Ugi reaction synthesis of oxindole-lactam hybrids as selective butyrylcholinesterase inhibitors

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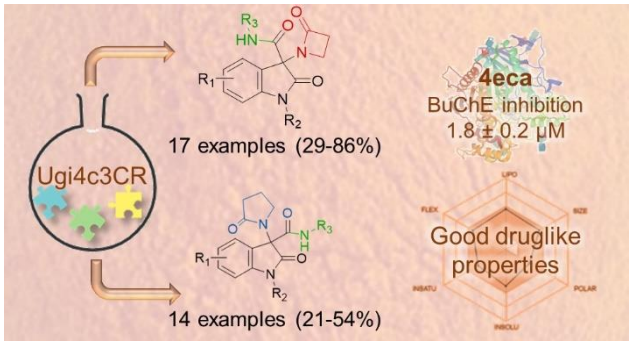
ABSTRACT

Molecular hybridization is a valuable approach in drug discovery. Combining it with multicomponent reactions is highly desirable, since structurally diverse libraries can be attained efficiently in an eco-friendly manner. In this work, isatin is used as key building block for the Ugi 4-center 3-component reaction synthesis of oxindole-lactam hybrids, under catalyst-free conditions. The resulting oxindole-β-lactam and oxindole-γ-lactam hybrids were evaluated for their potential to inhibit relevant central nervous system targets, namely cholinesterases and monoamine oxidases. Druglikeness evaluation was also performed, and compounds **4eca** and **5dab** exhibited great potential as selective butyrylcholinesterase inhibition, at the low micromolar range, with an interesting predictive pharmacokinetic profile. Our findings herein reported suggest oxindole-lactam hybrids as new potential agents for the treatment of Alzheimer's disease.

Keywords: isatin, multicomponent reactions, Ugi reaction, Alzheimer's disease, oxindole-lactam hybrids

ToC Graphic


Ugi4c3CR
17 examples (29-86%)
14 examples (21-54%)
4eca
BuChE inhibition
1.8 ± 0.2 μM
Good druglike
properties

Multicomponent reactions (MCRs) emerged as a key tool for diversity-oriented synthesis in Medicinal Chemistry. The opportunity to integrate in a one-step approach three or more reactants in a single chemical framework, not only allows the quick preparation of highly substituted libraries, with different substitution patterns, as well as contributing to faster identification of hit compounds, and even in the hit-to-lead optimization process. In recent years, several efforts have been made in order to integrate MCRs in drug discovery programs, and the number of publications reporting the application of these reactions in the synthesis of biologically active compounds have soared.[1-4] One more advantage of MCRs is their inherent sustainability, as they usually allow high atom economy, decreasing the number of required synthetic steps. These incredible chemical transformations are excellent tools for the successful implementation of the 12 principles of green chemistry.[5, 6]

Among the wide diversity of MCRs, the Ugi reaction, established by the Estonian-born German chemist Ivar Karl Ugi in 1959,[7] is one of the most relevant reactions for drug discovery settings, as it integrates the structural features of, classically, an aldehyde, a carboxylic acid, an amine and an isocyanide in a single product.[8, 9] This allows the generation of two new amide bonds, and it is well established that amides are the most commonly occurring functional group in bioactive molecules.[10-12] As many biological targets are proteins, peptidomimetics emerged as a very relevant class of compounds in drug discovery, for their potential interaction with multiple targets, in a polypharmacology context.[13, 14]

Another important concept in the realm of polypharmacology is molecular hybridization. By incorporating different pharmacophoric moieties in a single chemical framework, namely the so-called privileged structures, it is often observed a synergetic effect on the therapeutic potential of new drug candidates, often due to multitarget interaction activity.[15-20] Several research groups focused their attention on the application of isatin as an outstanding feedstock for the generation of libraries via MCRs.[21, 22] Oxindoles, often obtained from reactions using isatin as starting material,[23-25] and lactams, namely β- and γ-lactams.[26, 27] are well-known privileged structures present in a wide diversity of scaffolds, with a plethora of biological activities reported (**Figure 1**).[28-30]

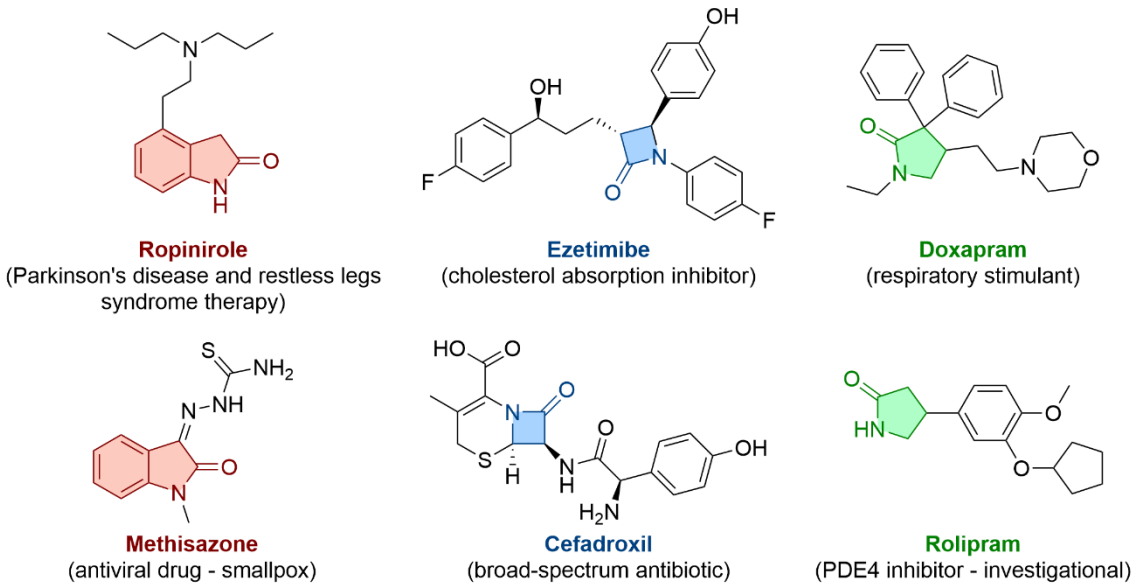


Figure 1. Examples of drugs bearing the oxindole, β- and γ-lactams heterocycles.

Herein we have explored the 4-center 3-componet Ugi reaction (U4c3CR) involving isatin to prepare a library of oxindole-β-lactams for developing druglike hybrid molecules, increasing the scope of *N*-unsubstituted isatins used as starting materials, and most importantly, accessing new libraries of oxindole-γ-lactam hybrids. The potential of these molecules on cholinesterase (ChE) inhibition has also been evaluated, showing promising results for further development of therapeutic drug candidates against Alzheimer's disease, especially as selective butyrylcholinesterase (BuChE) inhibitors. Selected compounds were also evaluated as potential inhibitors of monoamine oxidase (MAO A and MAO B).

Using the best reaction conditions reported by Rainoldi *et al*.,[31] two libraries were effectively generated, using the reagent scope depicted in **Figure 2**. A wide variety of isatins were suitable to perform the U4c3CR, as well as isocyanides, with the exception of 2-morpholinoethyl isocyanide (**2e**), which did not lead to the formation of the desired product. Four different amino acids were screened, with 3-aminopropanoic acid (or β-alanine (**3a**)) and 4-aminobutanoic acid (or γ-aminobutyric acid (**3b**)) affording the corresponding β- and γ-lactam derivatives, respectively. 5-Aminopentanoic acid (or 5-aminovaleric acid, (**3c**)) and 6-aminohexanoic acid (or ε-aminocaproic acid, (**3d**)) were also evaluated, however the corresponding δ- and ε-lactam derivatives were not formed, indicating that increasing the chain size of the amino acid derivative prevents the intramolecular cyclization and therefore the formation of larger lactam rings under the reaction conditions tested, which included performing the reaction in the presence of excess of amino acid and isocyanide components and known catalysts of the Ugi reaction, $InCl_3$ and $ZnCl_2$.

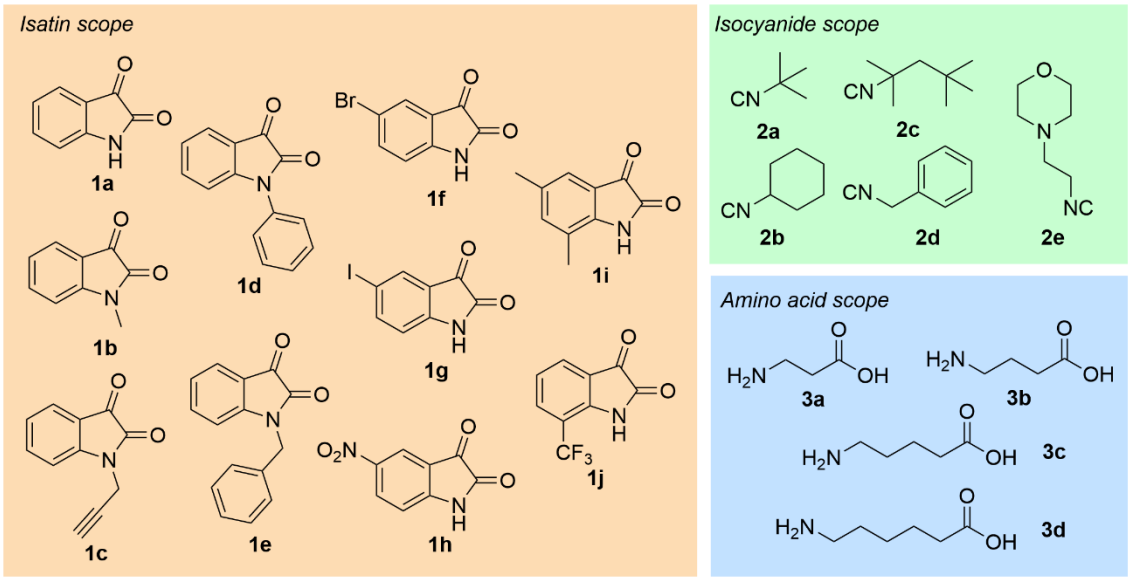
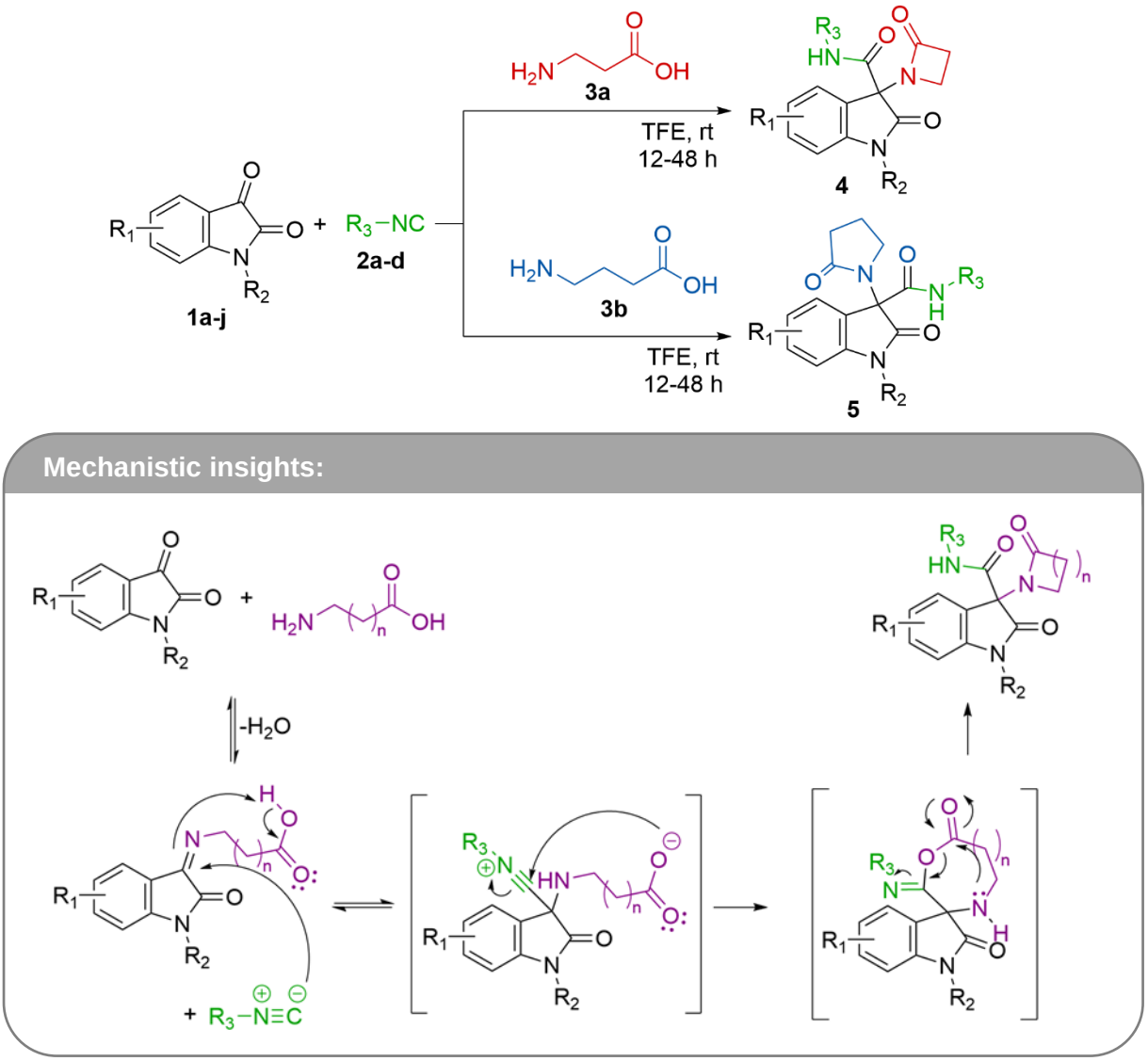


Figure 2. Isatin, isocyanide and amino acid components scope for the U4c3CR.

The overall synthetic approach is depicted in **Scheme 1**. This approach, promoted using acidic and protic 2,2,2-trifluoroethanol (TFE) as reaction media,[32] proved to be an efficient one-step methodology for synthesis of several β- and γ-lactam-oxindole hybrids (see supporting information for experimental details). The generated library, as well as the respective isolated yields, are shown in **Figure 3**.



Scheme 1. Synthetic approach for the U4c3CR.

It is noticeable that increasing the size of the lactam ring leads to an overall decrease of the yields. The reaction yield is mostly influenced by the substituents at the aromatic ring of isatin, while *N*-substituted isatins allow similar yields comparatively to the ones obtained using *N*-unsubstituted isatin. The different alkyl isocyanides tested did not lead to significant differences in the yields, although aliphatic isocyanides tend to display higher ones. The structural characterization of the 31 oxindole-lactam hybrids reported, including the four previously reported is given in the supporting information.[31]

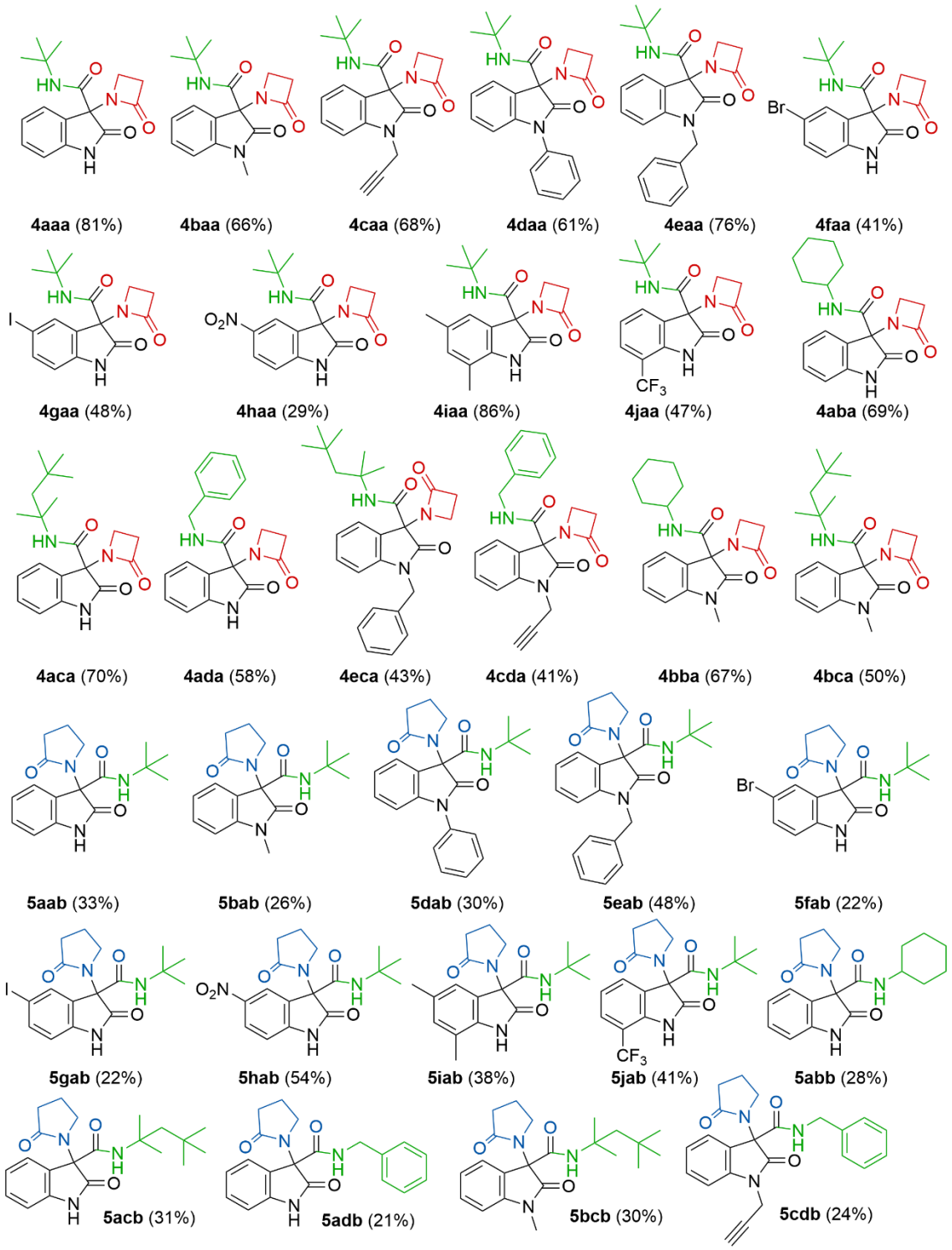


Figure 3. Library of oxindole-β-lactam and oxindole-γ-lactam hybrids.

Druglike properties of the synthesized compounds, as well as some of their most relevant physico-chemical properties for a good pharmacokinetic profile assessment were evaluated *in silico*. We selected SwissADME® to perform this evaluation, as this easy-to-use, free web tool is very versatile and provides a wide diversity of information in a very efficient way.[33] The other advantage of this suit is the availability of five key

druglikeness filters – Lipinski (Pfizer) rule of five,[34, 35] Ghose (Amgen) filter,[36] Veber (GSK) filter,[37] Egan (Pharmacopeia) filter,[38] and Muegge (Bayer) filter.[39]

Three important descriptors of the druglikeness compliance of new drug candidates are the number of hydrogen bond acceptors and receptors, as well as the number of rotatable bonds. The results obtained for all the new compounds are depicted in **Figure 4**. Gratifyingly, the key parameters (referred to above) for our library of β- and γ-lactam-oxindole derivatives fell within the specifications established for three of the key druglikeness filters, namely the Lipinski, Muegge and Veber filters.

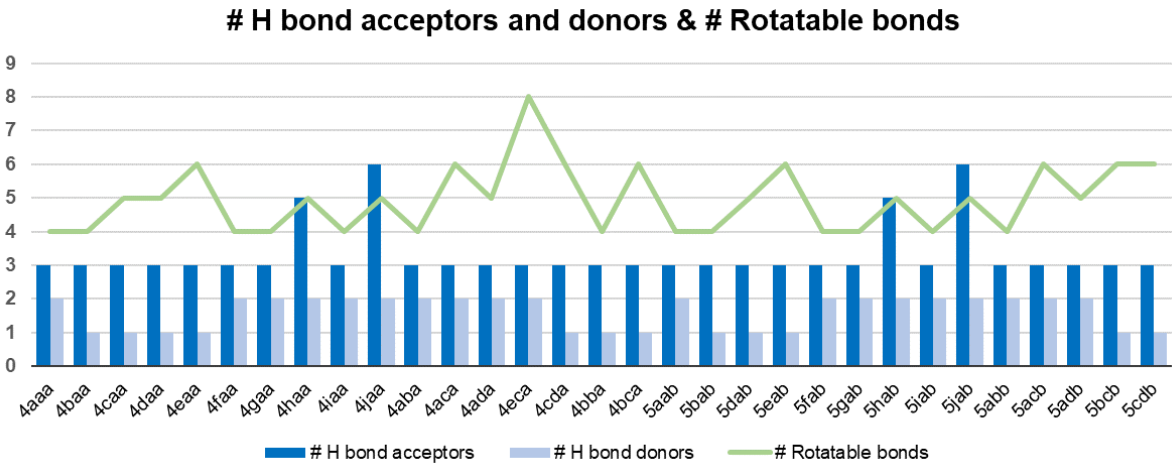


Figure 4. Calculated hydrogen bond acceptors, hydrogen bond donors, and rotatable bonds for the synthesized library of oxindole-lactam hybrids.

Molecular weight (MW) is another important property evaluated by several filters, including the Lipinski filter (MW≤500 Da), the Ghose rule (160≤MW≤480 Da), and the Muegge filter (200≤MW≤600 Da). All the compounds reported present MWs within the established intervals. Furthermore, and considering the central nervous system (CNS) distribution of the biological targets evaluated in this work, it is important to take into account that CNS drugs usually exhibit reduced MWs, usually in or below a 400-600 Da range. Marketed CNS-acting drugs display a MW mean value of 310 Da.[40] The lipophilicity (evaluated according to the calculated partition coefficient, CLogP) is another important feature to consider in the drug discovery process. Its value is evidenced by being one of the properties taken into account in four out of the five main filters - Lipinski filter (CLogP≤5), Ghose filter (-0.4≤CLogP≤5.6), Egan filter (CLogP≤5.88), and Muegge filter (-2≤CLogP≤5). Optimal blood-brain barrier (BBB) permeation is attained by compounds displaying a CLogP ranging between 1.5 and 2.7, with mean value of 2.1, with marketed CNS-acting drugs possessing a CLogP

mean value of 2.5.[40] **Figure 5** correlates these two features for the synthesized library, with all of them falling within the established parameters for all the filters.

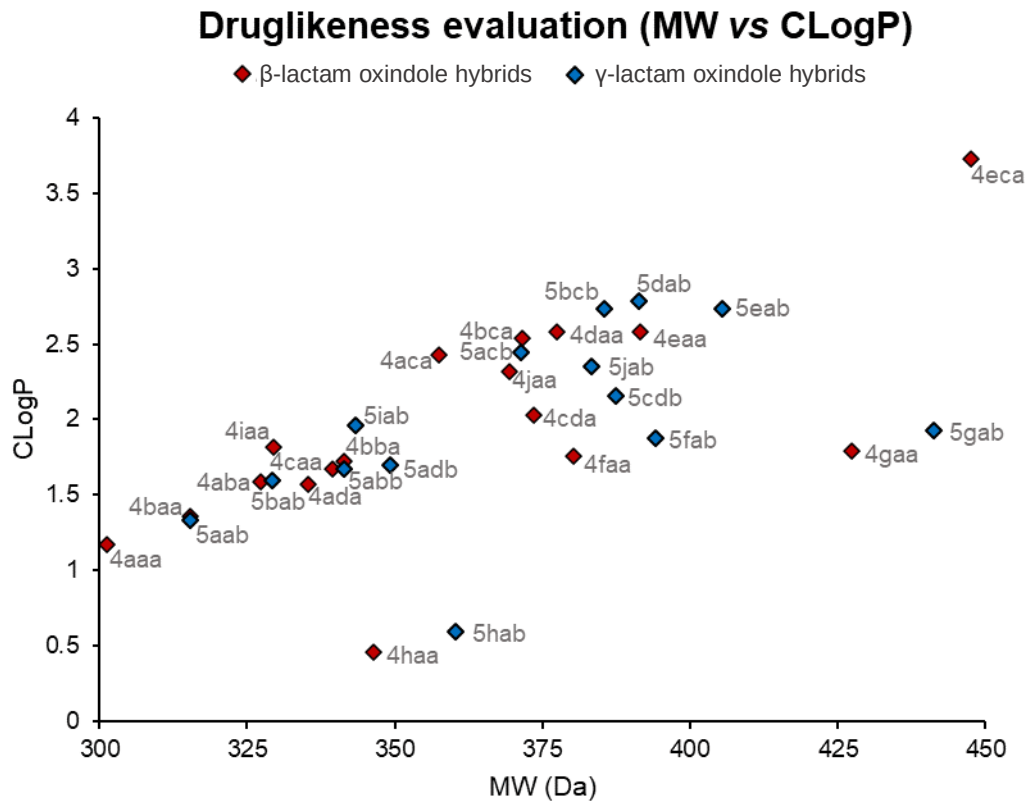


Figure 5. Relation between MW and CLogP of the synthesized compounds.

A BOILED-Egg (Brain Or IntestinaL EstimateD permeation method) model can also be assessed using SwissADME. This model predicts the behavior of the synthesized molecules in what concerns their ability to cross the gastrointestinal barrier via passive diffusion (white area), making them suitable candidates for oral administration, and also their ability to reach the central nervous system targets, by crossing the BBB (yolk/yellow area).[41] These calculations take into consideration two important properties – the lipophilicity (in this case, WLogP, calculated according to Wildman and Crippen method[42]), and the topological polar surface area (TPSA), a property which is considered in three out of five filters (Veber filter - TPSA≤140 Å^2; Egan filter - TPSA≤131.6 Å^2; and Muegge filter - TPSA≤150 Å^2). As depicted in **Figure 6**, all the synthesized compounds exhibit good predictive gastrointestinal absorption, making them suitable candidates for oral administration. However, BBB permeation as expected, is exclusively exhibited by some *N*-substituted oxindoles, including *N*-phenyl (**4daa**, **5dab**) and *N*-benzyl-oxindole (**4eaa**, **4eca**, **5eab**) derivatives. Those compounds with very greasy amide side-chains, like **4bca** and **5bcb** were predicted to easily cross the BBB.

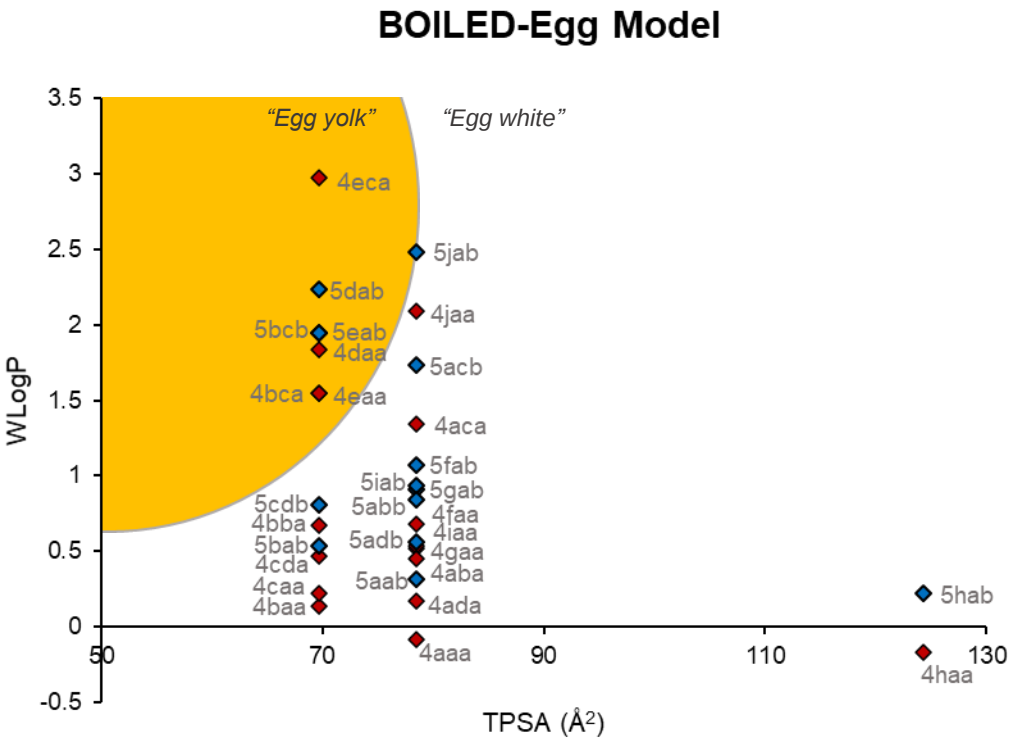


Figure 6. BOILED-Egg model for the oxindole-lactam hybrids synthesized *via* U4c3CR.

SwissADME also evaluates the presence of Pan-Assay Interference Compounds (PAINS), which possess the ability to interact with multiple targets, and therefore can be wrongly identified as hit compounds in one specific screening, however further development of such compounds is undesirable due to their off-target effects.[43, 44] None of our compounds were red-flagged in this regard. Furthermore, all the compounds comply with the five druglikeness filters, which makes them promising drug candidates for further development (see supporting information for experimental details).

ChE inhibition potential of the synthesized compounds was performed using model cholinesterases, namely AChE (*Electrophorus electricus*) and BuChE (equine serum) (**Table 1**) (see supporting information for experimental details). Concerning AChE inhibition, only one compound, **5hab**, showed moderate inhibitory activity (IC_{50}= 45 μM). However, more promising results were achieved against BuChE, indicating a great potential for these oxindole-lactam hybrids to act as selective BuChE inhibitors.

Table 1: ChE inhibition results (IC_{50} and K_i for the most promising compounds).

Inhibition of cholinesterases (IC_{50}, μM)[a.b]			
Compound	**AChE (*Electrophorus electricus*)**	**BuChE (equine serum)**	**K_i (μM)**
4aaa	>100	>100	-
4baa			
4caa			
4daa		22 ± 1	
4eaa		51 ± 2	
4faa		>100	
4gaa		80 ± 1	
4haa		>100	
4iaa		57 ± 5	
4jaa		>100	
4aba			
4aca		42 ± 5	
4ada		>100	
4eca		1.8 ± 0.2	K_{ia} = 1.8 ± 0.1 K_{ib} = 4.8 ± 0.6 (Mixed inhibition)
4cda		>100	-
4bba			
4bca			
5aab		54 ± 7	
5bab		>100	
5dab		6.2 ± 0.3	K_{ia} = 6.1 ± 1.1 K_{ib} = 18 ± 6 (Mixed inhibition)
5eab		32 ± 3	-
5fab		>100	
5gab		68 ± 13	
5hab	45 ± 5	94 ± 3	
5iab	>100	>100	
5jab			
5abb		44 ± 2	
5acb		8.4 ± 0.1	K_{ia} = 7.4 ± 1.8 K_{ib} = 15 ± 1 (Mixed inhibition)
5adb		71 ± 3	-
5bcb		18 ± 1	
5cdb		>100	
Galantamine	2.7± 0.2	3.9 ± 0.3	-

[a][S] = 112 μM; [b]A set of 5-6 different inhibitor concentrations was used, and the data were obtained in duplicate and expressed as the mean ± SD.

Title compounds can be categorized in three different groups according to their potency towards BuChE: inactive (IC_{50} > 100 μM), moderate inhibitors (18 μM < IC50 < 94 μM), or strong inhibitors (IC_{50} < 10 μM). The latter group of compounds (**4eca**, **5dab**, **5acb**) are the most promising ones, all of them behaving as mixed inhibitors,

that is, they can bind both the free enzyme and the E–I complex. Moreover, compound **4eca** was slightly more potent than galantamine (IC_{50}= 3.9 μM), one of the current cholinesterase inhibitors in clinical use against Alzheimer's disease, and used herein as a positive control.

Taking a closer look to the structures of the synthesized compounds and the exhibited activity, a pattern can be observed. The γ-lactam derivative is always more active than the β-lactam counterpart, which is either less active, or inactive (e. g., **5dab** *versus* **4daa**, **5acb** *versus* **4aca**, **5eab** *versus* **4eaa**, to name a few). We could also verify that oxindoles bearing substituents in the aromatic ring exhibit weak to no activity, and that alkyl isocyanides, in particular *t*-octyl isocyanide, tend to achieve more active compounds, while benzyl isocyanide leads to weak inhibitors or inactive compounds. The substitution at position 1 of the oxindole core also plays a role in the bioactivity shown by these derivatives. Propargyl and methyl derivatives leads to inactive compounds (except in the case of **5bcb**, which possesses good BuChE inhibition activity probably due to the combination of *t*-octyl and γ-lactam ring), whereas *N*-phenyl or *N*-benzyl oxindoles tend to exhibit promising activity. Integrating these results with the BOILED-Egg model, we verify that out of the three most active compounds, the two more active (**4eca** and **5dab**) predictably possess activity to cross the BBB, which is of great importance for the treatment of Alzheimer's disease. This is of major importance, as it can open the door for the development of new selective BuChE inhibitors with potential therapeutic application. Indeed, several efforts are being undertaken in recent years to achieve selective BuChE inhibitors, as such therapeutic option is still not available in clinical practice.[45-47] The BuChE role in the pathophysiology of Alzheimer's disease is also gaining attention, as recent evidence indicates this enzyme is present in high concentration in severe/late stages of Alzheimer's disease, whereas AChE depletes with disease evolution.[48-50]

In order to further study the potential of this library, selected compounds were evaluated against MAO A and MAO B enzymes, as these targets are involved in several pathologies affecting the CNS, including neurodegenerative diseases. As the propargyl moiety is present in many MAO inhibitors, we selected the compounds bearing this chain to perform this screening (**4caa**, **4cda** and **5cdb**) (see supporting information for experimental details). The results are summarized in **Table PBL2**.

Table 2. MAO A and MAO B one-point screening for compounds bearing the *N*-propargyl moiety (n=4).

	MAO A	**MAO B**
Compounds	Inhibition ± SD [%][a]	Inhibition ± SD [%][a]
Control	0.0 ± 0.8	0.0 ± 2.9
Clorgyline	100.4 ± 0.9	28.2 ± 14.0
Safinamide	-1.6 ± 3.3	97.0 ± 0.4
4caa	5.9 ± 7.1	7.4 ± 1.5
4cda	8.9 ± 0.7	7.6 ± 4.7
5cdb	13.2 ± 3.8	6.1 ± 7.0

[a] MAO inhibition was calculated as percentages related to control at a test concentration of 1 µM and given as mean ± SD of two independent experiments in duplicates.

Unfortunately, no relevant MAO inhibition was observed, with the best result being achieved by compound **5cdb**, displaying a 13.2% inhibition of MAO A at a concentration of 1 µM.

A series of β- and γ-lactam-oxindole hybrids were successfully synthesized using the versatile U4c3CR. ChE inhibition activity screening showed great potential for some of these compounds, in particular γ-lactam-oxindole hybrid, **4eca**, and β-lactam-oxindole hybrids, **5dab** and **5acb**, to inhibit selectively BuChE in the low micromolar range, and therefore more studies are currently being undertaken to further explore the potential of these compounds for the treatment of Alzheimer's disease. The hybrids exhibit great potential as new drug candidates, due to their predicted physico-chemical properties and excellent druglikeness profiles. Further studies are currently underway to explore the potential of these compounds as multi-target drug candidates.

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SUPPORTING INFORMATION

Experimental procedures for biological activity evaluation assays, as well as the general procedure for the synthesis of the described compounds, and respective characterization (including ^{1}H and ^{13}C NMR, ATR-FTIR, melting points, HRMS, as well as SwissADME® evaluation reports) are also available for this manuscript.

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