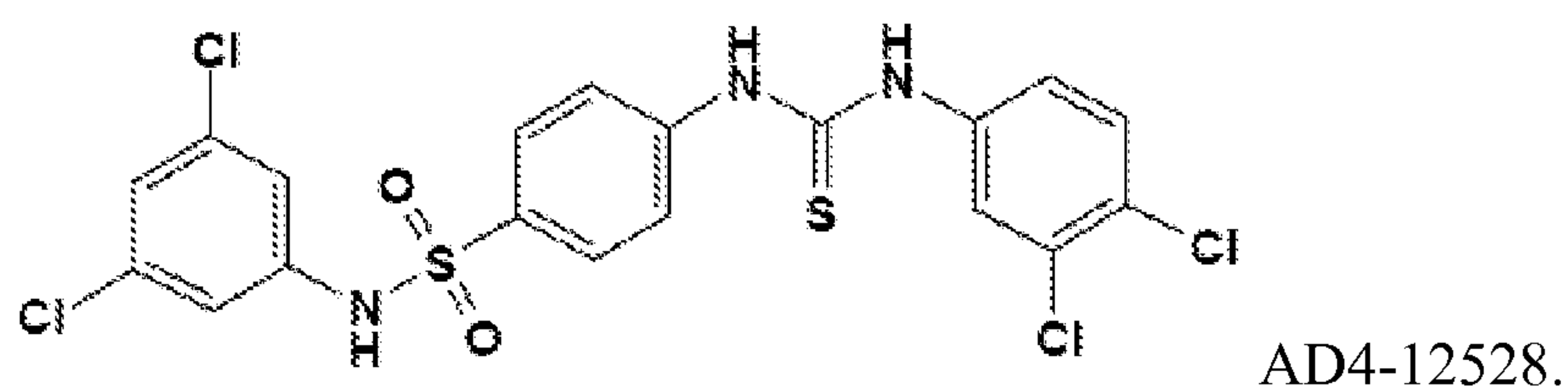
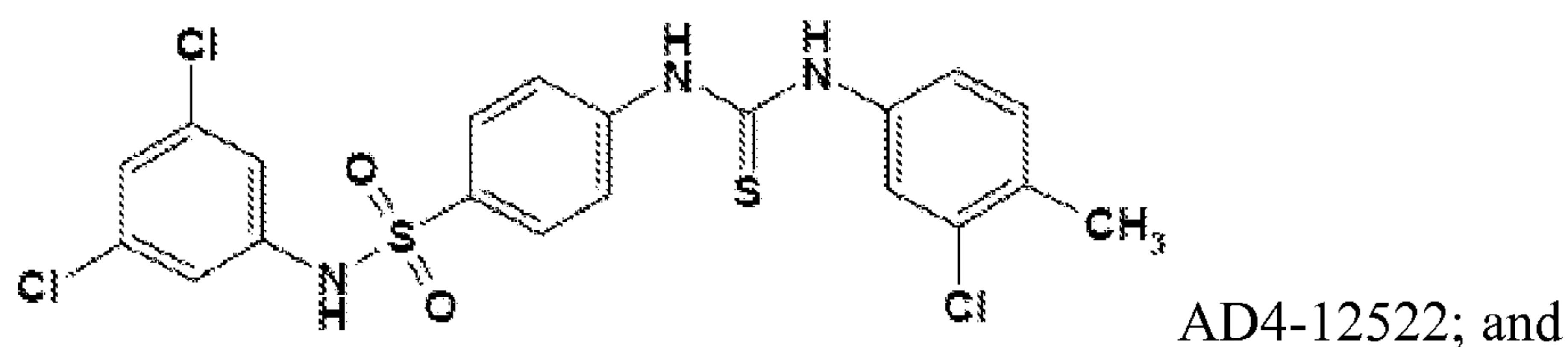
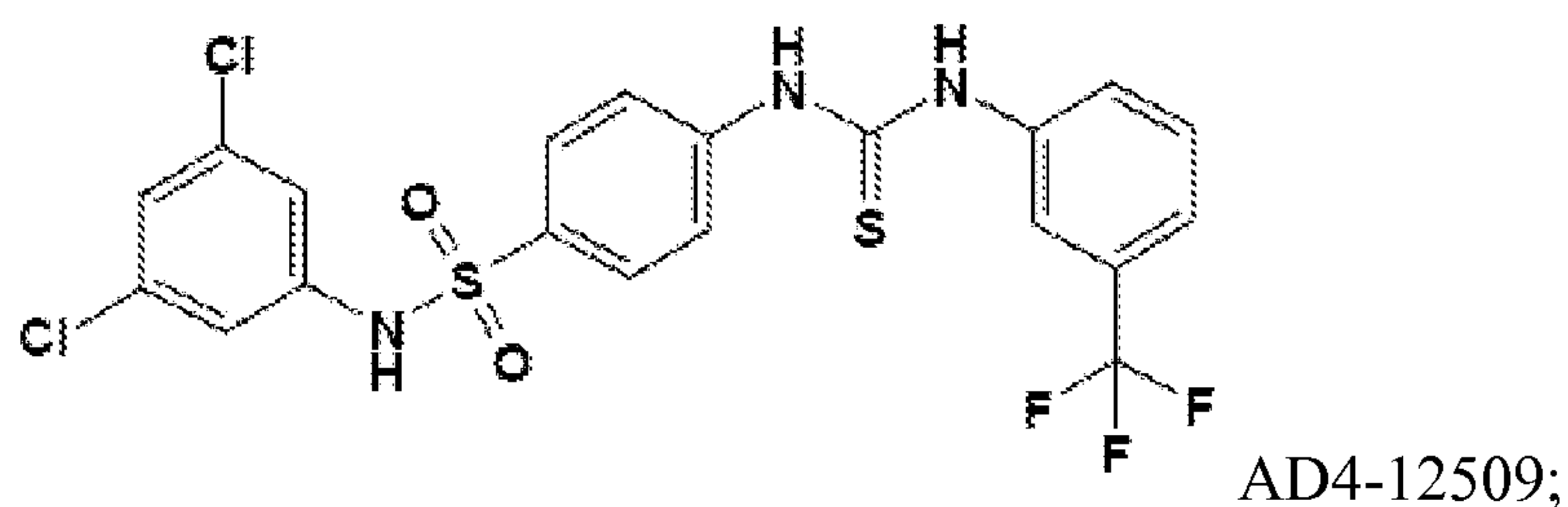
Claim 70. The compound of any one of claims 65-69 wherein R^{127} and R^{131} are independently selected from the group consisting of: hydrogen, methyl, and methoxy.

Claim 71. The compound of any one of claims 65-70 wherein R^{128} and R^{130} are independently selected from the group consisting of: hydrogen and chloro.

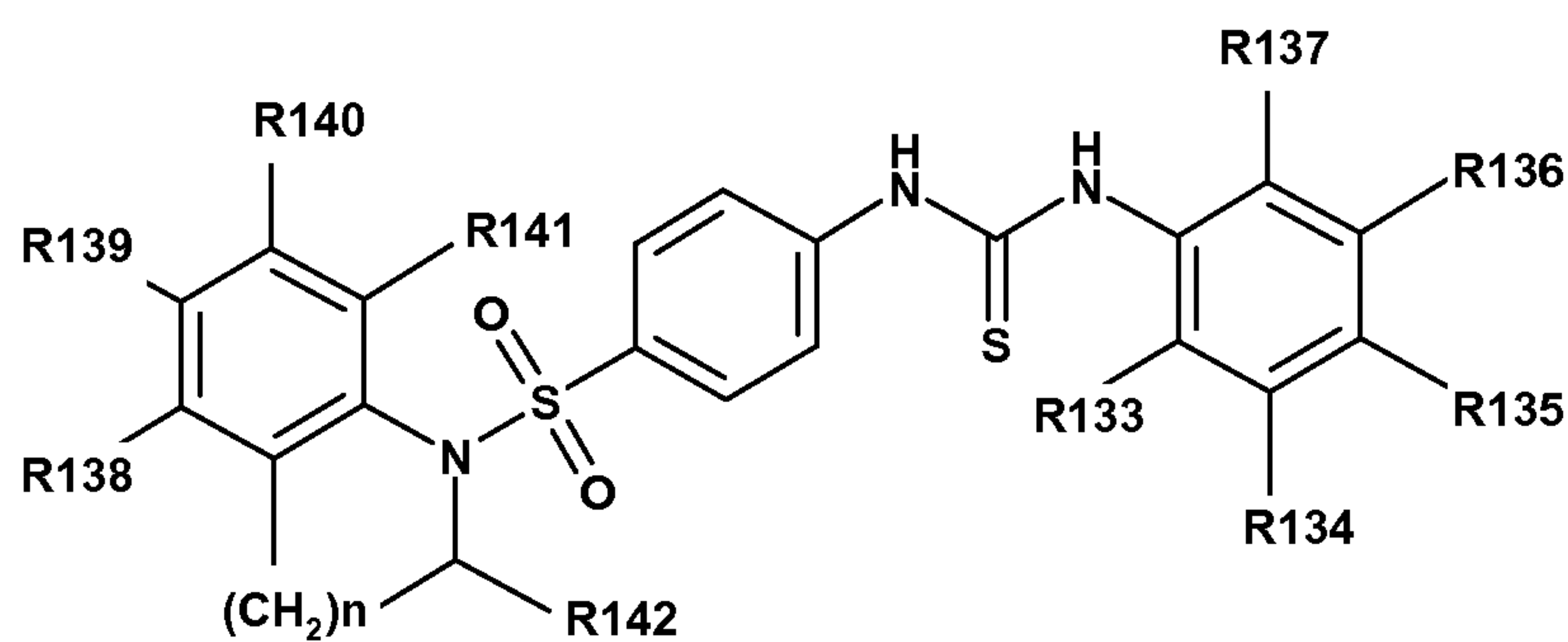
Claim 72. The compound of any one of claims 65-71 wherein R^{129} is selected from the group consisting of: hydrogen; chloro; methoxy; and methyl.

Claim 73. The compound of any one of claims 65-72 wherein R^{132} of Formula (32) is selected from the group consisting of: hydrogen; methyl; and ethyl.

Claim 74. The compound of claim 65 selected from the group consisting of:



Claim 75. A compound having a formula of:



Formula (33) (11091-like Type I)

or a stereoisomer or pharmaceutically acceptable salt thereof;

wherein

n is 1 or 2;

R^{133} and R^{137} are independently selected from the group consisting of:
hydrogen; halogen; and straight chain or branched C-1 to C-4 lower alkyl optionally
containing unsaturation;

R^{134} and R^{136} are independently selected from the group consisting of:
hydrogen; trifluoromethyl; and halogen;

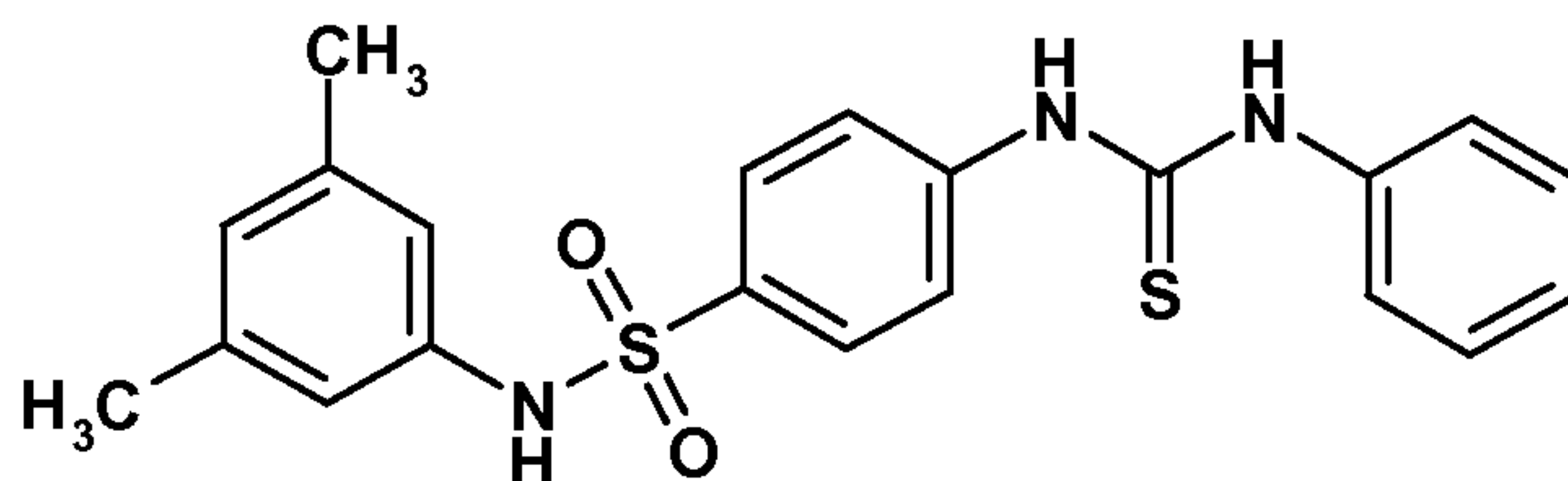
R^{135} selected from the group consisting of: hydrogen and halogen;

R^{138} , R^{140} , and R^{141} are hydrogen;

R^{139} is selected from the group consisting of: hydrogen and halogen;

R^{142} is selected from the group consisting of: hydrogen and straight chain or branched C-1 to C-4 lower alkyl optionally containing unsaturation; and

Formula (33) excludes a compound of Formula (31):



Formula (31).

Claim 76. The compound of claim 75 wherein R^{133} and R^{137} are independently selected from the group consisting of: hydrogen; methyl; and chloro.

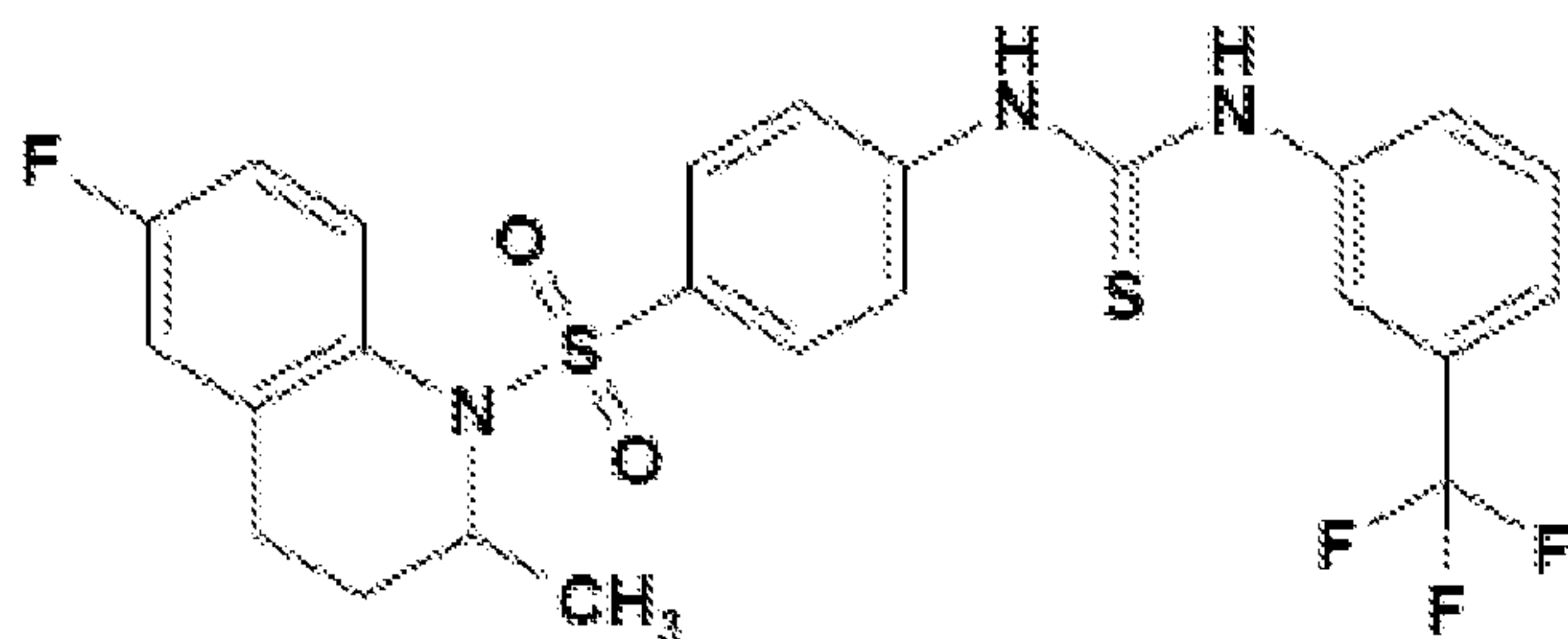
Claim 77. The compound of any one of claims 75-76 wherein R^{134} and R^{136} are independently selected from the group consisting of: hydrogen; trifluoromethyl; and chloro.

Claim 78. The compound of any one of claims 75-77 wherein R^{135} is chloro.

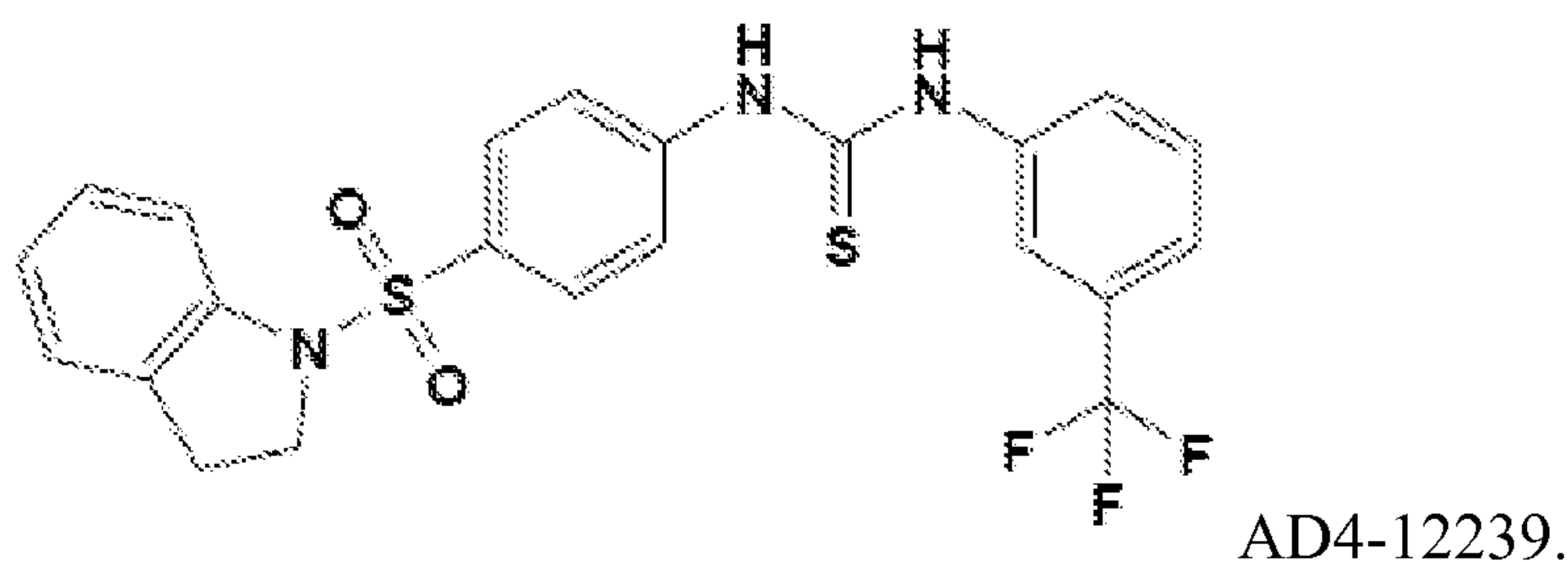
Claim 79. The compound of any one of claims 75-78 wherein R^{139} is selected from the group consisting of: fluoro and hydrogen.

Claim 80. The compound of any one of claims 75-79 wherein R^{142} is selected from the group consisting of: hydrogen or methyl.

Claim 81. The compound of claim 75 selected from the group consisting of:



AD4-12846; and



Claim 82. The compound of any one of claims 65-81 that inhibits EGFR activity comprising:

at least nine or more of functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11 of a Scheme V 11091-like pharmacophore;

wherein

functional group F(V)1 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 85.329$, θ (theta) = 34.962 , and Φ (phi) = 53.394 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)2 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 87.180$, θ (theta) = 35.618 , and Φ (phi) = 53.267 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)3 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.349$, θ (theta) = 34.560 , and Φ (phi) = 55.424 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)4 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.958$, θ (theta) = 35.296 , and Φ (phi) = 51.296 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)5 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.060$, θ (theta) = 34.984 , and Φ (phi) = 50.365 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)6 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.884$, θ (theta) = 36.166 , and Φ (phi) = 49.227 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)7 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.006$, θ (theta) = 35.523, and Φ (phi) = 49.076 and a spherical radius of about 1.5 Å;

functional group F(V)8 accepts a hydrogen bond to a side chain imidazole of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.100$, θ (theta) = 38.590, and Φ (phi) = 43.143 and a spherical radius of about 1.2 Å;

functional group F(V)9 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 84.418$, θ (theta) = 37.489, and Φ (phi) = 46.968 and a spherical radius of about 1.2 Å;

functional group F(V)10 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 82.486$, θ (theta) = 38.027, and Φ (phi) = 47.246 and a spherical radius of about 1.5 Å; and

functional group F(V)11 forms a favorable hydrophobic interaction with a side chain of receptors Val417 and Ile448 of SEQ ID NO: 1 and has coordinates of $r = 84.438$, θ (theta) = 40.753, and Φ (phi) = 48.557 and a spherical radius of about 1.2 Å;

functional group F11 forms a favorable hydrophobic interaction with a side chain of receptors Val417 and Ile448 of SEQ ID NO: 1 and has coordinates of $r = 84.438$, θ (theta) = 40.753, and Φ (phi) = 48.557 and a spherical radius of about 1.2 Å; and

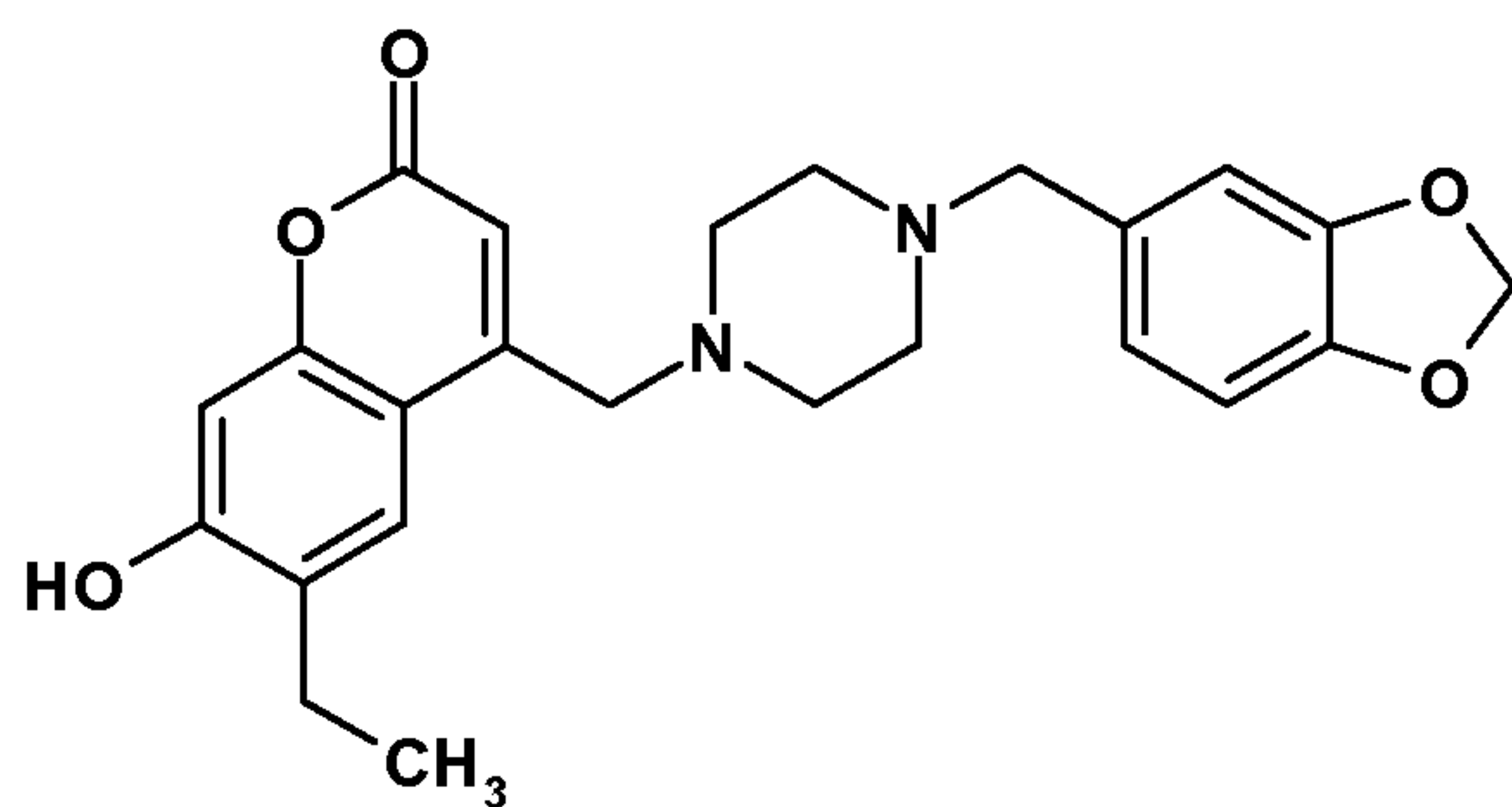
the compound substantially maintains a non-extended tether inactive configuration of EGFR or substantially prevents stabilization of an extended tether active configuration of EGFR.

Claim 83. A method of treating a proliferative disease, disorder, or condition comprising:

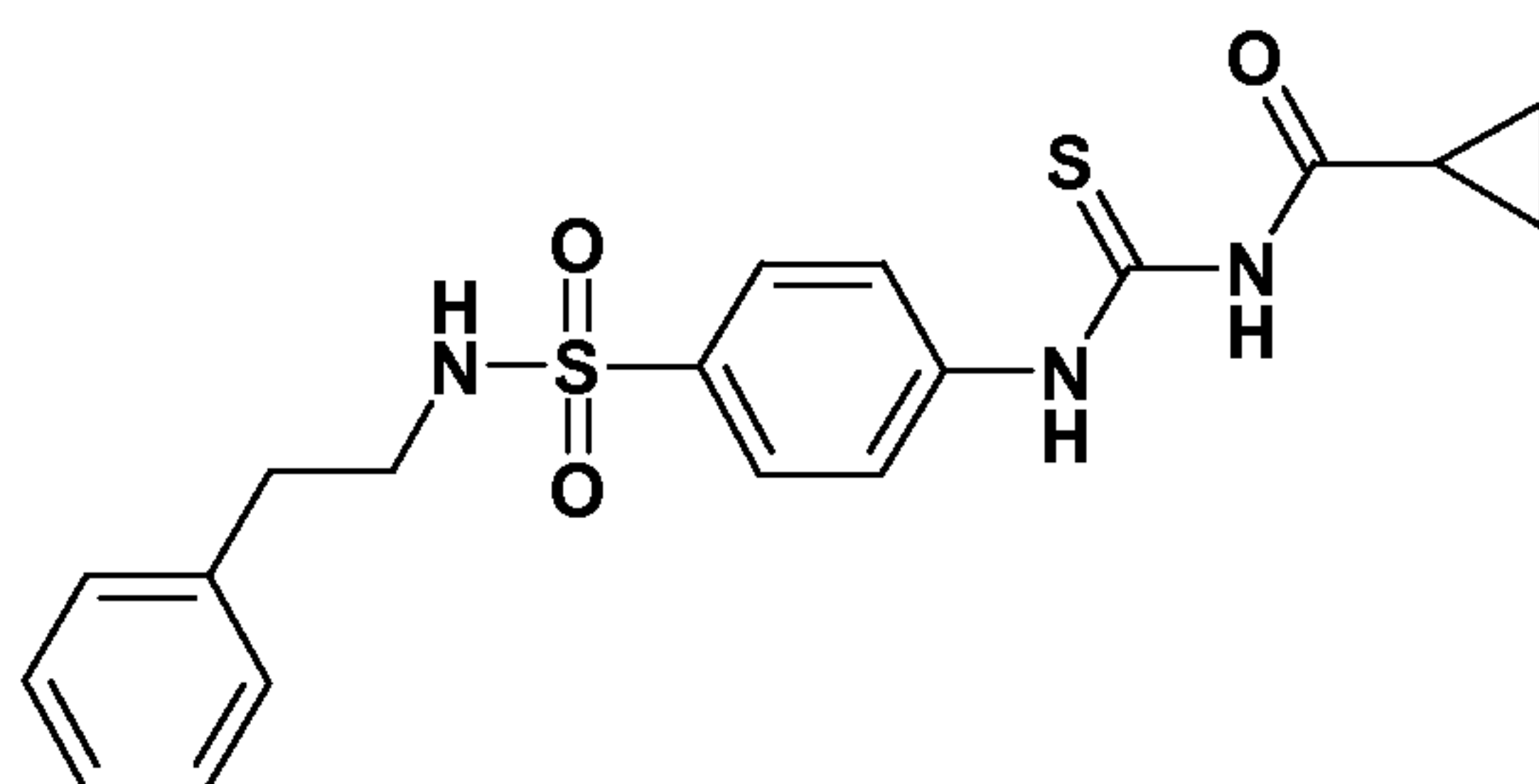
administering to a subject in need thereof a composition comprising a therapeutically effective amount of

(i) a compound of any one of claims 1-82, or a stereoisomer or pharmaceutically acceptable salt thereof;

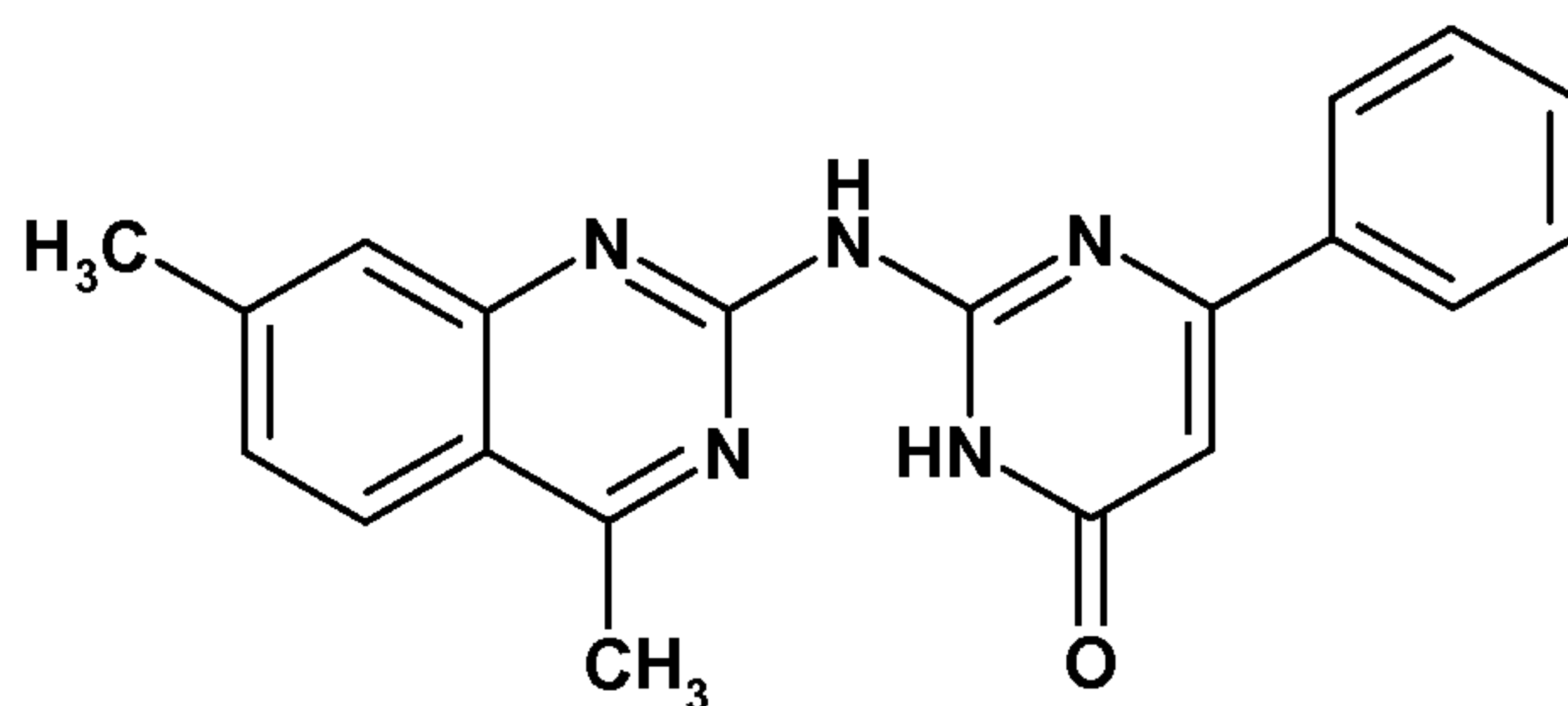
(ii) a compound selected from the group consisting of:



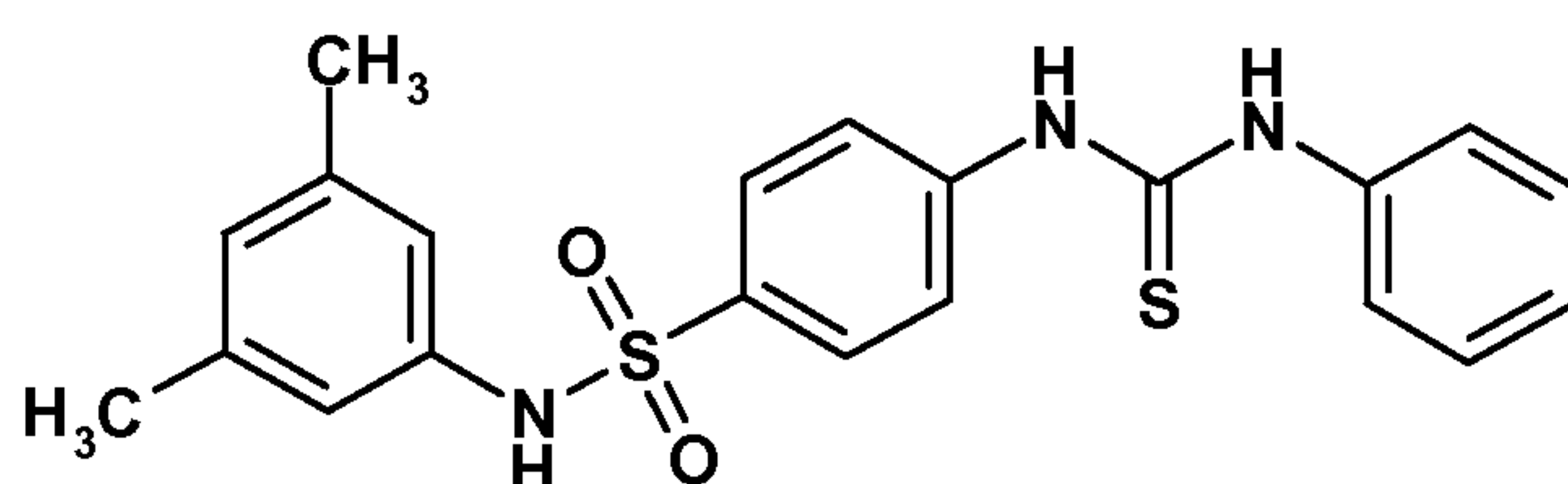
AD4-1734, Formula (12);



AD4-1886, Formula (17);



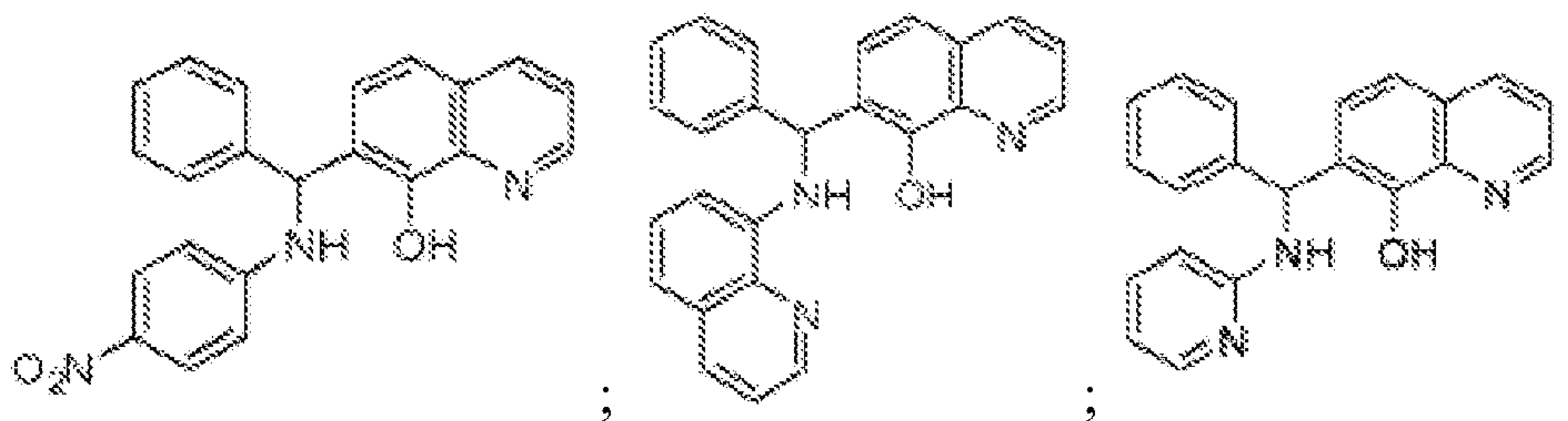
AD4-10381, Formula (24);

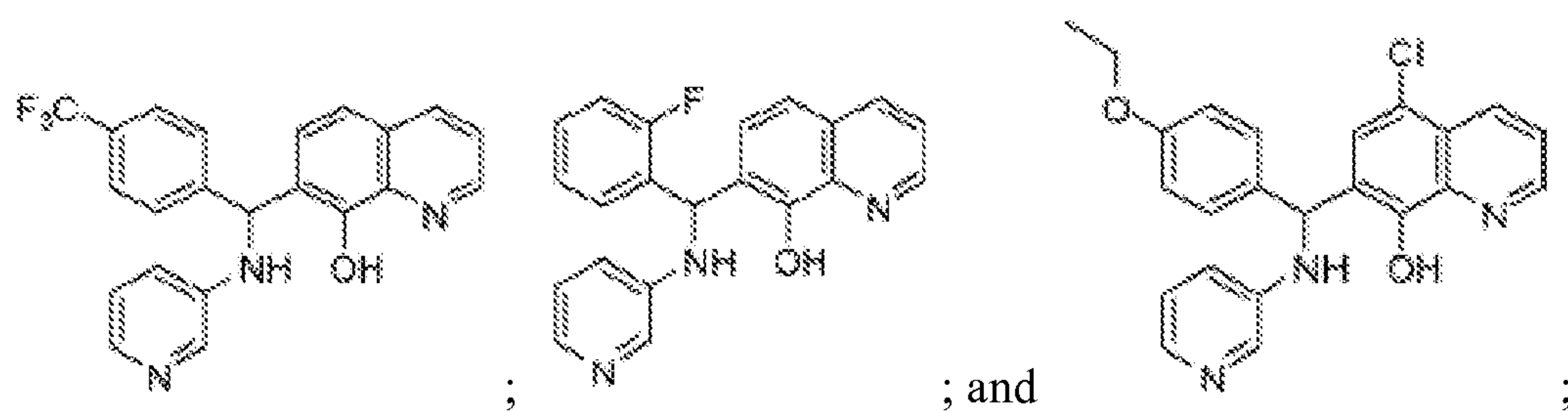


AD4-11091, Formula (31); or

a stereoisomer or pharmaceutically acceptable salt thereof; or

(iii) a compound selected from the group consisting of:

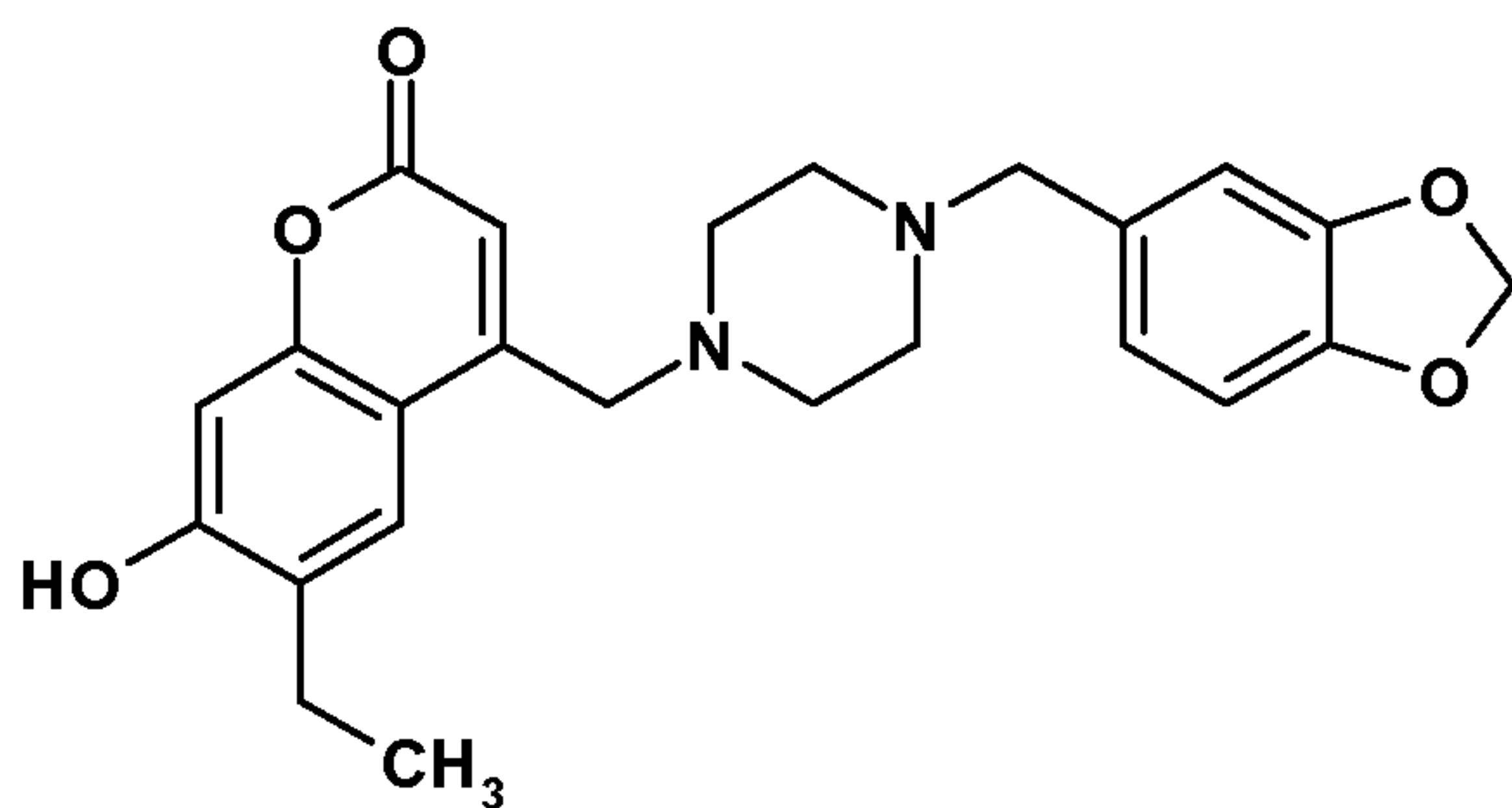




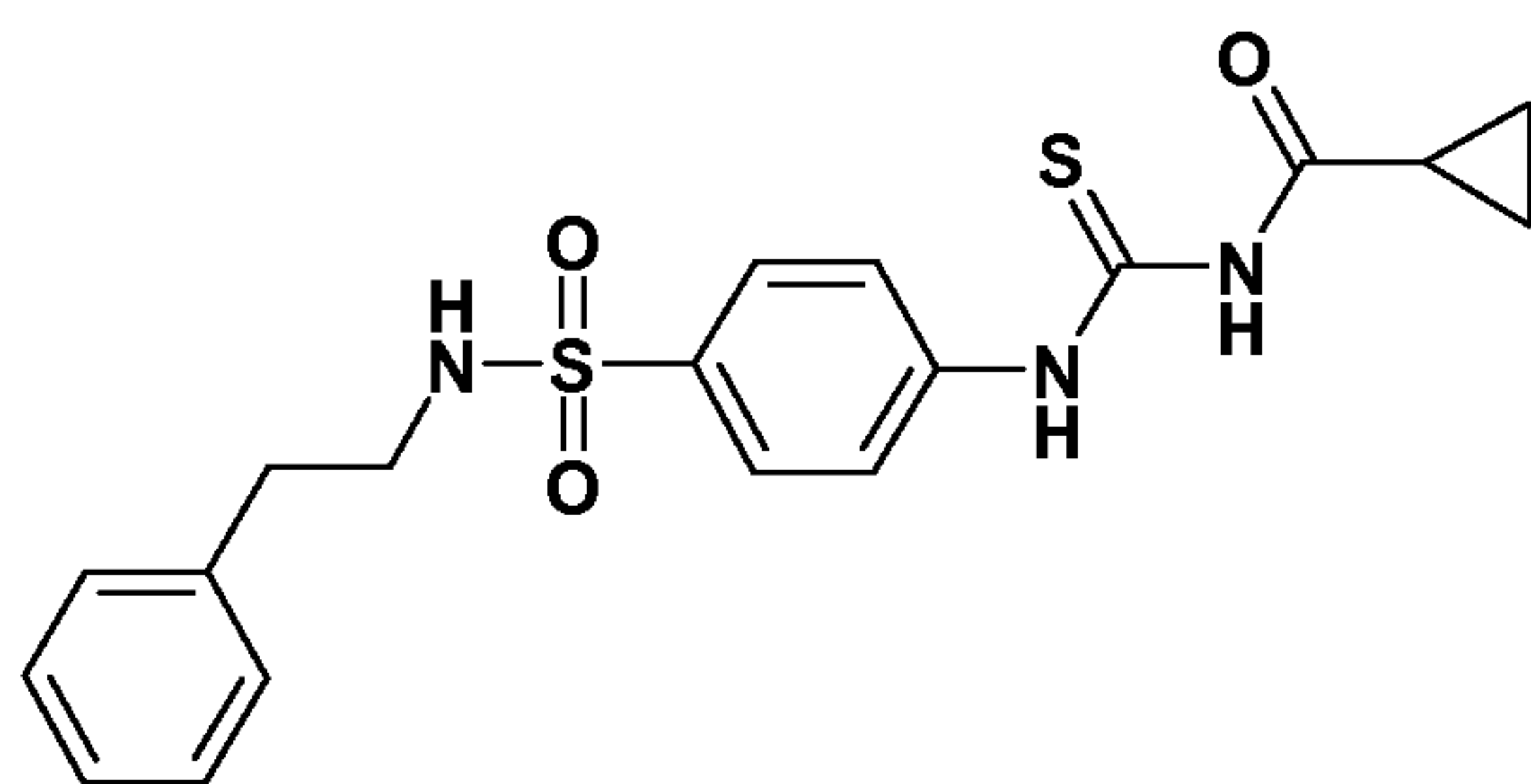
or a stereoisomer or pharmaceutically acceptable salt thereof.

Claim 84. The method of claim 83 wherein the composition comprises a compound selected from any one of claims 1-82, or a stereoisomer or pharmaceutically acceptable salt thereof.

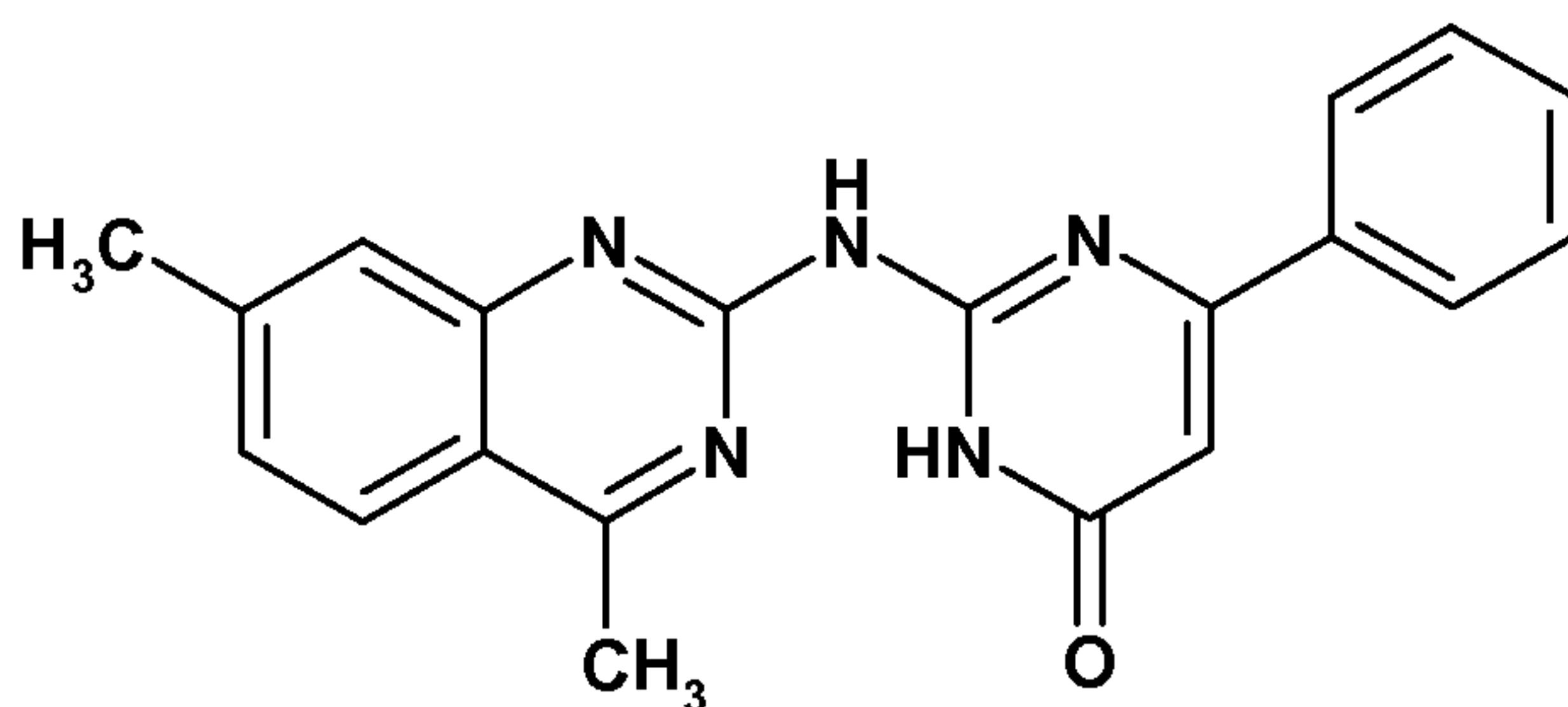
Claim 85. The method of any one of claims 83-84 wherein the composition comprises a compound selected from the group consisting of:



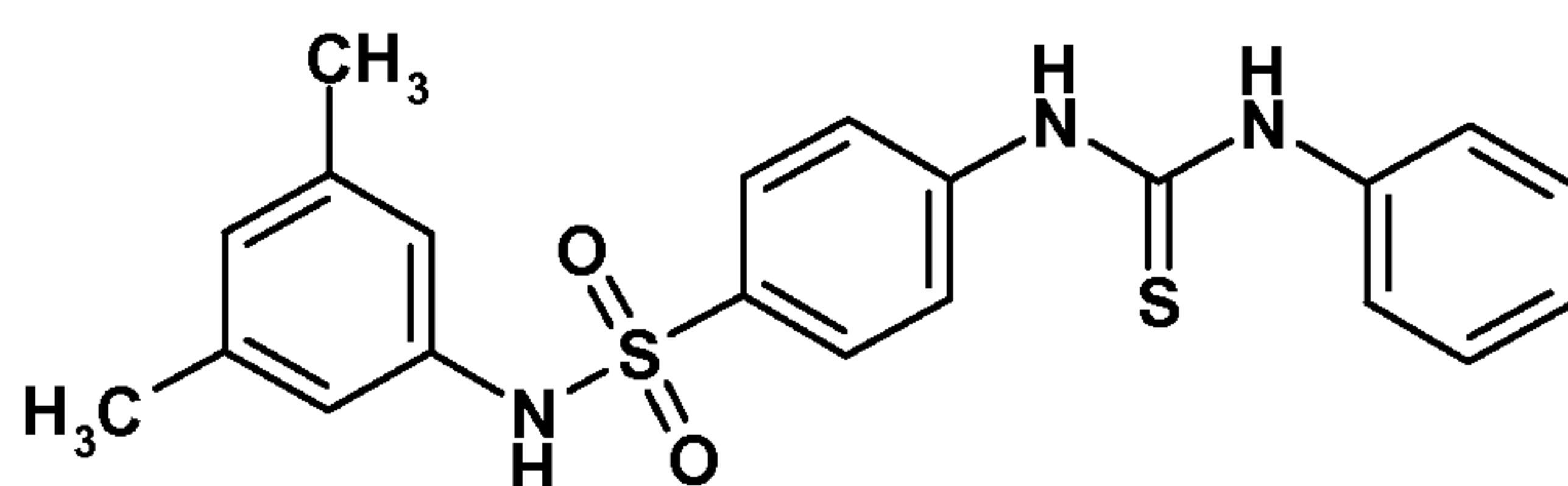
AD4-1734, Formula (12);



AD4-1886, Formula (17);



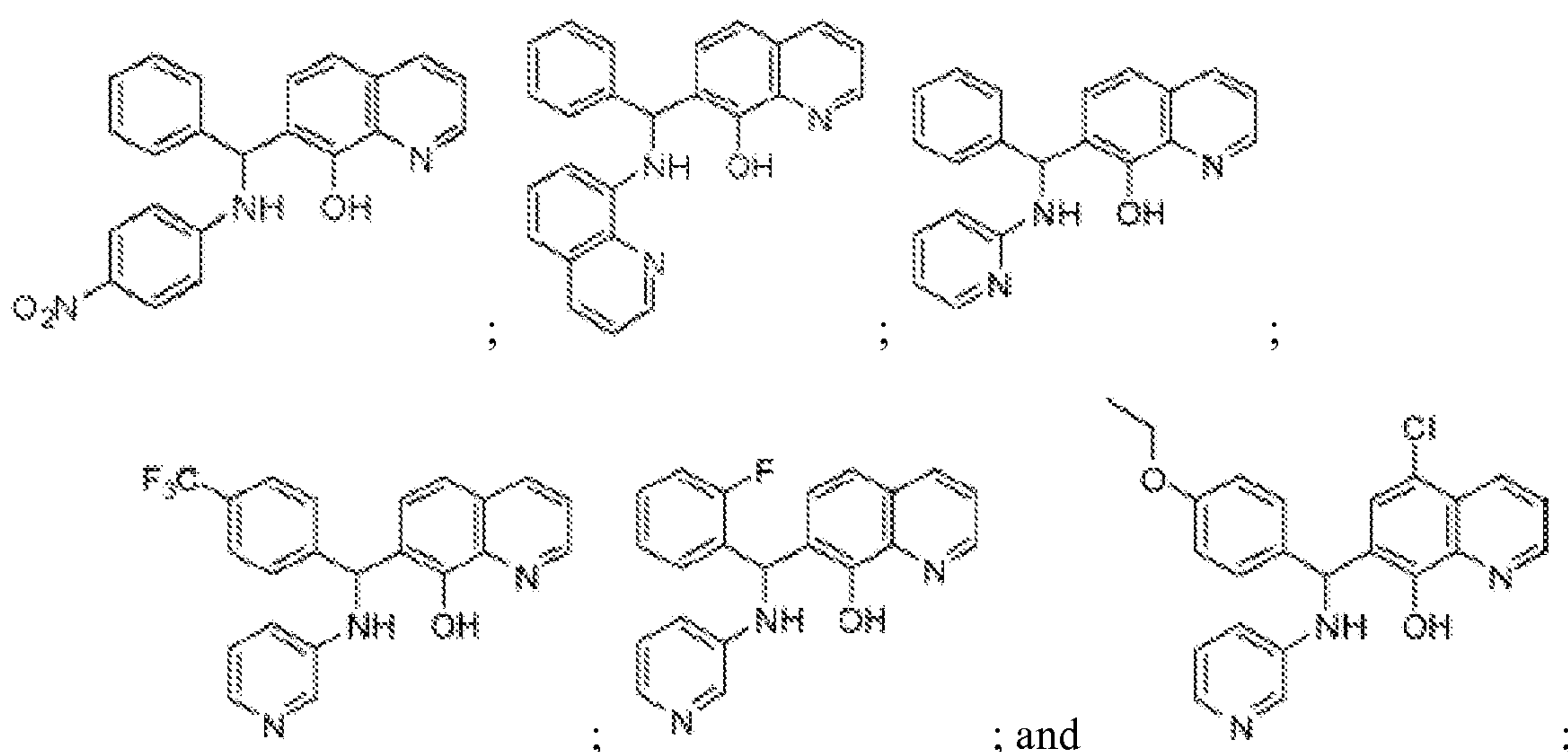
AD4-10381, Formula (24); and



AD4-11091, Formula (31),

or a stereoisomer or pharmaceutically acceptable salt thereof.

Claim 86. The method of any one of claims 83-85 wherein the composition comprises a compound selected from the group consisting of:



or a stereoisomer or pharmaceutically acceptable salt thereof.

Claim 87. The method of any one of claims 83-86 wherein the composition further comprises a pharmaceutically acceptable carrier or excipient.

Claim 88. The method of any one of claims 83-87 wherein the proliferative disease, disorder, or condition is selected from the group consisting of: cancer; a blood vessel proliferative disorder; a fibrotic disorder; a mesangial cell proliferative disorder; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by a viral infection.

Claim 89. The method of any one of claims 83-88 wherein the proliferative disease, disorder, or condition is a disease, disorder, or condition associated with EGFR.

Claim 90. The method of claim 89 wherein the proliferative disease, disorder, or condition associated with EGFR is selected from the group consisting of: cancer; a blood vessel proliferative disorder; a fibrotic disorder; a mesangial cell proliferative disorder; psoriasis; actinic keratoses; seborrheic keratoses; warts; keloid scars; eczema; and hyperproliferative diseases caused by a viral infection.

Claim 91. A method for identifying an epidermal growth factor receptor (EGFR) inhibitor comprising:

providing a pharmacophore comprising a scheme selected from the group consisting of: Scheme II, Scheme III, Scheme IV, or Scheme V as input to a 3-dimensional database;

comparing a three dimensional structure of a candidate compound to the three dimensional structure of the pharmacophore;

selecting a candidate compound with a three dimensional structure that substantially aligns with:

five or more functional groups of Scheme II (ADS-1734-like);

seven or more functional groups of Scheme III (AD4-1886-like);

eight or more of functional groups of Scheme IV (AD4-10381-like); or

nine or more of functional groups of Scheme V (AD4-11091-like);

wherein

similarity between the three-dimensional structure of the candidate compound and the three-dimensional structure of the pharmacophore is indicative of an ability of the candidate compound to inhibit EGFR by substantially maintaining a tethered inactive configuration of EGFR or substantially preventing stabilization of the untethered active configuration of EGFR;

Scheme II (ADS-1734-like) comprises functional groups F(II)1, F(II)2, F(II)3, F(II)4, F(II)5, F(II)6, F(II)7, F(II)8, and F(II)9; wherein

functional group F(II)1 donates an H-bond or forms a salt bridge to a carboxylate side chain of receptor Asp553 of SEQ ID NO: 1 and has coordinates of $r = 56.363$, θ (theta) = 94.368, and Φ (phi) = -17.752 and a spherical radius of about 1.2Å;

functional group F(II)2 is a donor and has coordinates of $r = 53.290$, θ (theta) = 101.494, and Φ (phi) = -23.244 and a spherical radius of about 1.0Å;

functional group F(II)3 forms a hydrophobic contact with a side chain of receptor Val568, imidazolidine side chain of receptor Pro552 and with a side chain of Met253 of SEQ ID NO: 1 and has coordinates of $r = 53.726$, θ (theta) = 97.830, and Φ (phi) = -18.377 and a spherical radius of about 1.7Å;

functional group F(II)4 forms a hydrophobic contact with side chain of receptor Val575, Met253, and with an imidazolidine ring of receptor Pro552 of SEQ ID NO: 1 and has coordinates of $r = 53.647$, θ (theta) = 103.844, and Φ (phi) = -20.990 and a spherical radius of about 1.4Å;

functional group F(II)5 donates an H-bond to a side chain hydroxyl of Thr570 of SEQ ID NO: 1 and has coordinates of $r = 51.093$, θ (theta) = 104.261, and Φ (phi) = -25.552 and a spherical radius of about 1.2Å;

functional group F(II)6 is a donor having directionality of F4 with respect to a backbone carbonyl of receptor Thr570 of SEQ ID NO: 1 and has coordinates of $r = 52.340$, θ (theta) = 103.980, and Φ (phi) = -27.461 and a spherical radius of about 1.5Å;

functional group F(II)7 accepts an H-bond from a receptor backbone NH of Ala573 of SEQ ID NO: 1 and has coordinates of $r = 51.383$, θ (theta) = 106.455, and Φ (phi) = -24.319 and a spherical radius of about 1.2Å;

functional group F(II)8 is an acceptor having directionality of F7 with respect to the backbone NH of receptor Ala573 of SEQ ID NO: 1 and has coordinates of $r = 52.861$, θ (theta) = 107.691, and Φ (phi) = -25.448 and a spherical radius of about 1.5Å; and

functional group F(II)9 donates an H-bond to a backbone carbonyl of Asp563 and forms a salt bridge to a side chain carboxylate of receptor Asp563 of SEQ ID NO: 1 and has coordinates of $r = 57.688$, θ (theta) = 99.198, and Φ (phi) = -21.588 and a spherical radius of about 1.2Å;

Scheme III (AD4-1886-like) comprises functional groups F(III)1, F(III)2, F(III)3, F(III)4, F(III)5, F(III)6, F(III)7, F(III)8, and F(III)9; wherein

functional group F(III)1 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 and accepts a hydrogen bond from the side chain NH₂ of receptor Gln384 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243, and Φ (phi) = 45.369 and a spherical radius of about 1.2Å;

functional group F(III)2 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 87.287$, θ (theta) = 40.739, and Φ (phi) = 51.781 and a spherical radius of about 1.2Å;

functional group F(III)3 accepts a hydrogen bond from a side chain OH of receptor Ser440 of SEQ ID NO: 1 and has coordinates of $r = 86.320$, θ (theta) = 41.915, and Φ (phi) = 52.323 and a spherical radius of about 1.5Å;

functional group F(III)4 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.870$, θ (theta) = 38.463, and Φ (phi) = 41.650 and a spherical radius of about 1.2Å;

functional group F(III)5 forms a favorable coulombic interaction with an imidazole side chain of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 82.241$, θ (theta) = 37.431, and Φ (phi) = 44.151 and a spherical radius of about 1.5Å;

functional group F(III)6 accepts a hydrogen bond from, or forms a salt bridge to, NH³⁺ of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 88.009$, θ (theta) = 37.822, and Φ (phi) = 54.903 and a spherical radius of about 1.2Å;

functional group F(III)7 accepts a hydrogen bond from, or forms a salt bridge to, NH³⁺ of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.513$, θ (theta) = 36.889, and Φ (phi) = 54.484 and a spherical radius of about 1.5Å;

functional group F(III)8 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 81.552$, θ (theta) = 41.243, and Φ (phi) = 45.369 and a spherical radius of about 1.2Å; and

functional group F(III)9 donates a hydrogen bond to a side chain carbonyl of receptor Gln408 of SEQ ID NO: 1 and has coordinates of $r = 79.652$, θ (theta) = 40.928, and Φ (phi) = 44.528 and a spherical radius of about 1.6Å;

Scheme IV (AD4-10381-like) comprises functional group F(IV)1, F(IV)2, F(IV)3, F(IV)4, F(IV)5, F(IV)6, F(IV)7, F(IV)8, and F(IV)9; wherein

functional group F(IV)1 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 49.686$, θ (theta) = 113.993 , and Φ (phi) = -17.014 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)2 accepts a hydrogen bond from receptor side chain OH of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 48.071$, θ (theta) = 115.388 , and Φ (phi) = -16.211 and a spherical radius of about $1.6\text{\AA}$;

functional group F(IV)3 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.781$, θ (theta) = 113.121 , and Φ (phi) = -17.520 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)4 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 52.021$, θ (theta) = 114.264 , and Φ (phi) = -15.878 and a spherical radius of about $1.5\text{\AA}$;

functional group F(IV)5 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 50.322$, θ (theta) = 111.253 , and Φ (phi) = -15.426 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)6 forms a hydrophobic contact with a side chain of receptor Met576 and imidazolidine ring of Pro242 of SEQ ID NO: 1 and has coordinates of $r = 51.575$, θ (theta) = 112.433 , and Φ (phi) = -13.827 and a spherical radius of about $1.5\text{\AA}$;

functional group F(IV)7 forms a hydrophobic contact with a side chain of receptor Leu243 and a side chain of Thr239 of SEQ ID NO: 1 and has coordinates of $r = 47.767$, θ (theta) = 112.521 , and Φ (phi) = -10.196 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)8 donates a hydrogen bond to a backbone carbonyl of His280 of SEQ ID NO: 1 and has coordinates of $r = 45.184$, θ (theta) = 112.328 , and Φ (phi) = -8.875 and a spherical radius of about $1.2\text{\AA}$;

functional group F(IV)9 forms a hydrophobic contact with a side chain of receptor Met244 and Leu243 of SEQ ID NO: 1 and has coordinates of $r = 49.512$, θ (theta) = 108.007 , and Φ (phi) = -8.504 and a spherical radius of about $1.2\text{\AA}$; and

functional group F(IV)10 forms a hydrophobic contact with a side chain of receptor Met244 of SEQ ID NO: 1 and has coordinates of $r = 50.431$, θ (theta) = 104.985 , and Φ (phi) = -7.420 and a spherical radius of about $1.8\text{\AA}$; and

Scheme V (AD4-11091-like) comprises functional group functional groups F(V)1, F(V)2, F(V)3, F(V)4, F(V)5, F(V)6, F(V)7, F(V)8, F(V)9, F(V)10, and F(V)11; wherein

functional group F(V)1 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 85.329$, θ (theta) = 34.962 , and Φ (phi) = 53.394 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)2 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 87.180$, θ (theta) = 35.618 , and Φ (phi) = 53.267 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)3 accepts a hydrogen bond from a side chain amino of receptor Lys465 of SEQ ID NO: 1 and has coordinates of $r = 86.349$, θ (theta) = 34.560 , and Φ (phi) = 55.424 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)4 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.958$, θ (theta) = 35.296 , and Φ (phi) = 51.296 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)5 donates a hydrogen bond to a side chain carbonyl of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.060$, θ (theta) = 34.984 , and Φ (phi) = 50.365 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)6 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 83.884$, θ (theta) = 36.166 , and Φ (phi) = 49.227 and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)7 donates a hydrogen bond to a side chain nitrogen of receptor Gln411 of SEQ ID NO: 1 and has coordinates of $r = 82.006$, θ (theta) = 35.523 , and Φ (phi) = 49.076 and a spherical radius of about $1.5\text{\AA}$;

functional group F(V)8 accepts a hydrogen bond to a side chain imidazole of receptor His409 of SEQ ID NO: 1 and has coordinates of $r = 85.100$, θ (theta) $= 38.590$, and Φ (phi) $= 43.143$ and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)9 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 84.418$, θ (theta) $= 37.489$, and Φ (phi) $= 46.968$ and a spherical radius of about $1.2\text{\AA}$;

functional group F(V)10 forms a favorable π - π interaction with a phenyl ring of receptor Phe412 of SEQ ID NO: 1 and has coordinates of $r = 82.486$, θ (theta) $= 38.027$, and Φ (phi) $= 47.246$ and a spherical radius of about $1.5\text{\AA}$; and

functional group F(V)11 forms a favorable hydrophobic interaction with a side chain of receptors Val417 and Ile448 of SEQ ID NO: 1 and has coordinates of $r = 84.438$, θ (theta) $= 40.753$, and Φ (phi) $= 48.557$ and a spherical radius of about $1.2\text{\AA}$.

Claim 92. The method of claim 91 wherein the pharmacophore comprises Scheme II.

Claim 93. The method of claim 91 wherein the pharmacophore comprises Scheme III.

Claim 94. The method of claim 91 wherein the pharmacophore comprises Scheme IV.

Claim 95. The method of claim 91 wherein the pharmacophore comprises Scheme V.

Claim 96. The method of any one of claims 91-95 further comprising:
determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation; and
constructing a pharmacophore, wherein the pharmacophore comprises a plurality of pharmacophoric features that approximates the identity and the spatial

orientation of the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation such that the pharmacophore structural features are complementary to the inactive EGFR configuration.

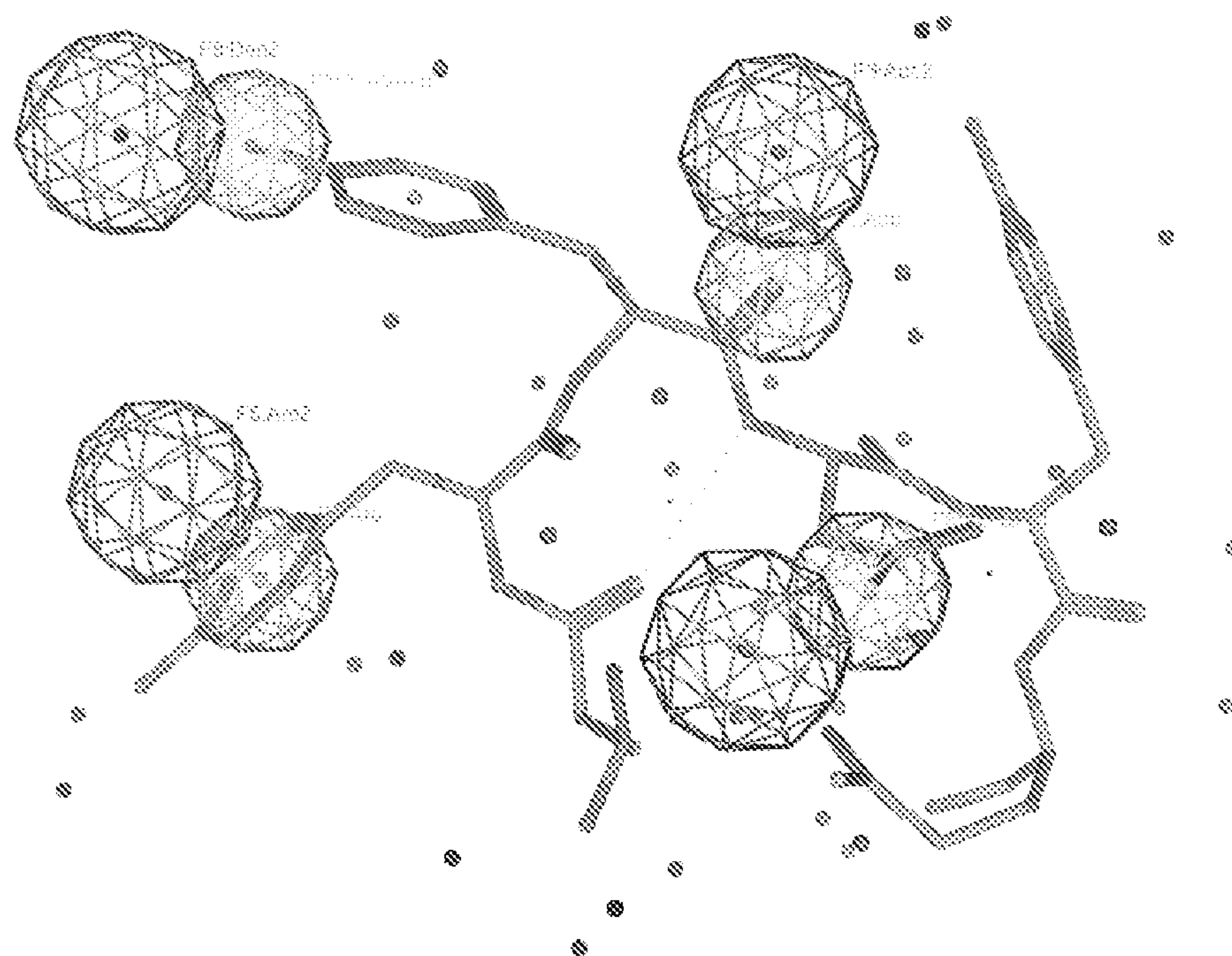
Claim 97. The method of claim 96, wherein determining identity and spatial orientation of at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation comprises analysis of X-ray crystallographic data derived from a crystalline form of EGFR in an inactive, tethered conformation.

Claim 98. The method of any one of claims 91-97 wherein at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of domain II of EGFR in a tethered inactive conformation.

Claim 99. The method of any one of claims 91-98 wherein at least one pharmacophoric feature approximates identity and spatial orientations of at least a portion of atoms of a cleft region between domain II and domain IV of EGFR in a tethered inactive conformation.

Claim 100. The method of any one of claims 91-99 further comprising:
determining a docking affinity of the candidate molecule for the at least a portion of atoms of EGFR associated with stabilizing a tethered configuration of domain II and domain IV of EGFR in an inactive conformation;
wherein docking affinity is quantified by energy gained upon interaction of the candidate molecule with the target biomolecule, energy required to attain the docked conformation relative to the lowest energy conformation, or a combination thereof.

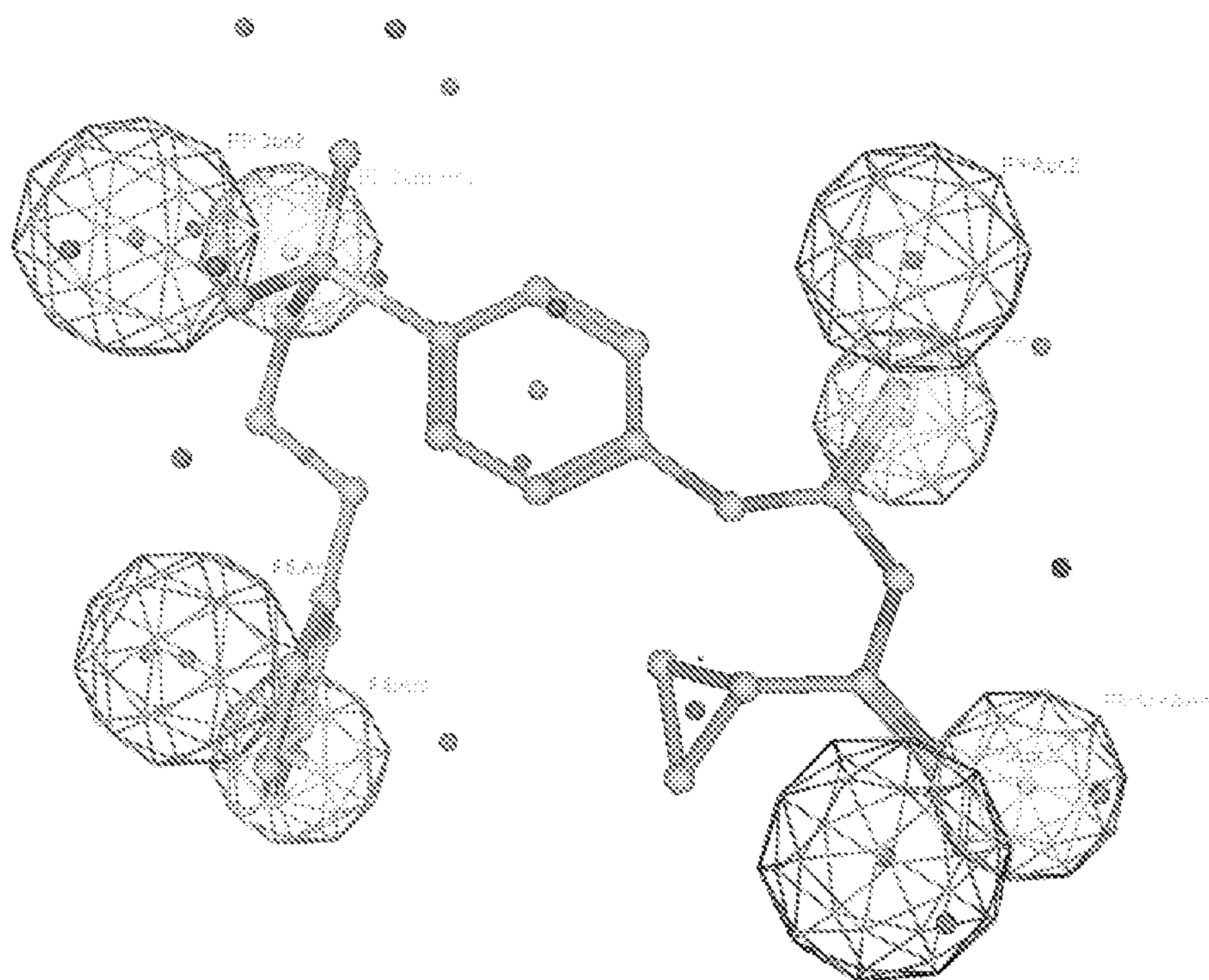
1/36



Pharmacophore 21_thr100_glu105

FIG. 1A

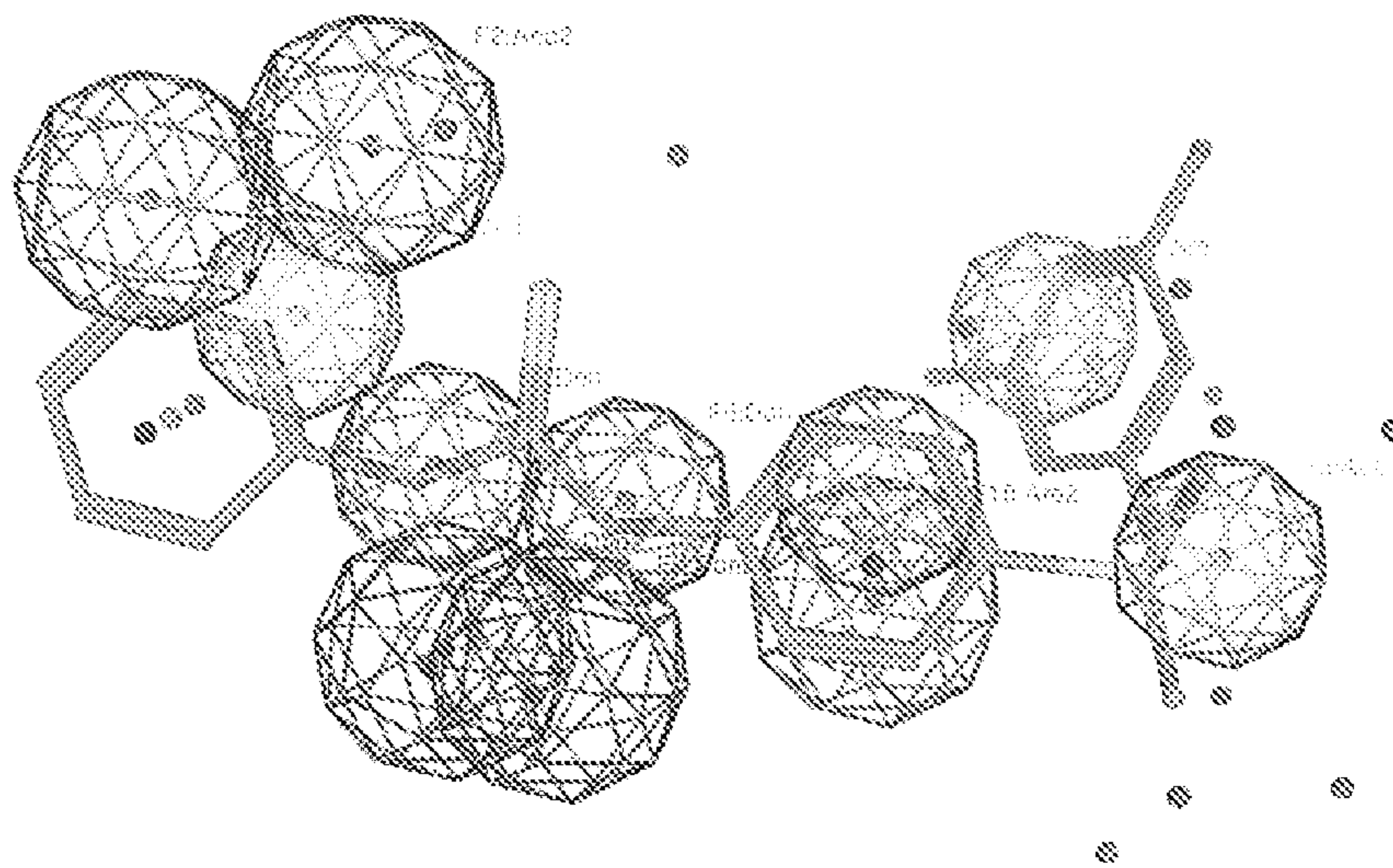
2/36



Pharmacophore 21_thr100_glu105 aligned to AD4-1886

FIG. 1B

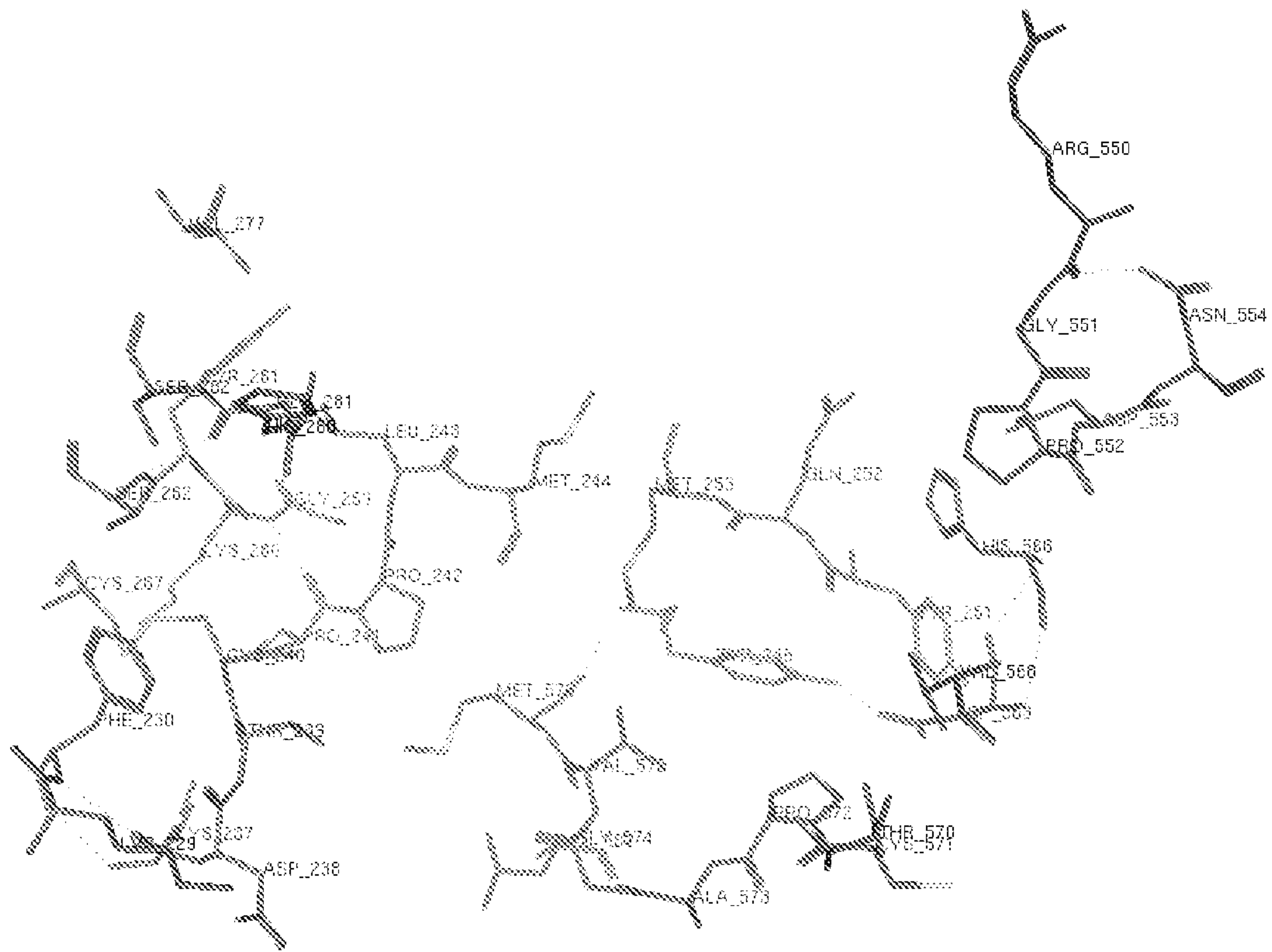
3/36



AD4-11091 aligned to the pharmacophore model, Pharm1886-6.

FIG. 2

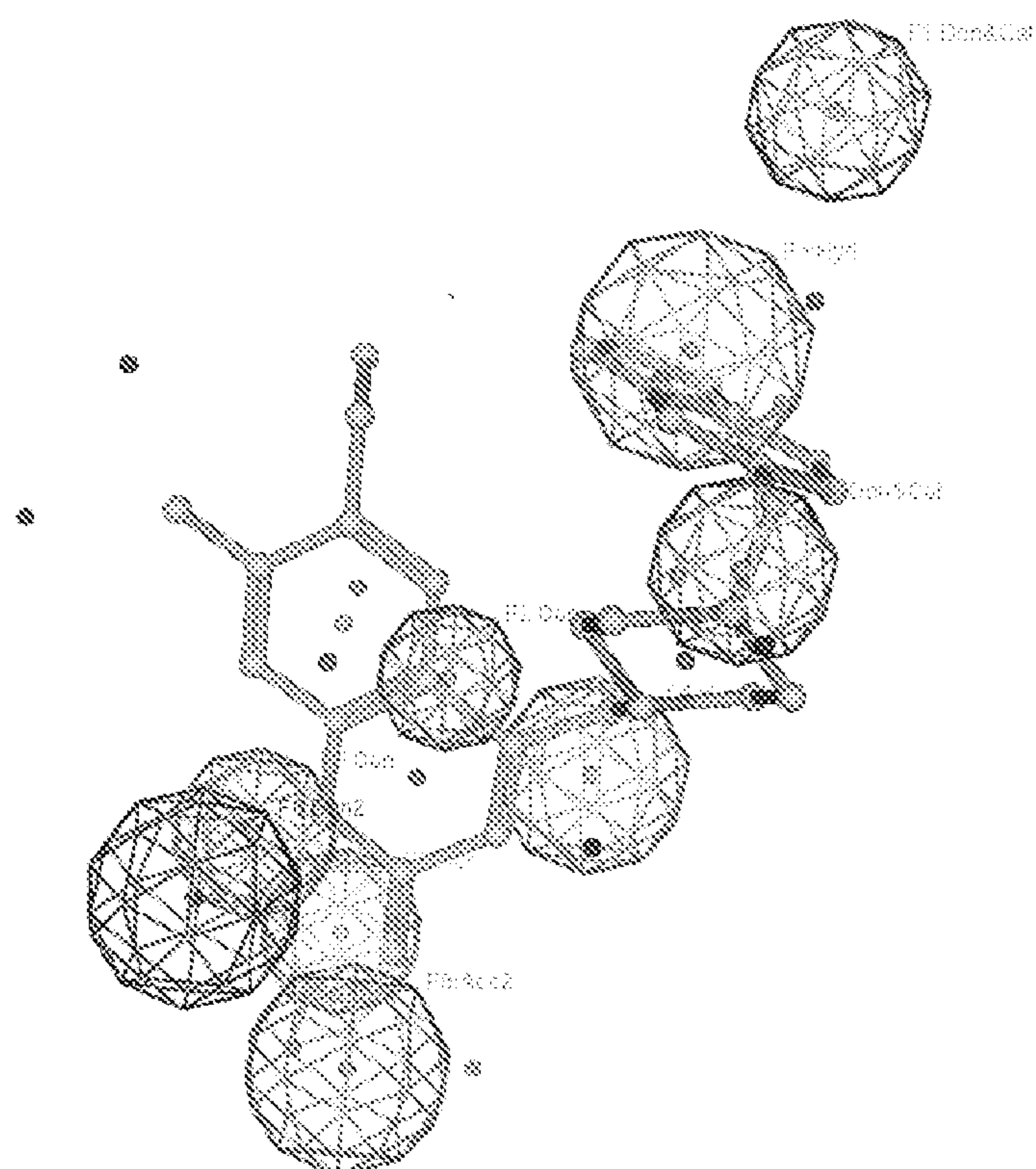
4/36



Binding site at the interface of Domain II and Domain IV of the Inactive Form of EGFr
(1NQL.pdb)

FIG. 3

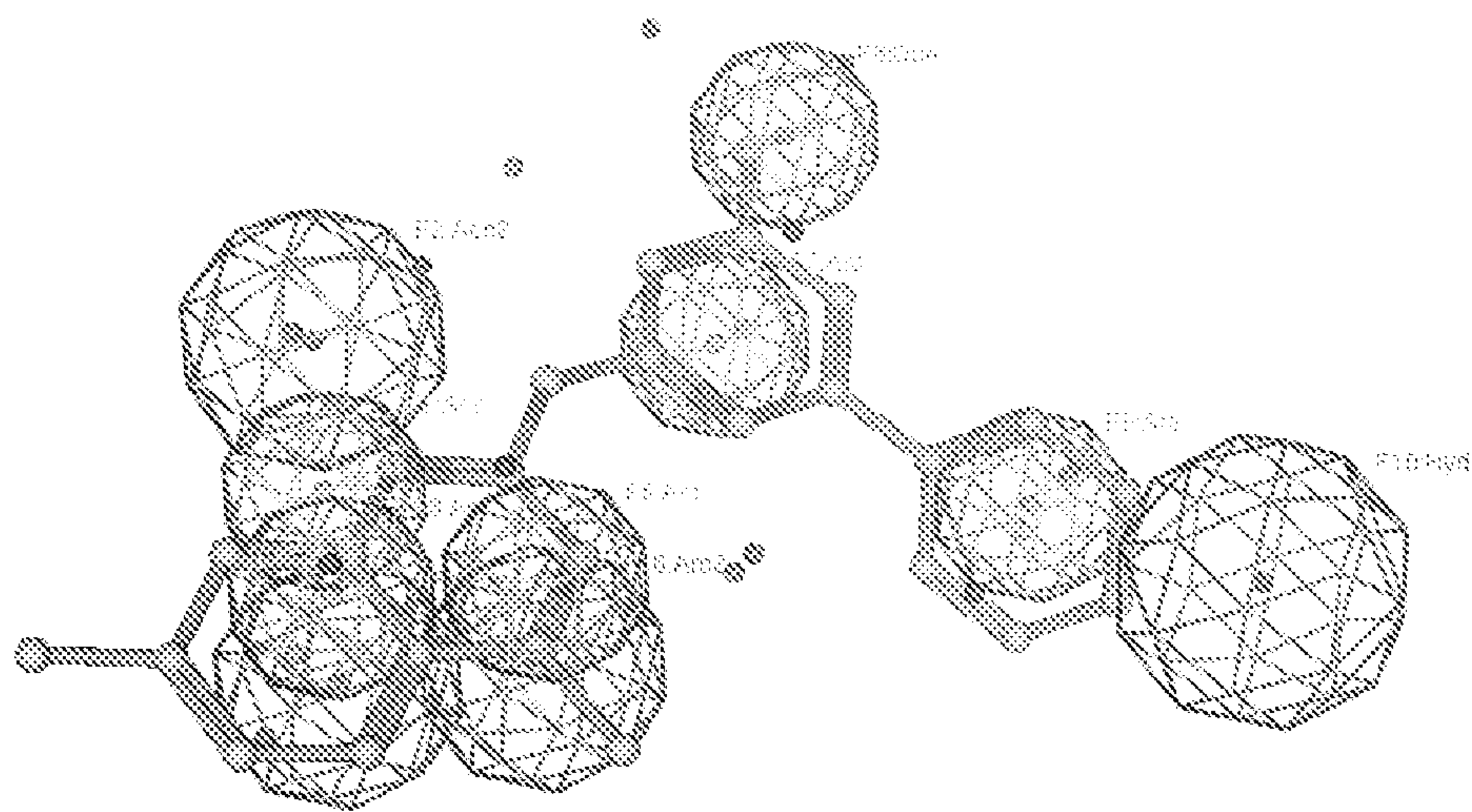
5/36



Pharmacophore model: Pharm-1nql-glue-6 aligned to the hit AD4-1734.

FIG. 4B

6/36



Pharmacophore model: DockPharm1505-2 aligned to the hit AD4-10381

FIG. 5

7/36

AD4-1886

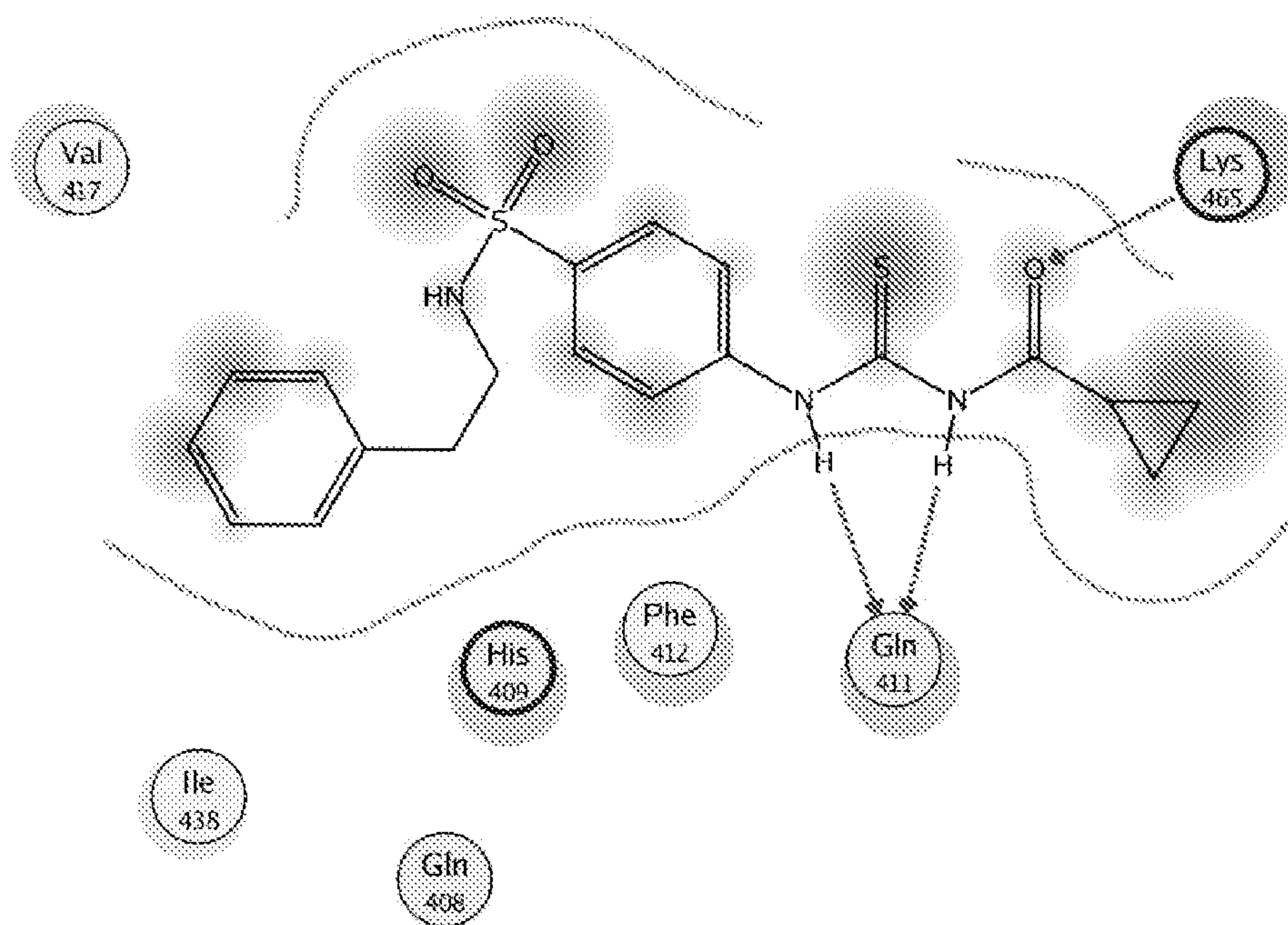


FIG. 6A

8/36

AD4-11883 (AD4-1886-like)

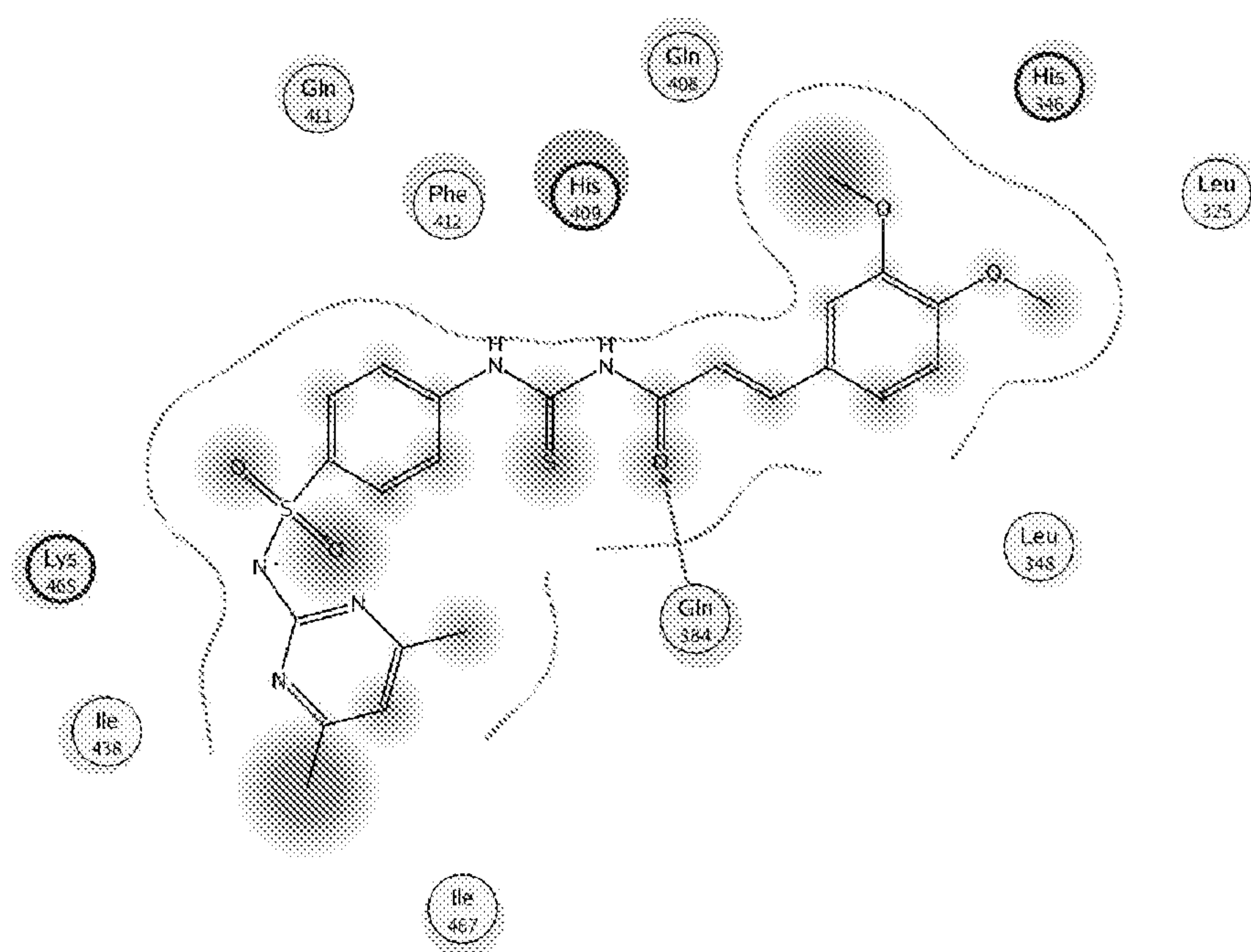


FIG. 6B

9/36

AD4-11975 (AD4-1886-like)

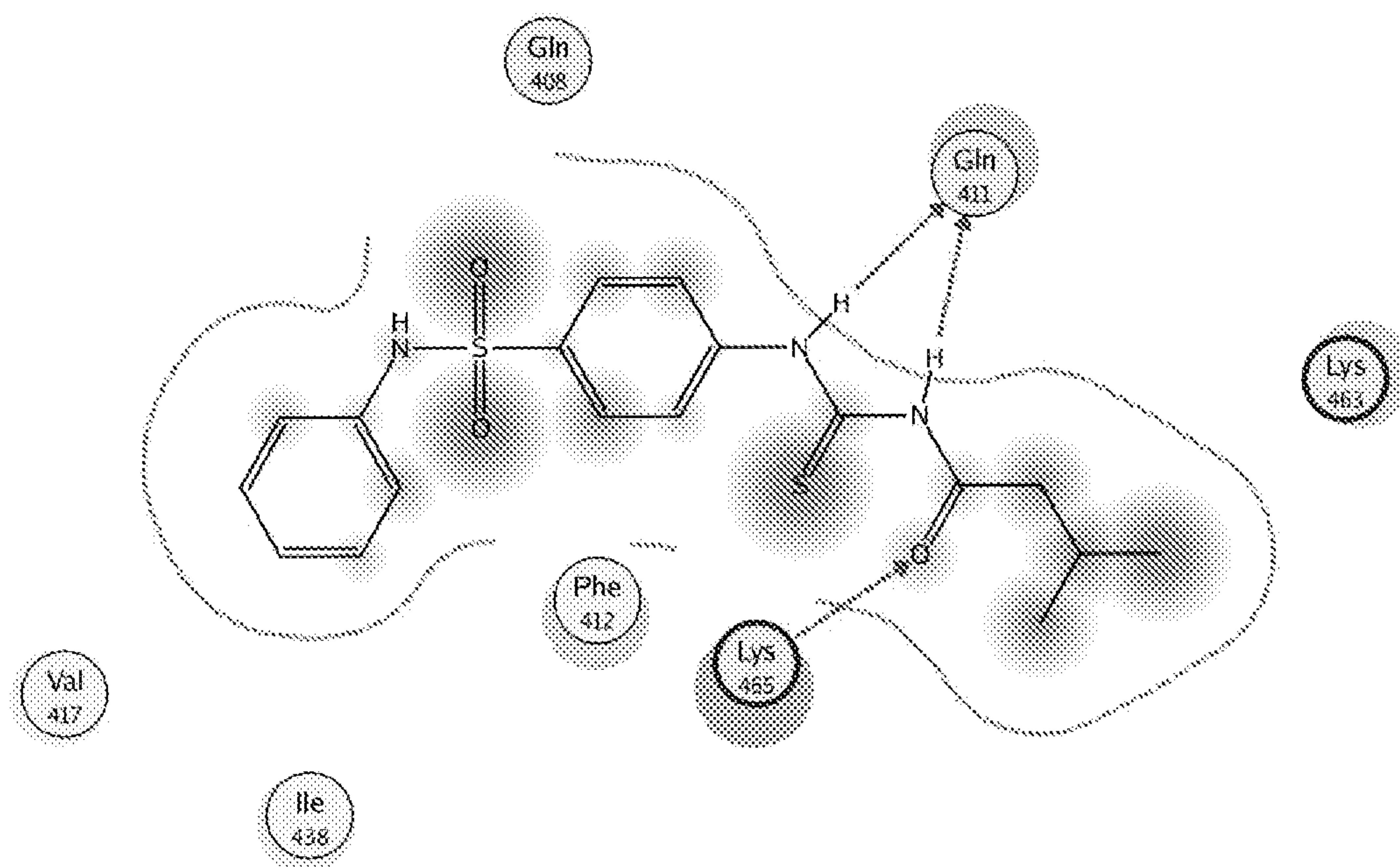


FIG. 6C

10/36

AD4-11409 (AD4-1886-like)

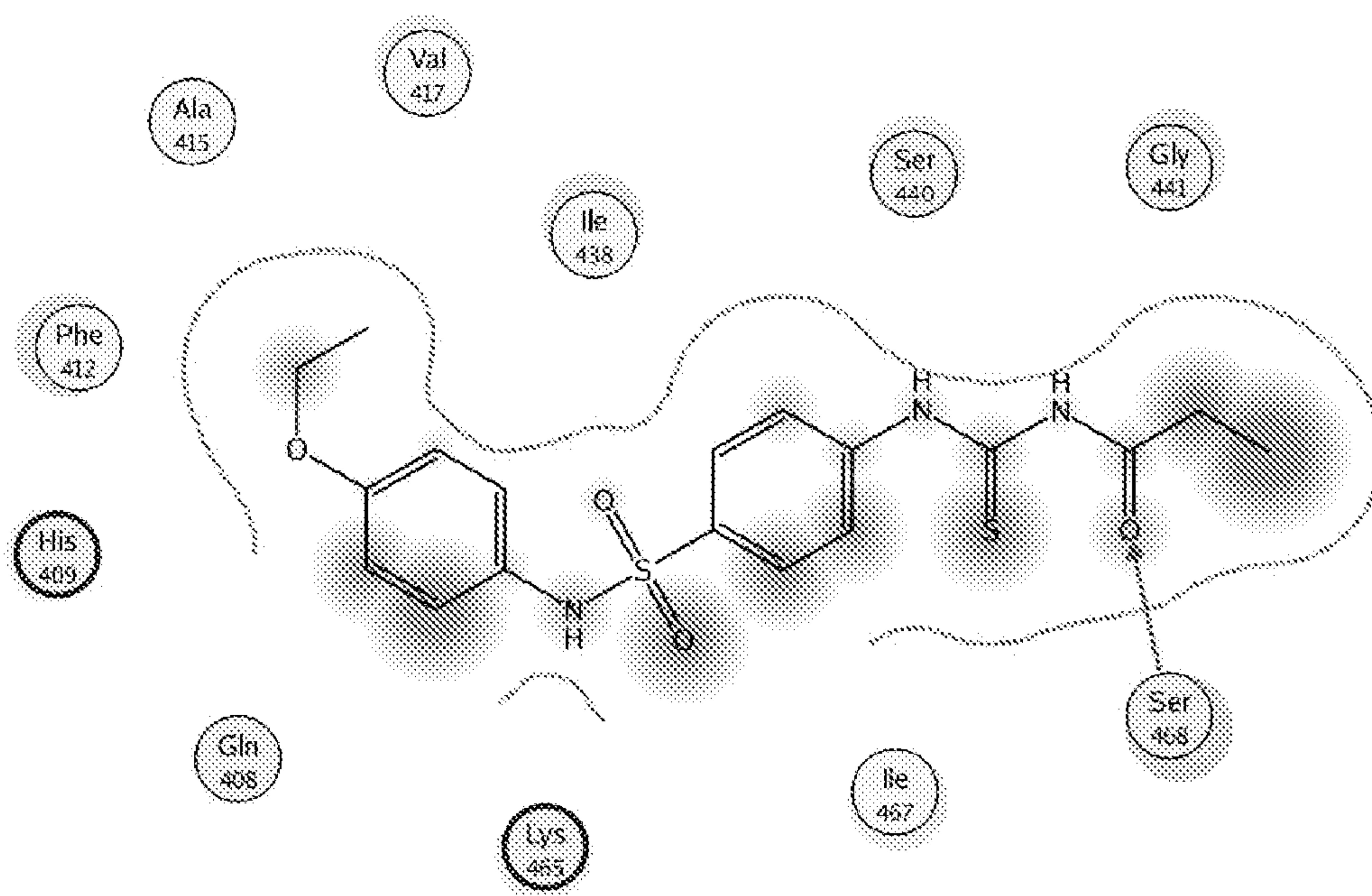


FIG. 6D

11/36

AD4-11638 (AD4-1886-like)

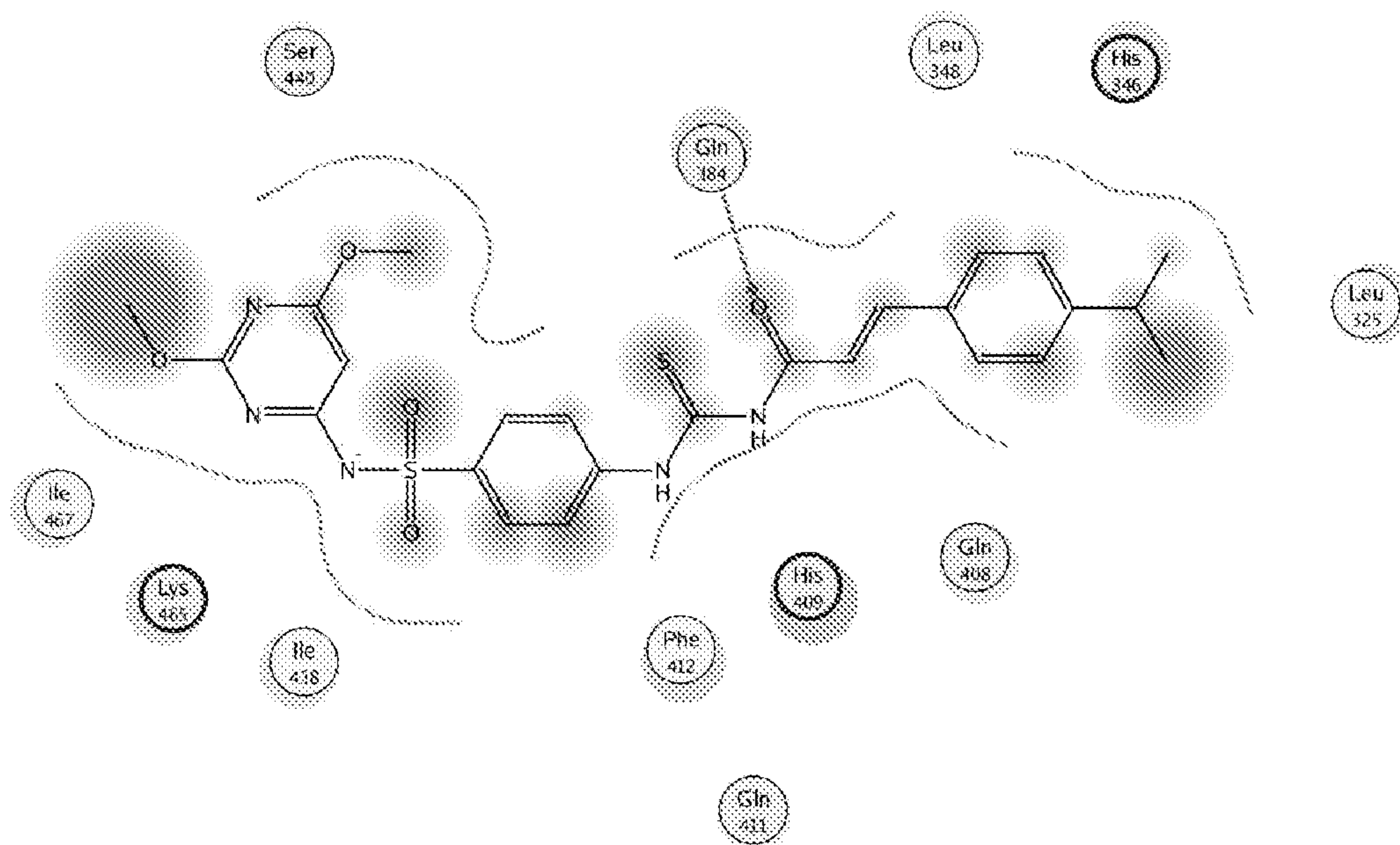


FIG. 6E

12/36

AD4-11645 (AD4-1886-like)

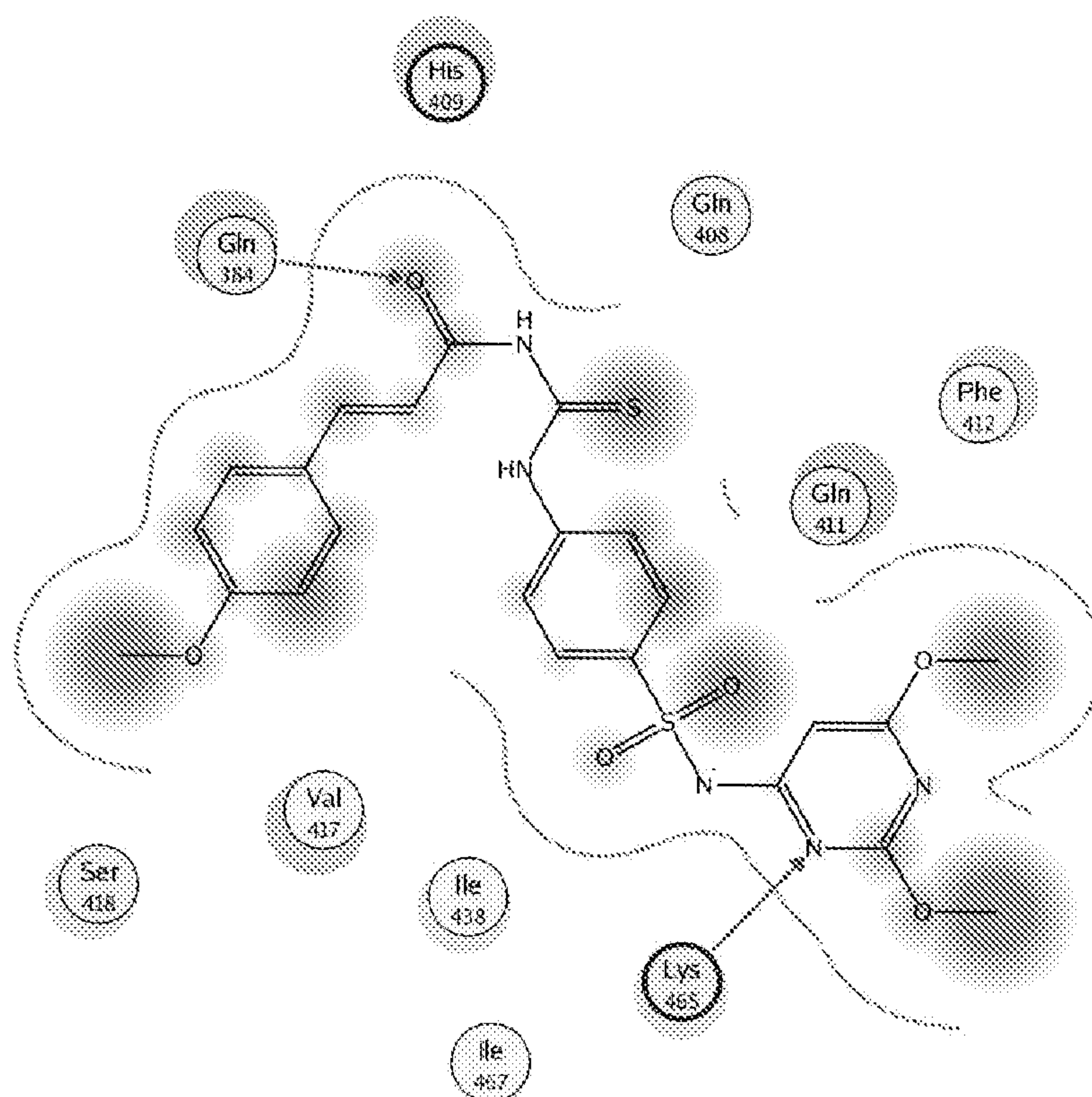


FIG. 6F

13/36

AD4-11091

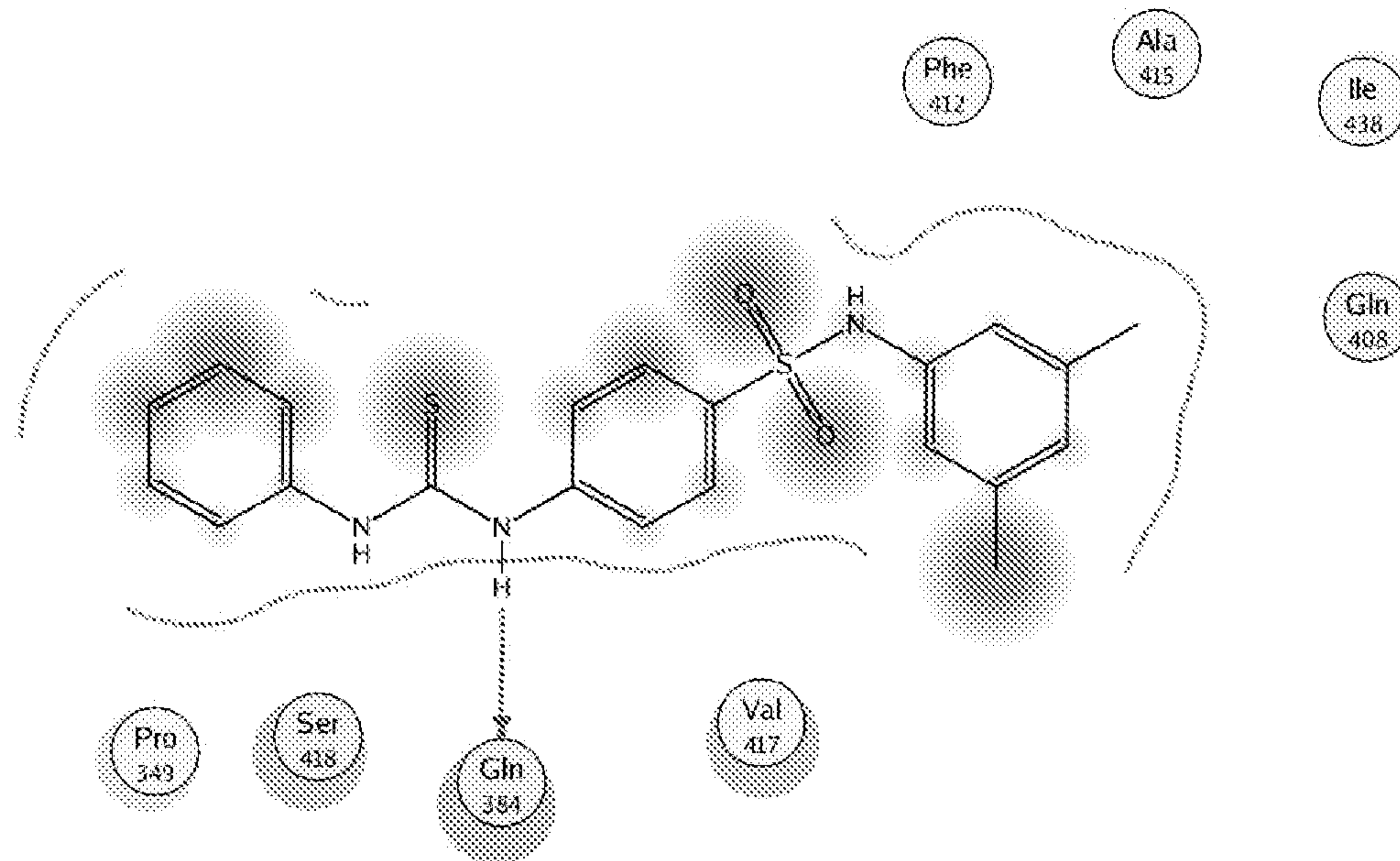


FIG. 7A

14/36

AD4-12509 (AD4-11091-like)

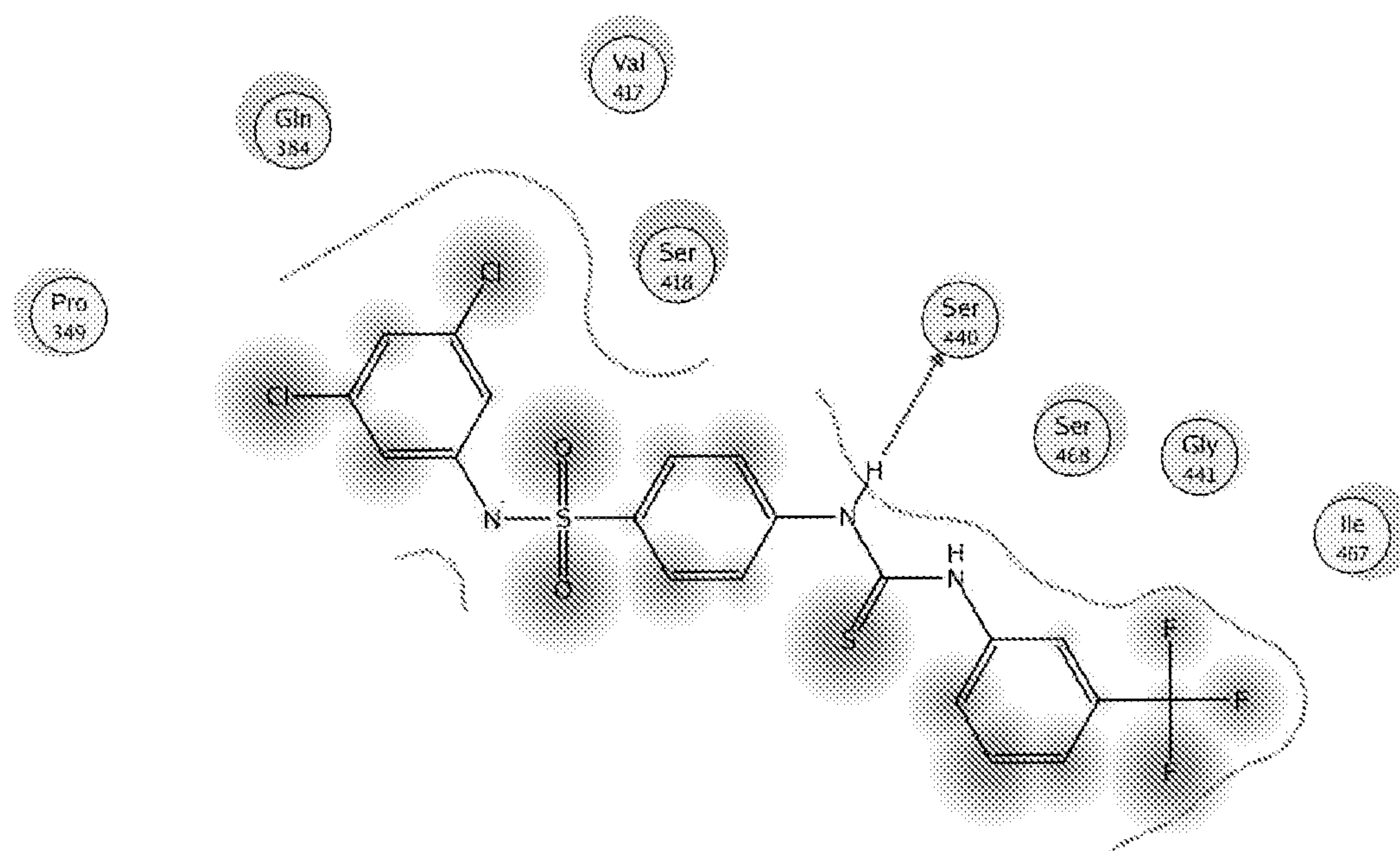


FIG. 7B

15/36

AD4-12423 (AD4-11091-like)

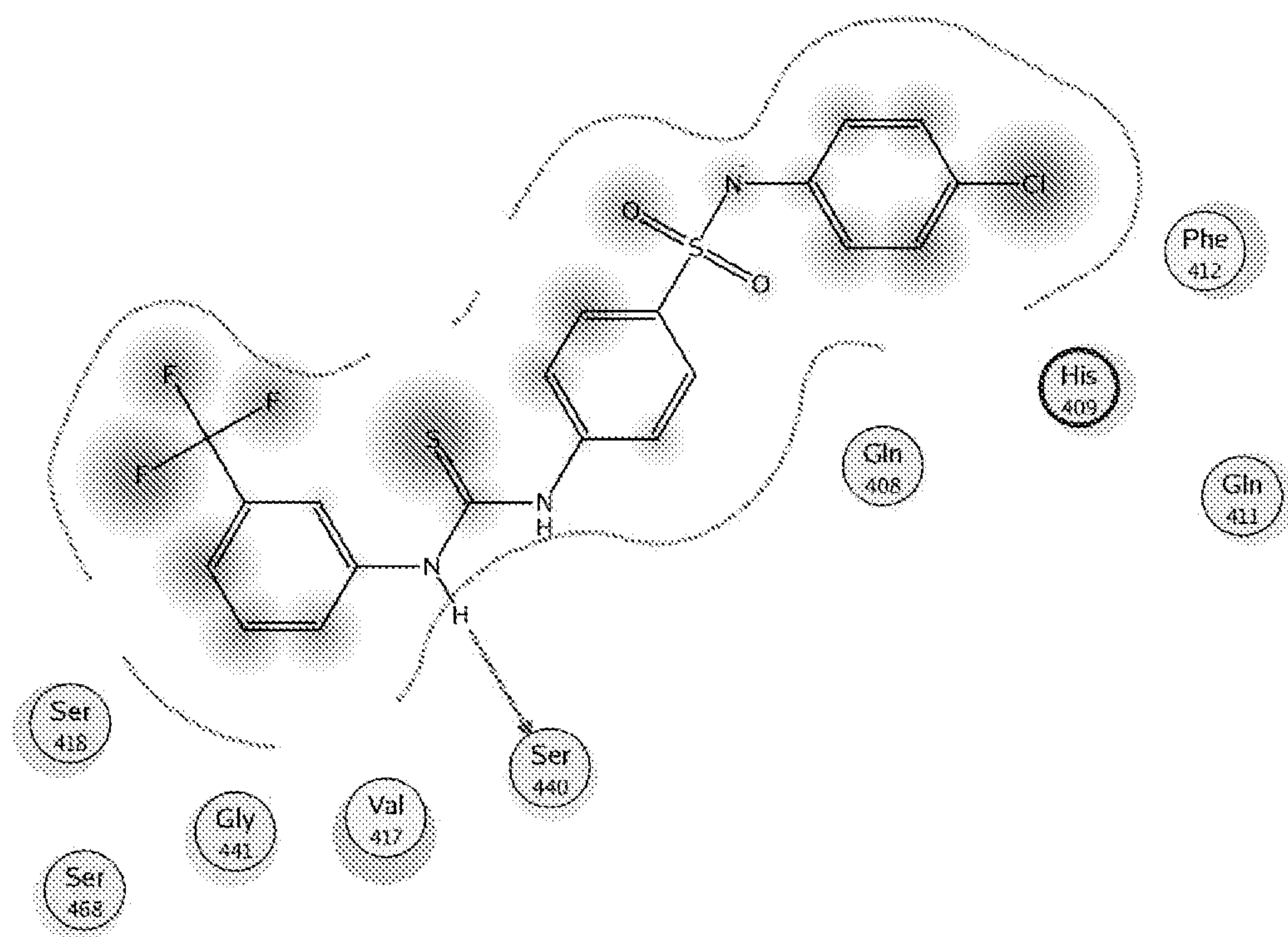


FIG. 7C

16/36

AD4-12528 (AD4-11091-like)

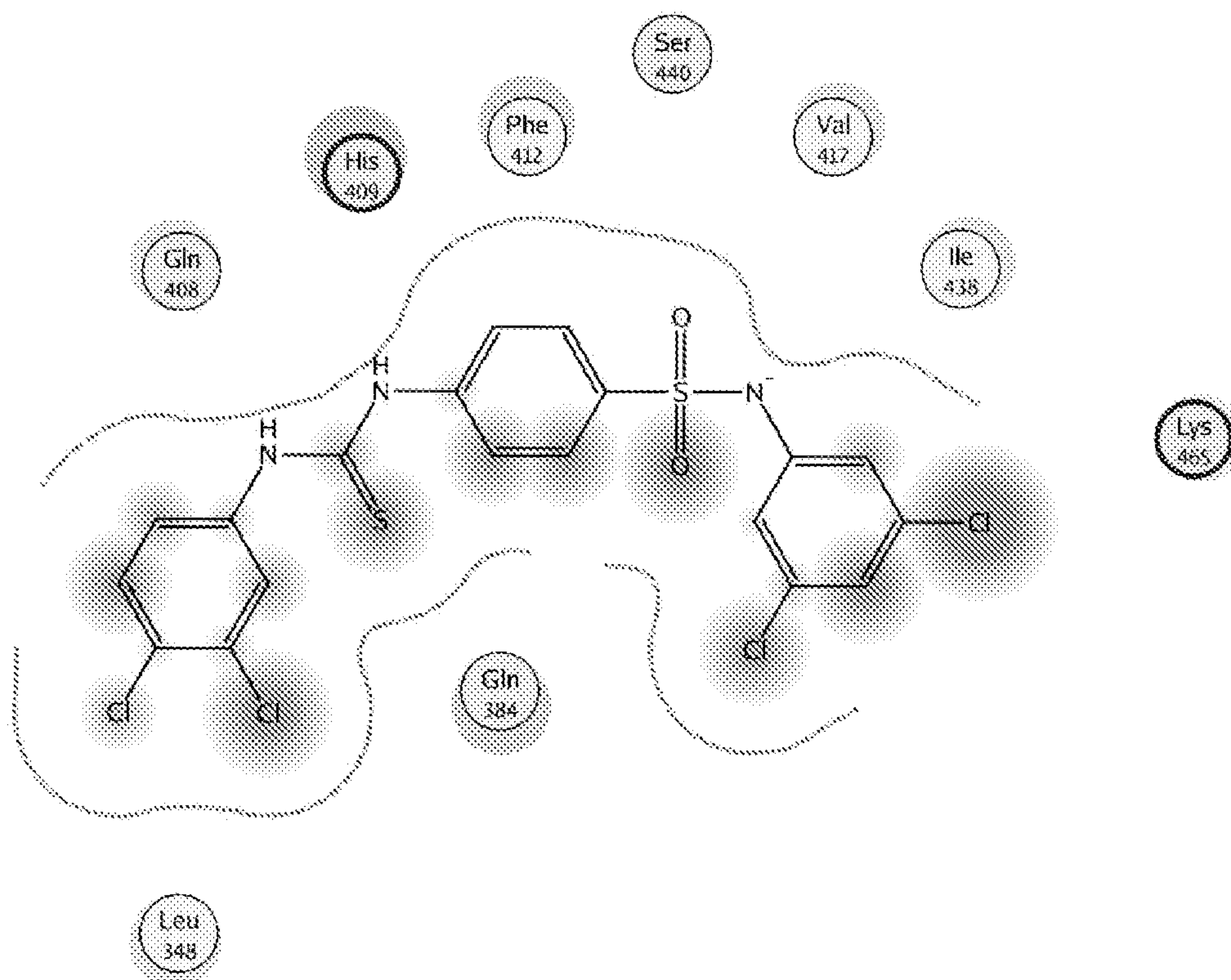


FIG. 7D

17/36

AD4-12522 (AD4-11091-like)

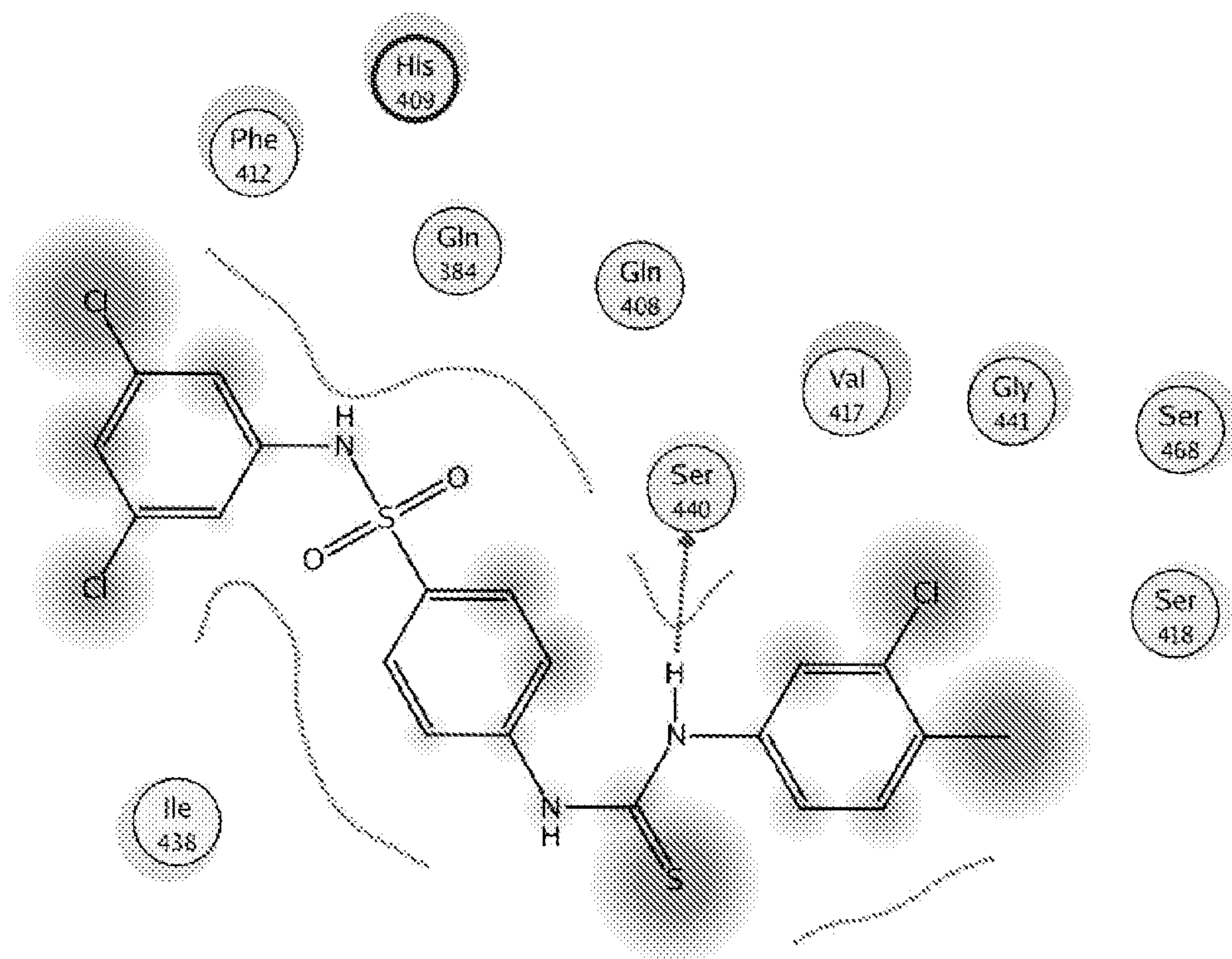


FIG. 7E

18/36

AD4-12504 (AD4-11091-like)

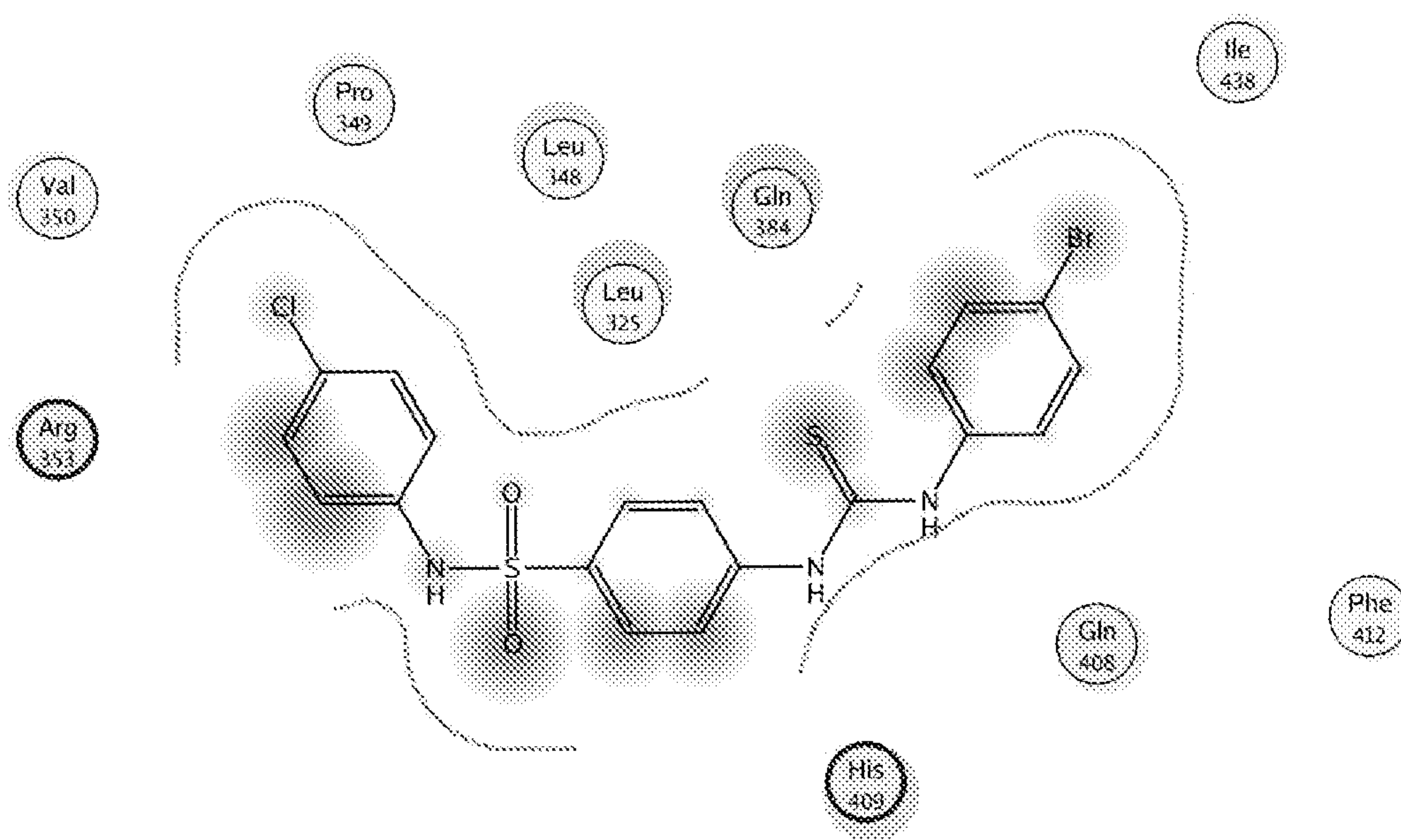


FIG. 7F

19/36

AD4-1734

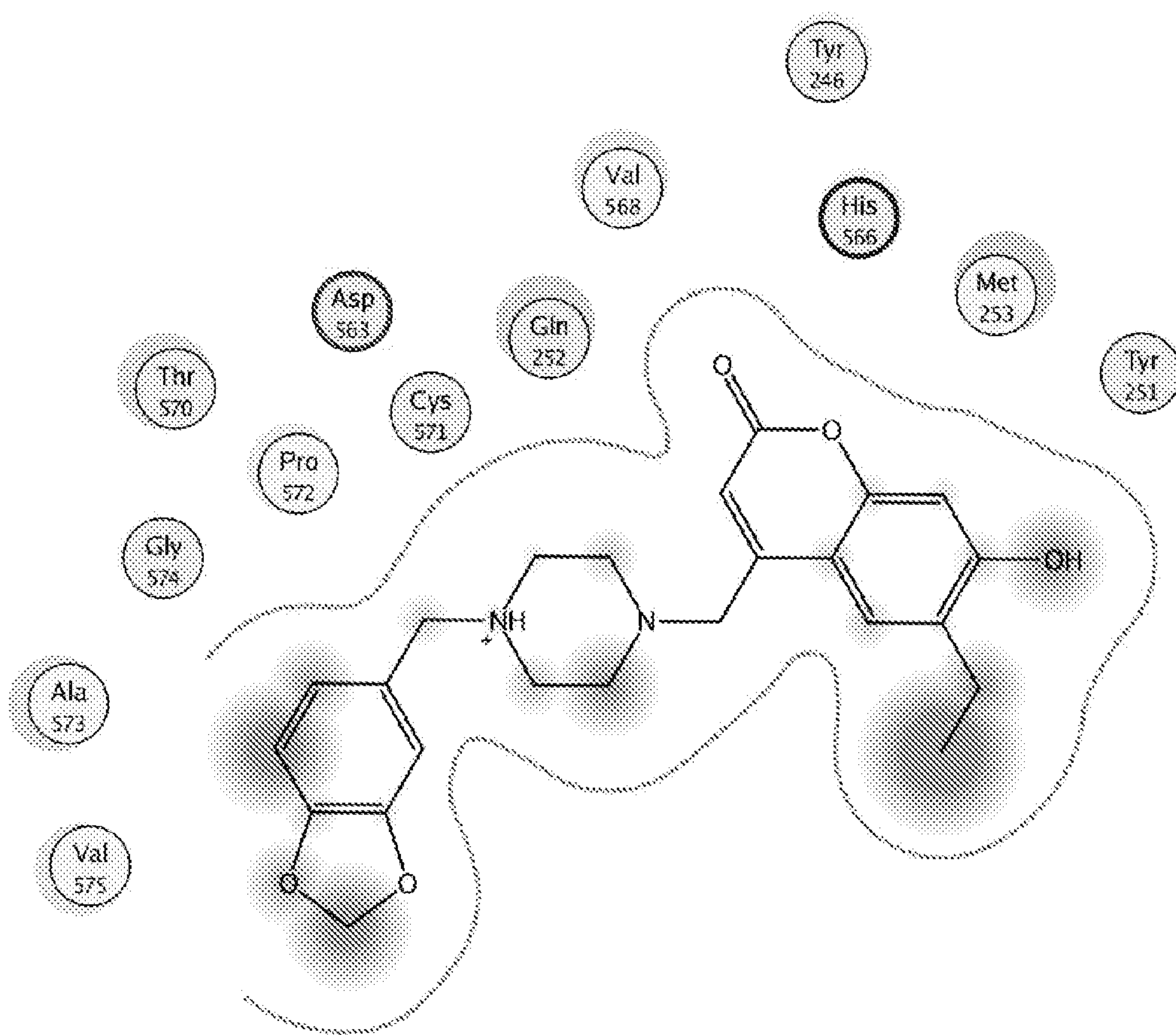


FIG. 8A

20/36

AD4-10631 (AD4-1734-like)

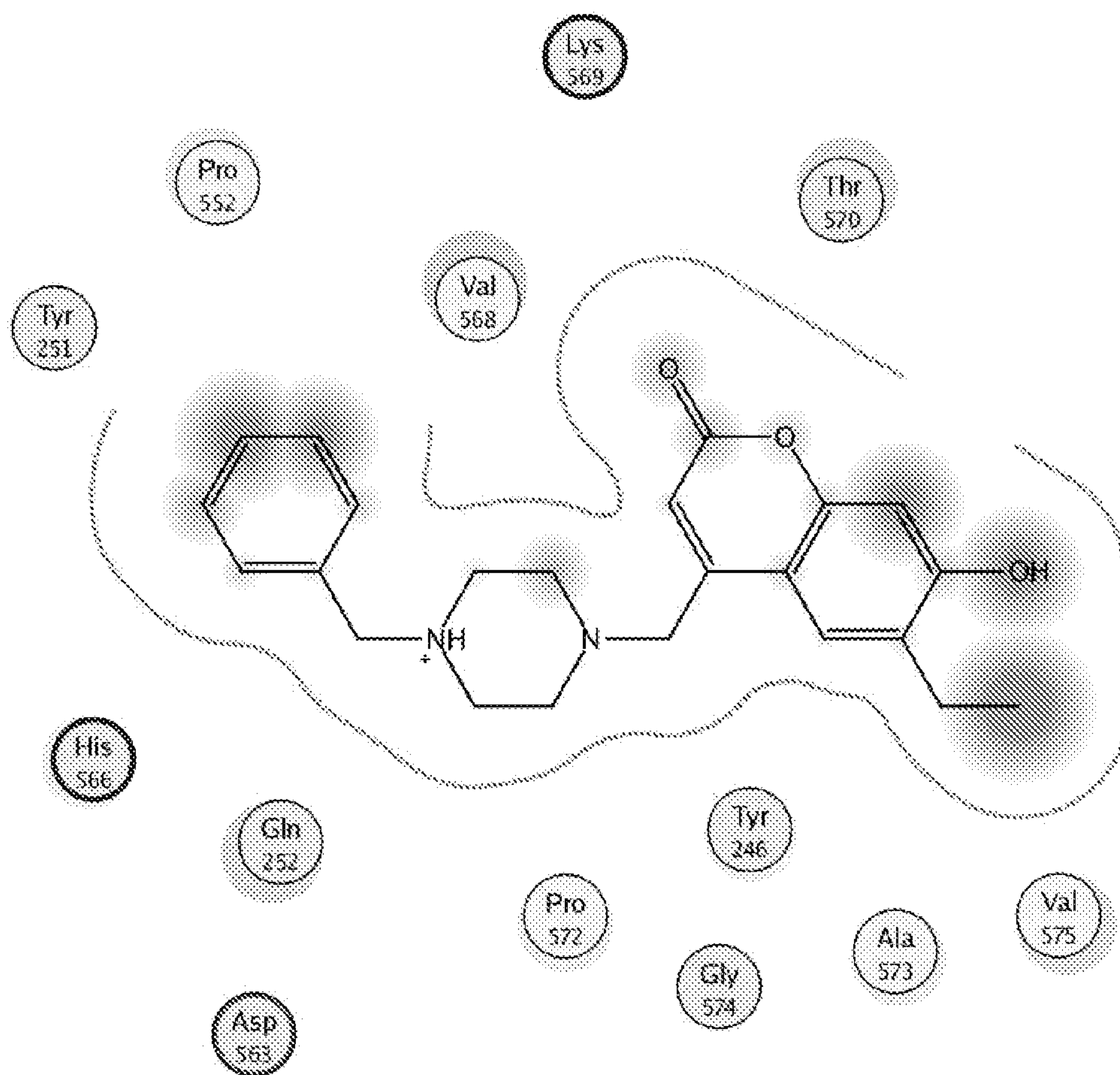


FIG. 8B

21/36

AD4-10188 (AD4-1734-like)

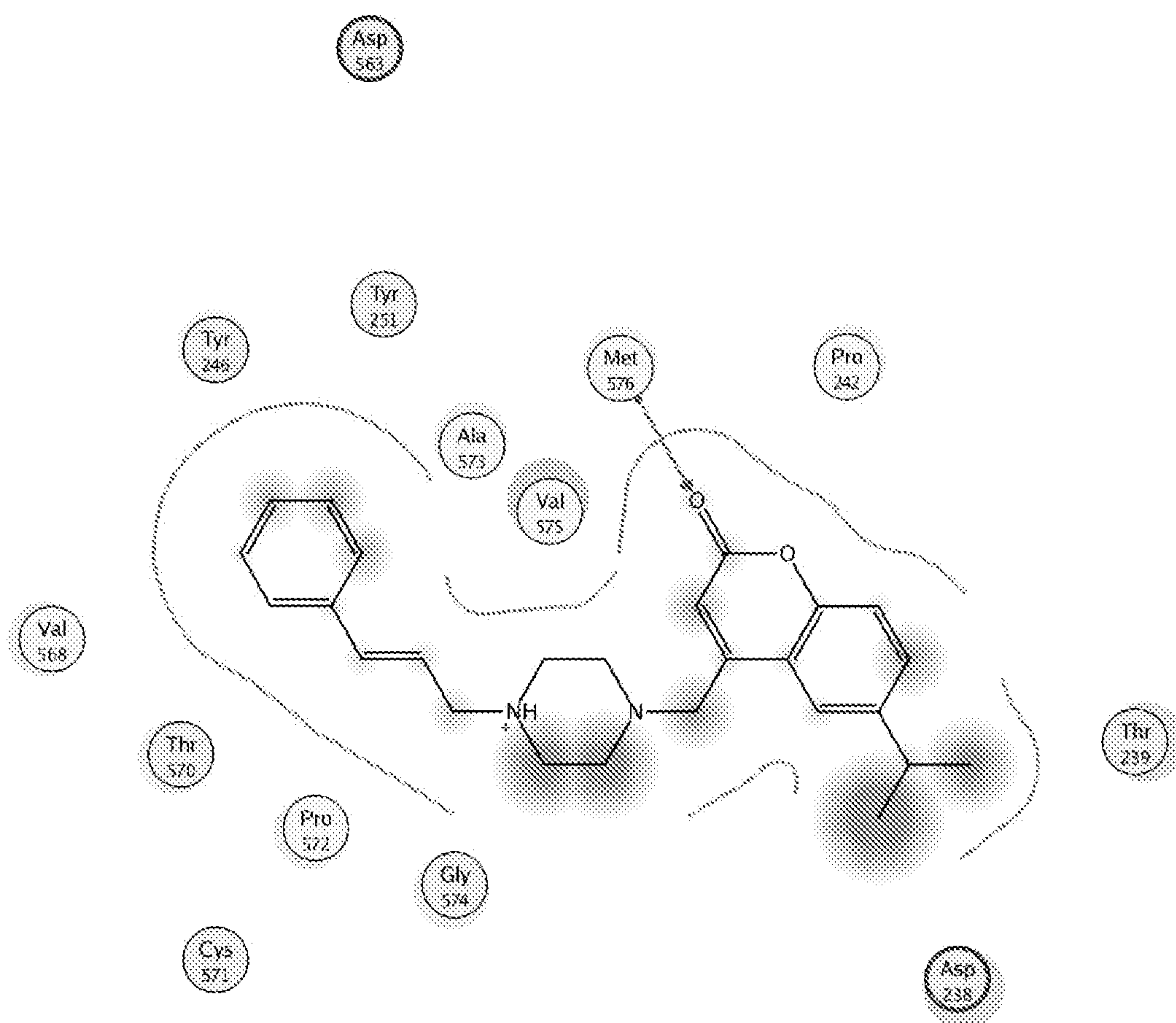


FIG. 8C

22/36

AD4-10186 (AD4-1734-like)

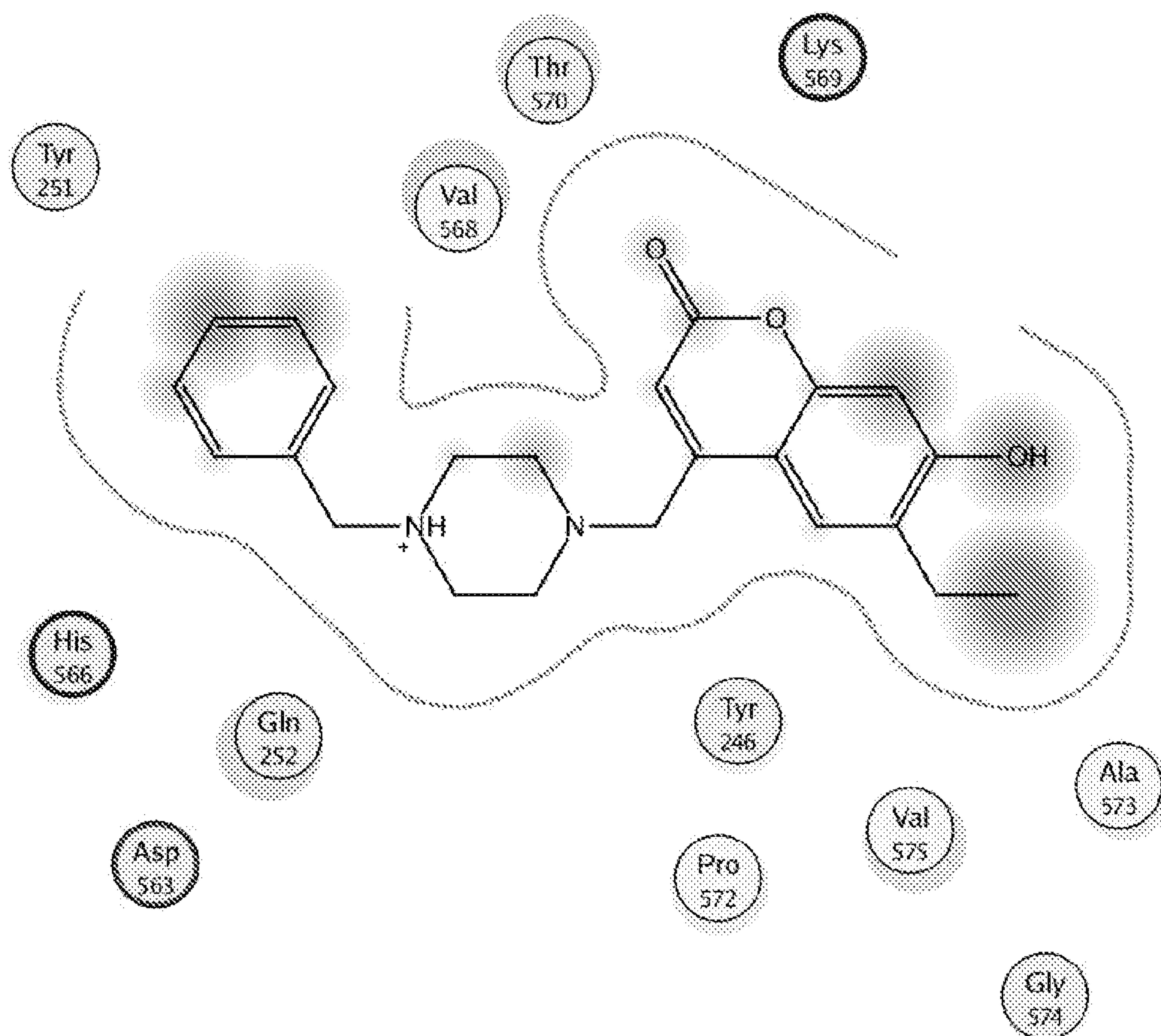


FIG. 8D

23/36

AD4-10633 (AD4-1734-like)

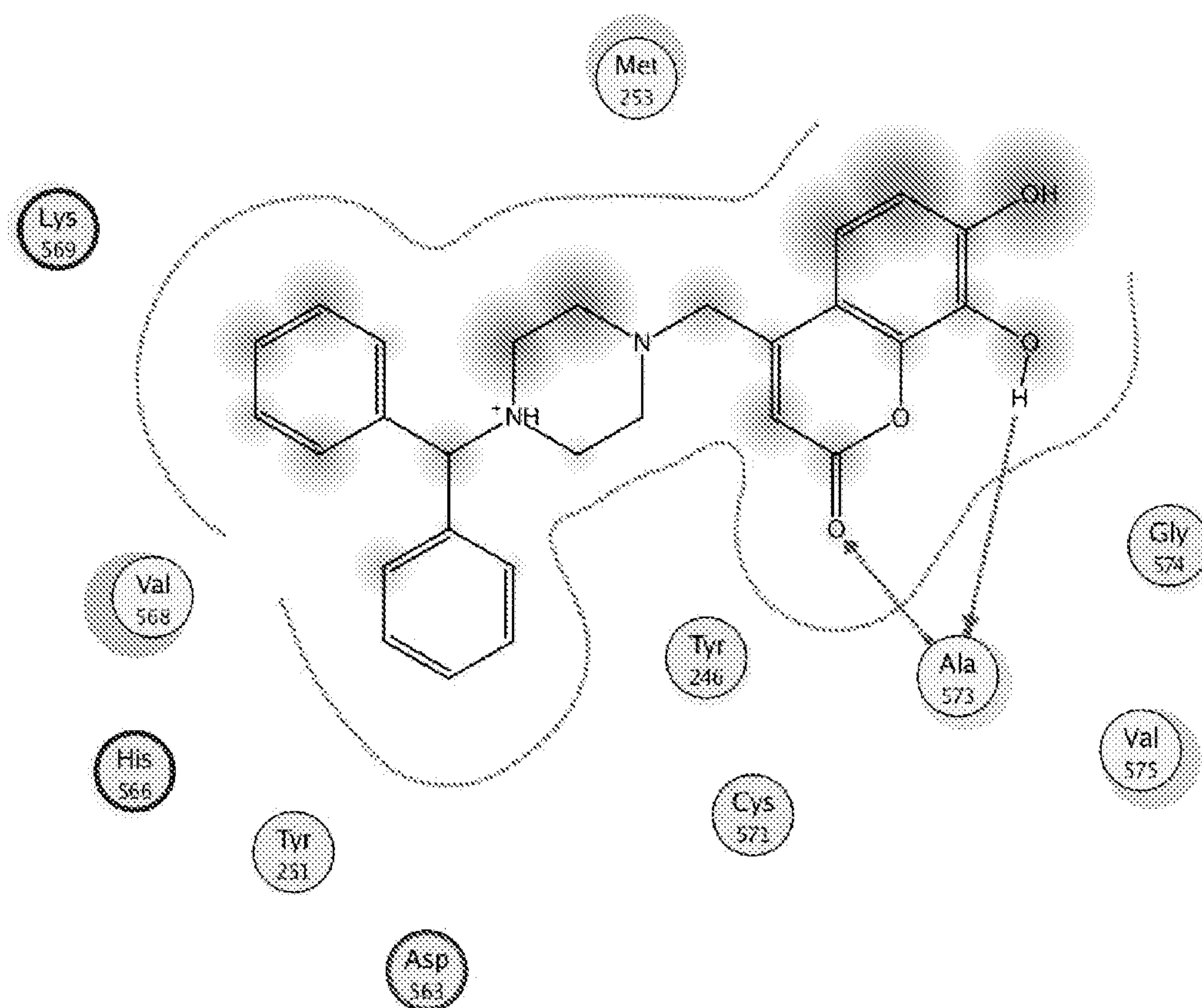


FIG. 8E

24/36

AD4-10174 (AD4-1734-like)

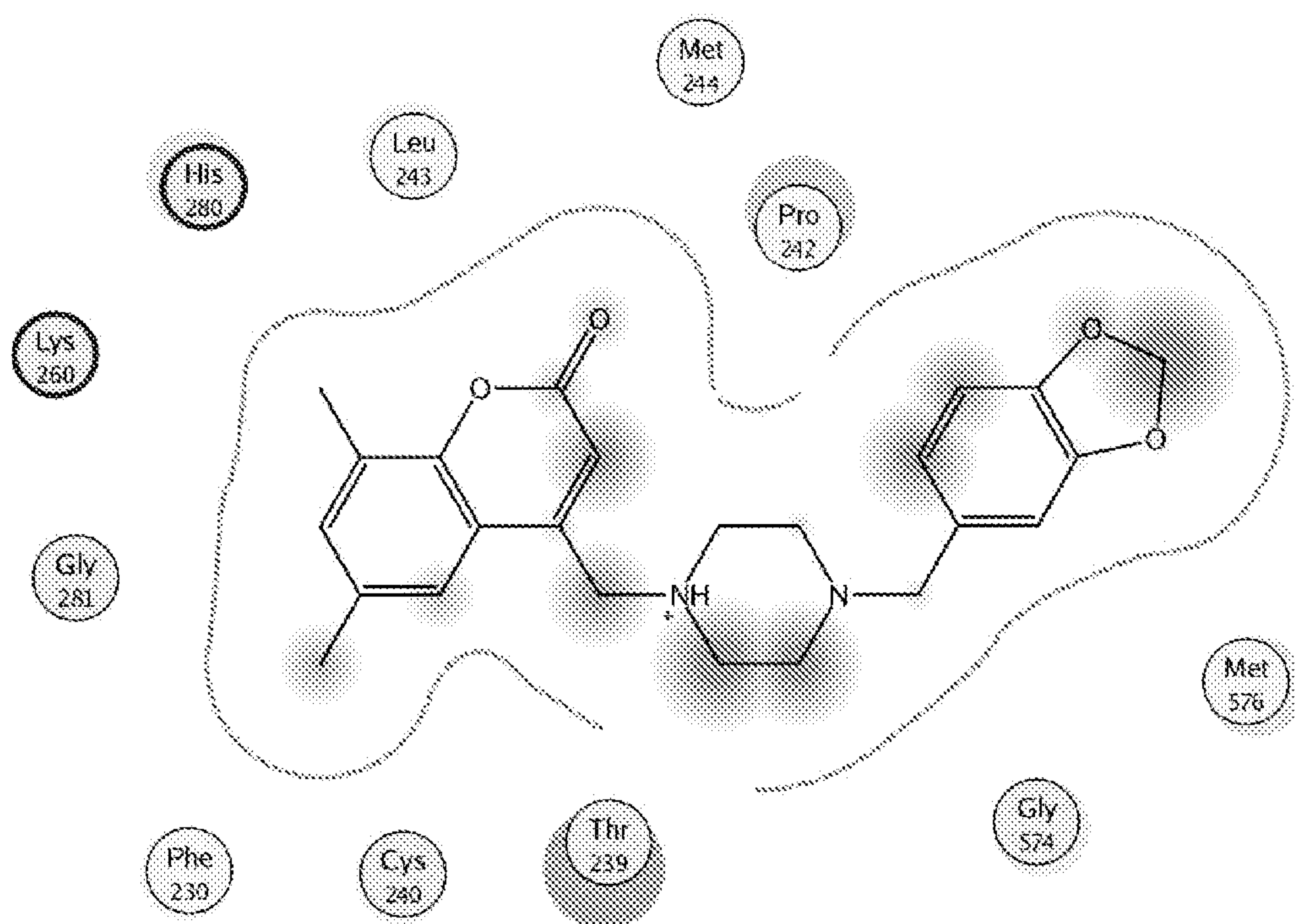


FIG. 8F

25/36

AD4-10381

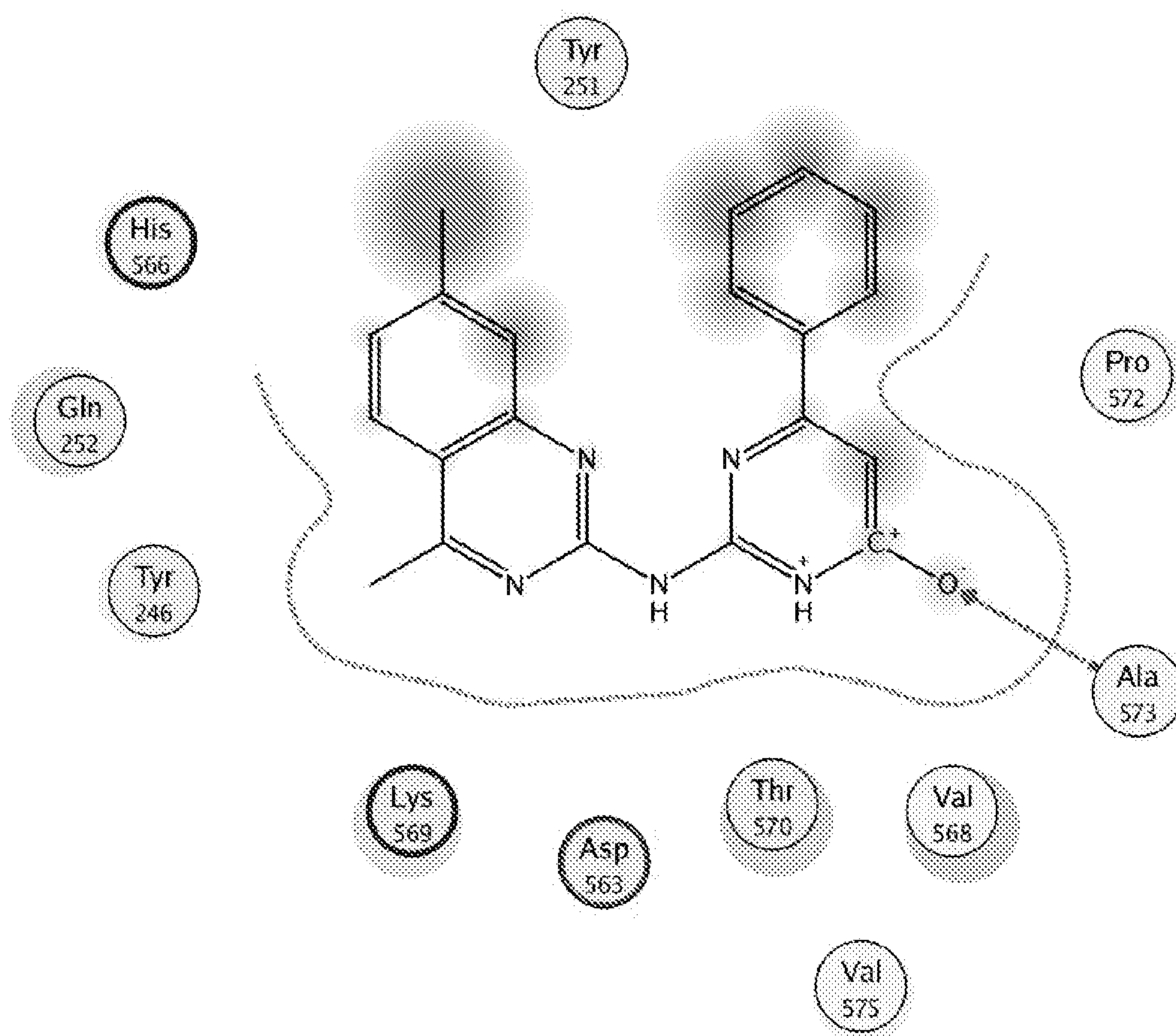


FIG. 9A

26/36

AD4-11340 (AD4-10381-like)

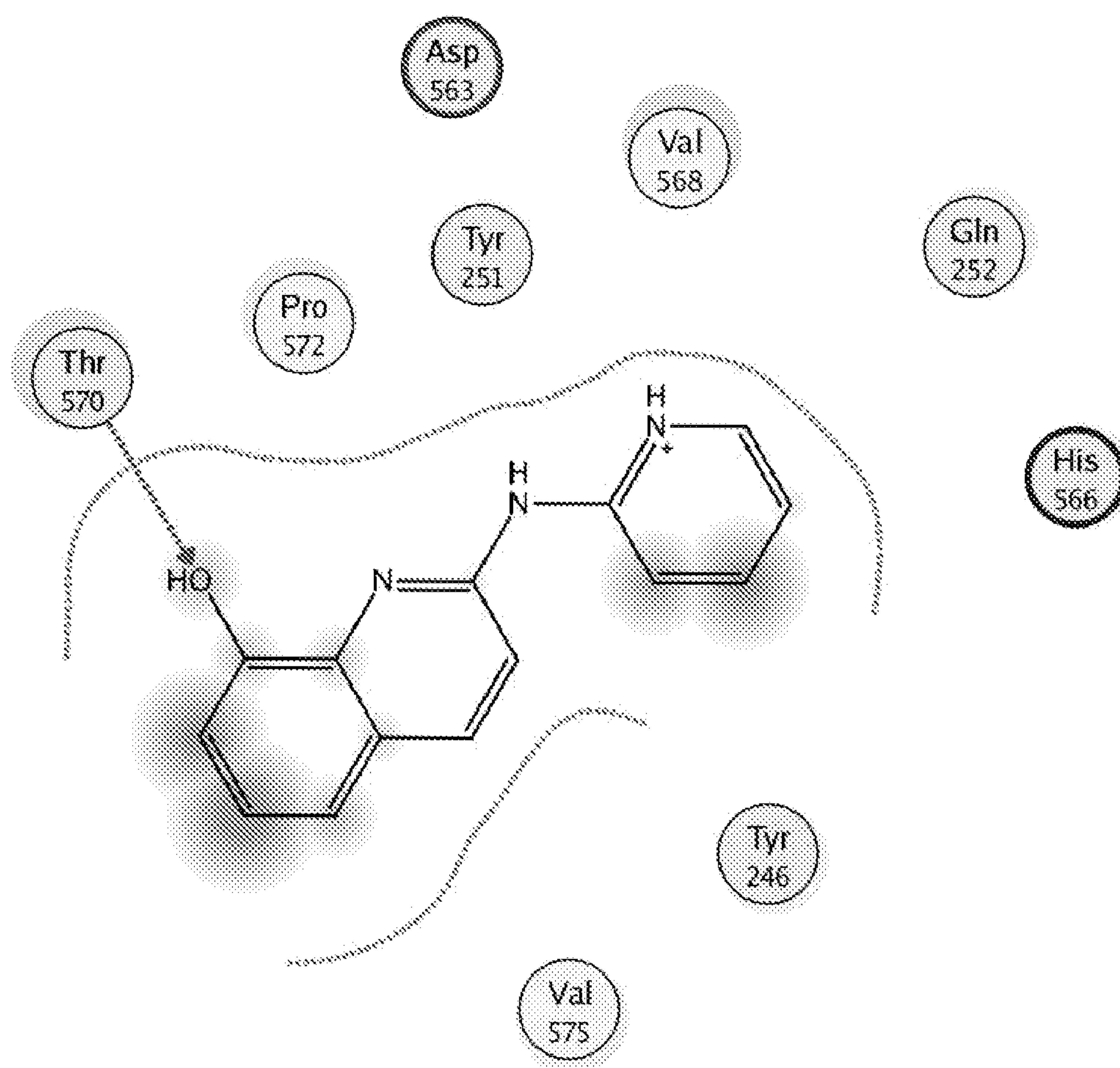


FIG. 9B

27/36

AD4-12632 (AD4-10381-like)

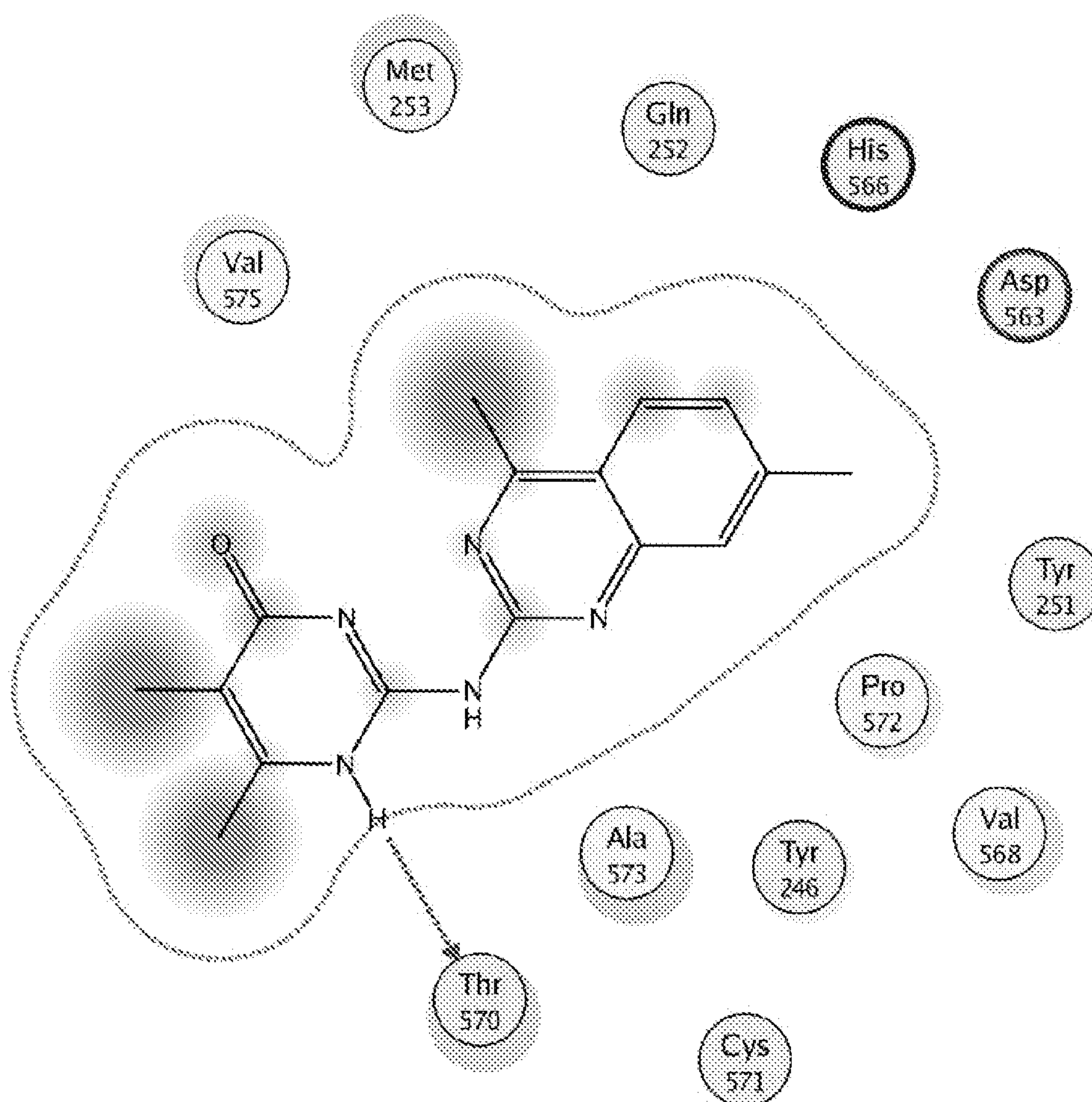


FIG. 9C

28/36

AD4-12681 (AD4-10381-like)

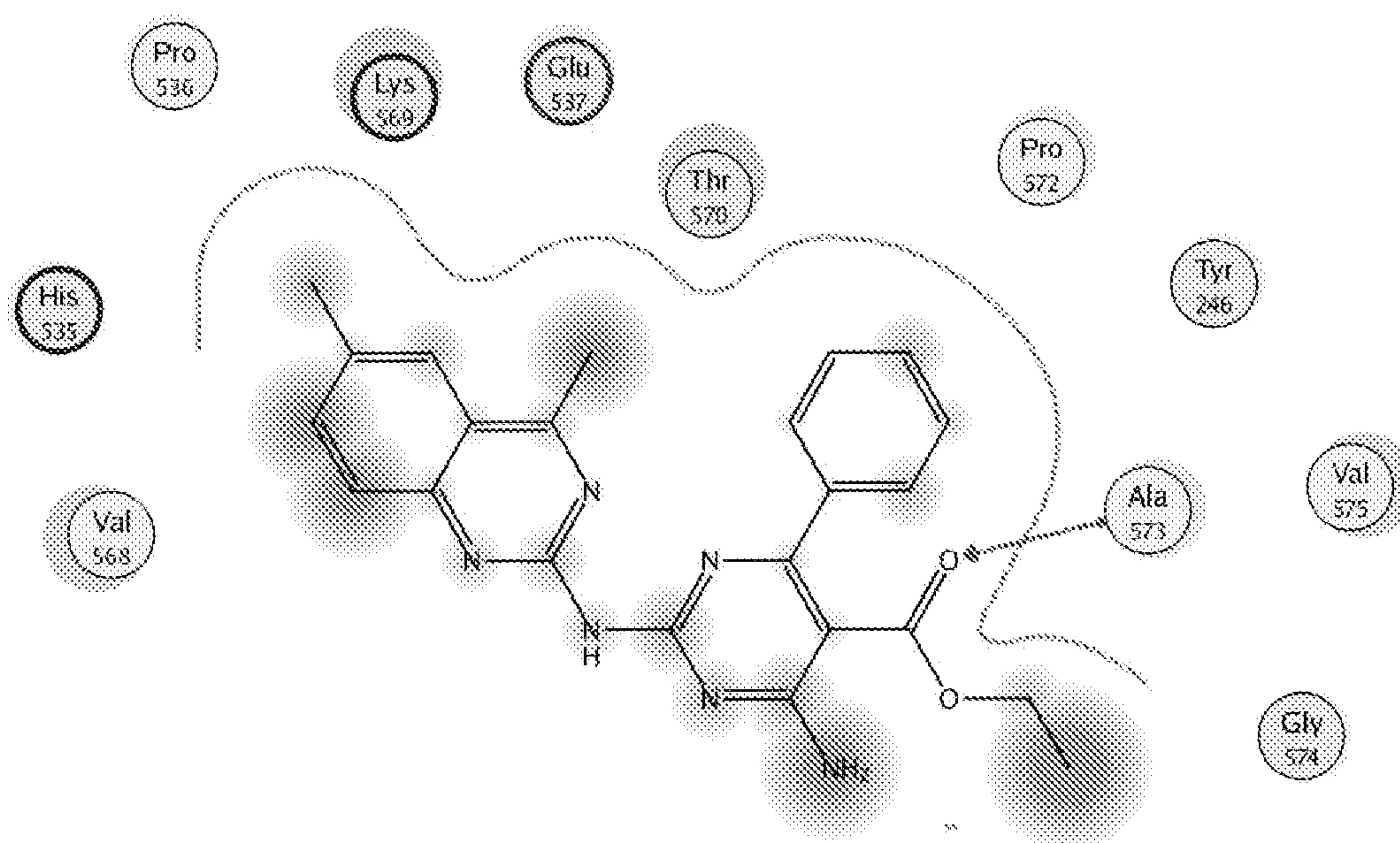


FIG. 9D

29/36

AD4-12732 (AD4-10381-like)

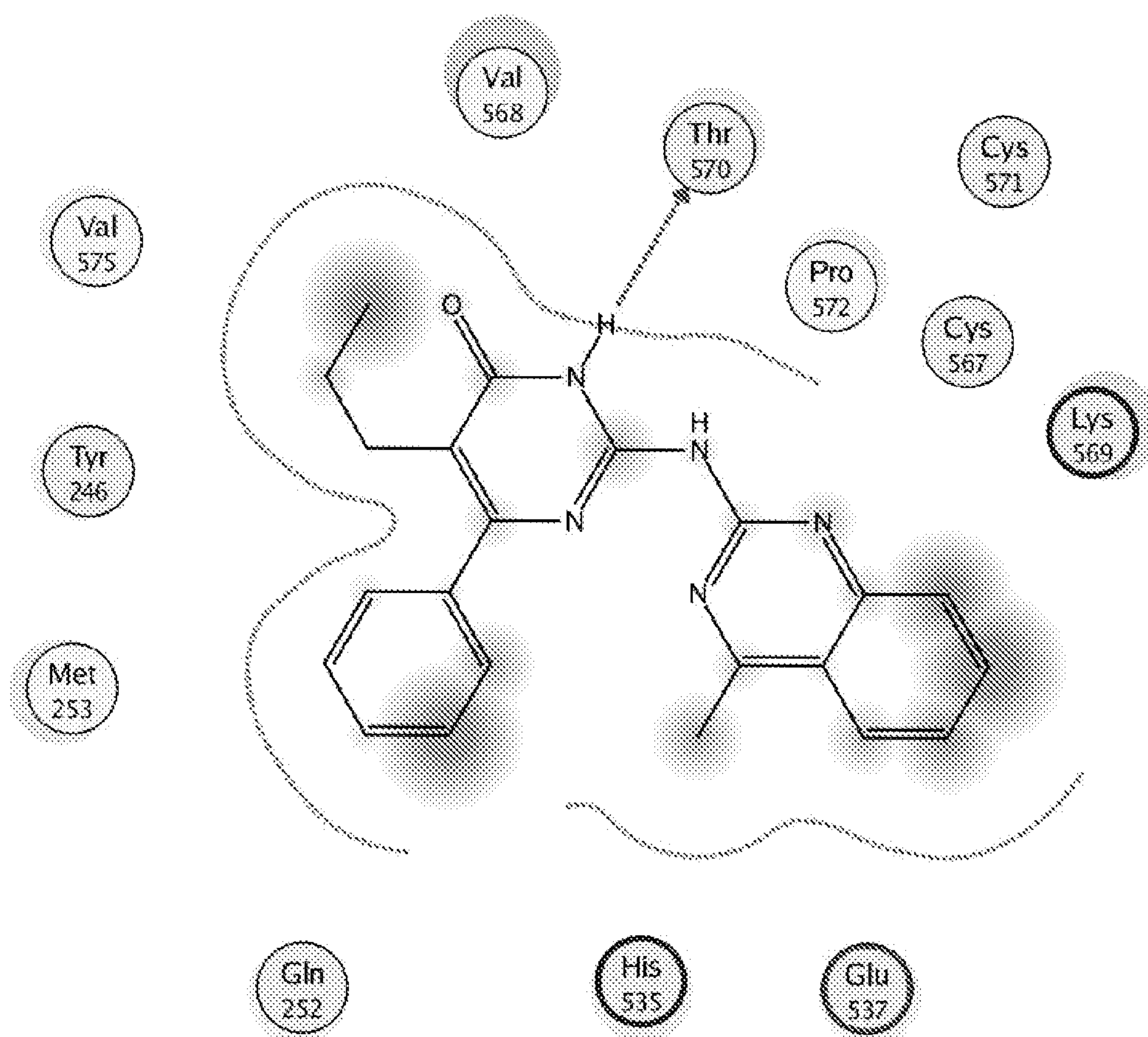


FIG. 9E

30/36

AD4-11511 (AD4-10381-like)

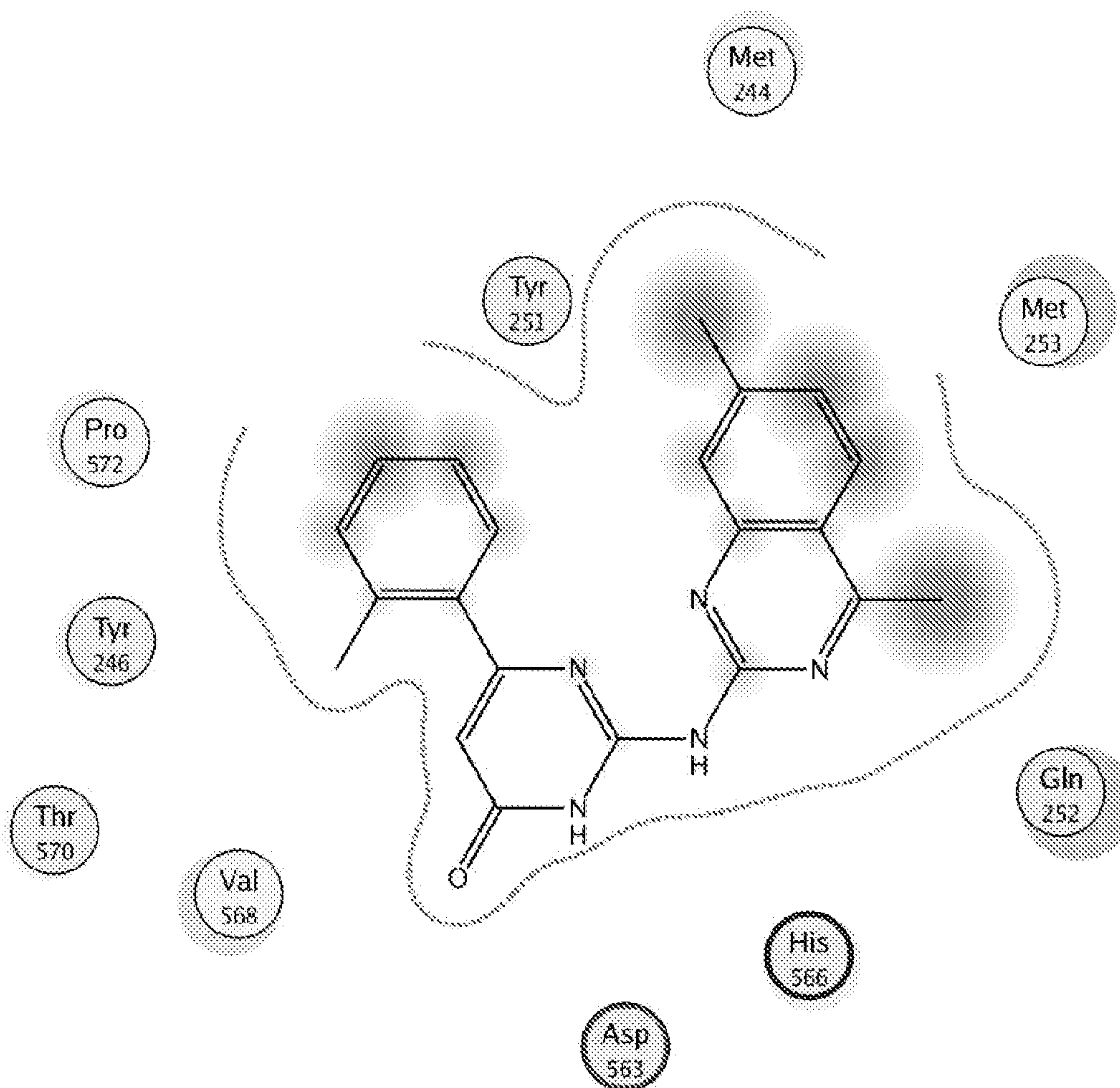


FIG. 9F

31/36

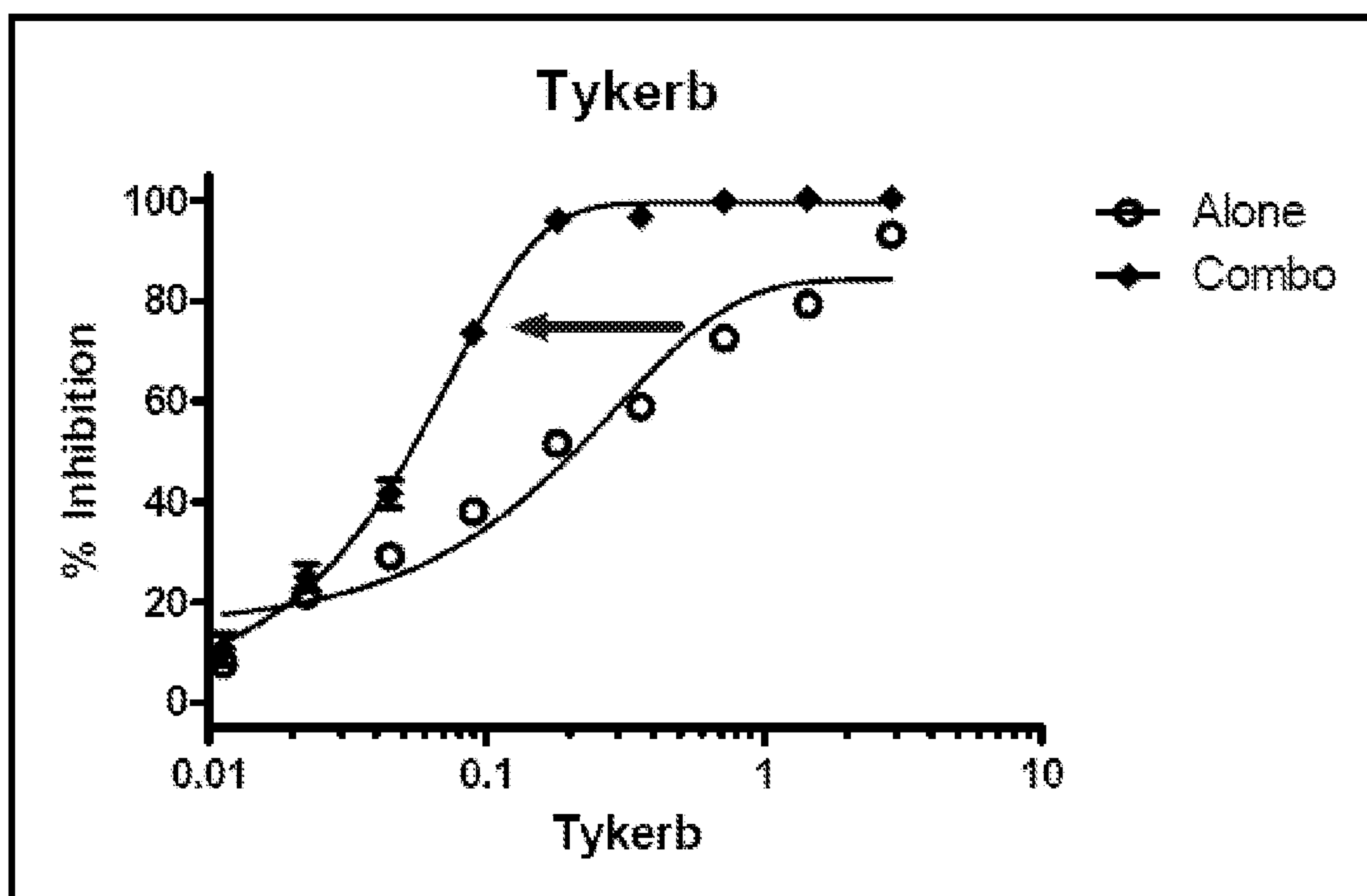


FIG. 10A

32/36

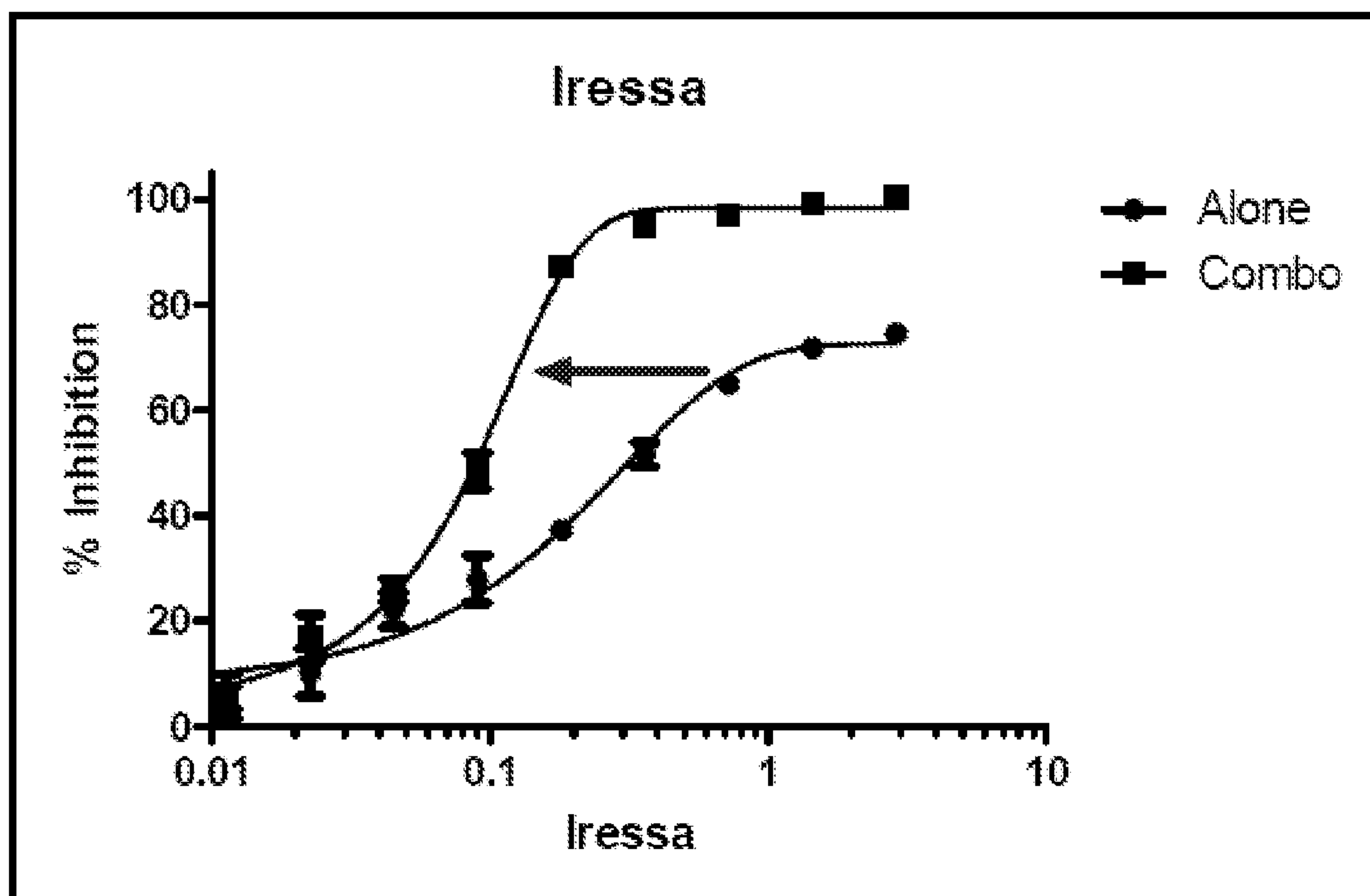


FIG. 10B

33/36

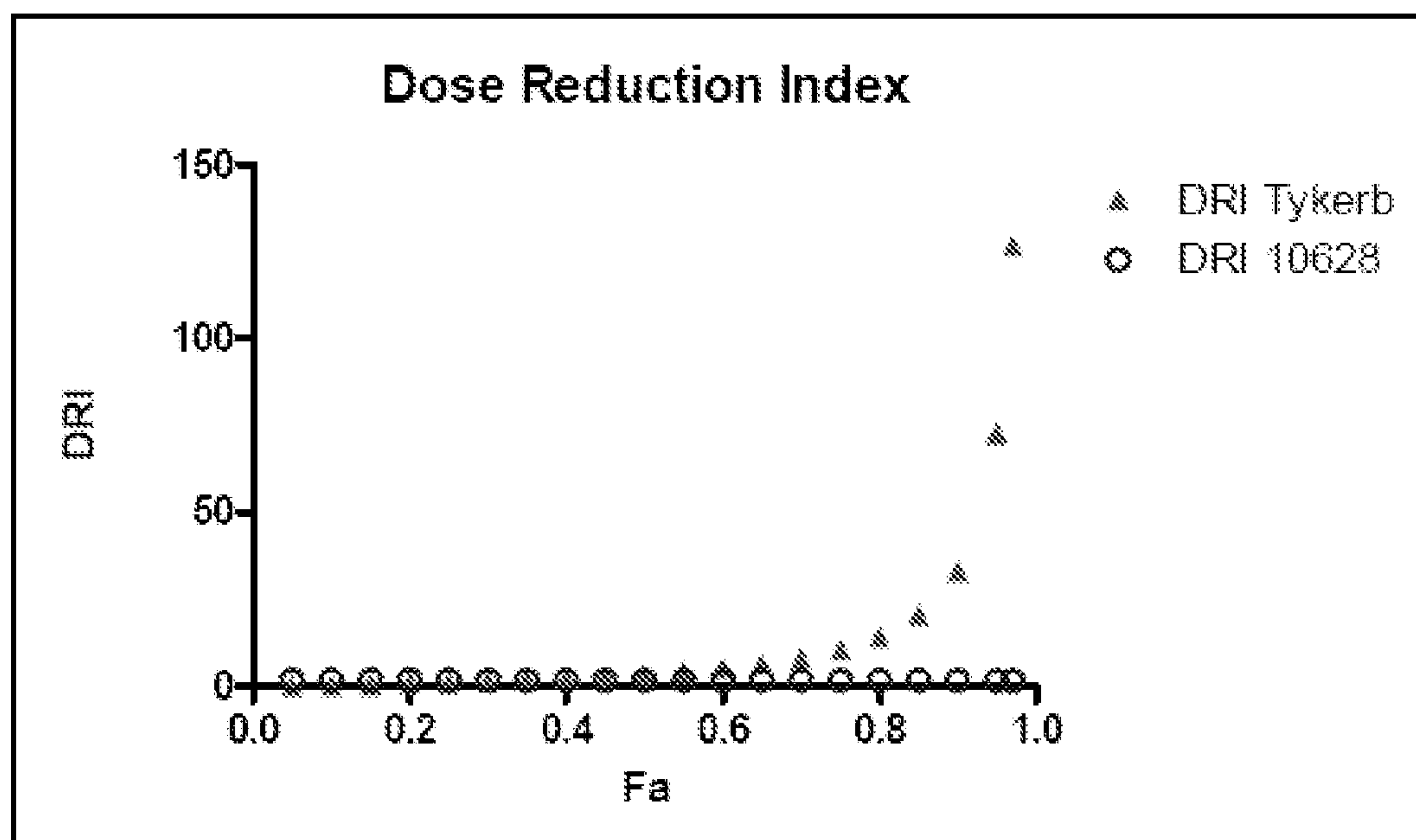


FIG. 11

34/36

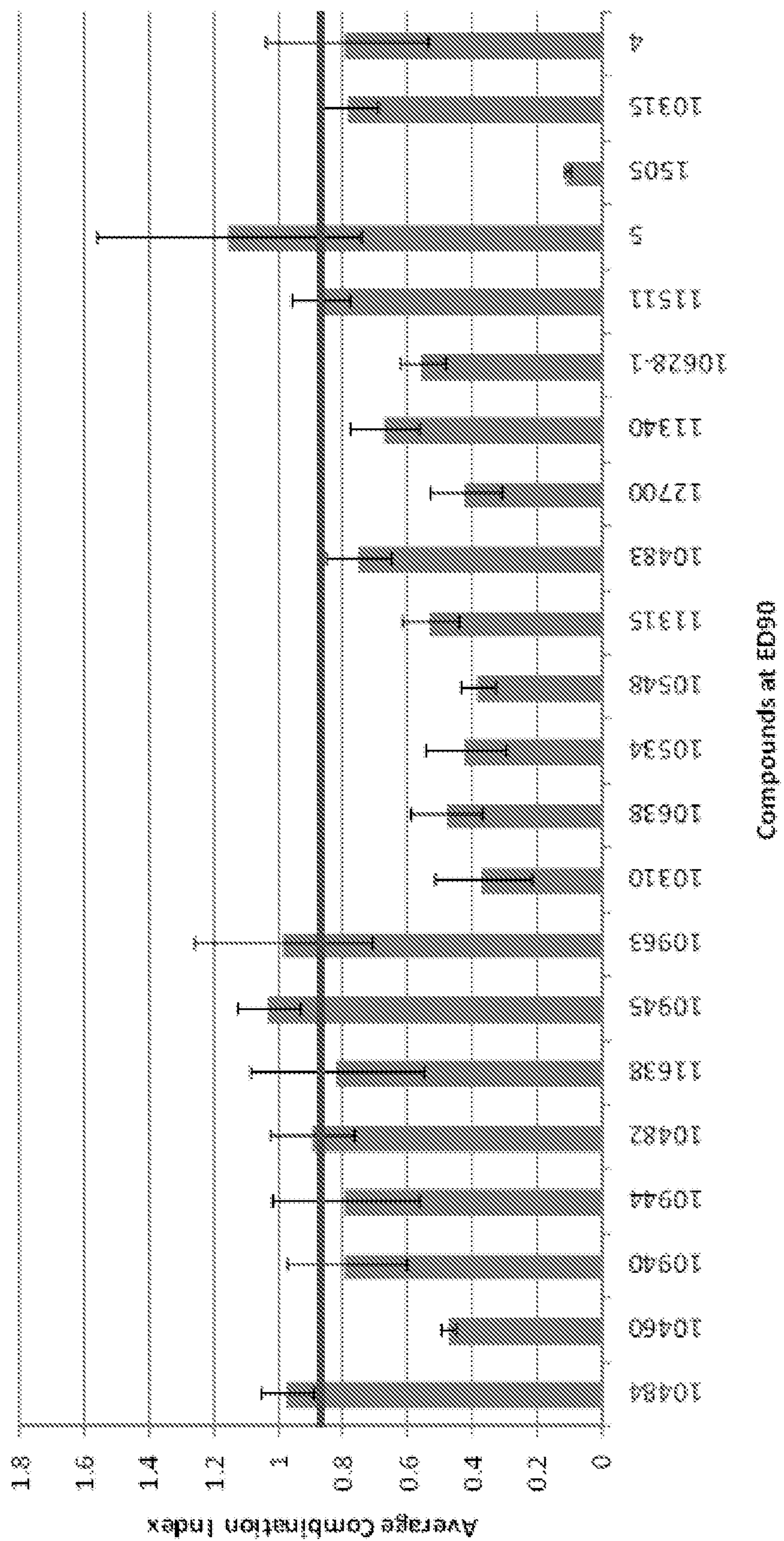


FIG. 12

35/36

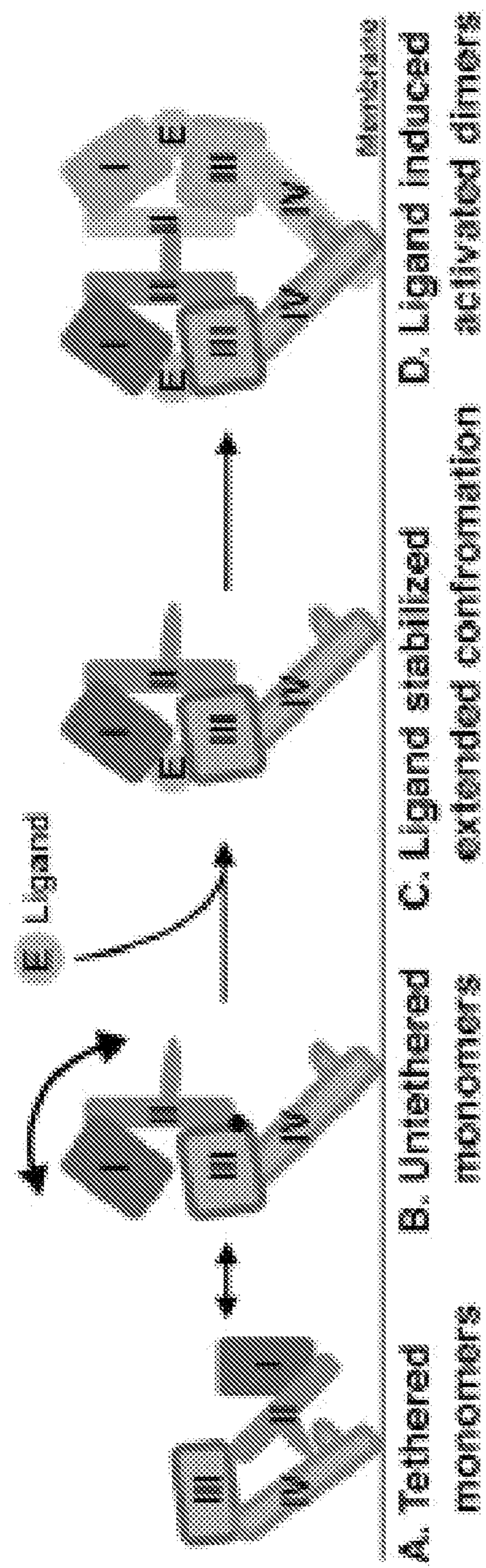


FIG. 13

36/36

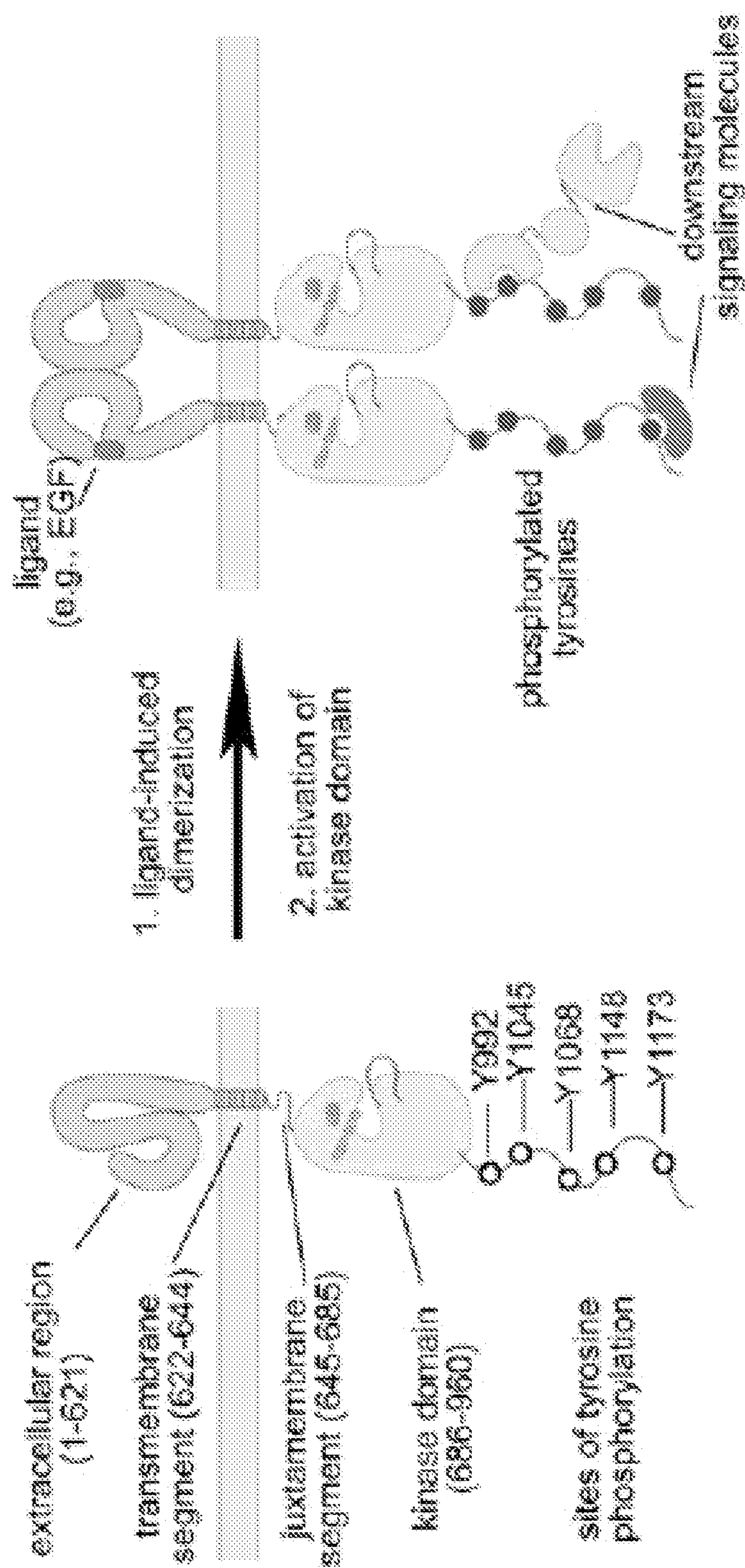


FIG. 14