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Dioxotriazine derivatives as a new class of $P2X_3$ receptor antagonists: Identification of a lead and initial SAR studies

Hiroyuki Kai [a,*], Tohru Horiguchi [a,*], Takayuki Kameyama [b], Kentaro Asahi [a], Takeshi Endoh [c], Sae Jikihara [d], Tsuyoshi Hasegawa [e], Satoru Tanaka [a], Azusa Nozu [a], Chie Takeyama [e], Maki Tomari [e], Fumiyo Takahashi [f], Naomi Tamura [g], Shigenori Yagi [a], Tetsuji Itoh [h], Yasuyoshi Isou [i]

[a] Laboratory for Medicinal Chemistry Research, Shionogi & Co., Ltd., 1-1 Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan
[b] Corporate Social Responsibility Department, Shionogi & Co., Ltd., 12F, Hankyu Terminal Bldg., 1-4 Shibata 1-chome, Kita-ku, Osaka 530-0012, Japan
[c] Shionogi Administration Service Co., Ltd., 1-1 Futaba-cho 3-chome, Toyonaka, Osaka 561-0825, Japan
[d] Corporate Planning Department, Shionogi & Co., Ltd., 1-8, Doshomachi 3-chome, Chuo-ku, Osaka 541-0045, Japan
[e] Shionogi TechnoAdvance Research & Co., Ltd., 1-1 Futaba-cho, 3-chome, Toyonaka, Osaka 561-0825, Japan
[f] Laboratory for Innovative Therapy Research & Disease Research, Shionogi & Co., Ltd., 1-1 Futaba-cho, 3-chome, Toyonaka, Osaka 561-0825, Japan
[g] Laboratory for Drug Discovery and Development, Shionogi & Co., Ltd., 1-1 Futaba-cho, 3-chome, Toyonaka, Osaka 561-0825, Japan
[h] Disease Care Strategy Department, Shionogi & Co., Ltd., 2F, Nissay Yodoyabashi East, 3-13, Imabashi 3-chome, Chuo-ku, Osaka 541-0042, Japan
[i] CMC R&D Division, Shionogi & Co., Ltd., 1-3, Kuiseterajima 2-chome, Amagasaki, Hyogo 660-0813, Japan

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ABSTRACT

$P2X_3$ receptor is an ATP-gated ion channel, mainly localized on peripheral sensory neurons. Currently, several clinical trials are being conducted with $P2X_3$ receptor antagonists for the treatment of chronic pain or cough. To identify a $P2X_3$ lead compound, we reexamined the HTS evaluation compounds and selected dioxotriazine derivatives from which we identified a hit compound. As a result of the hit-to-lead SAR, we obtained lead compound **1** which had a moderate inhibitory effect on $P2X_3$ receptors (IC_{50}, 128 nM). Further improvement of the potency and PK profiles of this lead compound finally led to the selected compound **74** ($P2X_3$ IC_{50}, 16.1 nM; $P2X_{2/3}$ IC_{50}, 2931 nM), which demonstrated a strong analgesic effect against allodynia on oral administration in the rat partial sciatic nerve ligation model of neuropathic pain (ED_{50}, 3.1 mg/kg).

$P2X_3$ receptor, a ligand-gated ion channel activated by ATP, is mainly localized in the peripheral sensory neuron.[1] $P2X_3$ receptor antagonists (Fig. 1) reportedly show analgesic effects in neuropathic and inflammatory pain models.[2] Therefore, many $P2X_3$ receptor antagonists are targets in the development of analgesics.[3] Among them, gefapixant showed alleviation of interstitial cystitis/bladder pain syndrome (IC/BPS) in a Phase 2 clinical trial. Additionally, gefapixant showed alleviation of refractory chronic cough in a Phase 2b clinical trial. Thus, expectations are high for the utility of $P2X_3$ receptor antagonists in the treatment of various diseases.[4]

We previously reported on pyrrolinone and pyrropyrazolone derivatives (Figure 1.) as a new class of selective $P2X_3$ antagonists,[5–7] which were derivatized from screening hits from the Shionogi compound library. Even though the selected compounds showed high oral absorbabilities and potent analgesic effects, clinical developments were suspended due to a potential risk of drug-drug interactions.

We reexamined the compounds obtained by HTS to explore novel hit compounds (Fig. 2. A). Among them, based on a chemist's flash of inspiration of the similarity between dioxotriazines and RO-3, a known $P2X_3$ antagonist,[8] we reevaluated dioxotriazines and found that they exhibit weak activity. One dioxotriazine was selected as a hit compound (IC_{50}, 1200 nM). The hit to lead SAR was done using parallel synthesis (Fig. 2. B), and compound **1** with improved potency (IC_{50}, 128 nM) and a modest PK profile on rat (Clt, 23.9 mL/min/kg; BA, 25.2%) was selected as a lead compound.

In this paper, we report further SAR study from compound **1**, which led to a new class of selective $P2X_3$ antagonists, which showed improved oral absorbabilities and potent analgesic effects.

Synthesis of compounds with various linkers at position 6 are summarized in Scheme 1–4.[9]

* Corresponding authors.
E-mail addresses: hiroyuki.kai@shionogi.co.jp (H. Kai), tooru.horiguchi@shionogi.co.jp (T. Horiguchi).

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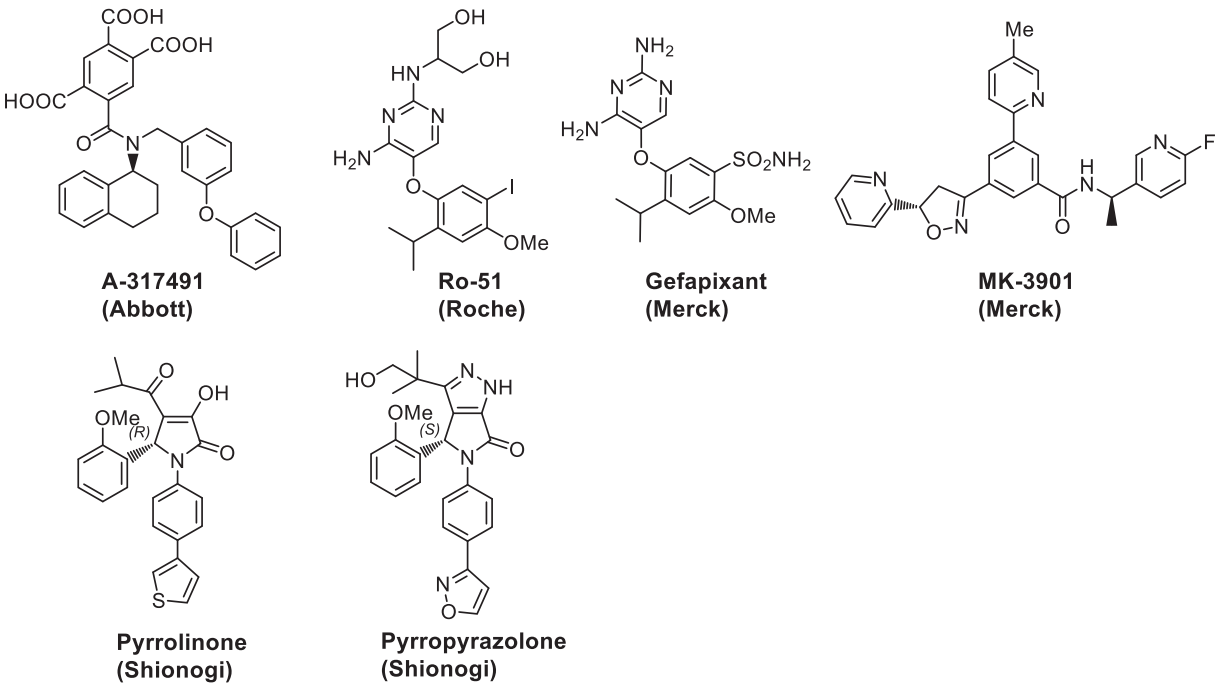


Fig. 1. Classical $P2X_3$ receptor antagonists and Shionogi's pyrrolinone and pyrropyrazolone.

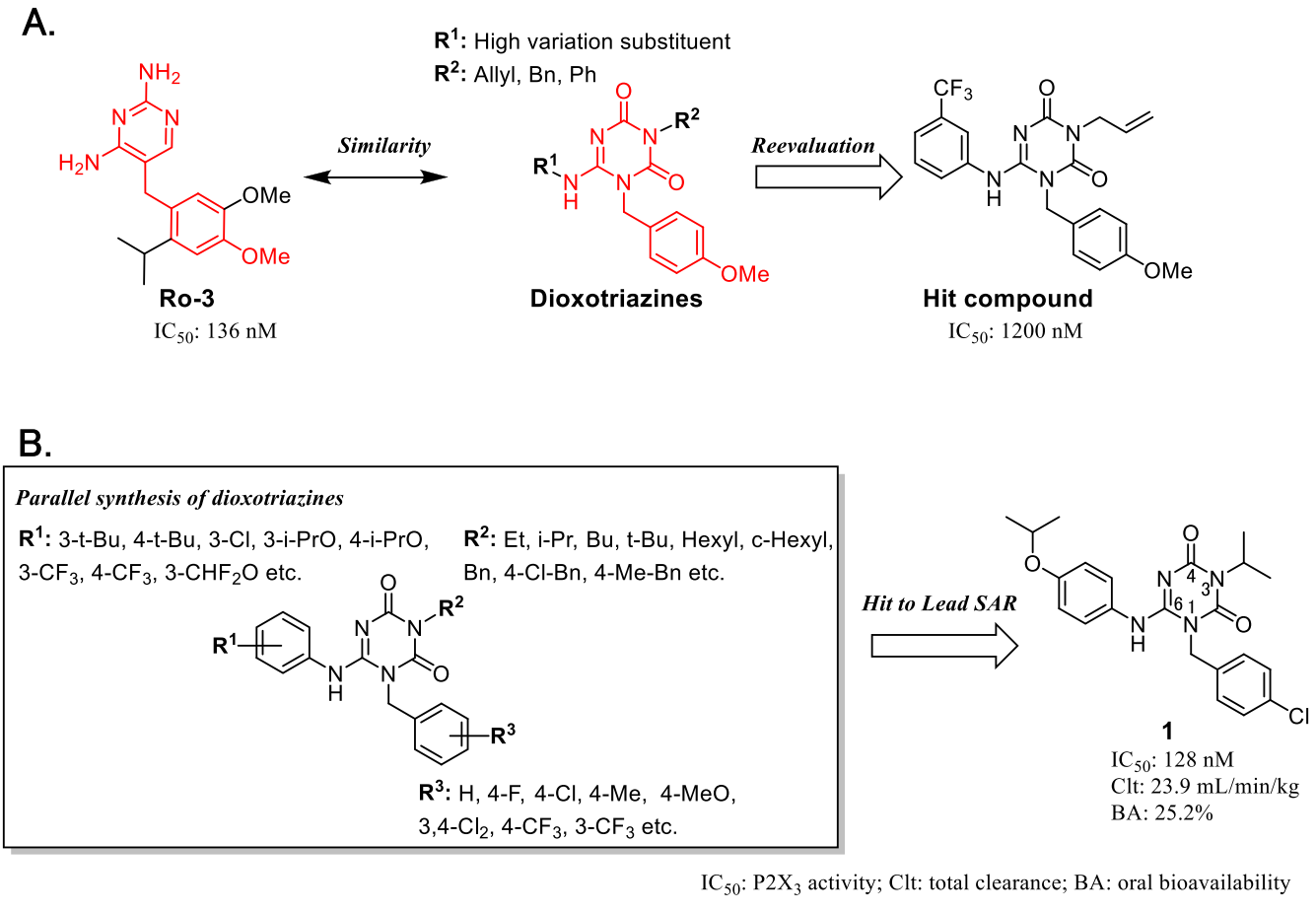
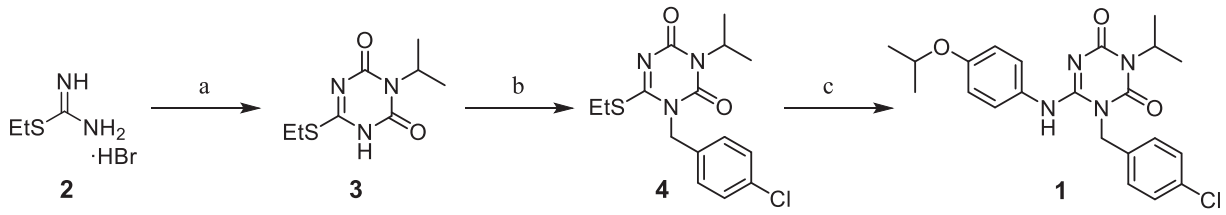
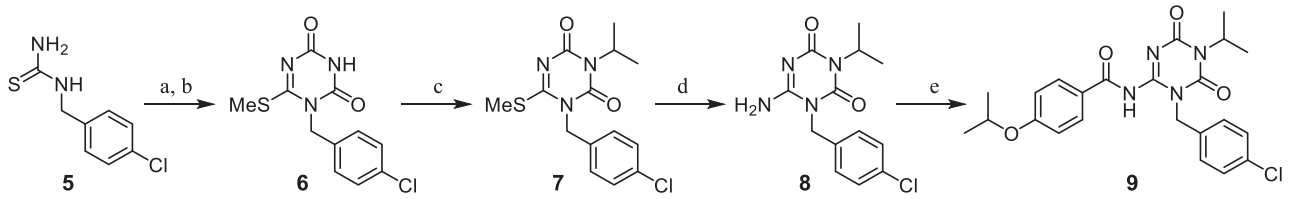


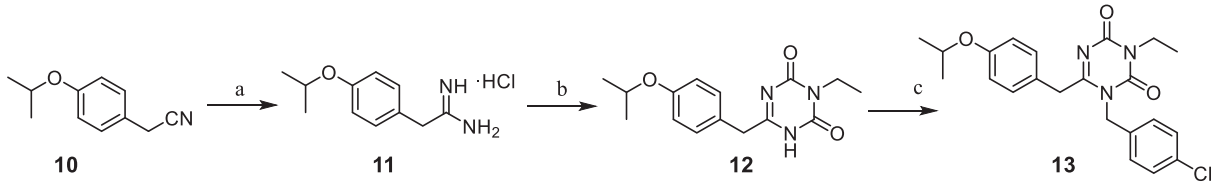
Fig. 2. Exploration of a novel hit compound and hit to lead SAR of dioxotriazines **A.** Dioxotriazine was selected as a hit compound, based on its structural resemblance to Ro-3**B.** Parallel synthesis of dioxotriazines gave lead compound **1** with improved potency and a modest PK profile on rat.



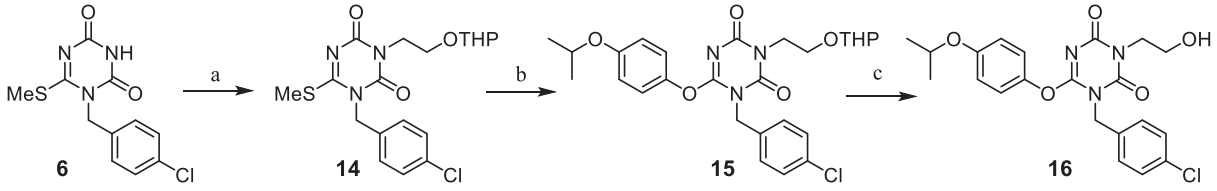
Scheme 1. Reagents and conditions for the synthesis of compound **1**: (a) i-PrNCO, DBU, DMF, 0 °C, 4 h then CDI, DBU, rt, overnight, 72%; (b) 4-Cl-PhCH$_2$Br, K_2CO_3, MeCN, reflux, 1.5 h, 100%; (c) 4-i-PrOPhNH$_2$, AcOH, 100 °C, 9 h, 76%.



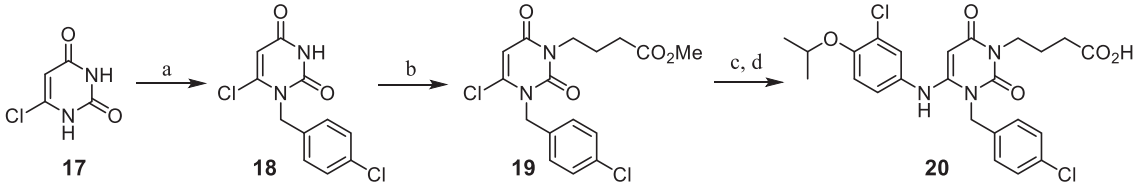
Scheme 2. Reagents and conditions for the synthesis of compound **9**: (a) MeI, MeOH, rt, 5 h; (b) ClCONCO, DIPEA, CH_2Cl_2, rt, 1 h, 58%; (c) i-PrBr, *t*-BuOK, DMF, 55 °C, 17 h, 61%; (d) microwave, $AcONH_4$, *t*-BuOH, 200 °C, 25 min, 69%; (e) 4-i-PrO-PhCOCl, Et_3N, DMF, rt, 4 h, 49%.



Scheme 3. Reagents and conditions for the synthesis of compound **13**: (a) NH_4Cl, $AlMe_3$, PhMe, 80 °C, overnight, 94%; (b) EtNCO, DBU, DMA, rt, 1 h then CDI, DBU, rt, 3 h, 58%; (c) 4-Cl-PhCH$_2$Br, DIPEA, DMA, 60 °C, 3 h, 39%.



Scheme 4. Reagents and conditions for the synthesis of compound **16**: (a) THPO(CH$_2$)$_2$OH, PPh_3, DIAD, THF, rt, 3 h, 78%; (b) microwave, 4-*i*-PrO-PhOH, Pyridine, 180 °C, 2 h, 45%; (c) PPTS, MeOH/CH_2Cl_2, rt, 4 h, 48%.



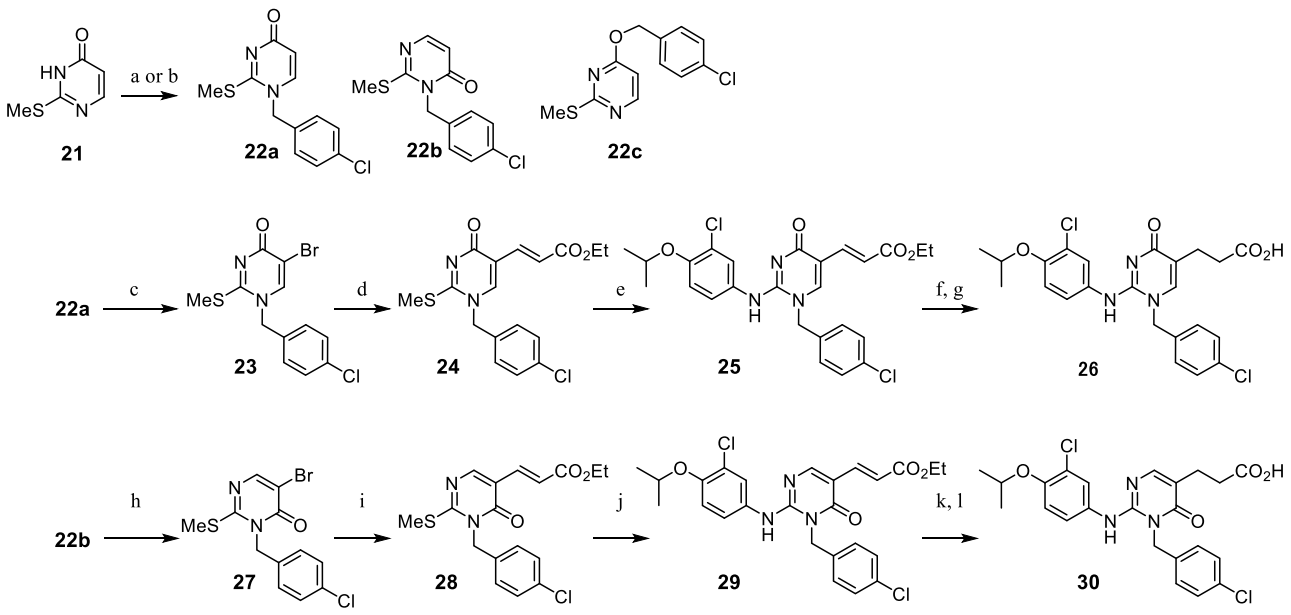
Scheme 5. Reagents and conditions for the synthesis of compound **20**: (a) 4-Cl-PhCH$_2$Br, NaH, LiBr, DMF, rt, overnight, 42%; (b) Br(CH$_2$)$_3$CO$_2$Me, NaH, DMF, 50 °C, 1 h, 88%; (c) 3-Cl-4-*i*-PrO-PhNH$_2$, Pd(OAc)$_2$, xantphos, Cs_2CO_3, Dioxane, 100 °C, 1 h; (d) 1 M−LiOH aq, MeOH/THF, rt, 4 h, 67%.

Scheme 1 shows the synthesis of compound **1**. Treatment of S-ethylisothiourea hydrobromide **2** with isopropyl isocyanate and 1,1′-carbonyldiimidazole in the presence of DBU in DMF afforded 6-(ethylthio)-triazinedione **3**.[10] Alkylation of **3** with 4-chlorobenzybromide and K_2CO_3 furnished intermediate **4**. The ethylthio group of **4** was substituted by the 4-isopropoxyanilino group by treatment with 4-isopropoxyaniline in AcOH at 100 °C to obtain compound **1**.

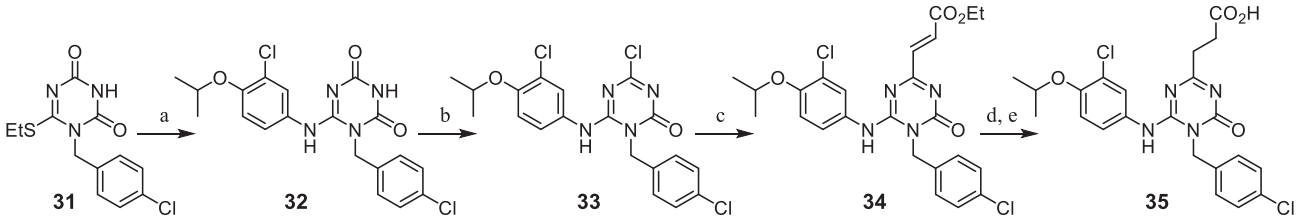
6-Benzeamide derivative **9** was prepared as depicted in Scheme 2. Methylation of 1-(4-chlorobenzyl)thiourea **5**, followed by treatment with ClCONCO gave triazine **6**.[11] Isopropylation at position 3 afforded intermediate **7**, a homolog of **4**. Nucleophilic substitution of the 6-methylthio group of **7** with ammonia required MW irradiation at 200 °C to give amine **8**,[12] which was coupled with 4-isopropoxy-benzoyl chloride to afford target compound **9**.

We also prepared 6-benzyl derivative **13** by the method shown in Scheme 3. Reaction of 2-(4-isopropoxyphenyl)acetonitrile **10** with NH_4Cl in the presence of $AlMe_3$ furnished amidine **11**.[13] Treatment of **11** with EtNCO afforded 6-benzyltriazine **12**, which is benzylated at position 1, to give the target 6-benzyl derivative **13**.

6-Aryloxy triazine derivative[14] **16** (Scheme 4) was prepared from the



Scheme 6. Preparation of **22a** and **22b:** (a) 4-Cl-PhCH$_2$Br, DIPEA, CH$_2$Cl$_2$, rt, overnight, **22a** (70%); (b) 4-Cl-PhCH$_2$Br, K$_2$CO$_3$, DMF, rt, overnight, **22b** (39%), **22c** (49%). Preparation of compound **26:** (c) NBS, CH$_2$Cl$_2$, reflux, 2 h, 71%; (d) [(*E*)-2- (Ethoxycarbonyl)vinyl]boronic acid, pinacol ester, PdCl$_2$ (dtbpf), K$_2$CO$_3$, THF, reflux, 2.5 h, 90%; (e) 3-Cl-4-*i*-PrO-PhNH$_2$, AcOH, *t*-BuOH, reflux, overnight, 87%; (f) H$_2$, 5% Pt/C sulfided, CHCl$_3$/DMF, rt, 4.5 h; (g) 1 M−LiOH aq, EtOH, rt, 4.5 h, 33%. Preparation of compound **30:** (h) 4-Cl-PhCH$_2$Br, K$_2$CO$_3$, DMF, rt, overnight; (i) NBS, CH$_2$Cl$_2$, rt, 2.5 h, 34%; (i) [(*E*)-2- (Ethoxycarbonyl)vinyl]boronic acid, pinacol ester, PdCl$_2$ (dtbpf), 2 M−K$_2$CO$_3$ aq, THF, reflux, 4 h, 55%; (j) 3-Cl-4-*i*-PrO-PhNH$_2$, AcOH, *t*-BuOH, reflux, 2 days, 44%; (k) H$_2$, 5% Pt/C sulfided, CHCl$_3$/DMF, rt, 4.5 h; (l) 1 M−LiOH aq, EtOH, rt, 4.5 h, 35%.



Scheme 7. Reagents and conditions for the synthesis of compound **35**: (a) 3-Cl-4-*i*-PrO-PhNH$_2$, AcOH, *t*-BuOH, reflux, overnight, 84%; (b) POCl$_3$, 50 °C, 2 h; (c) [(*E*)-2- (Ethoxycarbonyl)vinyl]boronic acid, pinacol ester, PdCl$_2$ (dtbpf), 1 M−K$_2$CO$_3$ aq, THF, reflux, 7 h, 30%; (d) H$_2$, 10% Pt/C, MeOH, rt, 0.5 h; (e) 1 M−LiOH aq, EtOH, rt, 4.5 h, 9%.

intermediate **6**. Mitsunobu reaction of **6** with THP-O(CH$_2$)$_2$OH gave intermediate **14**, and the 6-methio group of **14** was substituted with an aryloxy moiety under MW irradiation in pyridine at 180 °C to give intermediate **15**. Deprotection of the THP group of **14** with PPTS in MeOH yielded the target **16**

Synthesis of center ring analogs are shown in Schemes 5–7.

Benzylation and subsequent alkylation of 6-chlorouracil **17** (Scheme 5) afforded intermediate **18**.[15] Pd catalysis coupling of **18** and aniline,[16] followed by alkaline hydrolysis of the methyl ester **19** yielded the uracil analog **20**.

4-Oxo-1,4-dihydropyrimidine analog **26** and 6-Oxo-1,6-dihydropyrimidine analog **30** (Scheme 6) were prepared from a same starting material, 2-(methylthio)pyrimidin-4(3H)-one **21**. Benzylation of **21** with chlorobenzylbromide in the presence of DIPEA in CH$_2$Cl$_2$, followed by extraction and trituration of the product with EtOAc afforded 1-(4-chlorobenzyl)-2-(methylthio)pyrimidin-4(1H)-one **22a** as a major product (70%), while benzylation of **21** in the presence of K$_2$CO$_3$ in DMF afforded the isomers (**22b**, 39%) and (**22c**, 49%) mainly. Subsequent bromination of the **22a** afforded the bromide **23**. Suzuki coupling of **23** with vinyl boronic acid pinacol ester gave intermediate **24**. Nucleophilic substitution of the 6-methylthio group of **24** with aniline proceeded in a similar manner to the triazine derivative **4**, which afforded intermediate **25**. Catalytic hydrogenation of the olefin **25**, followed by alkaline hydrolysis of the Et ester, furnished the target **26**.

6-Oxo-1,6-dihydropyrimidine analog **30** was prepared from the **22b** by a method very similar to that for preparation of the 4-oxo-1,4-dihydropyrimidine analog **26**; however, nucleophilic substitution of the 6-methylthio group of **28** with aniline required long hours and resulted in a poor yield relative to that of **24**.

4-Alkyltriazine **35** (Scheme 7) was prepared from triazine **31**. Chlorination of **32** using POCl$_3$ afforded the 4-chloride **33**, which was converted to the target **35** by methods very similar to those in Schemes 6.

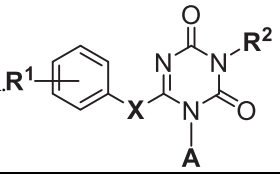
To evaluate the P2X receptors antagonistic activities of all compounds, IC$_{50}$ was calculated using stably expressed C6BU-1 cells transfected with the human P2X$_1$, $_2$, $_3$, $_4$ receptor genes, HEK-293 cells transfected with human P2X$_7$ receptor gene, or transiently expressed C6Bu-1 cell transfected with P$_2$X$_2$ and P2X$_3$ receptor genes.

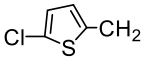
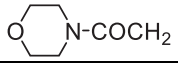
Table 1 shows the substituent effects of dioxotriazine derivatives on P2X$_3$ receptor antagonistic activity. First, the effect of the linker (X) at position 6 on the triazine ring was investigated, and the NH moiety was found to be crucial for the potency. The IC$_{50}$ value of compounds **1**, **36**, and **37** were 128 nM, 149 nM, and 83 nM, respectively, while other linkers (**9**: CONH, **13**: CH$_2$, **16**: O) greatly reduced the potency (IC$_{50}$ > 1000 nM).

For substituents on the phenyl group (R^1), compared with the 4-*i*-PrO

Table 1

SAR study of substituents X, R^2 and A.

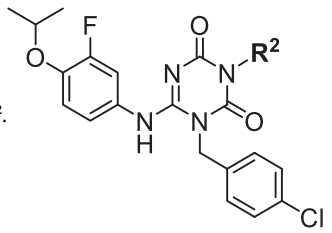


Compound	X	R^1	A	R^2	IC_{50} (nM) [a]
1	NH	4-*i*-PrO	4-Cl-PhCH_2	*i*-Pr	128
9	CONH	4-*i*-PrO	4-Cl-PhCH_2	*i*-Pr	>1000
36	NH	4-*i*-PrO	4-Cl-PhCH_2	Et	149
13	CH_2	4-*i*-PrO	4-Cl-PhCH_2	Et	>1000
37	NH	4-*i*-PrO	4-Cl-PhCH_2	$(CH_2)_2OH$	83
16	O	4-*i*-PrO	4-Cl-PhCH_2	$(CH_2)_2OH$	>3000
38	NH	3-CF_3	4-Cl-PhCH_2	*i*-Pr	389
39	NH	4-MeOCO	4-Cl-PhCH_2	*i*-Pr	420
40	NH	4-HOCO	4-Cl-PhCH_2	*i*-Pr	>3000
41	NH	4-*i*-PrNHCO	4-Cl-PhCH_2	*i*-Pr	520
42	NH	3-F-4-*i*-PrO	4-Cl-PhCH_2	*i*-Pr	45
43	NH	3-Cl-4-*i*-PrO	4-Cl-PhCH_2	*i*-Pr	31
44	NH	3-Cl-4-MeO	4-Cl-PhCH_2	*i*-Pr	450
45	NH	3-Cl-4-PhO	4-Cl-PhCH_2	*i*-Pr	953
46	NH	3-F-4-*i*-PrO	H	*i*-Pr	>3000
47	NH	3-F-4-*i*-PrO		*i*-Pr	144
48	NH	3-F-4-*i*-PrO		*i*-Pr	>3000
49	NH	3-F-4-*i*-PrO		*i*-Pr	>3000
50	NH	3-F-4-*i*-PrO	4-MeO-Ph	*i*-Pr	>3000
51	NH	3-F-4-*i*-PrO	4-Cl-Ph	*i*-Pr	>3000
52	NH	3-F-4-*i*-PrO	Me_2NCOCH_2	*i*-Pr	>3000
53	NH	3-F-4-*i*-PrO		*i*-Pr	>3000

a) IC_{50} was calculated using a stably expressed cell line (C6BU-1 cell) transfected with the human $P2X_3$ gene.

Table 2

SAR study of substituents R^2.



Compound	R^2	IC_{50} (nM)	Solubility [a] (μM)	Microsomal stab. (%)[b] Human	Rat
42	*i*-Pr	45	ND	36	49
54	H	415	>50	78	54
55	Et	38	10	86	42
56	Pr	13	1	36	35
57	CH_2Ph	48	ND	21	1
58	CH_2CN	93	19	68	41
59	$(CH_2)_2CN$	9.1	23	59	30
60	COMe	176	8	0	2
61	$(CH_2)_2OH$	38	>50	92	60
62	$(CH_2)_3OH$	14	>50	82	29
63	$(CH_2)_4OH$	18	20	59	36
64	$(CH_2)_2NHAc$	34	>50	45	1
65	$(CH_2)_3NHAc$	11	>50	47	15
66	CH_2COOH	629	>50	99	97
67	$(CH_2)_2COOH$	66	>50	92	99
68	$(CH_2)_3COOH$	78	>50	106	103
69	$(CH_2)_2CONH_2$	46	30	94	61
70	$(CH_2)_2CONHMe$	27	>50	86	30

ND: not detected.

[a]Solubilities were measured in sodium phosphate buffer (pH 6.8, 10 mmol) containing 1% DMSO.

[b]Percent of compound remaining after 30 min incubation with human (or rat) liver microsomes (0.5 mg protein/mL) at 37 °C with or without NADPH generating system.

(**1**), polar functional groups such as COOMe (**39**), COOH (**40**) and CONH-*i*-Pr (**41**) considerably reduced the potency, while introduction of F (**42**) or Cl (**43**) at position 3 on the 4-i-PrO-Ar of compound **1** improved the potency 3 to 4 times over compound **1**.

For substituents at position 1 (**A**), only benzyl (**42**) and its isostere thiophen (**47**) were potent, with others showing loss of the potency; the unsubstituted (**46**), alkyls (**48** and **49**), phenyls (**50** and **51**), and amides (**52**, **53**) showed no activity. This indicates that hydrophobicity and the length of the substituent are both necessary for the potency.

Next, we substituted an isopropyl moiety ($\mathbf{R^2}$) of compound **42** to examine the potency, solubility and stability on human and rat microsomes (Table 2). Removal of isopropyl to give the unsubstituted **54** decreased the potency to 1/10 (**42** vs **54**), and thus substitutions seem to be preferred for the potency. Ethyl was slightly more potent, and propyl was 3 times more potent than isopropyl (**42** vs **55**, **56**). Benzyl was equipotent to isopropyl (**42** vs **57**), which indicates a wider space around R^2. Reduced potency was noted with a relatively short polar group, e.g., acetyl directly connected to the nitrogen atom, and also CN and COOH with one carbon linkage (CH_2) (**60**, **58**, **66**). On the other hand, long polar groups (CN, OH, NHAc, COOH, $CONH_2$, CONHMe) at the end of chains longer than two carbons (CH_2CH_2, $CH_2CH_2CH_2$) all led to potent compounds (**59**, **61**, **62**, **63**, **64**, **65**, **67**, **68**, **70**). The fact that both lipophilic and hydrophilic groups were tolerated suggests that R^2 may exist in the solvent-exposed surface area of the protein. Among those investigated, the carboxylic acids (**67**, **68**) were slightly less potent than the other analogs, but they were more soluble and showed better stabilities in both human and rat microsomes.

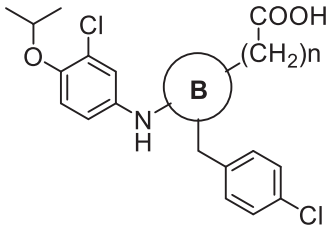
Interestingly, other center ring systems (B) were also tolerated (**20**, **26**, **30**, Table 3), and uracil was almost equipotent to triazine (**20** vs **72**). Also, even triazine **35** displayed modest potency in spite of the different orientation of the R^2 group.

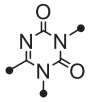
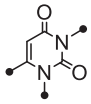
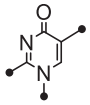
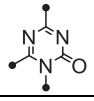
All tested ring analogs showed high oral absorbability (**71**, **72**, **20**, **26**), and triazine **72** with propanoic acid (n = 2, $-CH_2CH_2COOH$) at position 3 displayed the best potency and the highest stability in rat.

With triazine and uracil derivatives of high activity in hand,

Table 3

Effects of center ring systems (B).



Compound	B	n	IC_{50} (nM)	CLt (ml/min/kg) [b]	$t_{1/2}$ (hr) [b]	BA(%) [b]
71		1	20	1.7	2.1	71.2
72		2	13	0.5	6.0	43.2
20		2	34	2.1	2.2	61.5
26		1	152	8.3	1.8	79.9
30		1	219	NT	NT	NT
35		1	614	NT	NT	NT

CLt: total clearance; $t_{1/2}$: half-life; BA: oral bioavailability; NT: not tested.
[a]All compounds were administered at 0.5 mg/kg iv and 1.0 mg/kg po.
Compounds were administered as a mixture of three to five compounds.

optimization of the R^1–R^4 moieties was carried out, focusing on the polar R^2 groups, especially carboxylic acid analogs due to their better solubilities and stabilities (Fig. 3.).

Among those examined, compounds **73** and **74** were selected for further development because of their strong antagonist activity and well-balanced ADME profiles (Table 4). Compounds **73** and **74** showed better $P2X_3$ selectivity over $P2X_{2/3}$ than Ro-51.[17] Both **73** and **74** were more soluble in a neutral buffer JP2 (pH 6.8) than an acidic buffer JP1 (pH 1.2) probably due to their acidic nature attributed to the carboxylic moieties. Compound **73** and **74** did not inhibit five major species of CYP enzymes, which indicates their low risk of drug-drug interactions.

The selected compounds **73** and **74** showed good PK profiles and demonstrated a strong analgesic effect on oral administration against allodynia in the rat partial sciatic nerve ligation model of neuropathic pain. The analgesic activity of compounds **73** (ED_{50}: 4.1 mg/kg) and **74** (ED_{50}: 3.1 mg/kg) were more potent than that of Ro-51 (ED_{50} : >10 mg/kg).[18]

Unfortunately, clinical development of compounds **73** and **74** was suspended due to the predicted high dose in humans. Although not selected for clinical development, these compounds were evaluated from various aspects as tool compounds.

In conclusion, a new hit compound was identified by focusing on dioxotriazines in reexamination of the compounds obtained by HTS of the Shionogi compound library. The hit to lead SAR was performed by using parallel synthesis to find lead compound **1**. Replacement of the center ring part (B) did not cause loss of potency, and among them, triazine and uracil types showed high potency. The point of modification was the R2 substituent at position 3 on the triazine ring where the polar groups are expected to be effective for improvement of ADME and physical properties. As a result of lead optimization, compounds **73** and **74** were selected for their high $P2X_3$ selectivity, oral absorbability and strong analgesic efficacy.

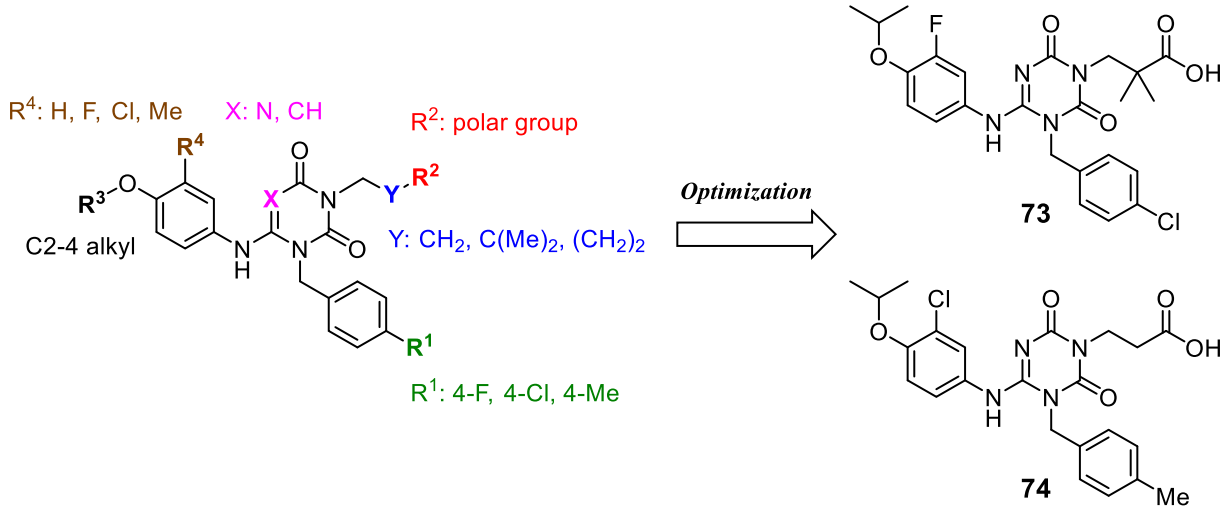


Fig. 3. Optimization of triazines and uracils High potent triazines and uracils were optimized on R^1, R^2, R^3, and R^4. Polar R^2 substituents were designed due to their good solubility and stability. **73** and **74** were nominated for further evaluation.

Table 4
Biological tests of selected compounds.

Compound	In vitro activity IC_{50} (nM)						Solubility[a] (μg/mL)		CYP inhibition IC_{50} (μM)				
	$P2X_3$	$P2X_{2/3}$	$P2X_1$	$P2X_2$	$P2X_4$	$P2X_7$	JP1	JP2	1A2	2C9	2C19	2D6	3A4
73	30.3	2366	all: >30000				< 0.1	6.0	all: >20				
74	16.1	2931	all: >30000				0.3	169	all: >20				
Ro-51	6.3	44.9	all: >10000				NT		NT				

Compound	Rat pharmacokinetics (iv, 1 mg/kg; po, 3 mg/kg)						Analgesic effects[b]	
	Clt (mL/min/kg)	$T_{1/2}$ (h)	AUC (po)(ng hr/mL)	Cmax (po)(ng/mL)	BA (%)	fu (%)	ED_{50} (mg/kg)	Emax % (mg/kg)
73	3.0	9.6	6990	1260	47.3	1.0	4.1	41% (10)
74	2.7	1.6	14,300	1610	71.8	0.9	3.1	40% (10)
Ro-51	42.0	1.1	369	118	30.3	7.4	>10	36% (100)

[a] JP1 (pH 1.2) measured in 1st Fluid for dissolution test and JP2 (pH 6.8) in 2nd Fluid for dissolution test in THE JAPANESE PHARMACOPOEIA.[19]
[b] Rat Seltzer model: Allodynia (von Frey).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2021.127833.

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