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Synthesis and Anti Hiv-1 Reverse Transcriptase Evaluation of A Series of N-Mono Substituted Thiourea Derivatives

Research Article

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Abstract

Thirteen kinds of *N*-monosubstituted thioureas have been synthesized from various primary amines through three different methods. The chemical structures of all the compounds have been characterized by the various spectral analyses. Four of them were evaluated for the anti-HIV-1 activity. The results showed that compound **1b**, showing the IC_{50} = 29.7 (μg/mL) to the strain of ROD of HIV-1, CC_{50} > 50 (μg/mL), SI (selectivity index) > 2, was the best one among the test compounds. As for other compound **1a, 1c** and **1d**, the SI of them was less than 1, which means that these compounds might be toxic at the therapeutic level. Both the steric, electronic and topologic descriptors of the molecules were calculated to assist understanding the basic relationship between the structure and the biological activity. The docking result of **1c** with HIV-1 reverse transcriptase (HIV-1 RT, PDB ID: 2HNZ) showed that there were still more unexploited rooms in the active site of the binding pocket of HIV-1 RT with compounds **1c**

Keywords: Mono-Substituted Thioureas; Anti-HIV Activity; Molecular Descriptors; SAR; CADD; DOCK.

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Introduction

As the important classes of compounds [76], thioureas could be used as the versatile reagents [56] and building blocks for constructing the S,N-containing heterocyclic compounds as well as the substrates for the further structural modification. Beside being oxidized into ureas [74] or alkylated into isothioureas [77], they were widely used to construct thiazole [5, 28, 61, 75, 87, 91, 92] 2-thiouracil [50], aminothiazole [25 28, 51, 62, 69, 75, 93, 95] aminobenzothiazoles [34, 90] iminothiazolines [2, 36, 54, 60] thiohydantoins [38, 39] 1, 3, 5-triazines [14], 2-aminooxazolidines [26], thiazolididiones [51, 53, 55], fused and spiro *N/S*-containing heterocycles [4]. The most interesting aspects of this type of compounds are the potent heterogeneous biological activities [58], such as anti-HIV activity [6], antituberculosis activity [35], cytokinin activity [8], promoting effect on wheat growth [94], reverses cross-links and restores biological activity in DNA, antimicrobial activity [13], anti-oxidant activity [1], anti-cancer activity [44, 73], tyrosinase inhibition [11, 84] and melanogenesis inhibition [83, 45]. The thiourea derivatives were also reported to be HIV non-nucleoside reverse transcriptase inhibitors (NNRTIs) for both the wild type [88], drug-resistant [49] and multidrug-resistant virus [86]. Among these active compounds, the anti-HIV agents have recently received much more attention than ever before, including the dual functional agents [12], PETTs [6, 64, 67, 80] , ITUs [15, 48].

N-monosubstituted thioureas are biologically attractive for containing the primary NH_2 group, serving as a hydrogen bond donor [30] to interact with the amino acid residues in the binding pocket of target enzymes [84] especially the reverse transcriptase (RT) during the HIV-1 life cycle. Therefore, as part of our research program for the possible anti-HIV-1 RT agents [19, 46, 50, 52, 54, 96] we would like to report the synthesis and the anti-HIV RT evaluation of a series of *N*-mono substituted thioureas. The target compounds were obtained from various substituted amines [4] mainly by the method shown in Scheme 1. This procedure usually required three steps from benzoyl chloride [2] and ammonium thiocyanate, via an intermediate of aroyl isothiocyanate [3] to afford primary amine [4] followed by the basic hydrolysis to give the target compounds (**1a-1j**) [66].

Although the above method was tedious in overall procedures, it might give the relatively high yield. Some other synthesis methods were also developed to obtain *N*-mono substituted thioureas efficiently. They might also involve using the toxic or special reagents, such as CS_2 [59], carbonothioic dichloride (thiophosgene) [42], hydrazine hydrate, [40] LiAlHSH [41] and TMS-Cl [9] *via* the harsh conditions, such as the high temperature, the long time and the tedious work-ups [40].

In some special circumstances, when above procedures described in the method A could not give the desired result; other alternative methods were used to achieve the target molecules such as Method B and Method C, which were illustrated in the following (Scheme 2-3), respectively.

It should be pointed out that the synthesis procedures have also been improved based on these traditionally reported methods. The chemical structures of all of these compounds have been characterized by the spectrum analysis as well as their physical data (Table 1).

The anti-HIV activities of N-monosubstituted thioureas were measured using the MTT method via comparing with four FDA-approved drugs (Nevirapine, Zidovudine, Dideoxycitidine and Dideoxyinosine). The cells were infected with HIV-1 wild-type virus (IIIB) strain cell line and HIV-2 strain (ROD). The results were reported as the half maximal (50%) inhibitory concentration (IC_{50}). Moreover, the cytotoxicity (CC_{50}) values of the compounds for each strain line were also determined. The selective index ($SI=CC_{50}/IC_{50}$) indicating the specificity of the antiviral effect, was given for both virus strains (Meng et al. 2003).

Due to the poor solubility of the molecules, only four compounds in the target molecules were tested for their anti-HIV activities. The result showed that compound **1b**, showing the IC_{50}=29.7μg/mL to the strain of ROD of HIV-1, CC_{50}>50μg/mL, SI (selectivity index) > 2, was the best one among all the test compounds. The SI of other compound **1a, 1c** and **1d** were all less than 1, meaning these compounds might be toxic at the therapeutic level (Table 2).

The steric, electronic and topologic descriptors of the compounds have been calculated using Chem3D Ultra (Cambridge software package) to find some relationships between the biological activities and the chemical structure features (Table 3).

Materials and Methods

Experimental Section

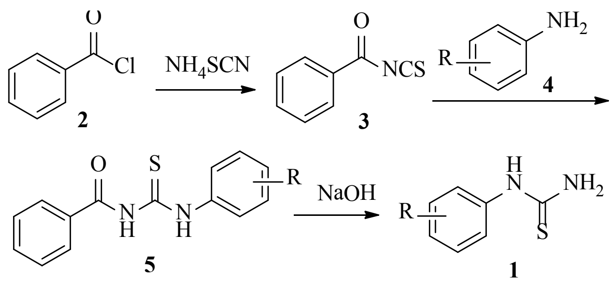
General Methods and Materials: All materials were obtained from the commercial suppliers and used as received. Melting points were taken on an X-4 digital melting point apparatus and were uncorrected. The elemental analyses were performed on a Carlo-Elba 1106 elemental analyzer. IR spectra were recorded on a Nicolet FI-IR 360 spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were determined on a Bruker AM-400 (400 MHz) spectrometer with TMS as an internal standard. Chemical shifts were reported in δ. Mass spectra were measured on a HP5988A instrument by direct inlet at 70ev. All materials were obtained from the commercial suppliers and used as received.

1. Synthesis

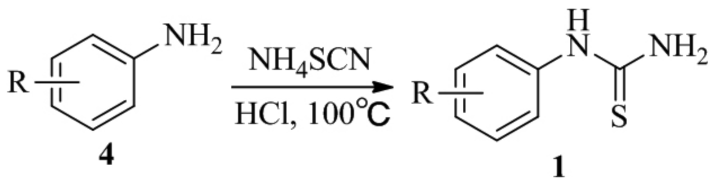
General procedures for synthesizing N-mono-substituted (1): The N-mono-substituted thioureas were synthesized according to the following three methods (A, B, C) based on the different substituents on the aromatic ring. The general synthetic procedures were described as follows in details. The related descriptors of the desired thioureas obtained via calculating with Cambridge software package were also listed thereafter.

Method A: Benzoyl chloride (7.20 g, 50.0 mmol) was added drop wise to a solution of NH_4SCN (4.20 g, 51.0 mmol) in dry acetone (25.0 mL). The mixture was stirred under refluxing for 15 mins. Heating was removed and appropriate substituted anilines (50.0

Scheme 1. Method A for synthesizing mono-N-substituted thioureas.



Scheme 2. Method B for synthesizing mono-N-substituted thioureas.



Scheme 3. Method C for synthesizing mono-N-substituted thioureas.

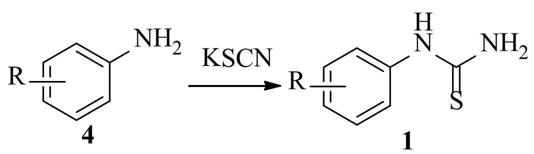


Table 1. The Information for preparing substituted thioureas (1) from amine (4).

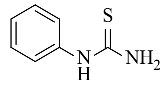
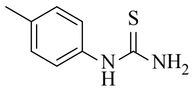
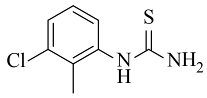
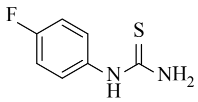
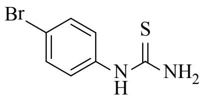
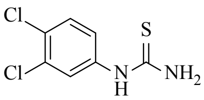
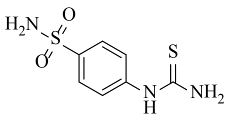
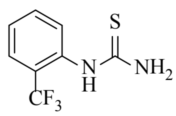
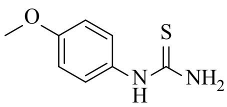
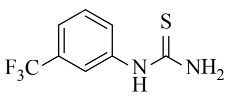
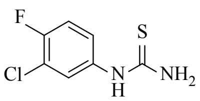
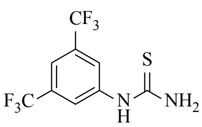
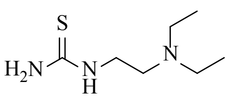
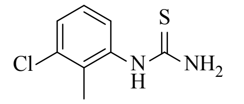
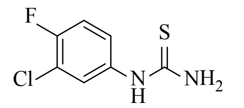
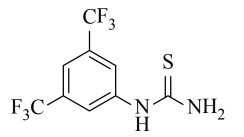
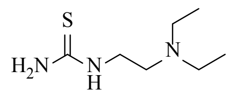
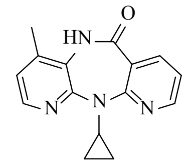
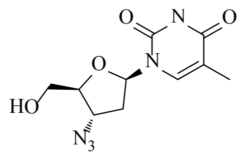
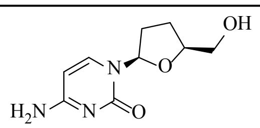
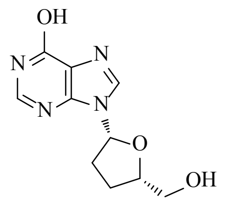
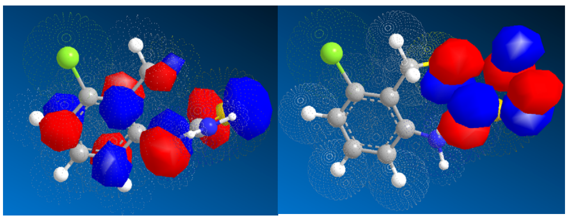
Entry	Chemical structure	No.	Mp (°C)	Form (Recrystallization solvent)	Yield (%)	Method
1		**1a**	178-179	White crystal from anhydrous ethanol	70.0	Method A
2		**1b**	201-202	White crystal from anhydrous ethanol	60.0	Method A
3		**1c**	190-192	White crystal from anhydrous ethanol	81.0	Method A
4		**1d**	173-175	White crystal from anhydrous ethanol	57.9	Method A
5		**1e**	198-200	White crystal from anhydrous ethanol	55.0	Method A
6		**1f**	220-222	White crystal from anhydrous ethanol	67.0	Method A
7		**1g**	212-214	White needle like crystal from anhydrous ethanol	40.0	Method A
8		**1h**	163-164	Yellow to white crystals from anhydrous ethanol	99.3	Method C
9		**1i**	236-238	White needle like crystals from anhydrous ethanol	85.7	Method A
10		**1j**	104-105	White crystals from anhydrous ethanol	70.5 49.2	Method A Method B
11		**1k**	193-195	White crystals from anhydrous ethanol	58.0	Method A
12		**1l**	150-156	White crystals from anhydrous ethanol	31.9	Method A
13		**1m**	122-124	White crystals from anhydrous ethanol	62.4	Method A

Table 2. Anti- HIV-1 RT activity evaluations of N-mono substituted thioureas.

No.	Chemical structure	Strain III B					Strain ROD		
		IC_{50}	SD	CC_{50} (SD)	SI	IC_{50}	SD	CC_{50} (SD)	SI
1c		>50.00	1.70	>50.00	X1	29.70		>50.00	>2
1k		>31.70	≥31.70		< or X1	>31.7		≥31.7	<or X1
1l		>19.38		>19.38 (0.58)	< 1	19.38		>19.38 (0.58)	< 1
1m		>50.00		>50.00	X1	>50.00		>50.00	X1
C1		0.047	0.016	>4	>86	>4		>4	<1
C2		0.0015	0.0002	>25	>16420	>25	0.0003		>15408
C3		0.29	0.05	>20	>69	>20	0.10	>20	>67
C4		2.89	0.43	>50	>17	4.59	0.81	>50	>11

C1-4: were the reference compounds (Nevirapine, Zidovudine, Dideoxycitidine and Dideoxyinosine) as the controlling group.

Figure 1. The HOMO (Left) and LUMO (Right) of the compound 1c.



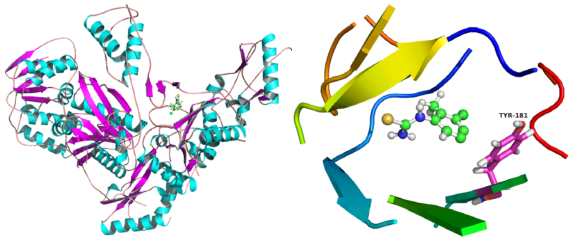
mmol) were added drop wise over a period of 15 mins. The reaction mixture was kept under refluxing for further 30 mins, and then cooled to room temperature before pouring into icy water (375 mL). The resulting precipitates were collected by filtration, washed with water or a cold mixture of water and methanol (1: 1) [28]. The yellow solids (various substituted benzoyl thioureas), were added to a solution of sodium hydroxide (NaOH, 7.50 g, 65.0 mL water) and stirred at 80°C for 30 mins [61]. The mixture was adjusted to pH=7 with hydrochloric acid (HCl, 10.0 %). The appeared precipitates were filtered and washed with water, recrystallized with ethanol and then dried to give the pure products (**1a-1g**, **1i-1j**) [66].

Method B: To a flask was added substituted aromatic amine (150 mmoL) and aqueous hydrochloride (1.0 N, 16.0 mL) or HCl (conc. 36%, 15.2 g, 15.0 mmol), after slightly heated, the mixture was added ammonium thiocyanate (NH_4SCN, 12.6 g, 165 mmol), and then the temperature of the mixture was raised to 90°C for 2 hrs, and then stop heating to stay for 16~18 hrs, until there was a first portion of the yellow solid appeared from the solution. The solid was filtered and then the filtration was concentrated to give a second part of the yellow solids. The two parts were combined and heated to 100°C for 8 hrs. After being triturated and washed with

Table 3. The calculated descriptors of N-monosubstituted thioureas.

No.	MW	LogP	PC	MR	Ovality	HBD	HBA	CAA	CMA	CSEV	BI	NRB	PSA	R	SC	TVC	HOMO (eV)	LUMO (eV)	TE (kcal/ mol)
1a	152.22	0.730	0.75	47.66	1.32	2	0	344.40	159.39	124.65	12491	2.00	38.05	3.00	1.00	0.006	-9.433	-0.709	5.024
1b	166.24	1.463	1.24	52.70	1.36	2	0	376.32	178.60	142.01	19887	2.00	38.05	4.00	0.00	0.005	-8.120	0.411	4.911
1c	200.69	2.082	1.7	57.51	1.35	2	0	385.19	186.87	153.47	28107	2.00	38.05	3.00	1.00	0.005	-7.281	0.353	-12.995
1d	170.21	1.184	1.19	47.88	1.14	2	1	230.29	92.28	68.25	19887	2.00	38.05	4.00	0.00	0.002	-8.113	0.388	5.067
1e	231.11	1.936	1.91	55.28	1.14	2	0	242.82	100.19	77.11	19887	2.00	38.05	4.00	0.00	0.005	-8.106	0.368	5.756
1f	221.11	2.286	2.46	57.27	1.14	2	0	243.41	100.87	78.2	29231	2.00	38.05	4.00	0.00	0.005	-8.104	1.222	8.553
1g	231.3	0.22	-0.71	61	1.17	3	2	255.39	106.72	81.81	63010	3.00	98.21	4.00	1.00	0.001	-7.588	0.367	9.866
1h	220.21	2.396	1.28	53.63	1.15	2	3	237.94	96.91	72.81	56878	3.00	38.05	3.00	1.00	0.000	-7.428	0.509	8.491
1i	182.24	1.179	0.85	54.12	1.18	2	1	256.58	106.43	80.37	30890	3.00	47.28	4.00	1.00	0.002	-7.125	0.435	2.046
1j	220.21	2.396	2.16	53.63	1.15	2	3	239.9	98.1	73.89	59991	3.00	38.05	4.00	0.00	0.000	-8.091	0.589	10.821
1k	204.65	1.803	2.01	52.68	1.14	2	1	236.97	96.67	73.38	29231	2.00	38.05	4.00	0.00	0.002	-8.106	1.398	8.918
1l	288.21	3.317	3.23	59.61	1.15	2	6	246.99	102.7	78.87	186087	4.00	38.05	4.00	0.00	0.000	-7.519	0.681	7.836
1m	175.29	0.486	53.62	0.71	1.15	2	1	226.89	88.83	64.22	40933	6.00	41.29	4.00	0.00	0.020	-8.984	6.923	17.618

Figure 2. Docking of 1c with HIV-1 RT (PDB ID: 2HNZ).



water (5.0 mL×3), the solid was obtained from filtration. Further recrystallization with a mixed solvent petroleum and ether (3:2), ethanol or THF (especial when 3,4-dichloroaniline was chosen as the material) [78] afforded the white crystal like products (**1j**) [25].

Method C: A solution of aromatic amine (0.017 mol) in ethanol (15.0 mL) was stirred at room temperature while concentrated hydrochloric acid (37.4%, 2.14 mL) was added dropwise. The formed suspension was heated to reflux until being dissolved, to which was added with a solution of potassium thiocynate (2.60 g, 25.5 mmol) in ethanol (5.00 mL). The reaction mixture was stirred at reflux for 18 h. The precipitate formed upon cooling was dried under vacuum and recrystallized from ethanol to yield the desired compounds (**1h**) [27].

***N*-Phenylthiourea (1a).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 70.0%. mp. 178-179°C, lit. 148-150°C [22], ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.49 (br, 2H, -NH_2), 7.10 (d, 2H, Ar*H*-2, 6), 7.31 (dd, 2H, Ar*H*-3, 5), 7.45 (dd, 1H, Ar*H*-4), 9.66 (br, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 123.01 (Ar-*C*-4), 124.38 (Ar-*C*-2, 6), 128.67 (Ar-*C*-3, 5), 139.06 (Ar-*C*-1), 180.99 (*C*=S).

***N*-(p-Toly) lthiourea (1b).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 60.0%. mp. 201-202°C, lit. 181-183°C [27], ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.25 (s, 3H, CH_3), 2.49 (br, ^{1}H, -N*H*), 7.11 (d, 2H, Ar*H*-2,6), 7.23 (d, 2H, Ar*H*-3, 5), 9.55 (br, 2H, NH_2); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 20.47 (4-CH_3), 123.33 (Ar-*C*-3,5), 129.16 (Ar-*C*-2,6), 133.70 (Ar-*C*-4), 136.40 (Ar-*C*-1), 180.94 (*C*=S).

***N*-(3-Chloro-2-methylphenyl) thiourea (1c).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 81.0%. mp. 190-192°C, lit. 153-154°C [66], ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.20 (s, 3H, CH_3), 3.32 (s, 2H, -NH_2), 7.19 (m, 1H, Ar*H*-5), 7.34 (d, 1H, Ar*H*-6), 7.54 (d, 1H, Ar*H*-6), 9.37 (br, 1H, N*H*); ^{13}C NMR (DMSO-d_6, 100 MHz): δ 15.05 (2-CH_3), 126.97 (Ar-*C*-6), 127.09 (Ar-*C*-4), 127.34 (Ar-*C*-5), 133.18 (Ar-*C*-3), 133.87 (Ar-*C*-1), 138.79 (Ar-*C*-2), 181.86 (*C*=S).

***N*-(4-fluorophenyl) thiourea (1d).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 57.9%. mp. 173-175°C, lit. 164°C [16]. ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.44 (s, 2H, -NH_2), 7.19 (d, 2H, Ar*H*-2, 6), 7.49 (d, 2H, Ar*H*-3, 5), 9.45 (br, 1H, N*H*).

***N*-(4-Bromophenyl) thiourea (1e).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 55.0%. mp. 198-200°C, lit. 171°C [23]. 1H NMR (DMSO-d_6, 400 MHz): δ 3.34 (s, 2H, -NH_2), 7.39 (d, 2H, Ar*H*-2, 6), 7.47 (d, 2H, Ar*H*-3, 5), 9.75 (br, 1H, N*H*).

***N*-(3,4-Dichlorophenyl) thiourea (1f).** Synthesis method: Method A. Recrystalization solvent: Anhydrous ethanol, Form: White needle like crystals, Yield 67.0%. mp. 220-222°C, lit. 216-217°C [78]. lit. 205-206°C [31], ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.33 (s, 2H, -NH_2), 7.19 (m, 1H, Ar*H*-2), 7.34 (d, 1H, Ar*H*-6), 7.54 (d, 1H, Ar*H*-5), 9.86 (br, 1H, N*H*); MS (m/z): 220 (M+H$^+$).

***N*-(*p* -Aminosulphonylphenyl) thiourea (1g, also call as 4-thioureido-benzenesulfonamide).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: Yellow to white needle like crystals, Yield 40.0%. mp. 212-214°C, lit. 206°C [79]. ^{1}H NMR (DMSO-d_6, 400 MHz): 3.33 (s, 2H, -NH_2), 7.64 (d, 2H, Ar*H*-2, 6), 7.72 (d, 2H, Ar*H*-3, 5), 9.96 (br, 1H, N*H*).

***N*-(2-Trifluoromethylphenyl) thiourea (1h).** Synthesis method: Method C, Recrystalization solvent: Anhydrous ethanol, Form: Yellow to white crystals, Yield 99.3 %. mp. 163-164°C, lit. 170°C, [71, 82, 87]. ^{1}H NMR (DMSO-d_6, 400 MHz): 6.56 (s, 2H, -NH_2), 7.01 (d, 1H, Ar*H*-6), 7.10 (dd, 1H, Ar*H*-4), 7.26 (dd, 1H, Ar*H*-5), 7.53 (d, 1H, Ar*H*-3), 8.91 (br, 1H, N*H*).

***N*-(*p*-Methoxyphenyl) thiourea (1i).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White needle like crystals, Yield 85.7%. mp. 236-238°C, lit. 206-209°C [25], 212-214°C [65], 198-200°C [20], 210°C [85], ^{1}H NMR (DMSO-d_6, 400 MHz): 3.72 (s, 3H, OCH_3), 6.87 (d, 2H, Ar*H*-2, 6), 7.13 (d, 2H, H-3, H-5), 7.29 (br, 2 H, NH_2), 9.02 (br, 1 H, N*H*).

***N*-(3-Trifluoromethylphenyl)thiourea (1j).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 70.5%. mp. 104-105°C, Synthesis method: Method B, Yield 49.2%, mp. 104-105°C, lit. 104-106°C (Kurzer and Canelle 1963), 103°C [21]. ^{1}H NMR (DMSO-d_6, 400 MHz): 6.09 (s, 2H, -NH_2), 6.45 (d, 1H, Ar*H*-6), 7.01 (s, 1H, Ar*H*-2), 7.09 (d, 1H, Ar*H*-4), 7.16 (dd, 1H, Ar*H*-5), 8.68 (br, 1H, N*H*).

***N*-(3-Chloro-4-fluorophenyl)thiourea (1k).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 58.0%. mp. 193-195°C, lit. Triclinic crystals [70]. ^{1}H NMR (DMSO-d_6, 400 MHz): 5.96 (s, 2H, -NH_2), 6.30 (d, 1H, Ar*H*-6), 6.65 (s, 1H, Ar*H*-2), 7.26 (d, 1H, Ar*H*-5), 8.59 (br, 1H, N*H*).

***N*-(3,5-Ditrifluoromethylphenyl) thiourea (1l).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White crystals, Yield 31.9%. mp. 150-156°C. The compound **1l** has been prepared by a streamlined method [7]. ^{1}H NMR (DMSO-d_6, 400 MHz): 6.06 (s, 2H, -NH_2), 6.96 (s, 2H, Ar*H*-2, 6), 7.37 (s, 1H, Ar*H*-4), 8.91 (br, 1H, N*H*).

***N*-(N',N'-Diethylaminoethylene) thiourea (1m).** Synthesis method: Method A, Recrystalization solvent: Anhydrous ethanol, Form: White needle crystals, Yield 62.4%. mp.122-124°C [24]. ^{1}H NMR (DMSO-d_6, 400 MHz): 1.10 (t, 6H, CH_3 × 2), 2.79 (m, 4H, CH_2×2), 2.56 (t, 4H, CH_2 × 2), 2.95 (t, 4H, CH_2 × 2), 8.69 (br, 1H, N*H*). Compounds **1m** has been prepared from thiourea and diethylaminoethyl chloride *via* dissolving sodium in alcohol [24].

2. Biological Activity: The general procedure for anti-HIV activity assay was described as the following. The anti-HIV activity and cytotoxicity of the compounds were evaluated against the wild-type HIV-1 strain IIIB and HIV-2 strain ROD in MT-4 cell cultures using the 3-(4, 5-dimethylthiazol- 2-yl)-2, 5-diphenyltetrazo lium bromide (MTT) method. Briefly, virus stocks were titrated in MT-4 cells and expressed as the 50% cell culture infective dose ($CCID_{50}$). MT-4 cells were suspended in a culture medium at 1×10^5 cells/mL and infected with HIV at a multiplicity of infection of 0.02. Immediately after the viral infection, 100 μL of the cell suspension was placed in each well of a flat-bottomed micro titer tray containing various concentrations of the test compounds. The tested compounds were dissolved in DMSO at 50 mM. After 4 days of incubation at 37°C, the number of viable cells was determined using the MTT method. Compounds were tested in parallel for the cytotoxic effects in the uninfected MT-4 cells.

3. Molecular modeling

3.1 Calculation of the molecular descriptors: The various molecule descriptors, including both the steric and the electric descriptors were calculated by the options available modules in Cambridge software package. The explanations and category of the calculated descriptors were listed as the following. General descriptors included molecular weight (MW). Hydrophobic descriptors include the Log value of the partition coefficient (LogP), partition coefficient (PC). Steric descriptors included the following: molecular refractory (MR), molecular shape index (Ovality), the number of hydrogen bond donor (HBD), the number of hydrogen bond acceptor (HBA). The descriptors calculated from CHEMPROPSTD included Connolly accessible area (CAA), Connolly molecular area (CMA) and Connolly solvent excluded volume (CSEV). Molecular topology index included a Balaban index (BI), number of rotatable bonds (NRB), polar surface area (PSA), radius (R), shape coefficient (SC) and total valence connectivity (TVC). Energy related index included the energy of the highest occupied molecular orbit (HOMO), the energy of the lowest unoccupied molecular orbit (LUMO) and the total energy after energy minimization by MM semi-empirical method (TE). To obtain these descriptors, molecular dynamics calculation and energy minimization were sequentially run on each of the molecule with default values (Step interval = 2.0 fs, Frame interval = 10 fs, Terminate after 10000 steps) at first, the molecular descriptors of **1a-1m** were then computed via Chem3D Ultra (Cambridge software), respectively.

3.2 Docking analysis: The dock studies were performed using the molecular modeling package SYBYLX.2.0 (Company 2011). Tripos force field and Gasteiger-Hückel partial atomic charges were used for minimizing the molecules [18]. The minimum energy difference of 0.001 kcal/mol was set as a convergence criterion. While considering Surflex-Dock was an well-known software to understand the interaction between the small molecules with the target protein [17, 32, 33, 63, 81], using an idealized active site called a protomol, which was built from the hydrogen-containing protein mol2 file [72, 89]. The construction was based on the amino acid residues that constitute the active site tuned to produce a small and buried docking target [37]. Dock analysis of compound **1c** with HIV-1 RT 2HNZ were carried out according to the normal procedures in Surflex-Dock workflow on a SYBYL-2.0 workstation using all the default values [47].

Result and Discussions

Discussion About the Synthesis Method

Method A was a conventional synthesis method to obtain *N*-monosubstituted thioureas from primary amines, involved using benzoyl chloride as the assisting material. While heating primary amine directly with ammonium thiocyanate in acidic water instead of using any irritating agent, the whole process of the Method B involves less reaction time and easier work-up than the traditional methods (Scheme 2) [25]. As for the Method C [27], while considering KSCN was often used as the starting product for the synthesis of CS_2, and therefore it can be used as a non-toxic agent to replace CS_2 for preparing *N*-mono-substituted thioureas, especially in a one-pot and supported reagent methods (Scheme 3) [3]. As a summary, both method B and method C were convenient for involving less reaction steps, but some time their yield were not as good as that of method A.

As for the biological activity evaluation result for N-monosubstituted thioureas were listed in Tables 2 together with the reference compounds. Some compounds were not evaluated for anti-HIV-1 activity due to their poor solubility. Four of the 13 compounds were screened with two infected virus strains. The result showed that only one compound **1c**, containing a methyl group at the othor-position of the phenyl group and a chloro atom at the meta-position of the phenyl group, showed the relative inhibitory activity against HIV-2 strain ROD (IC_{50}=29.70μg/mL, SI > 2), other compounds exhibit almost no activity against both wild-type HIV-1 strain IIIB and HIV-2 strain ROD.

The most active molecule (**1c**) of the series was subjected to MM minimization, and then the HOMO and LUMO of compound **1c** were calculated and shown in Figure 2. The properties of all the target molecules were calculated according the different kind of molecular descriptors listed as the following: steric descriptors including molecular weight (MW) and Connolly molecular area (CMA), etc. (Table 3).

As a summary, it was quite surprising that the compounds **1c** show activity again HIV-2, although the rest tested compounds could not inhibit both the wild type and the HIV-2 strain line virus.

The molecule weight of **1c** and other target molecule were much more less than that of the ligand of 2HNZ, in which the ligand is a PETT derivative with the name of 1-(2-(4-ethoxy-3-fluoropyridin-2-yl)ethyl)-3-(5-methylpyridin-2-yl) thiourea (Ren et al. 2006). This might lead to the active binding pocket of HIV-1 RT was less sterically fulfilled when interacting with **1c** (Both the left and right diagram, Figure 2). The phenyl ring of **1c** was almost perpendicular to the aromatic phenyl ring of Tyr181 in the HIV-1 RT BP, which was not favorable for enhancing aromatic π-π stacking effect for steric reasons. (The right diagram, Figure 2).

Conclusion

To summarize, it should be cautious when trying to change the structure feature of PETT from di-substituted thiourea into the mono-substituted thiourea structures for achieving the possible potential anti-HIV-1 RT reagents. The simplification in the structural skeleton might decrease the biological activity for their poor solubility and less compatibility in the active binding pocket of HIV-1 RT.

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