

RECENT DISCLOSURES OF CLINICAL CANDIDATES

HIGHLIGHTS FROM THE SOCIETY OF MEDICINES RESEARCH SYMPOSIUM, HELD DECEMBER 4, 2014 – NATIONAL HEART & LUNG INSTITUTE, LONDON, UK

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SUMMARY

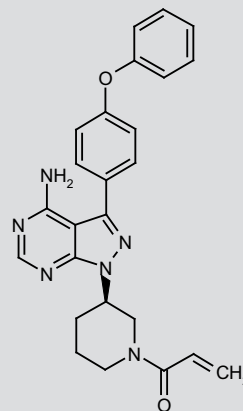
This symposium featured an international line-up of speakers presenting on the discovery and clinical development of novel therapeutic agents. The program included the Society of Medicines Research (SMR) Award Lecture, given by Dr. Betty Chang from Pharmacicyclics on the discovery and development of the marketed drug ibrutinib, as well as talks, several representing first U.K. disclosures, on Toll-like receptor 7 (TLR7) agonists, Na_v1.7 modulators, inhibitors of *Clostridium difficile*, Mdm2 inhibitors, muscarinic M₁ receptor agonists and β-secretase 1 (BACE1) inhibitors. The meeting was held at the National Heart and Lung Institute, Kensington, London, U.K. and was organized by the authors of this report.

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Key words: Ibrutinib – GSK-2245035 – CGM-097 – Ridinilazole

SMR AWARD LECTURE: BENCH TO BEDSIDE: FROM PCI-32765 TO IBRUTINIB TO IMBRUVICA

Dr. Betty Chang (Pharmacicyclics) charted the discovery of **ibrutinib** (PCI-32765) and its progression through clinical trials. Most of the early work about the function of Bruton tyrosine kinase (BTK) was gathered through studies of a genetic disease called agammaglobulinemia (XLA), where BTK is mutated and as a result is compromised in its expression leading to lack of mature B cells and no circulating immunoglobulins. At the mechanistic level activation of BTK by SYK and upstream kinases, phosphorylate and activate phospholipase C-γ (PLCγ) leading to Ca²⁺ mobilization and activation of NF-κB and mitogen-activated protein (MAP) kinase pathways (1). As a conse-



Ibrutinib

quence, BTK is an attractive target for selective B-cell inhibition therapy, such as the treatment for B-cell lymphoma, and autoimmune diseases, such as rheumatoid arthritis, lupus and glomerulonephritis. Aberrant breakpoint cluster region (BCR) signaling is a driver for B-cell malignancies, such as chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL) and Waldenström's macroglobulinemia (WM). As a consequence, BTK inhibition represents an attractive target for B-cell malignancies.

Ibrutinib represents the first in human BTK inhibitor. At the molecular level it forms a specific and irreversible bond with Cys-481 in BTK delivering potent inhibition ($IC_{50} = 0.5$ nM). The drug is orally available and once-daily dosing results in 24-hour sustained target inhibition. Preclinical proof of concept was established in lymphoma and other BTK-dependent models.

In terms of biochemical selectivity, ibrutinib inhibits a range of tyrosine kinases, many of them reversibly. However, due to the low AUC and fast clearance of the compound many of these kinases lack engagement in an in vivo setting. Due to existence of a Cys with homology to Cys-481, several other kinases are inhibited irreversibly, these include epidermal growth factor receptors (EGFRs) and interleukin-2-inducible T-cell kinase (ITK).

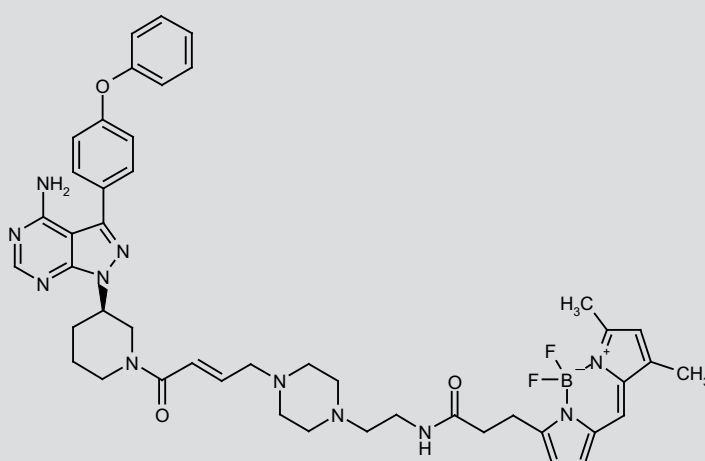
Ibrutinib is a potent inhibitor of adhesion and migration of B-lymphoma cells and sheds light on some of the early clinical observations (2). When CLL and MCL patients were on 29-day-on/7-day-off cycles in the first clinical study, the absolute lymphocyte count (ALC) went up and down each cycle. In contrast, when patients went on continuous drug treatment ALC did not fluctuate. The observations can be rationalized through different states of the adhesion and migration pathways, with continuous drug treatment driving an increase of ma-

lignant cells into the bloodstream. This process underpins the mechanism of action of ibrutinib through the egress of tumor cells into the peripheral blood with concomitant reduction of tumor burden and regression.

To investigate the pharmacodynamics effects of ibrutinib, a chemical derivative of ibrutinib with a fluorescent tag **I** was synthesized. The probe was shown to bind to BTK at Cys-481 and generate a single predominant band following SDS-Page treatment. Probe labeling is prevented by pretreatment with a BTK inhibitor. Results from a phase I study using the probe clearly demonstrated BTK occupancy at each dose level cohort in the study and confirmed sustained BTK occupancy in the absence of plasma levels of the drug (3).

A small percentage of patients relapse on long-term use of ibrutinib (> 1 year), which appears to be driven by the development of key mutations in BTK and subsequent activation of the BCR signal transduction pathway (4).

The clinical program for ibrutinib comprised several key trials. The first focused on refractory B-cell malignancies, where the overall response rate of 69% or more was above expectations (3). These results and the three breakthrough therapy designations that followed set the tone for additional positive and regulatory milestones. The early exciting results with ibrutinib in the phase I trial set the stage for a phase II CLL/SLL (small lymphocytic lymphoma) study involving treatment-naïve or relapsed/refractory patients. Patients were treated with oral once-daily ibrutinib (420 or 840 mg/day). For treatment-naïve, the overall survival rate was 97% after 30 months, with an estimated overall survival rate of 80% for relapsed/refractory patients. Encouragingly there appeared to be no increase in grade ≥ 3 adverse events by time to event onset. The phase III pivotal clinical study fo-



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cused on a head-to-head comparison of ibrutinib with ofatumumab in patients with previously treated CLL/SLL (5). Patients receiving ibrutinib realized a significant improvement in progression-free survival (median not reached vs 8.1 months for ofatumumab) and overall survival compared to patients receiving ofatumumab. Exposure-adjusted analysis showed no difference in any grade of infection and a 40% relative reduction in grade 3/4 infections comparing ibrutinib with ofatumumab (5). For MCL a phase II study was designed to investigate oral ibrutinib at a daily dose of 560 mg in 111 patients with relapsed or refractory MCL. Patients were enrolled into two groups, those who had previously received at least two cycles of bortezomib therapy and those who had received less than two cycles of bortezomib or no therapy. The primary endpoint was the overall response rate. Secondary endpoints were duration of response, progression-free survival, overall survival and safety. A response rate of 68% was observed, with a complete response rate of 21%. Prior treatment with bortezomib had no effect on the response rate. The estimated median progression-free survival was 13.9 months, with an estimated rate of overall survival of 58% at 18 months. The data suggested ibrutinib provided an opportunity for less intensive and more effective treatment regimens than those currently available for MCL. Safety data showed that grade 3 and 4 adverse events were uncommon with ibrutinib therapy. Beyond CCL and MCL, ibrutinib is currently in 13 phase III trials and 29 combination clinical trials, with drugs ranging from anti-programmed cell death protein (PD) checkpoint inhibitors, mTOR inhibitors, phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) inhibitors and obinutuzumab. Over and above U.S. and E.U. approvals to treat CCL and MCL patients, U.S. and E.U. filings have been submitted to treat WM.

THE DISCOVERY AND EARLY CLINICAL EVALUATION OF THE INTRANASAL TLR7 AGONIST GSK-2245035

Dr. Keith Biggadike (GlaxoSmithKline [GSK], Stevenage, U.K.) described the discovery and early clinical evaluation of the intranasal Toll-like receptor 7 (TLR7) agonist **GSK-2245035** for allergic asthma. Allergic asthma is driven by an aberrant T-helper 2 (Th2)-biased immune response to the exposure of innocuous allergens, resulting in the over production of Th2 cytokines that drive the differentiation, survival and functions of cells involved in the allergic cascade, such as mast cells, basophils and eosinophils. In addition Th2 cells trigger generation of allergen-specific IgE by B cells, which in turn mediate type I hypersensitivity reactions and local chronic allergic inflammation. As a hypothesis restoration towards a more balanced Th2/Th1 and regulatory T-cell response within an allergic airway could provide short-term beneficial impact regarding treatment of allergic asthma symptoms, and potentially provide a long-term modification of disease pathogenesis.

Human TLRs are a family of 10 transmembrane proteins, which on activation through recognition of pathogens or pathogen-derived products triggers both an innate and adaptive host defense mechanism that looks to eliminate the pathogen and preserve immune homeostasis. GSK focused on TLR7 stimulation given it is known to induce a strong Th1 interferon (IFN)-mediated immune response, and the receptor is predominantly expressed in plasmacytoid dendritic cells found in the airway mucosa, thereby allowing access to the target by intranasal (IN) administration. In addition to IFN- α release, activation of TLR7 can also lead to increased proinflammatory cytokines,

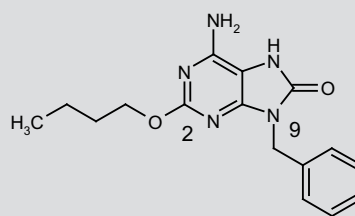
such as TNF- α . Hence, selectivity between the two responses represented a key goal of the program.

An initial high-throughput screening (HTS) campaign at GSK failed to deliver any novel TLR7 agonist lead structures. As a consequence a known class of TLR7 agonists, as exemplified by the 8-oxoadenine **[II]** (human PBMC pEC₅₀ INF- α 7), was taken and C-2/N-9 modifications investigated employing array chemistry.

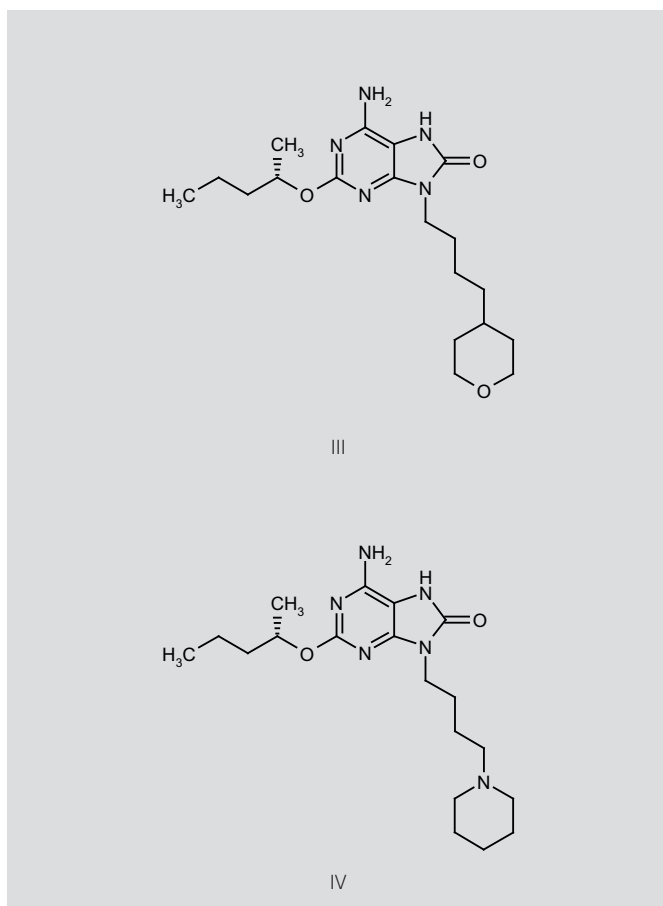
At C-2 *sec*-pentylloxy was identified as a novel potency enhancing substituent compared to an *n*-butylloxy. Replacement of the N-9 benzyl substituent with varying chain lengths carrying tetrahydrofuran and tetrahydropyran substituents delivered encouraging IFN- α potency with some selectivity over TNF- α induction. Compound **[III]** (human PBMC pEC₅₀ INF- α 7.4; pEC₅₀ TNF- α 6.8) represents an exemplar from the series. However, switching to a saturated nitrogen heterocycle in the N-9 side chain, as in compound **[IV]**, resulted in a dramatic enhancement of IFN- α potency (pEC₅₀ 9.4) without increasing stimulation of the unwanted cytokine TNF- α (pEC₅₀ 6).

Similar selectivity and potency boost was observed with a variety of appended basic piperidines and piperazines. Chain lengths of (CH₂)₄ or longer and 6 or 7 membered rings proved extremely beneficial. A reduction in basicity, such as the use of morpholine, delivered reduced IFN- α potency and an erosion of the selectivity window with TNF- α . From the optimization GSK-2245035 (structure undisclosed) was selected as the clinical candidate.

Compared to a PBMC readout GSK-2245035 demonstrated no loss of IFN- α potency or selectivity against TNF- α in a human whole blood setting (pEC₅₀ INF- α 9.05; pEC₅₀ TNF- α 5) (6). Induction of further additional cytokines, such as IFN- γ , IL-10 and IL-12p70, that are known to play a role in the downregulation of allergic inflammatory responses, were also observed (6). IN delivery of GSK-2245035 in Balb/C mice delivered a dose-dependent increase in serum levels of IFN- α and IP-10, with the latter being a much more sensitive biomarker of TLR7 activation. Given the comparable IFN- α /TNF- α cytokine profile between cynomolgus monkey and human whole blood, cynomolgus monkey was selected as the non-rodent species for safety assessment studies, which also incorporated PD markers of TLR7 activation. Intranasal dosing at 3 ng/kg resulted in upregulation of IFN-stimulated genes (ISG) in nasal scrape tissue, providing evidence for local engagement of TLR7 in the nose (6). To compensate for lack of any preclinical disease model, GSK-2245035 demonstrated inhibi-



II



tion of allergen (Timothy Grass or House Dust Mite) driven release of the Th2 cytokine IL-5 while enhancing Th1 cytokine release (IL-10 and IFN- α) (6). Dose selection for first-time-in-human (FTIH) studies was based on the concept of MABEL (minimum anticipated biological effect level), using cynomolgus monkey as the most relevant species. The MABEL was set at 3 ng/kg based on the observed upregulation of ISG expression in monkey nasal tissue. Scaling to human equivalent dose followed by applying a safety factor of 10 set the starting dose for FTIH of 2 ng.

The phase I study centered on a randomized, double-blind, placebo-controlled study to assess the safety, tolerability and pharmacokinetics of single escalating IN doses of GSK-2245035 in human volunteers (n = 33) and a cohort of seasonal allergic rhinitis (AR) patients (n = 28) during the pollen season. Target engagement was assessed via TLR7-inducible biomarkers in nasal scrapes, nasal lavage and serum. The highest dose in the human volunteer arm amounted to 100 ng, dropping to 40 ng in the AR patient group. All subjects completed the trial, with the only significant adverse events reported relating to TLR7-induced cytokine release syndrome (CRS). This appeared to be dose related in the human volunteer arm, kicking in at doses \geq 40 ng. In contrast, there appeared to be no dose relationship in the AR arm, with absence of any CRS events at the highest dose administered. All CRS events resolved completely within 48 hours. There were no signs

of nasal irritancy and only one subject gave a quantifiable serum PK concentration. Target engagement in AR patients was confirmed via increases of IP-10 levels in nasal lavage at doses of 20 and 40 ng, which returned to baseline at 96 hours, and upregulation of IFN responsive genes via nasal scrapes. On the back of this successful study a further randomized, double-blind, placebo-controlled study to evaluate the safety and tolerability of four repeat weekly 40- or 80-ng doses of IN GSK-2245035 in patients with symptomatic AR and mild asthma during the pollen season was initiated. One of the principle aims of the study was to determine whether repeat dosing resulted in tolerization or amplification of the PD response, as reflected by either local or systemic TLR7-inducible biomarkers. Although CRS events were reported, all were of mild intensity, resolved within 24-48 hours, and more importantly did not develop in the same subject following each weekly dosing, confirming lack of evidence of tolerization or amplification. A trial focused on evaluating the safety and tolerability of eight repeat doses of IN GSK-2245035, administered at 20 or 80 ng once weekly to patients with symptomatic AR during the pollen season, has completed with study results pending.

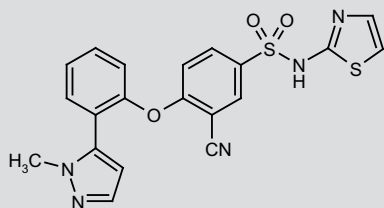
One interesting hypothesis was presented as to why introduction of a basic amine dramatically increased IFN- α release without affecting the TNF- α axis. It was suggested this stemmed from compartmentalization of the basic compound in the acidic late endosomal compartment (pH 4.5) where TLR7 activation occurred. In contrast, it was proposed TNF- α release was restricted to an early endosomal compartment where the pH environment was much less acidic (pH 6.5).

A NOVEL SUBTYPE SELECTIVE Na_v1.7 MODULATOR FOR THE TREATMENT OF PAIN

Dr. Ian Storer (Pfizer Neusentis, U.K.) described the discovery, lead optimization and early preclinical profile of PF-05241328, a selective Na_v1.7 modulator for the treatment of pain. The Na_v family of voltage-gated ion channels that control flow of Na⁺ ions across all membranes is comprised of 9 members. Structurally the ion channel comprises of a large α -subunit and several β -subunits which cycles through resting, open and inactivated states. Unselective Na_v blockers have been known for many years, but chronic use is limited by either CNS (Na_v1.1/1.2) or cardiovascular (Na_v1.5) side effects. Genetic mapping of several pain syndromes, such as inherited erythromelalgia (IEM) (7) and congenital indifference to pain (CIP) (8), identified Na_v1.7 as a potential target for modulation of pain and sparked the search for subtype selective inhibitors. The target profile adopted by Pfizer is outlined below:

- Na_v1.7 IC₅₀ < 30 nM with lipE (LLE) > 5 and LE > 0.35
- Good drug-like physicochemical properties (logD 2-3; Mol weight < 450)
- Selectivity > 100 x over other Na_v subtypes
- No significant CYP inhibition (DDI risk)
- Good PK with minimal in vitro to in vivo mismatch to aid dose prediction

An interesting compound identified lay in **PF-04856264**, which showed Na_v1.7 selectivity over Na_v1.3 and Na_v1.5. More importantly, by using mutated Na_v1.7 ion channels the binding site of the acidic compound was mapped to a novel voltage sensor domain (VSD) binding site, which was hypothesized to be a driver for subtype selectivity (9).

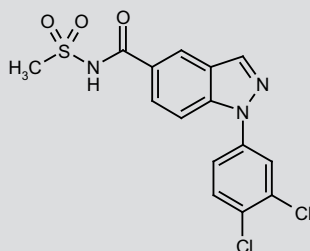


PF-04856264

The VSD binding site is lined with basic Arg residues offering the opportunity to form favorable binding interactions with the acidic fingerprint of the molecule. Unfortunately, PF-04856264 suffered poor PK and CYP inhibition issues. As a follow through a combination of file screening and design led to identification of the indazoleacetyl sulfonamide **PF-05078895**. This compound as an early lead demonstrated an encouraging subtype Na_v selectivity and drug-like properties, but appeared endowed with phosphodiesterase 5 (PDE5) activity.

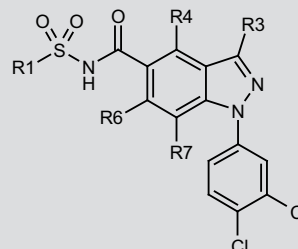
Further optimization focused on $\text{Na}_v1.7$ potency using LLE as a metric driver and dialing out PDE activity. Using the indazoleacetyl sulfonamide template **[V]** there appeared limited scope in terms of $\text{Na}_v1.7$ potency for R1 modification, with methyl or ethyl optimal. Core methyl substitution at R4, R6 and R7 was not tolerated. In contrast, small polar and lipophilic groups at R3 pushed the $\text{Na}_v1.7$ potency towards 100 nM with concomitant optimization of LLE.

Using modeling to help focus design, in conjunction with enabling parallel chemistry, it was discovered that replacement of the dichlorophenyl group with a methoxypyridine, as exemplified in **PF-05224064**,



Primary pharmacology: Na_v (IC_{50} μM): h1.7 = 1; h1.5 = 110
 Secondary pharmacology (IC_{50} μM): hERG > 22; PDE5 = 2.4; A_{2A} = 9.6
 Safety and DDI: IVMN & AMES negative; CYP inhibition IC_{50s} > 30 μM
 ADME: HLM < 8 $\mu\text{L}/\text{min}/\text{mg}$; RRCK 8.6×10^6 cm/s
 Physicochemical properties: logD = 1.3; solubility (pH 6.5) = 229 μM
 Molecular properties: Mol weight = 384; LLE = 5.3; LE = 0.35

PF-05078895

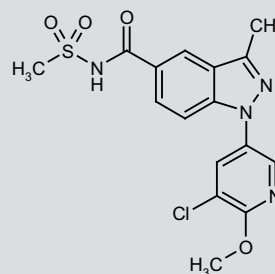


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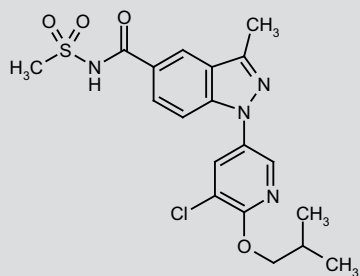
increased LLE significantly in addition to opening up further optimization opportunities. Although PF-05224064 remained an active $\text{Na}_v1.7$ inhibitor (IC_{50} 120 nM) it still carried unwanted PDE activity (PDE5 IC_{50} 460 nM).

To address PDE activity, a co-crystal of PF-05224064 in PDE5 was obtained and overlaid with other known PDE5 series. From the exercise it was hypothesized that increasing the size of the alkoxy group could remove PDE activity. Growing the methoxy to a 2-methylpropoxy gave **PF-05241328** and an increase in $\text{Na}_v1.7$ potency (IC_{50} 20 nM) and subtype selectivity. As predicted PF-05241328 delivered increased selectivity windows with respect to the PDE family and a safety, DDI and in vitro ADME profile that matched closely the early lead PF-05078895.

Based on its robust profile, PF-05241328 was profiled in rat and dog in vivo PK studies. Although in both cases the total clearance was low to moderate (rat Cl 6 mL/min/kg; dog Cl 16 mL/min/kg), factoring in plasma protein binding (ppb) (rat ppb 99.9%; dog ppb 99.5%) yielded free clearances of extreme values (rat Cl 3100 mL/min/kg;



PF-05224064



Primary pharmacology: Na_v (IC_{50} μ M): h1.7 = 0.02; h1.5 = 20; h1.1 = 1.4; h1.2 = 0.6; h1.3 = 1.9; h1.4 = 0.7; h1.6 = 2.4; h1.8 = 40
 Secondary pharmacology (IC_{50} μ M): hERG > 40; PDEs > 3; no hits in Cerep panel
 Safety and DDI: IVMN & AMES negative; CYP inhibition IC_{50} s > 30 μ M except 2C9 = 12 μ M
 ADME: HLM 14 μ L/min/mg; hhep 12 μ L/min/million; rhep 13 μ L/min/million; RRCK 4.2×10^6 cm/s
 Physicochemical properties: logD = 2.1; solubility (pH 6.5) = 150 μ M
 Molecular properties: Mol weight = 436; pKa = 3.5

PF-05241328

dog Cl 2605 mL/min/kg) and a miss match in the relationship between in vitro to in vivo. Human predictions would force a prohibitively high dose (\gg 1 g). The PK profile of PF-05241328 was typical of the series. In seeking an explanation, further studies of the acidic series revealed evidence of OATP and MRP transporter clearance driven mechanisms, typical of high molecular weight and amphiphilic acids. Unfortunately, human PK prediction with transporter-mediated Cl is extremely difficult, in part due to differences in transporter homology and expression between species. To address human clearance, PF-05241328, along with three other compounds (structures not disclosed but from a different chemical series), were taken to i.v./p.o. microdose studies (100 μ g dose). Although total clearance for PF-05241328 was 7 mL/min/kg, unbound clearance amounted to 3520 mL/min/kg, translating into extremely high dose predictions. As a consequence a decision was taken to abandon further development of PF-05241328. However, a compound from the microdosing PK of the other three compounds was identified and selected for further clinical evaluation.

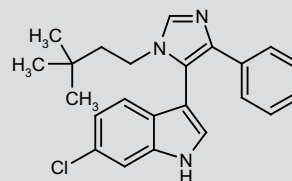
DISCOVERY OF CGM-097 – A HIGHLY POTENT AND SELECTIVE MDM2 INHIBITOR

After a short discussion of the rationale for targeting Mdm2 inhibition Dr. Philipp Holzer (Novartis, Switzerland) addressed some of the challenges of pursuing protein-protein interactions and the Novartis approach to identifying potent, selective compounds efficacious in in vivo models of cancer leading to the clinical candidate **CGM-097**.

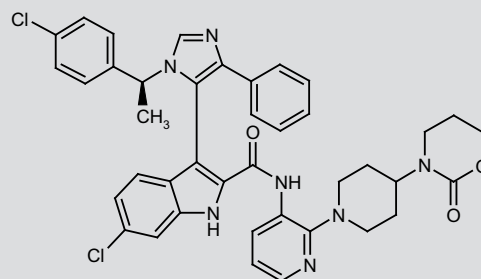
Mdm2 gain of function suppresses the activity of a key cell cycle regulator p53 in cancer by mediating its degradation. Thus, it has been proposed that inhibition of p53-Mdm2 binding will activate p53 and

will inhibit the growth of Mdm2-amplified cancer cells. A corollary of that is that p53 mutant cancer cells will be resistant to Mdm2 inhibition. Particular features of a protein-protein interaction that were highlighted as needing consideration were the type of secondary structures, strength of the interaction, buried surface area, residue conservation, the role of bound water, hydrogen bonding, the presence of salt bridges and the identification of binding hot spots in what are often large interaction surfaces. Early peptide scanning work starting from a 15-mer P53 N-terminal domain identified an 8-mer with a key 6-chlorotryptophan residue, which gave a 63-fold enhancement in activity relative to the corresponding tryptophan (10). The peptide work allowed generation of a three-point interaction hypothesis which, rather than connecting using an extensive peptide like backbone with consequent molecular weight and physicochemical liabilities, were connected via a minimal structural platform to give a pre-organized conformation of the interaction motifs. Based on this a 6-chloroindolyl imidazole was identified which could support the additional two interaction points. From this design strategy the ligand **[VI]** (IC_{50} TR-FRET 2.7 μ M) was identified, which following optimization, resulted in the highly potent indolylimidazole **[VII]** (IC_{50} TR-FRET 0.0012 μ M) (11). This compound was efficacious in cell assays (GI_{50} SJS-A-1 = 0.12 μ M), and at 175 mg/kg in a mouse xenograft SJS-A-1 model gave substantial reduction in tumor volume. Furthermore, a PD readout of increased p21 mRNA/GAPDH was also noted.

Seeking to extend chemical space a knowledge-based virtual screen of 50,000 cherry picked compounds exploiting the three hotspot 2D/3D pharmacophore was undertaken (12), resulting in the identifi-

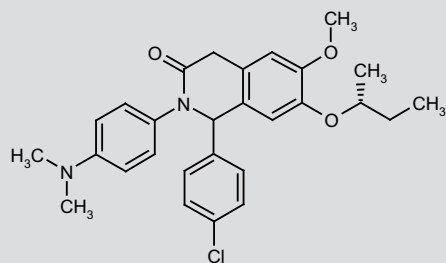


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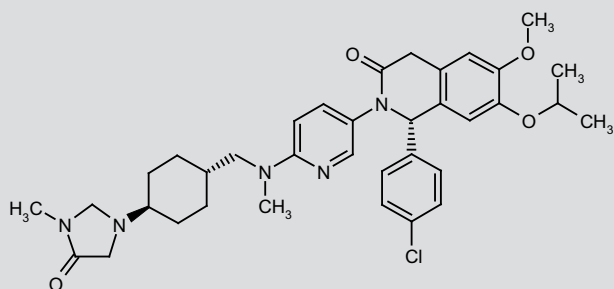


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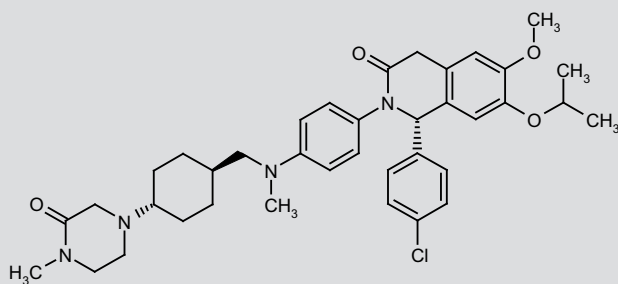
cation of a series of isoquinolinones. During the subsequent optimization of this series, X-ray crystallography indicated that there was a novel binding mode with some changes in protein organization (particularly involving His 96 interactions). Crystallography was used extensively to support lead optimization to low nanomolar potency ligands. Initial optimization gave **[VIII]** (IC_{50} TR-FRET 0.1 μ M) with modest cell activity (GI_{50} SJS-1 = 11.8 μ M). Introduction of a weakly basic center in the form of an oxoimidazolidine **[IX]** gave both improved target activity and much improved cell activity (IC_{50} TR-FRET 0.002 μ M, GI_{50} SJS-1 = 0.16 μ M). Unfortunately, compound **[IX]**, while stable in rat liver microsomes, was unstable in both monkey and human microsomes and had high in vivo monkey clearance. Further modification led to the clinical candidate **CGM-097** (IC_{50} TR-FRET 0.002 μ M, GI_{50} SJS-1 = 0.29 μ M) with excellent rat and monkey PK profiles and low human intrinsic clearance.



VIII



IX



CGM-097

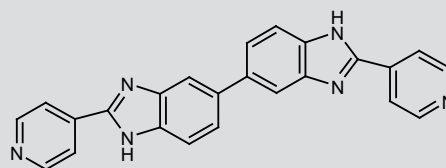
Detailed profiling of CGM-097 revealed cross species variability in inhibition attributed to the presence of Ile 54 and 57 in rat and mouse giving clashes not seen with the corresponding Leu of man and cynomolgus monkey. CGM-097 selectively inhibits p53/Mdm2 over p53/Mdm4, has negligible cell activity in p53 null cell lines and induces p53 accumulation and translocation to the nucleus. CGM-097 inhibits tumor growth in vivo in a p53-dependent manner in an HCT-116 mouse model. Furthermore, it activates p53-dependent transcription in vivo as measured by elevation of p21 mRNA. Finally, CGM-097 shows dose-dependent efficacy with complete regression demonstrated in a SJS-1 tumor-bearing rat model.

CGM-097 has been selected for clinical evaluation. Patients are pre-selected in clinical phase I for p53WT and recruitment is ongoing. Clinical indications of interest are those with a low prevalence of p53 mutation, with particular interest in tumors with Mdm2 amplification. There may be considerable potential for CGM-097 to be used in combination in selected indications.

RIDINILAZOLE: A SELECTIVE THERAPY FOR CLOSTRIDIUM DIFFICILE INFECTION

Dr. Richard Vickers (Summit, U.K.) gave an excellent overview of the *Clostridium difficile* field followed by a discussion of the profile of the phase II clinical stage compound **ridinilazole** (SMT-19969).

C. difficile is a spore-forming anaerobe that is the causative agent of pseudomembranous colitis. It is widely spread in the environment—particularly in rivers (80% give positive samples) and swimming pools (~50% positive samples). It is also present in 1-3% of healthy adults and in the majority of children under 12 months of age. Notwithstanding that it is seen by the CDC as one of the top three antimicrobial resistance (AMR) threats. Toxigenic strains produce toxins TcdA and TcdB that glycosylate host GTPases, while a third toxin, CDT, is produced by hypervirulent strains. The spores formed by *C. difficile* are highly resistant and persistent, and are a source of reinfection, a particular feature for this organism, making treatment particularly challenging. It is estimated that there are some 900,000 cases per annum in the U.S. and Europe. In the U.K. in 2007 approximately 41,000 cases were reported, with about 8,000 deaths. The great majority of infections—94%—are believed to occur in hospital although the infection may only manifest outside hospital. This is considered to be due to disturbed gut microbiota arising from antibiotic treatment in hospital in the presence of *C. difficile* spores. Mild symptoms



Ridinilazole

of illness include diarrhea and white blood cell count < 15 and necessitate antimicrobial treatment. Severe disease presents a more extensive range of potentially life-threatening symptoms—hypotension, fever $> 38.5\text{ }^{\circ}\text{C}$, white blood cell count ≥ 35 , ileus, fulminant colitis, megacolon—and can result in the need for colectomy and ileostomy.

Front-line agents for treating *C. difficile* are metronidazole and vancomycin, neither of which is entirely appropriate; for example, vancomycin should be reserved as a front-line treatment for multidrug-resistant bacteria such as multidrug-resistant *Staphylococcus aureus* (MRSA). A key issue is that while acute efficacy is good the risk of recurrence with both these agents is high. Recurrence has two consequences, firstly further recurrence becomes more probable, and secondly the disease becomes more severe with increased mortality. Fidaxomicin is a newly introduced drug which is superior to vancomycin against most strains, but may be associated with more limited efficacy against hypervirulent strains. However, all drugs damage the microbiota; studies with *C. difficile* susceptible versus resistant mice show that a diverse microbiome is consistent with a greater resistance to illness. From this fecal transplant has emerged as an approach to try to restore a more normal microbiota. This approach has an excellent 94% cure treatment from one clinical study, importantly with no recurrence. However, this approach is not quite as straightforward as it might appear, with identifying appropriate donors and avoiding infection transmission being just two of the issues. Finally, the FDA is urging the need for an IND, a challenge in its own right.

Moving on to ridinilazole, Dr. Vickers described the targeted spectrum of activity of this compound, which maintains similar high potency in vitro against all strains of *C. difficile* and unlike other agents, excellent selectivity with respect to both Gram +ve and -ve organisms indicative of likely conservation of the microbiota. Ridinilazole is bactericidal with > 5 log reduction in CFU/mL after 24 hours, and also significantly and dose-dependently reduces sporulation rates com-

pared to vancomycin. In in vivo models significant protection from acute and recurrent infection was achieved with consistent activity against multiple strains including the hypervirulent O27 strains. Furthermore, 100% survival was seen during acute infection, and 80-100% survival during the period of high risk of recurrence during the post-treatment period. A desirable low systemic exposure (below LOQ [1 ng/mL]) was found in plasma of ridinilazole-treated animals, while gastrointestinal concentrations were significantly above MIC, and a marked reduction in spores from fecal samples was observed. Almost all drug (97%) was excreted via the feces.

Phase I single and multiple ascending dose studies have been successfully completed with negligible systemic exposure demonstrated from 200 or 500 mg b.i.d. dosing, and fecal concentrations $> 1,000$ -fold MIC following a 200-mg b.i.d. dose. Furthermore in these subjects only clostridia bacteria were shown to be reduced in the gut microbiota (Fig. 1). SMT-19969 was awarded Qualified Infectious Disease Product status by the FDA granting priority review and a 5-year extension to market exclusivity. A 100-subject phase II clinical trial is currently in progress. The project was supported by SDDI and translational funding from the Wellcome Trust.

DISCOVERY OF STRUCTURALLY NOVEL, CENTRALLY ACTIVE, β -SECRETASE 1 INHIBITORS FOR THE TREATMENT OF ALZHEIMER'S DISEASE

Dr. Jack Scott described progress from the Merck & Co. β -secretase (BACE) inhibitor program, which has recently identified a clinical candidate that targets Alzheimer's disease (AD).

It is estimated that 22-30 million patients suffer from AD, which is anticipated to rise to some 35-50 million by the year 2030. Current therapies such as Aricept® (donepezil) and Namenda (memantine) provide modest benefits only.

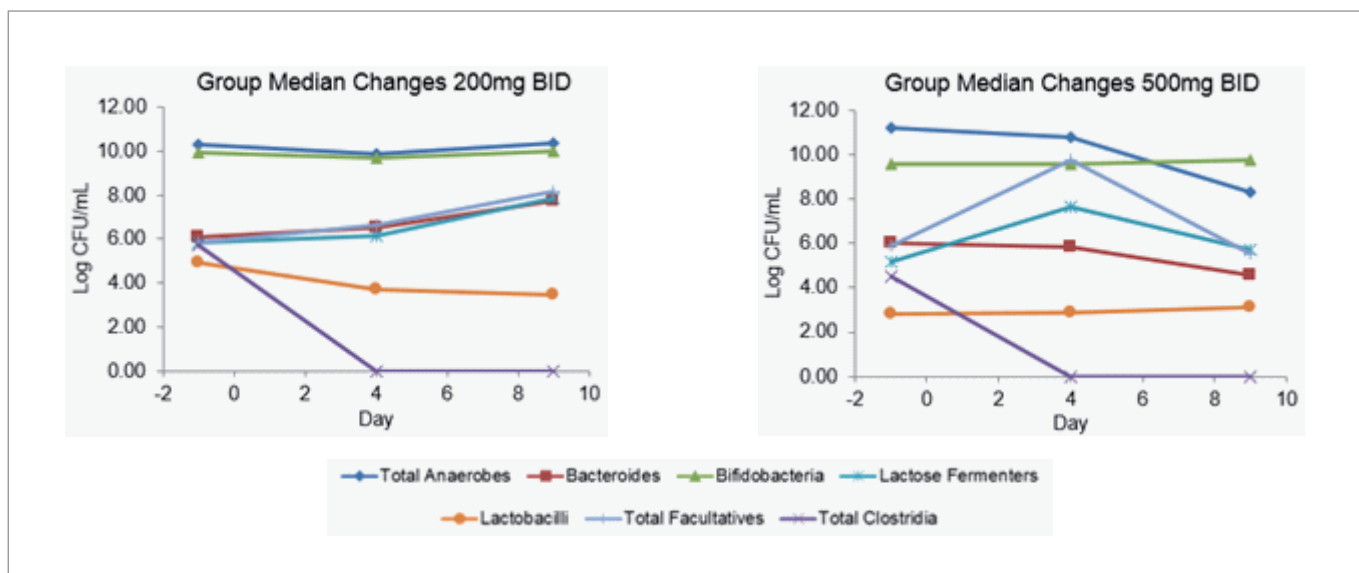


Figure 1. Changes in composition of gut microbiota as analyzed from fecal samples in phase I clinical studies of ridinilazole.

The hypothesis being explored with inhibitors of BACE1 is that β -amyloid plaques and tau tangles are produced as part of AD pathology, in which BACE1 acts on amyloid precursor protein at the first step of the amyloidogenic cascade. Inhibitors of BACE1 should reduce β -amyloid plaque production, and if the overall amyloid hypothesis is correct, thereby halt AD progression (13).

BACE1 is a membrane-bound aspartyl protease localized intracellularly in neurons in the brain inside an acidic endosomal compartment, with a relatively shallow and hydrophilic binding cleft, which has made identifying small-molecule drug candidates very challenging (14). Classic peptidomimetics have been reported in the literature but are P-glycoprotein substrates, limiting their CNS exposure (15). The Merck group therefore initiated their own HSQC NMR-based screen of a 10K-member fragment library with hits confirmed by X-ray crystallography. One of the most promising initial hits from this screen was an isothioureia series (16), which following some focused follow-up screening provided a small confirmed fragment hit with a BACE1 IC_{50} of around 200 μ M (Fig. 2.).

Crystallographic hit confirmation showed the amidine portion of the isothioureia formed a critical network of hydrogen bonds to the catalytic aspartates. Further designs sought to retain these features while tuning pKa and reducing hydrogen bond donors to provide a versatile iminohydantoin core (17). This provided an excellent starting lead for further elaboration. Optimization of the diphenyl substitution on the core led to sub-100 nM compounds at the cellular level. Further op-

timizations of the iminohydantoin to an iminopyrimidinone further improved functional activity (Fig. 3). An advanced lead, **[X]** (18), when dosed orally to rats was shown to translate into significant β -amyloid reduction in the cerebrospinal fluid and cortex with an ED_{50} of between 4 and 6 mg/kg.

The extensive structural information obtained throughout the program was used to propose further optimizations of the core template in which substitutions were designed to reach over into the prime side of the binding site (19). Further optimizations led to another advanced lead, **[XI]** (Fig. 4).

Together, **[X]** and **[XI]** provided excellent tool compounds to probe the pharmacology and efficacy of the BACE1 mechanism.

Merck have subsequently advanced a candidate, **MK-8931**, into clinical investigation. While the structure of MK-8931 was not disclosed, its early clinical data was described. The compound was well-tolerated in healthy volunteers and its pharmacokinetics were suitable for once daily dosing. It showed a profound reduction in cerebrospinal fluid β -amyloid peptides in AD patients following 7 days q.d. dosing that was dose dependent and sustained. Exposure-response profiling predicted that 12 and 40 mg MK-8931 would inhibit β -amyloid production by > 50% and > 75%, respectively, in the majority of AD patients. MK-8931 provides a unique opportunity to test the amyloid hypothesis of AD pathogenesis. MK-8931 is currently in two phase III trials (mild to moderate AD and prodromal).

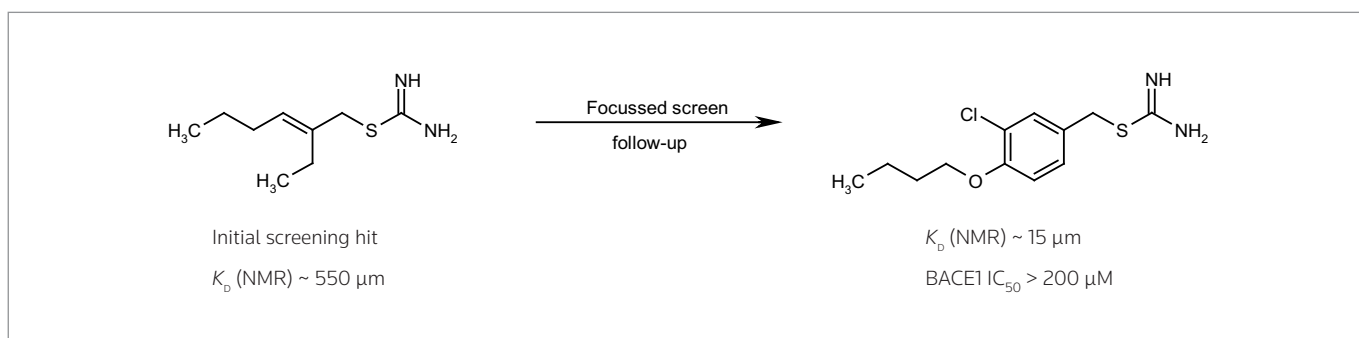


Figure 2. Screening and optimization of isothioureia BACE1 inhibitors.

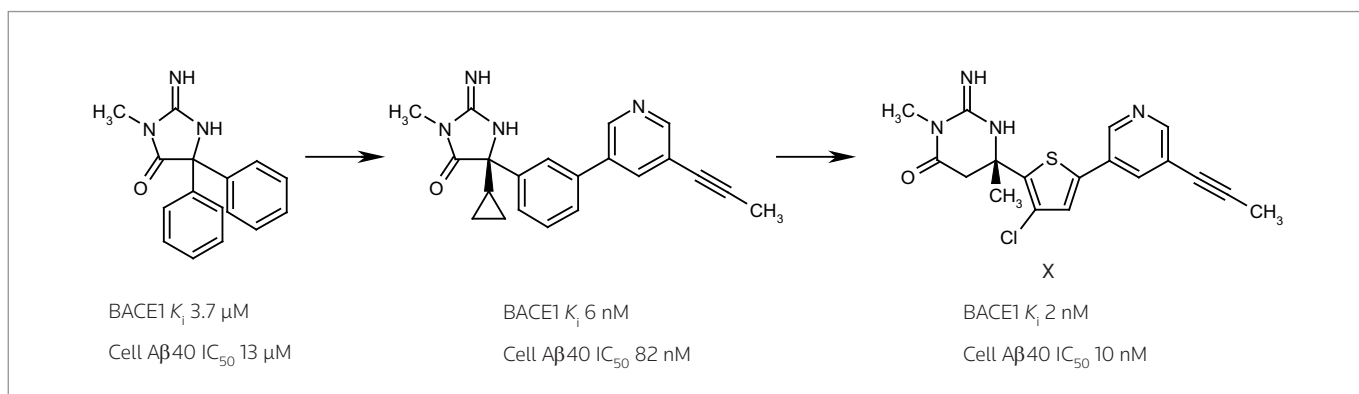


Figure 3. Optimization of iminohydantoin BACE1 inhibitors.

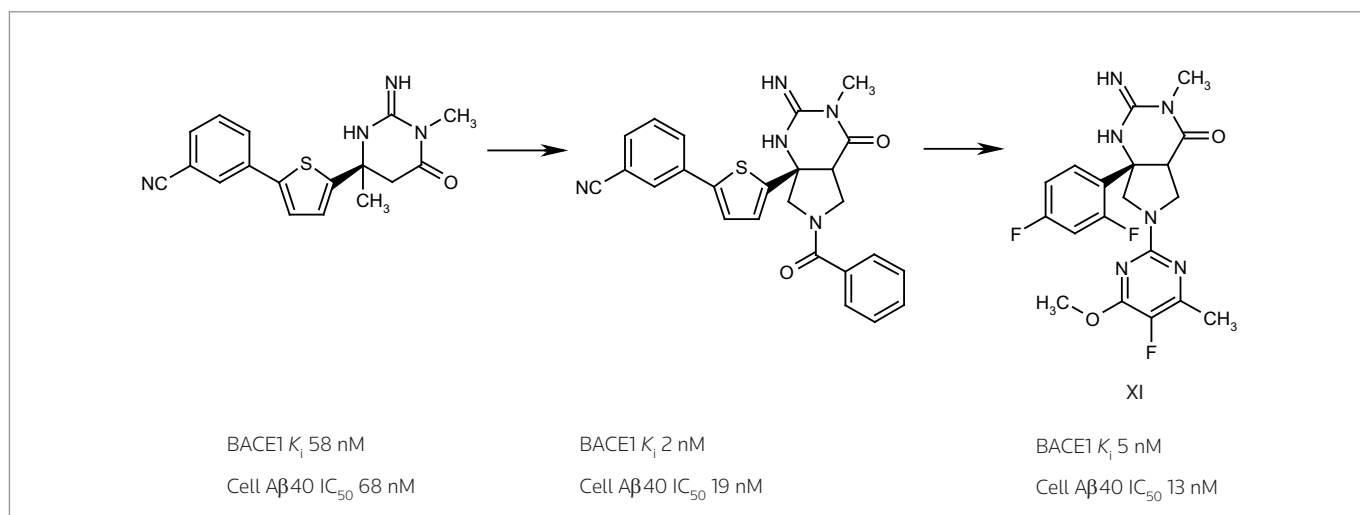


Figure 4. Further iminopyrimidinone optimization of BACE1 inhibitors.

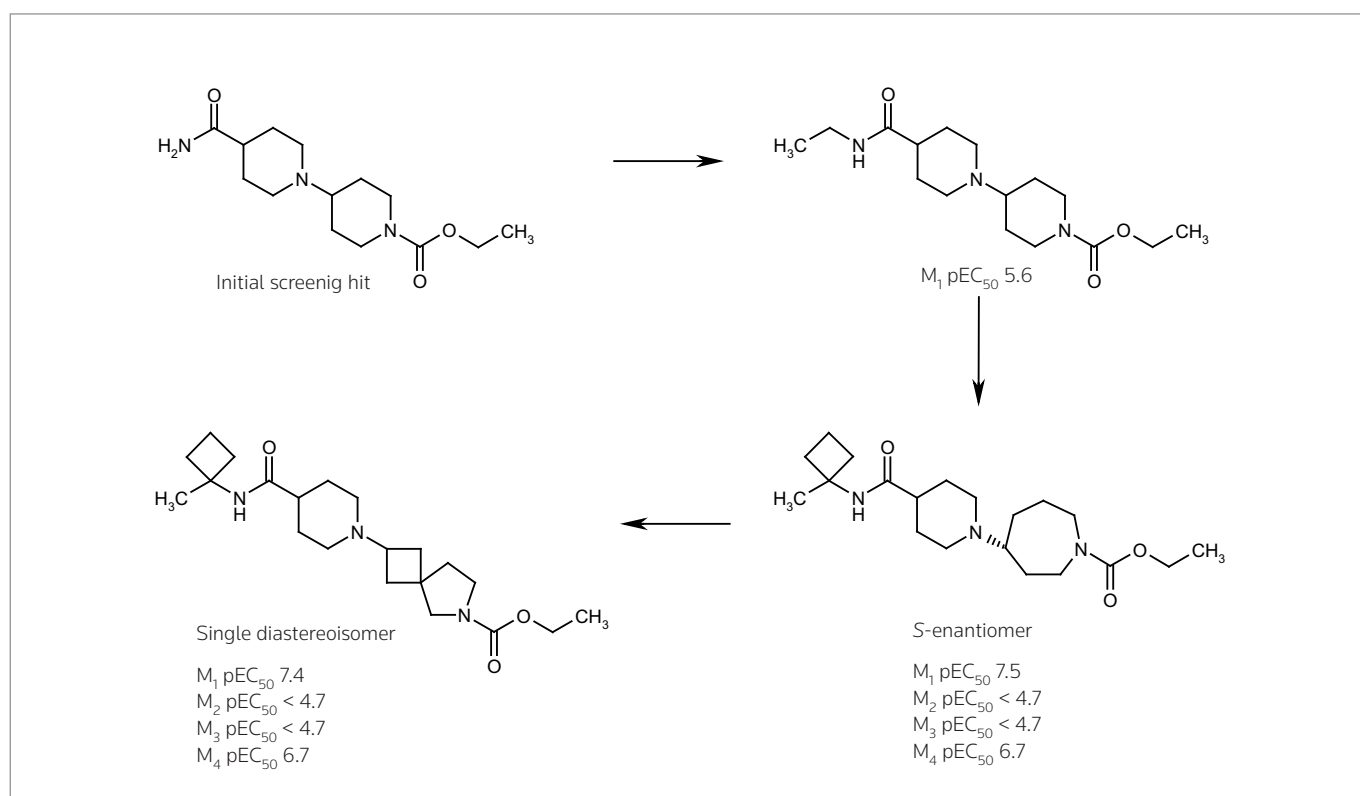


Figure 5. Identification of muscarinic acetylcholine M_1 receptor agonists.

THE DISCOVERY OF HIGHLY SELECTIVE MUSCARINIC M_1 RECEPTOR AGONISTS

Dr. Giles Brown (Heptares, U.K.) described a program that resulted in the identification of a highly subtype selective muscarinic M_1 receptor agonist for the treatment of AD using Heptares' stabilized receptor

technology. The cholinergic hypothesis explored with this approach is that in AD cholinergic neurons are lost in the basal forebrain. Acetylcholinesterase inhibitors prevent the breakdown of acetylcholine to deliver moderate efficacy. An alternate approach is to stimulate the muscarinic acetylcholine receptors either directly or allosterically (20).

It was highlighted that acetylcholinesterase inhibitors exhibit dose-limiting side effects which include gastrointestinal disturbances mediated largely by activation of the M₂ and M₃ receptor subtypes. Subtype selective M₁ receptor agonists would be expected to have fewer side effects and drive greater efficacy in AD patients than the current standard of care as the mechanism targets postsynaptic receptors and is independent of endogenous cholinergic drive.

The Heptares M₁ agonist project began with the construction of several stabilized muscarinic receptors in the agonist conformation. The insertion of an increasing number of stabilizing mutations improved the stability of the receptor in increasingly harsh detergents, making it suitable for crystallization with a range of competitor and in-house ligands of varying selectivity patterns. These structures, in concert with site-directed mutagenesis, were used to refine high confidence models for ligand binding to the M₁ receptor, which was then used to conduct virtual screening to identify hit molecules.

One of the most promising early hits was a bis-piperidine, confirmed through synthesis of close analogues to have micromolar-level activity. Further structure-activity relationship studies provided two advanced leads based on an azepine structure and a bicyclic spiro-fused system (21, 22) (Fig. 5). Both compounds showed good selectivity over the M₄ receptor (about 10-fold) and complete selectivity over the M₂ and M₃ subtypes.

The azepine compound (**HTL-9936**) was selected as the first development candidate from the program, based on a number of in vivo pre-clinical studies. For example, HTL-9936 reversed scopolamine-induced memory loss in a rat passive avoidance test and showed significant differences from baseline in an aged Beagle dog cognition test. Interestingly, some of the effects of HTL-9936 appeared to be synergistic with an acetylcholinesterase inhibitor. HTL-9936 has been scaled up to more than 1 kg scale, and is currently undergoing phase Ib clinical trials. A follow-on compound with differentiated pharmacokinetics is being progressed towards phase I in 2015.

DISCLOSURES

Rod Porter is owner of Rod Porter Consultancy, Steve Collingwood is a director of SPCR Consulting, Andrew Ratcliffe and David Pryde are employees of Redx Anti-infectives and Pfizer respectively. All authors are SMR committee members for which no remuneration is received.

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