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Discovery of a new HIV-1 inhibitor scaffold and synthesis of potential prodrugs of indazoles

Se-Ho Kim [a,*], Benjamin Markovitz [a], Richard Trovato [a], Brett R. Murphy [a], Harry Austin [a], Adam J. Willardsen [a], Vijay Baichwal [a], Scott Morham [b], Ashok Bajji [a]

[a] Myrexis Inc., 305 Chipeta Way, Salt Lake City, UT 84108, United States
[b] MesaGen LLC, 2500 South State Street, South Salt Lake, UT 84115, United States

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ABSTRACT

A new oxazole scaffold showing great promise in HIV-1 inhibition has been discovered by cell-based screening of an in-house library and scaffold modification. Follow-up SAR study focusing on the 5-aryl substituent of the oxazole core has identified **4k** (EC_{50} = 0.42 μM, TI = 50) as a potent inhibitor. However, the analogues suffered from poor aqueous solubility. To address this issue, we have developed broadly applicable potential prodrugs of indazoles. Among them, *N*-acyloxymethyl analogue **11b** displayed promising results (i.e., increased aqueous solubility and susceptibility to enzymatic hydrolysis). Further studies are warranted to fully evaluate the analogues as the potential prodrugs with improved physiochemical and PK properties

The HIV life cycle encompasses a variety of steps (e.g., attachment to the host cell, viral entry, proviral integration, gene expression to viral budding) and many stages in the life cycle have already been exploited in the search for better HIV inhibitors, resulting in the discovery of ~30 approved drugs for the treatment of this pandemic disease.[1] However, there is still an urgent need for new HIV drug discovery with novel modes of action due to the rapid emergence of drug resistance in HIV patients.[2]

With an aim to find a new chemical scaffold with HIV-1 inhibitory activity, we screened an in-house library consisting of small molecular weight (MW <500) compounds using an MT4 cell-based assay. An MT4 cytoprotection assay has the advantage of detecting compounds inhibiting all stages of HIV life cycles; this provides greater possibilities in identifying a novel chemical structure with a unique mechanism of action. Recently, such traditional cellular phenotypic screening approaches are gaining increased attention for drug discovery due to the advantage in discovering first-in class drugs as compared to target-based screening.[3] Furthermore, recent advances in chemical proteomic technology now enable faster, more efficient target identification after phenotypic screening.[4]

After screening of the library using the MT4 cytoprotection assay, we identified compounds **1**–**3** which have relatively good lead-like[5] characteristics (MW ⩽400, rings ⩽4, hydrogen-bond donors ⩽5, hydrogen-bond acceptors ⩽9, and log*P* 1.9–5.5), exhibited some cytoprotective effect (EC_{50} of 6–13 μM, MT4 cells) and can serve as a starting point for chemical modifications (Fig. 1). Structurally, compounds **1**–**3** have similar pharmacophore features. Each compound commonly contains an aminoindazole ring and an aryl ring, but its structure differs in core (piperidine, quinazoline, and urea for **1**, **2**, and **3**, respectively).

Initial screening results indicated that the modification of the core structure could be an effective lead optimization approach and this consideration prompted us to investigate a scaffold morphing strategy.[6] Consequently, oxazole **4a** was designed based on the structure of urea **3**, synthesized, and evaluated (Scheme 1). To our delight, oxazole **4a** exhibited improved potency and a wider therapeutic index (EC_{50} = 2 μM, TI >45). Encouraged by this result, we initiated a SAR study around this scaffold to find more potent and non-toxic HIV-1 inhibitors that can ultimately be used to identify the molecular target and understand the mechanism of HIV-1 inhibition.

Herein we report the synthesis and SAR study of the oxazole scaffold as well as the design and synthesis of potential prodrugs of indazoles that were developed in an effort to improve the aqueous solubility of the analogues.

A representative example depicted in Scheme 2 is the synthesis of analogue **4d**. We used an iminophosphorane-mediated cyclization for the 2-aminooxazole ring formation.[7] Isothiocyanate **6** was conveniently prepared by treatment of aminoimidazole **5** with

Abbreviations: CDKs, cyclin-dependent kinases; CKII, casein kinase 2; DMAP, dimethylaminopyridine; EDCI, *N*-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; LIMK, LIM kinases; PK, pharmacokinetics; TFA, trifluoroacetic acid.

* Corresponding author. Tel.: +1 801 733 9791; fax: +1 801 214 7991.
E-mail address: seho.kimkim@gmail.com (S.-H. Kim).

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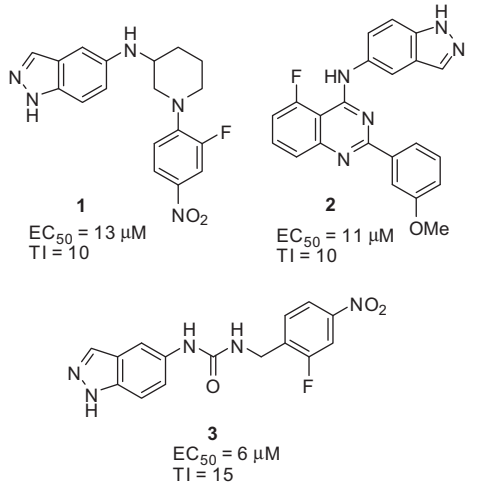
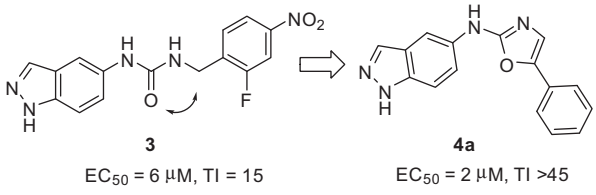


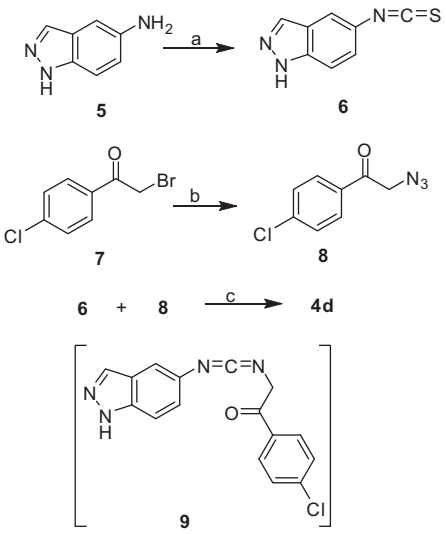
Figure 1. Hits identified through the MT4-cell cytoprotection assay.



Scheme 1. Core modification.

1,1′-thiocarbonyldi-2(*H*)-pyridone or thiocarbodiimidazole. Acyl bromide **7** was easily converted into acyl azide **8** by use of NaN_3. The synthesis of oxazole **4d** was achieved in good yield (85%) via intermediate **9** by heating a mixture of **6** and **7** in the presence of PPh_3. This protecting-group-free two-step procedure allowed for the rapid generation of related analogues.

The Topliss approach has been known as a popular tool in lead optimization for identifying optimal substituents. The approach involves systematic changes in electronic, steric and hydrophobic properties of substituents, and is particularly useful in cases where no structural information about the biological target is available.[8] In our initial study, a similar strategy was used to examine the effect of the substituents (Cl and OMe) on the 5-phenyl moiety in **4a** on the potency. Thus, *ortho*-, *meta*-, and *para*-subsituted phenyl analogues **4b**–**4k** were prepared. As summarized in Table 1, these analogues had markedly different potency. For chloro substituted phenyl analogues **4b**–**d**, the order of potency was *para*->*ortho*- >>*meta*-chlorophenyl analogues (EC_{50} = 3, 9, 54 μM, **4d**, **4b** and **4c**, respectively). The *para*-chlorophenyl analogue **4d** showed a similar potency to parent **4a** but exhibited significantly improved metabolic stability (%$R_{40\ min}$ = 83 for **4d** and **9** for **4a**, mouse liver microsome). These results clearly indicated that the *para*-position of the phenyl moiety is a major metabolic site. The opposite SAR trend was observed for methoxyphenyl analogues **4e**–**g**; *meta*-methoxyphenyl analogue **4f** showed anti-HIV-1 activity (EC_{50} = 2 μM) similar to that of **4a**,**d** whereas *ortho*- and *para*-methoxyphenyl analogues **4e**,**g** lacked the potency (EC_{50} = 100 μM). It was expected that combination of the favorable 4-chloro and 3-methoxy substituents might act synergistically for the in vitro anti-HIV-1 activity. Although the combination of the substitutents did not induce a significant synergistic effect, 4-chloro-3-methoxyphenyl analogue 4i exhibited comparable or slightly better potency (EC_{50} = 1.5 μM) to analogues **4d**,**f**. Interestingly, 3,4-dimethoxyphenyl analogue **4k** (EC_{50} = 0.42 μM, TI = 50)



Scheme 2. Reagents and conditions: (a) 1,1′-thiocarbonyldi-2(1*H*)-pyridone (or thiocarbodiimidazole), CH_2Cl_2, rt, 2 h, 64%; (b) NaN_3, acetone/water, 50 °C, 0.5 h, 92%; (c) PPh_3, dioxane, 90 °C, 0.5 h, 85%.

was found to be the most potent compound in this series. Methylenedioxyphenyl analogue **4h** maintained potency (EC_{50} = 2.7 μM) and 3,4-dichlorophenyl analogue **4j** resulted in a big loss in potency (EC_{50} = 35 μM). This brief SAR study revealed that anti-HIV-1 activity was quite sensitive to the substituents and their positions on the 5-aryl ring; the electron-donating methoxy group at 3-position and electron withdrawing chloro group at 4-position were tolerant. The potency of dimethoxyphenyl analogue **4k** could also be fine-tuned by modifying the alkoxy group.

Having identified leads showing good HIV-1 inhibitory activity, we decided to evaluate the PK properties of the analogues. Early PK studies guide decision making in drug discovery process and help reduce the attrition rate in drug development.[9] Initially, compound **4d** was selected for this study because it had reasonable HIV-1 inhibitory potency (EC_{50} = 3.0 μM), good lead like properties (MW = 310, log*P* = 3.3) along with an excellent metabolic stability (%$R_{40\ min}$ = 83, mouse liver microsome). However, the poor aqueous solubility of 4d (0.2 μM at pH 7.4 in phosphate buffer) was a major hurdle for preclinical PK evaluation and these analogues in general suffered from poor solubility properties.

To address this issue, we envisaged a prodrug approach. The indazole nitrogen of **4d**, rather than 2-amino group of the oxazole core, was considered to be a preferred site for attachment of a prodrug moiety because of easy chemical modifications and a stronger acidity of the N–H group that increases the self cleavage rate of a double prodrug.[10] There are relatively few precedents in the literature for prodrugs of indazoles. One is a tetrahydropyridinyl moiety.[11] The mechanism of cleavage of the tetrahydropyridinyl promoiety was proposed to partially mimic bioactivaton pathway that is catalyzed by monoamine oxidase B (MAO-B). Phosphate prodrugs[12] of indoles were also used to overcome formulation problems (e.g., phosphate prodrugs of PD154075[10], a highly potent and selective NK1 receptor antagonist). Inspired by many prodrug strategies designed for amines[13], we designed three types of potential prodrugs of indazoles: (a) double prodrugs containing *N*-acyloxymethyl group as a promoiety, (b) N-mannich bases, (c) *N*-acyl prodrugs (Scheme 3). In the following section, the synthesis and preliminary evaluation of the potential prodrugs are described.

Type A prodrugs are designed to form double prodrug systems that utilize *N*-acyloxymethyl or *N*-alkoxycarbonyloxymethyl