Bioorg. Med. Chem. Lett. 109 (2024) 129825

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Novel 4-[4-(4-methylpiperazin-1-yl)phenyl]-6-arylpyrimidine derivatives and their antitrypanosomal activities against *T.brucei*

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ARTICLE INFO

Keywords:
Antitrypanosomal
Pyrimidines
Antiparasitic
Kinetoplastid
Neglected tropical diseases
T.brucei

ABSTRACT

Human African trypanosomiasis, or sleeping sickness, is a neglected tropical disease caused by *Trypanosoma brucei rhodesiense* and *Trypanosoma brucei gambiense* and is invariably fatal unless treated. Current therapies present limitations in their application, parasite resistance, or require further clinical investigation for wider use. Our work, informed by previous findings, presents novel 4-[4-(4-methylpiperazin-1-yl)phenyl]-6-arylpyrimidine derivatives with promising antitrypanosomal activity. In particular, **32** exhibits an *in vitro* EC_{50} value of 0.5 μM against *Trypanosoma brucei rhodesiense*, and analogues **29**, **30** and **33** show antitrypanosomal activities in the <1 μM range. We have demonstrated that substituted 4-[4-(4-methylpiperazin-1-yl)phenyl]-6-arylpyrimidines present promising antitrypanosomal hit molecules with potential for further preclinical development.

Introduction

Neglected tropical diseases (NTDs) affect more than 1 billion people across the globe, leading to devastating health, social and economic consequences.[1] Disproportionately, NTDs are linked to vulnerable populations associated with impoverished communities, with public-health control further complicated due to the vector-borne nature and complex life cycles of pathogens.

Contributing to this burden are kinetoplastid parasites from the *Trypanosomatidae* family, including *Trypanosoma brucei rhodesiense* (*T.b.r*) and *Trypanosoma brucei gambiense* (*T.b.g*), which cause Human African Trypanosomiasis (HAT), also known as sleeping sickness.[2] Developments for treating *T.b.g* related HAT have been observed with fexinidazole, but first- and second-stage disease caused by *T.b.r* requires suramin or the arsenic containing melarsoprol which can cause reactive encephalopathy.[3,4] In addition, animal reservoirs harbouring this pathogen present the potential for future epidemics.[5] With the aforementioned, it is evident newer and safer medicines are needed for the treatment of kinetoplastid diseases.

The importance of pyrimidines and their potential as antikinetoplastids has been shown through our previous work.[6] As part of our on-going programme in drug development for NTDs,[7,8] we further explored the syntheses of this compound class and assessed antitrypanosomal activities against *T.b.r*. The compounds designed retained 1-methyl-4-phenylpiperazine at the 4-position of the pyrimidine ring, whilst the 2-position of was explored alongside electron withdrawing groups on the aryl A-ring (Figure 1).

The syntheses of substituted pyrimidines was achieved over 3 steps (scheme 1), in a similar manner to that reported by Robinson et al. [6]. First, 4-(4-methylpiperazin-1-yl)benzaldehyde (**1**) was generated and reacted with appropriate acetophenones to yield (2*E*-3-[4-(4-methylpiperazin-1-yl)phenyl]-1-arylprop-2-en-1-ones (**2**–**11**). Finally, the substituted α,β-unsaturated carbonyl compounds were converted to substituted 4-[4-(4-methylpiperazin-1-yl)phenyl]-6-arylpyrimidine derivatives (**12**–**39**), using either formamidine, acetamidine or guanidine.

Compounds were purified by recrystallisation or column chromatography and structures were fully in accord with their analytical and spectroscopic properties. Furthermore, **32** was determined by single crystal X-ray diffraction analysis (Figure 2).[9,10]

Compounds **12**–**39** were screened for their antitrypanosomal

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https://doi.org/10.1016/j.bmcl.2024.129825
Received 12 March 2024; Received in revised form 13 May 2024; Accepted 29 May 2024
Available online 31 May 2024

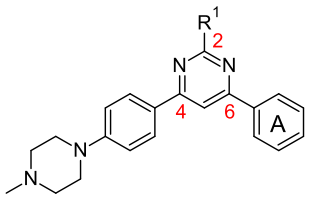


Figure 1. Exploration points of the pyrimidine structure.

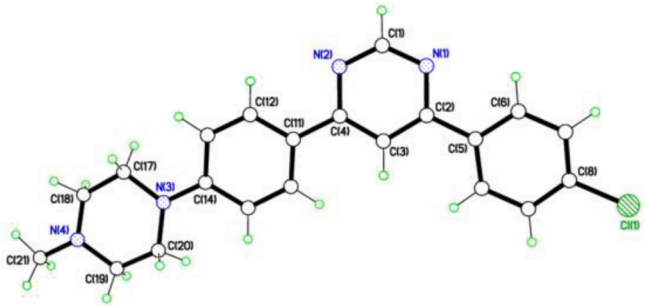


Figure 2. Molecular structure of one of two similar molecules in the asymmetric unit of 32.

activities (Table 1) against *Trypanosoma brucei rhodesiense* (*T.b.r*, STIB900) trypomastigotes.[11] Cytotoxicity was also assessed using L6 cells (rat skeletal myoblasts).

The use of formamidine in the syntheses of target molecules afforded the 2-H substituted pyrimidine core. Derivative **12**, bearing a phenyl A-ring, demonstrated low micromolar antitrypanosomal activity against *T.b.r* with an EC_{50} value of 3.8 μM, but no notable affect against L6 cells. Antitrypanosomal activity reduced with **15**, the 2-NO_2 substituted analogue and no notable EC_{50} values were recorded for the 2-bromo or 2-chlorophenyl derivatives **18** and **21**. Substitutions at the 3-position of the A-ring ring showed improvement in antitrypanosomal activity, with the 3-Br analogue **23** demonstrating an EC_{50} value of 1.6 μM, and the 3-Cl derivative **26** giving an EC_{50} value of 1.7 μM against *T.b.r*.

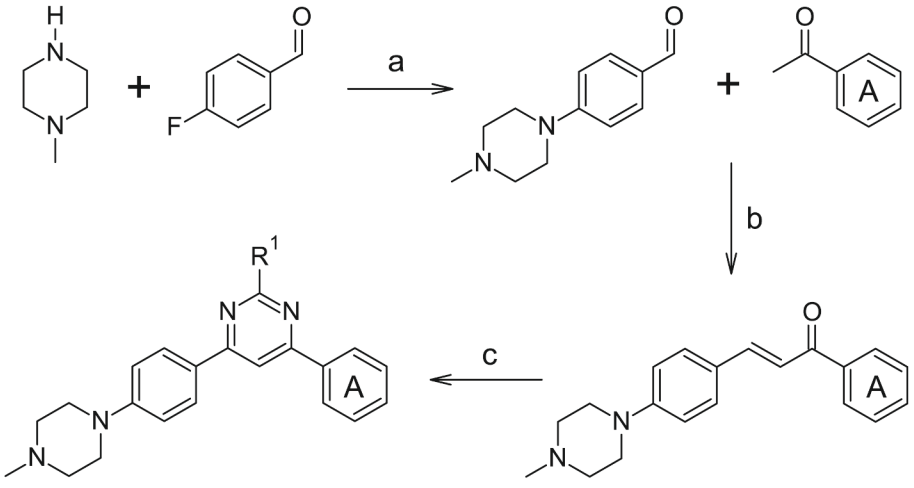
Exploration of the 4-position of the A-ring showed marked improvement, with the 4-Br analogue **29** and 4-Cl derivative **32** showing EC_{50} values of 0.6 and 0.5 μM against *T.b.r* respectively. Melarsoprol, which was used as the reference compound showed an expected EC_{50} value of 0.01 μM, which was 50-fold lower compared to **32**. This contrast can be extended to fexinidazole, as *in vitro* EC_{50} or EC_{90} values range between 0.76 to 3.31 μM as reported in the literature.[12] The 2,3 and 2,4-positions of the A-ring were explored with dichloro-substitutions, giving rise to compounds **35** and **37**. Both derivatives exhibited low micromolar activity against *T.b.r* with EC_{50} values of 1.8 and 2.4 μM, with no toxicity towards L6 cells.

Acetamidine derived pyrimidines yielded a 2-CH_3 substitution on the pyrimidine core. With a phenyl A-ring, **13** showed low micromolar activity against *T.b.r* with an EC_{50} value of 2.8 μM. In comparison, the 2-nitro analogue **16** showed a decrease in antitrypanosomal activity, but this improved with the 2-Br analogue **19** by 2-fold. The 3-Br substituted derivative **24**, alongside **27** bearing a 3-Cl substituent, further enhanced potency with EC_{50} values of 1.6 and 1.7 μM. Analogues **30** and **33**, bearing 4-Br and 4-Cl substituted A-rings proved beneficial as they demonstrated potent antitrypanosomal activity against *T.b.r*, with EC_{50} values of 0.9 and 0.6 μM. The incorporation of dichloro-substituted A-rings with **36** (2,3-Cl-Ph-) and **38** (2,4-Cl-Ph-) did not enhance antitrypanosomal activities.

The use of guanidine in the syntheses of compounds gave 2-NH_2 substituted pyrimidine cores.

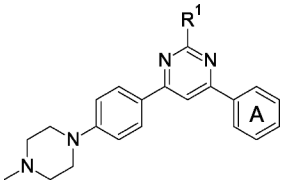
In comparison with **12** and **13**, the phenyl A-ring derivative **14** showed comparable low micromolar activity against *T.b.r* with an EC_{50} value of 3.6 μM. The 2-NO_2 substituted phenyl analogue **17**, as well as derivatives **20** and **22**, bearing 2-Br and 2-Cl-phenyl A-rings did not show improved potency. However, analogues **25** and **28** with 3-Br and 3-Cl-phenyl substituted A-rings markedly enhanced antitrypanosomal activities with EC_{50} values of 1.1 and 1.7 μM. Derivatives **31** and **34**, synthesised with 4-Br and 4-Cl substituted A-rings also demonstrated low micromolar activities against *T.b.r*, with EC_{50} values of 1.6 and 1.8 μM. Finally, the 2,4-Cl-phenyl analogue **39** demonstrated an EC_{50} value of 0.5 μM.

Overall, 1-methyl-4-phenylpiperazine containing pyrimidines syn-



Scheme 1. Reagents (a) K_2CO_3, DMF (b) $LiOH{\cdot}H_2O$, 1,4-dioxane (c) NaOEt, relevant amidine.

Table 1
Antitrypansomal activities and cytotoxicity evaluation of synthesised 4-[4-(4-methylpiperazin-1-yl)phenyl]-6-arylpyrimidines.



Compound	A-Ring	R^1	EC_{50} (μM)	
			T. b. rhodesiense	L6 cells
12	Ph-	–H	**3.8**	**42.7**
13	Ph-	$–CH_3$	**2.8**	**79.7**
14	Ph-	$–NH_2$	**3.6**	**30.0**
15	2-NO_2-Ph-	–H	**15.2**	**46.9**
16	2-NO_2-Ph-	$–CH_3$	**13.5**	**40.2**
17	2-NO_2-Ph-	$–NH_2$	**14.0**	**7.1**
18	2-Br-Ph-	–H	**9.7**	**39.6**
19	2-Br-Ph-	$–CH_3$	**4.3**	**35.2**
20	2-Br-Ph-	$–NH_2$	**10.3**	**11.6**
21	2-Cl-Ph-	–H	**5.8**	**42.6**
22	2-Cl-Ph-	$–NH_2$	**20.2**	**40.0**
23	3-Br-Ph-	–H	**1.6**	**64.5**
24	3-Br-Ph-	$–CH_3$	**1.6**	**21.1**
25	3-Br-Ph-	$–NH_2$	**1.1**	**33.6**
26	3-Cl-Ph-	–H	**1.7**	**36.3**
27	3-Cl-Ph-	$–CH_3$	**1.7**	**20.8**
28	3-Cl-Ph-	$–NH_2$	**1.7**	**79.7**
29	4-Br-Ph-	–H	**0.6**	**14.4**
30	4-Br-Ph-	$–CH_3$	**0.9**	**11.2**
31	4-Br-Ph-	$–NH_2$	**1.6**	**5.1**
32	4-Cl-Ph-	–H	**0.5**	**16.4**
33	4-Cl-Ph-	$–CH_3$	**0.6**	**5.3**
34	4-Cl-Ph-	$–NH_2$	**1.8**	**13.0**
35	2,3-Cl-Ph-	–H	**1.8**	**18.1**
36	2,3-Cl-Ph-	$–CH_3$	**3.6**	**37.3**
37	2,4-Cl-Ph-	–H	**2.4**	**>100**
38	2,4-Cl-Ph-	$–CH_3$	**10.1**	**>100**
39	2,4-Cl-Ph-	$–NH_2$	**0.5**	**7.4**
Melarsoprol			**0.01**	
Podophyllotoxin				**0.02**

thesised within this work are well-tolerated for antitrypanosomal activity against *T.b.r*, with limited toxicities observed against L6 cells (Figure 3). Substituted phenyl A-rings favoured –Br or –Cl at the 4-position, whilst retaining either –H or $–CH_3$ at the 2-position of the pyrimidine ring. However, this trend changed with **39**, which favoured a $–NH_2$ group at the 2-position of the pyrimidine ring whilst bearing a 2,4-

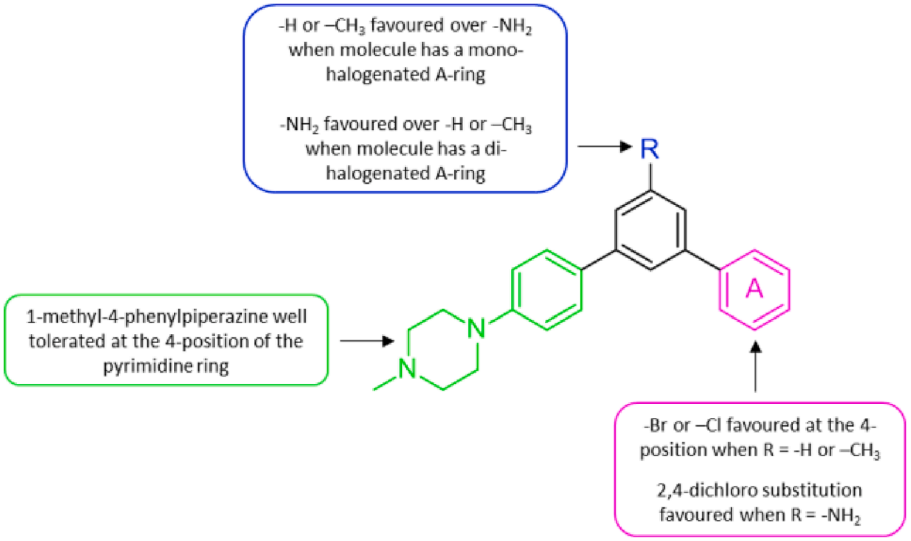


Figure 3. SAR of pyrimidines evaluated in this antitrypanosomal study.

dichloro substituted A-ring.

Compounds presented within this study were also screened against *Trypanosoma cruzi* (*T.c*, Tulahuen C4) and *Leishmania donovani* amastigotes (*L.d*, MHOM-ET-67/L82). However, no notable activities were observed.

CRediT authorship contribution statement

Annie E. Taylor: Investigation. **Moritz Hering:** Investigation. **Mark R.J. Elsegood:** Investigation. **Simon J. Teat:** Investigation. **George W. Weaver:** Investigation, Investigation. **Randolph R.J. Arroo:** Supervision. **Marcel Kaiser:** Investigation. **Pascal Maeser:** Supervision. **Avninder S. Bhambra:** Writing – review & editing, Writing – original draft, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgments

The Advanced Light Source is supported by the Director, Office of Science, Office of Basic Energy Sciences, of the U.S. Department of Energy under Contract No. DE-AC02-05CH11231. We thank De Montfort University for financial support and Loughborough University for facilities.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2024.129825.

References

[1]. dndi.org. (2022). Neglected Tropical Diseases (NTDs) | DNDi. [online] Available at: https://dndi.org/diseases/neglected-tropical-diseases/.
[2]. De Rycker M, Wyllie S, Horn D, Read KD, Gilbert IH. Anti-trypanosomatid drug discovery: progress and challenges. *Nature Rev Microbiol*. 2022. https://doi.org/10.1038/s41579-022-00777-y.
[3]. Cleghorn, L.A.T., Wall, R.J., Albrecht, S., MacGowan, S.A., Norval, S., Manu De Rycker, Woodland, A., Spinks, D., Thompson, S., Patterson, S., Victoriano Corpas Lopez, Dey, G., Collie, I.T., Hallyburton, I., Kime, R., Simeons, F.R.C., Stojanovski, L., Frearson, J.A., Wyatt, P.G. and Read, K.D. (2023). Development of a 2,4-Diaminothiazole Series for the Treatment of Human African Trypanosomiasis Highlights the Importance of Static–Cidal Screening of Analogues. doi:https://doi.org/10.1021/acs.jmedchem.3c00509.
[4]. www.who.int. (n.d.). *Trypanosomiasis, human African (sleeping sickness)*. [online] Available at: https://www.who.int/news-room/fact-sheets/detail/trypanosomiasis-human-african-(sleeping-sickness)#:~:text=Human%20African%20trypanosomiasis%2C%20also%20known.
[5]. Meisner J, Kato A, Lemerani MM, et al. The effect of livestock density on Trypanosoma brucei gambiense and T. b. rhodesiense: A causal inference-based approach. *PLoS Neglected Tropical Diseases*. 2022;16:e0010155–e. https://doi.org/10.1371/journal.pntd.0010155.
[6]. Robinson WJ, Taylor AE, Lauga-Cami S, et al. The discovery of novel antitrypanosomal 4-phenyl-6-(pyridin-3-yl) pyrimidines. *Eur J Med Chem*. 2021; 209, 112871.
[7]. Bhambra AS, Edgar M, Elsegood MRJ, et al. Design, synthesis and antitrypanosomal activities of 2,6-disubstituted-4,5,7-trifluorobenzothiophenes. *Eur J Med Chem*. 2016;108:347–353.
[8]. Bhambra AS, Ruparelia KC, Tan HL, et al. Synthesis and antitrypanosomal activities of novel pyridylchalcones. *Eur J Med Chem*. 2017;128:213–218.
[9]. Sheldrick GM. SHELXT– Integrated space-group and crystal-structure determination. *Acta Crystallographica Section A Found Adv*. 2015;71:3–8. https://doi.org/10.1107/s2053273314026370.
[10]. Sheldrick GM. Crystal structure refinement with SHELXL. *Acta Crystallographica Section C Struct Chem*. 2015;71:3–8. https://doi.org/10.1107/s2053229614024218.
[11]. Bernal FA, Kaiser M, Wünsch B, Schmidt TJ. Structure−activity relationships of cinnamate ester analogues as potent antiprotozoal agents. *ChemMedChem*. 2019; 15:68–78. https://doi.org/10.1002/cmdc.201900544.
[12]. EMA (2021). Fexinidazole Winthrop. [online] European Medicines Agency. Available at: https://www.ema.europa.eu/en/opinion-medicine-use-outside-EU/human/fexinidazole-winthrop.