ACS Medicinal Chemistry Letters

Featured Letter

Cite This: ACS Med. Chem. Lett. 2019, 10, 398−401

pubs.acs.org/acsmedchemlett

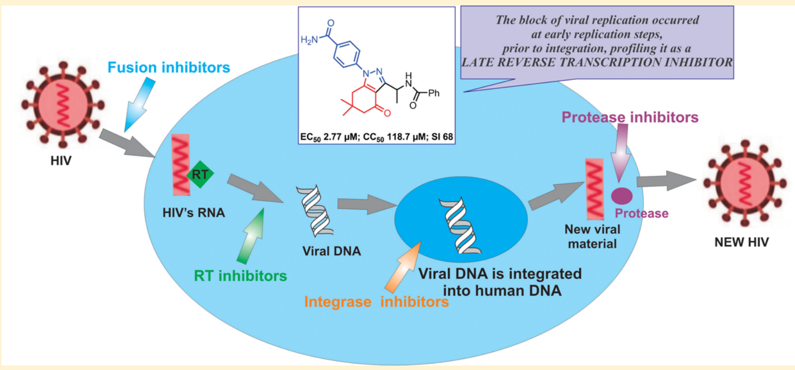
Synthesis and Anti-HIV Profile of a Novel Tetrahydroindazolylbenzamide Derivative Obtained by Oxazolone Chemistry

Angela Scala,*[,†] Anna Piperno,[†] Nicola Micale,[†] Frauke Christ,[‡] and Zeger Debyser[‡]

[†]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le F. Stagno D'Alcontres 31, Messina I-98166, Italy

[‡]Laboratory for Molecular Virology and Gene Therapy, Department of Pharmaceutical and Pharmacological Sciences, UZ St. Rafael, Kapucijnenvoer 33, BE-3000 Leuven, Belgium

Supporting Information



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 multistep strategy was adopted for the synthesis of the scaffold, consisting of a sequential ring-opening reaction of oxazol-5-(4*H*)-one with 1,3-diketone, followed by cyclocondensation with hydrazine and hydrolysis of the nitrile to the desired carboxamide.

KEYWORDS: *Tetrahydroindazole, azlactone, hydrazine, dimedone, reverse transcriptase*

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 that 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).

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 tetrahydroindolylbenzamide 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

Special Issue: Highlighting Medicinal Chemistry in Italy

Received: October 30, 2018
Accepted: December 15, 2018
Published: December 15, 2018

DOI: 10.1021/acsmedchemlett.8b00511
ACS Med. Chem. Lett. 2019, 10, 398−401

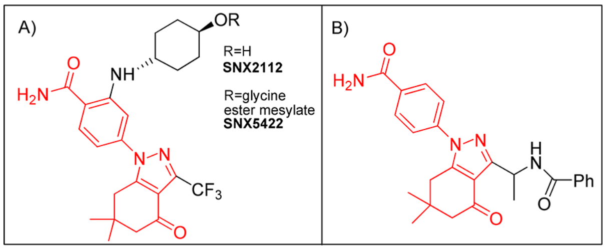


Figure 1. (A) Structures of SNX2112 and SNX5422; (B) the novel tetrahydroindazolylbenzamide derivative reported herein.

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-(4*H*)-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** to 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/dimethyl sulfoxide, 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).

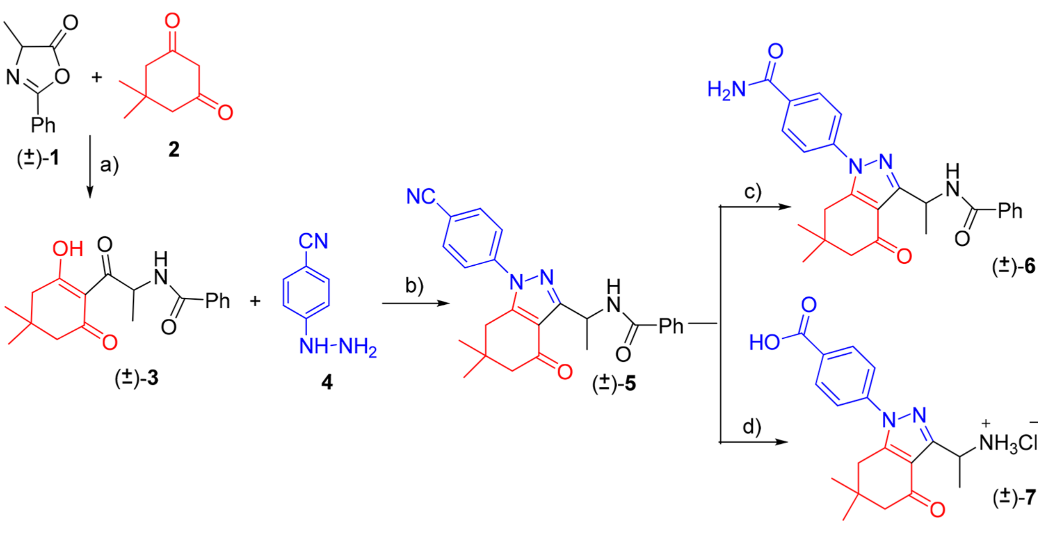
The structures of new compounds **5**, **6**, and **7** were determined on the basis of analytical and spectroscopic data (see Supporting Information).

To the best of our knowledge, the introduction of a functionalized side-chain at the C3-pyrazole carbon of the 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 2B). It rather profiled as a RT inhibitor with a stark reduction in formation of the reverse transcribed DNA, yet, to less extent than observed for AZT (Figure 2A). Next, both the formation of 2-LTRs and integrated provirus were reduced to background levels (Figure 2B,C, respectively), demonstrating once more that tetrahydroindazolylbenzamide **6** acts at early replication steps prior to

Scheme 1. Synthetic Strategy for the Preparation of Compounds 3−7[a]



[a]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) 6 N HCl, glacial CH_3COOH, reflux, 2 days.

DOI: 10.1021/acsmedchemlett.8b00511
ACS Med. Chem. Lett. 2019, 10, 398−401

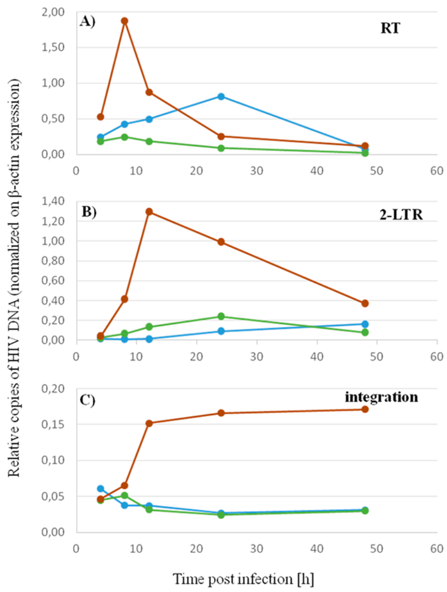


Figure 2. Analysis of the mechanism of action of **6** by QPCR analysis of HIV DNA species. Compounds were added at 50-fold EC_{50} value: control (DMSO, brown), compound **6** (light blue), and AZT (green). (A) Analysis of the RT activity by measurement of late RT transcripts. While AZT blocks reverse transcription completely, compound **6** shows a partial inhibition of reverse transcription. (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). (C) Analysis of the integration event. No provirus formation resulting from integration is observed.

integration. In time of addition experiments a similar profile was observed confirming that **6** profiles as a late reverse transcription inhibitor (data not shown).

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 at DOI: 10.1021/acsmedchemlett.8b00511.

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)

■ AUTHOR INFORMATION

Corresponding Author

*Tel: +39 (0)90 6765515. E-mail: ascala@unime.it.

ORCID

Angela Scala: 0000-0003-2171-9033

Anna Piperno: 0000-0001-6004-5196

Nicola Micale: 0000-0002-9294-6033

Author Contributions

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

Notes

The authors declare no competing financial interest.

Biography

Angela Scala received her degree in Pharmaceutical Chemistry and Technology in 2006 and her Ph.D. degree in Chemical Science in 2010 at the University of Messina (Italy). In 2014, she became Assistant Professor in Organic Chemistry at the University of Messina, where she is still working. Angela Scala began her research activity in 2007 working on the design, synthesis, and characterization of new carbo/heterocyclic compounds that interfere with viral replication or with cell proliferation. Successively, her research interests have been extended to the functionalization of biopolymers and nanomaterials for drug delivery applications.

■ 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 cytotoxic concentration; SI, selectivity index; QPCR, quantitative polymerase chain reaction; 2-LTR, two-long long terminal repeats; DNA, DNA; AZT, azidothymidine

■ REFERENCES

(1) De Castro, S.; Camarasa, M. J. Polypharmacology in HIV inhibition: can a drug with simultaneous action against two relevant targets be an alternative to combination therapy? *Eur. J. Med. Chem.* **2018**, *150*, 206–227.

(2) Franzese, O.; Barbaccia, M. L.; Bonmassar, E.; Graziani, G. Beneficial and Detrimental Effects of Antiretroviral Therapy on HIV-Associated Immunosenescence. *Chemotherapy* **2018**, *63*, 64–75.

(3) Soriano, V.; Fernandez-Montero, J. V.; Benitez-Gutierrez, L.; de Mendoza, C.; Arias, A.; Barreiro, P.; Pena, J. M.; Labarga, P. Dual antiretroviral therapy for HIV infection. *Expert Opin. Drug Saf.* **2017**, *16*, 923–932.

(4) Duerr, R.; Keppler, O.; Christ, F.; Crespan, E.; Garbelli, A.; Maga, G.; Dietrich, U. Targeting Cellular Cofactors in HIV Therapy. *Top. Med. Chem.* **2014**, *15*, 183–222.

(5) Scala, A.; Micale, N.; Piperno, A.; Rescifina, A.; Schirmeister, T.; Kesselring, J.; Grassi, G. Targeting of the leishmania mexicana cysteine protease CPB2.8DCTE by decorated fused benzo[*b*]thiophene scaffold. *RSC Adv.* **2016**, *6*, 30628–30635.

(6) Rescifina, A.; Scala, A.; Sciortino, M. T.; Colao, I.; Siracusano, G.; Mazzaglia, A.; Chiacchio, U.; Grassi, G. Decorated 6,60,7,70-tetrahydro-1H,10H-2,30-biindole scaffold as promising candidate for recognition of the CDK2 allosteric site. *MedChemComm* **2015**, *6*, 311–318.

(7) Scala, A.; Cordaro, M.; Grassi, G.; Piperno, A.; Barberi, G.; Cascio, A.; Risitano, F. Direct synthesis of C3-mono-functionalized oxindoles from N-unprotected 2-oxindole and their antileishmanial activity. *Bioorg. Med. Chem.* **2014**, *22*, 1063–1069.

(8) Scala, A.; Cordaro, M.; Mazzaglia, A.; Risitano, F.; Venuti, A.; Sciortino, M. T.; Grassi, G. Aldol-type compounds from water-soluble indole-3,4-diones. Synthesis, kinetics and antiviral properties. *Mol. Diversity* **2013**, *17*, 479–488.

(9) Scala, A.; Cordaro, M.; Risitano, F.; Colao, I.; Venuti, A.; Sciortino, M. T.; Primerano, P.; Grassi, G. Diastereoselective multicomponent synthesis and anti-HSV-1 evaluation of dihydrofuran-fused derivatives. *Mol. Diversity* **2012**, *16*, 325–333.

DOI: 10.1021/acsmedchemlett.8b00511
ACS Med. Chem. Lett. 2019, 10, 398–401

(10) Scala, A.; Cordaro, M.; Mazzaglia, A.; Risitano, F.; Venuti, A.; Sciortino, M. T.; Grassi, G. Synthesis and anti HSV-1 evaluation of novel indole-3,4-diones. *MedChemComm* **2011**, *2*, 172−175.
(11) Li, J.; Soroka, J.; Buchner, J. The Hsp90 chaperone machinery: Conformational dynamics and regulation by co-chaperones. *Biochim. Biophys. Acta, Mol. Cell Res.* **2012**, *1823*, 624−635.
(12) Butler, L. M.; Ferraldeschi, R.; Armstrong, H. K.; Centenera, M. M.; Workman, P. Maximizing the Therapeutic Potential of Hsp90 Inhibitors. *Mol. Cancer Res.* **2015**, *13*, 1445−1451.
(13) Smith, A. P.; Haystead, T. A. J. Hsp90: a key target in HIV infection. *Future Virol.* **2017**, *12*, 55−59.
(14) Orlemans, E. O. M.; Haynes, B. F.; Ferrari, G.; Haystead, T.; Kwiek, J. J. Use of tetrahydroindazolylbenzamide and tetrahydroindolylbenzamide derivatives for the treatment of Human Immunodeficiency Virus (HIV) and Acquired Immune Deficiency Syndrome (AIDS). From U.S. Pat. Appl. Publ. (2016), US 20160143884 A1, 20160526.
(15) Shim, J. S.; Rao, R.; Beebe, K.; Neckers, L.; Han, I.; Nahta, R.; Liu, J. O. Selective Inhibition of HER2-Positive Breast Cancer Cells by the HIV Protease Inhibitor Nelfinavir. *J. Natl. Cancer Inst.* **2012**, *104*, 1576−1590.
(16) Bernstein, W. B.; Dennis, P. A. Repositioning HIV protease inhibitors as cancer therapeutics. *Curr. Opin. HIV AIDS* **2008**, *3*, 666−675.
(17) Blumenthal, G. M.; Gills, J. J.; Ballas, M. S.; Bernstein, W. B.; Komiya, T.; Dechowdhury, R.; Morrow, B.; Root, H.; Chun, G.; Helsabeck, C.; Steinberg, S. M.; LoPiccolo, J.; Kawabata, S.; Gardner, E. R.; Figg, W. D.; Dennis, P. A. A phase I trial of the HIV protease inhibitor nelfinavir in adults with solid tumors. *Oncotarget* **2014**, *5*, 8161−8172.
(18) Cordaro, M.; Grassi, G.; Risitano, F.; Scala, A. A new construction of diversely funzionalized oxazoles from enolizable cyclic 1,3-dicarbonyls and 5(4H)-oxazolones. *Synlett* **2009**, *1*, 103−105.
(19) Piperno, A.; Scala, A.; Risitano, F.; Grassi, G. Oxazol-5-(4H)-Ones. Part 1. Synthesis and Reactivity as 1,3-dipoles. *Curr. Org. Chem.* **2014**, *18*, 2691−2710.
(20) Scala, A.; Piperno, A.; Risitano, F.; Cirmi, S.; Navarra, M.; Grassi, G. Efficient synthesis of highly substituted tetrahydroindazolone derivatives. *Mol. Diversity* **2015**, *19*, 473−480.
(21) Huang, K. H.; Veal, J. M.; Fadden, R. P.; Rice, J. W.; Eaves, J.; Strachan, J.; Barabasz, A. F.; Foley, B. E.; Barta, T. E.; Ma, W.; Silinski, M. A.; Hu, M.; Partridge, J. M.; Scott, A.; DuBois, L. G.; Freed, T.; Steed, P. M.; Ommen, A. J.; Smith, E. D.; Hughes, P. F.; Woodward, A. R.; Hanson, G. J.; McCall, W. S.; Markworth, C. J.; Hinkley, L.; Jenks, M.; Geng, L.; Lewis, M.; Otto, J.; Pronk, B.; Verleysen, K.; Hall, S. E. Discovery of novel 2-aminobenzamide inhibitors of heat shock protein 90 as potent, selective and orally active antitumor agents. *J. Med. Chem.* **2009**, *52*, 4288−4305.
(22) Song, H.; Lee, H.; Kim, J.; Park, S. B. Regioselective construction and screening of 1,3-disubstituted tetrahydroindazolones in enantiomerically pure pairs. *ACS Comb. Sci.* **2012**, *14*, 66−74.
(23) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and automated tetrazolium-based colorimetric assay for the detection of anti-HIV compounds. *J. Virol. Methods* **1988**, *20*, 309−321.

DOI: 10.1021/acsmedchemlett.8b00511
ACS Med. Chem. Lett. 2019, 10, 398−401