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ORIGINAL ARTICLE

Design, antihuman immunodeficiency activity and molecular docking studies of synthesized 2-aryl and 2-pyrimidinyl pyrrolidines

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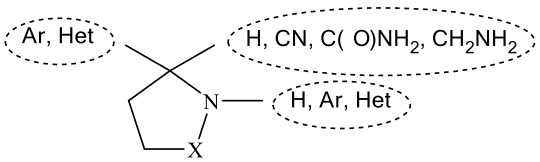
Abstract

A series of thirty-one new compounds were synthesized and evaluated for their anti-HIV-1 and cytotoxicity activity. Of these, twelve were found to be inhibitors of HIV replications in primary human lymphocytes with median effective concentration (EC_{50}) values < 20 μM. However, most of the compounds demonstrated cytotoxicity in different cells. Our structure activity relationship study identified different patterns. In the series of 2-aryl pyrrolidines, comparing the activity of the compounds containing 2-aryl substituents we observed that compounds **1c**, **1f–j**, **2f,g** with benzyloxyphenyl and isopropoxy groups were more potent. Compounds **1g–j**, **2f,g**, in which the 1-aryl moiety contained a methyl group in 3,5- or 4-positions also showed high activity. In the series of compounds containing the amide, aminomethyl and nitrile groups we observed an increase in activity with $C(O)NH_2 < CH_2NH_2 < CN$. In the series of 2-pyrimidinyl pyrrolidines, the best results were demonstrated with derivatives **5e** and **5f**, in which the presence of a benzyl fragment in 1st and aniline fragment in 6th positions of pyrimidine ring we observed an increase in anti-HIV activity. Molecular docking studies of synthesized compounds with HIV-1 reverse transcriptase enzyme were performed. Binding energies of ligands were estimated, and the interacting amino acids of HIV-1 reverse transcriptase protein were shown. Based on corroborative results of the molecular docking studies and in vitro experiments, we suggest that three groups of synthesized ligands (**1c**, **1f–i**), (**2f,g**), (**5e,f**, **7**) are of high interest for further research on new drugs against HIV.

Graphic abstract

General structure of synthesized 2-aryl and 2-pyrimidinyl pyrrolidines.



Keywords 2-Aryl pyrrolidines · Pyrimidinyl pyrrolidines · Docking studies · Binding energy · Anti-HIV-1 activity

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Introduction

Human immunodeficiency virus type 1 (HIV-1) is the etiological agent of acquired immunodeficiency syndrome (AIDS). HIV undergoes rapid genetic variation caused primarily by the enormous number of viruses produced daily in an infected individual and genetic variability due to the lack of DNA proofreading ability. The variation within individuals combined with the historic lack of human migration has led to the development of diverse HIV-1 subtypes. When combined with the selection of resistant viruses under drug treatment, these factors complicate the development of effective drugs.

More than half of the currently approved anti-AIDS drugs target the reverse transcriptase (RT) enzyme. The RT associated with HIV is actually the target for three FDA approved classes of inhibitors: nucleoside RT inhibitors (NRTIs), nucleotide RT inhibitors (NtRTIs) and non-nucleoside RT inhibitors (NNRTIs). The NRTIs and NtRTIs interact with the catalytic site (that is the substrate-binding site) of the enzyme, whereas the NNRTIs interact with an allosteric (that is non-catalytic) site located at a short distance from the catalytic site.

NNRTIs gained the greatest importance because of their specificity and low cytotoxicity. All non-nucleoside inhibitors bind to a hydrophobic pocket near the polymerase active site. NNRTIs were found to have more potential as a class of anti-HIV agents versus the NRTIs and nucleotide reverse transcriptase inhibitors (NtRTIs) because they differ structurally from the nucleoside analogues. NNRTIs usually do not interfere with the human cell cycle and are specific inhibitors of RT enzyme of HIV-1.

NNRTIs bind to HIV-1 RT in a hydrophobic pocket (NNIBP) that contains side chains of aromatic amino acid residues Tyr181, Tyr188, Phe227, Trp229 and Tyr318 and of hydrophobic amino acid residues Pro95, Leu100, Val106, Val108, Val179, Leu234 and Pro236 from the p66 subunit. Glu138 is the only amino acid residue of the p51 subunit that interacts with NNRTIs; Glu138 does not directly interact with all NNRTIs [1].

The era of NNRTIs began with the discovery of HEPT and TIBO as specific inhibitors of RT. With the discovery of α-anilinophenylacetamides (α-APAs), the era of flexible derivatives started and the most active compound in this series found is Loviride, the simplicity of its structure and the relative ease of its synthesis made the α-APA series attractive for lead optimization (Fig. 1) [2, 3].

Consequently, studies on the synthesis and biological activity of new NNRTIs are worth pursuing. Herein, we report our molecular docking and biological studies of new synthesized derivatives of 2-aryl and 2-pyrimidinyl pyrrolidines, which contain fragments of known reverse transcriptase inhibitors Loviride and HEPT. The molecular docking studies were performed in order to estimate binding energies of synthesized compounds and interacting amino acids of HIV-1 reverse transcriptase enzyme, and the obtained data were compared with the biological results of anti-HIV in vitro studies.

The purpose of our studies was to efficiently predict the potent anti-HIV activity via modeling, construction of new compounds and determination of the fragments responsible for the observed activity.

Experimental work

Anti-HIV-1 assay

Human peripheral blood mononuclear (PBM) cells were stimulated with phytohemagglutinin A for 2–3 days prior to use. HIV-1/LAI obtained from the Centers for Disease

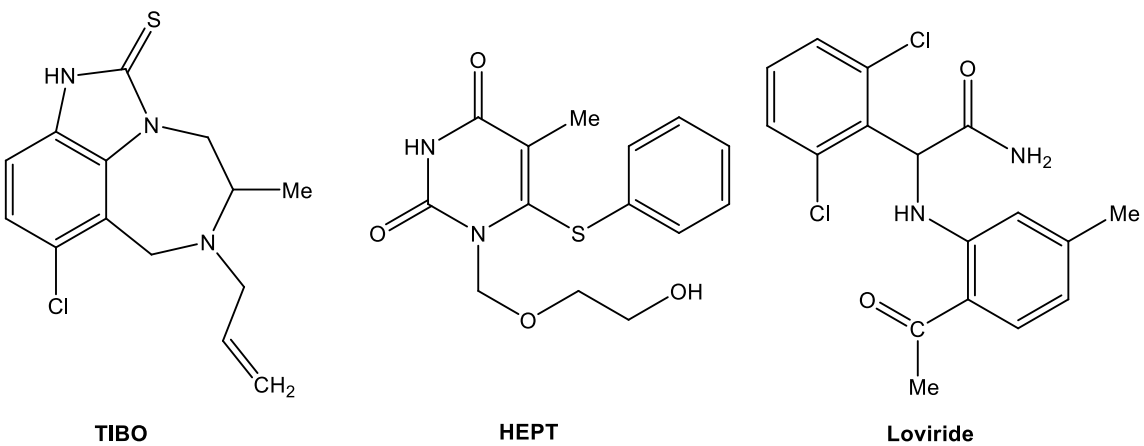


Fig. 1 Non-nucleoside reverse transcriptase inhibitors

Control and Prevention (Atlanta, GA) was used as the standard reference virus for the antiviral assays. The antiviral median effective concentration (EC_{50}) and 90% effective concentration (EC_{90}) were determined from the concentration–response curve using the median effect method [4].

Cytotoxicity assay

Compounds were evaluated for their potential toxic effects on uninfected PHA-stimulated human PBM cells, in CEM (T-lymphoblastoid cell line obtained from American Type Culture Collection, Rockville, MD) and Vero (African green monkey kidney) cells. The 50% inhibition concentration (IC_{50}) was determined from the concentration–response curve using the median effect method [5].

Molecular docking and ADME-Tox studies

Three-dimensional crystal structures with resolution < 2 Å of HIV-1 reverse transcriptase (RT) in complex with NNRTI compounds were downloaded from Protein Data Bank [6]. Therefore, four structures were chosen: 2ZD1, 6C0J, 4G1Q and 4KO0 [7–10]. The preparation of structures (addition of hydrogens, removal of water and co-crystallized compounds) and ligand files for docking were done with AutoDockTools4 and Avogadro [11, 12]. The docking of twenty-eight ligands with four structures of HIV-1 RT was performed with AutoDock Vina molecular docking software [13]. Grid boxes were generated using the co-crystallized ligands. Diagrams of ligand–protein interactions of docking results were drawn with LigPlot + software [14]. Figures were obtained using PyMOL 2.3 (The PyMOL Molecular Graphics System, version 2.0 Schrödinger, LLC).

ADME-Tox evaluation was carried out using admetSar [15] and SwissADME [16] web tools.

Results and discussion

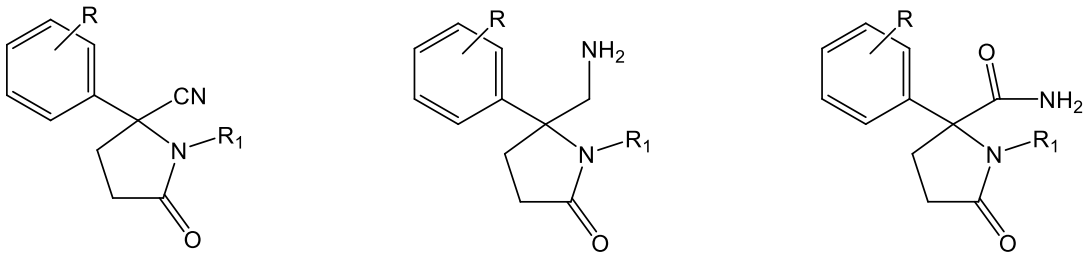
Chemistry

2-Aryl pyrrolidine derivatives

In the course of our studies directed to search for new NNRTI inhibitors, we have synthesized 2-arylpyrrolidines with different groups in various positions of the heterocyclic ring.

By acylation, corresponding 2-aryl-2-(arylamino)acetonitriles with 3-chloropropanoyl chloride and further intramolecular cyclization under phase transfer catalytic conditions were prepared 1,2-diaryl substituted pyrrolidine carbonitriles **1a**–**j** (Fig. 2) [17–21].

Different substituted pyrrolidine carbonitriles were reduced to corresponding aminomethyl pyrrolidines **2a**–**g** according to the method developed by us, using $NaBH_4$/PEG-400/$CoCl_2$ system [19, 21]. The pyrrolidine carboxamides **3a** and **3b** were synthesized from corresponding pyrrolidine carbonitriles via reaction with conc. H_2SO_4 (Fig. 2) [20].



1a-j **2a-g** **3a,b**

1a: R = 2,6-Cl_2, R_1 = 4-MeC_6H_4
1b: R = 2,6-Cl_2, R_1 = 3,5-$Me_2C_6H_3$
1c: R = 4-*i*PrO, R_1 = Ph
1d: R = 4-*i*PrO, R_1 = 4-MeC_6H_4
1e: R = 4-*i*PrO, R_1 = 2-naphthyl
1f: R = 4-$PhCH_2O$, R_1 = $PhCH_2$
1g: R = 4-$PhCH_2O$, R_1 = 4-MeC_6H_4
1h: R = 4-(2,4-$Cl_2C_6H_3CH_2O$), R_1 = 4-MeC_6H_4
1i: R = 4-(2,6-$Cl_2C_6H_3CH_2O$), R_1 = 4-MeC_6H_4
1j: R = 2-$PhCH_2O$, R_1 = 4-MeC_6H_4

2a: R = H, R_1 = 4-MeC_6H_4
2b: R = H, R_1 = $PhCH_2$
2c: R = 2,6-Cl_2, R_1 = 3,5-$Me_2C_6H_3$
2d: R = 4-*i*PrO, R_1 = Ph
2e: R = 4-$PhCH_2O$, R_1 = $PhCH_2$
2f: R = 4-$PhCH_2O$, R_1 = 4-MeC_6H_4
2g: R = 2-$PhCH_2O$, R_1 = 4-MeC_6H_4
3a: R = 4-Br, R_1 = 4-MeC_6H_4
3b: R = 2,6-Cl_2, R_1 = 3,5-$Me_2C_6H_3$

Fig. 2 Aryl pyrrolidine derivatives

2-Pyrimidinyl pyrrolidine derivatives

The next series of synthesized pyrrolidine derivatives refers to pyrimidinyl pyrrolidines. By condensation of 6-amino pyrimidinediones **4a–c** with pyroglutamic acid methyl ester or 2-pyrrolidone, in the presence of PCl_3 under Vilsmeier reaction conditions, 6-imino-5-tetrahydro-1*H*-2-pyrrolylidenehexahydro-2,4-pyrimidinediones **5a–g** were synthesized. For the reduction of 6-imino group of **5d**, we used the reducing system $NaBH_4$/1,4-dioxane/$CoCl_2$/PEG-400. The ester fragment was reduced to form hydroxymethylderivative **6**. Compound **7** was synthesized by acylation of **5c** with benzoyl chloride (Fig. 3) [22, 23].

Biology

Anti-HIV activity

The synthesized compounds appeared to have good anti-HIV activity, and among thirty-one compounds in this series, twelve exhibited micromolar activity with EC_{50} values < 20 µM. Compounds **1h,i** and **5f** proved to be the most potent compounds with EC_{50} value of 5.3, 2.0 and 0.48 µM, respectively; however, they were cytotoxic to all three cell lines and had narrow therapeutic windows. Only compound **1c** was non-toxic to PBM, CEM and Vero cells while displaying a reasonable EC_{50} value of 5.2 µM (Table 1) [21].

Cytotoxicity studies

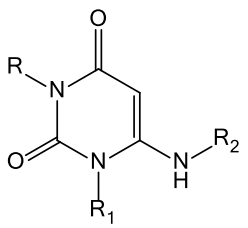
Compounds were evaluated for their cytotoxicity in human PBM, CEM and Vero cells to determine their spectrum of toxicity. CEM cells are a line of lymphoblastic cells originally derived from a child with acute lymphoblastic leukemia, whereas Vero cells are derived from African green monkey kidney cells.

Compounds **2b,c**, **3a,b**, **4a**, **4c**, **5a,b**, **5d** and **6** were non-toxic to all the cell systems used; however, they were also inactive or largely inactive versus HIV-1 (Table 1).

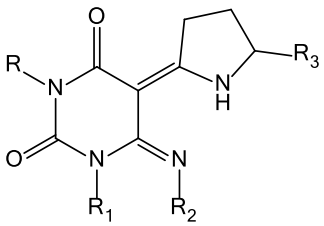
Molecular docking studies

Results of performed molecular docking experiments in the form of binding energy and interacting amino acids in a hydrophobic pocket of reverse transcriptase enzyme HIV-1 for each ligands of synthesized compounds and the biological results of anti-HIV studies are presented in Table 2.

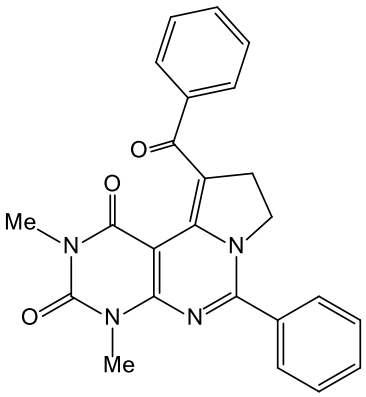
From the data obtained, it follows that for most derivatives of 2-aryl and 2-pyrimidinyl pyrrolidines, a direct correlation is observed between low binding energies and biological results of anti-HIV in vitro studies. As expected, the predicted and actually determined biological activity is directly related to the greatest decrease in binding energy. However, for some of the compounds studied, we did not observe a correlation between the decrease in energy and anti-HIV activity.



4a-c



5a-g, 6



7

4a: R = H, R_1 = $PhCH_2$, R_2 = Ph
4b: R = H, R_1 = $PhCH_2$, R_2 = 3,5-$Me_2C_6H_3$
4c: R = H, R_1 = Ph(CH_2)$_3$, R_2 = 3,5-$Me_2C_6H_3$
5a: R = R_1 = R_2 = R_3 = H
5b: R = R_1 = R_2 = H, R_3 = COOMe
5c: R = R_1 = Me, R_2 = R_3 = H
5d: R = R_1 = Me, R_2 = H, R_3 = COOMe
5e: R = H, R_1 = $PhCH_2$, R_2 = Ph, R_3 = H
5f: R = H, R_1 = $PhCH_2$, R_2 = 3,5-$Me_2C_6H_3$, R_3 = H
5g: R = H, R_1 = Ph(CH_2)$_3$, R_2 = 3,5-$Me_2C_6H_3$, R_3 = H
6: R = R_1 = Me, R_2 = H, R_3 = CH_2OH

Fig. 3 Pyrimidinyl pyrrolidine derivatives

Table 1 Anti-HIV-1 and cytotoxic activities of 2-aryl and 2-pyrimidinyl pyrrolidines

Compound	Anti-HIV-1 activity in human PBM cells (μM)[a]		Cytotoxicity (CC_{50}, μM)[a]		
	EC_{50}	EC_{90}	PBM	CEM	Vero
1a	24	> 100	≥ 100	48	> 100
1b	72	> 100	59	20	> 100
1c	5.2	30	96	> 100	> 100
1d	37	> 100	> 100	17	> 100
1e	22	71	74	19	> 100
1f	9.8	31	5.9	7.0	3.0
1g	17	> 100	> 100	30	54
1h	5.3	24	9.7	6.6	22
1i	2.0	52	14	17	53
1j	17	> 100	23	16	78
2a	33	> 100	54	54	> 100
2b	> 100	> 100	> 100	> 100	> 100
2c	> 100	> 100	> 100	≥ 100	> 100
2d	> 100	> 100	48	33	> 100
2e	33	> 100	16	24	13
2f	11	42	16	34	12
2g	12	> 100	45	48	> 100
3a	> 100	> 100	> 100	> 100	> 100
3b	> 100	> 100	> 100	> 100	> 100
4a	74	> 100	> 100	> 100	76
4b	4.7	14	> 100	50	33
4c	60	> 100	> 100	> 100	> 100
5a	> 100	> 100	> 100	> 100	> 100
5b	> 100	> 100	> 100	> 100	> 100
5c	57	> 100	49	> 100	> 100
5d	32	> 100	> 100	> 100	> 100
5e	8.9	46	16	28	35
5f	0.48	2.8	20	19	8.0
5g	45	> 100	65	> 100	7.9
6	> 100	> 100	> 100	> 100	> 100
7	4.5	> 100	11	> 100	> 100
AZT	0.0018	0.015	> 100	14	51

[a]All assays were performed in triplicate. Only the mean is shown

Table 2 Docking scores (ΔG°, kcal/mol) and anti-HIV-1 median effective concentration EC_{50} (μM) of synthesized 2-aryl and 2-pyrimidinyl pyrrolidines **1a–j**, **2a–g**, **3a–b**, **5a–g**, **6**, **7**

Ligands	PDB entry code[a]	Binding energy ΔG° (kcal/mol)	Anti-HIV-1 effective concentration EC_{50} (μM)
1a	6C0J	− 11.5	25
1b	4G1Q	− 11.1	72
1c	6COJ	− 9.70	5.2
1d	6COJ	− 9.80	37
1e	2ZD1	− 11.2	22
1f	2ZD1	− 11.0	9.8
1g	6C0J	− 11.5	17
1h	6C0J	− 11.8	5.3
1i	6C0J	− 11.0	2.0
1j	2ZD1	− 10.7	17
2a	4kO0	− 10.9	33
2b	4kO0	− 9.90	> 100
2c	6COJ	− 10.4	> 100
2d	6COJ	− 9.50	> 100
2e	6COJ	− 11.0	33
2f	6COJ	− 10.4	11
2g	2ZD1	− 10.5	12
3a	6COJ	− 10.3	> 100
3b	2ZD1	− 9.60	> 100
4a	2ZD1	− 10.7	74
4b	2ZD1	− 11.5	4.7
4c	4G1Q	− 12.3	60
5a	4KO0	− 7.30	> 100
5b	4KO0	− 8.80	> 100
5c	4KO0	− 7.30	57
5d	2ZD1	− 8.00	32
5e	4G1Q	− 10.8	8.9
5f	2ZD1	− 9.90	0.48
5g	6C0J	− 11.8	45
6	4G1Q	− 8.10	> 100
7	6COJ	− 10.7	4.5

[a]PDB entry codes of the structures that showed highest binding energy for compound in docking studies

Pyrrolidine carbonitriles **1a–j** interact with HIV-1 reverse transcriptase enzyme with high binding energies at (− 11.8 to − 9.70 kcal/mol). From this group, compounds **1c** (Fig. 4a), **1f–h** (Fig. 4b), **1i** (Fig. 4c) showed low values of EC_{50} (Table 2) and compound **1h** showed the highest docking binding energy among all studied ligands (− 11.8 kcal/mol). Binding of this group of compounds to the NNRTI-binding site is driven by hydrophobic interactions with Leu234, Tyr181, Val106, Trp229, Tyr188, Val179, Lys103 and Leu100. Only compound **1h** in the group showed the possibility of the formation of two weak electrostatic hydrogen bonds with Lys103 (distance of the bond 3.27 Å) and Tyr188 (distance of the bond 3.20 Å).

Aminomethyl pyrrolidines **2a–g** had good binding energies (from − 9.50 to − 11.0 kcal/mol) for HIV-1 reverse transcriptase enzyme. Compounds **2f** and **2g** (Fig. 4d) showed high binding energies to NNRTI-binding site of HIV-1 RT protein and low values of EC_{50}. These two compounds have similar binding energies − 10.4 kcal/mol (compound **2f**) and − 10.5 kcal/mol (compound **2g**). Studied compounds **2a–g** interact with Glu138, Tyr181, Tyr188, Trp229, Leu234, Leu100, Lys103, Val106 and Val179.

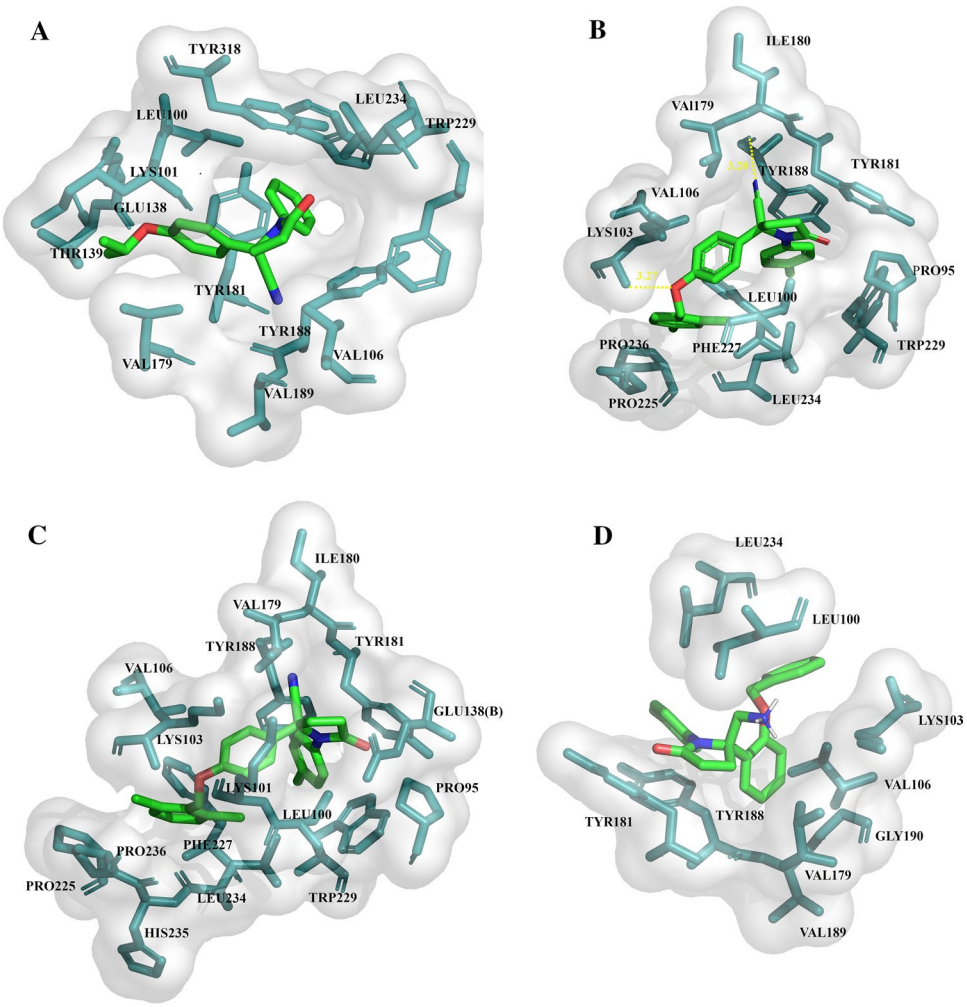


Fig. 4 Interaction of pyrrolidine carbonitriles **1a**–**j** and aminomethyl pyrrolidines **2a**–**g** with NNRTI-binding pocket of HIV-1 RT enzyme. For each compound, only interacting amino acids are presented. **a** 3D structure of the compound **1c**; **b** 3D structure of the compound **1h** (dotted line colored in yellow represents possible hydrogen bonding with Tyr188 and Lys103); **c** 3D structure of the compound **1i**; **d** 3D structure of the compound **2g**

Pyrrolidine carboxamides **3a,b** showed good binding energies; they are non-toxic to all the cell systems used, but inactive against HIV-1.

Within pyrimidinyl pyrrolidines **5a**–**g**, **6**, **7** with binding energies at − 11.8 to − 7.30 kcal/mol with HIV-1 reverse transcriptase, compounds **5e,f** and **7** showed good binding energies and low values of EC_{50} (Table 2).

Compound **5f** (Fig. 5a) binds with the NNRTI-binding site of HIV-1 RT protein with the binding energy of -9.90 kcal/mol and showed lowest EC_{50} values of 0.48 µM. Compound

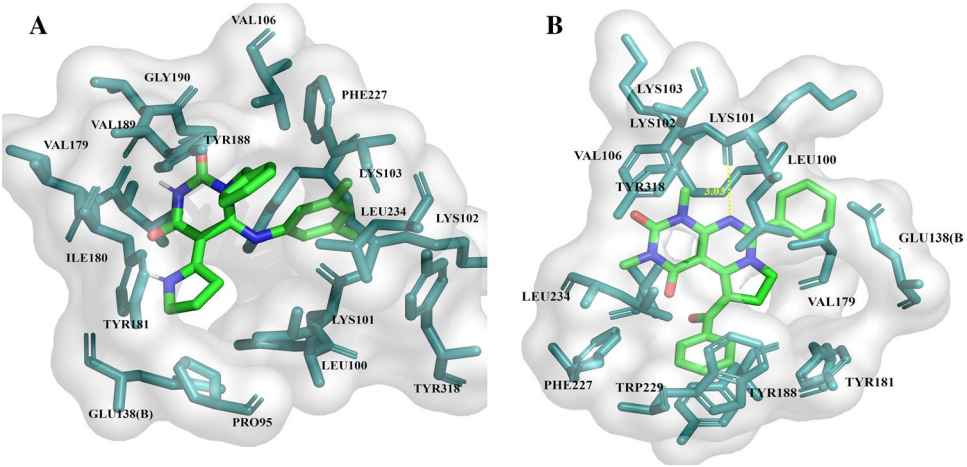


Fig. 5 Interaction of pyrimidinyl pyrrolidines **5f** and **7** with NNRTI-binding pocket of HIV-1 RT enzyme. **a** 3D structure of the compound **5f**; **b** 3D structure of the compound **7** (dotted line colored in yellow represents possible hydrogen bonding with Lys101)

7 (Fig. 5b) forms one weak hydrogen bond with Lys103 with the bond distance 3.03 Å. Common interacting amino acids of this group are Tyr188, Tyr181, Glu138, Val179, Lys103, Leu100, Tyr318, Leu234, Phe227 and Val106.

Based on the results of molecular docking, the following interacting amino acids: Leu234, Tyr181, Val106, Tyr188, Val179, Lys103 and Leu100 are common for all analyzed compounds and therefore considered important for interaction between NNRTIs and HIV-1 RT protein. Interaction of the all studied compounds with HIV-1 RT protein is mostly driven by hydrophobic forces.

In silico analysis of the compounds of three analyzed groups of synthesized ligands (**1c**, **1f–i**), (**2f,g**), (**5e,f**, **7**) showed their high affinity to HIV-1 RT and low values of EC_{50} protein and therefore are of high interest for further research on synthesis of new anti-HIV drugs. ADMET pharmacokinetic properties of mentioned compounds, were evaluated using admetSAR web tool and presented in table of supplementary materials. Based on SwissADME druglikeness evaluation, all mentioned compounds, except **1h** and **1i**, meet required properties, including Lipinski, Ghose, Veber, Egan and Muegge requirements. Compounds **1h** and **1i** have WLOGP > 5.6 and XLOGP3 > 5, which violates Ghose and Muegge requirements, respectively.

SAR studies

In the study of the relationship between chemical structure and biological activity, there were some patterns. In the series of 2-arylpyrrolidines, comparing the activity of the compounds containing 2-aryl substituents it can be seen that compounds **1c**, **1f–j**, **2f,g** with benzyloxyphenyl and isopropoxy groups are more active. Compounds **1g–j**, **2f,g** in which the 1-aryl moiety contained methyl group in 3,5- or 4-positions also showed high activity. In the series of compounds containing amide, aminomethyl and nitrile groups observed an increase in activity with $C(O)NH_2 < CH_2NH_2 < CN$ (Table 1) [21].

In the series of 2-pyrimidinyl pyrrolidines, the best results were demonstrated derivatives **5e,f**, in which the presence of a benzyl fragment in 1st, anilino fragment in 6th and pyrrolidine fragment in 5th positions of pyrimidine ring increases anti-HIV activity (Table 1).

Further structural modifications could be performed on the basis of the data obtained for these compounds to get worthwhile molecules with greater potential as anti-HIV-1 agents.

Conclusions

In summary, we herein report the anti-HIV-1 activity of new 2-aryl and 2-pyrimidinyl pyrrolidine derivatives. Twelve compounds were active against HIV-1 with EC_{50} values less than 20 μM, which indicates the potential of these compounds as anti-HIV-1 agents. Among these thirty-one compounds, **1h,i** and **5f** were the most potent anti-HIV-1 agents with EC_{50} values < 5 μM. They are weakly active compared to the AZT control, and the activity may be due to the toxicity observed in primary human lymphocytes. On these grounds, the structural modification of these compounds is expected to result in more potent candidates of anti-HIV drugs.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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