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Critique of Thesis presented by Mgr. Naděžda Cankařová, Palacký University

This thesis describes combinatorial solid-phase methodology to prepare, in Part I, numerous molecules based on an indazole core, and, in Part II, a diverse set of heterocyclic compounds, many obtained from a single generic polymer-supported α-acylamino intermediate. The ultimate goal of this work is to construct, via these combinatorial routes, a large diversity of molecules that can be tested for biological activity. The thesis begins with an extensive review of the literature regarding one of the major synthetic goals of this work: substituted indazoles. It then provides new synthetic routes to these indazoles, and to a wide variety of other substituted molecular scaffolds.

Some of the scaffolds presented are well represented in the literature, but in this work they are constructed in unique ways and by new methodology that permits ready variation. Other scaffolds occur much less frequently in the literature. This thesis describes enabling solid-phase combinatorial routes to all these scaffolds.

There are two major synthetic parts to this work. Part I describes routes to a unique structural type that was first obtained as an unexpected rearrangement product. From that observation a robust solid-phase synthetic sequence was subsequently developed that provides access to large numbers of molecules. Part II develops synthetic pathways to multiple scaffolds from a key intermediate, thus enabling maximum diversity from minimal starting materials.

This thesis presents an impressive range of diverse synthetic pathways to approximately 11 different substituted scaffolds. Some routes are developed in detail; a few have only one (**44(3,1,4)** and **46(5,1)**) or two (**47(1,1)** and **47(6,3)**) representative derivatives and thus, while promising, their combinatorial potential is not convincingly demonstrated.

Finally, this thesis addresses two important biological aspects: the testing of many of these compounds and the synthesis of biotin derivatives to assist the identification of the biological molecules with which some of these compounds may interact to cause their biological effect.

Critique [Many of the following comments and corrections, along with additional suggested changes, are shown in the pdf version of the thesis text sent with this critique]:

The formal organization of this thesis could be improved to more clearly communicate the goals, process and results. While the large number of different scaffolds investigated is a strength of this work, this diversity makes it especially important to organize the material, both in terms of rationale and presentation of results, in a way that is easy to follow. Although the introduction and discussion are substantially complete and relevant, the text, schemes and figures are not always organized in a way that the reader can easily follow.

There are two major parts to this work: the synthesis of 2*H*-indazole 1-oxides and related compounds, and the utilization of polymer-supported α-acylamino ketones to synthesize a diverse set of nitrogen heterocycles. Even though the candidate already designates these areas in the thesis, introducing before these respective parts the headers **Part I** and **Part II** would help the reader appreciate the very different nature of these two sections and focus on understanding them as largely independent pieces of work. One

source of confusion concerns the circumstances leading to the development of the Part I chemistry to 2*H*-indazole 1-oxides (p 50-52). Even though this is discussed in Part I, it appears this work may have been developed based on earlier exploratory chemistry to access the key intermediate **12** required for Part II. Yet the discussion begins with Scheme 45, providing **10** via the rearrangement route, *before* discussing the unexpected outcomes shown for the two cases in Scheme 46. One wonders if the candidate first prepared **8** in expectation of standard removal of the 2,4-nitroarylsulfonyl group to form **12** and then, when unexpected rearrangement was observed, take advantage of the rearrangement of **8** to build the robust route to numerous indazoles **10** described in Part I? Then, for Part II, did she go back to the 4-nitrobenzenesulfonyl derivative **11** to access originally desired **12**, and build the routes to diverse heterocycles possible from this key intermediate? If so, stating this clearly would make the story more interesting, the development more understandable, and the results and discussion for the two parts would fit more smoothly together.

Improving results tables: Because of the complexity of nomenclature and structures, more frequent repeat placement of generic structures would assist the reader. This would be especially helpful in the presentation of results, and could be accomplished by giving the generic structure, with R group substitution patterns shown, at the top of each table. Even though the reader can find the generic structures elsewhere in the text, having them placed directly at each table would makes it much easier to create the actual structure from the R groups shown. It would also be useful to have the yields included in the tables. This would permit the reader to quickly identify patterns and anomalies in yields, and consider if there might be something in the nature of the R substituents that contributed to this.

Some specific questions: 1. Page 55 implies a larger combinatorial set was prepared, with representative derivatives fully characterized. Where is the description of this larger set? All the molecules presented in this work appear to be fully characterized. 2. Page 55 presents some details of "purity" determination. Was this based on UV at a specific wavelength? How does it take into account variations in UV absorbance, inorganic contaminants, non-absorbing impurities, etc.? 3. Page 56: Here and elsewhere reference is made to the nature of resin-bound intermediates (e.g. "cleanly afforded resin-bound indazole oxides **9**"). Although these resin-bound intermediates are sometimes characterized by cleavage from resin it is important to realize that this can be misleading about the actual structure and purity of the intermediate *while resin-bound.* 4. Page 59: Why cyclization to stable **20(8,1)** a surprise? It would be expected that an amide be more stable than an imine. Any thoughts on why it was formed in such low yield? – Could cross linking at the diamine incorporation step be a hidden problem? 5. Page 75: As discussed earlier in this critique, was cleavage of the 4-nitro only looked at after problems with 2,4-dinitro derivative? 6. Page 84: Was there only one compound (**44(3,1,4)**) made from an amino acid (Fmoc-gly) and where is that description in the experimental? Why were other amino acids not tried? 7. Experimental section: give details of detection mode (frequency? Integrated diode array) for LC trace and integration. Are isolated yields based on initial loading? 8. Page 97: Again, as for comment #1, why last paragraph? No mention of other compounds is made other than those characterized in the experimental.

(Numerous other comments and corrections, none of a critical nature, are noted in an accompanying marked up copy of the thesis).

Summary: This thesis presents an impressive quantity and variety of solid-phase synthetic work providing numerous molecules with potential for biological activity. Most of the synthetic routes are documented with sufficient successful reagent variation to give confidence that large numbers of combinatorial libraries can be constructed from the general procedures. Some routes, however, have limited exemplification and will need future development to ensure wide generality. Given the large number of successfully employed routes (and the work this entails) it is understandable that there will be examples of more exploratory routes in need of further work.

In several instances (e.g. formation of **10** from **8**; formation of **14(8,1,2)**; formation of **44** via *route 1*) good observation and careful analysis led from unexpected results to fully developed procedures providing novel compounds. This is a hallmark of doctoral level studies that creatively explore unknowns revealed through experimentation, and capitalize on the knowledge that comes from that exploration. The quality and importance of the candidate's work is validated by her presence as co-author on five peer-reviewed publications based on these studies.

In consideration of the scope of the candidate's scientific work, its quality, and the acceptable presentation of discussion and results, this thesis is recommended for defense.

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