New Possibilities for Electron-rich Naphthyl Analogs to Facilitate Photocyclic Cycloaddition in the Synthesis of Drug Scaffolds

A Major Qualifying Project Report
Submitted to the Faculty
of the

WORCESTER POLYTECHNIC INSTITUTE

In partial fulfillment of the requirements for the
Degree of Bachelor of Science

by

_____
Nicholas G. Roumas

Submitted: February 13, 2013

Approved:

_____
Professor James P. Dittami
Advisor
Department of Chemistry

_____
Professor John C. MacDonald
Advisor
Department of Chemistry

Abstract

A photocyclic cycloaddition reaction via an ylide intermediate has opened the way for the synthesis and testing of numerous biologically active scaffolds. Research has demonstrated that the incorporation of electron-rich systems, most significantly naphthyl systems, increases the efficiency of the photocyclic reaction. In this project, new possibilities for electron-rich naphthyl analogs have been explored, incorporating heteroatoms into the bicyclic aromatic system. These new structures have been selected on the criteria of Lipinski's rules and feasibility of synthesis. A precursor to one of these structures was synthesized. Additionally, syntheses of other scaffold components were carried out.

Acknowledgments

I would like to thank Worcester Polytechnic Institute, the Department of Chemistry and Biochemistry, Professor James Dittami, Professor John MacDonald, and Alicia Morgan.

Table of Contents

I. Introduction

Recent work in the Dittami group has focused on the synthesis of novel biologically active (i.e. protein-binding) molecular scaffolds using a specific [3+2] photocyclization cycloaddition mechanism occurring via an ylide intermediate, shown in Figure 1. These reactions generally yield scaffolds resembling those of the important drugs morphine and huperzine, well-known for their activity as an analgesic and a memory-enhancing drug respectively.[1,2] Typically, in drug design, scaffolds with similar structures are more likely to exhibit similar activity.[3,4] By varying the substituents present on scaffold precursors, a variety of scaffolds can be realized. In order to determine what scaffolds to pursue, the Dittami group has looked to a procedure pioneered by the Shoichet research group at the University of California at San Francisco (UCSF). The Stoichet group has devised a computational method, called the Similarity Ensemble Analysis (SEA), that seeks to identify probable biologically active scaffolds based on their similarity to ligands that bind to their protein targets. Two algorithms are used to predict ligand-target associations. The Daylight algorithm is based on patterns of atom connectivity, while the Extended Connectivity Fingerprint 4 (ECFP4) algorithm is based on atomic environments in terms of concentric circular bond patterns within the molecule. Scaffolds are compared to known ligand sets and assigned an expectation value (*E*-value) that predicts the probability that the scaffold functions similarly to the ligand set. Lower *E*-values constitute a more strongly positive prediction. Based on the Stoichet group's predictions, five scaffolds, shown in Figure 2, have been selected as top priorities for synthesis. Once synthesized, scaffolds will be submitted for biological testing to validate these predictions.

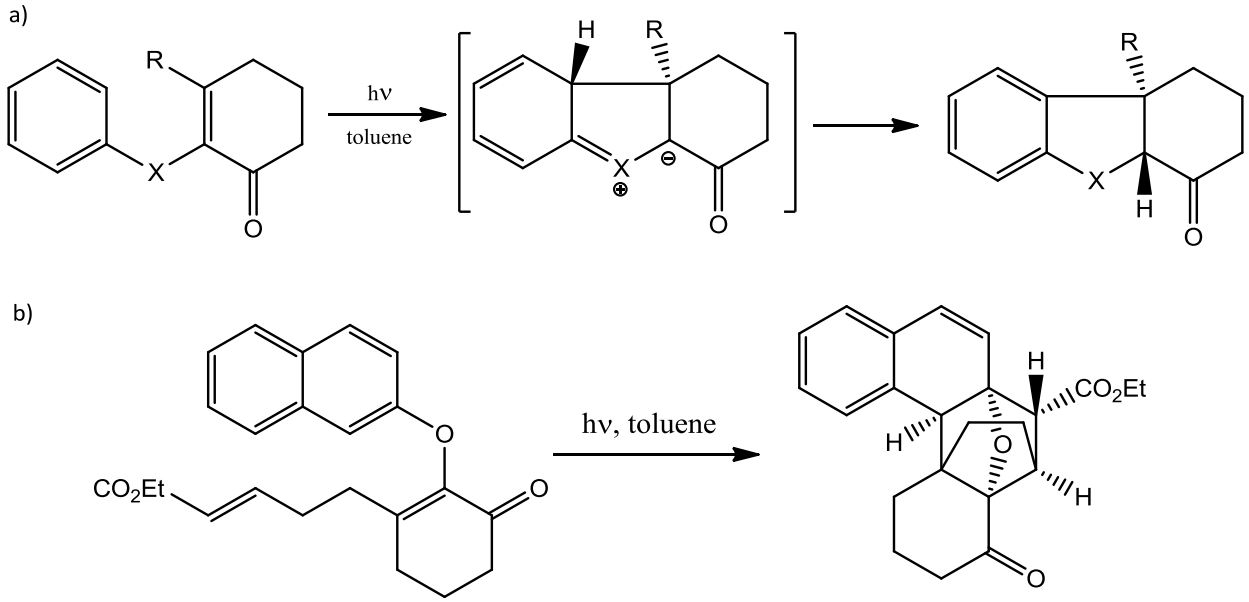


Figure 1: a) Photocyclization cycloaddition via an ylide intermediate.
b) Characteristic example of closure of the dipolarophile side chain.

Prior research has demonstrated that the [3+2] photocyclization cycloaddition reaction is most efficient when precursors incorporate an electron-rich aromatic system. In particular, naphthyl groups have been shown to favor formation of [3+2] products, perhaps due to the ability of such systems to donate electron density to the ylide.[5] In order to broaden the utility of this class of reactions, our group is exploring heteroaromatic naphthyl analogs as alternatives to the naphthyl system. We anticipate that the incorporation of heteroaromatic groups in place of naphthyl groups will significantly improve the efficiency of the photocyclization reaction due to the increased electron-donating capability of aromatic heterocycles compared to naphthalene. The inclusion of heteroatoms in the ring system may also yield new and interesting scaffold properties. In this project, we sought to identify a small library of bicyclic aromatic heterocycles that could be targeted for synthesis. Our selection of naphthyl analogs was based on two criteria: feasibility of synthesis, and the compliance of the products incorporating those groups with

Lipinski's rule of five, as described below.[6] Efforts to synthesize one of those compounds are described later in this report.

Drug design is a multistep process that requires potential drug compounds to operate effectively at a number of levels. At the first level, the compound needs to be synthetically accessible. Next, it needs to be efficient in its absorption, distribution, metabolism, and excretion (ADME) with regard to the host's body.[7] In the case of an orally administered drug, effective absorption consists in the passage of the drug from the intestine into the bloodstream. Distribution entails the ability to effectively pass through barriers in the body, for instance, the blood-brain barrier. Excretion is important in order to avoid undesirable effects from the buildup of the drug in the body. Finally, the compound needs to function properly as a ligand for its target protein. This project focused on designing synthetically feasible compounds that had a high probability of good ADME according to Lipinski's rules.

II. Background on method

The advent of High Throughput Screening (HTS) about 1989-1991 revolutionized the drug design process.[6,8] Previously, drug leads were arrived at after considerable investigation that involved in vivo or in vitro screening. HTS has enabled researchers to screen vastly greater volumes of compounds in vitro than had previously been feasible. In 1997, Christopher A. Lipinski published a cornerstone paper in which some of the consequences of HTS with respect to ADME-related parameters of screened compounds were examined.[6] These parameters were heavily relied upon in our method. It should be noted that Lipinski's rules predict ADME only, and not drug-likeness or drugability.[9] Lipinski found that compounds screened via HTS followed somewhat different trends from compounds screened in the pre-HTS era. By analyzing various

large databases of known drug compounds, Lipinski derived a "rule of five," which could predict whether a compound was likely to have good ADME using four simple parameters:

1. The compound has no more than 5 hydrogen-bond donors, counted as the number of nitrogen and oxygen atoms.

2. The compound has no more than 10 hydrogen-bond acceptors, counted as the sum of NH and OH groups.

Note that because donors contain hydrogen-bond-accepting atoms, they are necessarily H-bond acceptors as well.

3. The Molecular weight must be no greater than 500 Daltons.

4. The lipophilicity, expressed as the logarithm of the partition coefficient of the compound in 1-octanol and water (LogP), is no greater than 5. LogP may be estimated by various methods. In this report, when LogP is referred to without qualification, it has been determined by the commonly used Calculated LogP (CLogP). CLogP calculates LogP values based on the molecular fragments of which the entire compound is composed. CLogP may fail to provide a value, however, if unusual functional groups are present. In such cases, one may resort to Moriguchi LogP (MLogP), an atomic-based method which, although less accurate, will always provide a result. When MLogP is used, its value must not exceed 4.15 in Lipinski's rules.[6]

In 1999, a paper by Arup K. Ghose *et al.* modified some of Lipinski's rules, making them more precise.[10] Ghose determined a qualifying range for certain parameters within which 80% of compounds fell. Ghose's qualifying range of LogP is between-0.4 and 5.6, and of molecular weight, between 160 and 480.[10] Although Ghose's modifications to the Lipinski rules deserve a brief mention, they have not been used in this report, because the number of drug compounds

that fall outside either Lipinski's or Ghose's ranges is great enough that the precision of Ghose's numbers cannot be deemed very significant.

III. Method

We have researched a small library of compounds that will be suitable targets for synthesis. Our proposed structures consist in modifications of the naphthyl portion of the Dittami group's current top five scaffolds for synthesis, as shown in Figure 2. These compounds were considered "starting-point" structures for the selection of new naphthol analogs. The synthesis of each scaffold involves forming a bond at the 1- position of the naphthyl system.

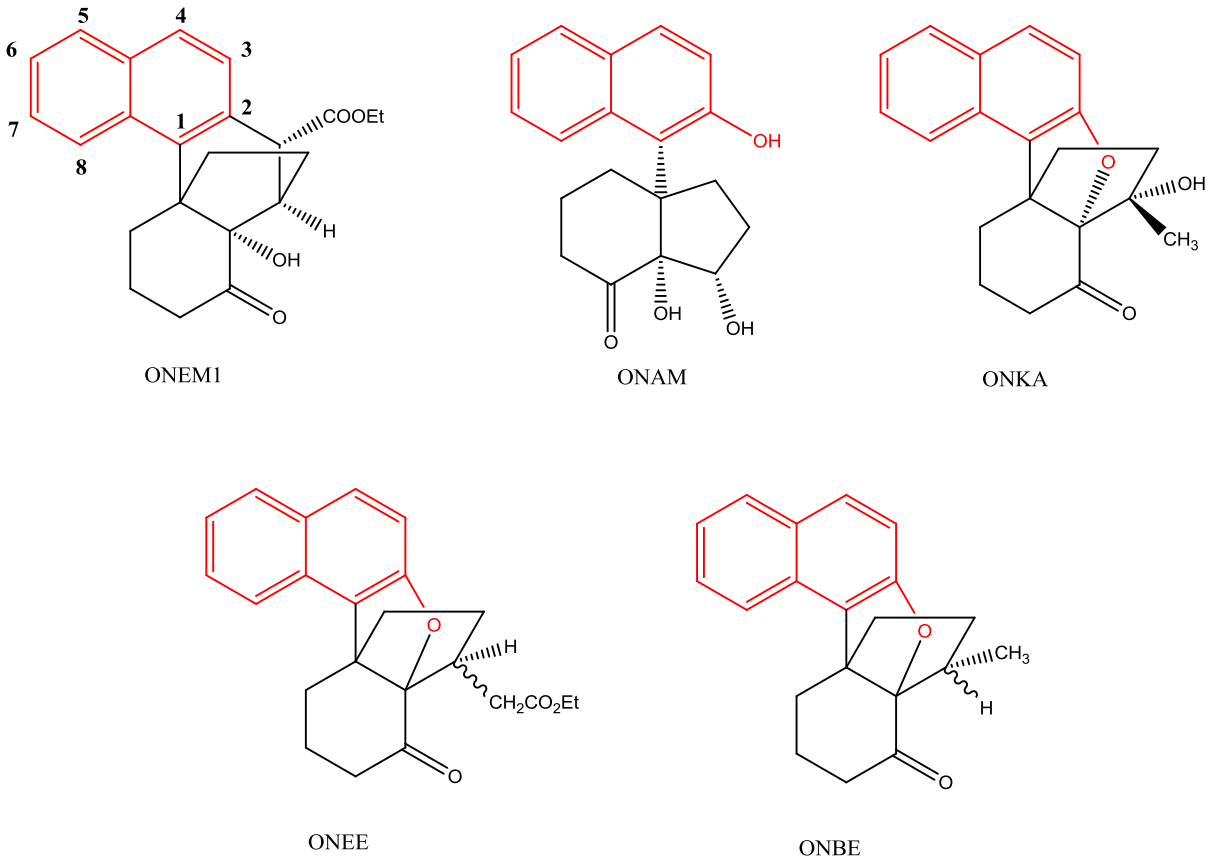


Figure 2: Starting-point structures. The naphthyl/naphthol group is highlighted.

All of the proposed bicyclic systems incorporate a hydroxyl group at the 2- position. In order to avoid interfering with the scaffold synthesis, placement of heteroatoms and substituent groups was limited to the 5-, 6-, 7- and 8- positions. Because the starting-point scaffolds each incorporate between two and four hydrogen-bond acceptors and between zero and three hydrogen-bond donors, the number of hydrogen-bond donors and acceptors (not including the hydroxyl group) in new naphthyl analogs is limited to at least six to eight and two to five, respectively. Most potential substitutes easily meet these criteria. The molecular weights of the starting-point compounds range from 292-364 Daltons. Given that the substitution of heteroatoms does not greatly affect molecular weight, this criterion is also essentially automatically fulfilled by all analogs.

Lipophilicity was calculated using the OSIRIS Property Explorer, produced by Actelion,[11] one of a number of web programs that calculates CLogP based on an input structure. It was found that the substitution of our preferred heteroaromatic naphthyl equivalents into the starting-point scaffolds reduced CLogP by no more than 1.9, while none of the starting-point scaffolds had a CLogP lower than 2.57, confirming that the scaffolds will remain within favorable LogP range with the substitution of the analogs.

Finally, we wished to limit our results to syntheses involving no more than two steps and relatively inexpensive starting materials.

IV. Proposed structures

Here we present the top five structures that were found to have the greatest potential as naphthyl analogs, as shown in Figure 3. The synthesis of 4-amino-3-nitrophenol, a precursor to structure **A**, was carried out, as reported in the experimental section. Synthetic schemes are shown in Figure 4, with procedures available in the papers referenced.

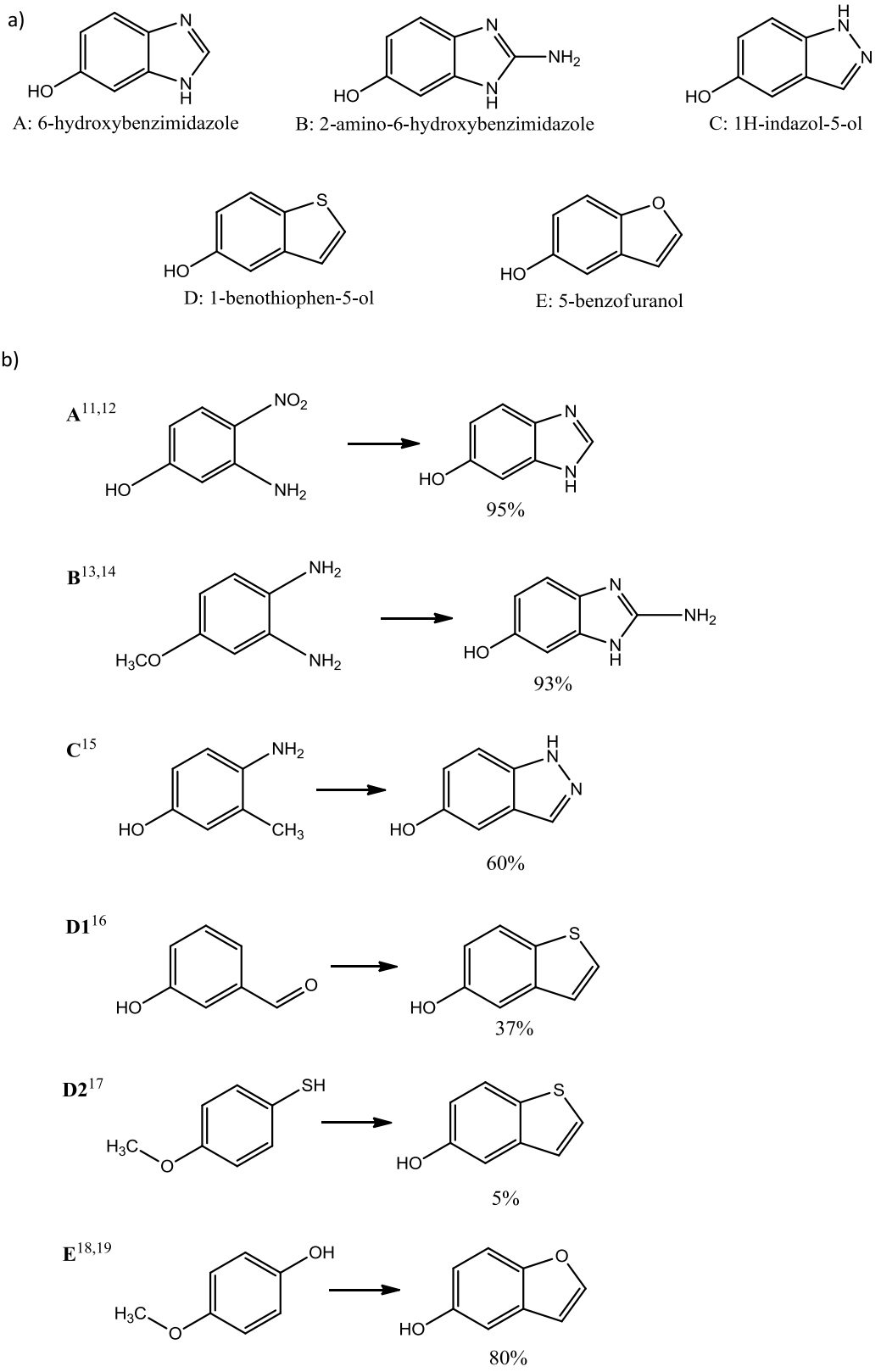
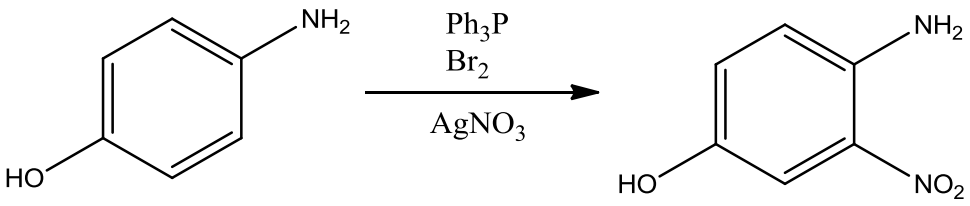


Figure 3: a) Top 5 proposed heteroatomic naphthyl analogs. b) Summary of synthetic schemes for the five proposed structures, with references. Two possibilities are given for the synthesis of D.

V. Experimental

Synthesis of 4-amino-3-nitrophenol [NR-I-31] (Scheme 1).[13]

Scheme 1.



To a round-bottomed flask was added triphenylphosphine (0.630 g, 2.4 mmol), 10 mL acetonitrile, bromine (0.13 mL, 0.403 g, 2.4 mmol), $AgNO_3$ (0.378 g, 2.4 mmol) and 4-aminophenol (0.218 g, 2.0 mmol), with stirring. The originally clear, colorless solution turned to a deep purple, then black, followed by precipitation. The mixture was filtered after about half an hour to remove AgBr precipitate, then evaporated. The residue was redissolved in dichloromethane and washed in 5% aqueous sodium bicarbonate (2x), water (2x), and brine. The organic phase was removed and dried over $MgSO_4$, then evaporated. Crude yield: 1.26 g.

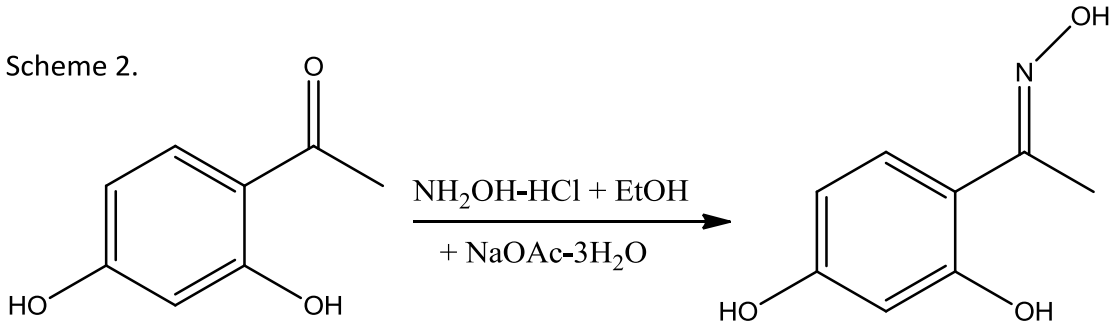
VI. A related synthesis

During our research, we carried out the synthesis of a non-naphthyl-analogous structure, 1-(2,4-dihydroxyphenyl)ethanone oxime. In the context of the final scaffold, the oxime product serves the same purpose as the naphthol analogs. Similarly to a bicyclic aromatic system, the aromatic oxime is expected to produce an efficient cycloaddition during scaffold synthesis due to its easily delocalized electronic system.[5]

VII. Experimental

Synthesis of 1-(2,4-dihydroxyphenyl)ethanone oxime [NR-I-29] (Scheme 2).

Scheme 2.



1-(2,4-dihydroxyphenyl)ethanone (5.00 g, 32.9 mmol), hydroxylamine hydrochloride (5.32 g, 153 mmol) and sodium acetate trihydrate (6.88 g, 55.5 mmol) were mixed together and 100 mL ethanol was added to the reaction vessel. The mixture was refluxed for 2 hours, monitored by TLC. Most of the solvent had to be evaporated off at this point to accelerate the reaction. Reflux continued for 1 hour. Solvent was evaporated and the product was redissolved in ethyl acetate, filtered, washed with water and brine, and dried over $MgSO_4$. Solvent was evaporated and an NMR sample was taken. Crude yield: 4.19 g (76%).

VIII. Syntheses of other segments of the scaffold

During our research, we synthesized two compounds that serve as the complementary segment to the naphthyl system in the scaffold—the scaffold's "other half," whose pendant chain functions as the dipolarophile of the ylide reaction. An example of the role of these compounds in scaffold synthesis is shown in Figure 4.

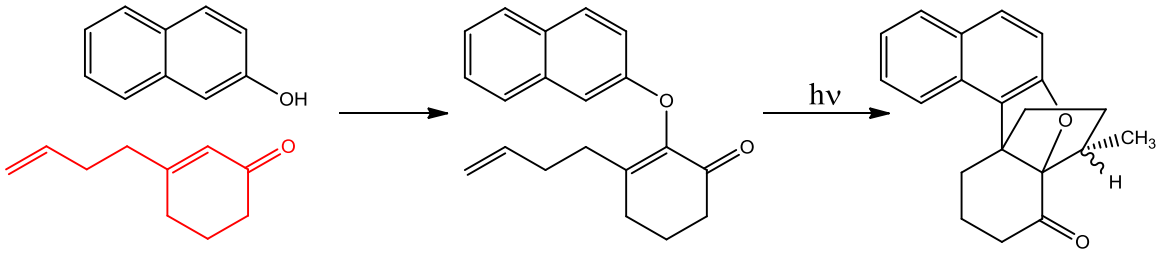
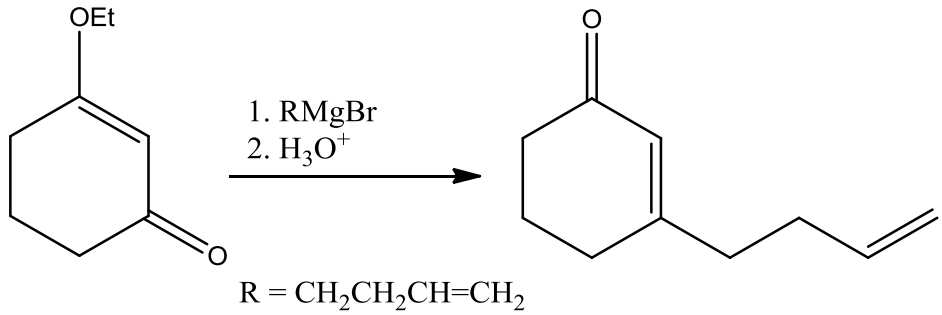


Figure 4: Synthetic process by which 3-(3-butenyl)-2-cyclohexenone [NR-I-7] (in red) can be combined with naphthol to produce the ONBE scaffold.

IX. Experimental

1. Synthesis of 3-(3-butenyl)-2-cyclohexenone [NR-I-7] (Scheme 3).

Scheme 3.

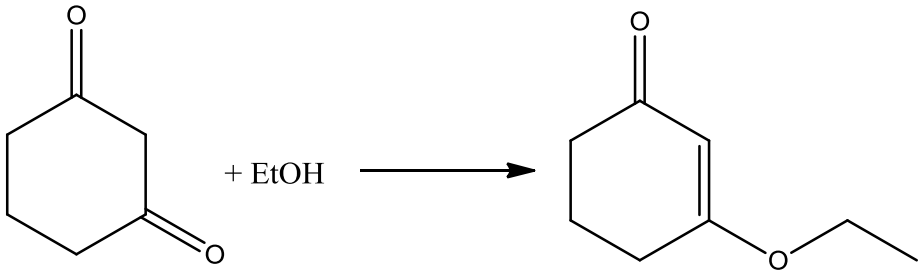


Into a dry flask was added Magnesium turnings (133 mg, 5.47 mmol) and 1 mL dried THF. To this initial mixture was added bromobutene (280 μL, 2.74 mmol) in 2 mL dried THF, followed by 1,2-dibromoethane (50 μL) to initiate the reaction. The solution produced bubbles and was exothermic to the touch. Upon auto-cooling to room temperature, 3-ethoxycyclohex-2-enone (384 mg, 369 μL, 2.74 mmol) in 2 mL THF was added to the solution. The reaction mixture again became warm to the touch, and was stirred at room temperature until it was judged complete as confirmed by TLC. Saturated aqueous ammonium chloride (1 mL) was added and excess THF was evaporated in vacuo. The crude product was partitioned between dichloromethane and saturated aqueous oxalic acid solution, dissolving the majority of the remaining Magnesium turnings and precipitate salts. The aqueous phase was further extracted

with 10 mL dichloromethane. The organic phases were combined and washed with 15 mL water and 2x10 mL brine. The solution was dried over NO_2SO_4, gravity filtered using a cotton filter funnel, and rotoevaporated, yielding a brown oil.

2. Synthesis of 3-ethoxycyclohex-2-enone [NR-I-17, from VK-I-5] (Scheme 4).

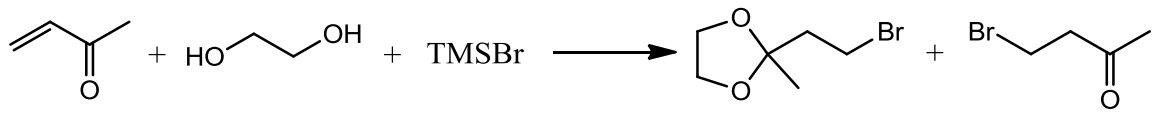
Scheme 4.



Into a round-bottomed flask was added cyclohexenone-1,3-dione (30.00 g, 268 mmol), ethanol (200 mL, 3425 mmol), p-toluenesulfonic acid monohydrate (2.843 g, 14.95 mmol) and toluene (600 mL).The resulting mixture was refluxed for 4 hours using a Dean Stark trap to remove water formed during the reaction. Reflux was stopped upon complete consumption of the starting material as determined by TLC. The mixture was brought to room temperature and washed with a 1% NaOH solution in brine, then water and brine, dried over Na_2SO_4, and vacuum distilled. The product was a colorless liquid Yield: 27.2 g (72.4%).

3. Synthesis of 2-(2-bromoethyl)-2-methyl-1,3-dioxolane [NR-I-19] (Scheme 5).

Scheme 5.



1 eq. 4 eq. 90% conv. 10% conv.

To a flame-dried flask kept under an inert atmosphere was added ethylene glycol (11.2 mL) followed by 3-butene-2-one (4.2 mL, 50 mmol), resulting in a clear yellow mixture. The mixture was cooled in an ice bath. TMS Br (7.92 mL, 60 mmol) was added dropwise, resulting in a biphasic mixture consisting of a clear colorless layer and an opaque yellow layer. The mixture was stirred at room temperature for 2 hours, gradually becoming opaque red. The reaction mixture was then poured onto 100 mL biphasic hexane and 50 mL 5% aqueous Na_2CO_3, washed with 5% $Na_2S_2O_3$, water, and brine, and dried over anhydrous K_2CO_3. The organic mixture was decanted and rotary evaporated. Yield 5.47 g (62%).

References

[1] Sun, Q. Q., Xu, S. S., Pan, J. L., Guo, H. M., Cao, W. Q. (1999) Huperzine-A capsules enhance memory and learning performance in 34 pairs of matched adolescent students. *Acta Pharmacol. Sin.* **20**, 601-603.

[2] Wang, B. S., Wang, H., Wei, Z. H., Song, Y. Y., Zhang, L., Chen, H. Z. (2009) Efficacy and safety of natural acetylcholinesterase inhibitor huperzine A in the treatment of Alzheimer's disease: an updated meta-analysis. *J. Neural Transm.* **116**, 457-465.

[3] Keiser, M. J., Setola, V., Irwin, J. J., Laggner, C., Abbas, A. I., Hufeisen, S. J., Jensen, N. H., Kuijer, M. B., Matos, R. C., Tran, T. B., Whaley, R., Glennon, R. A., Hert, J., Thomas, K. L. H., Edwards, D. D., Stoichet, B. K. and Roth, B. L. (2009) Predicting new molecular targets for known drugs. *Nature* **462**, 175-181.

[4] Ursu, O., Rayan, A., Goldblum, A. and Oprea, T. I. (2011) Understanding drug-likeness. *Wiley Interdis. Rev.: Comput. Mol. Sci.*, **1**, 760–781. doi: 10.1002/wcms.52.

[5] Dittami, J.P., Nie, X-Y., Nie, Hong, Ramanathan, H., Breining, S., Bordner, J., Decosta, D., Kiplinger, J., Reiche, P. and Ware, R. (1991) Intramolecular addition reactions of carbonyl ylides formed during photocyclization of aryl vinyl ethers. *J. Org. Chem.* **56**, 5572-5578.

[6] Lipinski, C. A., Lombardo, F., Dominy, B. W. and Feeney, P. J. (2001) Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. *Adv. Drug Delivery Rev.* **46**, 3-26.

[7] For an overview of ADME as it pertains to drug development, see: Wishart, D. S. (2007) Improving early drug discovery through ADME modelling: an overview. *Drugs in R & D* **8**, 349-362.

[8] Pereira, D. A., Williams, J. A. (2007) Origin and evolution of high throughput screening. *Br. J. Pharmacol.* **152**, 53-61.

[9] For reviews detailing some issues with the historical use of the Rule of 5, see: a) Bickerton, G. R., Paolini, G. V., Besnard, J., Muresan, S. and Hopkins, A. L. (2012) Quantifying the chemical beauty of drugs. *Nat. Chem.* **4**, 90-98. b) Abad-Zapatero, C. A. (2007) A sorcerer's apprentice and the Rule of Five: from rule-of-thumb to commandment and beyond. *Drug Discov. Today* **12**, 995-997.

[10] Ghose, A. K., Viswanadhan, V. N. and Wendoloski, J. J. (1999) A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J. Comb. Chem.* **1**, 55-68.

[11] Molecular Properties Prediction – OSIRIS Property Explorer. Thomas Sander, Actelion Pharmaceuticals Ltd. http://www.organic-chemistry.org/prog/peo/ (December 19, 2012).

[12] Van Vliet, D. S., Gillespie, P. and Scicinski, J. J. (2005) Rapid one-pot preparation of 2-substituted benzimidazoles from 2-nitroanilines using microwave conditions. *Tetrehedron Lett.* **46**, 6741-6743.

[13] Iranpoor, N., Firouzabadi, H., Nowrouzi, N. and Firouzabadi, D. (2006) Highly chemoselective nitration of aromatic amines using the Ph3P/Br2/AgNO3 system. *Tetrehedron Lett.* **47**, 6879-6881.

[14] Drinkwater, N., Vu, H., Lovell, K. M., Criscione, K. R., Collins, B. M., Prisinzano, T. E., Poulsen, S., McLeish, M. J., Grunewald, G. L. and Martin, J. L. (2010) Fragment-based screening by X-ray crystallography, MS and isothermal titration calorimetry to identify PNMT (phenylethanolamine N-methyltransferase) inhibitors. *Biochem. J.* **431**, 51-61. See Supplementary Experimental section at http://www.BiochemJ.org/bj/431/bj4310051add.htm.

[15] Galan, A. A., Chen, J., Du, H., Forsyth, T., Huynh, T. P., Johnson, H. W. B., Kearney, P., Leahy, J. W., Lee, M. S., Mann, G. et al. (2008) Preparation of 1H-imidazole-4,5-dicarboxamides as JAK-2 modulators. European Patent Foundation no. WO2008042282-A2.

[16] Iwakubo, M., Takami, A., Okada, Y., Kawata, T., Tagami, Y., Ohashi, H., Sato, M., Sugiyama, T., Fukushima, K. and Iijima, H. (2007) Design and synthesis of Rho kinase inhibitors (II). *Bioorg. Med. Chem.* **15**, 350-364.

[17] Misra, T., Ganguly, T., Kamila, S., Basu, C. and De, A. (2001) Synthesis and studies on spectroscopic as well as electron donating properties of the two alkoxy benzo[b]thiophenes. *Spectrochim. Acta, Part A* **57**, 2795-2808.

[18] Pérez-Silanes, S., Martínez-Esparza, J., Oficialdegui, A. M., Villanueva, H., Orús, L. and Monge, A. (2001) Synthesis of new 5-substituted benzo[*b*]thiophene derivatives. *J. Heterocyclic Chem.* **38**, 1025-1030.

[19] Bonini, C., Cristiani, G., Funicello, M. and Viggiani, L. (2006) Facile entry to 4- and 5-hydroxybenzofuran and to their amino derivatives. *Synth. Commun.* **36**, 1983-1990.

[20] Barker, P., Finke, P. and Thompson, K. (1989) Preparation and cyclization of aryloxyacetaldehyde acetals: A general synthesis of 2,3-unsubstituted benzofurans. *Synth. Commun.* **19**, 257–265.