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Synthesis and SAR study of imidazoquinolines as a novel structural class of microsomal prostaglandin E_2 synthase-1 inhibitors

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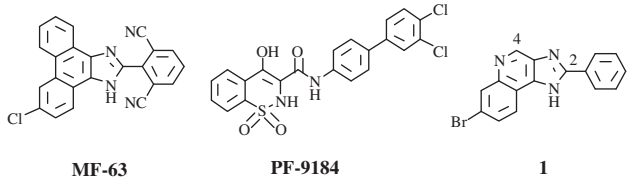
ABSTRACT

The imidazoquinoline derivative **1** was found as a novel mPGES-1 inhibitor. Optimization of **1** led to the identification of the 2-chlorophenyl group at the C(2)-position and the quinolone structure at the C(4)-position. Compound **33**, the most potent synthesized compound, showed excellent mPGES-1 inhibition (IC_{50} = 9.1 nM) with high selectivity (>1000-fold) over both COX-1 and COX-2.

Prostaglandin E_2 (PGE_2) is well known as an important lipid mediator with broad range of biological activities in various cells and tissues, and is biosynthesized by sequential conversion of arachidonic acid with various metabolic enzymes, such as cyclooxygenase-1 (COX-1), -2 (COX-2), and PGE synthases (PGES). Among these enzymes, PGES is a terminal enzyme in the biosynthesis of PGE_2 classified into three isoforms: microsomal PGES-1 (mPGES-1), -2 (mPGES-2), and cytosolic PGES (cPGES).[1] mPGES-1, which is functionally linked to COX-2, is inducible and responsible for the release of PGE_2 in response to inflammatory stimuli, such as IL-1β, TNF-α, and LPS.[2] Indeed, a previous study has shown that PGE_2 production by LPS is completely suppressed in peritoneal macrophages derived from mPGES-1 knockout mice.[3] This allowed the use of mPGES-1 knockout mice as models of various diseases, such as collagen induced arthritis, pain hypersensitivity, and neuropathic pain. The symptoms of such diseases were significantly relieved by suppression of PGE_2 production.[4a–c] As mPGES-1 knockout mice show increased prostacyclin (PGI_2) level in plasma with normal thromboxanes levels,[5] it is expected that cardiovascular system related side effects, such as thrombosis and myocardial infarction, observed with selective COX-2 inhibitors, would not manifest with mPGES-1 inhibitors. A recent study in human mPGES-1 knock-in mice has shown that a selective mPGES-1 inhibitor specifically reduced PGE_2 production in plasma without affecting that of other prostaglandins, such as TXB_2 and $PGF_1\alpha$.[6] Unlike COX-1/2 inhibitors, administration of selective mPGES-1 inhibitors resulted in no undesirable effects on mice gastrointestinal system.[6] Based on the information described above, it is believed that selective mPGES-1 inhibitors could be ideal agents for the treatment of inflammation, pain, and other PGE_2-related disorders.[7a–e]

To date, several selective mPGES-1 inhibitors have been reported. Among them, the phenanthreneimidazole derivatives were reported by Merck Frosst researchers and represented here by MF-63.[8a,8b] MF-63 inhibits mPGES-1 with an IC_{50} value in the subnanomolar range. Pfizer researchers reported the dioxobenzothiazinone derivative PF-9184,[8c] which inhibits mPGES-1 with an IC_{50} value of 16 nM (Fig. 1). However, no compound has reached clinical development. In our search for novel potent mPGES-1 inhibitors, we conducted a high-throughput screening (HTS) campaign[9a] of our chemical library and found the imidazoquinoline



MF-63 PF-9184 1

Figure 1. Structures of reported mPGES-1 inhibitors and compound **1**.

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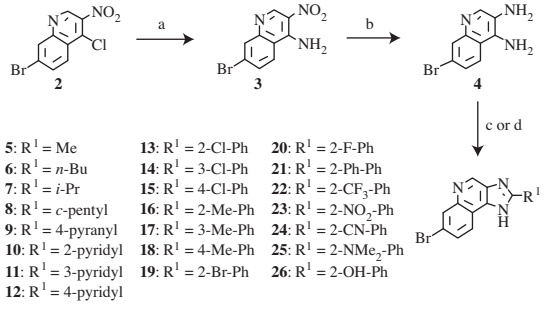
derivative **1** as a hit compound. Compound **1**, which has a simple structure favoring various substitutions, showed moderate inhibitory activity for mPGES-1 (60% at 10 μM). To improve the mPGES-1 inhibitory activity of **1**, we initiated a structure–activity relationship (SAR) study. Here, we report the synthesis and biological evaluation of a novel series of imidazoquinolines, in particular, chemical modification of the substituents at the C(2)- and the C(4)-positions of **1**.

Procedure for the preparation of compounds **5**–**26** having the C(2)-substituted imidazoquinoline ring system is shown in Scheme 1. Reaction of 7-bromo-4-chloro-3-nitroquinoline (**2**)[10] with 28% ammonia provided 4-amino-7-bromo-3-nitroquinoline (**3**). Reduction of **3** with iron powder in the presence of ammonium chloride provided 7-bromo-3,4-diaminoquinoline (**4**).

With the exception of **5** and **6**, the C(2)-substituted imidazoquinolines **7**–**26** were prepared by cyclization of **4** with appropriate aldehydes in the presence of sodium pyrosulfate, respectively. The methyl and the *n*-butyl derivatives **5** and **6** were prepared by heating of **4** with appropriate orthoformates in the presence of catalytic amount of *p*-toluenesulfonic acid, respectively. The tautomers of the synthesized imidazoquinoline derivatives in the imidazole moiety were not identified by the structural analysis. The synthetic pathway to the C(4)-substituted imidazoquinolines is shown in Scheme 2. Oxidation of **13** with *m*CPBA followed by chlorination of the N-oxide with phosphorous oxychloride afforded **27** in 63% yield. The C(4)-substituted imidazoquinolines **28**–**32** were prepared by reaction of **27** with appropriate alkoxides, respectively. Reaction of **27** with hydrochloric acid provided the corresponding the imidazoquinolone derivative **33**. The quinolone structure of **33** was established on the basis of the characteristic nuclear Overhauser effects (NOE; in DMSO), which were observed between the N-proton and the imidazoquinolone proton at the 6 position by NOESY experiments. We also observed carbonyl vibration at 1654 cm^{-1} by Fourier transform infrared spectrum, indicating the presence of the quinolone structure of **33** in the solid state.



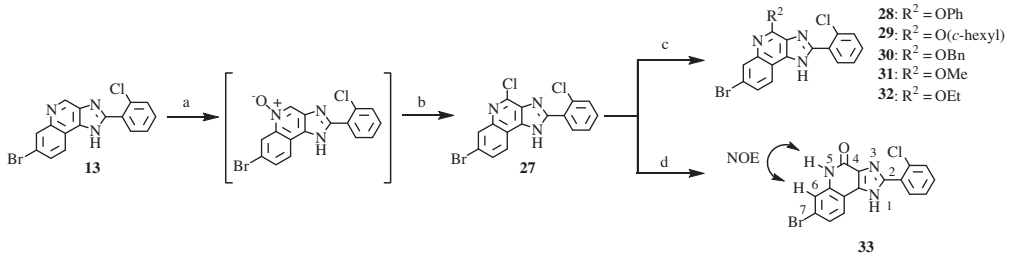
Scheme 1. Reagents and conditions: (a) 28% NH_3 aq, MeCN, 40 °C, 5 h, 87%; (b) Fe, NH_4Cl, EtOH/H_2O, reflux, 4 h, 86%; (c) R^1-$C(OEt)_3$, *p*-toluenesulfonic acid, NMP, 120 °C, 5 h, 50–56%; (d) R^1-CHO, $Na_2S_2O_7$, DMF, 140 °C, 4–8 h, 19–96%.

We initially investigated the effects of different substituents at the C(2)-position of **1** on mPGES-1 inhibitory activity (Table 1).[9b] Replacement of the phenyl group by several alkyl groups (**5**–**9**) resulted in a decrease in the inhibitory activity. However, among the synthesized compounds, those with cyclic alkyl group (**8** and **9**) showed slightly better inhibitory activity than those with straight or blanched alkyl group (**5**–**7**), suggesting that sterically large groups are preferable substituents at the C(2)-position. Compounds **10**–**12**, having 2-, 3-, and 4-pyridyl groups, respectively, showed decreased inhibitory activity (19%, 26%, and 22%, respectively, at 10 μM). These results indicate that a lipophilic aromatic group would be suitable at the C(2)-position of **1**.

To improve the inhibitory activity of **1** for mPGES-1, we investigated the effects of different substituents on the phenyl group (Table 2). Among the chlorine atom substituted compounds **13**–**15**, the *ortho*-chloro derivative **13** showed the most potent inhibitory activity for mPGES-1 compared with the *meta*-chloro and the *para*-chloro derivatives **14** and **15**. Compound **13** showed approximately 40-fold higher mPGES-1 inhibitory activity over the unsubstituted **1**. The same tendency was observed when the position of the electron-donating methyl group on the phenyl ring was changed from an *ortho*- to a *meta*- or a *para*-position (**16** vs **17** and **18**). These findings suggest that the substituent at the *ortho* position should be selected to cause steric repulsion between the phenyl group and the imidazoquinoline core. This steric repulsion can force the phenyl group to orientate at proper twist angle for potent inhibitory activity.

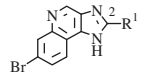
On the basis of these data, we performed a *ortho*-substitution study on the phenyl ring at the C(2)-position of the imidazoquinoline ring. As shown in Table 2, the bromine derivative **19** displayed a two-fold loss in the inhibitory activity compared with the chlorine derivative **13**. The fluorine derivative **20** showed great loss of the inhibitory activity (IC_{50} = 6970 nM). Furthermore, as shown by **21**, introduction of a bulky phenyl group also showed a 18-fold loss in the inhibitory activity over **13**. These results indicate that the *ortho*-substituents on the phenyl ring should be a moderate-sized substituent (e.g., the chlorine atom), which affects the above mentioned steric repulsion, to show a potent mPGES-1 inhibitory activity. The inhibitory activities of compounds having an electron-withdrawing group in the *ortho* position on the phenyl group (such as the trifluoromethyl, the nitro, and the cyano derivatives **22**–**24**) were not improved toward mPGES-1 response. The dimethylamino and the hydroxyl derivatives **25** and **26** showed significantly reduced inhibitory activity compared with the chlorine derivative **13**. These results suggest that the placement of a hydrophilic group on the phenyl group was not suitable to exert its mPGES-1 inhibitory activity.

To further improve the inhibitory activity for mPGES-1, we next focused on the substitution studies at the C(4)-position on the imidazoquinoline ring, keeping the 2-(2-chloro)phenyl group constant. As shown in Table 3, the phenanthreneimidazole derivative MF-63, used here as positive control, showed very potent inhibitory activity



Scheme 2. Reagents and conditions: (a) *m*CPBA, CH_2Cl_2, rt, 2 h; (b) $POCl_3$, reflux, 2 h, 63% in 2 steps; (c) R^2-Na or R^2-K, NMP or DMSO, 90–150 °C, 0.5–1 h, 33–97%; (d) cHCl, EtOH/H_2O, reflux, 8 h, 89%.

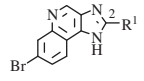
Table 1
Effects of substituents at the C(2)-position on mPGES-1 inhibitory activity



Compound	R^1	mPGES-1 % inhibition[a] at 10 μM
1	Ph	60
5	Me	27
6	*n*-Bu	22
7	*i*-Pr	13
8	*c*-Pentyl	39
9	4-Pyranyl	46
10	2-Pyridyl	19
11	3-Pyridyl	26
12	4-Pyridyl	22

[a] See Ref. 9b for details.

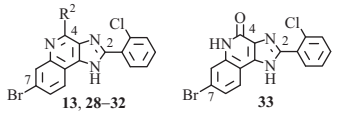
Table 2
Effects of substituents on the C(2)-phenyl group on mPGES-1 inhibitory activity



Compound	R^1	mPGES-1 IC_{50}[a] (nM)
1	Ph	9500
13	2-Cl-Ph	251
14	3-Cl-Ph	859
15	4-Cl-Ph	>10,000
16	2-Me-Ph	395
17	3-Me-Ph	1490
18	4-Me-Ph	>10,000
19	2-Br-Ph	506
20	2-F-Ph	6970
21	2-Ph-Ph	4620
22	2-CF_3-Ph	1000
23	2-NO_2-Ph	669
24	2-CN-Ph	687
25	2-NMe_2-Ph	6264
26	2-OH-Ph	4116

[a] See Ref. 9b for details.

Table 3
Effects of substituents at the C(4)-position on mPGES-1 inhibitory activity



13, **28**–**32** **33**

Compound	R^2	mPGES-1 IC_{50}[a] (nM)
MF-63	—	4.3
13	H	251
28	OPh	330
29	O(*c*-hexyl)	306
30	OBn	172
31	OMe	62
32	OEt	108
33	—	9.1

[a] See Ref. 9b for details.

for mPGES-1 (IC_{50} = 4.3 nM). Considering the structural similarity between MF-63 and **13**, we speculated that introduction of a hydrophobic bulky group at the C(4)-position of **13** would lead to improved inhibitory activity for mPGES-1. However, no improvement in mPGES-1 inhibitory activity was seen with the phenoxy, the cyclohexyloxy, and the benzyloxy derivatives **28**–**30**, which had IC_{50} values comparable to that of the unsubstituted **13**. These findings indicated that compounds having the imidazoquinoline scaffold would have different SAR from MF-63.

Next, we introduced several small substituents, such as a lower aliphatic alkoxy group and a hydroxyl group. The methoxy and the ethoxy derivatives **31** and **32** exhibited improved inhibitory activity compared with **13**. In particular, compound **31** led to a four-fold higher inhibitory activity for mPGES-1 over **13**. These findings indicate that a small alkoxy group is preferable for better inhibitory activity. Furthermore, we evaluated the inhibitory activity of the imidazoquinolone derivative **33**. Surprisingly, compound **33** showed significantly improved inhibitory activity, reaching 27-fold that of **13** and was almost equipotent to that of MF-63. Compound **33**,[11] the most potent synthesized compound in this study, was next evaluated for its mPGES-1 selectivity over COX-1 and COX-2, and exhibited less than 10% inhibition at 10 μM.[12] These findings indicate that compound **33** has over 1000-fold mPGES-1 selectivity over COX-1 and COX-2.

In summary, HTS screening of our chemical library resulted in the discovery of the imidazoquinoline derivative **1** as a novel mPGES-1 inhibitor. Optimization of **1** led to the identification of the 2-chlorophenyl group at the C(2)-position and the quinolone structure at the C(4)-position. Compound **33**, the most potent synthesized compound, showed excellent mPGES-1 inhibition (IC_{50} = 9.1 nM) with high selectivity (>1000-fold) over both COX-1 and COX-2. At present, we are now investigating the SAR studies of 7-substituted-imidazoquinolones. These studies will be reported in due course.

Acknowledgments

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9. (a) Takahashi, H.; Nagata, H.; Shiro, T.; Kase, H.; Tobe, M.; Shimonishi, M.; Ido, M. in preparation.; (b) Biological assay method for mPGES-1 inhibition: The test compound in DMSO (final 1%) was added to 15 mg/mL microsome in 100 mM Tris–HCl at pH 8.0, 5 mM EDTA, 500 mM phenol, 1 mM GSH, 10 mM Hematin. The microsome was prepared from mPGES-1 and COX-1 co-transfected HEK293 cells. Next, the mixture was pre-incubated at rt for 30 min. The reaction was started by the addition of 12.5 mM arachidonic acid dissolved in 0.1 N KOH. The reaction mixture was incubated at rt for 60 min. The reaction was stopped by addition of 1 N HCl. The reaction mixture was neutralized by 1 N NaOH and PGE_2 levels were measured by HTRF. The IC_{50} values were calculated by a logistic regression method.
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11. Compound **33**; mp 320–324 °C (dec); ^{1}H NMR (DMSO-d_6, 300 MHz) δ 13.90 (1H, s), 11.75 (1H, s), 8.02 (1H, d, J = 8.4 Hz), 7.79 (1H, , dd, J = 7.2, 1.8 Hz), 7.63–7.67 (2H, m), 7.49–7.59 (2H, m), 7.43 (1H, dd, J = 8.4, 1.8 Hz). ^{13}C NMR (DMSO-d_6, 75 MHz) δ 137.6, 132.1, 131.5, 130.2, 129.4, 127.4, 124.7, 123.6, 120.7, 118.2.; IR (ATR) ν 1654, 3392 cm^{-1}.; MS (ESI) m/z calcd for $C_{16}H_9BrClN_3O$ 375; found 376 (M+H); Anal. Calcd for $C_{16}H_9BrClN_3O{\cdot}0.25H_2O$; C, 50.69; H, 2.53; N, 11.08. Found: C, 50.59; H, 2.53; N, 11.01.
12. The inhibition assay for the COX-1 and -2 was performed by Ricerca Biosciences.