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Design of a series of bicyclic HIV-1 integrase inhibitors. Part 2: Azoles: Effective metal chelators

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ABSTRACT

Synthesis of a diverse set of azoles and their utilizations as an amide isostere in the design of HIV integrase inhibitors is described. The Letter identified thiazole, oxazole, and imidazole as the most promising heterocycles. Initial SAR studies indicated that these novel series of integrase inhibitors are amenable to lead optimization. Several compounds with low nanomolar inhibitory potency are reported.

Human immuno-deficiency virus type 1 (HIV-1) is the etiological agent that causes acquired immuno-deficiency syndrome (AIDS). The treatment for HIV-1 infected patients is known as highly active antiretroviral therapy (HAART) and typically consists of a combination of two nucleoside reverse transcriptase inhibitors (NRTIs) and either a non-nucleoside reverse transcriptase inhibitor (NNRTI) or a protease inhibitor (PI). Recently, however, a new drug (Raltegravir, Isentress™) targeting the third viral enzyme integrase (IN) has been approved by the FDA.[1] This drug interrupts the critical process of integration, whereby newly made viral DNA is inserted into the host cellular chromosome. IN achieves this via a two-step enzymic process in which the 3′ terminal two nucleotides on each newly made viral DNA strand are (3′-processing reaction). IN then facilitates the concerted integration of both trimmed ends of the viral DNA into cellular chromosomal DNA in a process called strand transfer. Within the active site of IN lies a highly conserved trio of basic amino acids (Asp-64, Asp-116, and Glu-152) that together co-ordinate two divalent metal ions (physiologically Mg^{2+}) that are critical for the architecture of the active site and enzyme catalysis. Raltegravir and related integration strand transfer inhibitors (INSTIs) are proposed to interrupt viral DNA binding of integrase by co-ordinating these metal ions via an hydrogen bond acceptor–donor–acceptor motif.[2] Recently we have disclosed the discovery of new bicyclic pyrimidine derivatives as potent inhibitors of HIV integrase.[3] It was demonstrated that varying the substitutions of the bicyclic rings provided an effective approach to optimize the interaction of the metal binding element with the protein. This strategy has resulted in the discovery of potent, nanomolar inhibitors of HIV integrase in both enzymatic and cell-based assays. We sought to further improve the activity and pharmacokinetic properties of these compounds in whole cell assays by replacing the amide group. The chelating scaffolds of these inhibitors consist of one phenolic hydroxyl group and two amide functionalities serving as co-ordinating components for divalent Mg^{2+} ions in the active site of the integrase protein. Since azoles are well known amide isosteres having been successfully utilized in design of potent inhibitors for HIV protease and apoptosis proteins,[4] we embarked upon studies to examine the possibility of using diverse azoles in place of the exo amide group to provide the essential chelation of the Mg^{2+} ions. We reasoned that changing the nature of the heterocycles from azoles to imidazoles or oxazoles, would offer additional means to fine-tune electronic properties, and thus metal binding capabilities of the scaffolds (Fig. 1). In addition, the heterocylic azoles would also allow modification of the flexible linker that connects the metal chelator and the *p*-fluoro substituted phenyl ring. Herein, we report our findings of successful utilization of azoles as effective metal chelators in designing a new class of potent HIV integrase inhibitors.

The key starting material for azoles is the methyl ester **3** of which the synthesis was described previously.[3] The ester group reacted readily with hydrazine hydrate to give the mono-hydrazide,

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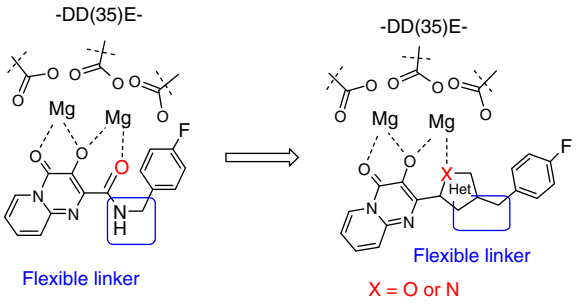
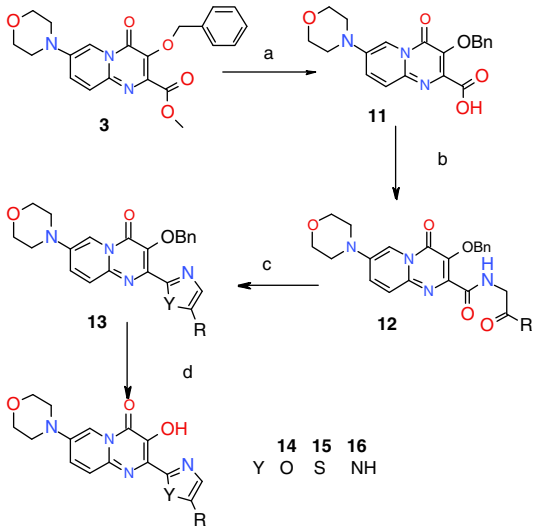


Figure 1. Design of azoles for amide replacement.

which was further acylated with an acid chloride to give intermediate **4** (Scheme 1).

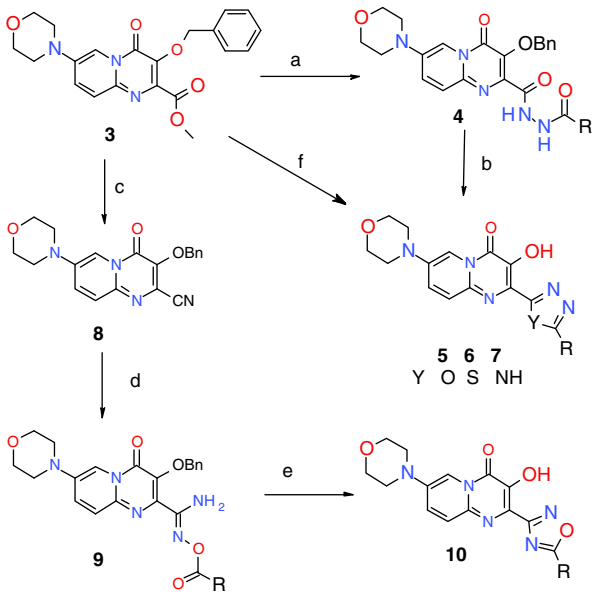
Cyclization of the hydrazide group followed by Bn deprotection with TMSI as Lewis acid furnished the final 1,3,4-oxadiazole **5**. Alternatively, using Lawesson's reagent as cyclizing agents led to 1,3,4-thiadiazole **6**. Triazole **7** was also prepared from ester **3** which readily underwent amidation with aqueous ammonium hydroxide. The resulting amide was reacted with Lawesson's reagent, and subsequently with MeI to provide an *S*-Me thioamide intermediate that cyclized with 4-F-phenyl acetohydrazide to form triazole **7**. Preparation of the asymmetrical 1,2,4-oxadiazole **10** required a different strategy. In the first step, ester **3** was converted to nitrile **8** via amidation with ammonia and dehydration with trichlorotriazine. Next, the nitrile group was reacted with hydroxylamine to give *N*-hydroxyamidine, which was acylated with an appropriate phenylacetyl chloride to afford precursor **9**. Formation of the 1,2,4-oxadiazole ring was achieved readily by refluxing intermediate **9** in toluene.

Similar cyclization approaches were also employed successfully for the preparation of oxazole **14**, thiazole **15**, or imidazole **16** (Scheme 2). The ester group of compound **3** was hydrolyzed with LiOH to give acid **11** which underwent amidation with a substituted 2-oxo-ethylamine to give the key intermediate **12**. Following the cyclization steps, removal of the Bn group was affected by TMSI in acetonitrile to complete the synthetic sequence.

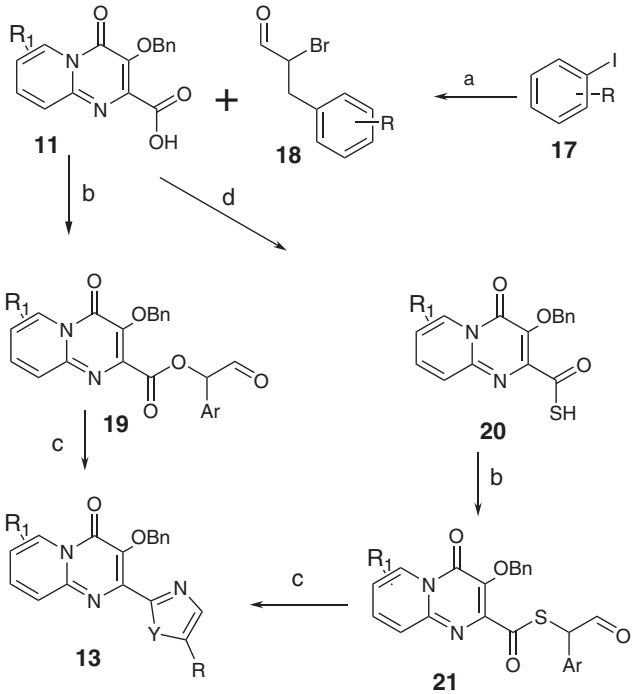


Scheme 2. Reagents and conditions: (a) LiOH, H_2O, THF, 80%; (b) EDCI·HCl, HOBt, Et_3N, NH_2–CH_2–C(O)–R, DMF; 74%; (c) Ph_3P, CCl_4, Et_3N for Y = O, 50%; Lawesson's reagent, toluene, reflux for Y = S, 35%; ammonium acetate, AcOH, xylene, reflux for Y = NH, 38%; (d) TMSI/CH_3CN, 54% for **14**, 30% for **15**, 76% for **16**.

Oxazole **14** and thiazole **15** rings can also be prepared from acid **11** and 2-bromo aldehyde **18** (Scheme 3). The latter was conveniently synthesized in two steps via Heck coupling of commercially available iodo-benzene derivatives **17**, followed by bromination. The acid group of **11** was alkylated with 2-bromo aldehyde **18** in the presence of a proton sponge to act as acid scavenger to afford ester **19**. Under similar conditions, thioacid **20**, which was prepared from **11** via reaction with sodium hydrosulfide and subsequent



Scheme 1. Reagents and conditions: (a) (i) hydrazine hydrate, MeOH, 81%; (ii) RCOCl, 94%; (b) Y = O,S, (i) Ph_3P, CCl_4, Et_3N for Y = O, 43%; Lawesson's reagent, toluene, reflux for Y = S, 35%; (ii) TMSI/CH_3CN, 41% for **5**, 35% for **6**; (c) (i) NH_3, H_2O, 50 °C, 59%; (ii) 2,4,6-trichloro-1,3,5-triazine, 88%; (d) (i) NH_2OH·HCl, $NaHCO_3$, 92%; EtOH, reflux; (ii) RCOCl, 97%; (e) (i) toluene, reflux, 75%; (ii) $FeCl_3$, CH_2Cl_2, 30%; (f) Y = N, (i) NH_3, H_2O, 50 °C, 59%; (ii) Lawesson's reagent, toluene, reflux, 45%; (iii) MeI, 50%; 4-F-phenyl acetohydrazide, AcOH, reflux, 55%; (iv) TMSI/CH_3CN, 50%.



Scheme 3. Reagents and conditions: (a) (i) All-OH, Heck coupling, 50%; (ii) TMSBr, Br_2, CH_2Cl_2, 75%; (b) proton sponge, CH_2Cl_2, reflux; (c) ammonium acetate, AcOH, toluene, reflux, 30–40% yield over steps (b) and (c); (d) CDI, NaSH, 60–8%.

acylation with **18** was converted to thioester **21**. Both **20** and **21** were cyclized readily under heating with ammonium acetate to the corresponding oxazole or thiazole **13**.

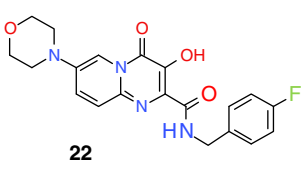
The inhibitory potency of the azole derivatives were assessed initially in an enzymatic assay using recombinant HIV WT integrase,[5] and compounds exhibiting IC_{50} values <1 μM were subjected to single-round HIV-1-based infectivity assays to determine the more therapeutically relevant EC_{50} values. The amide **22**, selected as the parent compound for our investigations displayed an IC_{50} of 775 nM and a reasonable EC_{50} of 215 nM in these assays. Gratifyingly, the easily accessible oxadiazoles **23** and **24**, triazole **25**, and thiadiazole **26** all displayed nanomolar potency akin to that of the parent amide. These results suggested that five-membered ring azoles are capable of functionally replacing an amide at this position. We postulated that in these azoles the nitrogen atom (highlighted in red, Table 1) furthest from the 4-F benzyl substituent would be positioned in a favorable geometry to chelate with the divalent Mg^{2+} ion. Changing the electronic properties of the azoles by inclusion of oxygen, nitrogen, or sulfur in the ring influenced the activity, although only with marginal effects. Similarly, the position of the non-chelating nitrogen and oxygen atoms were not crucial for potency (**23** vs **24**). The nature of the co-ordinating atom was critical as evidenced by compound **27**, which was inactive despite containing a similar asymmetrical oxadiazole as **24**. The data from these two compounds suggest that a nitrogen atom, and not an oxygen atom is the preferred metal chelator within the heterocyclic setting. This result is intriguing given the ability of the amide carbonyl oxygen to co-ordinate Mg^{2+} as demonstrated in the recent X-ray crystal structure of Raltegravir within the active site of the Foamy Virus integrase.[6]

It is worth noting that it has been previously reported that the nitrogen atom of the naphthyridine ring is able to co-ordinate Mg^{2+}, an observation that formed the basis of a scaffold for a series of potent HIV integrase inhibitors.[7] Replacement of the heteroatom neighbor to the chelating nitrogen proved most beneficial for the inhibitory activity. In particular, thiazole **28**, oxazole **29**, and imidazole **30** were all significantly more potent compared to the corresponding thiadiazole **26**, oxadiazole **23**, and triazole **25**, as well as the parent amide **22**.

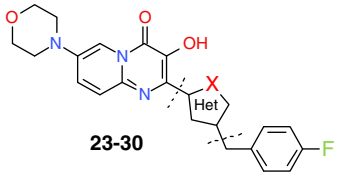
We next examined the SAR of other substituents of the bicyclic ring systems using the identified thiazole, oxazole, or imidazole as metal binding motifs. Introduction of halogen substitutions in position 9 improved the potency several fold resulting in low nanomolar inhibitors **31** and **32** (Table 2). Similarly the electron-donating cyclic sulfonamide group on the thiazole (**34**) was beneficial. Surprisingly the same group caused a significant reduction in activity when applied to oxazole and imidazole scaffolds (**35** and **36**) demonstrating the existence of a subtle difference between these heterocycles and the azole rings. In contrast, a range of nitrogen containing substitutions was well tolerated when combined with the imidazole as the amide isostere.

Sultam **37**, which is a close analog of sulfonamide **36**, exhibited a surprisingly good EC_{50} of 11 nM while cyclic carbamate **38**, cyclic lactam **39**, or amide **40** all showed a reasonable level of potency ranging from 39 nM to 86 nM in the cell-based assays. Among

Table 1
Enzymatic and in vivo activities of compounds **22**–**30**



22



23-30

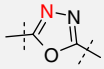
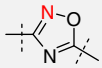
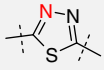
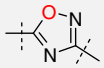
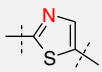
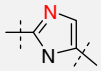
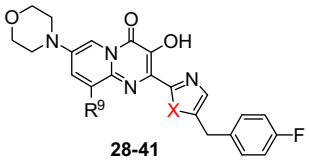
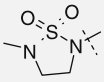
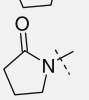
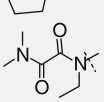
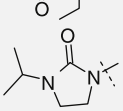
	Heterocycle	IC_{50} (nM)	EC_{50} spread (nM)	EC_{50} Luc (nM)
22	–	775	28	215
23		310	215	na
24		450	99	na
25		na	na	197.5
26		225	14	na
27		>10,000	na	>1000
28		20	16.5	32
29		59	6	21.5
30		45	na	62

Table 2
SAR of azoles as amide isotere



28-41

	R9	X	IC_{50} (nM)	EC_{50} spread (nM)	EC_{50} Luc (nM)
28	H	S	20	16.5	32
29	H	O	59	6	21.5
30	H	N	45	na	62
31	Br	S	42	4.35	14
32	I	O	170	2.6	9
33	Br	N	na	na	29
34		S	36	na	11.5
35		O	na	na	>1000
36		N	95	na	>1000
37		N	70	na	11
38		N	35	na	64
39		N	47	na	39
40		N	na	na	86
41		N	na	na	6

the studied substitutions, cyclic urea **41** appeared the most promising with an EC_{50} value of 6 nM. Importantly, the inhibitory activity of this compound displayed a small shift (<threefold) in cell-based assays conducted in a high (50%) serum environment (data not shown).

In summary we have presented data that supports the utility of azoles as a component of a metal binding motif in the acceptor–donor–acceptor pharmacophore of HIV-1 integrase inhibitors. We have examined eight different five-member ring azoles and established that they can efficiently serve as an amide isostere when placed adjacent to a 6,6 bicyclic pyrimidine core. Of the heterocycles tested, introduction of the thiazole, oxazole and imidazole into the scaffold resulted in the best anti-integrase and antiviral potencies. We further demonstrated that in contrast to that of the amide setting, within the heterocyclic environment a nitrogen atom rather than an oxygen atom appears to be the preferred heteroatom for metal co-ordination. Finally, the initial SAR presented here demonstrated that these compounds are highly potent leads amenable to further optimization to generate inhibitors of HIV integrase. A comprehensive lead optimization of these promising leads is underway and the results will be reported in due course.[8]

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