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Design, Synthesis, and Structure–Activity Relationships of 3,4,5-Trisubstituted 4,5-Dihydro-1,2,4-oxadiazoles as TGR5 Agonists

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Given its role in the mediation of energy and glucose homeostasis, the G-protein-coupled bile acid receptor 1 (TGR5) is considered a potential target for the treatment of type 2 diabetes mellitus and other metabolic disorders. By thorough analysis of diverse structures of published TGR5 agonists, a hypothetical ligand-based pharmacophore model was built, and a new class of potent TGR5 agonists, based on the novel 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazole core, was discovered by rational design. Three distinct synthetic methods for constructing 4,5-dihydro-1,2,4-oxadiazoles and extensive structure–activity relationship studies are reported herein. Compound (*R*)-**54n**, the structure of which was determined by single-crystal X-ray diffraction and quantum chemical solid-state TDDFT-ECD calculations, showed the best potency, with an EC_{50} value of 1.4 nM toward hTGR5. Its favorable properties in vitro warrant further investigation.

Introduction

Diabetes as a metabolic disorder is recognized as one of the major threats to human health. Statistics from the International Diabetes Federation (IDF) indicate that the number of people living with diabetes was 371 million in 2012, and will rise to 552 million by 2030 if no action is taken.[1] Type 2 diabetes mellitus (T2DM) accounts for at least 90% of the cases. T2DM, sometimes called non-insulin-dependent diabetes mellitus (NIDDM), is characterized by insulin resistance, relative insulin deficiency, and is often complicated with hyperlipidemia and inflammation. Although there are many drugs available with various mechanisms of action,[2] treatment results are generally unsatisfactory.[3] Therefore, there is still a need to find therapeutics with novel modes of action, especially compounds that can act in the early stages of diabetes, to combat the problem of metabolic disorders as a whole, and not exclusively through the decrease of blood glucose levels.

The G-protein-coupled bile acid receptor 1 (TGR5), first identified in 2002,[4] is a G_s-coupled GPCR and is expressed in the intestine, gall bladder, spleen, placenta, and ovary.[4,5] As one of the receptors of bile acids (BAs), which are being recognized as complex metabolic integrators and signaling factors with paracrine and endocrine functions,[6] TGR5 mediates many physiological activities. Through the BA–TGR5–cAMP–D2 signaling pathway,[7] BAs can increase energy expenditure in brown adipose tissue (BAT) by enhancing mitochondrial activity, the decrease of which is considered one of the early hallmarks of T2DM.[8] In addition to this, the secretion of glucagon-like peptide-1 (GLP-1), which has a wide variety of physiological effects[9] such as stimulating pancreatic insulin secretion, inhibiting the release of glucagon, decreasing appetite, and delaying gastric emptying from murine enteroendocrine L cells (STC-1), is increased significantly via stimulation of TGR5 by various agonists.[10] Five drugs based on GLP-1 are approved for use by the US Food and Drug Administration (FDA): exenatide[11] and liraglutide[12] (GLP-1 analogues), and sitagliptin,[13] saxagliptin,[14] and alogliptin[15] (dipeptidyl peptidase IV (DPP-IV) inhibitors). Their clinical efficacy has provided the perspective for applying such a strategy to the therapy of T2DM. It was also recently reported that the activation of TGR5 can inhibit atherosclerosis by decreasing macrophage inflammation and lipid loading.[16] On the basis of all the facets discussed above, TGR5 is becoming recognized as a potential target to treat diabetes, obesity, and other metabolic syndromes.[17]

The research groups of Pellicciari[18] and Wagner[19] have reported numerous projects in their search for potent and selective TGR5 agonists for further studies based on natural BAs and triterpenoid scaffolds, respectively. Among them, INT-777 (**1**, Figure 1),[18c,e] as one of the semisynthetic BA derivatives, exhibited much better properties in vitro and in vivo than others; it is currently in preclinical-phase studies as an antidiabetic drug candidate. In addition, a number of pharmaceutical companies including Takeda,[20] Kalypsys,[21] GSK,[22] Roche,[23] Exelixis,[24] and Pfizer[25] have focused their efforts toward finding small synthetic molecular agonists in this area, most of which

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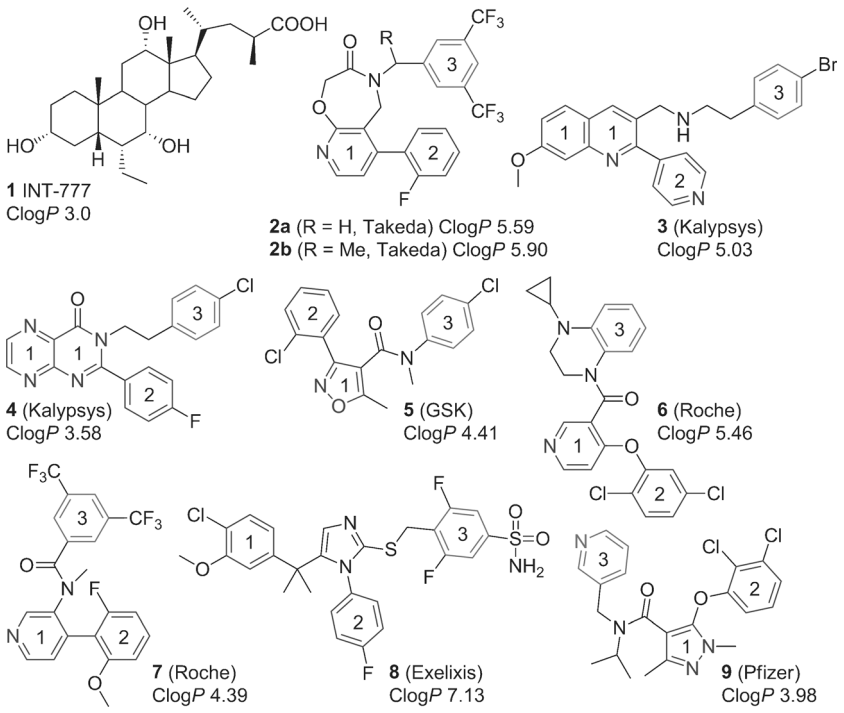

Figure 1. Structures and Clog *P* values of representative TGR5 agonists.

have been described in patents. Some representative structures are shown in Figure 1.

Herein we report our efforts to design a proprietary TGR5 agonist scaffold using Takeda's compounds **2a** and **2b** as templates. By thorough analysis of the structures of the published TGR5 agonists, a hypothetical ligand-based pharmacophore model for TGR5 agonists was proposed. We disclose the design, synthesis, and extensive structure–activity relationship (SAR) studies of 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazoles with a chemotype distinct from those previously reported.

Results and Discussion

The initial task in our research was to identify the common features among ligands with various scaffolds, based on the hypothesis that different ligands bind the same pocket of TGR5. The potent small-molecular agonists shown in Figure 1 have large Clog *P* values—particularly compounds **2a**, **2b**, **3**, **6**, and **8** (Clog $P > 5$)—which may indicate that higher lipophilicity is beneficial for activity. On this basis, we believed that the hydrophobic aromatic ring systems (indicated 1, 2, and 3) in Figure 1 may form a triangular backbone to anchor TGR5 by π–π stacking or hydrophobic interactions with certain residues of the receptor, and additional groups of the molecules orient the three aromatic moieties in a well-defined conformation or enhance the binding affinity through extra interactions, such as hydrogen bond or polar interactions, although the active binding conformations of these compounds are not yet known. Figure 2 shows the alignment of compound **2a** onto the hypothetical triangular hydrophobic pharmacophore model: the three hydrophobic sites (HYs 1–3) are mapped by the pyridine ring (HY1), 2-fluorophenyl ring (HY2), and 3,5-ditrifluoromethylphenyl ring (HY3). The distance between HY1 and HY2 is a little shorter than that between HY3 and HY1. The oxazepine ring of compound **2a** may just fix the three sites HY1, HY2, and HY3 into the correct orientation for binding with the receptor, while the oxygen and nitrogen atoms could be hydrogen bond acceptors. For agonists **3**–**9** in Figure 1, aromatic ring systems 1 and 2 may act as HY1 and HY2, respectively, while ring 3 acts as HY3 when biding to TGR5.

On the basis of the aforementioned logic, we planned to use compound **2a** as a template to keep the three aromatic rings

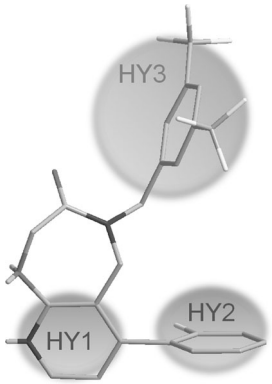

Figure 2. A hypothetical triangular hydrophobic pharmacophore model of TGR5 agonists represented by compound **2a**. A plausible conformer of **2a** was minimized at the MM2 level using the Chem3D software package.

and their plane distance unchanged by opening the oxazepine ring at the phenol bond and adding other groups to compensate for the loss of binding energy owning to the change of the overall conformation. Because the spatial orientation of the three aromatic moieties may change upon opening the oxazepine ring, a conformational constraint had to be introduced in a different way. It is a common strategy in drug design to use heterocycles as amide bioisosteres to fix conformations, modulate polarity, or to change bioavailability.[26] Furthermore, an additional methyl group was tolerated in compound **2b**, and hence, such a strategy was adopted in our design (Figure 3). Our medicinal chemistry strategy to identify a potent agonist with a novel scaffold was first to investigate the three different five-membered heterocyclic cores including

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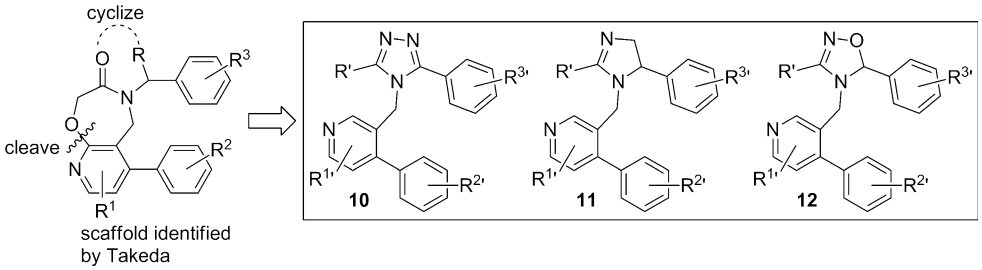


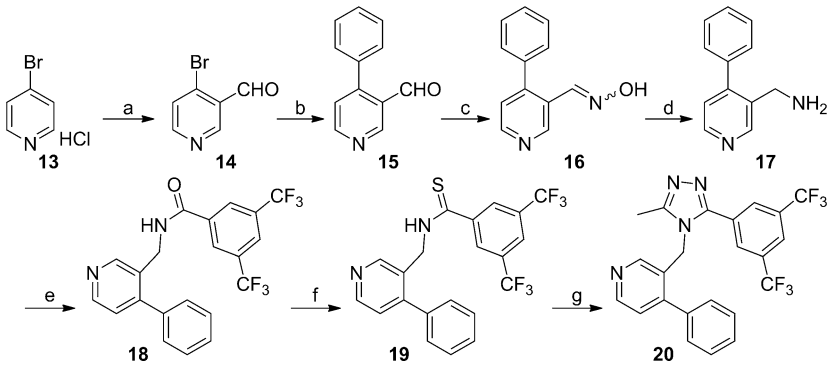
Figure 3. Our design strategy for novel scaffold of TGR5 agonists.

triazoles **10**, imidazolines **11**, and oxadiazoles **12** replacing the amide moiety of compounds **2a** and **2b**, followed by optimization of the 2-fluorophenyl moiety. We then envisaged a subsequent round of variation to find an appropriate position for the nitrogen atom in the central pyridine ring. Once the core structure was identified, modifications to the substituents of the trifluoromethylphenyl ring were carried out to study the effects of steric and electronic factors on activity. The synthetic routes are illustrated in Schemes 1–7.

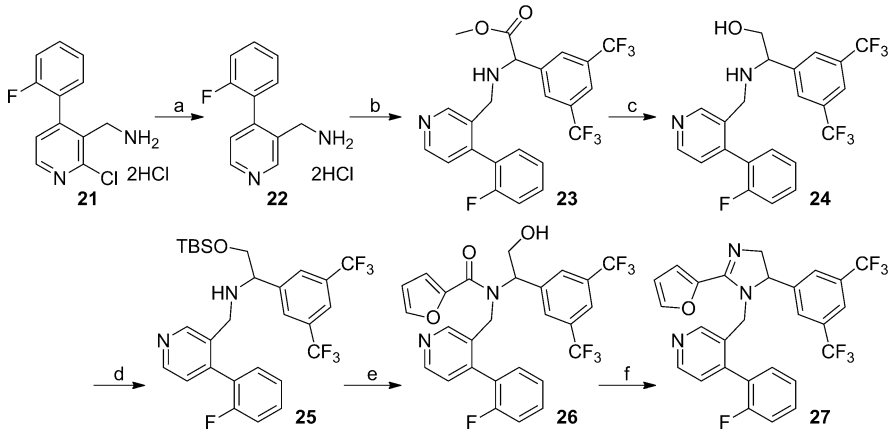
Synthesis

Syntheses of the three distinct five-membered heterocyclic cores were carried out as shown in Schemes 1–3 in completely different routes. Compound **15** was obtained from the commercially available **13** via electrophilic substitution with DMF[27] followed by Suzuki coupling.[28] Oxime **16** was transformed into **17** by catalytic hydrogenation under acidic conditions. Treatment of **18** with Lawesson's reagent[29] followed by cyclization with acethydrazide gave triazole **20** in moderate yield (Scheme 1).[30] Due to the harsh reaction conditions of step a in Scheme 1 and the instability of 4-bromonicotinaldehyde **14**,[31] a more efficient synthesis of the key intermediate amine **22** was developed. Amine **21** was obtained from 2′-fluoroacetophenone (see the Supporting Information). Dechlorination of **21** by catalytic hydrogenation gave **22**.[32] Compound **24** was prepared from **22** via alkylation[33] with methyl 2-(3,5-bis(trifluoromethyl)phenyl)-2-bromoacetate[34] followed by reduction. Selective protection of the hydroxy group on **24** with *tert*-butyldimethylsilyl chloride (TBDMSCl) gave **25**. Acylation[35] of **25** followed by deprotection afforded **26**. Finally, imidazoline **27** was prepared from **26** via Mitsunobu reaction followed by catalytic hydrogenation in one pot (Scheme 2).[36]

4,5-Dihydro-1,2,4-oxadiazoles **32a**–**k** were obtained from the corresponding hydroximoyl chlorides **30a**–**k** and imine **31** via 1,3-dipolar cycloaddition, which provides an efficient and short route to diverse 1,2,4-oxadiazoles[37] and 4,5-dihydro-1,2,4-oxadiazoles[38] (Scheme 3). Compounds **30a**–**k** were made from oximes **29a**–**k** by chlorination with NCS. For **29e** in particular, the chlorination was carried out under reflux in chloroform/methanol,[39] whereas for **29i** and **29j**, this process was carried out at room temperature to prevent over-chlorination.[40] However, in the case of oxime **29l**, none of the above chlorination conditions worked, because chlorination at position 2 of the furan ring was pre-



Scheme 1. *Reagents and conditions*: a) LDA, DMF, THF, −78 °C, 30 min, 29%; b) phenylboronic acid, K_2CO_3, $Pd(PPh_3)_4$, DME/H_2O, 85 °C, 3 h, 78%; c) NH_2OH·HCl, K_2CO_3, EtOH/H_2O, RT, 3 h, 91%; d) Pd/C, HCl, H_2, MeOH, RT, 4 h, 80%; e) 3,5-bis(trifluoromethyl)benzoic acid, HATU, DIEA, CH_3CN, RT, 5 h, 94%; f) Lawesson's reagent, toluene, reflux, 4 h, 66%; g) acethydrazide, silver benzoate, HOAc, CH_2Cl_2, RT, 24 h, 31%.



Scheme 2. *Reagents and conditions*: a) Pd/C, H_2, Et_3N, MeOH, RT, 3 h, 70%; b) 2-(3,5-bis(trifluoromethyl)phenyl)-2-bromoacetate, Et_3N, CH_3CN, 0 °C–RT, 3 h, 35%; c) $NaBH_4$, MeOH, 0 °C, 1 h, 71%; d) TBDMSCl, imidazole, DMF, RT, 1.5 h, 100%; e) 1. 2-furoyl chloride, *N*-ethylmorpholine, THF, reflux, overnight; 2. NH_4FBu_4·3H_2O, THF, RT, 1 h, 69%; f) 1. DIAD, PPh_3, DPPA, THF, RT, overnight; 2. Pd/C, H_2, MeOH, RT–reflux, 7 h, 34%.

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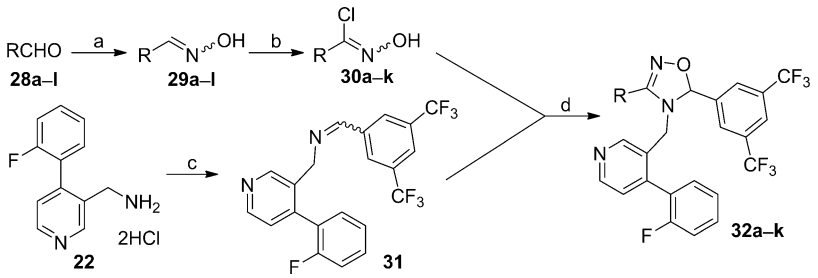
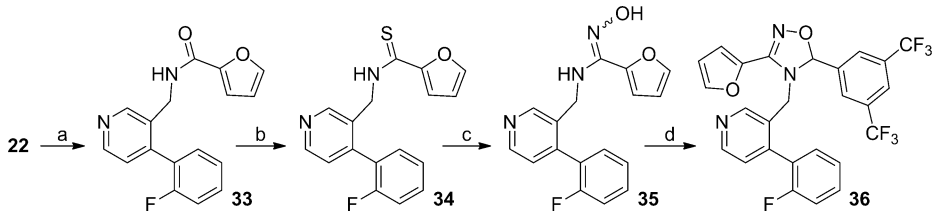


Scheme 3. *Reagents and conditions*: a) $NH_2OH{\cdot}HCl$, Na_2CO_3 or $NaHCO_3$, $EtOH/H_2O$, RT, 2 h, 65–98%; b) NCS, DMF, 40–50 °C, 3 h or NCS, pyridine, $CHCl_3$, 4 h or $CHCl_3$/MeOH, RT–40 °C, 3 h, 28–97%; c) 3,5-bis(trifluoromethyl)benzaldehyde, Et_3N, 4 Å molecular sieves, CH_2Cl_2, reflux, overnight; d) Et_3N, THF, −40 °C, overnight or toluene, RT–80 °C, 8 h; 11–58% for steps c and d.



Scheme 4. *Reagents and conditions*: a) 2-furoic acid, EDCI, HOBt, Et_3N, CH_2Cl_2, RT, overnight, 88%; b) Lawesson's reagent, toluene, reflux, 2 h, 63%; c) $NH_2OH{\cdot}HCl$, Et_3N, $Hg(OAc)_2$, CH_3CN, reflux, 8 h, 73%; d) 3,5-bis(trifluoromethyl)-benzaldehyde, $TsOH{\cdot}H_2O$, 1,4-dioxane, microwave 150 °C, 30 min, 14%.

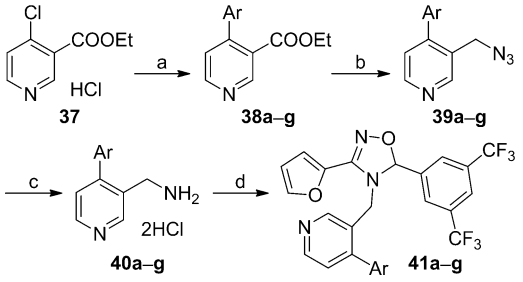
derivatives were synthesized as shown in Scheme 6, although the starting material is slightly different. Dehydration of **22** or **40g** with appropriate aldehydes followed by cycloaddition with oxime afforded various substituted products **54a**–**n** (Scheme 7). Aldehydes **56a** and **56b** were synthesized from the corresponding halogenated benzenes at low temperature.[48] Finally, the chiral racemic 4-(2,6-difluorophenyl)pyridine derivative **54n** was selected for chiral HPLC separation to obtain the enantiomers (*S*)- and (*R*)-**54n**.

Determination of the configuration and conformation of the enantiomers

To determine absolute configuration and conformation, the preferred over the double bond of the oxime, and only multichlorinated products could be obtained. Therefore, a novel route had to be explored.

Although the synthesis of 4,5-dihydro-1,2,4-oxadiazoles by condensation between amidoximes and aldehydes or ketones has been reported, the products are usually disubstituted.[41] By screening reagents and conditions, we successfully prepared trisubstituted 4,5-dihydro-1,2,4-oxadiazole **36** from amidoximes **35**, albeit in moderate yield (Scheme 4). Thioamide **34** was transformed into **35** via desulfurization with mercury(II) acetate followed by nucleophilic substitution with hydroxylamine hydrochloride.[42] The synthetic route was somewhat longer than that previously described, and an excess of the aldehyde was needed. Upon investigating the synthesis of similar heterocycles, an alternate method was developed. The nitrile oxide generated from **29l** in situ using a combination of *tert*-butyl hypochlorite and sodium iodide in the presence of 2,6-lutidine can be directly converted into **36** via cycloaddition with the imine by heating.[43] This was the first report for the synthesis of a 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazole in a manner that was adopted and optimized for the analogues below.

The synthetic route for esters **38a**–**g** was slightly altered from that described previously. As shown in Scheme 5, **37** was converted into ethyl 4-arylnicotinates by Suzuki coupling using different catalytic systems in excellent yields.[28,44] Reduction of **38a**–**g** with DIBAL-H[45] followed by Thompson's reaction[46] afforded azides **39a**–**g**, which were transformed into amines **40a**–**g** via Staudinger reaction.[47] The synthesis of **41a**–**g** was performed by using the above method elaborated by us under microwave heating. In a similar fashion, the pyridine moiety



Scheme 5. *Reagents and conditions*: a) $ArB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, DME/H_2O, 85 °C, overnight, 76–92%, or Et_3N, K_3PO_4, $Pd_2(dba)_3$, tBu_3P, toluene, reflux, 36 h, 73%; b) 1. DIBAL-H, CH_2Cl_2, −78 °C, 1.5 h; 2. DPPA, DBU, THF, reflux, 5 h, 41–79%; c) PPh_3, THF/H_2O, RT, overnight, 75–92%; d) 1. 3,5-bis(trifluoromethyl)benzaldehyde, Et_3N, 4 Å molecular sieves, CH_2Cl_2, reflux, overnight; 2. **29l**, NaI, 2,6-lutidine, *t*BuOCl, 1,4-dioxane, microwave 120 °C, 20 min, 5–19%.

aration of single crystals of (*S*)- and (*R*)-**54n** was endeavored for X-ray analysis, but our efforts were fruitless. However, a good single crystal of *rac*-**54n** was obtained by slow evaporation of its methanolic solution (see the Supporting Information), which afforded the solid-state X-ray geometry without absolute configuration. With knowledge of the solid-state X-ray geometry available, the solid-state TDDFT-ECD method could be applied to determine the absolute configuration and compare the solid-state and solution conformations.[49] DFT reoptimization of the X-ray geometry (see the Supporting Information) was carried out at the B3LYP/6-31G(d) level, which was followed by TDDFT calculations of the arbitrarily chosen (*S*)-**54n** using various functionals (B3LYP, BH&HLYP, PBE0) and the TZVP basis set. The computed ECD spectra of (*S*)-**54n** are in

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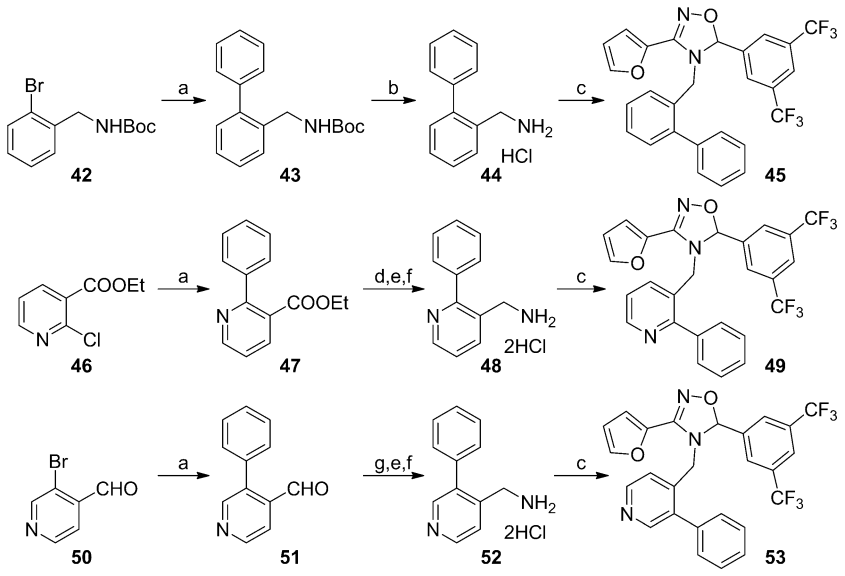
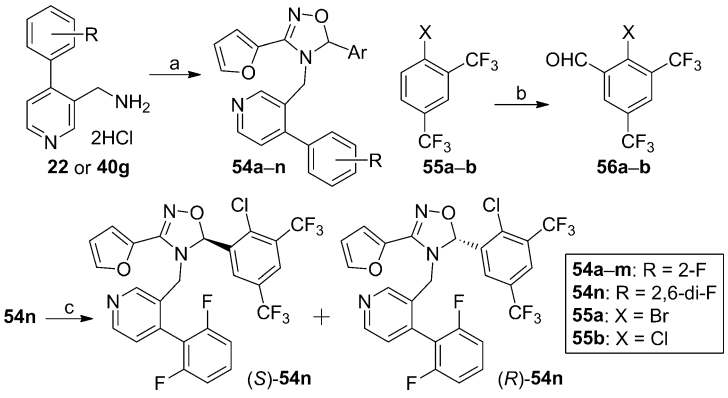


Scheme 6. *Reagents and conditions*: a) $PhB(OH)_2$, K_2CO_3, $Pd(PPh_3)_4$, DME/H_2O, 85 °C, 3–15 h, 66–84%; b) 4 N HCl/EA, RT, overnight, 93%; c) 1. 3,5-bis(trifluoromethyl)benzaldehyde, Et_3N, 4 Å molecular sieves, CH_2Cl_2, reflux, overnight; 2. **29l**, NaI, 2,6-lutidine, *t*BuOCl, 1,4-dioxane, microwave 120 °C, 20 min; 2–18%; d) DIBAL-H, CH_2Cl_2, −78 °C–0 °C, 1.5 h, 89%; e) DPPA, DBU, THF, reflux, 5 h, 73–84%; f) PPh_3, THF/H_2O, RT, overnight, 68–77%; g) $NaBH_4$, EtOH, RT, 1 h, 88%.



Scheme 7. *Reagents and conditions*: a) 1. ArCHO, Et_3N, 4 Å molecular sieves, CH_2Cl_2, reflux, overnight; 2. **29l**, NaI, 2,6-lutidine, *t*BuOCl, 1,4-dioxane, microwave 120 °C, 20 min; 4–18%; b) *n*BuLi, 2,2,6,6-tetramethylpiperidine, DMF, THF/*n*-hexane, −100 °C, 2 h, 32–36%; c) separation by chiral HPLC.

good agreement with the experimental microcrystalline solid-state ECD spectrum of the first-eluting enantiomer of **54n**, affording its absolute configuration as *S*. Moreover, the solution ECD spectrum of (*S*)-**54n** corroborated the solid-state spectrum, indicating that the solid-state conformer is also prevalent in solution (Figure 4).

Biological evaluation in vitro and discussion

The newly synthesized triazole **20**, imidazoline **27**, and oxadiazoles **32a–k**, **36**, **41a–g**, **45**, **49**, **53**, **54a–n**, (*S*)-**54n**, and (*R*)-**54n** were evaluated for their capacity for human TGR5 (hTGR5) agonism in vitro by using a procedure similar to that reported previously.[18] Compounds **1** and **2a** were reported to have respective EC_{50} values of 0.82 μM and 0.23 nM;[18c,20d] in our re-evaluated assay, these compounds showed EC_{50} values of 1.226 μM and 19.4 nM, respectively. The results are summarized in Tables 1–4.

Our initial point of variation was the five-membered heterocyclic core. Three kinds of five-membered heterocycles with slightly different substituents, including triazole **20**, imidazoline **27**, and oxadiazole **32a**, were constructed to establish an optimal core which can orient the three aromatic pharmacophores in the correct way (Table 1). Compound **20** ($EC_{50} > 10$ μM) completely lost activity, and increasing the number of methylene units between the triazole ring and the 3,5-bis(trifluoromethyl)phenyl group did not enhance potency (data not shown). Oxadiazole **32a** ($EC_{50} = 79$ nM, effect = 93%) was nearly 20-fold more potent than imidazoline **27** ($EC_{50} = 1.461$ μM), but the crucial structural difference lies solely with the oxazolidinone oxygen atom, as subsequent studies indicated that a furan ring is also tolerated. Therefore, it can be concluded that the oxygen atom may form a hydrogen bond with TGR5. Although **20** has a nitrogen atom that can also serve as a hydrogen bond acceptor like the oxygen atom at the same position of **32a**, its activity is significantly different. This may be attributed to the double bond of the triazole ring, which situates the 3,5-bis(trifluoromethyl)phenyl fragment in a suboptimal orientation. All the above investigations revealed that a hydrogen bond acceptor, like an oxygen atom, linked with a hydrophobic fragment such as a 3,5-bis(trifluoromethyl)phenyl group through an sp^3-hybridized carbon is a guarantee for potency. In this way, 4,5-dihydro-1,2,4-oxadiazole has the best type of conformational constraint to mimic the bioactive conformation of compound **2a** and was therefore selected for further investigation.

We next turned our attention to the newly introduced phenyl ring (Table 2). A range of electron-withdrawing and electron-donating substituents were introduced at various positions, but unfortunately no compound showed enhanced potency. Compounds **32b–e** were selected as examples. A complete loss of activity was observed for **32b**, bearing a 4-chloro substituent, whereas 3-chloro-substituted **32c** showed moderate activity ($EC_{50} = 2.909$ μM). A slight increase in potency was observed for 2-chloro-substituted **32d** ($EC_{50} = 0.921$ μM). To mimic the oxygen atom in the oxazepine ring of **2a**, 2-hydroxy **32e** was synthesized, yet a significant decrease in potency was displayed ($EC_{50} = 1.613$ μM). Even lower potency was observed

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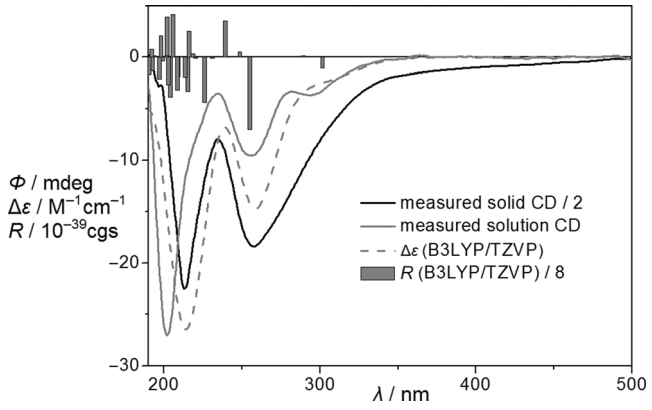
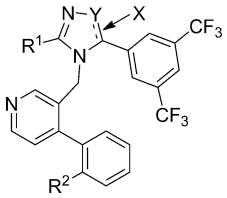


Figure 4. Solid-state and solution-phase experimental spectra of the first-eluting enantiomer of **54n** compared with the B3LYP/TZVP ECD spectra of (*S*)-**54n** calculated for the optimized X-ray geometry. Gray bars represent the calculated rotational strength (*R*) values.

if the phenyl was replaced by a benzyl group (**32k**, EC_{50} = 4.691 μM). These results indicate that the binding pocket of TGR5 that recognizes this aryl region is relatively narrow; there is insufficient space to accommodate larger substituents. Thus, the introduction of any other substituents was likely to be meaningless, and aromatic heterocycles were then adopted. All in the six-membered pyridine series **32f–h** showed moderate activity, with EC_{50} values <1 μM. A nitrogen atom at position 2 was slightly more beneficial for potency than at other positions (**32h** vs. **32f,g**). Five-membered heterocyclic analogues **32i,j** and **36**, with less bulk than the above six-membered cyclic series, displayed remarkably increased potencies. 2-Thienyl-substituted **32i** (EC_{50} = 65 nM) was slightly less potent than 3-thienyl-substituted **32j** (EC_{50} = 40 nM). Surprisingly, 2-furanyl-substituted **36** (EC_{50} = 15 nM, effect = 102%) possessed very good activity and was much more potent than **32a**. Therefore, 2-furanyl was shown to be an optimal substituent at position 3 of the 1,2,4-oxadiazole ring and was kept for further optimization.

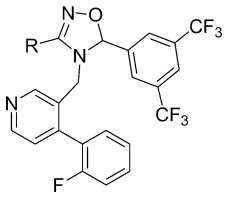
Table 1. Variation of the five-membered heterocyclic core.



Compd	R^1	R^2	X	Y	EC_{50} [μM][a]	ME [%][b]
20	methyl	H	double bond	N	8% at 10 μM	NT
27	2-furyl	F	single bond	CH	1.461 ± 0.341	121
32a	phenyl	F	single bond	O	0.079 ± 0.009	93
1	–	–	–	–	1.226 ± 0.357	100

[a] EC_{50} values against hTGR5 were determined from at least three independent tests at eight concentrations each; results are expressed as the mean ± SD. [b] ME = maximal effect, determined by using the effect of DMSO vehicle control set as 0%, and the effect of INT-777 at 20 μM set as 100%; $ME_{compd} = [data_{compd} - data_{DMSO}]/[data_{INT-777} - data_{DMSO}] \times 100\%$; NT: not tested.

Table 2. Variation of substituents at position 3 of 4,5-dihydro-1,2,4-oxadiazoles.



Compd	R	EC_{50} [μM][a]	ME [%][b]
32a	phenyl	0.079 ± 0.009	93
32b	4-chlorophenyl	15% at 10 μM	NT
32c	3-chlorophenyl	2.909 ± 1.513	98
32d	2-chlorophenyl	0.921 ± 0.238	112
32e	2-hydroxyphenyl	1.613 ± 0.732	90
32f	4-pyridyl	0.971 ± 0.087	100
32g	3-pyridyl	0.544 ± 0.148	100
32h	2-pyridyl	0.391 ± 0.149	97
32i	2-thienyl	0.065 ± 0.036	109
32j	3-thienyl	0.040 ± 0.001	100
32k	benzyl	4.691 ± 2.521	74
36	2-furanyl	0.015 ± 0.003	102

[a] EC_{50} values against hTGR5 were determined from at least three independent tests at eight concentrations each; results are expressed as the mean ± SD. [b] ME = maximal effect, determined by using the effect of DMSO vehicle control set as 0%, and the effect of INT-777 at 20 μM set as 100%; $ME_{compd} = [data_{compd} - data_{DMSO}]/[data_{INT-777} - data_{DMSO}] \times 100\%$; NT: not tested.

Our focus then turned to the 4-phenylpyridine moiety (Table 3). Systematic variation of the substituents on the 4-phenyl ring revealed interesting subtleties in the SARs. Removal of fluorine in **36** resulted in a slight decrease in activity (**41a**, EC_{50} = 34 nM). A clear decrease in potency was observed if the fluorine of **36** was replaced by either an electron-withdrawing substituent like chlorine (in **41b**) or an electron-donating substituent like methyl (in **41c**). At this point, we believed that weak electron-withdrawing substituents such as fluorine would be beneficial to activity. However, substitution with fluorine at position 4 (**41d**, EC_{50} = 47 nM) also caused a slight decrease in potency relative to **41a** (1.4-fold). This phenomenon initiated a thorough comparison of these compounds. Either electron-withdrawing or electron-donating substituents at position 2 gave better potency than at other positions (see the Supporting Information). This result might indicate that the activity is determined not only by electronic, but by steric effects as well. The substituent at position 2 is a key factor that influences the dihedral angle of the phenyl and pyridine rings, and the orientation of the phenyl ring would affect binding with the receptor. The bulkier the substituent, the greater the dihedral angle between the two aromatic rings, which can be inferred from NMR spectra as well (atropisomers appeared for **41b,c** and **41e**; see the Supporting Information). Accordingly, trifluoromethyl as a bulky and strong electron-withdrawing substituent significantly decreased activity (**41e**, EC_{50} = 2.189 μM). In comparing **36** with **41a–c** and **41e**, one might con-

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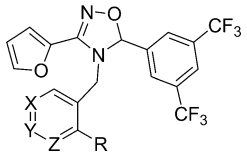
Table 3. Variation of substituents at position 4 of 4,5-dihydro-1,2,4-oxadiazoles.



Compd	X	Y	Z	R	EC_{50} [μM][a]	ME [%][b]
41a	N	CH	CH	phenyl	0.034 ± 0.009	99
41b	N	CH	CH	2-chlorophenyl	0.225 ± 0.024	99
41c	N	CH	CH	2-methylphenyl	0.092 ± 0.020	96
41d	N	CH	CH	4-fluorophenyl	0.047 ± 0.005	100
41e	N	CH	CH	2-trifluoromethylphenyl	2.189 ± 0.271	88
41f	N	CH	CH	3-thienyl	0.073 ± 0.009	91
41g	N	CH	CH	2,6-difluorophenyl	0.015 ± 0.001	97
45	CH	CH	CH	phenyl	7% at 10 μM	NT
49	CH	CH	N	phenyl	8% at 10 μM	NT
53	CH	N	CH	phenyl	18% at 10 μM	NT

[a] EC_{50} values against hTGR5 were determined from at least three independent tests at eight concentrations each; results are expressed as the mean ± SD. [b] ME = maximal effect, determined by using the effect of DMSO vehicle control set as 0%, and the effect of INT-777 at 20 μM set as 100%; $ME_{compd} = [data_{compd} - data_{DMSO}]/[data_{INT\text{-}777} - data_{DMSO}] \times 100\%$; NT: not tested.

clude that the two aromatic rings adopt a conformation with a smaller dihedral angle when binding with TGR5. In addition, the introduction of another fluorine atom did not lead to enhanced activity (**41g**, EC_{50} = 47 nM). On the other hand, because substitution at positions 3 or 4 does not contribute positively to activity, the size of the entire aromatic ring was taken into account, just like optimization of the substituents at position 3 described above. Therefore, a five-membered heterocycle was explored. 3-Thienyl-substituted **41f** retained most potency (EC_{50} = 73 nM), and was selected as the example. Considering the similarities of **2a** and **6**, replacement of 4-(2-fluorophenyl)pyridine with 4-(2,5-dichlorophenoxy)pyridine also gave encouraging results (see the Supporting Information), which can verify the previously proposed pharmacophore model to some extent. These results favor further modification, applying strategies that will be disclosed in the near future.

The pyridine moiety became the next focal point of our study. Complete loss of activity was observed when substituents were introduced on the pyridine ring (see the Supporting Information). Removal (**45**) or change in the position (**49** and **53**) of the nitrogen atom in the pyridine ring caused a dramatic drop in activity in all cases. All these results indicate that the pyridine portion is relatively well conserved, and that the nitrogen atom may act as a hydrogen bond acceptor when binding with TGR5.

Despite various attempts, no compound with significantly improved potency over that of **36** was obtained. Faced with this dilemma, our final task was the exploration of substituents of the 3,5-bis(trifluoromethyl)phenyl ring. Mono- or multi-substituted phenyl derivatives were investigated, and the results are listed in Table 4. Both simple replacement and removal of the two trifluoromethyl groups of **36** caused large decreases in activity (see the Supporting Information), whereas switching to mono-trifluoromethyl (**54a**, EC_{50} = 0.268 μM) yielded a slightly better result. The presence of a bulky and strong electron-withdrawing substituent at positions 3 or 5 is essential to activity. Regarding positions 2 and 4, chlorine as an electron-withdrawing substituent and methyl as the electron-donating substituent were initially explored. The results demonstrate that electron-withdrawing substituents favor potency at both of the two positions (**54b** vs. **54d**, **54c** vs. **54e**) and substituents at position 2 contributed more to the activity than those at position 4 (**54b** vs. **54c**, **54d** vs. **54e**). Systematic variations of electron-withdrawing groups at position 2 were then explored. Bromine (**54g**, EC_{50} = 62.2 nM) or trifluoromethyl (**54h**, EC_{50} = 68.0 nM) showed similar results to that of chlorine (**54b**, EC_{50} = 76.1 nM), whereas the weaker electron-withdrawing and smaller fluorine (**54f**, EC_{50} = 383 nM) caused a fivefold decrease in activity. On the above basis, all positions favored electron-withdrawing substituents. In other words, lower electron density on the phenyl ring is a vital factor that influences the binding affinity for TGR5. Hence, trifluoromethyl was the preferred choice for positions 3 or 5. Indeed, all three disubstituted derivatives (**54i–k**) displayed potencies similar to that of **36**. Trisubstituted benzenes were then constructed. Both the chloride **54l** (EC_{50} = 5.6 nM) and the bromide **54m** (EC_{50} = 5.7 nM) exhibited excellent activity. Moreover, replacement of 2-fluorophenyl with 2,6-difluorophenyl led to an unexpected improvement in potency (**54n**, EC_{50} = 3.5 nM).

Lastly, the best racemic compound **54n** was selected as an example to investigate the relationship between chirality and activity. Separation of the *S* and *R* enantiomers of *rac*-**54n** by chiral HPLC and subsequent individual pharmacological testing demonstrated that (*R*)-**54n** is the eutomer with significantly better activity ((*S*)-**54n** EC_{50} = 464 nM, (*R*)-**54n** EC_{50} = 1.4 nM).

The entirety of the SAR studies described above indicate that the hTGR5 binding pocket that accommodates 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazoles is endowed with the following: a small hydrophobic region that accepts the furanyl group at position 3, a long hydrophobic site that anchors the 4-substituted pyridine part at position 4, a large hydrophobic pocket that holds the 3,5-bis(trifluoromethyl)phenyl group at position 5, and two hydrogen bonding donors that recognize the oxygen and nitrogen atoms on the oxadiazole and pyridine rings, respectively, which form the key interactions that anchor the three hydrophobic regions in the correct orientation (Figure 5). Hence, another hydrophobic pharmacophore could be added to the hypothetical model proposed in Figure 2. This result is also consistent with that described by Sato and co-workers: steric complementarities and lipophilic profile contribute greatly to activity.[18b] Such SARs will be very

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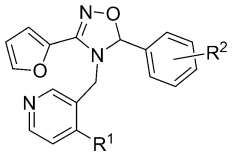
Table 4. Variation of substituents at positions 4 and 5 of 4,5-dihydro-1,2,4-oxadiazoles.



Compd	R^1	R^2	hTGR5		mTGR5	
			EC_{50} [μM][a]	ME [%][b]	EC_{50} [μM][a]	ME [%][b]
36	2-fluorophenyl	3,5-di(trifluoromethyl)	15.4 ± 2.8	102	988 ± 133	127
41 d	4-fluorophenyl	3,5-di(trifluoromethyl)	46.8 ± 5.4	100	832 ± 60	120
41 f	3-thienyl	3,5-di(trifluoromethyl)	72.9 ± 9.5	91	1574 ± 487	127
41 g	2,6-difluorophenyl	3,5-di(trifluoromethyl)	15.8 ± 0.8	97	916 ± 162	118
54 a	2-fluorophenyl	3-trifluoromethyl	268 ± 89	98	NT	NT
54 b	2-fluorophenyl	2-chloro	76.1 ± 18.1	99	NT	NT
54 c	2-fluorophenyl	4-chloro	534 ± 194	119	NT	NT
54 d	2-fluorophenyl	2-methyl	205 ± 142	147	NT	NT
54 e	2-fluorophenyl	4-methyl	604 ± 219	108	NT	NT
54 f	2-fluorophenyl	2-fluoro	383 ± 50	119	NT	NT
54 g	2-fluorophenyl	2-bromo	62.2 ± 12.9	96	NT	NT
54 h	2-fluorophenyl	2-trifluoromethyl	68.0 ± 20.0	99	NT	NT
54 i	2-fluorophenyl	2,5-di(trifluoromethyl)	34.9 ± 4.5	93	NT	NT
54 j	2-fluorophenyl	2-chloro-5-trifluoromethyl	39.1 ± 5.3	103	1095 ± 233	117
54 k	2-fluorophenyl	2-chloro-3-trifluoromethyl	17.2 ± 1.6	101	2453 ± 285	123
54 l	2-fluorophenyl	2-chloro-3,5-di(trifluoromethyl)	5.6 ± 1.4	90	644 ± 14	133
54 m	2-fluorophenyl	2-bromo-3,5-di(trifluoromethyl)	5.7 ± 0.6	90	724 ± 10	123
54 n	2,6-difluorophenyl	2-chloro-3,5-di(trifluoromethyl)	3.5 ± 0.8	84	380 ± 48	104
(*R*)-**54 n**	2,6-difluorophenyl	2-chloro-3,5-di(trifluoromethyl)	1.4 ± 0.2	96	249 ± 42	126
(*S*)-**54 n**	2,6-difluorophenyl	2-chloro-3,5-di(trifluoromethyl)	464 ± 161	99	1446 ± 569	114
1	–	–	1226 ± 357	100	358 ± 77	100
2 a	–	–	19.4 ± 4.6	106	126 ± 25	130

[a] EC_{50} values were determined from at least three independent tests at eight concentrations each; results are expressed as the mean ± SD. [b] ME = maximal effect, determined by using the effect of DMSO vehicle control set as 0%, and the effect of INT-777 at 20 μM set as 100%; $ME_{compd} = [data_{compd} - data_{DMSO}]/[data_{INT\text{-}777} - data_{DMSO}] \times 100\%$; NT: not tested.

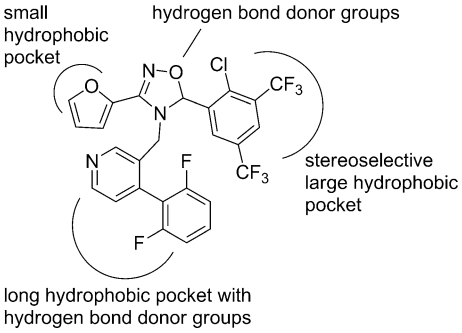


Figure 5. Structure–activity relationships of 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazoles as TGR5 agonists. The alignment of compound **54 n** is shown along with the features of the hTGR5 binding pocket.

useful guides in the design of novel potent and selective TGR5 agonists.

Some potent hTGR5 agonists were selected for screening against murine TGR5 (mTGR5) to identify one that is potent against both species for further evaluation in vivo. The results summarized in Table 4 show that the potency of tested compounds against mTGR5 is much weaker than that against hTGR5; an approximate 100-fold difference for certain analogues (**54 l**–**n**) was observed, and this may be attributed to the relatively low sequence similarity (83%) between the two species.[4b] Altogether, (*R*)-**54 n** possesses the best potency (hTGR5 EC_{50} = 1.4 nM, mTGR5 EC_{50} = 249 nM) in vitro. Due to the weak activity toward mTGR5, it is difficult to evaluate the acute effect on blood glucose in mouse. Therefore, evaluation in other species and more focused structural optimization on the activity toward mTGR5 are required.

Conclusions

In summary, by thorough analysis of diverse structures of published TGR5 agonists, we proposed a hypothetical ligand-based pharmacophore model. Using Takeda's compounds as the template, a novel class of potent TGR5 agonists based on the 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazole core was discovered by rational design. Simple and efficient methods for the synthesis of 4,5-dihydro-1,2,4-oxadiazoles were developed, and a large number of analogues were prepared, most of which possess good TGR5 activity, with EC_{50} values $<$ 100 nM. Extensive SAR explorations of this scaffold has indicated features of the TGR5 binding pocket that are potentially useful for the future design of novel potent and selective agonists. It was also shown that the configuration at position 5 of 4,5-dihydro-1,2,4-oxadiazole is important for activity; the absolute configuration of the separate enantiomers was determined by single-crystal X-ray diffraction and quantum chemical

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solid-state TDDFT-ECD methods. Compound (*R*)-**54n** is a TGR5 agonist similar to other previously reported agonists, with an EC_{50} value of 1.4 nM against hTGR5 and 249 nM against mTGR5. Our future work will focus on antidiabetic evaluations in vivo and further investigations in other species.

Experimental Section

Chemistry

General methods: All reagents were purchased from commercial suppliers and used without further purification unless otherwise stated. Yields were not optimized. Analytical and preparative thin-layer chromatography (TLC) was performed on $HSGF_{254}$ (0.15–0.2 mm thickness for analytical, 0.4–0.5 mm thickness for preparative; Yantai Jiangyou Company, Yantai, Shandong, China). Column chromatography was carried out on silica gel (200–300 mesh) or with pre-packed silica cartridges (4–40 g) from Bonna-Agela Technologies Inc. (Tianjin, China) and eluted with a Teledyne Isco CombiFlash R_f flash chromatography system. Microwave reactions were performed in a Biotage Initiator using the manufacturer-supplied vials, stir bars, and caps. ^1H and ^{13}C NMR spectra were recorded on Varian Mercury 300 or 400 spectrometers, using tetramethylsilane (TMS) or solvent peaks as an internal reference. Chemical shifts (δ) are reported in parts per million (ppm), and the signals are described as brs (broad singlet), d (doublet), dd (doublet of doublets), m (multiplet), q (quartet), s (singlet), t (triplet), and td (triplet doublet); coupling constant (*J*) values are given in hertz (Hz). Low-resolution mass spectra were determined on a Waters ZQ2000a or Agilent LC–MS systems that consisted of an Agilent 1260 infinity LC coupled to an Agilent 6120 Quadrupole mass spectrometer (ESI+) using an Agilent ZORBAX 1.8 μm SB-C_{18} column (2.1×50 mm) or a CAPCELL PAK C_{18} MGIII S3 (Shiseido Co., Ltd.) column (2.0(i.d.)×50 mm) with aqueous CH_3CN (10–90%) containing 0.05% formic acid monitored at λ 240 nm. High-resolution mass spectra were recorded on a Q-Tof Ultima Globe mass spectrometer (Micromass, Manchester, UK). HPLC analyses for all compounds tested in biological systems were performed on an Agilent 1200 series LC system (Agilent ChemStation, Agilent Eclipse XDB-C_{18}, 5 μm, 4.6×150 mm, 30 °C, UV 254 nm, 1.0 mL min^{-1}) with aqueous CH_3CN (50–90%) containing 0.05% formic acid for 30 min. The purities of all the assayed compounds were >95%. Preparative and analytical chiral HPLC was performed using a Shimadzu LC20 system with UV detector SPD-20A (CHIRALCEL OD-3, 0.46(i.d.)×15 cmL×3 μm, UV 254 nm, hexane/*i*PrOH=85:15) by Daicel Chiral Technologies Co. Ltd. (China). Optical rotations were measured on a Rudolph Autopol V polarimeter in solutions of $CHCl_3$. Structural analysis of the single crystal was carried out on a Bruker Smart Apex II instrument. CD spectra were recorded on a Jasco J-810 CD spectrometer.

General procedure A: Suzuki coupling

A flask was charged with halogenated aromatic hydrocarbon (1 equiv), boronic acid (1.2 equiv), K_2CO_3 (2 equiv), H_2O (1 mL mmol^{-1}), $Pd(PPh_3)_4$ (0.05 equiv), and DME under nitrogen. The mixture was stirred at 85 °C for 3–15 h until consumption of the halogenated aromatic hydrocarbon as monitored by TLC, then cooled to room temperature and filtered. The aqueous layer was extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated.

General procedure B: interconversion of ester to azide

DIBAL-H (1.5 M toluene solution, 3–4 equiv) was added dropwise to a stirred solution of the appropriate ester (1 equiv) in dry CH_2Cl_2 at −78 °C under nitrogen, and the mixture was stirred for 1.5 h until consumption of the ester as monitored by TLC. A solution of 1 M aqueous potassium sodium tartrate was added to quench the reaction, and the mixture was extracted with CH_2Cl_2. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated to give the crude product that was used for the next step without further purification. To a solution of the above crude alcohol (1 equiv) in anhydrous THF was added DBU (2.5 equiv) and DPPA (2 equiv) successively. The reaction mixture was stirred under reflux for 4–5 h until consumption of the alcohol as monitored by TLC, then cooled to room temperature, and the solvent was concentrated. The residue was diluted with H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated.

General procedure C: Staudinger reaction

To a stirred solution of the appropriate azide (1 equiv) in THF/H_2O was added PPh_3 (2 equiv) portionwise. The mixture was stirred at room temperature overnight and was then acidified with concentrated HCl. The solvent was concentrated, and the residue was dissolved in EtOAc and extracted with H_2O. The combined aqueous phases were washed with EtOAc five times until most of the non-salinized side products were removed. The aqueous solution was then neutralized with solid NaOH and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$. After filtration, the filtrate was acidified with a solution of 4 N HCl/EtOAc. The resulting mixture was filtered to give the corresponding amine hydrochloride as a solid.

General procedure D-I: 1,3-dipolar cycloaddition

To a solution of amine hydrochloride **22** (1.1 equiv) in dry CH_2Cl_2 under nitrogen was consecutively added Et_3N (2.3 equiv), 3,5-bis(-trifluoromethyl)benzaldehyde (1 equiv), and 4 Å molecular sieves. The reaction mixture was held at reflux overnight and then cooled to room temperature. Then the mixture was filtered, and the filtrate was concentrated to give the crude imine **31**. The residue was dissolved in dry toluene followed by addition of the appropriate hydroximoyl chloride (1.5–3 equiv). Et_3N (2.25–4.5 equiv) in toluene was added slowly by syringe at room temperature or at 80 °C under nitrogen to the above reaction mixture for ~8 h until consumption of most of the imine. The resulting mixture was quenched by saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated.

General procedure D-II: 1,3-dipolar cycloaddition

To a solution of an amine hydrochloride (1.1 equiv) in dry CH_2Cl_2 under nitrogen was consecutively added Et_3N (2.3 equiv), the appropriate aromatic aldehyde (1 equiv), and 4 Å molecular sieves. The reaction mixture was held at reflux overnight and then cooled to room temperature. Then the mixture was filtered, and the filtrate was concentrated to give the crude imine. The residue was dissolved in dry 1,4-dioxane and filtered to remove the triethylamine hydrochloride. The filtrate was then transferred into a 20 mL microwave tube, and oxime **29l** (4 equiv), NaI (4 equiv), 2,6-lutidine (4 equiv), and *tert*-butyl hypochlorite (4 equiv) were added. The re-

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sulting mixture was heated at 120 °C by microwave for 20–30 min and then cooled to room temperature. The solvent was concentrated, and the residue was dissolved in EtOAc. After washing with H_2O, the organic phase was dried over $MgSO_4$ and concentrated.

3-((3-(3,5-Bis(trifluoromethyl)phenyl)-5-methyl-4*H*-1,2,4-triazol-4-yl)methyl)-4-phenylpyridine (20): To a solution of **19** (110 mg, 0.25 mmol) in dry CH_2Cl_2 was consecutively added acethydrazide (22 mg, 0.3 mmol), PhCOOAg (150 mg, 0.5 mmol) and HOAc (43 μL, 0.75 mmol) under nitrogen. The mixture was stirred at room temperature for 24 h and concentrated to give a black oil. The residue was diluted with CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ and brine. The combined extracts were washed with brine, dried over $MgSO_4$ and concentrated. The crude was purified by preparative TLC (CH_2Cl_2/MeOH = 25:1) to give **20** (35 mg, 31%) as a white solid (mp: 183–185 °C): HPLC purity: 98.41%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.65 (d, J = 4.9 Hz, 1H), 8.09 (s, 1H), 7.93 (s, 1H), 7.79 (s, 2H), 7.49–7.38 (m, 3H), 7.23 (d, J = 4.9 Hz, 1H), 7.09 (m, 2H), 5.13 (s, 2H), 2.37 ppm (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 153.2, 152.1, 150.3, 148.9, 147.5, 136.3, 132.5 (q, J = 33.8 Hz, 2C), 129.2, 129.1 (3C), 128.4, 127.6 (2C), 127.1, 124.7, 124.0 (q, J = 271 Hz, 2C), 123.6, 121.2, 44.5, 11.1 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{23}H_{16}F_6N_4$: 463.1357, found: 463.1362.

(4-(2-Fluorophenyl)pyridin-3-yl)methanamine hydrochloride (22): To a solution of **21** (10 g, 32 mmol) in MeOH (80 mL) was added 10% Pd/C (3.3 g) and Et_3N (30 mL). The mixture was hydrogenated at room temperature for 3 h and filtered. The filtrate was concentrated and diluted with H_2O. After alkalinization, the mixture was extracted with EtOAc. The combined extracts were washed with brine, dried over $MgSO_4$ and acidified with a solution of 4 N HCl/EtOAc. The resulting mixture was filtered to give 6.16 g (70%) of the corresponding amine hydrochloride as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.73 (s, 1H), 8.56 (d, J = 4.9 Hz, 1H), 7.42 (m, 1H), 7.30–7.22 (m, 2H), 7.21–7.13 (m, 2H), 3.78 ppm (s, 2H); MS (ESI): m/z 203.1 $[M+H]^+$.

3-((5-(3,5-Bis(trifluoromethyl)phenyl)-2-(furan-2-yl)-4,5-dihydro-1*H*-imidazol-1-yl)methyl)-4-(2-fluorophenyl)pyridine (27): DPPA (467 μL, 2.17 mmol) was added to a mixture of **26** (240 mg, 0.434 mmol), PPh_3 (569 mg, 2.17 mmol), and DIAD (430 μL, 2.17 mmol) in dry THF under nitrogen. The reaction mixture was stirred at room temperature overnight until most of the starting material was consumed. The mixture was then concentrated, and the residue was purified by flash column chromatography (0–25% EtOAc in petroleum ether (PE) gradient) to afford crude *N*-(2-azido-1-(3,5-bis(trifluoromethyl)phenyl)ethyl)-*N*-((4-(2-fluorophenyl)pyridin-3-yl)methyl)furan-2-carboxamide (560 mg) as a yellow oil: LC–MS (ESI): m/z 578.2 $[M+H]^+$.

To a solution of the above azide (560 mg) in MeOH was added 10% Pd/C (70 mg). The resulting mixture was hydrogenated at room temperature for 3 h and then heated at reflux under air for another 4 h. The mixture was filtered, and the filtrate was concentrated. The residue was purified by flash column chromatography (0–4% MeOH in CH_2Cl_2 gradient) to afford **27** (80 mg, 34% for the two steps) as a white solid: HPLC purity: 95.65%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.53 (m, 2H), 7.70 (s, 1H), 7.54 (s, 1H), 7.47 (s, 2H), 7.32 (m, 2H), 7.09 (m, 2H), 7.05–6.97 (m, 2H), 6.93 (m, 1H), 6.53 (dd, J = 3.4, 1.7 Hz, 1H), 4.90 (d, J = 16.1 Hz, 1H), 4.46 (m, 1H), 4.39–4.21 (m, 2H), 3.67 ppm (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5 (d, J = 245.5 Hz), 155.9, 150.0, 148.9, 144.4, 144.3, 144.0, 143.2, 132.0 (q, J = 33.4 Hz, 2C), 130.7 (d, J = 8.2 Hz), 130.4, 130.2 (d, J = 2.5 Hz), 126.8 (2C), 124.6, 124.5 (d, J = 2.5 Hz), 124.4 (d, J = 49.2 Hz), 121.6, 115.7 (d, J = 21.6 Hz), 114.3, 111.7, 65.1, 63.8, 45.5 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{27}H_{18}F_7N_3O$: 534.1416, found: 534.1401.

5-(3,5-Bis(trifluoromethyl)phenyl)-4-((4-(2-fluorophenyl)pyridin-3-yl)methyl)-3-phenyl-4,5-dihydro-1,2,4-oxadiazole (32a): To a solution of amine **22** (303 mg, 1.5 mmol) in dry CH_2Cl_2 under nitrogen was added 3,5-bis(trifluoromethyl)benzaldehyde (248 μL, 1.5 mmol) and 4 Å molecular sieves. The reaction mixture was held at reflux for 5 h and then cooled to room temperature. Then the mixture was filtered, and the filtrate gave the crude imine **31**. Et_3N (312 μL, 1.5 mmol) was added dropwise to a stirred solution of **30a** (234 mg, 1.5 mmol) in dry THF at −40 °C under nitrogen. The resulting mixture was stirred at −40 °C for 15 min, and the above imine filtrate was added by syringe. Then the mixture was warmed to room temperature and stirred overnight. The mixture was added saturated aqueous NH_4Cl and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The residue was purified by flash column chromatography (0–25% EtOAc in PE gradient) to afford **32a** (475 mg, 58%) as a white solid (mp: 137–140 °C): HPLC purity: 97.30%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.55 (m, 2H), 7.81 (s, 1H), 7.57 (s, 2H), 7.55–7.37 (m, 6H), 7.26–7.21 (m, 1H), 7.12 (m, 2H), 6.97 (m, 1H), 6.13 (s, 1H), 4.42 (d, J = 14.9 Hz, 1H), 4.23 ppm (d, J = 14.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6 (d, J = 196.5 Hz), 158.1, 150.4, 149.7, 143.7, 141.2, 131.9 (q, J = 26.6 Hz, 2C), 131.3, 131.1, 130.5, 129.2 (3C), 128.0 (2C), 126.9 (2C), 125.0, 124.9 (d, J = 2.76 Hz), 124.7, 123.9, 123.2, 122.9 (q, J = 217.0 Hz, 2C), 116.1 (d, J = 17.2 Hz), 96.1, 47.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{18}F_7N_3O$: 546.1416, found: 546.1396.

General procedure for the preparation of 4,5-dihydro-1,2,4-oxadiazoles 32b–d

Compounds **32b**–**d** were prepared from the amine hydrochloride **22** (0.55 mmol) and an appropriate hydroximoyl chloride **30b**–**d** (0.75 mmol) by the general procedure D-I after purification by flash column chromatography (0–25% EtOAc in PE gradient).

5-(3,5-Bis(trifluoromethyl)phenyl)-3-(4-chlorophenyl)-4-((4-(2-fluorophenyl)pyridin-3-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (32b): White solid (mp: 126–129 °C, 133 mg, 46%); HPLC purity: 98.20%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.57 (d, J = 4.9 Hz, 1H), 8.54 (s, 1H), 7.81 (s, 1H), 7.55 (s, 2H), 7.49 (m, 1H), 7.39 (m, 2H), 7.34–7.23 (m, 3H), 7.15 (m, 1H), 7.10 (d, J = 5.0 Hz, 1H), 6.95 (m, 1H), 6.16 (s, 1H), 4.40 (d, J = 15.2 Hz, 1H), 4.20 ppm (d, J = 15.1 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.6 (d, J = 196.3 Hz), 157.3, 150.3, 149.9, 143.8, 141.0, 137.5, 131.9 (q, J = 26.9 Hz, 2C), 131.1 (d, J = 6.4 Hz), 130.5, 129.5 (2C), 129.2 (2C), 129.0, 126.9 (2C), 125.1, 125.0 (d, J = 2.6 Hz), 124.8 (d, J = 12.8 Hz), 123.3, 122.8 (q, J = 291.1 Hz, 2C), 122.4, 116.1 (d, J = 17.2 Hz), 96.2, 48.0 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{17}ClF_7N_3O$: 580.1027, found: 580.1018.

5-(3,5-Bis(trifluoromethyl)phenyl)-3-(3-chlorophenyl)-4-((4-(2-fluorophenyl)pyridin-3-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (32c): White solid (mp: 132–133 °C, 90 mg, 31%); HPLC purity: 99.48%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55 (m, 2H), 7.83 (s, 1H), 7.52 (m, 5H), 7.42–6.84 (m, 6H), 6.14 (s, 1H), 4.40 (d, J = 14.8 Hz, 1H), 4.25 ppm (d, J = 14.6 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.9 (d, J = 196.7 Hz), 157.0, 150.3, 149.9, 143.8, 140.9, 135.2, 131.9 (q, J = 26.9 Hz, 2C), 131.4, 131.2 (d, J = 6.4 Hz), 130.4, 128.9, 128.1 (2C), 126.9 (2C), 126.0, 125.7, 125.1, 124.9, 124.7 (d, J = 2.8 Hz), 123.3, 122.9 (q, J = 217.0 Hz, 2C), 116.1 (d, J = 17.2 Hz), 96.3, 47.8 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{17}ClF_7N_3O$: 580.1027, found: 580.1012.

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5-(3,5-Bis(trifluoromethyl)phenyl)-3-(2-chlorophenyl)-4-((4-(2-fluorophenyl)pyridin-3-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (32d): Light-yellow solid (80 mg, 28%); HPLC purity: 97.86%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.50 (d, J = 4.5 Hz, 1H), 8.37 (s, 1H), 7.83 (s, 1H), 7.72 (s, 2H), 7.60–7.30 (m, 5H), 7.20 (t, J = 7.5 Hz, 1H), 7.05 (m, 2H), 6.95 (t, J = 7.3 Hz, 1H), 6.10 (s, 1H), 4.21–4.03 ppm (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.4 (d, J = 196.3 Hz), 155.2, 150.2, 149.4, 143.5, 140.3, 134.0, 132.4 (2C), 131.9, 131.9 (q, J = 26.7 Hz, 2C), 131.1 (d, J = 6.4 Hz), 130.3 (2C), 128.8, 127.4 (2C), 124.9, 124.8 (d, J = 2.7 Hz), 124.6 (d, J = 12.8 Hz), 123.5, 123.2, 122.9 (q, J = 217.9 Hz, 2C), 116.0 (d, J = 17.2 Hz), 95.8, 44.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{28}H_{17}ClF_7N_3O$: 580.1027, found: 580.1024.

5-(3,5-Bis(trifluoromethyl)phenyl)-4-((4-(2-fluorophenyl)pyridin-3-yl)methyl)-3-(furan-2-yl)-4,5-dihydro-1,2,4-oxadiazole (36): To a solution of compound **35** (130 mg, 0.4 mmol) in dry 1,4-dioxane (1.5 mL) in a dry microwave vial was added 3,5-bis(trifluoromethyl)-benzaldehyde (1.5 mL, 8 mmol) and TsOH·H_2O (152 mg, 0.8 mmol). The mixture was stirred at 150 °C by microwave irradiation for 30 min until consumption of most of the starting material. After the solvent was concentrated, the residue was diluted with H_2O and extracted with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$ and concentrated. The crude was purified by flash column chromatography (0–25% EtOAc in PE gradient) followed by preparative TLC (CH_2Cl_2/MeOH = 50:1) to give **36** (30 mg, 14%) as a light-yellow gum: HPLC purity: 98.87%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.61–8.52 (m, 2H), 7.80 (s, 1H), 7.58 (m, 3H), 7.41 (m, 1H), 7.21 (t, J = 7.5 Hz, 1H), 7.15–7.06 (m, 2H), 7.04–6.95 (m, 1H), 6.89 (d, J = 3.5 Hz, 1H), 6.54 (dd, J = 3.4, 1.7 Hz, 1H), 6.07 (s, 1H), 4.52 ppm (q, J = 15.8 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.8 (d, J = 245.2 Hz), 150.5, 150.0, 149.7, 145.2, 143.7, 140.5, 139.0, 132.4 (q, J = 39.6 Hz, 2C), 131.3 (d, J = 8.1 Hz), 130.7, 129.5, 127.4 (2C), 125.0 (2C), 124.9 (d, J = 15.9 Hz), 123.7, 122.7 (q, J = 320.6 Hz, 2C), 116.2 (d, J = 21.6 Hz), 114.5, 112.1, 96.6, 46.8 ppm (d, J = 4.1 Hz); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{17}F_7N_3O_2$: 536.1209, found: 536.1224.

5-(3,5-Bis(trifluoromethyl)phenyl)-3-(furan-2-yl)-4-((4-phenylpyridin-3-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (41a): **41a** was prepared from **40a** (198 mg, 0.77 mmol) and 3,5-bis(trifluoromethyl)benzaldehyde (116 μL, 0.7 mmol) by general procedure D-II to give 61 mg (17%) of a light-yellow solid after purification by flash column chromatography (0–30% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc/NH_3-MeOH = 5:1:5%): HPLC purity: 96.15%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.55 (s, 1H), 8.51 (d, J = 5.0 Hz, 1H), 7.79 (s, 1H), 7.58 (dd, J = 1.8, 0.8 Hz, 1H), 7.52 (s, 2H), 7.46–7.33 (m, 3H), 7.09 (m, 3H), 6.83 (dd, J = 3.5, 0.7 Hz, 1H), 6.54 (dd, J = 3.5, 1.8 Hz, 1H), 6.05 (s, 1H), 4.68 (d, J = 15.5 Hz, 1H), 4.57 ppm (d, J = 15.5 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 152.3, 151.2, 151.1, 150.8, 146.4, 141.8, 140.3, 138.6, 133.3 (q, J = 33.5 Hz, 2C), 130.27 (2C), 130.23, 129.8 (2C), 129.5, 128.6 (2C), 125.8, 124.9, 124.3 (q, J = 270.7 Hz, 2C), 115.8, 113.4, 97.5, 47.6 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{18}F_6N_3O_2$: 518.1303, found: 518.1297.

4-(Biphenyl-2-ylmethyl)-5-(3,5-bis(trifluoromethyl)phenyl)-3-(furan-2-yl)-4,5-dihydro-1,2,4-oxadiazole (45): **45** was prepared from **44** (198 mg, 0.77 mmol) and 3,5-bis(trifluoromethyl)benzaldehyde (116 μL, 0.7 mmol) by general procedure D-II to give 46 mg (13%) of a gray solid after purification by flash column chromatography (0–10% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc = 10:1): HPLC purity: 95.65%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.75 (s, 1H), 7.58 (d, J = 1.1 Hz, 1H), 7.54 (s, 2H), 7.43–7.26 (m, 7H), 7.23–7.13 (m, 1H), 7.07 (m, 2H), 6.80 (d, J = 3.5 Hz, 1H), 6.53 (dd, J = 3.5, 1.8 Hz, 1H), 6.05 (s, 1H), 4.55 ppm (q, J = 15.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.1, 144.8, 142.1, 140.7, 139.9, 139.2, 132.2, 131.7 (q, J = 266.6 Hz, 2C), 130.4, 129.1, 129.0 (2C), 128.4 (2C), 128.1, 127.8, 127.5, 127.3 (2C), 123.2, 122.9 (q, J = 217.3 Hz, 2C), 114.0, 111.7, 96.0, 48.3 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{27}H_{19}F_6N_2O_2$: 517.1351, found: 517.1365.

5-(3,5-Bis(trifluoromethyl)phenyl)-3-(furan-2-yl)-4-((2-phenylpyridin-3-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (49): **49** was prepared from **48** (198 mg, 0.77 mmol) and 3,5-bis(trifluoromethyl)-benzaldehyde (116 μL, 0.7 mmol) by general procedure D-II to give 66 mg (18%) of a yellow solid after purification by flash column chromatography (0–25% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc/NH_3-MeOH = 7:1:5%): HPLC purity: 98.41%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.54 (dd, J = 4.7, 1.6 Hz, 1H), 7.79 (s, 1H), 7.70 (dd, J = 7.9, 1.5 Hz, 1H), 7.65–7.60 (m, 1H), 7.56 (s, 2H), 7.41 (m, 3H), 7.32–7.25 (m, 2H), 7.17 (dd, J = 7.9, 4.7 Hz, 1H), 6.96 (m, 1H), 6.58 (dd, J = 3.5, 1.8 Hz, 1H), 6.00 (s, 1H), 4.73 (d, J = 15.9 Hz, 1H), 4.57 ppm (d, J = 15.9 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.5, 149.9, 148.9, 145.0, 140.1, 138.9, 138.6, 137.0, 131.9 (q, J = 26.8 Hz, 2C), 128.9 (2C), 128.7, 128.5 (3C), 127.3 (2C), 123.5, 122.8 (q, J = 217.1 Hz, 2C), 122.3, 114.3, 112.0, 96.7, 47.7 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{18}F_6N_3O_2$: 518.1303, found: 518.1320.

5-(3,5-Bis(trifluoromethyl)phenyl)-3-(furan-2-yl)-4-((3-phenylpyridin-4-yl)methyl)-4,5-dihydro-1,2,4-oxadiazole (53): **53** was prepared from **52** (198 mg, 0.77 mmol) and 3,5-bis(trifluoromethyl)-benzaldehyde (116 μL, 0.7 mmol) by general procedure D-II to give 7 mg (2%) of a yellow gum after purification by flash column chromatography (0–30% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc/NH_3-MeOH = 8:1:5%, then PE/EtOAc = 3:1): HPLC purity: 97.24%; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.62 (d, J = 5.2 Hz, 1H), 8.48 (s, 1H), 7.68 (s, 1H), 7.62 (d, J = 5.1 Hz, 1H), 7.60 (dd, J = 1.7, 0.7 Hz, 1H), 7.46–7.34 (m, 3H), 7.30 (s, 2H), 7.19 (dd, J = 6.5, 2.9 Hz, 2H), 7.00 (dd, J = 3.5, 0.6 Hz, 1H), 6.58 (dd, J = 3.5, 1.8 Hz, 1H), 6.27 (s, 1H), 4.43 (d, J = 15.9 Hz, 1H), 4.22 ppm (d, J = 15.8 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 150.6, 149.9, 149.3, 145.0, 142.3, 138.7, 137.9, 136.7, 135.0, 131.9 (q, J = 26.7 Hz, 2C), 129.4 (2C), 128.7(2C), 128.4, 127.9(2C), 122.8 (q, J = 217.2 Hz, 2C), 121.9, 121.4, 114.4, 112.1, 94.0, 50.5 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{18}F_6N_3O_2$: 518.1303, found 518.1308.

4-((4-(2-Fluorophenyl)pyridin-3-yl)methyl)-3-(furan-2-yl)-5-(3-(trifluoromethyl)phenyl)-4,5-dihydro-1,2,4-oxadiazole (54a): **54a** was prepared from **22** (182 mg, 0.66 mmol) and 3-trifluoromethylbenzaldehyde (81 μL, 0.6 mmol) by general procedure D-II to give 52 mg (18%) of a yellow gum after purification by flash column chromatography (0–35% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc/NH_3-MeOH = 5:1:5%): HPLC purity: 97.90%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.57 (s, 1H), 8.51 (d, J = 4.9 Hz, 1H), 7.55 (m, 2H), 7.48–7.32 (m, 4H), 7.20 (t, J = 7.5 Hz, 1H), 7.08 (m, 2H), 7.00 (t, J = 7.4 Hz, 1H), 6.87 (d, J = 3.5 Hz, 1H), 6.52 (dd, J = 3.5, 1.8 Hz, 1H), 6.03 (s, 1H), 4.48 ppm (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 158.7 (d, J = 196.3 Hz), 150.1, 149.7, 149.1, 144.7, 143.3, 139.1, 138.2, 131.0 (d, J = 6.5 Hz), 130.8 (q, J = 26.0 Hz), 130.6, 130.5, 129.6, 129.2, 126.6, 124.7 (3C), 124.0, 123.7 (q, J = 224.3 Hz), 115.8 (d, J = 17.2 Hz), 113.9, 111.8, 97.3, 46.1 ppm (d, J = 3.3 Hz); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{25}H_{18}F_4N_3O_2$: 468.1335, found: 468.1331.

5-(2-Chloro-3,5-bis(trifluoromethyl)phenyl)-4-((4-(2,6-difluorophenyl)pyridin-3-yl)methyl)-3-(furan-2-yl)-4,5-dihydro-1,2,4-oxadiazole (54n): **54n** was prepared from amine 40g (258 mg, 0.88 mmol) and **56b** (222 mg, 0.8 mmol) by general procedure D-II to give 30 mg (6%) of a white solid (mp: 122–124 °C) after purifica-

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tion by flash column chromatography (0–25% EtOAc in PE gradient) followed by preparative TLC (PE/EtOAc/NH_3-MeOH = 7:1:5%, then PE/EtOAc = 4:1): HPLC purity: 99.79%; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 8.58 (d, J = 5.0 Hz, 1H), 7.88 (s, 2H), 7.57 (d, J = 1.6 Hz, 1H), 7.47–7.32 (m, 1H), 7.15 (d, J = 5.0 Hz, 1H), 7.05–6.89 (m, 3H), 6.58 (s, 1H), 6.54 (dd, J = 3.5, 1.8 Hz, 1H), 4.54 ppm (q, J = 16.1 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 159.2 (2C), 150.1, 149.9, 149.3, 145.2, 138.8, 138.7, 136.7, 135.3, 131.2 (t, J = 7.9 Hz), 130.2, 130.0 (m, 2C), 128.8, 125.7 (2C), 122.6 (q, J = 217.0 Hz), 121.8 (q, J = 218.1 Hz), 114.5, 113.7 (t, J = 16.0 Hz), 112.0 (2C), 111.8, 93.7, 47.5 ppm; HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{15}ClF_8N_3O_2$: 588.0725, found: 588.0730.

(*S*)-5-(2-Chloro-3,5-bis(trifluoromethyl)phenyl)-4-((4-(2,6-difluorophenyl)pyridin-3-yl)methyl)-3-(furan-2-yl)-4,5-dihydro-1,2,4-oxadiazole ((*S*)-54n) and **(*R*)-5-(2-Chloro-3,5-bis(trifluoromethyl)phenyl)-4-((4-(2,6-difluorophenyl)pyridin-3-yl)methyl)-3-(furan-2-yl)-4,5-dihydro-1,2,4-oxadiazole ((*R*)-54n)**: The two enantiomers were separated by chiral HPLC using a Shimadzu LC20 system with UV detector SPD-20A (CHIRALCEL OD-3, 0.46(i.d.) × 15 cmL × 3 μm, UV 254 nM, hexane/*i*PrOH = 85:15), Daicel Chiral Technologies Co. Ltd. (China).

(*S*)-54n: HPLC purity: 98.82%; 99.94% *ee*; $[\alpha]_D^{20} = -165.6^\circ$ (c = 0.33 g(100 mL)$^{-1}$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 8.57 (d, J = 5.0 Hz, 1H), 7.88 (s, 2H), 7.57 (s, 1H), 7.47–7.32 (m, 1H), 7.15 (d, J = 5.0 Hz, 1H), 7.06–6.85 (m, 3H), 6.57 (s, 1H), 6.54 (dd, J = 3.3, 1.7 Hz, 1H), 4.54 ppm (q, J = 16.1 Hz, 2H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{15}ClF_8N_3O_2$: 588.0725, found: 588.0734.

(*R*)-54n: HPLC purity: 98.86%; 99.89% *ee*; $[\alpha]_D^{20} = 162.0^\circ$ (c = 0.33 g(100 mL)$^{-1}$, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.68 (s, 1H), 8.57 (d, J = 4.9 Hz, 1H), 7.88 (s, 2H), 7.57 (s, 1H), 7.47–7.32 (m, 1H), 7.15 (d, J = 4.9 Hz, 1H), 7.06–6.87 (m, 3H), 6.57 (s, 1H), 6.54 (dd, J = 3.4, 1.7 Hz, 1H), 4.54 ppm (q, J = 16.1 Hz, 2H); HRMS (ESI): m/z $[M+H]^+$ calcd for $C_{26}H_{15}ClF_8N_3O_2$: 588.0725, found: 588.0732.

Computational studies

Geometry optimizations of the X-ray structures at B3LYP/6-31G(d) in vacuo followed by TDDFT calculations using various functionals (B3LYP, BH&HLYP, PBE0) and TZVP basis set were performed by the Gaussian 09 software package.[50] ECD spectra were generated as the sum of Gaussians with 2400 cm^{-1} half-height width (corresponding to ~12 nm at 220 nm), using dipole-velocity-computed rotational strengths.[51] For the solution conformers, mixed torsional/low-mode conformational searches were carried out by means of the Macromodel 9.9.223 software using Merck Molecular Force Field (MMFF) with an implicit solvent model for chloroform.[52]

In vitro TGR5 assays

hTGR5/CRE/HEK293 or mTGR5/CRE/HEK293 stable cell lines were obtained by transfection of HEK293 cells with human or mouse TGR5 expression plasmid (hTGR5-pcDNA 3.1 or mTGR5-pcDNA 3.1) and CRE-driven luciferase reporter plasmid (pGL4.29, Promega, Madison, WI, USA), and were used to assess the activity of test compounds by reporter gene assay. Briefly, cells were seeded into 96-well plates and incubated overnight in DMEM supplemented with 10% FBS in 5% CO_2 at 37 °C. Cells were then incubated with fresh medium containing various concentrations of test compounds or 20 μM compound **1** as a positive control for 5.5 h. Luciferase activity in cell lysate was determined using the Steady-Glo Luciferase Assay System (Promega, Madison, WI, USA) according to the manufacturer's instructions.

Abbreviations

T2DM: type 2 diabetes mellitus; BAs: bile acids; BAT: brown adipose tissue; DPP-IV: dipeptidyl peptidase IV; GLP-1: glucagon-like peptide; SAR: structure–activity relationship; cAMP: 3′,5′-cyclic adenosine monophosphate; D2: type 2 iodothyronine deiodinase; DMF: *N*,*N*-dimethylformamide; THF: tetrahydrofuran; LDA: lithium diisopropylamide; DIBAL-H: diisobutylaluminum hydride; DME: 1,2-dimethoxyethane; DMFDMA: *N*,*N*-dimethylformamide dimethyl acetal; DPPA: diphenylphosphoryl azide; DBU: 1,8-diazabicyclo-[5.4.0]undec-7-ene; EDCI: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride; HOBt: 1-hydroxybenzotriazole; NBS: *N*-bromosuccinimide; NCS: *N*-chlorosuccinimide; DIAD: diisopropyl azodicarboxylate; TBDMSCl: *tert*-butyldimethylsilyl chloride; HATU: 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; DIEA, *N*,*N*-diisopropylethylamine.

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Keywords: 4,5-dihydro-1,2,4-oxadiazoles · cycloaddition · metabolic disorders · structure–activity relationships · TGR5

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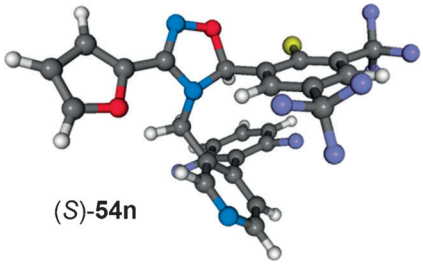
Binding pocket elaboration: A plausible pharmacophore model was used to design a novel class of TGR5 agonists based on a 3,4,5-trisubstituted 4,5-dihydro-1,2,4-oxadiazole core. New methods for constructing 4,5-dihydro-1,2,4-oxadiazoles were developed. An extensive structure–activity relationship study resulted in the identification of compound **54n**, which has excellent potency toward hTGR5. The configuration of the enantiomers was determined by solid-state TDDFT-ECD calculations.



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Design, Synthesis, and Structure–Activity Relationships of 3,4,5-Trisubstituted 4,5-Dihydro-1,2,4-oxadiazoles as TGR5 Agonists

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