Novel inhibitors of aspartate transcarbamoylase (atcase) and compositions, methods and uses related thereto

Groves, Matthew Robert; Wang, Chao; Lunev, Sergey; Zhang, Bidong; Dömling, Alexander Stephan Siegfried; Visser, Alida

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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
08 June 2023 (08.06.2023)

WIPO | PCT

(10) International Publication Number
WO 2023/101556 A1

(51) International Patent Classification:

C07D 307/68 (2006.01)	*A61P 35/00* (2006.01)
C07D 333/38 (2006.01)	*A61K 31/381* (2006.01)
C07D 333/68 (2006.01)	*A61K 31/4178* (2006.01)
C07D 409/04 (2006.01)	*A61K 31/4365* (2006.01)
C07D 409/06 (2006.01)	*A61K 31/4436* (2006.01)
C07D 409/12 (2006.01)	*A61K 31/4523* (2006.01)
C07D 495/04 (2006.01)	*A61K 31/496* (2006.01)
A61P 33/06 (2006.01)	*A61K 31/5377* (2006.01)

(21) International Application Number:
PCT/NL2022/050697

(22) International Filing Date:
02 December 2022 (02.12.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
21211903.6 02 December 2021 (02.12.2021) EP

(71) Applicants: **RIJKSUNIVERSITEIT GRONINGEN** [NL/NL]; Broerstraat 5, 9712 CP Groningen (NL). **ACADEMISCH ZIEKENHUIS GRONINGEN** [NL/NL]; Hanzeplein 1, 9713 GZ Groningen (NL).

(72) Inventors: **GROVES, Matthew Robert**; c/o Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen (NL). **WANG, Chao**; c/o Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen (NL). **LUNEV, Sergey**; c/o Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen (NL). **ZHANG, Bidong**; c/o Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen (NL). **DÖMLING, Alexander Stephan Siegfried**; c/o Faculty of Science and Engineering, Groningen Research Institute of Pharmacy, Antonius Deusinglaan 1, 9713 AV Groningen (NL). **VISSER, Alida**; c/o Faculty of Medical Sciences, Antonius Deusinglaan 1, 9713 AV Groningen (NL).

(74) Agent: **WITMANS, H.A.**; V.O., P.O. Box 87930, 2508 DH Den Haag (NL).

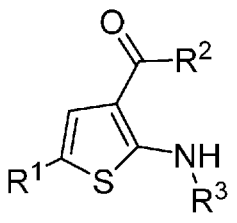
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CV, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IQ, IR, IS, IT, JM, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, 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, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, CV, 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, ME, 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))*

WO 2023/101556 A1

(54) Title: NOVEL INHIBITORS OF ASPARTATE TRANSCARBAMOYLASE (ATCASE) AND COMPOSITIONS, METHODS AND USES RELATED THERETO.



(I)

(57) Abstract: The invention relates to inhibitors of Aspartate Transcarbamoylase (ATCase) and compositions, methods and uses related thereto, such as the treatment of malaria, tuberculosis and proliferative diseases, e.g. cancer. Provided is an ATCase inhibitor compound of the Formula I or a pharmaceutically acceptable salt, solvent or hydrate thereof.

Title: Novel inhibitors of Aspartate Transcarbamoylase (ATCase) and compositions, methods and uses related thereto.

The invention relates to the field of medicinal chemistry. More specifically, it relates to inhibitors of Aspartate Transcarbamoylase (ATCase) and compositions, methods and uses related thereto, such as the treatment of malaria and proliferative diseases, e.g. cancer.

Malaria is one of the most serious and complex health problems affecting humanity, despite the work of the research community towards its eradication. Malaria is an infectious disease caused by four species of the protozoan parasite Plasmodium, *P. falciparum* being the most severe of the four. Attempts to develop protective vaccines against *P. falciparum* have only been partially successful (Regules *et al.*, 2011, Expert Review of Vaccines. 10 (5): 589–99). Therefore, more therapeutic options against malaria are needed.

Among the prior art anti-malaria drugs, some employ artemisinin derivatives (such as dihydroartemisinin, artesunate, artemether, arteether) in conjunction with piperaquine having a long half-life. GI tract side effects such as nausea and vomiting due to substantial amount of phosphates adversely affect the therapeutic effects, with incidence of up to 10% when the total amount for one course is divided into 3 doses, and reduced to 3-5% when divided into 4 doses. In addition, the prior art anti-malaria drugs suffer from the disadvantages of long processing period, high production cost, short shelf life, large dosage and the like.

Various classes of antimalarial drugs exist such as chloroquine sulphadoxine/pyrimethamine combination. These drugs have side effects and inconvenient dosing schedules which limit the compliance of patients. Moreover, resistance to many of the currently available antimalarial drugs is spreading rapidly, threatening people in areas where malaria is endemic.

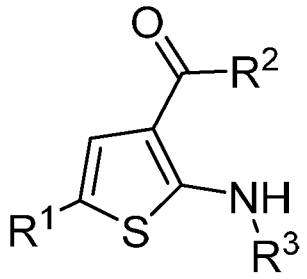
Unlike humans, malarial parasites lack pyrimidine salvage machinery, making de-novo pyrimidine biosynthesis pathway a promising target for drug discovery. Aspartate transcarbamoylase (EC 2.1.3.2) catalyzes condensation of carbamoyl-phosphate (CP) and l-aspartate to form N-carbamoyl-l-aspartate (CA) and phosphate. This is the second step of de-novo pyrimidine biosynthesis. The

crystal structure of *P. falciparum* aspartate transcarbamoylase (*Pf*ATC) *Pf*ATC in the liganded R-state as well as in complex with an inhibitor, 2,3-napthalenediol (IC_{50} value of 5.5 ± 0.9 µM), was previously reported (Lunev et al. Biochem. Biophys. Res. Comm., Vol. 497, Issue 3, 2018, Pg. 835-842).

Furthermore, human ATCase has been proposed as anticancer target. See for example Lei et al. (FEBS J. 2020 Aug;287(16):3579-3599) reporting that a loop region in ATCase serves as a gatekeeper for the active site, playing a new and unappreciated regulatory role in the catalytic cycle of ATCase. Based on virtual compound screening simultaneously targeting the new regulatory region and active site of human ATCase, two compounds were identified to exhibit inhibition of ATCase activity (IC_{50} values in the micromolar range), proliferation of multiple cancer cell lines, and growth of xenograft tumors. No effect of malarial cultures has been demonstrated.

Recognizing the therapeutic potential of targeting ATCase, the present inventors set out to identify novel ATCase inhibitor compounds to overcome at least part of the shortcomings in the prior art. Among others, they aimed at compounds that show high potency (in vitro), minimal or no significant cytotoxicity against human cells and high activity (nanomolar/ sub-micromolar IC_{50} values) against tumour cells and/or blood stage malarial cultures.

It has now unexpectedly been found that one or more of these goals are met by the provision of an aspartate transcarbamoylase (ATCase) inhibitor compound of the thiophene core structure



Formula I

wherein

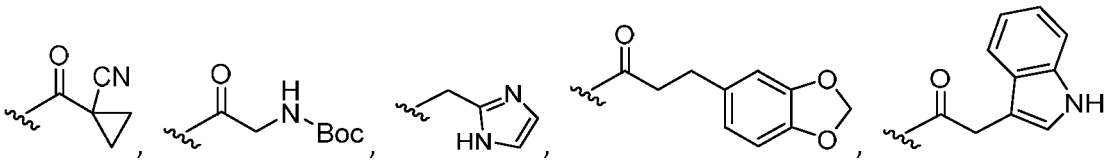
R^1 is selected from the group consisting of halogen, benzyl, C_3-C_6 branched alkyl, benzoheterocycle ring and an 5- or 6- membered (hetero)aromatic

moiety, optionally substituted with one or more of halogen, C_1-C_3 alkyl, CN, NH_2, acyl and C_4-C_6 heterocycloalkyl ring;

R^2 is OR', wherein R' is H or alkyl; or

R^2 is NR"R"', wherein R" and R"' are independently selected from H, -$(CH_2)_n$-OH wherein n = 1-3, -$(CH_2)_n$-A wherein n = 0-3, and -CH_2-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, and A is an optionally substituted C_4-C_6 (hetero)aromatic ring or an optionally substituted C_4-C_6 (hetero)cycloalkyl ring, provided that R" and R"' cannot both be H; or wherein R" and R"' together with the N-atom to which they are attached form a 5- to 7-membered heterocycloalkyl comprising 1 to 3 N-atoms, which heterocycloalkyl is optionally substituted;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_6 branched alkyl, *-tert-*butyloxycarbonyl (Boc) and



or a pharmaceutically acceptable salt, solvent or hydrate thereof.

An inhibitory compound and uses thereof as herein disclosed are not taught or suggested in the art.

US2003/0225155 discloses thiophene-based compounds that can be used as chelating agent in the treatment and prevention of diseases such as AIDS, cancers, untoward angiogenesis, pulmonary anthrax, malaria, inflammatory responses, Alzheimer's disease and other diseases. The thiophene ring carries a substituent of the structure -C(O)X, wherein X is the amino function of 2-hydrazine, 2-hydrazone, or 2-thiosemicarbazone. US2003/0225155 fails to teach a compound as disclosed in the present invention.

US2004/0014740 relates to anthelmintic compositions containing thiophene derivatives as active ingredients. However, these compounds are of a different structure. More in particular, US2004/0014740 fails to teach or suggests

compounds wherein the R^3 moiety is as defined herein above. Also, US2004/0014740 is silent about any inhibitory activity against ATCase.

US2004/0242673 relates to compounds that have a thiophene or furan skeleton which bind to phosphodiesterase 6D. Also disclosed are methods of using the compounds and/or compositions in the treatment of a variety of diseases and unwanted conditions in subjects. The substituents on the thiophene ring as defined in US2004/0242673 are distinct from those according to the present invention. Likewise, US2004/0242673 is silent about any inhibitory activity against ATCase.

The term "pharmaceutically acceptable salt" of a compound means a salt that is pharmaceutically acceptable and that possesses the desired pharmacological activity of the parent compound. Such salts, for example, include:

(1) acid addition salts, formed with inorganic acids such as hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid, and the like; or formed with organic acids such as acetic acid, propionic acid, hexanoic acid, cyclopentanepropionic acid, glycolic acid, pyruvic acid, lactic acid, malonic acid, succinic acid, malic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, 3-(4-hydroxybenzoyl)benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, 1,2-ethanedisulfonic acid, 2-hydroxyethanesulfonic acid, benzenesulfonic acid, 2-naphthalenesulfonic acid, 4-methylbicyclo-[2,2,2]oct-2-ene-1-carboxylic acid, glucoheptonic acid, 4,4'-methylenebis-(3-hydroxy-2-ene-1-carboxylic acid), 3-phenylpropionic acid, trimethylacetic acid, tertiary butylacetic acid, lauryl sulfuric acid, gluconic acid, glutamic acid, hydroxynaphthoic acid, salicylic acid, stearic acid, muconic acid, and the like; (2) salts formed when an acidic proton present in the parent compound either is replaced by a metal ion, e.g., an alkali metal ion, an alkaline earth ion, or an aluminum ion; or coordinates with an organic base. Acceptable organic bases include ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Acceptable inorganic bases include aluminum hydroxide, calcium hydroxide, potassium hydroxide, sodium carbonate, sodium hydroxide, and the like. Solvates contain either stoichiometric or non-stoichiometric amounts of a solvent, and are often formed during the process of

crystallization. Hydrates are formed when the solvent is water, or alcoholates are formed when the solvent is alcohol. Polymorphs include the different crystal packing arrangements of the same elemental composition of a compound. Polymorphs usually have different X-ray diffraction patterns, infrared spectra, melting points, density, hardness, crystal shape, optical and electrical properties, stability, and solubility. Various factors such as the recrystallization solvent, rate of crystallization, and storage temperature may cause a single crystal form to dominate.

As used herein, "ATCase" refers to the enzyme Aspartate transcarbamoylase (EC 2.1.3.2) which catalyzes condensation of carbamoyl-phosphate (CP) and l-aspartate to form N-carbamoyl-l-aspartate (CA) and phosphate. ATCase can be of any origin. In one aspect, it is of mammalian origin, preferably human origin (*Hu*ATCase or *Hs*ATCase). In another aspect, ATCase is of bacterial, pathogenic or parasitic origin. For example, a compound of the invention is advantageously used as inhibitor of ATCase from a virulent and antibiotic resistant bacterial pathogen, including: *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter spp.* This group of Gram-positive and Gram-negative bacteria, also referred to in the art as "ESKAPE" pathogens, can evade or 'escape' commonly used antibiotics due to their increasing multi-drug resistance (MDR).

In a specific embodiment, ATCase is of protozoan origin such as ATCase from a single-celled microorganism of the Plasmodium group, more in particular *P. falciparum* (*Pf*ATCase). In another specific embodiment, ATCase is of protozoan origin such as ATCase from a pathogenic bacterium, for example a bacterium of the family Mycobacteriaceae, more in particular ATCase of *Mycobacterium tuberculosis* (*Mt*ATCase).

"Aromatic" refers to substituted or unsubstituted unsaturated cyclic hydrocarbons of one or more rings and includes aryl structures such as phenyl, naphthalyl, phenanthrenyl, and anthracenyl. Aromatic examples include 6-membered (typified by benzene) and 5- membered (typified by furan, thiophene, pyrrole, and indole) rings.

"Heterocycle" refers to the presence of at least one non-carbon atom in a cyclic structure. Non-limiting examples include the presence of a nitrogen, oxygen, and sulfur atom to result in heterocyclic rings. Examples include pyridinyl, pyrimidinyl, pyrazinyl, pyridazinyl, pyrrolyl, imidazolyl, pyrazolyl, thienyl, furyl, tetrahydrofuryl, isoxazolyl, isothiazolyl, quinolinyl, isoquinolinyl, indolyl, benzimidazolyl, benzofuranyl, benzoxazolyl, benzisoxazolyl, benzpyrazolyl, benzothiofuranyl, cinnolinyl, pterindinyl, phthalazinyl, naphthypyridinyl, quinoxalinyl, quinazolinyl, purinyl and indazolyl.

In one embodiment, an inhibitor compound of the invention inhibits ATCase (e.g. using recombinant enzyme an *in vitro* assay) with an IC_{50} up to about 5 μM, 4 μM, 3 μM or 2 μM. Preferably, it inhibits ATCase with an IC50 value up to about 1 μM, more preferably below 1 μM such as about 900, 800, 700, or 500 nM. In a specific aspect, the inhibitor compound inhibits ATCase (e.g. *Hu*ATCase and/or *Pf* ATCase) with an IC_{50} value below 500 nM.

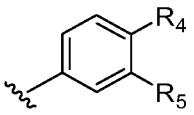
In a compound of the invention, substituent R^1 on the thiophene ring is selected from the group consisting of halogen, benzyl, C_3-C_6 branched alkyl, benzoheterocycle ring and an 5- or 6- membered (hetero)aromatic moiety, optionally substituted with one or more of halogen, C_1-C_3 alkyl, CN, NH_2, acyl and C_4-C_6 heterocycloalkyl ring.

In one embodiment, R^1 is of non-aromatic nature and selected from halogen (e.g. Br or Cl), and C_3-C_6 branched alkyl (e.g. propyl, isopropyl, butyl, butyl, sec-butyl or s-butyl, isobutyl, tert-butyl or t-butyl).

In another embodiment, R^1 is of aromatic nature and selected from benzyl, benzoheterocycle ring and an optionally substituted 5- or 6- membered (hetero)aromatic moiety. A benzoheterocycle ring is any polycyclic heterocycle consisting of a benzene ring fused to that of another heterocycle.

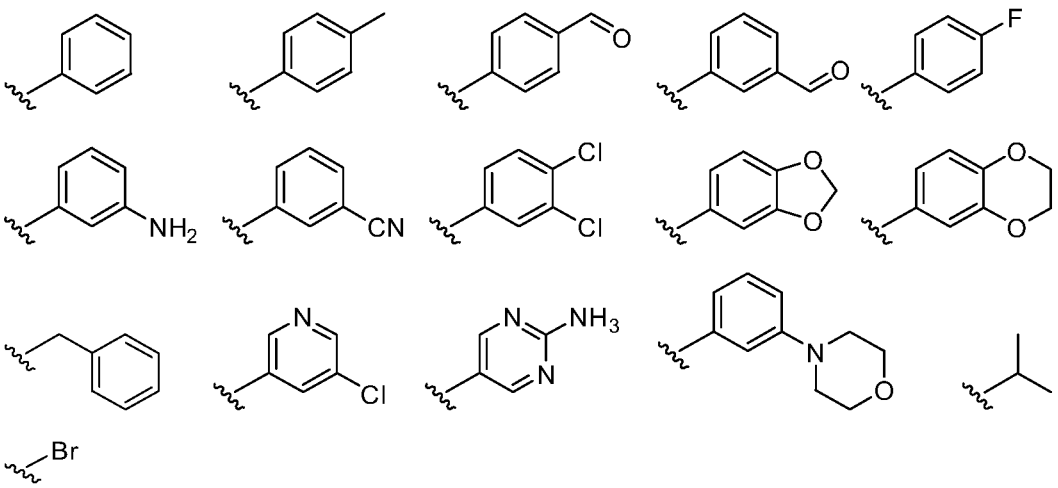
Preferred compounds include those wherein R^1 is an optionally substituted 6-membered (hetero)aromatic moiety, for example a substituted phenyl, pyridine or pyrimidine.

For example, provided is an inhibitor compound wherein R^1 is

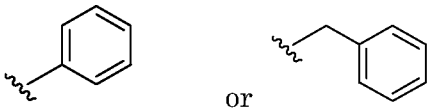


wherein R_4 and R_5 are independently selected from H, alkyl, halogen and amine; or wherein R_4 and R_5 together with the carbon atoms to which they are attached form a 5- to 7-membered carbocyclic or heterocyclic ring.

Exemplary inhibitor compounds according to the invention include those wherein R^1 is selected from the group consisting of

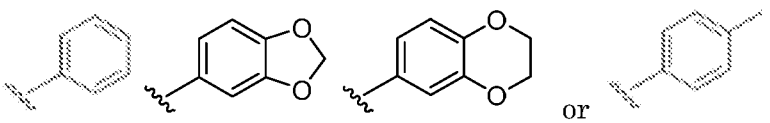


In one specific embodiment, R^1 is



.

In another specific embodiment, R^1 is



.

In an inhibitor compound as herein disclosed, moiety R^2 is OR' or NR"R"', wherein R' is H or (C1-C3) alkyl; and wherein R" and R"' are independently selected from H, $-(CH_2)_n$-OH wherein n = 1-3, $-(CH_2)_n$-A wherein n = 0-3, and $-CH_2$-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, and A is an optionally substituted C_4-C_6 (hetero)aromatic ring or an optionally substituted C_4-C_6 (hetero)cycloalkyl ring, provided that R" and R"' cannot both be H; or wherein R" and R"' together with the N-atom to which they are attached form a 5- to 7-membered heterocycloalkyl comprising 1 to 3 N-atoms, which heterocycloalkyl is optionally substituted.

In one embodiment, R^2 is OR' and R' is H or (C1-C3) alkyl, to provide a compound comprising a carboxylic acid or ester moiety. For example R^2 is -OH or $-OCH_3$ (see exemplary compounds BDA-01, -02 and -03, -64 to -69)

In another embodiment, R^2 is NR"R"', to provide an inhibitor compound comprising an organic amide or a carboxamide moiety. R" and R"' are independently selected from H, $-(CH_2)_n$-OH wherein n = 1-3, $-(CH_2)_n$-A wherein n = 0-3, and $-CH_2$-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, and A is an optionally substituted C_4-C_6 (hetero)aromatic ring or an optionally substituted C_4-C_6 (hetero)cycloalkyl ring, provided that R" and R"' cannot both be H; or wherein R" and R"' together with the N-atom to which they are attached form a 5- to 7-membered heterocycloalkyl comprising 1 to 3 N-atoms, which heterocycloalkyl is optionally substituted. Substituents on the heterocycloalkyl can for example be selected from $-CH_2$NHBoc, benzyl and substituted phenyl.

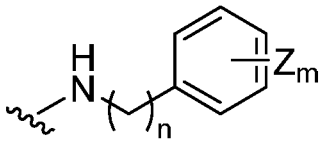
In one aspect, R" and R"' together with the N-atom to which they are attached form a 5- to 7-membered heterocycloalkyl comprising 1 to 3 N-atoms, which heterocycloalkyl is optionally substituted. For example, R" and R"' together with the N-atom to which they are attached form a 6-membered heterocycloalkyl comprising 1 to 3 N-atoms, preferably 1 or 2 N-atoms. See for example compounds BDA-09, -10, -63.

In another aspect, R" and R"' are independently selected from H, $-(CH_2)_n$-OH wherein n = 1-3, $-(CH_2)_n$-A wherein n = 0-3, and $-CH_2$-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, with the proviso that R" and R"' cannot both be H.

Preferably, A is an optionally substituted C_6 (hetero)aromatic ring or an optionally substituted C_6 (hetero)cycloalkyl ring.

In a preferred embodiment, one of R" and R"' is H and the other is as defined herein above.

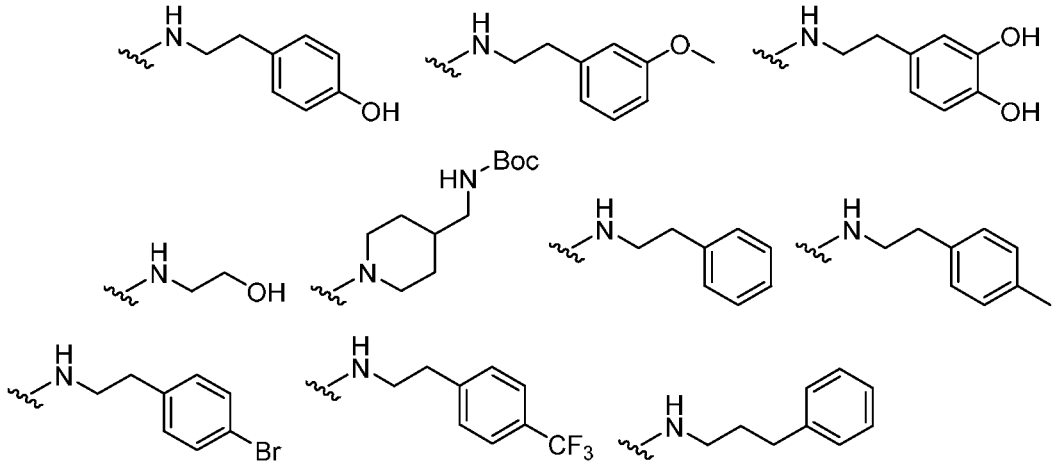
Provided is an inhibitor compound wherein moiety R^2 is of the formula

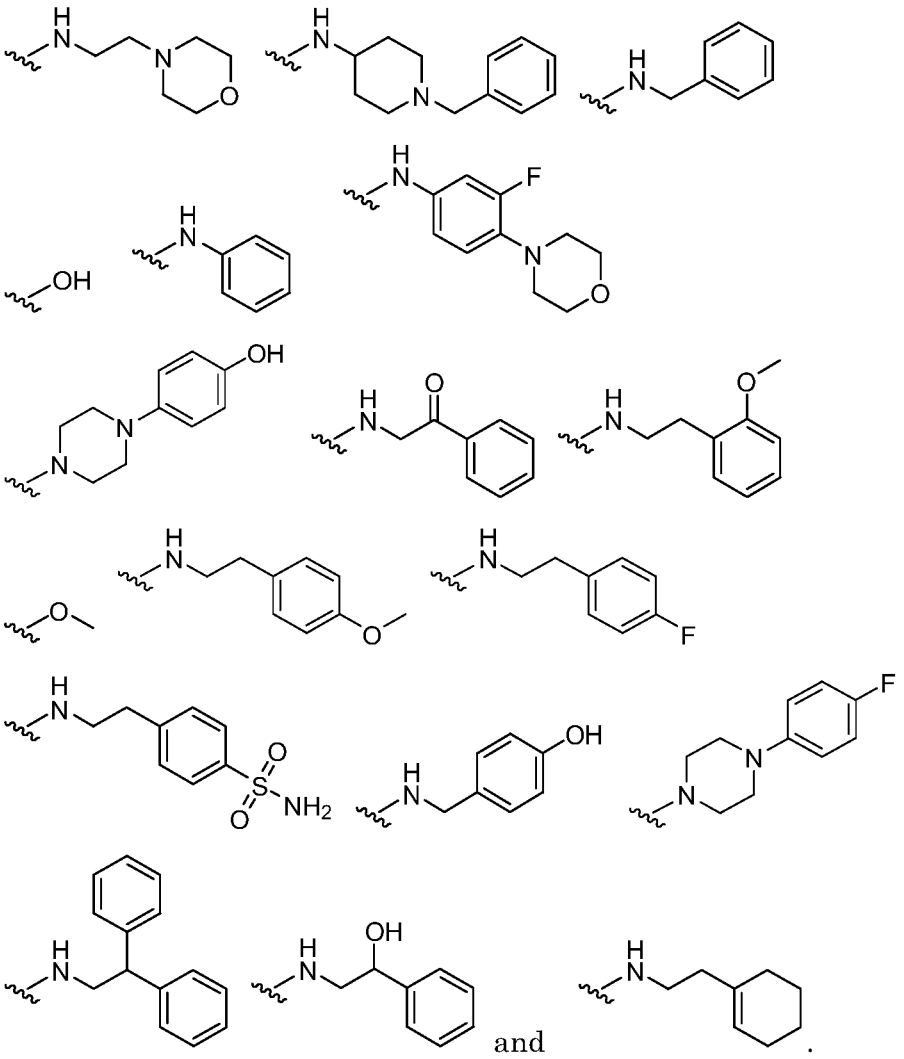


wherein n is 0-3, preferably n =2 ; m is 0-3 and Z is independently selected from halogen, -R^a, - OR^a, -OC(O) R^a, -CN, NO_2, -CO_2R^a, -OC(O)R^a, -S(O)R^a, -$S(O)_2$ R^a, -$S(O)_2NH_2$, and wherein R^a is selected from hydrogen, C_1-C_6 (hetero)alkyl.

In one embodiment, R^2 is optionally substituted -NH-$(CH_2)_2$-phenyl, -NH-$(CH_2)_2$-OH, NH-substituted piperidiny-4-yl or -NH-substituted piperidin-1-yl.

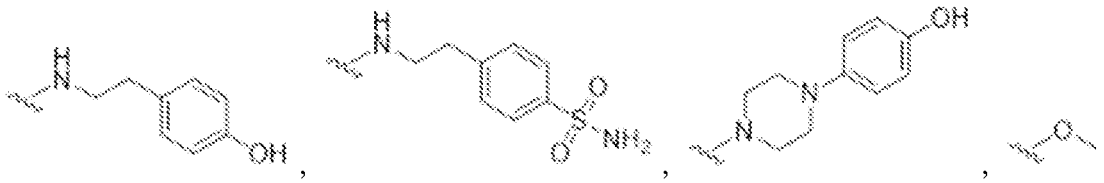
Preferred compounds include those wherein R^2 is selected from the group consisting of

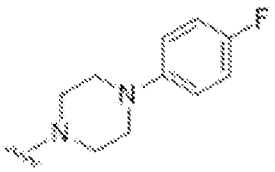
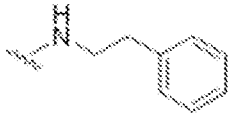
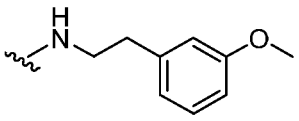
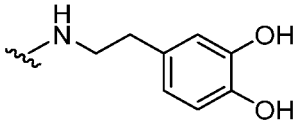


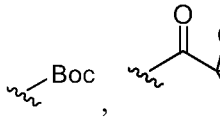


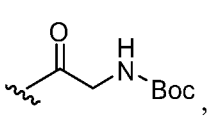
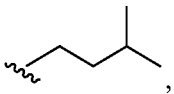
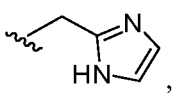
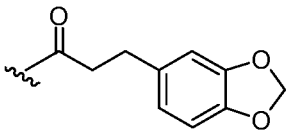
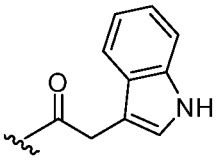
and .

R^2 may be suitably selected from the group consisting of



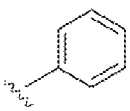
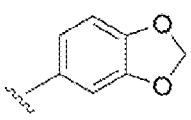
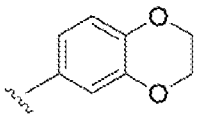
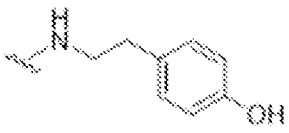
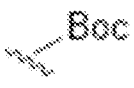





Preferred inhibitor compounds according to the invention are those wherein R_3 is selected from H,


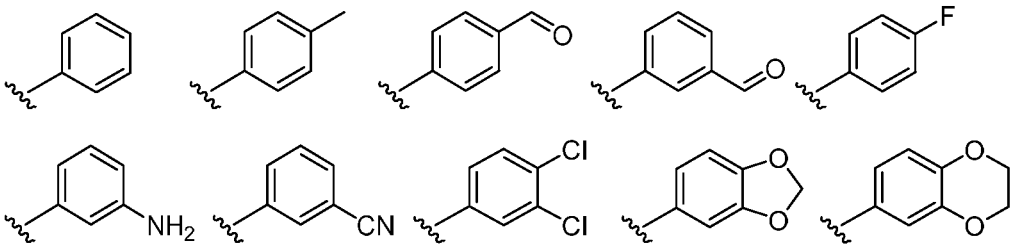
In a specific aspect, R^3 is H, Boc or a branched C_3-C_6 alkyl.

As will be understood and appreciated by a person skilled in the art, the invention encompasses compounds comprising any combination of exemplary, preferred or more preferred embodiments of R^1, R^2 and R^3 as herein disclosed.

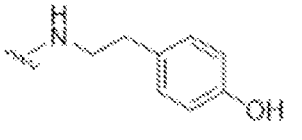
In a specific aspect, the invention provides a compound wherein R^1 is selected from






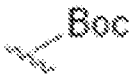
R^2 is and R^3 is .

In another preferred embodiment, R^1 is selected from the group consisting of



the R^2 moiety is

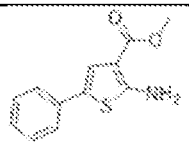
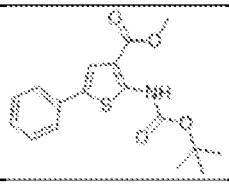
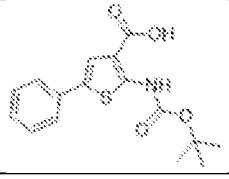


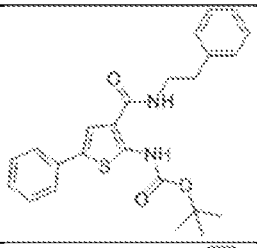
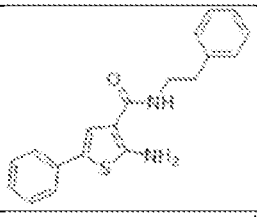
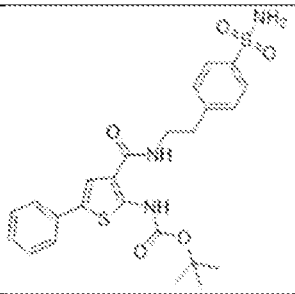
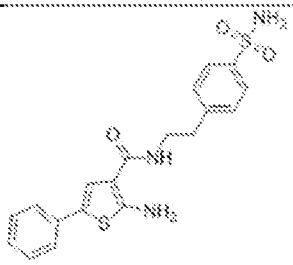
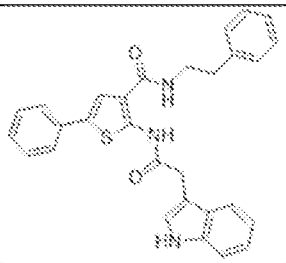
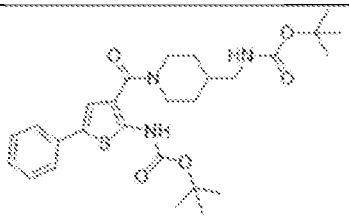
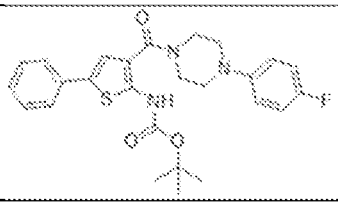
, and the R^3 moiety is  In a further embodiment, the invention provides an inhibitor compound wherein R^1 is an optionally substituted or condensed phenyl; R^2 is optionally substituted -NH-$(CH_2)_2$-phenyl, -NH-$(CH_2)_2$-OH, NH-substituted piperidiny-4-yl or -NH-substituted piperidin-1-yl; and/or wherein R^3 is H, Boc or a C_3-C_6 branched alkyl.

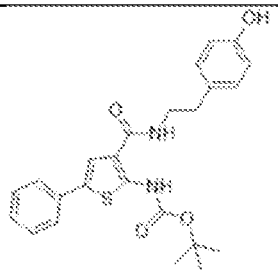
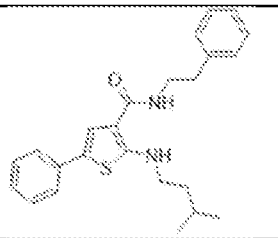
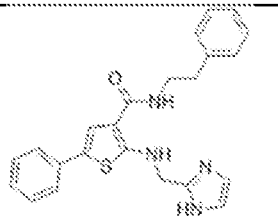
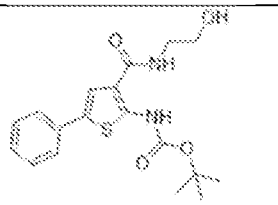
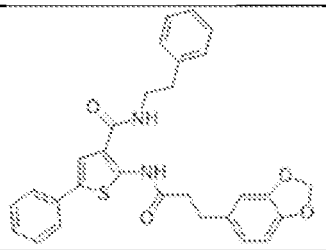
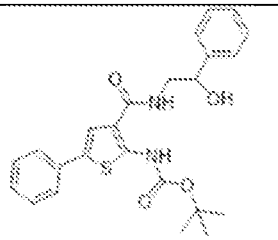
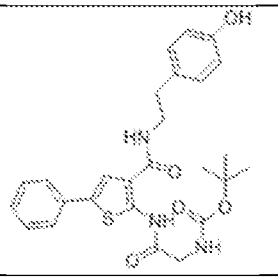
Preferred compounds may be selected from the compounds shown in Table 1.

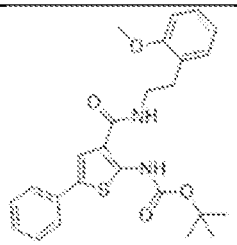
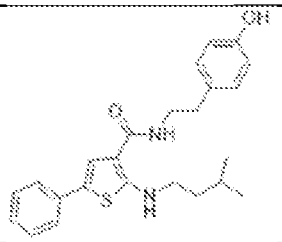
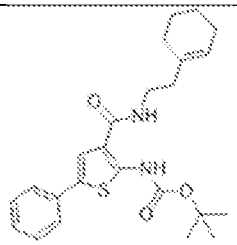
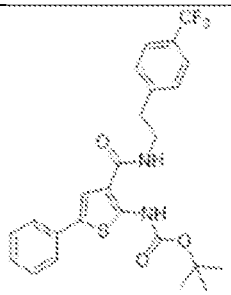
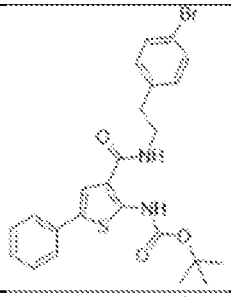
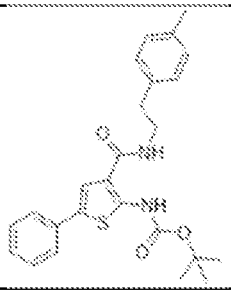
In one embodiment, the compound is a thiophene-compound selected from the compounds of Table 2, or a pharmaceutically acceptable salt, solvent or hydrate thereof.

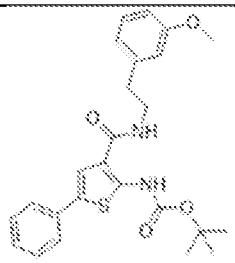
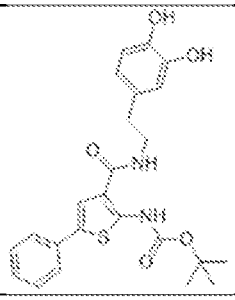
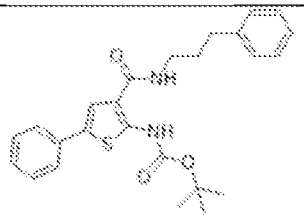
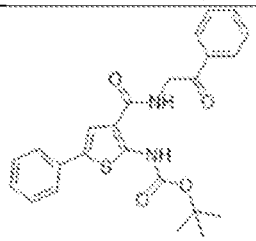
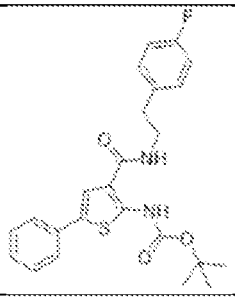
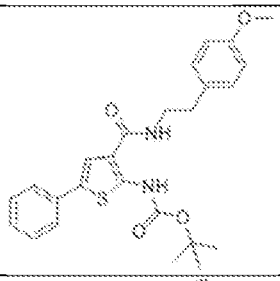
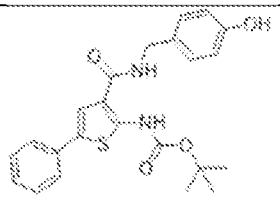
Table 2

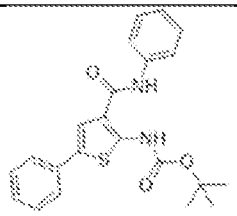
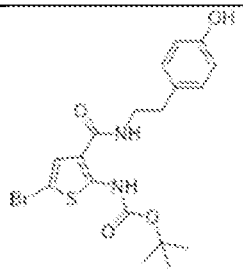
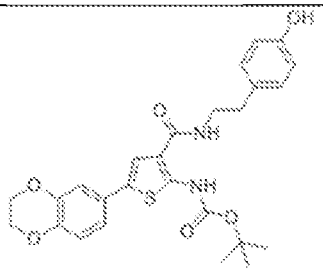
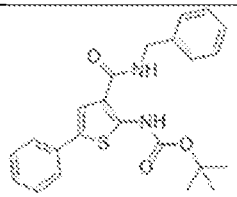
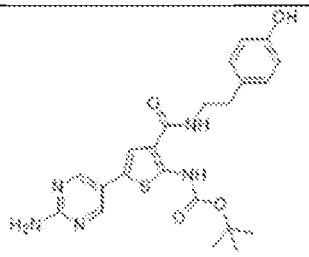
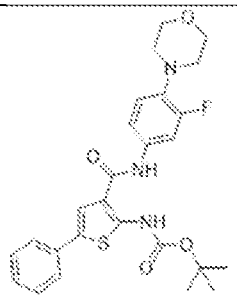
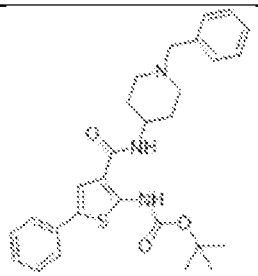
BDA-01	
BDA-02	
BDA-03	

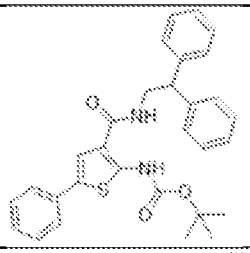
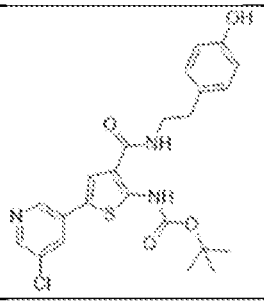
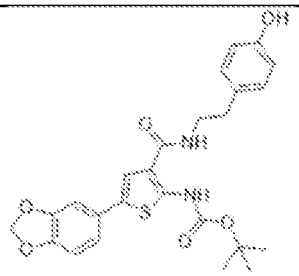
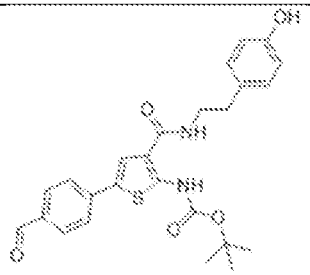
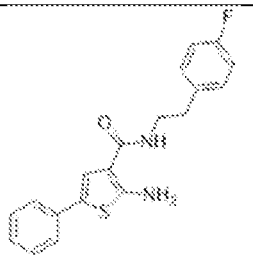
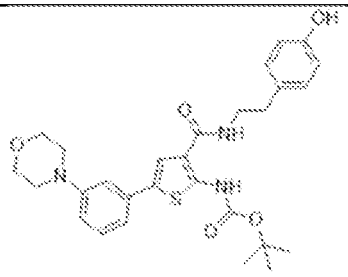
BDA-04	
BDA-05	
BDA-06	
BDA-07	
BDA-08	
BDA-09	
BDA-10	

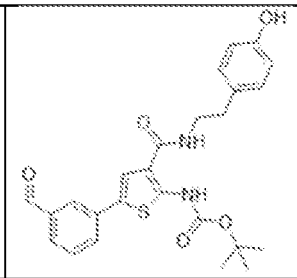
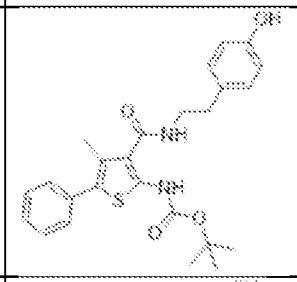
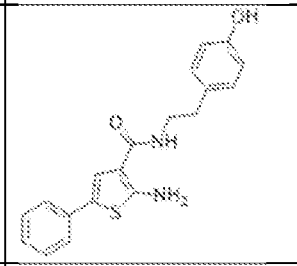
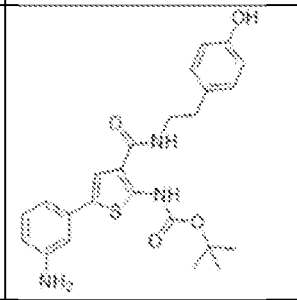
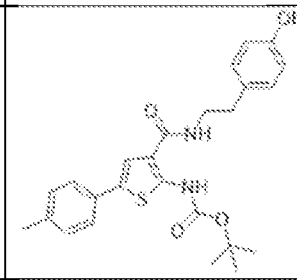
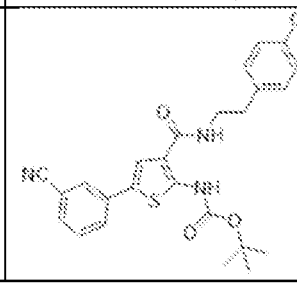
BDA-11	
BDA-12	
BDA-13	
BDA-14	
BDA-15	
BDA-16	
BDA-17	

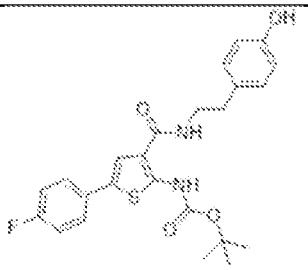
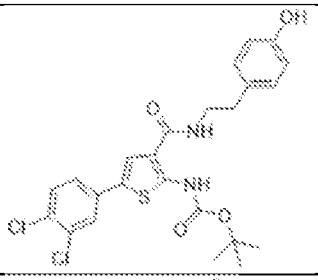
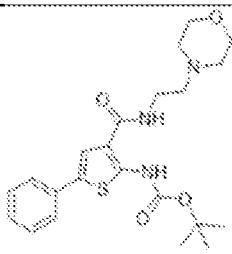
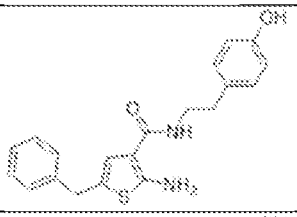
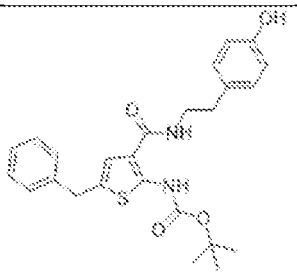
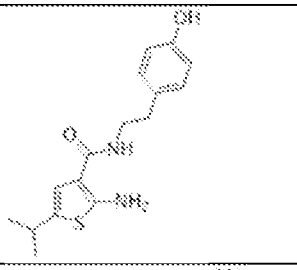
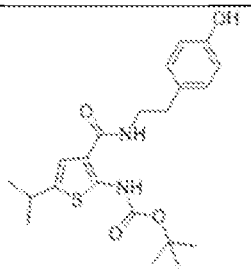
BDA-18	
BDA-19	
BDA-20	
BDA-21	
BDA-22	
BDA-23	

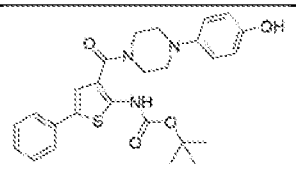
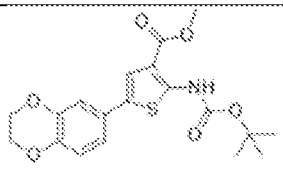
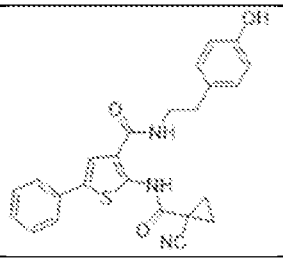
BDA-24	
BDA-25	
BDA-26	
BDA-27	
BDA-28	
BDA-29	
BDA-30	

BDA-31	
BDA-32	
BDA-33	
BDA-34	
BDA-35	
BDA-37	
BDA-38	

BDA-39	
BDA-40	
BDA-41	
BDA-45	
BDA-46	
BDA-47	

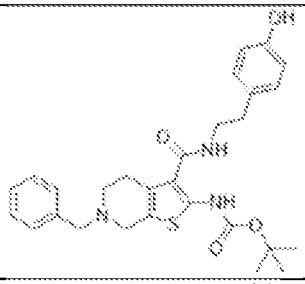
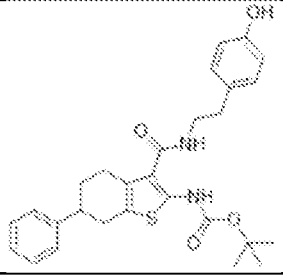
BDA-48	
BDA-49	
BDA-50	
BDA-51	
BDA-52	
BDA-53	

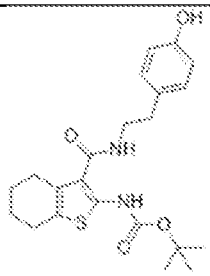
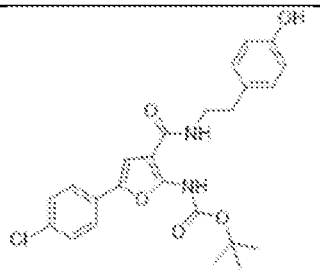
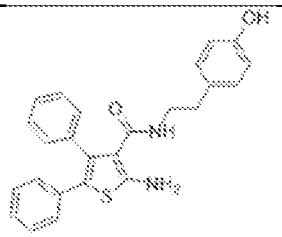
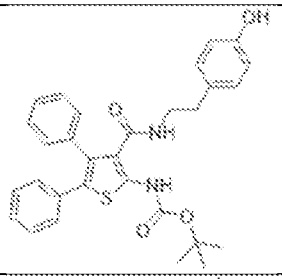
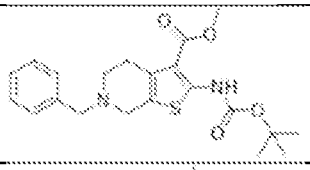
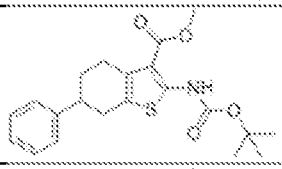
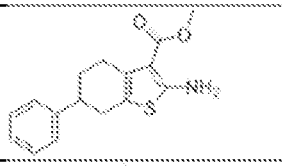
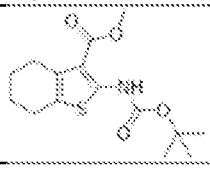
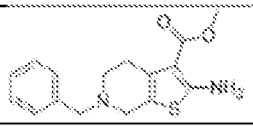
BDA-54	
BDA-56	
BDA-57	
BDA-58	
BDA-59	
BDA-61	
BDA-62	

BDA-63	
BDA-69	
BDA-70	

In another aspect, the invention provides in inhibitor compound comprising a condensed thiophene or furan core structure selected from those of Table 3, or a pharmaceutically acceptable salt, solvent or hydrate thereof.

Table 3

BDA-36	
BDA-42	

BDA-43	
BDA-44	
BDA-55	
BDA-60	
BDA-64	
BDA-65	
BDA-66	
BDA-67	
BDA-68	

In a specific aspect, the invention provides an inhibitor compound selected from the group consisting of BDA-04, 06, 07, 09, 10, 11, 12, 14, 17, 24, 25, 33, 38, 41, 45, 48, 52, 54, 63, 67, 69 and 70.

For example, the inhibitor compound is selected from the group consisting of BDA-04, -07, 09, 11, 12, 14, 24, 25, 33, 38, 41, 45, 48, 52 and 54, preferably from BDA11, BDA-33, BDA-41 and BDA-52.

In another embodiment, the inhibitor compound is selected from the group consisting of BDA-06, -10, -14, -17, -25, -54, -63, -67, -69 and -70, preferably BDA-06, BDA-14, BDA-69 and BDA-70.

The compounds of the present invention can be synthesized using techniques and materials known to those of skill in the art. Starting materials for the compounds of the invention may be obtained using standard techniques and commercially available precursor materials, such as those available from Aldrich Chemical Co. or Sigma Chemical Co. Suitable synthesis methods for preparing representative inhibitor compounds are herein disclosed below.

A compound of the invention can be screened for their ability to inhibit the activity of ATCase, i.e. identify compounds that decrease (inhibit) the function and/or activity of ATCase polypeptides or fragments, portions, or analogs thereof. The screening may be performed *in vitro* or *in vivo*. A preferred *in vitro* screening method comprises determining the inhibitory action against human (*Hu*) or *P. falciparum* (*Pf*) ATCase.

In some embodiments, a compound inhibits the activity of ATCase such that it may be administered to a subject, preferably human, in need of a decrease in the function and/or activity of ATCase. The invention thus provides for the treatment of a disease or undesirable condition mediated by unwanted ATCase activity, for example unwanted pyrimidine synthesis. Pyrimidine nucleotides play a significant role in tumor cell proliferation as precursors of RNA and DNA. There are two ways for the synthesis of pyrimidine: the salvage synthesis pathway and the de novo synthesis pathway. In resting or fully differentiated cells, pyrimidines are mainly

provided by the former. While in highly proliferative cells like tumor cells, the latter is usually highly active to meet the increased demand for nucleic acid precursors and other cellular components. Compared with normal proliferous cells, there is a significant imbalance of pyrimidine metabolism in cancer cells which is stringently linked with tumor transformation and progression.

The invention therefore also provides a pharmaceutical composition comprising a pharmaceutically effective amount of at least one inhibitor compound according to any one of the preceding claims, and a pharmaceutically acceptable carrier, vehicle or diluent. In a further embodiment, the invention provides an inhibitor compound as herein disclosed for use as a medicament.

The terms "effective amount" or "pharmaceutically effective amount" refer to a nontoxic but sufficient amount of the agent to provide the desired biological, therapeutic, and/or prophylactic result. That result can be reduction and/or alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system. For example, an "effective amount" for therapeutic uses is the amount of the compound having the thiophene skeleton as disclosed herein per se or a composition comprising the compound required to provide a clinically significant decrease in a disease. An appropriate effective amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation.

In one aspect, a compound inhibits the activity of *Pf* ATCase such that it may be administered to a subject, preferably human, in need of a decrease in the function and/or activity of Plasmodium ATCase, for example a subject suffering from or suspected to be suffering from malaria. The malaria may be caused by *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae, Plasmodium ovale* or *Plasmodium knowlesi.*

In another aspect, a compound inhibits the activity of human ATCase such that it may be administered to a subject, preferably human, in need of a decrease in the function and/or activity of human ATCase. For example, the compound is effective to reduce or inhibit the proliferation of cancer cells. Preferably, the inhibitor

compound shows a selective toxicity toward (human) tumor cells or tumour cell lines (e.g. H1299, A375, MCF-7, U2OS, REC1 and/or SUDHL2), but a much lower toxicity against healthy cells, e.g. human peripheral blood lymphocytes (PBLs). Cytotoxicity can be determined using *in vitro* or *in vivo* assays known in the art. Suitable in vitro assays include those measuring cell viability, membrane integrity, metabolic activity and the like, cell viability assays use a variety of markers as indicators of metabolically active (living) cells. Examples of markers commonly used include measuring ATP levels, measuring the ability to reduce a substrate, and detecting enzymatic/protease activities unique to living cells. Metabolic activity assays include Tetrazolium dye assays using MTT or a related tetrazolium salt. In a specific aspect, the resazurin reduction (RR) assay (also known as the alamarBlue or CellTiter-Blue assay) is used to determine cell viability/proliferation capacity in eukaryotic cells.

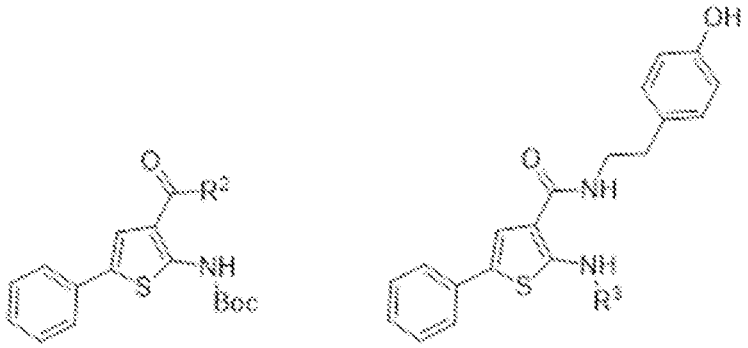
In one embodiment, the invention provides a compound for use in the treatment and/or prevention of malaria, i.e. as an anti-malaria agent. The thiophene-based compounds of the invention are able to inhibit the proliferation of *Plasmodium falciparum* in blood cells, showing a significant anti-malarial activity. They represent a new class of antimalarial agents with reduced side effects and might be less prone to induce Plasmodium resistance than other targeting agents whose ligands are small antigenic regions.

Hence, the invention also provides a method for treating malaria in a subject, comprising administering to the subject a therapeutically effective dose of a compound according to the invention, preferably wherein said compound is selected from the group consisting of BDA-11, BDA-24, BDA-25, BDA-12.

Preferably, the anti-malarial compound shows (in vitro) inhibition of Plasmodium ATCase with an IC50 value below 2μM, more preferably below 1 μM, such as below 900, 800, 700, 600, 500 or 400 nM. Most preferably, the compound shows (in vitro) inhibition of Plasmodium ATCase with an IC50 value 300 nM, 200 nM or even below 150 or 100 nM. Provided are anti-malaria compounds of Formula I wherein R^1 is phenyl, wherein R^2 is

and/or wherein R^3 is -*tert*- butyloxycarbonyl (Boc).

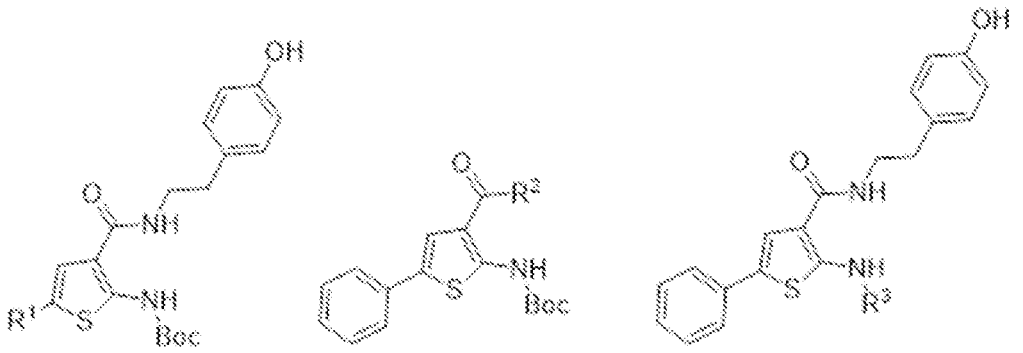
Provided is an anti-malaria compound according to one of the following core structures:



See Figure 5 showing representative compounds BDA-11, BDA-14, BDA-24 and BDA-25 indicating their suitability as anti-malarials while having limited impact (toxicity)on human cells.

In another embodiment, the invention provides a compound for use as an anti-cancer agent. Preferably, the anti-cancer compound shows (in vitro) inhibition of mammalian (e.g. human) ATCase with an IC_{50} value below 2 μM, more preferably below 1 μM, such as below 900, 800, 700, 600, 500 or 400 nM. Most preferably, the compound shows (in vitro) inhibition of human ATCase with an IC_{50} value 300 nM, 200 nM or even below 150 or 100 nM.

Provided is an anti-cancer compound according to one of the following core structures:



See Figure 6 showing the cellular properties of representative compounds BDA-11, BDA-14, BDA-25, BDA-33, BDA-41 and BDA-52 that are suitable as anti-cancer agents. Additionally, Figure 2 shows *in vitro* inhibitory properties of BDA-07, BDA-09, BDA-33, BDA-38, BDA-45, BDA-48 and BDA-54. Preferred exemplary anti-cancer compounds include BDA-45, BDA-48, BDA-54, BDA-38, BDA-9 and BDA-7, in particular BDA-41, BDA-33, BDA-11, BDA-52, BDA-45 and BDA-48.

In yet a further embodiment, the invention provides a compound for use in a method of preventing or treating a disease or disorder in a subject wherein a beneficial effect is obtained by inhibition of a Mycobacterium ATCase. For example, the Mycobacterium ATCas is *Mycobacterium tuberculosis* ATCase (*Mt*ATCase) or *Mycobacterium leprae* ATCase, preferably *Mt*ATCase. The disease may be tuberculosis or plague, preferably tuberculosis. Preferred compounds for inhibition of Mycobacterium ATCase include BDA-06, -10, -14, -17, -25, 54, -63, -67,- 69, and -70. In a preferred embodiment, the , more preferably BDA-06, -14 or -70. See Example.. Also provided is a method for treating or preventing tuberculosis in a human subject, comprising administering to the subject a therapeutically effective dose of a compound selected from the group consisting of BDA-06, -10, -14, -17, -25, 54, -63, -67,- 69, and -70, preferably BDA-06, -14 or -70.

In a still further embodiment, an inhibitor compound of the invention is suitably used to prevent or treat one or more Neglected tropical diseases (NTDs). These are a diverse group of tropical infections that are common in low-income populations in

developing regions of Africa, Asia, and the Americas. They are caused by a variety of pathogens, such as viruses, bacteria, protozoa, and parasitic worms (helminths).

Protozoan infections to be targeted by ATCase inhibition include those caused by Entamoeba histolytica and Naegleria fowleri (both pathogenic amoeba). Exemplary disease include Babesiosis, Balantidiasis, Chagas Disease, Giardiasis, Human African Trypanosomiasis, Leishmaniasis, Plasmodium vivax and other non-P. falciparum malarias.

Helminth infections to be targeted by ATCase inhibition include

Dracunculiasis, Echinococcosis, Food-borne Trematodiases, Loiasis, Lymphatic Filariasis, Onchocerciasis, and other food-borne helminthiases (Trichinosis, Anisakiasis, Gnathostomiasis), Schistosomiasis, Soil-transmitted Helminthiases (Ascariasis, Hookworm Diseases, Trichuriasis, Strongyloidiasis), Taeniasis-Cysticercosis, Toxocara and Baylisascaris (Visceral larva migrans)

Viral infections: Arboviral infections (Dengue, Chikungunya, Zika, Japanese encephalitis, Jungle yellow fever and others), Enterovirus 71 and related viruses HTLV-1, HTLV-2 and other non-HIV retrovirus infections, Rabies, Rift Valley fever, Viral hemorrhagic fevers.

Bacterial infections: Bartonella spp., Atypical mycobacteria (including Mycobacterium bovis and Buruli Ulcer), Cholera and other enteric bacteria (Shigella, Salmonella, E. coli), Leprosy, Leptospirosis, Melioidosis, Noma, Relapsing fever, Trachoma, Yaws and other nonvenereal treponematoses (Bejel and Pinta), Q fever.

Fungal infections: Mycetoma, chromoblastomycosis, and other deep tissue mycoses, Paracoccidioidomycosis.

Ectoparasites: Scabies, Myiasis

Also provided herein is a pharmaceutical composition comprising at least one inhibitor compound(s) according to the invention, and a pharmaceutically acceptable carrier, vehicle or diluent. Preferred pharmaceutical compositions comprise one or more compounds selected from the group consisting of BDA-04, 06, 07, 09, 10, 11, 12, 14, 17, 24, 25, 33, 38, 41, 45, 48, 52, 54, 63, 67, 69 and 70, preferably BDA-04, -07, 09, 11, 12, 14, 24, 25, 33, 38, 45, 48, 52, and 54.

The composition may include other ingredients such as a pharmacologically acceptable carrier, diluent or excipient. The pharmaceutical composition of the present invention can be administered in various forms. Examples of such an administration form include oral administration using tablets, capsules, granules, powders or syrups, or parenteral administration using injection, drop or suppository. Such a composition can be produced by any known method and comprises a carrier, a diluent and an excipient, which are commonly used in the pharmaceutical field. For example, as a carrier or excipient used for a tablet, lactose, magnesium stearate or the like is used. An injection solution can be prepared by dissolving, suspending or emulsifying the compound of the present invention or a salt thereof in a sterile aqueous or oily solution. Examples of aqueous solution used for an injection include a physiological salt solution and an isotonic solution containing glucose or another adjuvant, and the aqueous solution may be used in combination with an appropriate solution adjuvant such as alcohol, polyalcohol such as propylene glycol or a nonionic surfactant. Examples of the above-mentioned oily solution include sesame oil, soybean oil and so on, and the oily solution may be used in combination with a solution adjuvant such as benzyl benzoate or benzyl alcohol.

The dosage applied depends on symptom, age, body weight and others. In therapeutic use, the compounds of the invention are administered to a subject at dosage levels of from about 0.05 mg/kg to about 10.0 mg/kg of body weight per day. For a human subject of approximately 70 kg, a dosage of from 40 mg to 600 mg per day may be used as a non-limiting example. Preferred doses include about 1 mg/kg, about 2.5 mg/kg, about 5 mg/kg, and about 7.5 mg/kg. Lower or higher doses than those disclosed herein may be used, as required. Such dosages, however, may be altered depending on a number of variables, not limited to the activity of the compound used, the condition to be treated, the mode of administration, the requirements of the individual subject, the severity of the condition being treated, and the judgment of the practitioner. The foregoing ranges are merely suggestive, as the number of variables in regard to an individual treatment regime is large, and considerable excursions from these recommended values are not uncommon. The effective amount for use in humans can be determined from animal models. For example, a dose for humans can be formulated to achieve circulating, liver,

topical and/or gastrointestinal concentrations that have been found to be effective in animals.

The invention therefore provides a method of preventing or treating a disease or disorder wherein a beneficial effect is obtained by inhibition of ATCase, comprising administering to a subject in need thereof an inhibitory compound as herein disclosed. Also provided is a compound for use in a such method. In one embodiment, said ATCase is *Plasmodium falciparum* ATCase (*Pf*ATCase), and/or the disease is malaria. In another embodiment, said ATCase is human ATCase (*Hu*ATCase) and/or the disease is a proliferative disease such as cancer. In yet another embodiment, said ATCase is *Mycobacterium tuberculosis* ATCase (*Mt*ATCase) and/or the disease is tuberculosis.

LEGEND TO THE FIGURES

Figure 1: Inhibition dose-response of *Pf*ATC by exemplary inhibitory compounds.

Figure 2: Inhibition dose-response of *Hu*ATC by exemplary inhibitory compounds.

Figure 3: Dose-response profile of *Plasmodium falciparum* 3D7 cells using representative BDAs. Parasite viability was detected using light microscopy to assess parasitemia (population count) of parasites in human red blood stage cultures cultured in the presence of the indicated compounds.

Figure 4: Dose-response profile of 3D7-ATC and 3D7-MOCK cell lines versus BDAs. Effect of inhibitor compounds on 3D7 parasite viability in blood stage cultures was determined in the presence (filled squares) or absence (filled circles, plasmid control) of additional recombinantly expressed *Pf*ATC

Figure 5: Cell viability of cancer cell lines A375, H1299, MCF7, REC-1 and SUDHL-2, and normal blood cells (normal lymphocytes) in the presence of representative *Pf*ATCase inhibitory compounds. Cell viability was determined by resazurin reduction assay.

Figure 6: Cytotoxicity of representative compounds against the cancer cell lines A375, H1299, MCF7, REC-1 and SUDHL-2, and normal blood cells (PBMC/normal lymphocytes). Metabolic activity which is an indicator of cell viability was determined using Resazurin.

Figure 7: Proliferation inhibition of U2OS cells. A) Visible light microscopy images of U20S cells incubated with the indicated concentrations of, respectively, BDA-33, BDA-41 and BDA-52; B) Microscopy images of cell cultures; C)Visible light microscopy images of U20S cell cultures. Proliferation was inhibited by incubation with the indicated concentrations of BDA-33 and BDA-52

Figure 8: The BDA compound series inhibit *Mt*ATCase *in vitro*. A) IC_{50} values of an enzymatic assay of BDA series compounds (70 compounds) against *Mt*ATCase. Only compounds showing a measurable IC_{50} are displayed. Compounds taken forward are highlighted with an asterisk; B) IC_{50} values of an enzymatic assay of selected compounds BDA -06, -10, -14, -17, -25, -54, -63, -67, -69 and -70 against *Mt*ATCase repeated in triplicate are shown; C) An enzymatic assay of PALA against *Mt*ATCase indicates no inhibition; D) A comparison of an enzymatic IC_{50}s for the best 4 *Mt*ATCase inhibitors against *Mt*ATCase, *Pf*ATCase and *Hs*ATCase demonstrating species selectivity.

Figure 9: *In vivo* inhibition of *Mt*ATCase. A) Compound solubility in 7h9+OADC media was assessed using a Zetasizer ; B) MIC90 values of compounds BDA-06, -10, -14, -17, -25, -54, -63, -67, -69, and -70 at a concentration of 8 μM. C) Dose-response MIC90 assay of BDA-06 indicates a MIC90 of <4 mM. D) An XTT assay assessing cytotoxicity of BDA-06 against monocyte derived macrophages indicates a CC50 of 30 mM.

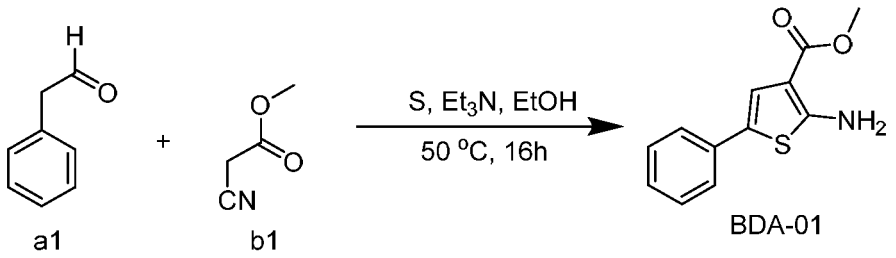
EXPERIMENTAL SECTION

General procedure for inhibitor compound synthesis

All chemicals were purchased from commercial suppliers and used without any purification unless otherwise noted. Nuclear magnetic resonance spectra were recorded. Chemical shifts for ^{1}H NMR were reported as δ values and coupling constants were in hertz (Hz). The following abbreviations were used for spin multiplicity: s = singlet, bs = broad singlet, d = doublet, t = triplet, q = quartet, quin = quintet, dd = double of doublets, ddd = double of doublet of doublets, m = multiplet. Chemical shifts for ^{13}C NMR reported in ppm relative to the solvent peak. Thin layer chromatography was performed on silica gel plates (0.20 mm thick, particle size 25 μm). Flash chromatography was performed using RediSep Rf Normal-phase Silica Flash Columns (Silica Gel 60 Å, 230-400 mesh). High resolution mass spectra were recorded using a LTQOrbitrap- XL (Thermo) at a resolution of 60000@m/z400.

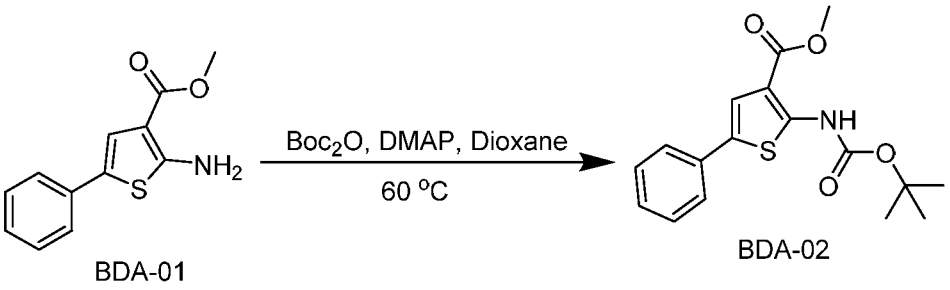
EXAMPLE 1:

General procedure 1:



A 100 mL round bottom flask was charged with 2-phenylacetaldehyde (**a1**, 6ml(6 g), 50 mmol, 1.0 eq), methyl 2-cyanoacetate (**b1**, 5ml (5.1 g), 55 mmol, 1.1 eq), sulfur (1.6 g, 60 mmol, 1.2 eq), and triethylamine (7ml(5.05 g), 50 mmol, 1.0 eq) in ethanol (70 mL). The reaction is heated 50 °C in an oil bath for 16 h. Then, the reaction was cooled down to room temperature. A batch of 120 mL ice water was poured into the mixture to yield a precipitate which was filtered and washed with cold ethanol to obtain 8.1 g (68%) of the title compound **BDA-01** as light yellow powder, M.P.= 178 - 182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (dd, J = 8.4, 1.2 Hz, 2H), 7.35 (t, J = 7.8 Hz, 2H), 7.26 (s, 1H), 7.23 (s, 1H), 6.05 (s, 2H), 3.86 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.80, 162.22, 133.94, 128.85, 126.65, 126.61, 124.70, 121.10, 107.61, 51.15. HRMS (ESI) m/z calculated for $C_{12}H_{12}NO_2S$ $[M+H]^+$: 234.0524; found $[M+H]^+$: 234.0521. Compounds **BDA-66** and **BDA-68** were synthesized according to this procedure using 4-phenylcyclohexan-1-one and 1-benzylpiperidin-4-one, respectively, as **a1** compound.

General procedure 2:

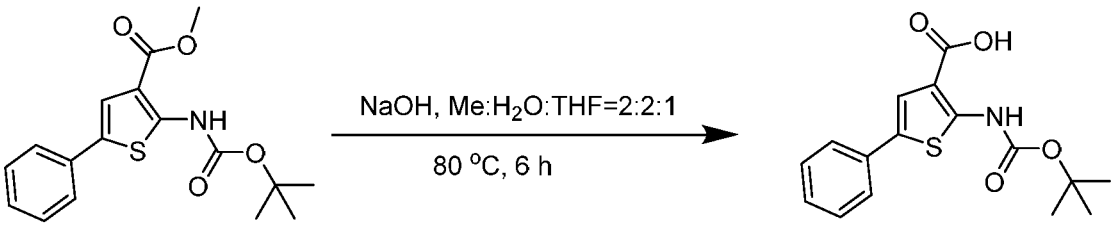


BDA-01 BDA-02

To a 50 mL round bottom flask were added dioxane (30 mL), compound **BDA-01**(1.16 g, 5 mmol, 1.0 eq), Boc_2O (1.2 g, 5.5 mmol, 1.1 eq) and DMAP (61 mg, 0.5mmol, 0.1 eq) and the reaction mixture was stirred at 60 °C for 4 h. Then $N_2H_4 \cdot H_2O$ (0.75g, 15mmol, 3.0 eq) was added and the mixture was stirred at 40 °C for an additional 1.5 h. After cooling to room temperature the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (EtOAc–heptane 5 → 50%) to yield the methyl ester of **BDA-02** (1.35 g, 81%), M.P.= 176 - 181 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.63 – 7.61 (m, 1H), 7.60 (s, 2H), 7.42 (t, J = 7.6 Hz, 2H), 7.35 (t, J = 7.3 Hz, 1H), 3.87 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.74, 152.12, 150.50, 133.76, 128.93, 127.26, 127.22, 125.17, 119.41, 111.77, 82.50, 51.68, 28.22. HRMS (ESI) m/z calculated for $C_{17}H_{20}NO_4S$ $[M+H]^+$: 334.1015; found $[M+H]^+$: 334.1013.

The compounds **BDA-64** and **BDA-65** were synthesized from **BDA-68 and BDA-66** respectively, in a similar fashion according to general procedure 2. **BDA-67** was synthesized from cyclohexanone according to general procedure 1 and 2.

General procedure 3:

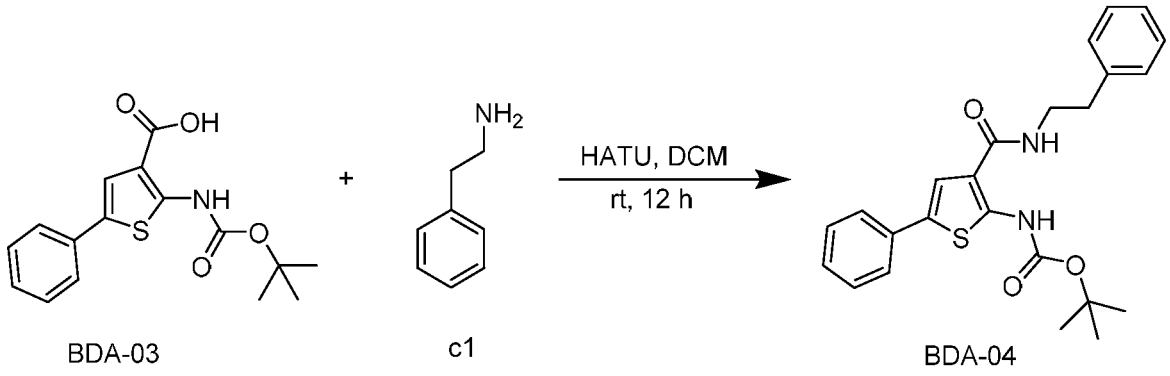


BDA-02 BDA-03

The ester **BDA-02**(1.67 g, 5 mmol, 1.0 eq) was subsequently subjected to a base hydrolysis at 80 °C for 3 h with a solution of NaOH (2.7 g, 13.5 eq) in 100 mL of a solvent mixture (MeOH–H_2O–THF = 2:2:1). The reaction was cooled to room temperature and the organic solvents were removed in vacuo. The aqueous layer

was acidified with 5% HCl to give a precipitate, which was triturated with methanol to afford compound **BDA-03** (1.2 g, 70%) as yellow solid, M.P.= 196 - 201 °C; ^{1}H NMR (500 MHz, DMSO) δ 12.58 (s, 1H), 7.54 (d, *J* = 8.2 Hz, 2H), 7.42 (s, 1H), 7.36 (t, *J* = 7.8 Hz, 2H), 7.21 (t, *J* = 7.3 Hz, 1H), 1.50 (s, 9H).^{13}C NMR (126 MHz, DMSO) δ 168.99, 152.14, 144.23, 134.99, 129.49, 129.01, 126.79, 124.65, 123.51, 122.97, 80.82, 28.42. HRMS (ESI) m/z calculated for $C_{16}H_{18}NO_4S$ $[M+H]^+$: 320.0937; found $[M+H]^+$: 320.0931.

General procedure 4:



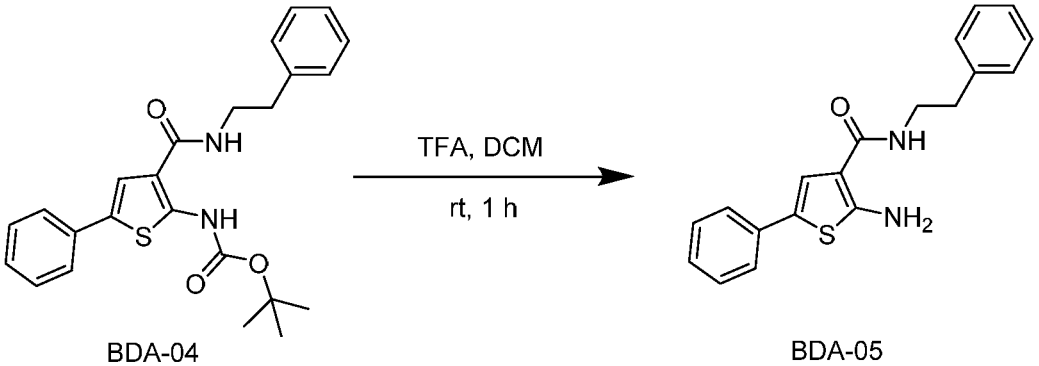
BDA-03 c1 BDA-04

To a solution of compound **BDA-03** (110mg, 0.34mmol, 1.0 eq) and DIPEA(180 ul, 1.02 mmol, 3.0 eq) in DCM (10 ml) was added HATU(155 mg, 0.408 mmol, 1.2 eq) and amine **c1**(50 mg, 0.408 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for overnight. The mixture was diluted with water and extracted with EtOAc. The organic layer was separated, and dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (5% → 50%) as an eluent to give **BDA-04** (106 mg, 74%) purple solid, M.P.= 168 - 173 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.97 (s, 1H), 7.53 (d, *J* = 8.2 Hz, 2H), 7.37 (t, *J* = 7.5 Hz, 4H), 7.29 (s, 1H), 7.27 (d, *J* = 7.1 Hz, 3H), 6.96 (s, 1H), 5.98 (s, 1H), 3.70 (q, *J* = 6.9 Hz, 2H), 2.96 (t, *J* = 7.0 Hz, 2H), 1.57 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.23, 152.46, 148.56, 138.77, 133.76, 132.83, 128.97, 128.85, 128.80, 127.31, 126.73, 125.17, 116.09, 113.67, 81.99, 40.71, 35.90, 28.26. HRMS (ESI) m/z calculated for $C_{24}H_{27}N_2O_3S$ $[M+H]^+$: 423.1738; found $[M+H]^+$: 423.1733.

The compounds **BDA-06, BDA-09, BDA-10, BDA-11, BDA-14, BDA-16, BDA-18, BDA-20, BDA-21, BDA-22, BDA-23, BDA-24, BDA-25, BDA-26, BDA-27, BDA-28, BDA-29, BDA-30, BDA-31, BDA-34, BDA-37, BDA-38, BDA-39, BDA-42, BDA-43, BDA-49, BDA-57, BDA-59, BDA-60, BDA-62** and **BDA-63** can essentially

also be synthesized using the appropriate amine compound **c1** according to general procedure 4.

General procedure 5:

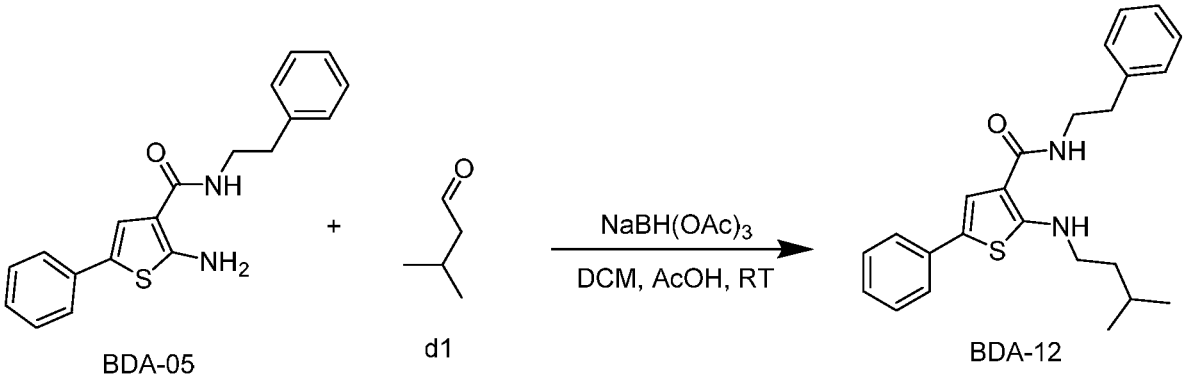


BDA-04 BDA-05

To a solution of compound **BDA-04**(60 mg, 0.142 mmol, 1.0 eq) in DCM (5 ml) was added TFA (2 mL) in DCM(2 mL) at RT for 1 h. The solvent was evaporated by high vacuum to give the final product **BDA-05** (45mg, 98%), M.P.= 198 - 202 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 7.7 Hz, 2H), 7.35 (q, *J* = 8.0 Hz, 4H), 7.31 – 7.19 (m, 5H), 6.88 (s, 1H), 5.92 (s, 2H), 3.67 (d, *J* = 6.3 Hz, 2H), 2.94 (d, *J* = 6.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.98, 160.54, 139.18, 134.05, 128.92, 128.71, 126.62, 126.55, 125.17, 124.64, 118.31, 109.71, 40.64, 36.13. HRMS (ESI) m/z calculated for $C_{19}H_{19}N_2OS$ $[M+H]^+$: 323.1147; found $[M+H]^+$: 323.1141.

The compound **BDA-07** was and synthesized essentially according to general procedure 5 starting from **BDA-06. BDA-50** was synthesized essentially according to general procedure 5 starting from **BDA-11. BDA-46, BDA-55, BDA-58 and BDA-61** were synthesized essentially according to general procedure 1 to 5, using 2-(4-fluorophenyl)ethan-1-amine, 1,2-diphenylethan-1-one, 3-phenylpropanal and 3-methylbutanal, respectively.

General procedure 6:

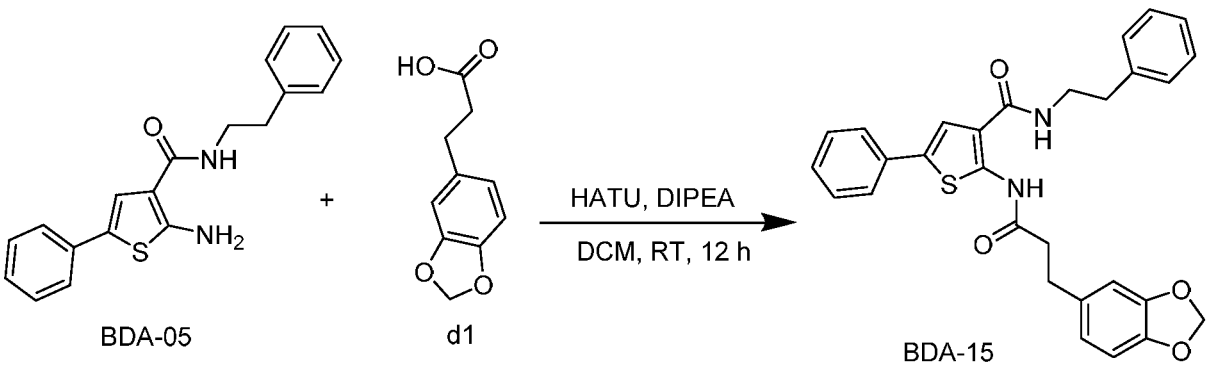


BDA-05 d1 BDA-12

To a solution of **BDA-05** (333 mg, 1.0 mmol, 1.0 eq) in dichloromethane (10 mL) and acetic acid (0.05 mL) were added 3-methylbutanal **d1** (87 mg, 1.0 mmol, 1.0 eq) and sodium triacetoxyborohydride (255 mg, 1.2 mmol, 1.2 eq). The reaction mixture was stirred for 18 h at room temperature and concentrated. The residue was dissolved in ethyl acetate, washed with aqueous sodium bicarbonate solution and with water, dried over anhydrous $MgSO_4$ and concentrated. The residual oil was purified on a silica gel flash chromatography column eluted with EtOAc–PE (5% → 80%) to afford the desire compound **BDA-12** as a slightly yellow oil (333 mg, 85% yield), M.P.= 198 - 203 °C; ^{1}H NMR (500 MHz, DMSO) δ 11.29 (s, 1H), 7.82 (s, 1H), 7.77 (d, J = 8.4 Hz, 1H), 7.55 (d, J = 7.1 Hz, 2H), 7.48 – 7.40 (m, 4H), 7.31 (s, 2H), 5.77 (s, 2H), 3.52 (q, J = 7.9, 7.3 Hz, 2H), 2.94 (t, J = 7.3 Hz, 2H), 2.51 (p, J = 1.8 Hz, 9H). ^{13}C NMR (126 MHz, DMSO) δ 165.22, 151.84, 147.14, 144.02, 142.59, 133.92, 131.49, 129.70, 129.61, 127.76, 126.35, 126.14, 125.06, 124.91, 119.38, 114.99, 82.15, 55.40, 35.22, 28.18. HRMS (ESI) m/z calculated for $C_{24}H_{28}N_3O_5S_2$ $[M+H]^+$: 502.1427; found $[M+H]^+$: 502.1424.

The compound **BDA-13** was started from 1H-imidazole-2-carbaldehyde and essentially synthesized according to general procedure 6. **BDA-19** was started from 3-methylbutanal and **BDA-50**, and essentially synthesized according to general procedure 6.

General procedure 7:



To a solution of compound **BDA-05** (333 mg, 1.0 mmol, 1.0 eq) and DIPEA(540 ul, 3.0 mmol, 3.0 eq) in DCM (10 ml) was added HATU(420 mg, 1.1 mmol, 1.1eq) and acid **d1**(195 mg, 1.0 mmol, 1.0 eq). The reaction mixture was stirred at room temperature for overnight. The mixture was washed with water and extracted with EtOAc. The organic layer was separated, dried over $MgSO_4$, filtered and

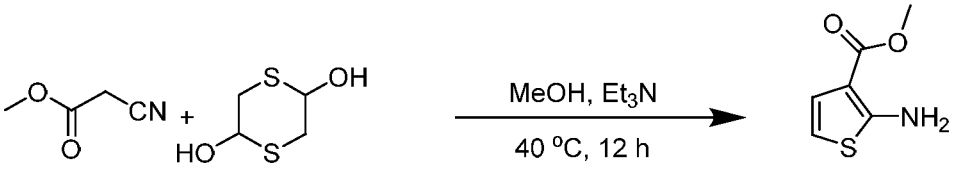
concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (5% → 50%) as an eluent to give **BDA-15** (318 mg, 64%) yellow solid, M.P.= 188 - 190 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.97 (s, 1H), 7.57 – 7.53 (m, 2H), 7.38 (q, *J* = 7.5 Hz, 4H), 7.31 (d, *J* = 7.6 Hz, 2H), 7.28 – 7.26 (m, 2H), 6.97 (s, 1H), 6.76 (d, *J* = 7.6 Hz, 2H), 6.72 (dd, *J* = 7.9, 1.7 Hz, 1H), 6.05 (t, *J* = 6.0 Hz, 1H), 5.94 (s, 2H), 3.71 (q, *J* = 6.9 Hz, 2H), 3.04 (t, *J* = 7.7 Hz, 2H), 2.96 (t, *J* = 6.9 Hz, 2H), 2.80 (t, *J* = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 169.22, 165.29, 147.71, 146.19, 146.03, 138.63, 134.29, 134.07, 133.68, 129.01, 128.83, 127.54, 126.79, 125.47, 121.22, 115.60, 115.08, 108.88, 108.35, 100.86, 40.71, 38.64, 35.81, 30.92. HRMS (ESI) m/z calculated for $C_{29}H_{27}N_2O_4S$ $[M+H]^+$: 499.1667; found $[M+H]^+$: 499.1662.

The compounds **BDA-08, BDA-17** and **BDA-70** were essentially also be synthesized according to general procedure 7, starting from 2-(1H-indol-3-yl)acetic acid, (tert-butoxycarbonyl)glycine, and 1-cyanocyclopropane-1-carboxylic acid, respectively.

EXAMPLE 2:

This example describes the synthesis of compound **BDA-33** using a series of consecutive steps. The same approach can be followed to prepare compounds **BDA-35, BDA-40, BDA-45, BDA-47, BDA-48, BDA-51, BDA-52, BDA-53, BDA-54, BDA-56** and **BDA-69** using the appropriate boric acid in step 6'.

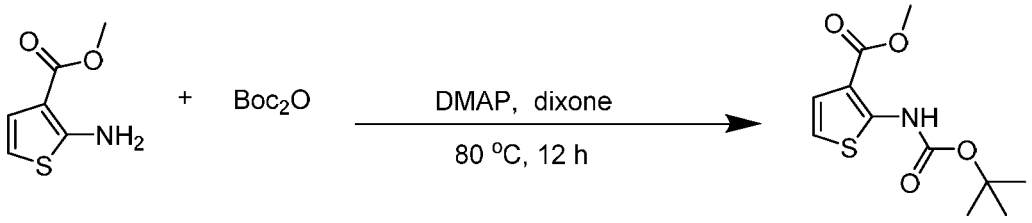
Step 1':



methyl 2-cyanoacetate(4.0 g , 40.0 mmol, 1.0 eq), 1,4-dithiane-2,5-diol(3.04 g , 20.0 mmol, 0.5 eq), and triethylamine(1.7 ml , 12.0 mmol, 0.3 eq) in 100 ml methanol are added into a 250 ml round-bottomed flask. The reaction mixture was heated at 40 °C for 12 h. Then the reaction was cooled to room temperature and extracted with DCM (200 x 3). The organic layer was dry with $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (5% → 80%) as an eluent to give

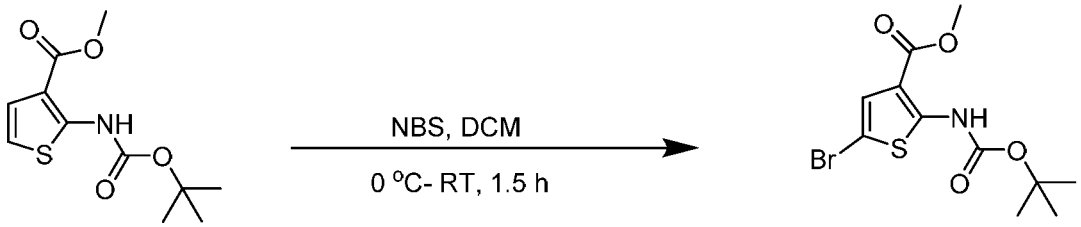
final compound (5.1 g, 80%) red solid. M.P.= 177 - 182 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.99 (d, J = 5.7 Hz, 1H), 6.21 (d, J = 5.8 Hz, 1H), 5.96 (s, 2H), 3.84 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.82, 162.76, 125.81, 107.03, 106.93, 51.00. HRMS (ESI) m/z calculated for $C_6H_8NO_2S$ $[M+H]^+$: 158.0247; found $[M+H]^+$: 158.0249.

Step 2':



To a 50 mL round bottom flask were added dioxane (40 mL), methyl 2-aminothiophene-3-carboxylate (4.7 g, 30.0 mmol, 1.0 eq), Boc_2O (7.2 g, 33.0 mmol, 1.1 eq) and DMAP (388 mg, 3.0 mmol, 0.1 eq) and the reaction mixture was stirred at 60 °C for 4 h. After cooling to room temperature the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (EtOAc–heptane 5 → 50%) to yield methyl 2-((tert-butoxycarbonyl)amino)thiophene-3-carboxylate (7.3 g, 95%), M.P.= 177 - 180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.36 (d, J = 6.6 Hz, 1H), 7.16 (d, J = 5.8 Hz, 1H), 3.84 (s, 3H), 1.42 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 162.25, 150.37, 146.67, 127.45, 127.21, 122.73, 83.31, 51.74, 27.78. HRMS (ESI) m/z calculated for $C_{11}H_{16}NO_4S$ $[M+H]^+$: 258.0739; found $[M+H]^+$: 258.0754.

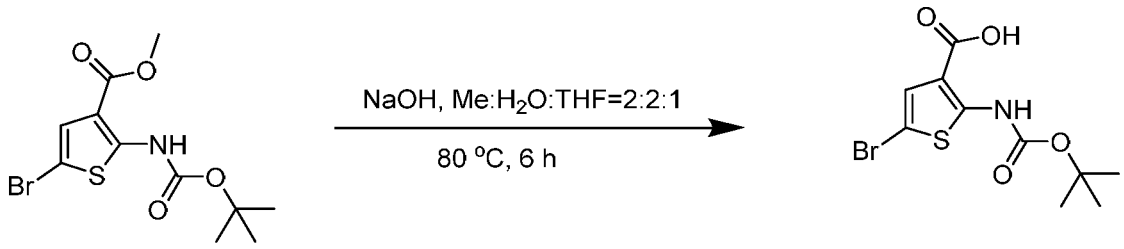
Step 3':



To a solution of methyl 2-((tert-butoxycarbonyl)amino)thiophene-3-carboxylate (1.6 g, 6.22 mmol, 1.0 eq) in dichloromethane (10 mL) and acetic acid (10 mL) were added NBS (1.33 g, 7.46 mmol, 1.2 eq) at 0 °C for 0.5 h. The reaction mixture was diluted with water and extracted with ethyl acetate (200 ml x 3). The organic layer was washed with saturated $NaHCO_3$ and finally with brine, dried with $MgSO_4$. The

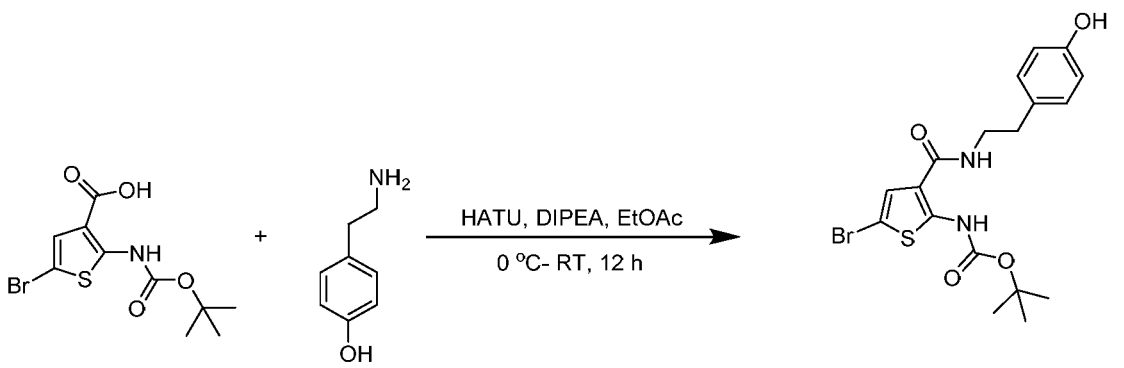
solvent was removed on a rotary evaporator, and the residue was purified by column chromatography on silica gel (EtOAc–heptane 10 → 50%) to yield the product (1.56 g, 75%) as red solid. M.P.= 177 - 180 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.04 (s, 1H), 7.15 (s, 1H), 3.87 (s, 3H), 1.55 (s, 9H). ^{13}C NMR (126 MHz, CDC_{l3}) δ 164.74, 152.15, 151.50, 126.01, 110.71, 102.55, 82.81, 51.75, 28.15. HRMS (ESI) m/z calculated for $C_{11}H_{15}BrNO_4S$ $[M+H]^+$: 335.9871; found $[M+H]^+$: 335.9883.

Step 4':



The methyl 5-bromo-2-((tert-butoxycarbonyl)amino)thiophene-3-carboxylate (740 mg, 2.2 mmol, 1.0 eq) was subsequently subjected to a base hydrolysis at 80 °C for 3 h with a solution of NaOH (594 mg, 14.85 mmol, 6.75 eq) in 30 mL of a solvent mixture (MeOH–H_2O–THF = 2:2:1). The reaction was cooled to room temperature and the organic solvents were removed in vacuo. The aqueous layer was acidified with 5% HCl to give a precipitate, which was filtered to afford the compound (465 mg, 65%) as red solid, M.P.= 198 - 203 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.85 (s, 1H), 7.21 (s, 1H), 1.58 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 168.05, 153.34, 148.42, 128.60, 126.25, 109.82, 84.02, 28.17. HRMS (ESI) m/z calculated for $C_{10}H_{13}BrNO_4S$ $[M+H]^+$: 321.9731; found $[M+H]^+$: 321.9745.

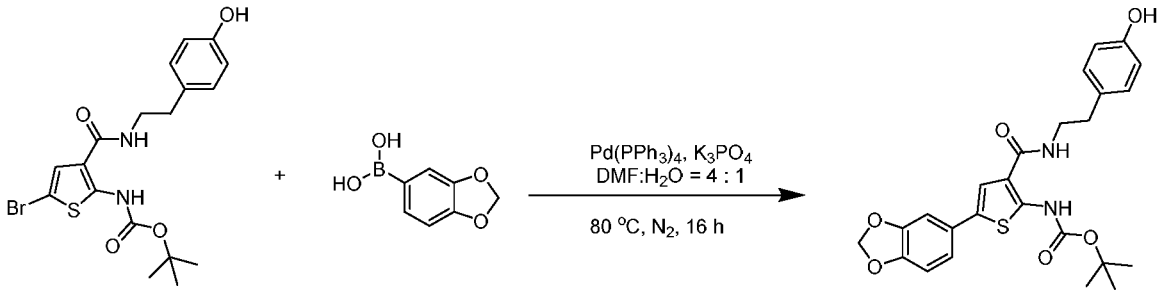
Step 5':



To a solution of compound 5-bromo-2-((tert-butoxycarbonyl)amino)thiophene-3-carboxylic acid (607 mg, 2.0 mmol, 1.0 eq) and DIPEA(1.2 ml, 6.0 mmol, 3.0 eq) in ethyl acetate (20 ml) was added HATU(420 mg, 1.1 mmol, 1.1eq) and 4-(2-

aminoethyl)phenol (302 mg, 2.2 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for overnight. The mixture was washed with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (10% → 75%) as an eluent to give the product (318 mg, 64%) as yellow solid, M.P.= 192 - 196 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.93 (s, 1H), 7.15 – 7.04 (m, 2H), 6.88 – 6.76 (m, 2H), 6.73 (s, 1H), 5.80 – 5.73 (m, 1H), 5.00 (s, 1H), 3.65 – 3.58 (m, 2H), 2.84 (t, J = 6.9 Hz, 2H), 1.54 (s, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.18, 154.43, 152.50, 149.51, 130.61, 129.91, 122.82, 115.68, 112.65, 103.17, 82.30, 40.83, 34.84, 28.19. HRMS (ESI) m/z calculated for $C_{18}H_{22}BrN_2O_4S$ $[M+H]^+$: 441.0427; found $[M+H]^+$:441.0434.

Step 6':



BDA-33

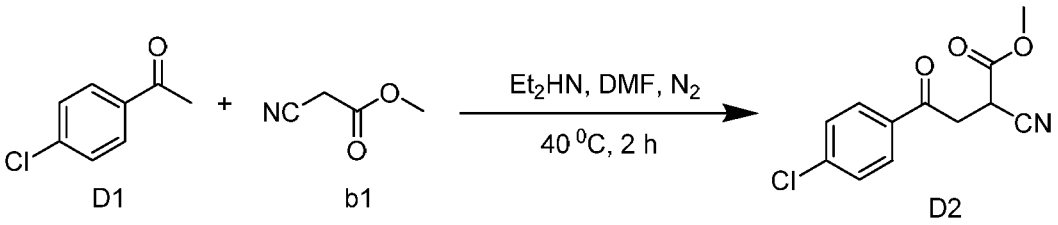
The compound tert-butyl (5-bromo-3-((4-hydroxyphenethyl)carbamoyl) thiophen-2-yl)carbamate(136 mg, 0.32 mmol, 1.0 eq), benzo[d][1,3]dioxol-5-ylboronic acid(64 mg, 0.38 mmol, 1.1 eq) and K_3PO_4 (424 mg, 2.0 mmol, 6.25 eq) were dissolved in 4:1 DMF /H_2O under the atmosphere of N_2. Then the $Pd(PPh_3)_4$ (12 mg, 0.001 mmol, 0.03 eq) was added and the reaction mixture was heated at 80 °C for 16 h. The solution was cooled and diluted with EtOAc(30 mL), extracted with EtOAc (3x 100 mL), then washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using MeOH /DCM (1% → 10%) as an eluent to give the final product **BDA-33** (105mg, 68%) as yellow solid, M.P.= 198 - 202 °C; ^{1}H NMR (500 MHz, DMSO) δ 11.26 (s, 1H), 9.20 (s, 1H), 8.38 (t, J = 5.5 Hz, 1H), 7.68 (s, 1H), 7.10 (d, J = 1.9 Hz, 1H), 7.06 – 7.00 (m, 3H), 6.97 (d, J = 8.2 Hz, 1H), 6.70 (d, J = 8.5 Hz, 2H), 6.07 (s, 2H), 3.44 – 3.37 (m, 2H), 2.73 (t, J

= 7.6 Hz, 2H), 1.51 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 165.09, 156.17, 151.83, 148.56, 147.19, 146.30, 131.48, 129.97, 129.78, 128.25, 118.79, 115.71, 115.56, 114.92, 109.40, 105.52, 101.79, 82.04, 41.29, 34.82, 28.35, 28.18. HRMS (ESI) m/z calculated for $C_{25}H_{27}N_2O_6S$ $[M+H]^+$: 483.1507; found $[M+H]^+$: 483.1532.

EXAMPLE 3:

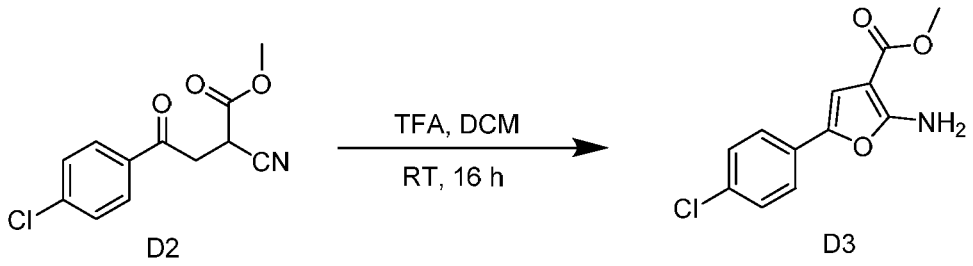
This example describes the synthesis of compound **BDA-44** using a series of consecutive steps.

Step 1":



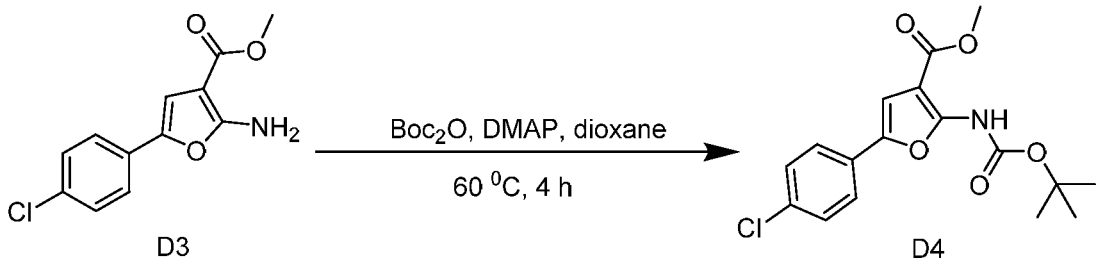
To a solution of 1-(4-chlorophenyl)ethan-1-one (1.54 g, 10.0 mmol, 1.0 eq) and methyl 2-cyanoacetate (0.9 ml, 10.0 mmol, 1.0 eq) in dry DMF(20 ml) was added diethylamine (2.0 ml, 20.0 mmol, 2.0 eq). The reaction mixture was stirred at room temperature for 2 h under N_2 atmosphere. The mixture was then diluted with DCM (100 mL), poured into water and washed with 2N HCl. The organic phase was extracted with DCM (100 mL x 3), then washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (10% → 75%) as an eluent to give the final product (1.7 g, 68%) as brown solid.

Step 2":



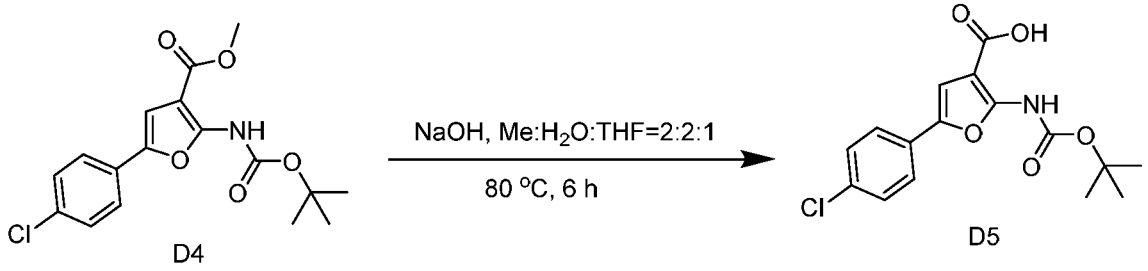
Trifluoroacetic acid (15 mL) was added in one portion to intermediate D2 (1.0 g, 4.0 mmol, 1.0 eq) in DCM (15 ml) at room temperature. The reaction was stirred for 16 h and the solvents removed under vacuum. The residue was was extracted with EtOAc (100 mL x 3), then washed with water and brine. The organic layer was dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (30% → 100%) as an eluent to give the final product (0.8 g, 80%) as brown solid.

Step 3":



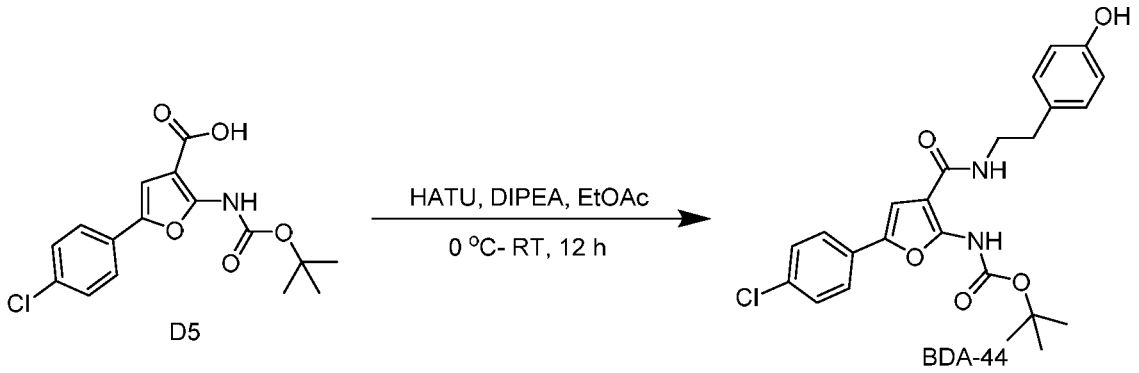
To a solution of intermediate D3 (0.75 g, 3.0 mmol, 1.0 eq) in dioxane (40 mL), Boc_2O (0.72 g, 3.3 mmol, 1.1 eq) and DMAP (39 mg, 0.33 mmol, 0.1 eq) were added. The reaction mixture was stirred at 60 °C for 4 h. After cooling to room temperature the solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel (EtOAc–heptane 15 → 65%) to yield product D4 (1.03 g, 98%).

Step 4":



The intermediate D4 (0.7 g, 2.0 mmol, 1.0 eq) was subsequently subjected to a base hydrolysis at 80 °C for 3 h with a solution of NaOH (480 mg, 12.0 mmol, 6.0 eq) in 20 mL of a solvent mixture (MeOH–H_2O–THF = 2:2:1). The reaction was cooled to room temperature and the organic solvents were removed under vacuum. The aqueous layer was acidified with 5% HCl to give a precipitate, which was filtered to afford the compound (540 mg, 80%) as red solid.

Step 5":



To a solution of intermediate D5 (337 mg, 1.0 mmol, 1.0 eq) and DIPEA(0.6 ml, 3.0 mmol, 3.0 eq) in DCM (10 ml) was added HATU(420 mg, 1.1 mmol, 1.1eq) and 4-(2-aminoethyl)phenol (165 mg, 1.2 mmol, 1.2 eq). The reaction mixture was stirred at room temperature for overnight. The mixture was washed with water and extracted with EtOAc. The organic layer was separated, washed with water and brine, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The residue was purified by column chromatography on silica gel using EtOAc–PE (10% → 85%) as an eluent to give the product **BDA-44** (351 mg, 75%) as yellow solid, M.P.= 190 - 195 °C; ^{1}H NMR (500 MHz, DMSO) δ 9.62 (s, 1H), 9.20 (s, 1H), 8.17 (t, J = 5.9 Hz, 1H), 7.59 (d, J = 8.7 Hz, 2H), 7.52 (d, J = 8.7 Hz, 2H), 7.29 (s, 1H), 7.03 (d, J = 8.5 Hz, 2H), 6.69 (d, J = 8.5 Hz, 2H), 3.36 (s, 2H), 2.70 (t, J = 7.5 Hz, 2H), 1.48 (s, 9H). ^{13}C NMR (126 MHz, DMSO) δ 162.77, 156.15, 151.32, 148.80, 145.72, 132.29, 129.95, 129.82, 128.76, 125.02, 115.58, 106.23, 105.55, 81.31, 34.89, 28.37, 28.20. HRMS (ESI) m/z calculated for $C_{24}H_{26}ClN_2O_5$ $[M+H]^+$: 457.1525; found $[M+H]^+$: 457.1528.

EXAMPLE 3: Inhibition of aspartate transcarbamoylase (ATCase).

This example demonstrates the *in vitro* inhibitory action of exemplary compounds against human (*Hu*) or *P. falcipareum* (*Pf*) aspartate transcarbamoylase (ATCase).

Materials

L-Aspartic acid sodium salt monohydrate >99% (NT), Carbamyl phosphate disodium salt >80% were purchased from Aldrich. Antipyrine, 98%, 2,3-Butanedione monoxime, 99% were obtained from Alfa Aesar. Tris base was obtained from Fisher bioreagents.

PfATCase assay

Wild type *Pf*TC-Met3 was cloned, expressed and purified to homogeneity according to Lunev et al.(Acta Crystallogr F Struct Biol Commun. 2016 Jul 1; 72(Pt 7): 523–5332016).

Enzymatic reactions were performed in a total volume of 150 µL in 50 mM Tris-Acetate buffer at pH 8.0; the final concentration of *Pf*ATC-Met3 was 50 nM. L-Aspartate (Asp) and carbamoyl-phosphate (CP) saturation curves of the enzymes were assayed using a fixed concentration of CP (2 mM) and L-aspartate (1mM). Small-molecule dose-response curves were measured using assay buffer supplemented with 2% (v/v) DMSO, 2 mM CP and 1mM aspartate. *Pf*ATC was pre-incubated with Asp and compounds for 10 min by putting the plate in a shaker at room temperature. The reactions were initiated by adding CP and quenched after 10 min with 100 µ L of stop mix (two volumes of Antipyrine (26.5 Mm 2,3-Dimethyl-1-phenyl-3-pyrazolin-5-one in 50% (v/v) sulfuric acid) and one volume of 2,3-Butanedione monoxime (80Mm 2,3-Butanedione monoxime in 5% (v/v) acetic acid). After plates were sealed with transparent sealing tape to prevent evaporation and incubated overnight in the dark place at room temperature. After incubation, the plates were heated at 95°C for 15 min, and kept for 30 min before measuring at 466 nm suing a Synergy H1 Hybrid Reader (BioTek). Analyses were performed using Microsoft Excel and Graph Pad Prism.

HuATCase cloning, expression and purification

The full-length human CAD gene (carbamoyl-phosphate synthetase 2, aspartate transcarbamylase, and dihydroorotase [Homo sapiens (human)] Gene ID: 790) was purchased from Eurofins and was amplified by PCR using Phusion High-Fidelity PCR Master Mix (New England Biolabs), using the forward primer (*hu*ATC_fwd; 5'-agggcgccATGCTGCACTCATTAGTGG-3') and reverse primer (*hu*ATC_rev; 5'-cgaattcgCTAGAAACGGCCCAGCAC-3'). The pETM-41 vector was obtained from European Molecular Biology Laboratory (EMBL) and the PCR reaction was performed using Phusion High-Fidelity PCR Master Mix(New England Biolabs), the forward primer sequence (pETM-41_fwd 5'-ccgtttctagCGAATTCGAGCTCCGTCG-3') and the reverse primer sequence (pETM-41_rev 5'-gcagcatGGCGCCCTGAAAATAAAG-3'). The final expression plasmid pETM-41-huATC-full was obtained by Gibson Assembly reaction using E2611 Gibson Assembly Master Mix purchasing from New England Biolabs, and it encoded the *hu*ATC with N-terminal His_6-tagged maltose-binding protein.

Protein expression and purification

His-tagged maltose-binding protein *hu*ATC was recombinantly expressed using *E. coli* BL21 star competent cells transformed with pETM-41-huATC-full expression plasmid. The optimal cell line and inductor concentration were chosen based on preliminary small-scale expression trials (data not shown). The culture was propagated in 1L of selective TB media supplemented with 50 μg ml^{-1} kanamycin, 35 μg ml^{-1} chloramphenicol at 310 K, followed by inducing with 0.1 M of IPTG 18h at 291 K according to expression trial results. Bacterial cells were harvested by centrifugation and resuspended in 35 ml lysis buffer [20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% (v/v) glycerol, 2 mM β-mercaptoethanol (BME)]. Cell lysis was performed by sonication on ice. The lysate was clarified by centrifugation at 45,000 X g (SS-34 rotor, Thermo Scientific) for 50 min. The supernatant was filtered using 0.45 μm filter membrane (Whatman) and applied onto a 5 ml Ni^{2+} HisTrap HP column (GE Healthcare, USA). Following washing with Lysis buffer supplemented with 40 mM imidazole, the *hu*ATCase protein was eluted by increasing the concentration of imidazole to 300 mM. Excess imidazole was removed and the His-tag was cleaved off by overnight dialysis against dialysis buffer [20 mM Tris-HCl

pH 7.0, 75 mM NaCl, 5% glycerol, 2 mM ß-mercaptoethanol (BME)], with inclusion of TEV protease within dialysis bag. Then, the sample was loaded onto a 5 ml HiTrap SP HP column (GE Healthcare, USA) and equilibrated in dialysis buffer. The elate was pooled and concentrated at 277 K to 2 mg ml^{-1} using Vivaspin Turbo 4 concentration column with a 10 kDa cutoff (Sartorius Stedim Biotech, Germany).

The concentrated sample was further purified by size-exclusion chromatography (SEC), the protein was concentrated to a volume of 1 ml and purified via SEC suing a HiLoad 16/60 Superdex 75 column (GE Healthcare) pre-equilibrated with SEC buffer [20 mM Tris-HCl pH 7.0, 100 mM NaCl, 2% glycerol, 0.2 mM tris(2-carboxyethyl)phosphine(TCEP)], using NGC liquid chromatography system (BioRad). The purified protein as a single peak and was pooled and concentrated to 2 mg ml^{-1} at 277 K. the final concentration was determined based on the protein theoretical absorbance at 280 mm [ABS 0.1% (1mg ml^{-1}) = 0.354]

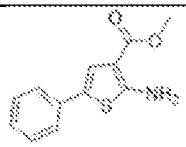
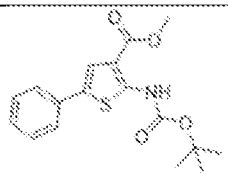
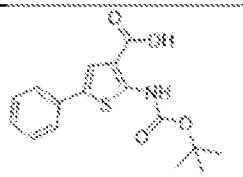
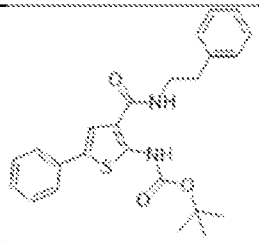
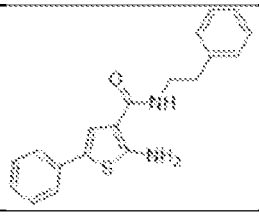
Hu ATCase Activity assay

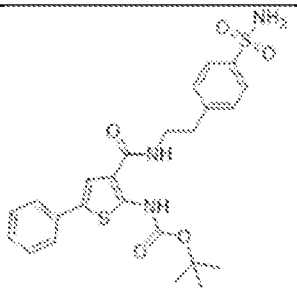
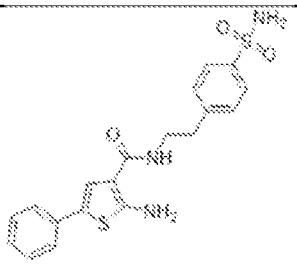
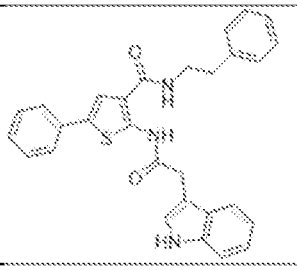
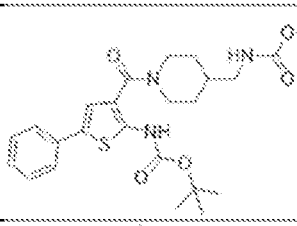
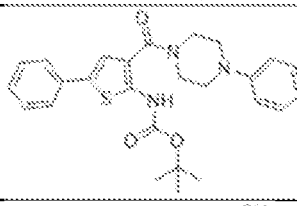
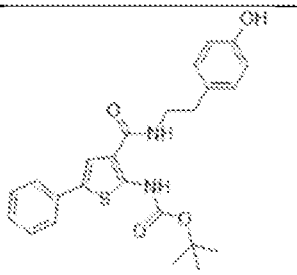
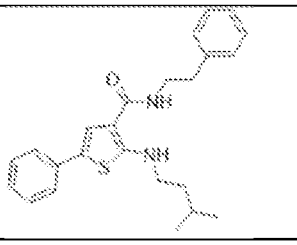
The enzymatic assay was performed as described herein above for enzymatic assay of *Pf*ATC with minor modifications. Briefly, the reaction was carried out at room temperature in a total volume of 150 µl of 10 mM L-aspartate and 5 mM carbamoyl phosphate saturated substrate solution in 50 mM Tris-Acetate buffer pH 8.3. *hu*ATC was pre-incubated with inhibitor and L-Asp for 10 min at room temperature on a shaker. The reaction was initiated by adding CP and stopped after 5 min with 10 ml color mix. Then the plate was covered by sealing tape and kept in dark place at room temperature and then heated to 95°C for 15min in dark and kept the plate in the dark for another 30 min before measuring the absorbance at 466 nm suing a Synergy H1 Hybrid Reader (BioTek). Analyses were performed using Microsoft Excel and Graph Pad Prism.

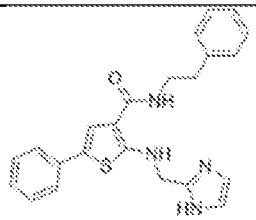
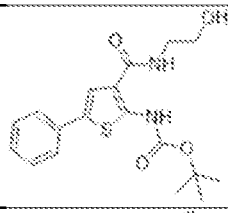
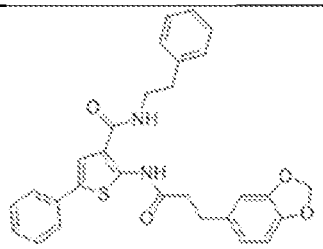
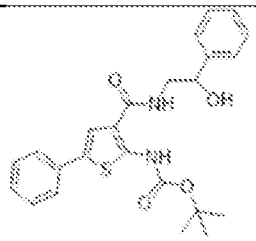
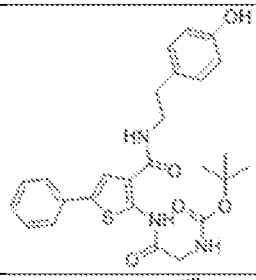
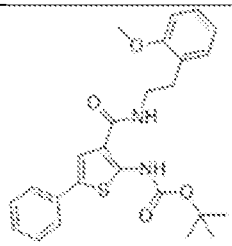
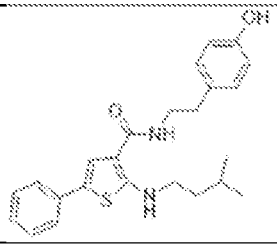
RESULTS

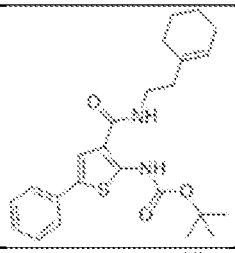
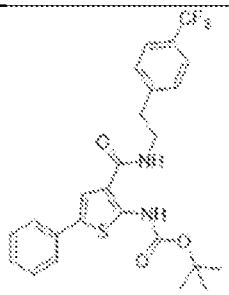
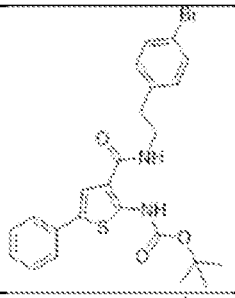
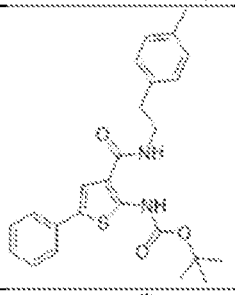
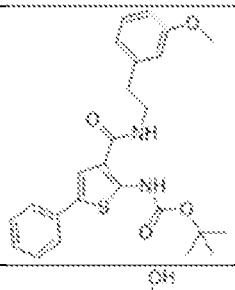
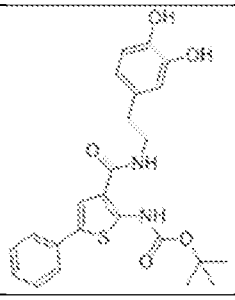
Figures 1 and 2 show representative inhibition dose-response curves that were obtained for exemplary BDA compounds against *Pf*ATCase and *Hu*ATCase, respectively. Table 1 provides the IC50 values for all compounds tested.

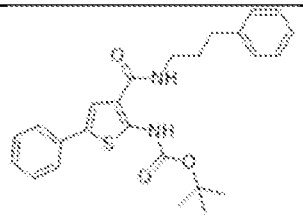
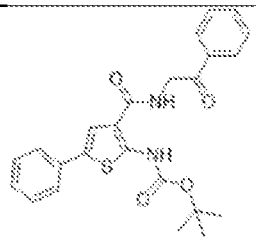
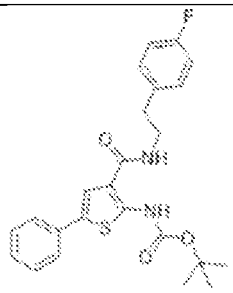
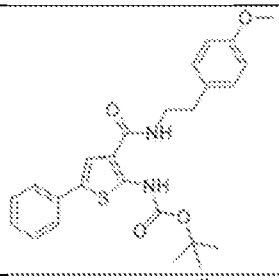
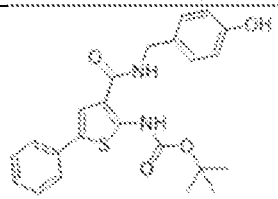
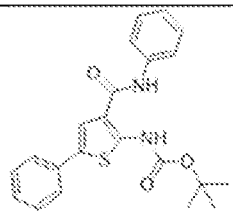
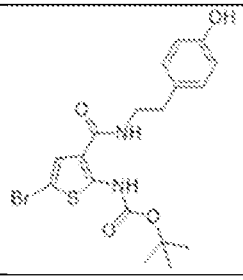
Table 1: Thiophene-containing compounds that are illustrative of formula I, along with the corresponding compound identification numbers. Shown are the structures and IC_{50} values (in nM) against recombinantly produced *Pf*ATCase and *Hu*ATCase.

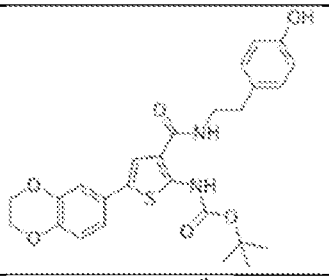
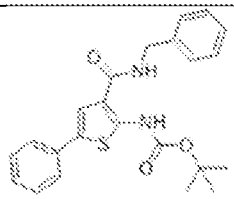
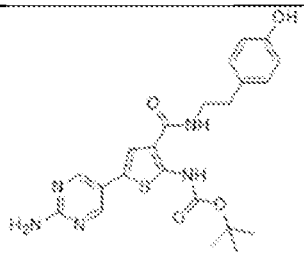
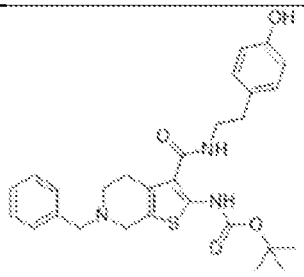
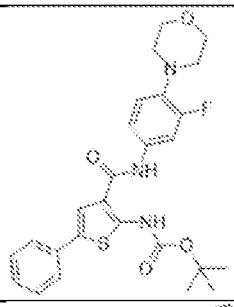
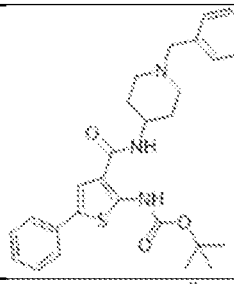
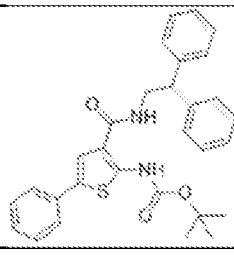
Cpd	Structure	*Pf*ATCase IC_{50}(nM)	*Hu*ATCase IC_{50}(nM)
BDA-01		1273	1539
BDA-02		1470	1504
BDA-03		1000	1630
BDA-04		77.2	2839
BDA-05		1270	765.1

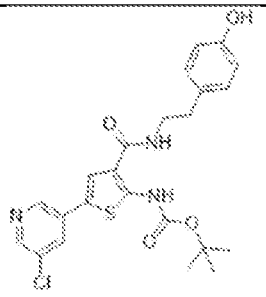
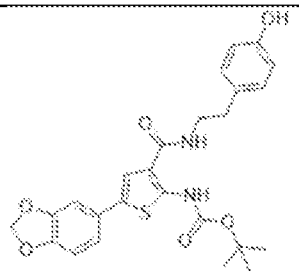
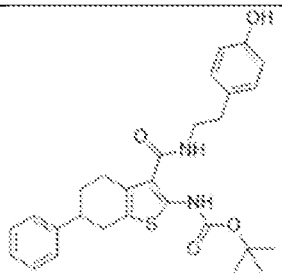
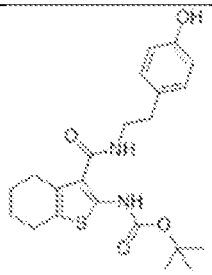
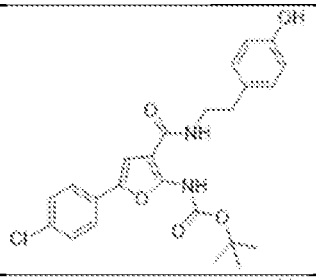
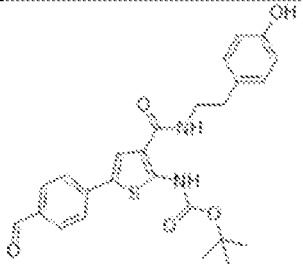
BDA-06		1873	287.0
BDA-07		996.5	115.9
BDA-08		14684	No
BDA-09		241.1	195.6
BDA-10		5058	298.4
BDA-11		45.65	115.9
BDA-12		158.9	655.2

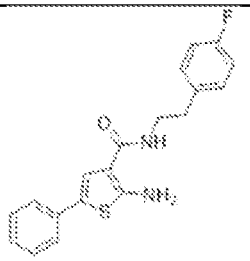
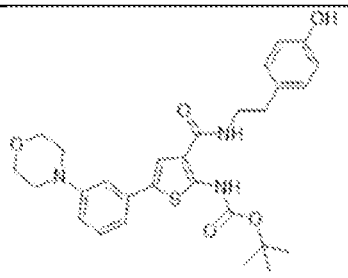
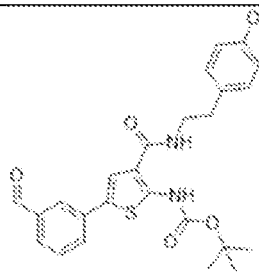
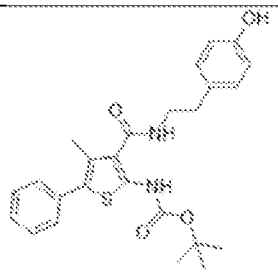
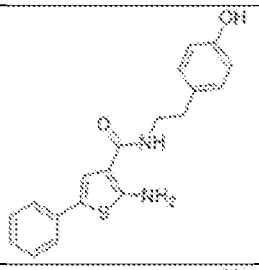
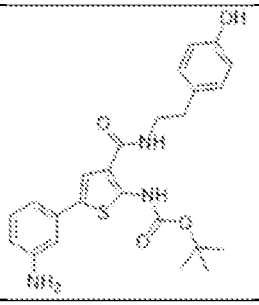
BDA-13		639.1	267.5
BDA-14		114.3	137.2
BDA-15		5223.7	No
BDA-16		6081	869.5
BDA-17		1149	308.0
BDA-18		1328	259.0
BDA-19		4073	678.0

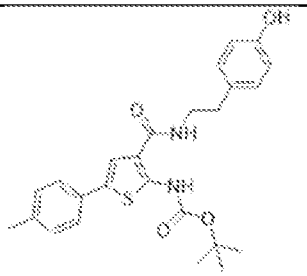
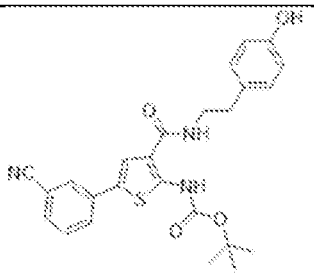
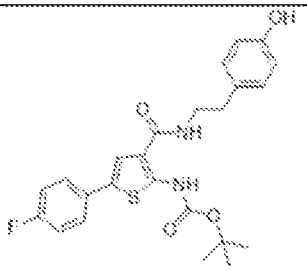
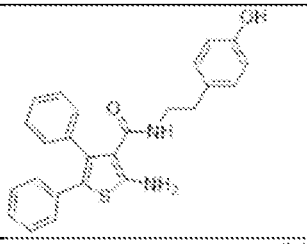
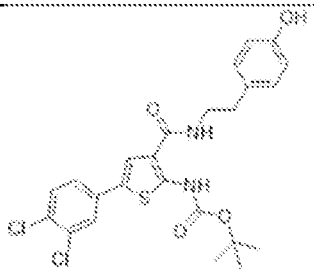
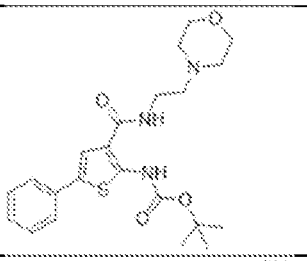
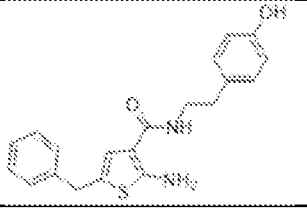
BDA-20		6311	1997
BDA-21		559.8	620.3
BDA-22		472.9	573.0
BDA-23		409.2	755.5
BDA-24		102.7	316.3
BDA-25		181.2	402.7

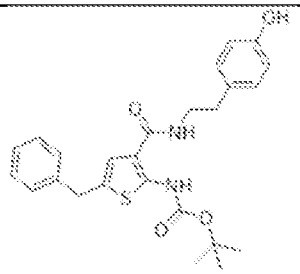
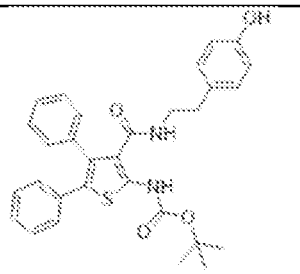
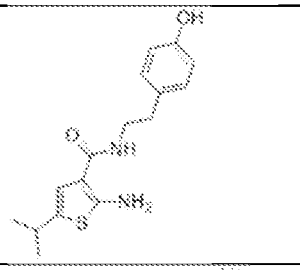
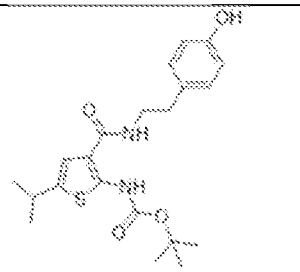
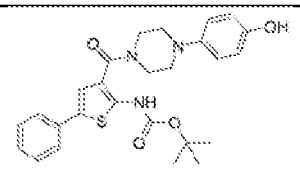
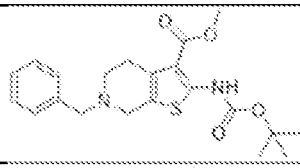
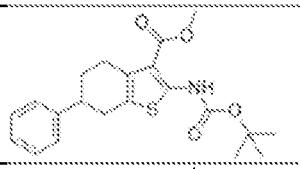
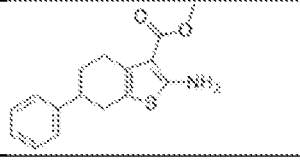
BDA-26		575.7	554.8
BDA-27		1230	369.3
BDA-28		1627	391.2
BDA-29		1616	585.1
BDA-30		2624	397.0
BDA-31		1048	332.0
BDA-32		2763	640.6

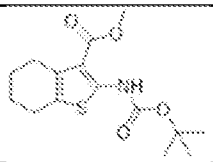
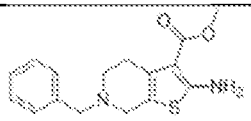
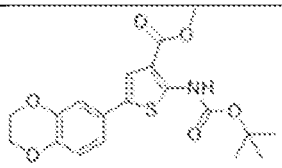
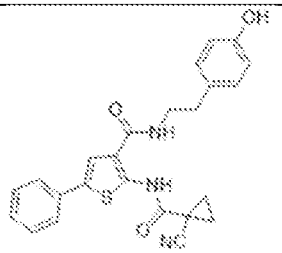
BDA-33		515.9	100.5
BDA-34		848.9	211.9
BDA-35		2077	233.9
BDA-36		No	3839
BDA-37		1080	348.7
BDA-38		805.2	184.8
BDA-39		5645	15441

BDA-40		No	11381
BDA-41		673	28.29
BDA-42		12212	2423
BDA-43		18357	2087
BDA-44		6112	829.9
BDA-45		3769	123.9

BDA-46		1639	740.5
BDA-47		1534	238.3
BDA-48		1665	124.8
BDA-49		5943	729.1
BDA-50		1139	360.1
BDA-51		1243	225.7

BDA-52		558.1	119.1
BDA-53		2720	382.9
BDA-54		844.9	157.9
BDA-55		No	No
BDA-56		2221	555.9
BDA-57		799.7	380.8
BDA-58		4833	1227

BDA-59		3109	2588
BDA-60		2013	1046
BDA-61		1977	862.4
BDA-62		2200	1125
BDA-63		1090	250.5
BDA-64		86943	703548
BDA-65		6422	5169
BDA-66		1292	764.1

BDA-67		1385	1009
BDA-68		No	58261
BDA-69		1048	1029
BDA-70		876.4	555.7

"No" means that no inhibitory activity was detectable.

As can be concluded from these data, majority of BDAs show a strong inhibition of both *Pf*ATC and *Hu*ATC. BDA-04, 09, 11, 12, 14, 24, 25 are highly potent inhibitors of *Pf*ATCase with IC_{50}s of 77.2 nM, 241.1 nM, 45.65 nM, 158.9 nM, 114.3 nM, 102.7 nM, 181.2 nM respectively. In contrast, BDA-07, 09, 11, 14, 33, 38, 45, 48, 52, 54 are particularly potent inhibitors of *Hu*ATCase, with IC_{50}s of 115.9 nM, 195.6 nM, 115.9 nM, 137.2 nM, 100.5 nM, 184.8 nM, 123.9 nM, 124.8 nM, 119.1 nM, 157.9 nM respectively.

EXAMPLE 4: Activity against *P. falciparum* 3D7

This example describes the anti-malarial activity of various BDA compounds using intact cells of the non-adapted 3D7 strain of *P. falciparum* cultured in human red blood cells. The canonical reference *P. falciparum* clone 3D7 was first published by Gardner et al. in 2002. The nuclear genome was described as 22.9 Mb and essentially complete, with 14 chromosomes, a G+C content of approximately 19%, 5268 genes and approximately 80 gaps.

To demonstrate anti-malarial activity of the compounds *P. falciparum* 3D7 cells were cultured in human red blood cells according to standard protocols (Trager et al., 1976. Science 193:673-675; Trager et al., 1977. Bull. W. H. O. 55:363-365).

The following three conditions were included:

1. Unmodified *P. falciparum* 3D7
2. *P. falciparum* 3D7 provided with a plasmid expressing *Pf*ATC ("ATC"). These experiments were performed to assess the protective impact against the compounds provided by additional *Pf*ATC present in the 3D7 cells.
3. *P. falciparum* 3D7 supplemented with an empty plasmid (ie. no additional *Pf*ATC. "MOCK"). These experiments were performed to control for the impact of the plasmid hosting additional *Pf*ATC described above.

To evaluate the cellular effect of BDAs on *P. falciparum* 3D7, unmodified parasites were cultured in the presence of BDA-04, BDA-11, BDA-16, BDA-18, BDA-19, BDA-21, BDA-22 and BDA-24. Impact of the compounds on the proliferation of *P. falciparum* 3D7 was assessed after 120 hours exposure to the compounds using light microscopy to count the parasite population ("parasitemia"). The results shown in Figure 3 indicates that the EC50 values (half maximal inhibitory concentration in human red blood cell culture) for BDA-04, BDA-11, BDA-16, BDA-18, BDA-19, BDA-21, BDA-22 and BDA-24 were 2.43, 3.37, 1.95, 1.85, 2.33, 2.41, 2.88 and 2.02 nM, respectively.

A similar series of experiments was performed to assess the dose-response profile of 3D7-ATC (3D7 supplemented with a plasmid expressing additional *Pf*ATC) and 3D7-MOCK (3D7 supplemented with the same plasmid without *Pf*ATC) cell lines against BDA-04, BDA-11, BDA-16, BDA-18, BDA-19, BDA-21, BDA-22 and BDA-24. The results of these experiments are shown in Figure 4, which demonstrate a protective effect of additional *Pf*ATC to cultures challenged with the inhibitor compounds (Figure 4, 3D7-ATC). The control experiment (Figure 4, 3D7-MOCK) demonstrates minimal impact on parasite proliferation arising from the presence of the plasmid. This data provide validation for *Pf*ATC as the cellular target of at least BDA-04, BDA-11, BDA-16, BDA-18, BDA-19, BDA-21, BDA-22 and BDA-24.

EXAMPLE 5: Activity against human tumor cells.

This example describes cytotoxicity studies of various BDA compounds using different human tumor cell lines, and normal human lymphocytes.

Cell lines and culture conditions

Cell lines H1299 (lung cancer, ATCC CRL5803), REC-1 (mantle cell lymphoma, ATCC CRL-3004) and SUDHL-2 (diffuse large B cell lymphoma, ATCC CRL-2956) were cultured in Roswell Park Memorial Institute medium 1640 (RPMI 1640; Lonza BioWhittaker, Walkersville, MD, USA) with 10% Fetal Bovine Serum (FBS; HyClone Thermo Scientific, Waltham, MA, USA), 1% Penicillin-Streptomycin (PS; Lonza BioWhittaker) and 1% Glutamine (Lonza BioWhittaker). A375 (melanoma, ATCC CRL-1619) and MCF7 (breast cancer, ATCC HTB-22) were cultured in DMEM with 10% FBS. All cell lines were cultured at 37°C with 5% CO_2 in a humidified atmosphere. The identity of the cell lines was checked at a regular base. Normal human lymphocytes were isolated from anonymous rest material from tonsillectomies.

Metabolic activity (Resazurin) assay

Cells were incubated in quadruplicate with increasing concentrations of BDA's for 72 hours. AlamarBlue (Thermo Fisher Scientific) was added eight hours prior to read-out (extinction 560nm, emission 590nm). All experiments were repeated 3 times. Data were normalized against untreated cells.

We performed cytotoxicity studies of representative compounds BDA-04, 06, 07, 11, 14, 24, 25, using 6 human cell types, including 5 cancer cell lines (A375, H1299, MCF7, REC-1, SUDHL-2) and normal blood lymphocyte cells (PBMC).

The data of Figure 5 show the impact of representative compounds BDA-11, BDA-33, BDA-41 and BDA-52 against a panel of human cell lines (A375, H1299, MCF7, REC-1, SUDHL-2) and normal blood leukocytes cells (PBMC). N-(phosphonoacetyl)-L-aspartate (PALA), a known strong inhibitor of ATCase, was used as control. Up to a concentrations of 100 μM, which is well in excess of the

EC50 values against blood stage malarial cultures (see Example 3), all compounds were well tolerated by the human cells.

The data of Figure 6 show the impact of BDA-11, BDA-33, BDA-41, BDA-52 against a panel of human cell lines. All compounds have a strong cytotoxic effect on the 5 cell lines screened (A375, H1299, MCF7, REC-1, SUDHL-2) and on normal blood leukocytes (PBMC or normal lymphocytes). The data of Figure 2 indicate that BDA-7, BDA-9, BDA-14, BDA-38, BDA-45, BDA-48 and BDA-54 show strong *in vitro* inhibition of human ATCase. This indicates these compounds (or derivatives thereof) have therapeutically relevant applications in the inhibition of human cell proliferation.

EXAMPLE 6: Inhibitors of HuATCase suppress proliferation of human bone osteosarcoma epithelial cells.

After having identified that BDA-33, 41 and 52 act as potent allosteric inhibitors of *Hs*ATC *in vitro* (see Example 5), the toxicity of these compounds was evaluated using an MMT assay. This indicated that these compounds were not cytotoxic at concentrations below 40 μM when cells were exposed for up to 24 h (data not shown). We then examined their effect on the proliferation of U2OS bone osteosarcoma epithelial cells as representative human cancer cell line. PALA was used as a comparison. The data show that BDA-41 inhibited U2OS cell proliferation in a dose-dependent manner (Fig. 7) with similar results also seen for BDA-33 and -52. In contrast, PALA showed almost no inhibition of the proliferation of these cell lines.

EXAMPLE 7: Inhibition of ATCase of *Mycobacterium tuberculosis*.

In this example, we assessed the inhibition of ATCase from *Mycobacterium tuberculosis* (*Mt*ATCase) by the BDA-compound series of the invention.

Experimental Section

MtbATCase Cloning, Expression and Purification

The *Mt*ATCase gene (aspartate carbamoyl transferase catalytic subunit [Mycobacterium tuberculosis complex] Gene ID: 886771) was ordered from Eurofins. The associated forward primer sequence for PCR experiments was 5'-tcagggcgccATGACTCCCAGGCACCTATTAAC-3' and the reverse primer sequence was 5'-ctcgaattcgTTAAGCCGCGCCCTCTTTG-3', the insert was cloned into pETM-41 generating an N-terminal His6-tagged maltose-binding protein fusion construct (European Molecular Biology Laboratory (EMBL)), using a forward primer sequence of 5'-cgcggcttaaCGAATTCGAGCTCCGTCG-3', and a reverse primer sequence of 5'-tgggagtcatGGCGCCCTGAAAATAAAG-3'. The PCR reaction both of *Mt*ATCase and pETM-41 was performed using Phusion High-Fidelity PCR Master Mix (New England Biolabs). The expression plasmid of pETM-41-*Mtb*ATCase was assembled through the Gibson Assembly reaction (ref) using E2611 Gibson Assembly Master Mix.

The *Mt*ATCase was recombinantly expressed using *E. coli* BL21 star. An overnight culture inoculated from a single colony was used to inoculate 1L TB media supplemented with 0.2% (w/v) D-glucose, 50 μg/ml kanamycin and 35 μg/ml chloramphenicol at 310 K, and shaken at 180 RPM. Expression was induced at an OD of 0.8-1.2 by the addition of 0.4 mM IPTG at 291 K and the cultures incubated for a further 17 hours at 291 K. Cells were harvested by centrifugation at 6K RPM, resuspended in 35 ml lysis buffer [20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% (v/v) glycerol, 5 mM ß-mercaptoethanol (BME)], cell lysis was performed by sonication and centrifugation at 16K rpm (SS-34 rotor, Thermo Scientific) for 45 min. The supernatant was incubated with amylose resin beads (BioLabs) for 2 hours, then washed with lysis buffer prior to elution in elution buffer [20 mM Tris-HCl pH 8.0, 0.5 M NaCl, 5% (v/v) glycerol, 5 mM BME, 10 mM maltose]. Then the protein was concentrated to 1 ml and purified by size-exclusion chromatography (SEC; HiLoad 16/60 Superdex 75 column) using SEC buffer [20 mM Tris-HCl pH 8.0, 300 mM NaCl, 5 mM BME). The purified protein was concentrated to 6.16 mg/ml, before storage at 193K.

Activity assay

Enzymatic reactions were performed in a total volume of 150 µL in 50 mM Tris-Acetate buffer at pH 8.0 and a final concentration of *Mt*ATCase of 50 nM. The concentration of L-Aspartate (Asp) and carbamoyl-phosphate (CP) were 20 mM and 25 mM. The concentration of BDA series compounds in dose-response experiments was started from 100 µM, and PALA from 1 mM. *Mt*ATCase was pre-incubated with Asp and compounds for 10 min in a shaker at room temperature, then CP was added and incubated for 5 min, the reaction was stopped by adding 100 µL of the colorimetric chemical mixture[27] (two volumes of Antipyrine in 50% (v/v) sulfuric acid and one volume of 2,3-Butanedione monoxime in 5% (v/v) acetic acid). Then the plate was incubated overnight in the dark at room temperature, followed with denaturation at 368 K for 15 min, samples were then measured at 466 nm using a Synergy H1 Hybrid Reader (BioTek).

Solubility Measurement using DLS assay

Compounds were analyzed for solubility in the 7H9 complete medium (BD Difco; Becton Dickinson; culture medium), supplemented with oleic acid-albumin-dextrose-catalase (OADC, 10%; BD) using the Dynamic Light Scattering (DLS) assay on a Zetasizer Nano ZS90 (Malvern Instruments Ltd, Worcestershire, UK). Analyses were performed by filling the disposable cuvettes (3.2 mL, 67.758, Sarstedt AG & Co, Nümbrecht, Germany) with 100ul of compounds with media at the testing concentration (64uM and lower). Culture medium free of compounds was used as a negative control. The Derived count rate values were obtained from the analyses indicating the degree of aggregation formation. The values were compared to that of the negative control which allowed the determination of the highest sample concentration at which the respective compound was entirely soluble, with no increase in the degree of aggregate formation.

Determination of in vitro Anti-Tubercular activity

7H9 complete medium (BD Difco; Becton Dickinson, Maryland, USA) supplemented with 10% OADC (BD), 0.2% glycerol, and 0.05% Tween80 as previously described[28] was used to culture Mycobacterium tuberculosis (Mtb) strain H37Rv (ATCC 25618) carrying a mCherry-expressing plasmid (pCherry10) 29 Cultures were harvested at mid-log phase and frozen in aliquots at −80 °C. On the

experiment day, these aliquots were thawed followed by centrifugation and the pellet was resuspended in 7H9 medium with 10%OADC (without glycerol and Tween80). This was further thoroughly resuspended by passing it through a syringe with a 26-gauge needle to avoid clumping of the bacteria. 2 × 105 CFU/20ul of the suspension was used to test the non-precipitating compounds, identified and determined by DLS, in triplicates for their anti-tubercular activity. The compounds were tested at 64uM initially; if found active were further tested by diluting them 2-fold (64uM to 1uM). For these assays, 96-well flat clear bottom black polystyrene microplates (Corning® CellBIND®, Merck, York, USA) were used. Each plate had Rifampicin (at 1ug/ml and 0.1ug/ml) (National Reference Center, Borstel) as a reference compound. Plates were sealed with an air-permeable membrane (Porvair Sciences, Wrexham, UK) in a 37 °C incubator with mild agitation (TiMix5, Edmund Bühler, Germany). The activity of compounds was determined after 7 days by measuring the bacterial growth as relative light units (RLU) from the fluorescence intensity obtained at an excitation wavelength of 575 nm and an emission wavelength of 635 nm (microplate reader, Synergy 2, BioTek Instruments, Vermont, USA). Two independent experiments (each in triplicates) were performed and all values were normalized to the bacterial growth (1% bacterial growth set to 100%) in each experiment. The graphs were obtained by the average of both experiments using GraphPad Prism version 9.4.1 for Windows (GraphPad Software, San Diego, California USA, www.graphpad.com). The first concentration of compounds at which 90% Mtb inhibition was observed was considered to be MIC90.

Measurement of in vitro cytotoxicity using XTT assay

XTT assay was used to determine the cytotoxicity of the best performing compound BDA-06. Human Monocyte-derived Macrophages (hMdM) were differentiated from peripheral blood mononuclear cells (PBMC) of healthy volunteers and cultured as previously described[30]. 5x104 cells/well in RPMI medium containing 10% (v/v) heat-inactivated Fetal Bovine Serum and 2 mmol/L l-glutamine (Biochrom, Berlin, Germany) were seeded in presence of 2-fold dilution concentrations (64uM to 1uM) of the compound for 24 hours with a final volume of 200ul/well. For this assay, 96-well clear flat bottom plates (Nunclon™ Delta Surface, ThermoScientific, Denmark) were used. Triton™ X-100 (Sigma-Aldrich, Missouri, USA; 1% and 0.1%

in RPMI medium) was used as a positive control. Cells were incubated with 200ul of Triton X-100 and incubated for 10 mins at 37°C. XTT dye from the kit (SERVA Electrophoresis GmbH; 50ul) was added to each well and resuspended thoroughly and further incubated for 3-4 hours at 37°C. Ultimately, the absorbance values were measured at 490 nm on a multi-well plate reader (Synergy 2, BioTek Instruments, Vermont, USA). Untreated cells were used as negative control. The Cytotoxic Concentration 50 (CC50) was determined by plotting a curve using GraphPad Prism version 9.4.1.

RESULTS

Figure 8A shows the results of the in vitro screening of ATCase inhibitors against *Mt*ATCase. The half maximal inhibitory concentration (IC_{50}) was measured using the absorbance of the product, carbamoyl-aspartate at 466 nm. The results of the activity assays demonstrated that most of BDA series showed inhibition in the high mM range. However, a significant subset of compounds displayed IC_{50} values in the single digit mM range. BDA-06, -10, -14, -17, -25, -54, -63, -67, -69 and -70 displayed promising IC_{50} values of 1.44 μM, 6.89 μM, 2.05 μM, 6.01 μM, 6.13 μM, 3.54 μM, 3.60 μM, 6.98 μM, 3.49 μM and 1.36 μM, respectively (Fig. 8B).

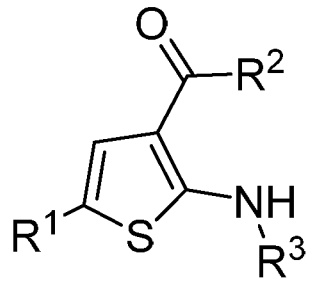
In addition, we performed the active assay using PALA, with the results showing that PALA surprisingly has no inhibition under these assay conditions (Figure 8C). In a follow up experiment, the 4 most potent *Mt*ATCase inhibitors BDA-06, -14, were also screened against *Pf*ATCase and *human* ATCase under the same conditions (Figure 8D).

The 10 best performing compounds from the *in vitro* assay were selected for further analysis. A solubility assay was performed using a Zetasizer at concentrations from 64 μM to 8 μM in 7h9+OADC media, the results showed that the compounds were soluble at concentrations up to 8μM (Figure 9A). MIC90 assays were then performed at 8 μM using the *Mtb* H37Rv cherry 10 fluorescent strain, and the antibiotic Rifampicin as a positive control (Figure 9B). The compound BDA-06 showed positive results and dose-response experiments from 8 μM to 0.625 μM

were carried out (Figure 9C), indicating that BDA-06 possesses a MIC90 of <4 mM. Initial cytotoxicity screens were then performed using monocyte derived macrophages, demonstrating a CC50 for BDA-06 of 30 mM.

Claims

1. An aspartate transcarbamoylase (ATCase) inhibitor compound of the Formula I



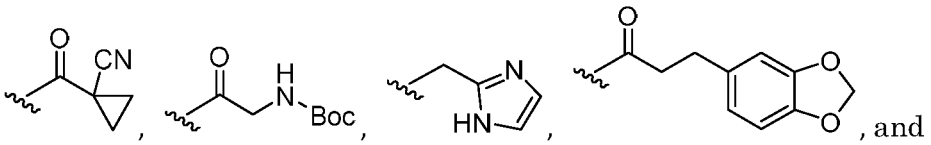
wherein

R^1 is selected from the group consisting of halogen, benzyl, C_3-C_6 branched alkyl, benzoheterocycle ring and an 5- or 6- membered (hetero)aromatic moiety, optionally substituted with one or more of halogen, C_1-C_3 alkyl, CN, NH_2, acyl and C_4-C_6 heterocycloalkyl ring;

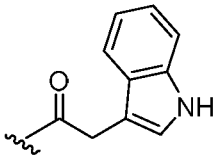
R^2 is OR', wherein R' is H or alkyl; or

R^2 is NR"R"', wherein R" and R"' are independently selected from H, -$(CH_2)_n$-OH wherein n = 1-3, -$(CH_2)_n$-A wherein n = 0-3, and -CH_2-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, and A is an optionally substituted C_4-C_6 (hetero)aromatic ring or an optionally substituted C_4-C_6 (hetero)cycloalkyl ring, provided that R" and R"' cannot both be H; or wherein R" and R"' together with the N-atom to which they are attached form a 5- to 7-membered heterocycloalkyl comprising 1 to 3 N-atoms, which heterocycloalkyl is optionally substituted;

R^3 is selected from H, C_1-C_6 alkyl, C_3-C_6 branched alkyl, *-tert-*butyloxycarbonyl (Boc),



, and

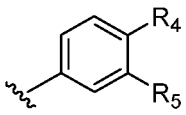


or a pharmaceutically acceptable salt, solvent or hydrate thereof.

2. Inhibitor compound according to claim 1, wherein R^1 is an optionally substituted 6- membered (hetero)aromatic moiety, preferably a substituted phenyl, pyridine or pyrimidine.

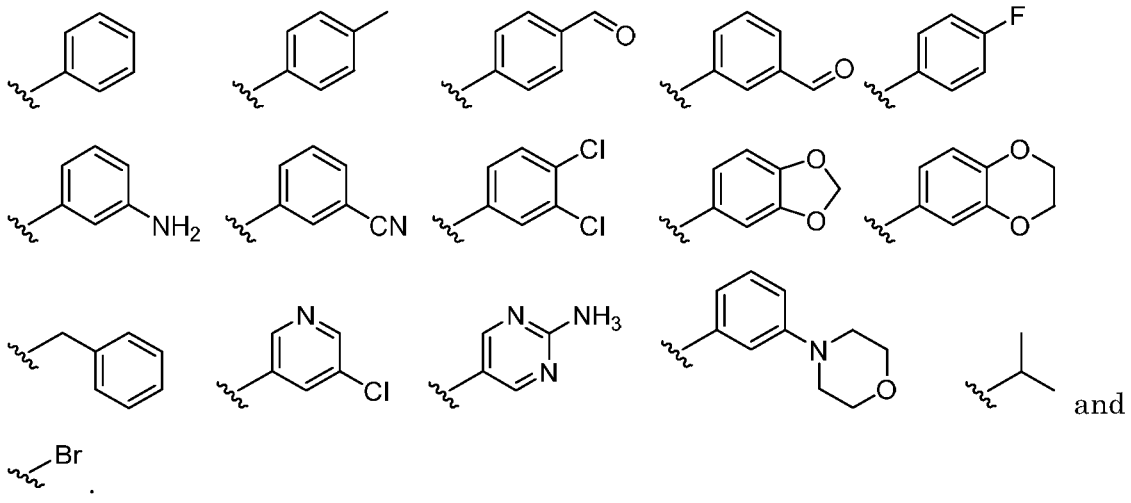
3. Inhibitor compound according to claim 2, wherein R^1 is an optionally substituted phenyl.

4. Inhibitor compound according to claim 3, wherein R^1 is



wherein R_4 and R_5 are independently selected from H, alkyl, halogen and amine; or wherein R_4 and R_5 together with the carbon atoms to which they are attached form a 5- to 7-membered carbocyclic or heterocyclic ring.

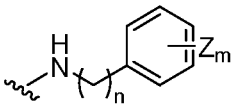
5. Inhibitor compound according to any one of the preceding claims, wherein R^1 is selected from the group consisting of



.

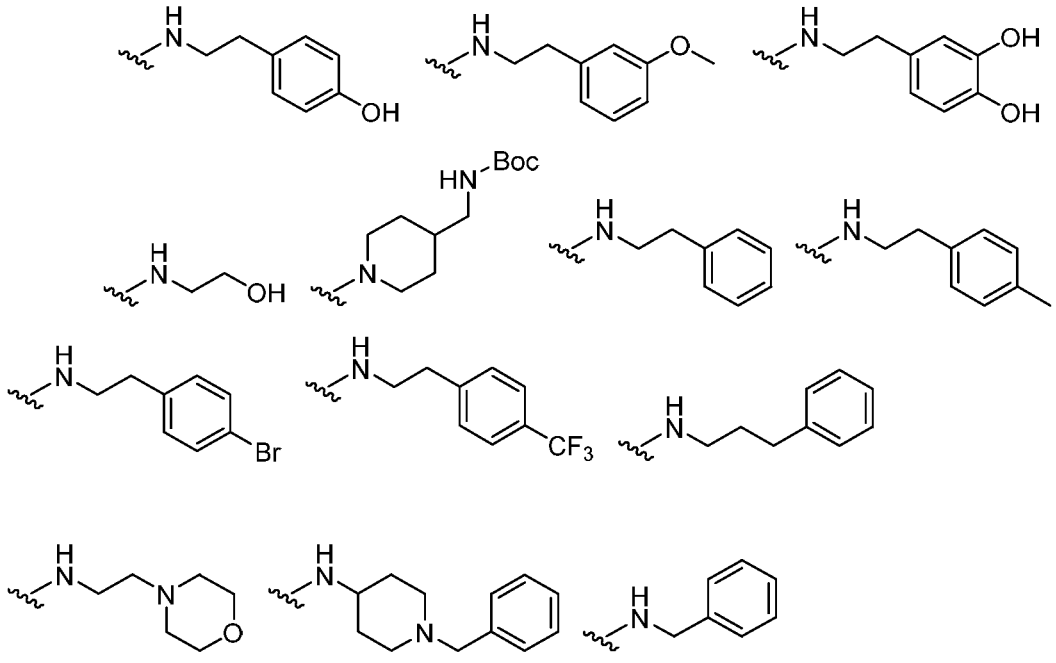
6. Inhibitor compound according to any one of the preceding claims, wherein R^2 is NR"R"', wherein R" and R"' are independently selected from H, -$(CH_2)_n$-OH wherein n = 1-3, -$(CH_2)_n$-A wherein n = 0-3, and -CH_2-Y-A; wherein Y is C(O), CH(OH) or $CH(C_6H_5)$, and A is an optionally substituted C_4-C_6 (hetero)aromatic ring or an optionally substituted C_4-C_6 (hetero)cycloalkyl ring, provided that R" and R"' cannot both be H;

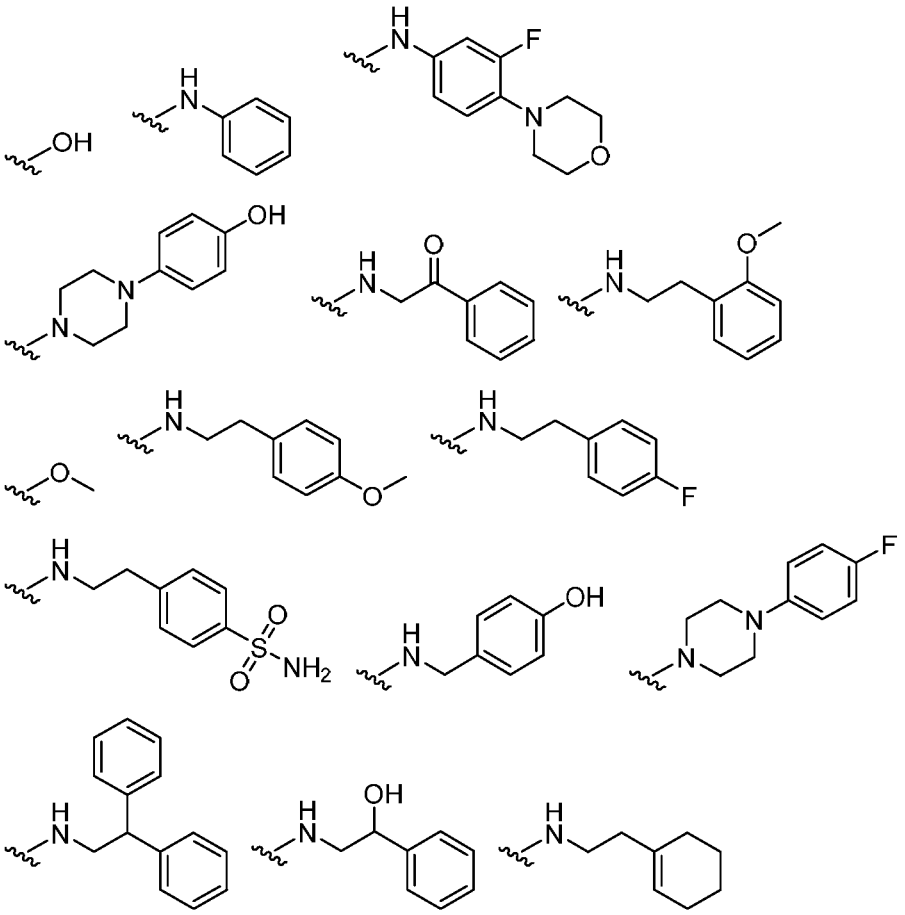
7. Inhibitor compound according to claim 6, wherein R^2 is of the formula



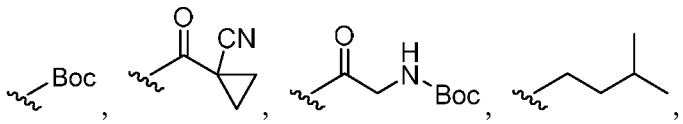
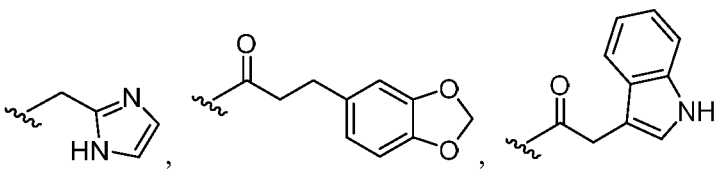
wherein n is 0-3; m is 0-3 and Z is independently selected from halogen, -R^a, - OR^a, -OC(O) R^a, -CN, NO_2, -CO_2R^a, -OC(O)R^a, -S(O)R^a, -$S(O)_2$ R^a, -$S(O)_2NH_2$, and wherein R^a is selected from hydrogen, C_1-C_6 (hetero)alkyl.

8. Inhibitor compound according to any one of the preceding claims, wherein R^2 is selected from the group consisting of



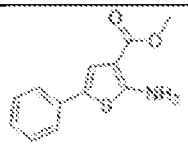
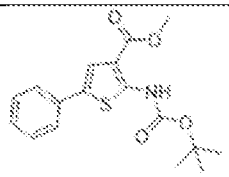
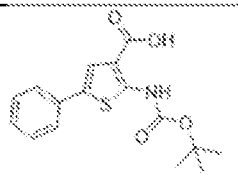
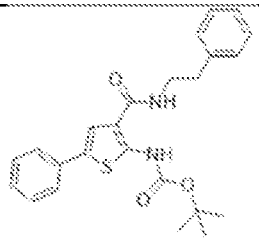
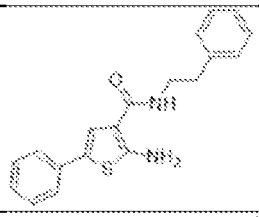
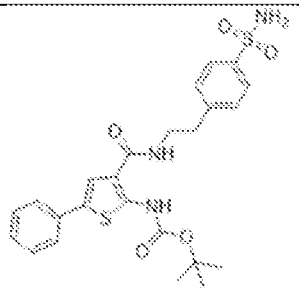
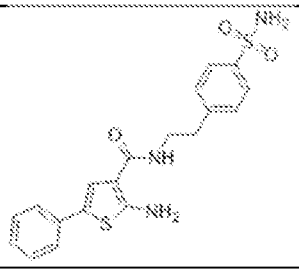


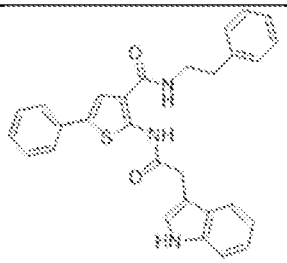
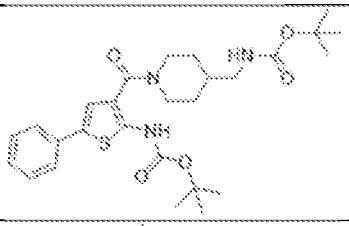
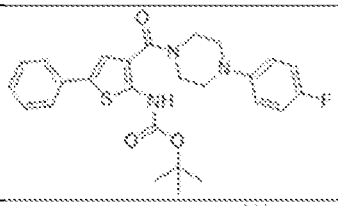
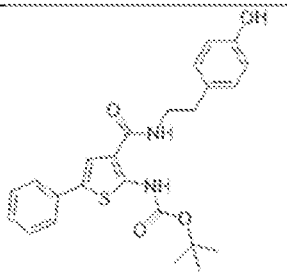
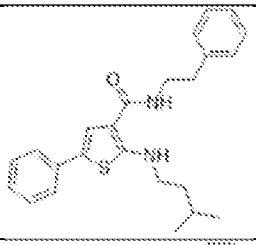
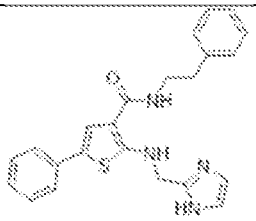
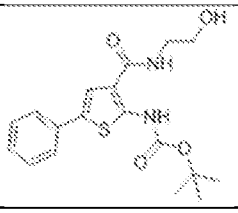
9. Inhibitor compound according to any one of the preceding claims, wherein R^2 is an optionally substituted -NH-$(CH_2)_2$-phenyl, -NH-$(CH_2)_2$-OH, NH-substituted piperidiny-4-yl or -NH-substituted piperidin-1-yl.

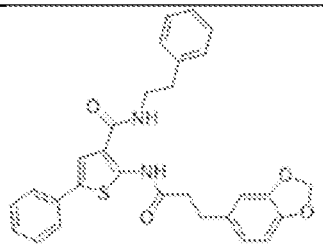
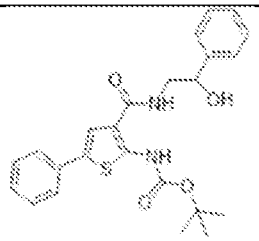
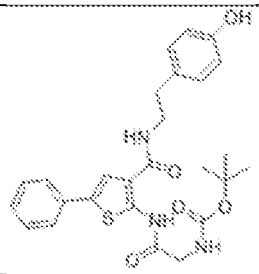
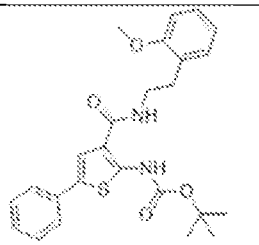
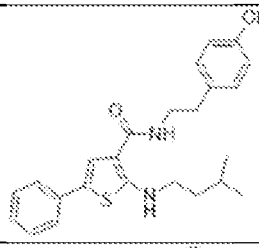
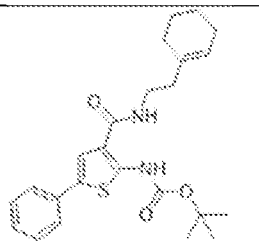
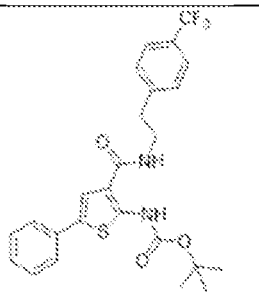
10. Inhibitor compound according to any one of the preceding claims, wherein R_3 is selected from H,  ,  ,

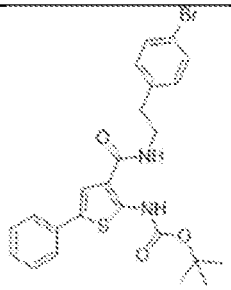
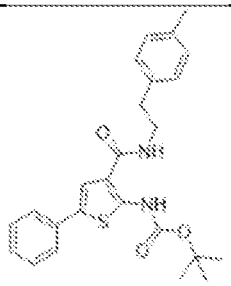
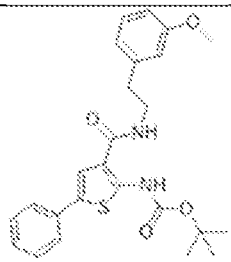
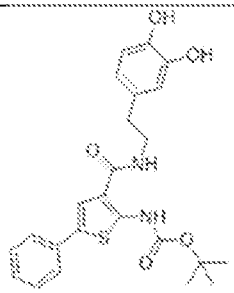
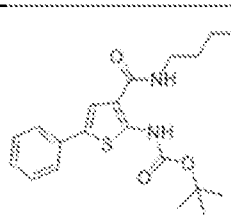
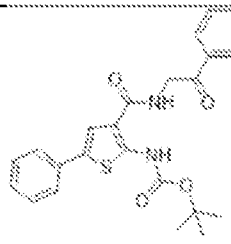
11. Inhibitor compound according to any one of the preceding claims, wherein R^1 is an optionally substituted or condensed phenyl, R^2 is an optionally substituted -NH-$(CH_2)_2$-phenyl, -NH-$(CH_2)_2$-OH, NH-substituted piperidiny-4-yl or -NH-substituted piperidin-1-yl, and R^3 is H, Boc or a C_3-C_6 branched alkyl.

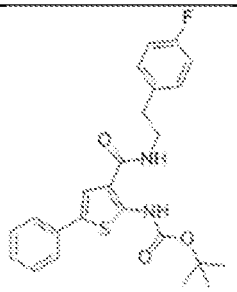
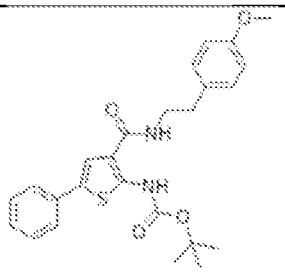
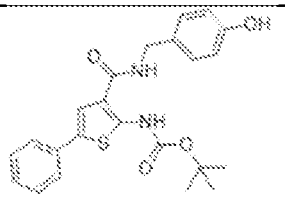
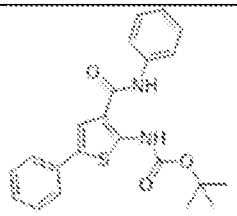
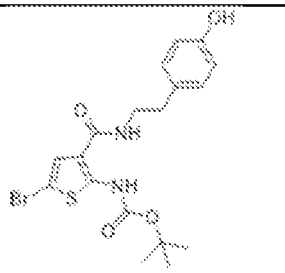
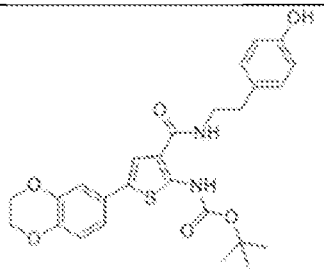
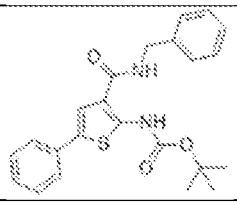
12. Inhibitor compound according to any one of the preceding claims, selected from the group consisting of

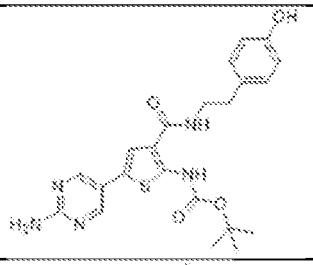
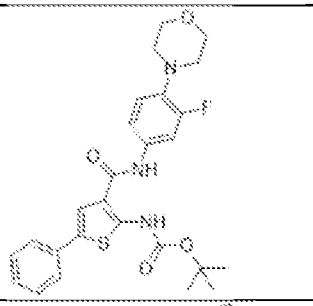
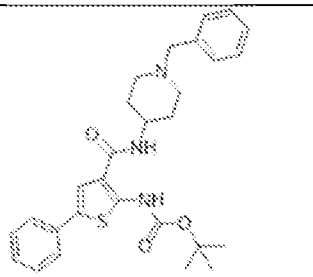
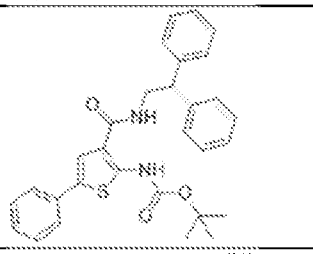
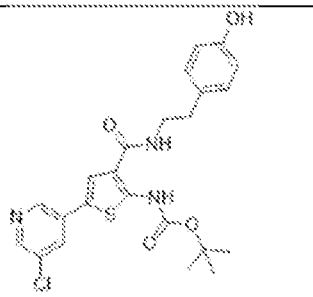
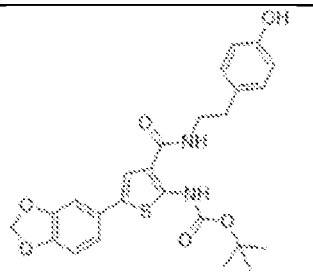
BDA-01	
BDA-02	
BDA-03	
BDA-04	
BDA-05	
BDA-06	
BDA-07	

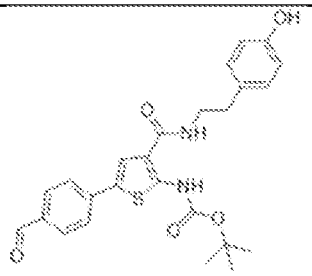
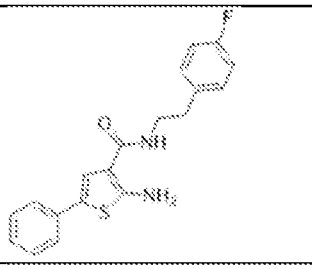
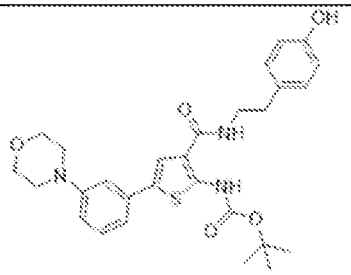
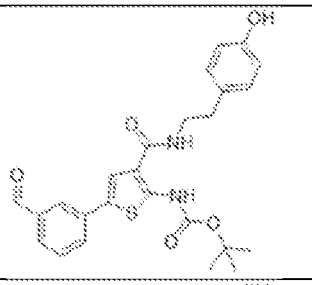
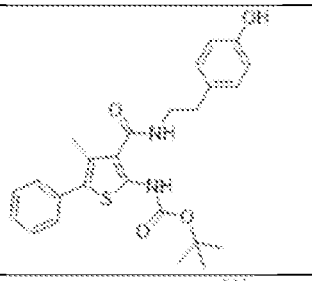
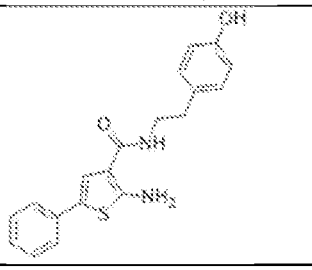
BDA-08	
BDA-09	
BDA-10	
BDA-11	
BDA-12	
BDA-13	
BDA-14	

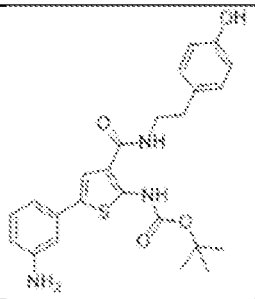
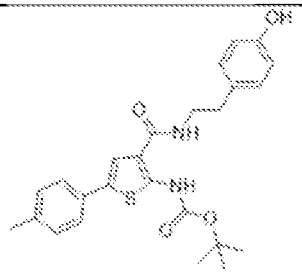
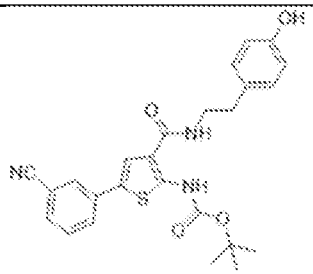
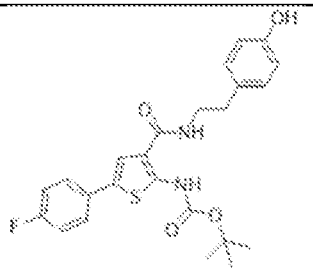
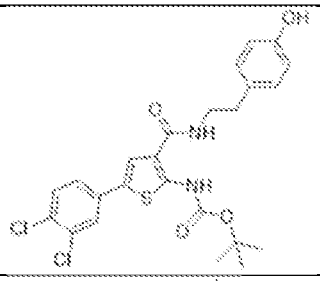
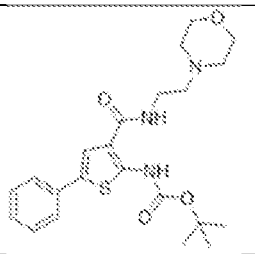
BDA-15	
BDA-16	
BDA-17	
BDA-18	
BDA-19	
BDA-20	
BDA-21	

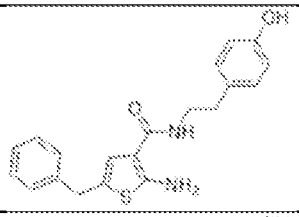
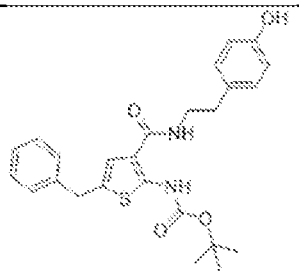
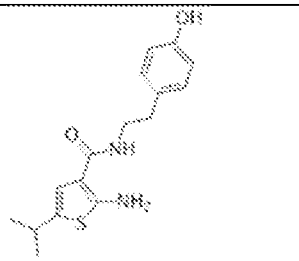
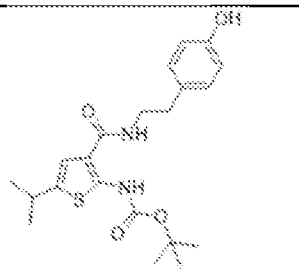
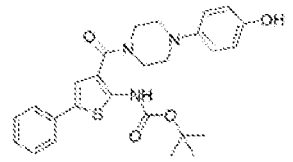
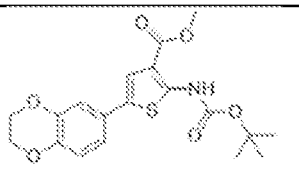
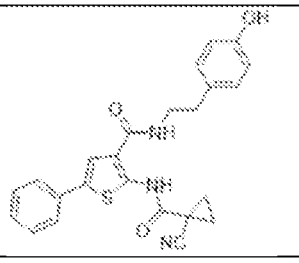
BDA-22	
BDA-23	
BDA-24	
BDA-25	
BDA-26	
BDA-27	

BDA-28	
BDA-29	
BDA-30	
BDA-31	
BDA-32	
BDA-33	
BDA-34	

BDA-35	
BDA-37	
BDA-38	
BDA-39	
BDA-40	
BDA-41	

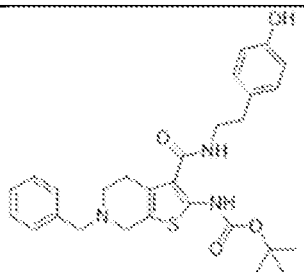
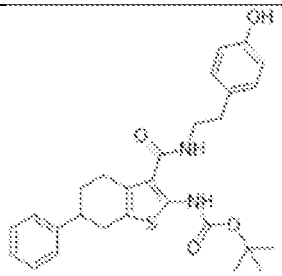
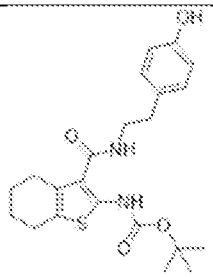
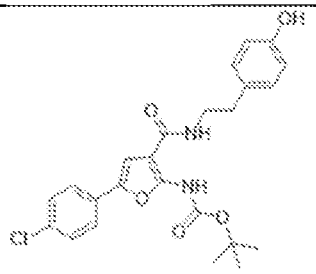
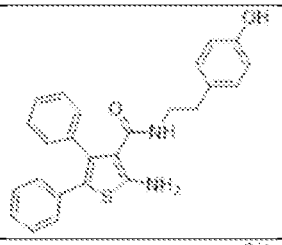
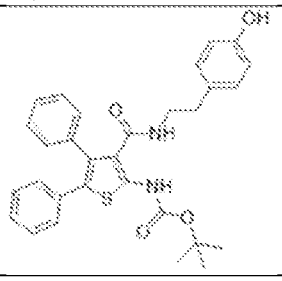
BDA-45	
BDA-46	
BDA-47	
BDA-48	
BDA-49	
BDA-50	

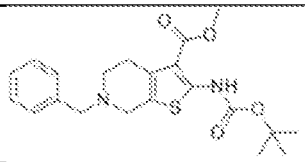
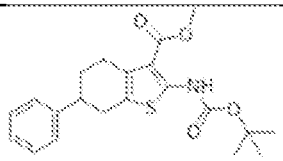
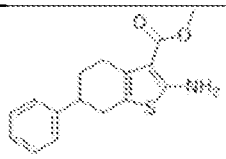
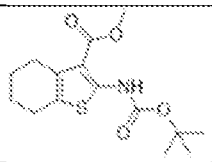
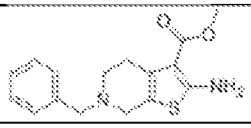
BDA-51	
BDA-52	
BDA-53	
BDA-54	
BDA-56	
BDA-57	

BDA-58	
BDA-59	
BDA-61	
BDA-62	
BDA-63	
BDA-69	
BDA-70	

or a pharmaceutically acceptable salt, solvent or hydrate thereof.

13. An ATCase inhibitor compound selected from the group consisting of

BDA-36	
BDA-42	
BDA-43	
BDA-44	
BDA-55	
BDA-60	

BDA-64	
BDA-65	
BDA-66	
BDA-67	
BDA-68	

or a pharmaceutically acceptable salt, solvent or hydrate thereof.

14. Inhibitor compound according to claim 12 or 13, selected from the group consisting of BDA-04, 06, 07, 09, 10, 11, 12, 14, 17, 24, 25, 33, 38, 41, 45, 48, 52, 54, 63, 67, 69 and 70, preferably BDA-04, -07, 09, 11, 12, 14, 24, 25, 33, 38, 45, 48, 52 and 54.

15. A compound according to any one claims 1-14, preferably according to claim 12, 13 or 14, for use as a medicament.

16. A pharmaceutical composition comprising at least one inhibitor compound(s) according to any one of the preceding claims, and a pharmaceutically acceptable carrier, vehicle or diluent.

17. Pharmaceutical composition according to claim 16, comprising one or more inhibitor compound(s) according to claim 12, 13 or 14.

18. A compound according to any one of claims 1-14, for use as an anti-malaria agent, preferably wherein said anti-malaria compound is selected from the group consisting of BDA-11, BDA-24, BDA-25, BDA-14.

19. A method for treating malaria in a subject, comprising administering to the subject a therapeutically effective dose of a compound according to any one of claims 1-14, preferably wherein said compound is selected from the group consisting of BDA-11, BDA-24, BDA-25, BDA-14.

20. A compound according to any one of claims 1-14 for use as an anti-cancer agent, preferably wherein said anti-cancer compound is selected from the group consisting of BDA-41, BDA-33, BDA-11, BDA-52, BDA-45, BDA-48, BDA-54, BDA-14, BDA-38, BDA-9 and BDA-7, more preferably BDA-41, BDA-33, BDA-11, BDA-52 and BDA-45.

21. A method for treating a proliferative disease, such as cancer, in a subject, comprising administering to the subject a therapeutically effective dose of a compound according to any one of claims 1-14, preferably wherein said compound is selected from the group consisting of BDA-41, BDA-33, BDA-11, BDA-52, BDA-45, BDA-48, BDA-54, BDA-14, BDA-38, BDA-9 and BDA-7, more preferably BDA-41, BDA-33, BDA-11, BDA-52 and BDA-45.

22. A compound according to any one of claims 1-14, for use in a method of preventing or treating a disease or disorder wherein a beneficial effect is obtained by inhibition of ATCase, preferably wherein said ATCase is human ATCase (*Hu*ATCase), more preferably wherein the disease is a proliferative disease such as cancer.

23. Compound for use in a method according to claim 22, wherein said ATCase is *Plasmodium falciparum* ATCase (*Pf*ATCase), preferably wherein the disease is malaria.

24. A compound according to any one of claims 1-14, for use in a method of preventing or treating a disease or disorder in a subject wherein a beneficial effect

is obtained by inhibition of a Mycobacterium ATCase, preferably *Mycobacterium tuberculosis* ATCase (MtATCase) or *Mycobacterium leprae* ATCase.

25. Compound for use according to claim 24, wherein the disease is tuberculosis or plague, preferably wherein the compound is selected from the group consisting of BDA-06, -10, -14, -17, -25, 54, -63, -67,- 69, and -70, more preferably BDA-06, -14 or -70.

26. A method for treating tuberculosis or plague in a subject, comprising administering to the subject a therapeutically effective dose of a compound according to any one of claims 1-14, preferably wherein said compound is selected from the group consisting of BDA-06, -10, -14, -17, -25, 54, -63, -67,- 69, and -70, more preferably BDA-06, -14 or -70.

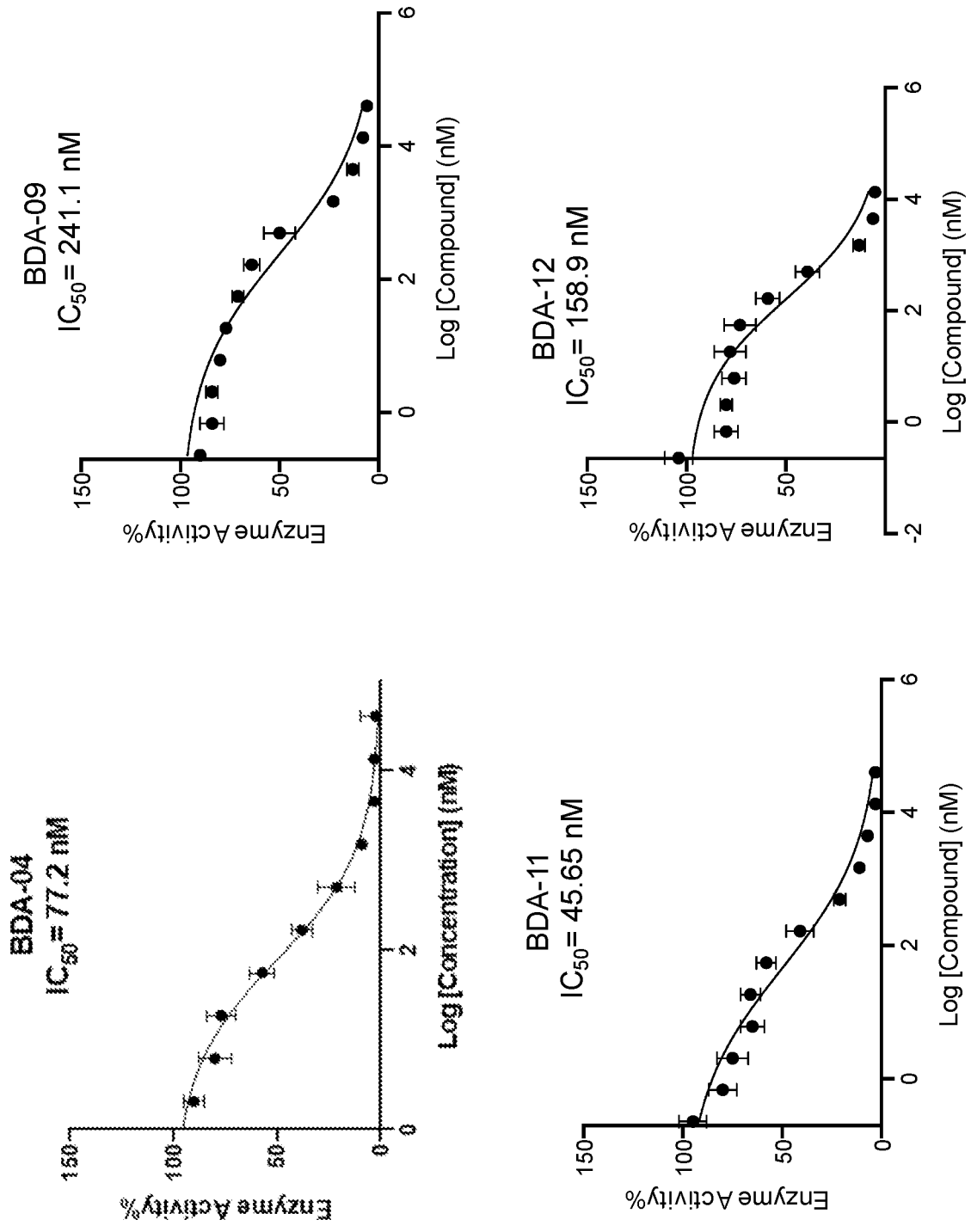
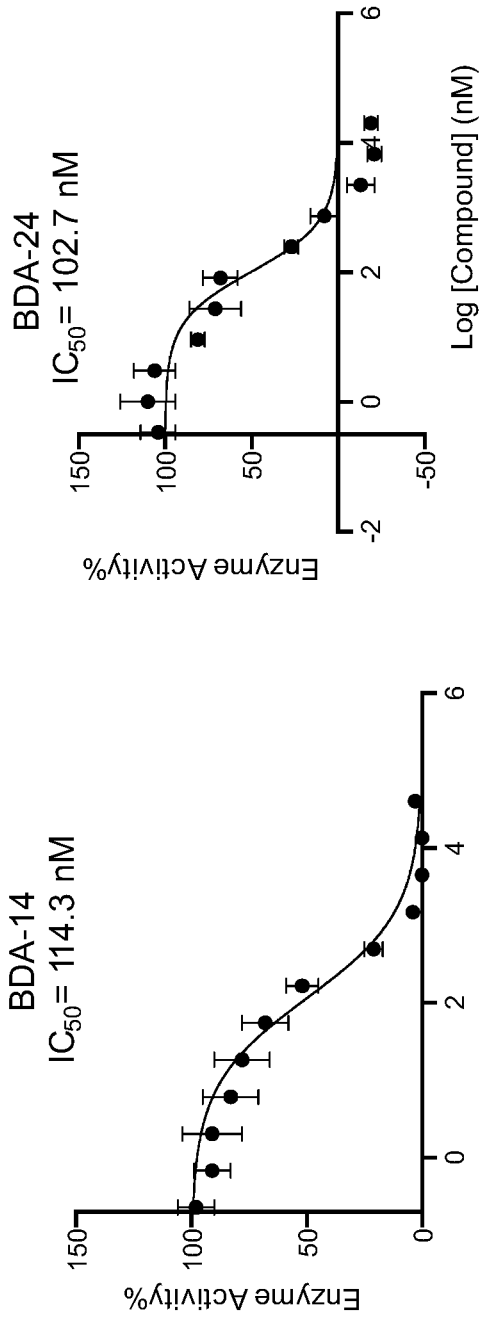

BDA-09
IC50= 241.1 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-12
IC50= 158.9 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-04
IC50= 77.2 nM
Enzyme Activity%
Log [Concentration] (nM)
BDA-11
IC50= 45.65 nM
Enzyme Activity%
Log [Compound] (nM)

Fig. 1


BDA-14
IC50= 114.3 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
0
-2
0
2
4
6

BDA-24
IC50= 102.7 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
-50
-2
0
2
4
6

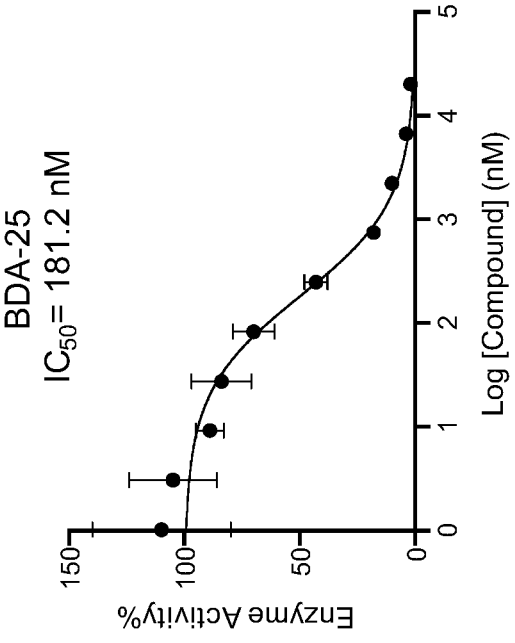

BDA-25
IC50= 181.2 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
0
0
1
2
3
4
5

Fig. 1, Cont'd

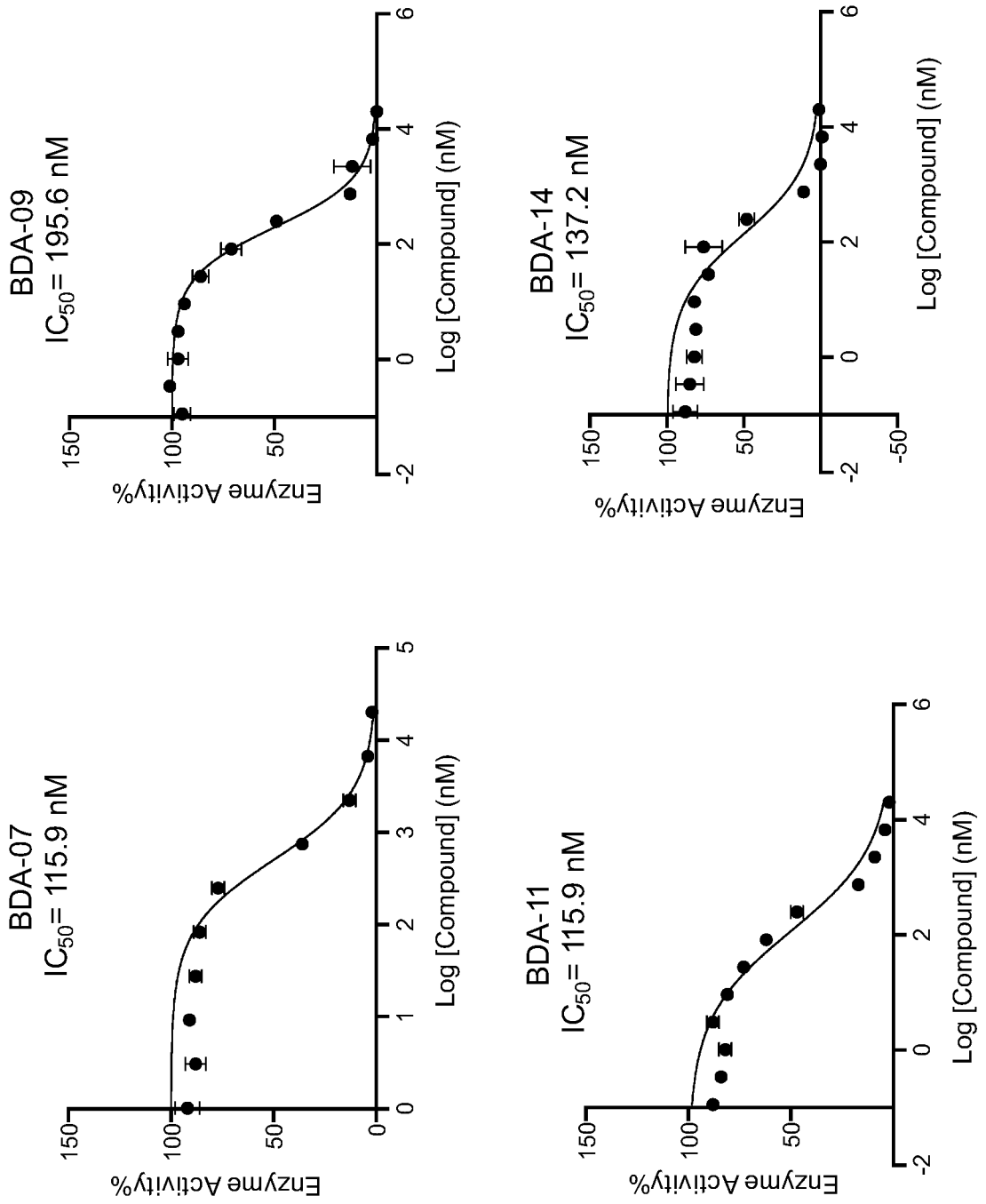

BDA-09
IC50= 195.6 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-14
IC50= 137.2 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-07
IC50= 115.9 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-11
IC50= 115.9 nM
Enzyme Activity%
Log [Compound] (nM)

Fig. 2

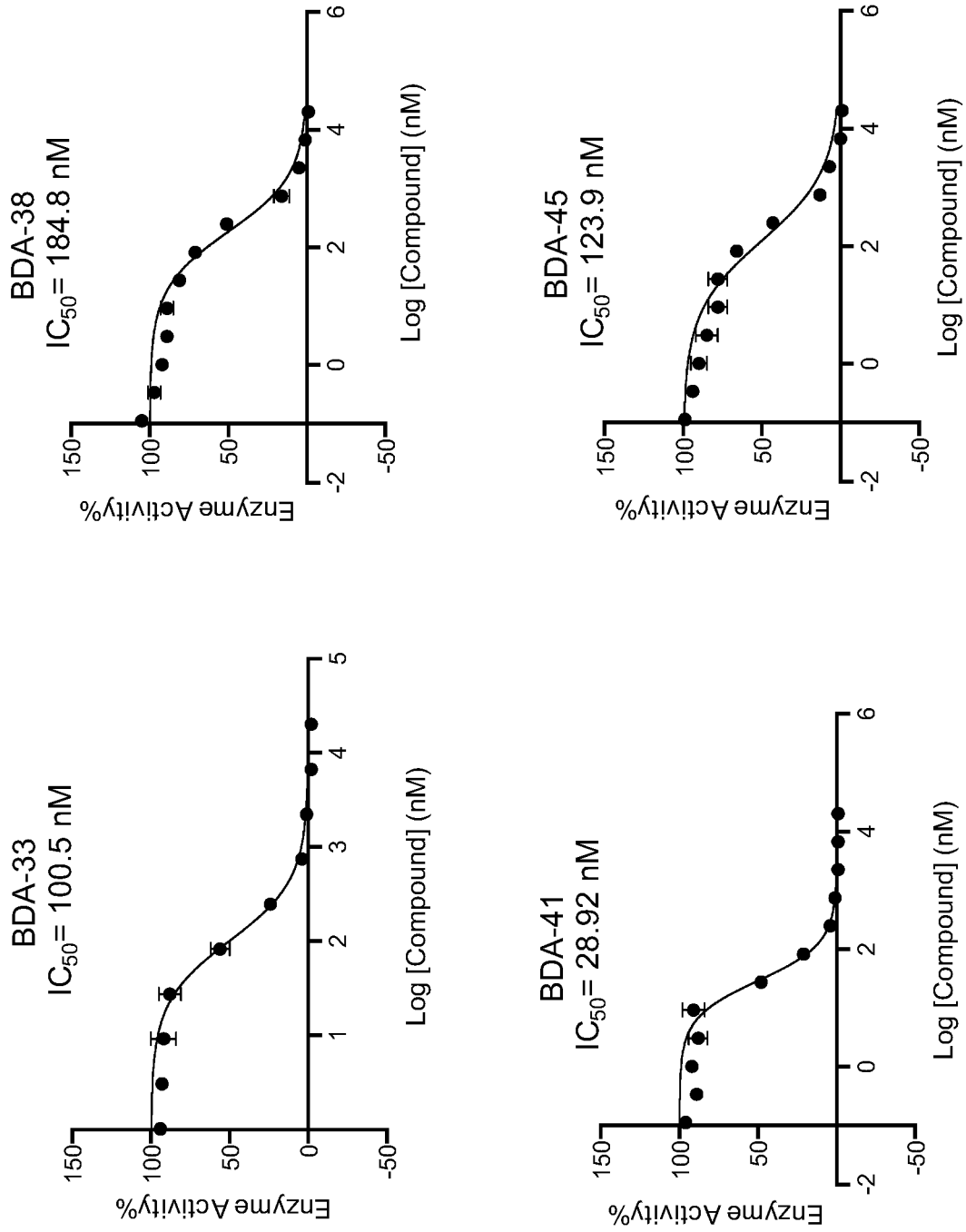
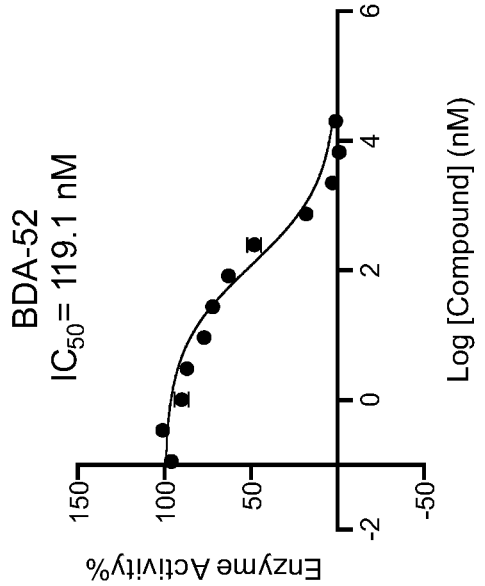
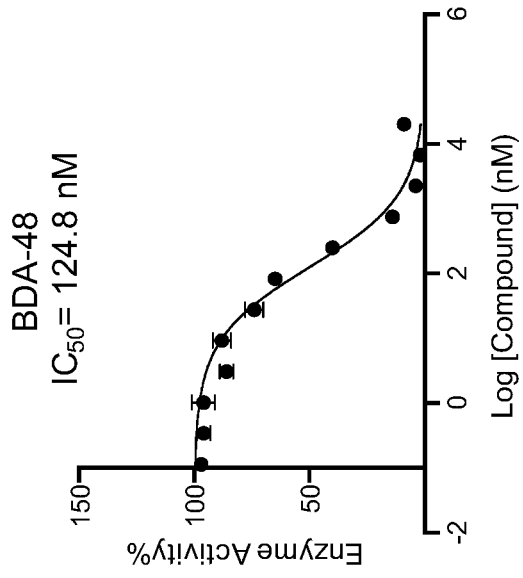

BDA-38
IC50= 184.8 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-45
IC50= 123.9 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-33
IC50= 100.5 nM
Enzyme Activity%
Log [Compound] (nM)
BDA-41
IC50= 28.92 nM
Enzyme Activity%
Log [Compound] (nM)

Fig. 2, Cont'd


BDA-52
IC50= 119.1 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
-50
-2
0
2
4
6


BDA-48
IC50= 124.8 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
-2
0
2
4
6

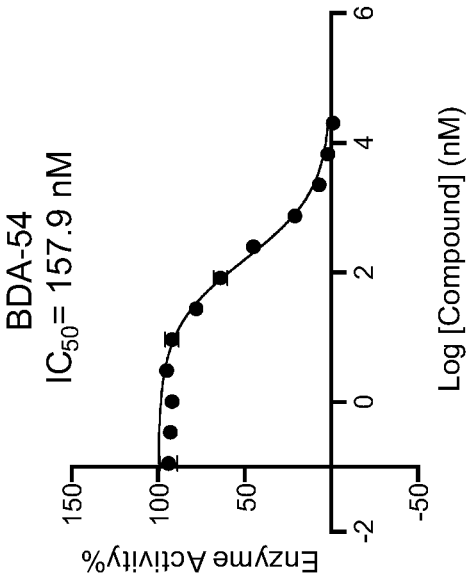

BDA-54
IC50= 157.9 nM
Enzyme Activity%
Log [Compound] (nM)
150
100
50
-50
-2
0
2
4
6

Fig. 2, Cont'd

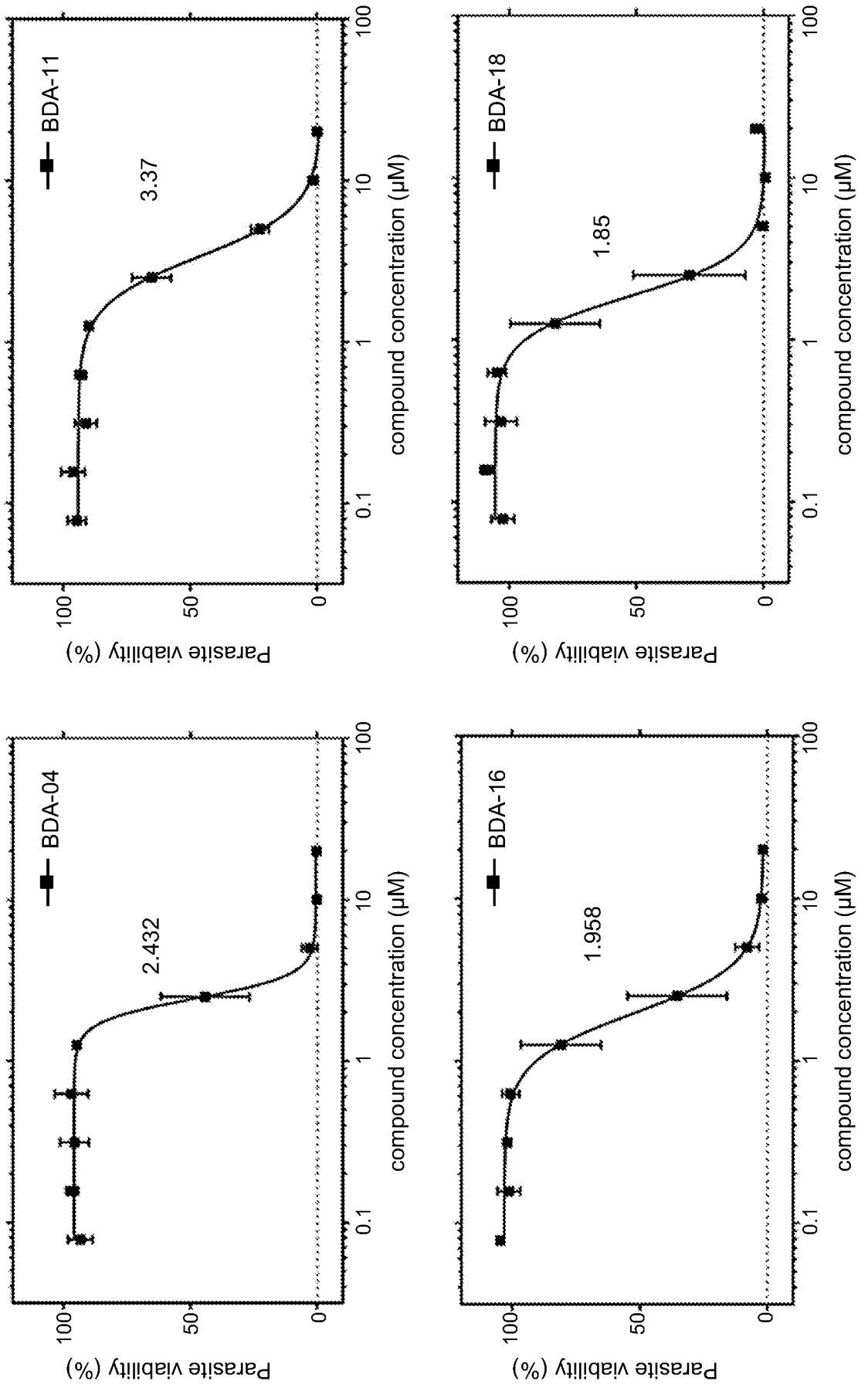

BDA-11
3.37
BDA-18
1.85
BDA-04
2.432
BDA-16
1.958
Parasite viability (%)
compound concentration (µM)
0
50
100
0.1
1
10
100

Fig. 3

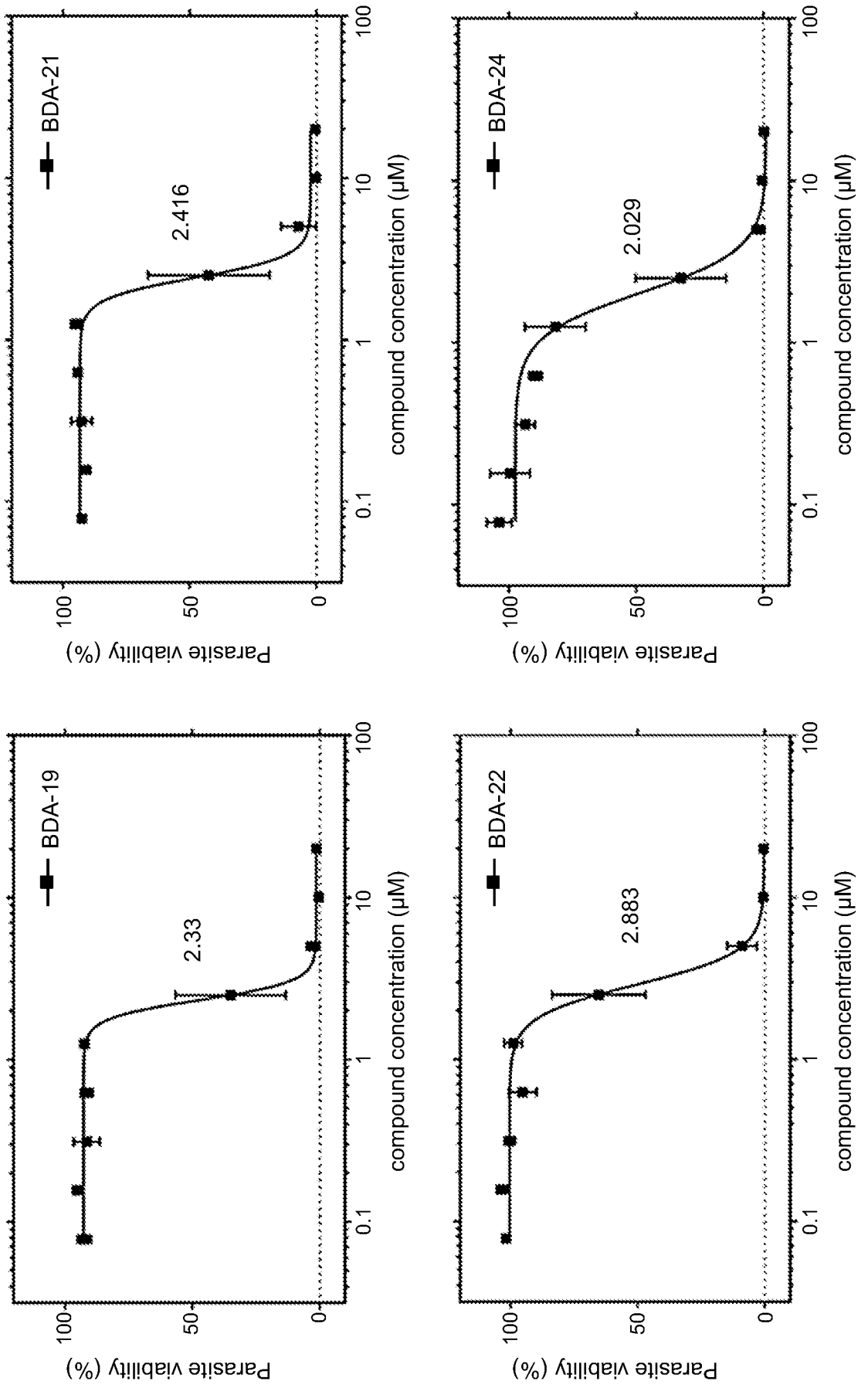

BDA-21
2.416
BDA-24
2.029
BDA-19
2.33
BDA-22
2.883
Parasite viability (%)
compound concentration (μM)
0
50
100
0.1
1
10
100

Fig. 3, Cont'd

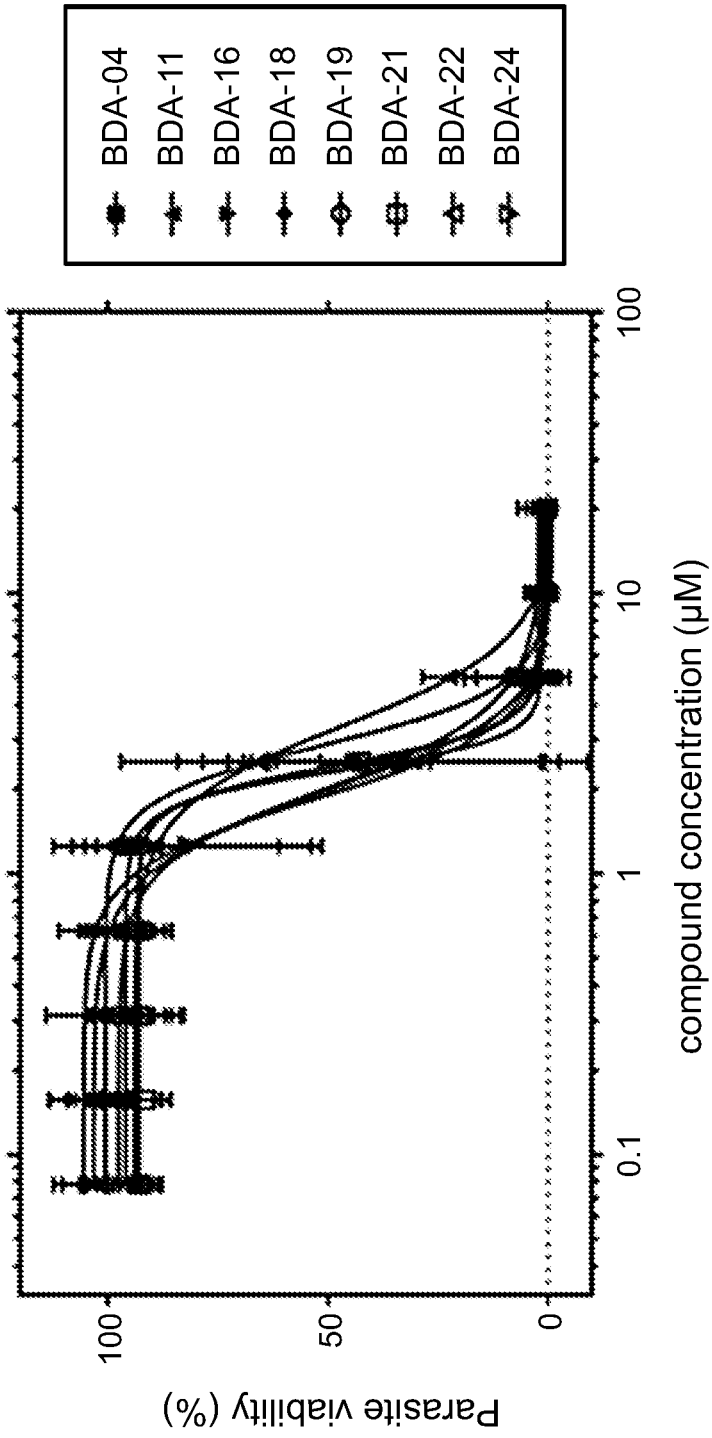

BDA-04
BDA-11
BDA-16
BDA-18
BDA-19
BDA-21
BDA-22
BDA-24
Parasite viability (%)
100
50
0
0.1
1
10
100
compound concentration (μM)

Fig. 3, Cont'd

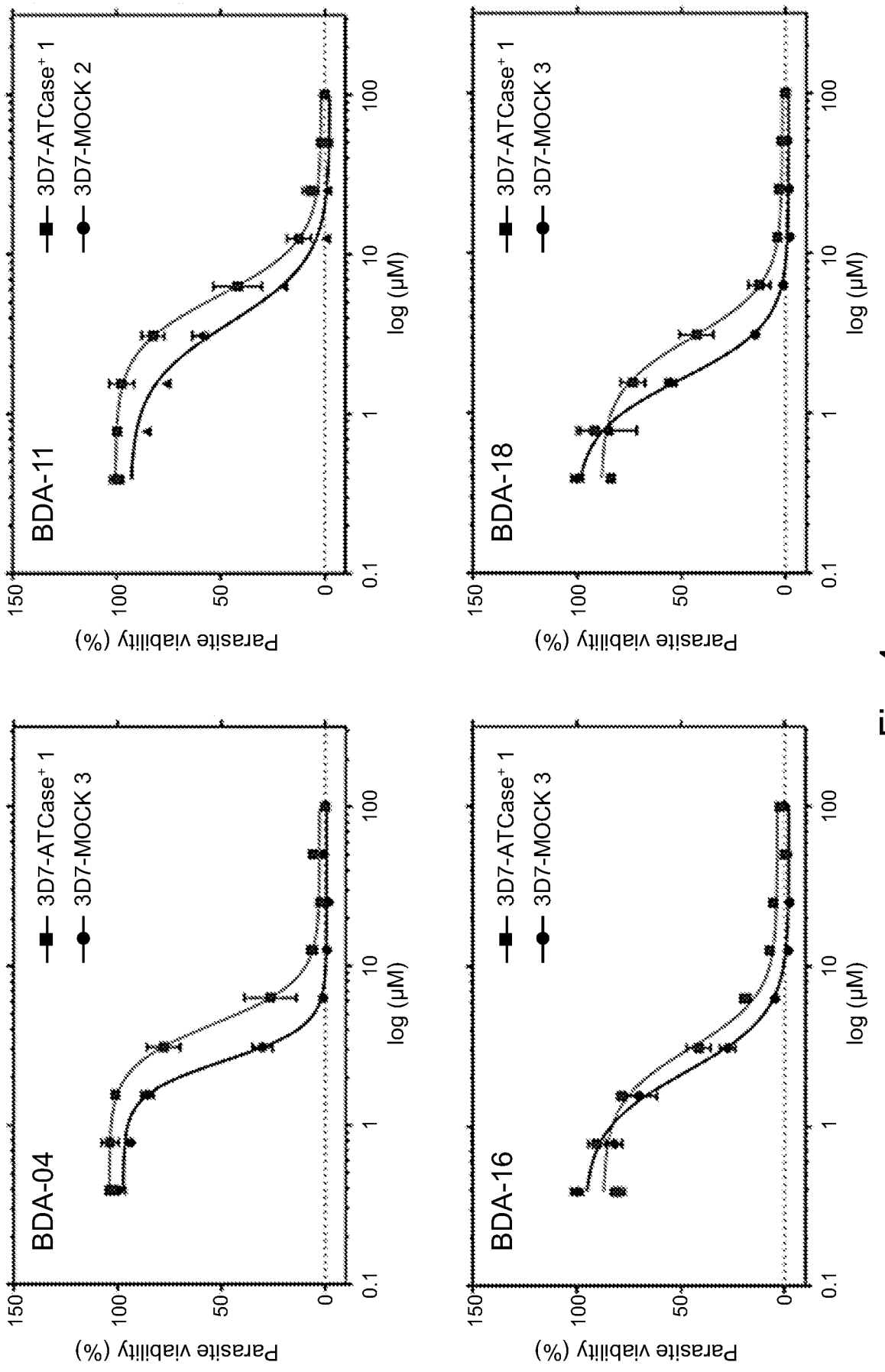

BDA-11
3D7-ATCase+ 1
3D7-MOCK 2
BDA-18
3D7-ATCase+ 1
3D7-MOCK 3
BDA-04
3D7-ATCase+ 1
3D7-MOCK 3
BDA-16
3D7-ATCase+ 1
3D7-MOCK 3
Parasite viability (%)
log (µM)
150
100
50
0
0.1
1
10
100

Fig. 4

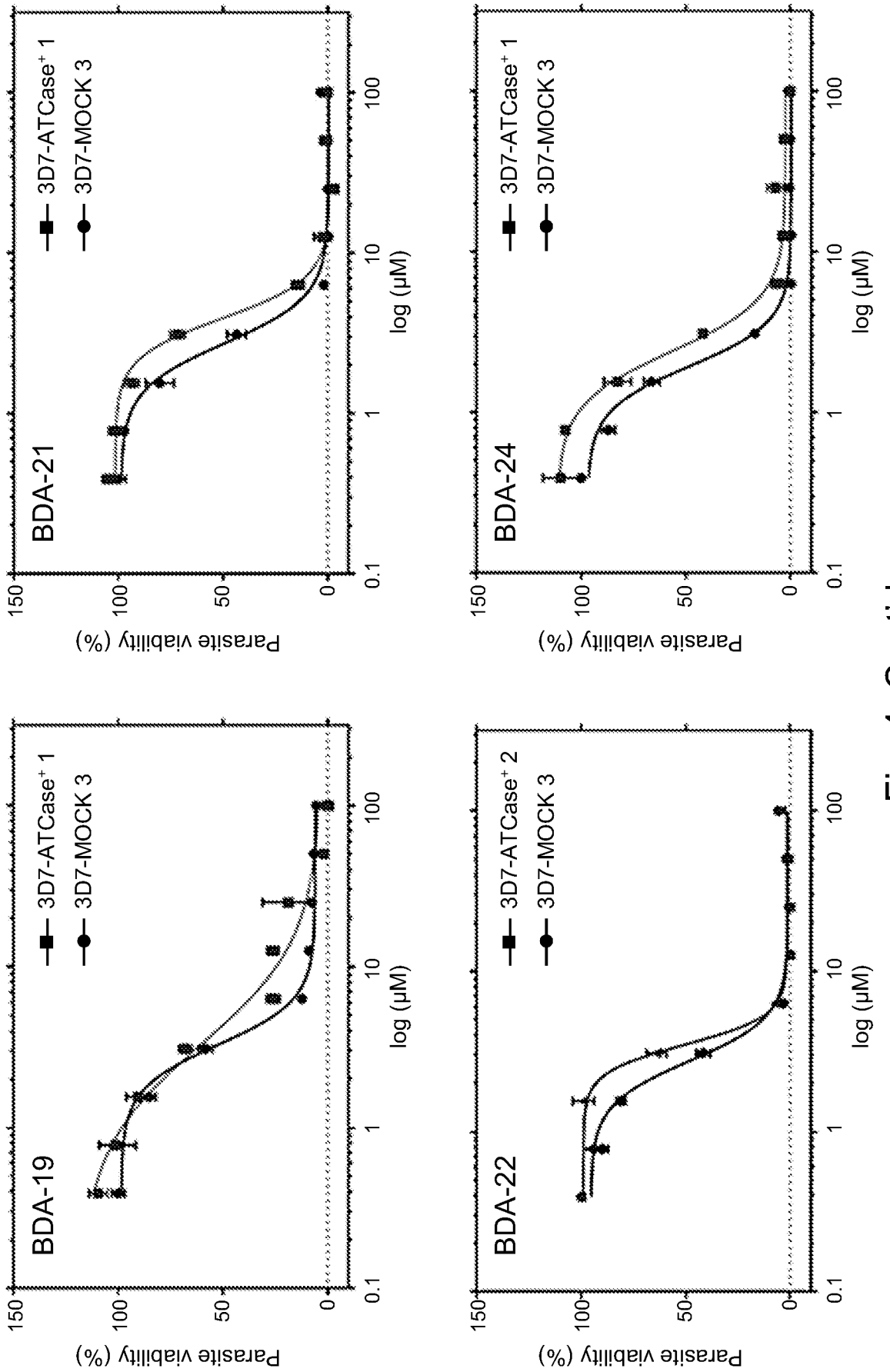

BDA-19
BDA-21
BDA-22
BDA-24
3D7-ATCase+ 1
3D7-ATCase+ 2
3D7-MOCK 3
Parasite viability (%)
log (µM)
150
100
50
0
0.1
1
10
100

Fig. 4, Cont'd

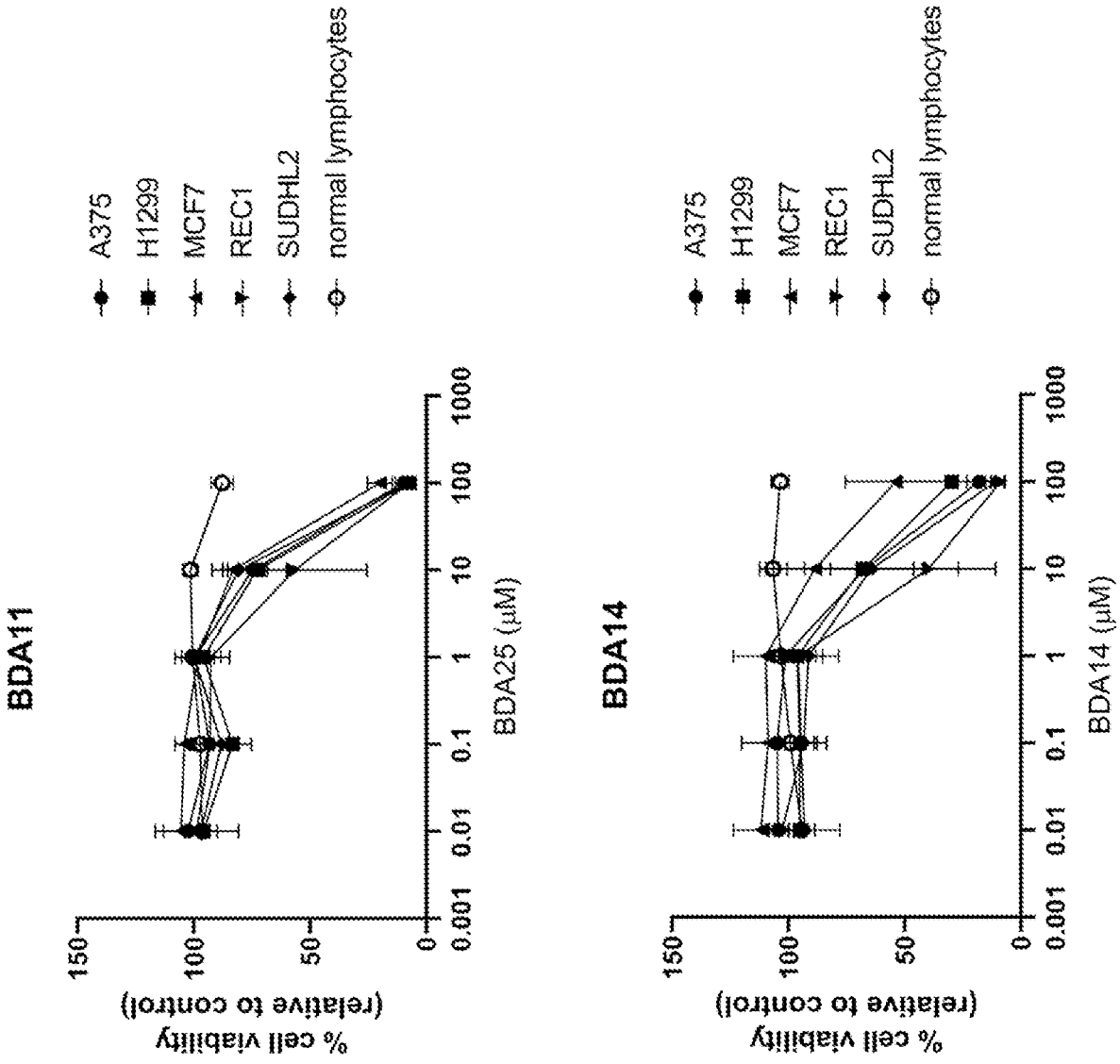
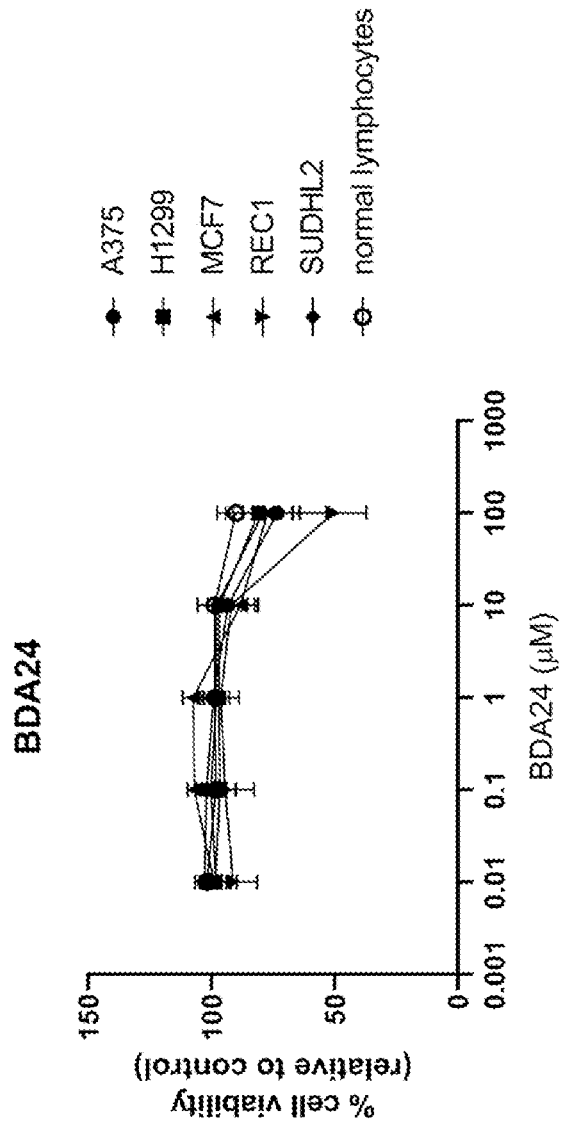

BDA11
% cell viability
(relative to control)
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
BDA25 (μM)
A375
H1299
MCF7
REC1
SUDHL2
normal lymphocytes
BDA14
% cell viability
(relative to control)
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
BDA14 (μM)
A375
H1299
MCF7
REC1
SUDHL2
normal lymphocytes

Fig. 5


BDA24
% cell viability
(relative to control)
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
BDA24 (μM)
A375
H1299
MCF7
REC1
SUDHL2
normal lymphocytes

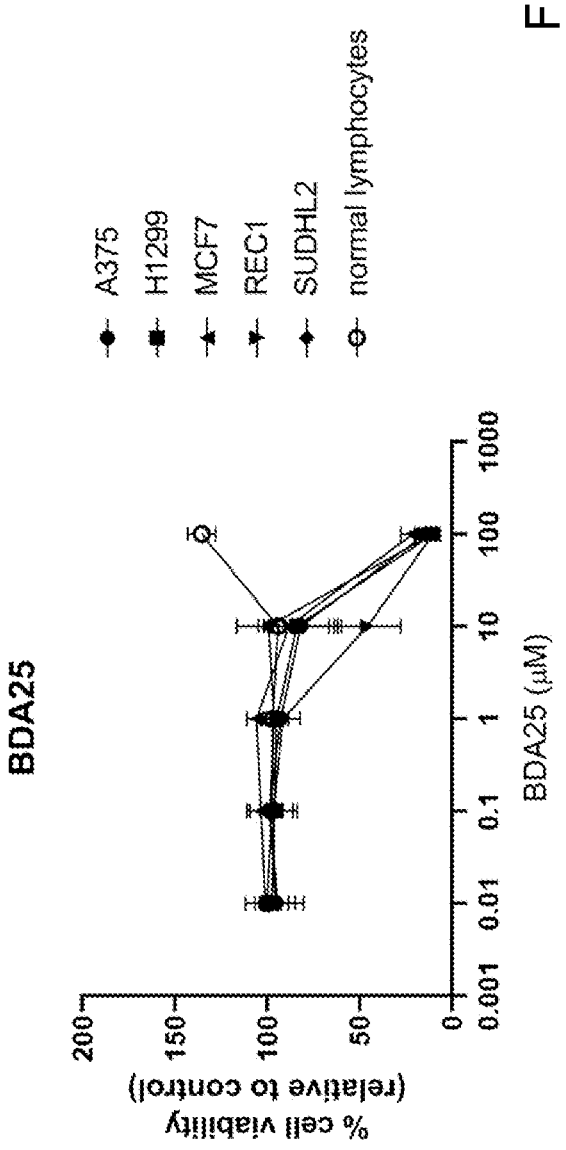

BDA25
% cell viability
(relative to control)
200
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
BDA25 (μM)
A375
H1299
MCF7
REC1
SUDHL2
normal lymphocytes

Fig. 5, Cont'd

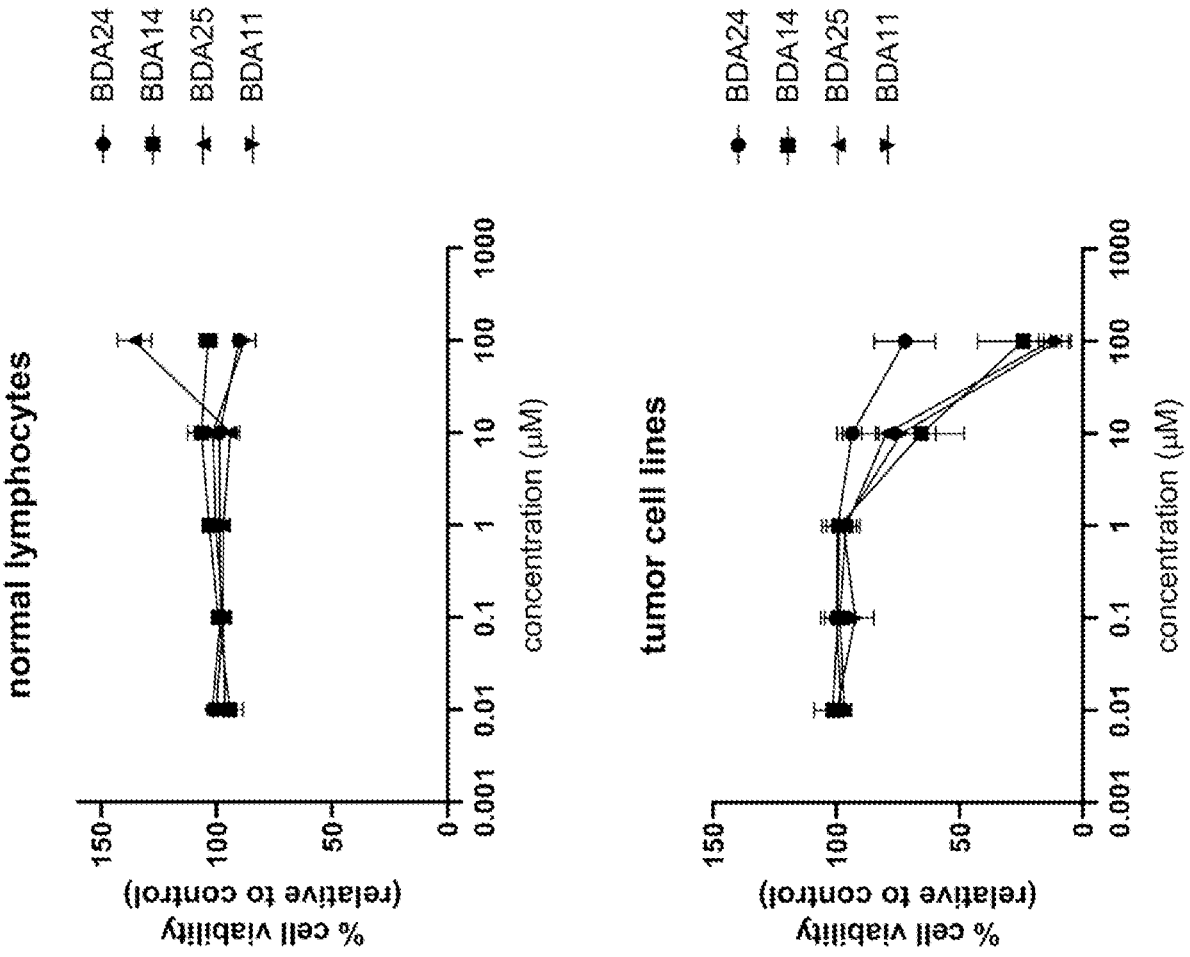

normal lymphocytes
BDA24
BDA14
BDA25
BDA11
% cell viability
(relative to control)
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
concentration (μM)
tumor cell lines
BDA24
BDA14
BDA25
BDA11
% cell viability
(relative to control)
150
100
50
0
0.001
0.01
0.1
1
10
100
1000
concentration (μM)

Fig. 5, Cont'd

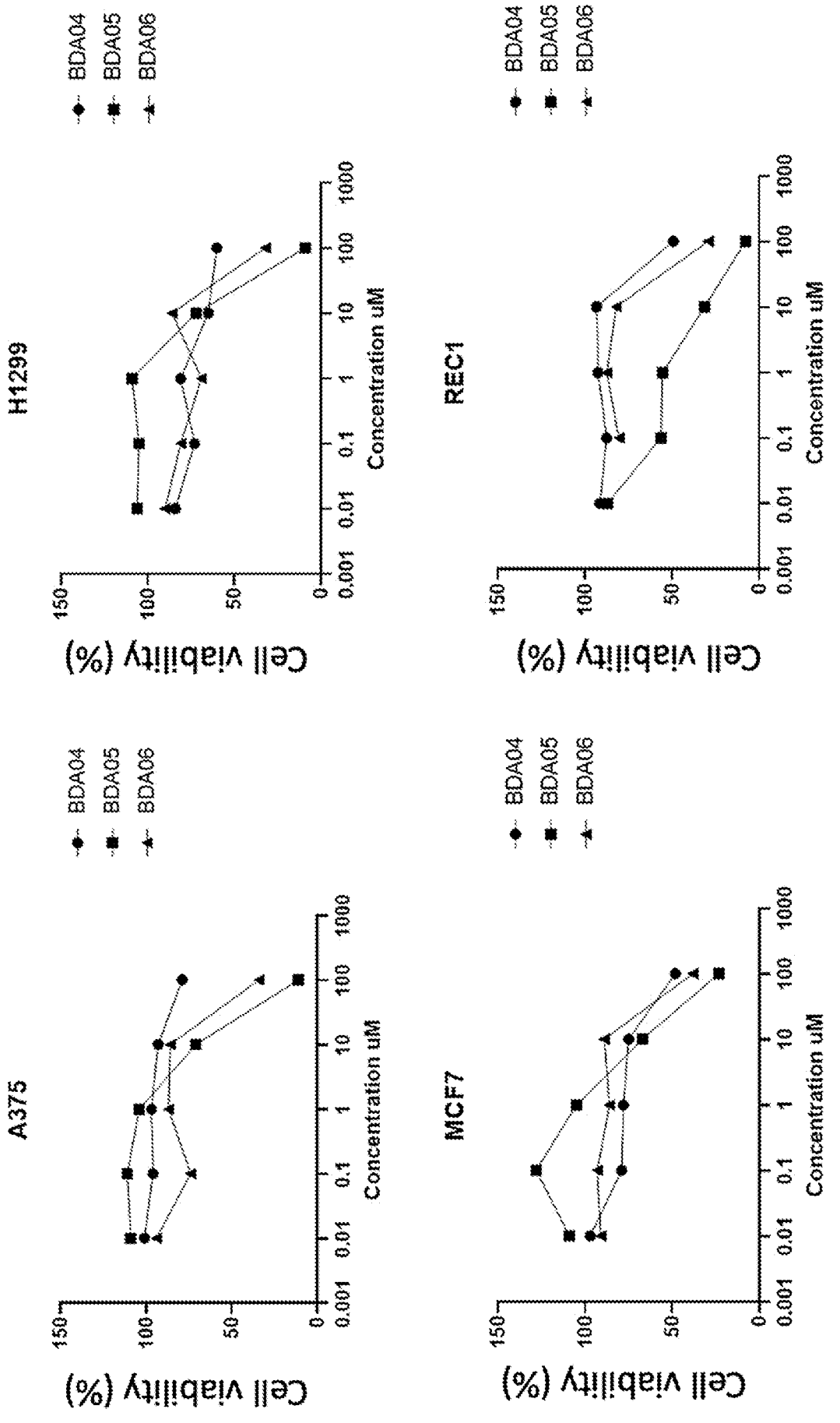

A375
H1299
REC1
MCF7
BDA04
BDA05
BDA06
Cell viability (%)
Concentration uM
0
50
100
150
0.001
0.01
0.1
1
10
100
1000

Fig. 6

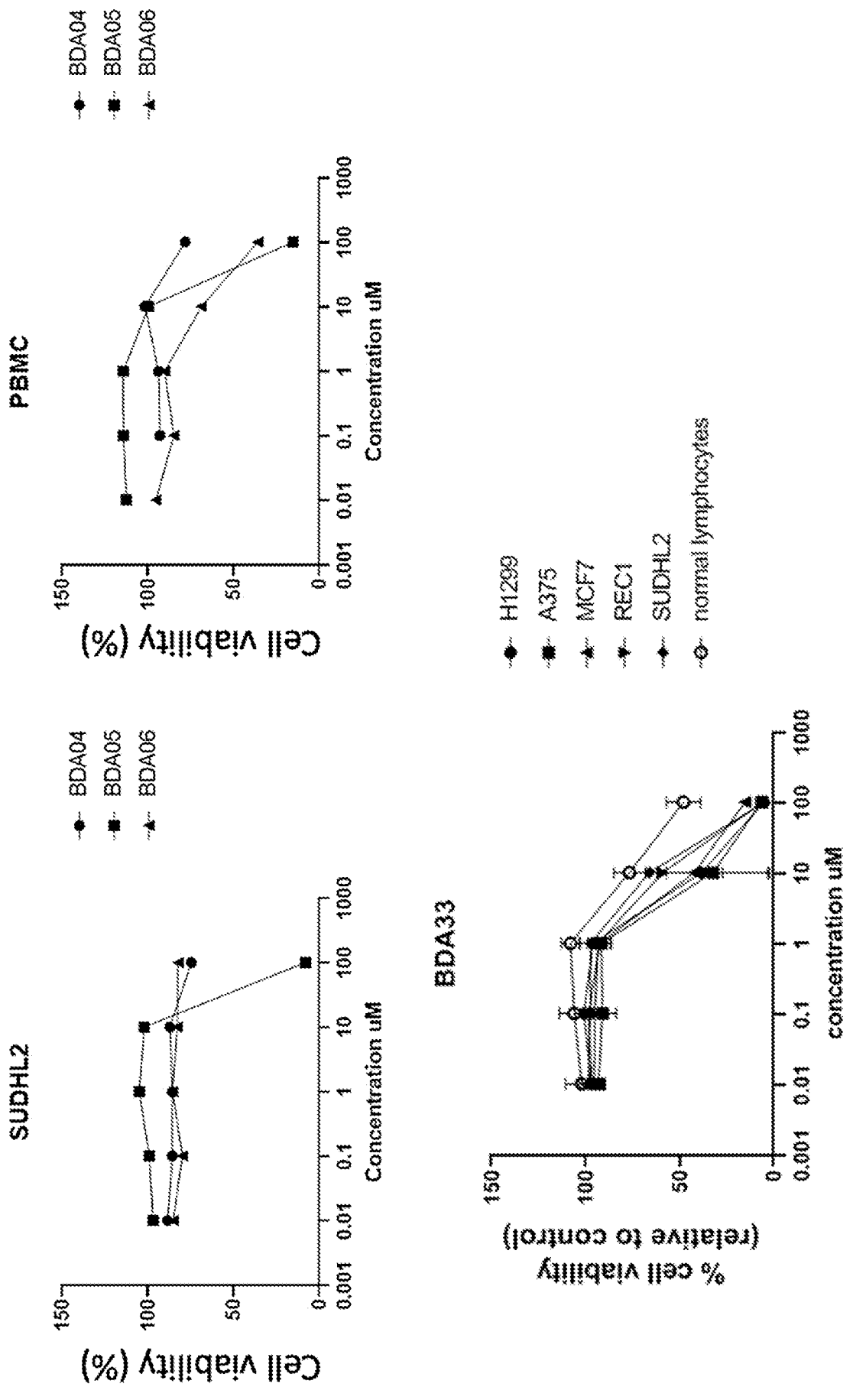

PBMC
BDA04
BDA05
BDA06
Cell viability (%)
Concentration uM
SUDHL2
BDA04
BDA05
BDA06
Cell viability (%)
Concentration uM
BDA33
H1299
A375
MCF7
REC1
SUDHL2
normal lymphocytes
% cell viability
(relative to control)
concentration uM

Fig. 6, Cont'd

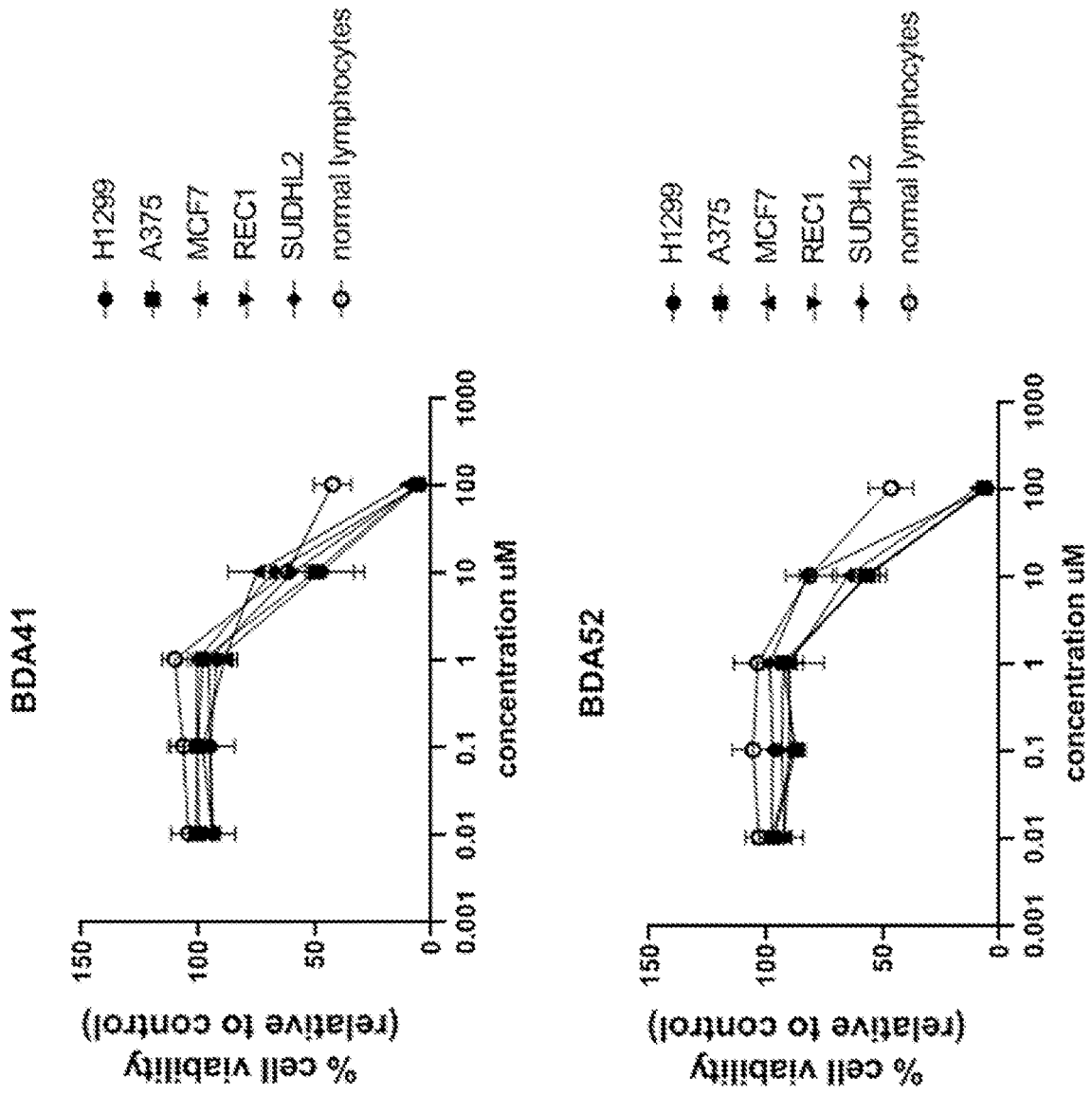

BDA41
BDA52
H1299
A375
MCF7
REC1
SUDHL2
normal lymphocytes
% cell viability
(relative to control)
concentration uM
150
100
50
0
0.001
0.01
0.1
1
10
100
1000

Fig. 6, Cont'd

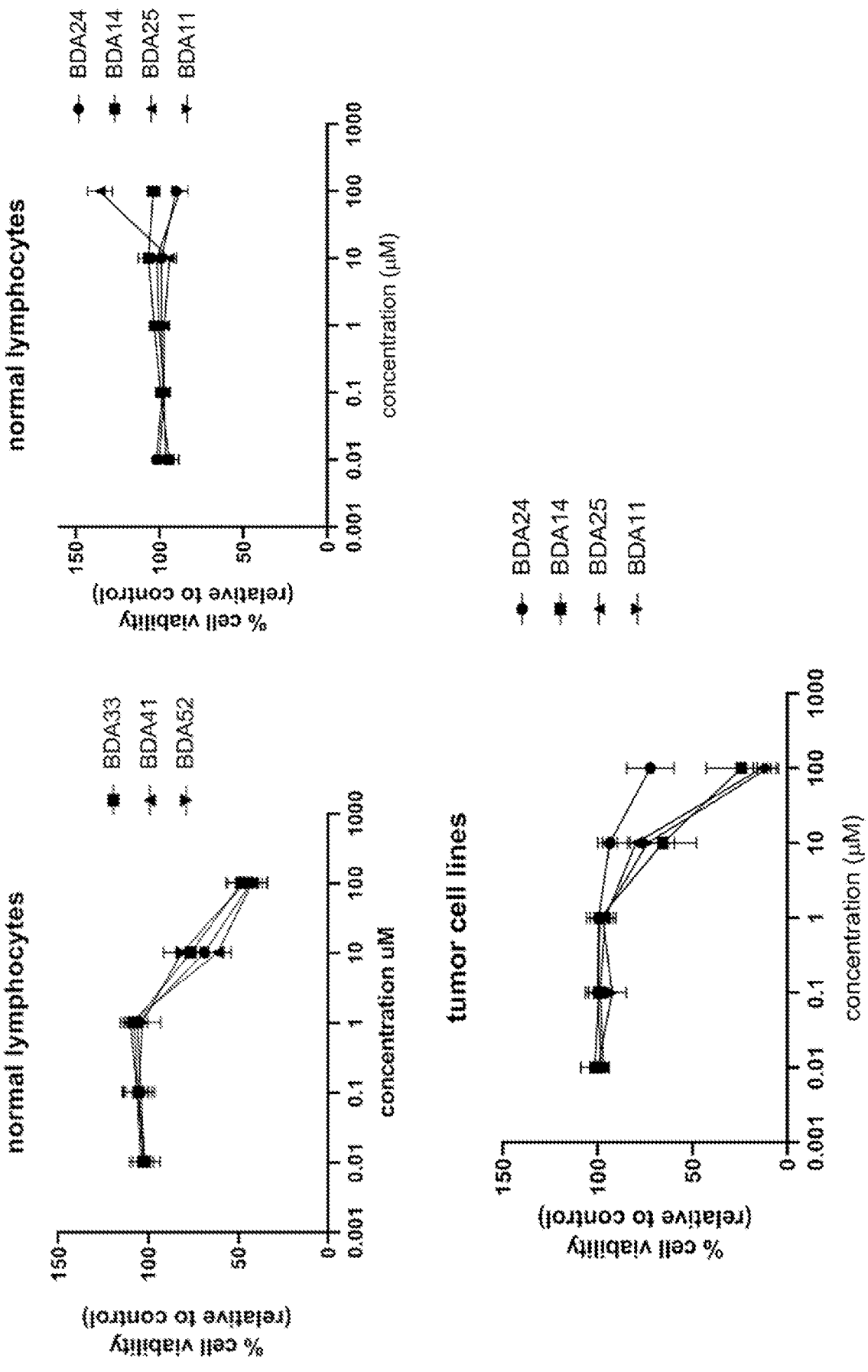

normal lymphocytes
BDA24
BDA14
BDA25
BDA11
% cell viability (relative to control)
concentration (µM)
normal lymphocytes
BDA33
BDA41
BDA52
% cell viability (relative to control)
concentration uM
tumor cell lines
BDA24
BDA14
BDA25
BDA11
% cell viability (relative to control)
concentration (µM)

Fig. 6, Cont'd

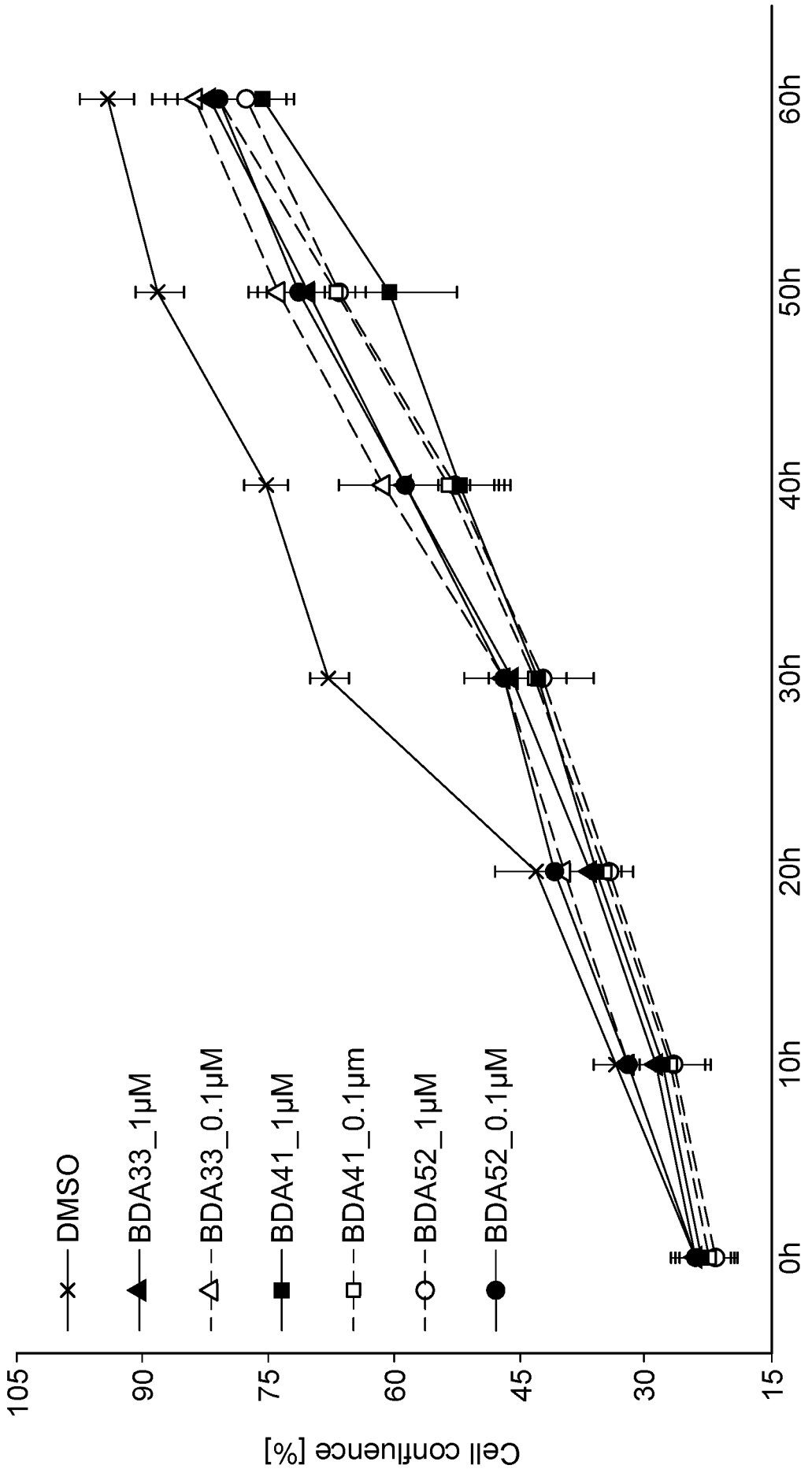

Cell confluence [%]
105
90
75
60
45
30
15
0h
10h
20h
30h
40h
50h
60h
DMSO
BDA33_1µM
BDA33_0.1µM
BDA41_1µM
BDA41_0.1µm
BDA52_1µM
BDA52_0.1µM

Fig. 7A

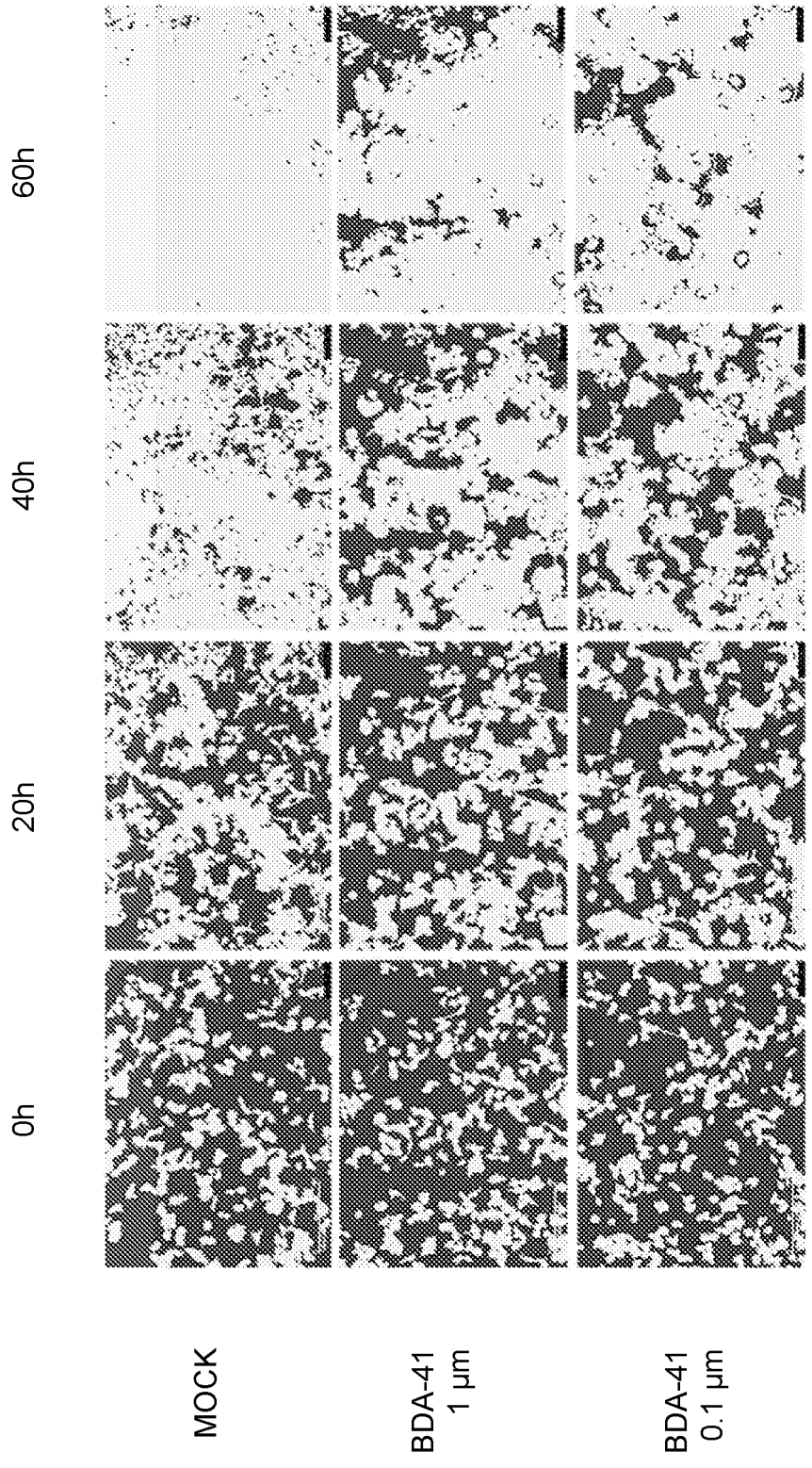


Fig. 7B

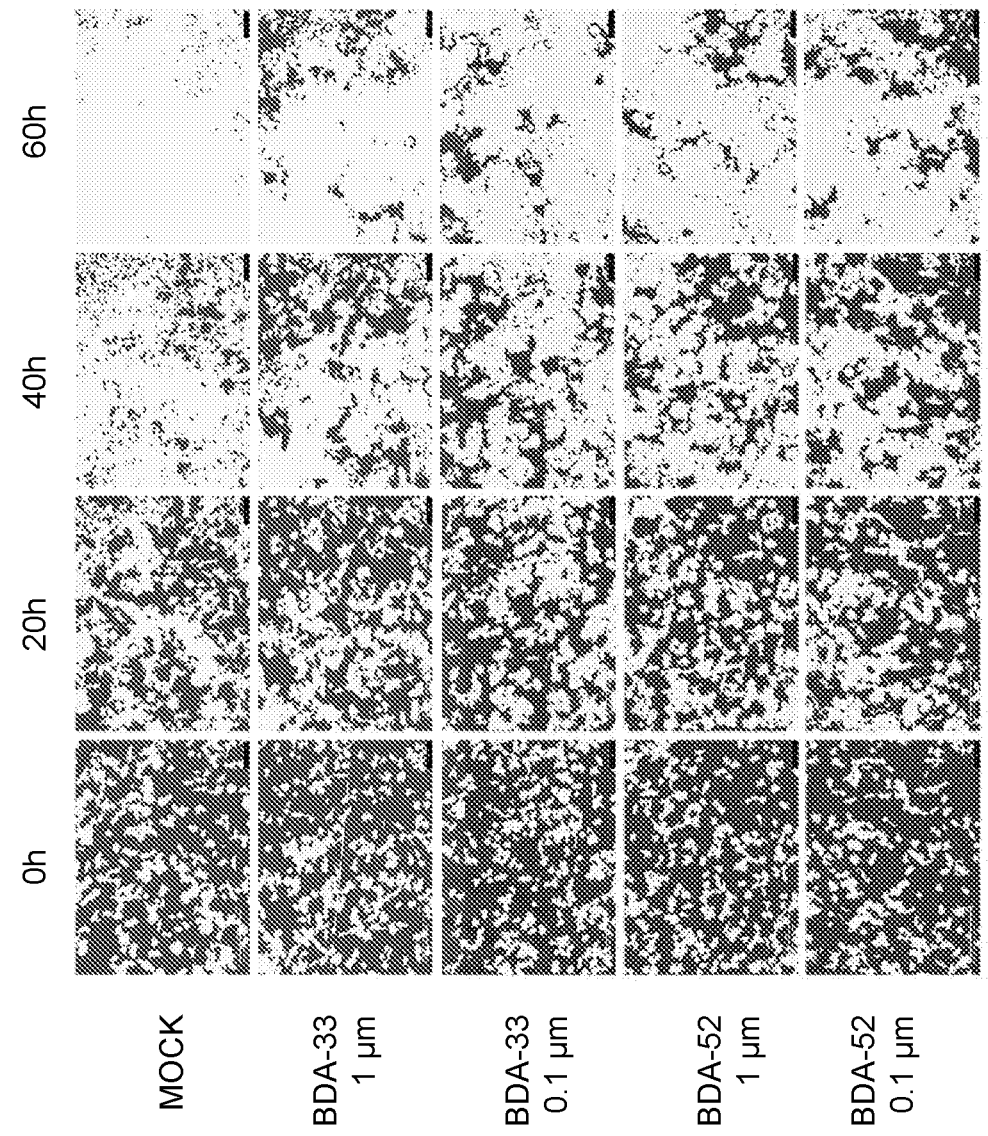


Fig. 7C

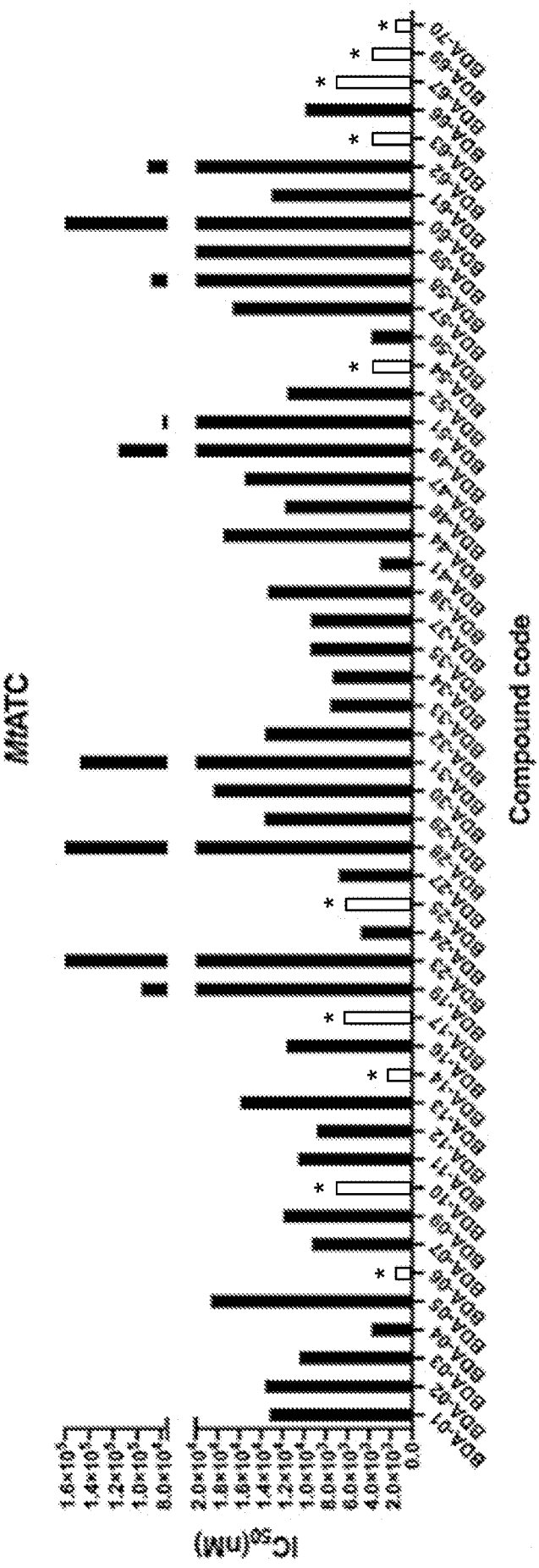
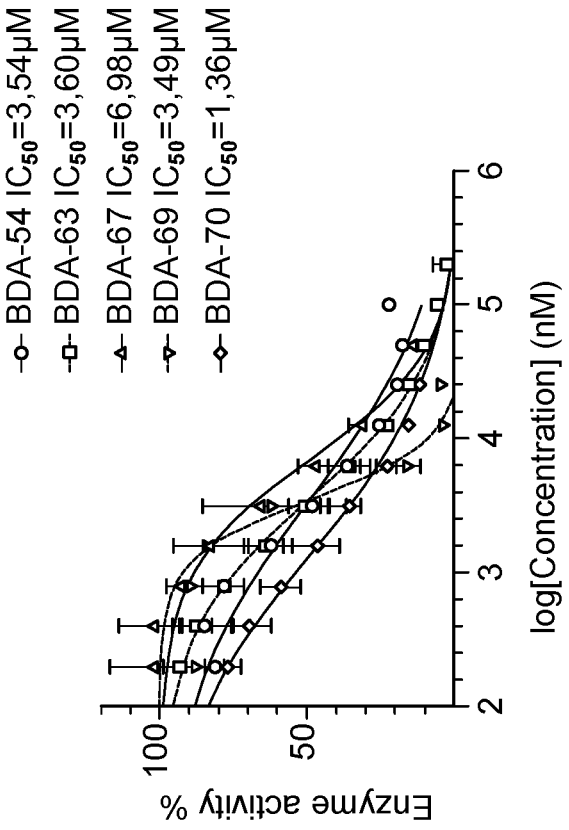


Fig. 8A


BDA-54 IC50=3,54µM
BDA-63 IC50=3,60µM
BDA-67 IC50=6,98µM
BDA-69 IC50=3,49µM
BDA-70 IC50=1,36µM
Enzyme activity %
100
50
2
3
4
5
6
log[Concentration] (nM)

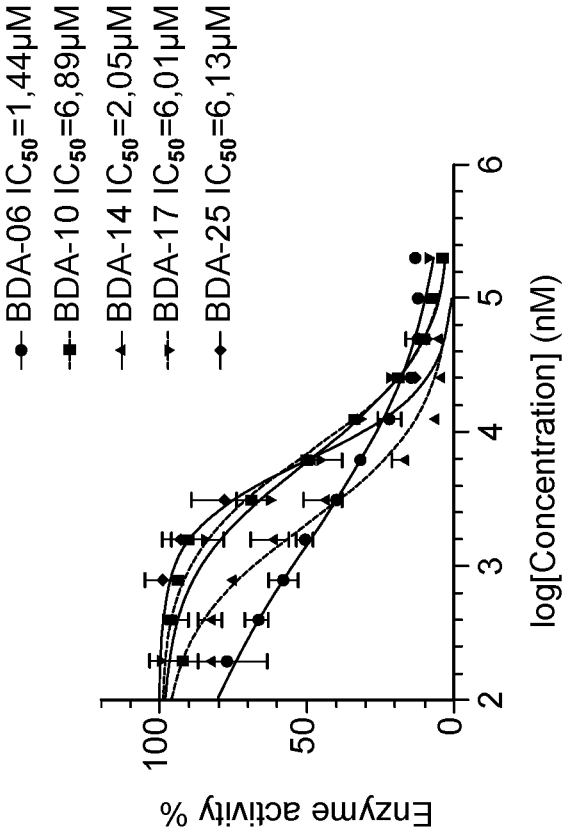

BDA-06 IC50=1,44µM
BDA-10 IC50=6,89µM
BDA-14 IC50=2,05µM
BDA-17 IC50=6,01µM
BDA-25 IC50=6,13µM
Enzyme activity %
100
50
0
2
3
4
5
6
log[Concentration] (nM)

Fig. 8B

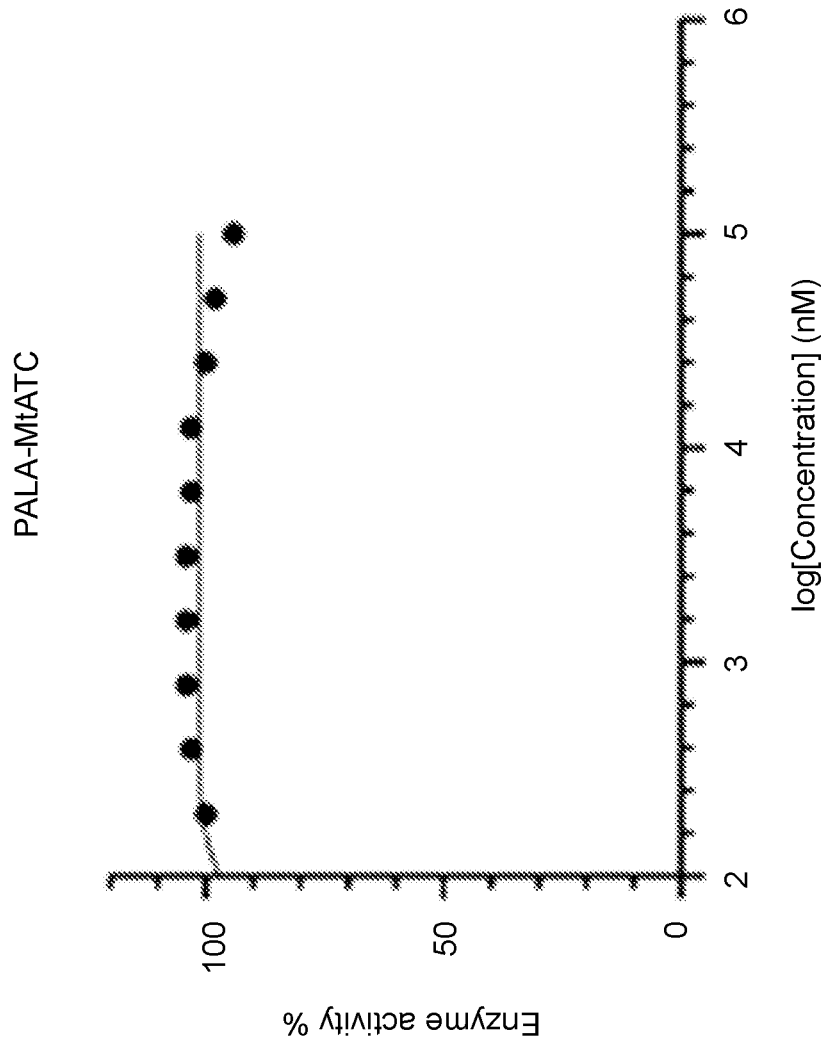

PALA-MtATC
Enzyme activity %
100
50
0
2
3
4
5
6
log[Concentration] (nM)

Fig. 8C

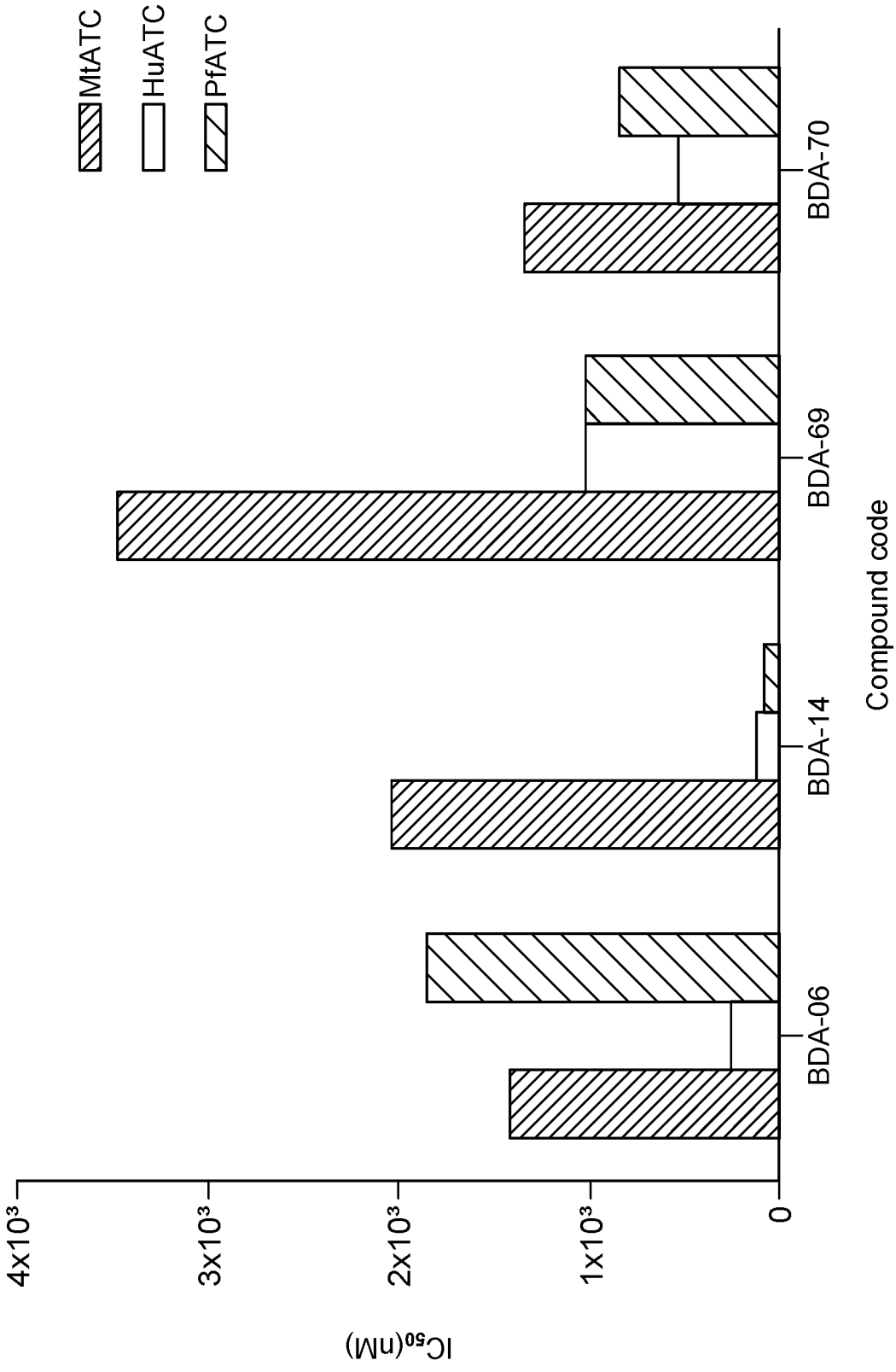

MtATC
HuATC
PfATC
4x10³
3x10³
2x10³
1x10³
0
IC50(nM)
BDA-06
BDA-14
BDA-69
BDA-70
Compound code

Fig. 8D

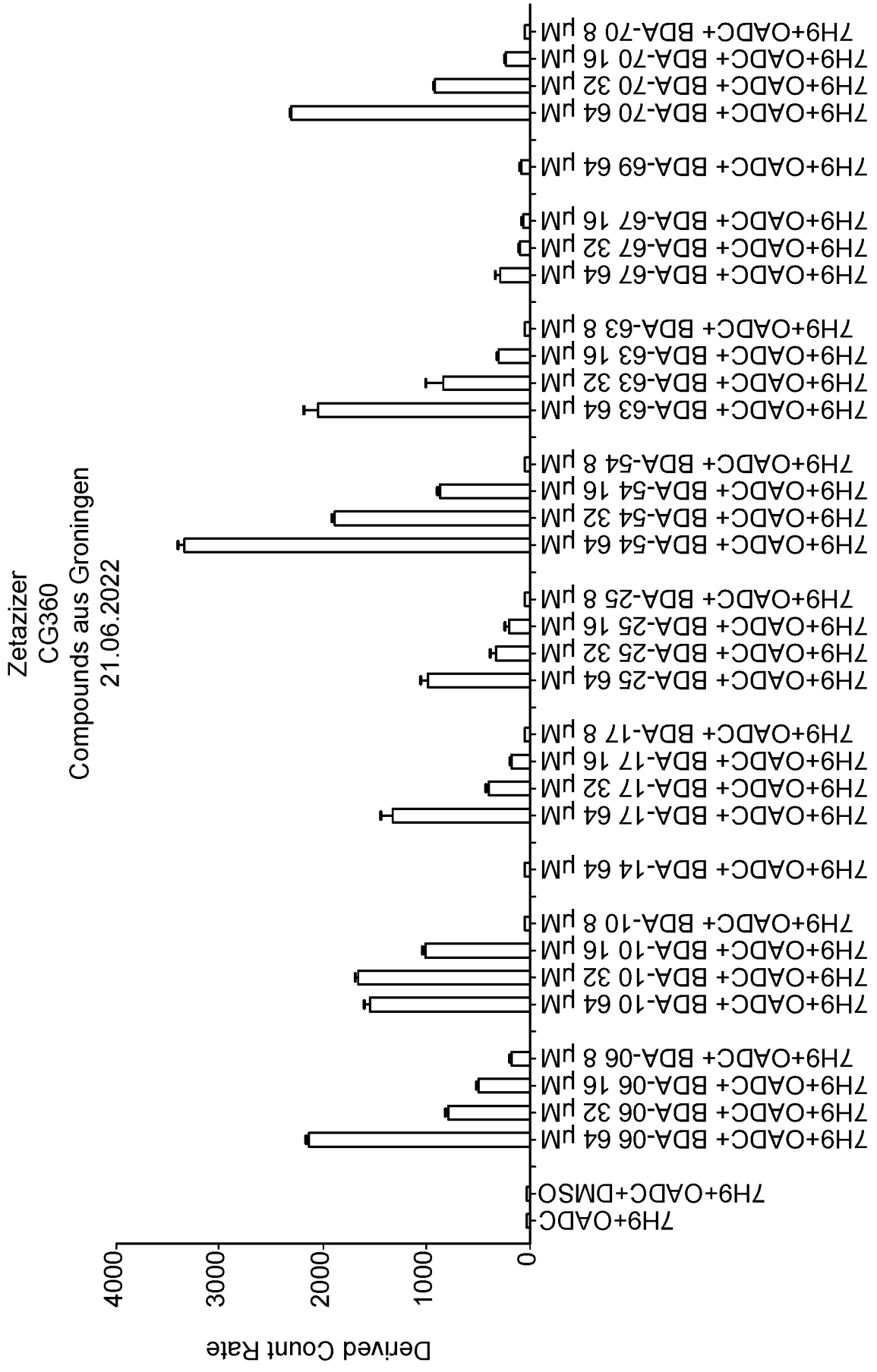

Zetazizer
CG360
Compounds aus Groningen
21.06.2022
Derived Count Rate
0
1000
2000
3000
4000
7H9+OADC
7H9+OADC+DMSO
7H9+OADC+ BDA-06 64 µM
7H9+OADC+ BDA-06 32 µM
7H9+OADC+ BDA-06 16 µM
7H9+OADC+ BDA-06 8 µM
7H9+OADC+ BDA-10 64 µM
7H9+OADC+ BDA-10 32 µM
7H9+OADC+ BDA-10 16 µM
7H9+OADC+ BDA-10 8 µM
7H9+OADC+ BDA-14 64 µM
7H9+OADC+ BDA-17 64 µM
7H9+OADC+ BDA-17 32 µM
7H9+OADC+ BDA-17 16 µM
7H9+OADC+ BDA-17 8 µM
7H9+OADC+ BDA-25 64 µM
7H9+OADC+ BDA-25 32 µM
7H9+OADC+ BDA-25 16 µM
7H9+OADC+ BDA-25 8 µM
7H9+OADC+ BDA-54 64 µM
7H9+OADC+ BDA-54 32 µM
7H9+OADC+ BDA-54 16 µM
7H9+OADC+ BDA-54 8 µM
7H9+OADC+ BDA-63 64 µM
7H9+OADC+ BDA-63 32 µM
7H9+OADC+ BDA-63 16 µM
7H9+OADC+ BDA-63 8 µM
7H9+OADC+ BDA-67 64 µM
7H9+OADC+ BDA-67 32 µM
7H9+OADC+ BDA-67 16 µM
7H9+OADC+ BDA-69 64 µM
7H9+OADC+ BDA-70 64 µM
7H9+OADC+ BDA-70 32 µM
7H9+OADC+ BDA-70 16 µM
7H9+OADC+ BDA-70 8 µM

Fig. 9A

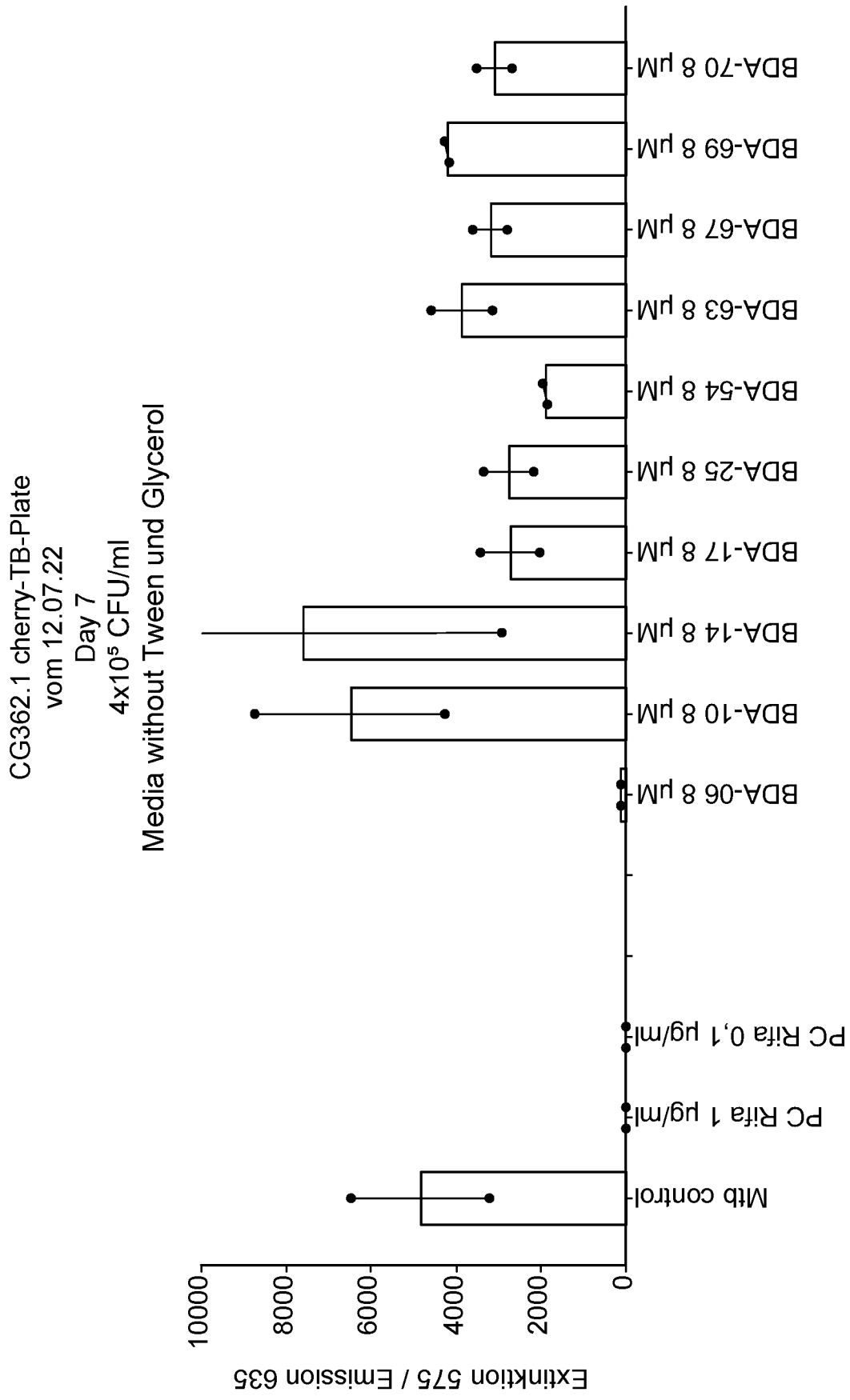

CG362.1 cherry-TB-Plate
vom 12.07.22
Day 7
4x10⁵ CFU/ml
Media without Tween und Glycerol
Extinktion 575 / Emission 635
10000
8000
6000
4000
2000
0
Mtb control
PC Rifa 1 µg/ml
PC Rifa 0,1 µg/ml
BDA-06 8 µM
BDA-10 8 µM
BDA-14 8 µM
BDA-17 8 µM
BDA-25 8 µM
BDA-54 8 µM
BDA-63 8 µM
BDA-67 8 µM
BDA-69 8 µM
BDA-70 8 µM

Fig. 9B

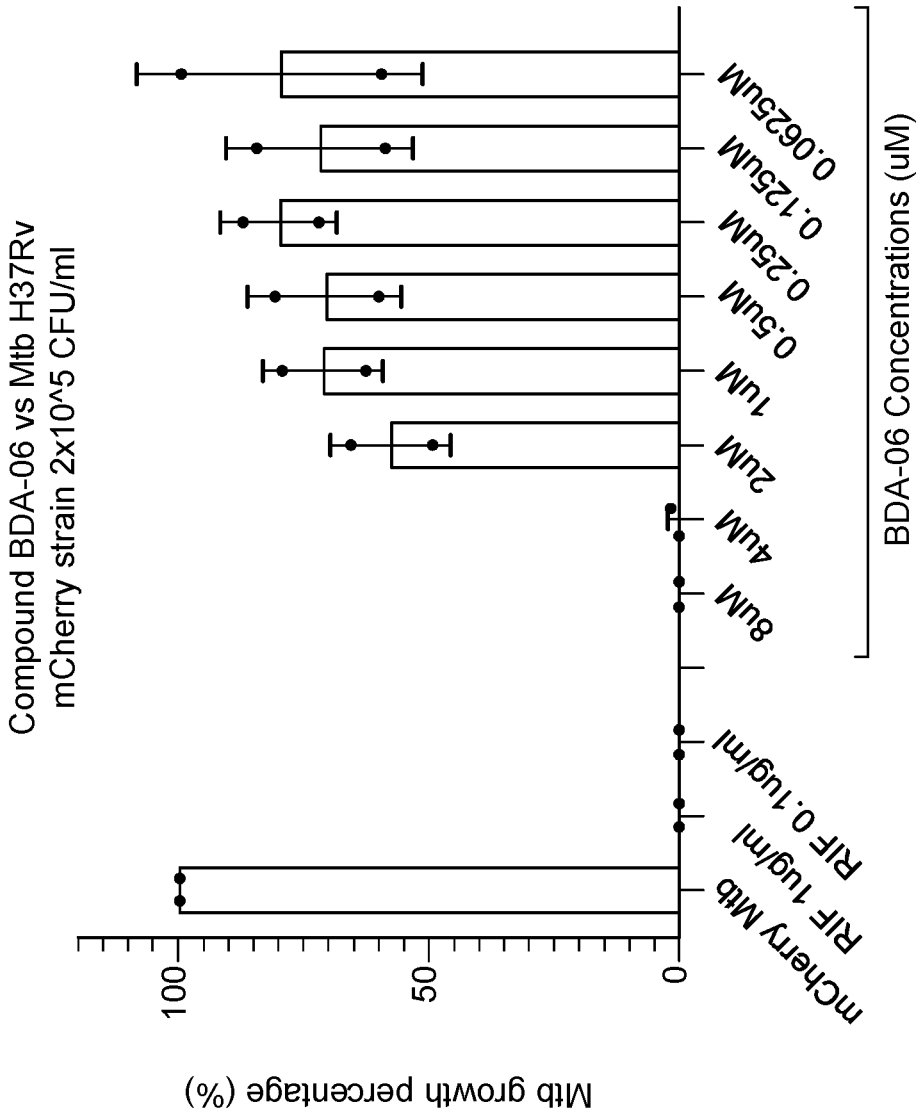

Compound BDA-06 vs Mtb H37Rv
mCherry strain 2x10^5 CFU/ml
Mtb growth percentage (%)
100
50
0
mCherry Mtb
RIF 1ug/ml
RIF 0.1ug/ml
8uM
4uM
2uM
1uM
0.5uM
0.25uM
0.125uM
0.0625uM
BDA-06 Concentrations (uM)

Fig. 9C

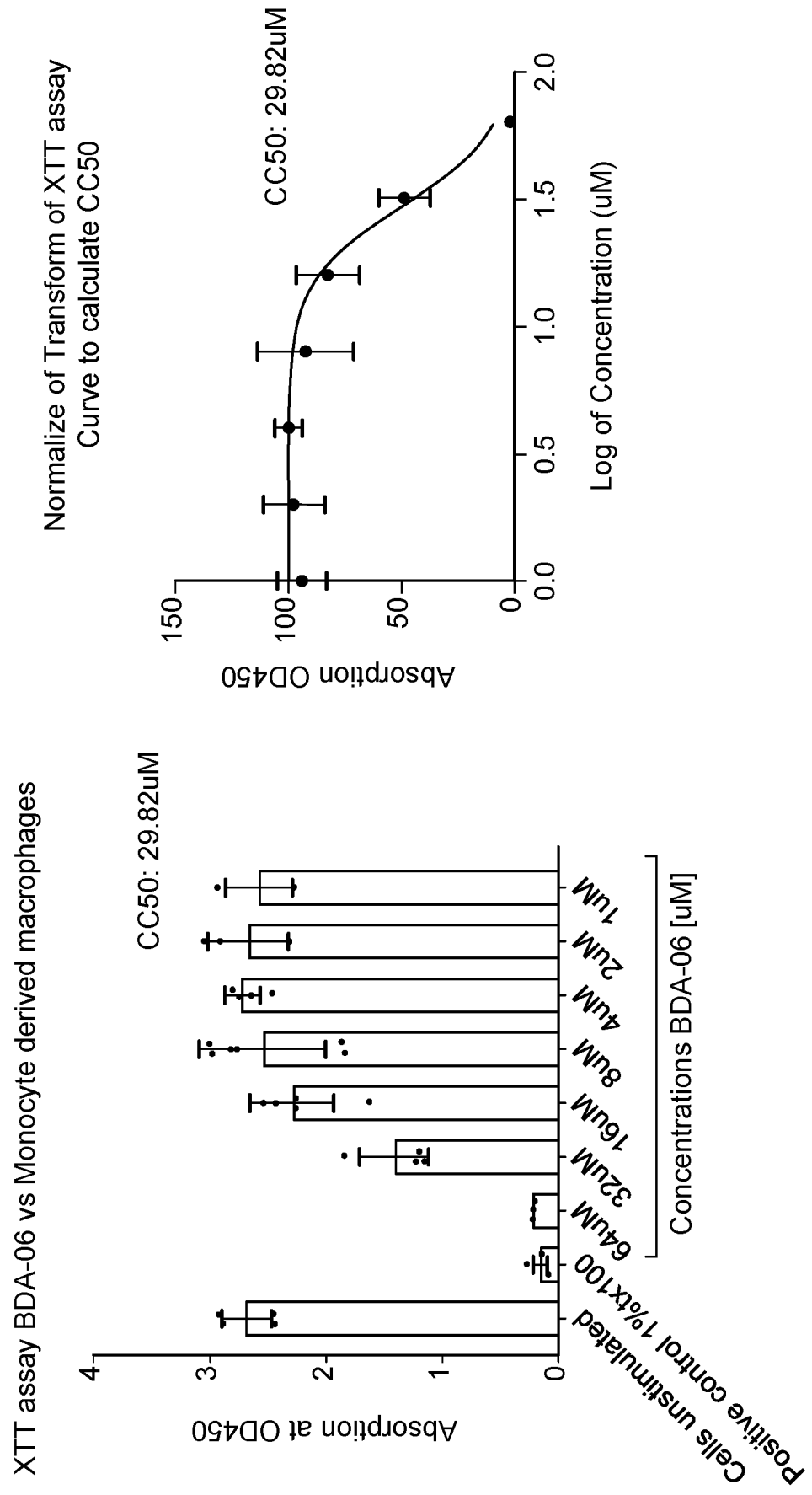

XTT assay BDA-06 vs Monocyte derived macrophages
CC50: 29.82uM
Absorption at OD450
0
1
2
3
4
Cells unstimulated
Positive control 1%x100
64uM
32uM
16uM
8uM
4uM
2uM
1uM
Concentrations BDA-06 [uM]
Normalize of Transform of XTT assay
Curve to calculate CC50
CC50: 29.82uM
Absorption OD450
0
50
100
150
0.0
0.5
1.0
1.5
2.0
Log of Concentration (uM)

Fig. 9D

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2022/050697

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D307/68 C07D333/38 C07D333/68 C07D409/04 C07D409/06
C07D409/12 C07D495/04 A61P33/06 A61P35/00 A61K31/381
A61K31/4178 A61K31/4365 A61K31/4436 A61K31/4523 A61K31/496
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

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

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2021/105335 A1 (CAPTOR THERAPEUTICS S A [PL]) 3 June 2021 (2021-06-03) pages 42-47: items 1) and 8); claims 1-42, 82, 87; table 1 (pages 31ff.); compounds 16, 40	1,15-26
X	WO 2013/190137 A2 (UNIV LEUVEN KATH [BE]; UNIV FERRARA [IT]) 27 December 2013 (2013-12-27) table on pages 47-48; claims; compounds (Ia); TJ109 and TR525,531,572,581,601	1,8, 15-26
X	US 2010/113418 A1 (FUKATSU KOHJI [JP] ET AL) 6 May 2010 (2010-05-06) claims 5-8, 18, 20, 22; examples 6, 7, 52, 53, 77, 108, 110, 143-145	1-5,10, 15-26

-/--

[X] Further documents are listed in the continuation of Box C.
[X] 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
13 January 2023	23/01/2023

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Stroeter, Thomas

1

Form PCT/ISA/210 (second sheet) (April 2005)

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 2010/160255 A1 (KAMATA MAKOTO [JP] ET AL) 24 June 2010 (2010-06-24) reference examples 75 (including the starting compound) and 76; paragraph [0908]; claims; examples 150,162,170,171,173, 176, 178, ...	1-5,10, 12,15-26
X	US 2019/119296 A1 (SUCHECK JR STEVEN [US] ET AL) 25 April 2019 (2019-04-25) paragraph [0212] - paragraph [0214]; figures 6, 7; compounds 5(a,b,c), 5(5,0,0) and 6(5,0,2) to 6(5,0,4)	1-11
X	Eleftheriadis Nikolaos ET AL: "S-1 Supplemental Information Design of a novel Thiophene Inhibitor of 15-Lipoxygenase-1 with both Anti-inflammatory and Neuroprotective Properties TABLE OF CONTENTS", , 1 January 2016 (2016-01-01), pages S1-S34, XP055915814, Retrieved from the Internet: URL:https://ars.els-cdn.com/content/image/1-s2.0-S022352341630558X-mmc1.pdf [retrieved on 2022-04-26] pages S-26 - pages S-27; compounds B3, C1, C3, C5, C8, D4, D5, D7, D8 & ELEFTHERIADIS NIKOLAOS ET AL: "Design of a novel thiophene inhibitor of 15-lipoxygenase-1 with both anti-inflammatory and neuroprotective properties", 9 July 2016 (2016-07-09), EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, ELSEVIER, AMSTERDAM, NL, PAGE(S) 786 - 801, XP029705965, ISSN: 0223-5234	1-11
X	SHIN YOUNGHWA ET AL: "A novel RPE65 inhibitor CU239 suppresses visual cycle and prevents retinal degeneration", BIOCHIMICA ET BIOPHYSICA ACTA. MOLECULAR BASIS OF DISEASE., vol. 1864, no. 7, 1 July 2018 (2018-07-01), pages 2420-2429, XP055915823, NL ISSN: 0925-4439, DOI: 10.1016/j.bbadis.2018.04.014 table 1; compound 223	1-8

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/NL2022/050697

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	XIAOYUN LU ET AL: "Design, synthesis and anti-tubercular evaluation of new 2-acylated and 2-alkylated amino-5-(4-(benzyloxy)phenyl)thiophene-3-carboxylic acid derivatives. Part 1", EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY, ELSEVIER, AMSTERDAM, NL, vol. 46, no. 9, 9 May 2011 (2011-05-09), pages 3551-3563, XP028278251, ISSN: 0223-5234, DOI: 10.1016/J.EJMECH.2011.05.018 [retrieved on 2011-05-19] schemes 1 and 2: compounds 8a-c, 11d, 11e, 11h, 11i, 11j, 12d, 12e, 12h, 12i, 12j and 14	1,2,8,10
X	WO 2009/124086 A2 (SIGA TECHNOLOGIES INC [US]; DAI DONGCHENG [US] ET AL.) 8 October 2009 (2009-10-08) claim 2; compound 5	13, 15-17, 22-24
X	HUNG THE DANG ET AL: "Syntheses and biological evaluation of 2-amino-3-acyl-tetrahydrobenzothiophene derivatives; antibacterial agents with antivirulence activity", ORGANIC & BIOMOLECULAR CHEMISTRY, vol. 12, no. 12, 1 January 2014 (2014-01-01), pages 1942-1956, XP055498237, ISSN: 1477-0520, DOI: 10.1039/C3OB42478B scheme 1: preparation of compound 11l via N-Boc protected ester (= BDA-65), step i (see also page 1944, right-hand column at the bottom); figure 1; compounds 4, 11	13
X	HUANG YIJUN ET AL: "1,4-Thienodiazepine-2,5-diones via MCR (I): Synthesis, Virtual Space and p53-Mdm2 Activity", CHEMICAL BIOLOGY & DRUG DESIGN, vol. 76, no. 2, 18 May 2010 (2010-05-18), pages 116-129, XP093013674, ISSN: 1747-0277, DOI: 10.1111/j.1747-0285.2010.00989.x preparation of 3a features the methyl ester (= BDA-67) as intermediate; see also page 123, left-hand column, paragraph 1; page 117, right-hand column, paragraph 2	13,14

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/NL2022/050697

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2021105335 A1	03-06-2021	EP 4065576 A1	05-10-2022
		WO 2021105335 A1	03-06-2021
WO 2013190137 A2	27-12-2013	AU 2013279275 A1	22-01-2015
		CA 2875944 A1	27-12-2013
		EP 2864312 A2	29-04-2015
		ES 2900519 T3	17-03-2022
		JP 6338577 B2	06-06-2018
		JP 2015521614 A	30-07-2015
		US 2015126559 A1	07-05-2015
		WO 2013190137 A2	27-12-2013
US 2010113418 A1	06-05-2010	EP 2123652 A1	25-11-2009
		JP WO2008102749 A1	27-05-2010
		US 2010113418 A1	06-05-2010
		WO 2008102749 A1	28-08-2008
US 2010160255 A1	24-06-2010	CA 2617042 A1	01-02-2007
		EP 1911753 A1	16-04-2008
		JP WO2007013691 A1	12-02-2009
		US 2010160255 A1	24-06-2010
		WO 2007013691 A1	01-02-2007
US 2019119296 A1	25-04-2019	US 2019119296 A1	25-04-2019
		WO 2017184947 A1	26-10-2017
WO 2009124086 A2	08-10-2009	NONE	