Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 605–611
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Laboratory note

Synthesis and DHFR inhibitory activity of a series of 6-substituted-2,4-diaminothieno[2,3-*d*]pyrimidines

Isaac O. Donkor [a,*], Hui Li [a], Sherry F. Queener [b]

[a] *Department of Pharmaceutical Sciences, University of Tennessee Health Science Center, Johnson Building, Room 327E, 847 Monroe Avenue, Memphis, TN 38163, USA*
[b] *Department of Pharmacology and Toxicology, Indiana University School of Medicine, Indianapolis, IN 46202, USA*

Received 30 December 2002; received in revised form 8 April 2003; accepted 10 April 2003

Abstract

A series of 6-aralkyl substituted 2,4-diaminothieno[2,3-*d*]pyrimidines in which the 6-aryl group is separated from the thieno[2,3-*d*]pyrimidine ring by two to five methylene groups were synthesized and studied as inhibitors of dihydrofolate reductase from *Pneumocystis carinii*, *Toxoplasma gondii*, *Mycobacterium avium*, and rat liver. Compounds in which the thieno[2,3-*d*]pyrimidine ring is separated from the 6-aryl substituent by three methylene groups were the most potent inhibitors of the series (with IC_{50} values ranging from 0.24 and 11.0 μM) but those with two methylene groups between the aromatic rings were the most selective agents.

Keywords: *Pneumocystis carinii*; Pneumonia; DHFR; Diaminothieno[2,3-*d*]pyrimidines; Opportunistic infections

1. Introduction

Pneumocystis carinii, *Toxoplasma gondii*, and *Mycobacteria* are among the opportunistic pathogens that afflict AIDS patients [1]. *P. carinii* causes pneumonia in AIDS patients; *T. gondii* causes toxoplasmic encephalitis; while *Mycobacteria*, including *Mycobacterium avium* complex (MAC) and *Mycobacterium tuberculosis* cause systemic infections in AIDS patients. Since 1996, the introduction of potent combinations of antiretroviral regimen known as HAART (highly active antiretroviral therapy) has resulted in a dramatic decrease in opportunistic infections associated with severe HIV infection in industrialized nations [2–5]. However, an appreciable number of patients remain unaware of their HIV status, refuse antiretroviral therapy or show poor adherence to HAART, and as such remain at high risk of developing severe opportunistic disorders due to their persistently low CD4+ cell count [6,7]. Also, the limited availability of HAART in the developing world implies that AIDS patients are still at risk from opportunistic infections [8]. Thus, despite the effectiveness of HAART therapy there is still the need for new agents to treat AIDS associated opportunistic infections.

Trimethoprim (**1**, TMP), trimetrexate (**2**, TMQ), and piritrexim (**3**, PTX) (Fig. 1) are lipophilic compounds that have been used clinically for the prophylaxis and treatment of *P. carinii* and *T. gondii* infections in AIDS patients [9]. The molecular target of TMP, TMQ and PTX is the enzyme dihydrofolate reductase (DHFR). DHFR plays a pivotal role in one-carbon metabolism, which is critical to the biosynthesis of DNA, RNA, and the essential amino acid methionine [10]. Several research groups have established programs aimed at discovering potent and pathogen selective DHFR inhibitors [11–18] as agents for treating AIDS associated opportunistic infections. As a part of this effort, we have synthesized eight new 6-substituted 2,4-diaminothieno[2,3-*d*]pyrimidines **4a**–**h** (Fig. 1) and studied them as inhibitors of DHFR isolated from rat liver, *P. carinii*, *T. gondii*, and *M. avium*.

* Corresponding author.
E-mail address: idonkor@utmem.edu (I.O. Donkor).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00101-6

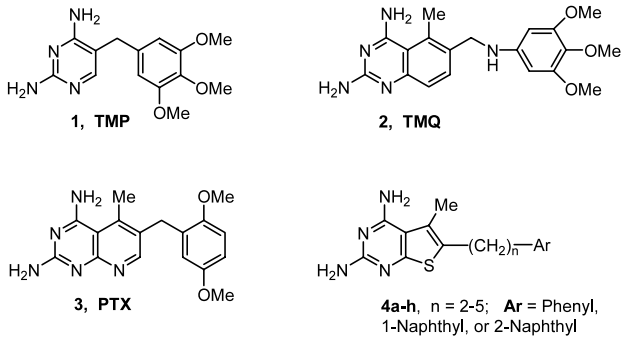

Fig. 1. Structures of known (**1**–**3**) and novel (**4a**–**h**) DHFR inhibitors.

2. Chemistry

Synthesis of compounds **4a**–**h** commenced with the synthesis of the required but commercially unavailable aldehydes **8a** and **8b** (Fig. 2) and **11a**–**d** (Fig. 3). The synthesis of aldehydes **8a** and **8b** involved a Wittig reaction followed by catalytic reduction and acidolysis. The ^{1}H-NMR spectra showed a mixture of *cis* and *trans* geometric isomers for **6a** but only the *trans* isomer was evident for **6b**. Aldehydes **11a**–**d** (Fig. 3) were derived from the appropriate carboxylic acids by coupling the acid with *N*,*O*-dimethylhydroxylamine in the presence of NMM using EDC as the coupling reagent. Partial reduction of the resulting Weinreb amides **10a**–**d** with $LiAlH_4$ gave the desired aldehydes in 97–100% yields. The aldehydes were transformed to α,β-unsaturated ketones **12a**–**h**, which were subjected to catalytic reduction to give ketones **13a**–**h**. The ketones were condensed with malononitrile to give ylidenemalononitriles **14a**–**h**. Ring closure with elemental sulphur in the presence of *i*-Pr_2NH afforded thiophene amino nitriles **15a**–**h** in low to moderate yields (15–54%). The reaction was sensitive to temperature and low yields were obtained at temperatures greater than 60 °C. Most of the thiophene amino nitriles were not stable even when stored under vacuum for a few weeks and had to be used immediately. Thermal fusion (120 °C internal temperature) of the thiophene amino nitriles with chloroformamidine hydrochloride for 30 min followed by treatment with *i*-Pr_2NH at 70–80 °C for 2 h afforded compounds **4a**–**h** [19–21].

3. Results and discussion

6-Substituted 2,4-diaminothieno[2,3-*d*]pyrimidines in which the 6-aryl substituents were either attached directly to the heterocyclic ring or separated from it by one or two methylene groups have been reported to inhibit DHFR isolated from *P. carinii* and *T. gondii* [19]. We synthesized compounds **4a**–**h** with two to five methylene groups between the 6-aryl substituent and the 2,4-diaminothieno[2,3-*d*]pyrimidine pharmacophore to investigate the optimal number of methylene groups between the aromatic rings that is required for potent and selective inhibition of *P. carinii* DHFR, *T. gondii* DHFR, and *M. avium* DHFR compared to rat liver DHFR. The IC_{50} values of the compounds for inhibition of the DHFRs were determined using previously reported procedures [22–24].

As shown in Table 1, the IC_{50} values for inhibition of *P. carinii* DHFR varied from 1.5 to 25 μM with selectivity ratios between 0.33 and 5.7. The length of the alkyl bridge influenced the inhibition of *P. carinii* DHFR. For example, the order of inhibition of *P. carinii* DHFR by compounds with 6-phenylalkyl substituents was **4d** > **4g** ≥ **4a** > **4h**. In this series **4d** with three methylene groups between the aromatic rings was over eightfold more potent than **4h**, which has a bridge length of five methylene groups. Similarly, among the derivatives with alkylnaphthalene substituents, compounds with a bridge length of three methylene groups were better inhibitors of the *P. carinii* enzyme than those with two methylene groups (**4e** > **4b**, and **4f** > **4c**). Compound **4e** with three methylene groups between the aromatic rings was the most potent inhibitor of *P. carinii* DHFR. Compound **4a** with a two methylene bridge between the aromatic rings was the most selective *P. carinii* inhibitor of the series. Compounds with 1-naphthyl substituents were better *P. carinii* inhibitors than their counterparts with 2-naphthyl groups (**4b** > **4c**, and **4e** > **4f**).

The IC_{50} values for inhibition of *T. gondii* DHFR ranged from 0.73 to 27.0 μM with selectivity ratios between 1 and 25. As was observed for the inhibition of *P. carinii* DHFR, the length of the alkyl bridge between the aryl substituent and the heterocyclic ring influenced the inhibitory potency and selectivity of the compounds towards inhibition of *T. gondii* DHFR. Compounds with a three methylene-bridge between the aromatic rings were the most potent inhibitors while those with a two methylene-bridge were the most selective members of the series. Thus, compound **4d** was the best *T. gondii* DHFR inhibitor but compound **4a** was the most selective inhibitor of *T. gondii* DHFR.

The IC_{50} values for inhibition of *M. avium* DHFR ranged from 0.24 to 3.0 μM. The bridge length

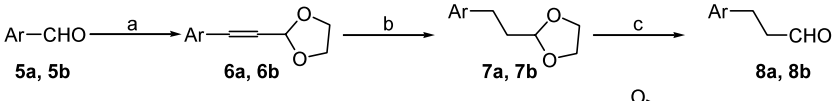

Fig. 2. Synthesis of compound **8a** and **8b** Ar = 1-naphthyl or 2-naphthyl; (a) $Ph_3P{=}CH$–[1,3-dioxolane]; (b) H_2/Pd–C; (c) 1 N HCl–AcOH (1:2).

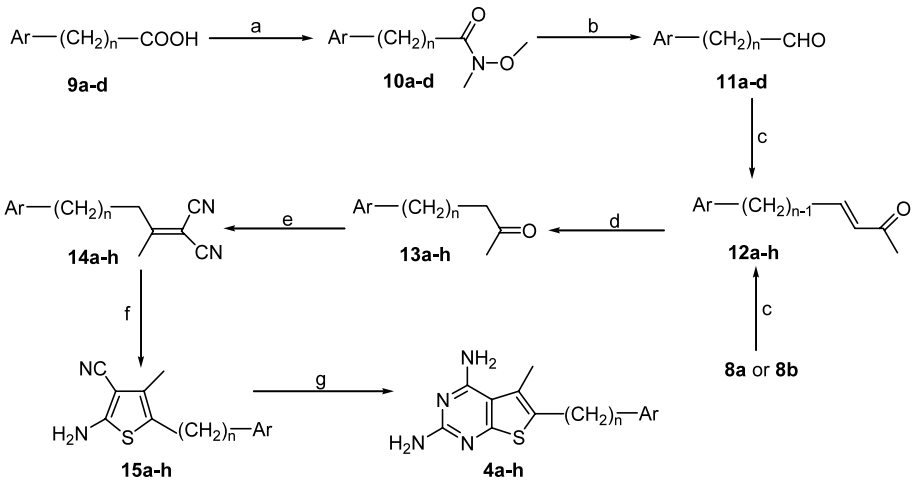

Fig. 3. Synthesis of compound **4a**–**h**. (a) $CH_3NHOCH_3 \cdot HCl$, NMM, EDC; (b) $LiAlH_4$; (c) $Ph_3P{=}CHCOCH_3$; (d) H_2/Pd–C; (e) malononitrile; (f) S/*i*-Pr_2NH; (g) $ClC(=NH \cdot HCl)NH_2$.

influenced *M. avium* DHFR inhibition in a similar manner to that observed for inhibition of the other two enzymes.

4. Conclusions

We have synthesized seven new 6-aralkyl substituted 2,4-diaminothieno[2,3-*d*]pyrimidines and studied them as inhibitors of DHFR isolated from AIDS associated opportunistic pathogens. The number of methylene groups between the 2,4-diaminothieno[2,3-*d*]pyrimidine ring and the 6-position aromatic substituent influenced the potency and the selectivity of the compounds. Compounds in which the thieno[2,3-*d*]pyrimidine ring was separated from the 6-aryl substituent by three methylene groups were the most potent DHFR inhibitors while those with two methylene groups between the aromatic rings were the most selective.

5. Experimental protocols

5.1. Chemistry

Thin layer chromatography (TLC) were performed on Riedel-deHaën silica gel plates. TLC spots were visualized under ultraviolet light (254 nm). Fisher silica gel S732-25 (170–400 mesh) was used for purification by column and flash chromatography. Melting points were

Table 1
Inhibitory concentration (IC_{50}, μM) and selectivity ratios of compounds **4a**–**h** against *P. carinii* DHFR (pc), *T. gondii* DHFR (tg) and *M. avium* DHFR (Mav) vs rat liver DHFR (rl)

Compound	*n*	Ar	rl	Pc	rl/pc	tg	rl/tg	Mav	rl/Mav
4a	2	Phenyl	80.0±20	14±1.0	5.7	3.2±0.3	25	2.1±0.2	38
4d	3	Phenyl	1.3±0.01	3.0±0.2	0.43	0.73±0.1	1.8	0.24±0.02	5.4
4g	4	Phenyl	5.0±2.0	12.0±2	0.42	3.9±0.8	1.3	0.68±0.08	7
4h	5	Phenyl	15.0±1.0	25.0±2	0.60	12.0±3.0	1.3	0.51±0.04	29
4b	2	1-Naphthyl	9.0±0.8	12.0±2	0.75	6.3±0.3	1.4	IA [a]	NA [b]
4e	3	1-Naphthyl	1.0±0.1	1.5±0.4	0.67	1.0±0.2	1.0	0.9±0.2	1
4c	2	2-Naphthyl	80.0±10	16.0±3	4.9	27.0±8	2.9	3.0±0.4	26
4f	3	2-Naphthyl	3.6±0.2	11.0±1.0	0.33	1.9±0.4	1.9	2.3±0.3	1.6
TMP			133 [c]	12 [c]	11.1	2.7 [c]	49	0.19 [d]	680
TMQ [c]			0.003	0.042	0.07	0.01	0.3		

Triplicate assays were performed as previously described [8,14,15].
[a] IA = inactive (0% inhibition at 41 μM).
[b] NA = not applicable.
[c] Data from Ref. [14].
[d] Data from Ref. [11].

determined with a Fisher-Johns melting point apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker ARX-300 MHz NMR spectrometer. The chemical shift values are reported as parts per million (ppm) relative to tetramethylsilane as internal standard; s = singlet, d = doublet, dd = doublet of doublet, t = triplet, dt = doublet of triplet, p = quintet, m = multiplet, bs = broad singlet. IR spectra were obtained on Perkin–Elmer 2000 FT-IR spectrophotometer. Analytical samples were dried over P_2O_5 for 24 h. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Analyses indicated by the symbols of the elements were within $\pm$0.4% of the theoretical values. Fractional moles of water or organic solvents found in some samples could not be removed by exhaustive drying (24 h in vacuo) and were all confirmed by their presence in the ^{1}H-NMR spectra. Chemicals were purchased from Sigma-Aldrich and Fisher Scientific, with the exception of Dess-Martin reagent, which was obtained from Omega, Canada.

*5.1.1. General procedure for the synthesis of aldehydes **8a** and **8b***

n-BuLi (2.5 M in hexanes, 4.8 mL, 12 mmol) was added under an atmosphere of nitrogen to a stirred mixture of (1,3-dioxolan-2-ylmethyl)-triphenylphosphonium bromide (11.6 mmol) in THF (25 mL) at 0 °C. The resulting deep red solution was stirred at 0 °C for 5 min and a solution of either 1-naphthaldehyde (11.6 mmol) or 2-naphthaldehyde (11.6 mmol) in THF (10 mL) was rapidly added. The mixture was stirred at RT overnight followed by refluxing for 4 h. The solvent was removed under reduced pressure and the residue was chromatographed on silica gel column with $CHCl_3$–hexanes (1:1) as eluant to give the unsaturated dioxolanes **6a** and **6b**, which were dissolved in EtOH (15 mL) and hydrogenated (20 psi) over 10% Pd–C in a Parr apparatus for 4 h. The catalyst was removed (Celite), and the filtrate was concentrated under reduced pressure. The residue was chromatographed on silica gel with hexanes–$CHCl_3$ (1:1) as eluant to give the saturated dioxolanes **7a** and **7b**. The dioxolanes were dissolved in 1 N HCl (10 mL), glacial AcOH (20 mL), and THF (30 mL) and stirred at RT for 2 days. The mixture was neutralized to pH 7 with 10 N NaOH followed by 1 N NaOH in an ice bath and extracted repeatedly with Et_2O. The Et_2O extracts were washed with brine, dried ($MgSO_4$), and evaporated to dryness to give the corresponding aldehyde, which was used without further purification.

*5.1.1.1. 3-Naphthalen-1-yl-propionaldehyde (**8a**).* Compound **8a** was synthesized as an oil. ^{1}H-NMR ($CDCl_3$) δ 2.88–2.93 (m, 2H), 3.13–3.19 (t, 2H), 7.32–7.84 (m, 7H), 9.88 (t, 1H).

*5.1.1.2. 3-Naphthalen-2-yl-propionaldehyde (**8b**).* Compound **8a** was synthesized as an oil. ^{1}H-NMR ($CDCl_3$) δ 2.84–2.89 (m, 2H), 3.09–3.14 (t, 2H), 7.33–7.79 (m, 7H), 9.85 (t, 1H).

*5.1.2. General procedure for the synthesis of aldehydes **11a–d***

The appropriate carboxylic acid (5 mmol) was dissolved in a mixture of $CHCl_3$ (10 mL) and THF (4 mL). EDC (5.4 mmol) was added and the solution was stirred for 30 min after which *N*,*O*-dimethylhydroxylamine hydrochloride (5.4 mmol) was added followed by NMM (5.4 mmol). The mixture was stirred overnight at RT, evaporated to dryness and the residue was stirred in $CHCl_3$–H_2O (1:1). The organic phase was washed with 1 N HCl, saturated $NaHCO_3$ solution, and saturated NaCl solution. After removal of the solvent the residue was passed through a bed of silica gel with $CHCl_3$ as the eluant. The $CHCl_3$ was removed and the resulting Weinreb amides **10a–d** (2.3 mmol) were dissolved in anhydrous Et_2O (20 mL), cooled, and LAH (2.9 mmol) was added. The mixture was stirred for 30 min at RT and EtOAc (5 mL) was added dropwise to destroy excess LAH followed by the addition of 5% $KHSO_4$ (10 mL) to quench the complex. The organic layer was separated and the aqueous phase was extracted with Et_2O. The combined Et_2O extracts were washed with 1 N HCl, saturated $NaHCO_3$ solution, and saturated NaCl solution. It was dried ($MgSO_4$), and evaporated under reduced pressure to afford aldehydes **11a–d**, which were used without further purification.

*5.1.2.1. 4-Phenylbutyraldehyde (**11a**).* Compound **11a** was obtained as a colourless liquid in 98% yield. ^{1}H-NMR ($CDCl_3$) δ 1.91–2.01 (p, 2H), 2.42–2.48 (dt, 2H), 2.63–2.68 (t, 2H), 7.16–7.29 (m, 5H), 9.75 (t, 1H).

*5.1.2.2. 5-Phenylpentaldehyde (**11b**).* Compound **11b** was synthesized as a colourless liquid in 97% yield. ^{1}H-NMR ($CDCl_3$) δ 1.62–1.69 (m, 4H), 2.42–2.46 (m, 2H), 2.61–2.66 (m, 2H), 7.15–7.28 (m, 5H), 9.75 (t, 1H).

*5.1.2.3. Naphthalen-1-yl-acetaldehyde (**11c**).* Compound **11c** was obtained as a light yellow liquid in quantitative yield. R_f 0.85 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 4.11 (d, 2H), 7.42–7.89 (m, 7H), 9.78 (t, 1H).

*5.1.2.4. Naphthalen-2-yl-acetaldehyde (**11d**).* Compound **11d** was obtained as a yellow oil in quantitative yield. R_f 0.85 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 3.86 (d, 2H), 7.47–7.87 (m, 7H), 9.83 (t, 1H).

*5.1.3. General procedure for the synthesis of ketones **13a–h***

A solution of the appropriate aldehyde (2.3 mmol) and 1-(triphenylphosphoranylidene)-2-propanone (2

mmol) in $CHCl_3$ (10 mL) was heated under reflux for 4 h. The mixture was cooled to RT and the solvent was removed under reduced pressure. The residue was purified by column chromatography on silica gel with hexanes–$CHCl_3$ (2:1) as the eluant to give α,β-unsaturated ketones **12a**–**h**. The ketones were dissolved in EtOH (15 mL) and hydrogenated (15 psi) over 10% Pd–C (50 mg) in a Parr apparatus for 2 h. The catalyst was removed (Celite), and the filtrate was concentrated to give saturated ketones **13a**–**h**.

*5.1.3.1. 5-Phenyl-pentan-2-one (**13a**).* Compound **13a** was obtained as a colourless oil in 69% yield. R_f 0.85 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.90 (p, 2H), 2.11 (s, 3H), 2.43 (t, 2H), 2.62 (t, 2H), 7.1–7.4 (m, 5H).

*5.1.3.2. 5-Naphthalen-1-yl-pentan-2-one (**13b**).* Compound **13b** was obtained as a yellow oil in 35% yield. R_f 0.3 (silica gel, 1:1 $CHCl_3$–hexanes); ^{1}H-NMR ($CDCl_3$) δ 2.04 (p, 2H), 2.13 (s, 3H), 2.51 (t, 2H), 3.08 (t, 2H,), 7.28–8.11 (m, 7H).

*5.1.3.3. 5-Naphthalen-2-yl-pentan-2-one (**13c**).* Compound **13c** was obtained as a yellow oil in 70% yield. R_f 0.65 (silica gel, 1:1 $CHCl_3$–hexanes); ^{1}H-NMR ($CDCl_3$) δ 1.97 (p, 2H), 2.08 (s, 3H), 2.43 (t, 2H), 2.76 (t, 2H), 7.28–7.81 (m, 7H, aromatic).

*5.1.3.4. 6-Phenyl-hexan-2-one (**13d**).* Compound **13d** was obtained as a colourless oil in 97% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.57–1.61 (m, 4H), 2.09 (s, 3H), 2.38–2.44 (m, 2H), 2.55–2.59 (m, 2H), 7.04–7.18 (m, 5H).

*5.1.3.5. 6-Naphthalen-1-yl-hexan-2-one (**13e**).* Compound **13e** was obtained as a colourless oil in quantitative yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.71–1.76 (m, 4H), 2.11 (s, 3H), 2.44–2.48 (t, 2H), 3.05–3.10 (t, 2H), 7.32–7.83 (m, 7H).

*5.1.3.6. 6-Naphthalen-2-yl-hexan-2-one (**13f**).* Compound **13f** was obtained as a colourless oil in 90% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.67–1.72 (m, 4H), 2.11 (s, 3H), 2.43–2.48 (t, 2H), 2.76–2.80 (t, 2H), 7.33–7.78 (m, 7H).

*5.1.3.7. 7-Phenyl-heptan-2-one (**13g**).* Compound **13g** was obtained as a colourless oil in 83% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.29–1.38 (m, 2H), 1.57–1.65 (m, 4H), 2.12 (s, 3H), 2.39–2.44 (t, 2H), 2.57–2.63 (t, 2H), 7.15–7.29 (m, 5H).

*5.1.3.8. 8-Phenyl-octan-2-one (**13h**).* Compound **13h** was obtained as a colourless oil in 57% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.30–1.33 (m, 4H), 1.54–1.61 (m, 4H), 2.12 (s, 3H), 2.38–2.43 (t, 2H), 2.56–2.62 (t, 2H), 7.15–7.27 (m, 5H).

*5.1.4. General procedure for the synthesis of ylidenemalononitriles **14a**–**h***

Malononitrile (4.5 mmol), glacial AcOH (0.15 mL), and solid NH_4OAc (0.1 g) were added to a solution of the appropriate ketone **13a**–**h** (1 mmol) in benzene (4 mL). The mixture was heated under reflux for 4 h and then cooled. After evaporation of the solvent under reduced pressure the residue was passed through a bed of silica gel using hexanes–$CHCl_3$ (1:1) as the eluant. Evaporation of the filtrate gave the corresponding ylidenemalononitrile.

*5.1.4.1. 2-(1-Methyl-4-phenyl-butylidene)-malononitrile (**14a**).* Compound **14a** was synthesized as a colourless oil in quantitative yield. R_f 0.5 (silica gel, 1:1 hexanes–$CHCl_3$); IR (NaCl) 2233 (CN); ^{1}H-NMR ($CDCl_3$) δ 1.87 (m, 2H), 2.21 (s, 3H), 2.57 (t, 2H), 2.66 (t, 2H), 7.0–7.3 (m, 5H).

*5.1.4.2. 2-(1-Methyl-4-naphthalen-1-yl-butylidene)-malononitrile (**14b**).* Compound **14b** was obtained as a yellow oil in 82% yield. R_f 0.9 (silica gel, $CHCl_3$); IR (NaCl) 2233 (CN); ^{1}H-NMR ($CDCl_3$) δ 2.03 (m, 2H), 2.20 (s, 3H), 2.70 (t, 2H), 3.17 (t, 2H), 7.30–7.95 (m, 7H).

*5.1.4.3. 2-(1-Methyl-4-naphthalen-2-yl-butylidene)-malononitrile (**14c**).* Compound **14c** was obtained as a yellow oil in 82% yield. R_f 0.5 (silica gel, $CHCl_3$–hexanes [1:1]); IR (NaCl) 2233 (CN); ^{1}H-NMR ($CDCl_3$) δ 1.98 (m, 2H), 2.25 (s, 3H), 2.65 (t, 2H), 2.87 (t, 2H), 7.30–7.86 (m, 7H).

*5.1.4.4. 2-(1-Methyl-5-phenyl-pentylidene)-malononitrile (**14d**).* Compound **14d** was obtained as a colourless oil in quantitative yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.56–1.72 (m, 4H), 2.23 (s, 3H), 2.60 (t, 2H), 2.66 (t, 2H), 7.15–7.32 (m, 5H).

*5.1.4.5. 2-(1-Methyl-5-naphthalen-1-yl-pentylidene)-malononitrile (**14e**).* Compound **14e** was obtained as a yellow oil in 73% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.64–1.72, 1.79–1.89 (m, 4H), 2.22 (s, 3H), 2.59–2.64 (t, 2H), 3.10–3.14 (t, 2H), 7.29–8.02 (m, 7H).

*5.1.4.6. 2-(1-Methyl-5-naphthalen-2-yl-pentylidene)-malononitrile (**14f**).* Compound **14f** was obtained as a yellow oil in quantitative yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.58–1.66, 1.73–1.81 (m, 4H), 2.21 (s, 3H), 2.58–2.63 (t, 2H), 2.80–2.85 (t, 2H), 7.43–7.80 (m, 7H).

*5.1.4.7. 2-(1-Methyl-6-phenyl-hexylidene)-malononitrile (**14g**).* Compound **14g** was obtained as a colourless oil in 87% yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.37–1.45 (m, 2H), 1.54–1.71 (m, 4H), 2.25 (s, 3H), 2.54–2.65 (dt, 4H), 7.15–7.28 (m, 5H).

*5.1.4.8. 2-(1-Methyl-7-phenyl-heptylidene)-malononitrile (**14h**).* Compound **14h** was obtained as a colourless oil in quantitative yield. R_f 0.8 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.17–1.22 (m, 4H), 1.38–1.45 (m, 4H), 2.08 (s, 3H), 2.36–2.46 (m, 4H), 7.08–7.13 (m, 5H).

*5.1.5. General procedure for the synthesis of thiophene amino nitriles **15a–h***

Diisopropylamine (5 mmol) was added dropwise to a mixture of ylidenemalononitrile (**14a–h**, 0.76 mmol) and elemental sulphur (5 mmol) in EtOH (25 mL) while stirring. The temperature was kept at 50–60 °C for 45 min. The mixture was then poured into 0.2 N HCl, extracted with EtOAc, and the organic portion was dried ($MgSO_4$) and evaporated under reduced pressure. The resulting brown oil was purified by column chromatography on silica gel with hexanes–$CHCl_3$ (1:1) as eluant and used directly or followed by recrystallized from a mixture of hexanes and Et_2O to give **15a–h**.

*5.1.5.1. 2-Amino-4-methyl-5-phenylethyl-thiophene-3-carbonitrile (**15a**).* Compound **15a** was obtained as an off-white solid in 10.3% yield. M.p. 84 °C; R_f 0.6 (silica gel, $CHCl_3$); IR (NaCl) 2198 (CN); ^{1}H-NMR ($CDCl_3$) δ 1.91 (s, 3H), 2.85 (m, 4H), 4.59 (bs, 2H), 7.05–7.28 (m, 5H).

*5.1.5.2. 2-Amino-4-methyl-5-(2-naphthalen-1-yl-ethyl)-thiophene-3-carbonitrile (**15b**).* Compound **15b** was obtained as a tan-brown solid in 12.3% yield. M.p. 155–157 °C; R_f 0.6 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.84 (s, 3H), 2.97 (t, 2H), 3.27 (t, 2H), 4.58 (bs, 2H), 7.2–8.1 (m, 7H).

*5.1.5.3. 2-Amino-4-methyl-5-(2-naphthalen-2-yl-ethyl)-thiophene-3-carbonitrile (**15c**).* Compound **15c** was obtained as an off-white solid in 54% yield after recrystallization from a mixture of Et_2O and hexanes. M.p. 145 °C; R_f 0.6 (silica gel, $CHCl_3$); IR (KBr) 2193 (CN); ^{1}H-NMR ($CDCl_3$) δ 1.93 (s, 3H); 2.96 (m, 4H), 4.98 (bs, 2H), 7.2–7.9 (m, 7H).

*5.1.5.4. 2-Amino-4-methyl-5-(3-phenyl-propyl)-thiophene-3-carbonitrile (**15d**).* Compound **15d** was obtained as an off-white solid in 21% yield. M.p. 86 °C; R_f 0.5 (silica gel, $CHCl_3$); IR (NaCl) 2199 (CN); ^{1}H-NMR ($CDCl_3$) δ 1.80–1.91 (p, 2H), 2.05 (s, 3H), 2.54–2.59 (t, 2H), 2.61–2.66 (t, 2H), 4.58 (bs, 2H), 7.15–7.31 (m, 5H).

*5.1.5.5. 2-Amino-4-methyl-5-(3-naphthalen-1-yl-propyl)-thiophene-3-carbonitrile (**15e**).* Compound **15e** was obtained as a yellow powder in 20% yield. M.p. 154 °C; R_f 0.5 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.94–2.01 (m, 2H), 2.04 (s, 3H), 2.63–2.68 (t, 2H), 3.06–3.12 (t, 2H), 4.57 (bs, 2H), 7.30–8.10 (m, 7H).

*5.1.5.6. 2-Amino-4-methyl-5-(3-naphthalen-2-yl-propyl)-thiophene-3-carbonitrile (**15f**).* Compound **15f** was obtained as a yellow powder in 32% yield. M.p. 152 °C; R_f 0.5 (silica gel, $CHCl_3$); ^{1}H-NMR ($CDCl_3$) δ 1.89–1.99 (p, 2H), 2.04 (s, 3H), 2.57–2.62 (t, 2H), 2.78–2.83 (t, 2H), 4.57 (bs, 2H), 7.41–7.79 (m, 7H, aromatic).

*5.1.5.7. 2-Amino-4-methyl-5-(4-phenyl-butyl)-thiophene-3-carbonitrile (**15g**) and 2-amino-4-methyl-5-(5-phenyl-pentyl)-thiophene-3-carbonitrile (**15h**).* Thiophene-3-carbonitriles **15g** and **15h** were obtained as brown oils but proved to be very unstable. Purification by silica gel chromatography and/or recrystallization from Et_2O and hexanes mixture failed. The compounds were therefore not characterized but used immediately to obtained target compounds **4g** and **4h**, respectively.

*5.1.6. General procedure for the synthesis of 6-substituted diaminothieno[2,3-d]pyrimidines **4a–h***

Chloroformamidine hydrochloride was obtained by bubbling HCl gas through a solution of NH_2CN in anhydrous Et_2O. The chloroformamidine hydrochloride (1.37 mmol) and the appropriate thiophene amino nitrile (**15a–h**, 0.35 mmol) were copulverized thoroughly under argon. The mixture was heated at 120 °C (internal) for 30 min. Melting and evolution of HCl were observed. After cooling to RT, the mixture was dissolved in MeOH–$CHCl_3$ (1:9) mixture and i-Pr_2NH (0.5 mL) was added and heated at 70–80 °C for 1.5 h. Chromatography of the residue followed by recrystallization from $CHCl_3$–MeOH (9:1) gave compounds **4a–h**.

*5.1.6.1. 5-Methyl-6-phenethyl-thieno[2,3-d]pyrimidine-2,4-diamine (**4a**).* Compound **4a** was synthesized as a white solid in 45% yield. M.p. 135 °C; R_f 0.5 (silica gel, 9:1 $CHCl_3$–MeOH); IR (NaCl) 3317, 3157, 1628, 1561, 1522, 1497, 1473, 1445, 1377, 1325, 1286, 786, 699 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.17 (s, 3H), 2.87–3.03 (m, 4H), 4.68 (bs, 2H), 5.10 (bs, 2H), 7.05–7.27 (m, 5H). Anal. ($C_{15}H_{16}N_4S$) C, H, N, S.

*5.1.6.2. 5-Methyl-6-(2-naphthalen-1-yl-ethyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4b**).* Compound **4b** was obtained as an off-white solid; yield 53%; M.p. 215–216 °C; R_f 0.6 (silica gel, 9:1 $CHCl_3$–MeOH); IR (KBr) 3390, 3165, 1608, 1561, 1528, 1472, 920, 778

cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.03 (s, 3H), 3.14 (t, 2H), 3.37 (t, 2H), 4.69 (bs, 2H), 5.07 (bs, 2H), 7.20–8.10 (7H). Anal. ($C_{19}H_{18}N_4S$) C, H, N, S.

*5.1.6.3. 5-Methyl-6-(2-naphthalen-2-yl-ethyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4c**).* Compound **4c** was obtained as an off-white solid in 77% yield. M.p. 230–231 °C; R_f 0.6 (silica gel, 9:1 $CHCl_3$–MeOH); IR (KBr) 3159, 2722, 1649, 1562, 1526, 1444 cm^{-1}; ^{1}H-NMR (DMSO) δ 2.18 (s, 3H), 2.99 (m, 4H), 6.04 (bs, 2H), 6.43 (bs, 2H), 7.30–8.00 (m, 7H). Anal. ($C_{19}H_{18}N_4S$) C, H, N, S.

*5.1.6.4. 5-Methyl-6-(3-phenyl-propyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4d**).* Compound **4d** was obtained as a white solid in 75% yield. M.p. 136–138 °C; R_f 0.6 (silica gel, 9:1 $CHCl_3$–MeOH); IR (NaCl) 3453, 3321, 3120, 2929, 1617, 1560, 1522, 1438, 1292, 925, 786, 698 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.96 (p, 2H), 2.33 (s, 3H), 2.65–2.76 (dt, 4H), 4.70 (bs, 2H), 5.16 (bs, 2H), 7.17–7.31 (m, 5H). Anal. ($C_{16}H_{18}N_4S$) C, H, N, S.

*5.1.6.5. 5-Methyl-6-(3-naphthalen-1-yl-propyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4e**).* Compound **4e** was obtained as a yellow solid in 84% yield. M.p. 236 °C; R_f 0.5 (silica gel, 9:1 $CHCl_3$–MeOH); IR (KBr) 3170, 2924, 1611, 1562, 1524, 1439, 1319, 1284, 778 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.05–2.15 (p, 2H), 2,34 (s, 3H), 2.81–2.86 (t, 2H), 3.11–3.16 (t, 2H), 4.67 (bs, 2H), 5.12 (bs, 2H), 7.33–8.10 (m, 7H). Anal. ($C_{20}H_{20}N_4S$) C, H, N, S.

*5.1.6.6. 5-Methyl-6-(3-naphthalen-2-yl-propyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4f**).* Compound **4f** was obtained as an off-white solid in 57% yield. M.p. 246 °C; R_f 0.5 (silica gel, 9:1 $CHCl_3$–MeOH); IR (KBr) 3135, 1654, 1636, 1617, 1560, 1541, 1522, 1507, 1457, 1437, 668 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 2.00–2.10 (p, 2H), 2,33 (s, 3H), 2.75–2.87 (t, 4H), 4.66 (bs, 2H), 5.12 (bs, 2H), 7.32–7.82 (m, 7H). Anal. ($C_{20}H_{20}N_4S$) C, H, N, S.

*5.1.7. 5-Methyl-6-(4-phenyl-butyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4g**)*

Compound **4g** was obtained as a yellow solid in 22% yield. M.p. 138 °C; R_f 0.5 (silica gel, 9:1 $CHCl_3$–MeOH); IR (NaCl) 3480, 3456, 3290, 3143, 2940, 1654, 1616, 1563, 1523, 1471, 1435, 787, 731, 713 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.68–1.76 (m, 4H), 2.38 (s, 3H), 2.63–2.77 (dt, 4H), 4.72 (bs, 2H), 5.18 (bs, 2H), 7.17–7.29 (m, 5H). Anal. ($C_{18}H_{22}N_4S$) C, H, N, S.

*5.1.8. 5-Methyl-6-(5-phenyl-pentyl)-thieno[2,3-d]pyrimidine-2,4-diamine (**4h**)*

Compound **4h** was obtained as a white solid in 10% yield. M.p. 140 °C; R_f 0.5 (silica gel, 9:1 $CHCl_3$–MeOH); IR (NaCl) 3630, 3393, 1700, 1684, 1654, 1636 cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ 1.39–1.45 (m, 2H), 1.61–1.71 (m, 4H), 2.38 (s, 3H), 2.60–2.74 (dt, 4H), 4.72 (bs, 2H), 5.18 (bs, 2H), 7.17–7.28 (m, 5H). Anal. ($C_{18}H_{22}N_4S$) C, H, N, S.

References

[1] H. Masur, J.E. Kaplan, K.K. Holmes, Ann. Intern. Med. 137 (2002) 435–478.
[2] F.J. Palella, K.M. Delaney, A.C. Moorman, M.O. Loveless, J. Fuhrer, G.A. Satten, D.J. Aschman, S.D Holmberg, N. Engl. J. Med. 338 (1998) 853–860.
[3] M. Floridia, V. Fragola, C.M. Galluzzo, G. Giannini, M.F. Pirrilo, M. Andreotti, C. Tomino, S. Vella, HIV Med. 3 (2000) 75–84.
[4] D.M. Forrest, E. Seminari, R.S. Hogg, B. Yip, J. Raboud, L. Lawson, P. Phillips, M.T. Schechter, M.V. O'Shaughnessy, J.S. Montaner, Clin. Infect. Dis. 27 (1998) 1379–1385.
[5] A. Morris, R.M. Wachter, J. Luce, J. Turner, L. Huang, AIDS 17 (2003) 73–80.
[6] R. Manfredi, F. Chiodo, Sex Transm. Infect. 76 (2000) 145–146.
[7] R. Manfredi, L. Calza, F. Chiodo, Eur. J. Clin. Microbiol. Infect. Dis. 20 (2001) 410–413.
[8] S.A. Marques, A.M. Robles, A.M. Tortorano, M.A. Tuculet, R. Negroni, R.P. Mendes, Med. Mycol. 38 (Suppl. 1) (2000) 269–279.
[9] M.E. Klepser, T.B. Klepser, Drugs 53 (1997) 40–73.
[10] B.I. Schweitzer, A.P. Dicker, J.R. Bertino, FASEB J. 4 (1990) 2441–2452.
[11] A. Rosowsky, C.E. Mota, J.E. Wright, J.H. Freisheim, J.J. Heusner, J.J. McCormack, S.F. Queener, J. Med. Chem. 36 (1993) 3103–3112.
[12] A. Gangjee, X. Guo, S.F. Queener, V. Cody, N. Galitsky, J.R. Luft, W. Pangborn, J. Med. Chem. 41 (1998) 1263–1271.
[13] A. Gangjee, X. Guo, S.F. Queener, V. Cody, N. Galitsky, J.R. Luft, W. Pangborn, J. Med. Chem. 41 (1998) 1263–1271.
[14] S.F. Queener, J. Med. Chem. 38 (1995) 4739–4759.
[15] A. Rosowsky, V. Cody, N. Galitsky, H. Fu, A.T. Papoulis, S.F. Queener, J. Med. Chem. 42 (1999) 4853–4860.
[16] W.J. Suling, L.E. Seitz, V. Pathak, L. Westbrook, E.W. Barrow, S. Zywno-Van-Ginkel, R.C. Reynolds, J.R. Piper, W.M. Barrow, Antimicrob. Agents Chemother. 44 (2000) 2784–2793.
[17] C.M. Shoen, O. Choromanska, R.C. Reynolds, J.R. Piper, C.A. Johnson, M.H. Cynamon, Antimicrob. Agents Chemother. 42 (1998) 3315–3316.
[18] J.R. Piper, C.A. Johnson, C.A. Krauth, R.L. Carter, C.A. Hosmer, S.F. Queener, S.E. Borotz, E.R. Pfefferkorn, J. Med. Chem. 39 (1996) 1271–1280.
[19] A. Rosowsky, C.E. Mota, J.E. Wright, J.H. Freisheim, J.J. Heusner, J.J. McCormack, S.F. Queener, J. Med. Chem. 36 (1993) 3103–3112.
[20] A. Rosowsky, K.K. Chen, M. Lin, J. Med. Chem. 16 (1973) 191–194.
[21] E.F. Elslager, P. Jocob, L.M. Werbel, J. Heterocyclic Chem. 9 (1972) 775–782.
[22] S.F. Queener, M.S. Bartlett, M.A. Jay, M.M. Durkin, J.W. Smith, Antimicrob. Agents Chemother. 31 (1987) 1323–1327.
[23] M.C. Broughton, S.F. Queener, Antimicrob. Agents Chemother. 35 (1991) 1348–1355.
[24] L.-C. Chio, S.F. Queener, Antimicrob. Agents Chemother. 37 (1993) 1914–1923.