



Trip Report for
“235th ACS National Meeting”
April 6–10, 2008

Ping Chen, Ph.D.; Jolicia F. Gauuan, Ph.D.; Junho Maeng, Ph.D.;
Gregory Martin, Ph.D.; Matthew Voss, Ph.D.

Medicinal Chemistry and Discovery R&D, Chemistry Departments

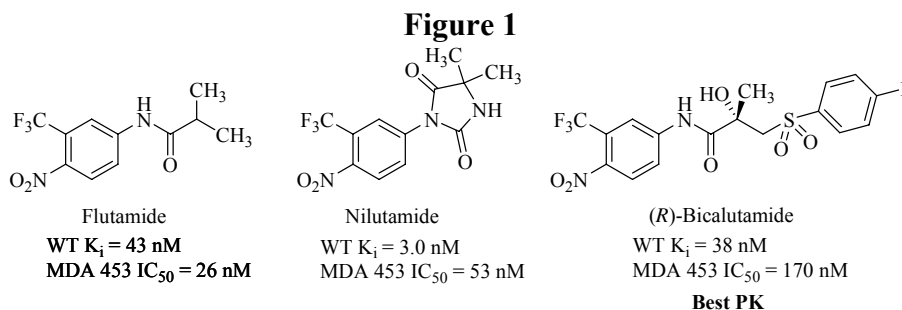
Abstract: *The 235th ACS National Meeting was held in New Orleans, LA from April 6–10, 2008. The following report is a collection of summaries from oral and poster presentations attended and viewed by the contributors during the meeting.*

“Design, Synthesis and Biological Profile of BMS-641988: A Novel AR Antagonist for the Treatment of Advanced Prostate Cancer”

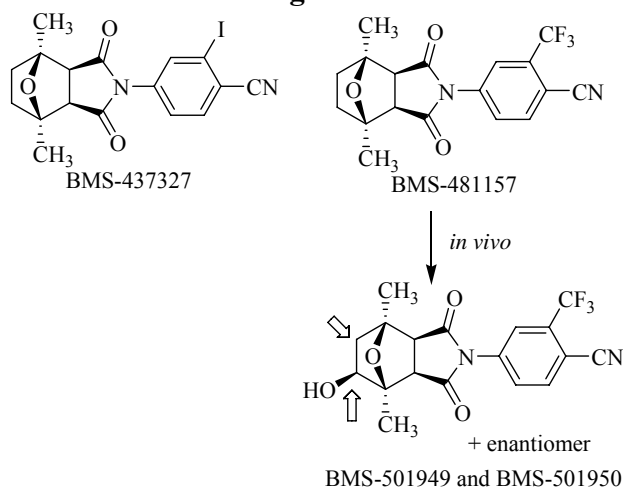
Mark Salvati, Bristol-Myers Squibb, Princeton, New Jersey

Prostate cancer (CaP) is the second leading cause of cancer fatality in men in the United States. In 2007, roughly 200,000 U.S. and 1.5 million worldwide new cases were diagnosed (27,000 deaths due to prostate cancer in the U.S.). The current standard of care for those diagnosed with advanced CaP is androgen deprivation therapy, which consists of surgical or chemical castration or by treatment with an antiandrogen. Initially, this mode of treatment is effective, but after 18 months approximately 50% of patients progress to “androgen-independent” disease. It has been found that the androgen receptor signaling pathway eventually gets reactivated in these cases. Dr. Mark Salvati explained in this presentation efforts at BMS to elucidate new androgen receptor (AR) antagonists as therapeutic agents. He commented on the initial lead, BMS-591305, and how it led to the discovery of BMS-641988 (SAR, molecular modeling and synthetic details were discussed).

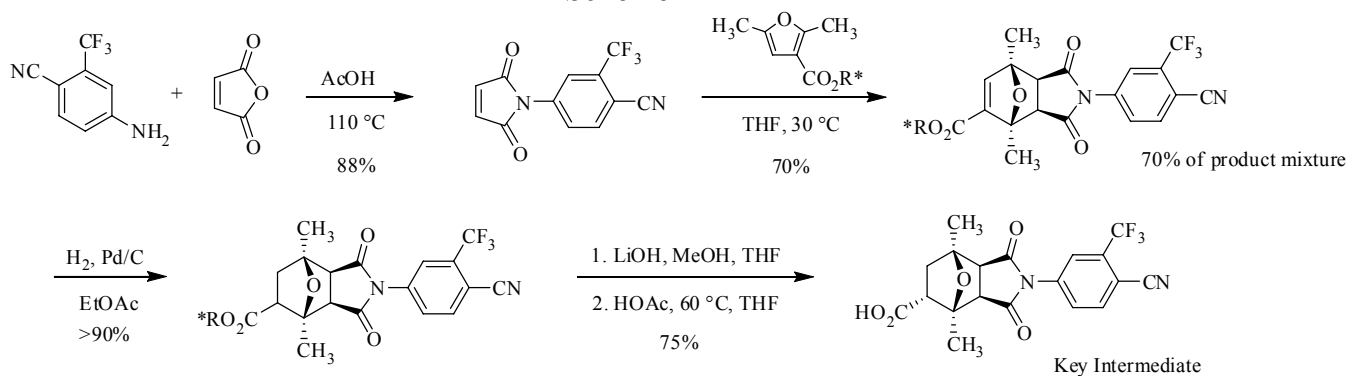
AR activation by testosterone and dihydrotestosterone is necessary for the proliferation and apoptosis prevention of prostate cells, therefore a full wild type and mutant type AR antagonist was desired. Current treatments for CaP include flutamide, nilutamide and (*R*)-bicalutamide (Casodex[®]) shown in Figure 1. The data shown are wild type AR K_i (the ability of program compounds to displace dihydrotestosterone) and MDA 453 IC_{50} (activity against a breast cancer cell line). Although Flutamide and Nilutamide have better efficacy, (*R*)-bicalutamide has by far the best PK profile and is most effective. However, BMS sought to develop a candidate with a full antagonist profile, with increased potency and increased activity against multiple mutant type AR variants.



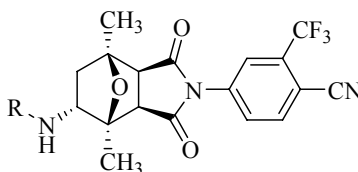
The program lead compounds included BMS-591305 and BMS-437327 shown in Scheme 2. BMS-437327 was dropped due to the liability of the iodo group on the phenyl ring and was replaced with a 3-trifluoromethyl group similar to the structures in Scheme 1. However, after further testing it was found that the metabolites of BMS-481157 (BMS-501949 and BMS-501950) were responsible for *in vivo* activity. Due to the CNS toxicity of BMS-501949 and BMS-501950, BMS began to explore the SAR of groups at the endo and exo positions of the six-membered ring of the fused system (shown by arrows in Figure 2). It was found that disubstitution at either carbon showed a loss in potency; but monosubstitution, especially an *endo* amine functionality, resulted in strong AR antagonism.

Figure 2

Synthesis of the main scaffold is shown in Scheme 1. Reaction to form the *N*- arylmaleimide proceeded in excellent yield as does the subsequent Diels-Alder reaction to produce the adduct containing the chiral auxiliary. The desired enantiomer was 70% of the Diels-Alder product mixture, and this material was then hydrogenated, followed by removal of the chiral auxiliary (all in good yields). An alternate route to the key intermediate acid was also employed using porcine pancreatic lipase to separate the enantiomeric mixture.

Scheme 1

A multitude of analogues were synthesized and tested, including esters, amides, and carbamates as well as reversed amides, reversed carbamates, sulfonamides and ureas. In general, the ester derivatives suffered from poor metabolic stability and moderate exposure *in vivo*, while amides were metabolically stable but showed poor exposure. Reversed amides, reversed carbamates and ureas were accessible via Curtius rearrangement from the acid intermediate. Table 1 shows some representative data for selected analogues. The K_i value represents binding affinity to the AR while the IC_{50} measures the ability of the compound to initiate transactivation in a whole cell.

Table 1

R	MDA 453 K _i (nM)	MDA 453 IC ₅₀ (nM)	Plasma Exposure (2 h post-dose, μM)
-COEt	2.9	23	9.1
-CO ⁱ Pr	10	35	2.9
-CO ₂ ⁱ Pr	3	9.9	165
-CONH(CH ₃) ⁱ Pr	15	71	18
-SO ₂ N(CH ₃) ₂	9	3	2.2
-SO ₂ CH ₃	3	13	4
-SO ₂ Et (BMS-641988)	4.8	14	3.6
-SO ₂ Ph	4	2	2.5

The reversed amides and carbamates showed good *in vitro* potency, although the latter suffered from high protein binding, therefore requiring higher doses for adequate exposure. Ureas required high plasma concentrations and suffered from low solubility, while the sulfamides were potent *in vitro* but not *in vivo*. The sulfonamides, however, showed good efficacy *in vitro* and *in vivo*, with very potent antagonist activity, requiring very low exposures. The lead compound chosen was BMS-641988, the ethylsulfonamide, due to its good PK/PD and *in vitro* safety profile. Also, in a CWR-22LD1 rodent xenograft (human tumor) study, BMS-641988 performed better than the lead clinical antiandrogen bicalutamide (Table 2). Most importantly, this compound offers >100× better binding to AR versus dihydrotestosterone. Dr. Salvati announced that BMS-641988 is now being evaluated in multiple ascending dose Phase I clinical trials in CaP patients.

Table 2

<i>In Vitro</i> Activity	BMS-641988	(<i>R</i>)-Bicalutamide
AR Binding (K _i)	1.7 ± 0.6 nM	38 ± 5 nM
MDA 453 Transactivation (IC ₅₀)	16 ± 3 nM	173 ± 67 nM
CWR22rv1 Transactivation (IC ₅₀)	270 ± 160 nM	3500 ± 650 nM

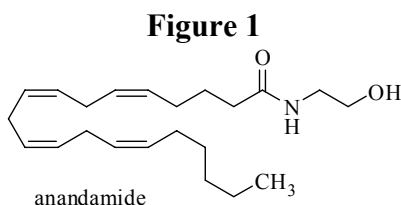
“Propylpiperidine-ketooxazole Based Inhibitors of Fatty Acid Amine Hydrolase”

J. G. Breitenbucher, Johnson & Johnson Pharmaceutical Research and Development L.L.C., San Diego, California

This presentation during the General Oral Session was given by Dr. Guy Breitenbucher, a scientist at J & J, who outlined recent progress in finding an efficient inhibitor of fatty acid amine hydrolase (FAAH) in a joint effort between J & J and the Scripps Research Institute in La Jolla.

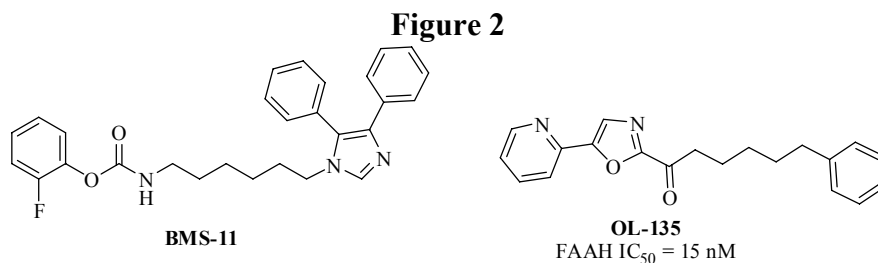
Neuropathic pain results from dysfunction or injury of the central nervous system, and is a disease that has led to a \$30 billion worldwide market for efficacious treatments. Some

medicines commonly used include Topamax, Ultram, Duragesic (fentanyl) and common agents like Tylenol and Motrin. It has been found that agonists of the CB1 cannabinoid receptor have shown promise in the treatment of emesis, appetite stimulation and neuropathic pain. CB1 agonism, however, can lead to severe CNS side effects such as motor impairment and dysphoria. In 1990, the CB1 receptor was successfully cloned and the discovery of the first endogenous CB1 agonist, anandamide (Figure 1), soon followed.

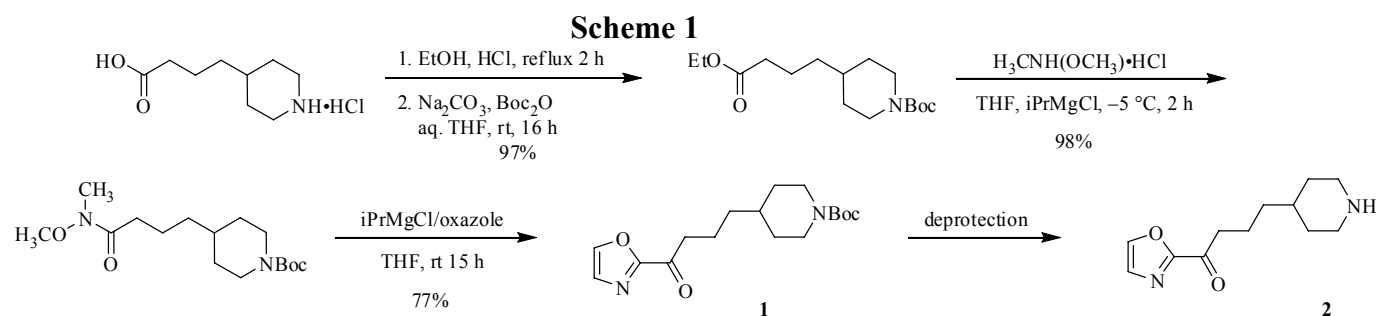


Several other lipid signaling agonists were also identified, with some exhibiting analgesic and anti-inflammatory properties. One enzyme that hydrolyzes these signaling molecules is fatty acid amine hydrolase (FAAH), which led researchers to consider the possibility of controlling the cannabinergic signaling by modulating endogenous agonists by blocking FAAH action. Indeed, animal studies of pain and anxiety have shown that selective FAAH inhibitors show efficacy without motor impairment associated with global CB1 receptor agonism.

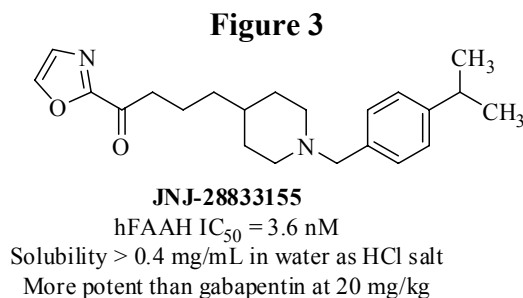
Two molecules discovered that inhibit FAAH are shown in Figure 2 (BMS-11 and OL-135). Scientists at Scripps Research Institute discovered that OL-135 is a competitive and fully reversible inhibitor of FAAH with an IC_{50} of 15 nM. However, this pyridyl-oxazole compound has a poor solubility and PK profile and is difficult to derivatize. Breitenbucher and colleagues at J & J set out to improve the drug properties of this chemotype.



In a first attempt to steer the SAR of this scaffold in the right direction, the pyridine group was removed. The resulting des-pyridyl derivative of OL-135 provided FAAH IC_{50} of 43 nM, showing that the loss of the pyridine does not affect the activity drastically. Molecular modeling suggested that compound **1** (Scheme 1) should provide potent inhibition of FAAH and would allow a reasonable starting point for preparing analogues.



Ketooxazole **1** had good activity (hFAAH IC₅₀ = 2.0 nM), but suffered from high clearance as would be expected for a *tert*-butyl carbamate. Removing the Boc group improved the clearance observed but the activity also dropped drastically (IC₅₀ = 5,700 nM). Many analogues were made by varying the substitution on the piperidine nitrogen, but most suffered from high clearance and hERG liabilities. However, several substituted benzyl derivatives that exhibited high clearance were free of hERG problems, showed no motor impairment in animal studies, had good solubility (as the HCl salts) and looked good in neuropathic pain models. The lead compound chosen from this series was JNJ-28833155, shown in Figure 3.



This compound was prepared by reductive amination of *p*-isopropylbenzaldehyde with the piperidine scaffold **2** in good yield. With good solubility, efficacy and reasonable clearance it progressed into nerve ligated rat models. Administered intraperitoneally, JNJ-28833155 fully reversed the tactile allodynia with ED₅₀ = 7.3 mpk. Additionally, this compound was more potent than Gabapentin in similar tests at 20 mpk. Breitenbucher said that favorable results from other studies on these types of compounds continue to move the series forward.

“Overview of Solubility in Drug Discovery; Impact, Measurement, and Structure Design”

Edward H. Kerns, Wyeth Research

The presentation was an excellent primer on the importance of solubility as a property to manage the drug discovery process. Solubility is one of the properties that affects drug exposure *in vivo* and is now recognized to affect most early-stage discovery experiments (HTS and binding experiments, enzyme and receptor assays, cellular assays) as well as later *in vivo* assays (animal and human).

Solution conditions (e.g. pH, water vs. buffer, co-solvent, co-solute, temperature), solid form (e.g. crystalline polymorph, amorphous, pre dissolved in solvent), and structural properties (e.g.

intrinsic solubility) are some of the factors that affect solubility properties. Examples were provided to demonstrate this impact on biological assays, absorption, and formulation.

Given that discovery experiments are usually conducted in aqueous media, either in high throughput screening or benchtop assays, it is ideal to have compounds that are completely in solution throughout the assay to affect full exposure to target, as precipitation of test compounds from the assay medium can result in “false data”. Low solubility *in vitro* causes confusion and lost productivity as poor HTS screen reliability results in low hit rate and the possibility of overlooking leads is increased, IC₅₀ curve shifts incorrectly reflect poor activity resulting in deprioritization of compounds, inconsistent trends from assay to assay result in confusion, toxicity may be under-estimated or stability may be over-estimated.

For most therapies, oral delivery and intestinal absorption are preferred, again underlining the importance of compounds with aqueous solubility. A compound with low aqueous solubility can result in incomplete absorption, which can lead to low exposure and low apparent efficacy (which often can lead to deprioritization of an otherwise useful compound), low bioavailability (%F) and non-linear PK making dose estimation unpredictable.

Exposure and PK in animals and humans is established by *in vivo* dosing experiments. It is therefore important that the most soluble active compounds are selected and the best dosing vehicle is used, otherwise ineffective dosing underestimates intrinsic efficacy.

Some strategies to improve solubility were discussed. Intrinsic solubility of a drug molecule can be improved by adding polar groups, reducing molecular weight, removing lipophilic groups, and adding H-bonding functionality. The pKa effects on solubility can be improved by adding ionizable groups. Ionizable or polar groups in a molecule could be masked as pro-drugs which are cleaved by intestinal hydrolases prior to absorption. Salt form and buffer pH affect dissolution rate and solubility of salts, respectively.

Some benchmarks for good solubility were cited:

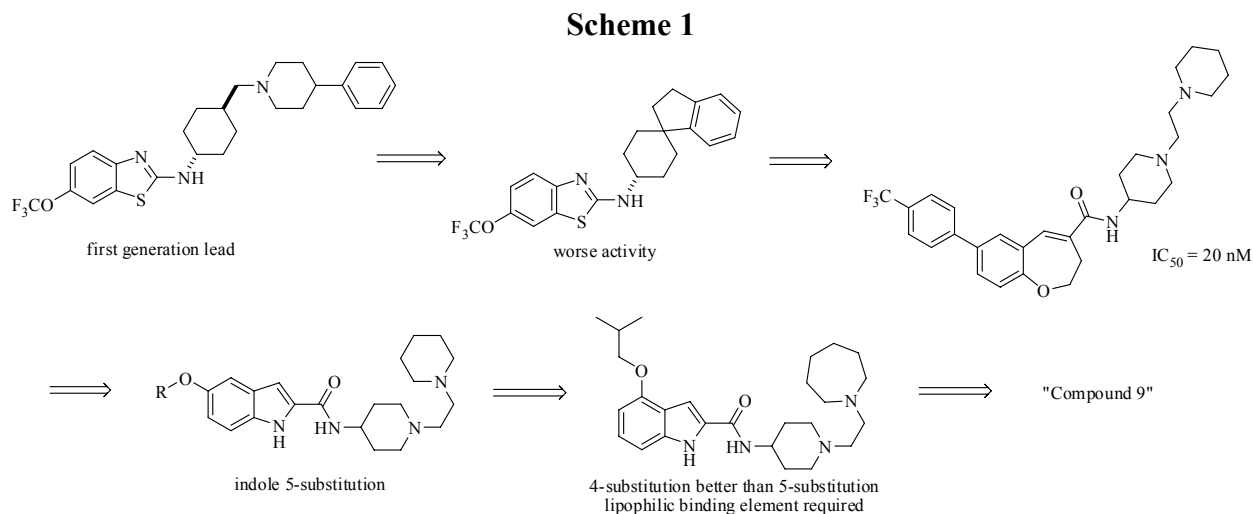
- Kinetic solubility of commercial drugs: 87% had solubility $\geq 65 \mu\text{g/mL}$ 7% had solubility $\leq 20 \mu\text{g/mL}$ (C. Lipinski, *et al.*, *Adv. Drug Deliv. Rev.* **1997**, *23*, 3–25)
- Minimum acceptable solubility (from “Minimum Absorbable Dose² for 1 mg/kg clinical dose and average permeability) $51 \mu\text{g/mL}$ (C. Lipinski, *J. Pharm. Tox. Meth.* **2000**, *44*, 235–249)
- Biopharmaceutics Classification System (BCS) Highest dose form soluble in 250 mL at pH 1–8:1 mg/kg dose = $280 \mu\text{g/mL}$ solubility (www.fda.gov/cder/guidance/2062dft.pdf)

“CCR2/CCR5 Antagonists: A new approach for the treatment of autoimmune diseases”

Wolfgang Miltz, Novartis

Novartis’ lead optimization program to develop a dual CCR2/CCR5 antagonist with application to multiple sclerosis was presented. The benzothiazole lead was first optimized through a series of modifications as shown in Scheme 1. Replacing the 4-aryl-piperidinyl moiety with a dihydrospiroindene moiety resulted in loss of activity, which suggested that the basic nitrogen may be required. Replacing the benzothiazole core with either a dihydrobenzooxepine or

benzimidazole motif gave reasonably active compounds. The 5-alkoxy indole series led to the 4-indole series which were found to have better activity.



SAR data along with a homology model designed for the project suggested that maintenance of a basic nitrogen interaction with Glu-291 along with lipophilic interactions at the tail region are required for binding activity. A "compound 9" was developed (structure not disclosed) that showed great promise, as demonstrated by the data shown in Table 1.

Table 1

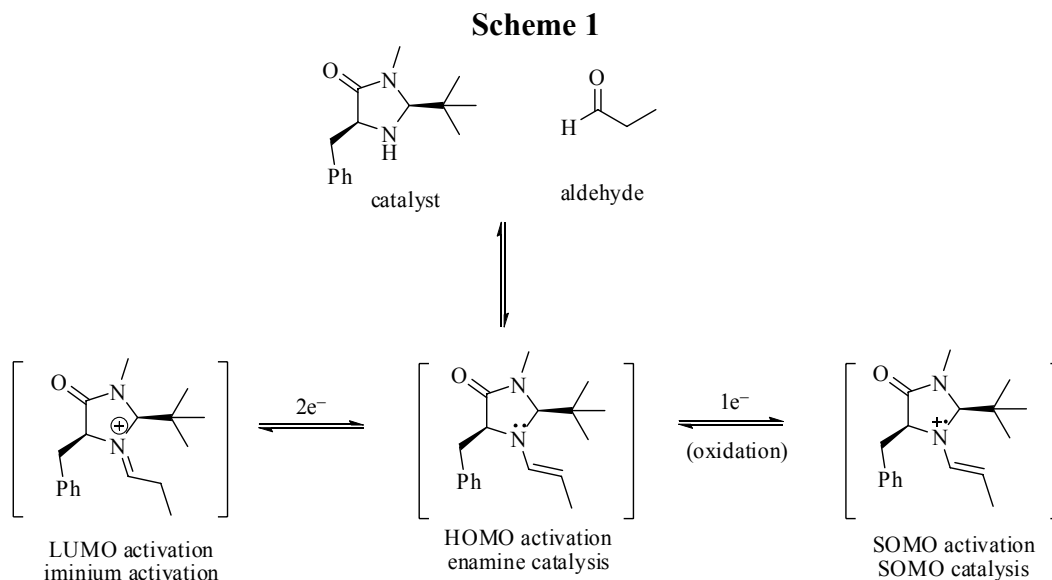
"Compound 9"		IC_{50} (nM)	ID_{90} (nM)
CCR2 occupancy	Human	13	77
	Monkey	8	116
	mouse	9	52
CCR5 occupancy	Human	33	305
Selectivity	h/m/r-CCR1 vs. hCCR4/4/7/9	>10000	
CCR2 binding	human	1	
	monkey	5	
	mouse	14	
	rodent	28	

Some issues that remained to be addressed with this compound include phospholipidosis, which was observed in a 2-week dosing toxicology study, and tissue retention.

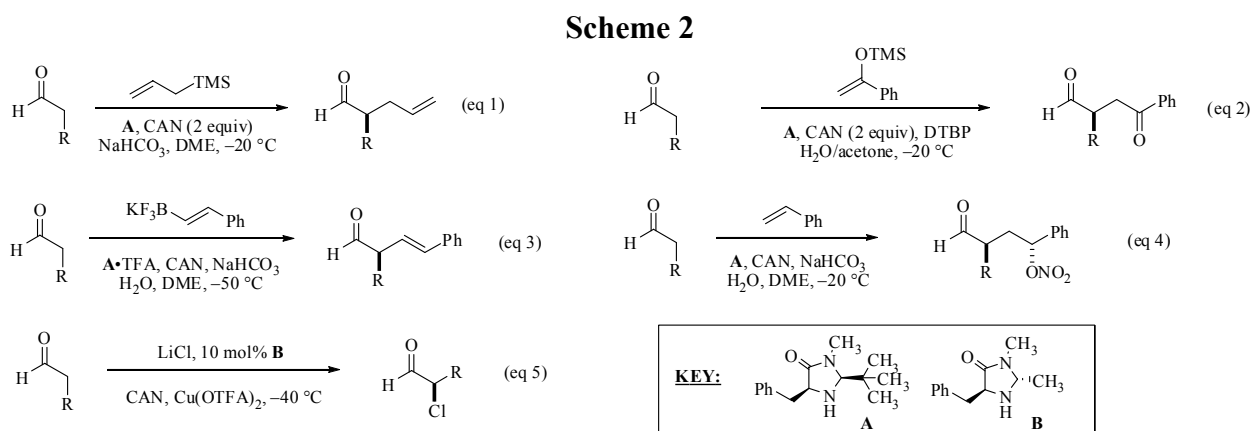
"New Catalysis Concepts"

David W. C. MacMillan, Princeton University

Research work on a relatively new mode of organo catalytic activation termed "Singly Occupied Molecular Orbital (SOMO) catalysis" was presented. According to the author, SOMO was founded upon the mechanistic hypothesis that one-electron oxidation of a transient enamine intermediate, derived from aldehydes and chiral amine catalysts, will render a three-pi-electron SOMO-activated species that can readily participate in a range of unique asymmetric bond construction (Scheme 1).



As shown in Scheme 2, this activation concept has been applied to the enantioselective allylic alkylation of aldehydes (eq 1), to asymmetric aldehyde α -enolation that allows access to γ -ketoaldehydes from simple aldehydes, enolsilanes, and a commercial catalyst (eq 2), to α -vinylation using vinylpotassium trifluoroborate salts (eq 3), to α -functionalization with activated olefins (eq 4) and to direct α -aldehyde chlorination (eq 5). Other applications discussed include organo SOMO cycloaddition, direct enantioselective pyrrole synthesis and intramolecular SOMO arylation.



“Overview of Newer Approaches to Treating Depression”

Nicholas J. Lodge, Bristol-Myers Squibb, Wallingford, Connecticut

Depression is a debilitating psychiatric illness that affects millions of people worldwide. Currently available pharmacological therapies for depression are based on the serendipitous discoveries of the tricyclic agents and monoamine oxidase inhibitors more than 50 years ago and act either through the prevention of monoamine metabolism or inhibition of monoamine reuptake. The current monoamine-based antidepressants typically require several weeks for onset of effect, only achieve remission rates of approximately 35% and exhibit a number of

tolerability issues. Thus, there continues to be a significant unmet medical need for antidepressants with improved efficacy, more rapid rate of response, and reduced side effects. Dr. Lodge talked about the current approaches to treating depression in his presentation.

A broad array of novel mechanisms, including agents that act on the stress axis, glutamate targets and two-pore potassium ion channels, are currently being explored as potential antidepressant routes with the promise of providing improved therapies. Additionally, systematic improvements in monoamine therapies also have the potential to deliver improved efficacy and reduced side effects. The following are some examples of the current studies.

Agomelatine is a potent agonist at melatonin receptors and an antagonist at 5-HT_{2C} receptors and has been tested in an animal model of depression. The antidepressant-like activity in this model most probably involves a combination of both its melatonin agonist and 5-HT_{2C} receptor antagonist properties. Amibegron (SR58611A), a selective beta-3 receptor agonist, also has antidepressant-like effects and is currently in a phase III study. The present experiment sought to confirm the antidepressant potential of amibegron by studying its effects in an animal model of depression. Saregutant (SR 48968) is a neurokinin-2 antagonist drug being developed as an antidepressant and anxiolytic by Sanofi-Aventis. Its mechanism of action is different from antidepressants currently available on the market. It works by blocking the effects of Neurokinin A at the NK-2 receptor. Phase III studies are currently being conducted.

CRF, AVP and Cortisol antagonists are being sought as therapies for stress and affective disorders. Current CRF₁ antagonists under investigation are R121919 (Janssen), 876009 (GSK, phase II) and CP-316,311 (Pfizer). SSR-149415 (Sanofi), a selective and orally active non-peptide antagonist of vasopressin V_{1b} receptor, is currently in clinical trials for treatment of anxiety and depression. Glucocorticoid antagonists are target for HPA axis dysregulation. Mifepristone is currently in phase III clinical trials. The 5-HT_{1B} receptor has attracted significant interest as a potential target for the development of therapeutics for the treatment of affective disorders such as anxiety and depression. SB-616234-A (GSK) is a novel, selective and orally bioavailable 5-HT_{1B} receptor antagonist. The TREK-1 protein is a background potassium channel regulated by various neurotransmitters including 5-HT. The regulation of the TREK-1 channel may alter mood, and that this particular potassium channel may be a potential target for new antidepressants. Deletion of TREK-1 resulted in increased 5-HT neurotransmission, reduced corticosterone elevation and amplified level of neurogenesis. TREK-1 knockout mice are insensitive to SSRIs. Ion channels like NMDA are another target for the treatment of depression. An AMPA receptor antagonist significantly attenuated antidepressant-like effect. Corlux (Corcept Therapeutics) is in Phase II clinical trials for the treatment of depression.

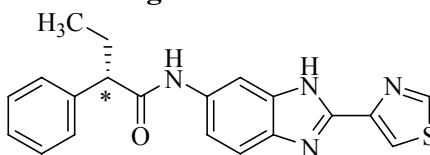
“Design, Synthesis and Biological Evaluation of Benzamide Derivatives as Tissue Selective Androgen Receptor Modulators”

B. Hanney, Y. Kim, J. Musselman, M. Krout, A. Schmidt, R. Vogel, C. Bai, S. McElwee-Witmer, H. Z. Zhang, F. Chen, C-T. Leu, D. Kimmel, C. Gibson, T. Prueksaritanont and R. Meissner, Merck Research Laboratories, Merck & Co., Inc., West Point, Pennsylvania.

In this poster by Barbara Hanney from Merck’s West Point laboratories, the results from a program to develop selective androgen receptor modulators (SARMs) for the treatment of postmenopausal osteoporosis were presented.

It has been shown that androgens stimulate bone formation in post-menopausal women, but unfortunately the administration of androgens to spur this growth also leads to unwanted side effects such as facial hair growth. This group sought a SARM that would increase bone density without the virilizing side effects. Compound **3** (Figure 1) exhibited a high binding affinity to the androgen receptor (AR) and moderate agonist activity in a cell transcription assay compared to the endogenous agonist dihydrotestosterone (DHT). However, the molecule was highly metabolized and the 5-aminothiabendazole portion was embryotoxic in a rat *in vivo* assay, leading the group to explore replacements for the right portion of **3**. A binding assay (ARBIND, which measures displacement of [³H]-1881 by ligands from endogenously expressed human AR in cell lysates) and an osteoanabolic assay (TAMAR, which measures trans-activation of a model promoter by endogenous human AR in tissue culture cells) were used to monitor the effects *in vitro*.

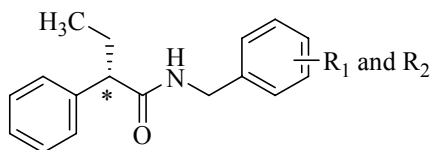
Figure 1



3

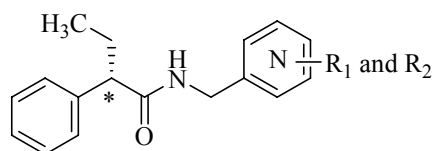
Tables 1 and 2 list data highlights from the study, using a substituted benzyl amide or a substituted methylpyridyl amide on the right-hand portion of **3**. Within the benzyl series, the 2-F-5-CF₃ analogue exhibited improved binding and potency, however congeners with a small alkyl group at the *ortho* position (still with the 5-CF₃ moiety) provided the best combination of potency and binding. Unfortunately, the best compounds in this series were highly protein bound (>99%), possibly limiting effective *in vivo* activity.

Table 1



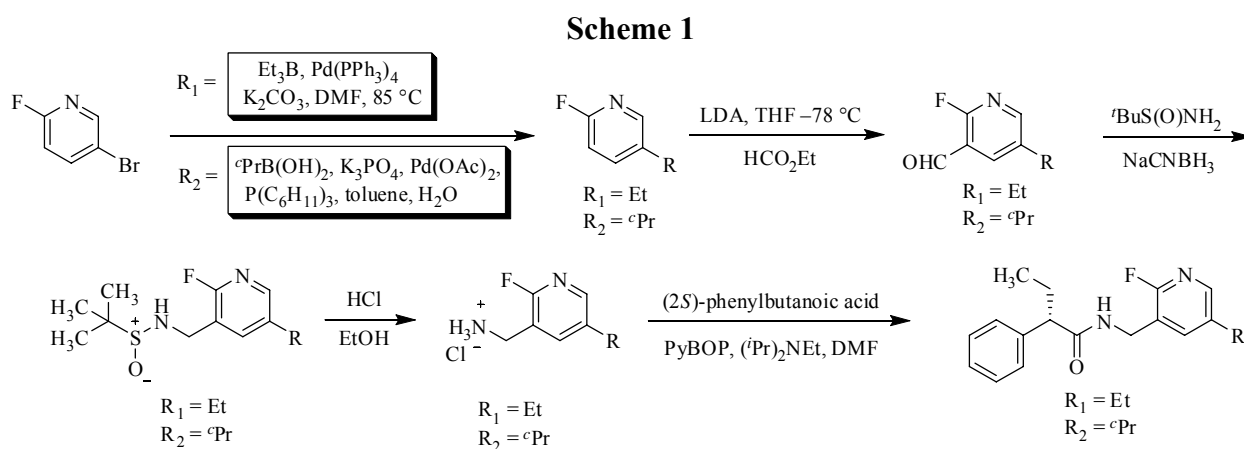
R ₁	R ₂	Chirality	ARBIND IC ₅₀ (nM)	TAMAR IP (nM)
H	H	N/A	>5000	---
2-F	5-CF ₃	<i>S</i>	33	652
2-F	5-Br	<i>S</i>	100	500
2-F	5-CH ₃	<i>S</i>	55	2000
2-F	5-C ₂ H ₅	<i>S</i>	19	1446
2-F	5- ^{<i>t</i>} Pr	racemic	71	384

In the pyridyl series, the 3-pyridyl benzyl group was best for binding and agonist activity. With respect to pyridyl substituents, a potency enhancement was seen with a 2-fluoro group, while the 5-cyclopropyl and 5-trifluoromethyl groups showed the best combination of agonist activity and binding. The group selected the 5-methyl substituted pyridyl for further *in vivo* efficacy study due to its activity and little change in binding in the presence of rat serum. Overall, the pyridyl derivatives exhibited reduced protein binding compared to the phenyl series.

Table 2

R ₁	R ₂	N	Chirality	ARBIND IC ₅₀ (nM)	TAMAR IP (nM)
H	5-CH ₃	3-N	racemic	978	---
2-F	5-CH ₃	3-N	S	41	364
2-F	5-C ₂ H ₅	3-N	S	8.2	265
2-F	5- ^c Pr	3-N	S	1.4	50
2-F	5-CF ₃	3-N	S	7.0	130

The syntheses of the 2-F-5-Et and cyclopropylpyridyl compounds are shown in Scheme 1 (no yields for the reactions were reported).



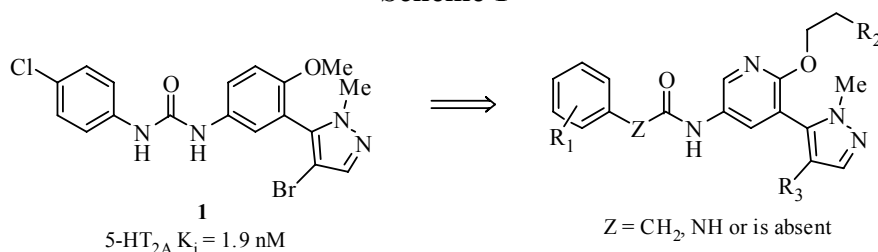
Further *in vivo* testing (OVX rat model) revealed that the 2-F-5-Et compound in the phenyl series displayed an increased osteoanabolic effect compared to DHT, but could not be tested at higher than 30 mpk due to solubility problems. On the other hand, the 2-F-5-methyl analogue in the 2-pyridyl series exhibited higher exposure due to its increased solubility, showed a good dose response and at the highest dose (60 mpk) the bone formation rate was comparable to DHT. Overall, good progress was made in finding more effective selective androgen receptor modulators and further work is in progress.

“Synthesis and SAR of Pyridinyl-Pyrazole Derivatives as Selective 5-HT_{2A} Inverse-Agonists for Platelet Aggregation”

P. I. Dosa, et al., Arena Pharmaceuticals, Inc.

Inverse-agonists of the 5-HT_{2A} receptor have been found to influence sleep patterns, alleviate negative symptoms in schizophrenia, and to inhibit the serotonin amplification of collagen or ADP-stimulated human platelet aggregation. The Arena research team presented their investigation of pyrimidinyl- and pyridinyl-pyrazole scaffolds for 5-HT_{2A} inverse-agonism.

Scheme 1



The research team synthesized the compound series, studied SAR and evaluated the effectiveness of several compounds at countering the serotonin amplification of ADP-stimulated human platelet aggregation. From their SAR, the pyrimidinyl-pyrazole series was found to be less potent than the pyridinyl-pyrazole series as 5-HT_{2A} inverse-agonists (Table 1).

Table 1. Heterocycle SAR

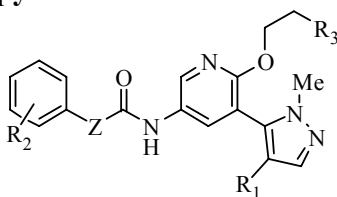
X	Y	Z	R ₁	R ₂	5-HT _{2A} K _i (nM)	5-HT _{2C} K _i (nM)
N	CH	CH	Br	4-Cl	52.0	446
N	CH	CH	Cl	4-Cl	135	803
N	N	CH	Br	4-Cl	55.2	>10,000
N	N	CH	Br	4-F	25.3	208
CH	CH	N	Br	4-Cl	0.54	16.6
CH	CH	N	Br	4-F	0.64	199

The team identified a series of potent and selective 5-HT_{2A} inverse-agonists, and several pyridinyl-pyrazole derivatives proved to be low nM inhibitors of serotonin amplification of ADP-stimulated human platelet aggregation (Table 2). Inclusion of an appropriate solubilizing group generally led to compounds with greater anti-platelet aggregation activity (Table 3).

Table 2. Pyridinyl-pyrazoles with no solubilizing group

Z	R ₁	R ₂	5-HT _{2A} K _i (nM)	Platelet Aggregation IC ₅₀ (nM)
NH	Br	4-Cl	0.54	162
NH	Br	2,4-F	1.5	64
NH	Cl	2,4-F	2.3	69
-	Br	3-CF ₃	0.51	99
-	Cl	3-CF ₃	0.87	95

Table 3. Pyridinyl-pyrazoles with an attached solubilizing group



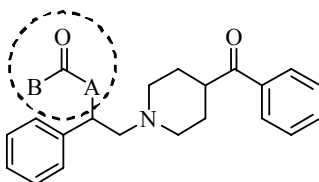
Z	R ₁	R ₂	R ₃	5-HT _{2A} K _i (nM)	Platelet Aggregation IC ₅₀ (nM)
CH ₂	H	4-Cl	Pyrrolidin-1-yl	0.98	4.8
CH ₂	H	4-Cl	Morpholin-4-yl	2.4	32
NH	H	4-Cl	Morpholin-4-yl	0.44	79
-	Br	4-CF ₃	Morpholin-4-yl	0.92	93
-	H	3-CF ₃	Morpholin-4-yl	3.8	38

“Structure Activity Relationship and Pharmacological Evaluation of Carbamic Acid Benzoyl Piperidine Analog: YKP 1358, Novel Atypical Antipsychotics”

Young-Gil Kim, et al., SK Holdings

Atypical antipsychotics have been used as effective treatment options with reduced risks for adverse events. Unfortunately, current atypical antipsychotics do not clearly solve the side effect issues such as extrapyramidal syndrome and metabolic disorder. These side effects may be due to low target receptor selectivity. It is known that stimulation of 5-HT₂ receptors results in antagonism of the catalepsy induced by treatment with a dopamine D₂ receptor antagonist. As a clean ligand for a given target receptor is required, the SK research team eventually identified the selective novel atypical antipsychotic YKP1358.

Table 1. SAR of lead compounds



α -Substituted analogs: carbamic acid, urea, carbonate

YKP	<i>In vitro</i> profile (IC ₅₀ nM)			TI (CAT/CAR) ^b
	5-HT _{2A}	D ₂	selectivity ^a	
Lead 1	8.3	91.5	11.0	79.4
Lead 2	17.0	105.1	6.2	2.8
Lead 3	12.2	477	39.1	5.0
Lead 4	8.2	60.8	7.4	3.5

^aselectivity: D₂/5-HT_{2A} binding affinity ratio. ^bCAR: Conditioned avoidance responses (efficacy), CAT: catalepsy (toxicity).

From SAR analysis of lead compounds, **Lead 1** (YKP1358) was identified as having the highest therapeutic index with potent pharmacological efficacy in various animal studies (Table 1). In phase I studies, YKP1358 showed good tolerance and excellent D₂ occupancy in a PET study. The development of YKP1358 is being continued by SK.

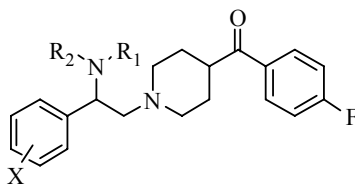
“Synthesis and Structure-Activity Relationship of a Series of Alpha-azole Substituted Phenyl Alkyl Amine as 5-HT_{2A}/D₂ Receptor Ligand: Potential Psychosis Treatment Agents”

Mi-Kyung Ji, et al., SK Holdings

Through HTS, the SK research team identified a series of alpha-azole substituted phenyl alkyl amines as 5-HT_{2A}/D₂ ligands as possible therapeutic agents for psychosis disorders.

A 4-fluorobenzoyl piperidine scaffold was found to be the best pharmacophore for a high binding at 5-HT_{2A} and D₂ receptors, and simple azoles had favorable profiles (Table 1). 4-*i*-Pr and 3,4-dimethyl substituents showed an acceptable binding affinity at 5-HT_{2A} and D₂ receptors. The research group found that the 1,2,4-triazole and imidazole derivatives showed the optimal binding affinity.

Table 1



No	X	NR ₁ R ₂	% Inhibition at 100 nM	
			5-HT _{2A}	D ₂
11	4- <i>i</i> -Pr	1,2,4-triazole	64.3%	65.1%
18	3,4-diMe	1,2,4-triazole	91.9%	55.1%
25	3,4-diMe	imidazole	79.3%	56.5%

The antipsychosis activity tests revealed compound **18** for good atypical efficacy in an apomorphine induced climbing test (APO) and DOI induced head twitch test (Table 2).

Table 2. Potential antipsychosis activity I

No	% Inhibition at 100 nM		APO ^a	DOI ^b
	5-HT _{2A}	D ₂		
11	64.3%	65.1%	3.1 ip	52% (0.3 ip)
18	91.9%	55.1%	4.5 ip	0.15 ip
25	79.3%	56.5%	30% (10 ip)	10% (0.3 ip)

^aapomorphine induced climbing test; % antagonism or ED₅₀; ^bDOI induced head twitch test; % antagonism or ED₅₀.

The compound **18** shows the antipsychotic efficacy in conditioned avoidance response (CAR) and phencyclidine induced locomotor activity (PCP-LMA) tests (Table 3).

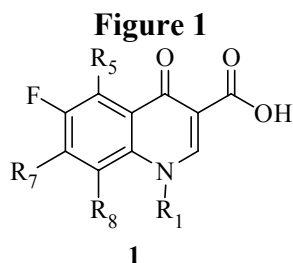
Table 3. Potential antipsychosis activity II

No	5-HT _{2A} IC ₅₀	D ₂ IC ₅₀	APO ED ₅₀	DOI ED ₅₀	CAR ED ₅₀	PCP-LMA ED ₅₀
18	8.2 nM	60.8 nM	4.5 ip	0.15 ip	2.8 ip	0.89 ip

“Microwave-Assisted Amination of Quinolone”

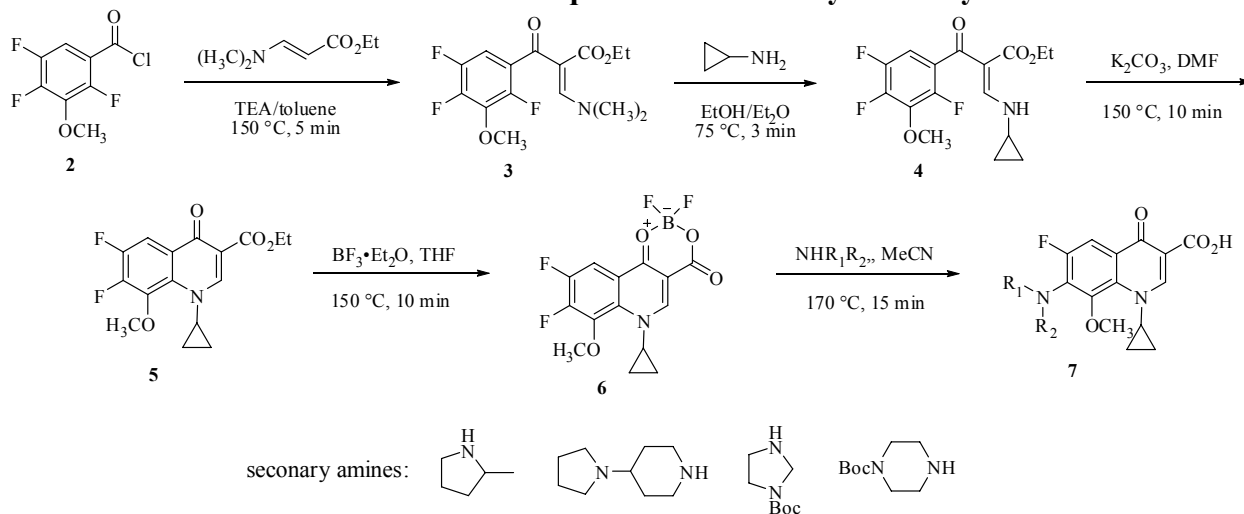
Shahnaz Ghassemi, Biotage, Discovery Chemistry Group

The fluoroquinolone antibacterials (**1**, Figure 1) have drawn much attention since it is discovered that the replacement of the C-6 hydrogen with a fluorine atom enhances antibacterial activity. The emergence and spreading of bacterial, parasitical and viral strains that are resistant to clinically used drugs has driven the search for new antibacterial and antiviral drugs. Norfloxacin is the first derivative with high activity, however flumequine was the first drug to demonstrate the advantage of a C-6 fluorine atom. The new version of this class of compounds consists of ofloxacin, ciprofloxacin and the most recent analogue tosufloxacin, all of which contain a piperazinyl or aminopyrrolidinyl moiety at carbon C-7 (R_7).



The conventional methods of synthesis and isolation for many anti-infective agents generally take weeks to carry out. Many disadvantages in the literature-reported preparations of these new targets include long reaction times and the use of strong acids (PPA, H_2SO_4 , etc.), bases (NaOEt, NaH, sodium 3-aminopropylamide, etc.) and harsh dehydrating agents ($ZnCl_2$, $AlCl_3$, etc.).

Scheme 1. MAOS 6-Fluoroquinolone-3-carboxylic acid syntheses



6-Fluoroquinolone-3-carboxylic acids (**7**) are conveniently prepared by the direct amination of 7-halo-6-fluoroquinolone-3-carboxylic acids with piperazine or pyrrolidine derivatives under thermal conditions. The literature published procedures for the synthesis require over one week with overall yield of 15-18%, in which the amination step requires reflux for one week. When MAOS (microwave-assisted organic synthesis) is used, the time required for this multiple synthesis is shortened to less than one hour, and the overall yield improves. Optimization of the microwave synthesis was performed on a single mode reactor (sealed vessel, 2-5 mL and later

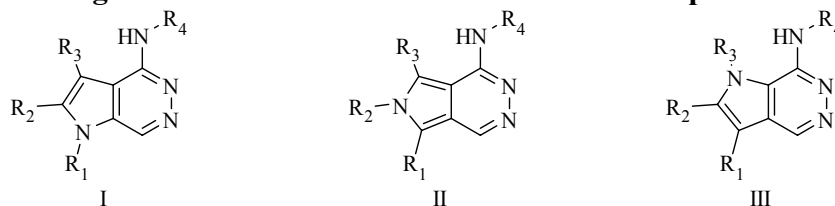
scaled up to 20 mL). Apart from the time saving, the chemistry was also modified for better results. Under conventional conditions, the direct amination does not occur with pyrrolidine derivatives. However, this problem can be overcome by converting the acid to corresponding, more reactive borate ester **6**. This step is easily done with MAOS in 10 min in 85% yield. This new methodology allowed for a rapid synthesis of fluoroquinolone analogs at the fraction of cost and time for biological testing.

“Synthesis and Biological Activity of Pyrrolo-pyridazine Derivatives”

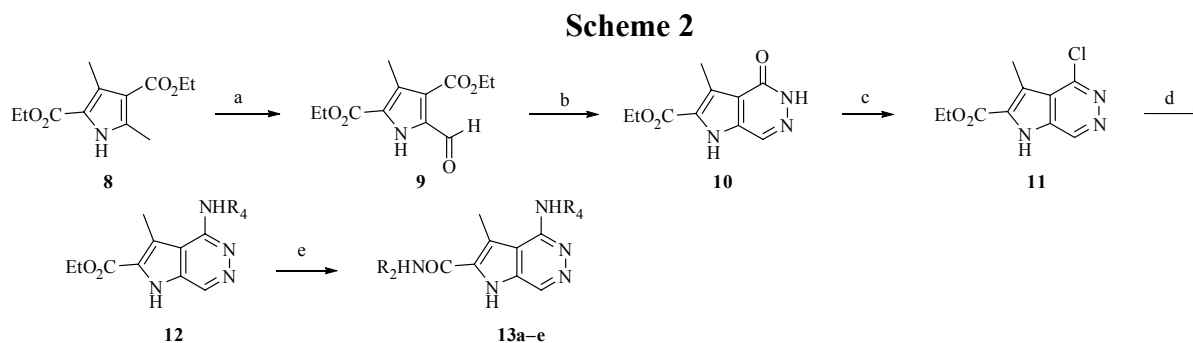
Peng Cho Tang; Jun Feng; Li Huang; Zhe Xu; Ling Cheng; Lei Zhang; Pingyan Bie and Bing Hu, Shanghai Hengrui Pharmaceutical Co. Ltd., Shanghai, China.

Receptor tyrosine kinases (RTKs) play a crucial role in signal transduction pathways that regulate cell differentiation, proliferation and angiogenesis. Inhibition of RTK activation has become a compelling approach toward the development of new anticancer agents. Human epidermal growth factor receptors EGFR, ErbB1 (a.k.a. HER-1) and ErbB2 (a.k.a. HER-2) are the members of the ErbB family of receptor tyrosine kinase which have been clinically validated as targets for cancer therapy. There are two pathways to anti-cancer agents (antibody-based and small molecule inhibitor-based) and three small molecule drugs gefitinib (Iressa), erlotinib (Tarceva), and lapatinib (Tykerb) have been approved for the treatment of non-small-cell lung cancer. EGFR inhibitors based on scaffolds that mimic the adenine moiety of ATP draw much attention as competitive inhibitors of ATP. This work describes the use of the pyrrolo-pyridazine scaffold (Figure 1) for a program developing competitive EGFR/HER-2 inhibitors.

Figure 1. General structure of the aimed compounds



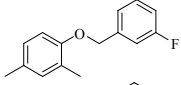
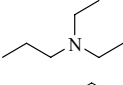
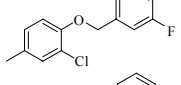
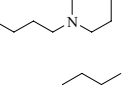
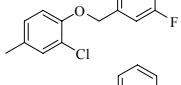
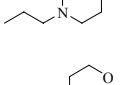
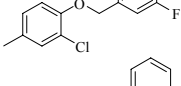
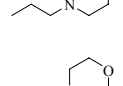
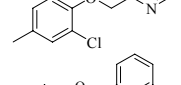
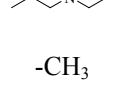
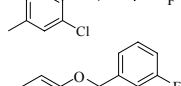
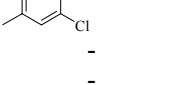

Scheme 2 outlines the synthesis of compound **I**, and compounds **II** and **III** were prepared by the same route. Inhibition of EGFR/HER-2 tyrosine kinase activity was evaluated in enzyme assays and in cellular assays using A431 (which over-expresses EGFR) and SK-BR-3 (which over-expresses HER-2).



Conditions and reagents: (a) 4 equiv CAN, THF/AcOH/H₂O, rt, 1.5 h, 65%; (b) 1.1 equiv NH₂NH₂, AcOH, 100 °C, 2 h, 79%; (c) 1 equiv POCl₃, CH₃CN, 80 °C, 2 h, 95%; (d) 1.5 equiv R₄NH₂, 2.5 equiv Et₃N, *i*-PrOH, 80 °C, 4–8 h, 50%; (e) 1. 1 M NaOH, EtOH, reflux, then 1 N HCl; 2. 1.5 equiv R₂NH₂, Et₃N, EDCI, HOBt, DMF, rt, 17–58%

As shown in Table 1, no activity was observed for EGFR with compounds **13b**, **13c** and **13e**, although compounds **13a** and **13d** showed significant HER-2 kinase inhibition. These two compounds were found to be >100-fold selective over the closely related EGFR kinase. It is expected that this type of compound could be explored for the selective HER-2 kinase activity. Further study is now in progress.

Table 1. Enzyme and cellular inhibitory activity assay

Compound	R ₄	R ₂	EGFR IC ₅₀ (μM)	A431 IC ₅₀ (μM)	HER-2 IC ₅₀ (μM)	SK-BR-3 IC ₅₀ (μM)
13a			1.28	1.16	0.007	0.96
13b			>10	0.98	ND	2.24
13c			>10	0.257	ND	0.843
13d			5-10	12.65	0.004	>5
13e			>10	0.867	ND	>5
IIa		-CH ₃	>10	1.21	ND	0.934
IIIa			>10	15.14	ND	0.62
Tarceva	-	-	0.015	0.42	1.52	1.89
Tykerb	-	-	0.019	0.14	0.003	0.124

“Design and Synthesis of Pyrazolone-Based Anaplastic Lymphoma Kinase (ALK) Inhibitors”

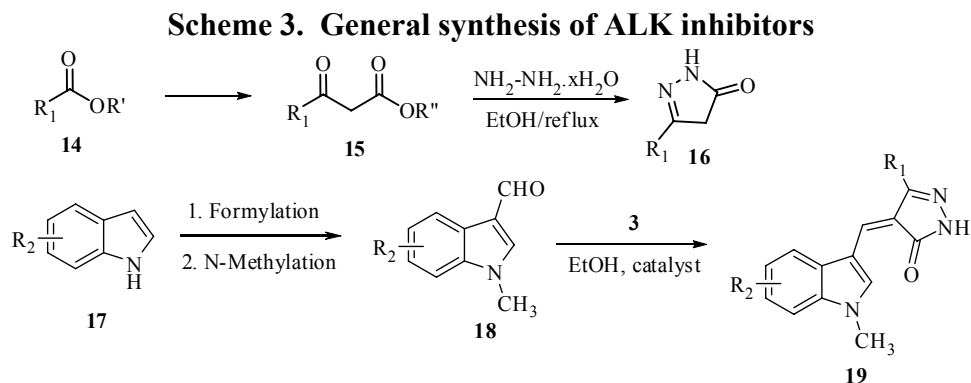
Rabindranath Tripathy, Robert McHugh, Thelma Angeles, Mark Albom, Mangeng Cheng and Bruce Dorsey, Cephalon, Inc., Discovery Research, West Chester, PA

Anaplastic lymphoma kinase (ALK) is one of the few tyrosine kinases implicated in oncogenesis in both non-hematological and hematological malignancies with ALK fusion protein that expresses tumors exhibiting “oncogene addiction”. Chromosomal translocations generate multiple ALK oncogenic fusion proteins in tumors. Over-expression of ALK and its putative ligands (pleiotrophin and midkine) has been confirmed in human cancers that include glioblastoma, melanoma, neuroblastoma, prostate, breast, colon, pancreatic and ovarian cancers. The current program is aimed to design ALK inhibitors as possible therapeutic interventions against such cancers by appropriately modifying the specific sites on the core pyrazolone structures, a class previously shown to inhibit VEGF-R2 kinase function.

Screening of the Cephalon internal library against ALK resulted in identification of several active pyrazolones. Based on structures originally designed for VEGF-R2 inhibition, an SAR investigation was focused on (a) modifications of the structure in favor of ALK and

identification of potent and cell permeable compounds; (b) identification of structural features responsible for improved PK profiles and (c) attachment of appropriate functionality to maintain potency and improve solubility.

The designed compounds were easily synthesized via Knoevenagel condensation of substituted pyrazolones with suitable aldehydes (generally indole-3-aldehydes or pyrrole-2-aldehydes) as shown in Scheme 3. The precursors were made from commercial materials in a few steps and provided for the rapid generation of compounds via variation of aldehyde and pyrazolone moieties.



A representative general structure of the final products with a thiazole heterocycle and biological evaluation data of analogues are listed in Table 2. Before this work, the original series produced a poor PK profile, which was probably due to rapid metabolism. The present results exhibit dramatic improvements in microsomal stability, and in particular resistance to metabolic degradation of benzyloxy groups at either the C-4 or C-5 position of the indole ring. Incorporation of a 4-benzyloxy group is well tolerated and analogues should provide reasonable PK profiles.

Table 2. Thiazole analogue biological evaluation results

Cmpd #	R ₂	ALK IC ₅₀ (nM)	Cellular IC ₅₀ Activity (nM)
20a	4-benzyloxy	46	300
20b	4-(1,3-oxazol-2-ylmethoxy)	27	300
20c	4-(3-pyridylmethoxy)	41	300
20d	4-(2-F-benzyloxy)	44	400
20e	4-(2,6-di-F-benzyloxy)	9	100
20f	4-(2-F-6-Cl-benzyloxy)	13	200
20g	4-(3-Cl-2,6-di-F-benzyloxy)	28	500

The indole *N*-methyl group was also changed to solubility-enhancing groups such as dimethylaminoethyl or dimethylaminopropyl moieties. Enhancement in solubility with high

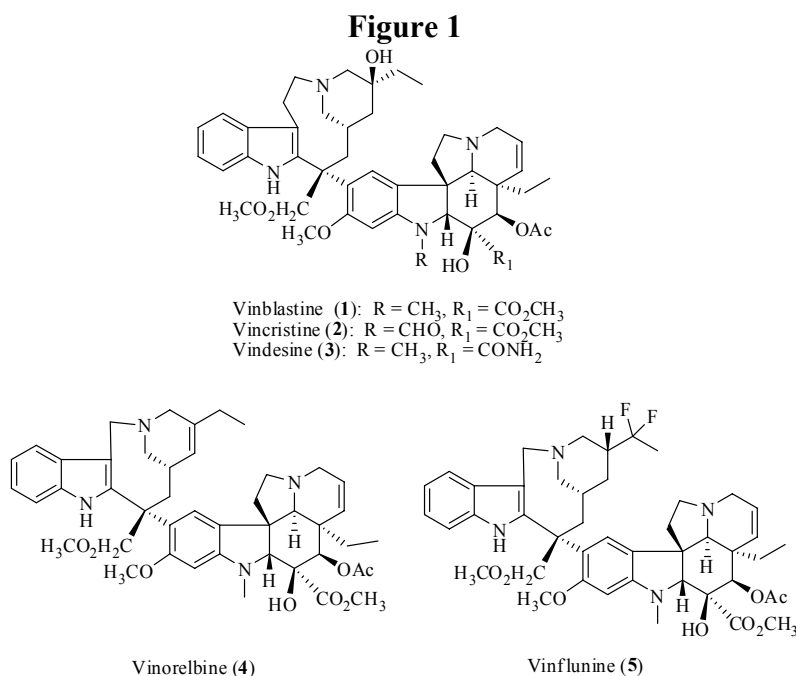
permeability was observed with some loss in potency (~6-9-fold). It is noted that while microsomal stability was improved, cell permeability, oral absorption and solubility continued to be problematic.

In summary, the work on pyrazolone-based kinase inhibitors has led to the identification of a number of heterocyclic-substituted pyrazolones as potent ALK inhibitors. In particular, several thiadiazoles, thiazoles and pyridines were shown to be active, and the pyridines offered the advantage of a potential increase in solubility through salt formation.

“Synthesis and Biological Evaluation of C-12’ Substituted Vinflunine Derivatives”

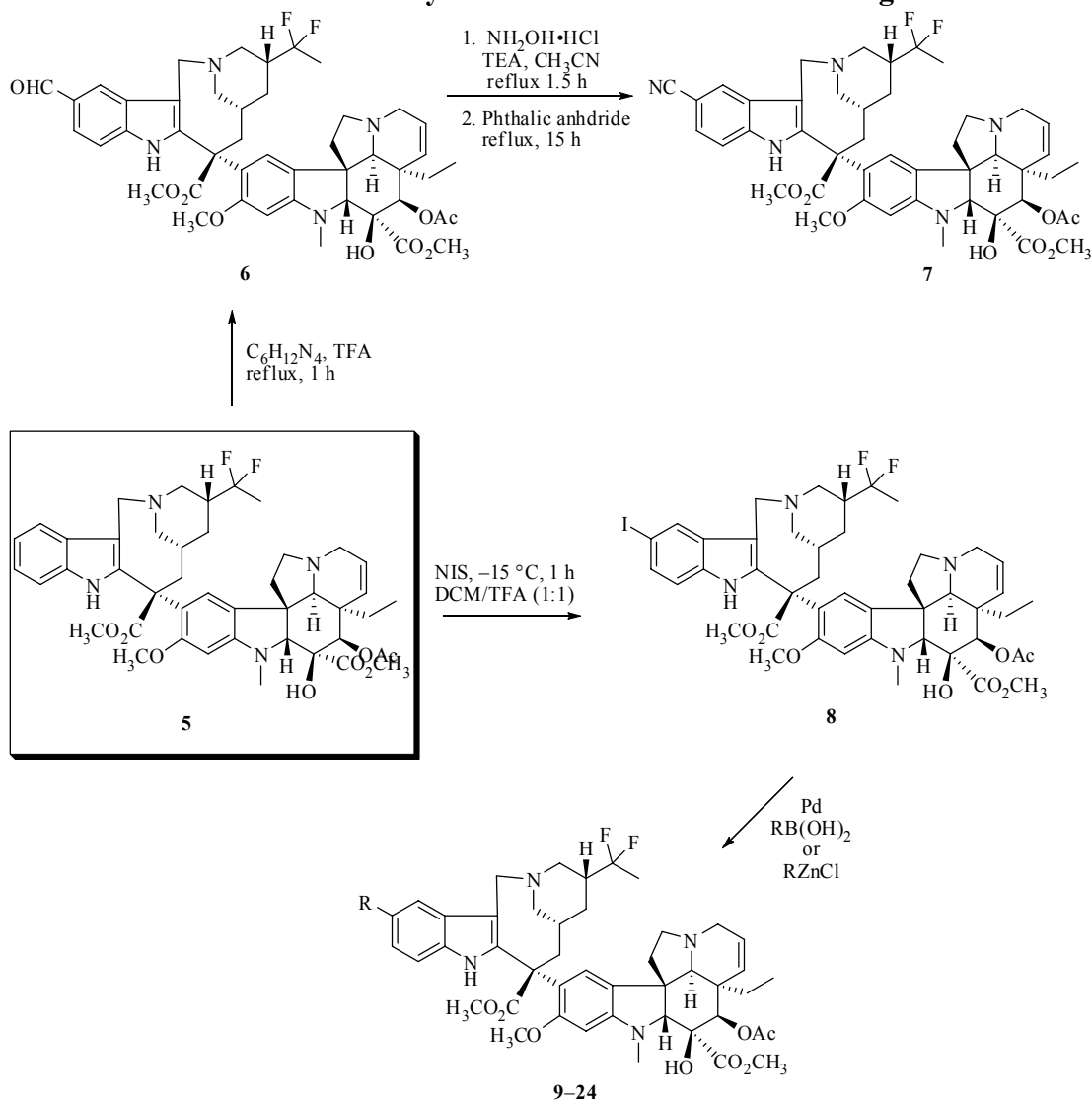
Lei Xin Sheng, Tang Peng Cho, Yu Xiang Da, Liu Zhen Hong, LV He Jun, Yin Long and Yang Fang Long, Shanghai Hengrui Pharmaceuticals, Shanghai, 2000245, China

The dimeric vinca alkaloids (Figure 1) are an important class of anticancer drugs with two natural products, vinblastine (**1**) and vincristine (**2**) and two semi-synthetic derivatives vindesine (**3**) and vinorelbine (**4**) currently on the market. Vinflunine (**5**) is a difluorinated derivative in late phase III clinical trials.



The authors disclosed a series of C-12’ analogues of vinflunine prepared from vinflunine-12’-carbaldehyde **6** or 12’-iodovinflunine **8** (Scheme 1). Aldehyde **6** was prepared by heating **5** with hexamethylenetriamine in TFA at 75 °C for one hour then was converted to cyano analogue **7** by oxime formation and dehydration with phthalic anhydride. The versatile iodide intermediate **8** was synthesized by treatment of **5** with NIS in a mixture of TFA/methylene chloride at -15 °C. Analogues **9** through **24** were prepared through a series of palladium-mediated Suzuki-Miyaura and Negishi reactions.

Scheme 1. Synthesis of C-12' vinflunine analogues



The yields for the final synthetic step and the biological activity of the vinflunine derivatives are presented in Table 1. The compounds were tested in a cell growth assay using the lung epithelial tumor cell line A549. Aryl (**9-16**), heteroaryl (**17-18**) and larger alkyl (**22**) substituents produced cell activity in the low micromolar range. Small non polar functional groups such as cyano (**7**), methyl (**19**), ethyl (**20**), cyclopropyl (**21**), ethynyl (**23**), and azide (**24**) derivatives were able to produce cytotoxicity below $1\ \mu\text{M}$ in the A549 cell assay. Of the compounds presented the methyl (**19**) and ethynyl (**23**) analogues demonstrated improved cell activity when compared to vinflunine. In the conclusion the authors indicated they were pursuing further biological testing with the compounds **23** and **24**.

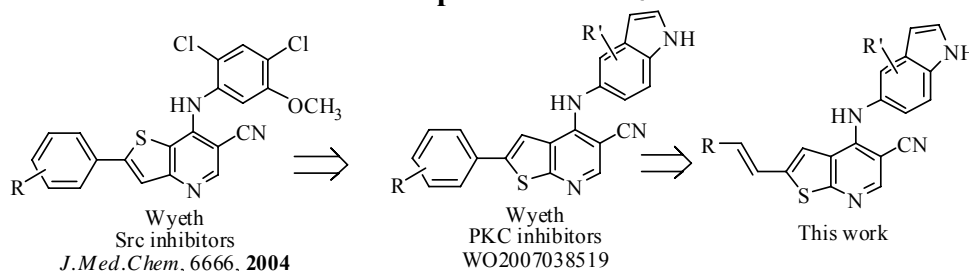
Table 1. *In vitro* cytotoxicity of vinflunine derivatives against A549

Compound	R	Yield%	IC ₅₀ μM	Compound	R	Yield%	IC ₅₀ μM
Vinflunine(5)	H	-	0.08	16	2-CF ₃ -Ph	39%	0.29
7	CN	47%	0.29	17	Furan-3-yl	39%	2.28
9	Ph	73%	2.16	18	Thiophen-3-yl	32%	3.00
10	4-Me-Ph	60%	0.56	19	Me	44%	0.037
11	4-Cl-Ph	53%	1.17	20	Et	31%	0.92
12	2-Cl-Ph	20%	1.01	21	Cyclopropyl	31%	0.12
13	2,4-di-F-Ph	57%	1.02	22	n-Bu	22%	1.88
14	4-NC-Ph	46%	2.50	23	Ethynyl	68%	0.05
15	4-F-Ph	80%	2.56	24	N ₃	68%	0.16

“2-Alkenyl thieno[2,3-*b*]pyridine-5-carbonitriles: Potent and Selective Inhibitors of PKCθ (MEDI-0036)”

L. Nathan Tumey, Diane H. Boschelli, Divya Chaudhary and Julie Lee, Wyeth Research

Protein kinase C theta (PKCθ) is a serine/threonine kinase expressed primarily in lymphocytes and mast cells. It is thought to play an essential role in T cell receptor (TCR)-mediated activation of transcriptional factors such as NFAT and AP-1. This ultimately leads to upregulation and activation of IL-2 and further inflammatory responses. PKCθ knockout mice show a diminished response in various T cell-mediated disease models suggesting that inhibition of PKCθ would be an effective anti-inflammatory therapy. Scheme 1 shows the development of a current series of inhibitors using leads from in-house screening against kinases. This presentation outlined the effect on SAR and kinase selectivity of 2-alkenyl substitution on the thieno[2,3-*b*]pyridine ring system.

Scheme 1. Development of PKCθ inhibitors

The synthesis of the initial series of analogues is described in Scheme 2. Thermal addition of 5-aminoindole **2** to compound **1** provided biarylamine **3**. Heck reaction with the corresponding vinyl compounds gave the initial series of analogues **4-11**. While Table 1 shows only moderate PKCθ binding activity some selectivity was demonstrated over PKCδ. PKCθ and PKCδ have a high sequence homology (75%) and PKCδ deficiency has been linked to B-cell proliferation in mice. The previously 2-arylthieno[2,3-*b*]pyridine series had difficulty in achieving a good selectivity ratio.

Scheme 2. Synthesis of initial analogues

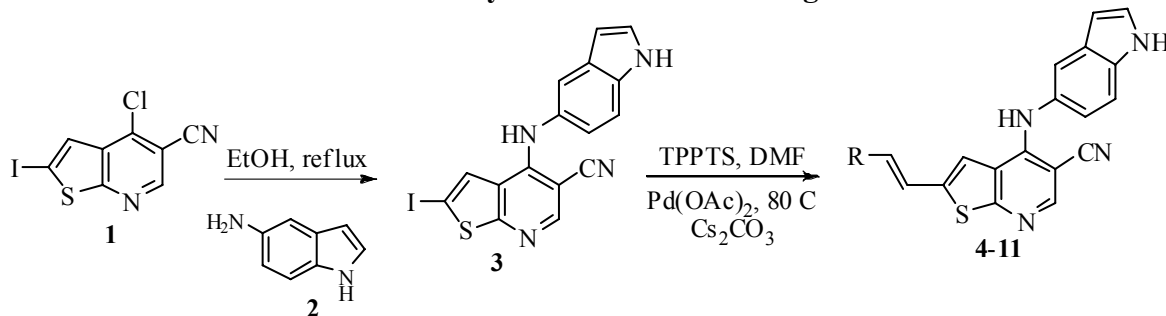


Table 1. Initial SAR

Compound	Q	PKC θ IC ₅₀ (nM)	PKC δ IC ₅₀ (nM)
4	Ph	440	2,800
5	3-F-Ph	460	1,700
6	4-F-Ph	2,500	nt
7	3-OCH ₃ -Ph	1,100	nt
8	4-OCH ₃ -Ph	480	1,400
9	CH ₂ Ph	3,700	nt
10	<i>n</i> -Bu	2,600	nt
11	CH ₂ CH ₂ OH	390	1,000

Continued work lead to the development of the alkenyl amide series described in Scheme 3. Thermal addition of aminoindoles **12** was followed by the Heck reaction with *tert*-butyl acrylate to provide alkenyl ester **14**. TFA deprotection afforded carboxylic acid **15** which was activated with EDCI in DMF and treated with a series of amines to provide alkenyl amides **16-33**. Consistent with previous SAR studies, it was found that the 4-methylindole analogue **17** was significantly more active than the hydrogen analogue **16** and maintained good selectivity over PKC δ (Table 2). Attempts to add solubilizing amides groups (compounds **20-24**) or non tertiary amides (compounds **25-28**) resulted in decreased PKC θ activity and selectivity over PKC δ . Small dialkyl amides **29** and **30** provided the most potent PKC θ activity with excellent selectivity over PKC δ .

Scheme 3. Synthesis of alkenylamide series

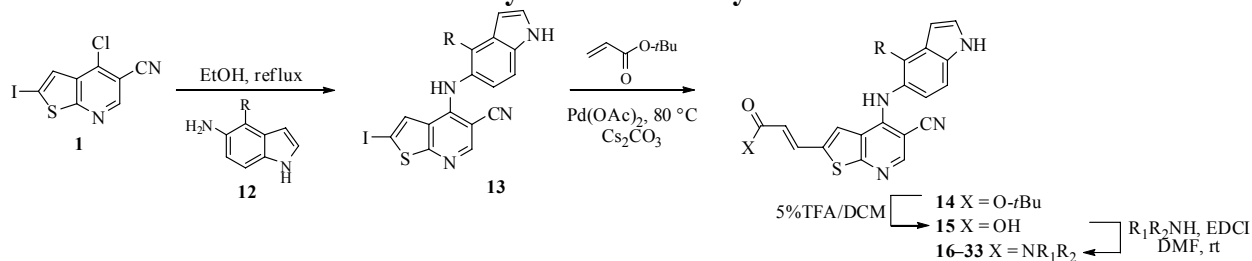
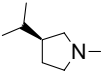
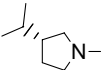
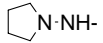
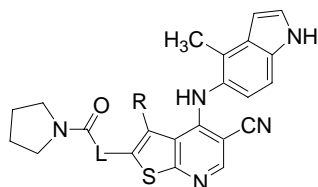


Table 2. Activity of alkenyl amide analogues

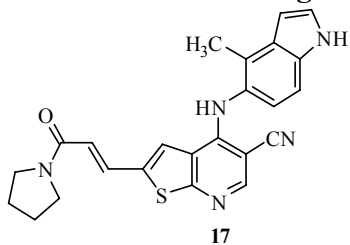
Compound	R	X	PKC θ IC ₅₀ [nM]	PKC δ IC ₅₀ [nM] (PKC θ / PKC δ)
16	H	pyrrolidine	130	18,000 (140x)
17	CH ₃	pyrrolidine	3.8	1,300 (340x)
18	CH ₃		180	430 (2x)
19	CH ₃		170	340 (2x)
20	CH ₃	(±)3-OH-pyrrolidine	41	450 (11x)
21	CH ₃	piperdine	48	450 (9x)
22	CH ₃	piperzine	88	200 (2x)
23	CH ₃	N-Me-piperizine	43	73 (2x)
24	CH ₃	Morpholine	53	4,100 (78x)
25	CH ₃	H ₂ N-	12	67 (6x)
26	CH ₃	EtNH-	15	500 (34x)
27	CH ₃	PhNH-	110	500 (4x)
28	CH ₃	MeO-(CH ₂) ₄ -NH-	48	1,800 (38x)
29	CH ₃	Me ₂ N-	1.6	2,100 (1300x)
30	CH ₃	Et ₂ N-	2.0	250 (120x)
31	CH ₃	Me ₂ N-CH ₂ -CH ₂ -N(Me)-	150	790 (5x)
32	CH ₃	Me ₂ N-NH-	100	420 (4x)
33	CH ₃	 N-NH-	370	330 (1x)

In Table 3, the SAR of the alkene linker was investigated. Reduction to alkane (**35**) gave a large decrease in activity and selectivity. Removal of the amide carbonyl (**36**) provided only a moderate loss of potency, but afforded a significant loss of selectivity. When the alkene was replaced with a benzene ring (**37**), again a moderate loss of potency was observed as previously noted the selectivity over PKC δ was severely compromised. Finally, it was noted that for compound **38**, methyl substitution at the 3-position of the thienopyridine ring did not compromise potency or selectivity.

Table 3. SAR on variations alkene linker

Compound	L	R	PKC θ IC ₅₀ nM	PKC δ IC ₅₀ nM (PKC θ /PKC δ)
34		H	3.8	1,300 (340 \times)
35		H	250	1,800 (7 \times)
36		H	22	100 (5 \times)
37		H	58	180 (3 \times)
38		CH ₃	3.5	380 (110 \times)

An expanded biological profile for compound **17** is given in Table 4. The analogue demonstrated good selectivity in a cell assay, reasonable half life in rat liver microsomes and a good profile against selected Cyp enzymes. Testing against an expanded panel of kinases showed excellent selectivity over several kinases, including other PKC isoforms. However, poor selectivity was noted over the SRC family of enzymes including FYN, HCK, LCK, and LYN.

Table 4. Biological profile for compound 17

PKC θ IC₅₀: 3.8 nM
 PKC δ IC₅₀: 1300 nM (340 \times)
 WT T cell: 170 nM
 PKC θ KO T cells > 1300 nM
 T_{1/2} Rat liver microsomes > 30 min
 Cyp 2D6 and 2C9 <50% inhibition at 3 μ M
 Cyp 3A4 61% inhibition at 3 μ M

Kinase	IC ₅₀ (nM)	Kinase	IC ₅₀ (nM)
PKC θ	>50,000	MK2	>50,000
PKC α	>100,000	VEGFR2	>50,000
PKC β	1,300	ERK2	>50,000
PKC δ	3,000	P38 α	>50,000
PKC ϵ	51,000	IKK α	>50,000
PKC η	>100,000	IKK β	>50,000
PKC ζ	>50,000	MET	>50,000
RSK1	>50,000	PGFR α	>50,000
Aurora B	>50,000	ITK	>20,000
CDK1/cyclin B	>50,000	SRC	330
CHK-1	>50,000	FYN	25
PKA	>50,000	HCK	26
ROCK1	>50,000	LCK	46
CK1 γ 1	>50,000	LYN	28

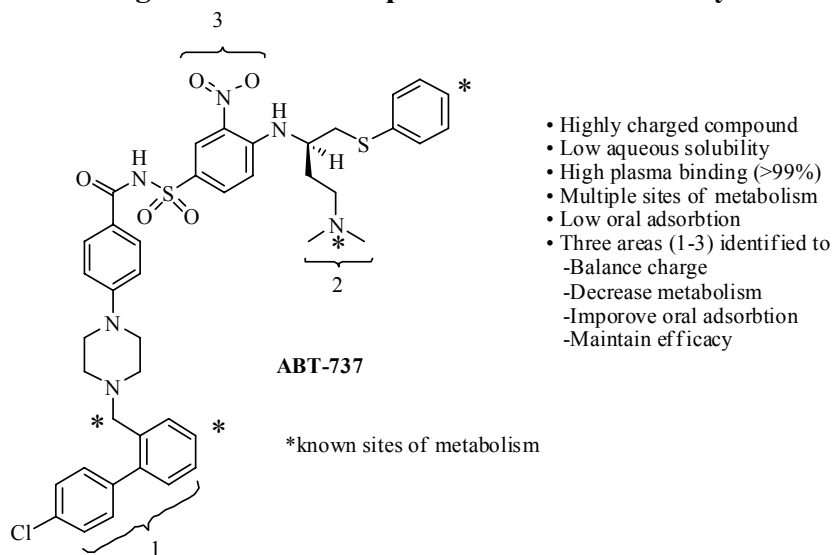
“ABT-263, An Orally Bioavailable Inhibitor of Bcl-2 Family Proteins (MEDI-0110)”

Hong Ding, Cheol-Min Park, Milan Bruncko, Christopher L. Lynch, Aaron R. Kunzer, Andrew M. Petros, Xiaohong Song, Xilu Wang, Michael D. Wendt, Paul M. Nimmer, Morey L. Smith, Stephen K Tahir, Haichao Zhang, Christin Tse, Saul H. Rosenberg and Steven W. Elmore, Global Pharmaceutical Research and Development, Abbott Laboratories

Bcl-2 is the prototype for a family of genes and the proteins they produce. Members of the gene family can be pro-apoptotic or anti-apoptotic (including Bcl-2). Over expression of Bcl-2 family proteins in cancer cells can lead to interference with programmed cell death and a poor prognosis for the cancer patient. Restoration of apoptosis in cancer cells could have broad therapeutic utility and possibly provide a single agent treatment for cancer.

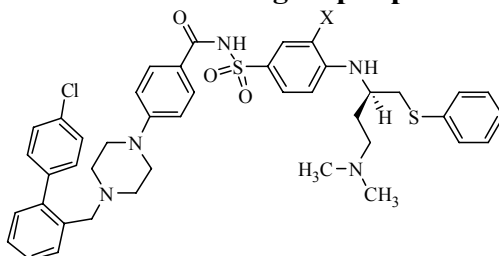
ABT-737 has high affinity for target proteins Bcl-2, Bcl-X, and Bcl-w as a single agent or in combination therapies. However, the molecule was highly protein bound, difficult to formulate and not bioavailable. Chemists at Abbott identified several sites for potential improvement to **ABT-737**. The goal, shown in Figure 1, was to reduce the overall charge of the molecule, create aqueous solubility and decrease metabolism.

Figure 1. Plan to improve oral bioavailability

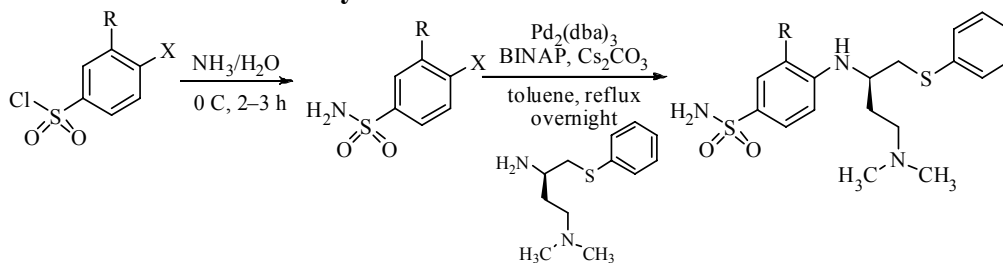


New compounds were studied in a number of assays including a competitive fluorescence assay to measure K_i , a growth inhibition assay with the H146 cell line and a mechanistic-based cell assay in which the interleukin 3 (IL-3)-dependent cell line FL5.12 has Bcl-2 or Bcl-X_L over-expressed, preventing apoptosis upon removal of IL-3. A compound's ability to reverse this effect should relate directly to inhibition of Bcl-2 or Bcl-X_L.

Table 1 shows the SAR for replacing the nitro group. Substituents that are strongly electron-withdrawing maintain activity (e.g. -CF₃, -COCF₃, -SO₂CH₃) while neutral groups such as methyl decrease activity in the FL5.12 cell assay. A critical observation was that replacement of the nitro group with trifluoromethyl improved oral bioavailability at 5 mpk in rat from 5.9 to 39%. The synthesis of the key sulfonamide intermediates is shown in Scheme 1.

Table 1. SAR of nitro group replacements

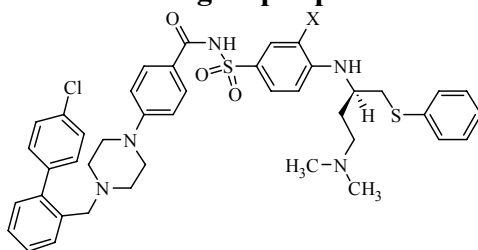
X	FL5.12 EC ₅₀ (nM, serum free)		H146 EC ₅₀ (nM)	FPA K _i (nM)	
	Bcl-X _L	Bcl-2	(10% HS)	Bcl-X _L	Bcl-2
NO ₂	9	4	86	<1	<1
H	15000	2700	NT	1.4	<1
F	6600	2000	NT	2.7	1.3
Cl	NT	NT	39500	<1	<1
CH ₃	13000	460	39200	<1	<1
CF ₃	250	37	3200	<1	<1
OCF ₃	230	805	NT	<1	<1
CN	230	49	1800	<1	<1
CONH ₂	4300	20000	NT	33	4
COCF ₃	290	55	2000	<1	<1
SO ₂ CH ₃	420	170	2000	<1	<1

Scheme 1. Synthesis of non-triflone dulfonamides

Seeking to improve the on-target cell potency while maintaining oral activity, the authors decided to investigate a series of lipophilic, strongly-electron withdrawing triflones (Table 2). From this series, the methyl triflate was identified as having the same on-target cell activity as the nitro analogue. Table 3 lists final structural modifications made to improve oral absorption and metabolic stability. To that end, replacement of the dimethylamine with morpholine and the biphenyl with cyclohexenylphenyl group improved both solubility and metabolic stability, providing the improved development candidate **ABT-263**.

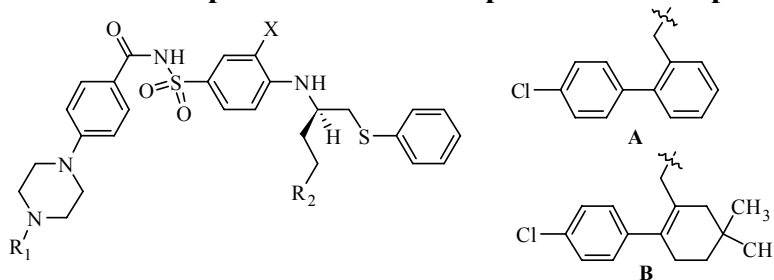
Synthesis of the triflone intermediates is given in Scheme 2 and the preparation of **ABT-263** is presented in Scheme 3.

Table 2. SAR of nitro group replacements: triflones



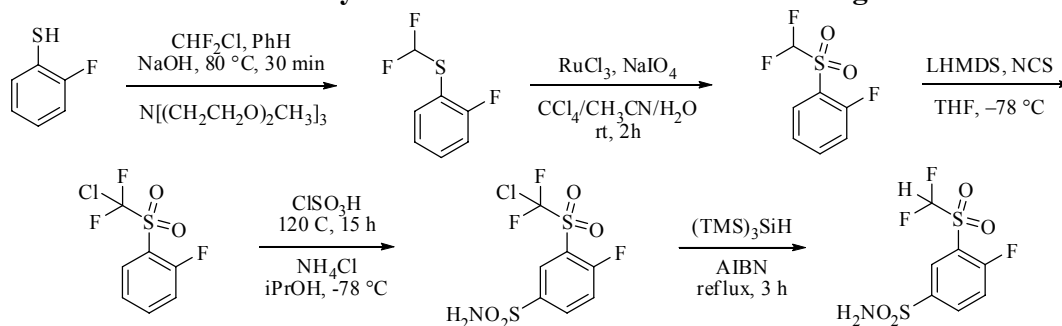
X	FL5.12 EC ₅₀ (nM, serum free)		H146 EC ₅₀ (nM)	FPA K _i (nM)	
	Bcl-X _L	Bcl-2	(10% HS)	Bcl-X _L	Bcl-2
NO ₂	9	4	86	<1	<1
SO ₂ CF ₃	15	4	39	<1	<1
SO ₂ C ₂ F ₅	69	25	630	<1	<1
SO ₂ <i>n</i> -C ₃ F ₇	2400	740	5100	<1	<1
SO ₂ <i>i</i> -C ₃ F ₇	3000	620	1940	<1	<1
SO ₂ CF ₂ H	NT	NT	180	<1	<1
SO ₂ CF ₂ Cl	NT	NT	21	<1	<1

Table 3. Multiple modifications improve oral absorption

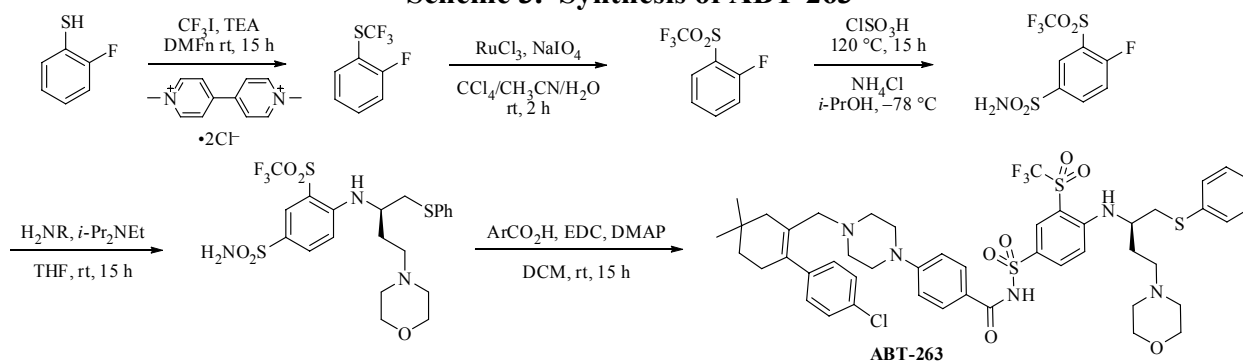


	R ₁	X	R ₂	Rat AUC (μM•h)	EC ₅₀ (μM)	AUC/EC ₅₀
ABT-737	A	NO ₂	NMe ₂	0.28 @ 5 mpk PO	0.086	3.3
	A	SO ₂ CF ₃	NMe ₂	0.83 @ 5 mpk PO	0.039	21.4
	A	SO ₂ CF ₃	morpholino	2.06 @ 5 mpk PO	0.083	24.9
ABT-263	B	SO ₂ CF ₃	morpholino	5.01 @ 5 mpk PO	0.110	45.5

Scheme 2. Synthesis of triflone sulfonamide analogues



Scheme 3. Synthesis of ABT-263



In summary, the Abbott team improved potency and oral absorption was obtained through SAR modification at 3 distinct sites (nitro, biphenyl, and dimethylamine) of **ABT-737** to provide **ABT-263**, which has high affinity for Bcl-X, Bcl-2, and Bcl-w ($K_i \leq 1$), has single agent activity against a subset of tumor cell lines including SCLC, lymphoma, and leukemia and is orally active in multiple *in vivo* xenograph models of SCLC. Oral dosing of **ABT-263** allows enhanced effectiveness in combination therapies and allows flexibility in dosing when combined with other agents and is currently in multiple phase I/IIA clinical trials for lymphoma, SCLC, and chronic lymphocytic leukemia as a joint development by Abbott Laboratories with Genentech, Inc.