Letter

Synthesis and anti-HIV profile of a novel tetrahydroindazolylbenzamide derivative obtained by oxazolone chemistry

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Synthesis and anti-HIV profile of a novel tetrahydroindazolylbenzamide derivative obtained by oxazolone chemistry

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ABSTRACT: A new tetrahydroindazolylbenzamide derivative has been synthesized, characterized and evaluated as HIV-inhibitor. The biological data revealed the ability to inhibit HIV proliferation with low cytotoxicity allowing for significant selectivity (EC_{50} 2.77 μM; CC_{50} 118.7 μM; SI=68). The compound did not inhibit the viral integrase as demonstrated by *in vitro* studies. QPCR experiments showed that the block of viral replication occurred at early replication steps, prior to integration, profiling it as a late reverse transcription inhibitor. An efficient multi-step strategy was adopted for the synthesis of the scaffold, consisting of a sequential ring-opening reaction of oxazol-5-(*4H*)-one with 1,3-diketone, followed by cyclocondensation with hydrazine and hydrolysis of the nitrile to the desired carboxamide.

The infection caused by the human immunodeficiency virus (HIV) induces a persistent and incurable disease with serious health and socio-economical impact.[1] HIV still causes approximately three million deaths annually. Despite the currently available drugs are unable to eradicate the virus, the combined antiretroviral treatment (cART) has markedly changed the evolution of the infection and transformed a deadly disease into a manageable chronic infection.[2] The pharmacological treatment of the HIV infection targets mostly HIV proteins such as reverse transcriptase (RT), protease, and integrase (IN) to specifically interfere with virus replication.[3] Nevertheless, the identification of cellular factors involved in the viral replication cycle has opened new avenues for the development of HIV inhibitors.[4] In the framework of our studies, dealing with the design of biologically active heterocycles,[5-10] we have discovered a new compound interfering *in vitro* with the HIV replication. It shares attractive structural features with some inhibitors of the heat-shock protein 90 (Hsp90), such as SNX-2112 and its prodrug SNX-5422, the latter currently in phase I clinical trials as antitumoral (Figure 1).

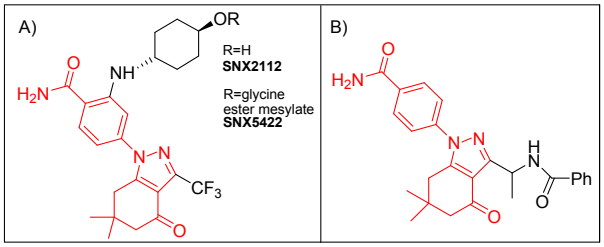


Figure 1. A) Structures of SNX2112 and SNX5422; B) the novel tetrahydroindazolylbenzamide derivative reported herein.

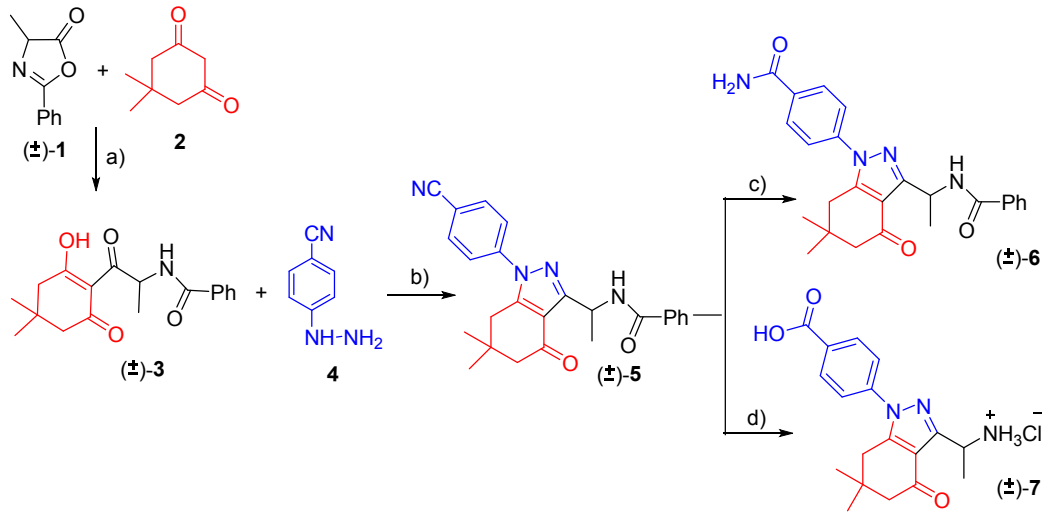
Hsp90 is a molecular chaperone involved in a variety of cellular processes that guides the folding, intracellular disposition, and proteolytic turnover of many key regulators of cell growth and differentiation.[11] Hsp90 has been identified as a therapeutic target in cancer and inhibitors of Hsp90 have proven to be effective at driving cancer cells into apoptosis.[12] Recently, Hsp90 inhibitors have also shown great promise in the treatment of HIV infection.[13] Tetrahydroindazolyl- and tetrahydroindolyl-benzamide derivatives were recently patented as Hsp90 inhibitors able to prevent integration of HIV viral DNA into host cells.[14] Additionally, nelfinavir and ritonavir, lead inhibitors of the HIV protease, were found to inhibit Hsp90 function in breast cancer cells, as an example of "drug repurposing".[15-17]

Herein, we report the synthesis and the HIV profile of a new tetrahydroindazolylbenzamide derivative prepared by ring opening reaction of 4-methyl-2-phenyl oxazol-5-(*4H*)-one **1** with dimedone **2**, followed by cyclocondensation of the resulted 1,3,3'-tricarbonyl derivative **3** with 4-cyanophenylhydrazine **4** and the subsequent hydration of the benzonitrile **5** into benzamide **6**. The synthetic procedure is depicted in Scheme 1.

Triketone **3** was conveniently prepared by an efficient chemo- and regioselective method, which entails the reaction of 5,5-dimethylcyclohexane-1,3-dione **2** with oxazolone **1** in acetonitrile under microwave irradiation.[18] The asymmetrical oxazolone **1** was obtained in turn by cyclodehydration of commercial *N*-benzoyl-D,L-alanine in acetic anhydride.[19] The cyclocondensation[20] of **3** with hydrazine **4** in refluxing ethanol efficiently yielded the tetrahydroindazolone **5**. The reaction proceeded regioselectively yielding only the 1,3-disubstituted regioisomer 5, due to the higher nucleophilicity of the external

nitrogen of arylhydrazine 4. Finally, the chemoselective conversion of the cyano group of **5** into amide derivative **6** was performed in a mixture ethanol/dimethylsulfoxide, by using sodium hydroxide and hydrogen peroxide as a catalyst. Alternatively, the nitrile moiety of **5** was converted into the corresponding carboxylic acid group by acid hydrolysis, with the simultaneous acid-promoted *N*-deprotection of the functionalized side-chain that led to the interesting amino acid derivative **7** as hydrochloride (Scheme 1).

Scheme 1. Synthetic strategy for the preparation of compounds 3-7



Reagents and conditions: (a) anhydrous CH_3CN, DBU, MW, reflux 85°C, 1 h; (b) abs. EtOH, reflux, 5 h; (c) EtOH, NaOH, DMSO, H_2O_2, rt, 24 h; (d) 6N HCl, glacial CH_3COOH, reflux, 2 days.

The structures of new compounds **5**, **6** and **7** were determined on the basis of analytical and spectroscopic data (See Supporting Information).

To the best of our knowledge, the introduction of a functionalized side-chain at the C3-pyrazole carbon of tetrahydroindazolylbenzamide scaffold is unprecedented, as only alkyl or aryl substituents (*e.g.* Me, Et, *i*-Pr, CF_3, cyclohexyl, Ph, thienyl, etc.) have been introduced so far.[14,21,22] In principle, using different amino acids as starting materials for the oxazolone formation, the proposed synthetic methodology might give efficient entry into facile diversification of the side-chain, allowing to maximize the molecular diversity of the final tetrahydroindazolylbenzamide. Moreover, the amino group could be further derivatized to improve the pharmaceutical properties including pharmacokinetic and druggability, *i.e.* by conjugation with biopolymers.

The anti-HIV activity of compounds **5**, **6** and **7** was evaluated *in vitro* in a classic MTT-MT4 assay[23] (see Supporting Information). The benzamide **6** showed a remarkable ability to inhibit HIV proliferation and low cytotoxicity (EC_{50} 2.77 µM; CC_{50} 118.7 µM) allowing for significant selectivity (SI=68); conversely, both the precursor benzonitrile **5** and the carboxylic derivative **7** did not show any antiviral activity, pointing out the essential role of the primary benzamide moiety at the N1-position of the pyrazole ring.

To further elucidate the mechanism of action, the effects of compound **6** on HIV integrase activity have been analyzed in an *in vitro* strand transfer assay. No activity was observed (data not shown) excluding HIV-IN as a drug target for this class of molecules. This finding was confirmed in cell culture by QPCR analysis (see Supporting Information). Indeed compound **6** did not show the increase in 2-LTRs typically seen for integration inhibitors (Figure 2, panel B). It rather profiled as a RT inhibitor with a stark reduction in formation of the reverse transcribed DNA, yet, to less extend than observed for AZT (Figure 2, panel A). Next, both the formation of 2-LTRs and integrated provirus were reduced to background levels (Figure 2, panel B and C, respectively), demonstrating once more that tetrahydroindazolylbenzamide **6** acts at early replication steps prior to integration. In time of addition experiments a similar profile was observed confirming that **6** profiles as a late reverse transcription inhibitor (data not shown).

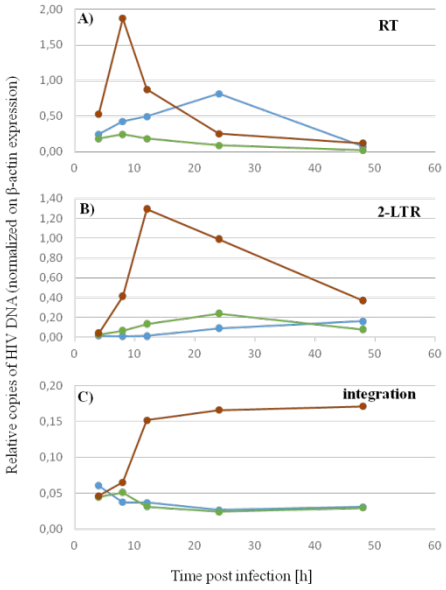


Figure 2. Analysis of the mechanism of action of **6** by QPCR analysis of HIV DNA species. Compounds were added at 50-fold EC_{50} value: control (DMSO, brown), compound **6** (light blue), and AZT (green). Panel A: Analysis of the RT activity by measurement of late RT transcripts. While AZT blocks reverse transcription completely, compound **6** shows a partial inhibition of reverse transcription. Panel B: Analysis of 2-LTRs circle formation as a measure of the block of integration. Alike AZT, compound **6** does not induce formation of 2-LTRs circles demonstrating that the antiviral activity is blocked at an earlier step (RT). Panel C: Analysis of the integration event. No provirus formation resulting from integration is observed.

In summary, the synthesis and the anti-HIV profile of a novel tetrahydroindazolylbenzamide derivative obtained by oxazolone chemistry have been reported. Compound **6** showed low cytotoxicity (CC_{50} 118.7), a remarkable anti-HIV activity (EC_{50} 2.77 μM) and significant selectivity (SI=68). The pivotal role of the primary benzamide moiety at the N1-position of the pyrazole ring emerged by the absence of antiviral activity for benzonitrile **5** and carboxylic derivative **7**. Preliminary studies carried out to elucidate the mechanism of action pointed out that it profiles as a late reverse transcription inhibitor.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website.
Materials and Methods; Synthetic procedures; Drug susceptibility assay; Quantification of different HIV-1 DNA species during HIV infection by real-time PCR; ^{1}H and ^{13}C NMR spectra (PDF file).

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Author Contributions

All authors have given approval to the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

HIV, human immunodeficiency virus; cART, combined antiretroviral treatment; RT, reverse transcriptase; IN, integrase; Hsp90, heat-shock protein 90; EC_{50}, half maximal effective concentration; CC_{50} half maximal citotoxic concentration; SI, selectivity index; QPCR, quantitative polymerase chain reaction; 2-LTR, two-long long terminal repeats; DNA, deoxyribonucleic acid; AZT, azidothymidine.

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