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Design of novel *N*-phenylnicotinamides as selective cyclooxygenase-1 inhibitors

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ABSTRACT

A series of *N*-phenylnicotinamides (**1**–**40**) were designed and evaluated in vitro for their COX inhibitory activities. Most of the synthesized compounds were proved to be potent and selective inhibitors of COX-1. Compound **28** showed the most potent COX-1 inhibitory activity (COX-1 IC_{50} = 0.68 ± 0.07 μM) and good selectivity (COX-2 IC_{50} >100 μM). This compound may be useful as a lead compound for superior COX-1 inhibitors. On the basis of the biological results, structure–activity relationships for the COX-1-inhibitory activities of the synthesized *N*-phenylnicotinamides were discussed concisely.

Inflammation is a major cause of pain and contributes to tissue injury and dysfunction, in particular for arthritis and inflammatory bowel syndrome.[1] Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, ibuprofen and indomethacin are widely used in the treatment of pain and inflammatory diseases.[2] The clinical efficacy of most NSAIDs is closely related to their inhibition of cyclooxygenases (COXs), which catalyze the bioconversion of arachidonic acid to prostaglandins (PGs).[3–5] Cyclooxygenase (COX) is a membrane-bound heme protein which exists in two distinct isoforms, a constitutive form (COX-1) and an inducible form (COX-2).[6,7] Recently, a novel COX-1 splice variant termed as COX-3 has been reported.[8]

The major side effect of NSAIDs, gastric damage on the stomach mucous membrane was thought to be due to their inhibition of COX-1.[9–11] Furthermore, it was thought that selective COX-2 inhibitors would be effective anti-inflammatory agents with reduced side effects.[12–14] However, very recently, rofecoxib, a COX-2-selective inhibitor, was withdrawn from the market because of possible association with an increased incidence of cardiovascular events, such as heart attack and stroke.[15]

Meanwhile, Langenbach et al. reported that COX-1 knockout mice did not spontaneously develop gastric lesions, as further evidence that inhibition of COX-1 alone is not sufficient to induce gastric damage.[16] The result was supported by Wallace et al. suggesting that NSAID-induced gastric damage is not solely caused by COX-1 inhibition.[17] Moreover, it has been suggested that COX-1 may play an important role in pain processing and sensitization of the spinal cord and gracile nucleus after surgery.[18] Therefore, COX-1-selective inhibitors are anticipated to be candidates as novel analgesic agents with reduced gastroenteric disturbance.

Only a few COX-1-selective inhibitors are currently available. These include mofezolac (**i**),[19] SC-560 (**ii**),[20] indomethacin (**iii**),[21] and FR122047 (**iv**)[22] (Fig. 1). Recently, Kakuta and coworkers

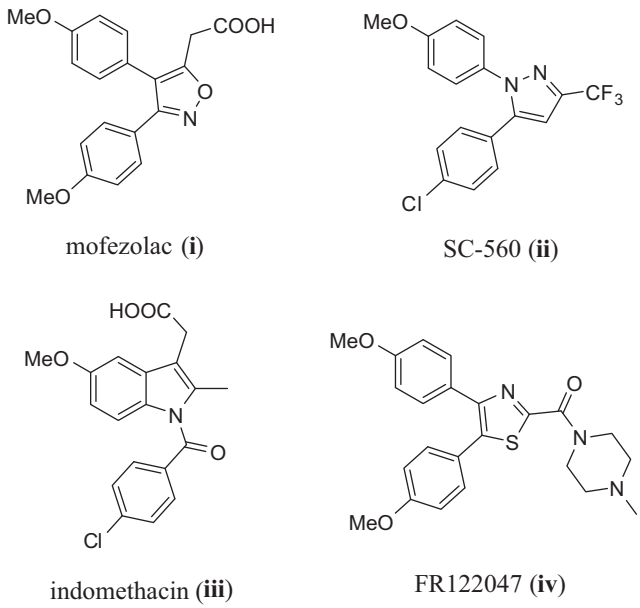

Figure 1. Chemical structures of representative COX-1 inhibitors.

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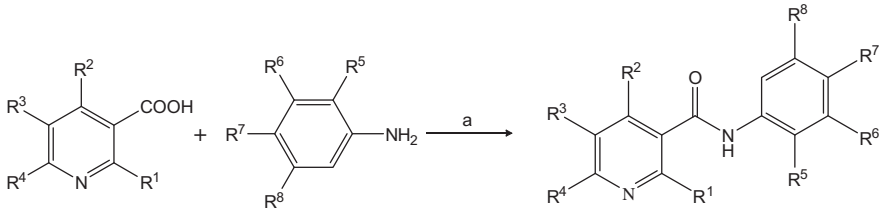
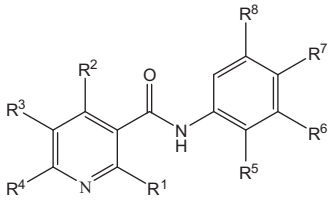


Scheme 1. General procedure for the synthesis of nicotinamides. Reagents and conditions: (a) EDC·HCl, CH_2Cl_2, reflux, 8–10 h.

designed a series of benzenesulfonanilide-type and benzamide-type compounds as potent COX-1-selective inhibitors.[23,24] Nicotinic acid derivatives were identified as therapeutic agents in several diseases, with the advantages of promoting digestive health and reducing gastrointestinal disorders.[25–27] Herein, we aimed to develop nicotinamide-type COX-1 inhibitors by combining nicotinic acid and anilines.

The synthesis of the title *N*-phenylnicotinamides followed the general reaction pathway outlined in Scheme 1. Compounds **1–40** were synthesized by coupling substituted anilines with equimolar quantities of substituted nicotinic acids, using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC·HCl) as condensing agent. The mixture was refluxed in anhydrous CH_2Cl_2 for 8–10 h.

Table 1
COX-inhibitory activity of compounds **1–41**



Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	% inhibition at 100 μM	
									COX-1	COX-2
1	OH	H	H	H	H	H	F	H	64	26
2	OH	H	H	H	H	H	Cl	H	60	19
3	OH	H	H	H	H	H	Br	H	47	12
4	OH	H	H	H	H	H	CH_3	H	28	10
5	OH	H	H	H	H	H	OCH_3	H	42	6
6	OH	H	H	H	H	Cl	H	Cl	35	7
7	H	H	Br	H	H	H	Cl	H	13	4
8	H	H	Br	H	H	H	Br	H	5	2
9	H	H	Br	H	H	H	OCH_3	H	0	0
10	H	H	Br	H	Cl	H	Cl	H	19	1
11	H	H	H	Cl	H	H	F	H	41	22
12	H	H	H	Cl	H	H	Cl	H	42	15
13	H	H	H	Cl	H	H	Br	H	18	24
14	H	H	H	Cl	H	H	CH_3	H	25	30
15	H	H	H	Cl	H	H	OCH_3	H	38	10
16	H	H	H	OH	H	H	F	H	78	35
17	H	H	H	OH	H	H	Cl	H	75	42
18	H	H	H	OH	H	H	Br	H	64	18
19	H	H	H	OH	H	H	CH_3	H	40	21
20	H	H	H	OH	H	H	OCH_3	H	53	6
21	H	H	H	CH_3	H	H	F	H	59	16
22	H	H	H	CH_3	H	H	Cl	H	45	22
23	H	H	H	CH_3	H	H	Br	H	35	14
24	H	H	H	CH_3	H	H	CH_3	H	10	0
25	H	H	H	CH_3	H	H	OCH_3	H	26	3
26	H	H	H	CH_3	Cl	H	Cl	H	38	7
27	H	H	H	CH_3	H	Cl	H	Cl	20	11
28	H	H	Cl	OH	H	H	F	H	90	44
29	H	H	Cl	OH	H	H	Cl	H	84	33
30	H	H	Cl	OH	H	H	Br	H	58	30
31	H	H	Cl	OH	H	H	CH_3	H	50	26
32	H	H	Cl	OH	H	H	OCH_3	H	64	37
33	H	H	Cl	OH	Cl	H	Cl	H	72	34
34	H	H	Cl	OH	H	Cl	H	Cl	63	22
35	H	H	Cl	Cl	H	H	F	H	45	13
36	H	H	Cl	Cl	H	H	Cl	H	42	18
37	H	H	Cl	Cl	H	H	Br	H	33	15
38	H	H	Cl	Cl	H	H	CH_3	H	37	7
39	H	H	Cl	Cl	Cl	H	Cl	H	40	9
40	H	H	Cl	Cl	H	Cl	H	Cl	34	14
Indomethacin									90	86

The in vitro biological activity of the compounds was assessed with a colorimetric COX (ovine) inhibitory screening assay kit (Cayman Chemical, catalog no. 760111) according to the supplier's protocol. Each experiment was initially performed at 100 μM of the test compound (final concentration). IC_{50} values were calculated for only potent compounds discovered in this assay system. Each experiment was performed at least three times, and the mean value was calculated. Table 1 shows the COX-inhibitory activities of the synthesized *N*-phenylnicotinamides at a concentration of 100 μM. Most of the synthesized compounds showed potent COX-1 inhibitory activities but weak to none COX-2 inhibitory activities.

Structure–activity relationships (SARs) were inferred from Table 1. In general, Compounds **1**–**6** and **16**–**34** with electronic-donating substituents (phenolic hydroxyl or methyl) on the pyridine ring showed more potent COX-1 inhibitory activities than compounds **7**–**15** and **35**–**40**, which only contains electronic-withdrawing halogen substituents (Cl or Br) on pyridine ring. The position of phenolic hydroxyl group on pyridine ring also influenced the activities of the *N*-phenylnicotinamides. Compounds **16**–**20** and **28**–**34** with *para*-phenolic hydroxyl group on pyridine ring were shown to be more active than compounds **1**–**6** with *ortho*-phenolic hydroxyl group on pyridine ring. It is worth mentioning that, compounds **28**–**34** with *meta*-chloro substituent and *para*-phenolic hydroxyl substituent on pyridine ring exhibited most potent COX-1 inhibitory activities.

Compounds **28**–**34** bearing the same substituents on pyridine ring exhibited distinct COX-1 inhibitory activities due to the difference of the substituents on the phenyl ring. The COX-1 inhibitory activities of compounds **28**–**32** with different *para*-substituents on phenyl ring increased in the following order: CH_3 (**31**) < Br (**30**) < OCH_3 (**32**) < Cl (**29**) < F (**28**). This result indicated that the *para* electronic-withdrawing halogen substituents on phenyl ring were benefit for the activity. Compound **33** with an *ortho*-chloro substituent and a *para*-chloro substituent on phenyl ring and compound **34** with two *meta*-chloro substituents on phenyl ring showed mild COX-1 inhibitory activities, which were less potent than compound **29**, which contained only one *para*-chloro substituent on phenyl ring. The similar rules were also found in the other series of compounds which contained the same substituents on pyridine ring but different substituents on phenyl ring.

IC_{50} values for potent compounds were calculated and listed in Table 2, which also indicted that these compounds showed potent COX-1-selective inhibitory activities. Among these compounds, compound **28** exhibited the most potent COX-1-selective inhibitory activity. The IC_{50} value of **28** (COX-1 IC_{50} = 0.68 ± 0.07 μM) was equivalent to the reference drug, indomethacin. The data in Table 2 also suggested that in the synthesized *N*-phenylnicotinamides, electronic-donating phenolic hydroxyl group on pyridine ring and electronic-withdrawing *para*-halogen substituents on phenyl ring were favorable for the COX-1-selective inhibitory activity.

Furthermore, to understand the binding mode in COX, a docking study was performed. The crystal structures of COX-1 (1PGF)[28] and COX-2 (6COX)[29] were obtained from the Protein Data Bank (http://www.rcsb.org). Molecular docking was performed with a focus on compound **28**, which showed the greatest COX-1-selective inhibitory activity. As shown in Figure 2, compound **28** binds to the catalytic site of COX-1. The amide group of compound **28** exhibited a hydrogen bond with Ser 530 in COX-1, supporting that compounds with *N*-phenylnicotinamide skeleton could be potent COX-1-selective inhibitors. In addition, the phenolic hydroxyl group of compound **28** exhibited a hydrogen bond with Val 344 in COX-1, supporting the importance of the phenolic hydroxyl group on these synthesized *N*-phenylnicotinamides. However, as shown in Figure 3, the molecular docking of compound **28** into COX-2 catalytic site showed no hydrogen bonds, which explains

Table 2
COX-inhibitory activity of some potent compounds

Compd	R^1	R^2	R^3	R^4	R^5	R^6	R^7	R^8	IC_{50} (μM)	
									COX-1	COX-2
1	OH	H	H	H	H	H	F	H	32 ± 3	>100
2	OH	H	H	H	H	H	Cl	H	28 ± 5	>100
16	H	H	H	OH	H	H	F	H	3.5 ± 0.6	>100
17	H	H	H	OH	H	H	Cl	H	9.2 ± 1.0	>100
18	H	H	H	OH	H	H	Br	H	30 ± 5	>100
20	H	H	H	OH	H	H	OCH_3	H	86 ± 15	>100
21	H	H	H	CH_3	H	H	F	H	70 ± 11	>100
28	H	H	Cl	OH	H	H	F	H	0.68 ± 0.07	>100
29	H	H	Cl	OH	H	H	Cl	H	2.7 ± 0.3	>100
30	H	H	Cl	OH	H	H	Br	H	74 ± 13	>100
31	H	H	Cl	OH	H	H	CH_3	H	95 ± 6	>100
32	H	H	Cl	OH	H	H	OCH_3	H	44 ± 10	>100
33	H	H	Cl	OH	Cl	H	Cl	H	16 ± 4	>100
34	H	H	Cl	OH	H	Cl	H	Cl	29 ± 6	>100
Indomethacin									0.15 ± 0.04	10 ± 2

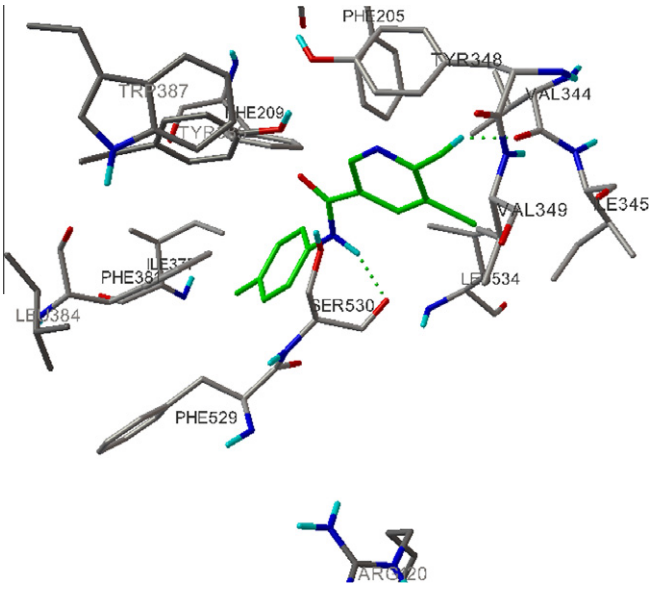


Figure 2. Potential binding mode of **28** in the COX-1 active site.

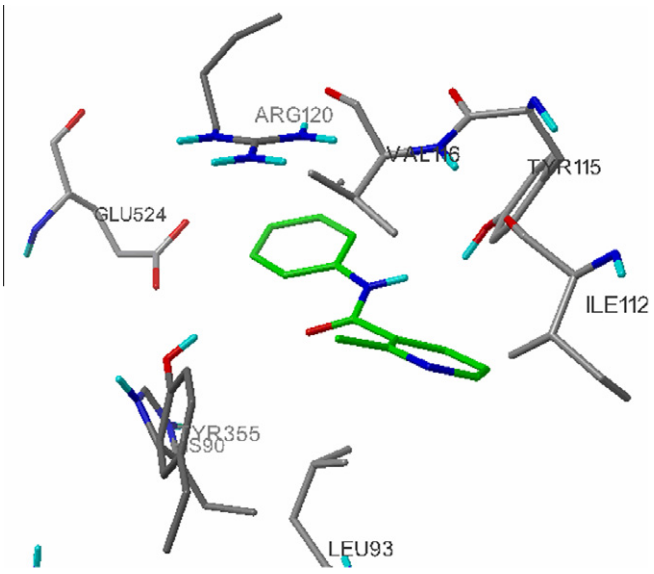


Figure 3. Potential binding mode of **28** in the COX-2 active site.

the observation of higher COX-1 inhibitory potency of this compound.

In summary, a series of *N*-phenylnicotinamides (**1**–**40**) were designed and evaluated in vitro for their COX inhibitory activities. Most of the synthesized compounds were proved to be potent and selective inhibitors of COX-1. Compound **28** showed the greatest COX-1 inhibitory activity (COX-1 IC_{50} = 0.68 ± 0.07 μM) and good selectivity (COX-2 IC_{50} >100 μM). This compound may be useful as a lead compound for superior COX-1 inhibitors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.11.062.

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