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New chalcones and thiopyrimidine analogues derived from mefenamic acid: microwave-assisted synthesis, anti-HIV activity and cytotoxicity as antileukemic agents

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Abstract: The development of new HIV non-nucleoside reverse transcriptase inhibitors offers the possibility of generating structures of increased potency. To this end, coupling of mefenamic acid (**4**) with 4-amino-acetophenone (**6**) in the presence of dicyclohexylcarbodiimide and dimethylaminopyridine (DMAP) reagents afforded 4-(acetyphenyl)-2-((2,3-dimethylphenyl)amino)benzamide (**7**). Analogously, treatment of mefenamyl chloride (**5**) prepared from **4** with **6** under microwave irradiation (MWI) afforded **7**. A new series of substituted chalconyl-incorporated amide derivatives of mefenamic acid **8–13** were synthesized from condensation of **7** with various substituted benzaldehydes via the Claisen–Schmidt reaction. Treatment of **8** and **11** with thiourea in a basic medium afforded the thiopyrimidine analogues **14** and **15**, respectively. The newly synthesized compounds were assayed against HIV-1 and HIV-2 in MT-4 cells. Compounds **9** and **11** showed cytotoxicity values of 2.17 and 2.06 μM, respectively, against mock-infected MT-4 cells (C type adult T leukemia cells), which considered to be promising antileukemic agents.

Keywords: anti-HIV activity; configuration; cytotoxicity; mefenamic acid; microwave-assisted synthesis.

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1 Introduction

Chalcones are well-known intermediates for synthesizing various heterocyclic compounds and constitute an important group of natural products. Some of them possess a wide range of biological activities such as antimicrobial [1], anticancer [2–9], antitubercular [10], anti-inflammatory [11, 12], anti-HIV [13, 14], antioxidant [15, 16], antimalarial [17, 18], antileishmanial [19] and antifungal agents [20]. The presence of a reactive α,β-unsaturated keto group in chalcones has been found to be responsible for their biological activity. Chalcones inhibit β(1,3)-glucan and chitin synthases, enzymes, that catalyze the biosynthesis of β(1,3)-glucan and chitin polymers of the fungal cell wall, respectively [21, 22]. Kolundžija et al. [23] have synthesized several chalcone analogues bearing an imine partial structure (**1**, Fig. 1) that were found to be potent *in vitro* against HeLa human colon carcinoma (LS174) and non-small-cell lung carcinoma (A549) cancer lines with IC_{50} values ranging from 1.76 to 6.11 μM. Recently, we have synthesized various steroidal bisaryl chalcones (e.g. **2**, Fig. 1) as inhibitors of CYP17 α-hydroxylase, an enzyme responsible for prostate cancer [24].

Several therapeutic strategies have been used for the treatment of HIV infection. Non-nucleoside reverse transcriptase inhibitors have gained a definitive and important place for the treatment of HIV due to their unique antiviral potency, high specificity and low toxicity. However, due to viral resistance to the currently available antiviral agents, there is a persistent need to develop new inhibitors with low toxicity and viral resistance as well as improved activity [25–27]. Wu et al. [28] have isolated chalcones and flavonoids from genus *Desmos*, where the chalcone analogue **3** (Fig. 1) exhibited a potent anti-HIV-1 activity with an EC_{50} value of 0.022 μM and a therapeutic index of 489.

Considering the significance of chalcones and in continuation of our ongoing work on the synthesis and anti-HIV activity of various heterocyclic compounds [29–33], herein we report the synthesis, anti-HIV activity and cytotoxicity

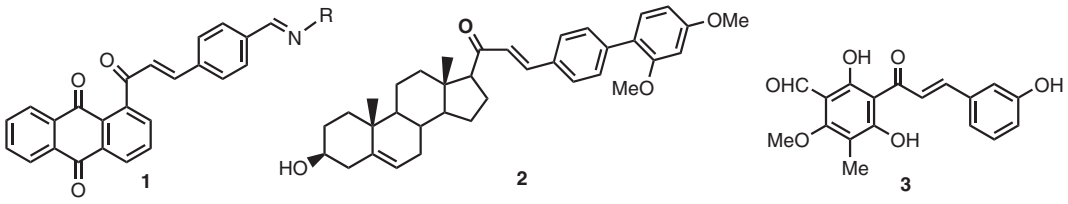


Fig. 1: Some potentially active anticancer and anti-HIV agents.

of new chalcone analogues derived from mefenamic acid (**4**), in addition to a quantum structure-activity relationship study, since **4** itself has demonstrated antiproliferative activity against colorectal cancer cells [34].

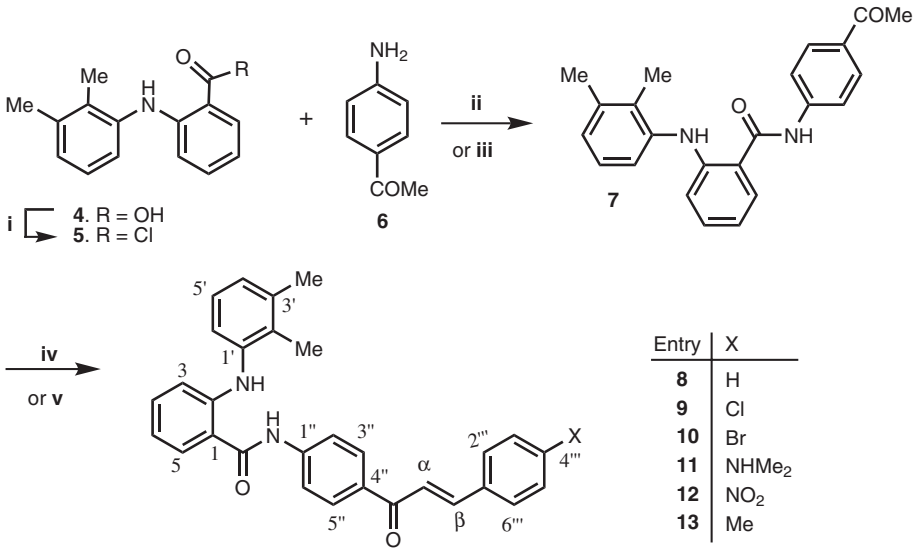
2 Results and discussion

2.1 Chemistry

The synthetic strategy followed toward the synthesis of different chalcone derivatives of mefenamic acid is outlined in Scheme 1. Thus, the acid chloride **5**, prepared by the treatment of mefenamic acid **4** with PCl_5 under microwave irradiation (MWI), was treated with *p*-aminoacetophenone **6** to give the amide analogue **7** (60%). Alternatively, the amide **7** was prepared, in 83% after chromatography, by the coupling of mefenamic acid **4** with **6** in the presence of *N*,*N*′-dicyclohexylcarbodiimide (DCC) and dimethylaminopyridine (DMAP) at 0°C and then at room temperature. The intermediate **7** was shared by all chalcone molecules synthesized. Treatment of **7** with the appropriate 4-substituted benzaldehydes (e.g. benzaldehyde, 4-chloro-, 4-bromo-, 4-dimethylamino-, 4-nitro and 4-tolualdehydes) in the presence of 20% NaOH for 6–8 min under MWI afforded, after chromatographic, the desired chalcone derivatives **8–13**, respectively, in 70–80% yield. Alternatively, the analogues **8–13** were prepared in 75–85% yield by refluxing of **7** with substituted benzaldehydes in the presence of ethanolic NaOH for 4–5 h.

The structures of **7** and **8–13** were assigned on the basis of their NMR (^1H, ^{13}C and 2D), which showed rather similar patterns for the aromatic scaffold.

The ^1H NMR spectra of **8–13** were characterized by the presence of additional aromatic and olefinic protons and carbon atoms, indicative for the formation of new chalcone analogues. The ^1H NMR spectra of **8–13** showed doublets at the regions $\delta = 8.40–8.31$ ppm ($J \sim 7.9$ Hz) assigned for H-3, while the doublets at the regions $\delta = 7.80–7.56$ and 8.40–8.06 ppm ($J = 15.0–16.2$ Hz) were assigned for the olefin protons Hα and Hβ of the chalcone moiety, respectively. The



Scheme 1: Reagents and conditions: (i) PCl_5, EtOH, room temperature, 3 h; (ii) method a: **5**, MWI, 5 min; (iii) method b: **4**, DCC, DMAP, CH_2Cl_2, room temperature, 16 h; (iv) method a: X–C_6H_4CHO, MWI, EtOH, 20% NaOH, 6–8 min; (v) method b: X–C_6H_4CHO, 20% NaOH, EtOH, room temperature, 4–5 h.

other aromatic and aliphatic protons have been fully analyzed (cf Experimental section). In the ^{13}C NMR spectra of **7** and **8–13**, the carbonyl carbon atoms (C=O and C_{amide}=O) appeared at the regions δ = 190.2–189.7 and 166.2–165.8 ppm, respectively. Carbon atoms of the chalcone residue (Cα and Cβ) resonated at δ = 122.1–119.0 and 149.2–139.3 ppm, respectively, while the resonances at δ = 121.2–120.1, 150.3–144.9 and 119.5–117.8 ppm were assigned to the aromatic carbon atoms 1, 2 and 3, respectively. The signals at the regions δ = 135.3–131.2, 118.4–116.3 and 128.4–128.0 ppm were attributed to carbon atoms 4, 5 and 6, respectively. Other aromatic carbon atoms as well as the substituents were fully analyzed (cf Experimental section).

Compound **10** was selected for further NMR experiments. The gradient heteronuclear multiple-bond correlation [35] NMR spectrum of **10** showed that six $^3J_{H,C}$ couplings were observed: H-6 at δ_H = 7.64 ppm with the C=O carbon atom at δ_C = 189.7 ppm, while the carbonyl carbon atom of the NHCO group at δ_C = 165.8 ppm coupled with both aromatic protons 3″-H + 5″-H at δ_H = 7.85 ppm, as well as with Hβ at δ_H = 8.09 ppm. Further, two $^3J_{H,C}$ couplings between Cβ at δ_C = 145.1 ppm and both aromatic carbon atoms 2‴-H + 6″-H at δ_H = 7.60 ppm were observed. Additionally, a $^3J_{H,C}$ coupling between Hα at δ = 7.58 ppm and the amide carbon atom at δ_C = 165.8 ppm was witnessed (Fig. 2).

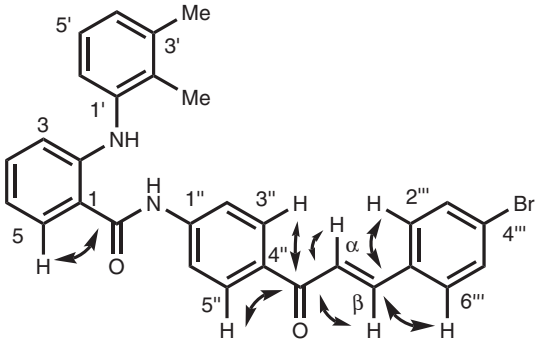


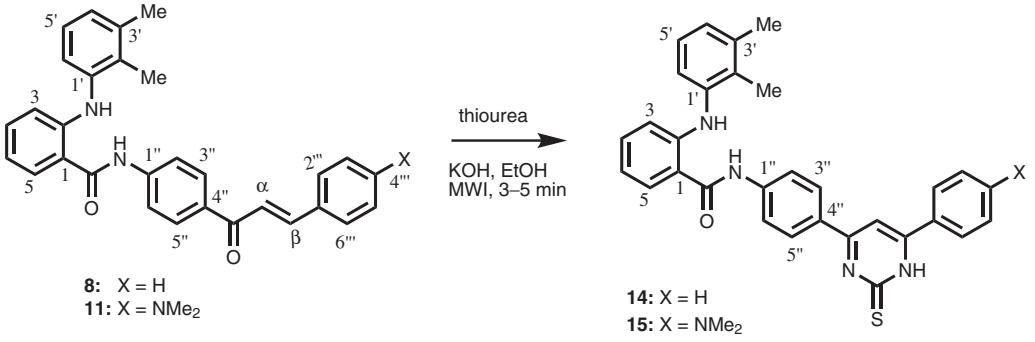
Fig. 2: $J_{H,C}$ correlations in the HMBC NMR spectrum of 10.

We selected the chalcones **8** and **11** as precursors for the synthesis of new thiopyrimidine derivatives, aiming at examining their anti-HIV activity as well as cytotoxicity against human lymphocyte MT-4 cells in comparison to those of the chalcone analogues **8–13**. Thus, the treatment of **8** and **11** with thiourea in 20% NaOH and EtOH under microwave irradiation (400 W, 180°C) for 3–6 min afforded, after neutralization with HCl and purification, the thiopyrimidine derivatives **14** and **15** in 65% and 60% yield, respectively (Scheme 2).

The structures of **14** and **15** were determined from their IR, ^1H and ^{13}C NMR spectra. In the ^1H NMR spectra, H-5 of the thiopyrimidine ring appeared as singlets at δ = 8.05 and 8.06 ppm, respectively, while the downfield doublets at δ = 8.49 and 8.48 ppm were assigned to 2-H of the aromatic ring. The other aromatic and substituent protons were analyzed (cf Experimental section). The ^{13}C NMR spectra showed signals at δ = 181.2 and 181.8 ppm, which were attributed to C=S of the thiopyrimidine ring, respectively. The resonances at δ = 175.0, 175.2 and 166.8, 166.9 ppm were assigned to C-4 of the thiopyrimidine moiety and to the carbonyl carbon atom of the amide group, respectively. C-5 and C-6 of the thiopyrimidine moiety resonated at δ = 163.9, 163.4 and δ = 109.6 and 110.1 ppm, respectively. The aromatic carbon atom C-1‴ was appeared at δ = 130.2 and 125.4 ppm, respectively, while $C_{4‴}$-NMe_2 of **15** resonated at δ = 153.9 ppm. The other aromatic and substituent carbon atoms were fully assigned (cf Experimental section).

2.2 *In vitro* anti-HIV activity

Compounds **8–15** were evaluated for their *in vitro* anti-HIV-1 (strain III_B) and anti-HIV-2 (strain ROD) activity, which were monitored by the inhibition of the virus-induced cytopathic effect in the human T-lymphocyte (MT-4) cells by MTT assay [36]. The results are listed in Table 1, in which



Scheme 2: Synthesis of 2-((2,3-dimethylphenyl)amino)-*N*-(4-(6-aryl-2-thioxo-1,2-dihydropyrimidine-4-yl)phenyl)benzamides.

Table 1: *In vitro* anti-HIV-1[a] and HIV-2[b] activity of chalcon analogues **8–13** and related derivatives **14** and **15.**

Compound	Virus strain	av. IC_{50} (μM)[c]	av. CC_{50} (μM)[d]	SI[e]
8	III_B	>125.00	>125.00	X1
	ROD	>125.00	>125.00	X1
9	III_B	>2.17	2.17	<1
	ROD	>2.17	2.17	<1
10	III_B	>12.68	12.68	<1
	ROD	>12.68	12.68	<1
11	III_B	>2.06	2.06	<1
	ROD	>2.06	2.06	<1
12	III_B	>13.18	13.18	<1
	ROD	>13.18	13.18	<1
13	III_B	>76.90	76.90	<1
	ROD	>76.90	76.90	<1
14	III_B	>13.90	>13.90	<1
	ROD	>13.90	>13.90	<1
15	III_B	>7.02	>7.02	<1
	ROD	>7.02	>7.02	<1
AZT	III_B	0.0019	>25	>13 144
	ROD	0.0018	>25	>14 245
3TC	III_B	0.51	>20	>39
	ROD	2.02	>20	>10

[a]Anti-HIV-1 activity measured against strain III_B.
[b]Anti-HIV-2 activity measured against strain ROD.
[c]Compound concentration required to achieve 50% protection of MT-4 cells from the HIV-1- and 2-induced cytopathogenic effect.
[d]Average CC_{50}: compound concentration that reduces the viability of mock-infected MT-4 cells by 50%.
[e]SI, Selectivity index (CC_{50}/IC_{50}). All data represent the mean values of at least two separate experiments.

the data for nevirapine (BOE/BIRG587) [37] and azidothymidine (AZT) [38] are included for comparison purposes. The cytotoxicity of the compounds was determined in parallel. All the compounds showed no activity against HIV-1 and HIV-2, except **9** and **11**, which showed IC_{50} >2.17 and >2.06 μM, respectively, with a selectivity index (SI) of <1, meaning no selectivity could be witnessed. The cytotoxicity of **9** and **11** against the human $CD4^+$ lymphocytes MT-4 (C type adult T leukemia cells) (CC_{50} = 2.17 and 2.06 μM, respectively) is remarkable and encourages to investigate the compounds further as lead structures.

3 Conclusions

A new series of substituted chalconyl-incorporated amide derivatives of mefenamic acid **8–13**, via the Claisen–Schmidt reaction, as well as two of their thiopyrimidine analogues **14** and **15**, were synthesized. All compounds were screened for their inhibitory activity against HIV-1 and HIV-2, where **9** and **11** showed cytotoxicity values of 2.17 and 2.06 μM, respectively, against mock-infected MT-4 cells (C type adult T leukemia cells). These analogues can be considered as promising antileukemic agents waiting for further structural modification.

4 Experimental section

4.1 General

Melting points are uncorrected and were measured on a Büchi melting point apparatus B-545 (Büchi Labortechnik AG, Switzerland). Microanalytical data were obtained with a Vario EL (Shimadzu, Japan). NMR spectra were recorded at 400 and 600 MHz (^1H) and at 150.91 MHz (^{13}C) (Bruker, Germany) with tetramethylsilane as the internal standard. Heteronuclear assignments were verified by ^1H-^{13}C HMBC experiments. Heteronuclear assignments were verified by ^1H-^{13}C HMBC experiments. Microwave-supported reactions were performed in a SmithSynthesizer (Personal Chemistry AB), monomode microwave cavity at 2.45 GHz, temperature control by automated adjustment of irradiation power in a range from 0 to 300 W in Biotage microwave reaction vials (2–5 mL) with Teflone septum and an aluminum crimp top.

4.2 2-((2,3-Diethylphenyl)amino)benzoyl chloride (mefenamyl chloride) (5)

To a solution of mefenamic acid (**4**) (241 mg, 1.00 mmol) in abs. EtOH (10 mL) was added slowly PCl_5 (208 mg, 1.00 mmol) and the mixture was heated under reflux for 2 h. The mixture was kept overnight at 0–4°C and the solid was filtered and directly used for the next step without further purification.

4.3 4-(Acetylphenyl)-2-((2,3-dimethylphenyl) amino)benzamide (7)

Method a: A mixture of **5** (78 mg, 1.00 mmol) and *p*-aminoacetophenone (41 mg, 3.00 mmol) was irradiated under microwave for 5 min. The mixture was cooled to room temperature and rinsed with $CHCl_3$ (2×10 mL) and the combined extract was washed successively with a solution of 2 M HCl (10 mL) and a solution of 5% $NaHCO_3$ (10 mL). The organic layer was dried ($MgSO_4$) and evaporated to dryness. The crude product was purified by washing

with pentane, dried and recrystallized from EtOH to give **7** (215 mg, 60%); m.p. 122–124°C. – ^{1}H NMR ([D$_6$]DMSO): δ = 10.25 (s, 1H, NH$_{amid}$), 8.40 (d, 1H, *J* = 7.9 Hz, 3-H), 8.06 (d, 1H, *J* = 15.0 Hz, Hβ), 7.87 (d, 2H, *J* = 7.8 Hz, 3″-H + 5″-H), 7.85 (d, 2H, *J* = 7.8 Hz, 2″-H + 6″-H), 7.64 (d, 1H, *J* = 7.7 Hz, 6-H), 7.59 (d, 1H, *J* = 15.0 Hz, Hα), 7.45 (m, 1H, 4-H), 6.99 (m, 1H, 5-H), 6.89 (m, 1H, 5′-H), 6.70 (d, 1H, *J* = 8.0 Hz, 4′-H), 6.32 (d, 1H, *J* = 8.0 Hz, 6′-H), 5.20 (s, 1H, *NH*Ph), 3.34 (s, 3H, C$_{3'}$-*Me*), 2.62 (COMe), 2.12 (s, 3H, C$_{2'}$-*Me*) ppm. – ^{13}C NMR ([D$_6$]DMSO): δ = 190.1 (*CO*Me), 166.2 (C$_{amide}$=O), 149.3 (C-2), 145.1 (C-1″), 139.1 (C-1′), 137.2 (C-3′), 135.5 (C-4″), 135.3 (C-4), 133.2 (C-2′), 130.4 (C-3″ + C-5″), 128.3 (C-6), 125.1 (C-6′), 123.1 (C-5′), 122.7 (C-2″ + C-6″), 120.1 (C-1), 117.8 (C-3 + C-4′), 116.3 (C-5), 21.3, 20.7 (2×*Me*Ph) ppm. – $C_{23}H_{22}N_2O_2$ (348.44): calcd. C 77.07, H 6.19, N 7.82; found C 76.91, H 6.02, N 7.59.

Method b: To a solution of appropriate carboxylic acid derivative (1.00 mmol) and phenol or amine derivative (1.00 mmol) in CH_2Cl_2 (10 mL), DMAP (0.20 mmol) and DCC (1.10 mmol) were added and the resulting solution was stirred for 16 h at room temperature. The reaction mixture was quenched with 0.5 N HCl and extracted with CH_2Cl_2. The organic phase was washed with a 1% $NaHCO_3$ solution and brine, dried over Na_2SO_4 and evaporated under vacuum. The residue was purified by flash column chromatography. Elution with CH_2Cl_2–MeOH (0–5%) as eluents afforded **7** (290 mg, 81%); m.p. and mixed m.p. with other physical properties were identical for those of the authentic sample prepared in method a.

4.4 General procedure for the preparation of chalcone derivatives (8–13)

Method a: To a mixture of **7** (358 mg, 1.00 mmol) in EtOH and 20% NaOH (40 mg, 1.00 mmol) was added substituted benzaldehyde (1.00 mmol) and the mixture was stirred under microwave irradiation for 6–8 min. The reaction progress was monitored by thin-layer chromatography (TLC). After completion of the reaction, the mixture was poured onto ice-cold water and neutralized with dil. HCl. The resulting solid was filtered, dried and recrystallized from EtOH.

4.4.1 N-(4-Cinnamoylphenyl)-2-((2,3-dimethylphenyl) amino)benzamide (8)

From benzaldehyde (106 mg). Yield: 335 mg (75%); m.p. 200–201°C. – IR (film): ν = 1470 – 3330 (NHPh), 3320 (NH$_{amid}$), 1690 (C=O$_{amid}$), (CH=CH), 1650 (C=O), 1585 (C=C$_{arom.}$) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 12.15 (s, 1H, NH$_{amid}$), 8.40 (d, 1H, *J* = 7.8 Hz, 3-H), 8.06 (d, 1H, *J* = 15.0 Hz, Hβ), 7.87 (d, 2H, *J* = 7.7 Hz, 3″-H + 5″-H), 7.85 (d, 2H, *J* = 7.7 Hz, 2″-H + 6″-H), 7.66 (d, 2H, 2‴-H + 6‴-H), 7.64 (d, 1H, *J* = 7.8 Hz, 6-H), 7.59 (d, 1H, *J* = 15.0 Hz, Hα), 7.40 (d, 2H, *J* = 7.9 Hz, 3‴-H + 5‴-H), 7.33 (m, 1H, 4‴-H), 7.45 (m, 1H, 4-H), 6.99 (m, 1H, 5-H), 6.89 (m, 1H, 5′-H), 6.70 (d, 1H, *J* = 7.8 Hz, 4′-H), 6.32 (d, 1H, *J* = 7.8 Hz, 6′-H), 5.20 (s, 1H, *NH*Ph), 3.34 (s, 3H, C$_{3'}$-*Me*), 2.12 (s, 3H, C$_{2'}$-*Me*) ppm. – ^{13}C ([D$_6$]DMSO): δ = 190.1 (C = O), 166.2 (C$_{amide}$=O), 149.3 (C-2), 146.1 (Cβ), 145.1 (C-1″), 139.1 (C-1′), 137.2 (C-3′), 136.4 (C-1‴), 135.5 (C-4″), 135.3 (C-4), 133.2 (C-2′), 130.4 (C-3″ + C-5″), 129.2 (C-3‴ + C-5‴), 128.5 (C-2‴ + C-6‴), 128.3 (C-6), 127.1 (C-4‴), 125.1 (C-6′), 123.1 (C-5′), 122.7 (C-2″ + C-6″), 120.7 (Cα), 120.1 (C-1), 117.8 (C-3 + C-4′), 116.3 (C-5), 21.3, 20.7 (2×*Me*Ph) ppm. – $C_{30}H_{26}N_2O_2$ (446.51): calcd. C 80.69, H 5.87, N 6.27; found C 80.62, H 5.83, N 6.19.

4.4.2 N-(4-(3-(4-Chlorophenyl)acryloyl)phenyl)-2-((2,3-dimethylphenylphenyl)amino)benzamide (9)

From 4-chlorobenzaldehyde (141 mg). Yield: 337 mg (70%); m.p. 201–203°C. – IR (film): ν = 3330 (NH$_{amide}$), 3325 (NHPh), 1680 (C=O), 1650 (C=O$_{amide}$), 1575 (C=C$_{arom.}$), 1465 (CH=CH) cm^{-1}. – ^{1}H ([D$_6$]DMSO): δ = 12.20 (s, 1H, NH$_{amide}$), 8.38 (d, 1H, *J* = 7.8 Hz, 3-H), 8.06 (d, 1H, *J* = 16.2 Hz, Hβ), 7.87 (d, 2H, *J* = 7.9 Hz, 3″-H + 5″-H), 7.85 (d, 2H, *J* = 7.9 Hz, H-2″ + 6″-H), 7.66 (d, 2H, *J* = 7.8 Hz, 2‴-H + 6‴-H), 7.64 (d, 1H, *J* = 7.9 Hz, 6-H), 7.56 (d, 1H, *J* = 16.2 Hz, Hα), 7.45 (m, 1H, 4-H), 7.44 (d, 2H, *J* = 7.8 Hz, 3‴-H + 5‴-H), 6.89 (m, 1H, 5′-H), 6.99 (d, 1H, *J* = 7.9 Hz, 6′-H), 6.39 (m, 1H, 5-H), 6.32 (d, 1H, *J* = 7.8 Hz, 4′-H), 5.20 (s, 1H, *NH*Ph), 2.34 (s, 3H, C$_{3'}$-*Me*), 2.12 (s, 3H, C$_{2'}$-*Me*) ppm. – ^{13}C ([D$_6$]DMSO): δ = 190.2 (C=O), 165.9 (CONH), 150.1 (C-2), 146.2 (Cβ), 145.0 (C-1″), 139.0 (C-1′), 137.2 (C-3′), 134.0 (C-4‴), 133.3 (C-4), 133.1 (C-1‴), 132.8 (C-4″), 131.5 (C-2′), 131.0 (C-3″ + C-5″), 129.4 (C-2‴ + C-6‴), 129.2 (C-6), 127.5 (C-3‴ + C-5‴), 126.5 (C-6′), 125.9 (C-5′), 123.6 (C-2″ + C-6″), 121.0 (Cα), 120.7 (C-1), 119.8 (C-4′), 119.3 (C-3), 116.5 (C-5), 21.3, 20.7 (2×*Me*Ph) ppm. – $C_{30}H_{25}O_2N_2Cl$ (480.97): calcd. C 74.91, H 5.24, N 5.83; found C 74.85, H 5.19, N 5.89.

4.4.3 N-(4-(3-(4-Bromophenyl)acryloyl)phenyl)-2-((2,3-dimethylphenyl)amino)benzamide (10)

From 4-bromobenzaldehyde (185 mg). Yield: 368 mg (70%); m.p. 211–213°C. – IR (film): ν = 3330 (NH$_{amide}$), 3320 (NHPh), 1681 (C=O), 1653 (C=O$_{amide}$), 1580 (C=C$_{arom.}$), 1460 (CH=CH) cm^{-1}. – ^{1}H NMR ([D$_6$]DMSO): δ = 11.96 (s, 1H, NH$_{amide}$), 8.32 (d, 1H, *J* = 7.8 Hz, 3-H), 8.09 (d, 1H, *J* = 16.2 Hz, Hβ), 7.85 (d, 2H, *J* = 8.1 Hz, 3″-H + 5″-H), 7.80 (d, 2H, *J* = 8.1 Hz, 2″-H + 6″-H), 7.64 (d, 1H, *J* = 7.8 Hz, 6-H),

7.60 (d, 2H, J = 7.9 Hz, 2‴-H + 6‴-H), 7.58 (d, 1H, J = 16.2 Hz, Hα), 7.46 (m, 1H, 4-H), 7.44 (d, 2H, J = 7.9 Hz, 3‴-H + 5‴-H), 7.02 (m, 1H, 5′-H), 6.91 (d, 1H, J = 8.0 Hz, 6′-H), 6.40 (m, 1H, 5-H), 6.31 (d, 1H, J = 7.8 Hz, 4′-H), 4.50 (s, 1H, *NH*Ph), 2.36 (s, 3H, $C_{3'}$-*Me*), 2.11 (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C ([D_6]DMSO): δ = 189.7 (C=O), 165.8 (CONH), 150.0 (C-2), 145.1 (Cβ), 144.1 (C-1″), 139.0 (C-1′), 137.8 (C-3′), 132.0 (C-1‴), 134.2 (C-4″), 133.5 (C-4), 133.0 (C-2′), 132.9 (C-3‴ + C-5‴), 131.4 (C-3″ + C-5″) 128.2 (C-2‴ + C-6‴), 128.0 (C-6), 127.5 (C-6′), 126.5 (C-5′), 122.3 (C-4‴), 122.1 (C-2″ + C-6″), 121.3 (Cα), 121.2 (C-1), 120.0 (C-4′), 119.1 (C-3), 117.9 (C-5), 21.7, 21.3 (2× *Me*Ph) ppm. – $C_{30}H_{25}O_2N_2Br$ (525.24): C 68.59, H 4.80, N 5.33; found C 68.44, H 4.75, N 5.21.

4.4.4 N-(4-(3-(4-(Dimethylamino)phenyl)acryloyl)phenyl-2-((2,3-dimethylphenyl)amino)benzamide (11)

From 4-dimethylaminobenzaldehyde (149 mg). Yield: 392 mg (80%); m.p. 212–213°C. – IR (film): ν = 3320 (NH_{amide}), 3315 (NHPh), 1680 (C=O), 1650 ($C{=}O_{amide}$), 1580 ($C{=}C_{arom.}$), 1460 (CH=CH) cm^{-1}. – ^{1}H ([D_6]DMSO): δ = 11.85 (s, 1H, NH_{amide}), 8.31 (d, 1H, J = 7.9 Hz, 3-H), 8.06 (d, 1H, J = 15.0 Hz, Hβ), 7.87 (d, 2H, J = 8.0 Hz, 3″-H + 5″-H), 7.85 (d, 2H, J = 8.0 Hz, 2″-H + 6″-H), 7.72 (d, 1H, J = 7.9 Hz 6-H), 7.66 (d, 2H, J = 7.8 Hz, 2‴-H + 6‴-H), 7.56 (d, 1H, J = 15.0 Hz, Hα), 7.44 (m, 1H, 4-H), 6.72 (d, 2H, J = 7.8 Hz, 3‴-H + 5‴-H), 6.99 (m, 1H, 5′-H), 6.89 (d, 1H, J = 7.9 Hz, 6′-H), 6.39 (m, 1H, 5-H), 6.32 (d, 1H, J = 7.7 Hz, 4′-H), 5.20 (s, 1H, *NH*Ph), 3.01 (2xs, 6H, NMe_2), 2.34 (s, 3H, $C_{3'}$-*Me*), 2.12 (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 190.2 (C=O), 165.9 (CONH), 150.3 (C-2 + C-4‴), 145.6 (C-1″), 144.5 (Cβ), 137.9 (C-1′ + C-3′), 133.1 (C-2′), 132.1 (C-4″), 131.2 (C-4), 131.8 (C-3″ + C-5″), 129.7 (C-2‴ + C-6‴), 129.4 (C-6), 127.2 (C-6′), 126.1 (C-5′), 124.7 (C-1‴), 122.2 (C-2″ + C-6″), 122.1 (Cα), 120.7 (C-1), 120.1 (C-4′), 118.6 (C-3), 117.5 (C-5), 116.7 (C-3‴ + C-5‴), 41.0 (NMe_2), 21.3, 20.7 (2× *Me*Ph) ppm. – $C_{32}H_{31}O_2N_3$ (489.60): calcd. C 78.45, H 6.38, N 8.49; found C 78.21, H 6.28, N 8.27.

4.4.5 2-((2,3-Dimethylphenyl)amino)-N-(4-(3-(4-nitrophenyl)acryoyl)phenyl)benzamide (12)

From 4-nitrobenzaldehyde (151 mg). Yield: 343 mg (70%); m.p. 220–222°C. – IR (film): ν = 3335 (NH_{amide}), 3323 (NHPh), 1680 (C=O), 1650 ($C{=}O_{amide}$), 1583 ($C{=}C_{arom.}$), 1467 (CH=CH) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 11.98 (s, 1H, NH_{amide}), 8.34 (d, 1H, J = 8.0 Hz, 3-H), 8.21 (d, 2H, J = 7.8 Hz, 3‴-H + 5‴-H), 8.20 (d, 1H, J = 16.2 Hz, Hβ), 8.03 (d, 2H, J = 7.8 Hz, 2‴-H + 6‴-H), 7.87 (d, 2H, J = 7.9 Hz, 3″-H + 5″-H), 7.85 (d, 2H, J = 7.9 Hz, 2″-H + 6″-H), 7.80 (d, 1H, J = 16.0 Hz, Hα), 7.65 (d, 1H, J = 7.9 Hz, 6-H), 7.46 (m, 1H, 4-H), 6.99 (m, 2H, 5-H + 6′-H), 6.89 (m, 2H, 5′-H + 6-H), 6.39 (d, 1H, J = 7.7 Hz, 4′-H), 5.21 (s, 1H, *NH*Ph), 2.34 (s, 3H, $C_{3'}$-*Me*), 2.11 (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 189.8 (C = O), 166.1 (CONH), 148.4 ($C_{4'''}$-NO_2), 147.1 (C-2), 145.2 (Cβ), 143.7 (C-1″), 138.4 (C-1′), 137.6 (C-3′), 134.3 (C-4″), 133.5 (C-4), 133.1 (C-2′), 131.5 (C-3″ + C-5″), 128.4 (C-2‴ + C-6‴), 128.0 (C-6), 127.2 (C-6′), 126.0 (C-5′), 124.1 (C-1‴), 122.2 (C-2″ + C-6″), 121.2 (C-3‴ + C-5‴), 120.7 (C-1), 119.8 (C-4′), 119.5 (C-3), 119.0 (Cα), 118.4 (C-5), 21.3, 20.7 (2× *Me*Ph) ppm. – $C_{30}H_{25}O_4N_3$ (489.36): calcd. C 73.63, H 8.18, N 5.11; found C 73.62, H 8.09, N 5.12.

4.4.6 2-((2,3-Dimethylphenyl)amino)-N-(4-(3-*p*-tolyl) acryoyl)phenyl)benzamide (13)

From 4-methylbenzaldehyde (120 mg). Yield: 355 mg (77%); m.p. 202–203°C. – IR (film): ν = 3320 (NH_{amide}), 3315 (NHPh), 1690 (C=O), 1655 ($C{=}O_{amide}$), 1582 ($C{=}C_{arom.}$), 1465 (CH=CH) cm^{-1}. – ^{1}H NMR ([D_6]DMSO): δ = 12.20 (s, 1H, NH_{amide}), 8.32 (d, 1H, J = 7.8 Hz, 3-H), 8.06 (d, 1H, J = 16.2 Hz, Hβ), 7.88 (d, 2H, J = 7.9 Hz, 3″-H + 5″-H), 7.85 (d, 2H, J = 7.9 Hz, 2″-H + 6″-H), 7.64 (d, 1H, J = 7.7 Hz, 6-H), 7.59 (d, 2H, J = 7.8 Hz, 2‴-H + 6‴-H), 7.56 (d, 1H, J = 16.0 Hz, Hα), 7.45 (m, 1H, 4-H), 7.33 (m, 1H, 5′-H), 7.18 (d, 2H, J = 7.8 Hz, 3‴-H + 5‴-H), 7.00 (m, 2H, J = 8.0 Hz, 6′-H), 6.91 (m, 1H, 5-H), 6.40 (d, 1H, J = 7.7 Hz, 4′-H), 5.20 (s, 1H, *NH*Ph), 2.34 (s, 3H, $C_{3'}$-*Me*), 2.30 (s, 3H, *Me*Ph), 2.12. (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C NMR ([D_6]DMSO): δ = 189.7 (C=O), 165.9 (NHCO), 150.0 ($C_{4'''}$-NMe_2), 144.9 (C-2), 139.3 (Cβ), 139.0 (C-1″), 137.6 (C-1′), 135.1 (C-3′), 133.5 (C-4), 133.0 (C-2′), 131.4 (C-3″ + C-5″), 128.5 (C-2‴ + C-6‴), 128.4 (C-6), 127.3 (C-6′), 125.9 (C-5′), 122.4 (C-2″ + C-6″), 122.1 (C-1‴), 121.5 (Cα), 120.7 (C-1), 120.1 (C-4′), 119.2 (C-3), 118.3 (C-5), 112.1 (C-3‴ + C-5‴), 21.3, 20.7 (2× *Me*Ph), 18.8 (NMe_2) ppm. – $C_{31}H_{28}O_2N_2$ (460.44): calcd. C 80.84, H 6.13, N 6.03; found C 80.73, H 6.12, N 6.02.

Method b: To a mixture of **7** (358 mg, 1.00 mmol) in EtOH and 20% NaOH (40 mg, 1.00 mmol) was added substituted benzaldehyde (1.00 mmol) and the mixture was heated under reflux for 4–5 h. The mixture was worked up as in method a to give **8–13** (75–85% yield).

4.5 General procedure for the synthesis of 2-((2,3-dimethylphenyl)amino)-N-(4-(6-aryl-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)benzamides (14 and 15)

A mixture of chalcone (1.00 mmol), thiourea (76 mg, 1.0 mmol) and 20% NaOH (112 mg, 2.0 mmol) in EtOH (10 mL) was heated under MWI for 3–6 min, and the

reaction was monitored by TLC. After cooling, the mixture was poured onto cold ice-water and acidified with dil. HCl, filtered, washed with water and dried. The product was recrystallized from EtOH to give the desired thiopyrimidine analogue.

4.5.1 2-((2,3-Dimethylphenyl)amino)-N-(4-(6-phenyl-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl) benzamide (14)

From **8** (447 mg). Yield: 329 mg (65%); m.p. 196–199°C. – IR (film): $\nu = 3321$ (NH_{amide}), 3310 (NHPh), 1653 ($C{=}O_{amide}$), 1606 (C=N), 1577, 1524 (2×C=C), 1150 (C=S) cm^{-1}. – ^1H ([D_6]DMSO): $\delta = 11.41$ (s, 1H, NH), 10.45 (s, 1H, NH), 8.49 (s, 1H, 5-$H_{pyrimidin}$), 8.05 (d, 1H, $J = 7.8$ Hz, 3-$H_{arom.}$), 7.84 (d, 2H, $J = 7.9$ Hz, 2‴-$H_{arom.}$ + 5‴-$H_{arom.}$), 7.82 (d, 2H, $J = 7.9$ Hz, 3″-$H_{arom.}$ + 5″-$H_{arom.}$), 7.79 (d, 2H, 2H, $J = 7.9$ Hz, 2″-$H_{arom.}$ + 5″-$H_{arom.}$), 7.64–7.25 (m, 8H, $H_{arom.}$), 7.16 (m, 2H, $H_{arom.}$), 2.25 (s, 3H, $C_{3'}$-*Me*), 2.11 (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C ([D_6]DMSO): $\delta = 181.2$ (C=S), 175.0 ($C_{pyrimid.}$-4), 166.8 (CONH), 163.9 ($C_{pyrimid.}$-6), 147.0 (C-2), 139.3 (C-1″), 137.2 (C-1′), 136.3 (C-3′), 134.8 (C-4), 131.9 (C-3′), 130.2 (C-1‴), 129.0 (C-3″ + C-5″), 128.2 (C-6 + C-4″), 127.8 (C-2‴ + C-4‴ + C-6‴), 127.0 (C-6′ + C-3‴ + C-5‴), 125.9 (C-5′), 122.8 (C-2″ + C-6″), 121.2 (C-1), 119.1 (C-4″), 118.3 (C-3), 116.7 (C-5), 109.6 ($C_{pyrimid.}$-5), 24.8 ($C_{2'}$-*Me*), 20.2 ($C_{3'}$-*Me*) ppm. – $C_{31}H_{26}N_4OS$ (506.64): calcd. C 74.08, H 5.21, N 11.15; found C 73.88, H 5.10, N 10.89.

4.5.2 N-(4-(6-(4-(Dimethylamino)phenyl)-2-thioxo-1,2-dihydropyrimidin-4-yl)phenyl)-2-((2,3-dimethylphenyl)amino)benzamide (15)

From **11** (490 mg). Yield: 327 mg (60%); m.p. 181–184°C. – IR (film): $\nu = 3325$ (NH_{amide}), 3312 (NHPh), 1646 ($C{=}O_{amide}$), 1610 (C=N), 1580, 1528 (2×C=C), 1155 (C=S) cm^{-1}. – ^1H ([D_6]DMSO): $\delta = 11.39$ (s, 1H, NH), 10.43 (s, 1H, NH), 8.48 (s, 1H, 5-$H_{pyrimid.}$), 8.06 (d, 1H, $J = 7.8$ Hz, 3-$H_{arom.}$), 7.84 (d, 2H, $J = 7.9$ Hz, 3″-$H_{arom.}$ + 5″-$H_{arom.}$), 7.80 (d, 2H, $J = 7.9$ Hz, 2″-$H_{arom.}$ + 6″-$H_{arom.}$), 7.65 (m, 1H, 5-$H_{arom.}$), 7.63 (m, 3H, 3-$H_{arom.}$ + 2‴-$H_{arom.}$ + 6‴-$H_{arom.}$), 6.10–6.92 (m, 6H, $H_{arom.}$), 2.69 (s, 6H, NMe_2), 2.24 (s, 3H, $C_{3'}$-*Me*), 2.11 (s, 3H, $C_{2'}$-*Me*) ppm. – ^{13}C ([D_6]DMSO): $\delta = 181.8$ (C=S), 175.2 ($C_{pyrimid.}$-4), 166.9 (CONH), 163.4 ($C_{pyrimid.}$-6), 153.9 (C_4‴-NMe_2), 146.4 (C-2), 140.9 (C-1″), 137.2 (C-1′), 136.1 (C-3′), 133.8 (C-4), 130.8 (C-4″), 128.9 (C-6), 128.2 (C-2‴ + C-6‴), 127.1 (C-6′ + C-3″ + C-5″), 125.4 (C-5′ + C-1‴), 123.2 (C-1), 122.6 (C-4′), 118.5 (C-2″ + C-6″), 117.6 (C-3), 115.8 (C-5), 112.6 (C-3‴ + C-5‴), 110.1 ($C_{pyrimid.}$-5), 41.3 (C-NMe_2), 25.5 ($C_{2'}$-*Me*), 20.4 ($C_{3'}$-*Me*) ppm. – $C_{33}H_{31}N_5OS$ (545.71): calcd. C 72.63, H 5.73, N 12.83; found C 72.41, H 5.60, N 12.61.

4.6 *In vitro* anti-HIV assay

Evaluation of the antiviral activity of the test compounds against HIV-1 strain III_B and HIV-2 strain (ROD) in MT-4 cells was performed using the MTT assay as previously described. Stock solution (10×final concentrations) of the test compound was added in 25 µL volumes to two series of triplicate wells to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial five-fold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman Instruments, Fullerton, CA, USA). Untreated control HIV- and mock-infected cell samples were included for each sample. HIV-1 (III_B) or HIV-2 (ROD) stock (50 µL) at 100–300 CCID50 (50% cell culture infectious dose) or culture medium was added to the microtiter tray. Mock-infected cells were used to evaluate the effect of test compounds on uninfected cells in order to assess the cytotoxicity of the test compound. Exponentially growing MT-4 cells were centrifuged for 5 min at 1000 rpm, and the supernatant was discarded. The MT-4 cells were resuspended at 6×105 cells/mL, and an amount of 50 µL volume was transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells were examined spectrophotometrically by the MTT assay. The MTT assay is based on the reduction of yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Across Organics, Geel, Belgium) by mitochondrial dehydrogenase of metabolically active cells to a blue purple formazan that can be measured spectrophotometrically. The absorbances were read in an eight-channel computer-controlled photometer (Multiscan Ascent Reader, Labsystem, Helsinki, Finland) at two wavelengths (540 and 690 nm). All data were calculated using the median optical density value of three wells.

5 Supplementary information

Energies of the analogues **8–15** and representations of the frontier orbitals in **8** and **14** are given as Supplementary Information available online (http://dx.doi.org/10.1515/znb-2016-0223).

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