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Evaluation of glycolamide esters of indomethacin as potential cyclooxygenase-2 (COX-2) inhibitors☆

Smriti Khanna,[a] Manjula Madan,[a] Akhila Vangoori,[b] Rahul Banerjee,[a] Ram Thaimattam,[a] S. K. Jafar Sadik Basha,[b] Mullangi Ramesh,[b] Seshagiri Rao Casturi[b] and Manojit Pal[a,*]

[a]*Department of Chemistry, Discovery Research, Dr. Reddy's Laboratories Ltd, Bollaram Road, Miyapur, Hyderabad 500049, India*
[b]*Department of Biology, Discovery Research, Dr. Reddy's Laboratories Ltd, Bollaram Road, Miyapur, Hyderabad 500049, India*

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Abstract—A number of novel indomethacin glycolamide esters were synthesized and tested for their cyclooxygenase (COX-1 and COX-2) inhibition properties in vitro. Many of these compounds proved to be selective COX-2 inhibitors, and subtle structural changes in the substituents on the glycolamide ester moiety altered the inhibitory properties as well as potencies significantly. Their in vitro data were rationalized through molecular modeling studies. Few of them displayed anti-inflammatory activity in vivo. Compound **32**, [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid 2-morpholin-4-yl-2-oxo ethyl ester, was identified as a promising compound in this class and its good anti-inflammatory activity was demonstrated in the in vivo model.
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1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) are useful for the treatment of pain and inflammation. They reduce the pain and swelling of joints by inhibiting prostaglandin synthesis via blocking the cyclooxygenation of arachidonic acid (AA) to prostaglandin G_2 (PGG_2) and their use suffers from undesirable gastric side effects, for example, mucosal damage and bleeding.[1] Since NSAIDs directly target cyclooxygenases (COXs),[2] the discovery of second and inducible isozyme (COX-2, responsible for inflammatory effects) in addition to constitutive isozyme (COX-1, responsible for cytoprotective effects) has opened the possibility of developing selective COX-2 inhibitors for therapeutic use as effective as NSAID without the gastric side effects. Accordingly, three COX-2 selective inhibitors that belong to 1,2-diarylheterocyclic class have been marketed in the US, for example, celecoxib,[3] rofecoxib,[4] and valdecoxib[5] (see structures **I**–**III** in Chart 1). Despite their initial commercial success, rofecoxib (Vioxx) has been withdrawn[6] recently because of evidence of increased cardiovascular risk in select, high-dose patients. Celecoxib, however, has not shown similar adverse cardiovascular effects.[7] While celecoxib and valdecoxib are shown to be effective and comparable to conventional NSAIDs in terms of analgesic, antipyretic, and anti-inflammatory activity, these sulfonamide-containing inhibitors can also inhibit carbonic anhydrase II[8] thereby raising concerns about enzyme specificity. Thus, development of alternative COX-2 inhibitors keeping a slight effect on COX-1 at therapeutic dosage is desirable.

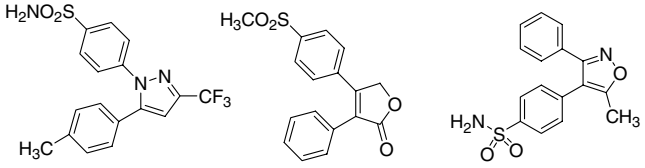

Chart 1.

Several strategies have been reported on the modification of the well-known nonselective agents for the design and development of novel COX-2 inhibitors. These include lengthening or derivatization of the carboxylic

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* Corresponding author. Tel.: +91 40 23045439; fax: +91 40 23045438/23045007; e-mail: manojitpal@drreddys.com

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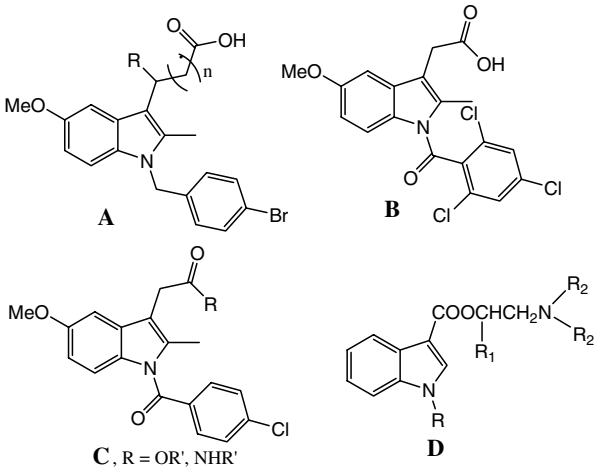


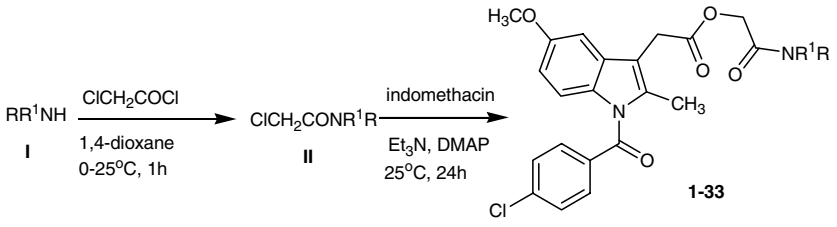
Figure 1. Structures of some indomethacin derivatives as COX-2 inhibitors.

side chain of indomethacin[9a,9b] (**A**–**D**, Fig. 1), development of 5-methylsulfonyl derivatives of indole-2-carboxylic acids (structurally related to indomethacin),[9c] and modification of the basic framework of zomepirac or flurbiprofen.[9d,e] The chemical structures of these COX-2 inhibitors including lumiracoxib clearly indicate that the basic framework of diarylheterocycles is not the exclusive prerequisite for COX-2 inhibition. This was supported by the recent report on the development of novel tetrahydro-2*H*-isoindoles as COX-2 inhibitor where 1,2-disubstitution by two aryl groups on a central core was missing.[9f] In an earlier effort on the development of COX-2 inhibitor from nonselective NSAID, Black and co-workers reported synthesis of a series of COX-2 inhibitors based on indomethacin[10] (**B**, Fig. 1). In this approach, the size of the indomethacin nucleus was increased by replacing the 4-chlorobenzoyl group by a bulky group to produce a compound that would still fit into COX-2 active site but not in COX-1 considering the hypothesis that COX-2 enzyme might have a larger active site than COX-1.[11] Recently Kalgutkar and co-workers described a general, biochemical-based strategy for the identification of COX-2 selective inhibitors from carboxylic acid NSAIDs,[12] for example, indomethacin[8b] (**C**, Fig. 1). It was observed that large alkyl, aryl, aralkyl and heterocyclic esters or amides of indomethacin showed high potency and selectivity. Further continuation of their study led to the identification of indomethacin alkanolamides as enantiospecific, selective COX-2 inhibitors.[13] More recently, a series of N-substituted indole-2-carboxylic acid esters have been studied as possible COX-2 selective inhibitors[14] (**D**, Fig. 1). These recent findings and our interest in the development of novel COX-2 inhibitors[15] as well as in the synthesis of indoles[16] prompted us to design potent and selective inhibitors of COX-2 based on indomethacin.

The crystal structures of indomethacin derivatives bound to COX enzymes wherein the carboxylic group of the ligand is oriented toward the narrow cross-section of a cone-shaped solvent accessible channel suggest that the esters of indomethacin can also be accommodated inside the active sites. This is also evident from the SAR studies on esters or amides of indomethacin reported by Kalgutkar and co-workers.[9b] Therefore, our strategy to develop new COX-2 inhibitors was based on derivatization of the carboxylic side chain of indomethacin. In the course of these investigations, we have synthesized and evaluated a number of novel indomethacin derivatives possessing substituted glycolamide ester ($-CO_2CH_2CONRR^1$) in place of acid side chain for cyclooxygenase activities. We felt that substituted glycolamide ester moiety might be worth exploring, as it is capable of forming additional hydrogen bonds directly or indirectly inside the solvent accessible channel. The results indicate that indeed many of these derivatives inhibited COX-2 selectively and subtle structural changes in the substituents of the glycolamide ester can alter the inhibitory properties as well as potencies significantly. The present class of indomethacin derivatives therefore represents a new variety of COX-2 inhibitors.

2. Chemistry

The synthesis of target compounds, that is, indomethacin glycolamide ester (**1**–**33**) is shown in Scheme 1. Easy access to these compounds was made possible by using a two-step process, that is, (a) preparation of 2-chloro N-substituted acetamide[17] followed by (b) the reaction of indomethacin with the resulting amide.[18] Thus, chloroacetyl chloride reacted with appropriate amine smoothly in 1,4-dioxane at 0–25 °C in the absence of a base to afford the desired acetamide derivatives in good yields. The second step, that is, reaction of the resulting 2-chloro N-substituted acetamide with indomethacin was carried out successfully in the presence of catalytic amount of *N*,*N*-dimethyl-4-aminopyridine using triethylamine as a base. The structure of all the compounds synthesized was confirmed by spectroscopic methods. In the IR spectra of all compounds, carbonyl-stretching bands belonging to ester and amide were seen at 1740 and 1670 cm^{-1}. In the ^1H NMR spectra, methylene protons of $-COOCH_2CO-$ group appeared at δ 4.5–4.8 ppm.



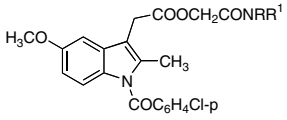
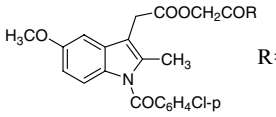
Scheme 1. Synthesis of indomethacin derivatives **1**–**33**.

3. Results and discussions

All the new compounds synthesized were tested in vitro initially at 10 μM for selectivity and potency against human COX-2 (expressed in *sf9* insect cells using baculovirus) and COX-1 (ram seminal vesicles) enzymes. On the basis of their in vitro efficacy, selected compounds were tested at 1.0 μM concentration as well. The IC_{50} values for COX-1 and COX-2 were determined[19] for selected/promising compounds. Celecoxib and indomethacin were used as reference compounds for the in vitro assay. Eight compounds were tested in vivo for anti-inflammatory activity at 30 mg/kg dose in the carrageenan-induced rat paw edema model.[20]

Biological evaluation of the title compounds showed that the conversion of the free acid group of non-selective inhibitor indomethacin to the N-substituted glycolamide ester ($-CO_2CH_2CONRR^1$) gives rise to compounds possessing varying degrees of COX-2 selectivity (see Table 1). Initially we examined the effect of various aryl and heteroaryl groups attached to the nitrogen of the glycolamide ester side chain, that is, R = H, R^1 = aryl or heteroaryl. Among the fluorinated aryl derivatives (**1**–**5**) compound

Table 1. In vitro and in vivo data for indomethacin derivatives

Compound			In vitro % inhibition at 10 μM[a]		In vivo % inhibition at 30 mg/kg (rat paw edema)[b]
	R^1	R	COX-1	COX-2	
1	3-Fluorophenyl	H	20	98, 63 (1)	56 ± 3
2	2-Fluorophenyl	H	0	47	14 ± 5
3	4-Fluorophenyl	H	38	51	80 ± 2
4	2,4-Difluorophenyl	H	0	86, 56 (1)	68 ± 4
5	2,3,4-Trifluorophenyl	H	20	71	19 ± 2
6	2-Chlorophenyl	H	0	100, 53 (1)	52 ± 3
7	4-Chlorophenyl	H	0	54	22 ± 2
8	2,6-Dichlorophenyl	H	0	51	n.d.
9	3-Chloro-4-fluorophenyl	H	0	98, 60 (1)	50 ± 4
10	2-Bromophenyl	H	0	24	n.d.
11	3-Bromophenyl	H	0	27	n.d.
12	4-Bromophenyl	H	11	29	19 ± 3
13	4-Bromo-2-methylphenyl	H	15	33	11 ± 2
14	2-Methyl-4-iodophenyl	H	0	4	39 ± 3
15	2,3-Dimethylphenyl	H	0	39	10 ± 3
16	3-Methoxyphenyl	H	42	55	26 ± 2
17	3,4-Dimethoxyphenyl	H	23	51	22 ± 1
18	Benzo[*d*][1,3]dioxol-5-yl	H	15	44	62 ± 3
19	2-Nitrophenyl	H	22	32	40 ± 5
20	3-Nitrophenyl	H	19	35	n.d.
21	2-Pyrimidinyl	H	23	54	21 ± 2
22	1,3-Thiazol-2-yl	H	24	47	n.d.
23	4-Methyl-1,3-thiazol-2-yl	H	20	74	n.d.
24	Benzo[*d*][1,3]thiazol-2-yl	H	32	80	n.d.
25	6-Methoxybenzo[*d*][1,3]thiazol-2-yl	H	0	69	n.d.
26	2-Methoxycarbonyl-3-thienyl	H	9	74	n.d.
27	5-Methyl-3-isoxazolyl	H	23	74	26 ± 3
28	*N*1-(5-carbamoyl-1-methyl-3-propyl-1*H*-4-pyrazolyl)-	H	0	86, 55 (1)	n.d.
29	Benzyl	H	18	79	40 ± 3
30	4-Methoxybenzyl	H	0	84	17 ± 1
31	Octyl	H	0	78, 48 (1)	59 ± 4
	 R=				
32	*N*-Morpholinyl		0, 0 (1)	93, 53 (1)	88 ± 3
33	*N*-Thiomorpholinyl		0	90, 55 (1)	44 ± 4
	Indomethacin		100	97	
	Celecoxib		0	100, 98 (1)	49 ± 2

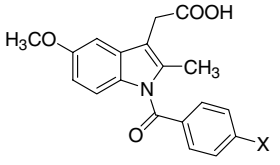
n.d., not determined.

[a] Human COX-2 (expressed in *sf9* insect cells using baculovirus) and COX-1 (ram seminal vesicles) enzyme. Figures in the brackets indicate concentration in micro molar. The result is the average of at least three determinations, and the deviation from the mean is <10% of the average value.

[b] The carrageenan-induced rat paw edema assay was carried out using six animals (male Wistar rats)/group following oral dose of the test compound.

4 possessing 2,4-difluorophenyl group showed good selectivity in COX-2 inhibition over COX-1. However, replacement of 2,4-difluorophenyl by a 2-chlorophenyl group increased the selectivity and potency further against COX-2 (compound **6**). Shifting of chloro or presence of additional chloro decreased the potency (compounds **7** and **8**). Presence of both chloro and fluoro on the benzene ring was found to be beneficial (compound **9**). Contrary to chloro and fluoro, other halogens such as bromo or iodo or mild electron-donating methyl groups in general were found to be less attractive (compounds **10**–**15** did not inhibit COX-2 significantly). The presence of a strong electron-donating group such as alkoxy (compounds **16**–**18**) or a strong electron-withdrawing group such as nitro (compounds **19**–**20**) on the benzene ring favored neither COX-2 selectivity nor potency. We then focused on replacing the aryl groups by heteroaryl moieties. A variety of heteroaryl moieties were examined and all of them induced moderate to good COX-2 selectivity (compounds **21**–**28**). The best compound however was found to be **28** which possessed *N*1-(5-carbamoyl-1-methyl-3-propyl-1*H*-4-pyrazolyl)- as a heterocycle in the side chain. We also examined the effect of alkyl and alkylaryl moieties in place of aryl/heteroaryl groups and the results were found to be encouraging (compounds **29**–**31**). Remarkably, selectivity and potency were observed when the cyclic amine such as morpholine or thiomorpholine moiety was introduced in place of primary amine in the glycolamide ester side chain (compounds **32**–**33**).

Indomethacin is a non-selective inhibitor of COX isozymes. The crystal structures of COX enzymes (4COX.pdb and 1PGG.pdb)[21] show that the binding poses of indomethacin derivatives are distinct in the two isoforms because of the structural differences in the active sites, in particular, the amino acid residue at position 523. Val at this position in COX-2 is replaced with Ile in COX-1. The smaller size of Val at 523-position in COX-2 leaves a side pocket, while Ile523 restricts access to the side pocket in COX-1. As a result, indomethacin moiety is flipped by about 180° (in 1PGG.pdb) such that the phenyl and carboxylate groups occupy similar environments in both isoforms (4COX.pdb and 1PGG.pdb). However, the orientation of the carboxylate group is significantly different in the two isoforms. In spite of these differences, the acid group, which is oriented toward the solvent-accessible channel, forms attractive interactions with Arg120 and Tyr355 in both isoforms. In the alternative binding pose, the orientation of iodo analogue of indomethacin (see structure **V** in Chart 2) in the active site of COX-1 (1PGF.pdb) is such that the acid group is almost buried and thus not exposed to the solvent-accessible channel while retaining crucial interactions with Arg120. Docking studies were carried out in order to understand the basis for COX-2 selectivity of compounds **6** and **32** using FlexX. The simulated binding poses of these molecules are similar to that of indomethacin inside the active site of the COX-2 crystal structure. However, FlexX failed to dock these molecules in the COX-1 active site. Hence, molecules **6** and **32** were manually positioned inside the active site of COX-1 such that the binding pose of each resembled that of iodoindomethacin in the COX-1.



IV; Indomethacin (X = Cl)

V; Iodoindomethacin (X = I)

Chart 2.

Molecules **6** and **32** show hydrogen bonding interactions with Ser530 in the active site of COX-2 (see Fig. 2 and Table 2). In both cases, the ester group is positioned in the solvent-accessible channel. In addition, the carbonyl O-atom of the ester group (of both **6** and **32**) is involved in bifurcated hydrogen bonding interactions with Arg120. Furthermore, molecule **6** shows additional hydrogen bonding interactions with Tyr355 and Lys83 involving the glycolamide oxygen and the morpholine

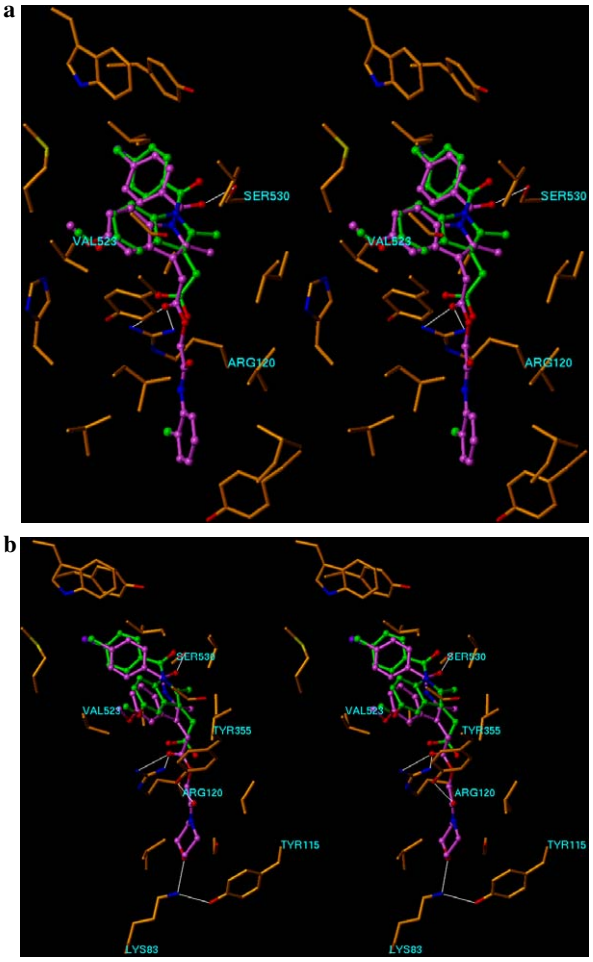
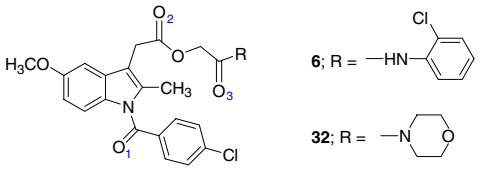


Figure 2. Stereoview of (a) **6** and (b) **32** in the active site of COX-2 (4COX.pdb). Molecules **6** and **32** are shown in pink C-atoms. The crystal structure conformation of indomethacin (green C-atoms, 4COX.pdb) is superimposed for reference. Residues within 4 Å radius around the ligand are shown in orange sticks, and hydrogen atoms are not shown for clarity. Hydrogen bonds are shown in white lines.

Table 2. Hydrogen bond geometries of molecules **6** and **32** inside the COX-2 active site



Compound	Functional group	Amino acid residue in the active site	Distance X–Y (Å) for X–H···Y	Angle X–H–Y (°)
6	C=O(1)	Ser530	2.63	160.88
	C=O(2)	Arg120	2.40	166.60
	C=O(3)	Arg120	3.01	125.70
32	C=O(1)	Ser530	2.70	161.61
	C=O(2)	Arg120	2.70	130.13
	C=O(2)	Arg120	2.40	153.58
	C=O(3)	Tyr355	2.53	166.83
	Morpholine	Lys83	2.65	167.25

group, respectively. However, in case of COX-1 docking experiments it indicated that there is little room to accommodate the ester group (1PGF.pdb) or the ester group would have steric clashes with Tyr355 and/or Arg120 (1PGG.pdb). In summary, molecules **6** and **32** fit comfortably inside the active site of COX-2, while showing steric clashes in COX-1. A closer analysis indicates that these differences are due to differences in the orientation of the indomethacin/ester group inside the COX active sites. These observations suggest that esters of indomethacin in general bind poorly to the COX-1 active site.

On the basis of in vitro data, few compounds were selected for further studies. The IC_{50} values for compounds **1**, **4**, **6**, **9**, **28**, and **31**–**33** are shown in Table 3 and all of them are found to be potent and selective inhibitors of COX-2. Some of them are either comparable or superior to celecoxib in terms of selectivity index (e.g., SI ~220 for **1** or 225 for **4** or 273 for **6** vs 219 for celecoxib). Notably, calculated log*p* values for all the eight compounds were higher than those of indomethacin and celecoxib except for **28** and **32** (Table 3), which contain hydrophilic groups. Compound **32** was better than **33** and comparable to indomethacin as well as celecoxib in terms of calculated log*p* value. Pharmacological studies were carried out to assess the in vivo anti-inflammatory activity of some of these compounds. The anti-inflammatory activity of these compounds was tested in the standard rat model of inflammation and compared with celecoxib. Encouragingly, a number of compounds showed fair to good inhibition in carrageenan-induced rat paw edema assay when dosed orally at 30 mg/kg (Table 1). Compounds **1**, **4**, **6**, **9**, **18**, **31**, and **32** showed more than 50% inhibition that was either comparable or better than celecoxib's 49% inhibition in the same assay. Maximum inhibition of 88% was achieved with the *N*-morpholino analogue **32**. The ED_{30} and/or ED_{50} of compounds **1**, **4**, **6**, **31**, and **32** are shown in Table 3. N,N-disubstituted glycolamide esters have previously been reported as potentially useful biolabile prodrug type for carboxylic acids.[18] Indeed, a quite rapid rate of hydrolysis of *N*,*N*-diethyl glycolamide ester of indomethacin was observed in 80% human plasma. However, the ease of plasma-catalyzed hydrolysis was altered with the change of substituents on amide nitrogen atom[22a] (e.g., R and R^1 of $–CO_2CH_2CONRR^1$ moiety) and monosubstituted or unsubstituted glycolamide esters were found to be more resistant than N, N-disubstituted glycolamide esters. Moreover, the

Table 3. In vitro and in vivo (rat paw edema assay[d]) data for indomethacin derivatives

Compound	IC_{50}[a] (μM)		log*p*[b]	Selectivity index (COX-1/COX-2)	ED_{30} (mg/kg)	ED_{50} (mg/kg)
	COX-1	COX-2				
1	>180[c]	0.82 ± 0.03	5.85 ± 0.1	>220	>10	nd
4	110 ± 5	0.49 ± 0.01	5.48 ± 0.1	~225	nd	12 ± 0.03
6	>150[c]	0.55 ± 0.03	5.57 ± 0.1	>273	10 ± 0.25	nd
9	>30[c]	0.83 ± 0.10	6.65 ± 0.1	~36	nd	nd
28	20 ± 2	0.73 ± 0.08	3.33 ± 0.1	~27	nd	nd
31	>50[c]	0.60 ± 0.10	7.30 ± 0.51	>83	nd	8.1 ± 0.07
32	25 ± 3	0.30 ± 0.05	4.14 ± 0.30	~83	nd	5.3 ± 0.11
33	>20[c]	0.44 ± 0.01	4.88 ± 0.30	>46	nd	nd
Indomethacin	0.067 ± 0.001	7.8 ± 0.11	4.18 ± 0.10	~0.0085	nd	2.4 ± 0.20
Celecoxib	15.33 ± 0.03	0.07 ± 0.005	4.37 ± 0.1	~219	nd	7.9 ± 0.14

nd, not determined.

[a] The result is the mean value of two determinations, and the deviation from the mean is <10% of the mean value.

[b] Calculated using SYBYL.

[c] Precipitation of compound observed beyond this concentration.

[d] ED_{30} and ED_{50} values were determined using a minimum of four dose points, six animals (male Wistar rats)/group followed by oral administration.

glycolamide esters of aspirin failed to deliver aspirin in human plasma when morpholine was the corresponding amine of the amide moiety.[22a] This structural dependence seems to hold true for the present compounds (**1**–**33**, Table 1) in rats, which is reflected by ED_{30} or ED_{50} values of compounds **1**, **4**, **6**, **31**, and **32**. Their relatively higher ED_{30} or ED_{50} values than indomethacin (Table 3) apparently indicated that these compounds are perhaps more resistant to enzymatic hydrolysis by plasma in rats. However, to gain further evidence stability studies were carried out using compounds **32** and **33** in the presence of 50 mM Tris buffer (pH 7.4) initially where 70% of **32** and 99% of **33** remained unchanged after 2 h incubation in buffer, respectively. In a separate study, these compounds were incubated for varying times with rat liver microsomes (RLM) in the presence of NADPH. After quenching with acetonitrile, the incubation mixtures were analyzed by HPLC and generation of any product that coeluted with indomethacin was examined. The RLM stability of these compounds (**32** and **33**) was found to be moderate (20–30%) and therefore it remained unclear that the in vivo activity of these compounds was due to their selective COX-1/COX-2 properties and not due to hydrolysis to indomethacin. Nevertheless, the ED_{50} of compound **32** was found to be 5.3 mg/kg after oral administration, which is comparable to that of celecoxib (7.9 mg/kg). Thus, compound **32** was identified as selective inhibitor of COX-2 with good anti-inflammatory activity. It was however not the most selective inhibitor in this chemical class and therefore may have the reduced cardiovascular liability of certain COX-2 inhibitors that showed very high selectivity for COX isozyme.[23a]

To test the ulcerogenicity of compound **32** and then compare with that of indomethacin, we conducted qualitative acute stomach ulceration study in swiss albino mice ($n = 5$). The UD_{30} (30% ulcerative dose) of compound **32** was found to be ~5-fold higher than 1.5 mg/kg/po noted for indomethacin after 6th hour of oral administration.[23b] Gastrointestinal toxicity of several glycolamide esters of non-steroidal anti-inflammatory drugs including indomethacin has been investigated earlier and all these esters showed markedly reduced GI toxicity than parent drugs in rats.[22b,22c] Temporarily masked acidic moiety of the parent drugs in the form of glycolamide ester that avoid the accumulation of the acid within gastric mucosal cells was thought to be the reason for their reduced GI toxicities. However, others[9b] and we in the present study have shown that converting the free acid side chain of indomethacin into simple or glycolamide esters afforded COX-2 inhibitors with moderate to high selectivity. Thus, the reduced GI toxicities shown by indomethacin esters are perhaps related to their COX-2 inhibiting properties rather than masked acidic carboxyl group. Nevertheless, the desired physicochemical properties along with high chemical stability can be obtained via glycolamide ester preparation of a carboxylic acid[18,22a] and therefore, the glycolamide esters of indomethacin described in the present study are attractive chemical class for the development of novel anti-inflammatory agents.

4. Conclusion

With the aim of developing a potent but moderately selective COX-2 inhibitor we have shown that in addition to the earlier report of converting it into simple ester or amide nonselective indomethacin can also be transformed into COX-2 inhibitors via converting the acid moiety to N-substituted glycolamide esters. Physical properties like hydrophilicity and hydrophobicity along with the size of the *N*-substituent played a key role in modulating COX-2/COX-1 selectivity. Some of these compounds synthesized were identified as good to moderately selective COX-2 inhibitors, and their in vitro data were rationalized through molecular modeling studies. While the discovery of COX-2 initiated a race to the development of the most selective inhibitors, the question now remains to be answered is whether a higher degree of selectivity confers any advantages. On the other hand, the design of a COX-2 'preferential' inhibitor keeping a slight effect on COX-1 at therapeutic dosage could theoretically limit the imbalance of prostacyclin/thromboxane A2. Therefore, the novel indomethacin glycolamide esters developed by us seem to have advantages over the other COX-2 inhibitors. Because of their distinctly different chemical structures they are not expected to show affinity for carbonic anhydrase isozymes like 1,2-diaryl sulfonamide class or cause marked increase in nonenzymatic generation of isoprostanes like diaryl methansulfones.[24] Compound **32** was identified as a promising candidate in this class and its good anti-inflammatory activity was demonstrated in the in vivo model.

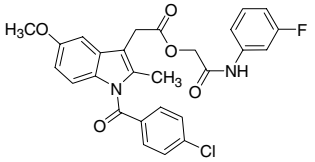
5. Experimental

5.1. Chemical methods

Unless stated otherwise, reactions were performed under nitrogen atmosphere. All the solvents used were commercially available and distilled before use. Reactions were monitored by thin-layer chromatography (TLC) on silica gel plates (60 F254), visualizing with ultraviolet light or iodine spray. Flash chromatography was performed on silica gel (230–400 mesh) using distilled petroleum ether, ethyl acetate, dichloromethane, chloroform, and methanol. ^{1}H NMR spectra were determined in $CDCl_3$ solution on 200 MHz spectrometers. Proton chemical shifts (δ) are relative to tetramethylsilane (TMS, $\delta = 0.00$) as internal standard and expressed in ppm. Spin multiplicities are given as s (singlet), d (doublet), t (triplet), and m (multiplet) as well as b (broad). Coupling constants (J) are given in hertz. Infrared spectra were recorded on a FT-IR spectrometer. Melting points were determined by using melting point apparatus and are uncorrected. MS spectra were obtained on a mass spectrometer. Chromatographic purity by HPLC was determined by using area normalization method and the condition specified in each case: column, mobile phase (range used), flow rate, detection wavelength, and retention times. Microanalyses were performed using a C H N S/O analyzer. Elemental data are within ±0.4% of the theoretical values. All yields reported are

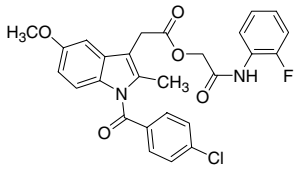
unoptimized. Celecoxib and Rofecoxib were prepared according to the literature procedure.[3,4b]

5.1.1. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3-fluoro phenylcarbamoyl) methyl ester (1).



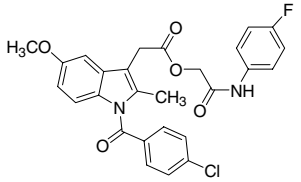
Yield 10%; off white solid; mp 152–154 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.60 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.18–7.0 (m, 2H), 6.93 (s, 1H), 6.86 (m, 1H), 6.75 (m, 3H), 6.61 (d, J = 8.3 Hz, 1H), 4.75 (s, 2H), 3.84 (s, 2H), 3.79 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3314, 1722 (C═O), 1644, 1616; Mass (m/z) 509 (M^+, 100%); HPLC 99.7%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 11.1 min; Elemental analysis found C, 63.79; H, 4.37; N, 5.45; $C_{27}H_{22}ClFN_2O_5$ requires C, 63.72; H, 4.36; N, 5.50.

5.1.2. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2-fluoro phenylcarbamoyl) methyl ester (2).



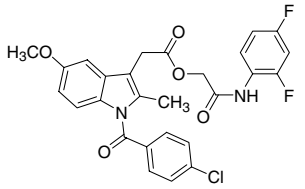
Yield 34%; off white solid; mp 133–135 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.18 (d, J = 7.8 Hz, 1H), 7.80 (s, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.19–7.01 (m, 3H), 6.96 (s, 1H), 6.82 (d, J = 9.3 Hz, 1H), 6.65 (d, J = 9.2 Hz, 1H), 4.75 (s, 2H), 3.85 (s, 2H), 3.79 (s, 3H), 2.47 (s, 3H); IR (KBr, cm^{-1}): 3308, 1721 (C═O), 1651, 1619; Mass (m/z) 509 (M^+, 30%), 139 (100%); HPLC 97.9%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 240 nm, retention time 8.96 min; Elemental analysis found C, 63.67; H, 4.36; N, 5.55; $C_{27}H_{22}ClFN_2O_5$ requires C, 63.72; H, 4.36; N, 5.50.

5.1.3. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-fluoro phenylcarbamoyl) methyl ester (3).



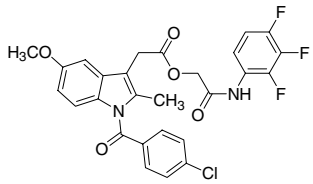
Yield 55%; white solid; mp 132–134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.61 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.05–7.04 (m, 2H), 6.93–6.90 (m, 4H), 6.76–6.70 (m, 2H), 4.71 (s, 2H), 3.85 (s, 2H), 3.76 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3340, 1735 (C═O), 1684, 1615; Mass (m/z) 509 (M^+, 45%), 139 (100%); HPLC 98.1%, column: Inertsil ODS 3 V (250 × 4.6) mm, mobile phase: 0.01 M KH_2PO_4/CH_3CN (30:70), pH 3.5, flow rate 1.0 mL/min, UV 235 nm, retention time 13.4 min; Elemental analysis found C, 63.60; H, 4.50; N, 5.57; $C_{27}H_{22}ClFN_2O_5$ requires C, 63.72; H, 4.36; N, 5.50.

5.1.4. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2,4-difluoro phenylcarbamoyl) methyl ester (4).



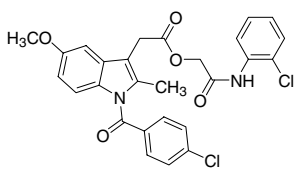
Yield 56%; off white solid; mp 131–134 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.08 (m, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 6.96 (s, 1H), 6.85–6.75 (m, 3H), 6.65 (d, J = 9.3 Hz, 1H), 4.74 (s, 2H), 3.84 (s, 2H), 3.71 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3251, 1737 (C═O), 1689; Mass (m/z) 527 (M^+, 33%), 139 (100%); HPLC 97.1%, column: ECLIPSE XDB C8 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (45:55), flow rate 1.0 mL/min, UV 215 nm, retention time 14.64 min; Elemental analysis found C, 61.60; H, 4.10; N, 5.20; $C_{27}H_{21}ClF_2N_2O_5$ requires C, 61.54; H, 4.02; N, 5.32.

5.1.5. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2,3,4-trifluoro phenylcarbamoyl) methyl ester (5).



Yield 45%; white solid; mp 142–145 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.81 (br s, 1H), 7.68 (d, J = 8.3 Hz, 2H), 7.58 (s, 1H), 7.48 (d, J = 8.3 Hz, 2H), 6.99–6.62 (m, 4H), 4.75 (s, 2H), 3.85 (s, 2H), 3.77 (s, 3H), 2.48 (s, 3H); IR (KBr, cm^{-1}): 3344, 1745 (C═O), 1707, 1679, 1610; Mass (m/z) 545 (M^+, 55%), 139 (100%); HPLC 99.3%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 225 nm, retention time 10.3 min; Elemental analysis found C, 59.41; H, 3.72; N, 5.10; $C_{27}H_{20}ClF_3N_2O_5$ requires C, 59.51; H, 3.70; N, 5.14.

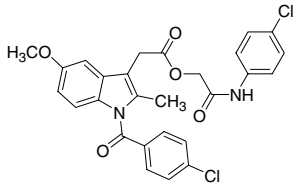
5.1.6. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2-chloro phenylcarbamoyl) methyl ester (6).



Yield 61%; white solid; mp 124–126 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.33 (d, J = 8.3 Hz, 1H), 8.22 (br s, 1H), 7.66 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.34 (d, J = 8.8 Hz, 2H), 7.12–6.65 (m, 4H), 4.78 (s, 2H), 3.87 (s, 2H), 3.80 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3253, 1739 (C═O), 1678, 1610; Mass (m/z) 525 (M^+,

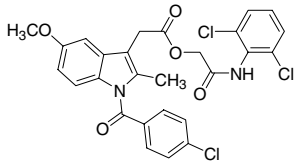
45%), 139 (100%); HPLC 97.8%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 8.6 min; Elemental analysis found C, 61.79; H, 4.23; N, 5.30; $C_{27}H_{22}Cl_2N_2O_5$ requires C, 61.72; H, 4.22; N, 5.33.

5.1.7. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-chloro phenylcarbamoyl) methyl ester (7).



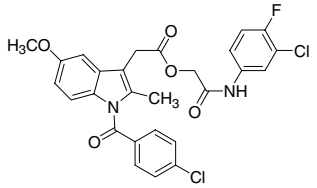
Yield 31%; off white solid; mp 159–162 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.6 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.17 (d, J = 8.8 Hz, 2H), 7.05 (s, 1H), 6.9 (m, 3H), 6.75 (d, J = 9.2 Hz, 1H), 4.71 (s, 2H), 3.84 (s, 2H), 3.77 (s, 3H), 2.46 (s, 3H); IR (KBr, cm^{-1}): 3334, 1734 (C═O), 1686; Mass (*m*/*z*) 525 (M$^+$, 100%); HPLC 99.2%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 250 nm, retention time 11.6 min; Elemental analysis found C, 60.67; H, 4.20; N, 5.40; $C_{27}H_{22}Cl_2N_2O_5$ requires C, 61.72; H, 4.22; N, 5.33.

5.1.8. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2,6-dichloro phenylcarbamoyl) methyl ester (8).



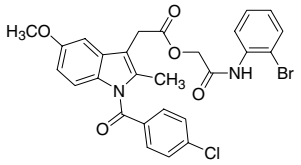
Yield 35%; white solid; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.62 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.32 (d, J = 8.8 Hz, 2H), 7.22–7.13 (m, 2H), 6.98 (d, J = 2.4 Hz, 1H), 6.77 (d, J = 8.8 Hz, 1H), 6.55 (d, J = 9.3 Hz, 1H), 4.81 (s, 2H), 3.85 (s, 2H), 3.74 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3422, 1742 (C═O), 1675, 1610; Mass (*m*/*z*) 560 (M$^+$, 55%), 139 (100%); HPLC 98.3%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 6.5 min; Elemental analysis found C, 57.80; H, 3.77; N, 5.12; $C_{27}H_{21}Cl_3N_2O_5$ requires C, 57.93; H, 3.78; N, 5.00.

5.1.9. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3-chloro-4-fluoro phenylcarbamoyl) methyl ester (9).



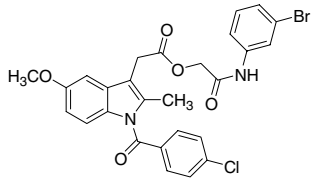
Yield 42%; off white solid; mp 158–160 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.62 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.25–6.73 (m, 7H), 4.70 (s, 2H), 3.83 (s, 2H), 3.74 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3286, 1721 (C═O), 1698; Mass (*m*/*z*) 545 (M+2, 30%), 544 (M+1, 30%), 543 (M$^+$, 42%), 139 (100%); HPLC 96.5%, column: ECLIPSE XDB-C8 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (40:60), flow rate 1.0 mL/min, UV 240 nm, retention time 14.0 min; Elemental analysis found C, 59.61; H, 3.90; N, 5.20; $C_{27}H_{21}Cl_2FN_2O_5$ requires C, 59.68; H, 3.90; N, 5.16.

5.1.10. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2-bromo phenylcarbamoyl) methyl ester (10).



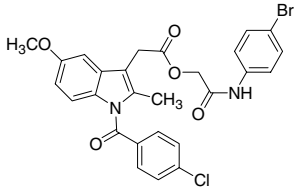
Yield 52%; off white solid; mp 135–136 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.29 (m, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.48 (m, 3H), 7.34 (m, 1H), 7.01 (m, 3H), 6.85 (d, J = 8.8 Hz, 1H), 6.67 (d, J = 8.8 Hz, 1H), 4.78 (s, 2H), 3.88 (s, 2H), 3.84 (s, 3H), 2.44 (s, 3H); IR (KBr, cm^{-1}): 3251, 1737 (C═O), 1675; Mass (*m*/*z*) 572 (M+2, 15%), 570 (M$^+$, 33%), 139 (100%); HPLC 96.4%, column: ECLIPSE XDB C8 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (45:55), flow rate 1.0 mL/min, UV 215 nm, retention time 18.39 min; Elemental analysis found C, 56.70; H, 3.85; N, 4.99; $C_{27}H_{22}BrClN_2O_5$ requires C, 56.91; H, 3.89; N, 4.92.

5.1.11. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3-bromo phenylcarbamoyl) methyl ester (11).



Yield 46%; off white solid; mp 152–154 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.62 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.26–6.75 (m, 8H), 4.72 (s, 2H), 3.85 (s, 2H), 3.76 (s, 3H), 2.46 (s, 3H); IR (KBr, cm^{-1}): 3253, 1744 (C═O), 1673; Mass (*m*/*z*) 572 (M+2, 15%), 570 (M$^+$, 15%), 139 (100%); HPLC 97%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 11.6 min; Elemental analysis found C, 56.71; H, 3.90; N, 4.90; $C_{27}H_{22}BrClN_2O_5$ requires C, 56.91; H, 3.89; N, 4.92.

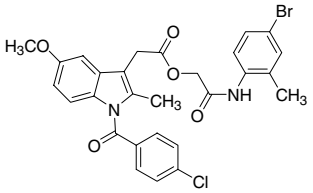
5.1.12. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-bromo phenylcarbamoyl) methyl ester (12).



Yield 42%; off white solid; mp 160–164 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, J = 8.8 Hz, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.33–6.78 (m, 8H), 4.71 (s, 2H), 3.84 (s,

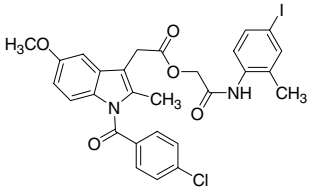
2H), 3.77 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3322, 1733 (C═O), 1684; Mass (*m*/*z*) 572 (M+2, 15%), 570 (M^+, 15%), 139 (100%); HPLC 96%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 8.75 min; Elemental analysis found C, 56.78; H, 3.87; N, 4.98; $C_{27}H_{22}BrClN_2O_5$ requires C, 56.91; H, 3.89; N, 4.92.

5.1.13. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-bromo-2-methyl phenylcarbamoyl) methyl ester (13).



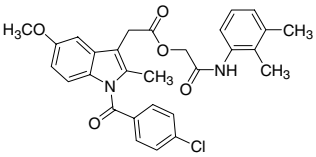
Yield 89%; off white solid; mp 168–170 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, J = 8.3 Hz, 2H), 7.44 (d, J = 8.8 Hz, 2H), 7.39–7.25 (m, 4H), 6.95–6.59 (m, 3H), 4.74 (s, 2H), 3.83 (s, 2H), 3.73 (s, 3H), 2.43 (s, 3H), 1.83 (s, 3H); IR (KBr, cm^{-1}): 3257, 1756 (C═O), 1673; Mass (*m*/*z*) 586 (M+2, 15%), 584 (M^+, 45%), 139 (100%); HPLC 97%, column: ECLIPSE XDB-C8 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (40:60), flow rate 1.5 mL/min, UV 210 nm, retention time 10.3 min; Elemental analysis found C, 57.66; H, 4.16; N, 4.75 $C_{28}H_{24}BrClN_2O_5$ requires C, 57.60; H, 4.14; N, 4.80.

5.1.14. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-iodo-2-methyl phenylcarbamoyl) methyl ester (14).



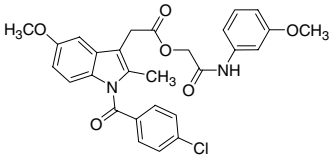
Yield 74%; light purple solid; mp 166-168 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.60 (d, J = 8.3 Hz, 2H), 7.48–6.61 (m, 9H), 4.75 (s, 2H), 3.84 (s, 2H), 3.75 (s, 3H), 2.45 (s, 3H), 1.82 (s, 3H); IR (KBr, cm^{-1}): 3276, 1728 (C═O), 1672; Mass (*m*/*z*) 630 (M^+, 8%), 505 (M^+-127, 15%), 139 (100%); HPLC 97.5%, column: ECLIPSE XDB-C8 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (40:60), flow rate 1.0 mL/min, UV 240 nm, retention time 16.5 min; Elemental analysis found C, 53.50; H, 3.83; N, 4.40 $C_{28}H_{24}ClIN_2O_5$ requires C, 53.31; H, 3.83; N, 4.44.

5.1.15. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2,3-dimethyl phenylcarbamoyl) methyl ester (15).



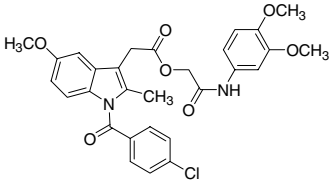
Yield 51%; off white solid; mp 152–155 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.56 (d, J = 8.3 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.09 (m, 3H), 6.98 (s, 1H), 6.78 (d, J = 8.7 Hz, 1H), 6.62 (d, J = 8.8 Hz, 1H), 4.76 (s, 2H), 3.83 (s, 2H), 3.72 (s, 3H), 2.44 (s, 3H), 2.25 (s, 3H), 1.82 (s, 3H); IR (KBr, cm^{-1}): 3282, 1741 (C═O), 1670; Mass (*m*/*z*) 519 (M^+, 40%), 180 (100%); HPLC 98.2%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 10.2 min; Elemental analysis found C, 67.34; H, 5.22; N, 5.44; $C_{29}H_{27}ClN_2O_5$ requires C, 67.11; H, 5.24; N, 5.40.

5.1.16. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3-methoxy phenylcarbamoyl) methyl ester (16).



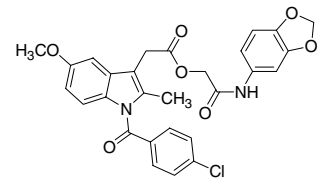
Yield 48%; off white solid; mp 142–144 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.60 (d, J = 8.8 Hz, 2H), 7.42 (d, J = 8.3 Hz, 2H), 7.27 (m, 2H), 7.08 (m, 2H), 6.79 (m, 2H), 6.31 (d, J = 7.8 Hz, 1H), 4.71 (s, 2H), 3.84 (s, 2H), 3.76 (s, 6H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3276, 1740 (C═O), 1672, 1609; Mass (*m*/*z*) 521 (M^+, 100%); HPLC 96.9%, column: ECLIPSE XDB C8 (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (40:60), flow rate 1.0 mL/min, UV 220 nm, retention time 8.69 min; Elemental analysis found C, 64.59; H, 4.83; N, 5.32; $C_{28}H_{25}ClN_2O_6$ requires C, 64.55; H, 4.84; N, 5.38.

5.1.17. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3,4-dimethoxy phenylcarbamoyl) methyl ester (17).



Yield 42%; purple colored solid; mp 168–170 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.20–6.63 (m, 7H), 6.14–6.10 (m, 1H), 4.69 (s, 2H), 3.83 (s, 9H), 3.75 (s, 2H), 2.44 (s, 3H); IR (KBr, cm^{-1}): 3233, 1719 (C═O), 1685, 1611; Mass (*m*/*z*) 551 (M+1, 100%); HPLC 99.6%, column: HICHROM RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 6.63 min; Elemental analysis found C, 63.39; H, 4.93; N, 5.00; $C_{29}H_{27}ClN_2O_7$ requires C, 63.22; H, 4.94; N, 5.08.

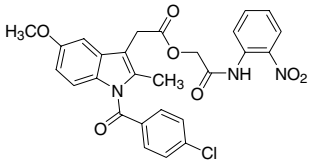
5.1.18. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid benzo[1,3]dioxol-5-ylcarbamoylmethyl ester (18).



Yield 30%; white solid; mp 155–160 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.58 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.3 Hz, 2H), 7.14 (s, 1H), 7.02 (m, 2H), 6.87 (d, J = 9.3 Hz, 1H), 6.70 (d,

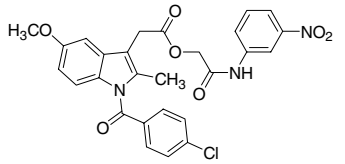
J = 11.2 Hz, 1H), 6.59 (d, J = 8.3 Hz, 1H), 6.22 (d, J = 8.3 Hz, 1H), 5.91 (s, 2H), 4.67 (s, 2H), 3.81 (s, 2H), 3.75 (s, 3H), 2.43 (s, 3H); IR (KBr, cm^{-1}): 3278, 1741 (C═O), 1670, 1610; Mass (*m/z*) 535 (M^+, 40%), 372 (70), 139 (100%); HPLC 96.9%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (50:50), flow rate 1.2 mL/min, UV 210 nm, retention time 23.2 min; Elemental analysis found C, 62.96; H, 4.32; N, 5.31; $C_{28}H_{23}ClN_2O_7$ requires C, 62.87; H, 4.33; N, 5.24.

5.1.19. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (2-nitro phenylcarbamoyl) methyl ester (19).



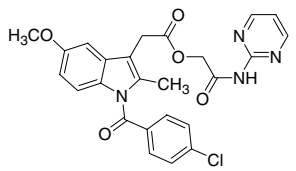
Yield 65%; light yellow solid; ^1H NMR (200 MHz, $CDCl_3$) δ 11.09 (s, 1H), 8.80 (d, J = 8.3 Hz, 1H), 8.26 (d, J = 8.8 Hz, 1H), 7.70 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.8 Hz, 2H), 7.26–6.63 (m, 5H), 4.81 (s, 2H), 3.95 (s, 2H), 3.79 (s, 3H), 2.44 (s, 3H); IR (KBr, cm^{-1}): 3338, 1743 (C═O), 1700, 1670, 1607; Mass (*m/z*) 536 (M^+, 50%), 139 (100%); HPLC 98.9%, column: Hichrom RPB 250 mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 225 nm, retention time 11.8 min; Elemental analysis found C, 60.45; H, 4.13, N, 7.90; $C_{27}H_{22}ClN_3O_7$ requires C, 60.51; H, 4.14; N, 7.84.

5.1.20. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (3-nitro phenylcarbamoyl) methyl ester (20).



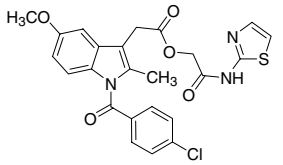
Yield 48%; pale yellow solid; mp 164–166 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.93 (d, J = 7.3 Hz, 1H), 7.66 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.8 Hz, 6H), 7.05–6.71 (m, 3H), 4.76 (s, 2H), 3.87 (s, 2H), 3.73 (s, 3H), 2.48 (s, 3H); IR (KBr, cm^{-1}): 3271, 1726 (C═O), 1703, 1667, 1605; Mass (*m/z*) 536 (M^+, 35%), 139 (100%); HPLC 96.3%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.2 mL/min, UV 240 nm, retention time 5.4 min; Elemental analysis found C, 60.57; H, 4.20, N, 7.70; $C_{27}H_{22}ClN_3O_7$ requires C, 60.51; H, 4.14; N, 7.84.

5.1.21. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid pyrimidin-2-ylcarbamoyl methyl ester (21).



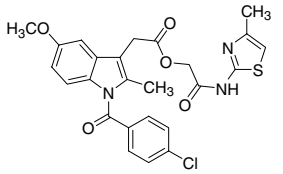
Yield 67%; off white solid; mp 205–207 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 8.54 (m, 3H), 8.38 (s, 1H), 7.68 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 7.03 (s, 1H), 6.88 (d, J = 9.2 Hz, 1H), 6.67 (d, J = 8.8 Hz, 1H), 5.14 (s, 2H), 3.84 (s, 5H), 2.43 (s, 3H); IR (KBr, cm^{-1}): 3442, 1742 (C═O), 1706, 1673; Mass (*m/z*) 493 (M^+, 20%), 138 (100%); HPLC 98.7%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: 0.01 M KH_2PO_4/CH_3CN (60:40), flow rate 1.0 mL/min, UV 235 nm, retention time 8.1 min; Elemental analysis found C, 60.78; H, 4.19, N, 11.50; $C_{25}H_{21}ClN_4O_5$ requires C, 60.92; H, 4.29; N, 11.37.

5.1.22. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid thiazol-2-ylcarbamoyl methyl ester (22).



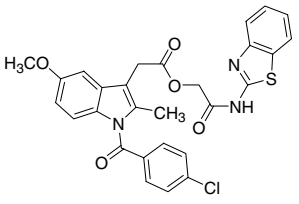
Yield 43%; white solid; mp 190–192 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 10.2 (br s, 1H), 7.69 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.39 (d, J = 3.4 Hz, 1H), 7.0 (d, J = 3.4 Hz, 1H), 6.97 (s, 1H), 6.85 (d, J = 8.8 Hz, 1H), 6.67 (m, 1H), 4.85 (s, 2H), 3.82 (s, 5H), 2.44 (s, 3H); IR (KBr, cm^{-1}): 1750 (C═O), 1710, 1669; Mass (*m/z*) 498 (M^+, 50%), 143 (100%); HPLC 96.0%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (40: 60), flow rate 1.0 mL/min, UV 260 nm, retention time 8.3 min; Elemental analysis found C, 57.91; H, 4.03, N, 8.48; $C_{24}H_{20}ClN_3O_5S$ requires C, 57.89; H, 4.05; N, 8.44.

5.1.23. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-methyl thiazol-2-ylcarbamoyl) methyl ester (23).



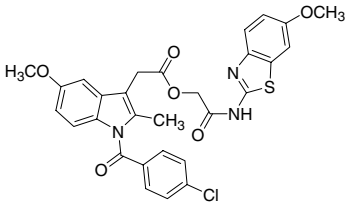
Yield 16%; off white solid; mp 180–183 °C; ^1H NMR (200 MHz, $CDCl_3$) δ 7.67 (d, J = 8.8 Hz, 2H), 7.46 (d, J = 8.8 Hz, 2H), 6.99 (s, 1H), 6.88 (d, J = 9.3 Hz, 1H), 6.62 (d, J = 9.3 Hz, 1H), 6.58 (m, 1H), 4.84 (s, 2H), 3.91 (s, 2H), 3.84 (s, 3H), 2.44 (s, 3H), 2.33 (s, 3H); IR (KBr, cm^{-1}): 3216, 1712 (C═O), 1639; Mass (*m/z*) 512 (M^+, 100%); HPLC 98.6%, column: Inertsil ODS 3V (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 6.52 min; Elemental analysis found C, 59.45; H, 4.37, N, 8.18; $C_{25}H_{22}ClN_3O_5S$ requires C, 58.65; H, 4.33; N, 8.21.

5.1.24. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid benzothiazol-2-ylcarbamoyl methyl ester (24).



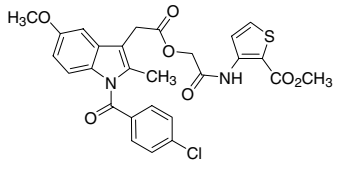
Yield 10%; off white solid; mp 188–190 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.82 (d, J = 8.3 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 7.8 Hz, 2H), 7.01 (s, 1H), 6.89 (d, J = 9.2 Hz, 1H), 6.74 (d, J = 7.8 Hz, 1H), 4.89 (s, 2H), 3.89 (s, 2H), 3.84 (s, 3H), 2.45 (s, 3H); IR (KBr, cm^{-1}): 3441, 1745 (C═O), 1714, 1690; Mass (*m*/*z*) 548 (M^+, 10%), 139 (100%); HPLC 98.6%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (25:75), flow rate 1.0 mL/min, UV 210 nm, retention time 6.65 min; Elemental analysis found C, 61.49; H, 4.00, N, 7.60; $C_{28}H_{22}ClN_3O_5S$ requires C, 61.37; H, 4.05; N, 7.67.

5.1.25. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (6-methoxy benzothiazol-2-ylcarbamoyl) methyl ester (25).



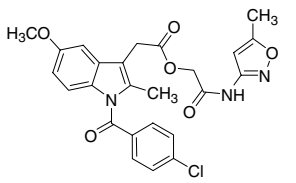
Yield 10%; yellow solid; mp 187–189 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.70 (d, J = 8.3 Hz, 2H), 7.58 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.04 (d, J = 9.2 Hz, 2H), 6.97 (s, 1H), 6.89 (d, J = 9.2 Hz, 1H), 4.87 (s, 2H), 3.89 (s, 2H), 3.84 (s, 6H), 2.47 (s, 3H); IR (KBr, cm^{-1}): 3413, 1745 (C═O), 1606; Mass (*m*/*z*) 578 (M^+, 20%), 139 (100%); HPLC 95.4%, column: ECLIPSE XDB (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (45:55), flow rate 1.0 mL/min, UV 210 nm, retention time 13.79 min; Elemental analysis found C, 60.43; H, 4.15, N, 7.30; $C_{29}H_{24}ClN_3O_6S$ requires C, 60.26; H, 4.18; N, 7.27.

5.1.26. 3-(2-{2-[1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetoxy} acetylamino) thiophene-2-carboxylic acid methyl ester (26).



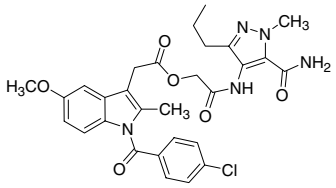
Yield 82%; off white solid; mp 165–168 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 10.95 (s, 1H), 8.12 (d, J = 5.4 Hz, 1H), 7.67 (d, J = 8.3 Hz, 2H), 7.48 (m, 3H), 7.05 (s, 1H), 6.86 (d, J = 9.2 Hz, 1H), 6.67 (d, J = 11.2 Hz, 1H), 4.79 (s, 2H), 3.98 (s, 2H), 3.90 (s, 3H), 3.79 (s, 3H), 2.46 (s, 3H); IR (KBr, cm^{-1}): 3325, 1748 (C═O), 1701, 1674; Mass (*m*/*z*) 555 (M^+, 100%); HPLC 98.8%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (25:75), flow rate 1.0 mL/min, UV 220 nm, retention time 9.9 min; Elemental analysis found C, 58.40; H, 4.20, N, 5.00; $C_{27}H_{23}ClN_2O_7S$ requires C, 58.43; H, 4.18; N, 5.05.

5.1.27. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (5-methyl isoxazol-3-ylcarbamoyl) methyl ester (27).



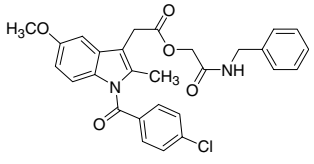
Yield 53%; off white solid; mp 175–178 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 8.32 (br s, 1H), 7.73 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.3 Hz, 2H), 6.96 (s, 1H), 6.85 (d, J = 8.8 Hz, 1H), 6.69 (m, 1H), 6.64 (s, 1H), 4.75 (s, 2H), 3.82 (s, 5H), 2.45 (s, 3H), 2.39 (s, 3H); IR (KBr, cm^{-1}): 1741 (C═O), 1714, 1677, 1635; Mass (*m*/*z*) 496 (M^+, 50%), 157 (100%); HPLC 98.6%, column: Inertsil ODS 3 V (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 220 nm, retention time 9.5 min; Elemental analysis found C, 60.47; H, 4.46, N, 8.50; $C_{25}H_{22}ClN_3O_6$ requires C, 60.55; H, 4.47; N, 8.47.

5.1.28. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (5-carbamoyl-1-methyl-3-propyl-1*H*-pyrazol-4-ylcarbamoyl) methyl ester (28).



Yield 42%; white solid; mp 168–170 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.14 (s, 1H), 6.98 (s, 1H), 6.78 (d, J = 9.2 Hz, 1H), 6.62 (d, J = 9.2 Hz, 1H), 4.75 (s, 2H), 3.98 (s, 3H), 3.84 (s, 2H), 3.74 (s, 3H), 2.45 (s, 3H), 2.15 (m, 2H), 1.44 (m, 2H), 0.84 (m, 3H); IR (KBr, cm^{-1}): 3330, 1730 (C═O), 1677; Mass (*m*/*z*) 580 (M^+, 30%), 223 (100%); HPLC 96.8%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (50: 50), flow rate 1.0 mL/min, UV 220 nm, retention time 18.3 min; Elemental analysis found C, 60.15; H, 5.20, N, 12.10; $C_{29}H_{30}ClN_5O_6$ requires C, 60.05; H, 5.21; N, 12.07.

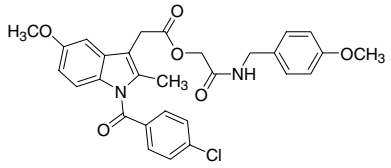
5.1.29. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid benzylcarbamoylmethyl ester (29).



Yield 23%; off white solid; mp 135–137 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, J = 8.3 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 7.24 (m, 4H), 7.05 (m, 1H), 6.95 (s, 1H), 6.82 (d, J = 9.2 Hz, 1H), 6.66 (d, J = 8.8 Hz, 1H), 6.01 (m, 1H), 4.64 (s, 2H), 4.25 (d, J = 5.9 Hz, 2H), 3.77 (s, 5H), 2.35 (s, 3H); IR (KBr, cm^{-1}): 3297, 1746 (C═O), 1662; Mass (*m*/*z*) 505 (M^+, 100%); HPLC 97.9%, column: Hichrom RPB (250 × 4.6) mm, mobile

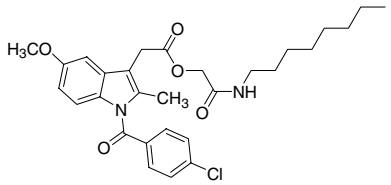
phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 7.83 min; Elemental analysis found C, 66.49; H, 5.00, N, 5.61; $C_{28}H_{25}ClN_2O_5$ requires C, 66.60; H, 4.99; N, 5.55.

5.1.30. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid (4-methoxy benzylcarbamoyl) methyl ester (30).



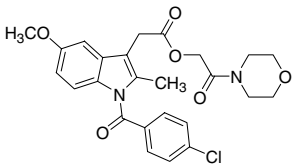
Yield 36%; white solid; mp 150–152 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.59 (d, J = 8.8 Hz, 2H), 7.45 (d, J = 8.3 Hz, 2H), 6.98 (d, J = 8.8 Hz, 2H), 6.93 (s, 1H), 6.85 (d, J = 9.2 Hz, 1H), 6.77 (d, J = 8.3 Hz, 2H), 6.67 (m, 1H), 5.96 (m, 1H), 4.62 (s, 2H), 4.18 (d, J = 5.9 Hz, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.75 (s, 2H), 2.34 (s, 3H); IR (KBr, cm^{-1}): 3295, 1735 (C=O), 1664; Mass (*m*/*z*) 535 (M$^+$, 20%), 139 (100%); HPLC 98.9%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (40: 60), flow rate 1.0 mL/min, UV 225 nm, retention time 10.3 min; Elemental analysis found C, 65.19; H, 5.07, N, 5.10; $C_{29}H_{27}ClN_2O_6$ requires C, 65.11; H, 5.09; N, 5.24.

5.1.31. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid octylcarbamoyl methyl ester (31).



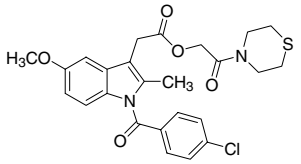
Yield 29%; white solid; mp 88–90 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, J = 8.8 Hz, 2H), 7.49 (d, J = 8.3 Hz, 2H), 6.98 (s, 1H), 6.89 (d, J = 8.8 Hz, 1H), 6.71 (d, J = 8.8 Hz, 1H), 5.64 (m, 1H), 4.57 (s, 2H), 3.83 (s, 3H), 3.78 (s, 2H), 3.03 (d, J = 5.9 Hz, 2H), 2.42 (s, 3H), 1.18 (m, 12H), 0.88 (t, J = 12.7 Hz, 3H); IR (KBr, cm^{-1}): 3302, 1743 (C=O), 1657; Mass (*m*/*z*) 527 (M$^+$, 100%); HPLC 98.3%, column: symmetry C18 (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.5 mL/min, UV 210 nm, retention time 10.9 min; Elemental analysis found C, 66.19; H, 6.61, N, 5.52; $C_{29}H_{35}ClN_2O_5$ requires C, 66.09; H, 6.69; N, 5.32.

5.1.32. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid 2-morpholin-4-yl-2-oxo ethyl ester (32).



Yield 24%; off white solid; mp 152–153 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.67 (d, J = 8.3 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 7.03 (s, 1H), 6.88 (d, J = 8.8 Hz, 1H), 6.67 (d, J = 9.3 Hz, 1H), 4.74 (s, 2H), 3.85 (s, 3H), 3.83 (s, 2H), 3.63 (br s, 4H), 3.34 (br s, 4H), 2.39 (s, 3H); IR (KBr, cm^{-1}): 3435, 1731 (C=O), 1661, 1603; Mass (*m*/*z*) 485 (M$^+$, 50%), 139 (100%); HPLC 97.7%, column: ECLIPSE XDB (150 × 4.6) mm, mobile phase: H_2O/CH_3CN (45:55), flow rate 1.0 mL/min, UV 225 nm, retention time 5.54 min; Elemental analysis found C, 61.99; H, 5.21, N, 5.68; $C_{25}H_{25}ClN_2O_6$ requires C, 61.92; H, 5.20; N, 5.78.

5.1.33. [1-(4-Chlorobenzoyl)-5-methoxy-2-methyl-1*H*-indol-3-yl]acetic acid 2-oxo-2-thiomorpholin-4-ylethyl ester (33).



Pale yellow solid; mp 164–165 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 7.66 (d, J = 8.3 Hz, 2H), 7.46 (d, J = 8.3 Hz, 2H), 7.02–6.65 (m, 3H), 4.73 (s, 2H), 3.84 (s, 2H), 3.82 (s, 3H), 3.59 (br s, 4H), 2.60 (br s, 4H), 2.39 (s, 3H); IR (KBr, cm^{-1}): 3439, 1733 (C=O), 1662; Mass (*m*/*z*) 503 (M+2, 25%), 502 (M+1, 40%), 501 (M$^+$, 55%), 139 (100%); HPLC 98.5%, column: Hichrom RPB (250 × 4.6) mm, mobile phase: H_2O/CH_3CN (30:70), flow rate 1.0 mL/min, UV 210 nm, retention time 6.7 min; Elemental analysis found C, 59.91; H, 5.00, N, 5.60; $C_{25}H_{25}ClN_2O_5S$ requires C, 59.93; H, 5.03; N, 5.59.

5.2. Docking studies

Docking studies were carried out using FlexX in both forms of COX. The crystal structures of sheep COX-1 with iodo analogue of indomethacin (1PGG.pdb and 1PGF.pdb) and murine COX-2 with indomethacin (4COX.pdb) were used in the study. The structural differences in the active sites of COX-1 and COX-2 from different species are negligible compared to those between COX-1 and COX-2 themselves. Docking studies were carried out using FlexX.[25] In the cases where FlexX failed, the bioactive conformations of iodoindomethacin (1PGG.pdb and 1PGF.pdb) was used to construct the 3D models using SYBYL.[25] Hydrogen atoms were added to the proteins while all the basic and acidic amino acid residues were considered to be charged. In addition, the acid group of ligands was considered negatively charged. The ligand–receptor complexes were minimized using MMFF94 force fields, and Powell method with a convergence gradient value of 0.001 kcal/mol, and a distance dependent dielectric constant value of 1. Atomic charges were computed using MMFF94 method. All amino acid residues within 6 Å radius around the ligand were minimized while treating the rest of the protein as an aggregate.

5.3. Biological assays

5.3.1. In vitro biochemical assays (spectrophotometric assay of COX-1 and COX-2). Microsomal fraction of ram seminal vesicles were used as a source of COX-1 enzyme[26] and microsomes from *sf-9* cells infected with

baculovirus containing human COX-2 c-DNA were used as a source of COX-2 enzyme.[27] Enzyme activity was measured using a chromogenic assay based on oxidation of *N*,*N*,*N*′,*N*′-tetramethyl-*p*-phenylenediamine (TMPD) during the reduction of PGG_2 to PGH_2 as per the procedure described by Copeland et al.,[28] with the following modifications. The assay mixture (1000 μL) contained 100 mM Tris pH 8.0, 3 mM EDTA, 15 μM hematin, 150 units enzyme and 8% DMSO. The mixture was pre-incubated at 25 °C for 15 min before initiation of enzymatic reaction in the presence of compound/vehicle. The reaction was initiated by the addition of 100 μM arachidonic acid and 120 μM TMPD. The enzyme activity was measured by estimation of the initial velocity of TMPD oxidation over the first 25 s of the reaction followed from increase in absorbance at 603 nM. The IC_{50} values were calculated using non-linear regression analysis.

5.3.2. In vivo screening methods (carrageenan-induced rat paw edema). Male Wistar rats (120–140 g) were fasted for 16 h before the experiment. Compounds were suspended in 0.25% carboxymethylcellulose and administered orally in volume of 10 mL/kg 2 h before carrageenan injection. Paw edema was induced in rats by intradermal injection of 50 μL of 1% lambda-carrageenan in saline into the plantar surface of the right hind paw. Paw volume was measured before and 3 h after carrageenan injection by plethysmometer (Ugo-Basile, Italy). Paw edema was compared with the vehicle control group and percent inhibition was calculated in comparsion to vehicle group. ED_{30} and ED_{50} values were calculated using linear regression plot.

5.3.3. RLM experiments. Rat liver microsomes were prepared from wistar rats by differential centrifugation and stored in 50 mM Tris buffer, pH 7.4, containing 25 mM Sucrose at −80 °C. Protein estimation was done by method of Bradford[29] using bovine serum albumin (BSA) as standard. Stability assay was done in the following sequence: A typical reaction mixture containing 50 mM Tris buffer, pH 7.4, compound, RLM and NADPH was incubated at 37 °C in water bath for different time periods. At the end of each time point the reaction was terminated by adding 4 mL of acetonitrile, vortexed, centrifuged at 2500 rpm for 3 min and supernatant was removed, dried under nitrogen at 50 °C. Dry residue was reconstituted in methanol/water (1:1) and injected on to HPLC.

5.3.4. Acute stomach ulceration study in Swiss albino mice. The study was carried out in overnight fasted male mice, which were grouped 5 per group. Graded doses of compound **32** and indomethacin were administered in a dose range from 1 to 10 mg/kg by oral gavage. Six hours later 5% pontamine sky blue dye was injected through lateral tail vein in a volume of 10 mL/kg. The mice were euthanized, stomachs were removed and cleaned with normal saline. The severity of ulcers was scored on a scale ranging from 0 to 5. The percentage ulcer was calculated from the average ulcer score of the group. The UD_{30} (the dose producing 30% ulcers) was calculated using regression analysis.

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