Bioorganic & Medicinal Chemistry 20 (2012) 6795–6802

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Discovery of novel 2-(3-(2-chlorophenyl)pyrazin-2-ylthio)-*N*-arylacetamides as potent HIV-1 inhibitors using a structure-based bioisosterism approach

Peng Zhan [a], Wenmin Chen [a], Zhenyu Li [a], Xiao Li [a], Xuwang Chen [a], Ye Tian [a], Christophe Pannecouque [b], Erik De Clercq [b], Xinyong Liu [a,*]

[a] *Department of Medicinal Chemistry, Key Laboratory of Chemical Biology (Ministry of Education), School of Pharmaceutical Sciences, Shandong University, 44, West Culture Road, 250012 Jinan, Shandong, PR China*
[b] *Rega Institute for Medical Research, KU Leuven, Minderbroedersstraat 10, B-3000 Leuven, Belgium*

ARTICLE INFO

Article history:
Received 13 August 2012
Revised 29 September 2012
Accepted 29 September 2012
Available online 6 October 2012

Keywords:
HIV reverse transcriptase
NNRTIs
Heterocycle
Synthesis
Anti-HIV-1 activity
SAR
Pyrazinylthioacetamide
Structure-based bioisosterism strategy

ABSTRACT

The present work is an extension of our ongoing efforts towards the development and identification of new molecules with anti-HIV activity which have previously led to the discovery of arylazolylthioacetanilides as highly active NNRTIs. In this article, a series of 2-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)-*N*-arylacetamide derivatives were synthesized and evaluated for in vitro anti-HIV activity. Most of the tested compounds exhibited moderate activities against wild-type HIV-1. Among them, compound **6k** showed significant activity against wild-type HIV-1 with an EC_{50} value of 1.7 μM, along with moderate activity against wild-type reverse transcriptase (RT). The preliminary structure–activity relationship (SAR) and docking calculations of this new series of compounds were also investigated, which may help designing more potent molecules.

1. Introduction

Non-nucleoside reverse transcriptase inhibitors (NNRTIs) play an essential role in the highly active antiretroviral therapy (HAART) for acquired immunodeficiency syndrome (AIDS) due to their potent antiviral activity, high specificity and low toxicity. Unfortunately, NNRTI potency is dramatically reduced by the emergence of drug-resistant HIV-1 mutants. Thus, the development of novel chemical entities with improved activity profiles has been a very active research field in recent years.[1–4]

Among the representatives of the NNRTIs, arylazolylthioacetanilides have interesting structural characteristics and highly anti-HIV-1 specific activities that have induced many kinds of structural modifications on the skeleton of the five-membered lead compounds.[5–11] Several triazolylthioacetanilide NNRTIs, such as VRX-480733[12] and RDEA806 (in phase IIa clinical trials)[13], have been or are currently being evaluated in clinical trials (Fig. 1).

In connection with our research program focusing on the preparation of new therapeutic agents, our group previously designed and synthesized a number of 'follow-on'-based[14] arylazolylthioacetanilide derivatives.[15–23] Most of these compounds displayed potent anti-HIV properties in cell lines infected with either wild-type or mutant HIV-1. In particular, 1,2,3-thiadiazole derivative **ZP7** displayed the most potent anti-HIV-1 activity (EC_{50} = 36 nm), inhibiting HIV-1 replication in MT-4 cells more effectively than NVP (by sevenfold) and DLV (by eightfold).[16] Molecular modeling studies demonstrated that the five-membered heterocycle portion of these inhibitors could be acting as a scaffold which orients the pharmacophores into the proper geometry for effective interactions with the target and as key structural element to form hydrogen bond with K103 of the RT pocket (as predicted by the modeling studies).[15] Therefore, there are differences in the electronic and conformational contribution of the heterocyclic groups to the binding of the inhibitors with the HIV-1 RT.[15] Supported by these promising results and with the aim of obtaining more potent compounds and establishing further SAR on this class of NNRTIs, we designed and synthesized a series of novel pyrazin-2-ylthioacetamide derivatives (**6a–6q**) (Fig. 2), based on the structure-based bioisosterism strategy in medicinal chemistry[24], in which the '–SCH_2CO–' linker was maintained in view of its paramount importance. Their anti-HIV activities against HIV-1 (III_B) and HIV-2 (ROD) as well as their HIV RT inhibitory activities were also presented.

* Corresponding author. Tel.: +86 531 88380270; fax: +86 531 88382731.
E-mail address: xinyongl@sdu.edu.cn (X. Liu).

0968-0896/$ - see front matter Crown Copyright © 2012 Published by Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.bmc.2012.09.058

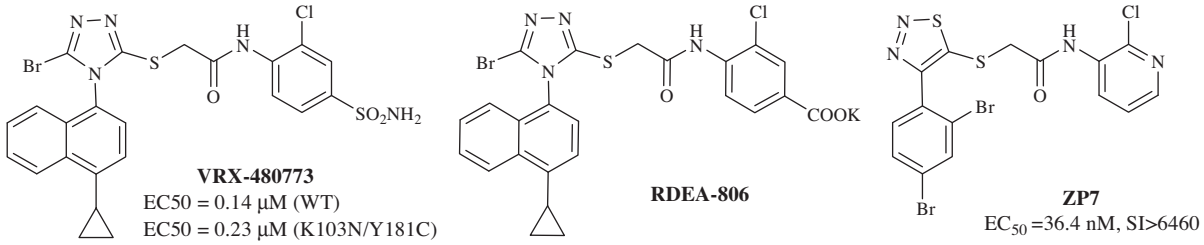


Figure 1. Azolylthioacetanilide-based NNRTIs.

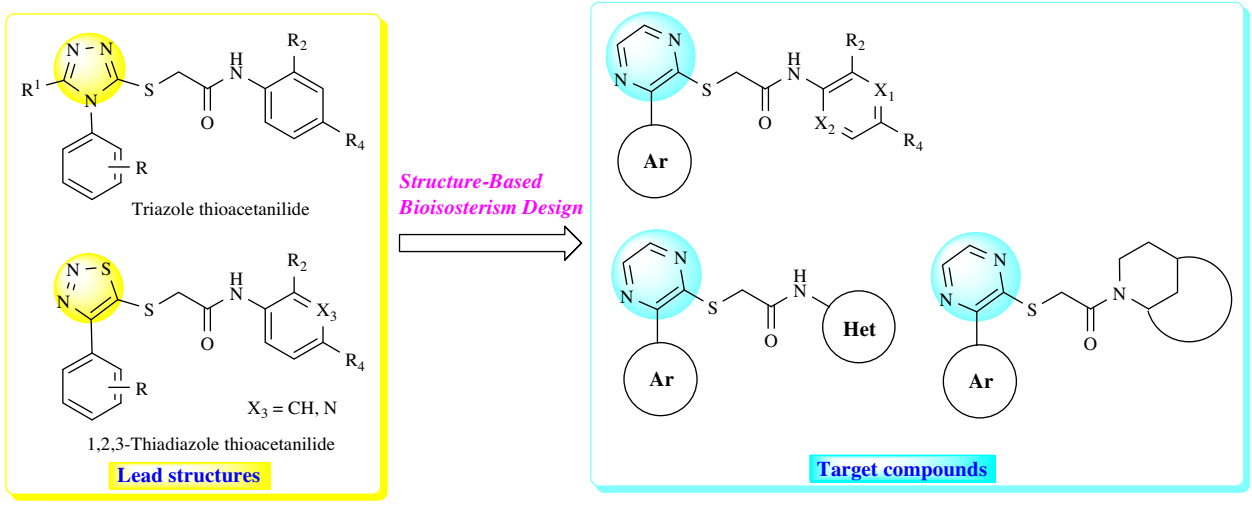
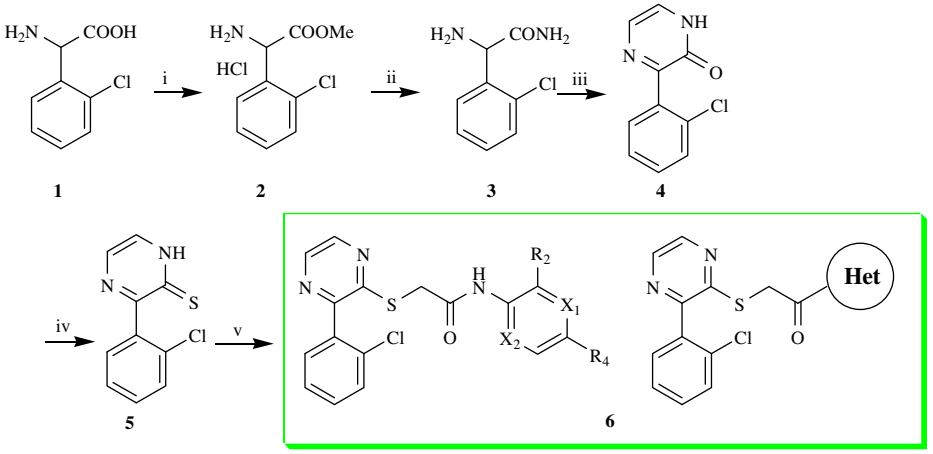


Figure 2. The structure-based bioisosterism replacement of azoles by pyrazine.

2. Results and discussion

2.1. Chemistry

To achieve the synthesis of the target compounds **6a–6q**, the steps outlined in Scheme 1 were adopted. The key intermediate, (+/−)-2-amino-2-(2-chlorophenyl) acetamide (**3**) was prepared from readily available racemic 2-chlorophenyl glycine (**1**) according to a reported procedure.[25] The synthesis of the pyrazinone **4** was achieved in one step by condensation of 2-chlorophenyl glycinamide with glyoxal.[26] Treatment of **4** with phosphorus pentasulfide in pyridine under reflux afforded 3-(2-chlorophenyl)pyrazine-2(1*H*)-thione (**5**). The final pyrazin-2-ylthioacetanilides (**6**) were synthesized by reaction of intermediate **5** with suitable 2-chloro-*N*-aryl-substituted acetamides in good yields, using the same conditions as for other alkyl halides. 2-Chloro-*N*-phenyl acetamides (or other alkyl halides) were synthesized according to the reported literature.[27] The synthesized compounds were characterized by the MS, IR and ^{1}H NMR spectral data together with TLC analysis.



Scheme 1. Reagents and conditions: (i) $SOCl_2$, MeOH, rt, overnight; (ii) $NH_3{\cdot}H_2O$, rt, overnight; (iii) glyoxal, 12.5 N NaOH; (iv) P_2S_5, Pyridine, reflux; (v) $ClCH_2CONHAr$ (other alkyl halides), Na_2CO_3 or NaOH, EtOH.

2.2. Biological activities

2.2.1. Anti-HIV activities evaluation

The newly synthesized pyrazin-2-ylthioacetamide derivatives were evaluated for anti-HIV activity by determining their ability to inhibit the replication of the HIV-1 III_B strain[28] and HIV-2 ROD strain[29] in MT-4 cell cultures[30] in comparison with nevirapine (NVP), zidovudine (azidothymidine, AZT), dideoxycytidine (DDC), delavirdine (DLV) and efavirenz (EFV) used as reference drugs. The methodology of the cell-based anti-HIV assay has been previously described.[31,32] The cytotoxicity of these compounds was determined in parallel. Comparisons of the antiviral inhibitory concentration (EC_{50}), cytotoxic concentration (CC_{50}), and SI (selectivity, given by the CC_{50}/EC_{50} ratio) values for different compounds are depicted in Table 1.

The experimental results indicated that 13 members of the tested pyrazin-2-ylthioacetamide derivatives were found to be active against HIV-1 (III_B) with EC_{50} values in the range of 1.7–9.0 μM, and none of the compounds was active against HIV-2 (ROD). In particular, the most potent compound was found to be **6k** having an EC_{50} value of 1.7 μM against HIV-1(III_B) with a selectivity index (SI) of 23. These results indicated that the pyrazine ring is an appropriate bioisosteric group instead of the five-membered heterocycles in the lead compounds.

Table 1 also reveals the potency order of the *ortho* substitution at the phenyl ring of the anilide moiety: NO_2 (**6k**, EC_{50} = 1.7 μM) > Br (**6e**, EC_{50} = 4.2 μM) > Cl (**6c**, EC_{50} = 4.6 μM) > F (**6b**, EC_{50} = 5.3 μM) > H (**6a**, EC_{50} >35 μM). It is very clear that significantly decreased potency was observed for **6a** characterized by the absence of the *ortho* substitution at the phenyl ring of the anilide moiety. Consistent with the observations in the arylazolylthioacetanilide series[15,17], these results indicated that the activity data of the pyrazin-2-ylthioacetamide derivatives are affected by the electronic nature or the steric demand of the *ortho* substitution.

In addition, it is worth noting that the anti-HIV activity was also strongly dependent on the nature of the *para* position of the anilide moiety. For instance, it has been shown that the introduction of 4-acetyl or a methoxy carbonyl group led to compounds with slightly improved or similar activity (**6h**, **6i**), whereas, when the methyl group or chlorine atom was introduced into the *para* position of the anilide moiety, the bioactivity was strikingly decreased (**6e**/**6f**, **6k**/**6l**, **6d**/**6c**) suggesting that the nature (probably hydrophobicity) of the methyl or chlorine atom did not accommodate the chemical environment in this region of RT.

In the 1,2,3-thiadiazole series, we[16] have demonstrated that the introduction of the pyridine moiety at the aromatic amine domain led to substantial improvement in potency and selectivity. Interestingly, this beneficial effect was also observed in this closely related pyrazin-2-ylthioacetamide series (as shown in **6c** and **6m**). Thus again, these results confirmed the idea that the introduction of structurally diverse heterocycles in this region could be a valid strategy to get novel molecules with increased or appreciable antiviral potency. Meanwhile, it was clear that the anti-HIV activities of the pyrazin-2-ylthioacetamides are very sensitive to these structural modifications.

From the structure–activity relationship (SAR) point of view, we found that the SAR features of the pyrazin-2-ylthioacetamides were highly consistent with the previously observed arylazolylthioacetanilide-typed NNRTIs.[15–21]

Moreover, in order to obtain comprehensive SAR indications and to identify more potent NNRTIs, the replacement of the N-substituted heterocycle acetamide moiety afforded 3,4-dihydro(iso)quinolin-2(1*H*)-yl ethanone derivatives **6p** and **6q**. It was also observed that 3,4-dihydroisoquinolin-2(1*H*)-yl ethanone derivative **6q** retained its anti-HIV activity in the submicromolar concentration range (EC_{50} = 4.0 μM).

Therefore, based on the above SAR conclusions and the fact that all the derivatives did not show any activity against HIV-2 (ROD) in MT-4 cells, it can be concluded that this new series of pyrazin-2-ylthioacetamide derivatives was specific for HIV-1 and could be classified as typical NNRTIs.

2.2.2. HIV-1 RT inhibition assay

With the aim to further confirm the drug target of pyrazin-2-ylthioacetamide derivatives, the selected title compound **6k** was tested in enzymatic assays against highly purified recombinant HIV-1 RT using poly(rC)-oligo(dG) as template primer.[33] As shown in Table 2, this compound exhibited moderate inhibition of enzymatic activity with an IC_{50} value of 50 μM, which was 18 fold higher than that of NVP (2.7 μM). This result effectively verified the same drug target for **6k**.

3. Molecular modeling

To better elucidate the HIV inhibitory potencies of the newly synthesized compounds at a molecular level and to understand the structural basis of their binding mode, a molecular modeling analysis of compound **6k** was performed by means of Autodock Vina [http://vina.scripps.edu]. The X-ray crystal structure of HIV-1 RT with benzophenone taken from PDB (3DLG) was used as the input structure for docking calculations because of the high degree of structural similarity between arylazolylthioacetanilides and benzophenones.[5] Default parameters were used as described in the Autodock Vina manual unless otherwise specified. The theoretical binding mode of **6k** to the NNIBP is shown in Figure 3.

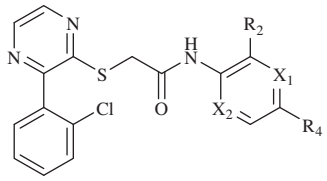
Results suggested that this class of compounds shares a similar binding mode with previous arylazolylthioacetanilides.[17] As illustrated in Figure 3, the 2-chlorophenyl ring of **6k** fitted into the aromatic-rich binding pocket, surrounded by the aromatic side chains of Tyr188, Phe227, and Trp229. Detailed analysis of the binding mode showed that one phenyl ring interacts favorably with the Tyr188 side chain, giving rise to a π–π stacking interaction. One N atom in the pyrazine ring and the nitro group at the phenyl ring of the anilide moiety form multiple key hydrogen bonds with their surrounding amino acid residues, which is important for the affinity between inhibitor and RT.

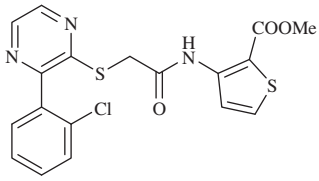
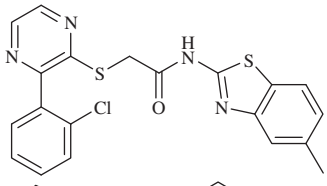
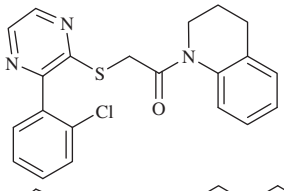
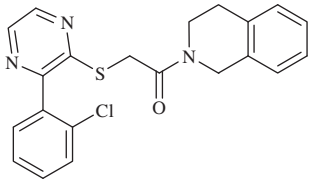
Taken together, our docking calculations allowed us to rationalize the activity profile of the pyrazin-2-ylthioacetamide against wild-type RT, which provided valuable information for further design of novel effective anti-HIV drugs.

4. Conclusion

In summary, in the present investigation, we have designed a series of novel 2-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)-*N*-arylacetamide derivatives as inhibitors of HIV-1 RT based on the structure-based bioisosterism approach and described the convenient and efficient method of synthesis for the first time. The preliminary SAR was discussed. Some derivatives proved to be highly effective in inhibiting HIV-1 replication at low micromolar concentrations. Among them, compound **6k** was identified as the most promising candidate with favorable inhibitory activity against the wild-type HIV-1. The RT inhibitory potency was also assayed. Although the IC_{50} value of the tested compound were suboptimal comparing to that of nevirapine, the valuable information from SAR analysis and molecular simulation encouraged us toward the rational design of new derivatives. Further structure modifications on the six-membered heterocycle section of arylazinylthioacetanilide scaffold are underway.

Table 1
Anti-HIV activity, cytotoxicity and selectivity indices of 2-(3-(2-chlorophenyl)pyrazin-2-ylthio)-*N*-arylacetamide derivatives (**6a**–**6q**)



Code	X_1	X_2	R_2	R_4	EC_{50}(μM)[a]		CC_{50} (μM)[b]		SI[c]	
					HIV-1 III_B	HIV-2 ROD	HIV-1 III_B	HIV-2 ROD	HIV-1 III_B	HIV-2 ROD
6a	CH	CH	H	H	>35	>33	35	33	<1	<1
6b	CH	CH	F	H	**5.3**	>91	48	91	9	<1
6c	CH	CH	Cl	H	**4.6**	>46	34	46	7	< 1
6d	CH	CH	Cl	Cl	**5.2**	>34	29	34	6	<1
6e	CH	CH	Br	H	**4.2**	>41	31	41	7	<1
6f	CH	CH	Br	Me	**4.3**	>35	31	35	9	<1
6g	CH	CH	Br	Cl	**5.1**	>29	26	29	5	<1
6h	CH	CH	Br	COMe	**2.5**	>31	30	31	12	<1
6i	CH	CH	Br	COOMe	**3.7**	>108	95	108	25	<1
6j	CH	CH	Br	COOEt	**9.0**	>62	45	62	5	<1
6k	CH	CH	NO_2	H	**1.7**	>55	39	55	23	<1
6l	CH	CH	NO_2	Me	**2.9**	>148	54	148	19	<1
6m	N	CH	Cl	H	**2.4**	>166	152	166	64	<1
6n					>68	>48	68	48	<1	<1
6o					>32	>30	32	30	<1	<1
6p					>33	>34	33	34	<1	<1
6q					4.**10**	>50	33	50	8	<1
NVP[d]					0.090		>15	>15	>168	
AZT[d]					0.021	0.004	249	249	12,221	56,907
DDC[d]					1.0	1.3	>95	>95	>93	>74
EFV[d]					0.005		>6.3	>6.3	>1187	
DLV[d]					0.032		>36	>36	>1096	

In bold are the values of active compounds.
[a] EC_{50}: concentration of compound required to achieve 50% protection of MT-4 cells against HIV-1-induced cytopathicity, as determined by the MTT method.
[b] CC_{50}: concentration required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method.
[c] SI: selectivity index (CC_{50}/EC_{50}). The SI values: X_1 stands for $\geqslant 1$ or <1.
[d] The data were obtained from the same laboratory (Rega Institute for Medical Research, K.U. Leuven, Belgium).

5. Experimental

5.1. Chemistry

All melting points were determined on a micromelting point apparatus and are uncorrected. Infrared spectra (IR) were recorded with a Nexus 470FT-IR Spectrometer. ^{1}H NMR spectra were recorded on a Bruker Avance 600 or 400 spectrometer at 600 or 400 MHz, using DMSO-d_6 as solvent and tetramethylsilane (TMS) as internal standard. Chemical shifts are reported in parts per million (δ), and signals are expressed as s (singlet), d (doublet), t (triplet), q (quartet) or m (multiplet). Mass spectra were taken on a LC

Table 2
Inhibitory activity of compound **6k** against HIV-1 RT

Compd	**6k**	**NVP**
IC_{50} (μM)[a]	50	2.7

[a] 50% of the inhibitory concentration of tested compound required to inhibit biotin deoxyuridine triphosphate (biotin-dUTP) incorporation into the HIV-1 RT by 50%.

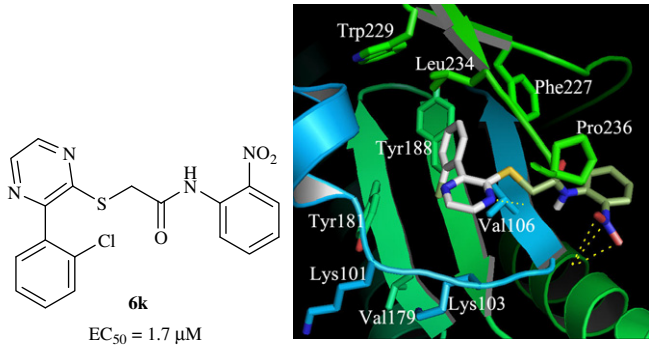


Figure 3. Predicted binding mode and molecular docking of compound **6k** into the allosteric site of HIV-1 RT (PDB code: 3DLG). The docking results are shown by PyMOL. Hydrogen bonds are indicated by dashed lines.

Autosampler Device: Standard G1313A instrument. TLC was performed on Silica Gel GF254 for TLC and spots were visualized by iodine vapors or by irradiation with UV light (254 nm). Flash column chromatography was performed on a column packed with Silica Gel 60 (200–300 mesh). Solvents were reagent grade and, when necessary, were purified and dried by standard methods. Concentration of the reaction solutions involved the use of rotary evaporator at reduced pressure.

5.2. General procedure for the synthesis of 2-(3-(2-chlorophenyl)pyrazin-2-ylthio) acetamides (6a–o)

5.2.1. General procedure for the synthesis of 3-(2-chlorophenyl)pyrazine-2(1*H*)-thione (5)

To a vigorously stirred mixture of the commercially available 2-amino-2-(2-chlorophenyl)acetic acid (**1**, 9.28 g, 0.05 mmol) and anhydrous MeOH (150 mL) at 0 °C was added thionyl chloride (4.4 mL) dropwise. The resulting solution was stirred at room temperature for 24 h (monitored by TLC), and then concentrated to 40 mL. The residue was triturated with diethyl ether to produce the white solid, which was collected and dried to afford the (+/−)-2-chlorophenylglycine methyl ester HCl salt (**2**) as gray powder. Compound **2** was used in the next step without any further purification.

The prepared methyl ester HCl salt was taken up in $NH_3{\cdot}H_2O$ (45 mL), and the reaction mixture was stirred at room temperature for 24 h. The solution was concentrated and the obtained solid was collected and recrystallized from ethyl acetate to afford the desired product (+/−)-2-amino-2-(2-chlorophenyl)acetamide (**3**) as white powder, which was pure enough for the condensation with glyoxal.[25]

To 25 g (16.6 mmol) of 40% aqueous glyoxal solution diluted with 25 mL of water was added 1.84 g. (0.010 mol) of finely powdered 2-amino-2-(2-chlorophenyl)acetamide (**3**). The mixture was cooled in an ice bath while kept stirring, was added dropwise 1.0 mL (0.0125 mol) of 12.5 N sodium hydroxide solution The resulting solution was allowed to stand at room temperature for a few minutes, which had set to a semisolid mass of finely-divided crystals (sodium salt of 3-(2-chlorophenyl)pyrazin-2-ol). After several hours, 1.0 mL of glacialacetic acid was added with stirring. The melicera precipitate was formed and extracted with ethyl acetate. The organic layer was dried over anhydrous sodium sulfate and filtered; then, the filtrate was removed under vacuo. The residue was purified by recrystallization from ethanol-petroleum ether to yield the 3-(2-chlorophenyl)pyrazin-2(1*H*)-one (4) as brown-yellow solid. Mp: 176–179 °C. MS (ESI): *m*/*z* 207.2 (M+1), 209.2 (M+3). $C_{10}H_7ClN_2O$ (Exact Mass: 206.02).

To a solution of 3-(2-chlorophenyl)pyrazin-2(1*H*)-one (**4**) (0.4 g, 1.93 mmol) in anhydrous pyridine (20 mL), phosphorous pentasulfide (1.0 g, 4.5 mmol) was added. The mixture was refluxed for 10 h. The pyridine was removed by evaporation under reduced pressure and the residue hydrolyzed by the addition of 100 mL water followed by heating the mixture in a steam bath until the evolution of hydrogen sulfide had ceased.

The mixture was allowed to cool to room temperature and extracted by ethyl acetate (3 × 15 mL). The combined orange solution was washed with 10% aqueous potassium hydroxide (3 × 15 mL). The extract was acidified with diluted hydrochloric acid, the saffron yellow precipitated solid was collected and dried to afford the 3-(2-chlorophenyl)pyrazine-2(1*H*)-thione (**5**) as reddish brown powder mp: 138–141 °C. MS (ESI): *m*/*z* 223.2 (M+1), 225.3 (M+3). $C_{10}H_7ClN_2S$ (Exact Mass: 222).

5.2.2. General procedure for the synthesis of 2-(3-(2-chlorophenyl)pyrazin-2-ylthio) acetamides (6a–o)

To the mixture solution of 3-(2-chlorophenyl)pyrazine-2(1*H*)-thione (**5**) (1.0 mmol, 0.22 g) and Na_2CO_3 (1.5 mmol) in ethanol (30 mL) were added $ClCH_2CONHPh$ (other alkyl halides) (1.0 mmol). The reaction mixture was stirred at room temperature overnight. Upon completion of the reaction, the solvent was evaporated, leaving a residue which was treated with methylene chloride (30 ml) and washed with water (3 × 30 ml). The organic layer was dried over anhydrous sodium sulfate, filtered off, and then the solvent was removed under vacuo. The residue was chromatographed on silica gel using ethyl acetate:petroleum ether. Pure fractions were collected and concentrated, giving the desired compounds (**6a–6q**) in good yield.

5.2.3. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-phenylacetamide (6a)

White powder, yield: 76.4%. Mp: 152–154 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.66 (*s*, 1H, NH), 8.51 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.47 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.74 (dd, 1H, J_1 = 0.6 Hz, J_2 = 8.4 Hz, PhH), 7.67–7.64 (m, 2H, PhH), 7.63 (dd, 1H, J_1 = 0.6 Hz, J_2 = 7.0 Hz, PhH), 7.55 (d, 2H, *J* = 7.8 Hz, Ph'H), 7.34 (t, 2H, *J* = 8.4 Hz, Ph'H), 7.10 (d, 1H, *J* = 7.0 Hz, Ph'H), 3.93 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3257 (υ_{NH}), 3085, 2957, 2924, 1647 ($\upsilon_{C=O}$), 1600, 1558, 1498, 1445 ($\upsilon_{N=N}$), 1331, 1239, 1117, 754 (υ_{C-S}). MS (ESI): *m*/*z* 356.3 (M+1), 358.3 (M+3). $C_{18}H_{14}ClN_3OS$ (Exact Mass: 355.05).

5.2.4. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(2-fluorophenyl)acetamide (6b)

White powder, yield: 72.8%. Mp: 107–109 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.64 (*s*, 1H, NH), 8.52 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.48 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.35 (t, 1H, *J* = 7.8 Hz), 7.53 (d, 1H, *J* = 7.8 Hz), 7.47–7.45 (m, 1H), 7.42–7.37 (m, 2H), 7.13–7.11 (m, 1H), 7.07–7.02 (m, 2H), 3.90 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3303 (υ_{NH}), 3118, 3064, 2925, 1693 ($\upsilon_{C=O}$), 1621, 1547, 1454 ($\upsilon_{N=N}$), 1361, 1330 (υ_{C-F}), 1132, 1070, 755, 751 (υ_{C-S}). MS (ESI): *m*/*z* 374.2 (M+1), 376.3 (M+3). $C_{18}H_{13}ClFN_3OS$ (Exact Mass: 373.05).

5.2.5. *N*-(2-Chlorophenyl)-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamide (6c)

White powder. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.29 (*s*, 1H, NH), 8.50 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.45 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.36

(dd, 1H, J_1 = 8.4 Hz, J_2 = 0.6 Hz), 7.55–7.54 (m, 1H), 7.48–7.45 (m, 1H), 7.43–7.38 (m, 2H), 7.38–7.33 (m, 1H), 7.32–7.25 (m, 1H), 7.04–7.02 (m, 1H), 4.38 (s, 2H, CH_2-S). MS (ESI): *m*/*z* 390.3 (M+1), 392.3 (M+2). $C_{18}H_{13}Cl_2N_3OS$ (Exact Mass: 389.02).

5.2.6. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(2,4-dichlorophenyl)acetamide (6d)

Yellow powder, yield: 64.8%. Mp: 148–150 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.31 (*s*, 1H, NH), 8.48 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.47 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.33 (d, 1H, *J* = 9.0 Hz, Ph'H), 7.54 (dd, 1H, J_1 = 9.0 Hz, J_2 = 0.6 Hz), 7.46 (dt, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz), 7.42 (dt, 1H, J_1 = 7.2 Hz, J_2 = 1.2 Hz), 7.37 (dd, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz), 7.48 (d, 1H, *J* = 2.4 Hz), 7.25–7.23 (m, 1H), 3.94 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3256 (υ_{NH}), 3073, 1693 ($\upsilon_{C=O}$), 1581, 1522, 1386 ($\upsilon_{N=N}$), 1359, 1301, 1132, 1071, 865, 826, 750 (υ_{C-S}). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 167.3 (C=O), 154.6, 150.9, 143.8, 139.9, 135.8, 134.3, 132.6, 131.8, 131.4, 130.2, 129.6, 129.4, 128.1, 128.1, 126.9, 126.5, 34.53 (CH_2-S). MS (ESI): *m*/*z* 424.3 (M+1), 426.3 (M+3), 428.2 (M+5). $C_{18}H_{12}Cl_3N_3OS$ (Exact Mass: 422.98).

5.2.7. *N*-(2-Bromophenyl)-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamide (6e)

White powder, yield: 58.4%. Mp: 120–122 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.11 (*s*, 1H, NH), 8.51 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.45 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.30 (d, 1H, *J* = 7.8 Hz), 7.54 (d, 1H, *J* = 7.8 Hz), 7.51–7.45 (m, 2H), 7.42–7.39 (m, 2H), 7.32–7.30 (m, 1H), 6.99–6.96 (m, 1H), 3.97 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3346 (υ_{NH}), 3055, 1688 ($\upsilon_{C=O}$), 1593, 1579, 1518, 1434 ($\upsilon_{N=N}$), 1359, 1296, 1117, 1072, 761 (υ_{C-S}), 750. MS (ESI): *m*/*z* 434.3 (M+1), 436.3 (M+3). $C_{18}H_{13}BrClN_3OS$ (Exact Mass: 432.97).

5.2.8. *N*-(2-Bromo-4-methylphenyl)-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamide (6f)

Light yellow brown powder, yield: 66.2%. Mp: 124–126 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.56 (*s*, 1H, NH), 8.15 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.92 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.65 (dd, 1H, J_1 = 7.8 Hz, J_2 = 0.6 Hz, Ph'H), 7.58–7.49 (m, 4H, PhH), 7.47 (d, 1H, *J* = 0.6 Hz, Ph'H), 7.16 (d, 1H, *J* = 7.8 Hz, Ph'H), 4.08 (s, 2H, CH_2-S), 2.27 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 166.9 (C=O), 154.7, 150.9, 143.9, 139.9, 137.0, 135.8, 133.8, 133.1, 132.6, 131.8, 131.4, 130.2, 129.1, 128.1, 126.1, 117.1, 34.53 (CH_2-S), 20.5 (CH_3). IR (KBr, cm^{-1}): 3348 (υ_{NH}), 3030, 2987, 2922, 1697 ($\upsilon_{C=O}$), 1684, 1574, 1519, 1431 ($\upsilon_{N=N}$), 1361, 1294, 1119, 1071, 765 (υ_{C-S}). MS (ESI): *m*/*z* 448.2 (M+1), 450.2 (M+3), 452.2 (M+5). $C_{19}H_{15}BrClN_3OS$ (Exact Mass: 446.98).

5.2.9. *N*-(2-Bromo-4-chlorophenyl)-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamide (6g)

Brown powder, yield: 59.4%. Mp: 140–142 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.12 (*s*, 1H, NH), 8.50 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.46 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.28 (d, 1H, *J* = 8.4 Hz, Ph'H), 7.54 (d, 1H, *J* = 8.4 Hz, PhH), 7.51 (d, 1H, *J* = 2.4 Hz), 7.47 (dt, 1H, J_1 = 7.8 Hz, J_2 = 1.2 Hz), 7.43–7.38 (m, 2H, PhH), 7.27 (dd, 1H, J_1 = 9.0 Hz, J_2 = 2,4 Hz, Ph'H), 4.95 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3277 (υ_{NH}), 3068, 1692 ($\upsilon_{C=O}$), 1573, 1517, 1383 ($\upsilon_{N=N}$), 1358, 1295, 1131, 1071, 866, 824, 750 (υ_{C-S}). $C_{18}H_{12}BrCl_2N_3OS$ (Exact Mass: 466.93).

5.2.10. *N*-(4-Acetyl-2-bromophenyl)-2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamide (6h)

White powder, yield: 66.7%. Mp: 159–160 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.34 (*s*, 1H, NH), 8.51 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.50 (d, 1H, *J* = 8.4 Hz, Ph'H), 8.47 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.14 (d, 1H, *J* = 2.4 Hz, Ph'H), 7.88 (dd, 1H, J_1 = 8.4 Hz, J_2 = 2.4 Hz, Ph'H), 7.55 (d, 1H, *J* = 7.8 Hz, PhH), 7.48 (dt, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz, PhH), 7.44–7.38 (m, 2H, PhH), 3.98 (s, 2H, CH_2-S), 2.57 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3282 (υ_{NH}), 3260, 2955, 2923, 2853, 1698 ($\upsilon_{C=O}$), 1673 ($\upsilon_{C=O}$), 1595, 1572, 1524, 1467, 1357 ($\upsilon_{N=N}$), 1309, 1266, 1128, 1070, 830, 750 (υ_{C-S}). MS (ESI): *m*/*z* 476.3 (M+1), 478.2 (M+3), 480.2 (M+5). $C_{20}H_{15}BrClN_3O_2S$ (Exact Mass: 474.98).

5.2.11. Methyl 3-bromo-4-(2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamido)benzoate (6i)

White powder, yield: 78.5%. Mp: 101–103 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.31 (*s*, 1H, NH), 8.51 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.47 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.46 (d, 1H, *J* = 3.6 Hz), 8.19 (d, 1H, *J* = 3.6 Hz), 7.96 (dd, 1H, J_1 = 8.4 Hz, J_2 = 3.6 Hz), 7.55–7.54 (m, 1H), 7.46 (dt, 1H, J_1 = 7.8 Hz, J_2 = 1.8 Hz), 7.43–7.38 (m, 2H), 3.98 (s, 2H, CH_2-S), 3.90 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3254 (υ_{NH}), 3058, 2997, 2945, 1719 ($\upsilon_{O-C=O}$), 1700 ($\upsilon_{NH-C=O}$), 1599, 1577, 1523, 1475, 1436, 1391 ($\upsilon_{N=N}$), 1360, 1288, 1272, 1227, 1119, 1072, 762 (υ_{C-S}), 749. MS (ESI): *m*/*z* 492.1 (M+1), 494.2 (M+3). $C_{20}H_{15}BrClN_3O_3S$ (Exact Mass: 490.97).

5.2.12. Ethyl 3-bromo-4-(2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamido)benzoate (6j)

Yellow powder, yield: 77.6%. Mp: 128–130 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.31 (*s*, 1H, NH), 8.51 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.47 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.46 (s, 1H, Ph'H), 8.19 (d, 1H, *J* = 1.8 Hz), 7.97 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 7.71 (d, 1H, *J* = 7.2 Hz), 7.47 (dt, 1H, J_1 = 8.4 Hz, J_2 = 1.8 Hz), 7.44–7.38 (m, 2H), 4.38 (s, 2H, CH_2-S), 4.37 (q, 2H, *J* = 1.2 Hz, CH_2), 1.38 (t, 3H, *J* = 1.2 Hz, CH_3). IR (KBr, cm^{-1}): 3257 (υ_{NH}), 3073, 3074, 2984, 2928, 2898, 1712 ($\upsilon_{O-C=O}$), 1705 ($\upsilon_{NH-C=O}$), 1599, 1579, 1521, 1477, 1439, 1391 ($\upsilon_{N=N}$), 1359, 1283, 1273, 1229, 1128, 1117, 1072, 761 (υ_{C-S}), 748. MS (ESI): *m*/*z* 506.2 (M+1), 508.2 (M+3), 510.2 (M+5). $C_{21}H_{17}BrClN_3O_3S$ (Exact Mass: 504.99).

5.2.13. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(2-nitrophenyl)acetamide (6k)

Yellow powder, yield: 48.6%. Mp: 107–109 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 11.12 (*s*, 1H, NH), 8.70 (dd, 1H, J_1 = 8.4 Hz, J_2 = 1.2 Hz, Ph'H), 8.53 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.44 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.17 (d, 1H, J_1 = 8.4 Hz, J_2 = 1.2 Hz, Ph'H), 7.65–7.63 (m, 1H), 7.55–7.54 (m, 1H), 7.47–7.41 (m, 3H), 7.21–7.18 (m, 1H), 3.79 (s, 2H, CH_2-S). IR (KBr, cm^{-1}): 3350 (υ_{NH}), 3093, 1697 ($\upsilon_{C=O}$), 1608, 1586, 1497 ($\upsilon_{as\ NO2}$), 1457 ($\upsilon_{N=N}$), 1436, 1344 ($\upsilon_{s\ NO2}$), 1284, 1118, 1070, 752 (υ_{C-S}), 743. MS (ESI): *m*/*z* 401.3 (M+1), 402.3 (M+3). $C_{18}H_{13}ClN_4O_3S$ (Exact Mass: 400.04).

5.2.14. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(4-methyl-2-nitrophenyl)acetamide (6l)

White powder, yield: 74.1%. Mp: 145–147 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 11.01 (*s*, 1H, NH), 8.57 (d, 1H, *J* = 8.4 Hz, Ph'H), 8.56 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.52 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.96 (s, 1H, Ph'H), 7.54 (d, 1H, *J* = 8.4 Hz, Ph'H), 7.47–7.41 (m, 4H, PhH), 3.99 (s, 2H, CH_2-S), 2.40 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMSO-d_6, ppm): 167.1 (C=O), 154.3, 150.9, 143.8, 141.2, 139.9, 135.8, 135.5, 132.6, 131.8, 131.4, 130.2, 129.6, 128.0, 125.4, 125.0, 34.6 (CH_2-S), 20.4 (CH_3). IR (KBr, cm^{-1}): 3327 (υ_{NH}), 3075, 2987, 2924, 2854, 1703, 1690 ($\upsilon_{C=O}$), 1574, 1497 ($\upsilon_{as\ NO2}$), 1456 ($\upsilon_{N=N}$), 1361, 1337 ($\upsilon_{s\ NO2}$), 1276, 1121, 766 (υ_{C-S}). MS (ESI): *m*/*z* 415.4 (M+1), 417.3 (M+3). $C_{19}H_{15}ClN_4O_3S$ (Exact Mass: 414.06).

5.2.15. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(2-chloropyridin-3-yl)acetamide (6m)

White powder, yield: 58.7%. Mp: 148–150 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 9.47 (*s*, 1H, NH), 8.73 (d, 1H, *J* = 7.8 Hz), 8.52 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.50 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.10 (d, 1H, *J* = 4.2 Hz), 7.55 (d, 1H, *J* = 8.4 Hz), 7.47 (t, 1H, *J* = 7.8 Hz), 7.42–7.38 (m, 2H), 7.25 (d, 1H, *J* = 9.6 Hz), 3.95 (s, 2H, CH_2-S). IR

(KBr, cm^{-1}): 3251 (υ_{NH}), 3075, 2996, 1691, 1583, 1526, 1455 ($\upsilon_{N=N}$), 1391, 1359, 1300, 1128, 1078, 1071, 801, 746 (υ_{C-S}). MS (ESI): *m*/*z* 391.3 (M+1), 393.2 (M+3). $C_{17}H_{12}Cl_2N_4OS$ (Exact Mass: 390.01).

5.2.16. Methyl 3-(2-(3-(2-chlorophenyl)pyrazin-2-ylthio)acetamido)thiophene-2-carboxylate (6n)

White powder, yield: 77.3%. Mp: 148–150 °C. ^{1}H NMR (DMSO-d_6, ppm) δ: 10.99 (*s*, 1H, NH), 8.52 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.40 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.13 (d, 1H, *J* = 5.4 Hz, thiophene-H), 7.55–7.52 (m, 2H), 7.47–7.40 (m, 3H), 4.02 (s, 2H, CH_2-S), 3.86 (s, 3H, Me). IR (KBr, cm^{-1}): 3211 (υ_{NH}), 3092, 2947, 2919, 2850, 1689 ($\upsilon_{O-C=O}$), 1662 ($\upsilon_{NH-C=O}$), 1569, 1442, 1420, 1376 ($\upsilon_{N=N}$), 1279, 1259, 1122, 1094, 1074, 783, 7445 (υ_{C-S}). MS (ESI): *m*/*z* 420.2 (M+1), 422.3 (M+3). $C_{18}H_{14}ClN_3O_3S_2$ (Exact Mass: 419.02).

5.2.17. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-*N*-(5-methylbenzo[d]thiazol-2-yl)acetamide (6o)

White powder, yield: 74.3%. Mp: 102–104 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 10.67 (*s*, 1H, NH), 8.58 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.52 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.70 (d, 1H, *J* = 7.8 Hz), 7.60 (s, 1H, Ph'H), 7.55 (d, 1H, *J* = 7.8 Hz), 7.48–7.38 (m, 3H), 7.12 (d, 1H, *J* = 8.4 Hz), 3.49 (s, 2H, CH_2-S), 2.43 (s, 3H, CH_3). IR (KBr, cm^{-1}): 3425 (υ_{NH}), 3138, 3053, 2920, 1695 ($\upsilon_{C=O}$), 1607, 1547, 1464, 1432, 1358 ($\upsilon_{N=N}$), 1273, 1154, 1119, 1071, 1015, 813, 757 (υ_{C-S}). MS (ESI): *m*/*z* 427.2 (M+1), 429.3 (M+3). $C_{20}H_{15}ClN_4OS_2$ (Exact Mass: 426.04).

5.2.18. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-1-(3,4-dihydroquinolin-1(2*H*)-yl)ethanone (6p)

White powder, yield: 67.3%. Mp: 102–104 °C. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.31 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 8.26 (d, 1H, *J* = 3.0 Hz, pyrazine-H), 7.50–7.16 (m, 8H), 4.16 (s, 2H, CH_2-S), 3.83 (s, 2H, CH_2), 2.75 (s, 2H, CH_2), 1.62 (s, 2H, CH_2). IR (KBr, cm^{-1}): 3041, 2947, 2888, 1653 ($\upsilon_{C=O}$), 1491, 1375 ($\upsilon_{N=N}$), 1358, 1198, 1169, 1071, 1015, 850, 759 (υ_{C-S}), 757. MS (ESI): *m*/*z* 396.2 (M+1), 398.2 (M+3). $C_{21}H_{18}ClN_3OS$ (Exact Mass: 395.09).

5.2.19. 2-(3-(2-Chlorophenyl)pyrazin-2-ylthio)-1-(3,4-dihydroisoquinolin-2(1*H*)-yl)ethanone (6q)

White powder. ^{1}H NMR ($CDCl_3$, ppm) δ: 8.37–8.32 (m, 2H), 8.26 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 8.22 (d, 1H, *J* = 2.4 Hz, pyrazine-H), 7.51–7.43 (m, 2H), 7.41–7.35 (m, 4H), 4.79 (s, 1H, CH), 4.73 (s, 1H, CH), 4.31 (s, 2H, CH_2-S), 3.83 (t, 1H, *J* = 6.0 Hz, CH), 3.74 (t, 1H, *J* = 6.0 Hz, CH), 2.94 (t, 1H, *J* = 6.0 Hz, CH), 2.85 (t, 1H, *J* = 6.0 Hz, CH). IR (KBr, cm^{-1}): 3046, 2925, 1644 ($\upsilon_{C=O}$), 1432, 1358 ($\upsilon_{N=N}$), 1119, 1071, 1014, 758 (υ_{C-S}), 730. MS (ESI): *m*/*z* 396.2 (M+1), 398.3 (M+3). $C_{21}H_{18}ClN_3OS$ (Exact Mass: 395.09).

5.3. Biological activity

5.3.1. In vitro *a*nti-HIV assay

The methodology of the anti-HIV assay has been previously described.[31,32] Stock solutions (10 × final concentration) of test compounds were added in 25-μL volumes to two series of triplicate wells in 96-well microtiter plates so as to allow simultaneous evaluation of their effects on mock- and HIV-infected cells at the beginning of each experiment. Serial fivefold dilutions of test compounds were made directly in flat-bottomed 96-well microtiter trays using a Biomek 3000 robot (Beckman instruments, Fullerton, CA). Untreated control HIV- and mock-infected cell samples were included for each sample.

HIV-1(III_B)[28] or HIV-2 (ROD)[29] stock (50 μL) at 100–300 CCID50 (cell culture infectious dose) or culture medium was added to either the infected or mock-infected wells of the microtiter tray. Mock-infected cells were used to evaluate the effect of the test compounds on uninfected cells in order to assess the cytotoxicity of the test compounds. Exponentially growing MT-4 cells[30] were centrifuged for 5 min at 1000 rpm and the supernatant was discarded. The MT-4 cells were resuspended at 6×10^5 cells/mL, and 50-μL volumes were transferred to the microtiter tray wells. Five days after infection, the viability of mock- and HIV-infected cells was examined spectrophotometrically by the MTT assay.

The MTT assay is based on the reduction of yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Acros 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, Labsystems, Helsinki, Finland), at two wavelengths (540 and 690 nm). All data were calculated using the median OD (optical density) value of three wells. The 50% cytotoxic concentration (CC_{50}) was defined as the concentration of the test compound that reduced the absorbance (OD540) of the mock-infected control sample by 50%. The 50% effective concentration (EC_{50}) was defined as the compound concentration required to inhibit virus-induced syncytium formation by 50%.

5.3.2. HIV-1 RT inhibition assay

Inhibition of HIV-1 RT was developed using nucleotides linked to microtiter plate wells with colorimetric detection of incorporated biotin-dUTP into homopolymer template primers.[33] The incorporated quantities of the biotin-dUTP into the enzyme represented the activity of HIV-1 RT. IC_{50} values corresponded to the concentration of the pyrazin-2-ylthioacetamide derivatives required to inhibit biotin-dUTP incorporation into the HIV-1 RT by 50%.

Acknowledgments

The financial support from the National Natural Science Foundation of China (NSFC No. 81102320, No. 30873133, No. 30772629, No. 30371686), Key Project of NSFC for International Cooperation (No. 30910103908), Research Fund for the Doctoral Program of Higher Education of China (No. 20110131130005, 20110131120037), Independent Innovation Foundation of Shandong University (IIFSDU, No. 2010GN044), Shandong Postdoctoral Innovation Science Research Special Program (No. 201002023), China Postdoctoral Science Foundation funded project (No. 20100481282, 2012T50584) and KU Leuven (GOA 10/014) is gratefully acknowledged.

We thank K. Erven, K. Uyttersprot and C. Heens for technical assistance with the anti-HIV assays.

The authors have declared no conflict of interest.

References and notes

1. Zhan, P.; Liu, X.; Li, Z. *Curr. Med. Chem.* **2009**, *16*, 2876.
2. Zhan, P.; Liu, X.; Li, Z.; Pannecouque, C.; De Clercq, E. *Curr. Med. Chem.* **2009**, *16*, 3903.
3. Zhan, P.; Chen, X.; Li, D.; Fang, Z.; De Clercq, E.; Liu, X. *Med. Res. Rev.* **2011**. http://dx.doi.org/10.1002/med.20241.
4. Zhan, P.; Liu, X. *Expert. Opin. Ther. Pattern* **2011**, *5*, 717.
5. Zhan, P.; Li, Z.; Liu, X.; De Clercq, E. *Mini-Rev. Med. Chem.* **2009**, *9*, 1014.
6. Wang, Z.; Wu, B.; Kuhen, K. L.; Bursulaya, B.; Nguyen, T. N.; Nguyen, D. G.; He, Y. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4174.
7. De La Rosa, M.; Kim, H. W.; Gunic, E.; Jenket, C.; Boyle, U.; Koh, Y. H.; Korboukh, I.; Allan, M.; Zhang, W.; Chen, H.; Xu, W.; Nilar, S.; Yao, N.; Hamatake, R.; Lang, S. A.; Hong, Z.; Zhang, Z.; Girardet, J. L. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 4444.
8. Muraglia, E.; Kinzel, O. D.; Laufer, R.; Miller, M. D.; Moyer, G.; Munshi, V.; Orvieto, F.; Palumbi, M. C.; Pescatore, G.; Rowley, M.; Williams, P. D.; Summa, V. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 2748.
9. O'Meara, J. A.; Jakalian, A.; LaPlante, S.; Bonneau, P. R.; Coulombe, R.; Faucher, A. M.; Guse, I.; Landry, S.; Racine, J.; Simoneau, B.; Thavonekham, B.; Yoakim, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3362.

10. Gagnon, A.; Amad, M. H.; Bonneau, P. R.; Coulombe, R.; DeRoy, P. L.; Doyon, L.; Duan, J.; Garneau, M.; Guse, I.; Jakalian, A.; Jolicoeur, E.; Landry, S.; Malenfant, E.; Simoneau, B.; Yoakim, C. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4437.
11. Gagnon, A.; Landry, S.; Coulombe, R.; Jakalian, A.; Guse, I.; Thavonekham, B.; Bonneau, P. R.; Yoakim, C.; Simoneau, B. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1199.
12. Moyle, G.; Boffito, M.; Stoehr, A.; Rieger, A.; Shen, Z.; Manhard, K.; Sheedy, B.; Hingorani, V.; Raney, A.; Nguyen, M.; Nguyen, T.; Ong, V.; Yeh, L. T.; Quart, B. *Antimicrob. Agents Chemother.* **2010**, *54*, 3170.
13. Li, D.; Zhan, P.; De Clercq, E.; Liu, X. *J. Med. Chem.* **2012**, *55*, 3595.
14. Zhao, H.; Guo, Z. *Drug Discovery Today* **2009**, *14*, 516.
15. Zhan, P.; Liu, X.; Cao, Y.; Wang, Y.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5368.
16. Zhan, P.; Liu, X.; Li, Z.; Fang, Z.; Li, Z.; Wang, D.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem.* **2009**, *17*, 5920.
17. Zhan, P.; Liu, X.; Fang, Z.; Li, Z.; Pannecouque, C.; De Clercq, E. *Eur. J. Med. Chem.* **2009**, *44*, 4648.
18. Zhan, P.; Liu, X.; Li, Z.; Fang, Z.; Pannecouque, C.; De Clercq, E. *Chem. Biodivers.* **2010**, *7*, 1717.
19. Zhan, P.; Liu, X.; Zhu, J.; Fang, Z.; Li, Z.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem.* **2009**, *17*, 5775.
20. Zhan, P.; Liu, X.; Fang, Z.; Pannecouque, C.; De Clercq, E. *Bioorg. Med. Chem.* **2009**, *17*, 6374.
21. Zhan, P.; Liu, H.; Liu, X.; Wang, Y.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. *Med. Chem. Res.* **2009**, *19*, 652.
22. Zhan, P.; Chen, X.; Li, X.; Li, D.; Tian, Y.; Chen, W.; Pannecouque, C.; De Clercq, E.; Liu, X. *Eur. J. Med. Chem.* **2011**, *46*, 5039.
23. Zhan, P.; Wang, L.; Liu, H.; Chen, X.; Li, X.; Jiang, X.; Zhang, Q.; Liu, X.; Pannecouque, C.; Naesens, L.; De Clercq, E.; Liu, A.; Du, G. *Arch. Pharm. Res.* **2012**, *35*, 1037.
24. (a) Patani, G. A.; LaVoie, E. J. *Chem. Rev.* **1996**, *96*, 3147; (b) Olesen, P. H. *Curr. Opin. Drug Discovery Dev.* **2001**, *4*, 471; (c) Lima, L. M.; Barreiro, E. J. *Curr. Med. Chem.* **2005**, *12*, 23; (d) Sheng, C.; Che, X.; Wang, W.; Wang, S.; Cao, Y.; Miao, Z.; Yao, J.; Zhang, W. *Eur. J. Med. Chem.* **2011**, *46*, 5276.
25. Maarten, W.; van der, M.; Michel, L.; Edith, G.; Wim, L. N.; Hugo, M.; van Enckevort, W. J. P.; Bernard, K.; Elias, V.; Richard, M. K. *Org. Process Res. Dev* **2009**, *13*, 1195.
26. (a) Jones, R. G. J. *Am. Chem. Soc.* **1949**, *71*, 78; (b) Semple, G.; Andersson, B. M.; Chhajlani, V.; Georgsson, J.; Johansson, M. J.; Rosenquist, A.; Swanson, L. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1141.
27. Baraldi, P. G.; Preti, D.; Tabrizi, M. A.; Fruttarolo, F.; Saponaro, G.; Baraldi, S.; Romagnoli, R.; Moorman, A. R.; Gessi, S.; Varani, K.; Andrea Borea, P. *Bioorg. Med. Chem.* **2007**, *15*, 2514.
28. Popovic, M.; Sarngadharan, M. G.; Read, E.; Gallo, R. C. *Science* **1984**, *224*, 497.
29. Barré-Sinoussi, F.; Chermann, J. C.; Rey, F.; Nugeyre, M. T.; Chamaret, S.; Gruest, J.; Dauguet, C.; Axler-Blin, C.; Vézinet-Brun, F.; Rouzioux, C.; Rozenbaum, W.; Montagnier, L. *Science* **1983**, *220*, 868.
30. Miyoshi, I.; Taguchi, H.; Kobonishi, I.; Yoshimoto, S.; Ohtsuki, Y.; Shiraishi, Y.; Agaki, T. *Gann. Monogr.* **1982**, *28*, 219.
31. Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. *J. Virol. Methods* **1988**, *20*, 309.
32. Pannecouque, C.; Daelemans, D.; De Clercq, E. *Nat. Protoc.* **2008**, *3*, 427.
33. (a) Suzuki, K.; Craddock, B. P.; Okamoto, N.; Kano, T.; Steigbigel, R. T. *J. Virol. Methods* **1993**, *44*, 189; (b) Wu, J.; Liu, X.; Cheng, X.; Cao, Y.; Wang, D.; Li, Z.; Xu, W.; Pannecouque, C.; Witvrouw, M.; De Clercq, E. *Molecules* **2007**, *12*, 2003.