CHAPTER 5

Bile Acid Receptor Modulators in Metabolic Diseases

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Contents

1. INTRODUCTION

Bile acids (BAs) have for a long time been viewed as detergents able to solubilize cholesterol, fatty acids, and liposoluble vitamins, thus facilitating the digestion, transportation, and gastrointestinal absorption of nutrients. BAs have also been shown to be involved in a large variety of cellular processes. Some recent discoveries have unveiled novel actions of BAs as signaling hormones endowed with a wide array of endocrine functions. In 1999, three research groups independently identified BAs as endogenous ligands for a nuclear receptor, the farnesoid X receptor (FXR) [1–3]. In early 2000, two research groups discovered that a novel class A G-protein-coupled receptor (GPCR), TGR5 (also known as GPBAR1, GPCR19, GPR131, BG37, M-BAR, Rup 43), can be activated by BAs [4,5].

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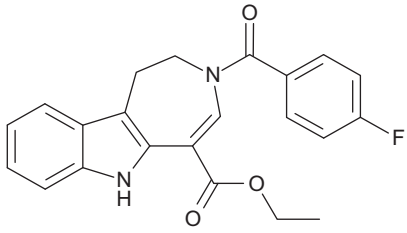
Highly expressed in the liver, intestine, kidney, adrenal glands, and adipose tissue, FXR is a master regulator of the synthesis and pleiotropic actions of endogenous BAs [6]. Activation of FXR by BAs or synthetic FXR agonists lowers plasma triglycerides by a mechanism involving repression of hepatic sterol regulatory element binding protein-1c (SREBP-1c) expression and the modulation of glucose-dependent lipogenic genes. Furthermore, FXR controls lipid and glucose metabolism through regulation of gluconeogenesis and glycogenolysis in the liver and through regulation of peripheral insulin sensitivity in striated muscle and adipose tissue [7–9]. Similar to effects in the liver, FXR agonists modulate lipid metabolism and promote anti-inflammatory and antifibrotic effects in the kidney, suggesting a potential use of FXR agonists to treat diabetic nephropathy and other fibrotic renal diseases [10].

TGR5 is expressed in brown adipose tissue, muscle, liver, intestine, gallbladder [11], and selected areas of the central nervous system [5]. TGR5 activation in intestinal enteroendocrine L cells stimulates secretion of the incretin glucagon-like peptide-1 (GLP-1) [12]. Activation of GLP-1 receptors by derivatives of exendin-4 or enhancement of GLP-1 half-life by dipeptidyl peptidase-4 inhibitors is clinically well-established therapeutic approaches for the treatment of type 2 diabetes [13]. By augmenting GLP-1 activity, these agents improve glycemic control in diabetic patients through increase of glucose-dependent insulin secretion and reduction of glucagon production. Treatment of mice fed a high fat diet with the TGR5 agonist oleanolic acid resulted in lower serum glucose and insulin levels and enhanced glucose tolerance [14]. In addition, administration of BAs to mice increased energy expenditure in brown adipose tissue, preventing obesity and insulin resistance *via* TGR5-mediated cAMP-dependent induction of type 2 iodothyronine deiodinase (D2), which locally stimulates thyroid hormone-mediated thermogenesis [15].

The intent of this report is to present the most recent publications on FXR agonists and to provide a comprehensive literature summary of TGR5 agonists.

2. FXR AGONISTS

Azepinol[4,5-*b*]indole **1** ($hEC_{50} = 600$ nM, efficacy (eff) = 100%) was identified as a FXR agonist lead from a high-throughput screening effort. Structure–activity relationship (SAR) studies around the azepine ring demonstrated that dialkyl substitution at C-1 led to a 30-fold improvement in potency. In addition, incorporation of an isopropyl ester yielded another ~3-fold boost in potency. Compound **2** represented the most potent FXR agonist within the series ($hEC_{50} = 4$ nM, eff = 149%) [16].



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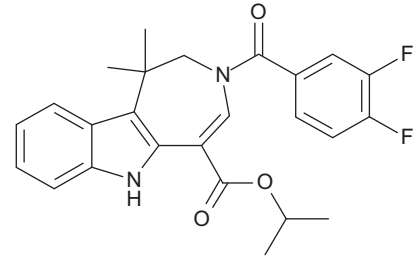
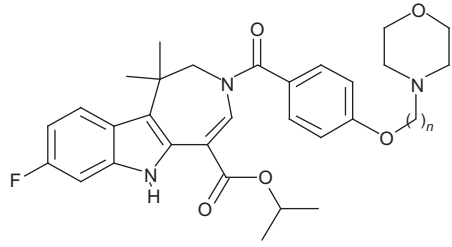


2

A rat pharmacokinetic (PK) study showed that compound **2** had good oral bioavailability ($F = 38\%$) and a long half-life ($t_{1/2} = 24$ h). Oral treatment of normal C57BL/6 mice with **2** administered at a dose of 10 mg/kg/d for 7 days yielded statistically significant reductions of triglycerides (TG, 24%) and total cholesterol (22%) levels. When administered to low-density lipoprotein receptor knockout ($LDLR^{-/-}$) mice fed a Western diet for 8 weeks, **2** lowered both TG (19% and 39% at doses of 1 and 3 mg/kg, respectively) and total cholesterol (23% and 50% at doses of 1 and 3 mg/kg, respectively). However, this molecule was poorly soluble. Guided by crystallographic data, the appended morpholine analogs **3a** and **3b** were identified and showed dramatic 400-fold improvements in equilibrium solubility measured in 0.5% methylcellulose/2% Tween-80 in water. However, bioavailability of **3a** and **3b** in rats was not improved compared to that of **2** ($F = 38\%$ and 25% for **3a** and **3b**, respectively) which is likely due to their high clearance (Cl = 52 and 64 mL/min/kg for **3a** and **3b**, respectively).

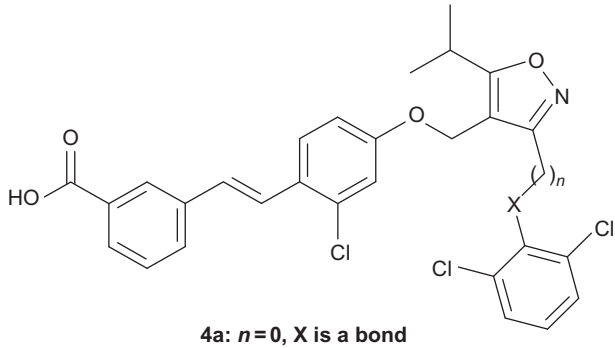


3a: *n* = 3
3b: *n* = 2

Both compounds showed potencies (**3a**: $mEC_{50} = 52$ nM, eff = 117%; **3b**: $mEC_{50} = 188$ nM, eff = 110%) at mouse FXR similar to that of compound **2** ($mEC_{50} = 152$ nM, eff = 174%). Oral administration of **3a** and **3b** to $LDLR^{-/-}$ mice caused a dose-dependent reduction of low-density lipoprotein cholesterol (LDLc). In female rhesus monkeys, **3a** given at a dose of 60 mg/kg/d po for 4 weeks resulted in a significant lowering of TG (~50%; absolute value was not reported), very low-density

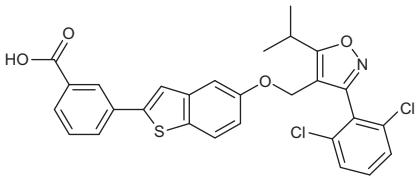
lipoprotein cholesterol (VLDLc ~ 50%; absolute value was not reported), and LDLc (63%) [17].

The potent FXR agonist, **4a** (GW4046, FXR transient transfection (TT) assay EC_{50} = 65 nM, eff = 100%), was unsuitable for further development due to several issues. These liabilities include poor rat PK (high clearance and low bioavailability), a potentially toxic stilbene pharmacophore, and stilbene-mediated UV light instability. SAR development at the 3- and 5-positions of the isoxazole ring revealed a preference for hydrophobic substituents [18]. In addition, some polarity was tolerated in the tether at the 3-position of the isoxazole linked to the phenyl group, for example, **4b** (FXR TT EC_{50} = 89 nM, eff = 89%). A rat PK study demonstrated that **4b** had an improved $t_{1/2}$ (2 h) and clearance (Cl = 20 mL/min/kg). Unfortunately, low oral bioavailability (F = 9%) was observed. It was hypothesized that the stilbene moiety could be the predominating detrimental structural feature irrespective of modifications elsewhere in these molecules.



4a: *n* = 0, X is a bond
4b: *n* = 1, X = O

In an attempt to address the perceived liability of the stilbene functional group, a series of conformationally constrained analogs were explored [19]. Benzothiophene analog **5** (FXR TT EC_{50} = 32 nM, eff = 87%) was equipotent with **4a**. Indole analog **6a** showed a slight attenuation of FXR activity (FXR TT EC_{50} = 210 nM, eff = 84%). In rat PK studies, compound **5** had very high clearance (Cl = 66 mL/min/kg), a short half-life ($t_{1/2}$ = 15 min), and poor bioavailability (F = 9%).



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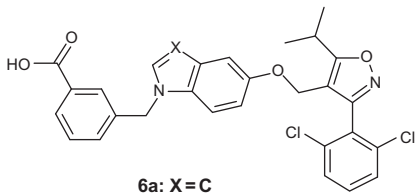
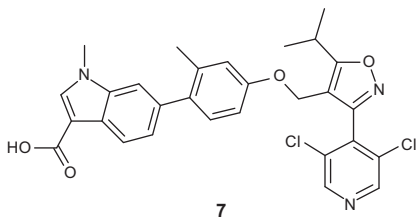


6a: X = C
6b: X = N

Indole **6a** had significantly lower clearance (Cl = 6.7 mL/min/kg); however, the half-life was modest ($t_{1/2}$ = 45 min) and the oral bioavailability was low (F = 12%). It was suggested that poor solubility of **6a** may contribute to its poor oral bioavailability. To improve solubility, a second nitrogen atom was incorporated into the indole ring to give benzimidazole **6b**. Although this compound was significantly less active at FXR (FRX TT EC_{50} = 5 μM, eff = 40%), a twofold improvement in bioavailability was achieved (F = 26%) with little alteration of clearance or half-life suggesting that low solubility of these GW 4064 analogs may limit their absorption.

GW4064 was also the starting point for a study by an independent research group. The co-crystal structure of GW4064 with FXR suggested the potential for favorable hydrogen bond interactions between the isoxazole 3-aryl group and several receptor residues such as Tyr373 and Ser336. Replacing the 2,6-dichlorophenyl with a 2,6-dichloro-4-pyridyl moiety attenuated both FXR binding and functional activity. However, combination of this pyridine moiety with an *N*-methyl indole ring, an optimized stilbene replacement, afforded **7** with good FXR binding affinity (94 nM) in a human scintillation proximity binding assay compared to 64 nM for GW4064 [20].



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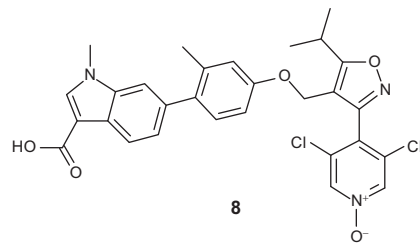
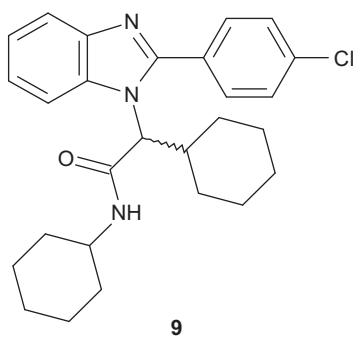


8

Oxidation of the pyridine to the corresponding *N*-oxide gave **8**, the most potent compound in this series, with a FXR binding affinity of 45 nM. Compared to other analogs reported in this study, **8** had the best permeability (PAMPA, 5.87 × 10^{-4} cm/s). Molecular docking suggested that the *N*-oxide oxygen most likely participates in an H-bond acceptor interaction with Tyr373 on Helix 7 and/or Ser336 on Helix 5 [20].

From a separate screening effort, benzimidazolyl acetamide **9** was discovered as a novel FXR agonist with binding affinity of 70 nM [21,22]. Attempts to improve the physical properties of this compound by replacing the cyclohexyl groups with more polar moieties proved unsuccessful. This result is consistent with the co-crystal structure of compound **9** and hFXR, where the cyclohexyl groups are oriented within highly lipophilic pockets.



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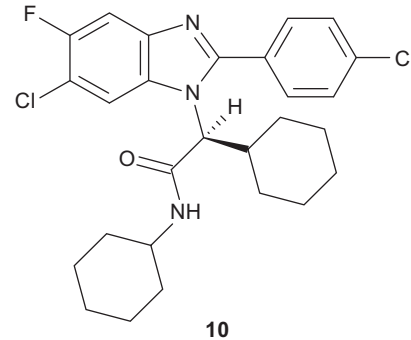
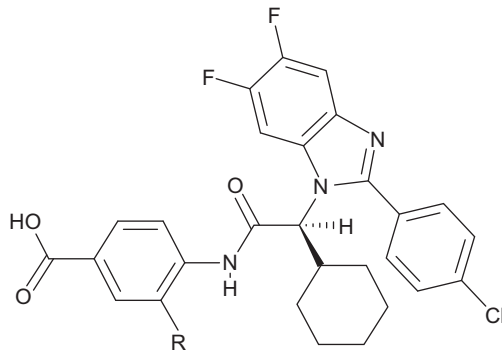


10

The lead molecule from this series, **10** (IC_{50} SPA = 13 nM), was evaluated in $LDLR^{-/-}$ mice. It significantly reduced total cholesterol (45%), LDL (48%), and TG (52%) when orally administered at a dose of 30 mg/kg/d for 5 days [21]. The poor physiochemical properties of **10**, namely high lipophilicity and low aqueous solubility, limited its potential for further development. In addition, this molecule inhibited the hERG potassium channel *in vitro* (IC_{50} = 1.6 μM). Further structural analysis revealed a more polar and yet unexplored pocket consisting of Gln267, Asn297, His298, Arg335, and three water molecules near the region where the *N*-cyclohexyl group binds [23]. Subsequent SAR efforts to replace this cyclohexyl group with a 4-carboxyphenyl ring yielded compound **11** without loss of receptor binding activity (FXR IC_{50} SPA = 50 nM) but with significantly improved solubility (88 vs. <1 μg/mL for **10**) and reduced hERG inhibition (IC_{50} > 20 μM) [23–25].

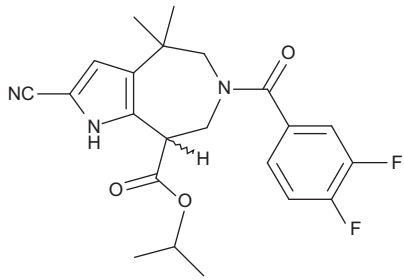


11: R = H
12: R = F

Compound **12**, a fluoro analog of **11**, showed further enhancement of FXR binding (IC_{50} SPA = 37 nM) and solubility (115 μg/mL) with no significant hERG activity (IC_{50} > 20 μM). As a result of its good murine

in vitro potency (IC_{50} = 290 nM, EC_{50} = 870 nM, eff = 38%) and PK properties in mice (Cl = 10 mL/min/kg, F = 33%), **12** was evaluated in $LDLR^{-/-}$ mice. After 5 days of treatment (10 mg/kg/d, po), statistically significant decreases in plasma total cholesterol (41%), LDLc (33%), and TG (59%) were observed [23].

There have been numerous recent patent applications disclosing FXR agonists with novel chemical scaffolds. In a 2009 U.S. patent application, hexahydropyrroloazepines exemplified by **13** were claimed as FXR agonists [26]. Using a Gal4/hFXR fusion protein expressed in the HEK293 cell line, compound **13** showed an EC_{50} of 280 nM. A related tetrahydropyrroloazepine series of FXR agonists was disclosed in a separate U.S. patent application [27]. Representative compound **14** showed an EC_{50} of 3 nM in the HEK293 cell assay.



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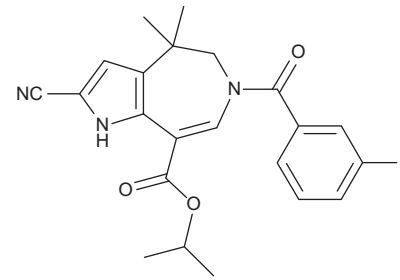
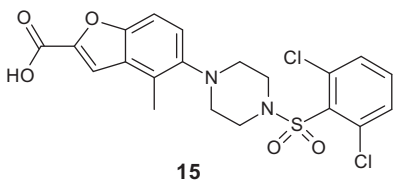


14

A class of benzofurane/benzothiophene/benzothiazole derivatives was described as FXR modulators. Representative compound **15** showed an EC_{50} of 1.95 μM in a hFXR transactivation assay in CV-1 cells [28,29]. Novel biaryl carboxylates were described in a 2009 patent application [30]. Compound **16** was exemplified and had an EC_{50} of <100 nM in a hFXR SRC-1 cofactor recruitment assay.



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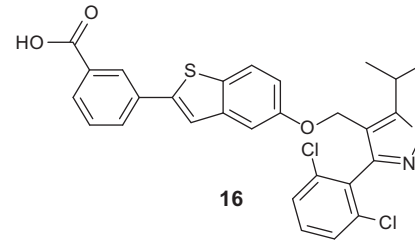
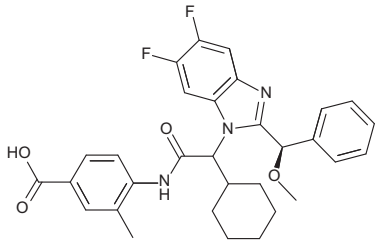


16

Another application described benzimidazole analogs represented by **17** which had an IC_{50} of 20 nM in a FXR binding assay [31,32].



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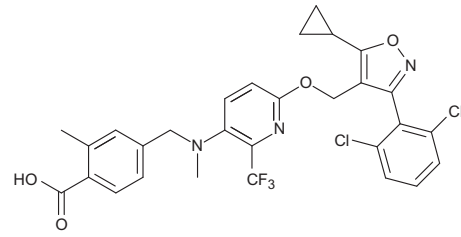


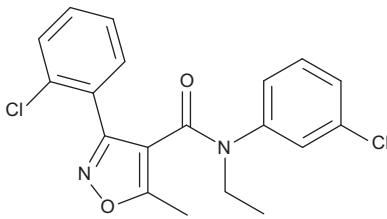
18

A series of benzoic acid analogs were reported to display FXR agonist activity. Compound **18** provided an example from this class and had an EC_{50} of 7.7 nM in a FRET functional assay [33].

3. TGR5 AGONISTS

3.1. Non-BA agonists

A high-throughput screen using a BacMam-transduced human osteosarcoma cell line (U2-OS) led to the discovery of isoxazole **19** as a TGR5 full agonist with a pEC_{50} of 5.3 (EC_{50} = 5.0 μM) and 100% response [34]. SAR optimization on the amide phenyl ring, exemplified by compound **20** (pEC_{50} = 7.5, EC_{50} = 32 nM), suggested that *para*-substitution was preferred [35]. In melanophore cells, compound **20** was equipotent at both human (pEC_{50} = 7.5, EC_{50} = 32 nM) and canine receptors (pEC_{50} = 7.2, EC_{50} = 63 nM). In a conscious dog model, intrajejunal injection of glucose (0.125 g/kg) with co-administration of **20** at a dose of 1 mg/kg afforded a significant improvement in hepatic portal vein GLP-1 secretion and reduction in portal vein glucose levels compared to vehicle [34].



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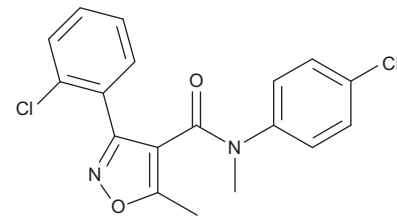
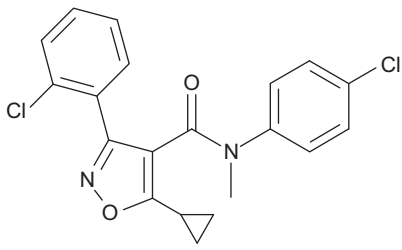


20

Compound **20** showed high *in vivo* clearance (Cl = 85 mL/min/kg) in rats and high intrinsic clearance (Cl_{int} = 48 mL/min/kg) in rat liver microsomes suggesting potential challenges to the developability of this series. In addition, **20** had measurable activity against two cytochrome P450 (CYP450) isoforms including 2C19 (pIC_{50} = 6.5, EC_{50} = 0.3 μM) and

3A4 ($pIC_{50} = 5.9$, $EC_{50} = 1.3$ μM). SAR development at the 5-position of isoxazole revealed that some increased steric bulk was well tolerated, exemplified by **21**. Compound **21** showed improved *in vitro* potency ($pEC_{50} = 8.4$, $EC_{50} = 4$ nM) and reduced *in vitro* clearance in rat ($Cl_{int} = 10$ mL/min/kg). Interestingly, replacement of the isoxazole with 1,2,3-triazole, **22**, afforded further reduction of intrinsic clearance ($Cl_{int} = 6.5$ mL/min/kg) as well as an improved CYP450 profile ($pIC_{50} < 5.7$, $EC_{50} > 2$ μM vs. all isoforms), while maintaining good *in vitro* potency ($pEC_{50} = 7.9$, $EC_{50} = 13$ nM) [35]. However, no *in vivo* pharmacodynamic activity was reported for this compound.



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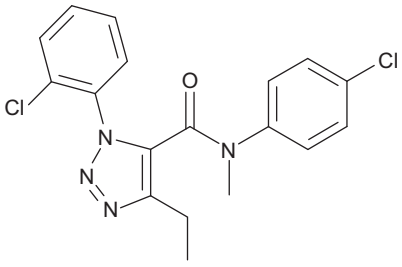
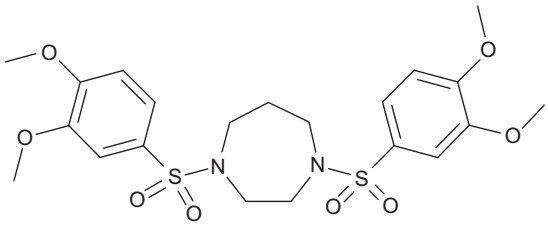


22

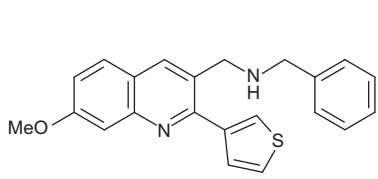
In a 2007 patent application, a series of bis-phenyl sulfonamides were disclosed as TGR5 agonists [36]. In melanophore cells transfected with human TGR5 (hTGR5), compound **23** showed agonist activity with a pEC_{50} (EC_{50}) between 6.0 (1 μM) and 6.9 (0.1 μM). Given the high TGR5 receptor expression in colon [5], it is hypothesized that maximum pharmacological effect can be achieved by local administration of drug. Thus, anesthetized CD rats were administered with **23** by intracolonic injection at a dose of 2.5 mg/kg which afforded a significant increase of plasma GLP-1 levels (measured by both active and total GLP-1). In a 16-day chronic study, conscious Goto-Kakizaki rats were dosed intracolonically with **23** (0.3 mg/kg QD). On day 16, an intravenous glucose tolerance test was performed. A significant glucose reduction was achieved in treated animals compared to vehicle control group.



23

A class of quinoline compounds represented by **24** was discovered as TGR5 agonists [37,38]. Compound **24** was first identified as a hit from a

high-throughput screen. In HEK293 cells expressing hTGR5, **24** increased cAMP production with an EC_{50} of approximately 10 μM. However, this compound was a significantly less potent agonist of mouse TGR5 ($EC_{50} > 10$ μM). Linker homologation to the phenethylamine and bromine substitution on the phenyl ring led to compound **25** affording a significant improvement in potency at both human ($hEC_{50} = 65$ nM) and mouse ($mEC_{50} = 3.2$ μM) receptors. Phenol deprotection gave analog **26**, which was considerably more active at the mouse receptor ($mEC_{50} = 0.28$ μM) but much less active at the human receptor ($hEC_{50} = 5.1$ μM). To test the hypothesis that activation of TGR5 stimulates GLP-1 release, **26** (30 mg/kg) was orally administered to diet-induced obese (DIO) mice. Following an oral glucose challenge, a statistically significant increase in plasma active GLP-1 levels was observed. Acute administration of **26** (30 mg/kg po) to DIO mice prior to an oral glucose tolerance test also resulted in a significant reduction of plasma glucose area under curve (AUC) [37]. In a separate study, treatment of C57BL/6 DIO mice with **26** for 2 weeks at doses of 3, 30, and 100 mg/kg bid reduced fasting glucose, post-prandial TG, and high-density lipoprotein (HDL) levels [38].



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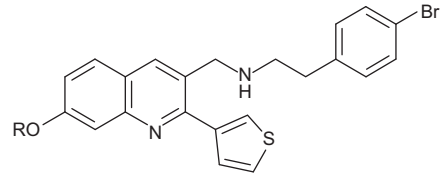
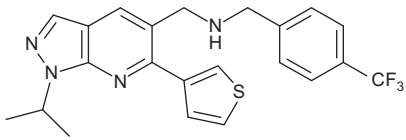


25: R = Me
26: R = H

Two separate publications described different types of arylpyridines as TGR5 agonists [39–40]. In HEK293 cells expressing hTGR5, compounds **27-29** increased cAMP production with EC_{50} values of less than 10 μM.



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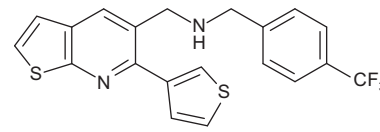

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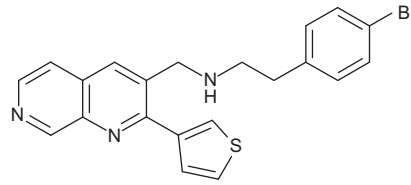
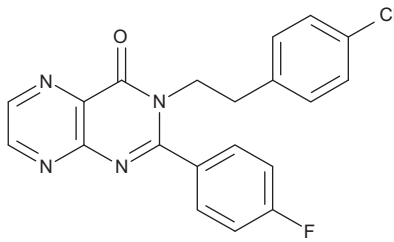


29

Recently, two patent applications were published describing different classes of pteridinone derivatives as potent TGR5 agonists [41,42]. Representative compounds **30** and **31** stimulated cAMP production with EC_{50} values of less than 10 μM in HEK293 cells expressing hTGR5.



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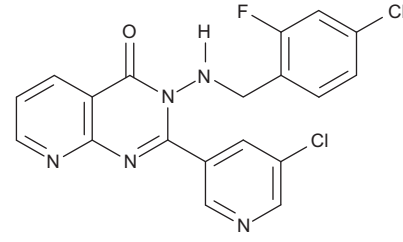
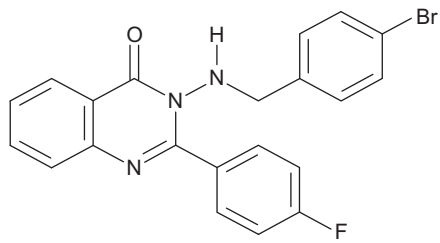


31

Compound **32** was reported to represent a novel class of quinazolinone TGR5 agonists [43]. In HEK293 cells expressing hTGR5, **32** stimulated cAMP production with an EC_{50} value of less than 10 μM. In an oral glucose tolerance test, mice that were dosed orally with 30 mg/kg of **32** demonstrated a 52% reduction in glucose AUC compared to a vehicle control group. This glucose reduction was accompanied by increased insulin (130%) and active GLP-1 (70%) levels compared to vehicle-treated animals [43].



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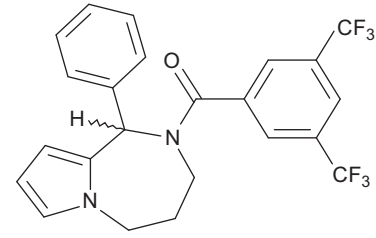
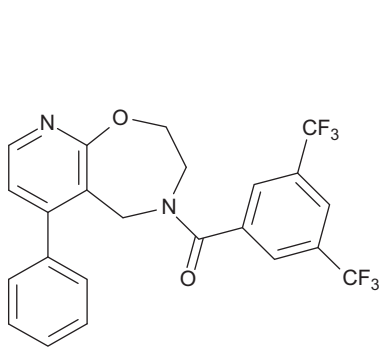


33

In a 2007 patent application, a family of diazepine derivatives was claimed as TGR5 agonists [44]. Representing this family, compound **33** was shown to stimulate cAMP secretion in a HEK293 cell line expressing hTGR5 with an EC_{50} value of less than 10 μM.

Related oxazepine compounds were also reported to be TGR5 agonists [45,46]. In CHO cells expressing hTGR5, compound **34** (1 μM) stimulated cAMP production by 100%. In NCI-H716 cells, an increase in cAMP production of 100% was observed in the presence of 10 μM of the *trans* isomer of compound **35**. In the same cell line, compound **35** (5 μM) was reported to increase GLP-1 secretion by 157%.



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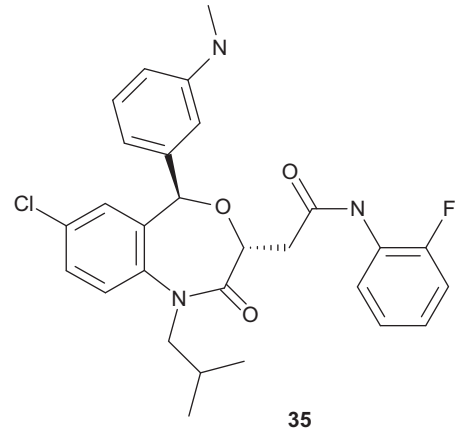
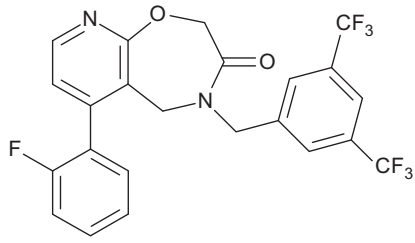


35

In a separate patent application [47], oxazepinone **36** (30 μM) was reported to stimulate GLP-1 secretion by 249% in NCI-H716 cells and by 360% in a rat bowel primary culture cell. To evaluate *in vivo* GLP-1 secretion and glucose-dependent insulin secretion, **36** was administered orally to male F344 rats at doses of 30 and 100 mg/kg. Animals treated with 100 mg/kg of **36** showed significant GLP-1 and insulin secretion after a glucose challenge compared to the vehicle group.



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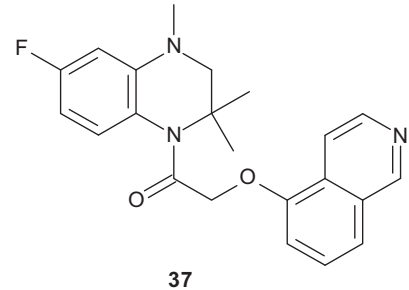
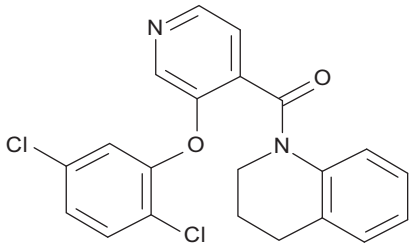


37

A set of heteroaryl acetamide derivatives was claimed as TGR5 agonists [48]. Among these, dihydroquinoxaline **37** (30 μM) elicited 251% GLP-1 secretion in rat bowel primary culture cells. In a rat intestine perfusion model, **37** (10 μM) gave a significant increase of portal vein GLP-1 concentration.

Recently, a series of aryl amides was discovered to have TGR5 agonist activity [49]. In CHO cells expressing hTGR5, compound **38** increased cAMP production with an EC_{50} of 7 nM.



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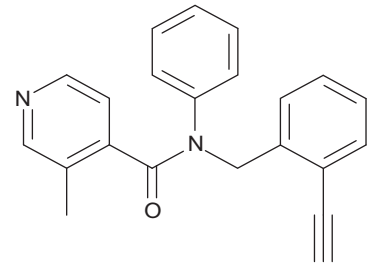
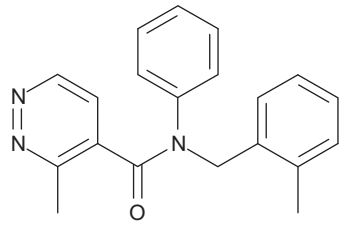


39

In a 2007 patent application, heterocyclic amides exemplified by compound **39** were described as TGR5 agonists [50]. No biological data were reported.

Another patent application from the same group disclosed a series of pyridazine/pyridine/pyran derivatives as TGR5 agonists [51]. Compounds **40** and **41** are shown as representative structures. However, no biological data were presented.



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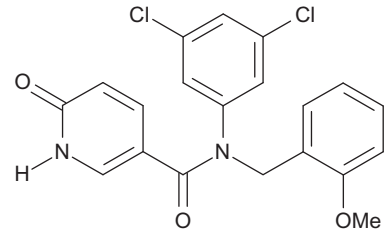
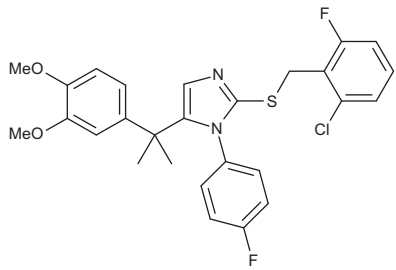


41

A recent patent application claimed a series of imidazole and triazole compounds exemplified by **42** and **43** [52]. In a hTGR5/CRE-luciferase assay, both compounds showed receptor activation with EC_{50} values of less than 100 nM. In mouse STC-1 cells under high glucose conditions, **42** effectively stimulated GLP-1 secretion with an EC_{50} of 17 nM. *In vivo*, a twofold increase in GLP-1 secretion was achieved when fasted C57BL/6 mice were treated with **42** at an oral dose of 30 mg/kg.



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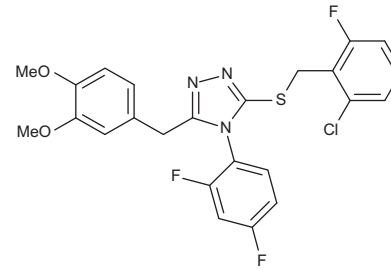
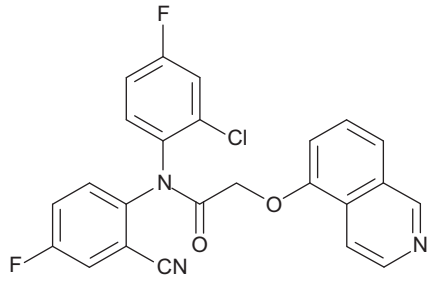


43

In a 2010 patent application, a class of isoquinolines was claimed to be TGR5 agonists [53]. Representative compound **44** stimulated cAMP production in HEK293 cells expressing hTGR5 with an EC_{50} of 7.37 μM. Another patent application from the same group claimed a series of isoquinolinyloxymethyl heteroaryl analogs exemplified by **45** [54]. In the same HEK293 cellular assay, compound **45** stimulated cAMP production with an EC_{50} of 229 nM.



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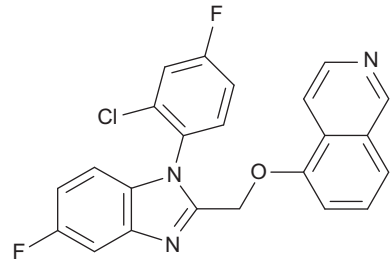
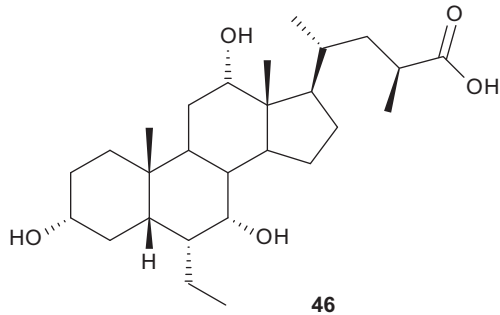


45

3.2. BA derivatives

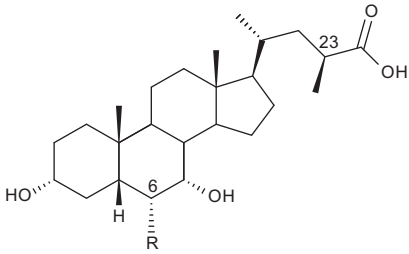
In 2009, a semisynthetic cholic acid (CA) derivative, 6α-ethyl-23(*S*)-methyl-CA (EMCA, INT-777, **46**), was reported to be a selective TGR5 agonist [55]. Initial SAR studies unveiled that the incorporation of a methyl group at the C-23 position of CA side chain afforded the selective, albeit not very potent, TGR5 agonist **47** which had an EC_{50} of 3.58 μM (FXR $EC_{50} > 100$ μM). The *S*-configuration at C23 was critical for TGR5 potency.



46

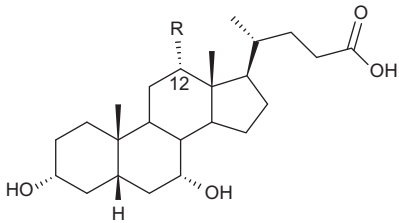
Improvement in TGR5 *in vitro* potency was noted by introduction of a small alkyl substituent at the C-6 position as in compound **48**. Unfortunately, this compound suffered from poor physical properties, namely low solubility and high albumin binding. Structurally, CA (**49**) differs from chenodeoxycholic acid (CDCA, **50**) at C-12 by having an additional α-hydroxyl group oriented on the polar side of the molecule.

This ''minor'' structural change accounts for the markedly different solubilities.



	TGR5 EC_{50} (efficacy %)	FXR EC_{50} (efficacy %)
47: R = H	3.58 μM (110%)	>100 μM (0%)
48: R = Et	0.095 μM (102%)	11.8 μM (73%)

Moreover, **49** is devoid of FXR activity ($EC_{50} > 100$ μM) while maintaining good TGR5 activity ($EC_{50} = 13.6$ μM). Introduction of the C-12 α-hydroxyl group into compound **48** afforded compound **46**, which showed potent TGR5 activity ($EC_{50} = 0.8$ μM, 166%) and excellent selectivity over FXR ($EC_{50} > 100$ μM). Compound **46** appeared to be stable to human stool broth culture, with more than 95% of compound unmodified after incubation for 12 h. It was believed that the 6α-ethyl group provided steric hindrance to the bacterial 7α-dehydroxylation process. This compound was resistant to conjugation since more than 90% of **46** was secreted into the bile in its parent form. The 23α-methyl group was thought to prevent carboxyl CoA activation and subsequent conjugation, thereby favoring the cholehepatic shunt pathway with ductular absorption and a potent choleretic effect.

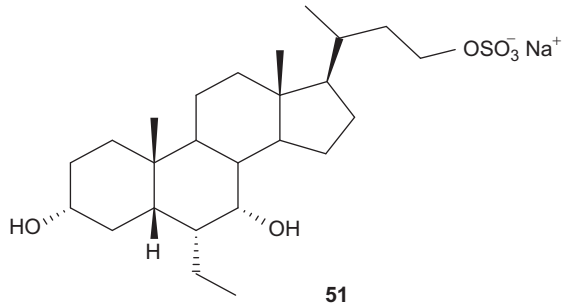


	Water solubility in 0.1 HCl	Albumin binding (%)
49: R = OH	270 μM	93
50: R = H	30 μM	54

Treatment of DIO C57BL/6 mice with compound **46** for 10 weeks at a dose of 30 mg/kg/d admixed with diet led to a significant increase in energy expenditure as determined by increases in O_2 consumption, CO_2 production, and respiratory quotient. In addition, liver function was improved as evidenced by reduction in liver steatosis. Significant reductions in plasma TG and nonesterified fatty acids were also observed. Treating these mice for 3 weeks at 30 mg/kg/d of **46** admixed with diet significantly improved glucose tolerance and insulin sensitivity [56].

4. FXR/TGR5 DUAL AGONISTS

In 2010, a FXR/TGR5 dual agonist, **51** (INT-767), was reported [57]. Using an AlphaScreen coactivator recruitment assay, the potency of **51** at FXR was 30 nM. In NCI-H716 cells, **51** stimulated intracellular cAMP secretion with an EC_{50} of 0.63 μM. Its TGR5 potency was comparable to that of the selective TGR5 agonist **46** (EC_{50} = 0.8 μM). Compound **51** also induced a dose-dependent increase of GLP-1 secretion from NCI-H716 cells.



51

In DBA/2J mice, a streptozotocin-induced type 1 diabetes model, plasma cholesterol levels were significantly higher in mice fed a western diet (WD) compared with standard chow. A 3-week treatment of these mice with **51** admixed at doses of 10 or 30 mg/kg/day in the WD resulted in a significant dose-dependent decrease of plasma total cholesterol levels and a significant decrease of TG levels only at the 30 mg/kg/d dose. The marked inhibition of total cholesterol induced by compound **51** treatment was correlated with normalization of LDL cholesterol levels; HDL cholesterol levels were not affected. In db/db mice, a model of type 2 diabetes, intraperitoneal administration of **51** for 2 weeks at doses of 10 and 30 mg/kg/day significantly and dose-dependently decreased plasma total cholesterol and TG levels.

5. CLINICAL STUDIES AND OUTLOOK

To date, only limited number of BA receptor agonists have been studied in humans. In 2009, a phase II trial result was reported on a FXR agonist, INT-747, in type 2 diabetes patients with comorbid fatty acid disease [58]. From this double-blind placebo-controlled study of 64 patients, INT-747 therapy (25 and 50 mg for 6 weeks) significantly improved insulin sensitivity, induced weight loss, and reduced liver damage. In 2010, a phase II study of a TGR5 agonist, SB-756050, for treatment of type 2 diabetes was completed [59]. Further development of this compound was discontinued after the highest dose failed to meet the predetermined efficacy threshold.

With the limited clinical data available, the full therapeutic potential of molecules that modulate BA receptors has yet to be realized. Based on the prolific patent literature around these targets, it is likely that additional molecules will advance into the clinic to test their therapeutic potential over the next few years.

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