The Ugi4CR as effective tool to access promising anticancer isatin-based α-acetoamide carboxamide oxindole hybrids

Carolina Marques, Aday González-Bakker and José M. Padrón

13 Dez. 2023

Full Research Paper

beilsteinJOC_SI.pdf; 9.0 MB

Carolina Marques - https://orcid.org/0000-0003-1697-7871; José M. Padrón - https://orcid.org/0000-0001-6268-6552
The Ugi4CR as effective tool to access promising anticancer isatin-based α-acetoamide carboxamide oxindole hybrids

Carolina S. Marques*1, Aday González-Bakker2, José M. Padrón2

Address:
1LAQV-REQUIMTE, University of Évora, Institute for Research and Advanced Studies, Rua Romão Ramalho, 59, 7000-641, Évora, Portugal
2BioLab, Instituto Universitario de Bio-Orgánica Antonio González (IUBO-AG) Universidad de La Laguna, PO Box 456, 38200, La Laguna, Spain

*Email: Carolina Silva Marques, carolsmarq@uevora.pt

Abstract

Considering early-stage drug discovery programmes, the Ugi four component reaction is a valuable, flexible, and pivotal tool, facilitating the creation of two new amide bonds in a one-pot fashion to effectively yield the desired α-aminoacyl amides. Here, we highlight the reputation of this reaction approach to access number and scaffold diversity of a library of isatin-based α-acetoamide carboxamide oxindole hybrids, promising anticancer agents, in a mild and fast sustainable reaction process. The library was tested against six human solid tumor cell lines, among them, non-small cell lung carcinoma, cervical adenocarcinoma, breast cancer and colon adenocarcinoma. The most potent compounds 8d, 8h and 8k showed GI50 values in the range of 1-10 μM.
Keywords

Ugi4CR; cancer; oxindole; GI₅₀; isatin

Introduction

In the last years, meticulous attention has been given by chemists regarding process formation of new bonds and synthesis of new scaffolds. In drug discovery and development, medicinal chemists struggle everyday towards the creation of new synthetic methods, driven by the increasing complexity of the molecules and taking into consideration economic and social aspects. Multicomponent reactions (MCRs) are remarkable tools which demonstrated great potential for more sustainable production of active pharmaceutical ingredients (API’s). These flexible and versatile one-pot transformations in which three or more reagents are combined to access a new complex scaffold with remarkable atom economy, cost and time-effective and mainly diminishing waste production is a conscientious boost for structural diversity and sustainability.[1-3] The well-known Passerini, Ugi, Mannich, Biginelli, Hantzsch and Strecker reactions are some examples of the classic MCRs, representing the easygoing generation of a collection of small-molecules essential for structure-activity studies (SAR). The isocyanide based Ugi reaction is one of the most resourceful tools and still broadly studied MCR, generating multifunctional libraries of α-amino acyl amide derivatives, or Ugi adducts, with stereochemistry control.[4-5] Unquestionable potential of application in the pharmaceutical industry is recognizable by the number of APIs obtained by this reaction approach.[6-7] Oxindole framework is a privilege unit, recognized massively by its extensive biological applications.[8-9] In the last few years we have been active in isatin modification using new synthetic approaches, anticipating the creation of new libraries of small-molecule hybrids with potential as cholinesterase
inhibitors[10-13], important to treat neurodegenerative diseases, and anticancer agents[14-16] (Figure 1).

Cancer is a complex, cureless and fatal disease, massively diagnosed worldwide. Being one of the leading causes of death worldwide, it is expected an increase of 47% with 28.4 million cases diagnosed, in 2040 [17-18]. Despite long years of research, there is still an urgent need to find novel, effective and safe drugs for cancer therapy.

Figure 1: (A) Accessing libraries of oxindole hybrids using commercially available isatin as starting material and (B) most potent oxindole derivatives as BuChE inhibitors and anticancer agents, by Marques et al.[10-16]

Recently, focusing on the design of more potent anticancer drug candidates using more sustainable synthetic processes, we report a new Ugi 4-component reaction approach for easy access to Ugi-derived isatin-peptoids in moderate to excellent yields (up to 99% yield). Some selected compounds were screened against five human solid
tumor cell lines: lung (A549), breast (HBL-100 and T-47D), cervix (HeLa) and colon (WiDr). Preliminary SAR studies have revealed the preference of the N-benzyl isatin structure over the 3,3-protected-oxindole, aliphatic chain on the acid component and small aliphatic chain on the aldehyde component to increase the antiproliferative activity. Also, benzyl isocyanide was favored over the aliphatic one (Scheme 1, (A)).

[16] Considering the value of amide groups in drug discovery [19], the feasibility of running the isatin-based Ugi reaction [16, 20-23] and the potential of the bis-amide-oxindole type derivatives as anticancer agents, a second family was synthesized, and bio evaluated (Scheme 1, (B)).

Scheme 1: (A) Library of isatin-based α-acetoamide carboxamide oxindole derivatives obtained using an Ugi four component reaction (Ugi4CR). (B) The synthetic strategy reported in this work.
Results and Discussion

Synthesis

Underlining sustainability and economically favored processes, a second family of \( \alpha \)-acetoamide carboxamide oxindole derivatives \( 5 \) was obtained using the previously optimized Ugi4CR approach \([16]\) (Scheme 2 and Figure 2). Taking into account the preliminary SAR studies reported for the first family of Ugi-derived isatin-peptoids, the second family was obtained using the 5-amino-1-benzyl-3,3-dimethoxyindolin-2-one \([12]\) \( 1 \) and the benzyl isocyanide \( 4 \), as amine and isocyanide components, respectively. Different carboxylic acids \( 2 \) and aldehydes/ketones \( 3 \) were evaluated using ZnF\(_2\) as catalyst (10 mol\%) and MeOH as the solvent (Scheme 2 and Figure 2).

![Figure 2: Carboxylic acid 2 and aldehyde/ketone 3 components for the Ugi4CR.](image)

A library of \( \alpha \)-acetoamide carboxamide oxindole hybrids \( 5 \) was obtained in moderate yields (26-63\%), at room temperature, in short time (2 hours), proving the efficiency and miscellany of this methodology. Aliphatic (\( 2a, 2c \) and \( 2m \)), aromatic (\( 2d, 2f, 2g, 2k \) and \( 2l \)), heterocyclic (\( 2e, 2h, 2i, 2n \) and \( 2o \)), alkyne \( 2b \) and alkene \( 2j \) carboxylic
acids were used successfully in this MCR, demonstrating great reaction scope (Scheme 2 and Figure 2). Remarkably, the best yields were obtained when heterocyclic carboxylic acid components like 1H-pyrrole-3-carboxylic acid 2n, 2-furoic acid 2o and 5-nitrofuran-2-carboxylic acid 2e were used. The corresponding products 5nb, 5ob and 5ec were achieved in 63, 51 and 49% yields, respectively. 1-Boc-azetidine-3-carboxylic acid 2i also gave the corresponding product 5ib in 54% yield. Considering the carbonyl component, 1-chloropropan-2-one 3a was used to access the corresponding Ugi-adduct 5aa in 42% yield (Scheme 2 and Figure 2). Interestingly, the N-benzyl-2-(N-(1-benzyl-3,3-dimethoxy-2-oxoindolin-5-yl)acetamido)-3-hydroxy-2-methylpropanamide 5aa was obtained rather than the predictable compound with the 3-chloride-2-methylpropanamide group. We believe that a nucleophilic substitution occurs due to the presence of acetic acid 2a as reaction component. Aliphatic aldehydes with small chain 3b and 3c were used successfully in the reaction approach, as expected. Also, the aromatic 2-chlorobenzaldehyde 3d was used and the desired compound 5ad was obtained in 36% yield (Scheme 2 and Figure 2).
Scheme 2: Library of α-acetoamide carboxamide oxindole hybrids 5 easy accessed via the Ugi4CR.

Like oxindole scaffold, 1,2,3-triazole is also considered a privilege unit in drug discovery since compounds having this structure have a broad spectrum of biological activities, and have been widely used to create anti-cancer drug candidates.[24-25] The copper-catalysed azide–alkyne cycloaddition (CuAAC) reaction, or commonly entitled “click” chemistry, is a widely and straightforward tool to access the 1,2,3-triazole ring.[26-27] Due to the presence of an alkyne group on the Ugi-adduct 5bb (Scheme 2) we decided to use the CuAAC reaction to introduce a 1,2,3-triazole unit into the scaffold. Benzyl azide 6, obtained using a previously reported procedure [27], was used in the CuAAC reaction. The α-acetoamide carboxamide 1,2,3-triazole
oxindole hybrid 7 was easily obtained in 61% yield using Cu(OAc)$_2$ as catalyst, ascorbic acid, DMF as solvent, and microwave reaction conditions (120ºC, 30 minutes) (Scheme 3).

Scheme 3: Microwave assisted CuAAC reaction to access $\alpha$-acetoamide carboxamide 1,2,3-triazole oxindole hybrid 7.

Resourcefulness of the Ugi4CR and preliminary SAR studies [16] lead us to synthesize a third library of oxindole-derivatives, using trifluoroacetic acid (TFA), in mild reaction conditions, to afford the corresponding $\alpha$-acetoamide carboxamide isatin hybrids 8 from the 3-protected oxindole counterparts 5 and 7, in moderate to good yields (Scheme 4). The best yield was obtained when 3-protected oxindole derivatives 5 possess an aromatic or heterocyclic unit substituted in the 5-amide position of the oxindole ring. Compounds 8c, 8d, 8e and 8m were obtained in 83, 72, 74 and 84% yield, respectively. Exception was noticed for the $N$-heterocycle units (pyridine and 1$H$-pyrrole) substituted in the same position, since compounds 8g, 8l and 8i were obtained in 56, 51 and 36% yield, respectively. The 1,2,3-triazole hybrid isatin compound 8n was obtained in 56% yield. Compounds 5ib and 5hb, with $N$-Boc protected-heterocycle units in the 5-amide position of the oxindole ring (Scheme 2) failed to afford the corresponding 3-deprotected isatin hybrids, since only decomposition by-products (not identified) were obtained in the reaction outcome.
Scheme 4: Library of α-acetoamide carboxamide isatin hybrids 8 easy accessed via deprotection reaction on the Ugi-adducts 5 and 7. TFA: trifluoroacetic acid.

**Antiproliferative activity**
Considering the antiproliferative potential activity of these compounds, we screened 14 α-acetoamide carboxamide isatin hybrids against six human solid tumour cell lines. The panel of cell lines comprised non-small cell lung carcinomas A549 and SW1573, cervical adenocarcinoma HeLa, breast cancer HBL-100 and T-47D, and colon adenocarcinoma WiDr. The half-maximal growth inhibitory concentration (GI\textsubscript{50}) values after 48 hours of exposure were calculated for each compound (Table S1). The standard anticancer drug cisplatin (CDDP) was used as positive control. The results are best viewed as GI\textsubscript{50} range plot (Figure 3). The compounds were classified in three groups according to the GI\textsubscript{50} range plot. The first group included the most active compounds \textit{8d}, \textit{8h} and \textit{8k}. These compounds exhibited antiproliferative effects in the range 1–10 µM against all cell lines. The second group comprised the less potent compounds, which were \textit{8a}, \textit{8b}, \textit{8g} and \textit{8i}. In this group, the GI\textsubscript{50} values were over 10 µM in all cell lines tested. Finally, the third group enclosed the compounds that displayed a larger GI\textsubscript{50} range with relevant activity against some cell lines (GI\textsubscript{50} <10 µM), but less potent against the others (GI\textsubscript{50} >10 µM). However, from the GI\textsubscript{50} values it is not easy to infer structure-activity relationships. Overall, the biological results point out the relevance of these isatin hybrids as privileged scaffolds for the development of new therapeutically relevant substances.
Figure 3: GI$_{50}$ range plot against human solid tumour cell lines of investigated $\alpha$-acetoamide carboxamide isatin hybrids. Green most potent, yellow intermediate, red less potent.
Conclusion

Two new families of $\alpha$-acetoamide carboxamide oxindole and isatin hybrids were synthesized efficiently using the sustainable and efficient Ugi4CR approach. Easy access to isatin from the 3-protected oxindole scaffold was demonstrated using mild reaction conditions. Flexibility of the carboxylic acid component and also the carbonyl one (ketone/aldehyde) was exhibited in the library of Ugi-adducts obtained in moderate to good yields, in a fast and clean reaction process. Among the library of $\alpha$-acetoamide carboxamide isatin hybrids, 14 were tested regarding their antiproliferative activity. Compounds 8d, 8h and 8k were found to be the most potent ones, with GI$_{50}$ values in the range of 1-10 $\mu$M. Further studies on the mode of action and lead-discovery are in progress and will be reported shortly.

Supporting Information

Supporting information file_SI

Experimental procedures, analytical data, NMR spectra and biological assays.

Acknowledgements

C. S. M. thanks the Norma transitória for funding through the program DL 57/2016 (project UIDB/50006/2020|UIDP/50006/2020). A.G.-B. thanks the Asociación Española Contra el Cáncer (AECC) de Santa Cruz de Tenerife for predoctoral grant PRDTF233958GONZ.
Funding

This work received financial support from PT national funds from Fundação para a Ciência e Tecnologia/Ministério da Ciência, Tecnologia e Ensino Superior (FCT/MCTES) 2022.02910.PTDC and the Spanish Government (Project PID2021-123059OB-I00 funded by MCIN/AEI/10.13039/501100011033 / FEDER, UE).

References


