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Triazinediones as prokineticin 1 receptor antagonists. Part 1: SAR, synthesis and biological evaluation

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ABSTRACT

A series of guanidine triazinediones were identified as potent PK1 receptor antagonists. A compound in this series inhibited the PK1 invoked prosecretory response in rat ileum tissue.

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The cysteine rich peptide prokineticin 1 (PK1), a member of the AVIT protein family, is the human homolog of a peptide originally isolated from snake venom (mamba intestinal toxin-1).[1] The peptide was named 'prokineticin' due to its ability to potently and selectively evoke contractions in mammalian intestinal smooth muscle.[2]

Immunostaining has demonstrated expression of PKR1 in approximately 25% of the myenteric neurons in mouse proximal colon.[3] PK1 applied directly to the isolated intact mouse colon inhibits spontaneous generation of large amplitude propulsive contractions.[3] We recently reported fluid enteropooling and transit stimulation effects of PK1 in the rat small intestine in vivo.[4] We have also found PK1 peptide to act as a novel potent intestinal secretagogue in vitro.[4] The distribution of PKR1 in the rat and mouse gastrointestinal (GI) tracts characterized via RT-PCR taken together with PK1-mediated functional effects suggest that it plays a significant role in modulating GI motility and secretomotor function.[2,5,6]

Dysmotility and altered secretomotor activity are functional hallmarks of GI diseases like irritable bowel syndrome (IBS) and inflammatory bowel disease (IBD).[7] Due to the pivotal role that PK1 and its receptor appear to play in the modulation of GI motility, novel agents that are designed to modulate the activation of PKR1 as antagonists could be highly efficacious in the treatment of functional bowel disorders that accompany IBS and IBD. Therefore, a program was initiated to discover a novel PK1 receptor antagonist.

A review of the literature revealed no reports of a small molecule inhibitor of the PK1 receptor.[8] A high throughput screening of our compound collection identified guanidine triazinedione **1** as a moderate PK1 receptor antagonist. (Fig. 1) Efforts were undertaken to increase potency and to explore structure–activity relationships (SAR) by optimizing R^1 and R^2 of scaffold **2**. (Fig. 2)

New analogs were synthesized via two different routes.[9] The first method was developed to explore changes to R^2, keeping R^1 constant (Scheme 1). Initially, the commercially available sulfonic acid salt of 2-methyl-isothiourea **3** was free-based with 3 N NaOH and the resulting compound reacted with commercially available isocyanates to provide intermediate **4**. Acylation of the isothiourea **4** with methylchloroformate under basic conditions yielded cyclization precursor **5**. Subsequently, **5** was cyclized under basic conditions to yield triazinedione **6**. Intermediate **6** was alkylated with alkyl halides under basic conditions or substituted with commercially available alcohols using Mitsunobu conditions to provide key intermediate **7**. Displacement of the thiomethyl group with

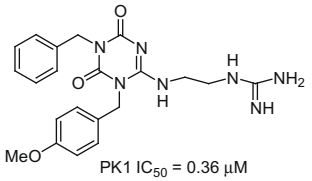

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Figure 1. Compound identified via HTS.

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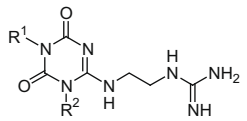

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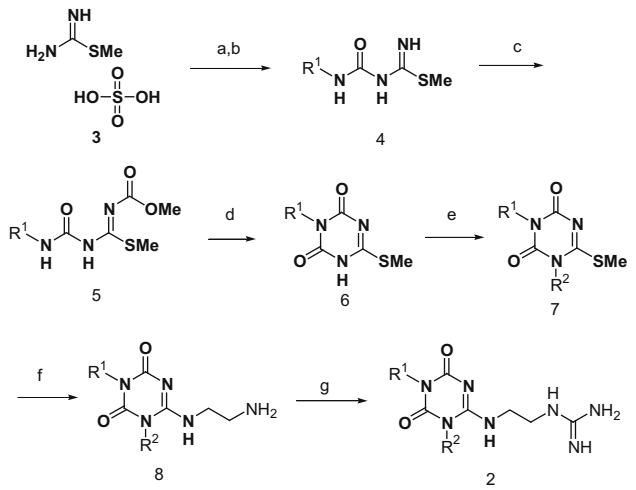
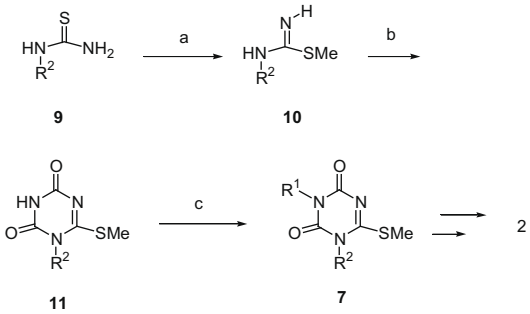
Figure 2. Scaffold **2**.



Scheme 1. Reagents and conditions: (a) 3 N NaOH; (b) R^1NCO, H_2O/MeOH/THF, 0 °C to rt; (c) methylchloroformate, NEt_3, CH_2Cl_2, −10 °C to rt; (d) NaOMe,MeOH; (e) R^2-OH, PPh_3, DEAD, THF or R^2–Cl, NaOMe, CH_3CN, heat; (f) ethylenediamine, toluene, 90 °C; (g) pyrazole-1-carboxamidine hydrochloride, DIEA, CH_3CN.

ethylenediamine in toluene at 90 °C yielded compound **8**.[10] To form the desired guanidine derivative **2**, the amine of **8** was reacted with pyrazole-1-carboxamidine hydrochloride and diisopropylethylamine in acetonitrile at room temperature.

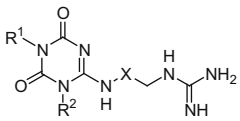


Scheme 2. Reagents: (a) MeI, MeOH; (b) ClCONCO, DIEA,CH_2Cl_2; (c) R^1–OH, PPh_3, DEAD, THF.

In order to synthesize target compounds varying R^1 and keeping R^2 constant, we utilized the reaction sequence shown in Scheme 2. Commercially available thiourea **9** was methylated with methyl iodide in methanol to provide isothiourea **10**. Upon addition of *N*-chlorocarbonyl isocyanate, isothiourea **10** was cyclized to give triazinedione **11**. Alkylation of the ring was accomplished either by utilizing Mitsunobu conditions as shown or alkyl halides under basic conditions to provide key intermediate **7**. Using the same procedures outlined in Scheme 1, **7** was converted to guanidine **2**. All final compounds were purified using reverse phase chromatography and isolated as trifluoroacetic acid (TFA) salts.

Compounds were tested by measuring the compounds ability to inhibit hPK1 induced calcium mobilization in GPR73 (hPKR1) expressing HEK 293 cells.[11] The results are shown in Table 1.

Table 1
The inhibition of prokineticin 1 by triazinediones with R^1 and R^2 modifications



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Compd #	R^1	R^2	X	PK1 IC_{50} (μM)
1	Benzyl	4-Methoxybenzyl	CH_2	0.336
13	Phenethyl	4-Methoxybenzyl	CH_2	2.50
14	*n*-Butyl	4-Methoxybenzyl	CH_2	2.18
15	2-Furyl	4-Methoxybenzyl	CH_2	2.59
16	4-Fluorobenzyl	4-Methoxybenzyl	CH_2	0.033
17	3,4-Dichlorobenzyl	4-Methoxybenzyl	CH_2	0.022
18	3-Methoxybenzyl	4-Methoxybenzyl	CH_2	3.69
19	2-Methoxybenzyl	4-Methoxybenzyl	CH_2	>10
20	4-Methoxybenzyl	4-Methoxybenzyl	CH_2	0.021
21	4-Hydroxybenzyl	4-Methoxybenzyl	CH_2	1.16
22	4-Methoxyphenethyl	4-Methoxybenzyl	CH_2	0.461
23	4-Cyanobenzyl	4-Methoxybenzyl	CH_2	0.318
24	3,4-Dichlorobenzyl	4-Methoxybenzyl	CH_2CH_2	1.21
25	4-Fluorobenzyl	4-Methoxyphenethyl	CH_2	2.86
26	4-Chlorobenzyl	Benzyl	CH_2	4.78
27	4-Chlorobenzyl	4-Methoxybenzyl	CH_2	0.019
28	4-Fluorobenzyl	4-Nitrobenzyl	CH_2	>10
29	3,4-Dichlorobenzyl	4-Propoxybenzyl	CH_2	0.515
30	3,4-Dichlorobenzyl	*n*-Hexyl	CH_2	6.51
31	4-Methoxybenzyl	4-Methoxy-methylcyclohexyl	CH_2	3.26
32	4-Methoxybenzyl	4-Hydroxybenzyl	CH_2	0.027
33	4-Chlorobenzyl	3-Furyl	CH_2	1.87
34	4-Methoxybenzyl	2-Methoxy-5-methylpyridyl	CH_2	0.051
35	3,4-Dichlorobenzyl	4-Difluoromethoxybenzyl	CH_2	0.047
36	Benzyl	4-Fluorobenzyl	CH_2	>10

When the benzyl group (R^1) of the original HTS hit (**1**) was substituted in the para position with an electron donating group such as methoxy (**20**), a significant increase in potency was achieved. Similar results were obtained when halogens were substituted in the para position (**16**, **17**, **27**). Methoxy substitutions in the meta or ortho positions (**18**, **19**) provided compounds with much less potency. When electron withdrawing functional groups, such as cyano (**23**), were substituted in the para position of the benzyl group, we observed similar activity to the HTS lead. Adding an extra carbon between the phenyl group and the triazinedione provided a less potent compound (**22**). Replacing the benzyl group with groups such as *n*-butyl (**14**) or furanyl (**15**) yielded less potent compounds.

When the *p*-methoxybenzyl group (R^2) of **27** was replaced with just a benzyl group (**26**), a significant loss of potency was observed. Interestingly, the methoxy group could be replaced with difluoromethoxy (**35**) but not propyloxy (**29**). Substituents such as para nitro (**28**) provided less potent compounds. The *p*-methoxy benzyl group was successfully replaced with the methoxy substituted pyridine (**34**) but not with *n*-hexyl (**30**) or 3-furyl (**33**). As was seen for R^1, adding an extra carbon between the triazinedione and the phenyl group (**25**) of R^2 did not yield greater potency. Unlike R^1, para substituted halogens like fluoro (**36**) substituted on the benzyl group R^2 were less potent. Also, para hydroxyl benzyl derivative worked well for R^2 but not for R^1 (**32** compared to **21**). The *p*-methoxy, *p*-hydroxyl or *p*-difluoromethoxy groups were the best benzyl substituents for R^2.

When an extra carbon was added to the linker between the heterocyclic ring and the guanidine (**24** compared to **17**), there was a significant loss in potency. The ethylenediamine linker was optimal.

To investigate the potential anti-secretory efficacy of selective small molecule PK1 receptor antagonists, we established a model of secretory diarrhea ex vivo in the Ussing-type flux chambers with mucosal exposure to PK1 peptide added cumulatively at 10 and 100 nM, respectively. Pre-treatment of isolated rat ileum mucosa with compound **16** (1 μM) added to the serosa 20 min prior to addition of PK1 peptide significantly suppressed the sustained increase in baseline short-circuit current (Isc) over time evoked by PK1 peptide by approximately 79.2% at 10 nM, and 60.7% at 100 nM (Fig. 3). These data suggest the potential for the efficacious use of PK1 receptor antagonists from this chemical class in gut disease states that have a significant secretory diarrhea component.

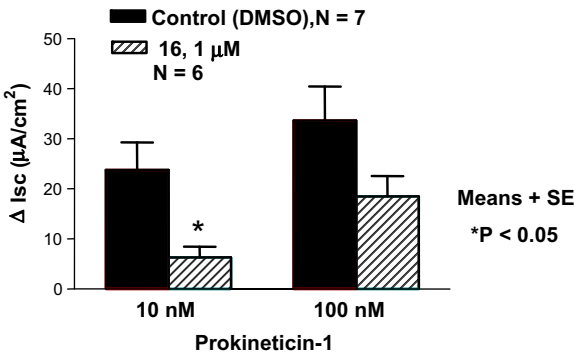

Figure 3. Effect of PK1 peptide tested at two concentrations, 10 and 100 nM, applied in a cumulative fashion to the basolateral side of mucosal tissues mounted in Ussing-type flux chambers on basal short-circuit current (Isc) in rat ileal mucosa in the absence (filled bars; $N = 7$ tissues) and the presence (hatched bars; $N = 6$ tissues) of **16** (1 μM). Compound **16** was added to the basolateral surface of the tissues 20 min before addition of the test concentrations of PK1 peptide used to evoke prosecretory response to these tissues. DMSO which was used to dissolve **16** served as a vehicle control in non-treated tissues.

In summary, through the discovery of an HTS hit, a series of Prokineticin 1 receptor antagonists were synthesized with excellent cellular functional activity. The SAR of the triazinedione scaffold was explored and novel compounds with eighteen times greater potency were identified. Benzyl groups with methoxy or halogens substituted in the para position were optimal for R^1, and the *p*-methoxy, *p*-hydroxyl or *p*-difluoromethoxy groups were the best benzyl substituents for R^2. These are the first reported small molecule inhibitors of the PK1 receptor. We further demonstrated the anti-secretory property of these inhibitors ex vivo by inhibiting the PK1 invoked prosecretory response in rat ileum tissue. Further publications will discuss SAR of the guanidine moiety.

Acknowledgments

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10. The reaction could also be done in the microwave in ethanol at 160 °C.
11. GPR73 expressing HEK 293 cells are grown on 96 well poly-D-lysine coated plate (Costar), in selective media at 37 °C and 5% CO_2. On day of experiment media is removed and replaced with Calcium plus dye solution (Molecular Devices). After equilibrations in dark 1st at 37 °C followed by rt, compound is added to the cells followed by addition of hPK1 ligand (at a previously determined EC_{50}). Fluorescence is measured on a Fuorometric Imaging Plate Reader (FLIPR), and IC_{50} determined using GraphPad Prism.