New 2-aminopyrimidine derivatives and their antitrypanosomal and antiplasmodial activities

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Abstract
Novel 2-aminopyrimidine derivatives were prepared from acyclic starting materials, benzylidene acetones and ammonium thiocyanates, via 5 steps, including ring closure, aromatization, S-methylation, oxidation to methylsulfonyl compounds, and formation of guanidines with suitable amines. The prepared compounds differ from each other by the substitutions of their amino group and of their phenyl ring. The 2-aminopyrimidines were tested by use of microplate assays for their in vitro activities against a causative organism of sleeping sickness, Trypanosoma brucei rhodesiense, as well as against a causative organism of malaria, Plasmodium falciparum NF54. Their cytotoxic properties were determined with L-6 cells (rat skeletal myoblasts). Some of the compounds exhibited quite good antitrypanosomal activity, and others showed excellent antiplasmodial activity. The influence of the structural modifications on these activities is discussed.

Graphic abstract

Keywords Antiplasmodial activity · Antitrypanosomal activity · Heterocycles · Drug research · Structure–activity relationships

Introduction

In the past two decades, over two billion of the world’s poorest people have been affected by neglected tropical diseases (NTDs). One of the 11 major NTDs studied is human African trypanosomiasis (HAT) [1]. HAT or sleeping sickness is caused by protozoa of the genus Trypanosoma like Trypanosoma brucei gambiense (Tbg) and Trypanosoma brucei rhodesiense (Tbr). The vector is the tsetse fly. Only one drug, melarsoprol, is available for the late-stage Tbr infection treatment [2]. This toxic arsenic compound causes severe side effects including a deadly encephalopathy in more than 5% of the patients [3]. Therefore it is an urgent...
need to develop new efficient antitrypanosomal compounds with less side effects.

In 2018 malaria globally affected 228 million people and caused 405,000 deaths [4]. The emergence and spread of resistance in *Plasmodium falciparum* malaria to artemisinin combination therapies in the Greater Mekong subregion poses a major threat to malaria control and elimination [5]. Since the last defense line, the artemisinines, might fall possibly, there is a great demand for antiplasmodial compounds with alternative mechanism of action.

Substituted pyrimidines were described early as antiplasmodial compounds [6–12]. Some 2-aminopyrimidines were reported to be active in low micromolar to submicromolar concentration [13]. We prepared new methyl-aryl-substituted 2-aminopyrimidines including compounds bearing partial structures of chloroquine which were connected to the nitrogen in ring position 2.

### Results and discussion

Starting from benzylidene acetones 1a–1d pyrimidine-thiones 2a–2d were prepared by reaction with ammonium thiocyanate in refluxing benzene/cyclohexanol [14]. Aromatization of the heterocyclic ring took place in boiling xylene in the presence of sulfur giving compounds 3a–3d. Subsequently the SH group was methylated using methyl iodide in chloroform yielding 4a–4d. The next step was an oxidation to the methylsulfonyl compounds 5a–5d with *m*-chloroperbenzoic acid in dichloromethane. The final formation of the target compounds 6–10 took place in dioxane or tetrahydrofuran in the presence of the various amines under microwave irradiation at 120 °C or under reflux. Structural modifications were restricted to the amino substituent including amino, a (pyrrolidin-1-yl) and (4-methylpiperazine-1-yl) groups of compounds 6–8. Moreover, partial structures of chloroquine were used as substituents for compounds 9 and 10. The chiral aliphatic amine moiety was connected with the pyrimidine core giving compounds 9 as racemates. In a further step, the quinoline residue was attached. Similar compounds, bearing an additional ester function have already been investigated [15]. Further variations concerned the substitution pattern of a phenyl ring (Scheme 1).

**Scheme 1.** Syntheses of compounds 2–10. Reagents and conditions: (i) benzene, cyclohexanol, water separator, reflux, 6 h, (ii) S, xylene, reflux, overnight, (iii) CH₃I, CHCl₃, r.t., overnight to 3 days, (iv) *m*-chloroperbenzoic acid, CH₂Cl₂, 0–20 °C, 2 h, (v) NH₃ conc. or amine, dioxane or THF, 85 °C, reflux overnight or microwave 120 °C 2–13 h. For the aromatization from 2a–2d to 3a–3d we observed the disappearance of the proton signal at 4.9 ppm of the CH group attached to the aromatic moiety in ¹H NMR spectra as well as the shift of the signal of the olefinic proton from 4.7 ppm to 7.3 ppm. Furthermore, the signals of the NH protons at 8.8 and 9.5 ppm
disappeared and a new signal was observed for the SH group at 13.5 ppm. In $^{13}$C spectra, the signal of the carbon attached to the aromatic moiety shifted from 54 to 165 ppm due to aromatization. $S$-Methylation to compounds 4a–4d caused appearance of an additional signal at 2.6 ppm for the methylthio group in $^1$H NMR spectra and at 13.8 ppm in $^{13}$C spectra. The subsequent oxidation to the methylsulfonyl group in 5a–5d shifted the signal of the attached methyl group from 2.6 ppm to 3.4 ppm in $^1$H NMR spectra and from 13.8 ppm to 39 ppm in $^{13}$C spectra. The replacement of the methylsulfonyl group of compounds 5a–5d by amino substituents shifted the signal for the C-2 2–6 ppm to lower frequencies. Moreover, we observed long-range couplings from protons of the amino substituent to C-2 in HMBC spectra of compounds 6–10 which confirmed the attachment of the amino groups to this ring position.

All 2-aminopyrimidine derivatives 6–10 were tested for their antiplasmodial activities against P. falciparum NF54 and for their antitrypanosomal potencies against Trypanosoma brucei rhodesiense STIB 200 as well as for their cytotoxicity against rat skeletal myoblasts (L-6 cells) in microplate assays. The results are presented in Table 1.

A series of 4-alkyl-6-[(hetero)aryl]pyrimidin-2-aminos was reported to possess promising antiplasmodial activity ($IC_{50} = 0.115–3.96 \mu \text{M}$); however, the significance of the results was not sustained by cytotoxicity data [13]. Our 2-amine compounds 6a–6d, 7a–7d, and 8a–8d were completely inactive ($IC_{50} = 11.2–270 \mu \text{M}$) against P. falciparum NF54. The far lower activity of our 6-methyl compound 6b ($IC_{50} = 139 \mu \text{M}$) compared to its 6-isopropyl analogue ($IC_{50} = 3.96 \mu \text{M}$) [13] may be explained by the use of different strain test methods. The substitution of the amino group with the side chain of chloroquine improved the antiplasmodial activity (9a–9c: $IC_{50} = 2.62–73.0 \mu \text{M}$) only slightly. Moreover, the selectivity indexes of compounds 6–9 ($SI_{PN} = 1.08–7.84$) were very low. High activity against P. falciparum NF54 ($IC_{50} = 0.04–0.14 \mu \text{M}$) and good selectivity ($SI_{PN} = 81–220$) was observed for the 2-aminopyrimidines 10a–10d, which exhibit a 4-aminquinoline partial structure linked to their amino nitrogen. The most active compound 10d was additionally tested against the multiresistant $K_1$ strain of P. falciparum and showed slightly decreased activity ($IC_{50} = 0.14 \mu \text{M}$) but is more active than chloroquine ($IC_{50} = 0.27 \mu \text{M}$) [16] against this strain.

Most of the new compounds exhibited weak or negligible antitrypanosomal activity ($IC_{50} = 6.20–214 \mu \text{M}$) or low selectivity ($SI_T = 0.68–5.78$) or both of them. However, moderate activity ($IC_{50} = 1.90, 2.40 \mu \text{M}$) and quite good selectivity ($SI_T = 17.4, 25.5$) were observed for compounds 8b, 8c with 4-methylpiperazinyl substitution. The most promising antitrypanosomal compounds 9b and 9c ($IC_{50} = 0.41, 1.03 \mu \text{M}; SI_T = 30.7, 30.9$) feature the side chain of chloroquine.

### Table 1: Antiprotozoal and cytotoxic activities of compounds 6–10 ($IC_{50}$ values in µM)

| Cpd   | L-6 cells | P. falciparum NF54 | T. b. rhodesiense |
|-------|-----------|--------------------|------------------|
|       | $IC_{50}^a$ | $IC_{50}^a$ | $SI_{PN}^b$ | $IC_{50}^a$ | $SI_T^c$ |
| 6a    | 304       | 270               | 1.12            | 172         | 1.77     |
| 6b    | 439       | 139               | 3.16            | 75.9        | 5.78     |
| 6c    | 270       | 112               | 2.41            | 92.3        | 2.93     |
| 6d    | 465       | 204               | 2.28            | 37.9        | 12.3     |
| 7a    | 215       | 86.9              | 2.47            | 214         | 1.00     |
| 7b    | 107       | 22.7              | 4.71            | 158         | 0.68     |
| 7c    | 178       | 22.7              | 7.84            | 210         | 0.85     |
| 7d    | 178       | 35.3              | 5.04            | 185         | 0.96     |
| 8a    | 89.4      | 41.0              | 2.18            | 7.00        | 12.8     |
| 8b    | 33.1      | 11.2              | 2.96            | 1.90        | 17.4     |
| 8c    | 61.1      | 11.8              | 5.18            | 2.40        | 25.5     |
| 8d    | 70.2      | 29.6              | 2.37            | 6.20        | 11.3     |
| 9a    | 59.3      | 16.5              | 3.59            | 6.43        | 9.22     |
| 9b    | 12.6      | 2.62              | 4.81            | 0.41        | 30.7     |
| 9c    | 31.8      | 5.52              | 5.76            | 1.03        | 30.9     |
| 9d    | 78.8      | 73.0              | 1.08            | 6.93        | 11.4     |
| 10a   | 19.6      | 0.14              | 8.62            | 2.28        |         |
| 10b   | 9.90      | 0.045             | 220             | 4.22        | 2.35     |
| 10c   | 14.3      | 0.10              | 4.90            | 2.92        |         |
| 10d   | 3.24      | 0.04              | 81              | 3.98        | 0.81     |
| Mel$^d$ | 7.78      |                  | 0.0039          | 1995        |         |
| CQ$^e$ | 116.9     | 0.007             | 16,700          |         |         |
| P$^f$ | 0.012     |                  |                 |         |         |

$^a$Values represent the average of four determinations (two determinations of two independent experiments) indicated in µM

$^b$Selectivity index for P. falciparum NF54 ($SI_{PN}$), expressed as ratio $[IC_{50}^{L6}/IC_{50}^{P.falciparum NF54}]$

$^c$Selectivity index for T. b. rhodesiense ($SI_T$), expressed as ratio $[IC_{50}^{L6}/IC_{50}^{T. b. rhodesiense}]$

$^d$Mel, melarsoprol

$^e$CQ, chloroquine diphosphate

$^f$P, podophyllotoxin

### Conclusion

Several new methyl-aryl-substituted 2-aminopyrimidines with differing amino and phenyl substitution have been prepared. The antitrypanosomal and antiplasmodial activities of the new compounds were determined. The most active antitrypanosomal compounds ($IC_{50} = 0.41, 1.03 \mu \text{M}$) exhibited the same side chain as chloroquine. Compounds possessing the 7-chloroquinoline partial structure of chloroquine showed excellent activity against P. falciparum NF54 ($IC_{50} = 0.04–0.14 \mu \text{M}$). The most
active compound was additionally tested against the multi-resistant \( K \) strain of \( P. falciparum \) and showed twice the activity of chloroquine against this strain. Therefore, this compound could be a lead for further optimization.

**Experimental**

Melting points were obtained on a digital melting point apparatus Electrothermal IA 9200. IR spectra: Bruker Alpha Platinum ATR FT-IR spectrometer (KBr discs). NMR spectra: Varian Inova 400 (300 K) 5 mm tubes, spectra were acquired in CDCl3 containing 0.03% TMS. Chemical shifts were recorded in parts per million (ppm), for \(^1\)H spectra TMS (0.00 ppm) was used as internal standard and for \(^13\)C spectra the central peak of the CDCl3 peak was used as the internal reference (77.0 ppm). Some spectra were acquired in DMSO-\(d_6\). Here the proton signal at 2.49 ppm served as internal reference as well as the central peak of the DMSO-\(d_6\) signal at 39.7 ppm. Abbreviations: aromatic \( H \), Ar\( H \); aromatic \( C \), Ar\( C \); quaternary aromatic \( C \), Ar\( C_q \). Signal multiplicities are abbreviated as follows: s, singlet; d, doublet; t, triplet; m, multiplet; q, quartet; br, broad. Coupling constants \( (J) \) are reported in Hertz (Hz). \(^1\)H and \(^13\)C resonances were assigned using \(^1\)H, \(^1\)H- and \(^1\)H, \(^13\)C-correlation spectra. \(^1\)H and \(^13\)C resonances are numbered as given in the formulae. Assignments marked with an asterisk are interchangeable.

HRMS: GCT-Premier, Waters (EI, 70 eV). Materials: column chromatography (CC): silica gel 60 (Merck 70–230 mesh, pore-diameter 0.6 nm), aluminium oxide (Alox) basic (Fluka for chromatography, 0.05–0.15 mm, Brockmann activity I, basic); Alox neutral 90 (Merck, 0.063–0.2 mm, activity I, neutral); thin-layer chromatography (TLC): TLC plates (Merck, silica gel 60 F\( 254 \), 0.2 mm, 200 × 200 mm); TLC plates (Merck, Alox 60 F\( 254 \) neutral, 200 × 200 mm); the substances were detected in UV light at 254 nm. Unless otherwise stated silica gel was used for separations (CC, TLC). Microwave-assisted reactions were carried out in a CEM Discover/Explorer system in sealed 10 cm\(^3\) standard vessels with temperature control. Syntheses of compounds 2a, 2b, and 2d were described previously [14]. Compund 3a was prepared following a reported procedure [17]. Its melting point (201–205 °C) corresponded well with the reported one (202–204 °C) [18]. The synthesis of 4a was already described and the melting point (214 °C) corresponded well with the reported one (213 °C) [19].

**6-Methyl-4-(4-methylphenyl)-3,4-dihydropyrimidine-2(1H)-thione (2c, C\( 12 \)H\( 14 \)N\( 2 \)S)** The reaction of 21.27 g of 1c (132.76 mmol) with 8.26 g of ammonium thiocyanate (108.5 mmol) was carried out in 400 cm\(^3\) of toluene in the presence of 4.12 g of cyclohexanol (41.16 mmol). The mixture was heated for 18 h at 160 °C oil bath temperature using a water separator filled with molecular sieves 4 Å. After cooling to r.t., the orange precipitate was collected by filtration, washed with ether and ethanol. Then it was dissolved in a mixture of hot ethanol/isopropanol (3:1), treated with charcoal and filtered. The filtrate was concentrated in vacuo. Thereafter it was allowed to stand overnight at r.t. to complete crystallization. The product was collected by filtration, washed with ethanol, and dried. Yield: 11.85 g of 2c (41%) as white crystals. \( R_f = 0.84 \) (benzene:CHCl\(_3\):EtOH = 4:4:1); m.p.: 207 °C; \(^1\)H NMR (DMSO-\(d_6\), 400 MHz): \( \delta = 1.69 \) (s, 3H, CH\(_3\)), 2.26 (s, 3H, ArCH\(_3\)), 4.68 (d, \( J = 1.8 \) Hz, 1H, H-5), 4.85 (s, 1H, H-4), 7.10 (d, \( J = 8.1 \) Hz, 2H, ArH), 7.15 (d, \( J = 7.7 \) Hz, 2H, ArH), 8.76 (s, 1H, H-3), 9.52 (s, 1H, H-1).
Preparation of pyrimidine-2-thiols 3b–3d

The aromatization of dihydropyrimidine-2(1H)-thiones 2b, 2c, and 2d took place overnight in refluxing xylene in the presence of sulfur. The solution was then allowed to cool to r.t. A precipitate was formed which was collected by filtration, washed with water to cool to r.t.. A precipitate was formed which was collected by filtration. The solid was stirred with 1 N NaOH to cool to r.t. A precipitate was formed which was collected by filtration, washed with water and recrystallized.

4-(4-Chlorophenyl)-6-methylpyrimidine-2-thiol (3b, C_{11}H_{9}ClN_{2}S) Reaction of 3.99 g of 2b (16.7 mmol) with 3.2 g of sulfur (0.1 mol) in 24 cm³ of xylene yielded 2.02 g of 3b (44%) as orange powder.

4-Methyl-6-(4-methylphenyl)pyrimidine-2-thiol (3c, C_{12}H_{12}N_{2}S) Reaction of 6.29 g of 2d (26.86 mmol) with 1.03 g of sulfur (32.23 mmol) in 40 cm³ of xylene yielded 5.73 g of 3c (82%) as buff powder.

Preparation of (methylsulfanyl)pyrimidine hydroiodides 4b–4d

To a stirred suspension of 3b, 3c, or 3d in CHCl₃ methyliodide (CH₃I) was added dropwise. Stirring was continued at r.t. for up to 3 days. Then the formed solid was collected by filtration. The filtrate was evaporated to dryness and the residue was crystallized by treatment with ethyl acetate to give a second portion of the product. The solids were combined and dried.
Preparation of (methanesulfonyl)pyrimidines 5a–5d

The methylthio-pyrimidine hydroiodides 4a, 4b, 4c, or 4d were dissolved in CH₂Cl₂. The resulting solution was cooled down to 0 °C and 3-chloroperoxybenzoic acid (77%) was added slowly with stirring and cooling. The color of the solution turned to violet due to the oxidation of iodide to iodine. The organic layer was washed once with saturated NaHCO₃ solution, once with an aqueous Na₂S₂O₃ solution, and finally with brine. Then it was dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo giving white solids which were recrystallized with a small amount of ethyl acetate giving colorless needles.

2-(Methanesulfonyl)-4-(4-methoxyphenyl)-6-methylpyrimidine (5c, C₁₃H₁₄N₂O₂S) Reaction of 5.61 g of 4c (15.66 mmol) in 575 cm³ of CH₂Cl₂ with 11.77 g of 3-chloroperoxybenzoic acid (52.52 mmol) yielded 2.996 g of 5c (73%). Rᵣ = 0.44 (cyclohexane:ethyl acetate = 1:3); m.p.: 146–147 °C; ¹H NMR (CDCl₃, 400 MHz): δ = 2.43 (s, 3H, ArCH₃), 2.69 (s, 3H, CH₃), 3.42 (s, 3H, SO₂CH₃), 7.32 (d, J = 8.1 Hz, 2H, ArH), 7.71 (s, 1H, H-5), 8.03 (d, J = 8.1 Hz, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 21.46 (ArCH₃), 24.34 (CH₃), 38.99 (SO₂CH₃), 117.84 (C-5), 127.34, 129.82 (ArC), 131.83, 142.75 (ArC₉), 165.11 (C-6), 165.82 (C-2), 170.08 (C-4) ppm; IR (KBr): v = 3013, 1591, 1575, 1513, 1494, 1444, 1398, 1356, 1324, 1301, 1228, 1137, 1094, 1012, 968, 840, 754 cm⁻¹; HRMS (EI+): m/z calcd. (C₁₃H₁₄N₂O₂S) [M⁺] 262.0776, found 262.0766.

Preparation of pyrimidin-2-amines 6a–6d

The pyrimidin-2-amines were prepared similar to a reported procedure [20]. Compounds 5a, 5b, 5c, or 5d were suspended in dioxane and concentrated aqueous NH₃ was
addition. The reaction mixture was subjected to microwave irradiation at 120 °C. The solvents were washed with water, dried over anhydrous Na2SO4, and filtered. The solvent was evaporated in vacuo giving a crystalline residue. The crude products were purified by means of sublimation at reduced pressure yielding the products as white needles.

4-Methyl-6-phenylpyrimidin-2-amine (6a, C11H11N3) Reaction of 200 mg of 5a (0.81 mmol) with 1.34 cm3 of concentrated aqueous NH3 (18 mmol) in 4.8 cm3 of dioxane for 2 h yielded 66 mg of 6a (44%). The melting point (169–170 °C) corresponded well with the reported one (169–171 °C) [21]. Rf = 0.63 (CH2Cl2:CH3OH = 20:1); 1H NMR (DMSo-d6, 400 MHz): δ = 2.29 (s, 3H, CH3), 6.58 (s, 2H, NH2), 7.02 (s, 1H, H-5), 7.54–7.47 (m, 3H, ArH), 8.02–8.05 (m, 2H, ArH) ppm; 13C NMR (DMSo-d6, 100 MHz): δ = 23.91 (CH3), 105.38 (C-5), 126.86, 128.80, 130.43 (ArC), 137.46 (ArCq), 163.75 (C-6), 163.90 (C-2), 168.39 (C-4) ppm; IR (KBr): ν = 3323, 3194, 1637, 1601, 1579, 1551, 1464, 1353, 1277, 770 cm−1; HRMS (EI +): [M+]+ 219.0563, found 219.0553.

4-Methyl-6-chloro-phenylpyrimidin-2-amine (6b, C11H10ClN3) Reaction of 200 mg of 5b (0.76 mmol) with 1.27 cm3 of concentrated aqueous NH3 (17 mmol) in 4.6 cm3 of dioxane for 3 h yielded 55.46 mg of 6b (30%). The melting point (198 °C) was similar to the reported one (204 °C) [22]. Rf = 0.54 (CH2Cl2:CH3OH = 9:1); 1H NMR (DMSo-d6, 400 MHz): δ = 2.27 (s, 3H, CH3), 6.62 (s, 2H, NH2), 7.03 (s, 1H, H-5), 7.52 (d, J = 8.4 Hz, 2H, ArH), 8.05 (d, J = 8.8 Hz, 2H, ArH) ppm; 13C NMR (DMSo-d6, 100 MHz): δ = 23.91 (CH3), 105.27 (C-5), 126.82, 128.86 (ArC), 135.19, 136.26 (ArCq), 162.42 (C-4), 163.86 (C-2), 168.68 (C-6) ppm; IR (KBr): ν = 3306, 3162, 1639, 1595, 1572, 1514, 1463, 1417, 1382, 1353, 1280 cm−1; HRMS (EI +): m/z calcd. (C11H11N3) [M+]+ 185.0953, found 185.0942.

4-(4-Chlorophenyl)-6-methylpyrimidin-2-amine (6c, C12H13N3Cl) Reaction of 215 mg of 5c (0.76 mmol) with 1.16 cm3 of concentrated aqueous NH3 (16.9 mmol) in 4.6 cm3 of dioxane for 3 h yielded 55 mg of 6c (33%). The melting point (202 °C) corresponded well with the reported one (204 °C) [24]. Rf = 0.74 (CH2Cl2:CH3OH = 9:1); 1H NMR (DMSo-d6, 400 MHz): δ = 2.26 (s, 3H, CH3), 3.80 (s, 3H, OCH3), 6.47 (s, 2H, NH2), 6.96 (s, 1H, H-5), 7.01 (d, J = 9.2 Hz, 2H, ArH), 8.01 (d, J = 8.8 Hz, 2H, ArH) ppm; 13C NMR (DMSo-d6, 100 MHz): δ = 23.90 (CH3), 55.46 (OCH3), 104.56 (C-5), 114.13, 128.39 (ArC), 129.71, 161.25 (ArCq), 163.33 (C-4), 167.79 (C-2), 167.98 (C-6) ppm; IR (KBr): ν = 3310, 3176, 1636, 1608, 1579, 1545, 1514, 1463, 1417, 1382, 1351, 1249, 1233, 1182, 1033, 820 cm−1; HRMS (EI +): m/z calcd. (C12H13N3ClO) [M+]+ 215.1059, found 215.1045.

Preparation of (pyrrolidin-1-yl)pyrimidines 7a–7d

The compounds 5a, 5b, 5c, or 5d were dissolved in dry THF and pyrrolidine was added. The reaction mixture was refluxed at 85 °C overnight or subjected to microwave irradiation. Water was added and the mixture was extracted five times with diethyl ether. The combined organic layers were washed neutral with water, dried over anhydrous Na2SO4, and filtered. The solvent was evaporated in vacuo giving pure compounds 7a–7d as white to yellowish needles. For analytical purposes they were recrystallized giving white needles.

4-Methyl-6-phenyl-2-(pyrrolidin-1-yl)pyrimidine (7a, C13H17N) Method 1: Reaction of 218 mg of 5a (0.88 mmol) in 45 cm3 of dry THF with 157 mg of pyrrolidine (2.21 mmol) yielded 200 mg of 7a (95%). Method 2: Reaction of 200 mg of 5a (0.81 mmol) in 4 cm3 of dry THF with 172 mg of pyrrolidine (2.42 mmol) yielded after 1 h microwave irradiation at 100 °C 148 mg of 7a (76%). Rf = 0.84 (CH2Cl2:CH3OH = 10:1); m.p.: 88 °C (ethyl acetate); 1H NMR (CDCl3, 400 MHz): δ = 1.90 (t, J = 6.6 Hz, 4H, (CH2)2), 2.33 (s, 3H, CH3), 3.60 (br, s, 4H, N(CH2)2), 6.72 (s, 1H, H-5), 7.34–7.36 (m, 3H, ArH), 7.97–7.99 (m, 2H, ArH) ppm; 13C NMR (CDCl3, 100 MHz): δ = 24.45 (CH3), 25.51 ((CH2)2), 46.63 (N(CH2)2), 104.33 (C-5), 126.93, 128.46, 129.98 (ArC), 138.10 (ArCq), 160.70 (C-2), 163.95 (C-6), 167.69 (C-4) ppm; IR (KBr): ν = 2971, 2928, 2857, 1587, 1555, 1512, 1482, 1458, 1374, 1336, 1233, 1183, 771, 693 cm−1; HRMS (EI +): m/z calcd. (C13H17N) [M+]+ 239.1422, found 239.1421.
4-(4-Chlorophenyl)-6-methyl-2-(pyrrolidin-1-yl)pyrimidine (7b, C_{15}H_{16}ClN_{3}) Reaction of 300 mg of 5b (1.06 mmol) in 5 cm³ of dry THF with 225 mg of pyrrolidine (3.16 mmol) yielded after 2 h microwave irradiation at 120 °C 87 mg of 7b (30%). Rf = 0.85 (CH₃Cl₂:CH₃OH = 10:1); m.p.: 87 °C (MeOH); ¹H NMR (CDCl₃, 400 MHz): δ = 1.97–2.01 (m, 4H, (CH₂)₂), 2.41 (s, 3H, CH₃), 3.65–3.68 (m, 4H, N(CH₂)₂), 6.76 (s, 1H, H-5), 7.41 (d, J = 8.4 Hz, 2H, ArH), 8.00 (d, J = 8.8 Hz, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 24.49 (CH₂), 25.52 (CH₃), 46.66 (N(CH₂)₂), 104.04 (C-5), 128.25, 128.68 (ArC), 136.03, 136.56 (ArC₈), 160.62 (C-2), 162.70 (C-4), 167.99 (C-6) ppm; IR (KBr): v = 2866, 1595, 1582, 1513, 1487, 1459, 1376, 1338, 1223, 1090, 1013, 806 cm⁻¹; HRMS (EI +): calcd. (C₁₅H₁₆ClN₃) [M⁺] 253.1579, found 253.1575.

4-Methyl-6-(4-methylphenyl)-2-(pyrrolidin-1-yl)pyrimidine (7c, C_{15}H₁₉N₃) Reaction of 400 mg of 5c (1.52 mmol) in 15 cm³ of dry THF with 651 mg of pyrrolidine (9.15 mmol) yielded 371 mg of 7c (96%). Rf = 0.60 (cyclohexane:ethyl acetate = 1:3); m.p.: 76 °C (ethyl acetate); ¹H NMR (CDCl₃, 400 MHz): δ = 1.96–1.99 (m, 4H, (CH₂)₂), 2.38 (s, 3H, ArCH₃), 2.39 (s, 3H, CH₃), 3.65–3.68 (m, 4H, N(CH₂)₂), 6.77 (s, 1H, H-5), 7.24 (d, J = 8.1 Hz, 2H, ArH), 7.96 (d, J = 8.1 Hz, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 21.34 (ArCH₃), 24.53 (CH₃), 25.53 (CH₂), 46.56 (N(CH₂)₂), 104.03 (C-5), 126.82, 129.18 (ArC), 135.36, 140.08 (ArC₈), 160.88 (C-2), 163.80 (C-6), 167.66 (ArC₈) ppm; IR (KBr): v = 2944, 2870, 1553, 1508, 1482, 1459, 1375, 1337, 1220, 1182, 1111, 803 cm⁻¹; HRMS (EI +): m/z calcd. (C₁₅H₁₉N₃) [M⁺] 253.1579, found 253.1575.

Preparation of 2-(4-methylpiperazin-1-yl)pyrimidines 8a–8d

The compounds 5a, 5b, 5c, or 5d were dissolved in dry THF and 1-methylpiperazine was added. The reaction mixture was refluxed at 100 °C overnight or subjected to microwave irradiation. Water was added and the mixture was extracted five times with diethyl ether. The combined organic layers were washed neutral with water, dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo giving a resin which was further purified.

4-Methyl-2-(4-methylpiperazin-1-yl)-6-phenylpyrimidine (8a, C_{16}H_{20}N₄) Reaction of 300 mg of 5a (1.21 mmol) in 12 cm³ of dry THF with 726 mg of 1-methylpiperazine (7.24 mmol) yielded a yellow resin which was purified by means of CC (CH₂Cl₂:CH₃OH = 40:1, Alox neutral) giving 245 mg of 8a (75%) as a yellow oil which crystallized upon cooling. Rf = 0.62 (CH₂Cl₂:CH₃OH = 40:1, Alox neutral); m.p.: 65 °C; ¹H NMR (CDCl₃, 400 MHz): δ = 2.36 (s, 3H, NCH₃), 2.40 (s, 3H, CH₃), 2.50–2.53 (m, 4H, H-3'), 3.96–3.98 (m, 4H, H-2'), 6.84 (s, 1H, H-5), 7.44–7.45 (m, 3H, ArH), 8.02–8.04 (m, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 24.50 (CH₃), 43.62 (C-2'), 46.21 (NCH₃), 55.04 (C-3'), 105.32 (C-5), 126.93, 128.51, 130.11 (ArC), 137.93 (ArC₈), 162.00 (C-2), 164.00 (C-6), 168.06 (C-4) ppm; IR (KBr): V = 2924, 2794, 1558, 1493, 1443, 1368, 1345, 1300, 1272, 1223, 1188, 1173, 1073, 1007, 994, 890, 815, 764, 687 cm⁻¹; HRMS (EI +): m/z calcd. (C₁₆H₂₀N₄) [M⁺] 268.1688, found 268.1693.

4-(4-Chlorophenyl)-6-methyl-2-(4-methylpiperazin-1-yl)pyrimidine (8b, C_{16}H₁₉ClN₄) Reaction of 375 mg of 5b (1.33 mmol) in 5 cm³ of dry THF with 787 mg of 1-methylpiperazine (7.86 mmol) yielded after 5 h microwave irradiation at 120 °C a yellow resin. It was purified by means of CC (CH₂Cl₂:CH₃OH = 10:1) giving 235 mg of 8b (58%) as a white amorphous solid. Rf = 0.43 (CH₂Cl₂:CH₃OH = 10:1); ¹H NMR (DMSO-d₆, 400 MHz): δ = 2.20 (s, 3H, NCH₃), 2.33 (s, 3H, CH₃), 2.34–2.36 (m, 4H, H-3'), 3.75–3.82 (m, 4H, H-2'), 7.11 (s, 1H, H-5), 7.53 (d, J = 8.4 Hz, 2H, ArH), 8.11 (d, J = 8.4 Hz, 2H, ArH) ppm; ¹³C NMR (DMSO-d₆, 100 MHz): δ = 24.36 (CH₃), 43.46 (C-2'), 46.06 (NCH₃), 54.67 (C-3'), 105.06 (C-5), 128.71, 128.93 (ArC), 135.41, 136.11 (ArC₈), 161.59 (C-2), 161.91 (C-4), 168.61 (C-6) ppm; IR (KBr): V = 2930, 2795, 1585, 1556, 1508, 1491, 1447, 1371, 1345, 1303, 1283, 1269, 1093, 1006, 995, 804 cm⁻¹; HRMS (EI +): m/z calcd. (C₁₆H₁₉ClN₄) [M⁺] 302.1298, found 302.1310.

4-Methyl-6-(4-methylphenyl)-2-(4-methylpiperazin-1-yl)pyrimidine (8c, C_{17}H₂₂N₄) Reaction of 400 mg of 5c
(1.52 mmol) in 3 cm³ of dry THF with 916 mg of 1-methylpiperazine (9.14 mmol) yielded after 7 h microwave irradiation at 120 °C a residue which was purified by means of CC (CH₂Cl₂:CH₃OH = 10:1) giving 334 mg of 8c (78%) as an orange oil which crystallized upon cooling. Rᵣ = 0.43 (CH₂Cl₂:CH₃OH = 10:1); m.p.: 73 °C; ¹H NMR (CDCl₃, 400 MHz): δ = 2.34 (s, 3H, NCH₃), 2.38 (s, 3H, CH₃), 2.39 (s, 3H, ArCH₃), 2.48–2.50 (m, 4H, H-3′), 3.94–3.96 (m, 4H, H-2′), 6.80 (s, 1H, H-5), 7.24 (d, J = 8.4 Hz, 2H, ArH), 7.93 (d, J = 8.1 Hz, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 21.33 (ArCH₃), 24.66 (C₆H₄), 43.67 (C-2′), 46.25 (NCH₃), 55.06 (C-3′), 104.94 (C-5), 126.81, 129.20 (ArC), 131.50, 140.25 (ArCq), 161.99 (C-2′), 163.91 (C-6), 167.82 (C-4′) ppm; IR (KBr): ν = 3268, 2936, 2788, 1610, 1586, 1570, 1557, 1504, 1447, 1371, 1345, 1300, 1285, 1272, 1008, 804 cm⁻¹; HRMS (EI +): m/z calcd. (C₁₇H₂₂N₄O) [M⁺] 282.1850, found 282.1844, identified 282.1850.

4-(4-Methoxyphenyl)-6-methyl-2-(4-methylpiperazin-1-yl)pyrimidine (8d, C₁₇H₂₂N₄O) Reaction of 250 mg of 5d (0.90 mmol) in 9 cm³ of dry THF with 540 mg of 1-methylpiperazine (5.39 mmol) yielded after 3 days refluxing at 100 °C a residue which was crystallized from ethyl acetate giving 141 mg of 8d (53%) as white needles. Rᵣ = 0.43 (CH₂Cl₂:CH₃OH = 10:1); m.p.: 93 °C; ¹H NMR (CDCl₃, 400 MHz): δ = 2.34 (s, 3H, NCH₃), 2.38 (s, 3H, CH₃), 2.48–2.50 (m, 4H, H-3′), 3.84 (s, 3H, OCH₃), 3.95 (br, s, 4H, H-2′), 6.77 (s, 1H, H-5), 6.95 (d, J = 8.4 Hz, 2H, ArH), 8.01 (d, J = 8.4 Hz, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 24.45 (CH₃), 43.67 (C-2′), 46.25 (NCH₃), 55.06 (C-3′), 104.46 (C-5), 113.78, 128.33 (ArC), 130.34, 161.30 (ArCq), 161.95 (C-2′), 163.44 (C-4′), 167.67 (C-6′) ppm; IR (KBr): ν = 3268, 2936, 2788, 1610, 1586, 1570, 1557, 1514, 1492, 1459, 1350, 1301, 1286, 1270, 1258, 1172, 1027, 1009, 813 cm⁻¹; HRMS (EI +): m/z calcd. (C₁₇H₂₂N₄O) [M⁺] 298.1794, found 298.1793.

Preparation of N-[5-(diethylamino)pentan-2-yl]pyrimidin-2-amines 9a–9d

The compounds 5a, 5b, 5c, or 5d were dissolved in dry THF and 2-amino-5-(diethylamino)pentane was added. The reaction mixture was subjected to microwave irradiation. Water was added and the mixture was extracted five times with diethyl ether. The combined organic layers were washed neutral with water, dried over anhydrous Na₂SO₄ and filtered. The solvent was evaporated in vacuo giving residues which were further purified.

N-[5-(Diethylamino)pentan-2-yl]-4-methyl-6-phenylpyrimidin-2-amine (9a, C₂₀H₂₉N₄) Reaction of 200 mg of 5a (0.81 mmol) in 4 cm³ of dry THF with 766 mg of 2-amino-(diethylamino)pentane (4.84 mmol) yielded after 12 h microwave irradiation at 120 °C a residue which was purified by means of CC (CH₂Cl₂:CH₃OH = 40:1, Alox neutral) giving 122 mg of 9a (46%) as yellow oil. Rᵣ = 0.24 (CH₂Cl₂:CH₃OH = 40:1, Alox neutral); ¹H NMR (CDCl₃, 400 MHz): δ = 1.03 (t, J = 7.1 Hz, 6H, H-2′), 1.26 (d, J = 6.5 Hz, 3H, H-1′), 1.54–1.65 (m, 4H, H-3′, H-4′), 2.38 (s, 3H, CH₃), 2.50–2.55 (m, 2H, H-5′), 2.56 (q, J = 7.1 Hz, 4H, H-1′), 4.23–4.28 (m, 1H, H-2′), 4.96 (d, J = 8.3 Hz, 1H, NH), 6.84 (s, 1H, H-5), 7.44–7.46 (m, 3H, ArH), 8.01–8.02 (m, 2H, ArH) ppm; ¹³C NMR (CDCl₃, 100 MHz): δ = 11.28 (C-2′′), 21.12 (C-1′′), 23.13 (C-4′′), 24.32 (CH₃), 35.11 (C-3′′), 46.48 (C-2′′), 47.61 (C-1′′), 52.71 (C-5′′), 105.75 (C-5), 126.92, 128.55, 130.13 (ArC), 137.85 (ArCq), 162.27 (C-2′′), 164.44 (C-6), 168.25 (C-4′′) ppm; IR (KBr): ν = 3268, 2965, 2954, 2925, 2858, 1574, 1551, 1495, 1458, 1373, 1346, 1203, 1069, 767, 693 cm⁻¹; HRMS (EI +): m/z calcd. (C₂₀H₂₉N₄) [M⁺] 326.2470, found 326.2471.
4.97 (d, J = 8.1 Hz, 1H, NH), 6.80 (s, 1H, H-5), 7.24 (d, J = 8.1 Hz, 2H, ArH) ppm; 13C NMR (CDCl₃, 100 MHz): δ = 11.56 (C-2'), 21.02 (C-1'), 21.33 (ArCH₃), 23.45 (C-4'), 24.24 (CH₃), 35.14 (C-3'), 46.50 (C-2'), 46.71 (C-1'), 52.84 (C-5'), 105.30 (C-5), 126.79, 129.21 (ArC), 135.01, 140.25 (ArC'), 162.21 (C-2'), 164.32 (C-6), 167.96 (C-4) ppm; IR (KBr): v = 2965, 1574, 1549, 1510, 1454, 1374, 1345, 1182, 806 cm⁻¹; HRMS (EI+): m/z calcd. (C₂₁H₂₆N₄) [M⁺] 340.2627, found 340.2632.

N-[5-(Diethylamino)pentan-2-yl]-4-(4-methoxyphenyl)-6-methylpyrimidin-2-amine (9d, C₁₂H₂₂N₂O₂) Reaction of 300 mg of 5d (1.08 mmol) in 3 cm³ of dry THF with 1.02 g (65%) as yellow oil. Rf = 0.35 (CH₂Cl₂:CH₃OH = 60:1, Alox neutral); 1H NMR (CDCl₃, 400 MHz): δ = 1.00 (t, J = 7.3 Hz, 6H, H-2, H-3, H-4, H-5), 2.35 (s, 3H, CH₃), 2.43–2.46 (m, 2H, H-1'), 2.51 (q, δ = 7.3 Hz, 4H, H-1''), 3.85 (s, 3H, OCH₃), 4.21–4.24 (m, 1H, H-2'), 4.96 (d, J = 8.1 Hz, 1H, NH), 6.77 (s, 1H, H-5), 6.96 (d, J = 8.8 Hz, 2H, ArH), 8.00 (d, J = 8.8 Hz, 2H, ArH) ppm; 13C NMR (CDCl₃, 100 MHz): δ = 24.04 (CH₃), 39.07 (C-2), 52.85 (C-5), 55.26 (OCH₃), 104.80 (C-5), 113.80, 128.33 (ArC), 130.25, 161.30 (ArC'), 162.16 (C-2), 163.85 (C-4), 167.81 (C-6) ppm; IR (KBr): v = 2964, 1780, 1741, 1674, 1604, 1565, 1503, 1455, 1367, 1345, 1205, 1171, 1033, 810 cm⁻¹; HRMS (EI+): m/z calcd. (C₁₂H₂₂N₂O₂) [M⁺] 356.2576, found 356.2585.

Preparation of N-[2-[[pyrimidin-2-ylamino]ethyl]-7-chloroquinolin-4-amine 10a–10d

The compounds 5a, 5b, 5c, or 5d were dissolved in dry THF and N-(2-aminoethyl)-7-chloroquinolin-4-amine was added. The reaction mixture was subjected to microwave irradiation. The solution was transferred into a separatory funnel and water was added. The mixture was extracted five times. The combined organic layers were washed with water, dried over anhydrous Na₂SO₄, and filtered. The solvent was evaporated in vacuo giving a residue which was further purified.

7-Chloro-N-[2-[4-(methyl-6-phenylpyrimidin-2-ylamino)ethyl]quinolin-4-amine (10c, C₂₃H₂₁ClN₃) Reaction of 226 mg of 5b (0.8 mmol) in 5 cm³ of dry THF with 714 mg of N-(2-aminoethyl)-7-chloroquinolin-4-amine (3.22 mmol) yielded after 5.5 h microwave irradiation at 120 °C and extraction with tert-butylmethyl ether a residue. This was purified by CC (CH₂Cl₂:CH₃OH = 40:1, Alox neutral) giving 285 mg of 10c (45%) as white foam.

7-Chloro-N-[2-[4-(4-chlorophenyl)-6-methylpyrimidin-2-ylamino]ethyl]quinolin-4-amine (10b, C₂₂H₁₉ClN₅) Reaction of 226 mg of 5b (0.8 mmol) in 5 cm³ of dry THF with 714 mg of N-(2-aminoethyl)-7-chloroquinolin-4-amine (3.22 mmol) yielded after 5.5 h microwave irradiation at 120 °C and extraction with tert-butylmethyl ether a residue. This was purified by CC (CH₂Cl₂:CH₃OH = 40:1, Alox neutral) giving 62 mg of 10b (18%) as a white-yellow amorphous solid. Rf = 0.26 (CH₂Cl₂:CH₃OH = 10:1, Alox neutral); 1H NMR (DMSO-d₆, 400 MHz): δ = 3.34–3.75 (m, 2H, H-5), 3.56–3.70 (m, 2H, H-2), 6.64–6.80 (m, 1H, ArH), 7.09 (s, 1H, H-5), 7.33–7.56 (m, 5H, 2NH, ArH), 7.76 (s, 1H, ArH), 8.03 (d, J = 8.3 Hz, 2H, ArH), 8.14 (br, s, 1H, ArH), 8.34–8.38 (m, 1H, ArH) ppm; 13C NMR (DMSO-d₆, 100 MHz): δ = 23.94 (CH₃), 38.81 (C-2'), 42.01 (C-3'), 98.98 (ArC), 105.47 (C-5), 117.63 (ArC), 124.12, 127.72, 128.70, 128.93 (ArC), 133.54, 135.32, 149.29, 150.33 (ArC'), 152.07 (ArC), 162.24 (C-4), 162.70 (C-2), 168.63 (C-6) ppm; IR (KBr): v = 3433, 3268, 2927, 1577, 1556, 1492, 1456, 1371, 1342, 1330, 1239, 1140, 1092, 1013, 808 cm⁻¹; HRMS (EI+): m/z calcd. (C₂₃H₁₉ClN₅) [M⁺] 423.1017, found 423.1056.
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In vitro assays

The in vitro growth inhibition assay of *P. falciparum* Kf was performed according to an established procedure [25]. The in vitro growth inhibition assay of *P. falciparum* NF54 and the in vitro growth inhibition assay of *Trypanosoma b. rhodesiense*, as well as the assay for the determination of cytotoxicity against L6-cells were performed as described earlier [26].

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