



(86) Date de dépôt PCT/PCT Filing Date: 2011/07/26
(87) Date publication PCT/PCT Publication Date: 2012/02/09
(85) Entrée phase nationale/National Entry: 2013/01/25
(86) N° demande PCT/PCT Application No.: US 2011/045390
(87) N° publication PCT/PCT Publication No.: 2012/018635
(30) Priorité/Priority: 2010/07/26 (US61/367,709)

(51) Cl.Int./Int.Cl. *C07C 311/39* (2006.01),
A61K 31/18 (2006.01), *A61P 25/16* (2006.01),
A61P 25/28 (2006.01)
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(54) Titre : DERIVES D'ARYLSULFONAMIDE, COMPOSITIONS EN CONTENANT ET LEURS METHODES
D'UTILISATION

(54) Title: ARYLSULFONAMIDE DERIVATIVES, COMPOSITIONS, AND METHODS OF USE

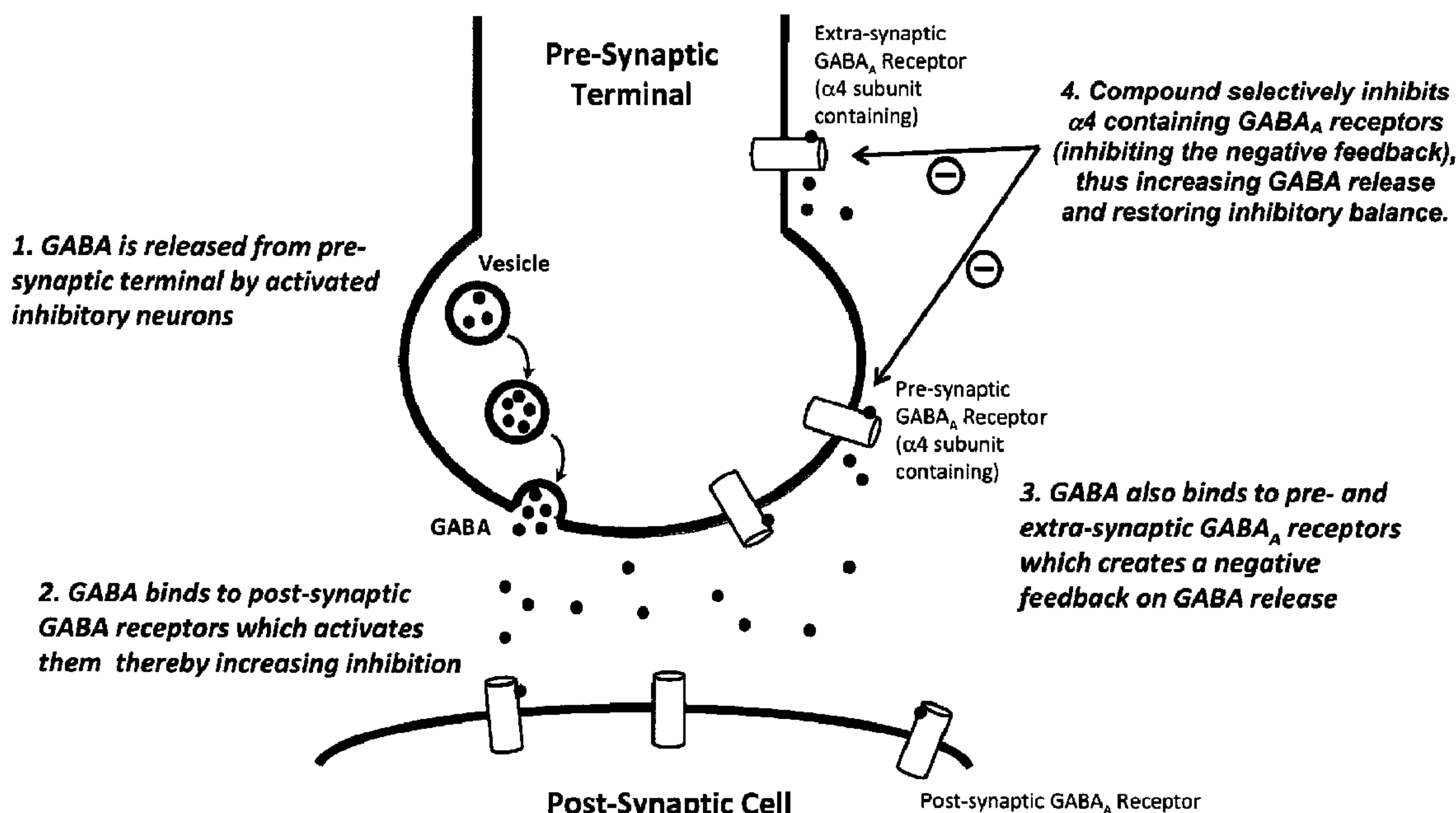


FIGURE 1

(57) **Abrégé/Abstract:**

The present invention provides acylsulfonamides including bumetanide derivatives and compositions comprising such analogs. The present invention also provides pharmaceutical compositions containing these compounds and methods for their use. All of these analogs are particularly useful for the treatment and/or prophylaxis of conditions that involve the Na⁺K⁺Cl⁻ co-transporter or GABA_A receptor including but not limited to addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, the improvement of cognitive function,

(57) Abrégé(suite)/Abstract(continued):

cognitive impairment, cognitive dysfunction, depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.

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

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
9 February 2012 (09.02.2012)

PCT

(10) International Publication Number
WO 2012/018635 A3

(51) International Patent Classification:

C07C 311/39 (2006.01) A61P 25/28 (2006.01)
A61K 31/18 (2006.01) A61P 25/16 (2006.01)

(21) International Application Number:

PCT/US2011/045390

(22) International Filing Date:

26 July 2011 (26.07.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/367,709 26 July 2010 (26.07.2010) US

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(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))

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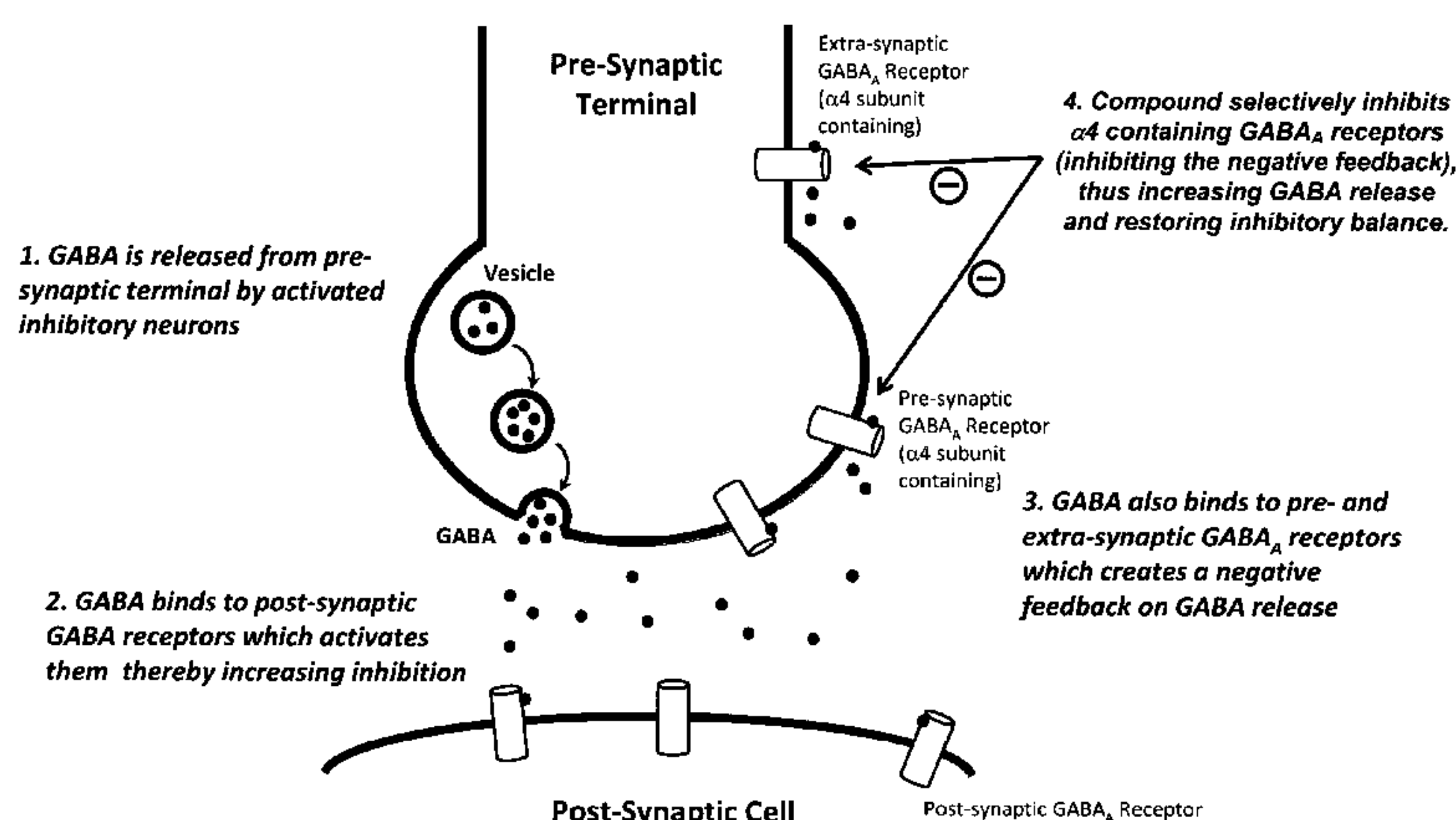


FIGURE 1

(57) **Abstract:** The present invention provides acylsulfonamides including bumetanide derivatives and compositions comprising such analogs. The present invention also provides pharmaceutical compositions containing these compounds and methods for their use. All of these analogs are particularly useful for the treatment and/or prophylaxis of conditions that involve the Na⁺K⁺Cl⁻ co-transporter or GABA_A receptor including but not limited to addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.

WO 2012/018635 A3

WO 2012/018635 A3

— *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))*

(88) Date of publication of the international search report:
19 April 2012

ARYLSULFONAMIDE DERIVATIVES, COMPOSITIONS, AND METHODS OF USE**Cross-Reference to Related Patent Applications**

[0001] This International Patent Application claims priority to U.S. Provisional Patent Application No. 61/367,709, filed July 26, 2010, the disclosure of which is herein incorporated by reference.

Field of the Invention

[0002] The present invention relates to arylsulfonamide derivatives, positional isomers, and prodrugs thereof, compositions comprising the same and methods of making and using the same. The present invention also relates to pharmaceutical compositions comprising these compounds and methods for using these compounds. Compounds described herein are particularly useful for the treatment and/or prophylaxis of diseases, disorders, and conditions that involve the $\text{Na}^+\text{K}^+\text{Cl}^-$ co-transporters (NKCC1 or NKCC2 or combinations thereof) including but not limited to addictive disorders, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), bipolar disorder, cancer, depression, edema, endothelial corneal dystrophy, epilepsy, glaucoma, inflammatory pain, ischemia, migraine, neuropathic pain, nociceptive pain, ocular diseases, pain, postherpetic neuralgia, and schizophrenia. Compounds described herein are also particularly useful for the treatment and/or prophylaxis of diseases, disorders, and conditions that involve GABA_A receptors including but not limited to Alzheimer's Disease, addictive disorders, anxiety disorders, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cognitive function (e.g., cognitive impairment, cognitive dysfunction), depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD) (nocturnal myoclonus), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.

Background of the Invention **$\text{Na}^+\text{K}^+\text{Cl}^-$ Co-Transporters**

[0003] In absorptive and secretory epithelia, transcellular ion transport depends on specific plasma membrane proteins for mediating ion entry and exit from cells. In basolateral membrane of almost all epithelia (with exception of choroidal plexus), sodium exit and potassium entrance occur through Na^+K^+ -ATPase, generating electrochemical gradients that constitute a driving force for Na^+ influx and K^+ efflux. Transport of these ions following their gradients can be accomplished by specific ion

channels, allowing membrane passage of ions alone or by transporters in which Na^+ or K^+ transport is accompanied by other ions or solutes by means of several different solute transporters. These membrane proteins are known as secondary transporters because ion translocation is not dependent on ATP hydrolysis but rather on gradients generated by primary transporters. A secondary transport mechanism for transcellular ion transport in epithelial cells involves cations (Na^+ or K^+) are coupled with chloride (Cl^-), with a stoichiometry of 1:1, and, therefore, the ion translocation produces no change in transmembrane potential. For this reason, these transporters are known as electroneutral cation-chloride coupled cotransporters. In addition to being heavily implicated in ion absorptive and secretory mechanisms, electroneutral cation-chloride coupled cotransporters play a key role in maintenance and regulation of cell volume in both epithelial and nonepithelial cells. Because Na^+ influx and K^+ efflux by electroneutral cotransporters are rapidly corrected by Na^+K^+ -ATPases, the net effect of its activity is Cl^- movement inside or outside cells. The change in intracellular chloride concentration is known to be accompanied by changes in cell volume. Finally, a variety of new physiological roles for electroneutral cotransporters are emerging (*e.g.*, regulation of intraneuronal Cl^- concentration and thus modulation of neurotransmission). Gamba (2005) Physiol. Rev. 85: 423–493.

[0004] Four groups of electroneutral cotransporter systems (also known as “symporters”) have been functionally identified based on cation(s) coupled with chloride, stoichiometry of transport process, and sensitivity to inhibitors. These systems include: (1) the benzothiadiazine (or thiazide)-sensitive Na^+Cl^- cotransporter; (2) the sulfamoylbenzoic (or bumetanide) sensitive $\text{Na}^+\text{K}^+2\text{Cl}^-$ cotransporters; (3) the sulfamoylbenzoic (or bumetanide) sensitive Na^+Cl^- cotransporters; and (4) the dihydroindenylloxy-alkanoic acid (DIOA)-sensitive K^+Cl^- cotransporter. Some overlap exists in sensitivity to inhibitors in the last two groups because $\text{Na}^+\text{K}^+2\text{Cl}^-$ and K^+Cl^- cotransporters can be inhibited by high concentration of DIOA or loop diuretics, respectively; however, affinity for inhibitor and the cation coupled with chloride clearly differentiate between both groups of transporters. Gamba (2005) Physiol. Rev. 85: 423–493. Loop diuretics (*e.g.*, bumetanide, furosemide, piretanide, azosemide, and torsemide) are antagonists of the $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransporter (*e.g.*, NKCC2) in the thick ascending limb of the loop of Henle and act to inhibit sodium and chloride reabsorption by competing for the Cl^- binding site. *See also* Russell (2000) Physiological Reviews 80(1): 211–275.

[0005] Major advances have been made in the past decade in molecular identification and characterization of solute carriers. As of 2005, the Human Genome Organization (HUGO) Nomenclature Committee Database recognizes 43 solute carriers (SLC) families, which include a total of 298 transporter genes encoding for uniporters (passive transporters), cotransporters (coupled transporters), antiporters (exchangers), vesicular transporters, and mitochondrial transporters. This amount of solute carrier genes represents ~1% of the total pool of genes that have been calculated to compose human genome. Gamba (2005) Physiol. Rev. 85: 423–493.

[0006] One isoform of the $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransporter (NKCC) NKCC1 is widely distributed throughout the body. NKCC1 transports sodium, potassium, and chloride into the cell. NKCC1 is also found throughout the nervous system where it is expressed on astrocytes, oligodendrocytes, and Schwann cells. Lenart, *et al.* (2004) The Journal of Neuroscience 24(43): 9585–9597. Another isoform, NKCC2, is found primarily in the kidney, where it serves to extract sodium, potassium, and chloride from the urine. Haas (1994) Am J Physiol Cell Physiol 267: C869–C885.

[0007] The mediators of transcellular Cl^- cotransport (Na-Cl cotransporter, NKCC1, NKCC2, KCC1, and KCC2) are all related members of the SLC12A family of cation/ Cl^- cotransporters; each takes advantage of inward Na^+ or outward K^+ gradients to move Cl^- into or out of cells, respectively. The importance of this family of transporters are underscored by their use as pharmacologic targets (thiazide diuretics act at NKCC, and loop diuretics act at NKCC2), and that their mutation results in diverse diseases. For example, disruption of NKCC1 in mice leads to hearing loss, altered pain perception, neuronal excitability, and altered blood pressure. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788.

[0008] The regulation of Cl^- transport into and out of cells also plays a critical role in the maintenance of intracellular volume and the excitability of GABA responsive neurons regulated by at least two ion cotransporters: Cl^- influx is mediated by the NKCC1 which mediates the Cl^- influx and KCC1 or KCC2 which mediates the Cl^- efflux. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788. The maintenance of intra- and extracellular electrolyte homeostasis are required for a wide range of essential physiologic processes, including general functions (*e.g.*, maintenance of proper cell volume), specialized cell functions (*e.g.*, control of neuronal excitability), and global functions (*e.g.*, regulation of blood pressure). This homeostasis is achieved via the regulated movement of Na^+ , K^+ , and Cl^- across cell membranes by ion channels, cotransporters,

exchangers, and pumps that execute transmembrane electrolyte flux. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788.

[0009] The predominant mechanism by which intracellular volume is maintained in cells in response to changes in extracellular tonicity is the raising or lowering of intracellular Cl^- concentration ($[\text{Cl}^-]_i$), thereby minimizing transmembrane water flux. $[\text{Cl}^-]_i$ is modulated by altering the balance between Cl^- entry and exit. The major mediator of Cl^- entry is NKCC1 and Cl^- exit is largely mediated by KCC1. These cotransporters are both regulated by extracellular tonicity: hypertonicity activates NKCC1 and inhibits KCC1, whereas hypotonicity has the opposite effect. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788.

[0010] An analogous system plays a key role in the control of neuronal excitability where, variation in $[\text{Cl}^-]_i$ in neurons is determined by mechanisms highly similar to those governing cell volume. Cl^- influx largely occurs via NKCC1, whereas Cl^- efflux is mediated via the neuronal-specific K-Cl cotransporter KCC2. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788.

GABA Receptors

[0011] Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system (CNS) where approximately 30% of all synapses use GABA as a transmitter. Thus, GABA receptors are crucial for proper cognitive function and balancing of excitatory and inhibitory signals in the brain. There are three classes of GABA receptors: GABA_A (ligand-gated ion channel), GABA_B (G protein-coupled receptor), and GABA_C (ligand-gated ion channel). Chloride flux into the cell results from the activation of GABA_A receptors by the binding of GABA molecules, hyperpolarizing the resting membrane potential, and decreasing the chances of the post-synaptic neuron propagating an action potential.

[0012] GABA_A receptors are pentameric and approximately 19 GABA receptor subunits have been cloned from mammals (6 α , 3 β , 3 γ , 1 δ , 1 ϵ , 1 θ , 1 π , and 3 ρ subunits). The heterogeneity of GABA subunits are further increased by alternate splicing (*e.g.*, $\gamma 2$ short and $\gamma 2$ long are the two major splice variants of the $\gamma 2$). In general, a functional GABA_A receptor requires 2 α subunits, 2 β subunits and a third “regulatory” subunit (usually γ or δ). WO 2009/100040. The specific subunit combination determines the pharmacological and ligand binding properties of the GABA_A receptor. The most abundant subunit combination found in the CNS are $\alpha_1\beta_2\gamma_2$. This subtype represents

approximately 40% of GABA_A receptors in the brain and it is expressed throughout the CNS and is located on post-synaptic cells. WO 2007/002359.

[0013] The importance of $[Cl^-]_i$ regulation has been recognized with the discovery that GABA neurotransmission is not uniformly inhibitory (*e.g.*, it is predominantly excitatory in the neonatal period.) If $[Cl^-]_i$ is below its equilibrium potential, Cl^- enters the cell, resulting in hyperpolarization and inhibition. If $[Cl^-]_i$ is above its equilibrium potential, GABA induces Cl^- efflux, depolarization, and neuronal excitation. Similarly, neurons of the suprachiasmatic nucleus show circadian variation in their response to GABA, demonstrating the ability to dynamically regulate $[Cl^-]_i$. Finally, GABA neurotransmission in the peripheral nervous system is predominantly excitatory.

[0014] GABA_A receptors are the targets of a wide range of therapeutic and clinically relevant compounds including benzodiazepines, barbiturates, neurosteroids, ethanol, certain intravenous anesthetics, and subtype specific modulators (*e.g.*, Zolpidem.) These compounds serve as anxiolytics, sedative/hypnotics, anti-epileptic drugs (AED), and memory enhancers. Many of these therapeutics show efficacy but cause side effects due to unwanted effects at α_1 and/or α_2 GABA_A variants or due to low therapeutic index. For example, benzodiazepines such as diazepam (VALIUM) are excellent anxiolytics but cause unwanted sedative effects. WO 2007/002359.

[0015] At a cellular level, GABA_A receptors are expressed both pre-synaptic, post-synaptic, and extra-synaptic sites (pre-synaptic and extrasynaptic being defined herein as parasynaptic to distinguish from post-synaptic) where they respond to large changes in GABA concentration caused by release of the neurotransmitter into the synaptic space, and extra-synaptically where the receptors respond to lower concentrations of GABA that “leak” from synaptic junctions. The post-synaptic receptors respond to acute changes in neuronal firing, pre-synaptic receptors are responsible for inhibition of GABA release in the setting of high GABA levels, whereas the extrasynaptic receptors are responsible for maintaining overall tone of neuronal networks. WO 2009/100040. Tonic inhibition is generated by the persistent activation of extrasynaptic (perisynaptic) GABA_A receptors and regulates the excitability of individual neurons and neural networks. Jia, *et al.* (2008) The Journal of Pharmacology and Experimental Therapeutics 326(2): 475–482.

[0016] Presynaptic GABA_A receptors situated at extrasynaptic sites may comprise $\alpha_4\beta\delta$ and $\alpha_6\beta\delta$ isoforms. The extrasynaptic $\alpha_4\beta\delta$ and $\alpha_6\beta\delta$ GABA_A receptor isoforms show marked sensitivity to GABA, alcohol, and anesthetics, suggesting that receptors may present a critical site for regulating synaptic function in the developing brain in both physiological and pathological situations. Xiao, *et*

al. (2007) J Physiol. 580(Pt.3):731–43. For example, temporal lobe epilepsy (TLE), Parkinson's disease (PD) and Huntington's disease (HD) are neurodegenerative disorders that involve disruptions in GABA signaling. TLE seizures reflect excess excitation, which may result from local inhibitory circuit dysfunction. PD devastates the input to striatal GABAergic neurons and HD destroys striatal GABAergic neurons. Directing GABA synthesis, degradation, release, transport or receptors may be useful in controlling GABA signaling in specific brain areas should benefit each of these diseases. Thus, new drugs targeting GABA synthesis, release, and binding may be useful for improved therapeutic treatments for epilepsy and both Parkinson's and Huntington's disease. Kleppner and Tobin (2001) Expert Opin Ther Targets. 5(2):219–39. *See also* Shumate, *et al.* (1998) Epilepsy Research (32): 114–128; Fritschy (2008) Frontiers in Molecular Neuroscience 1(5): 1–5; Roberts, *et al.* (2006) The Journal of Biological Chemistry 281(40): 29431–29435; and Roberts, *et al.* PNAS 102(33): 11894–11899.

Addictive Disorders

[0017] Addictive and/or compulsive disorders, such as eating disorders (including obesity), addiction/physical dependence to stimulants, narcotics (*e.g.*, cocaine, heroin) sedatives/hypnotics, and opioids including alcoholism and smoking are major public health problems that impact society on multiple levels. It has been estimated that substance abuse alone costs the United States more than \$484 billion per year.

[0018] The alcohol-sensitive $\alpha_4\beta\delta$ GABA_A receptor has also been postulated to be involved in alcohol addiction (alcoholism). For example, reduced expression of GABA_A receptors comprising an α_4 subunit in the nucleus accumbens (NAc) decreased the free consumption and preference for alcohol in rats. Further, the nucleus accumbens contributes to the rewarding and reinforcing effects of drugs including alcohol suggesting that the GABA_A receptor, specifically the $\alpha_4\beta\delta$ isoform, in the NAc is an important mediator of alcohol self-administration. Rewal, *et al.* (2009) The Journal of Neuroscience 29(2): 543–549. Although most GABA_A receptor subunit combinations can be activated by high (anesthetic) alcohol concentrations, so far only very specific GABA_A receptor subunit combinations (containing the δ as well as the β_3 subunit) exhibit dose-dependencies that mirror blood alcohol levels associated with mild to moderate intoxication in humans. These δ -subunit containing GABA_A receptors containing the δ subunit are located either outside or in the perimeter of synapses, but not in the sub-synaptic membrane. WO 2007/002359.

[0019] Current strategies for the treatment of addictive disorders include psychological counseling and support, use of therapeutic agents, or a combination of both. A variety of agents known to affect the central nervous system have been used in various contexts to treat a number of indications related directly or indirectly to addictive behaviors but a great need remains for improved addictive disorder therapeutics. GABA_A specific agents may be effective therapeutics for addictive behaviors.

Alzheimer's Disease

[0020] Alzheimer's disease (AD) is an age-related, non-reversible brain disorder that develops over a period of years and is the most common cause of dementia among people age 65 and older. Initially, people experience memory loss and confusion, which may be mistaken for the kinds of memory changes associated with normal aging. However, the symptoms of AD gradually lead to behavior and personality changes, a decline in cognitive abilities such as decision-making and language skills, and problems recognizing family and friends. AD ultimately leads to a severe loss of mental function. NINDS Alzheimer's Disease Information Page (2009).

[0021] AD results in neuron death in the brain. As neurons die throughout the brain, the affected regions begin to atrophy. By the final stage of AD, damage is widespread and brain tissue has shrunk significantly. Two major hallmarks associated with the AD disease processes in the brain are amyloid plaques and neurofibrillary tangles. Amyloid plaques comprise fragments of β -amyloid peptide mixed with a collection of additional proteins, and remnants of neurons. Neurofibrillary tangles (NFTs) are found inside neurons and comprise tau protein. NINDS Alzheimer's Disease Information Page (2009).

[0022] Currently there are no medicines that can slow the progression of AD. However, four FDA-approved medications are used to treat AD symptoms. Donepezil (Aricept), rivastigmine (Exelon), galantamine (Reminyl), and memantine (Namenda) are prescribed to treat AD symptoms. NINDS Alzheimer's Disease Information Page (2009). Further, treatment of an AD transgenic mouse model with picrotoxin, a GABA_A antagonist, showed improved cognitive functions in the mice. Yoshiike, *et al.* (August 21, 2008) PLoS One. 3(8):e3029. Additionally, the expression of NKCC1 has been found to be elevated in AD patients. Johanson, *et al.* (2004) Cerebrospinal Fluid Research 1:3. Unfortunately these medications will not stop or reverse AD, and they appear to help individuals for only a few months to a few years. Therefore novel therapies based on the regulation of GABA_A receptor activity may relieve the symptoms of AD.

Anxiety Disorders

[0023] Anxiety disorders are classified into several subtypes: anxiety, acute anxiety, panic disorder, social anxiety disorder, obsessive compulsive disorder (OCD), panic disorder, panic symptoms, post-traumatic stress disorder (PTSD), generalized anxiety disorder, and specific phobia. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 4th edition (1994).

[0024] As a group, the anxiety disorders have the highest prevalence in the U.S. of all psychiatric disorders and afflict 15.7 million people in the United States each year, and 30 million people in the United States at some point in their lives. Lepine (2002) J. Clin. Psychiatry, 63: Suppl 14:4–8; Kessler, *et al.* (1994) Arch Gen Psychiatry 51:8–19.

[0025] Several animal models are recognized in the art as being predictive of anxiolytic activity. These include the fear-potentiated startle model, described by Davis in Psychopharmacology 62:1; 1979, Behav. Neurosci. 100:814;1986 and TiPS, January 1992 Vol. 13, 35–41, the elevated plus model described by Lister in Psychopharmacol. 92:180–185; 1987, and the well-known punished-responding (conflict) model, described, in “Psychopharmacology of Anxiolytics and Antidepressants”, edited by S. E. File, pages 131–153, Raven Press, New York, 1991.

[0026] Anxiety disorders are generally treated with drugs and psychotherapy. The most commonly prescribed drugs for all anxiety types are benzodiazepines and selective serotonin reuptake inhibitors (SSRI). However, while these drugs show efficacy, both benzodiazepines and SSRIs show adverse effects during treatment. Denys and de Geus (August 2005) Curr Psychiatry Rep. 7(4): 252–7. Further, numerous side effects are associated with long-term use of SSRIs, such as sexual dysfunction and weight gain. Hirschfeld (2003) J. Clin. Psychiatry, 64: Suppl 18: 20–4. Additionally, existing drugs targeting postsynaptic type GABA_A receptors produce undesirable results because they indiscriminately target most of the GABA_A receptors in the brain. WO 2007/136838. In view of nonresponders and deleterious side effects, a great need exists for improved anxiety therapeutics.

Ascites

[0027] Ascites are excess fluid in the space between the tissues lining the abdomen and abdominal organs (the peritoneal cavity) typically caused by liver disease. Disorders that may be associated with ascites include: cirrhosis, hepatitis, portal vein thrombosis, constrictive pericarditis, congestive heart failure, liver cancer, ovarian cancer, protein-losing enteropathy, nephrotic syndrome, and

pancreatitis. Some agents are available for the treatment of ascites (*e.g.*, furosemide) but a great need remains for improved ascites therapeutics. *See Shiozaki, et al. (2006) J. Physiol. Sci.* 56(6): 401–406.

Attention Deficit Hyperactivity Disorder (ADHD)

[0028] ADHD is a problem with inattentiveness, over-activity, impulsivity, or a combination. ADHD is the most commonly diagnosed behavioral disorder of childhood. It affects about 3 - 5% of school aged children. ADHD is diagnosed much more often in boys than in girls. ADHD may run in families, but it is not clear exactly what causes it. Whatever the cause may be, it seems to be set in motion early in life as the brain is developing. Depression, lack of sleep, learning disabilities, tic disorders, and behavior problems may be confused with, or appear with, ADHD. Every child suspected of having ADHD should be carefully examined by a doctor to rule out possible other conditions or reasons for the behavior. Most children with ADHD also have at least one other developmental or behavioral problem. They may also have a psychiatric problem, such as depression or bipolar disorder. ADHD symptoms fall into three groups: lack of attention (inattentiveness), hyperactivity, and impulsive behavior (impulsivity). Some children suffer from primarily inattentiveness and others have a combination of these symptoms. ADHD is difficult to diagnosis but may be identified by a series of developmental, mental, nutritional, physical, and psychosocial examination. Attention deficit hyperactivity disorder (ADHD) (2011) PubMed Health.

[0029] Current treatment of ADHD is a combination of medications (*e.g.*, amphetamine-dextroamphetamine (ADDERALL)), dexamethylphenidate (FOCALIN), dextraamphetamine (DEXEDRINE, DEXTROSTAT), lisdexafetamine (Vyvanse), and methylphenidate (RITALIN) and behavior therapy. Attention deficit hyperactivity disorder (ADHD) (2011) PubMed Health. A recent study suggested that extended-release valproate (EVA), a GABA enhancer, improved hyperactivity and impulsivity in an ADHD study. Miyazaki, *et al. (2006) Brain and Development* 28(7): 470–472. Also, reduced feedback inhibition by striatal GABA neurons and interneurons was implicated in an animal model of ADHD. Viggiano, *et al. (2002) Behavioural Brain Research* 130(1–2): 181–189. However, the current treatment for ADHD leave a great need for improved ADHD therapeutics.

Autism Spectrum Disorders (Autism)

[0030] Autism spectrum disorder (ASD) is a range of complex neurodevelopment disorders, characterized by social impairments, communication difficulties, and restricted, repetitive, and

stereotyped patterns of behavior. Autistic disorder, sometimes called autism or classical ASD, is the most severe form of ASD, while other conditions along the spectrum include a milder form known as Asperger syndrome, a rare condition called Rett syndrome, and childhood disintegrative disorder and pervasive developmental disorder not otherwise specified (usually referred to as PDD-NOS).

Although ASD varies significantly in character and severity, it occurs in all ethnic and socioeconomic groups and affects every age group. Experts estimate that three to six people out of every 1,000 may develop ASD. Males are four times more likely to have ASD than females.

NINDS Autism Fact Sheet (2009).

[0031] Multiple lines of evidence, including genetic and imaging studies, suggest that the anterior cingulate cortex and gamma-amino-butyric acid (GABA) system may be affected in autism.

Compared to controls, the autistic patients show a significant decrease in the mean density of GABA_A receptors in the supragranular (46.8%) and infragranular (20.2%) layers of the anterior cingulate cortex (ACC) and in the density of benzodiazepine binding sites in the supragranular (28.9%) and infragranular (16.4%) lamina. In addition, a trend for a decrease in the density of benzodiazepine sites was found in the infragranular layers (17.1%) in the autistic group. These findings suggest that in the autistic group this down regulation of both benzodiazepine sites and GABA_A receptors in the ACC may be the result of increased GABA innervation and/or release disturbing the delicate excitation/inhibition balance of principal neurons as well as their output to key limbic cortical targets. These disturbances may underlie the core alterations in socio-emotional behaviors in autism spectrum disorders. Oblak, *et al.* (August 2009) Autism Res. 2009

Aug;2(4):205–19. Furthermore, various GABA_A subunit types, such as α_3 variant sequences and α_4 isoforms have been linked to autism spectrum disorders. WO 2009/100040. There is no cure for ASD, thus there exists a great need for therapeutics to treat autism spectrum disorders. Therefore, therapeutics that target the GABA_A receptor may be useful in treating autism spectrum disorders.

Bipolar Disorder

[0032] Bipolar disorder (manic-depressive illness) is a brain disorder that causes unusual shifts in a person's mood, energy, and ability to function. They can result in damaged relationships, poor job or school performance, and even suicide. About 5.7 million American adults or about 2.6 percent of the population age 18 and older have bipolar disorder in any given year. Bipolar disorder typically develops in late adolescence or early adulthood. However, some people have their first symptoms during childhood, and some develop them late in life. It is often not recognized as an illness, and

people may suffer for years before it is properly diagnosed and treated. National Institute of Mental Health “Bipolar Disorder” (2008) Complete Publication.

[0033] Bipolar disorder causes dramatic mood swings—from overly “high” and/or irritable to sad and hopeless, and then back again, often with periods of normal mood in between. Severe changes in energy and behavior go along with these changes in mood. The periods of highs and lows are called episodes of mania and depression. National Institute of Mental Health “Bipolar Disorder” (2008) Complete Publication.

[0034] Signs and symptoms of mania (or a manic episode) include: increased energy, activity, and restlessness; excessively “high,” overly good, euphoric mood; extreme irritability; racing thoughts and talking very fast, jumping from one idea to another; distractibility, difficulty concentrating; little sleep needed; unrealistic beliefs in one’s abilities and powers; poor judgment; spending sprees; a lasting period of behavior that is different from usual; increased sexual drive; drug abuse, particularly cocaine, alcohol, and sleeping medications; provocative, intrusive, or aggressive behavior; and/or denial that anything is wrong. A manic episode is diagnosed if elevated mood occurs with three or more of the other symptoms most of the day, nearly every day, for 1 week or longer. National Institute of Mental Health “Bipolar Disorder” (2008) Complete Publication.

[0035] Signs and symptoms of depression (or a depressive episode) include: lasting sad, anxious, or empty mood; feelings of hopelessness or pessimism; feelings of guilt, worthlessness, or helplessness; loss of interest or pleasure in activities once enjoyed, including sex; decreased energy, a feeling of fatigue or of being “slowed down”; difficulty concentrating, remembering, making decisions; restlessness or irritability; sleeping too much, or cannot sleep; change in appetite and/or unintended weight loss or gain; chronic pain or other persistent bodily symptoms that are not caused by physical illness or injury; and/or thoughts of death or suicide, or suicide attempts. A depressive episode is diagnosed if five or more of these symptoms last most of the day, nearly every day, for a period of 2 weeks or longer. National Institute of Mental Health “Bipolar Disorder” (2008) Complete Publication.

[0036] A mild to moderate level of mania is called hypomania. Hypomania may feel good to the person who experiences it and may even be associated with good functioning and enhanced productivity. Thus even when family and friends learn to recognize the mood swings as possible bipolar disorder, the person may deny that anything is wrong. Without proper treatment, however, hypomania can become severe mania in some people or can switch into depression.

[0037] In some people, however, symptoms of mania and depression may occur together in what is called a mixed bipolar state. Symptoms of a mixed state often include agitation, trouble sleeping, significant change in appetite, psychosis, and suicidal thinking. A person may have a very sad, hopeless mood while at the same time feeling extremely energized.

[0038] The classic form of the illness, which involves recurrent episodes of mania and depression, is called bipolar I disorder. Some people, however, never develop severe mania but instead experience milder episodes of hypomania that alternate with depression; this form of the illness is called bipolar II disorder. When four or more episodes of illness occur within a 12-month period, a person is said to have rapid-cycling bipolar disorder. Some people experience multiple episodes within a single week, or even within a single day. Rapid cycling tends to develop later in the course of illness and is more common among women than among men.

[0039] Medications known as “mood stabilizers” usually are prescribed to help control bipolar disorder (*e.g.*, lithium or valproic acid-DEPAKOTE/VALPROATE). In addition to medication, psychosocial treatments—including certain forms of psychotherapy, are often used to treat bipolar disorders. Depending on the medication, side effects include weight gain, nausea, tremor, reduced sexual drive or performance, anxiety, hair loss, movement problems, or dry mouth. Lithium treatment can cause low thyroid levels, resulting in the need for thyroid supplementation. Additionally, Valproate® may lead to adverse hormone changes in teenage girls and polycystic ovary syndrome in women who began taking the medication before age 20. Further, women suffering bipolar disorder who wish to conceive, or who become pregnant, face special challenges due to the possible harmful effects of existing mood stabilizing medications on the developing fetus and the nursing infant. National Institute of Mental Health “Bipolar Disorder” (2008) Complete Publication. Improved bipolar disorder therapeutics may be developed that act to increase GABA activity.

[0040] Postmortem and genetic studies have linked neuropsychiatric disorders including schizophrenia and bipolar disorder with GABAergic neurotransmission and various specific GABA_A receptor subunits. Further, GABA_A receptor-associated proteins involved in GABA_A receptor trafficking, targeting, clustering, and anchoring that often carry out these functions in a subtype-specific manner. Charych, *et al.* (2009) Neuropharmacology 57(5–6): 481–95. Therefore, GABA_A receptor specific therapeutics that improve inhibition may be beneficial because bipolar disease is a state of alterations of abnormal inhibition/excitation without adequate inhibition.

Depression

[0041] Depression is a common but serious illness, the most common are major depressive disorder and dysthymic disorder. Major depressive disorder, also called major depression, is characterized by a combination of symptoms that interfere with a person's ability to work, sleep, study, eat, and enjoy once-pleasurable activities. Major depression is disabling and prevents a person from functioning normally. An episode of major depression may occur only once in a person's lifetime, but more often, it recurs throughout a person's life. National Institute of Mental Health "Depression" (2008) Complete Publication.

[0042] The forms of depression include:

[0043] Dysthymic disorder, also called dysthymia, is characterized by long-term (two years or longer) but less severe symptoms that may not disable a person but can prevent one from functioning normally or feeling well. People with dysthymia may also experience one or more episodes of major depression during their lifetimes.

[0044] Psychotic depression, which occurs when a severe depressive illness is accompanied by some form of psychosis, such as a break with reality, hallucinations, and delusions. Postpartum depression, which is diagnosed if a new mother develops a major depressive episode within one month after delivery. It is estimated that 10 to 15 percent of women experience postpartum depression after giving birth.

[0045] Seasonal affective disorder (SAD), which is characterized by the onset of a depressive illness during the winter months, when there is less natural sunlight. The depression generally lifts during spring and summer. SAD may be effectively treated with light therapy, but nearly half of those with SAD do not respond to light therapy alone. Antidepressant medication and psychotherapy can reduce SAD symptoms, either alone or in combination with light therapy. National Institute of Mental Health "Depression" (2008) Complete Publication.

[0046] Depression can be treated with a number of methods. The most common treatments are medication and psychotherapy. Antidepressants work to normalize neurotransmitters, notably serotonin, norepinephrine, and dopamine. The newest and among the most popular types of antidepressant medications are called selective serotonin reuptake inhibitors (SSRIs). SSRIs include fluoxetine (Prozac®), citalopram (Celexa®), sertraline (Zoloft®), and several others. Serotonin and norepinephrine reuptake inhibitors (SNRIs) are similar to SSRIs and include venlafaxine (Effexor®) and duloxetine (Cymbalta®). SSRIs and SNRIs are more popular than the older classes of

antidepressants, such as tricyclics—named for their chemical structure—and monoamine oxidase inhibitors (MAOIs) because they tend to have fewer side effects. However, medications affect everyone differently—no one-size-fits-all approach to medication exists. National Institute of Mental Health “Depression” (2008) Complete Publication.

[0047] For all classes of antidepressants, patients can experience side effects. Antidepressants may cause mild and often temporary side effects in some people, but they are usually not long-term. The most common side effects associated with SSRIs and SNRIs include: headache, nausea, insomnia, nervousness, agitation, and sexual problems. National Institute of Mental Health “Depression” (2008) Complete Publication. Tricyclic antidepressants also can cause side effects including: dry mouth, constipation, bladder problems, sexual problems, blurred vision, and daytime drowsiness. Additionally, patients taking MAOIs must adhere to significant food and medicinal restrictions to avoid potentially serious interactions. They must avoid certain foods that contain high levels of the chemical tyramine, which is found in many cheeses, wines and pickles, and some medications including decongestants. MAOIs interact with tyramine in such a way that may cause a sharp increase in blood pressure, which could lead to a stroke. National Institute of Mental Health “Depression” (2008) Complete Publication.

[0048] GABA is involved in both clinical depression and in animal models of depression. Kram, *et al.* (2000) Neuroscience Research 38(2): 193–198. Therefore improved depression therapeutics based on the GABAergic system may provide better medication.

Epilepsy

[0049] Epilepsy is characterized by abnormal discharges of cerebral neurons and is typically manifested as various types of seizures. Epileptiform activity is identified with spontaneously occurring synchronized discharges of neuronal populations that can be measured using electrophysiological techniques. Epilepsy is one of the most common neurological disorders, affecting about 1% of the population. There are various forms of epilepsy, including idiopathic, symptomatic, and cryptogenic. Genetic predisposition is thought to be the predominant etiologic factor in idiopathic epilepsy. Symptomatic epilepsy usually develops as a result of a structural abnormality in the brain.

[0050] Status epilepticus are a particularly severe form of seizure, which is manifested as multiple seizures that persist for a significant length of time, or serial seizures without any recovery of consciousness between seizures. The overall mortality rate among adults with status epilepticus is

approximately 20 percent. Patients who have a first episode are at substantial risk for future episodes and for the development of chronic epilepsy. The frequency of status epilepticus in the United States is approximately 150,000 cases per year, with approximately 55,000 deaths being associated with status epilepticus annually. Sirven and Waterhouse (2003) American Family Physician 68: 469–476. Acute processes that are associated with status epilepticus include intractable epilepsy, metabolic disturbances (*e.g.*, electrolyte abnormalities, renal failure, and sepsis), central nervous system infection (meningitis or encephalitis), stroke, degenerative diseases, head trauma, drug toxicity, and hypoxia. The fundamental pathophysiology of status epilepticus involves a failure of mechanisms that normally abort an isolated seizure. This failure can arise from abnormally persistent, excessive excitation or ineffective recruitment of inhibition. Studies have shown that excessive activation of excitatory amino acid receptors can cause prolonged seizures and suggest that excitatory amino acids may play a causative role. Status epilepticus can also be caused by penicillin and related compounds that antagonize the effects of γ -aminobutyric acid (GABA).

[0051] Epilepsy is a chronic neurological condition characterized by recurrent seizures that is caused by abnormal cerebral nerve cell activity. Epilepsy is classified as idiopathic or symptomatic. A nerve cell transmits signals to and from the brain in two ways by (1) altering the concentrations of salts (sodium, potassium, calcium) within the cell and (2) releasing chemicals called neurotransmitters (*e.g.* gamma aminobutyric acid, GABA). The change in salt concentration conducts the impulse from one end of the nerve cell to the other. At the end, a neurotransmitter is released, which carries the impulse to the next nerve cell. Neurotransmitters either slow down or stop cell-to-cell communication (called inhibitory neurotransmitters) or stimulate this process (called excitatory neurotransmitters). Normally, nerve transmission in the brain occurs in an orderly way, allowing a smooth flow of electrical activity. Improper concentration of salts within the cell and over activity of either type of neurotransmitter can disrupt orderly nerve cell transmission and trigger seizure activity. Certain areas of the brain are more likely than others to be involved in seizure activity. The motor cortex, which are responsible for body movement, and the temporal lobes, including the hippocampus, which is involved in memory, are particularly sensitive to biochemical changes (*e.g.*, decreased oxygen level, metabolic imbalances, infection) that provoke abnormal brain cell activity.

[0052] Two molecules regulate cellular chloride levels: KCC2, which transports chloride out of cells, and NKCC1, which brings chloride in to the cells. Previous studies in rats had shown that

adult nerve cells mostly have KCC2, making their chloride concentrations lower inside than outside. Thus, when GABA receptors are activated, chloride tends to come in, with an inhibitory effect. In newborn rats, the situation is reversed: their nerve cells mostly have NKCC1, so chloride is actively transported inside, making initial chloride concentrations very high. As a result, GABA activation causes chloride to exit the cell, with an excitatory effect. *See e.g.*, Cohen (1981) J. Clin. Pharmacol. 21:537–542; Dzhalal, *et al.* (2005) Nat Med. 11: 1205–1213; Martinez, *et al.* (1998) Am. J. Clin. Nutr. 68:1354S–1357S, the disclosures of each of which is hereby incorporated by reference in their entireties. Accordingly, compounds described herein may inhibit seizure activity in the kainic acid induced seizure rat model.

[0053] A study demonstrated that NKCC antagonists may help treat seizures in newborns, which is difficult to control with existing anticonvulsants. Conventional anticonvulsants - phenobarbital and benzodiazepines are ineffective in newborns because their brains are biochemically different from adult brains. Conventional anticonvulsants work by mimicking the action of GABA, a natural inhibitory chemical in the brain, by activating GABA receptors on the surface of brain cells. In adult nerve cells, GABA activation opens up channels that allow chloride to move into the cell. The cell thereby acquires a negative charge and becomes less excitable, inhibiting seizure activity. But in newborns, chloride is already high, and therefore activating GABA receptors causes chloride to move out of nerve cells, creating a paradoxical excitatory reaction that may actually exacerbate seizures.

[0054] Traditional anti-epileptic drugs exert their principal effect through one of three mechanisms: (a) inhibition of repetitive, high-frequency neuronal firing by blocking voltage-dependent sodium channels; (b) potentiation of γ -aminobutyric acid (gamma-aminobutyric acid, GABA)-mediated postsynaptic inhibition; and (c) blockade of T-type calcium channels. Many current anti-epileptic drug therapies exert their pharmacological effects on all brain cells, regardless of their involvement in seizure activity. Common side effects are over-sedation, dizziness, loss of memory and liver damage. Furthermore, 20–30% of epilepsy patients are refractory to current therapy. Therefore there is a great need for improved epilepsy therapeutics to reduce both morbidity and mortality.

Glaucoma

[0055] Glaucoma is a group of diseases that occur when the normal fluid pressure inside the eyes slowly rises, damaging the eye's optic nerve and result in vision loss and blindness. Open-angle

glaucoma is the most common form and other types include: (1) low-tension or normal-tension glaucoma; (2) angle-closure glaucoma; (3) congenital glaucoma; (4) secondary glaucomas; and (5) pigmentary glaucoma including neovascular glaucoma. Glaucoma is usually detected through a comprehensive eye exam that includes: (a) visual acuity test; (b) visual field test; (c) dilated eye exam; (d) tonometry; and (e) pachymetry. Current glaucoma treatments include medicines, laser trabeculoplasty, conventional surgery, or a combination of any of these; however, there is a great need for improved glaucoma therapeutics. National Eye Institute Glaucoma Fact Sheet (2008).

Huntington's Disease

[0056] Huntington's disease (HD) results from neuronal degeneration leading to uncontrolled movements, loss of intellectual faculties, and emotional disturbance. HD is an autosomal dominant disease caused by a CAG expansion in the *Htt* gene that leads to a poly-glutamine expansion in the disease protein huntingtin. GABAergic interneurons are particularly sensitive to the accumulation of mutant huntingtin and die early in the development of HD. Some early symptoms of HD are mood swings, depression, irritability or trouble driving, learning new things, remembering a fact, or making a decision. As the disease progresses, concentration on intellectual tasks becomes increasingly difficult, and the patient may have difficulty feeding himself or herself and swallowing. The rate of disease progression and the age of onset vary from person to person. NINDS Publication "Huntington's Disease: Hope Through Research" (2009).

[0057] Huntington's disease (HD) is a neurodegenerative disorder that involves disruptions in GABA signaling. GABA_A is the major inhibitory neurotransmitter in the central nervous system (CNS). HD destroys striatal GABAergic neurons. Directing GABA_A synthesis, degradation, transport, or receptors can control GABA signaling and so drugs that target these aspects of GABA metabolism may be used for improved therapeutic treatments for Huntington's disease. Kleppner and Tobin (2001) Expert Opin Ther Targets. 5(2):219–39. Physicians prescribe a number of medications to help control emotional and movement problems associated with HD including tetrabenazine to treat Huntington's chorea (the involuntary writhing movements). However, the drugs used to treat the symptoms of HD have side effects such as fatigue, restlessness, or hyperexcitability. NINDS Publication "Huntington's Disease: Hope Through Research" (2009).

Insomnia

[0058] Insomnia is a symptom of sleep disorders, characterized by persistent difficulty falling asleep or staying asleep despite the opportunity. NHLBI Diseases and Conditions Index [Insomnia]

(2009). Although there are several different degrees of insomnia, three types of insomnia have been clearly identified: transient, acute, and chronic. Transient insomnia lasts from days to weeks. It can be caused by another disorder, by changes in the sleep environment, by the timing of sleep, severe depression, or by stress. Its consequences — sleepiness and impaired psychomotor performance — are similar to those of sleep deprivation. Acute insomnia is the inability to consistently sleep well for a period of between three weeks to six months. Chronic insomnia lasts for years at a time. It can be caused by another disorder, or it can be a primary disorder. Its effects can vary according to its causes. They might include sleepiness, muscular fatigue, hallucinations, and/or mental fatigue; but people with chronic insomnia often show increased alertness. NHLBI Diseases and Conditions Index [Insomnia] (2009). Current insomnia drug therapies that target the GABA_A receptor, hypnotics (*e.g.*, benzodiazepines) may have undesirable side effects, therefore a great need exists for improved insomnia therapeutics with reduced side effects.

Ischemia

[0059] Ischemia is a restriction in blood supply, generally due to factors in the blood vessels, with resultant damage or dysfunction of tissue due to inadequate oxygenation and lack of nutrients of the tissue. Insufficient blood supply causes tissue to become hypoxic, or, if no oxygen is supplied at all, anoxic. In contrast with hypoxia, a more general term denoting a shortage of oxygen (usually a result of lack of oxygen in the air being breathed), ischemia is an absolute or relative shortage of the blood supply to an organ. This can cause necrosis (*e.g.*, cell death). In aerobic tissues such as heart and brain, at body temperature necrosis due to ischemia usually takes about 3–4 hours before becoming irreversible. Later, more damage occurs due to the accumulation of metabolic wastes due to lack of adequate blood supply to the tissue. Complete cessation of oxygenation of such organs for more than 20 minutes typically results in irreversible damage.

[0060] Inhibition of NKCC1 activity with bumetanide and furosemide significantly reduces the infarct volume and cerebral edema following cerebral focal ischemia suggesting that NKCC1 antagonists may be useful in treating ischemia. Chen and Sun (2005) Neurol. Res. 27(3): 280–286. The typical treatment of ischemia involves “clot-buster” drugs (*e.g.*, Alteplase®) usually given for stroke and heart attack within this time period. However, restoration of blood flow after a period of ischemia can actually be more damaging than the ischemia because reintroduction of oxygen causes a greater production of damaging free radicals, resulting in reperfusion injury, and, eventually, necrosis. Therefore a great need exists for improved ischemia therapeutics.

Migraine

[0061] Migraine headaches afflict 10–20% of the U.S. population, with an estimated loss of 64 million workdays annually. Migraine headache is characterized by pulsating head pain that is episodic, unilateral or bilateral, lasting from 4 to 72 hours and often associated with nausea, vomiting, and hypersensitivity to light and/or sound. When accompanied by premonitory symptoms, such as visual, sensory, speech or motor symptoms, the headache is referred to as “migraine with aura,” formerly known as classic migraine. When not accompanied by such symptoms, the headache is referred to as “migraine without aura,” formerly known as common migraine. Both types evidence a strong genetic component, and both are three times more common in women than men. The precise etiology of migraine has yet to be determined. It has been theorized that persons prone to migraine have a reduced threshold for neuronal excitability, possibly due to reduced activity of GABA. GABA normally inhibits the activity of the neurotransmitters serotonin (5-HT) and glutamate, both of which appear to be involved in migraine attacks. The excitatory neurotransmitter glutamate is implicated in an electrical phenomenon called cortical spreading depression, which can initiate a migraine attack, while serotonin is implicated in vascular changes that occur as the migraine progresses.

[0062] It has been suggested that cortical spreading depression (CSD) underlies migraines including migraines with visual aura. It is also believed that CSD underlies migraine as part of the trigeminal pain circuit. CSD is characterized by a short burst of intense depolarization in the occipital cortex, followed by a wave of neuronal silence and diminished evoked potentials that advance anteriorly across the surface of the cerebral cortex. Enhanced excitability of the occipital-cortex neurons has been proposed as the basis for CSD. The visual cortex may have a lower threshold for excitability and therefore is most prone to CSD. It has been suggested that mitochondrial disorders, magnesium deficiency, and abnormality of presynaptic calcium channels may be responsible for neuronal hyperexcitability. Welch (1997) Seminars in Neurobiol. 17: 4. During a spreading depression event, profound ionic perturbations occur, which include interstitial acidification, extracellular potassium accumulation, and redistribution of sodium and chloride ions to intracellular compartments. In addition, prolonged glial swelling occurs as a homeostatic response to altered ionic extracellular fluid composition, and interstitial neurotransmitter and fatty acid accumulation. Studies have shown that furosemide inhibits regenerative cortical spreading depression in anaesthetized cats. Read, *et al.* (1997) Cephalagia 17: 826.

[0063] Drug therapy is tailored to the severity and frequency of migraine headaches. For occasional attacks, acute treatment may be indicated, but for attacks occurring two or more times per month, or when attacks greatly impact the patient's daily life, prophylactic therapy may be indicated. The side effects of acute and prophylactic treatment agents including serotonin acting agents, beta-blockers, tricyclic antidepressants, anticonvulsants, and botulinum toxin type A injections can limit their use. GABA modulates nociceptive input to the trigeminocervical complex mainly through GABA_A receptors. Storer, *et al.* (2001) Br J Pharmacol. 134(4): 896–904. Therefore GABA_A receptors may provide a target for the development of new therapeutic agents for both acute and prophylactic treatment of headaches including migraines.

Nociceptive pain

[0064] Nociceptive pain occurs in response to the activation of a specific subset of peripheral sensory neurons, the nociceptors. Nociceptors are the nerves that sense and respond to parts of the body that suffer from damage. They signal tissue irritation, impending injury, or actual injury. It is generally acute (with the exception of arthritic pain), self-limiting and serves a protective biological function by acting as a warning of on-going tissue damage. When activated, they transmit pain signals (via the peripheral nerves as well as the spinal cord) to the brain. The pain is typically well localized, constant, and often with an aching or throbbing quality. Examples include post-operative pain, sprains, bone fractures, burns, bumps, bruises, inflammation (from an infection or arthritic disorder), obstructions, and myofascial pain. Visceral pain is the subtype of nociceptive pain that involves the internal organs. It tends to be episodic and poorly localized.

[0065] Nociceptive pain is usually treated with opioids and/or non-steroidal anti-inflammatory drugs (NSAIDS) but due to low efficacy, unacceptable, even severe side effects, and addiction potential, their use can be limited. GABA_A receptors are a target for therapeutics to treat nociceptive pain. For example, Hara, *et al.* (2004) Anesth Analg 98:1380–1384 reports that the combination of GABA agonists and L-type calcium channel blockers may be used to reduce visceral pain. However, most GABA_A agonists are known to have side effects, including sedation, dizziness, euphoria, nausea, and blurred vision. Therefore a great need exists for nociceptive pain therapeutics.

Neuropathic pain

[0066] Neuropathic pain and nociceptive pain differ in their etiology, pathophysiology, diagnosis, and treatment. Neuropathic pain is a common type of chronic, non-malignant pain, which is the result of an injury or malfunction in the peripheral or central nervous system and serves no

protective biological function. It is estimated to affect more than 1.6 million people in the U.S. population. Neuropathic pain has many different etiologies, and may occur, for example, due to trauma, diabetes, infection with herpes zoster (shingles), HIV/AIDS (peripheral neuropathies), late-stage cancer, amputation (including mastectomy), carpal tunnel syndrome, chronic alcohol use, exposure to radiation, and as an unintended side-effect of neurotoxic treatment agents, such as certain anti-HIV and chemotherapeutic drugs.

[0067] In contrast to nociceptive pain, neuropathic pain is frequently described as “burning,” “electric,” “tingling,” or “shooting” in nature. It is often characterized by chronic allodynia (pain resulting from a stimulus that does not ordinarily elicit a painful response, such as light touch) and hyperalgesia (increased sensitivity to a normally painful stimulus), and may persist for months or years beyond the apparent healing of any damaged tissues.

[0068] Neuropathic pain is difficult to treat. Analgesic drugs that are effective against nociceptive pain (*e.g.*, opioid narcotics and non-steroidal anti-inflammatory drugs) are rarely effective against neuropathic pain. Similarly, drugs that have activity in neuropathic pain are not usually effective against nociceptive pain. The standard drugs that have been used to treat neuropathic pain appear to often act selectively to relieve certain symptoms but not others in a given patient (*e.g.*, relief of allodynia, but not hyperalgesia). Bennett (1998) Hosp. Pract. (Off Ed). 33: 95–98. Treatment agents typically employed in the management of neuropathic pain include tricyclic antidepressants (*e.g.*, amitriptyline, imipramine, desimipramine, and clomipramine), systemic local anesthetics, and anti-epileptic drugs (AED) (*e.g.*, phenytoin, carbamazepine, valproic acid, clonazepam, gabapentin, and pregabalin (LYRICA®)). See Lowther (2005) “Pharmacotherapy Update from the Department of Pharmacy” Vol. VIII, No. 5. Common side effects include over-sedation, dizziness, loss of memory and liver damage. Further, although traditionally not considered useful for the treatment of neuropathic pain, recent studies from genetically modified mice indicate that agents targeting only a subset of benzodiazepine (GABA_A) receptors may provide pronounced antihyperalgesic activity against inflammatory and neuropathic pain. Zeilhofer, *et al.* (2009) J Mol Med 87: 465–469. Furthermore, in a spinal cord injury (SCI) model of neuropathic pain, bumetanide, a NKCC1 antagonist, showed an analgesic effect suggesting that normal or elevated NKCC1 activity plays a role in the development and maintenance of SCI-induced neuropathic pain. Cramer, *et al.* (2008) Molecular Pain 4: 36. Therefore a great need exists for improved neuropathic pain therapeutics.

Postherpetic Neuralgia

[0069] Postherpetic neuralgia is a complication of shingles, a second outbreak of the varicella-zoster virus, which initially causes chickenpox. Postherpetic neuralgia results when nerve fibers are damaged during an outbreak of shingles. During an initial infection of chickenpox, some of the virus remains in the body, lying dormant inside nerve cells. Years later, the virus may reactivate, causing shingles. Once reactivated, the virus travels along nerve fibers causing pain. When the virus reaches the skin, it produces a rash and blisters. A case of shingles (herpes zoster) usually heals within a month. However, these damaged nerves may cause chronic, often excruciating pain that may persist for months — or even years — in the area where shingles first occurred. Some patients continue to feel pain long after the rash and blisters heal — a type of pain called postherpetic neuralgia. A variety of treatments for postherpetic neuralgia exist, although some do not experience complete relief from pain.

[0070] This complication of shingles occurs much more frequently in older adults. About 50 percent of adults older than 60 experience postherpetic neuralgia after shingles, whereas only 10 percent of all people with shingles do. The symptoms of postherpetic neuralgia are generally limited to the area of the skin where the shingles outbreak first occurred including sharp and jabbing, burning, or deep and aching pain; extreme sensitivity to touch and temperature change; itching and numbness; and headaches. In rare cases, patients might also experience muscle weakness or paralysis — if the nerves involved also control muscle movement. A great need exists for improved postherpetic neuralgia therapeutics.

Ocular Diseases (*e.g.*, vision disorders, ophthalmic diseases)

[0071] It is estimated that the lifetime costs for all people with vision impairment who were born in 2000 will total \$2.5 billion (2003 dollars). *See, generally*, Centers for Disease Control and Prevention, Economic Costs Associated with Mental Retardation, Cerebral Palsy, Hearing Loss, & Vision Impairment, United States, 2003, MMWR (2004) 53: 57–9. These costs include both direct and indirect costs. Direct medical costs, such as doctor visits, prescription drugs, and inpatient hospital stays, make up 6% of these costs. Direct nonmedical expenses, such as home modifications and special education, make up 16% of the costs. Indirect costs, which include the value of lost wages when a person dies early, cannot work, or is limited in the amount or type of work he or she can do, make up 77% of the costs. These estimates do not include other expenses, such as hospital outpatient visits, emergency department visits, and family out-of-pocket expenses. The actual

economic costs of vision impairment are, therefore, even higher than what is generally reported. U.S. Patent No. 7,251,528.

[0072] Both NKCC and KCC2 are expressed in the outer and inner plexiform layers and colocalized in many putative amacrine cells and in cells of the ganglion cell layer. However, the somata of putative horizontal cells displayed only NKCC immunoreactivity and many bipolar cells were only immunopositive for KCC2. In the outer retina, application of bumetanide, a specific inhibitor of NKCC activity, (1) increased the steady-state extracellular concentration of K^+ ($[K^+]_o$) and enhanced the light-induced decrease in the $[K^+]_o$, (2) increased the sPIII photoreceptor-dependent component of the ERG, and (3) reduced the extracellular space volume. In contrast, in the outer retina, application of furosemide, a specific inhibitor of KCC activity, decreased sPIII and the light-induced reduction in $[K^+]_o$, but had little effect on steady-state $[K^+]_o$. In the inner retina, bumetanide increased the sustained component of the light-induced increase in $[K^+]_o$. These findings thus indicate that NKCC and KCC2 control the $[K^+]_o$ and extracellular space volume in the retina in addition to regulating GABA- and glycine-mediated synaptic transmission. In addition, the anatomical and electrophysiological results together suggest that all of the major neuronal types in the retina are influenced by chloride cotransporter activity. Dmitriev, *et al.* (2007) Vis Neurosci 24(4): 635–45.

[0073] The bumetanide-sensitive $Na^+K^+2Cl^-$ cotransporter (NKCC) also clearly contributes to the Cl^- uptake into the pigmented epithelium (PE). This work reinforces the general consensus that active secretion of Cl^- is the major driving force of aqueous humor formation in mammalian eye and further substantiates the existence of species differences in the mechanism that accomplishes transepithelial Cl^- transport. Kong, *et al.* (2006) Invest Ophthalmol Vis Sci. 47(12): 5428–36.

[0074] Additionally, cation-chloride cotransporters are involved in retinal function by mediating neural computation in the retina. The directional responses of DS ganglion cells are mediated in part by the directional release of gamma-aminobutyric acid from starburst dendrites and that the asymmetric distribution of two cotransporters (K^+Cl^- cotransporter and $Na^+K^+Cl^-$ cotransporter) along starburst-cell dendrites mediates direction selectivity. Gavrikov, *et al.* (2003) Proc Natl Acad Sci USA 100(26): 16047–52.

[0075] Further, the function of retina depends on cation chloride transporters regulating GABA. In particular, different cation chloride cotransporters in retinal neurons allow for opposite responses to GABA. Thus, in the retina, the opposite effects of GABA on different cell types and on different

cellular regions are probably primarily determined by the differential targeting of these two chloride transporters. *See, e.g.,* Barbour, *et al.* (May 1991) J Physiol. 436: 169–193; Keller, *et al.* (1988) Pflugers Arch. 411(1): 47–52; and Vardi, *et al.* (2000) Journal of Neuroscience 20(20): 7657–63. *See, also,* Basu, *et al.* (1998) Invest Ophthalmol Vis Sci. 39(12): 2365–73; Cia, *et al.* (2005) J Neurophysiol. 93(3): 1468–75; Do, *et al.* (2006) Invest Ophthalmol Vis Sci. 47(6): 2576–82; Hunt, *et al.* (2005) Anat Rec A Discov Mol Cell Evol Biol. 287(1): 1051–66; MacLeish and Nurse (2007) J Neurophysiol. 98(1): 86–95; Mito, *et al.* (1993) Am J Physiol. 264(3 Pt 1): C519–26; Moody (1984) Annu Rev Neurosci 7: 257–78; Mroz and Lechene (1993) Hear Res. 70(2): 146–50; Schnetkamp (1980) Biochem Biophys Acta. 598(1): 66–90; and Uhl and Desel (1989) J Photochem Photobiol B. 3(4): 549–64.

[0076] Accordingly, a number of vision-threatening disorders of the eye presently do not have any effective therapies. One major problem in treatment of such diseases are the inability to deliver therapeutic agents into the eye and maintain them there at therapeutically effective concentrations. Therefore a great need exists for therapeutics to treat ocular diseases.

Parkinson's Disease

[0077] Parkinson's disease (PD) belongs to a group of conditions called motor system disorders, which result from the loss of dopamine-producing brain cells. The four primary symptoms of PD are tremor, or trembling in hands, arms, legs, jaw, and face; rigidity, or stiffness of the limbs and trunk; bradykinesia, or slowness of movement; and postural instability, or impaired balance and coordination. As these symptoms become more pronounced, patients may have difficulty walking, talking, or completing other simple tasks. PD usually affects people over the age of 50. Early symptoms of PD are subtle and occur gradually. Other symptoms may include depression and other emotional changes; difficulty in swallowing, chewing, and speaking; urinary problems or constipation; skin problems; and sleep disruptions. NINDS Parkinson's Disease Information Page (September 23, 2009).

[0078] At present, there is no cure for PD, but a variety of medications provide dramatic relief from the symptoms. Usually, patients are given levodopa combined with carbidopa. Carbidopa delays the conversion of levodopa into dopamine until it reaches the brain. Nerve cells can use levodopa to make dopamine and replenish the brain's dwindling supply. Although levodopa helps at least three-quarters of Parkinsonian cases, not all symptoms respond equally to the drug. Bradykinesia and rigidity respond best, while tremor may be only marginally reduced. Problems

with balance and other symptoms may not be alleviated at all. Anticholinergics may help control tremor and rigidity. Other drugs, such as bromocriptine, pramipexole, and ropinirole, mimic the role of dopamine in the brain, causing the neurons to react as they would to dopamine. An antiviral drug, amantadine, also appears to reduce symptoms. In May 2006, the FDA approved rasagiline (AZILECT®) to be used along with levodopa for patients with advanced PD or as a single-drug treatment for early PD. NINDS Parkinson's Disease Information Page (2009).

[0079] Parkinson's disease (PD) pathology also disrupts GABA signaling by destroying the input from the substantia nigra into striatal GABAergic neurons. Targeting GABA synthesis, degradation, transport, or receptors with new therapeutics may control GABA signaling, and therefore may be used for improved therapeutic treatments for Parkinson's disease. Kleppner and Tobin (2001) Expert Opin. Ther. Targets. 5(2):219–39.

Periodic Leg Movement Disorder

[0080] Periodic limb movement disorder (PLMD) (previously known as nocturnal myoclonus) is a sleep disorder where a patient limbs move involuntarily during sleep, and suffers problems related to the movement. PLMD differs from restless leg syndrome (RLS) in that RLS occurs while the patient is awake as well as when asleep, and when awake, there is a voluntary response to an uncomfortable feeling in the legs. In contrast, in PLMD the patient may often be unaware of the movements. Cleveland Clinic "Periodic Limb Movement Disorder" (2011).

[0081] Currently, several drugs have been used to treat PLMD including Sinemet (levodopa), anticonvulsant medications, benzodiazepines, and narcotics. Although medical treatment of PLMD often significantly reduces or eliminates the symptoms of these disorders, there is no cure for PLMD and medical treatment must be continued to provide relief. Cleveland Clinic "Periodic Limb Movement Disorder" (2011). A recent study found that valproate has a long-term beneficial effect on sleep consolidation in patients with PLMD. The principal mechanism of action of valproate is believed to be the inhibition of the transamination of GABA (*e.g.*, inhibiting GABA transaminase, leads to an increase in GABA). Ehrenberg, *et al.* (2000) Journal of Clinical Psychopharmacology 20(5): 574–578.

Restless Leg Syndrome (Restless Leg Disorder)

[0082] Restless leg syndrome (restless leg disorder) is a disorder where patients suffer from an urge or need to move the legs to stop unpleasant sensations occurring most often in middle-aged and older adults. The cause is not known in most patients but may occur more often in patients with

peripheral neuropathy, chronic kidney disease, Parkinson's disease, pregnancy, iron deficiency, or as a side-effect of some medications. Restless leg syndrome may result in a decreased quality of sleep (insomnia). Many patients may also have rhythmic leg movements during sleep hours, called periodic limb movement disorder (PLMD). PubMed Health Website "Restless Leg Syndrome" (2011).

[0083] There is no known cure for restless leg syndrome with current treatment aimed at reducing stress and muscle relaxation. Pramipexole or ropinirole (), Sinemet, or tranquilizers (*e.g.*, clonazepam) have been used to relieve the symptoms of restless leg syndrome. PubMed Health Website "Restless Leg Syndrome" (2011). For example, gabapentin (FANATREX), a GABA analogue, has been tested as a treatment for restless leg syndrome with some promising results. Imamura & Kushida (2010) Expert Opin Pharmacother. 11(11): 1925–32; *See also* Misra, *et al.* (2011) Neurology 76(4): 408. Thus, GABA based therapeutics may be useful in the treatment of restless leg syndrome (restless leg disorder).

Schizophrenia

[0084] Schizophrenia is a chronic, severe, and disabling brain disorder that affects about 1.1 percent of the U.S. population age 18 and older in a given year. People with schizophrenia sometimes hear voices others do not hear, believe that others are broadcasting their thoughts to the world, or become convinced that others are plotting to harm them. These experiences can make them fearful and withdrawn and cause difficulties when they try to have relationships with others. National Institute of Mental Health "Schizophrenia" website (2008).

[0085] Symptoms usually develop in men in their late teens or early twenties and women in the twenties and thirties, but in rare cases, can appear in childhood. They can include hallucinations, delusions, disordered thinking, movement disorders, flat affect, social withdrawal, and cognitive deficits. No cause of schizophrenia has been determined nor is there any curative therapy; however, antipsychotics are used in the treatment of symptoms. National Institute of Mental Health "Schizophrenia" website (2008).

[0086] Further, schizophrenia is associated with both decreased numbers and abnormalities in the distribution of GABAergic neurons in the cortex, particularly in the cortical laminae. Kaplan & Sadock's Comprehensive Textbook of Psychiatry (7th Ed) (2008). In the postmortem studies of schizophrenics, antipsychotic naive schizophrenics, and non schizophrenic controls, show a significant decrease in the number of GABA containing inter neurons, and a lessened amount of

GABA production within these inter neurons in both of the schizophrenic groups. Nestler (1997) Nature 385(6617): 578–9. Therefore therapeutic agents that target the GABA system may be useful in treating schizophrenia.

Tinnitus

[0087] Tinnitus is the perception of sound within the human ear in the absence of corresponding external sound. Tinnitus is not a disease but a symptom resulting from a range of underlying causes that can include ear infections, foreign objects or wax in the ear, nose allergies that prevent (or induce) fluid drain and cause wax build-up, and injury from loud noises. Tinnitus can also be caused by hearing impairment and as a side-effect of some medications. Some cases of tinnitus are medically unexplained.

[0088] Tinnitus can be perceived in one or both ears or in the head. It is usually described as a ringing noise, but in some patients it takes the form of a high pitched whining, buzzing, hissing, screaming, humming, singing or whistling sound, or as ticking, clicking, roaring, “crickets” or “tree frogs” or “locusts,” tunes, songs, or beeping. It has also been described as a “whooshing” sound, as of wind or waves. Tinnitus can be intermittent or it can be continuous in which case it can be the cause of great distress. In some individuals, the intensity of tinnitus can be changed by shoulder, head, tongue, jaw, or eye movements. To date, no satisfactory therapeutics exists for tinnitus.

[0089] Partial deafferentation produces a loss of tonic inhibition in the auditory system that may lead to inappropriate neuroplastic changes eventually expressed as the pathophysiology of tinnitus. The pathological down-regulation of GABA provides a potential mechanism for this loss of inhibition. For example, in an animal model of tinnitus, vigabatrin, a GABA agonist, completely and reversibly eliminated the psychophysical evidence of tinnitus. Brozoski, *et al.* (2007) J Assoc Res Otolaryngol. 8(1): 105–118. Further, the disruption of the *NKCC1* gene in mice causes hearing loss. Kahle, *et al.* (2004) Proc. Natl. Acad. Sci. USA 102(46): 16783–16788. Therefore, therapeutics targeting GABAergic system and/or NKCC1 may be useful in the treatment of tinnitus.

Withdrawal Syndrome

[0090] Withdrawal syndrome is generally associated with abnormal physical or psychological features that follow the abrupt discontinuation of a drug (*e.g.*, medications, recreational drugs, and/or alcohol) that has the capability of producing physical dependence. (*e.g.*, alcohol withdrawal syndrome, nicotine withdrawal syndrome, opioid withdrawal syndrome, benzodiazepine withdrawal syndrome, methadone withdrawal syndrome, SSRI discontinuation syndrome, hydrocodone

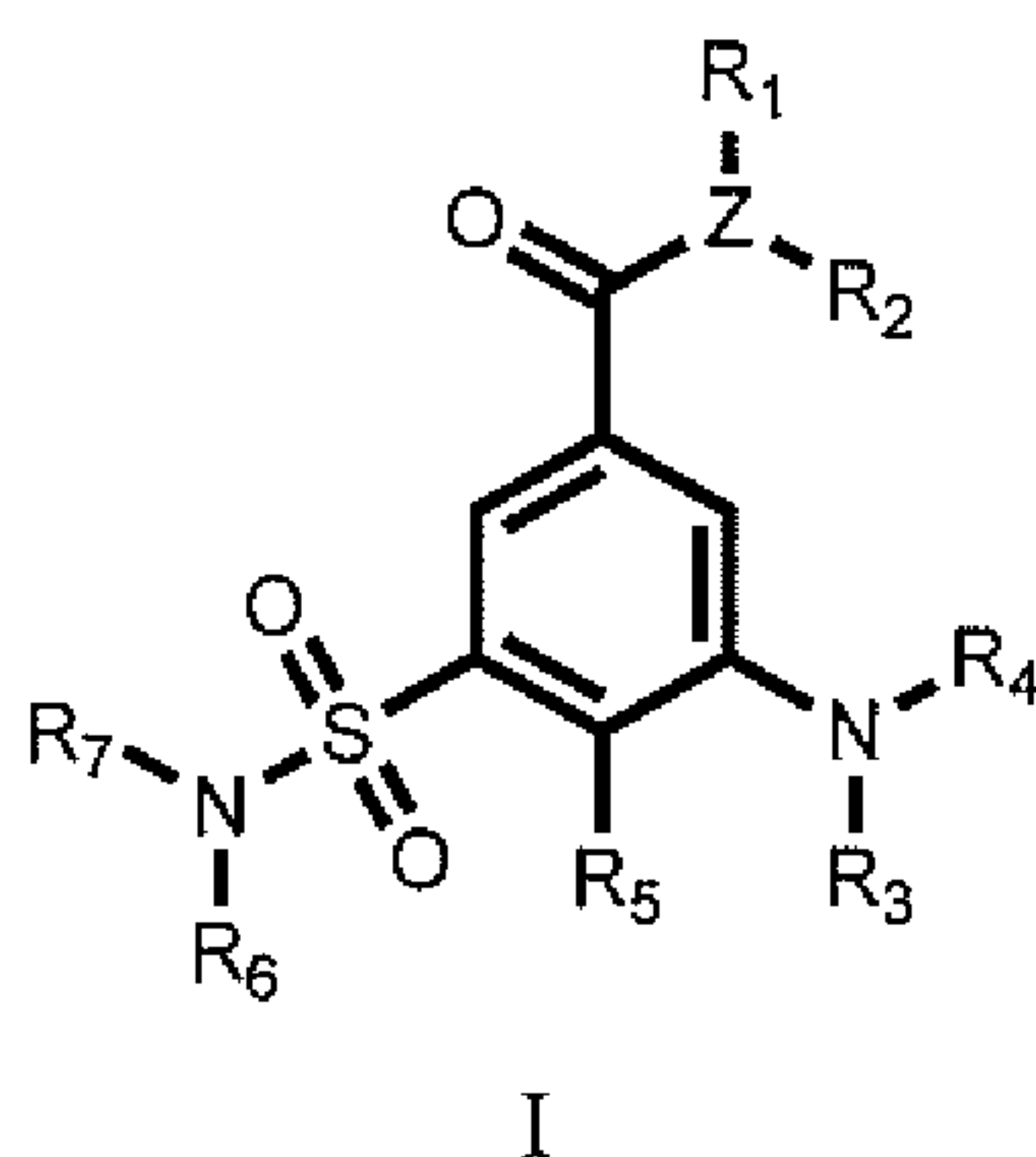
withdrawal syndrome). Common withdrawal symptoms include sweating, tremor, vomiting, anxiety, insomnia, and muscle pain. There are different stages of withdrawal. Generally, a person will start to feel worse and worse, hit a plateau, and then the symptoms begin to dissipate. However, withdrawal from certain drugs (*e.g.*, benzodiazepines, alcohol) can be fatal and therefore the abrupt discontinuation of any type of drug is not recommended. Further, many additions involve compounds which affect the GABAergic system (*e.g.*, alcohol and benzodiazepines.) Therefore, when a person ceases use of the compound, the GABAergic system is involved in the symptoms of withdrawal syndrome. Nutt and Lingford-Hughes (2008) British Journal of Pharmacology 154(2): 397–405. Therefore agents that act on the GABAergic system may provide therapeutics to treat withdrawal syndromes.

[0091] Accordingly, there is a continuing need for compositions and methods for treatment and/or prophylaxis of diseases, disorders, and conditions that involve the $\text{Na}^+\text{K}^+\text{Cl}^-$ co-transporters (*e.g.*, NKCC1 and NKCC2) including but not limited to addictive disorders, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), bipolar disorder, cancer, endothelial corneal dystrophy, edema, depression, epilepsy, glaucoma, ischemia, migraine, neuropathic pain, nociceptive neuralgia, ocular diseases, pain, postherpetic neuralgia, and schizophrenia. Additionally, there is a continuing need for compositions and methods for treatment and/or prophylaxis of diseases, disorders, and conditions that involve the GABA_A receptors including but not limited to Alzheimer's Disease, addictive disorders, anxiety disorders, autism spectrum disorders (autism), bipolar disorder, depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, neuropathic pain, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD) (nocturnal myoclonus), personality disorders, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.

Summary of the Invention

[0092] The present invention provides compounds according to Formulae I, II, III and IV, which are arylsulfonamides, including bumetanide derivatives, as provided herein:

[0093] Formula I:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

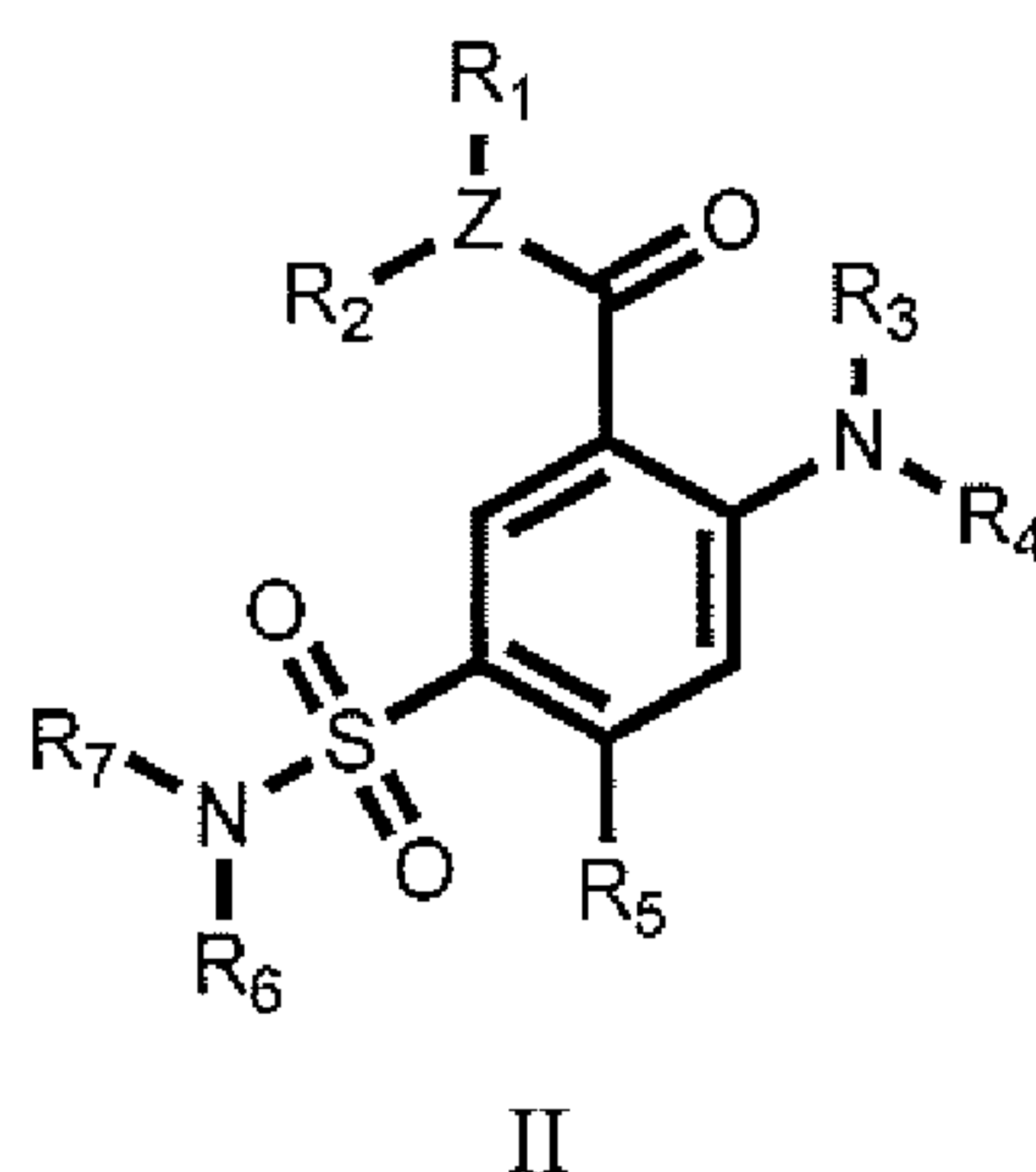
R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio; and

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents.

[0094] Formula II:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

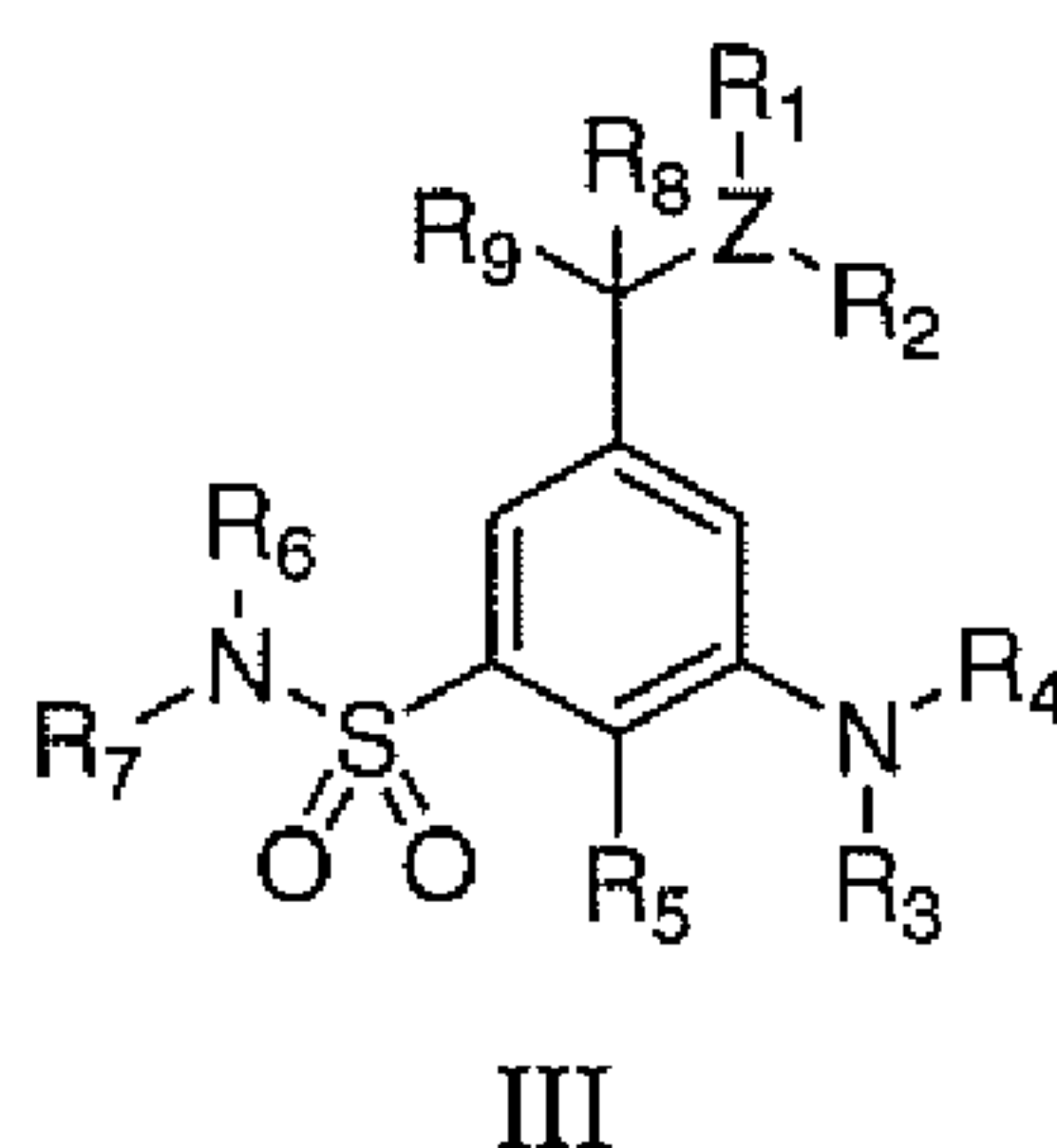
R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is halo, aryl, aryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio; and

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents.

[0095] Formula III:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

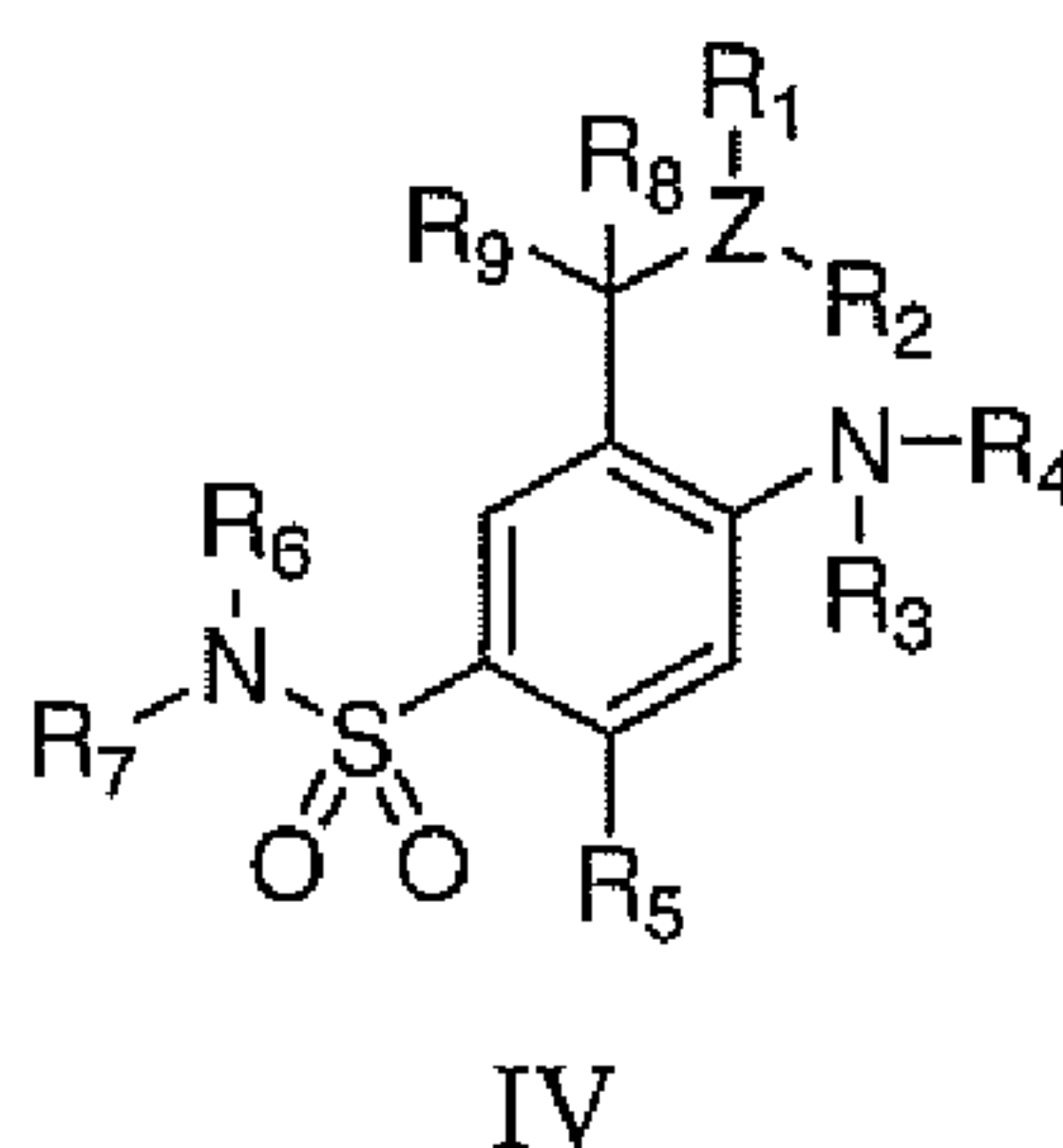
R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio;

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents; and

R₈ and R₉ are each independently hydrogen, alkyl, or R₈ and R₉ together with the atom to which they are attached, form a 3–6 membered substituted or unsubstituted cycloalkyl or heterocycloalkyl ring.

[0096] Formula IV:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

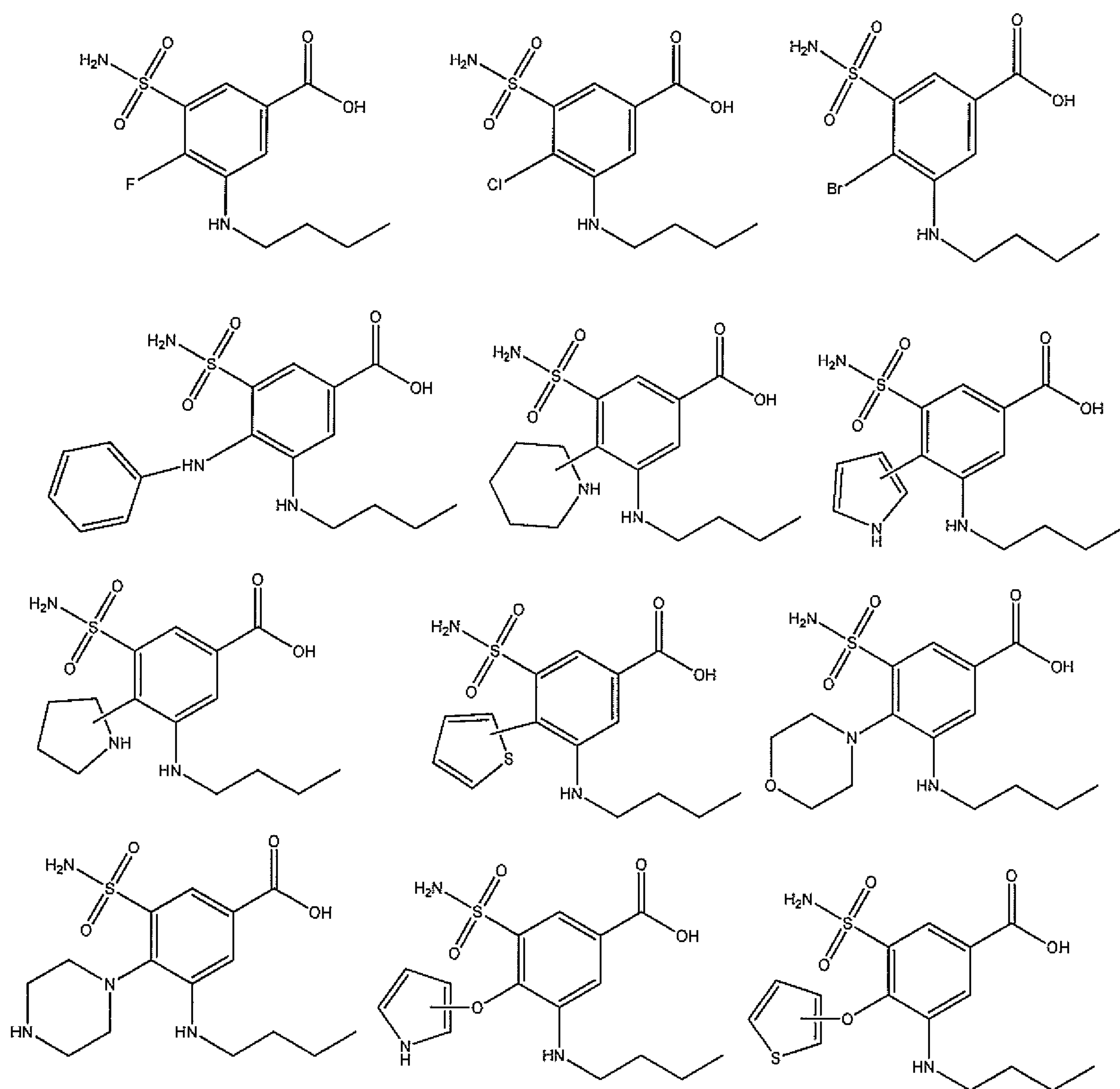
R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio;

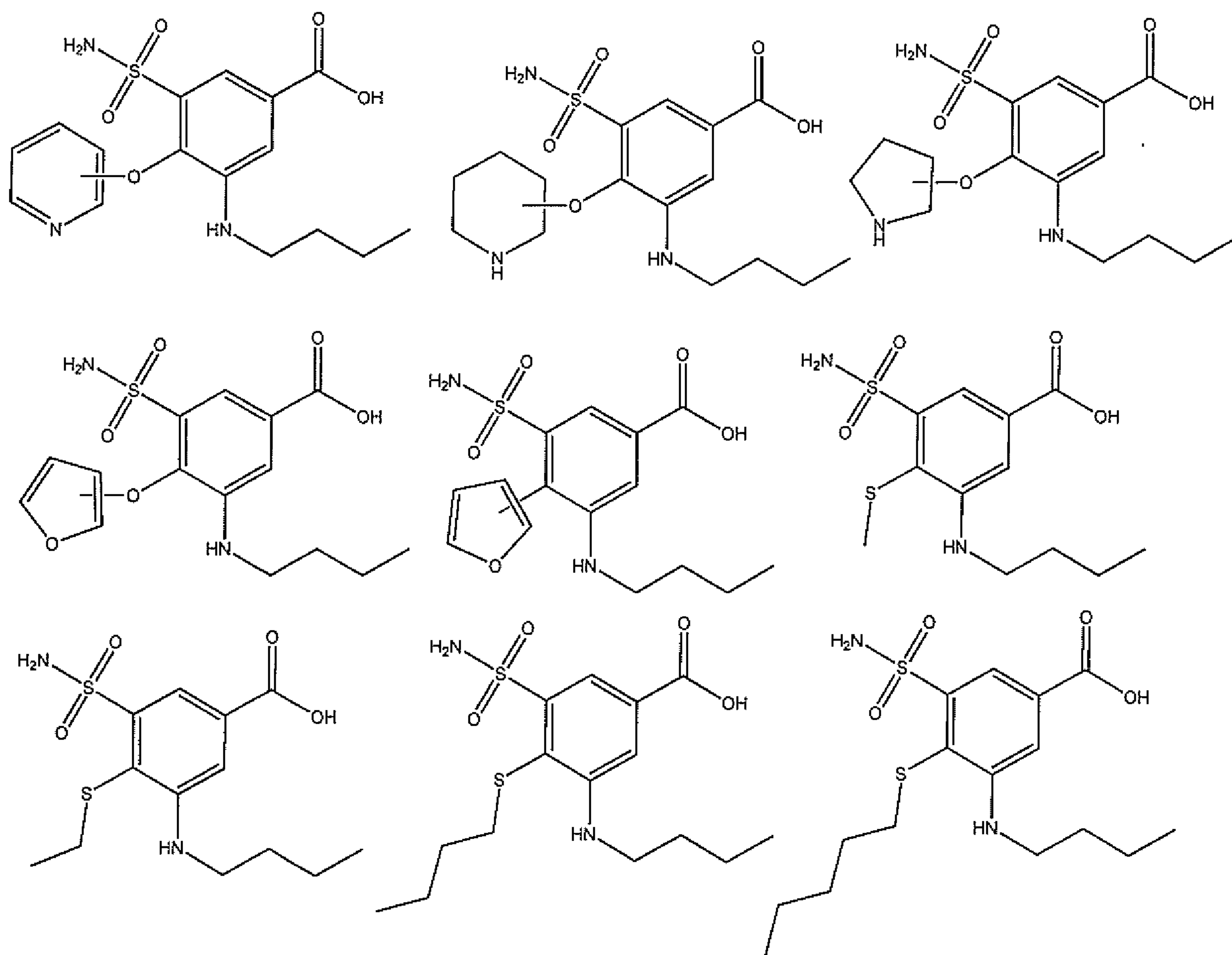
R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents; and

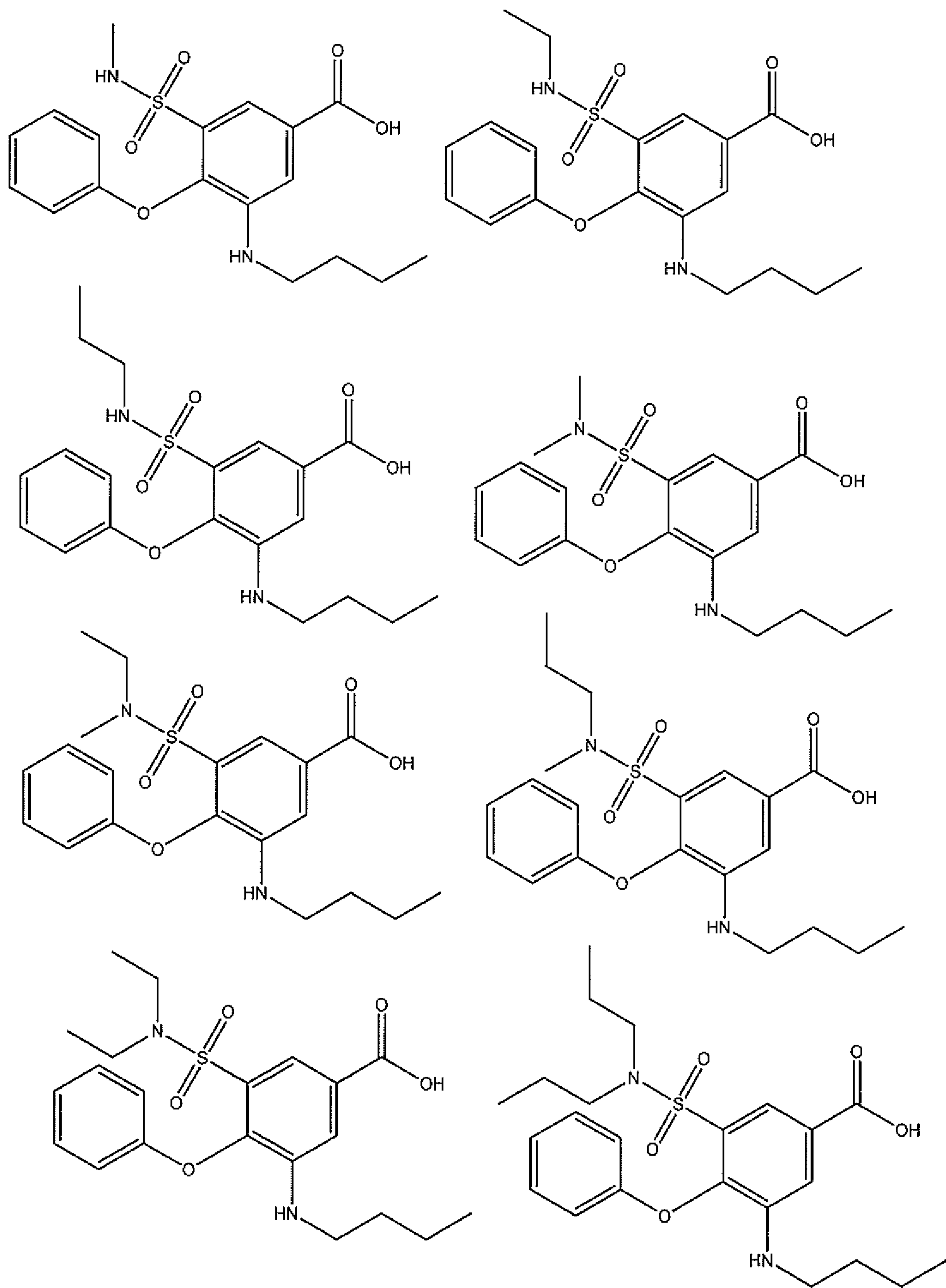
R₈ and R₉ are each independently hydrogen, alkyl, or R₈ and R₉ together with the atom to which they are attached, form a 3–6 membered substituted or unsubstituted cycloalkyl or heterocycloalkyl ring.

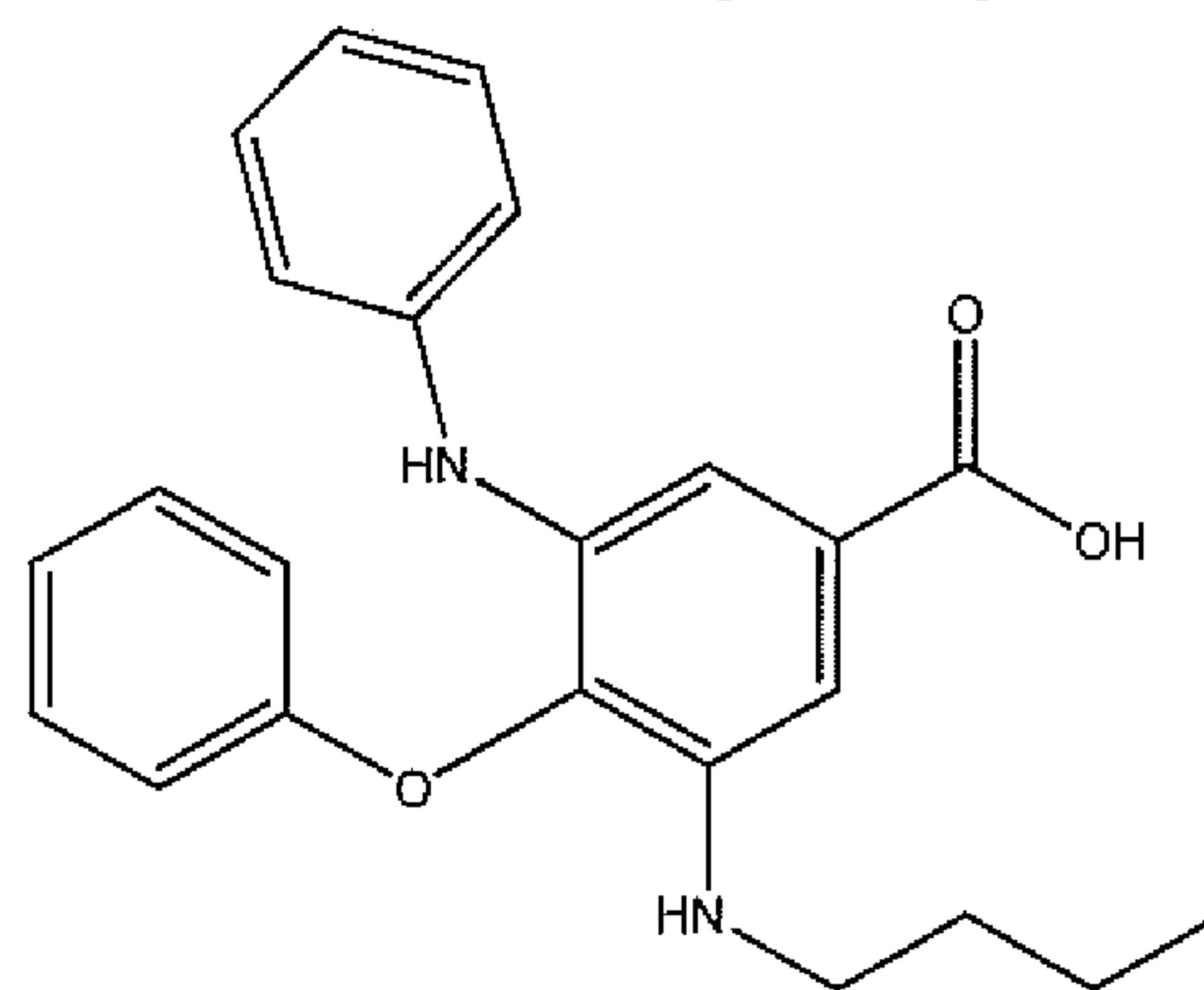
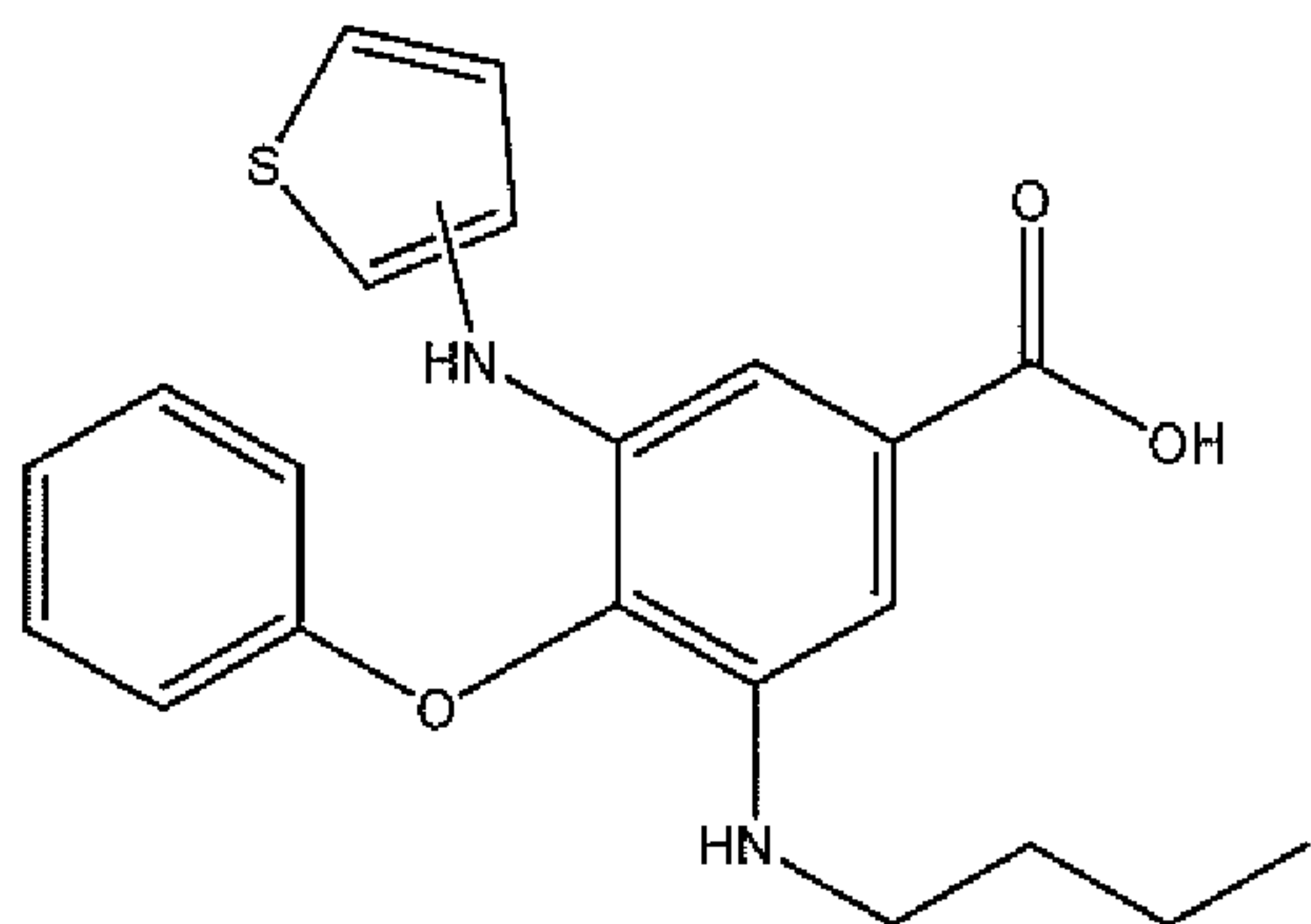
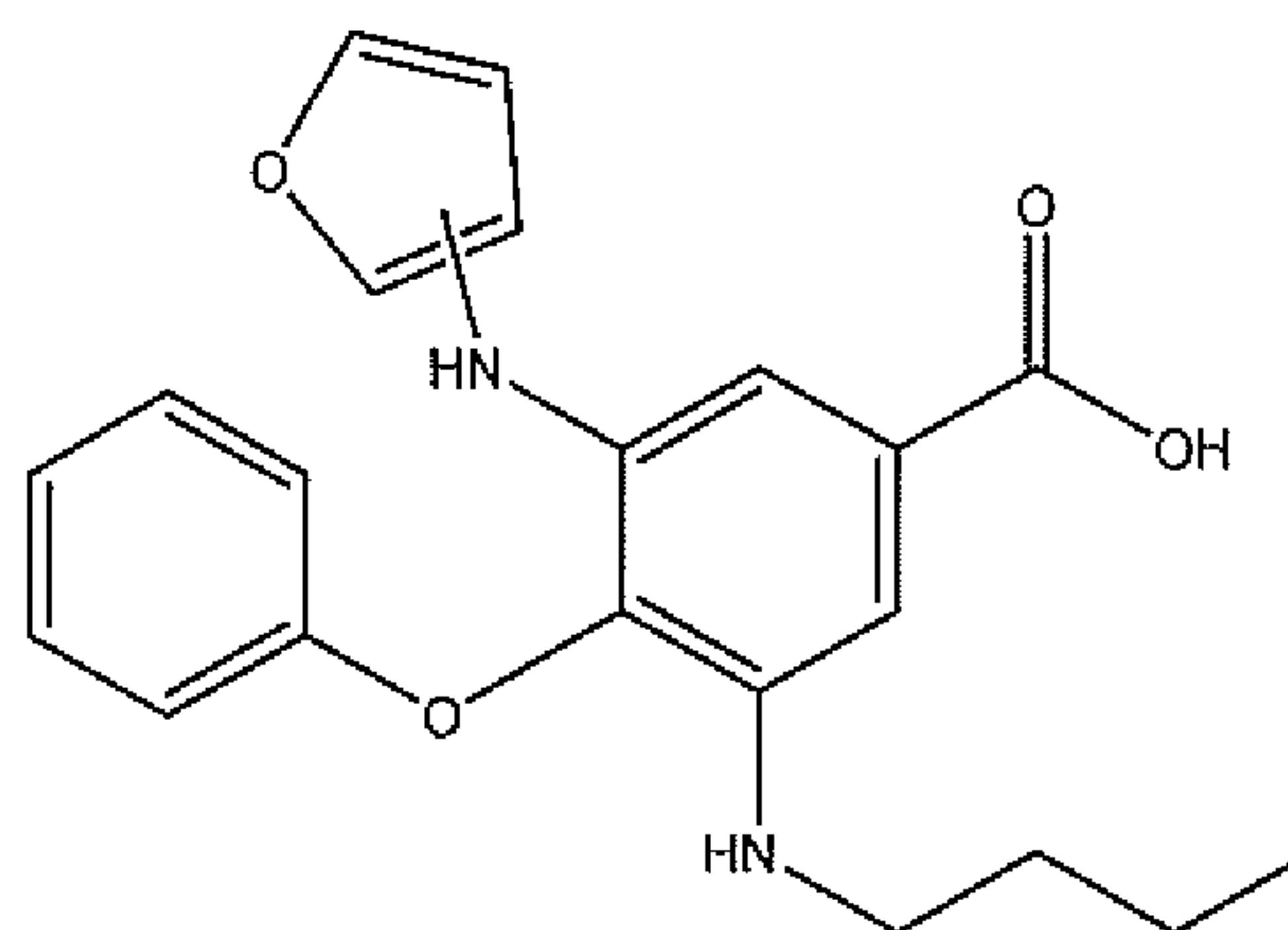
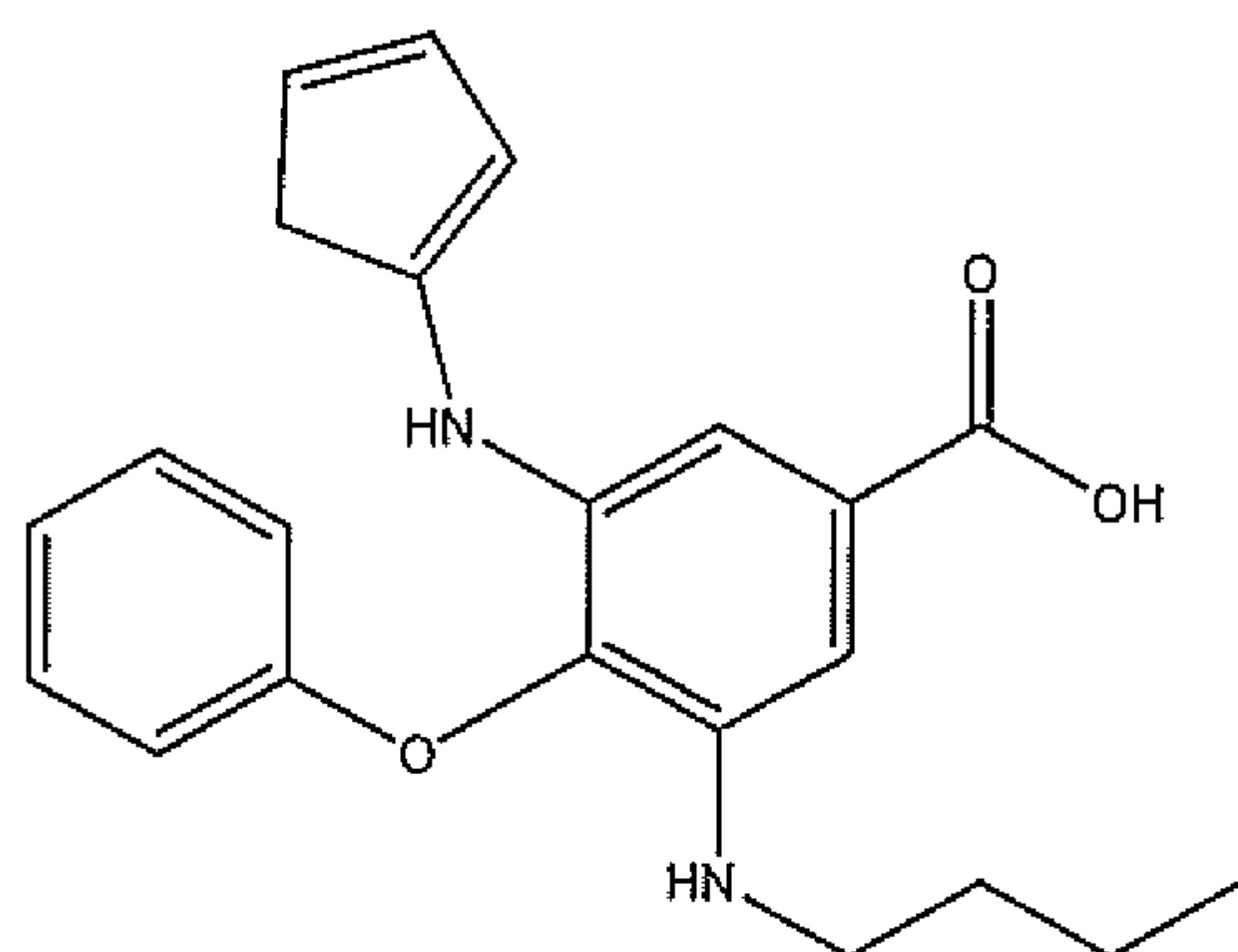
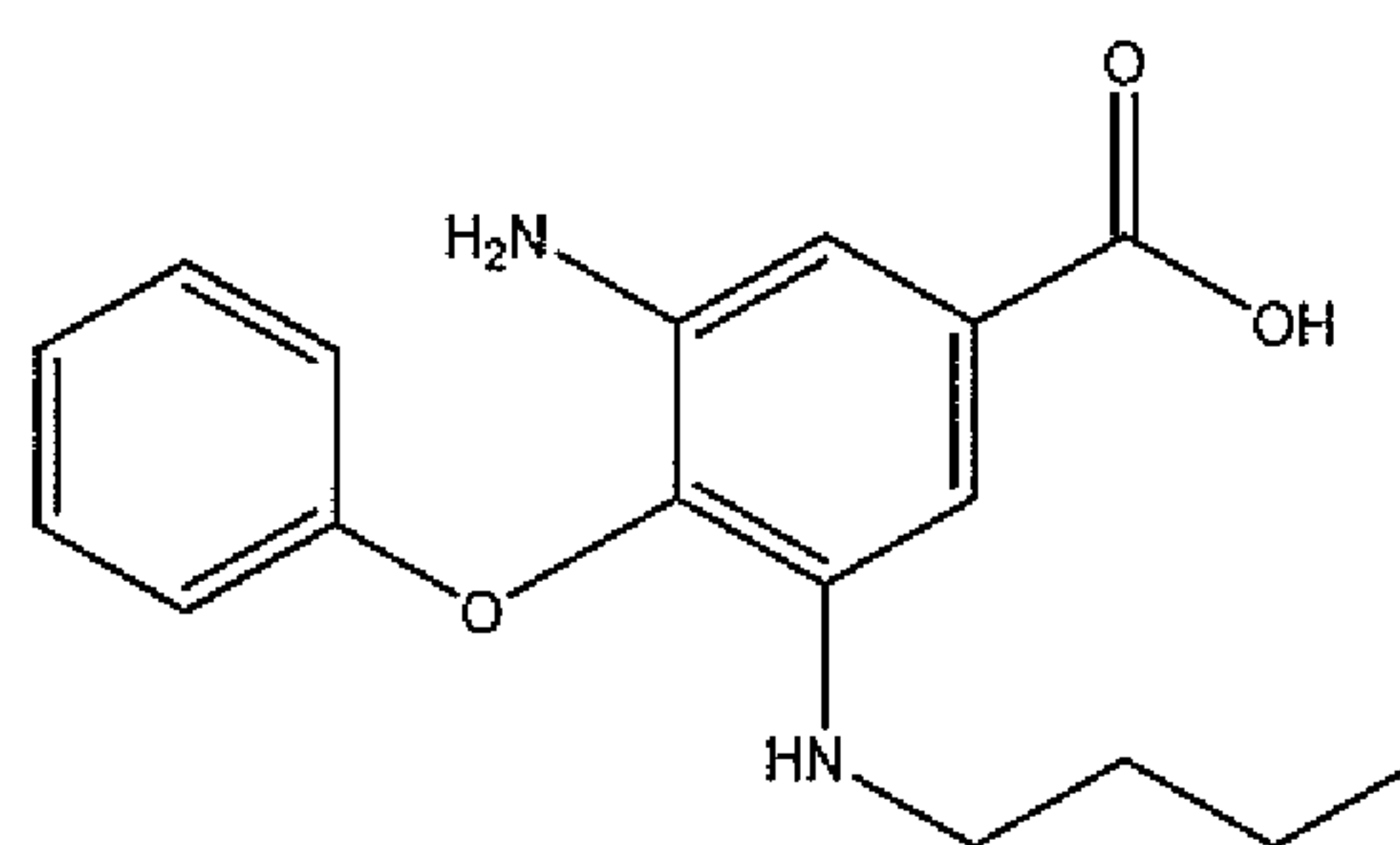
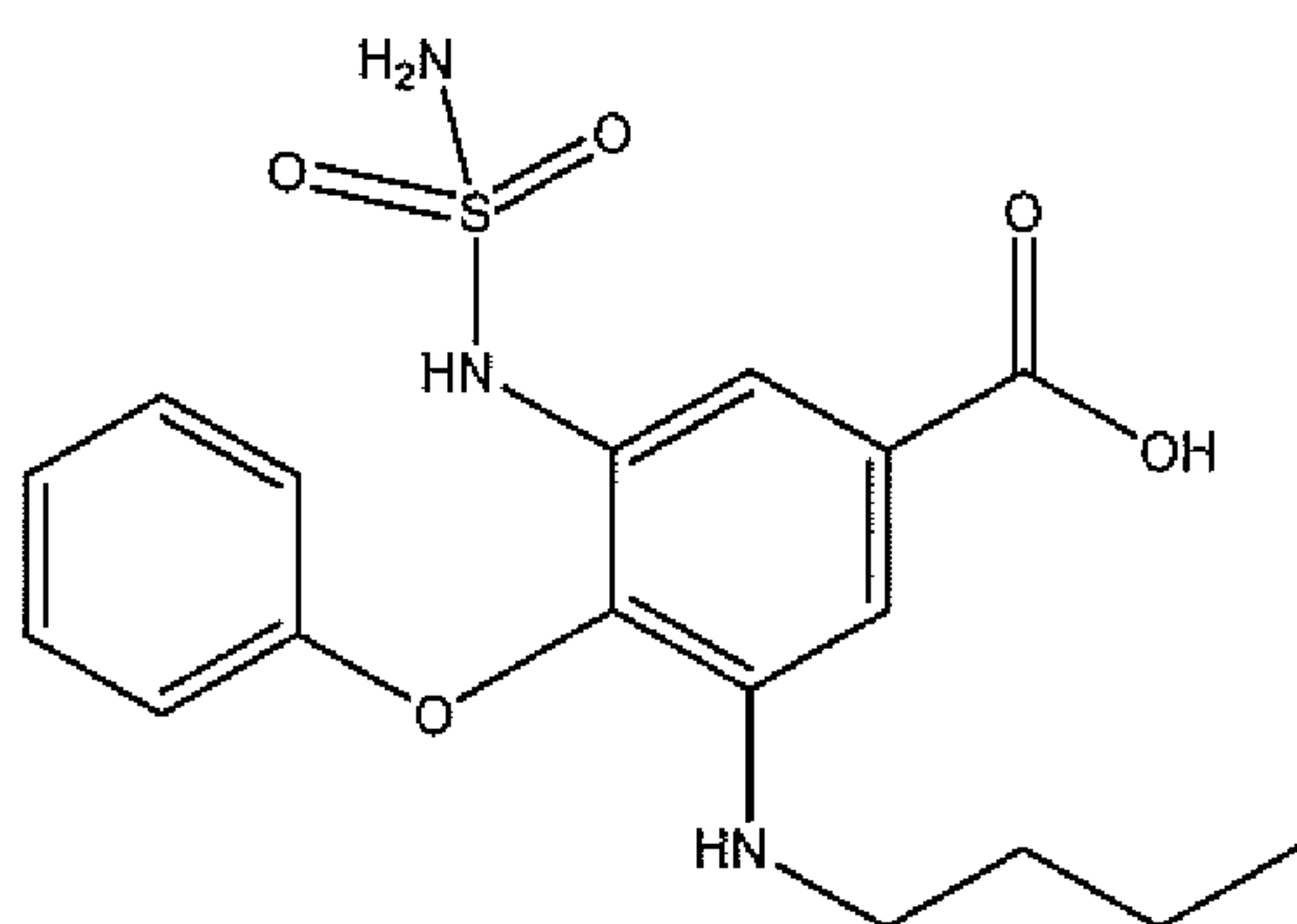
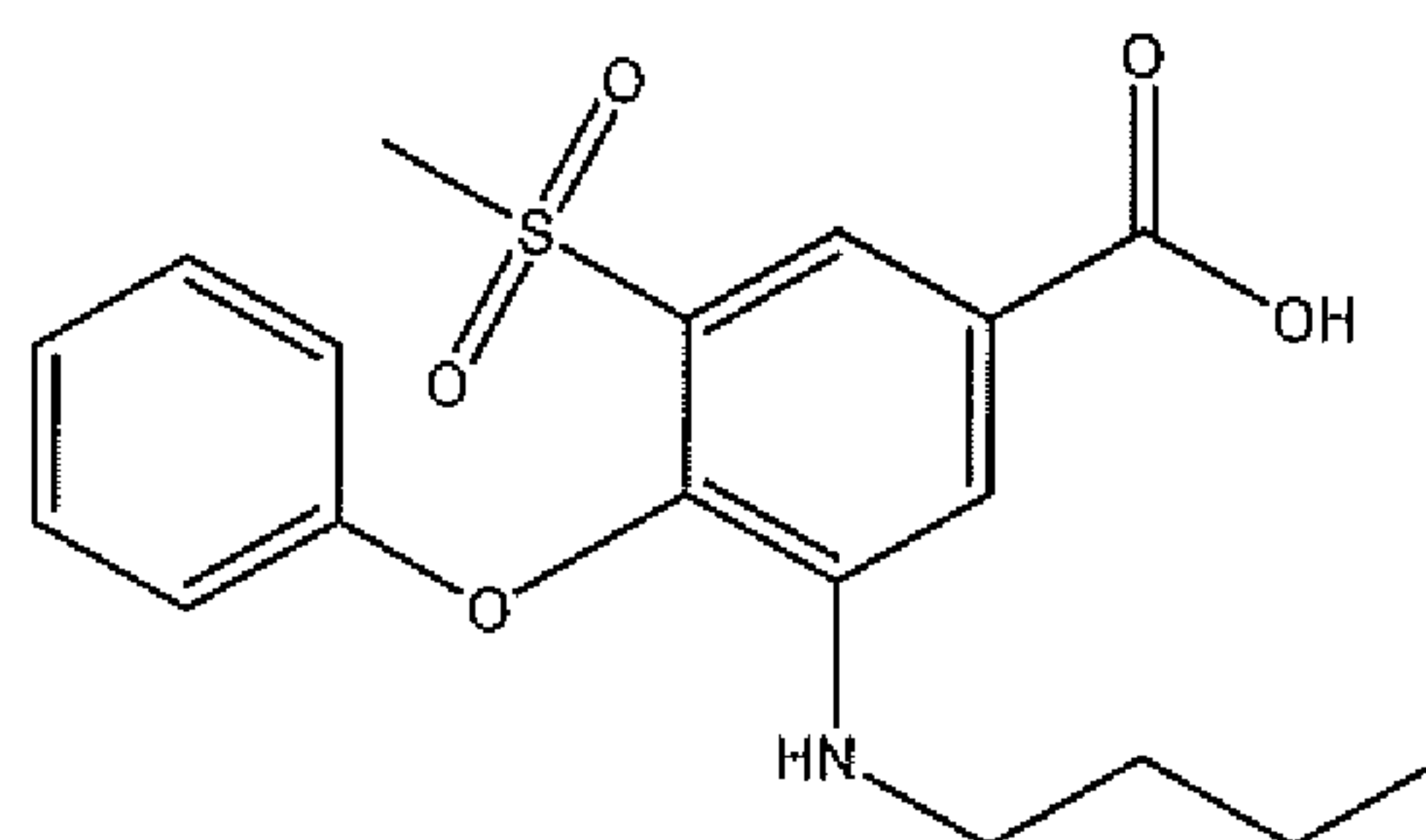
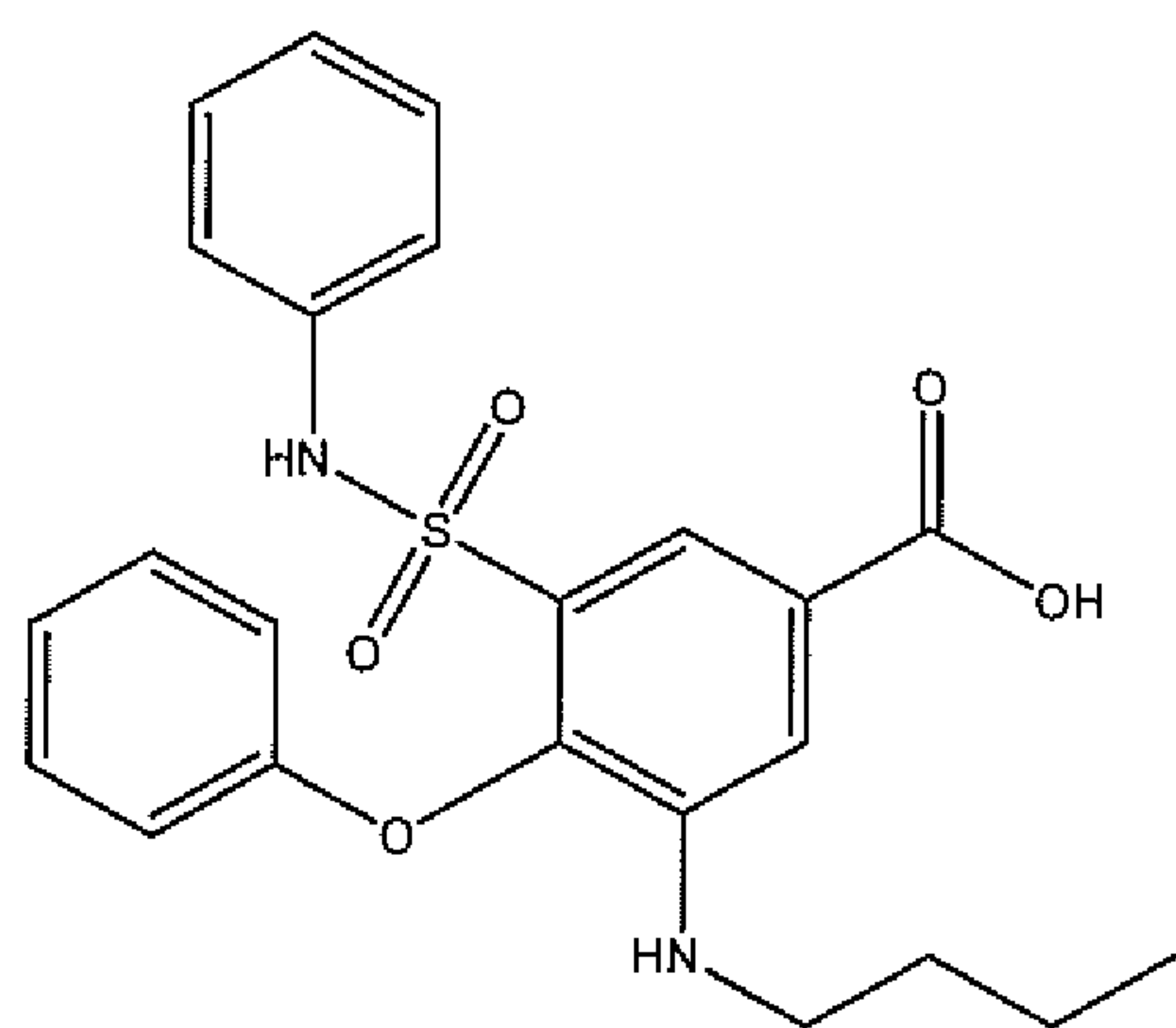
[0097] Embodiments of the present invention provide a pharmaceutical composition comprising a compound of Formulae I-IV, a pharmaceutically acceptable salt, solvate, tautomer, hydrate, or combination thereof and a pharmaceutically acceptable carrier, excipient, or diluent. Embodiments of the present invention provide methods of making the compounds including compounds described herein and further provide intermediate compounds formed through the synthetic methods described herein to provide the compounds of Formulae I-IV.

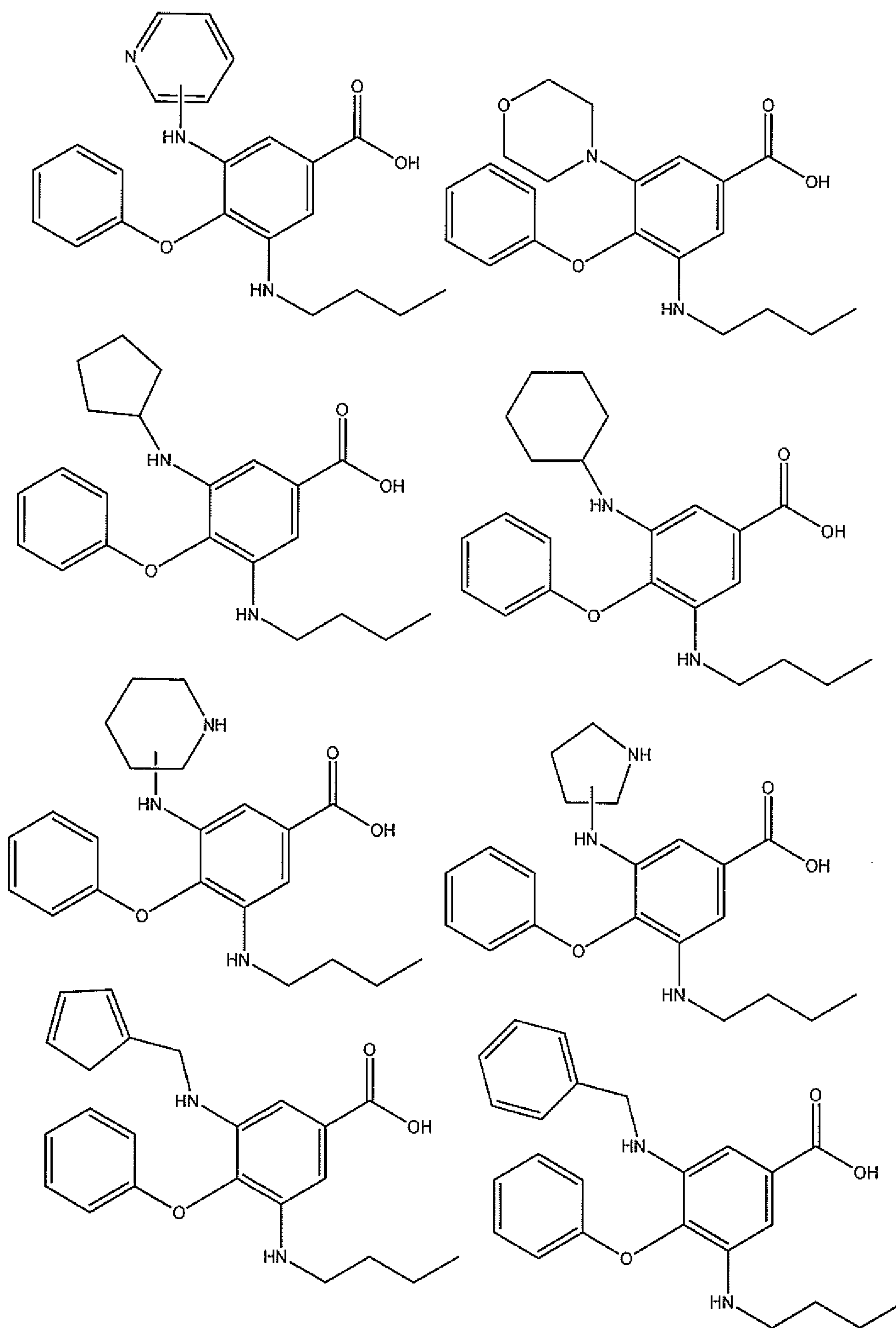
[0098] In some embodiments, the arylsulfonamides described herein exclude furosemide, bumetanide, and piretanide. In other embodiments, the arylsulfonamides described herein exclude one or more compounds disclosed in Examples 1–43 of U.S. Patent Application Publication No. 2007/0149526. In other embodiments, the arylsulfonamides described herein exclude one or more compounds disclosed in Examples 100–136 of WO 2010/085352. In yet other embodiments, the arylsulfonamides described herein exclude one or more compounds disclosed in European Journal of Medicinal Chemistry (1976) 11(5): 399–406; GB 2207129; Liebigs Annalen der Chemie (1979) (4): 461–9; American Journal of Physiology (1993) 265(5, Pt. 1): G942–G954; Journal of Medicinal Chemistry (1971) 14(5): 432–9; U.S. Patent No. 4,247,550; U.S. Patent No. 3,985,777; WO 2008/052190; U.S. Patent No. 7,282,519; International Journal of Pharmaceutics 60: 163–169 (1990); U.S. Patent No. 5,073,641; Revista Portuguesa de Farmacia 44: 164–169 (1994); Pharmacology 26: 172–80 (1983); U.S. Patent Application Publication No. 2007/0155729; European Journal of Pharmacology 344: 269–277 (1998); or JP 49/081334. In still other embodiments, the arylsulfonamides described herein exclude one or more compounds of the formulae:

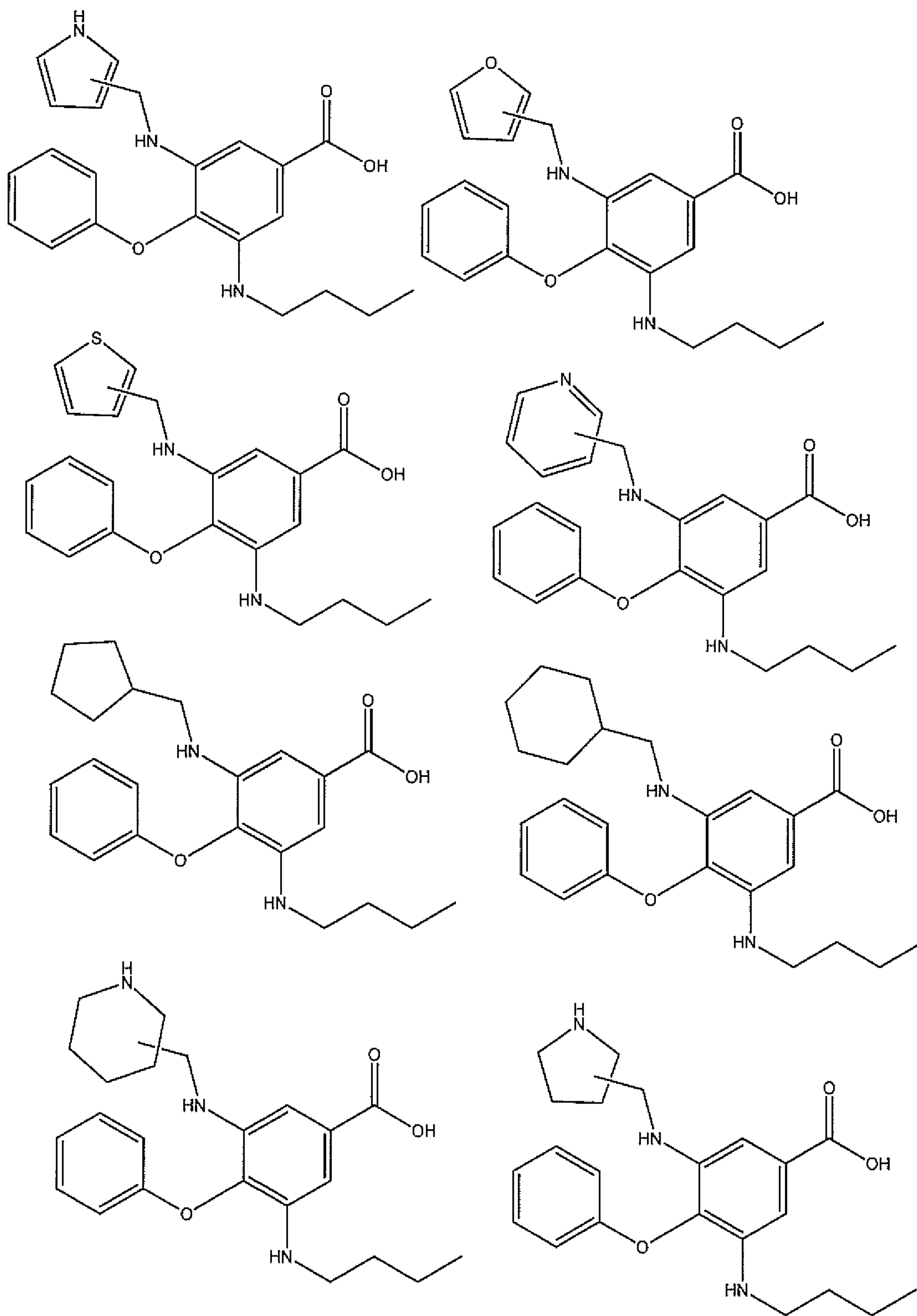


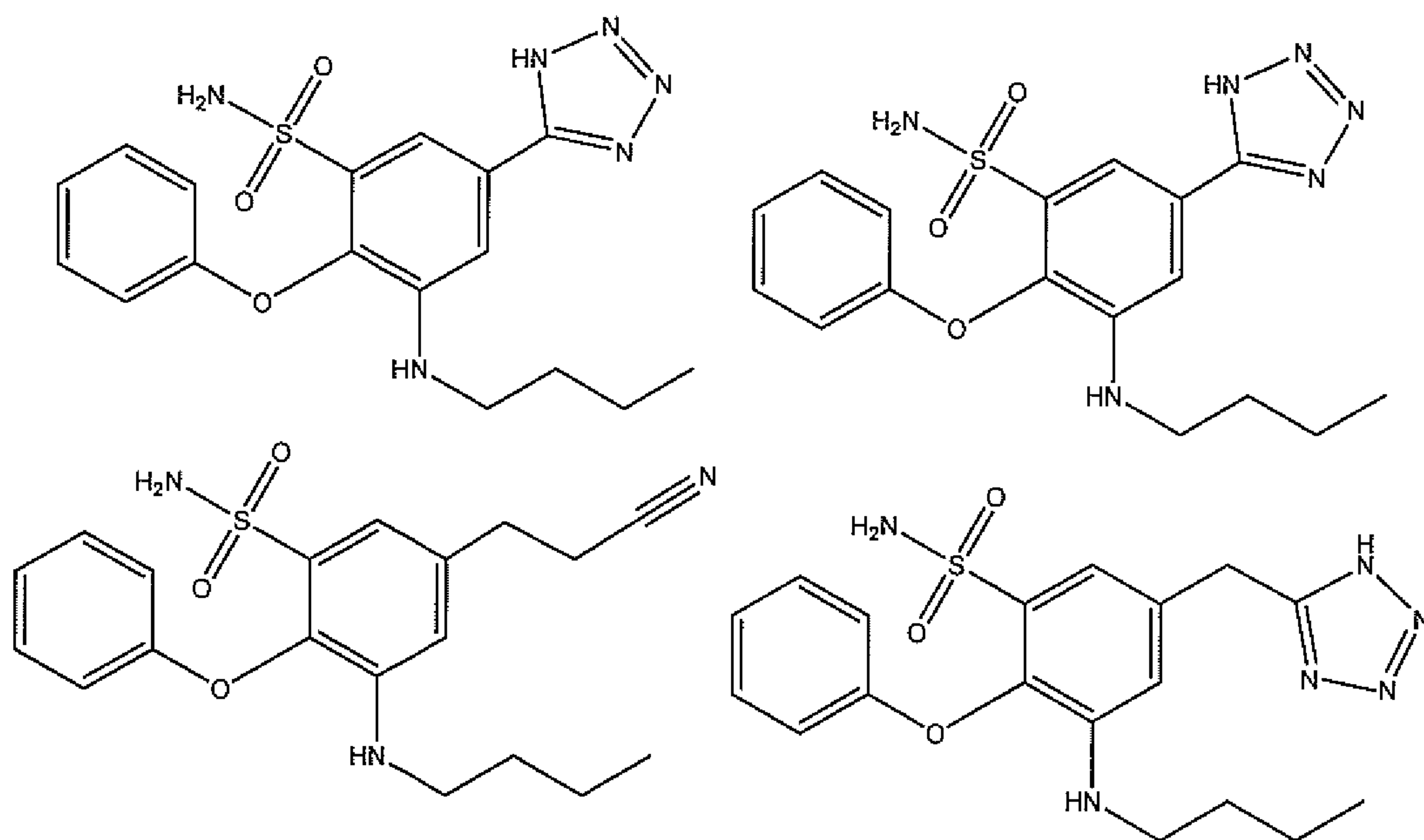


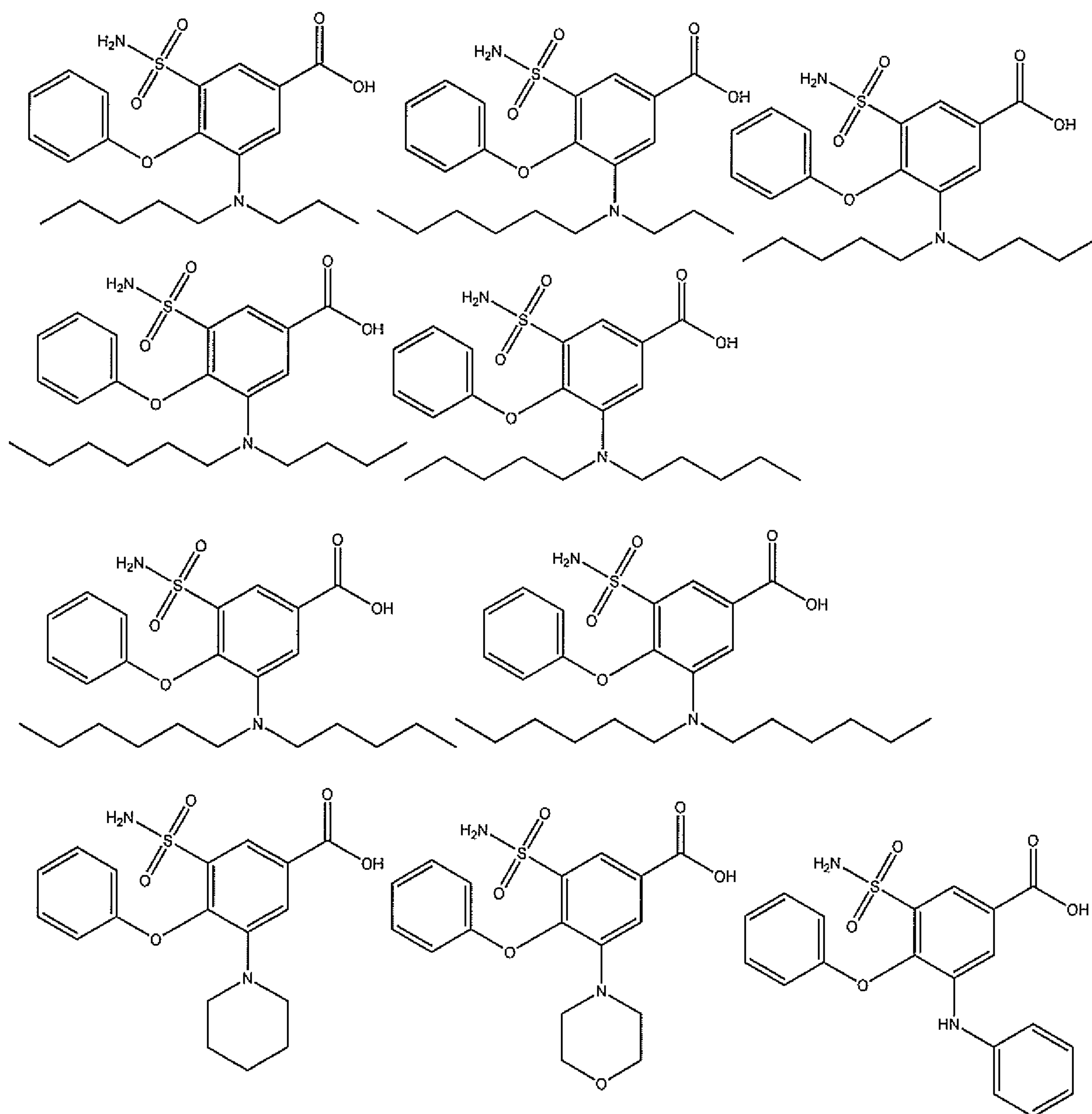


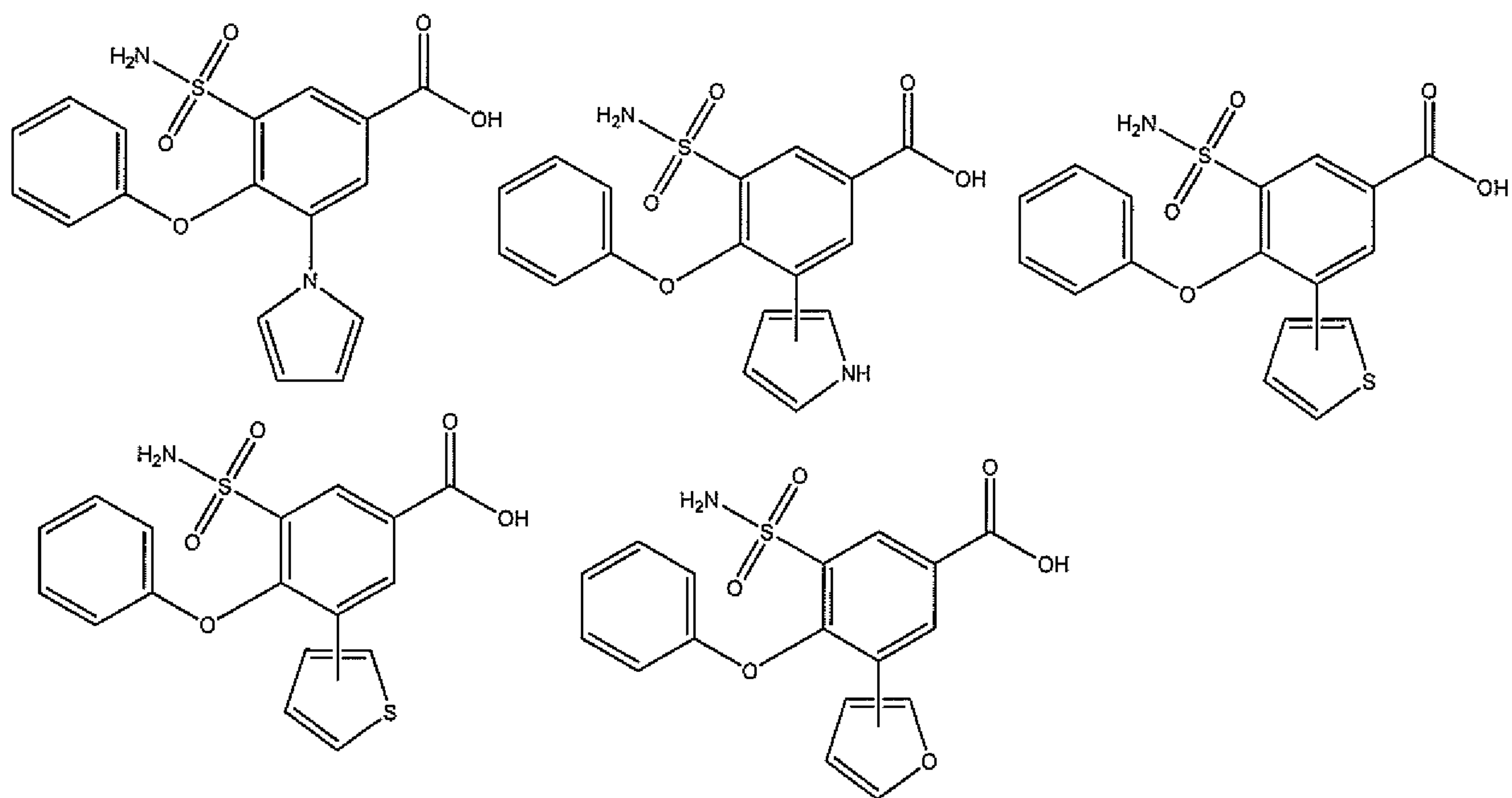


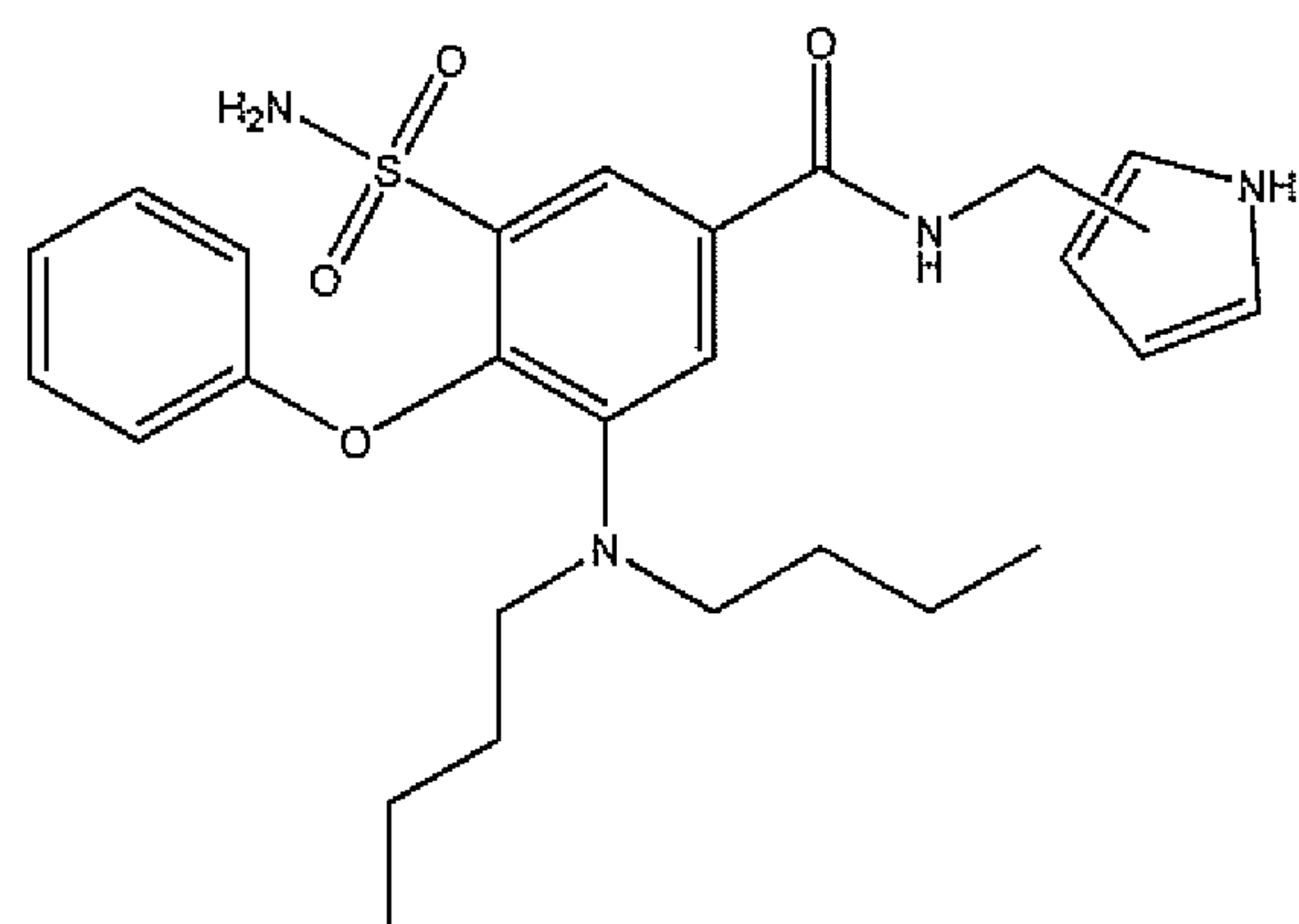


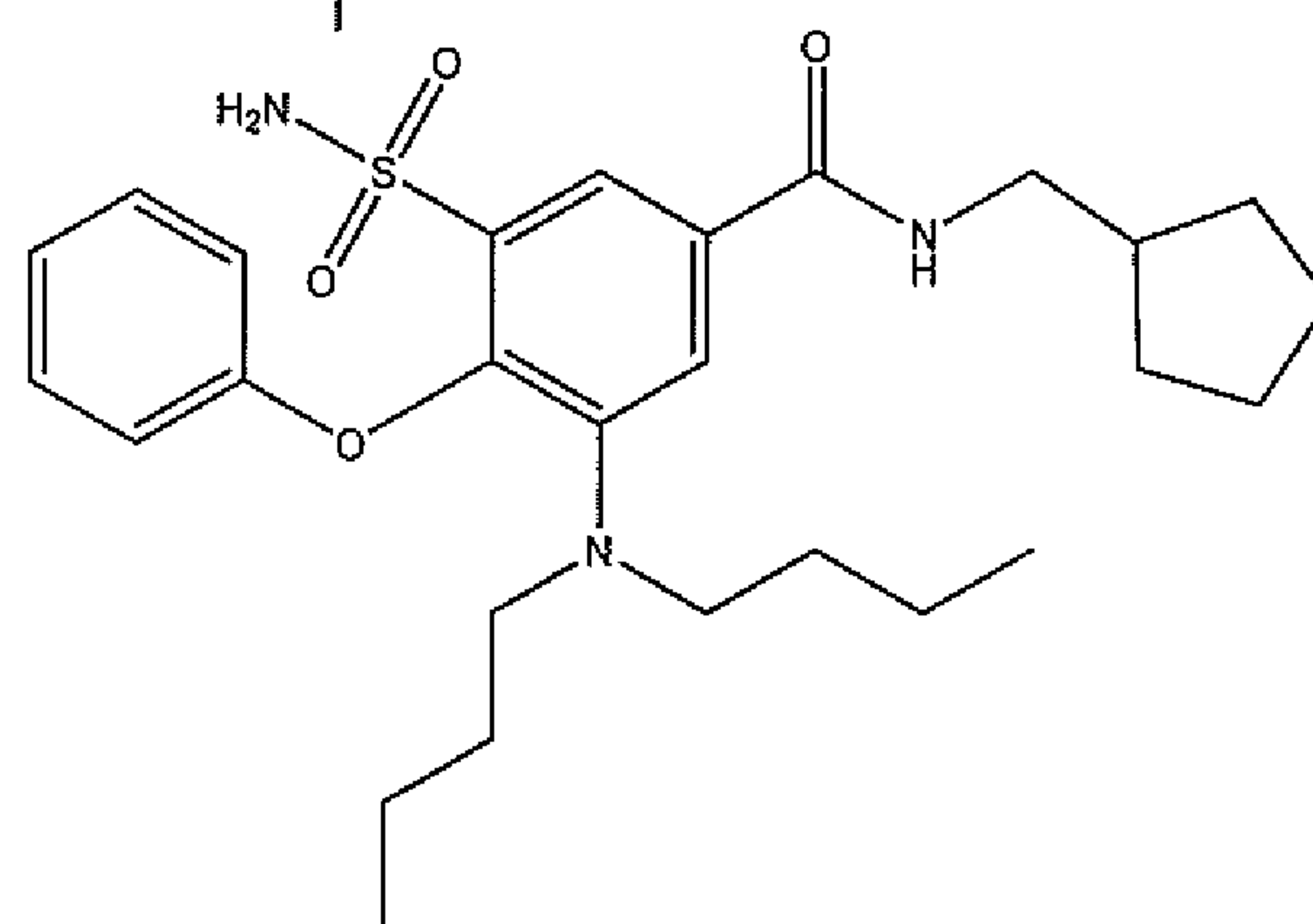
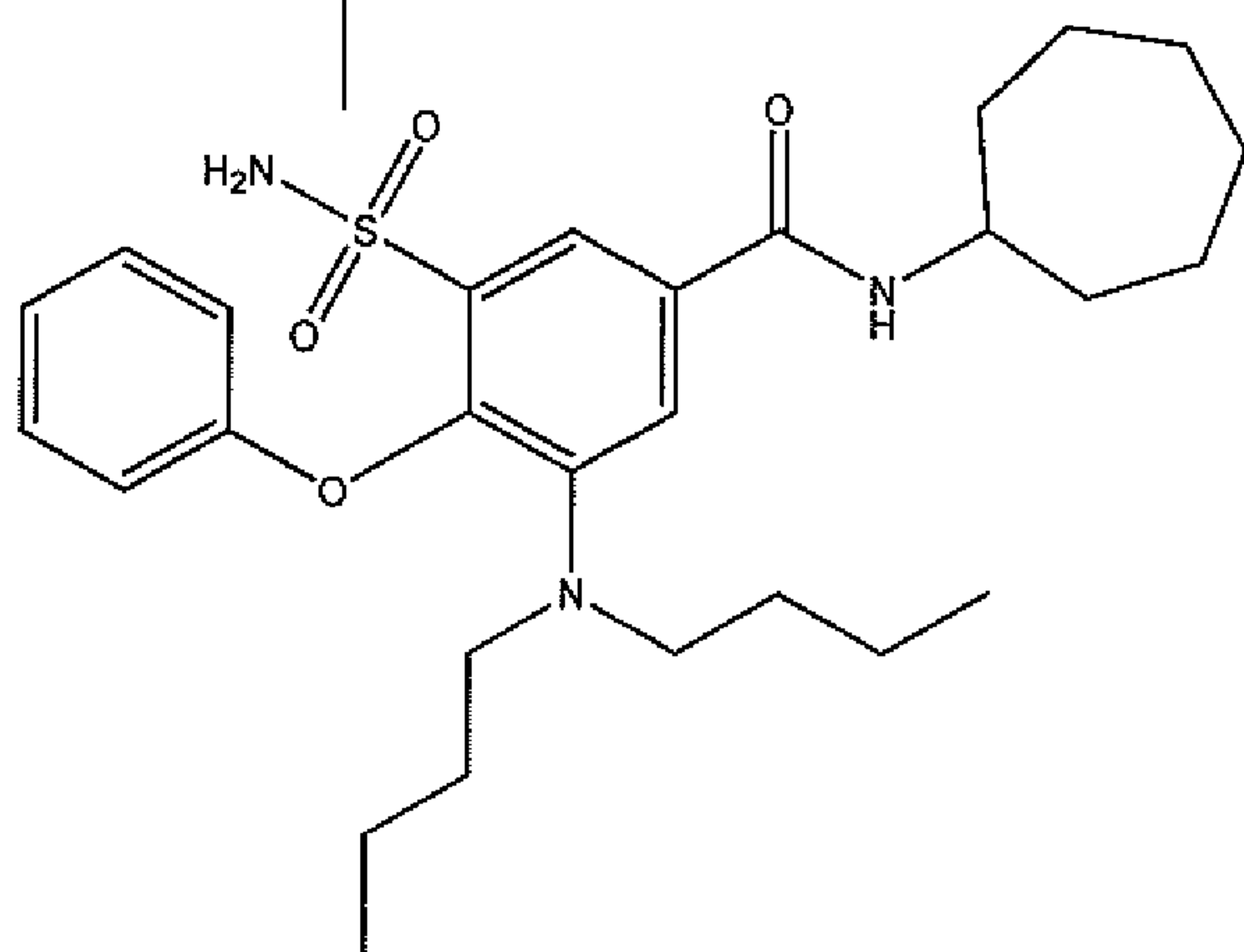
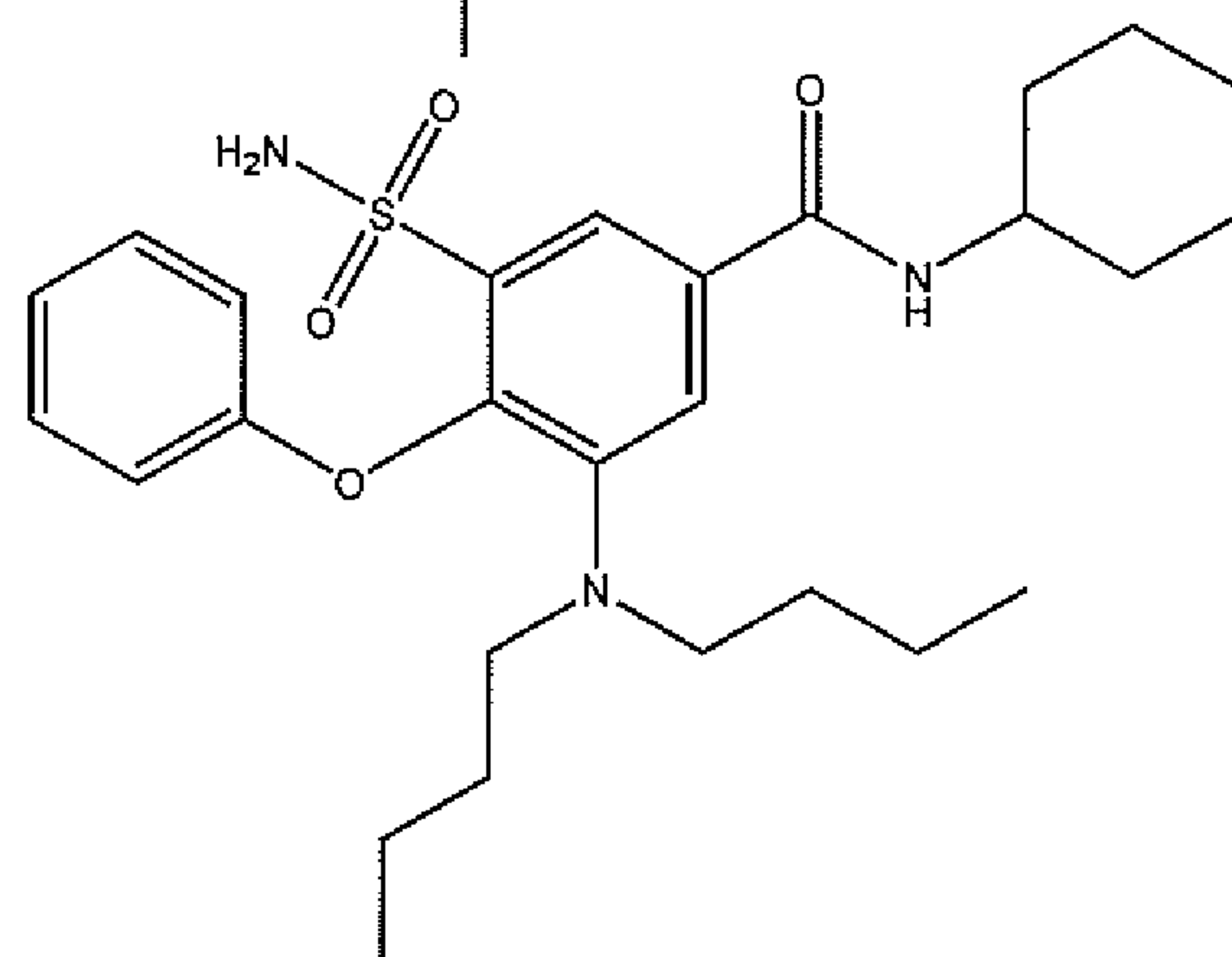
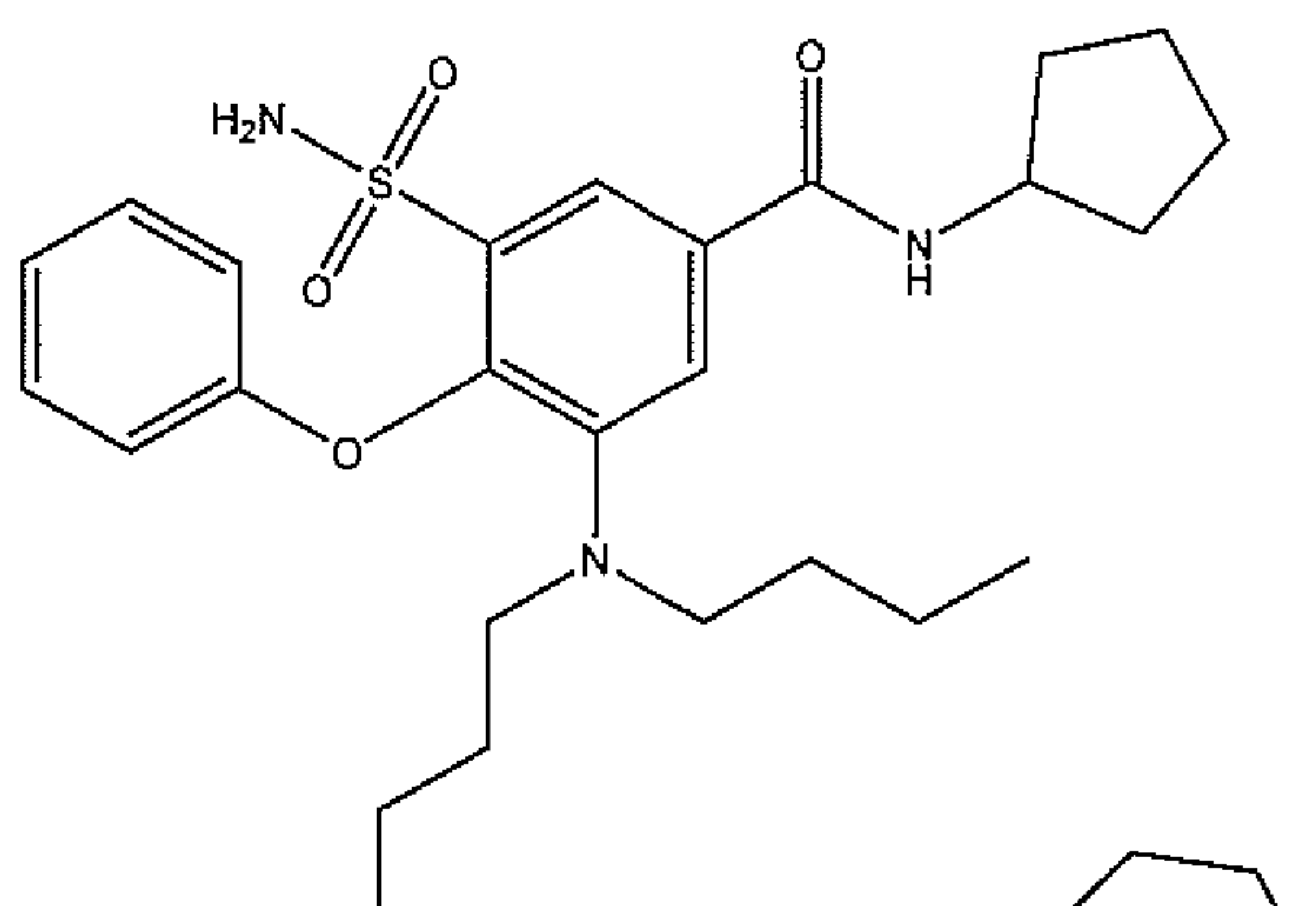
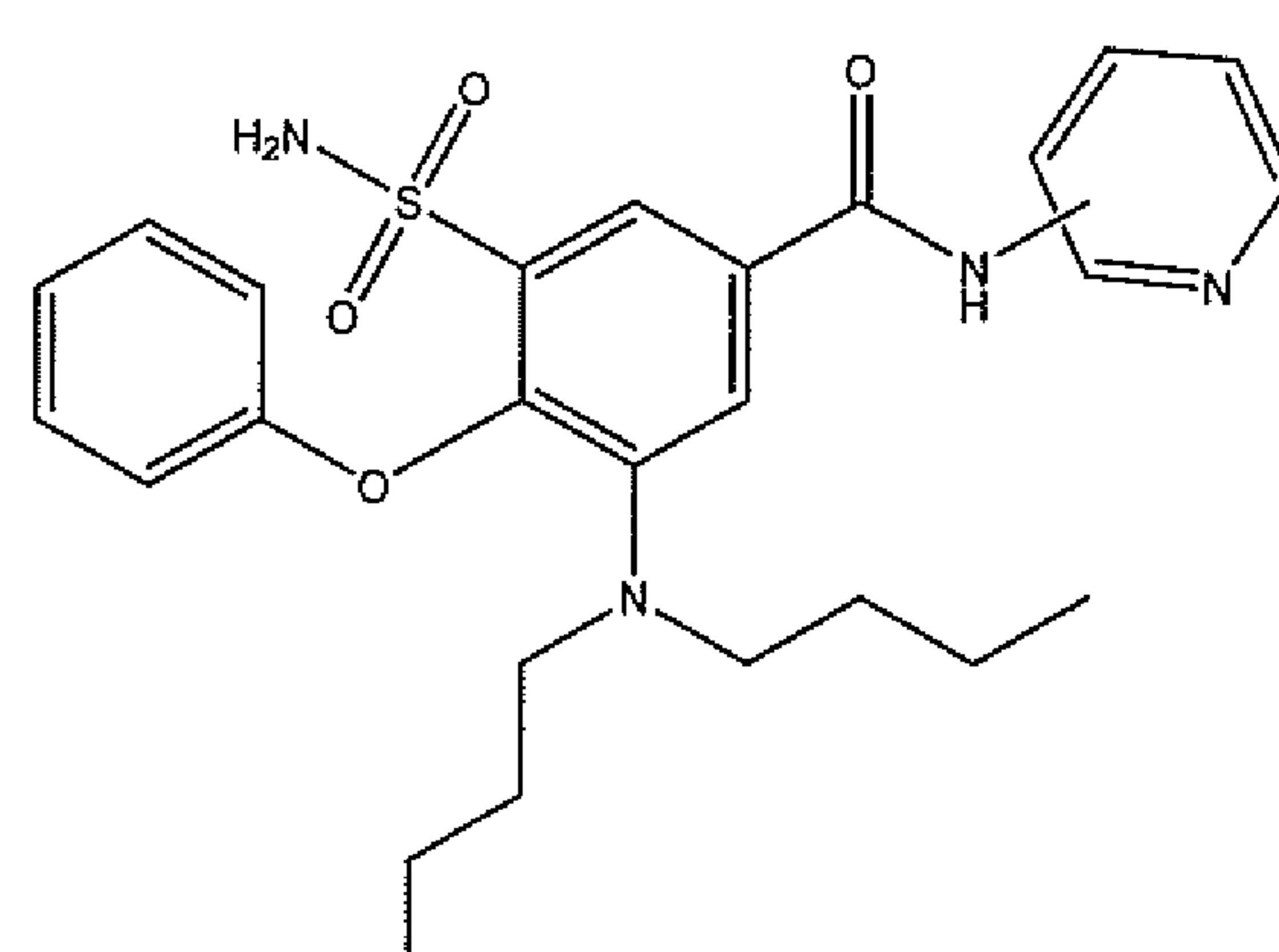
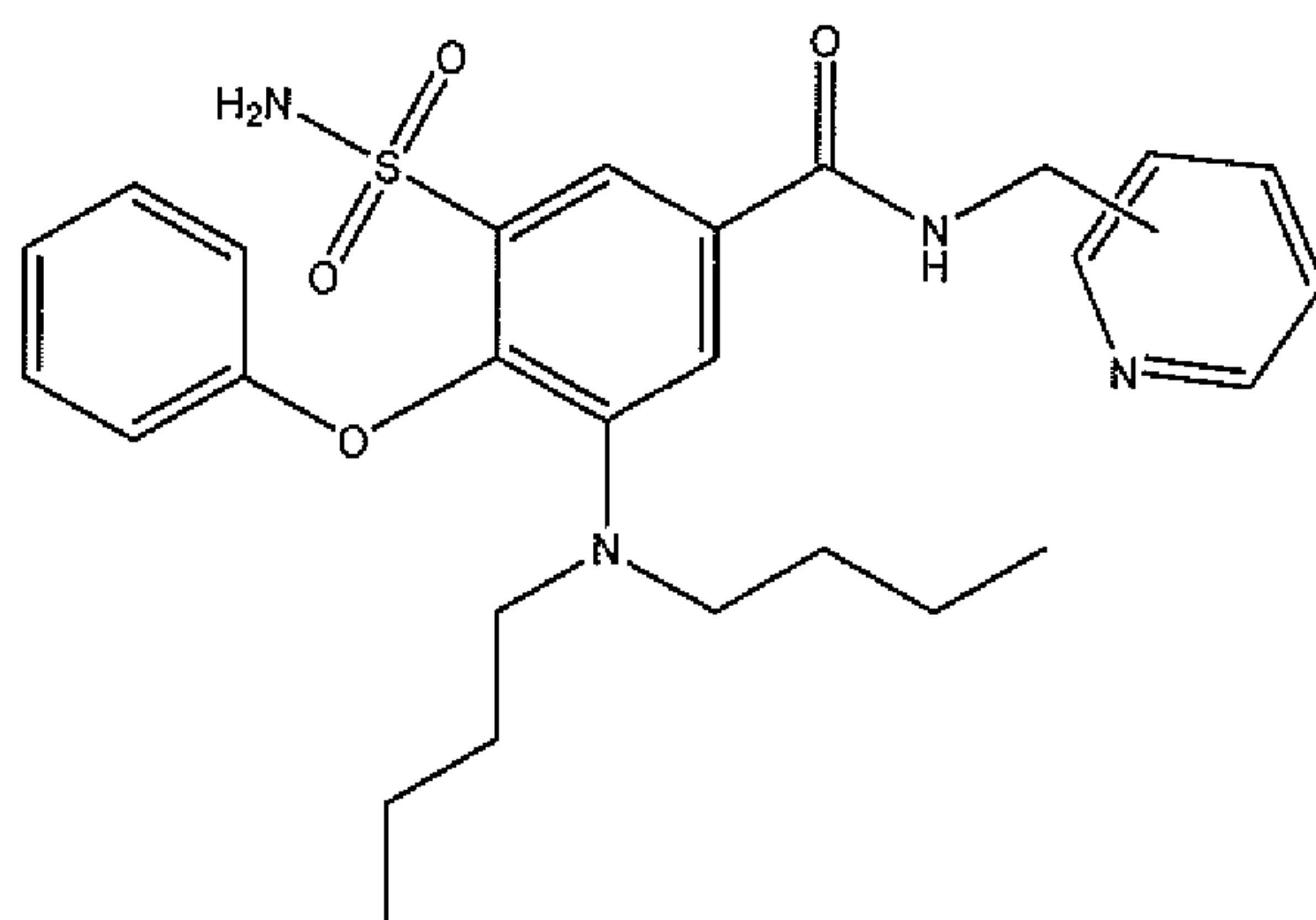
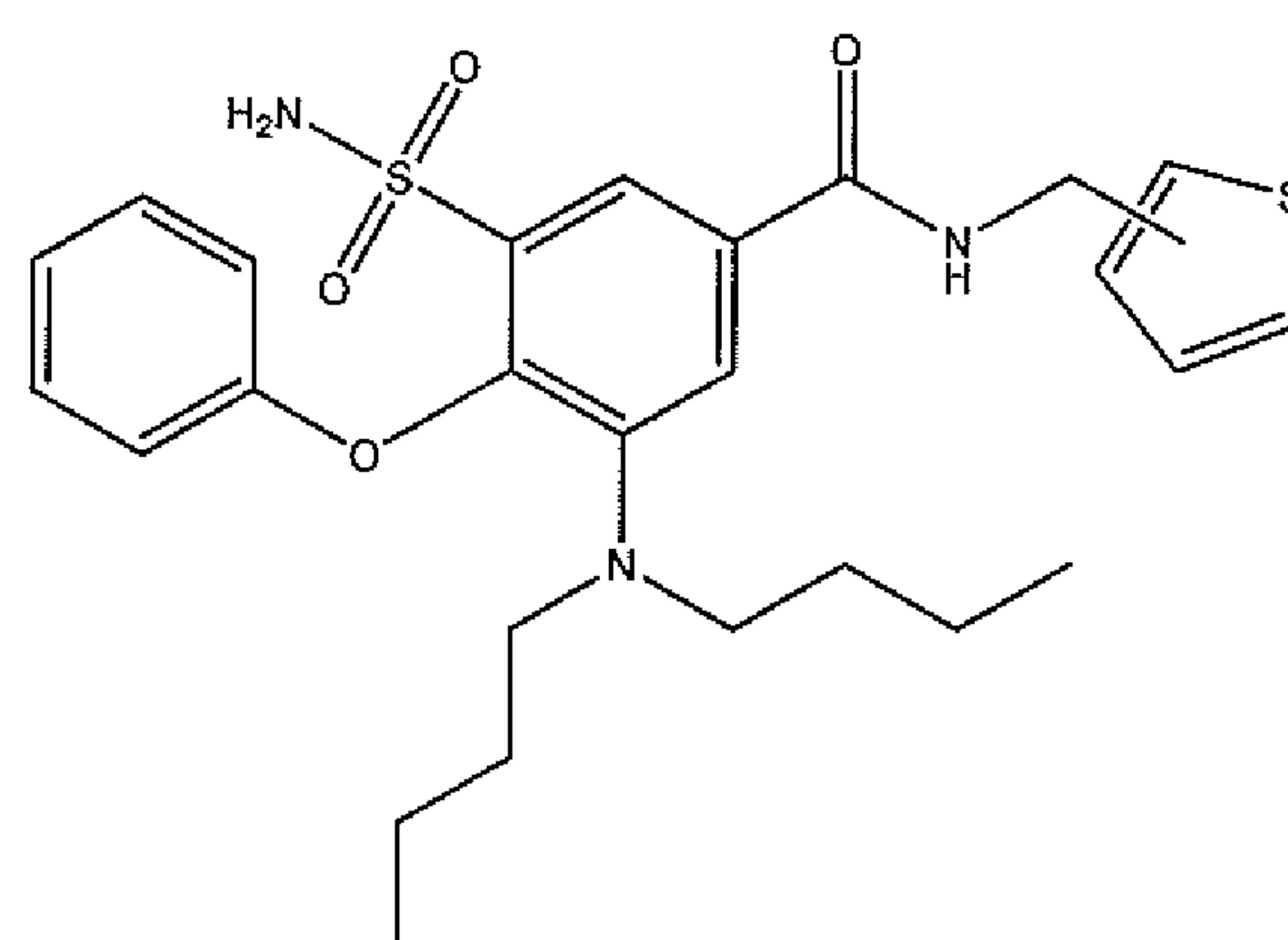
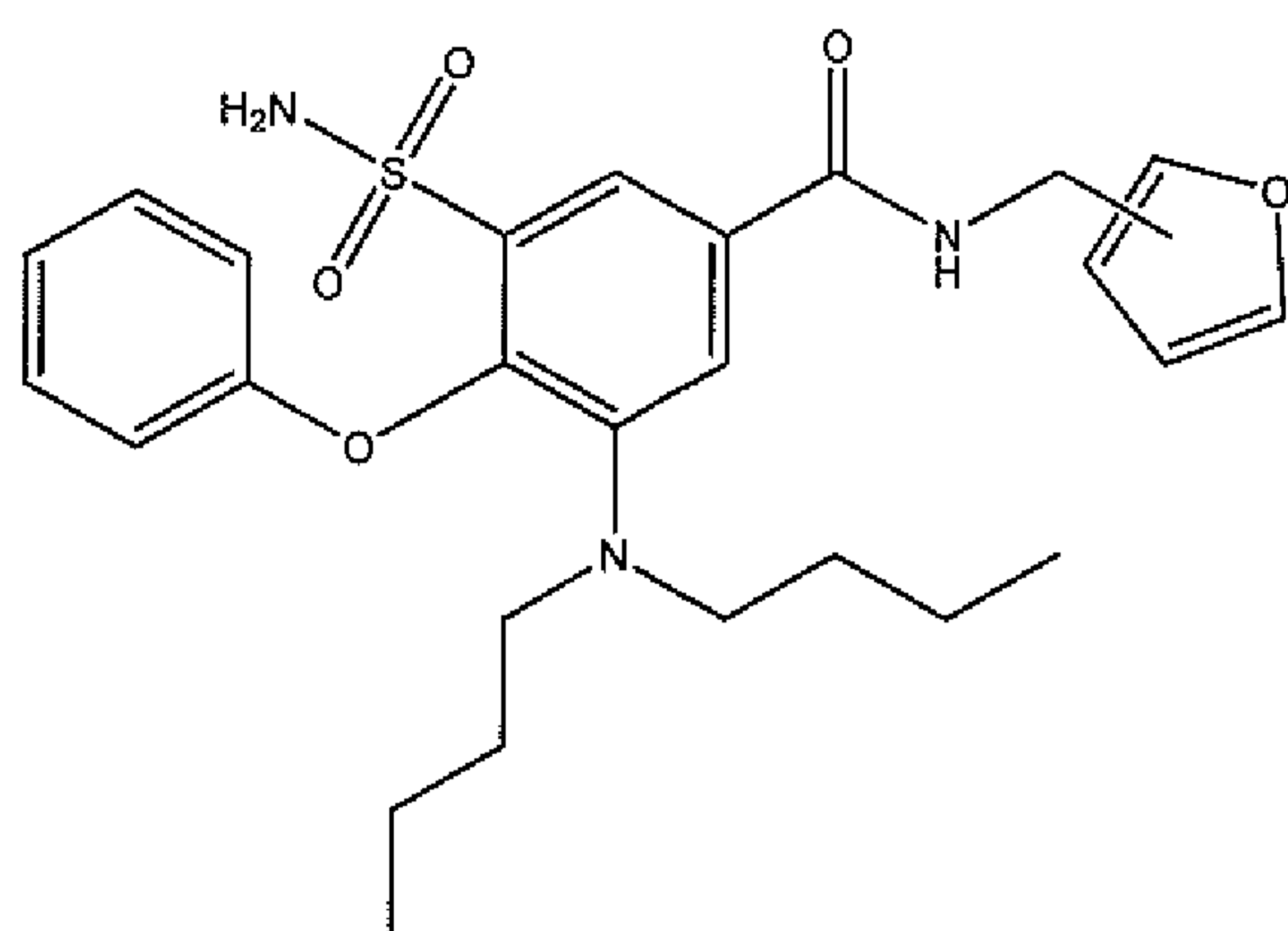


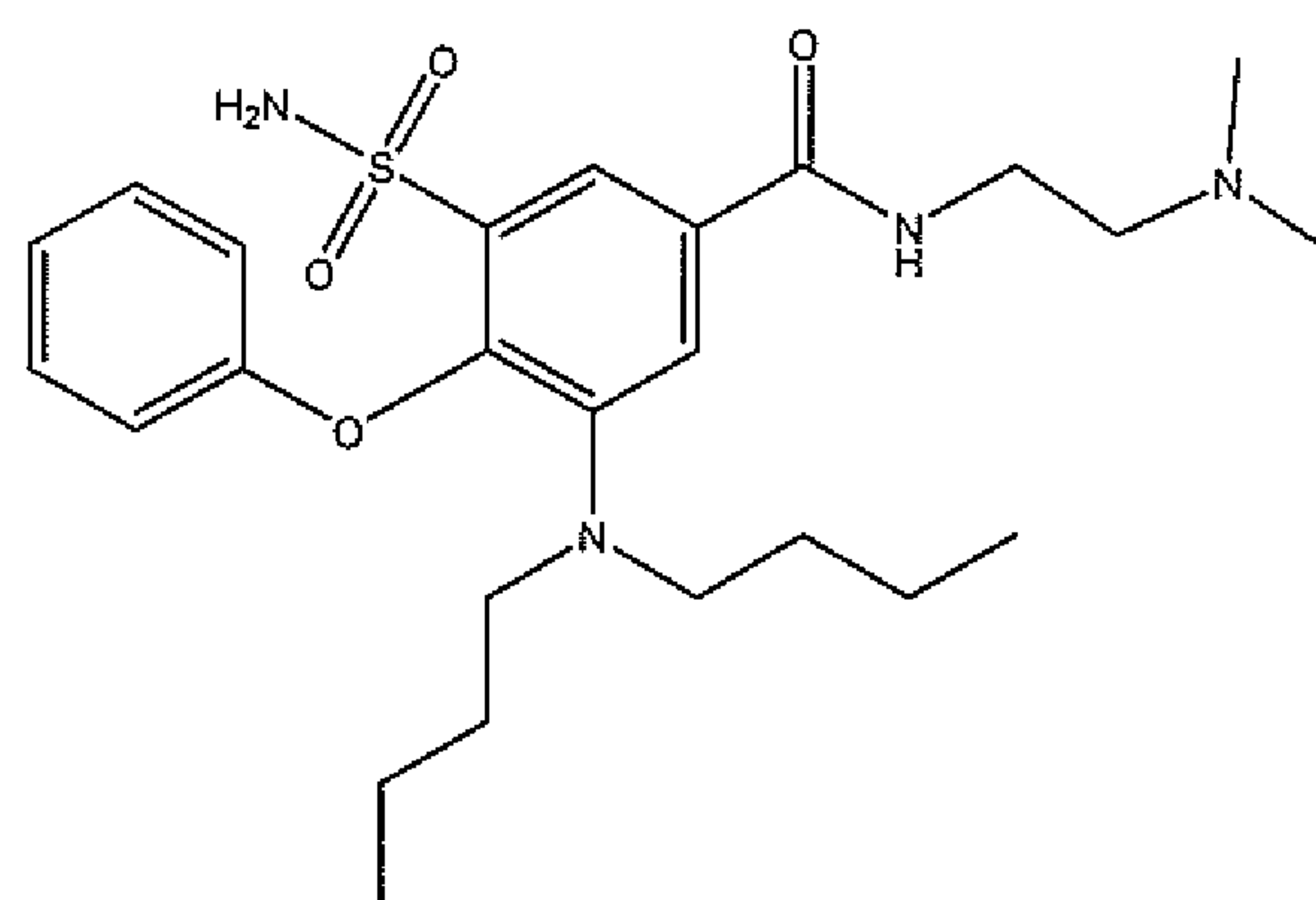
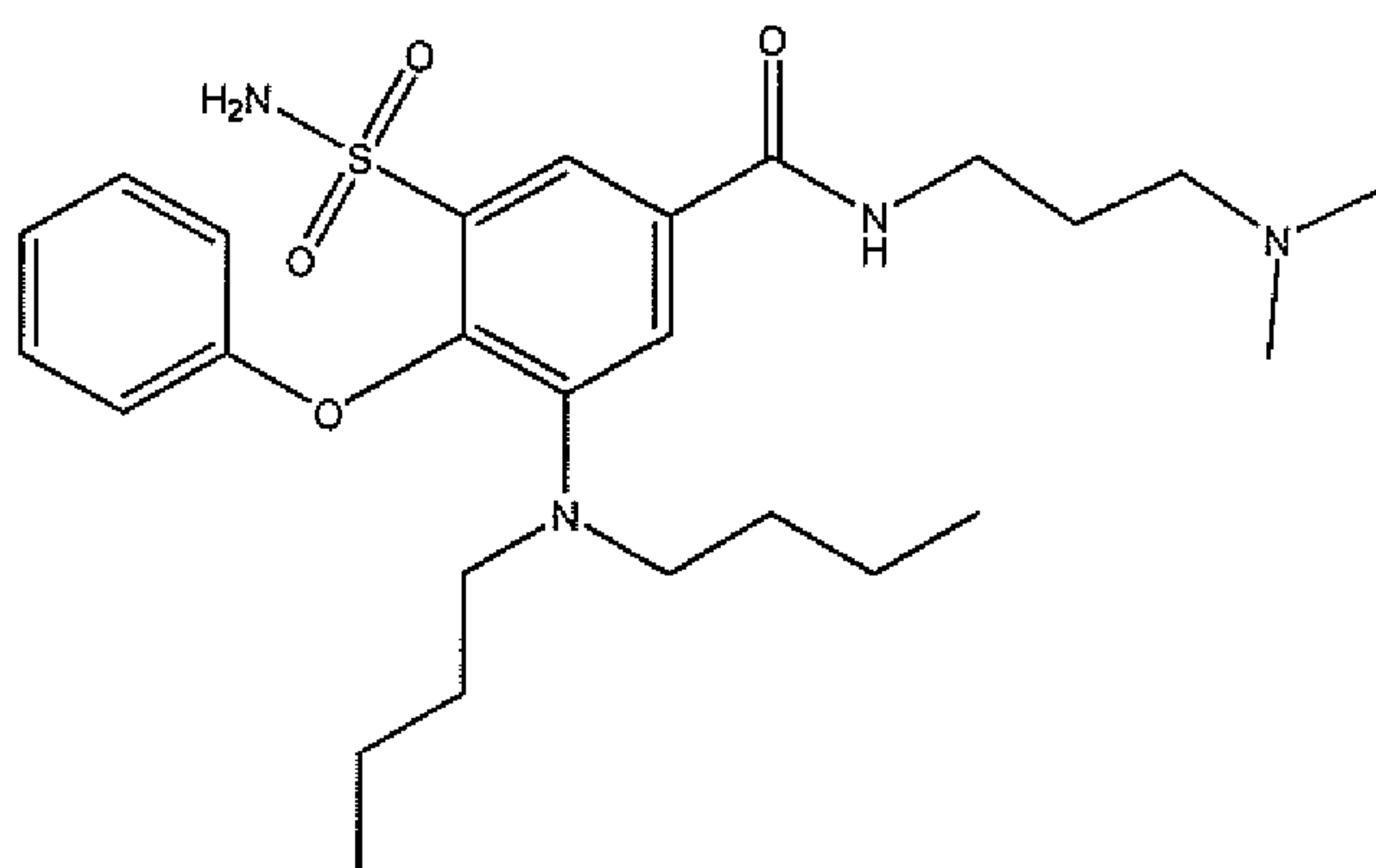
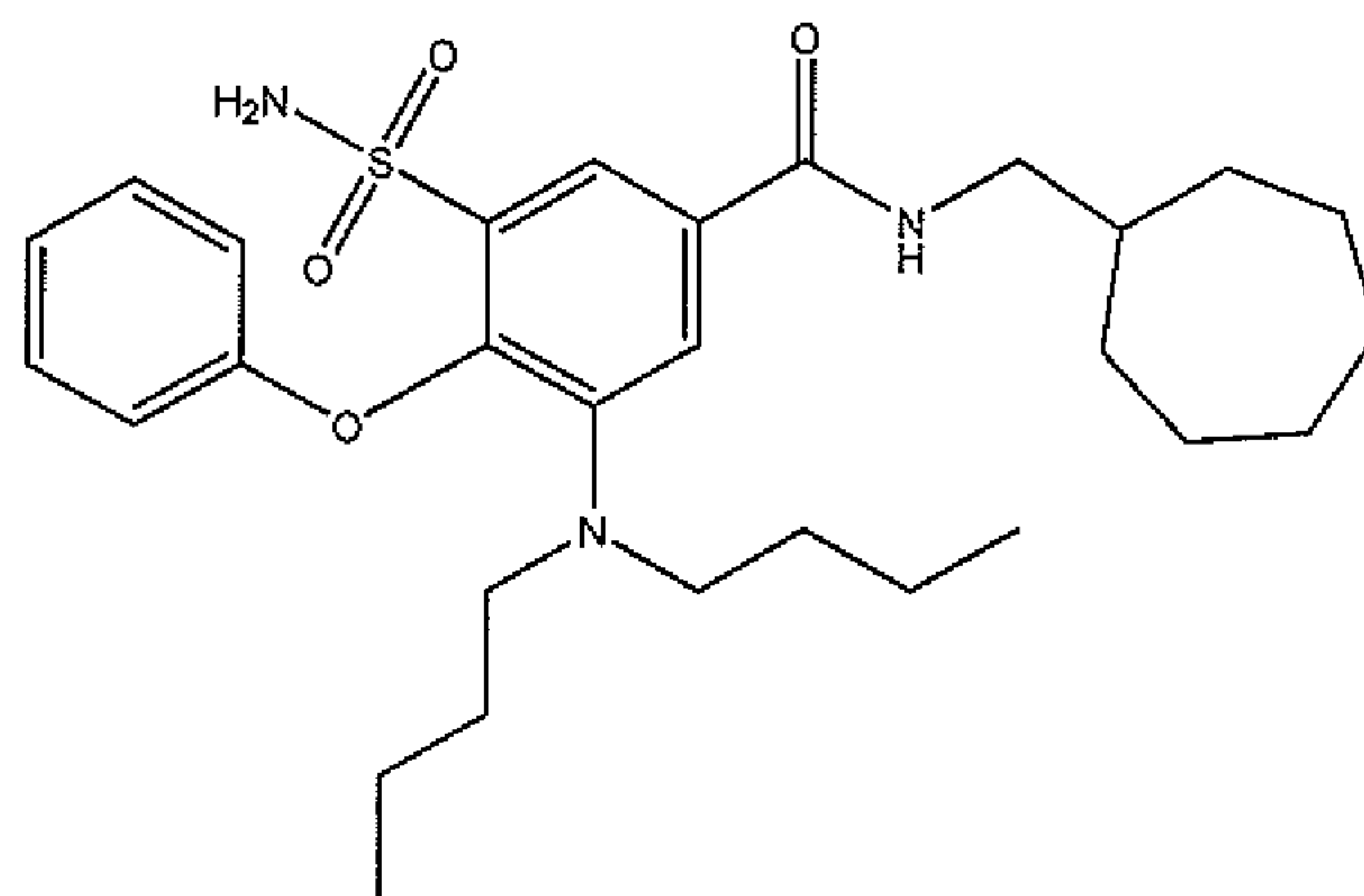
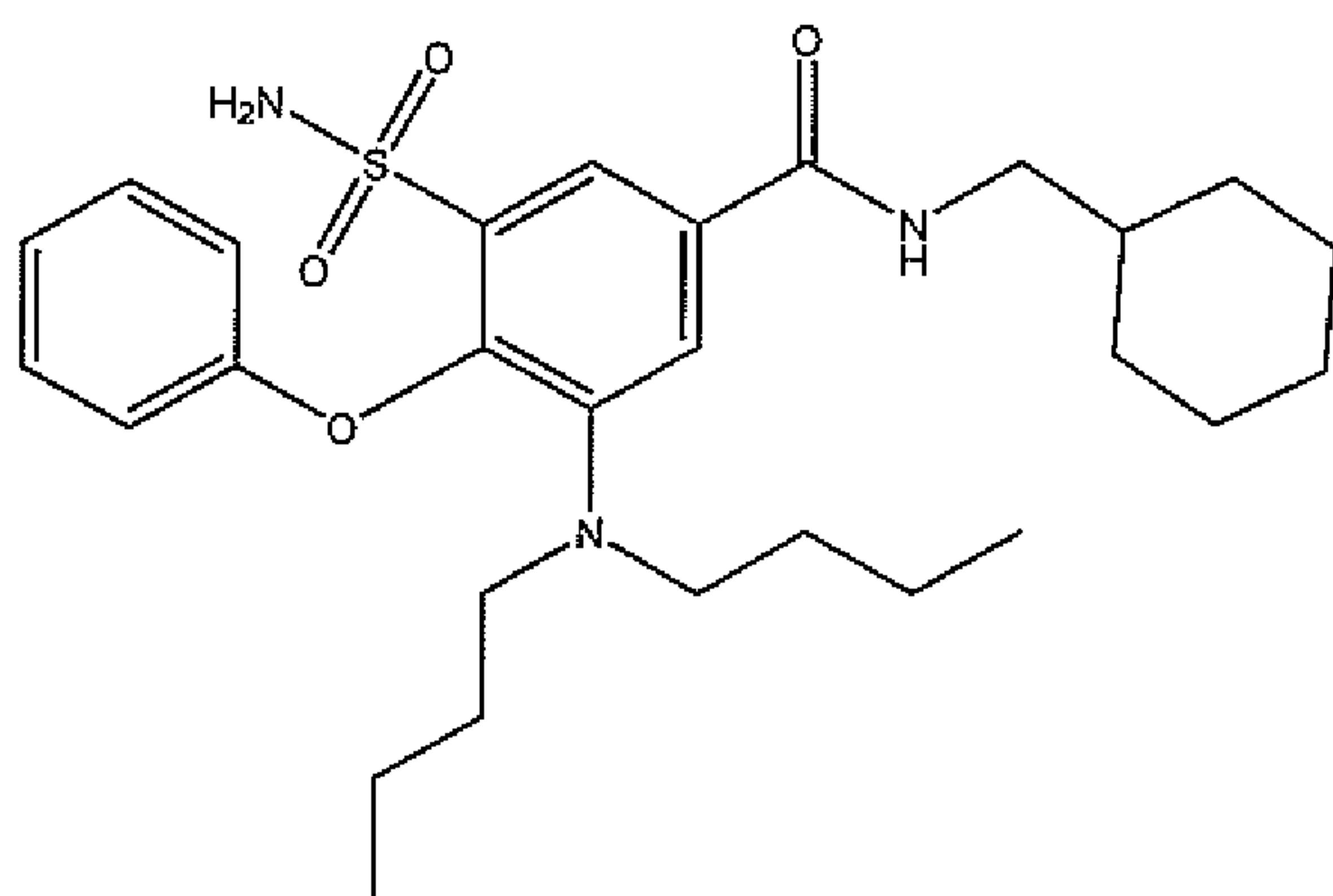


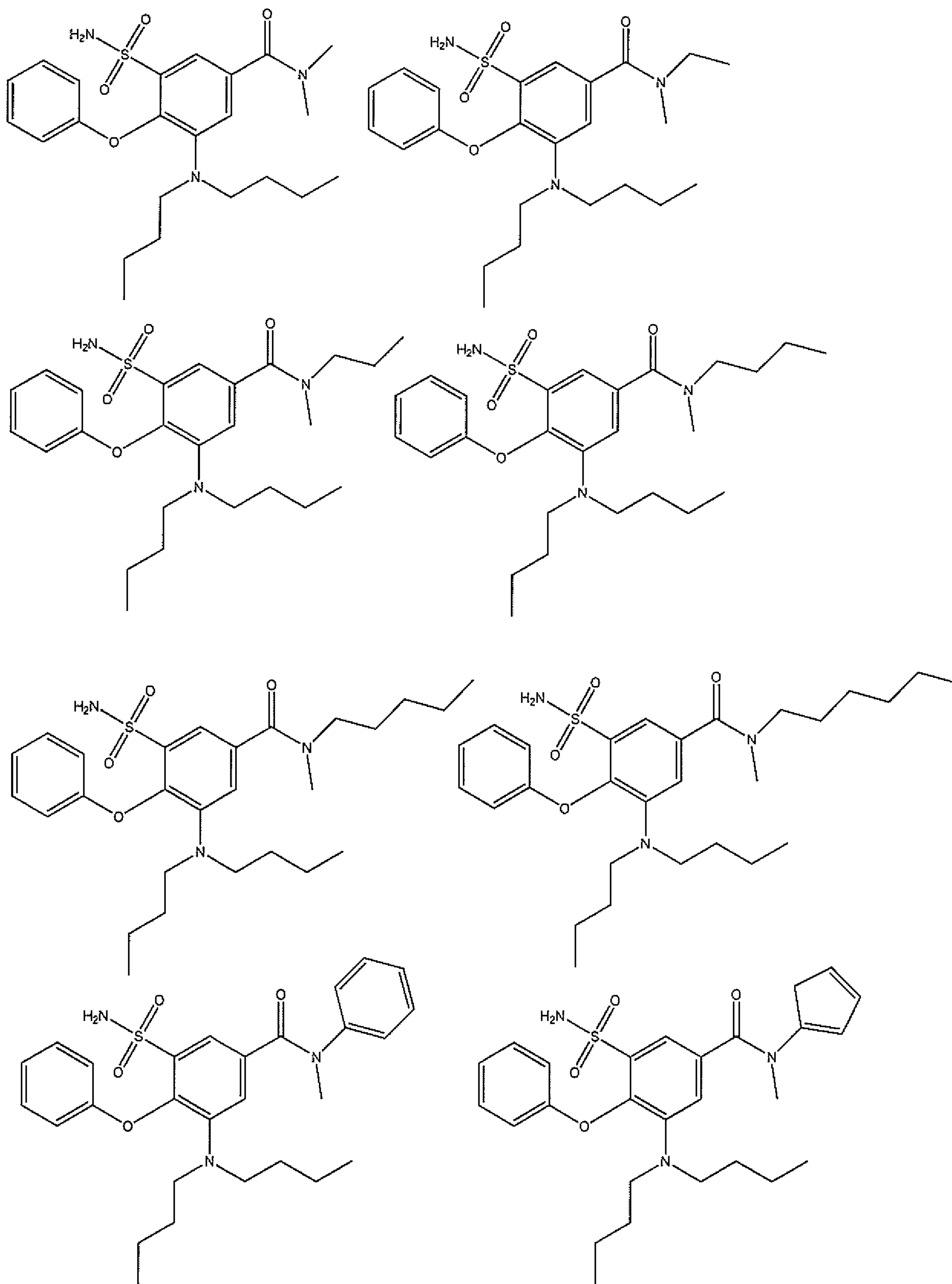


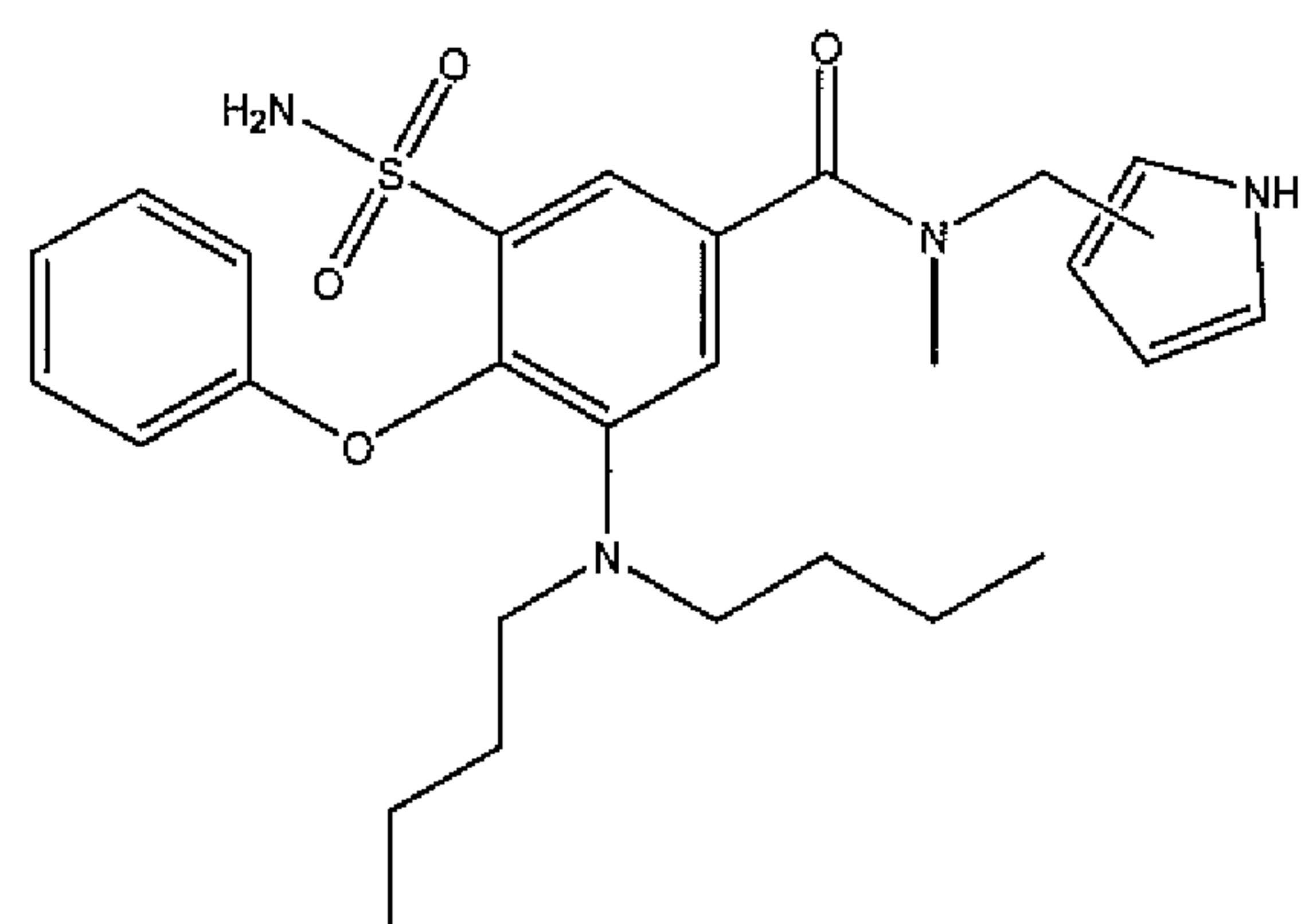


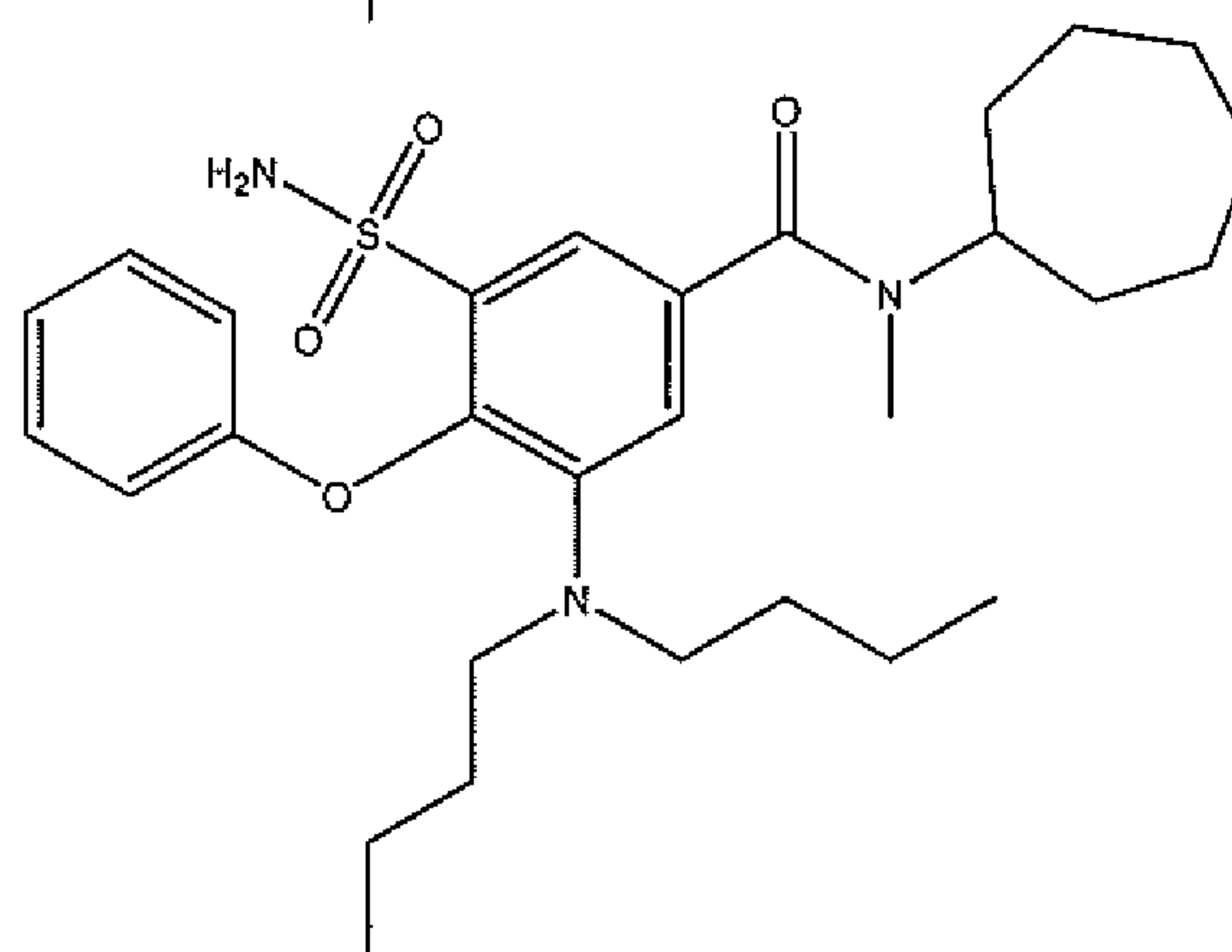
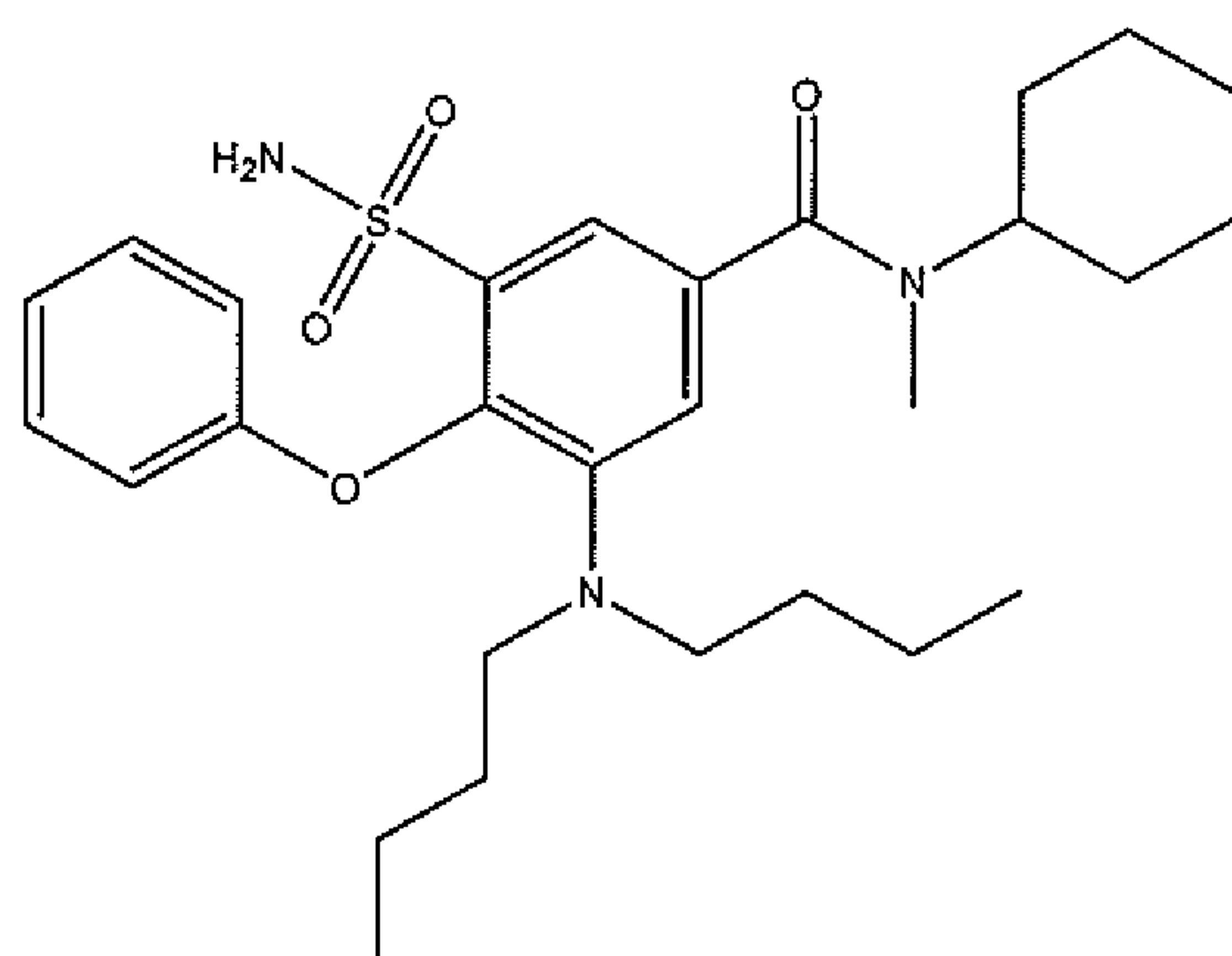
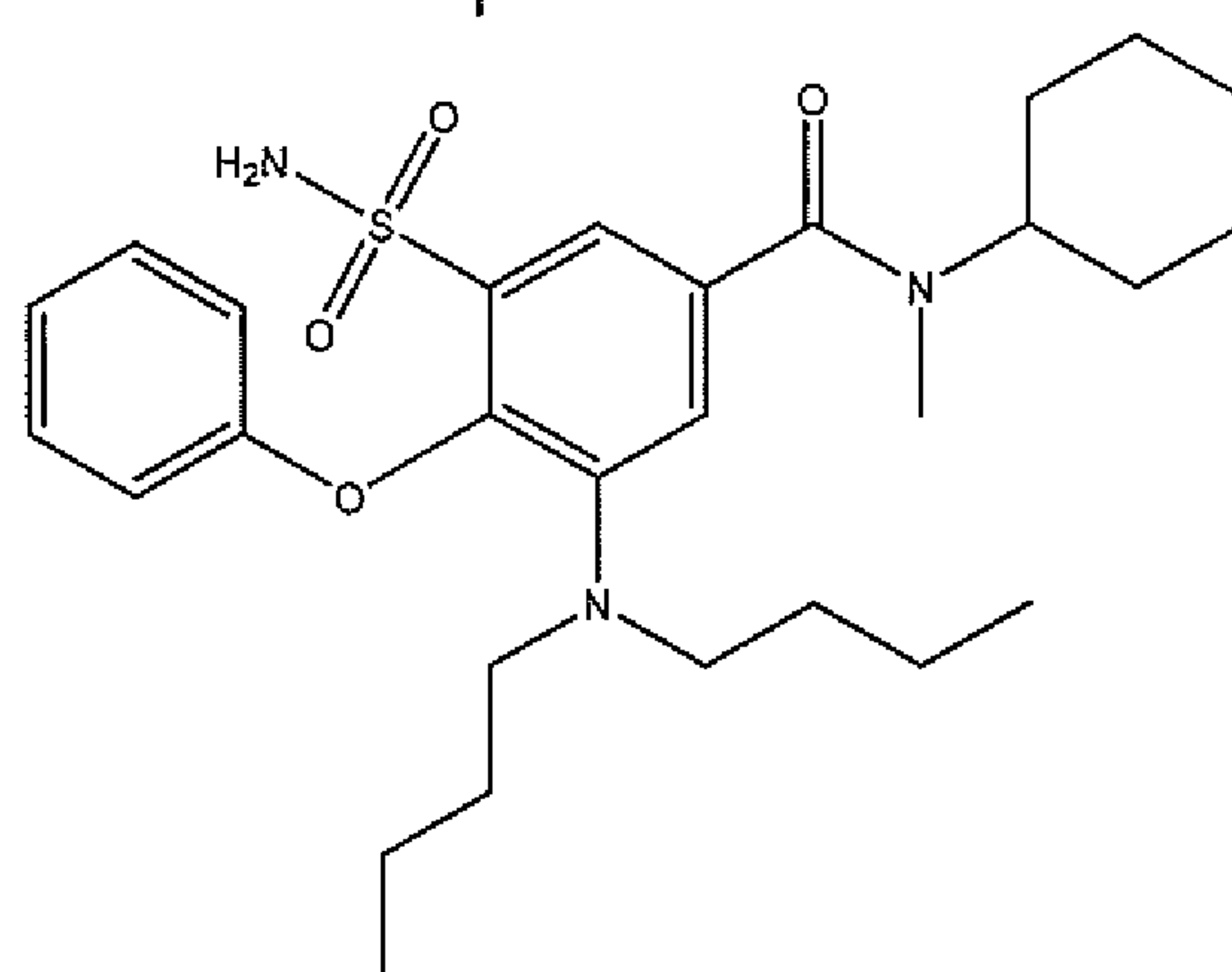
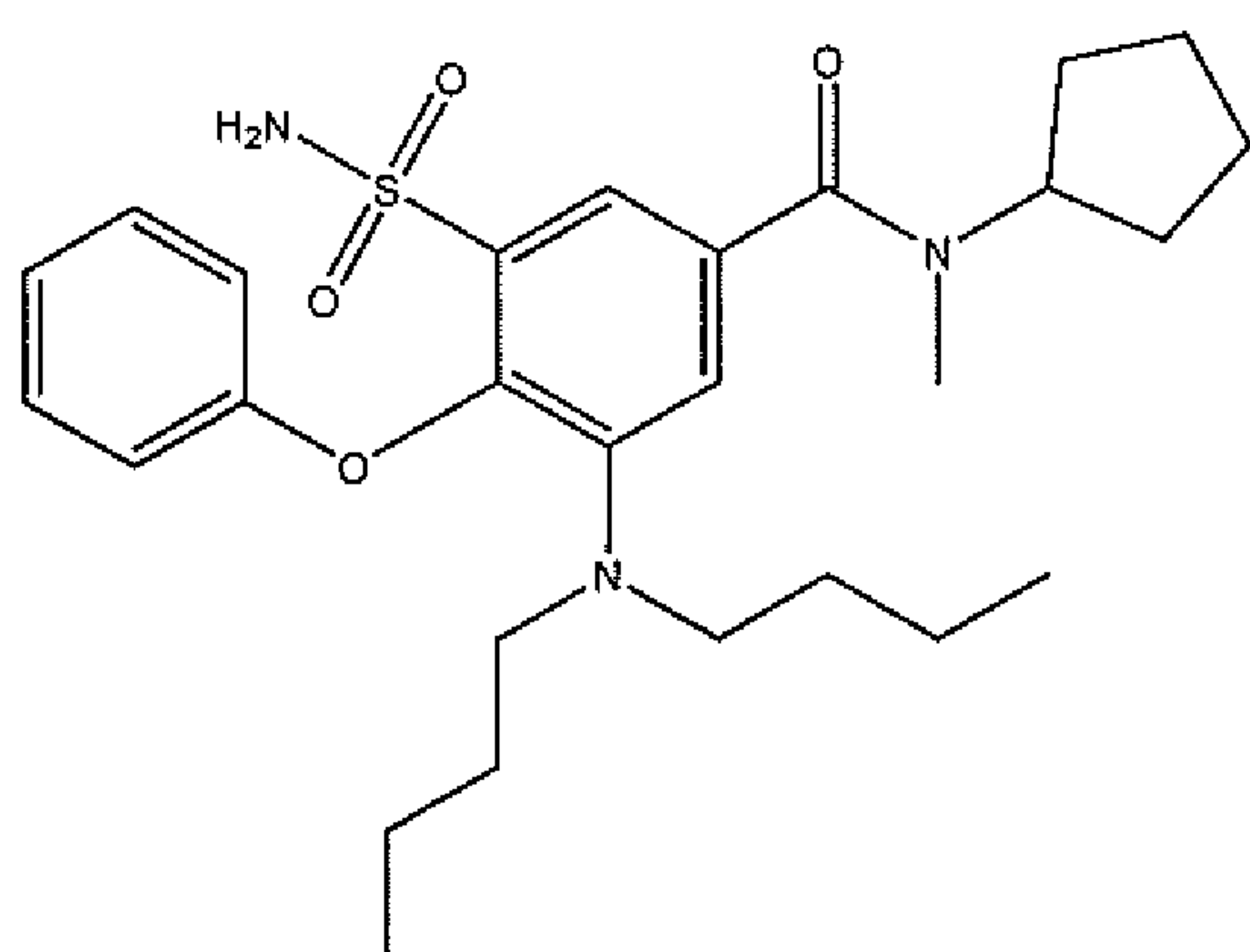
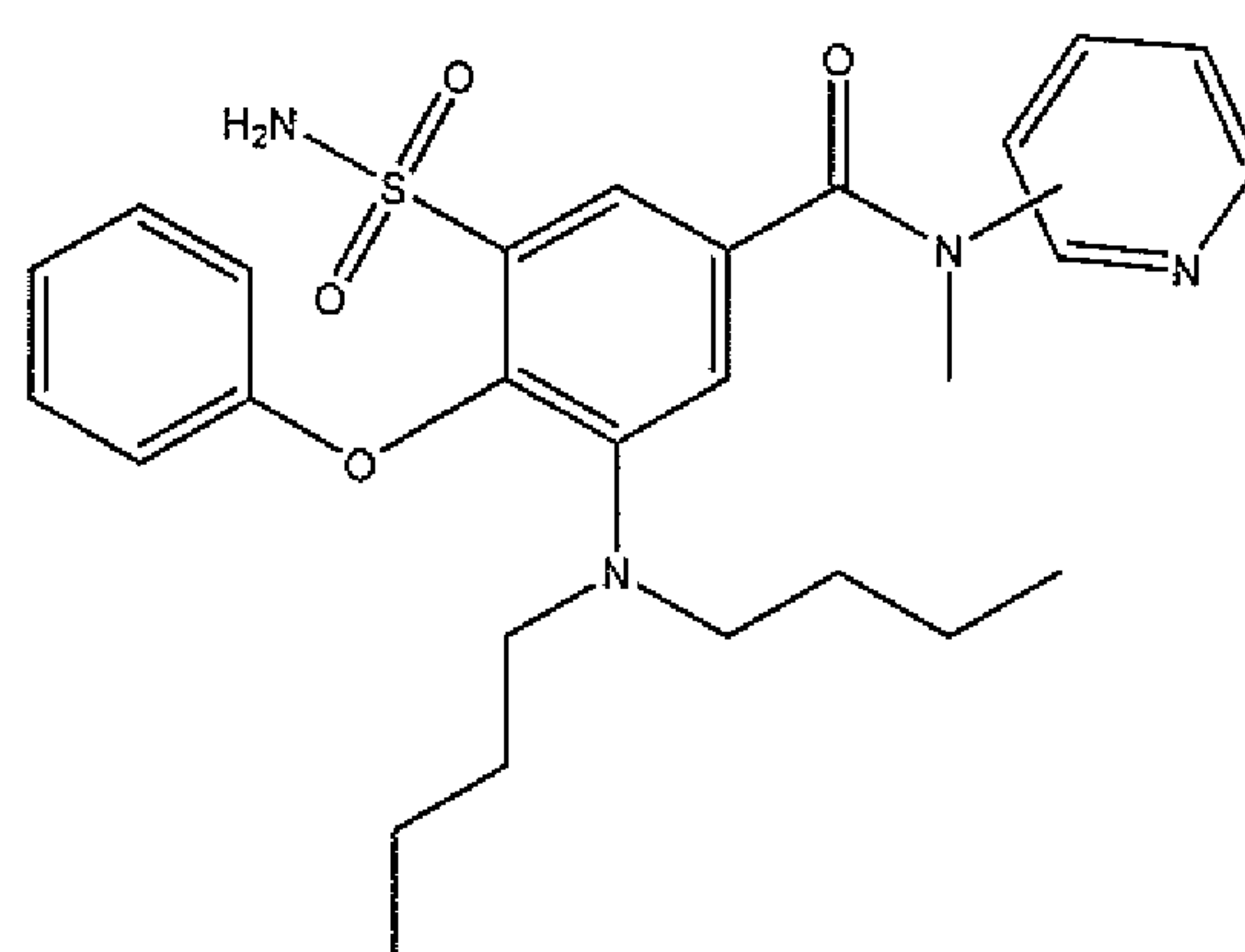
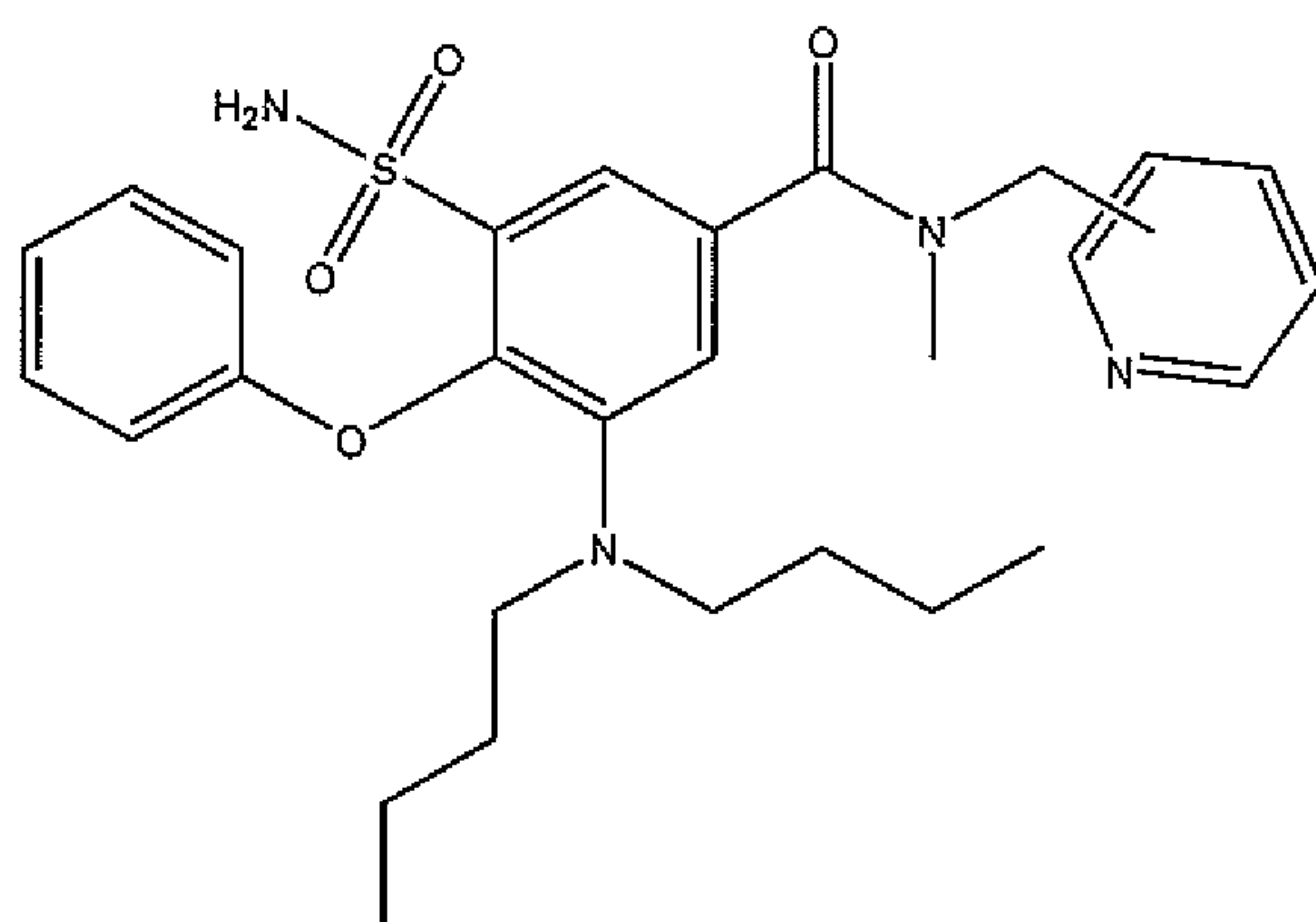
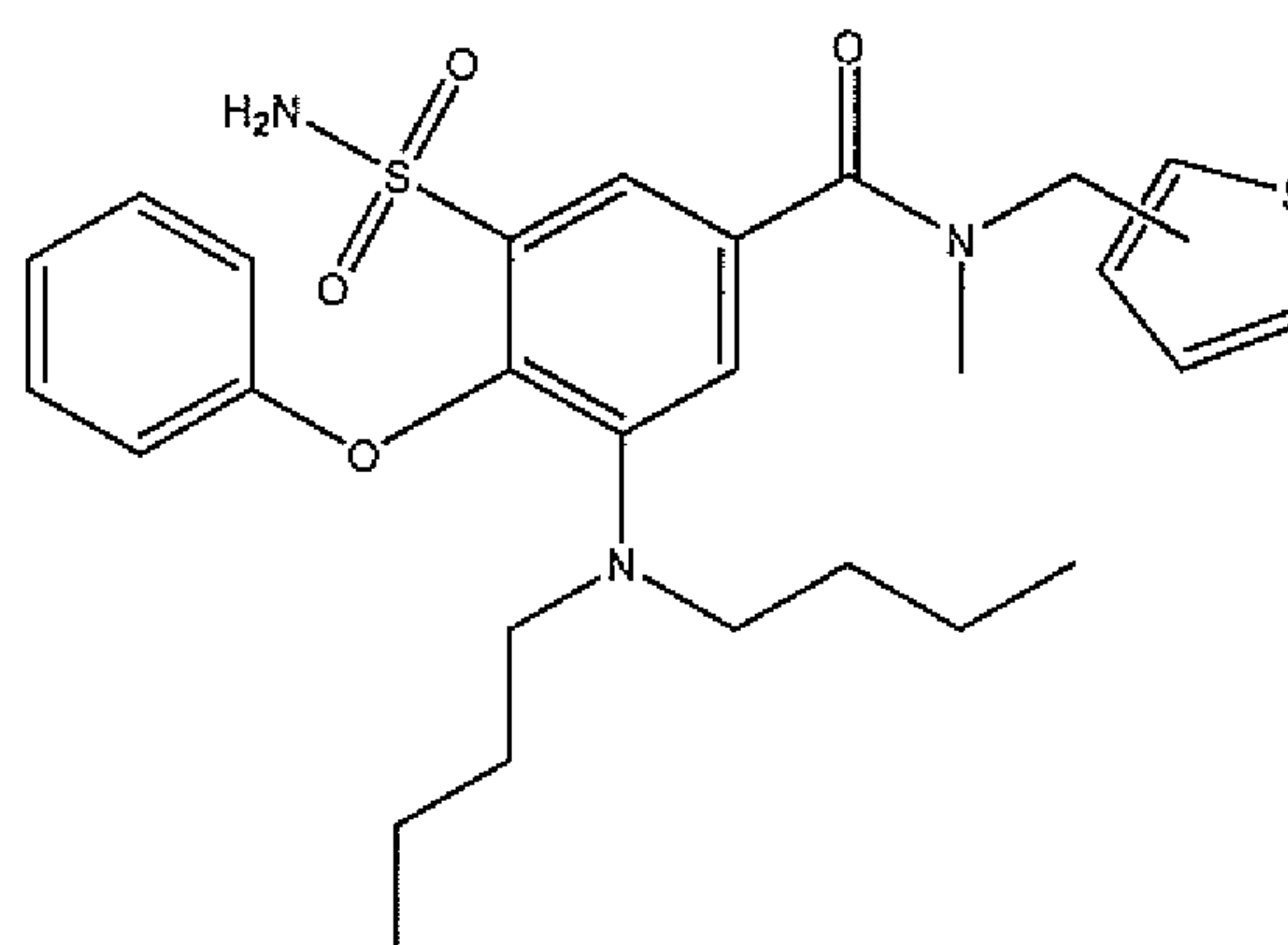
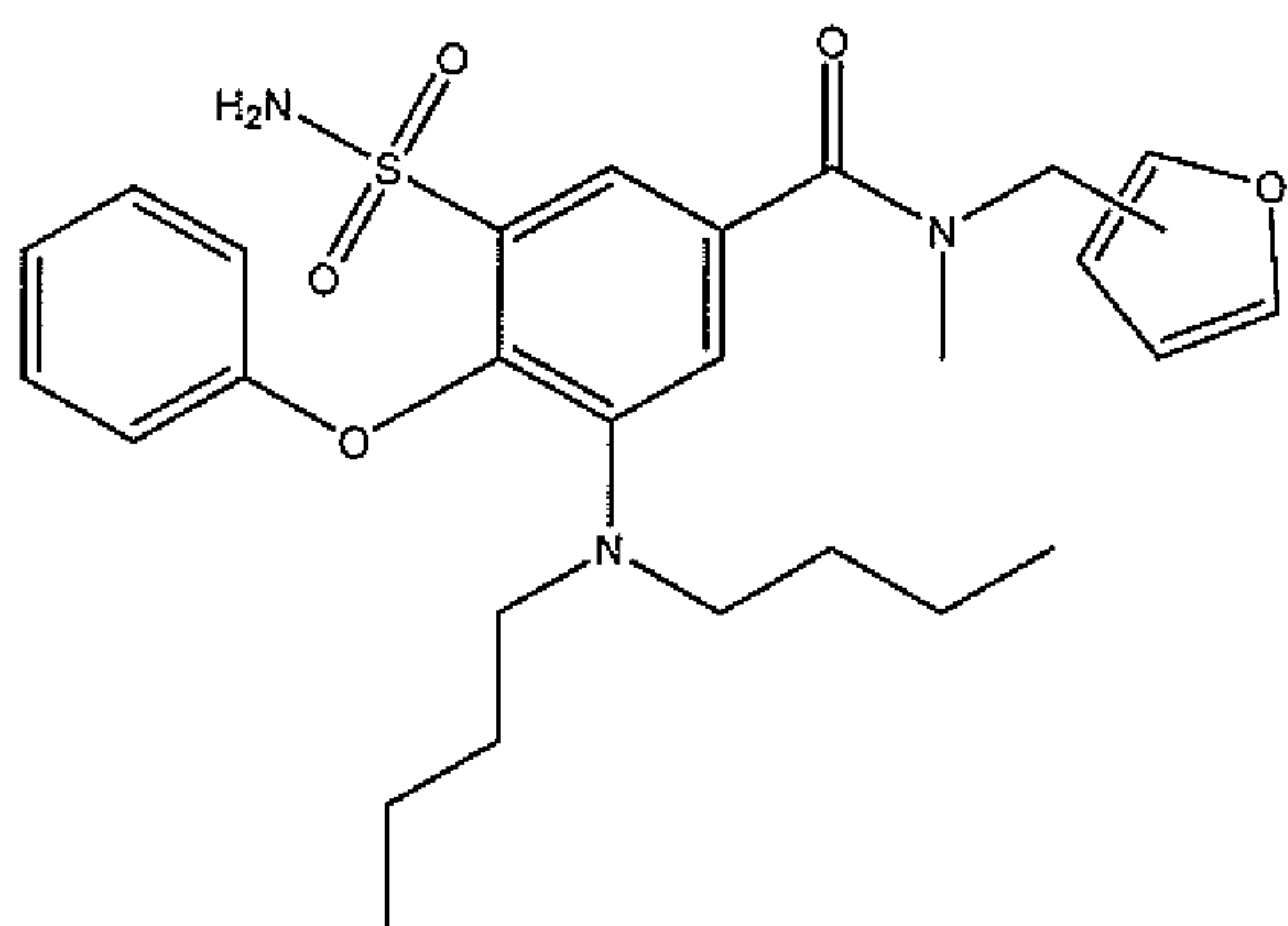


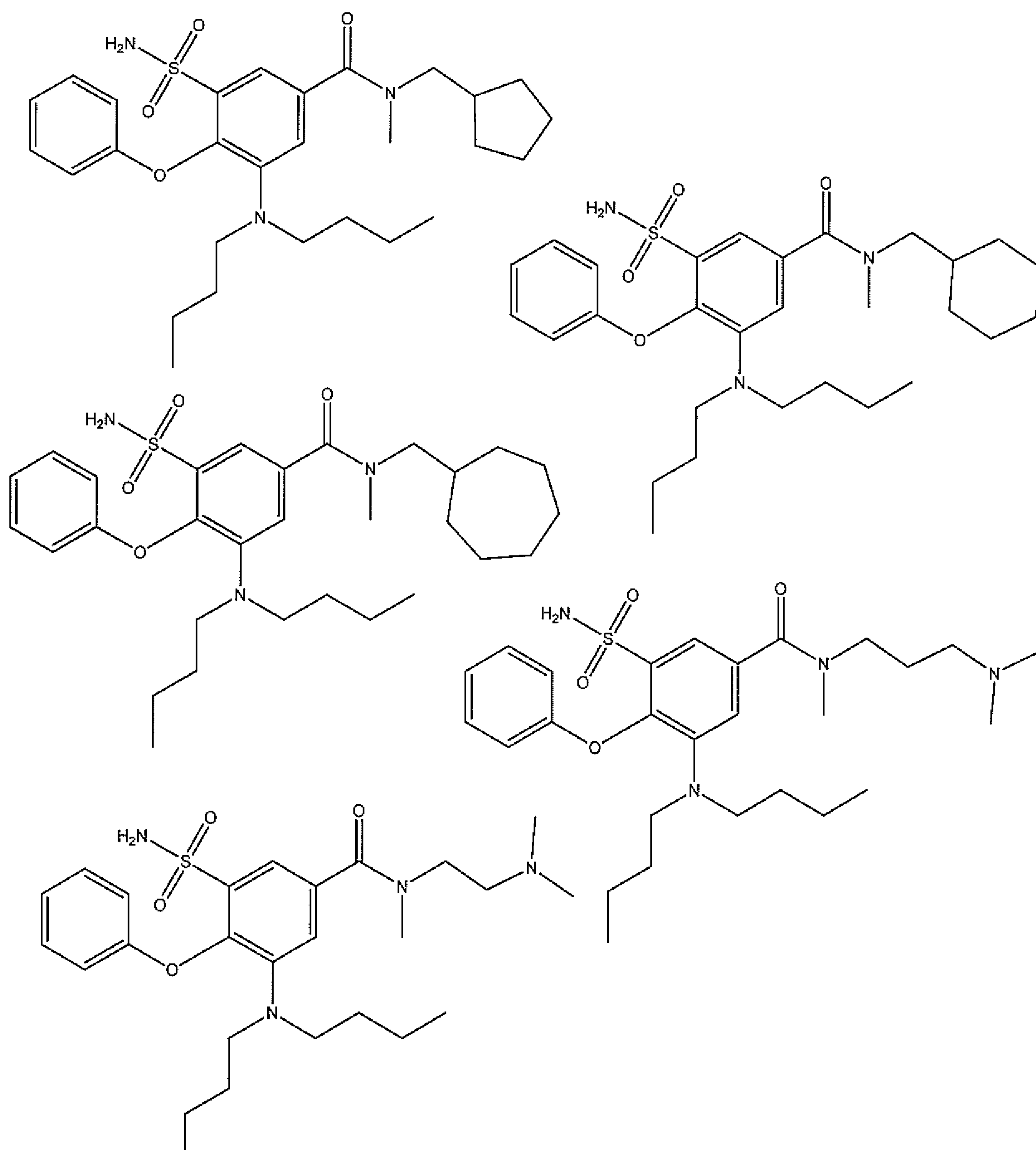


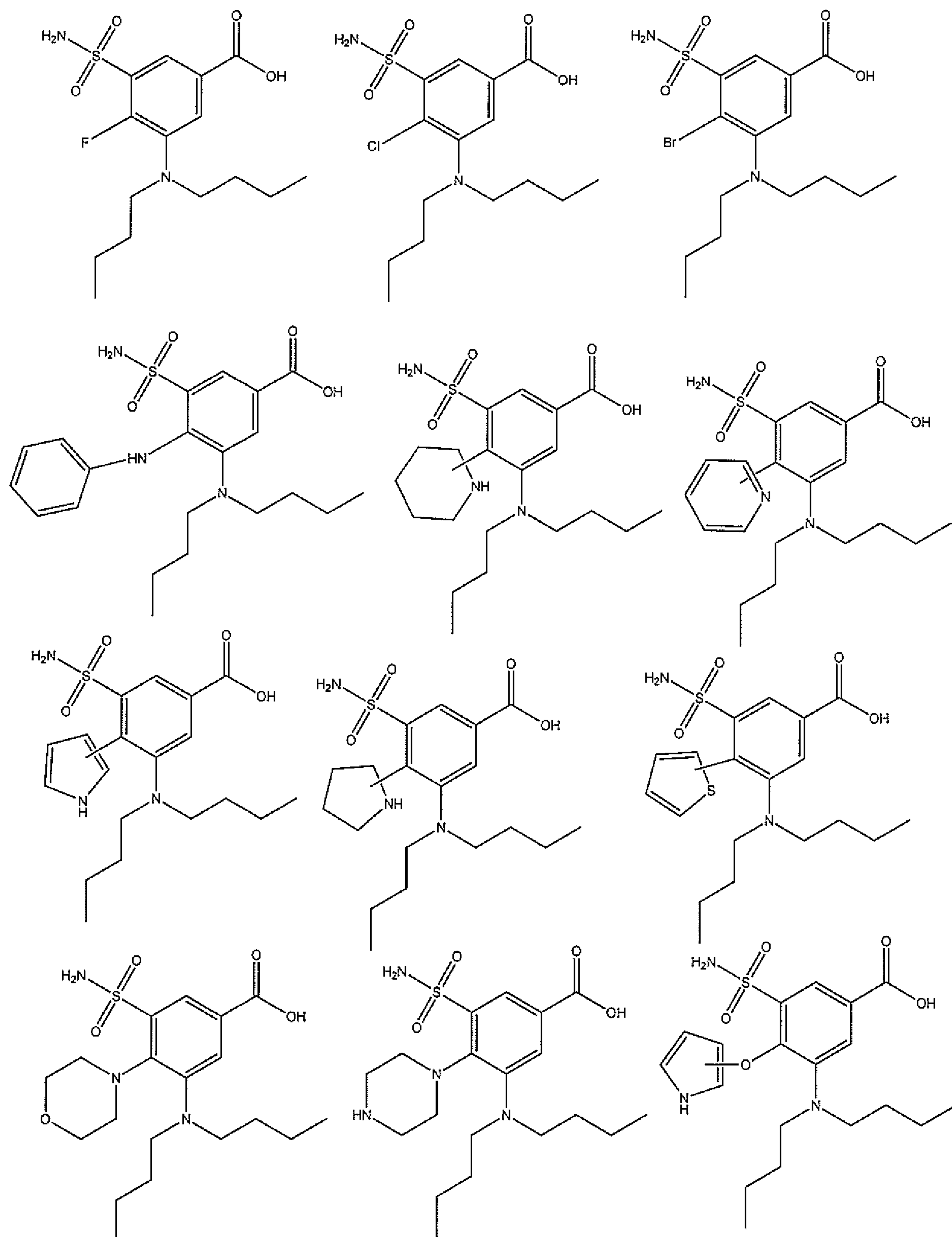


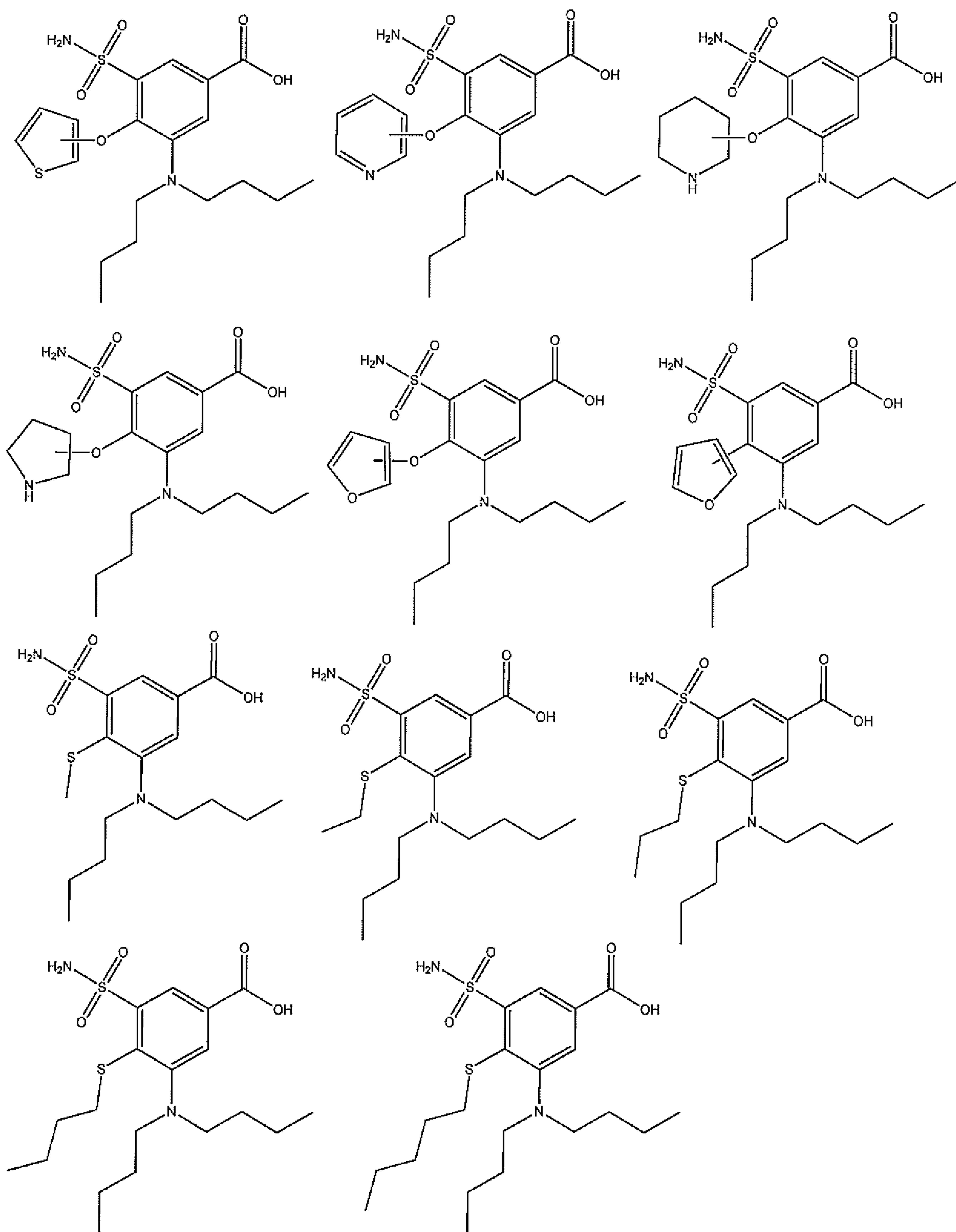


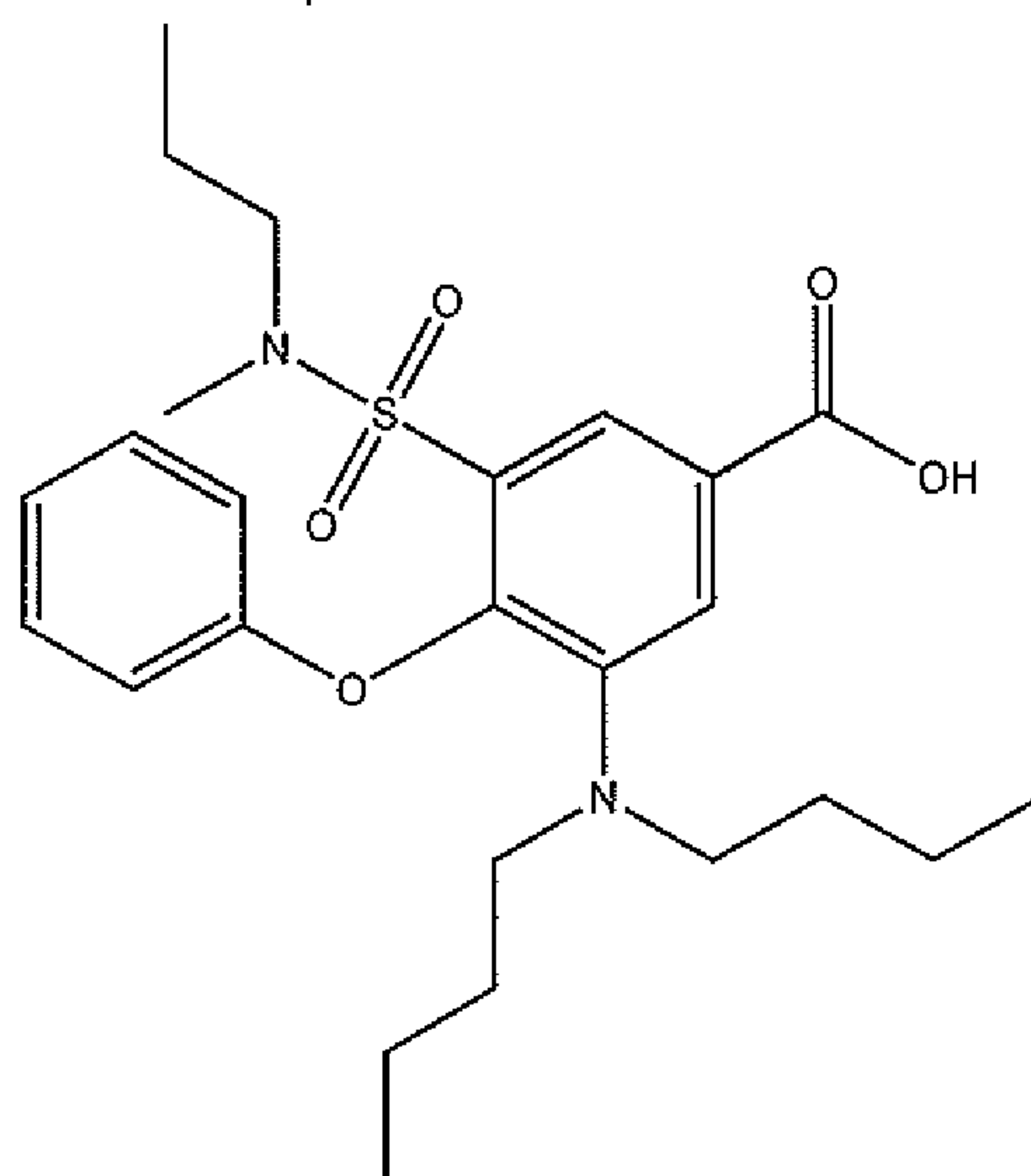
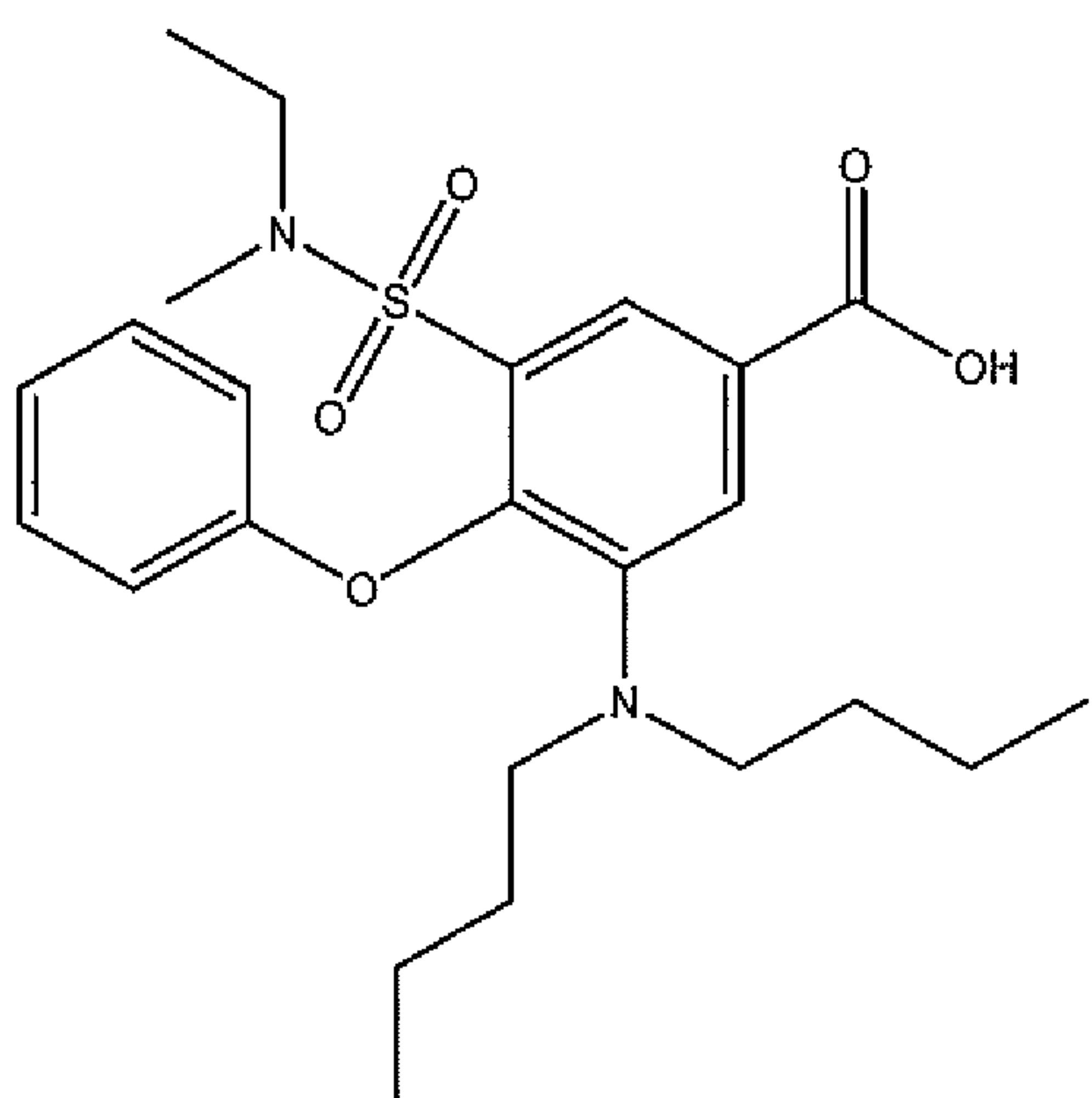
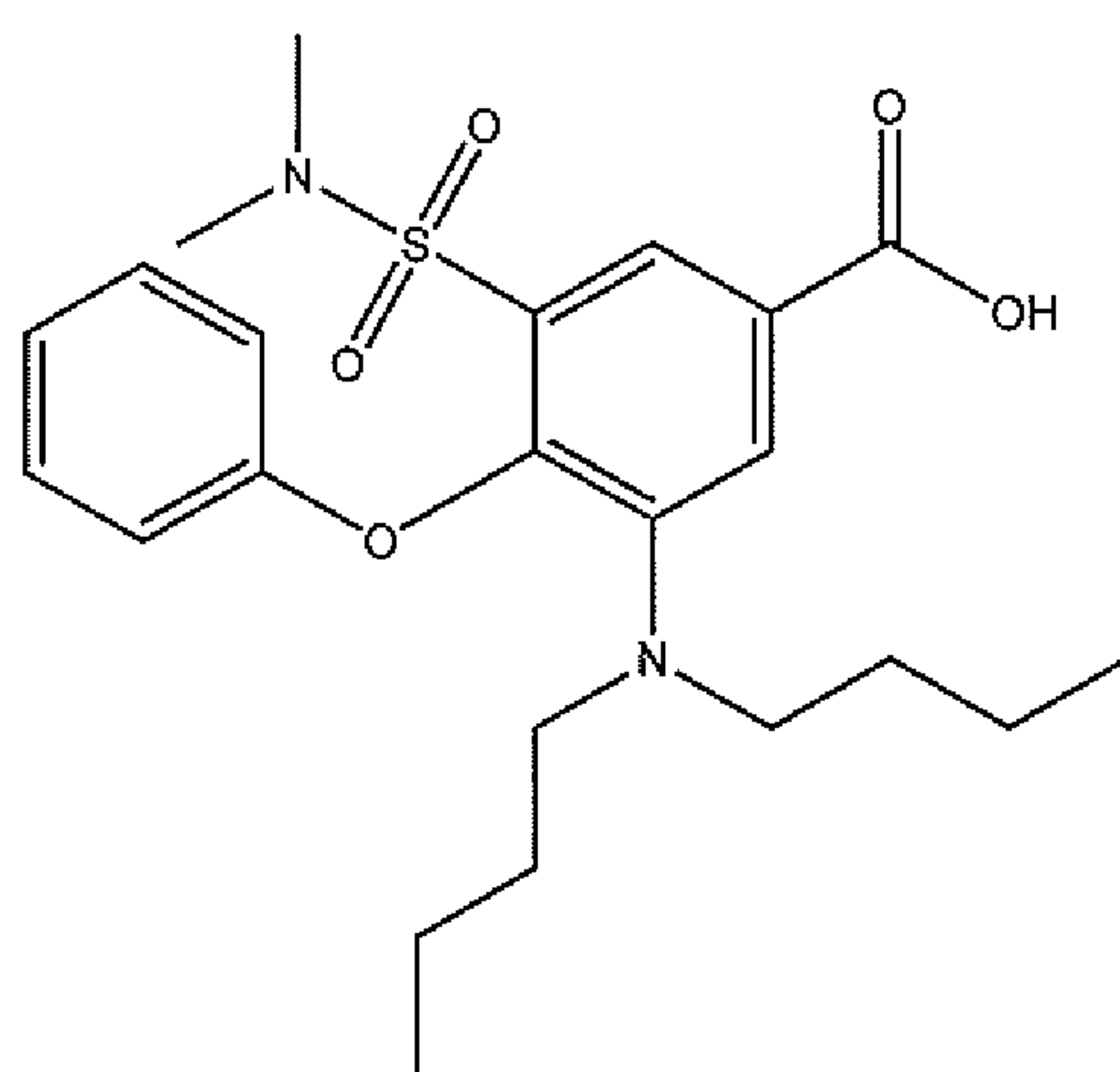
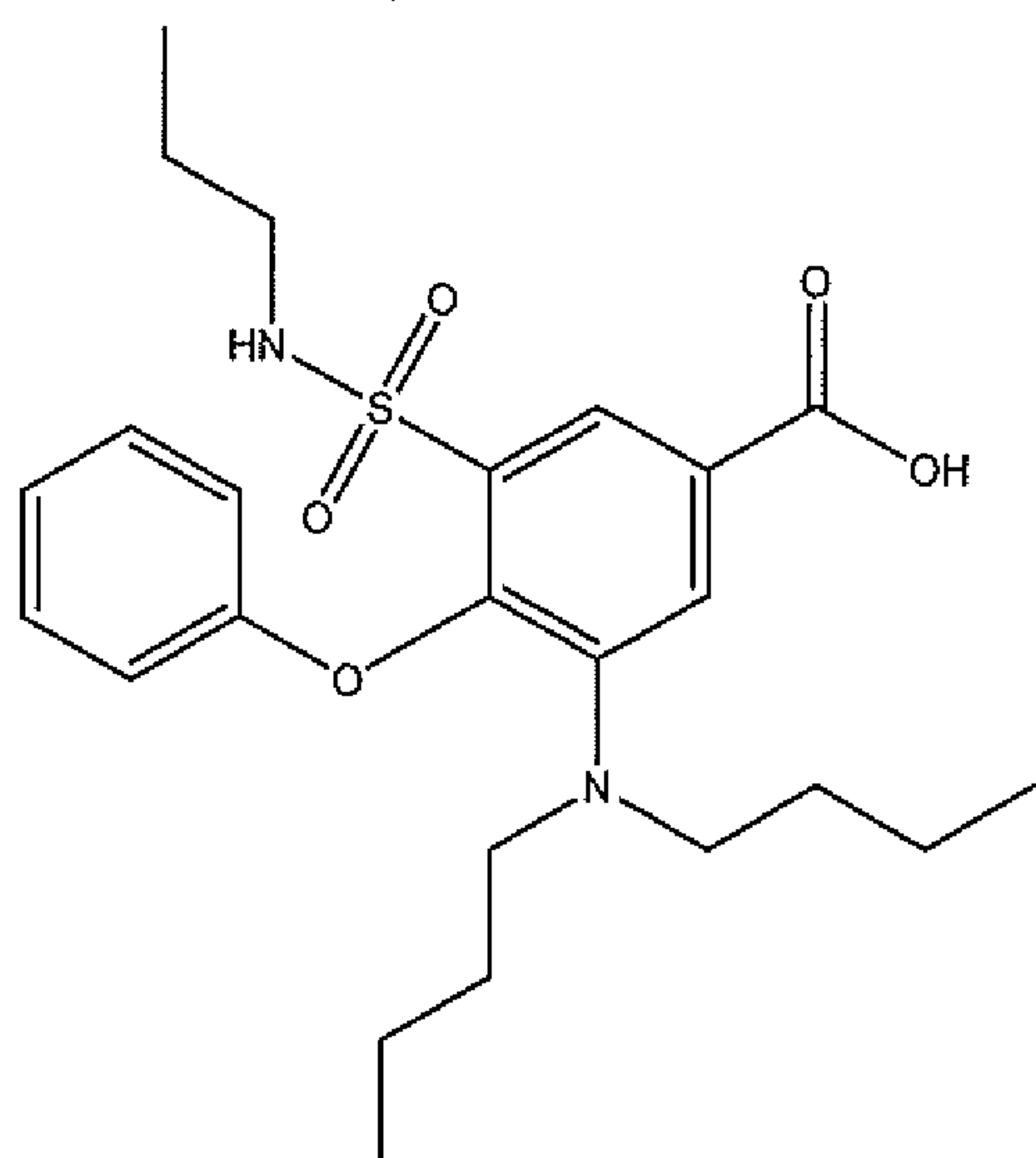
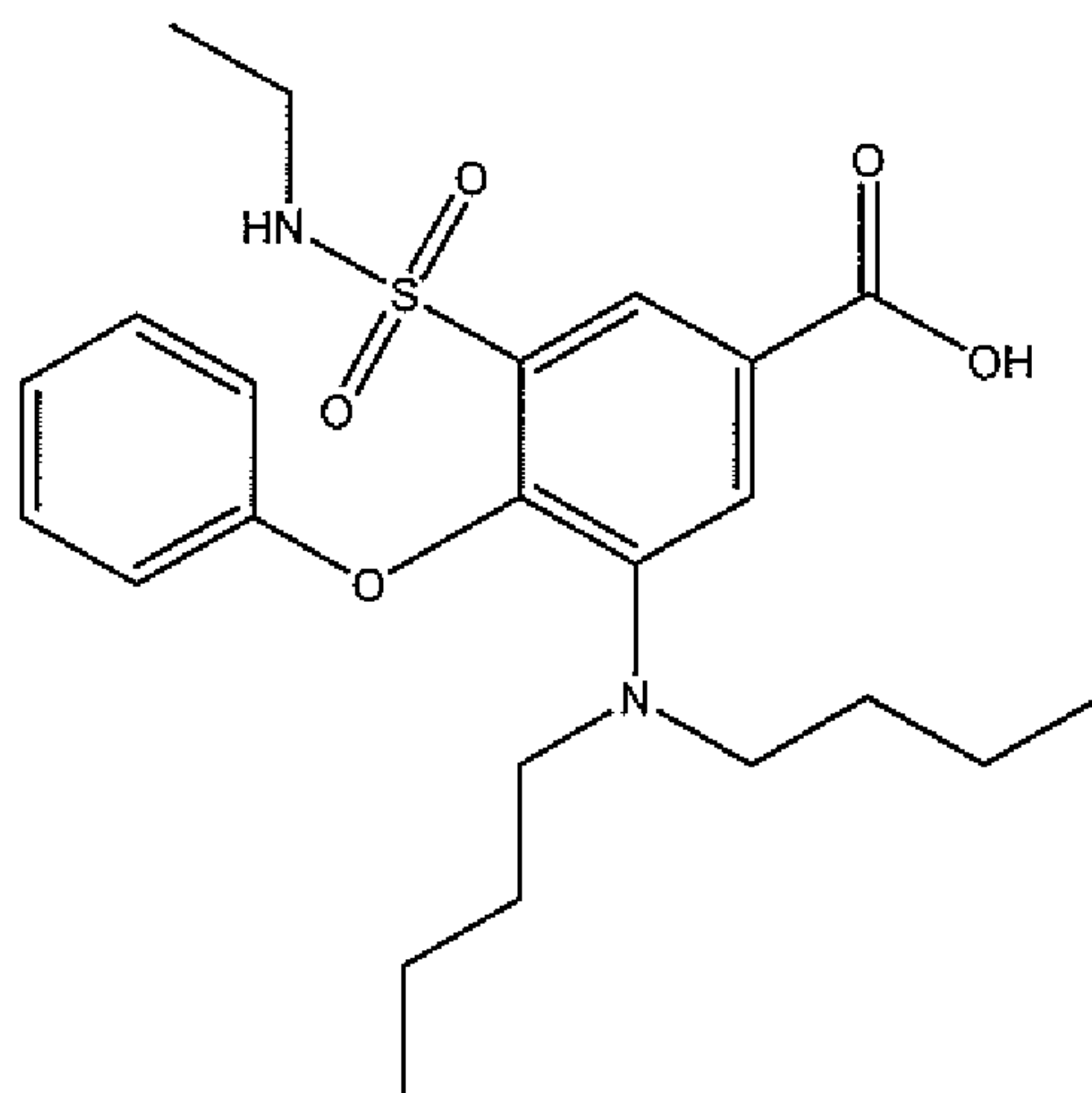
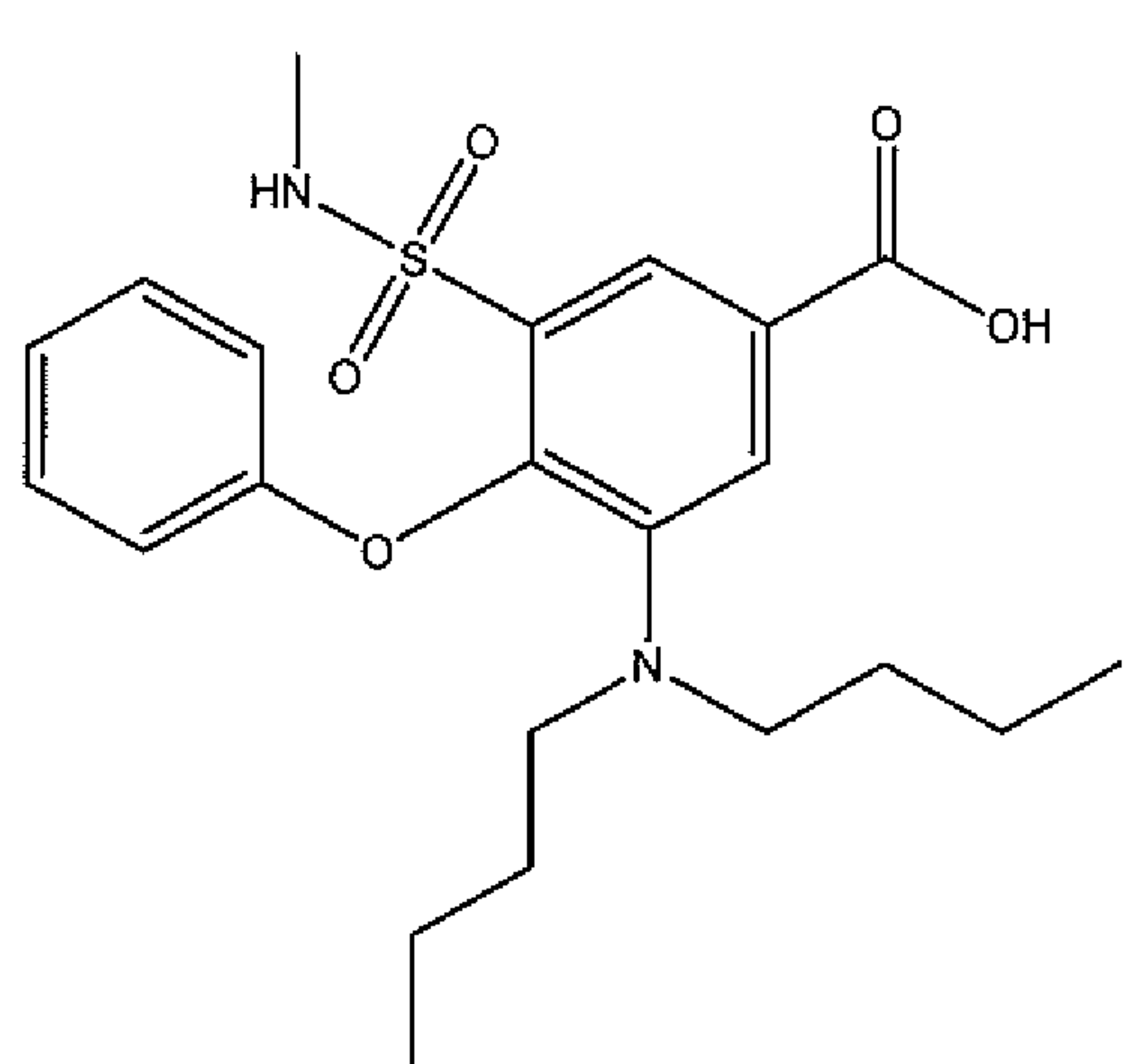


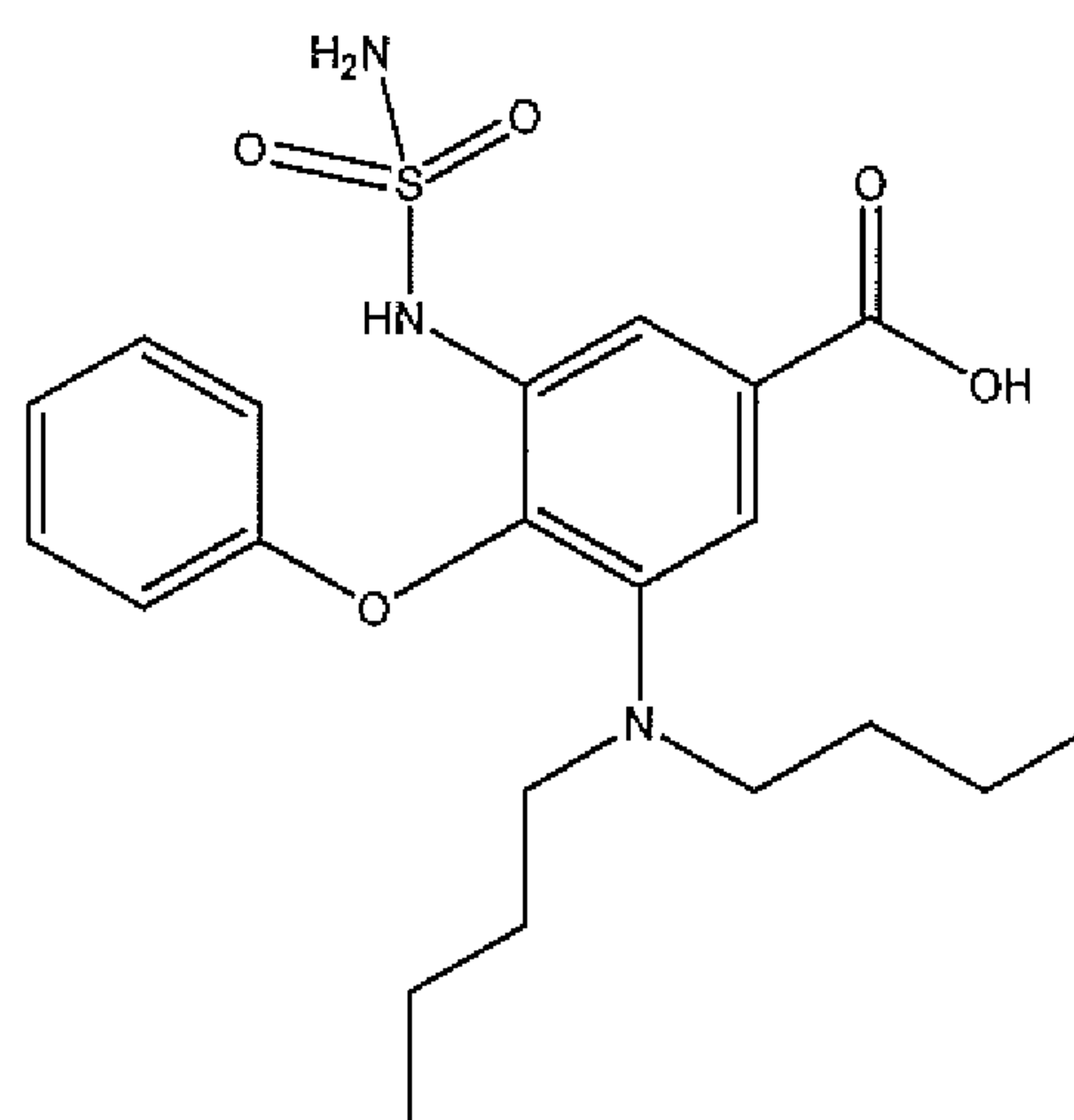
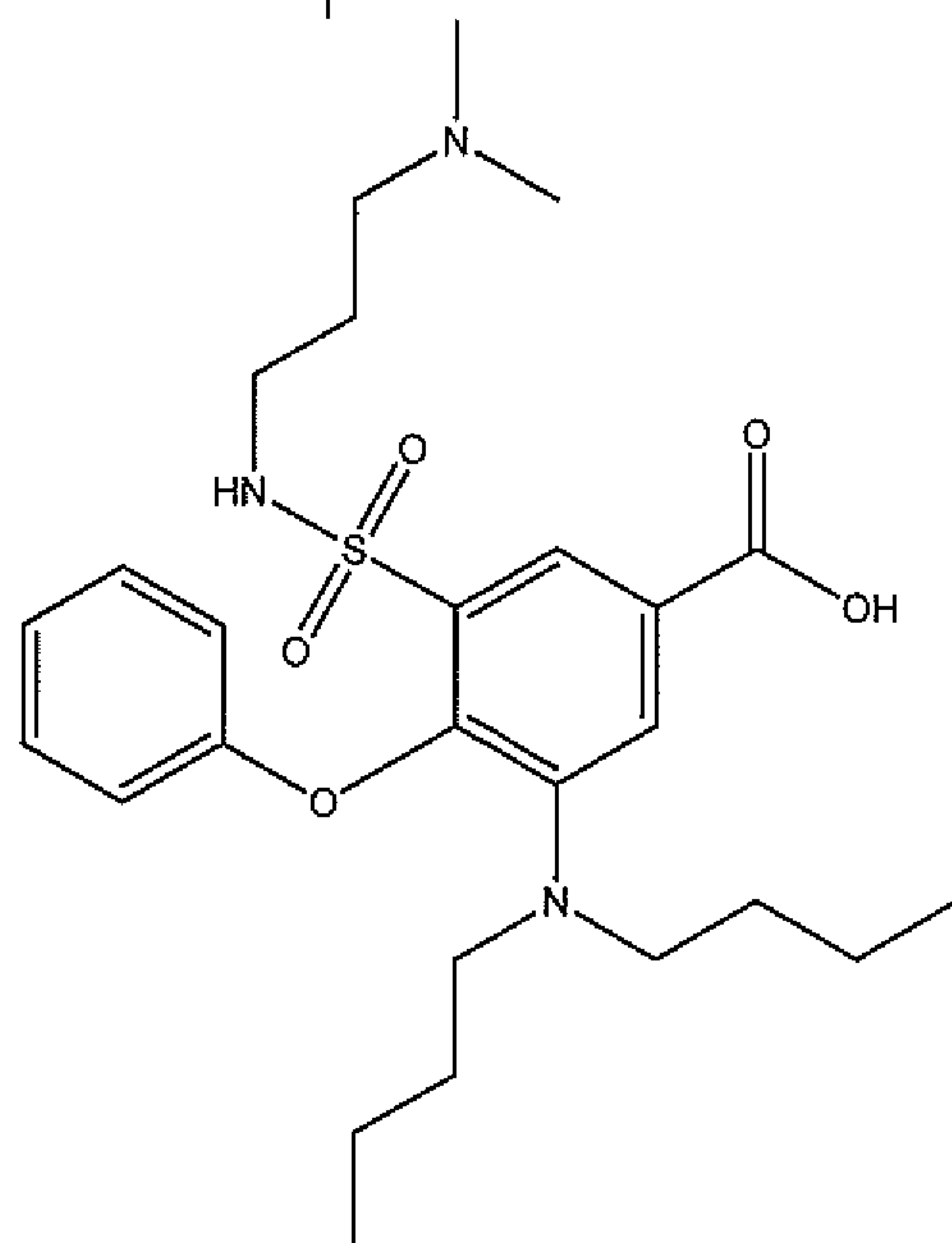
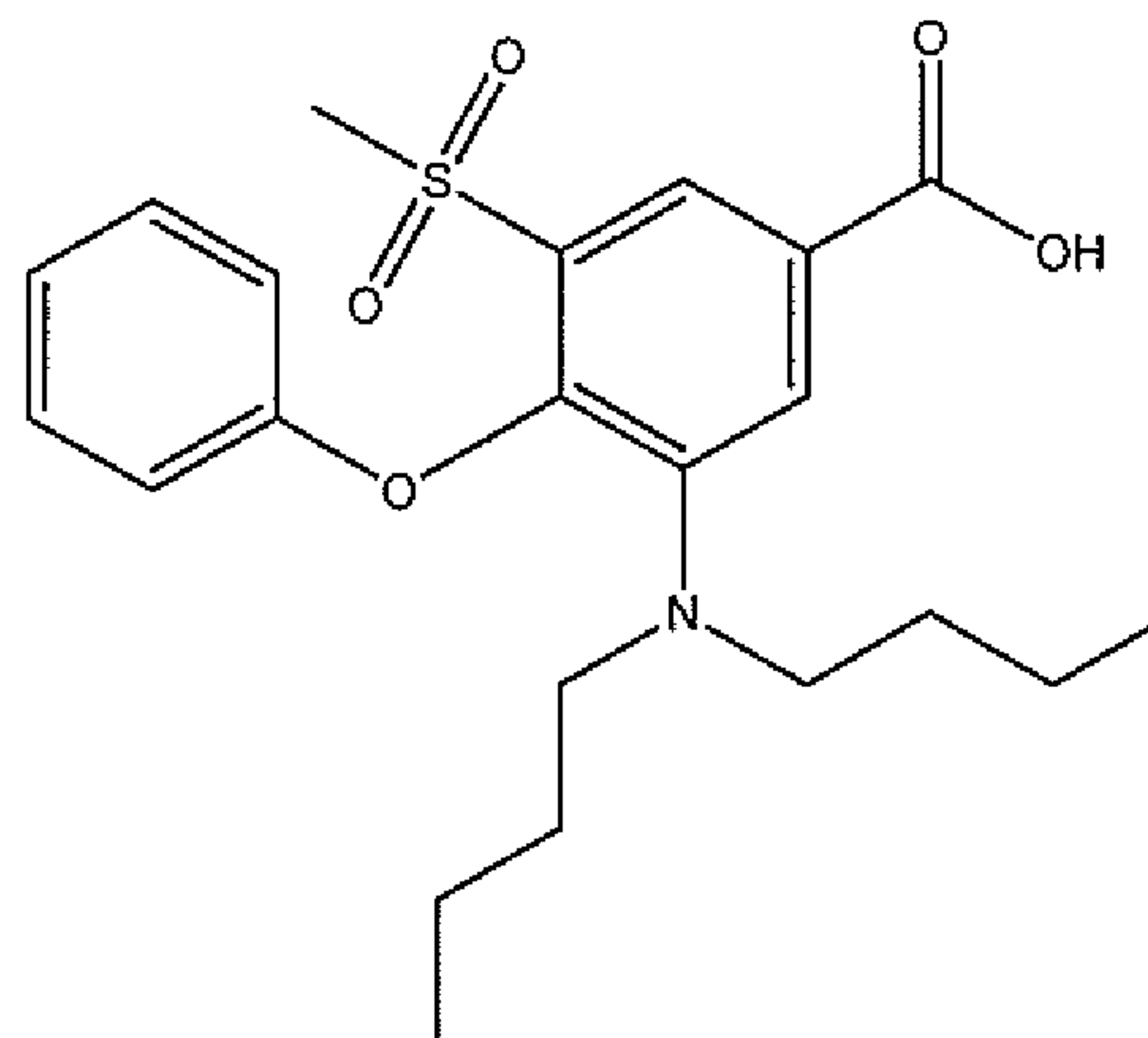
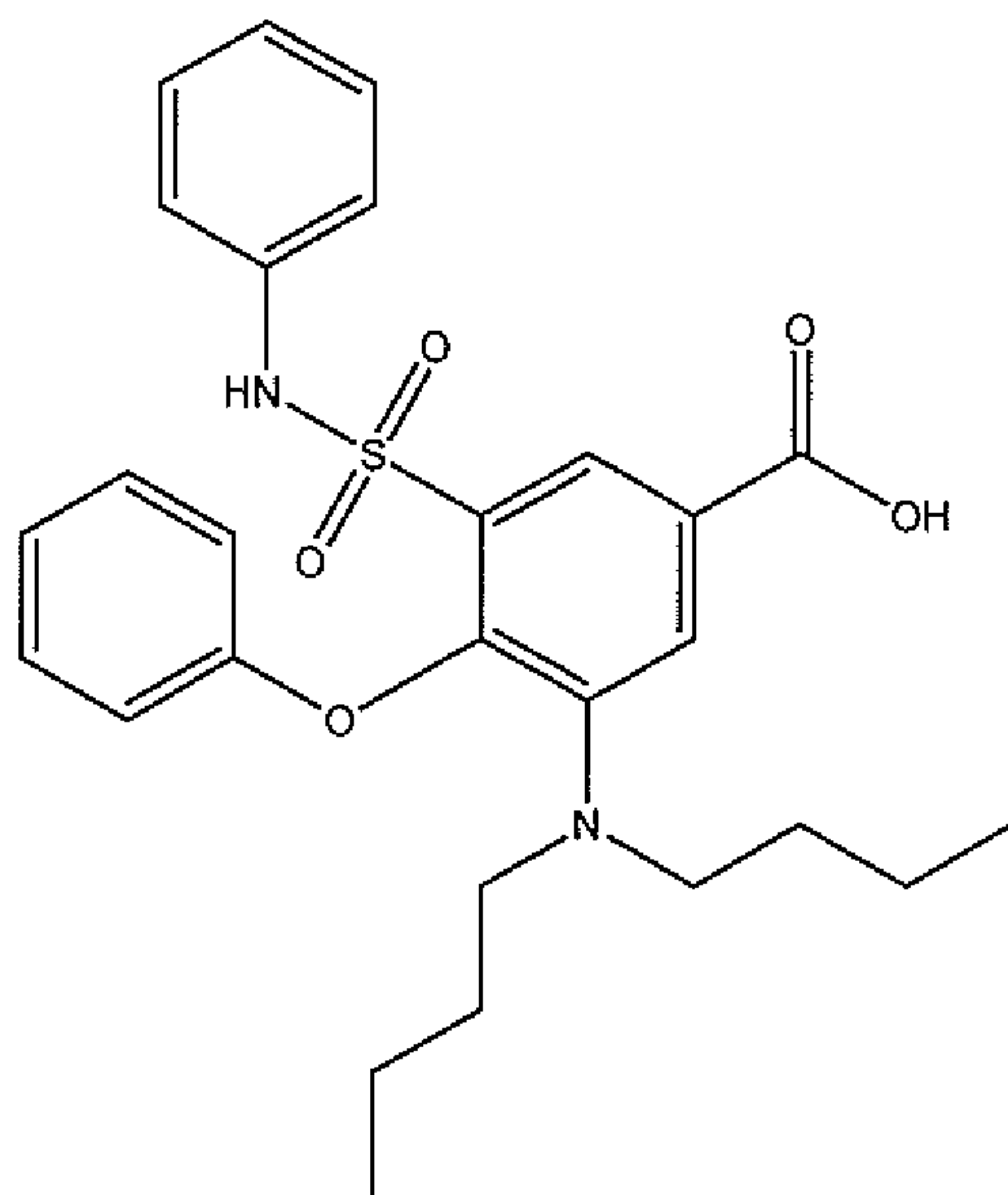
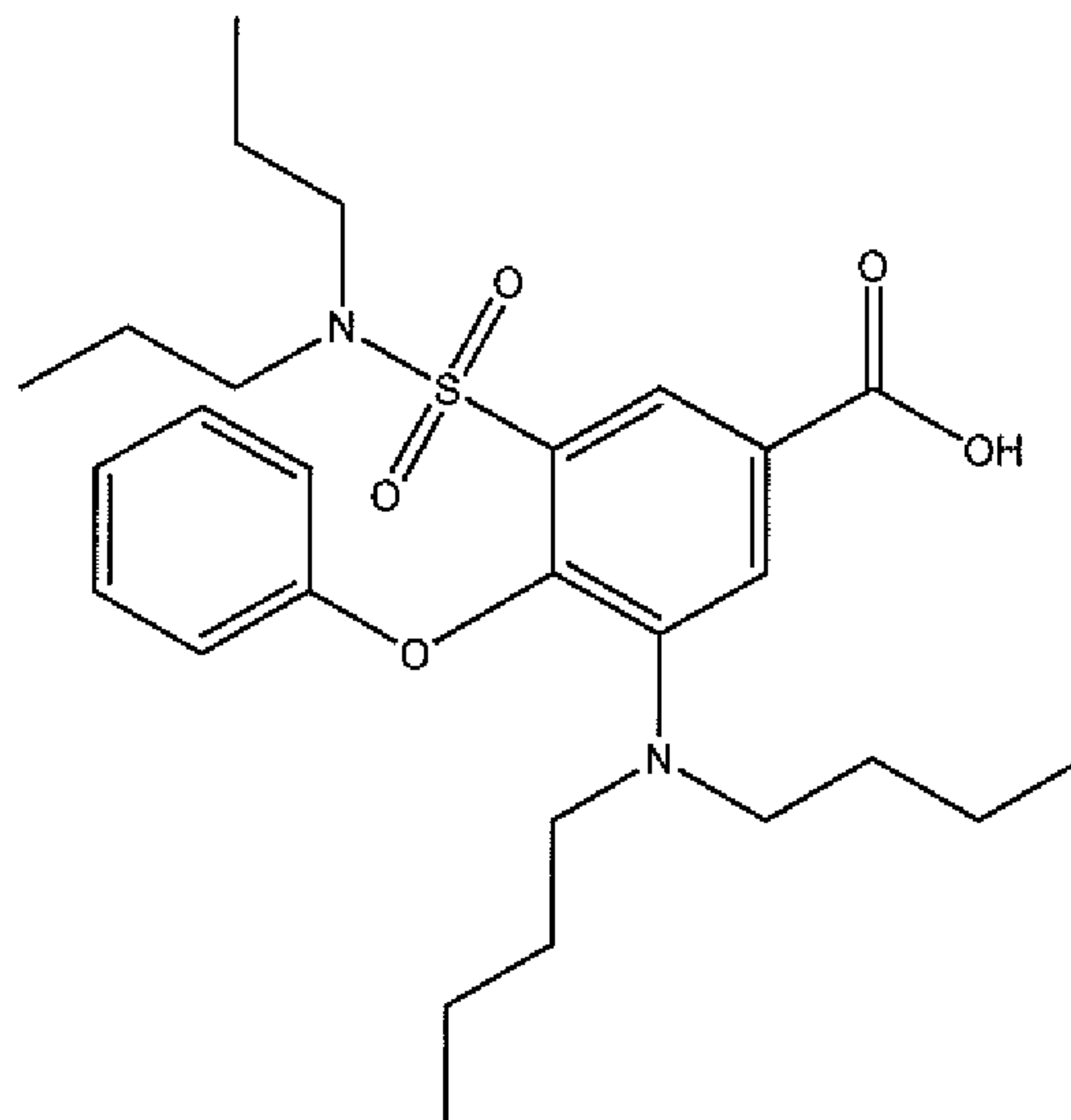
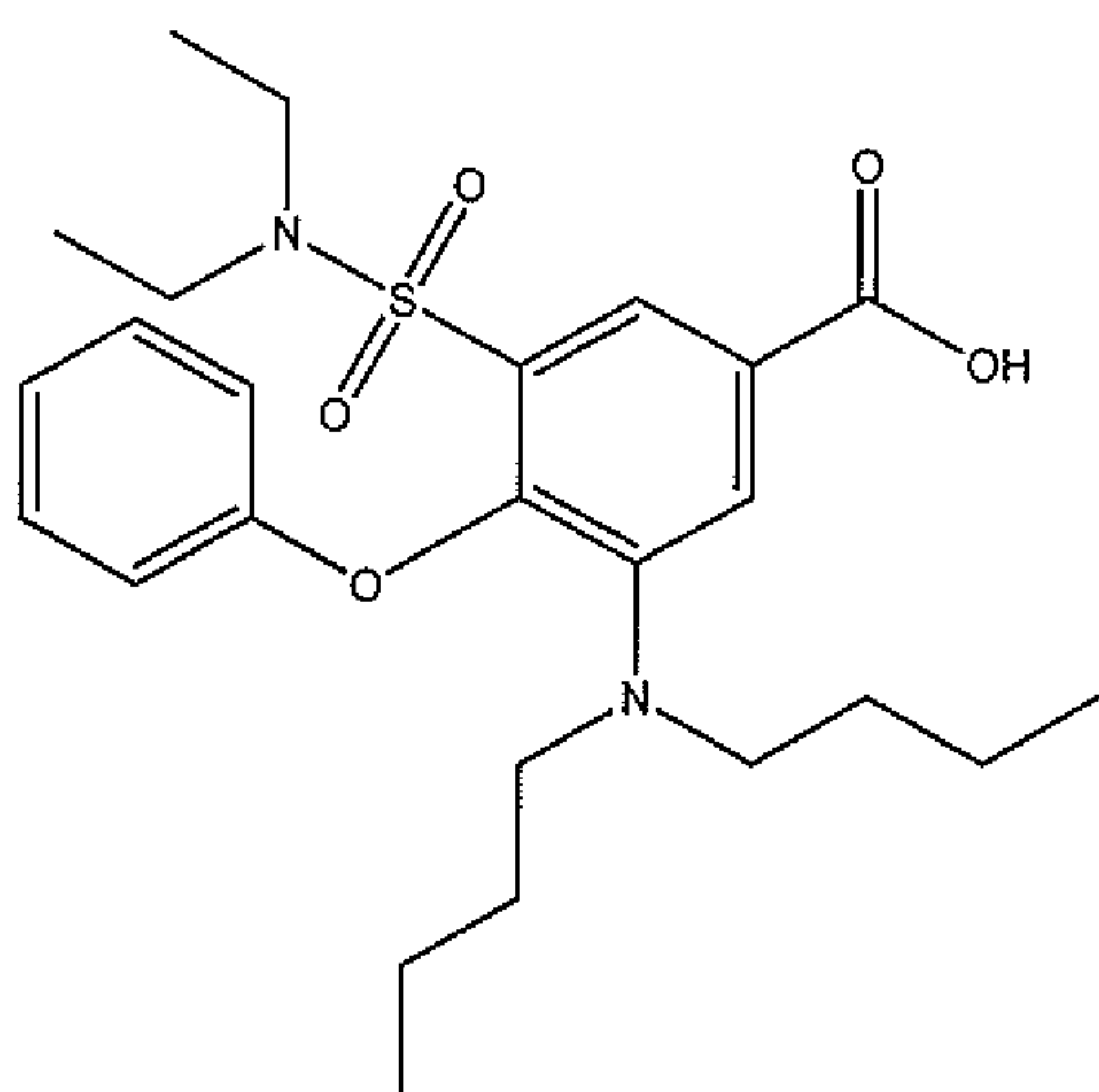


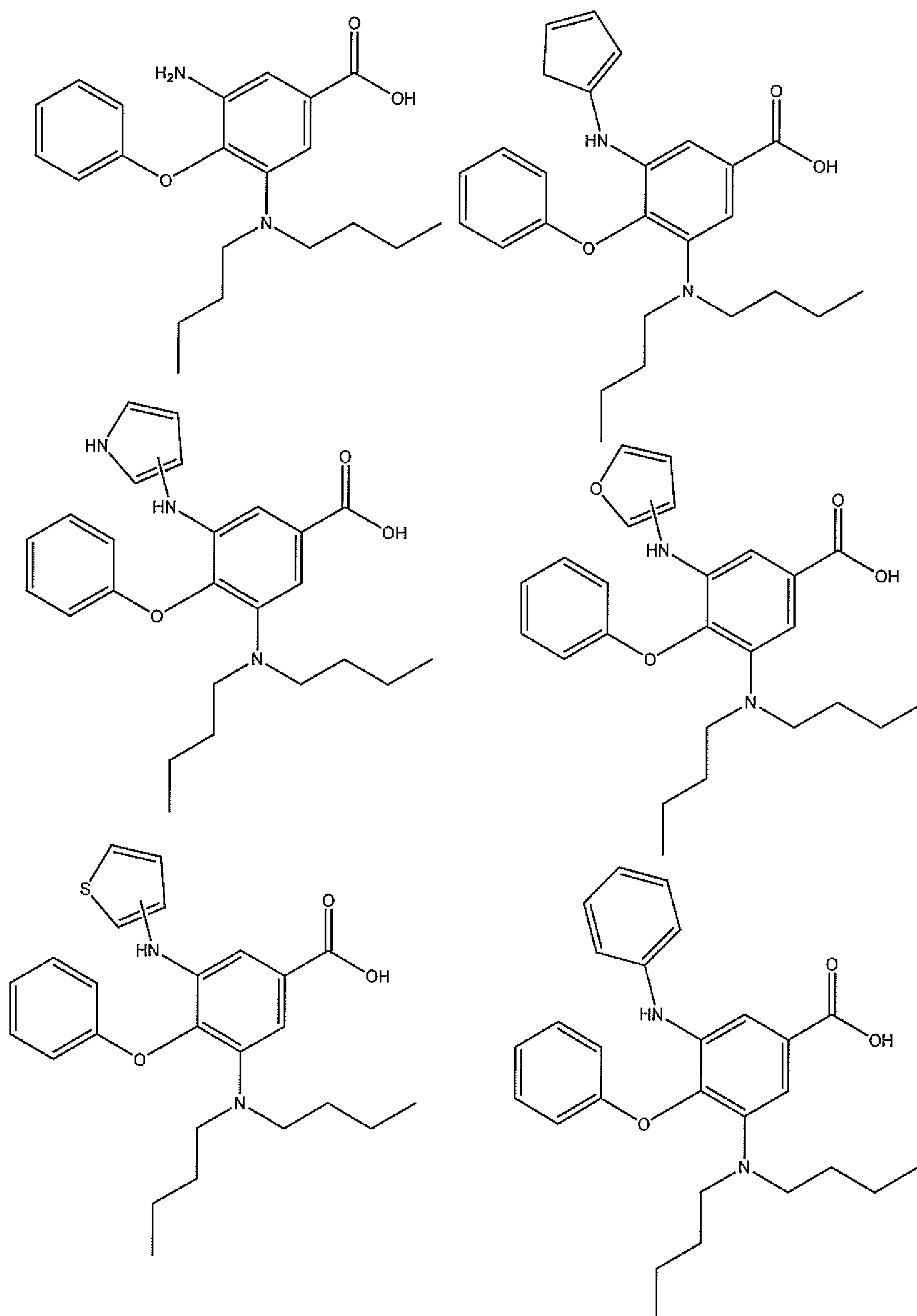


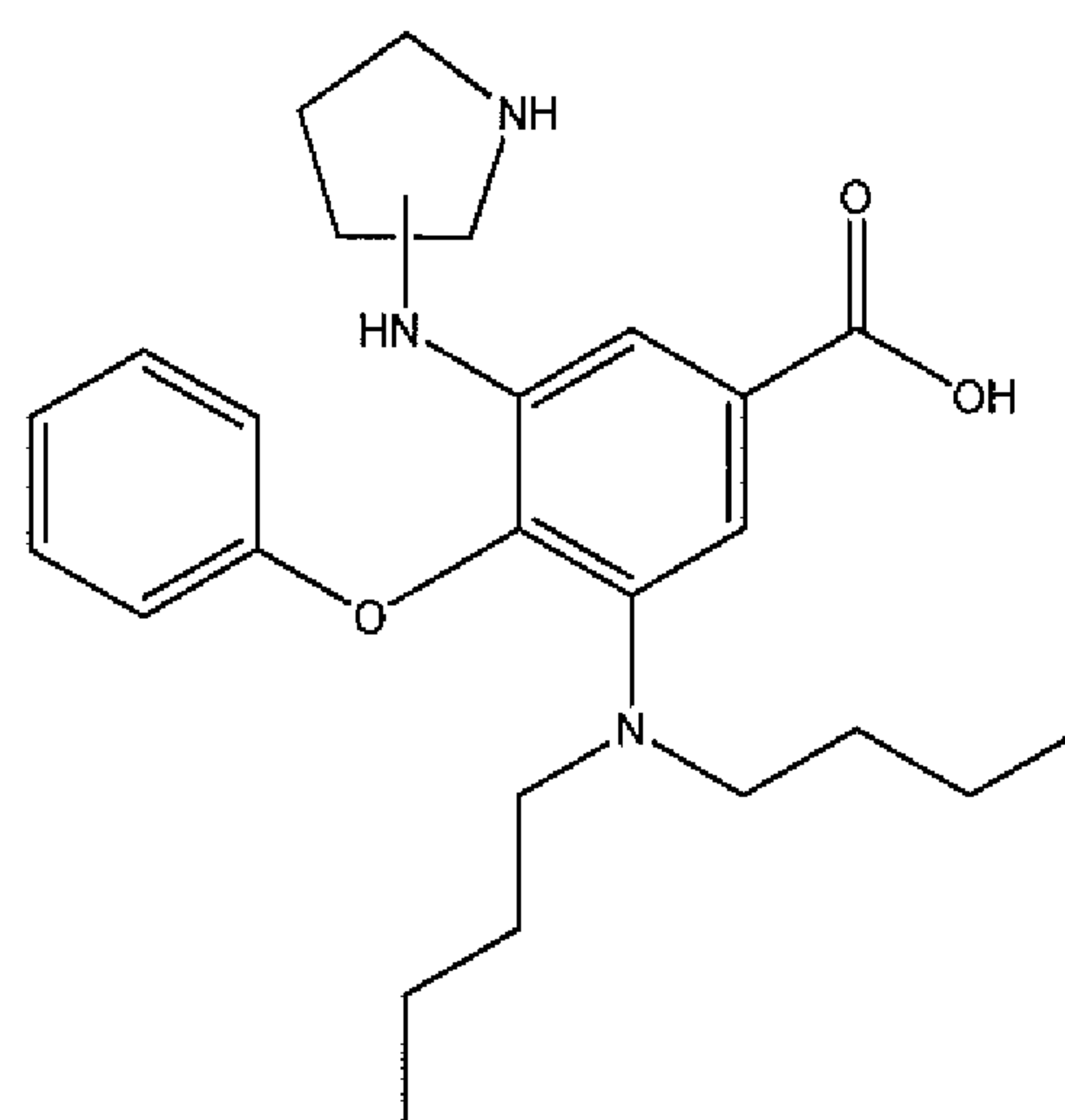
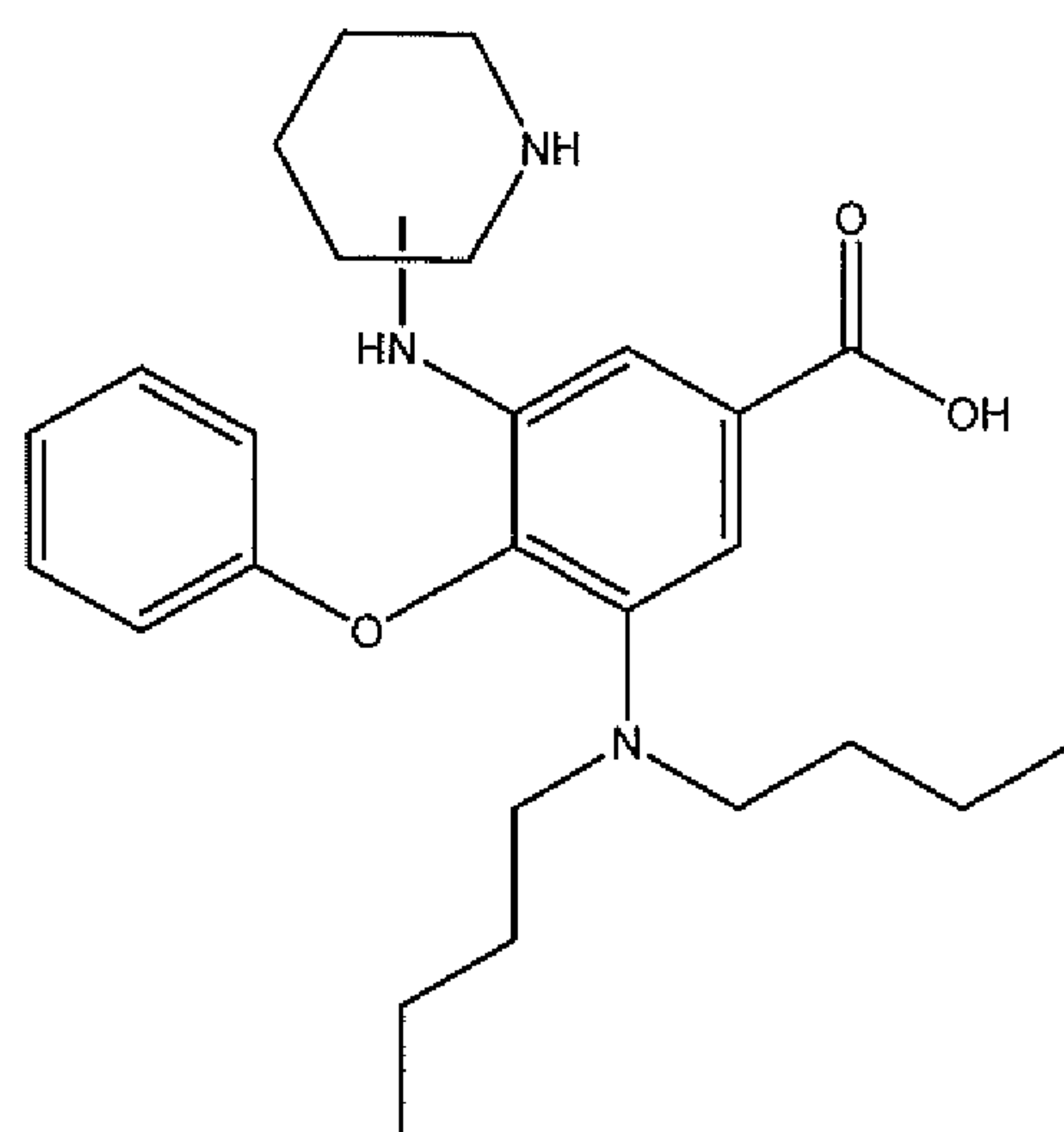
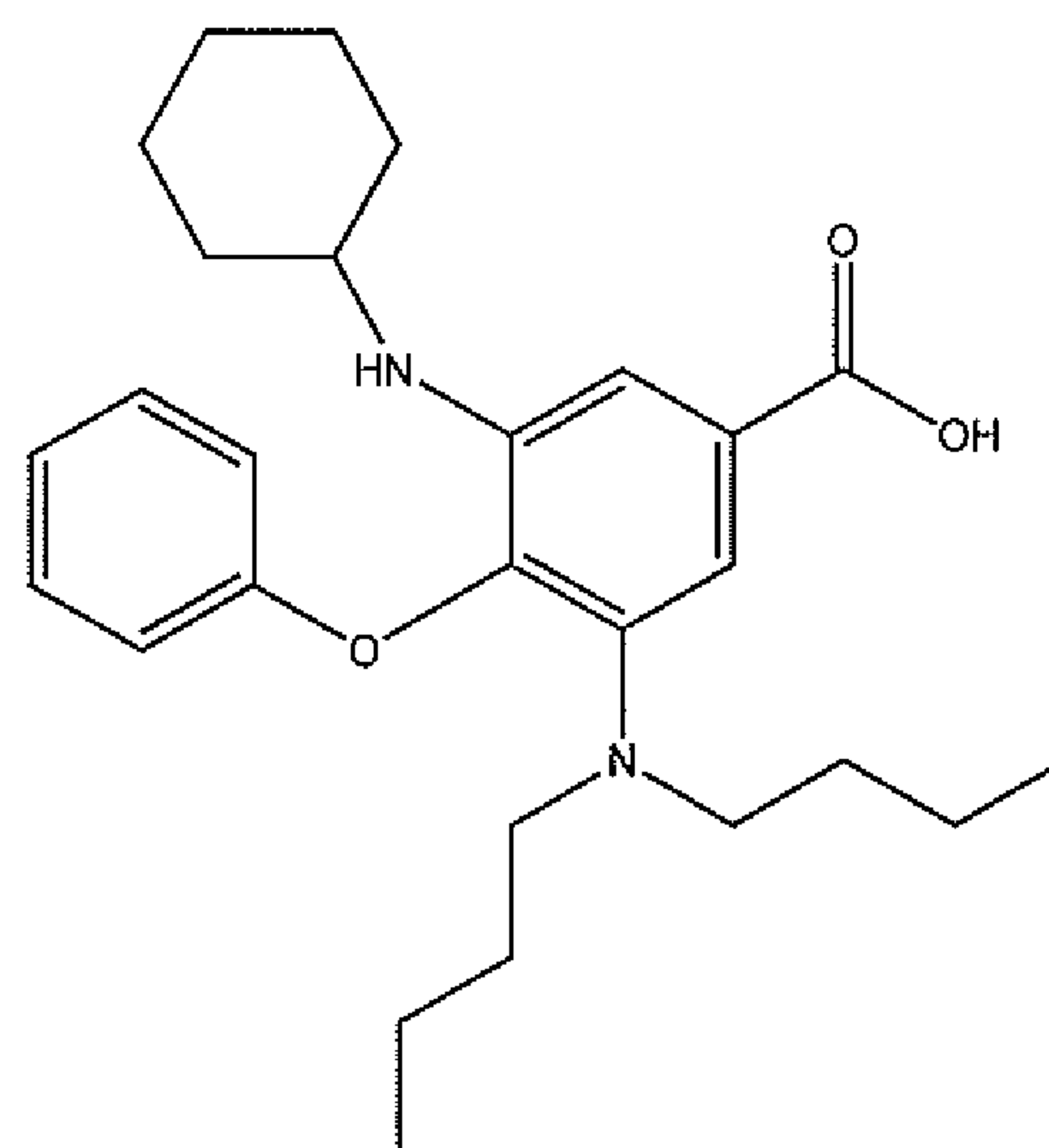
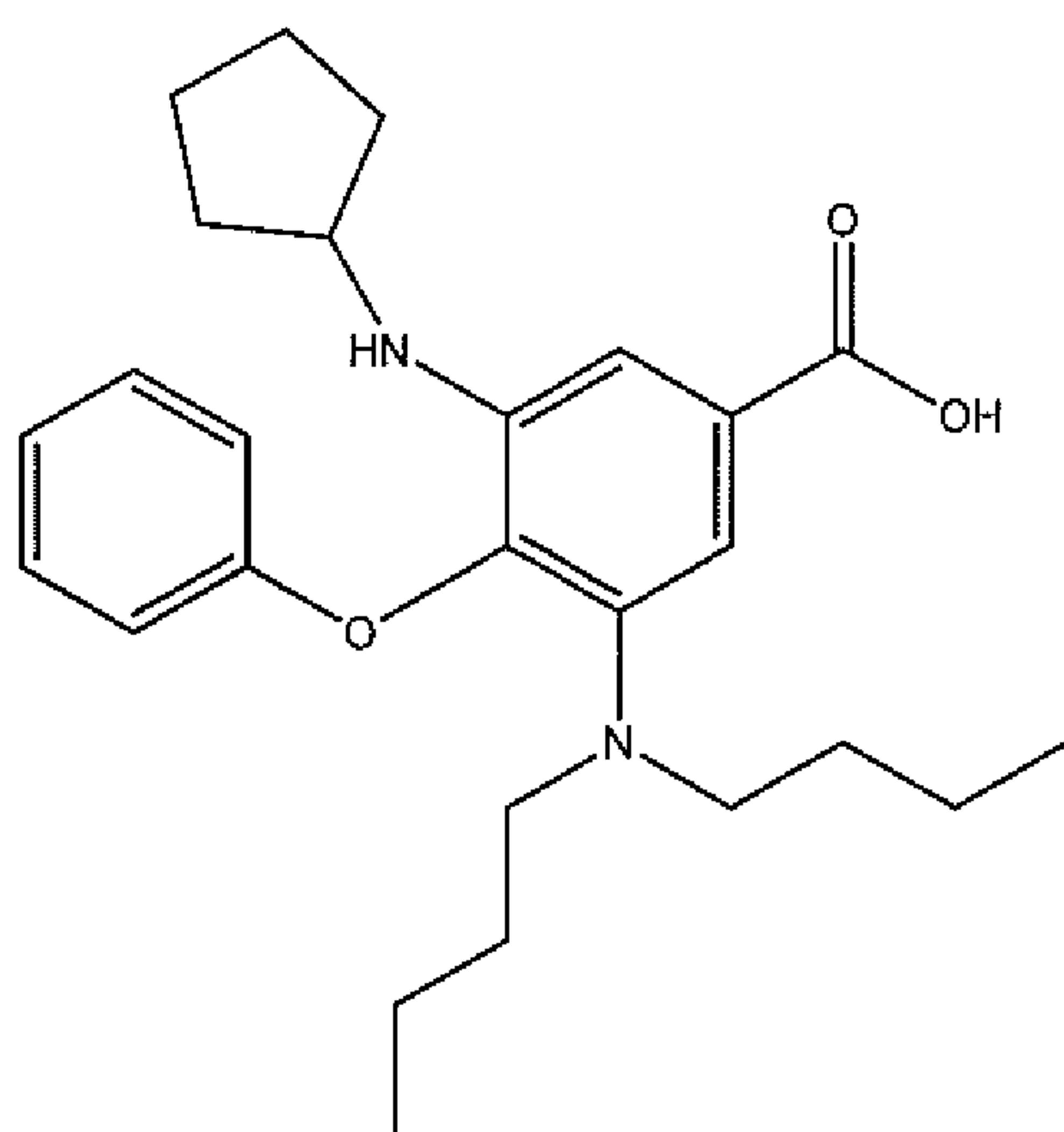
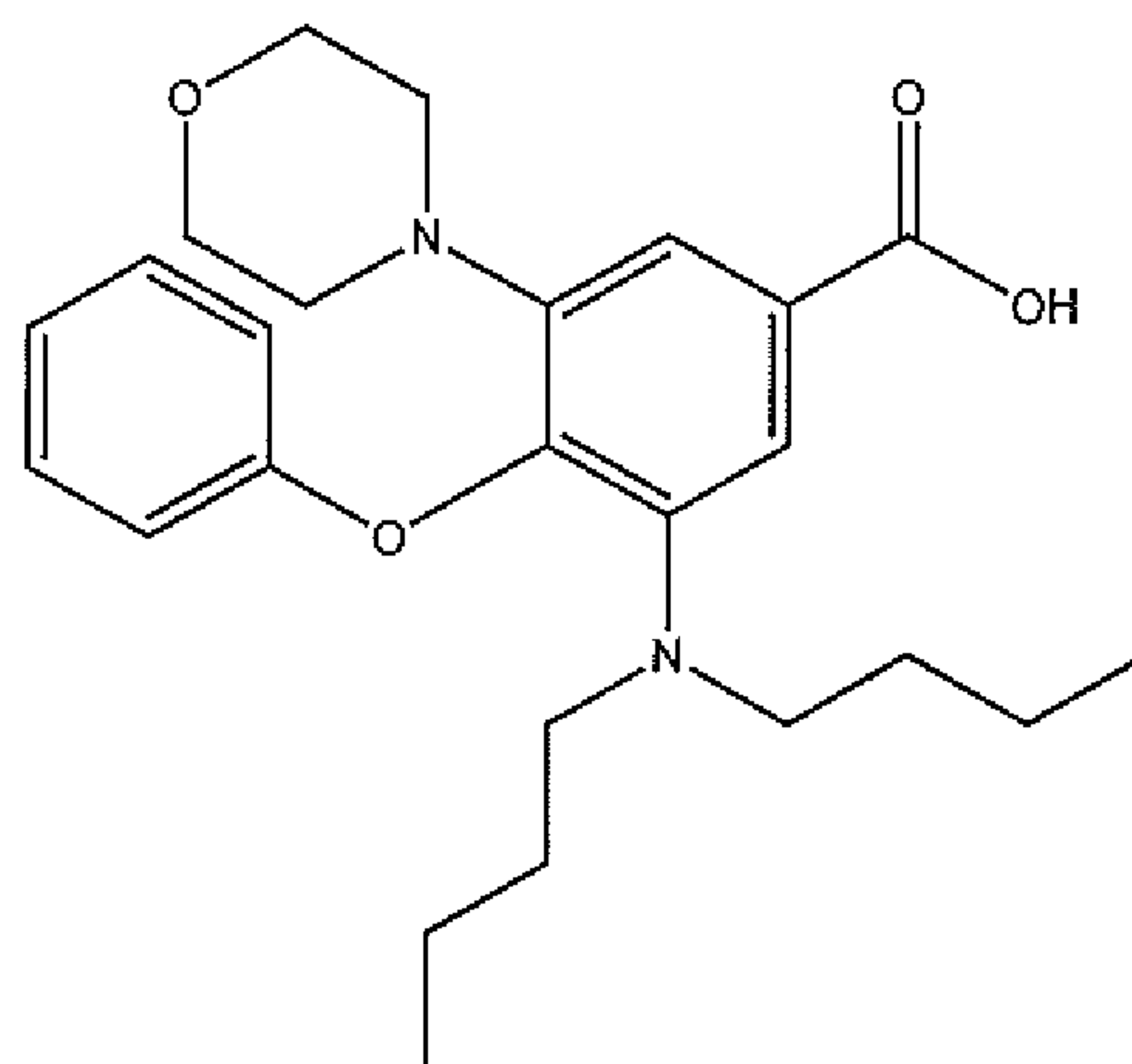
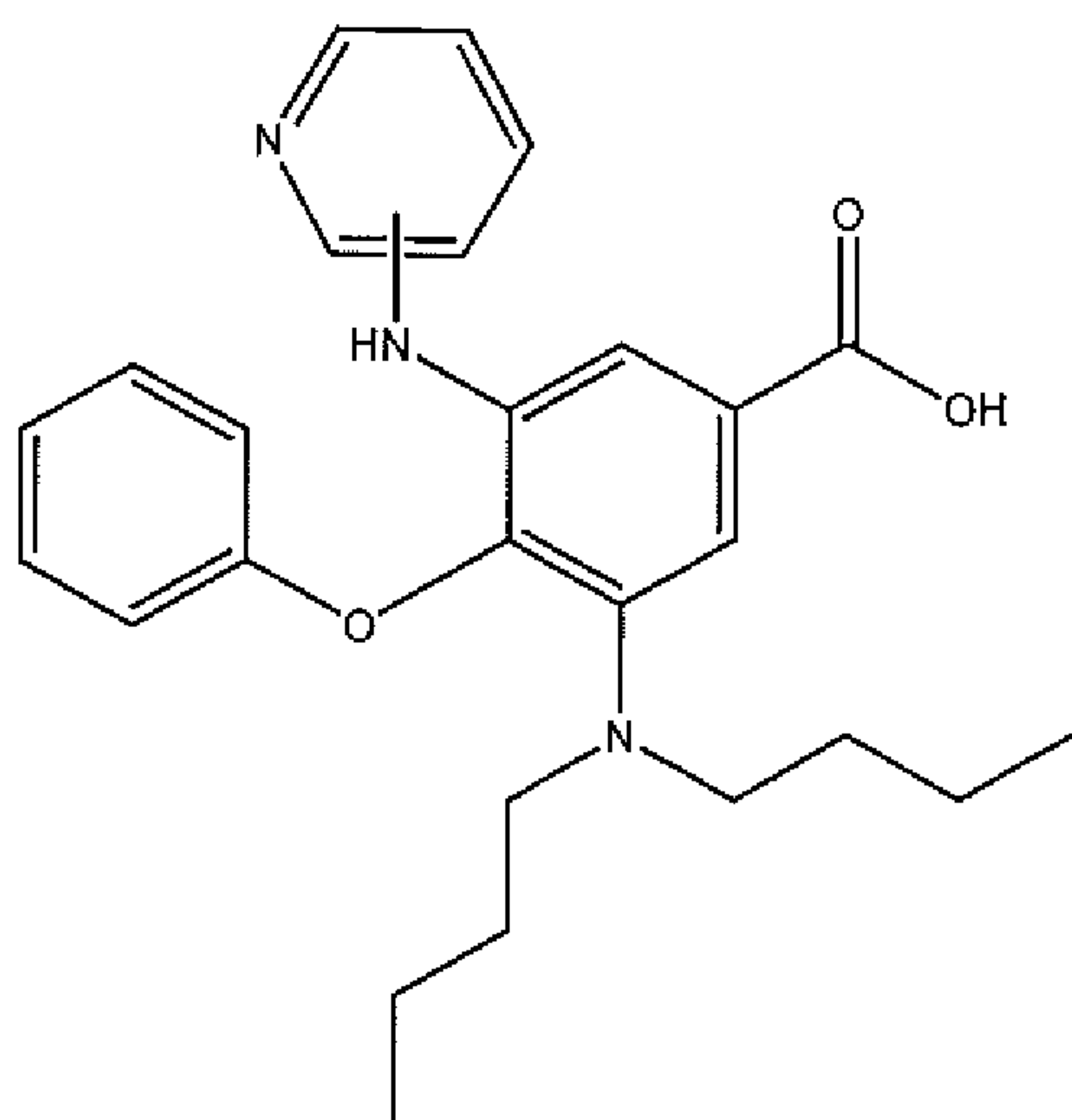


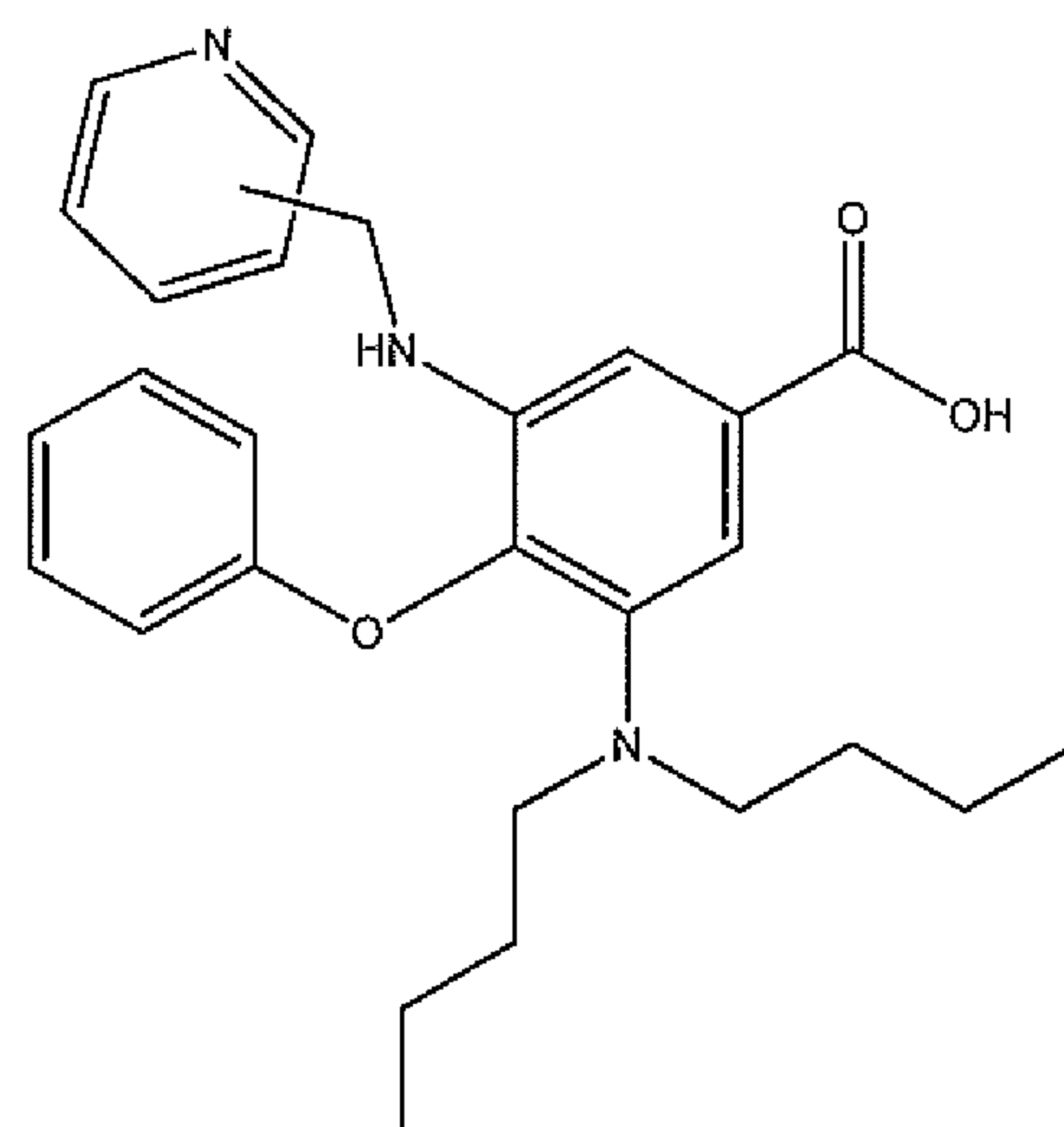
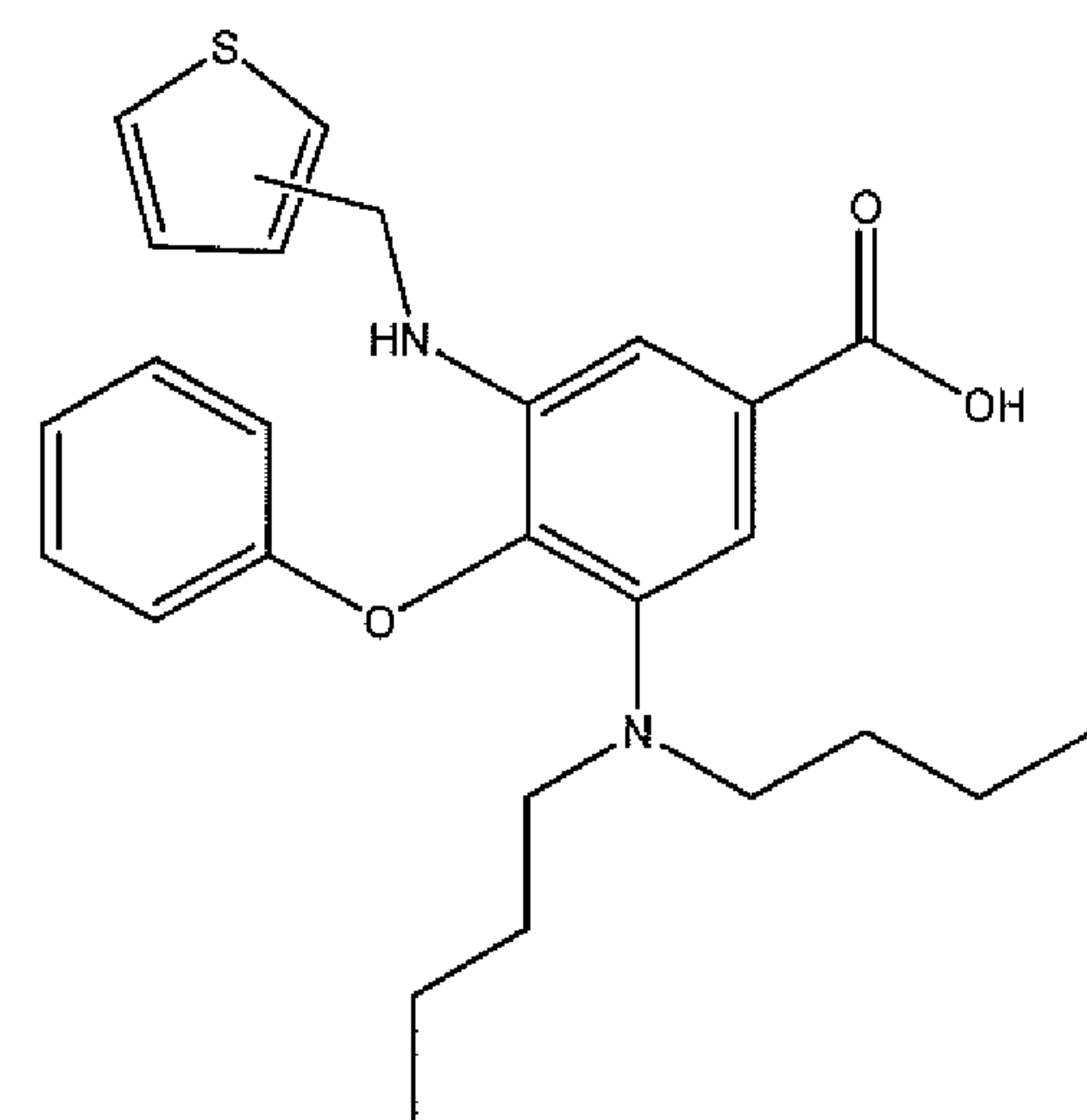
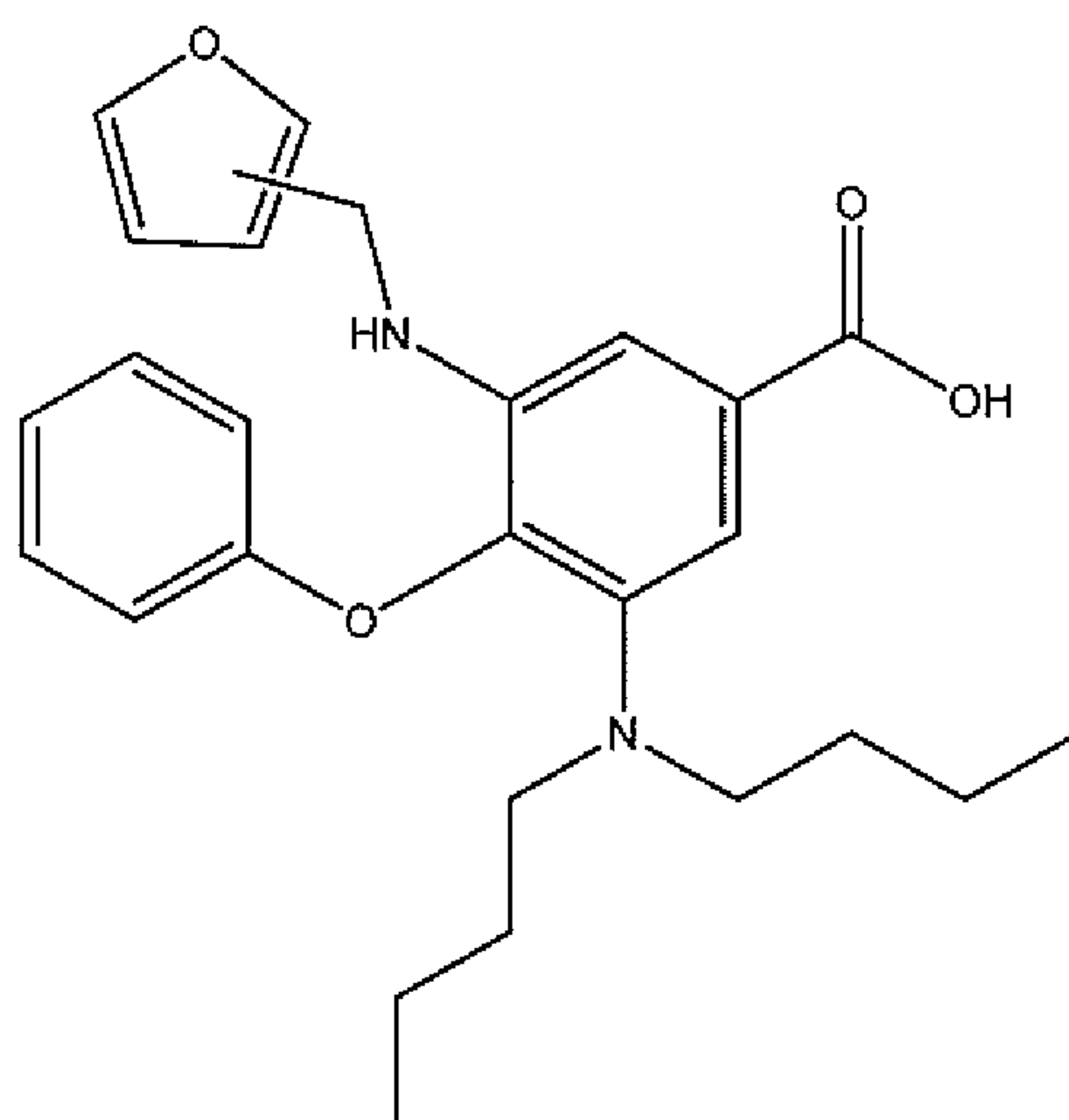
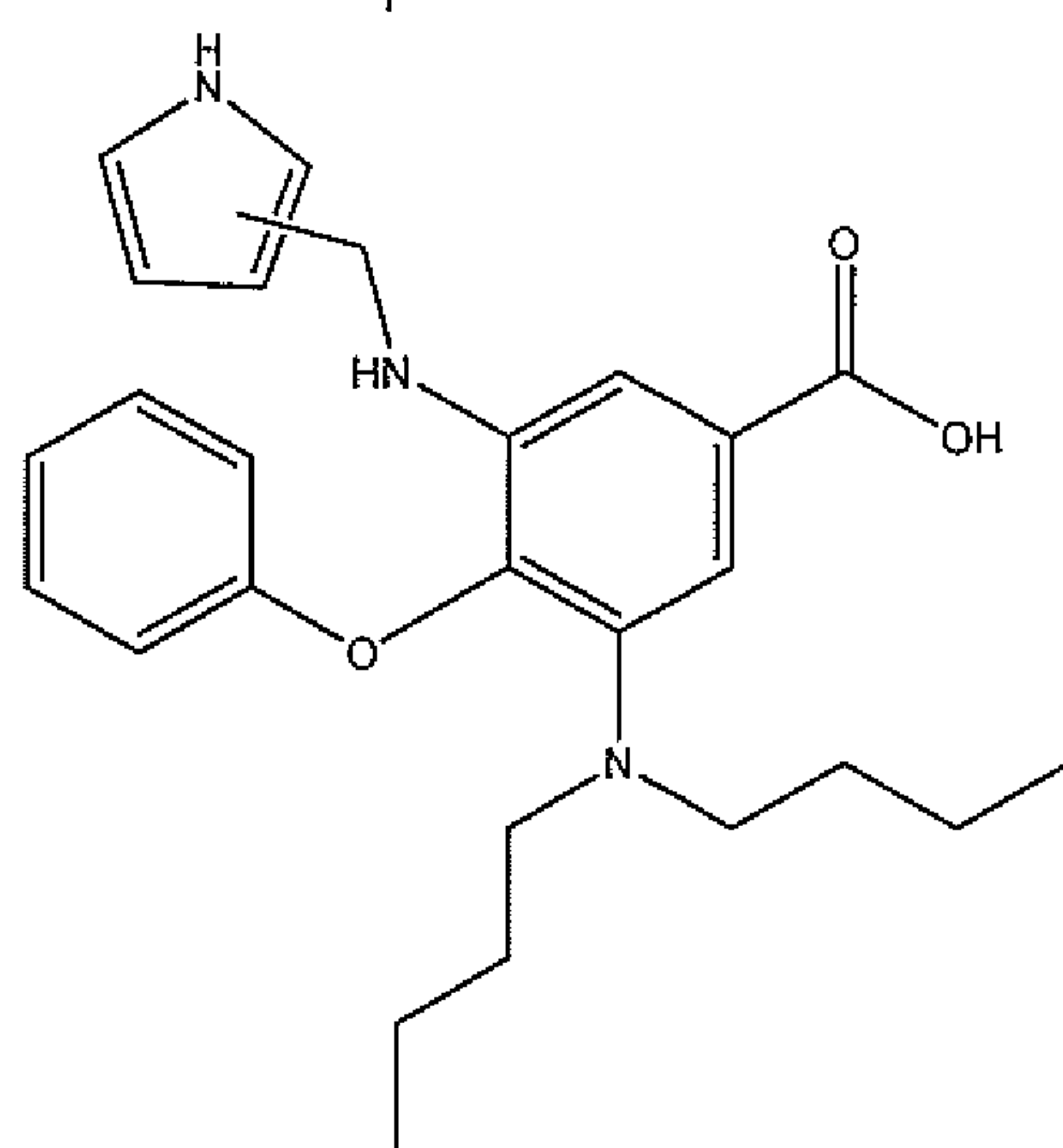
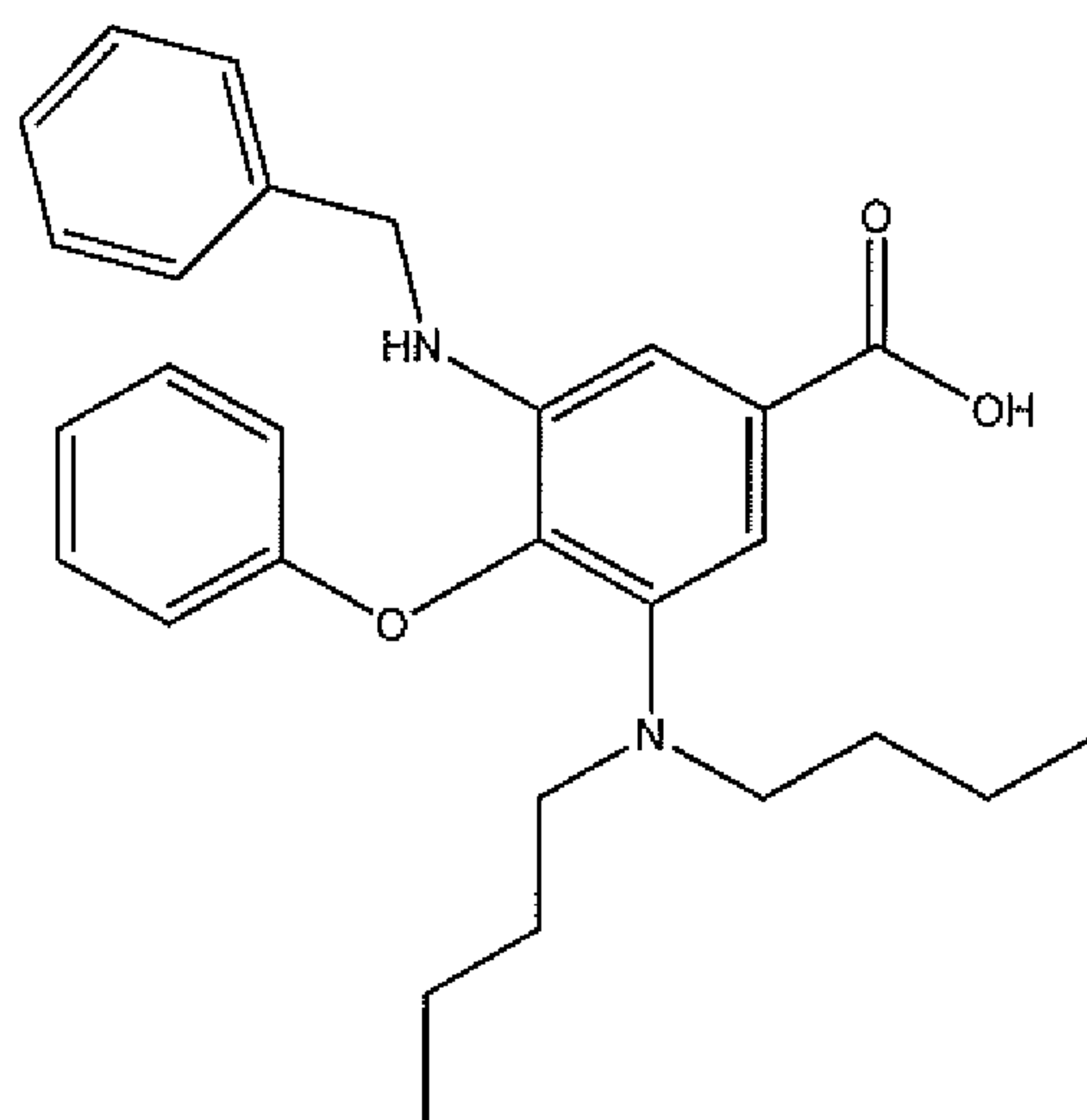
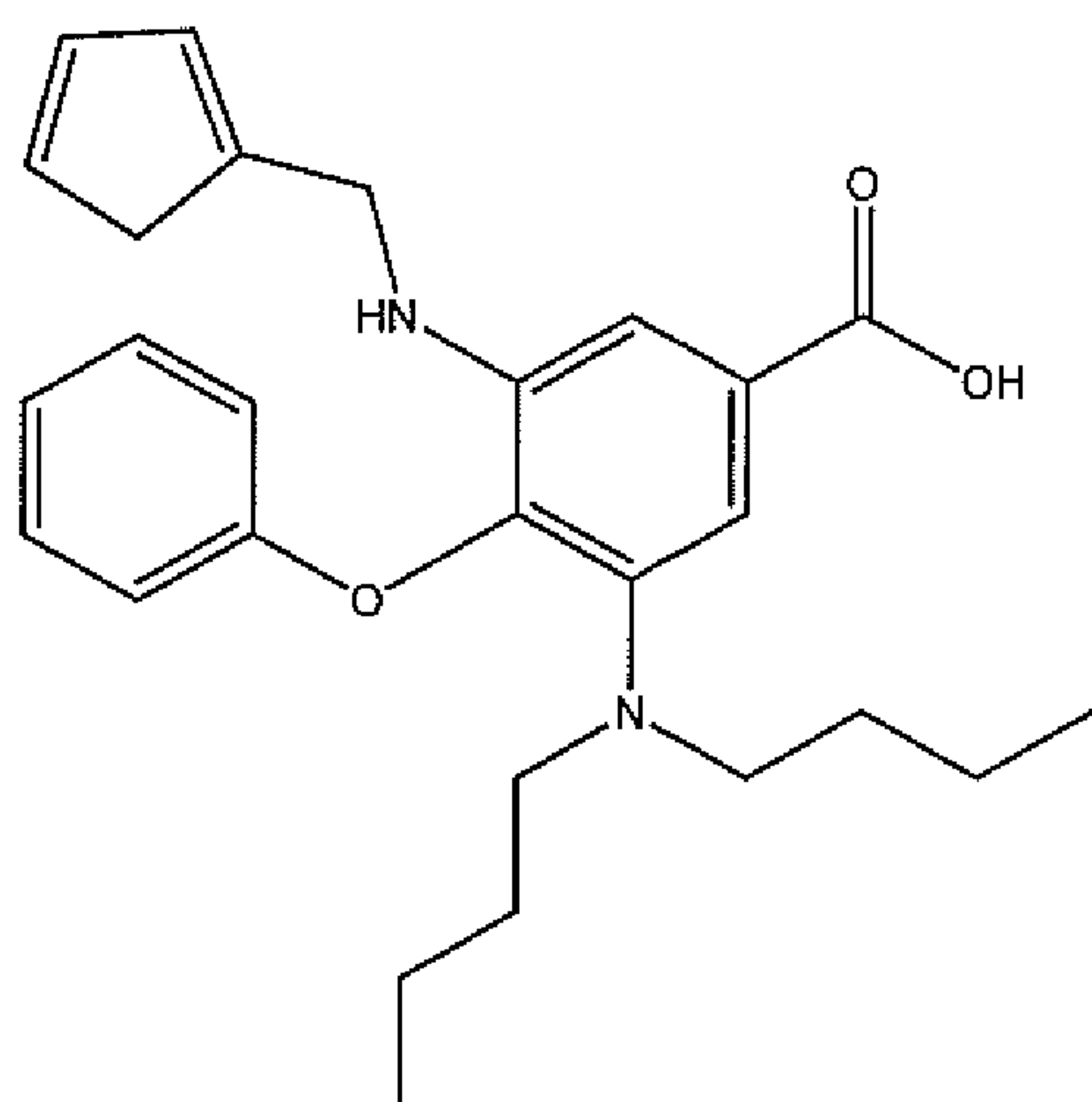


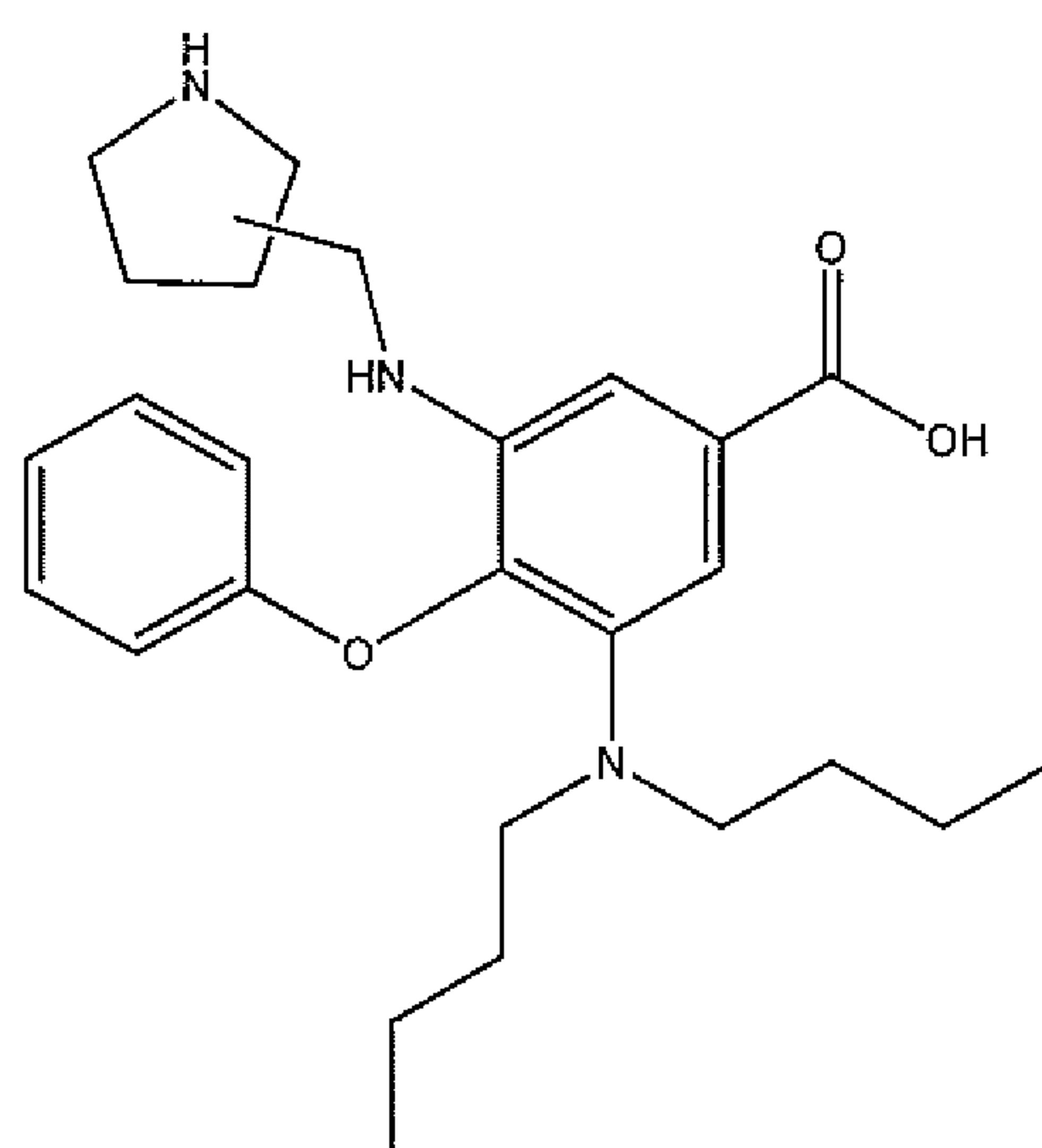
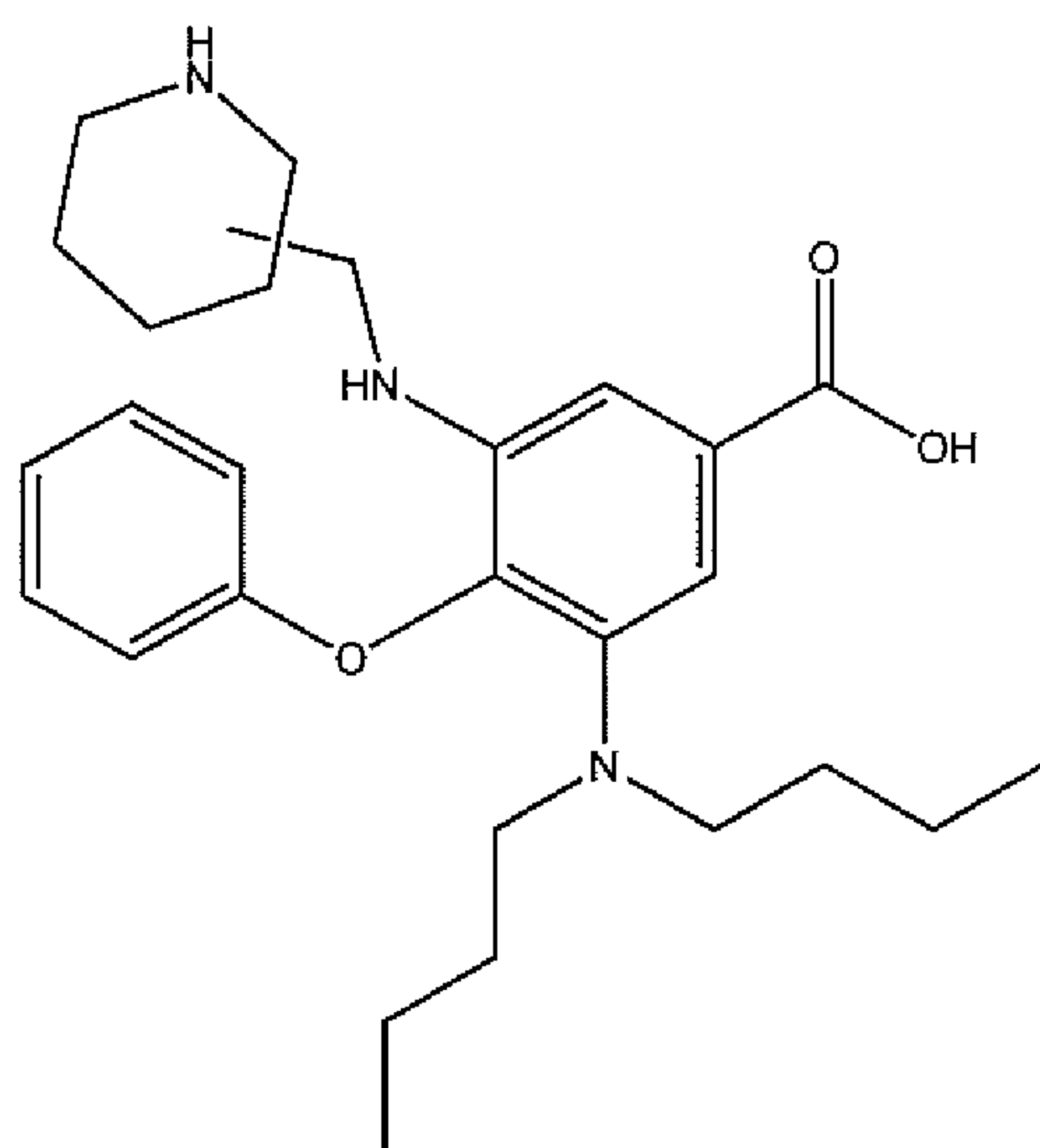
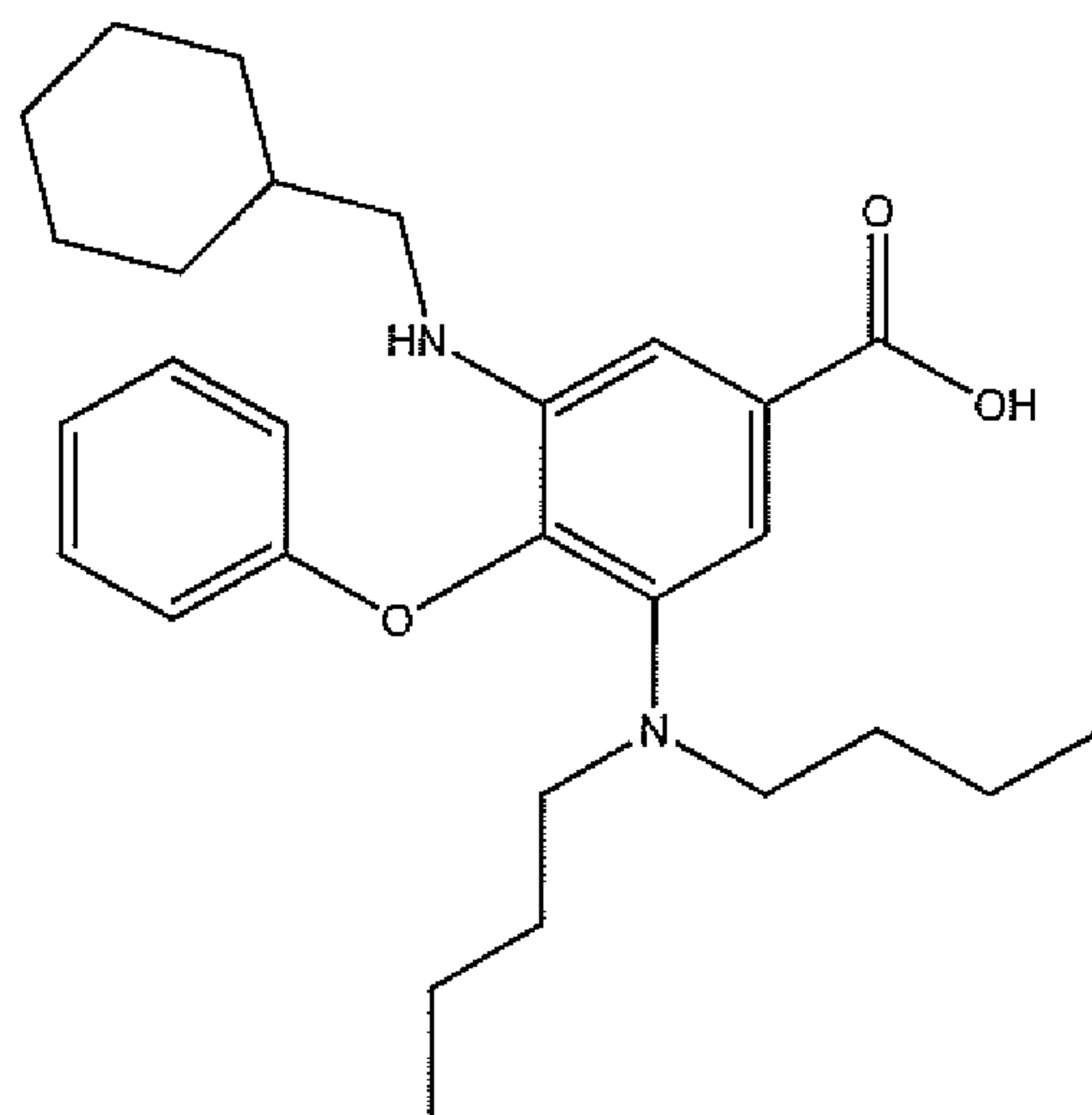
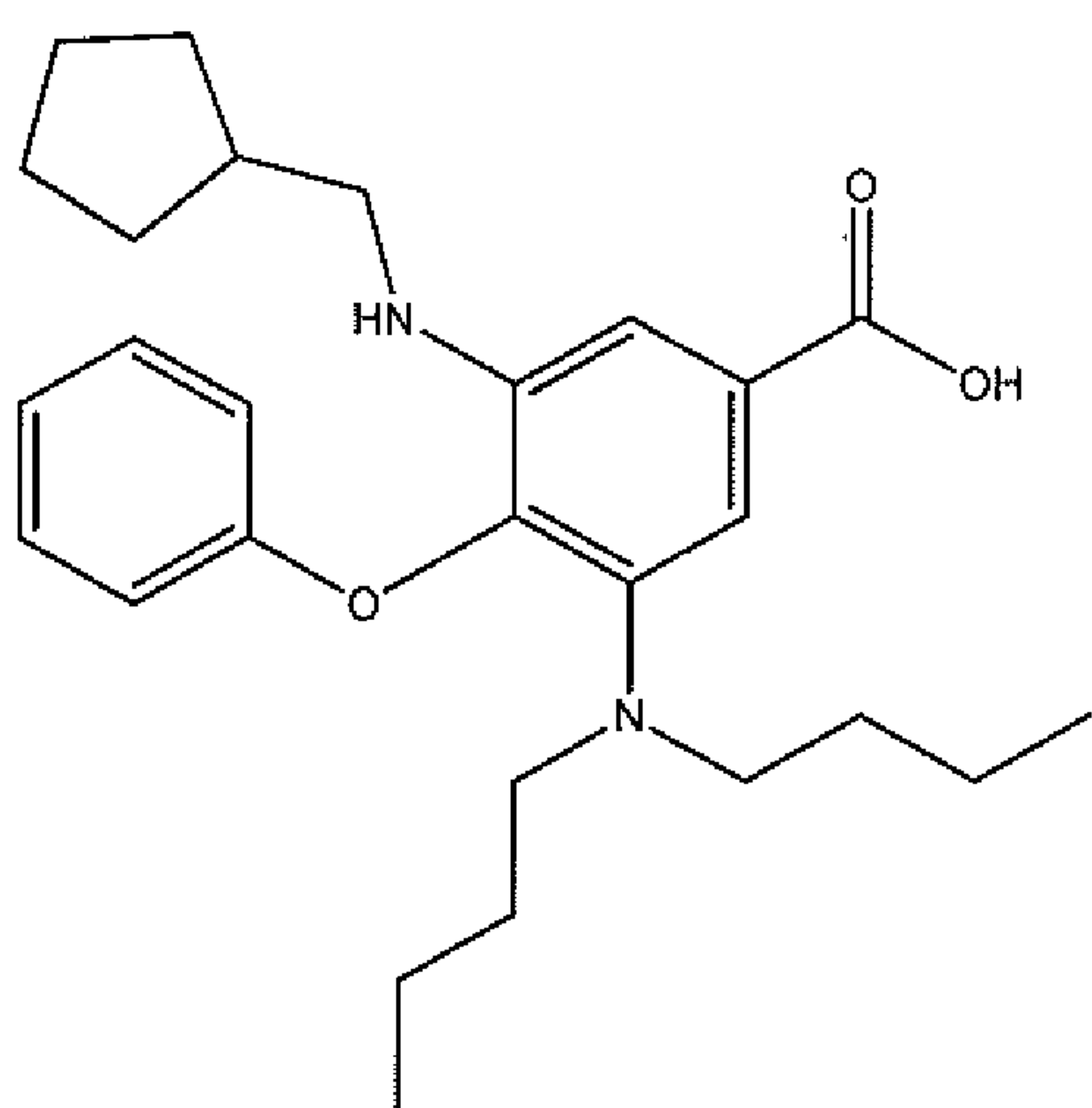


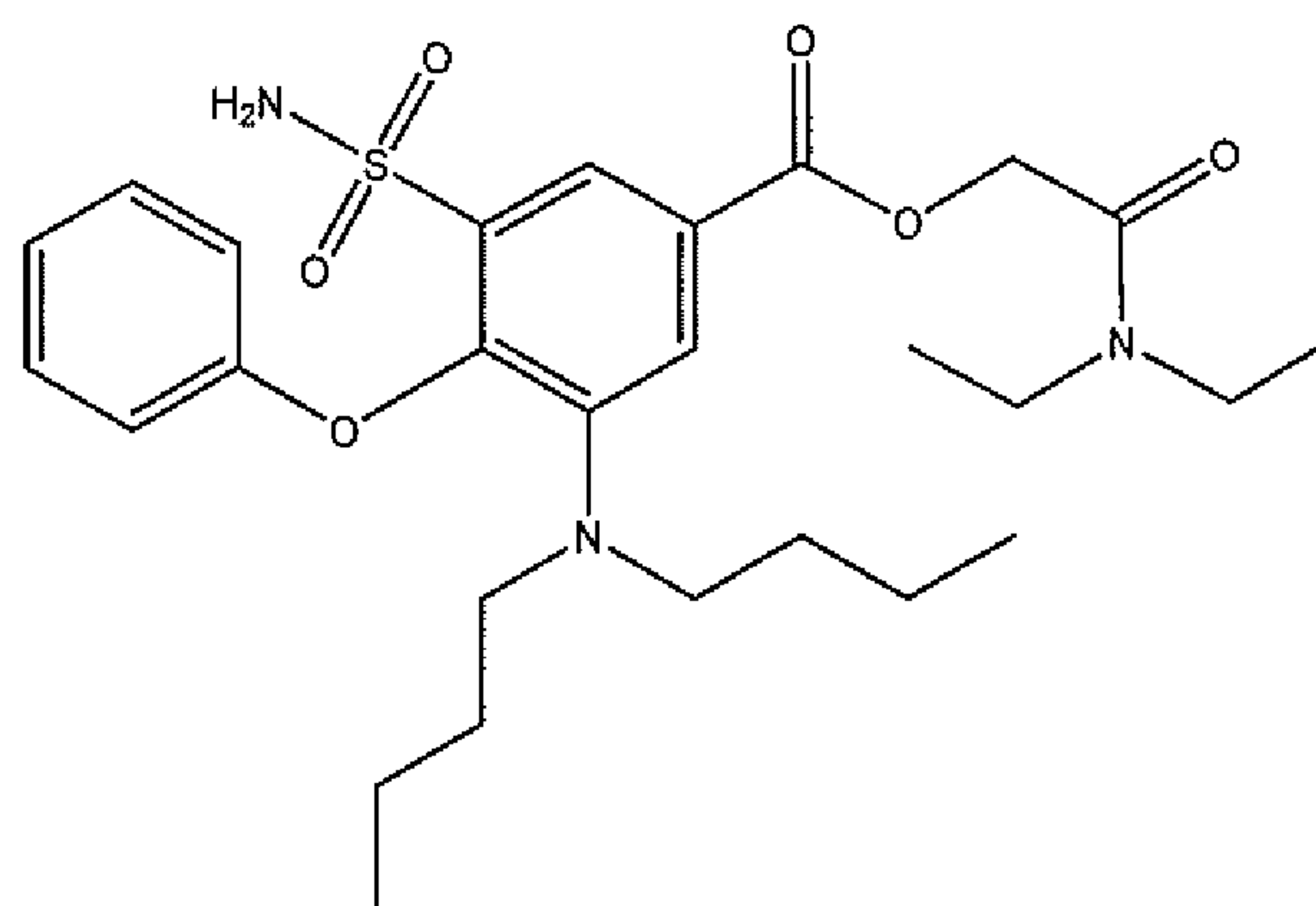
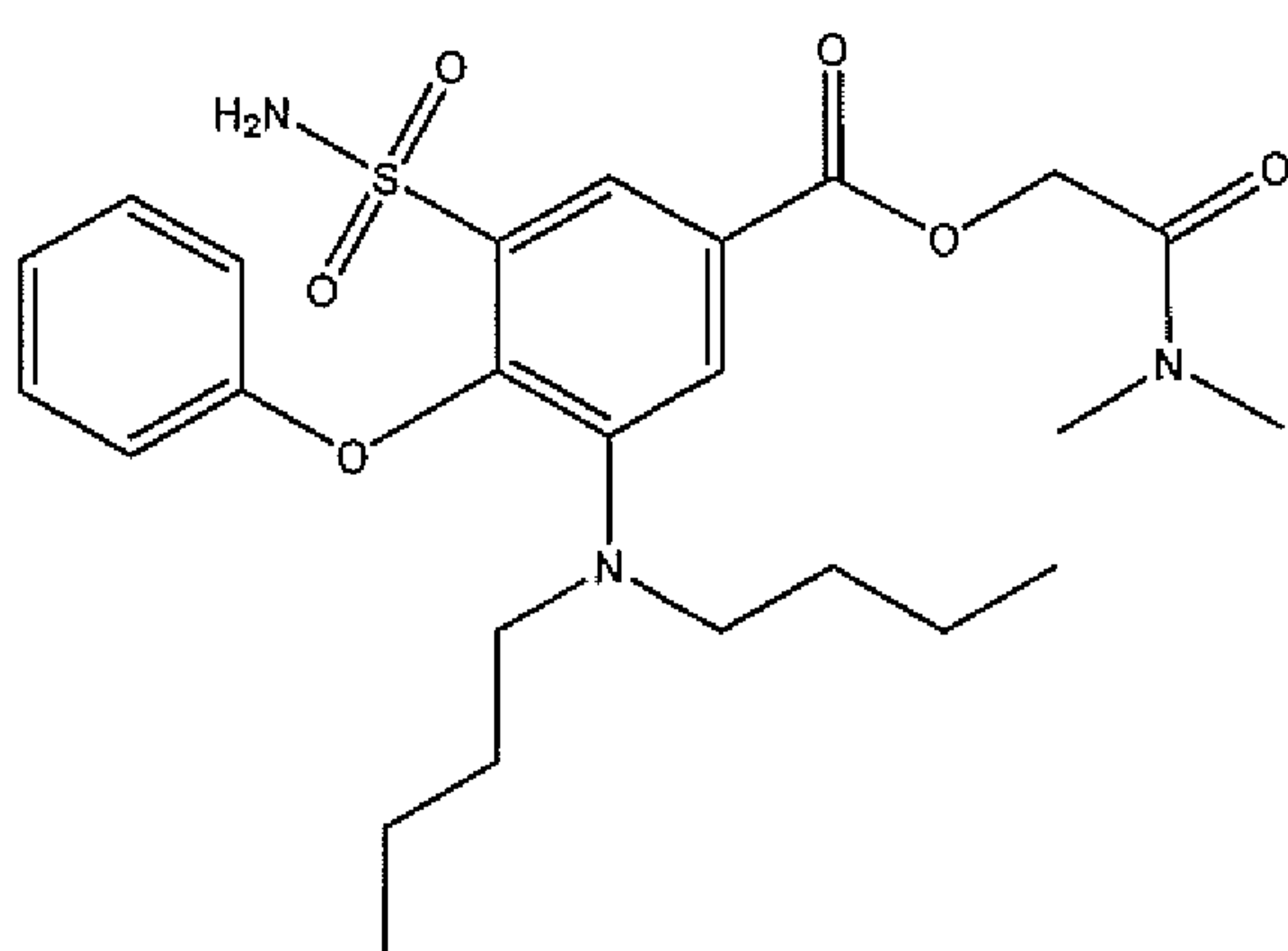
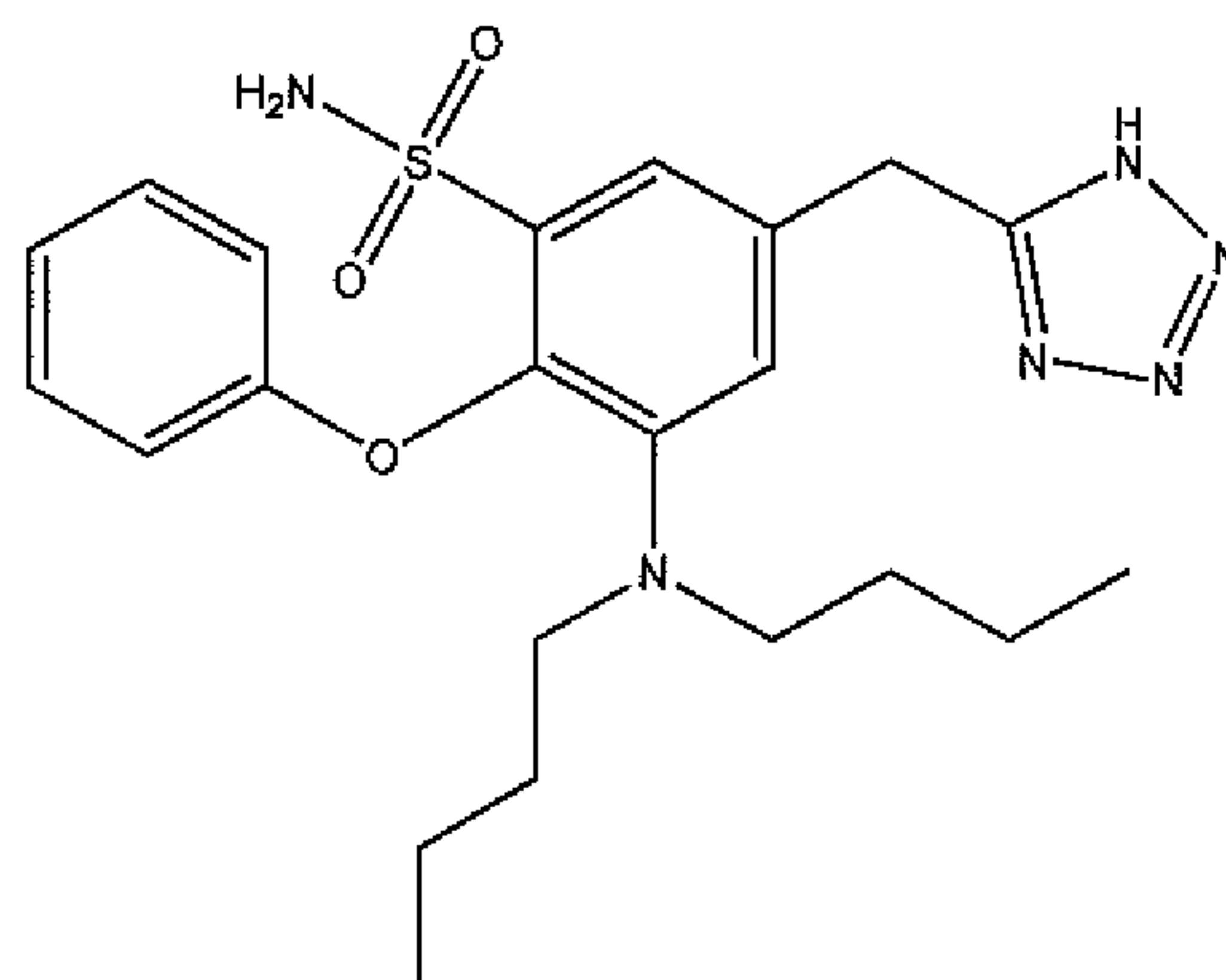
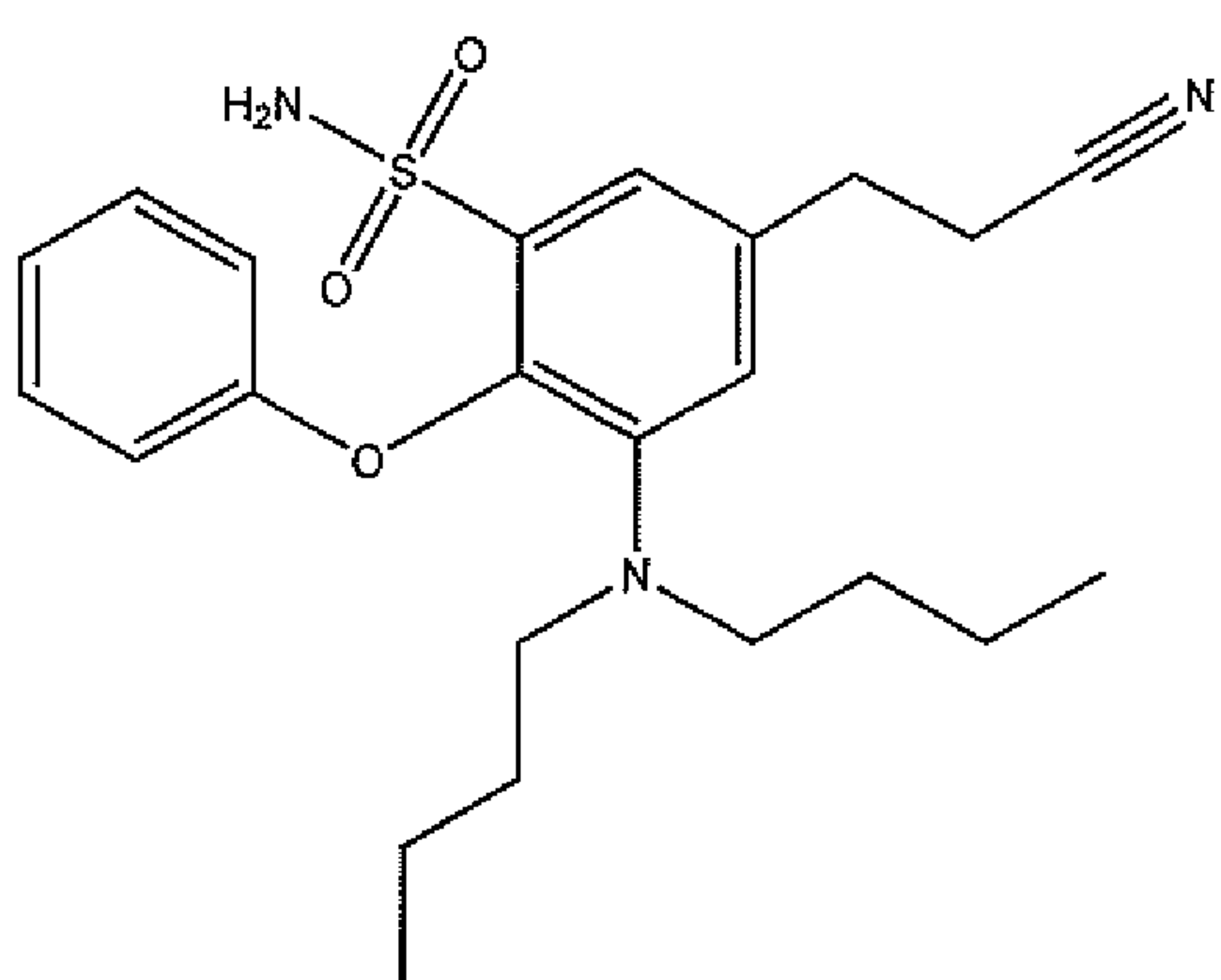
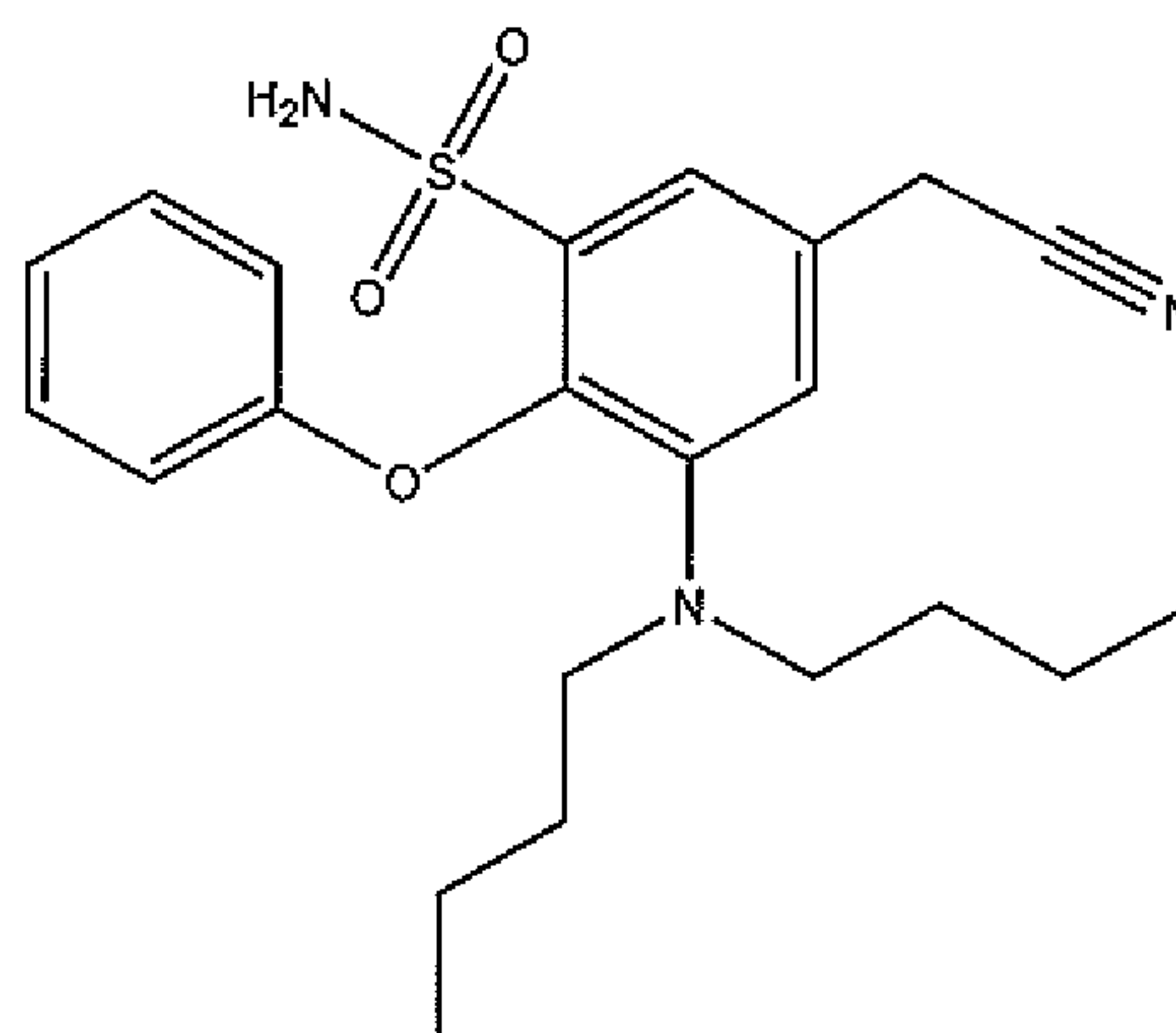
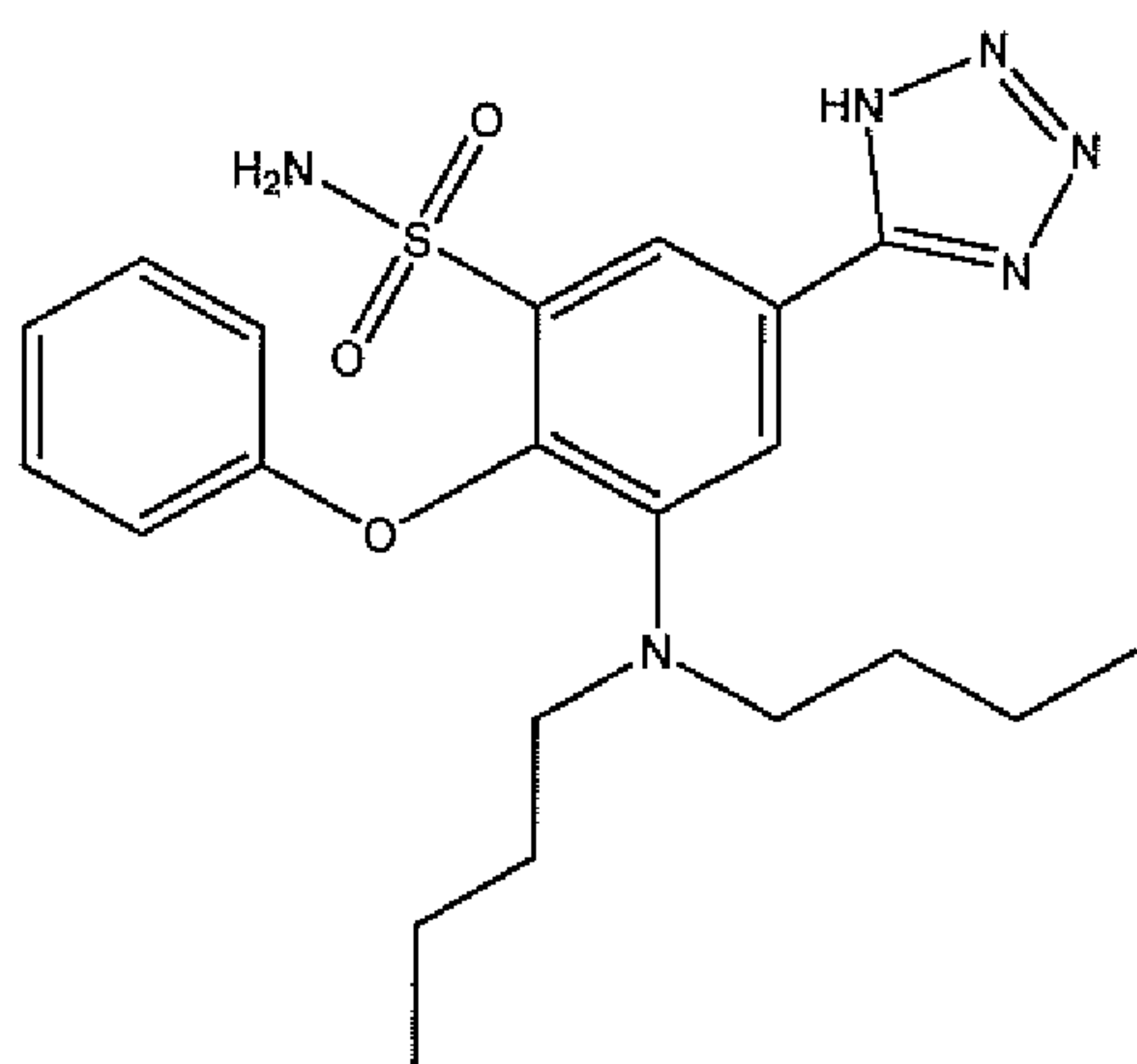


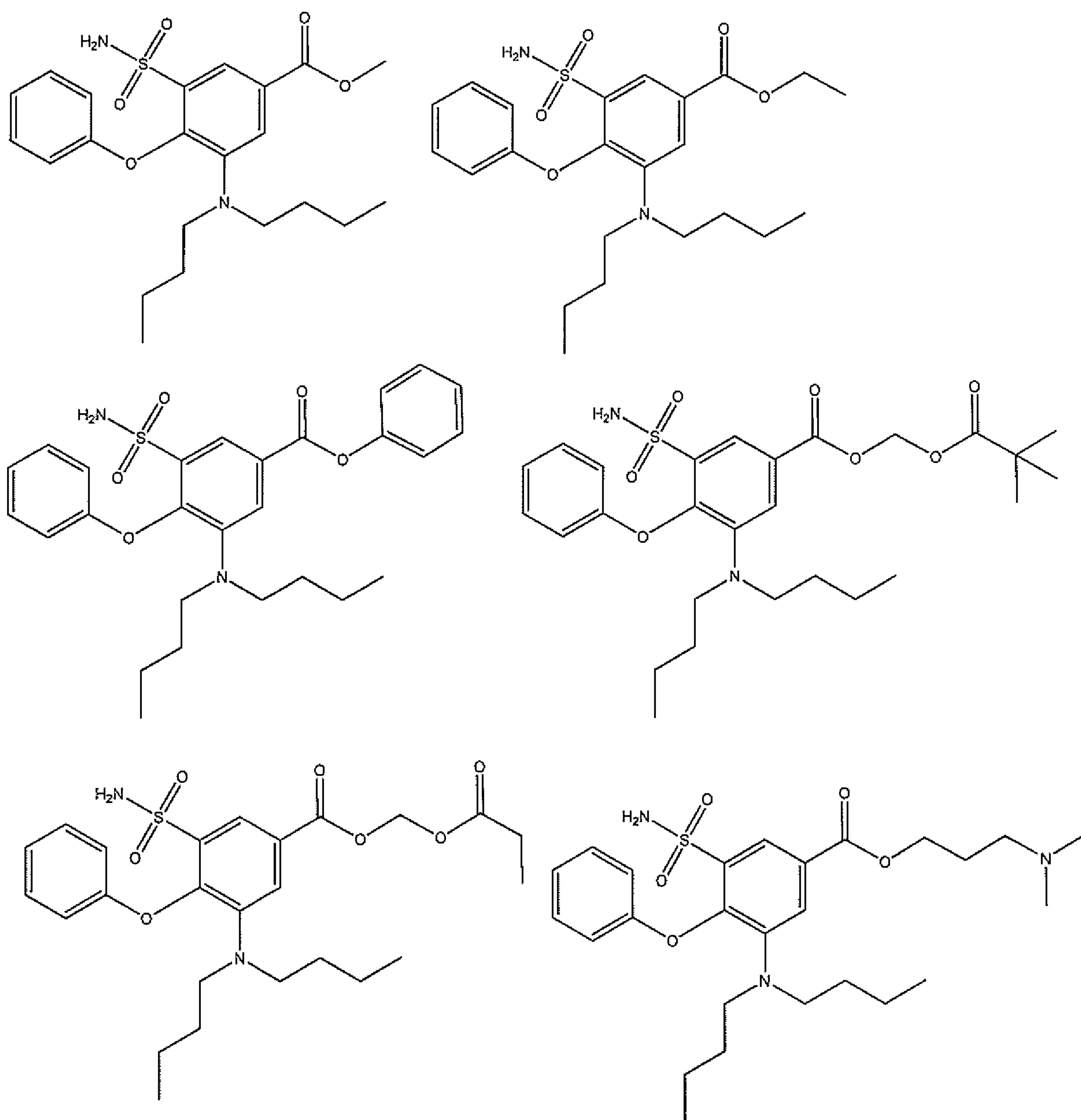




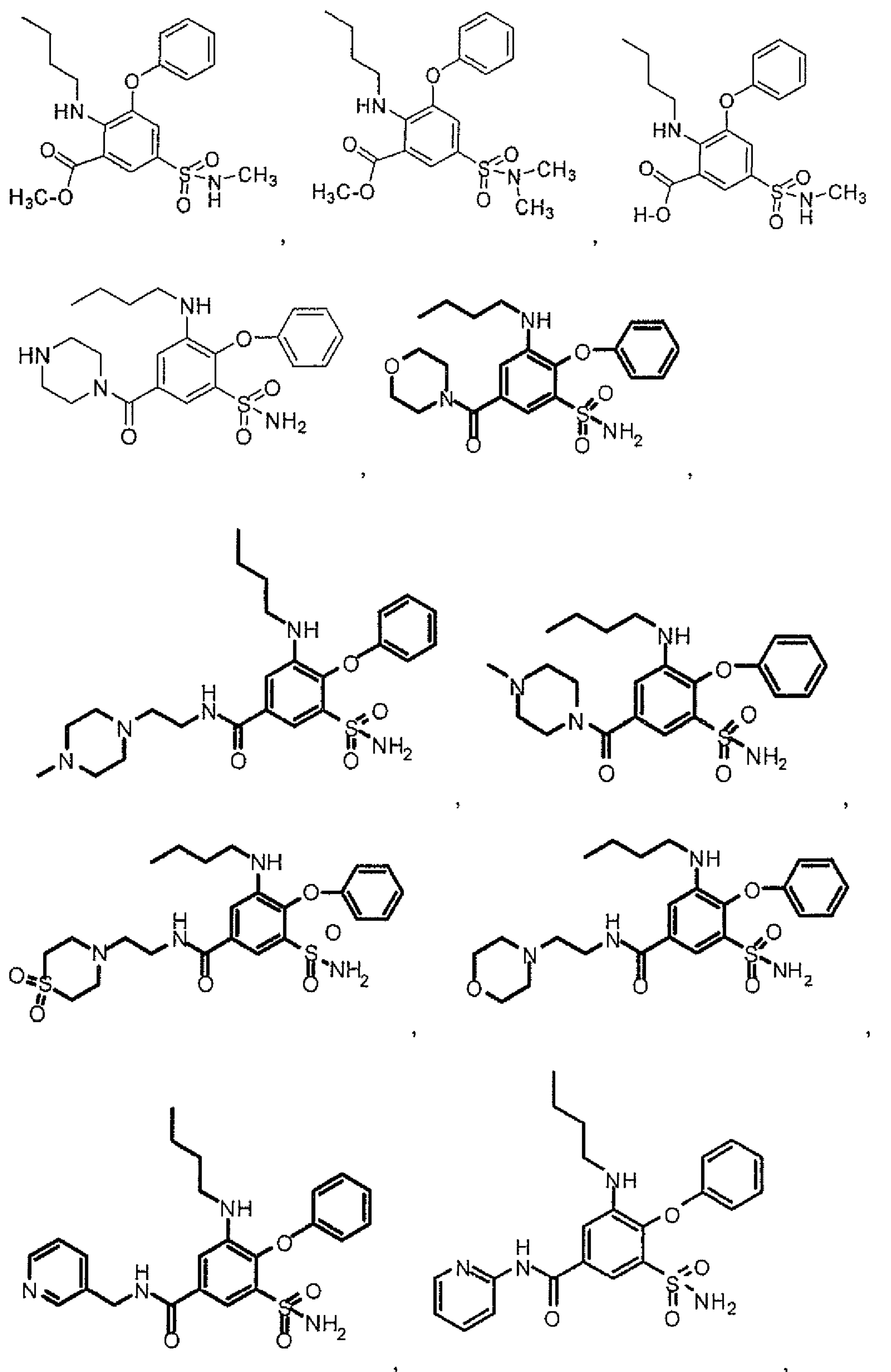


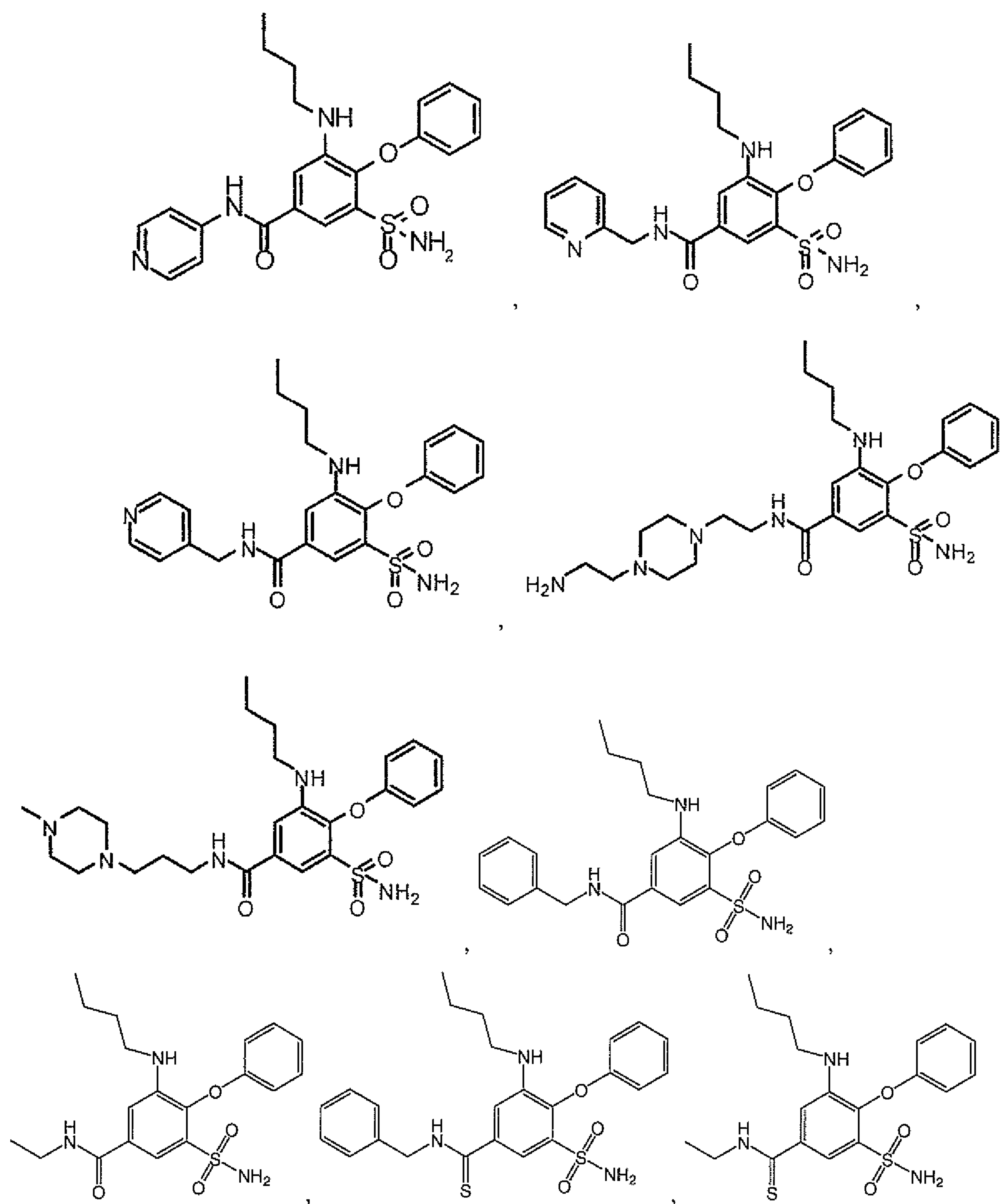


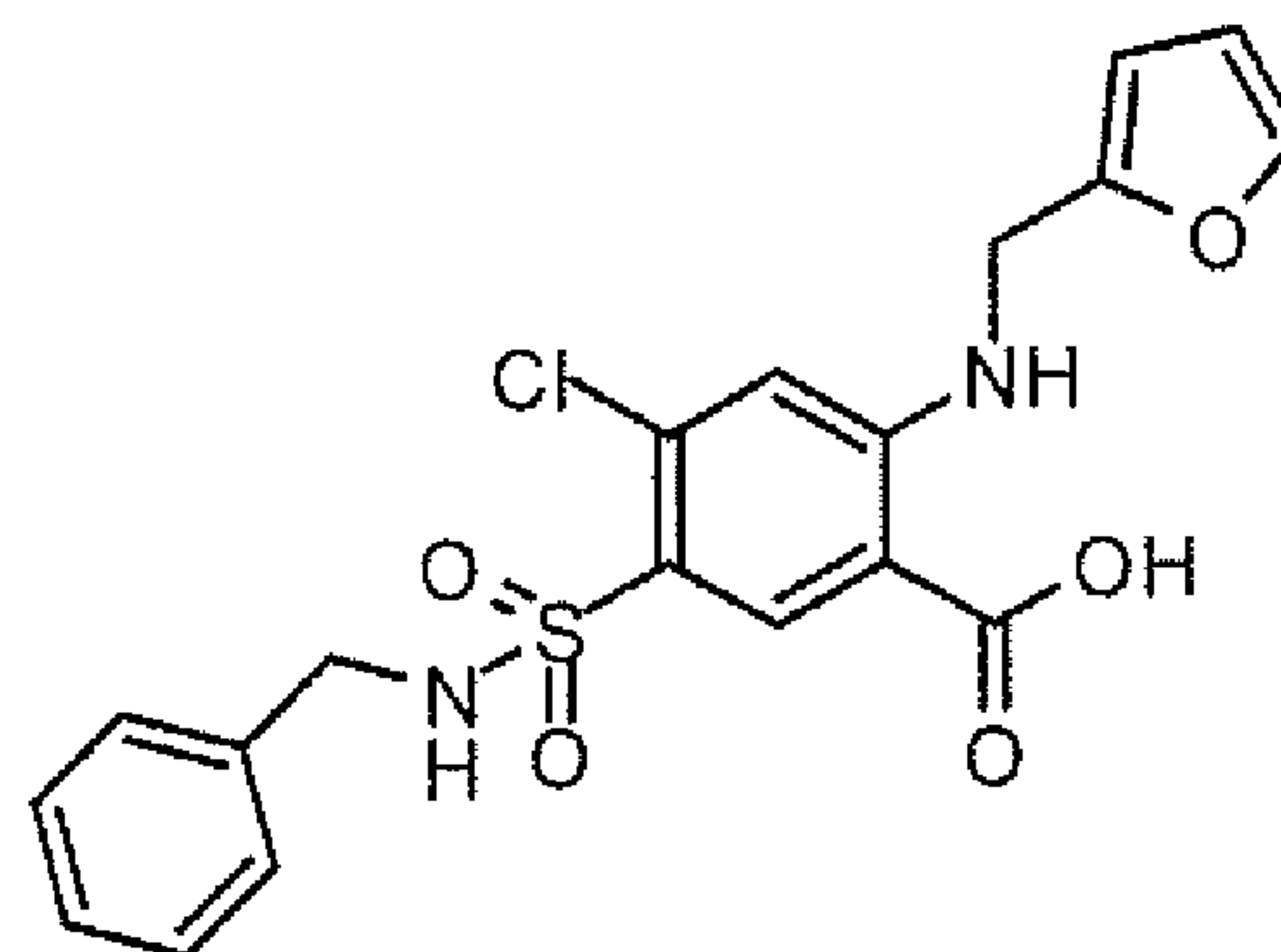
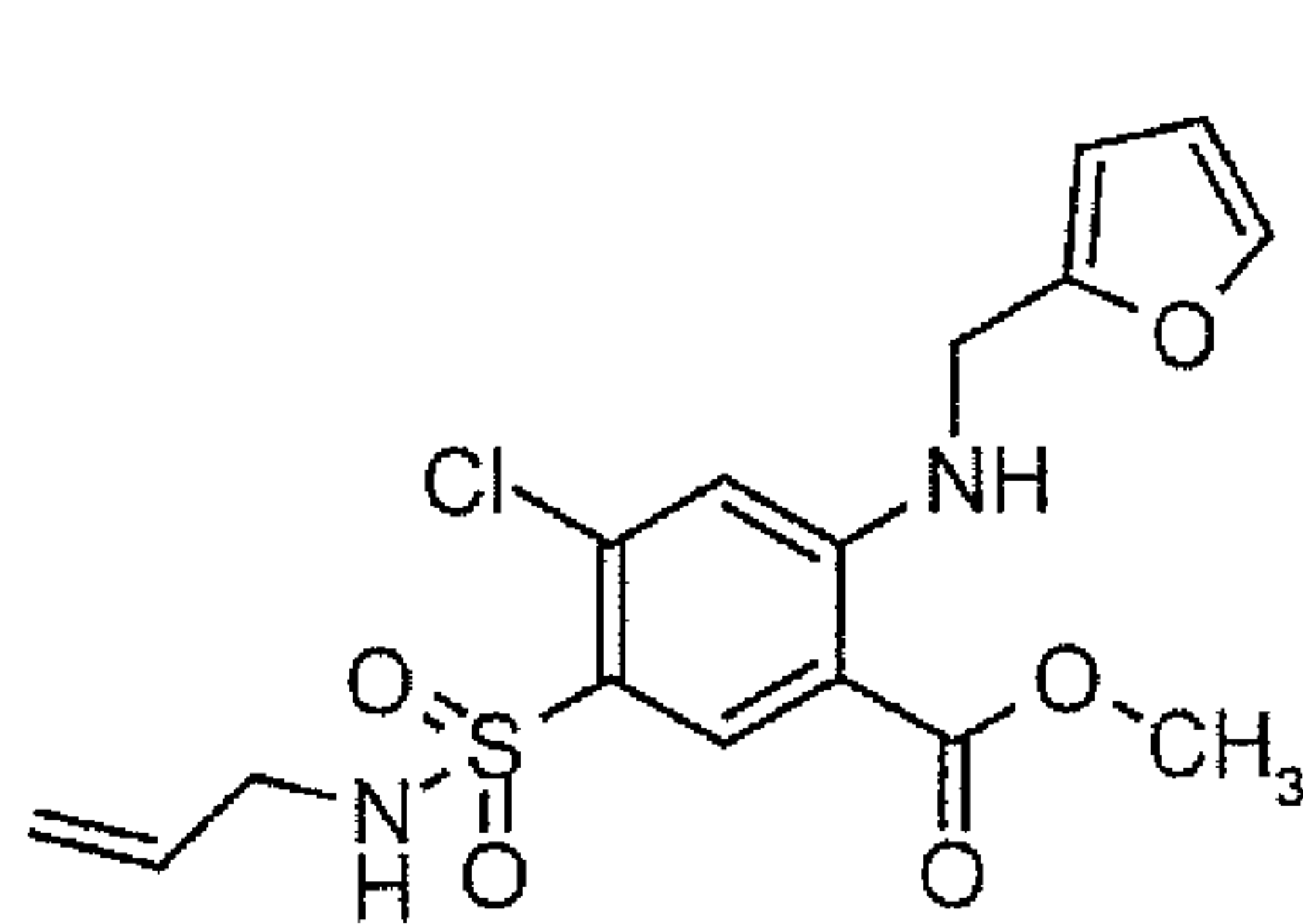
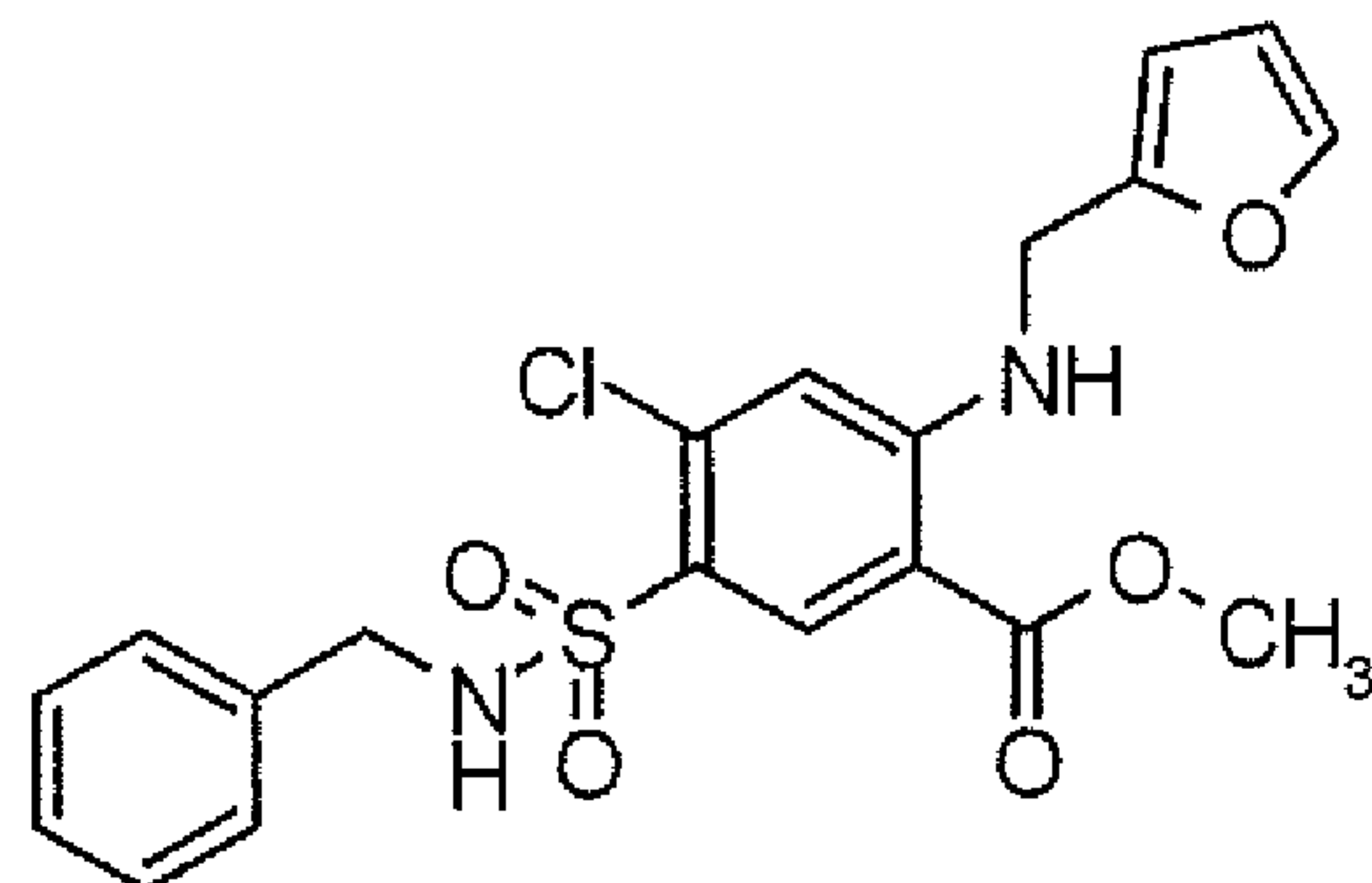
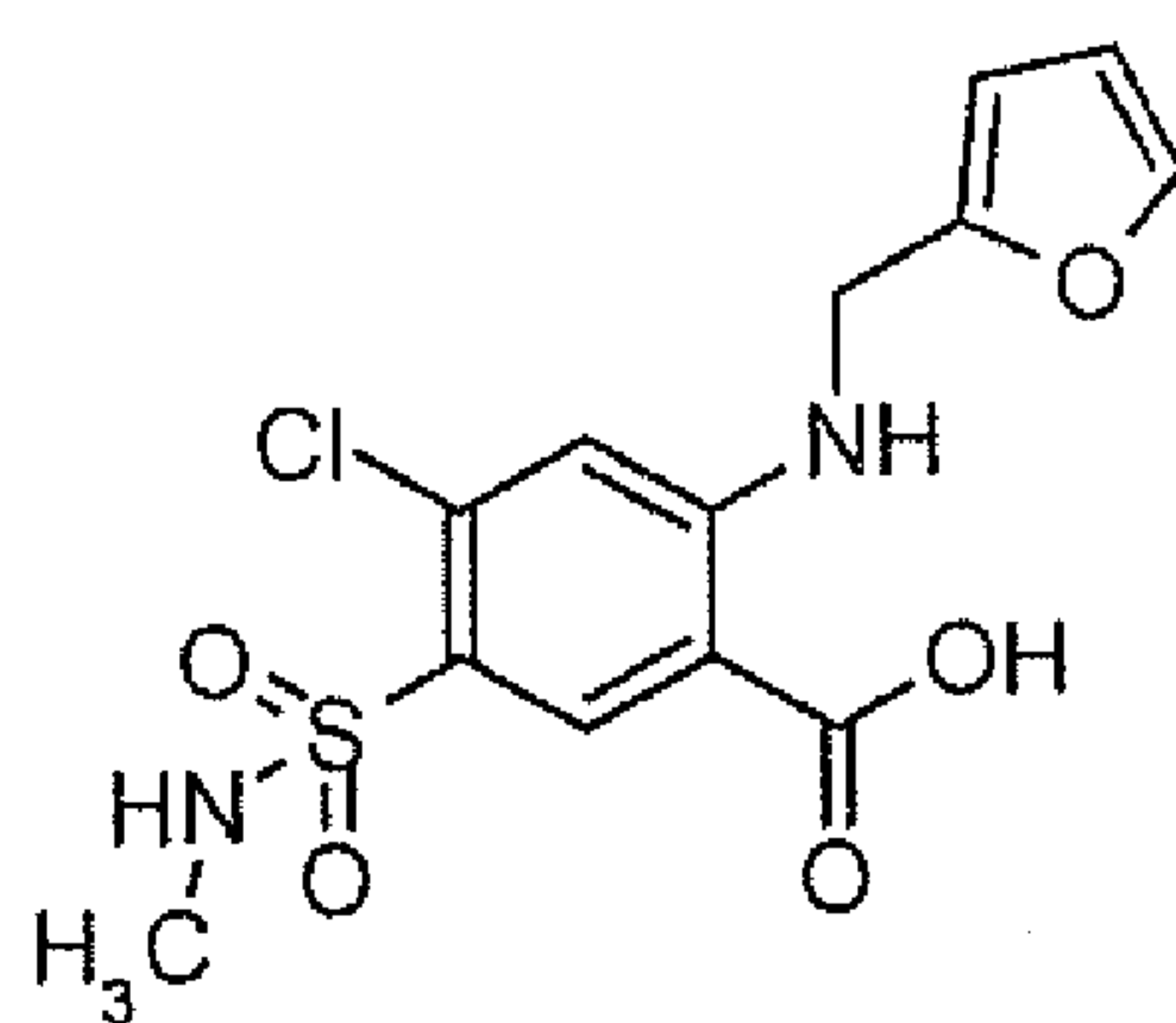
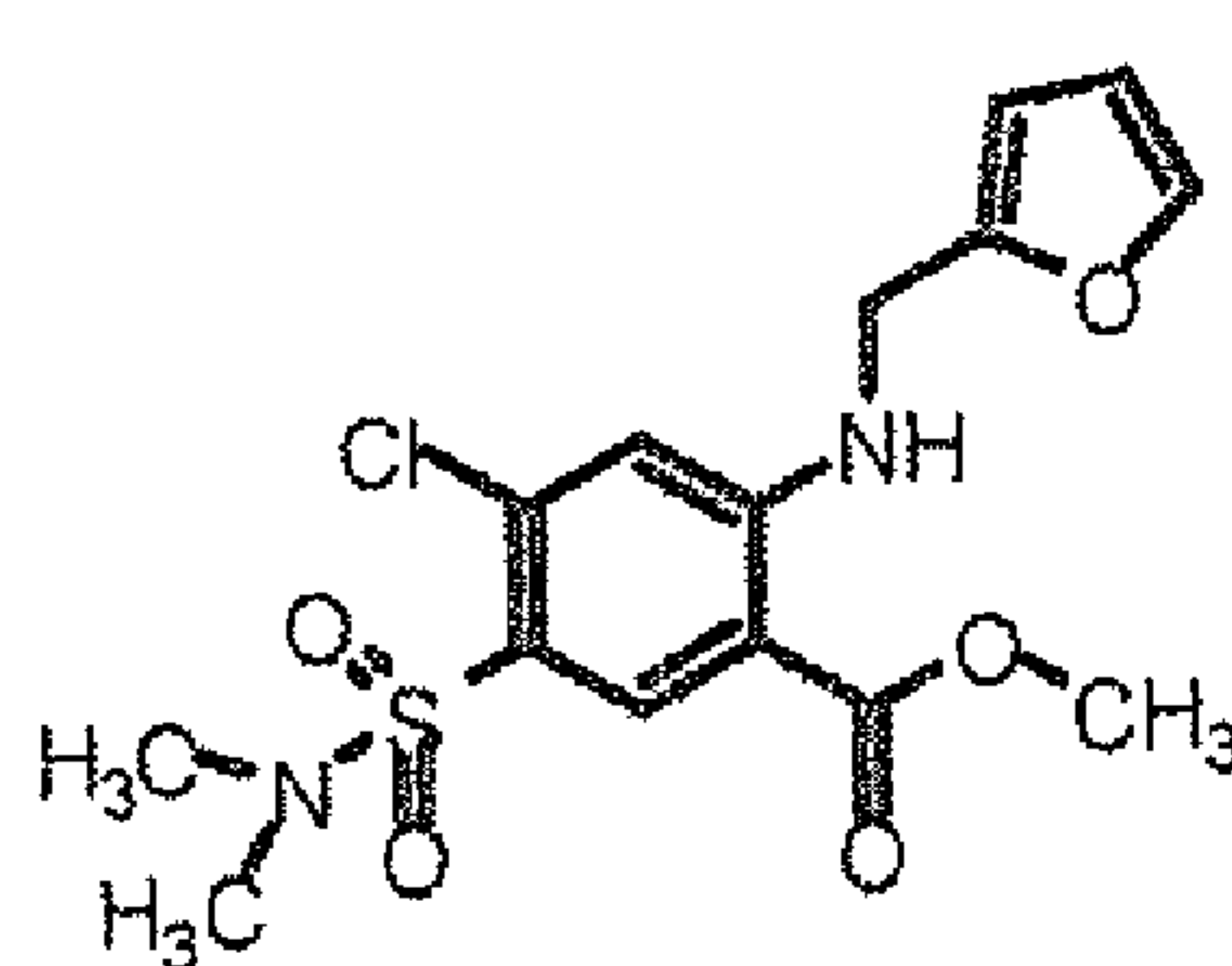
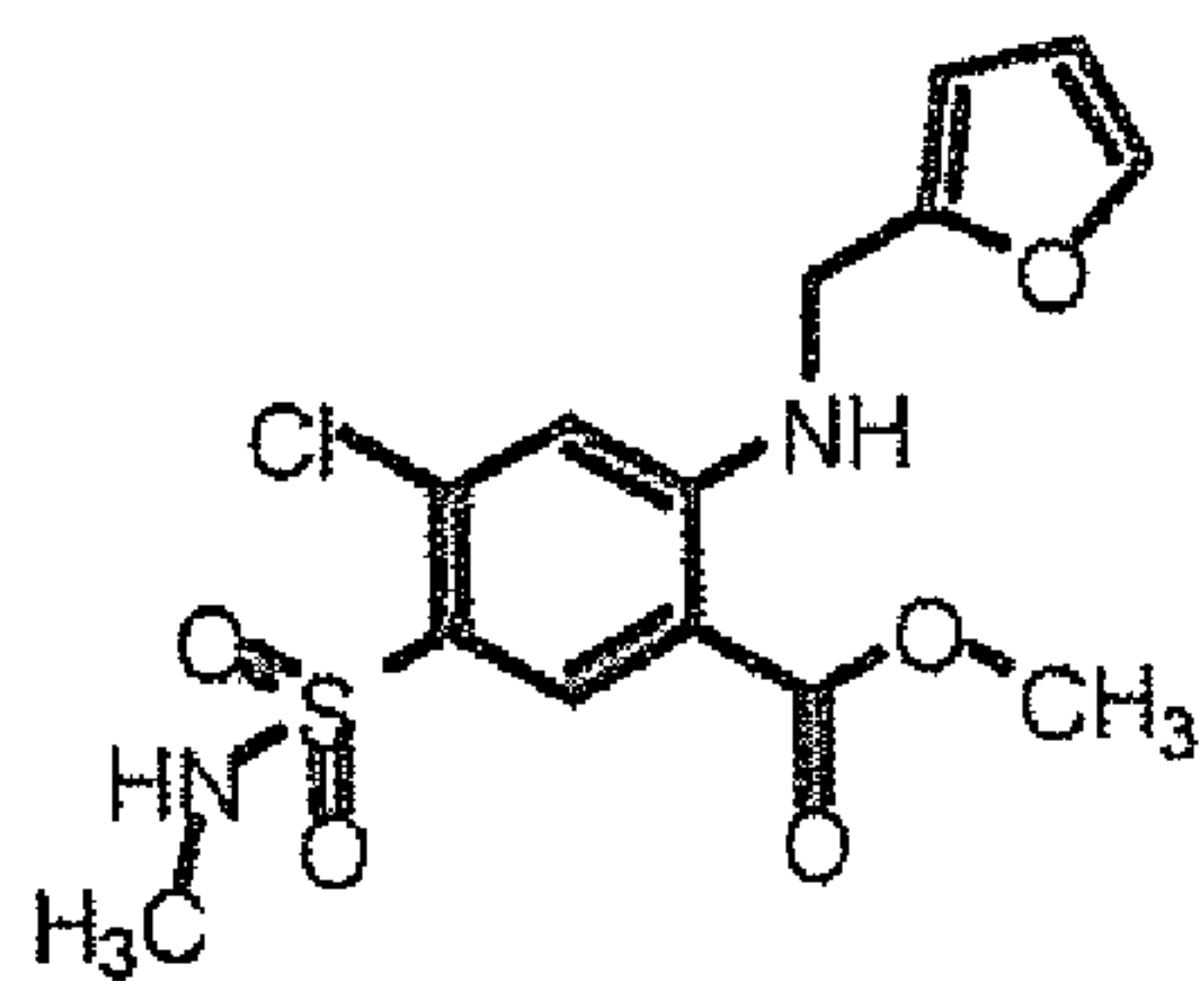
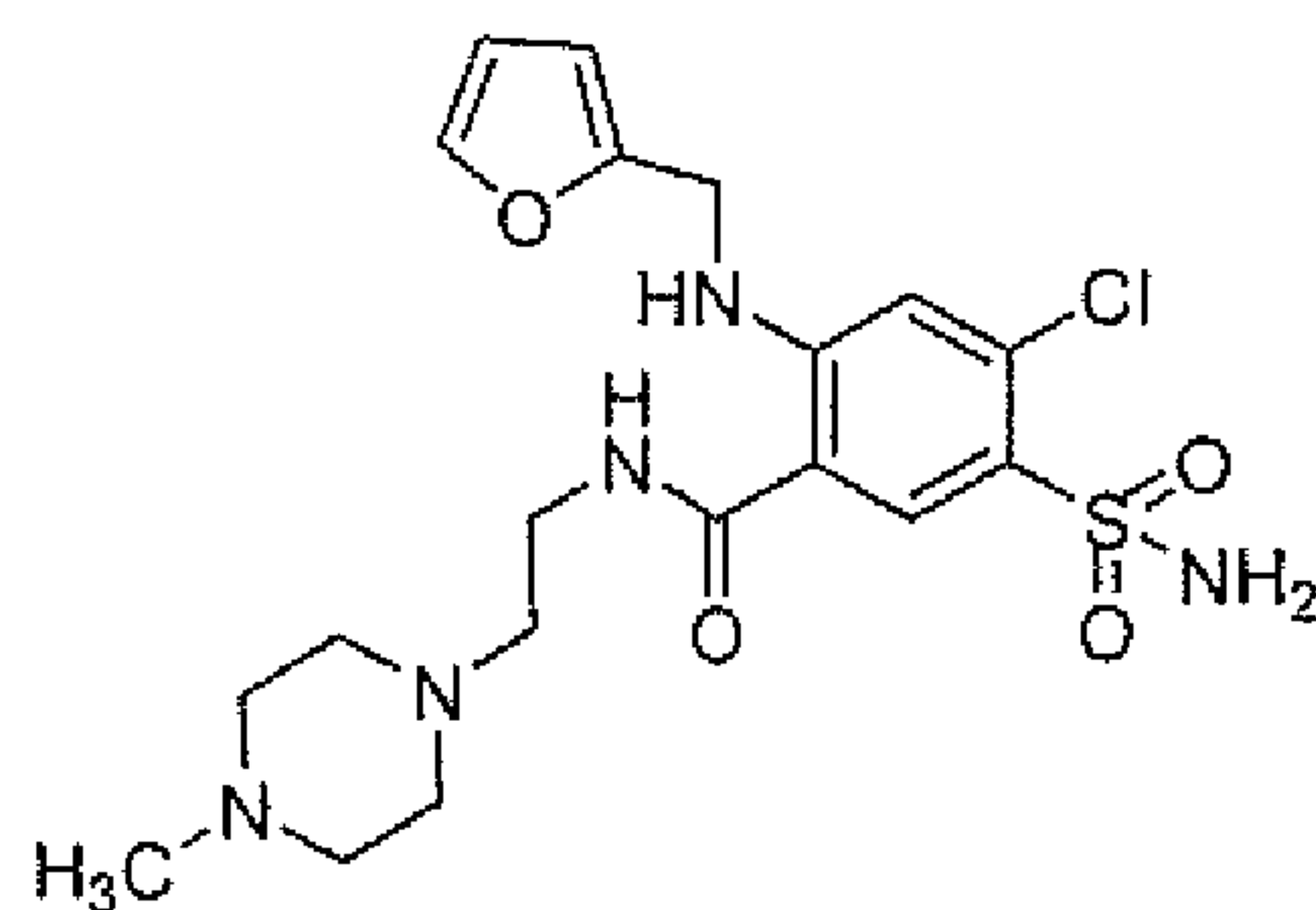
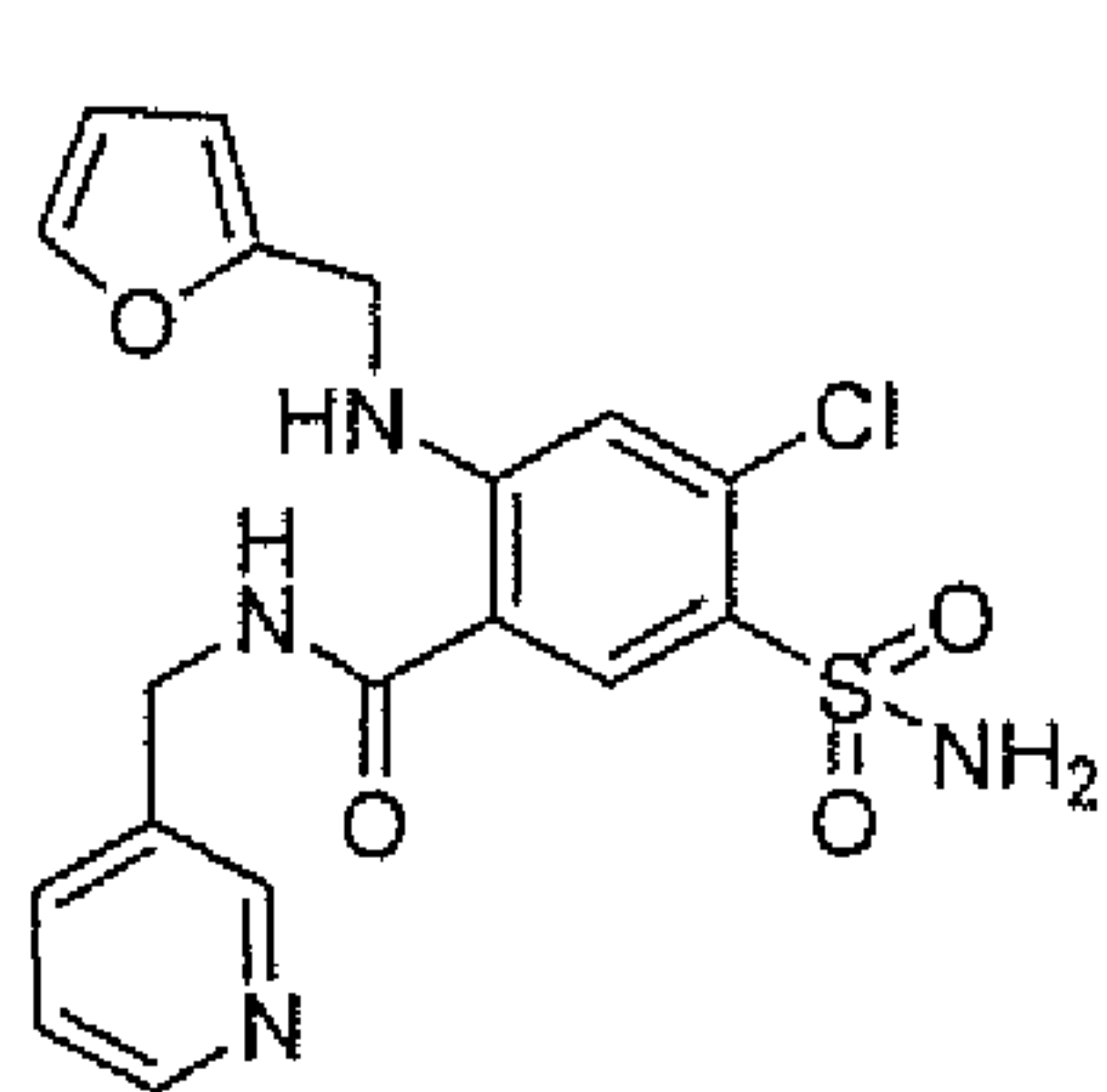


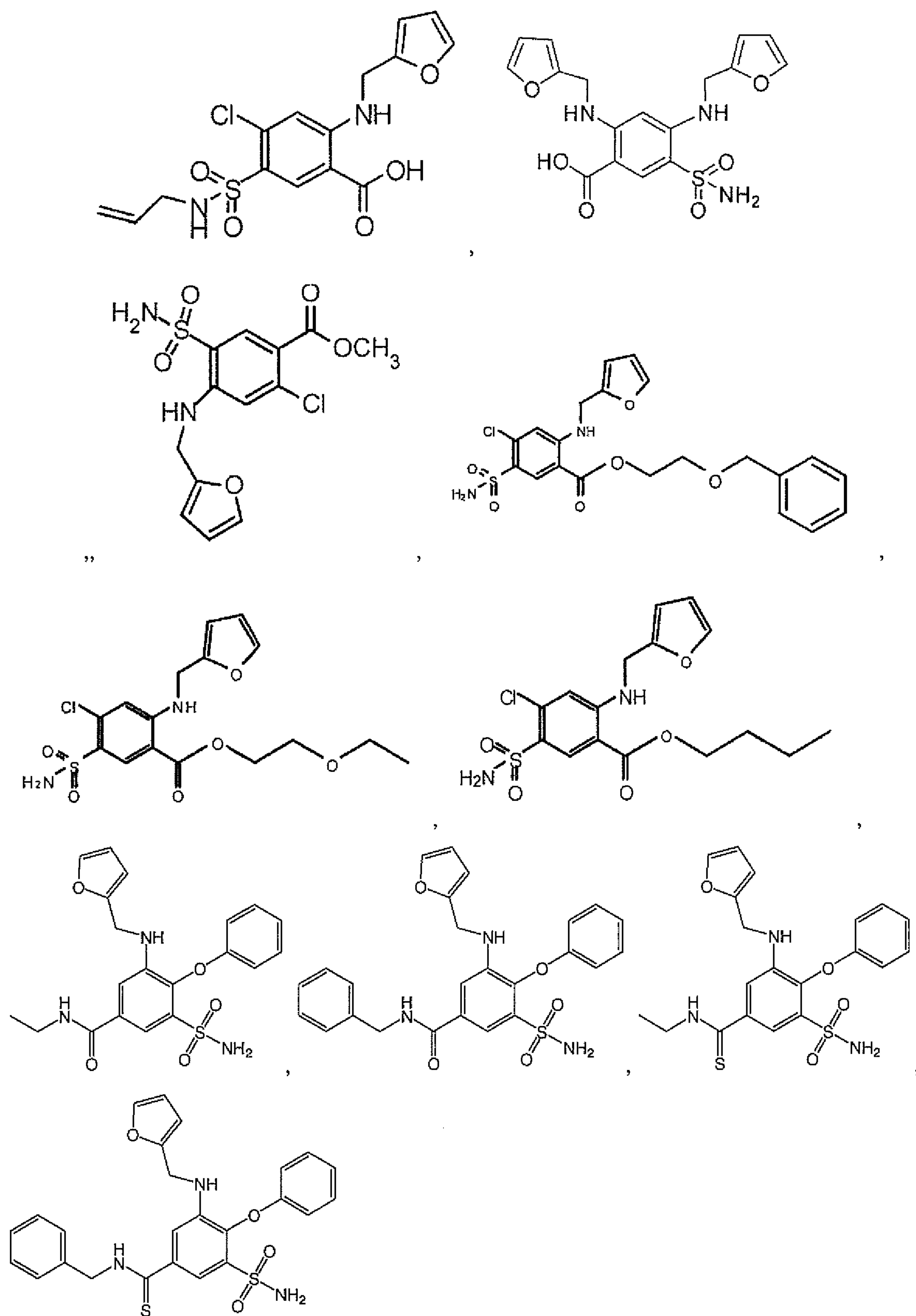


[0099] In still other embodiments, In still other embodiments, the sulfonamides of the Formulae I and II exclude one or more compounds of the formulae:









[0100] The compounds of the present invention antagonize NKCC1 and/or GABA_A receptors. The compounds of the present invention are useful in the treatment of conditions that involve NKCC1 and/or GABA_A receptors. In a preferred embodiment, these compounds are selective antagonists of NKCC1 and/or GABA_A receptors. In a preferred embodiment, these compounds are selective antagonists of GABA_A receptors. In a preferred embodiment, these compounds are selective antagonists of GABA_A receptors comprising an α_4 , α_5 , or α_6 subunit.

Methods of Use

[0101] In another embodiment, the invention relates to a method for treating addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, cognitive function (e.g., cognitive impairment, cognitive dysfunction), depression, edema, endothelial corneal dystrophy, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine with aura, migraine, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD) (nocturnal myoclonus), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, or withdrawal syndromes comprising administering an effective amount of a compound of Formulae I, II, III or IV:

[0102] In another aspect, the invention relates to a method of inhibiting the Na⁺K⁺Cl⁻ cotransporters comprising administering an effective amount of a compound of Formulae I, II, III or IV.

[0103] In yet another aspect, the invention relates to a method of inhibiting the NKCC1 (CCC1, BSC2) isoform of the Na⁺K⁺Cl⁻ cotransporters comprising administering an effective amount of a compound of the formula I, II, III or IV. In still another aspect, the invention relates to a method of inhibiting the NKCC2 (CCC2, BSC1) isoform of the Na⁺K⁺Cl⁻ cotransporters comprising administering an effective amount of a compound of the formula I, II, III or IV. In another aspect, the invention relates to a method of inhibiting both the NKCC1 (CCC1, BSC2) isoform and the NKCC2 (CCC2, BSC1) isoform of the Na⁺K⁺Cl⁻ cotransporters comprising administering an effective amount of a compound of the formula I, II, III or IV.

[0104] The present invention also provides methods of using the compounds of Formulae I-IV for treating disorders involving the Na⁺K⁺Cl⁻ co-transporters including but not limited to addictive disorders (e.g., compulsive disorders, eating disorders (e.g., obesity), addiction to narcotics/physical dependence, alcohol addiction, narcotic addiction, cocaine addiction, heroin addiction, opiate

addiction, alcoholism, and smoking); anxiety disorders (*e.g.*, anxiety, acute anxiety, panic disorder, social anxiety disorder, obsessive compulsive disorder (OCD), panic disorder, panic symptoms, post-traumatic stress disorder (PTSD), generalized anxiety disorder, and specific phobia); ascites (*e.g.*, peritoneal cavity fluid, peritoneal fluid excess, hydroperitoneum, abdominal dropsy, cancer related to ascites, tumors related to ascites); attention deficit hyperactivity disorder (ADHD); bipolar disorder (*e.g.*, manic-depressive illness, manic phase, depressive phase, mixed bipolar state, bipolar I disorder, bipolar II disorder, rapid-cycling bipolar disorder); cancer (*e.g.*, tumors, cancer related to ascites, tumors related to ascites); depression (*e.g.*, psychotic depression, postpartum depression, seasonal affective disorder (SAD), cortical spreading depression, dysthymia (mild depression)); edema (*e.g.*, central nervous system edema); endothelial corneal dystrophy (*e.g.*, post-chamber ocular diseases); epilepsy (*e.g.*, seizures, epileptic seizures, a seizure cluster, an acute seizure (*e.g.*, status epilepticus), seizure disorder, and other neurological disorders involving seizures (*e.g.*, cerebral palsy, Ohtahara Syndrome)); glaucoma (*e.g.*, increased intraocular pressure, angle-closure glaucoma, neovascular glaucoma, open-angle glaucoma); ischemia (*e.g.*, cardiac ischemia (myocardial ischemia), intestinal ischemia, mesenteric artery ischemia (acute mesenteric ischemia), hepatic ischemia, and cerebral ischemia (brain ischemia)); migraine (*e.g.*, migraine including headache, migraine variant, migraine headache, cervical migraine syndrome, acute confusional migraine, migraine with aura, migraine without aura); neuropathic pain (*e.g.*, diabetic neuropathy, nerve injury, nerve tract injury, neuropathic pain associated with visceral and/or somatic pain, peripheral neuropathy, chemotherapy-induced neuropathy, chemotherapy-induced peripheral neuropathy, neuralgia, polyneuropathy, mononeuropathy, mononeuritis multiplex, autonomic neuropathy, symmetrical peripheral neuropathy, radiculopathy, large fiber peripheral neuropathy, small fiber peripheral neuropathy, idiopathic neuropathic pain); nociceptive neuralgia; ocular diseases (*e.g.*, diseases of retina-retinal detachment and injury response; diseases of electrical transmission between various retinal elements such as rods, cones, amacrine and horizontal cells, activity of retinal ganglion cells, dysfunction of Müller (glial) cells, abnormal function of the retinal pigment epithelium; dysfunction of formation of the retina in development and the appropriate maintenance of neural connections following maturation and development; regulation of normal electrolyte homeostasis in various chorioretinal and vitreoretinal diseases; abnormal function of Müller cells in diabetic retinopathy; loss of normal electrical activity in degenerative diseases of retina, inherited and those of unknown etiology; inflammatory diseases and conditions of the eye

such as chorioretinitis, multiple sclerosis; infectious processes in the eye with abnormal inflammatory and injury responses; uveitis; abnormal function of Müller cells of retina and disease thereof; dysfunction of RPE-retinal pigment epithelium (*e.g.*, diseases of RPE); endothelial (posterior) corneal dystrophies, which result from primary endothelial dysfunction, (*e.g.*, Fuchs endothelial corneal dystrophy (FECD), posterior polymorphous corneal dystrophy (PPCD) and congenital hereditary endothelial dystrophy (CHED)); retinitis pigmentosa; age-related macular degeneration (*e.g.*, dry age-related macular degeneration, exudative age-related macular degeneration, and myopic degeneration); retinopathy (*e.g.*, diabetic retinopathy, proliferative vitreoretinopathy, and toxic retinopathy) and diseases of aqueous humor formation (*e.g.*, glaucoma)); pain (*e.g.*, chronic inflammatory pain, chronic musculoskeletal pain, pain associated with arthritis, pain associated to osteoarthritis, fibromyalgia, back pain, bone pain associated with cancer, cancer-associated pain, chemotherapy-induced neuropathy, chemotherapy-induced peripheral neuropathy, HIV-treatment induced neuropathy, HIV-treatment induced neuralgia, pain associated with digestive disease, pain associated with Crohn's disease, pain associated with autoimmune disease, pain associated with endocrine disease, pain associated with diabetic neuropathy, pain associated with shingles or herpes zoster, phantom limb pain, spontaneous pain, chronic post-surgical pain, chronic temporomandibular pain, causalgia, postherpetic neuralgia, AIDS-related pain, complex regional pain syndromes type I and II, trigeminal neuralgia, chronic back pain, pain associated with spinal cord injury and/or recurrent acute pain); postherpetic neuralgia (*e.g.*, shingles, herpes zoster); and schizophrenia. In a preferred embodiment, these compounds are selective antagonists of NKCC1.

[0105] The present invention also provides methods of using the compounds of Formulae I-IV for treating disorders involving a GABA_A receptor including but not limited to Alzheimer's Disease (AD), addictive disorders (*e.g.*, compulsive disorders, eating disorders (*e.g.*, obesity, anorexia nervosa, bulimia), addiction to narcotics/physical dependence, alcohol addiction, narcotic addiction, cocaine addiction, heroin addiction, opiate addiction, alcoholism, and smoking); anxiety disorders (*e.g.*, anxiety, acute anxiety, panic disorder, social anxiety disorder, obsessive compulsive disorder (OCD), panic disorder, panic symptoms, post-traumatic stress disorder (PTSD), generalized anxiety disorder, and specific phobia); autism spectrum disorders (autism); bipolar disorder (*e.g.*, manic-depressive illness, manic phase, depressive phase, mixed bipolar state, bipolar I disorder, bipolar II disorder, rapid-cycling bipolar disorder, bipolar I disorder, bipolar II disorder); depression (*e.g.*, psychotic depression, postpartum depression, seasonal affective disorder (SAD), cortical spreading

depression, dysthymia (mild depression)); epilepsy (*e.g.*, seizures, epileptic seizures, a seizure cluster, an acute seizure (*e.g.*, status epilepticus), seizure disorder, and other neurological disorders involving seizures (*e.g.*, cerebral palsy, Ohtahara Syndrome)); Huntington's Disease (HD) (*e.g.*, Huntington's chorea); insomnia, migraine (*e.g.*, migraine including headache, migraine variant, migraine headache, cervical migraine syndrome, acute confusional migraine, migraine with aura, migraine without aura, chronic migraine, transformed migraine); neuropathic pain (*e.g.*, diabetic neuropathy, cluster headache, nerve injury, nerve tract injury, neuropathic pain associated with visceral and/or somatic pain, peripheral neuropathy, chemotherapy-induced neuropathy, chemotherapy-induced peripheral neuropathy, HIV-treatment induced neuropathy, HIV-treatment induced neuralgia, neuralgia, polyneuropathy, mononeuropathy, mononeuritis multiplex, autonomic neuropathy, symmetrical peripheral neuropathy, radiculopathy, large fiber peripheral neuropathy, small fiber peripheral neuropathy, idiopathic neuropathic pain); nociceptive pain; pain (*e.g.*, acute pain, acute inflammatory pain, chronic inflammatory pain, chronic musculoskeletal pain, pain associated with arthritis, pain associated to osteoarthritis, fibromyalgia, back pain, bone pain associated with cancer, cancer-associated pain, chemotherapy-induced neuropathy, chemotherapy-induced peripheral neuropathy, pain associated with digestive disease, pain associated with Crohn's disease, pain associated with autoimmune disease, pain associated with endocrine disease, pain associated with diabetic neuropathy, pain associated with shingles or herpes zoster, phantom limb pain, spontaneous pain, chronic post-surgical pain, chronic temporomandibular pain, causalgia, postherpetic neuralgia, AIDS-related pain, complex regional pain syndromes type I and II, trigeminal neuralgia, chronic back pain, pain associated with spinal cord injury, incisional post operative, trauma associated, burns, recurrent acute pain, head pain, headache, nonmigrainous, specific non-migraine head pains, tic doloureux, postherpetic neuralgia, ice pick headache); Parkinson's disease, periodic limb movement disorder (PLMD) (nocturnal myoclonus), personality disorders, psychosis, restless legs syndrome (RLS), seizure disorders, personality disorders, schizophrenia, tinnitus, and withdrawal syndromes (*e.g.*, alcohol withdrawal syndrome, nicotine withdrawal syndrome, opioid withdrawal syndrome, benzodiazepine withdrawal syndrome, methadone withdrawal syndrome, SSRI discontinuation syndrome, hydrocodone withdrawal syndrome, cocaine withdrawal syndrome, heroin withdrawal syndrome).

[0106] The present invention further provides methods for treating a patient diagnosed with risk factors for a condition selected from the group consisting of addictive disorders, Alzheimer's

Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, cognitive function (e.g., cognitive impairment, cognitive dysfunction), depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD) (nocturnal myoclonus), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes comprising administering an effective amount of a compound of Formulae I, II, III, or IV.

[0107] Embodiments of the present invention provide kits including the compounds including compounds described herein. These kits may be used in the treatment methods disclosed herein. In another embodiment, the kits may include instructions, directions, labels, warnings, or information pamphlets.

[0108] Embodiments of the present invention provide uses of the compounds described herein for the preparation of a medicament for carrying out the aforementioned utilities.

[0109] In a preferred embodiment, compounds described herein show differential activity with stronger effect on the central nervous system and less diuretic effects. For example, compounds described herein may be used in long-term (maintenance) therapy without significant diuretic effect. Also, the arylsulfonamides described herein may be used in combination therapy with diuretics because of their lack of diuretic effect. Additionally, compounds described herein do not interfere with diuretics or cause severe side effects when administered in conjunction with or concurrently with a diuretic.

[0110] In another embodiment, the compounds described herein may be administered in combination with a second agent.

Enhanced Permeability

[0111] Embodiments of the present invention provide compounds capable of passage across the blood-brain barrier comprising a compound of Formulae I-IV, or a pharmaceutically acceptable salt, solvate, tautomer or hydrate thereof. In some embodiments, compounds of the present invention may have increased lipophilicity and/or reduced diuretic effects compared to the diuretic or diuretic-like compounds. The lipophilicity can be measured by determining the hydrophile-lipophile balance (HLB) or the partition coefficient (e.g., the distribution of a compound between water and octanol).

In further embodiments, compounds of the present invention may result in fewer undesirable side effects when employed in the regulatory, (*i.e.*, preventive, management), and/or treatment, methods described herein. In a preferred embodiment, compounds described herein show improved CNS pharmacologic properties and increased transit across the blood-brain barrier (BBB).

Weak Diuretic Effect

[0112] In some embodiments, the level of diuresis that occurs following administration of an effective amount of a compound provided herein as Formulae I-IV, is less than about 99%, 90%, 80%, 70%, 60%, 50%, 40%, 30%, 20% or 10% of that which occurs following administration of an comparable amount of a diuretic compound (*e.g.*, bumetanide, furosemide, piratanide, torsemide, azosemide). For example, the compound may have less of a diuretic effect than a diuretic compound (*e.g.*, bumetanide, furosemide, piratanide, torsemide, azosemide) when administered at the same mg/kg dose.

Selective Activity

Na⁺K⁺Cl⁻ co-transporters

[0113] The compounds of the present invention of Formulae I-IV described herein may be used for the regulation, including prevention, management and treatment, of a range of conditions including, but not limited to disorders that involve at least one of the Na⁺K⁺Cl⁻ co-transporters (*e.g.*, NKCC1, KNCC2) or K⁺Cl⁻ co-transporters (*e.g.*, KCC1, KCC2, KCC3, KCC4).

[0114] In a preferred embodiment, the invention comprises a method of inhibiting basolateral bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC1) comprising administering a composition comprising a compound described herein, wherein the inhibition of apical bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC2) is no more than 10%, 15%, 25%, or 50% of the effect on basolateral bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC1). In yet another embodiment, the invention comprises a method of inhibiting apical bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC2) comprising administering a composition comprising a compound described herein, wherein the inhibition of basolateral bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC1) is no more than 10%, 15%, 25%, or 50% of the effect on apical bumetanide-sensitive Na⁺K⁺Cl⁻ cotransporters (*e.g.*, NKCC2). Some preferred compounds described herein may not act on the GABA_A receptor or show only minimal activity on GABA_A receptors.

GABA_A receptors

[0115] Compounds of the present invention of Formulae I-IV described herein may be used for the regulation, including prevention, management and treatment, of a range of conditions including, but not limited to disorders that involve at least one of the GABA_A receptor.

[0116] In another embodiment, compounds described herein may show selective effect on a subset of GABA_A receptors in the CNS and less of the side-effects usually associated with agents that act on GABA_A receptors. For example, compounds described herein exhibit less sedation and less suppression of respiration, cognition, or motor function. In another embodiment, compounds described herein may show a selective effect on GABA_A receptors comprising an α_5 subunit or an α_6 subunit. In another embodiment, compounds described herein show a selective effect on GABA_A receptors comprising an α_4 subunit.

[0117] In one embodiment, the invention comprises a method for antagonizing parasynaptic (herein defined as pre- or extra-synaptic) GABA_A receptors comprising administering a composition comprising an effective amount of a compound of the Formulae I-IV or a pharmaceutically acceptable salt thereof. In another embodiment, the invention comprises a method for antagonizing parasynaptic GABA_A receptors comprising an α_4 , α_5 , or α_6 subunit comprising administering a composition comprising an effective amount of a compound of the Formulae I-IV or a pharmaceutically acceptable salt thereof.

[0118] Compounds described herein may have antagonistic effects on GABA_A receptors located parasynaptically. In one embodiment, compounds described herein may have antagonistic effects on GABA_A receptors comprising an α_4 , α_5 , or α_6 subunit located parasynaptically. In another embodiment, the invention comprises a method for antagonizing parasynaptic GABA_A receptors comprising an α_4 , α_5 , or α_6 subunit comprising administering a composition comprising a compound described herein, wherein the antagonism of GABA_A receptors with an α_1 , α_2 , or α_3 subunit is no more than 10%, 15%, 25%, or 50% of the effect on a GABA_A receptor with an α_4 , α_5 , or α_6 subunit.

[0119] Compounds described herein may preferentially bind GABA_A receptors. In one embodiment, compounds described herein may preferentially bind GABA_A receptors comprising an α_1 , α_2 , α_3 , α_4 , α_5 , or α_6 subunit. Preferential binding of the compounds of this invention may be reflected in the effective concentration (EC₅₀), *i.e.*, the concentration of the compound *in vitro* at which the antagonist effect is half the maximal antagonism demonstrated by the respective compound on the particular receptor. In particular, more preferred compounds of this invention will be those whose EC₅₀ for GABA_A receptors with an α_4 , α_5 , or α_6 subunit are no more than 10%,

15%, 25%, or 50% of the EC₅₀ of the same compound for GABA_A receptors having an α_1 , α_2 , or α_3 subunit.

[0120] Compounds described herein are effective in humans and animals to decrease seizures, decrease pain responses, and decrease migraine in humans and animal models. For example, compounds described herein may preferentially bind to GABA_A receptor subtypes and have an antagonistic effect on GABA_A receptors that is different from classic benzodiazepine and barbiturate mechanisms. Compounds described herein may not act on the Na⁺K⁺2Cl⁻ cotransporter (NKCC1 or NKCC2). Unlike a diuretic compound (*e.g.*, bumetanide, furosemide, piretanide, azosemide, and torsemide), compounds described herein may not elicit diuresis. For example, compounds described herein may not increase urine output, sodium excretion, or potassium excretion.

[0121] The foregoing and other objects and aspects of the present invention are explained in greater detail in reference to the drawing and description set forth herein.

Brief Description of the Drawings

[0122] FIGURE 1 is a schematic illustration of a possible mechanism for the action of compounds described herein that selectively antagonize parasynaptic GABA_A receptor isoforms in GABAergic interneurons. In this suggested mechanism, (1) GABA is released from the pre-synaptic terminal by activated inhibitory neurons, (2) GABA binds to post-synaptic GABA_A receptors that activates them thereby increasing inhibition (*e.g.*, hyperpolarization of the post-synaptic neuron), (3) GABA also binds to parasynaptic (*e.g.*, presynaptic and extrasynaptic) GABA_A receptors, (4) in one possible mechanism of action, compounds described herein selectively binds to parasynaptic α_4 variant GABA_A receptors (inhibiting the negative feedback loop), thus increasing GABA release. This leads to the restoration of the balance of excitation and inhibition by increasing the inhibitory stimulus applied to post-synaptic neurons.

[0123] FIGURE 2 illustrates the activating effect of 1 μ M clobazam, 0.1 μ M zolpidem, and 1 μ M diazepam on the current in α_1 containing GABA_A receptor isoforms at 10 μ M GABA.

[0124] FIGURE 3 illustrates the inhibiting effect of 10 μ M select compounds on the current in α_1 , α_4 , α_5 , and α_6 containing GABA_A receptor isoforms at 10 μ M GABA.

[0125] FIGURE 4 illustrates the inhibitory effect of select compounds at 10 μ M concentration on GABA_A receptor activity in the presence of 10 μ M GABA. 100% represents full activation of a GABA_A receptor. 50% to 20% value represents strong inhibition of GABA_A receptor activity. Bumetanide (BTX) was used as a negative control.

[0126] FIGURE 5 illustrate the inhibitory effect of 10 μ M of select compounds on $\alpha_4\beta_3\gamma_{2L}$ GABA_A receptor isoform activity in the presence of 10 μ M GABA. 100% represents full activation of the GABA_A receptor. 50% to 20% value represents strong inhibition of GABA_A receptor activity. Bumetanide (BTN) was used as a negative control.

[0127] FIGURE 6A-D illustrate the inhibitory effect of 10 μ M of select compounds on (A) $\alpha_4\beta_3\gamma_{2L}$, (B) $\alpha_6\beta_3\gamma_{2L}$, (C) $\alpha_1\beta_3\gamma_{2L}$, and (D) $\alpha_5\beta_3\gamma_{2L}$ GABA_A receptor isoforms activity in the presence of 10 μ M GABA. 100% represents full activation of the GABA_A receptor. 50% to 20% value represents strong inhibition of GABA_A receptor activity. Bumetanide (BTN) was used as a negative control.

[0128] FIGURE 7A-B illustrate the inhibitory effect of select compounds at 10 μ M concentration on (A) $\alpha_4\beta_3\gamma_{2L}$ and (B) $\alpha_6\beta_3\gamma_{2L}$ GABA_A receptor isoforms activity in the presence of 10 μ M GABA. 100% represents full activation of the GABA_A receptor. 50% to 20% value represents strong inhibition of GABA_A receptor activity. Bumetanide (BTN) was used as a negative control.

[0129] FIGURE 8A-D illustrate the inhibitory effect of 10 μ M of select compounds in the presence of 10 μ M GABA on (A) $\alpha_4\beta_3\gamma_{2L}$, (B) $\alpha_6\beta_3\gamma_{2L}$, (C) $\alpha_1\beta_3\gamma_{2L}$, and (D) $\alpha_5\beta_3\gamma_{2L}$ GABA_A receptor isoforms activity. 100% represents full activation of the GABA_A receptor. 50% to 20% value represents strong inhibition of GABA_A receptor activity. Bumetanide (BTN) was used as a negative control.

[0130] FIGURE 9A-J depicts the results from tail-flick assays.

[0131] FIGURE 10A-F depicts data on mIPSC frequency, mIPSC amplitude, mean mIPSC decay time, mean mIPSC rise time, and mean mIPSC half width for compound 3034.

[0132] FIGURE 11A-F depicts data on mIPSC frequency, mIPSC amplitude, mean mIPSC decay time, mean mIPSC rise time, and mean mIPSC half width for compound 6009.

[0133] FIGURE 12A-F depicts data on mIPSC frequency, mIPSC amplitude, mean mIPSC decay time, mean mIPSC rise time, and mean mIPSC half width for compound 7049.

Detailed Description

[0134] The foregoing and other aspects of the present invention will now be described in more detail with respect to embodiments described herein. It should be appreciated that the invention can be embodied in different forms and should not be construed as limited to the embodiments set forth herein. Rather, these embodiments are provided so that this disclosure will be thorough and complete, and will fully convey the scope of the invention to those skilled in the art.

Definitions

[0135] The terminology used in the description of the invention herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the invention. As used in the description of the embodiments of the invention and the appended claims, the singular forms “a,” “an” and “the” are intended to include the plural forms as well, unless the context clearly indicates otherwise. Also, as used herein, “and/or” refers to and encompasses any and all possible combinations of one or more of the associated listed items. Furthermore, “about,” as used herein when referring to a measurable value such as an amount of a compound, dose, time, temperature is meant to encompass variations of 20%, 10%, 5%, 1%, 0.5%, or even 0.1% of the specified amount. Unless otherwise defined, all terms, including technical and scientific terms used in the description, have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs.

[0136] “Administration” as used herein, refers broadly to any means by which a composition is given to a patient. A preferred route of administration is oral, and unless otherwise indicated, any reference herein to “administration” includes “oral administration.”

[0137] “Alkenyl” as used herein, refers broadly to a straight or branched chain hydrocarbon radical having one or more double bonds and containing from 2 to 20 carbon atoms. Examples of alkenyl groups include propenyl, butenyl, pentenyl, and the like. “Cycloalkenyl” or “cyclic alkenyl” as used herein refers to carbocycles containing no heteroatoms, and includes mono-, bi-, and tricyclic saturated carbocycles, as well as fused rings systems. Examples of cycloalkenyl groups include cyclopropenyl, cyclopentenyl, cyclohexenyl, cyclopentadienyl, cyclohexadienyl, and the like. Such alkenyl and cycloalkenyl groups may be optionally substituted as described herein.

[0138] “Alkyl” as used herein refers broadly to a straight or branched chain saturated hydrocarbon radical. “Alkyl” also refers broadly to cyclic (*i.e.*, cycloalkyl) alkyl groups. Examples of alkyl groups include, but are not limited to, straight chained alkyl groups including methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, and branched alkyl groups including isopropyl, tert-butyl, iso-amyl, neopentyl, iso-amyl, and the like. “Cycloalkyl” or “cyclic alkyl” as used herein refers to carbocycles containing no heteroatoms, and includes mono-, bi- and tricyclic saturated carbocycles, as well as fused ring systems. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, and the like. The cycloalkyl can be substituted or unsubstituted, and cyclic alkyl groups including cyclopropyl, cyclopentyl, cyclohexyl, cycloheptyl,

and the like. Such alkyl groups may be optionally substituted as described herein. When substituted, the substituents include, but are not limited to, cycloalkyl; hydroxy; alkoxy; aryl; heteroaryl; amino optionally substituted by alkyl; carboxy; amido; carbamoyl optionally substituted by alkyl; aminosulfonyl optionally substituted by alkyl; alkylsulfonyl; acyl; aroyl; heteroaroyl; acyloxy; aroyloxy; heteroaroyloxy; alkoxycarbonyl; nitro; cyano; halogen; perfluoroalkyl; and heterocycloalkyl; with multiple degrees of substitution being allowed on the alkyl group.

[0139] “Alkylcyano” refers broadly to a straight or branched chain, saturated or partially unsaturated hydrocarbon radical bonded to a cyano (*i.e.*, $C\equiv N$) group.

[0140] “Alkylhalo” refers broadly to a straight or branched chain, saturated or partially unsaturated hydrocarbon radical bonded to a halogen (*e.g.*, fluoro, chloro, bromo, and iodo).

[0141] “Alkaryl” or “arylalkyl” as used herein refers broadly to a straight or branched chain, saturated hydrocarbon radical bonded to an aryl group. Examples of alkaryl groups include, but are not limited to, benzyl, 4-chlorobenzyl, methylbenzyl, dimethylbenzyl, ethylphenyl, propyl-(4-nitrophenyl), and the like. Such alkaryl groups may be optionally substituted described herein.

[0142] “Alkylene” as used herein refers broadly to a straight or branched chain having two terminal monovalent radical centers derived by the removal of one hydrogen atom from each of the two terminal carbon atoms of straight-chain parent alkane.

[0143] “Aryl” or “Ar” as used herein refers broadly to an optionally substituted aromatic group or to an optionally substituted aromatic group fused to one or more optionally substituted aromatic groups, optionally substituted with suitable substituents including, but not limited to, alkyl; alkoxy; alkylsulfonyl; alkylsulfenyl; alkylsulfonyl; oxo; hydroxy; mercapto; amino optionally substituted by alkyl; amido; carboxy; carbamoyl optionally substituted by alkyl; aminosulfonyl optionally substituted by alkyl; acyl; aroyl; heteroaroyl; acyloxy; aroyloxy; heteroaroyloxy; alkoxycarbonyl; nitro; cyano; halogen; or perfluoroalkyl; multiple degrees of substitution being allowed. Examples of aryl include, but are not limited to, phenyl, 2-naphthyl, 1-naphthyl, and the like. In some embodiments, two adjacent hydroxy groups on an aromatic group can form a dioxolane.

[0144] “Alkoxy” as used herein alone or as part of another group, refers broadly to an alkyl group, as defined herein, appended to the parent molecular moiety through an oxy group. In some embodiments, the alkyl group can be interrupted by one or more heteroatoms (*e.g.*, O, S, or N). Examples of alkoxy include, but are not limited to, methoxy, ethoxy, propoxy, 2-propoxy, butoxy, tert-butoxy, pentyloxy, hexyloxy, ethyloxyethyl, and the like.

[0145] “Alkaryloxy” or “oxyalkaryl” as used herein refers broadly to the group -O-alkyl-aryl wherein Ar is aryl. Examples include, but are not limited to, benzyloxy, oxybenzyl, 2-naphthyloxy, and oxy-2-naphthyl.

[0146] “Alkaryloxyalkyl” or “alkyloxyalkaryl” as used herein refers broadly to the group -alkyl-O-alkyl-aryl wherein Ar is aryl. Examples include, but are not limited to benzyloxyethyl.

[0147] “Analog,” as used herein, refer broadly to the modification or substitution of one or more chemical moieties on a parent compound and may include derivatives, positional isomers, and prodrugs of the parent compound.

[0148] “Aryloxy” as used herein refers broadly to the group -ArO wherein Ar is aryl or heteroaryl. Examples include, but are not limited to, phenoxy, benzyloxy, and 2-naphthyloxy.

[0149] “Amino” as used herein refers broadly to -NH₂ in which one or both of the hydrogen atoms may optionally be replaced by alkyl, aryl or heteroaryl, where the alkyl, aryl, and heteroaryl groups is optionally substituted.

[0150] “Alkylthio” or “thioalkyl,” as used herein alone or as part of another group, refers broadly to an alkyl group, as defined herein, appended to the parent molecular moiety through a sulfur moiety. Representative examples of alkylthio include, but are not limited to, methylthio, thiomethyl, ethylthio, thioethyl, n-propylthio, thio-n-propyl, isopropylthio, thio-isopropyl, n-butylthio, thio-n-butyl, and the like.

[0151] “Arylthio” or “thioaryl,” as used herein refers broadly to the group -ArS wherein Ar is aryl. Examples include, but are not limited to, phenylthio, thiophenyl, 2-naphthylthio, and thio-2-naphthyl.

[0152] “Alkarylthio” or “thioalkaryl” as used herein refers broadly to the group -S-alkyl-aryl wherein Ar is aryl. Examples include, but are not limited to, benzylthio, thiobenzyl, 2-naphthylthio, and thio-2-naphthyl.

[0153] “Alkylheterocycloalkyl” as used herein refers to as used herein refers broadly to a straight or branched chain, saturated hydrocarbon radical bonded to a heterocycloalkyl group.

[0154] “Biocompatible polymer” as used herein refers broadly to a polymer moiety that is substantially non-toxic and does not tend to produce substantial immune responses, clotting or other undesirable effects. Accordingly to some embodiments of the present invention, polyalkylene glycol is a biocompatible polymer where, as used herein, polyalkylene glycol refers to straight or branched polyalkylene glycol polymers such as polyethylene glycol, polypropylene glycol, and polybutylene

glycol, and further includes the monoalkylether of the polyalkylene glycol. In some embodiments of the present invention, the polyalkylene glycol polymer is a lower alkyl polyalkylene glycol moiety such as a polyethylene glycol moiety (PEG), a polypropylene glycol moiety, or a polybutylene glycol moiety. PEG has the formula -HO(CH₂CH₂O)_nH, where n can range from about 1 to about 4000 or more. In some embodiments, n is 1 to 100, and in other embodiments, n is 5 to 30. The PEG moiety can be linear or branched. In further embodiments, PEG can be attached to groups such as hydroxyl, alkyl, aryl, acyl, or ester. In some embodiments, PEG can be an alkoxy PEG, such as methoxy-PEG (or mPEG), where one terminus are a relatively inert alkoxy group, while the other terminus are a hydroxyl group.

[0155] “Bioavailability”, as used herein, refers broadly to the availability of a drug to an animal following administration and may be used interchangeably with “systemic exposure” (*e.g.*, the bioavailability of a drug is expressed as the systemic exposure of a cell to drugs).

[0156] “Carboxy” as used herein refers broadly to the group -CO₂H.

[0157] “Cycloalkyl” as used herein refers to carbocycles containing no heteroatoms, and includes mono-, bi- and tricyclic saturated carbocycles, as well as fused ring systems. Examples of cycloalkyl include cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, and the like. The cycloalkyl can be substituted or unsubstituted.

[0158] “Effective amount” or “effective,” as used herein, refers broadly to a dose that causes a relief of symptoms of a disease or disorder as noted through clinical testing and evaluation, patient observation, and/or the like. “Effective amount” or “effective” further can further designate a dose that causes a detectable change in biological or chemical activity. The detectable changes may be detected and/or further quantified by one skilled in the art for the relevant mechanism or process. Moreover, “effective amount” or “effective” can designate an amount that maintains a desired physiological state, *i.e.*, reduces or prevents significant decline and/or promotes improvement in the condition of interest. As are generally understood in the art, the dosage will vary depending on the administration routes, symptoms, and body weight of the patient but also depending upon the compound being administered.

[0159] “Halo” as used herein refers broadly to bromo, chloro, fluoro, or iodo. Alternatively, the term “halide” as used herein refers broadly to bromide, chloride, fluoride, or iodide.

[0160] “Hydroxy” as used herein refers broadly to the group -OH.

[0161] "Heteroaryl" as used herein refers to an aromatic five- or six-membered ring where at least one atom consists of a heteroatom (*e.g.*, O, S, or N), and the remaining atoms are carbon. The five-membered rings have two double bonds, and the six-membered rings have three double bonds. The heteroaryl group can be monocyclic or bicyclic (fused or non-fused). Examples of monocyclic heteroaryl groups include furanyl, thiophene-yl, pyrrolyl, oxazolyl, thiazolyl, imidazolyl, pyrazolyl, isoxazolyl, isothiazolyl, oxadiazolyl, thiazolyl, thiadiazolyl, pyridinyl, pyridazinyl, pyrimidinyl, pyrazinyl, triazinyl, and the like. Examples of bicyclic heteroaryl groups include indoliziny, indolyl, isoindolyl, benzofuranyl, benzothiophene-yl, indazolyl, benzimidazolyl, benzthiazolyl, purinyl, quinolinyl, isoquinolinyl, cinnolinyl, phthalazinyl, quinazolinyl, quinoxalinyl, naphthyridinyl, pteridinyl, and the like. The heteroaryl group can be substituted or unsubstituted.

[0162] "Heterocycloalkyl" as used herein refers to a cycloalkyl group where at least one of the carbon atoms in the ring is replaced by a heteroatom (*e.g.*, O, S, or N). The heterocycloalkyl group can be monocyclic or bicyclic (fused or non-fused). Examples of monocyclic heterocycloalkyl groups include azetidiny, pyrrolidinyl, piperidinyl, homopiperidinyl, piperazinyl, morpholinyl, thiomorpholinyl, 1-oxothiomorpholinyl, 1,1-dioxothiomorpholinyl, tetrahydrooxazolyl, tetrahydroisoxazolyl, tetrahydroimidazolyl, tetrahydropyrazolyl, tetrahydrothiazolidinyl, tetrahydroisothiazolidinyl, tetrahydropyrimidinyl, tetrahydropyridazinyl, 4-piperadonyl, and the like. Examples of bicyclic non-fused heterocycloalkyl groups include quinuclidinyl, adamantyl, 2-azobicyclo[3.2.1]octyl, and the like. Examples of fused heterocycloalkyl groups include any of the aforementioned monocyclic heterocycloalkyl groups fused with another cycloalkyl or heterocycloalkyl group. Examples of non-fused heterocycloalkyl groups include spirocycles of any of the aforementioned monocyclic heterocycloalkyl groups with another cycloalkyl or heterocycloalkyl group. The heterocycloalkyl group can be substituted or unsubstituted. When substituted, the substituents include, but are not limited to, cycloalkyl; hydroxy; alkoxy; aryl; heteroaryl; amino optionally substituted by alkyl; carboxy; amido; carbamoyl optionally substituted by alkyl; aminosulfonyl optionally substituted by alkyl; alkylsulfonyl; acyl; aroyl; heteroaroyl; acyloxy; aroyloxy; heteroaroyloxy; alkoxycarbonyl; nitro; cyano; halogen; perfluoroalkyl; and heterocycloalkyl; with multiple degrees of substitution being allowed on the alkyl group.

[0163] "Increased" or "increase" as used herein, refers broadly to a quantified change in a measurable quality that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater relative to a control measurement, more preferably

an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater. In particular, the term “increase,” as used herein, refers broadly to make greater, as in number, size, strength, or quality; add to; and/or augment. “Increase,” as used herein, also encompasses expand, extend, prolong, augment, enlarge, grow, develop, and/or swell. “Increase,” as used herein, additionally encompasses where a given parameter (*e.g.*, level, amount, size, scope, duration, weight) is greater, as in number, size, strength, or quality, than it once was. Furthermore, the “increase” in any number, size, strength, or quality of a given parameter may be determined as between two or more time points, especially if before or after a treatment, event, or administration of an agent or composition. Further, “increase” refers broadly to significant or detectable, functionally, analytically, and/or clinically, changes in the number, size, strength, or quality of a given parameter in question.

[0164] “Mammal” as used herein, refers broadly to any and all warm-blooded vertebrate animals of the class Mammalia, including humans, characterized by a covering of hair on the skin and, in the female, milk-producing mammary glands for nourishing the young. Examples of mammals include but are not limited to alpacas, armadillos, capybaras, cats, chimpanzees, chinchillas, cattle, dogs, goats, gorillas, hamsters, horses, humans, lemurs, llamas, mice, non-human primates, pigs, rats, sheep, shrews, and tapirs. Mammals include but are not limited to bovine, canine, equine, feline, murine, ovine, porcine, primate, and rodent species. Mammal also includes any and all those listed on the Mammal Species of the World maintained by the National Museum of Natural History, Smithsonian Institution in Washington DC, hereby incorporated by reference in its entirety.

[0165] “N-oxide” or “amine N-oxide” as used herein refers broadly to a chemical structure having an N-O bond where the nitrogen is positively charged and the oxygen is negatively charged.

[0166] “N-substituted sulfonamide” as used herein refers broadly to a chemical structure having the -SO₂-NH(R) group. In this context, the R-group includes, but is not limited to lower alkyl (*e.g.*, C₁-C₅ alkyl), lower alkenyl (*e.g.*, C₂-C₆ alkenyl), alkaryl, aryl, cycloalkenyl, cycloalkyl, dialkylaminoalkyl, heterocycloalkyl, and heteroaryl.

[0167] “N,N-disubstituted sulfonamide” as used herein refers broadly to a chemical structure having the -SO₂-NRR' group. In this context, the R and R' are the same or different and are independently lower alkyl, lower alkenyl, alkaryl, aryl, cycloalkenyl, cycloalkyl, dialkylaminoalkyl, heterocycloalkyl, heteroaryl or taken together with the nitrogen atom to which they are attached

form a 4-8 member cycle which can be substituted or unsubstituted and can have one or more heteroatoms (*e.g.*, N, O, or S).

[0168] “Parasynaptic” as used herein, refers broadly to receptors (*e.g.*, GABA_A receptors) located outside or in the perimeter of the synapse (*e.g.*, synaptic cleft). Also, “parasynaptic” refers broadly to any receptors located perisynaptically, extrasynaptically, and presynaptically.

[0169] “Patient” as used herein, refers broadly to any animal who is in need of treatment either to alleviate a disease state or to prevent the occurrence or reoccurrence of a disease state. Also, “Patient” as used herein, refers broadly to any animal who has risk factors, a history of disease, susceptibility, symptoms, signs, was previously diagnosed, is at risk for, or is a member of a patient population for a disease. The patient may be a clinical patient such as a human or a veterinary patient such as a companion, domesticated, livestock, exotic, or zoo animal. Animals may be mammals, reptiles, birds, amphibians, or invertebrates.

[0170] A “pharmaceutical composition” refers to a chemical or biological composition suitable for administration to a subject (*e.g.*, mammal). Such compositions may be specifically formulated for administration via one or more of a number of routes, including but not limited to buccal, cutaneous, epicutaneous, epidural, infusion, inhalation, intraarterial, intracardial, intracerebroventricular, intradermal, intramuscular, intranasal, intraocular, intraperitoneal, intraspinal, intrathecal, intravenous, oral, parenteral, pulmonary, rectally via an enema or suppository, subcutaneous, subdermal, sublingual, transdermal, and transmucosal. In addition, administration can be by means of capsule, drops, foams, gel, gum, injection, liquid, patch, pill, porous pouch, powder, tablet, or other means of administration.

[0171] A “pharmaceutical excipient” or a “pharmaceutically acceptable excipient” is a carrier, usually a liquid, in which an active therapeutic agent is formulated. The excipient generally does not provide any pharmacological activity to the formulation, though it may provide chemical and/or biological stability, and release characteristics. Exemplary formulations can be found, for example, in Remington, The Science And Practice of Pharmacy (20th Ed.) (Gennaro, A. R., Chief Editor), Philadelphia College of Pharmacy and Science (2000).

[0172] As used herein “pharmaceutically acceptable carrier” or “excipient” includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents that are physiologically compatible. In one embodiment, the carrier is suitable for parenteral administration. Alternatively, the carrier can be suitable for intravenous, intraperitoneal,

intramuscular, sublingual, or oral administration. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances are well known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the invention is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[0173] “Pharmaceutically acceptable salt” as used herein, refers broadly to a salt form of a compound permitting its use or formulation as a pharmaceutical and which retains the biological effectiveness of the free acid and base of the specified compound and that is not biologically or otherwise undesirable.

[0174] “Prophylactically effective amount” as used herein, refers broadly to the amount of a compound that, when administered to a patient for prophylaxis of a disease or prevention of the reoccurrence of a disease, is sufficient to effect such prophylaxis for the disease or reoccurrence. The prophylactically effective amount may be an amount effective to prevent the incidence of signs and/or symptoms. The “prophylactically effective amount” may vary depending on the disease and its severity and the age, weight, medical history, predisposition to conditions, preexisting conditions, of the patient to be treated.

[0175] “Prophylaxis,” as used herein, refers broadly to a course of therapy where signs and/or symptoms are not present in the patient, are in remission, or were previously present in a patient. Prophylaxis includes preventing disease occurring subsequent to treatment of a disease in a patient. Further, prevention includes treating patients who may potentially develop the disease, especially patients who are susceptible to the disease (*e.g.*, members of a patient population, those with risk factors, or at risk for developing the disease).

[0176] “Protective,” as used herein, refers broadly to reducing the incidence or severity of the disease in a patient. Protective, as used herein, refers broadly to inhibiting the disease, arresting the development of the disease or its clinical symptoms, and/or causing regression of the disease or its clinical symptoms. Prevention also preferably includes preventing or reducing incidence or severity of disease in a patient.

[0177] “Protective effect amount,” as used herein, refers broadly to the amount of a compound that, when administered to a patient reduces the severity of the incidence of signs and/or symptoms, slows

the development of the incidence of signs and/or symptoms, prevents the development of the incidence of signs and/or symptoms. The “protective effective amount” may vary depending on the disease and its severity and the age, weight, medical history, predisposition to conditions, preexisting conditions, of the patient to be treated.

[0178] “Quaternary ammonium” as used herein refers broadly to a chemical structure having four bonds to the nitrogen with a positive charge on the nitrogen in the “onium” state, *i.e.*, “R₄N⁺” or “quaternary nitrogen,” wherein R is an organic substituent such as alkyl or aryl. The term “quaternary ammonium salt” as used herein refers broadly to the association of the quaternary ammonium cation with an anion.

[0179] “Signs” of disease, as used herein, refers broadly to any abnormality indicative of disease, discoverable on examination of the patient; an objective indication of disease, in contrast to a symptom, which is a subjective indication of disease.

[0180] “Symptoms” of disease as used herein, refers broadly to any morbid phenomenon or departure from the normal in structure, function, or sensation, experienced by the patient and indicative of disease.

[0181] “Subjects” as used herein, refers broadly to anyone suitable to be treated according to the present invention include, but are not limited to, avian and mammalian subjects, and are preferably mammalian. Mammals of the present invention include, but are not limited to, canines, felines, bovines, caprines, equines, ovines, porcines, rodents (*e.g.*, rats and mice), lagomorphs, primates, humans, and the like, and mammals in utero. Any mammalian subject in need of being treated according to the present invention is suitable. Human subjects are preferred. Human subjects of both genders and at any stage of development (*i.e.*, neonate, infant, juvenile, adolescent, adult) can be treated according to the present invention.

[0182] The present invention can also be carried out on animal subjects, particularly mammalian subjects such as mice, rats, dogs, cats, cattle, goats, sheep, and horses for veterinary purposes, and for drug screening and drug development purposes. The present invention can also be carried out on avians including chickens, ducks, turkeys, geese, quail, pheasant, ratites (*e.g.*, ostrich) and domesticated birds (*e.g.*, parrots and canaries), and birds *in ovo*. “Subjects” is used interchangeably with “patients.”

[0183] “Solvate” as used herein refers broadly to a molecular complex of a compound with one or more solvent molecules in a stoichiometric or non-stoichiometric amount. Such solvent molecules

are those commonly used in the pharmaceutical arts, *e.g.*, water, ethanol, and the like. A molecular complex of a compound or moiety of a compound and a solvent can be stabilized by non-covalent intra-molecular forces such as, electrostatic forces, van der Waals forces, and hydrogen bonds. The term “hydrate” refers to a complex in which the one or more solvent molecules are water including monohydrates and hemi-hydrates. Examples of solvates, without limitation, include compounds of the invention in combination with water, 1-propanol, 2-propanol, ethanol, methanol, DMSO, ethyl acetate, acetic acid, or ethanolamine.

[0184] “Substituted” as used herein refers broadly to replacement of one or more of the hydrogen atoms of the group replaced by substituents known to those skilled in the art and resulting in a stable compound as described below. Examples of suitable replacement groups include, but are not limited to, alkyl, acyl, alkenyl, alkynyl cycloalkyl, aryl, alkaryl, hydroxy, thio, alkoxy, aryloxy, acyl, amino, amido, carboxy, carboxyalkyl, thiocarboxyalkyl, carboxyaryl, thiocarboxyaryl, halo, oxo, mercapto, sulfinyl, sulfonyl, sulfonamido, amidino, carbamoyl, cycloalkyl, heterocycloalkyl, dialkylaminoalkyl, carboxylic acid, carboxamido, haloalkyl, dihaloalkyl, trihaloalkyl, trihaloalkoxy, alkylthio, aralkyl, alkylsulfonyl, arylthio, amino, alkylamino, dialkylamino, guanidino, ureido, nitro and the like. Substitutions are permissible when such combinations result in compounds stable for the intended purpose. For example, substitutions are permissible when the resultant compound is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and formulation into a therapeutic or diagnostic agent or reagent.

[0185] “Therapy” or “therapeutic” as used herein refers broadly to treating a disease, arresting or reducing the development of the disease or its clinical symptoms, and/or relieving the disease, causing regression of the disease or its clinical symptoms. Therapy encompasses prophylaxis, prevention, treatment, cure, regimen, remedy, minimization, reduction, alleviation, and/or providing relief from a disease, signs, and/or symptoms of a disease. Therapy encompasses an alleviation of signs and/or symptoms in patients with ongoing disease signs and/or symptoms (*e.g.*, pain, inflammation.) Therapy also encompasses “prophylaxis” and “prevention.” Prophylaxis includes preventing disease occurring subsequent to treatment of a disease in a patient or reducing the incidence or severity of the disease in a patient. The term “reduced,” for purpose of therapy, refers broadly to the clinical significant reduction in signs and/or symptoms. Therapy includes treating relapses or recurrent signs and/or symptoms (*e.g.*, of pain.) Therapy encompasses but is not limited to precluding the appearance of signs and/or symptoms anytime as well as reducing existing signs

and/or symptoms and eliminating existing signs and/or symptoms. Therapy includes treating chronic disease (“maintenance”) and acute disease.

[0186] Therapy can be for patients with risk factors, at risk patients in a susceptible population, patients with a history of disease, patients with symptoms, patients with signs, patients with signs but no symptoms, and patients with symptoms but no signs. Therapy can also be for patients without risk factors, not at risk, patients not in a susceptible population, patients with no history of disease, patients with no symptoms, patients without signs. Therapy can alleviate, allay, abate, assuage, curtail, decrease, ease, lessen, lighten, make better, make healthy, mitigate, mollify, pacify, relieve, rehabilitate, remedy, repair, and/or soothe a disease, disease signs, and/or disease symptoms.

[0187] “Treating” or “treatment,” as used herein, refers broadly to a course of therapy where signs and/or symptoms are present in the patient. The term “reduced,” for purpose of therapy, refers broadly to clinically significant reduction in signs and/or symptoms. Treatment includes treating chronic disease (“maintenance”) and acute disease. Treatment can be for patients with risk factors, at risk patients in a susceptible population, patients with a history of disease, and/or patients with symptoms, patients with signs. Treatment can alleviate, allay, abate, assuage, curtail, decrease, ease, lessen, lighten, make better, make healthy, mitigate, mollify, pacify, relieve, rehabilitate, remedy, repair, and/or soothe a disease, disease signs, and/or disease symptoms. By the terms “treating” or “treatment” of a disorder involving the $\text{Na}^+\text{K}^+\text{Cl}^-$ co-transporters, it is intended that the severity of the disorder or the symptoms of the disorder are reduced, or the disorder is partially or entirely eliminated, as compared to that which would occur in the absence of treatment. Treatment does not require the achievement of a complete cure of the disorder. By the terms “preventing” or “prevention” of the disorder involving the $\text{Na}^+\text{K}^+\text{Cl}^-$ co-transporters, it is intended that the inventive methods eliminate or reduce the incidence or onset of the disorder, as compared to that which would occur in the absence of treatment. Alternatively stated, the present methods slow, delay, control, or decrease the likelihood or probability of the disorder in the subject, as compared to that which would occur in the absence of treatment. Further, the terms “treating” or “treatment” of a disorder involving the GABA_A receptor, are intended that the severity of the disorder or the symptoms of the disorder are reduced, or the disorder is partially or entirely eliminated, as compared to that which would occur in the absence of treatment. Treatment does not require the achievement of a complete cure of the disorder.

Compounds

[0188] According to some embodiments, the present invention provides novel compounds. Thus, any of the R groups as defined herein can be excluded or modified in order to exclude a known compound and/or provide a novel compound. Also, any of the R groups as defined herein can be excluded from the compounds of the present invention, particularly with reference to denoting novel compounds of the present invention. Compounds of the present invention include compounds according to formula I, II, III or IV.

[0189] Embodiments of the present invention further provide intermediate compounds formed through the synthetic methods described herein to provide compounds of Formula I-IV. The intermediate compounds may possess utility as therapeutic agents for the range of indications described herein and/or reagents for further synthesis methods and reactions.

[0190] In some embodiments, the present invention encompasses the following compounds, including esters, amides, N-substituted sulfonamides and N,N-disubstituted sulfonamides thereof.

Synthetic Methods

[0191] Embodiments of the present invention provide methods of modifying compounds of the present invention to increase their lipophilicity. The lipophilicity can be measured by determining the hydrophile-lipophile balance (HLB) or the partition coefficient (*e.g.*, the distribution of a compound between water and octanol). In some embodiments, the compound is a diuretic or diuretic-like compound, and in particular embodiments, the compound is termed a “loop diuretic.” For a discussion of pharmacological properties of diuretics. *See generally*, Hardman, Limbird, and Gilman, (Eds.) (2001) Goodman & Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill Medical Publishing Division (10th Ed.) Further included are compounds that affect cation-chloride cotransporters. Furosemide and bumetanide are classic examples of NKCC antagonists.

[0192] Starting materials for synthesizing compounds of the present invention can further include compounds described in U.S. Patent No. 3,634,583; U.S. Patent No. 3,806,534; U.S. Patent No. 3,058,882; U.S. Patent No. 4,010,273; U.S. Patent No. 3,665,002; and U.S. Patent No. 3,665,002.

[0193] Compounds of the present invention can include isomers, tautomers, zwitterions, enantiomers, diastereomers, racemates, or stereochemical mixtures thereof. Compounds of the present invention can also comprise isosteres.

[0194] The term “isosteres” as used herein broadly refers to elements, functional groups, substituents, molecules, or ions having different molecular formulae but exhibiting similar or

identical physical properties. For example, tetrazole is an isostere of carboxylic acid because it mimics the properties of carboxylic acid even though they both have different molecular formulae. Typically, two isosteric molecules have similar or identical volumes and shapes. Other physical properties that isosteric compounds usually share include boiling point, density, viscosity, and thermal conductivity. However, certain properties are usually different: dipolar moments, polarity, polarization, size, and shape since the external orbitals may be hybridized differently.

[0195] The term “isomers” as used herein refers broadly to compounds having the same number and kind of atoms, and hence the same molecular weight, but differing with respect to the arrangement or configuration of the atoms in space. Additionally, the term “isomers” includes stereoisomers and geometric isomers. The terms “stereoisomer” or “optical isomer” as used herein refer to a stable isomer that has at least one chiral atom or restricted rotation giving rise to perpendicular dissymmetric planes (*e.g.*, certain biphenyls, allenes, and spiro compounds) and can rotate plane-polarized light. Because asymmetric centers and other chemical structure can exist in some of the compounds of the present invention, which may give rise to stereoisomerism, the invention contemplates stereoisomers and mixtures thereof. The compounds of the present invention and their salts can include asymmetric carbon atoms and may therefore exist as single stereoisomers, racemates, and as mixtures of enantiomers and diastereomers. Typically, such compounds will be prepared as a racemic mixture. If desired, however, such compounds can be prepared or isolated as pure stereoisomers, *i.e.*, as individual enantiomers or diastereomers, or as stereoisomer-enriched mixtures. Tautomers are readily interconvertible constitutional isomers and there is a change in connectivity of a ligand, as in the keto and enol forms of ethyl acetoacetate (including tautomers of any said compounds.) Zwitterions are inner salts or dipolar compounds possessing acidic and basic groups in the same molecule. At neutral pH, the cation and anion of most zwitterions are equally ionized.

Pharmaceutical Compositions

[0196] The compounds (*e.g.*, analogs, derivatives, and prodrugs) of the present invention or pharmacologically acceptable salts thereof may be formulated into pharmaceutical compositions of various dosage forms. To prepare the pharmaceutical compositions of the invention, at least one compound, or pharmaceutically acceptable salts thereof, as the active ingredient is intimately mixed with appropriate carriers and additives according to techniques well known to those skilled in the art

of pharmaceutical formulations. Remington, The Science And Practice of Pharmacy (20th Ed.) (Gennaro, A. R., Chief Editor), Philadelphia College of Pharmacy and Science (2000).

[0197] Pharmaceutically acceptable salts of the compounds described herein include the salt form of the compound permitting its use or formulation as a pharmaceutical and which retains the biological effectiveness of the free acid and base of the specified compound and that is not biologically or otherwise undesirable. Examples of such salts are described in Wermuth and Stahl, (Eds.) (2002) Handbook of Pharmaceutical Salts: Properties, Selection, and Use, Wiley-Verlag Helvetica Acta, Zürich, herein incorporated by references in its entirety. Examples of such salts include alkali metal salts and addition salts of free acids and bases. Examples of pharmaceutically acceptable salts, without limitation, include sulfates, pyrosulfates, bisulfates, sulfites, bisulfites, phosphates, monohydrogenphosphates, dihydrogen phosphates, metaphosphates, pyrophosphates, chlorides, bromides, iodides, acetates, propionates, decanoates, caprylates, acrylates, formates, isobutyrate, caproates, heptanoates, propiolates, oxalates, malonates, succinates, suberates, sebacates, fumarates, maleates, butyne-1,4-dioates, hexyne-1,6-dioates, benzoates, chlorobenzoates, methylbenzoates, dinitrobenzoates, hydroxybenzoates, methoxybenzoates, phthalates, xylenesulfonates, phenylacetates, phenylpropionates, phenylbutyrates, citrates, lactates, γ -hydroxybutyrates, glycollates, tartrates, methanesulfonates, ethane sulfonates, propanesulfonates, toluenesulfonates, naphthalene-1-sulfonates, naphthalene-2-sulfonates, and mandelates. In some embodiments, pharmaceutically acceptable salt includes sodium, potassium, calcium, ammonium, trialkylammonium, and tetraalkylammonium salts.

[0198] Similarly, compositions for liquid preparations include solutions, emulsions, dispersions, suspensions, syrups, and elixirs, with suitable carriers and additives including but not limited to water, alcohols, oils, glycols, preservatives, flavoring agents, coloring agents, and suspending agents. Typical preparations for parenteral administration comprise the active ingredient with a carrier such as sterile water or parenterally acceptable oil including but not limited to polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil, with other additives for aiding solubility or preservation may also be included. In the case of a solution, it can be lyophilized to a powder and then reconstituted immediately prior to use. For dispersions and suspensions, appropriate carriers and additives include aqueous gums, celluloses, silicates, or oils.

[0199] The pharmaceutical compositions according to embodiments of the present invention include those suitable for oral, rectal, topical, nasal, inhalation (*e.g.*, via an aerosol) buccal (*e.g.*, sub-

lingual), vaginal, topical (*e.g.*, both skin and mucosal surfaces, including airway surfaces), transdermal administration and parenteral (*e.g.*, subcutaneous, intramuscular, intradermal, intraarticular, intrapleural, intraperitoneal, intrathecal, intracerebral, intracranially, intraarterial, or intravenous), although the most suitable route in any given case will depend on the nature and severity of the condition being treated and on the nature of the particular active agent which is being used. Pharmaceutical compositions of the present invention are particularly suitable for oral, sublingual, parenteral, implantation, nasal, and inhalational administration. The carriers and additives used for such pharmaceutical compositions can take a variety of forms depending on the anticipated mode of administration.

[0200] Compositions for oral administration may be solid preparations including but not limited to tablets, sugar-coated tablets, hard capsules, soft capsules, granules, lozenges, and powders, with suitable carriers and additives being starches, sugars, binders, diluents, granulating agents, lubricants, and disintegrating agents. Tablets and capsules represent advantageous oral dosage forms for many medical conditions because of their ease of use and higher patient compliance.

[0201] Compositions for injection will include the active ingredient together with suitable carriers including organic solvents, propylene glycol-alcohol-water, isotonic water, sterile water for injection (USP), emulPhor[®]-alcohol-water, cremophor-EL[®] or other suitable carriers known to those skilled in the art. These carriers may be used alone or in combination with other conventional solubilizing agents such as ethanol, a glycol, or other agents known to those skilled in the art.

[0202] Where the compounds of the present invention are to be applied in the form of solutions or injections, the compounds may be used by dissolving or suspending in any conventional diluent. The diluents include but are not limited to physiological saline, Ringer's solution, an aqueous glucose solution, an aqueous dextrose solution, an alcohol, a fatty acid ester, glycerol, a glycol, an oil derived from plant or animal sources, and a paraffin. These preparations may be prepared according to any conventional method known to those skilled in the art.

[0203] Compositions for nasal administration may be formulated as aerosols, drops, powders, and gels. Aerosol formulations typically comprise a solution or fine suspension of the active ingredient in a physiologically acceptable aqueous or non-aqueous solvent. Such formulations are typically presented in single or multidose quantities in a sterile form in a sealed container. The sealed container can be a cartridge or refill for use with an atomizing device. Alternatively, the sealed container may be a unitary dispensing device such as a single use nasal inhaler, pump atomizer or an

aerosol dispenser fitted with a metering valve set to deliver an effective amount, which is intended for disposal once the contents have been completely used. When the dosage form comprises an aerosol dispenser, it will contain a propellant such as a compressed gas, air as an example, or an organic propellant including a fluorochlorohydrocarbon or fluorohydrocarbon.

[0204] Compositions suitable for buccal or sublingual administration include but are not limited to tablets, lozenges and pastilles, wherein the active ingredient is formulated with a carrier such as sugar and acacia, tragacanth or gelatin and glycerin.

[0205] Compositions for rectal administration include but are not limited to suppositories containing a conventional suppository base such as cocoa butter.

[0206] Compositions suitable for transdermal administration include but are not limited to ointments, gels, and patches.

[0207] The preferred forms of administration in the present invention are oral forms known in the art of pharmaceuticals. The pharmaceutical compositions of the present invention may be orally administered as a capsule (hard or soft), tablet (film coated, enteric coated or uncoated), powder or granules (coated or uncoated) or liquid (solution or suspension). The formulations may be conveniently prepared by any of the methods well-known in the art. The pharmaceutical compositions of the present invention may include one or more suitable production aids or excipients including fillers, binders, disintegrants, lubricants, diluents, flow agents, buffering agents, moistening agents, preservatives, colorants, sweeteners, flavors, and pharmaceutically compatible carriers.

[0208] For each of the recited embodiments, the compounds can be administered by a variety of dosage forms as known in the art. Any biologically-acceptable dosage form known to persons of ordinary skill in the art, and combinations thereof, are contemplated. Examples of such dosage forms include, without limitation, chewable tablets, quick dissolve tablets, effervescent tablets, reconstitutable powders, elixirs, liquids, solutions, suspensions, emulsions, tablets, multi-layer tablets, bi-layer tablets, capsules, soft gelatin capsules, hard gelatin capsules, caplets, lozenges, chewable lozenges, beads, powders, gum, granules, particles, microparticles, dispersible granules, cachets, douches, suppositories, creams, topicals, inhalants, aerosol inhalants, patches, particle inhalants, implants, depot implants, ingestibles, injectables (including subcutaneous, intramuscular, intravenous, and intradermal), infusions, and combinations thereof.

[0209] Other compositions known to those skilled in the art can also be applied for percutaneous or subcutaneous administration, such as plasters.

[0210] Further, in preparing such pharmaceutical compositions comprising the active ingredient or ingredients in admixture with components necessary for the formulation of the compositions, other conventional pharmacologically acceptable additives may be incorporated, including but are not limited to excipients, stabilizers, antiseptics, wetting agents, emulsifying agents, lubricants, sweetening agents, coloring agents, flavoring agents, isotonicity agents, buffering agents, and antioxidants. Additives include but are not limited to starch, sucrose, fructose, dextrose, lactose, glucose, mannitol, sorbitol, precipitated calcium carbonate, crystalline cellulose, carboxymethyl cellulose, dextrin, gelatin, acacia, EDTA, magnesium stearate, talc, hydroxypropylmethylcellulose, and sodium metabisulfite.

[0211] Compounds of the present invention may be used in conjunction with delivery systems that facilitate delivery of the agents to the central nervous system. For example, various blood brain barrier permeability enhancers may be used, if desired, to transiently and reversibly increase the permeability of the blood brain barrier to a treatment agent. Such BBB permeability enhancers include but are not limited to leukotrienes, bradykinin agonists, histamine, tight junction disruptors (*e.g.*, zonulin, zot), hyperosmotic solutions (*e.g.*, mannitol), cytoskeletal contracting agents, and short chain alkylglycerols (*e.g.*, 1-O-pentylglycerol). Oral, sublingual, parenteral, implantation, nasal and inhalational routes can provide delivery of the active agent to the CNS. In some embodiments, the compounds of the present invention may be administered to the CNS with minimal effects on the peripheral nervous system.

[0212] Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants.

[0213] In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an

agent which delays absorption, for example, monostearate salts and gelatin. Moreover, the compounds described herein can be formulated in a time release formulation, for example in a composition that includes a slow release polymer. The active compounds can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers may be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, polylactic acid and polylactic, polyglycolic copolymers (PLG). Many methods for the preparation of such formulations are known to those skilled in the art.

[0214] Other compounds which can be included by admixture are, for example, medically inert ingredients (*e.g.*, solid and liquid diluent), such as lactose, dextrosesaccharose, cellulose, starch or calcium phosphate for tablets or capsules, olive oil or ethyl oleate for soft capsules and water or vegetable oil for suspensions or emulsions; lubricating agents such as silica, talc, stearic acid, magnesium or calcium stearate and/or polyethylene glycols; gelling agents such as colloidal clays; thickening agents such as gum tragacanth or sodium alginate, binding agents such as starches, arabic gums, gelatin, methylcellulose, carboxymethylcellulose or polyvinylpyrrolidone; disintegrating agents such as starch, alginic acid, alginates or sodium starch glycolate; effervescing mixtures; dyestuff; sweeteners; wetting agents such as lecithin, polysorbates or laurylsulphates; and other therapeutically acceptable accessory ingredients, such as humectants, preservatives, buffers and antioxidants, which are known additives for such formulations.

[0215] In further embodiments, the present invention provides kits including one or more containers comprising pharmaceutical dosage units comprising an effective amount of one or more compounds of the present invention. Kits may include instructions, directions, labels, marketing information, warnings, or information pamphlets.

Prodrugs

[0216] The blood-brain barrier (BBB) is a physical barrier and system of cellular transport mechanisms between the blood vessels in the central nervous system (CNS) and most areas of the CNS itself. The BBB maintains homeostasis by restricting the entry of potentially harmful chemicals from the blood, and by allowing the entry of essential nutrients. However, the BBB can pose a formidable barrier to delivery of pharmacological agents to the CNS for treatment of disorders or maintaining or enhancing normal and desirable brain functions, such as cognition, learning, and memory. Prodrugs of the present invention are capable of passage across the blood-

brain barrier and may undergo hydrolysis by CNS esterases to provide the active compound.

Further, the prodrugs provided herein may also exhibit improved bioavailability, improved aqueous solubility, improved passive intestinal absorption, improved transporter-mediated intestinal absorption, protection against accelerated metabolism, tissue-selective delivery, less (or fewer) side effects, lessened or no deleterious drug interaction with other medications, and/or passive enrichment in the target tissue.

[0217] The term “prodrug” is intended to refer to a compound that is converted under physiological conditions, by solvolysis or metabolically to a specified compound that is pharmaceutically/pharmacologically active. The “prodrug” can be a compound of the present invention that has been chemically derivatized such that it retains some, all or none of the bioactivity of its parent drug compound and is metabolized in a subject to yield the parent drug compound. The prodrug of the present invention may also be a “partial prodrug” in that the compound has been chemically derivatized such that it retains some, all or none of the bioactivity of its parent drug compound and is metabolized in a subject to yield a biologically active derivative of the compound.

[0218] Moreover, as shown in the previously presented schemes, prodrugs can be formed by attachment of biocompatible polymers, such as those previously described including polyethylene glycol (PEG), to compounds of the present invention using linkages degradable under physiological conditions. *See also Schacht, et al. (1997) Poly(ethylene glycol) Chemistry and Biological Applications, American Chemical Society, San Francisco, CA 297–315.* Attachment of PEG to proteins can be employed to reduce immunogenicity and/or extend the half-life of the compounds provided herein. Any conventional PEGylation method can be employed, provided that the PEGylated agent retains at least some pharmaceutical activity.

[0219] The present invention further provides prodrugs comprising the compounds described herein. The prodrugs can be formed utilizing a hydrolyzable coupling to the compounds described herein. Ettmayer, *et al.* (2004) *J. Med. Chem.* 47(10): 2394 – 2404 Testa and Mayer (2003) Hydrolysis in Drug and Prodrug Metabolism: Chemistry, Biochemistry and Enzymology Wiley-Verlag Helvetica Chimica Acta, Zuerich (Chapters 1–12): 1–780.

Dosages

[0220] The amount of active compound in a therapeutic composition according to this invention may vary according to factors such as the disease state, age, gender, weight, patient history, risk factors, predisposition to disease, administration route, pre-existing treatment regime (*e.g.*, possible

interactions with other medications), and weight of the individual. Dosage regimens may be adjusted to provide the optimum therapeutic response. For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of therapeutic situation.

[0221] It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals. In therapeutic use for treatment of conditions in mammals (*e.g.*, humans) for which the compounds of the present invention or an appropriate pharmaceutical composition thereof are effective, the compounds of the present invention may be administered in an effective amount. The dosages as suitable for this invention may be a composition, a pharmaceutical composition or any other compositions described herein.

[0222] For each of the recited embodiments, the dose for a patient can be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 μg , as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 μg , as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 μg , as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 μg , as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, or 100 μg , as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 μg and all increments therein. Preferably, the dose for a patient can be about 0.05–5 μg and all increments therein. Alternatively, the dose for a patient can be about 1–10 μg and all increments therein. The dose for a patient can also be about 10–40 μg and all increments therein, about 6–24 μg and all increments therein, about 20–80 μg and all increments therein, about 40–80 μg and all increments therein, about 100–250 μg and all increments therein, or about 100–500 μg and all increments therein. More preferably, the dosage can be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, or 90 μg . Preferably, the dosage can be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, or 80 μg .

[0223] Alternatively, for each of the recited embodiments, the dose for a patient may be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 mg, as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6,

0.7, 0.8, 0.9, or 1.0 mg, as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg, as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg, as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg, as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 mg and all increments therein. Preferably, the dose for a patient may be about 0.05–5 mg and all increments therein. Alternatively, the dose for a patient may be about 1–10 mg and all increments therein. The dose for a patient may also be about 10–40 mg and all increments therein, about 6–24 mg and all increments therein, about 20–80 mg and all increments therein, about 40–80 mg and all increments therein, about 100–250 mg and all increments therein, or about 100–500 mg and all increments therein. More preferably, the dosage may be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, or 90 mg. Preferably, the dosage may be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, or 80 mg.

[0224] Alternatively, for each of the recited embodiments, the dose for a patient may be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 g, as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 g, as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 g, as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g, as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 g, as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 g and all increments therein. Preferably, the dose for a patient may be about 0.05–5 g and all increments therein. Alternatively, the dose for a patient may be about 1–10 g and all increments therein. The dose for a patient may also be about 10–40 g and all increments therein, about 6–24 g and all increments therein, about 20–80 g and all increments therein, about 40–80 g and all increments therein, about 100–250 g and all increments therein, or about 100–500 g and all increments therein. More preferably, the dosage may be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, or 90 g. Preferably, the dosage may be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, or 80 g.

[0225] For each of the recited embodiments, the dose for a patient can be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 $\mu\text{g/kg}$, as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 $\mu\text{g/kg}$, as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 $\mu\text{g/kg}$, as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 $\mu\text{g/kg}$, as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 $\mu\text{g/kg}$, as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 $\mu\text{g/kg}$ and all increments therein. Preferably, the dose for a patient can be about 0.05–5 $\mu\text{g/kg}$ and all increments therein. Alternatively, the dose for a patient can be about 1–10 $\mu\text{g/kg}$ and all increments therein. The dose for a patient can also be about 10–40 $\mu\text{g/kg}$ and all increments therein, about 6–24 $\mu\text{g/kg}$ and all increments therein, about 20–80 $\mu\text{g/kg}$ and all increments therein, about

40–80 $\mu\text{g/kg}$ and all increments therein, about 100–250 $\mu\text{g/kg}$ and all increments therein, or about 100–500 $\mu\text{g/kg}$ and all increments therein. More preferably, the dosage can be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, or 90 $\mu\text{g/kg}$.

[0226] Preferably, the dosage can be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, or 80 $\mu\text{g/kg}$.

Alternatively, for each of the recited embodiments, the dose for a patient may be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 mg/kg , as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 mg/kg , as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 mg/kg , as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 mg/kg , as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 mg/kg , as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 mg/kg and all increments therein. Preferably, the dose for a patient may be about 0.05–5 mg/kg and all increments therein. Alternatively, the dose for a patient may be about 1–10 mg/kg and all increments therein. The dose for a patient may also be about 10–40 mg/kg and all increments therein, about 6–24 mg/kg and all increments therein, about 20–80 mg/kg and all increments therein, about 40–80 mg/kg and all increments therein, about 100–250 mg/kg and all increments therein, or about 100–500 mg/kg and all increments therein. More preferably, the dosage may be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, 90, or 100 mg/kg . Preferably, the dosage may be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 80, 85, 90, or 100 mg/kg .

[0227] Alternatively, for each of the recited embodiments, the dose for a patient can be about 0.01, 0.02, 0.03, 0.04, 0.05, 0.06, 0.07, 0.08, 0.09, or 0.10 g/kg , as well as about 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, or 1.0 g/kg , as well as about 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 g/kg , as well as about 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, or 20 g/kg , as well as about 10, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95 or 100 g/kg , as well as about 100, 200, 300, 400, 500, 600, 700, 800, 900, or 1,000 g/kg and all increments therein. Preferably, the dose for a patient can be about 0.05–5 g/kg and all increments therein. Alternatively, the dose for a patient can be about 1–10 g/kg and all increments therein. The dose for a patient can also be about 10–40 g/kg and all increments therein, about 6–24 g/kg and all increments therein, about 20–80 g/kg and all increments therein, about 40–80 g/kg and all increments therein, about 100–250 g/kg and all increments therein, or about 100–500 g/kg and all increments therein. More preferably, the dosage can be about 0.5, 1, 2, 5, 6, 10, 12, 18, 20, 24, 30, 40, 50, 80, or 90 g/kg . Preferably, the dosage can be 0.5, 2, 6, 8, 10, 12, 18, 20, 30, 40, or 80 g/kg .

[0228] For each of the recited embodiments, the dosage is typically administered once, twice, or thrice a day, although more frequent dosing intervals are possible. The dosage may be administered every day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, and/or every 7 days (once a week). In one embodiment, the dosage may be administered daily for up to and including 30 days, preferably between 7–10 days. In another embodiment, the dosage may be administered twice a day for 10 days. If the patient requires treatment for a chronic disease or condition, the dosage may be administered for as long as signs and/or symptoms persist. The patient may require “maintenance treatment” where the patient is receiving dosages every day for months, years, or the remainder of their lives. In addition, the composition of this invention may be to effect prophylaxis of recurring symptoms. For example, the dosage may be administered once or twice a day to prevent the onset of symptoms in patients at risk, especially for asymptomatic patients.

[0229] For each of the recited embodiments, the patient can receive “pretreatment” with the compounds described herein wherein the compounds described herein are administered every day, every 2 days, every 3 days, every 4 days, every 5 days, every 6 days, and/or every 7 days (once a week). In one embodiment, the dosage can be administered daily for up to and including 30 days, preferably between 7–10 days. In another embodiment, the dosage can be administered twice a day for 10 days. If the patient requires treatment for a chronic disease or condition requiring prolonged treatment, the dosage of alkaline may be administered for as long as symptoms persist.

[0230] In one embodiment, the compounds described herein are administered in an initial dose of 20–80 mg on the first day of treatment and then at least two dosages of about 40 mg on the second day. In another embodiment the compounds described herein are administered in an initial dose of 0.5–2 mg on the first day of treatment and then at least two dosages of about 2 mg on the second day. In another embodiment, the compounds described herein are administered in an initial dose of 10–20 mg on the first day of treatment and then at least two dosages of about 20 mg on the second day. In yet another embodiment, the compounds described herein are administered in an initial dose of 5–10 mg on the first day of treatment and then at least two dosages of about 10 mg on the second day.

[0231] For administration via injection, in one embodiment the treatment begins as a course of 4 injections at 0, 12, 24, and 36 hours. The injections then may continue once, twice, or thrice a day for as long as signs and/or symptoms persists. Alternatively, the injections may be maintained to

prevent the recurrence of disease. Also, the injections may be administered as a prophylaxis for patients at risk, especially asymptomatic patients.

[0232] The dosage may be administered as a single dose, a double dose, a triple dose, a quadruple dose, and/or a quintuple dose. The dosages may be administered singularly, simultaneously, and sequentially.

[0233] For each of the recited embodiments, the dosage of the compounds described herein may be an effective amount of the compounds described herein, an amount effective for prophylaxis, and for acute treatment, or an amount effective for prevention. The dosage of the compounds described herein may be an amount of the compounds described herein effective to reduce signs or symptoms of a disease, an amount effective to prevent signs and/or symptoms of a disease, to reduce the severity of signs and/or symptoms of a disease, to eliminate signs and/or symptoms of a disease, to slow the development of signs or symptoms of a disease, to prevent the development of signs and/or symptoms of a disease, or effect prophylaxis of signs or symptoms of a disease.

[0234] The dosage form may be any form of release known to persons of ordinary skill in the art. The compositions of the present invention may be formulated to provide immediate release of the active ingredient or sustained or controlled release of the active ingredient. In a sustained release or controlled release preparation, release of the active ingredient may occur at a rate such that blood levels are maintained within a therapeutic range but below toxic levels over an extended period of time (*e.g.*, 4 to 24 hours). The preferred dosage forms include immediate release, extended release, pulse release, variable release, controlled release, timed release, sustained release, delayed release, long acting, and combinations thereof. The ability to obtain immediate release, extended release, pulse release, variable release, controlled release, timed release, sustained release, delayed release, long acting characteristics, and combinations thereof is known in the art.

[0235] It will be appreciated that the pharmacological activity of the compositions may be monitored using standard pharmacological models that are known in the art. Furthermore, it will be appreciated that the inventive compositions may be incorporated or encapsulated in a suitable polymer matrix or membrane for site-specific delivery, or may be functionalized with specific targeting agents capable of effecting site specific delivery. For instance, the dosage form may be made such that it preferably releases in the central nervous system or peripheral nervous system. These techniques, as well as other drug delivery techniques are well known in the art. Determination of optimal dosages for a particular situation is within the capabilities of those skilled in the art. *See*

e.g., Gennaro (2000) Remington, The Science And Practice of Pharmacy (20th Ed.) Philadelphia College of Pharmacy and Science.

[0236] In further embodiments, compounds according to the present invention may be administered 1.5 to 6 mg daily, for example, 1 tablet or capsule three times a day. In some embodiments, compounds according to the present invention may be administered 60 to 240 mg/day, for example, 1 tablet or capsule three times a day. In other embodiments, compounds according to the present invention may be administered 10 to 20 mg daily, for, example, 1 tablet or capsule once a day. In some embodiments, compounds according to the present invention may be administered 60 mg per day. In other embodiments, compounds according to the present invention may be administered 10 to 20 mg daily, for example, 1 tablet or capsule once a day. It should be noted that lower doses may be administered, particularly for intravenous administration. Moreover, administration of a lower dose than administered for the parent compound may prevent undesirable peripheral effects such as diuresis.

[0237] In additional further embodiments, compounds are administered at about 0.5, 1.0, or 2.0 mg; compounds are administered at about 20–80 mg or two 40 mg doses daily; compounds are administered 0, 200, 500, or 1,250 mg/kg, preferably at about 10–30 mg/kg or 200–500 mg/kg; compounds are administered at 5, 10, 20, 40, or 200 mg. More preferably, the compounds are administered orally and daily at about 1, 10, or 20 mg.

Routes of Administration

[0238] The compositions described herein may be administered in any of the following routes: buccal, epicutaneous, epidural, infusion, inhalation, intraarterial, intracardial, intracerebroventricular, intradermal, intramuscular, intranasal, intraocular, intraperitoneal, intraspinal, intrathecal, intravenous, oral, parenteral, pulmonary, rectally via an enema or suppository, subcutaneous, subdermal, sublingual, transdermal, and transmucosal. The preferred routes of administration are buccal and oral. The administration can be local, where the composition is administered directly, close to, in the locality, near, at, about, or in the vicinity of, the site(s) of disease, *e.g.*, inflammation, or systemic, wherein the composition is given to the patient and passes through the body widely, thereby reaching the site(s) of disease. Local administration can be administration to the cell, tissue, organ, and/or organ system, which encompasses and/or is affected by the disease, and/or where the disease signs and/or symptoms are active or are likely to occur. Administration can be topical with a local effect, composition is applied directly where its action is

desired. Administration can be enteral wherein the desired effect is systemic (non-local), composition is given via the digestive tract. Administration can be parenteral, where the desired effect is systemic, composition is given by other routes than the digestive tract.

Nutritional Compositions

[0239] The compositions of the compounds described herein may be used in (or consumed in) nutritional supplements; dietary supplements; medical foods; nutraceuticals; food-stuffs such as pharmaceutical-benefit foods (*e.g.*, “phoods”); beverages including fortified (*e.g.*, orange juice with calcium); traditional (*e.g.*, regular oatmeal, whole-grain breads), and “designer” products (*e.g.*, protein bars, smart spreads, smart bars, energy bars). The compounds described herein may be formulated in health bars, confections, animal feeds, cereals, dietary supplements, yogurts, cereal coatings, foods, nutritive foods, functional foods, and combinations thereof.

Second Agents

[0240] Second agents for treatment in combination with compositions of the present invention include, but are not limited to, phenytoin, carbamazepine, barbiturates, phenobarbital, mephobarbital, trimethadione, mephentyoin, paramethadione, phenethynylate, phenacetamide, metharbital, benzchlorpropamide, phensuximide, primidone, methsuximide, ethotoin, aminogluthethinide, diazepam, clonazepam, clorazepate, fosphenytoin, ethosuximide, valproate, felbamate, gabapentin, lacosamide, lamotrigine, retigabine, rufinamide, topiramate, vigabatrin, pregabalin, tiagabine, zonisamide, clobazam, thiopental, midazolam, propofol, levetiracetam, oxcarbazepine, CCPene, GYK152466, serotonin receptor agonists, ergotamine, dihydroergotamine, sumatriptan, propranolol, metoprolol, atenolol, timolol, nadolol, nifedipine, nimodipine, verapamil, aspirin, ketoprofen, tofenamic acid, mefenamic acid, naproxen, methysergide, paracetamol, clonidine, lisuride, ipرازochrome, butalbital, benzodiazepines, divalproex sodium and other similar classes of compounds. *See* U.S. Patent No. 6,495,601 and U.S. Patent Application Publication No. 2002/0082252.

[0241] For example, in addition to the composition described herein patients may also be treated with antidepressants (*e.g.*, tricyclic antidepressants [*e.g.*, amitriptyline (Elavil®), desipramine (Norpramin®), imipramine (Tofranil®), nortriptyline (Aventyl®, Pamelor®)] ; Serotonin and norepinephrine reuptake inhibitors (SNRIs) [*e.g.*, venlafaxine (Effexor®), duloxetine (Cymbalta®)]; norepinephrine and dopamine reuptake inhibitors (NDRIs) [*e.g.*, bupropion (Wellbutrin®)]; combined reuptake inhibitors and receptor blockers [*e.g.*, trazodone (Desyrel®), nefazodone

(Serzone®), maprotiline, mirtazpine (Remeron®)]; monamine oxidase inhibitors (MAOIs) [*e.g.*, isocarboxazid (Marplan®), phenelzine (Nardil®), tranlcypromine (Parnate®)] and selective serotonin reuptake inhibitors (SSRIs) [*e.g.*, citalopram (Celexa®), escitalopram (Lexapro®), fluoxetine (Prozac®), paroxetine (Paxil®, Pexeva®), sertraline (Zoloft®)] fluvoxamine (Luvox®), and amitriptyline); anticonvulsants to stabilize abnormal electrical activity in the nervous system caused by injured nerves (*e.g.*, gabapentin (NEURONTIN®), pregabalin (LYRICA®), carbamazepine (Tegretol®), lamotrigine (Lamictal®), topiramate (Topamax®), felbamate (Felbatol®), tiagabine (Gabitril®), diazepam rectal (Diastat®), phenobarbital, phenytoin (Dilantin®) primidone (Mysoline®), valproate (Depakote®), vigabatrin, oxcarbazepine (Trileptal®), zonisamide (Zonegran®), and levetiracetam (Keppra®)); steroids (*e.g.*, corticosteroid); analgesics (*e.g.*, acetaminophen (Tylenol®), codeine (Tylenol #2,3,4®), propoyl APA (Darvocet®), propoeylphene (Darvon®), fentanyl patch (duragesic®), hydromorphone (Palladone®), morphine (MS Contin®), oxycodone (Percocet®, OxyContin®, Percodan®), pentazocine (Talwin NX®), tramadol APAP (Ultracet®), tramadol (Ultram®), hydrocodone APAP (Vicodin®)); lithium, and non-steroidal anti-inflammatory drugs (NSAID) (*e.g.*, Tylenol®, Motrin®, salicylates (*e.g.*, acetylsalicylic acid (Aspirin), amoxiprin, benorylate/benorilate, choline magnesium salicylate, Diflunisal®, ethenzamide, faislamine, methyl salicylate, magnesium salicylate, salicyl salicylate, and salicylamide), arylalkanoic acids (*e.g.*, Diclofenac®, Aceclofenac®, Acemethacin®, Alclofenac®, Bromfenac®, Etodolac®, Indomethacin®, Nabumetone®, Oxametacin®, Proglumetacin®, Sulindac®, and Tolmetin®) 2-Arylpropionic acids (profens) (*e.g.*, Ibuprofen®, Alminoprofen®, Carprofen®, Dexibuprofen®, Dexketoprofen®, Fenbufen®, Fenoprofen®, Flunoxaprofen®, Flurbiprofen®, Ibuproxam®, Indoprofen®, Ketorolac®, Loxoprofen®, Naproxen®, Oxaprozin®, Pirprofen®, Suprofen®, Tiaprofenic acid); N-Arylanthranilic acids (fenamic acids) (*e.g.*, mefenamic acid, flufenamic acid, meclofenamic acid, tolfenamic acid, pyrazolidine derivatives, Phenylbutazone®, Ampyrone®, Azapropazone®, Clofezone®, Kebuzone®, Metamizole®, Mofebutazone®, Oxyphenbutazone®, Phenazone®, and Sulfinpyrazone®); and oxicams (*e.g.*, Piroxicam®, Droxicam®, Lornoxicam®, Meloxicam®, and Tenoxicam®).

[0242] Such second agents can be administered in the same formulation (*e.g.*, the same pill) or in a separate formulation as the compounds of the present invention. It is preferred that the second agents described above be co-administered with the compounds of the present invention.

The second agents described herein can be administered with the compounds of the present invention simultaneously, sequentially, prior to, or after administering of the compounds of the present invention. Where the administration of the second agents described herein is simultaneous, the second agent and the compounds of the present invention are administered together or within a very short time interval (*e.g.*, 5 minutes). Where the administration of the second agent is administered as pre-treatment, the second agent is administered prior to administration of the compounds of the present invention for any length of time contemplated herein.

Psychotherapy

[0243] The compounds, pharmaceutical compositions, and treatment regimes described herein may be used in combination with psychotherapy. In one embodiment, methods for the treatment of addictive disorder, anxiety disorders, bipolar disorders, and/or depression may further comprise psychotherapy.

[0244] Several types of psychotherapy—or “talk therapy”—can help people with depression. In some embodiments, the regimens are short-term (*e.g.*, 10 to 20 weeks) and other regimens are longer-term (*e.g.*, 1–10 years), depending on the needs of the individual. Two main types of psychotherapies—cognitive-behavioral therapy (CBT) and interpersonal therapy (IPT)—have been shown to be effective in treating neuropsychiatric disorders (*e.g.*, addictive disorders, anxiety disorders, bipolar disorders, and depression).

GABA_A Receptors in Disease

[0245] GABA_A receptors have a pentameric structure generally comprising two α subunits and two β subunits with a fifth regulatory subunit. Specific GABA_A subunits are expressed throughout the brain in distinct spatial and developmental patterns and display different responses to known pharmacological modulators. GABA_A α_1 variant receptors are believed to be the major postsynaptic receptors mediating action of GABA at most inhibitory synapses, and as such are responsible for not only the efficacious properties of drugs acting upon GABA_A α_1 variant receptors but also the sedative effects of these drugs. GABA_A α_2 and α_3 variant receptors are expressed in the hippocampus, thalamus, and other CNS locations, and are believed to mediate the anti-anxiety effects of the benzodiazepines. α_4 containing GABA_A receptors located in the hippocampus are thought to play a role in epilepsy. α_5 containing GABA_A receptors are expressed in the hippocampus and are thought to play a role in learning and memory. α_4 and α_6 containing GABA_A receptors are insensitive to benzodiazepines. Specific GABA_A subunits such as α_1 and α_4 show

increased expression in patients with epilepsy. α_4 variants of the GABA_A receptor are important in acting in a negative feedback loop on presynaptic GABA release, where stimulation of the α_4 variants GABA_A receptor acts to suppress GABA release.

[0246] The minor “regulatory” subunits ϵ and θ are expressed in particular CNS locations such as the cortex, the substantia nigra, amygdala and hypothalamus. Another minor regulatory subunit, π , is expressed outside the CNS in the uterus and breast tissue (overexpression of π has been observed in breast cancer). Another regulatory subunit, γ is a component of benzodiazepine-sensitive GABA_A receptors. The GABA_A subunits γ_2 and δ are believed to be involved in the pathologies of certain monogenetic forms of epilepsy. Also, the GABA_A α_2 and δ subunits have been implicated in alcohol consumption and addiction. WO 2009/100040.

[0247] The focus of pharmacological intervention in many disorders of the central and peripheral nervous system has been on reducing neuronal hyperexcitability. Most agents currently used to treat such disorders target synaptic activity in excitatory pathways by, for example, modulating the release or activity of excitatory neurotransmitters, potentiating inhibitory pathways, blocking ion channels involved in impulse generation, and/or acting as membrane stabilizers. Conventional agents and therapeutic approaches for the treatment of central and peripheral nervous system disorders thus reduce neuronal excitability and inhibit synaptic firing. One serious drawback of these therapies is that they are nonselective and exert their actions on both normal and abnormal neuronal populations. This leads to negative and unintended side effects, which may affect normal CNS functions, such as cognition, learning and memory, and produce adverse physiological and psychological effects in the treated patient. Common side effects include over-sedation, dizziness, loss of memory and liver damage. For example, classic anticonvulsant drugs and anti-nociceptive drugs decrease excitation or increase inhibition via non-selective GABAergic drugs (*e.g.*, benzodiazepines and barbiturates) indiscriminately act on multiple GABA_A receptor isoforms. While this yields good efficacy, the non-selective GABAergic drugs cause undesirable CNS side effects.

[0248] GABA_A receptors are localized at synaptic and extrasynaptic levels. Whereas synaptic GABA_A receptors are involved in phasic inhibition, extrasynaptic GABA_A receptors are responsible for tonic inhibition. Tonic inhibition is due to persistent inhibitory conductance that contributes to “signal integration” in the brain because it sets the threshold for action potential generation and shunts excitatory synaptic inputs. Thus, tonic inhibition plays a crucial role in regulating neuronal

excitability because it sets the threshold for action potential generation and integrates excitatory signals. This conductance is maintained by “ambient” GABA—the amount of neurotransmitter present in the extracellular space. Ambient GABA originates from spillover of the neurotransmitter released at neighboring synapses, from astrocytes, or from non-vesicular release. Further, GABA_A receptors are clustered at the synapse and extrasynaptic areas (*e.g.*, presynaptic cell). GABA_A receptor clustering acts as an additional regulating factor for tonic inhibition because clustered extrasynaptic GABA_A receptors can mediate larger tonic currents. Petrini, *et al.* (2004) The Journal of Biological Chemistry 279(44): 45833–45843.

[0249] α_4 GABA_A receptor variants are primarily located presynaptically or extrasynaptically (*e.g.*, on the pre-synaptic cell). *See* FIGURE 1. Activation of the α_4 GABA_A receptor variants leads to hyperpolarization of the pre-synaptic cell, decreasing GABA release and thus decreasing inhibition (*e.g.*, agonists specific for α_4 GABA_A receptor variants lead to a decrease in GABA release and subsequent decrease in the inhibitory signaling). In contrast, inhibition (antagonism) of α_4 GABA_A receptor variants decreases hyperpolarization of the presynaptic cell, thus allowing for GABA release to continue—prolonging and strengthening the inhibitory signal to the postsynaptic cell (*e.g.*, antagonists specific for α_4 GABA_A receptor variants lead to an increase GABA release and subsequent increase in the inhibitory signaling). In effect antagonists specific for α_4 GABA_A receptor variants achieve physiologic effects similar to those observed in current GABA agonists.

[0250] The activation of presynaptic GABA_A receptors depolarizes the presynaptic nerve terminals. The presynaptic actions of neurons can either depress or enhance neurotransmitter release, processes called presynaptic inhibition and presynaptic facilitation, respectively. Some of the best analyzed instances of presynaptic inhibition and facilitation are in the neurons of invertebrate animals and in mechanoreceptor afferent neurons (dorsal root ganglion cells) of vertebrates studied in dissociated cell tissue culture. These studies, and those in the intact spinal cord of mammals, indicate that there are at least two mechanisms for presynaptic inhibition. One is due to a synaptically mediated depression of the Ca²⁺ channel, leading to a decrease in the influx of Ca²⁺ into the terminal. The other is due to an increased conductance to Cl[−] that leads to a decrease (or short-circuiting) in the height of the action potential in the presynaptic terminal. As a result, less depolarization is produced, fewer Ca²⁺ channels are activated by the action potential, and therefore, less Ca²⁺ flows into the terminals. Activation of GABA_A presynaptic receptors is of this latter type. Antagonism of the receptor would then lead to presynaptic facilitation. Conversely, presynaptic facilitation is due

enhanced influx of Ca^{2+} . The neurotransmitter acts to depress a K^+ channel, thereby broadening the action potential and allowing the Ca^{2+} influx to persist for a longer period of time.

[0251] Proper neural activity depends on maintaining an appropriate balance between excitation and inhibition. Any tipping of the balance too far toward inhibition leads to sedation, and conversely, tipping it too far toward excitation may trigger a seizure. For example, extrasynaptic δ subunit-containing GABA_A receptors contribute to temporal lobe epilepsy by decreasing inhibitory input onto dentate granule cells and increasing the inhibition of inhibitory interneurons. Peng, *et al.* (2004) J. Neurosci. 24: 8629–8639. This increase in the inhibition of the inhibitory interneurons tips the balance too far towards excitation by lessening the inhibitory signaling, leading to seizures.

[0252] Presynaptic actions also tend to occur at points of sensory inflow. For example, presynaptic inhibition is found in the retina, spinal cord, and dorsal column nuclei. Presynaptic actions are important because they allow selective control of the actions of individual branches of a neuron. Axoaxonic synapses can inhibit or facilitate transmitter release by altering Ca^{2+} influx. Presynaptic inhibition may occur as a result of the activity of the postsynaptic cell, either a presynaptic inhibitory neuron, or a presynaptic facilitating neuron. In presynaptic inhibition, the result of the activity of the presynaptic inhibitor neuron is to cause a depression of the Ca^{2+} current accompanying the action potential of the presynaptic neuron. Because the decreased Ca^{2+} influx leads to a reduction in the amount of neurotransmitter released, the synaptic potential in the postsynaptic cell is depressed. In presynaptic facilitation, the activity of the presynaptic facilitating neuron causes a depression of the K^+ current in the presynaptic neuron leading to an increase in the duration of the action potential and therefore of the Ca^{2+} current. Consequently neurotransmitter release is increased and as a result, so is the amplitude of the synaptic potential in the postsynaptic cell. Kandel and Schwartz Principles of Neural Science 2nd Edition (1985) pages 128–131.

[0253] The α_4 GABA_A variant is expressed at high levels in the hippocampus, striatum and thalamus, where it contributes to parasynaptic GABA_A receptor mediated tonic inhibition. Further, α_4 expression is markedly altered by electroshock, alcohol exposure/withdrawal, steroid withdrawal, social isolation, and epilepsy. $\alpha_4\beta\delta$ subtypes are modulated by nonbenzodiazepine GABA_A ergic drugs like steroids, anesthetics, and ethanol. Chandra, *et al.* (October 10, 2006) Proc. Natl. Acad. Sci. 103(41): 15230–15235.

[0254] Several clinical conditions are thought to arise, in part, from the imbalance between neurotransmission of GABA including, but not limited to Huntington's Disease, Parkinson's disease,

periodic limb movement disorder (PLMD) (nocturnal myoclonus), spasticity, epilepsy, schizophrenia, bipolar disease, and tardive dyskinesia. For instance, GABA receptors have been implicated in sleep regulation and rhythmicity as well as the anxiolytic, amnestic, sedative, and anesthetic effects of alcohol. Jia, *et al.* (2008) The Journal of Pharmacology and Experimental Therapeutics 326(2): 475–482. Decreased GABA activity appears to contribute to the pathogenesis of these diseases. In addition, analgesia and satiety are thought to be regulated by GABA activity. Several diseases and conditions are due, at least in part, to an imbalance between excitation and inhibition in the central nervous system including but not limited to addictive disorders, Alzheimer's Disease, anxiety disorders, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes. Therefore, antagonists specific for α_4 GABA_A receptor variants, which lead to an increase GABA release and subsequent increase in the inhibitory signaling, may be used to treat these disease and conditions because they act to restore a balance between excitation and inhibition in the central nervous system by increasing inhibition. For example, antagonists specific for α_4 GABA_A receptor variants bind to extrasynaptic antagonist α_4 GABA_A receptor variants preventing their activation by ambient GABA. This prevents the hyperpolarization of the presynaptic cell allowing for prolonged GABA release into the synaptic cleft that leads to a longer, stronger inhibitory signal. This, in turn, provides a means by which the proper balance between excitation and inhibition in the central nervous system may be restored by increasing inhibition to counteract an excess of excitation or a lack of inhibition.

[0255] Compounds described herein selectively antagonize GABA_A receptors. In a preferred embodiment, the GABA_A receptor is a GABA_A receptor isoform comprising at least one α_4 subunit. In another embodiment, the GABA_A receptor is a GABA_A receptor isoform comprising at least one α_5 subunit. In one embodiment, the invention comprises compositions comprising compounds described herein with GABA_A receptor antagonist activity. In a further embodiment, the invention is drawn to pharmaceutical compositions comprising at least one compound with GABA_A receptor antagonist activity and a pharmaceutically acceptable excipient. In one embodiment, compounds

described herein have GABA_A receptor antagonist activity. In another embodiment, compounds described herein do not have an effect on GABA_B receptors.

[0256] GABA_A receptors may be located parasynaptically (*e.g.*, pre- and extra-synaptically) and account for control of frequency of IPSCs. Without being committed to a specific mechanism, the inventors believe that selected compounds described herein act at parasynaptic sites by inhibiting the negative feedback by GABA on the terminal of the synaptic bouton. Parasynaptic GABA_A receptors act to decrease GABA release when sufficient GABA is present in the synaptic cleft to bind to and activate these parasynaptic GABA_A receptors (*e.g.*, negative feedback loop). By inhibiting this negative feedback loop, compounds described herein may increase the GABA levels in the synaptic cleft and decrease neuronal firing. This increase of GABA restores the appropriate excitatory/inhibitory balance for normal neuronal activity.

[0257] Many of the compounds described herein may show high selectivity at the terminals of GABA interneurons. *In vitro* electrophysiology studies with selected compounds described herein may show selective activity at pre- and/or extra-synaptic (parasynaptic) terminals indicating increased GABA release (inhibition). *See, e.g.*, FIGURES 4–8. Without being bound to a particular theory of action, the inventors believe that selected compounds described herein may be GABA_A receptor antagonists that increase the number of inhibitory events as measured by Inhibitory Postsynaptic Current (IPSCs). For example, selected compounds described herein may increase the frequency of spontaneous IPSCs (a combination of both action potential and miniature events releasing GABA) and increase the frequency of miniature IPSCs (miniature events are due to tonic release of synaptic vesicles containing GABA into the pre-synaptic space).

[0258] The inventors surprisingly discovered that selected compounds described herein may increase GABA_A inhibitory drive, since the increased frequency indicates a pre-synaptic mechanism. The interval between miniature and spontaneous inhibitory post-synaptic currents (mIPSCs and sIPSCs, respectively) events may be substantially decreased in the presence of selected compounds described herein. The inventors believe that the pre-synaptic mechanism for increasing the release of GABA from the neurons may be due to selected compounds described herein antagonizing GABA_A receptors on the pre-synaptic cells preventing hyperpolarization of the pre-terminal cell. *See* FIGURE 1. This may then allow for additional GABA to be released into the synaptic cleft, leading to longer, and stronger GABA-mediated inhibition.

[0259] Selected compounds described herein may selectively antagonize specific GABA_A receptor isoforms (*e.g.*, α_4 variants). α_4 GABA_A receptor variants are found at parasynaptic sites and account for less than 1% of the GABA_A receptors in the mammalian brain. The activation of α_4 GABA_A receptor isoform can inhibit the release of GABA from a GABAergic neuron (*e.g.*, activation of a α_4 GABA_A receptor leads to the hyperpolarization of the synaptic terminal which slows the release of GABA from synaptic vesicles and allows GABA clearance mechanisms to lower the amount of GABA in the synaptic cleft leading to a decrease of GABA in the synaptic cleft). Further, the inventors discovered surprisingly that inhibition of parasynaptic α_4 GABA_A receptor isoforms leads to an increase in GABA release, which leads to increased inhibitory stimulation on the post-synaptic neuron. This specific parasynaptic action supports a possible mechanism for the lack of CNS depressant effects (*e.g.*, sedation) demonstrated by compounds described herein, even at very high systemic exposure (*e.g.*, after dosages > 100 mg/kg/day). This mechanism of action is diametrically opposed to the activation of GABA_A receptors by benzodiazepines which work at low GABA concentrations. For example, selected compounds described herein may show selective action on specific a GABA_A receptor isoform (*e.g.*, α_4 GABA_A variants) generates strong efficacy in hyperexcitable states (*e.g.*, epilepsy, migraine, pain) without generating typical CNS side effects such as sedation and decreased cognition.

[0260] In particular, compounds of Formulae I-IV described herein may be used for the regulation, including prevention, prophylaxis, diagnosis, prognostication, management, and treatment, of a range of conditions that involve the GABA_A receptor including but not limited to the disorders described herein.

[0261] In another embodiment, compounds described herein show selective effect on GABA_A receptors in the CNS and less side-effects usually associated with agents that act on GABA_A receptors. For example, compounds described herein exhibit less sedation, decreased respiration, decreased cognition, or decreased motor function.

[0262] For example, compounds described herein are effective in humans and animals to decrease seizures, decrease pain responses, and decrease migraine. Several of the compounds described herein preferentially binds to GABA_A receptor subtypes and has an antagonistic effect on GABA_A receptors that is different from classic benzodiazepine and barbiturate mechanisms. Unlike some compounds described herein, several compounds described herein do not act on the Na⁺K⁺2Cl⁻ cotransporter (NKCC1 or NKCC2). Some compounds described herein that are similarly ineffective

with NKCC1 or NKCC2 are contemplated. Compounds described herein may not elicit diuresis. For example, many of compounds described herein may not increase urine output, sodium excretion, or potassium excretion.

[0263] Overall, compounds described herein may be well-tolerated toxicologically and demonstrate no CNS side effects after oral administration. The inventors surprisingly discovered that selected compounds described herein may act to specifically increase neuronal inhibition via a novel mechanism of action (not NKCC dependent). The inventors surprisingly discovered that selected compounds described herein may act at interneuron terminals, that regulate neuronal firing, and therefore, these compounds may inhibit abnormal firing. More specifically, selected compounds described herein may increase pre-synaptic inhibition without depressing all GABA receptors. This highly selective mechanism of action is novel and contrasts with the broad, non-specific activity of benzodiazepines and barbiturates.

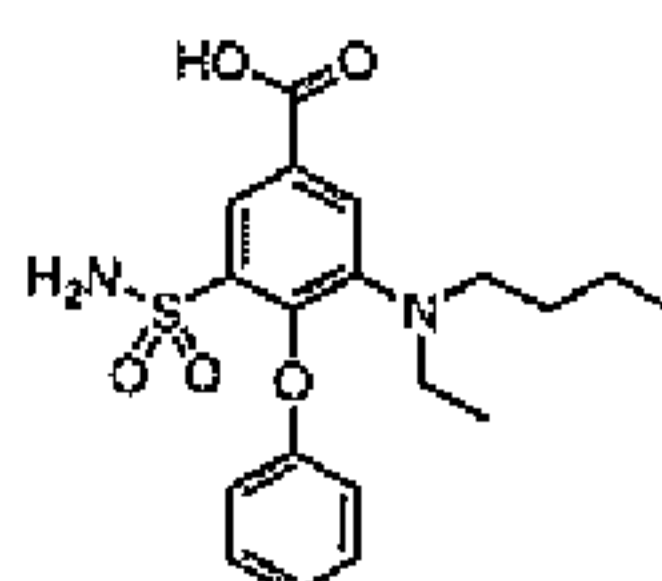
[0264] Benzodiazepines and barbiturates are known to be effective but are poorly tolerated because these compounds activate most GABA_A subtype receptors (*e.g.*, “fire-hose effect”). In contrast, selected compounds described herein may enhance inhibition via action at specific GABA_A receptor subtypes, preferentially α_4 variants of GABA_A. Due to this selectivity of selected compounds described herein may avoid the typical CNS side effects (*e.g.*, sedation) usually associated with known GABAergic compounds.

[0265] Further embodiments of the present invention will now be described with reference to the following examples. The examples contained herein are offered by way of illustration and not by any way of limitation. Exemplary syntheses for compounds according to the present invention are provided in, *e.g.*, U.S. Patent Application Publication No. 2007/0149526A1 and WO 2010/085352.

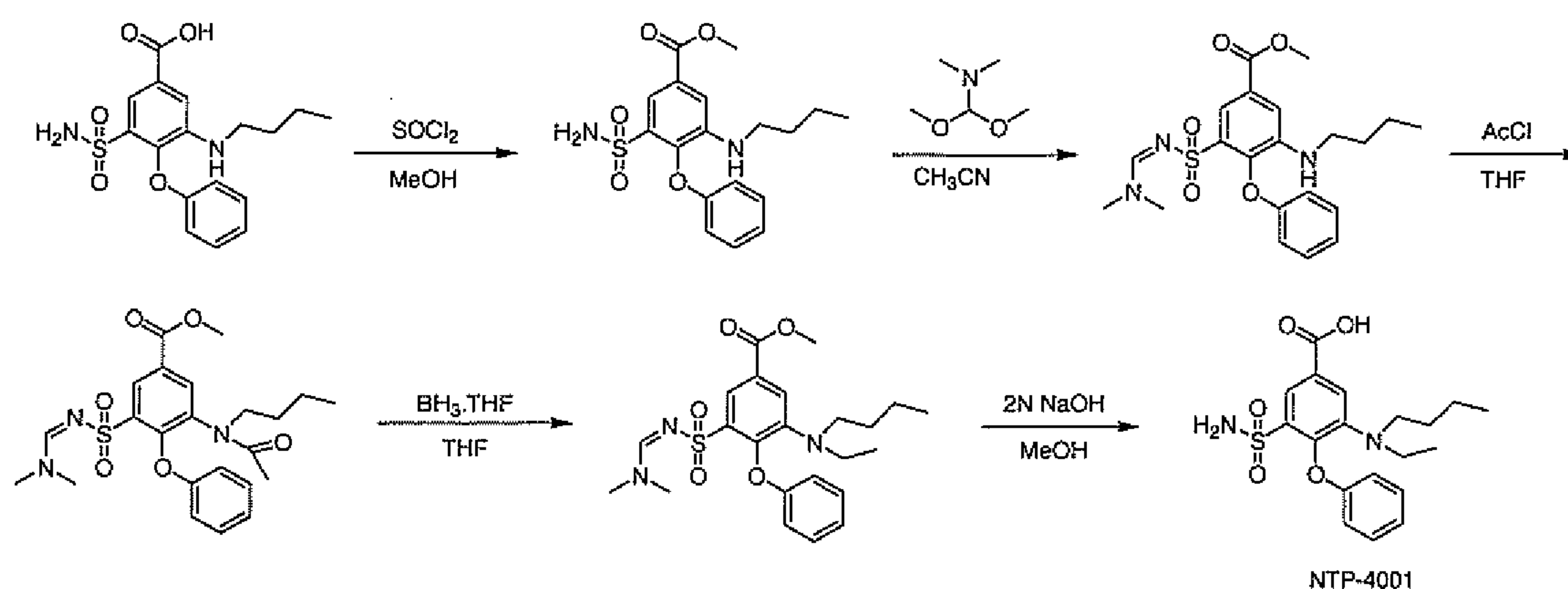
EXAMPLES

Example 1

3-(Butyl(ethyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid NTP-4001



General Method A



Step 1: 3-Butylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-butylamino-4-phenoxy-5-sulfamoyl benzoic acid (500 mg, 1.37 mmol) and methanol (50 mL). Thionyl chloride (490 mg, 4.12 mmol) was added slowly at room temperature and the reaction mixture was heated to 50°C overnight. The solvent was removed under reduced pressure and the residue re-dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution, water and brine. The organic solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (512 mg). MS *m/z*: 379 [M+1]⁺.

Step 2: 3-Butylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A reaction flask was charged with 3-butylamino-4-phenoxy-5-sulfamoyl benzoic acid methyl ester (509 mg, 1.346 mmol), acetonitrile (4 mL) and N,N-dimethyl formamide dimethyl acetal (0.19 mL, 1.413 mmol) and stirred at room temperature over night. The solvent was removed under reduced pressure and the resultant gummy residue was triturated with ice cold water to give white solid. The solid was filtered and dried to give the product (473 mg). MS *m/z*: 434 [M+1]⁺.

Step 3: 3-(Acetyl-butyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-butylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (100 mg, 0.230 mmol), acetyl chloride (0.018 mL, 0.254 mmol), diisopropylethyl amine (0.05 mL), THF (5 mL) and the reaction stirred at room temperature for 2 hours. The reaction mixture was poured into water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as colorless oil (110 mg). MS *m/z*: 476 [M+1]⁺.

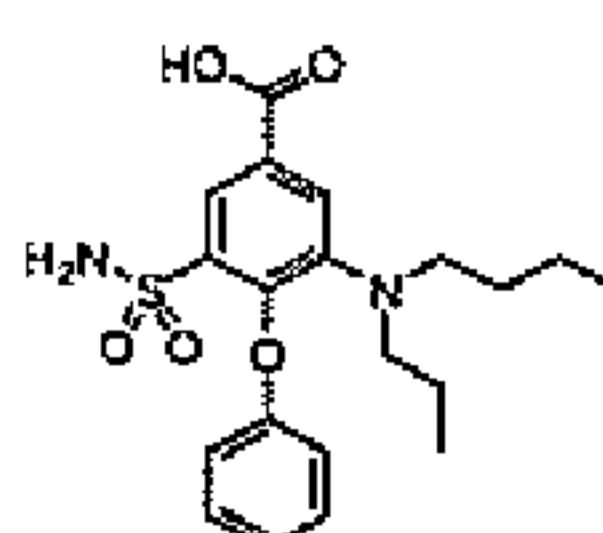
Step 4: 3-(Butyl-ethyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-(acetyl-butyl-amino)-5-

(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (110 mg, 0.23 mmol), THF (3 mL) and BH_3 .THF (1.0 M in THF) (4.6 mL, 4.6 mmol) was added slowly. The reaction was stirred at room temperature for 1 hour. The reaction was quenched by drop wise addition of water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as colorless oil (53 mg). MS m/z : 462 $[\text{M}+1]^+$.

Step 5: 3-(Butyl-ethyl-amino)-4-phenoxy-5-sulfamoyl-benzoic acid (NTP-4001). A reaction vial was charged with 3-(butyl-ethyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (50 mg, 0.11 mmol), 2N NaOH (3 mL), methanol (3 mL) and the reaction heated to 40°C for 3 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the desired product (20.3 mg). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 13.30 (bs, 1H), 8.02 (d, $J=1.8$ Hz, 1H), 7.72 (d, $J=2.18$ Hz, 1H), 7.39 (s, 2H), 7.21 (t, $J=7.5$ Hz, 2H), 6.97 (t, $J=7.2$ Hz, 1H), 6.76 (d, $J=7.8$ Hz, 2H), 3.06 (q, $J=6.9$ & 7.2 Hz, 2H), 2.94 (t, $J=6.9$ Hz, 2H), 1.17–1.08 (m, 2H), 1.00–0.88 (m, 2H), 0.76–0.68 (m, 6H). MS m/z : 391 $[\text{M}-1]^-$.

Example 2

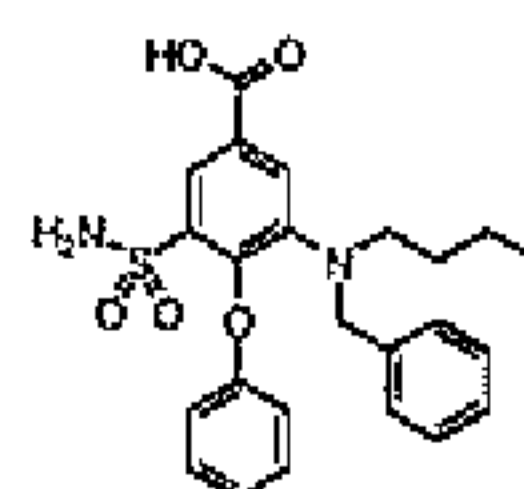
3-(Butyl(propyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid NTP-4002



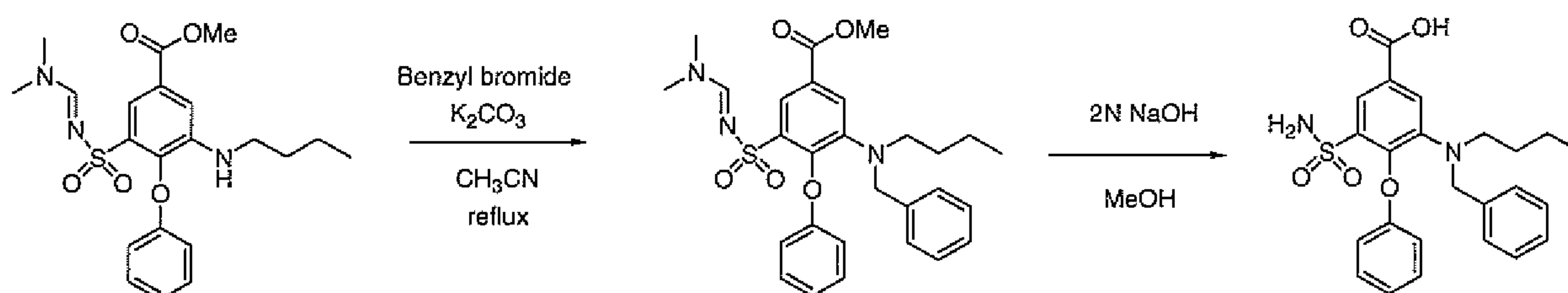
The title compound was prepared following General Method A and beginning with the appropriate acid chloride in step 3 to give the product as a white solid. MS m/z : 405 $[\text{M}-1]^-$.

Example 3

3-(Benzyl(butyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid NTP-4003



General Method B



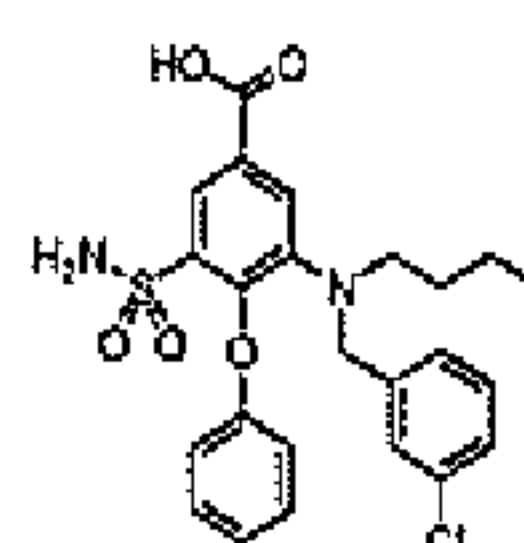
Step 1: Methyl 3-(benzyl(butyl)amino)-5-(N-((dimethylamino)methylene)sulfamoyl)-4-phenoxybenzoate. Methyl 3-(butylamino)-5-(N-((dimethylamino)methylene)sulfamoyl)-4-phenoxybenzoate (General Method A, 0.14 g, 0.286 mmol.), benzyl bromide (0.04 mL, 0.343 mmol.), potassium carbonate (60 mg, 0.429 mmol.), and acetonitrile (4 mL) were charged into a flask. The mixture was heated to reflux overnight. The excess potassium carbonate was removed by filtration over celite. The filtrate was evaporated and purified by chromatography (n-Hexane/Ethyl acetate=1/1) to give 0.106 g of the title compound as a white solid.

Step 2: 3-(benzyl(butyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid. Methyl 3-(benzyl(butyl)amino)-5-(N-((dimethylamino)methylene)sulfamoyl)-4-phenoxybenzoate (0.106 g, 0.203 mmol.), a 2N aqueous solution of sodium hydroxide (0.3 mL, 0.6 mmol.), and methanol (2 mL) were charged into a flask. The mixture was heated to 70°C overnight and then cooled to room temperature. 1N aqueous solution of hydrochloric acid (1 mL) was added to adjust pH to 2~3, and the mixture was extracted with ethyl acetate (3 X 2 mL), dried over magnesium sulfate, and evaporated to give 0.071 g of title compound as a white solid: ^1H NMR (400 MHz, CD_3OD) δ 8.23 (d, $J = 2.0$ Hz, 1H), 7.85 (d, $J = 2.0$ Hz, 1H), 7.36–7.26 (m, 2H), 7.18–7.06 (m, 4H), 6.92–6.86 (m, 2H), 6.80–6.72 (m, 2H), 2.97 (t, $J = 7.4$ Hz, 2H), 1.36–1.20 (m, 2H), 1.10–0.96 (m, 2H), 0.75 (t, $J = 7.4$ Hz, 3H). MS m/z : 453 $[\text{M}-1]^-$.

Example 4

3-(Butyl(3-chlorobenzyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid

NTP-4004



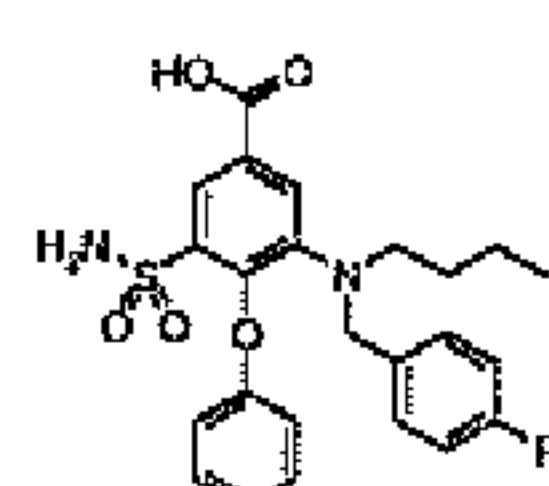
The title compound was prepared following General Method A but beginning with the appropriate benzyl bromide in step 2 to give the product as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 8.29 (d, $J = 2.0$ Hz, 1H), 7.95 (d, $J = 2.0$ Hz, 1H), 7.38–7.26 (m, 2H), 7.18–7.06 (m, 3H), 6.92–6.84 (m,

2H), 6.80–6.71 (m, 2H), 2.96 (t, $J = 7.4$ Hz, 2H), 1.38–1.20 (m, 2H), 1.10–0.98 (m, 2H), 0.75 (t, $J = 7.4$ Hz, 3H). MS m/z : 487 $[M-1]^-$.

Example 5

3-(Butyl(4-fluorobenzyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid

NTP-4005

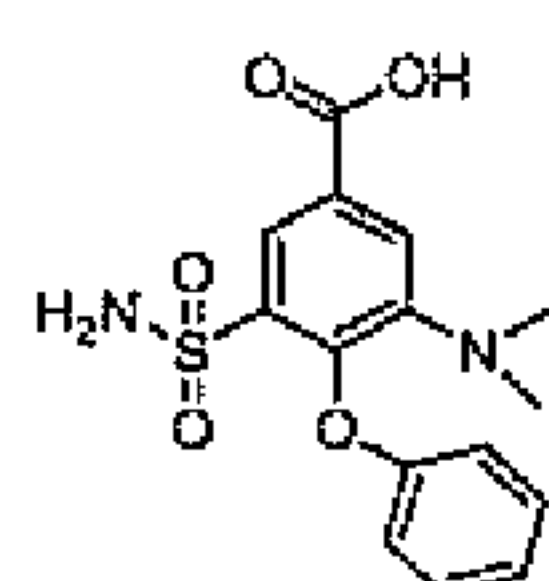


The title compound was prepared following General Method A but beginning with the appropriate benzyl bromide in step 2 to give the product as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 8.26 (d, $J = 2.0$ Hz, 1H), 7.87 (d, $J = 2.0$ Hz, 1H), 7.36–7.28 (m, 2H), 7.18–7.16 (m, 1H), 6.92–6.68 (m, 6H), 2.98 (t, $J = 7.4$ Hz, 2H), 1.33–1.20 (m, 2H), 1.10–0.98 (m, 2H), 0.76 (t, $J = 7.4$ Hz, 3H). MS m/z : 474 $[M+1]^+$.

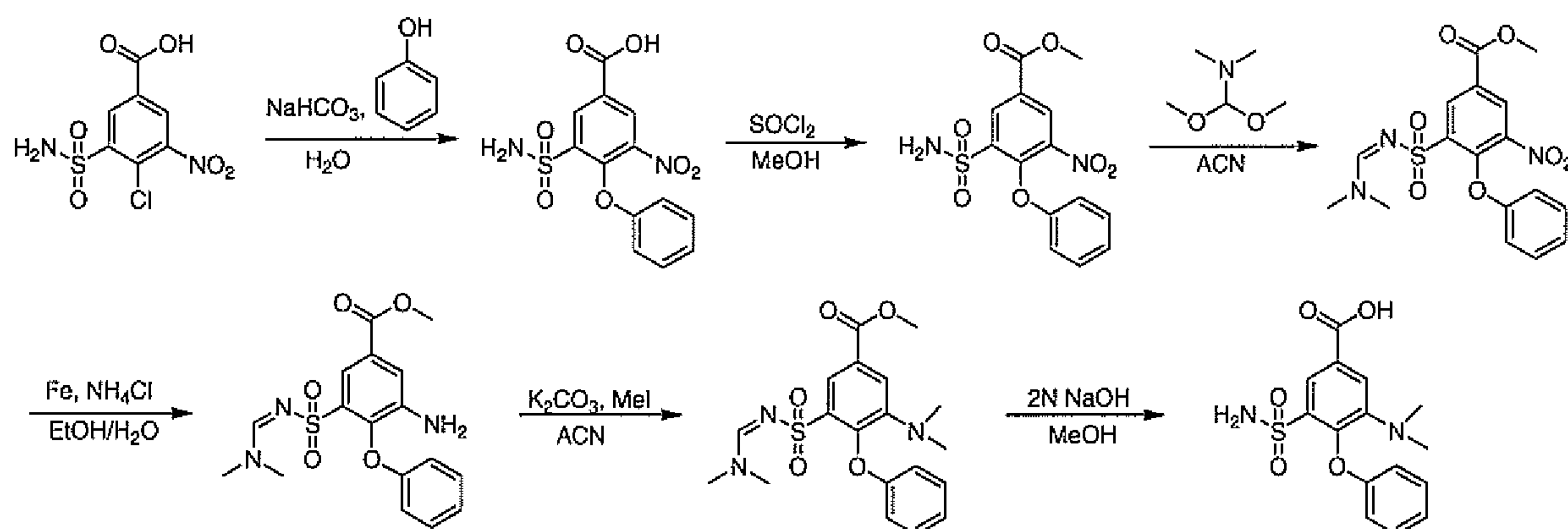
Example 6

3-(Dimethylamino)-4-phenoxy-5-sulfamoylbenzoic acid

NTP-4006



General Method C



Step 1: 3-Nitro-4-phenoxy-5-sulfamoyl benzoic acid. A round bottom flask was charged with 4-chloro-3-nitro-5-sulfamoyl-benzoic acid (2.0 g, 7.12 mmol), sodium bicarbonate (2.45 g, 29.2 mmol), phenol (1.47 g, 15.6 mmol) and water (20 mL) and heated at 85°C over night. The reaction

mixture was cooled to room temperature and acidified with 3N HCl. The product precipitated out which was filtered and dried to give the product as yellow solid (1.9 g). MS m/z : 337 [M-1]⁻.

Step 2: 3-Nitro-4-phenoxy-5-sulfamoyl benzoic acid methyl ester. A round bottom flask was charged with 3-nitro-4-phenoxy-5-sulfamoyl benzoic acid (1.9 g, 5.637 mmol) and methanol (50 mL). Thionyl chloride (2.012g, 16.91 mmol) was added slowly at room temperature and the reaction mixture was heated to 50°C overnight. The solvent was removed under reduced pressure and the residue redissolved in ethyl acetate and washed with saturated sodium bicarbonate solution, water and brine. The organic solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (1.72 g).

Step 3: 3-(Dimethylaminomethylene-sulfamoyl)-5-nitro-4-phenoxy-benzoic acid methyl ester. A reaction flask was charged with 3-nitro-4-phenoxy-5-sulfamoyl benzoic acid methyl ester (1.65 g, 4.68 mmol), acetonitrile (20 mL) and N,N-dimethyl formamide dimethyl acetal (0.65 mL, 4.917 mmol) and stirred at room temperature over night. The solvent was removed under reduced pressure and the resultant gummy residue was treated with ice cold water to give yellow solid. The solid was filtered and dried to give the product (1.9 g). MS m/z : 408 [M+1]⁺.

Step 4: 5-Amino-3-(dimethylaminomethylene-sulfamoyl)- 4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-(dimethylaminomethylene-sulfamoyl)-5-nitro- 4-phenoxy-benzoic acid methyl ester (1.0 g, 2.457 mmol), ethanol (50 mL) and the reaction mixture heated to 85°C. Ammonium chloride (1.3 g, 24.57 mmol) in water (25 mL) was added. Iron powder (541 mg, 9.828 mmol) was added in three portions 3 minutes apart. The heating was continued for another 1 h. The reaction mixture was cooled to 60°C and poured into dichloromethane (150 mL). The organic layer was separated and washed with water, brine and dried over sodium sulfate. The solvents were removed under reduced pressure to give the product as off white solid (690 mg). MS m/z : 378[M+1]⁺.

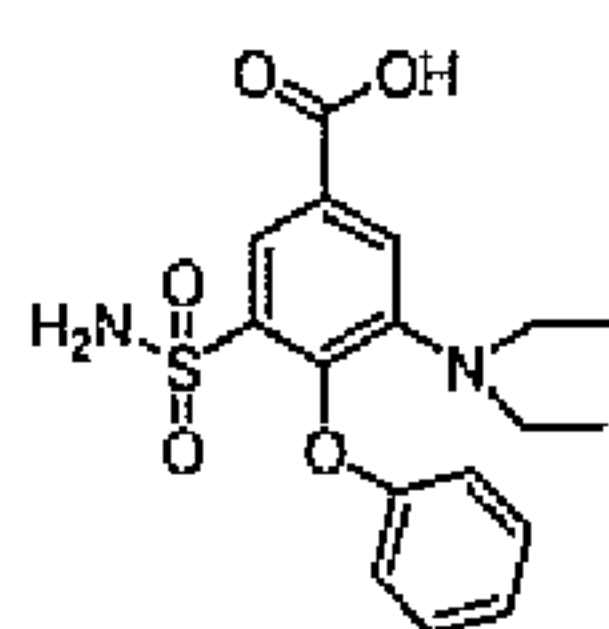
Step 5: 3-Dimethylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A pressure vial was charged with 5-amino-3-(dimethylaminomethylene-sulfamoyl)- 4-phenoxy-benzoic acid methyl ester (200mg, 0.530 mmol), potassium carbonate (440 mg, 3.18 mmol), methyl iodide (753 mg, 5.305 mmol), acetonitrile (10 mL) and the reaction heated at 77°C over night. The reaction was cooled and filtered and washed with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as white solid (70 mg). MS m/z : 406 [M+1]⁺.

Step 6: 3-Dimethylamino-4-phenoxy-5-sulfamoyl-benzoic acid (NTP-4006). A reaction vial was charged with 3-dimethylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (70 mg, 0.17 mmol), 2N NaOH (1 mL), methanol (2 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as white solid (49 mg). ¹H NMR (300 MHz, DMSO- *d*₆) δ 13.30 (bs, 1H), 8.00 (d, *J*=2.1 Hz, 1H), 7.70 (d, *J*=1.8 Hz, 1H), 7.39 (s, 2H), 7.23 (t, *J*=7.8 Hz, 2H), 6.98 (t, *J*=7.5 Hz, 1H), 6.76 (d, *J*=8.4 Hz, 2H), 2.65 (s, 6H). MS *m/z*: 337 [M+1]⁺.

Example 7

3-(Diethylamino)-4-phenoxy-5-sulfamoylbenzoic acid

NTP-4007

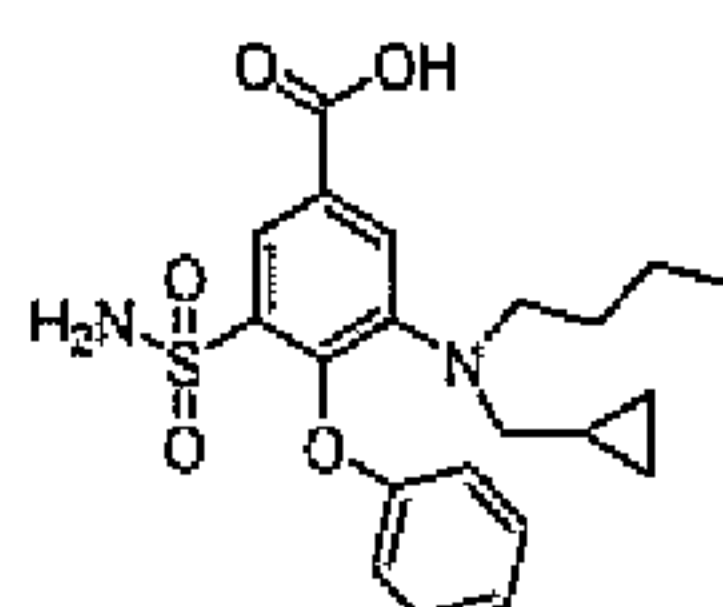


The title compound was prepared following General Method C but beginning with the appropriate alkyl iodide in step 5 to give the product as a white solid (21 mg). MS *m/z*: 365 [M+1]⁺.

Example 8

3-(Butyl(cyclopropylmethyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid

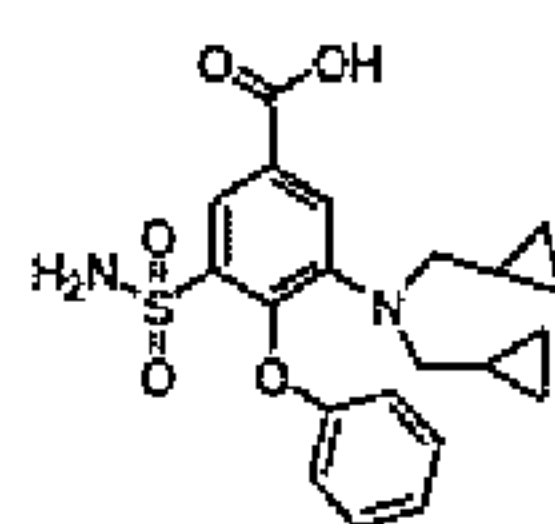
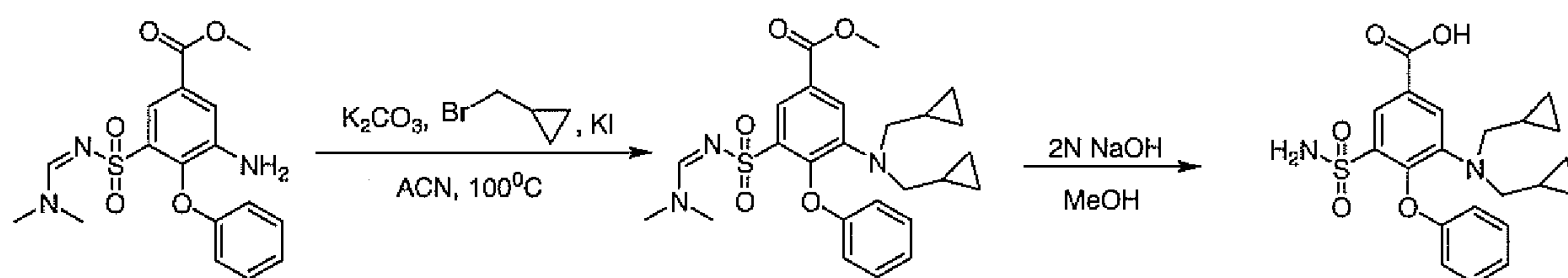
NTP-4008



The title compound was prepared following General Method B but beginning with the appropriate alkyl bromide and potassium iodide (1 eq) in step 2 and heating the reaction of step 2 in a sealed tube to 100°C for three days. ¹H NMR (300 MHz, DMSO- *d*₆) δ 13.28 (bs, 1H), 8.03 (d, *J*=1.8 Hz, 1H), 7.78 (d, *J*=2.1 Hz, 1H), 7.38 (s, 2H), 7.21 (t, *J*=7.5 Hz, 2H), 6.98 (t, *J*=7.2 Hz, 1H), 6.76 (d, *J*=7.5 Hz, 2H), 3.08 (t, *J*=6.9 Hz, 2H), 2.90 (d, *J*=6.6 Hz, 2H), 1.16–1.11 (m, 2H), 0.96–0.89 (m, 2H), 0.70 (t, *J*=7.5 Hz, 3H), 0.59 (m, 1H), 0.30–0.24 (m, 2H), 0.06–0.01 (m, 2H). MS *m/z*: 419 [M+1]⁺.

Example 9**3-(Bis(cyclopropylmethyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid**

NTP-4010

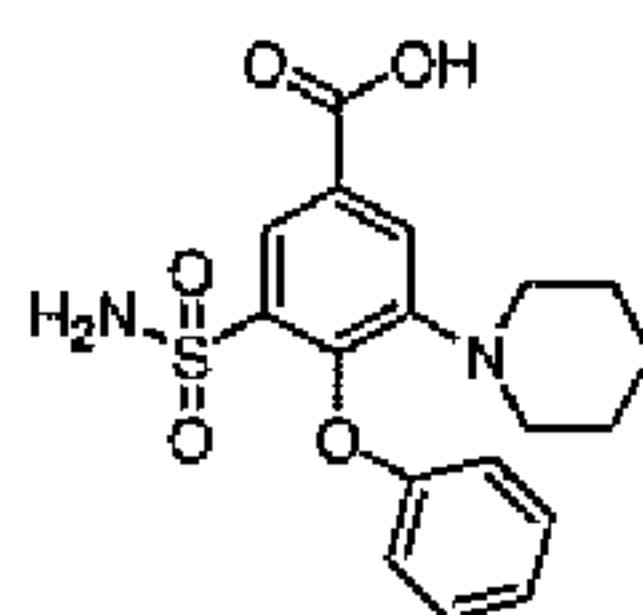
**General Method D**

Step 1: 3-(Bis-cyclopropylmethyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A pressure tube was charged with 3-amino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy benzoic acid methyl ester (250 mg, 0.663 mmol), potassium carbonate (549 mg, 3.978 mmol), cyclopropyl methyl bromide (269 mg, 1.989 mmol), potassium iodide (110 mg, 0.663 mmol), acetonitrile (4mL) and heated at 100°C for 3 days. The reaction gave a mixture of mono and di-alkylated products by LC/MS. The reaction was cooled and filtered over celite and the solvent removed under reduced pressure. The residue was purified by flash chromatography to give the product (80 mg). MS m/z : 486.1 $[M+1]^+$.

Step 2: 3-(Bis(cyclopropylmethyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid. A reaction vial was charged with 3-(bis-cyclopropylmethyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (75 mg, 0.15 mmol), 2N NaOH (1 mL), methanol (3 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as white solid (35 mg). ^1H NMR (300 MHz, DMSO- d_6) δ 13.28 (bs, 1H), 8.03 (d, $J=1.8$ Hz, 1H), 7.83 (d, $J=2.1$ Hz, 1H), 7.37 (s, 2H), 7.21 (t, $J=7.5$ Hz, 2H), 6.97 (t, $J=7.2$ Hz, 1H), 6.79 (d, $J=7.5$ Hz, 2H), 2.95(d, $J=6.3$ Hz, 4H), 0.61 (m, 1H), 0.28–0.22 (m, 2H), 0.09–0.04 (m, 2H). MS m/z : 417 $[M+1]^+$.

Example 10**4-Phenoxy-3-(piperidin-1-yl)-5-sulfamoylbenzoic acid**

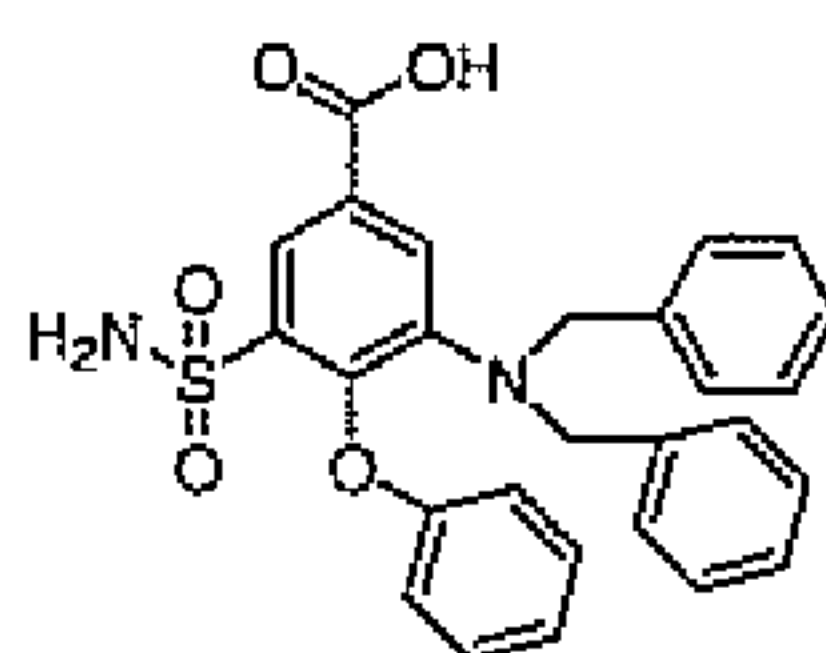
NTP-4011



The title compound was prepared following general method C but using 1,5-diiodopentane in place of methyl iodide in step 5. MS m/z : 377 $[M+1]^+$.

Example 11**3-(Dibenzylamino)-4-phenoxy-5-sulfamoylbenzoic acid**

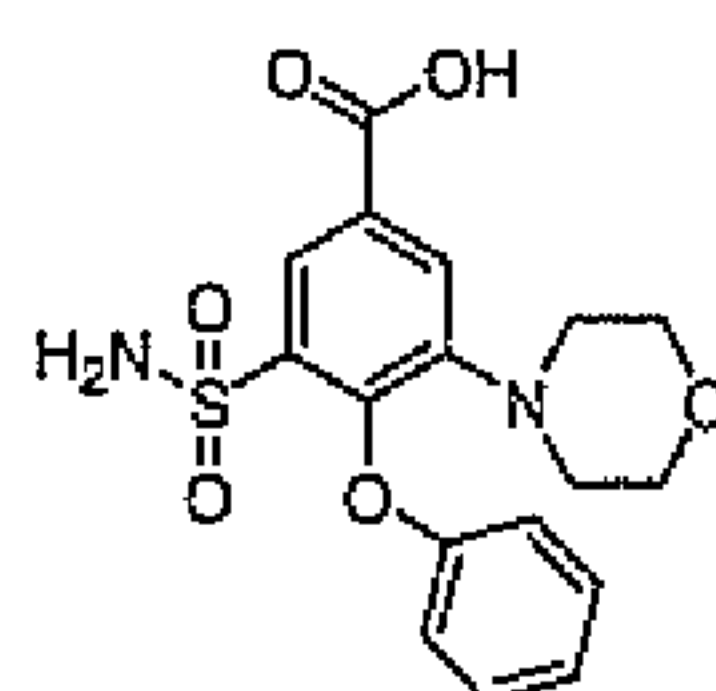
NTP-4012



The title compound was prepared following general method D but using benzyl bromide in place of (bromomethyl)cyclopropoane in step 1. MS m/z : 489 $[M+1]^+$.

Example 12**3-Morpholino-4-phenoxy-5-sulfamoylbenzoic acid**

NTP-4013

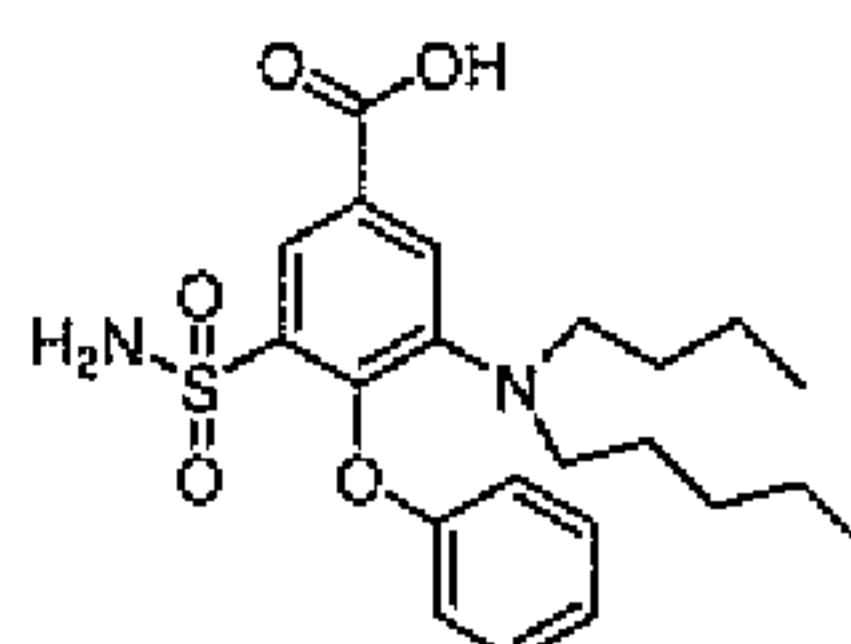


The title compound was prepared following General Method C but using 1-iodo-2-(2-iodoethoxy) ethane in place of methyl iodide in step 5. MS m/z : 379 $[M+1]^+$.

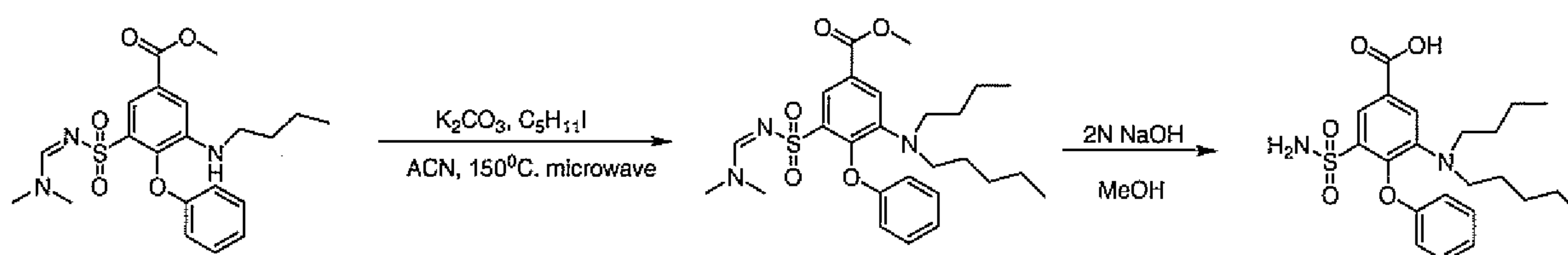
Example 13

3-(Butyl(pentyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid

NTP-4014



General Method E

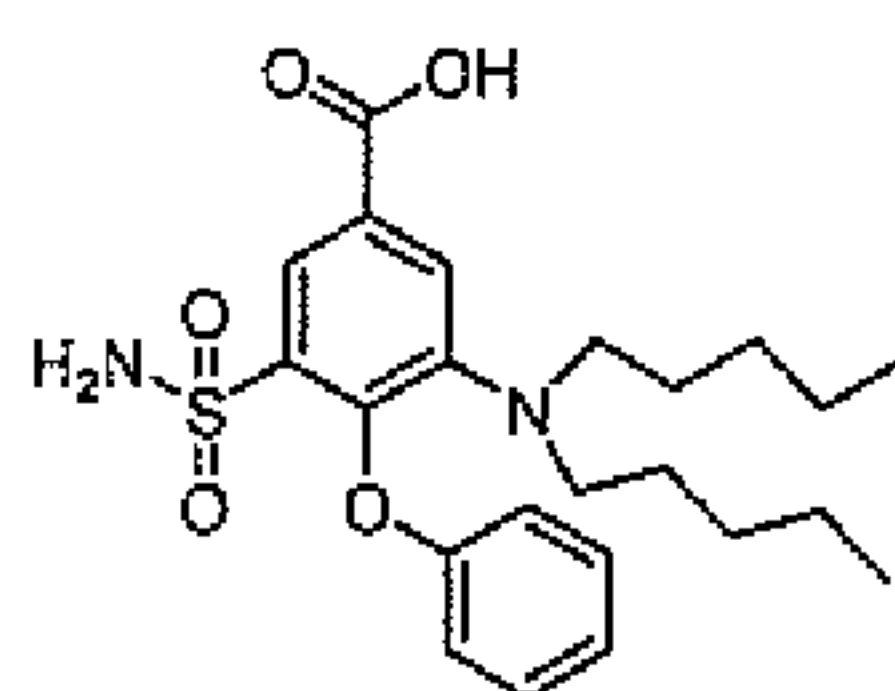
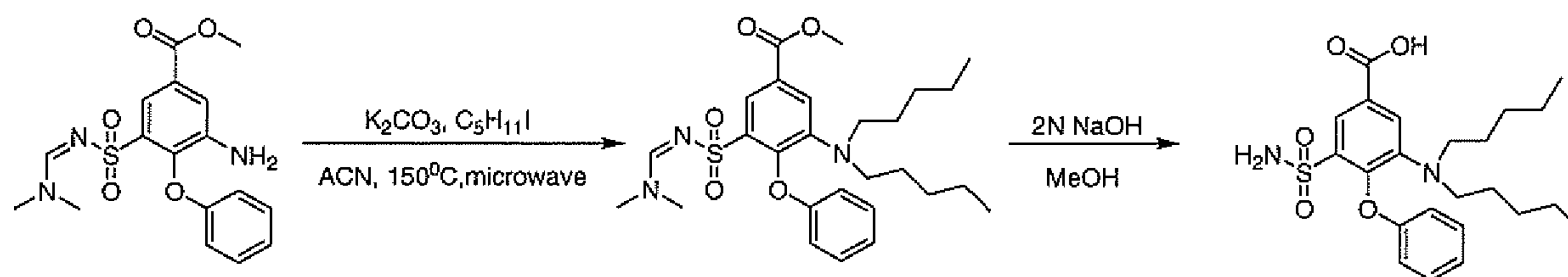


Step 1: 3-(Butyl-pentyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-butylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (General Method A step 2, 250 mg, 0.577 mmol), potassium carbonate (239 mg, 1.732 mmol), iodopentane (228 mg, 1.154 mmol), acetonitrile (5 mL) and the reaction heated at 150°C in a microwave reactor for 3 hours. The reaction was cooled to room temperature and filtered. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (140 mg). MS m/z : 504 $[M+1]^+$.

Step 2: 3-(Butyl-pentyl-amino)-4-phenoxy-5-sulfamoyl-benzoic acid (NTP-4014). A reaction vial was charged with 3-(butyl-pentyl-amino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (135 mg, 0.27 mmol), 2N NaOH (2 mL), methanol (4 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as yellow solid (78 mg). ^1H NMR (300 MHz, DMSO- d_6) δ 13.28 (bs, 1H), 7.99 (d, $J=1.8$ Hz, 1H), 7.71 (d, $J=2.1$ Hz, 1H), 7.34 (s, 2H), 7.20 (t, $J=7.5$ Hz, 2H), 6.97 (t, $J=7.2$ Hz, 1H), 6.72 (d, $J=7.5$ Hz, 2H), 3.01–2.95 (m, 4H), 1.15–1.07 (m, 6H), 0.97–0.89 (m, 4H), 0.76–0.69 (m, 6H). MS m/z : 435 $[M+1]^+$.

Example 14**3-Dipentylamino-4-phenoxy-5-sulfamoylbenzoic acid**

NTP-4015

**General Method F**

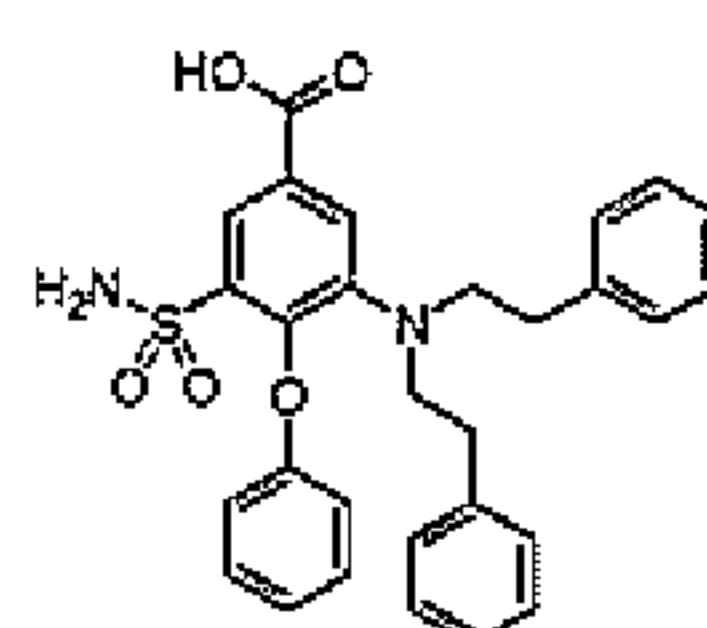
Step 1: 3-(Dimethylaminomethylene-sulfamoyl)-5-dipentylamino-4-phenoxy-benzoic acid methyl ester.

A microwave vial was charged with 3-amino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy benzoic acid methyl ester (175 mg, 0.501 mmol), potassium carbonate (415 mg, 3.008 mmol), iodopentane (397 mg, 2.01 mmol) and acetonitrile (4mL) and the reaction heated at 150°C in a microwave for 5 hours. A mixture of mono and d-alkylated products was present by LC/MS. The reaction was cooled to room temperature and filtered and washed with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid. The reaction was cooled and filtered over celite and the solvent removed under reduced pressure. The residue was purified by flash chromatography to give the product (80 mg). MS m/z : 518 $[M+1]^+$.

Step 2: 3-(Dipentylamino)-4-phenoxy-5-sulfamoylbenzoic acid (NTP-4015). A reaction vial was charged with 3-(dipentylamino)-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (93 mg, 0.18 mmol), 2N NaOH (1 mL), methanol (2 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as yellow solid (60 mg). ^1H NMR (300 MHz, DMSO- d_6) δ 7.99 (d, $J=1.5$ Hz, 1H), 7.72 (d, $J=1.5$ Hz, 1H), 7.37 (s, 2H), 7.20 (t, $J=7.5$ Hz, 2H), 6.97 (t, $J=7.2$ Hz, 1H), 6.73 (d, $J=7.5$ Hz, 2H), 2.99 (t, $J=7.5$ Hz, 4 H), 1.23–1.07 (m, 8H), 0.95–0.91 (m, 4H), 0.75 (t, $J=7.2$ Hz, 6H). MS m/z : 449 $[M+1]^+$.

Example 15**3-(diphenethylamino)-4-phenoxy-5-sulfamoylbenzoic acid**

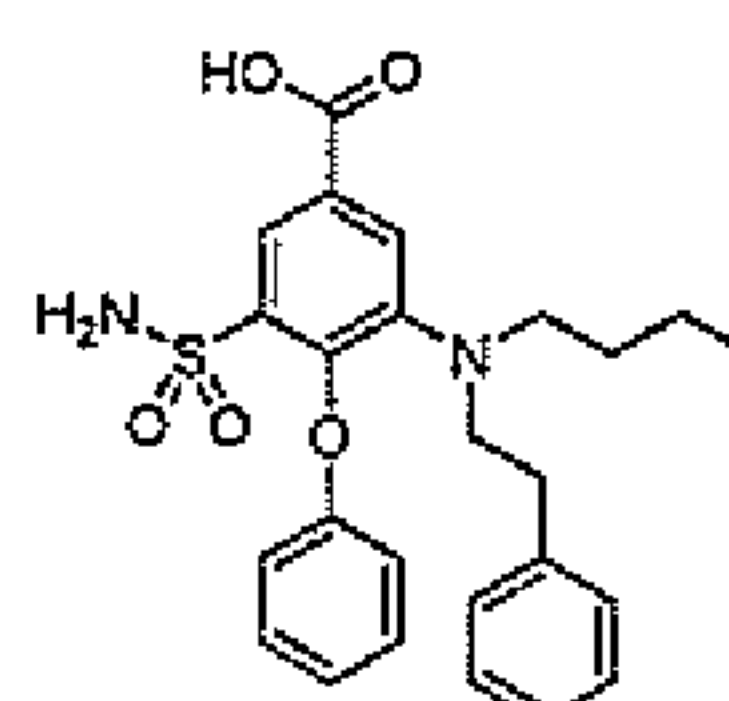
NTP-4016



The title compound was prepared following General Method F but using (2-iodoethyl)benzene in place of iodopentane in step 1. MS m/z : 517 $[M+1]^+$.

Example 16**3-(butyl(phenethyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid**

NTP-4017



The title compound was prepared following General Method E but using (2-iodoethyl)benzene in place of iodopentane in step 5. MS m/z : 469 $[M+1]^+$.

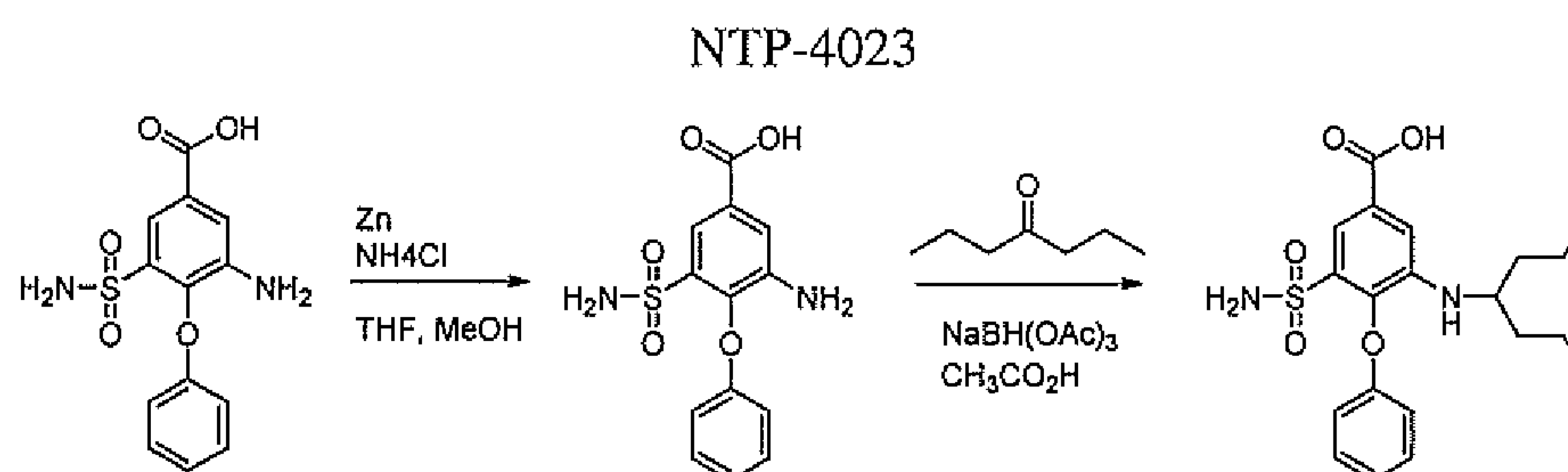
Following General Method D or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art and the following examples were prepared using appropriate benzy bromide in step 1.

Example Number	NTP-Number	Structure	M.W. calculated	MS m/z $[M+1]^+$
17	NTP-4018		557.48	558

18	NTP-4019		593.43	594
19	NTP-4020		524.54	525
20	NTP-4021		560.52	561
21	NTP-4022		593.43	594

Example 22

3-(heptan-4-ylamino)-4-phenoxy-5-sulfamoylbenzoic acid



Step 1: 3-amino-4-phenoxy-5-sulfamoylbenzoic acid.

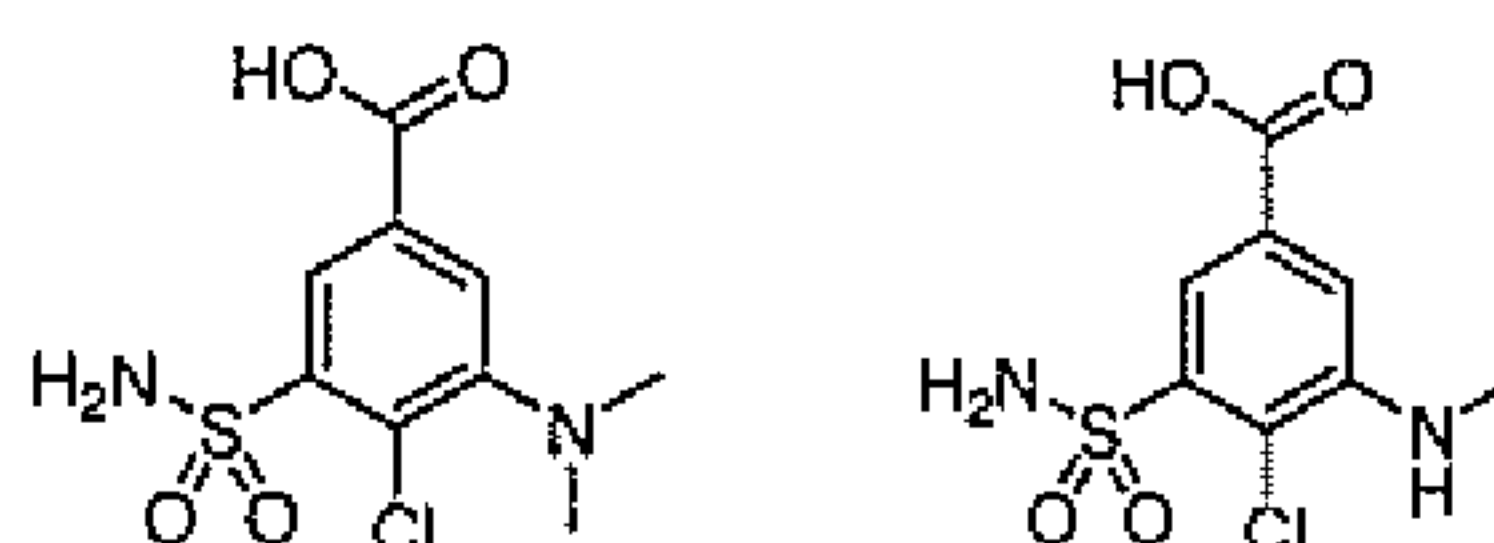
A round bottom flask was charged with 3-nitro-4-phenoxy-5-sulfamoylbenzoic acid (0.400 g, 0.00118 moles), Ammonium chloride (0.553 g, 0.01035 moles), and THF-MeOH (1:1, 10 mL). zinc (0.676 g, 0.01035 moles) was added under nitrogen and the reaction mixture was stirred at rt over night. TLC and LC/MS indicated completion of the reaction. The reaction mixture was filtered through Celite and the filter cake was washed with THF-MeOH (1:1). The filtrate was concentrated under reduced pressure to give a beige solid (360 mg). MS *m/z*: 307.0 [M+1]⁺.

Step 2: 3-(heptan-4-ylamino)-4-phenoxy-5-sulfamoylbenzoic acid

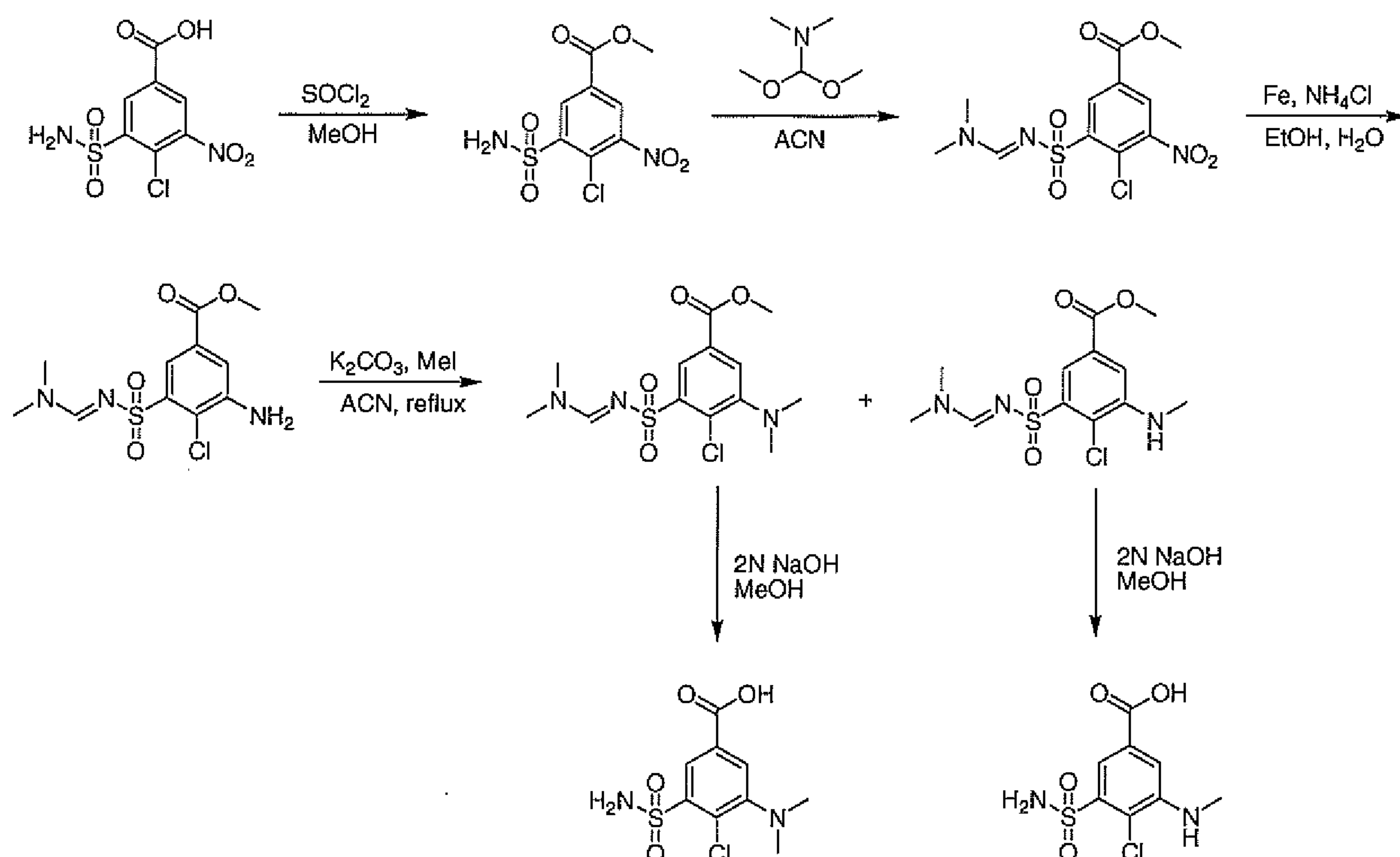
To a stirred solution of 3-amino-4-phenoxy-5-sulfamoylbenzoic acid (70.0 mg, 0.00023 moles) in Acetic acid (2 ml) was added heptan-4-one (0.95 ml, 0.00681 moles) and sodium sulfate (645 mg, 0.00454 moles), and the mixture was stirred at 70 °C for 4h. After cooling to rt, Sodium triacetoxyborohydride (0.481 g, 0.00227 moles) was added and the reaction was stirred at 40 °C overnight. LC-MS indicated that only trace starting material left. The reaction mixture was diluted with water and extracted with EtOAc (2 x). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The residue was purified by silica gel column (0-80% EtOAc/hexane) to afford the product as a white foam (30 mg, 38%). MS *m/z*: 407.0 [M+1]⁺; ¹H NMR (300 MHz, CDCl₃): δ 8.00 (s, 1H), 7.60 (s, 1H), 7.33 (t, 2H, J = 8.1 Hz), 7.12 (d, 1H, J = 7.5 Hz), 6.96 (d, 2H, J = 8.4 Hz), 4.99 (s, 2H), 3.74 (d, 1H, J = 7.5 Hz), 3.42 (m, 1H), 1.50-1.00 (m, 8H), 0.83 (t, 6H, J = 7.2 Hz).

Examples 23 and 24**4-Chloro-3-(dimethylamino)-5-sulfamoylbenzoic acid and 4-Chloro-3-(methylamino)-5-sulfamoylbenzoic acid**

NTP-5001 and NTP-5002



General Method G



Step 1: 4-Chloro-3-nitro-5-sulfamoyl benzoic acid methyl ester. A round bottom flask was charged with 4-chloro-3-nitro-5-sulfamoyl benzoic acid (3.0 g, 10.689 mmol) and methanol (50 mL). Thionyl chloride (3.82 g, 32.06 mmol) was added slowly at room temperature and the reaction mixture was heated to 50°C overnight. The solvent was removed under reduced pressure and the residue re-dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution, water and brine. The organic solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (3.1 g). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.72 (d, $J=2.1$ Hz, 1H), 8.65 (d, $J=2.1$ Hz, 1H), 8.14 (s, 2H), 3.93 (s, 3H).

Step 2: 4-Chloro-3-(dimethylaminomethylene-sulfamoyl)-5-nitrobenzoic acid methyl ester. A reaction flask was charged with 4-chloro-3-nitro-5-sulfamoyl benzoic acid methyl ester (500 mg, 1.696 mmol), acetonitrile (5 mL) and N,N-dimethyl formamide dimethyl acetal (0.24 mL, 1.781 mmol) and stirred at room temperature over night. The solvent was removed under reduced pressure and the resultant gummy residue was treated with ice cold water to give yellow solid. The solid was filtered and dried to give the product (520 mg). MS m/z : 350 $[\text{M}+1]^+$.

Step 3: 3-Amino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester. A round bottom flask was charged with 4-chloro-3-(dimethylaminomethylene-sulfamoyl)-5-nitrobenzoic acid methyl ester (500 mg, 1.428 mmol), ethanol (20 mL) and the reaction mixture

heated to 85°C. Ammonium chloride (756 mg, 14.28 mmol) in water (10 mL) was added. Iron powder (314 mg, 5.71 mmol) was added in three portions 3 minutes apart. The heating was continued for another 2 h. The reaction mixture was cooled to 60°C and poured into dichloromethane (150 mL). The organic layer was separated and washed with water, brine and dried over sodium sulfate. The solvents were removed under reduced pressure to give the product as off white solid (340 mg). MS *m/z*: 320 [M+1]⁺.

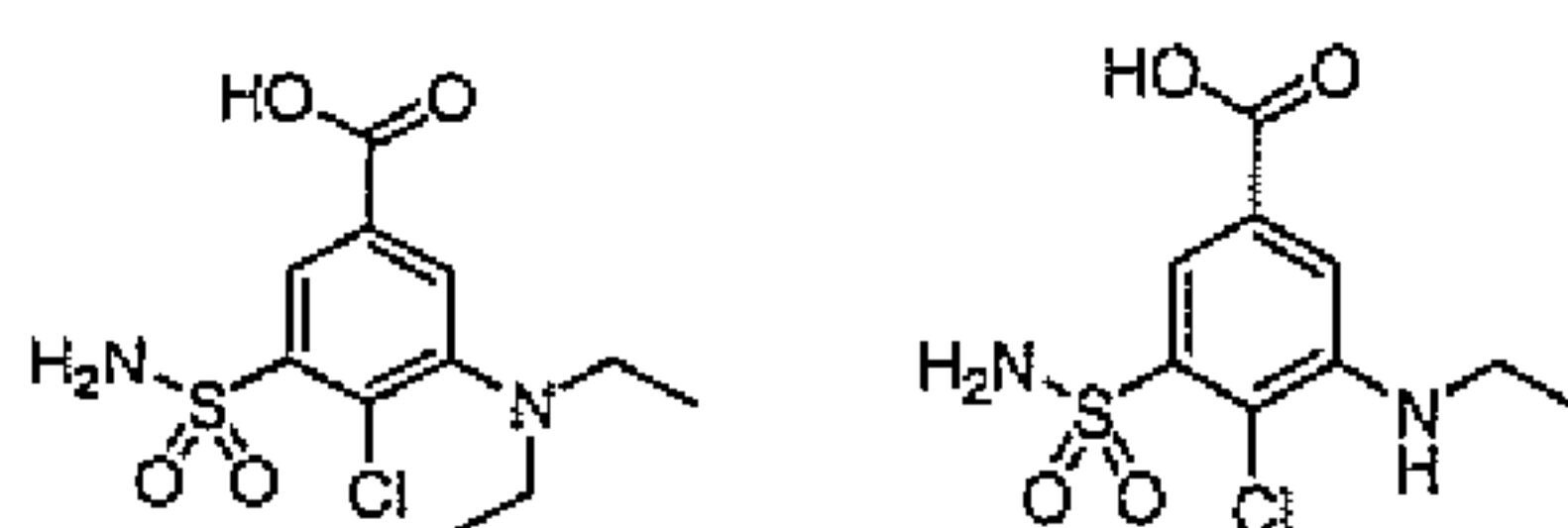
Step 4: 4-Chloro-3-dimethylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester and 4-chloro-3-(methylamino)-5-sulfamoylbenzoic acid. A pressure tube was charged with 3-amino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester (150 mg, 0.469 mmol), potassium carbonate (389 mg, 2.814 mmol), methyl iodide (400 mg, 2.8 mmol), acetonitrile (15 mL) and the reaction heated at 75°C over night. Both the mono and di-alkylated products were present by LC/MS. The reaction was cooled and filtered and washed with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give 4-chloro-3-dimethylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid as pale yellow solid (88 mg) and 4-chloro-3-(methylamino)-5-sulfamoylbenzoic acid as yellow solid (35 mg). MS *m/z*: 348 [M+1]⁺ and MS *m/z*: 334 [M+1]⁺.

Step 5: 4-Chloro-3-dimethylamino-5-sulfamoyl-benzoic acid (NTP-5001). A reaction vial was charged with 4-chloro-3-dimethylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (85 mg, 0.247 mmol), 2N NaOH (2 mL), methanol (4 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as white solid (49 mg). ¹H NMR (300 MHz, DMSO- *d*₆) δ 13.51 (bs, 1H), 8.16 (d, J=2.1 Hz, 1H), 7.81 (d, J=1.8 Hz, 1H), 7.70 (s, 2H), 2.79 (s, 6H). MS *m/z*: 279 [M+1]⁺.

Step 6: 4-Chloro-3-methylamino-5-sulfamoyl-benzoic acid (NTP-5002). The product was obtained following the procedure described for step 5 and starting with 4-chloro-3-(methylamino)-5-sulfamoylbenzoic acid to give the title compound as white solid (16 mg). ¹H NMR (300 MHz, DMSO- *d*₆) δ 13.51 (bs, 1H), 8.45 (d, J=2.1 Hz, 1H), 7.76 (d, J=1.8 Hz, 1H), 7.34 (s, 2H), 5.69 (m, 1H), 2.80 (d, J=5.1 Hz, 3H). MS *m/z*: 263 [M-1]⁻.

Examples 25 and 26**4-Chloro-3-(diethylamino)-5-sulfamoylbenzoic acid and 4-Chloro-3-(ethylamino)-5-sulfamoylbenzoic acid**

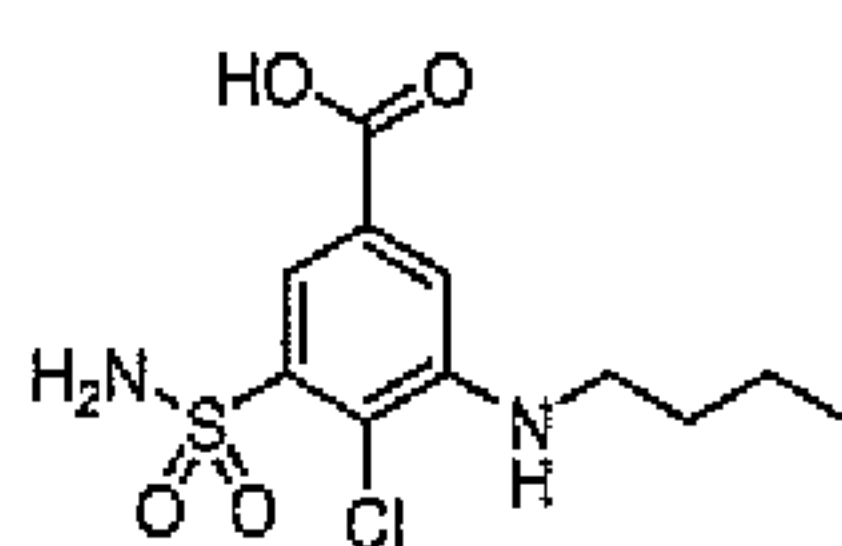
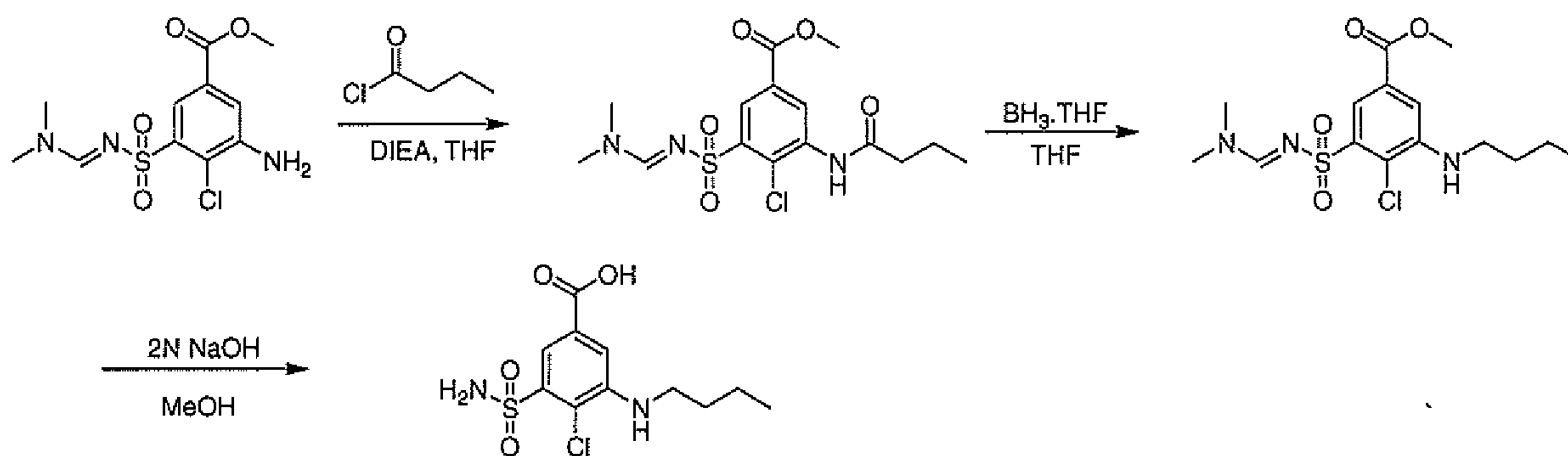
NTP-5003 and NTP-5004



The title compounds were prepared following General Method G and using the appropriate alkyl iodide in step 4. 4-chloro-3-(diethylamino)-5-sulfamoylbenzoic acid: MS m/z : 307 $[M+1]^+$. 4-chloro-3-(ethylamino)-5-sulfamoylbenzoic acid: MS m/z : 279 $[M+1]^+$.

Example 27**3-(Butylamino)-4-chloro-5-sulfamoylbenzoic acid**

NTP-5005

**General Method H**

Step 1: 3-Butyrylamino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester. A round bottom flask was charged with 3-amino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester (Method G, step 3, 500 mg, 1.56 mmol), butyryl chloride (0.20 mL, 1.876 mmol), diisopropylethyl amine (0.1 mL), THF (5 mL) and the reaction stirred at room temperature for 2 hours. The reaction mixture was poured into water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as light brown oil (650 mg). MS m/z : 390.2 $[M+1]^+$.

Step 2: 3-Butylamino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester: A round bottom flask was charged with 3-butyrylamino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester (600 mg, 1.539 mmol), THF (10 mL) and $\text{BH}_3\cdot\text{THF}$ (1.0 M in THF) (7.69 mL, 7.69 mmol) was added slowly. The reaction was stirred at room temperature for 1 hour. The reaction was quenched by drop wise addition of water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (340 mg). MS m/z : 376.3 $[\text{M}+1]^+$.

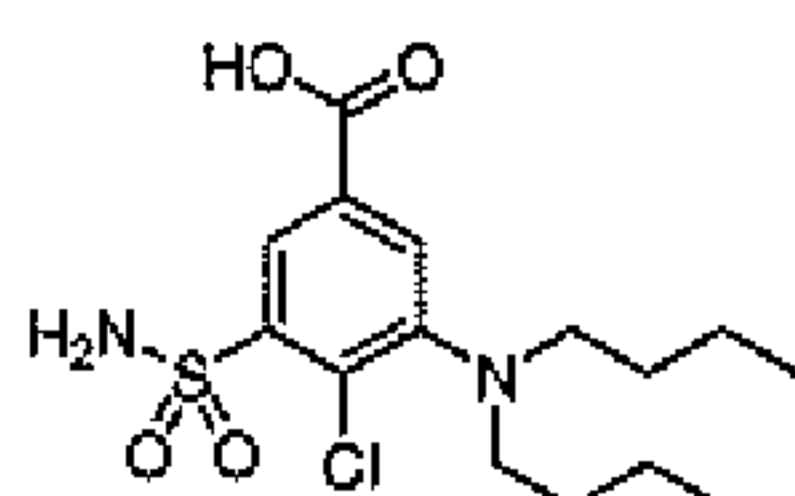
Step 3: 3-Butylamino-4-chloro-5-sulfamoyl-benzoic acid (NTP-5005).

A reaction vial was charged with 3-butylamino-4-chloro-5-(dimethylaminomethylene-sulfamoyl)-benzoic acid methyl ester (340 mg, 0.90 mmol), 2N NaOH (5 mL), methanol (5 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as white solid (105 mg). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 13.34 (bs, 1H), 7.72 (d, $J=2.1$ Hz, 1H), 7.59 (s, 2H), 7.33 (d, $J=1.8$ Hz, 1H) 5.99 (t, $J=5.4$ Hz, 1H), 3.20 (t, $J=6.3$ Hz, 2H), 1.59–1.52 (m, 2H), 1.40–1.1.33 (m, 2H), 0.92 (t, $J=6.6$ Hz, 3H). MS m/z : 307.1 $[\text{M}+1]^+$.

Example 28

4-Chloro-3-(dibutylamino)-5-sulfamoylbenzoic acid

NTP-5006

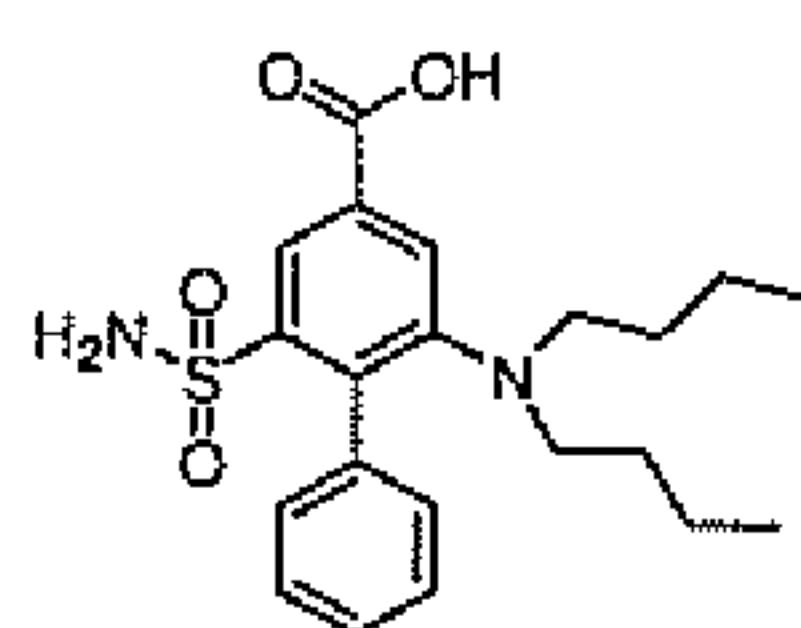


The title compound was prepared following General Method G and using the appropriate alkyl iodide in step 4. ^1H NMR (300 MHz, CD_3OD) δ 8.34 (m, 1H), 7.96 (m, 1H), 3.14 (t, $J=5.7$ Hz, 4H), 1.48–1.45(m, 4H), 1.38–1.31 (m, 4H), 0.90 (t, $J=6.9$ Hz, 6H). MS m/z : 363 $[\text{M}+1]^+$.

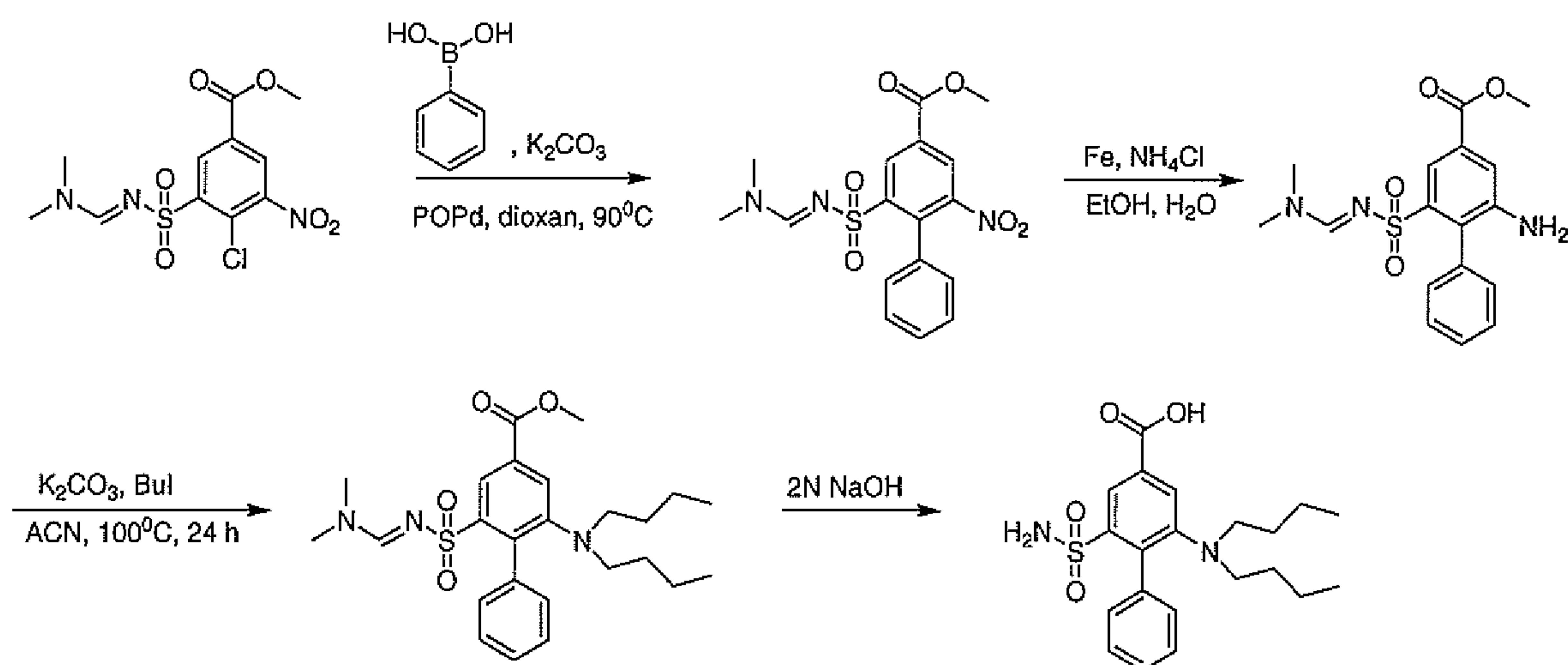
Example 29

2-(Dibutylamino)-6-sulfamoylbiphenyl-4-carboxylic acid

NTP-5007



General Method I



Step 1: 2-(Dimethylaminomethylene-sulfamoyl)-6-nitro-biphenyl-4-carboxylic acid methyl ester. A reaction vial was charged with 4-chloro-3-(dimethylaminomethylene-sulfamoyl)-5-nitrobenzoic acid methyl ester (Method G, step 2, 220 mg, 0.630 mmol), phenyl boronic acid (115 mg, 0.945 mmol), potassium carbonate (261 mg, 1.89 mmol), [(t-Bu)₂P(OH)]₂ PdCl₂ (POPd) (3 mg, 0.0063 mmol) and 1,4-dioxane (3 mL). The vial was evacuated with vacuum and flushed with nitrogen. The reaction was then heated at 90°C overnight. The reaction mixture was cooled to room temperature, diluted with ethyl acetate and washed with water. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product in 60% purity which was taken to next step without further purification (90 mg). MS *m/z*: 392 [M+1]⁺.

Step 2: 6-Amino-2-(dimethylaminomethylene-sulfamoyl)-biphenyl-4-carboxylic acid methyl ester. A round bottom flask was charged with 2-(dimethylaminomethylene-sulfamoyl)-6-nitro-biphenyl-4-carboxylic acid methyl ester (90 mg, 0.229 mmol), ethanol (10 mL) and the reaction mixture heated to 85°C. Ammonium chloride (122 mg, 2.29 mmol) in water (3 mL) was added. Iron powder (51 mg, 0.917 mmol) was added in three portions 3 minutes apart. The heating was continued for another 2 h. The reaction mixture was cooled to 60°C and poured into dichloromethane (100 mL). The organic layer was separated and washed with water, brine and dried over sodium sulfate. The solvents were removed under reduced pressure and the residue purified by flash chromatography to give the product as off white solid (60 mg). MS *m/z*: 362 [M+1]⁺.

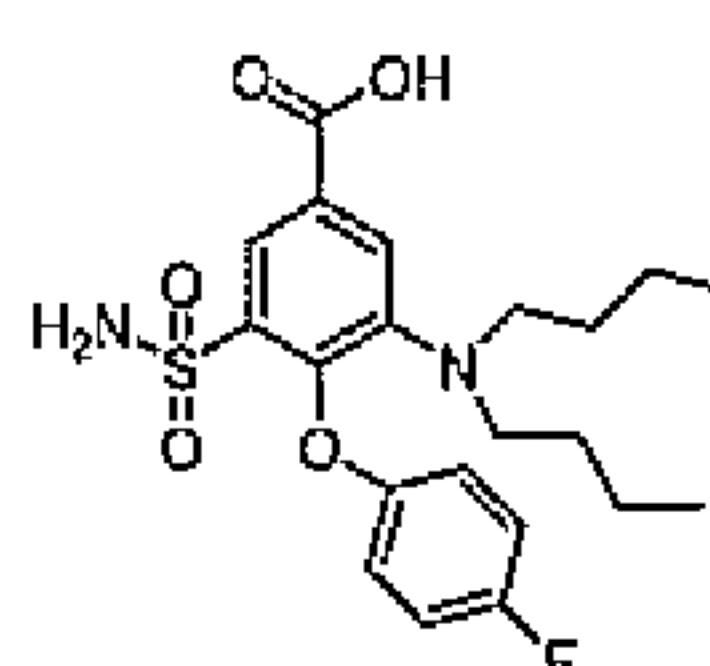
Step 3: 6-Dibutylamino-2-(dimethylaminomethylene-sulfamoyl)-biphenyl-4-carboxylic acid methyl ester. A pressure tube was charged with 6-amino-2-(dimethylaminomethylene-sulfamoyl)-biphenyl-4-carboxylic acid methyl ester (60 mg, 0.166 mmol), potassium carbonate (138 mg, 0.996 mmol), butyl iodide (122 mg, 0.664 mmol), acetonitrile (5 mL) and the reaction heated at 120°C over night. The reaction gave a mixture of mono and di-alkylated products. The reaction was cooled and filtered and washed with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product (28 mg). MS m/z : 474 $[M+1]^+$.

Step 4: 6-Dibutylamino-2-sulfamoyl-biphenyl-4-carboxylic acid (NTP-5007). A reaction vial was charged with 6-dibutylamino-2-(dimethylaminomethylene-sulfamoyl)-biphenyl-4-carboxylic acid methyl ester (28 mg, 0.059 mmol), 2N NaOH (1 mL), methanol (3 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as yellow solid (16 mg). ^1H NMR (300 MHz, DMSO- d_6) δ 13.41 (bs, 1H), 8.25 (d, $J=1.5$ Hz, 1H), 7.83 (d, $J=1.5$ Hz, 1H), 7.38–7.28 (m, 3H), 7.19 (d, $J=6.9$, 2H), 7.00 (s, 2H), 2.68 (m, 4H), 1.05–1.03 (m, 8H), 0.74 (t, $J=6.6$ Hz, 6H). EI MS m/z : 405 $[M+1]^+$.

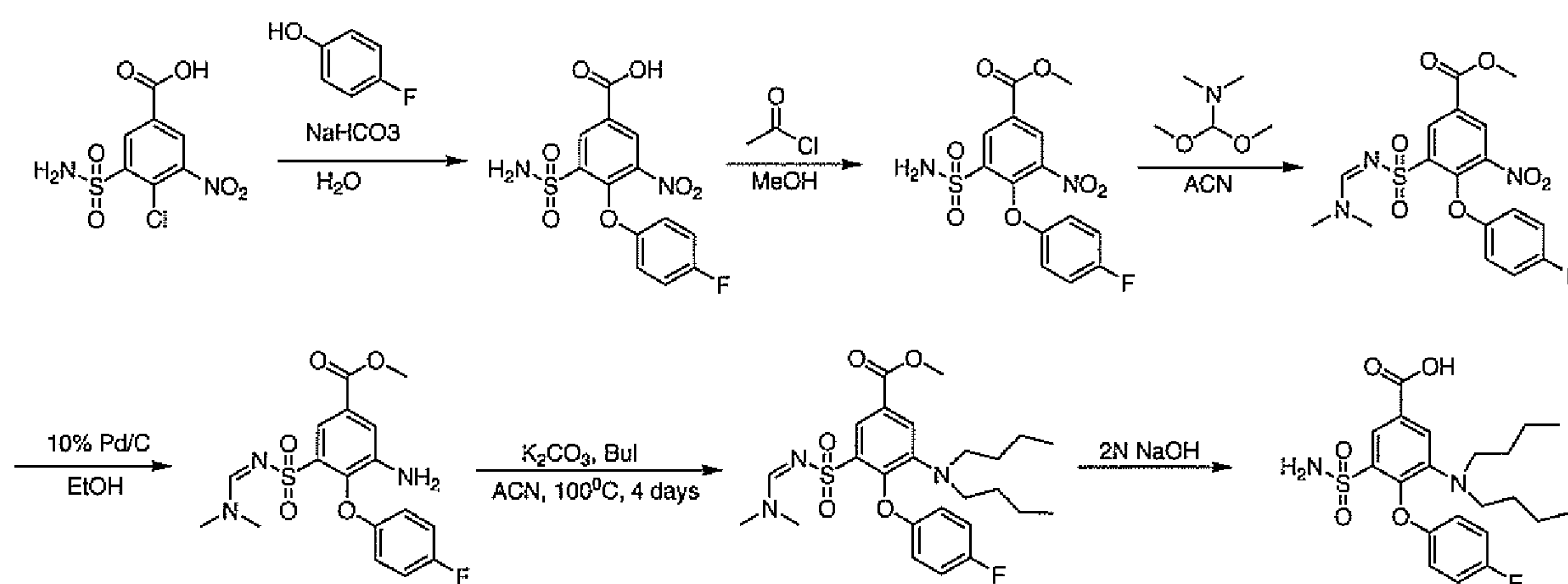
Example 30

3-(Dibutylamino)-4-(3-fluorophenoxy)-5-sulfamoylbenzoic acid

NTP-5009



General Method J



Step 1: 4-(4-Fluorophenoxy)-3-nitro-5-sulfamoylbenzoic acid. To a stirred solution of 4-Chloro-3-nitro-5-sulfamoylbenzoic acid (1 g, 3.56 mmol) in water was added 4-fluorophenol (0.8 g, 7.12 mmol) and NaHCO_3 (1.2 g, 14.24 mmol) at 0°C . The mixture was stirred at 100°C for 12 hours, then it was cooled to room temperature. The mixture was made acidic with 6 N HCl. The light yellow solid came out, then it was filtered to give a crude 4-(4-fluorophenoxy)-3-nitro-5-sulfamoylbenzoic acid, which was used in the next reaction without further purification.

Step 2: Methyl 4-(4-fluorophenoxy)-3-nitro-5-sulfamoylbenzoate. A solution of 4-(4-fluorophenoxy)-3-nitro-5-sulfamoylbenzoic acid (0.97 g, 2.72 mmol) in methanol was added acetyl chloride (0.42 g, 5.38 mmol) dropwise at 0°C . The mixture was stirred at 60°C for 12 hours. When all starting material disappeared, water was added slowly at 0°C . Methanol was removed, then the aqueous solution was extracted with ethyl acetate. The organic layers were combined, dried over anhydrous MgSO_4 , and evaporated. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 1/1) to afford methyl 4-(4-fluorophenoxy)-3-nitro-5-sulfamoylbenzoate (0.37 g).

Step 3: Methyl 3-(N-((dimethylamino)methylene)sulfamoyl)-4-(4-fluorophenoxy)-5-nitrobenzoate. To a solution of methyl 4-(4-fluorophenoxy)-3-nitro-5-sulfamoylbenzoate (0.37 g, 1 mmol) in acetonitrile was added *N,N*-Dimethylformamide dimethyl acetal (DMF/DMA, 0.178g, 1.5 mmol) at room temperature for 30 minutes. The reaction mixture was stirred at room temperature for 1 hour, and then quenched with water. The resulting mixture was extracted with ethyl acetate. The organic layer was separated and washed with 0.1 M HCl, dried over anhydrous MgSO_4 , and evaporated. The residue was purified by flash chromatography (n-hexane/ethyl acetate = 5/1) to afford the title compound (0.27 g).

Step 4: Methyl 3-amino-5-(N-((dimethylamino)methylene)sulfamoyl)-4-(4-fluorophenoxy)benzoate. A mixture of 3-(N-((dimethylamino)methylene)sulfamoyl)-4-(4-fluorophenoxy)-5-nitrobenzoate (190 mg, 0.447 mmol) and 10% Pd/C (19 mg, 10% w/w) in ethyl alcohol was stirred at 60 °C under H₂ atmosphere. After 3 hours the mixture was passed through a celite to remove the catalyst. The filtered catalyst was washed with ethyl alcohol (2x10 mL). The combined filtrates were washed with H₂O (2x30 mL) and brine (30 mL), dried over anhydrous MgSO₄, and evaporated under reduced pressure. The residue was purified by flash chromatography (*n*-hexane/ethyl acetate = 1/1) to afford the title compound (30 mg).

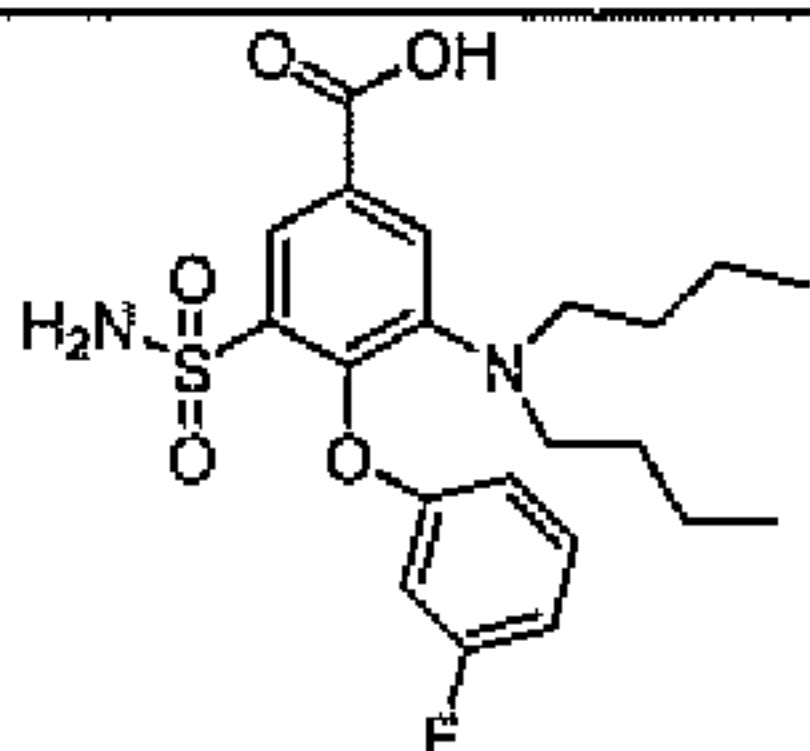
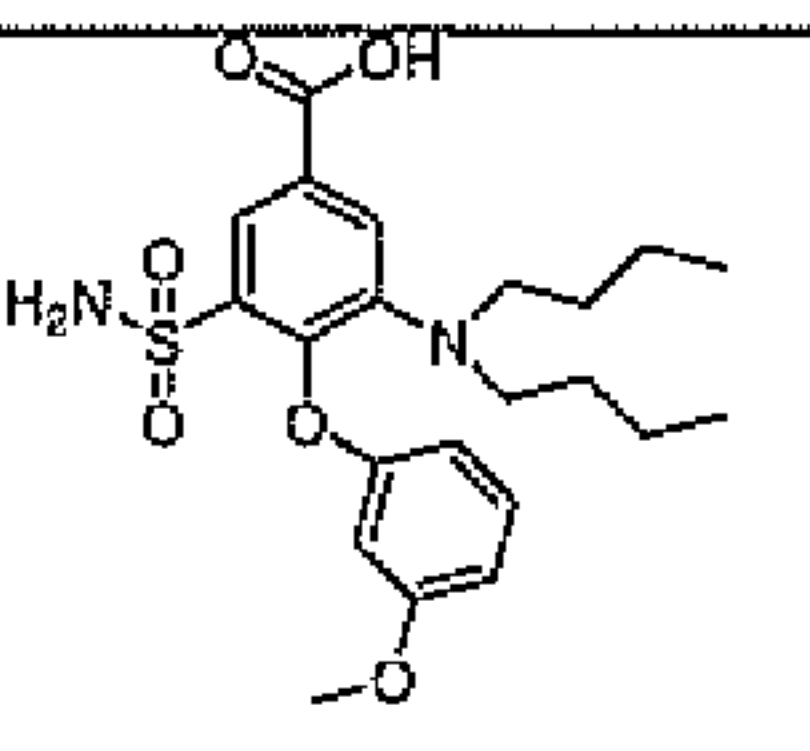
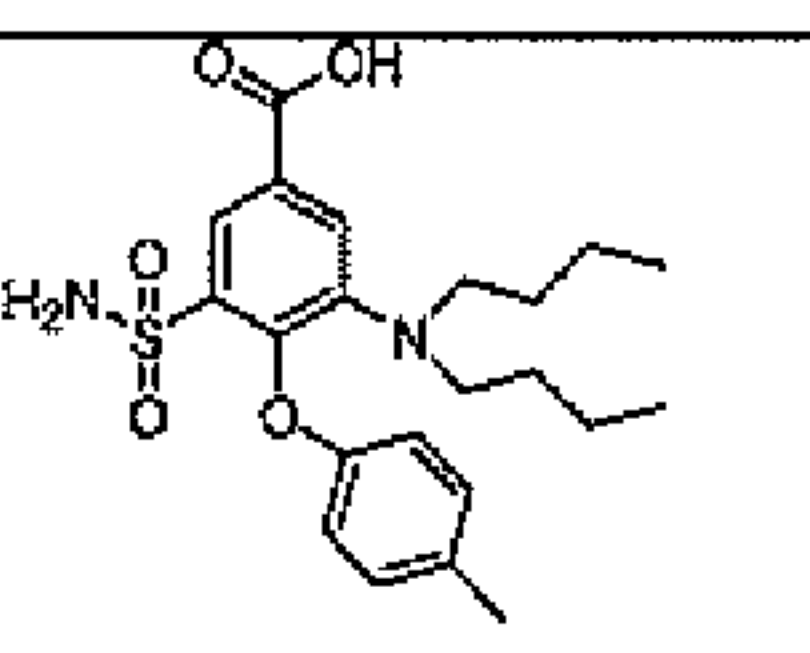
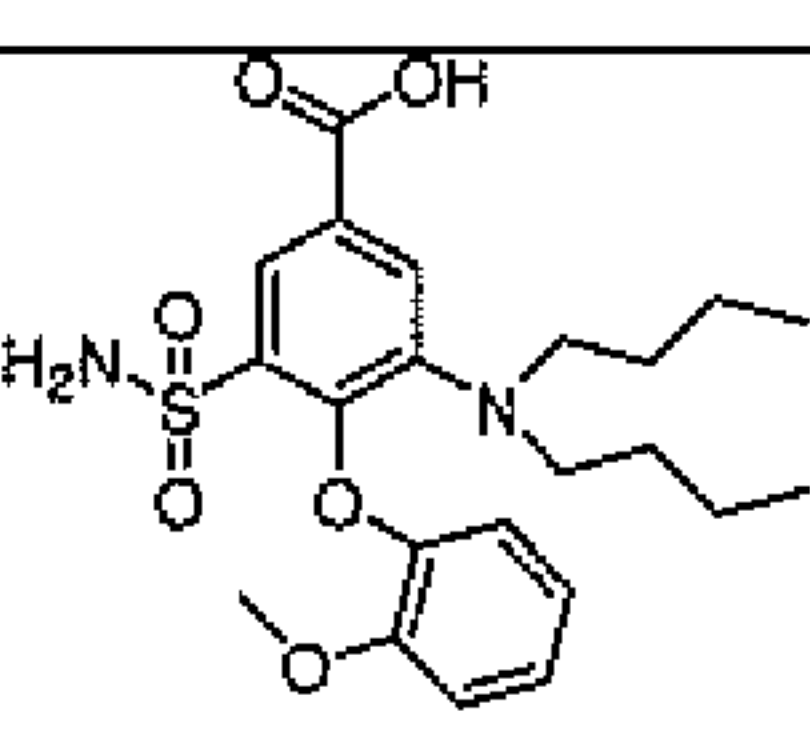
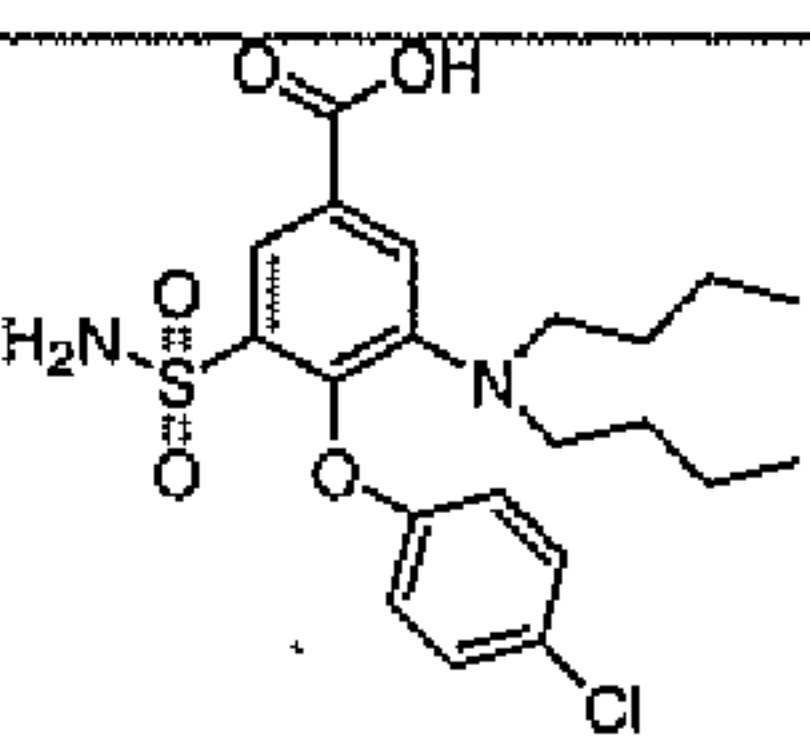
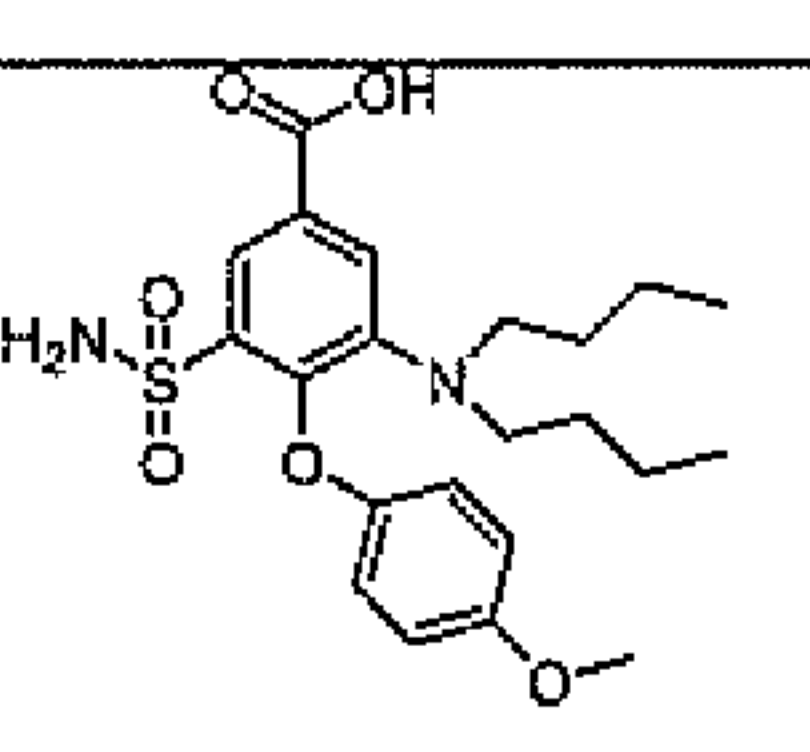
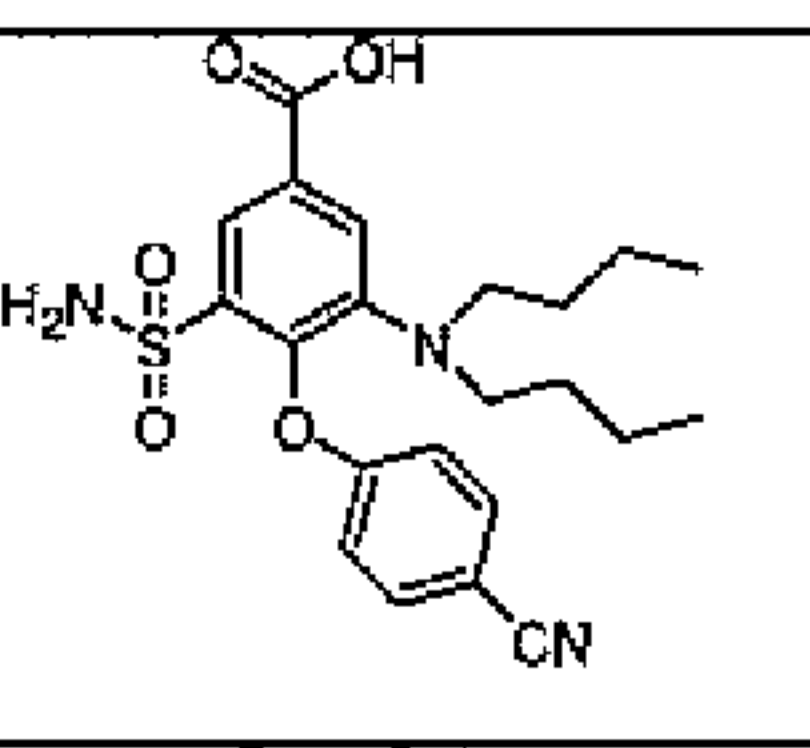
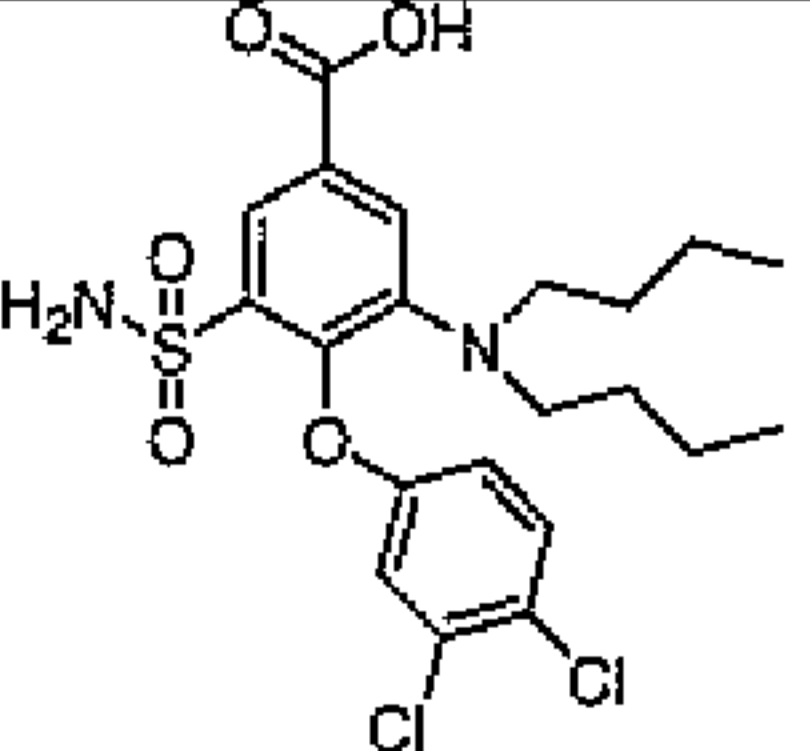
¹H NMR (400 MHz, DMSO-*d*₆) δ 7.91 (s, 1H), 7.71 (s, 1H), 7.63 (s, 1H), 7.18–7.11 (m, 2H), 6.74–6.71 (m, 2H), 5.37 (s, 2H), 3.86 (s, 3H), 2.92 (s, 3H), 2.55 (s, 3H).

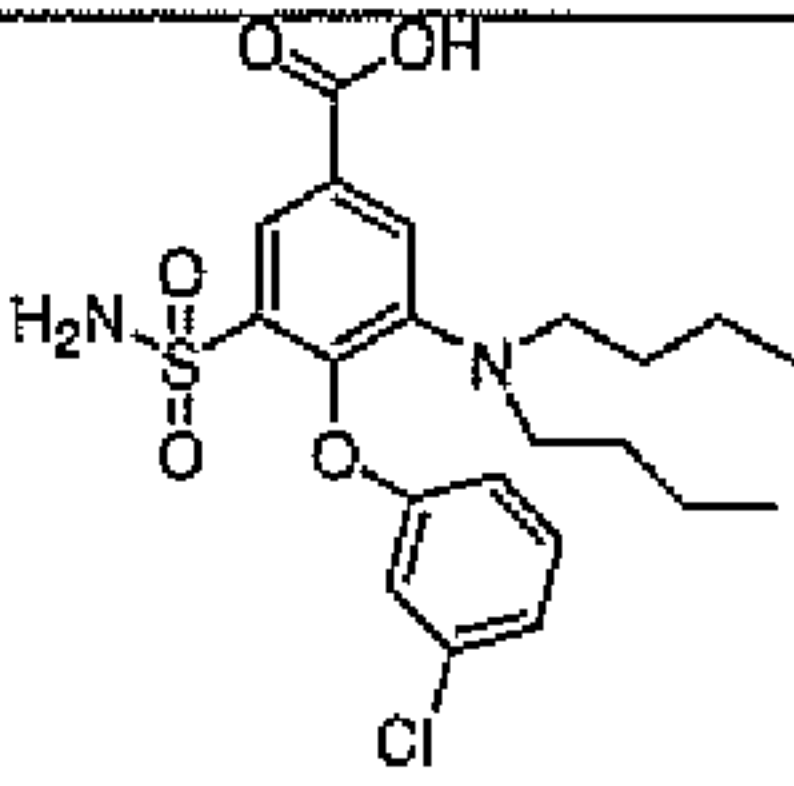
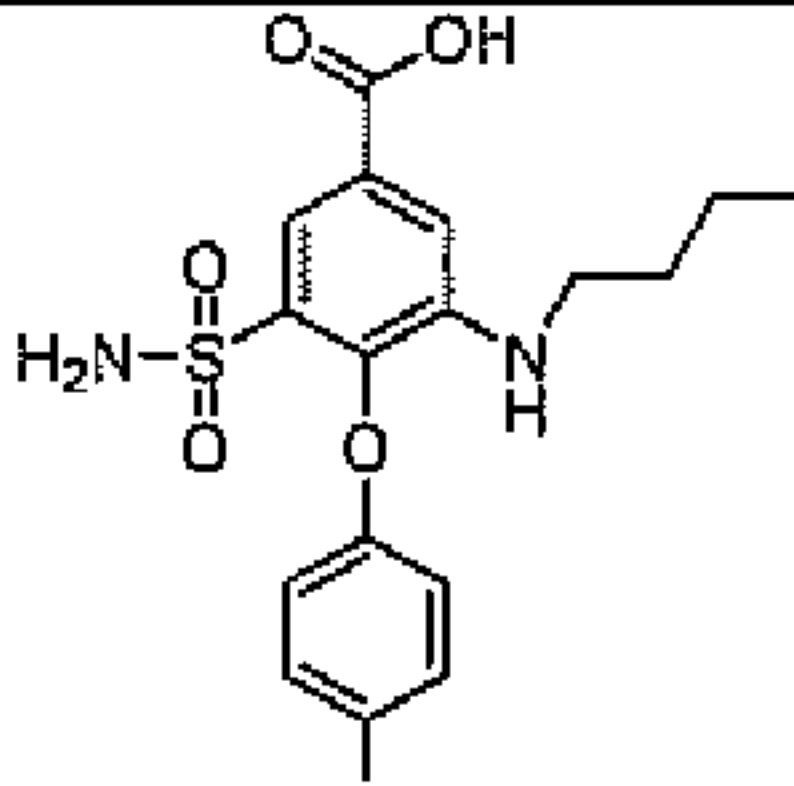
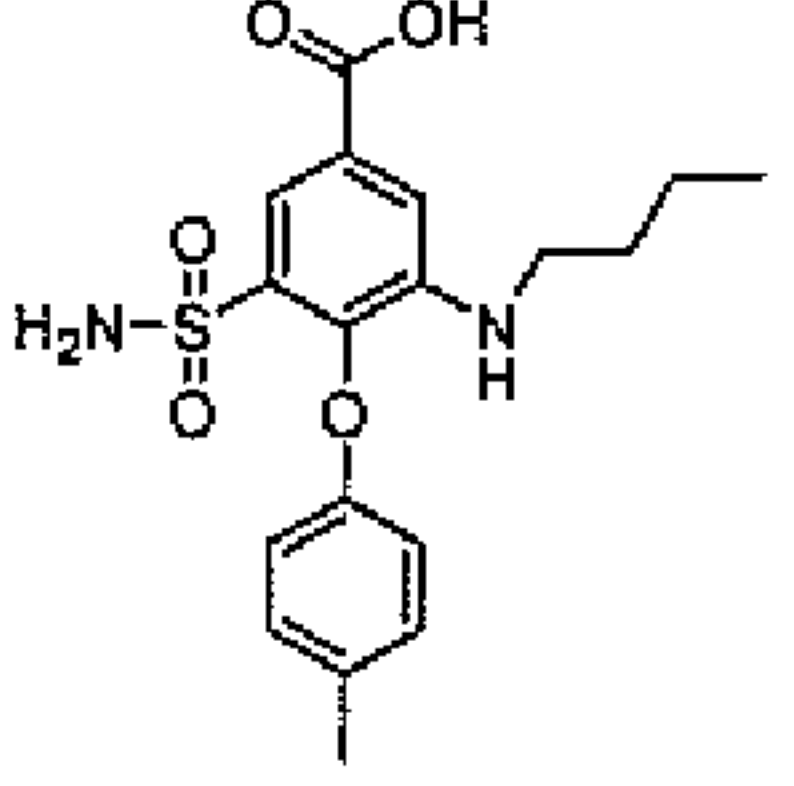
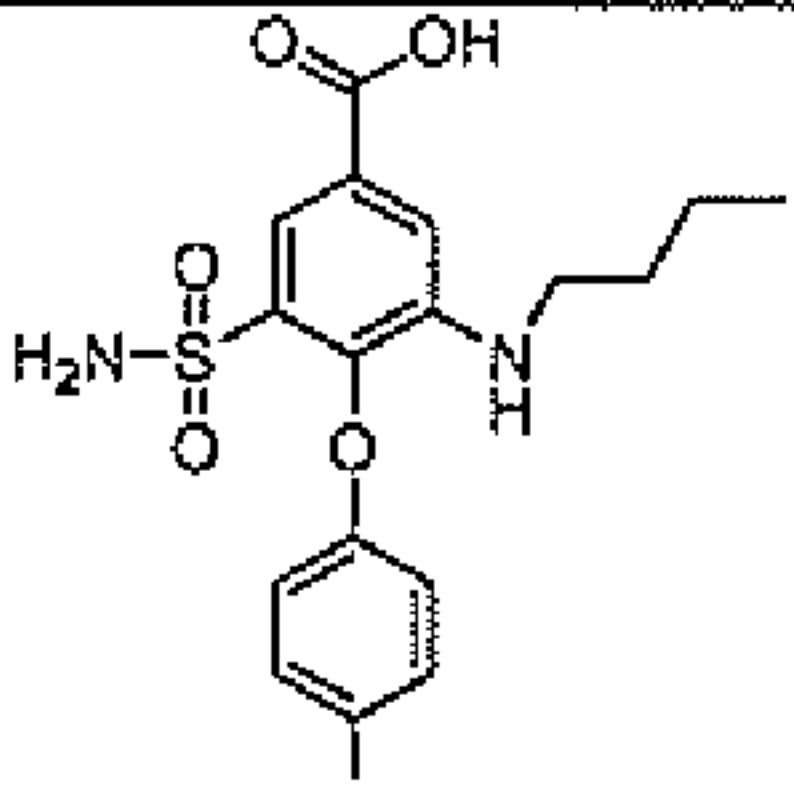
Step 5: 3-Dibutylamino-5-(dimethylaminomethylene-sulfamoyl)-4-(4-fluoro-phenoxy)-benzoic acid methyl ester. A pressure tube was charged with 3-amino-5-(dimethylaminomethylene-sulfamoyl)-4-(4-fluorophenoxy)-benzoic acid methyl ester (140 mg, 0.352 mmol), potassium carbonate (292 mg, 2.115 mmol), butyl iodide (259 mg, 1.408 mmol), acetonitrile (5 mL) and the reaction heated at 100°C for 4 days. The reaction gave a mixture of mono and di-alkylated products. The reaction was cooled and filtered and the solid washed with ethyl acetate. The filtrate was concentrated under reduced pressure and the residue purified by flash chromatography to give the product (51.5 mg).

MS *m/z*: 508 [M+1]⁺.

Step 6: 3-Dibutylamino-4-(4-fluoro-phenoxy)-5-sulfamoyl-benzoic acid (NTP-5009). A reaction vial was charged with 3-dibutylamino-5-(dimethylaminomethylene-sulfamoyl)-4-(4-fluoro-phenoxy)-benzoic acid methyl ester (51 mg, 0.101 mmol), 2N NaOH (1 mL), methanol (3 mL) and the reaction heated to 50°C for 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as pale yellow solid (37 mg). ¹H NMR (300 MHz, DMSO-*d*₆) δ 13.30 (bs, 1H), 8.00 (d, J=2.1 Hz, 1H), 7.73 (d, J=2.1 Hz, 1H), 7.42 (s, 2H), 7.08–7.03 (m, 2H), 6.78–6.73 (m, 2H), 3.00 (t, J=7.2 Hz, 4H), 1.21–1.11 (m, 4H), 1.01–0.91 (m, 4H), 0.73 (t, J=7.2Hz, 6H). MS *m/z*: 439 [M+1]⁺.

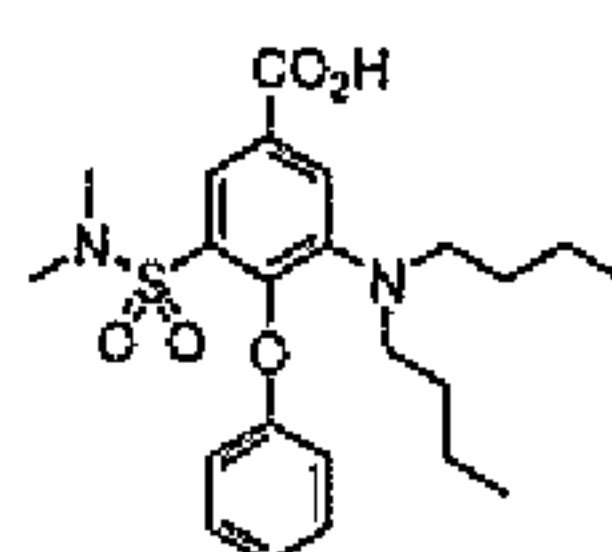
Following General Method J or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art and the following examples were prepared from commercially available reagents.

Example Number	NTP-Number	Structure	M.W. calculated	MS m/z [M+1] ⁺
31	NTP-5008		438.51	439
32	NTP-5010		450.55	451
33	NTP-5011		434.55	435
34	NTP-5012		450.55	451
35	NTP-5013		454.97	455
36	NTP-5014		450.55	451
37	NTP-5015		445.53	446
38	NTP-5016		489.41	489

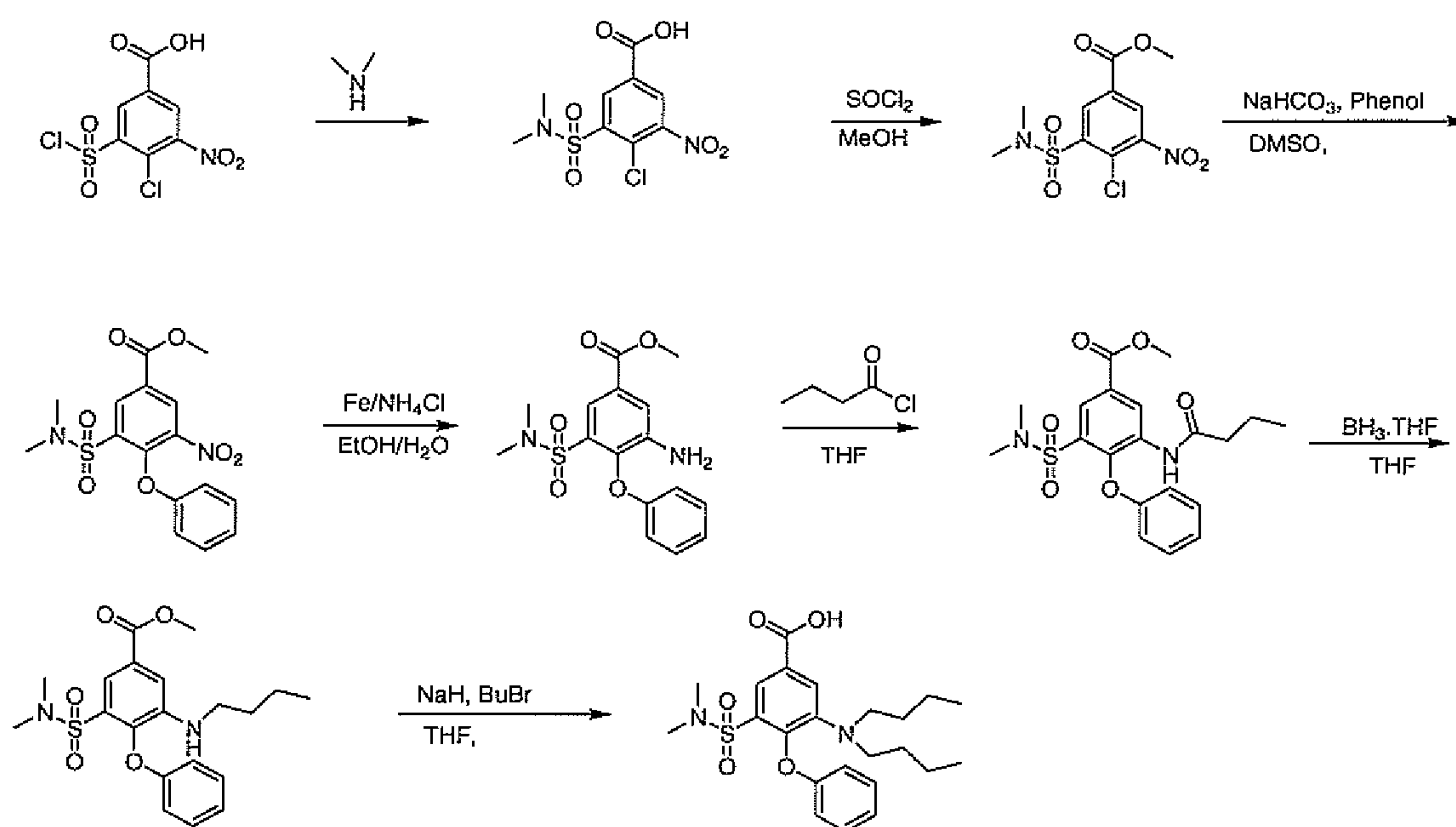
39	NTP-5017		454.97	455
40	NTP-5018		398.86	399
41	NTP-5019		378.44	379
42	NTP-5020		382.41	383

Example 43**3-(Dibutylamino)-5-(N,N-dimethylsulfonyl)-4-phenoxybenzoic acid**

NTP-6001



General Method K



Step 1: 4-Chloro-3-dimethylsulfamoyl-5-nitro-benzoic acid. A round bottom flask was charged with 4-chloro-3-chlorosulfonyl-5-nitro-benzoic acid (500g, 1.66 mmol), dimethylamine (2.0 M in THF, 1 mL, 1.99 mmol), diisopropylethylamine (0.36 mL, 1.99 mmol) and THF (3 mL) and the reaction was stirred at 45°C overnight. The solvent was removed under reduced pressure and the residue dissolved in ethyl acetate and washed with water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure to give the product as yellow solid (360 mg) which was used in the next reaction without further purification. MS *m/z*: 307 [M-1].

Step 2: 4-Chloro-3-dimethylsulfamoyl-5-nitro-benzoic acid methyl ester. A round bottom flask was charged with 4-chloro-3-dimethylsulfamoyl-5-nitro-benzoic acid (360 mg, 1.16 mmol) and methanol (10 mL). Thionyl chloride (152 mg, 1.283 mmol) was added slowly at room temperature and the reaction mixture was heated to 50°C overnight. The solvent was removed under reduced pressure and the residue re-dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution, water and brine. The organic solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as yellow solid which was used directly in the next reaction (340 mg).

Step 3: 3-Dimethylsulfamoyl-5-nitro-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 4-chloro-3-dimethylsulfamoyl-5-nitro-benzoic acid methyl ester (340 mg, 1.054

mmol), sodium bicarbonate (354 mg, 4.217 mmol), phenol (198 mg, 2.108 mmol) and DMSO (10 mL) and the reaction mixture heated to 80°C overnight. The reaction was cooled to room temperature and quenched with water. The product which precipitated out was filtered and dissolved in ethyl acetate and washed with brine. The solvent was removed under reduced pressure to give the product as pale yellow solid (340 mg). ¹H NMR (300 MHz, DMSO- *d*₆) δ 8.71 (d, *J*=1.8 Hz, 1H), 8.63 (d, *J*=2.1 Hz, 1H), 7.32 (t, *J*=5.7 Hz, 2H), 7.10 (t, *J*=7.5 Hz, 1H), 6.92 (d, *J*=8.4 Hz, 2H), 3.95 (s, 3H), 2.79 (s, 6H).

Step 4: 3-Amino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-dimethylsulfamoyl-5-nitro-4-phenoxy-benzoic acid methyl ester (340 mg, 0.894 mmol), ethanol (10 mL) and the reaction mixture heated to 85°C. Ammonium chloride (475 mg, 8.94 mmol) in water (5 mL) was added. Iron powder (197 mg, 3.578 mmol) was added in three portions 3 minutes apart. The heating was continued for another 1 h. The reaction mixture was cooled to 60°C and poured into dichloromethane (150 mL). The organic layer was separated and washed with water, brine and dried over sodium sulfate. The solvents were removed under reduced pressure to give the product as pale yellow solid (300 mg). MS *m/z*: 351 [M+1]⁺.

Step 5: 3-Butyrylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-amino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester (330 mg, 0.942 mmol), butyryl chloride (0.12 mL, 1.13 mmol), diisopropylethyl amine (0.1 mL), THF (5 mL) and the reaction stirred at room temperature for 2 hours. The reaction mixture was poured into water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as light brown oil (400 mg). MS *m/z*: 421 [M+1]⁺.

Step 6: 3-Butylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester. A round bottom flask was charged with 3-butyrylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester (330 mg, 0.785 mmol), THF (3 mL) and BH₃.THF (1.0 M in THF) (4 mL, 3.928 mmol) was added slowly. The reaction was stirred at room temperature for 1 hour. The reaction was quenched by drop wise addition of water and extracted with ethyl acetate. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as pale yellow solid (160 mg). MS *m/z*: 407 [M+1]⁺.

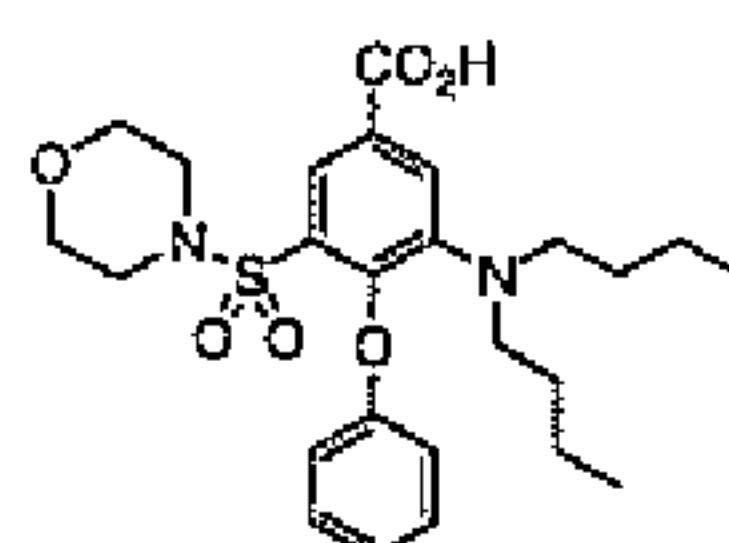
Step 7: 3-Dibutylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid (NTP-6001). A reaction vial was charged with 3-butylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester (30 mg,

0.073 mmol), THF (2 mL), sodium hydride (95%) (3.5 mg, 0.147 mmol) and butyl bromide (16 mg, 0.147 mmol) and the reaction stirred at 50° over night. The reaction was quenched with water and acidified with 3N HCl and extracted with ethyl acetate. The solvent was removed under reduced pressure to give the product as colorless viscous oil (17 mg). ¹H NMR (300 MHz, DMSO- *d*₆) δ 7.94 (d, J=1.8 Hz, 1H), 7.78 (d, J=1.8 Hz, 1H), 7.23 (t, J=7.5 Hz, 2H), 6.99 (t, J=7.2 Hz, 1H), 6.67 (d, J=7.5 Hz, 2H), 3.02 (t, J=7.2 Hz, 4H), 2.70 (s, 6H), 1.23–1.11(m, 4H), 0.98–0.93 (m, 4H), 0.72 (t, J=7.5 Hz, 6H). MS *m/z*: 449 [M+1]⁺.

Example 44

3-(Dibutylamino)-5-(morpholinosulfonyl)-4-phenoxybenzoic acid

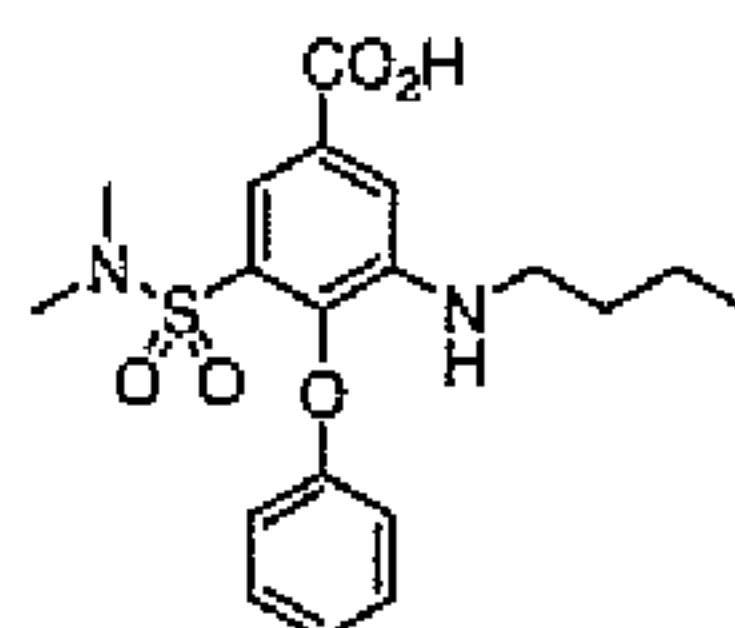
NTP-6002



Following General Method K or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art and the title compound was prepared from commercially available reagents. MS *m/z*: 491 [M+1]⁺.

Example 45**3-(Butylamino)-5-(N,N-dimethylsulfamoyl)-4-phenoxybenzoic acid**

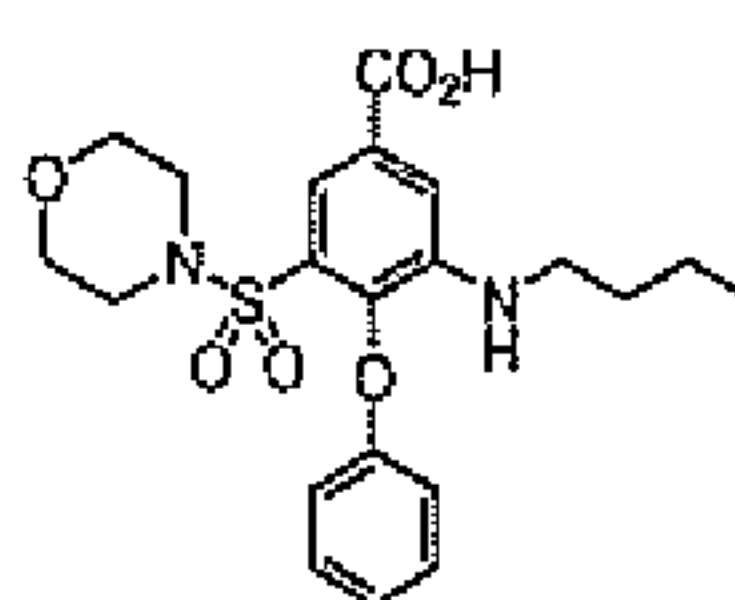
NTP-6003



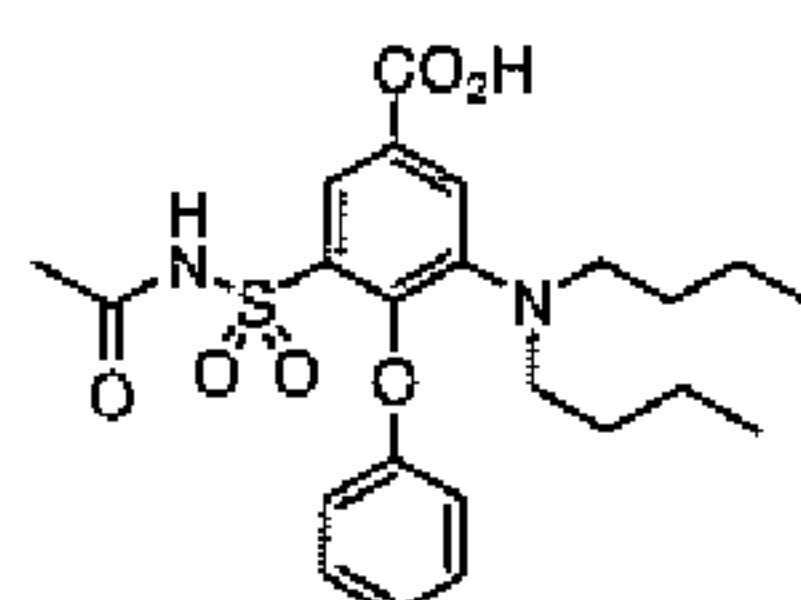
A reaction vial was charged with 3-butylamino-5-dimethylsulfamoyl-4-phenoxy-benzoic acid methyl ester (General Method K, step 6, 130 mg, 0.319 mmol), methanol (2 mL), THF (1 mL) and 1N LiOH (1 mL) and the reaction stirred room temperature 2 hours. The solvent was removed under reduced pressure and the aqueous layer was acidified with 3N HCl. The mixture was extracted with ethyl acetate and the solvent removed under reduced pressure to give the product as white solid (93 mg). ^1H NMR (300 MHz, DMSO- d_6) δ 13.25 (bs, 1H), 7.60 (d, $J=2.1$ Hz, 1H), 7.46 (d, $J=2.1$ Hz, 1H), 7.27 (t, $J=7.5$ Hz, 2H), 7.01 (t, $J=7.2$ Hz, 1H), 6.75 (d, $J=7.5$ Hz, 2H), 5.18 (t, $J=5.4$ Hz, 1H), 3.09–3.03(m, 2H), 1.39–1.34 (m, 2H), 0.77 (t, $J=7.2$ Hz, 6H). MS m/z : 393 $[\text{M}+1]^+$.

Example 46**3-(Butylamino)-5-(morpholinosulfonyl)-4-phenoxybenzoic acid**

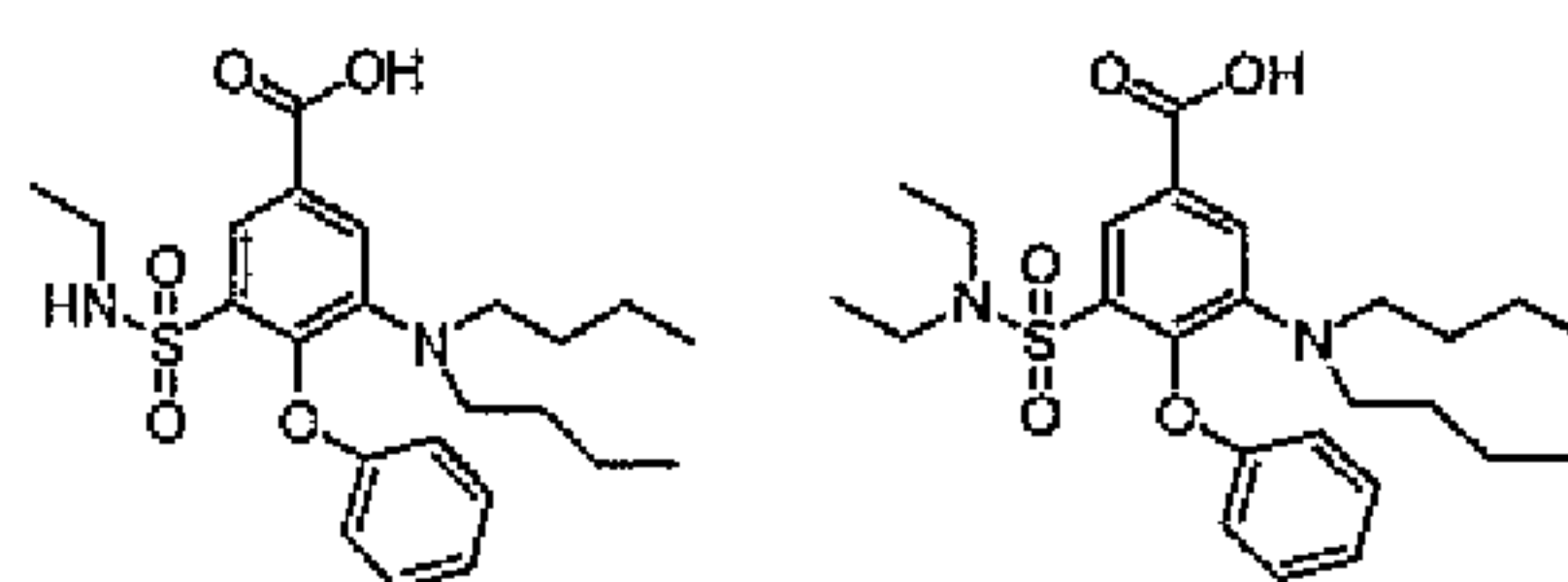
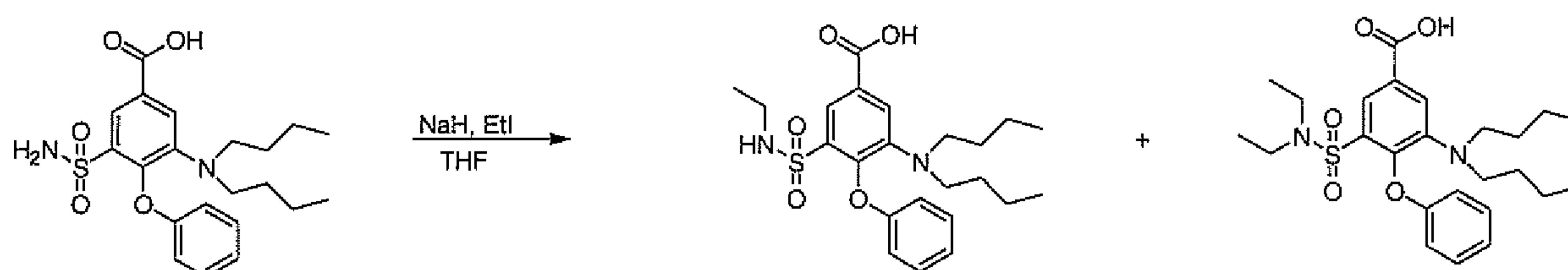
NTP-6004



The title compound was prepared in a manner similar to that used to prepare example 33. MS m/z : 435.1 $[\text{M}+1]^+$.

Example 47**3-(N-acetylsulfamoyl)-5-(dibutylamino)-4-phenoxybenzoic acid**
NTP-6005

Methyl 3-(dibutylamino)-4-phenoxy-5-sulfamoylbenzoate (0.16 g, 0.37 mmole), and Et₃N (0.1 mL, 0.74 mmole), and Ac₂O (0.04 mL, 0.44 mmole), and CH₂Cl₂ (2 mL) were charged into a flask. The mixture was stirred at room temperature for 2 hours. The reaction solution was quenched with water (10 mL). The methanol was removed on rotavapor, and the aqueous solution was extracted with EtOAc (2 X 10 mL). The organic layers were combined, dried over MgSO₄, and evaporated. The residue was purified by flash column (n-hexane/ EtOAc :1/1) to yield 0.11 g of a white solid. The solid (0.105 g, 0.221 mmol), 2N NaOH (0.22 mL, 0.442 mmol), and MeOH (2 mL) were charged into a flask. The mixture was heated to 40°C for 2 hours. The reaction solution was neutralized with 1N HCl (1 mL), and the mixture was extracted with EtOAc, dried over MgSO₄, and evaporated to yield 0.04 g of the title compound as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 8.28 (d, *J* = 2.0 Hz, 1H), 7.94 (d, *J* = 2.0 Hz, 1H), 7.35–7.20 (m, 2H), 7.10–7.02 (m, 1H), 6.80–6.70 (m, 1H), 3.20–3.02 (m, 4H), 1.54 (s, 3H), 1.40–1.15 (m, 4H), 1.10–1.09 (m, 4H), 0.79 (t, *J* = 7.2 Hz, 6H). MS *m/z*: 463 [M+1]⁺

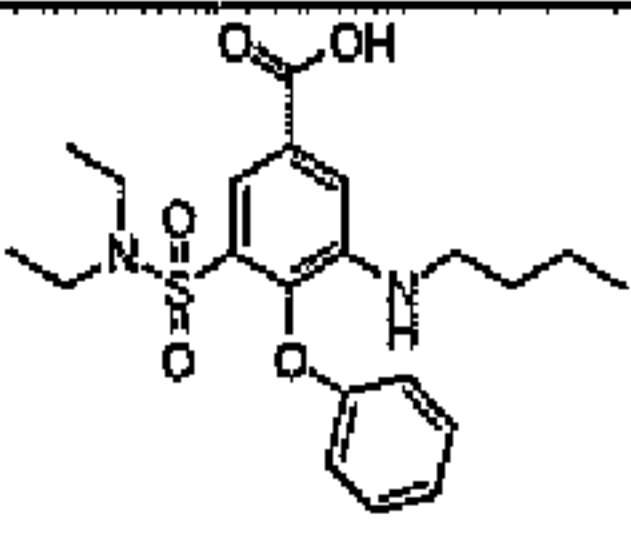
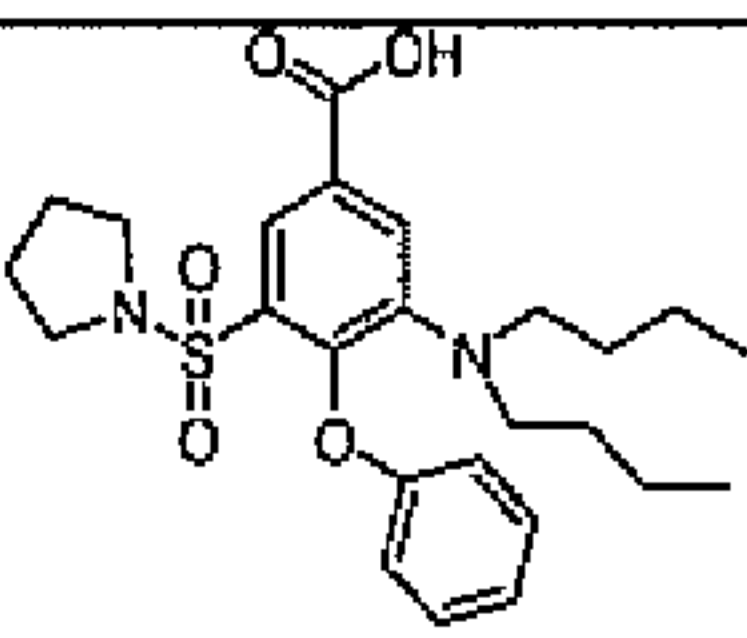
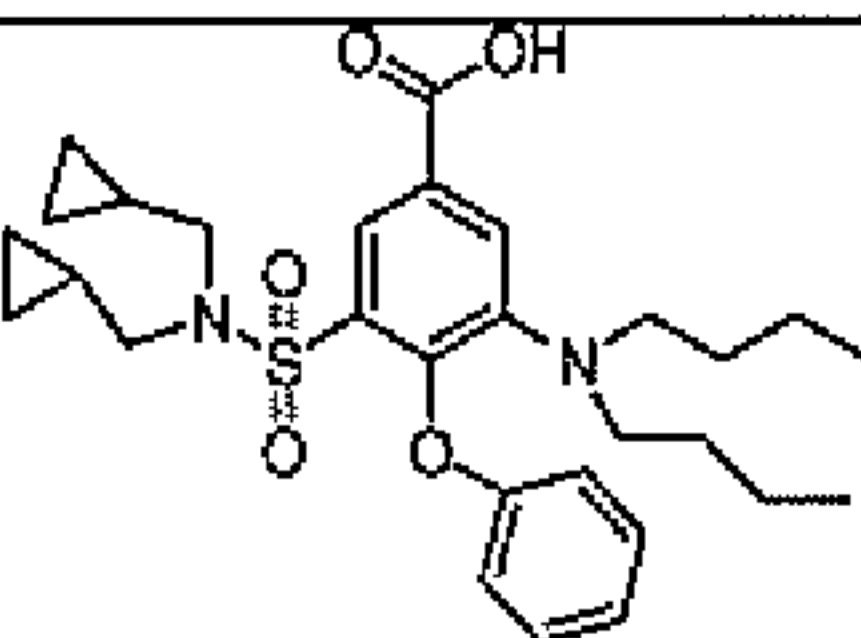
Examples 48 and 49**3-(Dibutylamino)-5-(N-ethylsulfamoyl)-4-phenoxybenzoic acid and 3-(Dibutylamino)-5-(N,N-diethylsulfamoyl)-4-phenoxybenzoic acid**
NTP-6006 and NTP-6008**General Method L**

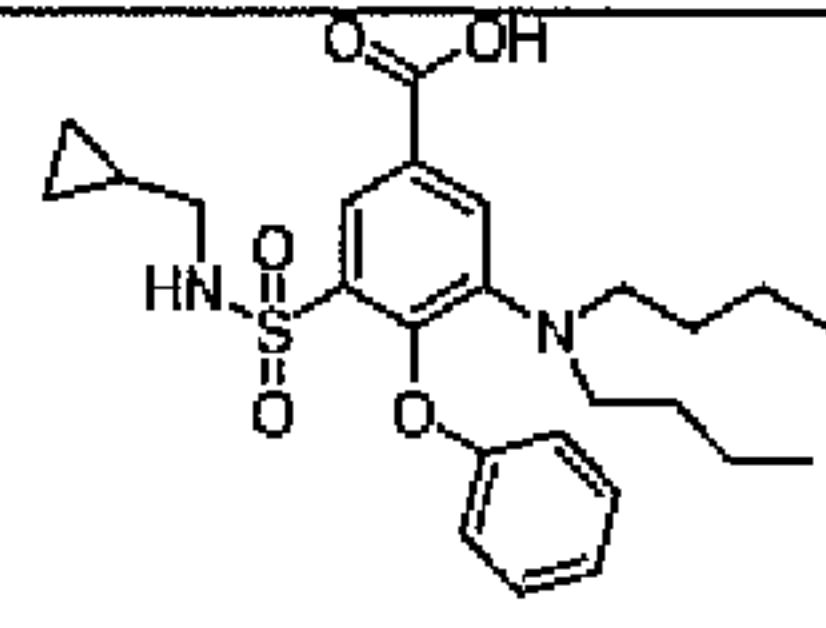
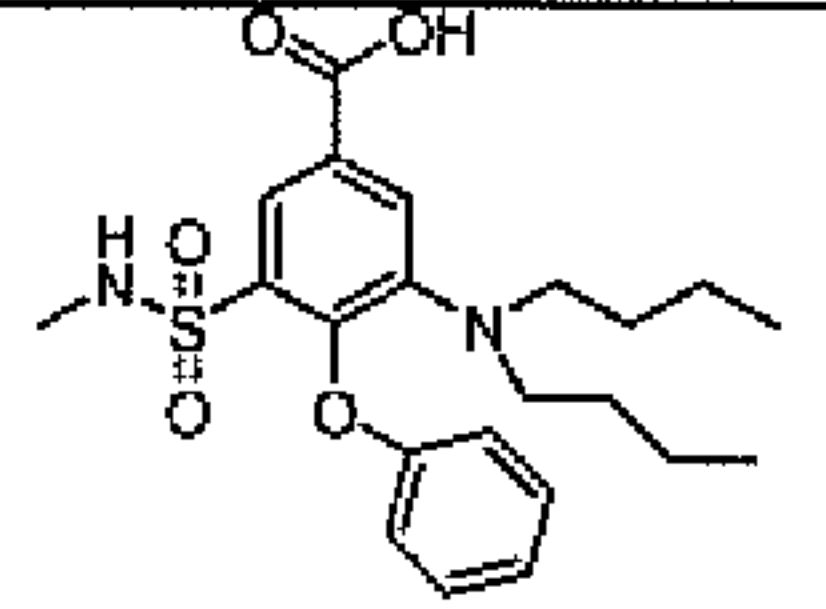
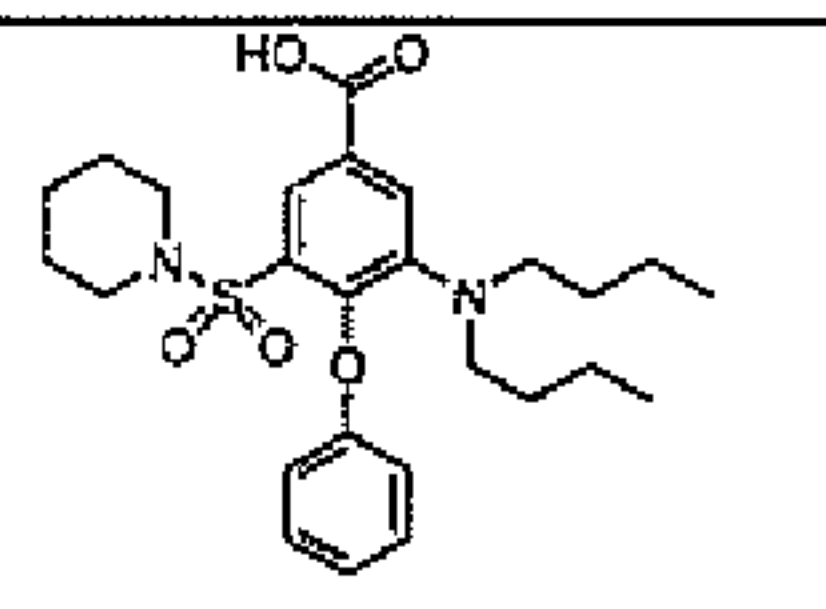
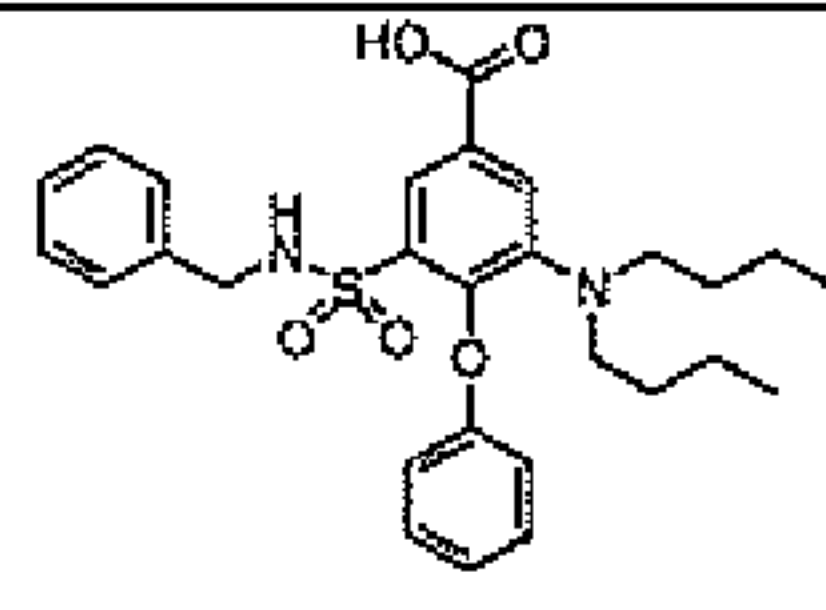
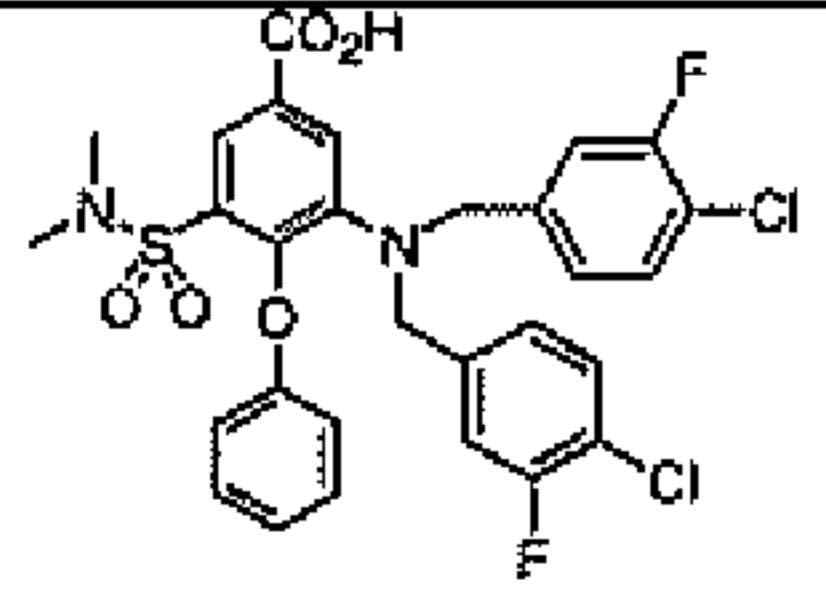
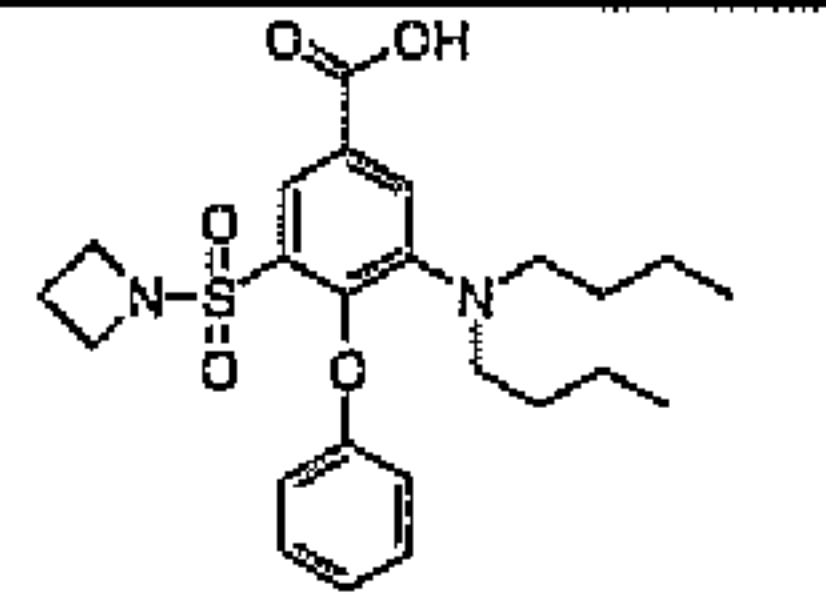
A round bottom flask was charged with 3-dibutylamino-4-phenoxy-5-sulfamoyl-benzoic acid (1.0g, 2.38 mmol), sodium hydride (95%) (171 mg, 7.134 mmol) and THF (15 mL). The reaction mixture was stirred for 15 minutes at room temperature and ethyl iodide (1.12 g, 7.134 mmol) was added and the reaction mixture was heated to 50°C overnight. The reaction mixture showed diethyl, mono ethyl and ethyl ester products by LCMS. The reaction mixture was quenched by addition of water and extracted with ethyl acetate. The organic layer was then washed with 3N HCl, water, brine and dried over Na₂SO₄. The solvent was removed under reduced pressure and the residue purified by flash chromatography to give 3-(dibutylamino)-5-(N-ethylsulfamoyl)-4-phenoxybenzoic acid (28 mg) and 3-(dibutylamino)-5-(N,N-diethylsulfamoyl)-4-phenoxybenzoic acid (132 mg) as white solids.

3-(Dibutylamino)-5-(N-ethylsulfamoyl)-4-phenoxybenzoic acid: MS m/z : 449 [M+1]⁺.

3-(dibutylamino)-5-(N,N-diethylsulfamoyl)-4-phenoxybenzoic acid: MS m/z : 477.2 [M+1]⁺.

Following General Method L or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art the following examples were prepared from commercially available reagents.

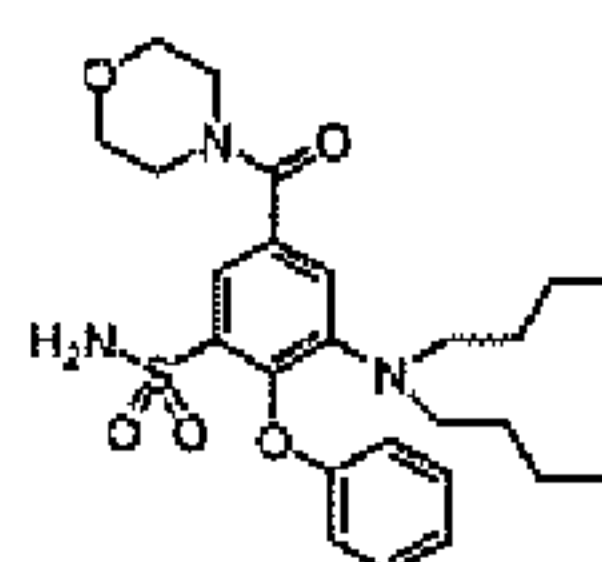
Example Number	NTP-Number	Structure	M.W. calculated	Mass Spec. m/z [M+1] ⁺
50	NTP-6007		420.52	421
51	NTP-6009		474.61	475
52	NTP-6010		528.70	529

53	NTP-6011		474.61	475
54	NTP-6012		434.55	435
55	NTP-6013		488.64	489
56	NTP-6014		510.64	511
57	NTP-6015		621.48	622
58	NTP-6016		460.59	

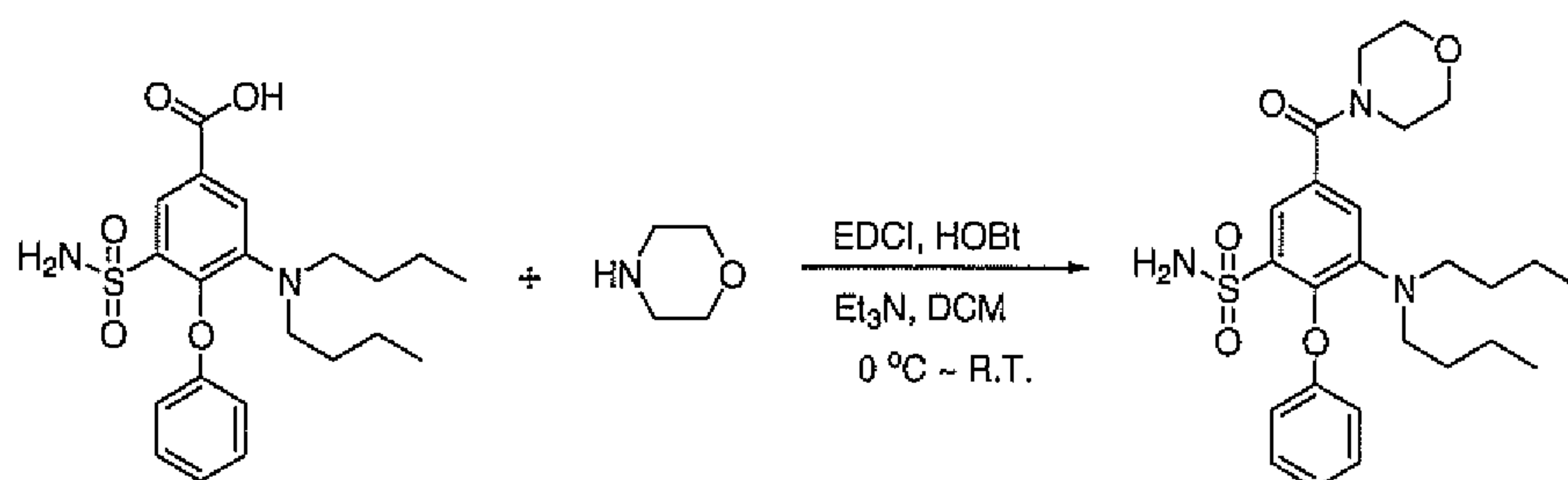
Example 59

3-(Dibutylamino)-5-(morpholine-4-carbonyl)-2-phenoxybenzenesulfonamide

NTP-3032



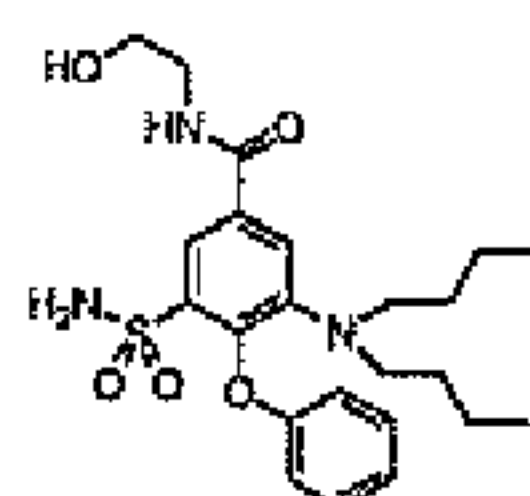
General Method M



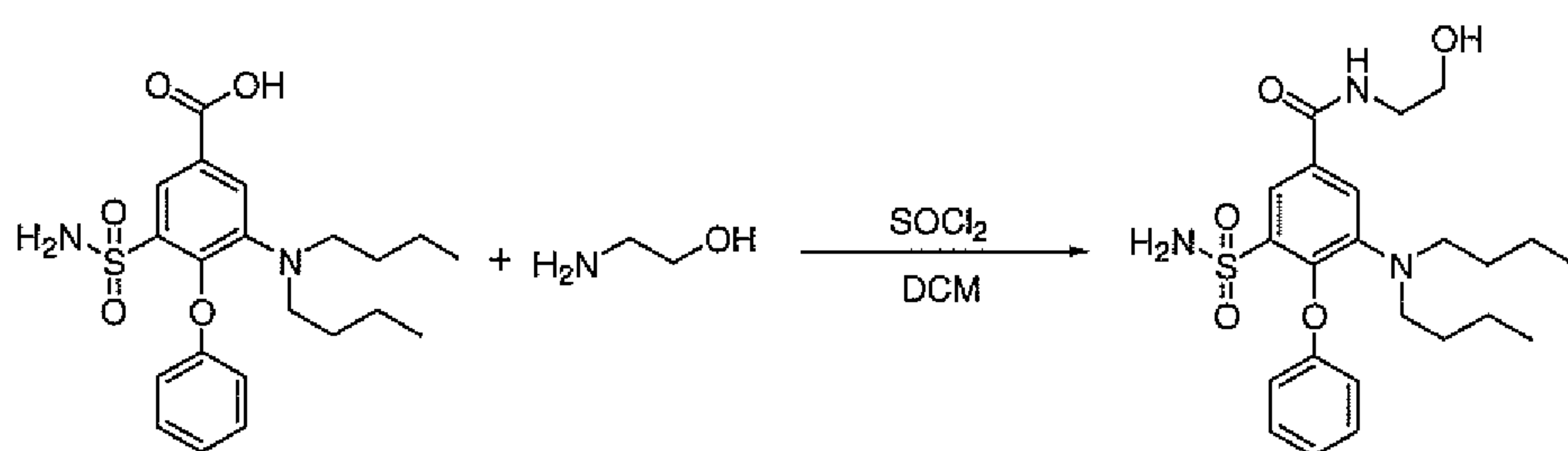
To a solution of 3-(dibutylamino)-4-phenoxy-5-sulfamoylbenzoic acid (100 mg, 0.238 mmol) in dichloromethane was added morpholine (41 mg, 0.476 mmol), triethylamine (48 mg, 0.476 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (54.7 mg, 0.286 mmol), and 1-hydroxybenzotriazole (48 mg, 0.357 mmol) at 0°C. The reaction was then stirred at 50°C for 1.5 hour, and quenched with a saturated aqueous solution of sodium bicarbonate. The resulting mixture was extracted with dichloromethane. The combined organic extracts were washed with 0.1 M solution of hydrochloric acid and a saturated aqueous solution of sodium bicarbonate, then dried over anhydrous MgSO_4 and concentrated in *vacuo*. The crude product was purified by flash chromatography (*n*-Hexane/Ethyl Acetate=5/1) to afford the title compound (70 mg).

^1H NMR (400 MHz, $\text{CD}_3\text{OD}-d_4$) δ 7.54 (s, 1H), 7.30 (s, 1H), 7.22 (t, $J = 8.0$ Hz, 2H), 7.00 (t, $J = 8.0$ Hz, 1H), 6.83 (d, $J = 8.0$ Hz, 2H), 3.81–3.43 (m, 8H), 3.08 (t, $J = 7.2$ Hz, 4H), 2.26–1.86 (m, 4H), 1.07–1.00 (m, 4H), 0.77 (t, $J = 7.2$ Hz, 6H). MS m/z : 490 $[\text{M}+1]^+$.

Example 60
3-(Dibutylamino)-N-(2-hydroxyethyl)-4-phenoxy-5-sulfamoylbenzamide
 NTP-3033



General Procedure N



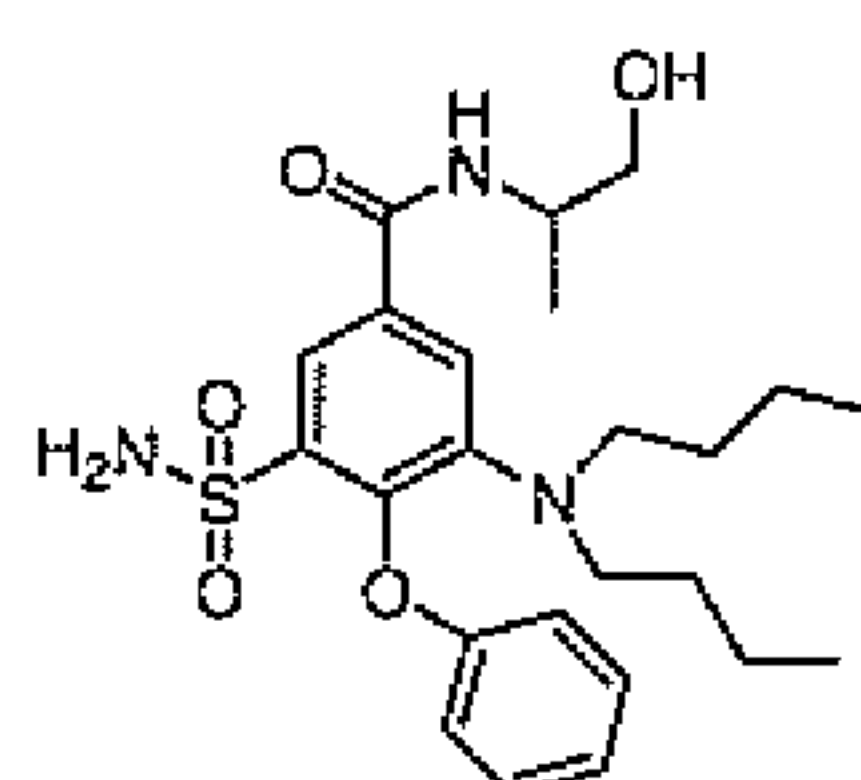
3-(Dibutylamino)-4-phenoxy-5-sulfamoylbenzoic acid (100 mg, 0.24 mmol) and dried dichloromethane (3 mL) were placed in 25 mL round-bottom flask with a magnetic stirring bar. Thionyl chloride (0.05 mL, 0.713 mmol) was added drop wise into the above solution at 0°. The mixture was stirred at room temperature for 1 hour. The solvent and excess thionyl chloride were removed on a rotary evaporator. Dichloromethane (3 mL) and 2-aminoethanol (29 mg, 0.48 mmol) were added to the residue at 0°C. The solution was kept stirring at room temperature for 1 hour. The solvent was removed and the crude product was purified by flash chromatography (*n*-

Hexane/Ethyl Acetate=5/1) to afford the title compound (40 mg). ^1H NMR (400 MHz, $\text{CD}_3\text{OD}-d_4$) δ 8.02 (d, $J=3.2$ Hz, 1H), 7.75 (d, $J=3.2$ Hz, 1H), 7.24 (t, $J=8.0$ Hz, 2H), 7.00 (t, $J=8.0$ Hz, 1H), 6.83 (d, $J=8.0$ Hz, 2H), 3.72 (t, $J=7.6$ Hz, 2H), 3.52 (t, $J=8.0$ Hz, 2H), 3.09 (t, $J=8.0$ Hz, 4H), 1.27–1.20 (m, 4H), 1.06–0.96 (m, 4H), 0.77 (t, $J=7.2$ Hz, 6H). MS m/z : 464 $[\text{M}+1]^+$.

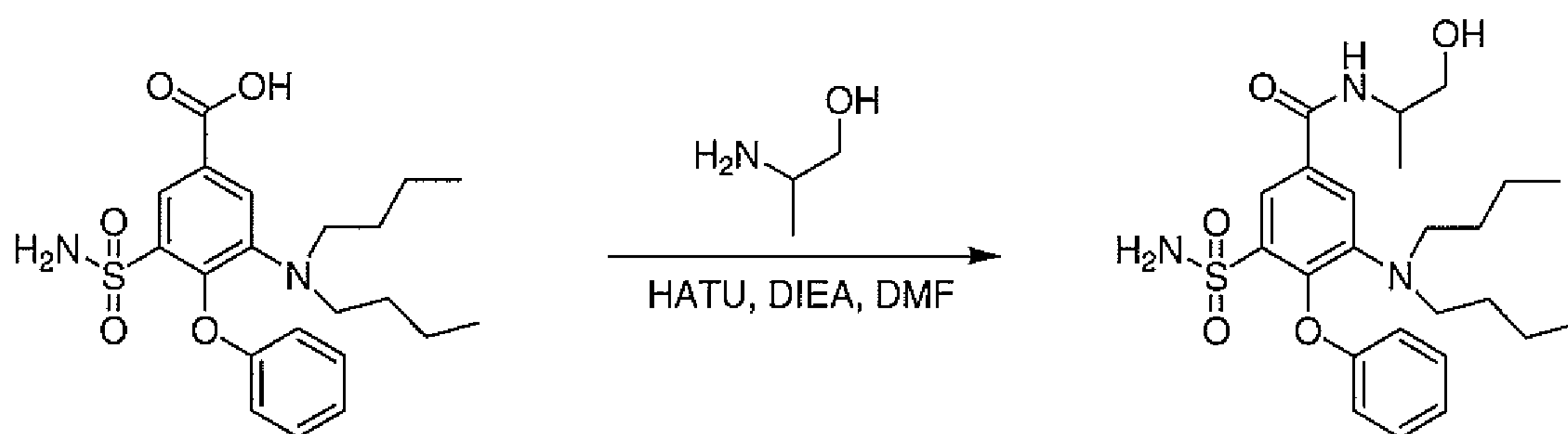
Example 61

3-(Dibutylamino)-N-(1-hydroxypropan-2-yl)-4-phenoxy-5-sulfamoylbenzamide

NTP-3045



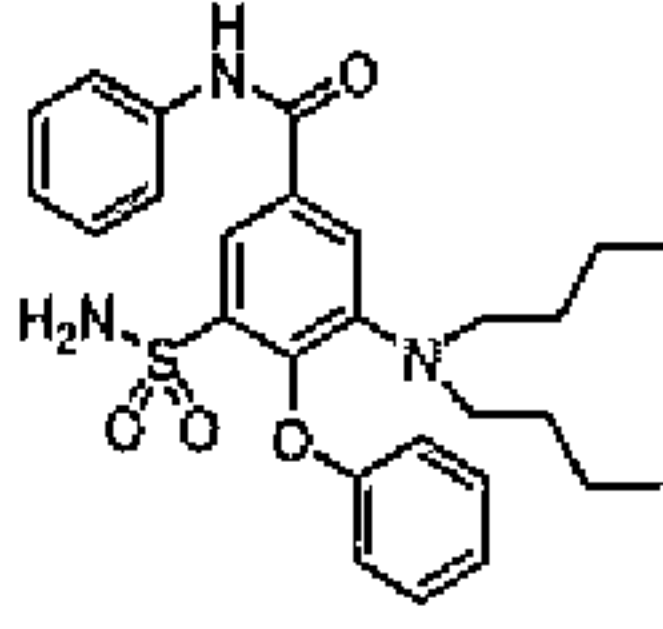
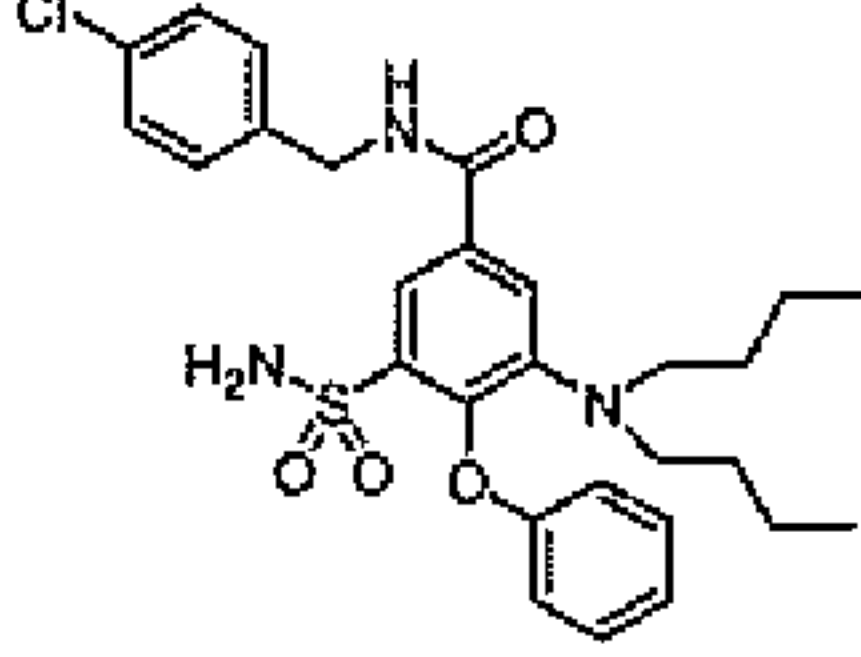
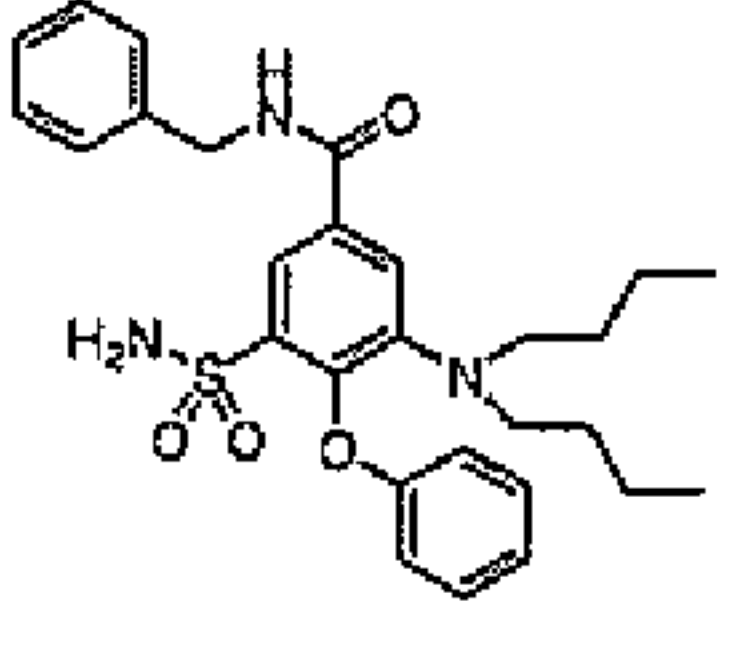
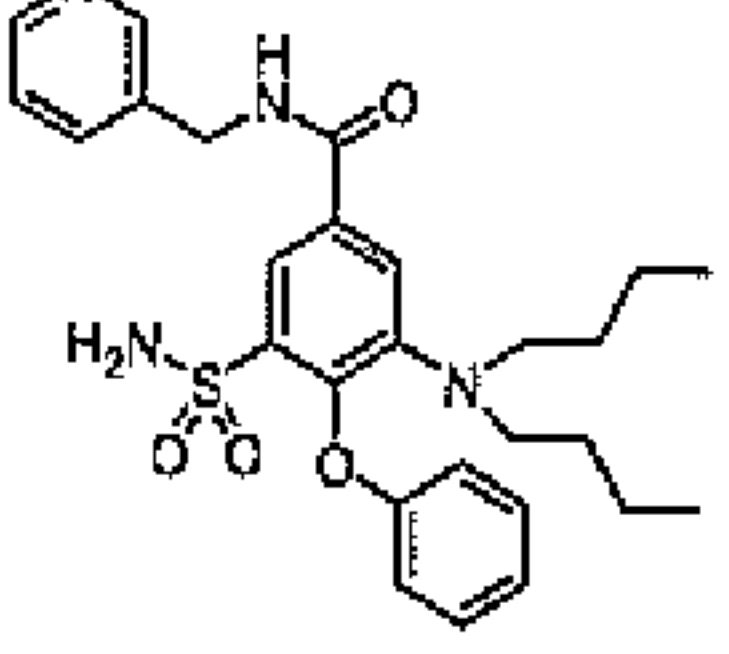
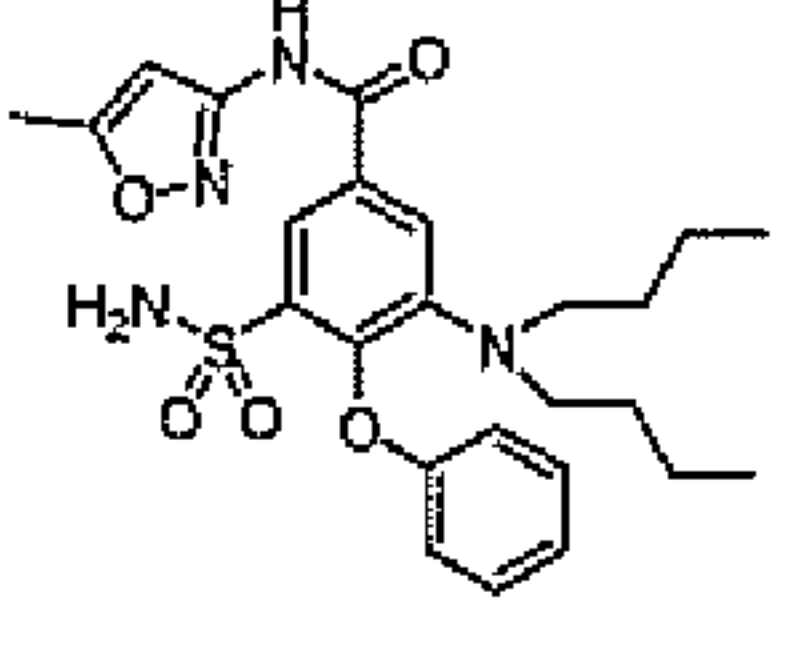
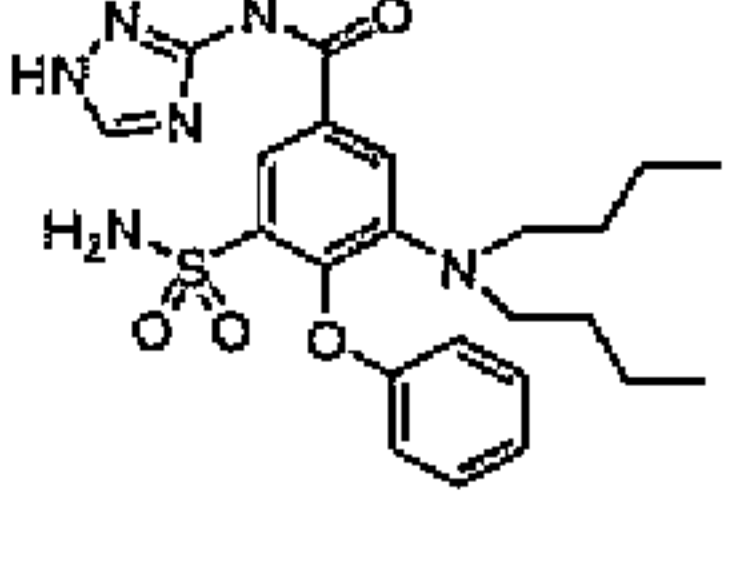
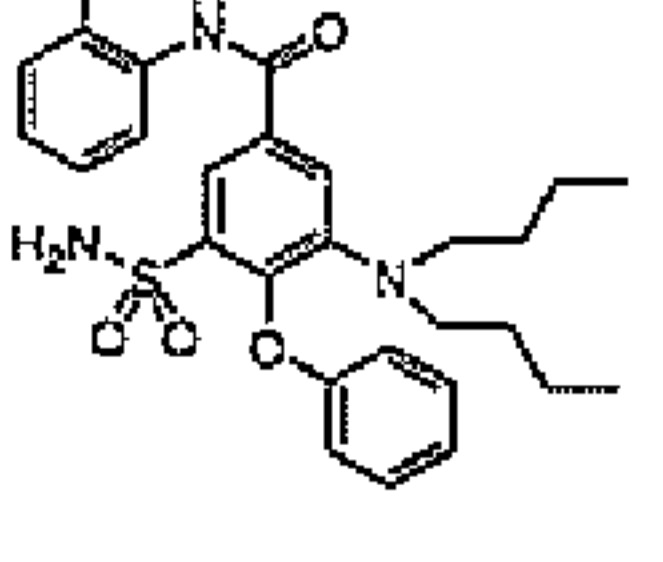
General Method O

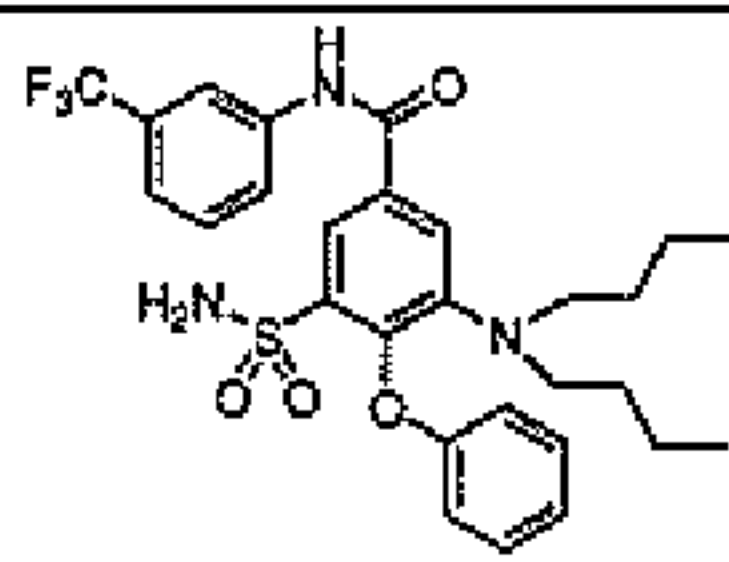
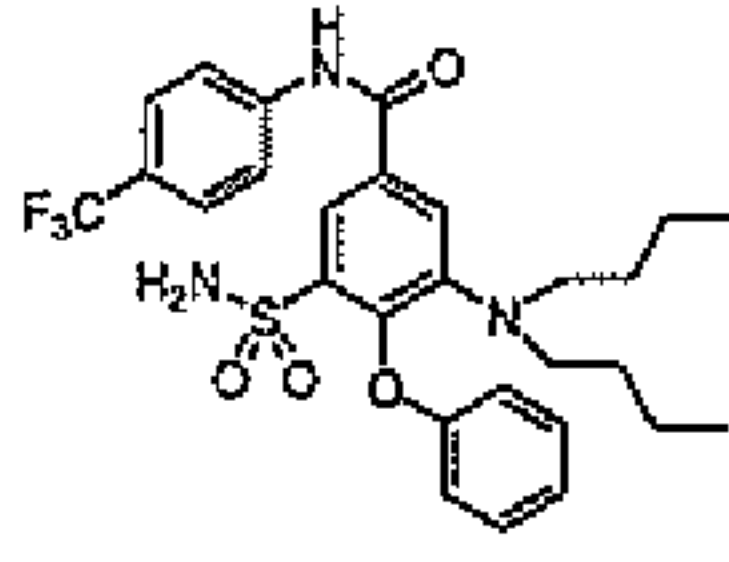
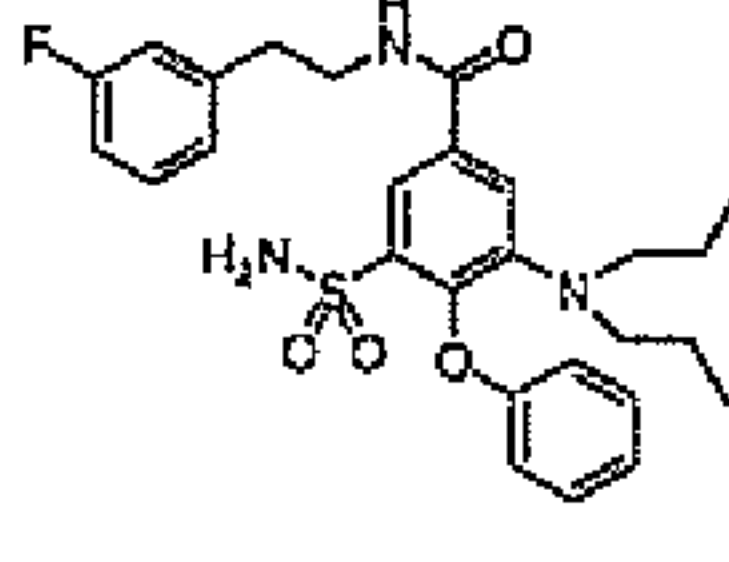
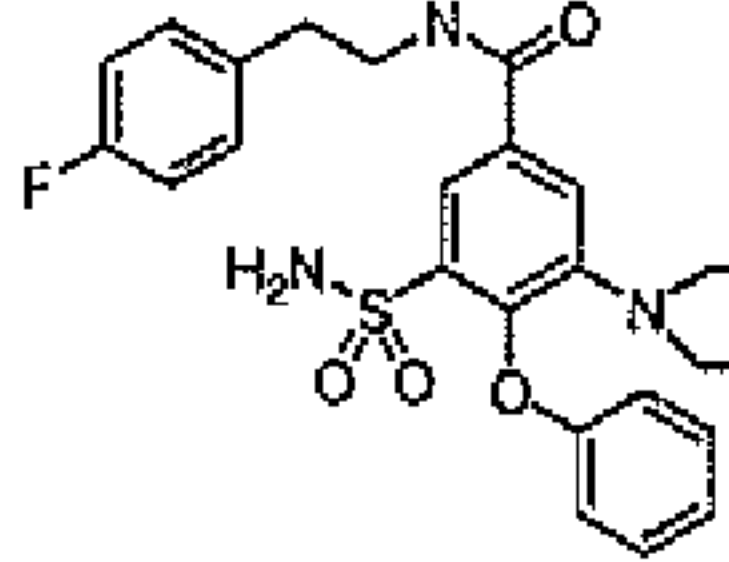
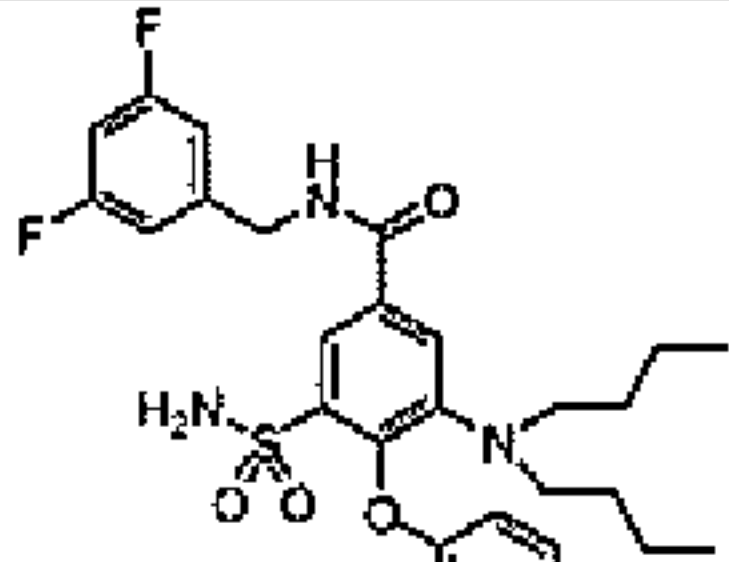
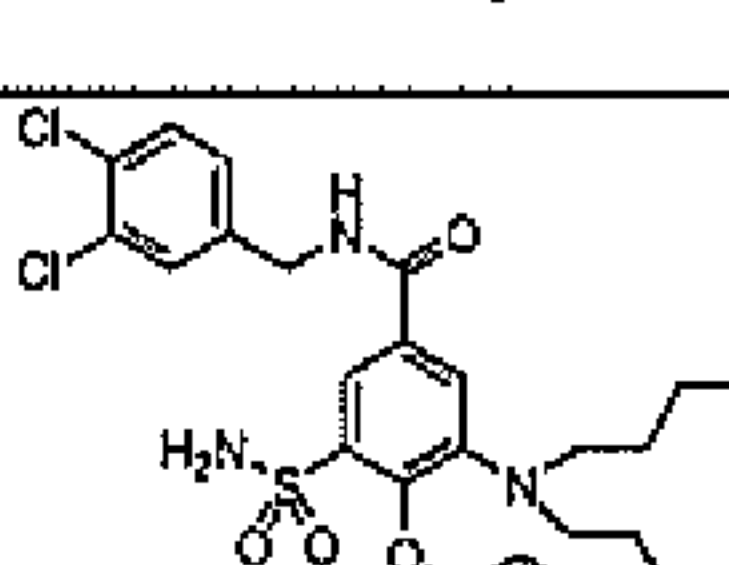
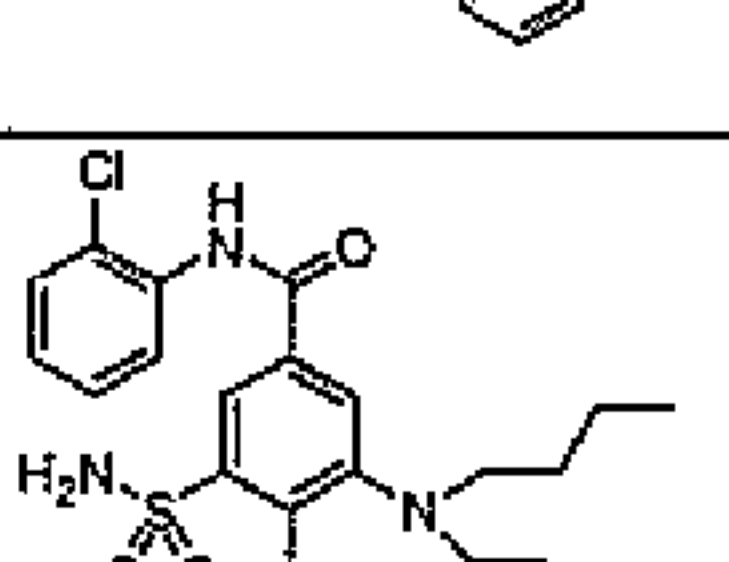
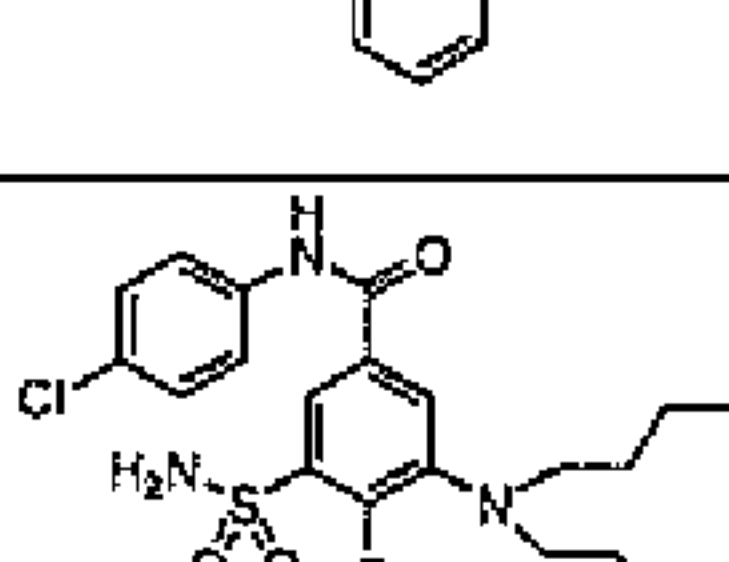
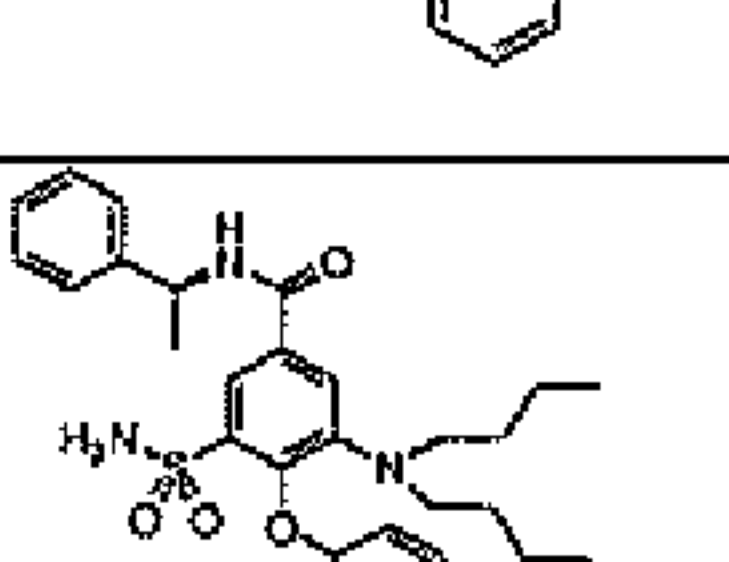
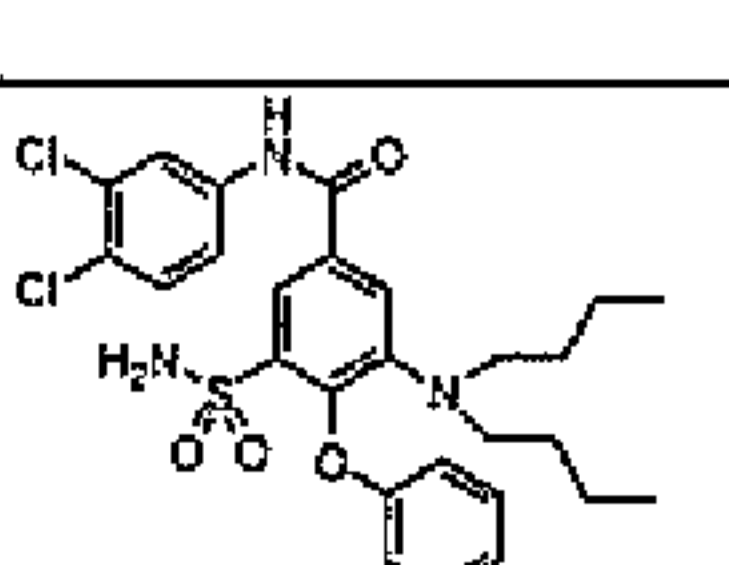


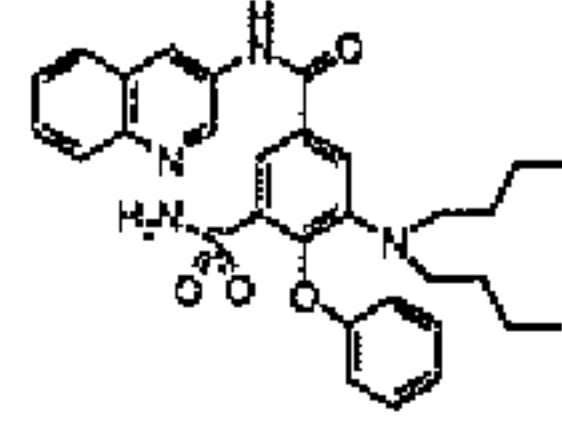
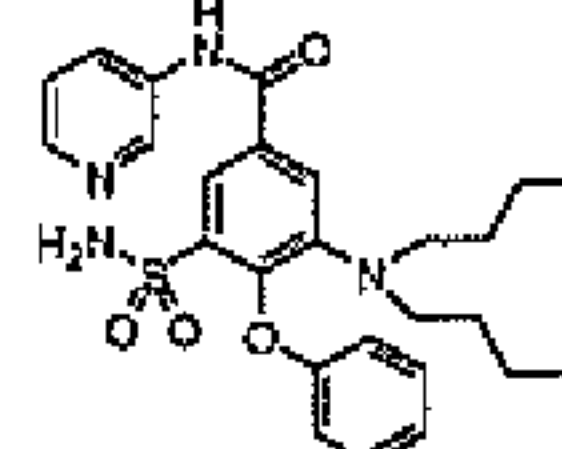
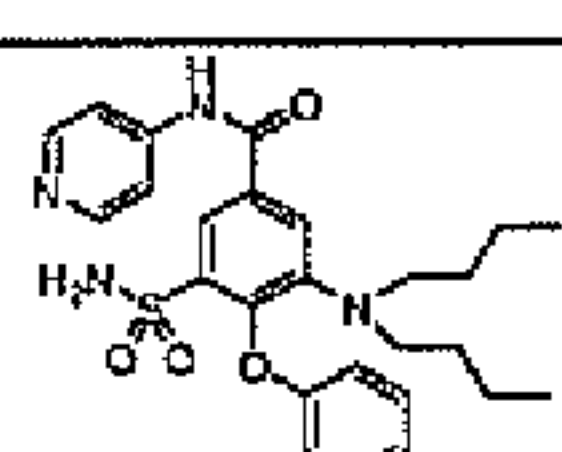
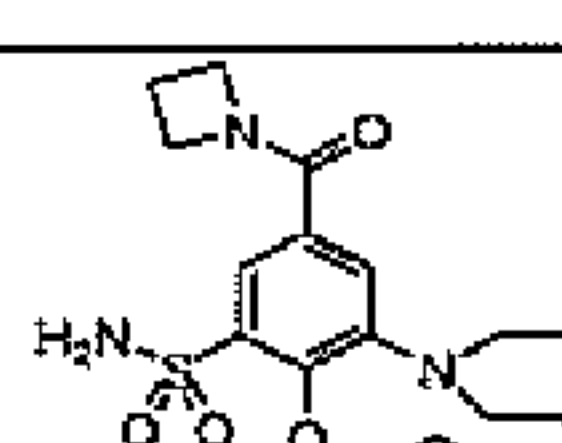
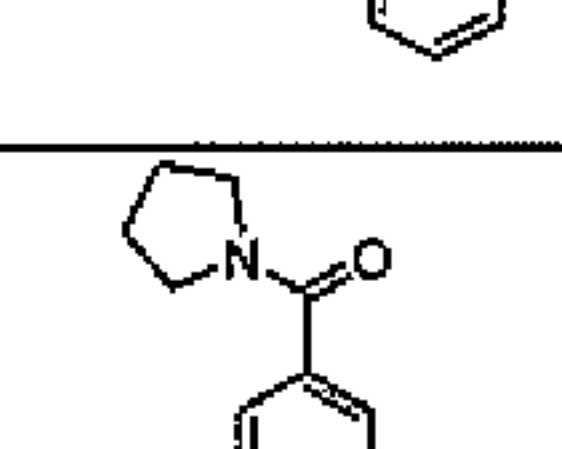
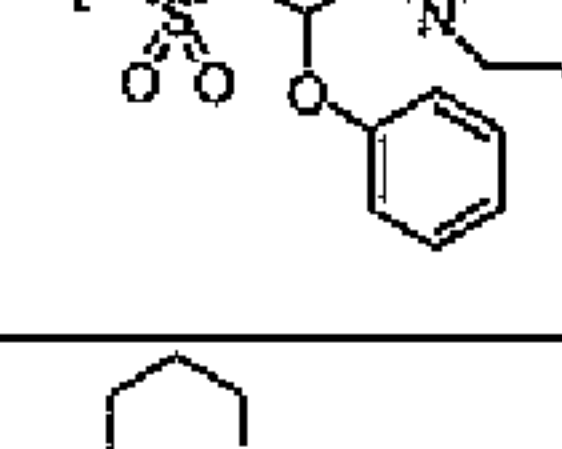
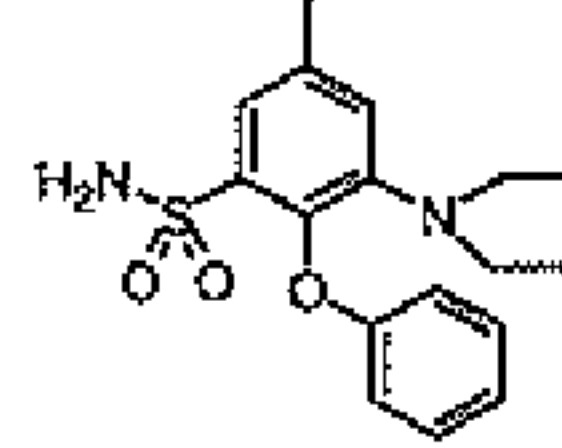
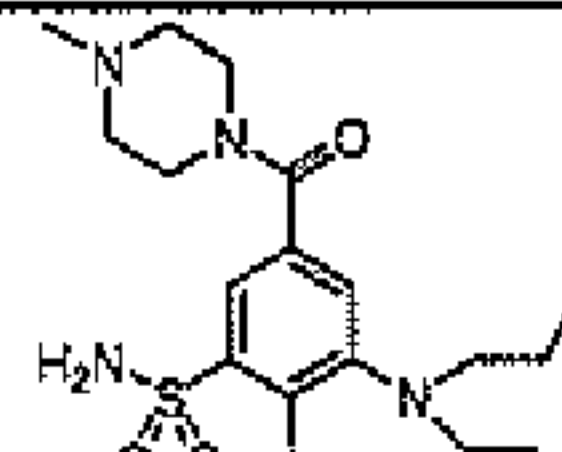
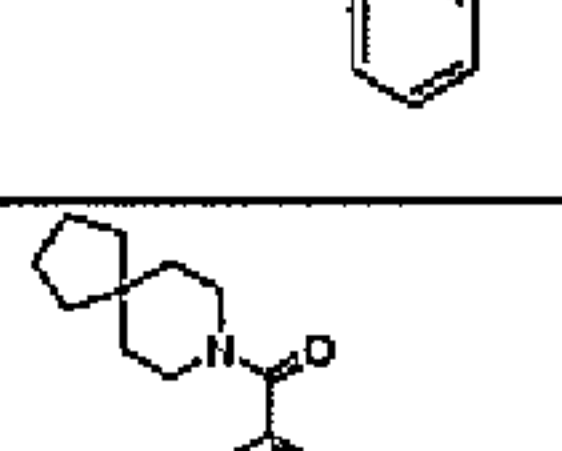
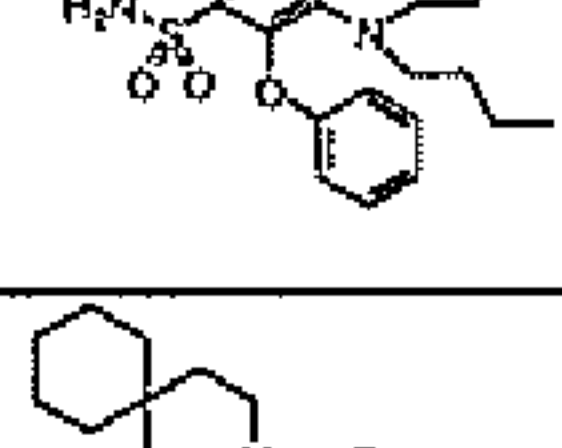
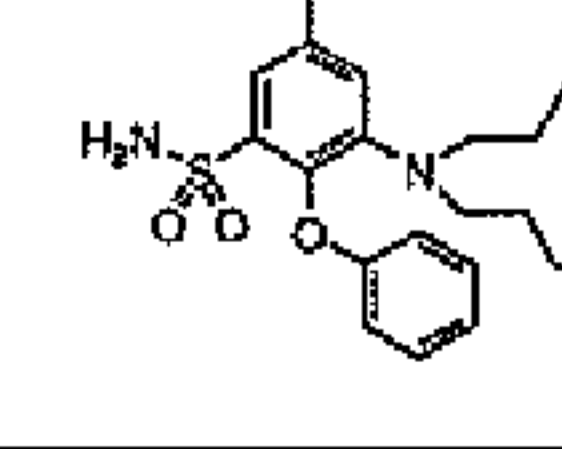
A reaction vial was charged with 3-dibutylamino-4-phenoxy-5-sulfamoyl-benzoic acid (70 mg, 0.166 mmol), 2-amino-1-propanol (15 mg, 0.1999 mmol), *O*-(7-azabenzotriazol-1-yl)-*N,N,N,N*-tetramethyl uranium hexafluorophosphate (76 mg, 0.199 mmol), *N,N*-diisopropyl ethyl amine (33 μL , 0.199 mmol), DMF (2 mL) and the mixture was stirred at room temperature overnight. The reaction was diluted with ethyl acetate and washed with water, brine and dried over Na_2SO_4 . The solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as white solid (69 mg). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 8.29 (d, $J=8.1$ Hz, 1H), 7.94 (d, $J=2.1$ Hz, 1H), 7.67 (d, $J=1.8$ Hz, 1H), 7.25 (s, 2H), 7.20 (t, $J=7.2$ Hz, 2H), 6.96 (t, $J=7.2$ Hz, 1H), 6.73 (d, $J=8.1$ Hz, 2H), 4.75 (t, $J=6.00$ Hz, 1H), 4.06–4.401 (m, 1H), 3.51–3.22 (m, 6H), 3.00 (t, $J=7.2$ Hz, 4H), 1.19–1.09 (m, 4H), 1.01–0.89 (m, 4H), 0.72 (t, $J=7.5$ Hz, 6H). MS m/z : 478 $[\text{M}+1]^+$.

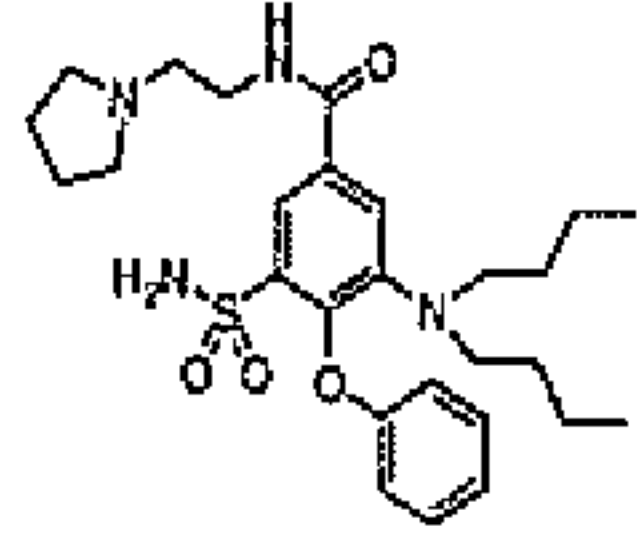
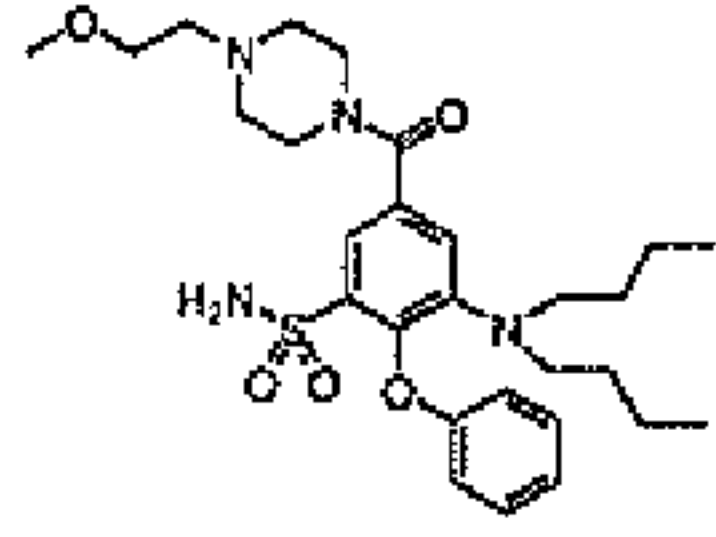
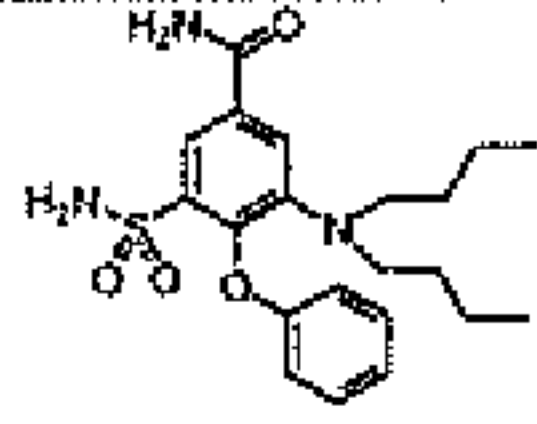
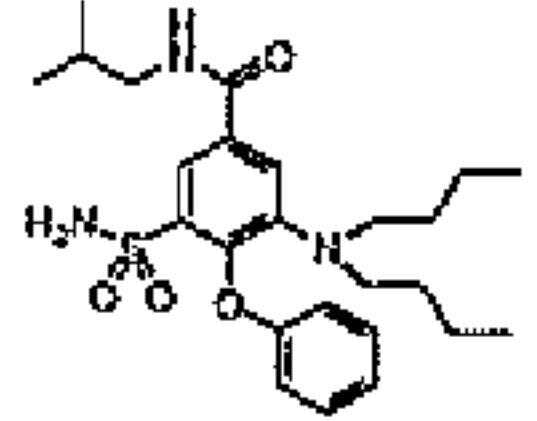
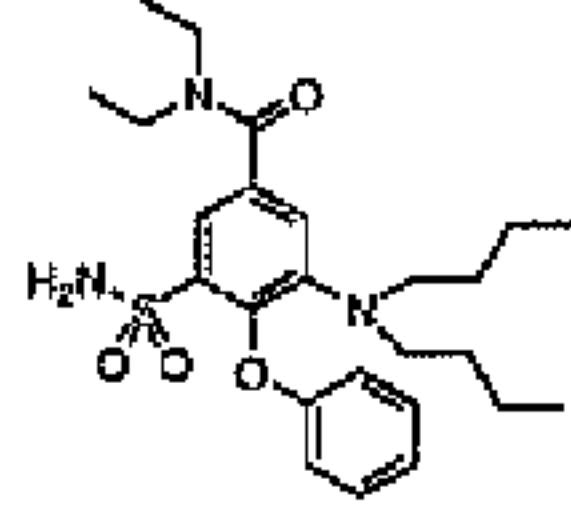
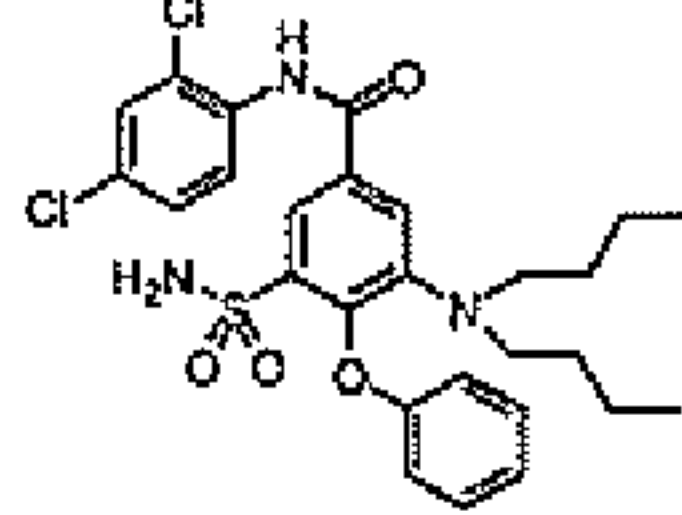
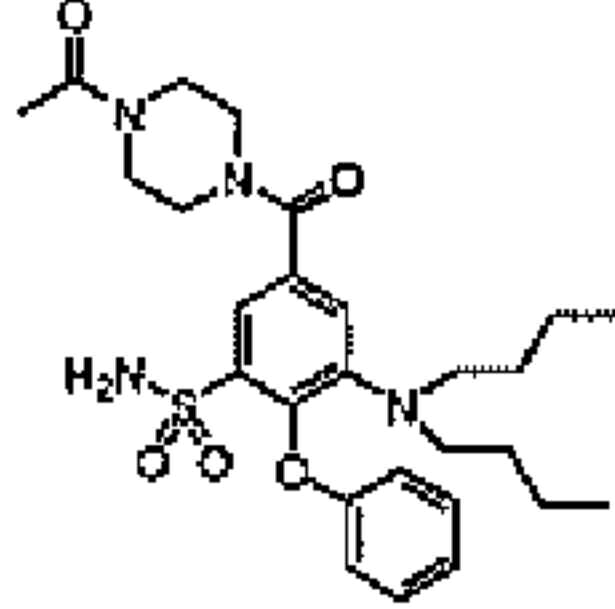
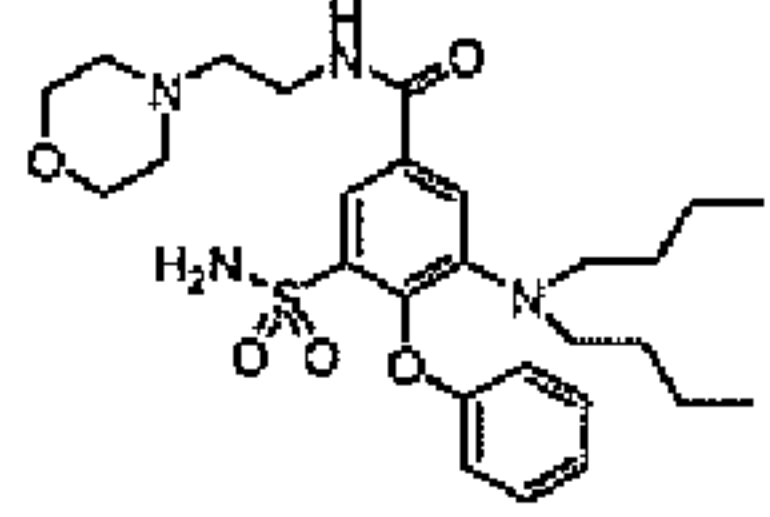
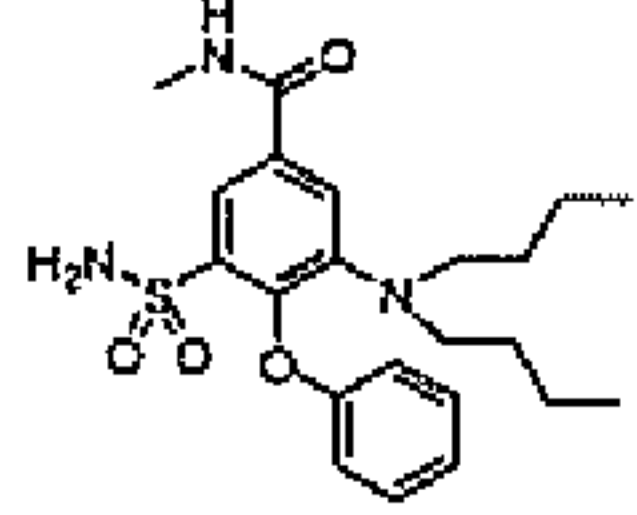
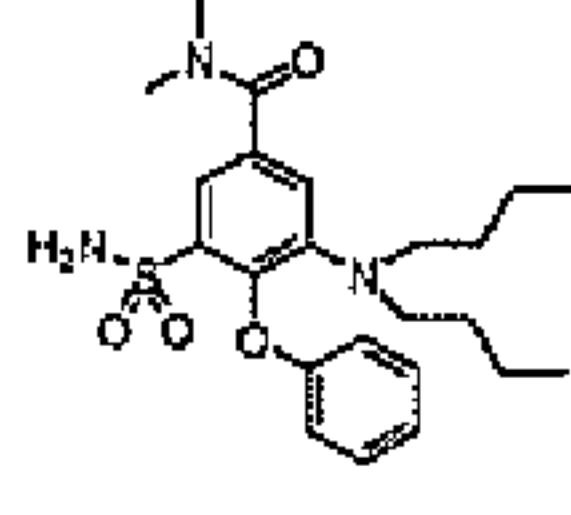
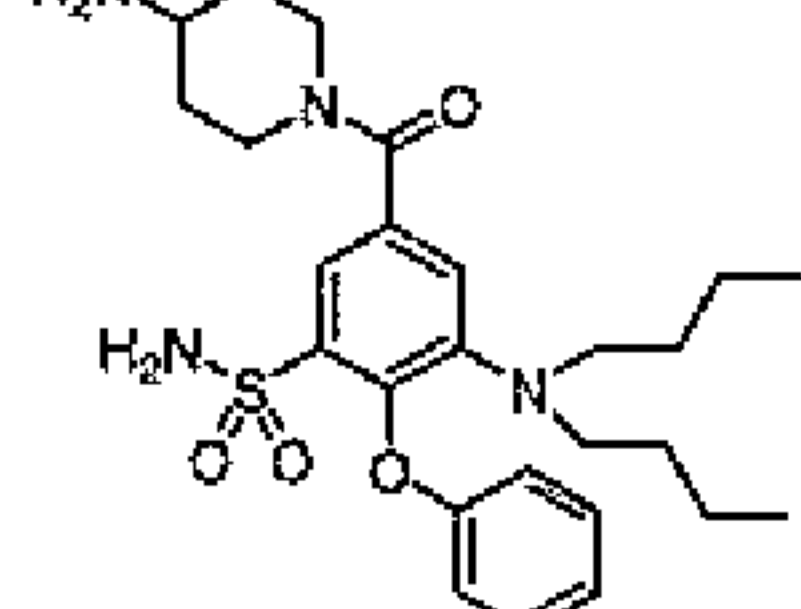
Following General Methods M through O or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples were prepared from

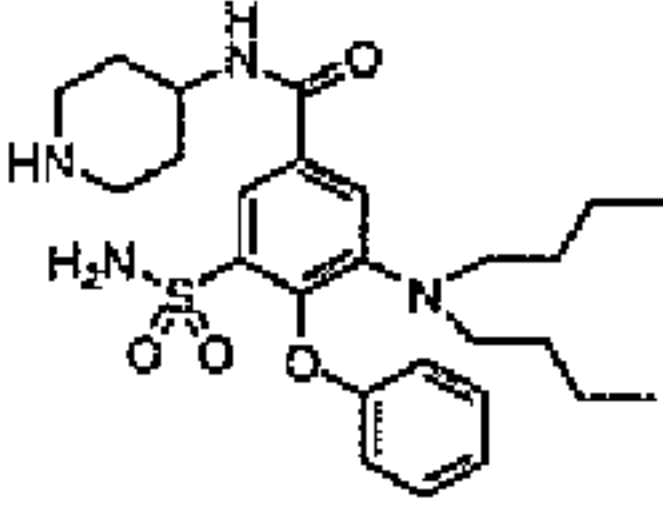
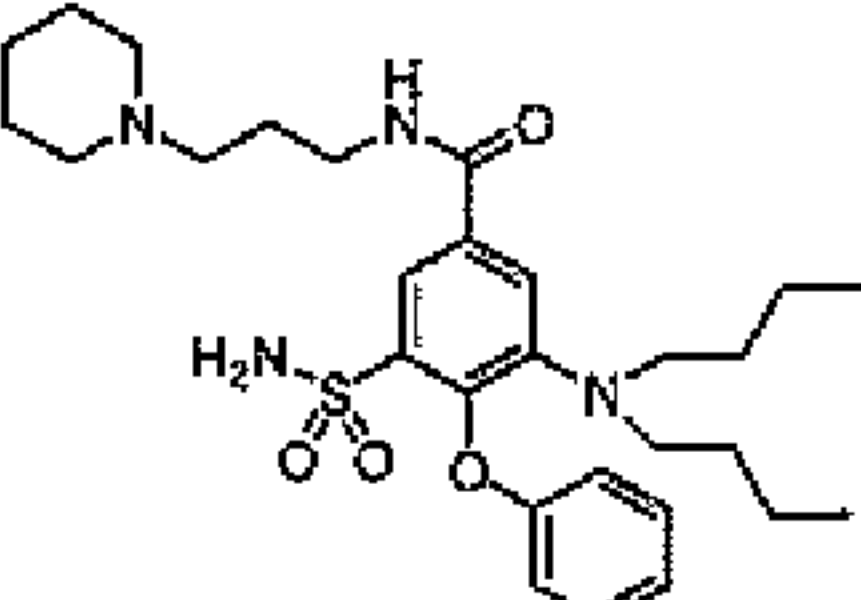
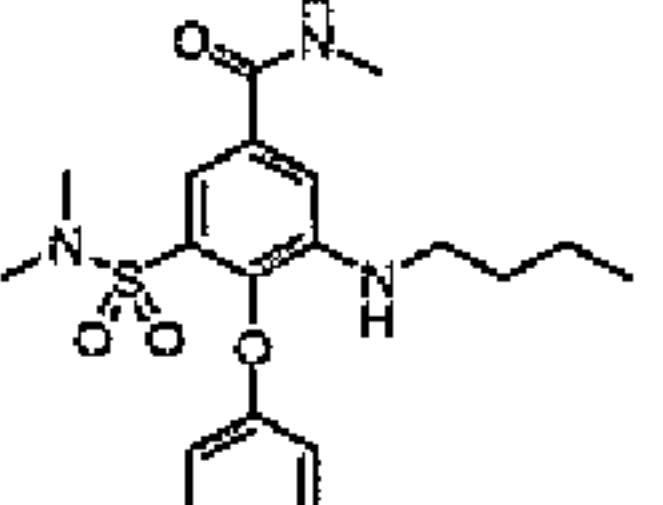
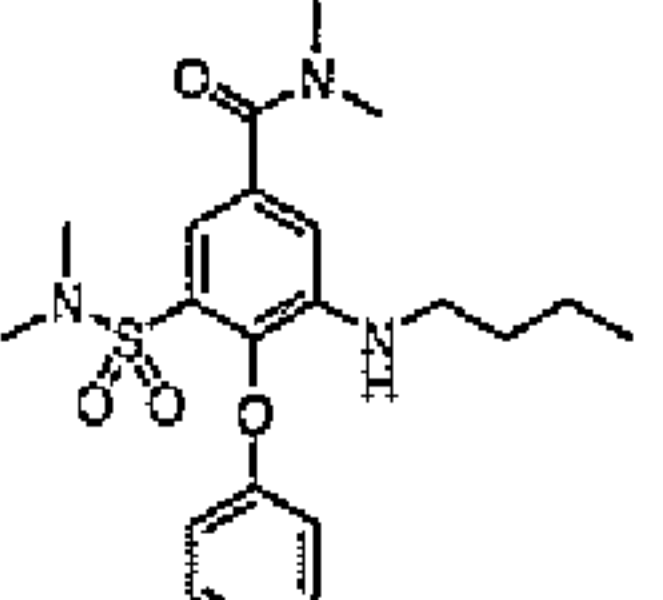
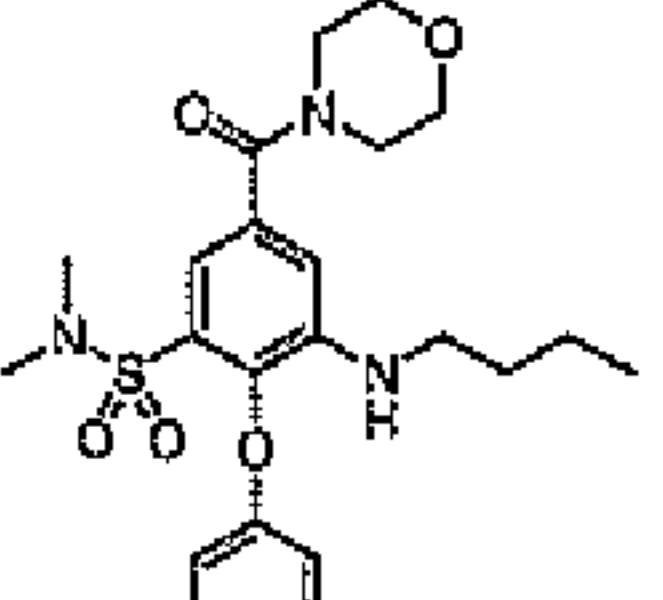
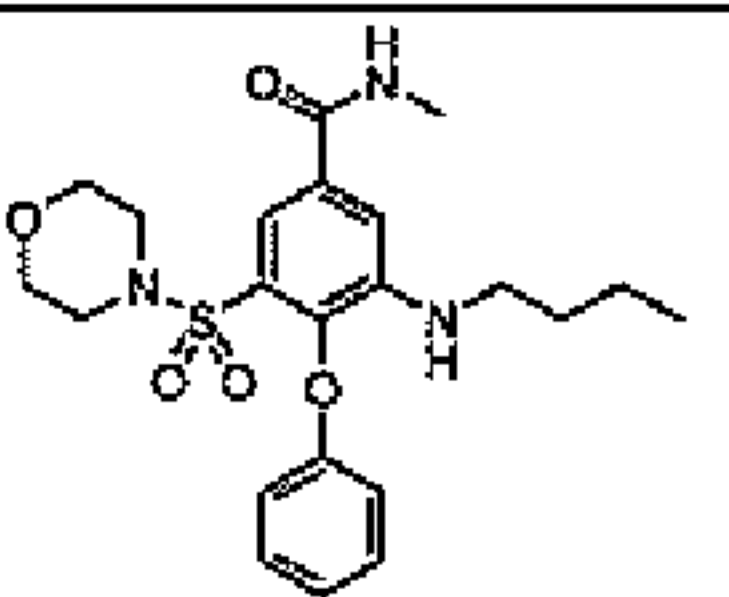
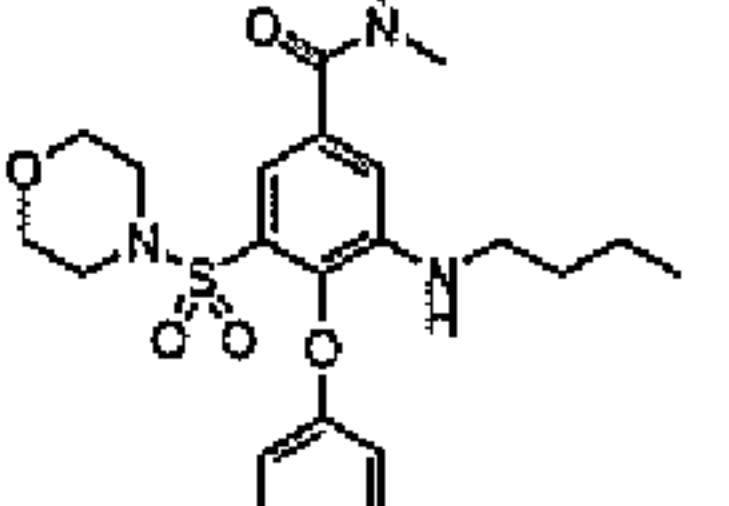
benzoic acids which can be prepared following General Methods A through P and commercially available reagents.

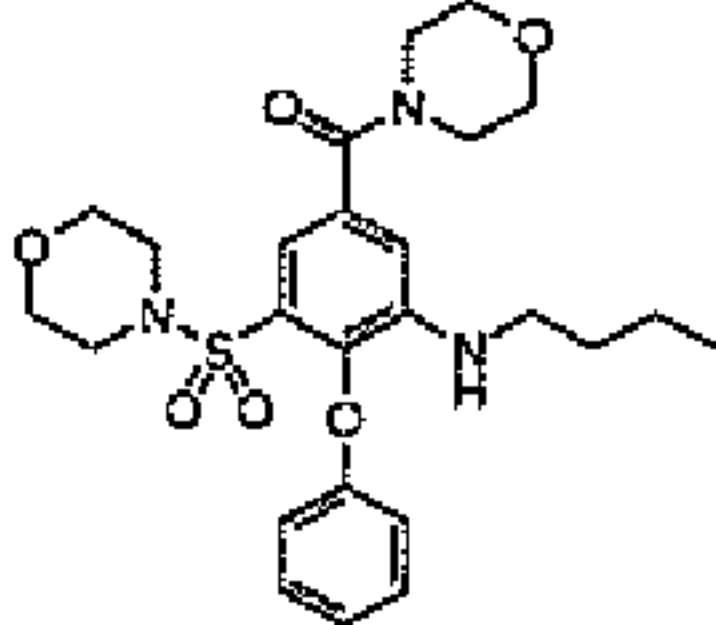
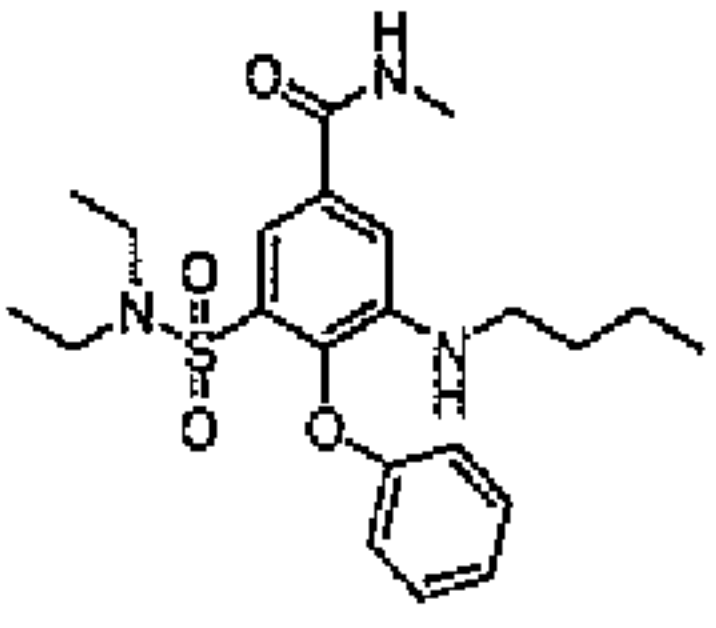
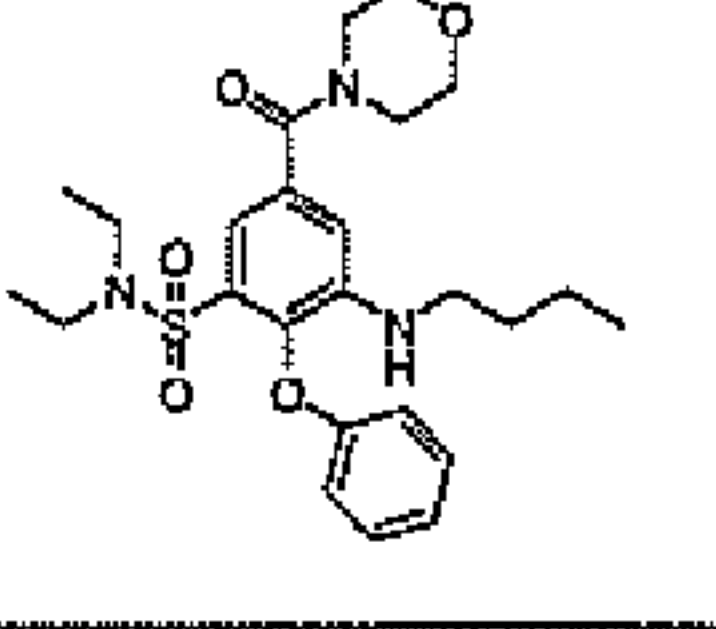
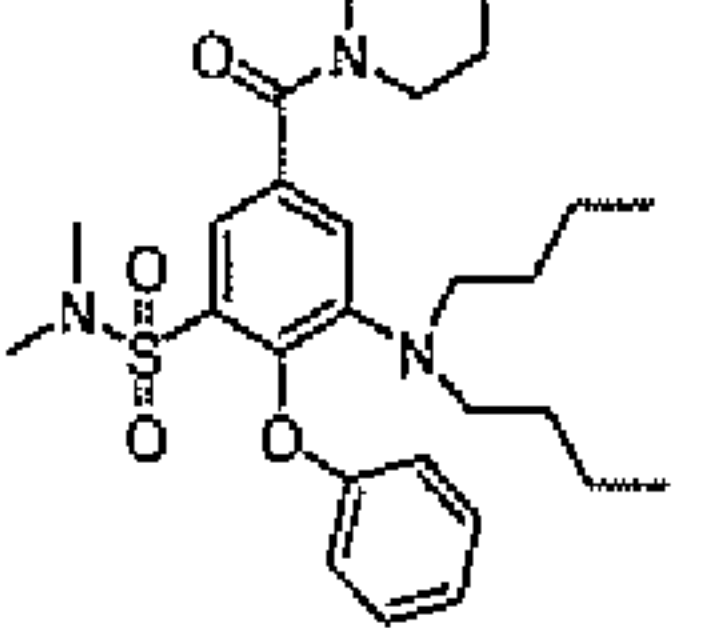
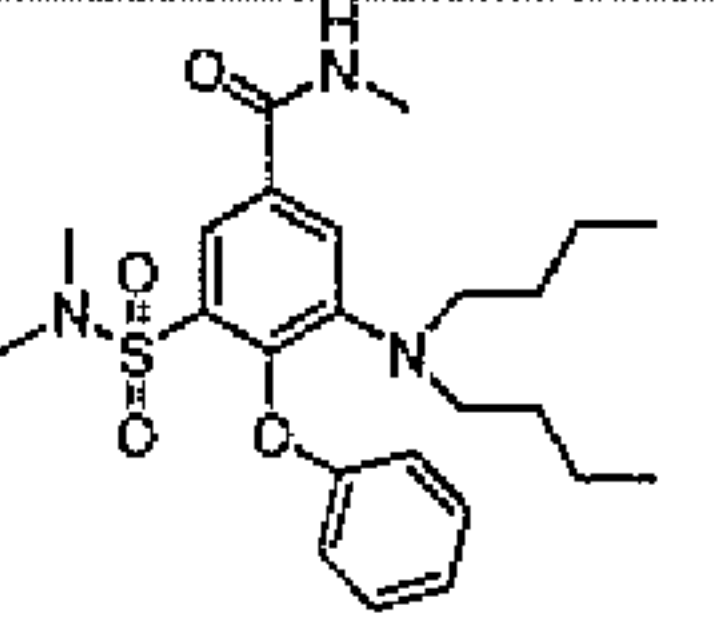
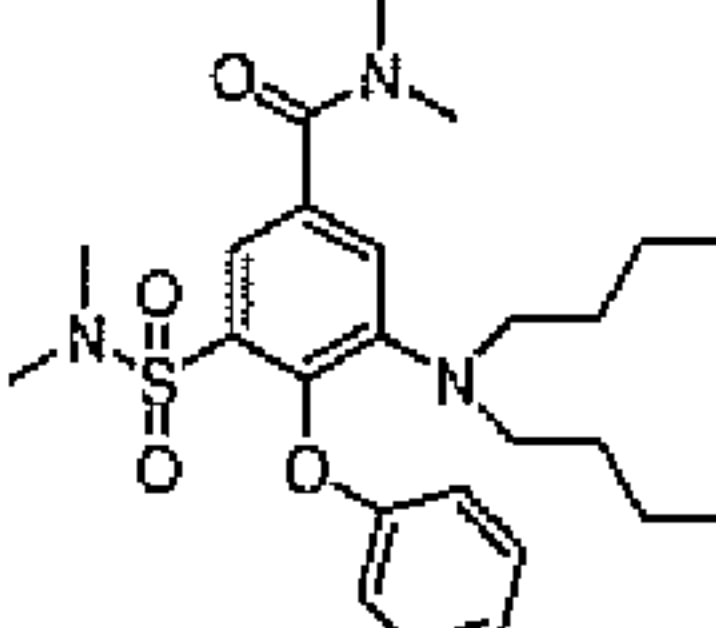
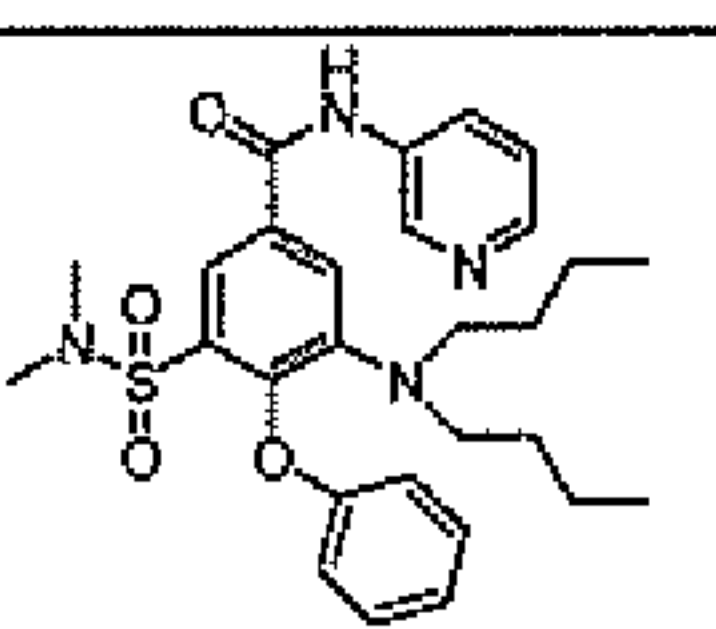
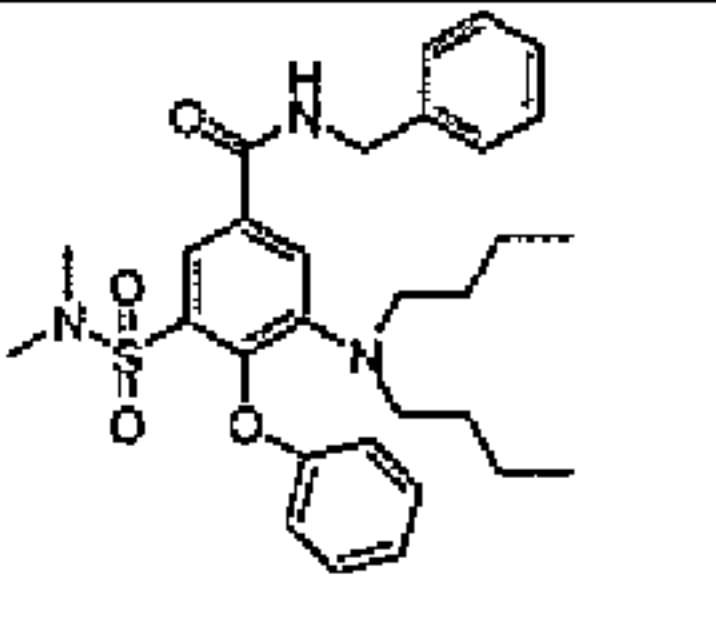
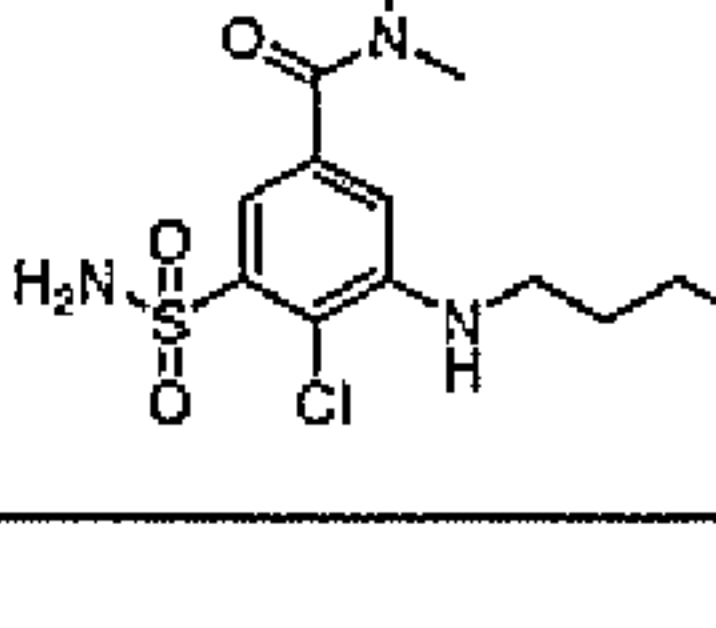
Example Number	NTP Number	General Method	Structure	M.W. calculated	Mass Spec. found <i>m/z</i>
62	NTP-3001	M		495.63	496[M+1] ⁺
63	NTP-3002	M		544.11	544
64	NTP-3003	M		509.66	510[M+1] ⁺
65	NTP-3005	M		510.65	511[M+1] ⁺
66	NTP-3006	M		500.61	501[M+1] ⁺
67	NTP-3007	N		486.59	487[M+1] ⁺
68	NTP-3008	N		563.63	564[M+1] ⁺

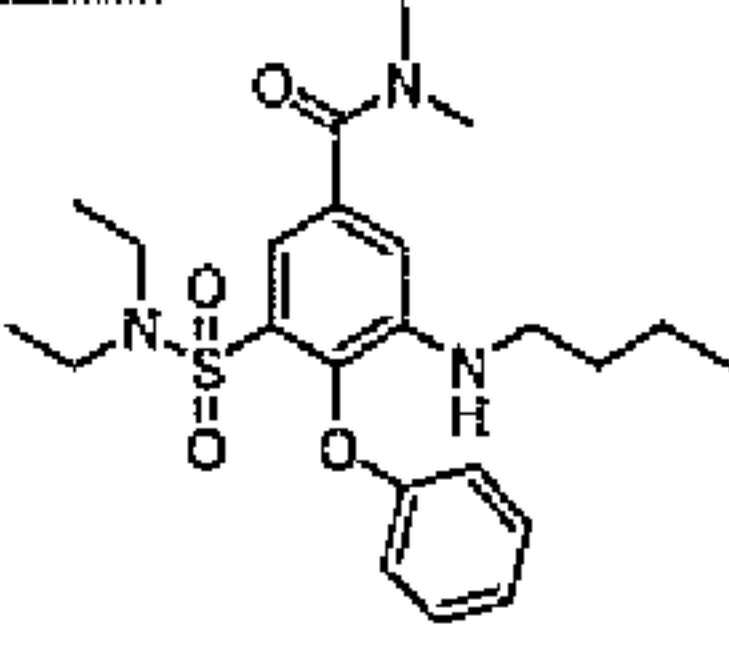
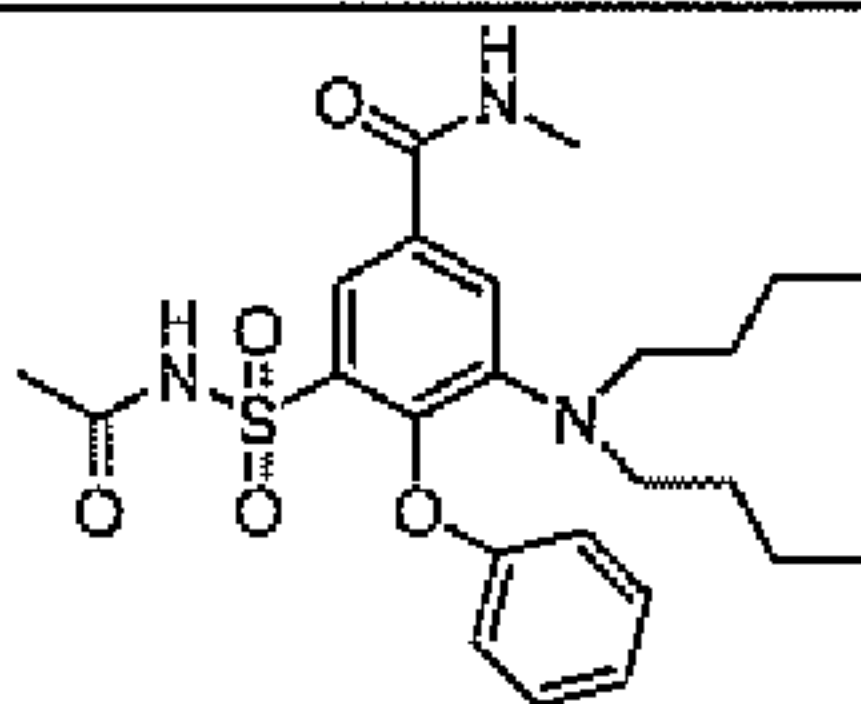
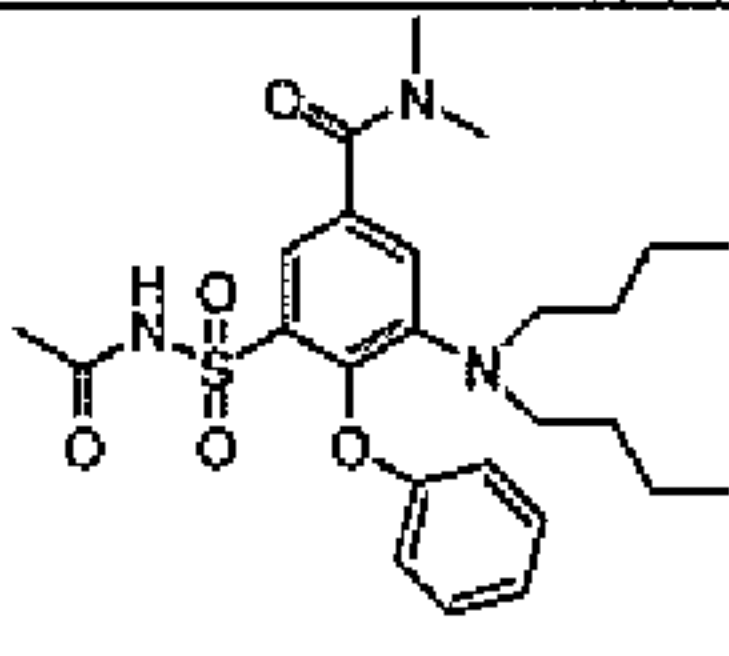
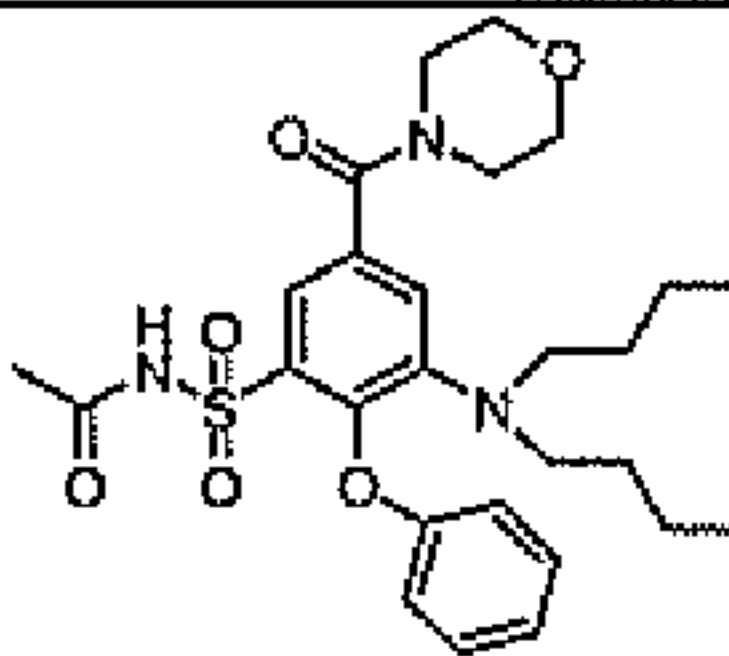
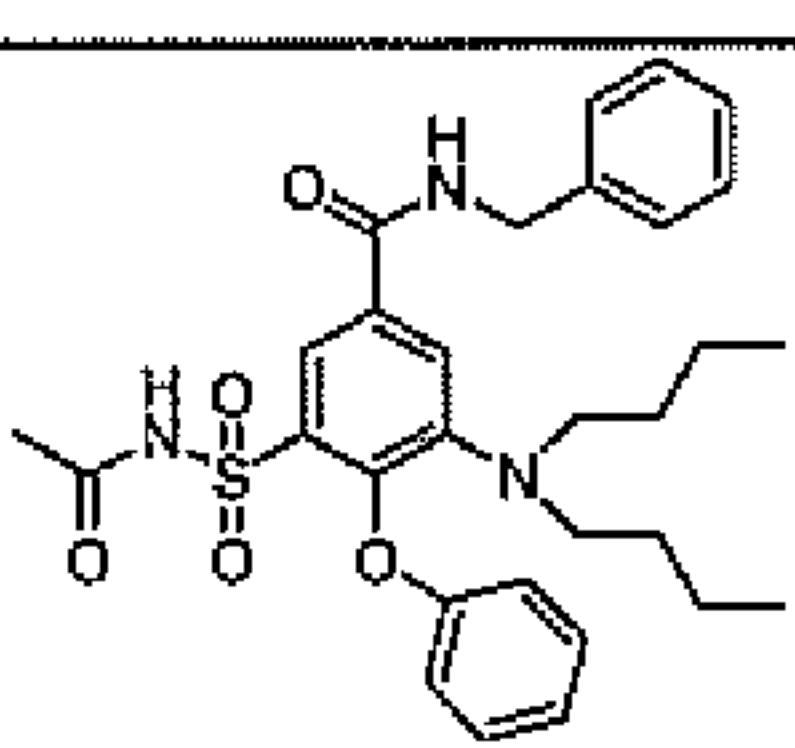
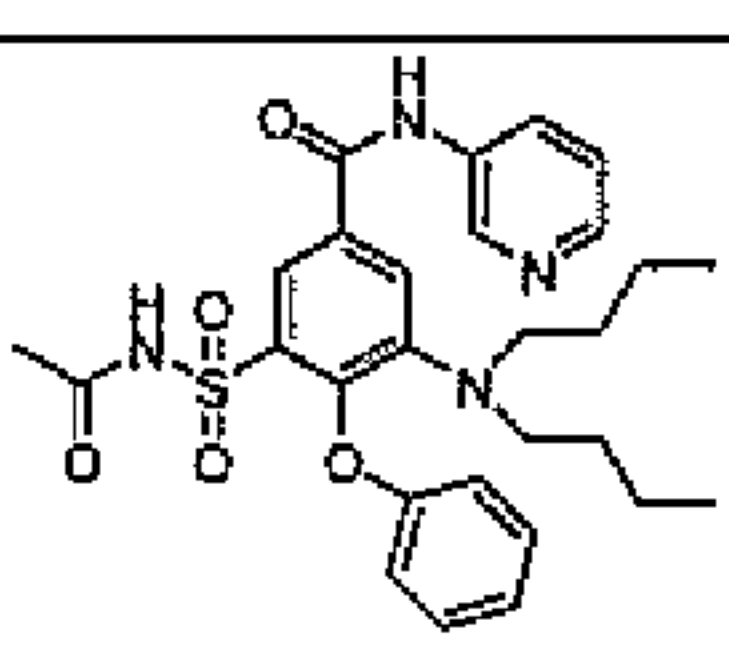
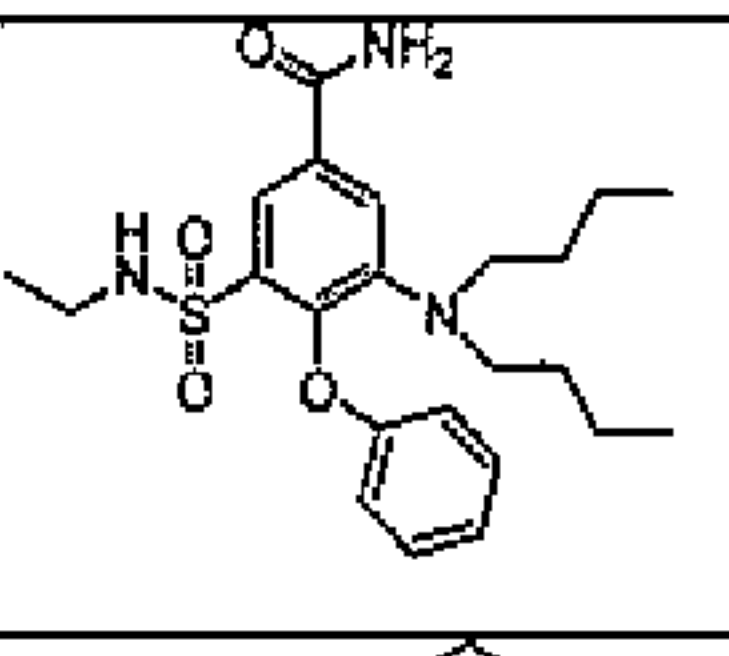
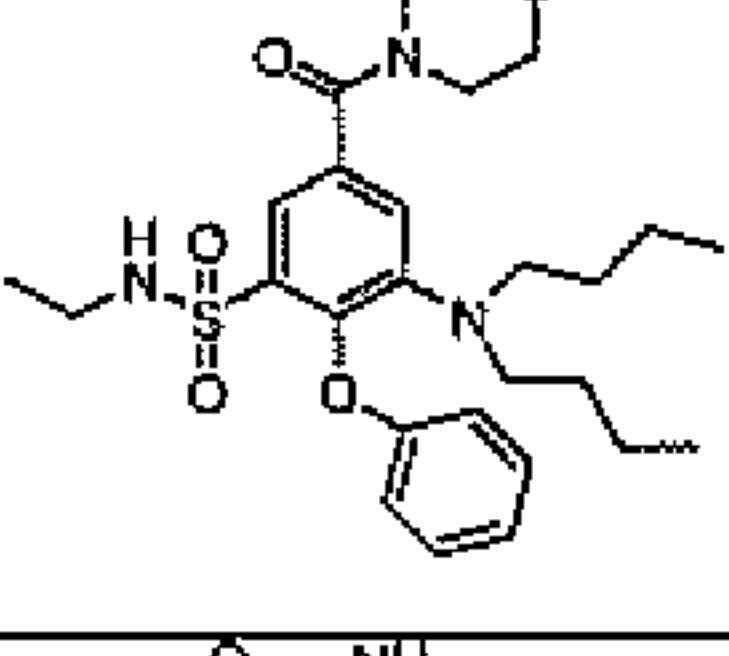
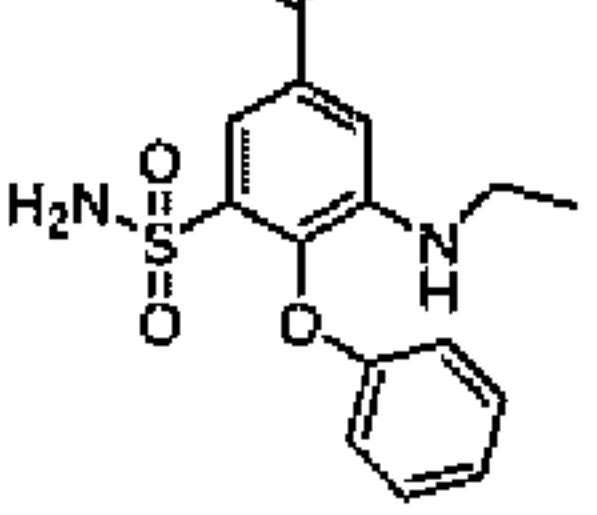
69	NTP-3009	M		563.63	564[M+1] ⁺
70	NTP-3010	N		563.63	564[M+1] ⁺
71	NTP-3011	M		541.68	542[M+1] ⁺
72	NTP-3012	M		541.68	542[M+1] ⁺
73	NTP-3013	M		545.64	546[M+1] ⁺
74	NTP-3014	M		578.55	578,580[M+2] ⁺
75	NTP-3015	N		530.08	530,532[M+2] ⁺
76	NTP-3016	M		530.08	530,532[M+2] ⁺
77	NTP-3017	M		523.69	524[M+1] ⁺
78	NTP-3018	M		564.52	564,566[M+2] ⁺

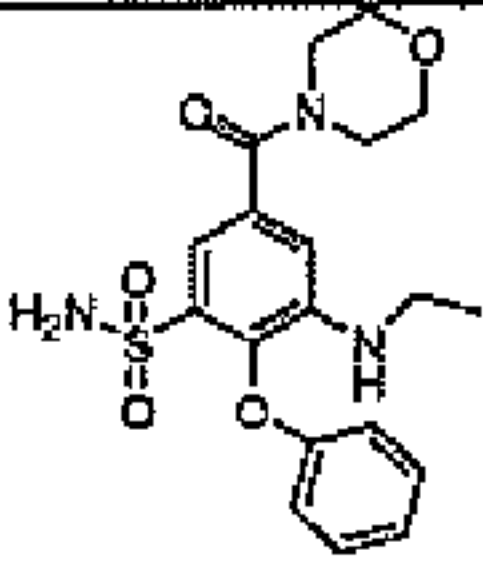
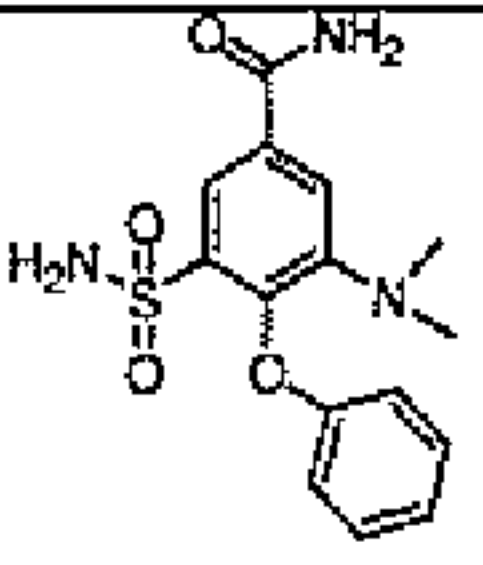
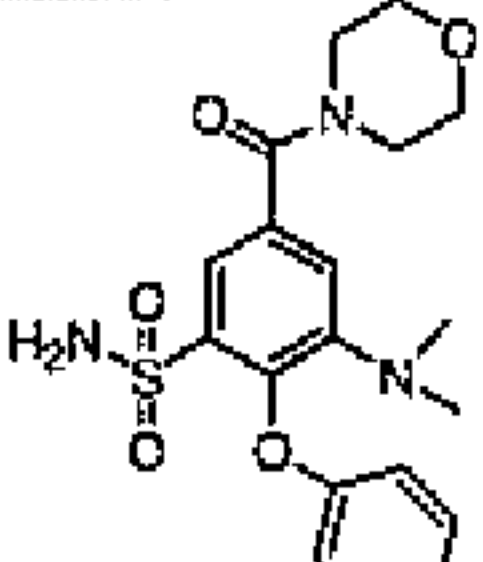
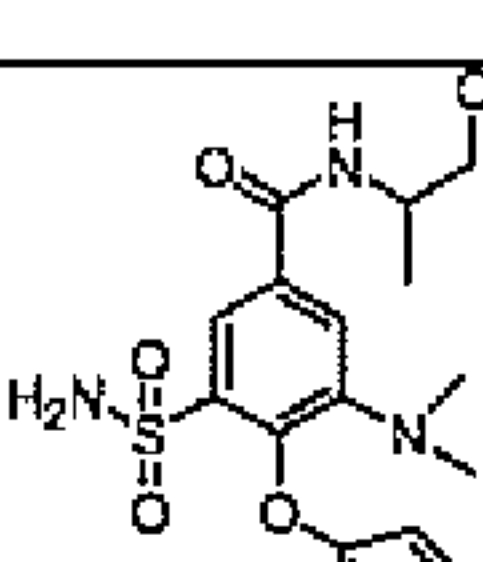
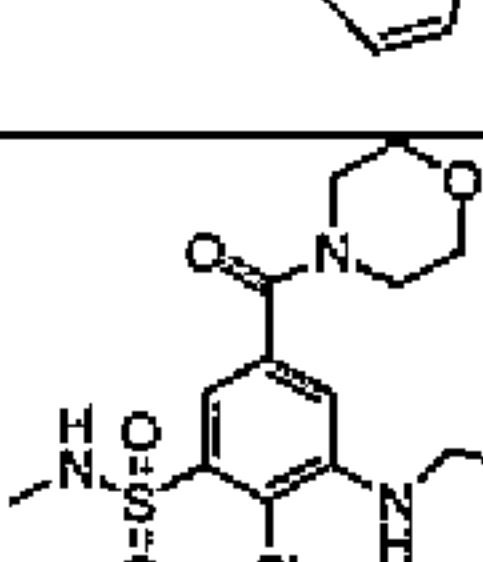
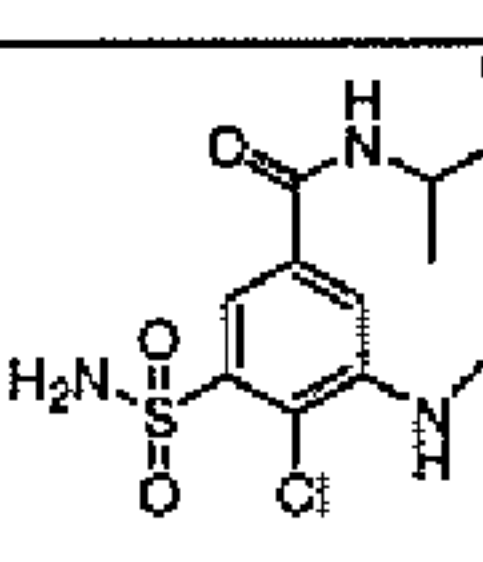
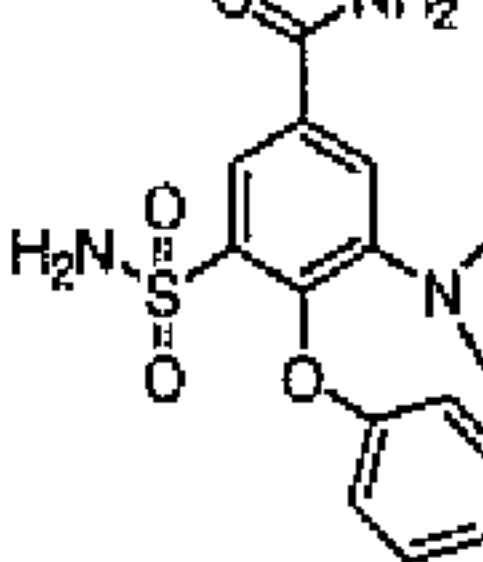
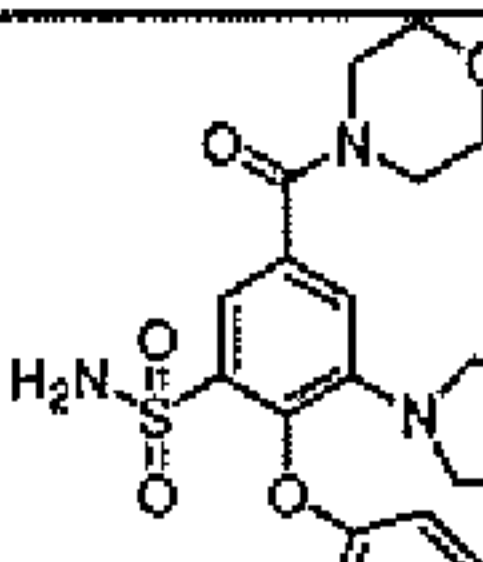
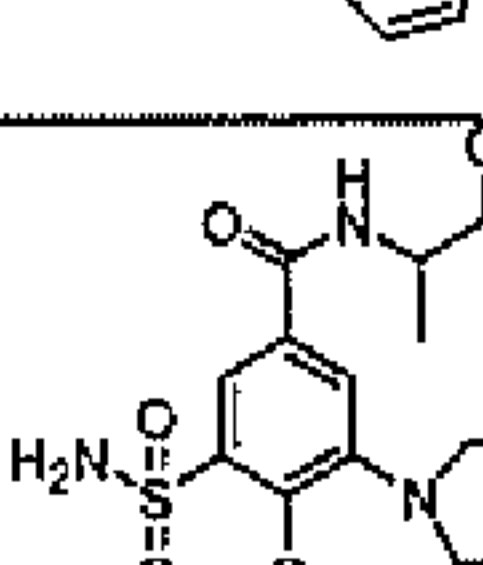
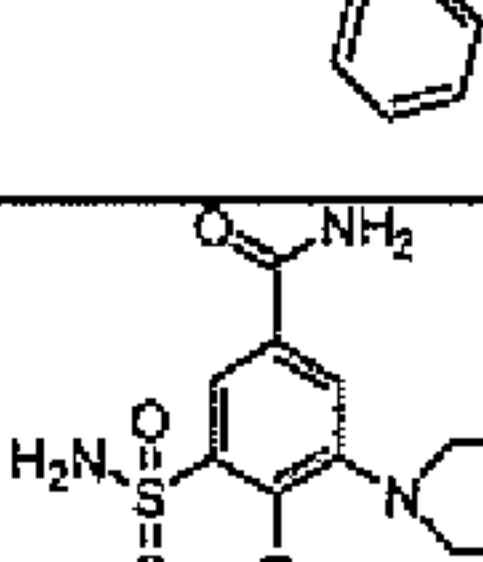
79	NTP-3019	N		546.68	547[M+1] ⁺
80	NTP-3020	N		496.62	497[M+1] ⁺
81	NTP-3021	N		496.62	497[M+1] ⁺
82	NTP-3022	N		459.60	460[M+1] ⁺
83	NTP-3023	M		473.63	474[M+1] ⁺
84	NTP-3024	M		487.65	488[M+1] ⁺
85	NTP-3025	M		502.67	503[M+1] ⁺
86	NTP-3026	N		541.75	542[M+1] ⁺
87	NTP-3027	M		555.77	556[M+1] ⁺
88	NTP-3028	N		528.71	529[M+1] ⁺
89	NTP-3029	M		542.73	543[M+1] ⁺

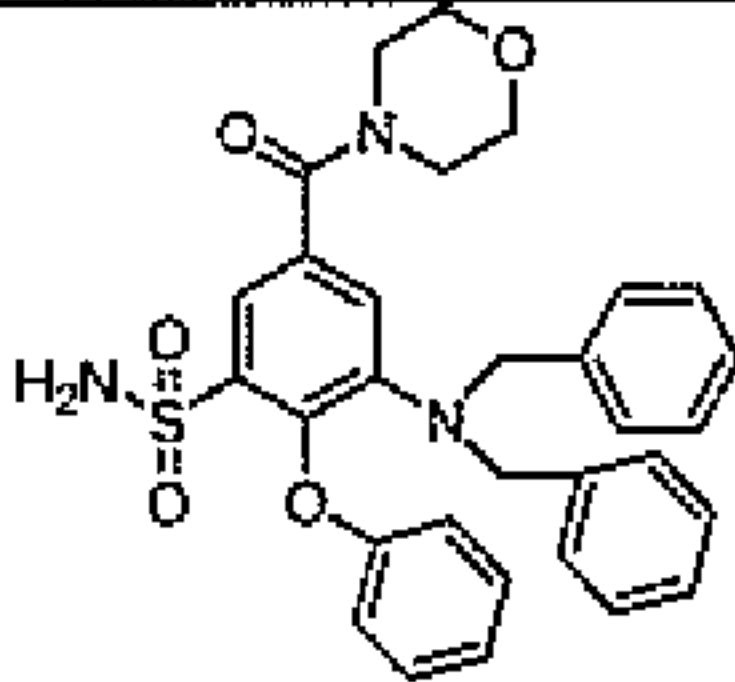
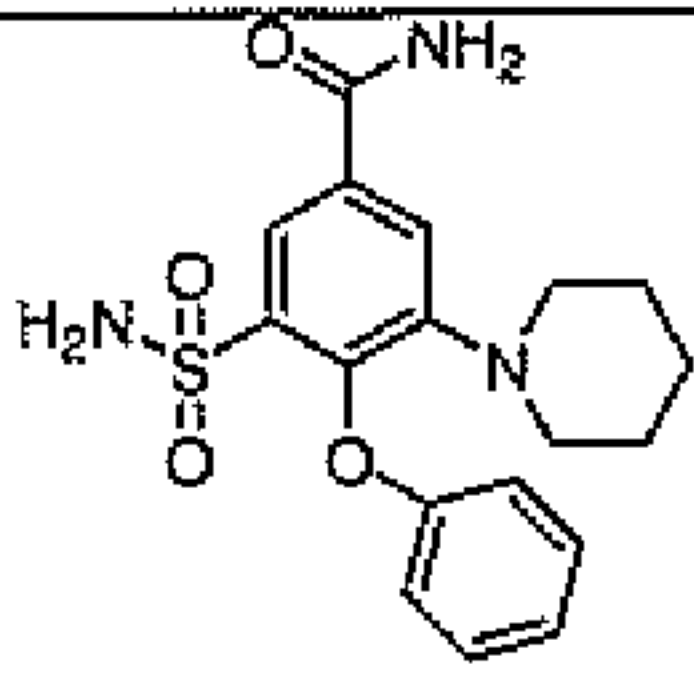
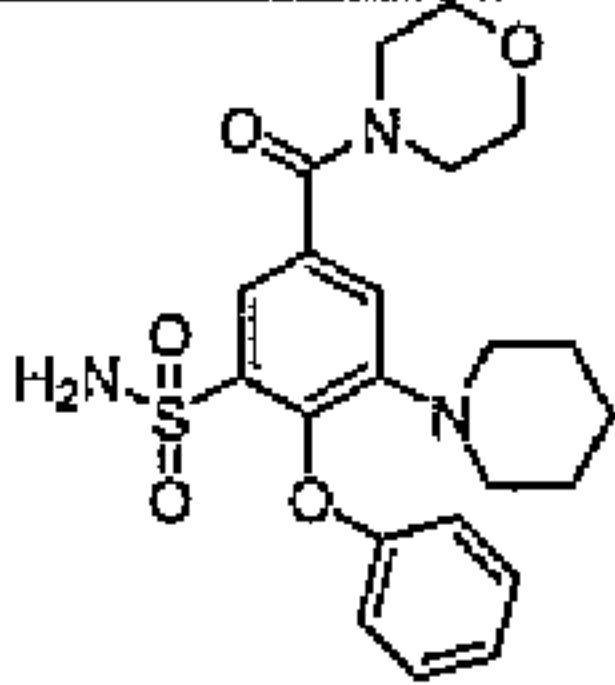
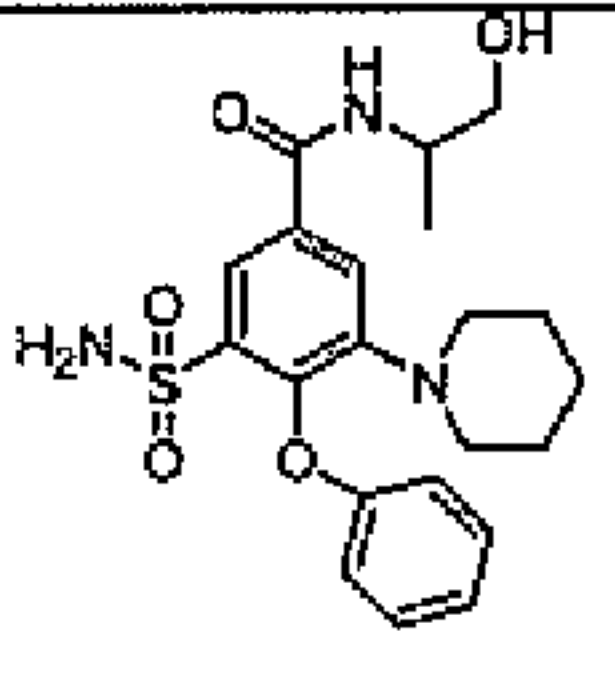
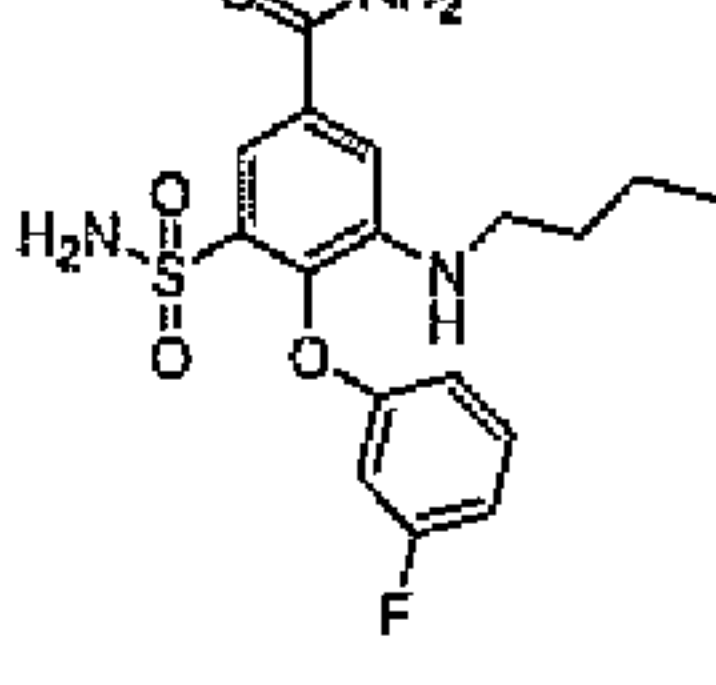
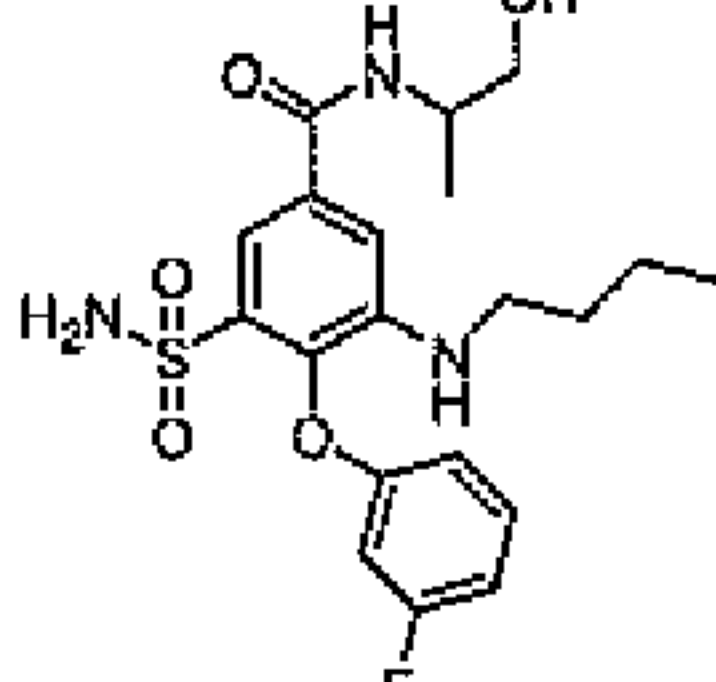
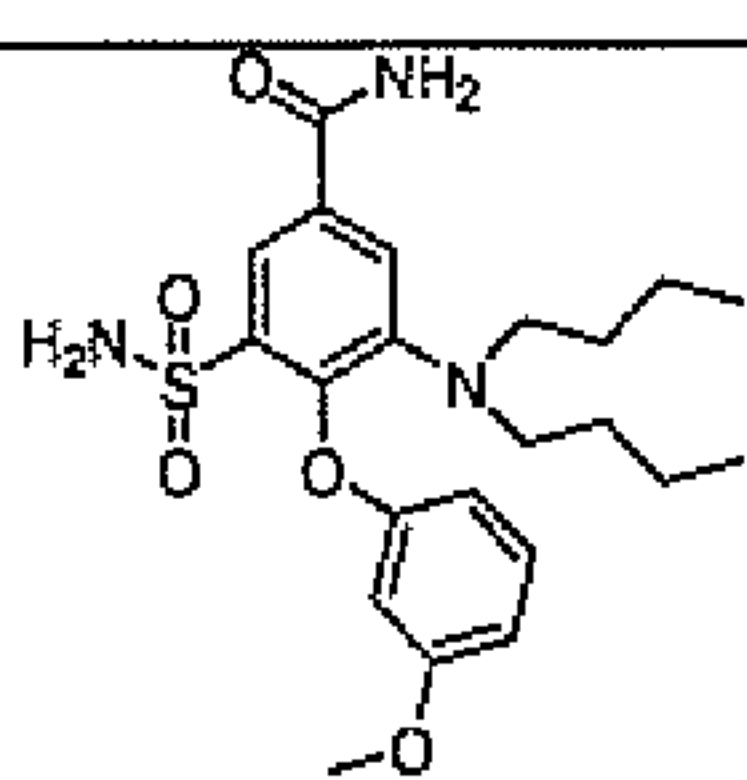
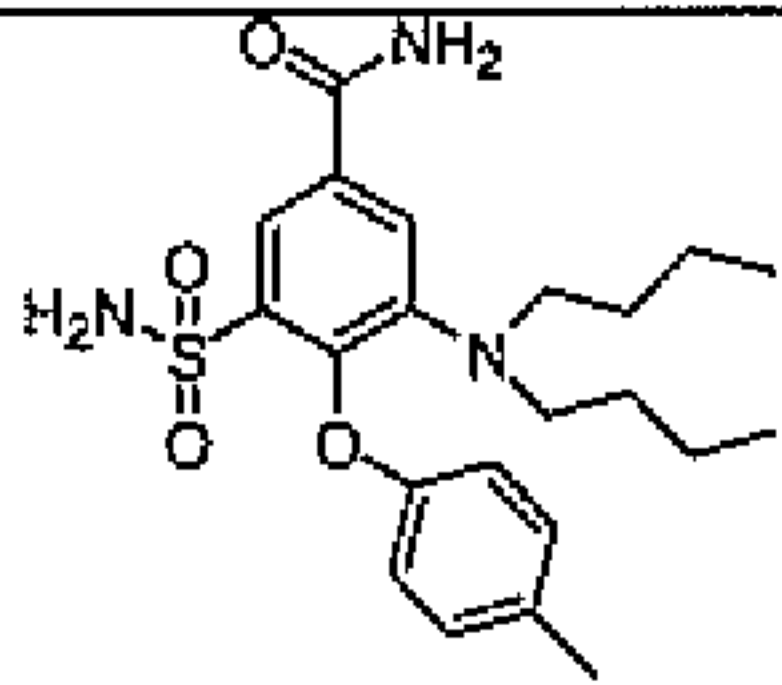
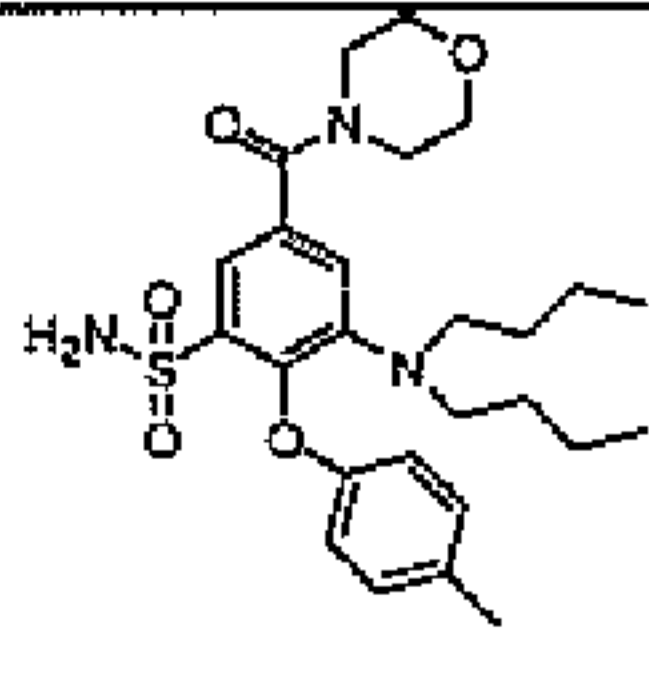
90	NTP-3030	N		516.7	517[M+1] ⁺
91	NTP-3031	M		546.72	547[M+1] ⁺
92	NTP-3034	M		419.19	420[M+1] ⁺
93	NTP-3035	M		475.64	476[M+1] ⁺
94	NTP-3036	N		475.64	476[M+1] ⁺
95	NTP-3037	N		564.52	564,566[M+2] ⁺
96	NTP-3038	M		530.68	531[M+1] ⁺
97	NTP-3039	N		532.70	533[M+1] ⁺
98	NTP-3040	N		433.56	434[M+1] ⁺
99	NTP-3041	N		447.59	448[M+1] ⁺
100	NTP-3042	M Followed by deprotection of the boc-		502.67	503[M+1] ⁺

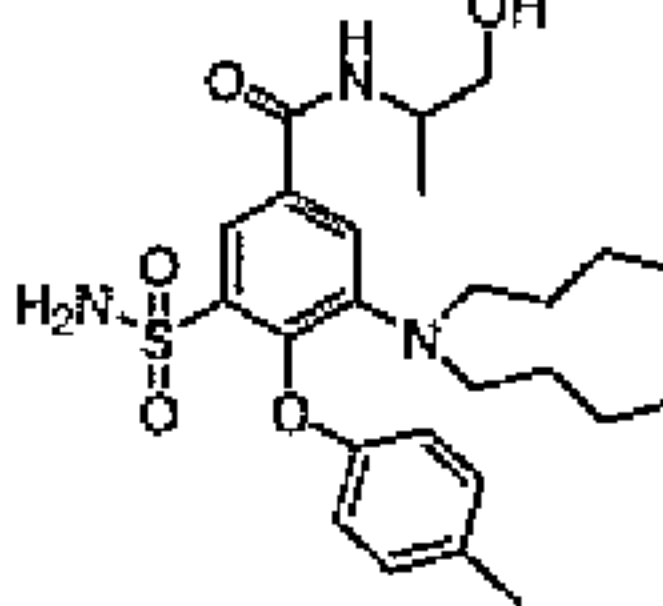
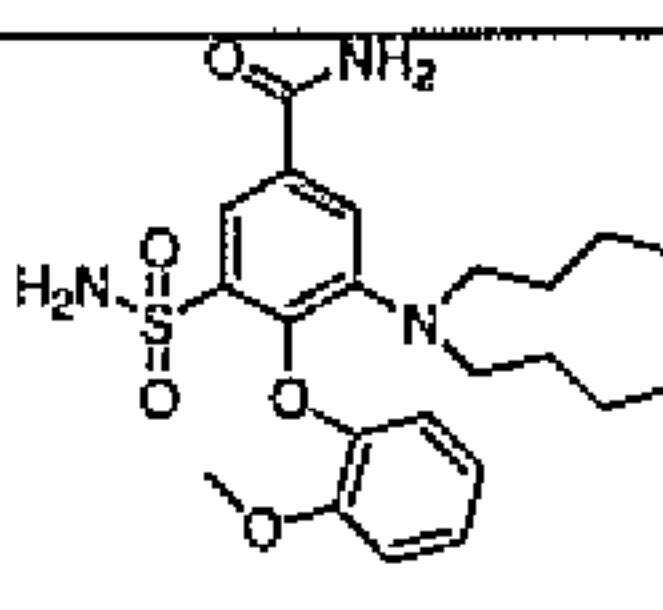
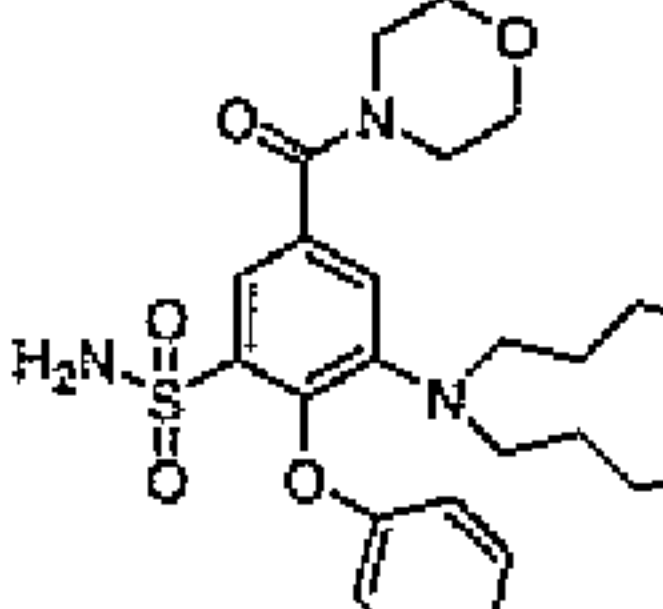
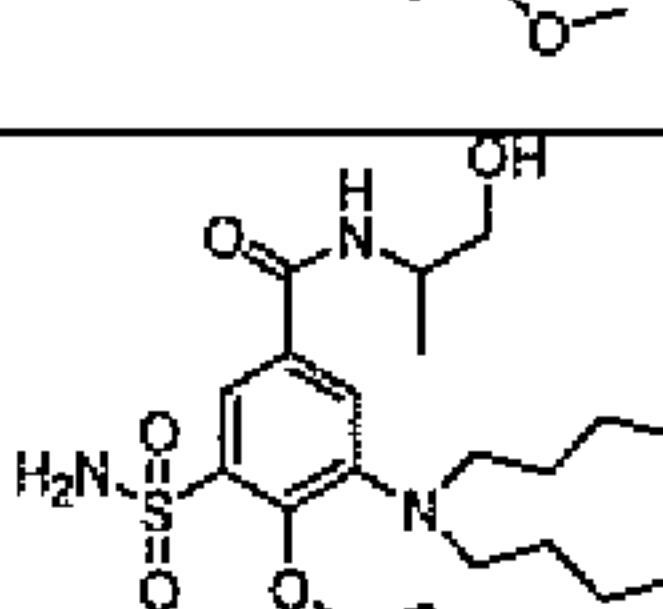
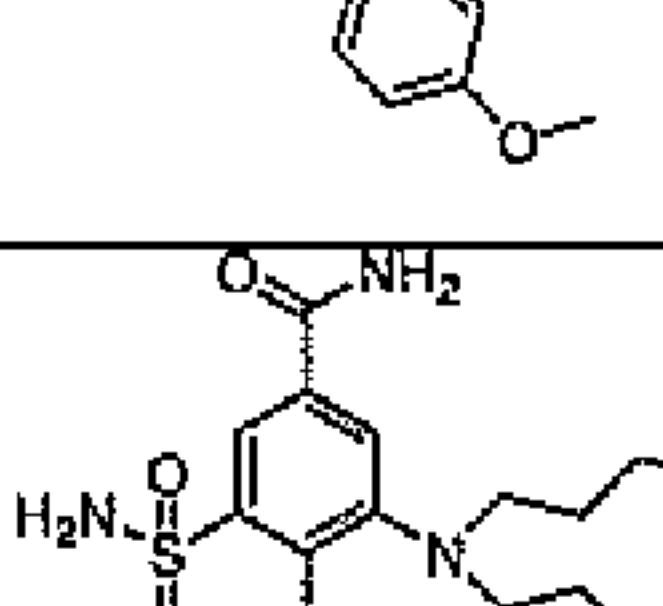
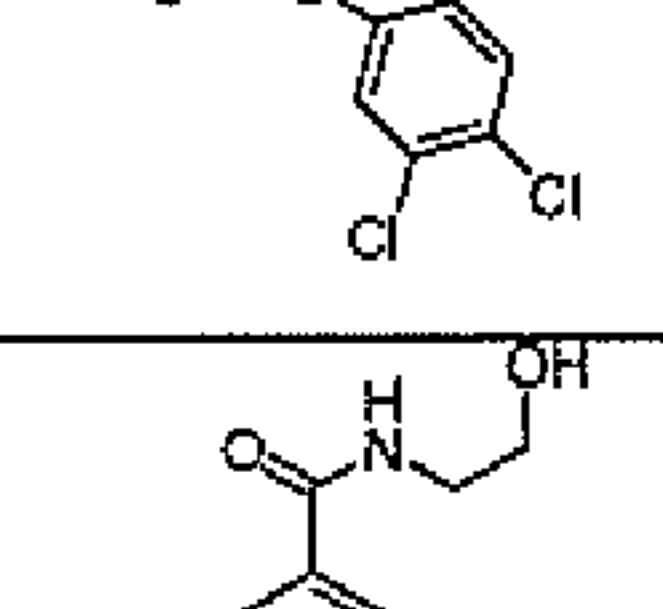
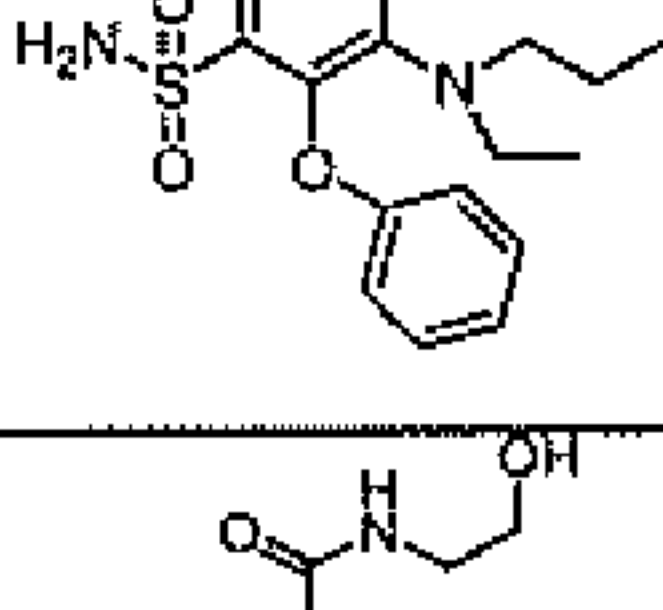
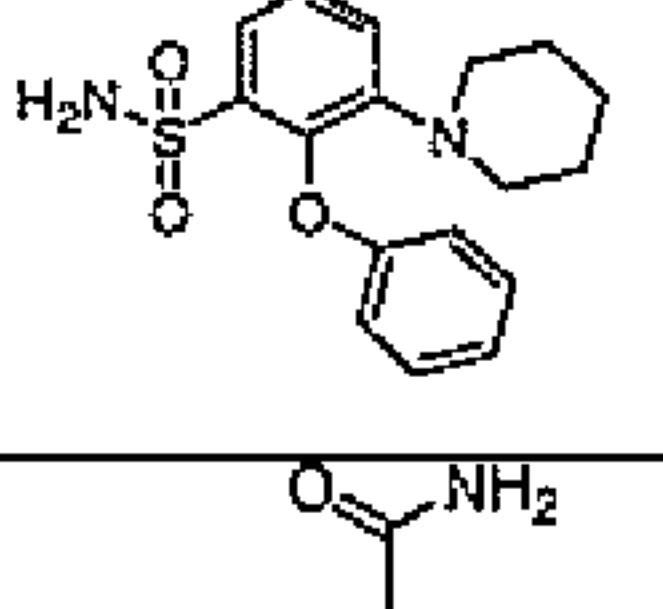
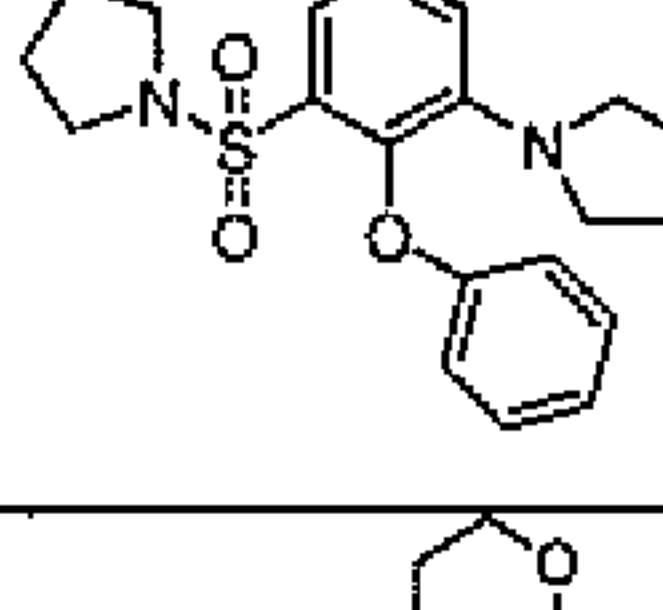
		protected primary amine with HCl			
101	NTP-3043	M Followed by deprotection of the boc- protected secondary amine with HCl		502.67	503[M+1] ⁺
102	NTP-3044	O		544.75	545[M+1] ⁺
103	NTP-7001	O		405.51	406[M+1] ⁺
104	NTP-7002	O		419.54	420[M+1] ⁺
105	NTP-7003	O		461.57	462 [M+1] ⁺
106	NTP-7004	O		447.55	448[M+1] ⁺
107	NTP-7005	O		461.57	462[M+1] ⁺

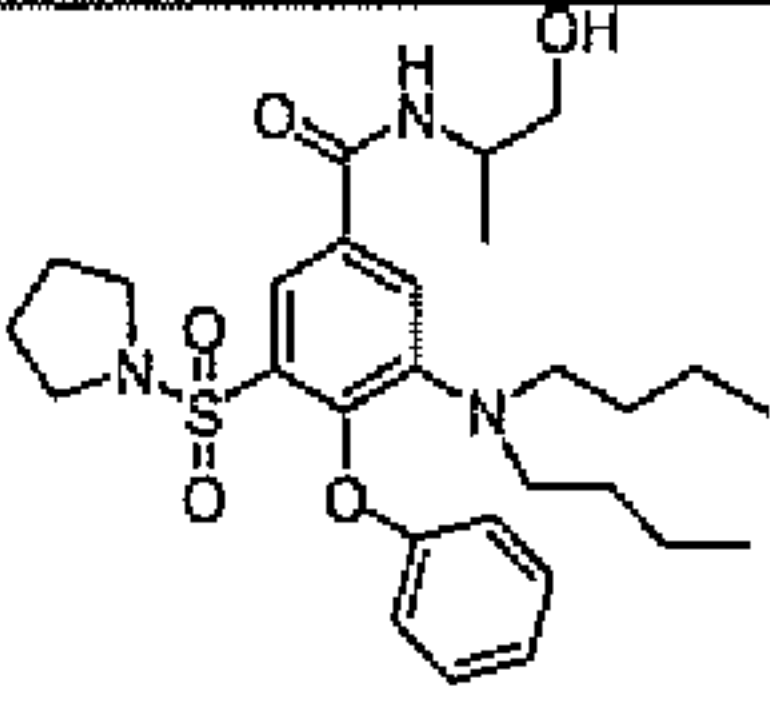
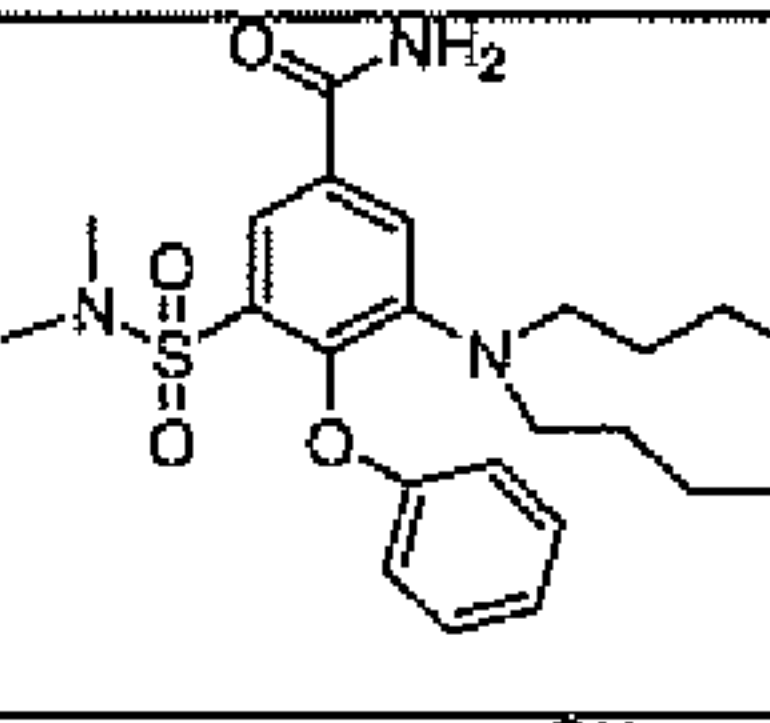
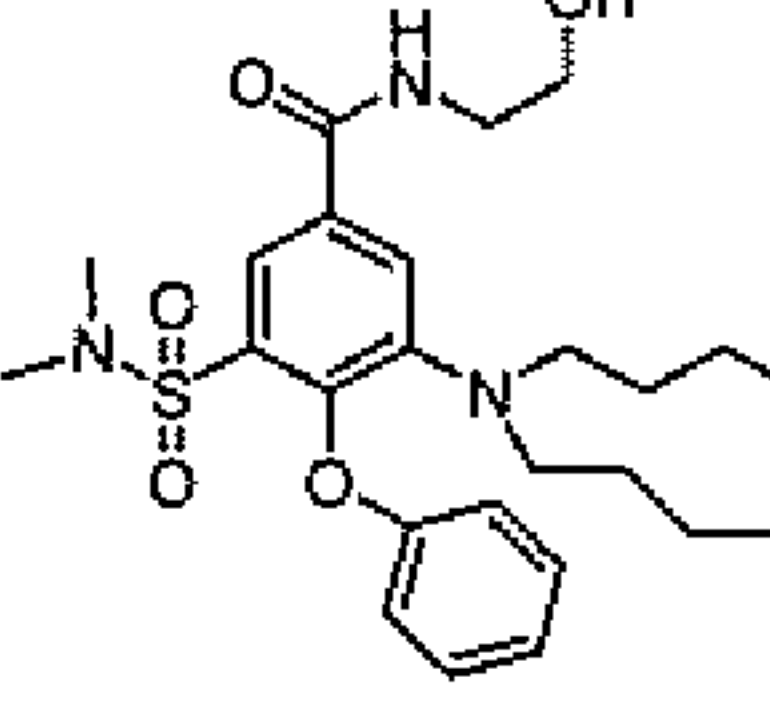
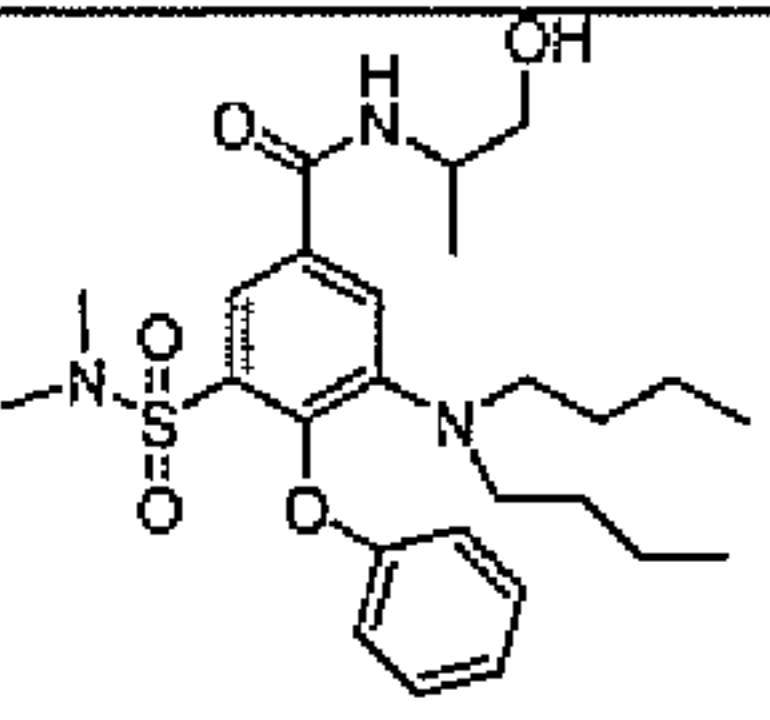
108	NTP-7006	O		503.21	504[M+1] ⁺
109	NTP-7007	O		433.56	434[M+1] ⁺
110	NTP-7008	O		489.63	490[M+1] ⁺
111	NTP-7009	O		517.68	518[M+1] ⁺
112	NTP-7010	O		461.62	462[M+1] ⁺
113	NTP-7011	O		475.64	476[M+1] ⁺
114	NTP-7012	O		524.67	525[M+1] ⁺
115	NTP-7013	O		537.71	538[M+1] ⁺
116	NTP-7014	O		333.83	334[M+1] ⁺

117	NTP-7015	O		447.22	448[M+1] ⁺
118	NTP-7016	N		475.60	476[M+1] ⁺
119	NTP-7017	N		489.63	490[M+1] ⁺
120	NTP-7018	M		531.67	532[M+1] ⁺
121	NTP-7019	N		551.70	552[M+1] ⁺
122	NTP-7020	N		538.66	539[M+1] ⁺
123	NTP-7021	O		447.59	448[M+1] ⁺
124	NTP-7022	O		517.68	518[M+1] ⁺
125	NTP-7023	O		335.38	336[M+1] ⁺

126	NTP-7024	O		405.47	406[M+1] ⁺
127	NTP-7025	O		335.38	336[M+1] ⁺
128	NTP-7026	O		405.47	406[M+1] ⁺
129	NTP-7027	O		393.46	394[M+1] ⁺
130	NTP-7028	O		375.87	376[M+1] ⁺
131	NTP-7029	O		363.86	364[M+1] ⁺
132	NTP-7030	O		391.48	392[M+1] ⁺
133	NTP-7031	O		461.57	462[M+1] ⁺
134	NTP-7032	O		449.56	450[M+1] ⁺
135	NTP-7033	O		487.57	488[M+1] ⁺

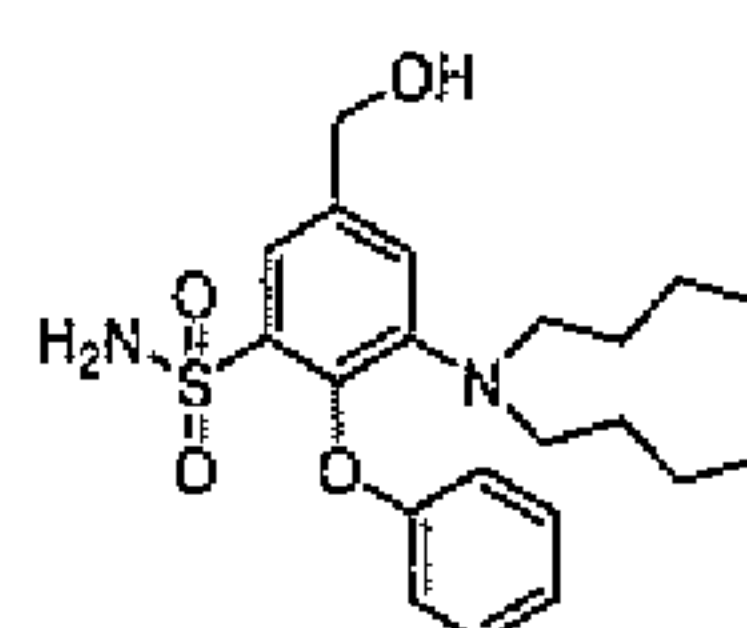
136	NTP-7034	O		557.67	558[M+1] ⁺
137	NTP-7035	O		375.45	376[M+1] ⁺
138	NTP-7036	O		445.57	446[M+1] ⁺
139	NTP-7037	O		433.52	434[M+1] ⁺
140	NTP-7038	O		381.42	382[M+1] ⁺
141	NTP-7039	O		439.50	440[M+1] ⁺
142	NTP-7040	O		449.56	450[M+1] ⁺
143	NTP-7041	O		433.56	434[M+1] ⁺
144	NTP-7042	O		503.55	504[M+1] ⁺

145	NTP-7043	O		491.64	492[M+1] ⁺
146	NTP-7044	O		449.56	450[M+1] ⁺
147	NTP-7045	O		519.65	520[M+1] ⁺
148	NTP-7046	O		507.64	508[M+1] ⁺
149	NTP-7047	O		488.43	488
150	NTP-7048	O		435.54	436[M+1] ⁺
151	NTP-7049	O		419.49	420[M+1] ⁺
152	NTP-7050	O		473.63	474[M+1] ⁺
153	NTP-7051	O		543.72	544[M+1] ⁺

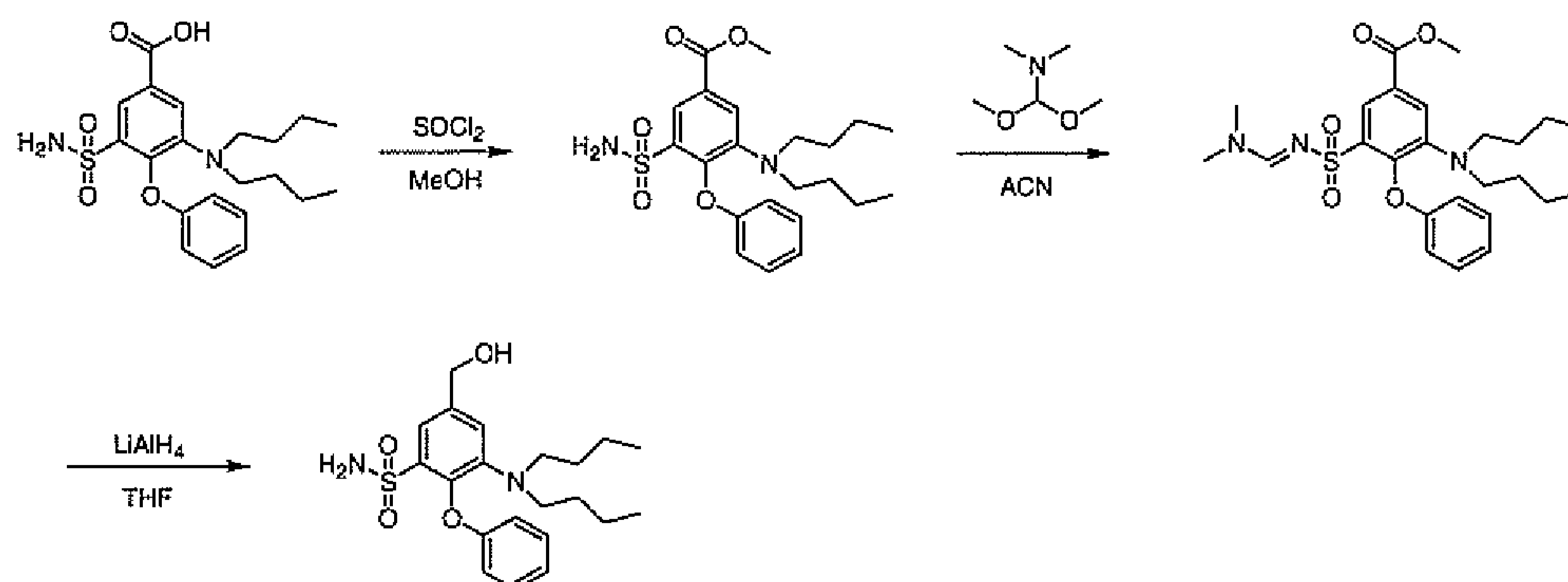
154	NTP-7052	O		531.71	532[M+1] ⁺
155	NTP-7053	O		447.59	448[M+1] ⁺
156	NTP-7054	O		491.64	492[M+1] ⁺
157	NTP-7055	O		505.67	506[M+1] ⁺

Example 158**3-Dibutylamino-5-hydroxymethyl-2-phenoxy-benzenesulfonamide**

NTP-10001



General method P



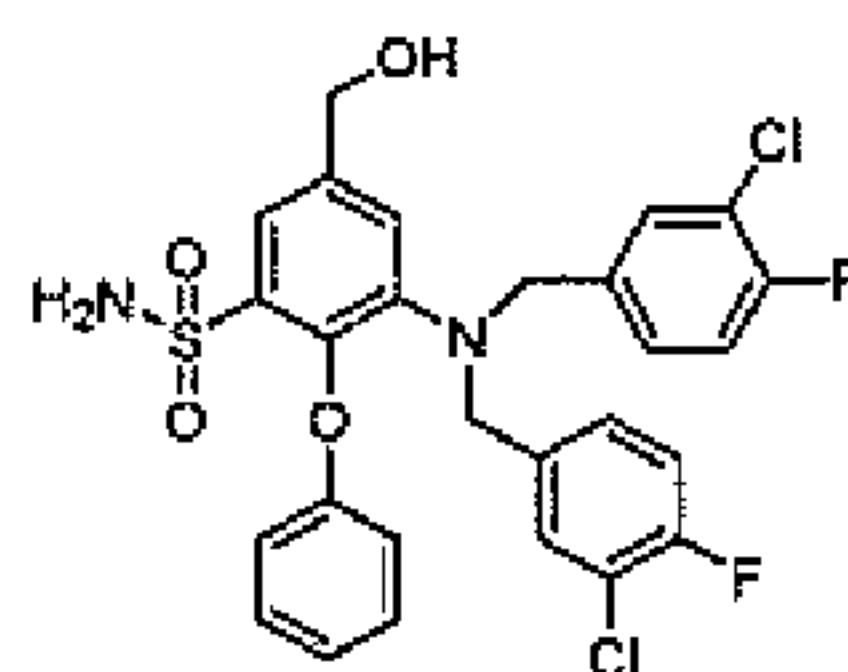
Step 1: 3-Dibutylamino-4-phenoxy-5-sulfamoyl-benzoic acid methyl ester. A round bottom flask was charged with 3-dibutylamino-4-phenoxy-5-sulfamoyl benzoic acid (1.82 g, 4.33 mmol) and methanol (50 mL). Thionyl chloride (1.50 g, 12.99 mmol) was added slowly at room temperature and the reaction mixture was heated to 50° overnight. The solvent was removed under reduced pressure and the residue re-dissolved in ethyl acetate and washed with saturated sodium bicarbonate solution, water and brine. The organic solvent was removed under reduced pressure and the residue purified by flash chromatography to give the product as white solid (1.75g). MS *m/z*: 435 [M+1]⁺.

Step 2: 3-Dibutylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester. A reaction flask was charged with 3-dibutylamino-4-phenoxy-5-sulfamoyl benzoic acid methyl ester (1.75 g, 4.032 mmol), acetonitrile (25 mL) and N,N-dimethyl formamide dimethyl acetal (0.56 mL, 4.233 mmol) and stirred at room temperature for 2 hours. The solvent was removed under reduced pressure and the resultant gummy residue was treated with ice cold water to give pale yellow solid. The solid was filtered and dried to give the product (2.1g). MS *m/z*: 490 [M+1]⁺.

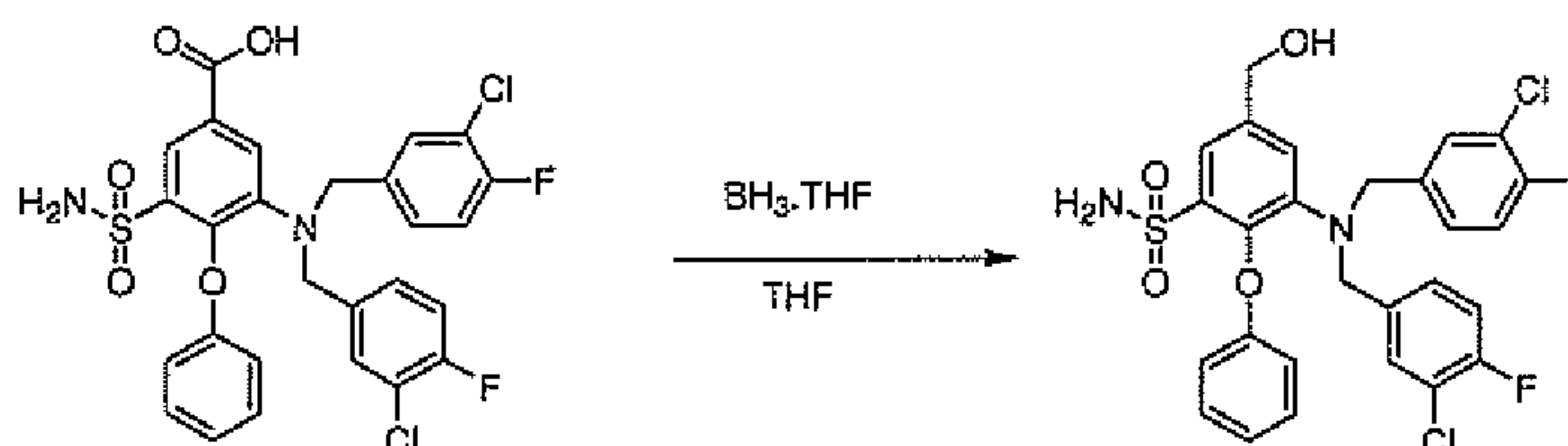
Step 3: 3-Dibutylamino-5-hydroxymethyl-2-phenoxy-benzenesulfonamide (NTP-10001). A round bottom flask was charged with 3-dibutylamino-5-(dimethylaminomethylene-sulfamoyl)-4-phenoxy-benzoic acid methyl ester (200 mg, 0.41 mmol), THF (5 mL) and lithium aluminum hydride (2M in THF) (0.25 mL, 0.49 mmol) was added and the reaction stirred at room temperature for 1 hour. Another portion of lithium aluminum hydride (2M in THF) (0.25 mL, 0.49 mmol) was added and the reaction was heated to 50°C for 1 hour. The reaction was cooled and quenched with cold water and diluted with ether. The reaction was filtered and the filtrate removed under reduced pressure. The residue was then purified by flash chromatography to give the product as white solid (33 mg). ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.41 (d, J=1.8 Hz, 1H), 7.21–7.16 (m, 3H) 7.13 (s, 2H), 6.93 (t, J=7.2 Hz, 1H), 6.71 (d, J=8.7 Hz, 2H), 5.35 (t, J= 5.7 Hz, 1H), 4.51 (d, J=5.7 Hz, 2H), 2.97 (t, J=7.2 Hz, 4H), 1.17–1.10 (m, 4H), 1.02–0.94 (m, 4H), 0.73 (t, J=7.5 Hz, 6H). MS *m/z*: 407 [M+1]⁺.

Example 159

3-(bis(3-chloro-4-fluorobenzyl)amino)-5-(hydroxymethyl)-2-phenoxybenzenesulfonamide
NTP-10002



General method Q

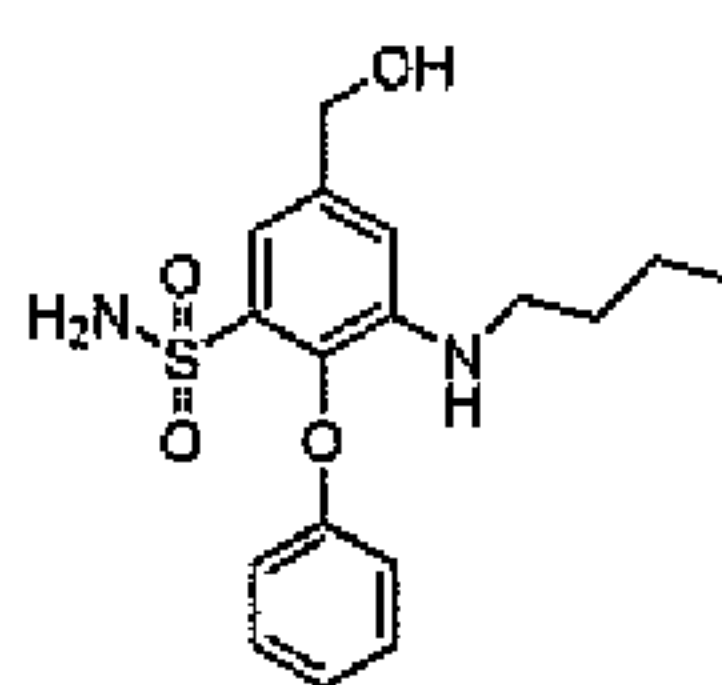


3-(bis(3-chloro-4-fluorobenzyl)amino)-5-(hydroxymethyl)-2-phenoxybenzenesulfonamide: A reaction vial was charged with 3-(bis(3-chloro-4-fluorobenzyl)amino)-4-phenoxy-5-sulfamoylbenzoic acid (75 mg, 0.126 mmol), tetrahydrofuran (4 mL) and $\text{BH}_3\cdot\text{THF}$ complex (1.0M in tetrahydrofuran) was added dropwise. The reaction mixture was stirred at room temperature for 1 hour. Water was added and the reaction mixture was extracted with ethyl acetate, washed with brine and dried over MgSO_4 . The solvents were removed under reduced pressure and the residue purified by flash chromatography to give the title product (48 mg). ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.52 (d, $J=2.1$ Hz, 1H), 7.34 (t, $J=7.5$ Hz, 2H), 7.26 (s, 2H), 7.22-7.11 (m, 4H), 6.91 (dd, $J=7.5$ & 2.1 Hz, 2H), 6.83-6.77 (m, 4H), 5.34 (t, $J=5.7$ Hz, 1H), 4.44 (d, $J=5.7$ Hz, 2H), 4.11 (s, 4H). MS m/z : 580.9 $[\text{M}+1]^+$.

Example 160

3-(butylamino)-5-(hydroxymethyl)-2-phenoxybenzenesulfonamide

NTP-10003



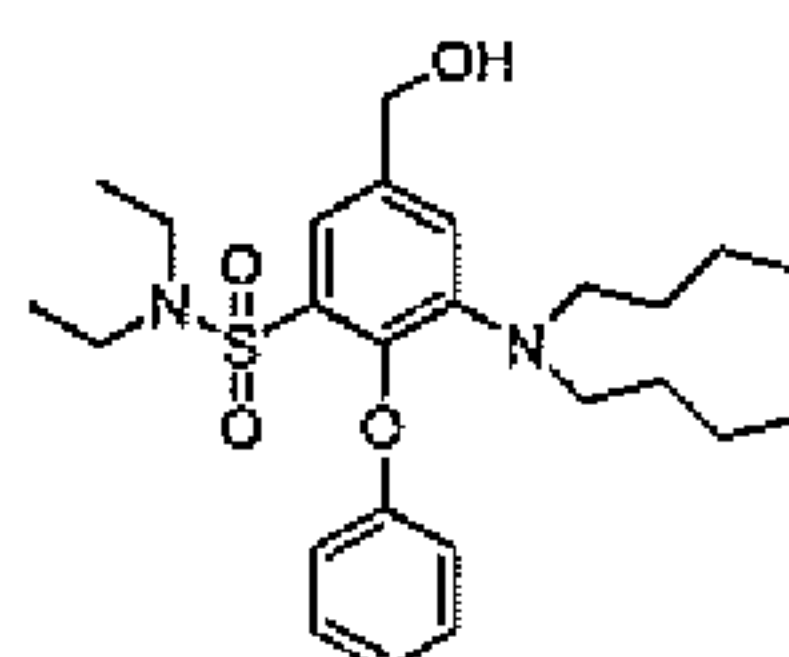
The title compound is made according to general method P, beginning with bumetanide in step 1. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ 7.25-7.19 (m, 2H), 7.07 (bs, 2H), 7.05 (d, $J=2.1$ Hz, 1H), 6.95 (t, $J=7.5$ Hz, 1H), 6.86 (s, 1H), 6.81 (d, $J=7.5$ Hz, 2H), 5.28 (t, $J=5.7$ Hz, 1H), 4.67 (t, $J=5.7$ Hz, 1H),

4.48 (d, J=6.0 Hz, 2H), 3.04-2.97 (m, 2H), 1.37–1.30 (m, 2H), 1.14–1.06 (m, 2H), 0.76 (t, J=7.5 Hz, 3H). MS *m/z*: 351.0 [M+1]⁺.

Example 161

3-(dibutylamino)-N,N-diethyl-5-(hydroxymethyl)-2-phenoxybenzenesulfonamide

NTP-10004

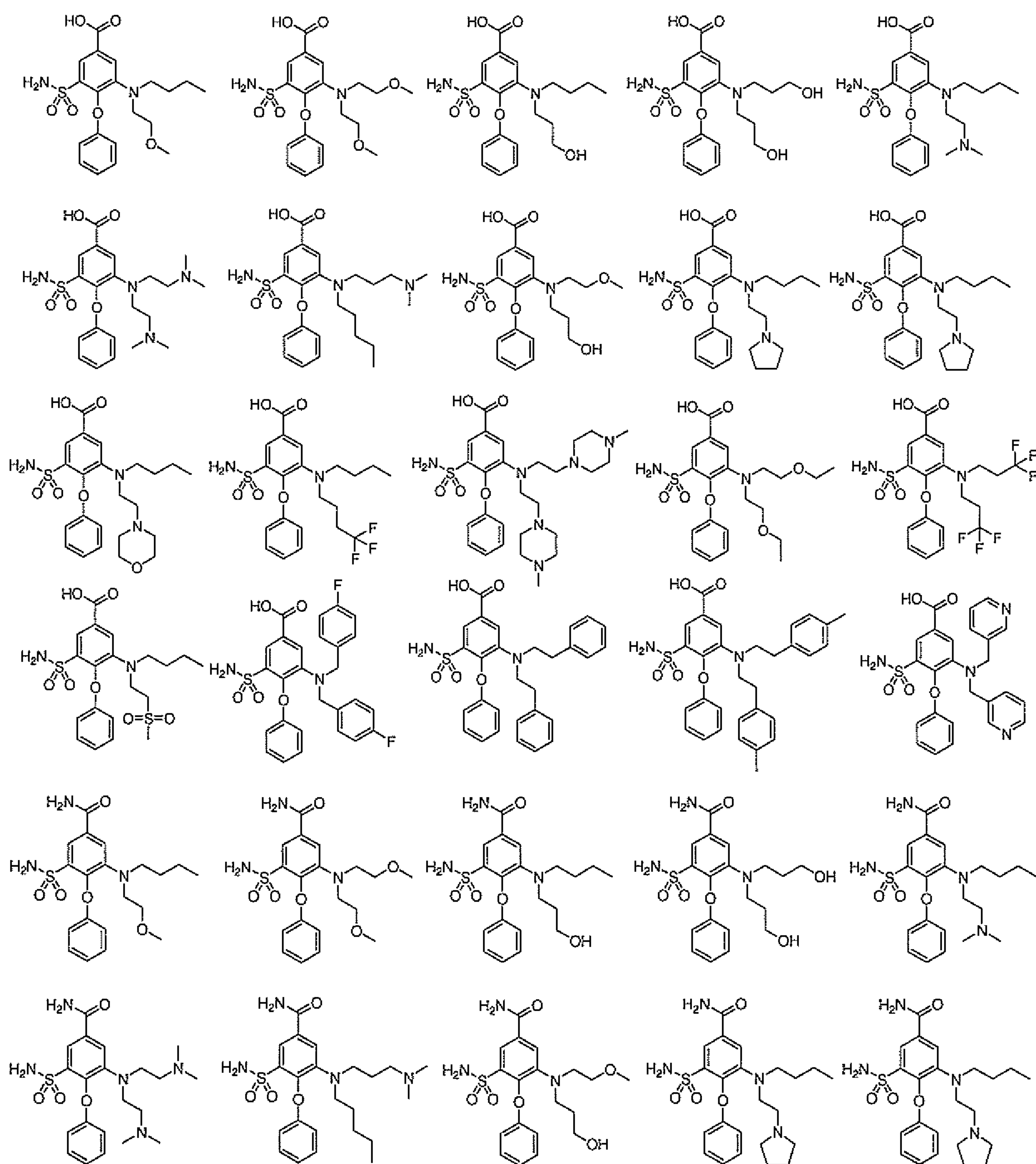


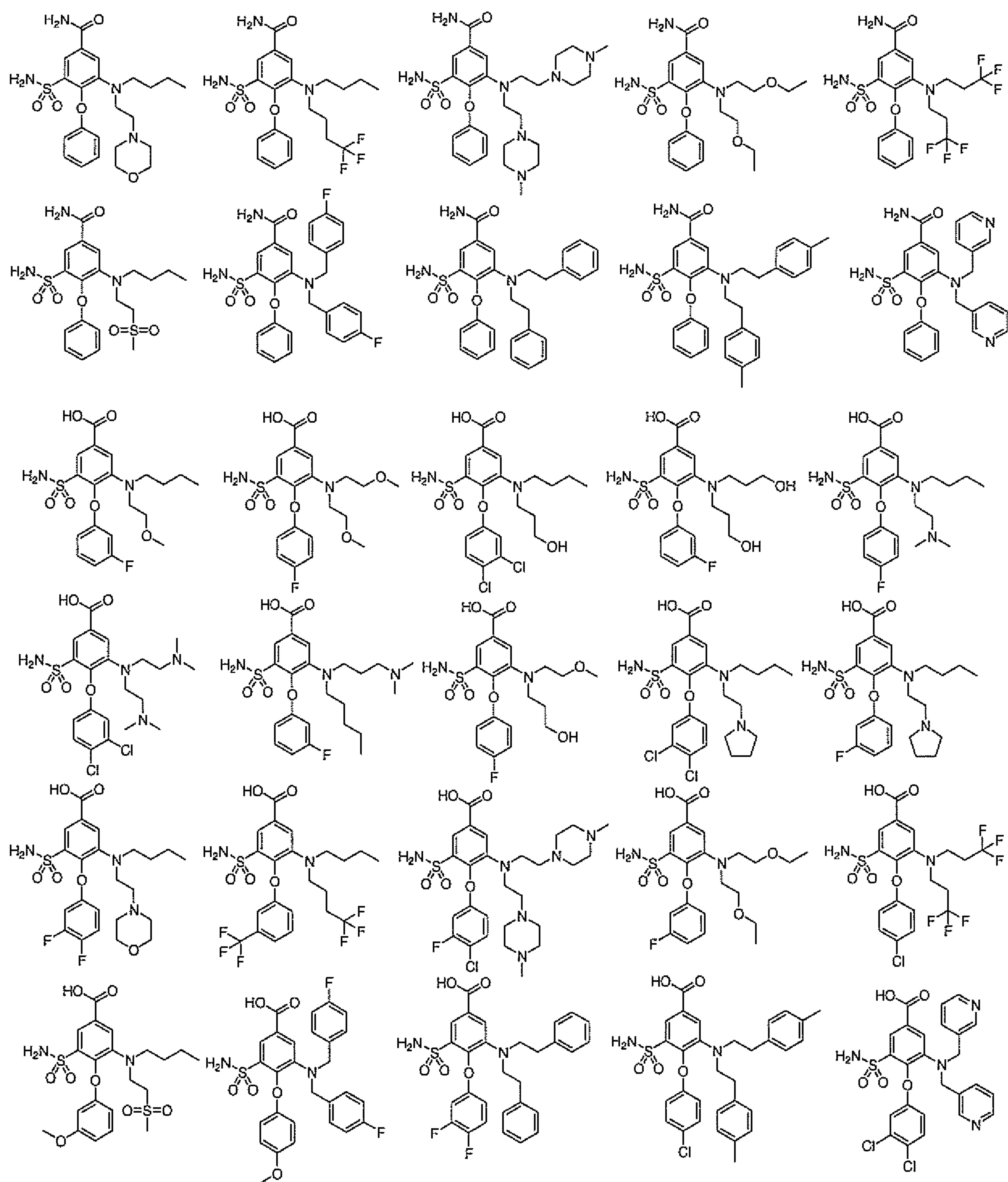
The title compound is made according to general procedure Q, beginning with the appropriate benzoic acid derivative. ¹H NMR (300 MHz, DMSO-*d*₆) δ 7.42 (d, J=1.2 Hz, 1H), 7.20 (t, J=7.8 Hz, 3H), 6.94 (t, J=7.5 Hz, 1H), 6.63 (d, J=7.8 Hz, 2H), 5.35 (t, J= 5.7 Hz, 1H), 4.52 (d, J=6.0 Hz, 2H), 3.24-3.13 (m, 4H), 2.95 (t, J=7.2 Hz, 4H), 1.18–1.08 (m, 4H), 1.01–0.90 (m, 10H), 0.70 (t, J=7.2 Hz, 6H). MS *m/z*: 463.3 [M+1]⁺.

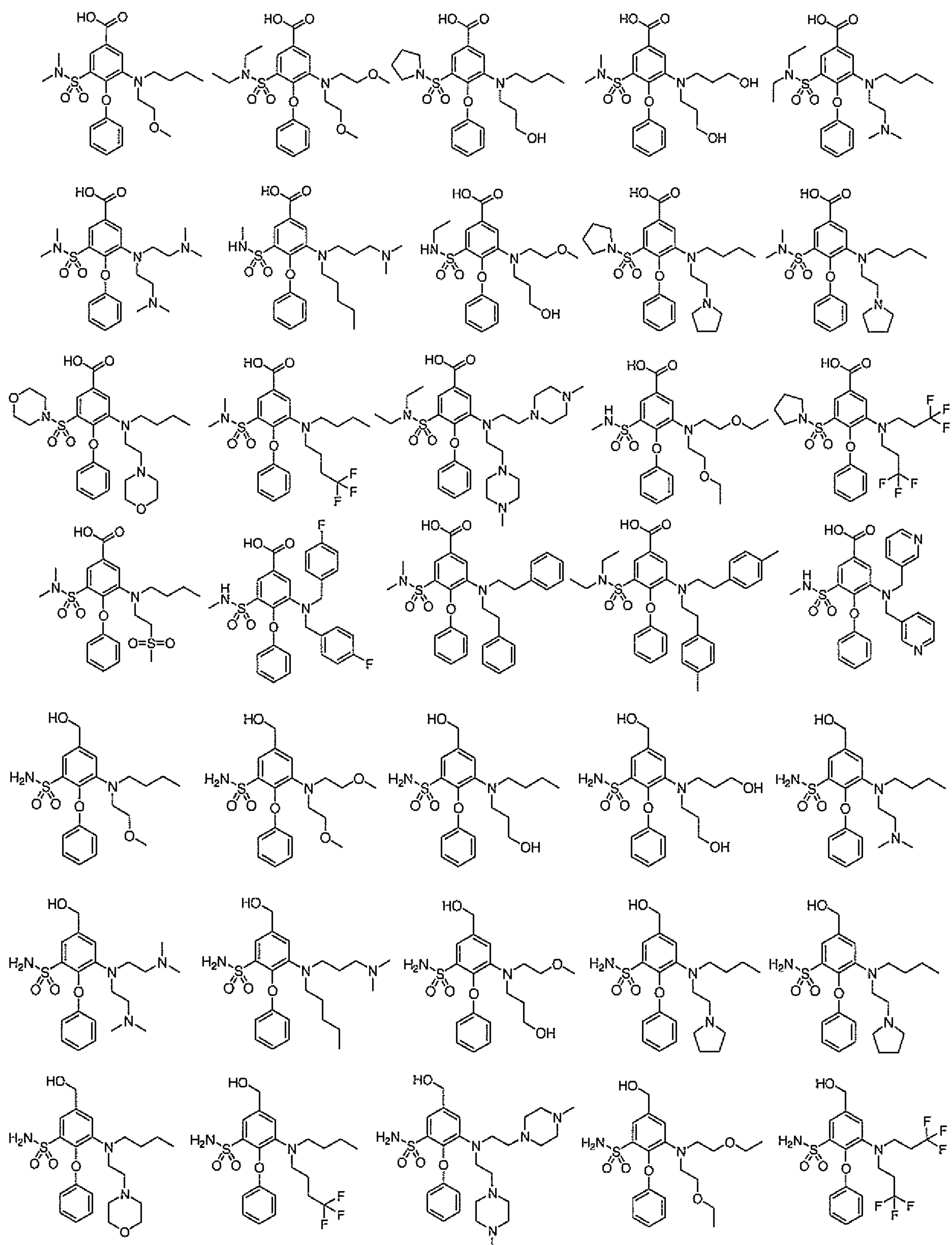
EXAMPLE 162

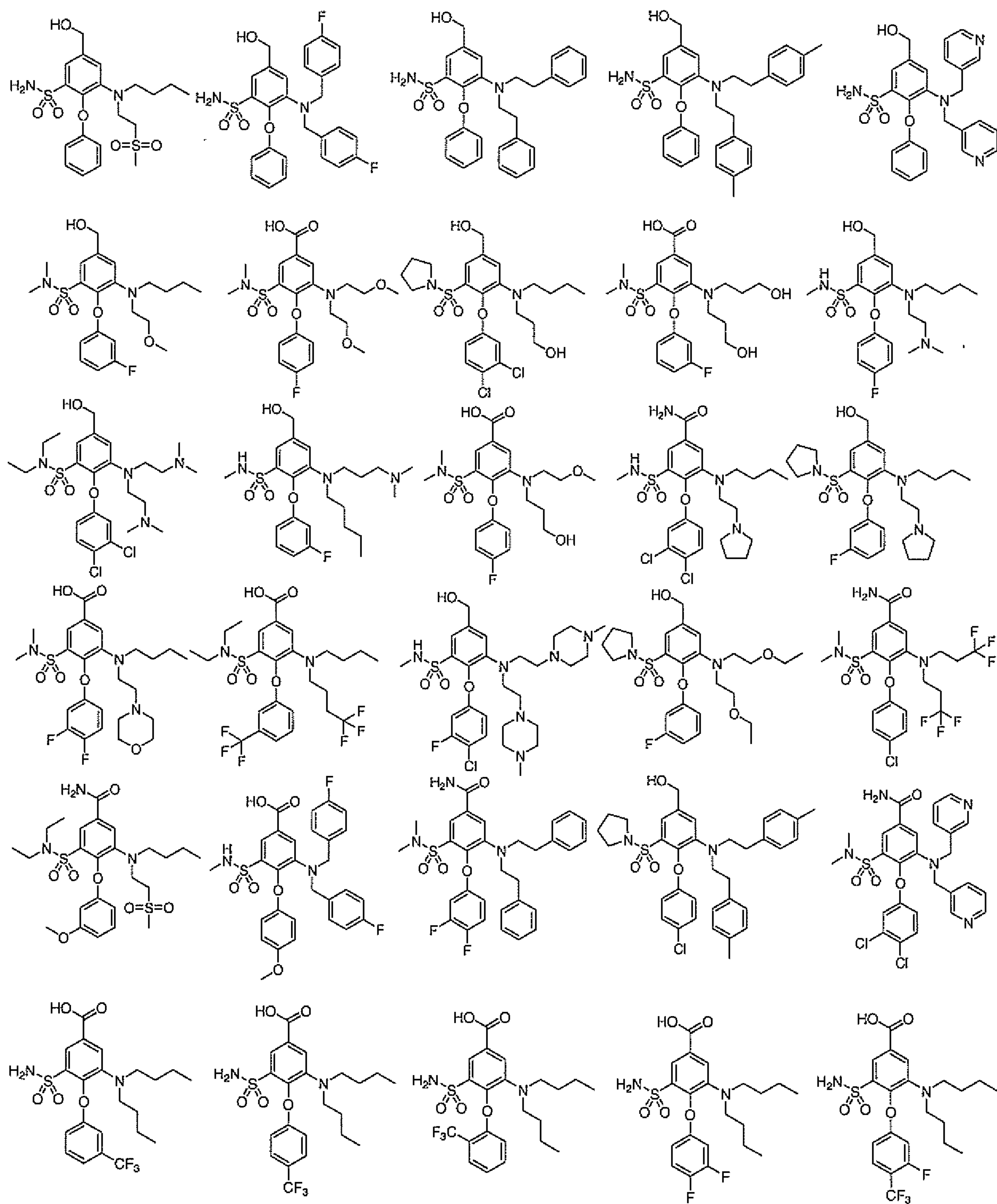
Additional Arylsulfonamide Compounds

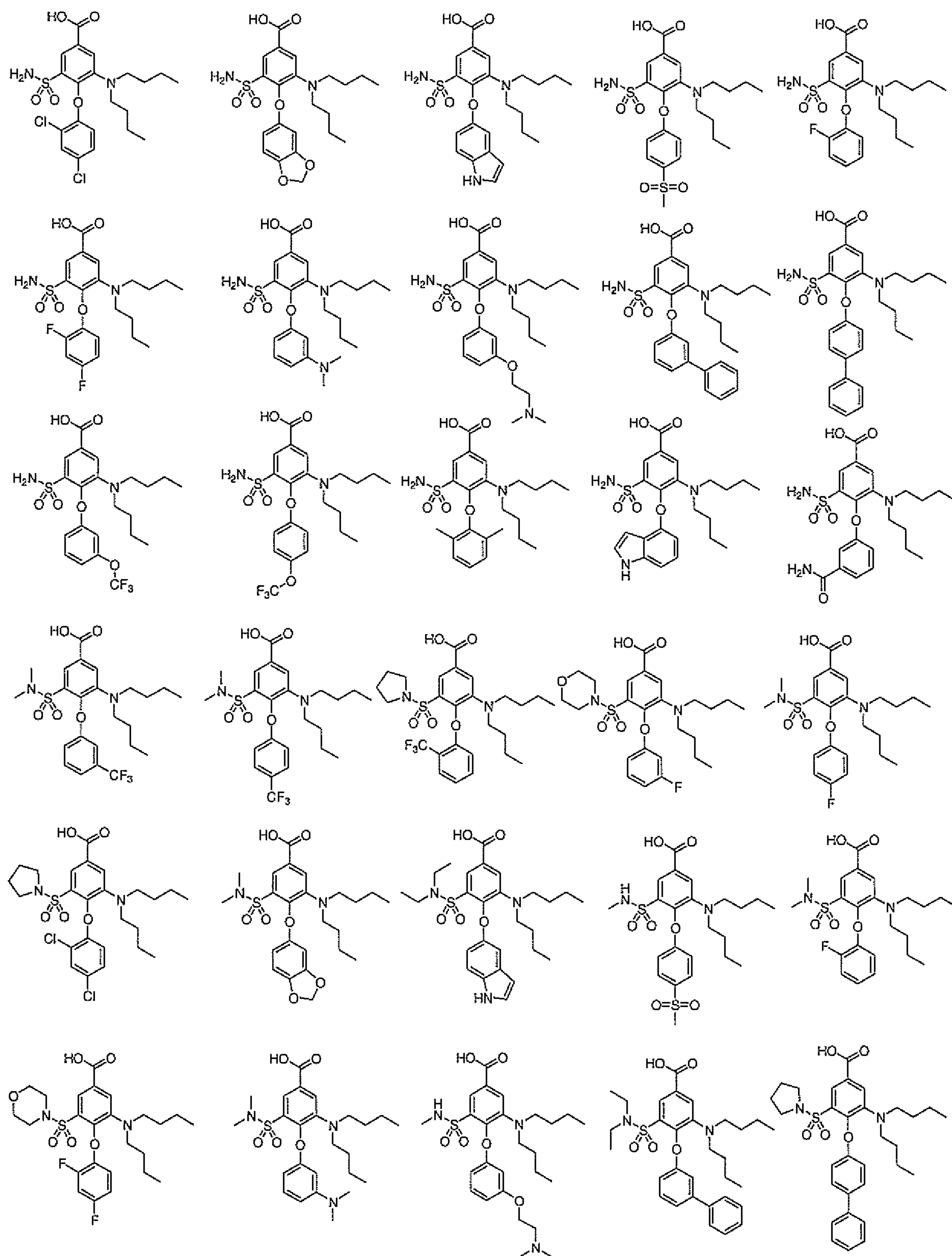
[0266] Additional arylsulfonamide compounds may be synthesized using standard methods. Following General Methods A through P or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples can be prepared from commercially available reagents.

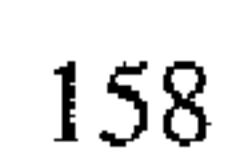


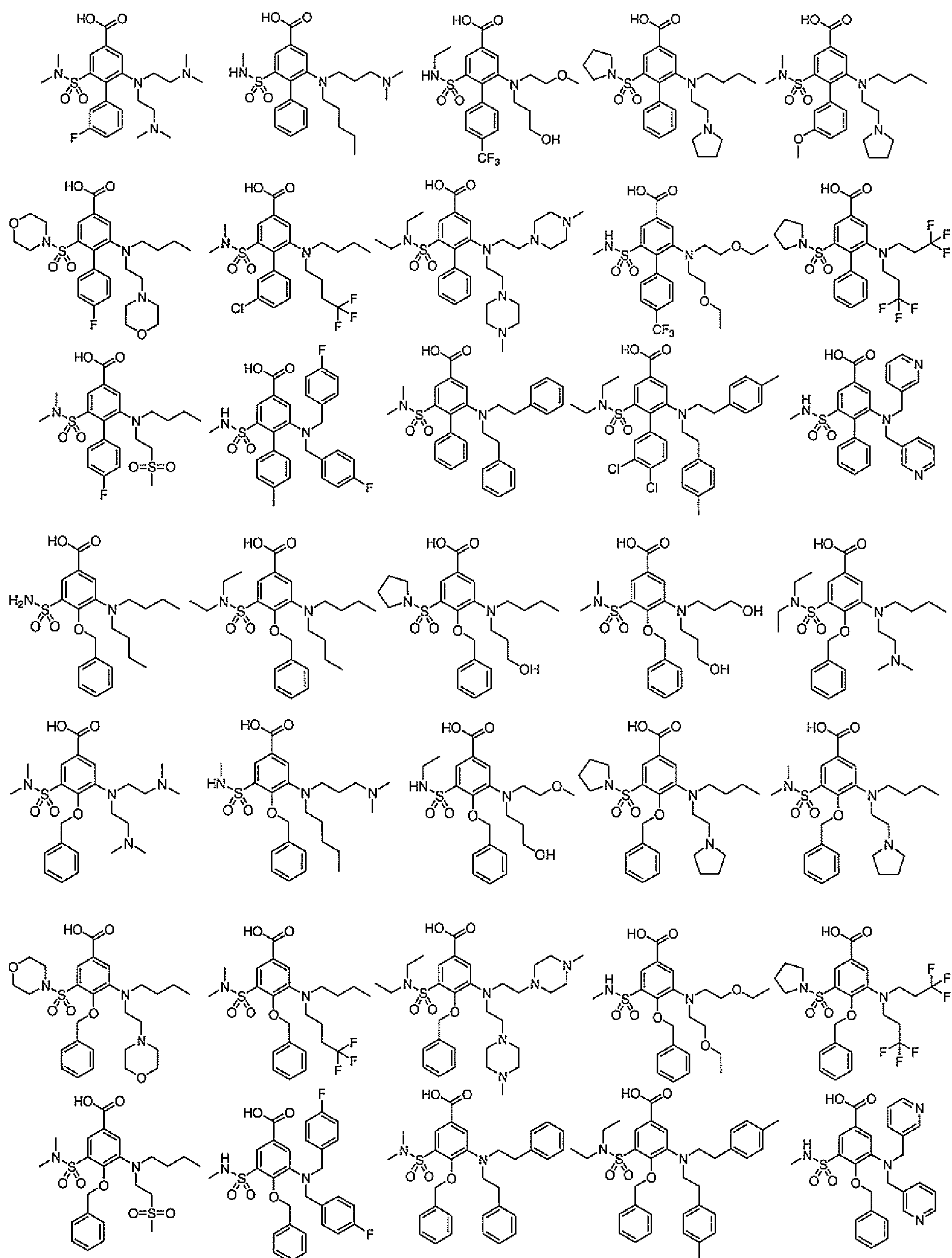






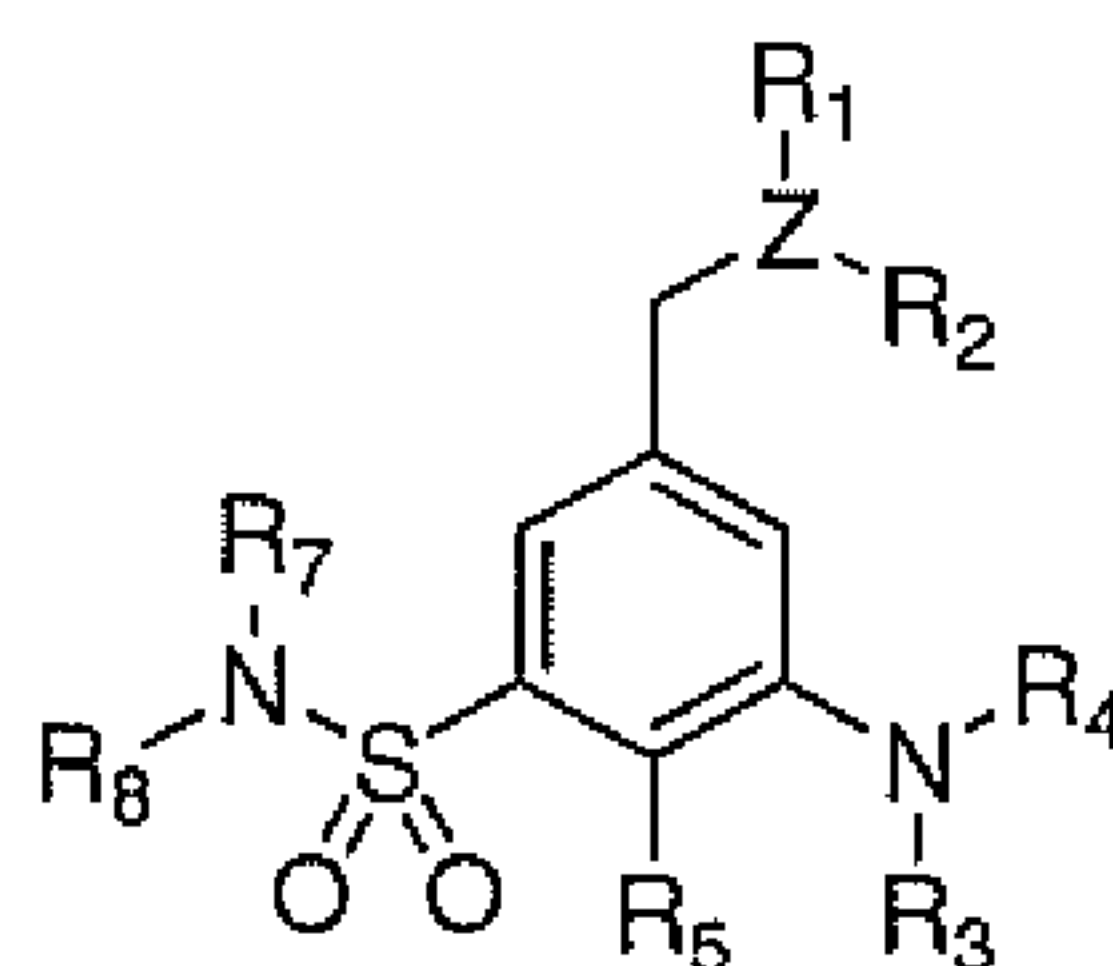




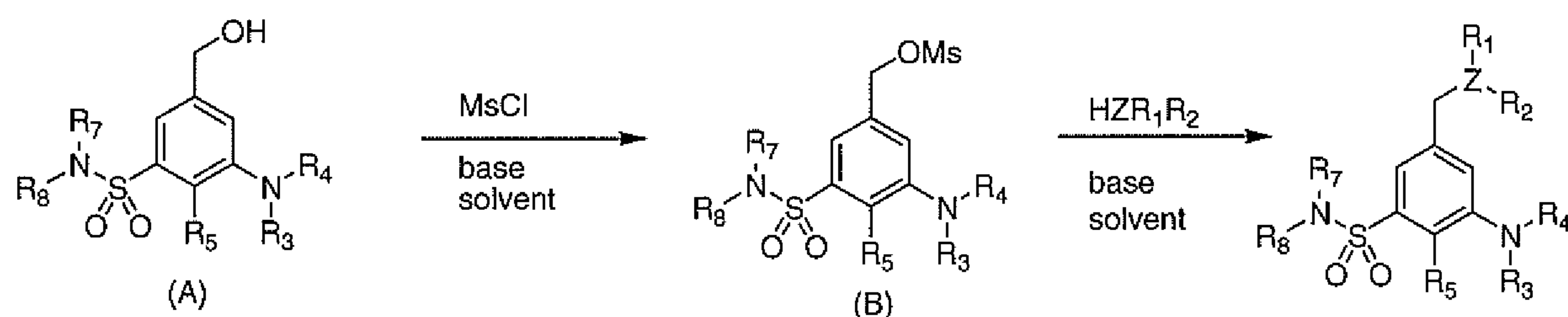


EXAMPLE 163**Benzylic Alcohol and Amine Compounds**

[0267] Additional arylsulfonamide compounds may be synthesized using standard methods. R_{1-8} are as defined herein. In addition to the above examples, compounds of the formula:

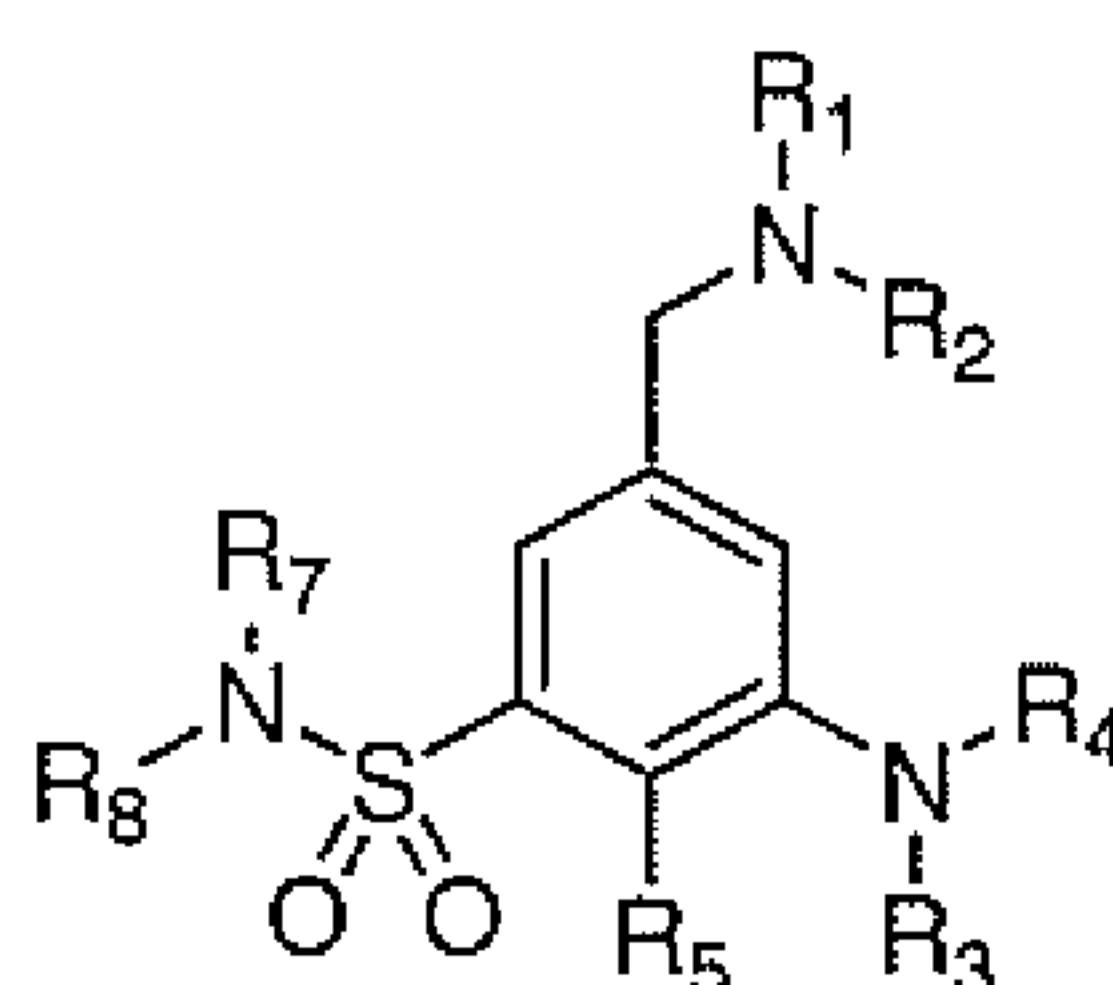


can be prepared following General Method Q or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples can be prepared from commercially available reagents.

General Method Q

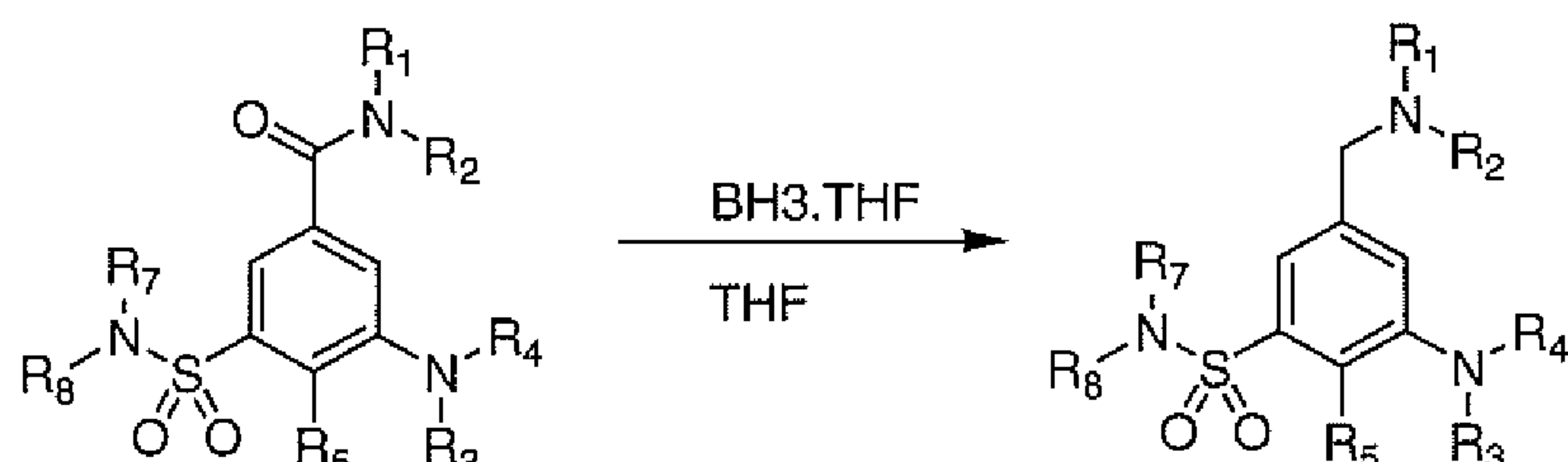
[0268] Primary alcohols (A) of the invention which can be made starting from benzoic acids of the invention following method P or with slight modifications thereof, can be converted to compounds with a suitable leaving group such as a mesylate, tosylate or triflate using procedures familiar to one of ordinary skill in the art such as reacting the primary alcohol with methanesulfonyl chloride in the presence of The converted alcohols can then be reacted with suitable alcohols or primary or secondary amines to provide in the presence of a suitable base such as triethylamine or diisopropylethylamine in a suitable solvent such as dichloromethane or N,N-dimethylformamide to provide compounds of the general formula.

[0269] Alternatively, compounds of the general formula:



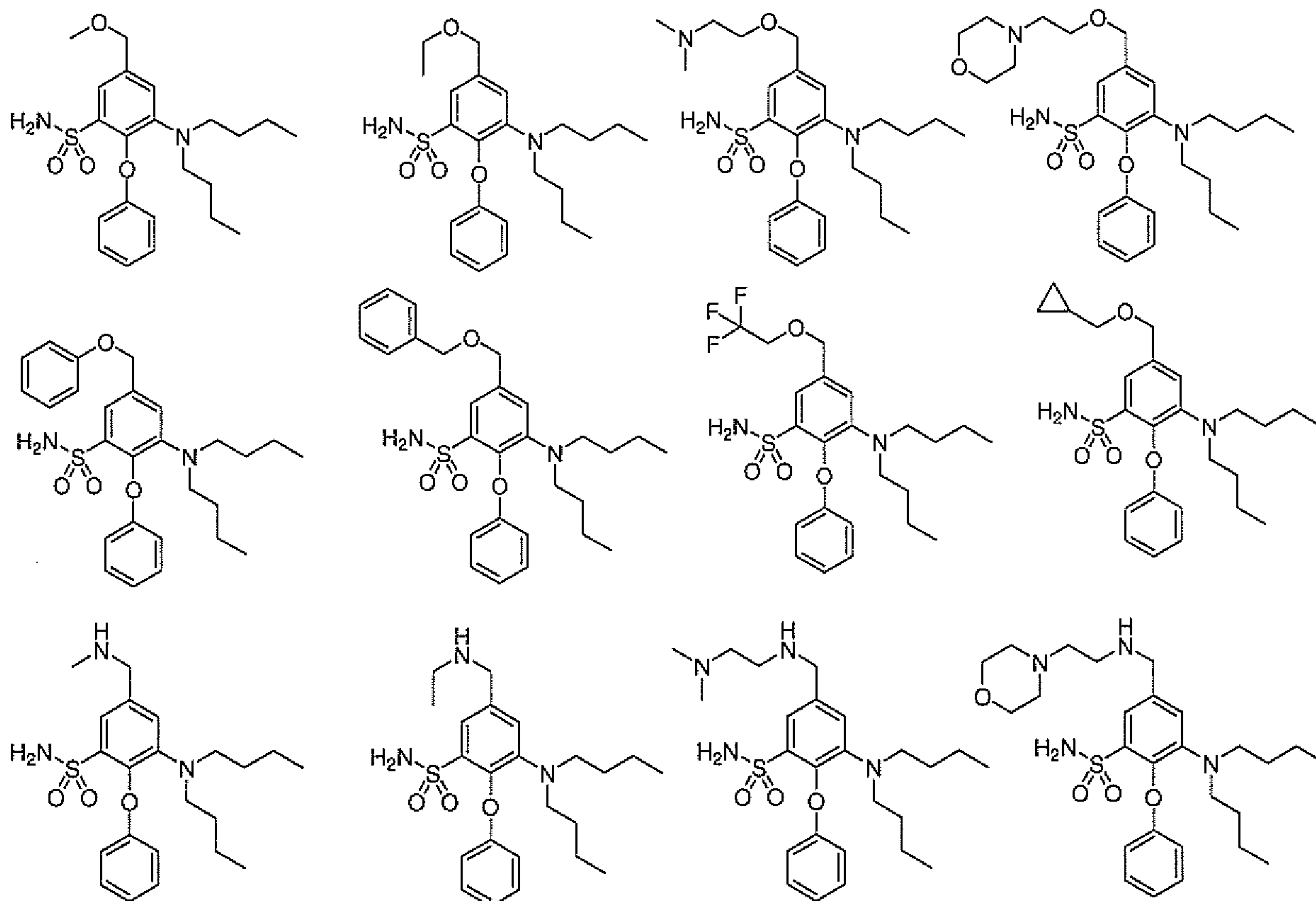
can be prepared following General Method R or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art.

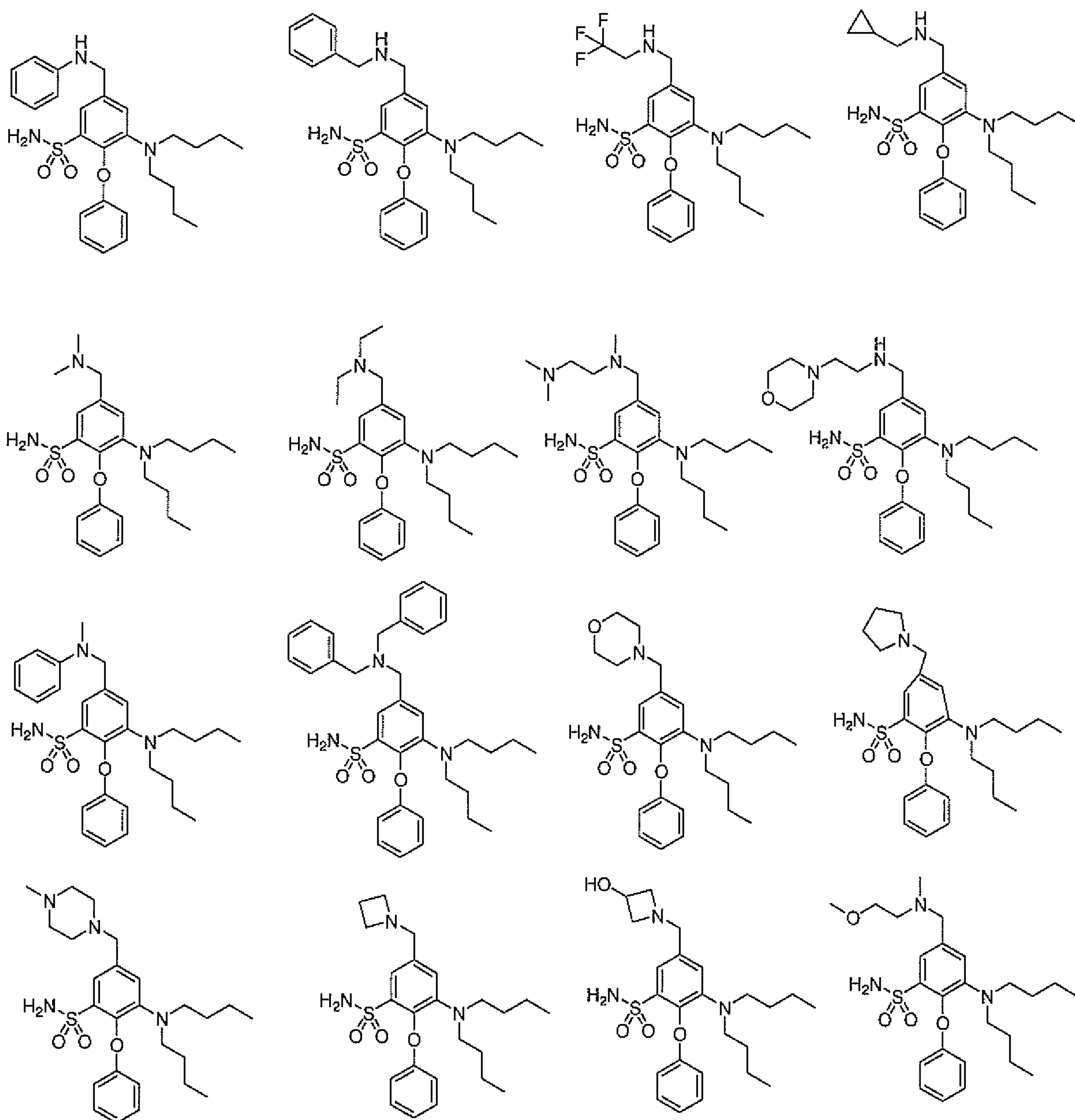
General Method R

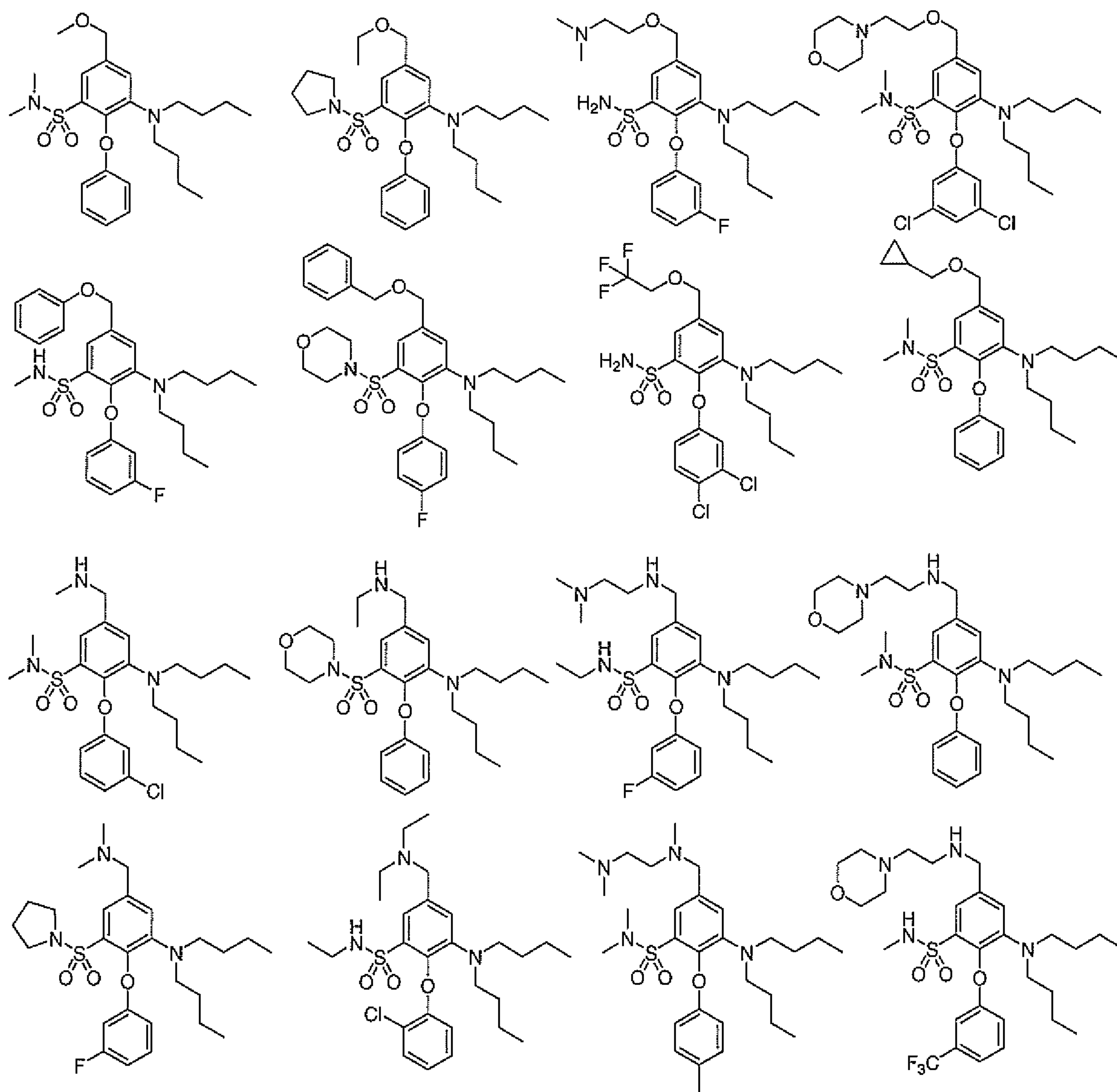


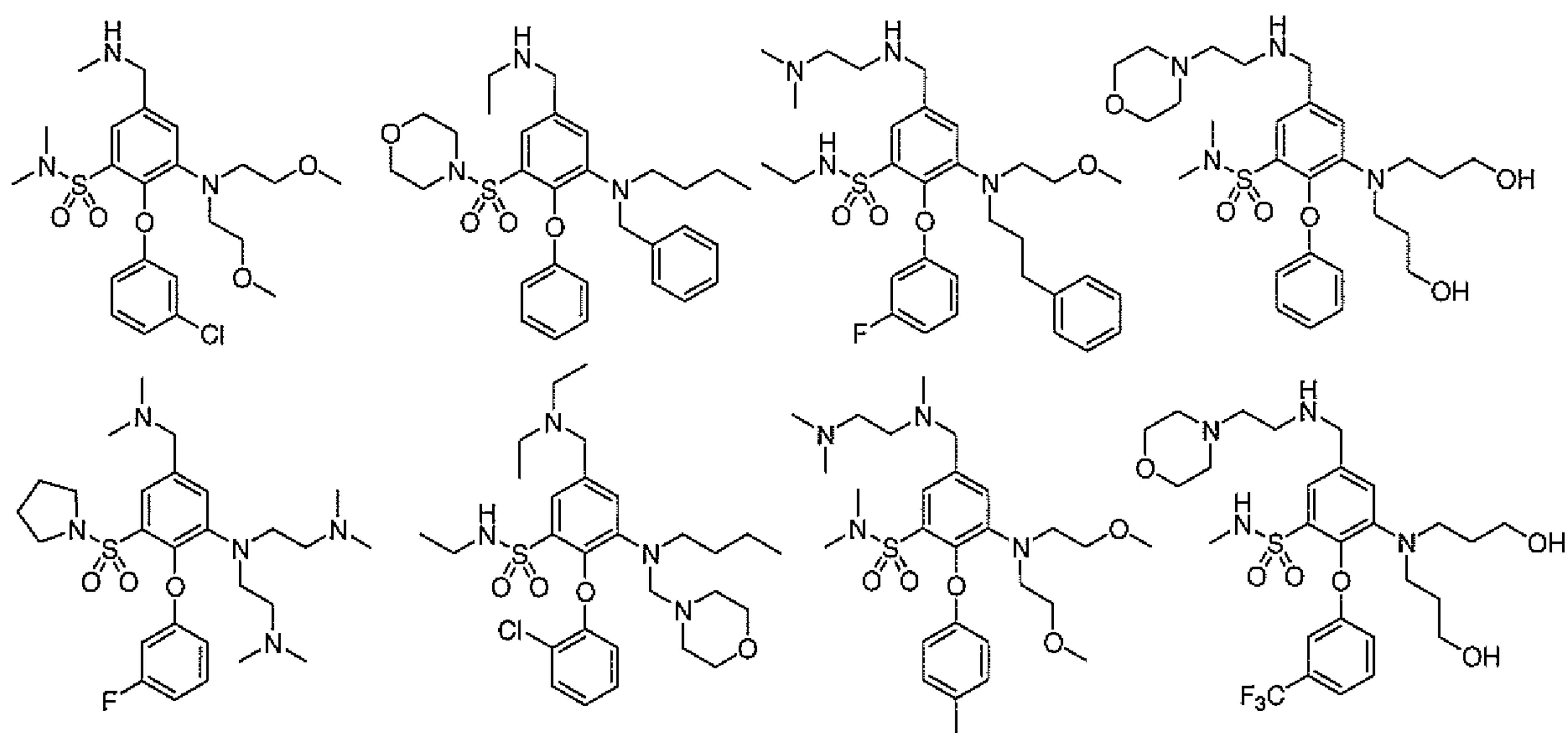
[0270] Amide compounds of the invention which can be prepared following Methods M through O or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art can be converted to primary and secondary amines by reaction with a suitable reducing agent such as borane-tetrahydrofuran in a suitable solvent such as tetrahydrofuran.

[0271] Following General Methods Q through R or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples can be prepared from commercially available reagents.

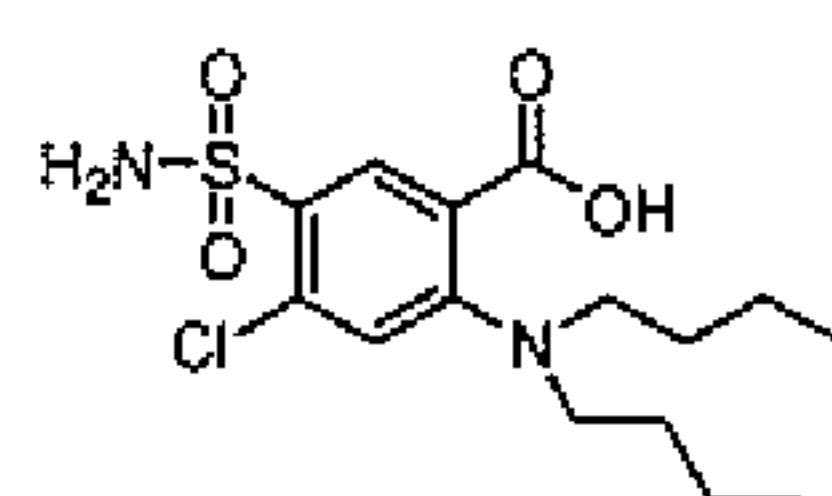




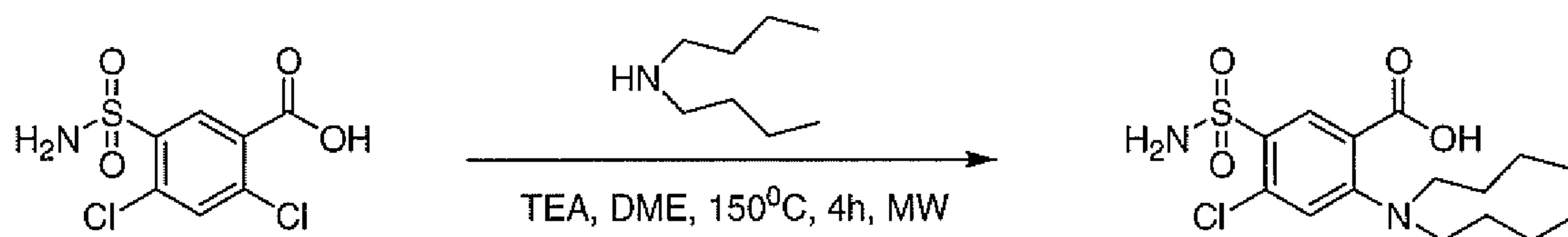




Example 164
4-chloro-2-(dibutylamino)-5-sulfamoylbenzoic acid
 NTP-9001



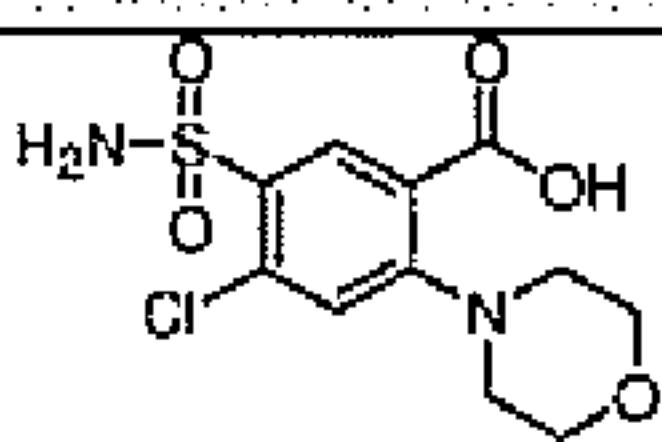
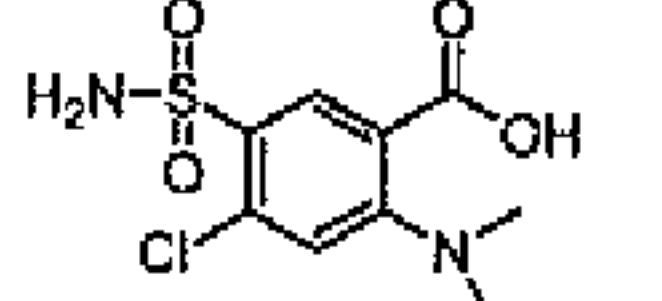
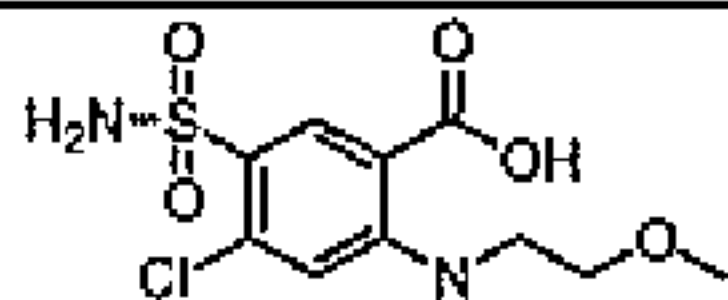
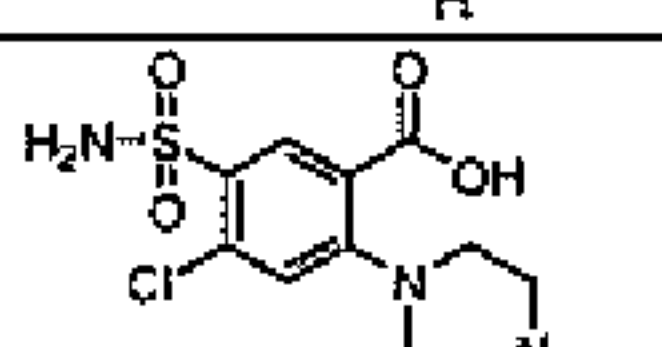
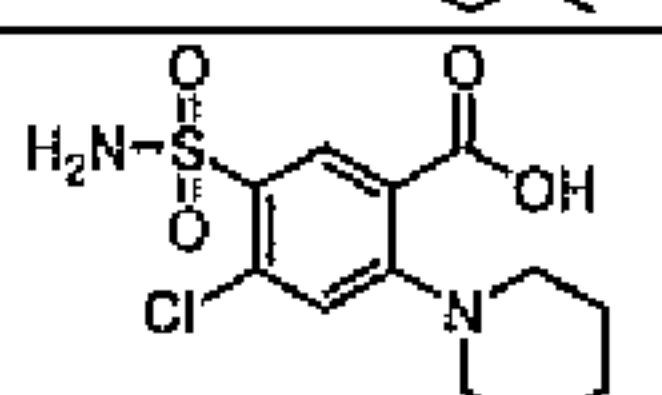
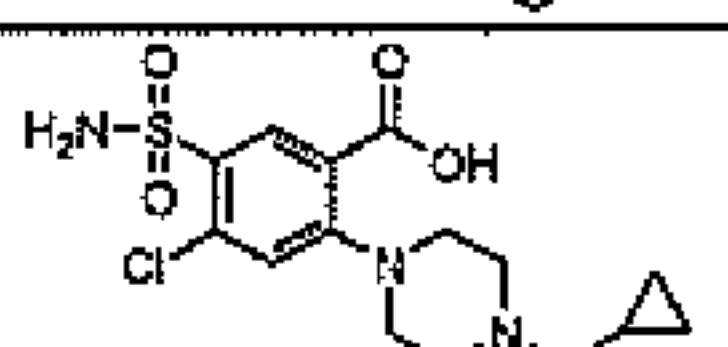
General Method S



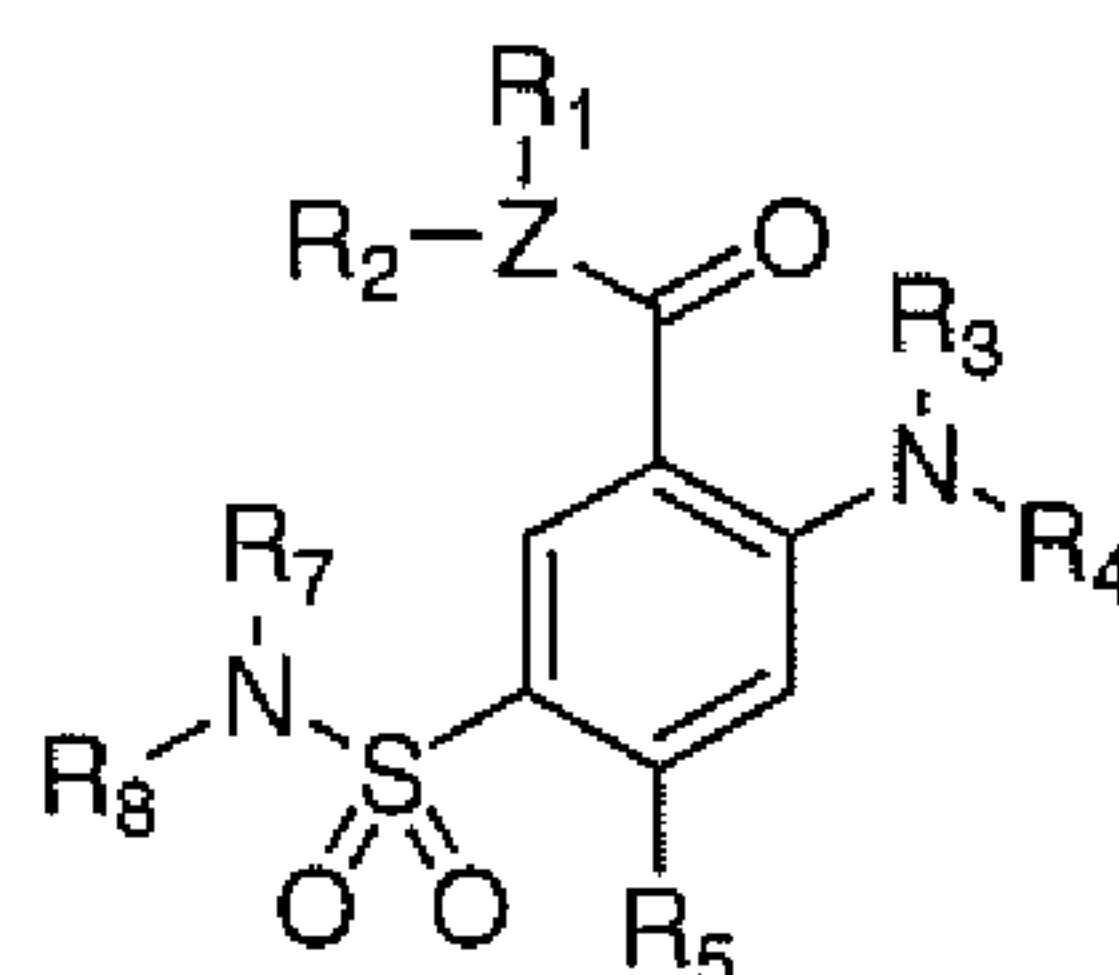
[0272] A microwave vial was charged with 2,4-dichloro-5-sulfamoyl-benzoic acid (200 mg, 0.741 mmol), triethylamine (0.4 mL), dibutyl amine (341 mg, 1.85 mmol) and dimethoxy ethane (2 mL) and the reaction mixture was heated in a microwave reactor at 150° for 4 hours. The reaction was cooled to room temperature, diluted with ethyl acetate and washed with water and brine. The organic solvents were removed under reduced pressure and the residue purified by flash chromatography to give the product as white solid (120 mg). ¹H NMR (300 MHz, DMSO-*d*₆): δ 8.18 (s, 1H), 7.49 (s, 2H), 7.42 (s, 1H), 3.22 (t, J=7.5 Hz, 4H), 1.44 – 1.38 (m, 4H), 1.27–1.18 (m, 4H), 0.83 (t, J=7.2 Hz, 6H). MS *m/z* : 363 (M+1)⁺.

[0273] Following General Methods S or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples were prepared from commercially available reagents.

TABLE 4

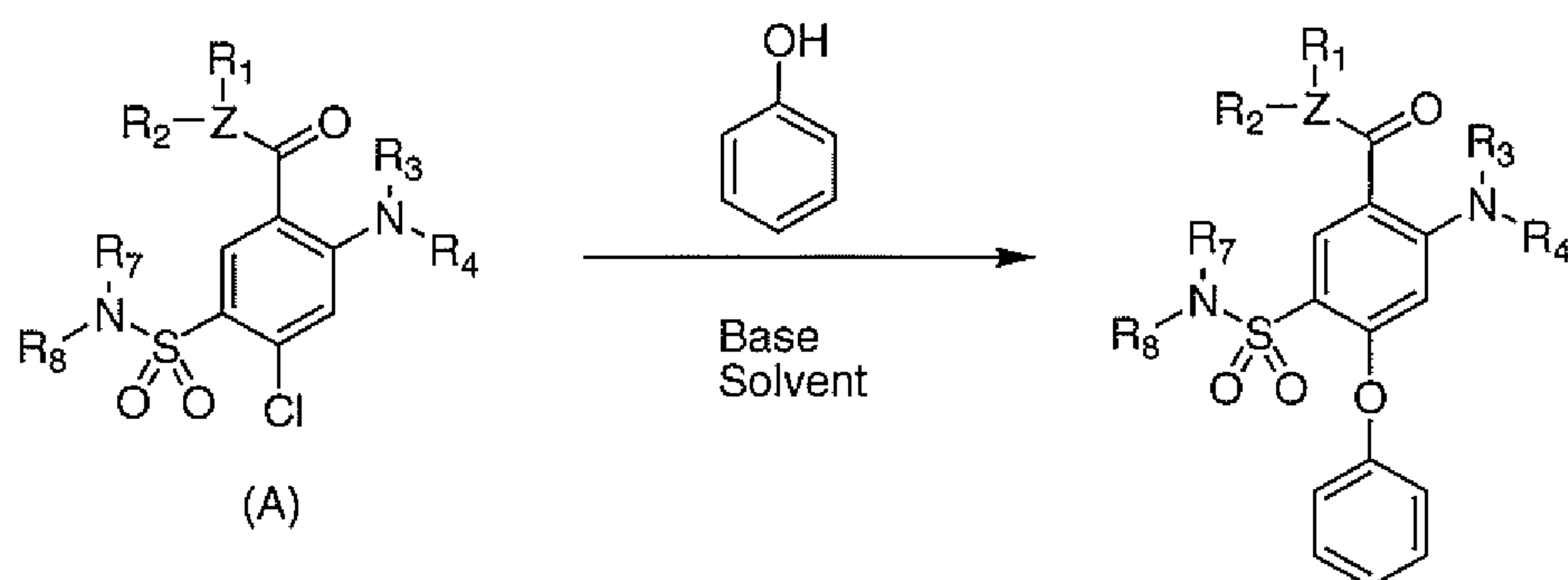
Example Number	NTP Number	Structure	M.W. calculated	Mass Spec. found (<i>m/z</i>)
165	NTP-9002		320.75	[M+1] ⁺
166	NTP-9003		278.71	279[M+1] ⁺
167	NTP-9004		308.74	309[M+1] ⁺
168	NTP-9005		333.79	334[M+1] ⁺
169	NTP-9006		318.78	319[M+1] ⁺
170	NTP-9007		373.86	374[M+1] ⁺

In addition the above examples, compounds of the formula:

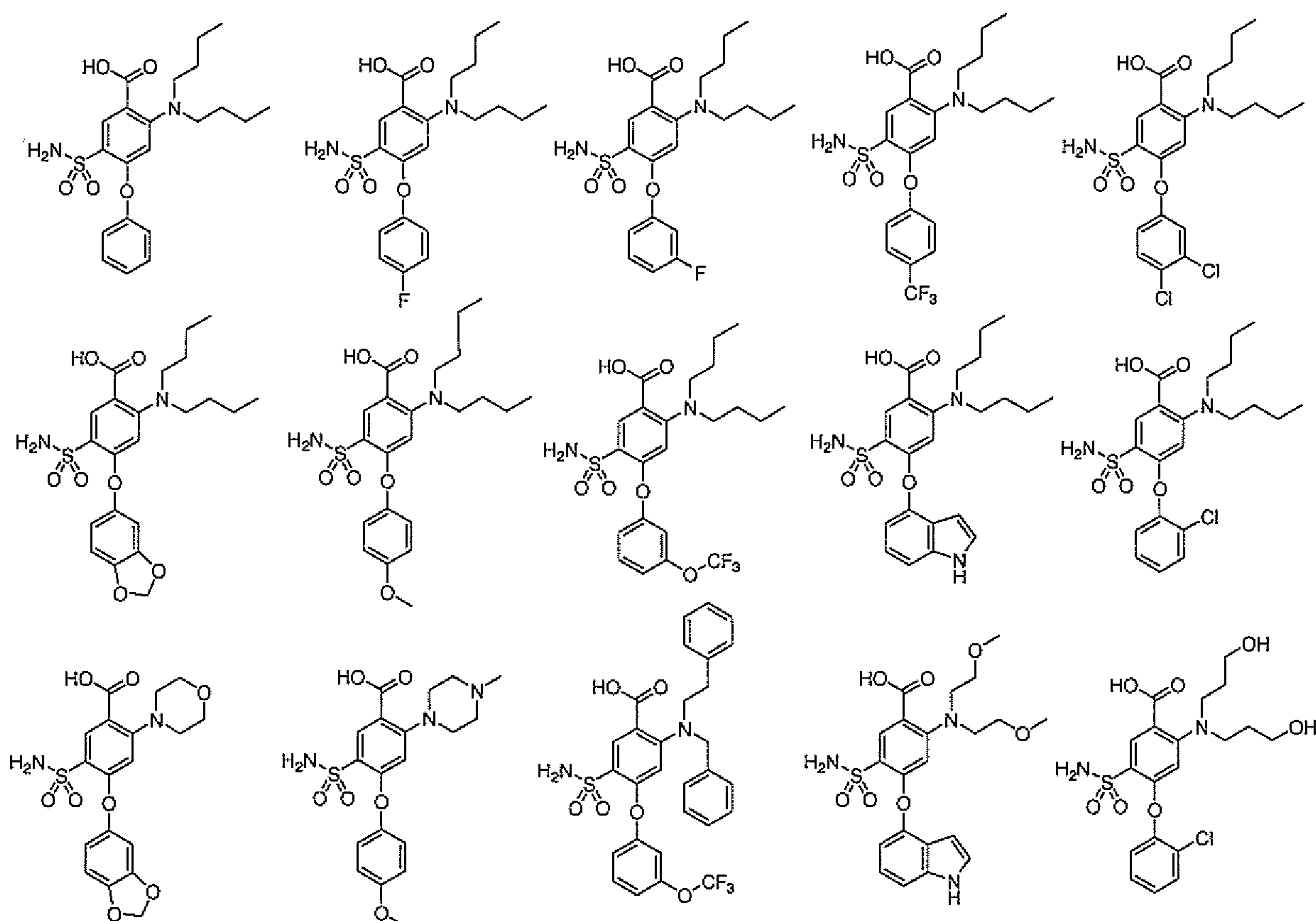


where R₅ is aryloxy, can be prepared following General Method T or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art, the following examples can be prepared from commercially available reagents.

General Method T



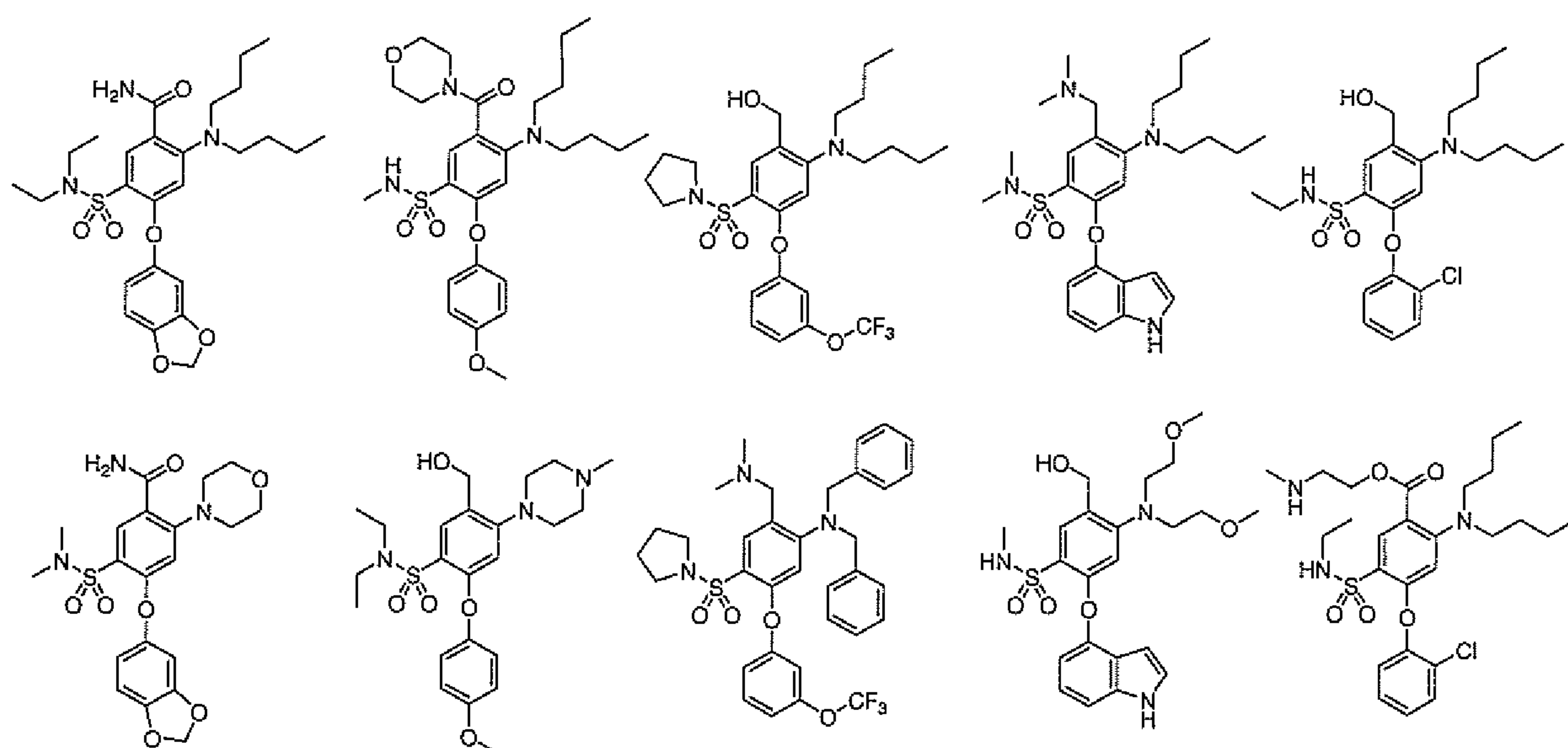
[0274] Aromatic chlorides (A) which can be prepared following General Method U or with slight modifications thereof, and following procedures familiar to one of ordinary skill in the art can further be reacted with substituted or unsubstituted aromatic phenols in the presence of a base such as potassium carbonate or sodium hydride in a suitable solvent such as water or N,N-dimethylformamide to produce compounds of the formula. Following General Methods S and T the following compounds can be prepared from commercially available reagents.



The image displays 20 chemical structures of substituted benzothiazine derivatives, arranged in a 5x4 grid. Each structure features a central benzothiazine core with various substituents. The substituents include:

- Phenyl rings:** Some structures have a phenyl ring attached to the benzothiazine core via an ether linkage.
- Fluorine (F) and Chlorine (Cl) atoms:** These halogens are attached to the phenyl rings in several structures.
- Trifluoromethoxy (OCF₃) and Methoxy (OMe) groups:** These groups are attached to the phenyl rings in several structures.
- Indole rings:** Some structures have an indole ring attached to the benzothiazine core via an ether linkage.
- Nitrogen-containing groups:** Some structures have a nitrogen-containing group (e.g., a morpholine ring) attached to the benzothiazine core.
- Sulfonamide groups:** Some structures have a sulfonamide group (SO₂NH-) attached to the benzothiazine core.

The structures are numbered 1 through 20, corresponding to the entries in the table above.



Example 171 Formulations for CNS-Targeted Drugs

[0276] Oral Preparations. The compounds as described herein may be formulated for oral administration. Exemplary oral preparations comprise a compound described herein in the range of about 10–60 mg of drug substance together with various inactive ingredients such as microcrystalline cellulose and other excipients, contained in a gelatin capsule. Alternatively, the active drug substance may be provided in tablet form, including about 10–60 mg of drug substance with microcrystalline cellulose, hydroxypropyl cellulose, magnesium stearate and other excipients.

[0277] Additionally, for oral administration, the compounds described herein may be used in the range of about 10–100 mg/kg, together with various inactive ingredients such as microcrystalline cellulose and other excipients, contained in a gelatin capsule. Alternatively, the compounds described herein may be provided in tablet form, including about 10–100 mg/kg with microcrystalline cellulose, hydroxypropyl cellulose, magnesium stearate and other excipients.

[0278] Intravenous Preparations. The compounds as described herein may be formulated for intravenous administration. In an exemplary intravenous formulation, each milliliter of sterile solution can include about 1–25 mg of a compound as described herein with about 20–40% propylene glycol, about 0–10% ethyl alcohol, optionally water, buffers (*e.g.*, about 5% sodium benzoate and benzoic acid as buffers), and preservatives (*e.g.*, about 1.5% benzyl alcohol as a preservative.)

[0279] Also, for intravenous administration, each milliliter of sterile solution can include about 1–25 mg/kg of a compound as described herein formulated with about 20–40% propylene glycol, about 0–10% ethyl alcohol, optionally water, buffers (*e.g.*, about 5% sodium benzoate and benzoic acid as buffers) and preservatives (*e.g.*, about 1.5% benzyl alcohol as a preservative.)

Example 172

In Vitro Pharmacology: Na⁺K⁺Cl[−] Cotransporter Assay

[0280] The effects of selected compounds were assessed for their effect on the Na⁺K⁺Cl[−] Cotransporter *in vitro*. See Gamba (2005) *Physiol. Rev.* 85: 423–493.

[0281] **Material.** The Normal Human Dermal Fibroblasts (NHDF), Fibroblast Basal Medium (FBM), FGM-2 bullet kit were purchased from LONZA (C-2511). For the experiments the 3 following buffers were made: Buffer A: 5 mM KCl, 0.8 mM MgSO₄, 5 mM Glucose, 25 mM HEPES/TRIS pH 7.4. Buffer B: Buffer A with 127, 3 mM Choline Chloride, 1.63 mM CaCl₂, 0.9 mM ouabaine (Sigma, O3125) and 1 ng/ml bFGF. Buffer C: Buffer A with 140 mM NaCl, 1.8 mM CaCl₂, 1 mM Ouabaine, bFGF 1 ng/mL and 5 μCi/mL [⁸⁶Rb] (Perkin Elmer, NEZ072).

[0282] **Method.** After 2 weeks of cell culture in the reconstituted cell culture medium (FBM with the FGM-2 bullet kit), cells were seeded in white 96 well cell culture plates at 3000 cells per well and incubated overnight at 37°C (5% CO₂). The following day, the cell culture medium was replaced by a medium with 0.2% FCS (low serum) and incubated for 48h at 37°C (5% CO₂). Then, bFGF was added in each well at a final concentration of 1 ng/mL and cells were incubated 1 hour at 37°C (5% CO₂). The cells were then washed in buffer B for 15 minutes at 37°C. The buffer B was then replaced by compounds diluted in Buffer C. After an incubation of 20 minutes at 37°C, each well was washed with a cold MgCl₂ (0.1M) solution. Subsequently, 40μL of scintillation fluid (Microscint-40, Canberra Packard) was added to each well. Plates were sealed and incubated overnight at RT. The radioactivity was counted in a 96-well plate counter. The uptake was measured as the percentage of Bumetanide-sensitive ⁸⁶Rb influx. All experiments were performed in duplicate. See also Chassande, *et al.* (1988) *Eur. J. Biochem.* 171: 425–433.

[0283] Bumetanide (30 μM) was used a positive control compound to establish a specific activity. The results were expressed as a percent of control specific activity [100X(⁸⁶Rb influx with compound–⁸⁶Rb influx with bumetanide)/⁸⁶Rb influx in Buffer C–⁸⁶Rb influx with bumetanide] and as a percent inhibition of control specific activity [100–(percent/control specific activity)] obtained

in the presence of the compounds listed herein in Table 18. These results were expressed as % inhibition at 10 μ M.

TABLE 5

Compound	% of Control Value	% Inhibition of Control Values
3001	124.6	<i>none</i>
3002	85.3	14.7
3003	95.6	4.4
3005	107.1	<i>none</i>
3006	98.9	1.1
3007	60.7	39.3
3008	96.5	3.5
3009	121.8	<i>none</i>
3010	120.3	<i>none</i>
3011	115.5	<i>none</i>
3012	95.4	4.6
3013	115.1	<i>none</i>
3014	125.2	<i>none</i>
3015	107.7	<i>none</i>
3016	117.6	<i>none</i>
3017	132.8	<i>none</i>
3018	131.3	<i>none</i>
3019	71.3	28.7
3020	122.4	<i>none</i>
3021	110.5	<i>none</i>
3022	150.5	<i>none</i>
3023	133.1	<i>none</i>
3024	47	53
3025	82.5	17.5
3026	101.4	<i>none</i>
3027	76.7	23.3
3028	79.5	20.5
3029	97.1	2.9
4002	75.5	24.5
3032	68.2	31.8
3033	90	10
3034	99.4	0.6
3035	93.1	6.9
3036	85.5	14.5
3037	103.9	<i>none</i>
3038	96.8	3.2
3039	108.7	<i>none</i>
3040	66.4	33.6
3041	71.2	28.8
3042	110.9	<i>none</i>
3043	102.6	<i>none</i>
4001	104.6	<i>none</i>

6001	115.7	<i>none</i>
6002	84.8	15.2
7001	115.7	<i>none</i>
7002	99.6	0.4
7003	64.6	35.4
7004	102.6	<i>none</i>
7005	117.9	<i>none</i>
7006	112.4	<i>none</i>

[0284] Compounds showing “% Inhibition of Control Values” between 20 and 50% were indicative of weak to moderate inhibition of NKCC activity. Compounds showing “% Inhibition of Control Values” lower than 20% were indicative of no significant difference between the compound as described herein and vehicle control values. Many of the compounds show little or no inhibition of NKCC, while some show moderate inhibition. None of these compounds would be expected to have diuretic activity comparable to bumetanide.

Example 173

Testing Compounds for Diuretic Effects including cumulative urine volume, sodium excretion, and potassium excretion

[0285] **Purpose.** Renal function assessments, including cumulative urine volume, sodium excretion, and potassium excretion is measured in animals administered compounds, and vehicle controls over 6 hours post-dose.

[0286] **Methods.** The animals are fasted overnight prior to dosing without access to drinking water prior to any pretreatment. Food and water are withheld until after the terminal sample collection or for the first 6 hours of blood sample collection, where applicable. Prior to test compound administration, all animals are pretreated with a single IP dose of PBx. At approximately 5 to 6 minutes prior to dosing, all animals may receive a single oral (PO) gavage dose of 0.9% Sodium Chloride for Injection, USP, at a dose volume of 15 mL/kg. The vehicle, diuretic (control) [(e.g., bumetanide, furosemide, piretanide, azosemide, and torsemide)], DMSO, and the test compounds are administered via a single IP dose at a dose volume of 1 mL/kg. Cumulative urine volume, sodium excretion, and potassium excretion may be measured.

[0287] Test compounds showing less cumulative urine volume, sodium excretion, and/or potassium excretion in comparison to a diuretic control may show less diuretic side effects when used clinically.

Example 174

In vitro Molecular Tests of Select Bumetanide Derivatives on GABA_A Receptor Isoforms

Experimental design for selectivity screen

[0288] The addition of GABA to GABAergic cells activates the recombinant expressed GABA_A receptors, creating an ion movement through the ion channel in the GABA_A receptor. The electrical current generated by the movement of chloride ions into the cells can be quantified.

[0289] Experiments are conducted examining the activity of α_4 -subunit containing GABA_A receptors activated by 10 μ M GABA in the presence and absence of 10 μ M test compound. HEK-293T cells are transiently transfected with rat or human GABA_A receptor subunits. Whole-cell patch clamp recording is performed at -50 mV unless otherwise indicated. Test compounds are diluted from a freshly made stock in DMSO, GABA is prepared from a frozen stock. For each experiment GABA or GABA + a test compound is applied for 5 seconds, and the electrical current generated by the movement of chloride ions into the cells are measured and recorded as a trace of current versus time.

[0290] In FIGURES 4–8, 100% response to GABA denotes no inhibition of GABA_A receptors. Any value below 100% was indicative of inhibition of GABA_A receptors by a test compound.

Compounds described herein were tested at 10 μ M GABA concentrations against α_1 , α_4 , α_5 , and α_6 GABA_A receptor isoforms. Many of the compounds described herein show inhibition of α_4 , α_5 , and α_6 GABA_A receptor activity but not α_1 GABA_A receptor activity. Therefore compounds described herein show activity as selective α_4 -subunit containing GABA_A receptor inhibitors.

Classic GABAergic drugs

[0291] Classic GABAergic drugs (*e.g.*, benzodiazepines) are non-selective agonists that increase both the amplitude and time course of inhibitory currents in GABA_A receptor. As shown in FIGURE 2, at (A) FRISIUM (clobazam) an anticonvulsant, (B) AMBIEN (zolpidem) a sleep aid, and (C) VALIUM (diazepam) an anxiolytic drug, all increase both the amplitude and time course of inhibitory currents in GABA_A receptor. GABA_A receptor agonists activate GABA_A receptors at low GABA concentrations, and while effective, also induce CNS side effects including sedation, decreased respiration, decreased cognition, and impaired motor function.

Acylsulfonamide Derivatives

[0292] Compounds described herein were tested at 10 μ M GABA concentrations against multiple GABA_A receptor isoforms (*e.g.*, $\alpha_1\beta_3\gamma_2$, $\alpha_4\beta_3\gamma_2$, $\alpha_5\beta_3\gamma_2$, $\alpha_6\beta_3\gamma_2$). Several of compounds described

herein selectively antagonize the α_4 GABA_A receptor isoform by inhibiting currents in the α_4 GABA_A receptor isoform. Several of the compounds may act as noncompetitive inhibitors. Compounds described herein may inhibit the currents in the parasynaptic α_4 GABA_A receptor isoform in GABAergic interneurons. Compounds described herein may inhibit α_4 GABA_A receptors *pre-synaptically*. Further, preferred compounds described herein will not active α_1 GABA_A receptors post-synaptically.

$\alpha_1\beta_3\gamma_2$ GABA_A receptor isoform

[0293] The α_1 subunit is the predominant α subunit in GABA_A receptors in the adult brain. The α_1 -containing receptors showed no significant activation in response many of the compounds described herein. In general, the amplitude of the current associated with GABA activation of these receptors is not affected by preferred compounds, with a mixture of increased decay time and decreased amplitude seen at the highest concentration (10 μ M) but no alteration at lower concentrations. This is in contrast to the significantly increased positive modulation seen with action of benzodiazepines and other classic GABA-ergic agents.

$\alpha_1/\alpha_4/\alpha_5/\alpha_6$ GABA_A receptor isoforms

[0294] In cells transfected with GABA_A receptor isoforms containing α_5 subunit subtype, 10 μ M NTP-3032, NTP-3033, NTP-3034, NTP-6002, and NTP-6008 inhibited the receptor compared to the control condition in the presence of 10 μ M GABA alone. The results are shown in FIGURE 3.

α_4/α_6 GABA_A receptor isoforms

[0295] Receptors containing the α_4 and α_6 subunits are inhibited by NTP-3032, NTP-3033, NTP-3034, NTP-6002, and NTP-6008 at 10 μ M. These results are shown in Figure 3. These compounds demonstrated marked inhibition of these receptor isoforms compared to the control condition in the presence of 10 μ M GABA alone. Additionally, these compounds demonstrated little effect at the post-synaptic α_1 -containing GABA_A receptors under the same experimental conditions.

[0296] Several of the compounds described herein (*e.g.*, 3032, 3033, 3034, 4012, 4015, 5007, 5008, 5009, 5010, 5011, 5013, 5016, 6001, 6002, 6006, 6008, 6009, 6011, 6012, 7021, and 10001) show selective inhibition on α_4 and α_6 GABA_A receptor isoforms.

[0297] Compounds described herein appear to have a different mechanism of action upon the GABA_A receptors than traditional GABA agonist drugs. The primary effect is an inhibition of

chloride current reducing total GABA “drive.” It is seen best at α_4 and α_6 , α_5 and the inhibition is consistent with noncompetitive antagonism, *i.e.*, open channel block.

[0298] The activity of compounds described herein may exhibit two features—inhibition of receptors containing specific subunits, *e.g.*, α_4 , and through a lack of positive modulation of receptors containing α_1 , *e.g.*, traditional GABAergic mechanisms such as benzodiazepines. Increased action via an increase of GABA release at synapses would lead to increased inhibition via a pre-synaptic mechanism and thus would be expected to lead to a decrease in anxiety and seizure frequency. Compounds whose activity shows these features may also reduce pain, especially neuropathic pain. Inhibition is the only effect observed at α_4 , α_5 , and α_6 -containing receptors. Both these effects may require a γ subunit, as δ -containing receptors are believed to be unaffected by compounds described herein.

Examples 175–179

Assessment of Therapeutic Potential of Compounds in Alleviating Anxiety

Example 175

Fear Potentiated Startle Paradigm

[0299] **Purpose.** To evaluate the effects of test compounds in models of anxiety in rats, test compounds are assessed in the fear potentiated startle paradigm (FPS) model of anxiety.

[0300] **FPS Design.** FPS model is a commonly used assessment of therapeutic value of anxiolytic compounds in the rat. Rats may receive a 30 min period of habituation to the FPS apparatus. 24-hr later baseline startle amplitudes are collected. The rats will be divided into two matched groups based on baseline startle amplitudes. Following baseline startle amplitude collection, 20 light/shock pairings are delivered on 2 sessions over 2 consecutive days (*i.e.*, 10 light/shock pairings per day). On the final day, one group of rats may receive an injection (*i.v.*) of a test compound and the other group may receive vehicle only. Immediately following injections, startle amplitudes are assessed during startle alone trials and startle plus fear (light followed by startle) trials. Fear potentiated startle (light+startle amplitudes minus startle alone amplitudes) are compared between the treatment groups.

[0301] Animals are trained and tested in four identical stabilimeter devices (Med-Associates). Briefly, each rat was placed in a small Plexiglas cylinder. The floor of each stabilimeter consists of four 6-mm-diameter stainless steel bars spaced 18 mm apart through which shock can be delivered. Cylinder movements result in displacement of an accelerometer where the resultant voltage is

proportional to the velocity of the cage displacement. Startle amplitude is defined as the maximum accelerometer voltage that occurs during the first 0.25 sec after the startle stimulus are delivered. The analog output of the accelerometer is amplified, digitized on a scale of 0–4096 units and stored on a microcomputer. Each stabilimeter is enclosed in a ventilated, light-, and sound-attenuating box. All sound level measurements are made with a Precision Sound Level Meter. The noise of a ventilating fan attached to a sidewall of each wooden box produces an overall background noise level of 64 dB. The startle stimulus are a 50 ms burst of white noise (5 ms rise-decay time) generated by a white noise generator. The visual conditioned stimulus (“CS”) used is illumination of a light bulb adjacent to the white noise source. The unconditioned stimulus is a 0.6 mA foot shock with duration of 0.5 sec, generated by four constant-current shockers located outside the chamber. The presentation and sequencing of all stimuli is under the control of the microcomputer.

[0302] FPS procedures consists of 5 days of testing; during days 1 and 2 baseline startle responses are collected, days 3 and 4 light/shock pairings are delivered, day 5 testing for fear potentiated startle was conducted.

[0303] **Matching.** On the first two days all rats are placed in the Plexiglas cylinders and 3 min later presented with 30 startle stimuli at a 30 sec interstimulus interval. An intensity of 105 dB is used. The mean startle amplitude across the 30 startle stimuli on the second day is used to assign rats into treatment groups with similar means.

[0304] **Training.** On the following 2 days, rats are placed in the Plexiglas cylinders. Each day following 3 min after entry 10 CS-shock pairings are delivered. The shock is delivered during the last 0.5 sec of the 3.7 sec CSs at an average intertrial interval of 4 min (range, 3–5 min).

[0305] **Testing.** Rats are placed in the same startle boxes where they are trained and after 3 min are presented with 18 startle-eliciting stimuli (all at 105 dB). These initial startle stimuli are used to again habituate the rats to the acoustic startle stimuli. Thirty seconds after the last of these stimuli, each animal may receive 60 startle stimuli with half of the stimuli presented alone (startle alone trials) and the other half presented 3.2 sec after the onset of the 3.7 sec CS (CS-startle trials). All startle stimuli are presented at a mean 30 sec interstimulus interval, randomly varying between 20 and 40 sec.

[0306] **Measures.** The treatment groups are compared based on the difference in startle amplitude between CS-startle and startle-alone trials (fear potentiation). In general, the compounds described herein may affect the startle amplitude where the greater the reduction in fear-potentiated startle, the

more anxiolytic the test compound. Therefore the compounds described herein may be used in methods of treating anxiety as shown in U.S. Patent Application Publication Nos. 2006/0089350; 2007/0149526; and 2009/0215754.

EXAMPLE 176

Contextual Fear Conditioning Model

[0307] Purpose. To evaluate the potential of the compounds described herein into alleviate intense anxiety in contextual fear conditioning in rats.

[0308] Design. Contextual fear conditioning involves pairing an aversive event, in this case moderate foot shock, with a distinctive environment. The strength of the fear memory is assessed using freezing, a species-typical defensive reaction in rats, marked by complete immobility, except for breathing. If rats are placed into a distinctive environment and are immediately shocked, they do not learn to fear the context. However, if they are allowed to explore the distinctive environment sometime before the immediate shock, they show intense anxiety and fear when placed back into the same environment. By procedurally dividing contextual fear conditioning into two phases, one can separately study effects of treatments on memory for the context (specifically a hippocampus based process) from learning the association between context and shock or experiencing the aversiveness of the shock (which depend upon emotional response circuitry including amygdala). Post-Traumatic Distress Syndrome (PTSD) in humans has been shown to be related to emotional response circuitry in the amygdala; for this reason contextual memory conditioning is a widely accepted model for PTSD.

[0309] At typical experiment may use 24 rats. Each rat may receive a single 5-min episode of exploration in a small, novel environment. 72-hr later they will placed into the same environment, and immediately they will receive a single, moderate foot-shock. 24-hours later, 12 of the rats may receive an injection (i.v.) of a test compound as described herein. The remaining 12 rats may receive an injection of the vehicle. Each rat may again be placed into the same environment for 8-min, during which time freezing will measured, as an index of Pavlovian conditioned fear.

[0310] Methods. In a typical experiment, 4 identical chambers (20 X 20 X 15 cm) are used. All aspects of the timing and control of events are under microcomputer control. Measurement of freezing is accomplished through an overhead video camera connected to the microcomputer and is automatically scored using a specialty piece of software, FreezeFrame. In Phase 1, rats are placed individually into the chambers for 5 minutes. Phase 2 begins 72 hours later, when again rats are

placed individually into the same chambers but they receive an immediate foot shock (*e.g.*, 1 mA for 2 s). Thirty seconds later they are removed from the chambers. Phase 3, 24 hours later, the rats are returned to the chambers for 8 min, during which time freezing, the index of conditioning fear is scored. Total freezing time will analyzed in a one-way ANOVA with drug dose as the within-groups factor.

[0311] Compounds described herein which show significant anxiolytic effect in this model system may be used in a method for treating anxiety or post-traumatic stress disorder. Exemplary use of this model system are shown in U.S. Patent Application Publication Nos. 2006/0089350 and 2009/0215754.

EXAMPLE 177

Elevated Plus Maze

[0312] **Design.** The elevated plus maze (EPM) is commonly used to assess anxiety levels in rodents. The EPM takes advantage of the fact that when a normal rat is feeling anxious in a novel environment it may seek out and hide in enclosed spaces. A normal rat may venture out into open spaces within the new environment only when it feels less anxious. Drugs like diazepam and buspirone show anxiolytic effects in this task, and hence rats treated with such drugs spend more time within the open areas of the maze.

[0313] This experiment may employ two groups of rats. A first group of the rats may receive an injection (i.v) of test compound and a second group may receive an injection of vehicle. Each rat may immediately be placed on the elevated plus maze. Time spent in the open arms of the maze will recorded and compared between treatment groups. If the test compound reduces anxiety in rat then the group that received the test compound may spend more time in the open arms than the rats that received vehicle.

[0314] The elevated plus maze may consist of two opposing open arms, 50X10 cm, crossed with two opposing enclosed arms of the same dimensions but with walls 40 cm high. Each of the four arms is connected to one side of a central square (10X10 cm) giving the apparatus a plus-sign appearance. The maze will elevated 50 cm above the floor in a normally illuminated room. The rats are placed individually on the central square of the plus maze facing an enclosed arm. The entire 3-min session is videotaped and later scored. The time spent and the number of entries into the open and closed arms, and the number of trips made to at least the midpoint down the open arms is

recorded. An arm entry is defined as placement of all four paws onto the surface of the arm. This model is described in U.S. Patent Application Publication No. 2006/0025387.

EXAMPLE 178 Marble Burying Test in the Mouse

[0315] This method detects anxiolytic/tranquillizing activity and was described by Broekkamp, *et al.* (1986) *Eur. J. Pharmacol.* 126: 223–229. Mice exposed to novel object (marbles) will bury them in the sawdust floor covering. Anxiolytics decrease the number of marbles buried at non-sedative doses.

[0316] Mice will individually placed in transparent plastic cages (33 x 21 x 18 cm) with 5 cm of sawdust on the floor 25 marbles grouped in the centre of the cage. The cage will covered with an inverted plastic cage. Each test cage, together with the marbles, will impregnated with mouse odor before-hand by leaving 10 mice in the cage for 15 minutes. These mice then play no further role in the experiment.

[0317] The number of marbles covered by sawdust (2/3 or more) will counted at the end of a 30 minute test. 12 mice will studied per group. The test will performed blind. Each of two (2) test substances will evaluated at 3 doses, administered i.p. 30 minutes before the test, and compared with a vehicle control group. Clobazam (8 mg/kg i.p.) under the same experimental conditions will used as reference substance. The experiment may include 8 groups.

TABLE 6: Anxiety Model Results for Seletected Compounds

Compound	Dose (mg/kg)	Number of Marbles covered by sawdust (mean $\pm$ s.e.m.)	Number of marbles covered by sawdust as compared to control
5011	50	6.3 ($\pm$ 2.4)	-71%
6001	50	12.8 ($\pm$ 2.7)	-41%
6008	50	8.8 ($\pm$ 3.2)	-59%
CLOBAZAM	8	2.8 ($\pm$ 2.0)	-87%

TABLE 7: Anxiety Model Results for Seletected Compounds

Compound	Dose (mg/kg)	Number of Marbles covered by sawdust (mean $\pm$ s.e.m.)	Number of marbles covered by sawdust as compared to control
2014	10.5	26.3 ($\pm$ 0.3)	-10%
2014	31.5	25.9 ($\pm$ 0.3)	-51%
2014	105.1	27.3 ($\pm$ 0.4)	-98%
4011	9.4	27.1 ($\pm$ 0.5)	-14%
4011	28.2	25.8 ($\pm$ 0.4)	-1%

4011	94.1	26.8 ($\pm$ 0.4)	-17%
CLOBAZAM	8	26.2 ($\pm$ 0.3)	-77%

[0318] Compounds described herein may decrease the number of marbles buried at non-sedative doses and thus may be useful as anxiolytics.

EXAMPLE 179 Light Dark Box Test

[0319] This method detects anxiolytic activity and was described by Crawley (1981) Pharmacol. Biochem. Behav. 15: 695–699. Anxiolytics increase the time spent in the light compartment and decrease the number of crossings.

[0320] Animals will placed into the light compartment of a 2-compartment box with one half light and open (25 x 27 x 27 cm) and the other half dark and closed (20 x 27 x 27 cm). The time spent in each compartment as well as the number of times the animal crosses from one side to the other will scored during a 3-minute test. 10 mice will studied per group. The test will performed partially blind (apart from positive control).

[0321] The compounds will evaluated at 3 doses, administered i.p. 30 minutes before the test, and compared vehicle control group. Clobazam (16 mg/kg i.p.) will administered 30 minutes before the test as a reference substance. The experiment may include 8 groups.

TABLE 8: Results for Light/Dark Box Test

Compound	Dose (mg/kg)	Time Spent in Light Compartment as compared to control	Time Spent in Number of Crossings as compared to control
2014	10.5	-20%	+8%
2014	31.5	+85%	-37%
2014	105.1	+87%	-84%
5009	11	0%	+16%
5009	32.9	+54%	-33%
5009	109.6	+98%	-72%
CLOBAZAM	16	+104%	-42%

[0322] Two selected compounds described herein (*e.g.*, 2014 and 5009) increase the time spent in the light compartment and decrease the number of crossings. Therefore, the compounds described herein may be useful as anxiolytics in the treatment of anxiety disorders.

Examples 180–183
Analgesic/Anti-inflammatory Activity Models

Experimental Models of Pain

[0323] Experimental models of pain include tests of response thresholds to high intensity stimuli (acute pain tests) and changes in spontaneous or evoked behavioral responses in animals with peripheral injury or inflammation (persistent pain models). Acute thermal pain is modeled by the hot-plate and tail-flick test, while persistent pain can be modeled by the formalin test. *See* Bannon and Malmberg “Models of Nociception: Hot-Plate, Tail-Flick, and Formalin Tests in Rodents.” Curr. Protoc. Neurosci. 41:8.9.1–8.9.16 for protocols for all three of these tests, including preparation of animals (rats or mice), administration of a compound being tested for its analgesic properties and data collection.

EXAMPLE 180
Formalin Paw Test (late phase)

[0324] The method described herein detects analgesic/anti-inflammatory activity, generally used to test compounds for pain relief, in particular diabetic neuropathy or nociceptive neuropathy. *See* Wheeler-Aceto, *et al.* (1991) Psychopharmacology 104: 35–44.

[0325] Mice are given an intraplantar injection of 5% formalin (25 μ l) into the posterior left paw. This treatment induces paw licking in control animals. Mice are briefly observed at 1 minute intervals between 15 and 50 minutes after the injection of formalin and the number of occasions that the mice are observed licking the injected paw is recorded. There are 10 mice per group and the test is performed “blind.”

[0326] Compounds as described herein will be evaluated at 3 doses each, administered i.p. 30 minutes before the test (*i.e.*, 15 minutes before formalin), and compared with a common vehicle control group. Morphine (8 mg/kg i.p.) or gabapentin (100 mg/kg i.p.) or venlafaxine (32 mg/kg i.p.), administered under the same experimental conditions, is used as reference substance. Data is analyzed by comparing treated groups with vehicle control using unpaired Mann-Whitney U tests.

[0327] The data recorded for each animal is the amount of time(s) spent licking the affected hind paw in a two minute period. These two minute periods occur at five minute intervals and continue for 45 minutes. Plotting the time spent licking versus time reveals the characteristic biphasic response. From this plot, the area under the curve (AUC) for each animal during both the acute and inflammatory stages are calculated. The AUC for each phase is shown for both control and drug-

treated animals. The AUC for each drug-treated animal is compared to the average result from the control group, yielding an average percent of control. Significant reductions in this number indicate a reduction in licking and a reduction of perceived pain.

[0328] Several compounds described were tested in a Formalin Paw Test at a dose of 250 μ mol/kg using vehicle 1 control (0.2% HPMC in physiological saline), Gabapentin (100 mg/kg i.p.) as a reference substance, and vehicle 2 (5% dimethylacetamide/50% PEG 400/45% H₂O).

TABLE 10: Analgesic effects of selected compounds

Compound	Licking Score (15–50 minutes after formalin)
NTP-4011	–55%
NTP-3034	–61%
NTP-6008	–99%
NTP-4005	–66%
NTP-2014	–98%
NTP-6002	–82%
Gabapentin	–76%
Vehicle 2	–90%

[0329] Several compounds described herein show a reduction in perceived pain and thus may be used in methods to treat and/or prevent (prophylactic) for pain.

EXAMPLE 181 **Taxol Induced Neuropathy Model**

[0330] Peripheral neuropathies are chronic conditions that arise when nerves are damaged by trauma, disease, metabolic insufficiency, or by certain drugs and toxins. The sensory disturbances associated with chemotherapeutic agents, such as paclitaxel (Taxol®), range from mild tingling to spontaneous burning, typically in the periphery such as the hands and feet. Symptoms become more intense with continued therapy and can lead to weakness, ataxia, numbness and pain, limiting the dose and/or treatment with the chemotherapeutic agent.

[0331] Gabapentin, 100 mg/kg, IP is able to mitigate the mechanical allodynia seen as a result of the Taxol-induced neuropathic pain. Similarly, rats treated with compounds described herein are believed to show a significant improvement in allodynia when compared to the vehicle control group.

[0332] Test articles are administered intraperitoneally in dose volumes of 10 mL/kg body weight. Preparations are made freshly for each day of administration.

[0333] The reference article, Gabapentin, is formulated in saline to a concentration of 100 mg/mL and delivered subcutaneously at a dose volume of 1 mL/kg body weight (for a dosing concentration of 100 mg/kg). Male Sprague Dawley rats will used.

[0334] **Allocation to Treatment Groups.** For inclusion into the study (first portion), the animals have a baseline thermal paw test, which is measured prior to Taxol injections. Animals with a thermal paw score greater than 15 seconds are excluded from study.

[0335] All animals that may receive Taxol® are tested for thermal hyperalgesia. Animals need to have at least a 20% drop from baseline for inclusion into the treatment segment of the study. All animals underwent a baseline pre-dose von Frey test, which is measured prior to Taxol injection. For inclusion into the study, the animals needed to have a baseline von Frey score above 12.

[0336] All animals administered Taxol® are tested for mechanical allodynia using von Frey. Animals receiving a score of 13 or below are allocated to treatment groups. The mechanical allodynia scores for each group are reviewed to ensure the mean values and standard deviations are homogeneous. Rats are allocated to treatment groups.

[0337] All animals are administered Taxol®, 2 mg/kg, IP at a dose volume of 1 mL/kg, on Days 1, 3, 5 and 7.

[0338] All animals may receive a single intraperitoneal injection of test compound. All animals may receive an IP injection of vehicle or test compound 30 min prior to mechanical allodynia testing. Animals are dosed at a volume of 10 mL/kg.

[0339] Control animals may receive an IP injection of Gabapentin 90 minutes prior to mechanical allodynia testing. Animals are dosed at a volume of 1 mL/kg.

[0340] **Behavioral Testing—Acclimation.** Twice prior to baseline testing, the animals underwent acclimation to the mechanical allodynia apparatus. This habituated the rats to the testing devices so they are calm at the time of testing.

[0341] **Mechanical Allodynia (von Frey).** All animals undergo von Frey testing for mechanical allodynia. On testing days, the animals are returned to the chambers and allowed approximately 15 minutes to explore their surroundings prior to testing. A filament is applied to the left hind paw.

[0342] The group mean results are analyzed versus the vehicle control group using a two way ANOVA, followed by a Bonferroni post-hoc test. Individual groups are tested pre and post dose using a paired t-test.

[0343] Therefore, compounds described herein act parasynaptically and will administered to treat neuropathic pain without the unwanted side effects usually associated with GABAergic compounds (*e.g.*, sedation from benzodiazepines). Further, treatment with compounds described herein are expected to have similar effects.

EXAMPLE 182

Tail Flick Test Model of Nociception

[0344] **Overview.** The mouse tail-flick assay is a well-accepted model of acute thermal pain in which a number of clinically relevant opioid analgesics produce moderate to full efficacy on several different pain-related measures. The mouse model also requires smaller amounts of drug and is a more rapid initial screen for antinociceptive activity compared to the chronic pain models. This model of pain is also described in U.S. Patent Application Publication Nos. 2006/0089550 and 2006/0025387.

[0345] **Tests for Antinociception.** Efficacy of the test compound may be assessed using the 52°C warm water tail-flick test. The latency to the first sign of a rapid tail-flick is taken as the behavioral endpoint (Jannsen et al., 1963). Each mouse is first tested for baseline latency by immersing its tail in the water and recording the time to response. Mice not responding within 5 seconds are excluded from further testing. Mice are then administered the test compound and tested for antinociception at various time points afterwards. Antinociception is calculated by the following formula: % Antinociception = $100 \times (\text{test latency} - \text{control latency}) / (10 - \text{control latency})$. A maximum score is assigned (100%) to animals not responding within 10 seconds to avoid tissue damage.

[0346] **Subjects:** Male CD-1 (25-35 g, Charles River) mice may be used for all studies. Mice will housed in groups of five in Plexiglas chambers with food and water available ad libitum. All animals are maintained on a 12 hr light/dark cycle (lights on at 7:00 AM) in a temperature- and humidity-controlled animal colony. Only animals in good health are acclimatized to laboratory conditions and are used in the study. The acclimation period to the vivarium will a minimum of 7 days. All animal experiments may be performed under an approved protocol in accordance with institutional guidelines and in accordance with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health.

[0347] **Drugs and Injections.** Morphine sulfate (Mallinckrodt, St. Louis, MO) may be dissolved in physiological saline. Injections will made using a 1-mL syringe with a 30-gauge needle at a volume of 10 ml/kg body weight. Animal body weights will measured on the morning of testing. Mice are

firmly grasped by the nape of the neck and the tail is tucked between the last two fingers and palm of the technician's hand. The back of the mouse is arched slightly backwards exposing the abdominal region. The needle is inserted through the skin and abdominal musculature into the peritoneal cavity just off of the midline. Mice are immediately returned to the holding cage until behavioral testing.

[0348] Total Number of Animals: Each experimental group may consist of 8 mice with a total of approximately 260 mice needed to complete the proposed studies (30 compounds x 1 dose/compound x 8 mice/group plus controls).

[0349] Housing: Automatically controlled environmental conditions are set to maintain temperature at 20–24°C with a relative humidity (RH) of 30–70%, a 12:12 hour light:dark cycle and 15-30 air changes/h in the study room. Temperature and RH are monitored daily. During acclimation and throughout the entire study duration, animals are housed within a limited access rodent facility and kept in groups of 5 mice/polypropylene cages (23 x 17 x 14 cm), fitted with solid bottoms and filled with wood shavings as bedding material. Individual cages are suspended in a stainless steel rack system with each cage having its own water sipping tube and food hopper. Mice may have *ad libitum* access to food (Harlan Teklad Global 2018) and water except during the formal drug administration and testing procedures.

[0350] Overall Experimental Design: On the morning of Day 1, mice are marked and weighed, and then baselined for thermal latencies in the 52°C tail-flick assay. Test compounds are then injected and the mice are rested for thermal latencies at 10, 20, 30, 45, 60, 90, 120 and 180 minutes post-injection (if a drug effect drops below 20% for the group average then the testing is halted for that group). All compounds may be dissolved in 0.2% hydroxypropylmethylcellulose (HPMC) in physiological saline to yield final concentrations of between 5-20 mg/ml (pH ~7.4). Tween 80 (1-2 drops) may be added to aid in solubilization of compounds. A similar vehicle will be used as a control. Morphine sulphate may be dissolved in physiological saline.

[0351] Evidence of the ability of compounds described herein to alleviate pain will be seen as a higher pain threshold than the group that received vehicle as tested. The results of selected compounds tested in the tail flick pain model (*e.g.*, 4012, 4018, 5009, 5011, 6002, 6008, 6009, 7009, 10001) are presented in FIGURE 9. The showing of increasing the pain threshold of the treated mice suggests that the compounds described herein may be useful as analgesics in the treatment of pain.

EXAMPLE 183
Chung model of neuropathy

[0352] Design. Spinal nerve ligation is performed under isoflourane anesthesia with animals placed in the prone position to access the left L4–L6 spinal nerves. Under magnification, approximately one-third of the transverse process is removed. The L5 spinal nerve is identified and carefully dissected free from the adjacent L4 spinal nerve and then tightly ligated using a 6–0 silk suture. The wound is treated with an antiseptic solution, the muscle layer is sutured, and the incision is closed with wound clips. Behavioral testing of mechanical paw withdrawal threshold takes place within a 3–7 day period following the incision. Briefly, animals are placed within a Plexiglas chamber (20X10.5X40.5 cm) and allowed to habituate for 15 min. The chamber is positioned on top of a mesh screen so that mechanical stimuli can be administered to the plantar surface of both hindpaws. Mechanical threshold measurements for each hindpaw are obtained using an up/down method with eight von Frey monofilaments (5, 7, 13, 26, 43, 64, 106, and 202 mN). Each trial begins with a von Frey force of 13 mN delivered to the right hindpaw for approximately 1 sec, and then the left hindpaw. If there is no withdrawal response, the next higher force is delivered. If there is a response, the next lower force is delivered. This procedure is performed until no response is made at the highest force (202 mN) or until four stimuli are administered following the initial response. The 50% paw withdrawal threshold for each paw is calculated using the following formula:
$$[X_{th}]_{log} = [vFr]_{log} + ky$$
where $[vFr]$ is the force of the last von Frey used, $k=0.2268$ which is the average interval (in log units) between the von Frey monofilaments, and y is a value that depends upon the pattern of withdrawal responses. If an animal does not respond to the highest von Frey hair (202 mN), then $y=1.00$ and the 50% mechanical paw withdrawal response for that paw is calculated to be 340.5 mN. Mechanical paw withdrawal threshold testing is performed three times and the 50% withdrawal values are averaged over the three trials to determine the mean mechanical paw withdrawal threshold for the right and left paw for each animal. This model is described in U.S. Patent Application Publication No. 2006/0025387.

EXAMPLES 184–186 SEIZURE MODELS

EXAMPLE 184

Mouse model of Mesial Temporal Lobe Epilepsy (MTLE)

[0353] Purpose. To test the anti-seizures properties of compounds described herein, compounds may be tested for their ability to suppress seizures in a kainate model of epilepsy.

[0354] Methods. Using stereotaxic techniques under general anaesthesia, intrahippocampal injection of kainate (1 nmole in 50 nl) will performed on adult mice (*e.g.*, C57/Bl6). Mice may then be implanted with a bipolar electrode in the dorsal hippocampus and 3 cortical monopolar electrodes. After 4 weeks following kainate injection, the mice will injected with test compounds (*e.g.*, two injections per week). Drug conditions will counter-balanced, the animals being used as their own controls. Digital EEG recordings will performed in freely moving animals for 20 minutes pre-injection and 20 minutes between 20 and 40 minutes post-injection. The effects of the injected compound will compared versus reference period. Several compounds described herein were tested in an MTLE model at a dose of at least about 20–75 mg/kg.

TABLE 9: MTLE Model Results for Selected Compounds

Compound	Dose (mg/kg)	Reduction in number of hippocampal discharges by more than 50%	Reduction in number of hippocampal discharges by less than 50%	Slight increase in number of hippocampal discharges
NTP-4007	23			X
NTP-5009	27	X		
NTP-4011	47		X	
NTP-4008	52	X		
NTP-6008	59	X		
NTP-6002	61	X		
NTP-3034	52		X	
NTP-4005	59	X		
NTP-5013	57	X		
NTP-7013	67	X		
NTP-3003	64	X		
NTP-3004	64		X	
NTP-4012	61	X		
NTP-3032	61	X		
NTP-4018	70	X		
NTP-4020	66	X		
NTP-5009	55	X		
NTP-5011	54	X		
NTP-6001	56	X		
NTP-6006	56	X		
NTP-6009	59	X		

NTP-7009	65	X		
NTP-7049	52	X		
NTP-10001	51	X		

[0355] Several compounds described herein decrease the cumulative duration and number of hippocampal discharges in this model of mesial temporal lobe epilepsy. Therefore, the compounds described herein may be used in therapeutic methods to treat seizures.

Example 185 Epileptiform Discharges in Hippocampal Slices

[0356] During these studies, spontaneous epileptiform activity may be elicited by a variety of treatments. Sprague-Dawley rats (males and females; 25–35 days old) will be decapitated, the top of the skull is rapidly removed, and the brain will be chilled with ice-cold oxygenated slicing medium. A slicing medium used will be a sucrose-based artificial cerebrospinal fluid (sACSF) consisting of 220 mM sucrose, 3 mM KCl, 1.25 mM NaH₂PO₄, 2 mM MgSO₄, 26 mM NaHCO₃, 2 mM CaCl₂, and 10 mM dextrose (295–305 mOsm). A hemisphere of brain containing hippocampus will be blocked and glued (cyanoacrylic adhesive) to the stage of a Vibroslicer (Frederick Haer, Brunswick, ME). Horizontal or transverse slices 400 μm thick are cut in 4°C, oxygenated (95% O₂; 5% CO₂) slicing medium. The slices are then immediately transferred to a holding chamber where they remain submerged in oxygenated bathing medium (ACSF) consisting of 124 mM NaCl, 3 mM KCl, 1.25 mM NaH₂PO₄, 2 mM MgSO₄, 26 mM NaHCO₃, 2 mM CaCl₂, and 10 mM dextrose (29–305 mOsm). The slices can be held at room temperature for at least 45 minutes before being transferred to a submersion-style recording chamber. In the recording chamber, the slices will be perfused with oxygenated recording medium at 34–35°C. All animal procedures should be conducted in accordance with NIH animal care guidelines. In most slice experiments, simultaneous extracellular field electrode recordings are obtained from CA1 and CA3 as described in U.S. Patent Nos. 6,495,601 and 7,214,711.

[0357] Hippocampal slices treated with the test compounds may show less epileptiform activity, indicative of anti-seizure properties. Compounds described herein may be used in methods to treat and/or prevent (prophylactic) for seizures, seizure disorders, epilepsy, epileptic seizures, and other neurodegenerative disorders (*e.g.*, those neurodegenerative disorders which involve seizures).

Example 186***In vitro* Hippocampal Recordings of miniature and spontaneous inhibitory post-synaptic currents (mIPSCs and sIPSCs)**

[0358] Ion flux in neuronal cells are measured using standard techniques. Kandel and Schwartz *Principles of Neural Science* 2nd Edition (1985), *see, e.g.*, pages 128–131. Recording is performed *in vitro* in hippocampal slices (CA1 pyramidal cell layer). For recording GABA_A-IPSCs, glutamatergic and GABA_B transmission is blocked by adding DNOX (50 μ M), AP-5 (50 μ M), and SCH50911 (20 mM) into the medium. The intracellular solution comprised CsCl and OX314.

[0359] Compounds may be tested for their ability to increase GABA_A inhibitory drive, such as a marked increase in spontaneous IPSCs or in miniature IPSCs in a hippocampal slice model where the compound consistently shows a significant decrease in the time between inhibitory events (*e.g.*, increased frequency of events).

[0360] Data demonstrating that the interval between miniature and/or spontaneous inhibitory post-synaptic currents (mIPSCs and sIPSCs, respectively) events are substantially decreased in the presence of the compound indicate a highly significant increase in the frequency of inhibitory events. Such data suggest a pre-synaptic mechanism increasing the release of GABA from the neurons by the action of compounds described herein.

[0361] **Hippocampal Slice Preparation**— Young adult male Sprague-Dawley rats weighing 220–250 g at time of use were housed in groups of 4 in an air conditioned room on a 12 hour light/dark cycle with food and water available *ad libitum*. On the day of experiments, animals were terminally anaesthetised using isofluorane, cervically dislocated, and decapitated. The brain was removed and 300–400 μ m thick hippocampal slices cut using a Leica VT1000S.

[0362] **Electrophysiological recording**— Slices were maintained in artificial cerebrospinal fluid (aCSF) at room temperature for 1 hour after slicing before commencing electrophysiological recordings. After this period, individual slices were transferred to a custom-built chamber continuously perfused with aCSF at a rate of 4–10 ml/min of the following composition (mM): NaCl, 127; KCl, 1.9; KH₂PO₄, 1.2; CaCl₂, 2.4; MgCl₂, 1.3; NaHCO₃, 26; D-glucose, 10; equilibrated with 95% O₂-5% CO₂. Whole-cell patch-clamp recordings were performed at room temperature (17–21°C) from hippocampal pyramidal CA1 neurons using an Axoclamp 700B amplifier using the ‘visualised’ version of the patch clamp technique. Patch pipettes had resistances of between 3 and 8 M Ω when filled with intracellular solution of the following composition (mM): caesium chloride,

150; EGTA-Na; HEPES, 10; Na₂ATP. All experiments were carried out in the presence of Tetrodotoxin (TTX, 1 μ M), NBQX (10 μ M), D-AP-5 (10 μ M) and CGP55845 (GABA_B antagonist, 200 nM) to eliminate action potential-dependent synaptic activity and isolate GABA_A receptor-dependent synaptic responses.

[0363] Test Compounds— 3034, 6008, 6009, and 7049 were prepared as 5 mM stock in DMSO and serially diluted to the required concentrations in aCSF immediately prior to use. TTX (1 mM), Bicuculline (10 mM), NBQX (10 mM), CGP55845 (10 mM) and AP5 (10 mM) were all obtained from Ascent Scientific and prepared as stock solutions in DMSO or ddH₂O as appropriate. All compounds were stored at –20°C prior to use. Test compounds were cumulatively applied for a minimum of 10 minutes.

[0364] Analysis— All analysis was conducted using Excel (Microsoft), Clampfit (MDS Technologies) and Mini-analysis (Lavasoft) software. mIPSC analysis was performed on 60s traces using a threshold of ~10 pA and an area of ~150 fC/s for synaptic currents.

[0365] Three select compounds (*e.g.*, 3034, 6009, 7049) were tested in synaptic transmission in adult rat hippocampal neurons *in vitro*. See FIGURES 10–12. Compounds 3034, 6009 increased mIPSC frequency in a concentration-dependent manner. Compounds 3034, 6008, 6009, and 7049 decreased mIPSC inter-event interval in a concentration manner. Compounds 3034, 6008, 6009, and 7049 had no effect on mIPSC amplitude. These data indicate an increase in presynaptic inhibition corresponding to the increased mIPSC frequency. The compounds did not increase the mIPSC amplitude indicative that the compounds do not affect post-synaptic inhibition. Further, this data is indicative of these compounds increasing GABA inhibitory drive.

[0366] Compounds described herein which act parasynaptically may be administered at high doses (*e.g.*, 100 mg/kg) without the unwanted side effects usually associated with GABAergic compounds (*e.g.*, sedation from benzodiazepines). Compounds may show anticonvulsant activity and may be useful as therapeutics for treating seizure disorders without these unwanted side effects.

EXAMPLE 187 6 Hz Psychomotor Test in the Mouse

[0367] The 6Hz psychomotor test method detects anticonvulsant activity and was described by Brown, *et al.* (1953) J. Pharmacol. Exp. Ther. 107: 273–283. Mice will administered a rectangular current (*e.g.*, 32 mA, rectangular pulse: 0.2 ms pulse width, 3 s duration, 6 Hz) via corneal electrodes connected to a constant current shock generator. The results for the number of seizures as reflected

by forelimb clonus and Straub-tail are recorded during 30 seconds following current administration. Forelimb clonus is scored as absent (0), mild (1) and strong (2) whereas Straub tail is rated as absent (0) or present (1).

[0368] Test substances will be evaluated at 1 dose, administered i.p. 30 minutes before the test and compared with a vehicle control group. 15 mice will be studied per group. The test will be performed blind. Diazepam (2 mg/kg i.p.) will be administered under the same experimental conditions and used as reference substance. Compounds which lower the number of seizures experienced by the mouse are indicative of compounds with anticonvulsant activity that may be useful in the treatment of seizures.

EXAMPLE 188

Amphetamine Hyperactivity Test in the mouse

[0369] This method detects antipsychotic and antiparkinson activity and was described by Costall, *et al.* (1977) Brain Res. 123: 89–111, and uses an activity meter similar to that described by Boissier and Simon (1965) Arch. Int. Pharmacodyn. 158: 212–221.

[0370] Amphetamine induces hyperactivity in this test situation. Hyperactivity is antagonized by classical and atypical antipsychotics acting on dopaminergic systems at the limbic level, and is potentiated by antiparkinson drugs. Mice were injected with d-amphetamine (3 mg/kg i.p.) and are immediately placed in the activity meter. The activity meter may consist of 24 covered Plexiglas cages (20.5 x 10.5 x 18 cm) contained within a darkened cabinet and connected to silent electronic counters. Each cage was equipped with four photocell assemblies (two at each end of the cage) 2.5 cm above the floor, in order to measure the number of movements by each animal (one per cage) in the horizontal plane. Seven additional photocell assemblies will be placed at even intervals 9.5 cm above the floor along the long wall to record rearing. The number of (horizontal) crossings by each animal (one per cage) from one pair of photocells to the other will be recorded by computer in 10-minute intervals for 30 minutes. A similar procedure will be utilized for recording of rearing, except that individual photobeam breaks are recorded. The scores for activity and rearing will be recorded by computer over 10-minute intervals and cumulated over a 30-minute period. 10 mice were studied per group. The test was performed blind.

[0371] Each compound was evaluated at a dose of 50 mg/Kg, administered i.p. 30 minutes before amphetamine, and compared with a vehicle control group. The experiment may also include a control group not treated with amphetamine. Haloperidol (0.25 mg/kg i.p.) may be administered

under the same experimental conditions as a reference substance. The experiment may include 9 groups.

TABLE 11: Hyperactivity Test Results

Compound	Number of Crossings (0–30') [mean ± s.e.m.]	Compared with Amphetamine Control	Number of rears (0–30') [mean ± s.e.m.]	Compared with Amphetamine Control
Amphetamine Control	313.7 (± 54.4)	+572%	80.7 (± 21.6)	–33%
2014	232.2 (± 38.8)	–26%	44.0 (± 15.7)	–45%
5009	159.2 (± 33.4)	–49%	32.7 (± 8.6)	–59%
6008	137.8 (± 25.2)	–56%	33.8 (± 14.4)	–58%
HALOPERIDOL	110.3 (± 21.0)	–65%	111.3 (± 34.3)	+38%

[0372] The selected compounds described herein were tested in the amphetamine-induced hyperactivity test and show a decrease in the number of crossings and number of rears. Therefore the compounds described herein show antipsychotic and antiparkinson activity and may be useful in the treatment of anxiety disorders, ADHD, and Parkinson's disease as well as other movement disorders that involve bradykinesia such as Huntington's Disease.

Example 189 Animal Model of Amphetamine Sensitization (Addiction)

[0373] **Purpose.** The therapeutic usefulness of compounds in the treatment of behavior disorders may be examined by measuring the ability of a compound to reverse the symptoms of amphetamine sensitization in rats.

[0374] **Method.** Amphetamine sensitization will induced in animals. Following sensitization, the rats are divided into two equal groups. One group receives treatment with compounds and the other half receives vehicle. All rats may then be given a challenge injection of amphetamine. Open field motor activity will monitored. If a compound reduces or blocks amphetamine sensitization, the group that received compounds prior to the amphetamine challenge exhibits shorter distances and fewer total rears.

[0375] Following three days of handling, the animals receive daily intraperitoneal (i.p.) injections of 1.5 mg/kg amphetamine hydrochloride (injection volume 1.0 ml/kg) for 5 days (amphetamine-amphetamine group). Amphetamine may be freshly diluted with saline (0.9%) every morning (injections performed between 10:00 and 12:00 h). The fifth day of treatment with amphetamine will followed by withdrawal for 48 h. Following the 48 hours withdrawal, eight, of the rats receive an injection of compounds (i.v) and eight receive an injection of vehicle (i.v). The rats then receive

a challenge injection of amphetamine (1.5 mg/kg) and are monitored for locomotor activity in an open field. All injections except the challenge injection are administered in the rats' home cage.

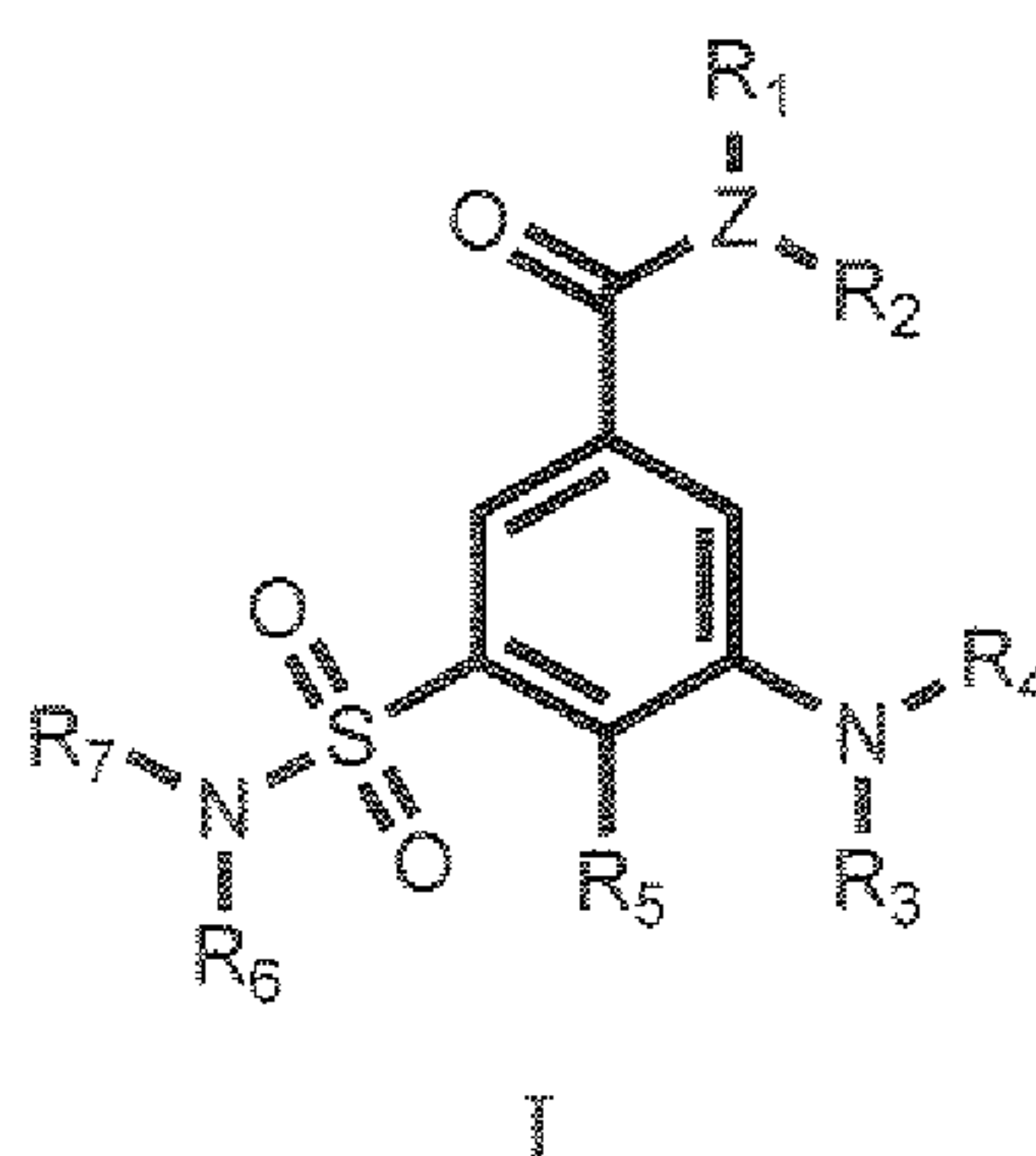
[0376] Locomotor activity will be measured in an open field for 120 min following the amphetamine challenge. Total distance traveled and number of rears are automatically recorded and compared between groups using one-way analysis of variance. This model is described in U.S. Patent Application Publication No. 2006/0025387.

[0377] Although the invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, it will be obvious that certain changes and modifications will be practiced within the scope of the appended claims. Modifications of the above-described modes for carrying out the invention that are obvious to persons of skill in medicine, pharmacology, microbiology, and/or related fields are intended to be within the scope of the following claims.

[0378] All publications (*e.g.*, Non-Patent Literature), patent application publications, and patent applications mentioned in this specification are indicative of the level of skill of those skilled in the art to which this invention pertains. All such publications (*e.g.*, Non-Patent Literature), patent application publications, and patent applications are herein incorporated by reference to the same extent as if each individual publication, patent, patent application publication, or patent application is specifically and individually indicated to be incorporated by reference.

WE CLAIM:

1. A compound of the formula I:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

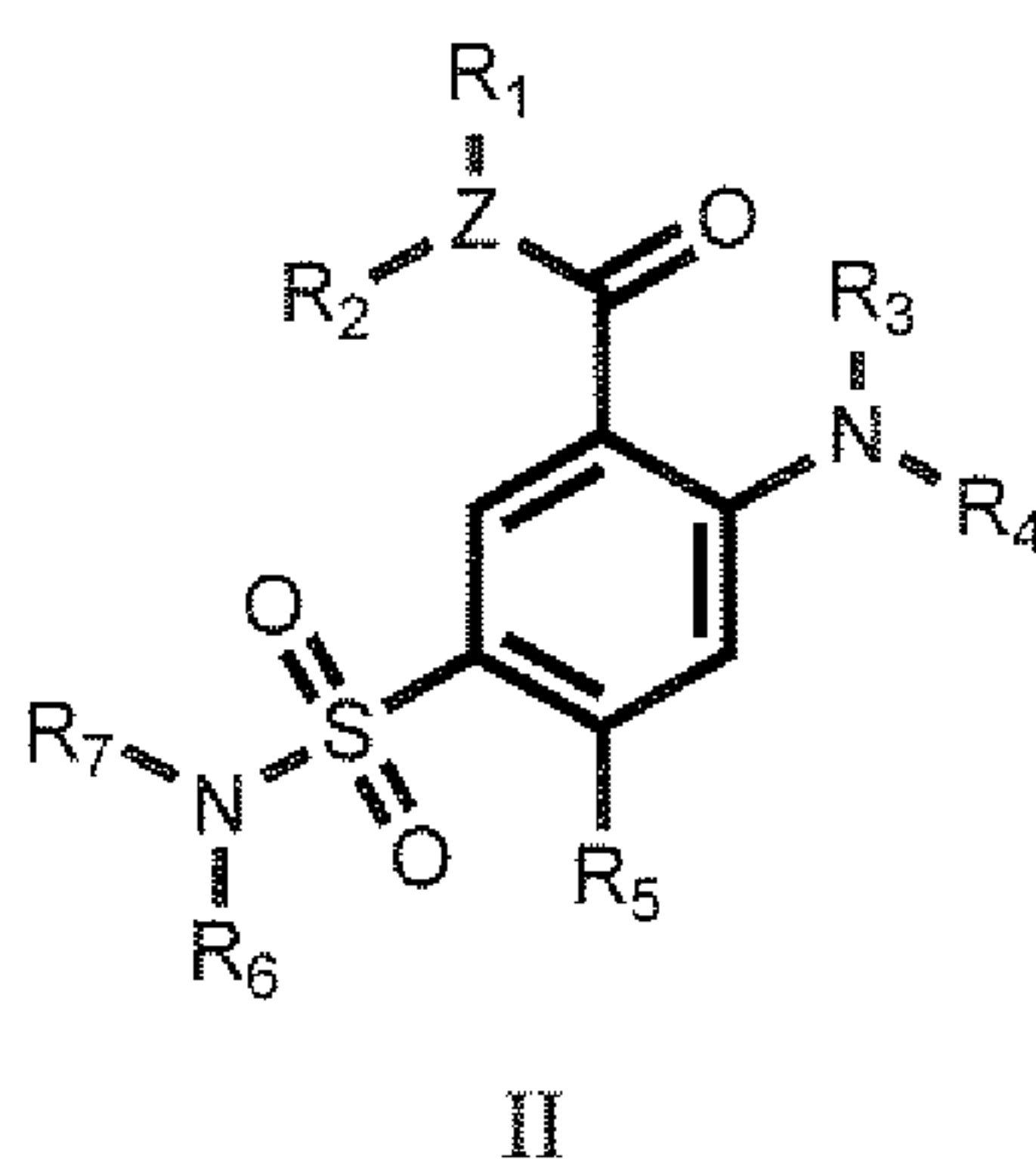
R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio; and

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents.

2. The compound of claim 1, wherein Z is nitrogen; and R₅ is aryloxy.
3. The compound of claim 2, wherein R₆ and R₇ are hydrogen.
4. The compound of claim 1, wherein Z is oxygen; and R₁ is hydrogen.

5. The compound of claim 4, wherein R_6 and R_7 are hydrogen.
6. The compound of claim 4, wherein R_5 is aryloxy or halo.
7. The compound of claim 1, wherein neither R_3 nor R_4 are hydrogen.
8. The compound of any one of claims 2–6, wherein R_4 is alkyl, preferably n-butyl.
9. A compound of the formula II:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

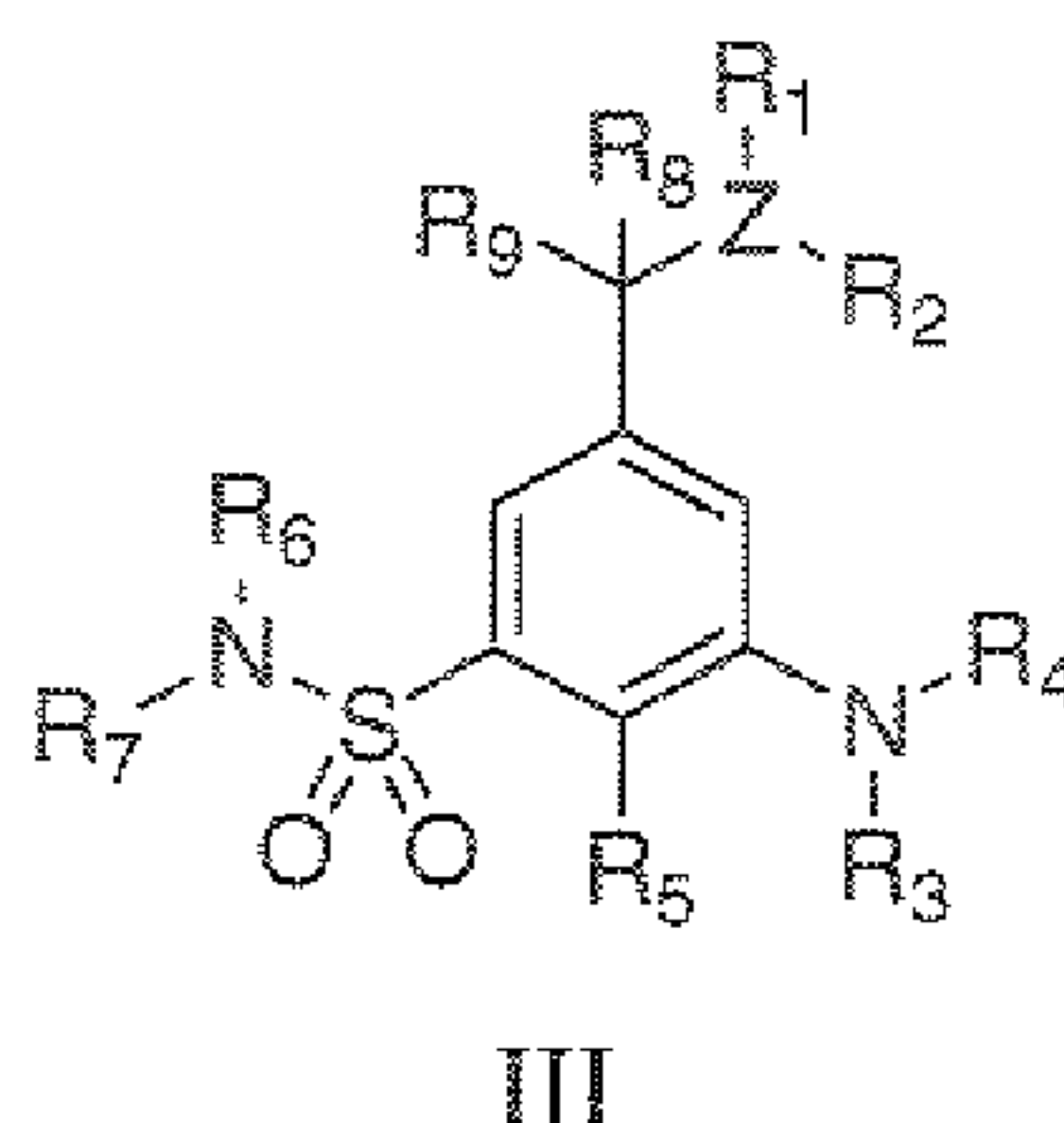
R_1 and R_2 are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R_1 and R_2 , together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R_2 is not present;

R_3 and R_4 are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R_3 and R_4 , together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R_5 is halo, aryl, aryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio; and

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents.

10. A compound of the formula III:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

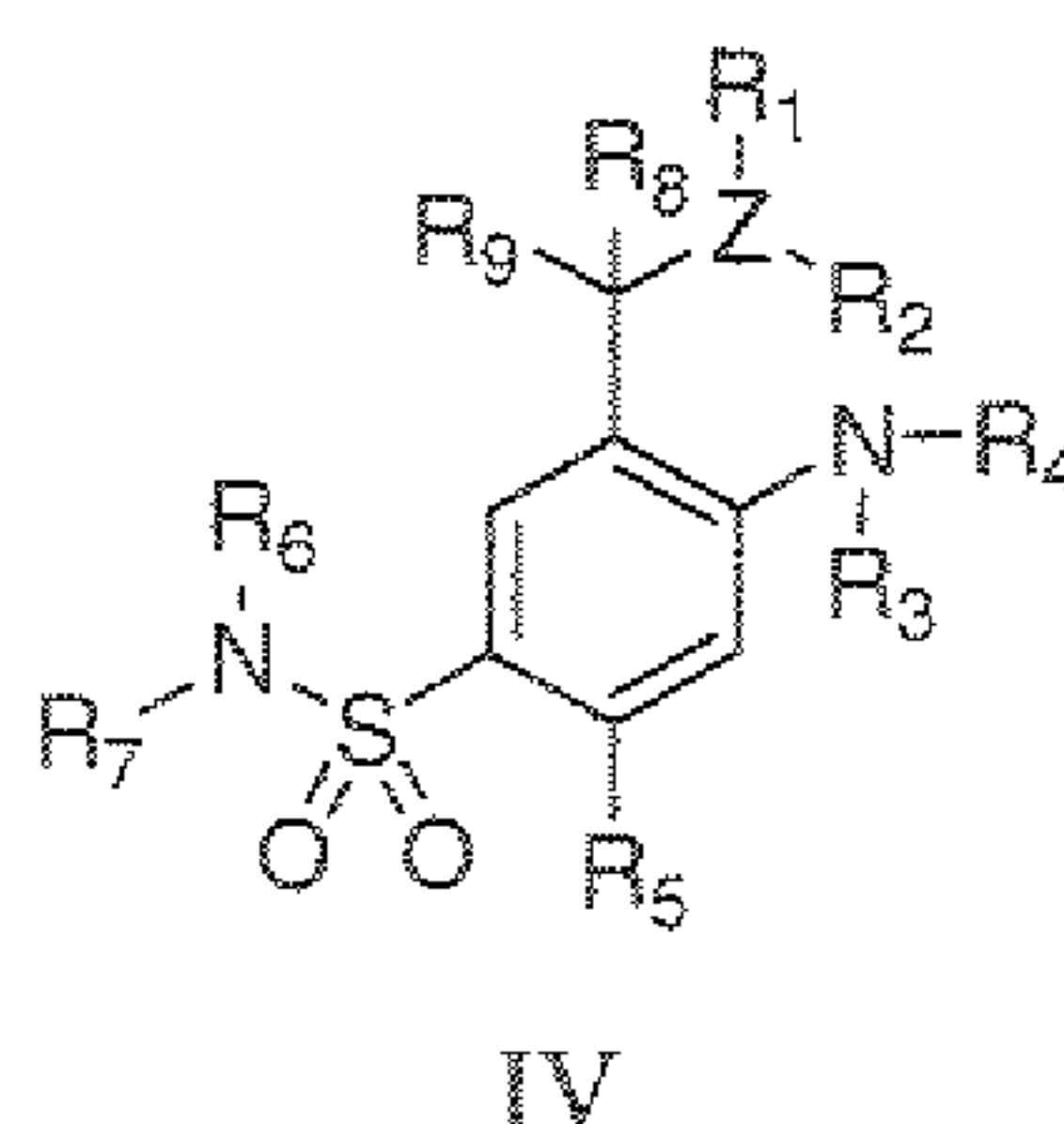
R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio;

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents; and

R₈ and R₉ are each independently hydrogen, alkyl, or R₈ and R₉ together with the atom to which they are attached, form a 3–6 membered substituted or unsubstituted cycloalkyl or heterocycloalkyl ring.

11. A compound of the formula IV:



or a pharmaceutically acceptable salt thereof,

wherein:

Z is oxygen or nitrogen;

R₁ and R₂ are each independently hydrogen, alkyl, aryl, arylalkyl, heteroaryl, heteroarylalkyl, heterocycloalkyl, or R₁ and R₂, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents, with the proviso that if Z is oxygen, then R₂ is not present;

R₃ and R₄ are each independently hydrogen, alkyl, cycloalkyl, cycloalkyl alkyl, aryl, arylalkyl, heteroaryl, or heteroarylalkyl, or R₃ and R₄, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

R₅ is alkoxy, halo, aryl, aryloxy, alkaryloxy, arylamino, heteroarylamino, heterocycloalkyl, heteroaryl, heteroaryloxy, heterocycloalkoxy, or alkythio;

R₆ and R₇ are each independently hydrogen, acyl, alkyl, cycloalkyl alkyl, aryl or arylalkyl, or R₆ and R₇, together with the atom to which they are attached, form a 4–7 membered heterocyclic ring that can have one or more additional heteroatoms and can have one or more substituents;

and

R₈ and R₉ are each independently hydrogen, alkyl, or R₈ and R₉ together with the atom to which they are attached, form a 3–6 membered substituted or unsubstituted cycloalkyl or heterocycloalkyl ring.

12. A pharmaceutical composition comprising a compound of any one of claims 1–11.

13. A pharmaceutical composition comprising a compound of any one of claims 1–11 and a pharmaceutically acceptable excipient.
14. A method for treating a condition involving NKCC comprising administering a composition comprising an effective amount of a compound of any one of claims 1–11.
15. The method of claim 14, wherein said condition is selected from the group consisting of selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.
16. The method of claim 14, wherein said compound preferentially inhibits basolateral bumetanide-sensitive $\text{Na}^+\text{K}^+\text{Cl}^-$ cotransporters (NKCC1).
17. The method of claim 16, wherein the inhibition of NKCC2 is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the effect on NKCC1.
18. The method of claim 14, wherein said compound has increased lipophilicity compared to a loop diuretic selected from the group consisting of bumetanide, furosemide, piretanide, azosemide, and torsemide.
19. The method of claim 18, wherein said lipophilicity is measured by partition coefficient.
20. A method for treating a condition involving GABA_A receptor comprising administering a composition comprising an effective amount of a compound of any one of claims 1–11.

21. The method of claim 20, wherein said condition is selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.
22. The method of claim 20, wherein said compound has reduced diuretic effects compared to a loop diuretic selected from the group consisting of bumetanide, furosemide, piretanide, azosemide, and torsemide.
23. The method of claim 20, wherein compound is an antagonist for a GABA_A receptor comprising an α_4 subunit.
24. The method of claim 20, wherein the antagonism of GABA_A receptors comprising an α_1 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the effect on a GABA_A receptor comprising an α_4 subunit.
25. The method of claim 20, wherein the effective concentration (EC₅₀) of the compound for GABA_A receptors comprising an α_4 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the EC₅₀ for the same compound for GABA_A receptors comprising an α_1 subunit.
26. The method of claim 20, wherein compound is an antagonist for a GABA_A receptor comprising an α_5 subunit.
27. The method of claim 20, wherein the antagonism of GABA_A receptors comprising an α_1 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the effect on a GABA_A receptor comprising an α_5 subunit.

28. The method of claim 20, wherein the effective concentration (EC_{50}) of the compound for $GABA_A$ receptors comprising an α_5 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the EC_{50} for the same compound for $GABA_A$ receptors comprising an α_1 subunit.
29. The method of claim 20, wherein compound is an antagonist for a $GABA_A$ receptor comprising an α_6 subunit.
30. The method of claim 20, wherein the antagonism of $GABA_A$ receptors comprising an α_1 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the effect on a $GABA_A$ receptor comprising an α_6 subunit.
31. The method of claim 20, wherein the effective concentration (EC_{50}) of the compound for $GABA_A$ receptors comprising an α_6 subunit is no more than 50%, preferably no more than 25%, more preferably no more than 15% of the EC_{50} for the same compound for $GABA_A$ receptors comprising an α_1 subunit.
32. A composition for treating a condition involving NKCC comprising administering a composition comprising an effective amount of a compound of any one of claims 1–11.
33. The composition of claim 32, wherein said condition is selected from the group consisting of selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.

34. A composition for treating a condition involving GABA_A receptor comprising administering a composition comprising an effective amount of a compound of any one of claims 1–11.
35. The composition of claim 34, wherein said condition is selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.
36. Use of a compound of formula I, II, III or IV for the manufacture of a medicament for treating a condition involving NKCC comprising administering a composition comprising an effective amount of a.
37. The use of claim 36, wherein said condition is selected from the group consisting of selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, ascites, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, cancer, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, endothelial corneal dystrophy, edema, epilepsy, glaucoma, Huntington's Disease, inflammatory pain, insomnia, ischemia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, ocular diseases, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.
38. Use of a compound of formula I, II, III or IV for the manufacture of a medicament for treating a condition involving GABA_A receptor comprising administering a composition comprising an effective amount of a compound of formula I, II, III or IV.

39. The use of claim 38, wherein said condition is selected from the group consisting of addictive disorders, Alzheimer's Disease, anxiety disorders, attention deficit hyperactivity disorder (ADHD), autism spectrum disorders (autism), bipolar disorder, the improvement of cognitive function, cognitive impairment, cognitive dysfunction, depression, epilepsy, Huntington's Disease, inflammatory pain, insomnia, migraine, migraine with aura, migraine without aura, neuropathic pain, nociceptive neuralgia, nociceptive pain, pain, Parkinson's disease, periodic limb movement disorder (PLMD), personality disorders, postherpetic neuralgia, psychosis, restless legs syndrome (RLS), schizophrenia, seizure disorders, spasticity, tinnitus, and withdrawal syndromes.
40. A method for improving cognition comprising administering a composition comprising an effective amount of a compound of any one of claims 1-11.
41. A method for treating cognitive impairment comprising administering a composition comprising an effective amount of a compound of any one of claims 1-11.
42. A composition for improving cognition comprising an effective amount of a compound of any one of claims 1-11.
43. A composition for treating cognitive impairment comprising an effective amount of a compound of any one of claims 1-11.

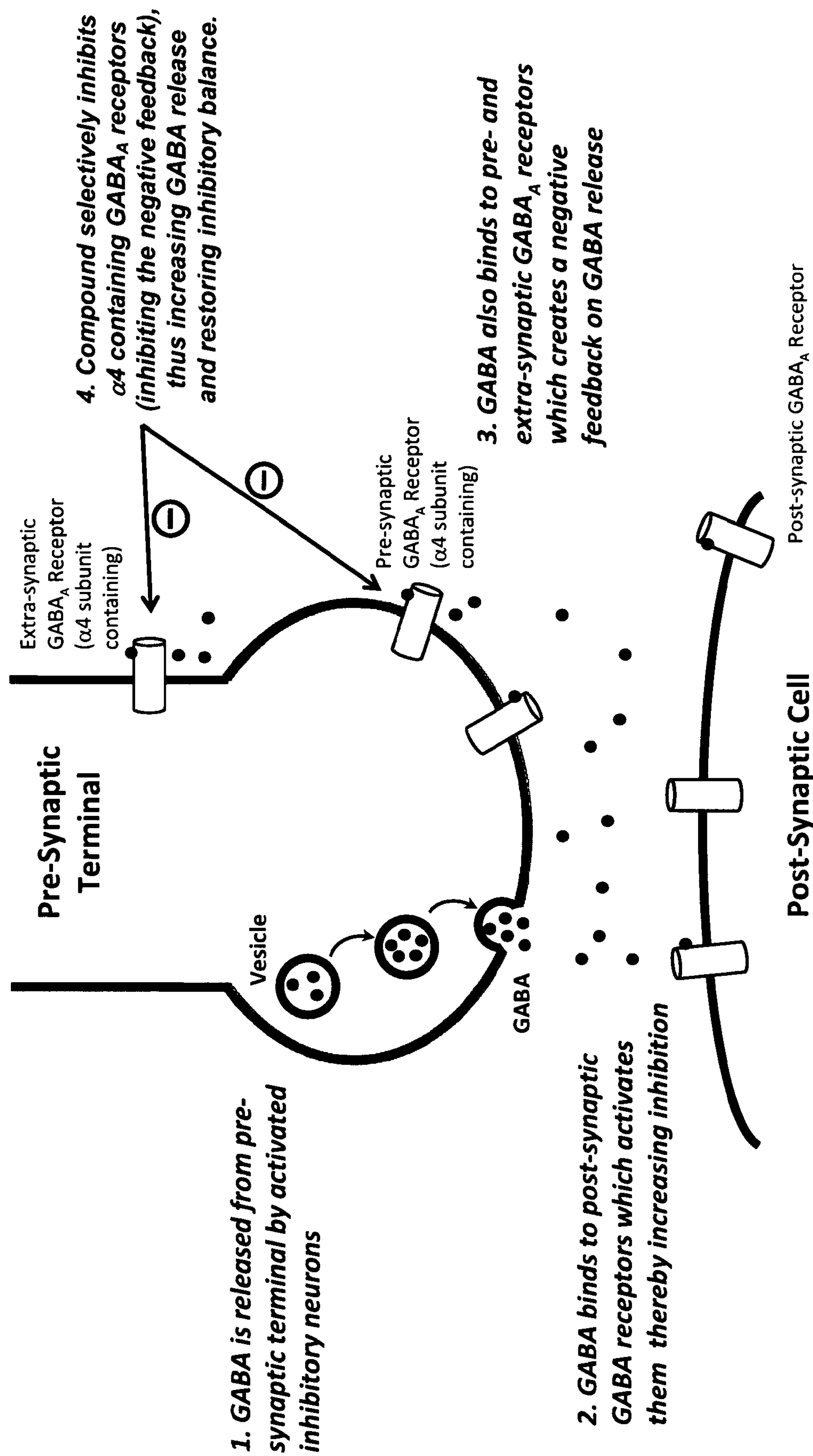


FIGURE 1

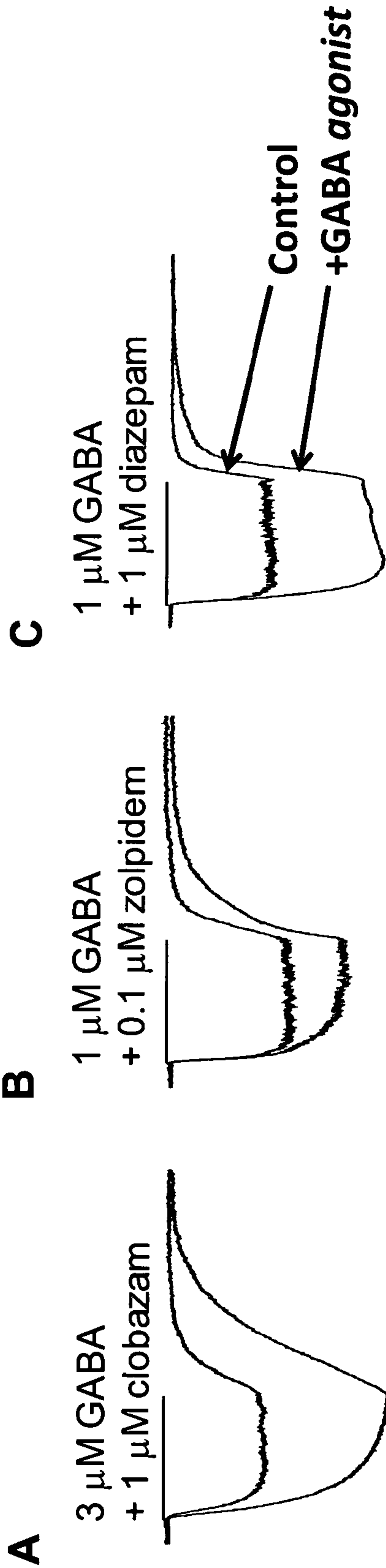


FIGURE 2A-C

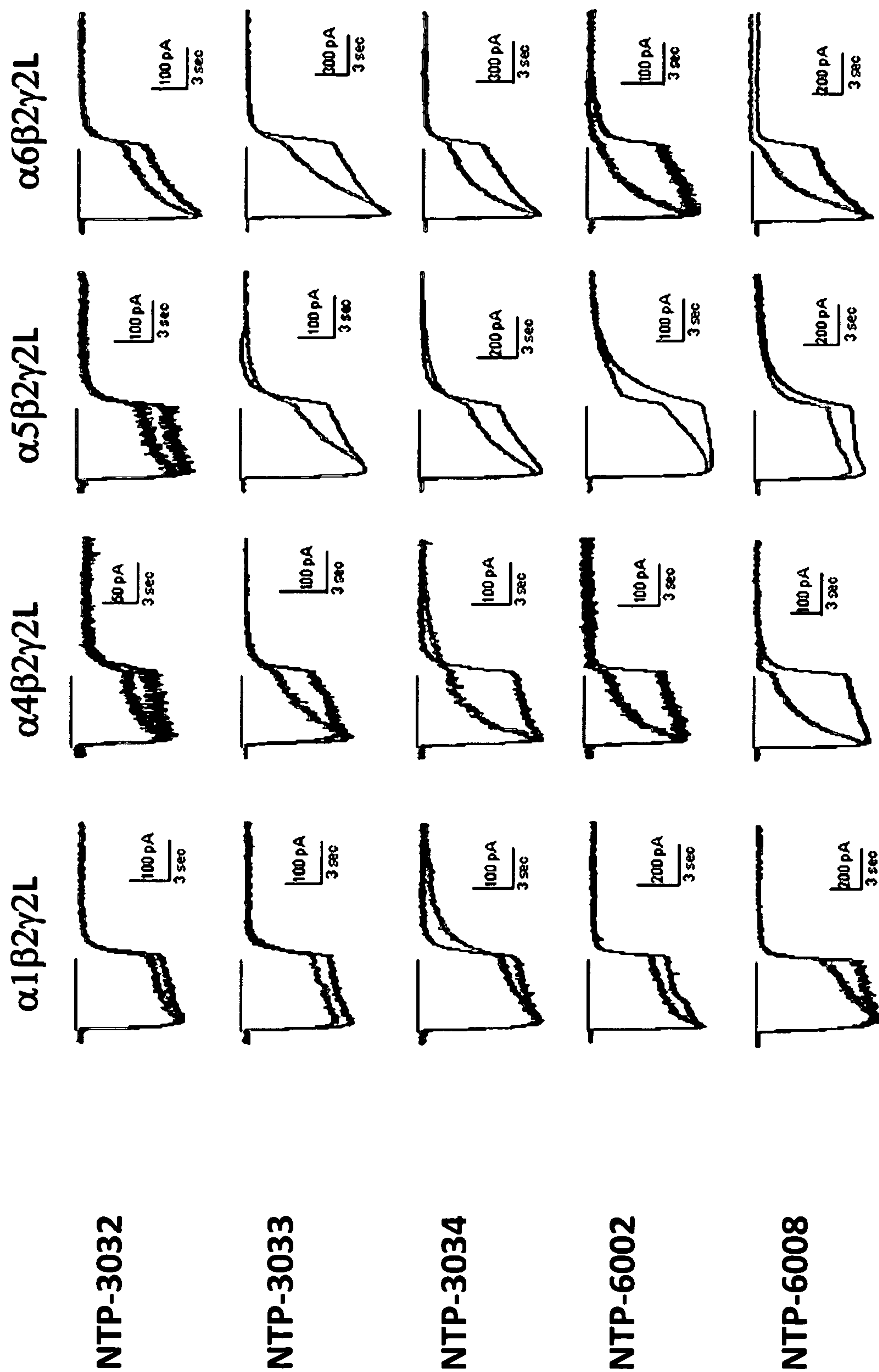


FIGURE 3

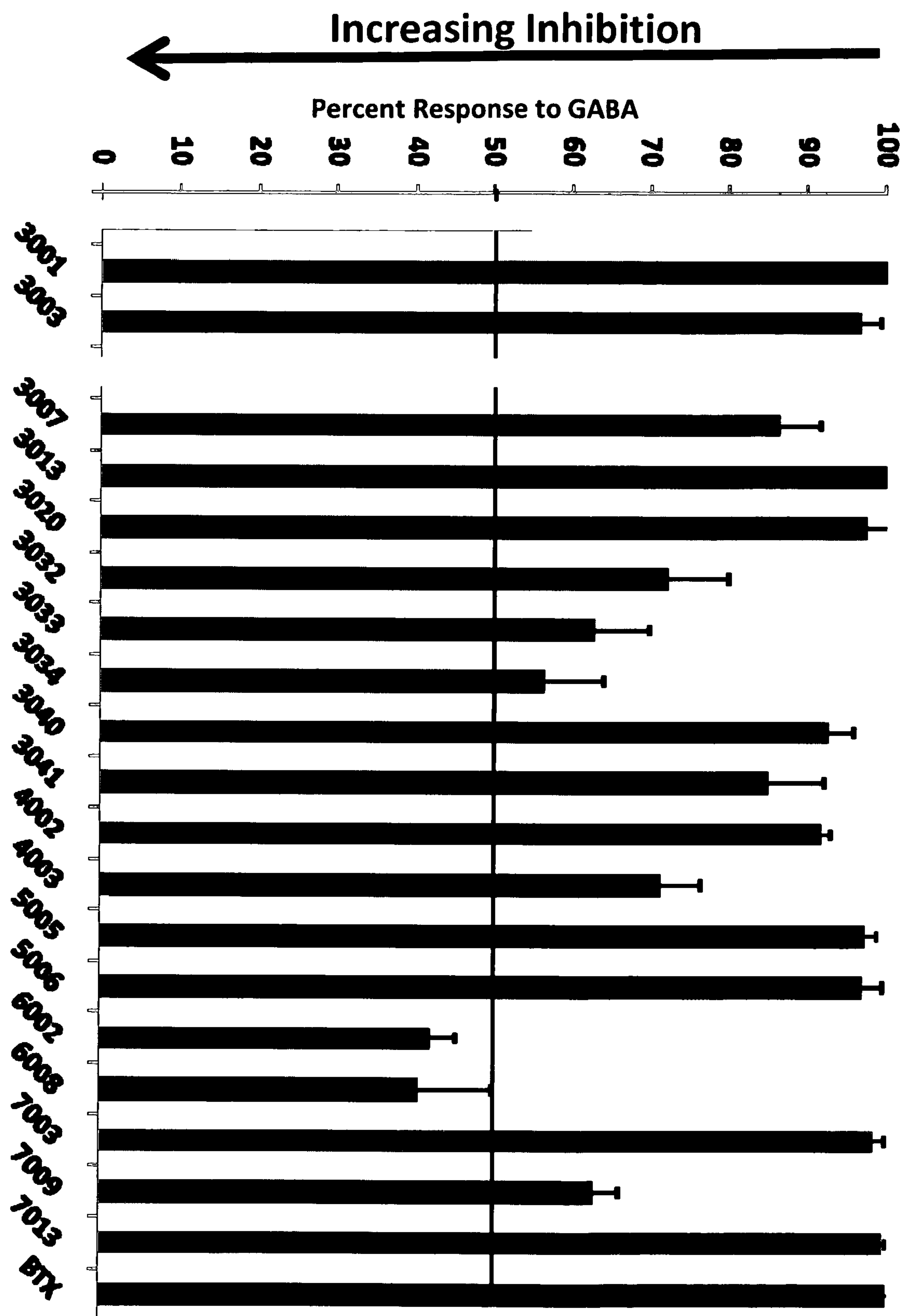


FIGURE 4

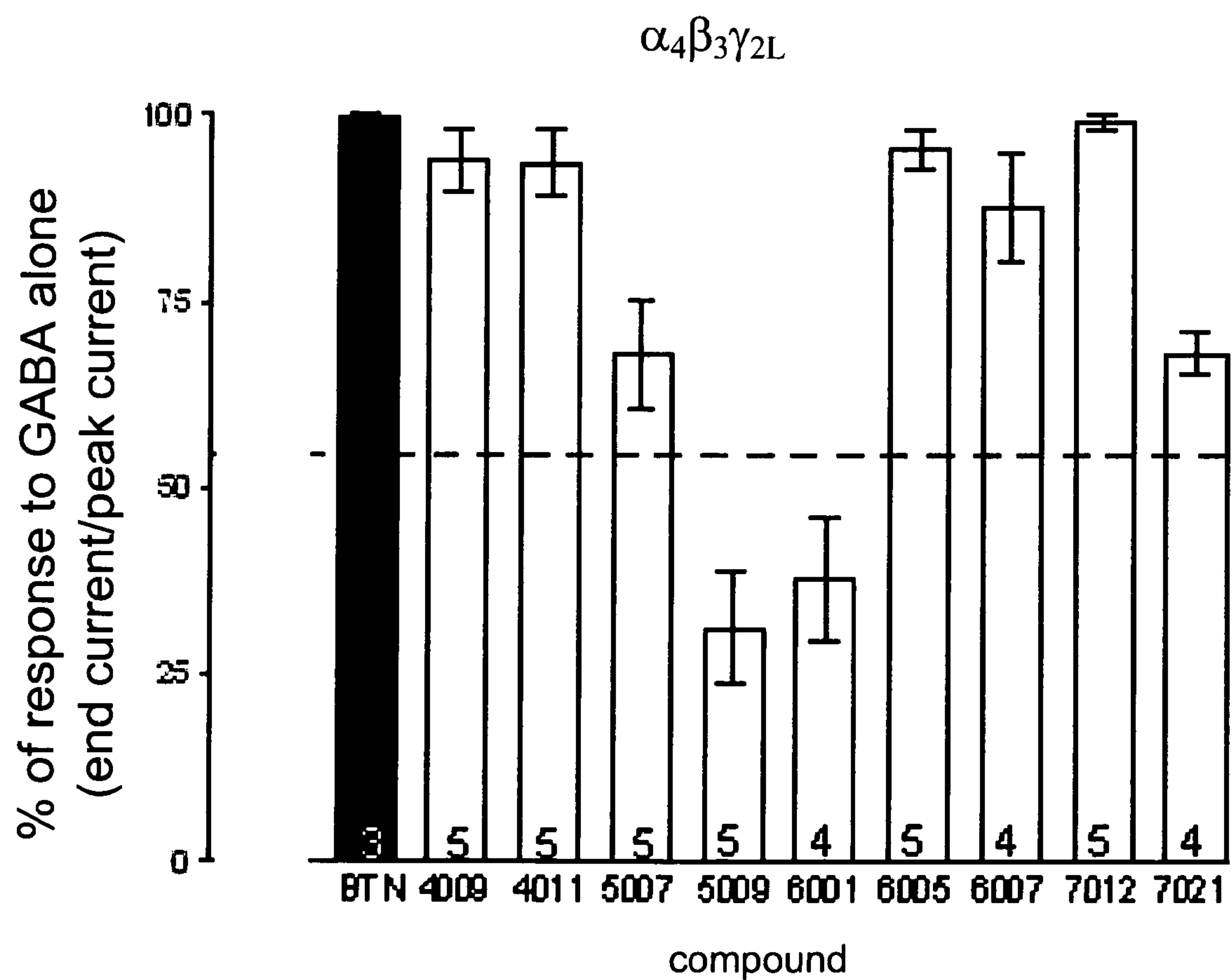


FIGURE 5

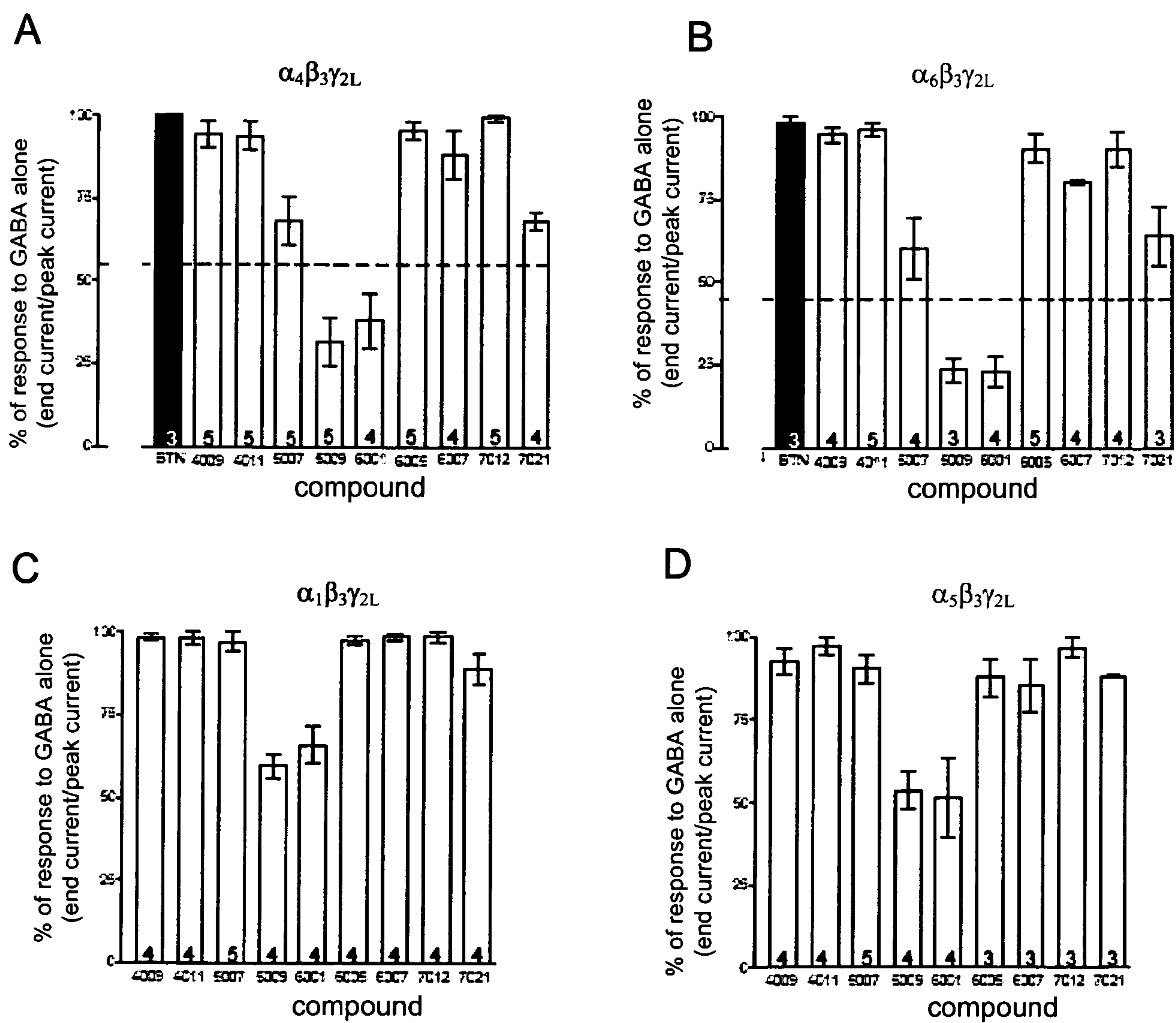
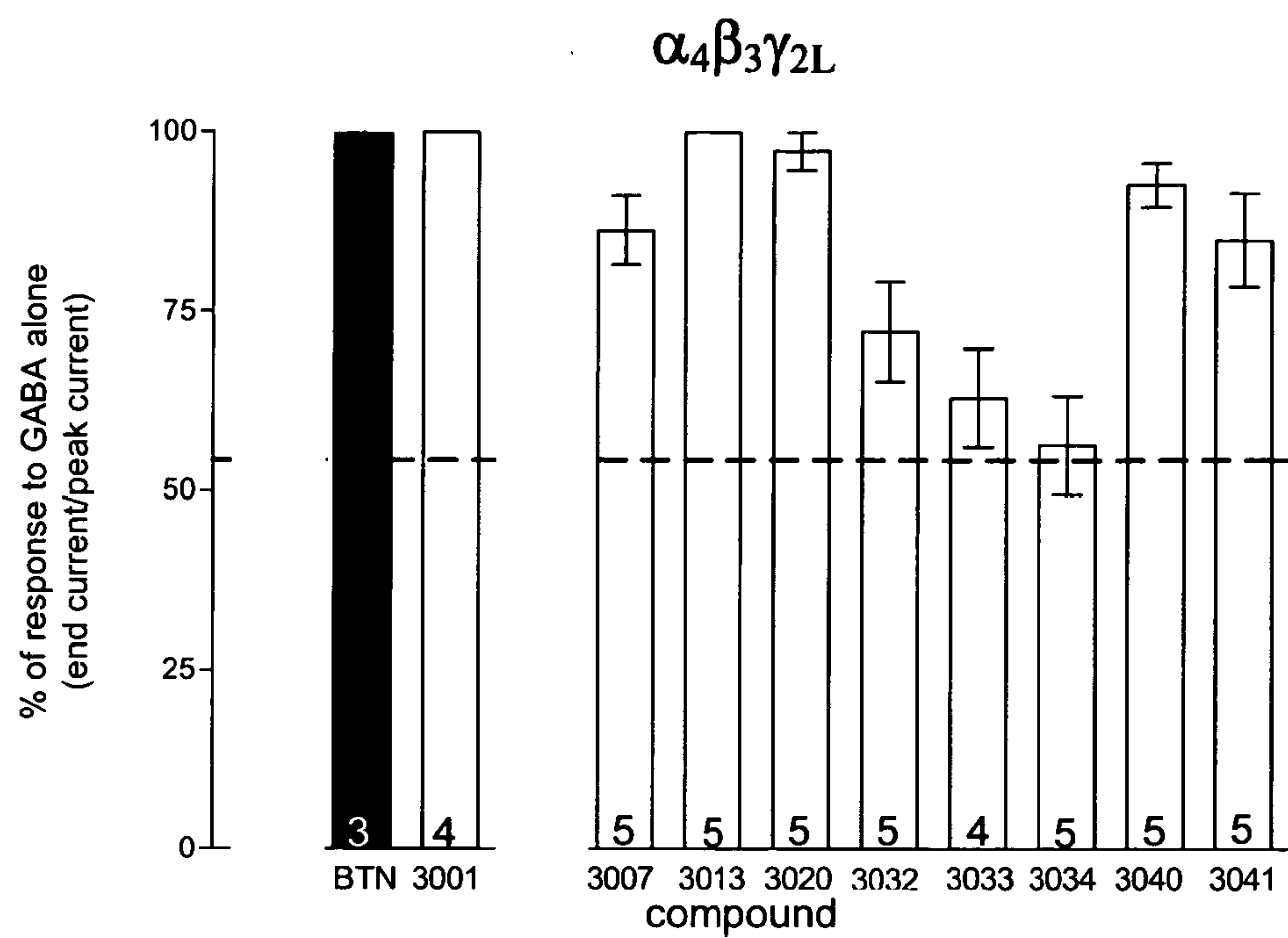


FIGURE 6

A



B

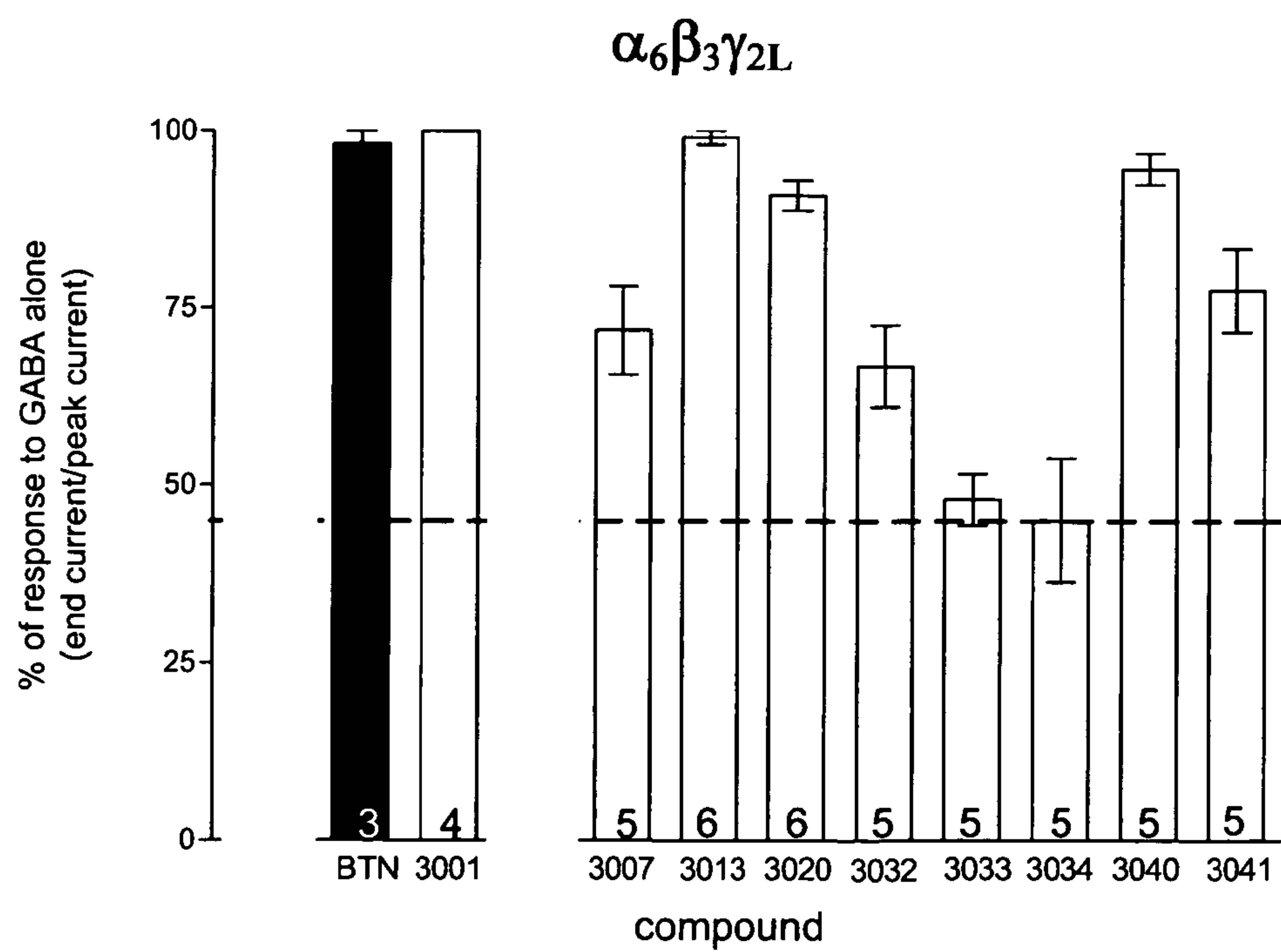


FIGURE 7

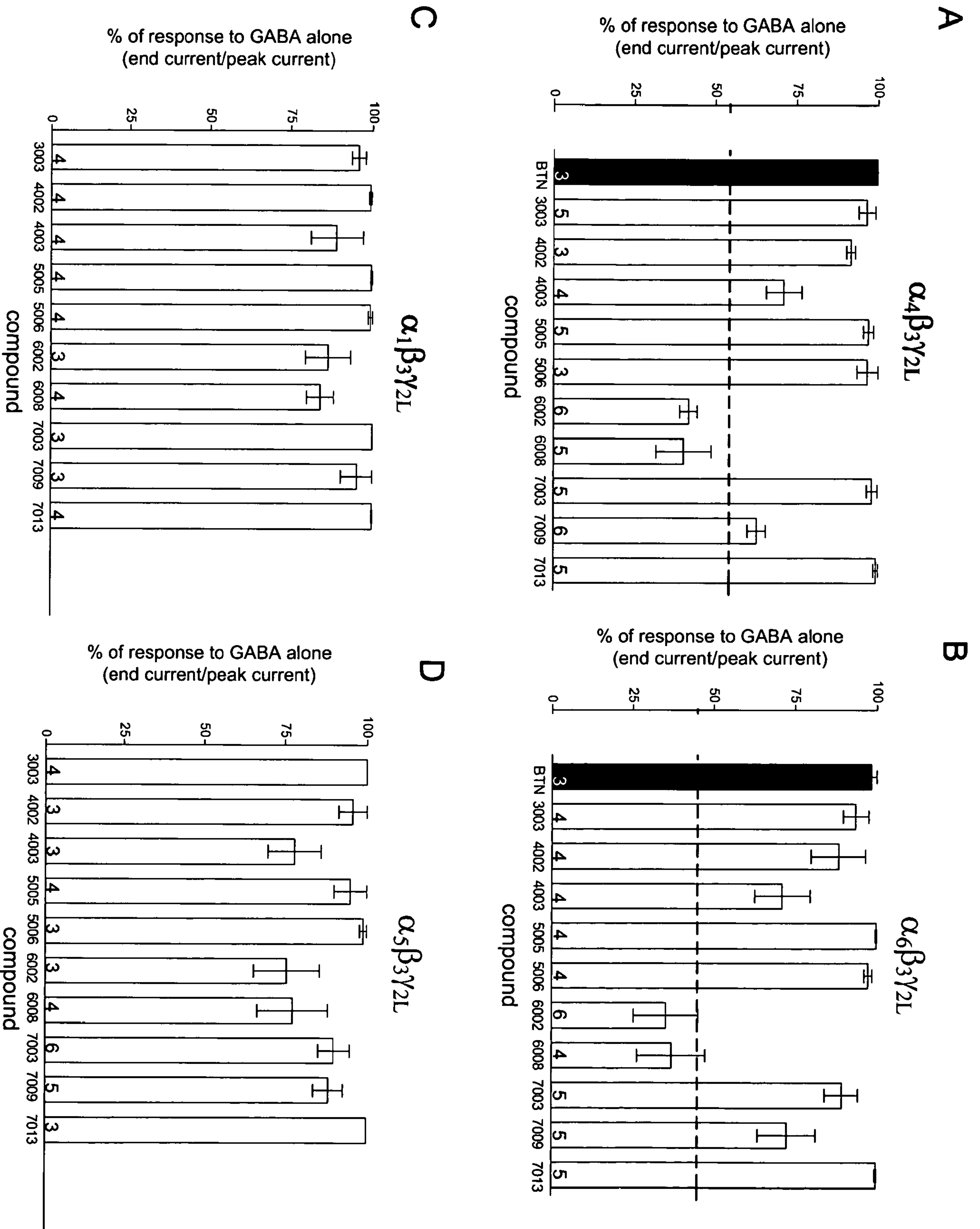


FIGURE 8A-D

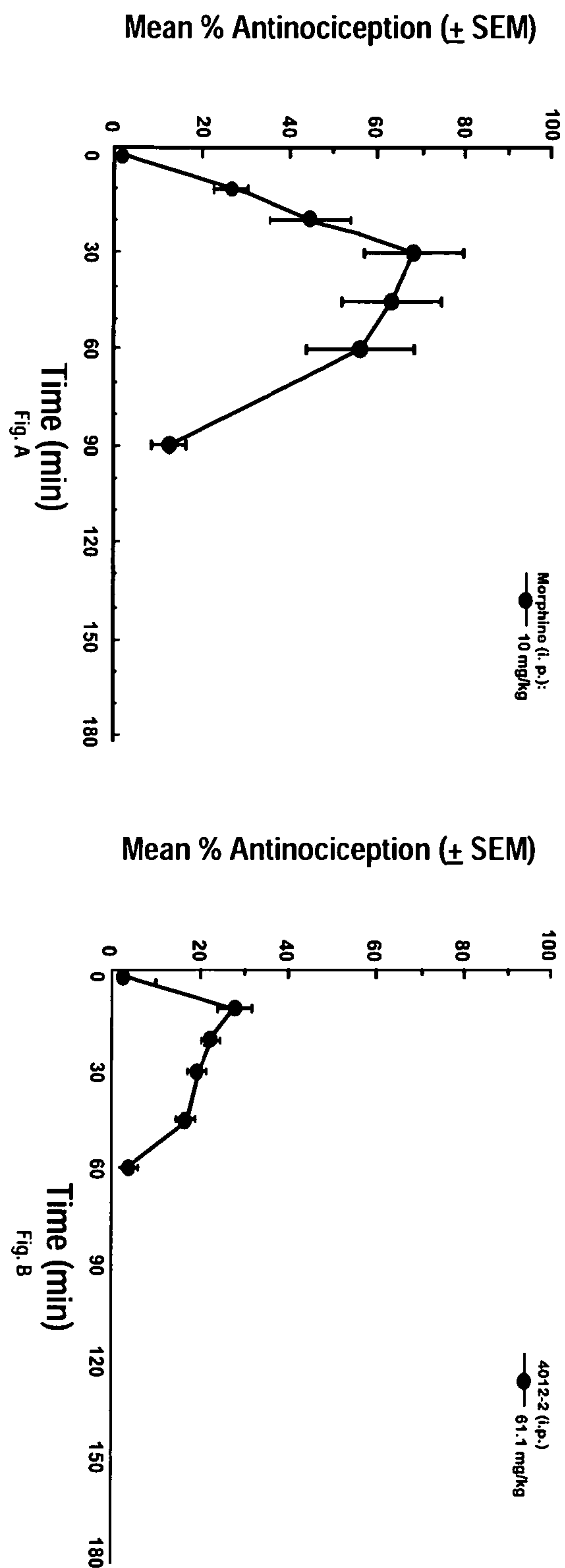


FIGURE 9A-C

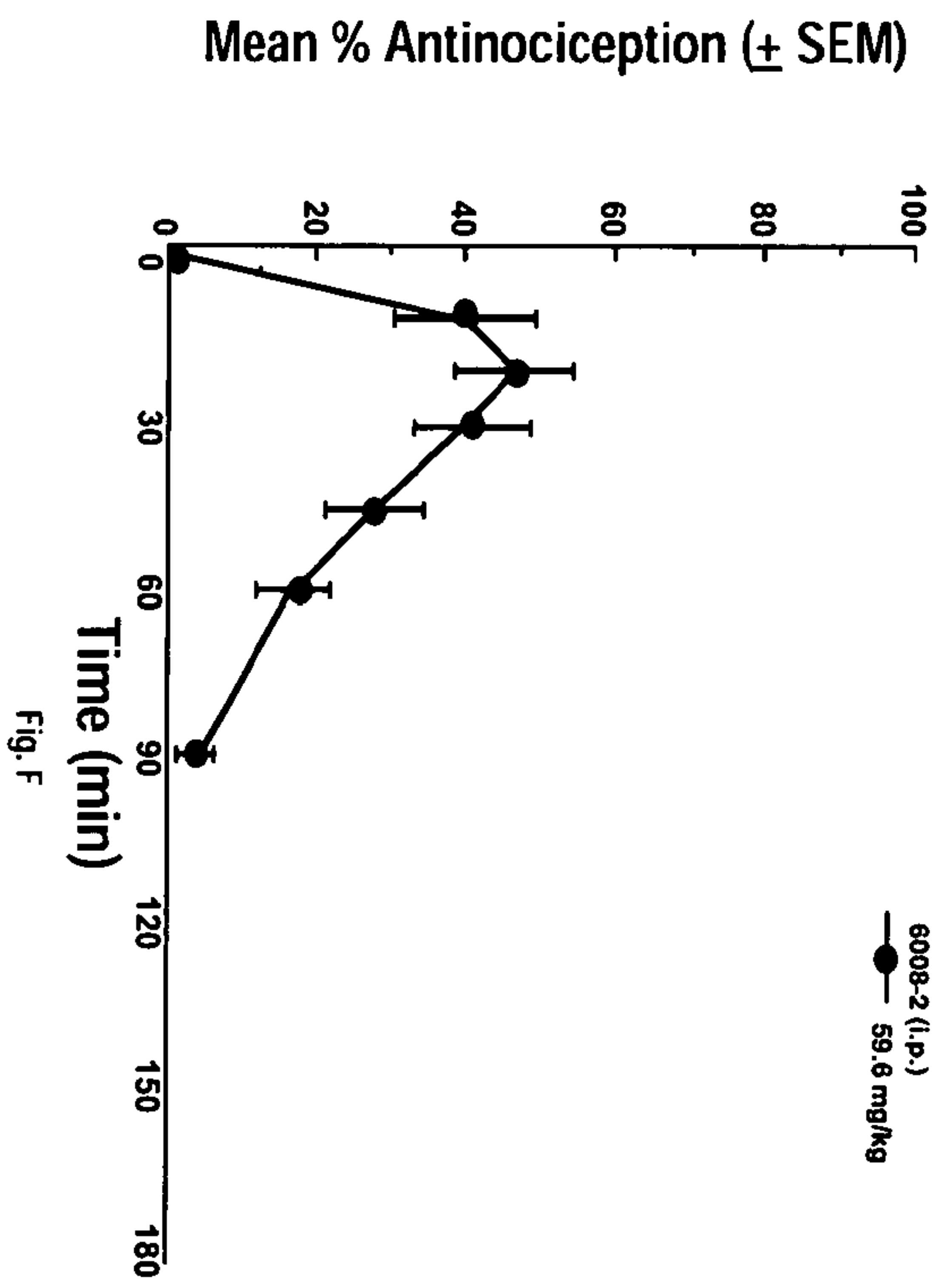
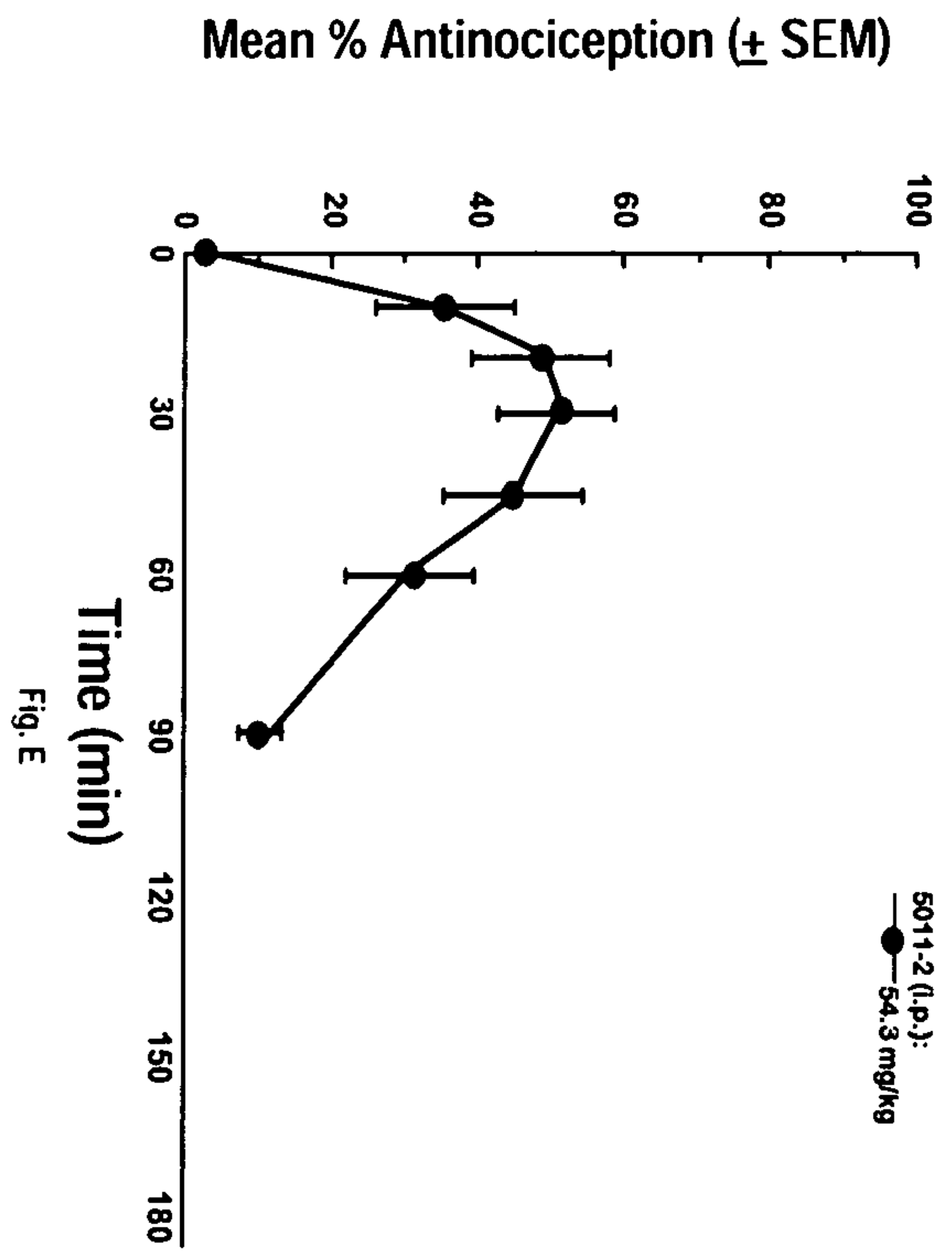
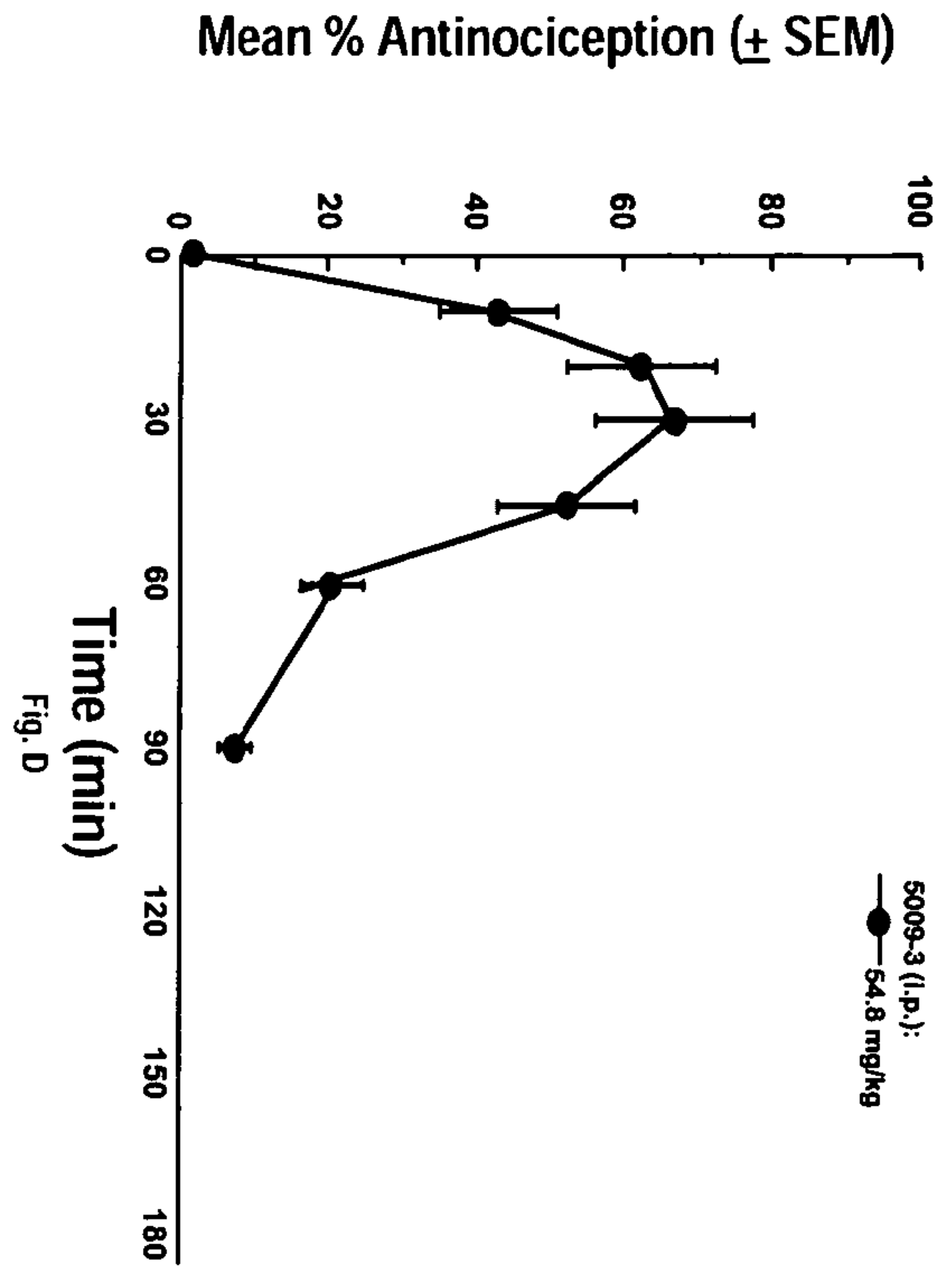


FIGURE 9D-F

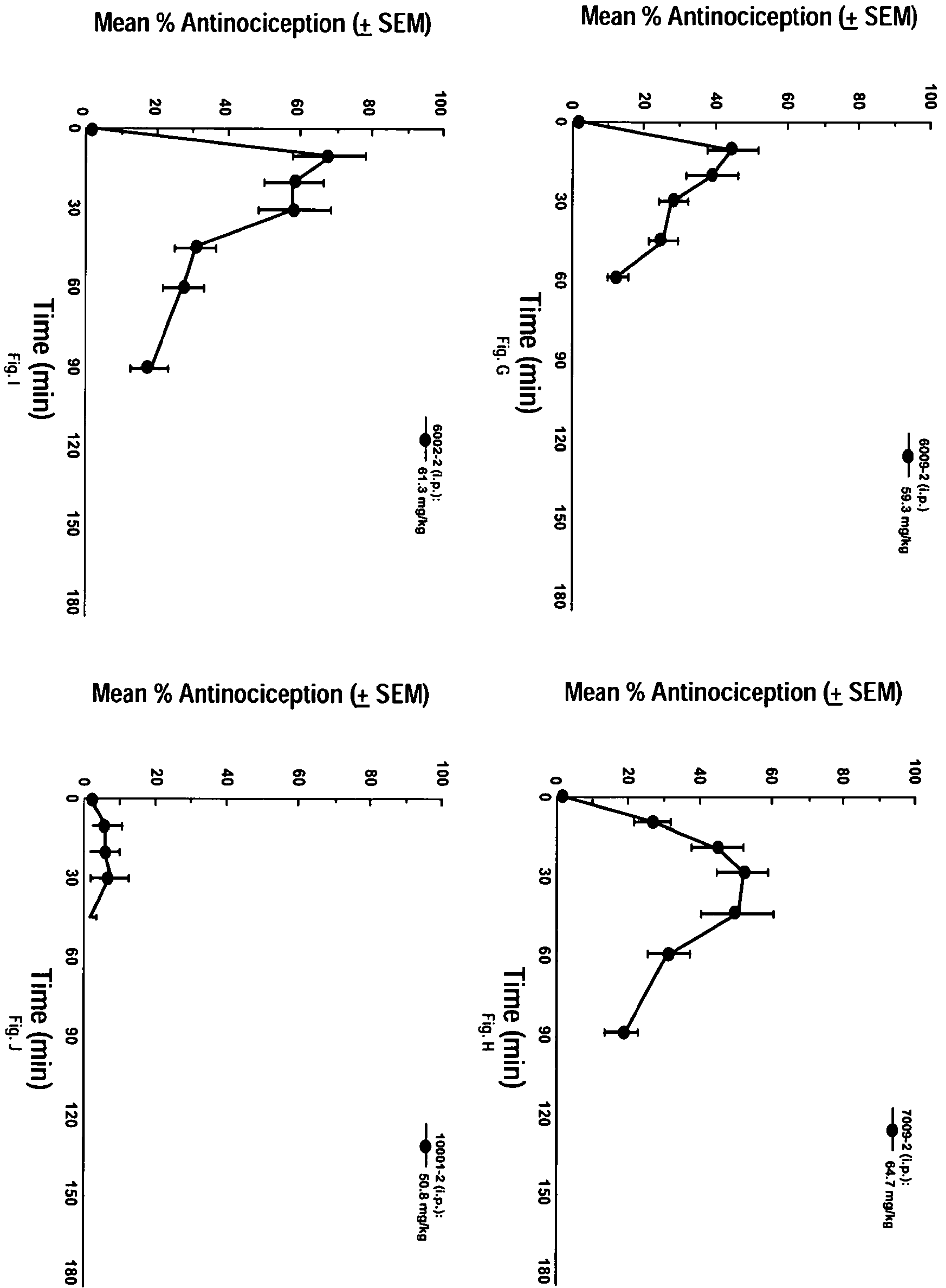
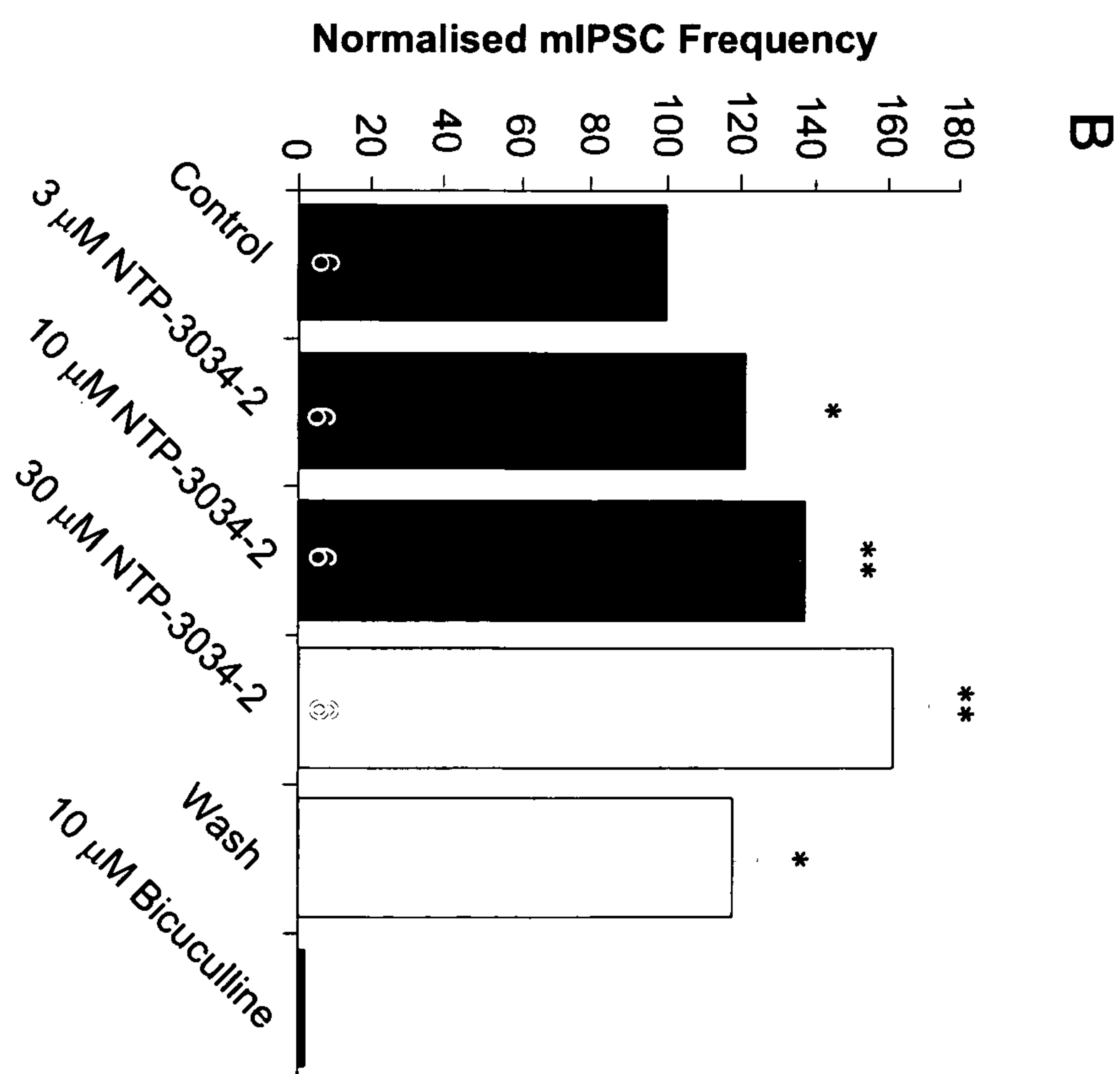
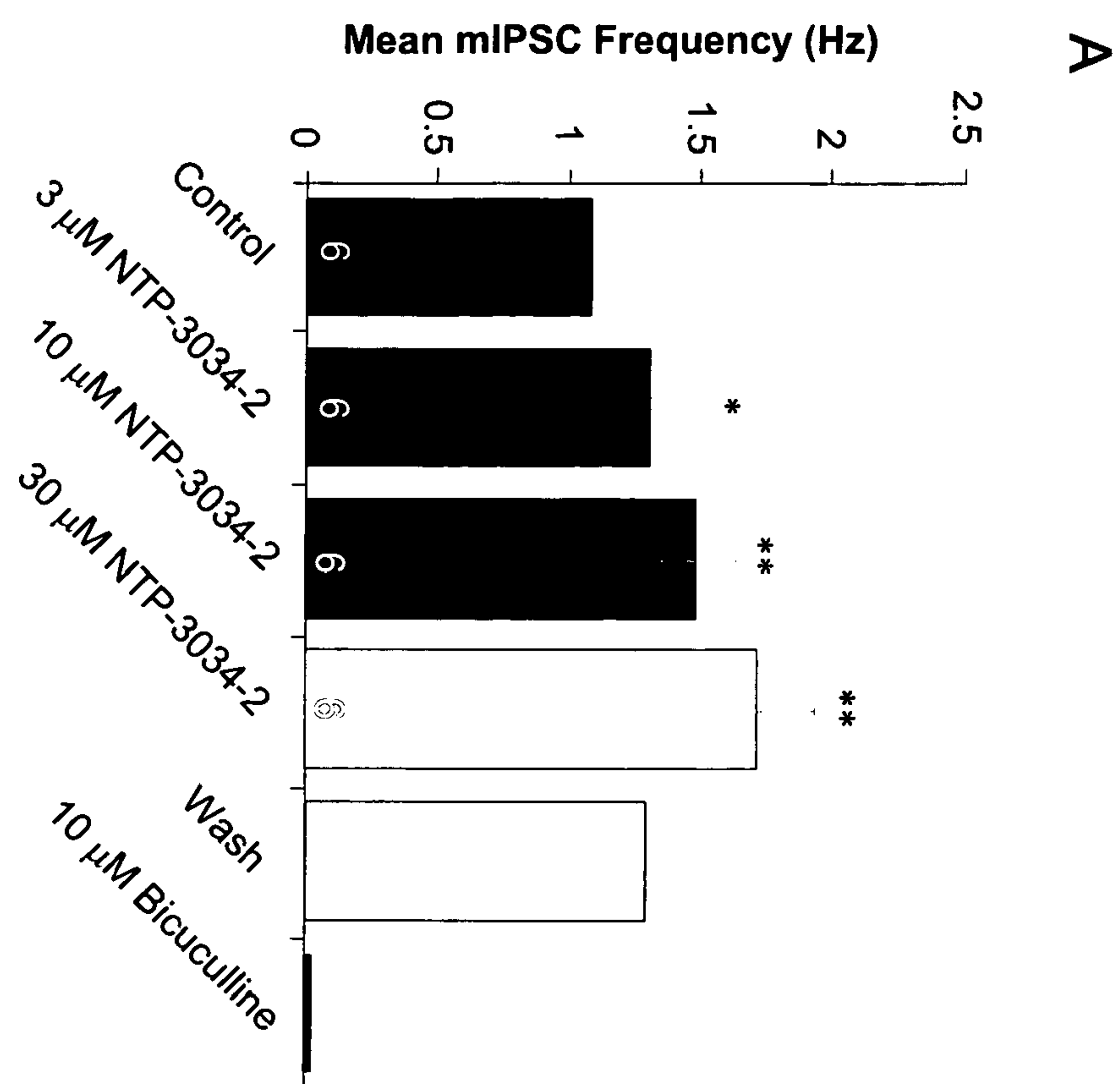


FIGURE 9G-J

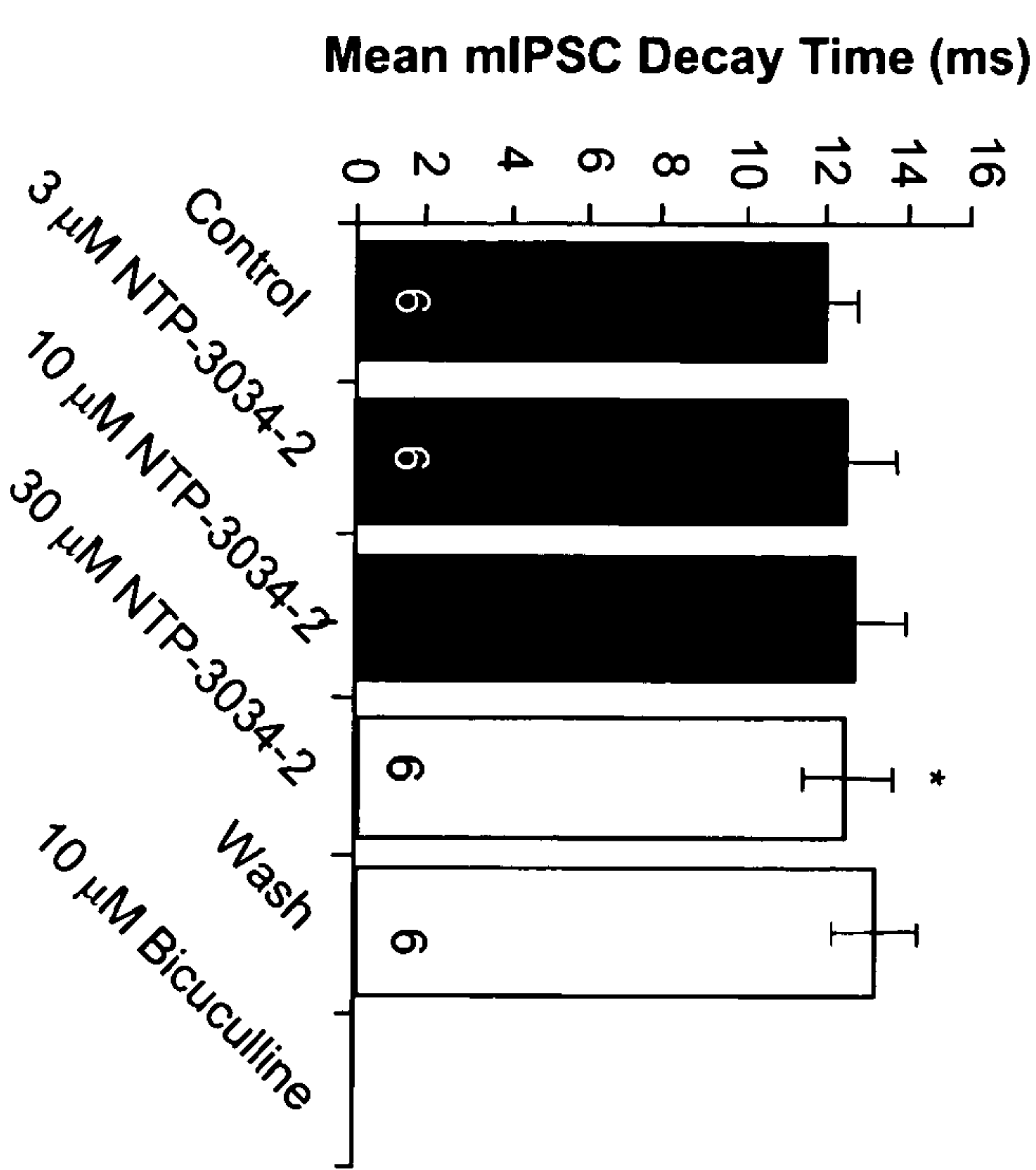
12/17



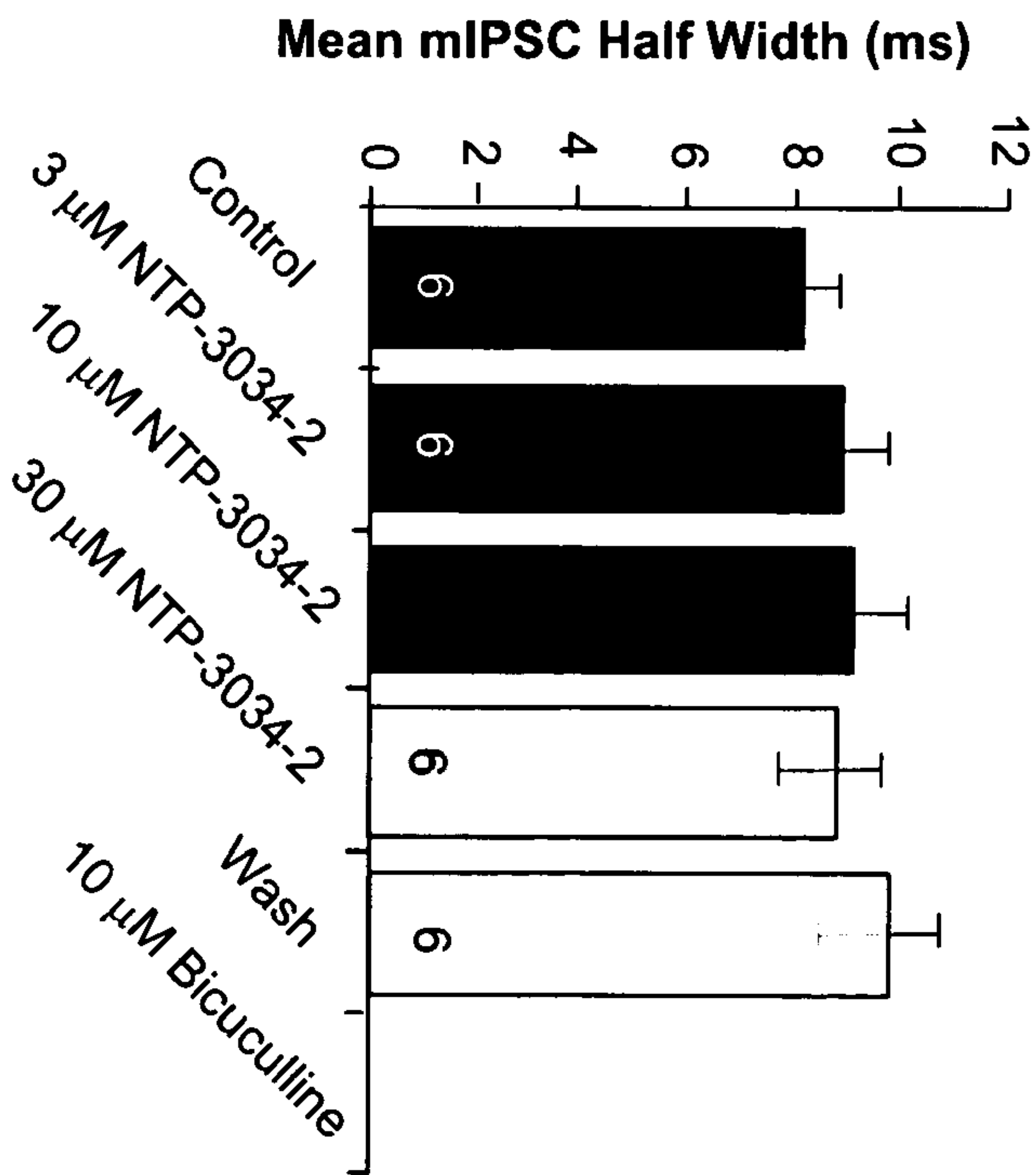
* P < 0.05
** P < 0.01

FIGURE 10A-B

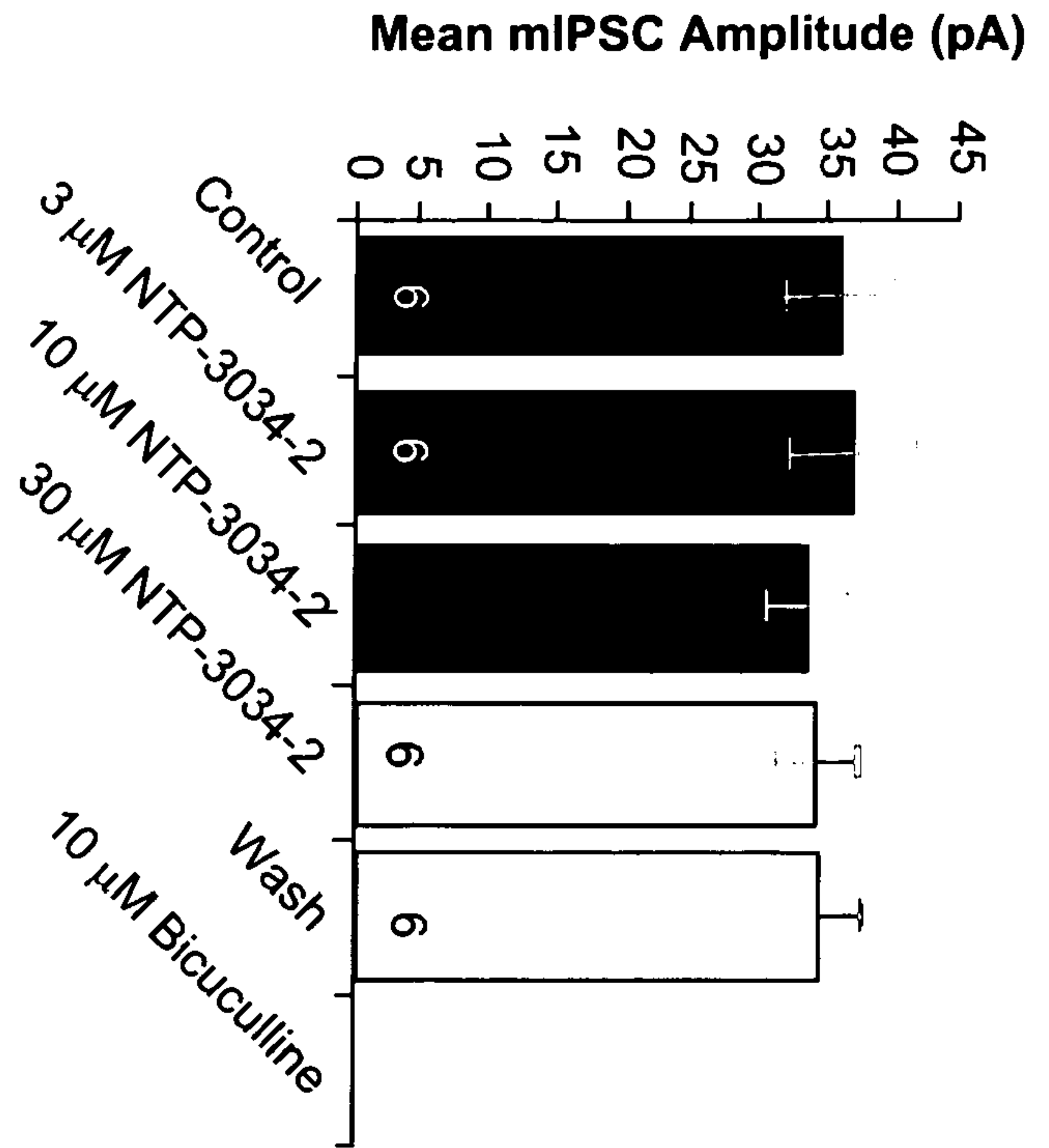
E



F



C



D

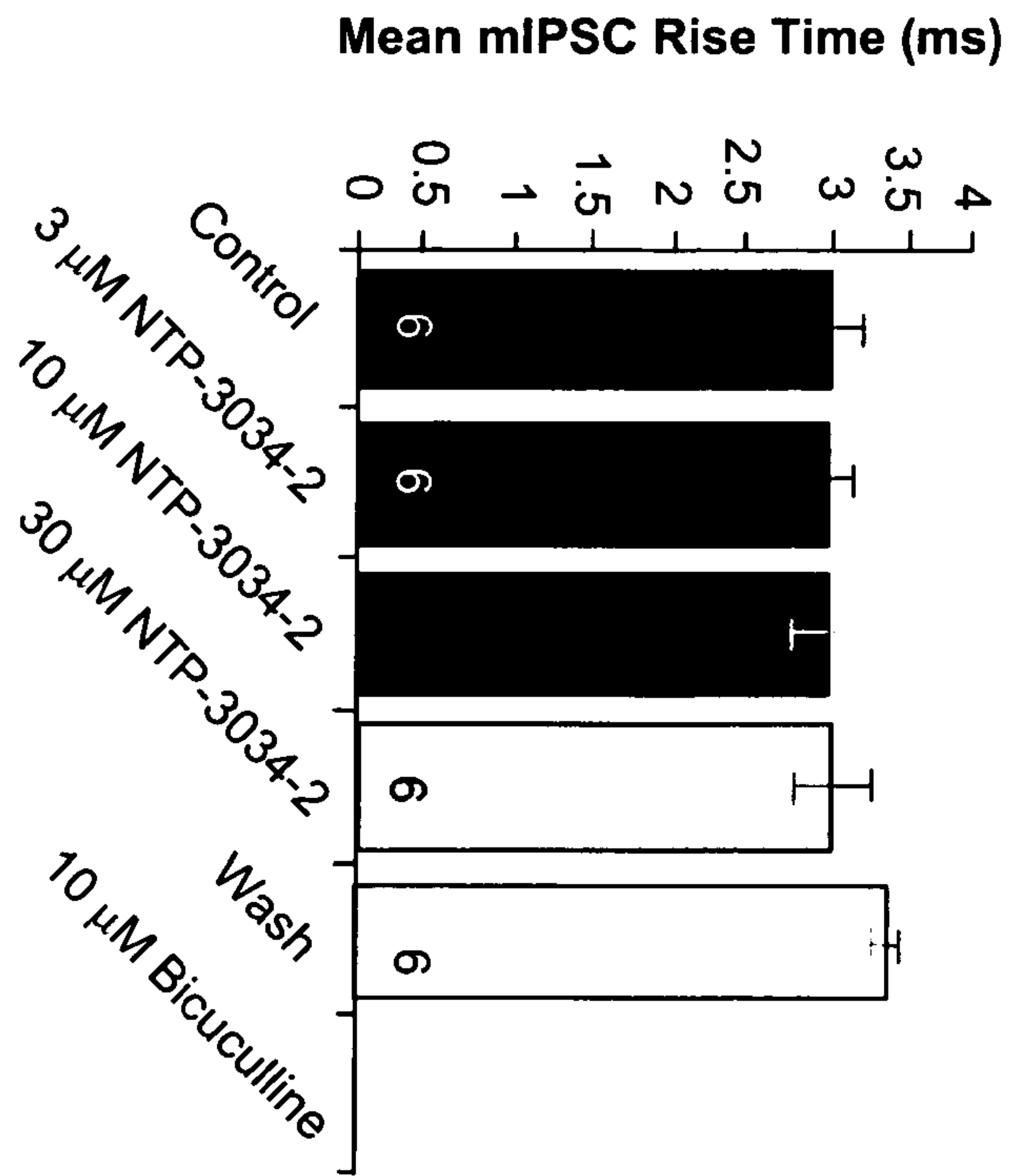
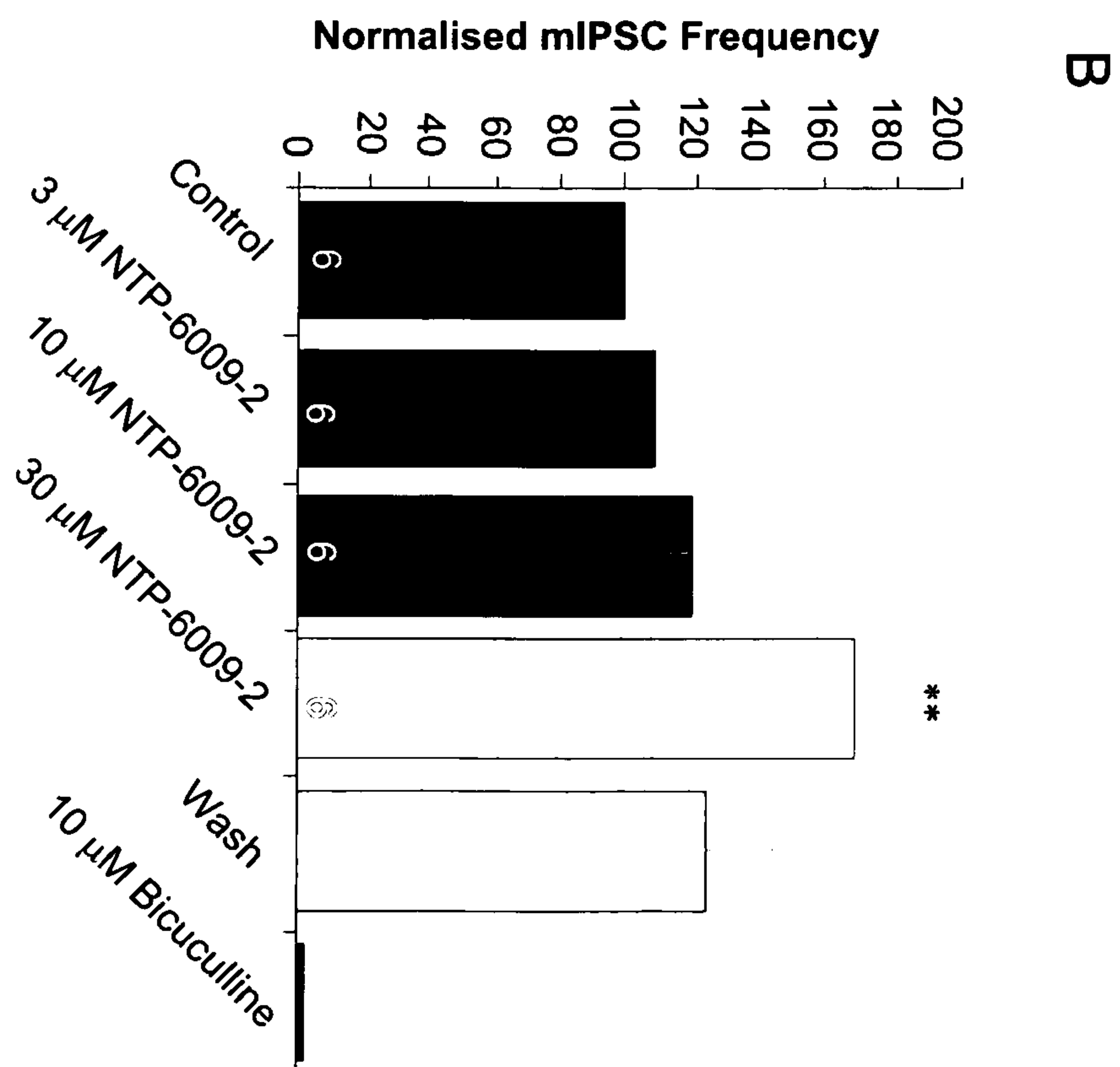
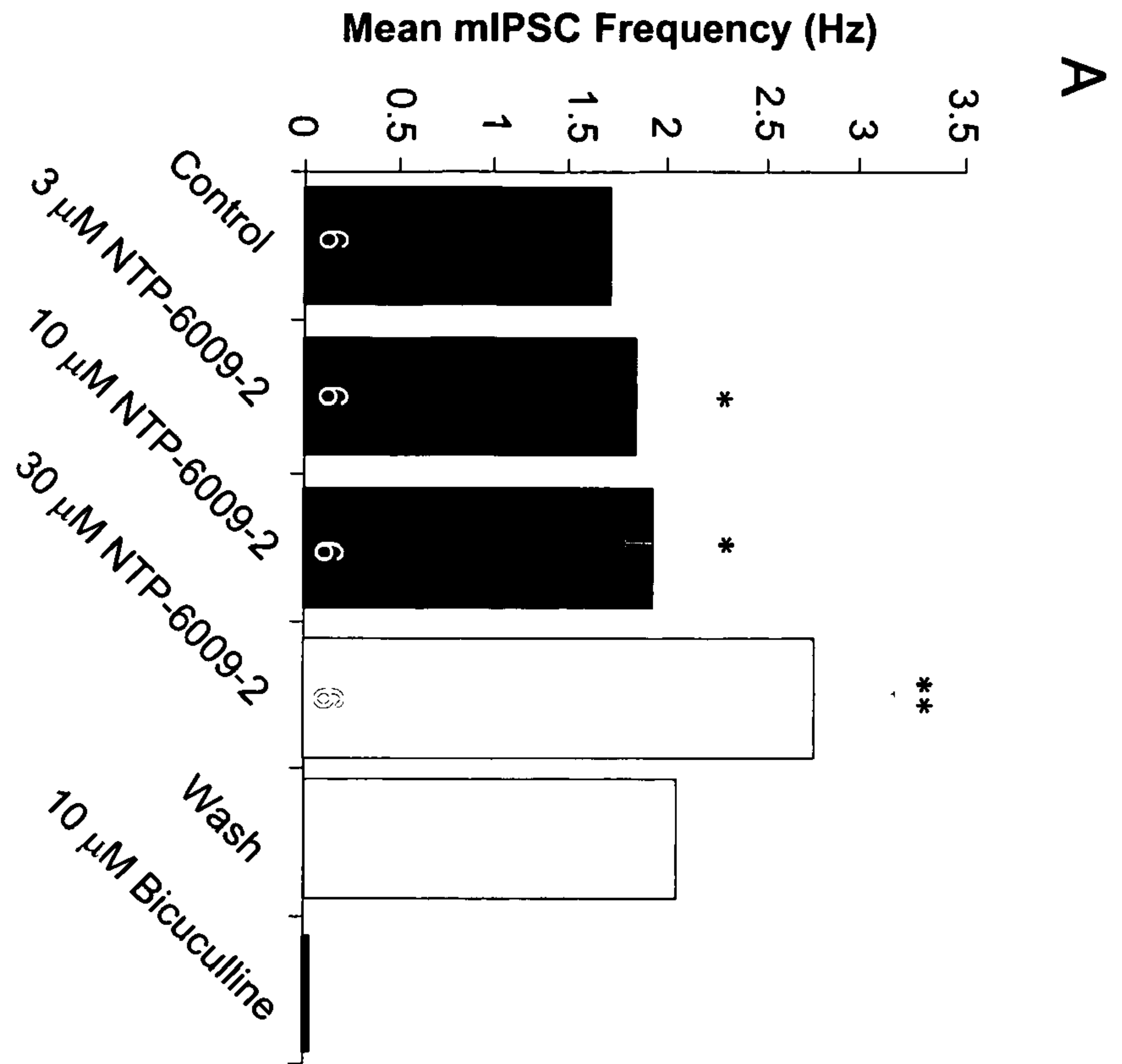


FIGURE 10C-F



* P < 0.05
** P < 0.01

FIGURE 11A-B

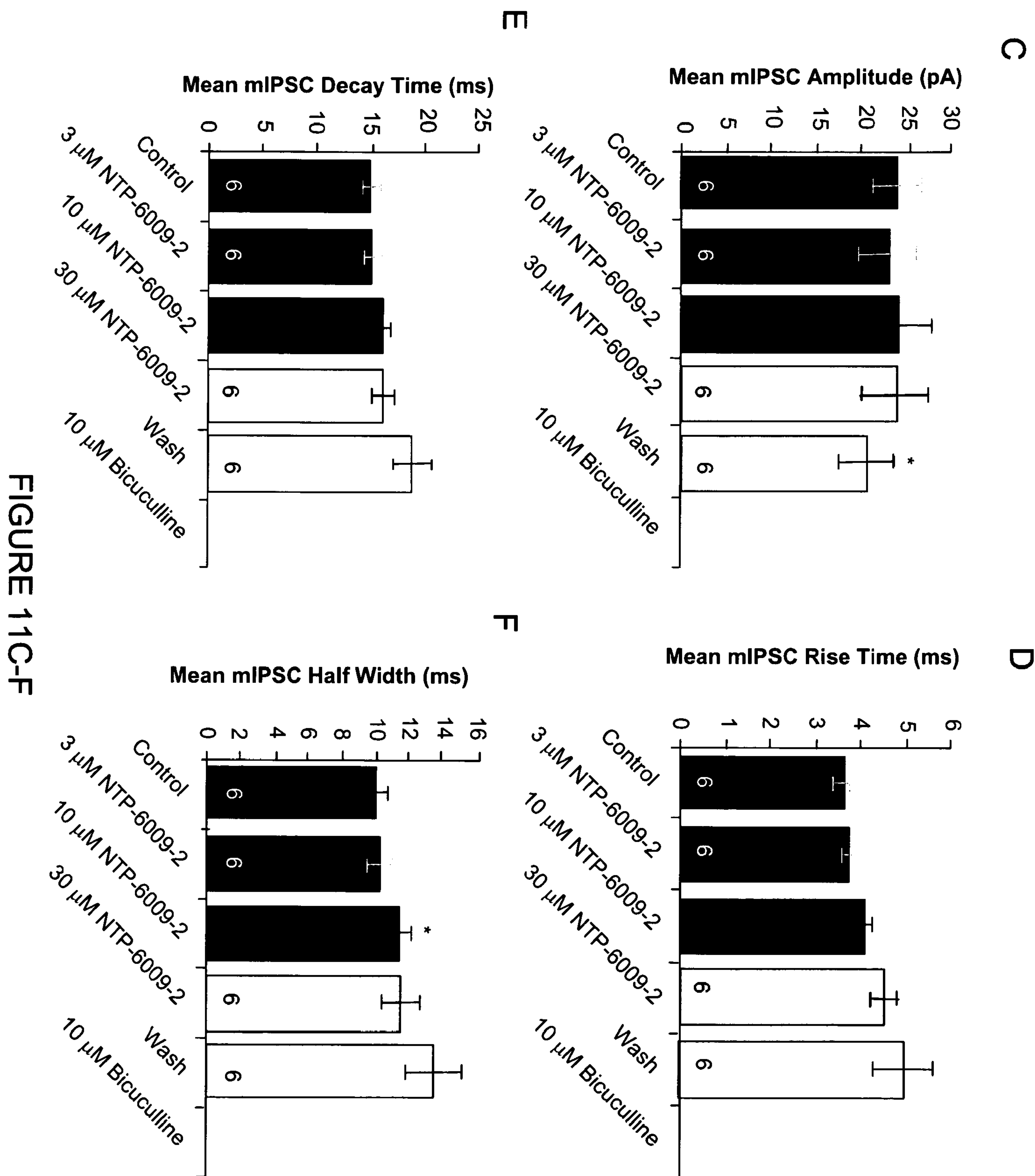
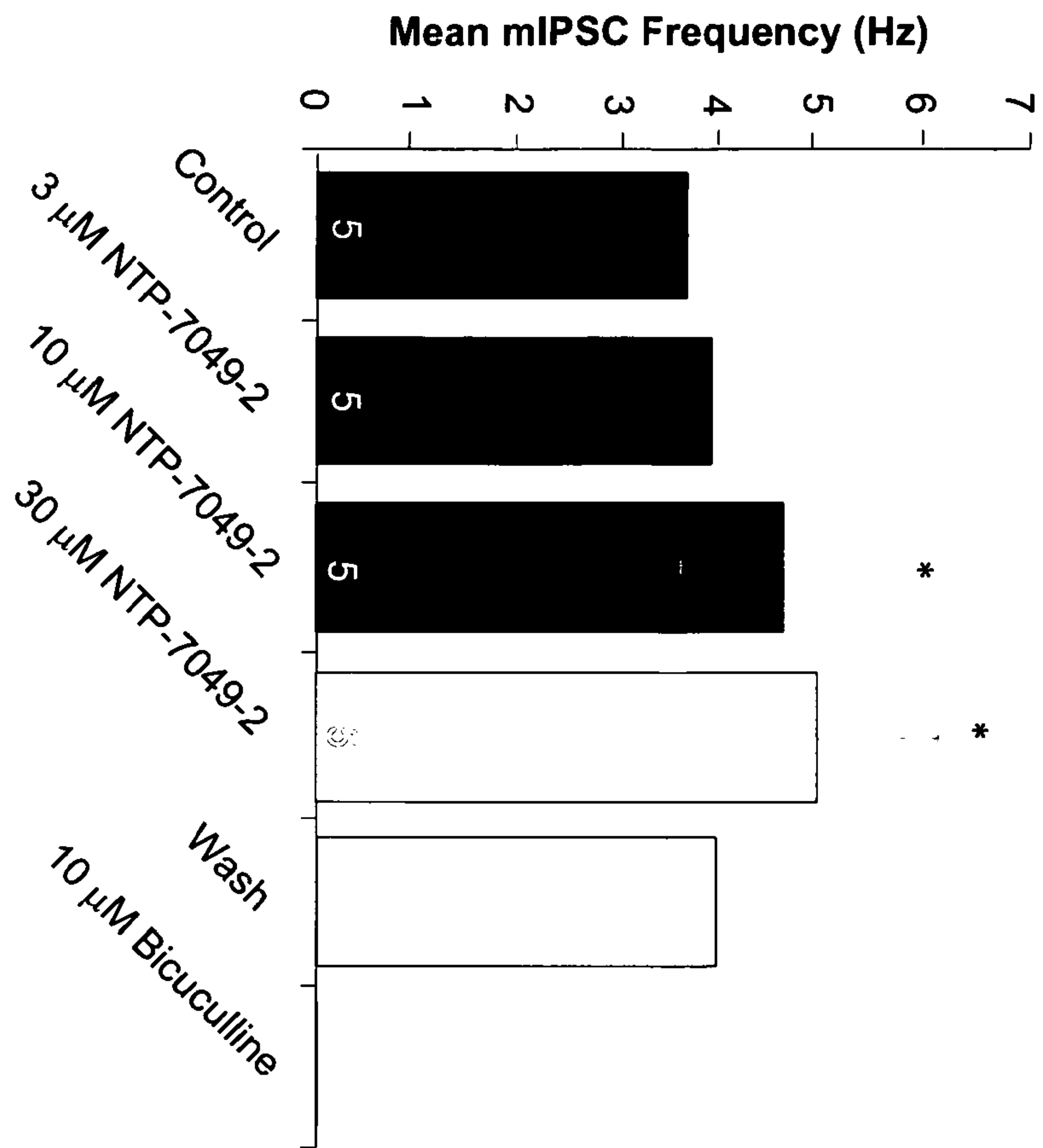


FIGURE 11C-F

A



B

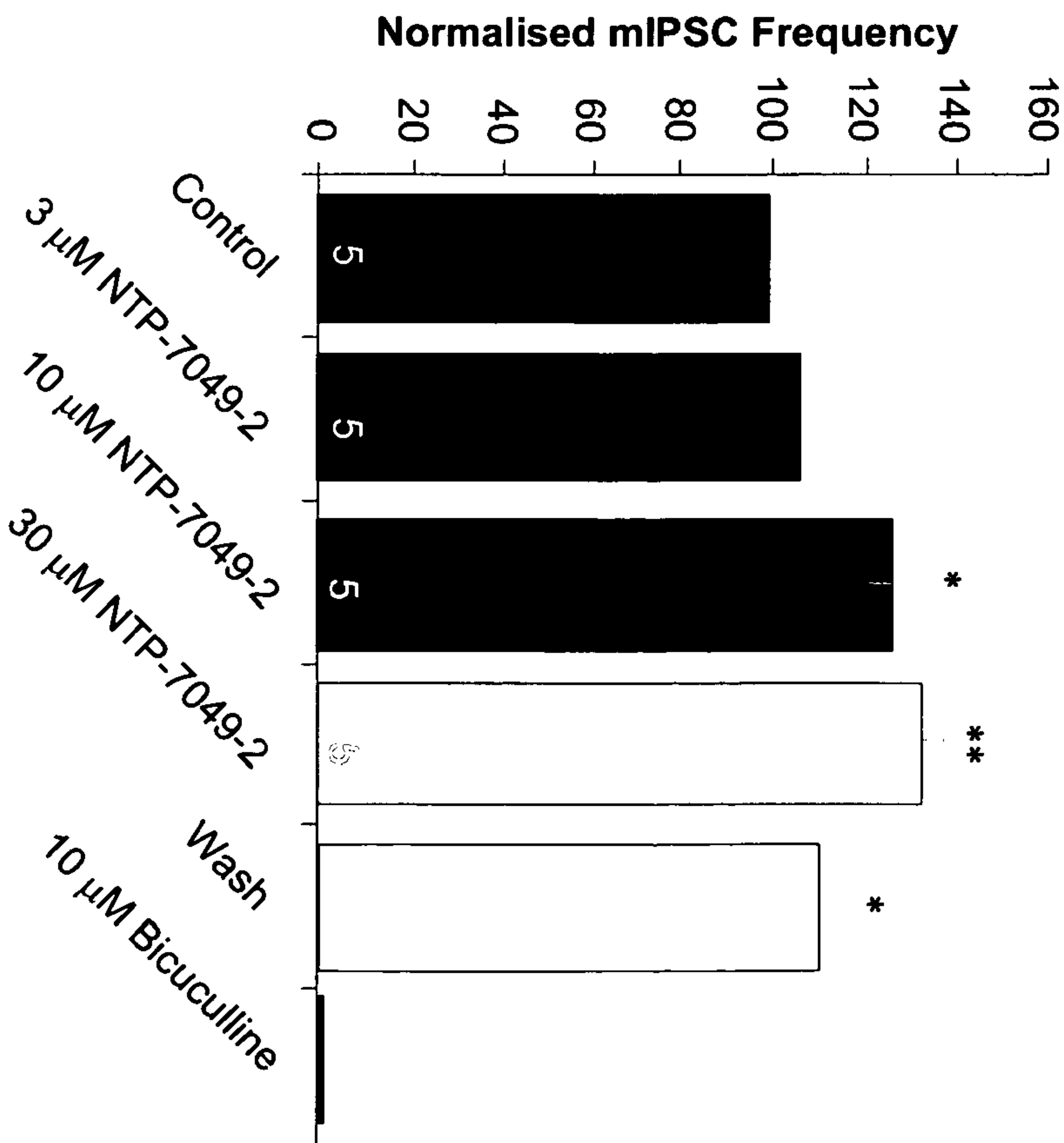


FIGURE 12A-B

* P < 0.05
** P < 0.01

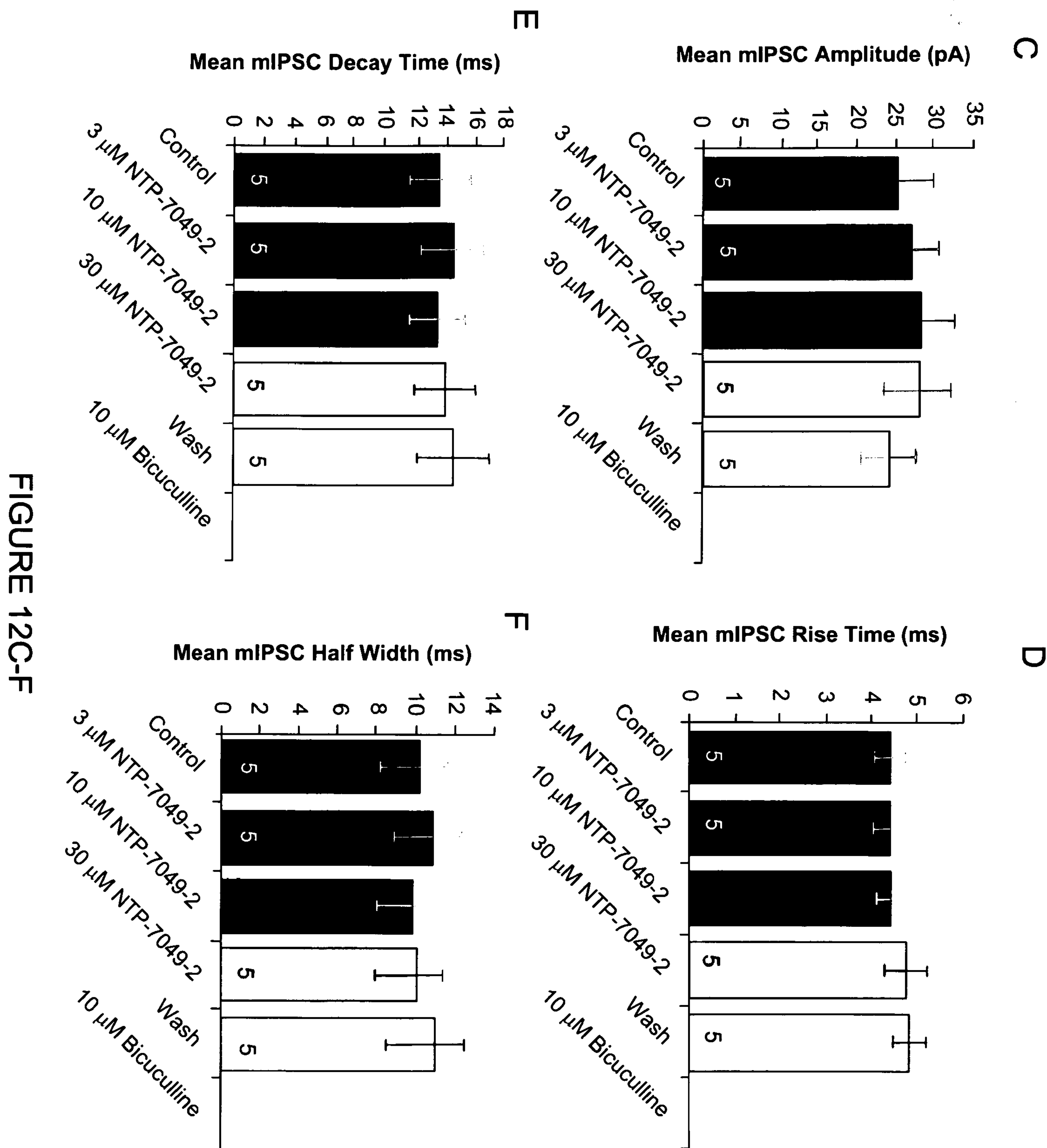


FIGURE 12C-F

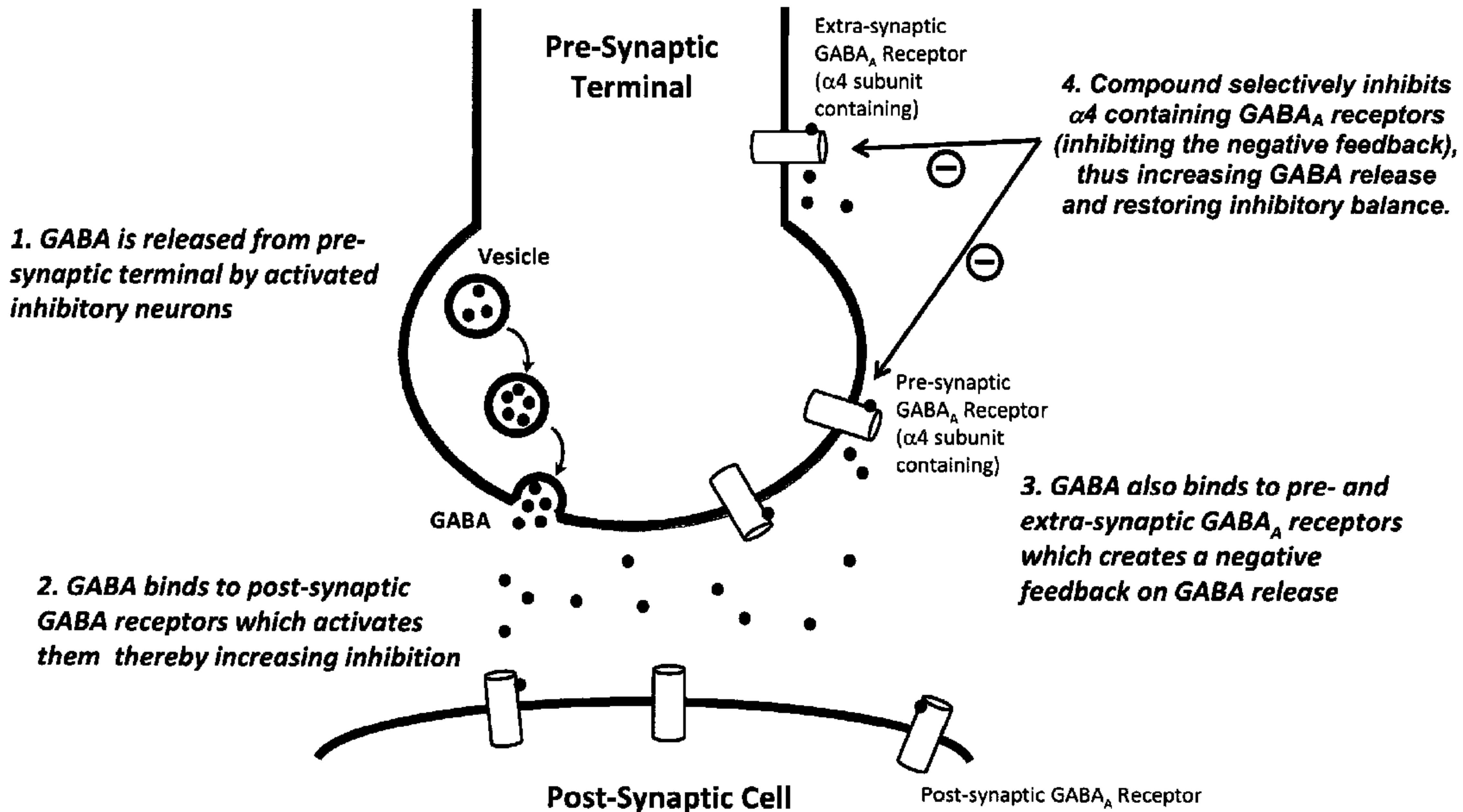


FIGURE 1