Solid-Phase Synthesis and Biological Activity of *2H*-Indazole 1-Oxides and Other Nitrogenous Heterocycles

Author: Mgr. Nadezda Cankarova
Oponent: Josef Vagner, PhD.

Overall:
This first part of this thesis describes experiments to introduce novel heterocyclic scaffolds capable of mimicking natural pharmacophores, such as an indole, one of the most prolific scaffolds in medicinal chemistry. The second part of thesis deals with a variety of heterocycles derived from important intermediates, resin-bound amino ketones. Major strengths of the thesis include the very high significance of the problem, a well-written and organized manuscript that describes a thoughtful and comprehensive approach supported by solid analytical data. Another strength lies in application of solid-phase methodology, an innovative approach for synthesis of heterocycles. Most of results have been already published in peer-reviewed journals.
Minor weaknesses of the thesis include minimal discussions of biological activites and chemistry-driven design of compounds. Relevant pharmacological targets such as indazoles, imidazoles and other heterocycles described in the thesis deserve detailed drug design and comprehensive screening. In academia, there are limited resources and drug discovery does not rely on high throughput screening of large portfolios of compounds but on rational and biology-driven molecular design. Independent of these weaknesses there is high enthusiasm for the outstanding synthetic work presented in the thesis.

Project subject:
Indazole is an aromatic heterocycle with a rare occurence in nature, but having significant potential as an aza mimic of indole (tryptophan, serotonin or 5-hydroxytryptamine). This family of nitrogen-containing heterocycles has attracted great interest because of its wide variety of biological properties as was documented in the thesis (pages 38-40). In my opinion, the impact of this family of compounds on drug discovery is larger than is described in the thesis. Some compounds are already available or in late stage of clinical trials. A first example is **Riociguat** (Fig 1, compound **I**, **BAY 63-2521**), a novel drug that is in clinical development by Bayer. Currently, Phase III clinical trails investigate the use of this drug as a new approach to treat two forms of pulmonary hypertension as the first drug of a novel class of sGC stimulators [1]. A second example is the Pyrazolo[4,3-d]pyrimidin-7-one scaffold is known as a active component of **Sildenafil** (Fig 1, **II**). Sildenafil (Viagra, Pfizer) and its naturally occuring analog, Acetildenafil, are potent and selective inhibitors of cGMP-specific phospodiesterase V (PDE5; degrades cGMP in cells), which results in vasodilatation. Sildenafil represents one of the most successful drugs in history of pharmaceutical industry with sales exceeding $2.3 billions in 2009. A third example, **Granisetron (III)**, is a serotonin (5-HT_3R) antagonist that was developed as antiemetic remedy [2]. Inclusion of these specific examples (or similar ones) would better emphasize the importance of the current work.

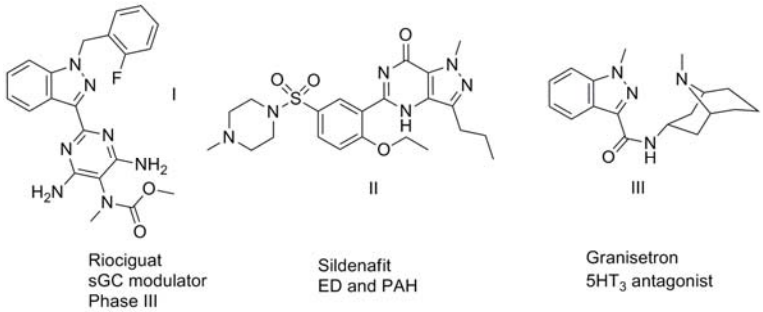

Figure 1

Methodology:
Beyond doubt, the most important achievements lie in the implementation of solid-phase chemistry in synthetic procedures and a discovery of unexpected formation of *2H*-indazole-1-one (Scheme 46, page 52). Solid-phase synthesis (SPS) is a synthetic technique that combines important requirements for modern organic synthesis: excess reagents help drive the reactions to completion, mere washing of the resin is required to remove impurities and excess reagents, resulting in a vast number of compounds produced and an increase in molecular diversity of chemical libraries. Novel chemistry presented in the thesis enabled quite unique synthetic routes leading to combinatorial arrays of compounds such as: indazoles, 3,4-dihydropyrazinoindazoles, imidazoles, dihydropyrrolo-2-ones, pyrazin-2-ones, triazepin-6-ones, morpholin-3-

ones, and isoquinolin-1-ones. Significantly, SPS of indazoles developed by the author in this thesis is the only available method known today. SPS of indazoles, and important intermediates aminoketones, illustrates very innovative and needed synthetic methodologies. I am convinced that many research groups will soon accept presented methodology.

Experimentals:
Presented chemistry, results and experimental procedures are adequately described. Representative compounds are fully characterized by high quality Nuclear Magnetic Resonance (NMR) and high-resolution Mass Spectroscopy (MS). Critical structures have been elucidated by 2D NMR analysis to unequivocally determine the structure (such as compound 15(10,1,1) in Fig 4 or compound 45(6,1). Compound arrays are prepared in miligram quantities, characterized by LC-MS, and purified by High Performance Liqiud Chromatography (HPLC) for screening. Experimentals are well organized and focused on preparation of desired compounds and their in vitro evaluation. The methodology and experimental planning are well designed and technically feasible. The majority of compounds and arrays have already been published in peer-reviewed journals.

Weaknesses:
The major criticism of the presented study is a lack of a clear proposition of how the heterocyclic scaffolds will be converted into drug-like molecules. Cytotoxic activity assays were performed on a variety of human cell lines. It is noted here that the most active, compound 10(8,4,4); page 66, contains a nitro group, yet the tested compound, 10(8,1,4), that exhibited no cytotoxic activity did not contain the nitro group. This is important because the metabolism of a nitro group occurs in cells via a reduction involving the commonly expressed enzyme CYP450 to an arylamine. The intermediates of this reduction reaction, hydroxylamine and nitrosamine, have been implicated as carcinogen and hepatotoxic (side effects observed for nitrazepam, clonazepan etc). An ideal cytostatic compound should be both tissue specific and cell specific (i.e. should only destroy targeted, malignant cells). Therefore, the nitro group that increases cell toxicity for all cell types, can have overriding effect. The cytotoxicity could be solely due to the nitro group contribution and not any effect of the indazole scaffold. Pyridines [3] are the most common isosteres known as suitable replacements for nitro-substituted aryls, although a carboxylate has been shown to function as a nitro isostere, too [4].
Obviously, in this work the nitro is one electron-withdrawing group (EWG) that enabled an original observation that led to efficient synthesis of *2H*-indazole-1-oxide and its para-derivatives. However, when cyclization of the indazole is completed, subsequent transformation of nitro to less toxic derivatives or use isosteres (both pyridine and carboxylate are also EWG), is possible. Having a nitro-free indazole scaffold would allow screening for biological activities broader than cytotoxic and antimicrobial activities. It is noted that biological assays are only as complementary information. Thus, this criticism does not diminish quality and importance of the overall thesis that is positioned well to add to synthetic methodology.

Formal deficiencies:
Page 13, Scheme 5; X is halogen or methoxy?
Page 18, Scheme 14, aryl is not specified. The reaction is likely form regioisomers, therefore it may be useful to discuss the mechanism
Page 26, Scheme 25, R^2 is not defined
Page 26, Scheme 25, R^2 is not defined
Page 31, Scheme 31; atypical reference (43 and 44) citation.
Page 53-54, Table 3; the legend is disconneted from the table
Page 78, Scheme 57, structure 49; what X does stand for? Fmoc or H?

Summary:
- Novel synthetic routes for biologically important heterocyles and their intermediates has been developed
- Most of procedures implemented the solid-phase synthesis, a modern methodology for synthesis of chemically diverse libraries of compounds.
- Results and experimental procedures are adequately described, including analytical data
- Arrays of compounds have been prepared in high yield and purity for biological screening
- Some compounds have been screened in *in vitro* assays

- Presented chemistry is highly innovative; this creates an excellent opportunity for further research in drug discovery

Recommendation:
I recommend the thesis for defense.

[1] Serap Gurat et al, *Current Pharmaceutical Design,* 2010*, 16,* 1619-1633.
[2] Hsu Eric S et al, *American Journal of Therapeutics* 2010, 17(5), 476-86.
[3] Renard J F et al, *J. Med. Chem.* 2009, 52, 5864–5871.
[4] Firestine S M et al, *J. Med. Chem.* 1993, 36, 3484–3486.

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Josef Vagner, PhD
Associate Research Professor
Director, The Ligand Discovery Laboratory
The Bio5 Institute, The University of Arizona
1657 E Helen Street, Tucson, AZ 85719, USA

Appendix

Questions:

1) Have you ever observed Smiles rearrangement (product of basic treatment of sulfonamide-based N-protecting groups, shown in Fig 2)?

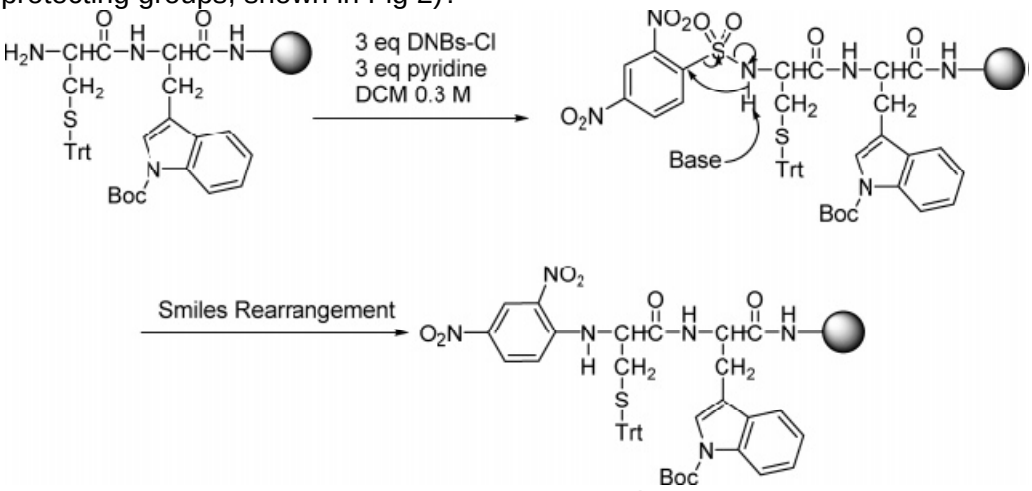
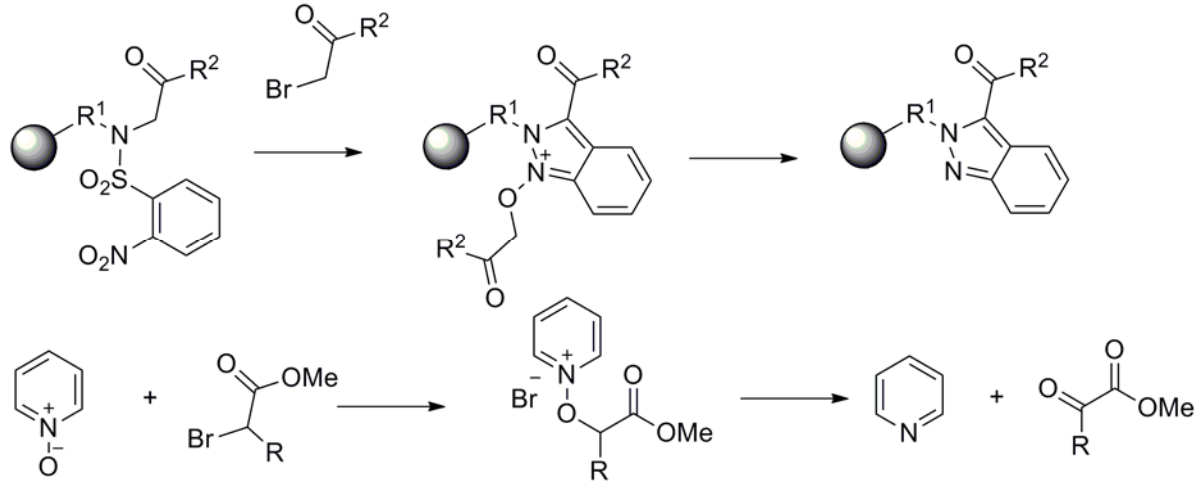


Figure 2

Figure 2, "Smiles Rearrangement Occurred in the Initial Synthesis of Peptide **1** under a Mild Sulfonylation Condition" was originally published in *J. Med. Chem.* **2006,** *49,* 6888-6896.

2) N-oxides are oxidative agents known to be easily O-acylated or O-alkylated with variety of electrophiles. As an example, α-bromoacids alkylate pyridine N-oxide to form α-ketoacids (Fig 3). Have you observed overalkylation (Fig 3) that may lead to premature deoxygenation of N-oxide.



3) Benzophenones (cmpds 10) are known as photoreactive probes used in affinity labeling of active sites of many proteins. Have you observed any reaction of benzophenones initiated by light? Have you tried to reduce benzophenone to less reactive derivatives?
4) Why there is no 2H-indazole compound (deoxygenated 10) decribed, only 3,4-dihydropyrazino [1,2b]indazole derivatives 17?