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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis and evaluation of unsymmetrical heterocyclic thioureas as potent β-glucuronidase inhibitors

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Abstract Thiourea analogs **1–20** were synthesized and evaluated for their in vitro β-glucuronidase inhibitory potential. The compounds **9** (0.86 ± 0.01 μM), **6** (1.24 ± 0.01 μM), **16** (1.64 ± 0.02 μM) and **15** (2.12 ± 0.02 μM) showed potent activity. Other analogs **1**–**5**, **7**, **8**, **10**, **11**, **13**, **17**, **20** showed better activity than standard drug D-saccharic acid 1,4-lactone (47.34 ± 0.21 μM) ranging 4.36–34.4 μM. All synthetic compounds were characterized by different spectroscopic methods. This study has identified a new class of potent inhibitors β-glucuronidase.

Keywords Thioureas · β-Glucuronidase inhibition · Heterocyclic

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Introduction

Urea derivatives have broad spectrum of biological activities as colchicine-binding antagonist, (Fortin *et al*., 2007, 2009) and CXCR3 antagonist, (Watson *et al*., 2008) anti-HIV, antiviral, antibacterial, analgesic and HDL-elevating properties (Sivan *et al*., 2013; Venkatachalam *et al*., 2004; Holešová *et al*., 2014; Flieger *et al*., 2012; Bloom *et al*., 2004; Seth *et al*., 2004; Lee *et al*., 2003). The urea analogs are found to be very selective, and they are well suited to interact with the viral proteases and showed no interaction with the human proteases. Since, after first report of these inhibitors, various derivatives have rapidly been synthesized, class of cyclic compounds may soon become a feasible choice to the currently available antiretroviral agents. The substituted cyclic urea shown below has shown an increase in antiretroviral potency (Bäckbro *et al*., 1997; Sham *et al*., 1996a; Hultén *et al*., 1997; Ala *et al*., 1998).

Cyclic urea derivatives are also cytotoxic on a large number of cancer cell lines and remain active on most chemo-resistant cells (Fortin *et al*., 2007; Liu *et al*., 2014). Chalcon reacts with isocyanate, thioisocyanate and amines to produce important industrial products belonging to urea and thiourea which posses a series of biological activities including herbicidal activities, inhibition of nitric oxide, antimicrobial, anti-HIV, antiviral, analgesic and HDL-elevating properties (Yang *et al*., 2012). Derivatives of 1-phenyl-3-{4-[(2*E*)-3-phenylprop-2-enoyl]-phenyl}-urea (**2**) exhibited anti-inflammatory and antimalarial activities (Santos *et al*., 2008).

Classes of heterocyclic ureas, the azacyclic ureas, are the potent inhibitors of HIV-1 protease; inhibition of HIV protease is an important approach for the intervention of HIV infection but possesses very low bioavailability (Chrusciel and Strohbach, 2004; Sham *et al*., 1996b).

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N-Acyl azide ureas **3** are excellent inhibitors of viral protease and have shown good antiviral activities against resistant strains (Zhao *et al*., 2005).

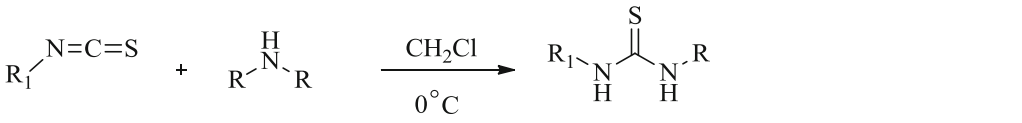
β-Glucuronidase is an enzyme which catalyzes the glucuronosyl-*O*-bonds cleavage (Sperker *et al*., 1997). This is also up-regulated in different pathological conditions, such as infection of urinary tract (Bank and Bailine, 1965; Roberts *et al*., 1967; Ronald *et al*., 1971; Kallet and Lapco, 1967), renal disease (Gonick *et al*., 1973), rejection of transplantation (Schapiro *et al*., 1968), epilepsy (Plum 1967), larynx and breast cancer (Boyland *et al*., 1957). Besides, this enzyme is also involved in inflammatory joint diseases, like rheumatoid arthritis (Caygill and Pitkeathly, 1966), and some hepatic diseases, and AIDS is also reported due to over-expression of this enzyme. Literature report showed that the bacterial *β*-glucuronidase inhibitor leads to a decrease in carcinogen-induced colonic tumors (Reddy, 1976). The current study is focused on the discovery of *β*-glucuronidase inhibitors of pharmacological importance. Some synthetic *β*-glucuronidase inhibitors reported in recent past (Jamil *et al*., 2014; Khan *et al*., 2014a, b, c; Cheng *et al*., 2015; Ahmad *et al*., 2012).

Our research group is continuously working on the chemistry and biology of new compounds (Aziz *et al*., 2014; Musharraf *et al*., 2012; Anouar *et al*., 2013), sulfur-containing compounds (Khan et al., 2008, 2009, 2010, 2011) and hydrazones (Khan *et al*., 2012; Taha *et al*., 2013, 2014).

Results and discussion

Chemistry

We previously reported that unsymmetrical urea derivatives are excellent antiglycating agent; therefore, we further explored this class for discovering other biological uses. The synthetic procedure which was adopted was quite simple and required very short time to complete. Urea derivatives **1–20** were synthesized from different amines treating them with isocyanate (2 mmol). The crude products were recrystallized with ethanol afforded the pure products (Scheme 1).



In continuation of our work on enzyme inhibition (Rahim *et al*., 2015; Taha *et al*., 2015a; Taha *et al*., 2015b; Khan *et al*., 2014d), the compounds **1–20** were evaluated for in vitro *β*-glucuronidase inhibitory potential (Table 1).

The compounds **9**, **6**, **15** and **16** showed 0.86 ± 0.01, 1.24 ± 0.01, 2.12 ± 0.02 and 1.64 ± 0.02 μM activity far better than standard drug D-saccharic acid 1,4-lactone 47.34 ± 0.21 μM, respectively. 3-Chlorophenyl-2-methylpiperazinethiourea (**9**) showed potent activity that may be due to 3-chlorophenyl ring which can be proved by comparing the activity of compound **5** (28.12 ± 0.35 μM) having phenyl ring with no substitution on 2-methylpiperazinethiourea. The second most active compound also has 3-chlorophenyl ring and 4-phenylpiperazinethiourea; when 3-chlorophenyl is replaced with phenyl as in compound **4**, the activity decreased almost two times. The compounds **15** and **16** have quinoline ring and phenyl and 3-chlorophenyl, respectively; the activity of 3-chlorophenyl is slightly better than phenyl substituted. Compounds **3** and **17** have morpholine and phenyl and 3-chlorophenyl thiourea, respectively; it was observed that both compounds have almost same inhibition potential.

The compounds **2**, **7**, **10**, **11** and **13** having piperidine ring with different substitutions showed good activities. The activity pattern is common, and 3-chlorophenylthiourea showed better activity than phenylthiourea. Phenyl4-methylpiprazinethiourea (**1**) showed less activity as compared to 3-chlorophenyl-4-ethylpiprazinethiourea (**8**) which may be due to 3-chlrophenyl. The compound **20** showed fewer activities than compounds **1** and **8** because 4-acyl group replaces ethyl group. Compounds **12**, **14**, **18** and **19** showed less than 50 % inhibition and so considered as inactive. Hence, we have discovered new potent derivatives of thiourea for *β*-glucuronidase inhibition. Further study on this compound could produce new molecules for the *β*-glucuronidase inhibition.

Experimental

Materials and methods

NMR experiments were performed on Avance Bruker AM 300 and 500 MHz machines. CHN analyses were carried out on a Carlo Erba Strumentazione-Mod-1106, Italy. Electron impact mass spectra (EI MS) were recorded on a Finnigan MAT-311A (Germany) mass spectrometer. Thin-layer chromatography (TLC) was monitored on pre-coated silica gel aluminum plates (Kieselgel 60, 254, E. Merck, Germany). Chromatograms were visualized by iodine vapors or UV at 254 and 365 nm.

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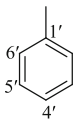
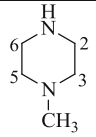
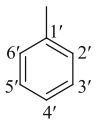
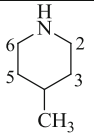
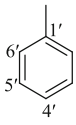
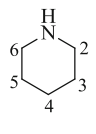
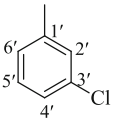
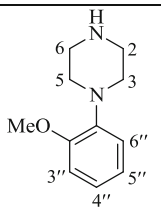
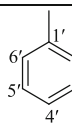
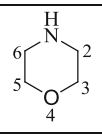
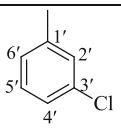
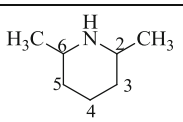
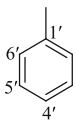
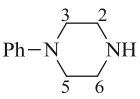
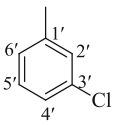
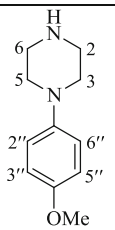
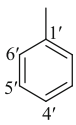
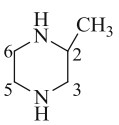
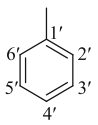
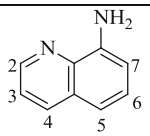
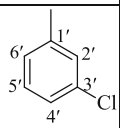
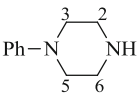
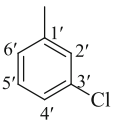
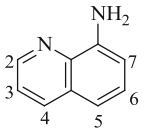
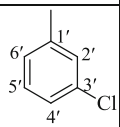
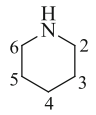
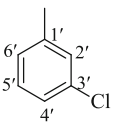
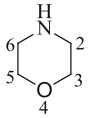
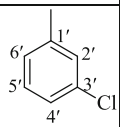
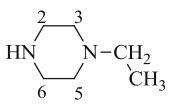
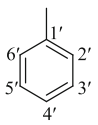
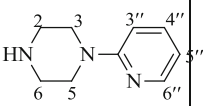
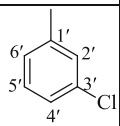
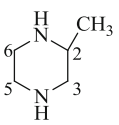
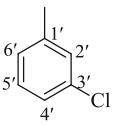
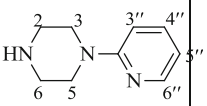
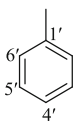
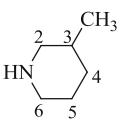
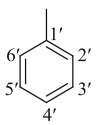
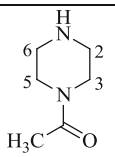
1			85	**11**			80
2			74	**12**			82
3			80	**13**			77
4			70	**14**			85
5			82	**15**			78
6			75	**16**			88
7			83	**17**			90
8			78	**18**			84
9			80	**19**			83
10			76	**20**			84

Scheme 1 Synthesis of heterocyclic thioureas **1–20**

Table 1 In vitro β-glucuronidase activity of compounds **1–20**

Compounds	IC_{50} (μM ± SEM[a])	Compounds	IC_{50} (μM ± SEM[a])
1	20.50 ± 0.26	**11**	31.40 ± 0.54
2	25.40 ± 0.30	**12**	NA[b]
3	8.34 ± 0.15	**13**	11.21 ± 0.26
4	4.36 ± 0.08	**14**	NA[b]
5	28.12 ± 0.35	**15**	2.12 ± 0.02
6	1.24 ± 0.01	**16**	1.64 ± 0.02
7	18.46 ± 0.24	**17**	8.42 ± 0.36
8	15.4 ± 0.46	**18**	NA[b]
9	0.86 ± 0.01	**19**	NA[b]
10	34.4 ± 0.42	**20**	47.34 ± 0.21
D-Saccharic acid 1,4-lactone[c]	48.4 ± 1.25 μM	–	–

[a] SEM is the standard error of the mean

[b] NA Not active

[c] D-saccharic acid 1,4-lactone, standard inhibitor for β-glucuronidase

General procedure for the synthesis of compounds 1–20

Thiourea derivatives **1–20** were synthesized from different heterocyclic amines by treating with isocyanate. Heterocyclic amines (2 mmol) dissolved in dichloromethane (10 mL), and temperature was maintained at 0 °C; then isocyanate (2 mmol) was added drop-wise with constant stirring. The formation of white solid after 1–1.5 h confirmed the completion of reaction. The resultant solid products **1–20** were filtered and washed with hexane. Recrystallization with ethanol afforded the yield between 60 and 85 %.

Biological assays

β-Glucuronidase (E.C. 3.2.1.31 from bovine liver, G-0251) and *p*-nitrophenyl-β-D-glucuronide (N-1627) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Assay for β-D-glucuronidase

β-D-Glucuronidase inhibition was determined by measuring the absorbance of the *p*-nitrophenol which is produced from the substrate at 405 nm. The total reaction volume was 250 μL. The reaction mixture contains 5 μL of test compound solution, 185 μL of 0.1 M acetate buffer and 10 μL of enzyme, and it was incubated at 37 °C for 30 min. The plates were read on a multiplate reader at 405 nm after the addition of 50 μL of 0.4 mM *p*-nitrophenyl-β-D-glucuronide. All assays were performed in triplicate.

4-Methyl-N-phenylpiperazine-1-carbothioamide **(1)** Yield: 85 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.90 (s, 1H, NH), 7.54 (dd, 2H, $J = 6.4$, $J = 1.7$ Hz, H-2′/H-6′), 7.23 (t, 2H, $J = 7.8$ Hz, H-3′, H-5′), 6.98 (t, 1H, $J = 8.4$ Hz, H-4′), 3.52 (t, 4H, $J = 10.1$ Hz, 2-CH_2/6-CH_2), 2.33 (t, 4H, $J = 10.0$ Hz, 3-CH_2/5-CH_2), 2.50 (s, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 138.4 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.3 (CH, C-4′), 126.6 (CH, C-2′), 126.6 (CH, C-6′), 56.7 (CH_2, C-2), 56.7 (CH_2, C-6), 51.6 (CH_2, C-3), 51.6 (CH_2, C-5), 46.5 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 235.

N-Phenylpiperidine-1-carbothioamide **(2)** Yield: 74 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.42, (s, 1H, NH), 7.46 (d, 2H, $J = 8.0$ Hz, H-2′/H-6′), 7.23 (t, 2H, J 8.0 Hz, H-3′/H-5′), 6.93 (t,1H, $J = 7.0$ Hz, H-4′), 3.44 (t, 2H, $J = 10.0$ Hz, 2-CH_2), 2.52 (t, 2H, $J = 11$ Hz, 6-CH_2), 1.58 (m, 6H, 3-CH_2/4-CH_2/5-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 187.3 (C=S, C), 138.4 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.5 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 54.5 (CH_2, C-2), 54.5 (CH_2, C-6), 25.7 (CH_2, C-3), 25.7 (CH_2, C-5), 24.9 (CH_2, C-4); EI MS: *m*/*z* (rel. abund%) 220.

N-Phenylmorpholine-4-carbothioamide **(3)** Yield: 80 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.38 (dd, 2H, $J = 8.0$, $J = 2.0$, Hz, H-2′/H-6′) 7.29 (t, 2H, $J = 8.0$ Hz, H-3′/H-5′), 7.07 (t, 1H, $J = 8.0$ Hz, H-4′), 3.75 (t, 4H, $J = 10.4$ Hz, 3-CH_2, 5-CH_2), 3.48 (t, 4H, $J = 10.3$ Hz, 2-CH_2/6-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.9 (C=S, C), 138.4 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.5 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 66.3 (CH_2, C-3), 66.3 (CH_2, C-5), 50.1 (CH_2, C-2), 50.1 (CH_2, C-6); EI MS: *m*/*z* (rel. abund%) 222.

N-4-Diphenylpiperazine-1-carbothioamide **(4)** Yield: 70 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.38, (dd, 2H, $J = 8.0$, $J = 2.0$ Hz, H-2′/H-6′), 7.31 (m, 4H, H-3′/H-5′/H-2″/H-6″), 7.06 (m, 3H, H-3″/H-5″/H-4′), 6.91 (t, 1H, $J = 8.0$ Hz, H-4″), 3.72 (t, 4H, $J = 10.0$ Hz, 2-CH_2/6-CH_2), 3.24 (t, 4H, $J = 10.1$ Hz, 3-CH_2,5-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 149.5 (C, C-1′), 138.4 (C, C-1′), 129.7 (CH, C-3′), 129.7 (CH, C-5′), 128.9 (CH, C-3′), 128.9 (CH, C-5′), 128.5 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 121.8 (CH, C-4′), 114.4 (CH, C-2′), 114.4 (CH, C-6′), 56.8 (CH_2, C-2), 56.8 (CH_2, C-6), 53.6 (CH_2, C-3), 53.6 (CH_2, C-5); EI MS: *m*/*z* (rel. abund%) 297.

3-Methyl-N-phenyl-4-(phenylcarbamothioyl)piperazine-1-carboxamide **(5)** Yield: 82 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.56 (s, 1H, NH), 8.52 (s, 1H, NH), 7.50 (m, 4H, H-2′/H-6′/H-2″/H-6″), 7.29 (m, 4H, H-3′/H-5′/H-3″/H-5″), 7.01-6.94 (m, 2H, H-4′/H-4″), 4.45(t, 1H, $J = 6.5$ Hz, 3a-CH), 4.16 (d, 1H, $J = 11$ Hz, 3b-CH), 3.97 (t, 4H, $J = 11$ Hz, 6-CH_2), 3.18 (t, 1H, $J = 11$ Hz, 5-CH2), 2.99 (m, 1H, 2-CH), 1.11 (d,3H, $J = 7.2$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.3 (C=S, C), 138.6 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.5 (CH, C-4′), 126.6 (CH, C-2′), 126.6 (CH, C-6′), 66.6 (CH, C-2), 57.5 (CH_2, C-3), 56.9 (CH_2, C-6), 47.8 (CH_2, C-5), 18.5 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 354.

N-(3-Chlorophenyl)-4-phenylpiperazine-1-carbothioamide **(6)** Yield: 75 %; ^{1}H NMR: (300 MHz, DMSO-d_6): δ 8.24 (s, 1H, NH), 7.86 (d, 1H, $J = 2.0$ Hz, H-2′), 7.49 (dt, 1H, $J = 8.0$, $J = 2.0$, Hz, H-6′), 7.32 (d, 1H, $J = 2.0$ Hz, H-4′), 7.29-7.22 (m, 2H, H-2″/H-6″), 7.04-6.98 (m, 3H, H-4″/H-6″/H-6′), 6.90 (t,1H, $J = 7.0$ Hz, H-4″), 3.74 (t, 4H, $J = 10.0$ Hz, H-3/H-5), 3.24 (t, 4H, $J = 10.0$ Hz H-2/H-6); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 149.4 (C, C-1′), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 129.5 (CH, C-3′), 129.5 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 121.8 (CH, C-4′), 114.4 (CH, C-2′), 114.4 (CH, C-6′), 56.8 (CH_2, C-2), 56.8 (CH_2, C-6), 53.4 (CH_2, C-3), 53.5 (CH_2, C-4); EI MS: *m*/*z* (rel. abund%) 331.

N-(3-Chlorophenyl)piperidine-1-carbothioamide **(7)** Yield: 83 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.50 (t,1H, $J = 4.0$ Hz, H-2′), 7.28 (dt, 1H, $J = 8.5$, $= 2.0$ Hz, H-4′), 7.28 (t, 1H, $J = 8.0$ Hz, H-5′), 6.99 (dt, 1H, J_6', $= 8.0$ - $J = 2.0$ Hz, H-6′), 3.49 (t, 4H, $J = 10.5$ Hz, H-2,6), 1.68-163 (m, 2H, H-4), 1.60-1.58 (m, 4H, H-3,5); ^{13}C NMR (75 MHz, DMSO-d_6): δ 187.1 (C=S, C), 138.6 (C, C-1′), 134.5 (C, C-3′), 130.6 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.7 (CH, C-6′), 54.3 (CH_2, C-2), 54.3 (CH_2, C-6), 25.5 (CH_2, C-3), 25.5 (CH_2, C-5), 24.7 (CH_2, C-4); EI MS: *m*/*z* (rel. abund%) 254.

N-(3-Chlorophenyl)-4-ethylpiperazine-1-carbothioamide **(8)** Yield: 78 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.52, (t,1H, $J = 2.0$ Hz, H-2′), 7.28 (ddd, 1H, $J = 8.0$, $J = 2.0$, $J = 2.0$ Hz, H-4′), 7.27 (t, 1H, $J = 8.5$ Hz, H-5′), 7.04 (ddd, 1H, $J = 8.0$, $J = 2.0$, $J = 2.0$ Hz, H-4′), 3.56 (t, 4H, $J = 10.0$ Hz, H-2/H-6), 2.56-2.52 (m, 6H, 2xH-3/H-5/, CH_2), 1.20 (d, 3H, $J = 8.0$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 138.6 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 57.2 (CH_2, C-2), 57.2 (CH_2, C-6), 56.6 (CH_2, C-3), 56.6 (CH_2, C-5), 49.7 (CH_2, C), 13.4 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 283.

N^{1}, *N*^{1}*4-Bis(3-chlorophenyl)-2-methyl-1,4-piperazinedicarboxamide* **(9)** Yield: 80 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.71 (s, 1H, NH), 8.62 (s, 1H, NH) 7.63 (s, 2H, H-2′/H-2″), 7.40 (d, 2H, $J = 8.0$ Hz, H-5′/H-5″), 7.24 (dt, 2H, $J = 8.0$, $J = 2.0$ Hz, H-4′/H-4″), 7.02 (dd, 2H, $J = 2.0$, Hz, $J = 2.0$, H-6′/H-6″), 4.39 (s, 1H, H-3a), 4.06 (d,1H, $J = 12.0$ Hz, H-3b), 3.97 (t, 2H, $J = 13.0$ Hz, H-5), 3.17 (t, 2H, $J = 13.0$ Hz, H-6), 2.98-2.91 (m, 1H, H-2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.3 (C=S, C), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.7 (CH, C-6′), 66.6 (CH, C-2), 57.7 (CH_2, C-3), 56.7 (CH_2, C-6), 47.8 (CH_2, C-5), 18.2 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 422.

3-Methyl-N-phenylpiperidine-1-carbothioamide **(10)** Yield: 76 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.36 (s, 1H, NH), 7.46 (dd, 2H, $J = 8.0$, $J = 1.5$, Hz, H-2′/H-6′), 7.32 (t, 2H, $J = 8.0$, $J = 8.0$ Hz, H-3′/H-5′), 6.99 (t, 1H, $J = 8.0$ Hz, H-4′), 4.08 (t, 2H, $J = 10.5$ Hz, 6-CH_2), 2.72 (dd 1H, $J = 6.0$, $J = 8.0$ Hz, H-2b) 2.52 (dd,1H, $J = 7.0$, $J = 6.5$, Hz, H-2a), 1.78-1.67 (m, 2H, H-4b/H-4a),1.59-1.38 (m, 2H, 5-CH_2), 1.16-1.05 (m,1H, H-3), 0.91 (d, 3H, $J = 6.6$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 187.3 (C=S, C), 138.4 (C, C-1′), 128.9 (CH, C-3′), 128.9 (CH, C-5′), 128.3 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 60.3 (CH_2, C-2), 54.6 (CH_2, C-6), 32.7 (CH_2, C-4), 28.8 (CH, C-3), 23.2 (CH_2, C-5), 18.6 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 234.

4-Methyl-N-phenylpiperidine-1-carbothioamide **(11)** Yield: 80 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.42 (s, 1H, NH), 7.46 (dd, 2H, $J = 8.0$, $J = 2.0$, Hz, H-2′/H-6′) 7.31 (t, 2H, $J = 8.0$ Hz, H-3′/H-5′), 7.06 (t, 1H, $J = 7.2$ Hz, H-4′), 4.07 (d, 2H, $J = 13.0$ Hz, 2-CH_2), 2.79 (t, 2H, $J = 13.0$ Hz, 6-CH_2) 1.66-1.58 (m, 3H, 4-CH/3-CH_2), 1.15-1.08 (m, 2H, 5-CH_2) 1.02 (d, $J = 13.0$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 187.1 (C=S, C), 138.4 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.3 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 51.8 (CH_2, C-2), 51.8 (CH_2, C-6), 34.2 (CH_2, C-3), 34.2 (CH_2, C-5), 32.2 (CH, C-4), 20.3 (CH_3, C); EI MS: *m*/*z* (rel. abund%) 234.

N-(3-Chlorophenyl)-4-(2-methoxyphenyl)piperazine-1-carbothioamide **(12)** Yield: 82 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 7.54, (t, 1H, $J = 2$ Hz, H-2′) 7.31 (ddd, 1H, $= J = 1.6$, $J = 8.0$ Hz, H-4′), 7.28 (t, 1H, $J = 8.0$ Hz, H-5′), 7.07-6.97 (m, 4H, H-3″/H-4″/H5″/H-6″), 6.96 (dt, 1H, $J = 8.4$, $J = 2.3$ Hz, H-6′), 3.89 (s, 3H, OCH_3), 3.71 (t, 4H, $J = 10.0$ Hz, 2-CH_2/6-CH_2), 3.08 (t, 4H, $J = 10.0$ Hz, 3-CH_2/5-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 162.3 (C, C-1″), 141.2 (C, C-2″), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 123.1 (CH, C-6″), 122.0 (CH, C-4″), 121.8 (CH, C-5″), 113.4 (CH, C-3″), 56.6 (CH_2, C-2), 56.6 (CH_2, C-6), 55.7 (OCH_3, C), 53.7 (CH_2, C-3), 53.7 (CH_2, C-5); EI MS: *m/z* (rel. abund%) 361.

N-(3-Chlorophenyl)-2,6-dimethylpiperidine-1-carbothioamide **(13)** Yield: 77 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.42 (s, 1H, NH), 7.72, (t,1H, $J = 2.0$ Hz, H-2′), 7.47 (dd, 1H, $J = 8.0$ Hz, H-4′), 7.28 (t, 1H, $J = 8.0$ Hz, H-5′), 7.07 (dt, 1H, $J = 8.0$, $J = 2$ Hz,H-6′),7.28 (t, $J = 8.0$ Hz, H-5′) 6.99 (dt, 1H, $J = 7.5$, $= 2.0$ Hz, H-4′), 4.36-4.30 (m, 2H, 4-CH_2), 1.58-1.54 (m, 1H, 2-CH), 1.51-1.46 (m, 4H, 3-CH_2/5-CH_2), 1.42-1.37 (m, 1H, 6CH), 1.14 (d, 3H, $J = 7$ Hz, CH_3), 1.04 (d, 3H, $J = 7$ Hz, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 187.1 (C=S, C), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 64.2 (CH, C-2), 64.2 (CH, C-6), 30.0 (CH_2, C-3), 30.0 (CH_2, C-5), 20.2 (CH_3, C), 20.2 (CH_3, C), 14.0 (CH_2, C-4); EI MS: *m/z* (rel. abund%) 284.

N-(3-Chlorophenyl)-4-(4-methoxyphenyl)piperazine-1-carbothioamide **(14)** Yield: 85 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.79, (s, 1H, H-NH), 7.68 (t,1H, $J = 2.0$ Hz, H-2′), 7.43 (dd, 1H, $J = 8.0$, $J = 2.0$ Hz, H-4′), 7.25 (t, 1H, $J = 8.0$ Hz, H-5′), 7.02 (d,1H, $J = 2.0$ Hz, H-2′), 7.01 (d, 2H, $J = 9$ Hz, H-2″/H-6″) 6.87 (d, 2H, $J = 6.0$ Hz, H-3″/H-5″), 3.69 (s, 3H, CH_2), 3.60 (t, 4H, $J = 9.5$ Hz, 3-CH_2/5-CH_2), 3.06 (t, 4H, $J = 2$-CH_2/6-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 152.9 (C, C-4″), 146.2 (C, C-1″), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 115.2 (CH, C-2″), 115.2 (CH, C-6″), 115.1 (CH, C-3″), 115.1 (CH, C-5″), 56.6 (CH_2, C-2), 56.6 (CH_2, C-6), 55.7 (OCH_3, C), 53.4 (CH_2, C-3), 53.4 (CH_2, C-5); EI MS: *m/z* (rel. abund%) 361.

1-Phenyl-3-(quinolin-8-yl)thiourea **(15)** Yield: 78 %; ^{1}H NMR: (500 MHz, Methanol-d_4): δ 8.82 (dd, 1H, $J = 2.0$, $J = 5.0$ Hz, H-2), 8.52 (dd, 1H, $J = 7.0$, $J = 2.0$ Hz, H-4), 8.29 (dd, 1H, $J = 8.0$, $J = 2.0$ Hz, H-7), 7.54-7.48 (m, 5H, H-3, H-5/H-6, H-2′/H-6′), 7.33 (*t*, 2H, $J = 7.6$ Hz, H-3/H-5), 7.06 (t 1H, $J = 7.6$ Hz, H-4′); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.8 (C=S, C), 148.5 (CH, C-2), 138.4 (C, C-1′), 137.1 (C, C-10), 136.6 (CH, C-4), 133.7 (C, C-9), 129.1 (C, C-5), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.3 (CH, C-4′), 127.4 (CH, C-7), 126.6 (CH, C-2′), 126.6 (CH, C-6′), 121.3 (CH, C-3), 116.6 (CH, C-8),113.7 (CH, C-6); EI MS: *m/z* (rel. abund%) 279.

1-(3-Chlorophenyl)-3-(quinolin-8-yl)thiourea **(16)** Yield: 88 %; ^{1}H NMR: (500 MHz, Methanol-d_4): δ 8.80 (dd, 1H, $J = 2.0$, $J = 4.5$ Hz, H-2), 8.52 (dd, 1H, $J = 6.0$, $J = 2.5$ Hz, H-3), 8.29 (dd, 1H, $J = 8.4$, $J = 1.7$ Hz, H-7), 7.74 (t, 1H, $J = 2.0$, Hz, H-2′), 7.57-7.49 (m, 3H, H-4/H-5/H-6), 7.38 (ddd, 1H, $J = 8.4$, $J = 2.0$, $J = 1.6$ Hz H-4′), 7.25 (t, 1H, $J = 8.0$ Hz, H-5′), 7.04 (ddd, 1H, $J = 7.0$, $J = 2.0$ $J = 1.6$ Hz, H-6); ^{13}C NMR (75 MHz, DMSO-d_6): δ 179.8 (C=S, C), 148.5 (CH, C-2), 138.4 (C, C-1′), 137.1 (C, C-10), 136.6 (CH, C-4), 134.7 (C, C-3′), 133.9 (C, C-9), 130.5 (CH, C-5′), 129.1 (C, C-5), 128.2 (CH, C-4′), 127.2 (CH, C-7), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 121.1 (CH, C-3), 116.4 (CH, C-8),113.7 (CH, C-6); EI MS: *m/z* (rel. abund%) 313.

N-(3-chlorophenyl)morpholine-4-carbothioamide **(17)** Yield: 90 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.71 (s, 1H, NH) 7.66 (t, 1H, $J = 2.0$ Hz, H-2), 7.41 (dt, 1H, $J = 8.0$, $J = 2.0$ Hz, H-4), 7.29 (t, 1H, $J = 8.0$ Hz, H-5), 7.02 (dd, 1H, $J = 7.8$, $J = 2.0$ Hz, H-6), 3.62 (t, 4H, $J = 5.0$ Hz, 2′-CH_2/6′-CH_2), 3.52 (t, 4H, $J = 5.0$ Hz, 3′-CH_2, 5′-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 138.4 (C, C-1′), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 66.3 (CH_2, C-3), 66.3 (CH_2, C-5), 50.1 (CH_2, C-2), 50.1 (CH_2, C-6); EI MS: *m/z* (rel. abund%) 256.

N-Phenyl-4-(pyridin-2-yl)piperazine-1-carbothioamide **(18)** Yield: 84 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.15 (dd, 1H, $J = 1.5$, $J = 5.0$ Hz, H-6″), 7.62 (ddd, 1H, $J = 5.0$, $J = 5.0$, $J = 1.5$ Hz, H-5″), 7.39 (d, 2H, $J = 8.5$ Hz, H-2′/H-6′), 7.25 (t, 2H, $J = 8.5$ Hz, H-3′/H-5′), 7.06 (t, 1H, $J = 7.6$ Hz, H-4′), 6.88 (d, 1H, $J = 8.4$ Hz, H-3″), 6.72 (dd, 1H, $J = 8.0$, $J = 1.5$ Hz, H-4″), 3.62 (t, 4H, $J = 9.2$ Hz, 2-CH_2/6-CH_2), 3.59 (t, 4H, $J = 9.2$ Hz, 3-CH_2/5-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.9 (C=S, C), 158.2 (C, C-2″), 148.0 (CH, C-6″), 138.6 (C, C-1′), 138.4 (CH, C-4″), 128.9 (CH, C-3′), 128.9 (CH, C-5′), 128.3 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 117.8 (CH, C-5″), 106.3 (CH, C-3″), 56.9 (CH_2, C-2), 56.9 (CH_2, C-6), 46.2 (CH_2, C-3), 46.2 (CH_2, C-5); EI MS: *m/z* (rel. abund%) 298.

N-(3-Chlorophenyl)-4-(pyridin-2-yl)piperazine-1-carbothioamide **(19)** Yield: 83 %; ^{1}H NMR: (500 MHz, DMSO-d_6): δ 8.12 (dd, 1H, $J = 5.0$, $J = 1.5$ Hz, H-6″), 7.62 (ddd, 1H, $J = 5.0$, $J = 5.0$, $J = 1.5$ Hz, H-5″), 7.55 (t, 1H, $J = 4.5$ Hz, H-2′), 7.29 (dd, 1H, $J = 7.0$, $J = 2.0$ Hz,

H-4′), 7.26 (t, 1H, $J = 8.0$ Hz, H-5′), 7.02 (ddd 1H, $J = 8.0$, $J = 1.8$, $J = 2.0$ Hz, H-6′), 6.86 (d, 1H, $J = 8.8$ Hz, H-3″), 6.74(dd, 1H, $J = 7.0$, $J = 5.0$ Hz, H-4″), 3.69 (t, 4H, $J = 10.0$ Hz, 2-CH_2/6-CH_2), 3.56 (t, 4H, $J = 10.0$ Hz, 3-CH_2/5-CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.7 (C=S, C), 158.2 (C, C-2″), 148.0 (CH, C-6″), 138.4 (C, C-1′), 138.2 (CH, C-4″), 134.5 (C, C-3′), 130.3 (CH, C-5′), 128.2 (CH, C-4′), 126.8 (CH, C-2′), 124.5 (CH, C-6′), 117.8 (CH, C-5″), 106.1 (CH, C-3″), 56.6 (CH_2, C-2), 56.6 (CH_2, C-6), 46.2 (CH_2, C-3), 46.2 (CH_2, C-5); EI MS: m/z (rel. abund%) 332.

4-Acetyl-N-phenylpiperazine-1-carbothioamide **(20)** Yield: 84 %; ^{1}H NMR: (500 MHz, Methanol-d_4): δ 7.37 (dd, 2H, $J = 7.6$, $J = 1.8$, Hz, H-2′/H-6′), 7.32 (t, 2H, $J = 8.5$ Hz, H-3′/H-5′), 7.07 (t, 1H, $J = 8.5$ Hz, H-4′), 3.67 (t, 4H, $J = 10.0$ Hz, 2-CH_2/6-CH_2), 3.56 (t, 4H, $J = 10.0$ Hz, 3-CH_2/5-CH_2), 2.12 (s, 3H, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6): δ 181.9 (C=S, C), 168.7 (C=O, C), 138.4 (C, C-1′), 129.1 (CH, C-3′), 129.1 (CH, C-5′), 128.5 (CH, C-4′), 126.4 (CH, C-2′), 126.4 (CH, C-6′), 56.7 (CH_2, C-2), 56.7 (CH_2, C-6), 49.4 (CH_2, C-3), 49.4 (CH_2, C-5), 21.2 (CH_3, C); EI MS: *m/z* (rel. abund%), 263.

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