



(51) International Patent Classification:

C07C 259/06 (2006.01) *A61K 31/4406* (2006.01)
C07D 231/12 (2006.01) *A61K 31/4409* (2006.01)
C07D 213/42 (2006.01) *A61P 33/06* (2006.01)
C07D 333/24 (2006.01) *A61P 35/00* (2006.01)
A61K 31/16 (2006.01) *A61P 35/02* (2006.01)
A61K 31/381 (2006.01)

(21) International Application Number:

PCT/AU2016/000248

(22) International Filing Date:

13 July 2016 (13.07.2016)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

2015902765 13 July 2015 (13.07.2015) AU

(71) Applicant: MONASH UNIVERSITY [AU/AU]; Wellington Road, Clayton Victoria 3800 (AU).

(72) Inventors: **McGOWAN, Sheena**; 13 Tottenham Grove, Mulgrave VIC 3170 (AU). **SCAMMELLS, Peter**; 31 Duggan Street, North Balwyn VIC 3014 (AU). **VINH, Natalie**; 233 Hawthorn Road, Vermont South VIC 3133 (AU). **DRINKWATER, Nyssa**; U12/236 Wattletree Road, Malvern VIC 3144 (AU). **LEE, Jisook**; 3/907 Dandenong Road, Malvern East VIC 3135 (AU).

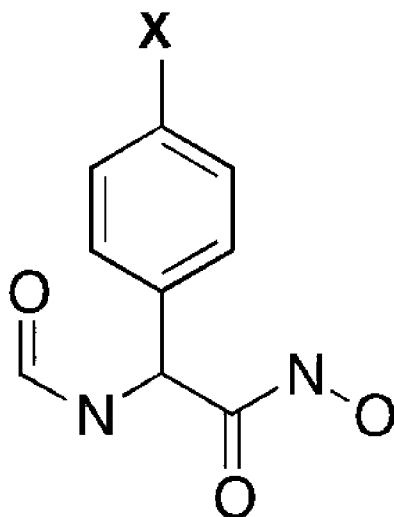
(74) Agent: SMOORENBURG PATENT & TRADE MARK ATTORNEYS; PO Box 515, Ringwood VIC 3134 (AU).

(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,

[Continued on next page]

(54) Title: NOVEL AMINOPEPTIDASE INHIBITORS AND METHODS OF USE



(57) Abstract: An aminopeptidase inhibitor compound comprising a biaryl, hydroxamic acid based core of formula: wherein X is a 5 or 6-membered ring, and including pharmaceutically acceptable salts and solvates thereof.



LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, **Published:**

SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, — *with international search report (Art. 21(3))*
GW, KM, ML, MR, NE, SN, TD, TG).

NOVEL AMINOPEPTIDASE INHIBITORS AND METHODS OF USE

FIELD OF INVENTION

[0001] The present invention relates to the field of pharmaceutical actives, particularly pharmaceutically active aminopeptidase inhibitors.

[0002] In one form, the invention relates to a method and actives for inhibiting aminopeptidases.

[0003] In another form the present invention is suitable for use in relation to disorders that involve aminopeptidases. In one particular aspect the present invention relates to anticancer and antimalarial therapeutics.

[0004] It will be convenient to hereinafter describe the invention in relation to aminopeptidase related disorders such as leukaemia and malaria, however it should be appreciated that the present invention is not limited to that use only and extends to other applications wherein it is useful to inhibit aminopeptidase.

[0005] Furthermore, it will be convenient to hereinafter describe the invention in relation to specific core chemical structures, however it should be appreciated that the present invention is not so limited and extends more broadly to entire structures and other classes of compounds that have the potential to inhibit aminopeptidases.

BACKGROUND ART

[0006] It is to be appreciated that any discussion of documents, devices, acts or knowledge in this specification is included to explain the context of the present invention. Further, the discussion throughout this specification comes about due to the realisation of the inventor and/or the identification of certain related art problems by the inventor. Moreover, any discussion of material such as documents, devices, acts or knowledge in this specification is included to explain the context of the invention in terms of the inventor's knowledge and experience and, accordingly, any such discussion should not be taken as an admission that any of the material forms part of the prior art base or the

common general knowledge in the relevant art in Australia, or elsewhere, on or before the priority date of the disclosure and claims herein.

[0007] Aminopeptidases catalyse the removal of amino acids from proteins or peptide substrates by cleaving the peptide bond next to a terminal amino acid containing a free amino group. They are thus found widely in subcellular organelles, in cytoplasm and as membrane components of both plants and animals. Aminopeptidases are important for the proper functioning of prokaryotic and eukaryotic cells, but very often are central players in the devastating human diseases like cancer, malaria and diabetes.

[0008] For example, aminopeptidase N occurs in humans and plays a role in the final digestion of peptides generated from the hydrolysis of proteins by gastric and pancreatic proteases. Aminopeptidases are involved in the metabolism of regulatory peptides of diverse cell types, such as those responsible for the processing of peptide hormones, such as angiotensin III and IV, neuropeptides and chemokines. Other activities include cleavage of antigen peptides bound to major histocompatibility complex class II molecules of presenting cells and degradation of neurotransmitters at synaptic junctions. Aminopeptidases are also implicated in activities such as regulation of IL-8 bioavailability in the endometrium, and therefore may contribute to the regulation of angiogenesis. It is also known to be a useful marker for acute myeloid leukaemia and plays a role in tumour invasion.

[0009] Because aminopeptidases are essential to many physiologically important processes such as protein maturation, degradation of peptides, and cell cycle control, inhibiting aminopeptidase disrupts protein turnover leading to an accumulation of peptides and a reduction in the cellular free amino acid content, which has a profound effect on cell survival and proliferation. Importantly, many tumour cells are dependent on specific amino acids and previous studies have shown that depletion of these amino acids has a greater effect on cancer cells than normal cells.

[0010] Therefore, inhibiting the aminopeptidase enzyme system has been receiving increasing attention as a therapy and there is an ongoing need to identify suitable compounds that can inhibit aminopeptidases.

[0011] This control can also be extended beyond humans and animals. For example, *P. falciparum* M1 and M17 (PfA-M1 and PfA-M17) are neutral aminopeptidases which are essential for malaria parasite growth and development.

[0012] Malaria is a parasitic disease that kills over half a million people each year, posing a huge burden to public health and remaining a persistent global health problem. Almost half of the global population, particularly those living in sub-Saharan Africa and south-east Asia, remain vulnerable to malaria. The disease is caused by five parasites of the genus *Plasmodium*, with *Plasmodium falciparum* (Pf) the most virulent. Although international efforts for treatment and eradication have made some headway, the emergence of drug-resistant malaria parasites threatens this progress. Pf has developed widespread resistance to commonly used antimalarial pharmaceuticals such as chloroquine, mefloquine, pyrimethamine, and sulfadoxine. Resistance to the artemisinins, the last line of defense, has emerged in five countries of the Greater Mekong subregion (Cambodia, Thailand, Myanmar, Vietnam and Laos).

[0013] If a resurgence of malaria is to be prevented, new therapeutics of diverse chemistry and different mechanisms of action are urgently required.

SUMMARY OF INVENTION

[0014] An object of the present invention is to provide potential new pharmaceutical actives.

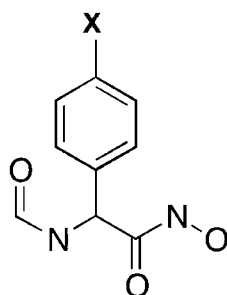
[0015] Another object of the present invention is to provide potential new aminopeptidase inhibitors.

[0016] Another object of the present invention is to provide potential new therapeutics for disorders that would be ameliorated by inhibition of aminopeptidases.

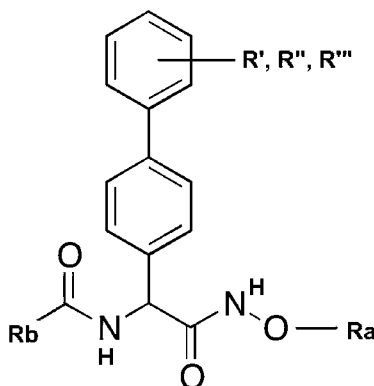
[0017] A further object of the present invention is to alleviate at least one disadvantage associated with the related art.

[0018] It is an object of the embodiments described herein to overcome or alleviate at least one of the above noted drawbacks of related art systems or to at least provide a useful alternative to related art systems.

[0019] In a first aspect of embodiments described herein there is provided an aminopeptidase inhibitor compound, and including its pharmaceutically acceptable salts and solvates, comprising a biaryl, hydroxamic acid based core (in which X is a 5 or 6 membered ring) of formula:

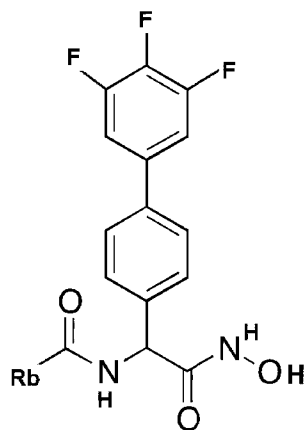


[0020] The hydroxamic acid moiety of the core is preferably substituted (Ra, Rb), and one of the aryl rings may be mono, bi or tri-substituted (R', R'', R''') such as, for example:



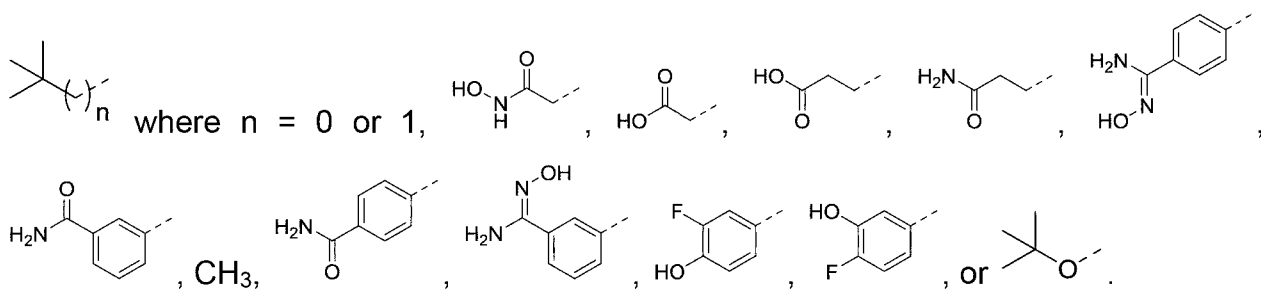
[0021] In a particularly preferred embodiment, R', R'' and R''' are fluorine and Ra is hydrogen, and Rb is chosen from the group comprising, -C(CH₃)₃, -CH₂C(CH₃)₃, -CH₂C(O)OH, -CH₂C(O)NH(OH), -(CH₂)₂C(O)OH, -(CH₂)₂C(O)NH₂, -(4-phenyl)C(NO₂)NH₂, -(4-phenyl)C(O)NH₂, -CH₃, -(3-phenyl)C(NO₂)NH₂, -(3-phenyl)C(O)NH₂, 3-fluoro-4-hydroxyphenyl, 4-fluoro-3-hydroxyphenyl or -OC(CH₃)₃.

[0022] That is, in a particularly preferred embodiment the aminopeptidase inhibitor compound is of formula:



and its pharmaceutically acceptable salts and solvates,

wherein Rb is chosen from the group comprising:

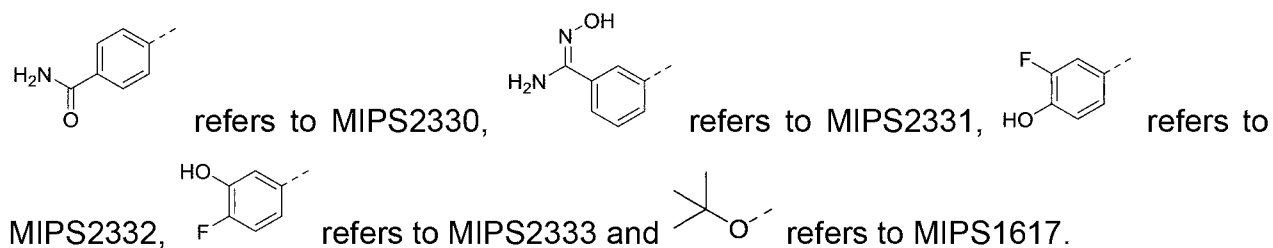


[0023] Where used herein, the term MIPS1700 refers to where n = 0,

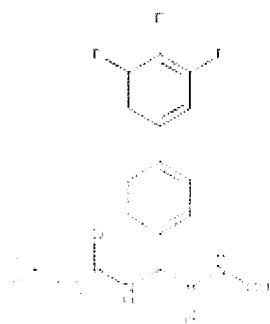
MIPS1778 refers to where n = 1, refers to MIPS1992, refers

to MIPS1993, refers to MIPS1994, refers to MIPS1995, refers

to MIPS1996 and refers to MIPS1997, CH₃ refers to MIPS1998,



[0024] In a particularly preferred embodiment, the aminopeptidase inhibitor compound is of formula:



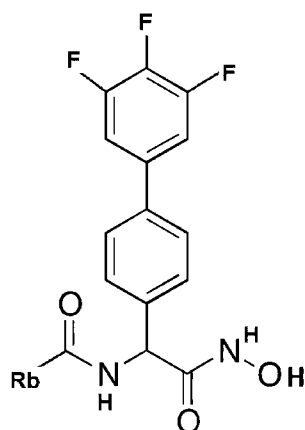
and its pharmaceutically acceptable salts and solvates,

wherein $n = 0$ or 1 .

[0025] In a particularly preferred embodiment, $n=0$ in the above structure, that is, an N-(2-(hydroxyamino)-2-oxoethyl)pivalamide.

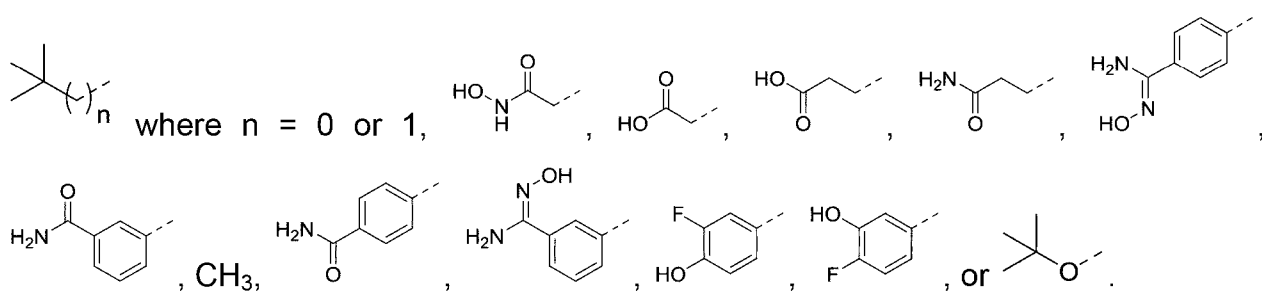
[0026] It has been found that these two hydroxamic acid-based compounds exhibit pharmaceutical activity, particularly as inhibitors of aminopeptidases.

[0027] In another aspect of embodiments described herein there is provided a compound of formula:



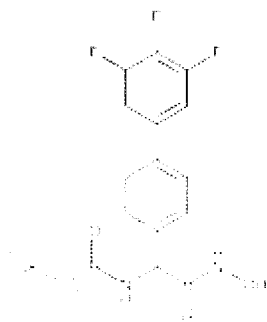
and their pharmaceutically acceptable salts and solvates,

wherein Rb is chosen from the group comprising:



when used as an aminopeptidase inhibitor.

[0028] In a particularly preferred embodiment, the aminopeptidase inhibitor used is a compound of formula:



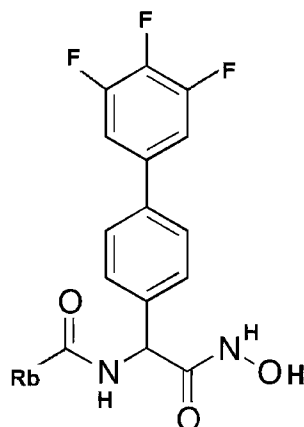
and its pharmaceutically acceptable salts and solvates, wherein $n = 0$ or 1 .

[0029] It has now been found that MIPS1700, MIPS1778, MIPS1992 to 1998, and MIPS2330 to 2333 exhibit activity in respect of aminopeptidases such as the malarial aminopeptidases M1 and M17. For example, they exhibit nanomolar activity against cultured *P. falciparum* parasites, which is within the range of current antimalarial agents.

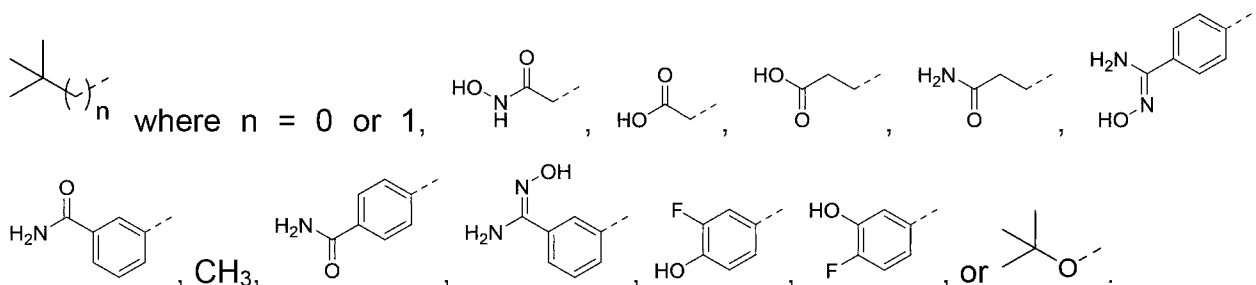
[0030] Importantly, compounds having the biaryl hydroxamic acid core have been shown to have dual activity, inhibiting both the M1 and M17 aminopeptidases. These compounds are therefore extremely attractive lead molecules that have the potential to be developed into novel antimalarial therapeutics.

[0031] Therefore, in another aspect of embodiments described herein there is provided a method of treating malaria, particularly malaria associated with *P. falciparum* parasites, using a compound having a biaryl hydroxamic acid core.

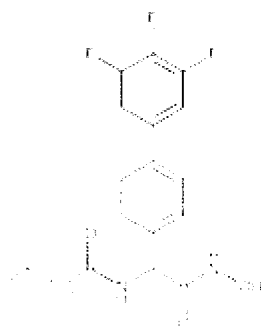
[0032] In a particularly preferred embodiment the method of treating malaria utilises a compound having a biaryl hydroxamic acid therapeutic, of formula:



wherein Rb is chosen from the group comprising:



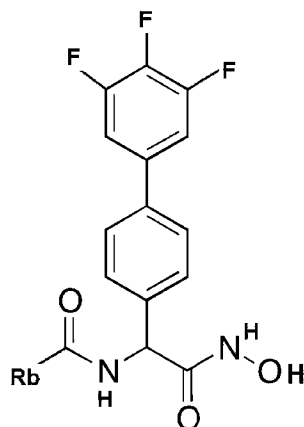
[0033] In a particularly preferred embodiment the method of treating malarial utilises the biaryl hydroxamic acid therapeutic, of formula:



and its pharmaceutically acceptable salts and solvates, wherein $n = 0$ or 1 .

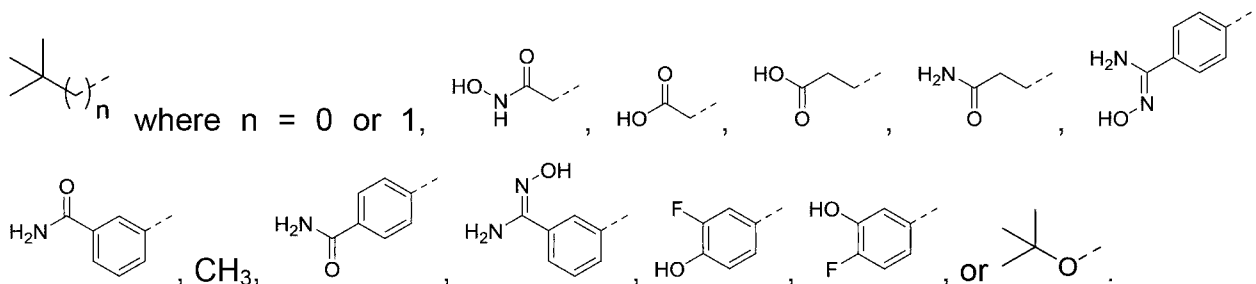
[0034] Compounds having a biaryl hydroxamic acid core, such as MIPS1700 and MIPS1778, have also been shown to exhibit pharmaceutical activity in respect of human aminopeptidase N, referred to herein as APN. APN inhibitors have exhibited clinical efficacy for the treatment of many cancer types including acute myeloid leukaemia and lung cancer. For example, structures MIPS1700 and MIPS1778 demonstrate an approximate 10 fold improvement over current clinical candidates (currently Bestatin and Tosedostat) and show sub-micromolar cellular efficacy against leukemic cell lines.

[0035] In another aspect of embodiments described herein there is provided a method of inhibiting an aminopeptidase, including the step of administering to a patient a therapeutic compound comprising a biaryl hydroxamic acid core. More particularly the therapeutic compound is of formula:

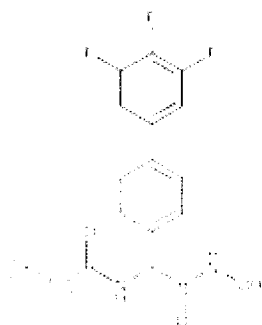


including its pharmaceutically acceptable salts and solvates,

wherein Rb is chosen from the group comprising:



[0036] Preferably the therapeutic compound is of formula:



and its pharmaceutically acceptable salts and solvates, wherein $n = 0$ or 1 .

[0037] In one embodiment the aminopeptidase is aminopeptidase N and the patient is suffering a cancer, such as leukaemia.

[0038] In another aspect of embodiments described herein there is provided the use of the aforementioned compounds for the preparation of a medicament formulated for an administrative route chosen from oral, nasal, buccal, parenteral, topical, depot or rectal.

[0039] In another aspect of embodiments described herein there is provided a method for the treatment of a human or animal subject comprising the step of administering an effective amount of the aforementioned compounds or a pharmaceutically acceptable salt or solvate thereof, and inhibiting aminopeptidase function for the treatment or prevention of a disorder.

[0040] Other aspects and preferred forms are disclosed in the specification and/or defined in the appended claims, forming a part of the description of the invention.

[0041] In essence, embodiments of the present invention stem from the realization that biaryl hydroxamic acid based compounds may be particularly suitable for binding to certain biomolecular targets.

[0042] Advantages provided by the present invention comprise the following:

- potential new treatments for aminopeptidase related disorders,
- potential new treatments for disorders that would be ameliorated by the inhibition of aminopeptidase N, such as cancer, including leukaemia,
- potential new therapeutics for disorders that would be ameliorated by the inhibition of aminopeptidases M1 and M17, particularly malaria.

[0043] Further scope of applicability of embodiments of the present invention will become apparent from the detailed description given hereinafter. However, it should be understood that the detailed description and specific examples, while indicating preferred embodiments of the invention, are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure herein will become apparent to those skilled in the art from this detailed description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0044] Further disclosure, objects, advantages and aspects of preferred and other embodiments of the present application may be better understood by those skilled in the relevant art by reference to the following description of embodiments taken in conjunction with the accompanying drawings, which are given by way of illustration only, and thus are not limitative of the disclosure herein, and in which:

[0045] FIG. 1 illustrates key compound structures; FIG. 1(a) is MIPS1700 when $n=0$ and MIPS1778 when $n=1$; FIG 1(b) is tert-butyl (1-(4-(1H-pyrazol-1-yl)phenyl)-2-

(hydroxyamino)-2-oxoethyl)carbamate; FIG. 1(c) is *N*-(1-(4-(1H-pyrazol-1-yl)phenyl)-2-(hydroxyamino)-2-oxoethyl)pivalamide

[0046] FIG 2 is a plot of % Inhibition against log concentration (nM) for MIPS1700 (FIG. 2(a)) and MIPS1778 (FIG. 2(b)) for 3D7 (1, ♦), Dd2 Parent (2, ■), Dd2 SpiroR (3, ▲) and HEK293 (4, ▼).

[0047] FIG. 3 is a scheme illustrating the synthesis of hydroxamic acid derivatives 9a-u and 10 a-v. Reagents and conditions: (a) i. cat. concd H₂SO₄ (aq), MeOH, reflux; ii. Boc₂O, THF, water, rt, or pivaloyl chloride, Et₃N, DCM, rt; (b) i. Boc₂O, 2M NaOH (aq), THF, water; ii. CDI, dry THF, rt; iii. NH₂OH.HCl; (c) i. dry MeOH, rt; ii. NH₂OH.HCl, 5M KOH/MeOH, dry MeOH (premixed), rt; (d) cat. PdCl₂(PPh₃)₂, boronic acid or boronate ester, degassed 1M Na₂CO₃ (aq), degassed THF, 100°C.

[0048] FIG. 4 is a scheme illustrating the synthesis of MIPS1778. Reagents and conditions: (a) cat. Concd H₂SO₄, MeOH, reflux; (b) 3,3-Dimethylbutyryl chloride, Et₃N, DCM, rt; (c) cat. PdCl₂(PPh₃)₂, boronic acid, degassed 1M Na₂CO₃ (aq), degassed THF, 100 °C; (d) i. dry MeOH, rt; ii. NH₂OH.HCl, 5M KOH/MeOH, dry MeOH (premixed), rt.

DETAILED DESCRIPTION

[0049] The following paragraphs describe the structure based drug design that has led to specific hydroxamic acid-based compounds, particularly those having a biaryl hydroxamic acid core, as potential therapeutic amniopeptidase inhibitors. Structure based drug design or rational design is the inventive process of finding new medications based on the knowledge of a biological target, obtained through methods such as x-ray crystallography. In essence, drug design involves the design of molecules that are complementary in shape and charge to the biomolecular target with which they interact and will therefore bind to it. 'Structure' based drug design relies on the knowledge of the three-dimensional structure of the biomolecular target.

[0050] The following paragraphs describe the design, synthesis and characterisation of novel compounds that inhibit essential Pf MAPs (metalloaminopeptidases) by evaluating proposed analogs within the binding cavity of enzymes *PfA*-M1 and *PfA*-M17

which are validated antimalarial therapeutic targets. Antimalarial drug resistance represents a major threat to global health. While progress towards a malaria vaccine continues, the continued development of antimalarial agents that work via novel mechanisms is absolutely required.

[0051] Using a comprehensive structure-guided medicinal chemistry approach, the hydroxamic acid-based compounds have been elaborated deep into the S1 pocket of both enzymes, which resulted in a series of potent, dual *PfA*-M1 and *PfA*-M17 inhibitors. Furthermore, it was determined that the compounds possess nanomolar anti-malarial activity against *Pf*-3D7 parasites, and excitingly, drug resistant strains Dd2 and Dd2 SpiroR. It has therefore been shown that dual inhibition of both *PfA*-M1 and *PfA*-M17 is an effective approach to treat drug-resistant parasite infections, and present a new series of antimalarial compounds with the potential to be developed into pre-clinical candidates.

[0052] Many of the clinical symptoms of malaria develop during the erythrocytic stage of infection. During this stage, multiple metabolic pathways are initiated within the parasites, which present a wide range of potential drug targets. Among the essential metabolic pathways that occur within erythrocytes, is haemoglobin digestion; host haemoglobin is degraded into free amino acids that are absolutely required for parasite survival. Interference with this pathway is therefore an attractive strategy for the development of novel antimalarial compounds. The final stage of this process is mediated by *PfA*-M1 as well as several other metalloaminopeptidases (MAPs) that are proposed to work in concert to remove of the N-terminal amino acid residue from peptide fragments.

[0053] *PfA*-M1 and *PfA*-M17 are from different enzyme families, and have very different structural arrangements; however, the active site of both enzymes is located within domain II, the catalytic domain. The active site of *PfA*-M1 is enclosed deep within the catalytic domain and is accessed by putative substrate entry and exit channels. In contrast, the six active sites of *PfA*-M17 are located on the edge of the catalytic domain, and exposed to solvent in the interior cavity of the hexameric assembly. Furthermore, *PfA*-M1 possesses one catalytic zinc ion (Zn^{2+}), whereas *PfA*-M17 has two Zn^{2+} and a catalytic carbonate atom.

[0054] In essence, embodiments of the present invention stem from the realization that despite these major differences between *PfA*-M1 and *PfA*-M17, the enzymes share similar architecture within the active sites and a single compound capable of inhibiting both enzymes could potentially slow the emergence of drug resistance parasites.

[0055] However, although previous inhibition studies have shown that cross inhibition of *PfA*-M1/*PfA*-M17 is achievable by targeting the catalytic zinc ion/s (Sivaraman, K. K. et al, *Journal of Medicinal Chemistry* 2013, 56, 5213-5217) differences in the compound binding profiles of the enzymes meant that compound elaboration tended toward improved inhibition of one enzyme target at the expense of the other.

[0056] The present invention stems from introduction of a hydroxamic acid moiety as a tighter zinc-binding group (ZBG) and elaborating into the S1' pocket to develop the first inhibitor series capable of potent dual inhibition of both *PfA*-M1 and *PfA*-M17. (Weik, S. et al. *Chemmedchem* 2006, 1, 445-457)

[0057] Two compounds were characterized as potent dual inhibitors of *PfA*-M1 and *PfA*-M17: tert-butyl (1-(4-(1H-pyrazol-1-yl)phenyl)-2-(hydroxyamino)-2-oxoethyl)carbamate (FIG. 1(b) designated herein as 2) and *N*-(1-(4-(1H-pyrazol-1-yl)phenyl)-2-(hydroxyamino)-2-oxoethyl)pivalamide (Figure 1(c) designated herein as 3). Both compounds bind the catalytic zinc ion/s through the hydroxamic acid moiety, and prevent growth of *Pf*-3D7 parasites (IC₅₀ of 2 = 783 nM; IC₅₀ of 3 = 227 nM) with no observable human cell cytotoxicity.

[0058] Improvement of the *PfA*-M1 and *PfA*-M17 inhibitory and *Pf* parasitocidal activity of the series was achieved by selecting the two optimized S1' binding moieties (previously identified in Mistry S. N. et al. *J. Med. Chem.*, 2014, 9168-9183) - the *N*-Boc group of 2 and *N*-pivaloyl group of 3, and using them as 'anchors' explored the S1 pocket with a range of substituted-phenyl and heteroaromatic rings. One of these series, the *N*-(2-(hydroxyamino)-2-oxoethyl)pivalamides, showed superior dual enzyme inhibitory activity compared to other *PfA*-M1 and *PfA*-M17 inhibitors, and resulted in greater inhibition of *Pf* growth in culture, including the multi-drug resistant strain Dd2. As such, the inhibitors described herein represented promising lead compounds for further development.

[0059] The hydroxamic acid-based compounds MIPS1700 and MIPS1778 (FIG. 1) have been identified as particularly promising. These compounds exhibit nanomolar activity against cultured *P. falciparum* parasites, which is within the range of current antimalarial agents. This is illustrated by the results in **Table 1** (illustrated graphically in FIGS. 2(a) and 2(b)) which indicate that MIPS1700 and MIPS1778 are extremely attractive lead molecules that have the potential to be developed into novel antimalarial therapeutics.

TABLE 1:

	<i>K_i</i> (nM)		<i>P. falciparum</i> IC ₅₀ (nM)			
	<i>PfA</i> -M1	<i>PfA</i> -M17	3D7	Dd2 Parent	Dd2 SpiroR	HEK293
MIPS1700	76	60	125.6 ± 1.5	188.5 ± 23.4	107.2 ± 19.8	NC
MIPS1778	107	12.2	11.3 ± 0.1	14.8 ± 0.1	14.4 ± 0.7	NC

NC = No curve, activity not sufficient to fit curve.

[0060] Having identified attractive lead molecules for antimalarial therapeutics, their other potential applications may be identified through drug re-profiling, also known as 'drug repurposing'.

[0061] A drug repurposing study has indicated that MIPS1778 also acts as potent inhibitor of human Aminopeptidase N (APN). APN inhibitors have shown clinical efficacy for the treatment of many cancer types including acute myeloid leukaemia and lung cancer. MIPS1770 and MIPS1778 also demonstrate an approximate 10-fold improvement over current clinical candidates, and show sub-micromolar cellular efficacy against leukaemic cell lines.

[0062] Table 2 records their activity against purified aminopeptidase N (in vitro).

TABLE 2:

Compound	IC ₅₀ (μM)
Bestatin	2.3
Tosedostat	2.6
MIPS1700	0.3
MIPS1778	0.2

SD were all $< \pm 0.002$

[0063] Table 3 records their anti-proliferative activity against human leukaemia cell lines.

TABLE 3:

No.	R ¹	R ²	IC ₅₀ (μM)			
			U937	HL60	SEMK2	THP1
9f	<i>tert</i> -butyloxycarbonyl	phenyl	15.2	6.8	0.94	19.3
9i	<i>tert</i> -butyloxycarbonyl	4-fluorophenyl	6.7	6.5	0.60	15.6
10f	trimethylacetyl	phenyl	18.5	10.6	4.0	64.4
10g	trimethylacetyl	2-fluorophenyl	9.8	7.9	3.0	26.1
10h	trimethylacetyl	3-fluorophenyl	17.8	7.2	3.1	16.1
10i	trimethylacetyl	4-fluorophenyl	13.8	7.3	4.6	14.3
10o	trimethylacetyl	3,4,5-trifluorophenyl	7.3	7.6	2.0	7.5
10p	trimethylacetyl	2,3,4,5,6-pentafluorophenyl	14.9	21.1	2.4	13.7
10q	trimethylacetyl	thiophen-3-yl	26.4	21.5	2.4	ND
MIPS1776	2-cyclopentylacetyl	3,4,5-trifluorophenyl	5.5	4.4	0.72	7.8
MIPS1777	2-cyclohexylacetyl	3,4,5-trifluorophenyl	6.5	7.3	0.74	7.4
MIPS1778	<i>tert</i> -butylacetyl	3,4,5-trifluorophenyl	14.7	7.5	0.65	6.7

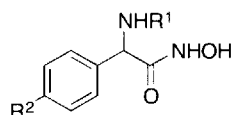
ND = no detectable activity

[0064] Tests for preliminary cytotoxicity in HEK293 cells did not result in any observable cytotoxicity up to 40 μM. Assessments of physicochemical and metabolism

data for both compounds indicate that MIPS1770 and MIPS1778 both display good PK properties.

[0065] Table 4 records selected activities against purified Pf aminopeptidases (K_i) and *P. falciparum* (IC₅₀).

TABLE 4:

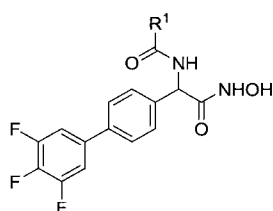


MIPS	R ¹	R ²	K _i (nM)		
			<i>rPfA</i> -M1	<i>rPfA</i> -M17	<i>Pf</i> IC ₅₀ [nM]
1776	2-cyclopentylacetyl	3,4,5-trifluorophenyl	147	43	163.0 ± 24.2
1777	2-cyclohexylacetyl	3,4,5-trifluorophenyl	102	64	552.6 ± 38.1
1778	tert-butylacetyl	3,4,5-trifluorophenyl	107	12.2	11.3 ± 0.1

Other Active Species

[0066] Other hydroxamic acid-based compounds which have been identified as particularly promising are listed in Table 5, listed with their activity as an APN inhibitor and activity against a malarial target:

TABLE 5



MIPS	R ¹	K _i ^(app) nM	
		APN	PfA-M1
1992		348.2 ± 33.2	257.2 ± 13.1
1993		187.6 ± 8.9	299.3 ± 19.8
1994		172.2 ± 9.2	263.0 ± 14.1
1995		497.2 ± 35.1	326.3 ± 20.7
1996		33.0 ± 3.7	495.2 ± 49.7
1997		71.2 ± 6.5	349.6 ± 31.6
1998	CH ₃	559.5 ± 50.4	1283 ± 118.9
2330		16.5 ± 1.8	350.4 ± 38.0
2331		31.6 ± 3.1	398.2 ± 37.7
2332		20.9 ± 0.9	450.5 ± 34.6
2333		11.0 ± 1.2	189.9 ± 17.4

EXPERIMENTAL RESULTS

CHEMISTRY

General Experimental

[0067] Chemicals and solvents were purchased from standard suppliers and used without further purification. Davisil silica gel (40-63 μm), for column chromatography was supplied by Grace Davison Discovery Sciences (Victoria, Australia), and deuterated

solvents were purchased from Cambridge Isotope Laboratories, Inc. (United States, distributed by Novachem PTY. Ltd., Victoria, Australia).

[0068] Unless otherwise stated, reactions were carried out at ambient temperature. Reactions are monitored by thin-layer chromatography on commercially available aluminium-backed plates (Merck Kieselgel 60 F₂₅₄). Visualisation was by examination under UV light (254 nm). General staining was varied out with ninhydrin solution in ethanol to visualise primary amines. A solution of Fe(III)Cl₃ (5% in 0.5 M HCl(aq)) was used to visualise hydroxamic acids. Organic solvents were evaporated *in vacuo* at ≤ 40°C.

[0069] ¹H NMR, ¹⁹F NMR and ¹³C NMR spectra were recorded on a Bruker Avance Nanobay III 400 MHz Ultrashield Plus spectrometer at 400.13, 376.46 and 106.6 MHz, respectively. Chemical shifts (δ) are recorded in parts per million with reference to the chemical shift of the deuterated solvent. Coupling constants (*J*) and carbon-fluorine coupling constants (*J*_{CF}) are recorded in hertz and the significant multiplicities are described as singlet (s), doublet (d), triplet (t), multiplet (m), doublet of doublets (dd), doublet of triplets (dt), and doublet of doublets of doublets (ddd). Spectra were assigned using appropriate DEPT, HSQC, and HMBC spectra.

[0070] LC-MS was run to verify the reaction outcome and purity using Agilent 6120 series single-quadrupole instrument coupled to an Agilent 1260 series HPLC instrument. The following buffers were used: buffer A, 0.1% formic acid in H₂O; buffer B, 0.1% formic acid in MeCN. The following gradient was used with a Poroshell 120 EC-C18 50 × 3.0 mm 2.7 μm column, a flow rate of 0.5 mL/min, and a total run time of 5 min: 0-1 min 95% buffer A and 5% buffer B, from 1-2.5 min up to 0% buffer A and 100% buffer B, held at this composition until 3.8 min, 3.8-4 min 95% buffer A and 5% buffer B. held until 5 min at this composition. Mass spectra were obtained in positive and negative ion modes with a scan range of 100-1000 *m/z*. UV detection was carried out at 214 and 254 nm.

[0071] High Resolution Mass Spectra were obtained by using Agilent 6224 TOF LC-MS Mass spectrometer coupled to an Agilent 1290 Infinity. All data were acquired and referenced via dual-sprayed electrospray ionisation source. Chromatographic separation was performed with Agilent Zorbax SB-C18 Rapid Resolution HT 2.1 × 50 nm, 1.8 μm

column using buffer A: 0.1% formic acid in H₂O and buffer B: 0.1% formic acid in MeCN. The gradient of buffer B changed from 5% to 100 % over 3.5 min with a flowrate of 0.5 mL/min.

[0072] Analytical HPLC was performed to assess purity of final compounds by using an Agilent 1260 Infinity Analytical HPLC with Zorbac Eclipse Plus C-18 Rapid Resolution 4.6 × 100 mm, 3.5 µm column. Buffer A: 0.1 % TFA in H₂O and buffer B: 0.1 % TFA in MeCN were used. Samples were run at gradient of 5% buffer B to 100% buffer B over 10 min at a flow rate of 1 mL/min.

[0073] Analogues of phenylglycine derivatives 2 and 3 were synthesized in which the pyrazole moiety was replaced with a variety of aromatic rings. The hydroxamic acid analogues were prepared by a facile four-step synthesis shown in FIG. 3. In this way, 2-amino-2-(4-bromophenyl)acetic acid (4b) or 2-amino-3-(4-bromophenyl)propanoic acid (4d) were efficiently elaborated to provide a number of derivatives in good overall yields.

[0074] Access to the target derivatives of 2, bearing pyrazole ring-replacements was achieved from commercially available 4b (FIG. 3). *N*-Boc protection of 4b was achieved in high yield following a procedure based on the protection of the homologous phenylalanine. Subsequent conversion to the desired hydroxamic acid derivative 9b proceeded smoothly in good yield using the previously described CDI-mediated activation method. (Mistry, S. N. et al. *Journal of Medicinal Chemistry* 2014, 57, 9168-83)

[0075] The remaining aryl derivatives were synthesized by conversion of 4b to the corresponding α-amino ester using previously described esterification conditions. (Mistry, S. N. et al. *Journal of Medicinal Chemistry* 2014, 57, 9168-83)

[0076] The crude product then underwent *N*-Boc protection to give 5b in quantitative yield over two steps. Parallel Suzuki coupling of 5b with a number of aromatic boronic acid or boronate ester partners, in the presence of PdCl₂(PPh₃)₂ at 100°C in degassed 1M Na₂CO₃ (aq)/THF readily afforded the desired 4-arylphenyl compounds 7a-p. Final conversion to the target hydroxamic acids 9f-u was successful.

[0077] The N-pivaloyl analogues were important target compounds that possess the tert-butyl “anchor” optimal for binding to the S1' pocket. Most reactions used for the synthesis of the N-Boc derivatives were similarly applied for the preparation of the N-pivaloyl analogues. 4b was esterified and subsequently reacted with Et₃N and pivaloyl chloride in DCM at room temperature to afford compound 6b in excellent yield (94%) over 2 steps. The Suzuki reaction was used to incorporate an additional aromatic ring, to give compounds 8a-q. Conversion of the methyl esters to the corresponding hydroxamic acids proceeded smoothly, affording the final compounds 10f-v in excellent yields. The transformation of the nitrile moieties to amidoxime groups, which was previously observed for the N-Boc series, was again observed for the corresponding members of the N-pivaloyl series (analogues 10s and 10t).

[0078] This synthetic strategy was also easily adapted to investigate the effect of inserting an extra methylene unit between the α -carbon (to the hydroxamic acid moiety) and phenyl ring. This homologous scaffold was anticipated to improve compound stability, by reducing the acidity of the α -proton, whilst enabling us to probe space in the S1 pockets of PfA-M1 and PfA-M17. Using 4d as the starting material, the established procedures for esterification, attachment of the N-Boc/pivaloyl group, Suzuki coupling and aminolysis were applied. In this study, the 4-fluoro (9c, 10c), 4-bromo (9d, 10d), 4-iodo (9e, 10e), 4-phenyl (9t, 10u) and 1-methylpyrazol-4-yl (9u, 10v) derivatives were synthesized for direct comparison to their more truncated counterpart.

[0079] The production of recombinant malaria neutral aminopeptidases PfA-M1 and PfA-M17 was undertaken in *Escherichia coli* as previously described. (McGowan, S. et al *Proceedings of the National Academy of Sciences of the United States of America* **2009**, 106, 2537-2542; McGowan, S. et al *Proceedings of the National Academy of Sciences of the United States of America* **2010**, 107, 2449-2454.)

[0080] Aminopeptidase assays were based on previously published protocols. (Stack, C. M. et al; *Journal of Biological Chemistry* **2007**, 282, 2069-2080.

PfA-M1 and PfA-M17 inhibitor-bound crystal structures

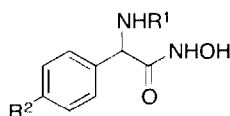
[0081] Nine *PfA*-M1 co-crystal structures (compounds 9b, 9f, 9q, 9m, 9r, 10b, 10o, 10q, 10s) and six *PfA*-M17 co-crystal structures (compounds 9b, 10b, 10o, 10q, 10r, 10s) were developed. Data were collected at 100 K using synchrotron radiation at the Australian Synchrotron using the macro crystallography MX1 beamline 3BM131 for *PfA*-M1 and the micro crystallography MX2 beamline 3ID1 for *PfA*-M17. The coordinates and structure factors will be available from the Protein Data Bank with PDB Accession codes, *PfA*-M1: 9b (4ZW3), 9f (4ZW5), 9q (4ZW6), 9m (4ZW7), 9r (4ZW8), 10b (4ZX3), 10o (4ZX4), 10q (4ZX5), 10s (4ZX6), and *PfA*-M17: 9b (4ZX8), 10b (4ZX9), 10o (4ZY2), 10q (4ZY0), 10r (4ZY1), 10s (4ZYQ).

[0082] In all structures clear electron density for the bound compounds was observed in the active sites. For *PfA*-M17, which has two copies of the hexamer in the asymmetric unit, the compound binding modes are largely conserved in all twelve active sites. Therefore, when describing *PfA*-M17 structures, it is intended to refer to Chain I, which has the clearest electron density.

PfA-M1 and PfA-M17 display different enantiomeric selectivities

[0083] Firstly, the effect of replacing the pyrazole ring of 2 and 3 with simple halogen substituted phenyl groups (9a, 9b and 10a, 10b) was investigated. **Table 6** lists relevant values for inhibition of *PfA*-M1 and *PfA*-M17 by hydroxamic acid compounds 9a-b, f-s and 10a-b, f-t.

TABLE 6:



No.	R ¹	R ²	K _i (μM)	
			<i>PfA</i> -M1	<i>PfA</i> -M17
2 ¹⁷	Boc	pyrazole	0.8	0.030
3 ¹⁷	C(O) ^t Bu	pyrazole	0.7	0.028
9a	Boc	F	5.3	1.7
9b	Boc	Br	0.027	0.079
9f	Boc	phenyl	37	0.055
9g	Boc	2-fluorophenyl	0.95	0.040
9h	Boc	3-fluorophenyl	0.95	0.13
9i	Boc	4-fluorophenyl	100	0.072
9j	Boc	2-(trifluoromethyl)phenyl	0.75	0.12
9k	Boc	3-(trifluoromethyl)phenyl	3.0	0.14
9l	Boc	4-(trifluoromethyl)phenyl	>500	0.47
9m	Boc	3,4,5-trifluorophenyl	19	0.20
9n	Boc	3-pyridyl	1.1	0.22
9o	Boc	4-pyridyl	0.91	0.54
9p	Boc	thiophen-3-yl	20	0.063
9q	Boc	1-methylpyraz-4-yl	1.9	0.056
9r	Boc	3-(amidoximo)phenyl	1.1	0.13
9s	Boc	4-(amidoximo)phenyl	0.40	0.018
10a	C(O) ^t Bu	F	3.8	0.028
10b	C(O) ^t Bu	Br	0.065	0.041
10f	C(O) ^t Bu	phenyl	1.6	0.007
10g	C(O) ^t Bu	2-fluorophenyl	1.6	0.011
10h	C(O) ^t Bu	3-fluorophenyl	0.53	0.004
10i	C(O) ^t Bu	4-fluorophenyl	6.0	0.033
10j	C(O) ^t Bu	2,4-difluorophenyl	2.5	0.005
10k	C(O) ^t Bu	2,6-difluorophenyl	4.8	0.011
10l	C(O) ^t Bu	3,4-difluorophenyl	1.4	0.006
10m	C(O) ^t Bu	3,5-difluorophenyl	0.40	0.089
10n	C(O) ^t Bu	2,4,6-trifluorophenyl	5.0	0.002
10o	C(O) ^t Bu	3,4,5-trifluorophenyl	0.076	0.060
10p	C(O) ^t Bu	2,3,4,5,6-pentafluorophenyl	1.6	0.008
10q	C(O) ^t Bu	thiophen-3-yl	0.64	0.009
10r	C(O) ^t Bu	1-methylpyrazol-4-yl	10	0.006
10s	C(O) ^t Bu	3-(amidoximo)phenyl	3.4	0.010
10t	C(O) ^t Bu	4-(amidoximo)phenyl	4.0	0.008

[0084] Irrespective of the S1' anchor used, the bromo-substituted compounds 9b and 10b demonstrated the highest *PfA*-M1 and *PfA*-M17 inhibitory activity (for *PfA*-M1, the K_i of 9b = 27 nM and 10b = 65 nM; for *PfA*-M17 the K_i of 9b = 79 nM and 10b = 41 nM, Table 5). Placement of a phenyl ring in the same position resulted in 9f and 10f. While

both **9f** and **10f** demonstrated good *PfA*-M17 inhibitory activity (K_i values of 55 nM and 6.5 nM respectively), only moderate *PfA*-M1 inhibition was observed (K_i values of 36.5 μ M and 1.6 μ M respectively, Table 5).

[0085] To inform elaboration of the series, the crystal structures of both *PfA*-M1 and *PfA*-M17 were solved with **9b** (**9b**:*PfA*-M1 at 2.0Å and **9b**:*PfA*-M17 at 2.7Å) and **10b** (**10b**:*PfA*-M1 at 2.0Å and **10b**:*PfA*-M17 at 2.6Å). Although a racemic mixture of both compounds was used for the crystallization experiments, the crystal structures show that both *PfA*-M1 and *PfA*-M17 demonstrate enantiomeric selectivity. Electron density in the active site of *PfA*-M1 demonstrates that only **S-9b** is bound in the crystal, whereas **R-10b** is preferentially bound. In contrast, only the *R*-enantiomer of both **9b** and **10b** could be fit to electron density in the *PfA*-M17 binding pocket.

[0086] Compounds **9b** and **10b** bear different linkers to the *tert*-butyl moieties, a carbamate on **9b** and an amide on **10b**, that alters the disposition of substituents in the *PfA*-M1 S1' cavity. The carbamate of **9b** forms three hydrogen bonds to the main chain of Gly460 and Ala461, which places the *tert*-butyl group in a position to form hydrophobic interactions with Val493 at the end of the pocket. Selection of the *R*-enantiomer of **10b** allows the equivalent *tert*-butyl group to occupy a similar position in the *PfA*-M1 pocket despite the shorter linker. Comparison of these *PfA*-M1 structures to **9b**:*PfA*-M17 and **10b**:*PfA*-M17 structures suggest that the different enantiomeric selectivity of the enzymes is due to spatial differences in the S1' cavities.

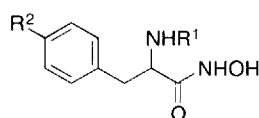
[0087] *PfA*-M1 has an enclosed S1' pocket, while the S1' cavity of *PfA*-M17 is exposed to solvent and is both larger and shallower than the *PfA*-M1 pocket. While the interaction between the *tert*-butyl substituents and Val493 in the *PfA*-M1 cavity is vital, the *tert*-butyl groups of neither **9b** nor **10b** form direct interactions with *PfA*-M17. Instead the linkers themselves dominate the protein-inhibitor interactions and bind similarly in both **9b**:*PfA*-M17 and **10b**:*PfA*-M17; the carbamate of **9b** forms water mediated hydrogen bonds with the main chain of Gly489 and Ala490, while the carbonyl of **10b** forms a direct hydrogen bond with the main chain amine of Gly489. Unfortunately, the different enantiomeric preference exhibited by *PfA*-M1 and *PfA*-M17 make it difficult to directly compare inhibitory activities between the *N*-Boc and *N*-pivaloyl series. As a

result, comparisons herein have been largely restricted to compounds within the same series.

[0088] Despite the different poses adopted by **9b** and **10b** within the S1' pockets, the position of the hydroxamic acid and bromophenyl moieties are conserved. Compounds **9b** and **10b** form identical zinc binding interactions with *PfA*-M1, and the bromophenyl positions overlay closely. Similarly, in *PfA*-M17, the hydroxamic acid and bromophenyl moieties of both **9b** and **10b** make identical active site interactions. In both *PfA*-M1 and *PfA*-M17, the hydroxamic acid of **10b** forms a dense network of metallo- and hydrogen bonding interactions with the zinc ion/s and surrounding residues, and additionally to the catalytic carbonate ion in *PfA*-M17. The bromophenyl moiety sits within the hydrophobic S1 pocket of both enzymes. In **10b**:*PfA*-M1, the bromine is ideally placed to interact with the aromatic ring of Tyr575, while the phenyl ring is aligned with the side chain of Val459. Similarly, in **10b**:*PfA*-M17, the bromophenyl interacts through hydrophobic interactions with Met396, Phe398 and Met392.

[0089] While the bromo-substituted compounds **9b** and **10b** represent some of the most potent dual *PfA*-M1 and *PfA*-M17 inhibitors to date, they provide little opportunity to build further into the S1 pocket. In an effort to probe further into the S1 pocket, an extra methylene was incorporated between the phenyl ring and the α -proton. However, regardless of the substituent incorporated (halogen, phenyl or 1-methylpyrazol-4-yl), homologues containing an extra methylene linker generally demonstrated poor inhibition. **Table 7** records values for inhibition of *PfA*-M1 and *PfA*-M17 by hydroxamic acid compounds 9c-e, t-u and 10c-e, u-v.

TABLE 7:



No.	R ¹	R ²	K _i (μM)	
			<i>PfA</i> -M1	<i>PfA</i> -M17
9c	Boc	F	4.3	20
9d	Boc	Br	38	7.7
9e	Boc	I	120	14
9t	Boc	phenyl	430	5.2
9u	Boc	1-methylpyrazol-4-yl	120	14
10c	C(O) ^t Bu	F	62	0.037
10d	C(O) ^t Bu	Br	87	0.42
10e	C(O) ^t Bu	I	15	0.016
10u	C(O) ^t Bu	phenyl	27	0.64
10v	C(O) ^t Bu	1-methylpyrazol-4-yl	39	0.74

[0090] Compounds bearing the N-Boc moiety were poor inhibitors of both *PfA*-M1 (K_i values of 4 – 430 μM, Table 3) and *PfA*-M17 (K_i values of 5 – 14 μM, Table 3), while the *N*-acyl series were reasonable *PfA*-M17 inhibitors (K_i values of 20 – 740 nM, Table 6) but poor *PfA*-M1 inhibitors (K_i values of 15 – 90 μM, Table 5). As a result, the compounds of this type were not pursued further.

[0091] Since the incorporation of an extra methylene linker did not offer improvements in potency, an attempt was made to determine whether the bromo- substituent could be replaced with a phenyl ring that, through further decoration, would allow access deep within the S1 pocket. While the bi-phenyl compounds **9f** and **10f**, were excellent inhibitors of *PfA*-M17 (*PfA*-M17 K_i of **9f** = 55 nM and **10f** = 6.5 nM, Table 2), they demonstrated substantially less activity against *PfA*-M1 than their corresponding bromophenyl analogues **9b** and **10b** (*PfA*-M1 K_i of **9f** = 36.5 μM and **10f** = 1.6 μM, Table 2).

[0092] To elucidate any structural reason for this loss of activity, the structure of **9f** in complex with *PfA*-M1 (2.1Å) was also determined. The **9f**:*PfA*-M1 structure showed that the biphenyl dihedral angle is approximately 60° in **9f**. In an energetically ideal state, biphenyl systems rapidly convert between two chiral conformations with dihedral angles of approximately 45°. Therefore, restricting the biphenyl system of **9f** to a non-ideal 60°

dihedral angle likely contributes to the reduced inhibitory activity of **9f**. Regardless, the observed conformation of the biphenyl places the second aromatic ring in a position to interact via edge-face π -stacking with Tyr575, carbonyl- π interactions with the main chain oxygen of Glu319, and hydrophobic contacts with Met1034.

[0093] Further, insight into the reduced activity of the biphenyl-substituted **9f** and **10f** is gained by comparison of the S1 pocket structure of **9f**:PfA-M1, **9b**:PfA-M1, and unliganded PfA-M1. In **9b**:PfA-M1 and unliganded PfA-M1, Glu572 sits on an active site helix, and the side chain has no set position. However, in **9f**:PfA-M1, the added phenyl ring of **9f** presses against Glu572, which occupies a position away from the pocket. To adopt this position, the main chain of Glu572 has undergone a substantial movement, which has shifted a single turn of the active site helix it lies on by $>1\text{\AA}$. Although aminopeptidases, including PfA-M1, are known to be capable of substantial active site flexibility, such main chain movements are likely to come at an energetic cost, which would account for the loss in PfA-M1 inhibitory activity of **9f** compared to **9b**. Despite the kinetic liability of the biphenyl group to both the **9f** and **10f**, its incorporation allows access to the hydrophilic end of the pocket, which is occupied by ordered water molecules in **9f**:PfA-M1, and which has not previously been accessible to the hydroxamic acid-based compound series.

Different S1' anchors result in different SAR

[0094] Substituting the α -carbon with a biphenyl system has allowed us access deeper into the S1 pocket of PfA-M1 and PfA-M17. Therefore, a variety of substituted-phenyl and heteroaromatic rings were used to probe the region. Despite having different S1' anchors, compound pairs 2 and 3, and 9b and 10b each demonstrate very similar PfA-M1 and PfA-M17 inhibitory activities. However, this trend is not conserved for the remainder of the 9f-s and 10f-t investigated here. Table 2 shows the binding affinities of the hydroxamic acid analogues to both PfA-M1 and PfA-M17, which show that in general, the *N*-Boc analogues are less active than their corresponding *N*-pivaloyl compounds. Although the *N*-Boc series 9f-s demonstrates reduced inhibitory activities, the series has allowed us to extract some notable SAR.

[0095] In the *N*-Boc 9f-s series, fluoro- and trifluoromethyl- substituents were tolerated in both the 2- and 3- positions of the phenyl ring (9g, 9h, 9j and 9k). When placed at the 4-position (9i, 9l), a substantial loss in binding to *PfA*-M1 was observed. In contrast, substitution at all of the positions was well tolerated by *PfA*-M17. Isosteric replacement of the phenyl ring with pyridyl (9n and 9o) and thiophenyl rings (9p) was also investigated. Both 3- and 4-pyridyl analogues 9n and 9o demonstrated reasonable inhibitory activity for *PfA*-M1, but lost activity against *PfA*-M17. The thiophenyl analogue 9p and the phenyl analogue 9f have similar inhibition activities.

[0096] The more polar substituents 9q-9s were able to maintain inhibitory activity against both enzymes, however even the most potent of these, 9s, which bears an amidoxime moiety in the 4-position of the phenyl ring, has not regained the activity of the bromophenyl substituted compound 9b (9s has K_i values of 400 nM for *PfA*-M1 and 18 nM for *PfA*-M17; 9b has K_i values of 27 nM for *PfA*-M1 and 79 nM for *PfA*-M17). Generally, replacement of the pyrazole ring of compound 2 did not improve binding to *PfA*-M17, and only the bromo analogue 9b demonstrated notable improvement for *PfA*-M1 binding. With the aim of finding a binding feature of the *N*-Boc series that could be exploited to improve the activity of the series, the crystal structures of *PfA*-M1 in complex with 9m, 9q and 9r was determined. However, the structures gave no added insight into the reason for the reduced activity of this series, and the series was therefore discontinued.

Phenyl substituted *N*-pivaloyl series probes new region of *PfA*-M1 and *PfA*-M17 S1 pocket

[0097] Similarly to the *N*-Boc series 9f-s, a variety of substituted-phenyl and heteroaromatic rings were used to probe the S1 pocket of *PfA*-M1 and *PfA*-M17 with the *N*-pivaloyl series 10f-t. Fluoro-substitution of the phenyl ring at the 2- (10g), 3- (10h) or 4- (10i) positions alone had little effect on inhibition of either *PfA*-M1 or *PfA*-M17. This trend is translated to the di-fluorinated compounds (10j-10m) that exhibited only minor differences in *PfA*-M1 and *PfA*-M17 inhibitory activity. However, while the 2,4,6-trifluorophenyl substituted 10n was a poor *PfA*-M1 inhibitor (K_i = 4.95 μ M) and an excellent *PfA*-M17 inhibitor (K_i = 2 nM), the 3,4,5-trifluorophenyl substituted 10o is a potent, nanomolar inhibitor of both *PfA*-M1 (K_i = 76 nM) and *PfA*-M17 (K_i = 60 nM).

Finally, compounds 10r-10t, which are substituted with more polar groups, potentially inhibited *PfA*-M17, but were less active against *PfA*-M1.

[0098] Overall, compound 10o represents the most exciting lead, having regained the potent, dual inhibition activity of the bromo-substituted compound 10b. Therefore, the crystal structures of 10o were determined in complex with *PfA*-M1 (1.9Å) and *PfA*-M17 (2.1Å). Since 10q also demonstrates reasonable dual inhibition, and represents a substantially different chemotype to 10o, the crystal structures of 10q:*PfA*-M1 (1.95Å) and 10q:*PfA*-M17 (2.2Å) were also solved.

[0099] When bound to *PfA*-M1, the position of the biaryl of 10o compares with that of 9f, despite the different S1' anchors. The moiety therefore makes the same interactions with Tyr575 (edge-face π -stacking), Met1034 (hydrophobic) and Glu319 (carbonyl- π) (Figure 3a). The fluoro-substituents sit deeper into the S1 pocket than any other hydroxamic-acid based inhibitor. In fact, the only other *PfA*-M1 inhibitor that has probed this region is the organophosphorus aminopeptidase inhibitor, Co4. Although the fluoro groups of 10o did not displace any of the ordered water molecules as anticipated from the 9f:*PfA*-M1 structure, they have entered into an intricate network of water-mediated hydrogen-bonds, in which the fluorine atoms act as acceptors. These additional interactions are likely to account for the improved *PfA*-M1 inhibitory activity of 10o over 10f.

[0100] The structure of 10q:*PfA*-M1 showed that 10q adopts a different binding pose compared to other inhibitors of the series, aligned to the opposite face of the S1 pocket. Whereas the biaryl dihedral angle of 9p and 10f is approximately 60°, the thiophene ring of 10q sits co-planar to adjacent phenyl ring. This allows the thiophene ring to maintain the π -stacking interactions with Tyr575, albeit in a face-face configuration, rather than edge-face. Interactions are also observed between the thiophene ring and Met1034 and the main chain carbonyl of Glu572.

[0101] The presence of two different *PfA*-M1 binding poses for 10f/10o and 10q demonstrate that there remains additional room for elaboration in the S1 pocket. Compounds 10s and 10t possess larger amidoxime moieties at the 3- and 4-positions of the phenyl ring, and while neither compound displayed potent *PfA*-M1 inhibition, the

crystal structure of 10s:*PfA*-M1 was determined to establish how the protein accommodates the larger amidoxime group. While the data showed clear electron density in the binding pocket, modeling of the compound in a single conformation could not satisfy the density. Therefore, the compound was modeled in two different conformations, that are comparable to the two different conformations adopted by 10f/10o and 10q.

[0102] Conformation A resembles the pose adopted by 10q, with the phenyl ring undergoing face-face π -stacking with Tyr575. In this position, the added hydroxamic acid substituent pushes against Glu572, pushing it further out of the pocket, and causing disorder of the loop 570-574. The amidoxime itself makes hydrogen bonds with the main chain oxygen of Glu572, and a water-mediated hydrogen bond with the Tyr575 carbonyl oxygen. In conformation B, the distal ring has rotated approximately 90°, which changes the geometry of the π -interaction with Tyr575 to an edge-face configuration, similar to that undergone by 10f and 10o. Conformation B also places the amidoxime substituent on the opposite side of the pocket compared to conformation A, where it now forms hydrogen bonds with Gln317 and Asn458 (both direct and water-mediated). Despite the favorable hydrogen bonding interactions observed in both conformations, it is clear that the induced conformational changes within the binding pocket come at an energetic cost, thereby accounting for the relatively weak inhibition of *PfA*-M1 ($K_i = 3.4 \mu\text{M}$).

[0103] The crystal structures of the same three compounds, 10o, 10q, and 10s bound to *PfA*-M17 were also determined (2.1Å, 2.2Å and 2.6Å respectively). In contrast to the varying poses adopted when bound to *PfA*-M1, all three compounds bind similarly to *PfA*-M17, in poses comparable to 10b:*PfA*-M17. In all of the *PfA*-M17 structures, the R-enantiomer is preferentially bound, with the first phenyl ring of 10b, 10o, 10q, and 10s placed in the same position. This allows the second rings (3,4,5-trifluorophenyl in 10o, 3-(amidoximo)phenyl in 10s, and thiophene in 10q) to extend deep into the pocket. The different sizes of the substituents is accommodated for in *PfA*-M17 by adjustments in the position of the Met392 side-chain, which flexes in and out of the pocket depending on the potential for interactions (with 10b, 10o and 10q) or repulsion (10s). The phenyl rings themselves sit against the hydrophobic side of the pocket lined by Met396, Phe398, Leu395. In order to determine how the more polar compound 10r is capable of potent *PfA*-M17 inhibition, the crystal structure of 10r:*PfA*-M17 (2.5Å) was also determined. The

structure showed that 10r binds similarly to 10o, 10q, and 10s, and provided no additional information with which to elaborate.

[0104] Compound 10o is one of the most potent, dual inhibitors of *PfA*-M1 and *PfA*-M17 described. In particular, 10o is potent against the purified *Pf* aminopeptidases in in vitro protease assays. Importantly it exhibits activity when tested against malaria parasites or human cells. Based on the K_i data, another potentially potent dual inhibitor is 10b.

[0105] Its ability to potently inhibit both enzymes is due to the dual nature of the 3,4,5-trifluorophenyl substituent. While the fluorine atoms of 10o interact with *PfA*-M1 through a dense hydrogen-bonding network, they interact with *PfA*-M17 strictly through hydrophobic interactions with Leu492, Phe583, and Met392.

[0106] Since the crystal structures of *PfA*-M1 and *PfA*-M17 indicate that the enzymes have a preference for the R-enantiomer of the *N*-pivaloyl series, the enantiomers of 10o were separated by semi-preparative chiral HPLC. K_i determination showed that both *PfA*-M1 and *PfA*-M17 do in fact have a clear preference for the R-enantiomer (for *PfA*-M1, the K_i of R-10o = 120 nM and S-10o = 28 μ M; for *PfA*-M17, the K_i of R-10o = 7nM and S-10o = 31.4 μ M). Finally, to confirm that the correct enantiomer identity had been assigned following separation, both enantiomers were separately soaked into *PfA*-M1 and solved the crystal structures. Electron density in the active site of R-10o:*PfA*-M1 was consistent with the structure of 10o:*PfA*-M1 in which the R-enantiomer was modelled, whereas no electron density indicative of compound binding was observed in the S-10o:*PfA*-M1 crystal structure. It was therefore confirmed that the enantiomeric preference exhibited by *PfA*-M1 and *PfA*-M17 within crystals also occurs in solution.

Dual *PfA*-M1 and *PfA*-M17 inhibitors are active against multi-drug resistant *Pf*

[0107] To determine the effect of our dual *PfA*-M1/*PfA*-M17 inhibitors on *Pf* in culture, an image-based assay was used to measure the growth inhibition on *Pf* strain 3D7 for selected compounds in the series. **Table 8** records the growth inhibition results for selected hydroxamic acid compounds.

TABLE 8:

	IC₅₀ ± SEM (nM) <i>Pf</i> 3D7	IC₅₀ ± SEM (nM) Dd2 Parent	IC₅₀ ± SEM (nM) Dd2 SpiroR
2 ¹⁷	783 ± 87		
3 ¹⁷	227 ± 4		
9b	293 ± 10		
9g	633 ± 24		
9q	978 ± 106		
9r	679 ± 33		
9s	334 ± 31		
10a	1530 ± 60	2010 ± 20	2090 ± 50
10b	169 ± 17	194 ± 14	163 ± 45
10f	95.7 ± 16.7	190 ± 12	219 ± 71
10g	131 ± 5	207 ± 10	210 ± 25
10h	162 ± 4	226 ± 10	316 ± 21
10i	126 ± 4	225 ± 29	193 ± 7
10j	109 ± 2	164 ± 46	216 ± 39
10k	142 ± 17	251 ± 35	246 ± 34
10l	139 ± 0.1	170 ± 52	195 ± 17
10m	144 ± 0.0	239 ± 34	232 ± 43
10n	125 ± 13	267 ± 47	450 ± 38
10o	126 ± 2	189 ± 23	107 ± 20
10p	130 ± 38	461 ± 42	408 ± 52
10q	103 ± 3	110 ± 7	100 ± 5
10s	469 ± 28	819 ± 48	627 ± 2
10t	249 ± 23	303 ± 35	384 ± 8

[0108] Lead compounds 2 and 3 were previously reported to inhibit Pf-3D7 with IC₅₀ values of 783 nM and 227 nM respectively. (Mistry, S. N. et al; *Journal of Medicinal Chemistry* **2014**, 57, 9168-83.)

[0109] The N-pivaloyl series demonstrates superior activity against *Pf*-3D7 growth compared to the *N*-Boc series. Without wishing to be bound by theory it is thought that this may be the result of better cellular penetration and stability. Significantly, within the *N*-pivaloyl series, incorporation of a biaryl system generally led to improved inhibition of *Pf* growth compared to the parent compound 3. The exceptions to this trend were compounds 10s and 10t, in which the amidoxime moieties likely interfere with cellular penetration. All of the fluorophenyl-substituted compounds performed well, inhibiting *Pf*-3D7 growth in the range of 95 – 160 nM. The potent dual inhibitor, 10o, demonstrated an IC₅₀ of 125.6 nM, while the best cellular inhibitors of the series included: 10f, bearing the unsubstituted biphenyl system (IC₅₀ = 95.7 nM), the 2,4-difluorophenyl substituted 10j (IC₅₀ = 108.5 nM), and 10q, which is substituted with the thiophen-3-yl moiety (IC₅₀ = 103.4 nM).

[0110] Given the potent inhibition of *Pf*-3D7 growth, it was also useful to determine how the compounds performed in other strains, including drug-resistant strains. Therefore, tests were carried out to gauge the effect of compound treatment on malarial strains Dd2 (chloroquine-, quinine-, pyrimethamine- and sulfadoxine-resistant) and NITD609-RDd2 clone#2 (abbreviated here on as Dd2 SpiroR), which is resistant to the same parent drugs as Dd2, but additionally resistant to spiroindolones, aminopyrazoles, dihydroisoquinolones and pyrazolamindes) (Table 4).

[0111] Again, the compounds demonstrated potent inhibition of parasite growth. The most potent inhibitors of *Pf*-3D7 growth also demonstrated potent activity against Dd2 Parent and Dd2 SpiroR, particularly 10o (IC₅₀ Dd2 Parent = 188.5 nM, IC₅₀ Dd2 SpiroR = 107.2 nM), and 10q (IC₅₀ Dd2 Parent = 109.6 nM, IC₅₀ Dd2 SpiroR = 99.9 nM). Finally, the compounds were also against human mammalian cell line HEK293, to predict whether it is likely to encounter human toxicity with the compound series. No toxicity to HEK293 cells was observed when treated with each of the compounds listed in Table 3 up to a 40 µM concentration over a period of 72 hours.

Synthesis Details for MIPS1778, MIPS1992 to 1998, MIPS2330 to 2333

[0112] Synthesis of MIPS1700 and several other compounds referred to above have been published in Drinkwater, N., Vinh, NB., Mistry, SN., Bamert, RS., Ruggeri, C.,

Holleran, JP., Loganathan, S., Paiardini, A. Charman, SA., Powell, AK., Avery, VM., McGowan, S. & Scammells, PJ. "Potent dual inhibitors of Plasmodium falciparum M1 and M17 aminopeptidases through optimization of S1 pocket interactions." (2016) Eur. J. Med. Chem. **110**, 43-64. Synthesis of MIPS1778, MIPS1992 to 1998, MIPS2330 to 2333 are outlined below.

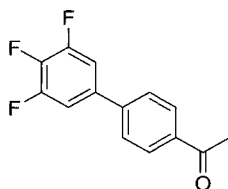
General Procedure A: Amide coupling using HCTU

[0113] The carboxylic acid (1.1 eq.) and HCTU (1.2-2.4 eq.) were dissolved in DMF (2 mL/mmol) and stirred for 30 min in a nitrogen flushed microwave vial. DIPEA (2.1-4.2 eq.) was added dropwise followed by methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (1.0 eq.) in DCM (2 mL/mmol). The reaction mixture was stirred at room temperature for 1-2 days. After completion, the reaction mixture was diluted with sat. NaHCO_3 (10 mL) and extracted with DCM (3 $\times$ 15 mL). The combined organic layers were washed with water (2 $\times$ 10 mL) and brine (15 mL). The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude was purified by column chromatography using either DCM/MeOH or PE/EtOAc as the eluent.

General Procedure B: Direct aminolysis of methyl ester to the hydroxamic acid

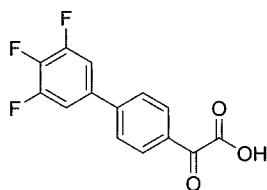
[0114] $\text{NH}_2\text{OH} \cdot \text{HCl}$ (4-8 eq.) was dispersed in anhydrous MeOH (1 mL) and sonicated for 10 s. 5M KOH/MeOH (5-10 eq.) was added and the mixture was stirred for 5 min. The methyl ester (1 eq.) was dissolved in anhydrous MeOH (3 mL/1 mmol) and added to the hydroxylamine mixture. The reaction was stirred at room temperature for 1-7 days which was monitored by LCMS and TLC with Fe(III)Cl_3 stain. Once the reaction was complete, the suspension was dry-loaded onto Isolute and purified by column chromatography (eluent DCM/MeOH 100:0 to 90:10 or DCM/MeOH/AcOH 99:0:1 to 90:9:1 for more polar compounds).

1-(3',4',5'-Trifluoro-[1,1'-biphenyl]-4-yl)ethan-1-one (11)

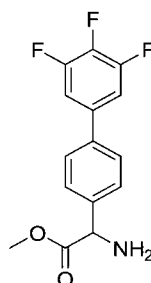


[0115] To a nitrogen flushed 500 mL round bottom flask was added 4'-iodoacetophenone (5.00 g, 20.3 mmol), 3,4,5-trifluorophenylboronic acid (4.65 g, 26.4 mmol), THF (150 mL) and 1 M Na₂CO₃ (50 mL). PdCl₂(PPh₃)₂ (428 mg, 0.610 mmol) was added and the mixture was heated at reflux for 2 h. The reaction mixture was concentrated under reduced pressure and extracted with Et₂O (3 × 50 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude product was purified using column chromatography (eluent PE/EtOAc 100:0 to 50:50) to afford 4.59 g (90%) of **11** as a yellow-brown solid; ¹H NMR (CDCl₃) δ 8.04 (d, *J* = 8.6 Hz, 2H), 7.60 (d, *J* = 8.6 Hz, 2H), 7.24 (dd, *J* = 8.7/6.4 Hz, 2H), 2.64 (s, 3H); ¹⁹F NMR (CDCl₃) δ -133.4 (d, *J* = 20.5 Hz), -160.9 (dd, *J* = 20.5 Hz); ¹³C NMR (CDCl₃) δ 197.6, 151.7 (ddd, *J*_{CF} = 250.4/10.1/4.3 Hz), 142.8–42.5 (m), 140.0 (dt, *J*_{CF} = 255.1/16.1 Hz), 136.9, 136.3–136.0 (m), 129.3, 127.2, 111.9–110.7 (m), 26.9; *m/z* MS (TOF ES⁺) C₁₄H₁₀F₃O [MH]⁺ calcd 251.1, found 251.0; LC-MS *t*_R = 3.5.

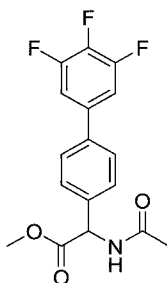
2-Oxo-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetic acid (**12**)



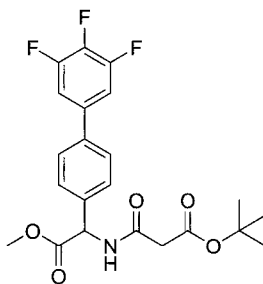
[0116] 1-(3',4',5'-Trifluoro-[1,1'-biphenyl]-4-yl)ethan-1-one (**11**) (4.59 g, 18.3 mmol) and SeO₂ (3.05 g, 27.5 mmol) were dissolved in anhydrous pyridine (130 mL). The mixture was sonicated for 30 sec and then heated at 110°C overnight under nitrogen. Once the reaction was complete, the mixture was filtered through CeliteTM and the filtrate was concentrated *in vacuo*. 1 M HCl (20 mL) was added and the compound was extracted with EtOAc (3 × 80 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to give 4.25 g (83%) of 2-Oxo-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetic acid (**12**) as a brown solid; ¹H NMR (*d*₆-DMSO) δ 8.05–7.93 (m, 4H), 7.84 (dd, *J* = 9.5/6.7 Hz, 2H). Exchangeable proton was not observed; ¹⁹F NMR (*d*₆-DMSO) δ -134.4 (d, *J* = 21.7 Hz), -161.3 (dd, *J* = 21.7 Hz); ¹³C NMR (*d*₆-DMSO) δ 188.0, 165.8, 150.7 (ddd, *J*_{CF} = 247.2/9.8/4.1 Hz), 142.9–142.8 (m), 139.1 (dt, *J*_{CF} = 248.4/14.2 Hz), 135.8–134.5 (m), 131.6, 130.2, 127.7, 116.5–105.3 (m); *m/z* MS (TOF ES⁻) C₁₄H₆F₃O₃ [M-H]⁻ calcd 279.0, found 279.0; LC-MS *t*_R = 3.3.

Methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (13)

[0117] 2-Oxo-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetic acid (**12**) (4.25 g, 15.2 mmol) and benzylamine (3.30 mL, 30.3 mmol) were dissolved in anhydrous DCE (150 mL) and stirred for 30 min. Na(OAc)₃BH (6.40 g, 30.4 mmol) was added and the mixture was stirred at room temperature overnight. Once the reaction was complete, water (30 mL) was added and the mixture was stirred for 5 min. DCE was removed *in vacuo* and the compound was extracted with EtOAc (3 × 50 mL). A precipitate formed in the organic layer, which was filtered to give 4.50 g of 2-(benzylamino)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetic acid as a yellow solid. The crude solid was dissolved in MeOH (120 mL) and conc. H₂SO₄ (2.58 mL, 48.5 mmol) was added dropwise. The reaction mixture was heated at reflux for 16 h and then concentrated under reduced pressure. Sat. NaHCO₃ was added (until a pH 8 was reached) and the mixture was extracted with Et₂O (3 × 150 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated under reduced pressure to afford 4.52 g of methyl 2-(benzylamino)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate. The crude ester was dissolved in MeOH (120 mL) before addition of 10% Pd/C (100 mg) and conc. HCl (1 mL). The mixture was stirred under hydrogen at room temperature overnight. Once complete, the reaction mixture was filtered through CeliteTM and washed with MeOH (20 mL). The filtrate was concentrated *in vacuo*. The crude product was purified using column chromatography (eluent PE/EtOAc 50:50 to 0:100). LCMS showed a trace amount of impurity, so the crude was further purified by trituration in Et₂O. The solid was filtered to give 2.50 g (72% over 3 steps) of methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**). ¹H NMR (CDCl₃) δ 7.47 (app. s, 4H), 7.20–7.10 (m, 2H), 4.67 (s, 1H), 3.72 (s, 3H), 2.30 (s, 2H); ¹⁹F NMR (CDCl₃) δ -134.0 (d, *J* = 20.5 Hz), -162.4 (dd, *J* = 20.6 Hz); ¹³C NMR (CDCl₃) δ 174.3, 151.5 (ddd, *J*_{CF} = 249.6/10.0/4.3 Hz), 140.5, 140.9–137.9 (m), 138.1–137.9 (m), 136.8 (td, *J*_{CF} = 7.8/4.7 Hz), 127.7, 127.3, 111.3–110.9 (m), 58.4, 52.6; *m/z* MS (TOF ES⁺) C₁₅H₁₃F₃NO₂ [MH]⁺ calcd 296.1, found 296.1; LC-MS *t*_R = 3.0.

Methyl 2-acetamido-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14a)

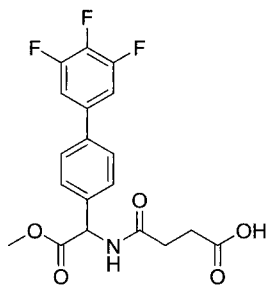
[0118] To a nitrogen flushed microwave vial was added compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (300 mg, 1.01 mmol) in anhydrous toluene (4 mL). Meldrum's acid (161 mg, 1.12 mmol) was added and the mixture was refluxed for 3 hr. After completion, the reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed several times with Et₂O to afford 172 mg (50%) of compound methyl 2-acetamido-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14a**) as a white solid. ¹H NMR (*d*₆-DMSO) δ 8.80 (d, *J* = 7.3 Hz, 1H), 7.89–7.64 (m, 4H), 7.48 (d, *J* = 8.3 Hz, 2H), 5.48 (d, *J* = 7.3 Hz, 1H), 3.63 (s, 3H), 1.91 (s, 3H); ¹⁹F NMR (*d*₆-DMSO) δ -134.8 (d, *J* = 21.8 Hz), -163.3 (dd, *J* = 21.7 Hz); ¹³C NMR (*d*₆-DMSO) δ 171.0, 169.3, 150.6 (ddd, *J* = 246.6/9.7/4.2 Hz), 138.4 (dt, *J* = 249.5/15.7 Hz), 136.9–136.8 (m), 136.7, 136.3 (td, *J* = 8.1/4.5 Hz), 128.4, 127.2, 117.8–108.1 (m), 55.8, 52.3, 22.2; *m/z* MS (TOF ES⁺) C₁₇H₁₅F₃NO₃ [MH]⁺ calcd 338.1, found 338.1; LC-MS *t*_R = 3.3.

tert-Butyl 3-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-3-oxopropanoate (14b)

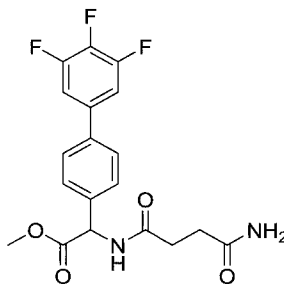
[0119] 3-(*tert*-Butoxy)-3-oxopropanoic acid (198 mg, 1.24 mmol) was coupled to compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (330 mg, 1.12 mmol) according to General Procedure A. The crude product was purified using

column chromatography (eluent PE/EtOAc 0:100 to 50:50) to afford 185 mg (38%) of *tert*-Butyl 3-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-3-oxopropanoate (**14b**) as an orange oil. ^1H NMR (d_6 -DMSO) δ 9.00 (d, J = 7.3 Hz, 1H), 7.80–7.67 (m, 4H), 7.50 (d, J = 8.3 Hz, 2H), 5.52 (d, J = 7.3 Hz, 1H), 3.65 (s, 3H), 3.31–3.21 (m, 2H), 1.39 (s, 9H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, J = 21.7 Hz), -163.3 (dd, J = 21.7 Hz); ^{13}C NMR (d_6 -DMSO) δ 170.6, 166.9, 165.5, 150.6 (ddd, J_{CF} = 246.6/9.8/4.2 Hz), 138.4 (dt, J_{CF} = 249.7/15.6 Hz), 136.9–136.8 (m), 136.6, 136.3 (m), 128.3, 127.2, 111.7–111.0 (m), 80.6, 55.8, 52.4, 43.2, 27.7; m/z MS (TOF ES $^-$) $\text{C}_{22}\text{H}_{21}\text{F}_3\text{NO}_5$ [$\text{M}-\text{H}$] $^-$ calcd 436.1, found 436.1; LC-MS t_{R} = 3.6.

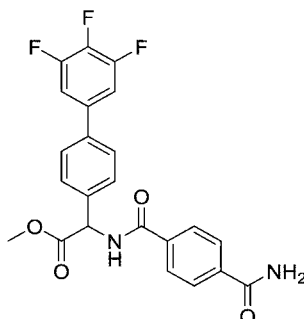
4-((2-Methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid (14c**)**



[0120] To a nitrogen flushed 50 mL round bottom flask was added compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (428 mg, 1.45 mmol) in anhydrous toluene (10 mL). Succinic anhydride (160 mg, 1.60 mmol) was added and the mixture was refluxed for 3 h. After completion, the reaction mixture was cooled to room temperature and the resulting precipitate was filtered and washed several times with Et₂O to afford 267 mg (49%) of 4-((2-Methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid (**14c**) as a white solid. The crude product was used without further purification. ^1H NMR (d_6 -DMSO) δ 12.11 (s, 1H), 8.83 (d, J = 7.3 Hz, 1H), 7.80–7.65 (m, 4H), 7.49 (d, J = 8.3 Hz, 2H), 5.49 (d, J = 7.3 Hz, 1H), 3.63 (s, 3H), 2.46–2.39 (m, 4H); ^{19}F NMR (d_6 -DMSO) δ -134.82 (d, J = 21.8 Hz), -163.24 (dd, J = 21.8 Hz); ^{13}C NMR (d_6 -DMSO) δ 173.7, 171.2, 171.0, 150.6 (ddd, J_{CF} = 246.4/9.6/4.0 Hz), 138.4 (m), 137.0 (t, J_{CF} = 2.1 Hz), 136.8, 136.3 (m), 128.4, 127.1, 111.5–111.2 (m), 55.8, 52.3, 29.6, 28.9; m/z MS (TOF ES $^+$) $\text{C}_{19}\text{H}_{17}\text{F}_3\text{NO}_5$ [MH] $^+$ calcd 396.1, found 396.1; LC-MS t_{R} = 3.3.

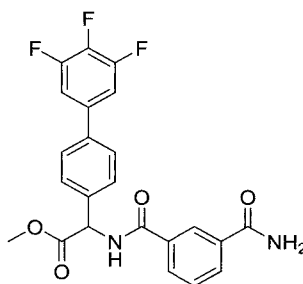
Methyl 2-(4-amino-4-oxobutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14d)

[0121] 4-((2-Methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid (**14c**) (272 mg, 0.688 mmol) and PyBOP (531 mg, 1.02 mmol) in DMF (10 mL) were stirred for 10 min in a dry 50 mL round bottom flask. DIPEA (0.2 mL, 1.02 mmol) and ammonium carbonate (332 mg, 3.45 mmol) were added to the mixture which was stirred overnight at room temperature. After completion, the mixture was diluted with water (10 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were washed with sat. NaHCO₃ (2 × 30 mL) and brine (30 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (PE/EtOAc 50:50 to 0:100) to afford 84 mg (31%) of methyl 2-(4-amino-4-oxobutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14d**) as a white solid. ¹H NMR (*d*₆-DMSO) δ 8.81 (d, *J* = 7.3 Hz, 1H), 7.87–7.60 (m, 4H), 7.49 (d, *J* = 8.3 Hz, 2H), 7.29 (s, 1H), 6.75 (s, 1H), 5.49 (d, *J* = 7.3 Hz, 1H), 3.63 (s, 3H), 2.47–2.25 (m, 4H); ¹⁹F NMR (*d*₆-DMSO) δ -134.8 (d, *J* = 21.8 Hz), -163.3 (dd, *J* = 21.8 Hz); ¹³C NMR (*d*₆-DMSO) δ 173.4, 171.7, 171.0, 150.6 (ddd, *J*_{CF} = 246.5/9.7/4.2 Hz), 138.4 (dt, *J*_{CF} = 249.6/15.7 Hz), 136.8 (2C, determined by HSQC and HMBC), 136.34 (td, *J*_{CF} = 8.2/4.2 Hz), 128.4, 127.2, 118.4–102.5 (m), 55.8, 52.3, 30.2, 30.1; *m/z* MS (TOF ES⁺) C₁₉H₁₈F₃N₂O₄ [MH]⁺ calcd 396.1, found 396.1; LC-MS *t*_R = 3.2.

Methyl 2-(4-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14e)

[0122] 4-Carbamoylbenzoic acid (246 mg, 1.49 mmol) was coupled to compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) using General Procedure A. The crude product was purified using column chromatography (PE/EtOAc 50:50 to 0:100) to afford 383 mg (64%) of methyl 2-(4-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14e**) as a light yellow solid. ^1H NMR (d_6 -DMSO) δ 9.38 (d, J = 7.2 Hz, 1H), 8.10 (s, 1H), 8.02–7.91 (m, 4H), 7.84–7.68 (m, 4H), 7.60 (d, J = 8.3 Hz, 2H), 7.52 (s, 1H), 5.77 (d, J = 7.1 Hz, 1H), 3.68 (s, 3H); ^{19}F NMR (d_6 -DMSO) δ -134.8 (d, J = 21.8 Hz), -163.2 (dd, J = 21.7 Hz); ^{13}C NMR (d_6 -DMSO) δ 170.8, 167.2, 165.9, 150.6 (ddd, J_{CF} = 246.4/9.5/4.1 Hz), 138.4 (dt, J_{CF} = 249.8/15.7 Hz), 136.9, 136.4 (td, J_{CF} = 8.2/5.5 Hz), 135.8–135.7 (m), 133.4, 132.2, 129.0, 127.7, 127.4, 127.1, 111.6–111.1 (m), 56.5, 52.5; m/z MS (TOF ES $^+$) $\text{C}_{23}\text{H}_{18}\text{F}_3\text{N}_2\text{O}_4$ $[\text{MH}]^+$ calcd 443.1, found 443.1; LC-MS t_{R} = 3.4.

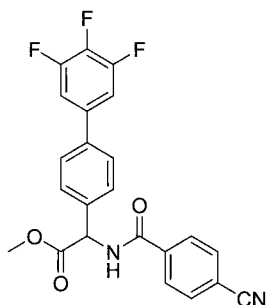
Methyl 2-(3-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14f)



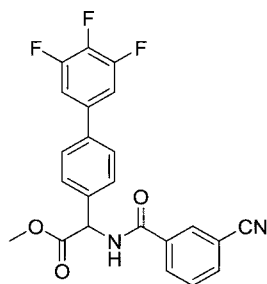
[0123] 3-Carbamoylbenzoic acid (205 mg, 1.24 mmol) was coupled to compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) using General Procedure A. The crude product was purified using column chromatography (PE/EtOAc 50:50 to 0:100) to afford 328 mg (66 %) of methyl 2-(3-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14f**) as a light yellow solid; ^1H NMR (d_6 -DMSO) δ 9.37 (d, J = 7.1 Hz, 1H), 8.42 (t, J = 1.6 Hz, 1H), 8.07 (s, 1H), 8.06–7.99 (m, 2H), 7.78–7.69 (m, 4H), 7.61 (d, J = 8.4 Hz, 2H), 7.56 (t, J = 7.8 Hz, 1H), 7.50 (s, J = 34.1 Hz, 1H), 5.78 (d, J = 7.1 Hz, 1H), 3.69 (s, 3H); ^{19}F NMR (d_6 -DMSO) δ -134.8 (d, J = 21.7 Hz), -163.3 (dd, J = 21.7 Hz); ^{13}C NMR (d_6 -DMSO) δ 170.9, 167.5, 166.2, 150.7 (ddd, J_{CF} = 246.6/9.7/4.1 Hz), 138.4 (dt, J_{CF} = 249.5/15.6 Hz), 136.9–136.8 (m), 136.7, 136.4 (td, J_{CF} = 8.1/4.4 Hz), 134.5, 133.6, 130.5, 130.4, 129.0, 128.4, 127.1, 126.9, 112.9–109.9 (m),

56.6, 52.5; m/z MS (TOF ES⁺) C₂₃H₁₈F₃N₂O₄ [MH]⁺ calcd 443.1, found 443.1; LC-MS t_R = 3.3.

Methyl 2-(4-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14g)

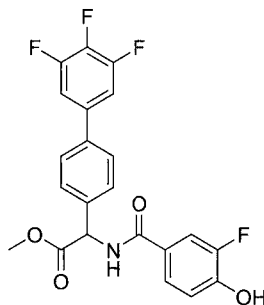


[0124] To a nitrogen flushed 50 mL round bottom flask was added 4-carbamoylbenzoic acid (167 mg, 1.01 mmol) in DCM (10 mL) and a catalytic amount of DMF (20 μ L). Oxalyl chloride (130 μ L, 1.51 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. DCM was concentrated *in vacuo* and a mixture of DIPEA (126 μ L, 1.31 mmol) and methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (300 mg, 1.01 mmol) in DCM (10 mL) were added. The mixture was stirred at room temperature for 30 min. The mixture was then diluted with water (15 mL) and extracted with DCM (3 $\times$ 10 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent PE/EtOAc 100:0 to 0:100) to afford 185 mg (43%) of methyl 2-(4-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14g**) as a yellow oil. ¹H NMR (CDCl₃) δ 7.98–7.73 (m, 4H), 7.54–7.45 (m, 4H), 7.34 (app. t, J = 6.6 Hz, 1H), 7.19–7.11 (m, 2H), 5.79 (d, J = 6.6 Hz, 1H), 3.81 (s, 3H); ¹⁹F NMR (CDCl₃) δ -133.7 (d, J = 20.5 Hz), -161.9 (dd, J = 20.5 Hz); ¹³C NMR (CDCl₃) δ 171.1, 164.9, 151.6 (ddd, J_{CF} = 250.0/10.1/4.3 Hz), 139.6 (dt, J_{CF} = 252.5/15.3 Hz), 139.0–138.8 (m), 137.4, 136.8–136.3 (m, 2C determined by HSQC and HMBC), 132.7, 128.2, 128.0, 127.7, 118.0, 115.8, 111.9–110.6 (m), 56.7, 53.5; m/z MS (TOF ES⁺) C₂₃H₁₆F₃N₂O₃ [MH]⁺ calcd 425.1, found 425.1; LC-MS t_R = 3.6.

Methyl 2-(3-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14h)

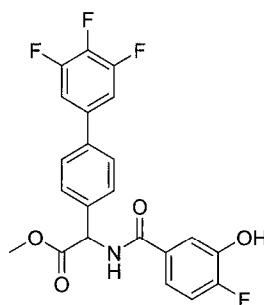
[0125] To a nitrogen flushed 50 mL round bottom flask was added 3-carbamoylbenzoic acid (167 mg, 1.01 mmol) in DCM (10 mL) and a catalytic amount of DMF (20 μ L). Oxalyl chloride (130 μ L, 1.51 mmol) was added dropwise and the mixture was stirred at room temperature for 1 h. DCM was concentrated *in vacuo* and a mixture of DIPEA (126 μ L, 1.31 mmol) and compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (300 mg, 1.01 mmol) in DCM (10 mL) were added. The mixture was stirred at room temperature for 30 min. The mixture was then diluted with water (15 mL) and extracted with DCM (3 $\times$ 10 mL). The combined organic layers were dried over Na₂SO₄, filtered and concentrated *in vacuo*. The crude product was purified by column chromatography (eluent PE/EtOAc 100:0 to 0:100) to afford 383 mg (89%) of methyl 2-(3-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14h**) as a clear oil. ¹H NMR (CDCl₃) δ 8.12 (s, 1H), 8.06 (d, *J* = 7.9 Hz, 1H), 7.80 (d, *J* = 7.8 Hz, 1H), 7.58 (t, *J* = 7.7 Hz, 1H), 7.54–7.48 (m, 4H), 7.44 (d, *J* = 6.7 Hz, 1H), 7.19–7.10 (m, 2H), 5.79 (d, *J* = 6.7 Hz, 1H), 3.81 (s, 3H); ¹⁹F NMR (CDCl₃) δ -133.7 (d, *J* = 20.6 Hz), -161.9 (dd, *J* = 20.6 Hz); ¹³C NMR (CDCl₃) δ 171.1, 164.7, 151.6 (ddd, *J*_{CF} = 250.0/10.1/4.2 Hz), 139.6 (dt, *J*_{CF} = 252.4/15.4 Hz), 138.9–138.7 (m), 136.6–136.5 (m), 136.4, 135.3, 134.7, 131.5, 131.2, 129.8, 128.2, 127.7, 117.9, 113.2, 112.0–110.2 (m), 56.8, 53.4; *m/z* MS (TOF ES⁺) C₂₃H₁₄F₃N₂O₃ [M-H]⁺ calcd 423.1, found 423.1; LC-MS *t*_R = 3.6.

Methyl- 2-(3-fluoro-4-hydroxybenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14i)



[0126] 3-Fluoro-4-hydroxybenzoic acid (194 mg, 1.24 mmol) was coupled to compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (330 mg, 1.12 mmol) using General Procedure A. After completion the reaction mixture was diluted with 1 M HCl (10 mL) and extracted with DCM (3 × 15 mL). The combined organic layers were washed with water (2 × 20 mL) and brine (20 mL). The organic layer was dried over Na₂SO₄, filtered and concentrated under reduced pressure. The crude product was purified by column chromatography (eluent DCM/MeOH 100:0 to 95:5) to afford 430 mg (89%) of a mixture of the desired product **14i** (64%), 2-fluoro-4-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)carbamoyl)phenyl 3-fluoro-4-hydroxybenzoate (23%) and 5-chloro-1*H*-benzo[d][1,2,3]triazol-1-ol (13%). The crude product was used without further purification. *m/z* MS (TOF ES⁻) C₂₂H₁₄F₄NO₄ [M-H]⁻ calcd 432.1, found 432.1; LC-MS *t*_R = 3.5.

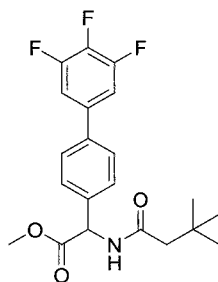
Methyl 2-(4-fluoro-3-hydroxybenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14j)



[0127] 4-Fluoro-3-hydroxybenzoic acid (233 mg, 1.49 mmol), was coupled to compound methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (400 mg, 1.35 mmol) according to General Procedure A. After completion the reaction mixture

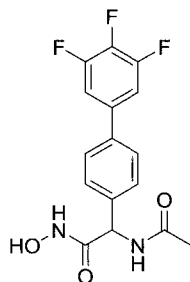
was diluted with 1 M HCl (10 mL) and extracted with DCM (3×15 mL). The combined organic layers were washed with water (2×20 mL) and brine (20 mL). The organic layer was dried over Na_2SO_4 , filtered and concentrated under reduced pressure. The crude was purified by column chromatography (eluent DCM/MeOH 98:2 to 90:10) to afford 577 mg (98%) of white solid. ^1H NMR showed the compound obtained was a mixture of the desired product methyl 2-(4-fluoro-3-hydroxybenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14j**) (53%) and 2-fluoro-4-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)carbonyl)phenyl 4-fluoro-3-hydroxybenzoate (31%) with 5-chloro-1*H*-benzo[d][1,2,3]triazol-1-ol (16%). This crude was used without further purification. m/z MS (TOF ES^-) $\text{C}_{22}\text{H}_{14}\text{F}_4\text{NO}_4$ $[\text{M}-\text{H}]^-$ calcd 432.1, found 432.1; LC-MS $t_{\text{R}} = 3.5$.

Methyl 2-(3,3-dimethylbutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (14k)



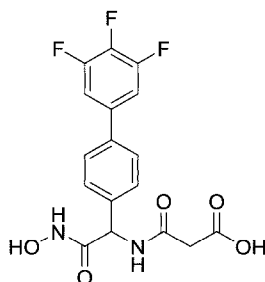
[0128] To a solution of compound Methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**13**) (149 mg, 0.50 mmol) in DCM (5 mL) was added triethylamine (153 μL , 1.1 mmol) followed by 3,3-dimethylbutyryl chloride (77 μL , 0.56 mmol). The reaction mixture was stirred at room temperature for 2 h. Water was added and the aqueous layer was extracted with DCM (3 times). The combined organic layers were dried over Na_2SO_4 , filtered and concentrated. The crude product was purified using FCC (DCM) to give methyl 2-(3,3-dimethylbutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14k**) (110 mg, 55%) as a white solid. ^1H NMR δ 7.50–7.41 (m, 4H), 7.20–7.10 (m, 2H), 6.47 (d, $J = 6.7$ Hz, 1H), 5.62 (d, $J = 6.9$ Hz, 1H), 3.75 (s, 3H), 2.13 (s, 2H), 1.03 (s, 9H); ^{19}F NMR δ -133.9 (d, $J = 20.6$ Hz), -162.2 (dd, $J = 20.6$ Hz); ^{13}C NMR δ 171.4, 171.2, 151.6 (ddd, $J_{\text{CF}} = 249.8/10.0/4.3$ Hz), 139.5 (dt, $J_{\text{CF}} = 252.3/15.3$ Hz), 138.66–138.33 (m), 137.2, 137.0–136.0 (m), 128.2, 127.5, 111.4–111.0 (m), 56.1, 53.1, 50.3, 31.2, 29.9; m/z MS (TOF ES^+) $\text{C}_{21}\text{H}_{23}\text{F}_3\text{NO}_3$ $[\text{MH}]^+$ calcd 394.2, found 394.2; LC-MS $t_{\text{R}} = 4.0$ min.

2-Acetamido-*N*-hydroxy-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetamide (MIPS1998)



[0129] Methyl 2-acetamido-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14a**) (101 mg, 0.300 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. The crude product was purified by column chromatography (eluent DCM/MeOH/AcOH 99:0:1 to 90:9:1) to give 64 mg (65%) of **MIPS1998** as a pink solid. ^1H NMR (d_6 -DMSO) δ 11.02 (s, 1H), 9.01 (s, 1H), 8.71 (d, J = 8.4 Hz, 1H), 7.70 (dd, J = 12.5/6.0 Hz, 4H), 7.49 (d, J = 8.3 Hz, 2H), 5.41 (d, J = 8.4 Hz, 1H), 1.91 (s, 3H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, J = 21.8 Hz), -163.5 (dd, J = 21.8 Hz); ^{13}C NMR (d_6 -DMSO) δ 169.1, 166.5, 150.7 (ddd, J_{CF} = 13.6/9.8/4.3 Hz), 139.4, 134.0–136.8 (m), 136.7–136.4 (m), 136.3–135.9 (m), 127.7, 126.8, 119.6–101.7 (m), 53.5, 22.4; m/z MS (TOF ES $^-$) $\text{C}_{16}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_3$ $[\text{M}-\text{H}]^-$ calcd 337.1, found 337.1; m/z HRMS (TOF ES $^+$) $\text{C}_{16}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_3$ $[\text{MH}]^+$ calcd 339.0951, found 339.0953; LC-MS t_{R} = 3.2; HPLC t_{R} = 5.8, > 95%.

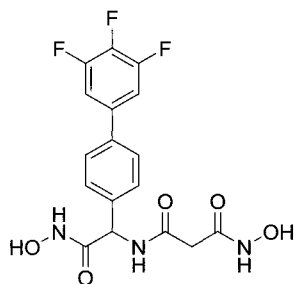
3-((2-(Hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-3-oxopropanoic acid (MIPS1993)



[0130] tert-Butyl 3-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-3-oxopropanoate (**14b**) (185 mg, 0.485 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. After 1 d, only partial

conversion had occurred and therefore $\text{NH}_2\text{OH}\cdot\text{HCl}$ (135 mg, 1.94 mmol) and 5 M KOH/MeOH (0.486 mL, 2.43 mmol) were added. The reaction mixture was stirred for a further 24 h. LC-MS indicated that tert-Butyl 3-((2-methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4I)ethyl)amino)-3-oxopropanoate was converted to the desired product **MIPS1993** and the dihydroxamic acid N^1 -hydroxy- N^3 -(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)malonamide (**MIPS1992**). These two compounds were isolated by column chromatography (eluent DCM/MeOH/AcOH 99:0:1 to 90:9:1). The desired hydroxamic acid was treated with 20% TFA/DCM (5 mL) and stirred overnight. The reaction mixture was concentrated *in vacuo* and extracted with EtOAc (3×5 mL). The organic layer was dried over Na_2SO_4 , filtered and concentrated *in vacuo*. The crude product was purified using column chromatography (eluent DCM/MeOH/AcOH 99:0:1 to 90:9:1) to afford 10 mg (6% over 2 steps) of compound **MIPS1993** as a light brown oil. ^1H NMR (d_6 -DMSO) δ 11.28 (s, 1H), 9.29 (d, $J = 8.2$ Hz, 1H), 7.86–7.58 (m, 4H), 7.49 (d, $J = 8.3$ Hz, 2H), 5.41 (d, $J = 8.2$ Hz, 1H), 3.26–3.06 (m, 2H). Two exchangeable protons were not observed; ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, $J = 21.7$ Hz), -163.5 (dd, $J = 21.8$ Hz); ^{13}C NMR (d_6 -DMSO) δ 170.2, 166.9, 166.3, 150.6 (ddd, $J_{\text{CF}} = 246.5/9.7/4.1$ Hz), 139.3, 138.3 (dt, $J_{\text{CF}} = 251.1/16.7$ Hz), 136.6 (td, $J_{\text{CF}} = 8.1/4.5$ Hz), 136.2–136.1 (m), 127.6, 126.8, 113.1–108.6 (m), 53.7, 43.3; m/z MS (TOF ES^-) $\text{C}_{17}\text{H}_{12}\text{F}_3\text{N}_2\text{O}_5$ $[\text{M}-\text{H}]^-$ calcd 381.1, found 381.0; m/z HRMS (TOF ES^+) $\text{C}_{17}\text{H}_{14}\text{F}_3\text{N}_2\text{O}_5$ $[\text{MH}]^+$ calcd 383.0849, found 383.0857; LC-MS $t_{\text{R}} = 3.2$; HPLC $t_{\text{R}} = 5.4$, > 95%.

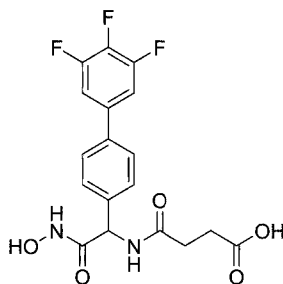
N^1 -hydroxy- N^3 -(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)malonamide (MIPS1992)



[0131] ^1H NMR (d_6 -DMSO) δ 10.64 (s, 2H), 9.08 (s, 1H), 8.94 (s, 1H), 8.84 (d, $J = 8.2$ Hz, 1H), 7.81–7.58 (m, 4H), 7.49 (d, $J = 8.3$ Hz, 2H), 5.39 (d, $J = 8.2$ Hz, 1H), 3.07 (s, 2H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, $J = 21.7$ Hz), -163.5 (dd, $J = 21.7$ Hz); ^{13}C NMR (d_6 -DMSO) δ 166.1, 165.9, 163.8, 150.6 (ddd, $J = 246.5, 9.7, 4.4$ Hz), 139.4, 138.3 (dt, J

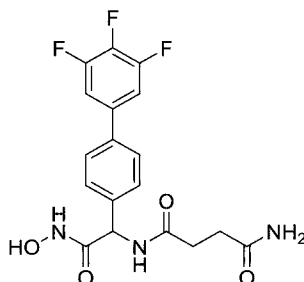
= 249.8/19.6 Hz), 136.6 (td, $J = 8.2/4.5$ Hz), 136.2–136.0 (m), 127.5, 126.8, 111.8–109.8 (m), 53.8, 40.34; m/z MS (TOF ES⁻) C₁₇H₁₃F₃N₃O₅ [M-H]⁻ calcd 396.1, found 396.0; m/z HRMS (TOF ES⁺) C₁₇H₁₅F₃N₃O₅ [MH]⁺ calcd 398.0958, found 398.0954; LC-MS $t_R = 3.1$; HPLC $t_R = 5.3$, > 95%.

4-((2-(Hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid (MIPS1994)



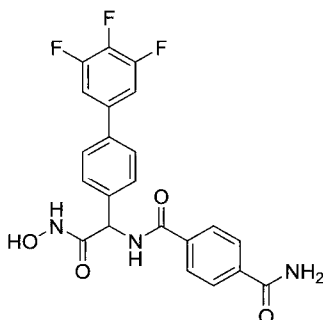
[0132] 4-((2-Methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid (**14c**) (267 mg, 0.675 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. After 1 d, only partial conversion had occurred, therefore NH₂OH.HCl (188 mg, 2.71 mmol) was added and heated at 40 °C for 2 h. The reaction progressed slowly and therefore the temperature was increased to 50 °C and stirred overnight. LC-MS showed degradation of **14c** to methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate. The reaction was stopped and methyl 2-amino-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate, 4-((2-Methoxy-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)amino)-4-oxobutanoic acid, and **MIPS1994** were isolated by column chromatography (eluent DCM/MeOH/AcOH 99:0:1 to 95:4:1). 15 mg (6%) of **MIPS1994** was obtained as a white solid. ¹H NMR (*d*₆-DMSO) δ 11.95 (s, 1H), 11.01 (s, 1H), 9.04 (s, 1H), 8.72 (d, $J = 8.3$ Hz, 1H), 8.08–7.61 (m, 4H), 7.49 (d, $J = 8.3$ Hz, 2H), 5.41 (d, $J = 8.3$ Hz, 1H), 2.49–2.02 (m, 4H); ¹⁹F NMR (*d*₆-DMSO) δ -134.9 (d, $J = 21.8$ Hz), -163.5 (dd, $J = 21.8$ Hz); ¹³C NMR (*d*₆-DMSO) δ 174.1, 171.1, 166.5, 150.7 (ddd, $J_{CF} = 246.4/9.7/4.1$ Hz), 139.4, 138.4 (dt, $J_{CF} = 248.9/15.1$ Hz), 136.6 (td, $J_{CF} = 8.0/4.3$ Hz), 136.3–136.0 (m), 127.7, 126.8, 111.5–111.0 (m), 53.6, 29.9, 29.2; m/z MS (TOF ES⁻) C₁₈H₁₄F₃N₂O₅ [M-H]⁻ calcd 395.1, found 395.0; m/z HRMS (TOF ES⁺) C₁₈H₁₆F₃N₂O₅ [MH]⁺ calcd 397.1006, found 397.1018; LC-MS $t_R = 3.2$; HPLC $t_R = 5.6$, 90%.

***N'*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)succinamide (MIPS1995)**



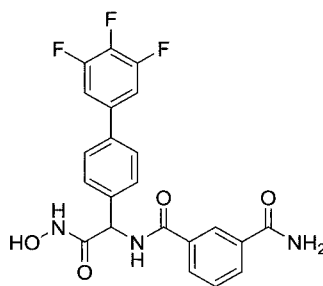
[0133] Methyl 2-(4-amino-4-oxobutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14d**) (84 mg, 0.213 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. The crude product was purified by column chromatography (eluent DCM/MeOH/AcOH 95:4:1 to 90:9:1) to give 43 mg (52%) of **MIPS1995** as a white solid. ^1H NMR (d_6 -DMSO) δ 11.00 (s, 1H), 9.01 (s, 1H), 8.66 (d, J = 8.3 Hz, 1H), 7.79–7.62 (m, 4H), 7.49 (d, J = 8.4 Hz, 2H), 7.27 (s, 1H), 6.74 (s, 1H), 5.41 (d, J = 8.3 Hz, 1H), 2.43 (t, J = 7.9 Hz, 2H), 2.28 (t, J = 7.2 Hz, 2H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, J = 21.7 Hz), -163.5 (dd, J = 21.8 Hz); ^{13}C NMR (d_6 -DMSO) δ 173.5, 171.4, 166.4, 150.6 (ddd, J_{CF} = 246.4/9.8/4.3 Hz), 139.4, 138.3 (dt, J_{CF} = 258.4/15.8 Hz), 136.6 (td, J_{CF} = 8.1/4.2 Hz), 136.2–135.9 (m), 127.7, 126.8, 115.9–102.5 (m), 53.6, 30.4, 30.3; m/z MS (TOF ES $^-$) $\text{C}_{18}\text{H}_{15}\text{F}_3\text{N}_3\text{O}_4$ [$\text{M}-\text{H}$] $^-$ calcd 394.1, found 394.1; m/z HRMS (TOF ES $^+$) $\text{C}_{18}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_4$ [MH] $^+$ calcd 396.1166, found 396.1173; LC-MS t_{R} = 3.2; HPLC t_{R} = 5.5, > 95%.

***N*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)terephthalamide (MIPS2330)**



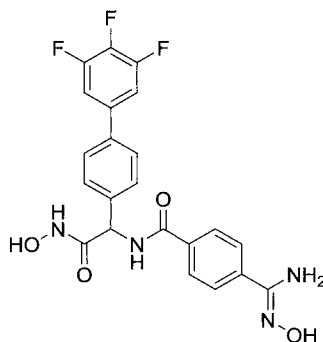
[0134] Methyl 2-(4-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14e**) (383 mg, 0.865 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. The crude product was purified by column chromatography (eluent DCM/MeOH 100:0 to 90:10) to afford 38 mg (10%) of **MIPS2330** as a white solid. ^1H NMR (d_6 -DMSO) δ 11.08 (s, 1H), 9.11 (d, J = 8.1 Hz, 1H), 9.07 (s, 1H), 8.09 (s, 1H), 8.01–7.89 (m, J = 10.7/5.3 Hz, 4H), 7.78–7.58 (m, 6H), 7.50 (s, 1H), 5.67 (d, J = 8.1 Hz, 1H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, J = 21.8 Hz), -163.5 (dd, J = 21.7 Hz); ^{13}C NMR (d_6 -DMSO) δ 167.2, 166.4, 165.8, 150.6 (ddd, J_{CF} = 246.5/9.9/4.3 Hz), 138.7, 138.2 (dt, J_{CF} = 262.9/14.7 Hz), 136.7, 136.5 (dt, J_{CF} = 6.4/5.2 Hz), 136.4–136.2 (m), 136.1, 128.1, 127.8, 127.3, 126.8, 114.4–104.6 (m), 54.4; m/z MS (TOF ES $^-$) $\text{C}_{22}\text{H}_{15}\text{F}_3\text{N}_3\text{O}_4$ $[\text{M}-\text{H}]^-$ calcd 442.1, found 442.0; m/z HRMS (TOF ES $^+$) $\text{C}_{22}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_4$ $[\text{MH}]^+$ calcd 444.1166, found 444.1172; LC-MS t_{R} = 3.2; HPLC t_{R} = 5.8, > 95 %.

***N*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)isophthalamide (**MIPS1997**)**



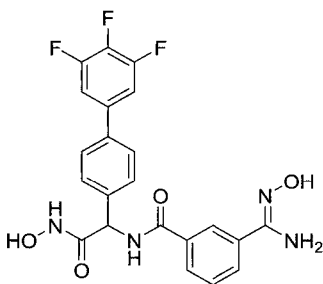
[0135] Methyl 2-(3-carbamoylbenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14f**) (328 mg, 0.741 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. The crude product was purified by column chromatography (eluent DCM/MeOH 95:5 to 90:10) to give 153 mg (47%) of **MIPS1997** as a white solid. ^1H NMR (d_6 -DMSO) δ 11.11 (s, 1H), 9.09 (s, 1H), 9.07 (d, J = 8.2 Hz, 1H), 8.41 (s, 1H), 8.07 (s, 1H), 8.05–7.95 (m, 2H), 7.77–7.67 (m, 4H), 7.62 (d, J = 8.4 Hz, 2H), 7.55 (t, J = 7.8 Hz, 1H), 7.50 (s, 1H), 5.68 (d, J = 8.1 Hz, 1H); ^{13}C NMR (d_6 -DMSO) δ 167.5, 166.4, 165.9, 150.6 (ddd, J_{CF} = 246.5/9.7/4.1 Hz), 140.2–136.8 (m), 138.9, 136.6 (td, J_{CF} = 8.0/4.3 Hz), 136.4–136.3 (m), 134.3, 133.9, 130.6, 130.4, 128.3, 128.1, 126.8, 126.8, 112.6–109.7 (m), 54.4; m/z MS (TOF ES $^-$) $\text{C}_{22}\text{H}_{15}\text{F}_3\text{N}_3\text{O}_4$ $[\text{M}-\text{H}]^-$ calcd 442.1, found 442.0; m/z HRMS (TOF ES $^+$) $\text{C}_{22}\text{H}_{17}\text{F}_3\text{N}_3\text{O}_4$ $[\text{MH}]^+$ calcd 443.1097, found 444.1172; LC-MS t_{R} = 3.2; HPLC t_{R} = 5.9, > 95%.

***N*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)-4-(*N*'-hydroxycarbamimidoyl)benzamide (MIPS1996)**



[0136] Methyl 2-(4-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14g**) (212 mg, 0.500 mmol) was converted to the amidoxime **MIPS1996** following General Procedure B. The reaction mixture was purified by column chromatography (eluent DCM/MeOH 100:0 to 95:5) to afford 34 mg (15%) of **MIPS1996** as a white solid. ^1H NMR (d_6 -DMSO) δ 11.05 (s, 1H), 9.81 (s, 1H), 9.07 (s, 1H), 9.00 (d, J = 8.1 Hz, 1H), 7.92 (d, J = 8.5 Hz, 2H), 7.79–7.66 (m, 6H), 7.62 (d, J = 8.4 Hz, 2H), 5.91 (s, 2H), 5.66 (d, J = 8.1 Hz, 1H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, J = 21.8 Hz), -163.5 (dd, J = 21.7 Hz); ^{13}C NMR (d_6 -DMSO) δ 166.5, 165.9, 150.6 (ddd, J_{CF} = 246.2/9.5/4.1 Hz), 150.2, 138.9, 138.3 (m), 136.7–136.4 (m), 136.3–136.2 (m), 136.1, 133.9, 128.1, 127.7, 126.8, 125.0, 111.2 (m), 54.4; m/z MS (TOF ES $^-$) $\text{C}_{22}\text{H}_{16}\text{F}_3\text{N}_4\text{O}_4$ $[\text{M}-\text{H}]^-$ calcd 457.1, found 457.1; m/z HRMS (TOF ES $^+$) $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_4\text{O}_4$ $[\text{M}+\text{H}]^+$ calcd 459.1275, found 459.1281; LC/MS t_{R} = 3.0; HPLC t_{R} = 5.5, 85%

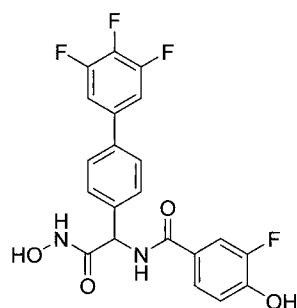
***N*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)-3-(*N*'-hydroxycarbamimidoyl)benzamide (MIPS2331)**



[0137] Methyl 2-(3-cyanobenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14h**) (372 mg, 0.883 mmol) was converted to the amidoxime **MIPS2331** following

General Procedure B. After stirring at room temperature for 1 d the nitrile was completely converted to the amidoxime but the ester was not converted to the hydroxamic acid according to LC-MS. The reaction was left for 3 d but only 50% had converted to the desired hydroxamic acid. $\text{NH}_2\text{OH}\cdot\text{HCl}$ (244mg, 3.51 mmol) and 5 M KOH/MeOH (0.876 mL, 4.38 mmol) were added. After further stirring for 1 d, the reaction was complete and the mixture was purified by column chromatography (eluent DCM/MeOH 100:0 to 90:10) to afford 132 mg (33%) of **MIPS2331** as white flakes. ^1H NMR (d_6 -DMSO) δ 11.11 (s, 1H), 9.72 (s, 1H), 9.08 (s, 1H), 8.98 (d, $J = 8.2$ Hz, 1H), 8.20 (s, 1H), 7.86 (dd, $J = 20.8/7.9$ Hz, 2H), 7.76–7.67 (m, 4H), 7.62 (d, $J = 8.4$ Hz, 2H), 7.46 (t, $J = 7.8$ Hz, 1H), 5.93 (s, 2H), 5.68 (d, $J = 8.1$ Hz, 1H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, $J = 21.7$ Hz), -163.5 (dd, $J = 21.8$ Hz); ^{13}C NMR (d_6 -DMSO) δ 166.4, 166.1, 150.6 (ddd, $J_{\text{CF}} = 246.5/9.7/4.2$ Hz), 150.3, 139.0, 138.3 (dt, $J_{\text{CF}} = 247.1/14.4$ Hz), 136.7–136.4 (m), 136.4–136.2 (m), 133.7, 133.3, 128.3 (2C, determined by HSQC and HMBC), 128.2, 128.0, 126.8, 124.7, 112.2–109.2 (m), 54.3; m/z MS (TOF ES^-) $\text{C}_{22}\text{H}_{16}\text{F}_3\text{N}_4\text{O}_4$ $[\text{M}-\text{H}]^-$ calcd 457.1, found 457.1; m/z HRMS (TOF ES^+) $\text{C}_{22}\text{H}_{18}\text{F}_3\text{N}_4\text{O}_4$ $[\text{MH}]^+$ calcd 459.1275, found 459.1276; LC-MS $t_{\text{R}} = 3.1$; HPLC $t_{\text{R}} = 5.5$, 95%.

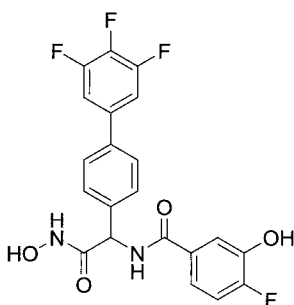
3-Fluoro-4-hydroxy-N-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)benzamide (MIPS2332)



[0138] Methyl 2-(3-fluoro-4-hydroxybenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14i**) (430 mg, 1.00 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. The reaction mixture was purified by column chromatography (eluent DCM/MeOH (100:0 to 90:10) to afford 30 mg (7%) of **MIPS2332** as a white solid. ^1H NMR (d_6 -DMSO) δ 11.05 (s, 1H), 10.53 (s, 1H), 9.05 (s, 1H), 8.82 (d, $J = 8.1$ Hz, 1H), 7.85–7.47 (m, 8H), 6.99 (t, $J = 8.7$ Hz, 1H), 5.63 (d, $J = 8.1$ Hz, 1H); ^{19}F NMR (d_6 -DMSO) δ -134.9 (d, $J = 21.8$ Hz), -136.6, -163.5 (dd, $J = 21.8$ Hz); ^{13}C NMR

(d_6 -DMSO) δ 166.5, 164.9 (d, J_{CF} = 2.0 Hz), 150.6 (ddd, J_{CF} = 246.5/9.8/4.2 Hz), 150.3 (d, J_{CF} = 241.0 Hz), 148.1 (d, J_{CF} = 12.1 Hz), 138.9, 138.3 (dt, J_{CF} = 249.1/15.9 Hz), 136.8–136.4 (m), 136.3–136.1 (m), 128.1, 126.7, 125.0 (d, J_{CF} = 2.8 Hz), 124.9 (d, J_{CF} = 5.3 Hz), 117.0 (d, J_{CF} = 2.9 Hz), 115.9 (d, J_{CF} = 19.6 Hz), 111.6–110.6 (m), 54.4; MS (TOF ES⁻) m/z C₂₁H₁₃F₄N₂O₄ [M-H]⁻ calcd 433.1, found 433.1; m/z HRMS (TOF ES⁺) C₂₁H₁₅F₄N₂O₄ [MH]⁺ calcd 435.0962, found 435.0968; LC-MS t_R = 3.2; HPLC t_R = 6.3, > 95%.

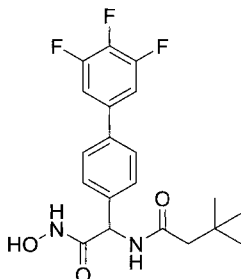
4-Fluoro-3-hydroxy-N-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)benzamide (MIPS2333)



[0139] Methyl 2-(4-fluoro-3-hydroxybenzamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14j**) (527 mg, 1.22 mmol) was converted to the corresponding hydroxamic acid according to General Procedure B. After stirring at room temperature for 1 d, 40% of the starting material had conversion to **MIPS2333**. The reaction was left for a further 6 days reaching 70% conversion according to LC-MS. The reaction was stopped and starting material and product were isolated by column chromatography (eluent DCM/MeOH (100:0 to 90:10) in which 68 mg (13%) of **MIPS2333** was obtained as pale yellow solid and 10 mg of the starting material was recovered. ¹H NMR (d_6 -DMSO) δ 11.04 (s, 1H), 10.11 (s, 1H), 9.04 (s, 1H), 8.87 (d, J = 8.1 Hz, 1H), 7.74–7.66 (m, 4H), 7.60 (d, J = 8.4 Hz, 2H), 7.49 (dd, J = 8.6/2.2 Hz, 1H), 7.41 (ddd, J = 8.4/4.3/2.2 Hz, 1H), 7.21 (dd, J = 11.0/8.5 Hz, 1H), 5.60 (d, J = 8.1 Hz, 1H); ¹⁹F NMR (d_6 -DMSO) δ -132.2, -134.9 (d, J = 21.7 Hz), -163.5 (dd, J = 21.7 Hz); ¹³C NMR (d_6 -DMSO) δ 166.5, 165.5, 153.1 (d, J_{CF} = 246.2 Hz), 150.6 (ddd, J_{CF} = 246.4/10.2/4.0 Hz), 144.6 (d, J_{CF} = 12.4 Hz), 138.9, 138.3 (dt, J_{CF} = 243.0/12.5 Hz), 136.6 (td, J_{CF} = 7.9/4.6 Hz), 136.3–136.1 (m), 130.6 (d, J_{CF} = 3.1 Hz), 128.1, 126.8, 119.3 (d, J_{CF} = 7.4 Hz), 117.7 (d, J_{CF} = 3.9 Hz), 115.8 (d, J_{CF} = 18.9 Hz), 111.9–110.6 (m), 54.4; m/z MS (TOF ES⁻) C₂₁H₁₃F₄N₂O₄ [M-H]⁻

calcd 433.1, found 433.1; m/z HRMS (TOF ES⁺) C₂₁H₁₅F₄N₂O₄ [MH]⁺ calcd 435.0962, found 435.0973; LC-MS t_R = 3.3; HPLC t_R = 6.4, > 95%.

***N*-(2-(hydroxyamino)-2-oxo-1-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)ethyl)-3,3-dimethylbutanamide (MIPS1778)**



[0140] Methyl 2-(3,3-dimethylbutanamido)-2-(3',4',5'-trifluoro-[1,1'-biphenyl]-4-yl)acetate (**14k**) (100 mg, 0.25 mmol) was converted to the title compound according to General Procedure B. The crude product was purified by column chromatography (eluent DCM/MeOH 100:0 to 90:10) to give 75 mg (75%) of **MIPS1778** as a white solid. ¹H NMR (DMSO-*d*₆) δ 11.09 (s, 1H), 9.10 (s, 1H), 8.62 (d, *J* = 8.3 Hz, 1H), 7.85–7.73 (m, 4H), 7.59 (d, *J* = 8.3 Hz, 2H), 5.53 (d, *J* = 8.3 Hz, 1H), 2.31–2.12 (m, 2H), 1.03 (s, 9H); ¹⁹F NMR (DMSO-*d*₆) δ -134.94 (d, *J* = 21.7 Hz), -163.55 (dd, *J* = 21.7 Hz); ¹³C NMR (DMSO-*d*₆) δ 170.7, 166.6, 150.61 (ddd, *J* = 246.3, 9.7, 4.1 Hz), 139.5, 139.8–136.7 (m), 136.74–136.32 (m), 136.0, 127.6, 126.7, 111.2 (dd, *J* = 15.9/5.5 Hz), 53.2, 47.9, 30.6, 29.7; m/z HRMS (TOF ES⁺) C₂₀H₂₂F₃N₂O₃ [MH]⁺ calcd 395.1577; found 393.1590; LC-MS t_R : 3.3 min.

[0141] While this invention has been described in connection with specific embodiments thereof, it will be understood that it is capable of further modification(s). This application is intended to cover any variations uses or adaptations of the invention following in general, the principles of the invention and including such departures from the present disclosure as come within known or customary practice within the art to which the invention pertains and as may be applied to the essential features herein before set forth.

[0142] As the present invention may be embodied in several forms without departing from the spirit of the essential characteristics of the invention, it should be understood that the above described embodiments are not to limit the present invention unless

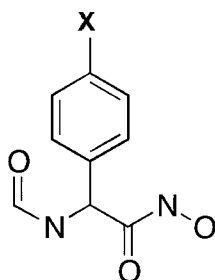
otherwise specified, but rather should be construed broadly within the spirit and scope of the invention as defined in the appended claims. The described embodiments are to be considered in all respects as illustrative only and not restrictive.

[0143] Various modifications and equivalent arrangements are intended to be included within the spirit and scope of the invention and appended claims. Therefore, the specific embodiments are to be understood to be illustrative of the many ways in which the principles of the present invention may be practiced. In the following claims, means-plus-function clauses are intended to cover structures as performing the defined function and not only structural equivalents, but also equivalent structures.

[0144] “Comprises/comprising” and “includes/including” when used in this specification is taken to specify the presence of stated features, integers, steps or components but does not preclude the presence or addition of one or more other features, integers, steps, components or groups thereof. Thus, unless the context clearly requires otherwise, throughout the description and the claims, the words ‘comprise’, ‘comprising’, ‘includes’, ‘including’ and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of “including, but not limited to”.

CLAIMS

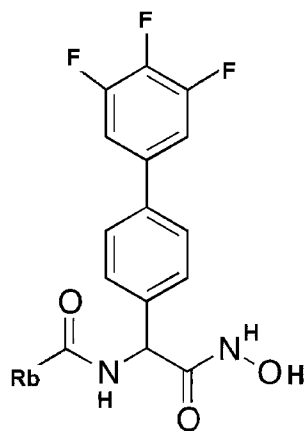
1. An aminopeptidase inhibitor compound comprising a biaryl, hydroxamic acid based core of formula:



wherein X is a 5 or 6-membered ring

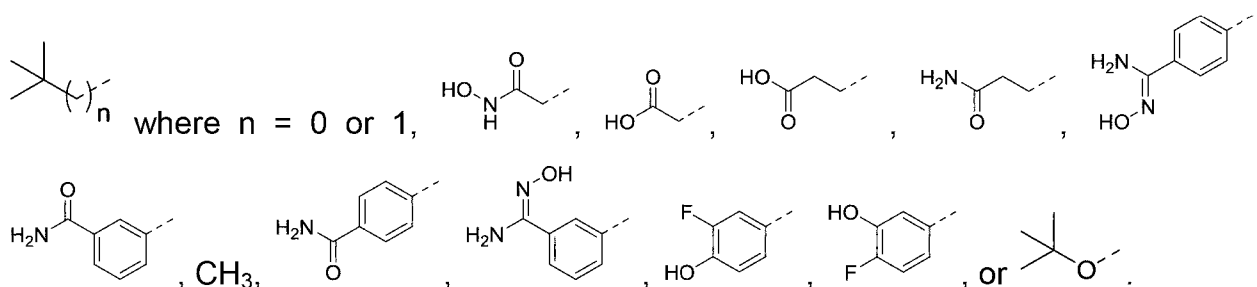
and including pharmaceutically acceptable salts and solvates thereof.

2. An amino peptidase inhibitor compound according to claim 1, the aminopeptidase inhibitor being of formula:

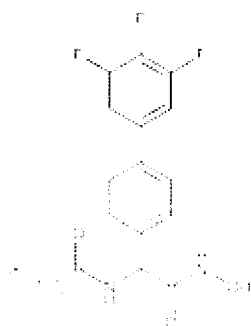


including its pharmaceutically acceptable salts and solvates,

wherein Rb is chosen from the group comprising:



3. An aminopeptidase inhibitor compound according to claim 1, the compound being of formula:



and its pharmaceutically acceptable salts and solvates,

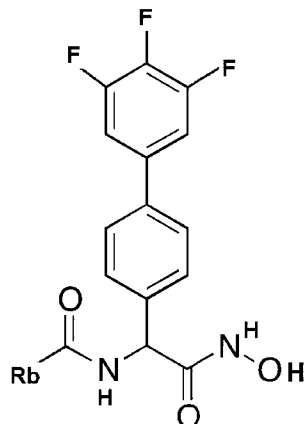
wherein $n = 0$ or 1 .

4. An aminopeptidase inhibitor compound according to claim 1 and having the structure of any one of compounds 9f, 9g, 9h, 9i, 9j, 9k, 9l, 9m, 9r, 9s, 10f, 10g, 10h, 10i, 10j, 10k, 10l, 10m, 10n, 10o, 10p, 10s as defined herein and their pharmaceutically acceptable salts and solvates.

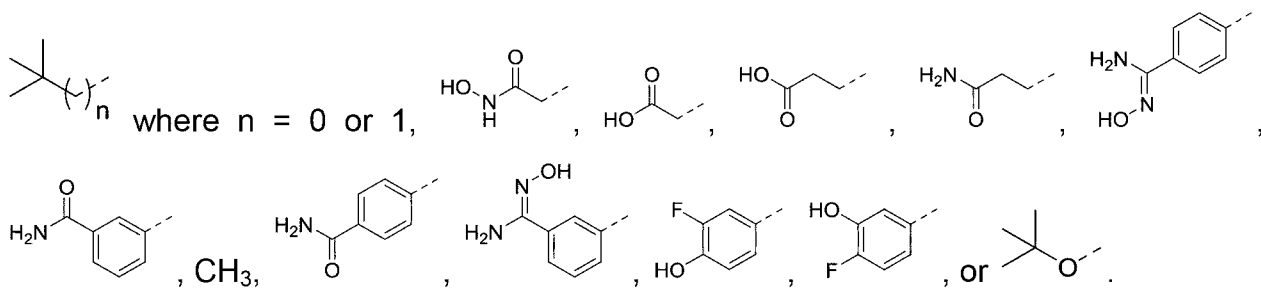
5. A compound of any one of the preceding claims when used to inhibit an aminopeptidase.

6. A compound according to any one of claims 1 to 4 when used to inhibit an M1 aminopeptidase, an M17 aminopeptidase, or both M1 and M17 aminopeptidase.

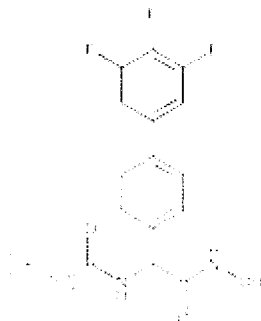
7. A method of inhibiting an aminopeptidase, including the step of administering to a patient a therapeutic compound comprising a biaryl hydroxamic acid core according to claim 1.
8. A method of inhibiting an aminopeptidase, according to claim 7 including the step of administering to a patient a therapeutic compound of formula:



wherein Rb is chosen from the group comprising:



9. A method of inhibiting an aminopeptidase, according to claim 7 comprising the step of administering to a patient a therapeutic compound of formula:



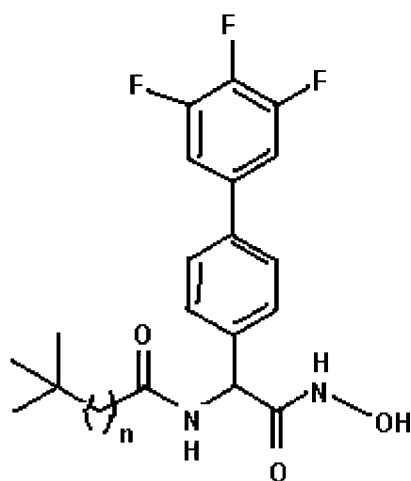
and its pharmaceutically acceptable salts and solvates, wherein $n = 0$ or 1 .

10. A method according to any one of claims 6, 7 or 8, wherein the aminopeptidase is related to malaria.

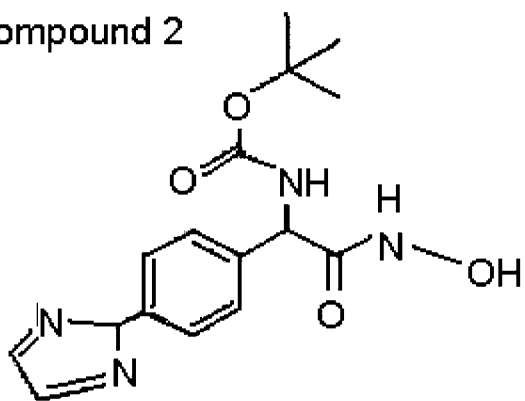
11. A method according to any one of claims 6, 7 or 8, wherein the aminopeptidase is related to leukaemia.

12. A method according to any one of claims 6, 7 or 8, wherein the aminopeptidase is related to a cancer.

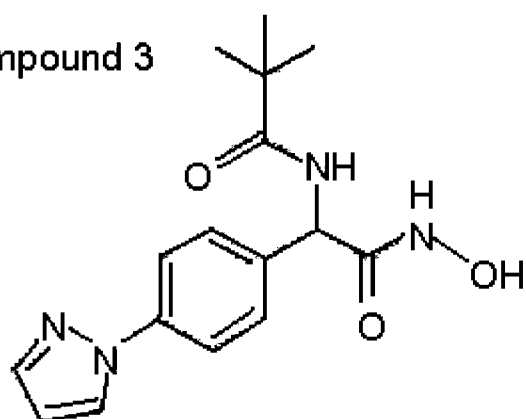
13. The use of a compound according to any one of claims 1, 2 or 3 for the preparation of a medicament formulated for use via an administrative route chosen from oral, nasal, buccal, parenteral, topical, depot or rectal.

**FIG 1(a)**

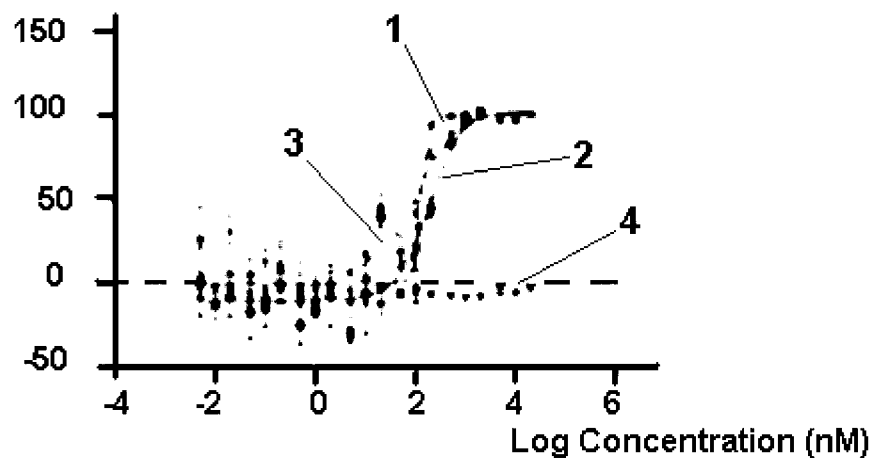
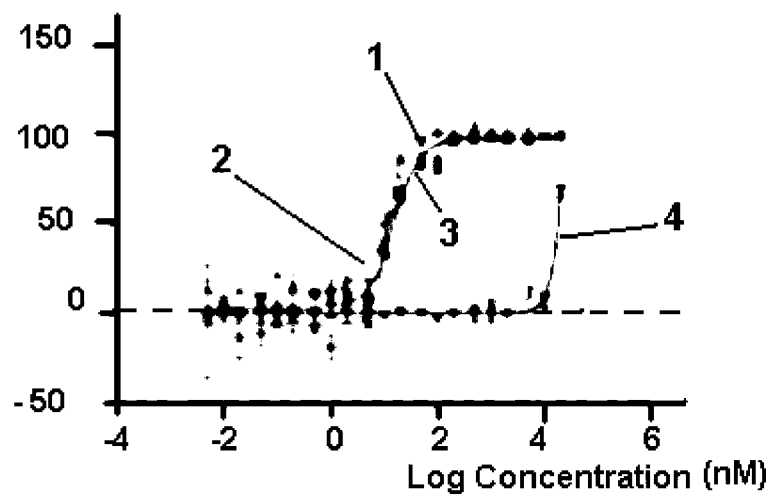
Compound 2

**FIG 1(b)**

Compound 3

**FIG 1(c)**

% Inhibition

**FIG 2(a)****FIG 2(b)**

3/3

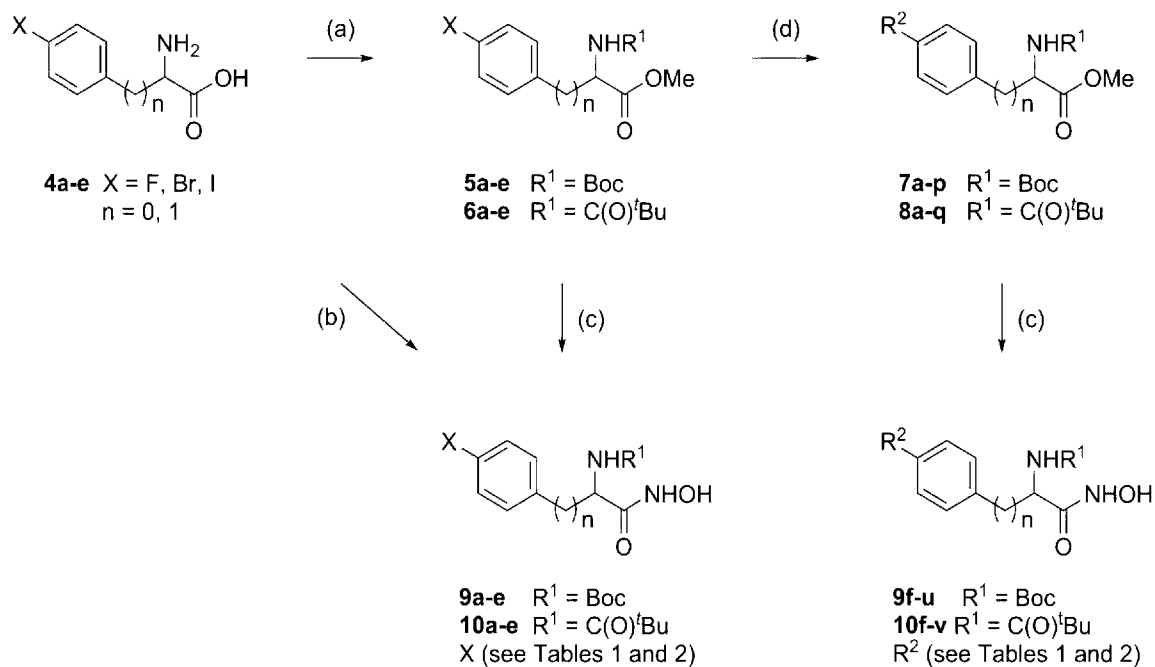


FIG. 3

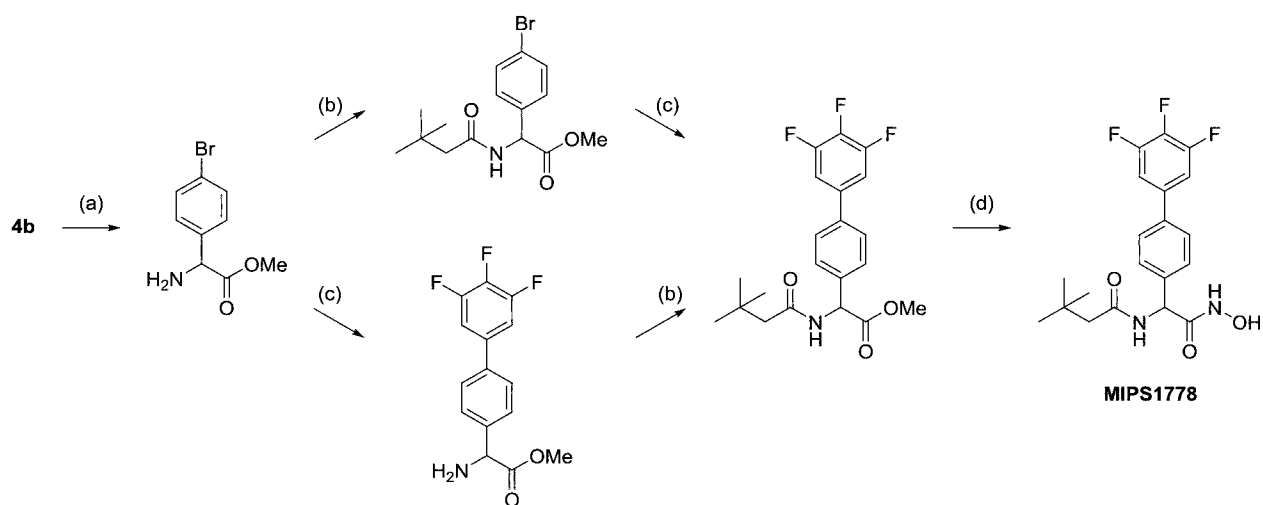


FIG. 4

INTERNATIONAL SEARCH REPORT

 International application No.
PCT/AU2016/000248

A. CLASSIFICATION OF SUBJECT MATTER

C07C 259/06 (2006.01) **C07D 231/12 (2006.01)** **C07D 213/42 (2006.01)** **C07D 333/24 (2006.01)** **A61K 31/165 (2006.01)**
A61K 31/381 (2006.01) **A61K 31/4406 (2006.01)** **A61K 31/4409 (2006.01)** **A61P 33/06 (2006.01)**
A61P 35/00 (2006.01) **A61P 35/02 (2006.01)**

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)

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)

STN: Registry and CAPlus; Structure search of sub-formula in claim 1; Keywords Aminopeptidase, Malaria, Cancer, Tumour, Leukaemia and like terms.

Espacenet and AusPat: Applicant and inventor names

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
	Documents are listed in the continuation of Box C	



Further documents are listed in the continuation of Box C



See patent family annex

* "A"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance	"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
"E"	earlier application or patent but published on or after the international filing date	"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
"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)	"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
"O"	document referring to an oral disclosure, use, exhibition or other means	"&"	document member of the same patent family
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 3 August 2016	Date of mailing of the international search report 03 August 2016
Name and mailing address of the ISA/AU AUSTRALIAN PATENT OFFICE PO BOX 200, WODEN ACT 2606, AUSTRALIA Email address: pct@ipaustalia.gov.au	Authorised officer Scott Makin AUSTRALIAN PATENT OFFICE (ISO 9001 Quality Certified Service) Telephone No. +61 2 6283 7971

INTERNATIONAL SEARCH REPORT		International application No.
C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		PCT/AU2016/000248
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	MISTRY, S.N. et al, "Two-Pronged Attack: Dual Inhibition of <i>Plasmodium falciparum</i> M1 and M17 Metalloaminopeptidases by a Novel Series of Hydroxamic Acid-Based Inhibitors", Journal of Medicinal Chemistry, 2014, vol. 57 (21), pp. 9168-9183 Abstract; Compounds 7, 13a-i, 13l-m, Table 1, p. 9170; Experimental	1, 5-7, 10-13
A	VELMOUROUGANE, G. et al, "Synthesis of New (-)-Bestatin-Based Inhibitor Libraries Reveals a Novel Binding Mode in the S1 Pocket of the Essential Malaria M1 Metalloaminopeptidase", Journal of Medicinal Chemistry, 2011, vol. 54 (6), pp. 1655-1666 Abstract; Compounds 1-6, Table 1, p. 1659; Schemes 1-3, pp. 1657-1658	1-13
A	ZHANG, X. et al, "Design, synthesis and preliminary activity evaluation of novel 3-amino-2-hydroxyl-3-phenylpropanoic acid derivatives as aminopeptidase N/CD13 inhibitors", Journal of Enzyme Inhibition and Medicinal Chemistry, 2013, vol. 28 (3), pp. 545-551 Abstract; Compounds 7a-7h, Table 1, pp. 547-548	1-13
A	NIU, M. et al, "Establishment of a screening protocol for identification of aminopeptidase N inhibitors", Journal of the Taiwan Institute of Chemical Engineers, 2015, vol. 49, pp. 19-26 Abstract; Introduction; Compound 2, Fig. 4, p. 22 and Hit 2, Table 3, p. 24	1-13
P,X	DRINKWATER, N. et al, "Potent dual inhibitors of <i>Plasmodium falciparum</i> M1 and M17 aminopeptidases through optimization of S1 pocket interactions", European Journal of Medicinal Chemistry, 2016, vol. 110, pp. 43-64 Whole document	1-13