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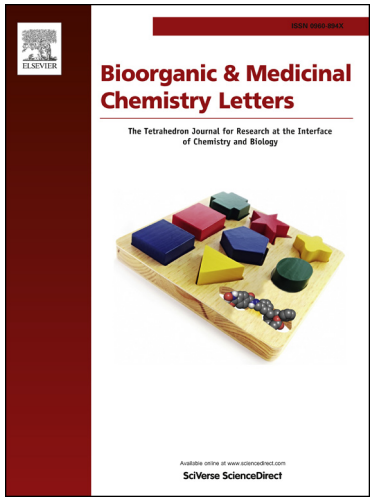

Hit-to-lead optimization of 2-(1H-pyrazol-1-yl)-thiazole derivatives as a novel class of EP_1 receptor antagonists

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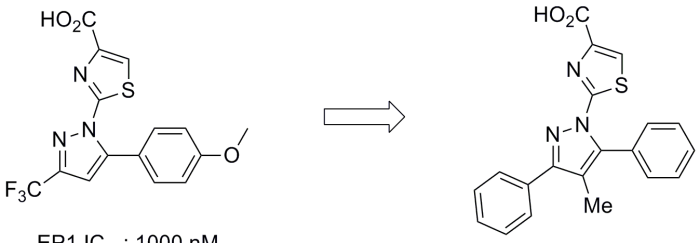

EP1 IC_{50}: 1000 nM

EP1 IC_{50}: 50 nM

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Pharmaceutical Research Center, Asahi Kasei Pharma Corporation, 632-1 Mifuku, Izunokuni-shi, Shizuoka 410-2321, Japan

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ABSTRACT

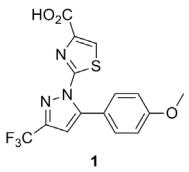
We describe a medicinal chemistry approach to generate a series of 2-(1H-pyrazol-1-yl)thiazole compounds that act as selective EP_1 receptor antagonists. The obtained results suggest that compound **12** provides the best EP_1 receptor antagonist activity and demonstrates good oral pharmacokinetics.

Prostaglandin E_2 (PGE_2) is a metabolite of arachidonic acid and is involved in various physiological actions such as cell protection, oxytocic activity, pain generation, peristaltic movement in the digestive tract, anti-hypertensive activity, and diuretic activity. PGE_2 is released from the urothelium or smooth muscle of the bladder, and intravesicular PGE_2 contributes to the pathophysiology of bladder overactivity[1, 2].

It is believed that PGE_2 acts on bladder sensation by contractile action of the detrusor smooth muscle and by stimulating the afferent nerves of the bladder[3, 4]. According to recent studies, there are four receptor subtypes of PGE_2: EP_1, EP_2, EP_3 and EP_4 receptor[5, 6]. Of these, the EP_1 receptor is the most abundant on the C fiber of the bladder[7]. It was found that antagonizing this receptor inhibits the voiding reflex[7]. It was also reported that the symptoms of overactive bladder accompanying prostatic hyperplasia were improved by inhibiting afferent neural activities. EP1 receptor antagonists inhibit afferent activity and are used to treat overactive bladder symptoms[8].

A number of companies are pursuing EP1 receptor antagonists, but very limited information on their clinical development is available. The few exceptions to this are AstraZeneca's ZD6416[9], Ono Pharmaceutical's ONO-8130[10] and Ono Pharmaceutical's ONO-8539[11].

Our starting compound was identified by screening our in-house compound library. 2-(1H-pyrazol-1-yl)thiazole **1** was identified as having potent EP_1 receptor antagonist activity by hEP1 reporter gene assay (Fig 1). Compound **1** was then tested for activity towards the other PGE_2 receptors (EP_2, EP_3 and EP_4) and showed a 3–8 fold EP_1 selectivity relative to EP_2, EP_3 and EP_4.



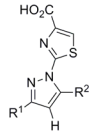
1

hEP1 reporter gene assay (hEP1 rep)
EP1 IC_{50}: 1000 nM
EP2 IC_{50}: >8000 nM
EP3 IC_{50}: >5000 nM
EP4 IC_{50}: >2700 nM

Figure 1. HTS hit compound

To obtain more potent compounds with higher EP_1 activity, we introduced different functional groups into compound **1** (Table 1). Regioisomer **2** showed improved EP_1 receptor antagonist activity. Compound **3** (R^1 = Ph) also showed increased activity, whereas **4** (R^1 = 4-Me-Ph) showed a loss of activity. These results suggested that the 4-methoxy group and 4-methyl group are not important for EP_1 receptor activity. However, the 4-phenol analog **5** and 3-phenol analog **6** showed moderate potency, whereas the 2-phenol **7** showed increased potency; these results indicated that *ortho* substitution is favored over *meta* or *para* substitution.

The *in vitro* ADME profile of **1**, **3** and **5** was determined (Table 2). Compound **5** was found to be intrinsically unstable in human plasma (human CLint: 500 ml/min/kg; rat CLint: 130 ml/min/kg). We checked the metabolites of **5** by LC/MS/MS and found that the major metabolite arises from glucoronidation. In contrast, compound **3** was stable in human and rat plasma, so we investigated modifications of compound **3**.



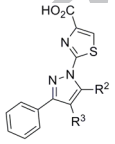
Comp.	R^1	R^2	hEP1 rep. IC_{50} (nM)
1	F_3C---	MeO-	1000
2	MeO-	F_3C---	430
3		F_3C---	320
4	Me-	F_3C---	3000
5	HO-	F_3C---	320
6	HO	F_3C---	580
7	OH	F_3C---	180

Table 1. Optimization of hit compound

Comp.	hEP1 rep. IC_{50} (nM)	CYP1A2 inh(%)	CYP2C9 inh(%)	CYP2C19 inh(%)	CYP2D6 inh(%)	CYP3A4 inh(%)	human CLint (ml/min/kg)	rat CLint (ml/min/kg)
1	1000	>10	>10	7.14	>10	>10	187	195
3	320	>10	>10	>10	>10	>10	28	ND
7	180	>10	>10	>10	>10	>10	500	130

Table 2. Profile of selected compounds

Starting from compound **3**, modification of the 4- or 5-position moiety was investigated (Table 3). The analogues with R^2 = Me (**8**) and R^2 = ^{t}Bu (**9**) showed a loss of EP1 antagonistic activity, whereas when R^2 was a phenyl group (**10**), EP1 activity decreased. When we prepared compound **11** (R^2 = CF_3, R^3 = Me) and **12** (R^2 = Ph, R^3 = Me), it was found that **12** showed the most potent EP_1 receptor antagonist activity.



Comp.	R^2	R^3	hEP1 rep. inhibition at 1.1μM	hEP1 rep. IC_{50} (nM)
3	F_3C---	H---		320
8	Me---	H---	14%	
9	Me, Me, Me	H---	1%	
10		H---	31%	
11	F_3C---	Me---		84
12		Me---		50

Table 3. Optimization of **3**

The central ring of compound **12** was also replaced with two types of imidazole rings (Table 4) to provide imidazole **13**, which exhibited decreased activity, and imidazole **14**, in which the replacement was tolerated. These results show that the pyrazole ring plays an important role in the expression of EP1 antagonistic activity.

Comp.	R^4	hEP1 rep. inhibition at 1.1μM	hEP1 rep. IC_{50} (nM)
12			50
13		7%	
14			500

Table 4. Optimization of the central ring

EP1 signals primarily through Gq, producing a transient rise in intracellular calcium. Therefore, compound **12** was tested using an intracellular Ca^{2+} release assay. In addition, compound **12** was tested for its ability to bind EP_2, EP_3 and EP_4 receptors. As shown in Table 4, **12** demonstrated excellent EP_1 receptor selectivity over other EP family receptors. The in vitro ADME profile of **12** was also evaluated. Compound **12** was found to be intrinsically stable in plasma (human CLint: 35 ml/min/kg; mouse CLint: 15 ml/min/kg). We delineated the metabolites of compound **12** by LC/MS/MS and observed several oxidized products. Compound **12** displayed excellent apparent permeability compared with atenolol as measured by flux through MDCK cells in transwell cultures (A/BPapp = 51.64 x 10^{-6} cm/s). Compounds **1**, **7** and **12** were tested using the Ames test and were found to be negative against both TA98 and TA100.

Comp.	hEP1 rep. IC_{50} (nM)	Ca assay IC_{50} (nM)	hEP1 bind. Ki (nM)	hEP2 bind. Ki (nM)	hEP3 bind. Ki (nM)	hEP4 rep. Ki (nM)
1	1000	5800	8700	>8000	>5000	>2700
7	180	800	530	>8000	>5000	>2700
12	50	500	290	>8000	>5000	>2700

Table 5. EP family profile of **1**, **7** and **12**

Comp.	CYP1A2 inh(%)	CYP2C9 inh(%)	CYP2C19 inh(%)	CYP2D6 inh(%)	CYP3A4 inh(%)	human CLint (ml/min/kg)	rat CLint (ml/min/kg)	MDCK Papp x 10^{-6}(cm)	Ames Test TA98, TA100
1	>10	>10	7.14	>10	>10	187	195	ND	negative
7	>10	>10	>10	>10	>10	500	130	22.76	negative
12	7.93	>10	4.71	>10	>10	35	15	51.64	negative

Table 6. *In vitro* ADMET profile of **1**, **7** and **12**

The pharmacokinetics of compounds **7** and **12** were examined via intravenous and oral administration to male Sprague–Dawley rats. The animals received a single administration of 3 mg/kg, i.v. or 10 mg/kg, p.o. Compound **12** exhibited good oral pharmacokinetics and oral bioavailability was 32%. This prolonged pharmacokinetic half-life suggests that the metabolic stability of **12** is improved (cf., Fig 2 and Fig 3).

The synthetic route for the synthesis of the 2-(1H-pyrazol-1-yl)thiazole derivatives **8**–**10** is highlighted in Schemes 1–3.

Compounds **1**–**7** are commercially available. As shown in Scheme 1, a S_NAr reaction of the known 3,5-disubstituted pyrazole derivatives **15a–c** and ethyl 2-bromothiazolecarboxylate using NaH in DMF at 150°C afforded the thiazoleacetates **16a–m**. Saponification of the resultant esters with NaOH provided **8–10** (Scheme 1).

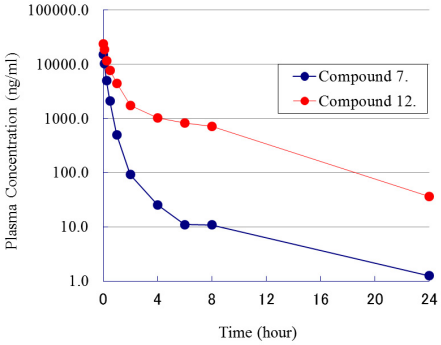


Figure 2. Change in plasma concentration of **7** and **12**. Each compound was dosed at 10 mg/kg, p.o. in rats.

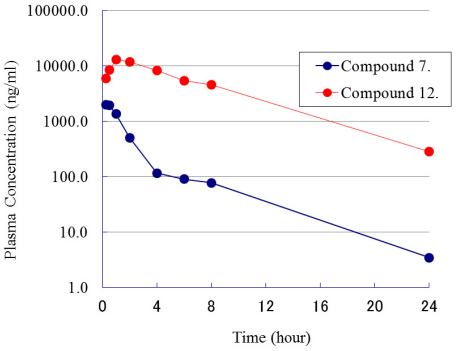
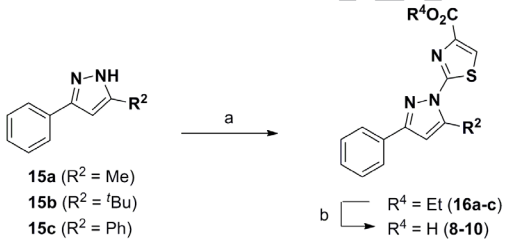
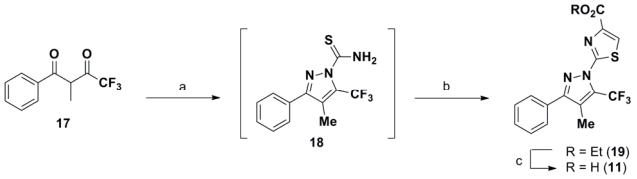


Figure 3. Change in plasma concentration of **7** and **12.** Each compound was dosed at 10 mg/kg, p.o. in rats.



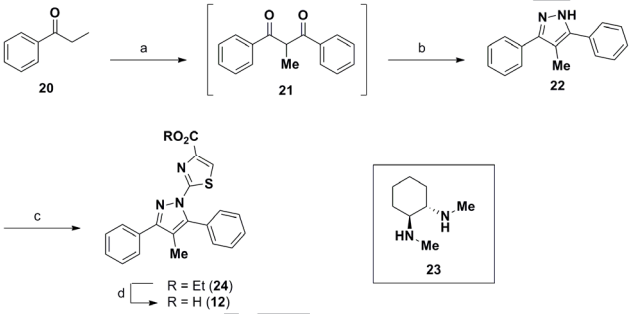
Scheme 1. Reagents and conditions: (a) ethyl 2-bromothiazole-carboxylate, NaH, DMF, 150°C, o.n. (**16a**: 44%, **16b**: 20%, **16c**: 18%); (b) 5M NaOH, EtOH, rt, o.n., (**8**: 55%, **9**: 63%, **10**: 60%).

As shown in Scheme 2, reaction of 4,4,4-trifluoro-2-methyl-1-phenyl butane-1,3-dione **17** and thiosemicarbazide in ethanol under reflux produced the pyrazolylthiourea **18**, which was then alkylated *in situ* with ethyl 2-bromopyruvate (Hanzsch reaction) to give 2-(1H-pyrazol-1-yl)thiazole **19** in 37% yield in a two-step, one-pot reaction. Finally, basic hydrolysis of **19** provided **11** (Scheme 2).



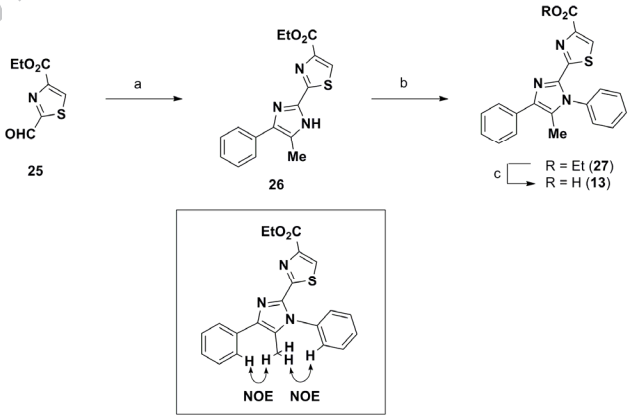
Scheme 2. Reagents and conditions: (a) thiosemicarbazide, EtOH, 80°C, 1 hour; (b) ethyl 2-bromopyruvate, EtOH, 80°C, (2 steps, 17%); 5M NaOH, EtOH, rt, o.n., 99%.

The synthetic route to compound **12** started from commercially available propiophenone **20** (Scheme 3), which was first α-acylated to provide the corresponding 1,3-diketone **21** (LiHMDS, BzCl). Subsequent condensation with $NH_2NH_2•H_2O$ afforded 3,5-diphenyl-4-methyl pyrazole **22**[12]. Buchwald *N*-arylation[13] of compound **22** was employed to install the thiazole-ring moiety, followed by hydrolysis to provide **12**[14].



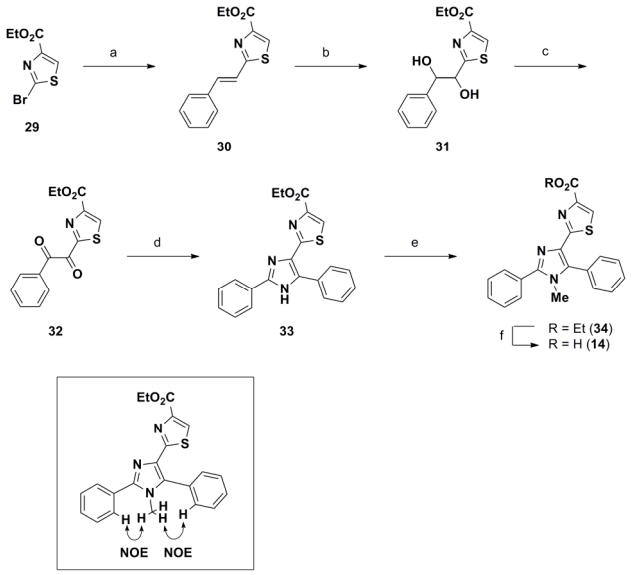
Scheme 3. Reagents and conditions: (a) LiHMDS, PhCOCl, toluene, rt, 1 min; (b) $NH_2NH_2•H_2O$, AcOH, rt, 30 min, (2 steps, 99%); (c) CuI, K_3PO_4, ethyl 2-bromo-thiazolecarboxylate, **23**, mesitylene, 185°C, (15%); (d) 5M NaOH, EtOH, rt, o.n., (82%).

The synthetic route providing compound **13** is illustrated in Scheme 4. Ethyl 2-formylthiazole-4-carboxylate was first condensed with 1-phenylpropane-1,2-dione and NH_4OAc to give the imidazole **26**. Buchwald *N*-arylation of compound **26** was used to install the *N*-phenyl moiety in **27**, followed by hydrolysis to give **13**. The NOESY correlation of the imidazole confirmed that the structure of **27** is as shown below.



Scheme 4. Reagents and conditions: (a) NH_4OAc, phenylpropane-dione, MeOH, rt, on, (74%); (b) CuI, K_3PO_4, iodobenzene, **23**, mesitylene, 185°C, (7%); (c) 5M NaOH, EtOH, rt, o.n., 71%.

Compound **14** was prepared using the route shown in Scheme 5. Suzuki-Miyaura coupling of compound **29** installed the vinyl moiety, and the styrenyl disubstituted olefin was converted by a two-step sequence (OsO_4-catalyzed dihydroxylation followed by oxidation with Dess-Martin periodinane) to the dione **32**. Dione **32** was condensed with benzaldehyde and NH_4OAc to give the imidazole **33**. *N*-alkylation of compound **33** effected the installation of the *N*-methyl moiety in **34**, followed by hydrolysis to afford **13**. The NOESY correlation of the imidazole confirmed the structure of **34** to be as shown below.



Scheme 5. Reagents and conditions: (a) *trans*-2-phenylvinyl-boronic acid, $Pd_2(dba)_3$, tri-*o*-tolylphosphine, K_3PO_4, DMF, 120ºC, o.n., (62%); (b) OsO_4, *N*-methylmorpholine, MeCN-H_2O(4:1), rt, (38%); (c) Dess-Martin periodinane, CH_2Cl_2, rt, (48%); (d) NH_4OAc, benzaldehyde, MeOH, rt, o.n., (41%); (e) MeI, NaH, DMF, rt, 1 hour, (91%); (f) 5M NaOH, EtOH, rt, o.n., (82%).

In conclusion, we present a 2-(1H-pyrazol-1-yl)thiazole as a novel class of EP_1 receptor antagonists. Compound **12** appeared to give the best EP_1 receptor antagonist activity and good selectivity over the other PGE_2 receptors. Our research effort focused on substituting the 2-phenol ring and identified compound **12** as having good oral pharmacokinetics. Compound **12** may serve as a lead compound for further development of overactive bladder drugs. Ongoing studies will be reported elsewhere.

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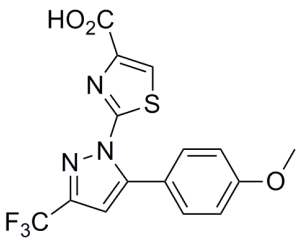
14. Characterization data for compound **12**: 2-(4-methyl-3,5-diphenyl-1H-pyrazol-1-yl)thiazole-4-carboxylic acid ; ^{1}H NMR (400 MHz, *DMSO-d_6*) ppm: 2.13 (3H, s), 7.46-7.56 (m, 8H), 7.78 (1H, s), 7.80 (1H, s), 8.20 (1H, s), ^{13}C NMR (100 MHz, *DMSO-d_6*) ppm: δ 9.69 (CH_3), 116.4 (C), 126.4 (CH), 127.6 (C and 2 carbons of CH), 128.1 (2 carbons of CH), 128.54 (CH), 128.6 (C), 128.69 (3 carbons of CH), 128.9 (CH), 130.3 (2 carbons of CH), 132.0 (C), 142.1 (C), 152.44 (C), 159.89 (C), 161.52 (C), FT-IR (KBr, cm^{-1}) 3071-2540 broad, 1686 (C=O), 1671, 1522, 1499; HRMS (ESI) calcd for $C_{20}H_{15}N_3O_2S$ [M+H]+ 362.0958, found 362.0960.



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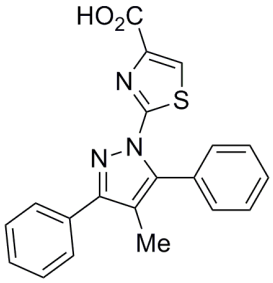

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