Drugs of the Future 2011, 36(11): 867-871

CCC: 0377-8282/2011
DOI: 10.1358/dof.2011.36.11.1688491

MEETING REPORT

ROYAL SOCIETY OF CHEMISTRY'S 22ND SYMPOSIUM ON MEDICINAL CHEMISTRY IN EASTERN ENGLAND, HATFIELD, UK, MAY 19, 2011

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CONTENTS

NOVEL PROSTANOID DP_1 RECEPTOR ANTAGONISTS

The identification of a novel series of prostanoid DP_1 receptor antagonists as potential treatments for allergic rhinitis was described by Matthew Crawford (GlaxoSmithKline). The progress of one series of compounds over a 12-month period was described, from the design phase to the identification of a pre-candidate, before the program was terminated for strategic reasons. Two other series of compounds had also been explored. The strategy was to design compounds based on laropiprant (MK-0524) and SAR-389644, focusing on physicochemical properties, conformational analyses and efficiency. The aim was to prioritize and select compounds that appeared most suitable, and then prepare the planned set before screening commenced. This strategy led to the preparation of a series of bicyclic scaffolds, comprising a heterocyclic 5-membered ring substituted by both alkyl and *p*-chlorobenzyl groups fused to the 2- and 3-positions of a cycloalkyl acetic acid. 2-[1-(4-Chlorobenzyl)-2-isopropyl-4,5,6,7-tetrahydro-1*H*-benzimidazol-7-yl]acetic acid was highlighted as the most promising of these, with the (±)-enantiomer showing nanomolar potency as a DP_1 receptor antagonist and good activity in platelet-rich plasma. However, in that assay system, compounds were typically only 10-fold selective over the prostanoid TP receptor, which was viewed as a potential risk for prolonged bleeding time issues. Further exploration of this template indicated that the 2-isopropyl substituent was optimal and a *p*-chlorobenzyl group was desirable, as potency was enhanced by switching to the 3,4-dichlorobenzyl derivative (p*K*i = 8.7) (**I**). Cycloheptane analogues (**II**) appeared to offer a marginal potency improvement, whereas cyclopentane analogues were somewhat less potent. The attachment of an acid substituent to the 7-position was also determined to be optimal.

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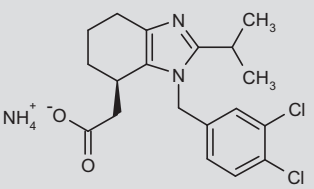
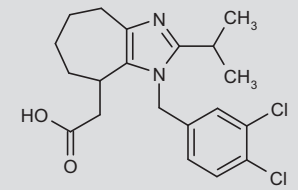
AN UPDATE ON THE SERINE/THREONINE-PROTEIN KINASE mTOR INHIBITOR AZD-8055

The immunosuppressant macrolide rapamycin exerts its biological effects primarily via inhibition of the atypical protein kinase mTOR. However, rapamycin does not completely inhibit the effects of the TORC1 complex and does not inhibit the TORC2 complex. Kurt Pike



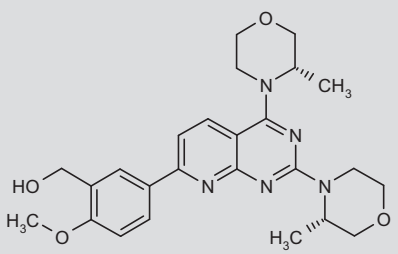
(I)



(II)

(AstraZeneca) described the identification and optimization of a series of selective mTOR inhibitors culminating in the development of compound **AZD-8055**, which inhibits both TORC1- and TORC2-mediated effects. The program originated at KuDOS Pharmaceuticals prior to its acquisition by AstraZeneca and involved the development of a pharmacophore model based on the similarity of the protein kinase mTOR to the phosphatidylinositol-4-phosphate 3-kinases (PI3Ks), and used the information available from LY-294002, PI-103 and NU-7441. However, although this led to the identification of a triamino-substituted triazine derivative with 300-nM potency, the less potent lead identified from a high-throughput screening (HTS) campaign was found to be more tractable.

Initial attempts to optimize this series led to the decision to pursue the less potent pyrido[2,3-*d*]pyrimidine-2,4-diamine leads. The original lead, with 1-μM potency, contained a 4-morpholino and a 2-(2-methylpiperidin-1-yl) substituent and was improved to generate **KU-63794**, with 16-nM potency, by introducing a 7-[3-(hydroxymethyl)-4-methoxyphenyl] substituent and switching the 2-substituent to a *cis*-2,6-dimethylmorpholine. KU-63794 had submicromolar activity in cellular assays and good selectivity relative to PI3Ks and DNA-dependent protein kinase (DNA-PK). The compound was also found to be effective against both mTORC1- and mTORC2-mediated effects and was more effective than rapamycin as an inhibitor of eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1) and Akt phosphorylation. Cellular potency was further enhanced by modifying both the 2- and 4-morpholino substituents to 3(*S*)-methylmorpholin-4-yl substituents to give AZD-8055, an ATP-competitive mTOR inhibitor with a potency of 0.8 nM. Improved potency and solubility trends were seen in studies using matched pairs with AZD-8055. Potency against three of the four PI3K forms, as well as serine-protein kinase ATM and DNA-PK, was in the low-micromolar range, while PI3K-δ was only weakly affected. In cellular assays, the extent of inhibition of S6 (TORC2) and 4E-BP1 (TORC1) phosphorylation was similar, with IC_{50} values of 27 and 23 nM, respectively. Good activity was also seen in xenograft models at doses of 2.5 and 5 mg/kg b.i.d. or 20 mg/kg q.d.

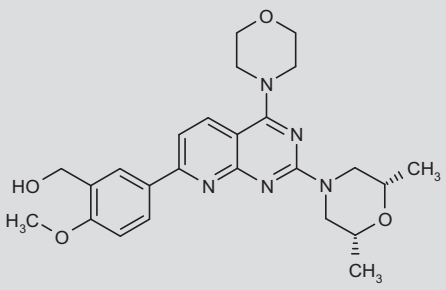


AZD-8055

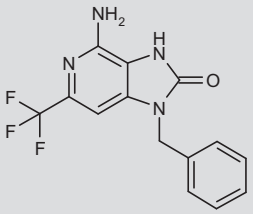
ALDEHYDE OXIDASE METABOLISM

Several enzymes are recognized to pose significant problems for drug development; however, the cytosolic enzyme aldehyde oxidase, which employs molybdenum ions as cofactors and is mainly expressed in the liver, has only recently been highlighted as a potential problem. The impact of this enzyme on the metabolism of drugs was highlighted by Thien Duc Tran (Pfizer). An example of Pfizer's Toll-like receptor 7 (*TLR7*) agonist program, which is based on the use of a 3-deazapurine scaffold, was provided. The initial lead, **PF-4171455**, was a reasonably potent agonist, but was very poorly soluble, with a moderately high logD value. Replacing the lipophilic trifluoromethyl and benzyl substituents with oxazole and 3-pyridylmethyl substituents, respectively, resulted in a 50-fold reduction in lipophilicity and a 66-fold increase in solubility, while maintaining potency. However, this change also had a major impact on drug clearance, with the second compound having a clearance that was much greater than liver blood flow compared with the clearance of PF-4171455, which was much less than liver blood flow in rats. This was shown to be attributable to the oxidation of the pyridine ring by aldehyde oxidase, which can be inhibited by raloxifene.

Aldehyde oxidase has been found not just to oxidize aldehydes, but also to be capable of oxidizing azaheterocycles and iminium compounds to lactones, as well as effecting reductive transformations of *N*-oxides, sulfoxides, nitro groups and ketones. It has previously been shown to be involved in the secondary metabolism of tolbutamide and in the ring oxidation of methotrexate. The actions of this enzyme also led to the abandonment of the clinical development of the inotropic agent carbazeran, when it was found that the drug had negligible bioavailability in man despite 68% bioavailability in dogs, because it was rapidly oxidized at the phthalazine ring by aldehyde oxidase to 4-oxocarbazeran. Dr. Tran indicated that the mouse is a



KU-63794



PF-4171455

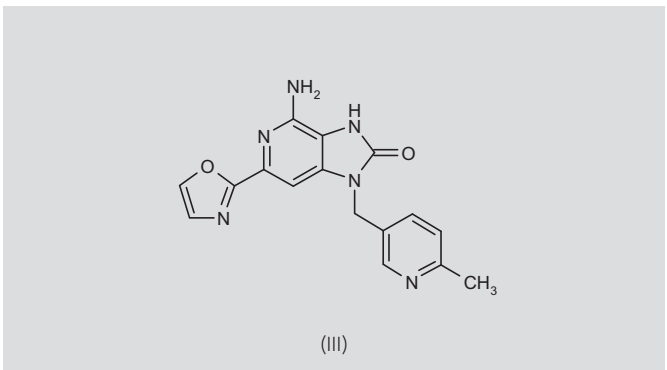
better species for predicting the impact of this enzyme, as the canine enzyme is rarely efficient at metabolizing drugs, with the exception of the oxidation of famciclovir (Famvir®) to penciclovir (Vectavir®).

To redress the problems in the Toll-like receptor 7 program, three approaches were found to attenuate this metabolic liability with a dramatic impact on plasma half-life values. Various analogues improved the half-life value from 102 minutes to greater than 1000 minutes, but both steric and electronic modulation could also be achieved. Substituting or replacing the oxazole substituent enhanced the half-life to over 1000 minutes, despite the increased bulk being remote from the metabolically liable center. Less surprisingly, the introduction of a substituent adjacent to the pyridine nitrogen attenuated the effect to varying degrees according to the electronic impact of the substituent (**III**). Another reason for this issue becoming more significant is the nature of compounds being prepared, and data from various diverse compound sets were presented showing that kinase inhibitors are more likely to be prone to the effects of this enzyme than either a fully diverse set of structures or a G protein-coupled receptor (GPCR)-biased set of compounds.

SERINE/THREONINE-PROTEIN KINASE CHK2 INHIBITORS

The checkpoint kinases Chk1 and Chk2 both play significant, but distinct, roles in the regulation of the cell cycle and represent possible targets for the development of new anticancer drugs that seek to disrupt the repair mechanism of cancer cells. Knockout of the serine/threonine-protein kinase Chk2 causes mice to become less susceptible to tumor development. The optimization of a series of Chk2 inhibitors leading to the selective inhibitor **CCT-241533** was described by John Caldwell (Imperial Cancer Research Technology). Two approaches were employed, an HTS campaign and a counter-screen of 78 kinase inhibitors. Optimization of compounds identified as potential leads from the latter revealed compounds with Chk2 selectivity based on a 2-phenyl-4-aminoquinazoline scaffold.

The original micromolar potent lead, with 10-fold selectivity over protein kinase D (PKD), contained 2-(*o*-hydroxyphenyl) and 4-(dimethylamino)pyrrolidinyl substituents. Optimization was hindered by the inability to co-crystallize the lead with the kinase. It was found that the phenol was essential for activity. Potency and crystallization were improved when the cyclic substituent at position 4 was modified (**IV**). The crystal structure showed the interaction to be with the hinge region of the ATP site of the active form of the kinase, which rationalized the importance of the phenolic hydroxyl group. Fluorination of the phenol ring was found to enhance potency, with 5-fluorination having the most dramatic effect and generating an inhibitor with a potency of 60 nM. This analogue had good selectivity over Chk1 but significant hERG liability. Exploration of quinazoline ring substitution indicated that 6- and/or 7-substitutions were acceptable for improving activity, with 6,7-dimethoxy substitution offering the greatest selectivity. The use of a 3-pyrrolidinylamino substituent at position 4 was found to be best for potency and hERG liability, while selectivity was enhanced by adding a 4-(1-hydroxy-1-methylethyl) substituent to the aforementioned substituent. The resulting compound, CCT-241533, had an IC_{50} value of 3 nM, showed 63-fold selectivity over Chk1 (IC_{50} = 190 nM) and had an IC_{50} value of 22 μM against the hERG channel. CCT-241533 also had good solubility, permeability and a 50% growth inhibition value of 1.4 μM in human colon adenocarcinoma HT-29 cells. In vivo, CCT-241533 did not act as a sensitizer to gemcitabine (Gemzar®) in mice bearing human colon adenocarcinoma SW620 tumor xenografts, in contrast to selective Chk1 inhibitors such as SAR-020106 (Cancer Research Technology/Institute of Cancer Research/Sareum). However, CCT-241533 protected against the effects of ionizing radiation and potentiated the actions of poly [ADP-ribose] polymerase (PARP) inhibitors.



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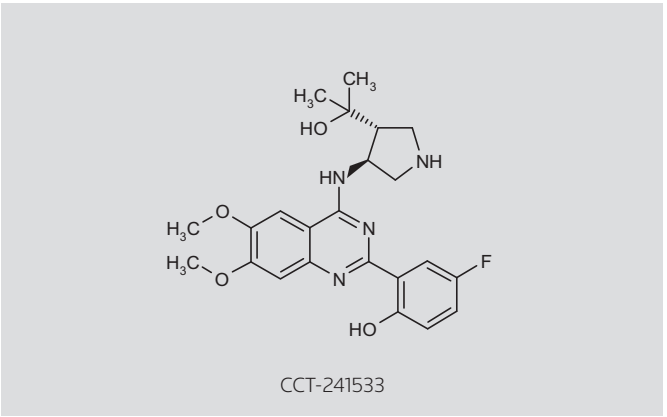
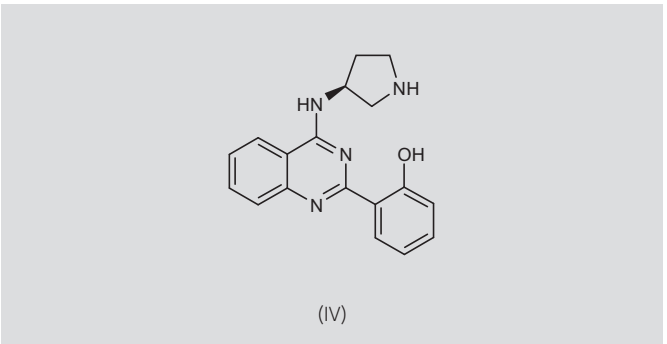


CCT-241533

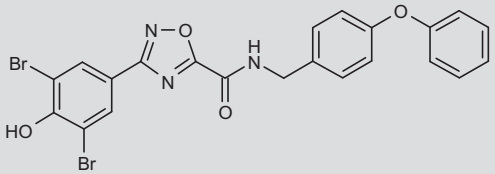


(IV)

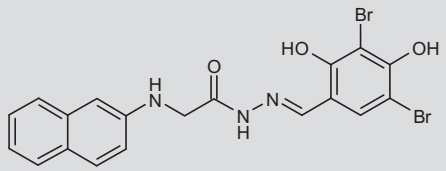
CFTR CHANNEL BLOCKERS

The cystic fibrosis transmembrane conductance regulator (CFTR) chloride channel is highly expressed in the gut epithelium, stimulat-

ed by the actions of cholera toxin, and thus plays a major role in the secretory diarrhea that is characteristic of cholera infection and the cause of the high mortality rate. Although the consequences of the disease can be alleviated by oral rehydration therapy, there is a need for an adjunct treatment that reduces diarrhea. Kevin Doyle (BioFocus) described a collaboration with the Institute for OneWorld Health directed towards the identification of selective CFTR blockers suitable for such use. These agents must be safe for pediatric use, stable, orally active for use over 2 or 3 days and have a low cost, while also having low oral bioavailability and a fast onset of action. This collaboration led to the identification of **iOWH-032**, which has recently commenced phase I trials. The small-molecule CFTR inhibitors **GlyH-101** and CFTRinh-172 were found to be active in animal models of secretory diarrhea, and were reported to act extra- and intracellularly, respectively. GlyH-101 was used as the basis for the design of compounds, replacing the hydrazone linker with a tricyclic ring. The 1,2,4-triazole and 1,2,4-oxadiazole rings appeared to offer the best activity, albeit slightly less than that of GlyH-101. Replacing the β-naphthylamine with isosteres such as 3-trifluoromethylbenzyl or 4-phenoxybenzyl provided compounds with comparable potency. Furthermore, the 3,5-dibromo-4-hydroxyphenyl group was found to be essential, although the use of chloro-substituted analogues offered comparable potency. The compounds were screened using an IonWorks Quattro device in conjunction with CHO cells expressing CFTR. The activity of the compounds was found to be variable in human colon carcinoma T84 cell monolayers; however, this assay was useful for assessing which compounds offered a fast onset of action. iOWH-032 was one of the few compounds that demonstrated activity in a murine intestinal closed loop model at 10 μg. It was further shown to be orally active in cecectomized rats, reducing fecal output at a dose of 5 mg/kg. As iOWH-032 could be economically prepared in three steps from readily available starting material, it was progressed into development.



iOWH-032

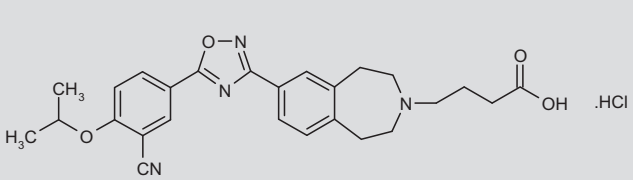


GlyH-101

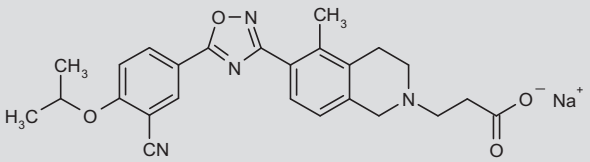
SELECTIVE LYSOPHOSPHOLIPID $S1P_1$ AGONISTS

Sphingosine 1-phosphate (S1P) acts on a family of five GPCRs to exert diverse biological effects, with $S1P_1$ and $S1P_3$ considered to be the two most significant S1P receptors. $S1P_1$ receptors are primarily expressed on lymphocytes and are implicated in cell trafficking and maintaining endothelial barrier function, while $S1P_3$ receptors are highly expressed in the heart, lung and spleen, and appear to have a cardiovascular function. The first-in-class $S1P_1$ receptor agonist fingolimod (Gilenya™) was recently approved for the treatment of multiple sclerosis and is a prodrug that is phosphorylated to generate the active phosphate. However, the phosphorylated version can also activate $S1P_3$ receptors, causing bradycardia and increased blood pressure. The agent also has a long half-life and behaves as a functional antagonist once internalized. Jack Brown (GlaxoSmithKline) described an effort directed toward identifying more selective $S1P_1$ receptor agonists that lack the sustained half-life of fingolimod and that behave as conventional agonists. This led to the identification of compounds with the desired profile, but comparable efficacy to fingolimod in animal models.

The program's starting point was based on the extensive series of biaryloxadiazoles described by Merck, which it was felt could be improved by reducing the high lipophilicity and enhancing their low polar surface area. To this end, a number of bicyclic scaffolds, including indazole, tetrahydroisoquinoline and tetrahydrobenzazepine, each bearing a propanoic acid substituent, were used to replace the 3-phenyl substituent. All the compounds described incorporated a 3-cyano-4-isopropoxyphenyl substituent as the other aryl group. Each ring system provided the desired degree of $S1P_1$ receptor selectivity, but the indazoles were found to retain relatively high lipophilicity. The tetrahydrobenzazepine (**V**) had the desired profile and was active in rat lymphocytes. This compound also had good pharmacokinetic properties despite poor solubility. However, the tetrahydroisoquinoline compounds had even lower predicted doses and a logP value of 1.9. Selectivity in this series was enhanced by introducing an 8-methyl substituent, whereas the propanoic acid was shown to have optimal potency relative to other alkanoic acids (**VI**). This compound had good pharmacokinetic properties. In rats, treatment and washout resulted in a return to normal in lymphocyte counts 48 hours after dosing, in contrast to the 7-day effects of fingolimod. The compounds were compared in a collagen-induced arthritis model, with a dose of 3 mg/kg showing comparable efficacy to a dose of 0.3 mg/kg of fingolimod. The compound was shown to behave as a normal agonist and to have no effect on rat heart rate.



(V)



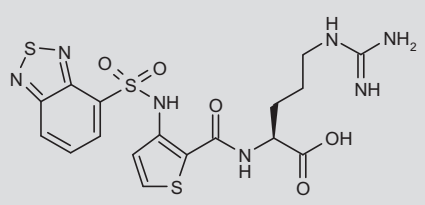
(VI)

L-Seryl-L-cysteinyl-L-lysyl-L-asparaginyl-L-threonyl-L-aspartyl-L-seryl-L-arginyl-L-cysteinyl-L-lysyl-L-alanyl-L-arginyl-L-glutaminyl-L-leucyl-L-glutamyl-L-leucyl-L-asparaginyl-L-glutamyl-L-arginyl-L-threonyl-L-cysteinyl-L-aspartyl-L-lysyl-L-prolyl-L-arginyl-L-arginine S-3.2-S-3.21: S-3.9-S-3.23-bis(disulfide)

EG-3287

NEUROPILIN-1 RECEPTOR ANTAGONISTS

The neuropilin-1 receptor (*NRP1*) is a transmembrane receptor expressed in neurons, as well as endothelial and tumor cells, that is a receptor for the vascular endothelial growth factor (VEGF) family and appears to play a key role in vascular development. Overexpression of neuropilin-1 receptors has been shown to promote tumor growth, while neuropilin-1 antibodies have been shown to inhibit tumor growth and angiogenesis in a synergistic manner when administered with bevacizumab (Avastin®). Neuropilin-1 is activated by ligands such as VEGF-A_{165}, in particular by the exon-7 and exon-8 domains, which do not interact with the VEGF receptor. Trevor Perrior (Domainex) described how small-molecule neuropilin-1 receptor antagonists under investigation by Ark Therapeutics, such as **EG-00229**, had been identified starting from the primary structure of those domains. The *N*-terminal portion of VEGF-A_{165}, termed $VEGF_{111-165}$, is relatively well defined by NMR and both the exon-7 and exon-8 domains were examined using molecular dynamics methods. This showed that **EG-3287**, the exon-8 domain residues 138-165, was an active antagonist with low micromolar potency; however, the exon-7 domain was inactive. Modification of the *N*- and *C*-termini of this sequence indicated that the *C*-terminus is key to activity. EG-3287 was shown to target VEGF-A signaling in human endothelial cells and to enhance the activity of cytotoxic drugs in a similar manner to that observed in *NRP1* knockdown studies. Alanine scanning of the *C*-terminal heptapeptide sequence revealed that Lys and Arg residues were important for activity and led to simplified tetrapeptides, of which Lys-Pro-Pro-Arg was the most active, with 14-μM potency.



EG-00229

The availability of X-ray structural data on neuropilin-1 permitted a detailed computational examination of the putative binding pocket, and also prompted an alanine scan on the full-length peptide sequence. This defined the arginine binding pocket and led to the exploration of many arginine derivatives, and the activity of various aryl-substituted *N*-benzoylarginines was examined, exploring the linkage between the two phenyl rings and the substitution of the terminal rings. The most promising derivatives included 4-nitro- and 4-aminophenylsulfonamido derivatives, leading to the exploration of alternative terminal aryl groups and the replacement of the benzoyl group by 3-aminothiophene-2-carboxylic acid, resulting in EG-00229, which demonstrated a K_d value of 200 nM when determined using isothermal titration calorimetry. EG-00229 also displayed cellular activity in the low micromolar range in human lung carcinoma A549 and prostate carcinoma DU 145 cells, and enhanced cytotoxicity. The nature of the interaction was studied by both X-ray and NMR, suggesting that the central ring is locked into a specific conformation. Ark Therapeutics has recently reported that a more potent derivative, EG-01257, has demonstrated proof of principle in a preclinical model of lung cancer, with the compound combining nanomolar potency with anticancer and antiangiogenic activity.

DISCLOSURES

The author states no conflicts of interest.

The website for this conference can be found at http://www.rsc.org/ConferencesAndEvents/conference/alldetails.cfm?evid=107845.