Bioorganic & Medicinal Chemistry Letters 20 (2010) 1363–1367

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Synthesis and structure–activity relationships of a series of 3-aryl-4-isoxazolecarboxamides as a new class of TGR5 agonists

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ARTICLE INFO

Article history:
Received 20 November 2009
Revised 24 December 2009
Accepted 4 January 2010
Available online 7 January 2010

Keywords:
SAR
TGR5
TGR5 agonists
GPCR
Solution-phase synthesis

ABSTRACT

A series of 3-aryl-4-isoxazolecarboxamides identified from a high-throughput screening campaign as novel, potent agonists of the human TGR5 G-protein-coupled receptor is described. Many analogues were readily accessible via solution-phase synthesis which resulted in the rapid identification of key structure–activity relationships (SAR), and the discovery of potent exemplars (up to $pEC_{50} = 9$). Details of the SAR and optimization of this series are presented herein.

Diabetes mellitus is an ever-increasing threat to human health. According to the International Diabetes Federation (IDF), in 2010 diabetes is expected to cause close to four million deaths in the 20–79 age group, accounting for 6.8% of global mortality for this age range. The estimated diabetes prevalence for 2010 is 285 million, and within 20 years this number is expected to rise to 438 million.[1] Those that suffer from type II diabetes (T2D) have too little insulin or cannot use insulin effectively. As a result, glucose levels build up in the blood and urine, and if left untreated, can cause life-threatening complications, including blindness, kidney failure, and heart disease. Despite the use of various hypoglycemic agents with or without co-therapy with insulin, current treatments often fail to achieve significant lowering of serum glucose to a level where the severity of diabetic complications is effectively reduced. Thus, there is a clear need for novel therapeutics targeting the underlying mechanism(s) of elevated serum glucose.

TGR5, also known as BG37, M-BAR, or hGPCR19, is a bile acid G-protein-coupled receptor primarily expressed in monocytes and macrophages, lung, spleen, and the intestinal tract. It has been suggested that bile acids induce GLP-1 secretion from primary intestinal cells by increasing intracellular cAMP levels via the TGR5 receptor.[2–4] Recent FDA approved therapies for T2D based on GLP-1 are GLP-1 receptor agonist peptides (GLP-1 mimetics) and DPP-IV inhibitors, which enhance insulin action by prolonging the half-life of endogenous GLP-1.[5] These approaches are effective because while type II diabetics show impaired GLP-1 secretion, they maintain normal responsiveness to GLP-1. However, the use of a peptide in clinical treatment is severely limited because of the requirement for parenteral administration and low in vivo stability. Therefore, a small molecule agonist that increases GLP-1 secretion from the gut during a meal is a potentially useful therapeutic for metabolic disorders such as type II diabetes and its associated complications.

We previously reported a series of 3-aryl-4-isoxazolecarboxamides exemplified by compounds **9** and **22** as novel, potent small molecule agonists of the human TGR5 receptor which demonstrated improved GLP-1 secretion in vivo via an intracolonic dose (1 mg/kg) co-administered with glucose (0.125 g/kg) in conscious dogs as compared to control.[6] Herein we describe the SAR and optimization of this isoxazolecarboxamide series from the high-throughput screening (HTS) hit **1** to the identification of lead compounds such as **48** and **61**.

High-throughput screening (HTS) of the GSK proprietary compound collection[7] in a BacMam transduced human osteosarcoma cell line (U2-OS) led to the identification of isoxazole **1** as a specific agonist with a pEC_{50} of 5.3 and 100% maximum response.[8] In addition, the compound had excellent physicochemical properties

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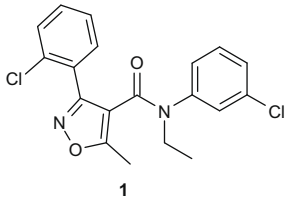
doi:10.1016/j.bmcl.2010.01.003

which made it of particular interest as a starting point for an optimization program designed to improve potency via exploration of the SAR (Fig. 1).

To explore this novel HTS hit, an efficient one- or two-step synthesis was initially utilized (Scheme 1). Commercially available 5-methyl-3-aryl-4-isoxazole-carbonyl chlorides **2** could be converted to the desired amides **3** in one step from commercially available, or prepared,[9] substituted *N*-alkylanilines. Alternatively, the acid chloride could be coupled with a substituted primary aniline, and then alkylated in a second step. Final compounds were typically purified by reverse-phase HPLC to purities of >95% (LC–MS, UV 214 nm detection) and then evaluated for pEC_{50} against the human TGR5 receptor in both a U2-OS and a melanophore cell line.[10] The results are detailed below. Initially the SAR of substitutions on the amide phenyl ring (R^1) was investigated (Table 1). The key finding was that *para*-substitution (**6**, **9**, **14**, **17**) was preferred over substitutions at either the *ortho*- (**4**, **7**, **12**, **15**) or *meta*-positions (**5**, **8**, **13**, **16**) as well as the unsubstituted parent compound **25**. Moreover, at the *para*-position, the chloro group (**9**), as well as the methyl substitution (**14**), were preferred over the strongly electron-rich methoxy group (**17**). Interestingly, some of these substituents could be combined to afford a further boost in potency, while other effects were not additive. For example, the 4-chloro-3-methyl disubstitution (**18**) lost some potency as compared to the monosubstituted 4-chloro analogue (**9**), with further reductions seen in the 4-chloro-2-methyl analogue (**19**). This same trend in potency reduction is seen with the dichloro combinations: 4-Cl (**9**) > 3,4-diCl (**20**) > 2,4-diCl (**21**). In contrast, when the 4-substitution was methyl, additional halo (**22–23**) and methyl substitution (**24**) resulted in a boost in potency in each case.

To further probe these findings, monosubstitution at the *para*-position of the aryl amide was further investigated with additional electron-withdrawing groups as well as alkyl groups with branching, both cyclic and acyclic (Table 2). The modifications in this region clearly showed that small lipophilic groups were preferred. Any alkyl substitutions larger than methyl, as with compounds **26–30**, demonstrated reduction in potency with increasing size. The CF_3 group (**31**) was well tolerated, and to a lesser extent the OCF_3 (**32**), but all other groups prepared (nitrile **33**, dimethylamino **34**, ketone **35**, sulfone **36** and ester **37**) lost potency, likely due to a combination of either steric and/or electronic effects.

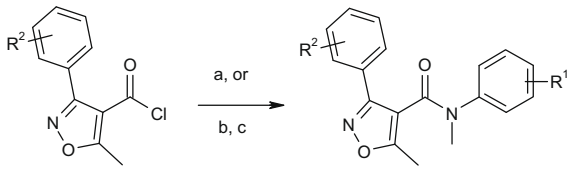
Variations on the isoxazole phenyl ring were next investigated to better understand the role of substitutions on this ring. These compounds were prepared where possible starting from commercially available 5-methyl-3-aryl-4-isoxazole-carbonyl chlorides as described in Scheme 1. Analogues could also be prepared according to Scheme 2. Starting from commercially available benzaldehydes, the chloro-oxime was prepared in two steps by treatment with hydroxylamine followed by *N*-chlorosuccinimide.[11] A microwave-assisted 1,3-dipolar cycloaddition of the chloro-oxime **3** with *N*-(4-chlorophenyl)-*N*-methyl-2-butynamide **38** afforded the desired product.[12] The butynamide **38** was readily prepared in large quantity from EDC/HOAt coupling of the appropriate acid with *para*-chloro-*N*-methylaniline. It is notable that 1,3-dipolar cycloadditions of alkynamides with chloro-oximes are rare in the litera-



1

TGR5 pEC_{50} = 5.3, 100 %
U2-OS Host pEC_{50} < 4.6
cLogP = 4.95
MW = 375
Solublity = 134 uM
AMP= 310 nm/sec
Passive Papp = 448 nm/sec

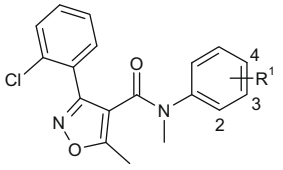
Figure 1. In vitro profile of HTS hit **1**.



2 3

Scheme 1. Synthesis of 3-aryl-4-isoxazolecarboxamides. Reagents and conditions: (a) various substituted *N*-alkylanilines, Et_3N, DCM, rt, or Et_3N, DME, μW 190 °C, 1 min; (b) various substituted anilines, Et_3N, DCM, rt, or Et_3N, DME, μW 190 °C, 1 min; (c) CH_3I, powdered KOH, DMSO, 50 °C, or rt.

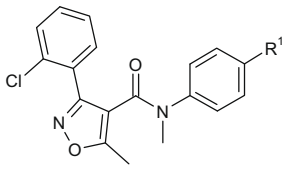
Table 1
pEC_{50} inhibition for amide phenyl substitution[a]



Compound	R^1	pEC_{50}
4	2-F	5.6
5	3-F	6.2
6	4-F	6.3 (±0.7)
7	2-Cl	5.7
8	3-Cl	6.2
9	4-Cl	7.5
10	4-Br	7.8
11	4-I	6.9
12	2-Me	6.4
13	3-Me	5.9
14	4-Me	6.9
15	2-OMe	5.6
16	3-OMe	5.9
17	4-OMe	6.4
18	4-Cl, 3-Me	7.2 (±0.9)
19	4-Cl, 2-Me	6.6
20	3,4-diCl	7.0
21	2,4-diCl	5.6
22	3-Cl, 4-Me	7.1
23	3,5-diBr, 4-Me	7.9
24	3,4-diMe	7.7
25	H	5.4

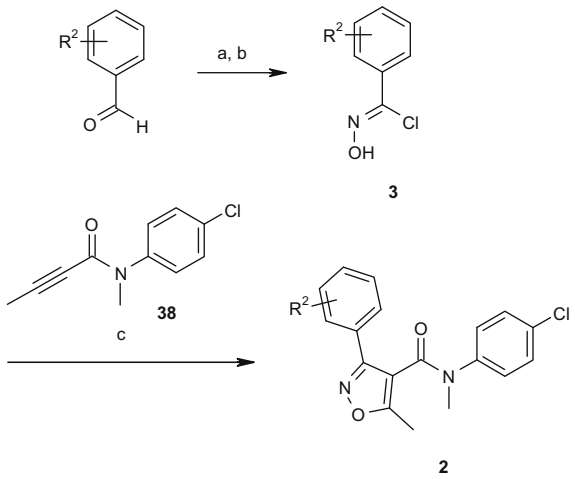
[a] pEC_{50} values given are means of at least two experiments in the melanophore assay. Standard deviation is less than ±0.6 except where noted in parentheses.

Table 2
pEC_{50} inhibition for amide phenyl *para*-substitution[a]



Compound	R^1	pEC_{50}
14	Me	6.9
26	Et	6.0
27	Pr	5.2
28	*i*Pr	5.9
29	*t*Bu	5.1
30	Cy	<4.6
31	CF_3	7.7
32	OCF_3	6.5
33	CN	6.5
34	NMe_2	5.4
35	COCH3	4.9
36	SO_2Me	<5.1
37	CO_2Me	5.1

[a] pEC_{50} values given are means of at least two experiments in the melanophore assay. Standard deviation is less than ±0.6.

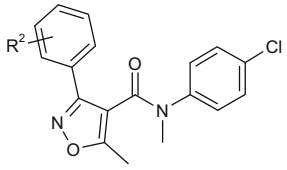


Scheme 2. Synthesis of substituted 3-aryl-4-isoxazolecarboxamides. Reagents and conditions: (a) hydroxylamine HCl, NaOAc, EtOH/H_2O (5:1), μW 150 °C, 1 min; (b) NCS, DMF, 16 h, heat then rt; (c) toluene, μW 160 °C, 10 min.

ture.[13] Although our yields for this cyclization were low (typically <10%), the simplicity of this convergent route was very attractive for our purposes.[14]

The results for this set of compounds (Table 3) suggested that *ortho*- and *meta*-substitutions were preferred over *para*-substitution for each substituent studied (**39–41**, **44–52**), except for the chloro substitutions in which the *meta*- and *para*-substitutions were equipotent (**9**, **42–43**). Some 2-chloro disubstitutions were also prepared (**54** and **55**) but these compounds lost potency as compared to the parent **9** and were not further pursued. Interestingly, compound **48** with a *meta*-methoxy substituent was the most potent compound prepared in the entire series (pEC_{50} = 9.0). Compounds were also prepared in which the isoxazole aryl ring was replaced with unsubstituted pyridines as well as thiophenes. All lost potency as compared to the unsubstituted phenyl parent compound (data not shown).

Table 3
pEC_{50} inhibition for isoxazole phenyl substitution[a]

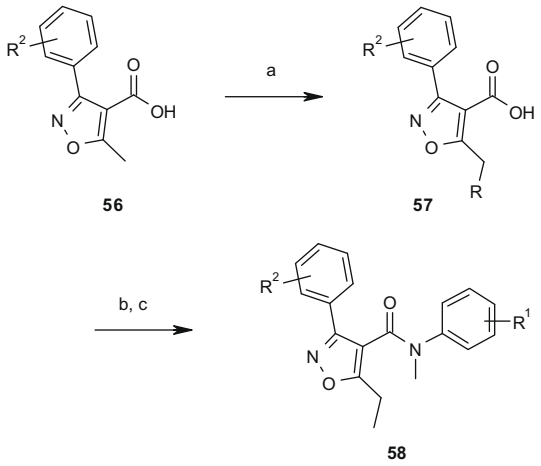


Compound	R^2	pEC_{50}
39	2-F	6.8 (±0.7)
40	3-F	8.1
41	4-F	6.3
9	2-Cl	7.5
42	3-Cl	7.9
43	4-Cl	7.9
44	2-Me	7.9
45	3-Me	7.1 (±0.9)
46	4-Me	6.0 (±0.9)
47	2-OMe	7.2 (±0.6)
48	3-OMe	9.0
49	4-OMe	5.3
50	2-CF3	7.5
51	3-CF3	6.5 (±0.9)
52	4-CF3	5.9
53	H	7.7 (±0.7)
54	2,6-diCl	6.2
55	2-Cl,6-F	6.2

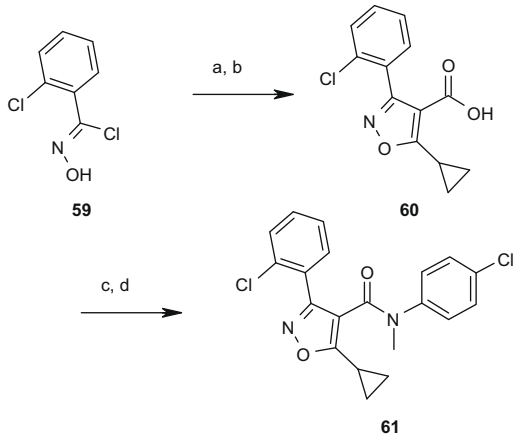
[a] pEC_{50} values given are means of at least two experiments in the melanophore assay. Standard deviation is less than ±0.6 except where noted in parentheses.

ADME studies on compound **9** suggested that the developability of this series needed to be addressed. While compound **9** had good physicochemical properties, there was measurable activity against two of the common cytochrome P450 (CYP450) isoforms, including 2C19 (pIC_{50} = 6.5) and 3A4 (vivid green) (pIC_{50} = 5.9). Moreover, in rats compound **9** showed very high in vivo clearance at levels exceeding hepatic blood flow[6] as well as high intrinsic clearance (Cl_{int} = 48 mL/min/g) in liver microsomes. In order to address these liabilities, variations of the isoxazole 5-methyl and *N*-methyl substituents were studied to find more metabolically stable analogues.

The isoxazole 5-alkyl analogues were synthesized as shown in Schemes 3 and 4. Treatment of acid **56** with 2.2 equiv of butyl lithium generated the dianion, which was then quenched with the appropriate alkyl halide to afford **57**.[15] Preparation of the acid chloride followed by acylation with the desired aniline afforded the desired 5-alkyl-substituted-4-isoxazole-carboxamides **58** (Scheme 3). The cyclopropyl analogue however, was prepared via an alternate route (Scheme 4). The chloro-oxime **59** was reacted with ethyl 3-cyclopropyl-3-oxopropanoate in triethylamine and toluene at room temperature to afford the desired cyclized 5-cyclopropyl-isoxazole ethyl ester.[16] Hydrolysis, acid chloride formation, and coupling with 4-chloro-*N*-methylaniline as before afforded the desired product **61**.

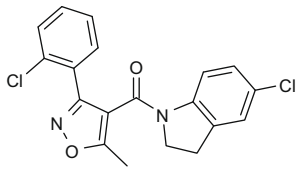


Scheme 3. Alkylation of isoxazole-5-methyl substituent. Reagents and conditions: (a) *n*-BuLi (2.2 equiv), then RI, −78 °C; (b) oxalyl chloride, cat. DMF; (c) *N*-methylaniline R^1, Et_3N, μW 190 °C, 1 min.



Scheme 4. Preparation of 5-cyclopropyl-*N*-methyl-4-isoxazole-carboxamide. Reagents and conditions: (a) ethyl 3-cyclopropyl-3-oxopropanoate, Et_3N, toluene, rt, 16 h; (b) KOH, EtOH, μW 100 °C, 2 min; (c) oxalyl chloride, cat. DMF; (d) *N*-methyl-4-Cl-aniline, Et_3N, μW 190 °C, 1 min.

The assay results for these compounds demonstrated a key finding that while the *N*-methyl amide (R^3) could not be varied without some loss of potency, the 5-methyl substituent on the isoxazole ring (R^4) appeared to tolerate more variability (Table 4). Replacement of either one of the methyl groups with hydrogen resulted in significant loss of potency (**62**, **67**) as compared to **9**. In addition, conformational constraint of the *N*-methyl group as seen in preparation of the 2,3-dihydro-1*H*-indole analogue (**73**) resulted in weak potency (pEC_{50} = 5.5).



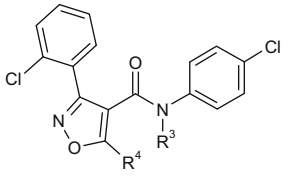
73
pEC_{50} = 5.5

Increasing bulk at R^3 corresponded with a trend in decreasing potency (**9**, **63**–**66**). However, increasing bulk at R^4 corresponded to a trend of increasing potency (**67**–**70**, **61**) by as much as one log unit (**61**, pEC_{50} = 8.4) as compared to **9**. Interestingly, some increased steric bulk at both positions concurrently (R^3 = R^4 = Et, **71**) was well tolerated, but further substitution (R^3 = R^4 = *i*Pr, **72**) caused potency loss.

ADME studies on compounds **9**, **68**, and **61** confirmed the hypothesis that the 5-methyl group was a source of metabolic liability in this series. The increasing substitution at R^4 from methyl (**9**) to ethyl (**68**) to cyclopropyl (**61**) (Table 5) resulted in reduced in vitro clearance in rats, though **61** unfortunately still had very high clearance. We therefore considered other potential sources of metabolic instability in this series.

It has been reported in the literature that other isoxazole templates also suffer from undesirable DMPK properties such as high clearance and poor exposure.[17] When we replaced the isoxazole ring with benzene or furan, the resulting compounds had reduced potency (by one log unit or greater) as compared to the parent compound (data not shown). Triazole **74**, however, was slightly more potent than isoxazole **75**, had equally good solubility, and had an improved P450 profile (Fig. 2). The intrinsic clearance was also further reduced as compared to compound **68** (Table 5).

Table 4
pEC_{50} Inhibition for alkyl variations at R^3 and R^4[a]



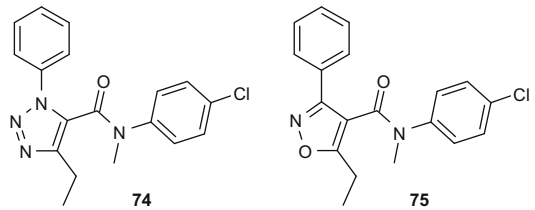
Compound	R^3	R^4	pEC_{50}
62	H	Me	<5.5
9	Me	Me	7.5
63	Et	Me	7.5
64	*i*Pr	Me	7.4
65	*n*-Pr	Me	6.5
66	*c*Pr	Me	5.5
67	Me	H	5.9
68	Me	Et	8.4
69	Me	*i*Pr	7.7
70	Me	*n*-Pr	8.0
61	Me	*c*Pr	8.4
71	Et	Et	8.0
72	*i*Pr	*i*Pr	6.1 (±1)

[a] pEC_{50} values given are means of at least two experiments in the melanophore assay. Standard deviation is less than ±0.5 except where noted in parentheses.

Table 5
In vitro rat DMPK results

Compds	pEC_{50}	Cl_{int}[a] (mL/min/g)
9	7.5	46
68	8.4	45
61	8.4	10
74	7.9	6.8

[a] Intrinsic clearance in rat liver microsomes.



74
pEC_{50}=7.9
P450 pIC_{50} <5.7 (all forms)

75
pEC_{50}=7.2
P450 2C19 pIC_{50} =6.6

Figure 2. 1,2,3-Triazole as an isoxazole replacement. pEC_{50} values are means of at least four experiments in the melanophore assay. Standard deviation is less than ±0.6.

In summary, SAR exploration of multiple regions of the HTS hit **1** led to a series of 3-aryl-4-isoxazolecarboxamides as novel, potent agonists of the human TGR5 G-protein-coupled receptor. Potent exemplars such as **9**, **48** (pEC_{50} = 9.0), and **61** (pEC_{50} = 8.4) were quickly identified. Triazole **74** (pEC_{50} = 7.9) showed the most promising improvements in the in vitro metabolic stability profile.

Acknowledgments

The authors thank Walter Johnson and Chad Quinn for their assistance with the LC–MS spectra, Victoria Magaard and Carl Bennett for their assistance with compound purification, and Minghui Wang for additional NMR support.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.01.003.

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7. The HTS used a MRE/CRE (multiple response element/cAMP response element) directed reporter gene assay measuring luciferase production in response to changes in cAMP via a Gs-protein coupled signaling pathway.
8. The compound had no response in the host cell line (pEC_{50} <4.6 in untransfected U2-OS cells) which confirmed the specificity.
9. Typical reaction conditions for preparation of substituted *N*-alkyl anilines: treatment of the aniline with the appropriate aldehyde or ketone (R), sodium triacetoxyborohydride ($Na(OAc)_3BH$), and acetic acid in dichloroethane (DCE) for 16 h at rt afforded the desired product. See also: Thorstensson, F.;

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10. For assay protocols as well as experimental procedures and spectral data for compounds **1**, **9**, and **22** see Ref. 6. Melanophore assay potencies were typically ~0.8–1 log unit more potent than the U2-OS cell data, and thus only one data set is reported.
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