GENERATION OF NOVEL SMALL MOLECULE LIBRARIES FOR DRUG DISCOVERY

by

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FOR DRUG DISCOVERY

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December 2010

ABSTRACT

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Pyrazoles are privileged structures in medicinal chemistry. These molecules have a large impact in pharmacology and have been used in several FDA approved drugs for their analgestic, anti-inflammatory, antibacterial, and antiarrhythmic effects. Due to their prevalence in biologically active scaffolds, the synthesis of these small molecules has been increasingly important in medicinal chemistry programs. Innovative pyrazoles have been synthesized and evaluated in various screens for biological activity, using novel methodology derived from the Frantz laboratory. These screens were conducted in a collaborative arrangement with Dr. Susan Mooberry, of the Department of Pharmacology at UTHSCSA, which tested against an anti-cancer assay, and with Eli Lilly and Co. Inc., against five separate screens, including: Alzheimer's disease, two cancer assays, diabetes, and bone formation. The novel methodology used in this pyrazole synthesis will give rise to an innovative class of pyrazoles and open new pathways to identifying new biological processes.

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CHAPTER 1: INTRODUCTION

Many drugs in today's pharmaceutical industry rely on the synthesis of novel small molecules with specific functionality towards biochemical receptors. One specific class of compounds that are biologically active across an array of therapeutic areas is substituted pyrazoles.[1] These molecules (Figure 1) are the focus of many medicinal chemistry programs, and have been widely studied in therapeutic areas such as hypoglycemic,[2] sedative-hypnotic,[3] anti-inflammatory,[4] analgesic,[5] and antimicrobial properties.[6] Some federal drug administration (FDA) approved drugs which include a pyrazole core are Celebrex, Viagra, and Acomplia.[7] For these reasons, the synthesis of pyrazoles continue to attract the efforts of organic chemists worldwide.[8]

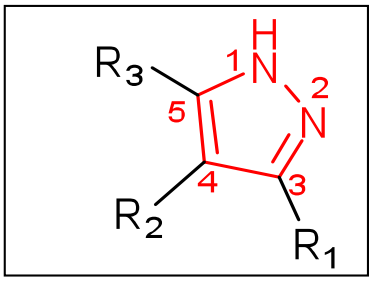
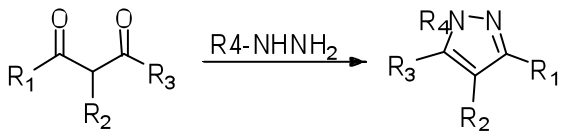


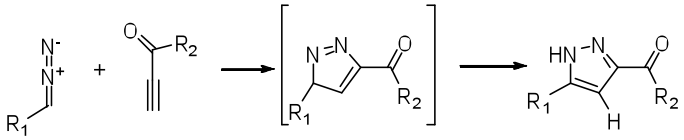
Figure 1. General pyrazole structure

The most utilized approaches to pyrazole synthesis are based on the basic reaction of hydrazines with β-difunctional compounds[9] (Reaction 1) and 1,3-dipolar cycloadditions of diazo compounds onto alkynes (Reaction 2). [10, 11] While these methodologies readily provide the pyrazole core, there are some limitations. Reaction 1 is limited by the inability to control the formation of regioisomers, and Reaction 2 does not allow for substitution at the 4-position. These limitations have stimulated synthetic chemists to discover a methodology which produces a poly-

Reaction1:



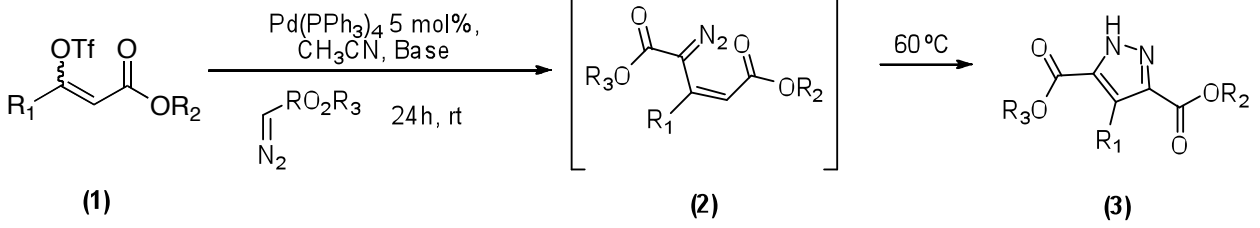
Reaction 2:



Scheme 1. Current methodology of pyrazole synthesis

substituted pyrazoles with absolute regiocontrol of all positions.

Recently, the Frantz group has developed a practical approach to the stereoselective synthesis of both (*Z*)- and (*E*) enol triflates **(1)**.[12] These are powerful building blocks that have a wide array of uses within organic chemistry and especially medicinal chemistry methodology. Currently, the Frantz laboratory is using these triflates to develop 3,4,5-trisubstituted pyrazoles **(3)** by the reaction of enol triflates **(1)** with diazoacetates under Pd-catalysis (Scheme 2). Upon heating intermediate **(2)**, an electrocyclization is induced and yields the targeted pyrazoles in a single flask. These structures are obtained from a regiospecific cross-coupling reaction that allows complete control of the substitution pattern around the pyrazole core while providing differentiation of esters off the 3- and 5-positions. ($R_2 \neq R_3$)



(1) **(2)** **(3)**

Scheme 2.Frantz lab pyrazole generation

These diverse pyrazoles are the target of this small molecule library due to the fact that they are currently unknown in the literature and have not been incorporated in any drug screens to date.This library was composed of novel small molecules and was tested in a collaborative effort with Dr. Susan Mooberry of the Department of Pharmacology at The University of Texas Health Science Center at San Antonio. In addition, we also worked in an academic/industrial partnership with Eli Lilly and Co., Inc. This partnership was created to stimulate the drug discovery within Eli Lilly and Co. and academic laboratories, and was used to test the pyrazoles against five different assays. The five assays to be tested against included anti-cancer (two

separate screens), Alzheimer's disease, diabetes, and bone formation through the Phenoypic Drug Discovery Program (see https://pd2.lilly.com/pd2Web/ for more details Dec, 2010).

CHAPTER 2: THESIS STATEMENT

2.1 Hypothesis 1

The 3,4,5-trisubusituted pyrazole libraries generated from the novel cross-coupling of enol triflates with diazoacetates will provide potential new small molecule leads. These leads will be determined by using high throughput screening (HTS) of several whole cell assays to determine biological activity.

2.2 Hypothesis 2

Through the evaluation of structure-activity relationships (SAR), more potent drug-like leads will be provided for further pre-clinical development.

CHAPTER 3: METHODS AND APPROACH

3.1 Specific Aims

The process of drug discovery and development is a complex, multi-disciplinary area of research that often involves several years of intense effort to identify a single preclinical candidate. This process begins with the synthesis and screening of thousands of small molecules and that will ultimately lead to one candidate suitable for further development. In this particular study, the use of a novel class of pyrazoles as part of a focused medicinal chemistry program will be evaluated. Due to the rich history of pyrazoles in drug discovery, and their prevalence among FDA approved drugs, there is a potential that these small molecules will have biological effects across several different assays. Through this process, many scaffolds with a constant pyrazole core will be developed to identify the structure-activity relationships of most importance against a specific class of biological targets. This research program was divided into two specific aims, which after completion were able to produce two strong hits that will be used for further drug development within a medicinal chemistry program. The aims outlined below were used to test both hypotheses (page 4).

Specific Aim 1: Design and synthesize novel pyrazole libraries that will be screened for biological activity on several different fronts.

Specific Aim 2: Evaluate biological data and design future small molecule libraries using structure-activity relationship studies to identify specific functionality responsible for biological reactivity.

3.2 Background and Significance

The biological activity of these novel small molecule pyrazoles was obtained from two important sources. The first source of data collection came from a collaborator at the University

of Texas Health Science Center at San Antonio, Dr. Susan Mooberry. The primary focus of her cell-based screening assay is to identify microtubule disrupting agents, which can be further screened against several cancer cell lines. Microtubule targeting agents are some of the most important in current cancer therapy, and are used to treat a wide range of tumor types. This spectrum includes: pediatric leukemia, testicular cancer in young men, ovarian and breast cancer, non-small lung cancer, prostate cancer, and head and neck tumors. These novel small molecules will help identify new agents that have a similar mechanism of action, but with superior properties; especially the ability to evade multidrug resistance. The two mechanisms of resistance, clinically documented for diverse microtubule targeting drugs, include the expression of the ATP-dependent membrane transport protein P-glycoprotein (Pgp) and the expression of the βIII isotype of tubulin.[13] The molecules screened in collaboration with Dr. Mooberry will fulfill the goal of synthetically designing tubulin-targeting drugs that possess these superior properties, and will serve as a viable candidate for pre-clinical drug synthesis.

The second source of data collection of biological activity of these novel molecules was obtained from the PD^2 Phenotypic Screening Panel (Figure 2) laboratories of Eli Lilly and Co. Due to the sheer number of molecules that must be screened in order to determine one viable pre-clinical candidate, many pharmaceutical companies have developed such collaborations with academic institutions to make these efforts more effective. The program developed by Eli Lilly and Co. allows for collaborating chemists to subject their small molecules to screening among the five assays of the phenotypic discovery panel. This collaborative effort has been established by the Frantz laboratory. The assays (Figure 2) tested within this collaboration include Alzheimer's disease and the apoE secretion, cancer growth in the cell cycle and G2/M phases, anti-angiogenesis with tube formation, tube area and nuclear staining, the insulin secretion associated with diabetes, and the Wnt pathway of bone formation. These assays allow the small

molecule, pyrazole libraries to be incorporated into many clinically relevant biological targets resulting in the possibility of having a large range of medical applications.

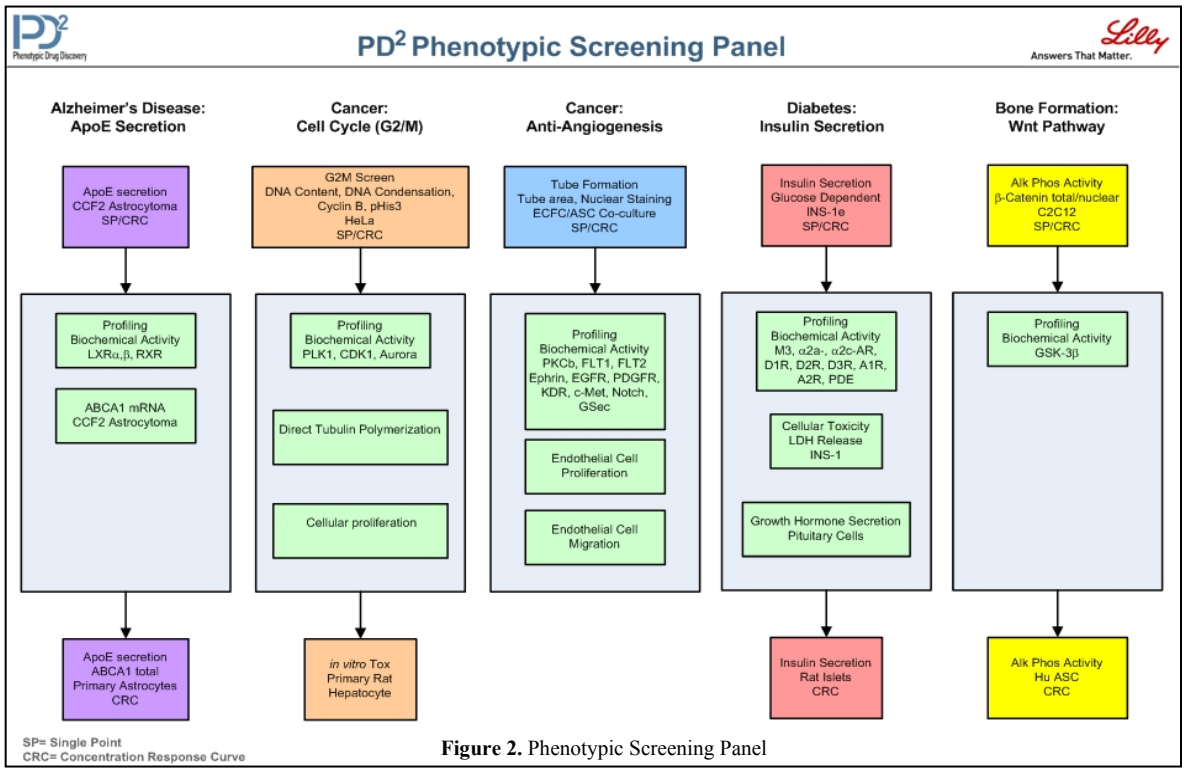


Figure 2. Phenotypic Screening Panel

Alzheimer's disease is a progressive degenerative dementia usually beginning with memory impairment and eventually evolves to a severe loss of cognitive function over an array of several areas. The Alzheimer's phenotypic module measures secretion of apolipoprotein E (apoE) from a human astrocyte cell line. Apolipoprotein E is an essential protein for the normal catabolism of triglyceride-rich liproprotein constituents, and is currently being studied for its role in Alzheimer's disease. The ε4 allele of the apolipoprotein E gene was identified as an important genetic risk factor for the development of the most prevalent form of Alzheimer's Disease (sporadic), [14] and played an active role in the subsequent studies that indicated the early suppression of amyloid-beta (Aβ) deposition was controlled by apoE.[15] This suppression occurs by facilitating the clearance of Aβ from the brain. Molecules that selectively enhance apoE

secretion through a non-LXRβ mechanism are of select interest to Eli Lilly and Co. Confirmed activity from the primary phenotypic assay are further tested in primary astrocytes and in biochemical nuclear hormone receptor assays.

Current cancer therapy has two main targeted routes. The first includes the blocking of the cell cycle during rapid growth and separation during mitosis (G2/M phases), and the second is through the inhibiting of blood vessel formation to tumors, known as, anti-angiogenesis. The two cancer assays of Eli Lilly and Co. focus on both of these methods. The cell cycle G2/M arrest phenotypic assay identifies compounds that arrest HeLa cells in G2 or M phase. This is a multiplexed, high throughput cell imaging identifying those compounds that are G2/M blockers and utilize a non-tubulin mechanism of action. The second phenotypic assay developed by Eli Lilly and Co. utilizes the co-culture of human endothelial progenitor cells with adipose-derived adult stem cells to measure angiogenesis. The compounds of interest within this assay inhibit angiogenesis but do not show inhibition of known angiogenic receptor tyrosine kinases.

The fourth assay of the academic/industrial partnership addresses the lack of insulin secretion that occurs when one suffers from Type I diabetes. Insulin is secreted by the beta cells of the pancreatic islets. It is a protein hormone that contains 51 amino acids and is required by almost all of the body's cells. Insulin helps regulate the blood/glucose levels throughout the body and is dependent upon the amount of glucose in the blood. This phenotypic module tests for compounds that enhance glucose-dependent insulin secretion. The preferred molecule would act only in the presence of glucose and would have a novel mechanism of action to stimulate the insulin secretion.

The final phenotypic assay, also referred to as bone formation, tests compounds for their ability to differentiate murine C2C12 cells involved in bone formation. This cell line has a multi-lineage potential, and can be differentiated to an osteoblast-like phenotype through β-catenin-

dependent stimulation of alkaline phosphatase activity. The goals of this assay are to identify compounds which increase osteoblast formation in human cell assays through a non-glycogen synthase kinase.

3.3 Preliminary Data

Some preliminary data received from the collaboration with Eli Lilly and Co. show biological activity within the anti-angiogenesis cancer assay as well as the G2/M cell cycle assay. Figure 3 shows a molecule (HA-135-167), that has demonstrated activity within the anti-angiogenesis assay. This pyrazole contains a tert-butyl acetate substitution at the 3-position and an ethyl acetate substitution at the 5-position. This particular molecule, after screening within the anti-angiogenesis assay of Eli Lilly & Co. had a CD31 IC_{50}-Value of 4.0 µM and a Nuc Area IC_{50}-Value of 9.84 µM, a viable hit within our small molecule library.

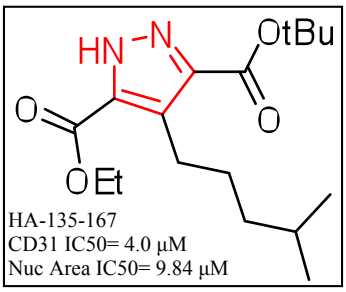


Figure 3. Preliminary Eli Lilly data and molecule structure.

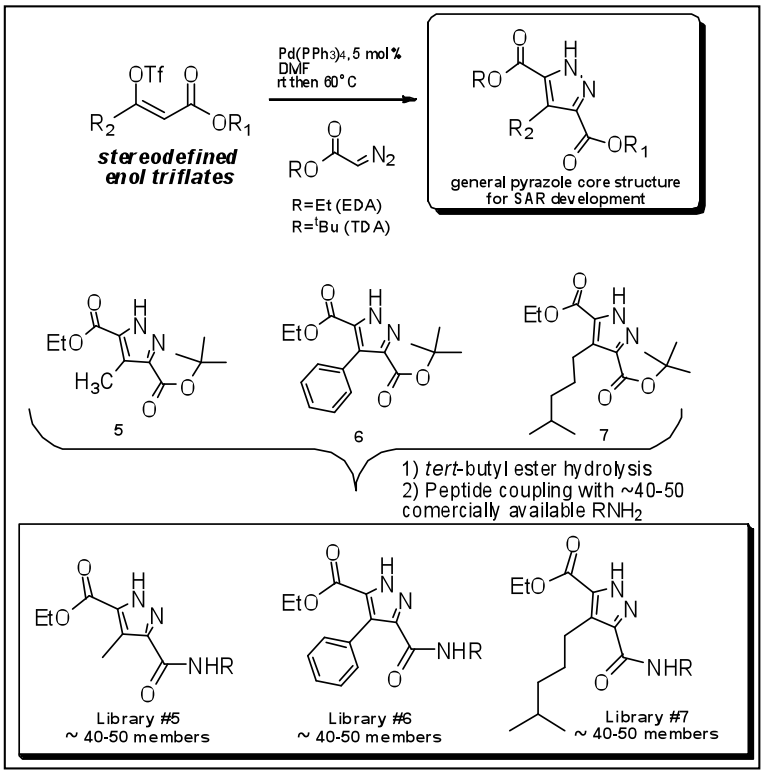
The anti-angiogenesis assay is a phenotypic assay that utilizes the co-culture of human endothelial progenitor cells (ECFCs) with adipose-derived adult stem cells (ADSCs) to measure angiogenesis. The ability of a molecule to inhibit angiogenesis without destroying feeder cells is a key component of their success and advancement to biochemical kinase and cell based assays. Eli Lilly and Co. is particularly interested in molecules with the ability to inhibit angiogenesis while keeping intact the receptor tyrosine kinase (RTK). Class I, EGF receptor family, tyrosine kinase receptors are associated with cell signaling. Excessive cell signaling from the EGF receptor family is linked to the development of many types of solid tumors, including sarcomas, carcinomas, and lymphomas, and is a main determining factor in using RTK inhibitors as an effective cancer therapeutic.

PECAm-1 (platelet endothelial cell adhesion molecule), also referred to as CD31, is a glycoprotein expressed on the cell surfaces of monocytes, neutrophils, platelets and a subpopulation of T cells, and is known to mediate endothelial cell-cell interactions that are involved in angiogenesis. These proteins are expressed on certain tumors, and relevant changes were observed during this whole cell pyrazole screening assay. The second value reported by Eli Lilly and Co., refers to the nuclear area of the cells involved in angiogenesis. These cells are known to have a much larger nuclear area than the same tissue cells under normal conditions, and this is the main component in determining the effect of a potential drug candidate.

This reported biological data was a great starting point for the created small molecule library and has three major advantages. The first advantage of this starting point is that these pyrazoles follow Lipinski's rule of five.[16] This rule describes molecular properties that potentially increase pharmokinetics within the human body, making the small molecules more likely to become viable drug candidates. The small molecules synthesized have a relatively small molecular weight, which allows diversification of the pyrazole core without over functionalizing the molecule, while maintaining lipophilicity, [17] and a low octonal-water partition coefficient.[18, 19] Additionally, the pyrazole library contains molecular properties which improve drug likeliness by containing no more than five hydrogen bond donors, and ten hydrogen bond acceptors. The second advantage of this starting point is the fact that the assays used are whole cell assays, as opposed to biochemical assays. Thus, the ability of these pyrazoles to penetrate the cell wall and inhibit their target gives them a significant advantage to those acting within a biochemical assay. The final advantage is the ability to easily design structure activity relationship studies with these libraries. This is made possible by the novel methodology used to synthesize the pyrazole core.

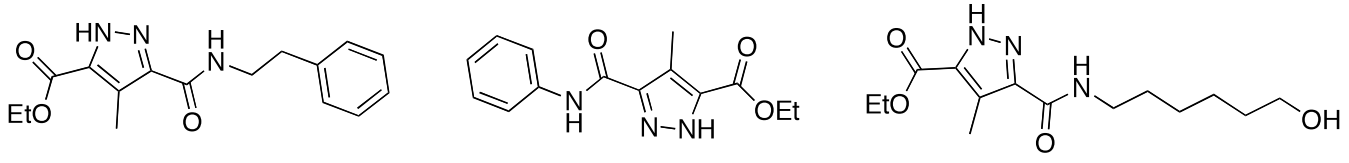
3.4 Research Design and Methods

The novel pyrazole libraries were synthesized utilizing the chemistry developed by the Frantz group, as outlined in Scheme 3. Starting with enol triflates, that are readily available using methodology previously published from the laboratory, [12] a cross-coupling reaction was completed in a one-pot synthesis. The starting material was cross-coupled with either ethyl diazoacetate (EDA) or tert-butyl diazoacetate (TDA) via Pd-catalysis to provide the essential pyrazoles.[20] Initially, three core pyrazoles (5-7, Scheme 3) that differed by substitution in the 4-position, were planned to be synthesized, and comprised the base for the small molecule library. However, due to time constraints, all three libraries were not fully explored, and the majority of pyrazoles synthesized only contained the core of Libraries #5 and #7 (Scheme 3). From these building blocks, novel small molecules were synthesized with a significant advantage over the existing approaches and their structure was based upon previous SAR studies and the current HTS screen, while maintaining the incorporation of targeted amine side chains. Based upon the methodology outlined in Scheme 3, the ability to selectively place substitution at the 4-position, while incorporating two differential ester groups in the 3- and 5-positions of the pyrazole core was obtained. This differentiation allowed regiospecific chemistry to occur, yielding flexibility in our



Scheme 3. Initial generation of highly substituted pyrazole libraries using new synthetic approaches developed by the Frantz laboratory.

medicinal chemistry program that has previously been unattainable. Primarily, the first library (Library #5) was explored and several commercially available amines were incorporated via peptide coupling. Three representative structures that were originally synthesized are depicted in Figure 4. Following incorporation of diverse functional groups, the pyrazoles were submitted to our collaborators for further evaluation and biological screening. The expansion of this library and those following it were dependent upon the biological data received from our various collaborators and specific structure activity relationship that was established.



MD-124-154 MD-124-155 MD-124-160

Figure 4. Library #5 preliminary structures.

The structure activity relationship (SAR) study was conducted under an iterative process which continually evaluated potential leads. From the biological data received, an initial pharmacophore model and structure activity relationship map to help refine future analogs was developed. It is with this process that ability to rationally design and synthesize these novel pyrazoles such that they have confirmed increased activity in each particular assay (i.e. IC_{50}, cell kill, and phenotype) was possible. This process can be visualized in Figure 5. Those pyrazoles that did not have a quantifiable increase in biological activity relative to the previous hit were not evaluated further, and structure design of this molecule was halted.

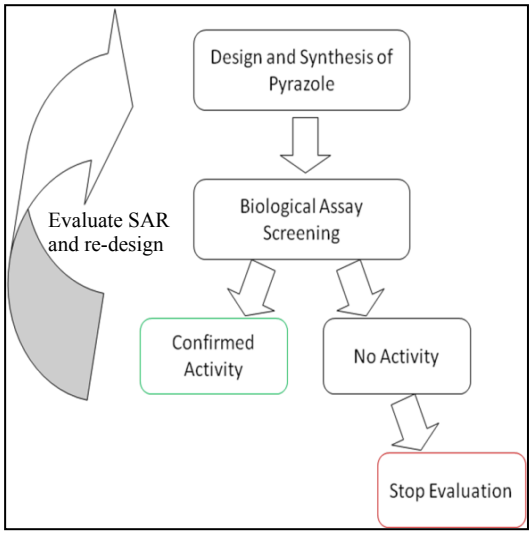


Figure 5. SAR Flowchart

The structures of the small molecules were purified by single and multi-solvent recrystallization or silica gel column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and LC/MS to determine purity (greater than 95%) and structural confirmation.

3.5 Biological Assay Design and Methods

In collaborative efforts with Dr. Mooberry's Laboratory, the biological testing of the novel small molecule library was completed. Using A-10 (CRL-1476) thoracic, aorta cell lines, the effect of the small molecules applied at varying concentrations was visualized after 18 hours of incubation. The cell line from American Type Culture Collection (ATCC) was stored in ATCC-formulated Dulbecco's Modified Eagle's Medium (DMEM) at 37˚C and was stained using and indirect immunofluorescent (IF) protocol to adequately see the microtubules of the A-10 cells. Dulbecco's Modified Eagle's Medium (DMEM) is comprised of 4 mM L-glutamine, 4500 mg glucose L^{-1}, and 1500 mg L^{-1} of sodium bicarbonate. The immunofluorescent (IF) protocol used for staining the cells used the following reagents: 100% methanol (CH_3OH), phosphate buffered saline (PBS), 10% bovine calf serum (BCS), phosphate buffered saline/bovine serum albumin (PBS/BSA 1.5 g BSA (albumin, Fraction V) and 150 mL PBS), 1:400 Primary (1˚) Antibody (Sigma T-4026 Tub 2.1 B-tubulin), 1:200 Secondary (2˚) Antibody (Sigma F-3008 IgG FITC conjugate), 0.1 μg mL^{-1} 4',6-diamidino-2-phenylindole (DAPI), and the ATCC formulated mounting media. Using the above listed reagents, the IF microtubule staining protocol was completed by first removing the media and immediately fixing the cells with 100% methanol for 5 minutes. Following fixation, the cells were washed 3 times at room temperature with PBS. Next, the cells were incubated in 10% BCS for 20 minutes. Following incubation, the cells were washed two times with PBS/BSA solution and then added to the 1:400

Primary (1˚) Antibody solution at 37˚C for 90-120 minutes. After the cells were allowed to incubate in the Primary antibody, they were washed again three times with PBS/BSA solution. The cells were then added to the 1:200 Secondary (2˚) Antibody solution at 37˚C for 60 minutes. After incubation, the cells were washed twice more with PBS/BSA solution and then once with PBS. Next, the cells were allowed to incubate in 0.1 μg mL^{-1} of DAPI for 7-10 minutes, followed by a PBS wash two times. Finally the slides were prepared for viewing under the IF microscope using the wet mount technique. If microtubule depolymerizing or stabilizing activity was apparent, the effective concentration (EC_{50}) was determined. Upon confirmation (n=3) of microtubule depolymerizing or stabilizing activity within the A-10 cell line, a secondary assay (i.e. dose response) in MDA-MB435 cells was evaluated. Following the same staining protocol as mentioned above, the inhibitory concentration (IC_{50}) was determined.

CHAPTER 4: RESULTS AND DISCUSSION

During this study a small molecule library of thirty four molecules was created. A complete list of synthesizied pyrazoles can be found in Appendix A. These novel pyrazoles were synthesized using the above mentioned EDC coupling chemistry and were tested for biological activity in a collaborative effort with Eli Lilly and Dr. Susan Mooberry of UTHSCSA. As anticipated, a hit molecule was successfully identified and has led to structurally designed subsequent analogs.

In collaborative efforts with Eli Lilly and Co., twenty-five novel pyrazoles were screened using their bioinformatics assay. Of the twenty-five screened, only two did not advance to the second level of biological testing, and the other twenty-three have been sent to Eli Lilly and Co. for further evaluation. The results of these assays have not yet been received.

Our second collaboration with Dr. Mooberry allowed for more frequent testing due to the relatively close nature of our laboratories. Using the A-10 cell line for initial screening for microtubule disrupting agents, twenty-seven of the small molecules were screened. Of the twenty-seven screened only two showed noticeable biological activity. From this data, a hit molecule (Figure 6) was identified, that contains a methyl group at the 4-positon and an ethyl acetate substitution at the 5-position. This particular molecule, after screening within the A-10 cell line had an EC_{50} value of 26.8 μM (n=3). Following primary screening, secondary validation was completed in the MDA-MB-435 cell line, and an IC_{50} value of 752.7 nM was obtained.

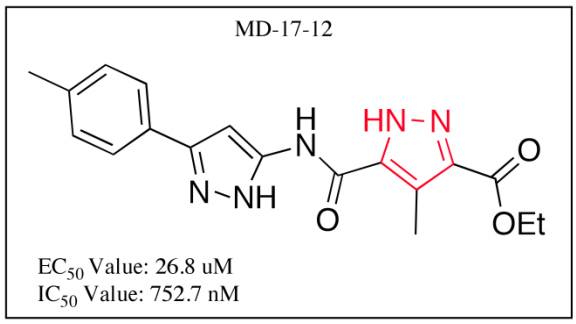


Figure 6. Biologically active microtubule depolymerizing molecule that was identified.

The current hit, MD-17-12 (Figure 6), caused depolymerizing activity within the A-10 cell line which is visualized in Figure 7. Compared to the normal A-10 cell, which was treated with DMSO and stained using the indirect immunofluorescent staining protocol, the cell treated with MD-17-12, showed about 35% depolymerization of the microtubules after 18 hours. While this is a quantifiable amount, increased activity would be necessary in order to consider this molecule a lead.

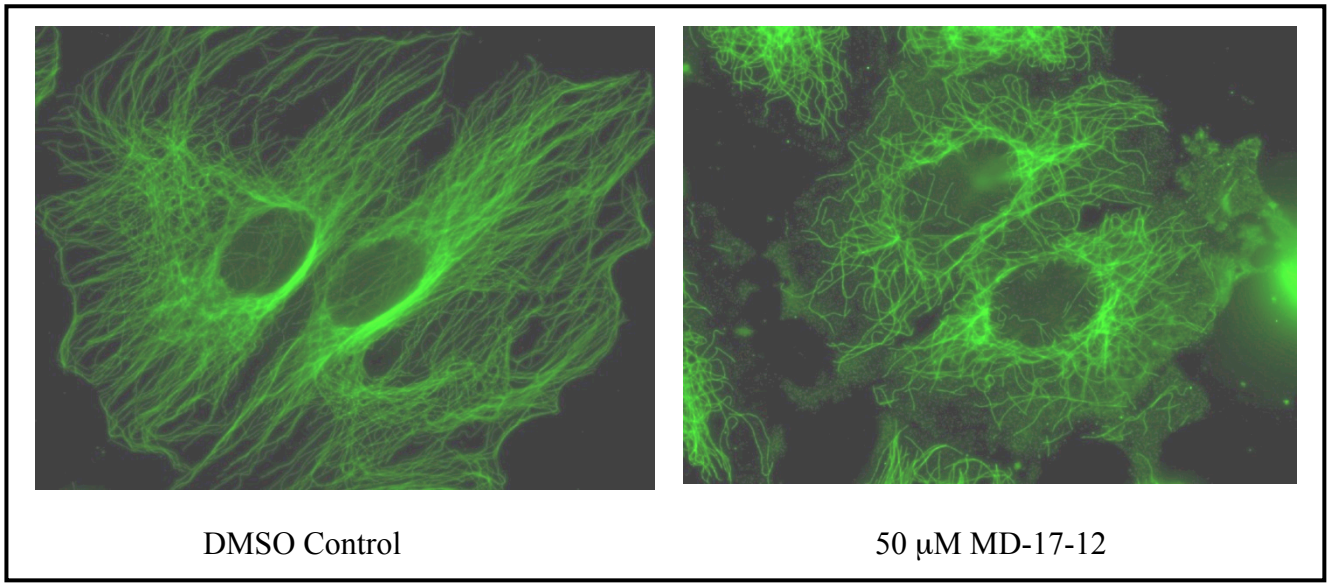


Figure 7. IF stained A-10 Cells with microtubule depolymerization after 18 hours of drug treatment.

In addition to identifing a hit pyrazole, Dr. Mooberry's Laboratory identified a more active small organic molecule. CB694, was identified from 10,000 small molecules in a cell-based screening assay (Figure 8), and acts as a tubulin-binding antimitotic agent.[21] CB694 proves to be effective in four drug resistant cancer cell lines. These cell lines include: HeLa, MDA-MB-435, SK-OV-3, and 1A9. In addition to relevant biological activity within the tumor cell assays, CB694 also showed antitumor activity *in vivo*, but was limited by its poor aqueous solubility. Using the same EDC coupling chemistry previously used to produce the

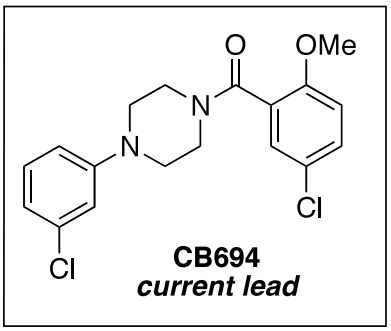
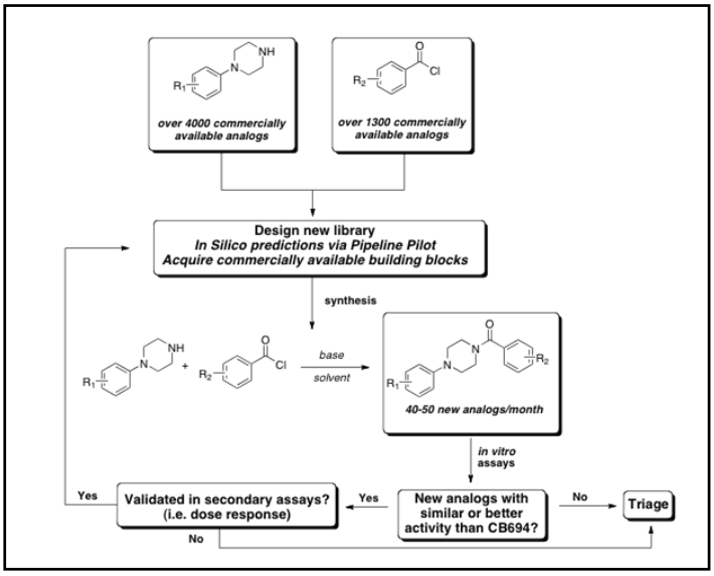


Figure 8. Current lead molecule with microtubule depolymerization activity.

novel pyrazole analogs, a small molecule library of CB694 analogs was created. This small molecule library was an iterative process and is outlined in Scheme 4. From over 4,000 commercially available piperazines, and 1,300 benzoic acid derivatives, a small molecule library of nine CB694 analogs was completed. The complete list of CB694 analogs is shown in Appendix B.



Scheme 4. Iterative process for the design, synthesis and analysis of CB694 analogs towards identifying a backup candidate to CB694.

Similar to the scheme of the pyrazole library synthesis, the scheme for the CB694 analogs follows an iterative process, in which active molecules play an important role in the structure determination and design of the next generation analogs. Additionally, CB694 and its analogs have also shown loss of cellular microtubules and the formation of abnormal mitotic spindles shown in Figure 9. In Figure 9, **(A)** depicts microtubules in untreated A-10 cells, **(B)** shows microtubule depolymerization after 18 hours of treatment with CB694, **(C)** depicts normal bipolar spindles of A-10 cells, while **(D)** shows multi-polar spindles after 18 hours of drug treatment at 37˚C.

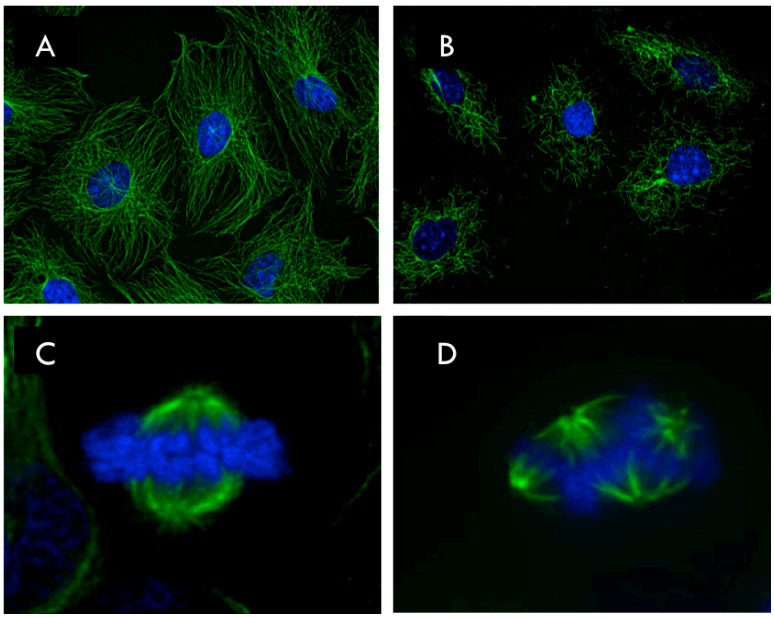


Figure 9. A-10 cells comparison between untreated and CB694 treated cells.

While developing the small molecule library of CB694 analogs, we discovered one molecule (MD-17-40), shown in Figure 10, which had relevant biological activity. This molecule had an EC_{50} value of 2.2 μM in the primary A-10 cellular assay. Additionally, MD-17-40 had an IC_{50} value of 81.5 nM in the MDA-MB-435 cancer cell line, with an overall EC_{50}/IC_{50} ratio of 27.2. MD-17-40 allowed us to evaluate the importance of the meta substitution of the *N*-phenyl piperazine for biological activity, however, it does not have better aqueous solubility than our original lead and will need further evaluation to gain more drug-like properties. Overall, the data obtained from the biological testing of the novel small molecule libraries will help identify a potential pre-clinical drug candidate.

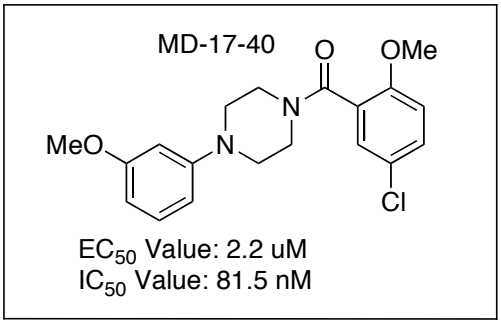


Figure 10. CB694 analog with relevant biological data.

CHAPTER 5: CONCLUSIONS

The new class of substituted pyrazoles produced a two hit molecules that will serve as a precursor for a medicinal chemistry research project within the Frantz laboratory. Although the data has not yet been received from Eli Lilly and Co., it is expected that relevant biological activity will be seen due to prevalent fact that this pharmacore model has shown prior biological activity and this library was designed based on the initial hit HA-135-167. Without data conformation, the impact of these novel pyrazoles on the drug discovery process is currently unknown, but with further testing and library development, this new class of pyrazoles could potentially lead to a pre-clinical candidate.

In addition to the biological activity expected from our collaboration with Eli Lilly, it can be concluded that these molecules remain important in the discovery and understanding of biological processes. Their overall effectiveness was demonstrated in the phenotypic assays completed in collaboration with Dr. Susan Mooberry. Additionally, it can be deduced that this novel class of pyrzaoles can act within the MDA-MB-435 cancer cell line via microtubule depolymerization and is validated by the primary phenotypic assay completed in the A-10 cell line. This tubulin-binding activity is very important in the determination of mechanism of induced apoptosis. While the biological data we received for both hit molecules MD-17-12 and MD-17-40 is a starting point, these molecules do not have enough activity at pharmaceutically acceptable concentrations to be considered a lead molecule or pre-clinical candidate. The results of this study allow for further development of a medicinal chemistry program within the Frantz laboratory, which can explore the possibility of converting these hits into a lead molecule with greater drug-like properties and the ability to overcome multi-drug resistance which could eventually lead to a pre-clinical candidate.

CHAPTER 6: IMPLICATIONS OF RESEARCH

This research serves as a backbone for a future medicinal chemistry program within the Frantz laboratory, with initial collaborations already in place. The biological activity demonstrated by these molecules will help develop a new scope of medicinal uses for such compounds, and elicit new methodology for obtaining other, highly substituted, pyrazoles. These small molecule libraries will serve as a base for the development of further small organic molecule libraries and will lead to a preclinical candidate that can serve in any of the tested areas.

REFERENCES

(1) Elguero, J.; Goya, P.; Jagerovic, N.; Silva, A. M. S. *Targets Heterocycl. Syst.***2002**, 6, 52-98.

(2) Bauer, V.J.; Dalalia, H.P.; Fanshawe, S.R.; Safir, S.R.; Tocus, E.C.; Benedict, A. *J. Med. Chem*. **1968**, 11, 981-984;

(3) Berer, J.C.; Iorio, L.C. *Annu. Rep. Med. Chem*. **1979**, 14, 22-30;

(4) Gandhale, D.N.; Patil, A.S.; Awate, B.G.; Naik, L.M. *Pesticides***1982**, 16, 27-28;

(5) Takagi, K.; Tanaka, M.; Murakami, Y.; Morita, H.; Aotsuka, T.; *Eur. J. Med. Chem.—Chim. Ther*, 1986, 21, 65-69;

(6) Pimerova, E.V.; Voronia, E.V.; *J. Pharm. Chem*. **2001**, 35, 18-21.

(7) Deng, X., Mani, N.; *J. Org. Chemistry*. **2008**, 73, 2412-2415

(8) Elguero, J.; *Comprehensive Heterocyclic Chemistry*;

(9) Katritzky, A. R., Rees, C.W., Scriven, E. F. V., Eds.; *Pergamon*: Oxford 1996; Vol. 5.

(10) Kost, A.N.; Grandberg, I.I.; *Adv. Heterocycl. Chem*. **1966**, 6, 347-429.

(11) Padwa, A. *1,3-Dipolar Cycloaddition Chemistry*, John Wiley & Sons: New York, 1984; Vol. I.

(12) Babinski, D.; Soltani, O.; Frantz, D. *Organic Letters*. **2008**, 10, 2901-2904.

(13) Seve, P.; Dumontet, C. *Lancet Oncol*. **2008**, 9, 168-175.

(14) Jiang, Q., *Neuron*. **2008**, 58, 681-693

(15) Cao, G.; *Current Alzheimer Research.***2007**, 4, 1567-2050.

(16) C.A. Lipinski; F. Lombardo; B.W. Dominy and P.J. Feeney; *Adv Drug Del Rev* **1997**, 23: 3–25.

(17) T. I. Oprea, A. M. Davis, S. J. Teague, P. D. Leeson; *J. Chem. Inf. Comput. Sci.* **2001***,* 41: 1308–1315.

(18) Leo A, Hansch C, and Elkins D.; *Chem Rev* **1971**, 6: 525–616.

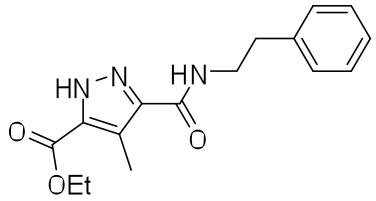
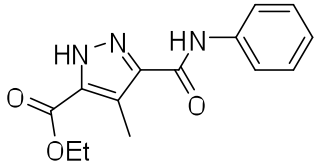
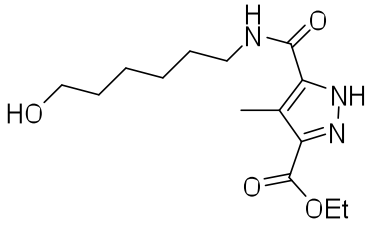
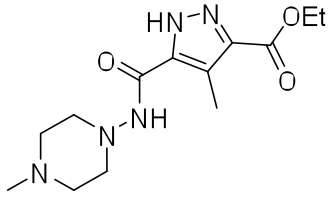
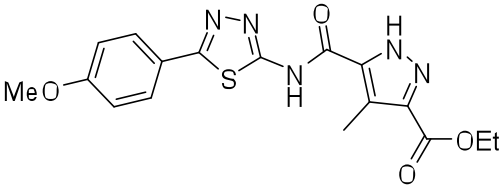
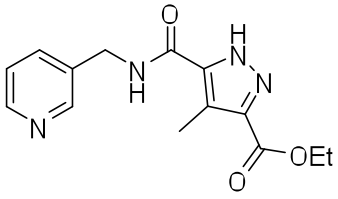
(19) Arup K. Ghose, Vellarkad N. Viswanadhan, and John J. Wendoloski; *J. Combin. Chem.* **1999**, 1: 55–68.

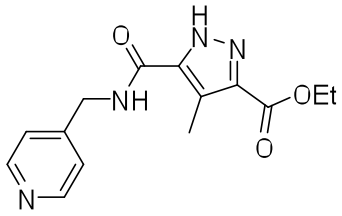
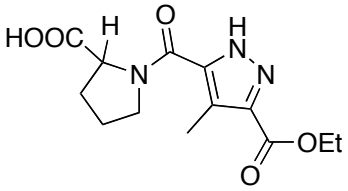
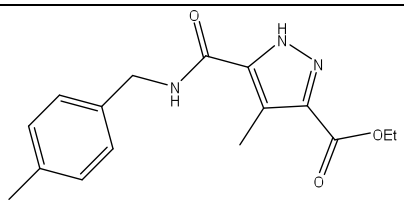
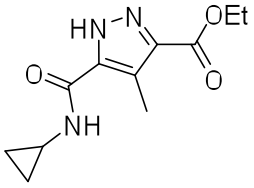
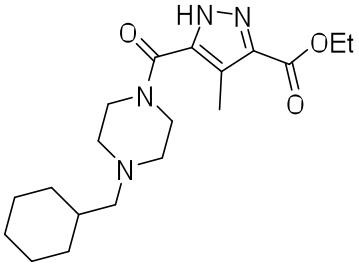
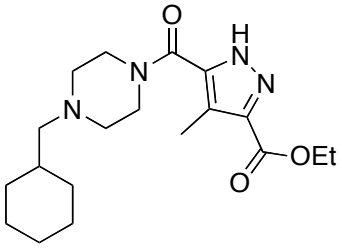
(20) For a similar Pd-mediated reaction with vinyl iodides, see: Peng, C.; Cheng, J.; Wang, J.; *J. Am. Chem. Soc*. **2007**, 129, 8708-8709.

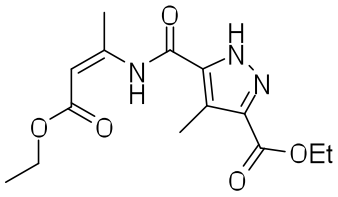
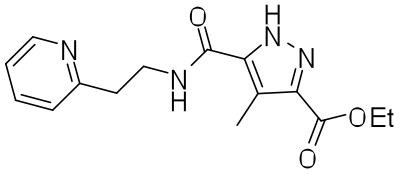
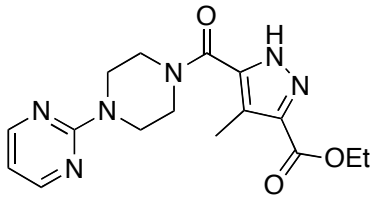
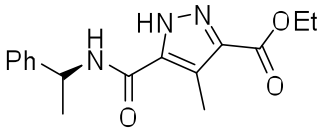
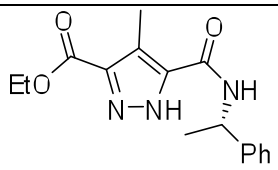
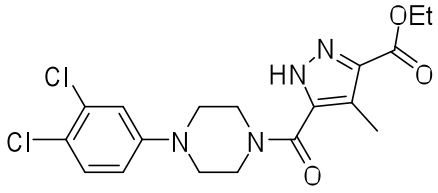
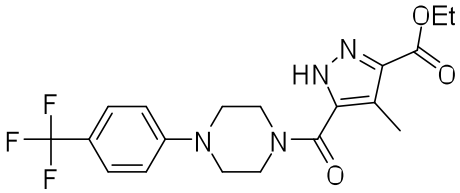
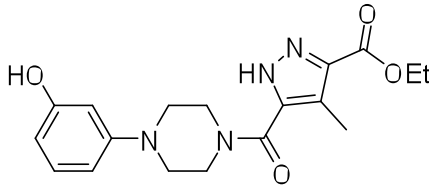
(21) Weiderhold K, Randall-Hlubeck D, Polin L, Hamel E, Mooberry S.; *Int. J. Cancer.* **2006,** 118: 1032-1040.

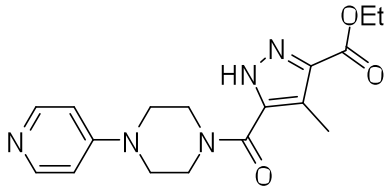
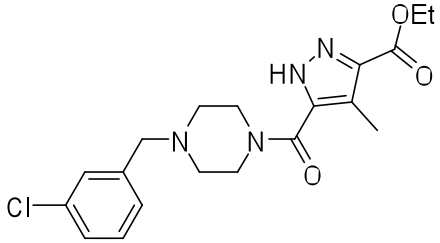
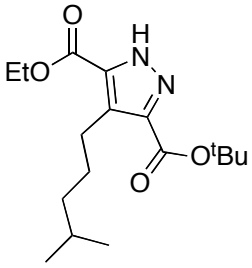
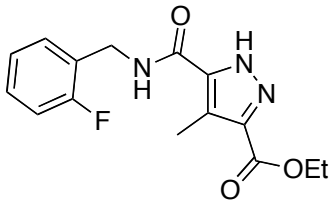
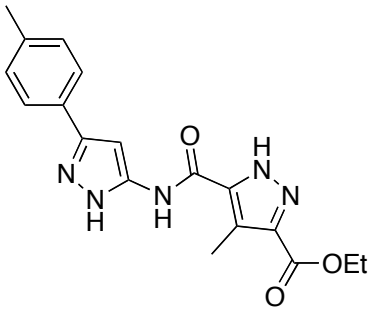
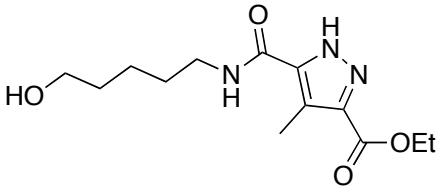
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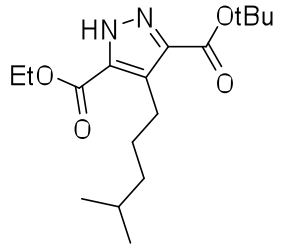
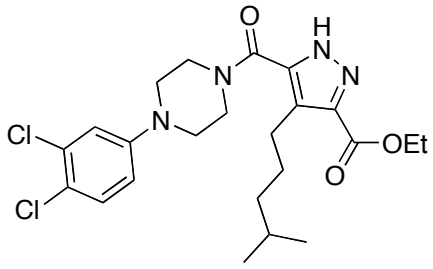
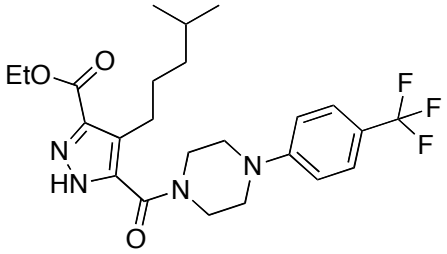
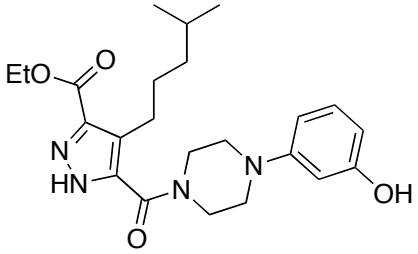
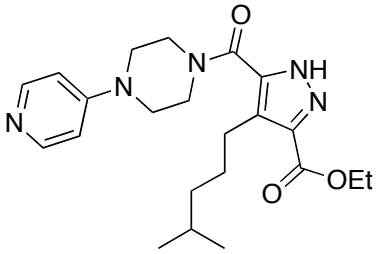
Small molecule library of 3,4,5-tri-substituted pyrazoles.

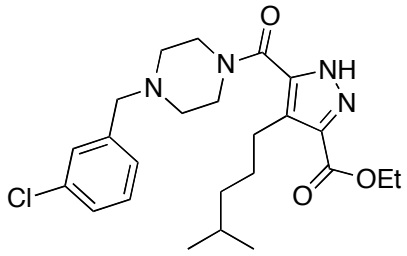
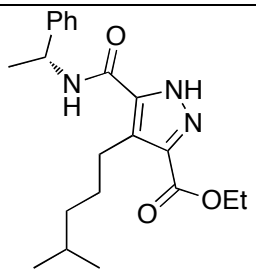
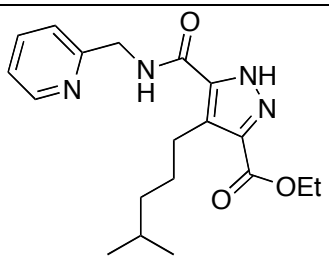
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MD-124-162		295.34
MD-124-163		387.41
MD-124-164		288.30

MD-124-165		288.30
MD-124-166		295.29
MD-124-167		301.34
MD-124-168		237.26
MD-124-169		376.84
MD-124-176		364.48

MD-124-178		311.33
MD-124-180		302.14
MD-124-182		346.38
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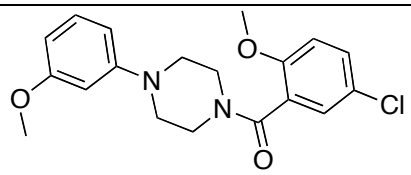
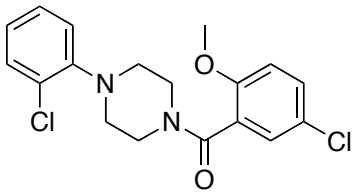
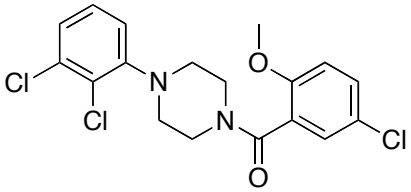
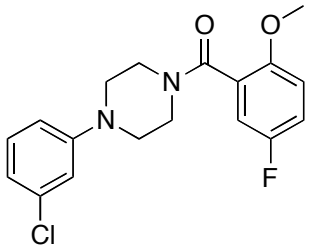
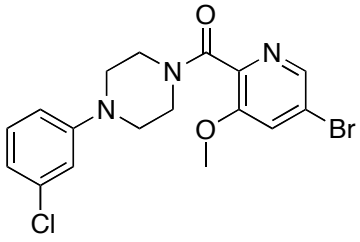
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MD-17-12		353.15
MD-17-14		283.32

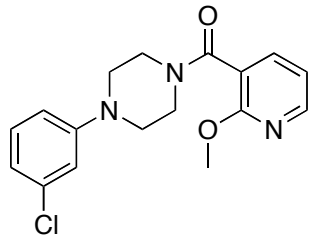
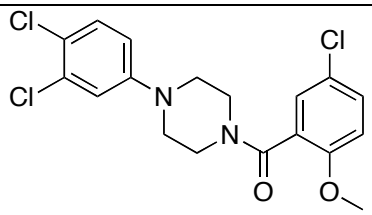
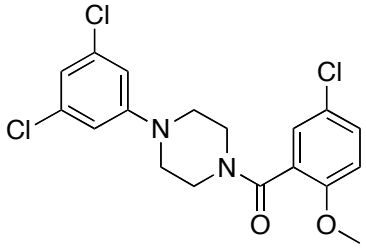
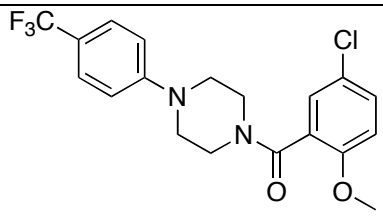
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MD-17-18		481.28
MD-17-20		480.52
MD-17-22		428.52
MD-17-24		413.51

MD-17-26		461.00
MD-17-28		371.47
MD-17-30		358.43

APPENDIX B

Small molecule library of CB694 analogs.

Reference	Structure	Molecular Weight (Da)
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MD-17-42		365.25
MD-17-44		399.70
MD-17-46		348.80
MD-17-48		410.69

MD-17-50		331.80
MD-17-54		399.70
MD-17-56		399.70
MD-17-58		398.81