



(51) International Patent Classification:

A61K 31/4545 (2006.01) A61K 31/70 (2006.01)
A61K 31/513 (2006.01)

(21) International Application Number:

PCT/US2018/042099

(22) International Filing Date:

13 July 2018 (13.07.2018)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/532,156 13 July 2017 (13.07.2017) US

(71) Applicant: PLEX PHARMACEUTICALS, INC.
[US/US]; Nancy Ridge Technology Center, 6330 Nancy Ridge Drive, Suite 105, San Diego, CA 92121 (US).

(72) Inventors: DE, Surya Kanta; 8676 Gold Coast Drive, San Diego, CA 92126 (US). PRASAD, Sridhar, G.; 12236 Ragweed Street, San Diego, CA 92129 (US). PETERMAN, Marshall, C.; 425 S. Ditmar Street, Oceanside, CA 92054 (US). SHARMA, Purnendu, Kumar; 1903 Anderson Road, Apt. #8, Oxford, MS 38655 (US).

(74) Agent: MAHANTA, Sanjeev, K. et al.; Posternak, Blankstein & Lund LLP, 800 Boylston Street, Prudential Tower, Boston, MA 02199 (US).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, 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, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, 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, KM, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

- as to applicant's entitlement to apply for and be granted a patent (Rule 4.17(ii))
- as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))
- of inventorship (Rule 4.17(iv))

(54) Title: COMBINATION THERAPY FOR TREATING CANCER

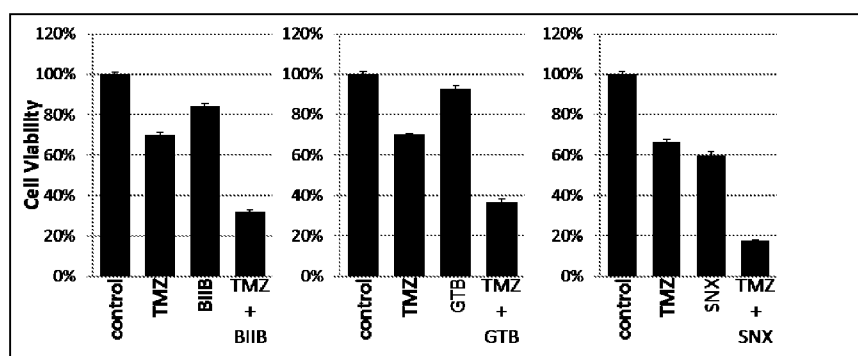


FIG. 5

(57) Abstract: A method of treating cancer comprising administering an effective amount of each of a chemotherapeutic agent and an Hsp90 inhibitor, either separately or as a covalent conjugate of the two, to provide a combination therapy having an enhanced therapeutic effect is described. The Hsp90 inhibitor is capable of also inhibiting TRAP1. Also disclosed are compositions including a chemotherapeutic agent and an Hsp90 inhibitor for nasal delivery.



Published:

— *with international search report (Art. 21(3))*

5

COMBINATION THERAPY FOR TREATING CANCER**BACKGROUND**

Glioblastoma (GBM) is one of the most challenging brain tumors to treat and GBM patients generally do not live more than two years after developing the cancer [1].

10 Worldwide, an estimated 238,000 patients are diagnosed with all forms of brain tumors each year and an estimated 174,000 die from these tumors [2, 3]. With the progress made in diagnosis and treatment of cancer, the 5-year relative overall survival (OS) rate for all cancers (not including GBM) diagnosed between 2003 and 2009 increased to 68% from 49% in 1975-77 [4]. On the contrary, the current standard of care for GBM, which is surgery followed by
15 treatment with temozolomide (TMZ) in combination with radiation, has led to an increase in the median OS to just 14.6 months with 26.5% of patients surviving only up to two years [5]. Avastin (bevacizumab), used as a second-line treatment, showed no difference in OS in a multi-center clinical trial [6-8].

Extensive genetic profiling of glioma has shown that GBM tumors harbor genetic
20 aberrations in three critical cell-signaling pathways: (a) RTK/RAS/PI3K (88%); (b) p53 (87%), and (c) RB (78%) [9-12]. Collectively, numerous oncogenes in these pathways are either mutated and/or amplified, including epidermal growth factor receptor (EGFR) and cyclin dependent kinases (CDK4 and CDK6), supporting signaling kinases as targets for chemotherapy of GBM [9-12]. However, inhibition of a single kinase has been found to be
25 ineffective due to the activation of alternative and compensatory signaling pathways [13]. In addition, development of resistance by GBM cells towards therapy-induced apoptosis further limits the effectiveness of current chemotherapeutic agents [14]. Several preclinical and clinical studies have established overexpression of Bcl-2 family of proteins as one of the underlying factors for drug-induced resistance in GBM [15].

30 Cytosolic Hsp90 and its mitochondrial paralog, TRAP1, are ATP-dependent molecular chaperones that are highly expressed in GBM in contrast to normal brain [16-21]. Hsp90 promotes GBM cell viability through the formation of a chaperone complex or interactome made of hundreds of client proteins, including cell-signaling proteins that have been shown to be critical for proliferation, growth, and survival of GBM tumors [22]. These
35 client proteins are highly dependent on Hsp90 activity for folding into an active conformation. TRAP1 is exclusively localized in mitochondria and is crucial for the Warburg effect in GBM [19]. Notably, TRAP1 inhibition induces tumor cell-death

5 independent of Bcl-2 family of proteins [23-25]. Furthermore, Hsp90 and TRAP1 share a high degree of sequence homology, including in the ATP-binding pocket [26].

Inhibition of Hsp90 function disrupts normal cycling of the chaperone complex, resulting in simultaneous degradation of multiple key oncogenic proteins [27]. The resulting widespread loss of these key oncogenic proteins is expected to increase the susceptibility of
10 GBM tumors to therapeutic intervention [28]. Of note, inhibition of Hsp90 in animal models of GBM has shown to: (i) enhance the radiosensitization effects of PARP and PI-3 kinase inhibitor [29-31] and (ii) improve the cytotoxic effect of radiation and TMZ [32].

The Hsp90 inhibitor NXD30001 leads to reduction of EGFR, Akt, Cdk4 and CyclinD1 expression, with associated cytotoxicity in GBM. Most importantly, in an EGFR-
15 driven genetically engineered mouse model of GBM, NXD30001 led to a significant increase in mean survival times [33]. In addition, multiple Hsp90 inhibitors, including 17-N-allylamino-17-demethoxygeldanamycin (17-AAG) and NVP-AUY992, have been shown to display anti-proliferative activity in a number of GBM cell lines, and shown to inhibit tumor growth along with induction of apoptosis in xenograft models [18, 34, 35-37]. However,
20 NXD30001 was found to accumulate in the brain only at very high doses (100 and 150 mg/kg) [49]. On the other hand, 17-AAG has unfavorable PK properties and there is no evidence that NVP-AUY992 crosses the blood brain barrier [37, 38]. Given these limitations, no Hsp90 inhibitor has been advanced into clinical trials for GBM.

TRAP1 is exclusively localized in the mitochondria and is intimately involved with
25 cellular processes associated with apoptosis [39]. Its depletion was shown to strongly decrease GBM cell proliferation, inhibit neurosphere recovery and secondary neurosphere formation, and most importantly, enhance the therapeutic effect of TMZ in neurosphere cultures [19]. In addition to the genetic evidence, pharmacological targeting of TRAP1 by Gamitrinibs (Gas; resorcinolic small molecules that specifically act on TRAP1) was shown to
30 suppress intracranial GBM growth in mice with no detectable weight loss, or organ/systemic toxicity [40]. However, GAs are sub-optimal with respect to the crossing of the blood brain barrier (MW>500Da, cLogP= -1.01 and PSA = 185.5Å²) [41]. The peptidomimetic Hsp90/TRAP1 inhibitor, shepherdin (MW>900Da), has been shown to cause a CypD-dependent irreversible collapse of mitochondrial membrane potential in primary brain tumor
35 cells (suggesting effective mitochondrial penetration), and to inhibit intracranial tumor growth in an animal model [20]. As noted before, mitochondrial TRAP1 inhibitor-mediated cell death and apoptosis occurs independent of antiapoptotic Bcl-2 pathways [25, 42],

5 suggesting that TRAP1 inhibitors potentially bypasses the commonly acquired Bcl-2 chemoresistance mechanism in GBM [43].

Many drug molecules that are potent vis-à-vis their respective targets and hold great promise as therapeutic agents fail in clinical trials due to problems associated with formulation, e.g., low bioavailability in the case of drugs that have to be localized to the
10 brain.

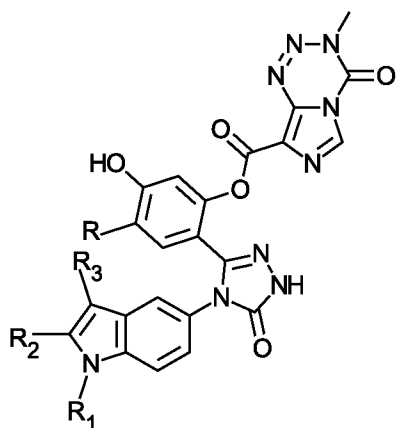
There is an unmet need for more efficacious treatment of cancer including cancers of the brain such as GBM.

SUMMARY

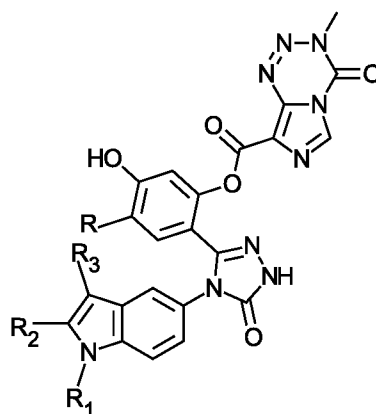
15 To meet this need, described herein is a technology that provides methods and compositions for treating cancer using a combination therapy. The combination therapy requires using a chemotherapeutic agent with an Hsp90 inhibitor. The chemotherapeutic agent and the Hsp90 inhibitor may be covalently linked. Therapy with chemotherapeutic agent often leads to induction of resistance caused at least in part due to overexpression of
20 Bcl-2 family of anti-apoptotic proteins. The present technology encompasses using Hsp90 inhibitor of the kind that both suppresses cancer promoting signaling pathways and inhibits TRAP1, thereby bypassing Bcl-2 mediated resistance to chemotherapeutic agents. Such Hsp90 inhibitors are also referred to as dual-acting Hsp90/TRAP1 inhibitors. Use of brain-permeable, mitochondria-permeating, dual-acting Hsp90/TRAP1 inhibitors in combination
25 with a chemotherapeutic agent, such as Temozolomide, make methods disclosed herein particularly suitable for treating tumors of the brain, such as Glioblastoma and related tumors. Further described are formulations for nasal delivery of chemotherapeutic agents and Hsp90 inhibitors, as well as examples of dual-acting Hsp90/TRAP1 inhibitors covalently linked to a chemotherapeutic agent and their formulation for nasal delivery.

30 Accordingly, in one aspect the present technology provides a method of treating cancer, the method comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof. The Hsp90 inhibitor is capable of also inhibiting TRAP1. The conjugate is selected from the group consisting of

35 (i) compounds with ganetespib as the Hsp90 inhibitor, having the formula



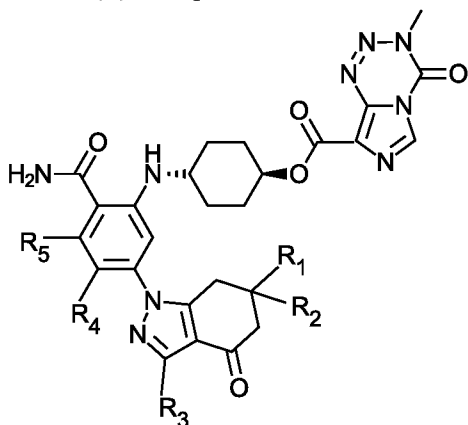
(I), and



(II), wherein

R, R₁, R₂, R₃, are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

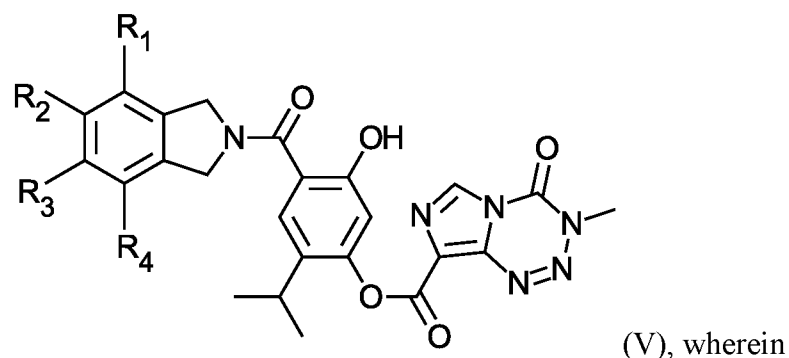
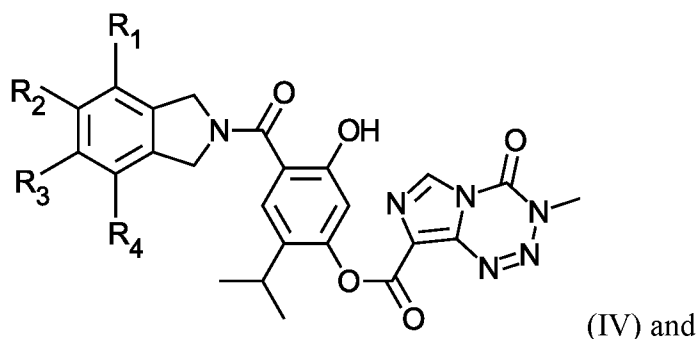
(ii) compounds with SNX-2112 as the Hsp90 inhibitor, having the formula



(III), wherein

R₁, R₂, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

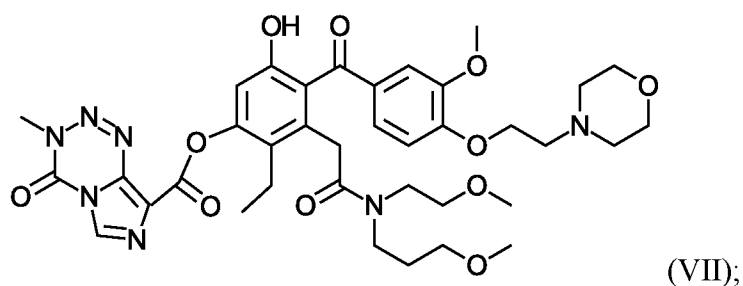
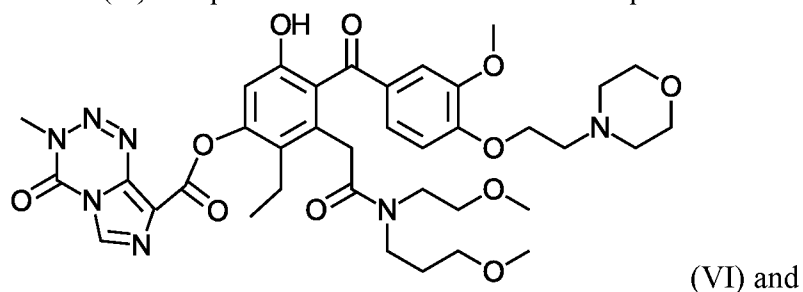
(iii) compounds with Onalespib as the Hsp90 inhibitor, having the formula



R₁, R₂, R₃, and R₄ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

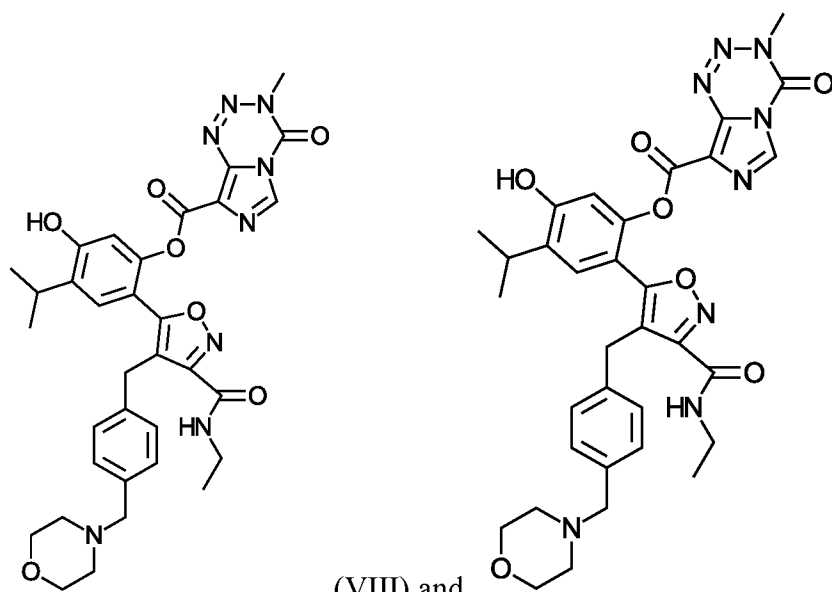
10

(iv) compounds with KW-2487 as the Hsp90 inhibitor having the formula

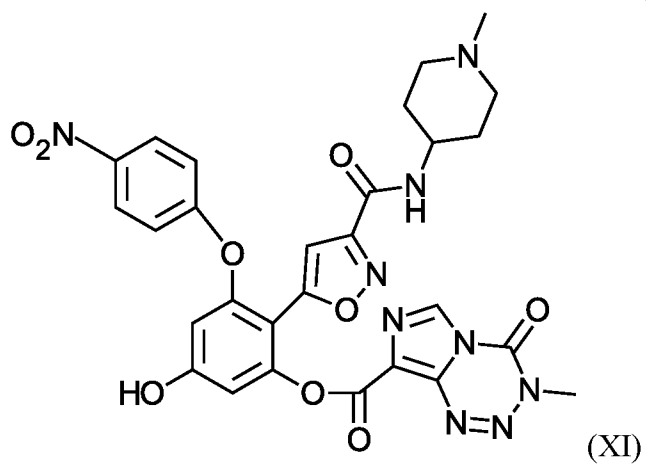
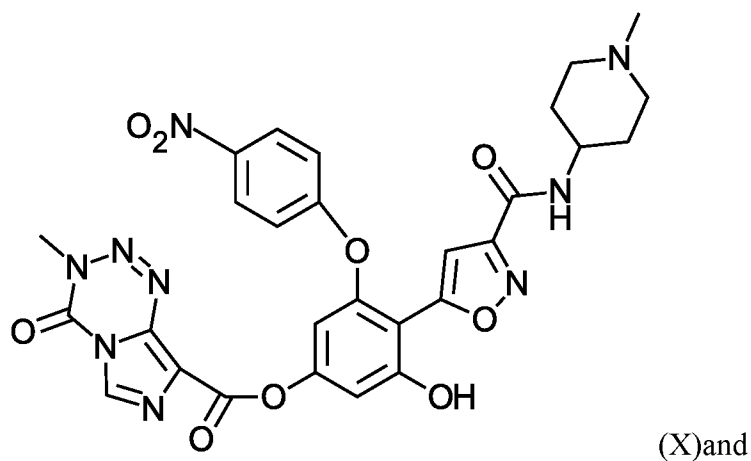


(v) compounds with Luminespib as the Hsp90 inhibitor, having the formula

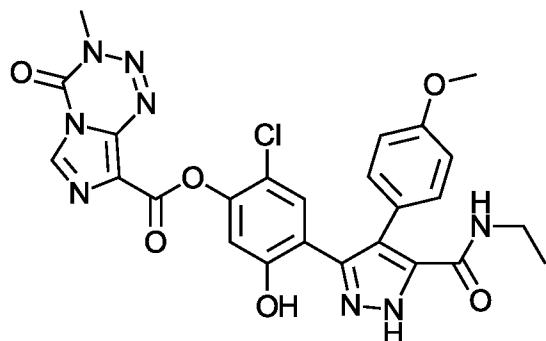
15



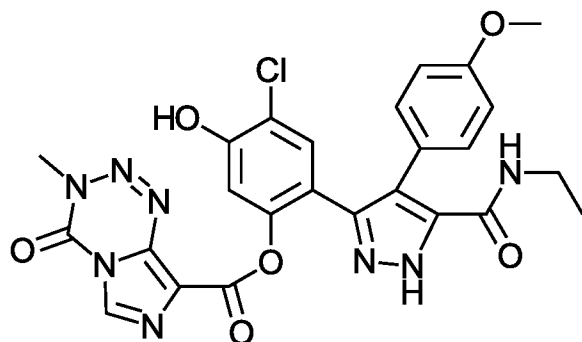
(v) compounds with NMS-E973 as the Hsp90 inhibitor, having the formula



(v) compounds with VER-50589 as the Hsp90 inhibitor, having the formula



(XII) and



(XIII).

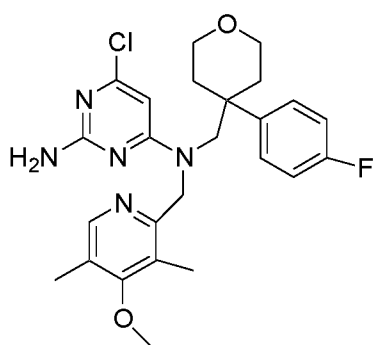
The method may be used to treat a variety of cancer including glioblastoma, primary malignant brain tumor, or malignant glioma.

In another aspect, the present technology provides a pharmaceutical composition for nasal administration. The composition comprises a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor having any one of the formulas I to XIII; a lipid and a non-ionic surfactant; and an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the conjugate. In a related aspect, the technology includes a method of treating cancer, the method comprising administering to a subject in need thereof the composition for nasal administration.

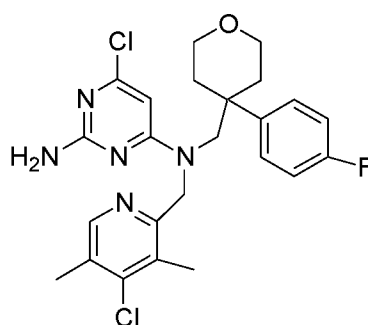
In a further aspect, provided herein is pharmaceutical composition comprising a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor having any one of the formulas I to XIII, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier. In a related aspect, the technology includes a method of treating cancer, the method comprising administering this composition to a subject in need thereof.

The present technology also includes a method of treating comprising administering to a subject in need thereof an effective amount of each of a chemotherapeutic agent and an Hsp90 inhibitor, which are not conjugated by a covalent bond. The method provides a combination therapy having enhanced therapeutic effect compared to the effect of the

5 chemotherapeutic agent or the Hsp90 inhibitor alone. Among the Hsp90 inhibitor that are used are those capable of also inhibiting TRAP1. The combination therapy produces a synergistic effect. Alternatively, the therapy may produce an additive effect. The chemotherapeutic agent may be a DNA alkylating agent, e.g., temozolomide (TMZ). Hsp90 inhibitor that may be used include Genetespib, Onalespib, SNX-2112, SNX-5422, XL-888, NMS-873, NMS-E973 (or PU-H71), NVB-BEP800, Luminespib, KW-2487, Tenespimycin (17-AAG), Alvespimycin (17-DMAG), VER-49009, CH5138303, VER-50589, BIIB021, VER-155008, Geldanamycin, HSP990 (NVP-HSP990), IPI-504 (retaspimycin hydrochloride), IPI-493, any of the Hsp90 inhibitors used to produce the conjugates defined by formulas I to XIII, or a compound having the formula CAP1088 or CAP1100 shown below



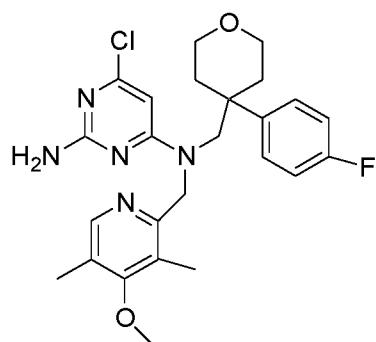
(CAP1088),



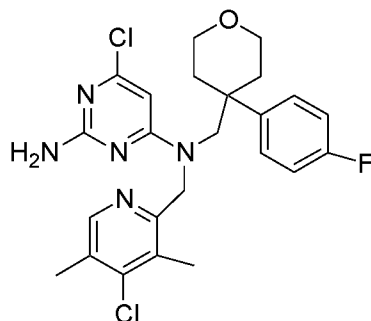
(CAP1100).

The chemotherapeutic agent and the Hsp90 inhibitor may be administered simultaneously either as a single composition or as separate compositions. The two may also be administered sequentially.

20 The present technology also includes a pharmaceutical composition for nasal administration. The composition includes (i) a chemotherapeutic agent, (ii) an Hsp90 inhibitor, or (iii) a combination of a chemotherapeutic agent and an Hsp90 inhibitor; each combined with a lipid and a non-ionic surfactant and an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the chemotherapeutic agent and the Hsp90 inhibitor. The Hsp90 inhibitors that may be used in this composition include Genetespib, Onalespib, SNX-2112, SNX-5422, XL-888, NMS-873, NMS-E973 (or PU-H71), NVB-BEP800, Luminespib, KW-2487, Tenespimycin (17-AAG), Alvespimycin (17-DMAG), VER-49009, CH5138303, VER-50589, BIIB021, VER-155008, Geldanamycin, HSP990 (NVP-HSP990), IPI-504 (retaspimycin hydrochloride), IPI-493, any of the Hsp90 inhibitors used to produce the conjugates defined by formulas I to XIII, or a compound having the formula CAP1088 or CAP1100 shown below



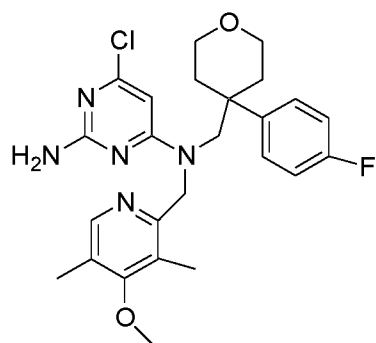
(CAP1088),



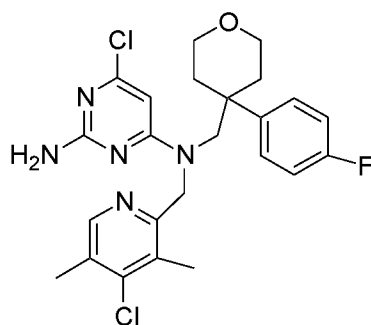
(CAP1100). The

absorption promoting agent may be a cationic polymer, e.g., chitosan. The technology also includes a method of treating cancer requiring administering to a subject in need thereof a composition for nasal administration as described in this paragraph. The cancer to be treated can be Glioblastoma, primary malignant brain tumor, or malignant glioma.

The present technology also contemplates a method of treating cancer, comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof in combination with a second Hsp90 inhibitor, a second chemotherapeutic agent, or both. The second Hsp90 inhibitor and the second chemotherapeutic agent may be the same as the ones used for making the covalently-linked conjugate or different. The Hsp90 inhibitors that may be used in this method include Genetespi, Onalespi, SNX-2112, SNX-5422, XL-888, NMS-873, NMS-E973 (or PU-H71), NVB-BEP800, Luminespi, KW-2487, Tenespimycin (17-AAG), Alvospimycin (17-DMAG), VER-49009, CH5138303, VER-50589, BIIB021, VER-155008, Geldanamycin, HSP990 (NVP-HSP990), IPI-504 (retaspimycin hydrochloride), IPI-493, any of the Hsp90 inhibitors used to produce the conjugates defined by formulas I to XIII, or a compound having the formula CAP1088 or CAP1100 shown below



(CAP1088),



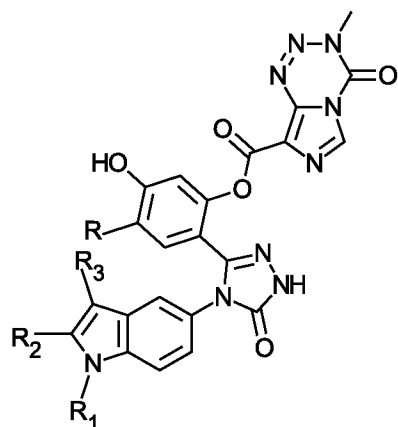
(CAP1100).

Using the methods described above, cancers which have developed resistance to a chemotherapeutic agent may also be treated.

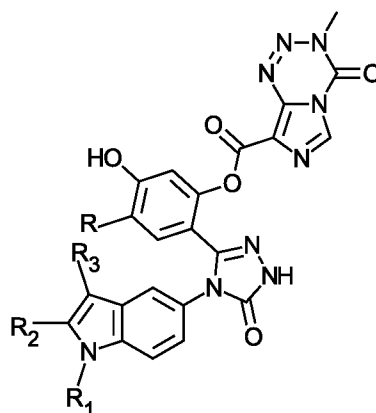
5 The present technology can be further summarized by the following list of embodiments.

1. A method of treating cancer, comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof,
 10 wherein the Hsp90 inhibitor is capable of also inhibiting TRAP1, and wherein the conjugate is selected from the group consisting of

(i) compounds with Ganetespib as the Hsp90 inhibitor, having the formula



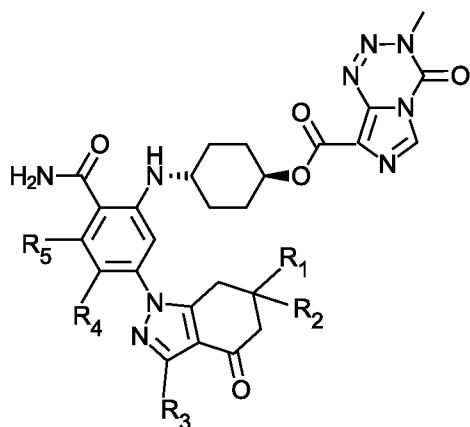
(I), and



(II), wherein

R, R₁, R₂, R₃, are independently selected from the group consisting of hydrogen; halogen;
 15 (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

(ii) compounds with SNX-2112 as the Hsp90 inhibitor, having the formula

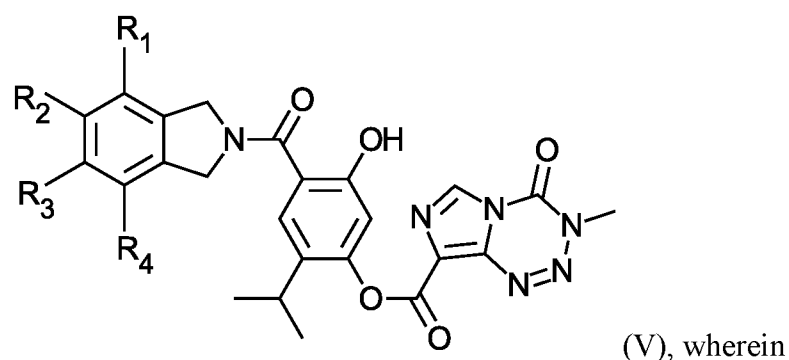
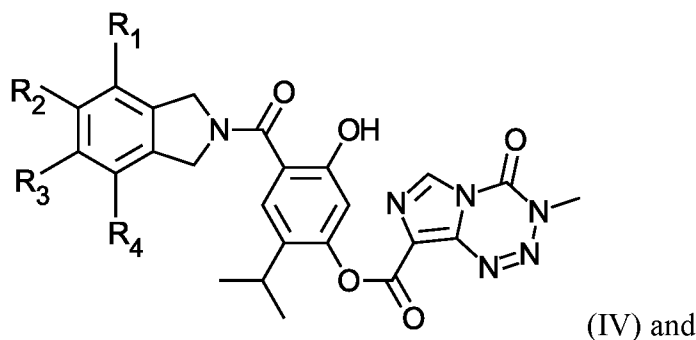


(III), wherein

20 R₁, R₂, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-

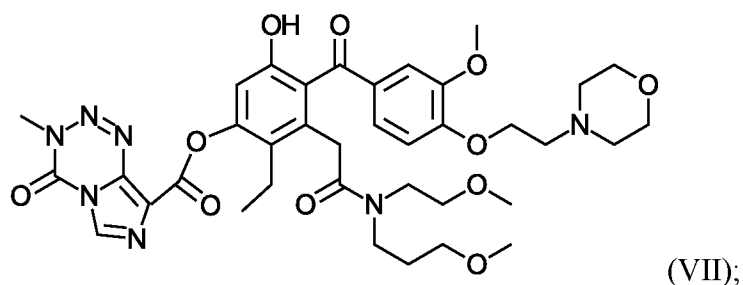
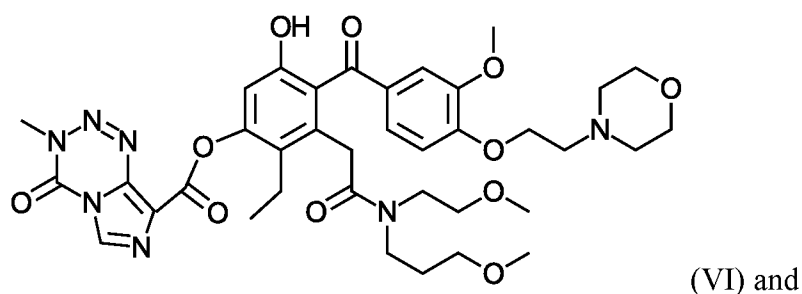
- 5 C₇cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

(iii) compounds with Onalespib as the Hsp90 inhibitor, having the formula

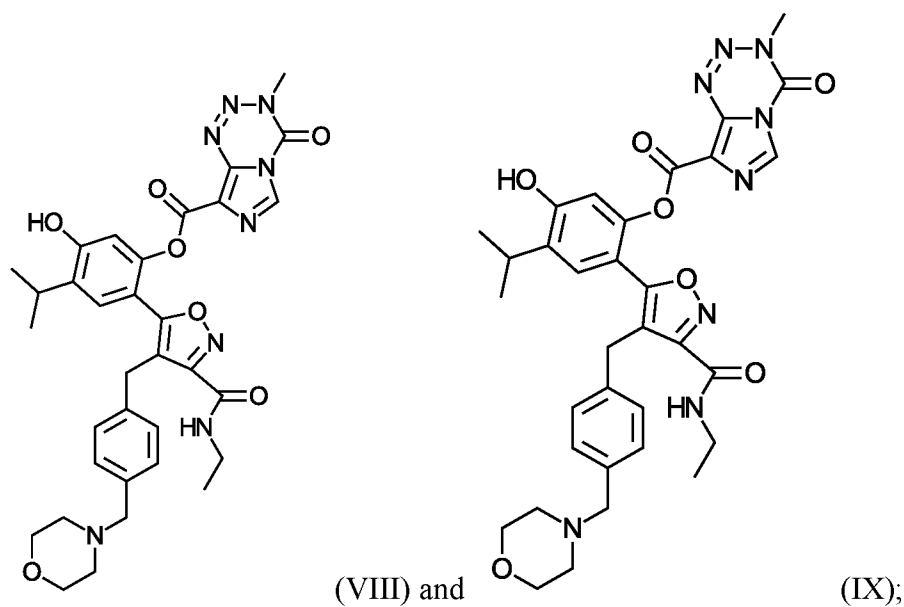


- 10 R₁, R₂, R₃, and R₄ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

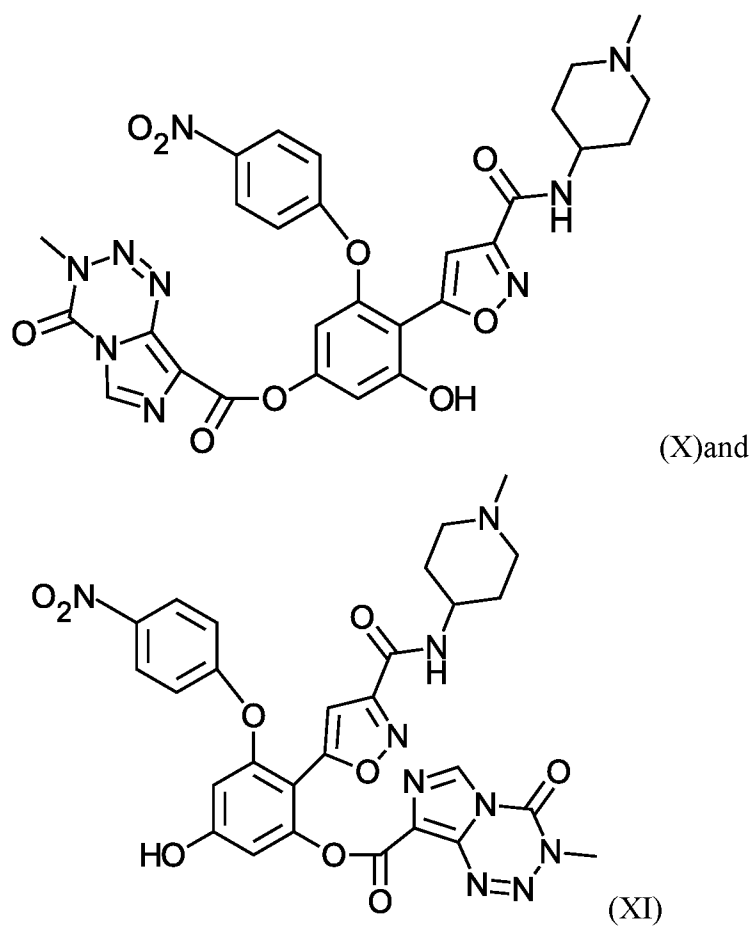
(iv) compounds with KW-2487 as the Hsp90 inhibitor having the formula



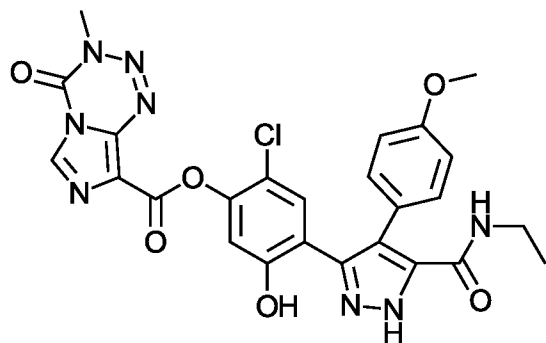
- 5 (v) compounds with Luminespib as the Hsp90 inhibitor, having the formula



- (vi) compounds with NMS-E973 as the Hsp90 inhibitor, having the formula

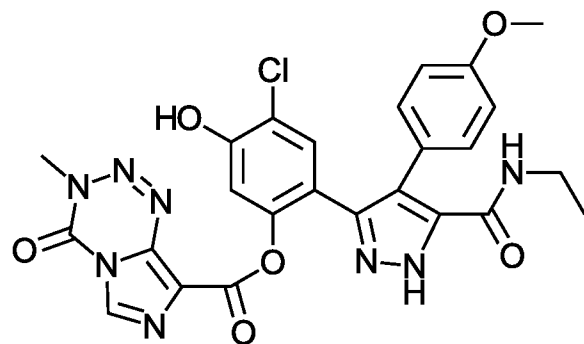


- 10 (vii) compounds with VER-50589 as the Hsp90 inhibitor, having the formula



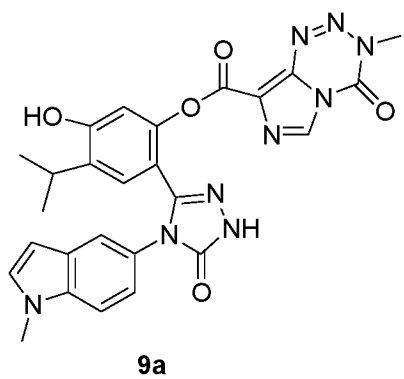
5

(XII) and

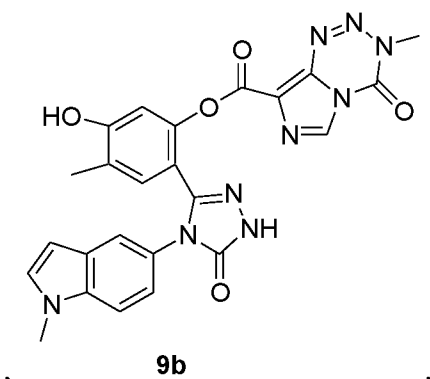


(XIII).

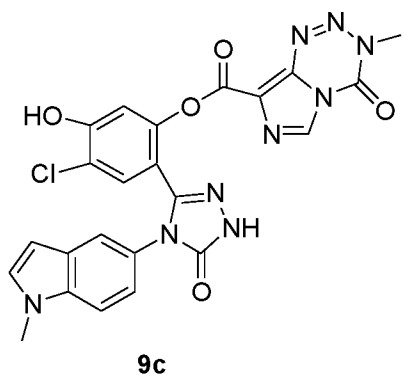
2. The method of embodiment 1, wherein the compounds having formula (I) are selected from the group consisting of



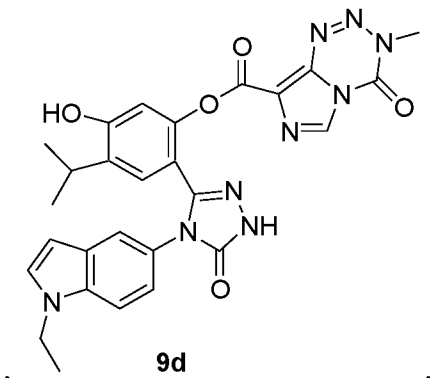
9a



9b

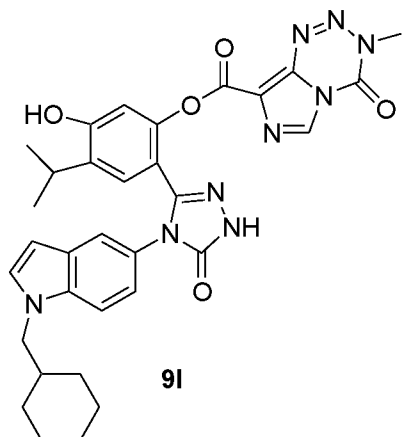
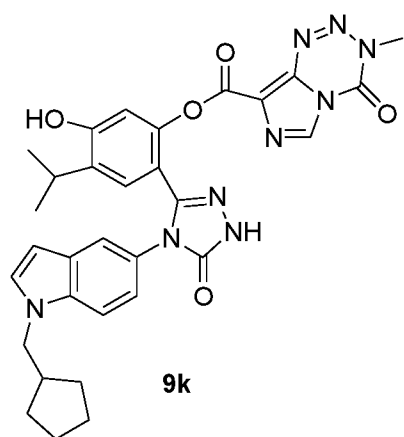
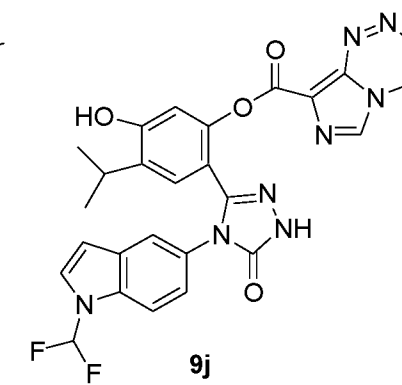
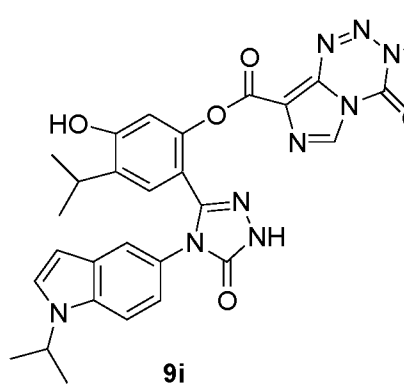
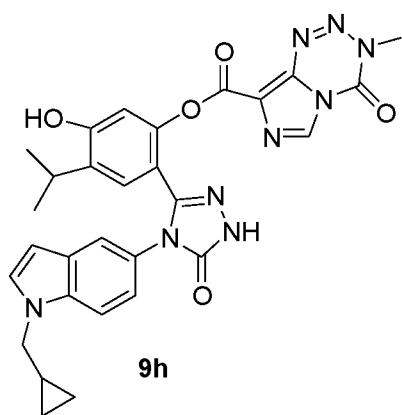
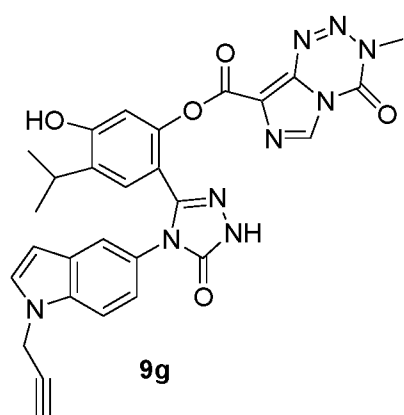
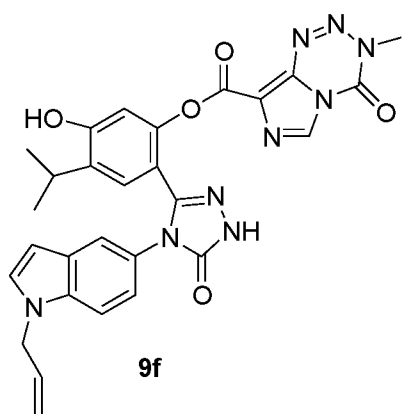
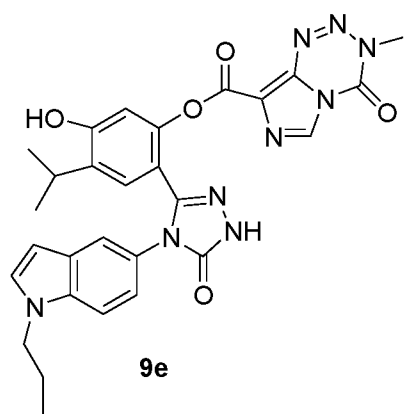


9c

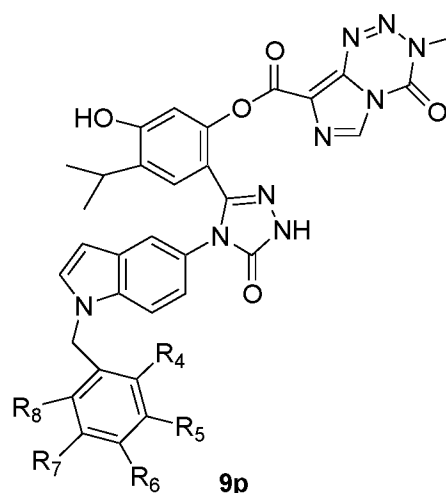
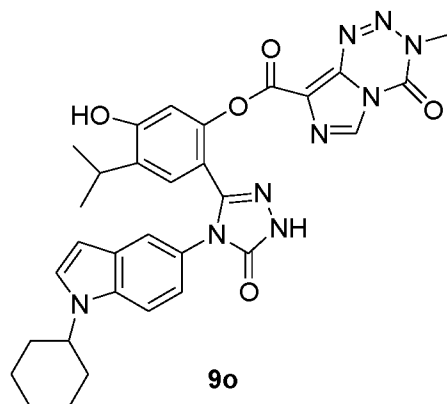
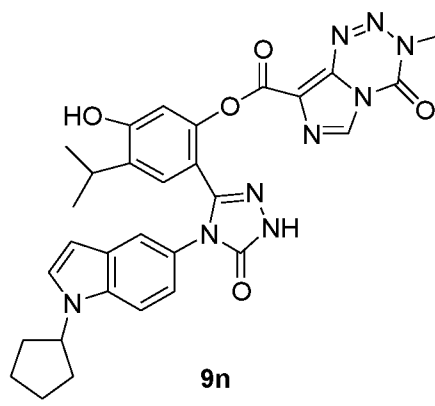
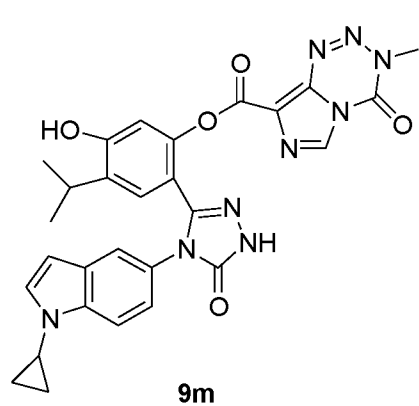


9d

10



5

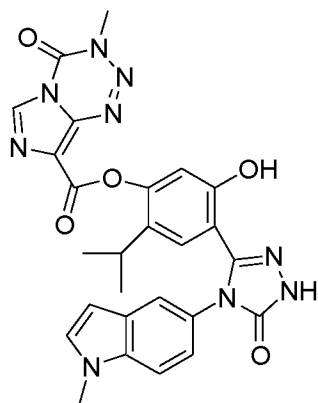


, and

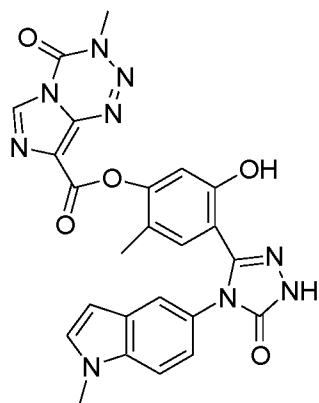
wherein R_4 , R_5 ,

R_6 , R_7 , and R_8 , are independently selected from the group consisting of hydrogen, lower alkyl, (C₁-C₄)alkoxy, (C₄-C₇)cyclopropyl, (C₂-C₆)alkenyl, and (C₂-C₆)alkynyl, each optionally substituted with one or more halogen atoms.

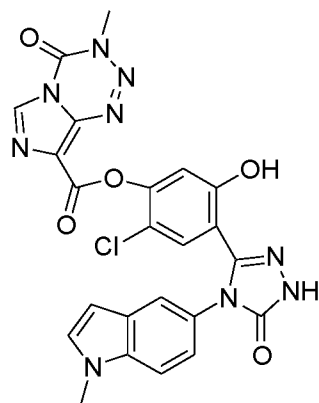
- 10 3. The method of embodiment 1, wherein the compounds having formula (II) are selected from the group consisting of



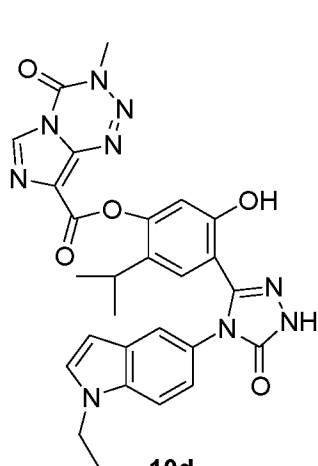
10a



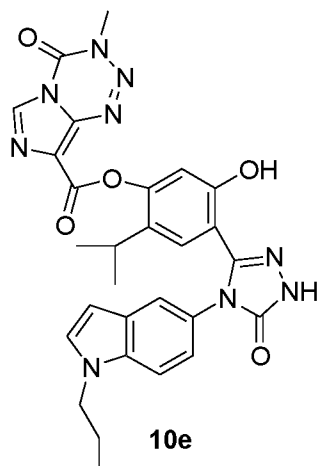
10b



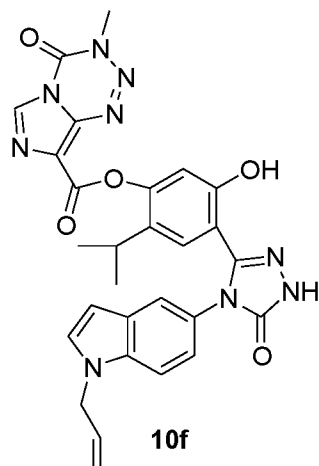
10c



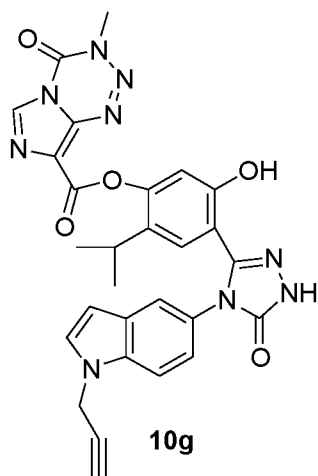
10d



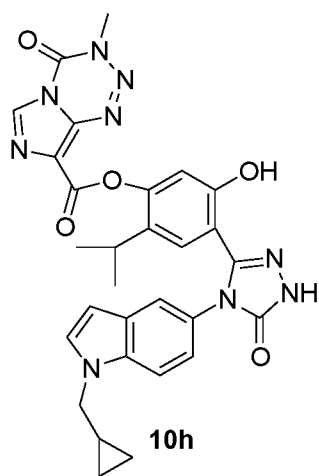
10e



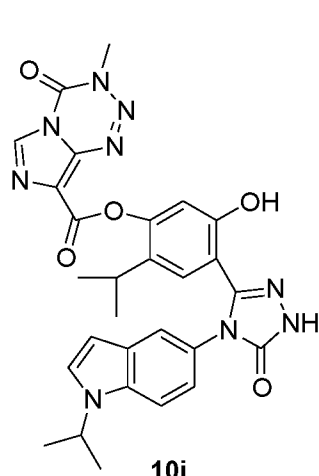
10f



10g

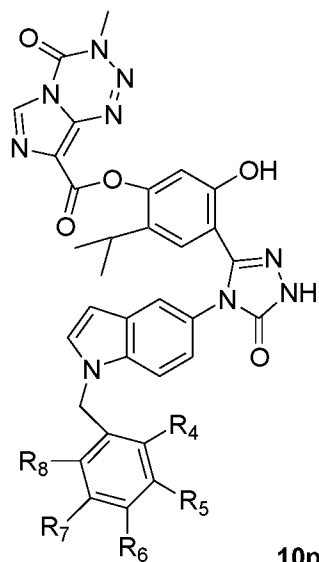
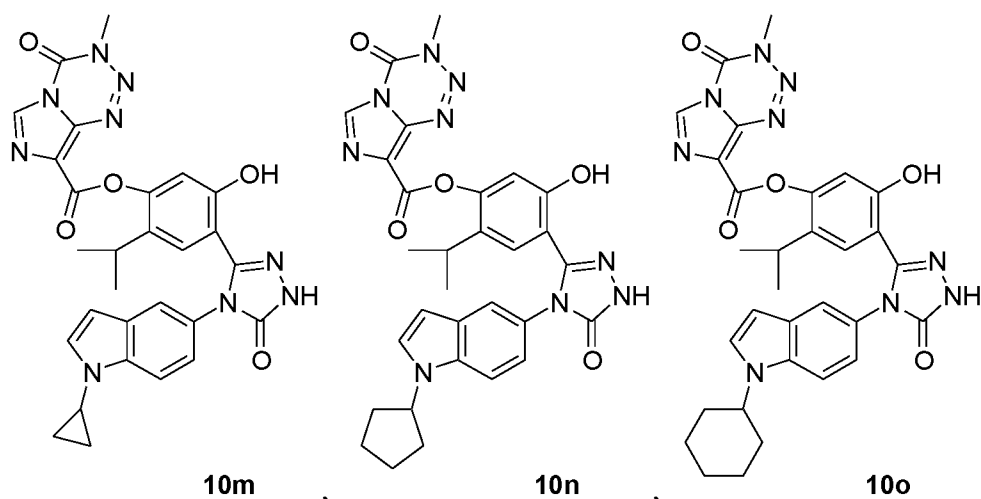
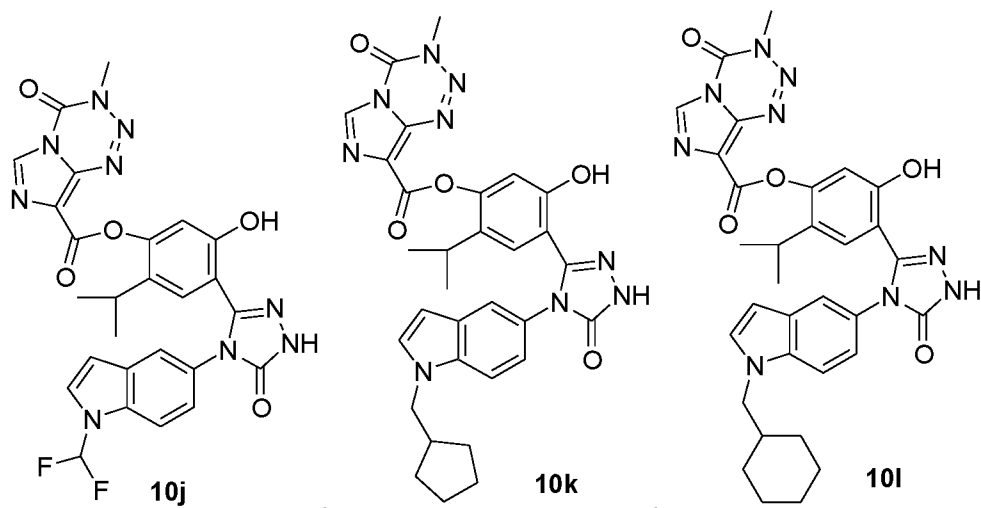


10h



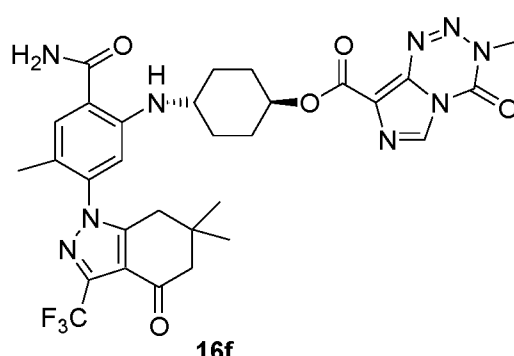
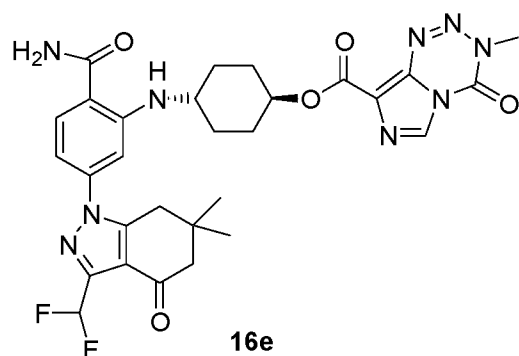
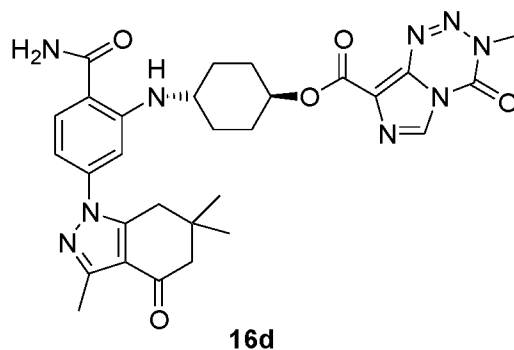
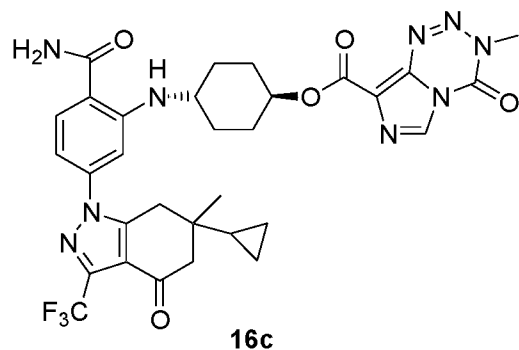
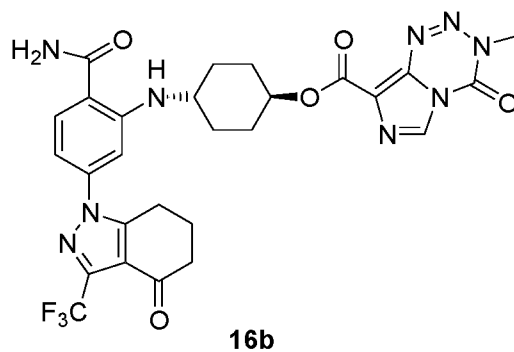
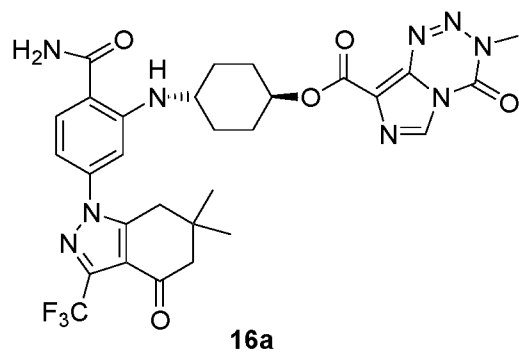
10i

5

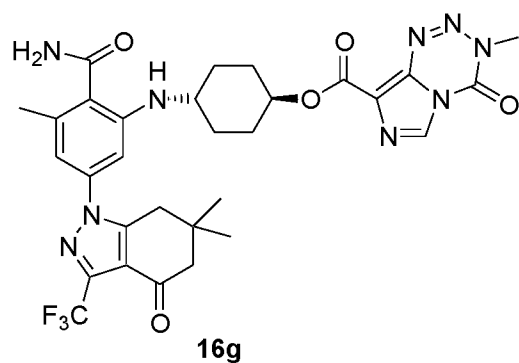


wherein R_4 , R_5 , R_6 , R_7 , and R_8 are independently selected from the group consisting of hydrogen, lower alkyl, (C_1-C_4) alkoxy, (C_4-C_7) cyclopropyl, (C_2-C_6) alkenyl, and (C_2-C_6) alkynyl, each optionally substituted with one or more halogen atoms.

- 5 4. The method of embodiment 1, wherein the compounds having formula (III) are selected from the group consisting of

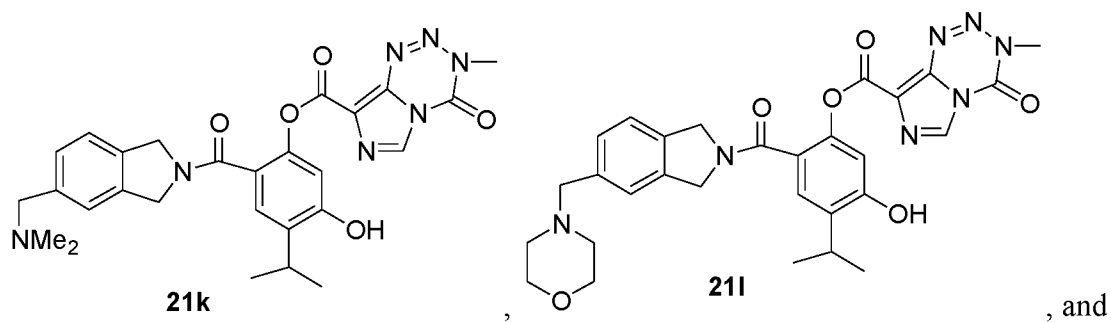


, and

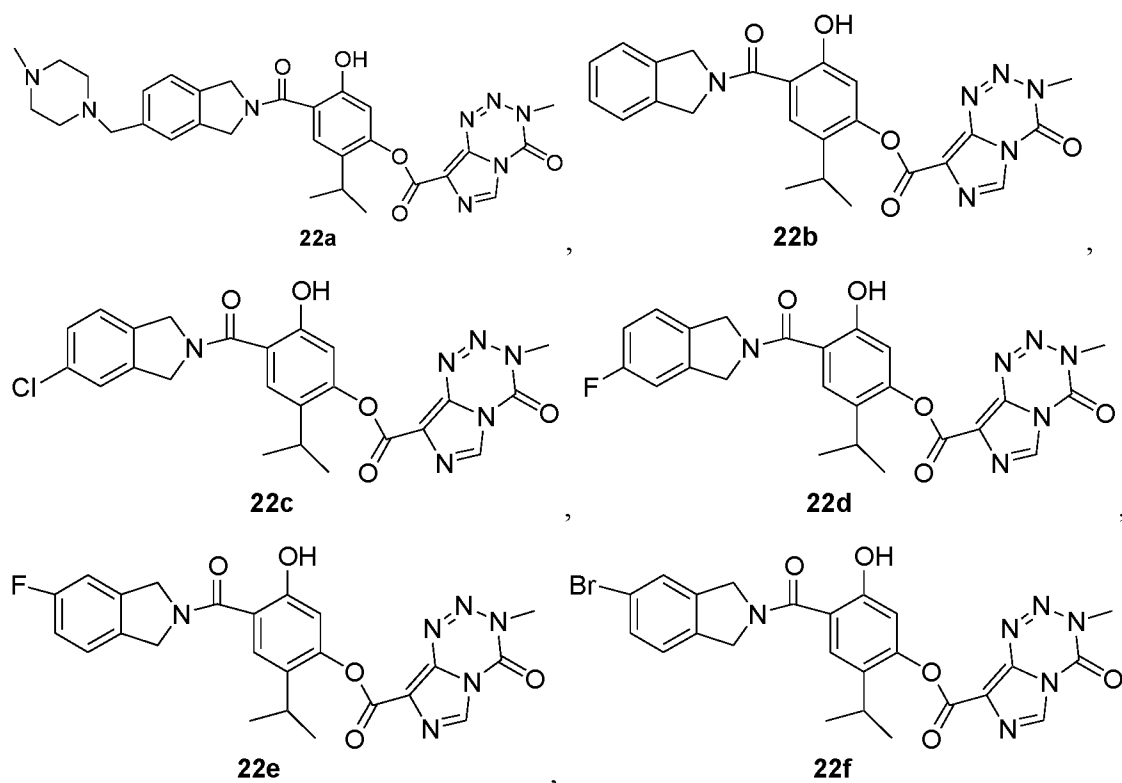


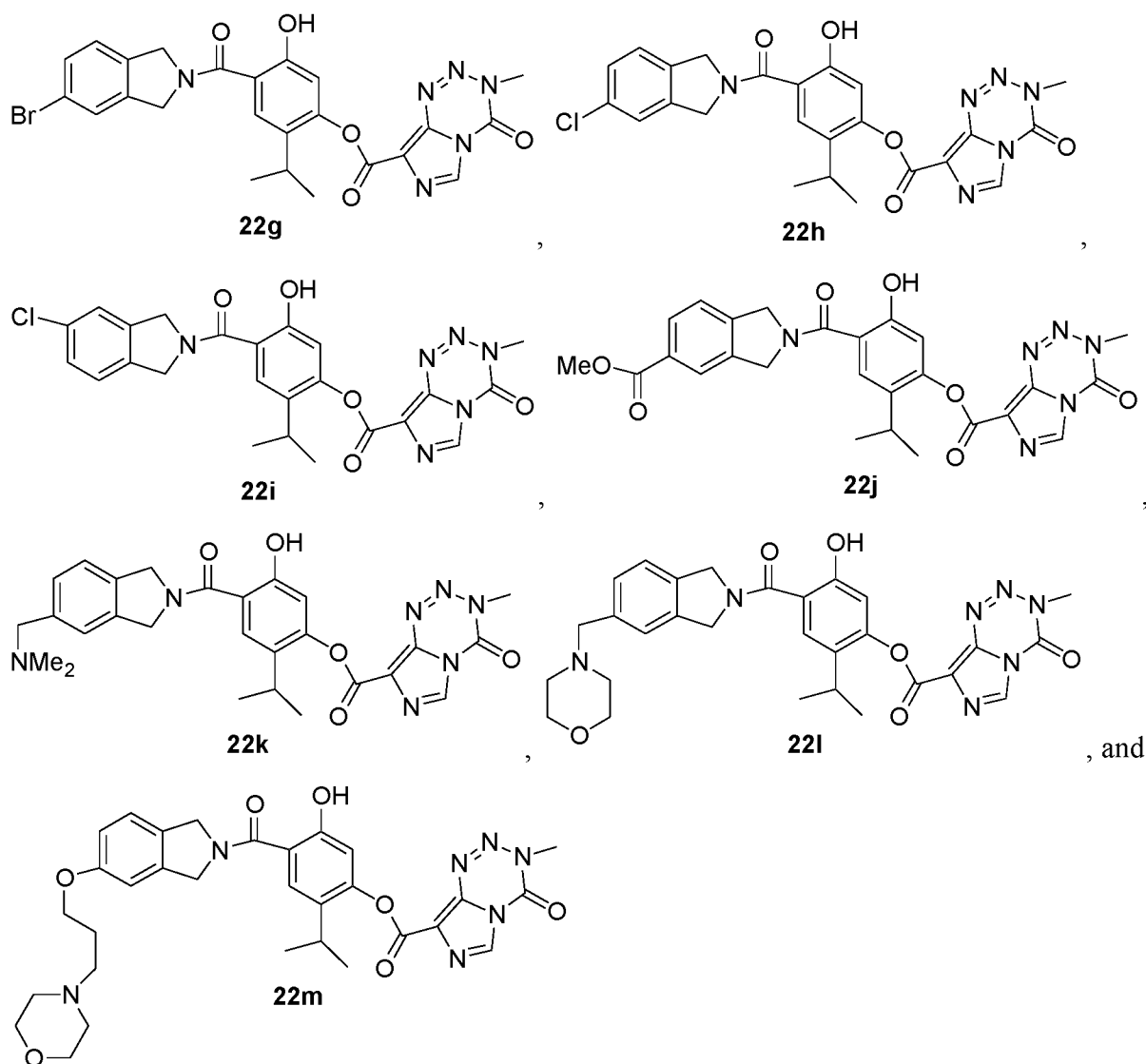
10

5. The method of embodiment 1, wherein the compounds having formula (IV) are selected from the group consisting of



6. The method of embodiment 1, wherein the compounds having formula (V) are selected from the group consisting of





7. The method of any of embodiments 1-6, wherein the cancer is glioblastoma, primary malignant brain tumor, or malignant glioma.
8. The method of any of embodiments 1-6, wherein the cancer is glioblastoma.
9. A pharmaceutical composition for nasal administration comprising a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90 inhibitor of any of embodiments 1-6; a lipid and a non-ionic surfactant; and an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the conjugate.
10. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is a compound having formula (I) or (II).
11. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is a compound having formula (III).

5

12. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is a compound having formula (IV) or (V).

13. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is a compound having formula selected from (VI) to (XIII).

10 14. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 2.

15. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 3.

15 16. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 4.

17. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 5.

18. The pharmaceutical composition of embodiment 9, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 6.

20 19. The composition of embodiment 9, wherein the absorption promoting agent is a cationic polymer.

20. The composition of embodiment 19, wherein the cationic polymer is chitosan.

21. A pharmaceutical composition comprising a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90 inhibitor of any of embodiments 1-6, or a
25 pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is a compound having formula (I) or (II).

23. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is a compound having formula (III).

30 24. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is a compound having formula (IV) or (V).

25. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is a compound having formula selected from (VI) to (XIII).

26. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 2.

27. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 3.

28. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 4.

29. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 5.

30. The pharmaceutical composition of embodiment 21, wherein the covalently-linked conjugate is selected from one of the compounds of embodiment 6.

BREIF DESCRIPTION OF DRAWINGS

Figure 1 is a bar graph showing enhanced death of U87 human malignant glioma cells following four days of treatment with different concentrations of temozolomide (TMZ) in combination with varying concentrations of the HSP90 inhibitor Ganetespib (GTB).

Figure 2 is a bar graph showing enhanced death of LN229 human malignant glioma cells following four days of treatment with different concentrations of TMZ in combination with varying concentrations of the HSP90 inhibitor GTB.

Figure 3 is a bar graph showing enhanced death of U87 human malignant glioma cells following four days treatment of with different concentrations of TMZ in combination with varying concentrations of the HSP90 inhibitor SNX-2112 (SNX).

Figure 4 is a bar graph showing enhanced death of U87 human malignant glioma cells following four days of treatment of with different concentrations of TMZ in combination with varying concentrations of the HSP90 inhibitor SNX.

Figure 5 is a bar graph showing enhanced death of LN229 human malignant glioma cells following six days of treatment with TMZ (50uM) in combination with the HSP90 inhibitor BIIB (100nM), GTB (5 nM), or SNX (25 nM).

Figure 6 is a bar graph showing enhanced death of LN229 human malignant glioma cells following seven days of treatment with TMZ in combination with the HSP90 inhibitor BIIB (100nM), GTB (5 nM), or SNX (25 nM).

5 Figure 7 is a bar graph demonstrating concentration dependent increase in total AKT1 expression in human malignant glioma LN229 cells upon treatment with TMZ. Inhibition of HSP90 activity by a HSP90 inhibitor (GTB or SNX) suppresses TMZ-induced AKT1 expression.

10 Figures 8A, 8B and 8C are bar graphs showing accumulation of dual-acting Hsp90/TRAP1 inhibitors in mitochondria. Figure 8A shows accumulation of CAP01088 in mitochondria isolated from human HEI193 neurofibromatosis type-2 (NF2) schwannoma cell lines compared with the levels in the culture medium. Figure 8B shows dose-dependent decrease in tetramethylrhodamine ethyl ester (TMRE) fluorescence in HEI193 cells caused by treatment with CAP01088 (grey) and ganetespib (black) for 2 hours indicating
15 depolarization of mitochondrial membrane. Figures 8C shows mitochondrial membrane depolarization as measured by TMRE fluorescence in U251MG cells following treatment with CAP01100 (yellow) or CAP01088 (green) for 4 hours. Each bar shows mean $\pm$ SEM and *p* value (*t* test).

20 Figure 9A is a bar graph showing enhanced death of LN229 human malignant glioma cells following treatment for 72 hours with various concentrations of the chemotherapeutic agent, TMZ, covalently linked to an Hsp90 inhibitor SNX-2112. Also, shown are cell death caused by the agent and the inhibitor individually at the same concentrations. Figure 9B is a bar graph showing enhanced death of U87 human malignant glioma cells following treatment for 72 hours with various concentrations of the chemotherapeutic agent, TMZ, covalently
25 linked to an Hsp90 inhibitor SNX-2112. Also, shown are cell death caused by the agent and the inhibitor individually at the same concentrations.

30 Figure 10A is a bar graph showing accelerated Caspase 3/7 activation mediated cell death of human U87 malignant glioma tumor cells treated with a combination of TMZ and an Hsp90 inhibitor (Ganestinib or SNX-2112). Figure 10B is a bar graph showing accelerated Caspase 3/7 activation mediated cell death of LN229 malignant glioma tumor cells treated with a combination of TMZ and an Hsp90 inhibitor (Ganestinib or SNX-2112). In each case, Caspase 3/7 activity was measured after treatment with the individual agents or the combination in varying concentrations for 48 hours.

5

DETAILED DESCRIPTION

Disclosed first in detail herein is a method of treating cancer using a combination therapy. The method includes administering to a patient suffering from cancer an effective amount of each of a chemotherapeutic agent and an Hsp90 inhibitor. The Hsp90 inhibitor is capable of also inhibiting TRAP1. The combination therapy produces an enhanced
10 therapeutic effect compared to the effect of the chemotherapeutic agent or the Hsp90 inhibitor alone.

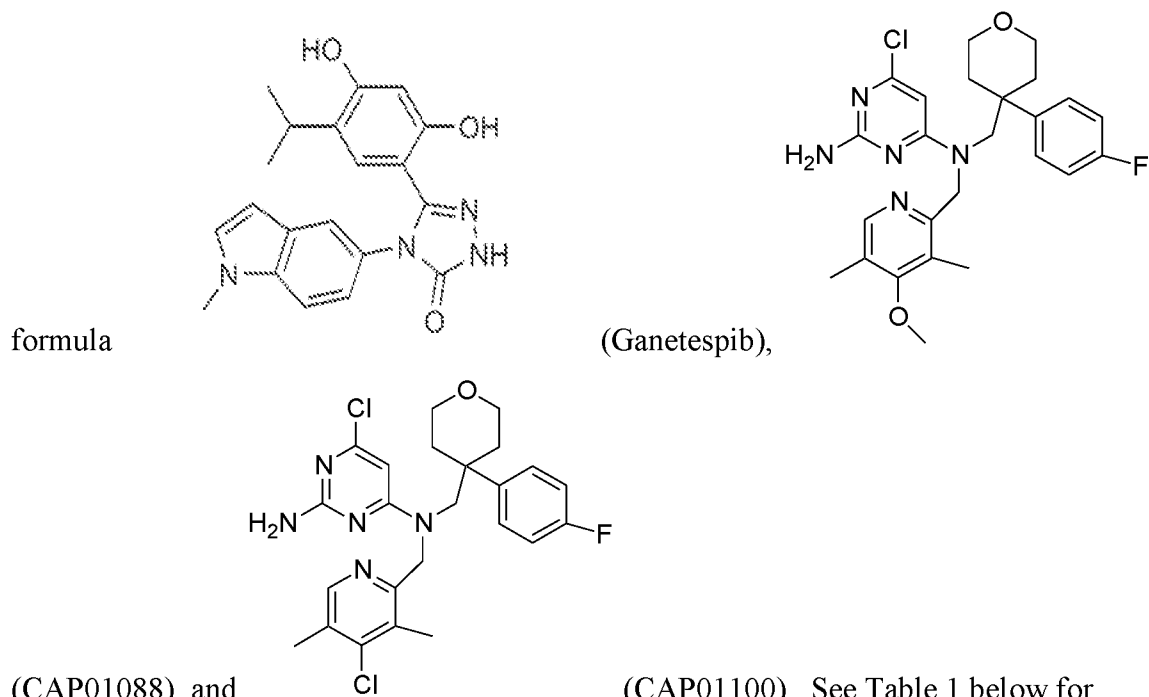
Effectiveness of current chemotherapeutic agents is often limited by the development of resistance to the agents by the cancer cells. Overexpression of the Bcl-2 family of antiapoptotic proteins is one of the underlying factors for drug-induced resistance. This drug-
15 induced resistance to driven in part by overexpression of Bcl-2 family of proteins is also an obstacle in the treatment of GBM and related cancers.

Hsp90 promotes cell viability with an interactome made of hundreds of client proteins, including oncogenic proteins that are the key drivers of cell proliferation, growth and survival. These client proteins are highly dependent on Hsp90 activity for folding into an
20 active conformation. Inhibition of Hsp90 function results in simultaneous degradation of multiple key oncogenic proteins. As to chemotherapeutic agents, nearly all rely on endogenous physiological pathways for cell death in order to kill cancer cells. As such, over-activation of genes that block these pathways, e.g., genes coding for Bcl-2 family of proteins, can lead to tumor cells becoming resistant to the cytotoxic effects of the drugs. One way of
25 overcoming this resistance is to activate cell death pathways that are not affected by the Bcl-2 family of proteins. Inhibition of TRAP1 provides one such pathway.

Accordingly, the above-referred method of treating cancer requires use of an Hsp90 inhibitor that also inhibits TRAP1 in combination with a chemotherapeutic agent.

In certain embodiments, the combination therapy produces a synergistic effect. For example,
30 see Figures 1 and 2. Chemotherapeutic agents frequently used in cancer therapy are DNA alkylating agents, and as such they may be used in the above described method. Exemplary DNA alkylating agents that may be used include, but are not limited to, the following: temozolomide (TMZ); MeOSO₂(CH₂)₂-leixitropsin (Me-Lex); Cis-diamminedichloroplatinum II (cis-DDP); mitomycin bioreductive alkylating agents;
35 quinones; STZ (streptozotocin); cyclophosphamide; nitrogen mustard family members such as chloroambucil; pentostatin (purine analogs); fludarabine; bendamustine hydrochloride

- 5 which is the active ingredient of Ribomustin (alkylating group in common with the nitrogen mustard family, also an antimetabolites); chloroethylating nitrosoureas (lomustine, fotemustine, cystemustine); dacarbazine (DTIC); and procarbazine. Exemplary Hsp90 inhibitors that also inhibit TRAP1 for use in this method include compounds having the



Cmpd. ID	Mol. Wt. (Da)	PSA (Å ²)	cLogP	Biochemical potency (nM)			
				Hsp90		TRAP1	
				K _i	IC ₅₀	K _i	IC ₅₀
CAP01088	486.0	86.4	4.71	10	40.9	33.4	249
CAP01100	490.4	77.2	5.33	5.5	22.2	11.5	85.5

Ganetespi	364.4	97.1	3.64	4.8	20	11.9	89.0
-----------	-------	------	------	-----	----	------	------

5

Different types of cancer may be treated using this method. Among these, Glioblastoma is particularly suited for treatment by this method given that depletion of TRAP1 leads to a marked decrease in GBM cell proliferation, inhibition of neurosphere recovery and secondary neurosphere formation, and notably, enhancement of the therapeutic effect of TMZ in neurosphere cultures [19]. Other brain cancers that are suitable for treatment with the above method include primary malignant brain tumor and malignant glioma.

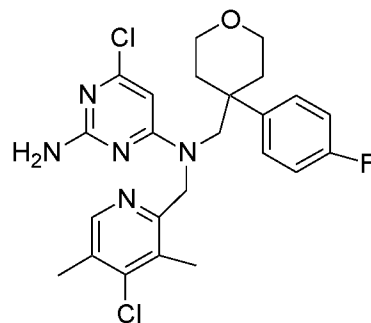
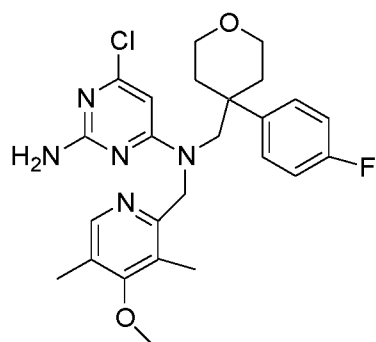
The chemotherapeutic agent and the Hsp90 may be administered simultaneously in a single composition or as separate compositions. Alternatively, the chemotherapeutic agent and the Hsp90 inhibitor may be administered sequentially.

Included within the scope of the technology is a pharmaceutical composition for nasal administration that includes (i) a chemotherapeutic agent, or (ii) an Hsp90 inhibitor, or (iii) a combination of a chemotherapeutic agent and an Hsp90 inhibitor; each with an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the chemotherapeutic agent, the Hsp90 inhibitor, or the combination. Intranasal delivery is attractive as an alternative route for administration of drugs and biomolecules that are susceptible to enzymatic or acidic degradation, first-pass hepatic metabolism, and for overcoming problems of low brain bioavailability due to blood-brain barrier. As such, the composition is useful for treating cancers of many types including Glioblastoma, primary malignant brain tumor, and malignant glioma. Nasally administered drugs have the advantage of access to a relatively large surface area for permeation (afforded by the large number of microvilli present in the nasal cavity), a porous endothelial membrane, and a highly-vascularized epithelium. Lipid and non-ionic surfactant based elastic vesicular delivery systems, which include an agent that promotes absorption to mucus membrane (e.g., a chitosan-based hybrid vesicular system), show enhanced permeability compared to conventional forms of dosing (solutions, suspensions, sprays, snuffs, emulsions, and gels). These vesicular delivery systems include both stabilizing and destabilizing molecules within a single bilayer wall, which provides elasticity and also allows the system to act as a barrier

5 modulating agent. Intranasal route has proven to be a promising means for use of these delivery systems and allows intimate and prolonged contact between the drug carrier and the mucus membrane. In addition to enhanced permeability, the vesicles of the lipid and non-ionic surfactant based elastic vesicular delivery systems are highly stable and possess a high capacity for drug loading along and a controlled release profile, all of which when taken into
10 consideration, make them a good choice as carriers for both hydrophilic and lipophilic drug molecules.

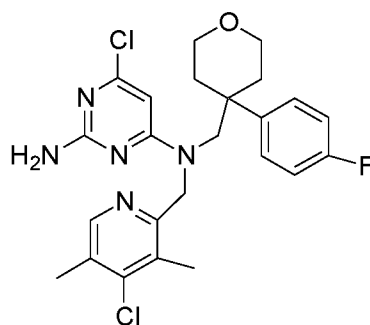
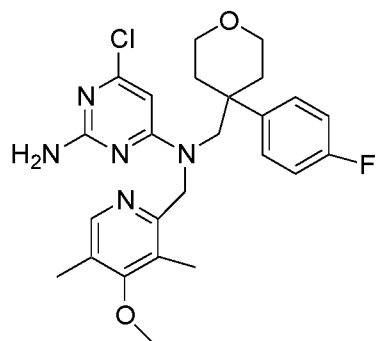
Absorption promoting agents contemplated to be used in the composition for nasal administration described herein include a cationic polymer, a surface active agent, a chelating agent, a mucolytic agent, a cyclodextrin, and combinations thereof. Further, cationic
15 polymers that may be used include other polycationic carbohydrates such as but not limited to inorganic or organic salts of chitosan and modified forms of chitosan (especially more positively charged ones), polyaminoacids such as polylysine, polyquaternary compounds, protamine, polyamine, DEAE-imine, polyvinylpyridine, polythiodiethyl-aminomethylethylene (P(TDAE)), polyhistidine, DEAE-methacrylate, DEAE-acrylamide,
20 poly-p-aminostyrene, polyoxethane, co-polymethacrylates (e.g. copolymers of HPMA, N-(2-hydroxypropyl)-methacrylamide, GAFQUAT (U.S. Pat. No. 3,910,862) and polyamidoamines. The polycationic substances used in the technology typically have a molecular weight of 10 000 or more. The chitosan (or salt thereof) preferably has an intrinsic viscosity of at least 400 ml/g, more preferably at least 500, 750 or 1000 ml/g.

25 Hsp90 inhibitor that may be used in the above described composition for nasal administration include those selected from the group consisting of Genetespib, Onalespib, SNX-2112, SNX-5422, XL-888, NMS-873, NMS-E973, or PU-H71, NVB-BEP800, Luminespib, KW-2487, Tenespimycin (17-AAG), Alveospimycin (17-DMAG), VER-49009, CH5138303, VER-50589, BIIB021, VER-155008, Geldanamycin, HSP990 (NVP-HSP990),
30 IPI-504 (retaspimycin hydrochloride), IPI-493, and a compound having the formula



5 (CAP1088), or the formula
(CAP1100).

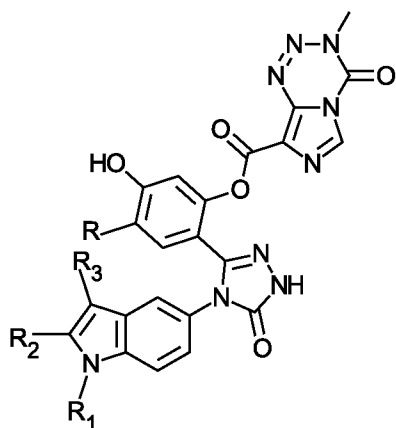
In some embodiments, the Hsp90 inhibitor used in the composition is capable of also inhibiting TRAP1, i.e., they are dual-acting Hsp90/TRAP1 inhibitors. Exemplary Hsp90 inhibitors of this kind include Ganetespib, or a compound having the structure



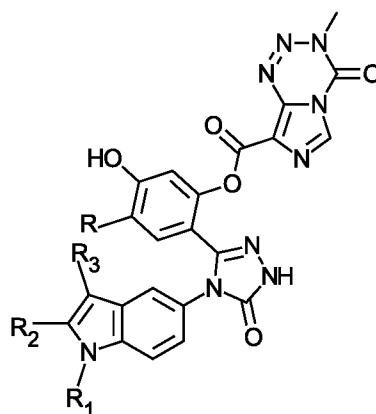
10 (CAP1088), or (CAP1100).

The technology described herein further provides compounds obtained by covalently linking an Hsp90 inhibitor and a chemotherapeutic agent, e.g., TMZ, or a pharmaceutically acceptable salt thereof. Such compounds offer certain advantages including that of a single formulation. Accordingly, the present technology provides a method of treating cancer, the method comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof. The Hsp90 inhibitor is capable of also inhibiting TRAP1. The conjugate is selected from the group consisting of

(i) compounds with ganetespib as the Hsp90 inhibitor, having the formula



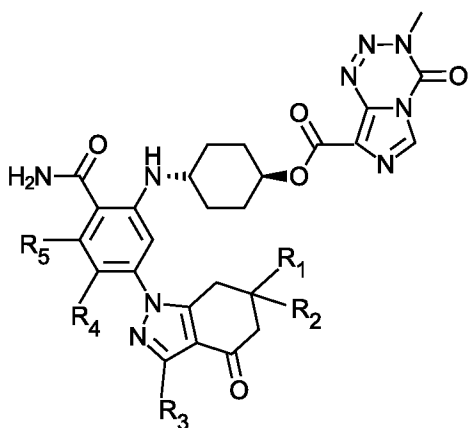
(I), and



(II), wherein

R, R₁, R₂, R₃, are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

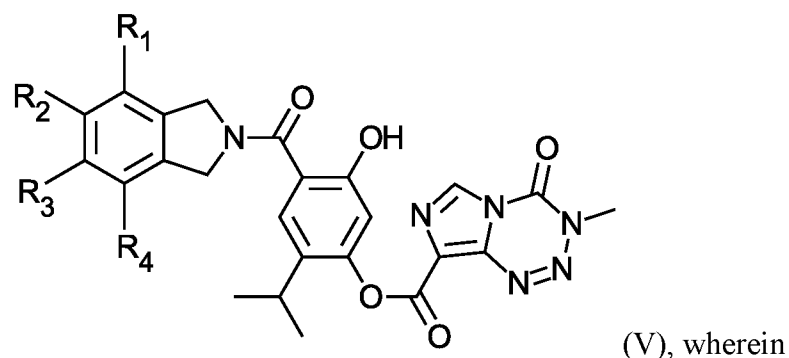
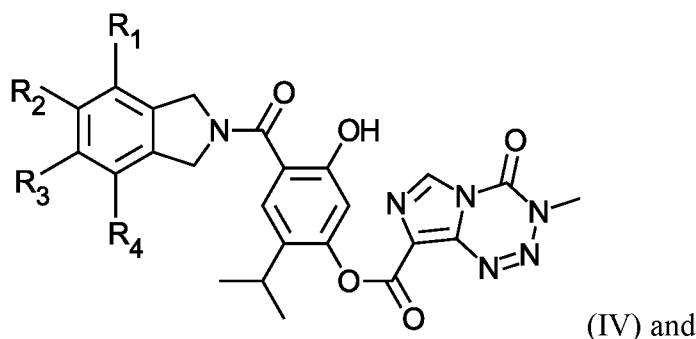
(ii) compounds with SNX-2112 as the Hsp90 inhibitor, having the formula



(III), wherein

R₁, R₂, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

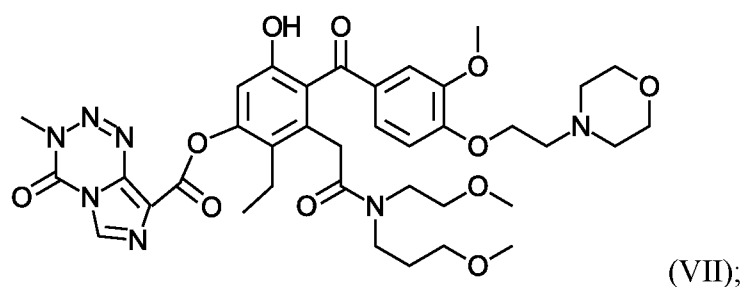
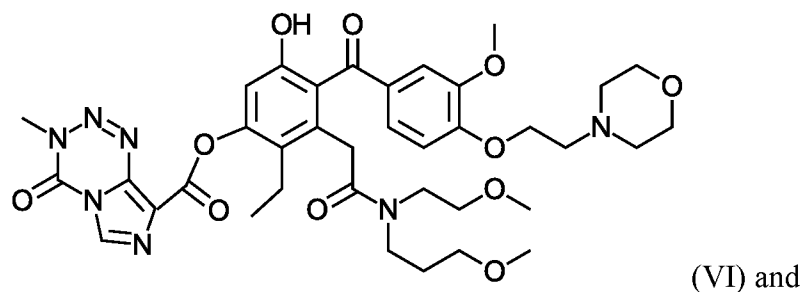
(iii) compounds with Onalespib as the Hsp90 inhibitor, having the formula



R₁, R₂, R₃, and R₄ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

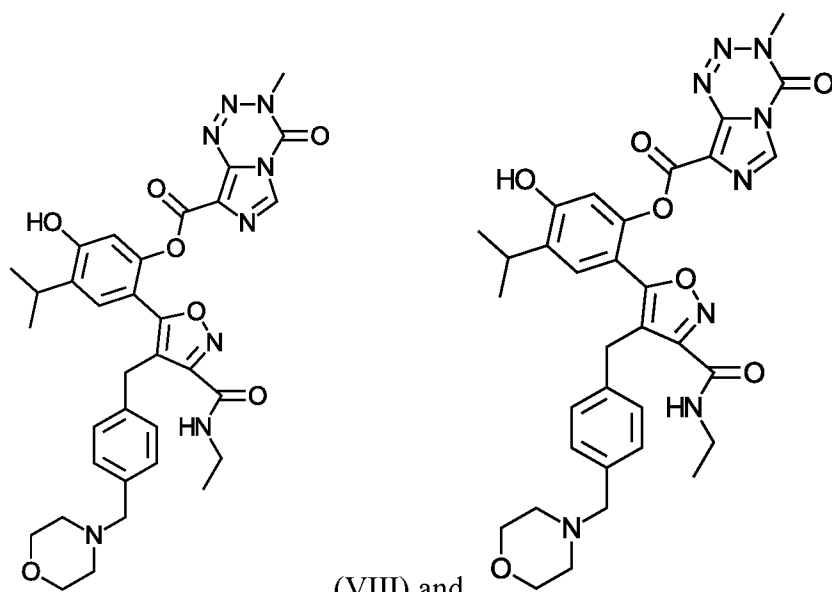
10

(iv) compounds with KW-2487 as the Hsp90 inhibitor having the formula



(v) compounds with Luminespib as the Hsp90 inhibitor, having the formula

15

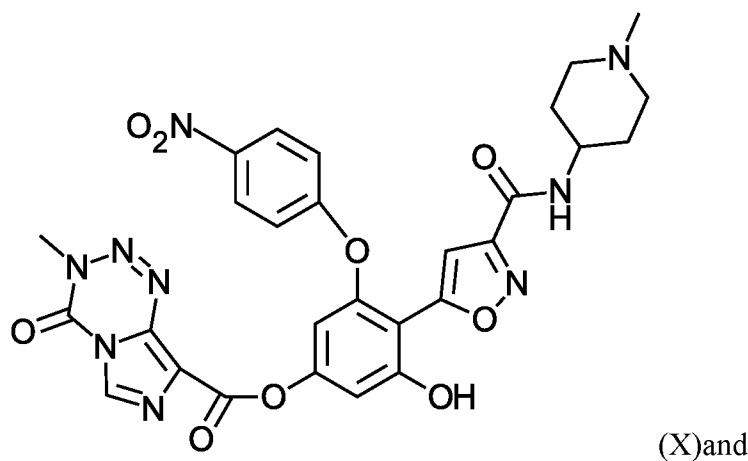


5

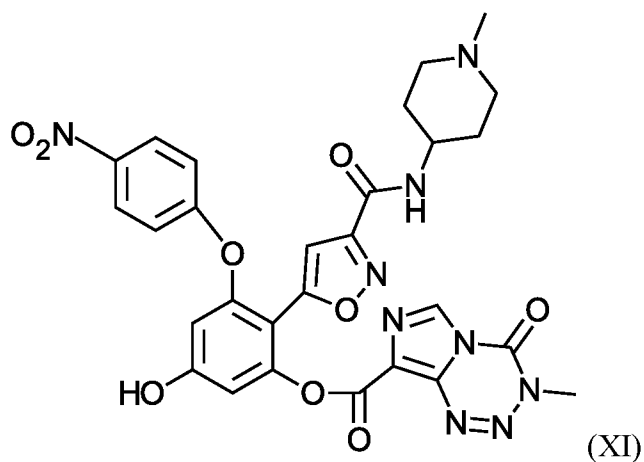
(VIII) and

(IX);

(v) compounds with NMS-E973 as the Hsp90 inhibitor, having the formula

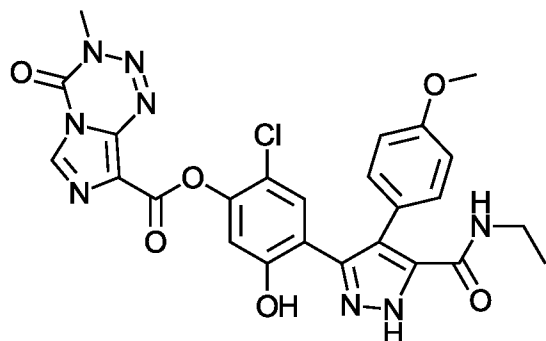


(X)and

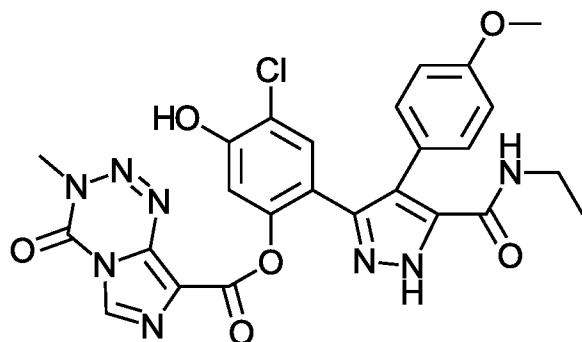


(XI)

(v) compounds with VER-50589 as the Hsp90 inhibitor, having the formula



(XII) and



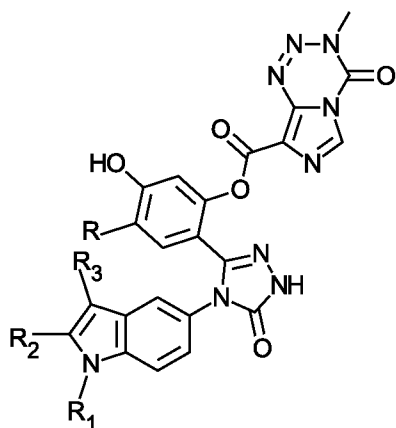
(XIII).

The covalently linked compounds described above may be used for preparing a pharmaceutical composition that comprises the compound or a pharmaceutically acceptable salt thereof and a pharmaceutically acceptable carrier. Such compositions, containing an effective amount of the compound may be used for treating cancer including cancers of the brain such as Glioblastoma, primary malignant brain tumor, or malignant glioma.

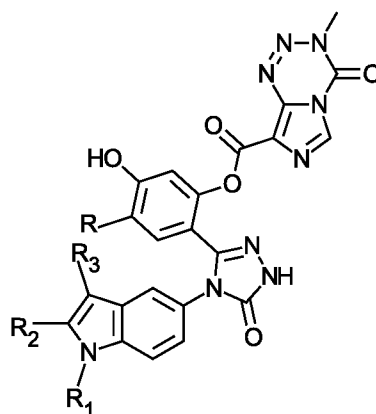
Further, these covalently linked compounds may be included in a pharmaceutical composition for nasal administration that also includes an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the compound. The composition may further include a lipid and a non-ionic surfactant. As described above, many different types of absorption promoting agents may be used including a cationic polymer such as chitosan, and such compositions may be used for treating cancer in an efficacious manner.

In a first embodiment, the present technology provides a method of treating cancer. The method requires administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof. The Hsp90 inhibitor is capable of also inhibiting TRAP1, and the conjugate is selected from the following:

- (i) compounds made with Ganetespib as the Hsp90 inhibitor, having the formula



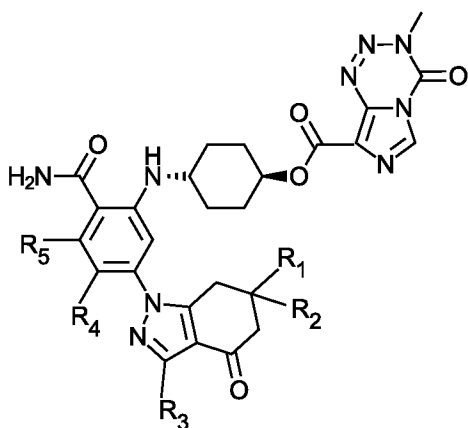
(I), and



(II), wherein

R, R₁, R₂, R₃, are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

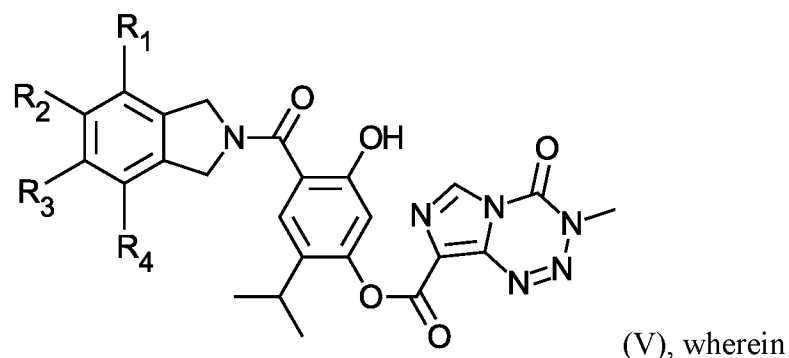
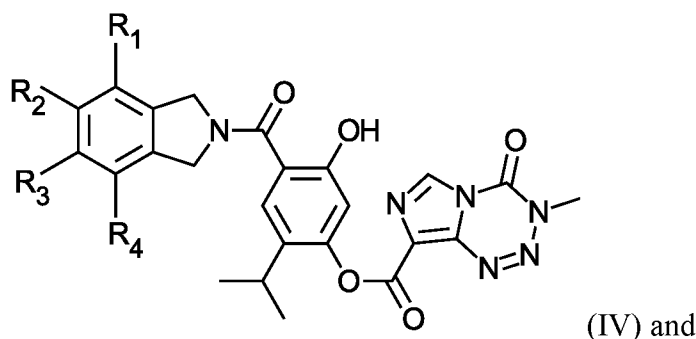
(ii) compounds made with with SNX-2112 as the Hsp90 inhibitor, having the formula



(III), wherein

R₁, R₂, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

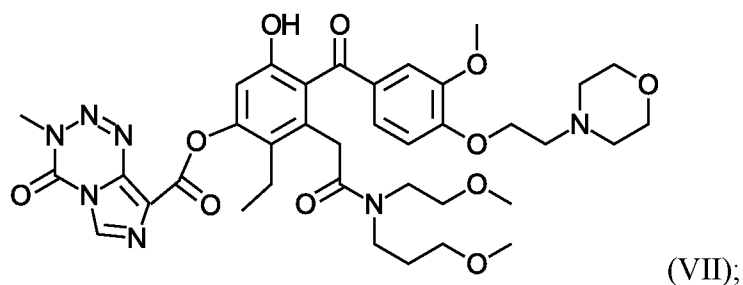
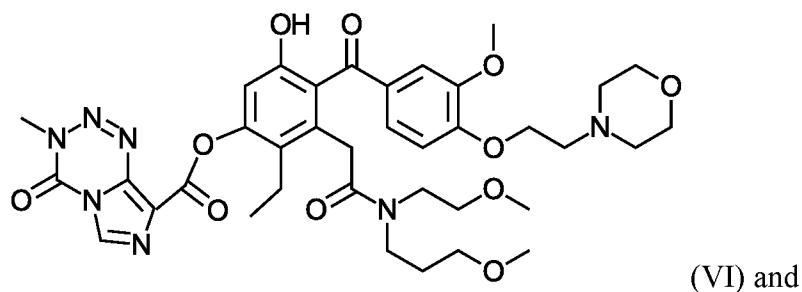
(iii) compounds made with Onalespib as the Hsp90 inhibitor, having the formula



R₁, R₂, R₃, and R₄ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

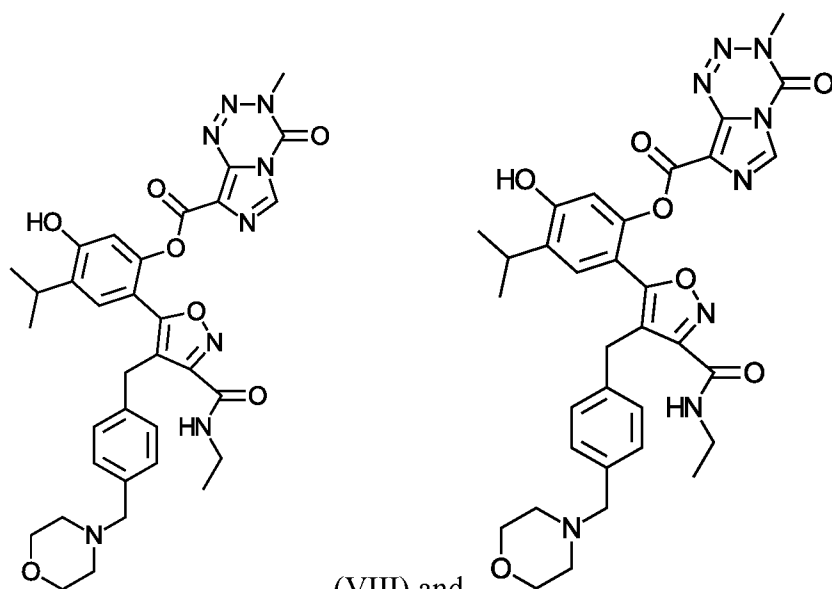
10

(iv) compounds made with KW-2487 as the Hsp90 inhibitor having the formula

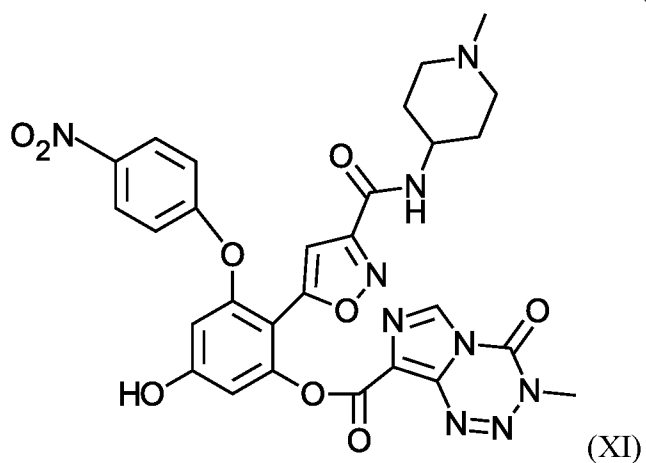
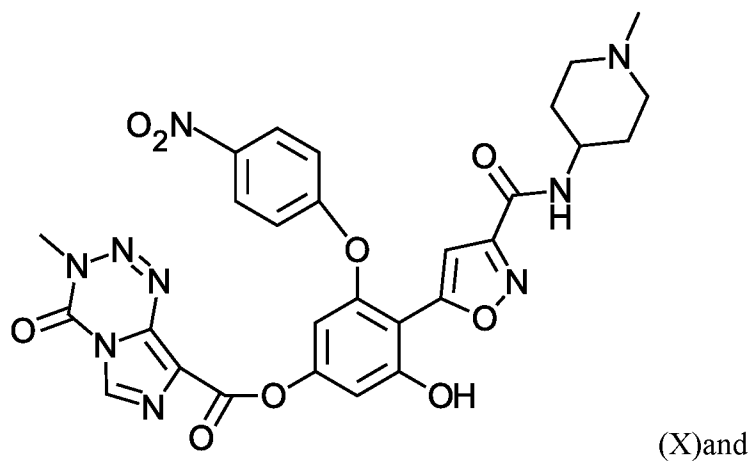


(v) compounds made with Luminespib as the Hsp90 inhibitor, having the formula

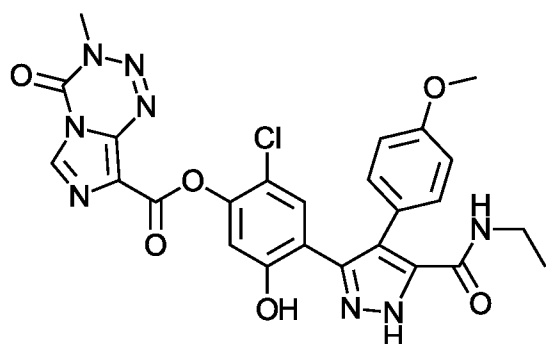
15



(vi) compounds made with NMS-E973 as the Hsp90 inhibitor, having the formula

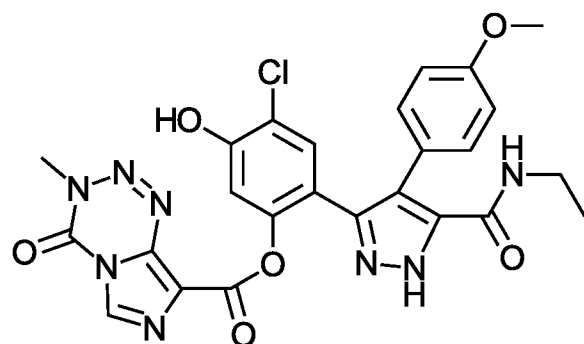


(vii) compounds made with VER-50589 as the Hsp90 inhibitor, having the formula



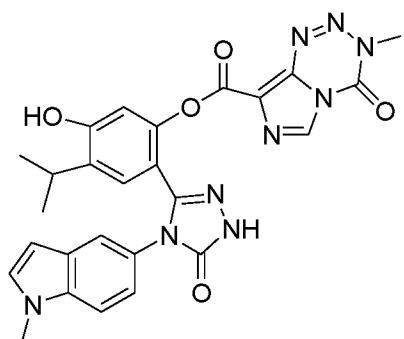
5

(XII) and

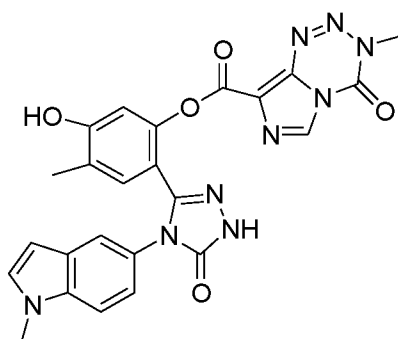


(XIII).

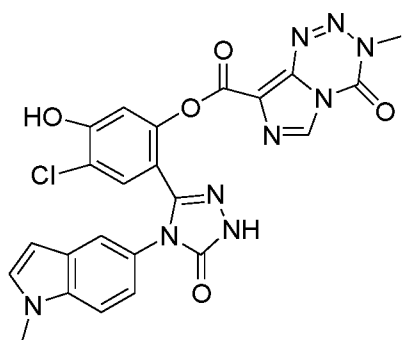
In a second embodiment of the present technology, the compounds having formula (I) are selected from the group consisting of



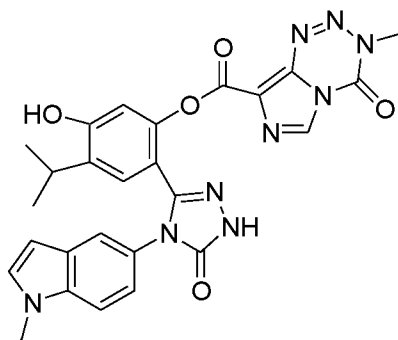
9a



9b

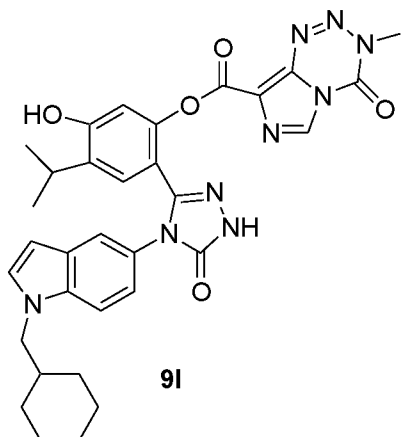
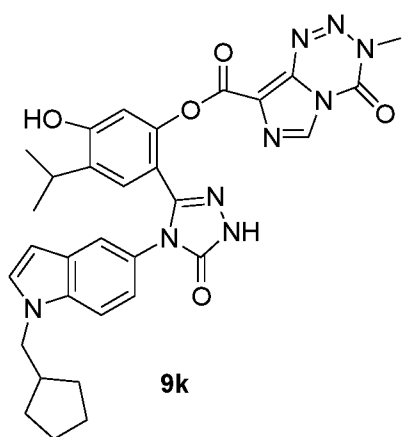
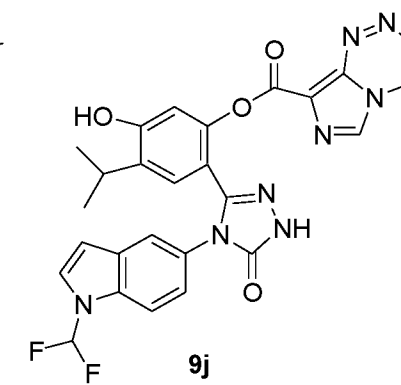
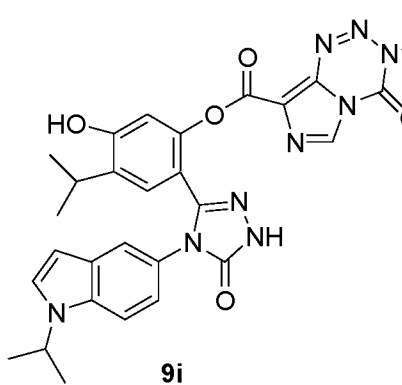
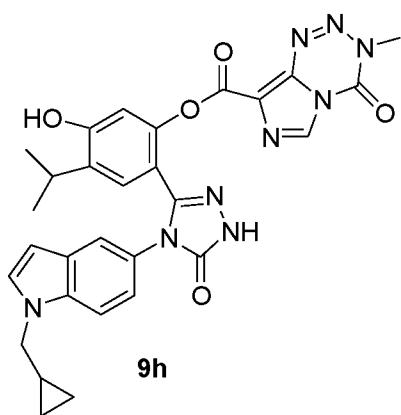
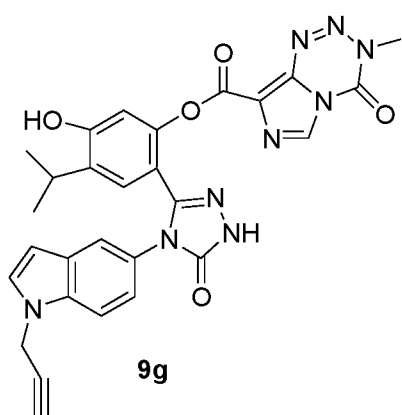
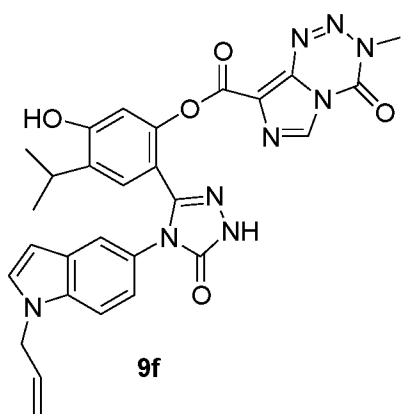
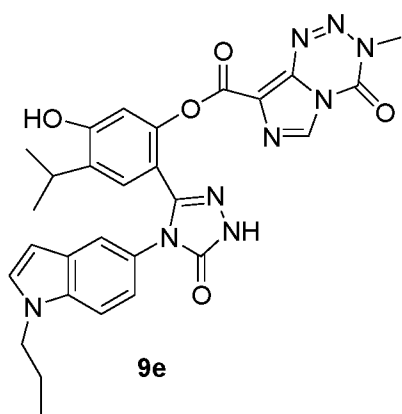


9c

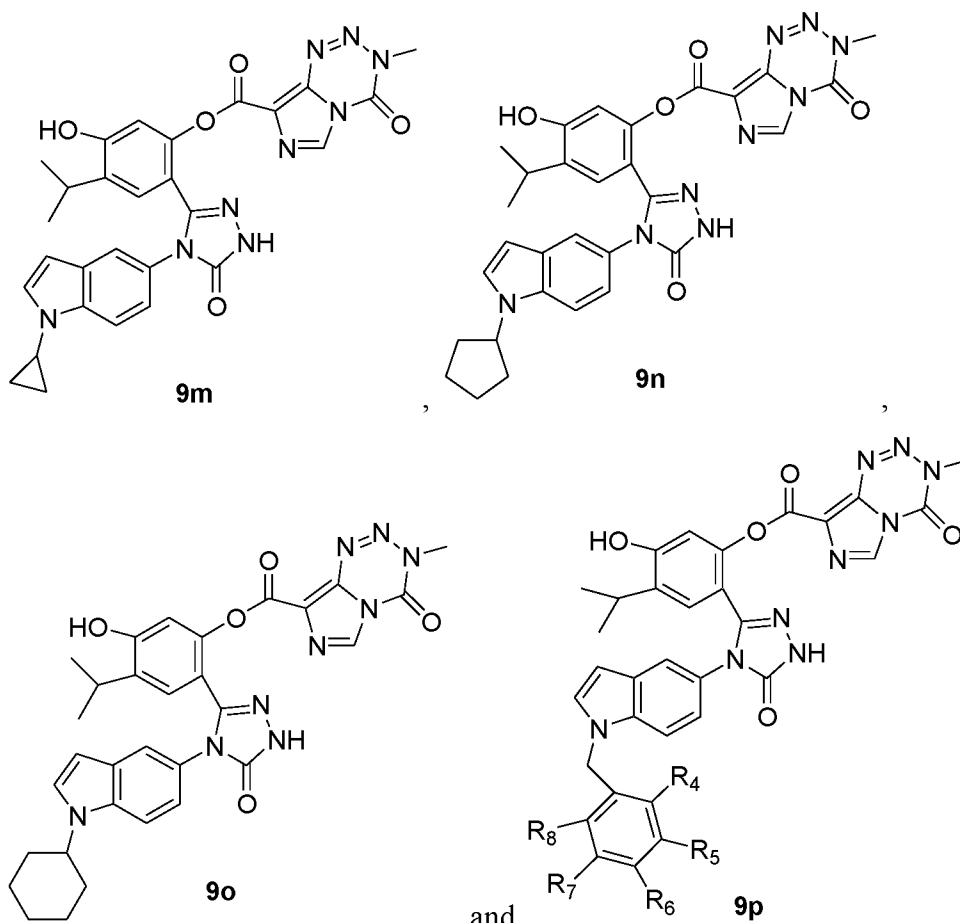


9d

10



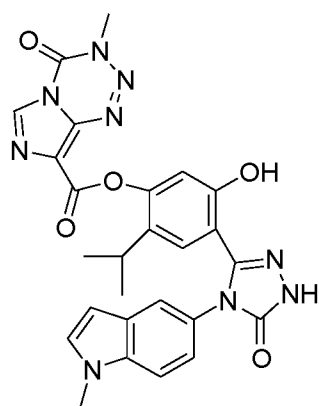
5

wherein R₄, R₅,

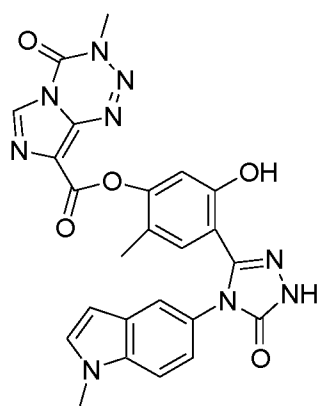
R₆, R₇, and R₈, are independently selected from the group consisting of hydrogen, lower alkyl, (C₁-C₄)alkoxy, (C₄-C₇)cyclopropyl, (C₂-C₆)alkenyl, and (C₂-C₆)alkynyl, each optionally substituted with one or more halogen atoms.

10

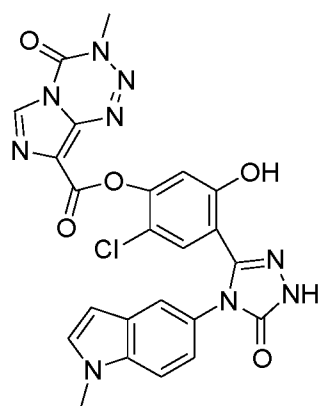
In a third embodiment, the compounds having formula (II) are selected from the group consisting of



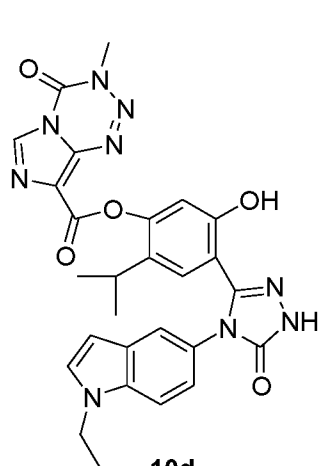
10a



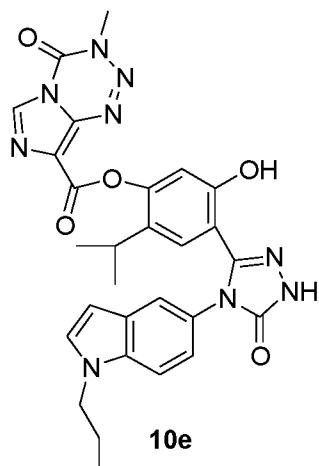
10b



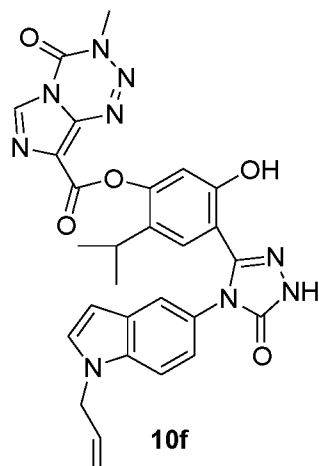
10c



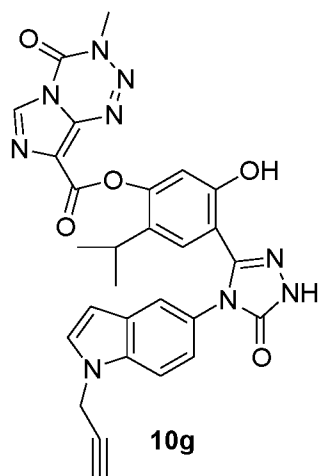
10d



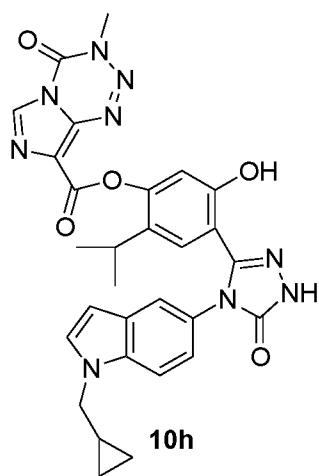
10e



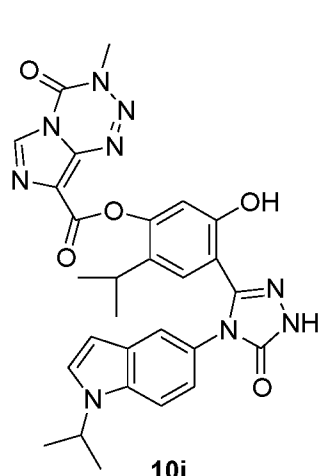
10f



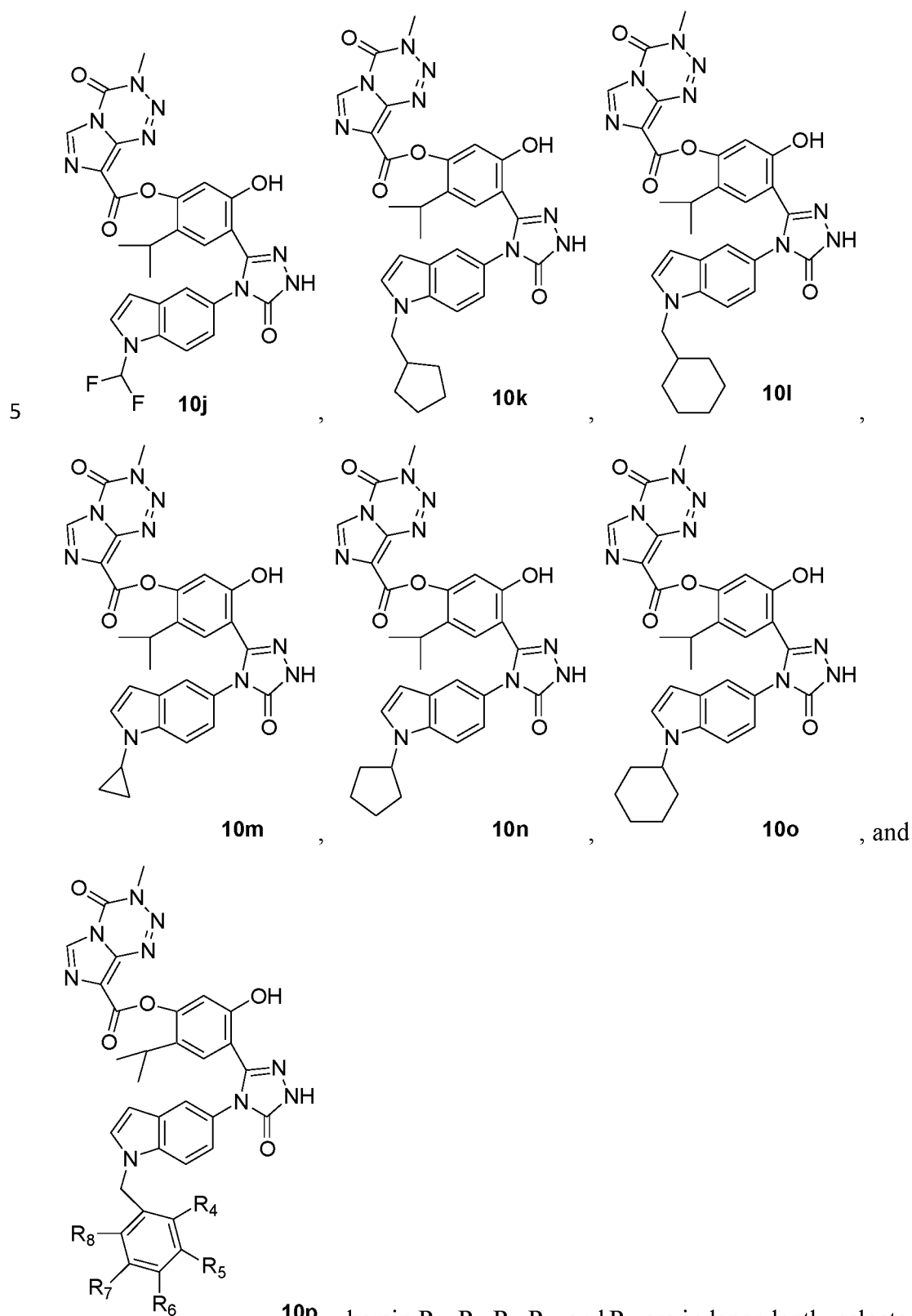
10g



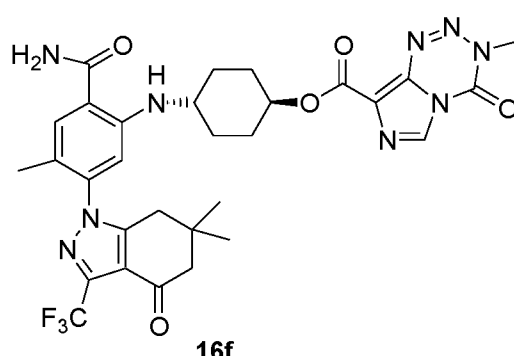
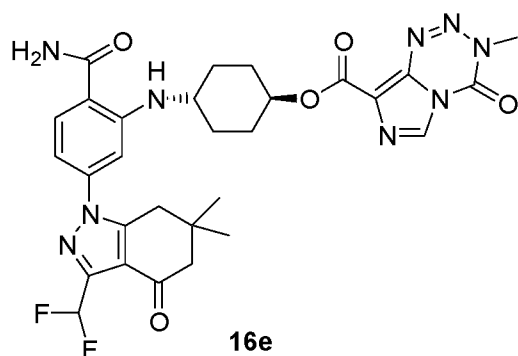
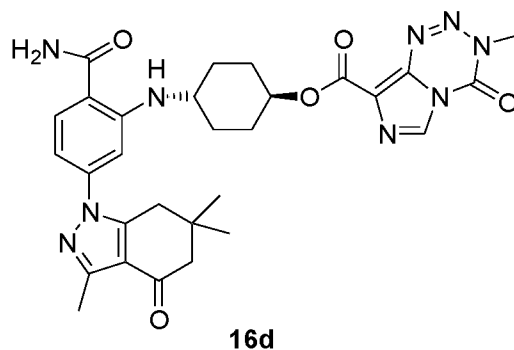
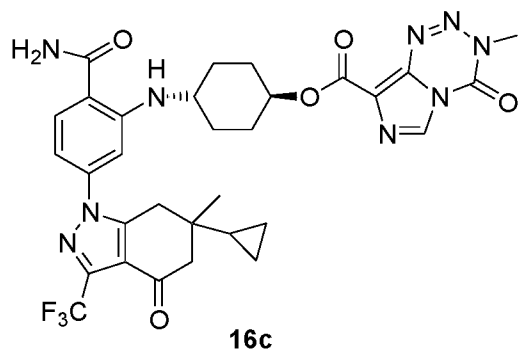
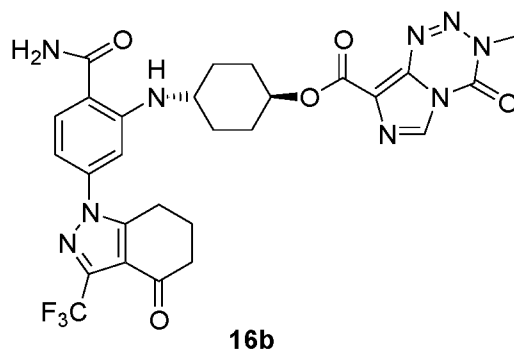
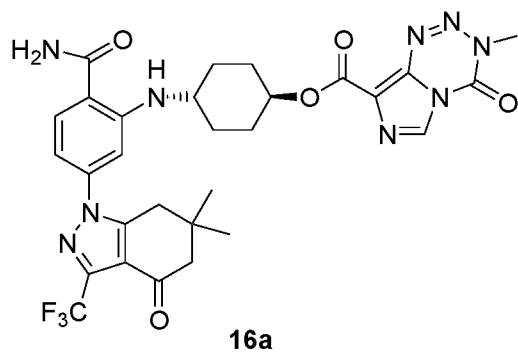
10h



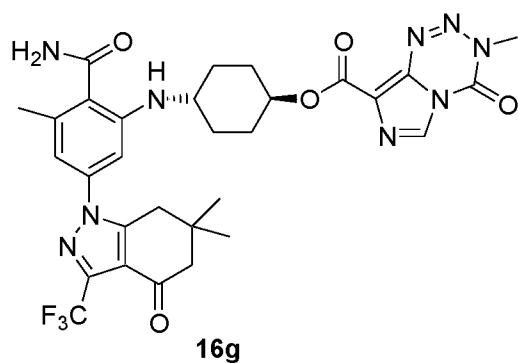
10i



5 In a fourth embodiment, the compounds having formula (III) are selected from the group consisting of

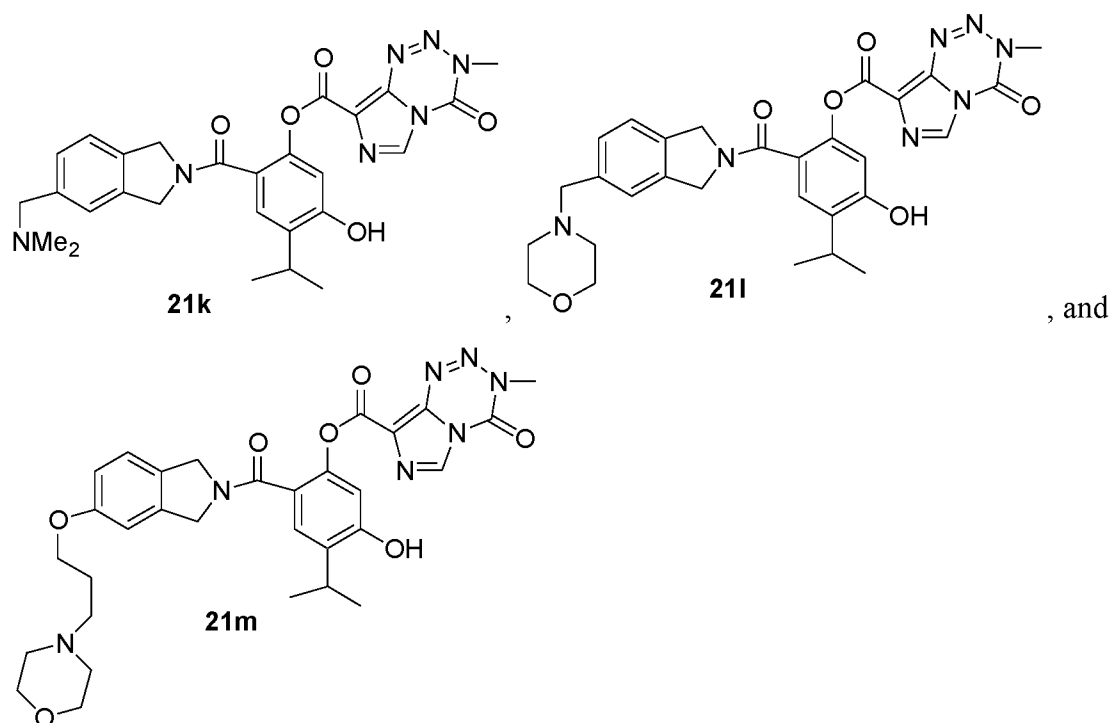


, and

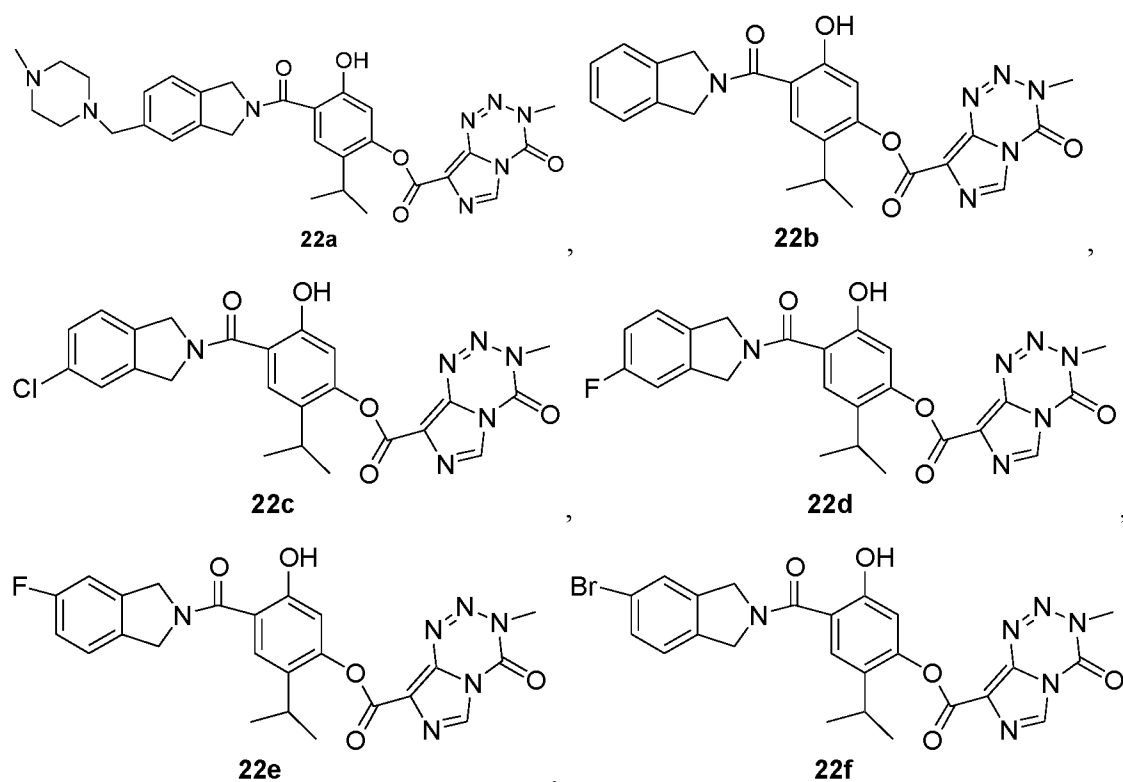


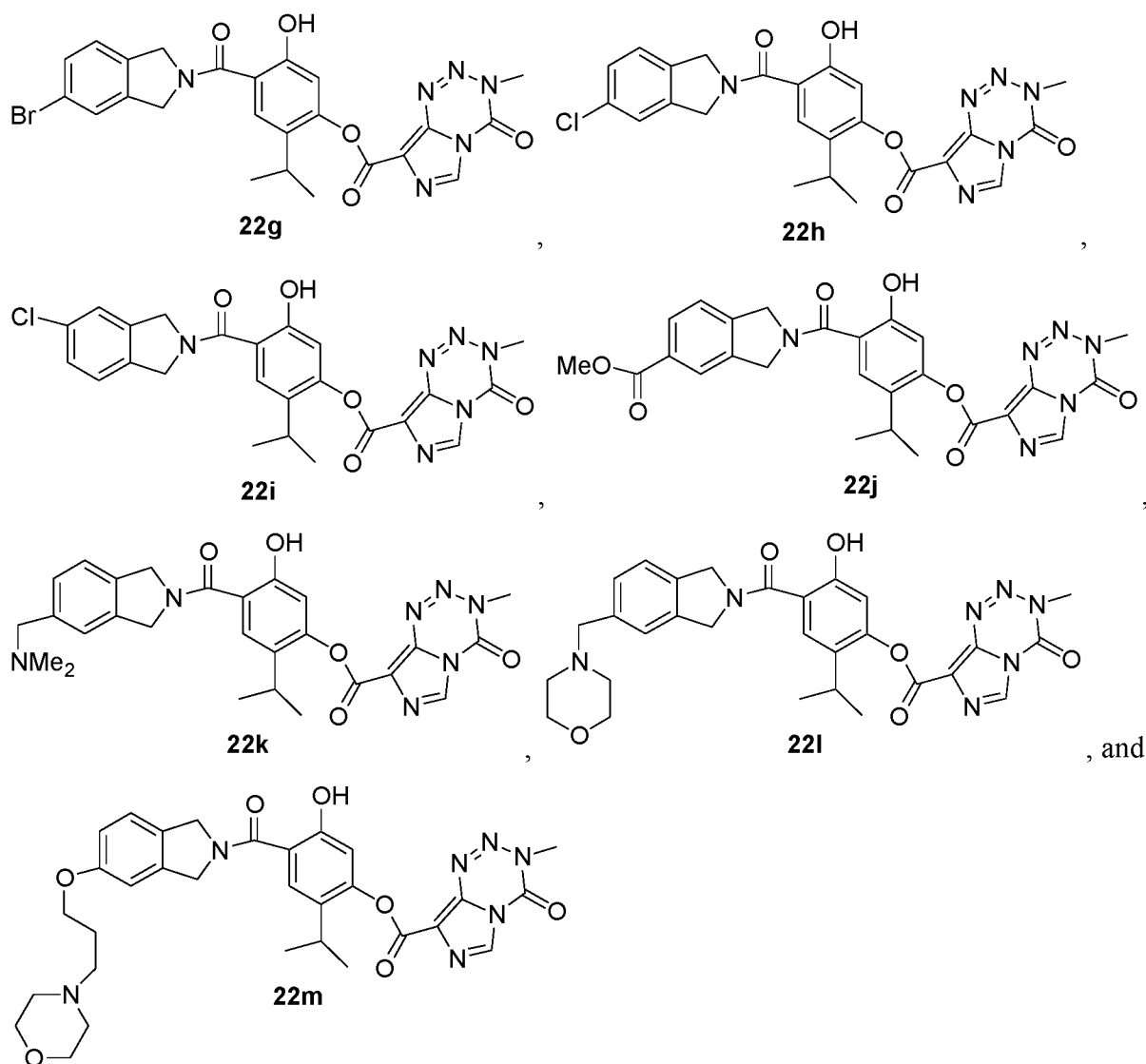
10

In a fifth embodiment, the compounds having formula (IV) are selected from the group consisting of



In a sixth embodiment, the compounds having formula (V) are selected from the group consisting of





10 In a seventh embodiment, the present technology is directed to the above six embodiments, wherein the cancer is glioblastoma, primary malignant brain tumor, or malignant glioma.

In an eighth embodiment, the present technology is directed to the above six embodiments, wherein the cancer is glioblastoma.

15 In a ninth embodiment, the present technology provides a pharmaceutical composition for nasal administration comprising a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor of any of the above first six embodiments; a lipid and a non-ionic surfactant; and an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the conjugate.

5 In a tenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is a compound having formula (I) or (II).

 In an eleventh embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is a compound having formula (III).

10 In a twelfth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is a compound having formula (IV) or (V).

 In a thirteenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is a compound having formula selected
15 from (VI) to (XIII).

 In a fourteenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is one of the compounds of the second embodiment.

 In a fifteenth embodiment is provided the pharmaceutical composition of the ninth
20 embodiment, wherein the covalently-linked conjugate is one of the compounds of the third embodiment.

 In a sixteenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is one of the fourth embodiment.

 In a seventeenth embodiment is provided the pharmaceutical composition of the ninth
25 embodiment, wherein the covalently-linked conjugate is one of the fifth embodiment.

 In an eighteenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the covalently-linked conjugate is one of the sixth embodiment.

 In a nineteenth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the absorption promoting agent is a cationic polymer.

30 In a twentieth embodiment is provided the pharmaceutical composition of the ninth embodiment, wherein the cationic polymer is chitosan.

 In a twenty-first embodiment, the present technology provides a pharmaceutical composition comprising a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90

- 5 inhibitor of any of the first six embodiments, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

In a twenty-second embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is a compound having formula (I) or (II).

- 10 In a twenty-third embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is a compound having formula (III).

- In a twenty-fourth embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is a compound having
15 formula (IV) or (V).

In a twenty-fifth embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is a compound having formula selected from (VI) to (XIII).

- In a twenty-second embodiment is provided a pharmaceutical composition of the
20 twenty-first embodiment, wherein the covalently-linked conjugate is one of the compounds of the second embodiment.

In a twenty-second embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is one of the third embodiment.

- 25 In a twenty-second embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is one of the fourth embodiment.

- In a twenty-second embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is one of the fifth
30 embodiment.

In a twenty-second embodiment is provided a pharmaceutical composition of the twenty-first embodiment, wherein the covalently-linked conjugate is one of the sixth embodiment.

5 Contemplated within the scope of the present technology are all of the above described methods for treating cancer in situations where the cancer has developed resistance to a chemotherapeutic agent.

 As used herein the terms "subject" and "patient" may be used interchangeably, and means a mammal in need of treatment, e.g., companion animals (e.g., dogs, cats, and the
10 like), farm animals (e.g., cows, pigs, horses, sheep, goats and the like) and laboratory animals (e.g., rats, mice, guinea pigs and the like). Typically, the subject is a human in need of treatment.

Pharmaceutical Compositions

15 This technology also provides a pharmaceutical composition comprising at least (i) an Hsp90 inhibitor, or (ii) a chemotherapeutic agent, or (iii) a combination of an Hsp90 inhibitor and a chemotherapeutic agent, or (iv) a compound formed by covalently linking an Hsp90 inhibitor and a chemotherapeutic agent, or a pharmaceutically-acceptable salt thereof; and a pharmaceutically-acceptable carrier.

20 The phrase "pharmaceutically-acceptable carrier" as used herein means a pharmaceutically-acceptable material, composition or vehicle, such as a liquid or solid filler, diluent, excipient, solvent or encapsulating material, involved in carrying or transporting the subject pharmaceutical agent from one organ, or portion of the body, to another organ, or portion of the body. Each carrier must be "acceptable" in the sense of being compatible with
25 the other ingredients of the formulation and not injurious to the patient. Some examples of materials which can serve as pharmaceutically-acceptable carriers include: sugars, such as lactose, glucose and sucrose; starches, such as corn starch and potato starch; cellulose, and its derivatives, such as sodium carboxymethyl cellulose, ethyl cellulose and cellulose acetate; powdered tragacanth; malt; gelatin; talc; excipients, such as cocoa butter and suppository
30 waxes; oils, such as peanut oil, cottonseed oil, safflower oil, sesame oil, olive oil, corn oil and soybean oil; glycols, such as butylene glycol; polyols, such as glycerin, sorbitol, mannitol and polyethylene glycol; esters, such as ethyl oleate and ethyl laurate; agar; buffering agents, such as magnesium hydroxide and aluminum hydroxide; alginic acid; pyrogen-free water; isotonic saline; Ringer's solution; ethyl alcohol; phosphate buffer solutions; and other non-toxic
35 compatible substances employed in pharmaceutical formulations.

5 As set out above, certain embodiments of the present pharmaceutical agents may be provided in the form of pharmaceutically-acceptable salts. The term "pharmaceutically acceptable salt," in this respect, refers to the relatively non-toxic, inorganic and organic acid addition salts of compounds (i.e., (i) a chemotherapeutic agent, or (ii) an Hsp90 inhibitor, (iii) a combination of (i) and (ii), and (iv) a compound formed by linking (i) and (ii)) of the present technology. These salts can be prepared *in situ* during the final isolation and purification of the compounds of the technology, or by separately reacting a purified compound of the technology in its free base form with a suitable organic or inorganic acid, and isolating the salt thus formed. Representative salts include the hydrobromide, hydrochloride, sulfate, bisulfate, phosphate, nitrate, acetate, valerate, oleate, palmitate, stearate, laurate, benzoate, lactate, phosphate, tosylate, citrate, maleate, fumarate, succinate, tartrate, naphthylate, mesylate, glucoheptonate, lactobionate, and laurylsulphonate salts and the like. (See, for example, Berge *et al.*, (1977) "Pharmaceutical Salts," *J. Pharm. Sci.* 66:1-19).

 The pharmaceutically acceptable salts of the subject compounds include the conventional nontoxic salts or quaternary ammonium salts of the compounds, e.g., from non-toxic organic or inorganic acids. For example, such conventional nontoxic salts include those derived from inorganic acids such as hydrochloride, hydrobromic, sulfuric, sulfamic, phosphoric, nitric, and the like; and the salts prepared from organic acids such as acetic, butionic, succinic, glycolic, stearic, lactic, malic, tartaric, citric, ascorbic, palmitic, maleic, hydroxymaleic, phenylacetic, glutamic, benzoic, salicylic, sulfanilic, 2-acetoxybenzoic, fumaric, toluenesulfonic, methanesulfonic, ethane disulfonic, oxalic, isothionic, and the like.

 In other cases, the compounds (i.e., (i) a chemotherapeutic agent, or (ii) an Hsp90 inhibitor, (iii) a combination of (i) and (ii), and (iv) a compound formed by linking (i) and (ii)) of the present technology may contain one or more acidic functional groups and, thus, are capable of forming pharmaceutically-acceptable salts with pharmaceutically-acceptable bases. The term "pharmaceutically-acceptable salts" in these instances refers to the relatively non-toxic, inorganic and organic base addition salts of compounds of the present technology. These salts can likewise be prepared *in situ* during the final isolation and purification of the compounds, or by separately reacting the purified compound in its free acid form with a suitable base, such as the hydroxide, carbonate or bicarbonate of a pharmaceutically-acceptable metal cation, with ammonia, or with a pharmaceutically-acceptable organic primary, secondary or tertiary amine. Representative alkali or alkaline earth salts include the

5 lithium, sodium, potassium, calcium, magnesium, and aluminum salts and the like. Representative organic amines useful for the formation of base addition salts include ethylamine, diethylamine, ethylenediamine, ethanolamine, diethanolamine, piperazine and the like. See, for example, Berge *et al.*, *supra*.

10 Wetting agents, emulsifiers and lubricants, such as sodium lauryl sulfate, magnesium stearate, and polyethylene oxide-polybutylene oxide copolymer as well as coloring agents, release agents, coating agents, sweetening, flavoring and perfuming agents, preservatives and antioxidants can also be present in the compositions.

Formulations of the present technology include those suitable for oral, nasal, topical (including buccal and sublingual), rectal, vaginal and/or parenteral administration. The 15 formulations may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. The amount of active ingredient which can be combined with a carrier material to produce a single dosage form will vary depending upon the host being treated and the particular mode of administration. The amount of active ingredient, which can be combined with a carrier material to produce a single dosage form 20 will generally be that amount of the compound which produces a therapeutic effect. Generally, out of 100%, this amount will range from about 1% to about 99% of active ingredient, preferably from about 5% to about 70%, most preferably from about 10% to about 30%.

Methods of preparing these formulations or compositions include the step of bringing 25 into association a compound (i.e., (i) a chemotherapeutic agent, or (ii) an Hsp90 inhibitor, (iii) a combination of (i) and (ii), and (iv) a compound formed by linking (i) and (ii)) of the present technology with the carrier and, optionally, one or more accessory ingredients. In general, the formulations are prepared by uniformly and intimately bringing into association a compound of the present technology with liquid carriers, or finely divided solid carriers, or 30 both, and then, if necessary, shaping the product.

Formulations of the technology suitable for oral administration may be in the form of capsules, cachets, pills, tablets, lozenges (using a flavored basis, usually sucrose and acacia or tragacanth), powders, granules, or as a solution or a suspension in an aqueous or non-aqueous liquid, or as an oil-in-water or water-in-oil liquid emulsion, or as an elixir or syrup, or as 35 pastilles (using an inert base, such as gelatin and glycerin, or sucrose and acacia) and/or as mouth washes and the like, each containing a predetermined amount of a compound of the

5 present technology as an active ingredient. A compound of the present technology may also be administered as a bolus, electuary or paste.

In solid dosage forms of the technology for oral administration (capsules, tablets, pills, dragees, powders, granules and the like), the active ingredient is mixed with one or more pharmaceutically-acceptable carriers, such as sodium citrate or dicalcium phosphate,
10 and/or any of the following: fillers or extenders, such as starches, lactose, sucrose, glucose, mannitol, and/or silicic acid; binders, such as, for example, carboxymethylcellulose, alginates, gelatin, polyvinyl pyrrolidone, sucrose and/or acacia; humectants, such as glycerol; disintegrating agents, such as agar-agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, sodium carbonate, and sodium starch glycolate; solution retarding
15 agents, such as paraffin; absorption accelerators, such as quaternary ammonium compounds; wetting agents, such as, for example, cetyl alcohol, glycerol monostearate, and polyethylene oxide-polybutylene oxide copolymer; absorbents, such as kaolin and bentonite clay; lubricants, such as talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate, and mixtures thereof; and coloring agents. In the case of capsules,
20 tablets and pills, the pharmaceutical compositions may also comprise buffering agents. Solid compositions of a similar type may also be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugars, as well as high molecular weight polyethylene glycols and the like.

A tablet may be made by compression or molding, optionally with one or more
25 accessory ingredients. Compressed tablets may be prepared using binder (for example, gelatin or hydroxybutylmethyl cellulose), lubricant, inert diluent, preservative, disintegrant (for example, sodium starch glycolate or cross-linked sodium carboxymethyl cellulose), surface-active or dispersing agent. Molded tablets may be made by molding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent.

30 The tablets, and other solid dosage forms of the pharmaceutical compositions of the present technology, such as dragees, capsules, pills and granules, may optionally be scored or prepared with coatings and shells, such as enteric coatings and other coatings well known in the pharmaceutical-formulating art. They may also be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxybutylmethyl
35 cellulose in varying butortions to provide the desired release profile, other polymer matrices, liposomes and/or microspheres. They may be sterilized by, for example, filtration through a bacteria-retaining filter, or by incorporating sterilizing agents in the form of sterile solid

5 compositions, which can be dissolved in sterile water, or some other sterile injectable medium immediately before use. These compositions may also optionally contain opacifying agents and may be of a composition that they release the active ingredient(s) only, or preferentially, in a certain portion of the gastrointestinal tract, optionally, in a delayed manner. Examples are embedding compositions, which can be used include polymeric
10 substances and waxes. The active ingredient can also be in micro-encapsulated form with one or more of the above-described excipients.

Liquid dosage forms for oral administration of the compounds of the technology include pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups and elixirs. In addition to the active ingredient, the liquid dosage forms may contain
15 inert diluents commonly used in the art, such as, for example, water or other solvents, solubilizing agents and emulsifiers, such as ethyl alcohol, isobutyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, butylene glycol, 1,3-butylene glycol, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor and sesame oils), glycerol, tetrahydrofuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures
20 thereof. Additionally, cyclodextrins, e.g., hydroxybutyl-.beta.-cyclodextrin, may be used to solubilize compounds.

Besides inert diluents, the oral compositions can also include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, coloring, perfuming and preservative agents.

25 Suspensions, in addition to the active compounds, may contain suspending agents as, for example, ethoxylated isostearyl alcohols, polyoxyethylene sorbitol and sorbitan esters, microcrystalline cellulose, aluminum metahydroxide, bentonite, agar-agar and tragacanth, and mixtures thereof.

Formulations of the pharmaceutical compositions of the technology for rectal or
30 vaginal administration may be presented as a suppository, which may be prepared by mixing one or more compounds of the technology with one or more suitable nonirritating excipients or carriers comprising, for example, cocoa butter, polyethylene glycol, a suppository wax or a salicylate, and which is solid at room temperature, but liquid at body temperature and, therefore, will melt in the rectum or vaginal cavity and release the active pharmaceutical
35 agents of the technology .

5 Formulations of the present technology which are suitable for vaginal administration also include pessaries, tampons, creams, gels, pastes, foams or spray formulations containing such carriers as are known in the art to be appropriate.

 Dosage forms for the topical or transdermal administration of a compound of this technology include powders, sprays, ointments, pastes, creams, lotions, gels, solutions,
10 patches and inhalants. The active compound may be mixed under sterile conditions with a pharmaceutically-acceptable carrier, and with any preservatives, buffers, or butellants which may be required.

 The ointments, pastes, creams and gels may contain, in addition to an active compound of this technology, excipients, such as animal and vegetable fats, oils, waxes,
15 paraffins, starch, tragacanth, cellulose derivatives, polyethylene glycols, silicones, bentonites, silicic acid, talc and zinc oxide, or mixtures thereof.

 Powders and sprays can contain, in addition to a compound of this technology, excipients such as lactose, talc, silicic acid, aluminum hydroxide, calcium silicates and polyamide powder, or mixtures of these substances. Sprays can additionally contain
20 customary butellants, such as chlorofluorohydrocarbons and volatile unsubstituted hydrocarbons, such as butane and butane.

 Transdermal patches have the added advantage of providing controlled delivery of a compound of the present technology to the body. Such dosage forms can be made by dissolving, or dispersing the pharmaceutical agents in the buter medium. Absorption
25 enhancers can also be used to increase the flux of the pharmaceutical agents of the technology across the skin. The rate of such flux can be controlled, by either providing a rate controlling membrane or dispersing the compound in a polymer matrix or gel.

 Ophthalmic formulations, eye ointments, powders, solutions and the like, are also contemplated as being within the scope of this technology.

30 Pharmaceutical compositions of this technology suitable for parenteral administration comprise one or more compounds of the technology in combination with one or more pharmaceutically-acceptable sterile isotonic aqueous or nonaqueous solutions, dispersions, suspensions or emulsions, or sterile powders which may be reconstituted into sterile injectable solutions or dispersions just prior to use, which may contain antioxidants, buffers,
35 bacteriostats, solutes which render the formulation isotonic with the blood of the intended recipient or suspending or thickening agents.

5 In some cases, in order to prolong the effect of a drug, it is desirable to slow the absorption of the drug from subcutaneous or intramuscular injection. This may be accomplished

by the use of a liquid suspension of crystalline or amorphous material having poor water solubility.

10 The rate of absorption of the drug then depends upon its rate of dissolution, which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally-administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. One strategy for depot injections includes the use of polyethylene oxide copolymers wherein the vehicle is fluid at room temperature and solidifies at body
15 temperature.

Injectable depot forms are made by forming microencapsule matrices of the subject compounds in biodegradable polymers such as polylactide-polyglycolide. Depending on the ratio of drug to polymer, and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly
20 (orthoesters) and poly (anhydrides). Depot injectable formulations are also prepared by entrapping the drug in liposomes or microemulsions, which are compatible with body tissue.

When the compounds of the present technology are administered as pharmaceuticals, to humans and animals, they can be given per se or as a pharmaceutical composition containing, for example, 0.1% to 99.5% (more preferably, 0.5% to 90%) of active ingredient
25 in combination with a pharmaceutically acceptable carrier.

The compounds (i.e., (i) a chemotherapeutic agent, (ii) an Hsp90 inhibitor, (iii) a combination of (i) and (ii), and (iv) a compound formed by linking (i) and (ii)) and pharmaceutical compositions of the present technology can be employed in combination therapies, that is, the compounds and pharmaceutical compositions can be administered
30 concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. The particular combination of therapies (therapeutics or procedures) to employ in a combination regimen will take into account compatibility of the desired therapeutics and/or procedures and the desired therapeutic effect to be achieved. It will also be appreciated that the therapies employed may achieve a desired effect for the same disorder
35 (for example, the compound of the present technology may be administered concurrently

5 with another agent for treating the same disorder), or they may achieve different effects (e.g., control of any adverse effects).

The compounds of the technology may be administered intravenously, intramuscularly, intraperitoneally, subcutaneously, topically, orally, or by other acceptable means. The compounds may be used to treat arthritic conditions in mammals (i.e., humans,
10 livestock, and domestic animals), birds, lizards, and any other organism, which can tolerate the compounds.

The also provides a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the technology. Optionally associated with such container(s) can be a notice in the form
15 prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

As used herein, the term "alkyl" means a saturated straight chain or branched non-cyclic hydrocarbon having from 1 to 10 carbon atoms. Representative saturated straight
20 chain alkyls include methyl, ethyl, n-propyl, n-butyl, n-pentyl, n-hexyl, n-heptyl, n-octyl, n-nonyl and n-decyl; while saturated branched alkyls include isopropyl, sec-butyl, isobutyl, iert-butyl, isopentyl, 2-methylbutyl, 3-methylbutyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-methylhexyl, 3-methylhexyl, 4-methylhexyl, 5-methylhexyl, 2,3-dimethylbutyl, 2,3-dimethylpentyl, 2,4-dimethylpentyl, 2,3-dimethylhexyl, 2,4-dimethylhexyl, 2,5-dimethylhexyl, 2,2-dimethylpentyl, 2,2-dimethylhexyl, 3,3-dimethylpentyl, 3,3-dimethylhexyl, 4,4-dimethylhexyl, 2-ethylpentyl, 3-ethylpentyl, 2-ethylhexyl, 3-ethylhexyl, 4-ethylhexyl, 2-methyl-2-ethylpentyl, 2-methyl-3-ethylpentyl, 2-methyl-4-ethylpentyl, 2-methyl-2-ethylhexyl, 2-methyl-3-ethylhexyl, 2-methyl-4-ethylhexyl, 2,2-diethylpentyl, 3,3-diethylhexyl, 2,2-diethylhexyl, 3,3-diethylhexyl and the like. The term
25 "(C₁-C₆)alkyl" means a saturated straight chain or branched non-cyclic hydrocarbon having from 1 to 6 carbon atoms. Representative (C₁-C₆)alkyl groups are those shown above having from 1 to 6 carbon atoms. Alkyl groups included in compounds of this invention may be optionally substituted with one or more substituents.

As used herein, the term "alkynyl" means a saturated straight chain or branched non-cyclic hydrocarbon having from 2 to 10 carbon atoms and having at least one carbon-carbon
35 triple bond. Representative straight chain and branched alkynyls include acetylenyl,

5 propynyl, 1-butyryl, 2-butyryl, 1-pentyryl, 2-pentyryl, 3-methyl-1-butyryl, 4-pentyryl, 1-hexyryl, 2-hexyryl, 5-hexyryl, 1-heptyryl, 2-heptyryl, 6-heptyryl, 1-octyryl, 2-octyryl, 7-octyryl, 1-nonyryl, 2-nonyryl, 8-nonyryl, 1-decyryl, 2-decyryl, 9-decyryl, and the like. Alkynyl groups may be optionally substituted with one or more substituents.

As used herein, the term "alkynyl" means a saturated straight chain or branched non-cyclic hydrocarbon having from 2 to 10 carbon atoms and having at least one carbon-carbon triple bond. Representative straight chain and branched alkynyls include acetylenyl, propynyl, 1-butyryl, 2-butyryl, 1-pentyryl, 2-pentyryl, 3-methyl-1-butyryl, 4-pentyryl, 1-hexyryl, 2-hexyryl, 5-hexyryl, 1-heptyryl, 2-heptyryl, 6-heptyryl, 1-octyryl, 2-octyryl, 7-octyryl, 1-nonyryl, 2-nonyryl, 8-nonyryl, 1-decyryl, 2-decyryl, 9-decyryl, and the like.
15 Alkynyl groups may be optionally substituted with one or more substituents.

As used herein, the term "cycloalkyl" means a saturated, mono- or polycyclic alkyl radical having from 3 to 20 carbon atoms. Representative cycloalkyls include cyclopropyl, 1-methylcyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl, -cyclodecyl, octahydro-pentalenyl, and the like. Cycloalkyl groups may be optionally substituted with one or more substituents.
20

As used herein, the term "haloalkyl" means an alkyl group in which one or more (including all) the hydrogen radicals are replaced by a halo group, wherein each halo group is independently selected from -F, -Cl, -Br, and -I. The term "halomethyl" means a methyl in which one to three hydrogen radical(s) have been replaced by a halo group. Representative haloalkyl groups include trifluoromethyl, bromomethyl, 1,2-dichloroethyl, 4-iodobutyl, 2-fluoropentyl, and the like.
25

As used herein, an "alkoxy" is an alkyl group which is attached to another moiety via an oxygen linker.

As used herein, an "haloalkoxy" is a haloalkyl group which is attached to another moiety via an oxygen linker.
30

As used herein, the term an "aromatic ring" or "aryl" means a hydrocarbon monocyclic or polycyclic radical in which at least one ring is aromatic. Examples of suitable aryl groups include, but are not limited to, phenyl, tolyl, anthracenyl, fluorenyl, indenyl, azulenyl, and naphthyl, as well as benzo-fused carbocyclic moieties such as 5,6,7, 8-tetrahydronaphthyl. Aryl groups may be optionally substituted with one or more substituents.
35

- 5 In one embodiment, the aryl group is a monocyclic ring, wherein the ring comprises 6 carbon atoms, referred to herein as "(C₆)aryl."

As used herein, the term "alkylene" refers to an alkyl group that has two points of attachment. The term "(C₁-C₆)alkylene" refers to an alkylene group that has from one to six carbon atoms. Straight chain (C₁-C₆)alkylene groups are preferred. Non-limiting examples of
10 alkylene groups include methylene (-CH₂-), ethylene (-CH₂CH₂-), n-propylene (-CH₂CH₂CH₂-), isopropylene (-CH₂CH(CH₃)-), and the like. Alkylene groups may be optionally substituted with one or more substituents.

As used herein, the term "heterocyclyl" means a monocyclic (typically having 3- to 10-members) or a polycyclic (typically having 7- to 20-members) heterocyclic ring system
15 which is either a saturated ring or an unsaturated non-aromatic ring. A 3- to 10-membered heterocycle can contain up to 5 heteroatoms; and a 7- to 20-membered heterocycle can contain up to 7 heteroatoms. Typically, a heterocycle has at least one carbon atom ring member. Each heteroatom is independently selected from nitrogen, which can be oxidized (e.g., N(O)) or quaternized; oxygen; and sulfur, including sulfoxide and sulfone. The
20 heterocycle may be attached via any heteroatom or carbon atom. Representative heterocycles include morpholinyl, thiomorpholinyl, pyrrolidinonyl, pyrrolidinyl, piperidinyl, piperazinyl, hydantoinyl, valerolactamyl, oxiranyl, oxetanyl, tetrahydrofuranyl, tetrahydropyranyl, tetrahydropyrindinyl, tetrahydropyrimidinyl, tetrahydrothiophenyl, tetrahydrothiopyranyl, and the like. A heteroatom may be substituted with a protecting group known to those of
25 ordinary skill in the art, for example, the hydrogen on a nitrogen may be substituted with a tert-butoxycarbonyl group. Furthermore, the heterocyclyl may be optionally substituted with one or more substituents. Only stable isomers of such substituted heterocyclic groups are contemplated in this definition.

As used herein, the term "halogen" or "halo" means -F, -Cl, -Br or -I.

- 30 In addition, alkyl, cycloalkyl, alkylene, a heterocyclyl, and any saturated portion of an alkenyl, cycloalkenyl, alkynyl, aralkyl, and heteroaralkyl groups, may also be substituted with =O, or =S.

As used herein, the term "lower" refers to a group having up to four atoms. For example, a "lower alkyl" refers to an alkyl radical having from 1 to 4 carbon atoms, "lower
35 alkoxy" refers to "-O-(C₁-C₄)alkyl and a "lower alkenyl" or "lower alkynyl" refers to an alkenyl or alkynyl radical having from 2 to 4 carbon atoms, respectively.

5 Unless indicated otherwise, the compounds of the invention containing reactive functional groups (such as (without limitation) carboxy, hydroxy, thiol, and amino moieties) also include protected derivatives thereof. "Protected derivatives" are those compounds in which a reactive site or sites are blocked with one or more protecting groups. Examples of suitable protecting groups for hydroxyl groups include benzyl, methoxymethyl, allyl, 10 trimethylsilyl, tert-butyldimethylsilyl, acetate, and the like. Examples of suitable amine protecting groups include benzyloxycarbonyl, tert-butoxycarbonyl, tert-butyl, benzyl and fluorenylmethyloxy-carbonyl (Fmoc). Examples of suitable thiol protecting groups include benzyl, tert-butyl, acetyl, methoxymethyl and the like. Other suitable protecting groups are well known to those of ordinary skill in the art and include those found in T. W. Greene, 15 Protecting Groups in Organic Synthesis, John Wiley & Sons, Inc. 1981.

As used herein, the terms "treatment," "treat," and "treating" refer to reversing, alleviating, delaying the onset of, or inhibiting the progress of a disease or disorder, or one or more symptoms thereof, as described herein. In some embodiments, treatment may be administered after one or more symptoms have developed, *i.e.*, therapeutic treatment. In 20 other embodiments, treatment may be administered in the absence of symptoms. For example, treatment may be administered to a susceptible individual prior to the onset of symptoms (*e.g.*, in light of a history of symptoms and/or in light of genetic or other susceptibility factors), *i.e.*, prophylactic treatment. Treatment may also be continued after symptoms have resolved, for example to prevent or delay their recurrence.

25 The term "cancer" or "tumor" are well known in the art and refer to the presence, *e.g.*, in a subject, of cells possessing characteristics typical of cancer-causing cells, such as uncontrolled proliferation, immortality, metastatic potential, rapid growth and proliferation rate, decreased cell death/apoptosis, and certain characteristic morphological features.

As used herein, "cancer" or "tumor" refers to all types of cancer or neoplasm or 30 malignant tumors found in humans, including, but not limited to: leukemias, lymphomas, melanomas, carcinomas and sarcomas. As used herein, the terms "cancer," "neoplasm," and "tumor," are used interchangeably and in either the singular or plural form, refer to cells that have undergone a malignant transformation that makes them pathological to the host organism. Primary cancer cells (that is, cells obtained from near the site of malignant 35 transformation) can be readily distinguished from non-cancerous cells by well-established techniques, particularly histological examination. The definition of a cancer cell, as used herein, includes not only a primary cancer cell, but also cancer stem cells, as well as cancer

- 5 progenitor cells or any cell derived from a cancer cell ancestor. This includes metastasized cancer cells, and in vitro cultures and cell lines derived from cancer cells.

In certain embodiments cancers treatable according to the methods described herein include a carcinoma, sarcoma, lymphoma, melanoma, and leukemia. In one embodiment, the treatable cancers include pancreatic cancer, breast cancer, liver cancer, skin cancer, lung
10 cancer, colon cancer, prostate cancer, thyroid cancer, bladder cancer, rectal cancer, endometrial cancer, kidney cancer, bone cancer, brain cancer (e.g. glioblastoma multiforme), cervical cancer, stomach cancer, mouth and oral cancers, neuroblastoma, testicular cancer, uterine cancer, and vulvar cancer. In a further embodiment, the skin cancer is selected from the group consisting of melanoma, squamous cell carcinoma, basal cell carcinoma, and
15 cutaneous T-cell lymphoma (CTCL). In another embodiment, the oncological disorder is triple negative breast cancer.

The term "sarcoma" generally refers to a tumor which is made up of a substance like the embryonic connective tissue and is generally composed of closely packed cells embedded in a fibrillar or homogeneous substance. Examples of sarcomas which can be treated with a
20 colloidal dispersion of CoQ10 in an IV formulation include, for example, a chondrosarcoma, fibrosarcoma, lymphosarcoma, melanosarcoma, myxosarcoma, osteosarcoma, Abemethy's sarcoma, adipose sarcoma, liposarcoma, alveolar soft part sarcoma, ameloblastic sarcoma, botryoid sarcoma, chloroma sarcoma, chorio carcinoma, embryonal sarcoma, Wilms' tumor sarcoma, endometrial sarcoma, stromal sarcoma, Ewing's sarcoma, fascial sarcoma,
25 fibroblastic sarcoma, giant cell sarcoma, granulocytic sarcoma, Hodgkin's sarcoma, idiopathic multiple pigmented hemorrhagic sarcoma, immunoblastic sarcoma of B cells, lymphoma, immunoblastic sarcoma of T-cells, Jensen's sarcoma, Kaposi's sarcoma, Kupffer cell sarcoma, angiosarcoma, leukosarcoma, malignant mesenchymoma sarcoma, parosteal sarcoma, reticulocytic sarcoma, Rous sarcoma, serocystic sarcoma, synovial sarcoma, and
30 telangiectatic sarcoma.

The term "melanoma" is taken to mean a tumor arising from the melanocytic system of the skin and other organs. Melanomas which can be treated with the colloidal dispersions of CoQ10 in IV formulation include, for example, acral-lentiginous melanoma, amelanotic melanoma, benign juvenile melanoma, Cloudman's melanoma, S91 melanoma, Harding-
35 Passey melanoma, juvenile melanoma, lentigo maligna melanoma, malignant melanoma, nodular melanoma, subungal melanoma, and superficial spreading melanoma.

5 The term "carcinoma" refers to a malignant new growth made up of epithelial cells
tending to infiltrate the surrounding tissues and give rise to metastases. Carcinomas which
can be treated with compositions, as described herein, include, for example, acinar
carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma
adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma,
10 basal cell carcinoma, carcinoma basocellul are, basaloid carcinoma, basosquamous cell
carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma,
cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid
carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en
cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct
15 carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid
carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere,
carcinoma fibrosum, gelatiniform carcinoma, gelatinous carcinoma, giant cell carcinoma,
carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix
carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline
20 carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ,
intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-
cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous
carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma,
melanotic carcinoma, carcinoma molle, merkel cell carcinoma, mucinous carcinoma,
25 carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma
mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell
carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal
carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell
carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian
30 carcinoma, scirrhou carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma
simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell
carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string
carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell
carcinoma, carcinoma tuberosum, tuberos carcinoma, verrucous carcinoma, and carcinoma
35 villosum.

Any suitable anti-cancer chemotherapeutic agent can be used in either a covalent
conjugates or individually in a combination in the of the methods of treating cancer described

5 herein. For instance, in particular embodiments, the chemotherapeutic agent is
mechlorethamine, cyclophosphamide, ifosfamide, melphalan, chlorambucil, thiotepa,
hexamethylmelamine, busulfan, carmustine, lomustine, semustine, streptozocin, decarbazine,
vincristine, vinblastine, etoposide, teniposide, paclitaxel, docetaxel, daunorubicin, idarubicin,
doxorubicin, epirubicin, dactinomycin, plicamycin, mitomycin C, bleomycin, mitoxantrone,
10 fluorouracil, floxuridine, fludarabine, mercaptopurine, thioguanine, cytarabine, azacytidine,
cladribine, pentostatin, cisplatin, carboplatin, mitotane, procarbazine, or amsacrine.

 In other particular embodiments, the chemotherapeutic agent is mechlorethamine,
cyclophosphamide, ifosfamide, melphalan, chlorambucil, thiotepa, hexamethylmelamine,
busulfan, carmustine, lomustine, semustine, streptozocin, decarbazine, vincristine,
15 vinblastine, etoposide, teniposide, paclitaxel, docetaxel, daunorubicin, idarubicin,
doxorubicin, epirubicin, dactinomycin, plicamycin, mitomycin C, bleomycin, mitoxantrone,
methotrexate, fluorouracil, floxuridine, fludarabine, mercaptopurine, thioguanine, cytarabine,
azacytidine, cladribine, or pentostatin.

 In other particular embodiments, the anti-cancer chemotherapeutic agent is amsacrine,
20 azacytidine, bleomycin, busulfan, capecitabine, carboplatin, carmustine, chlorambucil,
cisplatin, cladribine, cyclophosphamide, cytarabine, dactinomycin, daunorubicin,
decarbazine, docetaxel, doxorubicin, epirubicin, estramustine, etoposide, floxuridine,
fludarabine, fluorouracil, gemcitabine, hexamethylmelamine, idarubicin, ifosfamide,
irinotecan, lomustine, mechlorethamine, melphalan, mercaptopurine, methotrexate,
25 mitomycin C, mitotane, mitoxantrone, oxaliplatin, paclitaxel, pemetrexed, pentostatin,
plicamycin, procarbazine, raltitrexed, semustine, streptozocin, temozolamide, teniposide,
thioguanine, thiotepa, topotecan, trinitrexate, valrubicin, vincristine, vinblastine, vindesine,
or vinorelbine.

 In some embodiments, the anti-cancer chemotherapeutic agent is an antimetabolite.
30 For instance, it can be methotrexate, fluorouracil, floxuridine, fludarabine, mercaptopurine,
thioguanine, cytarabine, azacytidine, cladribine, pentostatin, pemetrexed, raltitrexed,
trimetrexate, capecitabine, or gemcitabine.

 The technology further provides a method of treating a subject, such as a human,
suffering from one of the abovementioned disorders or diseases.

5 The technology further relates to the use of provided compounds for the production of pharmaceutical compositions which are employed for the treatment and/or prophylaxis and/or amelioration of the diseases and disorders mentioned herein.

Without further elaboration, it is believed that one skilled in the art can, based on the above description, utilize the present technology to its fullest extent. The following specific
10 examples are, therefore, to be construed as merely illustrative, and not limitative of the remainder of the disclosure in any way whatsoever. All publications cited herein are incorporated by reference in their entirety.

EXAMPLES

15 Example 1. Measurement of cell viability

Glioblastoma cell lines, LN229 and U87, were seeded into 96-well cell culture dishes at densities of 1,000 or 2,000 cells per well in DMEM, 10% FBS, and the antibiotics, penicillin and streptomycin. The day after seeding the cells, compounds (TMZ and Hsp90 inhibitors) dissolved in DMSO were added to the cell culture medium to achieve the
20 indicated final concentrations. After treatment with the compound(s) for the indicated amount of time, cell viability was measured by monitoring change in absorbance upon addition of WST-8 (Dojindo) or change in fluorescence upon the addition of 20 µg/ml resazurin. Percentage viability was calculated for each treatment compared to treatment with control (vehicle DMSO). Results are shown as average (with standard error) of measurements from
25 triplicate samples. See Figs. 1-6, 9A, and 9B]

Example 2. AKT1 ELISA

The glioblastoma cell line, LN229, was seeded into 6-well cell culture dishes at a density of 120,000 cells per well in DMEM, 10% FBS, and the antibiotics, penicillin and
30 streptomycin. The day after seeding the cells, compounds, dissolved in DMSO, were added to the cell culture dishes to achieve the indicated final concentrations. After 48 hours of treatment with the compound(s), cells were lysed in 100 µl per well of 20 mM Tris-HCl, 1% NP-40, 137 mM NaCl, 10% glycerol, 2 mM EDTA, 2.5 mM sodium pyrophosphate, 5 mM sodium fluoride, 1 mM activated sodium orthovanadate, pH 7.2, and HALT protease inhibitor
35 cocktail (Thermo Fisher). Total cellular AKT1 was measured by ELISA using the total AKT1 DuoSet IC ELISA kit (R&D Systems) according to the manufacturer's protocol. Total

5 cellular protein for each treatment was measured by BCA assay (Thermo Fisher). The proportion of AKT1 to total cellular protein content in each treatment was calculated and the average and standard error from three experimental replicates were graphed. See Fig. 7

Example 3. Caspase 3/7 Activity Assay

10 Glioblastoma cell lines LN229 and U87 were seeded into 96-well cell culture dishes at a density of 2,000 cells per well in DMEM, 10% FBS, and penicillin-streptomycin. The day after seeding the cells, compound(s) dissolved in DMSO were added to the cell culture medium to achieve the indicated final concentrations. After 48 hours of treatment with the compound(s), caspase 3/7 activity was measured by the addition of a pro-luminescent caspase
15 3/7 substrate, Caspase-Glo ® (Promega), according to the manufacturer's protocol. Relative fold change in caspase 3/7 activity was graphed for the average from triplicate samples. See Figs. 10A and 10B.

Example 4. Accumulation of dual-acting HSP90/TRAP1 inhibitors in mitochondria

20 As previously noted, the rationale for using a dual-acting Hsp90/TRAP1 inhibitor for treatment of GBM is two-fold: (i) it induce simultaneous degradation of multiple oncogenic proteins and (ii) it overcomes the effects of Bcl-2 overexpression, a cause of acquired resistance. TRAP1 inhibitor-mediated apoptosis is known to occur independent of antiapoptotic Bcl-2 pathways. Therefore, effective TRAP1 inhibition requires penetration
25 through mitochondrial membranes to gain access to TRAP1 in the mitochondrial matrix. Based on studies using whole-cell and isolated mitochondria, CAP01088 and Ganetespib were observed to cause reduction in the mitochondrial membrane potential suggesting that the inhibitors accumulated in mitochondria in concentrations sufficient to inhibit TRAP1 (Figs. 8 A & 8B). In human malignant glioma cells (U251MG), CAP01088 treatment caused
30 significant depolarization of the mitochondrial membrane (Fig. 8C).

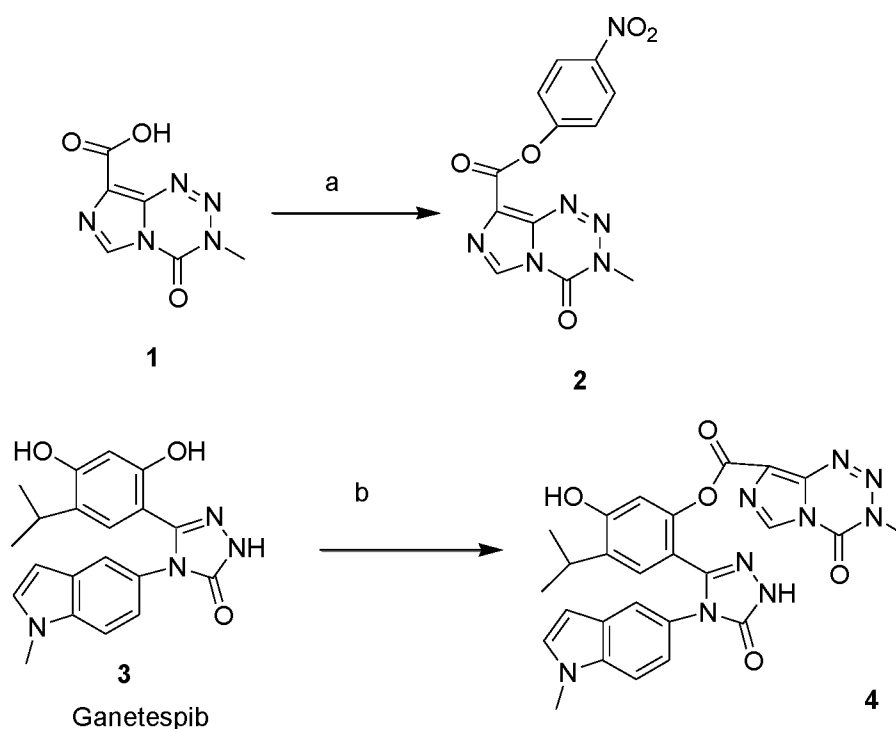
Example 5. Conjugation (covalent linking) of Temozolomide with HSP90 inhibitors

General

Unless otherwise indicated, all anhydrous solvents were commercially obtained and
35 stored in Sure-seal bottles under nitrogen. All other reagents and solvents were purchased as

the highest grade available and used without further purification. Thin-layer chromatography (TLC) analysis of reaction mixtures was performed using Merck silica gel 60 F254 TLC plates, and visualized using ultraviolet light. NMR spectra were recorded on Bruker 500 MHz instrument. Chemical shifts (δ) are reported in parts per million (ppm) referenced to ^1H (Me_4Si at 0.00). Coupling constants (J) are reported in Hz throughout. Mass spectral data were acquired on Shimadzu LCMS-2010EV for low resolution, and on an Agilent ESI-TOF for either high or low resolution. Purity of all compounds was obtained in a HPLC Breeze from Waters Co. using an Atlantis T3 $3\mu\text{m}$ 4.6×150 mm reverse phase column. A linear gradient of solvents A and B from 95% A and 5% B to 5% A and 95% B in 15 min a flow rate of 1 ml/min followed by 5 min of 100% B (Solvent A: H_2O with 0.1% TFA; Solvent B: acetonitrile with 0.1% TFA) was used for elution. Compounds were detected at $\lambda=254$ nm or 214 nm. Purity of key compounds was established by elemental analysis performed on a Perkin Elmer series II-2400 instrument. Combustion analysis was performed by NuMega Resonance Labs, San Diego, CA, USA.

Conjugation of Temozolomide acid with Ganetespib



Scheme 1 (reagents and conditions: a - HATU, DIEA, THF-DCM; b - 2, K_2CO_3 , THF)

Synthesis of 4-nitrophenyl-3-methyl-4-oxo-3H-4H-imidazo[4,3-d][1,2,3,5]tetrazine-8-carboxylate (compound 2)

15 Synthesis of 5-hydroxy-2-[4-(1-methyl-1H-indol-5-yl)-5-oxo-4,5-dihydro-1H-1,2,4-tetrazol-3-yl]-4-(propan-2-yl)-phenyl-3-methyl-4-oxo-3H,4H-imidazo[4,3-d]tetrazine-8-carboxylate (compound **4**)

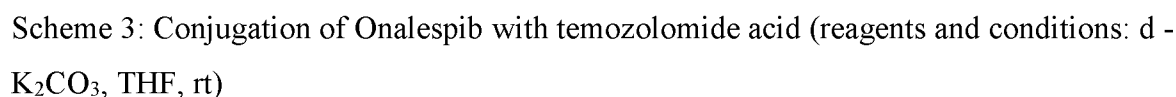
Chemical reaction scheme showing the synthesis of SNX-2112 (6) from intermediate 5 and compound 1.

Intermediate 5 (labeled **5**) is a benzimidazole derivative with a trifluoromethyl group and a cyclohexylcarbamate group. It reacts with compound 1 (a 1,2,4,5-tetrazine-3,6-dione derivative) in the presence of catalyst **c** to form SNX-2112 (6).

SNX-2112 (6) is the final product, where the cyclohexylcarbamate group of intermediate 5 is linked via an ester bond to the 3-position of the tetrazine ring of compound 1.

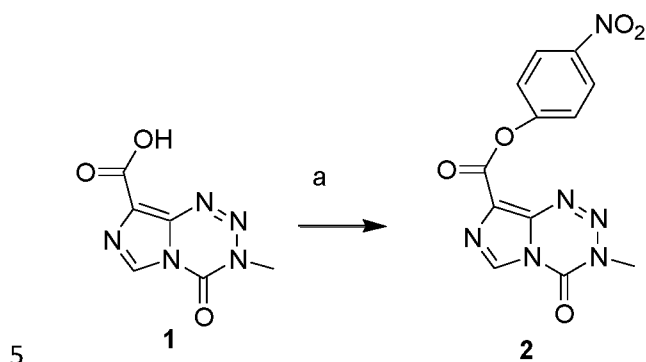
65

- A mixture of compound **5** (50 mg, 0.107 mmol), compound **1** (23 mg, 0.117 mmol), EDC (41 mg, 0.214 mmol), DMAP (2 mg, 0.017 mmol) in CH₂Cl₂ (6 mL) was stirred at room temperature for 24 h. Water was added to the reaction mixture and was extracted with CH₂Cl₂, organic layer was washed with saturated NaHCO₃ solution in water (50 mL x 3), brine (50 mL x 2), dried over MgSO₄, and concentrated. The residue was purified over silica gel column using 2-10% MeOH in DCM to afford a pure product **6** (20 mg, 29%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.03 (s, 6 H), 1.23 (s, 2 H), 1.42-1.49 (m, 2 H), 1.70-1.78 (m, 2 H), 2.04-2.12 (m, 4 H), 2.44 (s, 2 H), 2.99 (s, 2 H), 3.57-3.59 (m, 1 H), 3.88 (s, 3 H), 5.06-5.11 (m, 1 H), 6.74 (dd, *J* = 8.5 and 1.8 Hz, 1 H), 6.93 (d, *J* = 1.5 Hz, 1 H), 7.35 (br s, 1 H), 7.80 (d, *J* = 8.5 Hz, 1 H), 8.00 (br. S, 1 H), 8.54 (d, *J* = 7 Hz, 1 H), 8.83 (s, 1 H); MS *m/z* 664 (M+Na)⁺, 642 (M+H)⁺.

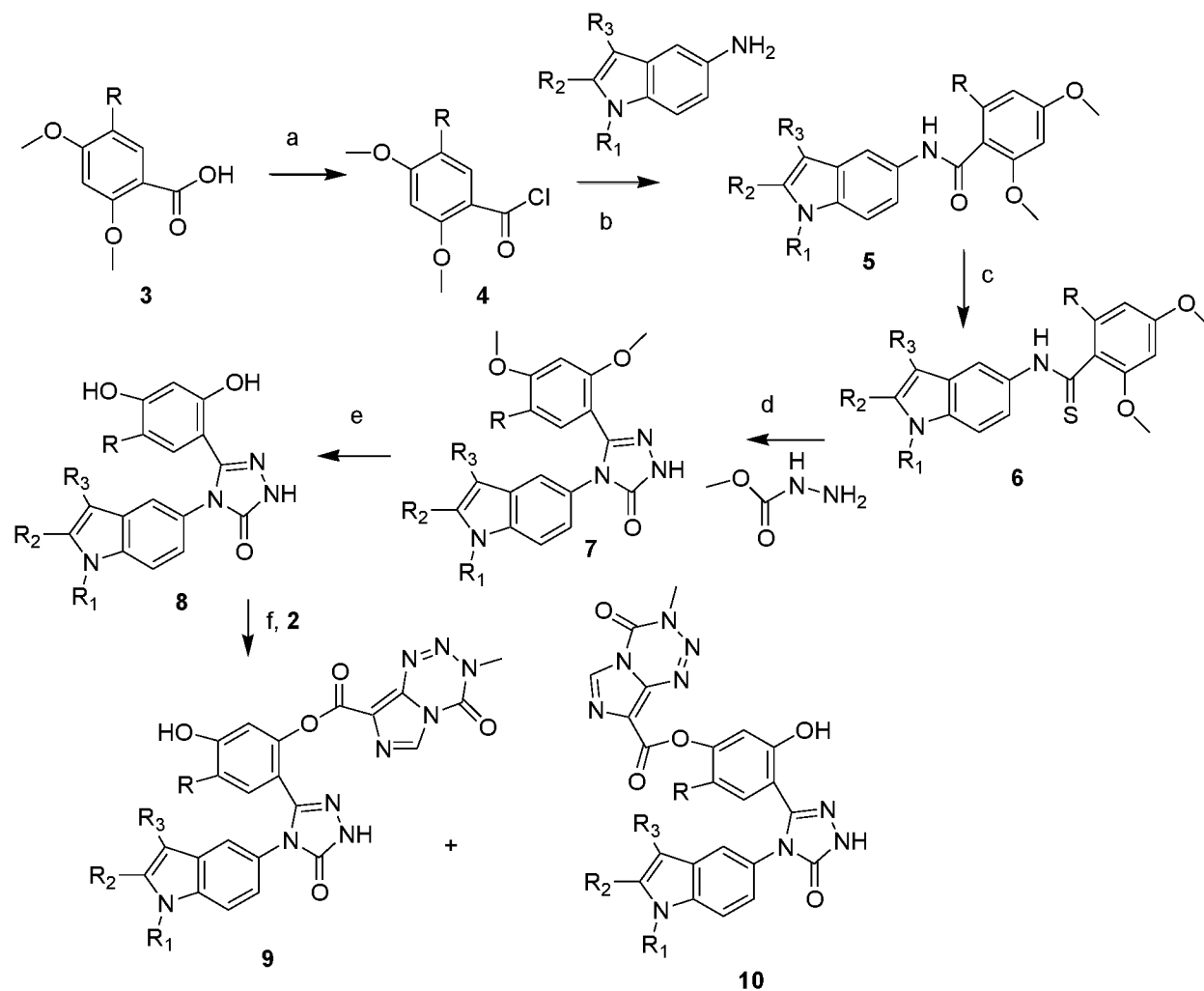


Onalespib and temozolomide are covalently linked using Scheme 3 shown above.

66



Scheme 1: Reagents and Conditions: (a) HATU, DIEA, THF, CH₂Cl₂, rt.



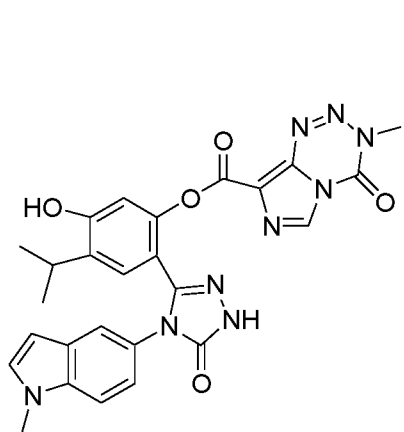
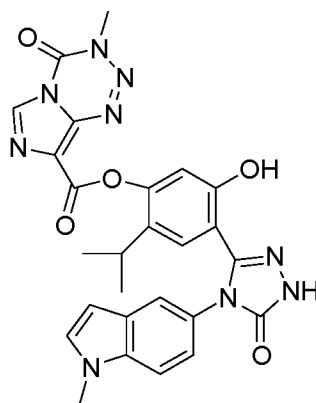
R = H, Me, Et, *n*-Pr, isopropyl, Chloro

10 R₁ = Me, Et, *n*-Pr, CHF₂, methyl-cyclopropane, allyl, Propargyl, Cyclopropyl, cyclopentyl, cyclohexyl, etc.

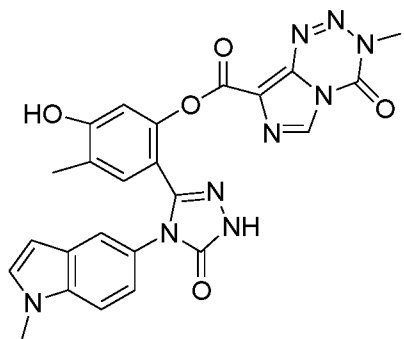
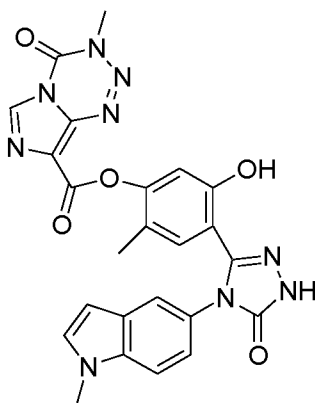
R₂ = H, Me,

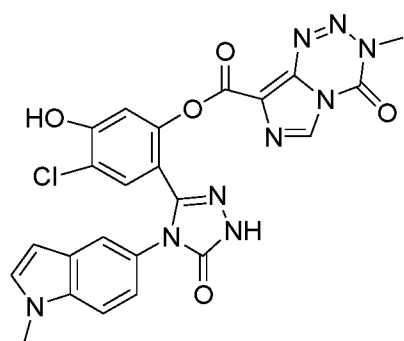
R₃ = H, Me, Ph, Cl,

- 5 Scheme 2: General Synthesis of two drugs conjugation: Reagents and conditions: (a) Thionyl chloride, DMF (cat), CH₂Cl₂, reflux; (b) Et₃N, CH₂Cl₂, rt.; (c) Lawesson's Reagent; (d) HgCl₂, Pyridine, Dioxane, reflux; (e) Pyridine hydrochloride, NaI, 200 °C; (f) compound **2**, K₂CO₃, THF, rt.

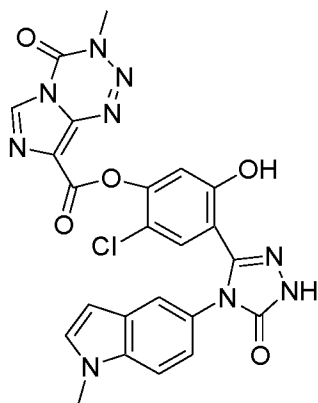
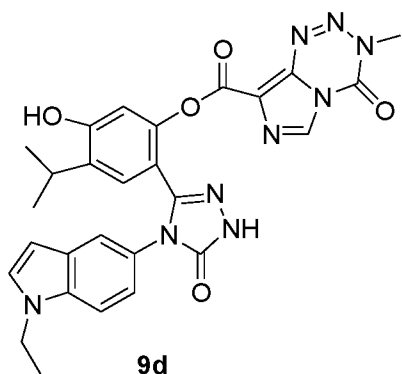
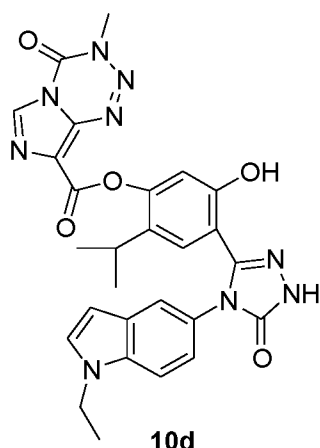
**9a****10a**

10

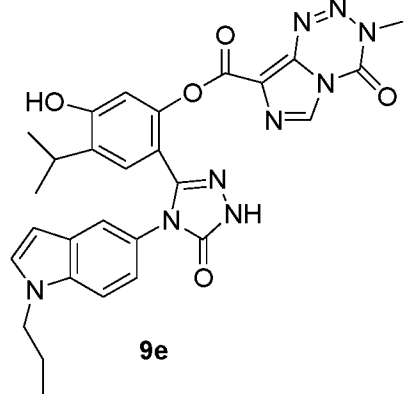
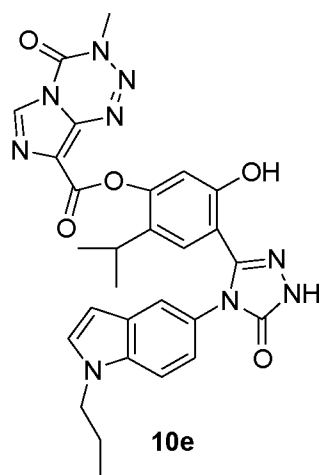
**9b****10b**

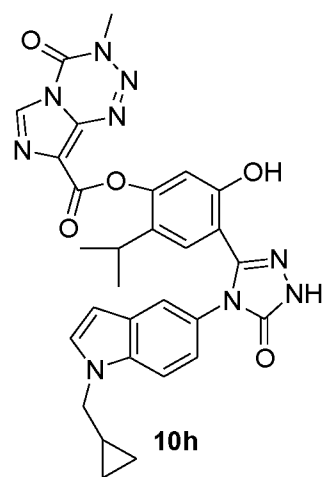
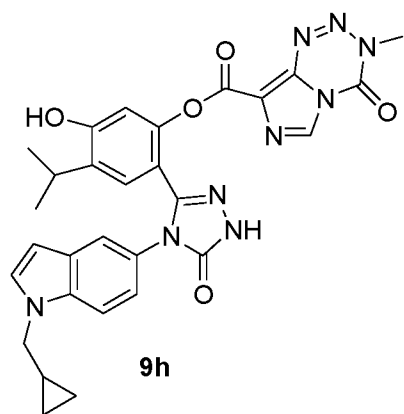
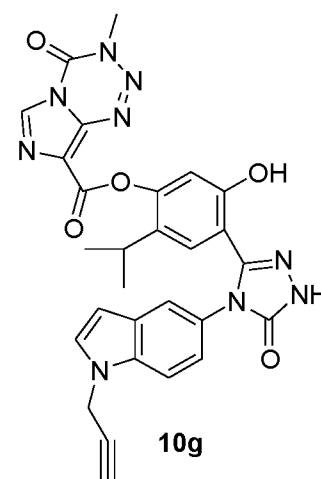
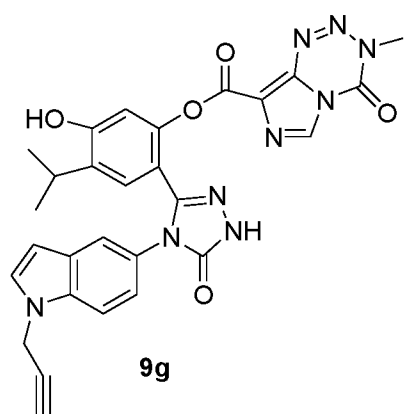
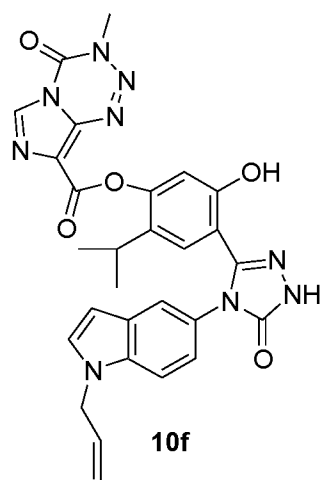
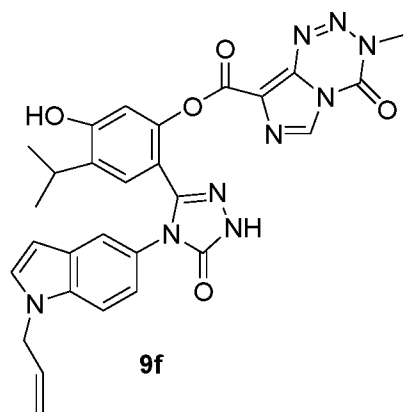


5

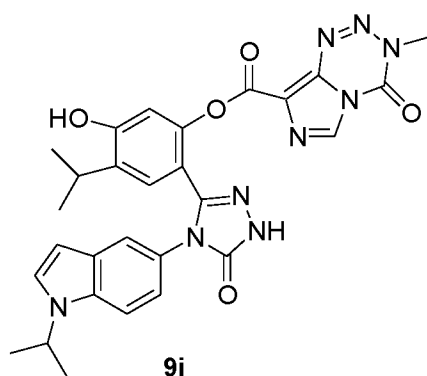
9c**10c****9d****10d**

10

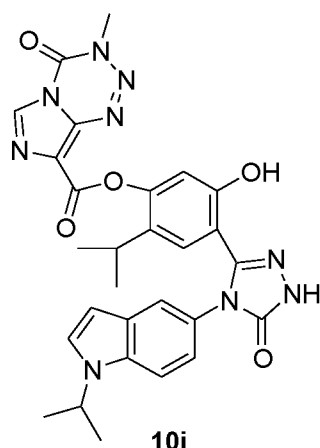
**9e****10e**



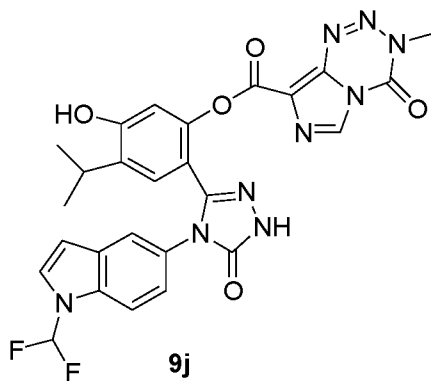
5



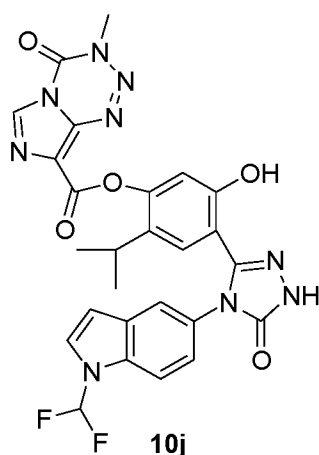
9i



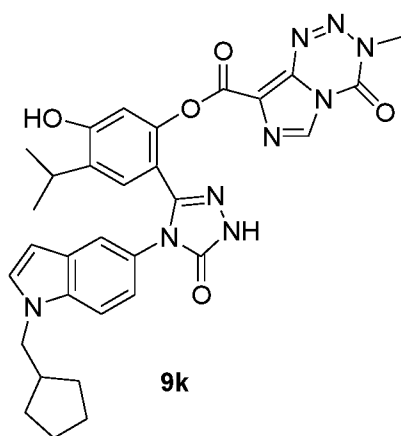
10i



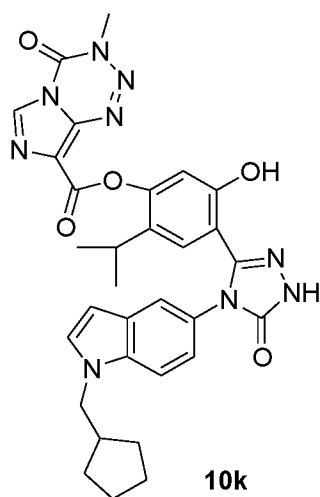
9j



10j

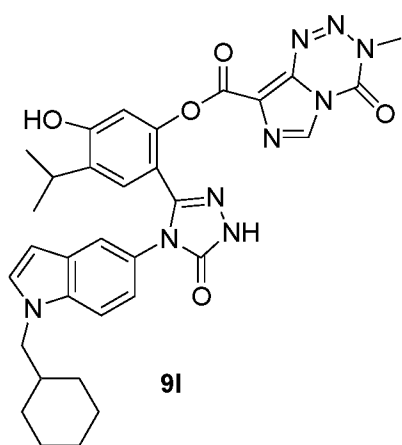


9k

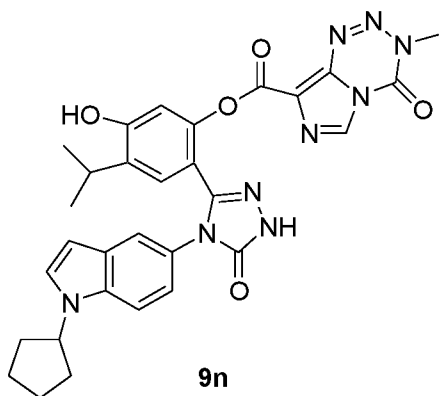
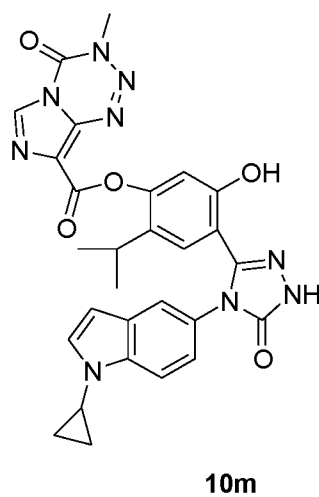
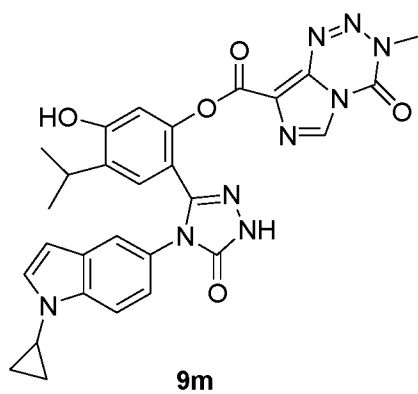
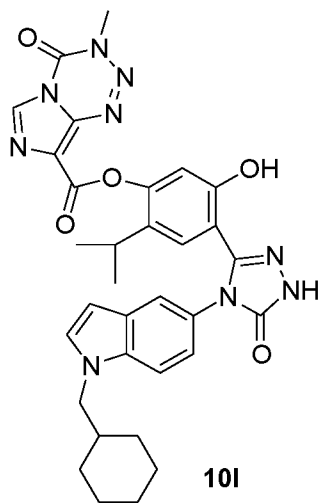


10k

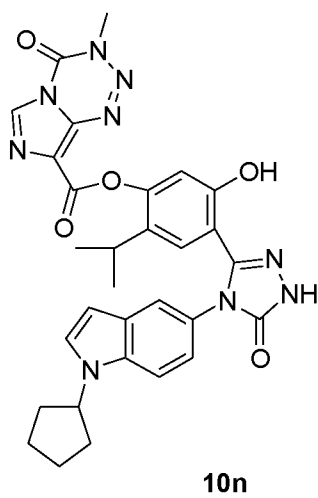
10



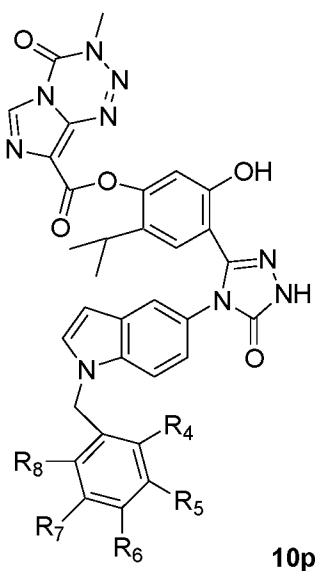
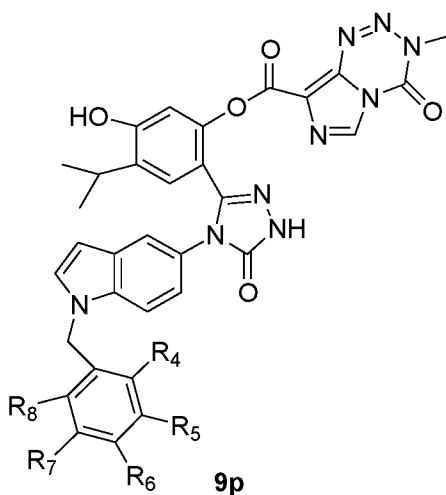
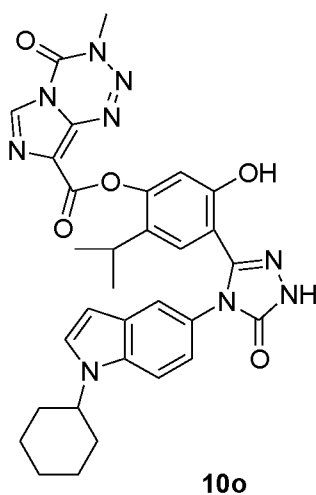
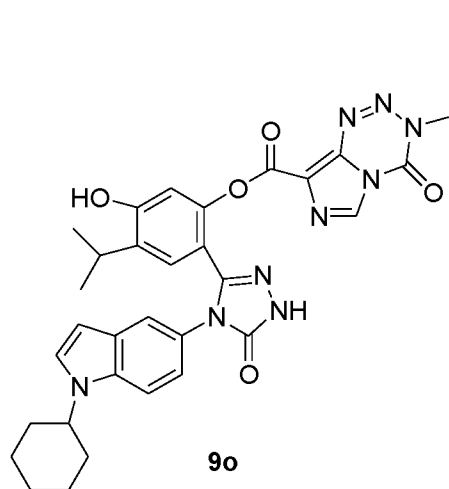
5



10



5



10 R_4 , R_5 , R_6 , R_7 , and R_8 , are independently selected from the group consisting of hydrogen, lower alkyl, (C_1-C_4) alkoxy, (C_4-C_7) cyclopropyl, (C_2-C_6) alkenyl, and (C_2-C_6) alkynyl, each optionally substituted with one or more halogen atoms.

Synthetic Procedures:

15 Synthesis of 4-nitrophenyl-3-methyl-4-oxo-3*H*-4*H*-imidazo[4,3-*d*][1,2,3,5]tetrazine-8-carboxylate (compound 2)

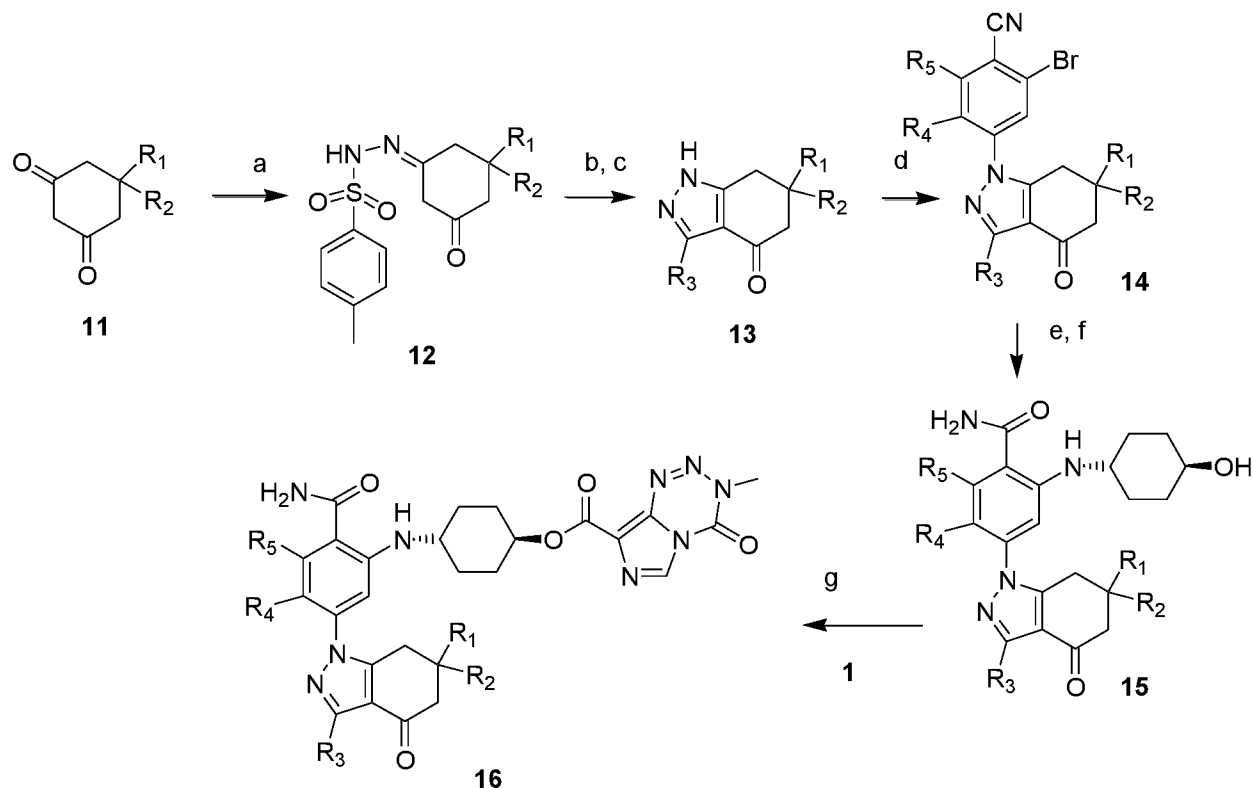
Temozolomide acid (compound 1) (49 mg, 0.25 mmol) was mixed with 5 mL of THF and 5 mL of DCM in a round bottom flask and was cooled to 0 °C. 4-Nitrophenol (35 mg, 0.25 mmol) was added followed by *N,N*-diisopropylethylamine (20 mg, 0.3 mmol) and HATU

- 5 (105 mg, 0.28 mmol). The mixture was stirred at room temperature for about 5 hours. Water was added and the product was extracted with DCM (30 ml x3), the combined organic phase was washed with brine, dried over Na₂SO₄, filtered and solvent was removed under reduced pressure. A pale yellow solid was obtained, which is used in the next step without further purifications.

10

Synthesis of 5-hydroxy-2-[4-(1-methyl-1*H*-indol-5-yl)-5-oxo-4,5-dihydro-1*H*-1,2,4-tetrazol-3-yl]-4-(propan-2-yl)-phenyl-3-methyl-4-oxo-3*H*,4*H*-imidazo[4,3-*d*]tetrazine-8-carboxylate (compound 10a, major)

- Compound **2** (41 mg, 0.13 mmol) was mixed with compound **8** (47 mg, 0.13 mmol) and K₂CO₃ (18 mg, 0.13 mmol) in 5 mL of anhydrous THF. The mixture was stirred at room temperature overnight. Cooled to 0 °C and 1N HCl aqueous solution was used to adjust the pH to acidic. The mixture was then extracted with DCM (30 mL x3), the combined organic phase was washed with brine, dried over Na₂SO₄, filtered and solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel (DCM: MeOH /100:0 to 90:10). The pure product was collected and dried under vacuum. 14 mg of pale yellow solid was obtained (**compound 10a**) (yield 20%). MS: *m/z* 564 (M+ Na)⁺, 542 (M+H)⁺.



5

Scheme 3: Reagents and Conditions: (a) TsNHNH₂, Cat. *P*-TsOH, toluene, reflux; (b) (R₃CO)₂O, Et₃N, THF, 60 °C; (c) NaOH, MeOH, water, rt; (d) 2-Bromo-4-Fluorobenzonitriles, NaH, DMSO, rt, (e) Pd(OAc)₂, DPPF, NaOtBu, *trans*-4-aminocyclohexanol, 110 °C, microwave; (f) EtOH, NaOH, DMSO, H₂O₂, rt; (g) compound **1**, EDC, DMAP, CH₂Cl₂, rt.

10

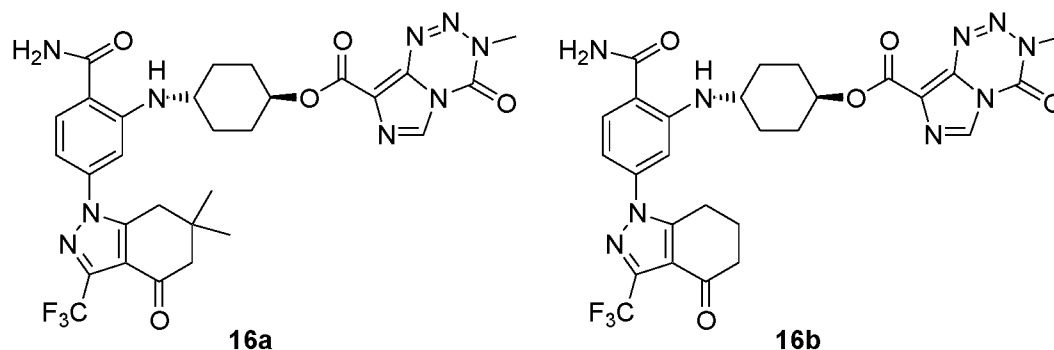
Synthesis of 4-([2-carbamoyl-5-[6,6-dimethyl-4-oxo-3-(trifluoromethyl)-4,5,6,7-tetrahydro-1*H*-indazol-1-yl]amino)cyclohexyl-3-methyl-4-oxo-3*H*,4*H*-imidazo[4,3-*d*][1,2,3,5]tetrazine-8-carboxylate (Compound **16a**)

15

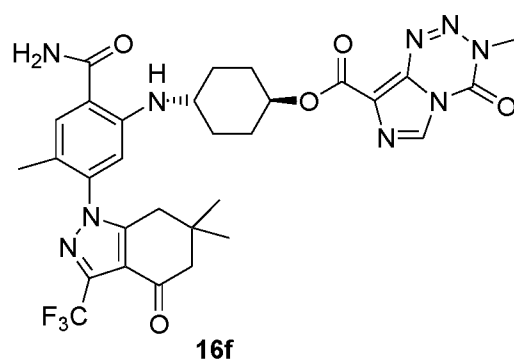
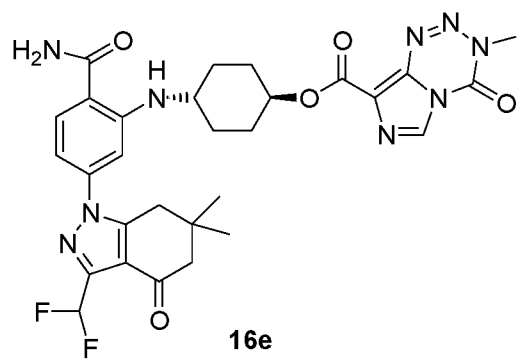
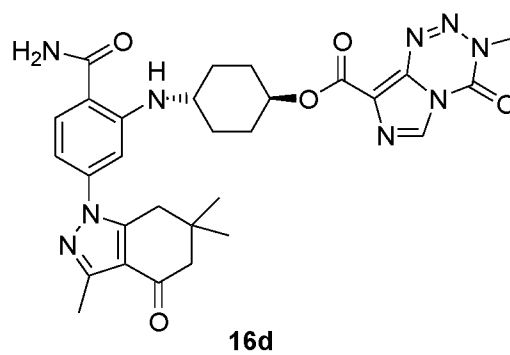
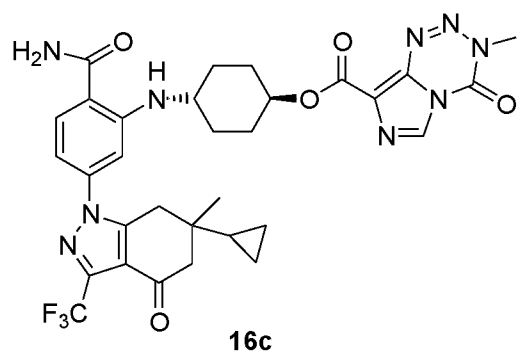
A mixture of compound **15a** (50 mg, 0.107 mmol), compound **1** (23 mg, 0.117 mmol), EDC (41 mg, 0.214 mmol), DMAP (2 mg, 0.017 mmol) in CH₂Cl₂ (6 mL) was stirred at room temperature for 24 h. Water was added to the reaction mixture and was extracted with CH₂Cl₂, organic layer was washed with saturated NaHCO₃ solution in water (50 mL x 3), brine (50 mL x 2), dried over MgSO₄, and concentrated. The residue was purified over silica gel column using 2-10% MeOH in DCM to afford a pure product **16a** (20 mg, 29%). ¹H NMR (500 MHz, DMSO-*d*₆) δ 1.03 (s, 6 H), 1.23 (s, 2 H), 1.42-1.49 (m, 2 H), 1.70-1.78 (m, 2 H), 2.04-2.12 (m, 4 H), 2.44 (s, 2 H), 2.99 (s, 2 H), 3.57-3.59 (m, 1 H), 3.88 (s, 3 H), 5.06-5.11 (m, 1 H), 6.74 (dd, *J* = 8.5 and 1.8 Hz, 1 H), 6.93 (d, *J* = 1.5 Hz, 1 H), 7.35 (br s, 1 H), 7.80 (d, *J* = 8.5 Hz, 1 H), 8.00 (br s, 1 H), 8.54 (d, *J* = 7 Hz, 1 H), 8.83 (s, 1 H); MS *m/z* 664 (M+Na)⁺, 642 (M+H)⁺.

25

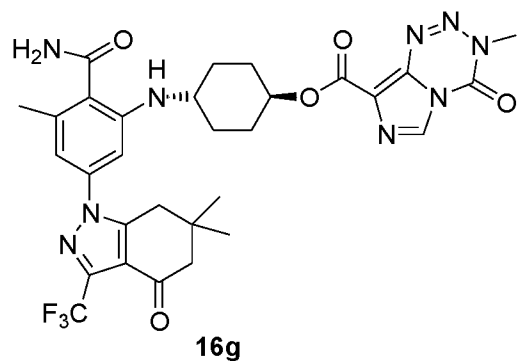
Using Scheme 3 and appropriate starting materials and reagents, compounds **16a-16** were synthesized.

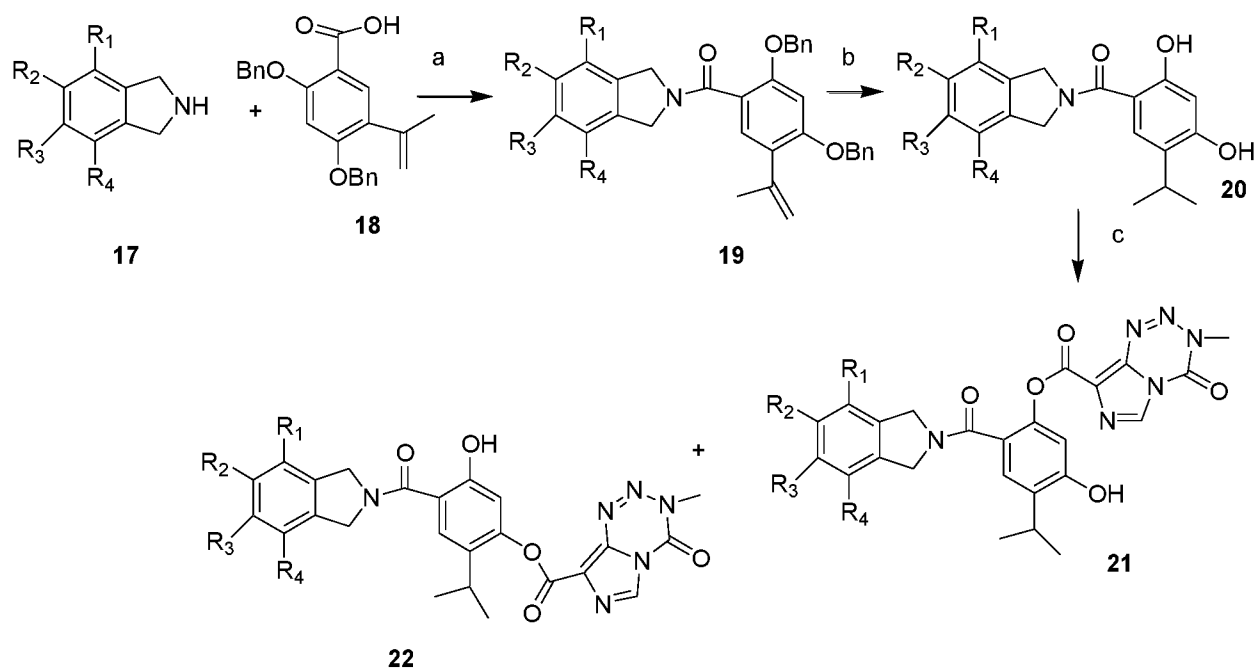


30

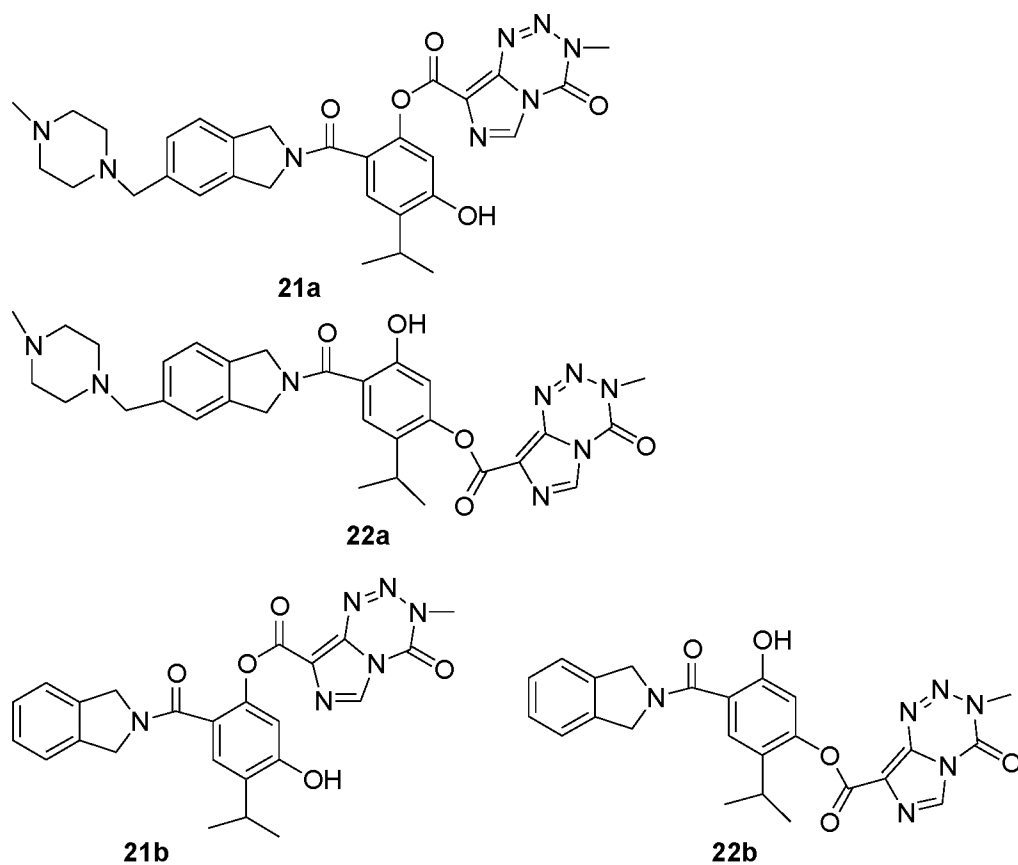


10

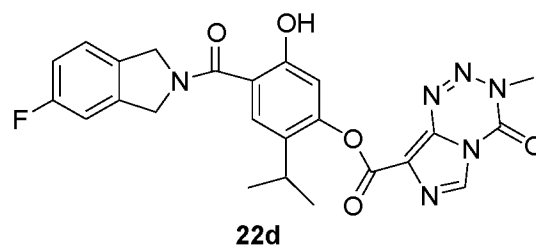
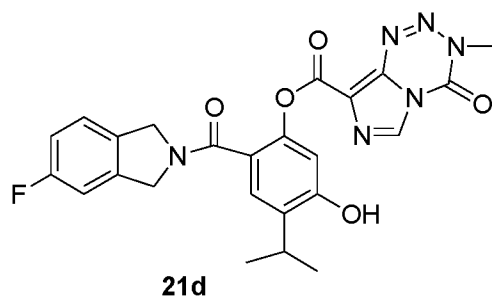
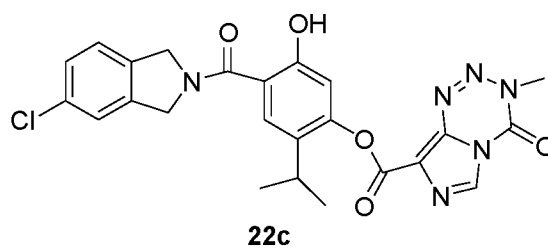
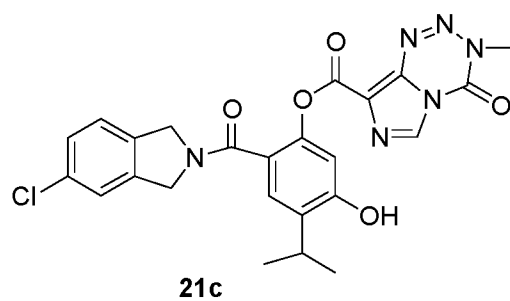




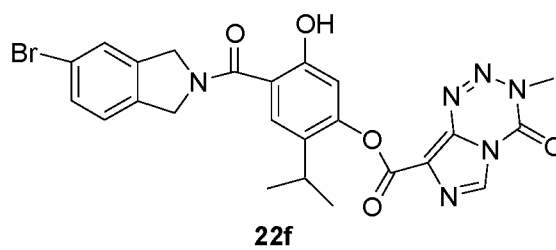
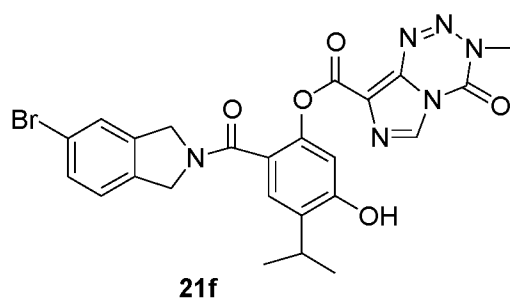
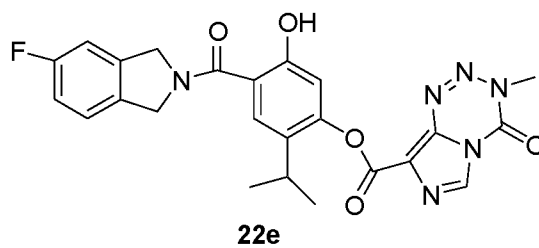
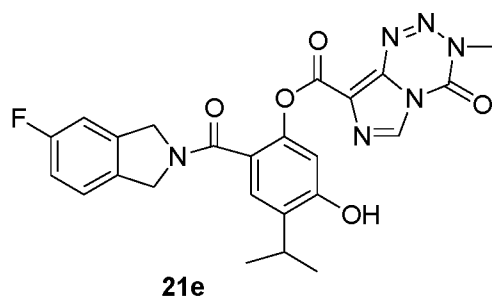
Scheme 4: Reagents and conditions: (d) EDC, HOBt, DIEA, DMF, 16 h, rt; (b) 10% Pd/C, EtOH, H₂; (c) compound 2, K₂CO₃, THF, rt.

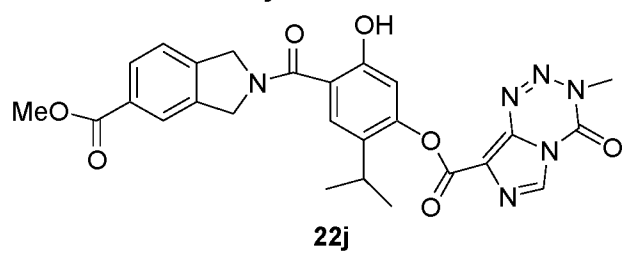
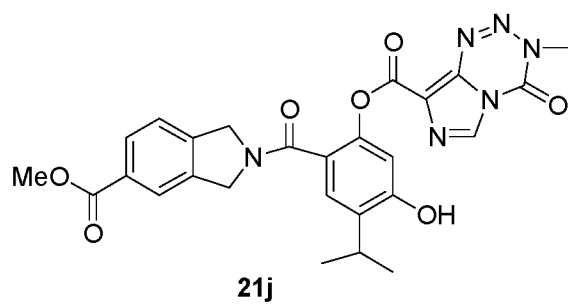
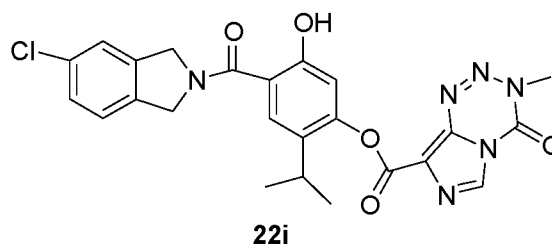
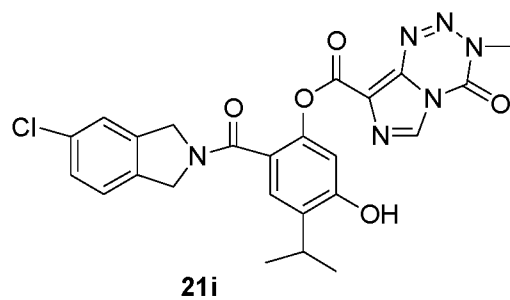
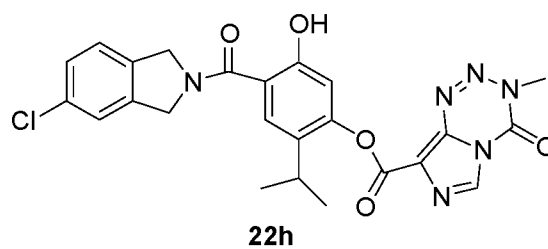
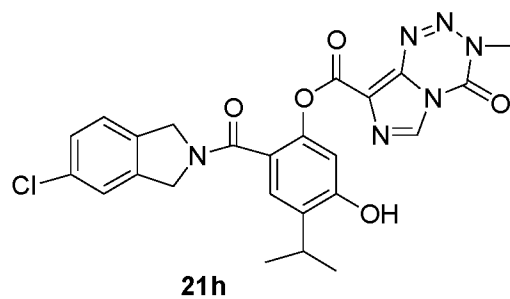
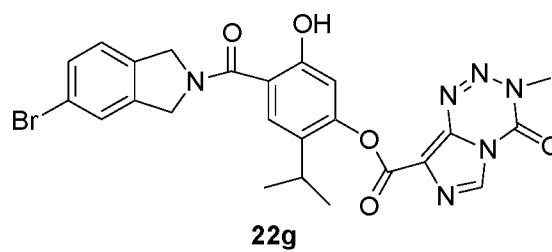
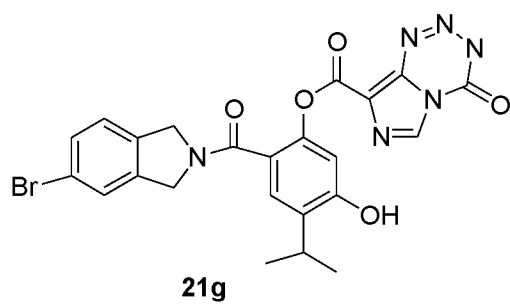


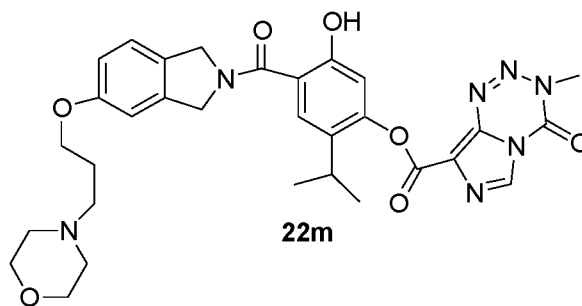
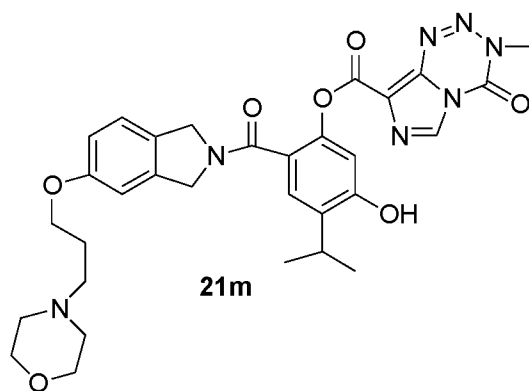
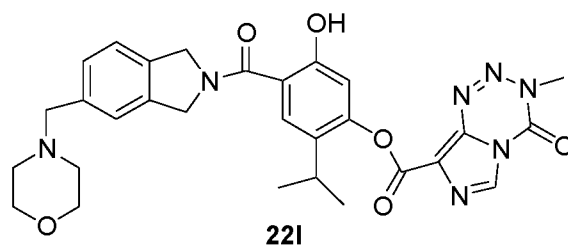
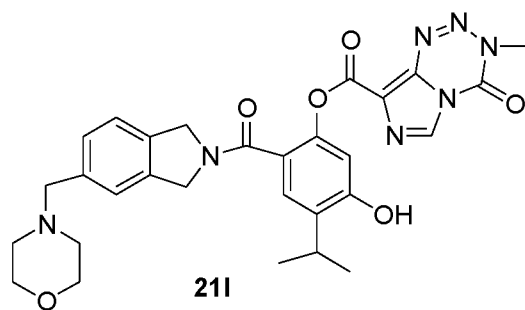
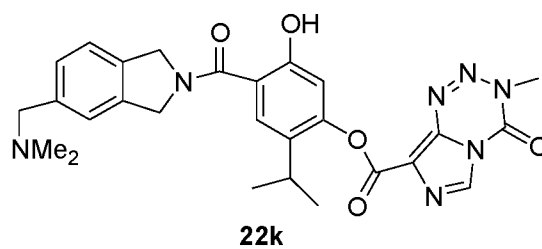
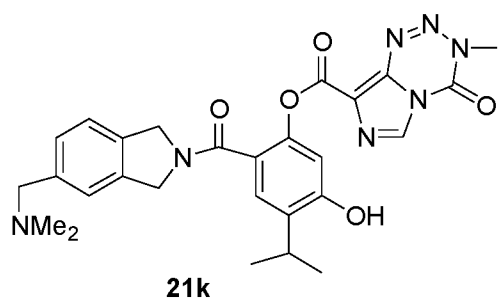
5

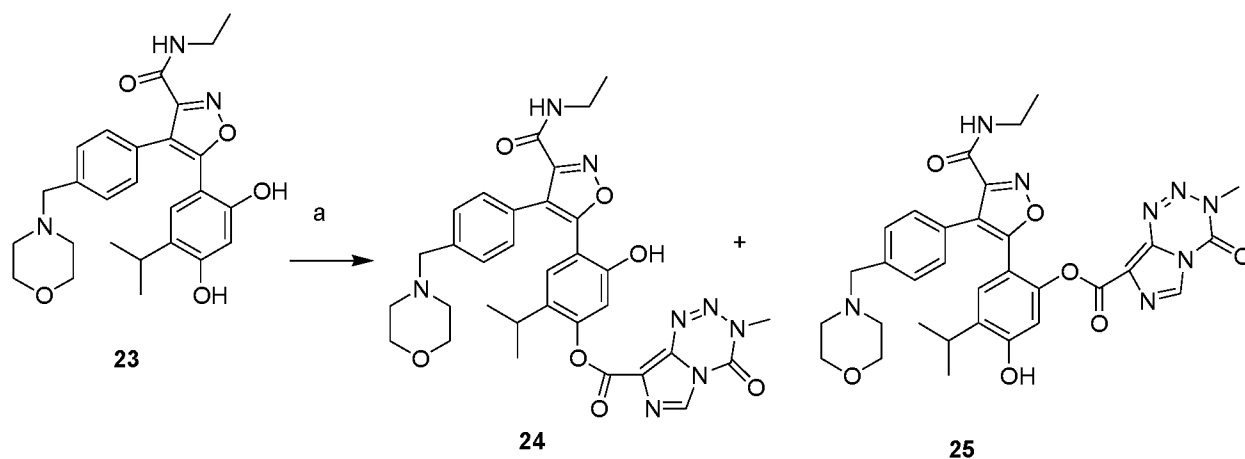


10

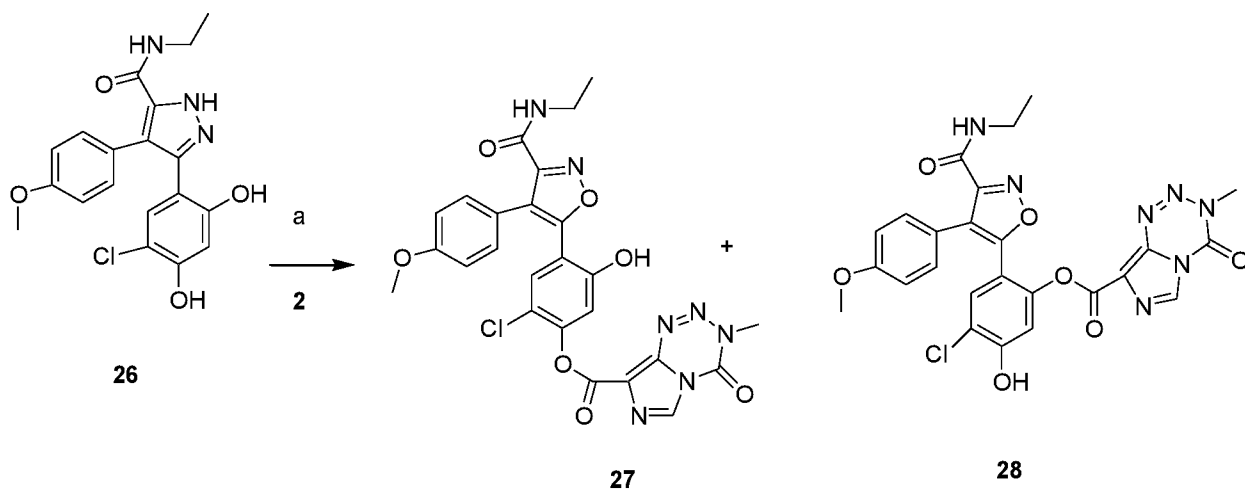




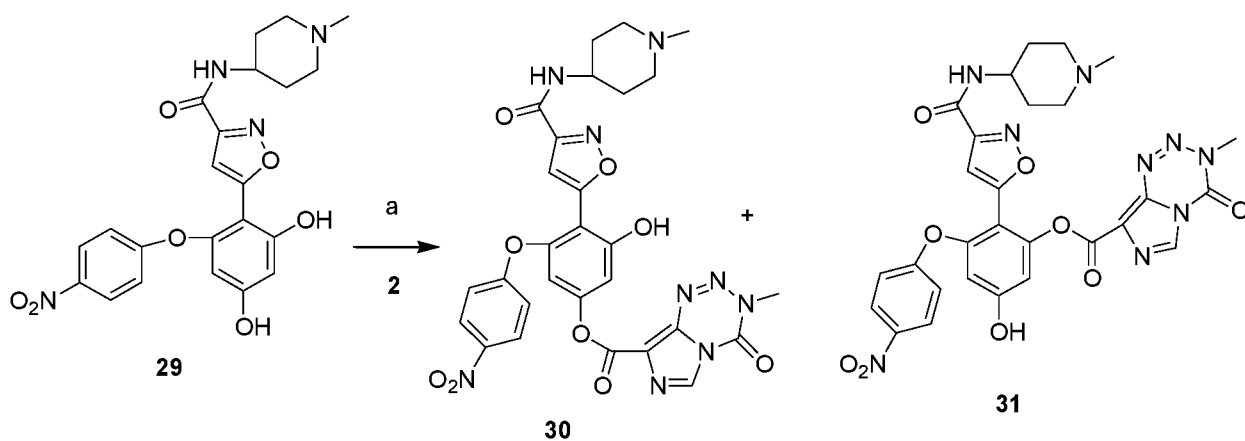




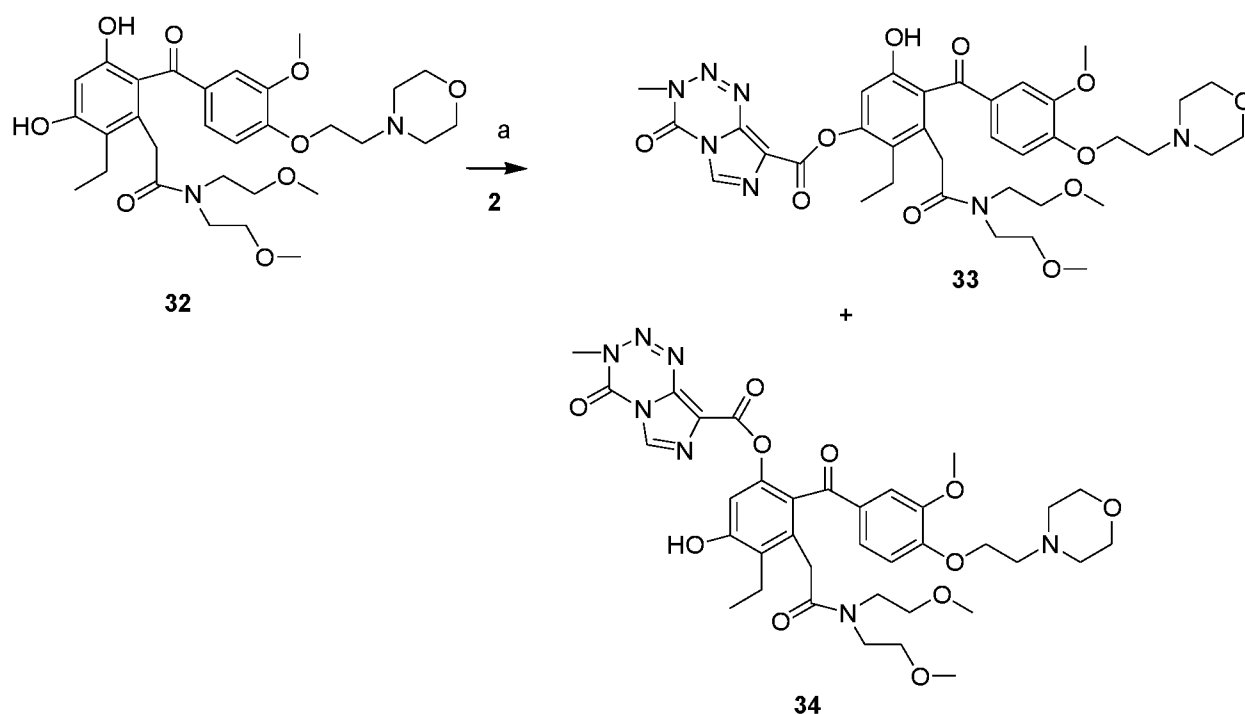
Scheme 5: Reagents and conditions: (a) Compound 2, K_2CO_3 , THF, rt.



Scheme 6: Reagents and conditions: (a) Compound 2, K_2CO_3 , THF, rt



Scheme 7: Reagents and conditions: (a) Compound 2, K_2CO_3 , THF, rt



Scheme 8: Reagents and conditions: (a) Compound 2, K_2CO_3 , THF, rt

Example 6. Enhancement of cell death by covalently conjugated TMZ and SNX-2112

The effect of covalently linked TMZ and SNX-2112 was determined by measuring cell viability (Example 1) upon treatment of cells with the covalently linked compound. As shown in Fig. 9A, enhanced death of LN229 human malignant glioma cells was observed following treatment with various concentrations of the compound for 72 hours. Further, as shown in Fig. 9B, enhanced death of U87 human malignant glioma cells was observed following treatment of the cells with the compound for 72 hours.

Example 7. Formulation of compounds for the nose to brain delivery using the chitosan-based hybrid vesicular system

The compounds (TMZ and Hsp90 inhibitors), individually and in combination, are encapsulated in phosphatidylcholine, cholesterol, and non-ionic surfactant-based vesicles (e.g., vesicles made from Brij 76 and span 60) in varying ratios, and are hybridized with chitosan to form different suspensions. These suspensions are used to prepare various formulations using thin film hydration followed by sonication. Formulations are designed and developed using central composite design (CCD) and optimized with response surface methodology. They are characterized with respect to stability, pH, osmolarity, drug loading,

5 entrapment efficiency, size and surface charge, etc., using methods such as column chromatography, dynamic light scattering, TEM/SEM, HPLC and LC-MS/MS. Based on the above parameters, formulations having a combination of desirable characteristics are selected for studying transport across bovine nasal mucosa and *in vitro* kinetics.

10 Example 8. Investigation of drug delivery enhancement efficiency of hybrid chitosan vesicular system and its in-vitro cytotoxicity

Transport studies across bovine nasal mucosa (olfactory region) are performed using a select few formulations as identified above. Specifically, effects of the hybrid chitosan vesicular system on drug transport across nasal epithelial layer are studied using a suitable
15 analytical technique such as LCMS or fluorescent labeling. The ability to open cell junctions in the nasal epithelial membrane is investigated by measuring trans-epithelial electrical resistance. These studies are performed to quantify the drug permeated by the vesicles to enhance delivery across the nasal epithelium. Transport rates are compared with that obtained using active agents formulated in a buffer solution. Kinetic parameters such as
20 passive permeation flux (J_{max}), steady state permeation flux (J_{ss}), and lag time are evaluated *in vitro* across bovine nasal mucosa.

Example 9. Investigation of *in-vitro* efficacy of drug formulations for delivery by intranasal route on PC-12, LN229, U87, and SHSY-5y neuronal cells

25 PC-12, LN229, U87, and SHSY-5y neuronal cells are used as models to perform efficacy of various drug formulations comprising hybrid chitosan vesicles (see Example 7). Effects on the viability of these neuronal cells are studied to confirm potency of the formulations.

30 Example 10. *In vivo* evaluation for nose to brain drug delivery – microdialysis

In vivo evaluation of vesicular system is be carried out in male Sprague–Dawley rats (250–300 g, Envigo, CA) under anesthesia [ketamine (80 mg/kg) and xylazine (10 mg/kg) (intraperitoneal injection)]. The rats are be divided into groups based on the type and/or duration of administration of the formulations: (i) solution (administered only once), (ii)
35 solution (administered daily once for 6 days based on *in vitro* studies) and (iii) vesicular

5 formulation (administered only once). Each of these group of rats is further divided into three subgroups ($n = 3$) each. To the rats of subgroup 1, vehicle control is administered. To the rats of subgroup 2, chitosan solution containing vesicular system of the optimized batch (at a dose of equivalent effective drug concentration) is administered via intranasal route. To the rats of subgroup 3 rats chitosan solution containing vesicular system at the same dose is
10 administered by subcutaneous injection. The formulations are administered intra-nasally (subgroups 1 & 2) by placing the anesthetized rats on their back and by inserting the soft polymer capillary connected to a micro-syringe into the posterior segment of the nose. After 6 days, the rats are anesthetized and the drug concentration in the brain are sampled by micro-dialysis. Briefly, the rats are secured on the stereotaxic frame (Harvard Instruments, Holliston, MA) and the probe (CMA 12) implanted into the hippocampal region (anterior-posterior = 5.6 mm, medio-lateral = 5 mm, dorso-ventral = 7 mm, from bregma). Initially, the micro-dialysis probes is equilibrated by perfusing KRB buffer at the rate of 2 $\mu\text{L}/\text{min}$ using a microinjection pump for a period of 1 hour with the aid of a microinjection pump. Following i.v. or intranasal administration of drug solution (10 mg in 100 μL), microdialysis
20 fractions are collected at 15, 30, 60, 90, 120, 180, 240, 300, and 360 min and quantified using LCMS/MS. In case of rats belonging to groups 3 and 4, blood samples are collected at 15, 30, 60, 90, 120, 180, 240, 300, and 360 min from cannulated rat (jugular vein) using heparinized capillary tubes. The microdialysis samples are collected and assayed for biomarkers and drug by sensitive analytical instrument like LCMS/MS and enzyme-linked
25 immunosorbent assay.

Calibration of microdialysis probe: Microdialysis probes (CMA 12) are calibrated *in vitro* by immersing the probes in dialysis media containing drug combinations (concentrations 25, 50, and 75 ng/mL). A syringe pump controller (BASi, West Lafayette, IN) is used to continuously perfuse the KRB buffer at a flow rate of 2.0 $\mu\text{L}/\text{min}$. The probes
30 are equilibrated for a period of 1 hour and samples collected at regular intervals for a period of 6 hours. The percentage recovery is calculated as the ratio of dialysate concentration to its concentration in the dialysis medium surrounding the probe.

Extraction of drug and analysis: Blank CSF/plasma is obtained from untreated rats and spiked with known concentrations of drug combinations and the calibration curve plotted
35 using data obtained from an analytical method, e.g., LCMS. The rat CSF/plasma samples containing drug is extracted initially by vortexing CSF/plasma diluted with a specific solvent. Next, extraction solvent is added to the mixture and vortexed for 30 min followed by

5 centrifugation at 4°C for a period of 10 min at 1000g. The bottom organic phase (5 mL) of the centrifuged product is collected and phosphate buffer (pH 7) added to back extract (30 min) the drug, which is then centrifuged at 4°C for a period of 10 min. The separated (back extracted) phase is quantified by LCMS/MS for drug content.

10 Example 11. *In vivo* toxicity and efficacy of combination therapy

Initial tolerability studies are performed in general in FVB mice using cohorts of five mice tested with various doses and schedules suggested by the PK studies. After 2 weeks of dosing, complete blood counts and chemical analysis are performed under the supervision of a trained professional Toxicologist. All organs are harvested and processed in parallel for
15 determining Hsp70 levels and fixed in formalin. Based on the chemical analyses, specific organ toxicities are evaluated by histopathology. Additional studies in nude mice having established orthotopic tumors are used to confirm the tolerability of selected regimens. In the same experiment, orthotopic tumors are assessed for pharmacodynamic effects of various treatment strategies on Hsp70 upregulation and client protein suppression (EGFR, PDGFR,
20 Akt and c-Met RTK) by immunohistochemistry. In addition, plasma and brain samples are processed at the end of the two week dosing regimens to determine the brain to plasma ratios of the administered agent. In this manner, one or two tolerable dosing regimens for each compound (i.e., (i) a chemotherapeutic agent, or (ii) an Hsp90 inhibitor, (iii) a combination of (i) and (ii), and (iv) a compound formed by linking (i) and (ii)) with optimal client protein
25 suppression for subsequent efficacy testing are identified.

The efficacy of two different dosing regimens for any given combination of agents (a chemotherapeutic agent and an Hsp90 inhibitor) is compared in a single orthotopic PDX (patient-derived tumor graft) model selected based on the relative *in vitro* efficacy. Mice with established orthotopic tumors are randomized into seven groups of 15 mice each and
30 treated with placebo or one of the two treatment regimens for each combination of agents. After two weeks of treatment, five mice in each group are euthanized by paraformaldehyde perfusion and brain tumors processed for immunohistochemistry for Hsp70 and relevant client proteins (EGFR, PDGFR, Akt, and c-Met RTK) to evaluate the extent of Hsp90 suppression. The remaining 10 mice are dosed until reaching a moribund state at which time
35 they are euthanized, their brains processed for paraffin embedding, and then sectioned to evaluate the tumor volume.

Using the above described procedure, combination of (i) TMZ and ganestepib and of (ii) TMZ and SNX-2112 are tested for their ability to bring about a reduction in tumor volume. The doses of TMZ, ganestepib, and SNX-2112 are prepared taking into consideration the maximum tolerated dose of 75mg/m²/day, 200mg/m²/day, and 100mg/m²/day. A reduction in tumor volume is observed using each of these combinations together with improvement in overall survival (OS) and progression-free survival (PFS).

Statistical analysis: *In vivo* efficacy is evaluated by the log-rank test and survival results presented graphically using Kaplan-Meier plots.

REFERENCES

1. Lee CY. Strategies of temozolomide in future glioblastoma treatment (2017). *Onco Targets Ther.* **9**(10):265-270. [PMID: 28123308]
2. http://www.roche.com/backgrounder_glioblastoma_concise_guide.pdf: Glioblastoma (cancer affecting the brain) A guide for journalists on glioblastoma and its treatment.
3. Ferlay J, Shin HR, Bray F, et al.: GLOBOCAN 2008: Cancer Incidence and Mortality Worldwide in 2008. Lyon, France: IARC CancerBase No. 10.
4. Kohler BA, Sherman RL, Howlader N, Jemal A, Ryerson AB, Henry KA, Boscoe FP, Cronin KA, Lake A, Noone AM, Henley SJ, Ehemann CR, Anderson RN, Penberthy L. Annual Report to the Nation on the Status of Cancer, 1975-2011, Featuring Incidence of Breast Cancer Subtypes by Race/Ethnicity, Poverty, and State (2015). *J Natl Cancer Inst.* **107**(6). [PMID: 25825511]
5. Rich JN, Bigner DD. Development of novel targeted therapies in the treatment of malignant glioma (2004). *Nat Rev Drug Discov.* **3**(5):430-446. [PMID: 15136790]
6. Yung WK, Albright RE, Olson J, Fredericks R, Fink K, Prados MD, Brada M, Spence A, Hohl RJ, Shapiro W, Glantz M, Greenberg H, Selker RG, Vick NA, Rampling R, Friedman H, Phillips P, Bruner J, Yue N, Osoba D, Zaknoen S, Levin VA. A phase II study of temozolomide vs. procarbazine in patients with glioblastoma multiforme at first relapse (2000). *Br J Cancer.* **83**(5):588-593. [PMID: 10944597]
7. Gilbert MR, Dignam JJ, Armstrong TS, Wefel JS, Blumenthal DT, Vogelbaum MA, Colman H, Chakravarti A, Pugh S, Won M, Jeraj R, Brown PD, Jaeckle KA, Schiff D, Stieber VW, Brachman DG, Werner-Wasik M, Tremont-Lukats IW, Sulman EP, Aldape KD, Curran WJ Jr, Mehta MP. A randomized trial of bevacizumab for newly diagnosed glioblastoma (2014). *N Engl J Med.* **370**(8):699-708. [PMID: 24552317]
8. http://www.delmarpharma.com/DMPI_SCIR-2014-11-19.pdf

- 5 9. Cancer Genome Atlas Research Network. Comprehensive genomic characterization defines human glioblastoma genes and core pathways (2008). *Nature*. **455**(7216):1061-1068. [PMID: 18772890]
- 10 10. Misra A, Pellarin M, Nigro J, Smirnov I, Moore D, Lamborn KR, Pinkel D, Albertson DG, Feuerstein BG. Array comparative genomic hybridization identifies genetic subgroups in grade 4 human astrocytoma (2005). *Clin Cancer Res*. **11**(8):2907-2918. [PMID: 15837741]
- 15 11. Parsons DW, Jones S, Zhang X, Lin JC, Leary RJ, Angenendt P, Mankoo P, Carter H, Siu IM, Gallia GL, Olivi A, McLendon R, Rasheed BA, Keir S, Nikolskaya T, Nikolsky Y, Busam DA, Tekleab H, Diaz LA Jr, Hartigan J, Smith DR, Strausberg RL, Marie SK, Shinjo SM, Yan H, Riggins GJ, Bigner DD, Karchin R, Papadopoulos N, Parmigiani G, Vogelstein B, Velculescu VE, Kinzler KW. An integrated genomic analysis of human glioblastoma multiforme (2008). *Science* **321**(5897):1807-1812. [PMID: 18772396]
- 20 12. Kotliarov Y, Steed ME, Christopher N, Walling J, Su Q, Center A, Heiss J, Rosenblum M, Mikkelsen T, Zenklusen JC, Fine HA. High-resolution global genomic survey of 178 gliomas reveals novel regions of copy number alteration and allelic imbalances (2006). *Cancer Res*. **66**(19):9428-9436. [PMID: 17018597]
- 25 13. Patel M, Vogelbaum MA, Barnett GH, Jalali R, Ahluwalia MS. Molecular targeted therapy in recurrent glioblastoma: current challenges and future directions (2012). *Expert Opin Investig Drugs*. **21**(9):1247-66. [PMID: 22731981]
- 30 14. Haar CP, Hebbar P, Wallace GC 4th, Das A, Vandergrift WA 3rd, Smith JA, Giglio P, Patel SJ, Ray SK, Banik NL. Drug resistance in glioblastoma: a mini review (2012). *Neurochem Res*. **37**(6):1192-1200. [PMID: 22228201]
- 35 15. Kouri FM, Jensen SA, Stegh AH. The role of Bcl-2 family proteins in therapy responses of malignant astrocytic gliomas: Bcl2L12 and beyond (2012). *ScientificWorldJournal*. 838916. [PMID: 22431925]
- 40 16. Hermisson M, Strik H, Rieger J, Dichgans J, Meyermann R, Weller M. Expression and functional activity of heat shock proteins in human glioblastoma multiforme (2000). *Neurology* **54**(6):1357-1365. [PMID: 10746610]
- 45 17. Alexiou GA, Karamoutsios A, Lallas G, Ragos V, Goussia A, Kyritsis AP, Voulgaris S, Vartholomatos G. Expression of heat shock proteins in brain tumors (2014). *Turk Neurosurg*. **24**(5):745-749. [PMID: 25269047]
- 50 18. Sauvageot CM1, Weatherbee JL, Kesari S, Winters SE, Barnes J, Dellagatta J, Ramakrishna NR, Stiles CD, Kung AL, Kieran MW, Wen PY. Efficacy of the HSP90 inhibitor 17-AAG in human glioma cell lines and tumorigenic glioma stem cells (2009). *Neuro Oncol*. **11**(2):109-121. [PMID: 18682579]
19. Wu J, Liu Y, Cho K, Dong X, Teng L, Han D, Liu H, Chen X, Chen X, Hou X, Peng F, Bi Y, Shen C, Zhao S. Downregulation of TRAP1 sensitizes glioblastoma cells to

- 5 temozolomide chemotherapy through regulating metabolic reprogramming (2016). *Neuroreport*. **27**(3):136-144. [PMID: 26716385]
20. Siegelin MD, Plescia J, Raskett CM, Gilbert CA, Ross AH, Altieri DC. Global targeting
of subcellular heat shock protein-90 networks for therapy of glioblastoma (2010). *Mol*
10 *Cancer Ther*. **9**(6):1638-1646. [PMID: 20501802]
21. Li S, Lv Q, Sun H, Xue Y, Wang P, Liu L, Li Z, Li Z, Tian X, Liu YH. Expression of
TRAP1 Predicts Poor Survival of Malignant Glioma Patients (2015). *J Mol Neurosci*.
15 **55**(1):62-68. [PMID: 25189320]
22. Echeverría PC, Bernthaler A, Dupuis P, Mayer B, Picard D. An interaction network
predicted from public data as a discovery tool: application to the Hsp90 molecular
chaperone machine (2011). *PLoS One*. **6**(10):e26044. [PMID: 22022502]
23. Altieri DC, Stein GS, Lian JB, Languino LR. TRAP-1, the mitochondrial Hsp90 (2012).
20 *Biochim Biophys Acta*. **1823**(3):767-773. [PMID: 21878357]
24. Kang BH, Altieri DC. Compartmentalized cancer drug discovery targeting
mitochondrial Hsp90 chaperones (2009). *Oncogene*. **28**(42):3681-3688. [PMID:
25 19648961]
25. Siegelin MD. Inhibition of the mitochondrial Hsp90 chaperone network: a novel,
efficient treatment strategy for cancer? (2013). *Cancer Lett*. **333**(2):133-146. [PMID:
30 23376257]
26. Gewirth DT. Paralog Specific Hsp90 Inhibitors - A Brief History and a Bright Future
(2016). *Curr Top Med Chem*. **16**(25):2779-2791. [PMID: 27072700]
27. Jhaveri K, Modi S. Ganetespib: research and clinical development (2015). *Onco*
35 *Targets Ther*. **8**:1849-1858. [PMID: 26244021]
28. Powers MV, Workman P. Inhibitors of the heat shock response: biology and
pharmacology (2007). *FEBS Lett*. **581**(19):3758-3769. [PMID: 17559840]
29. van Ommeren R, Staudt MD1, Xu H1, Hebb MO. Advances in HSP27 and HSP90-
targeting strategies for glioblastoma (2016). *J Neurooncol*. **127**(2):209-219. [PMID:
40 26842818]
30. Dungey FA, Caldecott KW, Chalmers AJ. Enhanced radiosensitization of human
glioma cells by combining inhibition of poly(ADP-ribose) polymerase with inhibition
45 of heat shock protein 90 (2009). *Mol Cancer Ther*. **8**(8):2243-2254. [PMID: 19671736]
31. Wachsberger PR, Lawrence YR, Liu Y, Rice B, Feo N, Leiby B, Dicker AP. Hsp90
inhibition enhances PI-3 kinase inhibition and radiosensitivity in glioblastoma (2014).
50 *J Cancer Res Clin Oncol*. **140**(4):573-582. [PMID: 24500492]
32. Choi EJ, Cho BJ, Lee DJ, Hwang YH, Chun SH, Kim HH, Kim IA. Enhanced cytotoxic
effect of radiation and temozolomide in malignant glioma cells: targeting PI3K-AKT-

- 5 mTOR signaling, HSP90 and histone deacetylases (2014). *BMC Cancer*. **13**(14):17. [PMID: 24418474]
33. Zhu H, Woolfenden S, Bronson RT, Jaffer ZM, Barluenga S, Winssinger N, Rubenstein AE, Chen R, Charest A. The novel Hsp90 inhibitor NXD30001 induces tumor
10 regression in a genetically engineered mouse model of glioblastoma multiforme (2010). *Mol Cancer Ther*. **9**(9):2618-2626. [PMID: 20643786]
34. Neckers L, Workman P. Hsp90 molecular chaperone inhibitors: are we there yet? (2012). *Clin Cancer Res*. **218**(1):64-76. [PMID: 22215907]
35. Sydor JR, Normant E, Pien CS, Porter JR, Ge J, Grenier L, Pak RH, Ali JA, Dembski MS, Hudak J, Patterson J, Penders C, Pink M, Read MA, Sang J, Woodward C, Zhang Y, Grayzel DS, Wright J, Barrett JA, Palombella VJ, Adams J, Tong JK. Development
20 of 17-allylamino-17-demethoxygeldanamycin hydroquinone hydrochloride (IPI-504), an anti-cancer agent directed against Hsp90 (2006). *Proc Natl Acad Sci U S A*. **103**(46):17408-17413. [PMID: 17090671]
36. Gaspar N, Sharp SY, Eccles SA, Gowan S, Popov S, Jones C, Pearson A, Vassal G, Workman P. Mechanistic evaluation of the novel HSP90 inhibitor NVP-AUY922 in
25 adult and pediatric glioblastoma (2010). *Mol Cancer Ther*. **9**(5):1219-1233. [PMID: 20457619]
37. Jhaveri K, Taldone T, Modi S, Chiosis G. Advances in the clinical development of heat shock protein 90 (Hsp90) inhibitors in cancers (2012). *Biochim Biophys Acta*.
30 **1823**(3):742-755. [PMID: 22062686]
38. Eccles SA, Massey A, Raynaud FI, Sharp SY, Box G, Valenti M, Patterson L, de Haven Brandon A, Gowan S, Boxall F, Aherne W, Rowlands M, Hayes A, Martins V, Urban F, Boxall K, Prodromou C, Pearl L, James K, Matthews TP, Cheung KM, Kalusa A, Jones K, McDonald E, Barril X, Brough PA, Cansfield JE, Dymock B, Drysdale MJ, Finch H, Howes R, Hubbard RE, Surgenor A, Webb P, Wood M, Wright L, Workman P. NVP-AUY922: a novel heat shock protein 90 inhibitor active against xenograft
35 tumor growth, angiogenesis, and metastasis (2008). *Cancer Res*. **68**(8):2850-2860. [PMID: 18413753]
39. Chae YC, Caino MC, Lisanti S, Ghosh JC, Dohi T, Danial NN, Villanueva J, Ferrero S, Vaira V, Santambrogio L, Bosari S, Languino LR, Herlyn M, Altieri DC. Control of
40 tumor bioenergetics and survival stress signaling by mitochondrial HSP90s (2012). *Cancer Cell*. **22**(3):331-344. [PMID: 22975376]
40. Chae YC, Angelin A, Lisanti S, Kossenkov AV, Speicher KD, Wang H, Powers JF, Tischler AS, Pacak K, Flidner S, Michalek RD, Karoly ED, Wallace DC, Languino LR, Speicher DW, Altieri DC. Landscape of the mitochondrial Hsp90 metabolome in
45 tumours (2013). *Nat Commun*. **4**:2139. [PMID: 23842546]
41. Siegelin MD, Dohi T, Raskett CM, Orlowski GM, Powers CM, Gilbert CA, Ross AH, Plescia J, Altieri DC. Exploiting the mitochondrial unfolded protein response for cancer
50 therapy in mice and human cells (2011). *J Clin Invest*. **121**(4):1349-1360. [PMID: 21364280]

- 5 42. Leav I, Plescia J, Goel HL, Li J, Jiang Z, Cohen RJ, Languino LR, Altieri DC. Cytoprotective mitochondrial chaperone TRAP-1 as a novel molecular target in localized and metastatic prostate cancer (2010). *Am J Pathol.* **176**(1):393-401. [PMID: 19948822]
- 10 43. Fels C, Schäfer C, Hüppe B, Bahn H, Heidecke V, Kramm CM, Lautenschläger C, Rainov NG. Bcl-2 expression in higher-grade human glioma: a clinical and experimental study (2000). *J Neurooncol.* **48**(3):207-216. [PMID: 11100818]

OTHER EMBODIMENTS

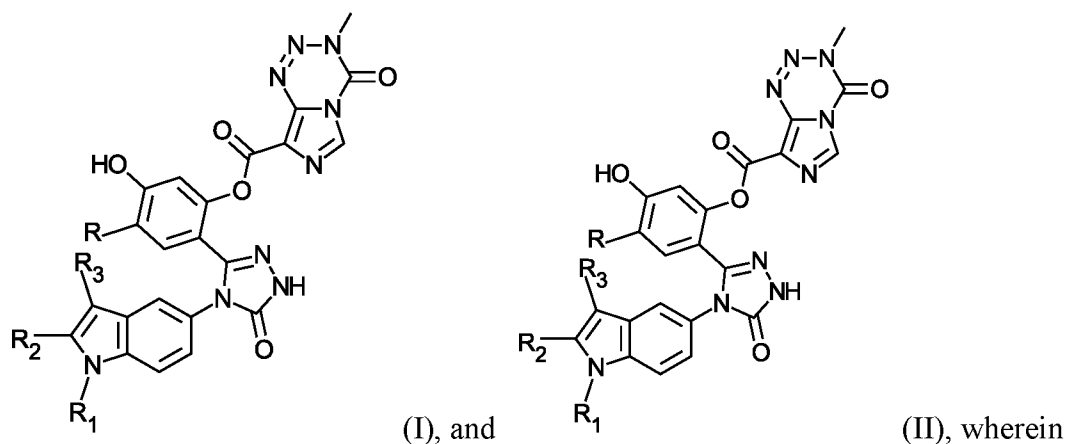
All of the features disclosed in this specification may be combined in any combination. Each feature disclosed in this specification may be replaced by an alternative feature serving the same, equivalent, or similar purpose. Thus, unless expressly stated
20 otherwise, each feature disclosed is only an example of a generic series of equivalent or similar features. From the above description, one skilled in the art can easily ascertain the essential characteristics of the present invention, and without departing from the spirit and scope thereof, can make various changes and modifications of the invention to adapt it to various usages and conditions. Thus, other embodiments are also within the scope of the
25 following claims

CLAIMS

What is claimed is:

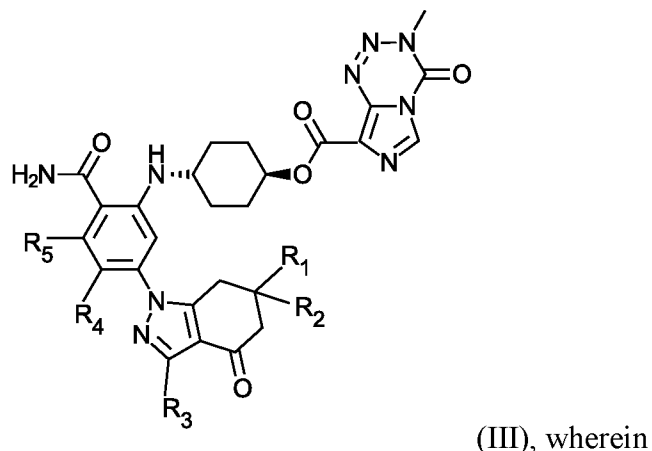
1. A method of treating cancer, comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof, wherein the Hsp90 inhibitor is capable of also inhibiting TRAP1, and wherein the conjugate is selected from the group consisting of

(i) compounds with ganetespib as the Hsp90 inhibitor, having the formula



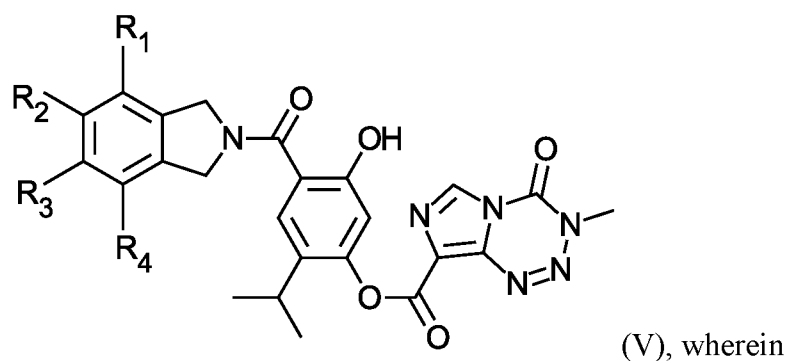
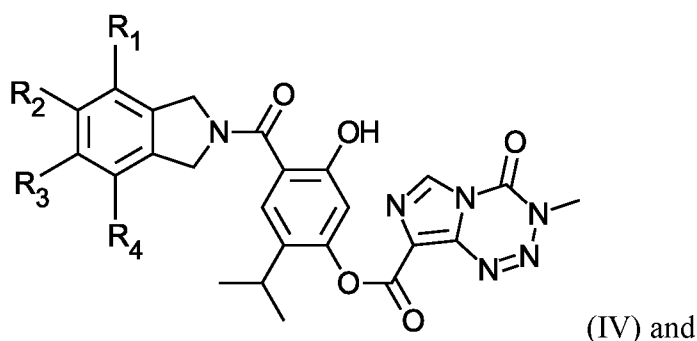
R, R₁, R₂, R₃, are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

(ii) compounds with SNX-2112 as the Hsp90 inhibitor, having the formula



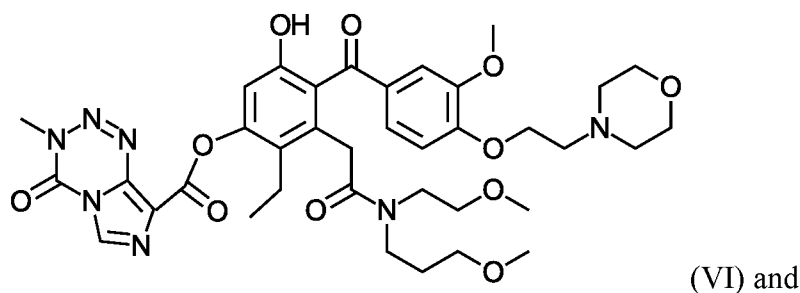
R₁, R₂, R₃, R₄ and R₅ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

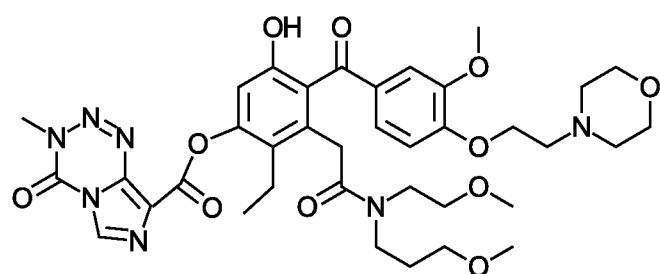
(iii) compounds with Onalespib as the Hsp90 inhibitor, having the formula



R₁, R₂, R₃, and R₄ are independently selected from the group consisting of hydrogen; halogen; (C₁-C₄)alkyl; halo(C₁-C₄)alkyl; (C₁-C₄)alkoxy; halo(C₁-C₄)alkoxy; (C₁-C₇)cycloalkyl; halo(C₁-C₇)cycloalkyl; (C₁-C₇)cycloalkyl(C₁-C₄)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms;

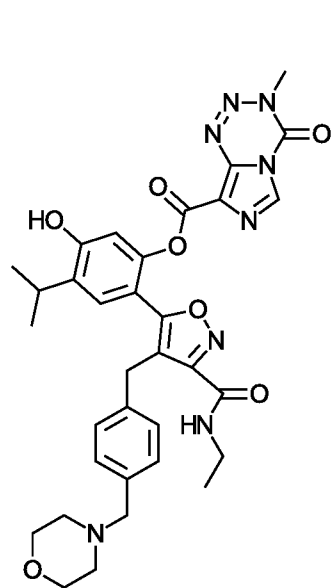
(iv) compounds with KW-2487 as the Hsp90 inhibitor having the formula



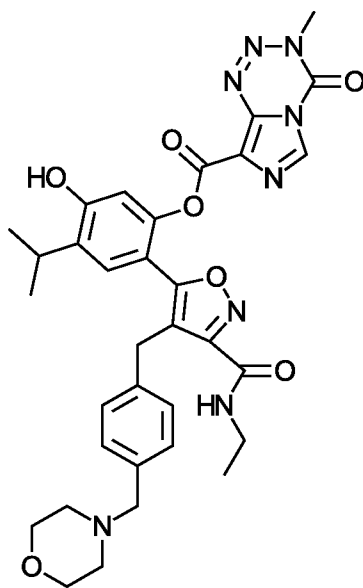


(VII);

(v) compounds with Luminespib as the Hsp90 inhibitor, having the formula

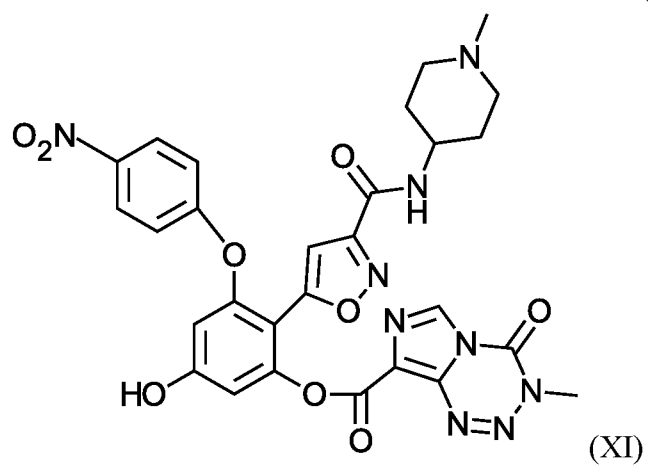
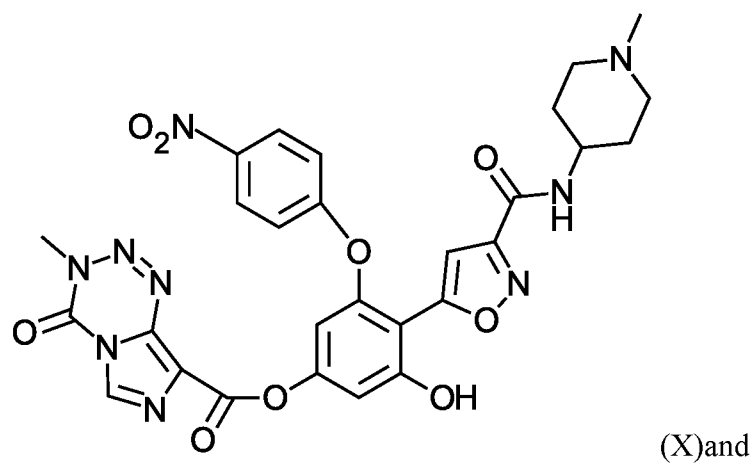


(VIII) and

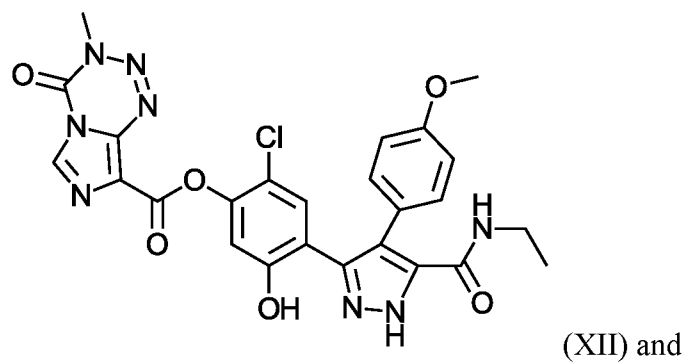


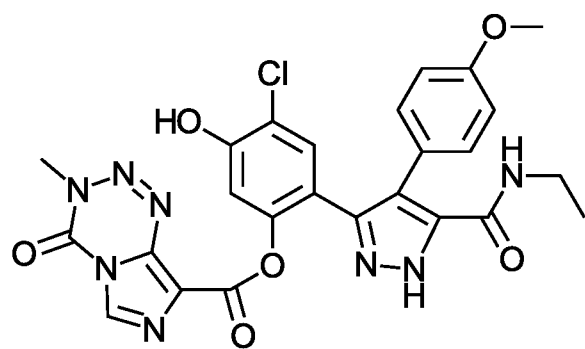
(IX);

(v) compounds with NMS-E973 as the Hsp90 inhibitor, having the formula



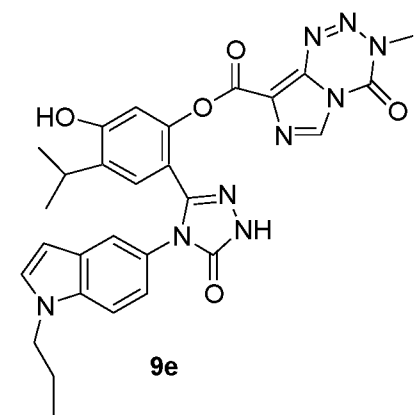
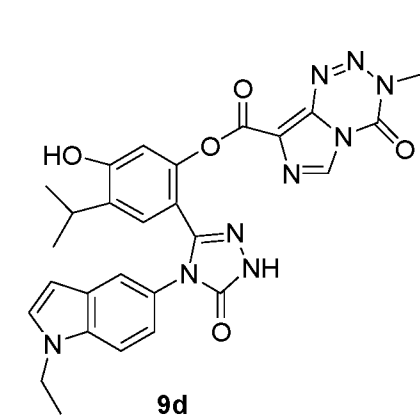
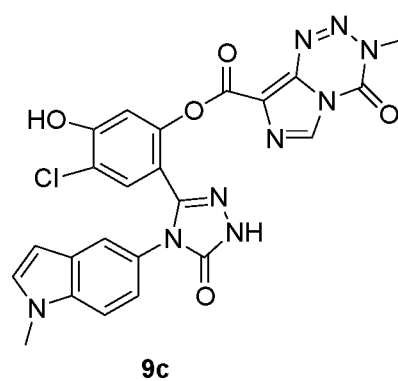
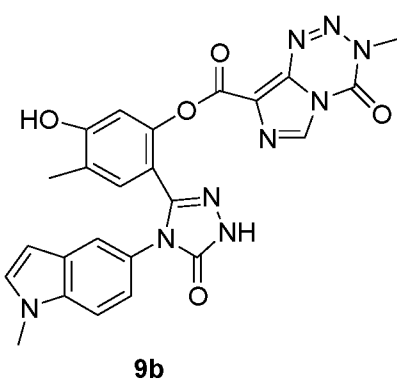
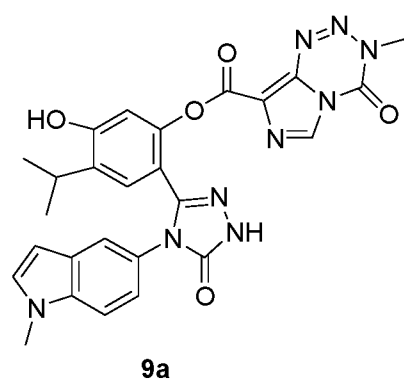
(v) compounds with VER-50589 as the Hsp90 inhibitor, having the formula

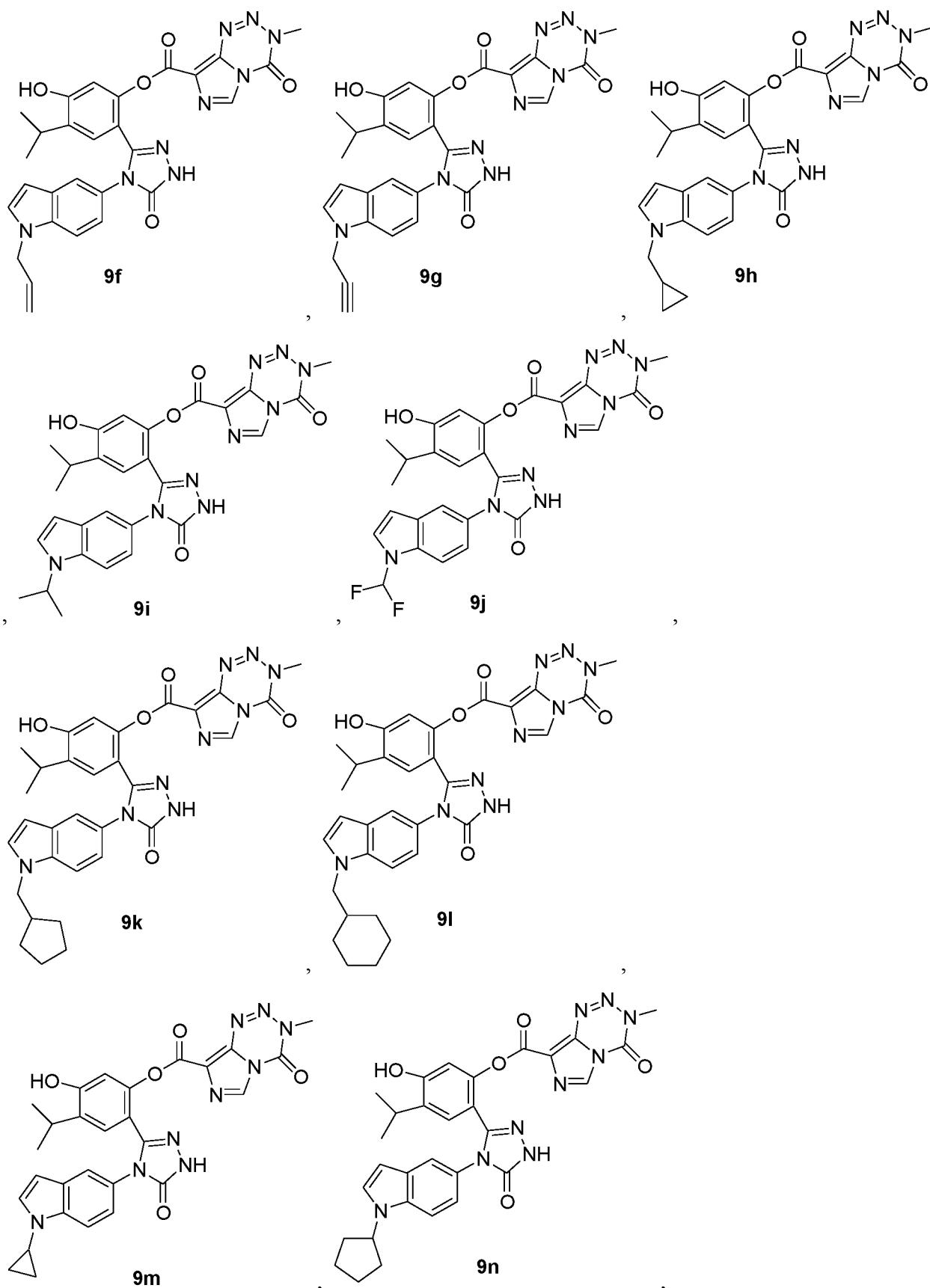


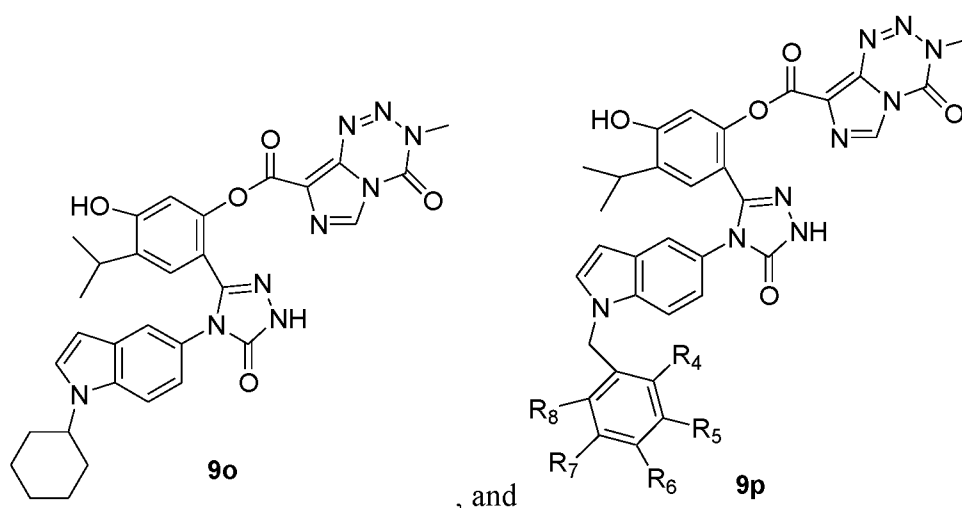


(XIII).

2. The method of claim 1, wherein the compounds having formula (I) are selected from the group consisting of



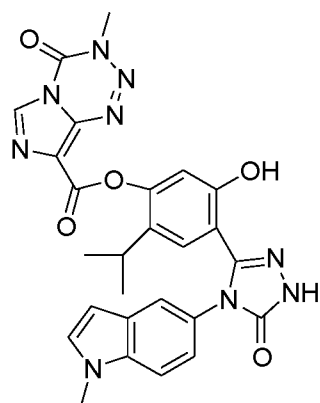




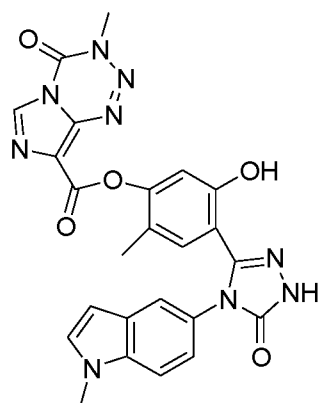
wherein R₄, R₅, R₆,

R₇, and R₈, are independently selected from the group consisting of hydrogen, lower alkyl, (C₁-C₄)alkoxy, (C₄-C₇)cyclopropyl, (C₂-C₆)alkenyl, and (C₂-C₆)alkynyl, each optionally substituted with one or more halogen atoms.

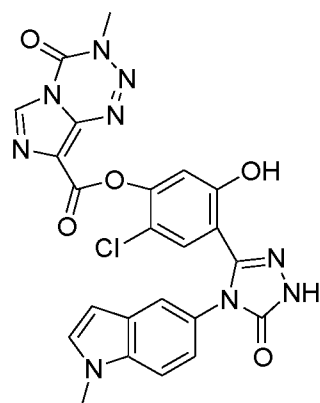
3. The method of claim 1, wherein the compounds having formula (II) are selected from the group consisting of



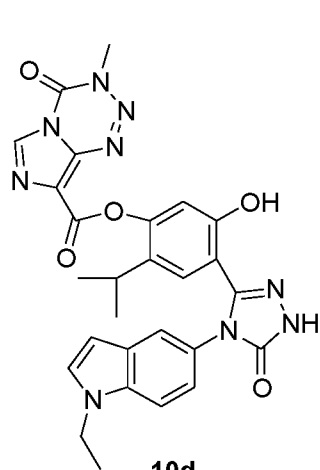
10a



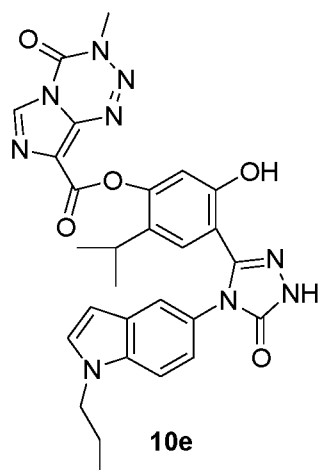
10b



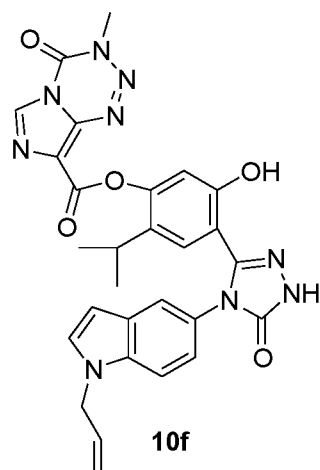
10c



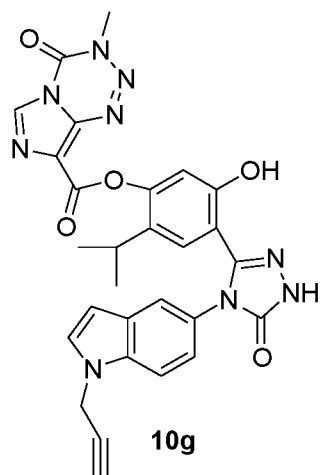
10d



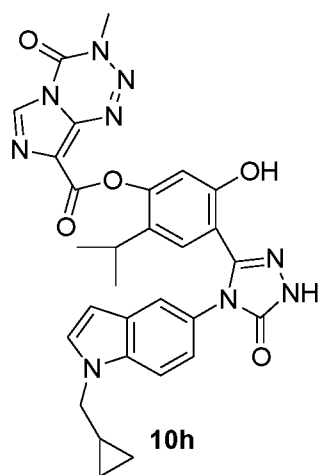
10e



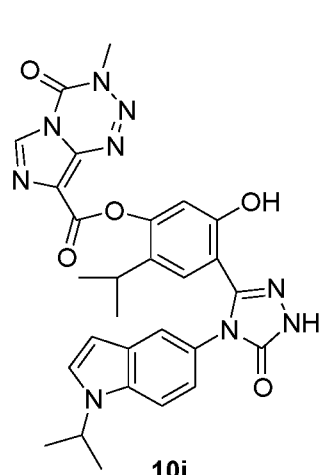
10f



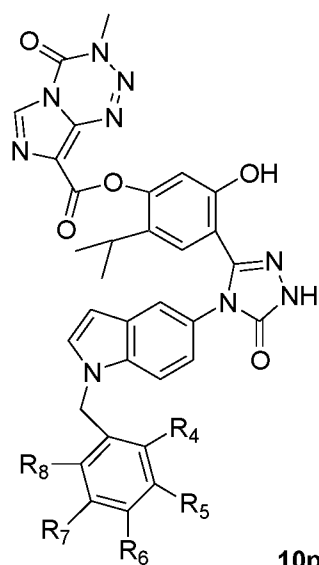
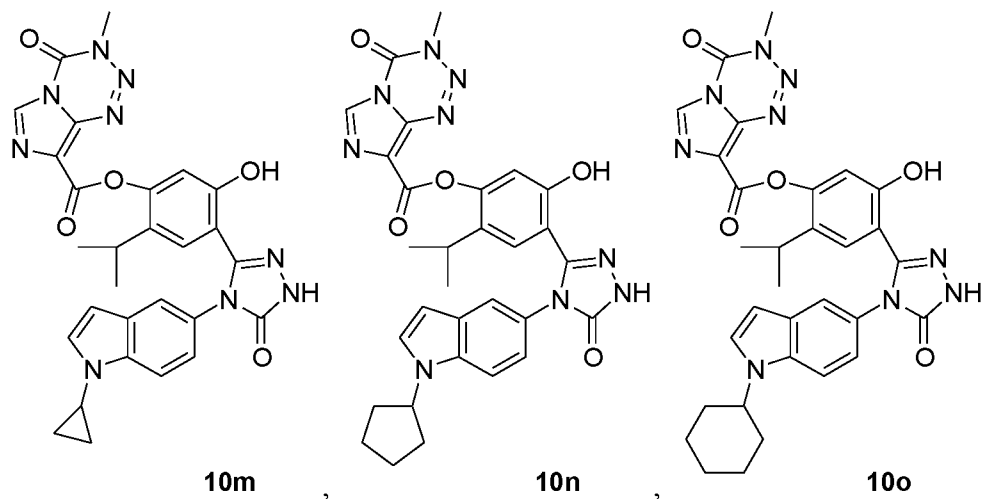
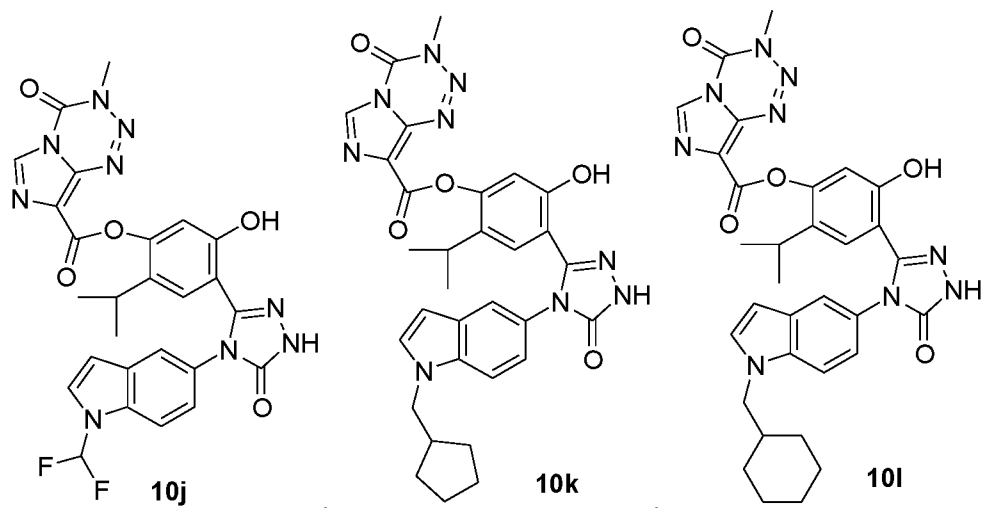
10g



10h



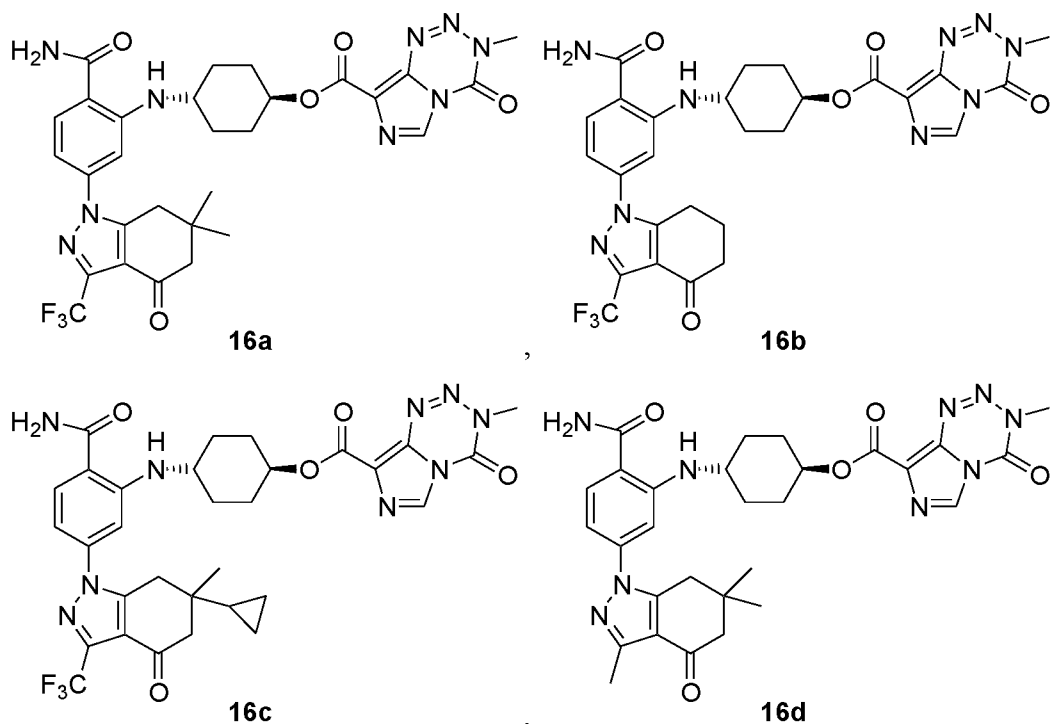
10i

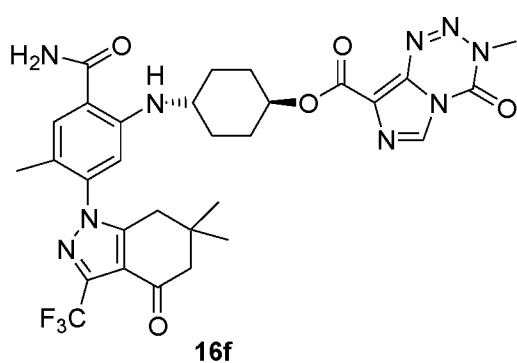
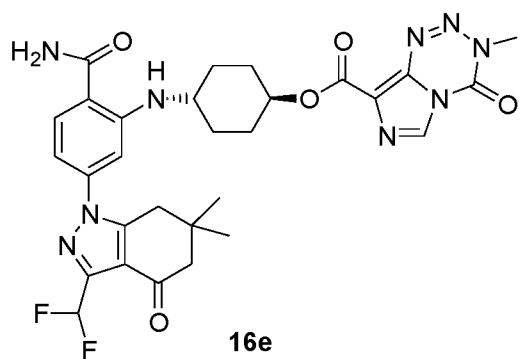


10p wherein R₄, R₅, R₆, R₇, and R₈, are independently selected from the

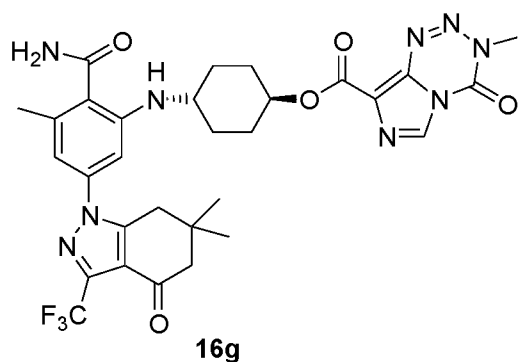
group consisting of hydrogen, lower alkyl, (C₁-C₄)alkoxy, (C₄-C₇)cyclopropyl, (C₂-C₆)alkenyl, and (C₂-C₆)alkynyl, each optionally substituted with one or more halogen atoms.

4. The method of claim 1, wherein the compounds having formula (III) are selected from the group consisting of

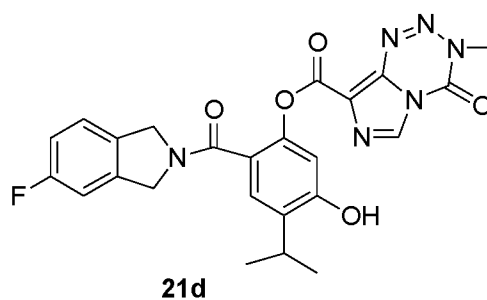
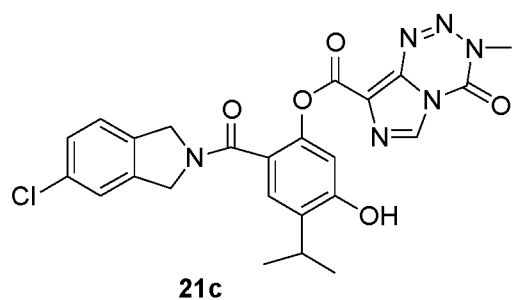
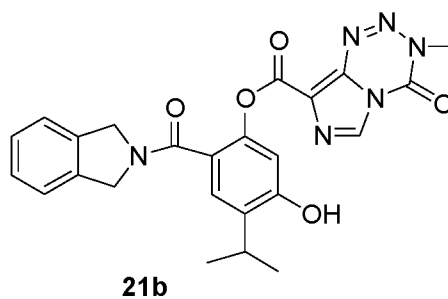
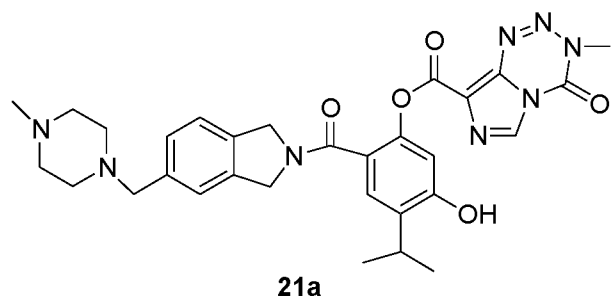


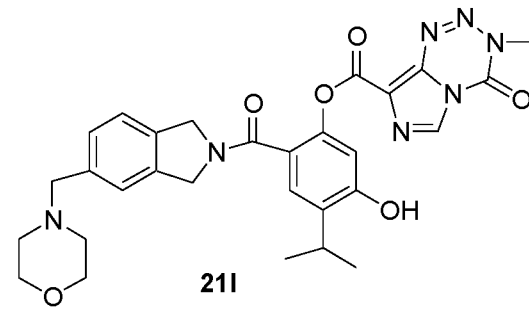
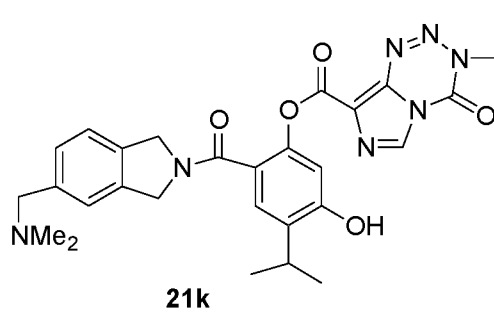
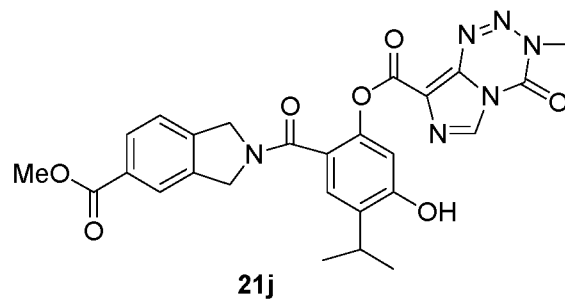
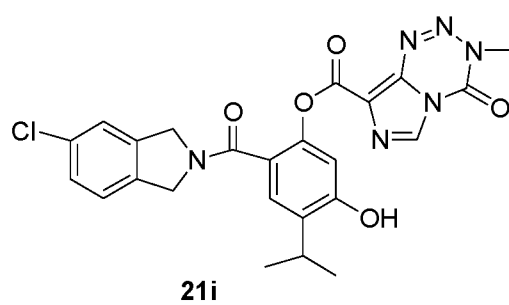
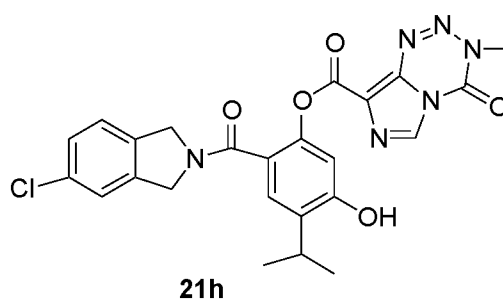
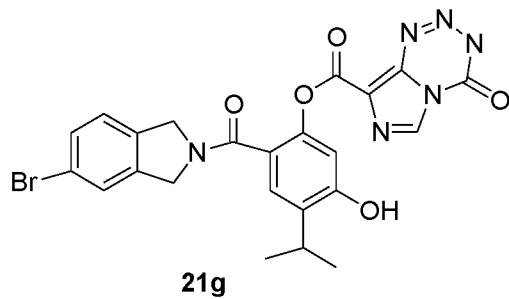
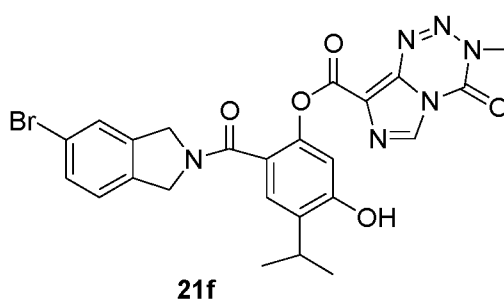
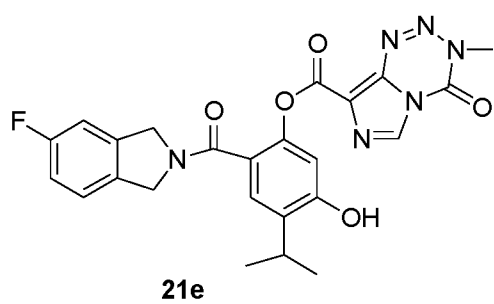


, and

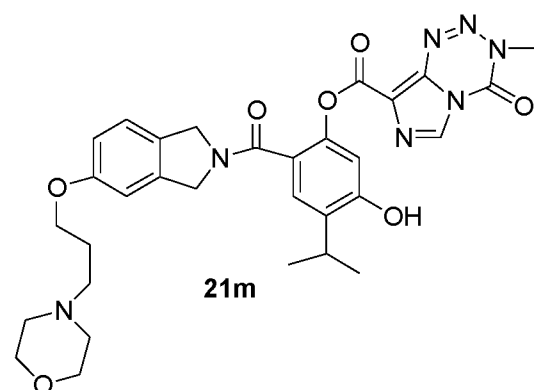


5. The method of claim 1, wherein the compounds having formula (IV) are selected from the group consisting of

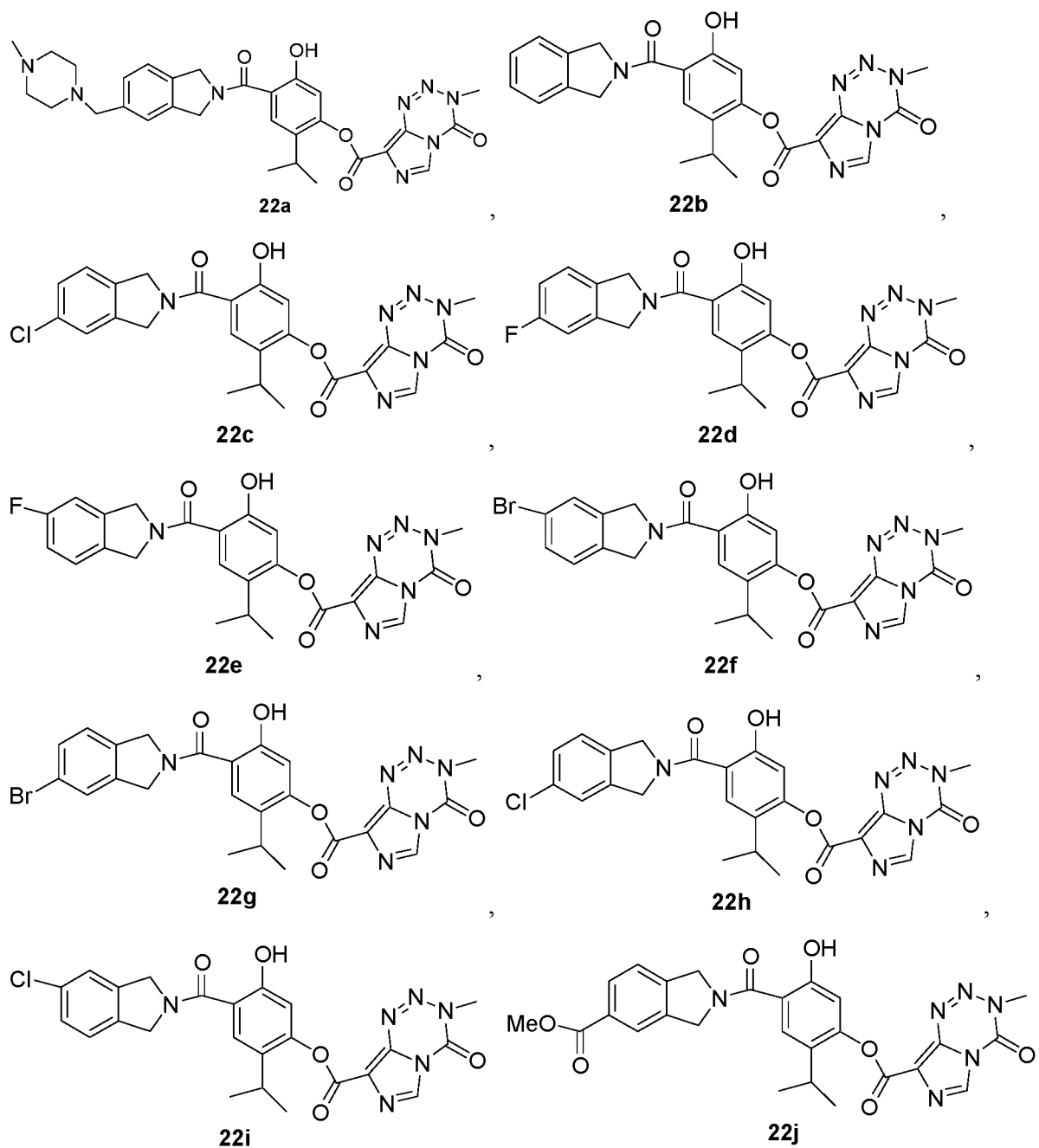


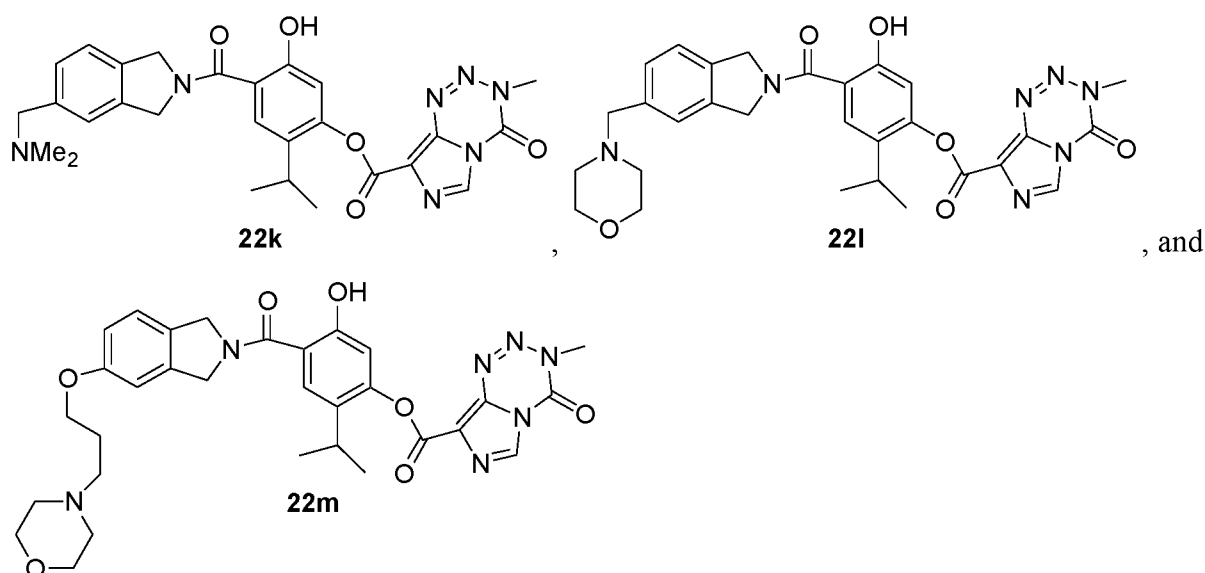


, and



6. The method of claim 1, wherein the compounds having formula (V) are selected from the group consisting of





7. The method of any of claims 1-6, wherein the cancer is glioblastoma, primary malignant brain tumor, or malignant glioma.
8. The method of any of claims 1-6, wherein the cancer is glioblastoma.
9. A pharmaceutical composition for nasal administration comprising a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90 inhibitor of any of claims 1-6; a lipid and a non-ionic surfactant; and an effective amount of an absorption promoting agent to allow nasal absorption of a pharmacologically effective amount of the conjugate.
10. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is a compound having formula (I) or (II).
11. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is a compound having formula (III).
12. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is a compound having formula (IV) or (V).

13. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is a compound having formula selected from (VI) to (XIII).
14. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is selected from one of the compounds of claim 2.
15. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is selected from one of the compounds of claim 3.
16. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is selected from one of the compounds of claim 4.
17. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is selected from one of the compounds of claim 5.
18. The pharmaceutical composition of claim 9, wherein the covalently-linked conjugate is selected from one of the compounds of claim 6.
19. The composition of claim 9, wherein the absorption promoting agent is a cationic polymer.
20. The composition of claim 19, wherein the cationic polymer is chitosan.
21. A pharmaceutical composition comprising a covalently-linked conjugate of temolozomide (TMZ) and an Hsp90 inhibitor of any of claims 1-6, or a pharmaceutically acceptable salt thereof, and a pharmaceutically acceptable carrier.

22. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is a compound having formula (I) or (II).
23. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is a compound having formula (III).
24. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is a compound having formula (IV) or (V).
25. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is a compound having formula selected from (VI) to (XIII).
26. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is selected from one of the compounds of claim 2.
27. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is selected from one of the compounds of claim 3.
28. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is selected from one of the compounds of claim 4.
29. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is selected from one of the compounds of claim 5.
30. The pharmaceutical composition of claim 21, wherein the covalently-linked conjugate is selected from one of the compounds of claim 6.

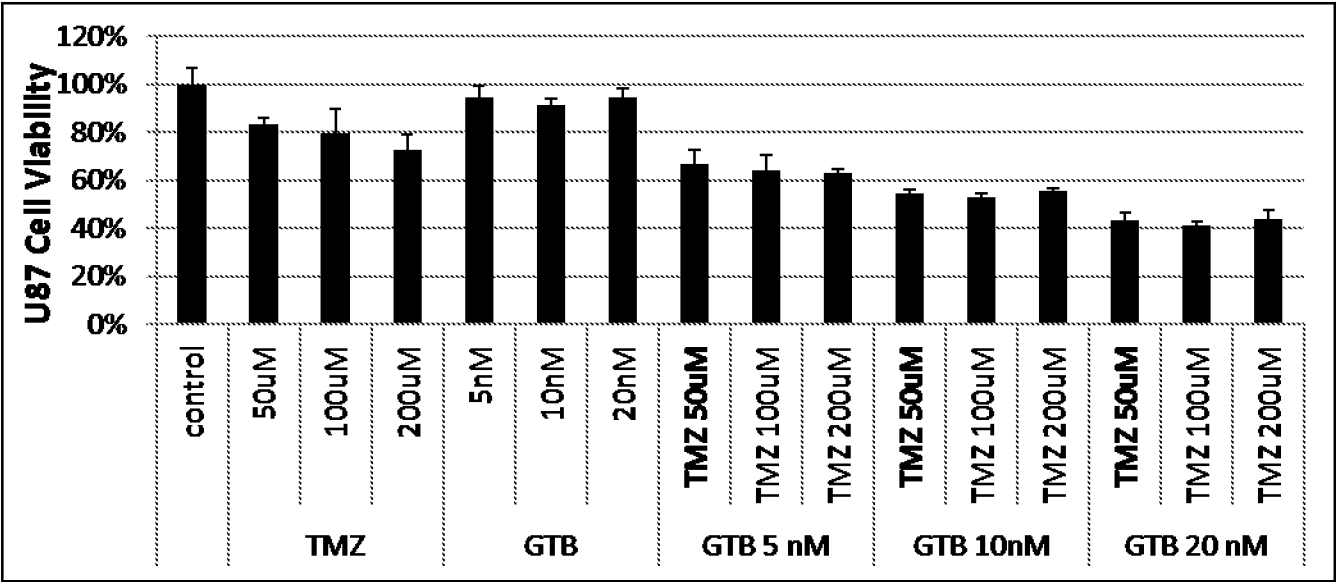


FIG. 1

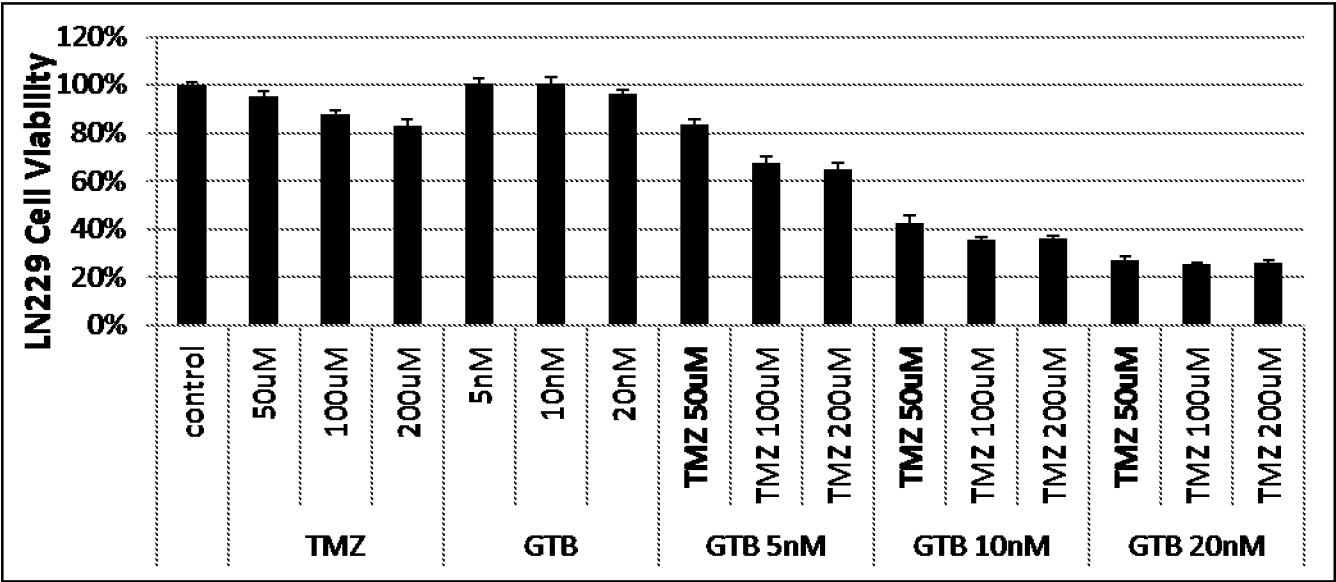


FIG. 2

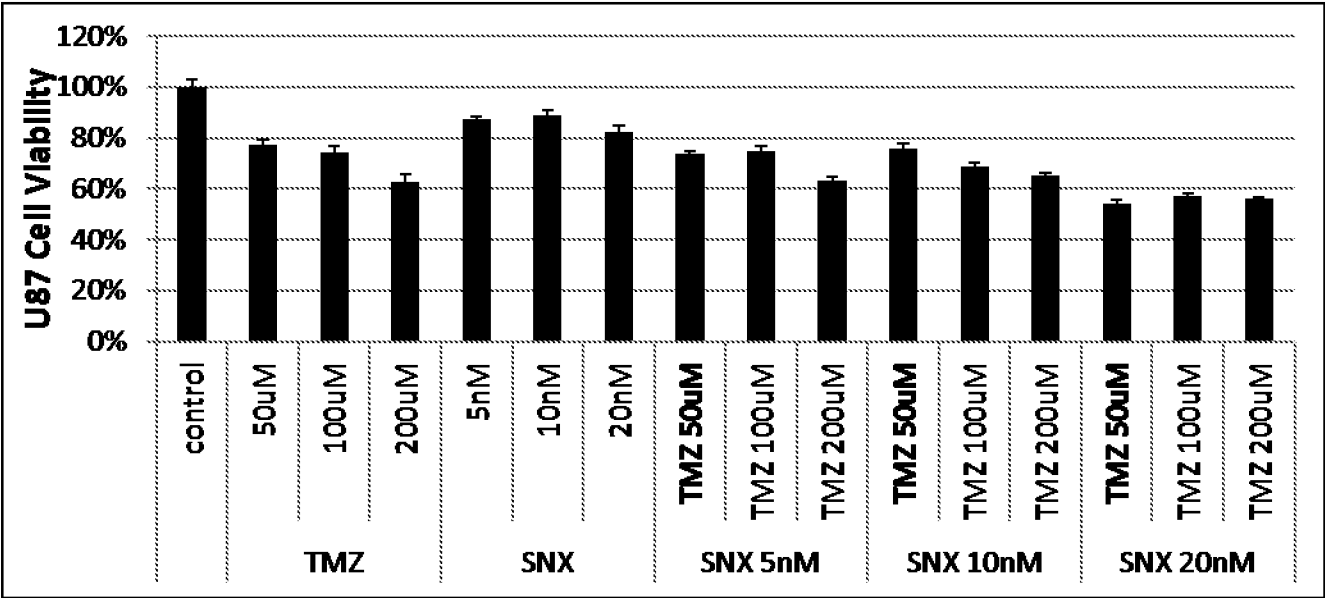


FIG. 3

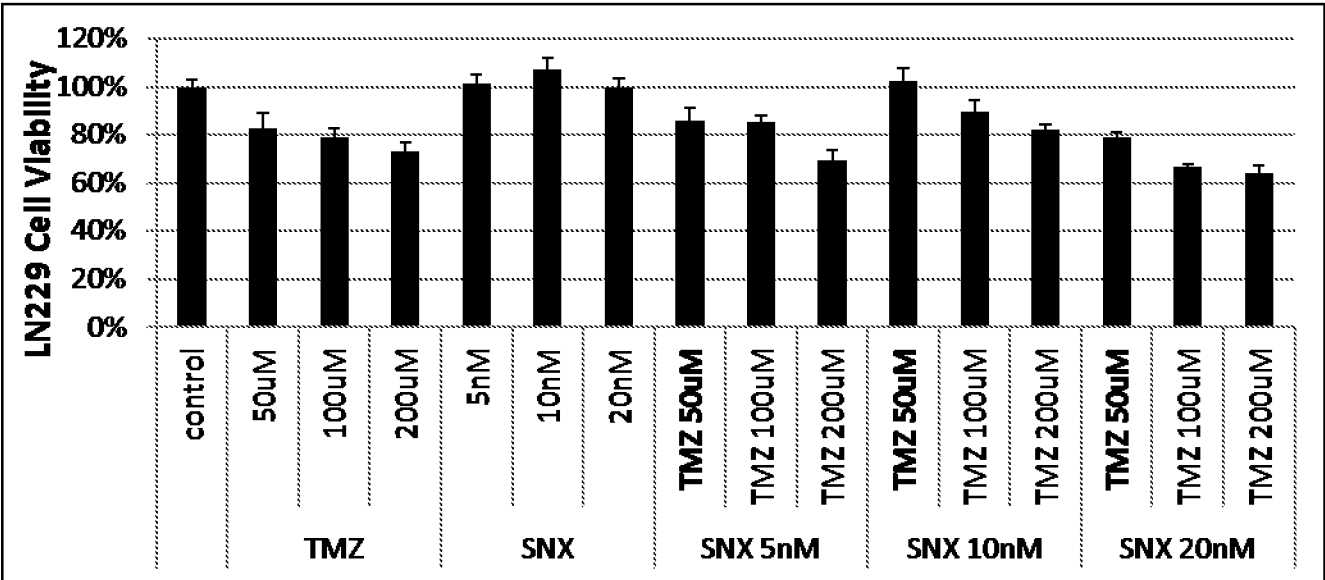


FIG. 4

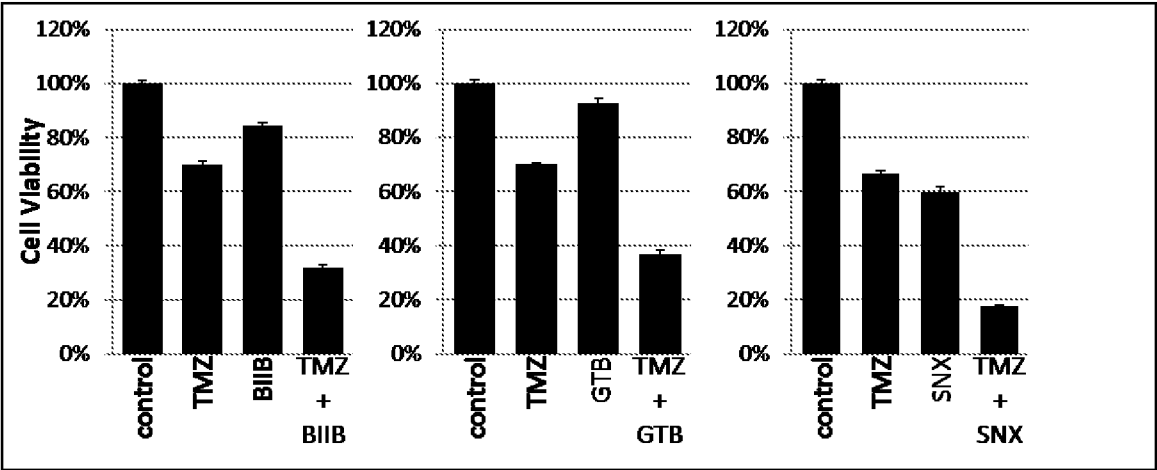


FIG. 5

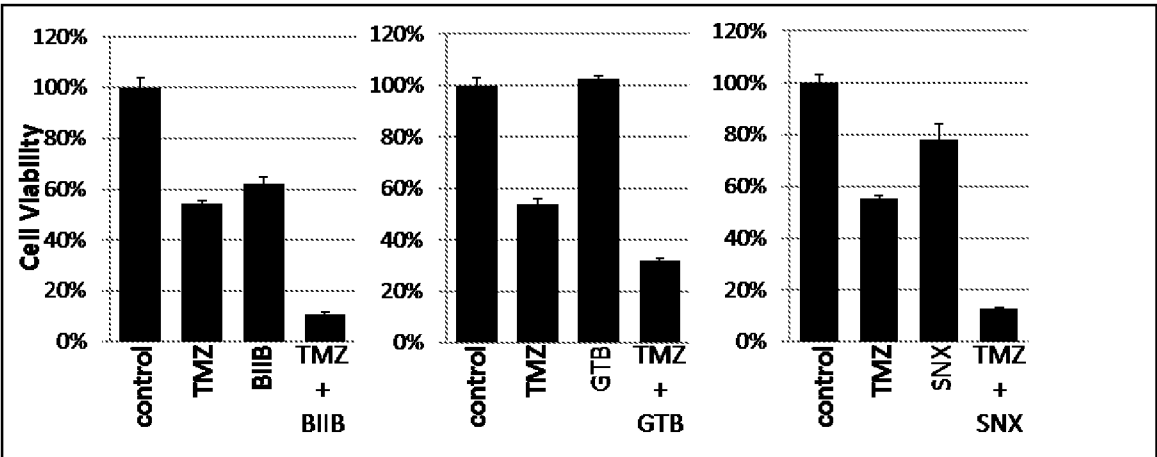


FIG. 6

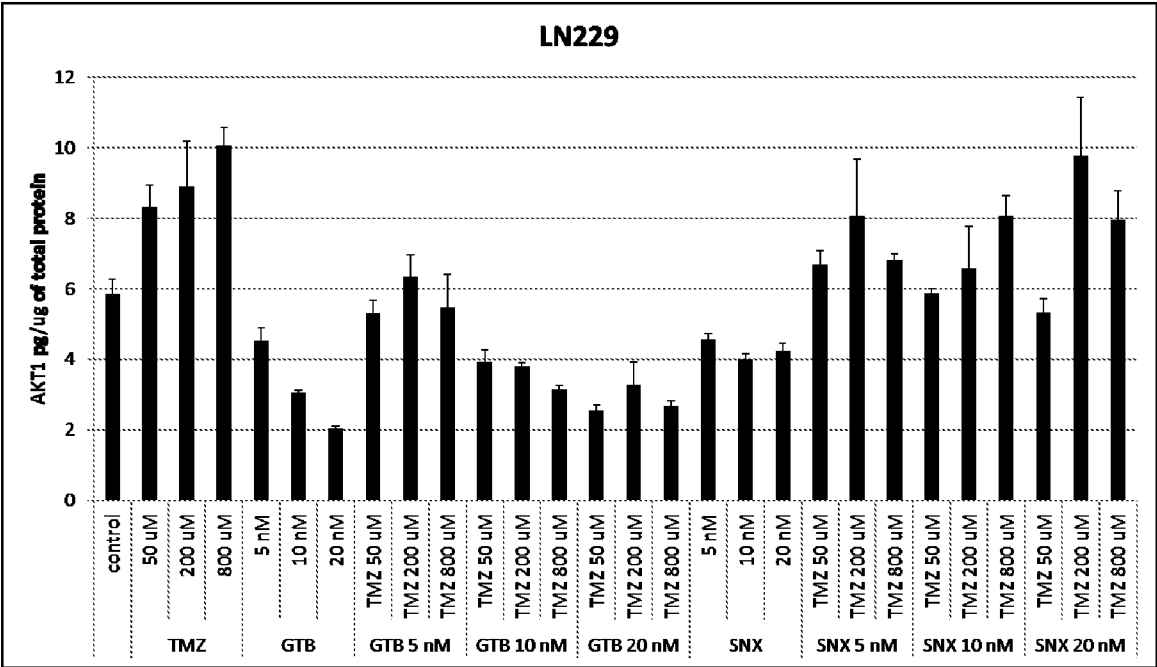


FIG. 7

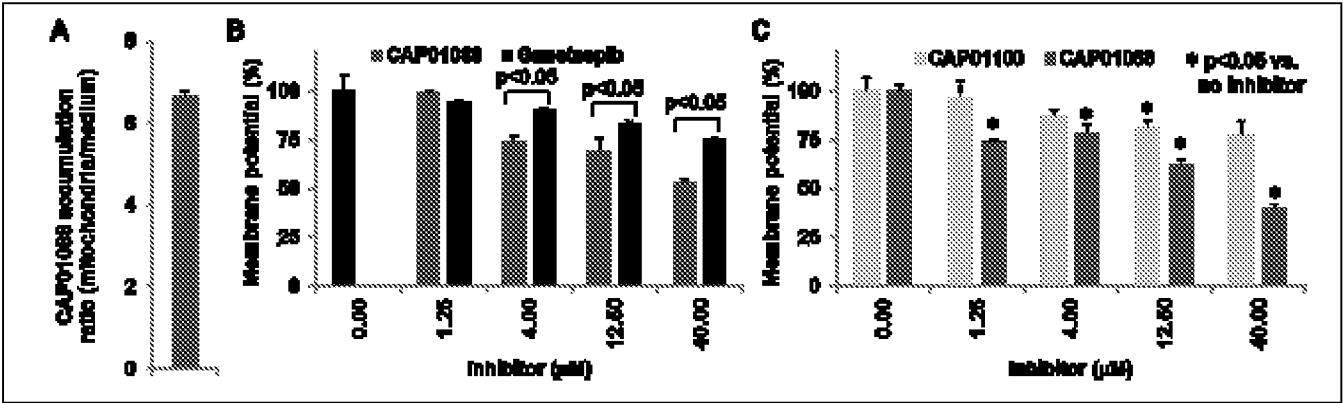


FIG. 8

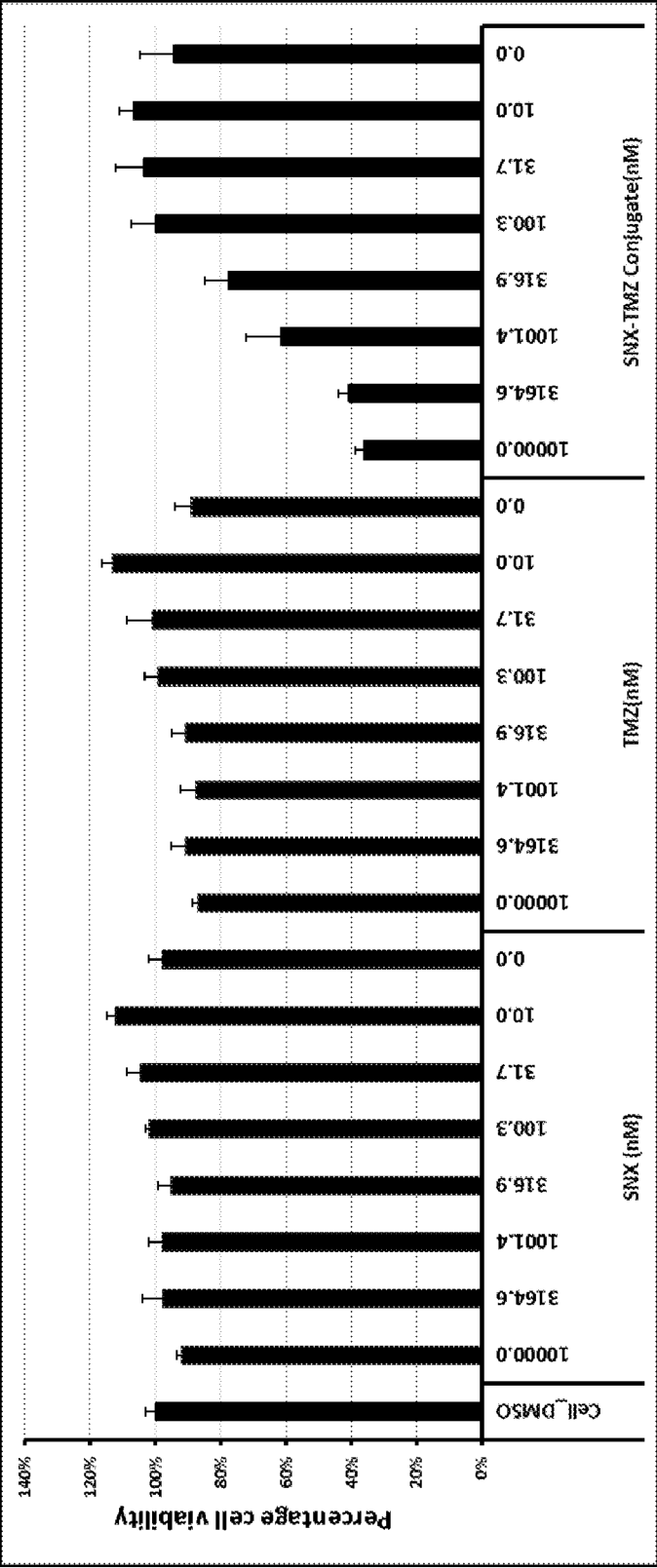


FIG. 9A

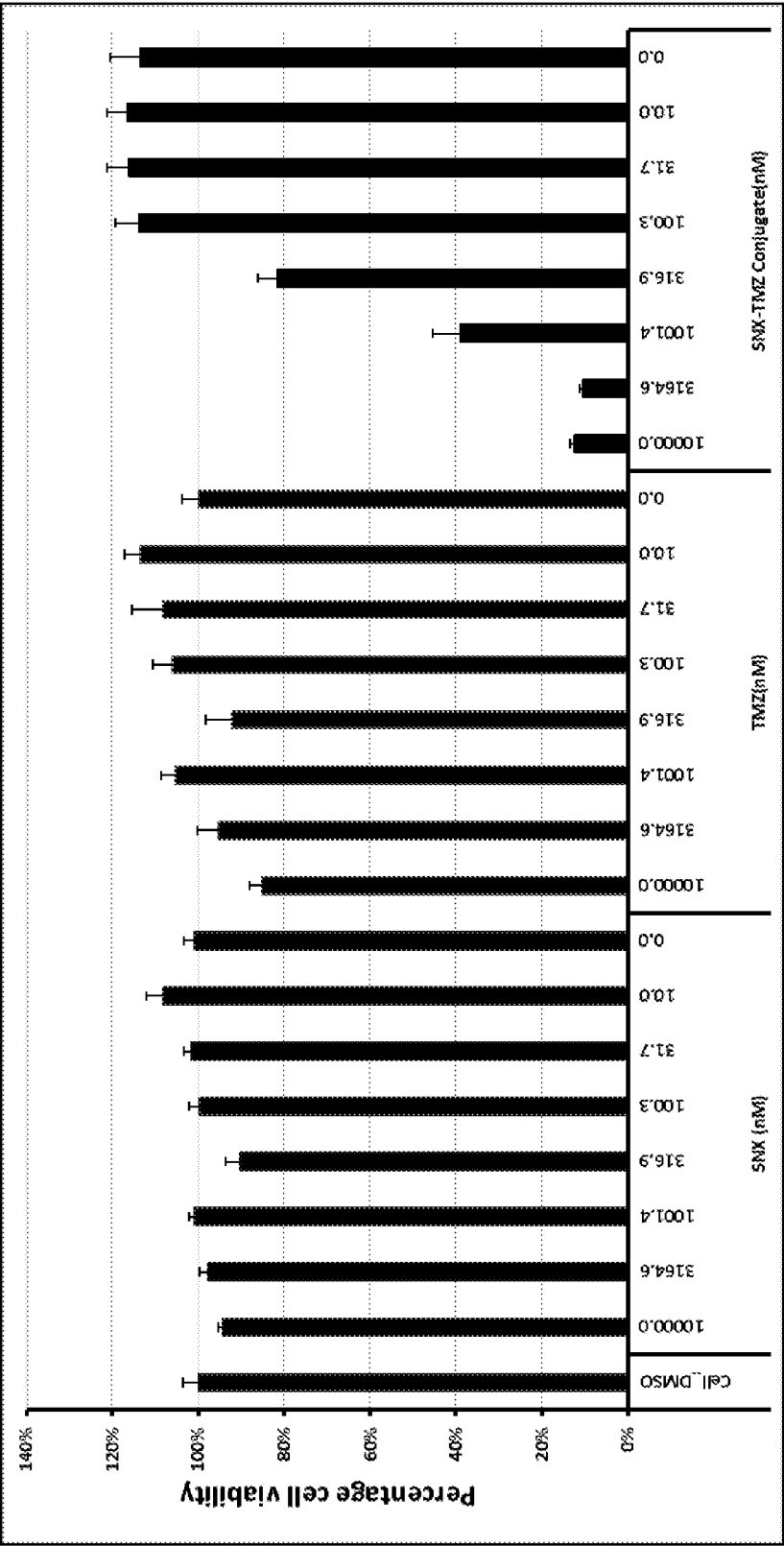


FIG. 9B

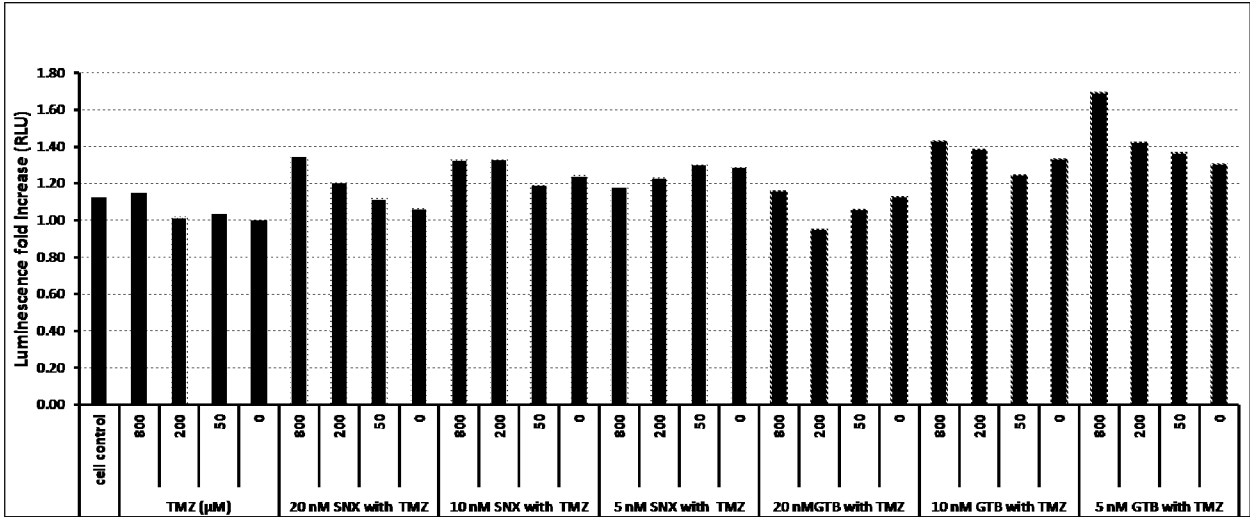


FIG. 10A

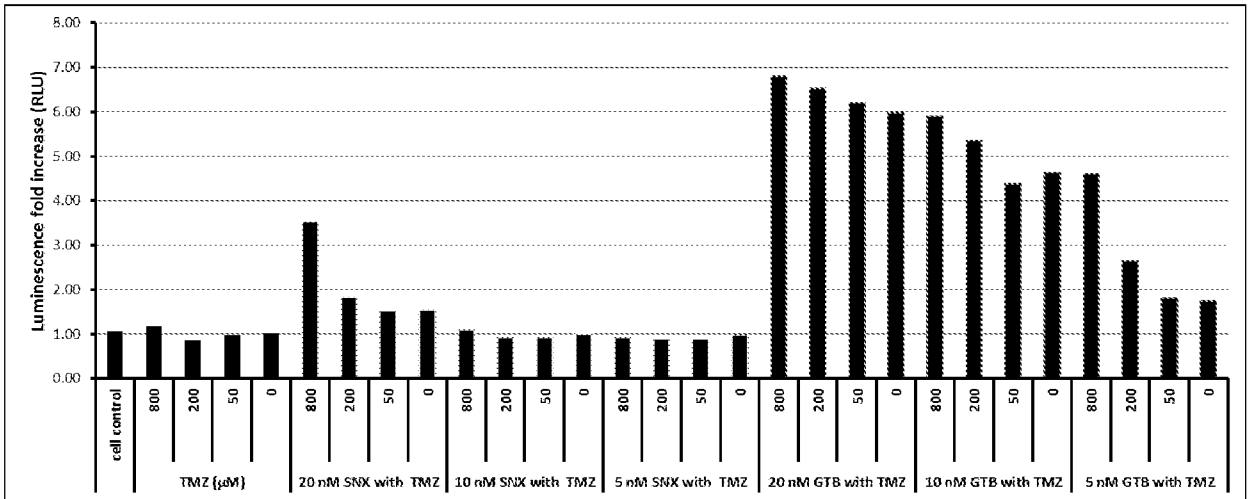


FIG. 10B

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/42099

A. CLASSIFICATION OF SUBJECT MATTER
 IPC - A61K 31/4545, 31/513, 31/70 (2018.01)
 CPC - A61K 31/4545, 31/513, 31/704

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2017/0128580 A1 (MADRIGAL PHARMACEUTICALS, INC.) 11 May 2017; abstract; figure 1; paragraphs [0006], [0031], [0034]-[0035], [0044], [0148], [0245]-[0246]	1, 7-10, 19-22
Y	US 2012/0328555 A1 (PATIL, R et al.) 27 December 2012; abstract; paragraphs [0003], [0005]-[0006], [0040]	1, 7, 9-10, 19-22
Y	US 2011/0250170 A1 (PEDRETTI, M et al.) 13 October 2011; abstract	1, 8
Y	US 2015/0202321 A1 (AHMAD, FJ et al.) 23 July 2015; paragraphs [0029], [0032], [0034], [0037], [0071], [0139], [0148]-[0149]	9-10, 19-20

☐ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

28 August 2018 (28.08.2018)

Date of mailing of the international search report

01 NOV 2018

Name and mailing address of the ISA/

Mail Stop PCT, Attn: ISA/US, Commissioner for Patents

P.O. Box 1450, Alexandria, Virginia 22313-1450

Facsimile No. 571-273-8300

Authorized officer

Shane Thomas

PCT Helpdesk: 571-272-4300

PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US18/42099

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☒ Claims Nos.: 14-18, 26-30
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

-Please See Within the Next Supplemental Box-

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1, 7-10, 19-22

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/US18/42099

***-Continued from Box No. III Observations where unity of invention is lacking ***-

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Groups I+, Claims 1-13 (in-part), 19-25 (in-part); compound that is a covalently-linked conjugate of temozolomide and ganetespib having the Formula I (first exemplary compound structure), and methods associated therewith.

The compound, compositions and methods will be searched to the extent the compound is a covalently-linked conjugate of temozolomide and ganetespib having the Formula I (first exemplary compound structure). Applicant is invited to elect additional compound(s), with fully specified structure (e.g. no optional or variable atoms or substituents) for each, to be searched. Additional compound(s) will be searched upon the payment of additional fees. It is believed that claims 1 (in-part), 7-10 (in-part), and 19-22 (in-part) encompass this first named invention and thus these claims will be searched without fee to the extent that they encompass a compound that is a covalently-linked conjugate of temozolomide and ganetespib having the Formula I (first exemplary compound structure). Applicants must specify the claims that encompass any additionally elected compound structure(s). Applicants must further indicate, if applicable, the claims which encompass the first named invention, if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be a compound that is a covalently-linked conjugate of temozolomide and SNX-2112 having the Formula III (first exemplary elected compound structure).

Groups I+ share the technical features including: a method of treating cancer, comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate of temozolomide (TMZ) and an Hsp90 inhibitor, or a pharmaceutically acceptable salt thereof, wherein the Hsp90 inhibitor is capable of also inhibiting TRAP1, and wherein the conjugate is a compound with ganetespib as the Hsp90 inhibitor, having the formula (I), as shown, wherein R, R1, R2, R3, are independently selected from the group consisting of hydrogen; halogen; (C1-C4)alkyl; halo(C1-C4)alkyl; (C1-C4)alkoxy; halo(C1-C4)alkoxy; (C1-C7)cycloalkyl; halo(C1-C7)cycloalkyl; (C1-C7)cycloalkyl(C1-C4)alkyl; and aryl and heteroaryl optionally substituted with lower alkyl or one or more halogen atoms.

However, these shared technical features are previously disclosed by US 2017/0128580 A1 (MADRIGAL PHARMACEUTICALS, INC.) (hereinafter 'Madrigal') in view of US 2012/0328555 A1 to Patil, et al. (hereinafter 'Patil').

Madrigal discloses a method of treating cancer (method of treating cancer; abstract), comprising administering to a subject in need thereof a composition comprising an effective amount of a covalently-linked conjugate (method comprises administering to a subject a composition comprising an effective amount of a covalently-linked conjugate; abstract; paragraphs [0031], [0034]-[0035]) of an Hsp90 inhibitor, wherein the Hsp90 inhibitor is capable of also inhibiting TRAP1, and wherein the conjugate is selected from the group consisting of (i) compounds with ganetespib as the Hsp90 inhibitor, having the formula, as shown, wherein R, R1, R2, R3, are independently selected from the group consisting of hydrogen; and (C1-C4)alkyl (conjugate comprises TRAP inhibitor, such as ganetespib (Hsp90 inhibitor capable of inhibiting TRAP1) as shown in figure 1 (R is C1-C4 alkyl, R1 and R2 are hydrogen, and R3 is C1-C4 alkyl), wherein prodrug moiety is attached at various locations of ganetespib compound; abstract; figure 1; paragraphs [0006], [0044]), but Madrigal does not disclose wherein the conjugate comprises temozolomide. However, Patil discloses wherein a conjugate comprises temozolomide (administering a conjugate comprising temozolomide; abstract; paragraphs [0005]-[0006]). It would have been obvious to a person of ordinary skill in the art, at the time of the invention, to have modified the method, as previously disclosed by Madrigal, in order to have provided wherein a conjugate comprises temozolomide, as previously disclosed by Patil, for providing pharmaceutical compositions and compounds comprising multiple moieties capable of treating cancerous tumors in a patient (Madrigal; abstract; Patil; abstract).

Since none of the special technical features of the Groups I+ inventions is found in more than one of the inventions, and since all of the shared technical features are previously disclosed by the Madrigal and Patil references, unity of invention is lacking.