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Synthesis and evaluation of anti-HIV activity of isatin β-thiosemicarbazone derivatives

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Abstract—On the basis of pharmacophoric modelling studies of existing NNRTIs, a series of isatin β-thiosemicarbazone derivatives was synthesized and evaluated for their anti-HIV activity in HTLV-III_B strain in the CEM cell line. Three compounds showed significant anti-HIV activity, whereupon compound **6** was found to be the most active compound with an EC_{50} value of 2.62 μM and a selectivity index of 17.41, while not being cytotoxic to the cell line at a CC_{50} value of 44.90 μM. Other tested compounds exhibited marked activity below their toxicity threshold.

1. Introduction

The human immunodeficiency (HIV) epidemic continues to have enormous human health consequences. During 2004, around five million adults and children became infected with HIV and by the end of the year, an estimated 39.4 million people worldwide were living with HIV/AIDS. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) have gained a definitive place in the treatment of HIV-1 infections,[1] in addition to the nucleoside reverse transcriptase inhibitors (NRTIs) and protease inhibitors (PIs), since they do not function as chain terminators and do not bind to the dNTP site,[2–4] making them less likely to interfere with the normal function of other DNA polymerases and therefore less toxic for the treatment of HIV-infected patients. In this respect, isatin β-thiosemicarbazone derivatives were found to demonstrate a range of antiviral activities (Moloney leukaemia virus, vaccinia virus), as reported by earlier studies.[5–8] The present study was an attempt to develop new anti-HIV drugs based on various structural modifications of thiosemicarbazone derivatives and 3D-pharmacophoric mapping of the existing NNRTIs.

2. Chemistry

2.1. Design

Earlier studies have shown that the overall structure of a widely diverging class of NNRTIs on a closer scrutiny exhibits several common features that are reminiscent of a butterfly with a hydrophilic centre as a 'body' and two hydrophobic moieties representing the 'wings.'[9,10] The 'butterfly-like' conformation has also been proven by a crystallographic analysis of nevirapine.[11] A 3D-pharmacophore model has been derived taking into account five well-known NNRTIs, as shown in Figure 1, to identify a set of pharmacophoric elements required for biological activity from structurally diverse ligands, as well as to guide in the design of new and more potent compounds. The selected ligands were geometrically optimized based on an internal strain energy and the essential structural components, such as atoms, centroids of collection of atoms, electron lone pair positions, etc., were matched in the three-dimensional space of the energetically accessible conformations of the ligands, to arrive at a 3-point pharmacophore model proposed in Figure 2.

The proposed isatin thiosemicarbazone analogue was designed based on the derived pharmacophoric model with the thiosemicarbazo moiety (=N–NH–CS–N<) constituting the 'body' and the aryl ring of isatin and bulky diethyl moiety constituting the 'wings' and was found to fulfil the specification of the pharmacophoric distance map by complying within the defined range,

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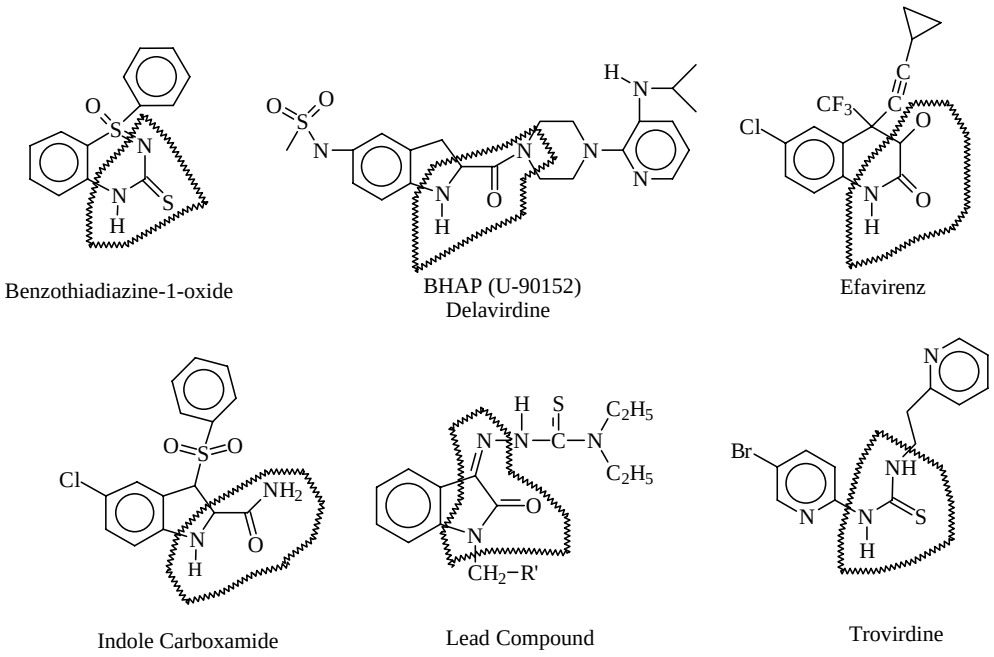


Figure 1. Schematic representation of a butterfly-like conformation of the existing NNRTIs and the lead compound.

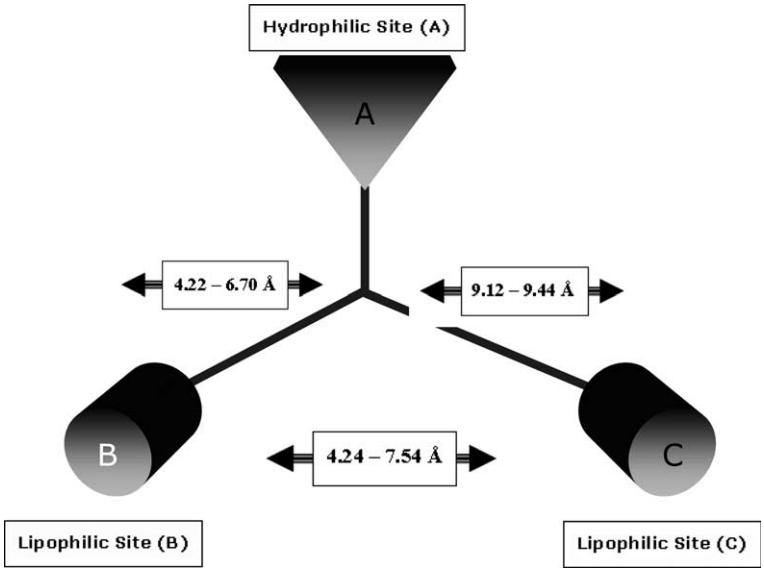


Figure 2. Schematic representation of a butterfly-like configuration of NNRTIS and the pharmacophoric distance map.

as shown in Table 1. Molecular superposition technique, namely least-squares superimposition study has shown that the RMS fit value of 0.187 shows a good correlation between selected points in the lead compound structure and corresponding points in the reference molecule, that is, Efavirenz (Fig. 3).

2.2. Synthesis

The synthesis of thiosemicarbazone derivatives was carried out in three steps, as shown in Scheme 1. First, to a solution of diethylamine (0.01 mol) in THF (10 ml) were added potassium hydroxide (0.01 mol) and carbon disulfide (0.75 ml),[12] and the mixture was stirred at 15–20 °C for 1 h to form a potassium salt of dithiocarbamate. To the stirred mixture was added hydrazine hydrate (0.01 mol) and the stirring was continued at 60 °C for 1 h to obtain *N*,*N*-diethyl thiosemicarbazide. This mixture was condensed with isatin in the presence of glacial acetic acid to form 1*H*-indole-2,3-dione-3-(*N*,*N*-diethyl thiosemicarbazone) (Schiff base). The N-Mannich bases were synthesized further by condensing the acidic imino group of isatin derivatives with formaldehyde and various secondary amines. The purity of the synthesized compounds was checked by TLC and elemental analyses; and the compounds of this study were identified by spectral data. In general, IR spectra[14] showed a C=N (azomethine) peak at 1640 cm^{-1} and, CH_2

Table 1. The pharmacophoric distance model of bioactive NNRTIs and lead compound by molecular mechanics (MM3) force fields

Compound	AB (Å)		BC (Å)		CA (Å)	
	Lower limit	Upper limit	Lower limit	Upper limit	Lower limit	Upper limit
Delavirdine	4.328 ± 0.04	6.705 ± 0.15	4.256 ± 0.19	7.542 ± 0.35	9.156 ± 0.04	9.382 ± 0.04
Trovirdine	4.235 ± 0.01	6.635 ± 0.02	4.289 ± 0.08	7.269 ± 0.16	9.168 ± 0.04	9.426 ± 0.04
Indole carboxamide	4.359 ± 0.01	6.705 ± 0.20	4.562 ± 0.14	7.478 ± 0.07	9.125 ± 0.04	9.434 ± 0.04
Efavirenz	4.425 ± 0.05	6.689 ± 0.16	4.247 ± 0.23	7.211 ± 0.21	9.145 ± 0.04	9.440 ± 0.04
Benzothiadiazine-1-oxide	4.512 ± 0.02	6.459 ± 0.03	4.269 ± 0.14	7.545 ± 0.01	9.129 ± 0.04	9.406 ± 0.04
Range	4.22–6.70		4.24–7.54		9.12–9.44	
Lead compound	4.26 ± 0.18	6.57 ± 0.12	4.27 ± 0.09	6.76 ± 0.15	9.16 ± 0.01	9.33 ± 0.02

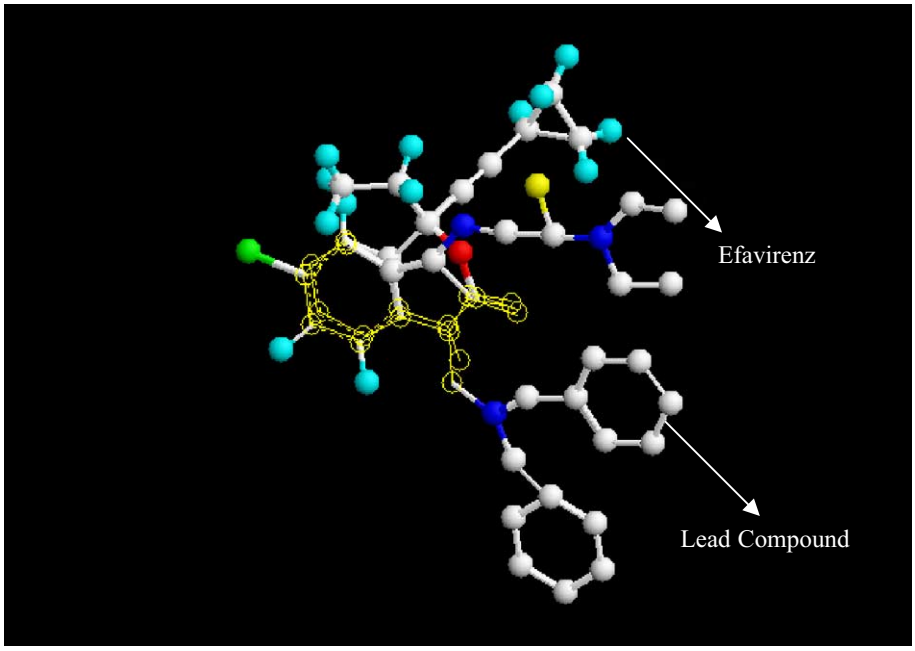
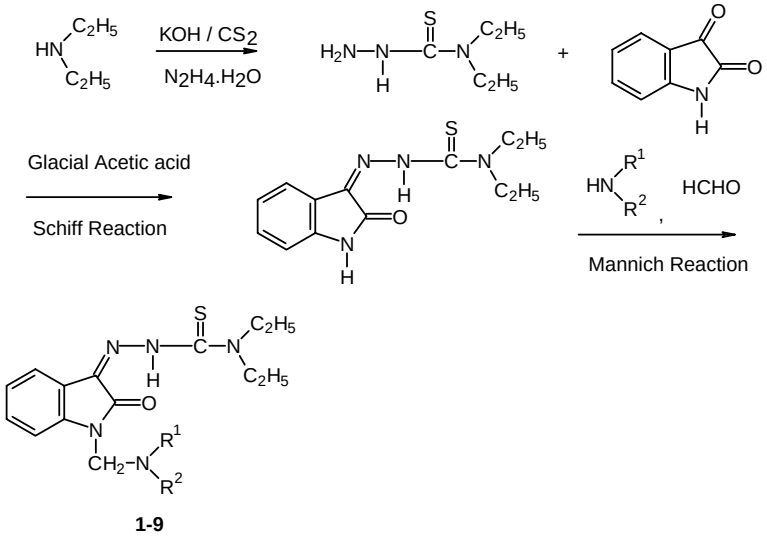


Figure 3. Superimposition and RMS fit of the proposed lead compound and Efavirenz (RMS value = 0.187).



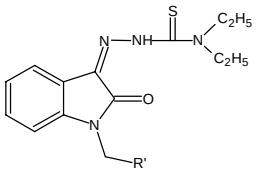
Scheme 1. Protocol for synthesis.

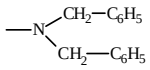
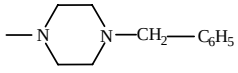
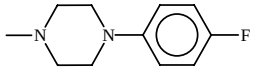
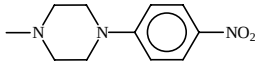
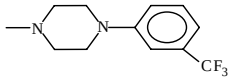
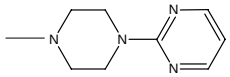
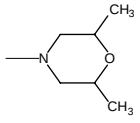
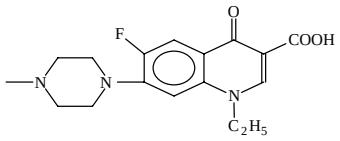
(Mannich methylene) peak at 2860 and 2840 cm^{-1}. In the ^1H NMR spectra, the signals of the respective protons of the prepared compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ 4.8–5.1 ppm corresponding to the $-NCH_2N-$ group. The elemental analysis results were within ±0.4% of the theoretical values.

2.3. Anti-HIV activity

The synthesized compounds were screened for anti-HIV activity on the replication of HIV-1 (HTLV-III_B) in the CEM cell line (Table 2).[13] The synthesized thiosemicarbazone derivatives inhibited the cytopathic effect of HIV-1 (III_B), with EC_{50} values ranging from 2.62 μM to >14.50 μM. Compounds **3**, **6** and **9** showed significant anti-HIV activity with an EC_{50} value in the range of 2.62–3.40 μM. Compound **6** exhibited the highest activity with an EC_{50} value of 2.62 μM and a selectivity index of more than 17, while not being cytotoxic to the cell line with a CC_{50} value of 44.90. Other compounds did not show any marked anti-HIV activity below their toxicity threshold. All the synthesized compounds were found to be less active than the standard drug Efavirenz.

Table 2. Physical constants and biological activity of compounds (**1**–**9**) against HIV-1 (HTLV-III_B) in the CEM cell line



Compound	R′	Molecular weight	Melting point	% yield	Anti-HIV-1 activity (μM)		
					EC_{50}[a]	CC_{50}[b]	SI[c]
1		485	52	72	>8.29	8.29	<1
2		333	66	74	>2.86	2.86	<1
3		464	78	69	3.40	14.00	4.12
4		468	58	67	>14.50	14.50	<1
5		495	92	70	>11.90	11.90	<1
6		518	64	71	2.62	44.90	17.14
7		452	62	75	>12.80	12.80	<1
8		401	85	70	>13.30	13.30	<1
9		607	198	76	3.12	37.50	12.02
Efavirenz	—				0.78	>200	>256

[a] 50% effective concentration or concentration required to inhibit HIV-1 induced cytopathicity in cell lines by 50%.
[b] 50% cytotoxic concentration or concentration required to reduce the viability of mock-infected cell lines by 50%.
[c] Selectivity index or ratio of CC_{50} to EC_{50}.

3. Conclusion

On the basis of the 3D-pharmacophoric-distance map based on the structures of existing NNRTIs, the thiosemicarbazone derivatives were found to comply to be within the defined range for anti-HIV activity. Least-squares superimposition technique also established a good correlation between thiosemicarbazone and Efavirenz with a RMS fit value in the agreeable range. Further investigation on structure–activity relationships and appropriate modification among the synthesized thiosemicarbazone derivatives is likely to provide more effective HIV-1 inhibitors with improved efficacy.

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14. Spectral data for compound **6**. IR (KBr): 3200, 2860, 2840, 1640, 1620, 1580, 1015 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm): 1.0 (t, 6H, $2CH_3$), 2.59 (t, 4H, CH_2NCH_2), 3.4 (t, 4H, CH_2NCH_2), 3.49 (q, 4H, $2CH_2$), 4.80 (s, 2H, NCH_2N), 7.01–7.58 (m, 8H, Ar-H), 11.26 (s, 1H, –NH, D_2O exchangeable).