

# Identification of Tetrahydropyrido[4,3-*d*]pyrimidine Amides as a New Class of Orally Bioavailable TGR5 Agonists

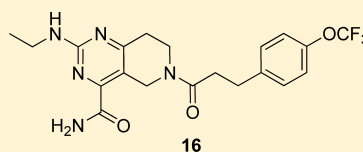
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## Supporting Information

**ABSTRACT:** Takeda G-protein-coupled receptor 5 (TGR5) represents an exciting biological target for the potential treatment of diabetes and metabolic syndrome. A new class of high-throughput screening (HTS)-derived tetrahydropyrido[4,3-*d*]pyrimidine amide TGR5 agonists is disclosed. We describe our effort to identify an orally available agonist suitable for assessment of systemic TGR5 agonism. This effort resulted in identification of **16**, which had acceptable potency and pharmacokinetic properties to allow for in vivo assessment in dog. A key aspect of this work was the calibration of human and dog in vitro assay systems that could be linked with data from a human ex vivo peripheral blood monocyte assay that expresses receptor at endogenous levels. Potency from the human in vitro assay was also found to correlate with data from an ex vivo human whole blood assay. This calibration exercise provided confidence that **16** could be used to drive plasma exposures sufficient to test the effects of systemic activation of TGR5.

**KEYWORDS:** GPCR, TGR5, agonist, diabetes, GLP-1



human TGR5 EC<sub>50</sub> = 75 nM  
NCI-H716 EC<sub>50</sub> = 145 nM  
ex vivo PBMC EC<sub>50</sub> = 110 nM

TGR5 (Takeda G-protein-coupled receptor 5) is a class A G-protein-coupled receptor (GPCR) expressed in liver, skeletal muscle, intestine, brown adipose tissues, and monocytes.<sup>1–4</sup> Activation of the TGR5 receptor upon ligand binding results in Gα<sub>s</sub>-coupled activation of adenylate cyclase. The subsequent downstream signaling cascade is believed to drive multiple effects that are cell type-dependent including (1) enhanced glucagon-like peptide-1 (GLP-1) release from intestinal cells potentially offering improved glycemic control through potentiation of glucose-dependent insulin secretion;<sup>5</sup> (2) nuclear effects, such as endothelial nitric oxide synthase activation and increased nitric oxide production in liver cells;<sup>6</sup> and (3) reduction of macrophage inflammation and lipid loading leading to inhibition of atherosclerosis.<sup>7,8</sup> Activation of TGR5 can also induce type 2 iodothyronine deiodinase in brown adipose tissue. In mice, this leads to increased energy expenditure, but the picture is less clear for humans.<sup>9,10</sup> For these reasons, TGR5 agonists may be useful agents to not only treat diabetes with concurrent management of glucose levels and body weight but also potentially address other aspects of metabolic syndrome.

Bile acids are the purported endogenous agonists for TGR5. Of the several bile acids that act as ligands, taurine-conjugated lithocholic acid, lithocholic acid, deoxycholic acid, chenodeoxycholic acid, and cholic acid have all been shown to dose dependently induce accumulation of cyclic adenosine 5'-monophosphate (cAMP) in TGR5-transfected Chinese ham-

ster ovary (CHO) cells.<sup>11</sup> Significant effort has been devoted to synthetic bile acids, such as INT-777 (**1**).<sup>12,13</sup> In addition, several other nonbile acid agonists have been reported in the literature (**2–4**),<sup>14–16</sup> which offer the potential for higher selectivity over other bile acid-mediated pathways (Figure 1). Previous work with **1** demonstrated in vivo modulation of

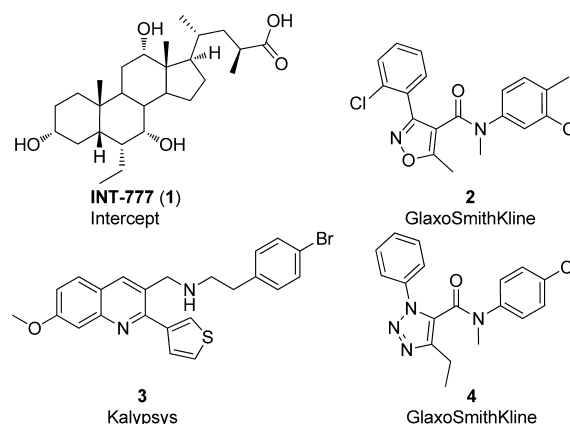


Figure 1. Previously disclosed TGR5 agonists.

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GLP-1 secretion and increased energy expenditure in a diet-induced obese mouse model. Furthermore, **2** was shown to potentiate GLP-1 secretion in an in vivo dog model when dosed in an intracolonic manner and coadministered with a glucose challenge. However, in view of the physicochemical property space and data generated from evaluation of these ligands, few appeared to offer the chance of attaining reasonable free drug concentrations in animal models to allow for the assessment of the full range of systemic effects associated with activation of TGR5. Thus, our goal for this work was the identification of a suitable compound capable of fully testing the effects of systemic TGR5 agonism to progress to in vivo studies. Because the potency of GPCR agonists in overexpressed cell systems is heavily system-dependent, we were very mindful to set appropriate goals for the level of TGR5 potency required in our tool compound.<sup>17</sup> Thus, an additional goal for this work became the establishment of a quantitative pharmacological relationship between biological systems that express TGR5 at endogenous levels and overexpressed assay systems commonly used in the literature.<sup>18</sup> Furthermore, we set the objective that the “endogenous” assay system used for this calibration should be one with an output linked to a clinically relevant end point [e.g., tumor necrosis factor- $\alpha$  (TNF- $\alpha$ )].

Literature reports and our evaluation of receptor homology among potential preclinical species suggested that careful selection of the animal model for in vivo evaluation would be required. Specifically, the relatively low homology between human and rodent TGR5 receptors coupled with the divergent structure–activity relationships (SARs) of human and rodent TGR5 activity for nonbile acid agonists<sup>15</sup> suggested that a nonrodent preclinical species would be necessary. Dog TGR5 agonist activity, however, is generally in line with human TGR5 agonist activity.<sup>14</sup> Thus, we sought to identify a potent, orally available TGR5 dog agonist that, if successful, might also act as a lead for a human agent. A key requirement would be the ability to rigorously test the safety and efficacy of systemic TGR5 activation by a nonbile acid agonist. Our medicinal chemistry effort toward such a tool compound is described herein.

A suitable starting point for generation of a tool compound was identified through high-throughput screening (HTS) of the Pfizer compound collection. The HTS assay system was tuned for high sensitivity and a large dynamic range, which is ideal for screening compounds with a wide variation in potency. In this case, a doxycycline-regulated promoter system was used to overexpress the human TGR5 receptor in a CHO cell line (induced assay). Elevation of cAMP was used as the detection system. A previously undisclosed chemotype, as represented by compound **5**, was discovered using this process. The MW, Log *D*, and topological polar surface area (TPSA) were acceptable, as were permeability (Ralph Russ canine kidney, RRCK)<sup>19</sup> and safety {[<sup>3</sup>H]-dofetilide binding, cytochrome P450 (CYP) inhibition} assessments. However, **5** showed very high intrinsic clearance in human and rat liver microsomes (HLM and RLM), indicating that significant effort would be required to achieve a reasonable clearance profile that could translate into a systemically available tool compound. Early on, a small cohort of compounds, including **5**, was subjected to further in vitro characterization in mouse-, rat-, and dog-induced cell lines, thus confirming the similarity between dog/human activity and the disparity for rodent/human activity for this series (Table 1).

With lead **5** in hand, a screening cascade was implemented to facilitate identification of an orally bioavailable tool compound

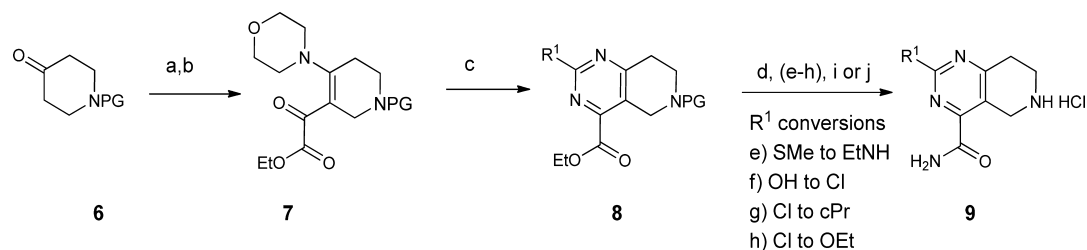
**Table 1. Pharmacological, Absorption, Metabolism, and Safety Properties of **5****

<b>5</b> ( $R^1 = \text{EtNH}$ , $R^2 = \text{iPr}$ )	
MW	395.5, eLog <i>D</i> 3.40, TPSA 101
hTGR5 induced $EC_{50}$ (nM)	2.5
mTGR5 induced $EC_{50}$ (nM)	5620
rTGR5 induced $EC_{50}$ (nM)	3930
dTGR5 induced $EC_{50}$ (nM)	6
RRCK ( $P_{app}$ , $10^{-6}$ cm/s) <sup>a</sup>	20.2
RLM CLint ( $\mu\text{L}/\text{min}/\text{mg}$ )	>564
HLM CLint ( $\mu\text{L}/\text{min}/\text{mg}$ )	>317
CYP (pct inh at 3 $\mu\text{M}$ )	1A2, 2D6, 2C9, 3A4 < 20%
[ <sup>3</sup> H]-dofetilide (pct inh at 10 $\mu\text{M}$ )	9%

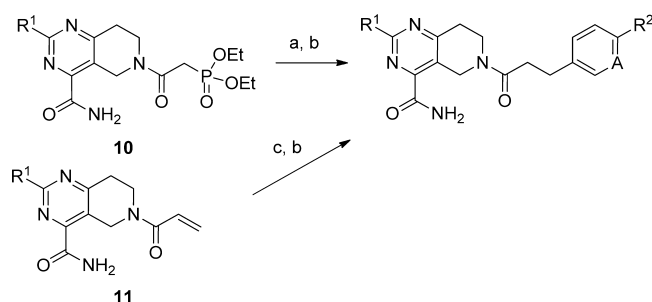
<sup>a</sup>RRCK = modified Madin–Darby permeability assay.

suitable for in vivo dog studies. Because both TGR5 agonist activity and metabolic fate in liver microsomes for human and dog were found to be generally aligned, the screening cascade initiated with parallel assessment of activity (human TGR5, induced assay) and HLM stability as the first decision points. Compounds with higher potency and lower intrinsic clearance relative to **5** were next profiled against in vitro cAMP assays described above without doxycycline (uninduced assay)<sup>20</sup> for both human and dog TGR5, as well as assessment of clearance in dog liver microsomes (DLM). Thus, the uninduced assay is based on cells expressing basal receptor numbers. The initial optimization process was designed to improve clearance by reduction of lipophilicity, minimize metabolic soft spots, and maximize TGR5 potency in the induced assay.

Compound **5** was divided into three fragments: the 2-substituent of the pyrimidine (A), the acyl linker (B), and the aryl group (C) for the purpose of exploring the SARs. Construction of the various tetrahydropyrido[4,3-*d*]pyrimidine cores is shown in Scheme 1. The enamine generated from *N*-Boc 4-piperidinone **6** was acylated with ethyl oxalyl chloride to give **7**, then cyclized with an amidine, urea, or 2-methylisothiourea to provide the pyrimidines **8**. Subsequent manipulation of the 2-substituent of the pyrimidine followed by deprotection provided **9** with a range of small alkyl, alkylamino, or ether groups at  $R^1$ . Variation of the amide substituent, using library or singleton chemistry, allowed for combination of cores with different  $R^1$  groups with a diverse set of acids. These were coupled under standard amide bond formation conditions [O-(7-azabenzotriazol-1-yl)-*N,N,N',N'*-tetramethyluronium hexafluorophosphate (HATU) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI)]. Expansion of the fragment C region was performed using two complementary, two-step parallel chemistry procedures. As outlined in Scheme 2, common intermediates **10** or **11** were combined with aldehydes (Horner–Emmons–Wadsworth) or aryl halides (Heck) followed by reduction to furnish the desired analogues. The library-based strategy, described above, allowed for modulation of the linker and aryl moieties to rapidly assess the B/C region of the molecule on a defined range of 2-substituted tetrahydropyrido[4,3-*d*]pyrimidine cores.

Scheme 1. Synthesis of Tetrahydropyrido[4,3-d]pyrimidine Core(s)<sup>a</sup>

<sup>a</sup>Reagents and conditions: PG = Boc or Cbz. (a) Morpholine, toluene, 110 °C. (b) Ethyl oxalyl chloride, Et<sub>3</sub>N, CH<sub>2</sub>Cl<sub>2</sub>. (c) R<sup>1</sup>-amidine, 2-methylisothiourea or urea, Et<sub>3</sub>N, EtOH, reflux. (d) Ammonia, MeOH. (e) Conversion of R<sup>1</sup> = SMe into R<sup>1</sup> = EtNH: (1) 3-chloroperoxybenzoic acid, CH<sub>2</sub>Cl<sub>2</sub>; (2) EtNH<sub>2</sub>, THF. (f) Conversion of R<sup>1</sup> = OH into R<sup>1</sup> = Cl: POCl<sub>3</sub>, acetone. (g) Conversion of R<sup>1</sup> = Cl into R<sup>1</sup> = cPr: cPrZnBr, PEPPSI<sup>TM</sup>. IPr, <sup>21</sup> THF. (h) Conversion of R<sup>1</sup> = Cl into R<sup>1</sup> = OEt: NaH/EtOH. (i) 4 N HCl, dioxane. (j) Pd/C, H<sub>2</sub>, MeOH.

Scheme 2. Synthesis of Phenpropanamides<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) Aldehyde, base. (b) Et<sub>3</sub>SiH, EtOH, cat. 5% Pd/C. (c) Aryl halide, (tBu<sub>3</sub>P)<sub>2</sub>Pd, dioxane, 95 °C.

The SAR trends for changes to the core and the B/C region could be gleaned from these libraries and are represented by selected compounds shown in Table 2. Compounds that were potent (EC<sub>50</sub> < 10 nM) in the induced assay were advanced to screening in the uninduced version of the human and dog assays. The uninduced EC<sub>50</sub> values for all analogues were right-shifted 50–150-fold relative to the induced EC<sub>50</sub> values as anticipated from standard receptor theory.<sup>17</sup> The correlation between human and dog potency was maintained. Even in the uninduced assay system, which should allow greater differentiation of agonist efficacy, all compounds were full agonists relative to control.<sup>22</sup> In general, minor changes to the original structure resulted in large shifts in agonist potency. We hypothesize that minor structural changes can alter the optimal agonist conformation of the ligand resulting in functionally significant changes to receptor conformation. Aromatic moieties linked by an ethylene spacer to the amide carbonyl

Table 2. Potency and Properties of Selected Analogues<sup>a</sup>

compd	R <sup>1</sup>	R <sup>2</sup>	A	EC <sub>50</sub> (nM) <sup>b</sup>			CLint <sup>c</sup>		
				induced hTGR5	uninduced hTGR5	uninduced dTGR5	HLM	DLM	eLog D
1				166	5540	4960	<8.0		1.85 <sup>d</sup>
2				24	316	445	>320		3.90
3				154	3220	2060	249		5.10
4				47	1420	584	>320	630	2.90
12	EtNH	H	CH	257	8320	N.T.	79		1.40
13	EtNH	CH <sub>3</sub>	CH	78	3780	5800	168		2.00 <sup>e</sup>
14	EtNH	CH <sub>2</sub> CH <sub>3</sub>	CH	9.2	215	828	238		2.40
5	EtNH	CH(CH <sub>3</sub> ) <sub>2</sub>	CH	3.6	158	213	>317	139	3.40
15	EtNH	OCH <sub>3</sub>	CH	137	2430	4280	96	27	1.05
16	EtNH	OCF <sub>3</sub>	CH	2.3	75	135	184	115	3.00
17	EtNH	SCF <sub>3</sub>	CH	1.6	48	112	>298		3.50
18	EtNH	CH <sub>2</sub> CF <sub>3</sub>	CH	4.7	154	233	141	68	2.60
19	EtNH	CF <sub>2</sub> CH <sub>3</sub>	CH	2.7	146	382	211		2.20
20	EtNH	CF <sub>2</sub> CH <sub>3</sub>	N	43	2990	3200	18	18	1.40
21	Et	OCF <sub>3</sub>	CH	2.0	83	220	174		2.90
22	EtO	OCF <sub>3</sub>	CH	3.3	112	303	115		2.90
23	cPr	OCF <sub>3</sub>	CH	2.8	43	407	217		3.30

<sup>a</sup>NT, not tested. <sup>b</sup>Most values are reflective of  $n \geq 3$  experiments. Please see the Supporting Information for the number of experiments and associated error. All values are for full agonists relative to a standard. <sup>c</sup>Intrinsic clearance  $\mu\text{L}/\text{min}/\text{mg}$ . <sup>d</sup>Measured Log  $D$  by shake-flask method. <sup>e</sup>Calculated Log  $D$  using a model based on Cubist modeling methodology [Rulequest Research, www.rulequest.com]. The training set for the model was based on >45000 measured eLog  $D$  values.<sup>24</sup>

were highly preferred. Changes made to the linking ethylene group (truncation, heteroatom interruption, and alkyl substitution) resulted in compounds with diminished potency (data not shown). Para substitution ( $R^2$ ) of the aromatic ring (phenyl, pyridyl) was preferred. Significant boosts in potency were noted in increasing the size of the para substituent from H (**12**) to  $\text{CH}_3$  (**13**) and then  $\text{CH}_3\text{CH}_2$  (**14**). Resulting increases in microsomal clearance from these structural changes correlate with higher Log  $D$  values. The potency increase in adjustment of the para substituent from OMe (**15**) to  $\text{OCF}_3$  (**16**) also warrants further discussion. Conformational analysis revealed that the ethyl of **14** and the  $\text{OCF}_3$  of **16** lie orthogonal to the plane of the phenyl ring, while the methoxy of **15** lies in the plane of the phenyl,<sup>23</sup> suggesting that the out of plane substituent could contribute to attaining a favorable agonist conformation. Additional fluoro-containing para substituents provided **17–19** that were equipotent with **16** but, despite having lower lipophilicity, suffered from high metabolic clearance. Incorporation of a pyridine led to a greater reduction of Log  $D$  for **20**. However, the resulting improvement in microsomal clearance for **20** came at the expense of a 20–40-fold decrease in potency relative to **19** and **16**, respectively. Conservative changes to the pyrimidine substituent ( $R^1$ ) to give **21** (Et), **22** (EtO), and **23** (cPr) were tolerated. More extensive changes to  $R^1$ , smaller or larger, usually resulted in an order of magnitude drop in potency and provided no metabolic stability advantage (data not shown).

With potent TGR5 agonists against both induced and uninduced assays in hand, we next sought to establish a correlation between the primary cAMP assays in cells recombinantly expressing human TGR5 with human cell lines that natively express TGR5. This would serve to improve confidence in functional potency determinations and ensure that adequate levels of TGR5 potency had been achieved within our series. Compound **16** was selected for characterization in human NCI-H716 cells (gut-derived) and peripheral blood mononuclear cells (PBMCs) isolated from whole human blood to assess its TGR5 cAMP activities (Table 3). Of note, an

**Table 3. Activation of TGR5 with 16 in Multiple Assay Systems**

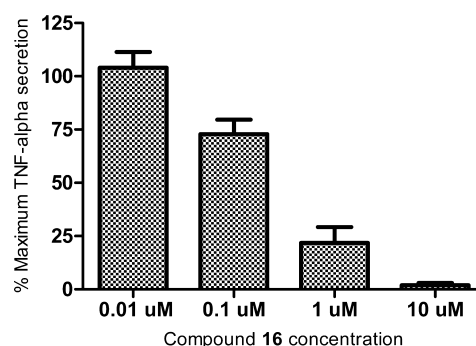
<b>16</b>	$\text{EC}_{50}$ (nM) <sup>a</sup>
induced hTGR5	2.3 ± 1.5
uninduced hTGR5	75 ± 72
NCI-H716 (cAMP)	145 ± 73
ex vivo PBMC (cAMP)	110 ± 67

<sup>a</sup>Values are means of >3 experiments ± standard deviations.

apparent correlation between the potency from the uninduced assay with the potencies from NCI-H716 cell and ex vivo human PBMC cAMP assays was established, while the induced assay appears to significantly overpredict potency.

An ex vivo whole blood assay was also developed. After a 30 min compound pretreatment, stimulation of human whole blood with lipopolysaccharides (LPS, 10 ng/mL, 4–5 h of stimulation), the suppression of TNF- $\alpha$  release in a dose-dependent manner was demonstrated with compound **16** (Figure 2). Similar results were noted for a compound from a distinct and highly dissimilar chemotype.<sup>25</sup>

Having established a correlation between our uninduced TGR5 assay and pharmacology in native systems, we then turned our attention toward identifying a tool compound for



**Figure 2.** Inhibition of TNF- $\alpha$  secretion, ex vivo whole blood LPS challenge. Data based on an average of three donors. Normalized to maximum TNF- $\alpha$  secreted.

use in vivo that may be able to achieve levels of exposure consistent with these findings. To better understand the clearance profiles of compounds **14** and **16**, in vitro metabolite identification studies using HLM in the presence and absence of reduced nicotinamide adenine dinucleotide phosphate (NADPH) cofactors were undertaken (Figure S2 in the Supporting Information). The major metabolite of **14** was a styrene derivative from hydroxylation at the benzylic carbon followed by elimination. With this information in hand, we replaced the 4-ethyl group in **14** with the metabolically blocked 4- $\text{OCF}_3$  group in **16** with the hope of reducing intrinsic clearance. While this change led to increased potency, there was no improvement in intrinsic clearance. A metabolite identification study of **16** showed changes corresponding to oxidation of the tetrahydropyrido[4,3- $d$ ]pyrimidine core; several hydroxylated metabolites (and peaks corresponding to elimination of water from these species) were found.<sup>26</sup> From these experiments, we concluded that there is no single metabolic soft spot to block within this series and that reduction in clearance would most likely be achieved through modulation of overall lipophilicity. However, our analogue program had demonstrated the challenges of achieving TGR5 potency at very low Log  $D$ .

The combination of potency, HLM clearance, and inability to block potential metabolic liabilities pointed to the selection of a compound that offered the best balance of attributes as a means to progress to in vivo studies. The best compounds at the extremes, that is, the most potent or the most metabolically stable, were assessed in DLM. While weaker in potency, compound **20** was selected on the basis of its lower HLM clearance. Compounds **16** and **18** were selected for further evaluation because of their potency. The in vitro human and dog microsomal intrinsic clearance are captured in Table 2. These compounds had moderate to high clearance in HLM and somewhat lower clearance in DLM.

On the basis of the balanced in vitro profile (uninduced  $\text{EC}_{50}$ , DLM, and Log  $D$ ), compound **16** was further assessed in an advanced battery of in vitro assays as well as in vivo dog pharmacokinetics study (Table 4). Of note was the high selectivity over CYP enzymes, selectivity over a wide panel of enzymes, receptors, and ion channels, and lack of activity against the farnesoid X receptor (FXR). In the dog pharmacokinetics study, **16** exhibited moderate clearance, low volume of distribution, short half-life, and good bioavailability.

On the basis of hTGR5 potency, its correlation to ex vivo assays linked to systemic TGR5 activation, dTGR5 potency, moderate in vivo dog clearance, and an acceptable in vitro

**Table 4. Pharmacological, ADME, and Safety Properties of 16**

MW	437.4
cLog P	2.30
shake flask Log D	3.03
TPSA (Å <sup>2</sup> )	110
microsomal intrinsic CL <sub>int</sub> (μL/min/mg)	
dog	115
human (fu, mic 0.52)	184
CYP IC <sub>50</sub> values	
1A2, 2C9, 2C19, 2D6, 3A4	all >30 μM
CYP metabolism phenotyping <sup>a</sup>	
2C19	5%
2C9	30%
3A4	65%
thermodynamic solubility	
pH 6.5, phosphate buffered saline, 12 μg/mL (27 μM)	
plasma protein binding (% free, fu)	
dog	2.6
human	8.3
permeability [P <sub>app</sub> (cm/s)]	
RRCK	AB 14.7 × 10 <sup>-6</sup>
MDRI-MDCK	AB 12.4 × 10 <sup>-6</sup> BA 19.2 × 10 <sup>-6</sup>
Genetox	Ames assay negative
hERG <sup>b</sup> inhibition (patch clamp)	IC <sub>50</sub> = 4.6 μM
CEREP panel (82 assays)	IC <sub>50</sub> > 10 μM
FXR (antagonist and agonist mode) <sup>27</sup>	IC <sub>50</sub> > 10 μM
in vivo pharmacokinetics	
CL (mL/min/kg)	dog, 8.05
V <sub>ss</sub> (L/kg)	0.43
t <sub>1/2</sub> (h)	0.84
F (%)	41

<sup>a</sup>Approximate percentage of drug-metabolizing isoform responsible for metabolism. <sup>b</sup>hERG = human ether-a-go-go-related gene.

safety profile, **16** was advanced to a 7 day toxicology study in dog. Of significant note, treatment-dependent changes in heart rate and blood pressure were noted for **16** as well as for another compound from a distinct and highly dissimilar chemotype,<sup>25</sup> with no treatment-related changes to electrocardiogram waveform parameters. These data infer that a mechanism-based toxicity associated with systemic exposure of this compound is likely involved.

In conclusion, a potent and orally available TGR5 agonist **16** was identified starting from HTS hit **5**. Compound **16** demonstrated an adequate dog in vivo pharmacokinetic profile that allowed for assessment of the effect of this systemically available TGR5 activator. A more detailed description of the in vitro and in vivo biological effects of **16** will be reported separately.

## ■ ASSOCIATED CONTENT

### 📄 Supporting Information

Experimental procedures, analytical data, and the description of in vitro and ex vivo assay systems. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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## Notes

The authors declare no competing financial interest.

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## ■ ABBREVIATIONS

ADME, absorption, distribution, metabolism, excretion; cAMP, cyclic adenosine 5'-monophosphate; CHO, Chinese hamster ovary; CYP, cytochrome P450; DLM, dog liver microsomes; EDCl, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; FXR, farnasoid X receptor; GLP-1, glucagon like peptide-1; GPCR, G-protein-coupled receptor; HATU, O-(7-azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate; hERG, human ether-a-go-go-related gene; HLM, human liver microsomes; HTS, high-throughput screening; LPS, lipopolysaccharides; MDCK Madin–Darby canine kidney; NADPH, reduced nicotinamide adenine dinucleotide phosphate; PBMC, peripheral blood mononuclear cells; PEPPSI-IPr, pyridine-enhanced precatalyst preparation stabilization and initiation-isopropyl; RLM, rat liver microsomes; RRCK, Ralph Russ canine kidney; SARs, structure–activity relationships; TGR5, Takeda G-protein-coupled receptor 5; TNF-α, tumor necrosis factor-α; TPSA, topological polar surface area

## ■ REFERENCES

- (1) Tiwari, A.; Maiti, P. TGR5: An Emerging Bile Acid G-protein Coupled Receptor Target for the Potential Treatment of Metabolic Disorders. *Drug Discovery Today* **2009**, *14*, 523–530.
- (2) Zhong, M. TGR5 as a Therapeutic Target for Treating Obesity. *Curr. Top. Med. Chem.* **2010**, *10*, 386–396.
- (3) Pols, T. W.; Noriega, L. G.; Nomura, M.; Auwerx, J.; Schoonjans, K. The Bile Acid Membrane Receptor TGR5 as an Emerging Target in Metabolism and Inflammation. *J. Hepatol.* **2011**, *54*, 1263–1272.
- (4) Xu, Y. Bile Acid Receptor Modulators in Metabolic Diseases. *Annu. Rep. Med. Chem.* **2011**, *46*, 69–87.
- (5) Katsuma, S.; Hirasawa, A.; Tsujimoto, G. Bile Acids Promote Glucagon-like Peptide-1 Secretion Through TGR5 in a Murine Enterendocrine Cell Line STC-1. *Biochem. Biophys. Res. Commun.* **2005**, *329*, 386–390.
- (6) Keitel, V.; Reinehr, R.; Gatsios, P.; Rupprecht, C.; Görg, B.; Selbach, O.; Häussinger, D.; Kubitz, R. The G-protein Coupled Bile Salt Receptor TGR5 is Expressed in Liver Sinusoidal Endothelial Cells. *Hepatology* **2007**, *45*, 695–704.
- (7) Pols, T. W.; Nomura, M.; Harach, T.; Lo Sasso, G.; Oosterveer, M. H.; Thomas, C.; Rizzo, G.; Gioiello, A.; Adorini, L.; Pellicciari, R.; Auwerx, J.; Schoonjans, K. TGR5 Activation Inhibits Atherosclerosis by Reducing Macrophage Inflammation and Lipid Loading. *Cell. Metab.* **2011**, *14*, 747–757.
- (8) Wang, Y.-D.; Chen, W.-D.; Yu, D.; Forman, B. M.; Huang, W. The G-Protein-Coupled Bile Acid Receptor, Gpbar1 (TGR5), Negatively Regulates Hepatic Inflammatory Response Through Antagonizing Nuclear Factor Kappa Light-Chain Enhancer of Activated B Cells (NF-κB) in Mice. *Hepatology* **2011**, *54*, 1421–1432.
- (9) Watanabe, M.; Houten, S. M.; Matak, C.; Christoffolete, M. A.; Kim, B. W.; Sato, H.; Messaddeq, N.; Harney, J. W.; Ezaki, O.; Kodama, T.; Schoonjans, K.; Bianco, A. C.; Auwerx, J. Bile Acids Induce Energy Expenditure by Promoting Intracellular Thyroid Hormone Activation. *Nature* **2006**, *439*, 484–489.
- (10) Brufau, G.; Bahr, M. J.; Staels, B.; Claudel, T.; Ockenga, J.; Böker, K. H. W.; Murphy, E. J.; Prado, K.; Stellaard, F.; Manns, M. P.;

Kuipers, F.; Tietge, U. T. F. Plasma Bile Acids are not Associated with Energy Metabolism in Humans. *Nutr. Metab. (London)* **2010**, *7*, 73.

(11) Sato, H.; Macchiarulo, A.; Thomas, C.; Gioiello, A.; Une, M.; Hofmann, A. F.; Saladin, R.; Schoonjans, K.; Pellicciari, R.; Auwerx, J. Novel Potent and Selective Bile Acid Derivatives as TGR5 Agonists: Biological Screening, Structure-Activity Relationships, and Molecular Modeling Studies. *J. Med. Chem.* **2008**, *51*, 1831–1841.

(12) Pellicciari, R.; Gioiello, A.; Macchiarulo, A.; Thomas, C.; Rosatelli, E.; Natalini, B.; Sardella, R.; Pruzanski, M.; Roda, A.; Pastorini, E.; Schoonjans, K.; Auwerx, J. Discovery of 6 $\alpha$ -Ethyl-23(S)-methylcholic Acid (S-EMCA, INT-777) as a Potent and Selective Agonist for the TGR5 Receptor, a Novel Target for Diabetes. *J. Med. Chem.* **2009**, *52*, 7958–7961.

(13) Thomas, C.; Gioiello, A.; Noriega, L.; Strehle, A.; Oury, J.; Rizzo, G.; Macchiarulo, A.; Yamamoto, H.; Matak, C.; Pruzanski, M.; Pellicciari, R.; Auwerx, J.; Schoonjans, K. TGR5-Mediated Bile Acid Sensing Controls Glucose Homeostasis. *Cell. Metab.* **2009**, *10*, 167–177.

(14) Evans, K. A.; Budzik, B. W.; Ross, S. A.; Wisnoski, D. D.; Jin, J.; Rivero, R. A.; Vimal, M.; Szewczyk, G. R.; Jayawickreme, C.; Moncol, D. L.; Rimele, T. J.; Armour, S. L.; Weaver, S. P.; Griffin, R. J.; Tadepalli, S. M.; M. R. Jeune, M. R.; T. W. Shearer, T. W.; Z. B. Chen, Z. B.; Chen, L.; Anderson, D. L.; Becherer, J. D.; De Los Frailes, M.; Colilla, F. J. Discovery of 3-Aryl-4-isoxazolecarboxamides as TGR5 Receptor Agonists. *J. Med. Chem.* **2009**, *52*, 7962–7965.

(15) Herbert, M. R.; Siegel, D. L.; Staszewski, L.; Cayanan, C.; Banerjee, U.; Dhamija, S.; Anderson, J.; Fan, A.; Wang, L.; Rix, P.; Shiau, A. K.; Rao, T. S.; Noble, S. A.; Heyman, R. A.; Bischoff, E.; Guha, M.; Kabakibi, A.; Pinkerton, A. B. Synthesis and SAR of 2-Aryl-3-aminomethylquinolines as Agonists of the Bile Acid Receptor TGR5. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5718–5721.

(16) Budzik, B. W.; Evans, K. A.; Wisnoski, D. D.; Jin, J.; Rivero, R. A.; Szewczyk, G. R.; Jayawickreme, C.; Moncol, D. L.; Yu, H. Synthesis and Structure-Activity Relationships of a Series of 3-Aryl-4-isoxazolecarboxamides as a New Class of TGR5 Agonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1363–1367.

(17) Kenakin, T. Agonists: The Measurement of Affinity and Efficacy in Functional Assays. In *A Pharmacology Primer: Theory, Applications, and Methods*, 3rd ed.; Academic Press: New York, 2009; Chapter 5, pp 81–100.

(18) A comparison of reported TGR5 potency with values from our induced and uninduced assays is shown in the Supporting Information. Several literature assays are based on recombinant cell lines that appear to have TGR5 receptor levels higher than the native system. The reported potencies for selected agonists are generally aligned with our induced assay system.

(19) Di, L.; Whitney-Pickett, C.; Umland, J. P.; Zhang, H.; Zhang, X.; Gebhard, D. F.; Lai, Y.; Federico, J. J.; Davidson, R. E.; Smith, R.; Reyner, E. L.; Lee, C.; Feng, B.; Rotter, C.; Varma, M. V.; Kempshall, S.; Fenner, K.; El-Kattan, A. F.; Liston, T. E.; Troutman, M. D. Development of a New Permeability Assay Using Low-efflux MDCKII Cells. *J. Pharm. Sci.* **2011**, *100*, 4974–4985.

(20) This lower expressing system provided a more conservative estimate of agonist potency and intrinsic activity. Detailed in vitro TGR5 pharmacology will be reported elsewhere.

(21) Valente, C.; Belowich, M. E.; Hadei, N.; Organ, M. G. Pd-PEPPSI Complexes and the Negishi Reaction. *Eur. J. Org. Chem.* **2010**, 4343–4354.

(22) Please see the Supporting Information.

(23) Manteau, B.; Genix, P.; BreLOT, L.; Vors, J.-P.; Pazenok, S.; Giornal, F.; Leuenger, C.; Leroux, F. R. A General Approach to (Trifluoromethoxy)pyridines: First X-ray Structure Determinations and Quantum Chemistry Studies. *Eur. J. Org. Chem.* **2010**, 6043–6066.

(24) Lombardo, F.; Shalaeva, M. Y.; K. A. Tupper, K. A.; Gao, F. Elog<sub>D</sub><sup>oct</sup>: A Tool for Lipophilicity Determination in Drug Discovery. 2. Basic and Neutral Compounds. *J. Med. Chem.* **2001**, *44*, 2490–2497.

(25) Futatsugi, K.; Bahnck, K. B.; Brenner, M. B.; Buxton, J.; Chin, J. E.; Coffey, S. B.; Dubins, J.; Flynn, D.; Gautreau, D.; Guzman-Perez, A.; Hadcock, J. R.; Hepworth, D.; Herr, M.; Hinchey, T.; Janssen, A.

M.; Jennings, S. M.; Jiao, W.; Lavergne, S. Y.; Li, B.; Li, M.; Munchhof, M. J.; Orr, S. T. M.; Piotrowski, D. W.; Roush, N. S.; Sammons, M.; Stevens, B. D.; Storer, G.; Wang, J.; Warmus, J. S.; Wei, L.; Wolford, A. C. Optimization of Triazole-based TGR5 Agonists Towards Orally Available Agents. *Med. Chem. Commun.*, published online September 26, **2012**; DOI: 10.1039/c2md20174g.

(26) A subset of compounds that incorporated methyl, fluoro, or deuterio substituents onto the core were designed to address metabolic soft spots. The substituted compounds that were assessed had lower potency and equivalent or higher clearance relative to that of **16**. A full description of deuteration and metabolic fate of compounds related to **16** will be published in due course. For metabolic blocking in a related core, see the following: Nagle, A.; Wu, T.; Kuhen, K.; Gagaring, K.; Borboa, R.; Francek, C.; Chen, Z.; Plouffe, D.; Lin, X.; Caldwell, C.; Ek, J.; Skolnik, S.; Liu, F.; Wang, J.; Chang, J.; Li, C.; Liu, B.; Hollenbeck, T.; Tuntland, T.; Isbell, J.; Chuan, T.; Alper, P. B.; Fischli, C.; Brun, R.; Lakshminarayana, S. B.; Rottmann, M.; Diagona, T. T.; Winzeler, E. A.; Glynne, R.; Tully, D. C.; Chatterjee, A. K. Imidazolopiperazines: Lead Optimization of the Second-Generation Antimalarial Agents. *J. Med. Chem.* **2012**, *55*, 4244–4273.

(27) Assays conducted at Caliper Life Sciences (catalog #100-1051 and 100-1052).