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Toward novel HIV-1 integrase binding inhibitors: Molecular modeling, synthesis, and biological studies

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Abstract—The identification of a novel hit compound as integrase binding inhibitor has been accomplished by means of virtual screening techniques. A small family of structurally related molecules has been synthesized and biologically evaluated with one of the compounds showing an $IC_{50} = 12\ \mu M$.

Currently, there are four FDA-approved classes of drugs to combat HIV infection: nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors, and one fusion inhibitor.[1,2] Application of these agents in combating HIV has led to remarkable success in inhibition of HIV-1 replication, reduction of viral load, and decline in morbidity and mortality. However, their adverse effects together with the emergence of resistant HIV mutants[3,4] have highlighted the need to develop novel antiviral agents with a different mechanism of action. Accordingly, significant effort is presently devoted to the development of inhibitors of integrase, the third viral enzyme,[5–7] which is responsible for the integration of the viral DNA into the chromosomes of the host cell, a process that occurs in two temporally and spatially separated reactions known as 3′-processing and strand transfer.

Although a large number of compounds have been reported to inhibit HIV-1 IN in biochemical assays,[8–11] no drug active against this enzyme has been approved by the FDA so far. The β-diketo acids (DKAs), acting as specific inhibitors of the strand-transfer step (INSTIs), provided the first proof of principle for HIV-1 IN inhibitors as antiviral agents[8] followed by a series of metabolically stable compounds characterized by the incorporation of the diketo acid moiety into more complex heterocyclic frames. Among them, S-1360 and

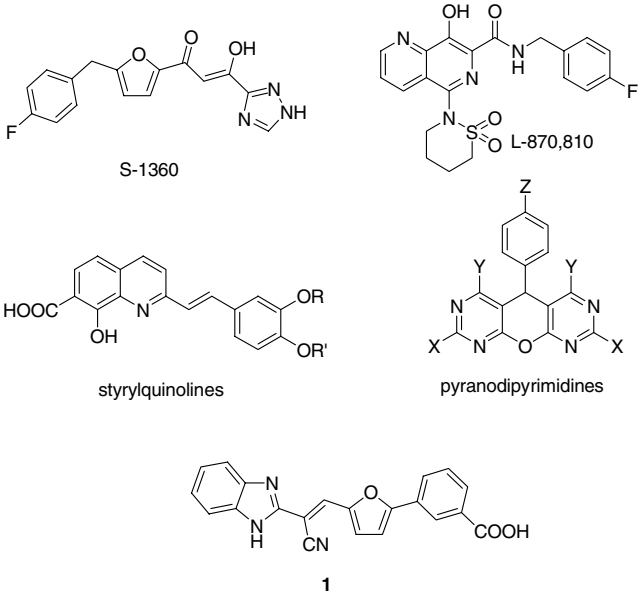

Chart 1. Known integrase inhibitors and our hit compound **1**.

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L-870,810 (Chart 1) represent the first generation of IN inhibitors that have entered clinical studies.[12–14]

Differently to DKAs and naphthyridine analogs, styrylquinolines (SQs)[15,16] and pyranodipirimidines (PDP)[17,18] (Chart 1) are two classes of compounds characterized by the ability to inhibit the 3′-processing reaction in the low micromolar range and they are referred to as integrase binding inhibitors (INBIs).

Within our ongoing efforts in the study of new antiviral agents,[19–22] and with the aim of identifying a novel class of IN inhibitors acting at the level of the IN–DNA complex formation, we set up a virtual screening protocol based on the application of different sequential filters which took into account both the structural information coming from known inhibitors of the 3′-processing step and 3D structural data of the enzyme.[23]

A database of over 200,000 compounds (Asinex gold collection) was initially screened using the electron–ion interaction potential (EIIP) technique[24] leading to the selection of approximately 96,000 compounds. Next, Lipinski's rule-of-five was applied on all retrieved compounds and the molecules that violated this rule were eliminated. Finally, in consideration that most of the known IN inhibitors are characterized by a rigid structure, only compounds having a number of rotatable bond <10 were selected giving rise to a cluster of 40,000 compounds.

Subsequently, a three-dimensional ligand-based pharmacophoric model was generated starting from 30 molecules known to inhibit the 3′-processing step with IC_{50} values <1 μM. It should be noted that, while several HIV-1 IN pharmacophores have already been reported,[25–28] based on the structure of strand-transfer selective inhibitors, no pharmacophoric model based on 3′-processing inhibitors has been described so far.

The pharmacophoric model was used as search query on previously selected compounds and the molecules that fitted all the features of the hypothesis (ca. 15,000) were retrieved. Finally, the binding mode of all retrieved compounds was investigated by computational docking using the 3D structure of the enzyme. The docking calculations were performed into the IN core domain, in the region of interaction with the DNA, which has been widely explored by mutational studies[29–31] and photocross-linking experiments.[32] On the basis of the docking scores, 12 compounds were selected and their antiviral activity was evaluated in in vitro assays. Among them, compound **1**, characterized by a completely new scaffold (Chart 1), showed an interesting anti-IN activity (IC_{50} = 164 μM) and was therefore chosen as a hit compound for further development.

On this basis, we decided to investigate a number of compounds structurally related to **1** with the aim of identifying molecules endowed with a better pharmacological profile (validation of the molecular scaffold) and of exploring their structure–activity relationships. Seven additional compounds (**2**–**8**) were chosen on the basis of a 2D substructure search and were purchased from the ASINEX database,[23] while 22 novel compounds (**9**–**30**) were easily prepared starting from commercially available building blocks.

The synthesis of the new compounds was guided by the following considerations: (i) exploration of different substitution patterns on the phenyl ring with the aim of studying their effect on the activity of the molecule, (ii) substitution of the cyano group with a hydrogen, a methyl or an amide group in order to understand its influence on the molecule toxicity, and (iii) bioisosteric replacement of the benzimidazole moiety with benzoxazole and benzothiazole to test the importance of the H-bond donor NH group of the benzimidazole on the activity of the molecule.

The new compounds could be easily accessed in a two-step sequence consisting of an initial coupling reaction followed by a condensation, starting from the appropriate furan aldehyde derivative (Fig. 1).

In particular, 5-bromo-2-furaldehyde (Scheme 1) was reacted with the appropriate arylboronic acid in the presence of $Pd(OAc)_2/PPh_3$ as the catalyst and Na_2CO_3 as the base in a $PrOH/H_2O$ mixture.[33]

The reaction rate was highly accelerated by the use of microwaves in sealed tube giving the desired compounds in 10–15 min. Alternatively, 2-furaldehyde was coupled, according to the Meerwin arylation procedure,[34,35] with the aryldiazonium tetrafluoborate salt of the appropriate aniline, in the presence of copper (II) chloride as the catalyst to give, after 48 h at room temperature, the target aldehyde as a solid after filtration of the reaction mixture.

The condensation step was performed according to two different methodologies on the basis of the final compound to be obtained.

Thus, a Knoevenagel condensation between 2-cyanomethylbenzimidazole (or cyanomethylbenzothiazole/2-cyanomethylbenzoxazole) and the appropriate 5-aryl-2-furaldehyde in the presence of piperidine (or Et_3N) afforded the CN substituted derivatives as colored solids after simple filtration of the reaction mixture.[36] A Wittig reaction was instead used to prepare the unsubstituted derivatives starting from the functionalized aldehyde and the triphenylphosphonium salt of chloromethyl-

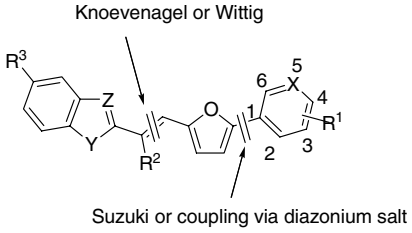
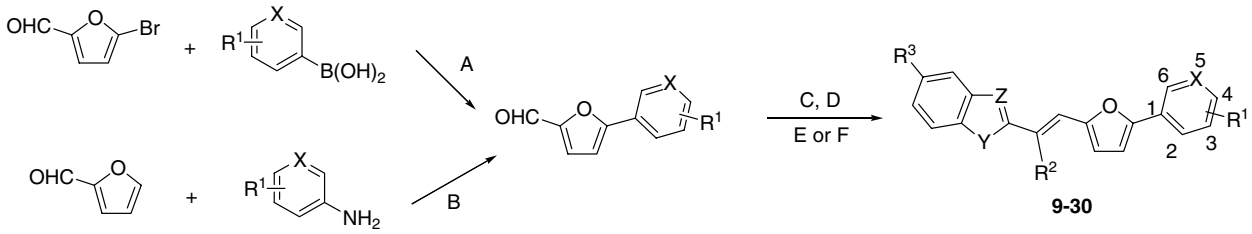


Figure 1. Retrosynthetic analysis.



Scheme 1. Reagents and conditions: (A) $Pd(OAc)_2$, PPh_3, Na_2CO_3, PrOH, H_2O, MW, 100 °C, 10 min, sealed tube; (B) i—$NaNO_2$, HBF_4, H_2O; ii—$CuCl_2$, acetone; (C) 2-cyanomethylbenzimidazole, piperidine, EtOH; (D) i—2-chloromethylbenzimidazole, PPh_3, CH_3CN; ii—NaOMe, MeOH; (E) 2-cyanomethylbenzothiazole or 2-cyanomethylbenzoxazole, Et_3N, EtOH; (F) 2-ethylbenzimidazole, AC_2O, reflux.

benzimidazole in the presence of a slight excess of NaOMe.[37] In this case *E*/*Z* mixtures were obtained consisting almost exclusively in the *E* isomer: recrystallization afforded the *E* isomer pure enough for the biological evaluation.

Compounds **16**, **20**–**22**, and **24**–**30** were prepared in a single synthetic step by condensing a commercially available functionalized aldehyde with the appropriate activated methylene compound.

Compound **23** was obtained starting from the corresponding nitrile derivative **20** by treatment with concentrated H_2SO_4 at 60 °C.

The results obtained from the biological evaluation of compounds **1**–**30** are summarized in Table 1.

Starting from our hit compound **1**, the introduction of a chlorine or a hydroxyl group on the 4 position of the phenyl ring gave compounds **4** and **8** approximately two fold more active. Moving the COOH moiety from the meta to the para position led to a completely inactive compound **6**; the activity was restored when an OH group was introduced in the meta position (**3**), thus underlining the importance, in this position, of a group able to interact with the enzyme through hydrogen bond formation.

Good results were also obtained when different electron-withdrawing groups such as Cl, CF_3, and NO_2 groups were introduced on the phenyl ring (**2**, **22**).

The substitution of the CN group with a H on the ethylene bridge proved to be irrelevant for both the activity and the cytotoxicity. In particular, in the series of

Table 1. Integrase inhibitory activity of hit compound **1** and its analogs

Compound	X	Y	Z	R^1	R^2	R^3	Method	Overall IC_{50} ± SD (μM)[a]
1	C	NH	N	3-COOH	CN	H	—	163.92 ± 94.22
2	C	NH	N	2-Cl, 5-NO_2	CN	H	—	58.68 ± 2.02
3	C	NH	N	3-OH, 4-COOH	CN	H	—	70.28 ± 16.37
4	C	NH	N	3-COOH, 4-Cl	CN	H	—	90.36 ± 4.26
5	C	NH	N	2-COOH, 4-Cl	CN	CH_3	—	36.16 ± 31.94
6	C	NH	N	4-COOH	CN	H	—	>281.40
7	C	NH	N	2-CH_3, 5-COOH	CN	H	—	39.80 ± 24.01
8	C	NH	N	3-COOH, 4-OH	CN	H	—	68.69 ± 11.34
9	C	NH	N	4-Cl	CN	H	B + C	>289
10	C	NH	N	3-CH_3, 4-OCH_3, 5-CH_3	CN	H	A + C	>271
11	C	NH	N	4-NHBoc	CN	H	A + C	>235
12	C	NH	N	2-CH_3, 3-NO_2	CN	H	B + C	85.84 ± 24.51
13	C	NH	N	2-NO_2	CN	H	B + C	>281
14	N	NH	N	4-OCH_3	CN	H	A + C	>292
15	C	NH	N	2-COOH, 4-F	CN	H	B + C	>268
16	C	NH	N	3-NO_2	H	H	D	12.02 ± 0.97
17	C	NH	N	2-NO_2	H	H	B + D	>302
18	C	NH	N	3-CH_3, 4-OCH_3, 5-CH_3	H	H	A + D	>291
19	C	NH	N	2-CH_3, 3-NO_2	H	H	B + D	>290
20	C	NH	N	2-NO_2, 4-Cl	H	H	D	>273
21	C	NH	N	3-NO_2	CN	H	C	>281
22	C	NH	N	2-Cl, 5-CF_3	CN	H	C	31.32 ± 22.47
23	C	NH	N	2-NO_2, 4-Cl	$CONH_2$	H	—	>244
24	C	NH	N	2-NO_2, 4-Cl	CH_3	H	F	>263
25	C	NH	N	2-CF_3, 6-Cl	H	H	D	>257
26	C	S	N	2-CF_3, 6-Cl	CN	H	E	>232
27	C	S	N	2-NO_2, 4-Cl	CN	H	E	>245
28	C	S	N	3-NO_2	CN	H	E	>268
29	C	O	N	2-CF_3, 6-Cl	CN	H	E	>241
30	C	O	N	3-NO_2	CN	H	E	>280

[a] Values are means of three experiments. Integrase inhibition was determined in overall integration assay, assaying both 3′ processing and strand-transfer activities in the presence of Mg^{2+}[23].

unsubstituted derivatives, the only compound endowed with interesting anti-IN activity (and also the best compound among those tested) was **16** (IC_{50} = 12 μM) characterized by the presence of a nitro group in the meta position of the phenyl ring. Going from benzimidazole (**1**–**25**) to benzothiazole (**26**–**28**) and benzoxazole (**29**–**30**) the inhibitory activity is completely lost thus underlying the importance of the NH of benzimidazole in the interaction with the enzyme.

All compounds were evaluated for their anti-HIV activity using MT-4/MTT experiments.[18] None of the compounds was able to inhibit HIV replication at subtoxic concentrations.

In conclusion, a novel hit compound has been identified as IN inhibitor by means of virtual screening techniques. Starting from it, a library of structurally related compounds has been rapidly generated and submitted to biological evaluation with the aim of identifying potential INBI endowed with positive pharmacological profile. Among all the tested compounds, **16** proved to be the most interesting one, with an IC_{50} = 12 μM.

Further studies on this new family of IN inhibitors are ongoing in our laboratories.

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