



**Trip Report for
“9th Tetrahedron Symposium”
Berkeley, CA
July 22-25, 2008**

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Abstract: *The Tetrahedron Symposium is an annual conference specializing in challenges in organic and bioorganic chemistry. The majority of the speakers and attendees were from academia, but a sizeable number from industry also attended. Reviews of selected presentations and posters from the Symposium are provided here.*

“Discovery and Optimization of Diamine Analogues as Potent Inhibitors of Leukotriene A4 Hydrolase”

Andreas Cleve, et al., Bayer-Schering Pharma AG, Berlin, Germany and Berlex Biosciences, Richmond, CA

Leukotrienes are known mediators of inflammation. In particular, Leukotriene B₄ (LTB₄) is a known pro-inflammatory mediator, with the capability of inducing the amplification of several inflammatory diseases, such as irritable bowel disease (IBD) and others. It is known that LTB₄ is produced biosynthetically from LTA₄ via a hydrolase enzyme called LTA₄-h. The goal of this program was to find an inhibitor of LTA₄-h that would result in reduced inflammation as less of LTA₄ would be converted to LTB₄.

Since LTA₄-h has a known crystal structure, it was a logical candidate to perform a high-throughput screen to identify hits which could be optimized to a lead development candidate. Such an HTS screen identified compound **ZK-1** (Figure 1), which the first compound to have an IC₅₀ of <1 micromolar. Initial PK and PD data were also favorable, as **ZK-1** was almost entirely bioavailable (F = 92%) with low intrinsic clearance and an acceptable half-life. In this manner, many of the ‘tougher’ biological parameters were already met, and the traditional ‘starting points’ for medicinal chemistry activities, such as potency and selectivity at the target were the major points of optimization. This unexpected discovery helped to considerably speed up the overall timeline of this program using traditional medicinal chemistry SAR analogue techniques.

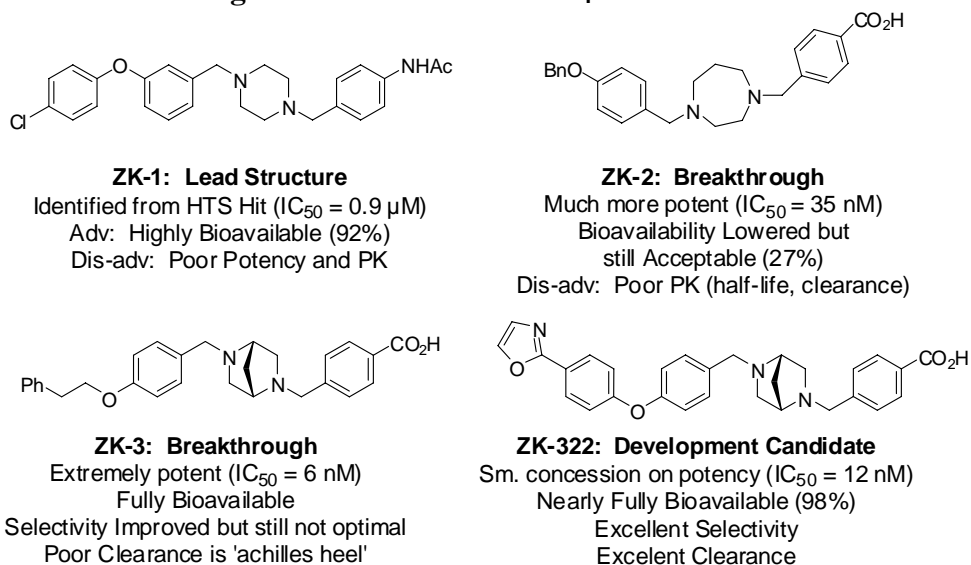
The SAR was ‘attacked’ simultaneously using three areas of diversity: at the left-hand phenolic ether, the central cyclic diamine and the right-hand acetamide. Although the author conceded this was done on a trial-and-error basis (no focus on specific analogues at first), the SAR was assembled fairly rapidly. It was also postulated that simultaneous optimization of the several areas of the overall molecule could result in the ‘ideal’ compound that incorporated the optimal functional group from each area (*i.e.*, a compound which ‘checks all the boxes’), provided the SAR behaved in an additive manner.

Results allowed the right-hand side of the molecule to be optimized first, where a *para*-carboxylic acid moiety was found to be best. From the initial HTS hit **ZK-1** (contained acetamide, IC₅₀ = 900 nM) the potency was routinely lowered to <100 nM. Many other functionalities (primary alcohol, methyl ester, methyl ketone, etc.) had IC₅₀'s >1 μM. It was also found that the acid moiety has its best potencies without other substituents on the right-hand phenyl ring, as adding a chloro or methoxy group typically decreased potency by a factor of 2-4-fold.

The central diamine moiety was tougher to optimize, as trial and error revealed that diamines which contained both nitrogens within the heterocycle were preferred over diamines with only one or no nitrogens as part of the ring. The resulting new inhibitor was **ZK-2**, a seven-membered diamine heterocycle with greatly improved potency, but at the expense of very poor PK, as the half-life, clearance and bioavailability all drastically regressed to a point where all previous advantages of **ZK-1** were eliminated. Fortunately, a new diamine which incorporated a [2.2.1]-bridged 1,5-diazabicyclic system, essentially a bridged piperazine, addressed these drawbacks. The analogue **ZK-3** was found which increased the potency to the single-digit nanomolar range

and restored bioavailability (which had decreased in **ZK-2**) to essentially a fully bioavailable compound. The selectivity of **ZK-3** was also improved (activity at other receptors was not performed on **ZK-2**) to micromolar levels, but not entirely acceptable (goal was >10 μM , essentially non-active) to eliminate potential off-target activity.

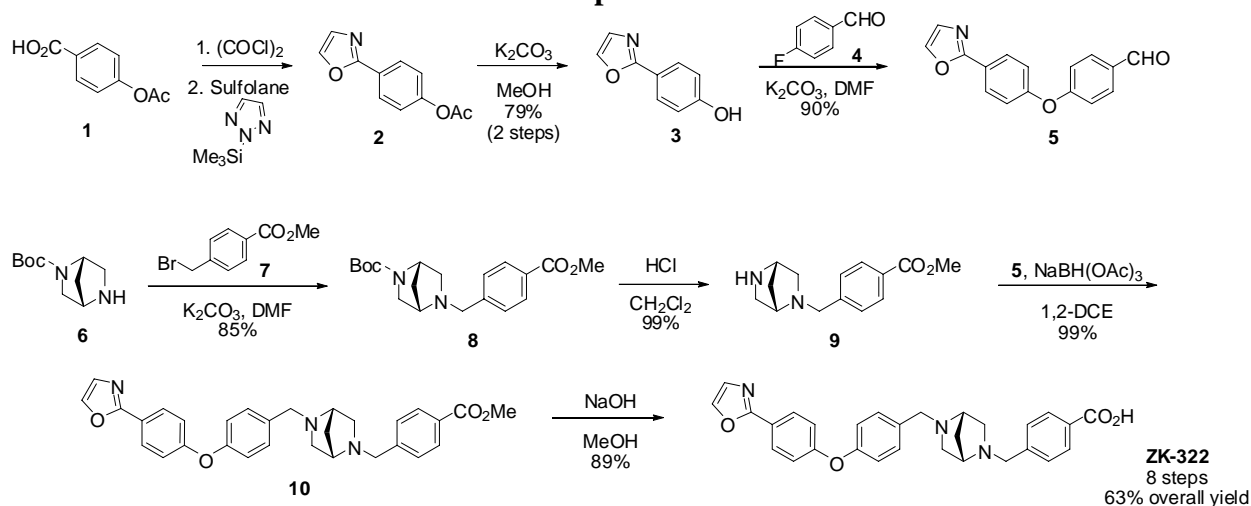
Figure 1. Leukotriene LTA₄-h inhibitors.



Development of the left-hand side of the molecule was intended to address the remaining flaws of **ZK-3**: selectivity and PK clearance. Various groups were substituted onto the left-hand phenol to slow the clearance. The 4-(2-oxazolyl)-phenoxy group (as incorporated into **ZK-322**) addressed the metabolic stability and also was able to be inactive at the other receptors. A modest decrease in potency was seen, but $\text{IC}_{50} = 12 \text{ nM}$ was still very acceptable. Thus, **ZK-322** was chosen at the development candidate for the LTA₄-h Inhibitor program.

The synthesis of **ZK-322** is short, convergent and high yielding (Scheme 2). 4-Acetylbenzoic acid (**1**) is converted to its acid chloride, then treated with sulfolane and (trimethylsilyl)triazole to afford the oxazole **2**. The acetyl group is removed and then an *ipso* substitution with 4-fluorobenzaldehyde is performed to afford fragment **5** in 72% yield over 3 steps. The opposite fragment is prepared from the 2-Boc-(1*S*, 4*S*)-2,5-diazabicyclo[2.2.1]heptane (**6**, skeleton prepared from *trans*-4-hydroxy-L-proline), which is reacted with 4-carbomethoxybenzyl bromide to afford intermediate **8**. The material is Boc-deprotected, then subjected to a reductive amination with intermediate **5** to afford the methyl ester of **ZK-322**, which is easily saponified to the desired drug candidate. The overall synthesis is 63% yield for 8 steps, and has been demonstrated on 500- to 1000-gram batches. At the time of the conference, **ZK-322** was slated to be entering clinical trials.

Scheme 2. Preparation of ZK-322.



“Synthesis of Natural Products of Biological Intrigue”

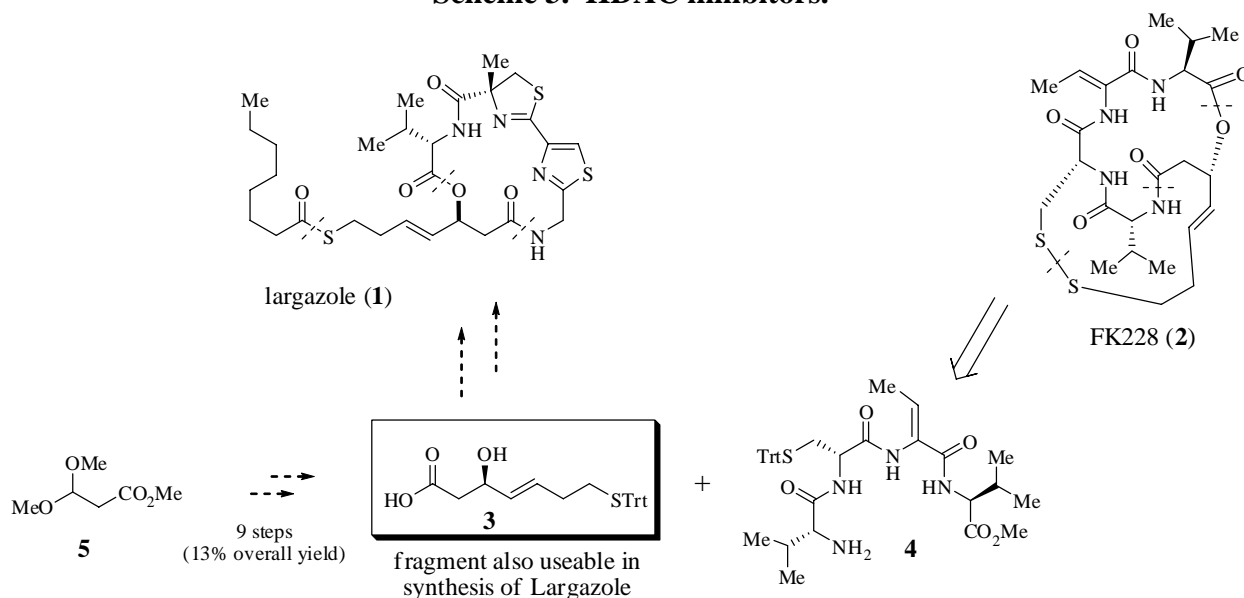
Prof. Robert M. Williams, Colorado State University, Fort Collins, CO

As Prof. Williams' research group specializes in the total synthesis of natural products, several examples of recent work in his group were presented. Of particular interest was the Williams' group synthesis of largazole, a macrocyclic peptide-containing natural product from the marine cyanobacterium *Symploca sp.* It is of particular interest due to its biological properties, as largazole has shown remarkable selectivity in a number of human- and murine-derived cancer cell lines, and has shown tumor growth inhibition of 2-10-fold vs. control as a histone deacetylase inhibitor (HDACi). HDAC inhibitors have been of interest in modern cancer therapy, and largazole may exhibit far lower toxicity levels than the first generation of HDAC inhibitors that have undesirable side effects. For these reasons and the inherent synthetic challenge of natural product total synthesis, academic researchers have aggressively researched synthetic approaches toward largazole.

Interestingly, the molecular structure of largazole was only elucidated and published in January 2008 by Luesch and coworkers (Taori, K., *et al.*, *J. Am. Chem. Soc.* **2008**, *130*, 1806), yet in the intervening six months, no less than three distinct total syntheses of largazole have appeared in the literature, one of which was published by the Luesch research group (Ying, Y., *et al.*, *J. Am. Chem. Soc.* **2008**, *130*, 8466). Presented here is Prof. Williams' approach (which recently appeared as Bowers, A.; West, N.; Taunton, J.; Schreiber, S. L.; Bradner, J. E.; Williams, R. M. *J. Am. Chem. Soc.* **2008**, *130*, 11219). For the third synthesis, see Nasveschuk, C. G., *et al.*, *Org. Lett.* **2008**, *10*, 3595.

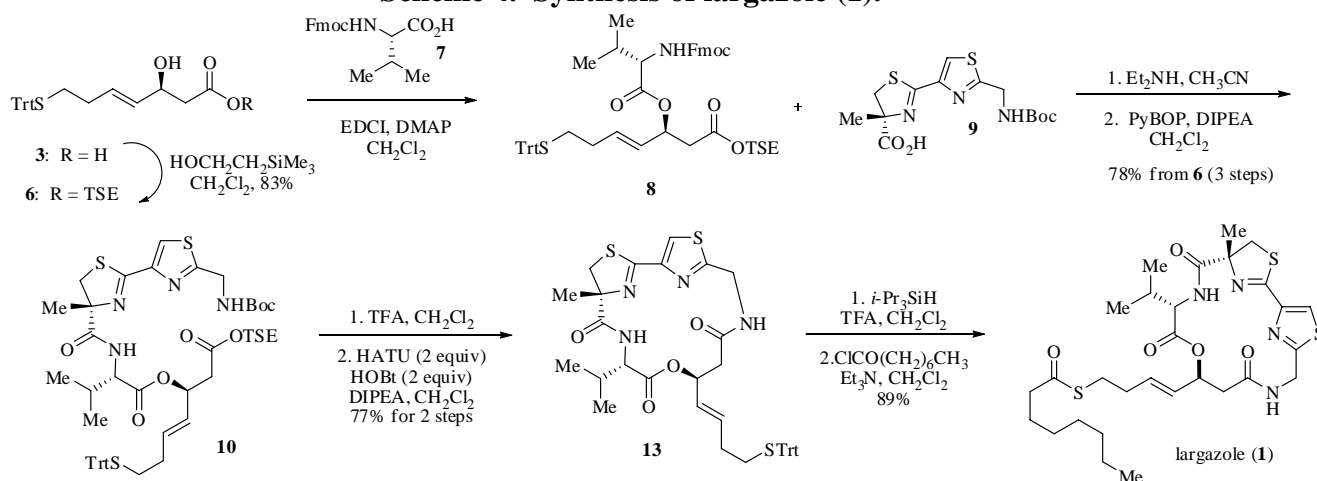
The Williams group has recently studied the total syntheses of other HDAC inhibitors, including FK228, another macrocyclic natural product which contains a disulfide bond (Johns, D.M., Greshock, T. J., Noguchi, Y., Williams, R. M. *Org. Lett.* **2008**, *10*, 613). Structural similarities to largazole were immediately noticed, and a key fragment (**3**) used in the synthesis of FK228 was also useable for the synthesis of largazole, which saved significant time in its overall preparation (Scheme 3).

Scheme 3. HDAC inhibitors.

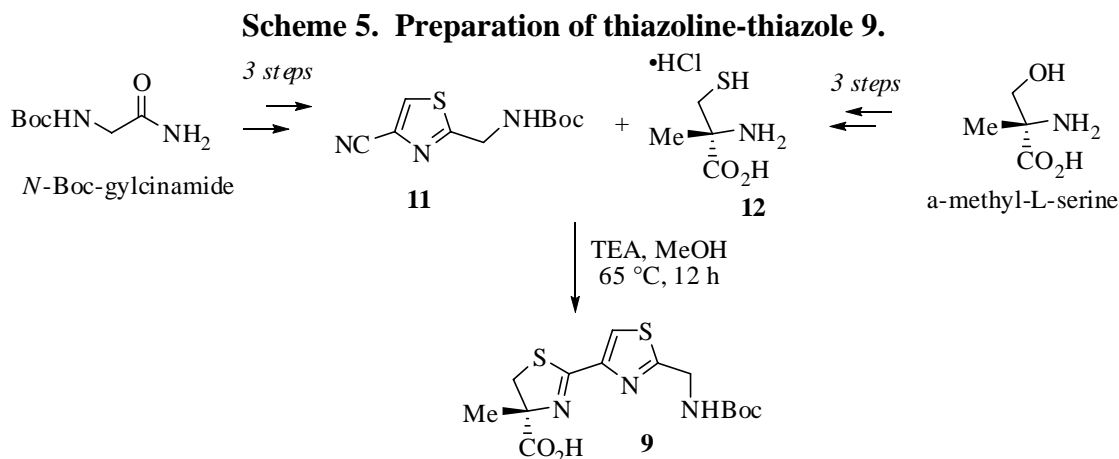


The synthesis of **3** is described in Williams' recent paper (*Org. Lett.* **2008**, *10*, 613) and is accomplished in nine steps from 3,3-dimethoxy propionate in 13% overall yield. The synthesis of largazole (**1**), when starting from fragment **3**, is surprisingly short and efficient (Scheme 4). The carboxylic acid moiety of **3** was protected as a 2-(trimethylsilyl)ethyl ester, and subsequently coupled with an *N*-protected valine residue to afford **8**. The amine was deprotected with diethylamine, and was immediately coupled with the thiazoline-thiazole fragment **9** (which prepared from 2-(*N*-Boc-aminomethyl)-4-cyanothiazole **11** and α -methyl-cysteine HCl salt **12**) to afford the amide **10**. TFA was used to perform a simultaneous de-protection of the terminal amine and ester functionalities of **10**, and subsequent coupling to the macrocycle **13** was accomplished at high dilution using HATU/HOBt. Finally, triisopropylsilane was used to cleave the trityl group and afford thiol **14** (Williams refers to this compound as "largazole thiol"), which was acylated with *n*-octanoyl chloride to afford largazole.

Scheme 4. Synthesis of largazole (1).



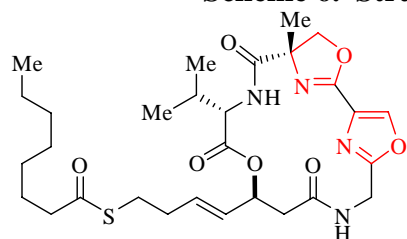
Interestingly, the thiazoline-thiazole **9** was prepared as shown in Scheme 5 from 2-(*N*-Boc-aminomethyl)-4-cyanothiazole **11** (prepared in three steps from *N*-Boc-glycinamide: Videnov, G., *et al.*, *Angew. Chem. Int. Ed. Engl.* **1996**, 35, 1503) and α -methyl-cysteine HCl salt (**12**, prepared in three steps from α -methyl-L-serine: Smith, N. D. and Goodman, M. *Org. Lett.* **2003**, 5, 1035).



Prof. Williams' group tested largazole *in vitro*, and was surprised to find comparatively weak IC_{50} and K_i values in comparison to naturally-occurring largazole. Closer investigation of the available HDAC inhibitors (*e.g.* FK228) revealed that these natural products all contained a disulfide bond, which has been postulated (Yurek-George, A., *et al.*, *J. Med. Chem.* **2007**, 50, 5720) to be cleaved *in vivo*. In the case of FK228, the resultant dithiol form binds to Zn^{+2} of the HDAC metalloenzyme, which results in the inhibitory effect. Therefore, Williams postulated that perhaps largazole itself is merely a prodrug for largazole thiol **14**. Subsequent testing of thiol **14** confirmed this hypothesis, as **14** was extremely potent ($K_i < 0.1$ nM) against several HDAC enzymes, and even more potent than FK228. Williams concluded that cellular lipases or esterases found *in vivo* are responsible for the cleavage of the octanoyl thioester moiety, resulting in the formation of active species **14**.

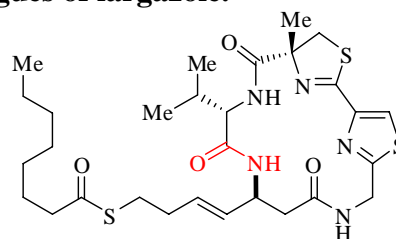
At the time of this presentation, the Williams group was also preparing analogues of largazole to develop a small SAR and hopefully determine which functional groups may be responsible for modulating the inhibitory effect (Scheme 6). In-progress analogues included replacement of the macrocyclic lactone linkage with an amide, essentially making the ring more peptide-like. This compound was tested and was shown to be as potent as largazole itself in the HDAC1 enzyme. However, attempts to prepare an *N*-methylated peptide-isostere were unsuccessful. Another interesting analogue which was prepared replaced the thiazoline-thiazole rings with oxazoline and oxazole rings, respectively. This synthesis was accomplished simply by preparing an analogue of fragment **9** with the analogous oxazoline-oxazole ring system and substituting this fragment in the total synthesis. Biological activity results for the oxazoline-oxazole analogue were still in testing.

Scheme 6. Structural analogues of largazole.



Replacement of thiazoline-thiazole rings with oxazoline-oxazole ring system

Compound synthesized; still in biological testing



Replacement of macro-lactone cyclization with amide (peptide-like) isostere

Compound synthesized and tested; nearly equipotent with Largazole at the HDAC1 Enzyme

“BCL-2 Family Inhibitors for Treatment of Cancer via Cell Apoptosis”

Stephen W. Fesik, Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL

Nearly all major pharmaceutical companies have competing research programs in the area of oncology, often investigating many different forms of cancer treatments simultaneously. Finding small-molecule anti-cancer compounds has been generally difficult, since often the cancer-causing agent (*i.e.*, that compound which causes cells to die) is protein-like, and drug companies have in turn focused their research on developing other protein-like molecules to interact with or inhibit the original protein.

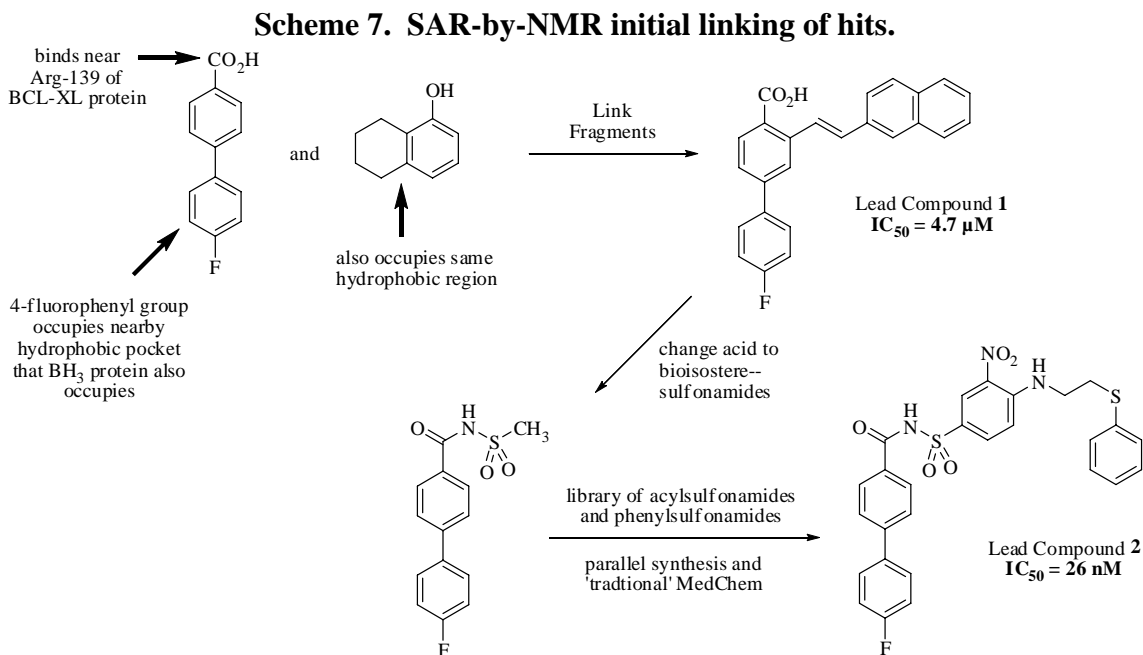
A family of proteins classified as BCL-2 (from B-Cell Lymphoma-2) have been identified as contributory in a number of different cancers, including melanoma (skin cancer), breast, prostate and small-cell and non-small-cell lung cancers. However, the BCL-2 family of protein has often proved resistant to conventional cancer therapies, due to the fact that some of the identified BCL-2 proteins can promote apoptosis (*i.e.*, programmed cell death) while others are *anti*-apoptotic. It has been found that these proteins can interact with each other (Kelekar, A.; Thompson, C. B. *Trends in Cell Biology*, **1998**, 8, 324; Huang, D. C.; Strasser, A. *Cell* **2000**, 103, 839) whereby the anti-apoptotic proteins are inhibited, and cell death of cancerous cells can occur over time. In other words, this type of apoptosis is beneficial to the patient. The pro-apoptotic proteins have been identified by Abbott researchers as the “BH3” region of the BCL-2 family of proteins (Petros, A. M.; Olejniczak, E. T.; Fesik, S. W. *Biochem. Biophys. Acta* **2004**, 1644, 83). Therefore, the goal of this research program was to find a small-molecule inhibitor (SMI) of the BH3 region in such a modulated way that unwanted inhibition of cell apoptosis is prevented.

The technique employed by the Abbott researchers is a proprietary method called “SAR by NMR” which has been previously utilized on several drug discovery programs. Briefly, small molecules are screened against the protein by observing ^{15}N or ^1H NMR shifts of amides present in the protein’s peptide chains, facilitated by ^{15}N isotopic labeling of the protein. When shifts are observed, it indicates that the small molecule is binding to that particular site of the protein. Therefore, it is necessary to know the *active* binding site of the protein to begin using this method to identify active molecules.

However, the novelty of SAR by NMR lies in that a second round of screening is also performed at a nearby peptide site, again by looking for changes in ^{15}N or ^1H NMR shifts. Therefore, when two 'lead' compounds have been selected, *i.e.*, screening at both sites has identified the functional groups which exhibit optimal binding, the fragments of the lead compounds can then be linked by traditional organic chemistry synthesis. Therefore, the only compounds which are prepared by the medicinal chemists have been pre-selected to be very efficacious at the binding site. This method therefore saves a drug discovery team a considerable amount of time and expense, since a focused set of analogues are the only ones prepared. Therefore, Abbott can design analogues to immediately undertake the problems of pharmacokinetics, drug metabolism, etc. which are also necessary to optimize before a candidate for development can be identified.

For this particular indication, a protein in the BCL-2 family called BCL-X_L has been identified as an anti-apoptotic protein, and is inhibited by the BH3 region of the separate pro-apoptotic BCL-2 proteins. Fortunately Abbott researchers have also elucidated a crystal structure of BCL-X_L and were able to selectively build in ('edit') the isotopic labels into the protein, thereby allowing the SAR-by-NMR technique to be used (Muchmore, S. W., *et al.*, *Nature* **1996**, 381, 335).

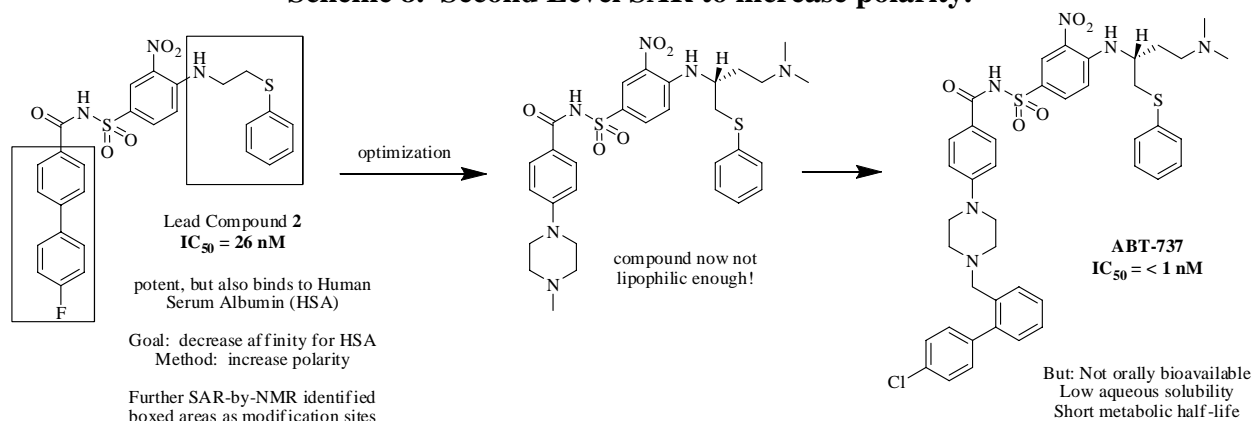
The results of the chemical screening found that 4-(4-fluorophenyl)benzoic acid was bound near an arginine residue (Arg-139) while 5,6,7,8-tetrahydronaphthalen-1-ol was bound to another site in close proximity, isoleucine-85. Additionally, it was found that the 4'-fluorophenyl group occupied a nearby hydrophobic pocket which is also occupied by 2 peptide residues (Asp-83 and Leu-78) from BH3 which have shown affinity for the BCL-X_L protein. These binding affinities were only in the sub-millimolar range (400 μM and 200 μM , respectively) but the linking of the two fragments typically has a multi-fold benefit to the binding affinity when an ideal-length linker is used to connect the fragments. So, with these interactions known and using the linking strategy, the lead molecule **1** was identified with micromolar efficacy against human cancerous tumor cell lines (basis for all IC₅₀ measurements) as shown in Scheme 7.



With a suitable starting point for SAR development, traditional medicinal chemistry techniques were then employed to further enhance the biological properties. Substitution of the benzoic acid moiety with a methylsulfonamide bioisostere provided an initial jump in potency, and a small library of acyl- and phenylsulfonamides was assembled. This was accomplished by using fairly standard parallel synthesis efforts, and the best compound from this library was Lead Compound **2**, which increased potency down to the sub-100 nanomolar range.

Therefore, the basic challenge of finding potent analogues was very rapidly addressed via a combination of the SAR-by-NMR technique, parallel synthesis and traditional medicinal chemistry efforts. However, further *in vitro* testing found that Compound **2** could also bind very tightly to human serum albumin (HSA), which would severely hinder the affinity of compound **2** to bind to the BCL-X_L protein. Further modifications in structure were needed to reduce the binding to HSA. NMR-based structural analysis compared the conformations of **2** when bound to BCL-X_L vs. bound to HSA. It was found that portions of the molecule differed in their immediate domains, e.g. when complexed with HSA, compound **2** was surrounded by lipophilic peptide residues, and thus the immediate groups/areas to modify **2** were identified. Knowing where to modify the molecule, the task was then to find which substituents best modified binding for HSA but without affecting affinity for BCL-X_L. It was found that basic groups increased the polarity of the compound and specifically a (dimethylamino)ethyl group was attached as a second appendage to the aniline moiety, while the fluorophenyl group was replaced with a substituted piperazine (Scheme 8).

Scheme 8. Second Level SAR to increase polarity.

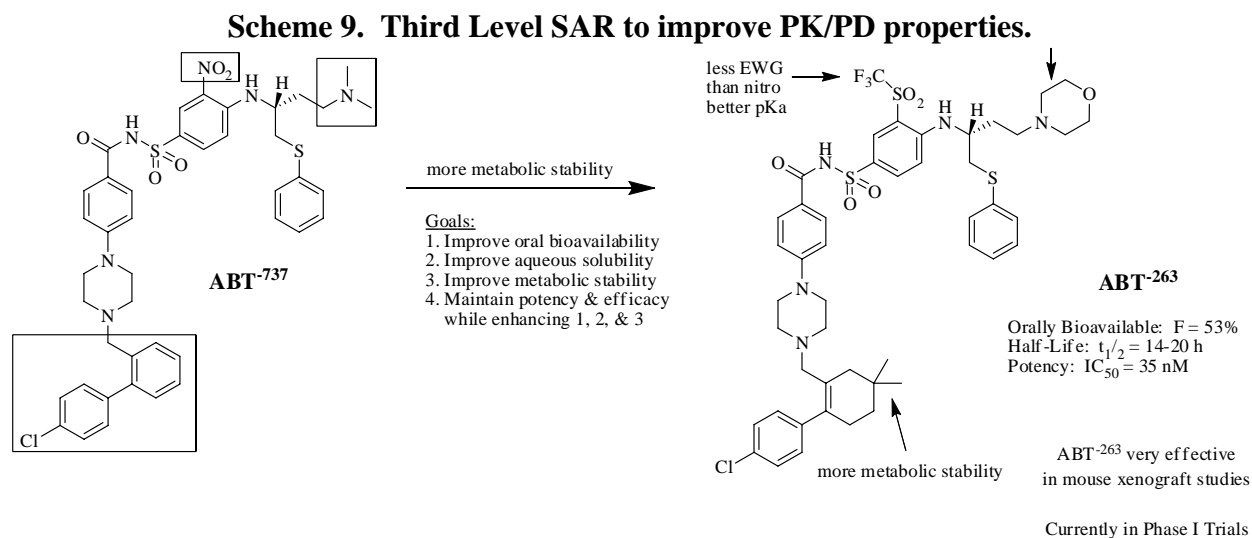


Further NMR study indicated a deep lipophilic pocket in the BCL-2 protein, which resulted in the addition of the 2'-(4-chlorophenyl)benzyl group off the piperazine ring, resulting in **ABT-737** as Abbott's first developmental candidate (Scheme 8). However, serious drawbacks to **ABT-737** still persisted: the compound has low aqueous solubility, was NOT orally bioavailable, and contains multiple sites of potential metabolism which would likely shorten the duration and half-life of this compound as a drug. These deficiencies were set to be changed by further SAR. Since **ABT-737** is already a very high molecular weight, improvement of parameters, such as aqueous solubility, were expected to be difficult to change. However, it was found that very small modifications could vastly modulate the overall properties of the molecule; thus the dimethylamino group was instead converted to *N*-morpholino in an effort to improve

metabolic properties. However, this was offset by decreases in cellular potency (likened driven by the new compound again binding to HSA). But the morpholino group could be retained if other changes were made to regain the cellular efficacy and/or oral bioavailability.

A series of compounds were prepared to replace the nitro group, for several reasons. It was assumed the pK_a of the acylsulfonamide was too low due to the powerful electron-withdrawing nitro group, plus it was feared that the nitro group could metabolize to potentially toxic groups (*e.g.*, primary anilines). Of the groups listed, trifluoromethylsulfonyl was shown to best mimic the nitro group for several parameters, while providing a modulating effect on pK_a (Park, C. M., *et. al.*, *J. Med. Chem. ASAP Alerts*, dated October 10, 2008). Finally, the 4-chlorobiphenyl moiety was another suspected site of metabolism. The Abbott researchers decided that cycloalkenes would be good mimics since the rigidity of the ring system would be maintained (as long as the double bond was located between the methylene and the second phenyl ring) while being much more stable metabolically. Again, several different ring sizes were examined and the 6,6-dimethylcyclohex-2-enyl group was optimal, though it was noted that nearly all ring sizes were fairly well tolerated.

When all of these modifications were incorporated, the resulting candidate compound was **ABT-263** (Scheme 9). This compound combined robust antitumor activity with key improvements in pharmacokinetics (PK) and pharmacodynamics (PD). Most importantly, this compound was screened against 67 different human cancerous cell lines, and has demonstrated remarkable effects on several specific cell lines, including human small-cell lung cancer.



A recently published xenograft study on mice has recently shown not only inhibition of tumor growth, but that *complete regression of tumors* is possible using **ABT-263**. It is also important to note that this study was performed on tumors which had grown to various states before administration of **ABT-263**, in an attempt to simulate the possibilities of tumor detection at early, moderate and advanced stages in eventual human patients. In certain test animals, tumors did not re-grow even after administration of **ABT-263** was stopped. Additional studies on other cancer cell lines, such as multiple myeloma cells, did see some animal subjects re-grow their tumors after administration was stopped, though to roughly 50% size of the original tumor. Such

encouraging *in vivo* test results allowed Abbott to ‘fast-track’ **ABT-263** into clinical trials, where Phase I studies are currently in progress.