**PhD Course in: RESEARCH METHODS IN SCIENCE AND TECHNOLOGY**
**CHEMISTRY (area of Chemical Sciences, ERC sectors PE5 and LS7)**

**RESEARCH PROJECT**

**Title: INDOLE-3-CARBINOL (I3C) DERIVATIVES: DESIGN, SYNTHESIS AND POTENTIAL BIOLOGICAL APPLICATIONS**

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## STATE OF ART

Indole-3-carbinol (I3C) is a natural occurring alkaloid produced by the degradation of glucobrassicin, a glucosinolate contained in various cruciferous vegetables[1] and hydrolyzed by specific β-thioglucosidase present in the plant,[2] such as the myrosinase.[3] This process is carried out as reported in Figure 1 through the formation of unstable intermediates thiohydroxymate-*O*-sulfonate and 3-indolylmethylisothiocyanate, which is finally converted into the desired product.

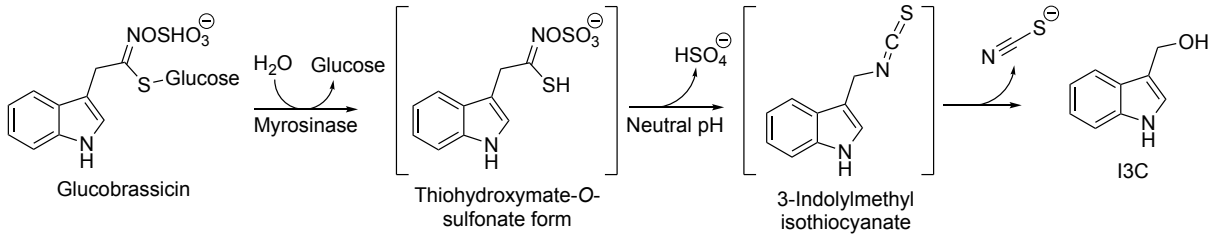



**Figure 1**. The formation of I3C starting from glucobrassicin.

I3C is often present in dietary supplements,[4] since its implementation has been related with a lower risk towards several diseases. Indeed, I3C is the subject of on-going biochemical and pharmacological research for its possible advantageous effects.[5]

In this research project classical medicinal chemistry design will be performed by modifications of the lead compound I3C. In particular, the indole ring will be functionalized by the introduction of various substituents with different properties such as those related to electronegativity, lipophilicity and steric hindrance. To this aim, some designing strategies will be applied to highlight the possibility to carry out a lead optimization and, therefore, derivatives with a better pharmacodynamic and pharmacokinetic profile than I3C. Furthermore, also the insertion of electron withdrawing group replacing indole hydrogen can improve *in vivo* stability of the reference compound. It will also be subjected to modifications based on the strategy by conjunction, which will lead to an increase in the size of I3C (2,3' and 3,3'-dimerization). These interventions will be important to understand if the analogues of molecules originated by metabolic processes that determine the *in vivo* transformation of I3C (3,3'-diindolylmethane, CTr, CTet, etc.) can also give satisfactory results in the *in vitro* and *in vivo* models.[6] For the same purpose, the indole ring will then be modified through classical isosteric and bioisosteric approaches and also the side chain will be subjected to modifications based on the homologation strategy (lower and upper) and on functionalization with the involvement of the hydroxyl group as well. The realization of the molecules will be carried out through common synthetic

strategies and/or ad hoc developed procedures, and according to the knowledge related to the properties of the scaffold and functional groups (Figure 2).

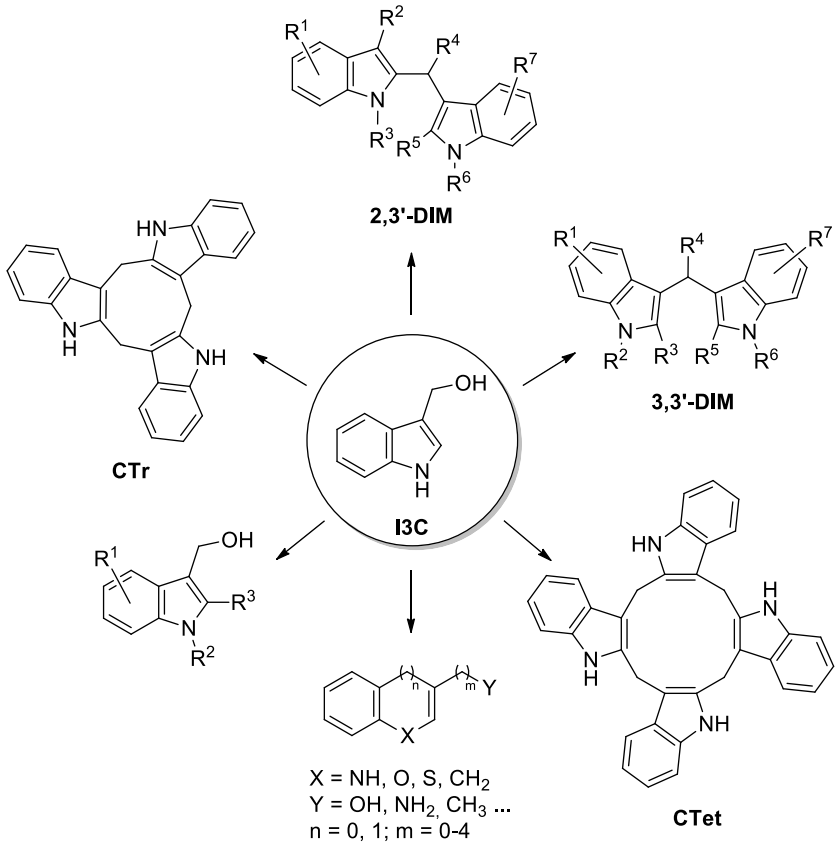
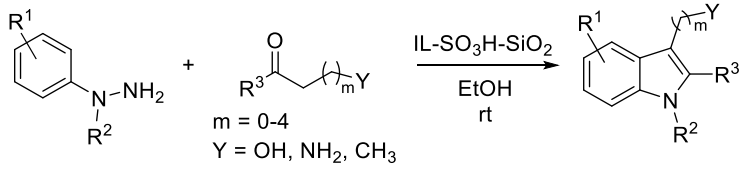



**Figure 2**. Overview of I3C derivatives.

## OBJECTIVES AND METHODOLOGY

The first objective of this research project will be to develop new synthetic methodologies starting from indole. On one hand, it will be important to find synthetic reaction that led to high yields and possibly respecting the standards of green chemistry; on the other hand, it will be important to screen different compounds and evaluate their biological activity. To this aim, the compounds will be characterized by NMR, ESI/MS and IR, and tested in various experimental models.

One of traditional method to obtain indoles is Fisher indole synthesis, which requires the use of stoichiometric amounts of Bronsted or Lewis acids. However, this reaction brings low yields and by-products. 4-Methylbenzenesulfonic acid-based ionic liquid on silica gel (IL-$SO_3H$-$SiO_2$)[7] could potentially be employed in heterogeneous catalysis to promote the reaction (Scheme 1). This is an important perspective for the project here presented.

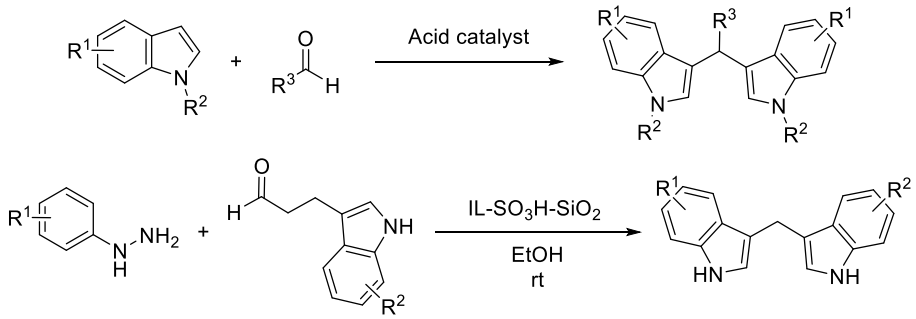



**Scheme 1**. A synthetic strategy to obtain I3C derivatives based on indole ring.

Potential advantages are mild and practical reaction conditions, high yields, easy isolation of products, and excellent recyclability of the catalyst.

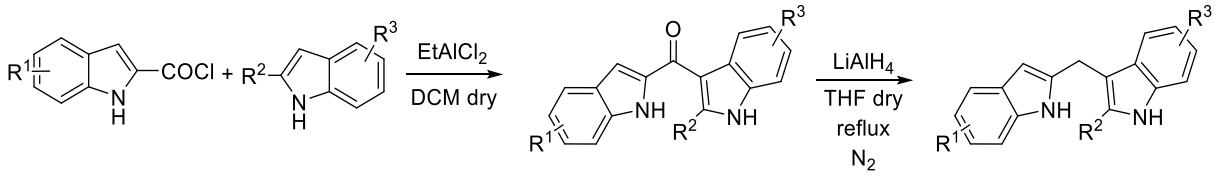
Another important perspective for the project concerns the linkage of two molecular building blocks (2,3'- and 3,3'-dimerization) in a facile, selective, high-yielding reaction under mild reaction conditions with few or no by-products.

For 3,3'-dimers the good nucleophilic property on indole 3-position could be exploited (Scheme 2).[8] Another route is to employ the beforementioned Fischer's synthesis (Scheme 2):



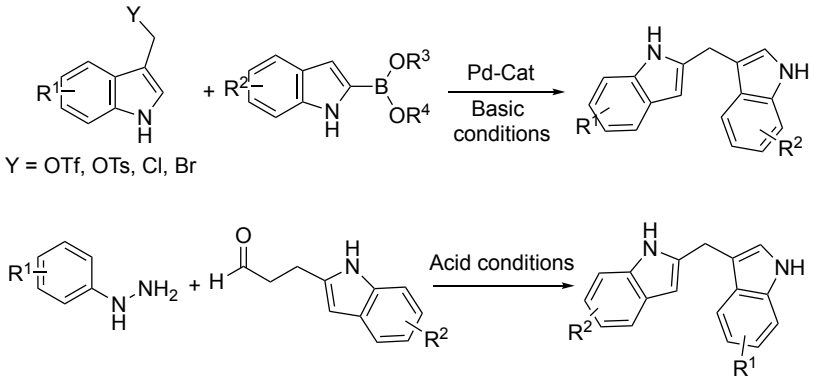

**Scheme 2**. Synthetic strategies to obtain 3,3'-derivatives of I3C.

The true challenge is to synthesize 2,3'-dimers because 2-position of indole is usually less reactive. Such as an example Wahlström *et al.* proposed the following synthesis for 2,3'-derivatives (Scheme 3).[9]



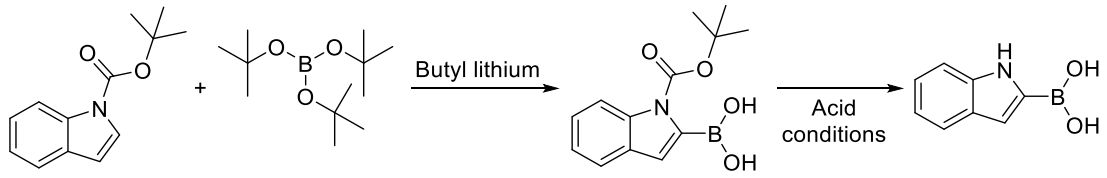

**Scheme 3**. Synthetic strategy to obtain 2,3'-derivatives of I3C.

So, it will be crucial to functionalize the 2-position (C-H activation/metalation are possibilities).[10] Two methods for the synthesis of 2,3'-dimers are proposed in the Scheme 4:



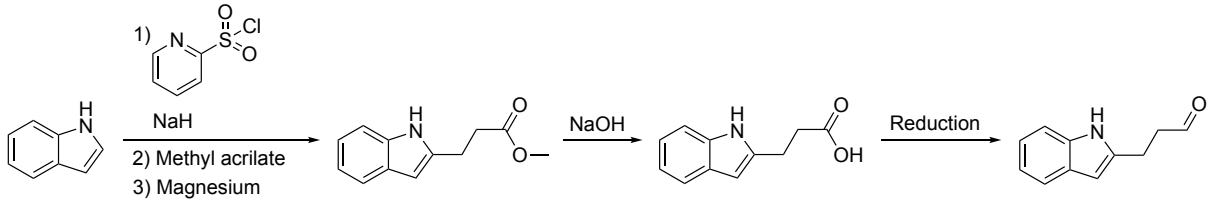

**Scheme 4**. Alternative synthetic strategies to obtain 2,3'-derivatives of I3C.

Palladium (0) catalyzed cross-coupling C-C bound forming the reaction between arylboronic acid and aryl halides (Suzuki-Miyaura Cross-Coupling) is surely a possibility to explore the use of protecting group strategies in all the proposed methodologies. One situation to obtain the arylboronic derivatives is to add *n*-butyllithium and tri-*i*-propyl borate to a solution of 1,1-dimethylethyl-1*H*-indole-1-carboxylate followed by removing of the protective group (Scheme 5).[11]




**Scheme 5**. Exemplifying synthetic methodology by using protecting groups.

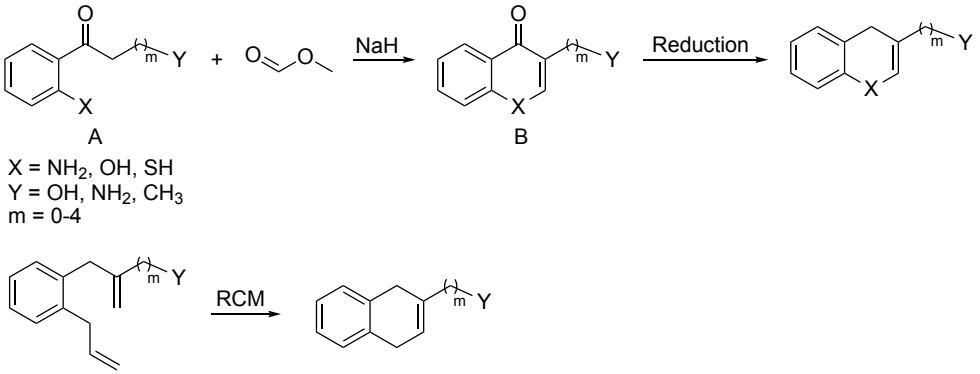
Instead, the method for synthesis of 3-(1*H*-indol-2-yl)propanal[10] is proposed in the Scheme 6:




**Scheme 6.** Synthetic multistep procedure to obtain 3-(1*H*-indol-2-yl)propanal starting from indole.

Furthermore, the proposed methodologies use a protective group strategy. In the first step indole nitrogen is protected with 2-pyridinesulfonyl chloride then the deprotection is carried out by magnesium in methanol. Subsequently, ester is hydrolyzed by sodium hydroxide and finally the carboxylic acid is reduced to aldehyde.

The indole ring will be also modified through bioisosteric approaches and the side chain will be subjected to modifications based on the homologation strategy (lower and upper) and on functionalization with the involvement of the hydroxyl group as well. For this purpose, the following synthesis is proposed (Scheme 7):[12]




**Scheme 7**. Synthetic strategy to obtain I3C derivatives based on bioisosteric approaches.

In the first synthetic step, sodium hydride is added to a solution of compound A in methyl formate to obtain compound B. The reaction is then quenched by the addition of water, ethanol and ethyl acetate, and the carbonyl group is finally reduced to the alkyl one. In the second synthetic step a ring closing metathesis (RCM) is proposed to obtain the desired product. This reaction is metal-catalyzed and proceeds through a metallacyclobutane intermediate. Since the only major by-product is ethylene, these reactions may also be considered atom economic, which is an increasingly important concern in the development of green chemistry.

## PLANNING OF THE RESEARCH PROJECT

*FIRST YEAR*

1) Design and synthesis of I3C classical isosteres and bioisosteres.

2) Widely physico-chemical characterization.

3) Following the lessons.

*SECOND YEAR*

1) Use of strategy by conjunction, which will lead to an increase in the size of I3C (2,3'- and 3,3'-dimerization).

2) Widely physico-chemical characterization.

3) Biological tests to determinate efficacy and other properties.

*THIRD YEAR*

1) Use of palladium (0) catalyzed cross-coupling C-C bound between aryl boronic acid and aryl halides.

2) and 3): See second year.

4) Writing thesis.

## EXPECTED RESULTS

This section focuses on obtaining new I3C derivatives from sustainable, easily available and renewable reagents avoiding. The products will have a potential biocompatible, biodegradable, and non-toxic profile and will be employed in different fields such as antitumor, antiviral, antioxidant and so on.

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