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SAR of 2-amino and 2,4-diamino pyrimidines with in vivo efficacy against *Trypanosoma brucei*

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ABSTRACT

A series of 2,4-diaminopyrimidines was investigated and compounds were found to have in vivo efficacy against *Trypanosoma brucei* in an acute mouse model. However, in vitro permeability data suggested the 2,4-diaminopyrimidenes would have poor permeability through the blood brain barrier. Consequently a series of 4-desamino analogs were synthesized and found to have improved in vitro permeability.

Human African Trypanosomiasis (HAT), commonly referred to as sleeping sickness, is a neglected CNS disease, that is, fatal in 100% of untreated individuals.[1] The disease infects 50,000–150,000 people per year and threatens to infect another 50 million in sub-Saharan Africa.[1] Current treatments for HAT are either inadequate, impractical to administer in sub-Saharan Africa or suffer from overt toxicity.[2–6] Clearly, new treatments for HAT are needed.

HAT is caused by *Trypanosoma brucei gambiense* (*T.b.g.*) and *Trypanosoma brucei rhodesiense* (*T.b.r.*), while a closely related animal pathogen *Trypanosoma brucei brucei* (*T.b.b.*) is utilized as an in vitro model.[7,8]

We previously identified 2,4-diaminopyrimidines as potential kinase inhibitors of *T.b.b.* using a chemical proteomics approach.[9] Herein we describe the SAR investigation of 2,4-diamino pyrimidines along with new 4-*des*-amino pyrimidines. Additionally in vivo efficacy results of both series are discussed along with DMPK and physicochemical data.

As part of our screening cascade, we were interested in identifying noncytotoxic compounds active against *T.b.b.* Sub-micromolar compounds with greater than 50 fold selectivity against the L929 mammalian cell line were further screened for aq solubility and S9 metabolic stability against S9 microsomes.

SAR investigation of the amino piperdine substituent off the 2 position of the pyrimidine ring led to compounds with double digit nanomolar potencies (Table 1). Unsubstituted analog **1** was inactive, while installation of hydrogen bond accepting functional groups **2–10** were found to be active against *T.b.b.* Acyl analog **2** was observed to have an IC_{50} of 3.3 μM while its corresponding sulfonyl analog **3** was observed to have a *T.b.b.* IC_{50} of 0.66 μM, cytotox IC_{50} of 11 μM, aqueous solubility of 50 μM and S9 metabolic half life greater than 350 min. Benzyl carbomyl **4** was observed to be an order of magnitude more potent, than its acyl analog, IC_{50} 0.34 μM with an acceptable selectivity ratio of 58. Unfortunately, solubility and S9 stability were unacceptable at 6 μM and 32 min. The benzyl sulfonyl **5** had an IC_{50} of 0.37 μM, however it also had poor solubility and S9 metabolic stability. Isosteric urea analog **6** however, was found to be an order of magnitude more potent, IC_{50} = 45 nM, while achieving a *T.b.b.* IC_{50} to cytotox IC_{50} ratio of greater than 160 fold. Aqueous solubility for phenyl urea **6** was acceptable at 25 μM and the S9 metabolic half-life was 96 min. Substitution of the phenyl urea with a benzimidazole **7** maintained potency in the double digit nanomolar range, and had about the same cytotox and S9 stability values, although solubility fell to 13 μM. N-methylation of the benzimidazole **8** resulted in loss of activity while benzothiazole analog **9** had an IC_{50} of 0.29 μM. Thiazole **10** was an order of magnitude less active, IC_{50} = 3.1 μM, than the benzothiazole **9**.

With phenyl urea analog **6** representing the best balance between potency, cytotoxicity, aq solubility and S9 metabolic

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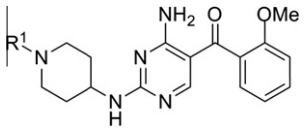
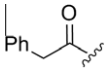
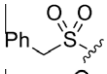
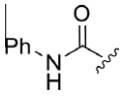
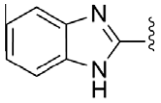
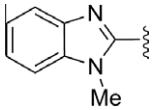
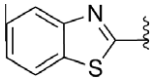
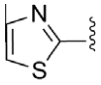
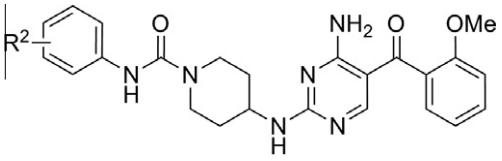
Table 1
SAR investigation of N-substituted 4-aminopyrimidine analogs



#	R^1	*T.b.b.* IC_{50} (μM)	L929 Cytotox. IC_{50} (μM)	Aq soln (μM)	S9 $t_{½}$ (min)
1	H	>14	NT	NT	NT
2		3.3	>27	NT	NT
3		0.66	11	50	>350
4		0.34	20	6	32
5		0.37	9.1	2	8
6		0.045	7.5	25	96
7		0.088	7.6	13	96
8		1.53	>22	NT	NT
9		0.29	15	NT	40
10		3.1	>24	NT	NT

NT = Not taken.

Table 2
SAR investigation of phenyl substituted ureas towards *T.b.b.*



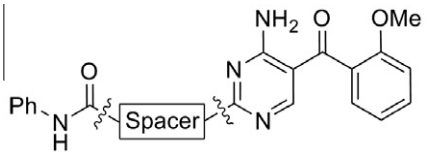
#	R^2	*T.b.b.* IC_{50} (μM)	L929 Cytotox. IC_{50} (μM)
11	2-F	0.13	4.1
12	3-F	0.043	9.3
13	4-F	0.065	4.6
14	2-Me	0.41	10
15	3-Me	0.11	7.1
16	4-Me	0.11	2.9
17	2-OMe	0.19	1.2
18	3-OMe	0.29	6.9
19	4-OMe	0.36	3.4

stability, a more detailed SAR investigation with respect to substitution on the phenyl ring was initiated (Table 2). Installation of a fluorine into the aryl group at the *ortho* position **11**, resulted in loss of potency with an IC_{50} of 0.13 μM. Movement of the fluorine atom to the *meta* **12** and *para* **13** positions regained activity with IC_{50}s of 43 and 65 nM, respectively. These compounds also had aq solubilities of 13 and 25 μM and S9 half-lives of 169 and 152 min, respectively. Installation of a more electron neutral methyl group into the aromatic ring at the *ortho* position **14** resulted in a loss of activity with an IC_{50} of 0.41 μM. Moving the methyl to the *meta* **15** and *para* **16** positions both gave potencies of 0.11 μM. Lastly installation of an electron rich methoxy at the ortho position **17** also resulted in a loss of potency with an IC_{50} of 0.19 μM. Moving the methoxy to the *meta* **18** and *para* **19** positions resulted in IC_{50}s of 0.29 and 0.36 μM, respectively. These results suggest that small groups (F) are tolerated in the *meta* and *para* positions, but substitution at the *ortho* position diminishes activity.

The effects of the linker between the pyrimidine ring and *N*-phenyl carbomyl substituent was investigated (Table 3). A 4-amino(syn benzamide) spacer **20** was inactive while the corresponding anti isomer **21** had an IC_{50} of 4.6 μM. Removing the rigidity of the highly active 4-aminopiperdine linker in **6** but maintaining the same carbon spacers provided linear urea **22** which was two orders of magnitude less active, IC_{50} = 4.5 μM. Changing the piperdine ring to a piperazine ring **23** resulted in loss of activity. The corresponding 3-amino pyrrolidine **24** was observed to have an IC_{50} of 5.5 μM.

The SAR of the *o*-methoxy benzoyl substituent was investigated (Table 4). Both compounds **3** and **6** were utilized as reference standards to examine the effects of changes to the benzoyl ring. Removal of the *o*-methoxy substituent **25** led to about an order

Table 3
SAR investigation of spacer between pyrimidine ring and *N*-phenyl carbomyl substituent



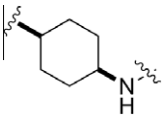
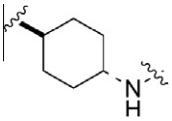
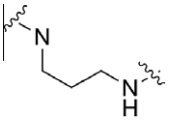
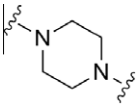
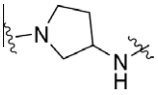
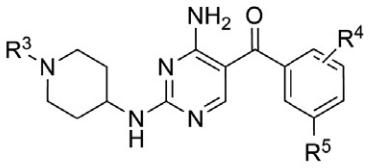
#	Spacer	*T.b.b.* IC_{50} (μM)	L929 cytotox. IC_{50} (μM)
20		>11	>22
21		4.6	4.6
22		4.5	11
23		>12	>27
24		5.5	13

Table 4
SAR investigation of benzoyl substituent towards *T.b.b.*



3, R^3 = Ms, R^4 = 2-OMe, R^5 = H, IC_{50} = 0.66μM
6, R^3 = Ph urea, R^4 = 2-OMe, R^5 = H, IC_{50} = 0.045 μM

#	R^3	R^4	R^5	IC_{50} (μM)
25	Ms	H	H	7.4
26	Ms	2-Me	H	1.8
27	Ms	3-OMe	H	10
28	Ms	4-OMe	H	>12
29	Ms	2-OMe	5-F	0.17
30	Ph urea	2-OMe	5-F	0.030
31	Ph urea	2-Et	H	0.17
32	Ph urea	2-OEt	H	0.26

of magnitude loss in activity. Substitution with an *o*-methyl group **26** resulted in about a 3 fold loss in activity. Moving the *o*-methoxy group to the *meta* position **27** led to a significant loss in activity, IC_{50} = 10 μM while the corresponding *para* isomer **28** was inactive. Addition of a 5-fluoro substituent to the *o*-methoxy benzoyl led to a modest increase in potency **29** and **30**. Substitution of the *o*-methoxy group with an isosteric ethyl group **31** resulted in about a 4 fold loss in activity. Interestingly maintaining the electronics but extending the alkyl chain by one carbon **32** resulted in about a 6 fold loss of activity. These results seem to indicate that a 2-methoxy substituent might be best.

The in vivo efficacy and in vitro permeability of select compounds with sub-micromolar potencies against *T.b.b.* were investigated (Table 5). Compounds **29**, **3** and **6** cured 100% of mice in a 30 day nonCNS acute mouse model for HAT. Examination of the MDR1/MDCK permeability data revealed that the compounds had modest to poor permeability.[10] Additionally, permeability was enhanced by the P-gp inhibitor GF120918, as revealed by the higher absorption quotients (AQ).[11] This suggests **29**, **3** and **6** were P-gp substrates.[10,11] The inability of these compounds to cross the blood brain barrier suggests that they are unlikely to exhibit efficacy against the CNS stage 2 of HAT. Presumably the high polar surface area (PSA) was responsible for the modest permeability.

While the potency and efficacy of **6** was promising, the S9 metabolic half-life was a concern we wished to improve. Incubation of **6** with S9 microsomes followed by HPLC/MS of the metabolites revealed loss of a methyl group was the primary oxidative byproduct followed by hydroxylation of the molecule. The methyl group of the 2-methoxybenzoyl substituent is the only methyl in **6**. Unfortunately, changes to the 2-methoxybenzyoyl resulted in loss of potency (Table 4).

To circumvent the poor permeability and P-gp liabilities, a series of *des*-amino pyrimidines were investigated (Table 6). Substitution of the primary amine in phenyl urea analog **6** with a hydrogen **33** resulted in a 7 fold loss of activity IC_{50} = 0.33 μM. However the in vitro permeability and P-gp liabilities were circumvented and the S9 metabolic half-life was modestly extended. Compound **33** also cured one of three mice in the nonCNS acute in vivo efficacy model. Substitution of the hydrogen with a methyl group **34** resulted in a 36 fold loss in potency against *T.b.b.* *Des*-amino benzimidazole analog **35** was observed to retain sub-micromolar potency against *T.b.b.*, while the metabolic S9 half-life dropped to 45 min. The in vitro permeability liabilities remained however. Substitution of the primary amine in mesyl analog **3** with a hydrogen **36** resulted in the loss of sub-micromolar potency, IC_{50} = 1.8 μM. Replacement of the hydrogen with a methyl **37** also resulted in significant loss of potency, IC_{50} >12 μM, similar to **34**. Finally the 4-H *des*-amino analog **38** had sub-micromolar potency and improved permeability while retaining some degree of in vivo efficacy.

The synthetic route for accessing the 4-amino pyrimidine compounds investigated utilized known intermediate **39** $R^9 = NH_2$ and Ar = 2-methoxyphenyl (Scheme 1).[12] Displacement of the sulfoxide with a 1-N substituted 4-aminopiperdine under microwave conditions provided the resultant 1-N (heterocyclic, mesyl or Boc) 4-aminopiperdine intermediate **40**.[12] Removal of the Boc group was accomplished with trifluoroacetic acid in CH_2Cl_2 to provide the secondary amine after basic workup. Addition of an isocyanate or sulphonyl chloride to the secondary amine then provided ureas or alkyl sulphonamides **41**.[12] Synthesis of the *des*-amino intermediates **39** R^9 = Me or H was carried out in a similar fashion as described in the literature with the corresponding *des*-amino pyrimidine starting materials.[12] Synthesis of **20–24** were carried out in a similar fashion starting with **39** ($R^9 = NH_2$) and using the appropriate functionalized amine in step A of Scheme 1.

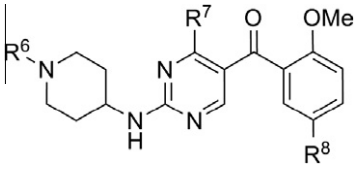
In conclusion, the SAR of 2-amino and 2,4-diamino pyrimidines were investigated for activity against *T.b.b.* 2,4-Diamino pyrimidines **29**, **3** and **6** cured 100% of mice tested in a nonCNS in vivo acute HAT mouse model. However, the in vitro permeability of these compounds predicted poor permeability across the blood brain barrier. A series of 4-*des*-amino pyrimidines were evaluated and found to have circumvented the in vitro permeability issues caused by the primary amine. However, these compounds did not provide complete efficacy in the nonCNS in vivo acute HAT mouse model.

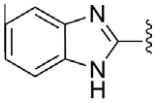
Table 5
In vivo efficacy in nonCNS acute HAT mouse model

#	Cures 20 mg/kg IP	Cures 20 mg/kg PO	S9 $t_{1/2}$ (min)	P_{app}[a] (nm/sec)	AQ0	PSA
29	100%	100%[b]	>350	532/272	0.49	136
3	100%	100%	>350	NT	NT	136
6	100%	100%	96	410/151	0.63	122

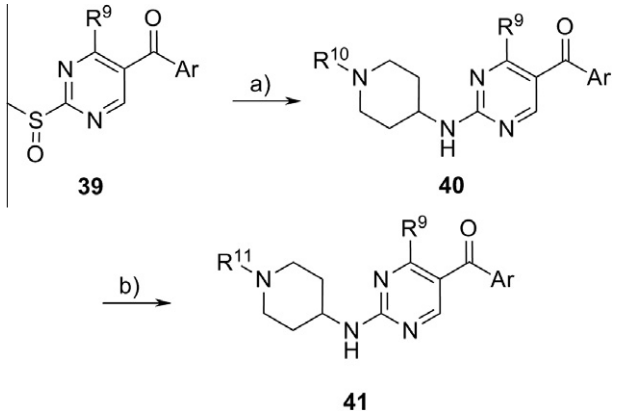
Administered to a group of three mice in a 20% ethanol, 30% aq 0.5% CMC and 50% PEG400 solution.
NT = Not taken.
[a] Values reported as with the P-gp inhibitor GF120918/without the P-gp inhibitor GF120918.
[b] Only two mice in group.

Table 6
Activity of *des*-amino pyrimidines in the nonCNS acute HAT mouse model



#	R^6	R^7	R^8	*T.b.b.* IC_{50} (μM)	S9 $t_{1/2}$ (min)	P_{app}[a]	AQ	PSA	Cures
33	Ph urea	H	H	0.33	168	552/398	0.14	96	33%
34	Ph urea	Me	H	1.6	73	NT	NT	96	NT
35		H	H	0.31	45	496/114	0.77	96	NT
36	Ms	H	H	1.8	223	NT	NT	110	NT
37	Ms	Me	H	>12	434	NT	NT	110	NT
38	Ms	H	F	0.57	NT	687/561	0.18	110	66%

Administered to a group of three mice in a 20% ethanol, 30% aq 0.5% CMC and 50% PEG400 solution.
[a] Values reported as with the P-gp inhibitor GF120918/without the P-gp inhibitor GF120918.



Scheme 1. Synthetic route employed. $R^9 = NH_2$, Me or H R^{10} = Heterocycle, Ms or Boc, R^{11} = urea or alkyl sulfonyl (a) 1N-substituted 4-aminopiperdine microwave 110 °C, 1:9 THF/IPA; (b) for R^2 = Boc (i) TFA/CH_2Cl_2, 25 °C, (ii) isocyanate in THF or alkyl sulfonyl chloride, NEt_3 in THF, 25 °C.

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