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Discovery of 3-Aryl-4-isoxazolecarboxamides as TGR5 Receptor Agonists

Karen A. Evans,*,† Brian W. Budzik,† Sean A. Ross,‖ David D. Wisnoski,† Jian Jin,† Ralph A. Rivero,† Mythily Vimal,‡ George R. Szewczyk,‖ Channa Jayawickreme,§ David L. Moncol,§ Thomas J. Rimele,§ Susan L. Armour,§ Susan P. Weaver,§ Robert J. Griffin,‖ Sarva M. Tadepalli,‖ Michael R. Jeune,‖ Todd W. Shearer,‖ Zibin B. Chen,‖ Lihong Chen,‖ Donald L. Anderson,‖ J. David Becherer,‖ Maite De Los Frailes,⊥ and Francisco Javier Colilla⊥

†*Discovery Medicinal Chemistry, Discovery Research, GlaxoSmithKline Pharmaceuticals, 1250 South Collegeville Road, P.O. Box 5089, Collegeville, Pennsylvania 19426-0989,* ‡*Discovery Medicinal Chemistry, Discovery Research, GlaxoSmithKline Pharmaceuticals, New Frontiers Science Park, Third Avenue, Harlow, Essex, CMI9 5AD, U.K.,* §*Discovery Research,* ‖*Metabolic Diseases Center of Excellence for Drug Discovery, Five Moore Drive, P.O. Box 13398, Research Triangle Park, North Carolina 27709-3398, and* ⊥*Department of Screening and Compound Profiling, Discovery Research, GlaxoSmithKline Pharmaceuticals, C/Santiago Grisolia 4, 28760 Tres Cantos, Madrid, Spain*

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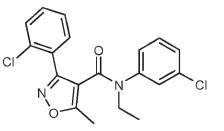
Abstract: A series of 3-aryl-4-isoxazolecarboxamides identified from a high-throughput screening campaign as novel, potent small molecule agonists of the human TGR5 G-protein coupled receptor is described. Subsequent optimization resulted in the rapid identification of potent exemplars **6** and **7** which demonstrated improved GLP-1 secretion in vivo via an intracolonic dose coadministered with glucose challenge in a canine model. These novel TGR5 receptor agonists are potentially useful therapeutics for metabolic disorders such as type II diabetes and its associated complications.

Type II diabetes, also known as diabetes mellitus, is now internationally recognized as one of the major threats to human health in the 21st century. According to the International Diabetes Federation (IDF), diabetes is expected to cause 3.8 million deaths worldwide in 2007, roughly 6% of total world mortality, about the same as HIV/AIDS and malaria combined.[1] Those that suffer from type II diabetes have too little insulin or cannot use insulin effectively. As a result, glucose levels build up in the blood and urine and, if left untreated, can cause life-threatening complications, including blindness, kidney failure, and heart disease.

Current oral therapies include insulin secretagogues, such as sulfonylureas; glucose-lowering effectors, such as metformin; activators of the peroxisome proliferator-activated receptor-γ (PPAR-γ), such as the thiazolidinediones; and α-glucosidase inhibitors.[2] There are, however, deficiencies associated with currently available treatments including hypoglycemic episodes, weight gain, gastrointestinal problems, edema, and loss of responsiveness over time.

TGR5, also known as BG37, M-BAR, or hGPCR19, is a bile acid G-protein-coupled receptor primarily expressed in monocytes and macrophages, lung, spleen, and the intestinal tract. In response to bile acids, namely, lithocolic acid (LCA[a]), TGR5 has been shown to cause a dose dependent elevation in intracellular concentrations of cAMP in cells that express the receptor.[3] Bile acids and compounds that affect TGR5 have also been shown to increase glucagon like peptide-1 (GLP-1) secretion from primary intestinal cells.[4] It has therefore been suggested that bile acids induce GLP-1 secretion by increasing intracellular cAMP levels via the TGR5 receptor.[5]



1
TGR5 pEC_{50} = 5.3, 100 %
U2-OS Host pEC_{50} < 4.6
MW = 375
Solublity = 134 uM
AMP= 310 nm/sec
Passive Papp = 448 nm/sec

Figure 1. In vitro profile of HTS hit **1**.

GLP-1 is a peptide secreted from enteroendocrine L cells and has a wide variety of physiological effects.[6] Recent FDA approved therapies for type II diabetes based on GLP-1 are GLP-1 receptor agonist peptides (GLP-1 mimetics) and DPP-IV inhibitors, which enhance insulin action by prolonging the half-life of endogenous GLP-1.[7] These approaches are effective because while type II diabetics show impaired GLP-1 secretion, they maintain normal responsiveness to GLP-1. However, the use of a peptide in clinical treatment is severely limited because of the requirement for parenteral administration and low in vivo stability. Therefore, a small molecule agonist that increases GLP-1 secretion from the gut during a meal is a potentially useful therapeutic for metabolic disorders such as type II diabetes and its associated complications. A number of bile acid derivatives and triterpenoid TGR5 agonists have been disclosed recently in the peer-reviewed literature.[8] In addition, a variety of small molecule agonists are also known but are currently described only in the patent literature.[9,10] Herein is presented the discovery of a series of 3-aryl-4-isoxazolecarboxamides as novel, potent small molecule agonists of the human TGR5 G-protein-coupled receptor.

High-throughput screening (HTS) of the GSK proprietary compound collection using a MRE/CRE (multiple response element/cAMP response element) directed reporter gene assay measuring luciferase production in response to changes in cAMP (via a Gs-protein-coupled signaling pathway)[11] in a BacMam transduced human osteosarcoma cell line (U2-OS) led to the identification of isoxazole **1** (Figure 1) as an agonist with a pEC_{50} of 5.3 and 100% maximum response.[12] The compound elicited no response in the host cell line (pEC_{50} < 4.6 in untransfected U2-OS cells) which confirmed its specificity. In addition, the compound has an excellent physicochemical profile that made it of particular interest as a starting

*To whom correspondence should be addressed. Phone: 1-610-917-7764. Fax: 1-610-917-6151. E-mail: karen.a.evans@gsk.com.

[a]Abbreviations: 7TM, seven-transmembrane; BA, bile acid; LCA, lithocholic acid; GPCR, G-protein-coupled receptor; FXR, farnesoid X receptor; T2D, type II diabetes; HTS, high-throughput screening; GLP-1, glucagon-like peptide 1; CYP450, cytochrome P450; PK, pharmacokinetic; MDCK, Madin−Darby canine kidney.

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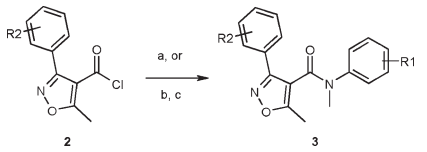
Scheme 1. Synthesis of 3-Aryl-4-isoxazolecarboxamides[a]



[a] (a) Various substituted *N*-methylanilines, Et_3N, DCM, room temp; (b) various substituted anilines, Et_3N, DCM, room temp; (c) CH_3I, powdered KOH, DMSO, 50 °C.

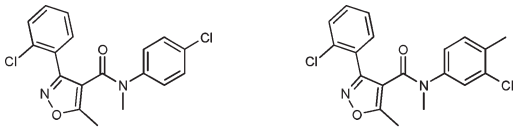
point for optimization: low molecular weight (MW < 400), good aqueous solubility (134 uM), and high permeability in both artificial membrane[13] (310 nm/s) and MDCK cells[14] (passive P_{app} = 448 nm/s).

To explore this novel HTS hit, an efficient one- or two-step synthesis was utilized to explore substitutions at R1 and R2 (Scheme 1). Commercially available 5-methyl-3-aryl-4-isoxazolecarbonyl chlorides **2** were converted to the desired amides **3** in one step from commercially available, or prepared, substituted *N*-alkylanilines.[15] Alternatively, the acid chloride was coupled with an aryl-substituted primary aniline and then alkylated in a second step.

Our lead optimization work rapidly led to the discovery of more potent compounds. Compound **6** (R1 = 4-Cl, R2 = 2-Cl) showed improved potency in the U2-OS cell assay (pEC_{50} = 6.8) and in melanophore cells (pEC_{50} = 7.5).[16] Likewise, **7** (R1 = 3-Cl-4-Me, R2 = 2-Cl) showed analogous potency (U2-OS cells pEC_{50} = 6.3; melanophore cells pEC_{50} = 7.1). Compounds **6** and **7** were therefore further profiled in vitro and in vivo (Table 1) to quickly ascertain whether a small molecule agonist from this series would have utility in improving GLP-1 secretion and thereby improving glucose disposal. These exemplars were fully specific for the human TGR5 receptor in the U2-OS and melanophore cell lines and also highly selective against other 7TM receptors. Compound **6** was profiled against more than 100 in-house and external 7TM, ion channel, enzyme, transporter, and nuclear hormone receptor selectivity assays, including FXR, another bile acid receptor, and showed significant response only in secretion of the pro-inflammatory cytokine TNFalpha (pIC_{50} = 6.8) in human primary monocytes following stimulation with LPS (lipopolysaccharide).[17] This result is not unexpected, as the TGR5 receptor is expressed in monocytes. In addition, **6** and **7** have good physicochemical properties and no measurable activity against three of the common cytochrome P450 (CYP450) isoforms (1A2, 2C9, and 2D6) or hERG dofetilide binding (pIC_{50} < 4.3). In rat pharmacokinetic (PK) studies, however, both compounds showed high in vivo clearance (**6**, Cl = 85 mL/min/kg; **7**, Cl = 148 mL/min/kg) and intrinsic clearance (**6**, Cl_{int} = 48 mL/min/g; **7**, Cl_{int} = 54 mL/min/g) which provided a reasonable explanation for the observed poor exposure. Because the TGR5 receptor is expressed in the GI tract at levels that increase corresponding with L-cell population density, we believe that agonists such as **6** and **7** possessing poor systemic exposure are good tool compounds for directly targeting the TGR5 receptor in the GI tract via local administration (vide infra) rather than systemic exposure. Our hypothesis was that for this receptor, systemic exposure was not necessary to achieve the desired effect of stimulating GLP-1 secretion in vivo.

To test this hypothesis, **6** and **7** were studied in an acute conscious dog animal model to evaluate GLP-1 secretion. The conscious canine in vivo model was found to be more robust than an anesthetized rat in vivo model for measurement of GLP-1 levels. Moreover, the in vitro melanophore data (Table 1) suggested that the compounds were a full log unit less potent at the rat receptor but were equipotent at the human and canine receptors. The dog PK also showed a very similar profile to the observed rat PK, in vivo and in vitro for **7**, with reasonable half-life, high clearance, and poor oral exposure. Therefore, in the conscious dog model, an intrajejeunal injection of glucose (0.125 g/kg) was coadministered

Table 1. Profile of Isoxazolecarboxamides **6** and **7**



	compd **6**		compd **7**	
	U2-OS (human)	melanophore (human, rat, canine)	U2-OS (human)	melanophore (human, rat, canine)
	In Vitro Potency[a]			
TGR5 pEC_{50}	6.8	7.5 5.7 7.2	6.3	7.1 6.2 7.5
host specificity pEC_{50}	< 4.3	4.7	< 4.3	4.5
	Rat in Vivo PK Parameters			
	$T_{1/2}$ = 6.6 h[b] Cl = 85 mL/min/kg V_{ss} = 7.1 L/kg F = 1%		$T_{1/2}$ = 8.7 h[c] Cl = 148 mL/min/kg V_{ss} = 40 L/kg F < 1%	
	Dog in Vivo PK Parameters[d]			
	not determined		$T_{1/2}$ = 5.2 h Cl = 39 mL/min/kg V_{ss} = 8.6 L/kg F = 0.3%	
	In Vitro PK Parameters[e]			
rat	Cl_{int} = 48 mL/min/g		Cl_{int} = 54 mL/min/g	
dog	Cl_{int} = 90 mL/min/g		Cl_{int} = 111 mL/min/g	
	CYP450 pIC_{50}			
	6.5 (2C19) 5.9 (3A4), < 5.0 (1A2, 2C9 and 2D6)		6.5 (2C19) 5.9 (3A4), < 5.0 (1A2, 2C9 and 2D6)	
	Permeability			
	422 nm/s		257 nm/s	
	Solubility			
	246 μM		112 μM	
	hERG Dofetilide Binding			
	pIC_{50} < 4.3		pIC_{50} < 4.3	

[a] Standard deviation is less than ±0.7. [b] Rat PK parameters: n = 4, male CD rats; iv, 2.5 mg/kg; po, 5 mg/kg; vehicle formulation 5% DMSO/2% EtOH/20% SBECD. [c] Rat PK parameters: n = 3; iv, 3 mg/kg, formulated in 5% DMSO/10% Solutol/85% 25 mM citrate buffer (pH 4); po, 30 mg/kg, formulated in 0.5% HPMC/0.1% Tween-80. [d] Dog PK parameters: n = 3; iv, 3 mg/kg, formulated in 5% DMSO/10% Solutol/85% saline; po, 30 mg/kg, formulated in 0.5% HPMC/0.1% Tween-80. [e] Liver hepatocytes preparation.

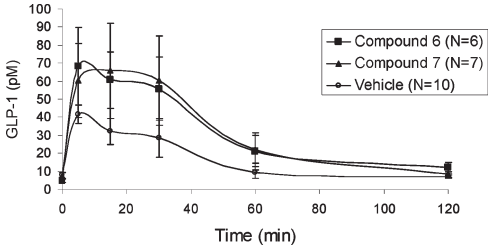


Figure 2. Effect of **6** and **7** on GLP-1 secretion in vivo via an intrajejeunal dose (1 mg/kg) coadministered with a glucose challenge at $t = 0$ (0.125 g/kg) in conscious dogs. Vehicle formulation is 0.5% HPMC/0.1% Tween-80. Data from time points at 5 and 60 min are statistically significant in their difference from vehicle control.

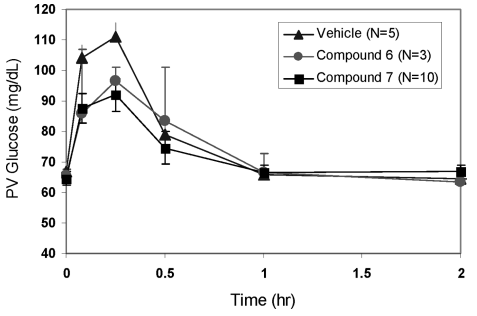


Figure 3. Effect of **6** and **7** on portal vein glucose levels.

Table 2. Dog Exposure Levels for Compounds **6** and **7**[a]

compd	vein sampling location	plasma concn C_{max} (ng/mL)	AUC_{0-2h} (ng·h/mL/mg/kg)	hEC_{50} (ng/mL)
6	portal (local)	514 ± 256	142 ± 80	11
6	cephalic (systemic)	22 ± 37	ND	11
7	portal (local)	852 ± 814	226 ± 210	30
7	cephalic (systemic)	9 ± 8	ND	30

[a] ND = not detected. Standard deviations are noted.

with compound (1 mg/kg) using a nanomilled suspension in 0.5% HPMC/0.1% Tween-80, and portal and cephalic vein blood samples were taken at 5, 15, 30, 60, and 120 min. The results of this experiment clearly demonstrated that **6** and **7** showed significant improvements in hepatic portal vein GLP-1 secretion (Figure 2) and reduction in portal vein glucose levels (Figure 3) compared to vehicle for the entire duration of the study.

Compound exposure levels at C_{max} from the portal vein were 50-fold and 30-fold over hEC_{50} for **6** and **7**, respectively (Table 2). Significant differences were observed in samples taken from the cephalic vein, however, with minimal plasma exposures observed, suggesting that systemic exposure is not required for acute efficacy, and possibly a local intracolonic mechanism for TGR5 stimulated GLP-1 release.

In conclusion, novel and highly selective small molecule TGR5 receptor agonists have been discovered. Isoxazole carboxamides **6** and **7** have excellent physicochemical properties, and their in vivo efficacy results in the acute canine model via intracolonic administration demonstrate that these novel TGR5 receptor agonists are potentially useful therapeutics for metabolic disorders such as type II diabetes and its associated complications. A full account of the synthesis and structure–activity relationships of this novel series will be the subject of a future publication.

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Supporting Information Available: Synthetic procedures and characterization data for all compounds; procedures for primary assays and in vivo canine model. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) Bological assay results are the mean of at least two determinations with standard deviation of less than ±0.7 unless otherwise noted.
(13) The artificial phospholipid membrane technique is similar to the widely used Caco-2 cell monolayer permeation technique. In short, egg phosphatidyl choline (1.8%) and cholesterol (1%) are dissolved in *n*-decane. A small amount of the volatile mixture is applied to the bottom of the microfiltration filter inserts. Phosphate buffer (0.05 M, pH 7.05) is quickly added to the donors and receivers,

and the lipids are allowed to form self-assembled lipid bilayers across the small holes in the filter. Permeation experiment is initiated by spiking the compounds of interest to the donor sides, and the experiment is stopped at a predetermined elapsed time. The samples are withdrawn and transferred to appropriate vials for analysis by HPLC with UV detection (215 nm).

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(15) Typical reaction conditions are treatment of the aniline with the appropriate aldehyde or ketone (R), sodium triacetoxyborohydride ($Na(OAc)_3BH$), and acetic acid in dichloroethane (DCE) for 16 h at room temperature. See also the following: Thorstensson, F.; Kvarnstrom, I.; Musil, D.; Nilsson, I.; Samuelsson, B. Synthesis of novel thrombin inhibitors. Use of ring-closing metathesis reactions for synthesis of P2 cyclopentene- and cyclohexenedicarboxylic acid derivatives. *J. Med. Chem.* **2003**, *46*, 1165–1179.

(16) All lead optimization compounds (post-HTS) were tested in two TGR5 assays using both U2-OS cells and melanophore cells, with specificity screening in the corresponding host cell line. Experimental protocols are described in the Supporting Information.

(17) Sample profiling results by target class. (a) 7TM: 5-HT1A, $pEC_{50} < 5.5$; M1, $pEC_{50} < 4.8$. (b) Ion channel: Ca^{2+} channel (verapamil site), $<10\%$ inhibition at 1 μM; K + V channel, $<10\%$ inhibition at 1 μM; Na^+ channel, 16% inhibition at 1 μM. (c) Nuclear receptors: FXR, $pEC_{50} < 5$.