Synthesis, antibacterial and potential anti-HIV activity of some novel imidazole analogs

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A series of 1-(2-methyl-4-nitro-imidazol-1-yl)-3-arylami-nopropan-2-ones (**2a–e**), 2-methyl-5-nitro-1-{2-[arylme-thoxy]ethyl}-1*H*-imidazoles (**5a–d**), and *N*-(3-hydroxy-phenyl)-2-(substituted imidazol-1-yl)alkanamides (**8a–e**) were synthesized with the aim to develop novel imidazole analogs with broad-spectrum chemotherapeutic properties. Title compounds were evaluated for their anti-HIV and antibacterial activities.

Keywords: imidazole, reverse transcriptase, non-nucleoside reverse transcriptase, anti-HIV, antibacterial

HIV-1 (Human Immunodeficiency Virus Type-1) is a pathogenic retrovirus of the lentivirus family and causative agent of AIDS or AIDS Related Complex (ARC) (1–3). HIV infection targets the monocytes expressing surface CD4 receptors and produces profound defects in cell-mediated immunity (4). Overtime infection leads to severe depletion of CD4 T-lymphocytes (T-cells) resulting in opportunistic infections (OIs) such as bacterial, fungal, viral, protozoal and neoplastic diseases and ultimately death (5). An ideal anti-HIV agent should suppress HIV replication and should also be able to combat other opportunistic infections, like tuberculosis, hepatitis and other bacterial infections.

Imidazoles have been known as antiviral agents, one of the examples being capravirine (6). Silvestri *et al*. (7) and De Martino *et al*. (8, 9) synthesized a number of 1-{2--(diarylmethoxy)ethyl]-2-methyl-5-nitroimidazole (DAMNI) analogs as novel HIV-1 reverse transcriptase (RT) inhibitory agents active at submicromolar concentration, with the racemic 1-2-[(thiophen-2-yl)phenylmethoxy]ethyl]-2-methyl-5-nitroimidazole (EC_{50}, 0.03 µmol^{-1}) being the most potent among all the analogs (Fig. 1), exhibiting higher activity than efavirenz against the viral RT carrying the K103N mutation. Similarly, *N*-ami-

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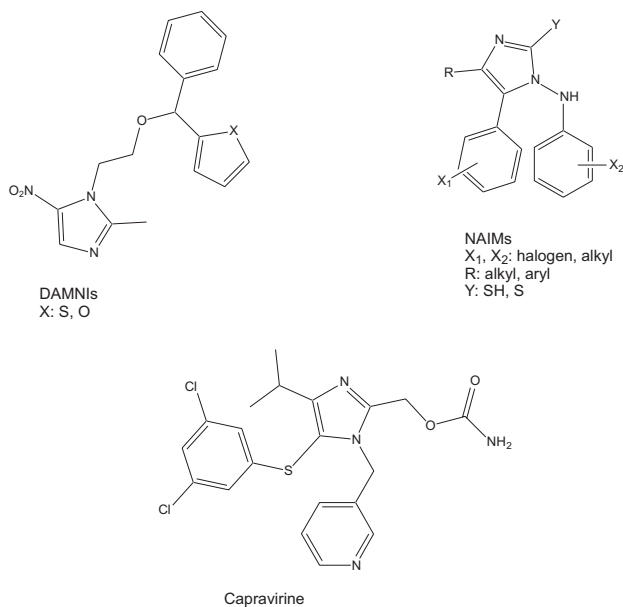* Correspondence; e-mail: swastikaganguly@bitmesra.ac.in

Fig. 1. Structural formulae of some clinically established imidazole drugs.

noimidazoles (NAIMs) have also been reported to inhibit replication of the WT virus as well as an HIV-1 strain that contained both the K103N and Y181C mutations (10) (Fig. 1).

Earlier works in our laboratory have identified various imidazole derivatives exhibiting broad-spectrum chemotherapeutic properties (11, 12). In continuation of our effort to develop imidazoles as broad-spectrum chemotherapeutic agents and encouraged by the work of de Martino *et al.* (9), we undertook the present study to synthesize and evaluate novel imidazoles and nitroimidazoles that could suppress HIV replication and also inhibit opportunistic microorganisms. Docking studies with HIV-1 RT (PDB ID 1FK9) were also performed in order to investigate the binding pattern of the compounds showing inhibitory activity on HIV-1 RT.

EXPERIMENTAL

Melting points are uncorrected and were measured on an IEC Precision melting point apparatus (Indian Equipment Corporation, India). Elemental analyses were performed on a Vario EL III M/s Elementar C, H, N, S, O analyzer (Germany). The ^{1}H NMR spectra were measured with a Bruker Avance digital spectrophtometer (Switzerland) (300 MHz) in DMSO-*d*6 and chemical shifts were assigned in δ ppm. The FAB mass spectra were recorded on a JEOL SX 102/DA-6000 mass spectrometer/data system using argon/xenon (6 kV, 10 mA) as the FAB gas (Japan).

Metronidazole was obtained from ARISTO Pharmaceuticals Pvt. Ltd. (India) and 2-methylimidazole was obtained from Ozone International, India.

1-(3′-Chloroacetonyl)-2-methyl-4-nitroimidazole (**1**), 2-[chloro(phenyl)methyl]thiophene (**3a**), 3-(2-chloro-2-phenylethyl)thiophene (**3b**), 2-[chloro(phenyl)methyl]pyrazine (**3c**) and 2-[chloro(phenyl)methyl]benzenethiol (**3d**) were prepared according to previously reported procedures (13). ω-Chloroalkanamides (**6**) were also prepared according to a previously reported method (14).

Physicochemical and spectral data for the synthesized compounds are given in Tables I and II. Synthetic routes are presented in Schemes 1–3.
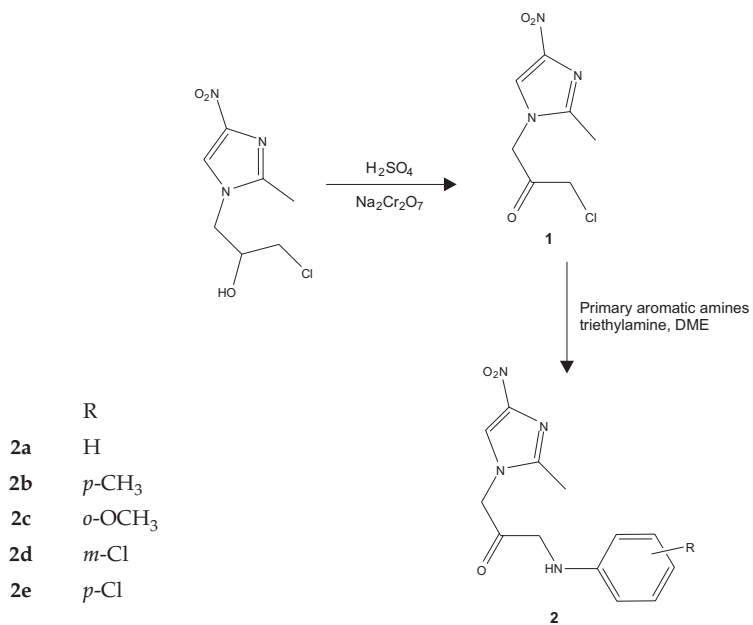
Table I. Physical and analytical data of synthesized compounds

Compd.	Molecular formula	M_r	M. p. (°C)	Yield (%)	Elemental analysis calcd./found (%)		
					C	H	N
2a	$C_{13}H_{14}N_4O_3$	273.11	132–133	60	56.93 56.89	5.14 5.10	20.43 20.39
2b	$C_{14}H_{16}N_4O_3$	287.12	129–130	57	58.32 58.35	5.59 5.67	19.43 19.36
2c	$C_{14}H_{16}N_4O_4$	303.12	153–154	65	55.26 55.16	5.30 5.35	18.41 18.46
2d	$C_{13}H_{13}ClN_4O_3$	307.06	140–141	79	50.58 50.68	4.24 4.27	18.15 18.18
2e	$C_{13}H_{13}ClN_4O_3$	307.07	135–136	65	50.58 50.54	4.24 4.19	18.15 18.28
5a	$C_{17}H_{17}N_3O_3S$	342.10	100–102	54	59.46 59.49	4.99 4.95	12.24 12.19
5b	$C_{17}H_{18}N_3O_3S$	343.11	125–126	40	59.28 59.22	5.27 5.33	12.20 12.24
5c	$C_{17}H_{17}N_5O_3$	338.13	135–137	50	60.17 60.10	5.05 5.03	20.64 20.35
5d	$C_{19}H_{19}N_3O_3S$	368.11	140–141	45	61.77 61.89	5.18 5.15	11.37 11.33
8a	$C_{12}H_{13}N_3O_2$	230.11	140–142	86	62.33 62.31	5.67 5.55	18.17 18.13
8b	$C_{13}H_{15}N_3O_2$	244.12	118–120	88	63.66 63.75	6.16 6.11	17.13 17.25
8c	$C_{13}H_{15}N_3O_2$	244.12	100–102	83	63.66 63.46	6.16 6.19	17.13 17.10
8d	$C_{12}H_{12}N_4O_4$	275.09	135–137	90	52.17 52.25	4.38 4.45	20.28 20.17
8e	$C_{13}H_{14}N_4O_4$	289.10	118–120	85	53.79 53.75	4.86 4.90	19.30 19.35
8f	$C_{13}H_{14}N_4O_4$	289.10	118–120	82	53.79 53.82	4.86 4.93	19.30 19.32

Table II. Spectral data of synthesized compounds

Compd.	IR (KBr) (cm^{-1})	^{1}H NMR (DMSO-d6) (δ, ppm)
2a	3325 (N-H), 1656 (C=O), 1500 (N=O), 1294 (C-N)	nr
2b	3369 (N-H), 1616 (C=O), 1529 (N=O), 1294 (C-N)	nr
2c	3336 (N-H), 1664 (C=O), 1506 (N=O), 1290 (C-N), 1251 (C-O-C)	8.22 (s, 1H, H-5), 6.90–6.51 (t, 1H, H-4',5'; d, 1H, H-3',6'), 4.14 (t, 2H, N-CH$_2$), 3.94 (s, 1H, -CH$_3$ of OCH$_3$), 3.91 (t, 2H, -CH$_2$-NH-Ar), 3.32 (s, 1H, aromatic C-N**H**) 2.33 (s, 3H, Me at C-2)
2d	3381 (N-H), 1600 (C=O), 1543 (N=O), 1294 (C-N), 781 (C-Cl)	8.2 (s, 1H, H-5), 7.039–6.638 (s, 1H, H-2'; d,1H, H -4',6'; t, 1H, H-5'), 5.38 (t, 2H, -CH$_2$- NH-Ar), 4.163 (t, 2H, N-CH$_2$), 3.94 (s, 1H, aromatic C-N**H**), 2.34 (s, 3H, Me at C-2)
2e	3375 (N-H), 1645 (C=O), 1541 (N=O), 1340 (C-N), 746 (C-Cl)	nr
5a	2947 (C-H), 1188 (–C-O-C), 1533 (N=O)	8.02 (s, 1H, H-5), 7.33 (brs, 8H, Ar-H), 4.35 (t, 2H, N-CH$_2$), 3.70–3.65 (m, 2H, O-CH$_2$), 2.45 (s, 3H, C**H$_3$**)
5b	2652 (C-S), 1193 (C-O-C), 1537 (N=O), 850 (C-N),	nr
5c	1188 (–C-O-C), 1533 (N=O), 850 (C-N)	8.02 (s, 1H, H-5), 7.43–7.27 (m, Ar-H), 4.35 (t, 2H, N-CH$_2$), 3.68-3.67 (d, 2H, O-CH$_2$), 2.45 (s, 3H, C**H$_3$**)
5d	2592 cm^{-1} (S-H), 1188 (–C-O-C), 1523 (N=O),	8.02 (s, 1H, H-5), 7.38–7.27 (m, 5H, Ar-H), 4.33 (t, 2H, N-CH$_2$), 3.70–3.65 (m, 2H, O-CH$_2$), 2.46 (s, 3H, C**H$_3$**)
8a	3590 (O-H), 3125 (N-H), 1643 (C=O), 1408 (C-N), 1567 (C=N)	10.13 (s, 1H, CON**H**), 9.95 (s, 1H, Ar-O**H**), 7.27–7.00 (m, 4H, Ar-**H**), 6.97 (s, 1H, **H**-5'), 6.73 (s, 1H, **H**-4'), 4.66 (s, 2H, C**H$_2$**CONH), 2.08 (s, 3H, Me-2')
8b	3601 (O-H), 3225 (N-H), 1639 (C=O), 1408 (C-N), 1548 (C=N)	9.58 (s, 1H, CON**H**), 9.43 (s, 1H, Ar-O**H**), 7.38–7.12 (m, 4H, Ar-**H**), 6.83 (s, 1H, **H**-5'), 6.73 (s, 1H, **H**-4'), 4.80 (q, 1H, C**H**(CH$_3$)CONH), 2.6 (s, 3H, Me-2'), 1.82 (d, 3H, CH(C**H$_3$**)CONH)
8c	3551 (O-H), 3212 (N-H), 1650 (C=O), 1378 (C-N), 1499 (C=N).	nr
8d	3663 (O-), 3175 (N-H), 1628 (C=O), 1418 (C-N), 1548 (C=N)	nr
8e	3590 (O-H), 3125 (N-H), 1643 (C=O), 1408 (C-N), 1548 (C=N)	9.83 (s, 1H, CON**H**), 9.46 (s, 1H, Ar-O**H**), 7.81 (s, 1H, **H**-4'), 7.38–6.61 (m, 4H, Ar-**H**), 4.82 (q, 1H, C**H**(CH$_3$)CONH), 2.51 (s, 3H, Me-2'), 1.80 (d, 3H, CH(C**H$_3$**)CONH
8f	3580 (O-H), 3175 (N-H), 1678 (C=O), 1418 (C-N), 1548 (C=N)	–

s – singlet, d – doublet, t – triplet, q – quartet, m – multiplet, nr – not recorded

	R
2a	H
2b	*p*-CH$_3$
2c	*o*-OCH$_3$
2d	*m*-Cl
2e	*p*-Cl

Scheme 1

	Ar
3a, 5a	
3b, 5b	
3c, 5c	
3d, 5d	

Scheme 2
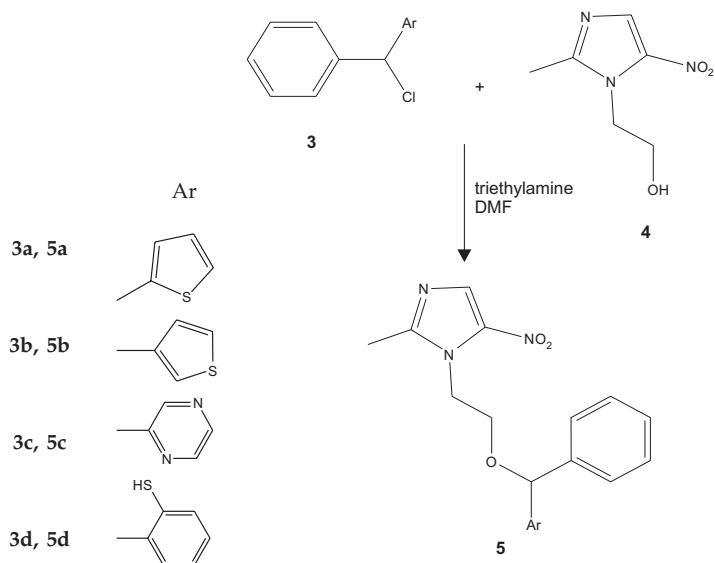
	R	R$_1$	Z
8a	CH$_3$	H	CH$_2$
8b	CH$_3$	H	CH(CH$_3$)
8c	CH$_3$	H	CH$_2$CH$_2$
8d	CH$_3$	NO$_2$	CH$_2$
8e	CH$_3$	NO$_2$	CH(CH$_3$)
8f	CH$_3$	NO$_2$	CH$_2$CH$_2$
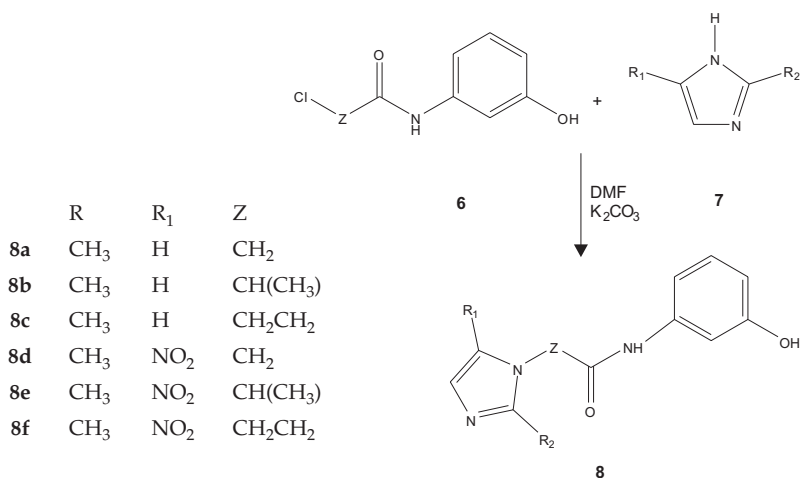
Scheme 3

Syntheses

1-(2-Methyl-4-nitro-imidazol-1-yl)-3-arylamino-propan-2-ones (2a–e). General procedure. – A mixture of **1** (0.92 mmol), triethylamine (1.43 mmol) and appropriate aromatic amines (0.92 mmol) in 2 mL *N,N*-dimethylformamide (DMF) was refluxed for 5 h with stirring. After cooling, 20 mL of cold water was added to the reaction mixture. The precipitate obtained was filtered, air-dried and recrystallized from ethyl acetate.

2-Methyl-5-nitro-1-{2-[phenyl(thiophen-2-yl)methoxy]ethyl}-1H-imidazole (5a) (13). – A mixture of 2-[chloro(phenyl)methyl]thiophene (**3a**), triethylamine (0.75 mmol) and 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (**4**) (0.19 mol) in 2 mL anhydrous DMF was stirred at 50 °C overnight. After cooling, the mixture was diluted with 15 mL of water and extracted with ethyl acetate (2 × 30 mL). The organic layer was then dried over anhydrous sodium sulphate. The solvent was then evaporated to yield a precipitate, which was then recrystallized from methanol/ethyl acetate (2:8) mixture.

Analogously, 2-methyl-5-nitro-1-{2-[1-phenyl-2-(thiophen-3-yl)ethoxy]ethyl}-1*H*-imidazole (**5b**), 2-methyl-5-nitro-1-{2-[phenyl(pyrazine-2-yl)methoxy]ethyl}-1*H*-imidazole (**5c**) and 2-methyl-5-nitro-1-{2-[phenyl(benzenethiol-2-yl)methoxy]ethyl}-1*H*-imidazole (**5d**) were prepared from 3-(2-chloro-2-phenylethyl)thiophene (**3b**), 2-[chloro(phenyl)methyl]pyrazine (**3c**) and 2-[chloro(phenyl)methyl]benzenethiol (**3d**), respectively.

N-(3-hydroxyphenyl)-2-(substituted imidazol-1-yl)alkanamides (8a–f). General procedure. – To a solution of appropriate 2-chloro-*N*-(3-hydroxyphenyl)alkanamide (**6**) (0.7 mmol) in 2 mL DMF, the substituted imidazole (**7**) (0.7 mmol) and anhydrous potassium carbonate (0.050 g) were added. The reaction mixture was heated at 60–70 °C for 6–8 h with

continuous stirring. The mixture was then poured onto crushed ice and then into water with stirring to yield a precipitate, which was further recrystallized in ethanol giving pale white crystals.

Biological assays

Antimicrobial evaluation. – Antimicrobial activity of the synthesized compounds was determined *in vitro* using the 2-fold serial broth dilution method in duplicates (15) against pathogenic microorganisms *Escherichia coli* NCIM 2810, *Salmonella typhi* NCIM 2501 (Gram-negative bacteria), *Staphylococcus aureus* NCIM 2901 and *Bacillus subtilis* MTCC 441 (Gram-positive bacteria). Metronidazole was used as the standard. Preparation of the standard and test stock solution was carried out by dissolving metronidazole and each of the synthesized compounds (**2a–e**, **5a–d** and **8a–f**) in dimethylsulfoxide (DMSO) to give a concentration of 4 mg mL^{-1}.

Fresh nutrient broth cultures were prepared by inoculating and incubating the desired microorganism at 37 °C for 24 h. These fresh cultures were standardized using the McFarland standard. 0.4 mL of 4 mg mL^{-1} test stock solution in DMSO was transferred to the first sterile test tube containing 3.6 mL of 1.1x (where x was the single strength concentration of nutrient broth medium) nutrient broth to prepare 400 µg mL^{-1} as starting dose, and the remaining test tubes 2–9 were serially diluted with double-strength nutrient broth to obtain the desired concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.56 µg mL^{-1}, respectively. The test tube with no compound but with equal volume of solvent DMSO (10 %) served as solvent control. One test tube with no compound and no vehicle but only with nutrient medium served as a negative control to ensure the growth property of the medium. Finally, to these test tubes (serially diluted test tubes containing the synthesized compound and positive control as metronidazole, solvent control test tubes and the negative control test tube), 0.1 mL suspension of 10^8 cfu mL^{-1} of the desired microorganism was added and further incubated at 37 °C for 24 hours. The lowest concentration, which apparently caused complete inhibition of the growth of test organisms, was considered the *MIC* value of the test compound and was expressed in µg mL^{-1}.

Anti-HIV-1 activity. – All the synthesized compounds were evaluated for inhibitory activity on HIV-1 RT using the HIV-1 RT RNA dependent DNA polymerase activity assay. Efavirenz was used as the standard. Fixed time point assays were used to determine HIV-1 RT-associated RNA-dependent DNA polymerase activity, as reported previously (16). Assays were carried out using poly (rA)-oligo (dT18) as the template/primer (T/P) substrate. Oligo (dT18) primers were synthesized with a biotin label on the 5′-terminus. DNA polymerase reactions were carried out in 50 mmol L^{-1} Tris-HCl pH 7.5 (37 °C), 50 mmol L^{-1} KCl, 10 mmol L^{-1} MgCl$_2$ containing 600 nmol L^{-1} T/P, 1 µmol L^{-1} of [^{3}H] TTP, and variable inhibitor concentrations. Reactions were initiated by the addition of 25 nmol L^{-1} of RT, incubated for 20 min at 37 °C and then quenched with 0.5 mol L^{-1} EDTA. Streptavidin scintillation proximity assay beads (GE Healthcare, USA) were then added to each reaction, and the extent of radionucleotide incorporation was determined by scintillation spectrometry using a 1450 Microbeta Liquid Scintillation Counter (Perkin Elmer, USA).

Molecular modelling and ligand and protein structure

All computational studies were carried out using the Glide 5.0 (Schrödinger Inc., USA) software package installed in a single machine running on a 3.4 GHz Pentium 4 processor with 1 GB RAM and 160 GB Hard Disk with Red Hat Linux Enterprise version 5.0 as the Operating System. Ligand structures were drawn using the Ligprep utility of Glide. These structures were then geometry optimized by using the Optimized Potentials for Liquid Simulations-2005 (OPLS- -2005) force field (17, 18) with the steepest descent followed by truncated Newton conjugate gradient protocol. Partial atomic charges were computed using the OPLS-2005 force field.

The geometry of the non-nucleoside inhibitory binding pocket (NNIBP) of the wild type RT strain was taken from the structure of the HIV-1 RT/EFAVIRENZ complex filed in the RCSB Protein Data Bank (entry code 1FK9). Water molecules were removed from the complex. The protein was optimized for docking using the protein preparation wizard of Schrödinger Inc. Partial atomic charges were assigned according to the Optimized Potentials for Liquid Simulations (OPLS) force field.

Validation of the docking protocol

The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the Extra Precision Glide docking procedure was validated by removing efavirenz from the binding site and re-docking it to the NNIBP of HIV-1 RT.

We found very good agreement between the localization of the inhibitor efavirenz upon docking and from the crystal structure (Fig. 2). The relative mean square deviations (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of efavirenz equaled 1.026 Å. This indicated the reliability of the docking method in reproducing the experimentally observed binding mode for HIV-1 RT.

RESULTS AND DISCUSSION

Chemistry

Compounds **2a–e** were prepared using 1-(3'-chloroacetonyl)-2-methyl-4-nitroimidazole **1** as starting material in the reaction with different primary aromatic amines in the presence of triethylamine (reaction time varied from 4 to 5 h). Spectral data are displayed in Table II. The reaction sequence is given in Scheme 1.

The IR and mass spectra were reported for all the newly synthesized compounds **2a–e** while ^{1}H NMR spectra were assigned for compounds **2c** and **2d**. In general, infrared spectral data (v, cm^{-1}) revealed bands at 3325–3381 (NH), 1600–1656 (C=O), 1500–1543 (N=O) and 1290–1340 (CN). The ^{1}H NMR spectra of **2c** and **2d** showed a singlet of one proton at δ 8.22 and 8.20 ppm assigned to the proton at the fifth position of the imidazole nucleus, respectively. The peaks at δ 6.90–6.51 ppm were assigned to the aromatic protons for compound **2c** while the peaks at δ 6.638–7.039 ppm were assigned to the aro-
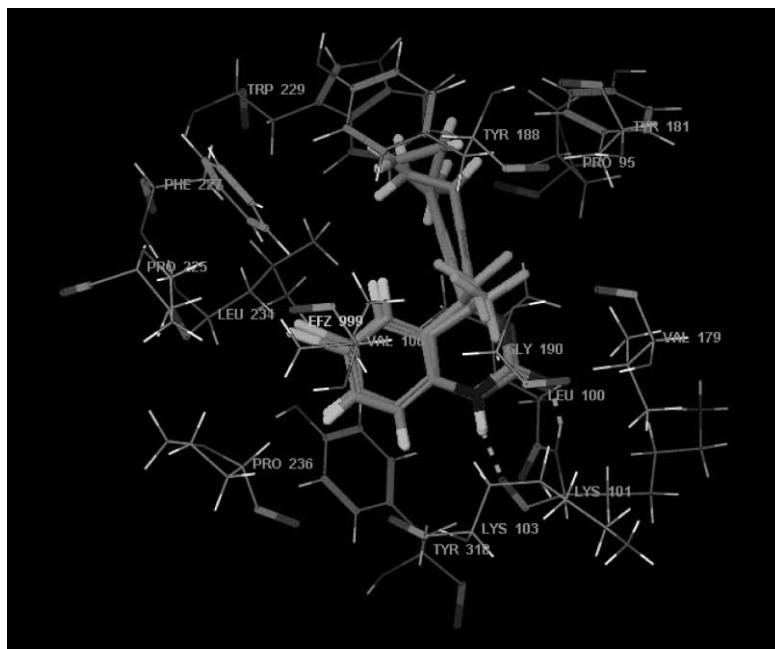
Fig. 2. Validation of docking by Glide. Superimposition of experimental bound (cocrystallized) conformation of efavirenz (gray) and that predicted by Glide (ball and stick model). Active site amino acid residues are represented as lines. Hydrogen bond interaction is shown in dotted lines.
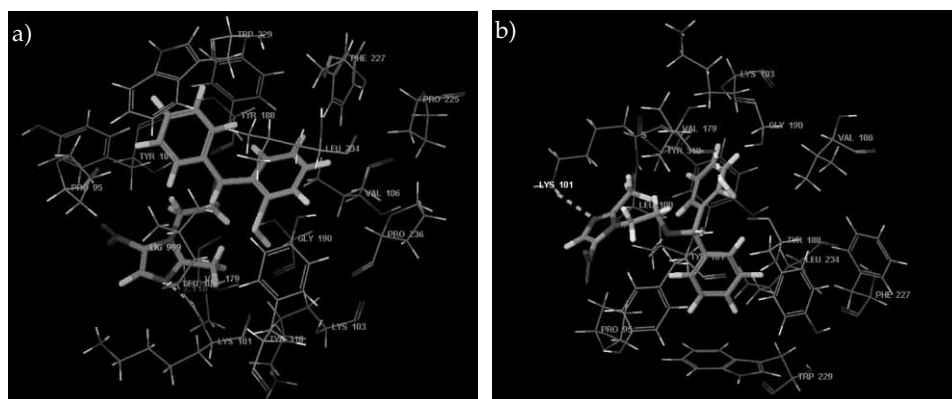

Fig. 3. a) XP-Glide predicted pose of R configuration of the most active compound **5d**. b) XP-Glide predicted pose of S configuration of compound **5d**. Active site amino acid residues are represented as lines while the inhibitor is shown as stick model. Hydrogen bond interaction is shown in dotted lines.

matic protons in the substituted *m*-chloroaniline ring of compound **2d**. Triplets of two methylene protons at δ 4.14 and 3.94 were assigned to N–CH_2 group and -CH_2-NH-Ar group, respectively, for compound **2c**. Similarly, triplets of two methylene protons at δ 4.16 and 5.38 ppm were assigned to the N–CH_2 group and -CH_2-NH-Ar group, respectively, for compound **2d**. A singlet at δ 3.94 ppm was observed for the methyl protons of the methoxy group in the *o*-anisidine ring for compound **2c**. Another singlet at δ 3.32 ppm was observed for the aromatic C-N**H** group of the linker for both compounds **2c** and **2d**. A singlet at δ 2.33 2.34 ppm was observed for methyl protons at the second position of imidazole ring for compounds **2c** and **2d,** respectively.

2-Methyl 5-nitroimidazole analogs **5a–d** were prepared by treating compounds **3a–d** with 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole (**4**) in anhydrous dimethyl formamide at 50 °C following the procedure of Martino *et al.* (9). The reaction sequence is given in Scheme 2.

In general, infrared spectral data revealed bands at 1523–1537 (N=O), 1188–1193 (C-O-C), and 850 cm^{-1} (C-N). The ^{1}H NMR spectrum of compounds **5a**, **5c** and **5d**, showed a singlet of 3 protons assigned to imidazole at δ 2.45 ppm and a singlet of one proton at δ 8.02 ppm assigned to the imidazole nucleus proton at the fifth position. Compounds **5a** and **5d** showed a triplet of two protons at δ 4.37–4.33 ppm and 3.70–3.65 ppm assigned to the N-CH_2 and O-CH_2 group respectively. Similarly compound **5c** also showed a triplet of two protons at δ 4.37–4.33 and 3.68–3.67 ppm assigned to the N-CH_2 and O-CH_2 groups respectively. Compound **5a** also showed a broad singlet at 7.33–7.24 ppm assigned to the aromatic protons. Similarly compounds **5c** and **5d** showed a multiplet at δ 7.4–7.27 ppm assigned to the aromatic protons.

Analogs **8a–8f** were synthesized by treating appropriate ω-chloroalkanamides **6** with substituted imidazoles **7** under constant stirring in DMF in the presence of potassium carbonate at 60–70 °C.

The ^{1}H NMR spectra of compounds **8a** and **8b** showed a singlet of one proton at δ 10.13 and 9.58 ppm assigned to the proton of the CON**H** group, respectively. Another singlet of one proton was observed at δ 9.95 and 9.43 ppm assigned to the hydroxyl proton attached to the aromatic group for compounds **8a** and **8b,** respectively. Compounds **8a** and **8b** also showed multiplets of four protons in the range of δ 7.27–7.00 and 7.38–7.12 ppm, respectively, for all the aromatic protons at positions 2, 4, 5, and 6 of the phenyl ring. Compounds **8a** and **8b** also showed a singlet at δ 6.73 ppm assigned to the proton at the 4th position of the imidazole ring, while another singlet at δ 6.97 and 6.83 ppm was observed for the proton at 5th position of the imidazole ring, respectively, for both compounds. Compound **8a** showed a quartet for the proton of C**H**(CH$_3$)CONH at δ 4.80 ppm while **8b** showed a singlet for two protons of the linker group C**H$_2$**CONH at δ 4.66 ppm. A broad singlet was observed for the methyl protons at the 2nd position of the imidazole ring at δ 2.08 and 2.60 ppm, respectively, for both **8a** and **8b**. A doublet was observed for compound **8b** at δ 1.82 ppm for three protons of the methyl group in CH(C**H$_3$**)CONH linker. The ^{1}H NMR spectrum of compound **8e** showed a broad singlet for one proton of the CON**H** amide bond at δ 9.83 ppm while another singlet was observed for the hydroxyl proton over phenyl ring at δ 9.46 ppm. A singlet was observed for the single proton at the 4th position of the imidazole ring at δ 7.81 ppm. A multiplet for all the aryl protons was obtained in the range of δ 7.38–6.61 ppm. A quartet was observed for a single proton at δ 4.82 ppm for the group C**H** (CH$_3$) CONH. A broad singlet

was observed for the methyl protons at the 2nd position of the imidazole ring at δ 2.51 ppm. A doublet was observed at δ 1.80 ppm for the methyl protons attached to the linker, CH(**CH$_3$**)CONH.

Biological activity

All the compounds were screened for their *in vitro* antibacterial activity. The results are given in Table III. Among all the series of compounds **2a–e**, **5a–d** and **8a–f**, compounds **8a–f** showed a significant degree of activity against all the bacterial strains compared to metronidazole, with compounds **8a**, **8d** and **8f** being the most active. Compound **8b** showed significant activity against *S. typhi, B. subtilis* and *S. aureus* and the highest activity against *E. coli.* Compound **8c** showed very high activity against *E. coli, B. subtilis* and *S. aureus*, but moderate activity against *S. typhi.*

In the series **5a–d**, compound **5c** showed considerable activity against *B. subtilis* while compound **5d** showed similar activity against *S. typhi.* Both of these compounds showed weak to moderate activity against *E. coli* and *S. aureus.*

In the series **2a–e**, compound **2c** showed a significant degree of activity against *E. coli* and *S. aureus* and weak activity against the other bacterial strains. Compounds **2b** and **2d** showed moderate activity against all the strains of bacteria while compound **2a** showed a moderate degree of activity against *E. coli* and *S. aureus* and weak activity against the other strains of bacteria. However, it was interesting to note that almost all

Table III. MIC (μg mL^{-1}) data for synthesized compound

Compd.	E. coli	S. typhi	B. subtilis	S. aureus
2a	200	400	400	200
2b	100	200	200	200
2c	100	400	400	100
2d	200	200	200	200
2e	200	200	400	200
5a	> 400	200	> 400	400
5b	400	200	200	> 400
5c	200	400	100	200
5d	400	100	> 400	200
8a	50	100	50	50
8b	50	100	100	100
8c	100	200	100	50
8d	50	50	50	50
8e	100	100	50	200
8f	100	50	50	50
Metronidazole	200	> 400	> 400	> 400

[a] Solvents: DMSO, water

the compounds showed higher or comparable degree of activity compared to metronidazole.

All the synthesized compounds were also evaluated for their HIV-1 RT-associated RNA dependent DNA polymerase activity. Compounds **5a**, **5b** and **5d** showed a weak HIV-1 RT inhibitory activity with IC_{50} values of 11.9 ± 4.2 µmol L^{-1}, 19.6 ± 4.8 µmol L^{-1} and 12.3 ± 3.9 µmol L^{-1}, respectively (Table IV). However, all the compounds showed lower activity then efavirenz.

In our study of imidazoles and nitroimidazoles, we found that compounds **5a–d** exhibited promising antibacterial activities while showing weak non-nucleosidal reverse transcriptase activity.

Molecular modelling

Based on the fact that among compounds **5a–d**, **5d** showed relatively high NNRTI activity, we performed *in silico* docking studies to investigate the interaction of **5d** with the NNRTI-binding pocket of the wt HIV-1 RT (PDB entry code 1FK9) using the Extra Precision (XP) mode of the Glide software to gain some structural insight into the interaction of these inhibitors with RT. Validation of the Glide software was carried out by modeling the interaction between efavirenz and HIV-1 RT. Superimposition of the experimental bound (co-crystallized) conformation of efavirenz and that predicted by Glide is shown in Fig. 2. Glide successfully reproduced the experimental binding conformations of efavirenz in the NNRTI-binding pocket of HIV-1 RT with an acceptable

Table IV. In vitro *anti-HIV-activity of synthesized compounds*

Compd.	wt HIV-1 RT IC_{50} (µmol L^{-1})
2a	> 100
2b	> 100
2c	> 100
2d	> 100
2e	> 100
5a	11.9 ± 4.2
5b	19.6 ± 4.8
5c	>100
5d	12.3 ± 3.9
8a	> 100
8b	> 100
8c	> 100
8d	> 100
8e	> 100
8f	> 100
Efavirenz	0.094 ± 0.006

RDDP activity assessed using recombinant purified HIV-1 RT

root-mean-square deviation (RMSD) of 1.026 Å. The docking score of efavirenz was obtained as –10.12. The docking studies of compound **5d** showed that introduction of a chiral centre did not have much effect on the binding of (*R*)-**5d** or (*S*)-**5d** and both of these configurations bound to the NNIBP in a similar way. However, the docking score for (*R*)-**5d** was –12.16 while the score for *S* configuration amounted to –9.07. A closer investigation of the (*R*)-**5d** binding mode revealed that the one phenyl moiety of compound (*R*)-**5d** was oriented towards the hydrophobic pocket formed by the side chain of Tyr 188, Tyr 181, Leu 234 and Trp 229. The thiophenol moiety occupied a more lipophilic pocket formed by the side chain of Val 106, Phe 227, Tyr 318, Gly 190 and Pro 225.The ethylene bridge interacted with the side chain of Val 179, Gly 190 and Leu 100. The basic nitrogen of imidazole nucleus made a hydrogen bond with the NH of Lys 101 (distance Nimidazole···NHLys101 = 2.11 Å) (Fig. 3a). In case of (*S*)-**5d**, it was interesting to note that it made two hydrogen bonds, one hydrogen bond between the basic nitrogen of imidazole and the NH of Lys 101 (Nimidazole···NHLys101 = 2.43 Å) and another hydrogen bond between the –SH and carbonyl oxygen of Val 179 (SH$_{thiophenol}$···C=O$_{Val179}$ = 2.05 Å) (Fig. 3b).

CONCLUSIONS

A number of novel imidazole and nitroimidazole analogs were synthesized with the objective to develop novel anti-HIV agents with broad-spectrum chemotherapeutic properties. The results of anti-HIV and antibacterial activities indicate that 2-methyl-5-nitro-1-{2-[phenyl(thiophen-2-yl)methoxy]ethyl}-1*H*-imidazole (**5a**), 2-methyl-5-nitro-1-{2-[1-phenyl-2-(thiophen-3-yl)ethoxy]ethyl}-1*H*-imidazole (**5b**), 2-methyl-5-nitro-1-{2-[phenyl(pyrazine-2-yl)methoxy]ethyl}-1*H*-imidazole (**5c**) and 2-methyl-5-nitro-1-{2-[phenyl(benzenethiol-2-yl)methoxy]ethyl}-1*H*-imidazole (**5d**) revealed moderate antibacterial and weak anti-HIV activity. *N*-(3-hydroxyphenyl)-2-(substituted imidazol-1-yl) alkanamides (**8a–f**) also exhibited significant antibacterial activities. It can be concluded that these series of imidazoles and nitroimidazoles might be useful as starting moieties that can be further exploited synthetically for the discovery of analogues with more potent anti-HIV activity with broad-spectrum chemotherapeutic properties.

Acronyms. – HIV – Human Immunodeficiency Virus, AIDS – Acquired Immunodeficiency Syndrome, ARC – AIDS related complex, NNRTI – non-nucleoside reverse transcriptase inhibitor, K103N – Lys 103, V106A – Val 106, L100I – Leu 100, DAMNI – diarylmethoxynitroimidazoles, RT– reverse transcriptase, EC_{50} – micromolar concentration of the compounds required to achieve 50 % protection of MT-4 cells from HIV-1 induced cytopathogenicity, NAIMS – *N*-aminoimidazoles, Y181C – Tyr 181, PDB ID – Protein Data Bank Identity, FAB – fast atom bombardment, MIC – minimum inhibitory concentration, RNA – ribonucleic acid, DNA – deoxyribonucleic acid, T/P (template/primer),

TTP – thymidine 5'-triphosphate, OPLS – Optimized Potentials for Liquid Simulations, NNIBP – non-nucleoside inhibitory binding pocket, RMSD – relative mean square distance, wt – wild type, XP – extra precision.

REFERENCES

1. R. C. Gallo, S. Z. Salahuddin, M. Popovic, G. M. Shearer, M. Kaplan, B. F. Haynes, T. J. Palker, R. Redfield, J. Oleske and B. Safai, Frequent detection and isolation of cytopathic retroviruses (HTLV-III) from patients with AIDS and at risk for AIDS, *Science* **224** (1984) 500–503; DOI: 10.1126/science.6200936.

2. F. S. Barre, J. C. Chermann, F. Rey, M. T. Nugeyre, S. Chamaret, J. Gruest, C. Dauguet, C. Axler--Blin, F. Vezinet-Brun, C. Rouzioux, W. Rozenbaum and L. Montagnier, Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS), *Science* **220** (1983) 868–871; DOI: 10.1126/science.6189183.

3. E. De Clercq, Toward improved anti-HIV chemotherapy: Therapeutic strategies for intervention with HIV infections, *J. Med. Chem.* **38** (1995) 2491–2517; DOI: 10.1021/jm00014a001.

4. E. De Clercq, New developments in anti-HIV chemotherapy, *Biochem. Biophys. Acta* **1587** (2002) 258–275; DOI: 10.1016/S0014-827X (01)01007-2.

5. D. Sriram, R. B. Tanushree and P. Yogeeswari, Design, synthesis and biological evaluation of novel non-nucleoside HIV-1 reverse transcriptase inhibitors with broad-spectrum chemotherapeutic properties, *Bioorg. Med. Chem.* **12** (2004) 5865–5873; DOI: 10.1016/j.bmc.2004.08.028.

6. J. J. Ren, C. Nicolas, L. E. Bird, T. Fujiwara, H. Sugimoto and D. Stuart, Binding of the second generation non–nucleoside inhibitor S-1153 to HIV-1 reverse transcriptase involves extensive main chain hydrogen bonding, *J. Biol. Chem.* **275** (2000) 14316–14320; DOI: 10.1074/jbc.275.19.14316.

7. R. Silvestri, M. Artico, S. Massa, T. Marceddu, F. De Montis and P. La Colla, 1-[2-(Diphenyl-methoxy) ethyl]-2-methyl-5-nitroimidazole: a potent lead for the design of novel NNRTIs, *Bioorg. Med. Chem. Lett.* **10** (2000) 253–256; DOI: 10.1016/S0960-894X (99)00664-2.

8. R. Silvestri, M. Artico, G. De Martino, R. Ragno, S. Massa, R. Lodda, C. Mugioni, A. Giulia Loi, P. La Colla and A. Pani, Synthesis, biological evaluation and binding mode of novel 1-[2-(diarylmethoxy)ethyl]-2-methyl-5-nitroimidazoles targeted at the HIV-1 reverse transcriptase, *J. Med. Chem.* **45** (2002) 1567–1576; DOI: 10.1021/jm010904a.

9. G. De Martino, G. Regina, A. D. Pasquali, A. Bergamini, C. Ciaprini, A. Sinistro, G. Maga, E. Crespan, M. Artico and R. Silvestri, Novel 1-[2-(diarylmethoxy) ethyl]-2-methyl-5-nitroimidazoles as HIV-1 non-nucleoside reverse transcriptase inhibitors. A structure-activity relationship investigation, *J. Med. Chem.* **48** (2005) 4378–4388; DOI: 10.1021/jm050273a.

10. I. M. Lagoja, C. Pannecouque, Z. Debyser, J. Balzarini, A. V. Aerschot, M. Witvtouw and P. Herdewijn, *N*-Aminoimidazole derivatives inhibiting retroviral replication via a yet unidentified mode of action, *J. Med. Chem.* **46** (2003) 1546–1553; DOI: 10.1021/jm0211117.

11. S. Ganguly, M. Sankaran, N. Prasanthi, O. Alptürk, B. Herman and N. S. Cremer, Synthesis and anti-HIV-1 activity of a novel series of aminoimidazole analogs, *Lett. Drug Des. Discov.* **7** (2010) 318–323; DOI: 10.2174/157018010791163424.

12. S. Ganguly, V. Gopalakrishnan and R. Chandra, Synthesis and antibacterial activity of some new 1-substituted 2-methyl-4-nitroimidazoles, *Int. J. Chem. Sci.* **7** (2009) 204–210.

13. M. Hoffer and E. Grunberg, Synthesis and antiprotozoal activity of 1-(3-chloro-2-hydroxypropyl)-substituted nitroimidazoles, *J. Med. Chem.* **17** (1974) 1019–1020; DOI: 10.1021/jm00255a026.

14. Z. Soyer, F. S. Kilic, K. Erol and V. Pabuccuoglu, Synthesis and anticonvulsant activity of some ω-(1H-imidazol-1-yl)-N-phenylacetamide and propionamide derivatives, *Farmaco* **59** (2003) 595–600; DOI: 10.1016/j.farmac.2003.07.011.

15. S. T. Selvi, V. Nadaraj, S. Mohan, R. Sasi and M. Hema, Solvent free microwave synthesis and evaluation of antimicrobial activity of pyrimido [4,5-*b*] and pyrazolo [3,4-*b*] quinolines, *Bioorg. Med. Chem.* **14** (2006) 3896–3903; DOI: 10.1016/j.bmc.2006.01.048.

16. D. V. Nissley, J. Radzio, Z. Ambrose, C. W. Sheen, N. Hamamouch, K. L. Moore, G. Tachedjian and N. S. Cremer, Characterization of novel non-nucleoside reverse transcriptase (RT) inhibitor resistance mutations at residues 132 and 135 in the 51 kDa subunit of HIV-1 RT, *Biochem. J.* **404** (2007) 151–157; DOI: 10.1042/BJ20061814.

17. A. L. Hopkins, J. Ren, R. M. Esnouf, B. E. Wilcox, E. Y. Jones, C. Ross, T. Miyasaka, R. T. Walker, H. Tanaka, D. K. Stammers and D. I. Stuart, Complexes of HIV-1 reverse transcriptase with inhibitors of the HEPT series reveal conformational changes relevant to the design of potent non-nucleoside inhibitors, *J. Med. Chem.* **39** (1996) 1589–1600; DOI: 10.1021/jm960056x.

18. W. L. Jorgensen, D. S. Maxwell and J. Tirado-Rives, Development and testing of the OPLS all-atom force field on conformational energetics and properties of organic liquids, *J. Am. Chem. Soc.* **118** (1996) 11225–11236; DOI: 10.1021/ja9621760.

S A Ž E T A K

Sinteza, antibakterijsko i potencijalno anti-HIV djelovanje nekoliko novih analoga imidazola

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Sintetizirana je serija 1-(2-metil-4-nitro-imidazol-1-il)-3-arilamino-propan-2-ona (**2a-e**), 2-metil-5-nitro-1-{2-[arilmetoksi]etil}-1*H*-imidazola (**5a-d**) i *N*-(3-hidroksifenil)-2-(supstituiranih imidazol-1-il)alkanamida (**8a-e**) s ciljem dobivanja novih derivata imidazola sa širokim kemoterapijskim svojstvima. Navedeni spojevi ispitani su na anti-HIV i antibakterijsko djelovanje.

Ključne riječi: imidazol, reverzna transkriptaza, nenukleozidna reverzna transkriptaza, anti-HIV, antibakterijski

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