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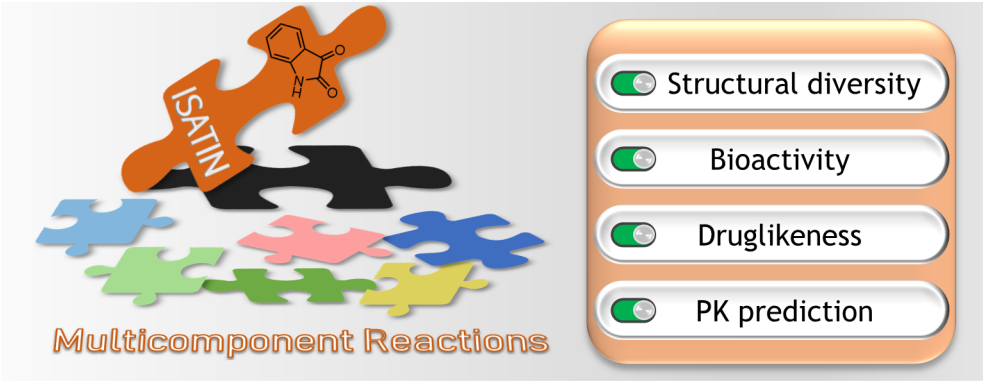

The application of isatin-based multicomponent-reactions in the quest for new bioactive and druglike molecules

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ABSTRACT

Oxindole derivatives are known for their great interest in the field of Medicinal Chemistry, as they display vast biological activities. Recent efforts concerning the preparation of oxindole derivatives using isatin-based multicomponent reactions (MCRs) constitute a great advance in generating druglike libraries fast and with wide scaffold diversity. In this review, we address those recent developments, exploring the synthetic pathways and biological activities described for these compounds, namely antitumor, antibacterial, antifungal, antiparasitic, antiviral, antioxidant, anti-inflammatory and central nervous system (CNS) pathologies. To add a new depth to this work, we used a well-established web-based free tool (SwissADME) to evaluate the most promising scaffolds in what concerns their druglike properties, namely by evaluating their compliance with some of the most valuable rules applied by medicinal chemists in both academia and industrial settings (Lipinski, Ghose, Veber, Egan, Muegge). The aim of this review is to endorse isatin-based MCRs as a valuable synthetic approach to attain new hit compounds bearing the oxindole privileged structure, while critically exploring these scaffolds druglike properties.

Keywords: multicomponent reactions, isatin, oxindole, bioactive compounds.

Introduction

The quest for bioactive molecules is the cornerstone of Medicinal Chemistry. Whether it happens *via* drug repurposing, structure-based design, privileged scaffold modifications and screening, the options are endless as regards the approaches that medicinal chemists can undertake to achieve their goals. With the ever-expanding toolbox medicinal chemists have available, it is necessary to keep in mind the multiple issues a molecule can experience from its first identification until it reaches the market. With high attrition rates, and with the growing number of new approved drugs that have deviated from the classical chemical space explored this century, pharmaceutical industries are attempting more holistic approaches when addressing drug discovery processes.[1-5]

The scientific community, as well as regulatory authorities, are more aware of the environmental impact of their actions, and Medicinal Chemistry is no exception to this. Green Chemistry applied to the field of Medicinal Chemistry is therefore a thriving field and is creating many solutions to make drug discovery and development processes more sustainable.[6] Although multicomponent reactions (MCRs) have been around since the second half of the 19th century, their role in drug discovery became pivotal in the most recent decades. By allowing the incorporation of three or more reactants into one single scaffold, MCRs unlock diversity-oriented synthesis in a faster and more eco-friendly manner, due to its high atom economy and shorter synthetic pathways when compared to more classical approaches.[7-10] Furthermore, besides the prodigious impact MCRs possess in early-stage drug discovery, they are becoming more popularly applied in the synthesis of active pharmaceutical ingredients (APIs), with the intention to reduce synthetic pathways, waste production, resources usage and, overall, improve sustainability scores for these processes.[11]

A common approach used by synthetic chemists in drug discovery is to explore the reactivity of privileged structures, incorporating them into more complex frameworks and screen their bioactive potential.[12, 13] One important privileged structure is the oxindole scaffold, present in many bioactive compounds from natural and synthetic origins, and even in APIs (**Figure 1**).[14-20]

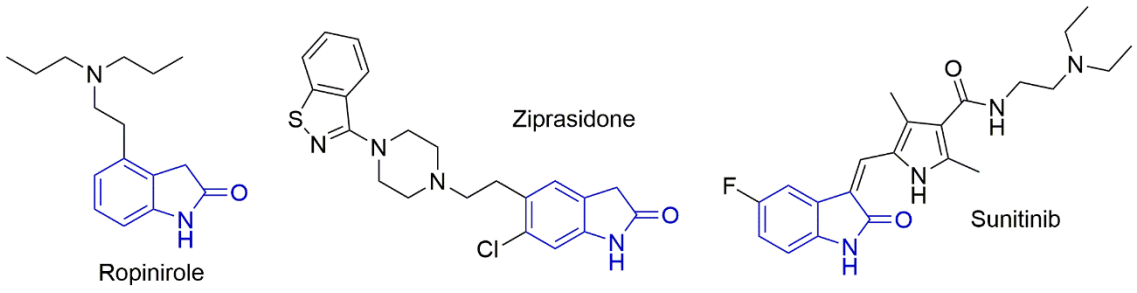


Figure 1: Examples of APIs bearing the oxindole scaffold.

Isatin is widely used as the starting point to generate oxindole-based libraries. Due to its unique reactivity, in particular at the carbonyl group at position 3, it allows a wide range of chemical transformations, including asymmetric catalytic reactions and MCRs.[21-24] Recent literature reviews are highly focused on the synthetic approaches to achieve spiro-oxindole derivatives, including via the application of MCRs to achieve such a goal.[25-28] However, a thorough literature survey focusing on the bioactivity and druglikeness of new oxindole-based molecules obtained through MCRs is long overdue.

In this work, we aim to discuss the most recent reports in the application of isatin-based MCRs in the synthesis of a wide diversity of oxindole-based libraries, highlighting the different biological activities reported. Being aware of the impact of high attrition rates in the process of drug discovery,[29, 30] we decided to take this systematic review to a new stage, complementing the reported synthesis and biological activity evaluation with *in silico* assessment of physicochemical and pharmacokinetic properties of the most promising compounds. Many promising molecules fail in later stages of the drug discovery and development pipeline due to ADMET (Absorption, Distribution, Metabolism, Excretion and Toxicity) issues and therefore the early detection of these phenomena is a milestone for medicinal chemists in both the academic lab and in a pharmaceutical industry setting. Several tools, filters and rules have been developed in order to predict and filter compounds, which structures could indicate poor druglikeness or indicate a likelihood to be Pan Assay interference compounds (Pan Assay Interference Structures (PAINS)) during bioactivity screening.[31-35] To encourage the widespread usage of such tools, several groups created easy to use and accessible platforms, so other researchers can explore the potential of such tools. Among them, we can highlight ADME-Space,[36] ADMETlab,[37] DRUDIT,[38] SwissADME,[39] just to name a few of the most recent examples. As many of these platforms present similar outputs, we decided to use the free web-based SwissADME tool in this work. This easy-to-use tool outputs include a wide range of physicochemical properties, lipophilicity evaluation, prediction of important pharmacokinetic parameters, determination of the presence of PAINS in the small-molecules evaluated, as well as druglikeness determination according to five important and well established rules – Lipinski (Pfizer) rule of five, or simply Lipinski filter,[40, 41] Ghose filter,[42] Veber (GSK) filter,[43] Egan (Pharmacia) filter,[44] and Muegge (Bayer) filter.[45] The main features/"conditions" of these rules/filters are summarized in **Table 1**. Throughout this work, the most relevant molecules evaluated received a color code based on their result – red if they are not compliant with the rule, green if they comply with the rule and therefore exhibit druglikeness. Nonetheless,

medicinal chemists need to be aware that the indications provided by these rules/filters are indicative, but not conclusive, as many examples of successful marketed drugs are beyond the compliance with such rules/filtes.[46-48]

Table 1: Main features of the five druglikeness rules evaluated throughout this work (MW=molecular weight; CLogP=calculated partition coefficient; MR=molar refractivity; TPSA=topological polar surface area).

DRUGLIKENESS				
Lipinski	*Ghose*	*Veber*	*Egan*	*Muegge*
MW ≤ 500 Da CLogP ≤ 5 #H-bond donor ≤ 5 #H-bond acceptor ≤ 10	160 ≤ MW ≤ 480 Da -0.4 ≤ CLogP ≤ 5.6 40 ≤ MR ≤ 130 20 ≤ #atoms ≤ 70	#Rotable bonds ≤ 10 TPSA ≤ 140	CLogP ≤ 5.88 TPSA ≤ 131.6	200 ≤ MW ≤ 600 Da -2 ≤ CLogP ≤ 5 TPSA ≤ 150 #Rings ≤ 7 #Carbons > 4 #Heteroatoms > 1 #Rotable bonds ≤ 15 #H-bond donor ≤ 5 #H-bond acceptor ≤ 10

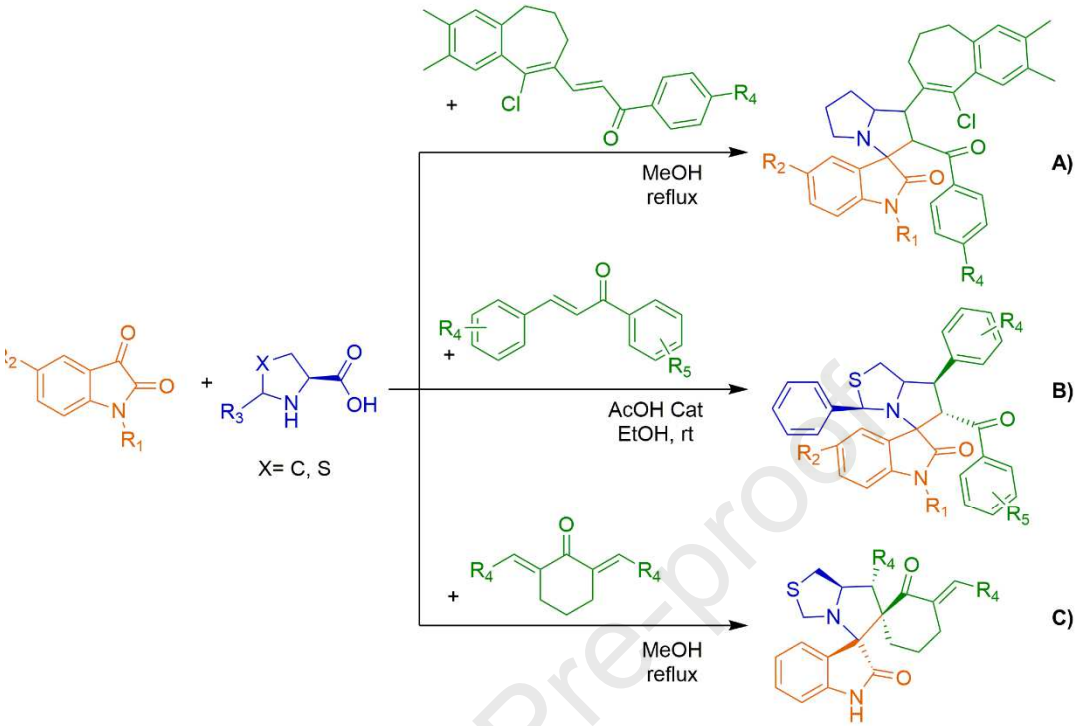
SwissADME also provides a BOILED-Egg model, which is a visual representation of the gastrointestinal (GI) absorption and blood-brain barrier (BBB) permeation of small-molecules, relevant for druglike compounds aiming to be administered orally,[49] summarizing the main features of a molecule in a bioavailability radar.

1. Anticancer Activity

Cancer is the second leading cause of mortality worldwide, with approximately 9.6 million deaths in 2018, from 18.1 million diagnosed patients around the world. According to World Health Organization (WHO), it is expected to rise by about 70% over the next two decades. Nearly one in six deaths is due to cancer.[50, 51] This alarming data, and despite more sophisticated understanding of the disease and bigger efforts for its early detection, overall mortality rates from cancer have not diminished impressively and are not expected to decrease in a near future. Many natural and synthetic anticancer agents are available on the market, however these drugs are always associated with grievous side effects. The design and discovery of effective and selective antitumor agents continues to be a huge challenge and proof of that is the massive number of reports of new promising scaffolds and their antiproliferative activity every year. The oxindole core is a structural framework found in several number of natural and synthetic compounds with a wide range of bioactivities, where anticancer emerges as one of the most significant ones.

Several research groups reported the synthesis and consequent anticancer activity evaluation of spiro-oxindole based molecules, using MCRs with isatin as starting material. In the following paragraphs the reader can find the corresponding synthesis and antiproliferative results of the most potent scaffolds, based on a spiro-oxindole framework, organized according to the number of components in the MCR, taking into consideration the structure and size of the spiro-ring, and also concerning the bioassay and the cell line studied. Examining the MCR itself and starting the triage with the use of three components as starting materials, a relatively high number of reports were found in the literature, with the purpose to obtain a spiropyrrolidine-oxindole scaffolds. Nagarapu and co-workers report the synthesis of some novel of hexahydrospiro[indoline-pyrrolizin]-one derivatives in good yields (18 examples - 83-92%) using substituted isatins, *L*-proline and the synthesized (*E*)-3-(9-chloro-2,3-dimethyl-6,7-dihydro-5*H*-benzo[7]annulen-8-yl)-1-phenylprop-2-en-1-one intermediates, in methanol under reflux temperature (**Scheme 1A**).[52] The synthetic approach consisted of a 1,3-dipolar cycloaddition reaction of azomethine ylides, generated *in situ* from isatin derivatives and proline derivatives, to activated dipolarophiles, which was also applied by other groups to get access to similar spiropyrrolidine-oxindole derivatives with some structural differences in the framework, depending on the dipolarophile used. For instance, the group of Kumar used chalcone derivatives (α,β-unsaturated ketones) as dipolarophiles in a similar 3-MCR approach to get a family of diastereoselective spiropyrrolidine-oxindole system (**Scheme 1B**).[53] Using acetic acid as catalyst, the reaction proceed smoothly under mild conditions (18 examples – 60-80% yield). Also, Barakat and co-workers reported a similar 3-MCR, using 2,6-bis[(*E*)-arylmethylidene]cyclohexanones to get access to a complex new family of di-spiro heterocycles incorporating pyrrolidine and oxindole rings (14 examples – 83-95% yield) (**Scheme 1C**).[54] These last two families of spiropyrrolidine-oxindole derivatives were obtained in a regioselective manner. The results of the antiproliferative activity evaluation of the most promising compounds are depicted in **Scheme 1D and 1E**. After testing in a HeLa cell line (derived from human cervical cancer cells), despite the higher IC_{50} values generally obtained, compared to the positive control doxorubicin, both compounds **1** and **2** showed promising results with IC_{50} values of 3.8 μM for both.[52] In contrast, the assays in breast cancer cell lines (MCF-7, T47D and MDA-MB231) identified compound **4** from Kumar's group as a promising candidate, displaying IC_{50}´s of 3.7, 4.7, and 4.2 μM, respectively,[53] and which were more potent that the positive controls used. No relevant antiproliferative activity was reported against other screened cell lines (A549 - human lung adenocarcinoma cells; SK-N-SH -

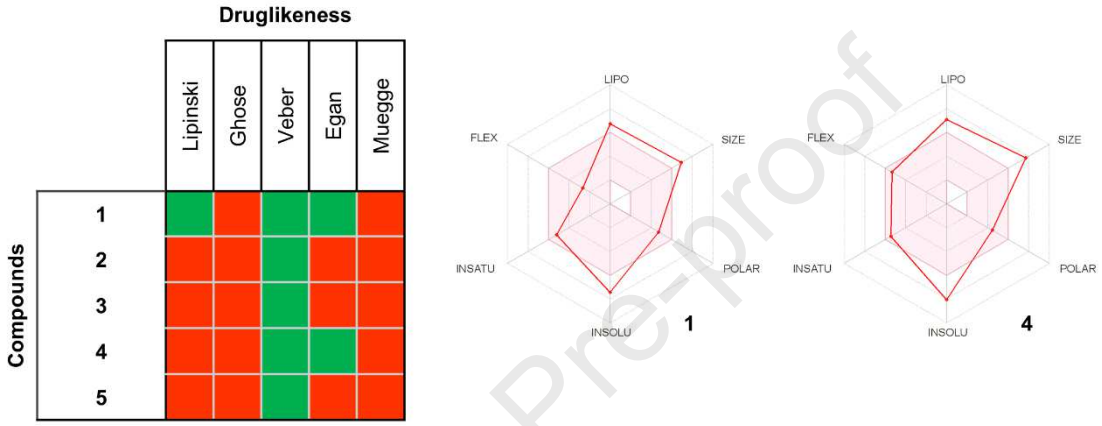
human neuroblastoma cells; HepG2: hepatocellular carcinoma cells; K562: human leukemia cells).



Compounds	R_2	n	IC_{50} (μM) A549	SK-N-SH	HeLa	HepG2	MCF-7	T47D	MDA-MB231	K562
1	NO_2	-	5.6	11.0	**3.8**	20.0	7.3	-	-	-
2	I	-	15.9	5.0	**3.8**	8.7	8.3	-	-	-
3	F	1	-	-	-	-	5.4	6.2	5.8	-
4	Cl	0	-	-	-	-	**3.7**	**4.7**	**4.2**	-
5	H	-	-	-	-	-	15.3	-	-	14.7
Doxorubicin			0.22	6.3	1.8	5.4	6.3	-	-	-
Tamoxifen	-		-	-	-	-	13.7	14.2	14.5	-
5-Fluorouracil			-	-	-	-	78.3	-	-	38.6

Scheme 1: 3-MCR for the synthesis of spiropyrrolidine-oxindole derivatives (A-C); D) Structures (D) and IC_{50} (E) of the most promising compounds.

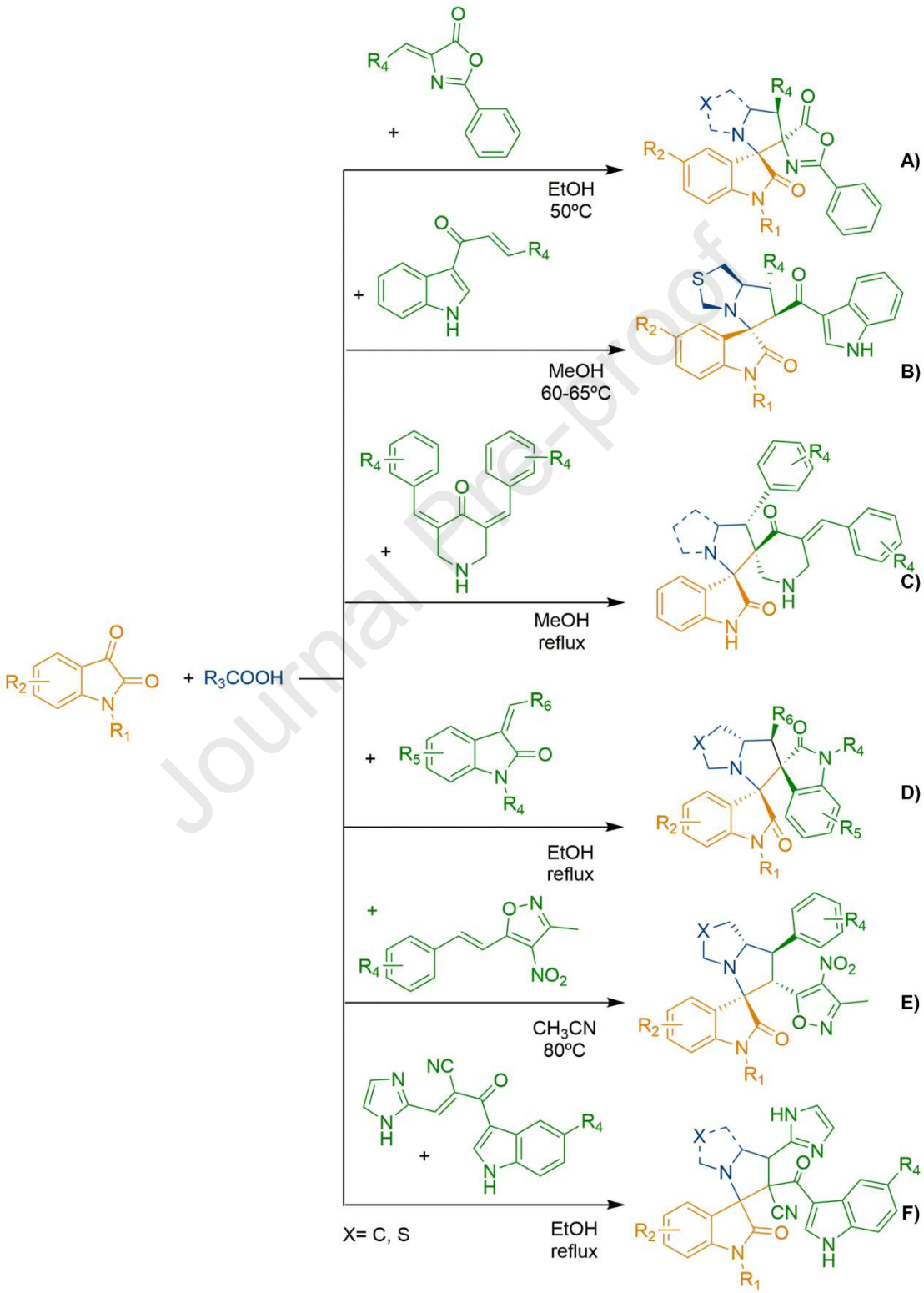
Analysis of the corresponding physicochemical properties and druglikeness of the described compounds, showed that only compound **1** was in agreement with Lipinski's rule, showing good orally bioavailability (**Scheme 2**). Despite having predictive high gastrointestinal absorption, the poor druglikeness (driven mostly by lipophilicity, solubility and size issues - see **Scheme 2** bioavailability radar of the most active compounds on human breast and cervical cancer cell lines) and inexistent BBB permeation lead us to conclude that these scaffolds will likely offer future drawbacks in the drug development pipeline.



Scheme 2: Druglikeness of the most active compounds and bioavailability radars for compounds **1** and **4**.

Taking some promising *in vitro* results and the knowledge that spirooxindole scaffolds have been identified as promising anti-cancer agents with ability to bind to several cellular receptors,[55-57] several researchers are currently working on the design of new hybrid structures, making structural modifications of the framework to get new compounds with high antiproliferative activity and improved safety profiles. The 3-MC 1,3-dipolar cycloaddition reaction was the chosen procedure due to being a direct method for obtaining spiropyrrolidine based oxindole derivatives in a one-pot fashion. The reason behind the appearance of so many reports in the literature is due to the simplicity of this methodology, allied with mild reaction conditions and short reaction times. The robust 1,3-dipolar cycloaddition reaction between azomethine ylides (thermally generated *in situ* from isatin derivatives and proline, thioproline or sarcosine) and electron deficient olefins, has been used by several groups to build new libraries of spiropyrrolidine-oxindole derivatives for antiproliferative bioassay studies. Ouyang and co-workers used substituted benzylidene-2-phenyloxazolone derivatives to develop a library (15 examples) of regio- and stereoselective oxazolones fused with spirooxindole-pyrrolidines in good yields (79-91%) (**Scheme 3A**).[58] Barakat and co-

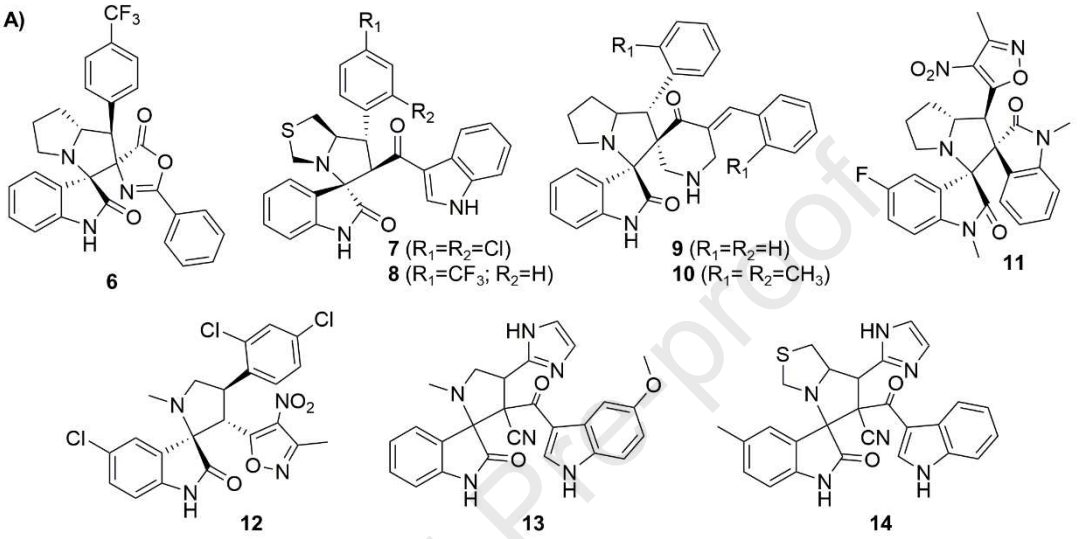
workers used a similar protocol to report a library of new thiazolo-pyrrolidine-spirooxindole linked with 3-acylindole scaffolds, also obtained in good yields (14 examples – 71-89% yield) (**Scheme 3B**).[59] Parang, Ali and co-workers also found this approach to be well-suited to the preparation of a novel library of piperidone grafted spiropyrrolidine-oxindole derivatives using 3,5-bis[(*E*)-arylmethylidene]tetrahydro-4(1*H*)-pyridinones as 1,3-dipolarophile (42 examples – 85-95% yield) (**Scheme 3C**).[60] The group of Liu and Zhou used 3-methyl-4-nitro-5-isatylidenyl-isoxazoles and 3-methyl-4-nitro-5-alkenyl-isoxazoles as 1,3-dipolarophiles for the successful synthesis of polycyclic 3,3'-pyrrolidinyl-dispirooxindoles (**Scheme 3D and 3E**). The introduction of four stereocenters in the scaffold (33 examples – in 62-85% yields considering a fused oxindole unit and 40 examples in 64-90% yields considering a isoxazole) with good diastereoselectivity (up to >20:1) was the motivation that this new isoxazole-fused spiropyrrolidine oxindoles may be potential leads against several cancer cells lines.[61, 62] Perumal and co-workers used a complex indole-type intermediate linked to an imidazole unit as the 1,3-dipolarophile to afford novel spiropyrrolidine-oxindole derivatives in good to excellent yields (29 examples – 82-95% yield) (**Scheme 3F**).[63]



Scheme 3: The 1,3-dipolar cycloaddition of azomethine ylides with electron deficient olefins applied in the synthesis of spiropyrrolidine-oxindole derivatives.

The design of new hybrid architectures is the principal objective of many groups for the generation of druglike molecules. All l the new families of spiropyrrolidine-oxindole derivatives mentioned above were screened for their cytotoxic activities against a wide spectrum of cell-lines. The most promising compounds are depicted in **Scheme 4**.

A)



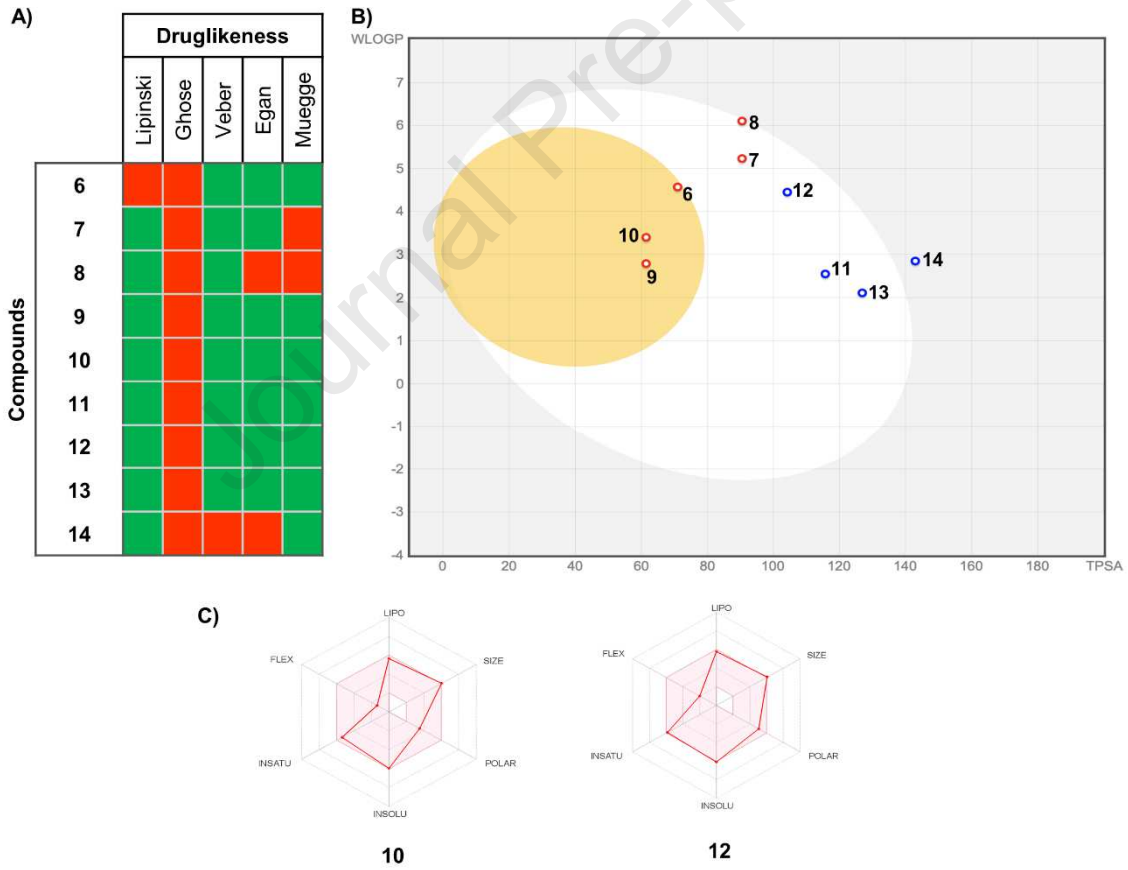
B)

Compounds	IC_{50} (μM)											
	MCF-7	MDA-MB-231	MDA-MB-468	HepG2	HCCC-9810	HuH7	HCT-116	PC-3	CCRF-CEM	SK-OV-3	K562	A549
6	8.1	**4.2**	**3.4**	8.7	7.2	7.9	-	-	-	-	-	-
7	-	-	-	7	-	-	9	6	-	-	-	-
8	-	-	-	**5.5**	-	-	**7**	**6**	-	-	-	-
9	-	-	-	-	-	-	-	-	25.2	38.9	-	-
10	-	-	-	-	-	-	-	-	**3.6**	35.8	-	-
11	-	-	-	-	-	-	-	51.6	-	-	25.0	39.7
12	-	-	-	-	-	-	-	13.1	-	-	**10.7**	21.5
13	-	-	-	-	-	-	-	-	-	-	-	66.3[a]
14	-	-	-	-	-	-	-	-	-	-	-	66.3[a]
Gefitinib	3.3	4.1	3.9	>10	>10	>10	-	-	-	-	-	-
Sorafenib	5.5	5.1	4.9	6.7	>10	7.1	-	-	-	-	-	-
Cisplatin	-	-	-	5.5	-	-	12.6	5[b] 25.8[c] 27.9[d]	-	-	23.7[c] 23.2[d]	21.2[c] 25.2[d]

Scheme 4: Structures of the most promising compounds (A) and respective antiproliferative activity (B) ([a] % activity at 25 μL/mL; [b] value reported in [59]; [c] value reported in [61]; [d] value reported in [62]) (MDA-MB-468: human breast cancer cells; HCCC-9819 and HuH7: hepatocellular carcinoma cells; HCT-116: colorectal cancer

cells; PC-3: prostate cancer cells; CCRF-CEM: lymphoblastic leukemia cells; SK-OV-3: human ovarian adenocarcinoma cells).

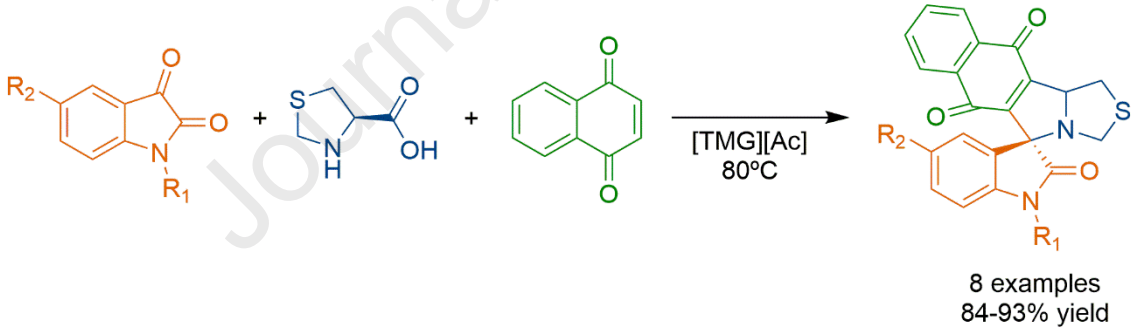
Upon analysis of the druglikeness predictions of the top nine described spiropyrrolidine-oxindoles (**Scheme 5A**) we can conclude that all the compounds (with the exception of **6**) are in agreement with Lipinski's rule of five. The violation of some remaining rules (especially the Ghose's rule) is related to the higher values of molar refractivity and in some cases high molecular weight. The Boiled-Egg model (**Scheme 5B**) shows high GI absorption for almost all of the compounds (except for compound **14**), predicting good overall oral bioavailability. Taking into account the bioassays performed in some of the carcinoma cell lines and all the physicochemical property data that was obtained, we conclude that compounds **10** and **12** could be lead compounds for the design of more potent and selective anticancer agents (**Scheme 5C**).



Scheme 5: Druglikeness rules compliance (A), Boiled-Egg model for the most active compounds (B) and bioavailability radars for compounds **10** and **12** (C).

Dandia, Jain and co-workers proposed that the design of hybrid heterocyclic systems with spirooxindole fused pyrrolo[1,2c]-thiazole skeletons and naphthoquinone

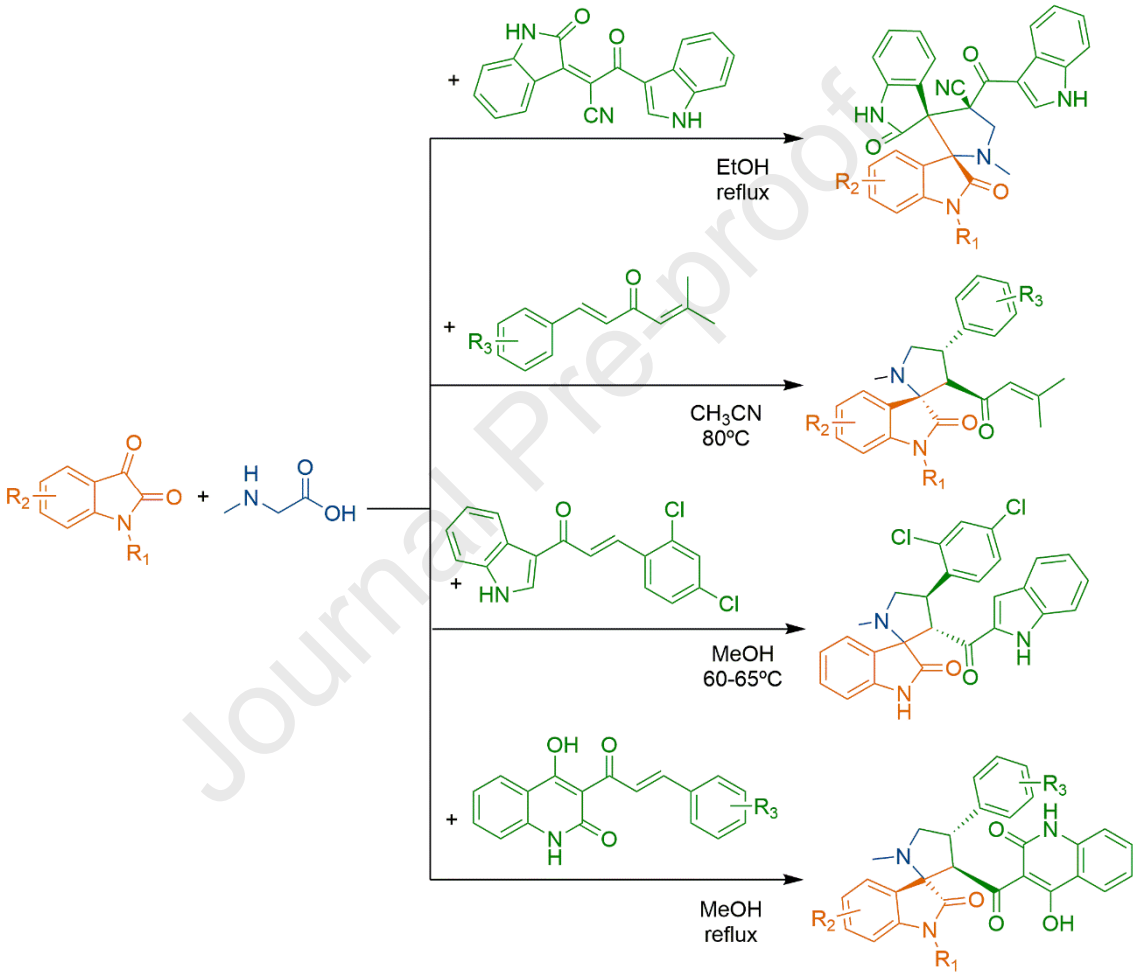
units could increase their biological activities. Consequently they reported the synthesis of a new family of spiroheterocyclic compounds, incorporating those three pharmacophoric components using a guanidium based ionic liquid (IL) (1,1,3,3-tetramethylguanidine acetate [TMG][Ac]) as green solvent.[64] Decarboxylative condensation of the substituted isatin derivative with *L*-thioproline affords the corresponding azomethine ylide which subsequently undergoes a 1,3-dipolar cycloaddition reaction with the 1,4-naphthoquinone and subsequent tautomerization and rapid oxidation under atmospheric conditions resulting in the formation of the described spiro[benzo[*f*]thiazolo[4,3-*a*]isoindole-5,3'-indoline]-2',6,11-trione derivatives (**Scheme 6**). Despite the poor substrate scope, good recyclability of the IL, mild reaction conditions and easy work-up are the main advantages of this procedure. With this small family in hand, the authors reported a preliminary study on a DNA cleavage assay, where the inhibitory potency of the compounds was evaluated. All the samples showed complete cleavage of DNA (in an agarose gel electrophoresis experiment), indicating that they should demonstrate antiproliferative activity. Small-molecule interactions with DNA continues to be a hot-topic in the field of anticancer drug development. This is because molecules that target DNA are more likely to achieve better outcomes with volunteers in cancer clinical trials when used with other drugs with different mechanisms of action.[65]



Scheme 6: Synthesis of spiro[benzo[*f*]thiazolo[4,3-*a*]isoindole-5,3'-indoline]-2',6,11-trione derivatives using a guanidium based ionic liquid as green solvent.

Several authors used sarcosine along with isatin derivatives as starting materials to form the azomethine ylide intermediates for 1,3-dipolar cycloaddition 3-MCR leading to spiropyrrolidine-oxindole derivatives with novel complex frameworks (**Scheme 7**). Perumal's group used this strategy to obtain a family of spiropyrrolidine-oxindole derivatives in good yields (22 examples – 80-92% yield) using 3-(1*H*-indol-3-yl)-3-oxo-2-(2-oxoindolin-3-ylidene)propanenitrile as dipolarophile (**Scheme 7A**).[66] The group of Zhou and Lin used dienones as dipolarophiles to access a family of novel turmerone motif-fused spiropyrrolidine oxindole derivatives in good yields (**Scheme 7B**) (25

examples – 70-93% yield). The desired products bearing adjacent chiral carbon centers were smoothly obtained also with good diastereoselectivity (>20:1).[67] Barakat and co-workers reported the synthesis of one spirooxindole analogue based on the same 1,3-dipolar cycloaddition reaction of isatin, sarcosine and the olefin (*E*)-3-(2,4-dichlorophenyl)-1-(1*H*-indol-3-yl)prop-2-en-1-one (**Scheme 7C**). In just 2 hours the desired compound was obtained with 73% yield.[68] Mohan and co-workers used a similar strategy to obtain 4-hydroxyquinolin-2(1*H*)-one grafted spiropyrrolidine hybrids (7 examples – 46-92% yield) (**Scheme 7D**).[69]

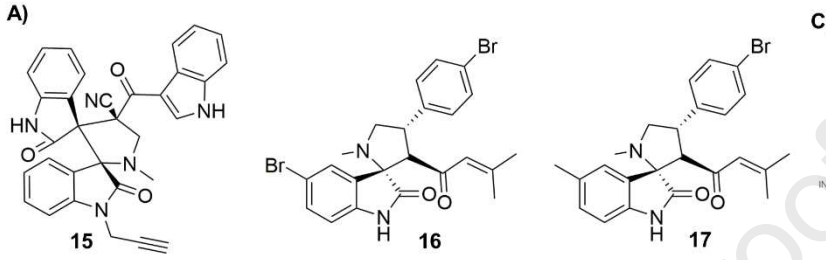


Scheme 7: The 1,3-dipolar cycloaddition of azomethine ylides formed *in situ* from isatin derivatives and sarcosine, with different electron deficient olefins used for the synthesis of new families of spiropyrrolidine-oxindole derivatives.

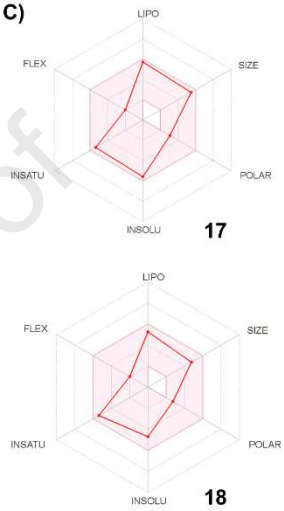
It should be noted that the despite the structural complexity of the compounds described above, their druglikeness predictions were very encouraging (**Scheme 8**). Overall, the selected compounds **15-20** presented excellent compliance with these rules, particularly **17** and **18**, which were compliant right across the board, making them good orally available drug candidates (**Scheme 8B**). The bioavailability radar for the

most promising scaffolds was also displayed on **Scheme 8C**).[67] This is quite relevant as these compounds showed better antiproliferative profiles in bioassay studies with A549 and K562 cell lines (human lung cancer and leukemia) than the bench-mark, cisplatin.

A)



C)



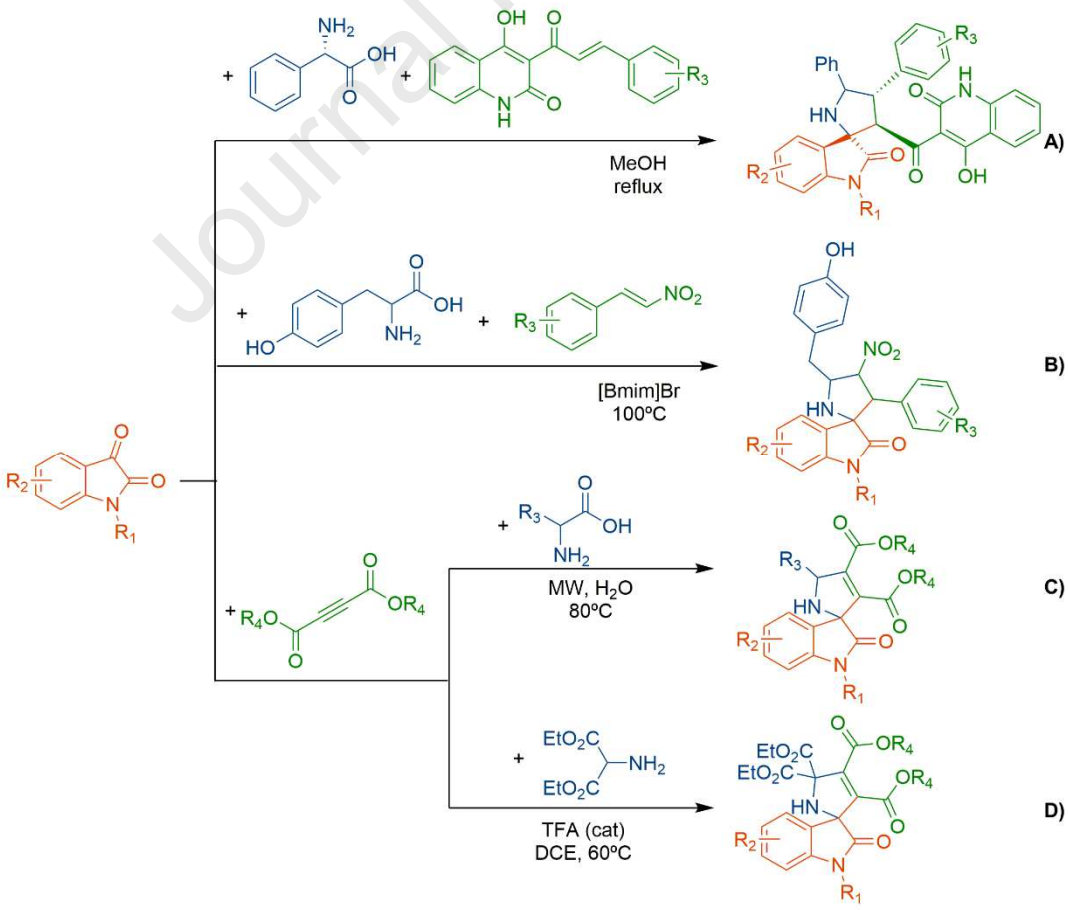
B)

Compounds	IC_{50} (μM)						Druglikeness				
	A549	K562	HepG2	MCF-7	HCT-116	HeLa	Lipinski	Ghose	Veber	Egan	Muegge
15	50	-	-	-	-	-					
16	22.9	5.7	-	-	-	-					
17	21.1	5.8	-	-	-	-					
18	19.1	7.2	-	-	-	-					
19	-	-	>50	15	<1	-					
20	-	-	-	-	-	10.7					
Cisplatin	25.7	28.9	9	9	3	-					
Doxorubicin	-	-	-	-	2.4	1.8					

Scheme 8: The structures of the most promising compounds (A), respective antiproliferative activity, druglikeness (B) and bioavailability radar for **17** and **18** (C).

Mohan and co-workers also explored a variation of the 1,3-dipolar cycloaddition reaction discussed so far, replacing the sarcosine by phenylglycine, using the same reaction conditions (**Scheme 9A**). Three new oxindole-fused 4-hydroxyquinolin-2(1*H*)-one grafted spiro-phenyl substituted pyrrolidine hybrids were obtained in good yields (63-83%) and also screened in the antiproliferative assay.[69] Using tyrosine as the amino acid, the β-nitrostyrene derivatives as dipolarophiles and common and

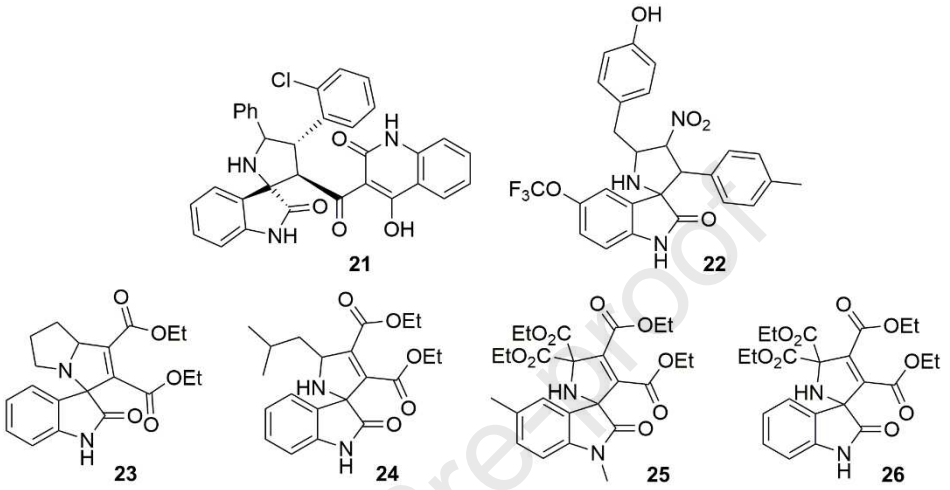
commercially available ionic liquid 1-butyl-3-methylimidazolium bromide ([Bmim]Br) as green reaction medium, Kumar *et al*. reported very recently a new family of spiropyrrolidine-oxindole derivatives with very good yields (12 examples – 88-94% yield) (**Scheme 9B**).[70] Increasing the scope of the amino acids and using electron-deficient alkynes as dipolarophiles, Meshram and co-workers reported the synthesis and corresponding cytotoxicity properties of a new family of spirooxindole derivatives (**Scheme 9C**). Several advantages of this 1,3-dipolar cycloaddition strategy using isatin derivatives, amino acids and but-2-ynedioates could be pointed out, such as good reaction yields (70-93%), suitable scope (28 examples), as well as a catalyst- and base-free approach. The use of microwave radiation significantly decreases the reaction time (10 minutes) and allowed the use of water as solvent in this synthetic transformation.[71] Using the same alkyne derivatives, the group of Zhang and Shi also reported the synthesis of a new family of spirooxindole-based 2,5-dihydropyrrole derivatives using the 1,3-dipolar cycloaddition 3-MCR strategy (**Scheme 9D**). Despite long reaction times (16 to 36 hours) and the need for a catalyst (trifluoracetic acid), excellent scope and moderate to excellent yields (36 examples – 52-99% yield) were accomplished for this new family of compounds.[72]



Scheme 9: 1,3-Dipolar cycloaddition 3-MCR strategy for the synthesis of several families of spirooxindole derivatives.

Similar to the previous examples, these new libraries of spirooxindole derivatives were also evaluated for their cytotoxic properties in some human cancer cell lines. Their druglikeness was also predicted (**Scheme 10).**

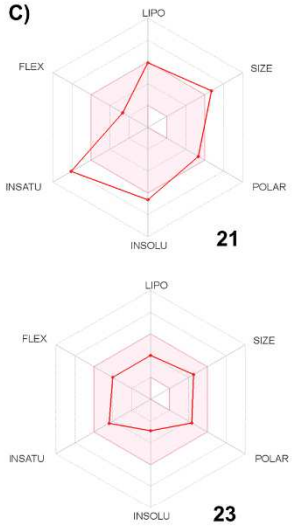
A)



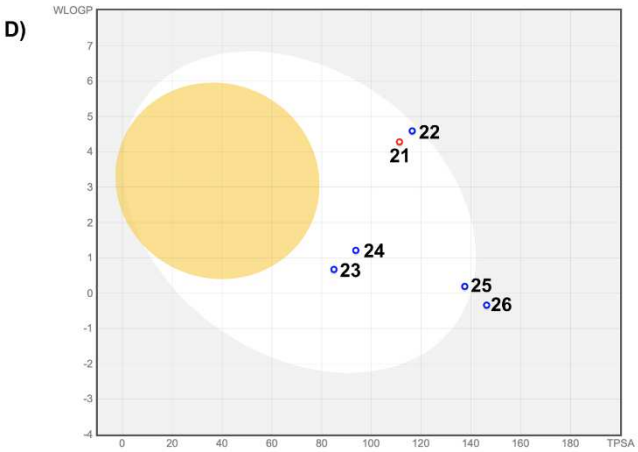
B)

Compounds	IC_{50} (μM)					Druglikeness				
	HCT-116	A549	Jurkat	MCF-7	HeLa	Lipinski	Ghose	Veber	Egan	Muegge
21	7.1	-	-	-	-					
22	-	29.6	42.9	-	-					
23	-	2.0	-	3.3	8.3					
24	-	4.5	-	5.0	>50					
25	-	-	-	20.8[a]	-					
26	-	-	-	28.0[b]	-					
Camptophecin	-	32.1	22	-	-					
Doxorubicin	2.4	1.8	-	1.6	1.9					
Sunitinib	-	7.9	-	25.4	>30					

C)



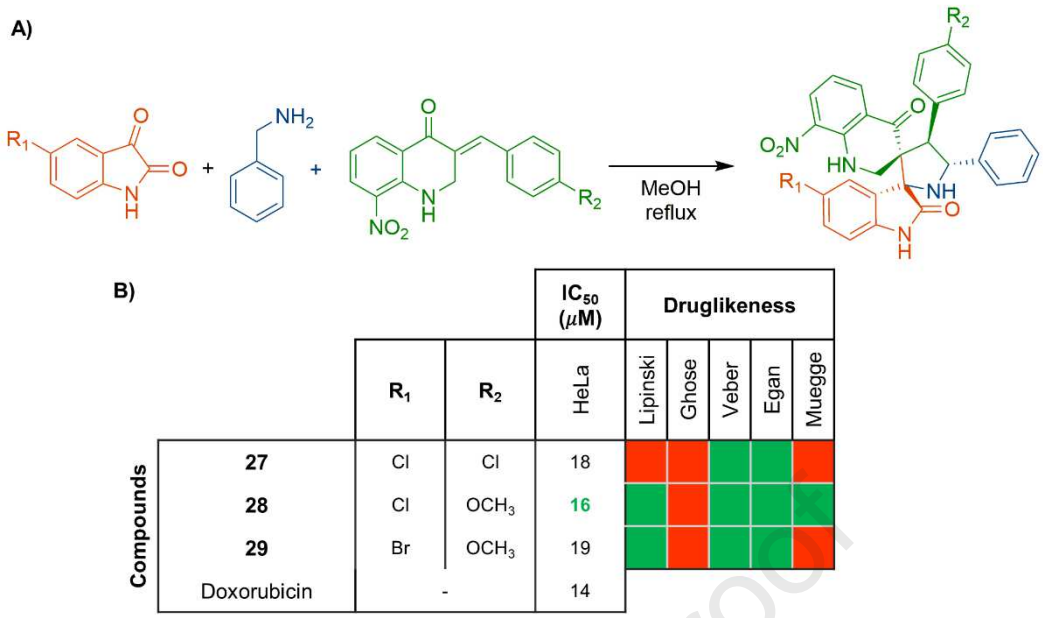
D)



Scheme 10: The structures of the most promising compounds (A) and antiproliferative activity evaluation, druglikeness (B), bioavailability radar for **21** and **23** (C) and Boiled-Egg model (D) ([a] % activity at 10 µL/mL; [b] % activity at 100 µL/mL).

Spiro-substituted-pyrrolidine oxindole derivatives (**Scheme 10**) apparently showed similar physicochemical and druglikeness profiles to their unsubstituted counterparts (**Scheme 8**), with the exception of compound **25** which does not fulfil the requirement of four of the five druglikeness rules. In terms of preliminary evaluation of the cytotoxicity, compound **25** (and also **26**) displayed poor inhibition in MCF-7 (**Scheme 10B**). Compounds **23** and **24** exhibited potent cytotoxicity against human cancer cell lines MCF-7, A549 and HeLa, compared with the control, doxorubicin. Total agreement with the druglikeness profile and high GI could indicate potential oral bioavailability (**Scheme 10C**). The problems around solubility and high molecular weight could make their BBB permeability an issue (see Boiled-Egg model in **Scheme 10D**).

Very recently, Mohan and co-workers described the one-pot 3-MC 1,3-dipolar cycloaddition reaction between azomethine ylides (formed *in situ* from the condensation of 5-substituted isatin derivatives and benzylamine) and (*E*)-3-arylidene-2,3-dihydro-8-nitro-4-quinolones (**Scheme 11A**). Despite moderate scope (12 examples), high yields (90-96%) and mild reaction conditions are certainly the main advantages of this protocol. The use of cheap and easily available benzylamine (rather than expensive amino acids) is also an advantage of this synthetic protocol for accessing complex spiropyrrolidine oxindole frameworks. They were screened against HeLa cells, and three compounds (**27-29**) were found to be the most active (**Scheme 11B** These compounds were non-cytotoxic against normal cells (human RBC cells).[73] However, the druglikeness profile was not very encouraging, since some of the compounds were non-compliant against some of the rules, like, solubility, high molecular weight and high molar refractivity, which can cause low oral availability and subsequent pharmacokinetic issues.

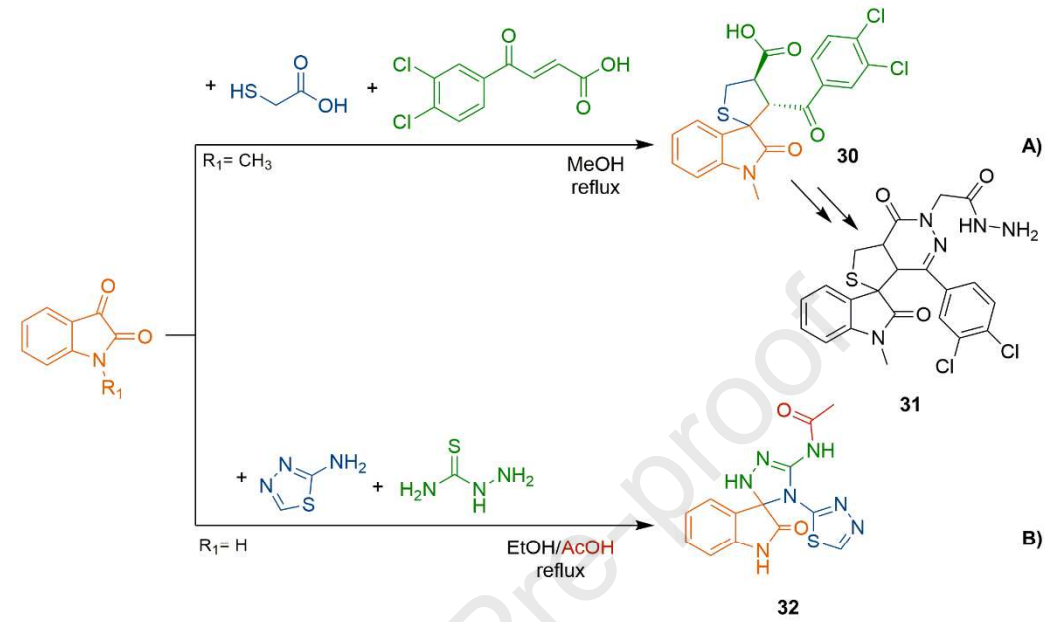


Scheme 11: Synthesis of new dispirooxindole analogues containing pyrrolidine 8-nitroquinolone unit (A) and the corresponding cytotoxic properties of the best inhibitors together with druglikeness profile (B).

Rizk and co-workers reported the synthesis of spiropyrrolidine-based thiophene oxindole derivatives using the 1,3-dipolar cycloaddition 3-MCR approach with thioglycolic acid instead of the commonly used amino acids (**Scheme 12A**). Despite the poor reaction scope, the desired spiro derivative (see as example **30**) was obtained in good yield (80%). In an attempt to expand the complexity of the framework it is worth noting that the corresponding 2-(4'-(3,4-dichlorophenyl)-1-methyl-1',2-dioxo-7',7a'-dihydro-1'*H*-spiro[indoline-3,5'thieno[3,4-*d*]pyridazin]-2'(4a'*H*)-yl)acetohydrazide **31**, was obtained in good yield (78%).[74] Also, the group of Hamama used the MCR approach with isatin, 1,3,4-thiadiazol-2-amine and hydrazinecarbothioamide in acetic acid (and ethanol) to access the *N*-(2-oxo-4′-(1,3,4-thiadiazol-2-yl)-2′,4′-dihydrospiro[indoline-3,3′-[1,2,4]triazole]-5′-yl)acetamide **32** in good yield (75% yield, **Scheme 12B**).[75]

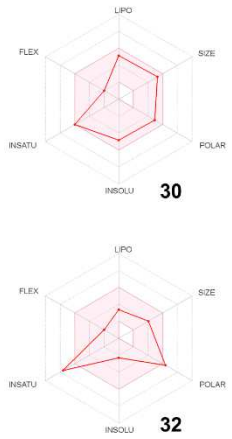
The cytotoxicity of compounds **30** and **31** was evaluated in MCF-7 and human lung fibroblast WI-38 cell lines. Despite the poor cytotoxicity results obtained for **30**, the druglike properties using the SwissADME tool (the results are depicted in **Scheme 12C**). showed that it was compliant with all five rules, and the bioavailability radar predicted good oral bioavailability. In the case of **31,** more potent cytotoxicity was in evidence for the same cell lines (**Scheme 12C**).[74] The druglikness profile for compound **32**, reported by Hamama and co-workers, with a complex spiro-2-(1,5-dihydro-4*H*-1,2,4-triazol-4-yl)-1,3,4-thiadiazole unit was far from ideal, missing the compliance with two of the five rules analyzed (**Scheme 12C**). On the other hand an

encouraging ED_{50} of 40μg/cm^3 was determined using the Ehrlich ascites carcinoma cells (EAC) cell line (exactly the same as the control 5-flourouracil).,[75] This implies that careful modifications of this scaffold should improve oral bioavailability.



C)

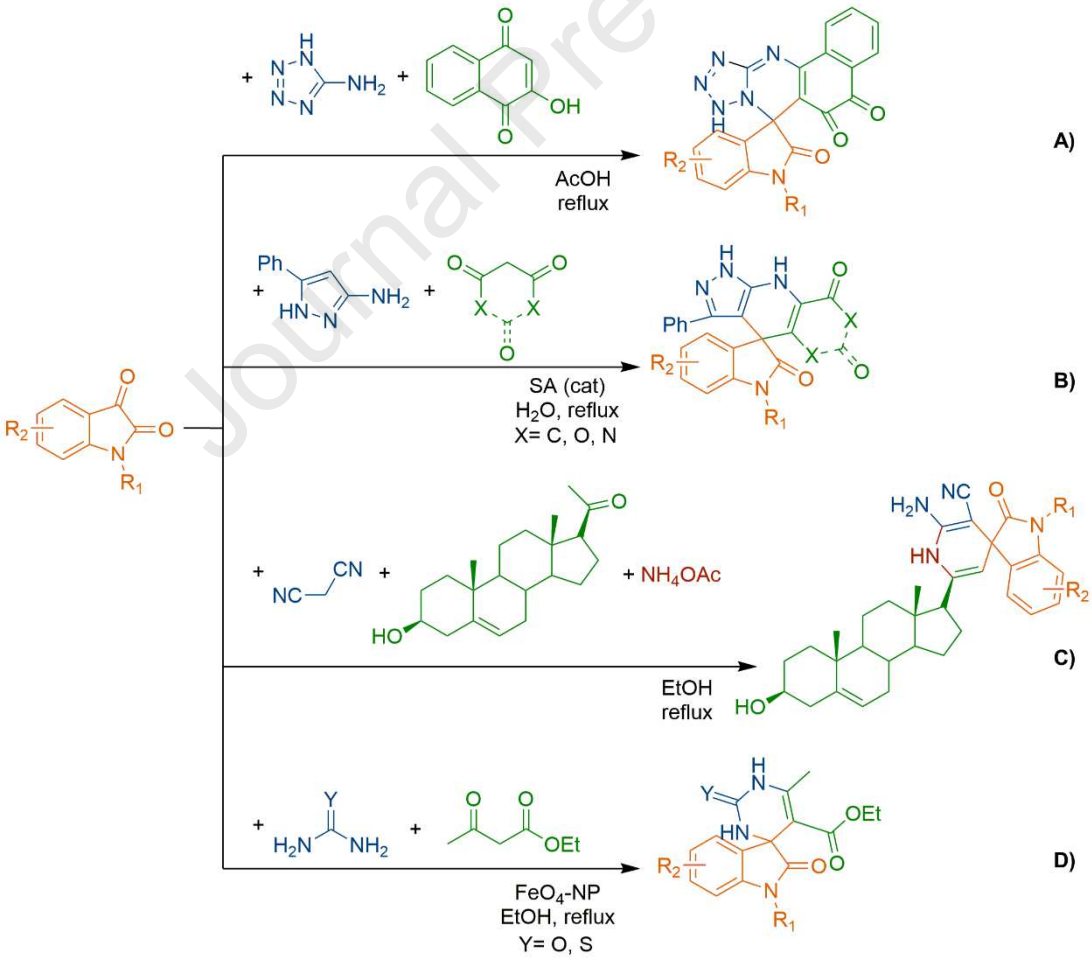
Compounds	IC_{50} (μM) MCF-7	IC_{50} (μM) WI-38	ED_{25} (μg/cm^3) EAC	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
30	36.3	55.1	-					
31	8.2	11.2	-					
32	-	-	40					
Doxorubicin	4.2	6.7	-					
5-Fluorouracil	-	-	40					



Scheme 12: The synthetic path for the 1,3-diploar cycloaddition 3-MCR using different reagents (A and B) and respective antiproliferative activity, druglikeness and bioavailability radar (**30** and **32**) (C).

Several authors reported the synthesis and corresponding antiproliferative bioassays of novel families of 6-ring spirooxindole scaffolds using 3- and 4-MCR approaches. For instance, Wu and co-workers reported the synthesis of spirooxindole-*O*-naphthoquinone-tetrazolo[1,5-*a*]pyrimidine hybrids with good scope and in general moderate yields (14 examples – 31-69% yield) using isatin derivatives, 2-hydroxy-1,4-naphthoquinone and 5-aminotetrazole (**Scheme 13A**).[76] Kamal *et al*. explored a green methodology to access pyrazolopyridine-based spirooxindoles by the 3-MCR

between isatin derivatives, 5-phenyl-1*H*-pyrazol-3-amine and tetronic acid (furan-2,4(3*H*,5*H*)-dione) or barbituric acid (pyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione) (**Scheme 13B**). The reaction showed good affording the products with good to excellent yields (34 examples – 77-99% yield), using water as reaction medium and sulfamic acid (SA, with chemical formula H_2NSO_3H) as reusable catalyst.[77] Liu and co-workers reported a 4-MCR protocol using pregnenolone, isatin derivatives, malononitrile and ammonium acetate to access steroidal dihydropyridinyl spirooxindoles with good yields and good scope (15 examples – 73-83% yield) (**Scheme 13C**). Mechanistically, it is expected that the reaction undergoes a Knoevenagel condensation and consequent Michael addition, ending with isomerization and cyclization steps to afford the desired products.[78] Mathew and co-workers used the well-known 3-MC Biginelli reaction approach to obtain a family of novel spirooxindole-dihydropyrimidinones in moderate yields (10 examples – 68-76% yield) (**Scheme 13D**). Metal nanoparticles (FeO_4-NP) were used as the catalyst used to perform this chemical transformation.[79]

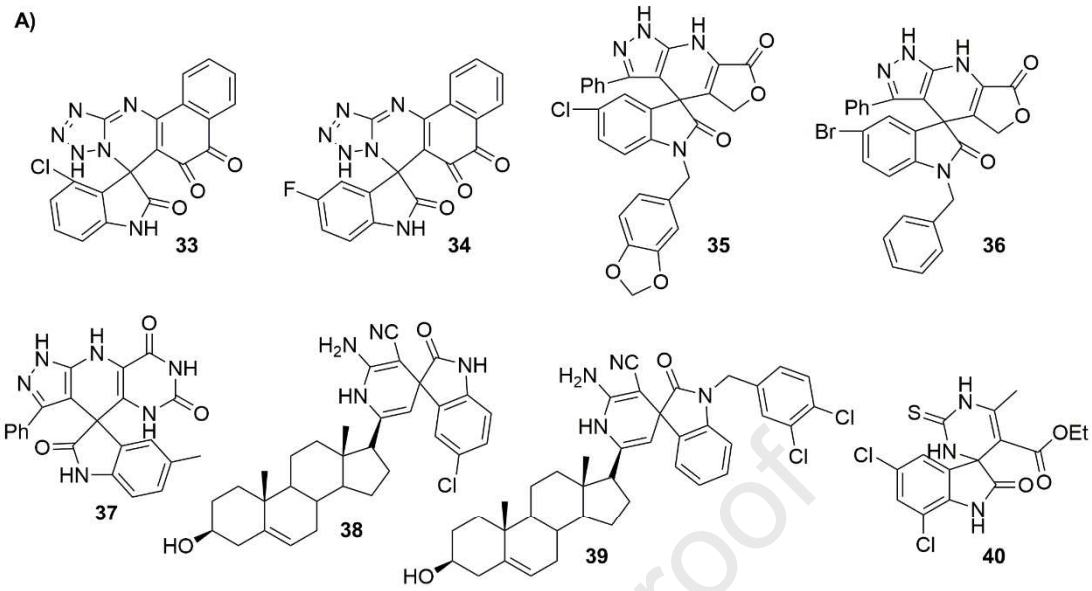


Scheme 13: Synthetic paths to the synthesis of pyrimidine, pyrazolopyridine, dihydropyridinyl and dihydropyrimidinone-based spirooxindole derivatives.

The chemical structures, as well as the antiproliferative results with the A549 cell line, are depicted in **Schemes 14A** and **14B,** as well as their respective druglikeness profile and physicochemical properties (**Scheme 14C**). The most promising compound was **37**, bearing a spiro-pyrazolopyridine unit.[77] Compound **39**, a steroidal dihydropyridinyl spirooxindole, exhibits remarkable antiproliferative activity in the human esophageal cancer cell line, EC-109, yet the druglikeness profile cannot be ignored.[78] Major issues with solubility, lipophilicity and molecular weight making it less likely to have appropriate orally bioavailability (see bioavailability radar in **Scheme 14C**). Also, low GI and inexistent BBB permeability indicate that it would make a poor drug candidate.

Furthermore, the druglikeness predictions revealed that compounds **33**, **34** and **40** fulfil the requirements of Lipinski, Ghose, Veber, Egan and Muegge rules and only compound **39** presents poor GI absorption according to the Boiled-Egg model. The *in vitro* screening for compound **40** performed in MCF-7 and HepG2 cell lines, showed lower potency although its favorable druglikeness predictions indicate that it might be structurally modified to improve its cytotoxicity. Compounds **33** and **34** showed an unfavorable PAINS profile, due to the presence of quinone and the iminone moieties.

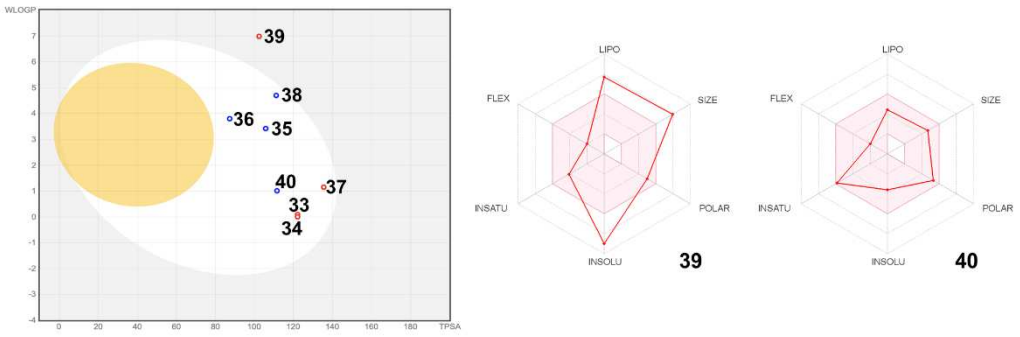
A)



B)

Compounds	IC_{50} (μM)								Druglikeness				
	HepG2	MDA-MB-231	A549	DU-145	EC-109	SMMC-7721	MGC-803	MCF-7	Lipinski	Ghose	Veber	Egan	Muegge
33	3.0	-	-	-	-	-	-	-					
34	2.9	-	-	-	-	-	-	-					
35	-	0.3	19.9	15.2	-	-	-	-					
36	-	12.5	15.8	8.5	-	-	-	-					
37	-	63.2	7.8	9.6	-	-	-	-					
38	-	-	11.5	-	3.8	9.6	4.2	-					
39	-	-	>128	-	0.3	>128	>128	-					
40	28.4	-	-	-	-	-	-	21.3					
Tanshinone IIA	23.9	-	-	-	-	-	-	-					
5-Flourouracil	-	5.8	7.5[a] 12.0[b]	6.4	10.6	9.7	7.6	-					
Cisplatin	17.3	-	-	-	-	-	-	12.6					

C)

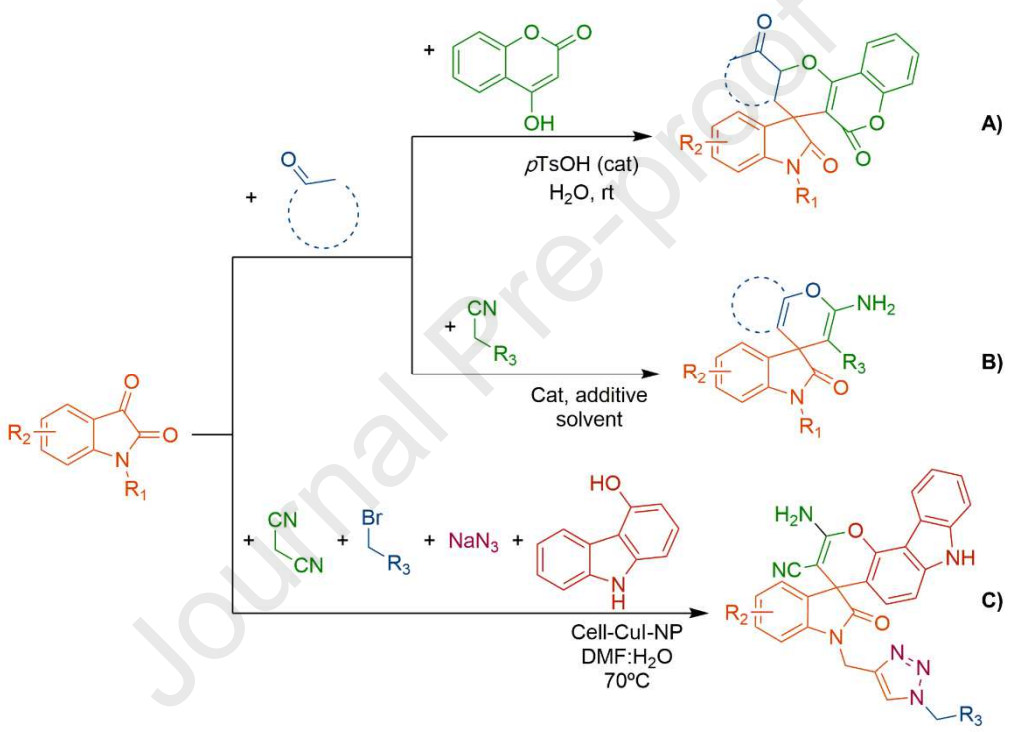


Scheme 14: The structures of the most promising compounds (A) and respective antiproliferative activity evaluation and druglikeness (B); Boiled-Egg model and bioavailability radar for **39** e **40** (C) ([a] value reported in [77]; [b] value reported in [78]).

At this point it was clear that isatin-derived compounds possessing cyclic structures attached at position 3 have been widely targeted over the last decade, in part due to their fascinating complex scaffolds and mainly to their bioactivity. Like pyrrolidine-derived spiro rings, also pyranone-spirooxindole hybrids were also synthesized using efficient and eco-friendly methods. In the context of this review, we found some reports on the synthesis and antiproliferative evaluation of these interesting frameworks, some of which are described below.

Dhayabaran and co-workers described the synthesis of novel 10,10-dimethyl-9,10,11,11*a*-tetrahydro-6*H*-spiro[chromeno[4,3*b*]chromene-7,3′-indoline]-2′,6,8(7*aH*)-triones using a 3-MCR approach involving isatin derivatives, cyclic ketones and 4-hydroxycoumarin under green conditions (**Scheme 15A**). The use of mild conditions, water as solvent, short reaction times and no chromatographic work-up are the main advantages of this methodology. The desired spirooxindole derivatives were synthesized in good to excellent yields (14 examples – 74-98% yield).[80] The group of Anbhule described the well-known 3-MCR involving isatin derivatives, malononitrile and 1,3-indanone to obtain a family of 2-amino-3-cynospiro(5*H*-indeno[1,2*b*]pyran-4,3-indoline)-2'5-diones with good reaction scope and good to excellent yields (14 examples – 83-96% yield, **Scheme 15B**). Briefly, the reaction proceeds through the preliminary construction of the Knoevenagel intermediate (formed between isatin and malononitrile) which will act as Michael acceptor for the 1,3-indanone. After cyclization and isomerization, the desired spirooxindole product was obtained in mild reaction conditions, in a catalyst-free approach using a mixture of water and DMF as solvents.[81] Using a similar synthetic protocol, Song and co-workers reported the synthesis of (*E*)-8'-arylidene-5',6',7',8'-tetrahydrospiro[oxindole-3,4'-pyrano[3,2*c*]pyridin] derivatives *via* one-pot 3-MCR of isatins, malononitrile and (*E*)-3-arylidene-1-methylpiperidin-4-ones using piperidine as catalyst and ethanol as an environmentally benign solvent (**Scheme 15B**). Very good substrate scope and moderate to excellent yields were obtained through this synthetic transformation (20 examples, 60-98% yield).[82] Also the group of Perumal reported a similar strategy to easily prepare a library of spiropyrano[3,2*b*]pyran-4(8*H*)-ones using isatin derivatives, active methylenes (like malononitrile) and kojic acid (**Scheme 15B**). Using $Cu(OTf)_2$ as an efficient catalyst, a very good scope and good yields were obtained for this family (19 examples – 77-93% yield).[83] The group of Kidwai explored the use of dimedone, 1,3-cyclohexanedione, 4-hydroxycoumarin, barbituric acid, thiobarbituric acid and 1-phenyl-3-methyl-2-pyrazolin-5-one as active methylene compounds in the 3-MCR approach along with isatin derivatives and malononitrile (**Scheme 15B**). They found out that the use of iodide as a Lewis acid catalyst improved significantly the yields of the

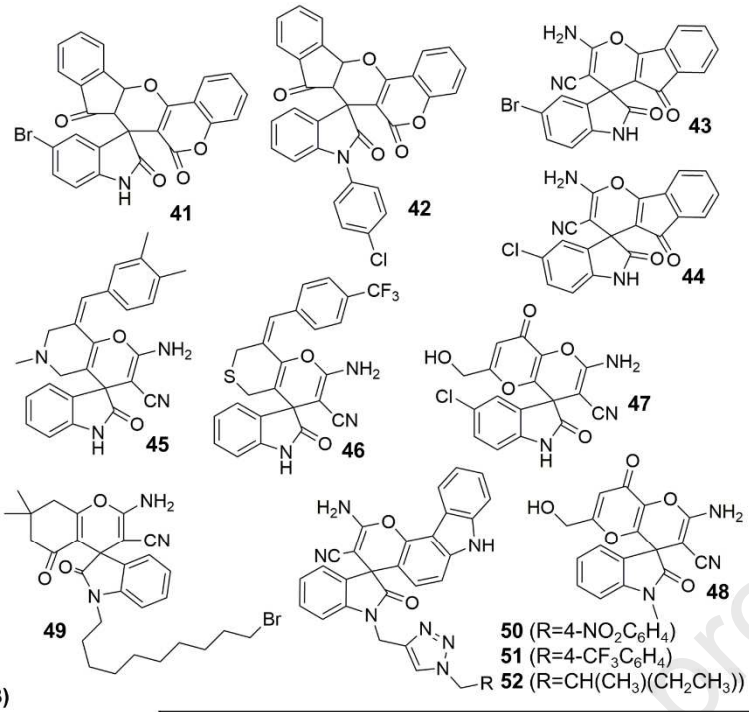
desired spirooxindole compounds and the remarkable scope of the reaction (42 examples) together with the use of water as solvent makes this synthetic 3-MCR an appealing strategy.[84] Sarkar and co-workers reported the synthesis of spiro-chromenocarbazole tethered 1,2,3-triazoles via a one-pot 5-MCR approach between *N*-propargyl isatin derivatives, malononitrile, 4-hydroxycarbazole, aralkyl halides and sodium azide using cellulose-supported CuI nanoparticles (Cell-CuI-NP) as heterogeneous catalyst in a mixture of DMF and water as solvent (**Scheme 15C**). A vast library of spiro-chromenocarbazole tethered 1,2,3-triazoles derivatives was produced under smooth reaction conditions and good yields (27 examples – 81-92% yield).[85]



Scheme 15: Synthetic pathways to prepare spiro-based pyran-oxindole derivatives using 3- and 5-MCR approaches.

Like all the previous compounds described in this section, also the spiro-based pyran-oxindole derivatives underwent a phenotypic screening campaign against some cancer cell lines to evaluate their cytotoxic behavior. The results are displayed in **Scheme 16**.

A)



C)

B)

Compounds	IC_{50} (μM)									Druglikeness				
	PC-3	LNCaP	MBD-MB-435	MCF-7	HeLa	HepG2	MDA-MB-231	A549	U87	Lipinski	Ghose	Veber	Egan	Muegge
41	0.03	1.9	-	-	-	-	-	-	-					
42	0.08	1.3	-	-	-	-	-	-	-					
43	-	-	1.8	>100	-	-	-	-	-					
44	-	-	2.1	>100	-	-	-	-	-					
45	-	-	-	-	76.1[d]	70.2[d]	64.9[d]	-	-					
46	-	-	-	-	87.1[d]	73.1[d]	61.3[d]	-	-					
47	-	-	-	-	-	-	-	51.4	-					
48	-	-	-	-	-	-	-	51.1	-					
49	-	-	-	-	-	-	-	-	2.5					
50	-	-	-	2.1	6.3	-	51.2	-	-					
51	-	-	-	>100	3.5	-	61.9	-	-					
52	-	-	-	24.9	14.9	-	3.8	-	-					
Bicalutamide	1.3	1.5	-	-	-	-	-	-	-					
Tamoxifen	-	-	0.1	-	-	-	-	-	-					
Doxorubicin	-	-	0.09	0.09[a] 4.6[b]	83.2[c,d] 2.7[b]	79.1[d]	76.0[c,d] 0.6[b]	-	-					
Crizotinib	-	-	-	-	-	-	-	-	-					
Carmustine	-	-	-	-	-	-	-	-	3.9					
Paclitaxel		-	-	0.003	0.006	-	0.1	-	-					

Scheme 16: Structures of the most promising compounds (A) and respective antiproliferative activity screening and druglikeness (B); bioavailability radar for **45**, **48** and **50** (C) ([a] value reported in [81]; [b] value reported in [85]; [c] value reported in [82]; [d] *In*

vitro percentage growth inhibition (GI %) caused by the test compounds at dose of 20 μmol/L).

At first sight, analysis of the *in vitro* screening against several human cancer cell lines, show that several compounds display relevant biological activity (with IC_{50} ranging from 6.3 μM to 30 nm for the most active compounds) and should be object of further studies. For instance, compounds **41** and **42**, exhibited very good values of IC_{50} in human prostate cancer cell lines PC-3 and LNCaP.[80] The same can be stated for compounds **43** and **44**, in what concerns the bioactivity against human breast cancer cell line MBD-MB-435.[81] In fact, verifying the druglikeness profile for these two compounds, they fulfil perfectly the five rules. Despite promising antiproliferative results obtained in breast cancer cell lines MCF-7 and MDA-MB-231 and human cervical cancer cell HeLa, compound **50** failed to comply with the five rules, and issues concerning molecular weight, polarity, solubility, molar refractivity, among others, pointed out that this scaffold might not be the best lead compound. In our opinion, compounds **45** and **48** should be screened further in other tumor cell lines. The Boiled-Egg model for all these compounds is depicted in **Figure 2**. Although none of the compounds are predicted to be capable of crossing the BBB – which would be crucial in the case they are used in neurological disorders - the vast majority are predicted to cross the GI barrier (except compounds **46** and **50-52**).

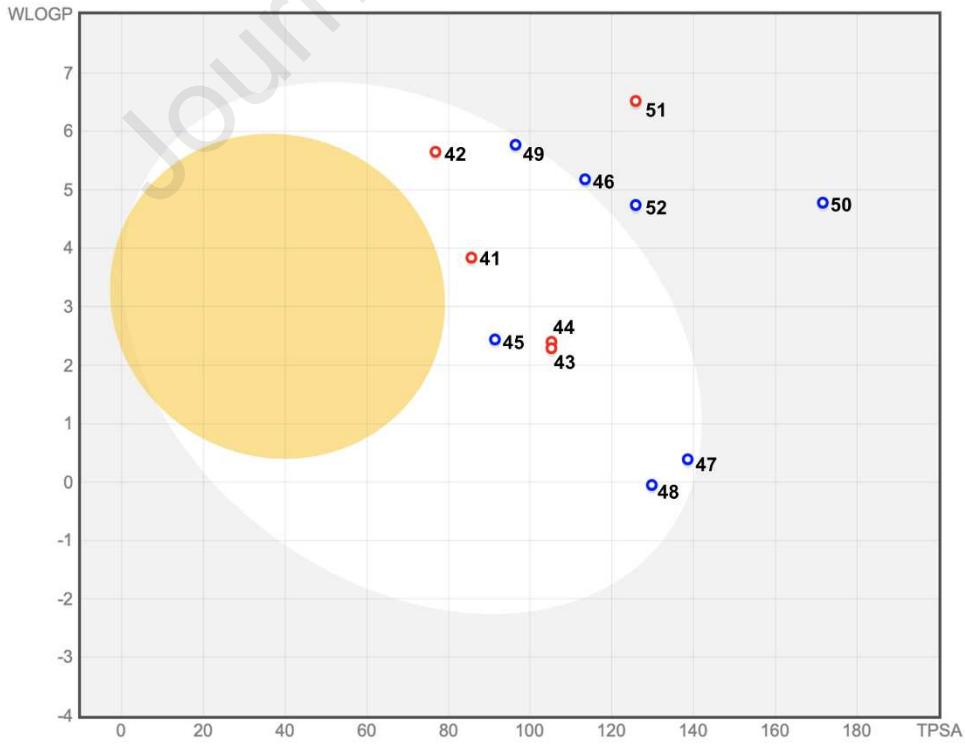
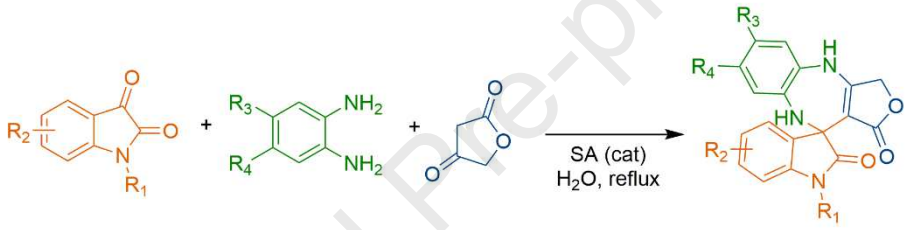


Figure 2: Boiled-Egg model for the most active spiro-based pyran-oxindole derivatives.

Kamal and co-workers decided to report a similar 3-MCR approach to obtain spiro-benzodiazepine derivatives with facile one-pot formation of C-C and C-N bonds, with consequent formation of a 7-member ring size spirooxindole (**Scheme 17A**). Using isatin derivatives, *o*-phenylenediamines and tetronic acid, the inexpensive sulphamic acid (NH_2SO_3H, SA) as catalyst and water as reaction medium a library of 4,9-dihydrospiro[benzo[*b*]furo[3,4*e*][1,4]diazepine-10,3'-indoline]-1,2'(3*H*)-dione derivatives was obtained in good yields and good reaction scope (21 examples – 77-90% yield). The cytotoxicity of the synthesized compounds was evaluated on the selected human cancer cell lines, that included A549, MCF-7, DU-145 and HeLa. The results exhibited promising cytotoxicity activities for compounds **53-55** (**Scheme 17B**).[86] Druglikeness predictions revealed full compliance with all the five rules by the three most active derivatives, showing also good GI absorption, with compound **54** predictively displaying the ability to cross the BBB (**Scheme 17C**).

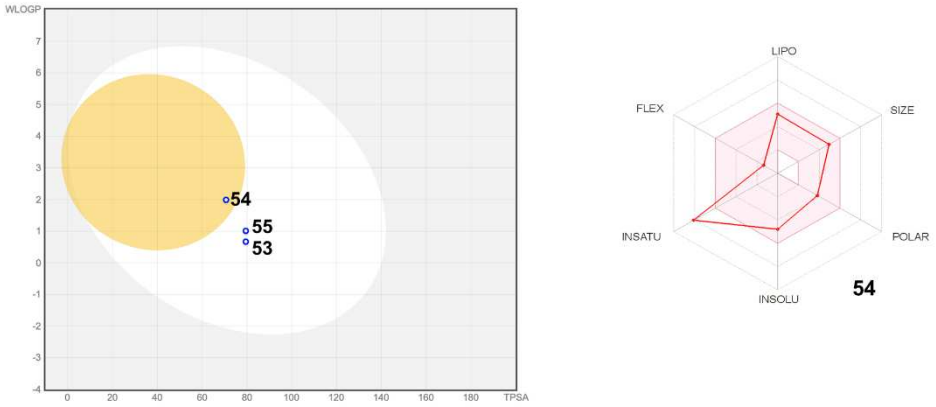
A)



B)

Compounds	R_1	R_2	R_3	R_4	IC_{50} (μM) HeLa	A549	MCF-7	DU-145	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
53	H	3-CH_3	H	H	9.6	**1.5**	**2.1**	**1.3**					
54	Bn	H	H	H	**2.3**	**1.3**	**2.2**	**1.1**					
55	H	H	Cl	H	5.4	**2.9**	9.5	**1.7**					
Doxorubicin	-	-	-	-	1.0	1.5	1.4	1.8					
Etoposide	-	-	-	-	1.0	2.2	1.6	2.1					

C)



Scheme 17: Synthetic path for the 3-MCR of spiro-benzodiazepine derivatives (A); antiproliferative activity screening and druglikeness for the most active compounds (B); Boiled-Egg model and bioavailability radar for compound **54** (C).

From all these compounds, it is noteworthy the scaffold diversity attained *via* MCRs. Nonetheless, these compounds have in common a spirooxindole nature, with some of the most active being unsubstituted at position 1 (**6**, **23**, **19**, **41**, **53** and **55**), although some good results were obtained for *N*-substituted derivatives (**35**, **39**, **42** and **54**). Multiple spirocenters (**6**) or adjacent chiral centers (**19**) led to important anti-tumor activity, but one of the most common features is the presence of a tricyclic or pentacyclic structure at position 3 of the oxindole core, as observed for compounds **35**, **41**, **42**, **53**, **54** and **55**.

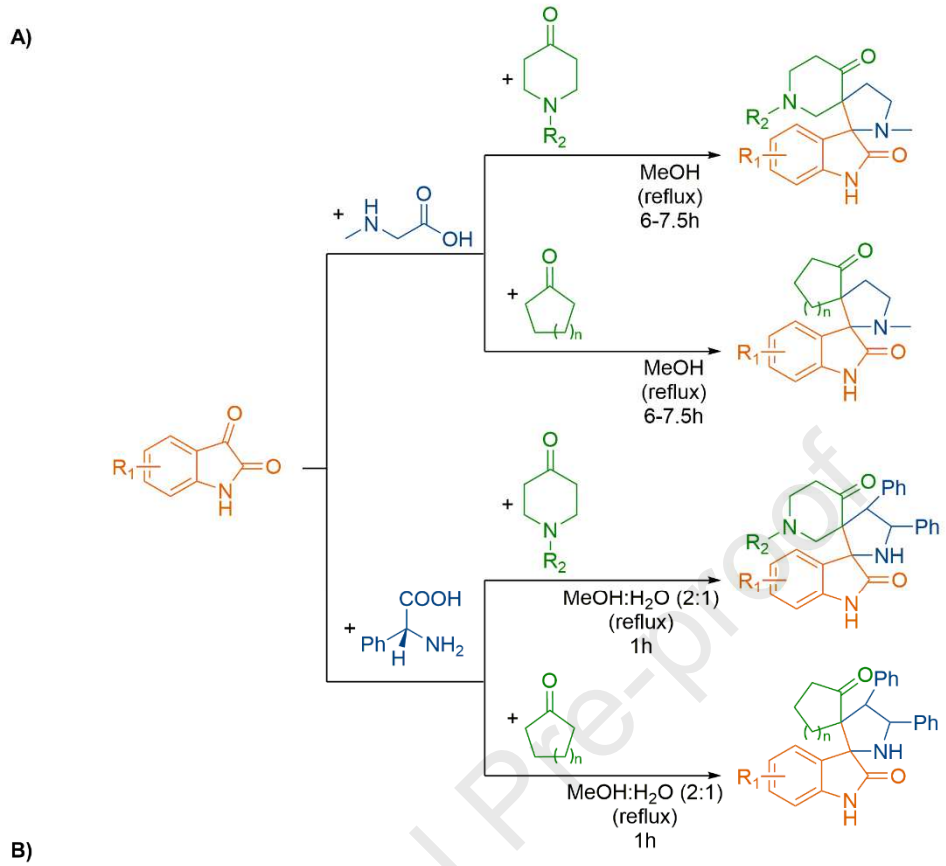
2. Antimicrobial and antiviral activity

2.1. Antibacterial activity

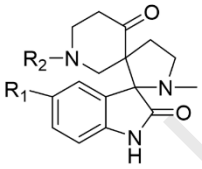
Tuberculosis is a very complex infectious disease caused by *Mycobacterium tuberculosis*, which represents the leading bacteria-related cause of death worldwide, and it is facing many challenges, including lack of therapeutic compliance, multidrug resistance, and clinical manifestations in immunosuppressed patients. Despite the increasing number of drugs and vaccines currently in the preclinical and clinical trials pipeline, the quest for new compounds with higher selectivity, efficacy and better pharmacokinetic and toxicological profiles persists.[87-89] Indeed, a recent report by the World Health Organization (WHO) places this pathogen as a top priority infectious agent for the discovery and development of new drug candidates.[90]

Several 3-MCR in methanol or methanol/water mixtures have been recently described to obtain new anti-tubercular agents. Kumar *et al*. reported the synthesis of highly functionalized dispiropyrrolidine derivatives (38 examples) in moderate yields (32-52%) (**Scheme 18A**). These compounds were obtained through the 3-MCR between isatin, cyclic mono ketones and sarcosine or phenylglycine. The minimum inhibitory activity (MIC) of the vast majority of the compounds was determined against *M. tuberculosis* H37Rv (MTB) using agar dilution method. The values obtained for several compounds (**56-60**) were in the same range as the ones obtained for the positive controls isoniazid, ciprofloxacin, ethambutol and pyrazinamide, marketed drugs for the treatment of tuberculosis (**Scheme 18B**).[91]

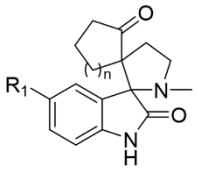
A)



B)



Comp.	R_1	R_2	MIC (μM)
56	CH_3	N=O	4.75
57	Cl	N=O	4.47
58	Br	N=O	**1.98**
Isoniazid	-		0.36
Ciprofloxacin	-		4.71
Ethambutol	-		7.64
Pyrazinamide	-		50.77

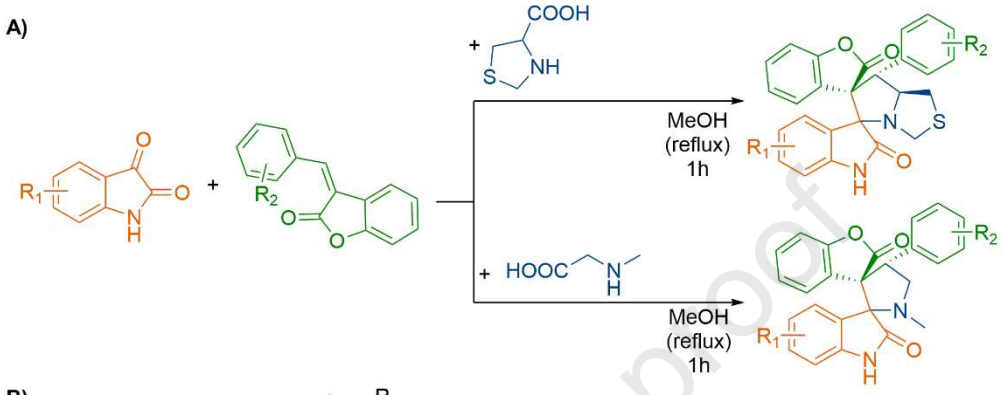


Comp.	n	R_1	MIC (μM)
59	2	Br	4.29
60	3	Br	4.13

Scheme 18: 3-MCR synthesis of dispiropyrrolidine derivatives with antitubercular activity using isatin, cyclic mono ketones and sarcosine or phenylglycine (A) and structure and MICs of the most bioactive compounds (B).

Other research groups dedicated their attention to the three-component 1,3-dipolar cycloaddition reaction, obtaining new spirooxindole derivatives with antitubercular activity. Mhiri *et al.* explored the reaction between isatin, (*Z*)-3-arylidenebenzofuran-2-ones and 1,3-thiazolane-4-carboxylic acid or sarcosine (**Scheme 19A**). A library of 28 derivatives was prepared in very good yields (71-89%) and short reaction times and their activity against MTB accessed, using the agar dilution method and the previously mentioned four positive controls, as well as

rifampicin. Ten compounds (**61-70**) displayed MICs similar or lower than the one presented by the positive controls, showcasing the potential of these frameworks as new antitubercular agents. Furthermore, the cytotoxicity of the most active compounds (**61** and **62**) was also evaluated using RAW 264.7 (Mouse monocyte macrophages) cells, and displayed low cytotoxicity (27.5% and 20.7%, respectively, at 50 μM) (**Scheme 19B**).[92]



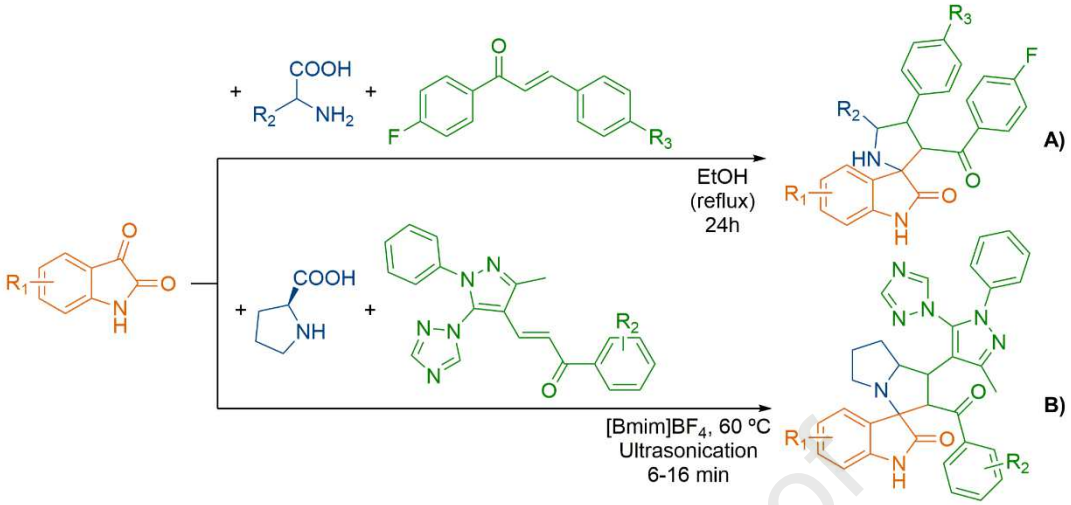
Comp.	R_1	MIC (μg/mL)	Cytotoxicity (%inhibition) RAW 264.7 cells
69	SCH_3	3.125	28.74
70	NO_2	3.125	29.5

Comp.	R_1	R_2	MIC (μg/mL)	Cytotoxicity (%inhibition) RAW 264.7 cells
61	H	OCH_3	**1.56**	27.53
62	H	NO_2	**1.56**	20.74
63	H	Cl	3.125	32.3
64	H	Br	3.125	28.5
65	Br	Cl	3.125	34.62
66	Br	NO_2	3.125	43.1
67	Br	Br	3.125	32.4
68	NO_2	H	3.125	35.23
Isoniazid	-		0.05	-
Rifampicin	-		0.10	-
Ethambutol	-		1.56	-
Ciprofloxacin	-		3.13	-
Pyrazinamide	-		6.25	-

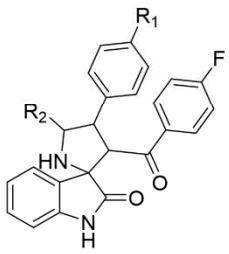
Scheme 19: Three-component 1,3-dipolar cycloaddition using (*Z*)-3-arylidenebenzofuran-2-ones as dipolarophiles (A) and structure, MIC and cytotoxicity evaluation of the most active compounds (B).

The same type of reaction was later explored by Sapnakumari *et al.*, but this time using chalcones as dipolarophile, combining them with isatin and different amino acids (**Scheme 20A**). A small library of 8 compounds was successfully achieved in moderate yields (52-71%) and their *in vitro* activity against *M. tuberculosis* evaluated using microplate alamar blue assay (MABA), suing pyrazinamide and streptomycin as positive controls. Compounds **71-73** displayed significant activity, four times higher

than the one described for pyrazinamide. The remaining compounds displayed activities similar to the positive controls indicating, once again, the potential of spirooxindoles as antitubercular agents (**Scheme 20C**).[93] A similar approach was undertaken by Pogaku *et al*., combining isatin, *L*-proline and chalcones to synthesize a library of 1,2,4-triazol-1-yl-pyrazole based spirooxindolopyrrolizidine derivatives (20 examples) in overall very good yields (78-92%) (**Scheme 20B**). The main advantage of this methodology is its short reaction times, promoted by using a reusable ionic liquid (IL) as reaction medium and ultrasonication as activation technique. The MABA assay was used to determine the MIC against MTB, using ethambutol as positive control. One compound in particular (**75**), proved to be the most active against *M. tuberculosis*, although compounds **76-81** displayed antitubercular activity similar to the positive control (**Scheme 20D**). Cytotoxicity was evaluated using RAW 264.7 cells, and low cytotoxicity was displayed by most compounds, including **75** (19.76%) at 25 μM.[94]

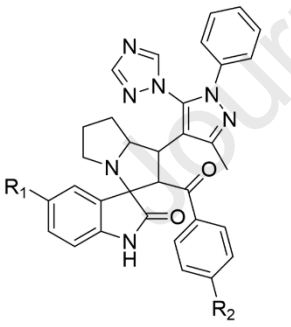


C)



Comp.	R_1	R_2	MIC (μg/mL)
71	H	H	**0.8**
72	Br	CH_2OH	**0.8**
73	Cl	$CH_2CH(CH_3)_2$	**0.8**
74	H	$CH_2CH(CH_3)_2$	6.25
Pyrazinamide		-	3.125
Streptomycin		-	6.25

D)

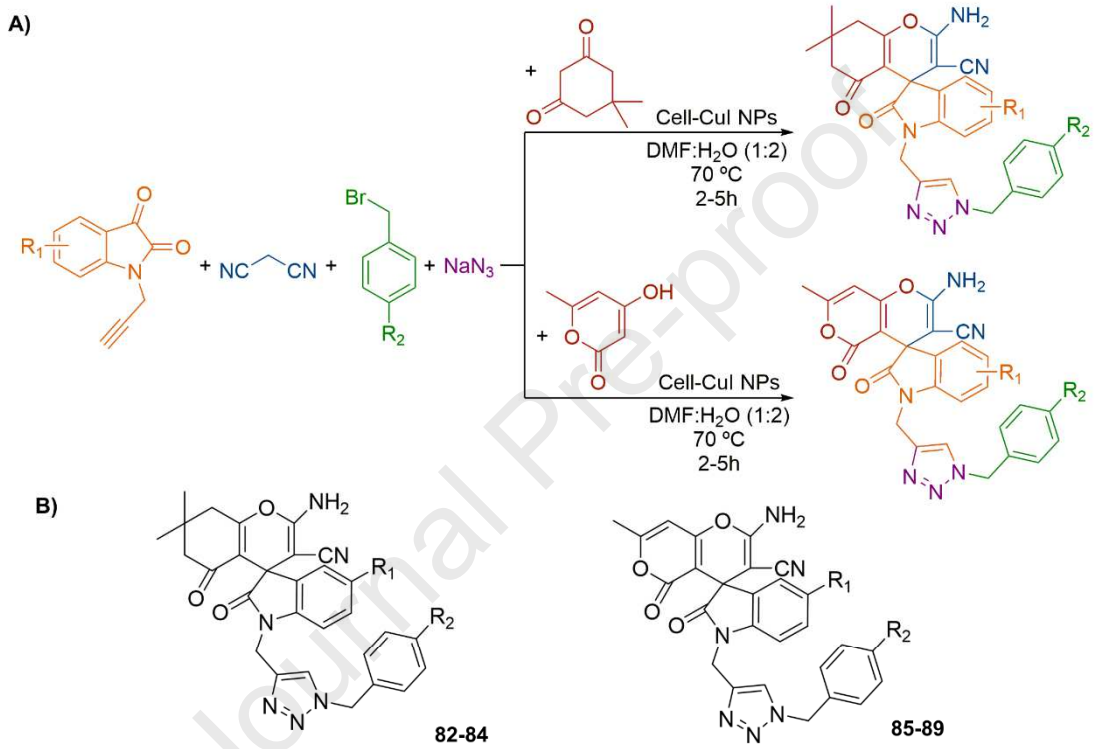


Comp.	R_1	R_2	MIC (μg/mL)	Cytotoxicity (%inhibition) RAW 264.7 cells
75	Cl	H	**0.78**	19.76
76	H	Br	1.56	27.15
77	Cl	Cl	1.56	17.91
78	Cl	Br	1.56	26.43
79	Cl	NO_2	1.56	24.94
80	Br	Cl	1.56	22.36
81	Br	Br	1.56	21.97
Ethambutol	-		1.56	-

Scheme 20: Three-component 1,3-dipolar cycloaddition using chalcones as dipolarophiles (A and B); structure, MIC and cytotoxicity evaluation of the most active compounds (C and D).

A totally different approach is the one recently described by Chavan *et al.*, exploring a five-component reaction to prepare a library of 1,2,3-triazolylspirochromene derivatives (32 examples) from *N*-propargyl isatin, malononitrile, arylalkyl bromide, sodium azide and dimedone or 4-hydroxy-6-methyl-2*H*-pyran-2-one (**Scheme 21A**). This methodology, which allowed the preparation of the desired compounds in very good yields (76-94%) and short reaction times (up to 5 hours), was enabled by

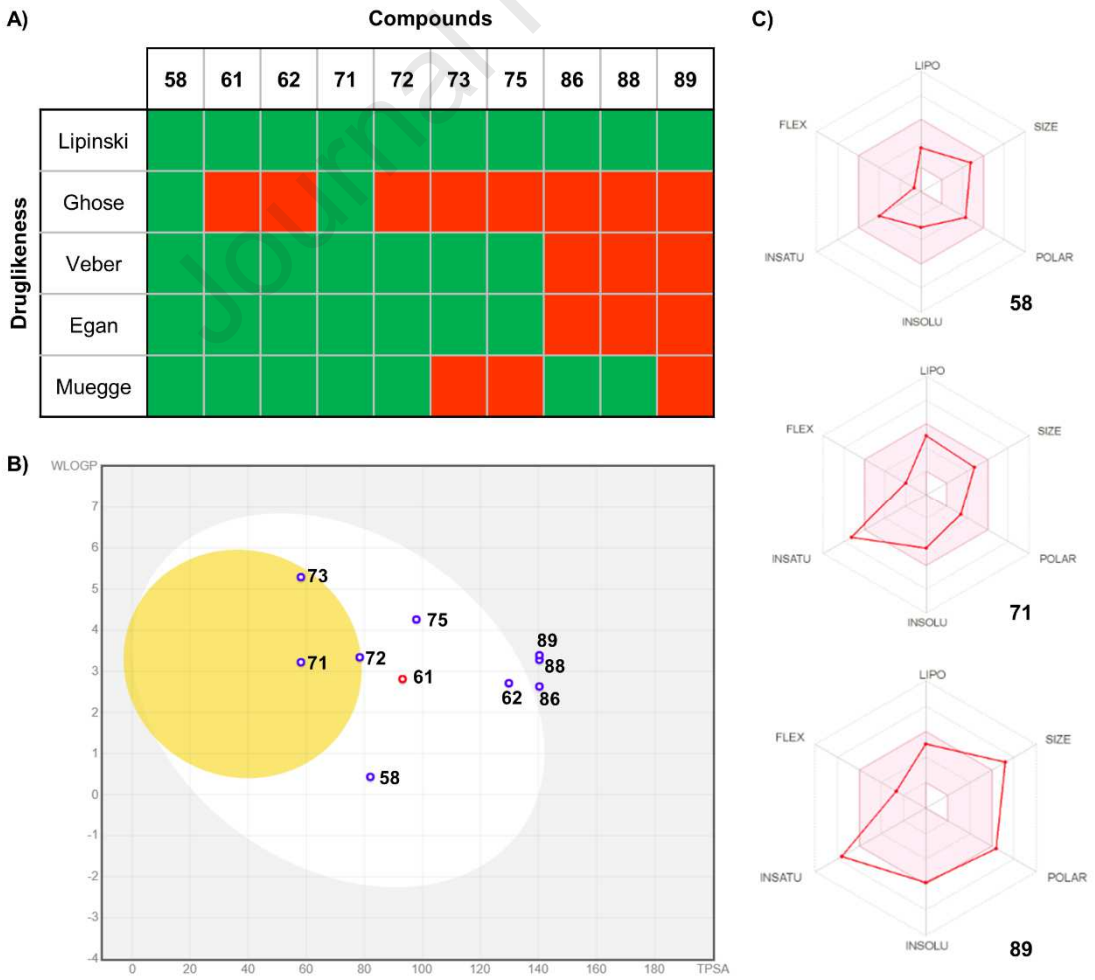
applying cellulose-supported CuI nanoparticles (Cell-CuI NPs) as an heterogeneous and reusable catalyst. The obtained compounds were then tested against MTB, using the XTT reduction menadione assay (XRMA), and *M. bovis* (BCG), using the nitrate reductase assay, both in active and dormant stage, and evaluating two parameters – MIC and IC_{50}, using rifampicin as positive control. Compounds **82-84** and **85-89** displayed interesting bioactivity and good selectivity (**Scheme 21B**), as they did not demonstrate relevant cytotoxicity towards three different cell lines (MCF-7, HCT116 and A549).[95]



			MTB				BCG			
			Active stage		Dormant stage		Active stage		Dormant stage	
Comp.	R_1	R_2	MIC (μg/mL)	IC_{50}	MIC (μg/mL)	IC_{50}	MIC (μg/mL)	IC_{50}	MIC (μg/mL)	IC_{50}
82	Cl	Cl	>50	15.45	8.07	3.06	**2.87**	**1.33**	**3.09**	**0.6**
83	Br	CH_3	29.23	3.24	8.89	5.1	**2.87**	**1.33**	6.23	1.33
84	Br	Cl	>50	22.81	10.60	5.89	4.32	1.8	**3.16**	**1.24**
85	H	CH_3	13.99	2.13	7.3	1.71	7.42	1.68	5.36	1.97
86	H	Cl	**5.21**	**1.63**	**4.39**	**1.43**	**2.97**	**0.7**	**4.35**	**2.06**
87	Cl	CH_3	>50	17.67	11.59	7.93	5.24	2.53	5.23	1.26
88	Cl	Cl	**5.39**	**1.84**	**4.81**	**2.32**	**3.04**	**0.45**	**7.73**	**1.56**
89	Br	Cl	**6.63**	**2.71**	**6.8**	**3.07**	**4.12**	**2.11**	**3.97**	**1.14**
Rifampicin	-		0.51	0.002	0.75	0.05	0.45	0.005	0.81	0.075

Scheme 21: 5-MCR for the synthesis of antitubercular agents A) Synthetic pathway; B) Structure, MIC and IC_{50} of the most active compounds.

Evaluating the inherent pharmacokinetic and druglike properties of the ten compounds which exhibited more interesting antitubercular activity *in vitro*, it is noteworthy that none of these compounds present structures that could make them possible PAINS. With regard to the drug-like-properties of these compounds, it was observed that the top ten most active compounds comply with the Lipinski's rule of five (**Scheme 22A**). The violations of the remaining rules are connected to high molecular weight presented by some of these frameworks, high molecular refractivity predicted and high topological polar surface area (TPSA). The BoiledEgg GI absorption and BBB permeation prediction model also shows that most of these compounds (except **86**, **88** and **89**) present high GI absorption, relevant for oral bioavailability (**Scheme 22B**). The bioavailability radars of the two compounds which performed better in what concerns druglikeness rules compliance (**58** and **71**) show that despite the significant differences in these two structures, they present a good bioavailability score. On the other hand, the bioavailability radar for the worst performing compound (**89**) indicates that most of the physical-chemical properties remain close to the optimal value, and therefore, some tweaking of the molecular structure could lead to a more druglike compound (**Scheme 22C**).



Scheme 22: Top 10 performing antitubercular agents (*in vitro*) A) Druglikeness rules compliance; B) Boiled-Egg model; C) Bioavailability radar for **58**, **71** and **89**.

Besides tuberculosis, several other bacterial infections deserve attention, namely due to an alarming increase in multidrug resistance displayed by several pathogens. The attrition rate in introducing new antibiotic drugs to the market is very high, and the introduction of new classes of antibiotics used in clinical practice has been alarmingly low over the past decades. As a matter of fact, from 1986 to 2017, there have been no new classes of antibiotic introduced to the antibiotic pipeline, until the discovery of teixobactin, a secondary metabolite produced by some bacteria species which displays relevant bactericidal activity without displaying induced resistance (in an *in vitro* setting). Nonetheless, this compound is still in early stage preclinical development, and therefore the need to discover other new promising antibiotic drug candidates is imperative to tackle the "discovery void" of the past decades.[96-100] The WHO priority list of antibiotic-resistant bacteria, which takes into consideration multiple criteria, indicates the existence of three tiers of pathogens requiring particular attention in drug discovery and development, as shown in **Figure 3**.

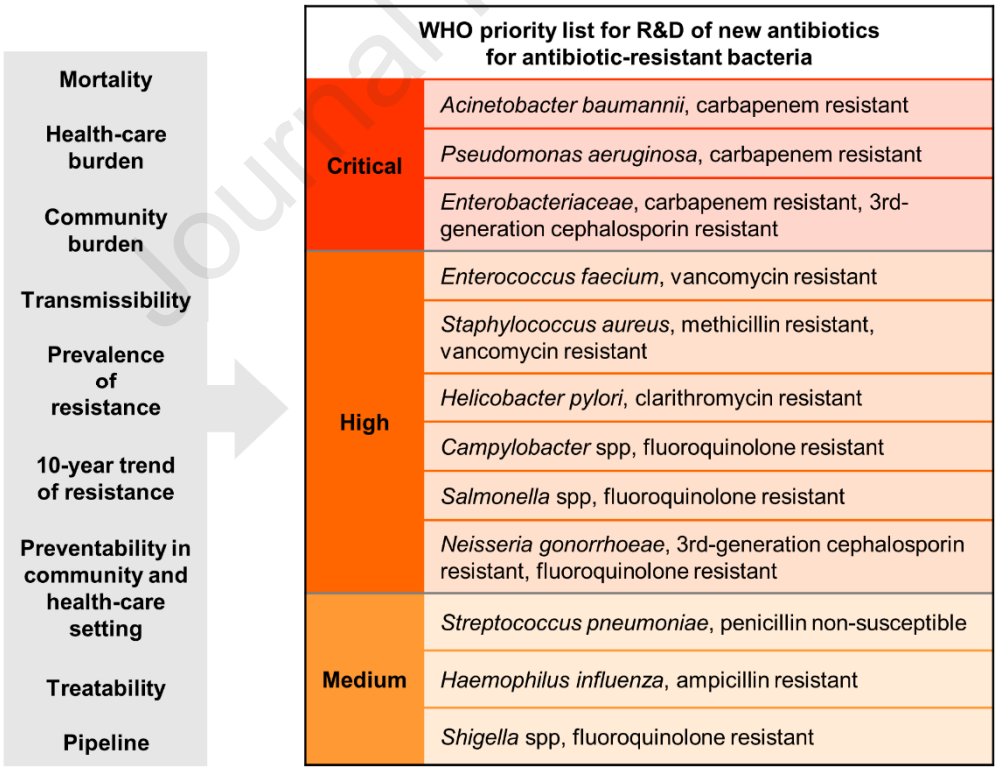


Figure 3: Criteria and pathogens of the WHO priority list for R&D of new antibiotics (adapted).[90]

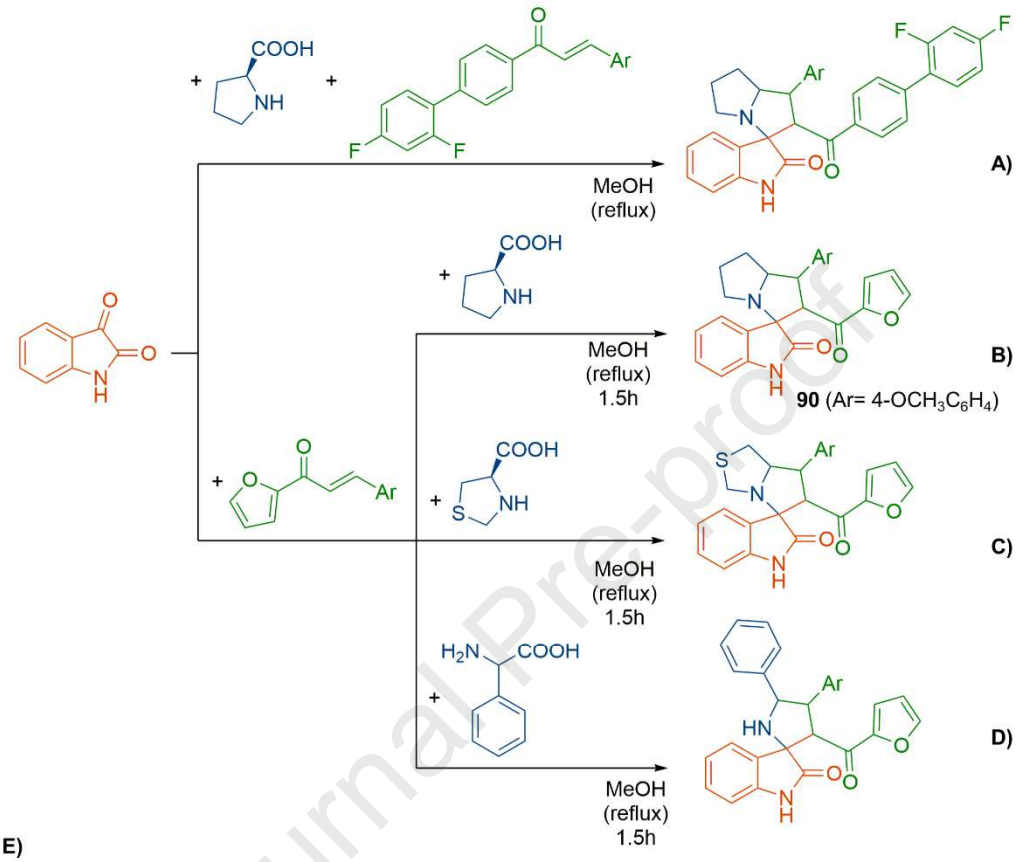
This urgent need led several groups to perform antibacterial screening of newly synthesized compounds, including MCR-obtained oxindole derivatives, namely spiro-oxindole derivatives.[101] Some of the products previously described for their antitubercular activity, were also tested for their antibacterial activity towards Gram-positive and Gram-negative bacteria. The library described in **Scheme 20A** was tested against *Staphylococcus aureus* (ATCC 6538) and *Escherichia coli* (ATCC 8739), with most compounds presenting similar antibacterial activity towards *E. coli* as the positive control ciprofloxacin, with a MIC of 20 μg/mL. This *in vitro* observation was further complemented using molecular docking studies of the newly synthesized compounds in potential prokaryotic targets. Compound **74** presents the best score in targeting the methionine tRNA synthase (showing some selectivity, as this compound did not exhibit good antitubercular activity), while compound **73** *in silico* binds more efficiently to the glucosamine-6-phosphate synthase, indicating potential mechanisms of action for these compounds.[93] The 5-MCR products shown in **Scheme 21A** were also evaluated for their antibacterial activity towards two Gram-negative bacteria, *Escherichia coli* (NCIM 2688) and *Pseudomonas aeruginosa* (NCIM 2036), and two Gram-positive bacteria, *Bacillus subtilis* (NCIM 2079) and *Staphylococcus aureus* (NCIM 2010). The MIC_{90} was determined, using ampicillin and kanamycin as positive control, and the most promising results are depicted in **Table 2** (structures can be checked in **Scheme 21**). Compounds **82** and **84** display interesting antibacterial activity towards *B. subtilis* and *S. aureus*, respectively, exhibiting lower or comparable MIC_{90} as the positive controls.

Table 2: Antibacterial activity of compounds **82**, **84**, **85**, **87** and **89**.

Compound	MIC_{90} (μg/mL)			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
82	**1.78**	5.29	3.55	**1.14**
84	2.86	**1.57**	**1.09**	3.18
85	**1.97**	3.08	4.27	**2.85**
87	2.65	4.88	**2.41**	7.19
89	2.75	**2.33**	3.86	5.27
Ampicillin	1.46	4.36	1	10.32
Kanamycin	1.62	0.49	>30	1.35

The 1,3-dipolar cycloaddition approach involving isatin, amino acids and α,β-unsaturated ketones (namely chalcone-like compounds) was already described in this work for antitubercular agents (**Scheme 20**). This approach was also used to

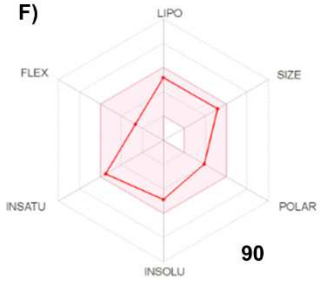
synthesize compounds with potential antibacterial activity. Fathimunnisa *et al*. promoted the reaction between isatin, L-proline and (2*E*)-1-[4-(2,4-difluorophenyl)phenyl]3-arylprop-2-en-1-ones to achieve spirooxindoles (8 examples) in overall good yields (69-89%) (**Scheme 23A**). The antibacterial activity was evaluated, using clinical isolates of *S. aureus*, *B. subtilis*, *P. aeruginosa*, *E. coli*, *Klebsiella pneumoniae* and *Proteus mirabilis*. The results show some antibacterial activity for a few of the obtained derivatives, however the MIC values obtained were above the ones displayed by the positive control, ciprofloxacin.[102] A similar 3-component approach was applied by Wu *et al*., using isatin, furanyl-substituted chalcones and L-proline (**Scheme 23B**), thioproline (**Scheme 23C**) or phenylglycine (**Scheme 23D**). The library (21 examples) was obtained in overall very good yields (65-90%) and their bioactivity screened against two Gram-positive bacteria, *Staphylococcus aureus* (ATCC 29213) and methicillin resistant *S. aureus* (MRSA) (ATCC 43300) and three Gram-negative bacteria, *E. coli* (ATCC 25922), *K. pneumoniae* (ATCC 700603) and *P. aeruginosa* (ATCC 27853). Although most compounds present low or no antibacterial activity, one compound, **90**, presented good selectivity and antibacterial activity against *P. aeruginosa*.[103] Since this bacteria exhibits a wide range of antibiotic resistance mechanisms, this makes treatment of these infections increasingly challenging.[104] The researchers studied further the selectivity and antibiotic activity of this spirooxindole derivative, by determining its MIC against three other strains of P. aeruginosa isolated from clinical practice, a quinolone-susceptible *P. aeruginosa* (No.010), and two strains of multidrug-resistance *P. aeruginosa* (MDRP 025 and MDRP 034). Noteworthy, this compound exhibited better activity towards the MDRP strains, when compared to three clinically available antibiotics, used as positive controls (norfloxacin, levofloxacin and ciprofloxacin) (**Scheme 23E**). Docking studies also suggest that compound **90** possibly interacts with multiple enzymes which are known antibiotic targets, such as lanosterol demethylase, dihydrofolate reductase and topoisomerase II, via hydrogen bonds involving the furanyl oxygen atom.[103] Looking in more detail to the features of compound **90**, SwissADME prediction shows that this chemical framework complies will the five druglikeness rules with no PAINS features detected. Further, its bioavailability radar, shown in **Scheme 23F**, indicates that the compound exhibits good potential to be administered *per os*, due to its high gastrointestinal absorption.



E)

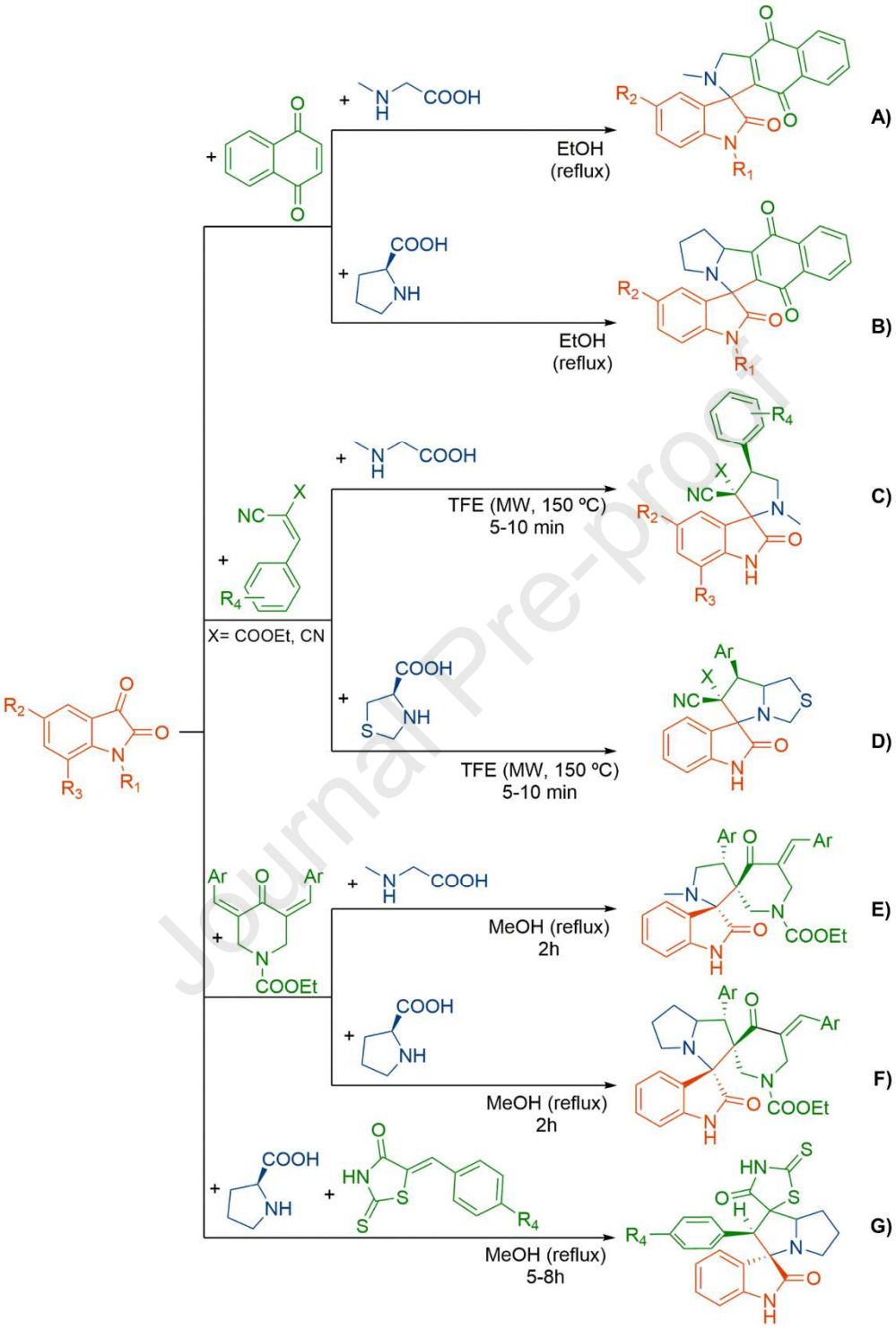
Bacteria	Compounds (MIC µg/mL)			
	90	Norfloxacin	Levofloxacin	Ciprofloxacin
S. aureus	64	-	-	-
MRSA	128	-	-	-
E. coli	128	-	-	-
K. pneumoniae	128	-	-	-
P. aeruginosa	16	2	1	0.25
P. Aeruginosa 010	16	2	2	0.5
MDRP 025	**16**	>64	>64	32
MDRP 034	**32**	>64	>64	32

F)



Scheme 23: Three-component 1,3-dipolar cycloaddition using α,β-unsaturated diketones as dipolarophiles (A-D); antibacterial activity (E) and bioavailability radar of compound **90** (F).

By using different dipolarophiles, the 1,3-dipolar cycloaddition is a very valuable methodology to attain a wide diversity of scaffolds, as summarized in **Scheme 24**.



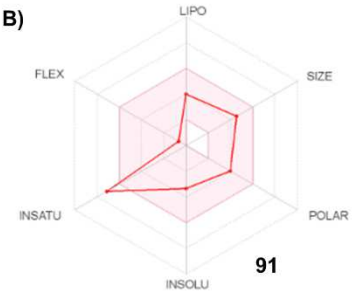
Scheme 24: Three-component 1,3-dipolar cycloaddition using various dipolarophiles for the synthesis of antibacterial compounds.

For example, by using 1,4-naphthoquinone, Bhaskar *et al*. explored the 1,3-dipolar cycloaddition combining this framework with isatin and sarcosine (**Scheme 24A**) or *L*-proline (**Scheme 24B**) to prepare a library of spirooxindole derivatives (22 examples) in excellent yields (87-96%). The compounds were then screened for antibacterial activity against four Gram-positive bacteria (*S. aureus* (MTCC 96), MRSA, *Micrococcus luteus* and *Enterobacter aerogenes* (MTCC 111)) and four Gram-negative bacteria (*Proteus vulgaris* (MTCC 1771), *K. pneumoniae* (MTCC 109), *Salmonella typhimurium* (MTCC 1251) and *Salmonella paratyphi-B*). While the *L*-proline derived spirooxindoles did not display relevant antibacterial activity, some of the sarcosine-based compounds exhibited relevant bactericidal effect, accessed using disc diffusion assay for all the compounds, and then determination of the MIC for the most promising compounds. Among them, compound **91** stood out as the most active compound against both Gram-positive and negative bacteria, in many cases exhibiting higher activity than the positive controls, streptomycin and ciprofloxacin. Compounds **92** and **93** also exhibited promising results, as shown in **Scheme 25**.[105]

A)

Compounds	R1	R2	MIC µg/mL: Gram positive bacteria: *S. aureus*	MRSA	*M. luteus*	*E. aerogens*	Gram negative bacteria: *P. vulgaris*	*K. Pneumoniae*	*S. typhimurium*	*S. paratyphi B*
91	$COCH_3$	H	**31.25**	**62.5**	**15.62**	**15.62**	**<15.62**	**15.62**	**15.62**	**<15.62**
92	CH_3	CH_3	**15.62**	125	125	62.5	125	250	**15.62**	500
93	H	Br	**15.62**	250	62.5	62.5	125	500	**31.25**	500
Streptomycin	-		6.25	>100	6.25	25	6.25	6.25	30	-
Ciprofloxacin			<0.78	>100	>100	<0.78	<0.78	<0.78	>100	6.25

B)



C)

Druglikeness	91	92	93
Lipinski			
Ghose			
Veber			
Egan			
Muegge			

Scheme 25: Antibacterial activity of compounds **91-93** (A); bioavailability radar of compound **91** (B); and druglikeness (C).

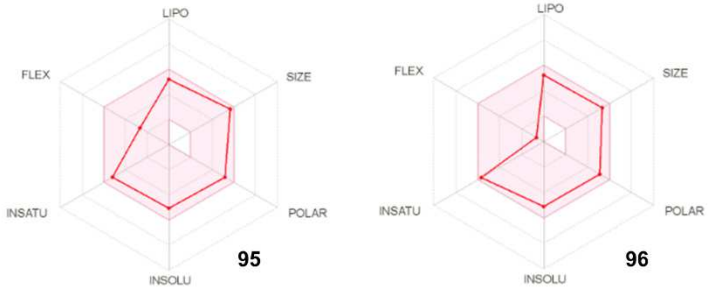
By combining isatin, sarcosine or 1,3-thiazoles-4-carboxylic acid with 2-cyano-3-phenyl-acrylic acid ethyl ester or 2-benzylidene-malononitrile, Dandia *et al*. reported the

synthesis of a small library of spiropyrrolidine (8 examples)/thiapyrrolizidine (4 examples) oxindole derivatives, under microwave irradiation (**Scheme 24C** and **24D** respectively). This methodology allowed the preparation of these derivatives in overall very good yields (88-92%) and short reaction times. The *in vitro* antibacterial activity of these spirooxindoles was then evaluated against *E. coli* (ATCC 9637), *P. aeruginosa* (ATCC BAA-427), *S. aureus* (ATCC 25923) and *K. pneumoniae* (ATCC 27736). When compared to the positive controls gentamicin and ampicillin, the MIC values were higher for all the compounds in the first three bacterial strains, however, surprisingly selectivity against *K. pneumoniae* was observed. Among all the compounds, the four thiapyrrolizidine derivatives (**94-97**) exhibited lower MIC than the two positive controls, with compound **96** displaying a remarkable MIC value of 5 ng/mL (**Scheme 26**). The authors further explore the *in silico* binding of this promising scaffold with New Delhi metallo-β-lactamase-1 (NDM-1) protein,[106] a well-established and widespread resistance mechanism of *K. pneumoniae* and one of the main causes of antibiotic resistance in infections caused by this bacterium.[107] The potential to establish several bonds between this target protein and the oxindole moiety, namely two H-bonds, one electrostatic and one hydrophobic interaction, besides van-der-Walls interactions, suggests that this is a potential target for compound **96**.[106]

A)

Compounds	Ar	X	MIC (μg/mL) *S. aureus*	MIC (μg/mL) *K. pneumoniae*	MIC (μg/mL) *P. aeruginosa*	MIC (μg/mL) *E. coli*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
94	2-thiophenyl	COOEt	>50	0.09	>50	>50					
95	2-Cl,6-FC_6H_3	COOEt	>50	0.045	>50	>50					
96	3,4-$Cl_2C_6H_3$	CN	>50	**0.005**	>50	>50					
97	4-$NO_2C_6H_4$	CN	>50	0.19	>50	>50					
Ampicillin	-		0.19	0.39	50	50					
Gentamicin	-		0.39	0.78	0.78	0.78					

B)



Scheme 26: Antibacterial activity and druglikeness of compounds **94-97** (A); bioavailability radar of the most active compounds **95** and **96**.

Hassaneen *et al*. also explored a three component 1,3-dipolar cycloaddition involving isatin, ethyl 3,5-*bis*[phenylmethylidene]-4-oxopiperidine-*N*-carboxylate as the dipolarophile and sarcosine (**Scheme 24E** - 10 examples) or L-proline (**Scheme 24F** - 10 examples). This synthetic approach, which allowed the preparation of these new spiro compounds in very good yields (79-95%) in straightforward conditions, was followed by antibacterial screening against two Gram-positive bacteria (*Streptococcus pneumoniae* (RCMB 010010) and *B. subtilis* (RCMB 010067)) and two Gram-negative bacteria (*P. aeruginosa* (RCMB 010043) and *E. coli* (RCMB 010052)), using ampicillin and gentamicin as positive controls for Gram-positive and Gram-negative bacteria, respectively. The compounds exhibited antibacterial activity against most of these bacteria, often displaying a MIC lower than the one exhibited by the positive control, except for *P. aeruginosa*, to which only compound **98** presented antibacterial activity comparable to gentamicin. For the remaining bacterial strains, the most active compounds were **98-101**, displaying promising MICs and IC_{50}s (**Table 3**), in particular compound **101**.[108]

Table 3: Antibacterial activity and druglikeness of compounds **98-101** (NA – no activity).

Compounds	Ar	MIC (µg/mL)				Druglikeness				
		S. pneumoniae	*B. subtilis*	*P. aeruginosa*	*E. Coli*	Lipinski	Ghose	Veber	Egan	Muegge
98	4-ClC_6H_4	0.12	0.015	15.63	**1.95**					
99	4-$NO_2C_6H_4$	1.95	0.06	NA	15.63					
100	4-$OCH_3C_6H_4$	0.98	0.015	NA	15.63					
101	2,4-$F_2C_6H_3$	**0.015**	**0.015**	NA	**1.95**					
Ampicillin	-	0.015	0.007	NA	NA					
Gentamicin		NA	NA	15.63	1.95					

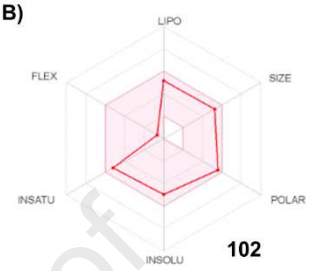
Switching the dipolarophile to 5-arylidine-2-thioxothiazolidin-4-one, Barakat *et al*. synthesized two new polycyclic (**102** and **103**) spirooxindole derivatives (85-89% yields) *via* a 1,3-dipolar cycloaddition (**Scheme 24G**). The antibacterial activity against the same four bacteria tested in the previous example was accessed, through the determination of the MIC value. In the case of the four bacteria, the compounds exhibited similar or even lower antibacterial activity when compared with the positive control. Despite the structural resemblance between both compounds, *in silico* studies

indicated that **103** binds in a different manner to the protein target aminoglycoside phosphotransferase than compound **102**, leading to a possible justification to differences in antibacterial activity (**Scheme 27**).[109]

A)

Compounds		R_4	MIC (μg/mL)				Druglikeness				
			S. pneumoniae	*B. subtilis*	*P. aeruginosa*	*E. Coli*	Lipinski	Ghose	Veber	Egan	Muegge
	102	CH_3	19	28	16	18.5					
	103	H	23	30	15	19					
	Ampicillin	-	25	33	NA	NA					
	Gentamicin		NA	NA	19	19					

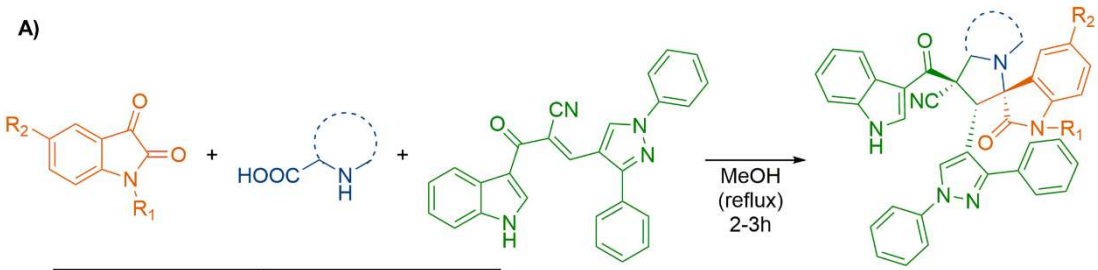
B)



Scheme 27: Antibacterial activity and druglikeness of compounds **102** and **103** (A) and bioavailability radar of compound **102** (B).

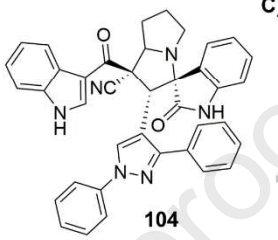
Another example of 1,3-dipolar cycloaddition involving isatin, various amino acids (sarcosine, proline and thioproline; 8 examples) and (*E*)-3-(1,3-diphenyl-1*H*-pyrazol-4-yl)-2-(1*H*-indole-3-carbonyl)acrylonitrile as dipolarophile (**Scheme 28A**) was reported by Kathirvelan *et al*., with the aim of preparing new antibacterial agents. The activity against a considerable number of pathogens was evaluated (*Shigella flexneri* (MTCC 1457), *M. luteus* (MTCC 106), *E. aerogenes* (MTCC 111), *S. aureus* (MTCC 96), *K. pneumoniae* (MTCC 109), *S. epidermidis* (MTCC 3615), *P. vulgaris* (MTCC 1771), *S. typhimurium* (MTCC 1251) and *S. aureus* (MRSA)), and while most compounds exhibited some level of growth inhibition, their MIC values were considerably lower than the ones displayed by positive control, streptomycin. The best results, displayed in **Scheme 28B**, were attained by compound **104**.[110] Concerning the predictive physical-chemical properties of this molecule, unfortunately it possesses low gastrointestinal absorption and poor druglikeness, probably due to its high molecular weight and lipophilicity (**Scheme 28C**).

A)

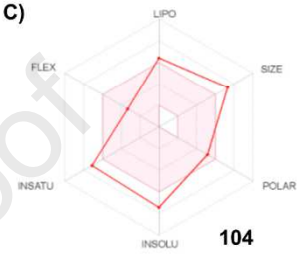


B)

Bacteria	MIC µg/mL	
	104	Streptomycin
S. typhimurium-B	250	30
K. pneumoniae	31.25	6.25
P. vulgaris	125	-
S. flexneri	31.25	6.25
M. luteus	31.25	6.25
E. aerogenes	62.5	25
S. epidermidis	62.5	6.25
S. aureus	31.25	6.25
MRSA	31.25	6.25



C)



Scheme 28: Synthetic route for the preparation of 3,2'-spiropyrrolidine-oxindole derivatives (A), antibacterial activity (B) and structure and bioavailability radar of compound **104** (C).

Looking closely to the structures of the most active compounds obtained via 1,3-dipolar cycloaddition and their respective physical-chemical properties prediction and druglikeness, some details need to be taken in consideration. Firstly, several of these compounds present excellent drug-like-properties and we would like to highlight the case of compound **96**, which is one of the most active of all these derivatives and highly selective against *K. pneumoniae*. Other compounds, such as **91-93**, despite being compliant with these rules, present a major drawback, which is the presence of the quinone moiety, a well-known PAINS. Rhodanines, present in the scaffold of compounds **102** and **103** are also well-established PAINS and therefore their potential as antibiotic agents might be jeopardized.

Remarkably, out of these thirteen compounds, only one (**97**) does not present a predictive good gastrointestinal absorption, according to the boiled-egg model (**Figure**

4). This feature makes these oxindole-derivatives obtained *via* 3-component 1,3-dipolar cycloaddition highly promising as potential antibiotic agents to be administered *per os*.

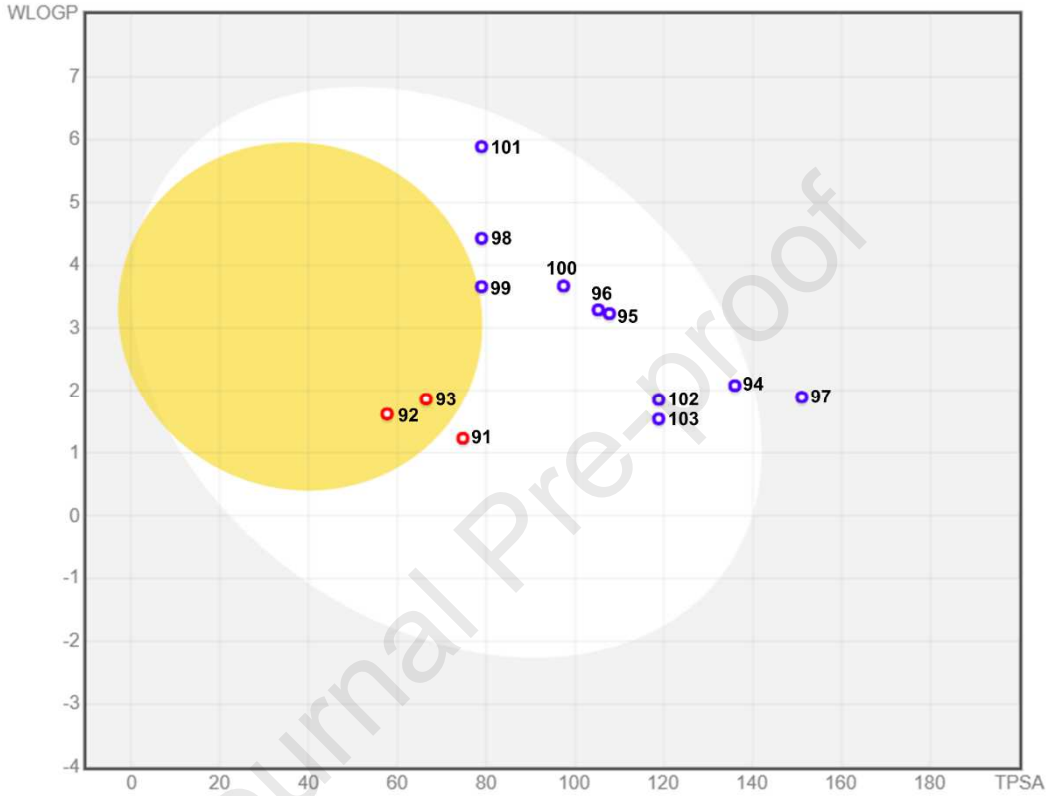
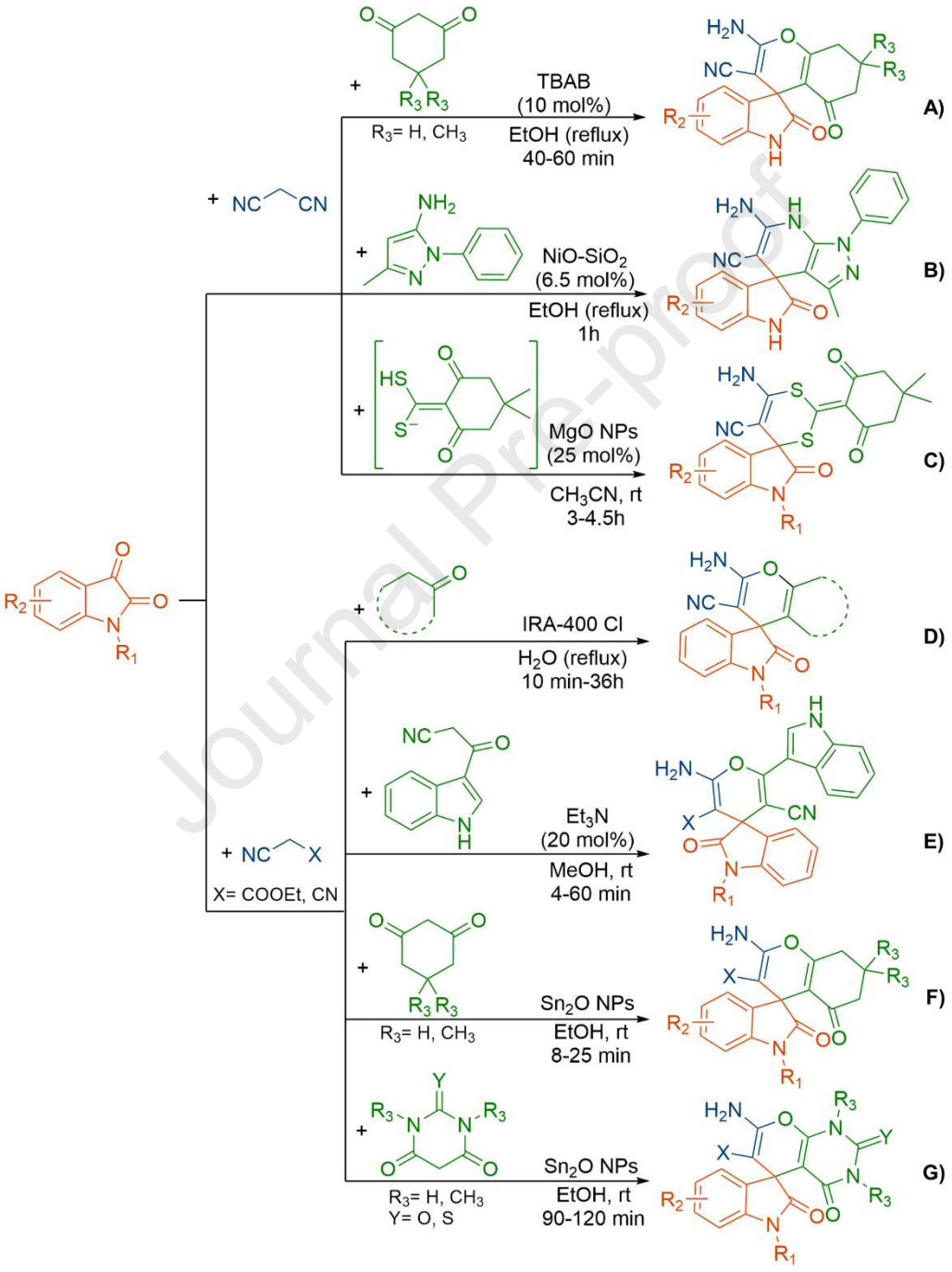


Figure 4: Boiled-egg model for the top 13 oxindole-derivatives with antibacterial activity obtained *via* 1,3-dipolar cycloaddition.

Knoevenagel/Michael addition MCRs are often applied in the context of Medicinal Chemistry, and there are also some recent examples of this approach in the synthesis of new isatin-based antibacterial drug candidates (**Scheme 29**).

Ramadoss *et al*. developed a small library of six derivatives (86-93% yield), using tetrabutylammonium bromide (TBAB) as the catalyst for the three-component reaction between isatin, malononitrile and cyclic 1,3-diketones (**Scheme 29A**). The obtained compounds were screened in what concerns their antibacterial activity against *E. coli*, *P. aeruginosa* and *K. pneumoniae*, but the compounds revealed to be substantially less active than the positive control ciprofloxacin.[111] The heterogeneous-catalyzed three component reaction between isatin, malononitrile and 3-methyl-1-phenyl-1*H*-pyrazol-5-amine (**Scheme 29B**), using NiO_2-SiO_2 as the acid catalyst, was explored in the preparation of spirooxindole-fused pyrazolo pyridine derivatives (13 examples) in very

good yields (85-95%). The determination of the growth inhibition zone induced by these compounds against *K. pneumoniae*, *S. aureus*, *E. coli* and *B. subtilis* showed weak antibacterial activity, with all the results being lower than the ones obtained for positive control streptomycin.[112]



Scheme 29: Knoevenagel/Michael addition based 3MCRs applied in the synthesis of isatin-based antibacterial agents.

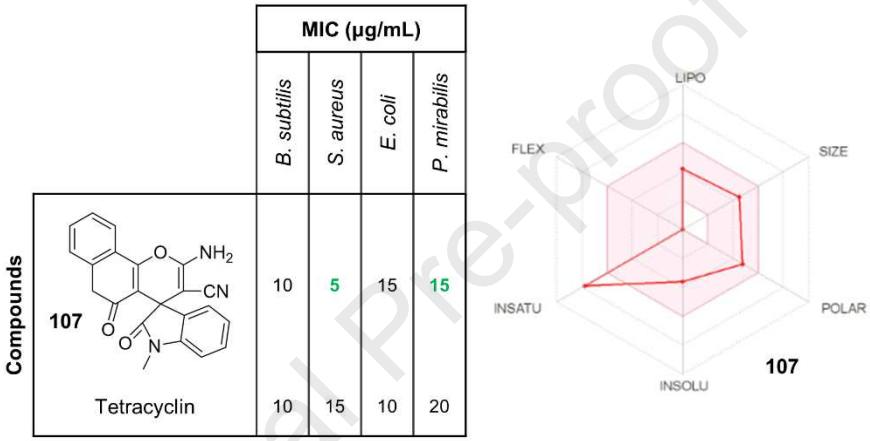
A different approach was reported by Moghaddam-Manesh *et al*., through the reaction between isatin, malononitrile and *in situ* generated (4,4-dimethyl-2,6-dioxo-cyclohexylidene)(mercapto)methanethiolate, from the reaction between dimedone and carbon disulfide (**Scheme 29C**). The generated small library (6 examples) was obtained in overall very good yields (81-93%), through a MgO NPs catalyzed reaction and their antibacterial activity evaluated, assessing the inhibition zone diameter (IZD), MIC and minimum bactericidal concentration (MBC) against five Gram-negative bacteria strains (*P. aeruginosa* PTCC 1310, *E. coli* PTCC 1399, *Shigella dysenteriae* PTCC 1188, *P. mirabilis* PTCC 1776 and *Salmonella enterica subsp. enterica* PTCC 1709) and three Gram-positive bacteria strains (*S. aureus* PTCC 1189, *Staphylococcus epidermidis* PTCC 1435 and *Rhodococcus equi* PTCC 1633). The most promising results were attained by compounds **105** and **106**, especially against *P. mirabilis* and *S. epidermidis* (**Table 4**), with MICs similar or slightly higher than the ones attained by positive control penicillin. Nonetheless, the compounds showed lower antibiotic activity towards all the strains tested when compared with the other positive control, gentamicin.[113] Nonetheless, the compliance with the main druglikeness rules falls short for these compounds, namely due to molecular weight and TPSA related concerns. Furthermore, a prediction of low oral bioavailability for both compounds can also indicate pharmacokinetic issues later on in the development pipeline.

Table 4: Most promising antibacterial results for compounds **105** and **106** and respective druglikeness rules compliance.

Compounds	R_1	R_2	MIC (µg/mL)		Druglikeness				
			P. mirabilis	*S. epidermidis*	Lipinski	Ghose	Veber	Egan	Muegge
105	2,4-$Cl_2C_6H_3CH_2$	H	16	8					
106	H	5-Cl	16	2					
Penicillin	-	-	16	1					
Gentamicin			0.5	0.5					

Harichandran *et al.* explored the reactivity of isatins, activated methylenes and different 1,3-dicarbonyl compounds, using amberlite IRA-400 Cl resin as heterogeneous catalyst and water as reaction medium (**Scheme 29D**).

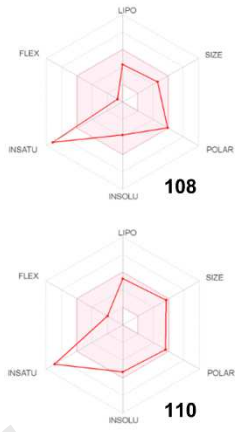
Six out of the sixteen compounds in this library of spirooxindole derivatives (21-98% yield) were then screened *in vitro* against four pathogenic bacterial strains (*B. subtilis* MTCC 441, *S. aureus* MTCC 96, *E. coli* MTCC 1689 and *P. vulgaris* MTCC 742). The most active compound, **107**, was obtained using *N*-methyl-isatin, malononitrile and 4-hydroxynaphthalen-2(1*H*)-one as starting materials and present comparable antibacterial activity, or in some cases even higher than the positive control, tetracyclin (**Scheme 30**).[114] This bioactive compound also displays a good predicted pharmacokinetic profile, as it complies with the five druglikeness rules and presents, theoretically, good oral bioavailability, making it a promising drug candidate.



Scheme 30: Antibacterial activity of compound **107** and respective bioavailability radar.

The triethylamine promoted three component reaction between isatins, activated methylenes and 3-cyanoacetyl indole (**Scheme 29E**) allowed the preparation of a library of spiro-oxindole derivatives bearing the indole moiety (12 examples, 72-89% yield). The antibacterial activity of these compounds was then accessed through the disc diffusion method, using *S. aureus*, *S. epidermidis* and *B. subtilis* as the targeted pathogenic bacteria. Compounds **108**-**110** presented antibacterial activity comparable to the one exhibited by the positive controls, gentamicin and chloramphenicol (**Scheme 31**).[115] The three compounds exhibit a high degree of compliance with the five rules of druglikeness, except for compound **110**, the most active, which exhibits one violation of the Ghose rule. The potential of these compounds is also highlighted by the prediction that they can cross the gastrointestinal barrier, and therefore present good bioavailability when administered *per os*.

Compounds		R_1	X	Zone of Inhibition (diameter, mm)			Druglikeness				
				S. aureus	*S. epidermidis*	*B. subtilis*	Lipinski	Ghose	Veber	Egan	Muegge
	108	H	CN	**29**	30	28					
	109	CH_3	CN	27	28	26					
	110	Bn	CN	**29**	**31**	**30**					
	Gentamicin	-	-	26	28	30					
	Chloramphenicol			29	31	32					

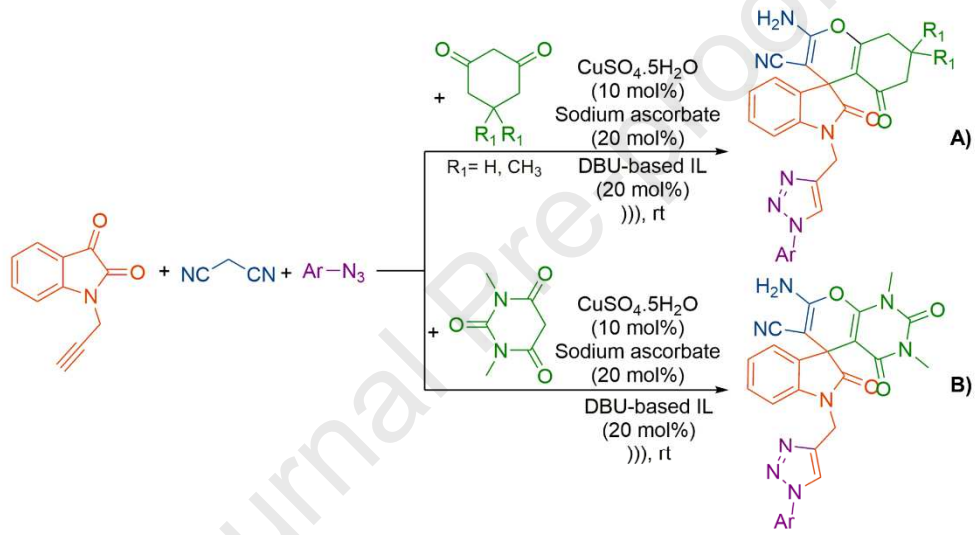


Scheme 31: Antibacterial activity of compounds **108**-**110**, and bioavailability radar for the two most active, **108** and **110**.

Recently, Moradi *et al*. explored the synthesis of spiro-oxindole derivatives through the three-component reaction involving isatin, activated methylenes and dicarbonyl compounds (including dimedone, cyclohexanedione and barbituric acid derivatives), under environmentally friendly conditions, using SnO_2 NPs as heterogeneous catalyst and ethanol as solvent at room temperature (**Scheme 29F and 29G**). The reaction allowed the preparation of 15 derivatives in very good yields (80-96%) and five compounds out of these were screened against seven bacterial strains (*S. aureus* ATCC 29737, *S. epidermidis* ATCC 12228, *E. coli* ATCC 10536, *K. pneumoniae* ATCC 10031, *S. dysenteriae* PTCC 1188, *P. vulgaris* PTCC 1182 and *S. paratyphi-A* ATCC 5702). The respective inhibition zones and MICs were evaluated, using tetracycline as positive control. However, the compounds were inactive against most bacterial strains, except for *S. epidermidis*, but the MIC was quite high for all the compounds (in the mg/mL range).[116]

A different approach was attempted by Singh *et al*., exploring a one pot Knoevenagel/Michael addition/azide-alkyne Huisgen cycloaddition reaction under ultrasonic irradiation using DBU-based ionic liquids as the reaction media (**Scheme 32**). The final derivatives (15 examples), bearing spiro-oxindole, 2-amino-4*H*-pyran and 1,2,3-triazole moieties, were obtained in excellent overall yields (88-94%). The antibacterial activity of these compounds was tested, first by determining the diameter of growth inhibition zone, where all the compounds proved to be ineffective towards Gram-negative bacteria (E. coli MTCC 1652 and P. aeruginosa MTCC 741), but some level of antibacterial activity was observed against two Gram-positive bacteria strains (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121). The authors decided to further explore

this selectivity towards Gram-positive bacteria, by determining the respective MICs and MBCs. The best results were achieved by compounds **111** (obtained via **Scheme 32A**) and **112** (obtained via **Scheme 32B**), although still less active than the positive control ciprofloxacin (**Scheme 32C**).[117] Noteworthy, the use of 1,3-dimethyl barbituric acid in the preparation of compound **112**, instead of dimedone (for compound **111**), leads to a considerably less favorable druglike features for the molecules, namely due to molecular weight, number of H-bond donors and acceptors and TPSA, which is further evidenced by the predicted non-gastrointestinal absorption. The presence of a *p*-bromophenyl moiety in the triazole seems to be relevant for the observed antibacterial activity, since the two more active compounds displayed this aromatic substituent.



C)

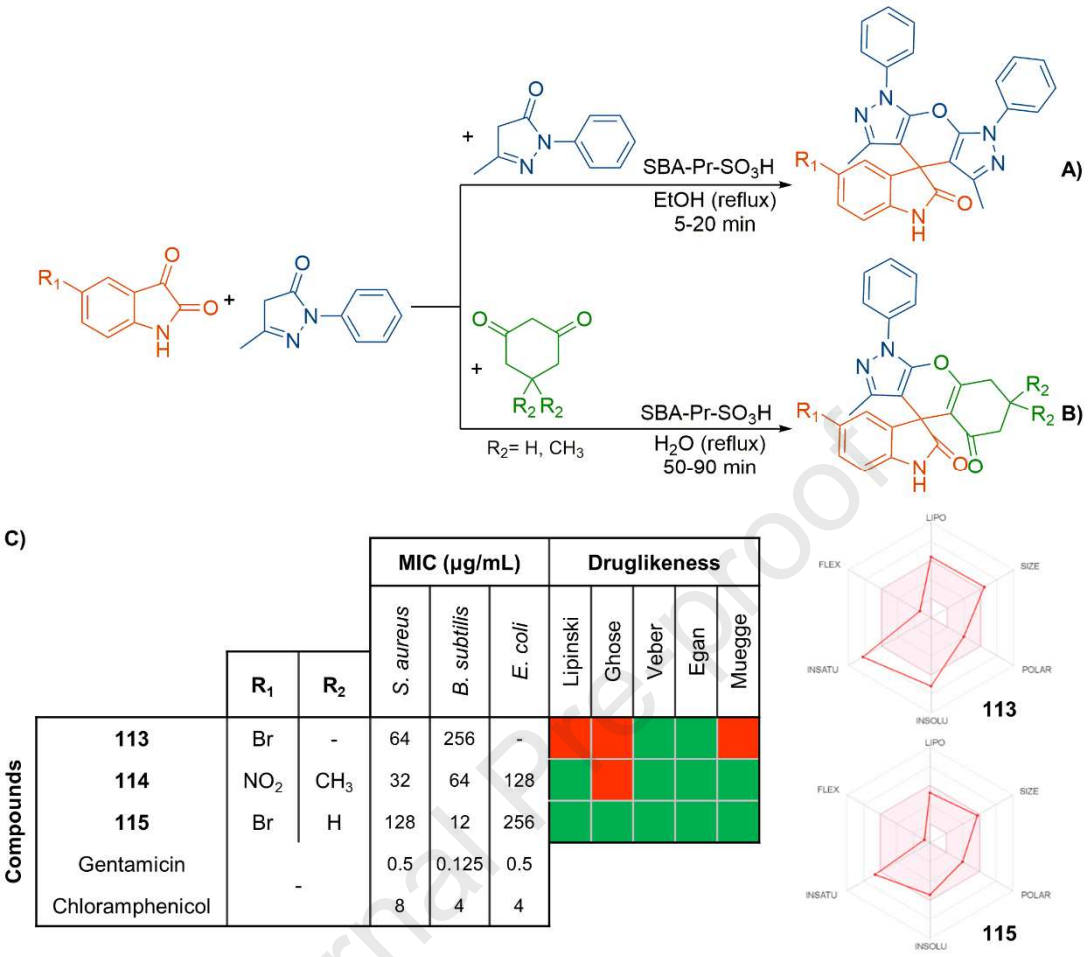
Compounds	R_1	Ar	MIC (µg/mL) *S. aureus*	MIC (µg/mL) *B. subtilis*	MBC (µg/mL) *S. aureus*	MBC (µg/mL) *B. subtilis*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
111	CH_3	4-BrC_6H_4	32	16	128	64					
112	-	4-BrC_6H_4	32	16	64	64					
Ciprofloxacin	-		6.25	6.25	6.25	6.25					

Scheme 32: Knoevenagel/Michael addition/azide-alkyne Huisgen cycloaddition based 4MCRs applied in the synthesis of isatin-based antibacterial agents (A-B) and respective MIC and MBC values and druglikeness evaluation (C).

Soorki and co-workers explored the application of sulfonic acid functionalized SBA-15 nanoporous material (SBA-Pr-SO_3H) to promote the synthesis of spiro-

oxindoles in two different contexts. In the first example, a *pseudo* 3 component reaction involving isatin and two equivalents of pyrazolone was carried out (**Scheme 33A**). The reaction showed low scope (6 examples) but afforded the products in overall very good yields (80-93%). Their antibacterial activity was accessed *in vitro* against two Gram-negative bacteria (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 85327) and two Gram-positive bacteria (*S. aureus* ATCC 25923 and *B. subtilis* ATCC 465). As in the previous example, all the compounds were ineffective against Gram-negative bacteria, and only two were active against *B. subtilis*. Antibacterial activity against *S. aureus* was observed for the six compounds, with the best MIC being achieved by compound **113**, although still less effective than with the positive controls; chloramphenicol and gentamicin (**Scheme 33C**).[118] In the second example, the same heterogeneous catalyst was applied, this time in a three-component reaction involving isatin, pyrazolone and dimedone or 1,3-cyclohexanedione (**Scheme 33B**). The small library (8 examples) was attained in good yields (76-85%) and their antibacterial activity was determined against the same four bacteria strains. This time, three of the derivatives displayed weak activity against *E. coli*, but no activity against *P. aeruginosa* was observed. In the case of the Gram-positive bacteria, once again all the derivatives displayed antibacterial activity against *S. aureus*, and just half of them had activity against *B. subtilis*, including the lowest MIC of the screening for compound **115** (**Scheme 33C**).[119]

Despite the structural similarities between the most active compounds, it is noteworthy that replacing the second pyrazolone moiety in compound **113** by a 1,3-cyclohexanedione in compound **115** leads to a major change in druglikeness, mostly due to molecular weight and lipophilicity related issues. Furthermore, the three compounds listed in **Scheme 33C** are predicted to show high gastrointestinal permeation, with **115** even exhibiting features that would allow permeation through the BBB.

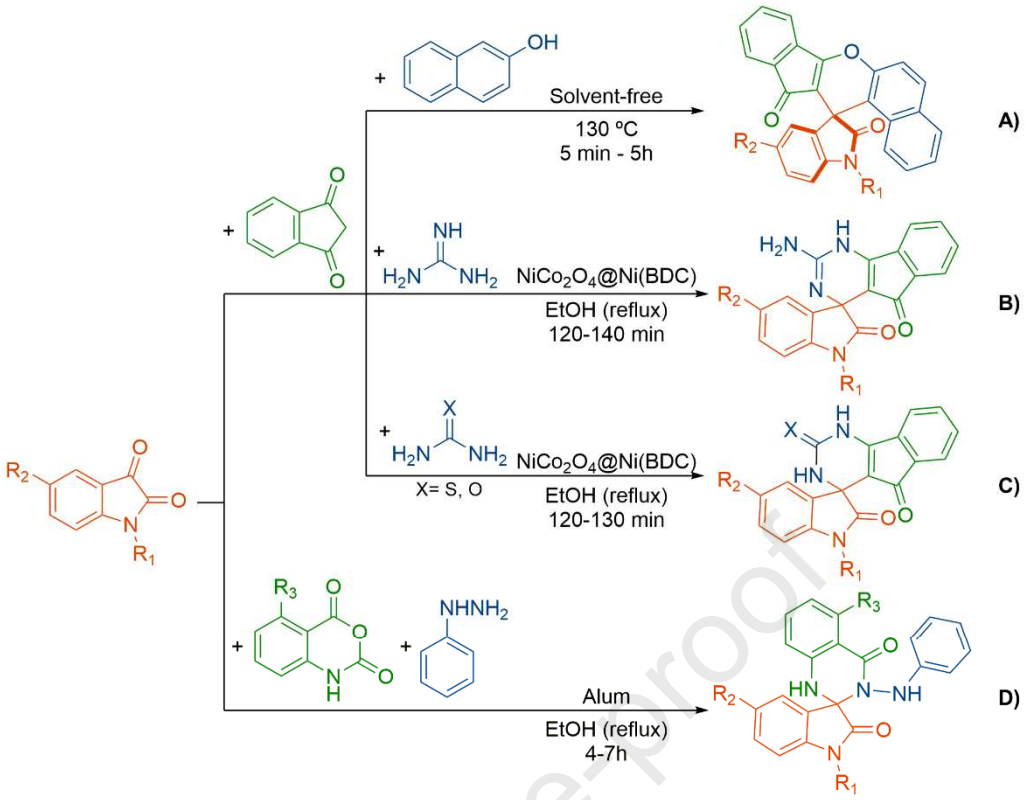


Scheme 33: SBA-Pr-SO_3H-promoted synthesis of spiro-oxindole derivatives (A-B); *In vitro* antibacterial screening and bioavailability radars for the most promising compounds (C).

Other spiro-oxindole derivatives were obtained using alternative synthetic methodologies and are summarized in **Scheme 34**. Soorki and co-workers explored the reaction between isatin, 1,3-indandione and 2-naphtol to achieve five spironaphthopyrano[1,2*b*]indeno-7,3'-indolines (50-92% yield) under solvent and catalyst-free conditions (**Scheme 34A**). The *in vitro* activity against the same four bacteria described in the last example was evaluated, but the MICs achieved showed weak antibacterial activity (the best result was 128 μg/mL).[120] Farhadi *et al*. used a $NiCo_2O_4$@Ni(BDC) (terephthalic acid) nanocatalyst to promote the reaction between isatin, 1,3-indandione and guanidine (3 examples; 93-98% yield - **Scheme 34B**) or (thio)urea (2 examples; 95-97% yield - **Scheme 34C**). These compounds were inactive against *E. coli* ATCC 25922 but most of them showed some inhibitory activity against *P. aeruginosa* ATCC 27853 accessed with disc diffusion method. The best results were

achieved when 5-bromo and 5-chloro isatins were used with guanidine to afford the corresponding spiro-oxindole derivatives against *S. aureus* ATCC 25932, leading to a larger halo of inhibition (17 and 18 mm, *versus* 16 mm in the positive control ciprofloxacin). Unfortunately, no MICs were evaluated.[121]

A different approach reported by Soorki and co-workers consisted in the Alum-catalyzed three component reaction involving isatin, isatoic anhydride and phenyl hydrazine (**Scheme 34D**) to afford a library of 3'-(phenylamino)-1'*H*-spiro[indoline-3,2'-quinazoline]-2,4'(3'*H*)-dione derivatives (13 examples, 60-97% yield). The resulting compounds were then subjected to a thorough *in vitro* antibacterial screening against ten bacterial strains (*B. subtilis* ATCC 465, *Bacillus pumilus* PTCC 1114*, M. luteus* PTCC 1110, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12228, *Streptococcus mutans* PTCC 1601, *E. coli* ATCC 25922, *Enterococcus faecalis* ATCC 29737, *P. aeruginosa* ATCC 85327, *K. pneumoniae* ATCC 29655). While five of these compounds were inactive in all the bacteria tested, compounds **116-119** proved to possess antibacterial activity higher or comparable to the positive controls (tetracyclin and gentamicin) (**Scheme 34E**). Remarkably, these four promising compounds comply with the five analyzed druglikeness rules and predictably exhibit good gastrointestinal absorption, making them good candidates to proceed in the drug discovery pipeline.

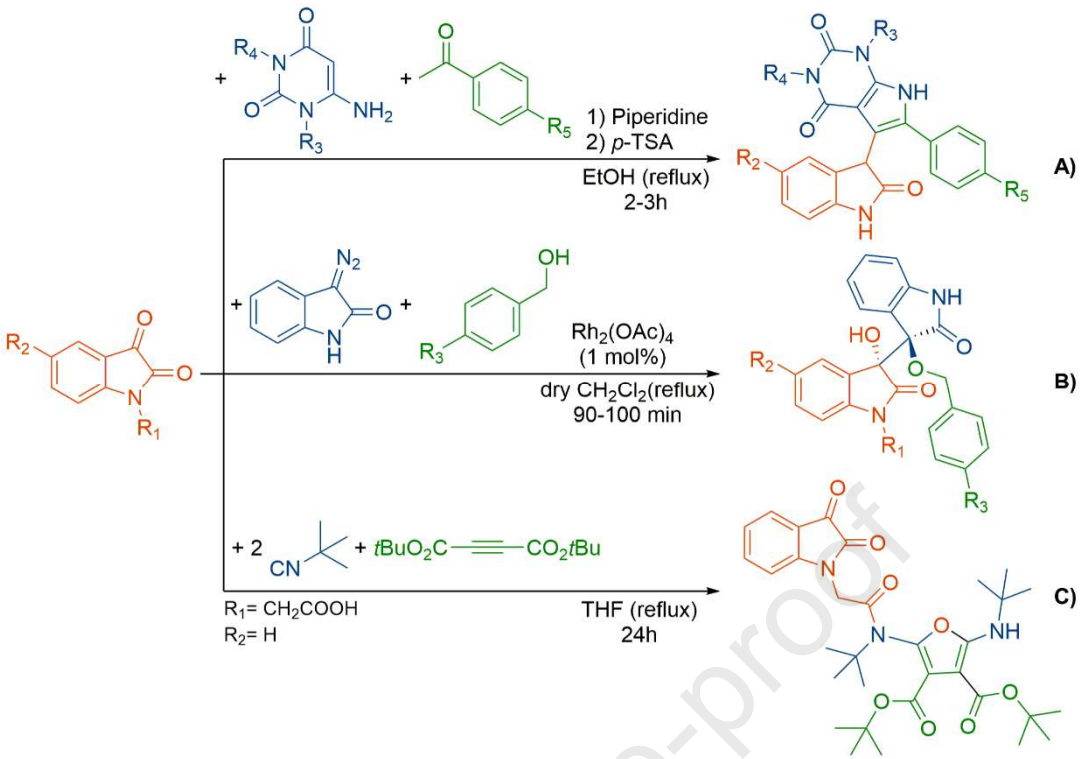


E)

Compounds	R_1	R_2	R_3	MIC (µg/mL)									
				B. subtilis	*B. pumilus*	*M. luteus*	*S. aureus*	*S. epidermidis*	*S. mutans*	*E. coli*	*E. faecalis*	*P. aeruginosa*	*K. pneumoniae*
116	H	NO_2	H	4	**4**	8	4	4	**<2**	4	16	32	**8**
117	H	Br	Cl	4	**<2**	4	**2**	2	**<2**	**<2**	**<2**	8	**4**
118	H	H	Cl	8	**4**	8	**2**	4	8	32	128	ND	ND
119	H	NO_2	Cl	**<2**	**<2**	**2**	**2**	2	8	**<2**	8	16	16
Tetracycline	-			4	8	4	4	<2	2	ND	8	ND	16
Gentamicin				ND	ND	ND	ND	ND	ND	4	ND	8	ND

Scheme 34: Synthesis of spiro-oxindole derivatives with potential antibacterial activity (A-D). Most promising results of the antibacterial screening (E) (ND=not determined).

Despite the fact that most oxindole-derivatives reported in the literature consisted of spiro-oxindoles, some relevant publications describe synthetic methodologies to afford non-spiro derivatives. The most recent examples concerning MCRs are summarized in **Scheme 35**.



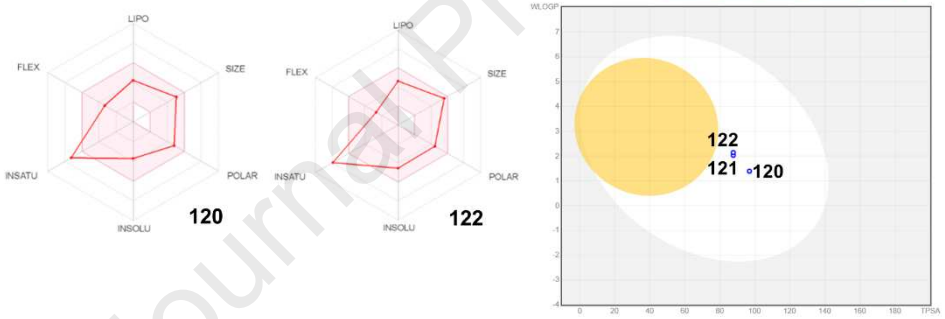
Scheme 35: Synthesis of non-spiro-oxindole derivatives with potential antibacterial activity.

Rad-Moghadam and Azimi reported the synthesis of a library of oxindolylpyrrolo[2,3*d*]pyrimidine derivatives (16 examples) using a tandem three component reaction, which required pH variation to succeed (**Scheme 35A**). This efficient methodology allowed the preparation of the desired derivatives in very good to excellent yields (82-95%), which were then screened to verify their antibacterial potential. The selected bacteria strains consisted of two Gram-negative (*E. coli* ATCC 25922 and *P. aeruginosa* ATCC 85327) and two Gram-positive bacteria (*B. subtilis* ATC465 and *S. aureus* ATCC 25923). The results showed that while several derivatives were inactive against *E. coli*, several of them (8 compounds) were more effective against *P. aeruginosa* than the positive control, norfloxacin. The vast majority of the compounds presented antibacterial activity against the two Gram-positive bacteria tested, however, for all the cases, the MIC values obtained were in the range of mg/mL.[122]

A rhodium(II)-catalyzed three component reaction was established by Lakshmi *et al.*, *via* the reaction between isatin, 3-diazooxindole and benzyl alcohols (**Scheme 35B**), affording bisoxindole derivatives (10 examples) in overall very good yields (82-90%). The antibacterial activity of these structurally interesting compounds was then evaluated, performing in vitro tests against five bacterial strains (*S. aureus* NCIM5021, *E. coli* NCIM 2931, *P. vulgaris* NCIM 2813, *S. typhi* NCIM 2501 and *P. aeruginosa*

NCIM 5029). The library proved to possess relevant antibacterial activity, in the μM-nM range, with compounds **120**-**122** exhibiting the most promising results (**Scheme 36**).[123] Besides the excellent antibacterial activity observed, these compounds share a total compliance with the five evaluated druglike rules. Furthermore, and as visualized in the bioavailability radars of compounds **120** and **122** (which are very similar due to their structural resemblance) and in the Boiled-Egg model, these compounds theoretically exhibit good gastrointestinal absorption, and therefore these bisoxindole derivatives appear as great candidates for further development in the drug discovery pipeline.

					MIC (μM)					Druglikeness				
		R_1	R_2	R_3	*S. aureus*	*P. aeruginosa*	*E. coli*	*P. vulgaris*	*S. typhi*	Lipinski	Ghose	Veber	Egan	Muegge
Compounds	**120**	H	H	OCH_3	0.075	**0.038**	**0.038**	0.075	**0.038**					
	121	H	H	Cl	0.149	0.074	0.074	0.149	0.074					
	122	H	H	Br	0.067	0.067	**0.034**	0.067	**0.034**					

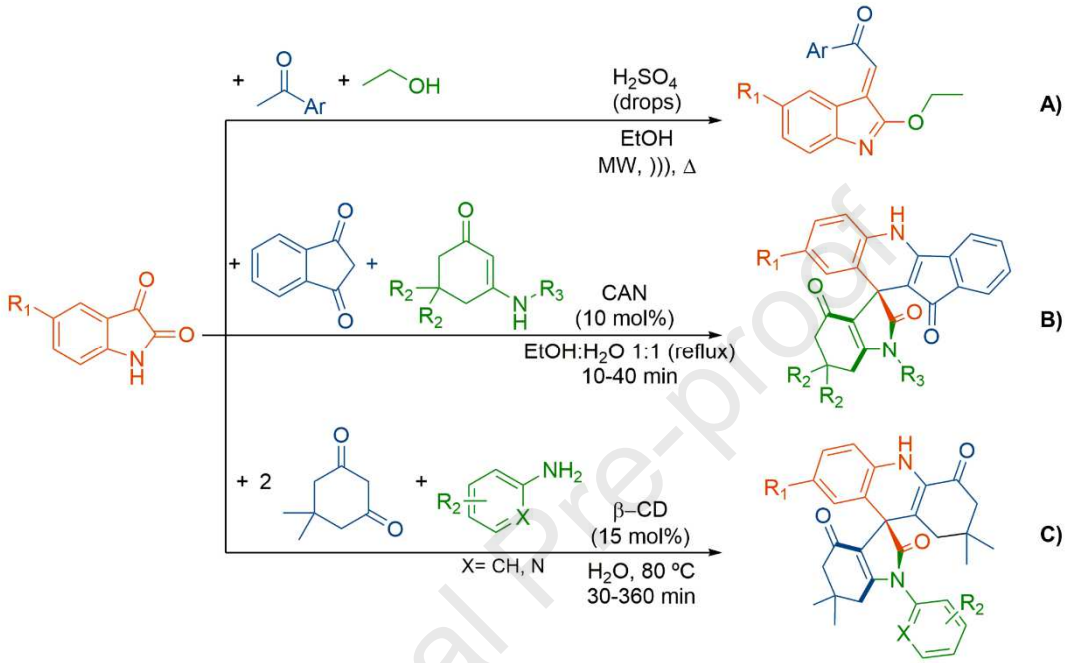


Scheme 36: Antibacterial activity, druglikeness and pharmacokinetic evaluation of the most promising bisoxindole derivatives prepared by Lakshmi *et al.*.

A very different approach to the previously mentioned was the one reported by Baharfar *et al*., using a pseudo-four component isocyanide-based reaction to prepare one compound bearing the isatin and 2,5-diaminofuran moieties. Unlike the previously reported compounds, in this case, the reaction is not promoted at the C3 position of the isatin core, as 2-(2,3-dioxoindolin-1-yl)acetic acid is used as starting material (**Scheme 35C**). The antibacterial activity of this compound against *E. coli* PTCC 1330, *P. aeruginosa* PTCC 1074, *S. aureus* ATCC 35923, and *B. subtilis* PTCC 1023 was accessed using disk diffusion assay. However, the results showed that the compound exhibited, in average, two times less activity than positive controls gentamicin and chloramphenicol.[124] Evaluation of the druglike properties and pharmacokinetic profile also does not show great potential for this compound, as it fails to comply with the five

rules and exhibits poor pharmacokinetic profile, with poor to no gastrointestinal absorption predicted.

Less common is the application of isatin in MCRs to obtain non-oxindole derivatives. Among the recent reported examples, we can highlight three concerning the generation of libraries which were screened against different bacteria, summarized in **Scheme 37**.

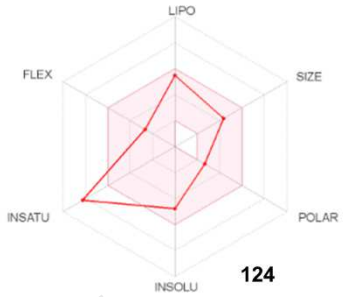


Scheme 37: Recent examples of isatin-based multicomponent reactions applied for the synthesis of non-oxindole derivatives with potential antibacterial activity.

Ashok *et al.* explored different activation techniques for the three-component reaction between isatin, aromatic ketones and ethanol, which played a dual role as both reagent and solvent (**Scheme 37A**). The reaction, catalyzed by sulfuric acid, allowed the preparation of the desired 2-(2-ethoxy-5-substituted-indol-3-ylidene)-1-aryl-ethanones (19 examples) under conventional heating, ultrasound irradiation and microwave irradiation, with the last one allowing higher yields (86-92%) and short reaction times. The antibacterial activity against *E. coli* and *S. aureus* was then evaluated, using disc diffusion method at three different concentrations (25, 50 and 100 µg/mL). All the compounds showed some level of antibacterial activity, with compounds **123**-**125** proving to be the most effective, as even at the lowest concentration they exhibited similar or higher activity than the positive control ampicillin (**Scheme 38**).[125] The SwissADME analysis of the most active compounds show that they display an overall good pharmacokinetic profile prediction, exception for compound **125**, which is not in accordance with the Muegge rule, due to a higher LogP than desirable, due to the inclusion of the naphthyl moiety. Furthermore, the three compounds predictably

exhibit good gastrointestinal absorption, as well as BBB permeation; making them a good starting point for further exploration in what concerns their potential as drugs.

Compounds		R_1	Ar	Zone of Inhibition (diameter, mm) *E. coli*	Zone of Inhibition (diameter, mm) *S. aureus*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
	123	H	4-ClC_6H_4	6	6					
	124	Br	C_6H_5	6	7					
	125	Br	1-naphthyl	6	6					
	Ampicillin		-	6	6					



Scheme 38: Antibacterial activity of the most active 2-(2-ethoxy-5-substituted-indol-3-ylidene)-1-aryl-ethanones and bioavailability radar for compound **124**.

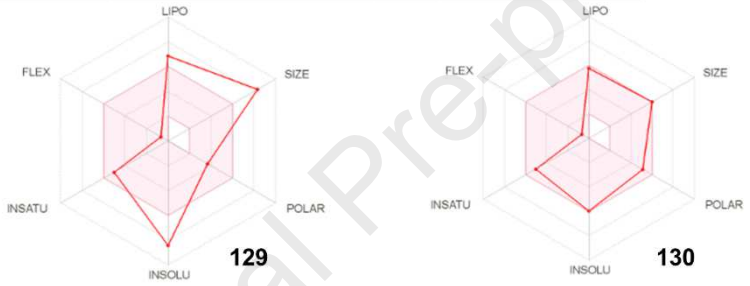
A library of spiro[indolo-3,10'-indeno[1,2-*b*]quinolin]-2,4,11'-trione derivatives (22 examples; 83-95% yield) was prepared *via* a three component reaction involving isatin, enaminones and 1,3-indandione, catalyzed by ceric ammonium nitrate (CAN) (**Scheme 37B**). The antibacterial activity against two Gram-positive bacteria strains (*S. aureus* MTCC 96 and *B. subtilis* MTCC 121) and two Gram-positive bacteria strains (*E. coli* MTCC 1652 and *P. aeruginosa* MTCC 741) was evaluated, using disk diffusion method and MIC determination. While all the derivatives showed to possess some activity against *E. coli* and the Gram-positive strains, they could not inhibit the growth of *P. aeruginosa*. The MIC values obtained showed that some compounds, and in particular IVc, possesses good antibacterial activity, although weaker than the one displayed by positive control ciprofloxacin (**Table 5**).[126] Despite the ability to cross the gastrointestinal barrier according to the predictive model, the compounds do not comply with all the druglike rules, in particular with the Ghose rule, in particular due to molecular weight and lipophilicity constrains.

Table 5: Antibacterial activity of the most promising spiro[indolo-3,10'-indeno[1,2-*b*]quinolin]-2,4,11'-trione derivatives.

Compounds		R_1	R_2	R_3	MIC (μg/mL)			Druglikeness				
					S. aureus	*B. subtilis*	*E. coli*	Lipinski	Ghose	Veber	Egan	Muegge
	126	H	CH_3	4-ClC_6H_4	16	8	64					
	127	NO_2	H	4-ClC_6H_4	32	32	128					
	128	NO_2	CH_3	C_6H_5	16	16	128					
	Ciprofloxacin	-			6.25	6.25	6.25					

Recently, the same four bacterial strains were applied in the antibacterial evaluation of a library of spiro[acridine-9,3′-indole]-2′,4,4′(1′*H*,5′*H*,10*H*)-trione derivatives (20 examples), synthesized *via* a *pseudo* four component reaction involving isatin, substituted anilines and dimedone (2 equivalents), using a sustainable methodology (**Scheme 37C**). Catalytic amounts of β-cyclodextrin (β-CD) allowed the preparation of the desired compounds (78-95% yield), using water as reaction media, while the hydrophobic interior of the catalyst provided the formation of efficient host-guest complexes through a supramolecular catalytic system. The best results of the antibacterial screening are summarized in **Scheme 39**. Remarkably, while so far most of the compounds seemed to be selective towards Gram-positive bacteria, in the case of compounds **129** and **132** they display more activity against Gram-negative bacteria. On the other hand, in the case of *B. subtilis* the two compounds, **130** and **131**, showed better results than their positive controls.[127] The substitution pattern of the aniline moiety not only plays a major role in the antibacterial activity, but also in the pharmacokinetic prediction profile of these derivatives. The depicted bioavailability radar for compound **129**, combined with its poor druglikeness, indicate that the presence of the tribromoaniline moiety leads to a substantial deterioration of the predicted pharmacokinetic profile, including the inability to permeate the gastrointestinal tract. The remaining four compounds are compliant with all the rules (except Ghose rule due to molecular weight concerns) and theoretically are able to cross the gastrointestinal barrier, being eligible candidates to be orally delivered antibiotics.

Compounds	R_1	R_2	X	MIC (µg/mL) *S. aureus*	*B. subtilis*	*E. coli*	*P. aeruginosa*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
129	H	2,4,6-Br_3	CH	100	62.5	37.5	50					
130	H	2-SH	CH	225	**25**	187.5	150					
131	H	H	N	125	**50**	62.5	NA					
132	H	2-F	CH	NA	75	37.5	50					
133	H	2-OCH_3	CH	75	75	175	100					
Ampicillin	-			250	250	100	100					
Ciprofloxacin	-			50	50	25	25					



Scheme 39: Antibacterial activity of the most promising spiro[acridine-9,3′-indole]-2′,4,4′(1′*H*,5′*H*,10*H*)-trione derivatives and respective bioavailability radar for compounds **129** and **130**.

2.2. Antifungal activity

Often overlooked by Health authorities and the scientific community, fungal infections present an unneglectable socio-economic burden nowadays. With an estimated mortality of more than 1.5 million worldwide, and an incidence of over one billion, fungal infections affect mostly immunosuppressed patients, a population which is continuously growing thanks to clinical and therapeutic advances.[128, 129] The existent therapeutic arsenal is becoming powerless, with the emergence of drug resistance, as well as due to toxicity and side-effects. This leads to the need of further drug discovery and development efforts in the field of antifungal agents, supported by the better understanding of fungal pathogenesis and identification of new druggable targets.[130-134]

Several of the compounds described in the antibacterial activity section of this work were also evaluated in what concerns their antifungal activity. For this reason, in this section, no observation will be made on the synthetic routes to achieve these

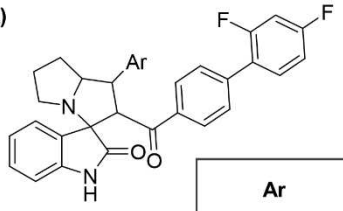
isatin-based MCRs products with promising antifungal activity, and focus will be given to their structures, bioactivity and pharmacokinetic/druglike features. Among the already described derivatives, some were tested against yeast strains, *Aspergillus spp*. and, less commonly *Rhizopus spp*. and *Fusarium spp*., but no or weak activity was observed (often the isatin-derived compounds were less active at least by two-fold when compared to the positive control). This was observed with some of the libraries already described in this review (**Schemes 28A, 29A-C, 37B-C**) and therefore no further detail will be given to these compounds regarding their antifungal activity.

Among the opportunistic infections caused by fungi, we will start to highlight the development of compounds targeting yeasts. These unicellular organisms can generate different clinical manifestations, from easy-to-treat mucocutaneous localized infections to more invasive and life-threatening conditions. Among the different yeast species, *Candida spp*. are among the most common fungal infections detected in clinical practice, that can lead to candidemia, a possible deadly form of the infection characterized by the presence of the yeast in the bloodstream. The worldwide distribution of *Candida spp*. in nosocomial infections leading to candidemia evolves with time and with the global region. While in the north of Europe (Iceland, Finland, Norway, Denmark) *C. albicans* is the most common candidemia agent (56-70%), followed by *C. glabrata* (13-21%), in the south of Europe (Spain), these percentages drop to 45% and 13% respectively. On the other hand, *C. parapsilosis*, which in the north of Europe corresponds to a small percentage of the cases (3.7-5.8%), in Spain can reach up to 25%. In the USA, the proportion of *C. albicans* is even lower (38%), with *C. glabrata* representing a large portion of the cases (29%). *C. tropicalis* and *C. krusei* have also been detected in several candidemia cases, although with lower incidence (up to 10% and 8% respectively).[135]

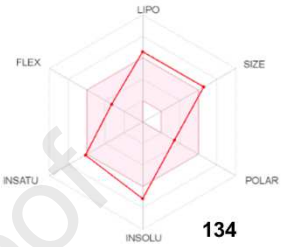
With this epidemiological problem in mind, it would be expected that some research groups would focus their attention on the quest for new antifungal drug candidates for treating yeast infections. The derivatives obtained by Fathimunnisa *et al*. (see **Scheme 23A**) have been screened against three *Candida spp* (*C. albicans*, *C. tropicalis* and *C. glabrata*), with one of them (**134**) displaying similar antifungal activity against the three strains using amphotericin-B as positive control. Compounds **135** and **136** also displayed interesting antifungal activity, as summarized in **Scheme 40A**.[102] Despite their predictable gastrointestinal absorption, as indicated by the bioavailability radar and druglikeness compliance, the molecular weight, as well as the predicted lipophilicity for these derivatives indicate their poor druggability. Singh *et al*. verified the antifungal activity of their derivatives (see **Scheme 32A**) against C. albicans (MTCC 227) and also against *Saccharomyces cerevisiae* (MTCC 170), a less common

pathogen in clinical practice, but which can also lead to invasive fungal infections. Two derivatives (**137** and **138**) displayed interesting antifungal activity against this species of yeast, proving to be more active than the positive control amphotericin-B.[117] Despite the structural resemblance between the two compounds, SwissADME predicts no gastrointestinal absorption for compound **137**, while **138** presents a more favorable pharmacokinetic profile (**Scheme 40B**).

A)

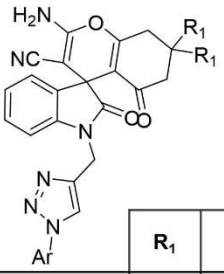


Compounds	Ar	MIC (µg/mL) *C. albicans*	*C. tropicalis*	*C. glabrata*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
134	2-$OCH_3C_6H_4$	12.5	12.5	12.5					
135	4-ClC_6H_4	12.5	25	25					
136	4-$CH_3C_6H_4$	12.5	25	25					
Amphotericin-B		12.5	12.5	12.5					

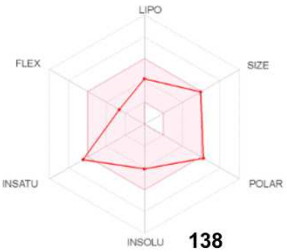


134

B)



Compounds	R_1	Ar	MIC (µg/mL) *C. albicans*	*S. cerevisiae*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
137	CH_3	4-$OCH_3C_6H_4$	128	64					
138	H	4-$OCH_3C_6H_4$	128	64					
Amphotericin-B		-	100	100					

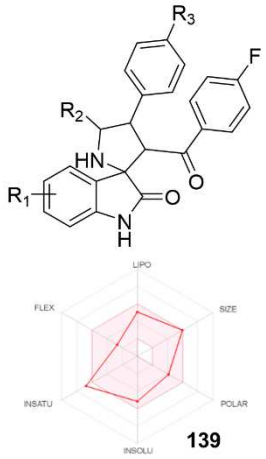


138

Scheme 40: Evaluation of the activity of different spiro-oxindoles against yeast strains.

Other common manifestation of fungal infections are the ones caused by mold and, among these, *Aspergillus spp*. play a major etiological role. The clinical symptoms can range from simple infections to more life-threatening conditions, usually involving complications in the respiratory system, and highly dependent on the subject immune system status.[136, 137] For these reasons, authors often include an *Aspergillus spp*. in their antibiotic screening procedure. For example, the already described druglike compounds **108** and **109** were evaluated against *A. niger* and led to a zone of inhibition similar to the one observed for the positive control, nystatin (28 and 27 mm, respectively, *versus* 28 mm).[115] The compounds prepared by Sapnakumari *et al*. – which were already described in this work (see **Scheme 20A**) for their excellent antitubercular activity - were also evaluated against *A. niger* and *A. flavus*, with several

of these derivatives displaying antifungal activity similar to the positive control, fluconazole (**Scheme 41**).[93] Despite good oral bioavailability for the five derivatives depicted in **Scheme 41**, only **139** was compliant with the main druglikeness rules, as shown in the bioavailability radar.



Compounds	R_1	R_2	R_3	MIC (µg/mL) *A. niger*	MIC (µg/mL) *A. flavus*	Druglikeness Lipinski	Ghose	Veber	Egan	Muegge
139	H	CH_2OH	Cl	20	20					
140	H	CH_2OH	Br	20	30					
141	H	$CH_2CH(CH_3)_2$	H	20	20					
142	H	$CH_2CH(CH_3)_2$	Cl	20	20					
143	H	$CH_2CH(CH_3)_2$	Br	30	20					
Fluconazole	-			20	20					

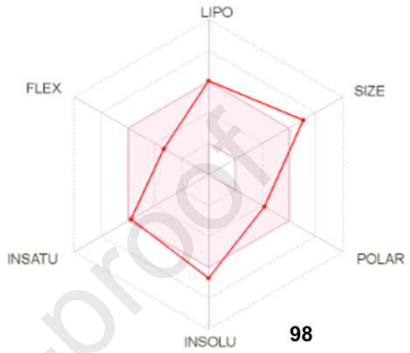
Scheme 41: Evaluation of the antifungal activity of spiro-oxindole derivatives against *Aspergillus spp*.

Several research groups chose to make a wider screening of antibiotic activity, which also included unicellular and pluricellular fungi as their target microbes. For example, compound **91**, already described for its relevant antibacterial activity, was also evaluated against three different fungal strains – *Malassezia pachydermatis* (a yeast which rarely infects humans, but with some prevalence in veterinary fungal infections), *C. albicans* MTCC 227, and *Botrytis cinerea* (a necrotrophic fungi which can, in predisposed individuals, lead to hypersensitivity pneumonitis after inhalation of the mold). Compound **91** exhibited similar activity against *M. pachydermatis* (MIC=15.62 µg/mL) with fluconazole (12.5 µg/mL) and ketoconazole (15 µg/mL) as the positive controls. Against *C. albicans*, the result was also promising (31.25 µg/mL versus 25 µg/mL for ketoconazole and >100 µg/mL for fluconazole).[105]

Other examples of spiro-oxindole derivatives already described in this work that were subjected to antifungal activity screening were compounds **98-103**. The selected fungi were *Aspergillus fumigatus* RCMB 02568, *Syncephalastrum racemosum* RCMB 05922 (a filamentous fungus, which can rarely lead to complications designated mucormycosis, an opportunistic infection), *Geotrichum candidum* RCMB 05097 (although part of the human microbiome, this fungus can cause geotrichosis, which can affect different organs), and *Candida albicans* RCMB 05036. The antifungal activity of these compounds is summarized in **Scheme 42**.[108, 109] In the case of compounds

102 and **103**, docking studies of the compounds with the well-established antifungal target, lanosterol 14α-demethylase, showed that compound **102** established four hydrogen bonds with the enzyme, while compound **103** only established one. This might explain the higher antifungal activity displayed by compound **102**.[109]

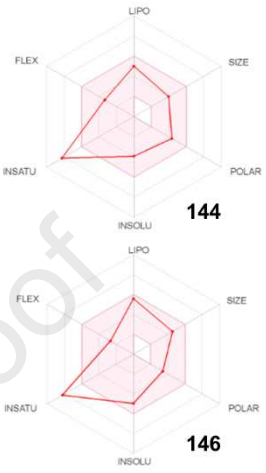
Compounds	MIC (µg/mL)			
	A. fumigatus	*S. racemosum*	*G. candidum*	*C. albicans*
98	**0.015**	**1.95**	**0.015**	**0.06**
99	1.95	**0.49**	0.12	NA
100	1.95	**0.98**	0.49	NA
101	7.81	**1.95**	0.03	NA
Amphothericin-B	0.015	1.95	0.015	0.06
	Zone of inhibition (diameter, mm)			
102	18	19	27	**27**
103	16	14	24	25
Fluconazole	25	24.1	30	28
Amphothericin-B	23	22.1	29	26



Scheme 42: Antifungal screening of several spiro-oxindole derivatives, and bioavailability radar of the most active compound, **98**.

Among the examples of isatin-based MCRs derived from non-oxindole derivatives, only the library reported by Ashok *et al*. (see **Scheme 37A**) was tested against two strains of fungus – *A. niger* and *Candida metapsilosis*. Using the disc diffusion assay, at concentrations of 25, 50 and 100 µg/mL, several compounds exhibited good antifungal activity. The best results are summarized in **Scheme 43** (at concentration of 25 µg/mL). Noteworthy, the compounds exhibiting higher antifungal activity were not the same as those that displayed higher antibacterial activity, indicating selectivity within the same family of compounds.[125] All the compounds predictably possess good pharmacokinetic profiles, complemented with good gastrointestinal absorption, and comply with the five druglikeness rules. These predictions, combined with the synthetic accessibility and selectivity towards fungal species versus bacterial strains, makes these compounds an excellent starting point for new antifungal drug candidate development.

Compounds		R_1	Ar	Zone of Inhibition (diameter, mm)		Druglikeness				
				A. niger	*C. metapsilosis*	Lipinski	Ghose	Veber	Egan	Muegge
	144	H	4-$NO_2C_6H_4$	11	11					
	145	H	2-OHC_6H_4	10	11					
	146	Br	2-OHC_6H_4	11	11					
	Grieseofulvin		-	10	10					



Scheme 43: Antifungal activity of the most active 2-(2-ethoxy-5-substituted-indol-3-ylidene)-1-aryl-ethanones and bioavailability radar for compounds **144** and **146**.

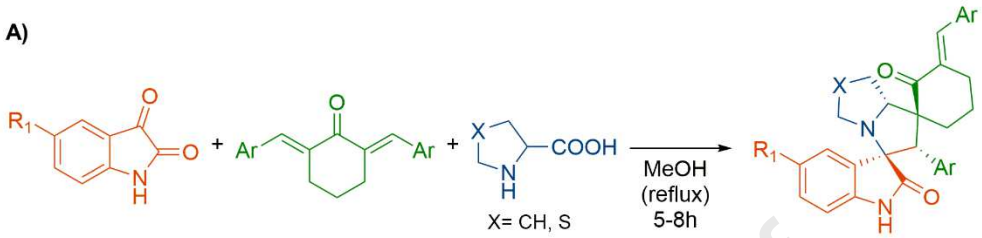
2.3. Antileishmanial activity

Leishmaniasis is a parasitic disease caused by several species of the genus *Leishmania*, with two main clinical manifestations, designated visceral and cutaneous leishmaniasis. It is classified by the WHO as a neglected tropical disease and despite some therapeutic options available, the diversity of infectious species and host symptoms makes the discovery of new drug candidates an urgent priority.[138, 139]

In the recent literature, only one example of isatin-based MCRs explores the antileishmanial activity of the synthesized products, although multiple examples can be found of oxindole-based antileishmanial active compounds.[140, 141] Lotfy *et al*. explored a 3-MCR involving isatin, cyclohexanone-based chalcones and *L*-proline or thioproline (**Scheme 44A**). The resulting library (17 examples, up to 96% yield) was evaluated *in vitro* against *Leishmania major* promastigotes, with compound **147** displaying relevant antileishmanial activity (IC_{50} = 6.8 ± 0.54 μM), even when compared with positive control drugs amphotericin B (0.49 ± 0.03 μM) and pentamidine (5.22 ± 0.70 μM) (**Scheme 44B**). Interestingly, this same compound was also exhibited more antiproliferative activity against HeLa cell line (IC_{50} = 4.8 ± 0.1 μM, *versus* the positive control doxorubicin, IC_{50} = 1.2 ± 0.4 μM). Nonetheless, this compound also exhibited high cytotoxicity against a normal mouse fibroblast cell line 3T3 (IC_{50} = 19.1 ± 2.6 μM, *versus* the positive control cycloheximide, IC_{50} = 0.3 ± 0.02 μM).[142] Nonetheless, the

compound presents poor compliance with the five druglikeness rules, as well as predictive poor GI absorption, making it a not so suitable drug candidate, especially for oral administration.

A)



B)

Compounds	R_1	Ar	X	MIC (μM): Antileishmanial activity	Druglikeness: Lipinski	Ghose	Veber	Egan	Muegge
147	H	3-$NO_2C_6H_4$	CH	6.8 ± 0.54					
Amphotericin B		-		0.49 ± 0.03					
Pentamidine				5.22 ± 0.7					

Scheme 44: Synthesis of spiro-oxindoles *via* 3-MCR (A), antileishmanial activity and druglikeness of the most active compound (B).

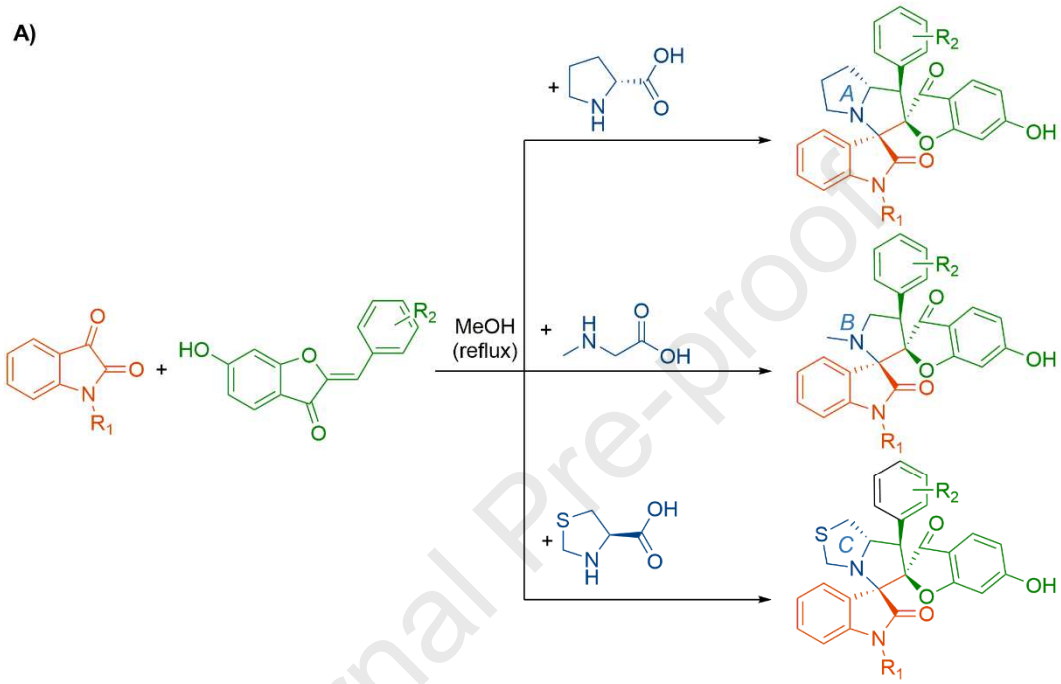
2.4. Antiviral activity

The quest for new antiviral agents is also the main focus area of several research groups working in drug discovery, and several oxindole-based molecules exhibit promising antiviral activity. The antiviral importance of oxindole-bearing compounds dates back to the last century, as methisazone (Marboran®) was applied as a prophylaxis treatment for smallpox infection, until the discovery of a vaccine and implementation of successful vaccination programs which led to its eradication in 1980.[143, 144]

Recently, Zhang *et al*. explored the [3+2]-cycloaddition 3-MCR to prepare a library of dispiroheterocycles from isatins, aurones and *D*-proline (14 examples – 45-83% yield), sarcosine (11 examples – 35-72% yield) or (*R*)-thiazolidine-4-carboxylic acid (5 examples – 61-78% yield) (**Scheme 45A**). The antiviral activity of these compounds was evaluated against tobacco mosaic virus (TMV) (**Scheme 45B**). Compound **149** displayed similar antiviral activity to the commercially available natural compound ningnanmycin,[145] which can induce tobacco plant defense mechanisms against TMV.[146] This shows the potential of MCR obtained oxindole derivatives as

potent agrochemicals. Furthermore, the fact that this molecule follows most of the already mentioned druglikeness rules (except the Ghose filter), with good oral bioavailability and even BBB passage, makes these chemical frameworks an interesting starting point for further antiviral activity evaluation, including human-infecting viruses.

A)



B)

Compounds	Substituents			Inactivation Effect (%)	Curative Effect (%)	Protection Effect (%)
	R_1	R_2	*A/B/C*			
148	CH_3	4-OCH_3	*B*	55.1	47.3	51.2
149	Bn	H	*B*	**57.1**	**52.3**	**50.9**
150	Br	H	*C*	53.8	49.7	43.2
Ningnanmycin	-			58.8	55.9	57.1

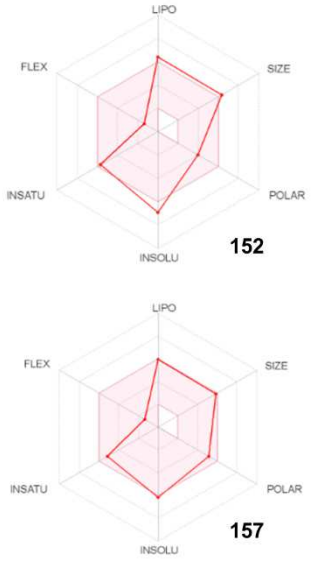
Scheme 45: Dispiroheterocycle derivatives obtained via 3-MCR with antiviral activity against TMV. A) Synthetic route; B) Inactivation, curative and protection effects of the most active compounds.

3. Antioxidant and anti-inflammatory activities

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the therapeutic classes most used worldwide. Used in clinical practice for pain relief and to ameliorate the pro-inflammatory processes associated to several pathologies, making them a very important drug class. Moreover, some oxindole derivatives have emerged as potential NSAID lead candidates.[147]

The already described library of spiro-oxindoles prepared by Lofty *et al*. (see **Scheme 44A**) was evaluated in the oxidative burst assay to evaluate their anti-inflammatory properties using Ibuprofen as the positive control. Gratifyingly, while four out of the seventeen compounds were inactive, eight exhibited anti-inflammatory activity higher than ibuprofen. The best results are summarized in **Scheme 46**. Docking studies with the most active compounds and cyclooxigenase-2 (COX-2), identified this as a possible target for these molecules, as they possess multiple interaction points, *via* hydrogen and halogen bonds, as well as hydrophobic and π-cation interactions.[142] In the case of the druglike features of these molecules, it is noteworthy that several are non-compliant with most of the rules, mostly due to their molecular weight and predicted lipophilicity. For example, molecules **151**, **156** and **158** present low gastrointestinal absorption, and therefore might present a poor pharmacokinetic profile or require alternative formulations and/or administration routes. Both compounds **152** and **157** showed good predicted bioavailabilities (see the Radar, **Scheme 46**), but of the most active compounds (IC_{50} < 5 µM) only compound **157** was compliant with four out of the five druglikeness rules, thus making it the best candidate for drug development.

Compounds	R_1	Ar	X	IC_{50} (µM)	Druglikeness: Lipinski	Ghose	Veber	Egan	Muegge
151	H	4-$CF_3C_6H_4$	S	5.8					
152	H	3-FC_6H_4	S	2.4					
153	H	3-BrC_6H_4	S	5.0					
154	H	2-furyl	S	10.4					
155	H	4-FC_6H_4	CH	8.9					
156	H	2,4-$Cl_2C_6H_3$	CH	4.2					
157	H	2-thiophenyl	CH	4.9					
158	H	2-naphthyl	CH	9.2					
Ibuprofen	-			11.2					



Scheme 46: Anti-inflammatory activity of spiro-oxindole derivatives, druglikeness and bioavailabity radar for compounds **152** and **157**.

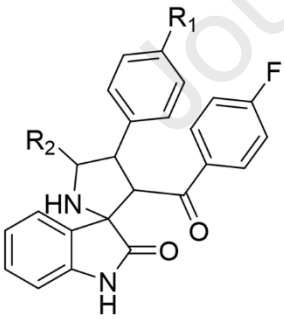
The antioxidant activity was determined for compound **32** (see **Scheme 12B**) using the 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) assay and the bleomycin-dependent DNA damage test. In the first assay, this oxindole-thiadiazole hybrid displayed moderate antioxidant activity (48.13% inhibition, with IC_{50} = 1048 µg/mL) when compared with the positive control, ascorbic acid (88.55% inhibition, IC_{50}

= 544.1 µg/mL). In the DNA damage assay, **32** exhibited similar pro-antioxidant action as ascorbic acid.[75]

The antioxidant activity of the spirooxindole-dihydropyrimidinone derivatives described by Maddela *et al*. (see **Scheme 14A**) was also assessed using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method and the H_2O_2 assay, with compound **40** exhibiting comparable antioxidant activity to the positive control, ascorbic acid (**40** presented IC_{50} = 20.13 µg/mL for DPPH assay and 23.27 µg/mL for H_2O_2 assay, whereas ascorbic acid showed IC_{50} = 19.16 µg/mL for the DPPH assay and 20.66 µg/mL for H_2O_2 assay).[79]

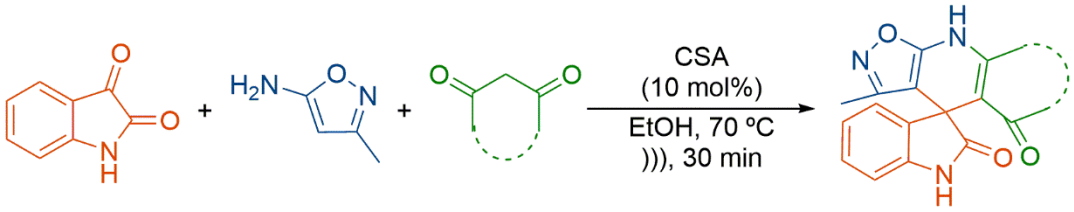
The DPPH radical scavenging assay was also applied by Sapnakumari *et al*. to evaluate the potential antioxidant activity of the library of spiro-oxindoles already mentioned in this work (see **Scheme 20A** and **20C**). At concentrations of 1 mg/mL, three compounds (**159**, **160** and **72**) exhibited slightly lower activity than the standard, glutathione (**Table 6**).[93] Another library already addressed in this work (see **Scheme 29C**) was also subjected to the DPPH scavenging assay and the results compared to ascorbic acid. Nevertheless, the activities presented by the spiro-oxindoles were moderate, with ascorbic acid exhibiting almost twice the antioxidant activity, with IC_{50}s in the range 12.19 and 14.32 µg/mL, with ascorbic acid displaying an IC_{50} of 3.94 µg/mL.[113]

Table 6: Compounds with potential antioxidant activity reported by Sapnakumari *et al*.



Comp.	R_1	R_2	DPPH scavenging (%)
159	Cl	H	88.71
160	Cl	CH_2OH	89.06
72	Br	CH_2OH	87.43
Glutathione	-		92.02

A sustainable methodology, involving a (±)-camphor-10-sulfonic acid (CSA)-catalyzed three component reaction between isatin, 5-amino-3-methylisoxazole and β-diketones using ultrasound activation was recently reported (**Scheme 47**). A small library (4 examples) was obtained with short reaction times and very good yields (76-90%). The antioxidant activity of these compounds was evaluated, using the DPPH radical scavenging assay, metal chelating activity and reducing power. Unfortunately, the results were poorer than those obtained with the reference compounds.[148]



Scheme 47: 3-MCR applied to the synthesis of spiro-oxindoles with moderate antioxidant activity.

4. Activity against CNS diseases

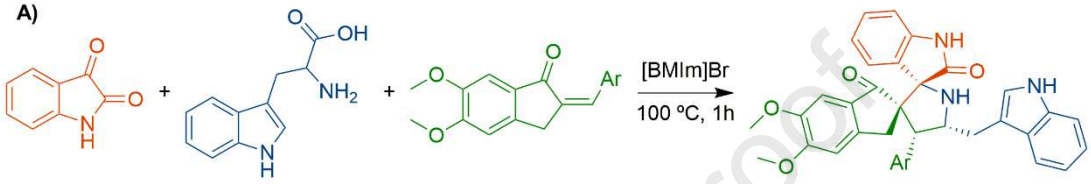
Alzheimer's Disease (AD) is one of the most challenging diseases for modern societies, with the global number of people living with AD or other types of dementia increasing by two-fold between 1990 and 2016. The main causes for this growth are related to the growth and ageing of the world population. The burden of this disease is therefore quite high, and the socioeconomic impact, as well as the healthcare implications, troublesome.[149] The complex pathophysiology of AD lead to the identification of several possible therapeutic targets, and many research groups and pharmaceutical industries devoted their resources to find possible solutions, which made several small molecules and monoclonal antibodies reaching clinical trials, although with several pitfalls registered in recent years, namely in the amyloid-targeting field.[150] In the current therapeutic landscape, cholinesterase inhibitors (ChEIs) – donepezil, rivastigmine and galantamine – are prescribed for treating the symptoms only, however their tolerability, safety and pharmacokinetic profile still justify the quest for new and safer therapeutic options.[151]

Several oxindoles already have displayed both cholinesterase inhibition and neuroprotection, making the oxindole unit a very relevant scaffold to be explored in this field.[152-154] Not surprisingly, some of these molecules have been accessed by multicomponent reactions from isatin. Kumar *et al*. explored a three-component [3+2]-cycloaddition reaction between isatin, 2-arylmethylidene-5,6-dimethoxy-2,3-dihydro-1*H*-inden-1-one and tryptophan, to afford a library of spiropyrrolidines (10 examples), in overall good yields (74-86%), using an IL ([BMIm]Br) as reaction medium (**Scheme 48A**). The *in vitro* cholinesterase inhibition activity of these derivatives was evaluated, using electric eel AChE enzyme and human BChE enzyme (most active compounds and positive control results shown in **Scheme 48B**), through the reduction of dithiobisnitrobenzoic acid (DTNB). Eight out of the ten derivatives presented IC_{50}s lower than 10 μM for AChE, and seven for the BChE. Remarkably, compounds **161**-**163** exhibited IC_{50}s lower than 3 μM for AChE, and **164** for BChE. The presence of a nitro group at the *meta* position afforded a derivative which possesses higher activity than the one displayed by the positive control, galantamine. Molecular docking studies

indicate the possibility of **163** interacting with the peripheral anionic site (PAS) of the AChE enzyme, blocking the gorge of the active site, disabling substrate insertion and respective hydrolysis, leading to the observed *in vitro* bioactivity.[155]

We then predicted the druglikeness and pharmacokinetic profiles of this molecule. The oral bioavailability radar depicted in **Scheme 48C**, indicates that the physical-chemical properties of compound **163** are not within the requirements for good oral bioavailability, but the proximity to the colored area might indicate that fine tuning of structural features might lead to a more druglike molecule.

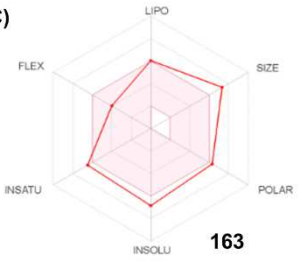
A)



B)

Compounds	Ar	AChE inhibition IC_{50} (μM)	BChE inhibition IC_{50} (μM)
161	2-$OCH_3C_6H_4$	2.76 ± 0.12	24.00 ± 0.18
162	3-$OCH_3C_6H_4$	2.31 ± 0.11	8.32 ± 0.16
163	3-$NO_2C_6H_4$	**1.89 ± 0.09**	28.45 ± 0.27
164	4-$CH_3C_6H_4$	14.24 ± 0.28	**2.24 ± 0.13**
Galantamine	-	2.09 ± 0.07	19.27 ± 0.17

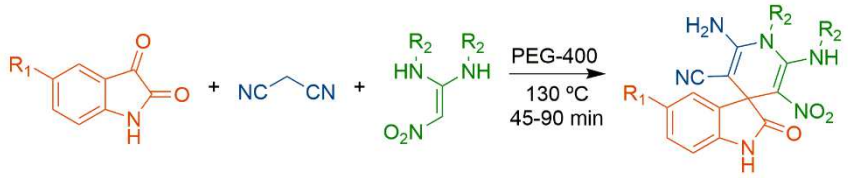
C)



Scheme 48: Spiropyrrolidine derivatives obtained via 3-MCR with cholinesterase inhibition activity (A); AChE and BChE inhibition (B); and oral bioavailability radar of compound**163** (C).

Hasaninejad and co-workers developed a library of spiro-oxindole-dihydropyridine derivatives (16 examples) in good yields (67-79%), *via* a 3-MCR involving isatins, malononitrile and *N,N*'-substituted-2-nitroethene-1,1-diamines, using PEG-400 as a sustainable and biodegradable reaction medium (**Scheme 49A**). The *in vitro* cholinesterase activity was measured using the previously mentioned DTNB assay using, once again, galantamine as positive control. Four compounds displayed an AChE enzyme inhibition activity lower than 1.1 μM (see **Scheme 49B**), with compound **167** displaying higher activity than galantamine, with high selectivity towards AChE when compared to BChE (35.024).[156] Intrigued with these results, we decided to perform an *in silico* prediction of several properties of this promising molecule, as it was done for compound **163**, with the oral bioavailability radar presenting a better score than compound **163**, indicating a higher likelihood of compound **167** to be administered *per os* (**Scheme 49C**).

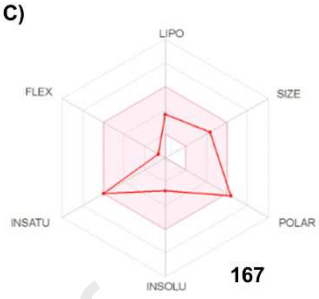
A)



B)

Compounds	Substituents		AChE inhibition IC_{50} (μM)	BChE inhibition IC_{50} (μM)
	R_1	R_2		
165	H	$-(CH_2)_3-$	1.02	8.93
166	CH_3	$-(CH_2)_3-$	0.94	12.34
167	F	$-(CH_2)_3-$	**0.42**	14.71
168	F	$-CH_2C(CH_3)_2CH_2-$	1.07	15.92
Galantamine		-	0.61	6.12

C)



Scheme 49: Spiro-oxindole-dihydropyridine derivatives obtained via 3-MCR (A) with cholinesterase inhibition activity (B); and oral bioavailability radar of compound **167** (C).

We decided to further explore the prediction of pharmacokinetic properties of these eight compounds with higher *in vitro* AChE inhibition activity and compare them with galantamine (**Table 7**).

Table 7: Pharmacokinetics and druglikeness compliance of isatin-derived MCR adducts with cholinesterase inhibition activity and galantamine (G).

	Pharmacokinetics and Medicinal Chemistry				Druglikeness				
	GI absorption	BBB permeation	Pgp substrate	PAINS	Lipinski	Ghose	Veber	Egan	Muegge
161	High	No	Yes	0 alerts					
162									
163	Low								
164	High								
165	Low								
166									
167									
168	High								
G		Yes							

While the commercialized drug displays a linear pharmacokinetic profile, with about 90% bioavailability after oral administration and easily crosses the BBB[157] (in accordance to the prediction of SwissADME), the most active oxindole derivatives **163** and **167** present low gastrointestinal absorption. Furthermore, none of these new AChE

inhibitors predictively is able to cross the BBB, which constitutes a great drawback, as they are unable to reach their physiological target, as clearly shown in the boiled-egg model (**Figure 5**). This is a major red flag, as many AD drug candidates fail to evolve in the drug development pipeline and even clinical trials due to pharmacokinetic attrition, especially lack of BBB permeation.[158, 159] Regarding the druglikeness of these compounds, the physical-chemical properties prediction dictates that most of them are compliant with three out of the five rules evaluated, whilst galantamine satisfies the five rules.

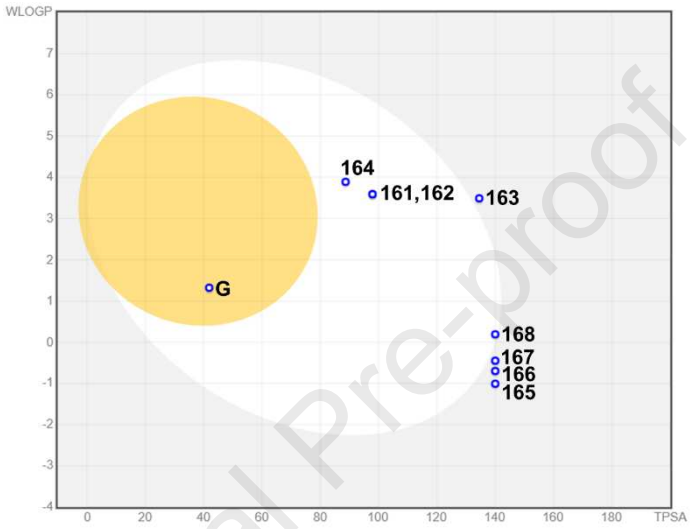


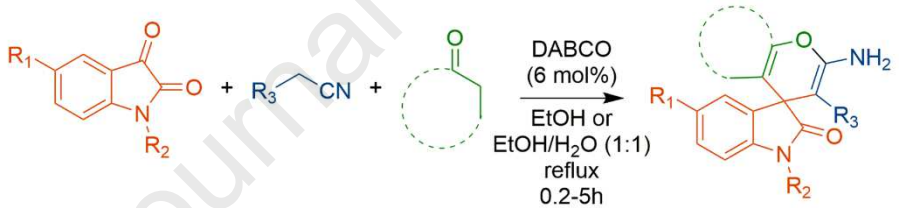
Figure 5: Boiled-egg model of BBB permeation and GI absorption (G=galantamine).

Another neurodegenerative disease with a massive socio-economic impact is Parkinson's disease (PD), with its global burden more than doubling over the past generation, caused mostly by the same demographic shift as reported for AD.[160, 161] Characterized by the loss of dopaminergic neurons, most of the current therapeutic options are focused on preserving dopamine levels in the brain, by administration of levodopa (dopamine precursor), catechol-*O*-methyltransferase (COMT) inhibitors, since COMT is responsible for the degradation of dopamine (e.g. tolcapone and entacapone), or monoamine oxidase (MAO) inhibitors, which also breaks down dopamine in the basal ganglia, and dopamine agonists (such as ropinirol, an oxindole based drug). Other therapeutic options are mostly focused on the treatment of symptoms. However, several new potential druggable targets have been reported in recent years, with multiple drug candidates emerging in the PD therapeutics pipeline, since the existent options do not cure the disease, and often only delay the symptoms for a certain amount of time.[162, 163] In this context, the NAD^+-dependent protein lysine deacylases that constitute the sirtuin family, appears as a possible target for several age-related diseases, including PD. There are seven human sirtuins

described, with a multitude of physiological functions, which determines their potential as targets for multiple diseases, such as type 2 diabetes, cancer, cardiovascular diseases, inflammatory diseases, and neurodegenerative disorders.[164, 165] For the last one, and for PD in particular, sirtuin 2 is the most relevant member of this family, since its age-related levels increment can mediate several routes of the PD pathogenesis, from α-synuclein aggregation, microtubule function, oxidative stress, inflammation, autophagy and even a possible connection with dopaminergic neurons death.[166]

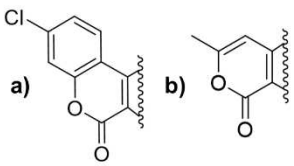
Hasaninejad and co-workers prepared a library of spiro-oxindole derivatives (25 examples) in excellent yields (84-98%), through a DABCO-catalyzed 3-MCR (**Scheme 50A**). Docking studies were then performed, in order to evaluate the affinity values of the spiro-oxindole derivatives with the target sirtuin 2. Out of the twenty-five derivatives, the three compounds (**169**-**171**) displaying a greater affinity were selected to proceed to *in vitro* studies, using a high-performance liquid chromatography (HPLC)-based methodology, using fluorogenic histone deacetylase substrate MAL to determine the inhibitory activity, and using sirtinol as positive control. The three compounds exhibited lower activity when compared to sirtinol, by almost two-fold, with compound **169** being slightly more active than the other two (**Scheme 50B**).[167]

A)



B)

Compounds	Substituents				Sirtuin 2 inhibition IC_{50} (μM)
	R_1	R_2	R_3		
169	CH_3	H	CO_2Et	a)	118
170	H	Bn	CO_2Et	b)	126
171	Br	H	CO_2Et	b)	124
Sirtinol	-				67

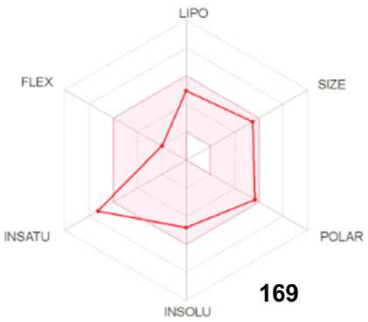


Scheme 50: Spiro-oxindole derivatives obtained via 3-MCR (A) with sirtuin 2 inhibition activity (B).

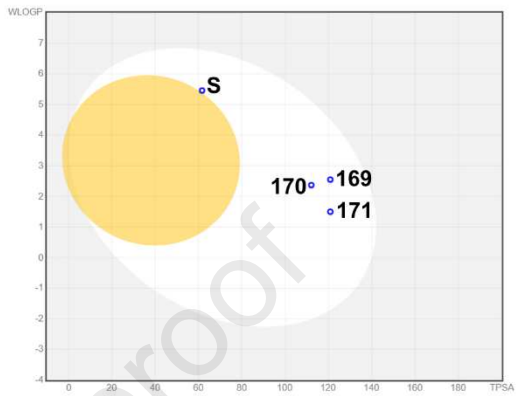
The oral bioavailability radar for compound **169** is depicted in **Scheme 51A**, and besides the insaturation of the molecule, all the other parameters fit within the colored area, showing the potential of this compound for an oral administration route. This is further confirmed in the Boiled-Egg model (**Scheme 51B**), with the three described compounds exhibiting high gastrointestinal absorption. Compliance with the main druglikeness rules is also verified with this predictive model, but the main drawback is,

once again, the lack of BBB permeation, and therefore inability to reach the therapeutic target (**Scheme 51C**). Drug delivery systems to enable BBB permeation, or evaluation of these compounds against different sirtuin subtypes and pathophysiological processes might be a suitable approach for further development of these druglike molecules.

A)



B)



C)

	Pharmacokinetics and Medicinal Chemistry				Druglikeness				
	GI absorption	BBB permeation	Pgp substrate	PAINS	Lipinski	Ghose	Veber	Egan	Muegge
169	High	No	Yes	0 alerts					
170									
171									
S									

Scheme 51: Pharmacokinetics and druglikeness of spiro-oxindole with sirtuin 2 inhibition activity. Bioavailability radar for *per os* administration (A); Boiled-egg model of BBB permeation and GI absorption (B); and pharmacokinetic parameters and druglikeness (C) (S=sirtinol).

Summary and Outlook

MCRs are a valuable synthetic approach to attain new druglike oxindole derivatives in a time-efficient and often eco-friendly manner. In this review, we have discussed the most recent developments in isatin-based MCRs and their respective biological activities. The emergence of new tools, such as SwissADME, allows researchers to perform an early selection of their hit compounds, predicting possible pharmacokinetic and pharmacodynamic advantages/disadvantages of these potential drug candidates. Furthermore, it is noteworthy that phenotypic assays constitute the most common approach for biological activity screening of new oxindole derivatives, contrary to target-based screening. This is mostly due to the wide scaffold diversity

obtained using MCRs and to the privileged status of the oxindole core as an important general pharmacophore. From the examples given in this review, we realize that MCRs are therefore a precious tool to increase the output of new druglike molecules, allowing faster hit identification and even hit-to-lead optimization in a variety of disease areas.

Conflict of Interest

There are no conflicts to declare.

Acknowledgements

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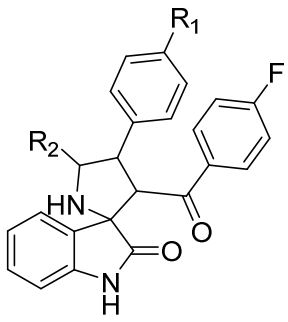
DRUGLIKENESS				
Lipinski	*Ghose*	*Veber*	*Egan*	*Muegge*
MW ≤ 500 Da CLogP ≤ 5 #H-bond donor ≤ 5 #H-bond acceptor ≤ 10	160 ≤ MW ≤ 480 Da -0.4 ≤ CLogP ≤ 5.6 40 ≤ MR ≤ 130 20 ≤ #atoms ≤ 70	#Rotable bonds ≤ 10 TPSA ≤ 140	CLogP ≤ 5.88 TPSA ≤ 131.6	200 ≤ MW ≤ 600 Da -2 ≤ CLogP ≤ 5 TPSA ≤ 150 #Rings ≤ 7 #Carbons > 4 #Heteroatoms > 1 #Rotable bonds ≤ 15 #H-bond donor ≤ 5 #H-bond acceptor ≤ 10

Compound	MIC_{90} (μg/mL)			
	E. coli	*P. aeruginosa*	*S. aureus*	*B. subtilis*
82	**1.78**	5.29	3.55	**1.14**
84	2.86	**1.57**	**1.09**	3.18
85	**1.97**	3.08	4.27	**2.85**
87	2.65	4.88	**2.41**	7.19
89	2.75	**2.33**	3.86	5.27
Ampicillin	1.46	4.36	1	10.32
Kanamycin	1.62	0.49	>30	1.35

Compounds	Ar	MIC (μg/mL)				Druglikeness				
		S. pneumoniae	*B. subtilis*	*P. aeruginosa*	*E. Coli*	Lipinski	Ghose	Veber	Egan	Muegge
98	4-ClC_6H_4	0.12	0.015	15.63	**1.95**					
99	4-$NO_2C_6H_4$	1.95	0.06	NA	15.63					
100	4-$OCH_3C_6H_4$	0.98	0.015	NA	15.63					
101	2,4-$F_2C_6H_3$	**0.015**	**0.015**	NA	**1.95**					
Ampicillin	-	0.015	0.007	NA	NA					
Gentamicin		NA	NA	15.63	1.95					

Compounds	R_1	R_2	MIC (μg/mL) *P. mirabilis*	MIC (μg/mL) *S. epidermidis*	Druglikeness: Lipinski	Ghose	Veber	Egan	Muegge
105	2,4-$Cl_2C_6H_3CH_2$	H	16	8					
106	H	5-Cl	16	2					
Penicillin	-	-	16	1					
Gentamicin			0.5	0.5					

Compounds	R_1	R_2	R_3	MIC (μg/mL)			Druglikeness				
				S. aureus	*B. subtilis*	*E. coli*	Lipinski	Ghose	Veber	Egan	Muegge
126	H	CH_3	4-ClC_6H_4	16	8	64					
127	NO_2	H	4-ClC_6H_4	32	32	128					
128	NO_2	CH_3	C_6H_5	16	16	128					
Ciprofloxacin	-			6.25	6.25	6.25					



Comp.	R_1	R_2	DPPH scavenging (%)
159	Cl	H	88.71
160	Cl	CH_2OH	89.06
72	Br	CH_2OH	87.43
Glutathione	-		92.02

	Pharmacokinetics and Medicinal Chemistry				Druglikeness				
	GI absorption	BBB permeation	Pgp substrate	PAINS	Lipinski	Ghose	Veber	Egan	Muegge
161	High	No	Yes	0 alerts					
162									
163	Low								
164	High								
165	Low								
166									
167									
168	High								
G		Yes							

Highlights

Recent developments of oxindole derivatives synthesized through isatin-based multicomponent reactions

Biological activity of isatin-based oxindoles derivatives

Evaluation of druglike properties using web-based free tool SwissADME

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: