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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 December 2014 (24.12.2014)

WIPO | PCT

(10) International Publication Number
WO 2014/205272 A1

(51) International Patent Classification:
C07D 487/04 (2006.01) *A61K 31/519* (2006.01)

(21) International Application Number:
PCT/US2014/043265

(22) International Filing Date:
19 June 2014 (19.06.2014)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
61/837,568 20 June 2013 (20.06.2013) US

(71) Applicant: **OREGON HEALTH & SCIENCE UNIVERSITY** [US/US]; 0690 Sw Bancroft St, Portland, Oregon 97238 (US).

(72) Inventors: **XIAO, Xiangshu**; 5240 NW 152nd Place, Portland, Oregon 97229 (US). **CHEN, Jingjin**; 1001 SW Woods St, Apt 4, Portland, Oregon 97239 (US). **LI, Bingbing**; 5240 NW 152nd Place, Portland, Oregon 97229 (US).

(74) Agent: **JACKSON, Jeffrey**; 0690 SW Bancroft St, Portland, Oregon 97239 (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, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, 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, 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).

Published:

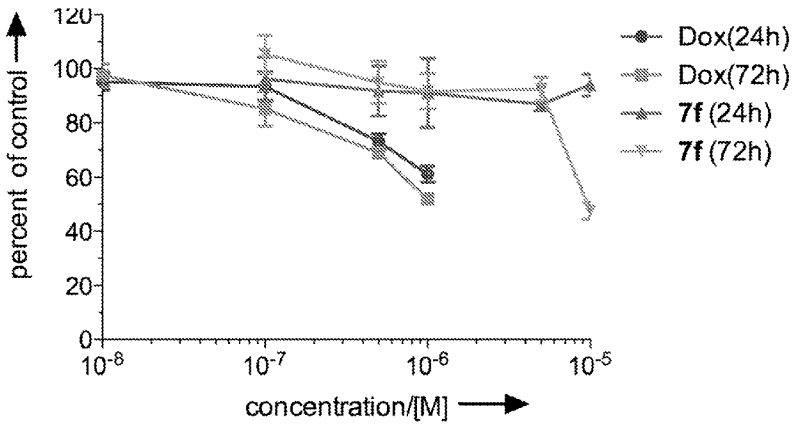
— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

WO 2014/205272 A1

(54) Title: PYRROLOQUINAZOLINE COMPOUNDS

Figure 2



(57) Abstract: Disclosed herein are acylated derivatives of 7H-pyrrolo[3,2-f]quinazoline-1,3-diamine and pharmaceutical compositions comprising said derivatives.

TITLE

PYRROLOQUINAZOLINE COMPOUNDS

FIELD

Generally, the disclosure relates to compounds that may be used in pharmaceutical compositions. More specifically, the disclosure relates to 7H-pyrrolo[3,2-*f*]quinazoline-1,3-diamine derivatives.

ACKNOWLEDGEMENT OF GOVERNMENT SUPPORT

This invention was made with support of the United States government under the terms of Grant Number RO1GM087305, awarded by the National Institutes of Health. The United States government has rights to this invention.

BACKGROUND

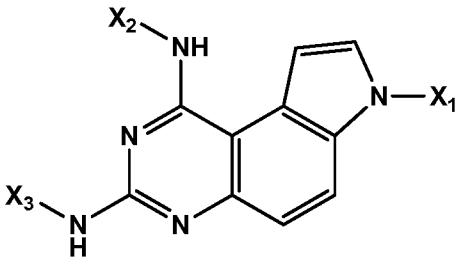
7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine (Compound **1** herein) and its derivatives, originally synthesized as antifolates in the 1970s (US Patent 4,118,561, (1978)) have been shown to possess a variety of biological activities including antibacterial, anticancer and antiparasitic activity (Gamo FJ *et al*, *Nature* **465**, 305-310 (2010); Kuyper LF *et al*, *J Med Chem* **39**, 892-903 (1996); Li Q *et al, Antimicrob Agents Chemother* **51**, 2898-2904 (2007)). Antiviral activity against herpes simplex virus (HSV) has also been reported (Dicker IB *et al*, *Antiviral Res* **28**, 213-224 (1995)).

The biochemical targets for these compounds include dihydrofolate reductase (DHFR) from various species, thrombin receptors, and protein tyrosine phosphatase 1B (PTP1B) McCormack JJ *et al*, *Biochem Pharmacol* **28**, 3227-3229 (1979); Ahn HS *et al*, *Bioorg Med Chem Lett* **9**, 2073-2078 (1999) Nadal-Wollbold F, *Eur J Pharmacol* **644**, 188-194 (2010); WO 2004101568 (2004); Cheung AW *et al*, *Bioorg Med Chem Lett* **22**, 7518-7522 (2012)). The wide spectrum bioactivity of Compound **1** is specific because a survey of the target-based and phenotypic screening assays involving Compound **1** in PubChem

(http://pubchem.ncbi.nlm.nih.gov/) show it is only active in 35/528 or 6.6% of the assays suggesting that this particular chemotype is a privileged scaffold that is intrinsically useful for different biological targets (Evans BE *et al*, *J Med Chem* **31**, 2235-2246 (1988) and Welsch SA *et al*, *Curr Opin Chem Biol* **14**, 347-361 (2010)).

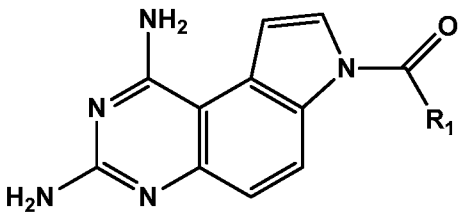
SUMMARY

Disclosed herein are compounds of formula (I)

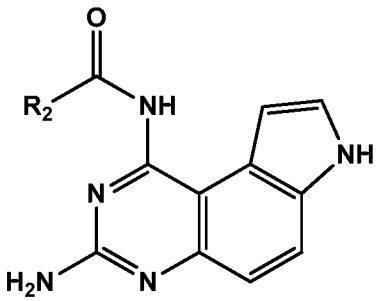


wherein X_1, X_2, and X_3 are independently H or acyl provided that X_1, X_2, and X_3 are not all H.

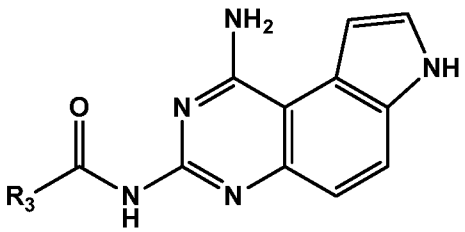
Examples include the compounds of formula (II)



wherein R_1 may be any of lower alkyl, ether, or aryl. In still further examples of the compounds, R_1 may be methyl, ethyl, propyl, isopropyl, silyl ether, or benzyl, substituted benzyl, naphthyl or substituted naphthyl. Further examples include the compounds of formula (III)



wherein R_2 may be any of lower alkyl, ether, or aryl. In still further examples of the compounds, R_2 may be methyl, ethyl, propyl, isopropyl, silyl ether, benzyl, substituted benzyl, naphthyl, or substituted naphthyl. Further examples include the compounds of formula (IV)



wherein R_3 may be any of lower alkyl, ether, or aryl. In still further examples of the compounds, R_3 may be methyl, ethyl, propyl, isopropyl, silyl ether, benzyl, substituted benzyl, or naphthyl or substituted naphthyl. Additionally disclosed are pharmaceutical compositions comprising the disclosed compounds.

It is an object of the invention to provide compounds with surprisingly improved potency over 7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine.

It is an object of the invention to provide compounds with different molecular targets than 7*H*-Pyrrolo[3,2-*f*]quinazoline-1,3-diamine.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the chemical structures of compounds **1**, **2a**, **4a**, and **7a** (top) and their corresponding molecular electrostatic potential (MEP) surfaces (bottom). MEP surfaces were calculated at an HF/6-31G** level of theory and mapped onto their electron densities. The Mulliken atomic charges, also calculated at HF/6-31G** level of theory on *N1*, *N3*, and *N7* of compound 1 are indicated in the parentheses. All the surfaces were normalized from -50 kcal/mol to +50 kcal/mol.

Figure 2 is a plot showing the effect of compound **7f** on normal human mammary epithelial cells (HMEC.) HMEC were treated with the indicated concentrations of doxorubicin or **7f** for 24 or 72 hours as indicated. Cells treated for 24 hours were further incubated in drug free media for 48 hours. The number of viable cells was determined by an MTT assay.

Figure 3 is a plot showing the activity of compound **7f** on human DHFR.

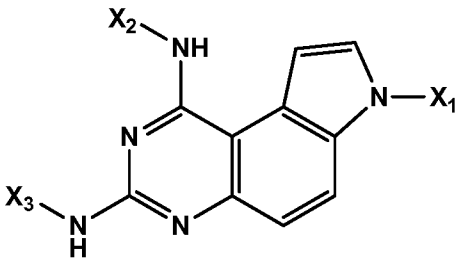
Figure 4 is a plot showing the activity of compound **7f** on CREB mediated gene transcription in HEK 293T cells. HEK 293T cells were transfected with a CREB renilla luciferase reporter (CRE-RLuc). Then the cells were treated with increasing concentrations of **7f** for 30 min before the addition of forskolin at a final concentration of 10 μM. The cells were further incubated for 5 hours before cell lysis and renilla luciferase activity measurement. The renilla luciferase activity was normalized to the protein concentration of the cell lysates and was expressed as relative luciferase unit (RLU)/μg of proteins.

Figure 5A is a schematic diagram of chemoproteomics experiments described in Example 38 below. MDA-MB-468 cells were treated with probe **10** with or without compound **7f**. Then the cells were irradiated by UV followed by cell lysis. The lysates were clicked with a biotin-N_3. The biotinylated proteins were pulled down with streptavidin beads. The bound proteins were trypsin-digested for LC-MS/MS analysis or eluted for Western analysis.

Figure 5B is an image of a Western blot showing that **7f** binds LMNA and LMNB1. The proteins from streptavidin-pulldown prepared as shown in Figure 5A were subjected to Western blot with antibodies specific for the proteins indicated by the arrows to the right of the figure.

DETAILED DESCRIPTION

Disclosed herein are compounds of formula (I)



wherein X_1, X_2, and X_3 are independently H or acyl provided that X_1, X_2, and X_3 are not all H.

The following explanations of terms and methods are provided to better describe the present compounds and compositions, and to guide those of ordinary skill in the art in the practice of the present disclosure. It is also to be understood that the terminology used in the disclosure is for the purpose of describing particular embodiments and examples only and is not

intended to be limiting. As used herein, the singular terms "a," "an," and "the" include plural referents unless context clearly indicates otherwise. Similarly, the word "or" is intended to include "and" unless the context clearly indicates otherwise. Also, as used herein, the term "comprises" means "includes." Hence "comprising A or B" means including A, B, or A and B. Variables such as X_1, X_2, X_3, R_1, R_2, and R_3, used throughout the disclosure are the same variables as previously defined unless stated to the contrary.

"Administration of" and "administering a" compound refers to providing a compound or a pharmaceutical composition comprising a compound as described herein. The compound or composition can be administered by another person to the subject or it can be self-administered by the subject.

The term "acyl" refers to a C=O group which is attached to two other moieties through the carbon atom. As used herein, it is attached to one of the moieties via a covalent bond with a nitrogen atom. The other groups may be alkyl, lower alkyl, alkenyl, alkynyl, ether, silyl ester, aryl, heterocylic, heteroaliphatic, heteroaryl, and the like. The acyl group may be substituted by any other substitutent including halo, cyano, nitro, oxo, thioxo, trimethylsilanyl, t-butylsilyl ether, or any other.

The term "alkyl" refers to a branched or unbranched saturated hydrocarbon group, such as methyl, ethyl, n-propyl, isopropyl, n-butyl, isobutyl, t-butyl, pentyl, hexyl, heptyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like. A "lower alkyl" group is a saturated branched or unbranched hydrocarbon having from 1 to 10 carbon atoms. Alkyl groups may be "substituted alkyls" wherein one or more hydrogen atoms are substituted with a substituent such as halogen, cycloalkyl, alkoxy, amino, hydroxyl, aryl, or carboxyl.

The term "aryl" refers to any carbon-based aromatic group including, but not limited to, benzyl, naphthyl, phenyl, and oxazole. The term "aryl" also includes heteroaryl, which is defined as an aromatic group that has at least one heteroatom incorporated within the ring of the aromatic group. Examples of heteroatoms include, but are not limited to, nitrogen, oxygen, sulfur, and phosphorous. The aryl group can be substituted with one or more groups including, but not limited to, alkyl, alkynyl, alkenyl, aryl, halide, nitro, amino, ester, ether, ketone,

aldehyde, hydroxy, carboxylic acid, cyano, amido, haloalkyl, haloalkoxy, or alkoxy, or the aryl group can be unsubstituted.

"Derivative" refers to a compound or portion of a compound that is derived from or is theoretically derivable from a parent compound.

The terms "pharmaceutically acceptable salt" or "pharmacologically acceptable salt" refers to salts prepared by conventional methods that include basic salts of inorganic and organic acids, including but not limited to hydrochloric acid, hydrobromic acid, sulfuric acid, phosphoric acid, methanesulfonic acid, ethanesulfonic acid, malic acid, acetic acid, oxalic acid, tartaric acid, citric acid, lactic acid, fumaric acid, succinic acid, maleic acid, salicylic acid, benzoic acid, phenylacetic acid, mandelic acid and the like. "Pharmaceutically acceptable salts" of the presently disclosed compounds also include those formed from cations such as sodium, potassium, aluminum, calcium, lithium, magnesium, zinc, and from bases such as ammonia, ethylenediamine, N-methylglutamine, lysine, arginine, ornithine, choline, N,N'-dibenzylethylenediamine, chloroprocaine, diethanolamine, procaine, N-benzylphenethylamine, diethylamine, piperazine, tris(hydroxymethyl)aminomethane, and tetramethylammonium hydroxide.

These salts may be prepared by standard procedures, for example by reacting the free acid with a suitable organic or inorganic base. Any chemical compound recited in this specification may alternatively be administered as a pharmaceutically acceptable salt thereof. Pharmaceutically acceptable salts are also inclusive of the free acid, base, and zwitterionic forms. Descriptions of suitable pharmaceutically acceptable salts can be found in Handbook of Pharmaceutical Salts, Properties, Selection and Use, Wiley VCH (2002). When compounds disclosed herein include an acidic function such as a carboxy group, then suitable pharmaceutically acceptable cation pairs for the carboxy group are well known to those skilled in the art and include alkaline, alkaline earth, ammonium, quaternary ammonium cations and the like. Such salts are known to those of skill in the art. For additional examples of "pharmacologically acceptable salts," see Berge *et al*., *J. Pharm. Sci*. 66, 1 (1977).

The term "ether" refers to a group with an R-O-R structure wherein R represents any chemical moiety. Silyl ethers have a $(R)_3$-Si-O-R structure wherein R represents any chemical moiety. Examples of silyl ethers include tert-butyldimethylsilyl ether, tert-butyldiphenyl silyl ether, diphenylmethyl silyl ether, and tri(isopropyl) silyl ether.

Protected derivatives of the disclosed compounds also are contemplated. A variety of suitable protecting groups for use with the disclosed compounds are disclosed in Greene and Wuts Protective Groups in Organic Synthesis; 5 3rd Ed.; John Wiley & Sons, New York, 1999. In general, protecting groups are removed under conditions which will not affect the remaining portion of the molecule. These methods are well known in the art and include acid hydrolysis, hydrogenolysis and the like. One preferred method involves the removal of an ester, such as cleavage of a phosphonate ester using Lewis acidic conditions, such as in TMS-Br mediated ester cleavage to yield the free phosphonate. A second preferred method involves removal of a protecting group, such as removal of a benzyl group by hydrogenolysis utilizing palladium on carbon in a suitable solvent system such as an alcohol, acetic acid, and the like or mixtures thereof. A t-butoxy-based group, including t-butoxy carbonyl protecting groups can be removed utilizing an inorganic or organic acid, such as HCl or trifluoroacetic acid, in a suitable solvent system, such as water, dioxane and/or methylene chloride. Another exemplary protecting group, suitable for protecting amino and hydroxyl functions amino is trityl. Other conventional protecting groups are known and suitable protecting groups can be selected by those of skill in the art in consultation with Greene and Wuts Protective Groups in Organic Synthesis; 3rd Ed.; John Wiley & Sons, New York, 1999.

Particular examples of the presently disclosed compounds include one or more asymmetric centers; thus these compounds can exist in different stereoisomeric forms. Accordingly, compounds and compositions may be provided as individual pure enantiomers or as stereoisomeric mixtures, including racemic mixtures. In certain embodiments the compounds disclosed herein are synthesized in or are purified to be in substantially enantiopure form, such as in a 90% enantiomeric excess, a 95% enantiomeric excess, a 97%

enantiomeric excess or even in greater than a 99% enantiomeric excess, such as in enantiopure form.

The compounds disclosed herein may be included in pharmaceutical compositions (including therapeutic and prophylactic formulations), typically combined together with one or more pharmaceutically acceptable vehicles or carriers and, optionally, other therapeutic ingredients.

Such pharmaceutical compositions can formulated for administration to subjects by a variety of mucosal administration modes, including by oral, rectal, intranasal, intrapulmonary, intravitrial, or transdermal delivery, or by topical delivery to other surfaces including the eye. Optionally, the compositions can be administered by non-mucosal routes, including by intramuscular, subcutaneous, intravenous, intra-arterial, intra-articular, intraperitoneal, intrathecal, intracerebroventricular, or parenteral routes. In other examples, the compound can be administered ex vivo by direct exposure to cells, tissues or organs originating from a subject.

To formulate the pharmaceutical compositions, the compound can be combined with various pharmaceutically acceptable additives, as well as a base or carrier useful in the dispersion of the compound. Desired additives include, but are not limited to, pH control agents, such as arginine, sodium hydroxide, glycine, hydrochloric acid, citric acid, and the like. In addition, local anesthetics (for example, benzyl alcohol), isotonizing agents (for example, sodium chloride, mannitol, sorbitol), adsorption inhibitors (for example, Tween®80), solubility enhancing agents (for example, cyclodextrins and derivatives thereof), stabilizers (for example, serum albumin), and reducing agents (for example, glutathione) can be included.

When the composition is a liquid, the tonicity of the formulation, as measured with reference to the tonicity of 0.9% (w/v) physiological saline solution taken as unity, is typically adjusted to a value at which no substantial, irreversible tissue damage will be induced at the site of administration. Generally, the tonicity of the solution is adjusted to a value of about 0.3 to about 3.0, such as about 0.5 to about 2.0, or about 0.8 to about 1.7. The compound can be dispersed in a carrier, which can include a hydrophilic compound having a capacity to disperse the compound, and any desired additives. The base can be selected from a wide range of

suitable compounds, including but not limited to, copolymers of polycarboxylic acids or salts thereof, carboxylic anhydrides (for example, maleic anhydride) with other monomers (for example, methyl (meth)acrylate, acrylic acid and the like), hydrophilic vinyl polymers, such as polyvinyl acetate, polyvinyl alcohol, polyvinylpyrrolidone, cellulose derivatives, such as hydroxymethylcellulose, hydroxypropylcellulose and the like, and natural polymers, such as chitosan, collagen, sodium alginate, gelatin, hyaluronic acid, and nontoxic metal salts thereof. Often, a biodegradable polymer is selected as a base or vehicle, for example, polylactic acid, poly(lactic acid-glycolic acid) copolymer, polyhydroxybutyric acid, poly(hydroxybutyric acid-glycolic acid) copolymer and mixtures thereof.

Alternatively or additionally, synthetic fatty acid esters such as polyglycerin fatty acid esters, sucrose fatty acid esters and the like can be employed as carriers. Hydrophilic polymers and other vehicles can be used alone or in combination, and enhanced structural integrity can be imparted to the vehicle by partial crystallization, ionic bonding, cross-linking and the like. The carrier can be provided in a variety of forms, including fluid or viscous solutions, gels, pastes, powders, microspheres, and films for direct application to a mucosal surface.

The compound can be combined with the base or vehicle according to a variety of methods, and release of the compound can be by diffusion, disintegration of the vehicle, or associated formation of water channels. In some circumstances, the compound is dispersed in microcapsules (microspheres) or nanoparticles prepared from a suitable polymer, for example, 5 isobutyl 2-cyanoacrylate (see, for example, Michael *et al.*, *J. Pharmacy Pharmacol*. 43, 1-5, 1991), and dispersed in a biocompatible dispersing medium, which yields sustained delivery and biological activity over a protracted time. Alternatively, the compound may be combined with a mesoporous silica nanoparticle including a mesoporous silica nanoparticle complex with one or more polymers conjugated to its outer surface.

The pharmaceutical compositions of the disclosure can alternatively contain as pharmaceutically acceptable vehicles substances as required to approximate physiological conditions, such as pH adjusting and buffering agents, tonicity adjusting agents, wetting agents and the like, for example, sodium acetate, sodium lactate, sodium chloride, potassium chloride,

calcium chloride, sorbitan monolaurate, and triethanolamine oleate. For solid compositions, conventional nontoxic pharmaceutically acceptable vehicles can be used which include, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharin, talcum, cellulose, glucose, sucrose, magnesium carbonate, and the like.

Pharmaceutical compositions for administering the compound can also be formulated as a solution, microemulsion, or other ordered structure suitable for high concentration of active ingredients. The vehicle can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, liquid polyethylene glycol, and the like), and suitable mixtures thereof. Proper fluidity for solutions can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of a desired particle size in the case of dispersible formulations, and by the use of surfactants. In many cases, it will be desirable to include isotonic agents, for example, sugars, polyalcohols, such as mannitol and sorbitol, or sodium chloride in the composition. Prolonged absorption of the compound can be brought about by including in the composition an agent which delays absorption, for example, monostearate salts and gelatin.

In certain embodiments, the compound can be administered in a time release formulation, for example in a composition which includes a slow release polymer. These compositions can be prepared with vehicles that will protect against rapid release, for example a controlled release vehicle such as a polymer, microencapsulated delivery system or bioadhesive gel. Prolonged delivery in various compositions of the disclosure can be brought about by including in the composition agents that delay absorption, for example, aluminum monostearate hydrogels and gelatin. When controlled release formulations are desired, controlled release binders suitable for use in accordance with the disclosure include any biocompatible controlled release material which is inert to the active agent and which is capable of incorporating the compound and/or other biologically active agent. Numerous such materials are known in the art. Useful controlled-release binders are materials that are metabolized slowly under physiological conditions following their delivery (for example, at a mucosal surface, or in the presence of bodily fluids). Appropriate binders include, but are not

limited to, biocompatible polymers and copolymers well known in the art for use in sustained release formulations. Such biocompatible compounds are non-toxic and inert to surrounding tissues, and do not trigger significant adverse side effects, such as nasal irritation, immune response, inflammation, or the like. They are metabolized into metabolic products that are also biocompatible and easily eliminated from the body.

Exemplary polymeric materials for use in the present disclosure include, but are not limited to, polymeric matrices derived from copolymeric and homopolymeric polyesters having hydrolyzable ester linkages. A number of these are known in the art to be biodegradable and to lead to degradation products having no or low toxicity. Exemplary polymers include polyglycolic acids and polylactic acids, poly(DL-lactic acidco- glycolic acid), poly(D-lactic acid-co-glycolic acid), and poly(L-lactic acid-coglycolic acid). Other useful biodegradable or bioerodable polymers include, but are not limited to, such polymers as poly(epsilon-caprolactone), poly(epsilon-aprolactone-CO-lactic acid), poly(epsilon.-aprolactone-CO-glycolic acid), poly(beta-hydroxy butyric acid), poly(alkyl-2-cyanoacrilate), hydrogels, such as poly(hydroxyethyl methacrylate), polyamides, poly(amino acids) (for example, L-leucine, glutamic acid, L-aspartic acid and the like), poly(ester urea), poly(2-hydroxyethyl DL-aspartamide), polyacetal polymers, polyorthoesters, polycarbonate, polymaleamides, polysaccharides, and copolymers thereof. Many methods for preparing such formulations are well known to those skilled in the art (see, for example, Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978). Other useful formulations include controlled-release microcapsules (U.S. Patent Nos. 4,652,441 and 4,917,893), lactic acid-glycolic acid copolymers useful in making microcapsules and other formulations (U.S. Patent Nos. 4,677,191 and 4,728,721) and sustained-release compositions for water-soluble peptides (U.S. Patent No. 4,675,189).

The pharmaceutical compositions of the disclosure typically are sterile and stable under conditions of manufacture, storage and use. Sterile solutions can be prepared by incorporating the compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated herein, as required, followed by filtered sterilization. Generally,

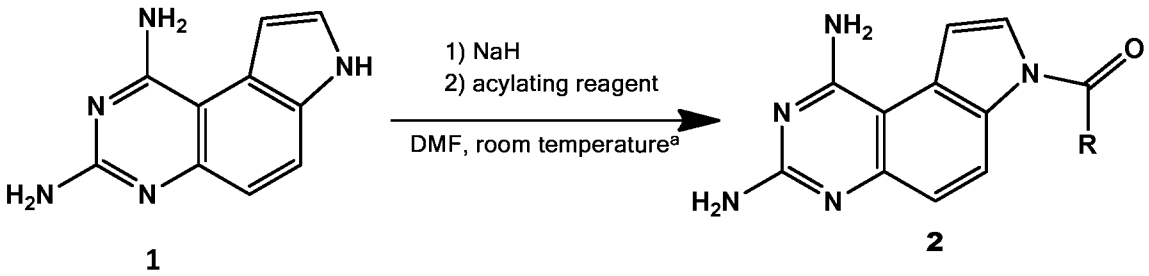
dispersions are prepared by incorporating the compound and/or other biologically active agent into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated herein. In the case of sterile powders, methods of preparation include vacuum drying and freeze-drying which yields a powder of the compound plus any additional desired ingredient from a previously sterile-filtered solution thereof. The prevention of the action of microorganisms can be accomplished by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like.

EXAMPLES

The following examples are illustrative of disclosed methods. In light of this disclosure, those of skill in the art will recognize that variations of these examples and other examples of the disclosed method would be possible without undue experimentation.

Example 1 – Selective N^7-acylation of Compound 1

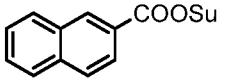
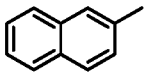
Acylation preceded by the following reaction:



1 2

Table 1 – N^7 acylation of compound 1.

Acylating reagent structure	Acylating reagent ID	R Group	Product ID	% Yield
$(CH_3CO)_2O$	**3a**	Methyl	**2a**	78
$(CH_3CH_2CO)_2O$	**3b**	Ethyl	**2b**	78
$(CH_3CH_2CH_2CO)_2O$	**3c**	Propyl	**2c**	73
$[(CH_3)_2CHCO]_2O$	**3d**	Isopropyl	**2d**	78
TBSO(CH2)$_4$COOSu	**3e**	TBSO$(CH_2)_4$	**2e**	68

	**3f**		**2f**	91

Compound **1** was treated with NaH (1.1 equivalents) in DMF for 1 hour. Then an acylating reagent (1.1 equivalents was added.) The yields refer to isolated yields.

It is predicted that the proton attached to N^7 is most acidic, however, the pKas of the protons attached to N^1 and N^3 are probably comparable. To further investigate this point, the structure of compound **1** was optimized at HF/6-31G** level of theory and the Mulliken atomic charges (Mulliken RS*, J Chem Phys* **23**, 1833-1840 (1955); incorporated by reference herein) were calculated (Xiao X *et al*, *J Med Chem* **48**, 3231-3238 (2005); incorporated by reference herein.) Consistent with the prediction, N^7 is the least negatively charged among the three ionizable nitrogen atoms (Figure 1). N^1 is slightly less charged than N^3, suggesting that the order of pKa is $N^7 < N^1 \leq N^3$. Therefore, it was speculated that N^7-H could be selectively deprotonated and acylated.

Compound **1** was prepared from 5-aminoindole using a reported procedure with slight modifications in 82% yield (Jones ML *et al*, *J Heterocycl Chem* **31**, 1681-1683 (1994)). The synthesized compound **1** was deprotonated by NaH followed by treatment with acetic anhydride (**3a**), resulting in compound **2a** obtained in 78% yield (Table 1). The diagnostic loss of N^7-H at 11.55 ppm and loss of a triplet at 7.43 ppm attributed to C^8-H in compound **1** supported the hypothesis that the acetyl group was attached to N^7. A few other anhydrides (**3b**, **3c**, and **3d** in Table 1) were used as acylating reagents and the corresponding N^7-acylated products were obtained in comparable yields (Table 1).

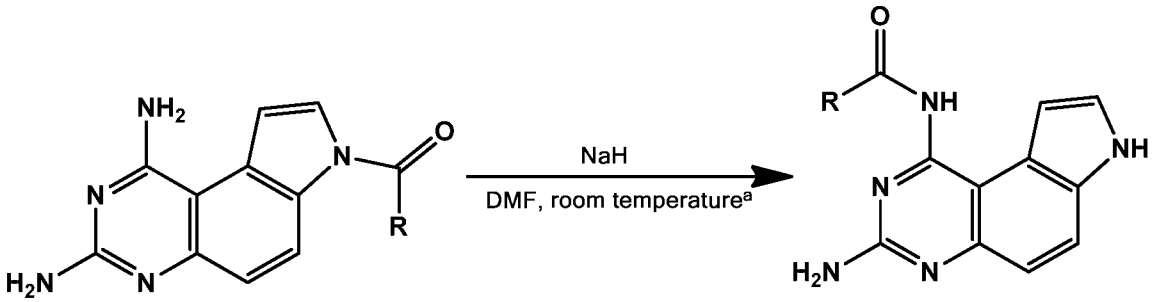
Due to the limited commercial availability of anhydrides and the loss of an acyl equivalent during reactions using anhydrides, the utility of N-hydroxysuccinimide was investigated (NHS) esters as the acylating regents. Both aliphatic and aromatic carboxylic NHS esters were found to react smoothly to give the N^7 acylated compounds in good to excellent yields (entries 5-6, Table 1). The TBS group in **3e** was well tolerated.

The discovery of NHS esters as efficient acylating agents substantially expands the variety of N^7 acylated compounds that can be prepared through this route. In general, the N^7 acylated

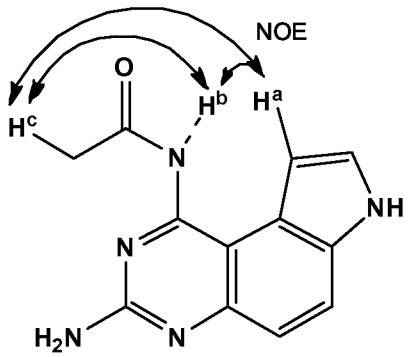
compounds of Series **2** are sparingly soluble in common organic solvents or water. Therefore, most of the products were not purified by column chromatography, but they were all found to be >95% pure based on ^{1}H NMR analyses. In the case of **2a**, **2b**, and **2c**, the solubility was so limited that high-quality ^{13}C NMR spectra could not be obtained.

Example 2 – Synthesis of N^1-acylated compounds of Series 4

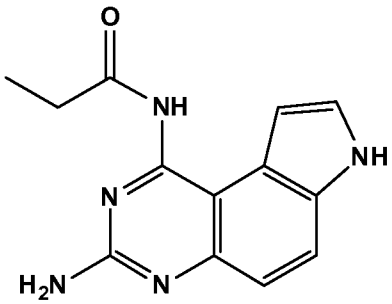
Acylation of compounds of Series 4 was performed by the following reaction:



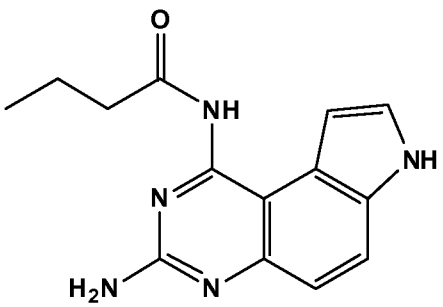
These yielded the compounds of Series 4:



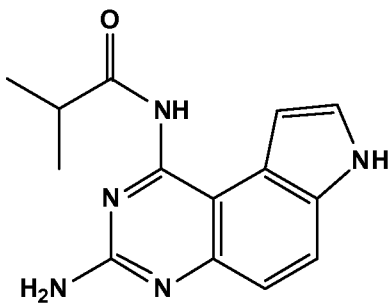
4a, 24%



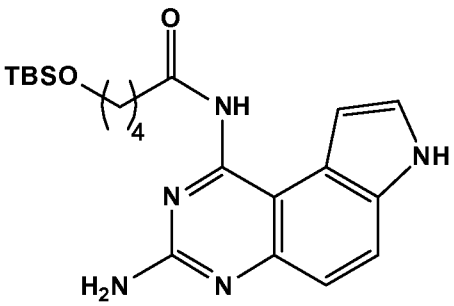
4b, 32%



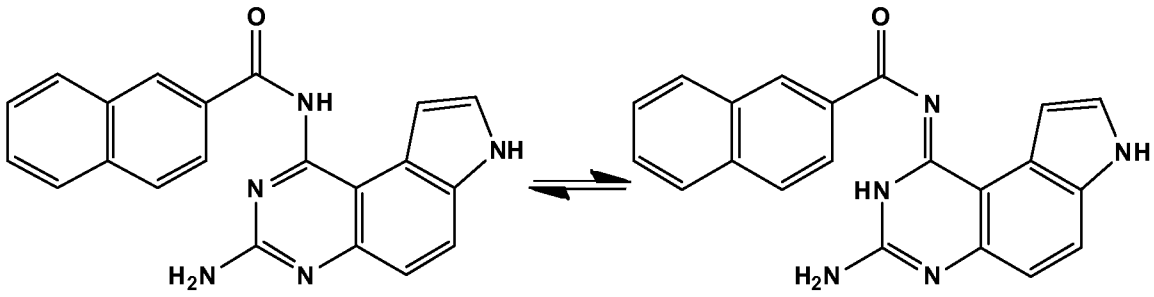
4c, 32%



4d, 27%



4e, 38%



4f, 29% 4f'

[a] The reactions were carried out with compounds of series **2** from Table 1 (1.0 equivalents) and NaH (1.1 equivalents) in DMF. The yields refer to isolated yields.

4f and **4f'** are a 1:1 mixture of two tautomers in DMSO-d_6.

N^1 and N^3 were predicted to be more nucleophilic than N^7, however, all attempts to direct acetylation of either N^1 or N^3 in compound **1** with Ac_2O failed to provide selectively mono-*N*-acetylated products. After considerable experimentation, it was found that treatment of **2a** with NaH resulted in N^1-acetylated product **4a** in 24% yield. The regioselectivity of this reaction was confirmed by the positive nuclear Overhauser effect (NOE) between Ha and Hb, Ha and Hc observed in **4a**. Without being bound by theory, the mechanism for this transformation presumably involves an intermolecular acetyl transfer from N^7 of one molecule to N^1 of the other molecule followed by cleavage of N^7-acetyl group from the latter molecule. The major byproduct generated from this reaction was the deacetylated compound **1**, which was isolated in 74% yield.

The combined yields of **1** and **4a** accounted for nearly quantitative recovery of **2a**. The absence of N^3-acylated product from this reaction supported the prediction of pK_a order of N^1 < N^3 (Figure 1) and illustrated that subtle differences in pK_a can be synthetically exploited. All the

aliphatic acylated substrates **2a**-**2e** were successfully converted into N^1-acylated products **4a**-**4e** in 24-38% isolated yields (Table 2). In the case of aromatic acylated compound **4f**, it was obtained in 29% yield existing as a 1:1 mixture of two clearly NMR-distinguishable tautomers **4f** and **4f'** in DMSO-d_6. This tautomeric mixture becomes a single tautomer **4f** upon treatment with an aqueous NaOH solution. In addition, all the active protons in **4f** disappeared in its ^{1}H NMR due to H-D exchange with HDO generated from the reaction of NaOH with DMSO-d_6. For the same reason, the signals from the residual solvents in both ^{1}H NMR and ^{13}C NMR spectra were very complicated.

It was also found that different bases exerted a great effect on the yield of this acyl transfer reaction. For example, LDA resulted in a 0% yield of **4a** while a 45% yield of **4a** was obtained if LiHMDS was used as a base (see Table 2). Similarly, a 50% yield of **4f** and **4f'** resulted when LiHMDS was used as the base.

Table 2: The effect of different bases on the yield of compound 4a from compound 2a.

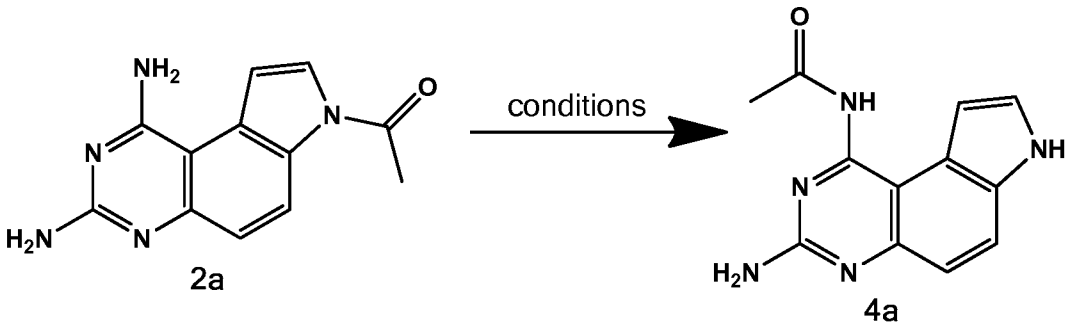


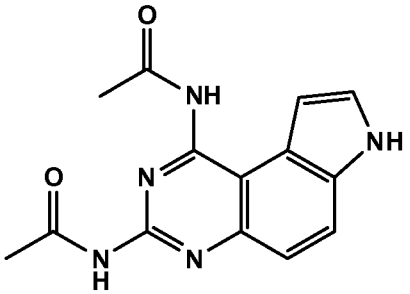
Table 2 – effect of bases on yields of compounds of series **4**

Base	Temp (°C)	Yield (%)[a]
NaH	25	24
LDA	25	0
NaO^{t}Bu	25	25
LiHMDS	25	35[b]
LiHMDS	0	45
LiHMDS	-20	40

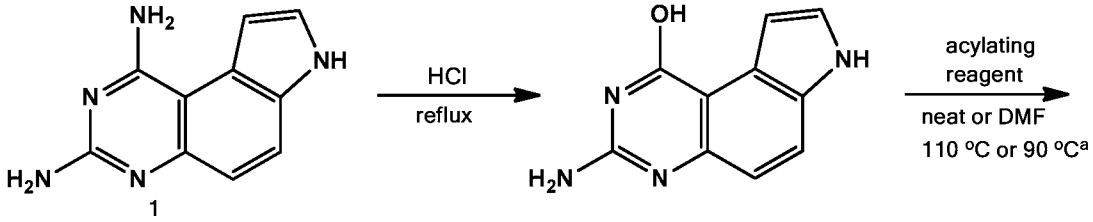
a - Isolated yields

b - Containing about 10% of 4a' as assessed by ^{1}H NMR.

Structure of compound 4a'



Example 3 – Synthesis of N^3-acylated compounds of Series 7



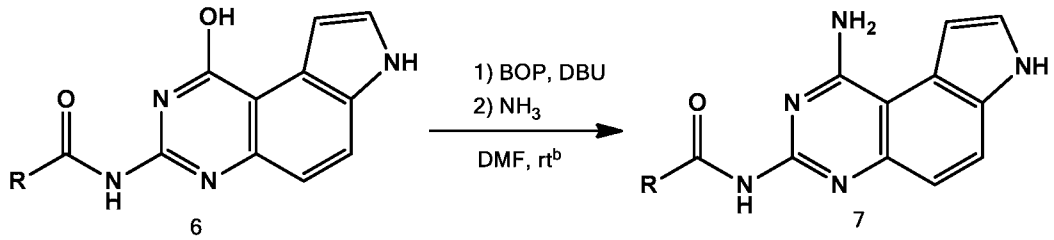
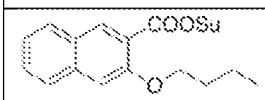
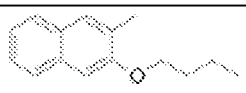


Table 3 N^3 acetylated compounds:

Acylating reagent structure	Acylating reagent ID	R Group	6	Yield 6	7	Yield 7
$(CH_3CO)_2O$	**3a**	Methyl	**6a**	78	**7a**	48
$(CH_3CH_2CO)_2O$	**3b**	Ethyl	**6b**	78	**7b**	44
$(CH_3CH_2CH_2CO)_2O$	**3c**	Propyl	**6c**	73	**7c**	37
$[(CH_3)_2CHCO]_2O$	**3d**	Isopropyl	**6d**	78	**7d**	40
TBSO(CH2)4COOSu	**3e**	$TBSO(CH_2)_4$	**6e**	68	**7e**	50
	3f		**6f**	91	**7f**	25
	**3g**		**6b**	79	**7g**	48

[a] carried out with compound **5** (1.0 equivalents) and an anhydride used neat or an NHS ester (1.5 equivalents) in DMF

[b] carried out with compound **6** (1.0 equivalents) BOP (1.3 equivalents) and DBU (1.5 equivalents for four hours.) After that, 7N NH_3 in methanol was added.

Yields are isolated yields.

To achieve selective N^3 acylation, a more elaborate and indirect scheme was designed (Table 3). The N^1 amine was temporarily converted into a less nucleophilic hydroxyl group to give compounds of series **5** in quantitative yield through acid hydrolysis (Guan J *et al*, *Antimicrob Agents Chemother* **49**, 4928-4933 (2005) and Trattner RB *et al*, *J Org Chem* **29**, 2674-2677 (1964); both of which are incorporated by reference herein.) Then the nucleophilic N^3 in the compounds of series **5** were selectively acylated by treating with either an anhydride or NHS ester to provide compounds of series **6** in good to excellent yields (Table 3). The TBS ether was well tolerated and product **6e** was obtained in 62% yield. Aromatic carboxylic NHS ester **3f** was compatible with this acylation step and the desired compound **6f** was generated in 56% yield. With the acylated intermediates of series **6** in hand, the N^1 amine was regenerated using an SNAr displacement reaction between ammonia (NH3/MeOH) and activated benzotriazole adducts generated between the compounds of series **6** and BOP (Wan ZK *et al*, *J Org Chem* **72**, 10194-10210 (2007)) to provide compounds **7a**, **7b**, **7c**, **7d**, **7e**, **7f** and **7g** in moderate to good yields. Therefore, the hydroxyl group in **5** served as a temporary protecting group for the N^1 amine.

Example 4 – Activity of Compounds of Series 4 and Series 7

The newly synthesized selectively mono-N-acylated compounds of series **4** and series **7** were evaluated as potential anticancer agents because compound **1** had been previously shown to display anticancer activity by inhibiting DHFR (Kuyper LF *et al*, *J Med Chem* **39**, 892-903 (1996)). Compounds of series **2** were not evaluated due to their poor solubility in DMSO. Two triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and MDAMB-468) were selected to evaluate potential anticancer activity of the compounds of series **4** and series **7** by an MTT assay (Li BX *et al*, *Bioorg Med Chem* **20**, 6811-6820 (2012); incorporated by reference herein.)

TNBC represents a unique subtype of breast cancer clinically characterized by the lack of expression of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2). Subjects with triple negative breast cancer often have a poor prognosis (Kang SP *et al, Curr Opin Obstet Gynecol* **20**, 40-46 (2008) and The Cancer Genome Atlas Network *Nature*, **490**, 61-70 (2012)). Current treatment options for TNBC are limited and novel agents are needed (Shastry M and Yardley DA, *Curr Opin Obstet Gynecol* **25**, 40-48 (2013); incorporated by reference herein). The antiproliferative activity of compounds of series **4** and series **7** in MDA-MB-231 and MDAMB-468 cells is presented in Table 4.

Table 4 – Antiproliferative activities of compounds of series **4** and series **7** in MDA-MB-231 and MDA-MG-468

Compound	GI_{50} (μM)	
	MDA-MB-231	**MDA-MB-468**
1	4.13 ± 0.54	3.34 ± 0.93
4a	32.62 ± 13.97	56.46 ± 17.97
4b	18.66 ± 2.24	20.28 ± 7.40
4c	26.86 ± 12.90	26.27 ± 7.40
4d	> 100	> 100
4e	15.64 ± 7.66	11.11 ± 1.73
4f	8.46 ± 2.56	8.91 ± 1.28
7a	27.17 ± 11.40	53.54 ± 29.54
7b	21.43 ± 9.86	24.41 ± 3.33
7c	25.52 ± 9.93	27.37 ± 4.52
7d	39.66 ± 22.46	29.80 ± 8.41
7e	2.43 ± 0.13	2.24 ± 0.40
7f	1.60 ± 0.51	0.44 ± 0.14
7g	0.65 ± 0.43	0.10 ± 0.079

GI_{50} values represent the concentration that limits the growth of the cancer cells by 50% during a 72 hour incubation period. These are presented as mean ± standard deviation of the mean of at least two independent experiments performed in duplicate.

In general, the compounds of series **7** are more potent than the compounds of series **4** (**4d**-**4f** vs **7d**-**7g**). Although most of the compounds are less potent than the parent compound **1**, compound **7f** and **7g** were more potent than compound **1** in both MDA-MB-231 (GI_{50} = 1.60

μM) and MDA-MB-468 (GI_{50} = 0.44 μM) cells. In addition, compound **7f** was found to be not toxic to normal human mammary epithelial cells (HMEC) up to 5 μM after a 72-h incubation period (Figure 2). This compares favorably to an approved cytotoxic chemotherapeutic agent such as doxorubicin (Dox). Dox has a GI_{50} = 0.12 μM in MDA-MB-468 cells, but is toxic to normal HMEC cells at a concentration as low as 0.1 μM. These results indicate that **7f** is a potential novel nontoxic anti-TNBC agent.

Compound **1** was known to be a human DHFR inhibitor based on the binding orientation of an N7-alkylated 1 in DHFR from Candida albicans and its structural similarity to human DHFR (Whitlow AJ *et al*, *J Biol Chem* **272**, 30289-30298 (1997); incorporated by reference herein). From that, it would be predicted that the bulky naphthyl group in compound **7f** would not be accommodated in the human DHFR binding pocket. Indeed, it was found that **7f** did not inhibit human DHFR up to a 10 μM concentration (Figure 3). Therefore, the potent antiproliferative activity of **7f** in TNBC cells is surprisingly independent of DHFR inhibition.

Other references show that small molecule inhibitors of CREB (cyclic-AMP response element binding protein) also have activity against cancer (Li BX *et al* 2012 *supra* and Xiao X *et al*, *Curr Cancer Drug Targets* **10**, 384-391 (2010) which is incorporated by reference herein.) However, **7f** was also unable to inhibit CREB-mediated gene transcription up to a 10 μM concentration using the CREB reporter assay in HEK 293T cells described in Li BX *et al*, *ChemBioChem* **10**, 2721-2724 (2009) which is incorporated by reference herein (Figure 4).

Example 5 – Experimental Procedures

The solvents used for each reaction were purified from the Glass Contout solvent purification system. Melting points were determined in capillary tubes using Mel-Temp and are uncorrected. NMR spectra were recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR). Chemical shifts (δ) are reported in ppm relative to the residual $CHCl_3$ (1H, 7.26 ppm, 13C, 77.0 ppm) or DMSO (1H, 2.50 ppm, 13C, 39.5 ppm). The following abbreviations were used to describe the splitting pattern of individual peaks if applicable: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. The coupling constants (J) were reported in Hertz (Hz). Silica gel flash chromatography was performed using 230– 400 mesh silica gel (EMD). The mass spectra were

obtained from an LTQ Orbitrap Discovery mass spectrometer (Thermo Scientific, West Palm Beach, FL) with electrospray operated either in positive or negative mode. All final compounds were confirmed to be of > 95% purity based on HPLC (Waters) analysis using an XBridge C18 column (4.6 x 150 mm) and detected at 254 nm (due to the poor solubility, compound 2a-2d were not evaluated by HPLC). The mobile phases for HPLC are water and acetonitrile, both of which contain 0.1% TFA (for compounds **2e**, **4e**, and **7e** which contain a TBS group, 0.01% TFA was used due to instability of these compounds in 0.1% TFA).

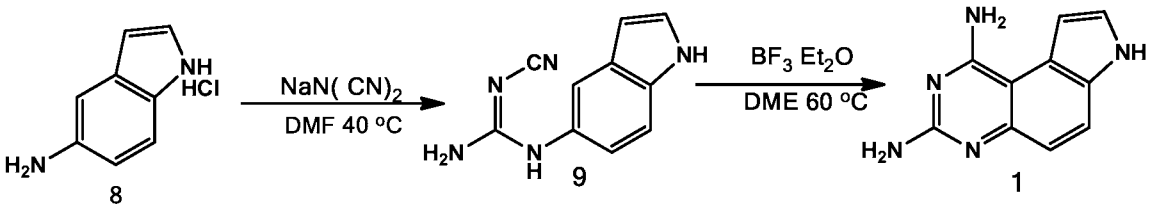
In vitro human DHFR inhibition: The human DHFR assay was done with DHFR assay kit (Sigma) following the manufacturer's instructions with minor modifications. Different concentrations of a test compound were incubated with human DHFR (0.1875 mU) and NADPH (60 μM) in the 1X assay buffer for 2 min at room temperature. Then DHF (50 μM) was added to initiate the reduction reaction, which was immediately monitored by absorbance at 340 nm every 12 s for 3 min. The final reaction volume was 100 μL and the final DMSO concentration was 1%. The reaction velocities were calculated as the slopes from the absorbance-time curves.

MTT assays: TMTT assays for MDA-MB-231, MDA-MB-468 and HMEC were performed as described in Li *et al*, 2012 supra.

Inhibition of CREB-mediated gene transcription: Inhibition of CREB mediated transcription in HEK 293T cells by a CREB reporter assay was performed as described in Li and Xiao, 2009 *supra*.

Molecular Modeling: Molecular modeling work was conducted in the Schrödinger modeling suite (Portland, OR). The structures were optimized at HF/6-31G** level of theory in Jaguar. The structural minima were confirmed by the absence of any negative vibrational frequencies. The MEP surfaces were generated by mapping the electrostatic potentials onto the electron densities and were normalized from -50 kcal/mol to +50 kcal/mol.

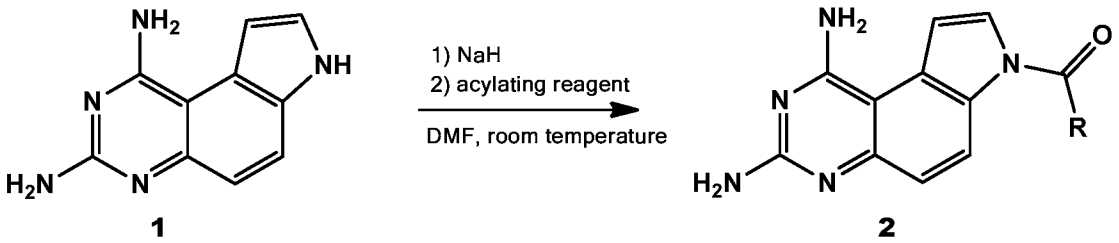
Example 6 - Synthesis of Compound 1



$NaN(CN)_2$ (7.3 g, 81.5 mmol) was added to a stirred solution of **8** (5.5 g, 32.6 mmol, prepared by treating a methanolic solution of 5-aminoindole with 1.5 equiv HCl in Et_2O) in DMF (55 mL). The reaction mixture was stirred at 40 °C for 4 hours. DMF was removed and the residue was treated with H_2O (50 mL) overnight. The gray solid was collected by filtration and dried in vacuum for 1 d to give compound **9** (6.3 g, 97% yield), which was used for the next step without further purification. The characterization data were consistent with literature reported values: 1H NMR (400 MHz, DMSO-*d*6) δ 11.12 (s, 1 H), 8.86 (s, 1 H), 7.46 (s, 1 H), 7.35-7.33 (m, 2 H), 6.94 (dd, *J* = 8.8 Hz, 2.0 Hz, 1 H), 6.74 (s, 2 H), 6.40 (s, 1 H). Boron trifluoride (18.8 mL, 152 mmol) was added dropwise to a stirred suspension of **9** (6.3 g, 31.6 mmol) in DME (600 mL) at 60 °C. The resulting mixture was stirred at 60 °C for 4 h. Then the solvent was removed and the residue was suspended in MeOH (60 mL) and treated with NH_4OH (40 mL) for 2 h. The solvents were removed in *vacuo* and the residue was purified by column chromatography on silica gel, eluting with 3:1 DCM:MeOH with 1% NH4OH to give a yellow solid, which was treated with 1 N NaOH (50 mL) at room temperature for overnight. Then the solid was collected to give compound **1** as a white to pale yellow solid (5.2 g, 89% yield). The characterization data were consistent with literature reported values: ^{1}H NMR (400 MHz, DMSO-*d*6) δ 11.55 (s, 1 H), 7.64 (d, J = 8.8 Hz, 1 H), 7.43 (t, J = 2.8 Hz, 1 H), 7.03-7.00 (m, 2 H), 6.65 (brs, 2 H), 5.65 (s, 2 H); 13C NMR (100 MHz, DMSO-d6) δ 162.0, 159.1, 150.1, 130.4, 124.8, 120.0, 119.1, 119.0, 102.5, 102.0.

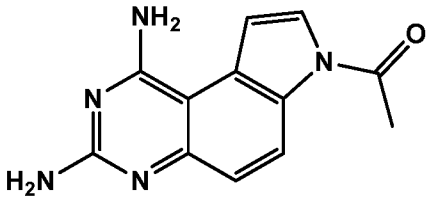
Example 7 – Common procedure for N^7 acylation

The following reaction shows the common reaction for the synthesis of compounds of series **2,** detailed in Examples 8-13 below. For acylating reagents see Table 1 above.



Example 8 – Synthesis of compound 2a

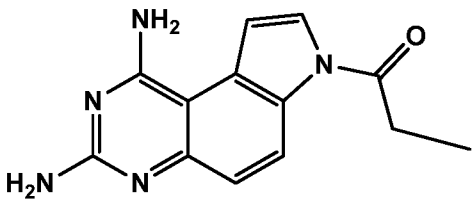
Compound 2a



NaH (20.0 mg, 60% in mineral oil, 0.50 mmol) was added to a stirred solution of compound **1** (90.0 mg, 0.45 mmol) in dry DMF (5 mL) at 25 °C under an Ar atmosphere. The reaction mixture was stirred for 1 h, when Ac_2O (47.3 µl, 0.50 mmol) was added and the mixture was stirred for another 3 h. The solvent was removed and the residue was treated with water. The solid was collected by filtration and dried in vacuum. Then it was treated with DCM (2 mL) and collected by filtration to give the desired product 2a as a yellowish solid (85.0 mg, 78% yield): mp 202-204 °C. 1H NMR (400 MHz, DMSO-d6) δ 8.56 (d, J = 8.8 Hz, 1 H), 7.99 (d, J = 3.6 Hz, 1 H), 7.39 (d, J = 4.0 Hz, 1 H), 7.18 (d, J = 9.2 Hz, 1 H), 6.90 (s, 2 H), 5.92 (s, 2 H), 2.69 (s, 3 H); HRMS (ESI) Calcd for $C_{12}H_{12}N_5O^+$ (M + H)+ 242.10364; Found 242.10313.

Example 9 – Synthesis of compound 2b

Compound **2b**

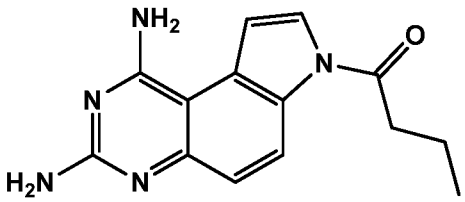


From 20.0 mg (0.10 mmol) of compound **1**, compound **2b** (20.0 mg, 78% yield) was obtained as a yellowish solid: mp 210-212 °C; 1H NMR (400 MHz, DMSO-d6) δ 8.59 (d, J = 9.2 Hz, 1 H), 8.03 (d, J = 3.6 Hz, 1 H), 7.39 (d, J = 4.0 Hz, 1 H), 7.18 (d, J = 9.2 Hz, 1 H), 6.90 (s, 2 H),

5.92 (s, 2 H), 3.12 (q, J = 7.2 Hz, 2 H), 1.19 (t, J = 7.6 Hz, 3 H); HRMS (ESI) Calcd for $C_{13}H1_4N_5O^+$ (M + H)+ 256.11929; Found 256.11893.

Example 10 – Synthesis of compound 2c

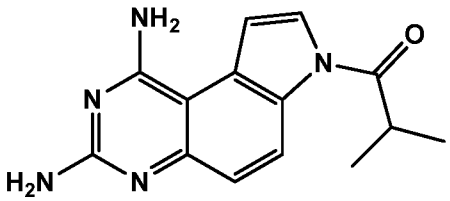
Compound **2c**



From 65.0 mg (0.33 mmol) of **1**, compound **2c** (65.0 mg, 73% yield) was obtained as a yellowish solid: mp 196-198 oC; 1H NMR (400 MHz, DMSO-*d*6) δ 8.59 (d, *J* = 9.2 Hz, 1 H), 8.05 (d, *J* = 3.6 Hz, 1 H), 7.39 (d, *J* = 4.0 Hz, 1 H), 7.18 (d, *J* = 8.8 Hz, 1 H), 6.90 (brs, 2 H), 5.92 (s, 2 H), 3.07 (t, *J* = 7.2 Hz, 2 H), 1.73 (sextet, *J* = 7.2 Hz, 2 H), 0.99 (t, *J* = 7.2 Hz, 3 H); HRMS (ESI) Calcd for C14H16N5O+ (M + H) + 270.13494; Found 270.13507.

Example 11 – Synthesis of compound 2d

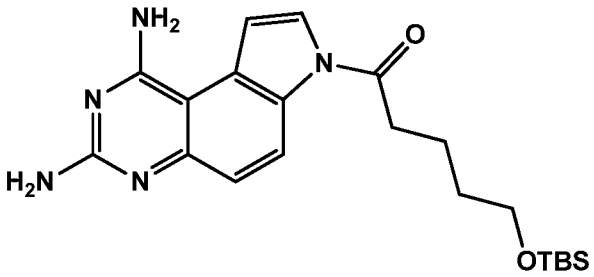
Compound **2d**



From 20.0 mg (0.10 mmol) of compound **1**, compound **2d** (21.0 mg, 78% yield) was obtained as a yellowish solid: mp 200-202 ^{o}C. 1H NMR (400 MHz, DMSO-d6) δ 8.59 (d, J = 9.2 Hz, 1 H), 8.14 (d, J = 4.0 Hz, 1 H), 7.41 (d, J = 3.6 Hz, 1 H), 7.18 (d, J = 9.2 Hz, 1 H), 6.90 (s, 2 H), 5.92 (s, 2 H), 3.63 (septet, J = 6.8 Hz, 1 H), 1.24 (d, J = 6.8 Hz, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 176.5, 162.3, 160.0, 151.5, 129.4, 126.5, 124.0, 122.2, 121.8, 108.1, 102.6, 32.9, 19.6; HRMS (ESI) Calcd for $C_{14}H_{16}N_5O^+$ (M + H)+ 270.13494; Found 270.13498.

Example 12 – Synthesis of compound 2e

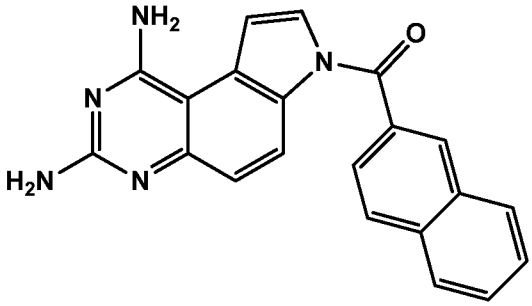
Compound **2e**



DCC (262 mg, 1.27 mmol) was added to a stirred solution of 5-(tert-Butyldimethylsilyloxy)pentanoic acid (247 mg, 1.06 mmol), NHS (146 mg, 1.27 mmol) and DMAP (14 mg, 0.117 mmol) in dry THF (5 mL) at 0 °C. The resulting mixture was stirred for 24 h at room temperature. The solid was filtered off and the solvent was removed *in vacuo*. The residue was purified by column chromatography on silica gel, eluting with 2:1 Hexane:EtOAc to give 3e (280 mg, 80% yield) as a colorless oil: 1H NMR (400 MHz, CDCl3) δ 3.64 (t, J = 6.4 Hz, 2 H), 2.84-2.83 (m, 4 H), 2.64 (t, J = 7.6 Hz, 2 H), 1.85-1.78 (m, 2 H), 1.65-1.58 (m, 2 H), 0.88 (s, 9 H), 0.04 (s, 6 H); 13C NMR (100 MHz, CDCl3) δ 169.3, 168.8, 62.5, 31.8, 30.8, 26.1, 25.7, 21.4, 18.4, -5.2; HRMS (ESI) Calcd for $C_{15}H_{28}NO_5Si^+$ $(M + H)^+$ 330.17313; Found 330.17282. From 100 mg (0.50 mmol) of **1**, following the representative procedure above, however, carboxylic NHS ester $TBSO(CH_2)_4COOSu$ **3e** was used instead of anhydride and when the reaction was complete, the solvent was removed and the residue was purified by column chromatography on silica gel, eluting with THF to give the desired compound **2e** (135 mg, 65% yield) as a yellowish solid: mp 173-175 °C. 1H NMR (400 MHz, DMSO-d6) δ 8.58 (d, J = 9.2 Hz, 1 H), 8.04 (d, J = 4.0 Hz, 1 H), 7.39 (d, J = 4.0 Hz, 1 H), 7.18 (d, J = 9.2 Hz, 1 H), 6.92 (brs, 2 H), 5.94 (s, 2 H), 3.64 (t, J = 6.4 Hz, 2 H), 3.11 (t, J = 6.8 Hz, 2 H), 1.78-1.72 (m, 2 H), 1.65-1.55 (m, 2 H), 0.86 (s, 9 H), 0.03 (s, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 172.5, 162.2, 159.9, 151.4, 129.3, 126.6, 123.9, 122.0, 121.7, 107.8, 102.7, 62.3, 34.6, 31.6, 25.9, 20.8, 18.0, -5.3; HRMS (ESI) Calcd for $C_{21}H_{32}N_5O_2Si^+$ $(M + H)^+$ 414.23198; Found 414.23187.

Example 13 – Synthesis of compound 2f

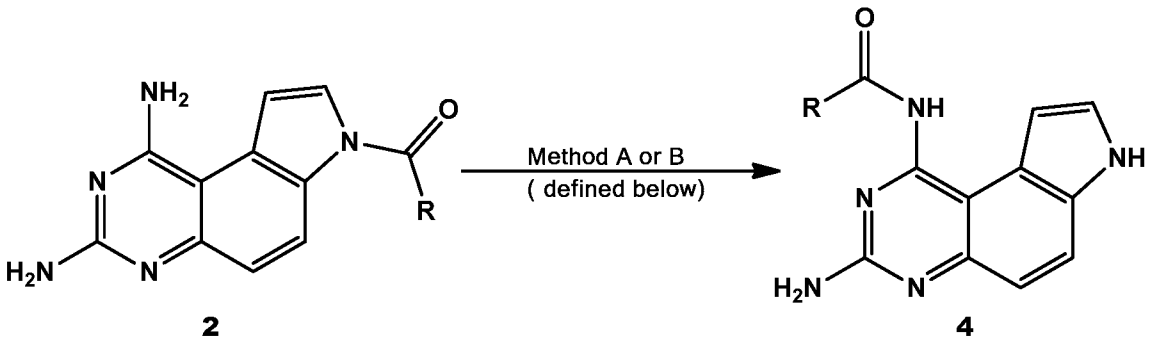
Compound **2f**



NHS (748 mg, 6.5 mmol) and EDCI · HCl (1.25 g, 6.5 mmol) was added to a stirred solution of 2-naphthoic acid (861 mg, 5.0 mmol) in dry DMF (8 mL). The resulting mixture was stirred overnight. Then the solvent was removed and the residue was treated with H_2O (15 mL). The white solid was collected by filtration to give compound **3f** (1.31 g, 97 % yield): mp 148-150 ^{o}C. 1H NMR (400 MHz, DMSO-d6) δ 8.87 (s, 1 H), 8.25 (d, J = 8.4 Hz, 1 H), 8.17 (d, J = 8.8 Hz, 1 H), 8.09 (d, J = 8.4 Hz, 1 H), 8.04 (d, J = 8.4 Hz, 1 H), 7.78 (t, J = 7.6 Hz, 1 H), 7.70 (t, J = 7.2 Hz, 1 H), 2.93 (s, 4 H). 13C NMR (100 MHz, DMSO-d6) δ 170.5, 162.0, 135.9, 132.5, 132.0, 130.0, 129.8, 129.4, 128.0, 127.7, 124.5, 121.7, 25.6. From 100 mg (0.50 mmol) of 1, following the representative procedure above, however, carboxylic NHS ester succinimidyl 2-naphthoate **3f** was used instead of anhydride. Compound **2f** (160 mg, 91% yield) was obtained as a yellowish solid: mp 188-190 ^{o}C. 1H NMR (400 MHz, DMSO-d6) δ 8.50 (d, J = 9.2 Hz, 1 H), 8.43 (s, 1 H), 8.16-8.13 (m, 2 H), 8.08 (d, J = 8.0 Hz, 1 H), 7.87 (d, J = 8.8 Hz, 1 H), 7.72 (t, J = 8.0 Hz, 1 H), 7.67 (t, J = 7.6 Hz, 1 H), 7.61 (d, J = 3.6 Hz, 1 H), 7.44 (d, J = 3.2 Hz, 1 H), 7.25 (d, J = 9.2 Hz, 1 H), 6.94 (s, 2 H), 5.98 (s, 2 H); 13C NMR (100 MHz, DMSO-d6) δ 168.5, 162.3, 160.0, 151.6, 134.5, 131.9, 131.0, 130.6, 129.8, 129.3, 128.7, 128.6, 128.5, 127.9, 127.3, 125.5, 124.4, 122.0, 121.5, 108.2, 102.7; HRMS (ESI) Calcd for C21H16N5O+ (M + H)+ 354.13494; Found 354.13490.

Example 14 – Common procedure for N^1 acylation reaction

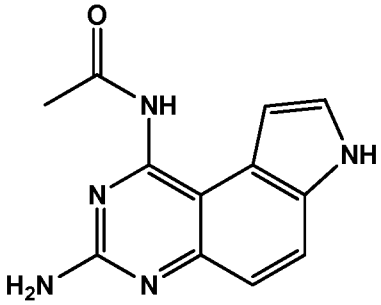
The following reaction shows the common reaction for the synthesis of compounds of series **4**, detailed in Examples 15-20 below. Method A and method B for each compound are described in the corresponding example.



2 4

Example 15 – Synthesis of Compound 4a

Compound **4a**



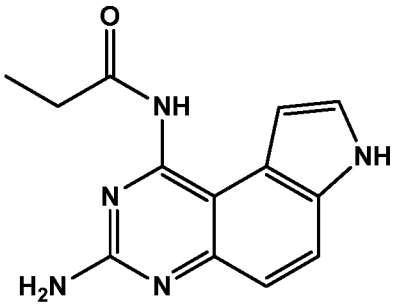
Method A: NaH (6.0 mg, 0.15 mmol) was added to a stirred suspension of compound **2a** (33 mg, 0.137 mmol) in dry DMF (3 mL) under argon atmosphere at 25 ^{o}C. The reaction mixture was stirred at 25 ^{o}C for 2 h, when a few drops of water was added to quench the reaction. Then the solvents were removed and the residue was purified by column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA to give a sticky solid, which was treated with water (1 mL) at room temperature for 1 hour. Then the solid was collected by filtration to give the desired product **4a** (8.0 mg, 23% yield) as a yellowish solid: mp 236-238 ^{o}C. 1H NMR (400 MHz, DMSO-d6) δ 11.61 (s, 1 H), 10.22 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.43 (brs, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 6.70 (brs, 1 H), 6.38 (s, 2 H), 2.20 (s, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 169.7, 159.0, 157.0, 152.1, 130.6, 124.8, 121.2, 119.9, 118.8, 109.2, 103.5, 23.5; HRMS (ESI) Calcd for $C_{12}H_{12}N_5O^+$ $(M + H)^+$ 242.10364; Found 242.10361.

Method B: A suspension of compound **2a** (40 mg, 0.166 mmol) in dry DMF under Ar was cooled to 0 ^{o}C. LiHMDS (182 µl, 1M, 0.182 mmol) was added dropwise to the suspension. The

reaction mixture was stirred for 40 min at 0 °C and then a few drops of water were added to quench the reaction. Then the solvents were removed in vacuo and the residue was purified by column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA to give compound 4a (18 mg, 45% yield) as a yellowish solid.

Example 16 – Synthesis of Compound 4b

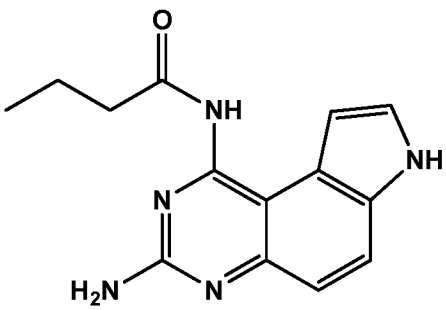
Compound **4b**



Method A. From 53.0 mg (0.21 mmol) of **2b**, and after column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA, compound **4b** (17.0 mg, 32% yield) was obtained as a yellowish solid: mp 226-228 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.59 (s, 1 H), 10.19 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.42 (t, J = 2.8 Hz, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 6.68 (brs, 1 H), 6.39 (s, 2 H), 2.53 (q, J = 7.6 Hz, 2 H), 1.12 (t, J = 7.6 Hz, 3H); 13C NMR (100 MHz, DMSO-d6) δ 173.1, 159.1, 157.1, 152.1, 130.6, 124.8, 121.2, 119.9, 118.8, 109.3, 103.6, 29.0, 9.4; HRMS (ESI) Calcd for $C_{13}H_{14}N_5O^+$ $(M + H)^+$ 256.11929; Found 256.11941.

Example 17 – Synthesis of Compound 4c

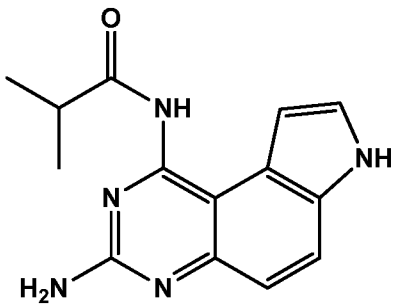
Compound **4c**



Method A. From 47.0 mg (0.175 mmol) of **2c**, and after column chromatography on silica gel, eluting with 15:1 DCM:MeOH containing 1% DIPEA, compound **4c** (15.0 mg, 32% yield) was obtained as a yellowish solid: mp 224-226 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.59 (s, 1 H), 10.17 (s, 1 H), 7.80 (d, J = 8.8 Hz, 1 H), 7.42 (t, J = 2.8 Hz, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 6.70 (brs, 1 H), 6.36 (s, 2 H), two protons were buried in residual DMSO signal, 1.65 (sextet, J = 7.6 Hz, 2 H), 0.96 (t, J = 7.2 Hz, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 172.2, 159.1, 157.1, 152.1, 130.6, 124.7, 121.2, 119.9, 118.8, 109.4, 103.6, 37.7, 18.2, 13.9; HRMS (ESI) Calcd for $C_{14}H_{16}N_5O^+$ $(M + H)^+$, 270.13494; Found 270.13497.

Example 18 – Synthesis of compound 4d

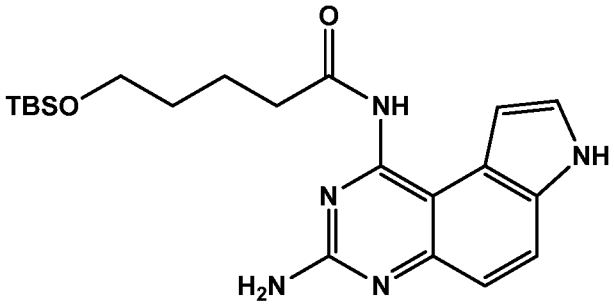
Compound **4d**



Method A. From 52.0 mg (0.193 mmol) of compound **2d**, and after column chromatography on silica gel, eluting with 10:1 DCM:MeOH containing 1% DIPEA, compound **4d** (15.0 mg, 27% yield) was obtained as a yellowish solid: mp 232-234 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.59 (s, 1 H), 10.21 (s, 1 H), 7.80 (d, J = 9.2 Hz, 1 H), 7.43 (t, J = 2.8 Hz, 1 H), 7.17 (d, J = 9.2 Hz, 1 H), 6.70 (brs, 1 H), 6.41 (s, 2 H), 2.85 (septet, J = 6.8 Hz, 1 H), 1.18 (d, J = 7.2 Hz, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 175.9, 159.1, 157.3, 152.2, 130.6, 124.7, 121.2, 119.9, 118.8, 109.7, 103.6, 34.3, 19.3; HRMS (ESI) Calcd for $C_{14}H_{16}N_5O^+$ $(M + H)^+$, 270.13494; Found 270.13467.

Example 19 – Synthesis of compound 4e

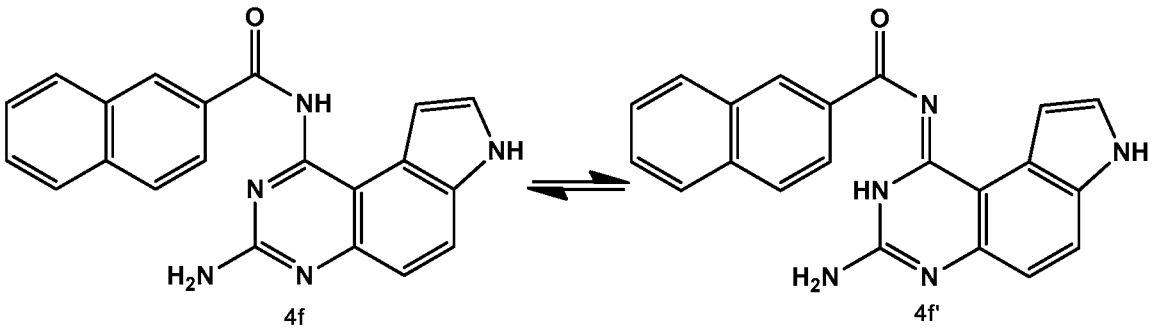
Compound **4e**



Method A. From 261 mg (0.631 mmol) of **2e**, and after column chromatography on silica gel, eluting with 20:1 DCM:MeOH containing 1% DIPEA, compound **4e** (100 mg, 38% yield) was obtained as a yellowish solid: mp 172-174 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.61 (s, 1 H), 10.23 (s, 1 H), 7.82 (d, J = 9.2 Hz, 1 H), 7.42 (s, 1 H), 7.17 (d, J = 8.8 Hz, 1 H), 6.70 (s, 1 H), 6.48 (brs, 2 H), 3.61 (t, J = 6.0 Hz, 2 H), two protons were buried in residual DMSO signal, 1.73-1.62 (m, 2 H), 1.58-1.52 (m, 2 H), 0.86 (s, 9 H), 0.03 (s, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 172.3, 159.1, 157.1, 152.2, 130.7, 124.7, 121.3, 120.0, 118.9, 109.4, 103.6, 62.3, 35.5, 32.0, 25.9, 21.3, 18.0, -5.2; HRMS (ESI) Calcd for C21H32N5O2Si+ (M + H)+ 414.23198; Found 414.23145.

Example 20 – Synthesis of compound 4f and 4f'

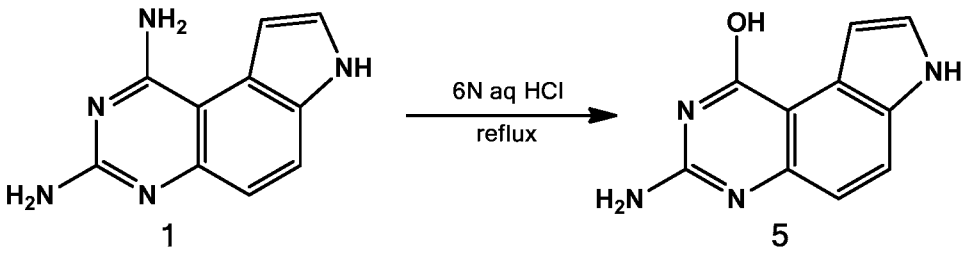
Compound **4f** and **4f'**



Method B. From 80 mg (0.226 mmol) of **2f**, and after column chromatography on silica gel, eluting with 20:1 DCM:MeOH containing 1% DIPEA, a yellow solid was obtained (40 mg, 50% yield), which exists as a 1:1 tautomeric mixture of 4f and 4f' in DMSO-d6: mp 230-232 oC. 1H NMR (400 MHz, DMSO-d6) δ 14.37 (s, 1 H), 11.64 (s, 1 H), 11.57 (s, 1 H), 11.11 (s, 1 H), 8.93

(s, 1 H), 8.78 (s, 1 H), 8.44 (d, J = 8.4 Hz, 1 H), 8.14-8.02 (m, 7 H), 7.87-7.83 (m, 2 H), 7.78 (brs, 1 H), 7.71-7.62 (m, 5 H), 7.30 (brs, 1 H), 7.25-7.23 (m, 3 H), 7.09 (d, J = 8.4 Hz, 1 H), 6.57 (s, 1 H), 6.50 (s, 2 H); 13C NMR (100 MHz, DMSO-d6) δ 177.7, 166.4, 159.3, 158.7, 157.8, 152.3, 149.7, 148.4, 135.8, 134.7, 132.4, 131.6, 131.2, 130.7, 129.7, 129.3, 128.8, 128.4, 128.2, 127.9, 127.8, 127.0, 126.7, 126.3, 125.6, 125.0, 124.4, 123.0, 121.5, 120.1, 118.9, 118.5, 110.6, 107.7, 105.4, 103.4; HRMS (ESI) Calcd for $C_{21}H_{32}N_5O_2Si^+$ $(M + H)^+$ 354.13494; Found 354.13475. When a mixture of **4f** and **4f'** in DMSO-d6 (375 µl) was treated with aq. NaOH (125µl, 0.4 N), it became **4f** and all the active protons disappeared due to the H-D exchange: 1H NMR (400 MHz) 8.61 (s, 1 H), 8.33 (dd, J = 8.4 Hz, 1.2 Hz, 1 H), 7.97-7.86 (m, 3 H), 7.64 (d, J = 8.8 Hz, 1 H), 7.53-7.48 (m, 2 H), 7.15 (d, J = 2.8 Hz, 1 H), 7.00 (d, J = 2.4 Hz, 1 H), 6.94 (d, J = 8.8 Hz, 1 H); 13C NMR (100 MHz) 171.6, 166.6, 159.2, 148.9, 139.4, 134.1, 133.6, 133.2, 129.3, 128.4, 128.1, 127.4, 127.3, 127.1, 126.6, 123.6, 120.7, 117.0, 110.9, 104.9.

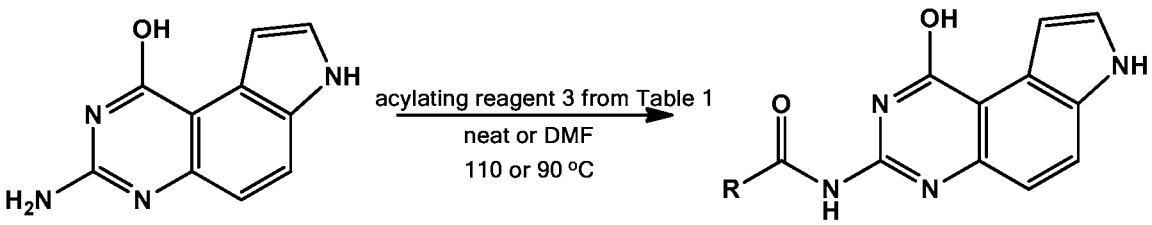
Example 21 - Synthesis of Intermediate compound 5 from compound 1



A suspension of compound **1** (200 mg, 1.0 mmol) in 6 N aq. HCl (10.0 mL) was heated under reflux overnight. The reaction mixture was adjusted to pH = 10-11 with 10.0 N NaOH and the resulting black solution was stirred for 1 hour. Then the pH was adjusted to between 6 and 7 and the precipitate was collected by filtration to give the desired product 5 (200 mg, 99% yield) as a brown solid: mp 284-286 °C; 1H NMR (400 MHz, DMSO-d6) δ 11.34 (s, 1 H), 10.95 (brs, 1 H), 7.64 (d, J = 8.8 Hz, 1 H), 7.41 (t, J = 2.4 Hz, 1 H), 7.10 (s, 1 H), 6.97 (d, J = 8.4 Hz, 1 H), 6.17 (s, 2 H); 13C NMR (100 MHz, DMSO-d6) δ 162.7, 150.9, 142.9, 131.8, 127.0, 123.9, 119.2, 115.2, 107.6, 102.5; HRMS (ESI) Calcd for $C_{10}H_9N_4O^+$ $(M + H)^+$ 201.07709; Found 201.07708.

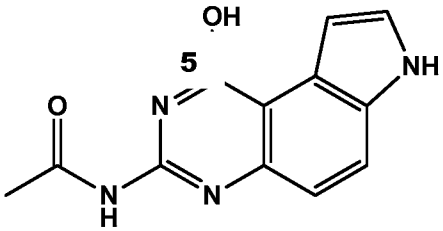
Example 22 – Common procedure for the reaction of compound 5 with anhydride or carboxylic NHS ester to prepare N3 acylated intermediates of series 6.

The following reaction shows the common reaction for the synthesis of intermediate compounds of series **6**, detailed in Examples 23-28 below.



Example 23 – Synthesis of intermediate compound 6a

Compound **6a**

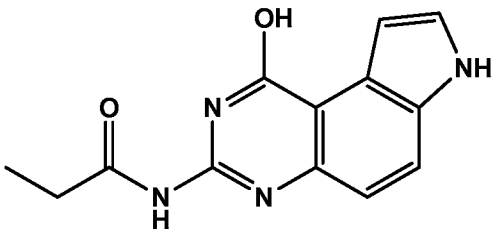


6

A suspension of compound **5** (50 mg, 0.25 mmol) in Ac_2O (3 mL) was stirred at 110 °C for 1.5 h. The reaction mixture was cooled to room temperature. The excess of acetic anhydride was removed and the residue was treated with DCM (3 mL). The solid was collected by filtration to give the desired product 6a (50 mg, 83% yield) as a brown solid, which was used for the next step without further purification: mp 266-268 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.95 (s, 1 H), 11.61 (s, 1 H), 11.49 (s, 1 H), 7.83 (d, J = 8.4 Hz, 1 H), 7.55 (t, J = 2.8 Hz, 1 H), 7.23-7.20 (m, 2 H), 2.17 (s, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 173.4, 160.4, 144.8, 144.7, 132.8, 127.3, 123.7, 119.6, 119.0, 111.1, 103.0, 23.8; HRMS (ESI) Calcd for $C_{12}H_9N_4O_2$ $(M - H)^-$ 241.07310; Found 241.07294.

Example 24 – Synthesis of intermediate compound 6b

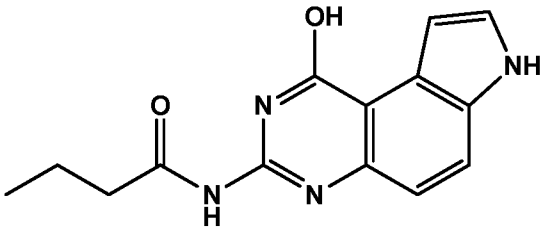
Compound **6b**



From 30 mg of compound **5**, compound **6b** (32 mg, 84% yield) was obtained as a brown solid: mp 262-264 °C. 1H NMR (400 MHz, DMSO-d6) δ 12.01 (s, 1 H), 11.58 (s, 1 H), 11.46 (s, 1 H), 7.83 (d, J = 8.8 Hz, 1 H), 7.55 (t, J = 2.4 Hz, 1 H), 7.26-7.18 (m, 2 H), 2.47 (q, J = 7.2 Hz, 2 H), 1.09 (t, J = 7.6 Hz, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 176.9, 160.4, 144.9, 144.8, 132.7, 127.3, 123.7, 119.6, 119.0, 111.1, 103.0, 29.4, 8.9; HRMS (ESI) Calcd for $C_{13}H_{13}N_4O_2$ + $(M + H)^+$ 257.10330; Found 257.10318.

Example 25 – Synthesis of intermediate compound 6c

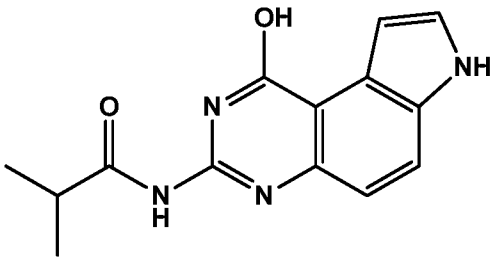
Compound **6c**



From 35 mg of compound **5**, compound **6c** (40 mg, 85% yield) was obtained as a brown solid: mp 270-272 °C. 1H NMR (400 MHz, DMSO-d6) δ 12.01 (s, 1 H), 11.65 (s, 1 H), 11.45 (s, 1 H), 7.83 (d, J = 9.2 Hz, 1 H), 7.55 (t, J = 2.4 Hz, 1 H), 7.26-7.21 (m, 2 H), 2.44 (t, J = 7.6 Hz, 2 H), 1.63 (sextet, J = 7.2 Hz, 2 H), 0.93 (t, J = 7.2 Hz, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 176.1, 160.4, 144.9, 144.8, 132.8, 127.3, 123.7, 119.6, 119.0, 111.1, 103.0, 37.9, 18.1, 13.5; HRMS (ESI) Calcd for $C_{14}H_{15}N_4O_2$ + $(M + H)^+$ 271.11895; Found 271.11874.

Example 26 – Synthesis of intermediate compound 6d

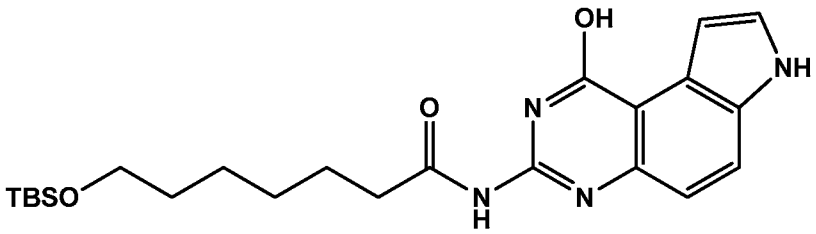
Compound **6d**



From 30 mg of compound **5**, compound **6d** (27 mg, 67% yield) was obtained as a brown solid: mp 240-242 °C. 1H NMR (400 MHz, DMSO-d6) δ 12.01 (s, 1 H), 11.65 (s, 1 H), 11.45 (s, 1 H), 7.84 (d, J = 8.8 Hz, 1 H), 7.55 (t, J = 2.8 Hz, 1 H), 7.28-7.20 (m, 2 H), 2.77 (septet, J = 7.2 Hz, 1 H), 1.13 (d, J = 7.2 Hz, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 180.1, 160.3, 145.0, 144.8, 132.8, 127.3, 123.7, 119.5, 119.0, 111.1, 103.0, 34.8, 19.0; HRMS (ESI) Calcd for $C_{14}H_{15}N_4O_2$ + $(M + H)^+$, 271.11895; Found 271.11881.

Example 27 – Synthesis of intermediate compound 6e

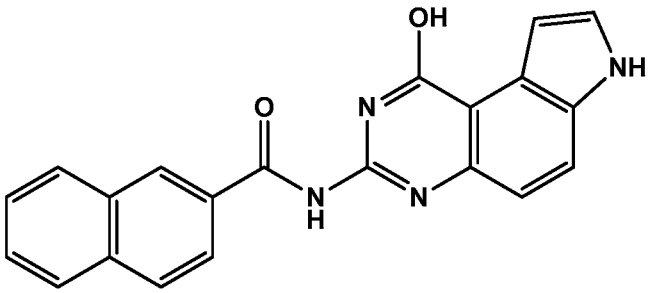
Compound **6e**



A mixture of compound **5** (100.0 mg, 0.5 mmol) and compound **3e** (247 mg, 0.75 mmol) in dry DMF (5 mL) was stirred at 90 °C for 4 h. Then the solvent was removed and the residue was purified by column chromatography on silica gel, eluting with 2:1 DCM:EtOAc containing 1% DIPEA to give compound 6e, which was further washed with Et_2O (3 mL) to give the desired compound as a white solid (130 mg, 62% yield): mp 188-190 °C. 1H NMR (400 MHz, DMSO-d6) δ 12.0 (s, 1 H), 11.6 (s, 1 H), 11.5 (s, 1 H), 7.83 (d, J = 8.8 Hz, 1 H), 7.55 (brs, 1 H), 7.23-7.21 (m, 2 H), 3.60 (t, J = 6.0 Hz, 2 H), 2 protons were buried in residual DMSO signal, 1.70-1.60 (m, 2 H), 1.55-1.45 (m, 2 H), 0.86 (s, 9 H), 0.03 (s, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 176.2, 160.4, 144.9, 144.8, 132.8, 127.3, 123.7, 119.6, 119.0, 111.1, 103.0, 62.1, 35.7, 31.6, 25.9, 21.0, 18.0, -5.2; HRMS (ESI) Calcd for $C_{21}H_{31}N_4O_3Si^+$ $(M + H)^+$ 415.21599; Found 415.21555.

Example 28 – Synthesis of intermediate compound 6f

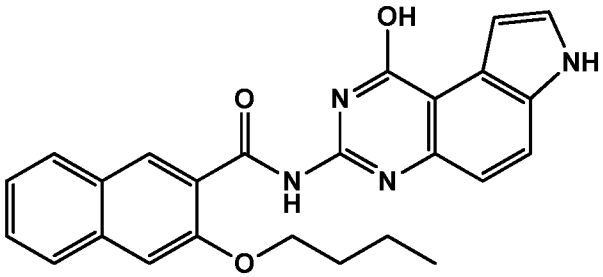
Compound **6f**



Following the same procedure as that described for **6e**. From 40 mg of **5**, and after column chromatography on silica gel, eluting with 3:1 EtOAc:DCM containing 1% DIPEA, compound **6f** (40 mg, 67% yield) was obtained as a yellowish solid: mp 226-228 °C. 1H NMR (400 MHz, DMSO-d6) δ 12.5 (brs, 1 H), 11.9 (brs, 1 H), 11.7 (s, 1 H), 8.8 (s, 1 H), 8.16 (d, J = 8.0 Hz, 1 H), 8.10-8.01 (m, 3 H), 7.89 (d, J = 8.8 Hz, 1 H), 7.69-7.60 (m, 3 H), 7.34 (d, J = 8.4 Hz, 1 H), 7.25 (s, 1 H); 13C NMR (100 MHz, DMSO-d6) δ 160.7, 134.8, 132.9, 132.1, 129.7, 129.4, 128.4, 128.0, 127.9, 127.7, 127.0, 124.8, 123.9, 119.8, 102.9; HRMS (ESI) Calcd for $C_{21}H_{15}N_4O_2$ + (M + H)$^+$ 355.11895; Found 355.11893.

Example 29 – Synthesis of compound 6g

Compound 6g

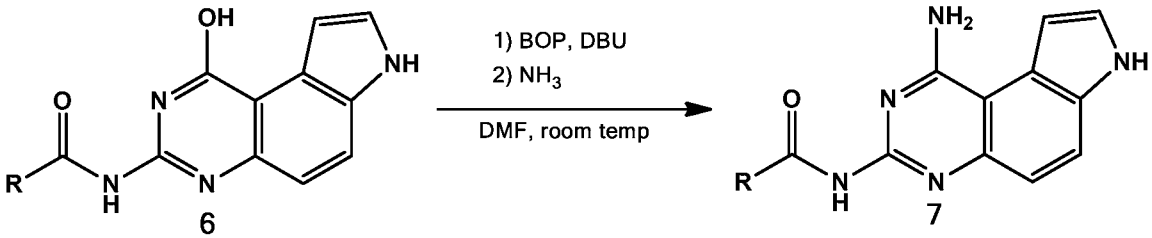


Synthesis of 3-Butoxy-*N*-(1-hydroxy-7*H*-pyrrolo[3,2-*f*]quinazolin-3-yl)-2-naphthamide (6g). From 100 mg of **5**, **6g** was obtained as a yellow solid (168 mg, 79% yield). mp 280-282 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 12.06 (s, 1 H), 11.67 (s, 1 H), 11.37 (s, 1 H), 8.48 (s, 1 H), 8.03 (d, *J* = 8.4 Hz, 1 H), 7.90 (d, *J* = 8.4 Hz, 1 H), 7.87 (d, *J* = 8.8 Hz, 1 H), 7.62-7.57 (m, 3 H), 7.47-7.43 (m, 1 H), 7.26-7.24 (m, 2 H), 4.28 (t, *J* = 6.0 Hz, 2 H), 1.90 (quintet, *J* = 6.0 Hz, 2 H), 1.62 (sextet, *J* = 7.2 Hz, 2 H), 1.01 (t, *J* = 7.2 Hz, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ 167.1, 160.4, 153.5, 144.6,

144.3, 136.0, 132.9, 132.0, 128.9, 128.8, 127.4, 127.3, 126.5, 124.7, 123.7, 122.5, 119.6, 119.2, 111.3, 107.9, 103.7, 68.8, 30.8, 19.1, 13.9.

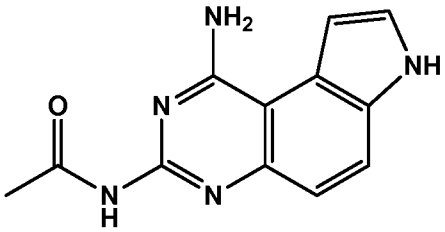
Example 30– Common procedure for the synthesis of N3 acylated compounds of series 7 from intermediate compounds of series 6.

The following reaction shows the common reaction for the synthesis of compounds of series **7**, detailed in Examples 30-35 below.



Example 31 – Synthesis of compound 7a

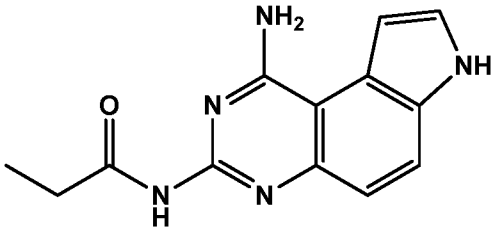
Compound **7a**



BOP (83.1 mg, 0.188 mmol) and DBU (32.3 µl, 0.216 mmol) were added to a stirred solution of 6a (35.0 mg, 0.144 mmol) in dry DMF (3 mL). The resulting reaction mixture was stirred for 4 h, when NH3 (7 N in MeOH, 0.82 mL, 5.7 mmol) was added. The reaction mixture was stirred at 25 ^{o}C for 16 h. The solvents were removed and the residue was purified by column chromatography on silica gel, eluting with 1.5:1 EtOAc:THF containing 1% DIPEA to give a yellow solid, which was further treated with DCM (2 mL) and collected by filtration to give the desired compound **7a** (17.0 mg, 48%) as a yellowish solid: mp 260-262 ^{o}C. 1H NMR (400 MHz, DMSO-d6) δ 11.79 (s, 1 H), 9.76 (s, 1 H), 7.84 (d, J = 8.4 Hz, 1 H), 7.56 (brs, 1 H), 7.26 (d, J = 8.8 Hz, 1 H), 7.23 (brs, 1 H), 7.11 (brs, 2 H), 2.25 (s, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 169.7, 161.9, 152.5, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 102.5, 24.6; HRMS (ESI) Calcd for $C_{12}H_{12}N_5O^+$ $(M + H)^+$ 242.10364; Found 242.10359.

Example 32 – Synthesis of compound 7b

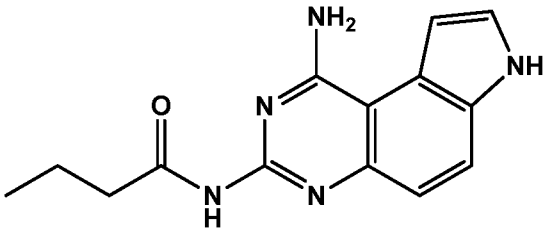
Compound **7b**



From 32 mg of **6b**, and after column chromatography on silica gel, eluting with 1.5:1 EtOAc:THF containing 1% DIPEA, compound **7b** (14 mg, 44% yield) was obtained as a yellowish solid: mp 228-230 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.79 (s, 1 H), 9.73 (s, 1 H), 7.83 (d, J = 8.8 Hz, 1 H), 7.56 (t, J = 2.4 Hz, 1 H), 7.27 (d, J = 8.8 Hz, 1 H), 7.24 (brs, 1 H), 7.10 (brs, 2 H), 2.56 (q, J = 7.6 Hz, 2 H), 1.07 (t, J = 7.6 Hz, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 172.8, 161.9, 152.4, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 104.8, 102.5, 29.5, 9.6; HRMS (ESI) Calcd for $C_{13}H_{14}N_5O^+$ $(M + H)^+$ 256.11929; Found 256.11913.

Example 33- Synthesis of compound 7c

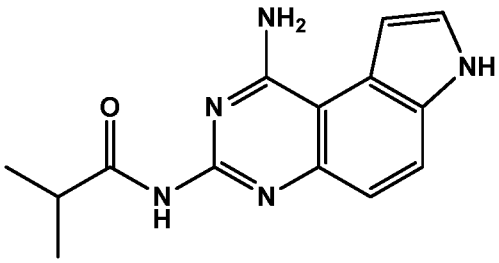
Compound **7c**



From 35 mg of **6c**, and after column chromatography on silica gel, eluting with 1.5:1 EtOAc:THF containing 1% DIPEA, compound **7c** (13 mg, 37% yield) was obtained as a yellowish solid: mp 262-264 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.79 (s, 1 H), 9.74 (s, 1 H), 7.83 (d, J = 8.8 Hz, 1 H), 7.56 (t, J = 2.4 Hz, 1 H), 7.27 (d, J = 9.2 Hz, 1 H), 7.24 (brs, 1 H), 7.09 (brs, 2 H), two protons were buried in residual DMSO signal, 1.60 (sextet, J = 7.2 Hz, 2 H), 0.93 (t, J = 7.6 Hz, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 171.8, 161.9, 152.4, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 104.8, 102.5, 38.1, 18.4, 13.8; HRMS (ESI) Calcd for $C_{14}H_{16}N_5O^+$ $(M + H)^+$ 270.13494; Found 270.13474.

Example 34 – Synthesis of compound 7d

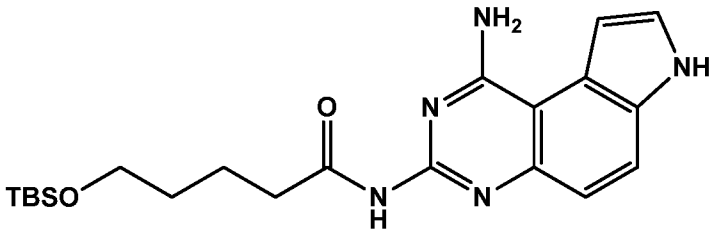
Compound **7d**



From 35 mg of **6d**, and after column chromatography on silica gel, eluting with 2:1 EtOAc:THF containing 1% DIPEA, compound **7d** (14 mg, 40% yield) was obtained as a yellowish solid: mp 288-290 oC. 1H NMR (400 MHz, DMSO-d6) δ 11.79 (s, 1 H), 9.79 (s, 1 H), 7.83 (d, J = 9.2 Hz, 1 H), 7.56 (t, J = 2.8 Hz, 1 H), 7.27 (d, J = 9.2 Hz, 1 H), 7.24 (brs, 1 H), 7.08 (brs, 2 H), 2.92 (brs, 1 H), 1.08 (d, J = 6.8 Hz, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 175.3, 162.0, 152.4, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 105.0, 102.6, 34.1, 19.5; HRMS (ESI) Calcd for $C_{14}H_{16}N_5O^+$ (M + H)$^+$ 270.13494; Found 270.13484.

Example 35 – Synthesis of compound 7e

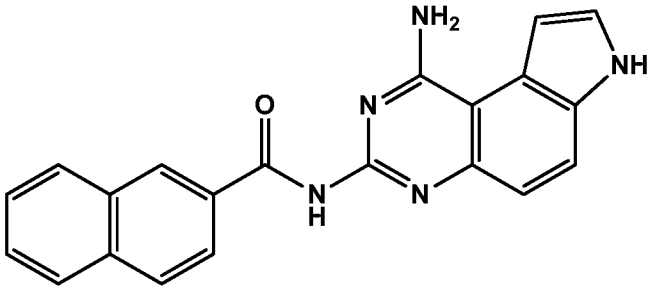
Compound **7e**



From 80 mg of **6e**, and after column chromatography on silica gel, eluting with 4:1 EtOAc:THF containing 1% DIPEA, compound 7e (40 mg, 50% yield) was obtained as a light green solid after treating with NH_4OH (3 mL) and hexanes (3 mL) successively: mp 140-142 ^oC. 1H NMR (400 MHz, DMSO-d6) δ 11.77 (s, 1 H), 9.74 (s, 1 H), 7.83 (d, J = 8.8 Hz, 1 H), 7.56 (t, J = 2.4 Hz, 1 H), 7.26 (d, J = 9.2 Hz, 1 H), 7.23 (s, 1 H), 7.08 (brs, 2 H), 3.60 (t, J = 6.0 Hz, 2 H), two protons were buried in residual DMSO signal, 1.65-1.57 (m, 2 H), 1.54-1.47 (m, 2 H), 0.85 (s, 9 H), 0.02 (s, 6 H); 13C NMR (100 MHz, DMSO-d6) δ 172.0, 162.9, 152.4, 148.4, 131.9, 125.6, 120.0, 119.6, 119.4, 104.9, 102.6, 62.4, 35.9, 32.0, 25.9, 21.5, 18.0, -5.2; HRMS (ESI) Calcd for $C_{21}H_{32}N_5O_2Si^+$ (M + H)$^+$ 414.23198; Found 414.23145.

Example 36 – Synthesis of compound 7f

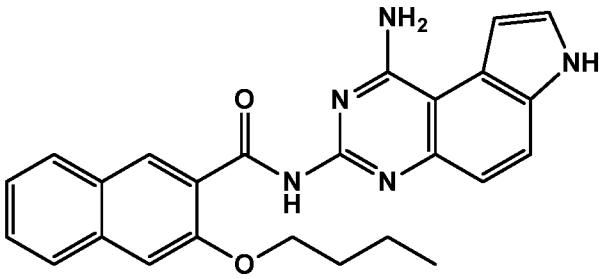
Compound **7f**



From 40 mg of 6f, and after column chromatography on silica gel, eluting with 20:1 EtOAc:THF containing 1% DIPEA, compound 7f (10 mg, 25% yield) was obtained as a yellow solid: mp 234-236 °C. 1H NMR (400 MHz, DMSO-d6) δ 11.85 (s, 1 H), 10.61 (brs, 1 H), 8.65 (s, 1 H), 8.08-7.99 (m, 4 H), 7.90 (d, J = 8.8 Hz, 1 H), 7.66-7.60 (m, 3 H), 7.39-7.20 (m, 4 H); 13C NMR (100 MHz, DMSO-d6) δ 162.3, 152.8, 134.4, 132.4, 132.3, 132.2, 129.2, 128.5, 127.9, 127.8, 127.7, 126.7, 125.9, 124.8, 119.8, 119.4, 105.3, 102.7; HRMS (ESI) Calcd for $C_{21}H_{16}N_5O^+$ $(M + H)^+$ 354.13494; Found 354.13478.

Example 37 – Synthesis of N-(1-Amino-7H-pyrrolo[3,2-f]quinazolin-3-yl)-3-butoxy-2-naphthamide 7g

Compound **7g**



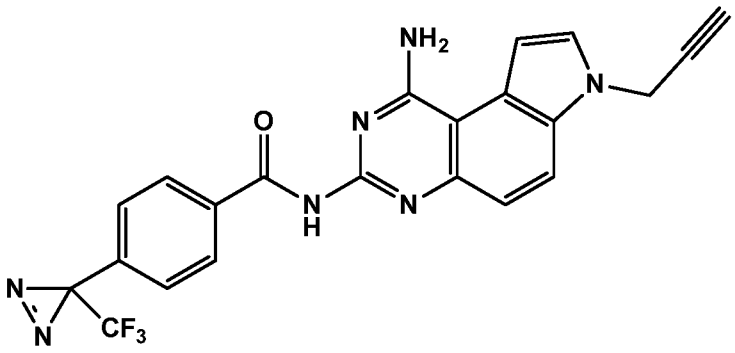
From 120 mg of **6g** and after column chromatography on silica gel, eluting with 2:1 DCM:THF, **7g** was obtained as a yellow solid (58 mg, 48% yield). mp 205-207 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ 11.88 (s,1 H), 10.62 (s, 1 H), 8.38 (s, 1 H), 8.00 (d, J = 8.0 Hz, 1 H), 7.90-7.87 (m, 2 H), 7.63 (t, J = 6.4 Hz, 1 H), 7.59-7.55 (m, 1 H), 7.52 (brs, 1 H), 7.44-7.30 (m, 5 H), 4.21 (brs, 2 H), 1.83 (brs, 2 H), 1.53 (brs, 2 H), 0.93 (t, J = 7.6 Hz, 3 H). ^{13}C NMR (100 MHz, DMSO-d_6) δ

164.1, 162.1, 153.7, 151.4, 145.9, 135.4, 132.3, 131.2, 128.6, 128.1, 127.6, 126.5, 126.3, 125.3, 124.4, 120.1, 119.4, 118.7, 107.5, 104.9, 102.5, 68.5, 30.6, 18.9, 13.8.

***Example 38 – Identification of nuclear lamins as potential molecular targets of* 7f**

Unbiased chemical proteomics experiments involving photocrosslinking MDA-MB-468 cells treated with probe compound **10**, clicking with a biotin-azide (biotin-N_3), streptavidin pulldown and mass spectroscopic analyses identified nuclear lamins as the molecular targets of **7f**. Nuclear lamins are type V intermediate filament proteins. In humans, there are three lamin genes (*LMNA*, *LMNB1* and *LMNB2*) encoding four major proteins: LMNA, LMNC, LMNB1 and LMNB2. To further confirm that lamins are the targets, the biotinylated proteins prepared as shown in Figure 5A were pulled down and analyzed by Western blot with individual antibodies (Figure 5B). This analysis clearly showed that LMNA/C and LMNB1 were pulled down and competed by **7f**.

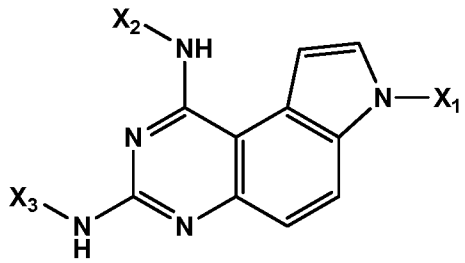
Probe compound **10**:



CLAIMS

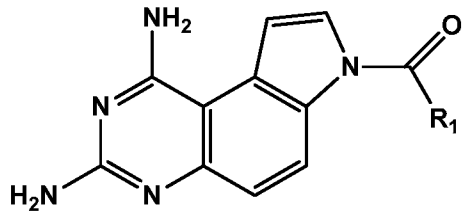
What is claimed is:

1. A compound of formula (I):



wherein X_1, X_2, and X_3 are independently H or acyl provided that not all of X_1, X_2, and X_3 are H.

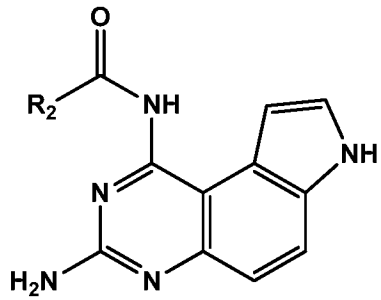
2. The compound of claim 1 wherein the compound is of formula (II),



and wherein R_1 is lower alkyl, ether, or aryl.

3. The compound of claim 2 wherein R_1 is methyl, ethyl, propyl, isopropyl, silyl ether, phenyl, substituted phenyl, naphthyl, or substituted naphthyl.

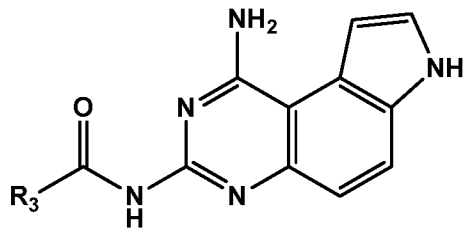
4. The compound of claim 1 comprising a compound of formula (III)



wherein R_2 is lower alkyl, ether, or aryl.

5. The compound of claim 4 wherein R_2 is methyl, ethyl, propyl, isopropyl, silyl ether, phenyl, substituted phenyl, naphthyl or substituted naphthyl.

6. The compound of claim 1 comprising a compound of formula (IV)



wherein R3 is lower alkyl, ether, or aryl.

7. The compound of claim 6 wherein R_3 is methyl, ethyl, propyl, isopropyl, silyl ether, phenyl, substituted phenyl, naphthyl, or substituted naphthyl.

8. The compound of claim 7 wherein R_3 is an ether substituted naphthyl.

9. The compound of claim 8 wherein R_3 is selected from an unsubstituted naphthyl and a 3-butoxy naphthyl.

10. A pharmaceutical composition comprising a therapeutically effective amount of the compound of any of claims 1-9 and a pharmaceutically acceptable salt.

11. The pharmaceutical composition of claim 10 further comprising a pharmaceutically acceptable carrier.

Figure 1

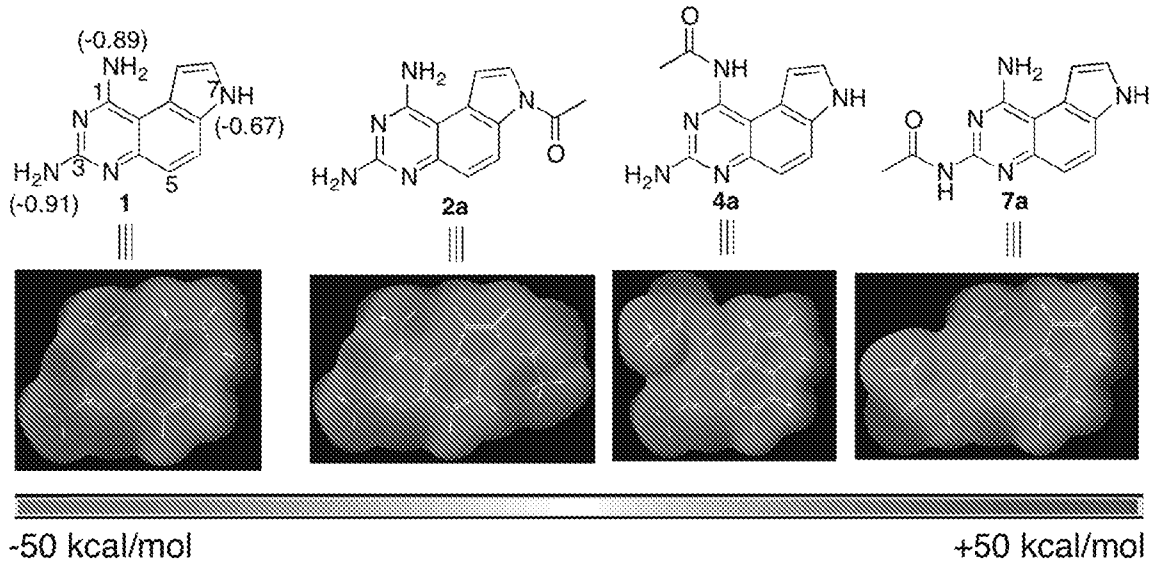


Figure 2

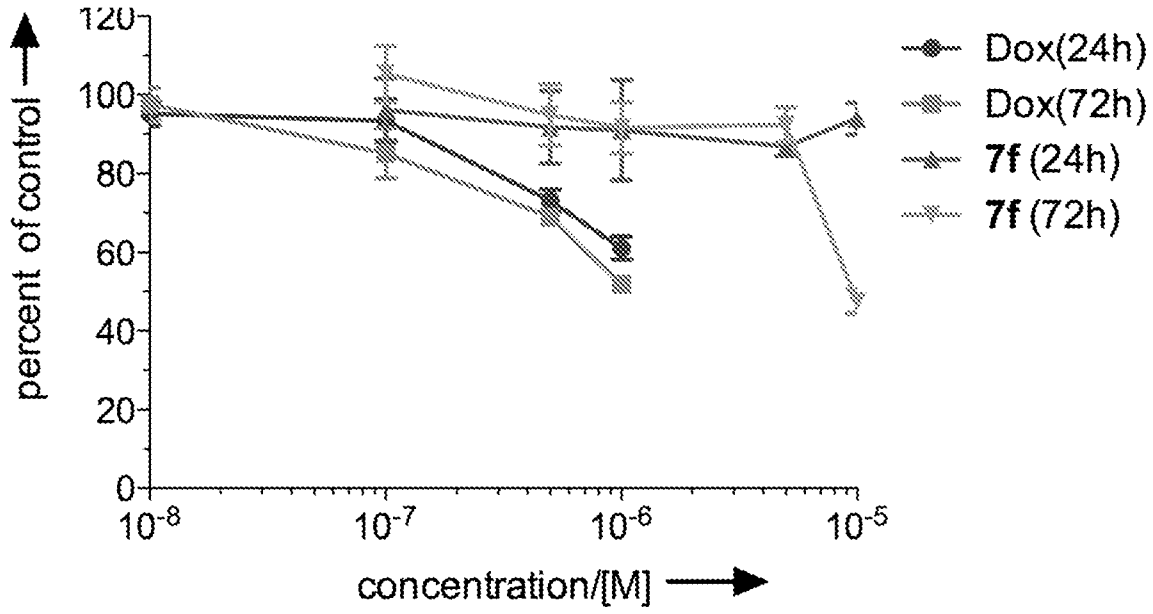


Figure 3

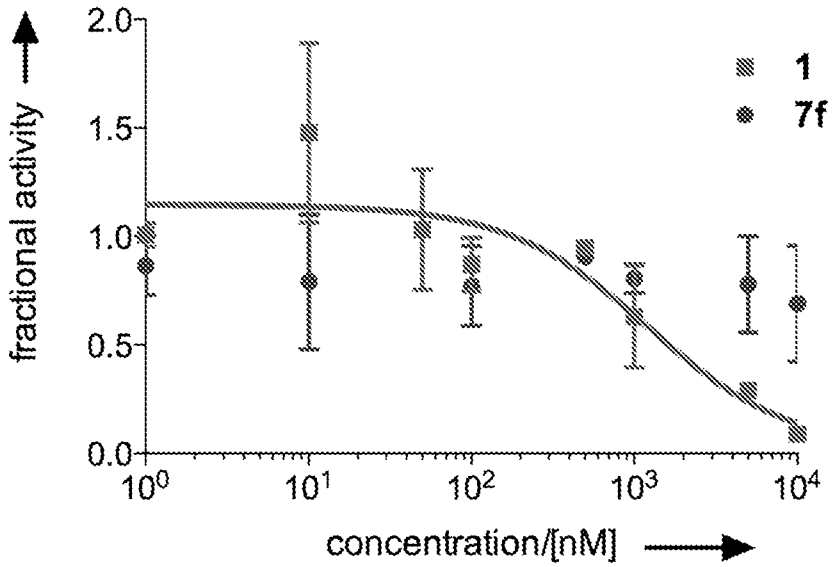


Figure 4

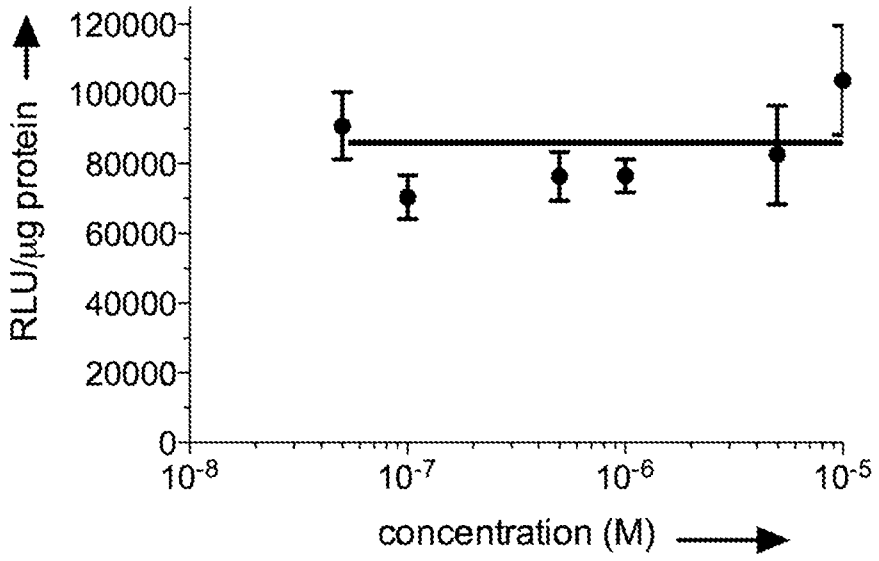


Figure 5A

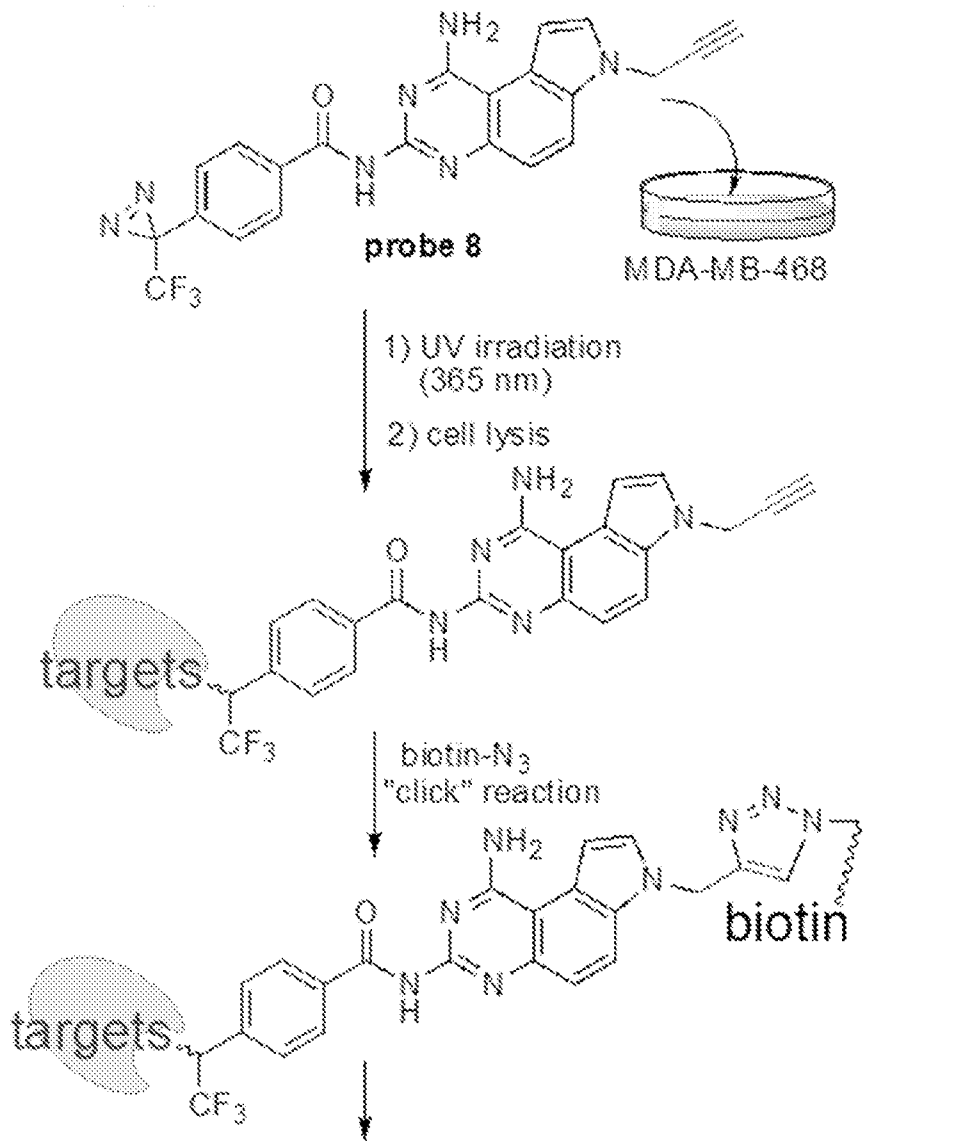
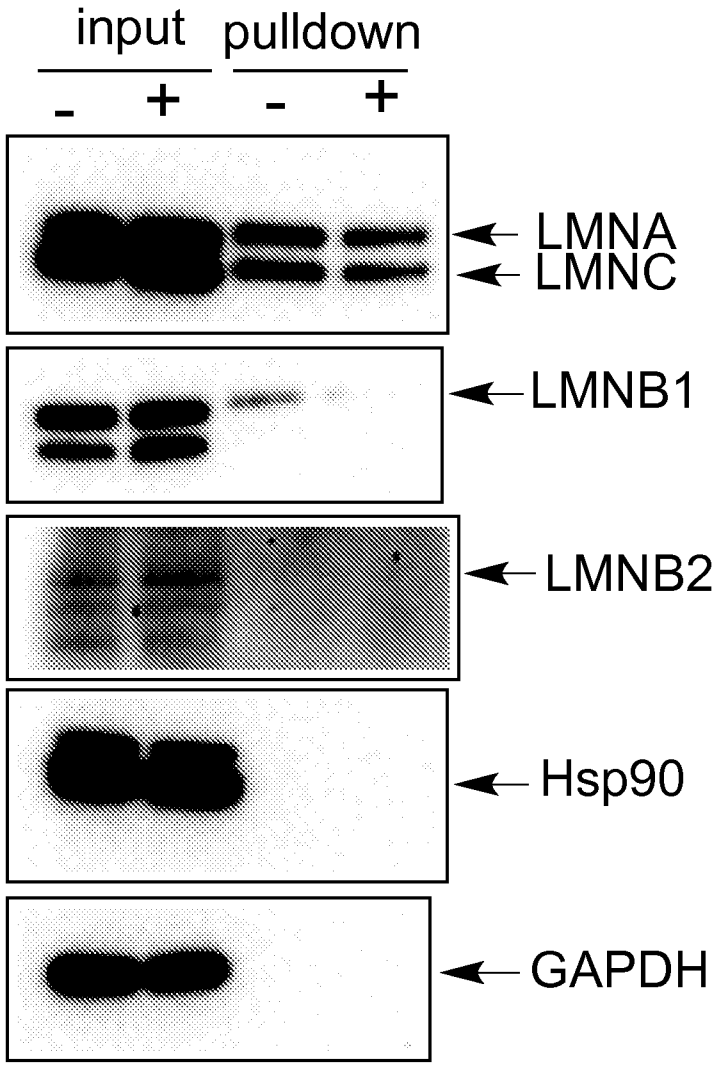

probe 8
MDA-MB-468
1) UV irradiation (365 nm)
2) cell lysis
targets
biotin-N3 "click" reaction
biotin
targets
Streptavidin pulldown followed by mass spectroscopy or Western analysis

Figure 5B



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/043265

A. CLASSIFICATION OF SUBJECT MATTER

C07D 487/04(2006.01)i, A61K 31/519(2006.01)i

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)
C07D 487/04; A61K 31/505; A01N 43/90; A01N 43/54; A61K 31/519

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
Korean utility models and applications for utility models
Japanese utility models and applications for utility models

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Google, eKOMPASS(KIPO internal) & Keywords: 7H-pyrrolo[3,2-f]quinazoline-1,3-diamine derivatives, anticancer, dihydrofolate reductase(DHFR) inhibitor

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 7253177 B2 (Ai J. Lin et al) 07 August 2007 See Table 2, Compound 3a,3c, WR227825	1-11
X	JP 2003-342276 A (MITSUI CHEMICALS INC) 03 December 2003 See Formula 1	1-9
A		10,11
A	Lee F. Kuyper et al. 'High-Affinity Inhibitors of Dihydrofolate Reductase: Antimicrobial and Anticancer Activities of 7,8-Dialkyl-1,3-diaminopyrrolo [3,2-f] quinazolines with Small Molecular Size', Journal of Medicinal Chemistry, 1996, Vol. 39, No. 4, pp. 892-903 See Table 5	1-11
A	Man Chin Chung et al. 'Prodrugs for the Treatment of Neglected Diseases', Molecules, 2008, Vol. 13, No. 3, pp. 616-677 See Figure 28	1-11
A	Anshuman Dixit et al, 'Development of CoMFA, advance CoMFA and CoMSIA models in pyrroloquinazolines as thrombin receptor antagonist', Bioorganic and Medicinal Chemistry, 2004, Vol. 12, No. 13, pp. 3591-3598 See Table 1	1-11

☐ 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	Date of mailing of the international search report
30 October 2014 (30.10.2014)	**30 October 2014 (30.10.2014)**

Name and mailing address of the ISA/KR
International Application Division
Korean Intellectual Property Office
189 Cheongsa-ro, Seo-gu, Daejeon Metropolitan City, 302-701,
Republic of Korea
Facsimile No. +82-42-472-7140

Authorized officer
KIM, Byung Sook
Telephone No. +82-42-481-3342

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No.
PCT/US2014/043265

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 7253177 B2	07/08/2007	US 2006-094736 A1	04/05/2006
JP 2003-342276 A	03/12/2003	None	