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Quality by design (QbD) of amide isosteres: 5,5-Disubstituted isoxazolines as potent $CRTh_2$ antagonists with favorable pharmacokinetic and drug-like properties

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ABSTRACT

Isoxazoles are frequently used amide isosteres, as shown in the context of discovery of $CRTh_2$ antagonists from amide **1** to isoxazole **2**. However, persistent agonism and poor solubility in isoxazole series presented challenges to its further development. Based on the concept of quality by design (QbD), 5,5-disubstituted isoxazolines **3** were introduced. The chirality at 5 position of isoxazolines controlled the switch between two modes of actions, which led to a novel series of pure antagonists. This non-planar motif also conferred a change of shape of these molecules, which avoided flat structures and improved their physical properties.

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The unmet medical needs in the treatment of chronic respiratory diseases such as asthma, COPD and allergic rhinitis have prompted the research and development of $CRTh_2$ receptor antagonists as novel therapy.[1] $CRTh_2$ (chemoattractant receptor-homologous molecule expressed on Th_2 cells) receptors[2] are one of the two types of G-protein coupled receptors (GPCRs) that mediates the biological actions of prostaglandin D_2 (PGD_2).[3] PGD_2 is a hormone-like metabolite in the arachidonic acid pathway, known to be involved in allergic inflammation. The $CRTh_2$ receptors are expressed on eosinophils, basophils, Th2 cells, macrophages and neutrophiles.[4] It has been shown that the PGD_2 induced chemotaxis, degranulation and cytokine production are exclusively exerted through $CRTh_2$ receptors. Therefore, modulation of $CRTh_2$ receptors is postulated to reduce excessive allergic responses and produce beneficial therapeutic effects.[5] In addition, Indomethacin,[6] (Fig. 1) a nonselective inhibitor of COX-1 and COX-2, has demonstrated an agonist effect on $CRTh_2$ receptors. Ramatroban,[7] (Fig. 1) a thromboxane A_2 antagonist, which was approved for allergic rhinitis, was thought to have efficacy derived from a moderate $CRTh_2$ activity. These proven pharmacological interventions lent further credibility to the targeting of $CRTh_2$ receptors for the treatment of respiratory diseases. The last decade has seen the rapid expansion of small molecule $CRTh_2$ antagonist discovery research, which has been reviewed extensively.[8] Several $CRTh_2$ antagonists have advanced to clinical trials.[1a] The Merck team has recently published the discovery of MK-7246 that also entered clinical trials[9] (Fig. 1).

To fully explore the potential opportunities of $CRTh_2$ antagonism therapeutics, a backup medicinal chemistry program was initiated. At the inception of the program, our focus was drawn to a screening derivative amide **1** (Fig. 2). These types of compounds generally demonstrated good $CRTh_2$ potency. The major concern for this series was the potential amide hydrolysis thought to be linked to unsatisfactory pharmacokinetic profiles. Therefore, a number of heterocycle replacements were investigated and the

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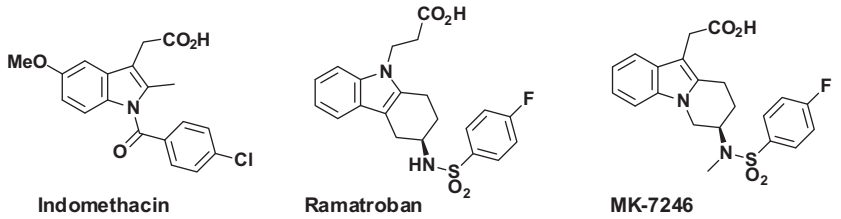


Figure 1. Representative $CRTh_2$ modulators.

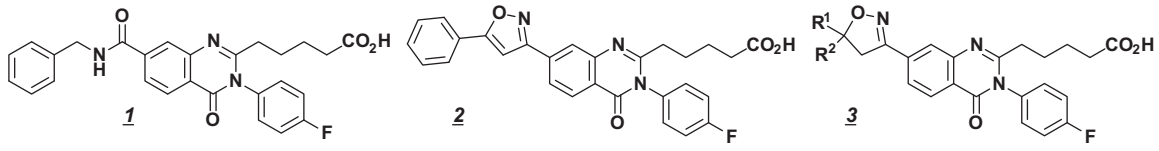


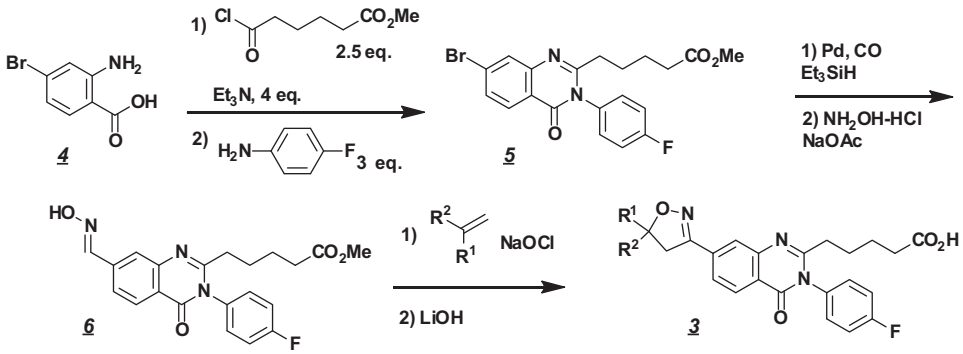
Figure 2. Leads and lead optimization targets.

isoxazole **2** (K_i $CRTh_2$ = 2.6 nM, partial agonist) emerged as a key lead. Drawbacks of **2** included partial agonist behavior and low solubility. To address these issues, we consciously applied quality by design (QbD) principle to the next round of amide replacement optimization. The QbD concept emphasizes that quality should be built into a product by design with an understanding of the product and process by which it is developed and produced.[10] QbD is a scientific risk-based, holistic and proactive approach. This approach seeks to identify and understand the risks involved in each steps and how best to mitigate these risks.[11] The QbD approach has been implemented by US Food and Drug Administration (US FDA) in pharmaceutical pre-market processes, particularly in drug manufacturing.[12] This concept has also been introduced in drug discovery processes as well.[13] It was postulated that, by design-in the druglikeness to discovery-stage analogs and preclinical candidates, the risk of development failures could be mitigated and the eventual success rate could be improved.[14] Focusing on quality amide bond mimics,[15] we envisioned adoption of 5,5-disubstituted isoxazolines **3** as replacement of amide **1** (Fig. 2). Compared to isoxazole **2**, the druglikeness of isoxazolines **3** identified by QbD included steric bulkiness and chiral preference to influence agonism/antagonism behavior, di-substitutions of desirable non-flat structures[16] that would likely improve solubility.[17] This paper will present the synthesis and pharmacological and physicochemical characterizations of isoxazoline amide mimics **3** as potent, selective $CRTh_2$ antagonists with excellent pharmacokinetic profiles. The successful completion of this study also demonstrated the importance of QbD concept in discovery medicinal chemistry.

The synthesis of the isoxazolines **3** started with commercial 4-bromo-2-aminobenzoic acid. It was treated with excess Et_3N and methyl 6-chloro-6-oxohexanoate followed by 4-fluoroaniline at 80 °C overnight to afford the bromoquinazolinone core **5**. Formylation of the bromide followed by hydroxylamine treatment provided ample quantity of oxime **6**. Subsequent 2+3 cycloaddition yielded isoxazoline methylesters which were hydrolyzed to produce the final compounds **3**. The entire synthesis was straight-forward and ready to scale up (Scheme 1).

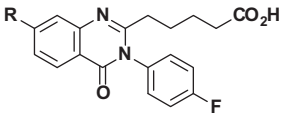
The initial design strategy was to keep one phenyl group as R_1 and insert another R_2 at the 5 position of the isoxazoline. The first round of SAR was aimed at securing an optimal R_2 group with balanced potency and drug-like properties. The methyl substituted analog **3a** showed comparable $CRTh_2$ binding activity as isoxazole **2** with moderate cAMP antagonism as a racemic mixture. With increasingly bulkier R_2 groups, the analogs **3b**–**3d**, albeit potent in the binding assay,[18] showed reduced cAMP activity,[18] and began to trend toward agonism. With the intention of improving aqueous solubility, adoption of polar groups such as hydroxymethyl or methoxymethyl in **3e**–**3f** resulted in decreased $CRTh_2$ binding potency. The diphenyl analog **3g**, though marginally potent in the binding assay, showed weak activity in the cAMP assay. The indane analog **3h** proved to be the most potent in the $CRTh_2$ binding assay, but decisively an agonist in the cAMP assay. In conclusion, the optimal R_2 substitution was the methyl group, which showed both good $CRTh_2$ and cAMP binding activities (Table 1).

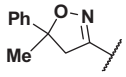
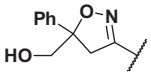
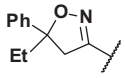
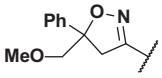
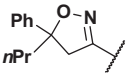
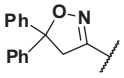
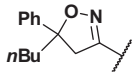
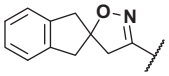
Concentrating efforts on the methyl analogs provided several advantages. The addition of the methyl group reinforces chiral bias by analogy of the Thorpe–Ingold effect.[19] This methyl would also



Scheme 1. Synthesis of 5,5-disubstituted isoxazolines.

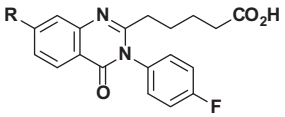
Table 1
$CRTh_2$ activities of 5-phenylisoxazolines

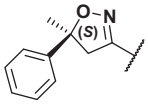
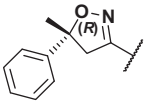
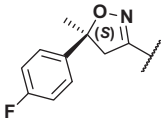
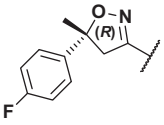
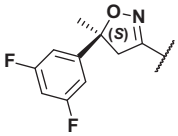
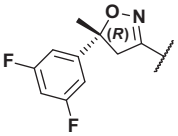
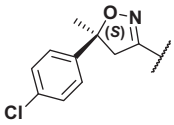
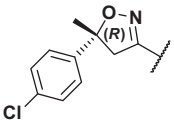
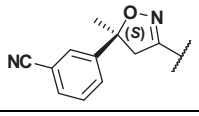
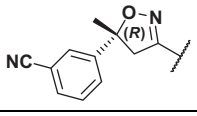


Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)	Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)
3a		4.7	18.6 Antagonist	**3e**		2.5	58.5 antagonist
3b		1.8	38.7 Antagonist	**3f**		1.9	79.7 antagonist
3c		1.8	73.4 Antagonist	**3g**		13.7	33.7 antagonist
3d		1.6	Partial agonist	**3h**		1.1	Agonist

Data represented the average values of duplicates or triplicates 18.

Table 2
SAR of 5-methyl-phenylisoxazolines



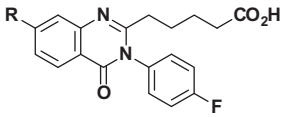
Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)	Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)
3a-(***S***)		3.7	8.1 Antagonist	**3a**-(***R***)		24.9	Partial agonist
3i-(***S***)		4.1	10.2 Antagonist	**3i**-(***R***)		6.0	Agonist
3j-(***S***)		3.4	7.8 Antagonist	**3j**-(***R***)		10.5	Partial agonist
3k-(***S***)		2.6	10.5 Antagonist	**3k**-(***R***)		2.0	Agonist
3l-(***S***)		3.0	5.2 Antagonist	**3l**-(***R***)		22.3	ND

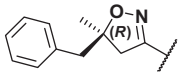
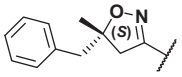
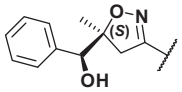
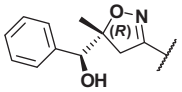
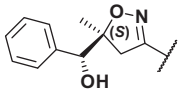
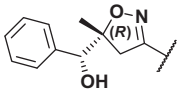
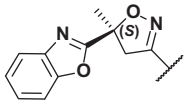
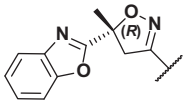
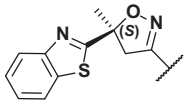
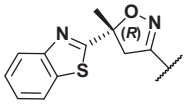
Data represented the average values of duplicates or triplicates.

prevent isoxazolines from potential oxidative metabolism of the benzyl oxime ether. Finally, the quaternary carbon substitution would break the planar linear aromatic structures to improve physical properties.[12] A number of 5-methylisoxazolines with a small array of 5-phenyl derivatives were synthesized using the chemistry shown above (Scheme 1). The enantiomeric mixtures were separated by chiral HPLC. In each enantiomeric series the order of HPLC elution and their bioassay profiles were consistent. This ensured straight forward assignment of their absolute stereochemistry.[20]

The enantiomerically pure 5,5-disubstituted isoxazolines **3** were generally very potent in the $CRTh_2$ binding assay (Table 2), particularly in the (*S*) enantiomeric series. The cAMP data showed more intriguing results. The (*S*) enantiomer series **3a**-(***S***), **3i**-(***S***), **3j**-(***S***), **3k**-(***S***) and **3l**-(***S***) exhibited potent antagonist activity, where the (*R*) enantiomers **3a**-(***R***), **3i**-(***R***), **3j**-(***R***), **3k**-(***R***) and **3l**-(***R***) were partial or full agonists (Table 2). As mentioned earlier, one of the drawbacks of the isoxazole **2** was its agonism. Similar crossover of mode of actions has been a problem for other $CRTh_2$ chemotypes.[21] In the methylphenyl disubstituted isoxazolines series,

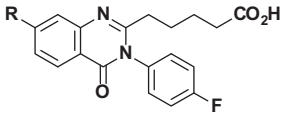
Table 3
SAR of 5-methyl-extended phenyl analogs

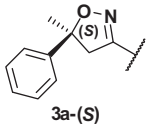
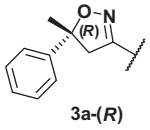
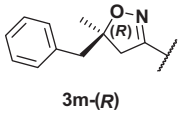
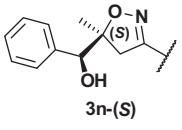
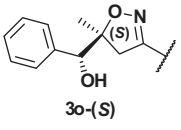
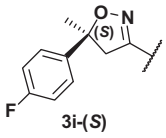
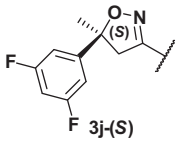
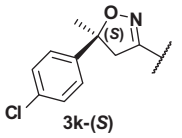
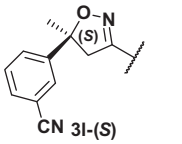
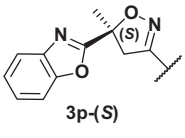


Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)	Entry	R	K_i ($CRTh_2$) (nM)	cAMP IC_{50} (nM)
3m-(***R***)		7.5	15.2 Antagonist	**3m**-(***S***)		7.4	13.7 antagonist
3n-(***S***)		3.7	4.7 Antagonist	**3n**-(***R***)		41.7	ND
3o-(***S***)		2.5	5.7 Antagonist	**3o**-(***R***)		18.3	ND
3p-(***S***)		1.9	9.7 Antagonist	**3p**-(***R***)		19.6	ND
3q-(***S***)		2.3	17.2 Antagonist	**3q**-(***R***)		5.6	Agonist

Data represented the average values of duplicates or triplicates.

Table 4
Representative pharmacokinetic profiles of 5,5-disubstituted isoxazolines



Entry	 3a-(*S*)	 3a-(*R*)	 3m-(*R*)	 3n-(*S*)	 3o-(*S*)
Rat (SD) AUC (0–6 h) 10 mpk, po; nM·h	16895.0	590.8	36390	11180.3	31158.1
Hepatocyte clearance (ml/min/M cell); r/m/h*	1.0/4.2/0.9	2.2/ND/1.5	4.7/23.3/3.1	2.0/2.8/<1	<1/2.7/0.5
Entry	 3i-(*S*)	 3j-(*S*)	 3k-(*S*)	 3l-(*S*)	 3p-(*S*)
Rat (SD) AUC (0–6 h) 10 mpk, po; nM·h	6537.7	1670.9	3408.8	1083.8	11095.6
Hepatocyte clearance (ml/min/M cell); r/m/h*	4.1/11.7/1.8	<1/13.4/0.7	3.7/17.7/3.6	2.6/5.2/0.9	<1/5.1/1.3

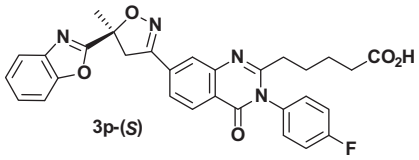
* r/m/h: rat/monkey/human.

the chirality provided a clean-cut control of agonism/antagonism and a facile solution to the problem.

Based on the most potent indane analog **3h**, the extended phenyl substitutions were further investigated. Analogs such as **3m**–**3q** were synthesized (Table 3). Unlike the phenyl analogs, the SAR in the extended series did not provide a clean cut picture. While both enantiomers of the benzyl analogs **3m**-(***R***) and **3m**-(***S***) were potent in the $CRTh_2$ binding assay, the cAMP assay confirmed that both were antagonists. The hydroxylbenzyl analogs took another trend. The (*S*) enantiomers **3n**-(***S***) and **3o**-(***S***) were potent in both $CRTh_2$ binding and cAMP assays as antagonists and the opposite enantiomers **3n**-(***R***) and **3o**-(***R***) were not potent in $CRTh_2$ binding assay. The more rigid benzooxazole and benzathiazole analogs also presented a complex picture. The benzooxazole enantiomer **3p**-(***S***) was potent in $CRTh_2$ binding and cAMP assay, but the enantiomer **3p**-(***R***) was not in binding assay. On the other hand, benzothiazole enantiomers **3q**-(***S***) and **3q**-(***R***) were potent in binding assay, but **3q**-(***R***) followed an earlier trend to be an agonist (Table 3).

All the in vitro data so far proved that 5,5-disubstituted isoxazolines were good amide mimics as $CRTh_2$ antagonists. Several analogs with excellent binding potency and pure antagonism profiles were identified. One of the key compound **3p**-(***S***) was subjected to additional investigations. This compound showed

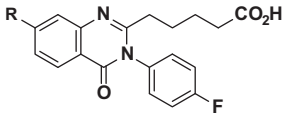
Table 5
Pharmacokinetic profiles of key compound **3p**-(***S***)

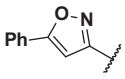
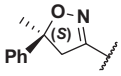
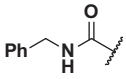
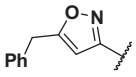
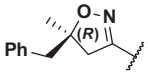
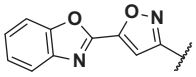
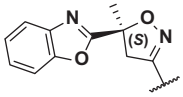


3p-(*S*)

Species	AUC μM h	F (%)	Clearance (ml/min/kg)	V_d(ss) (L/kg)	$T_{0.5}$ (h)
Rat (wistar) 10 mpk, po	335	86	0.87	0.21	4.9
Dog (beagle) 3 mpk, po	8.4	56	2.8	0.51	7.5
Monkey (cyno) 3 mpk, po	15.7	41	3.7	0.40	8.4

Table 6
Physicochemical properties of representative 5,5-disubstituted isoxazolines



Entry	R	HPLC log*D*	Soluibility pH 7 (μg/ml)	Soluibility FASSIF (μg/ml)	Calculated Papp (10^{-6} cm/s)
2		1.97	8	17	31
3a-(***S***)		1.91	170	90	26
1		0.98	202	ND	9.0
7		2.00	85	54	32
3m-(***R***)		1.98	>200	98	29
8		1.99	6	17	12
3p-(***S***)		1.81	169	125	13

IC50 of 6.8 nM in Eosinophil shape change assay[18] in human whole blood assay.

After securing the favorable in vitro biological data, the next logical step was to assess the pharmacokinetic and ADME properties of these compounds. Historically there have been two reports that utilized isoxazolines as amide bond isosteres with very limited profiling,[22] our research provided large numbers of diverse analogs to present a full picture. The drug-like properties of isoxazoline compounds were systematically evaluated. These properties would likely determine the prospective of isoxazolines as drug candidates (Table 4).

The pharmacokinetic (PK) studies[23] in Sprague-Dawley (SD) rats indicated that the isoxazoline compounds generally exhibited moderate to excellent exposures (Table 4). Interestingly, the more potent enantiomer **3a**-(***S***) showed much better AUC than **3a**-(***R***). All compounds had low hepatocyte clearance in multiple species, except **3i**-(***S***), **3j**-(***S***), **3k**-(***S***) and **3m**-(***R***) in monkeys, confirming a general trend of metabolic stability. Among these analogs, **3p**-(***S***) also showed good exposure and oral bioavailability in rodents, monkeys and dogs (Table 5). Collectively, the data shown demonstrated that 5,5-disubstituted isoxazolines could have excellent pharmacokinetic profiles.

To further assess the drug-like profile of isoxazolines, several physical properties such as HPLC log*D* and solubility were measured experimentally. Apparently, the addition of a methyl group at the 5-position did not alter the HPLC log*D* significantly. The differences between **2** and **3a**-(***S***), **7** and **3m**-(***R***) and **8** and **3p**-(***S***) were fairly small. On the other hand, the solubility data of 5-methyl substituted isoxazolines vis-à-vis the isoxazoles revealed consistently much improved solubility at pH 7 and in FASSIF at pH 6.5. Particularly noteworthy, for the more rigid analogs, **8** versus **3p**-(***S***) at pH 7, the solubility improved as much as 25fold. A likely reason is that the quaternary center disrupted planer structure and π-stacking force, leading to better solubility.[17] What is more interesting, as an amide isostere, was that compound **3a**-(***S***) showed similar solubility to the corresponding benzylamide **1**. The amide, however, has much lower log*D*, which may be related to poor permeability of peptide drugs (Table 1). Indeed, the calculated Apparent Permeability (P_{app}) showed amide **1** was nearly threefold worse than isoxazoline **3a**-(***S***). This demonstrated an additional

favorable property that enabled isoxazolines as privileged amide bond isosteres (Table 6).

In summary, a useful strategy of using 5,5-disubstituted isoxazolines as amide isosteres in the $CRTh_2$ antagonist program was presented. Based on the principles of quality by design (QbD),[10–14] several salient features of druglikness were built into isoxazolines. These included a simple one step synthesis resulting in a single chiral center, which led to chirally differentiated non-flat molecules. This chirality was utilized successfully to control the agonism/antagonism switch. Extensive evaluations of pharmacokinetic and physical properties were conducted to assess their likelihood of being drug candidates. The disubstituted isoxazoline $CRTh_2$ antagonists were metabolically stable and possessed good pharmacokinetic profiles. These structures had similar solubility and possibly better permeability compared to amide **1**. Indeed, when applicable, 5,5-disubstituted isoxazolines could be powerful isostere replacements of amide bonds. The QbD concept utilized in this study demonstrated the importance of front-loading druglikeness to medicinal chemistry design. This approach should be widely applicable to the discovery of high quality drug candidates and eventual human medicines.

Acknowledgments

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