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Amide Derivatives of Meclofenamic Acid as Selective Cyclooxygenase-2 Inhibitors

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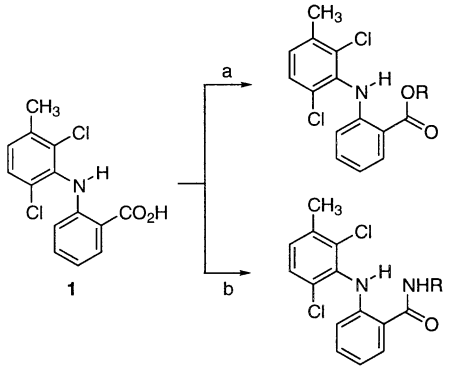
Abstract—This paper describes SAR studies involved in the transformation of the NSAID meclofenamic acid into potent and selective cyclooxygenase-2 (COX-2) inhibitors via neutralization of the carboxylate moiety in this nonselective COX inhibitor.

Prostaglandins, particularly PGE_2, are important mediators of inflammation and pain and also provide cytoprotection in the stomach and intestine. The rate-limiting step in prostaglandin biosynthesis is the conversion of arachidonic acid to prostaglandin H_2 (PGH_2), in a reaction catalyzed by cyclooxygenase (COX).[1] COX activity originates from two distinct and independently regulated enzymes, termed COX-1 and COX-2. COX-1 is the constitutive isozyme and plays a role in cytoprotection and in maintaining normal renal function. COX-2 is inducible and short-lived; its expression is stimulated in response to a proinflammatory insult. COX-2 plays a major role in prostaglandin biosynthesis in inflammatory cells and in the central nervous system.[2] Nonsteroidal antiinflammatory agents (NSAIDs) inhibit the two isozymes to different extents, and this feature accounts for their shared therapeutic properties and side effects.[3] The differential tissue distribution of COX-1 and COX-2 has provided a rationale for the development of selective COX-2 inhibitors as nonulcerogenic, antiinflammatory and analgesic agents.[4,5] Two selective COX-2 inhibitors, celecoxib and rofecoxib are currently marketed as anti-inflammatory agents and two others (valdecoxib and etoricoxib) await FDA approval.

We[6,7] and others[8] recently described a biochemically-based strategy for the facile conversion of carboxylate-containing NSAIDs. Thus, derivatization of the carboxylate moiety in indomethacin to esters and amides produces selective COX-2 inhibitors. The facile nature of this strategy is evident from the observation that a single chemical derivatization step (amidation or esterification) generates an impressive array of potent and highly selective COX-2 inhibitors with oral antiinflammatory activity. In this paper, we wish to disclose the details of the SAR studies on amide derivatives of the NSAID meclofenamic acid as COX-2-selective inhibitors.

The desired ester and amide derivatives of meclofenamic acid (**1**) were prepared as shown in Scheme 1. Treatment of **1** with the appropriate alcohol or amine in the presence of bis(2-oxo-3-oxazolidinyl)phosphonic chloride and triethylamine afforded the desired products in 60–75% yield.

IC_{50} values for the inhibition of purified human COX-2 or ovine COX-1 by test compounds (Table 1) were



Scheme 1. Reagents and conditions: BOP-Cl, Et_3N; (a) ROH; (b) RNH_2.

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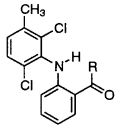
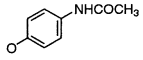
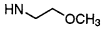
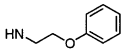
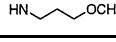
determined by the thin-layer chromatography (TLC) assay. Briefly, hematin-reconstituted COX-1 (44 nM) or COX-2 (66 nM) isozymes[9] in 100 mM Tris–HCl, pH 8.0 containing 500 μM phenol were incubated with several concentrations of test compounds at 25 °C for 20 min. The cyclooxygenase reaction was initiated by the addition of [1-^{14}C]-arachidonic acid (50 μM) at 37 °C and continued for 30 s. Control experiments in the absence of inhibitor indicated ~25–30% conversion of fatty acid substrate to products which was sufficient for assessing the inhibitory properties. Under these conditions, meclofenamic acid displayed nonselective time- and concentration-dependent inhibition of the COX isozymes (Table 1), whereas the acidic sulfonamide, NS-398[4] and the diarylheterocycle, SC-66299[10] displayed selective COX-2 inhibition [NS-398: IC_{50} (COX-1) > 66 μM, COX-2: IC_{50} = 0.12 μM; SC-66299: IC_{50} (COX-1) > 66 μM; IC_{50} (COX-2) = 0.050 μM]. Unlike indomethacin esters, alkyl or aryl esters of meclofenamic acid were either nonselective or COX-1-selective inhibitors as highlighted with the methyl- and the 4-acetamidophenyl ester derivatives **2** and **3**, respectively, whereas the primary amide derivative of meclofenamic acid (RCOOH→RCONH$_2$) displayed potent, but nonselective COX inhibition. Therefore, further SAR analysis was restricted to *N*-(substituted) amide derivatives only.

Extension of the alkyl chain length (methyl→octyl) in the alkylamide series significantly improved COX-2 inhibitory potency, while incorporation of terminal halogens in the alkyl substituent (e.g., 3-chloropropyl derivative **6**) improved COX-2 selectivity. Introduction of a terminal hydroxyl group in the alkyl substituent, however, led to significant losses in COX-2 potency and selectivity. COX-2 inhibitory potency and selectivity was regained by functionalization of the terminal hydroxyl group in the ethanol amide derivative **8** as shown with the methoxyethyl phenoxyethyl and methoxypropyl analogues **9**–**11**, respectively (Table 1). In terms of selectivity, the phenoxyethyl analogue **10** is the most selective COX-2 inhibitor in the meclofenamic acid series. Interestingly, the corresponding ester derivative of the phenoxyethyl amide **10** was inactive as a COX inhibitor.

Apart from the secondary alkylamide derivatives, some *O*-substituted hydroxamate analogues **12**–**15** also displayed selective COX-2 inhibition (Table 2). Incorporation of a 4-nitro group in the phenyl ring of **14** generated **15**, which demonstrated the most potent and selective COX-2 inhibitory properties in the series. Replacement of the oxygen atom in the hydroxylamide moiety in **14** with a methylene (**16**) or a –NH (**17**) group led to dramatic losses in COX-2 potency and selectivity. Previous studies by the Parke–Davis group have indi-

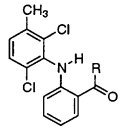
Table 1. IC_{50} values for COX-1 and -2 inhibition by alkyl ester and amide derivatives of meclofenamic acid

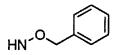
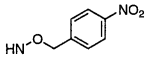
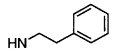
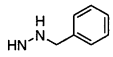


Compd	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
1	OH	0.05	0.04	0.8
2	OCH_3	17.0	4.0	0.2
3		> 20	> 20	—
4	$NHCH_3$	> 2.0[c]	> 2.0[d]	—
5	$NH(CH_2)_7CH_3$	0.05	0.06	1.3
6		0.06	2.4	40
7		0.07	2.0	28
8		0.6	1.0	1.7
9		0.14	3.0	21
10		0.15	66	440
11		0.25	11	44

[a]Values are average from two separate experiments.
[b]IC_{50} COX-1/IC_{50} COX-2.
[c]35% inhibition of COX-2 at this concentration.
[d]26% inhibition of COX-1 at this concentration.

Table 2. IC_{50} values for COX-1 and -2 inhibition by hydroxamate derivatives of meclofenamic acid



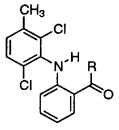
Compd	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
12	$NHOCH_3$	0.5	55	110
13	$NHOC(CH_3)_3$	8.0	66	8.0
14		1.0	66	66
15		0.2	66	300
16		4.5	4.0	0.9
17		5.0	6.3	1.3

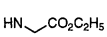
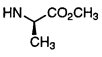
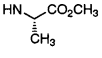
[a]Values are average from two separate experiments.
[b]IC_{50} COX-1/IC_{50} COX-2.

cated the dual COX and 5-lipoxygenase inhibitory properties of hydroxamate derivatives of meclofenamic acid such as **12**.[11] Furthermore, **12** demonstrated nonulcerogenic, antiinflammatory properties in animal models. Our results suggest that the nonulcerogenic, antiinflammatory properties of **12** are due to the selective inhibition of COX-2. The potency of hydroxamate derivatives **12**–**15** as 5-lipoxygenase inhibitors remains to be determined.

SAR analysis on the selective COX-2 inhibitory profile of meclofenamic acid-amino acid conjugates **18**–**24** was also undertaken (Table 3). Esterification of the carboxylate moiety in the amino acid portion of these conjugates resulted in an overall increase in COX-2 potency and selectivity, whereas conjugates with a free carboxylic acid group[12] were less potent and selective as COX-2 inhibitors. Comparison of the COX-2 selectivity ratios for the phenoxyethyl analogue **10** and the aminoacid conjugates **18** and **19** suggests some SAR trends for selective COX-2 inhibition by meclofenamic acid amides. For instance, bulky alkyloxy (ethyl vs methyl) (**19**) or aryloxy (**10**) substituents seem to be detrimental for COX-1 inhibition. Whether the COX-2 selectivity ratio can be further increased by incorporating functionalities in the aryl ring in **10** remains to be determined.

Table 3. IC_{50} values for COX-1 and -2 inhibition by amino acid conjugates of meclofenamic acid



Compd	R	IC_{50} (μM)[a]		Selectivity[b]
		COX-2	COX-1	
18		0.07	1.2	17
19		0.2	4.0	20
20		0.4	0.3	0.7
21		0.8	2.6	3.2
22		6.0	33	5.5
23		2.7	6.0	3.2
24		8.0	>33	>4.0

[a]Values are average from two separate experiments.
[b]IC_{50} COX-1/IC_{50} COX-2.

Simple aromatic amide derivatives of meclofenamic acid including 4-fluorophenyl-, 4-methoxyphenyl-, and 3-pyridyl amides also were included in the study, but these compounds did not display significant inhibition of the COX isozymes when tested at 30 μM. Examples of tertiary amide derivatives such as the piperidinyl- and the morpholino amide derivatives were also evaluated for selective COX-2 inhibitory properties. Although the piperidinyl amide displayed some COX-2 selective inhibition [IC_{50} (COX-2) = 1.0 μM; IC_{50} (COX-1) = 20 μM], the morpholino amide derivatives was inactive against the COX isozymes at the concentration range tested (20 μM). Additional examples are needed to fully characterize the SAR around tertiary amide derivatives of meclofenamic acid.

The ability of meclofenamic acid amides to inhibit COX-2 in intact cells was assayed in RAW264.7 macrophages in which COX-2 activity was induced by pathologic stimuli. The macrophages were exposed to lipopolysaccharide and γ-interferon to induce COX-2 and then treated with several concentrations of the phenoxyethyl amide and the 4-nitrobenzylhydroxamate derivatives **10** and **15**, respectively. The IC_{50} values for inhibition of prostaglandin D_2 (PGD_2) by **10** and **15** were 1.8 and 0.6 μM, respectively. The results from these studies indicate that meclofenamic acid amides, in addition to inhibiting purified COX-2, are also good inhibitors of COX-2 activity in intact inflammatory cells.

The present study extends our carboxylate derivatization strategy to include meclofenamic acid as a representative example from the fenamate class of NSAIDs. Unlike the indomethacin series, wherein both esters and amides were potent and selective COX-2 inhibitors, preliminary SAR studies on neutral meclofenamic acid analogues suggest that only the amide derivatives are capable of COX-2-selective inhibition. This may be due to subtle differences in the binding of the ester and amide derivatives of meclofenamic acid in the COX-2 active site.

Site-directed mutagenesis studies have also provided useful insights into the molecular basis for COX-2 inhibition by meclofenamic acid amides. As observed with the indomethacin ester and amide derivatives,[6,7] a representative meclofenamic acid amide (**10**) inhibited the Arg120Ala murine COX-2 mutant with potency comparable to wild-type enzyme [IC_{50} (wt) = 0.4 μM; IC_{50} (Arg120Ala) = 0.55 μM]. These results are consistent with our prediction that ester and amide derivatives of NSAIDs do not require the positively charged Arg120 for selective COX-2 inhibition.[6] Both meclofenamic acid and amide **10** were unable to inhibit the Tyr355Phe COX-2 mutant, suggesting that H-bonding of the carboxylate or the amide linkage with the hydroxyl group in Tyr355 is critical for COX-2 inhibition. Regions of the COX-2 protein that account for the selectivity of celecoxib (or rofecoxib),[13] proved unimportant for selective COX-2 inhibition by **10**, which was able to inhibit the Val523Ile mutant at comparable potency to wild-type enzyme. Since Tyr355, which is important for COX-2 inhibition by meclofenamic acid amides, is conserved within the two COX isozymes,

COX-2 selectivity must arise from the interactions of the individual amide substituent with residues below the constriction formed by Arg120 and Tyr355 at the mouth of the COX-2 active site. An interesting finding in these studies was the inhibition of the Arg120Ala but not the Tyr355Phe COX-2 mutants by meclofenamic acid [IC_{50} (wt) = 0.2 μM; IC_{50} (Arg120Ala) = 0.4 μM]. In contrast, indomethacin was unable to inhibit either mutant.[6] Therefore, the statement that all carboxylic acid-containing NSAIDs require Arg120 for COX inhibition[14] must be viewed with caution, as in the present study, COX-2 inhibition by meclofenamic acid seems dependent on Tyr355 and not Arg120.

Although several meclofenamic acid amide derivatives were potent and selective COX-2 inhibitors, further optimization is necessary to increase COX-2 selectivity of the present compounds. Since these derivatives are generated in a single step from the parent NSAID, combinatorial chemistry approaches may provide an easier access to more potent and selective meclofenamic acid analogues.

Acknowledgements

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