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Laboratory note

Synthesis and receptor docking studies of *N*-substituted indole-2-carboxylic acid esters as a search for COX-2 selective enzyme inhibitors

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Abstract – A series of *N*-substituted indole-2-carboxylic acid esters have been prepared by replacing the benzoyl group of indomethacin with a benzyl and a phenyl group. The carbocyclic acid side chain was extended via creating an ester structure by using several dialkylaminoalkyl groups. The receptor docking studies were performed to investigate the docking mode of each compound by using DOCK 4.0. All the compounds were shown to be docked at the site where intact flurbiprofen was embedded for COX-1 and s-58 (1-phenylsulphonamide-3-trifluoromethyl-5-*para*-bromophenylpyrazole) for COX-2. It was predicted that *N*-phenyl-indole-2-carboxylic acid piperazine ester **22** can be a fairly strong COX-2 selective compound which was compared to the others. Other predicted COX-2 selective compounds included are N–H indole-2-carboxylic acid diethyl **30** and piperazine **34** esters. In view of these findings, compounds **22**, **30** and **34** were chosen for the in vitro biological assays. © 2001 Éditions scientifiques et médicales Elsevier SAS

docking / indole esters / COX-2 selectivity / COX-1 and COX-2 inhibition

1. Introduction

The discovery of a second isoform of cyclooxygenase (cyclooxygenase-2), which is expressed in inflammatory cells and the central nervous system, but not in the gastric mucosa, offers the possibility of developing anti-inflammatory and analgesic agents that lack the gastrointestinal side effects of currently available nonsteroidal anti-inflammatory drugs [1].

Both isoforms of the enzyme cyclooxygenase (COX-1 and COX-2), responsible for prostaglandin synthesis, have enabled us to develop drugs capable of sparing the gastric mucosa. The inducible COX-2 enzyme is responsible for some aspects of pain and inflammation in arthritis while the constitutive COX-1 enzyme appears responsible for most of the gastroprotective prostaglandin synthesis in the stomach and duodenum. Drugs selective in their inhibition for COX-2 probably act by binding to a pocket in enzyme that is present in COX-2 but not in COX-1 [2, 3]. Preclinical and clinical studies suggest that COX-2 inhibitors are highly promising agents for the treatment of pain and inflammation, and for the prevention of cancer [1]. As a result of this critical finding, a substantial discovery effort has been underway in the pharmaceutical industry to identify selective and orally active COX-2 inhibitors, because they may provide the desired anti-inflammatory and analgesic profiles without the deleterious side effects commonly associated with the existing NSAIDs [4, 5].

Recently a number of selective inhibitors of COX-2 were shown to possess anti-inflammatory activity with little or no gastric side effects [6–8]. As a complement to this work, Black et al. [9] thought that it should be possible to modify a conventional, nonselective NSAID to obtain COX-2 selectivity, and thus take advantage of a structural class with a well-established safety profile. They synthesised a series of COX-2 selective inhibitors based on the nonselective NSAID

Abbreviations: NSAIDs, nonsteroidal antiinflammatuvar drugs.
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indomethacin. In earlier studies, it was found that the COX-2 enzyme might have a larger active site than COX-1 [1]. Based on this hypothesis, they thought that it may be possible to increase the size of the indomethacin nucleus to produce a compound that would still fit into the COX-2 active site but not into the COX-1 active site, thus generating the desired selectivity. Replacement of the 4-chlorobenzoyl group in indomethacin with a 2,4,6-trichlorobenzoyl group led to the formation of a reasonably COX-2 selective compound L-748,780 [9] (*figure 1*). To pursue this method, a number of indole acetic acid analogues from Merck's sample collection were examined, resulting in the identification of benzoyl indole derivatives **1** and **2** as highly selective COX-2 inhibitors (*figure 1*). Several alkyl-substituted propanoic acids **3** of indomethacin were prepared by same authors, and it was found that the one with an alkyl-substituted side chain is the most promising (*figure 1*).

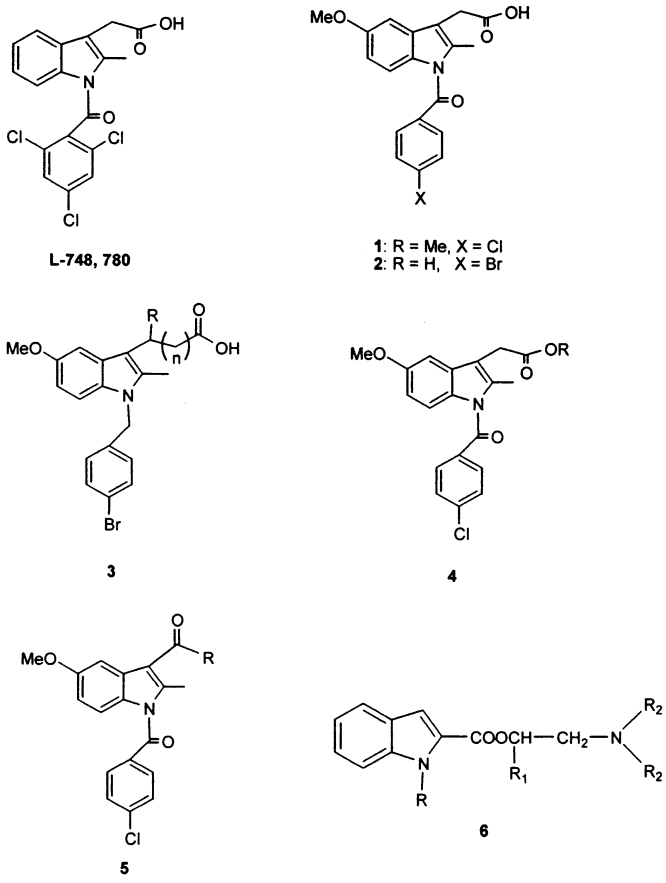
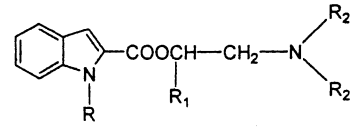


Fig. 1. Structures of designed compounds as COX-2 selective inhibitors.

Table I. Structural formulas of compounds **5**–**34**.



Compound	Type of salt	R	R_1	R_2
5	HCl	CH_2Ph	H	CH_3
6	HCl	CH_2Ph	H	C_2H_5
7	HCl	CH_2Ph	H	pyrrole
8	HCl	CH_2Ph	H	pyrimidine
9	HCl	CH_2Ph	CH_3	CH_3
10	CH_3I	CH_2Ph	H	CH_3
11	CH_3I	CH_2Ph	H	C_2H_5
12	CH_3I	CH_2Ph	H	pyrrole
13	CH_3I	CH_2Ph	H	pyrimidine
14	CH_3I	CH_2Ph	CH_3	CH_3
17	HCl	Ph	H	CH_3
18	HCl	Ph	H	C_2H_5
19	HCl	Ph	H	pyrrole
20	HCl	Ph	H	pyrimidine
21	HCl	Ph	CH_3	CH_3
22	HCl	Ph	H	piperazine
23	CH_3I	Ph	H	CH_3
24	CH_3I	Ph	H	C_2H_5
25	CH_3I	Ph	H	pyrrole
26	CH_3I	Ph	H	pyrimidine
27	CH_3I	Ph	CH_3	CH_3
29	HCl	H	H	C_2H_5
30	CH_3I	H	H	C_2H_5
31	HCl	H	H	pyrrole
32	HCl	H	H	pyrimidine
33	HCl	H	CH_3	CH_3
34	HCl	H	H	piperazine

Recently, Kalgutkar et al. [10] synthesised some ester **4** and amide **5** derivatives of indomethacin. They found that large alkyl, aryl aralkyl and heterocyclic esters or amides of indomethacin exhibit high potency and selectivity (*figure 1*).

These recent findings of indomethacin derivatives with a high degree of selectivity for COX-2 have led us to design potent and selective inhibitors **6** (see all structures of the compounds in *table I*) based on these structures (*figure 1*).

Docking simulations are widely used for screening of compound libraries to identify new drug leads, employing a simple model for rapid testing of thousands of compounds. Docking simulations are also useful for lead enhancement, using more detailed models to analyse the atomic interactions between inhibitors and target macromolecules [11].

Undoubtedly, a detailed three-dimensional (3D) picture of interactions between, synthesised indomethacin derivatives, COX-1 and COX-2 enzyme active sites would help us to determine the mechanism of activity.

In this study, the synthesised indole derivatives were docked in the cyclooxygenase channel using an in-house DOCK 4.0 program, in order to determine differences in binding modes of compounds in the cyclooxygenase channel.

2. Docking study

The docking program used was DOCK 4.0 developed by Kuntz et al. [12]. INSIGHT II (Molecular Simulation Incorporated) was used for visualisation and molecular modelling of the compounds. The 3D coordinates of COX-1 and COX-2 were obtained through the Internet at http://pdb.protein.osaka-u.ac.jp/pdb, the Research Collaboratory for Structural Bioinformatics (RCSB). Upon acquiring the COX-1 and COX-2 coordinates through the Internet, water molecules and ions were removed before the docking, The docking results were evaluated based on the method described in Ref. [13].

3. Results and discussion

3.1. Chemistry

The protection of the NH group of indoles is important in synthetic indole chemistry and several methods for the protection of indole nitrogen have been developed [14–16]. Among these, the benzyl group is found to be the most stable protecting group. In this study we have synthesised a 1-benzyl indole-2-carboxylic acid derivative by using the method of Murakami et al. [17]. The *N*-benzyl indole-2-carboxylic acid was prepared in good yields from the corresponding sodium salt of indole-2-carboxylic acid with benzyl chloride in dimethylformamide (*figure 2*).

Indole containing aryl substituents at the nitrogen are not easily accessible by previous synthetic methods [18]. In the 1970s, Ullmann's reaction (copper-catalysed condensation using aryl halides) proved to be an efficient

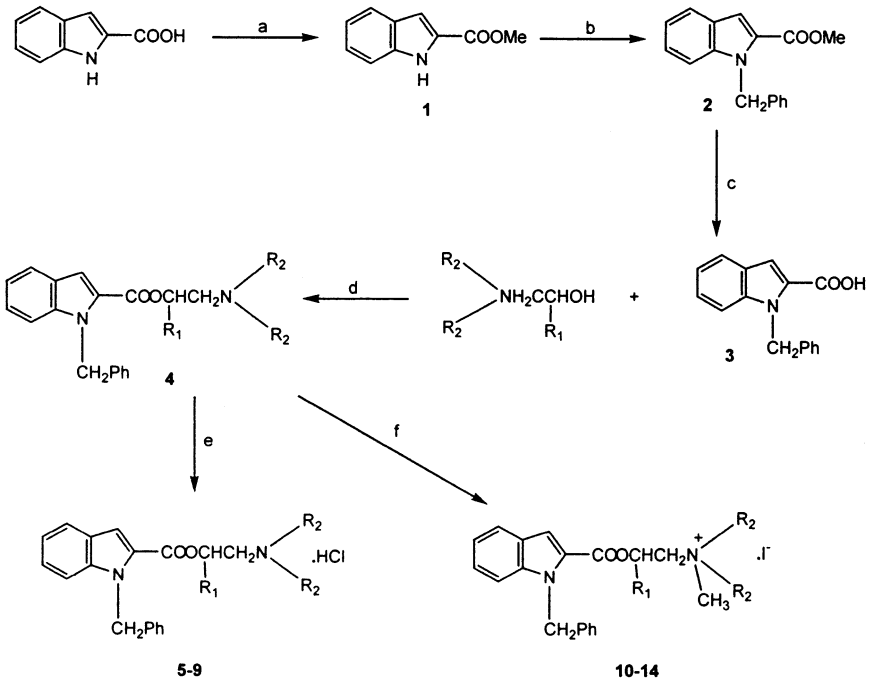


Figure 2. Synthesis of N-benzyl-indole-2-carboxylic acid eters. Reagents: (a) HCl gase/MeOH, rt; (b) $BrCH_2Ph$, NaH, DMF, rt; (c) 10% NaOH/MeOH, reflux; (d) 1, 1′-carbonyldiimidazole/DMF; (e) HCl gase/anhydrous diethylether, rt; (f) Mel/anhydrous diethylether, rt.

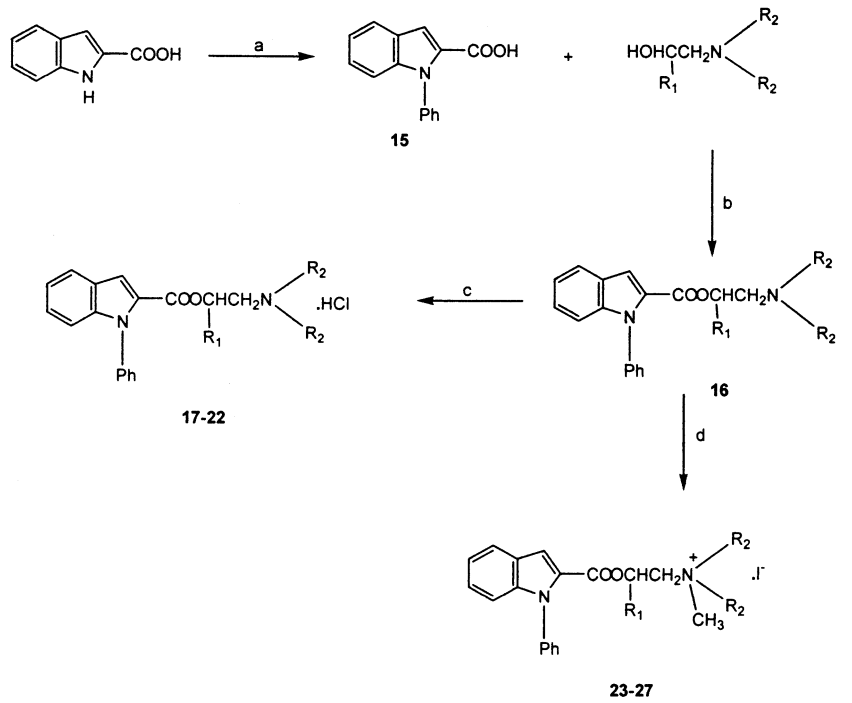


Figure 3. Synthesis of N-phenyl-indole-2-carboxylic acid esters. Reagents: (a) BrPh, anhydrous K_2CO_3, DMF, 154°C; (b) 1,1′-carbonyldiimidazole/DMF, rt; (c) HCl gase/anhydrous diethylether, rt; (d) Mel/anhydrous diethylether, rt.

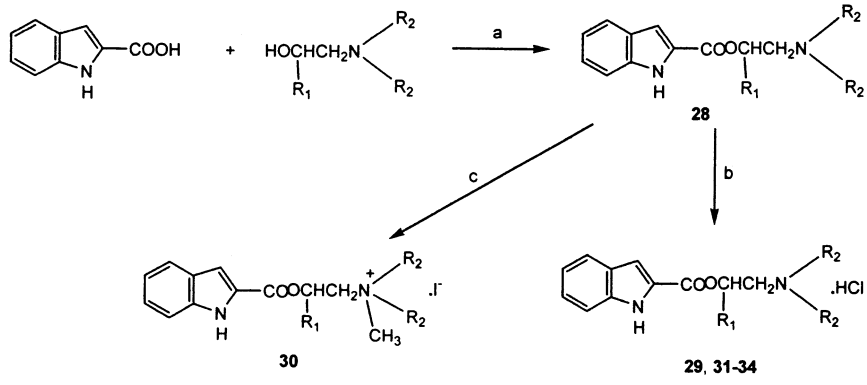


Figure 4. Synthesis of indole-2-carboxylic acid esters. Reagents: (a) 1,1′-carbonyldiimidazole, DMF, rt; (b) HCl gase/anhydrous diethylether, rt; (c) Mel/anhydrous diethylether, rt, in dark.

method for the synthesis of *N*-arylazoles and was used with success for the *N*-arylation of indole [19, 20]. In this work bromobenzene was chosen as the arylating agents for the synthesis of *N*-phenyl-indole-2-carboxylic acid derivatives. In this arylation, good yields of *N*-arylindole-2-carboxylic acids were obtained (*figure 3*). The catalyst used for the arylations was copper(II) oxide. It was reported that *N*′,*N*-dimethylformamide was the best solvent [21]. Therefore *N*,*N*-dimethylformamide was used as the reaction solvent to obtain good yield.

N-Phenyl or *N*-benzyl indole-2-carboxylic acid esters (*table I*) were prepared by the reaction of *N*-phenyl or *N*-benzyl indole-2-carboxylic acid with the coupling reagent 1,1′-carbonylbis(1*H*-imidazole) [22]. Ester synthesis by using this reagent was utilised because it provided an efficient way to synthesise and isolate the desired esters in high yield and purity (*figures 2–4*).

Spectroscopic methods and microanalysis confirmed the structure of the compounds mentioned. The purity of the compounds was checked by TLC on silica gel HF 254+366 nm and they have sharp melting points (m.p.). The UV spectra of the compounds show main intensive absorption bands between 291 and 301 nm. In the IR spectra of all the compounds, carbonyl-stretching bands belonging to ester were seen in addition to the carbonyl band at 1705–1725 cm^{-1}. In the ^{1}H-NMR spectra, benzylic protons at the indole ring were seen as a singlet at 5.8–5.9 ppm. In all the quaternary ammonium compounds, which have methyl halogen salts, methyl protons were seen as singlets at 2.9–3.2 ppm. The derivatives containing methyl substitution at the neighbouring protons to the ester carbonyl atom showed for the –CH– proton a quartet between 5.3 and 5.5 ppm and for the $–CH_3–$ proton a doublet at 1.20–1.35 ppm. The results of elemental analysis and mass spectroscopic analysis also confirmed the structures deduced.

3.2. Computational receptor docking studies

In the current discussion of this paper two different types of enzymes, COX-1 which should not be inhibited by a proposed compound, and COX-2, which should be inhibited by the proposed compound, are involved. Therefore, an ideal docking result of the proposed compound should be such that 'a proposed compound docked more firmly or inhibited more strongly the enzyme COX-2 than the enzyme COX-1.' Secondly, in order to perform a computer docking study an appropriate enzyme should be obtained. Protein Data Bank

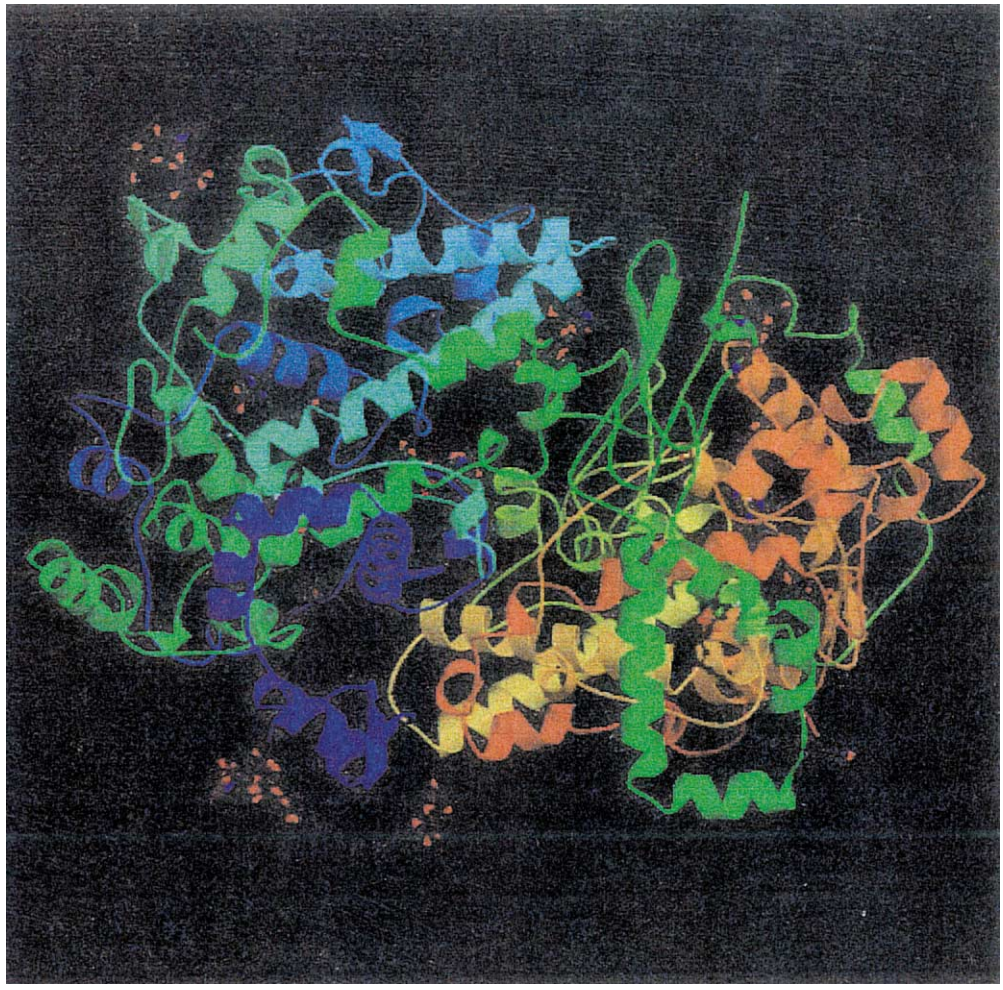


Figure 5.

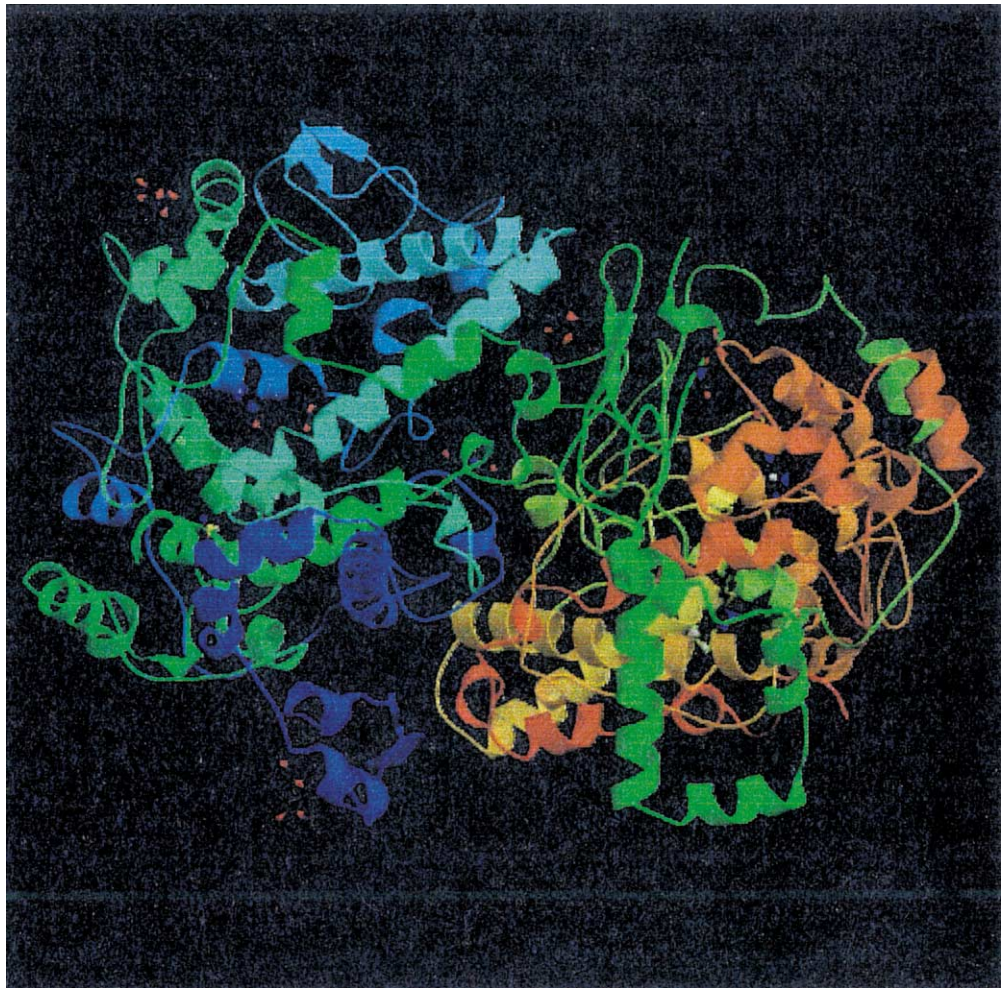


Figure 6.

(URL: http://www.rcsb.org/pdb/index.html) is one of the relevant Internet web sites where one can obtain over 12 000 protein structures. For COX-1 the one with pdb code 1cqe was used. The pdb file 1cqe is a complex of COX-1 with four ligands. The motif of the complex is shown in *figure 5*. In the COX-1 portion of the complex, five sheets, ten strands, 32 helices, 45 beta turns, seven gamma turns, one betabulge, five betahairpins, and five disulphide bridges are identified (*Figure 5*). Four types of ligands are clarified as NAG–NAG (*N*-acetyl-D-glucosamine), HEM (protoporphyrin IX containing Fe [Heme], BOG (b-octylglucoside), and FLP (flurbiprofen). Since flurbiprofen is a known nonsteroidal anti-inflammatory drug (NSAID), the vicinity where this flurbiprofen is situated should be an active site. Therefore, flurbiprofen was taken out of the pdb file of this COX-1 complex and treated as a so-called model inhibitor for the docking study.

For the COX-2 complex pdb code 6cox was selected and used for the docking study. The motif of the complex is shown in *Figure 6*. Three types of ligand were clarified as HEM (protoporphyrin IX Fe [Heme], NAG (*N*-acetyl-D-glucosamine), and s58 (1-phenylsulphonamide-3-trifluoromethyl-5-rabromophenylpyrazole).
Since s58 seems to play a role of inhibitor, the vicinity where this s58 is situated should be an active site. Therefore, s58 was taken out of the pdb file of this COX-2 complex and treated as the so-called model inhibitor for the docking study.

The next question is how one can judge from the computational docking result whether 'a proposed compound docked more firmly or inhibited more strongly

Table II. Docking results of indomethacin derivatives.

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
5	01. −31.01	17.N-2 with O-1 of Leu A321 (2.60)	−20.36	01. −35.95	23.N-2 with O-1 of Phe A350 (2.75)	444.65
	02. −30.90	18.N-2 with O-1 of Leu A321 (2.60)	−3.67	02. −35.88		
	03 −30.59	21.N-2 with O-2 of Ser A499 (2.60)	11.83	03. −35.53		
6	01. −4.38	15.N-1 with O-1 of Met A491 (2.69)	−2.59	01. −11.15	1.N-2 with O-1 of Leu A321 (2.97)	−11.15
	02. −4.22	15.N-2 with H-1 of Met A494 (1.73)	−2.59	02. −1014	5.N-2 with N-1 of Leu A321 (2.82)	−4.59
	03. −4.18	15.N-2 with O-2 of Ser A490 (0.37)	−2.59	03. −6.15	9.N-2 with H-1 of Tyr A324 (2.23)	−1.90
		12.N-2 with O-1 of Pro A497 (2.72)	−3.22			
		12.N-2 with O-1 of Ala A496 (2.03)	−3.22			
		13.N-2 with O-1 of Ile A492 (2.83)	−2.77			
		13.N-2 with O-1 of Met A491 (2.01)	−2.77			
7	01. −4.32	1. N-2 with O-1 of Ile A492 (2.20)	−4.32	01. −26.57	15. N-2 with O-1 of Tyr A324 (2.81)	34.69
	02. −4.26	1. O-1 with H-1 of Ser A499 (2.37)	−4.32	02. −22.44	15. N-2 with H-1 of Tyr A324 (1.94)	34.69
	03. −4.16	1. O-2 with N of Pro A497 (2.25)	−4.32	03. −21.86	19. N-2 with H-1 of Arg A89 (1.67)	71.69
		20.N-2 with O-3 of Glu A493 (2.19)	−2.6		19. N-2 with H-1 of Arg A89 (2.50)	71.69
		20.N-2 with H-1 of Arg A89 (0.82)	−2.6		14. N-2 with H-1 of Tyr A324 (2.25)	31.68
		20.N-1 with O-1 Tyr A324 (2.70)	−2.6			
		3. N-2 with O-1 Ile A492 (2.33)	−4.16			
		3. N-2 with O-1 Met A494 (2.83)	−4.16			
8	01. −3.22	9. N-1 with O-1 of Ser A499 (2.57)	−1.09	01. −8.56	6. N-2 with H-1 of Tyr A324 (2.36)	−1.00
	02. −2.75	9. N-2 with O-1 of Gly A495 (2.20)	−1.09	02. −5.21	7. N-2 with O-1 of Met A491 (2.51)	−0.45
	03. −2.46	8. N-2 with O-1 of Ser A322 (2.78)	−1.1	03. −4.69	9. N-2 with H-1 of His A58 (2.16)	2.45
		12.N-2 with O-1 of Val A318 (2.59)	−0.92			
9	01. −3.13 02. −3.10 03. −3.09	23.N-2 with O-1 of Gln A161 (2.96)	−1.96	01. −1.61	18.N-2 with O-1 of Leu A321 (2.68)	33.33
		23.N-2 with O-1 of Leu A321 (2.59)	−1.96	02. −0.88	18.N-2 with O-2 of Ser A499 (2.86)	33.33
		23.N-2 with O-2 of Ser A322 (2.42)	−1.96	03. −0.72	12.N-2 with H-1 of His A58 (2.14)	13.28

Table II. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		23.O-2 with H-1 of Tyr A324 (2.36)	−1.96		16.N-2 with O-1 of Ser A499 (2.63)	19.43
		14.N-2 with O-1 of Ile A492 (0.79)	−2.58			
		14.N-2 with O-1 of Met A491 (2.81)	−2.58			
		14.N-2 with H-1 of Ala A496 (1.38)	−2.58			
		6.N-2 with O-1 of Val A318 (2.27)	−2.9			
		6.N-2 with O-1of Ser A322 (2.24)	−2.9			

Note. (1) Energy represents the total binding energy between a ligand and a protein. (2) Details of circled numbers in H-bond column are explained in *figures. 4 and 9.* (3) Front numbers in the first energy column and in the H-bond column represent the docking result numbers which are in the order of the lowest energy first.

the enzyme COX-2 than the enzyme COX-1.' Akaho et al. proposed a method [13], and the modified version by the current authors are shown as follows:

1. After docking of a proposed compound against COX-1 and COX-2, examine the top 25 docking results which are arranged in the order of the one with the lowest energy first, and pick up for COX-1 and COX-2, respectively, to three docking results with negative binding energy (eliminate the one with positive binding energy) in the order to the one with more hydrogen bonds first creating List A.
2. Pick up from the List A the one with the highest number of hydrogen bonds (e.g. two hydrogen bonds) with the lowest binding energy. If this docking result belongs to COX-2 then one can say that a compound is COX-2 selective, or it is docked more firmly or inhibited more strongly with the enzyme COX-2 than the enzyme COX-1, visa versa.
3. If both COX-1 and COX-2 docking results show the same in terms of the number of hydrogen bonds and the energy, then pick up the one whose number of hydrogen bonds is the highest (e.g. two hydrogen bonds) with the next lowest binding energy. If this docking result belongs to COX-2 then one can say that a compound is COX-2 selective, or it is docked more firmly or inhibited more strongly with the enzyme COX-2 than the enzyme COX-1, visa versa.
4. As the next priority selection step, pick the one which has next highest number of hydrogen bonds (e.g. one hydrogen bond) with the lowest binding energy. If this docking result belongs to COX-2 then one can say that a compound is COX-2 selective, or it is docked more firmly or inhibited more strongly with enzyme COX-2 than the enzyme COX-1, visa versa.
5. If both COX-1 and COX-2 docking results show the same in terms of the number of hydrogen bonds and the energy, then pick up the one whose number of hydrogen bonds is next (e.g. one hydrogen bond) with the next lowest binding energy. If this docking result belongs to COX-2 then one can say that a compound is COX-2 selective, or it is docked more firmly or inhibited more strongly with the enzyme COX-2 than the enzyme COX-1, visa versa.

With this criteria in mind docking between indomethacin derivatives and COX-1 and COX-2 were performed. The protein structures of COX-1 and COX-2 were obtained from the Protein Data Bank previously mentioned. Based on the above criteria, dock results of synthesised indomethacin derivatives were evaluated and are shown in *tables 2–5.* Atoms responsible for H-bonds are shown in *figures 7–11.* After docking, compound **22** formed three H-bonds against COX-2, while its binding energy was −17.49 kcal mol^{-1}. Since its binding energies against COX-1 were positive it was eliminated from the evaluation list. Therefore, it was predicted that compound **22** was a COX-2 selective NSAID. The first location of the H-bond formed was between the piper-

Table III. Docking results of indomethacin derivatives.

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
29	01. −2.52	2.N-2 with H-1 of Leu A500 (2.37)	−2.51	01. −29.89	17.O-2 with H-1 of Tyr A324 (2.21)	−23.60
	02. −2.51	2.N-2 with H-1 of Ser A499 (2.09)	−2.51	02. −28.95	17.N-2 with H-1 of Arg A89 (2.44)	−23.60
	03. −2.47	2.N-2 with O-1 of Gly A495 (2.29)	−2.51	03. −28.64	24.N-2 with O-1 of Gly A488 (2.67)	325.32
		2.N-2 with O-1 of Ala A496 (1.11)	−2.51		24.O-1 with H-1 of Phe A487 (2.24)	325.32
		1.N-2 with O-1 of Gly A495 (2.38)	−2.52		25.N-2 with O-1 of Leu A321 (2.57)	445.94
		1.N-2 with O-1 of Ala A496 (1.13)	−2.52		25.N-2 with O-1 of Gln A161 (2.70)	445.94
		1.N-2 with H-1 of Leu A500 (2.13)	−2.52			
		15.N-2 with O-1 of Leu A321 (1.46)	−1.6			
		15.O-1 with H-1 of Tyr A324 (2.33)	−1.6			
10	01. 0.95	16.N-1 with O-1 of Tyr A324 (2.42)	1.62	01. −23.66	13.O-2 with H-1 of Arg A89 (2.44)	2.57
	02. 1.02	16.O-1 with H-1 of Tyr A324 (2.03)	1.62	02. −23.64	14.O-2 with H-1 of Arg A89 (2.44)	5.50
	03. 1.21	7.N-1 with O-1 of Tyr A324 (2.42)	1.36	03. −23.37	15.O-2 with H-1 of Arg A89 (2.44)	7.96
		18.N-1 with O-1 of Ser A499 (2.25)	1.68			
11	01. 0.64	15. N-1 with O-1 of Gly A495 (2.98)	0.95	01. −35.13	24. O-2 with H-1 of Arg A89 (2.29)	29.46
	02. 0.55	15. O-1 with H-1 of Ala A496 (2.38)	0.95	02. −34.56		
	03. 0.60	3. O-1 with H-1 of Tyr A324 (1.78)	0.6	03. −31.85		
		6.N-1 with O-1 of Met A491 (2.62)	0.67			
30	01. 3.34	20.N-1 with O-1 of Ser A499 (2.74)	4.95	01. −22.73	10.O-2 with H-1 of Asp A484 (2.41)	−17.76
	02. 3.66	21.N-1 with O-1 of Ile A492 (2.71)	5.02	02. −22.01	12.O-2 with H-1 of Asp A484 (2.41)	−17.07
	03. 3.73			03. −21.90	13.O-2 with H-1 of Asp A484 (2.41)	−17.04
23	01. 3.43	13.N-1 with S of Met A491 (3.68)	4.40	01. −23.05		
	02. 3.57	13.N-1 with O-1 of Met A491 (2.89)	4.40	02. −22.85		
	03. 3.60	14.N-1 with S of Met A491 (3.68)	4.44	03. −18.72	no hydrogen bond	
		14.N-1 with O-1 of Met A491 (2.89)	4.44			
		25.N-1 with O-1 of Ile A492 (2.89)	5.14			

Table III. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		25.N-1 with O-1 of Met A491 (2.60)	5.14			
24	01. 2.31	2.O-1 with H-1 of Tyr A324 (2.25)	2.92	01. −7.75		
	02. 2.92	4.O-2 with H-1 of Tyr A324 (2.25)	3.20	02. −5.00	no hydrogen bond	
	03. 3.11	7.O-1 with H-1 of Tyr A324 (2.25)	3.33	03. 1.36		
12	01. −1.99	19.N-1 with O-1 of Tyr A324 (1.32)	0.57	01. −30.65	25.O-1 with H-1 of His A58 (2.42)	947.68
	02. −1.89	19.O-1 with H-1 of His A59 (2.80)	0.57	02. −30.30		
	03. −1.05			03. −29.35		
25	01. −0.74	1.N-1 with O-1 of Met A491 (2.88)	−0.74	01. −25.01		
	02. −0.47	1.O-2 with H-1 of Met A494 (2.46)	−0.74	02. −24.95		
	03. −0.38	1.O-2 with H-1 of Gly A495 (1.98)	−0.74	03. −22.20		
		4.N-1 with O-1 of Met A491 (2.88)	−0.29			
		4.O-2 with H-1 of Met A494 (2.46)	−0.29		no hydrogen bond	
		4.O-2 with H-1 of Gly A495 (1.98)	−0.29			
		6.N-1 with O-1 of Met A491 (2.88)	−0.17			
		6.O-2 with H-1 of Met A494 (2.46)	−0.17			
		6.O-2 with H-1 of Gly A495 (1.98)	−0.17			
13	01. −1.31	23.O-2 with H-1 of Gly A495 (2.01)	0.55	01. −22.88		
	02. −1.29	23.O-2 with H-1 of Met A494 (1.06)	0.55	02. −20.83		
	03. −0.96	23.N-1 with O-1 of Met A491 (1.72)	0.55	03. −19.58	no hydrogen bond	
		11.N-1 with O-1 of Tyr A354 (2.90)	0.33			
		11.N-1 with O-1 of Ser A499 (2.86)	0.33			
		15.O-1 with H-1 of Tyr A324 (1.05)	0.46			
26	01. −1.80	21.O-1 with H-1 of Ala A496 (2.34)	0.97	01. −22.08		
	02. −1.53	21.O-2 with H-1 of Ala A496 (2.47)	0.97	02. −8.11	no hydrogen bond	
	03. −1.09	21.O-2 with H-1 of Gly A495 (1.49)	0.97	03. −4.51		

Table III. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		9.N-1 with O-1 of Met A491 (2.91)	−0.43			
14	01. −2.87	23.O-1 with H-1 of Tyr A89 (1.88)	−1.80	01. −15.98		
	02. −2.87	23.O-2 with H-1 of Arg A324 (2.33)	−1.80	02. −15.93	no hydrogen bond	
	03. −2.69	21.O-2 with H-1 of Gly A495 (2.11)	−1.82	03. −15.76		
27	01. −2.20	20.N-1 with O-1 of Met A491 (2.67)	−1.77	01. −23.70		
	02. −2.15	23.O-2 with H-1 of Trp A356 (2.29)	−1.72	02. −22.59	no hydrogen bond	
	03. −1.97	25.N-1 with O-1 of Ser A499 (2.90)	−1.59	03. −22.42		

Note. (1) Energy represents the total binding energy between a ligand and a protein. (2) Details of circled numbers in H-bond column are explained in *figures. 5, 7, and 9.* (3) Front numbers in the first energy column and in the H-bond column represent the docking result numbers which are in order of the lowest energy first.

azine hydrogen of compound **22** and the carbonyl oxygen of Phe A487 with its distance being 1.93 Å (*figure 13*). The second location of the H-bond formed was between the carbonyl oxygen of compound **22** and the amine hydrogen of Phe A487 with its distance being 2.22 Å. It was also found that compounds **30** (*figure 14*) and **34** (*figure 15*) had more H-bonds against COX-2 than COX-1 and predicted to be a COX-2 selective NSAID (*figure 12*).

3.3. Biological assays

Compounds **22**, **30** and **34** were tested for the inhibition of purified human COX-2 and ovine COX-1 by using thin layer chromatography (TLC) assay [23]. These compounds were chosen for the biological assays because of their significant binding capability to the COX-2 enzyme, according to molecular docking results. Biological activity tests were applied by comparing the COX-2 inhibitory effects of these compounds with indomethacin. Unfortunately, none of them showed any inhibitory effect concentration up to 50 μM. The results are shown in *table VI*. Although the present studies indicate that the compounds containing large alkyl, aryl, aryalkyl, and heterocyclic esters and amides exhibit high potency and selectivity [23], the IC_{50} values of our test compounds suggest that not all carboxylate-containing NSAIDs will be converted into COX-2 inhibitors by esterification.

In our studies, molecular docking results indicate the binding capability of the compounds to the enzyme active site and the results, which we have obtained from the activity tests, show COX-2 enzyme inhibitory capacity. Therefore, as it is mentioned in general, it is not always possible to obtain positive correlation between activity and docking results.

4. Experimental protocols

Indole-2-carboxylic acid, 1,1′-carbonyldiimidazole, 2-hydroxyethylpiperazine from Fluka; benzyl bromide, bromobenzene, anhydrous $CaCl_2$, anhydrous K_2CO_3, NaOH, isopropanol, hexanes, ether, methanol, acetone, ethylacetate, hydrogen chloride, ethanol from Merck and dimethylaminoethanol, 2-hydroxyethylpyrolidine, dimethylamino-2-propanol, 2-hydroxyethylpiperidin, 2-hydroxyethylpiperazine, dimethylamine, potassium bromide, sodium hydride were purchased from Aldrich.

Melting points were recorded in a Buchi SMP 20 melting point apparatus. ^{1}H-NMR spectra were consistent with molecular structures and were recorded in

Table IV. Docking results of indomethacin derivatives.

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
17	01. −2.10	25.N-1 with O-1 of Met A491 (2.19)	−0.19	01. −36.52	25.N-2 with O-1 of Tyr A324 (2.56)	78.43
	02. −1.65	25.N-2 with O-2 of Ser A490 (2.19)	−0.19	02. −36.49	25.O-2 with H-1 of Tyr A324 (2.48)	78.43
	03 −1.63	25.N-2 with S of Met A494 (2.68)	−0.19	03 −36.30	21.N-2 with H-1 of His A58 (2.50)	−16.14
		25.O-2 with H-1 of Met A494 (2.25)	−0.19			
		25.O-2 with H-1 of Gly A495 (2.31)	−0.19			
		1.N-2 with O-1 of Gly A495 (2.64)	−2.10			
		1.N-2 with O-1 of Met A494 (2.93)	−2.10			
		1.N-2 with O-1 of Met A491 (2.88)	−2.10			
		19.N-2 with O-1 of Met A491 (1.55)	−1.05			
		19.N-2 with S of Met A491 (2.87)	−1.05			
18	01. −3.67	13.N-2 with O-2 of Ser A322 (2.53)	−2.44	01. −25.89	22.N-2 with O-1 of Tyr A324 (2.58)	32.38
	02 −3.38	13.N-2 with O-1 of Leu A321 (2.76)	−2.44	02. −25.22	22.N-2 with H-1 of Arg A482 (1.97)	32.38
	03. −3.31	13.N-2 with O-1 of Ser A322 (2.66)	−2.44	03. −23.69	22.N-2 with H-1 of Tyr A324 (1.82)	32.38
		17.N-2 with O-1 of Met A491 (2.56)	−2.18		23.O-2 with N of Pro A483 (2.52)	76.41
		17.N-2 with O-1 of Ile A492 (2.02)	−2.18		23.O-1 with N-2 of His A58 (2.96)	76.41
		17.N-1 with O-1 of Tyr A324 (2.59)	−2.18		17.N-2 with H-1 of Tyr A324 (2.50)	−9.50
		8.N-2 with O-1 of Met A494 (1.46)	−2.80			
		8.N-1 with O-1 of Gly A495 (2.74)	−2.80			
19	01. −2.80	3.N-2 with O-1 of Ser A499 (2.55)	−2.59	01. −32.66	7.N-2 with H-1 of Arg A89 (2.08)	−20.68
	02. −2.62	3.N-2 with O-1 of Gly A495 (1.80)	−2.59	02. −32.23	7.O-2 with H-1 of Arg A89 (2.46)	−20.68
	03. −2.59	3.N-1 with S of Met A491 (3.24)	−2.59	03. −26.50	1.O-2 with H-1 of Arg A89 (2.46)	−32.66
		3.N-1 with O-1 of Met A491 (2.94)	−2.59		3.O-2 with H-1 of Arg A89 (2.46)	−26.50
		19.N-2 with S of Met A491 (2.38)	−1.82			
		19.N-2 with O-1 of Leu A353 (2.20)	−1.82			
		19.N-2 with H-1 of Trp A356 (2.43)	−1.82			

Table IV. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		19.N-1 with O-1 of Met A491 (2.17)	−1.82			
		12.N-2 with O-1 of Val A318 (0.90)	−2.06			
		12.N-2 with O-1 of Ser A322 (2.70)	−2.06			
		12.N-1 with O-1 of Tyr A324 (2.73)	−2.06			
20	01. −3.10	1.N-1 with O-1 of Tyr A354 (2.83)	−3.10	01. −30.25	20.N-2 with O-1 of Tyr A354 (2.61)	66.53
	02. −3.04	1.N-2 with O-1 of Tyr A354 (1.60)	−3.10	02. −29.43	20.N-2 with O-1 of Ser A499 (2.95)	66.53
	03. −2/61	1.N-2 with O-1 of Tyr A317 (2.31)	−3.10	03. −7.82	7.N-2 with H-1 of His A58 (2.13)	7.21
		24.N-2 with O-1 Phe A498 (2.78)	−1.13		11.N-2 with O-1 of Ser A499 (2.71)	47.22
		24.N-2 with O-2 Ser A499 (1.51)	−1.13			
		24.N-2 with O-1 of Ser A499 (2.97)	−1.13			
		13.N-2 with O-1 of Tyr A324 (1.73)	−1.74			
		13.N-2 with H-1 of Arg A89 (2.35)	−1.74			
21	01. −2.84	22.N-1 with O-1 of Met A491 (2.60)	−2.21	01. −31.67	6.O-1 with H-1 of Arg A89 (2.34)	−25.73
	02. −2.82	22.N-2 with O-1 of Ile A492 (2.81)	−2.21	02. −31.22	10.N-2 with H-1 of His A58 (2.24)	−20.28
	03. −2.77	22.N-2 with O-1 of Glu A489 (2.31)	−2.21	03. −31.20	21N-2 with O-1 of Tyr A324 (2.84)	23.47
		10.N-2 with O-1 of Ala A496 (2.71)	−2.56			
		10.N-2 with O-1 of Gly A495 (2.65)	−2.56			
		11.N-2 with O-1 of Ile A492 (2.96)	−2.52			
		11.N-2 with O-2 of Ser A90 (2.37)	−2.52			
22	01. 0.01	2.N-2 with O-1 of Met A491 (3.00)	0.12	01. −24.34	4.H-1 with O-1 of Phe A487 (1.93)	−17.49
	02. 0.12	2.N-2 with O-1 of Gly A495 (2.77)	0.12	02. −20.71	4.H-1 with N-6 of Phe A487 (2.22)	−17.49
	03. 0.57	2.N-2 with O-1 of Met A494 (2.65)	0.12	03. −20.39	4.N-3 with H-1 of Phe A487 (2.17)	−17.49
		2.H-1 with O-1 of Ala A496 (2.25)	0.12		18.H-1 with N of Phe A350 (2.30)	−3.33
		2.H-1 with N of Ser A499 (2.11)	0.12		18.H-1 with O-1 of Phe A487 (1.82)	−3.33
		15.N-2 with O-2 of Ser A322 (2.61)	1.41		18.N-3 with H-1 of Phe A487 (2.17)	−3.33

Table IV. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		15.N-2 with O-1 of Ser A322 (2.18)	1.41		1.N-2 with H-1 of His A58 (2.50)	−24.34
		15.H-5 with N of Gln A319 (2.48)	1.41		1.N-3 with H-1 of Phe A487 (2.12)	−24.34
		15.N-3 with H-1 of Ser A322 (1.24)	1.41			
		15.N-3 with H-1 of Gly A323 (1.96)	1.41			
		5.N-2 with O-1 of Leu A321 (2.02)	0.99			
		5.N-2 with O-2 of Ser A322 (2.95)	0.99			

Note. (1) Energy represents the total binding energy between a ligand and a protein. (2) Details of circled numbers in H-bond column are explained in *figures 6 and 9*. (3) Front numbers in the first energy column and in the H-bond column represent the docking result numbers which are in order of the lowest energy first.

a Bruker AC 400 spectrometer. Elemental analyses were performed in a LECO-932 CHNS-O Elemental Analyser. The IR values were determined with a Pye Unicam 1025 spectrophotometer. The UV spectral analyses were measured in a Shimadzu 240 B. High resolution mass spectra were run in a Fisions instrument, VG Platform II LC MS spectrometer.

4.1. Chemistry

4.1.1. Methyl indole-2-carboxylate (**1**)

Indole-2-carboxylic acid (25.0 g, 0.15 mol) was dissolved in 10% HCl in MeOH and refluxed at 65 °C for 1 h. The solvent was evaporated under vacuum and neutralised by K_2CO_3. Crystallisation by ethanol gave pale white crystals (18.0 g, 66.29%): m.p. 152 °C.

4.1.2. 1-Benzyl methyl indole-2-carboxylate (**2**)

Compound **1** (17.5 g, 0.10 mol) and 4.4 g (0.18 mol) 50% NaH were dissolved in 10.0 mL of DMF and stirred at room temperature (r.t.) 30 min. Benzyl bromide (12.1 mL, 0.10 mol) was added dropwise and stirred at r.t. for 48 h. Then the reaction mixture was poured into ice-water. Neutralisation with acetic acid gave an oily compound which was crystallised by ethanol–water to gave pale yellow crystals (16.0 g, 60.30%): m.p. 83 °C.

4.1.3. 1-Benzyl indole-2-carboxylic acid (**3**)

Compound **2** (15.0 g, 0.56 mol) was dissolved in 50 mL of MeOH and was added to 50 mL of 10% NaOH solution. The reaction mixture was stirred at 65 °C for 2 h. The reaction mixture was cooled down to r.t. and neutralised by AcOH to give a white precipitate. Crystallisation with MeOH–water gave white crystals (13.0 g, 91.60%): m.p. 191 °C.

4.1.4. 1-Benzyl indole-2-dialkylaminoalkyl carboxylate hydrogen chloride salts (**5**–**9**)

Compound **3** (1.0 g, 3.9 mmol) was dissolved in 10.0 mL of DMF. 1,1′-Carbonyldiimidazole (0.6 g, 3.9 mmol) was added in portions. The solution was stirred in ambient temperature for 1 h. The dialkylaminoalcohols (3.9 mmol) was added and heated at 50–60 °C for 4–8 h. At the end of the reaction, the reaction mixture was cooled to r.t. and was neutralised with saturated NaOH solution. The oily residue was extracted with ether and was washed with 5% NaCl solution and then water. The organic phase was dried under anhydrous $CaCl_2$ and filtered. The filtrate was evaporated to dryness and the residue was dissolved in anhydrous diethylether, which was saturated with HCl gas to give the desired final compounds.

5: Yield 0.6500 g, 46.62%; m.p. 128 °C; MS; m/z: 322 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 2.85 (s, 6H, N(CH_3)$_2$), 3.40 (t, 2H, CH_2N(CH$_3$)$_2$), 4.65 (t, 2H, COOCH_2), 5.85 (s, 2H, CH_2Ph), 7.05–7.75 (m, 10H, aromatic protons), 12.85 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{20}H_{22}N_2O_2$·HCl) C, H, N, O.

Table V. Docking results of indomethacin derivatives.

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
31	01. −1.83	5.N-2 with O-1 of Met A494 (2.96)	−1.78	01. −31.43	25.N-2 with O-1 of Leu A321 (2.36)	645.36
	02. −1.82	5.N-2 with O-2 of Ser A490 (2.72)	−1.78	02. −30.70	25.N-2 with O-1 of Gln A161 (2.82)	645.36
	03. −1.82	5.N-2 with O-1 of Met A491 (2.90)	−1.78	03. −28.36	1.O-2 with H-1 of His A58 (2.47)	−31.43
		5.N-1 with O-1 of Met A491 (1.80)	−1.78		2.O-2 with H-1 of His A58 (2.47)	−30.70
		5.N-1 with O-1 of Ile A492 (2.13)	−1.78			
		5.N-1 with H-1 of Ala A496 (2.12)	−1.78			
		1.N-1 with O-1 of Ile A492 (2.13)	−1.83			
		1.N-1 with O-1 of Met A491 (1.80)	−1.83			
		1.N-2 with O-1 of Met A494 (2.52)	−1.83			
		1.N-1 with H-1 of Ala A496 (2.12)	−1.83			
		11.N-2 with O-1 of Ile A492 (2.65)	−1.59			
		11.N-2 with O-1 of Met A491 (1.01)	−1.59			
		11.N-2 with H-1 of Gly A495 (1.69)	−1.59			
32	01. −1.50	25. N-2 with H-2 of Tyr A354 (2.26)	0.48	01. −29.77	5. O-1 with H-1 of Phe A487 (2.45)	−26.89
	02. −1.43	25. N-2 with O-1 of Phe A350 (1.18)	0.48	02. −27.98	6.N-2 with H-1 of His A58 (2.40)	−25.68
	03. −1.38	25. N-2 with O-1 of Glu A349 (2.64)	0.48	03. −27.19	8. O-1 with H-1 of Phe A487 (2.45)	−25.21
		1.N-1 with O-1 of Met A491 (2.80)	−1.50			
		1.N-2 with S of Met A491 (3.65)	−1.50			
		6.N-2 with O-1 of Tyr A354 (1.93)	−1.30			
		6.N-2 with O-1 of Tyr A317 (2.85)	−1.30			
33	01. −2.00	8.N-2 with O-2 of Tyr A354 (2.80)	−1.76	01. −27.53	25.N-2 with S of Met A491 (2.53)	574.93
	02. −1.97	8.N-2 with O-1 of Leu A353 (2.90)	−1.76	02. −27.25	25.N-2 with H-1 of Trp A356 (1.72)	574.93
	03. −1.92	8.N-1 with S of Met A491 (3.63)	−1.76	03. −27.25	21.N-2 with H-1 of Tyr A324 (2.49)	−12.96
		3.N-2 with S of Met A491 (3.67)	−1.92		23.O-1 with H-1 of Phe A487 (2.47)	2.38
		3.N-2 with O-1 of Met A491 (2.74)	−1.92			
		9.N-2 with O-1 of Gly A495 (2.12)	−1.73			

Table V. (*Continued*)

Indomethacin derivatives	Against COX-1			Against COX-2		
	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})	Energy (kcal mol^{-1})	H-bond (distance A)	Energy (kcal mol^{-1})
		9.N-2 with O-1 of Met A494 (2.58)	−1.73			
34	01. 1.43	6.N-1 with O-1 of Ser A499 (2.17)	1.60	01. −29.09	1.O-2 with H-1 of His A58 (2.50)	−29.09
	02. 1.48	6.N-2 with O-1 of Ser A499 (2.35)	1.60	02. −28.31	1.O-1 with H-1 of Phe A487 (2.47)	−29.09
	03. 1.49	6.N-2 with O-1 of Ala A496 (2.46)	1.60	03. −28.06	4.O-2 with H-1 of His A58 (2.50)	−25.61
		6.H-1 with N-6 of Phe A498 (1.72)	1.60		4.O-1 with H-1 of Phe A487 (2.47)	−25.61
		8.N-2 with O-1 of Tyr A324 (1.06)	1.63		5.O-2 with H-1 of His A58 (2.50)	
		8.O-2 with H-1 of Tyr A324 (2.32)	1.63		5.O-1 with H-1 of Phe A487 (2.47)	
		8.N-3 with H-1 of Arg A89 (2.25)	1.63			
		12.N-2 with H-1 of Ala A496 (1.45)	1.81			
		12.N-2 with O-1 of Ile A492 (1.07)	1.81			
		12.H-1 with N-3 of Arg A89 (1.84)	1.81			

Note: (1) Energy represents the total binding energy between a ligand and a protein. (2) Details of circled numbers in H-bond column are explained in *figures 8 and 9*. (3) Front numbers in the first energy column and in the H-bond column represent the docking result numbers which are in order of the lowest energy first.

6: Yield 0.9686 g, 63.05%; m.p. 144–145 °C; MS; *m*/*z*: 350 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 1.30 (s, 6H, N(CH$_2$*CH$_3$*)$_2$), 3.15 (q, 4H, CH$_2$N(*CH$_2$*CH$_3$)$_2$), 3.40 (t, 4H, *CH$_2$*N(CH$_2$CH$_3$)$_2$), 4.75 (t, 2H, COO*CH$_2$*), 5.80 (s, 2H, *CH$_2$*Ph), 6.90–7.80 (m, 10H, aromatic protons), 12.30 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{22}H_{26}N_2O_2$·HCl) C, H, N, O.

7: Yield 0.5529 g, 36.18%; m.p. 136–138 °C; MS; *m*/*z*: 348 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 0.70–5.10 (m, 12H, pyrrolidine and *CH$_2$*-pyrrolidine), 5.80 (s, 2H, *CH$_2$*Ph), 6.90–7.80 (m, 10H, aromatic protons), 12.45 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1725. Anal. ($C_{22}H_{24}N_2O_2$·HCl) C, H, N, O.

8: Yield 0.5669 g, 35.79%; m.p. 154 °C; MS; *m*/*z*: 362 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 0.70–3.60 (m, 12H, piperidine and *CH$_2$*-piperidine), 4.90 (t, 2H, COO*CH$_2$*), 5.80 (s, 2H, *CH$_2$*Ph), 7.05–7.75 (m, 10H, aromatic protons), 12.20 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{23}H_{26}N_2O_2$·HCl) C, H, N, O.

9: Yield 0.5212 g, 35.20%; m.p. 124–125 °C; MS; *m*/*z*: 336 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 1.30 (d, 3H, COOCH(*CH$_3$*), 2.80 (s, 6H, N(*CH$_3$*)$_2$), 3.40 (t, 2H, *CH$_2$*N(CH$_3$)$_2$), 5.40 (m, 1H, COO*CH*(CH$_3$), 5.85 (s, 2H, *CH$_2$*Ph), 7.05–7.80 (m, 10H, aromatic protons), 9.12 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{21}H_{24}N_2O_2$·HCl·0.1H$_2$O) C, H, N, O.

*4.1.5. 1-Benzyl indole-2-dialkylaminoalkyl carboxylate methyl iodide salts (**10**–**14**)*

The intermediate esters were synthesised as described for compounds **5**–**9**. The solution of esters in ether was treated with alkyl iodide (1.20 mmol) at r.t. and was stirred overnight with care to protect from light. The quaternary methyl iodide compounds were obtained as a thick white precipitate.

10: Yield 0.6208 g, 33.68%; m.p. 219–221 °C; MS; *m*/*z*: 322 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 3.20 (s, 9H, N(*CH$_3$*)$_3$), 3.80 (t, 2H, *CH$_2$*N(CH$_3$)$_3$), 4.75 (t, 2H, COO*CH$_2$*), 5.85 (s, 2H, *CH$_2$*Ph), 7.05–7.75 (m, 10H,

aromatic protons), 12.85 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{20}H_{22}N_2O_2 \cdot CH_3I$) C, H, N, O.

11: Yield 0.8165 g, 41.79%; m.p. 200–201 °C; MS; *m/z*: 350 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.30 (s, 6H, $N(CH_2CH_3)_2$), 3.20 (s, 3H, N^+–CH_3), 3.60 (q, 4H, $N(CH_2CH_3)_2$), 4.00 (t, 2H, $CH_2N(CH_2CH_3)_2$), 4.70 (t, 2H, $COOCH_2$), 5.80 (s, 2H, CH_2Ph), 6.80–7.90 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{22}H_{26}N_2O_2 \cdot CH_3I$) C, H, N, O.

12: Yield 0.6594 g, 33.87%; m.p. 230–231 °C; MS; *m/z*: 348 [M^+], ^{1}H-NMR (DMSO-d_6): δ 2.20–2.45 (t, 4H, pyrrolidine), 3.20 (s, 3H, N^+– CH_3), 3.45–3.60 (t, 4H, pyrrolidine potons), 4.00 (t, 2H, CH_2-pyrrolidine), 4.80 (t, 2H, $COOCH_2$), 5.80 (s, 2H, CH_2Ph), 6.90–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1705. Anal. ($C_{22}H_{24}N_2O_2 \cdot CH_3I$) C, H, N, O.

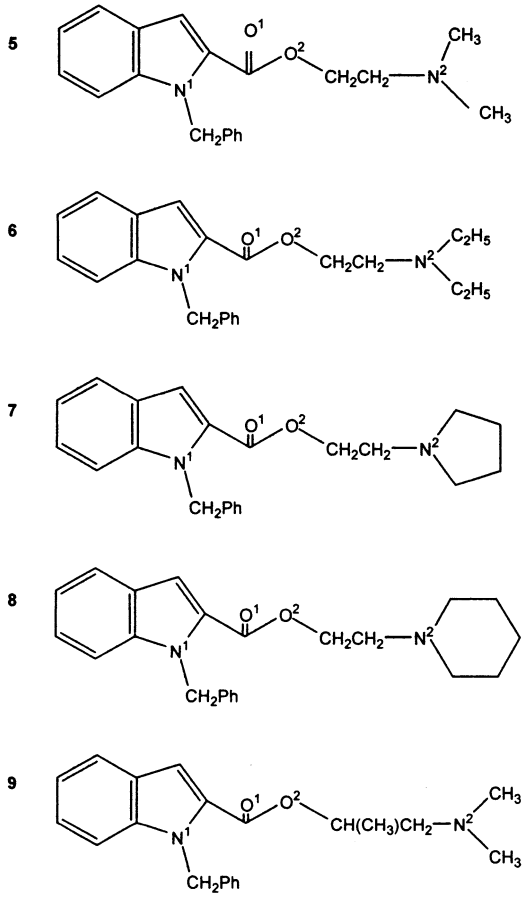


Figure 7. Indomethacin derivatives (compounds **5-9**) studied for docking mode and atoms responsible for H-bonding with COX. Note: Details of atoms with superscripts 1 and 2 are indicated in Table II.

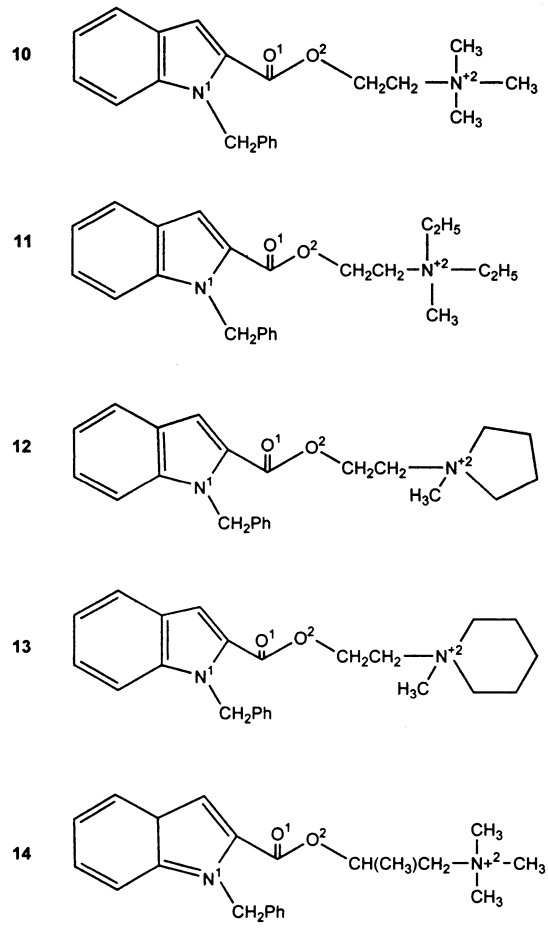


Figure 8. Indomethacin derivatives (compounds **10-14**) studied for docking mode and atoms responsible for H-bonding with COX. Note: Details of atoms with superscripts 1 and 2 are indicated in Table III.

13: Yield 0.5293 g, 26.43%; m.p. 234–236 °C; MS; *m/z*: 362 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.60–2.10 (t, 4H, piperidine protons), 3.20 (s, 3H, N^+–CH_3), 3.60 (t, 4H, piperidine protons), 4.00–4.12 (t, 4H, piperidine protons and CH_2-piperidine), 4.80 (t, 2H, $COOCH_2$), 5.80 (s, 2H, CH_2Ph), 7.05–7.75 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{23}H_{26}N_2O_2 \cdot CH_3I$) C, H, N, O.

14: Yield 0.7752 g, 40.82%; m.p. 212 °C; MS; *m/z*: 336 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.35 (d, 3H, $COOCH(CH_3)$, 3.15 (s, 9H, $N(CH_3)_3$), 3.40 (t, 2H, $CH_2N(CH_3)_3$), 5.50 (m, 1H, $COOCH(CH_3)$, 5.85 (s, 2H, CH_2Ph), 7.05–7.75 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1705. Anal. ($C_{21}H_{24}N_2O_2 \cdot CH_3I$) C, H, N, O.

*4.1.6. 1-Phenyl indole-2-carboxylic acid (**15**)*

Indole-2-carboxylic acid (8.0 g, 0.05 mol) was dissolved in 10.0 mL of DMF and then 7.0 g of anhydrous K_2CO_3, 0.25 g CuO and 5.2 mL (0.05 mol) bromobenzene were added. The reaction mixture was refluxed for 24 h at 154 °C. At the end of reaction, the mixture was cooled and added into ice-water. The water layer was washed with $CHCl_3$ (3×100 mL). The water layer was acidified with concentrated HCl and let to stand overnight. The precipitated *N*-phenyl indole-2-carboxylic acid was filtered off and purified by crystallisation from MeOH–H_2O (4.95 g, 42.03%): m.p. 176 °C.

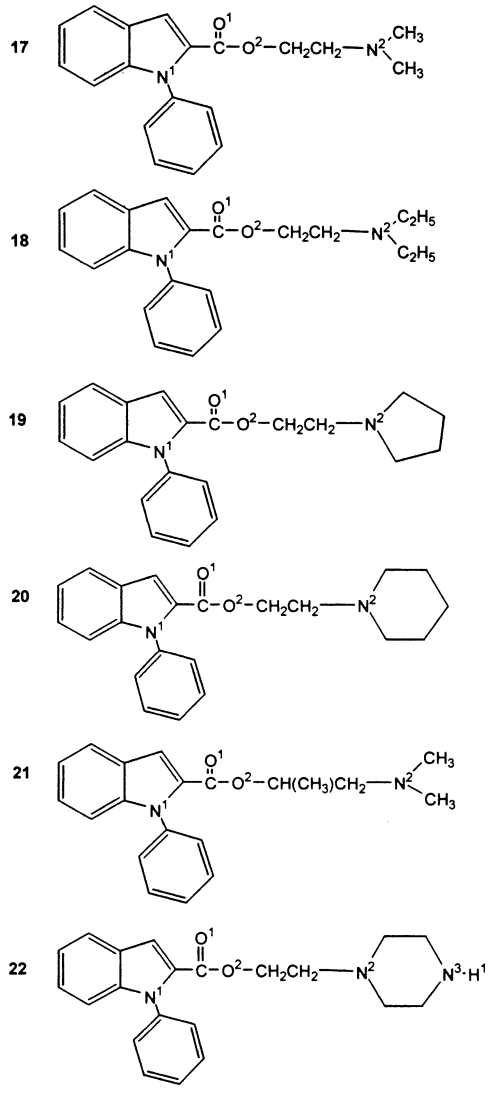


Figure 9. Indomethacin derivatives (compounds **17-22**) studied for docking mode and atoms responsible for H-bonding with COX. Note: Details of atoms with superscripts 1 and 2 are indicated in Table IV.

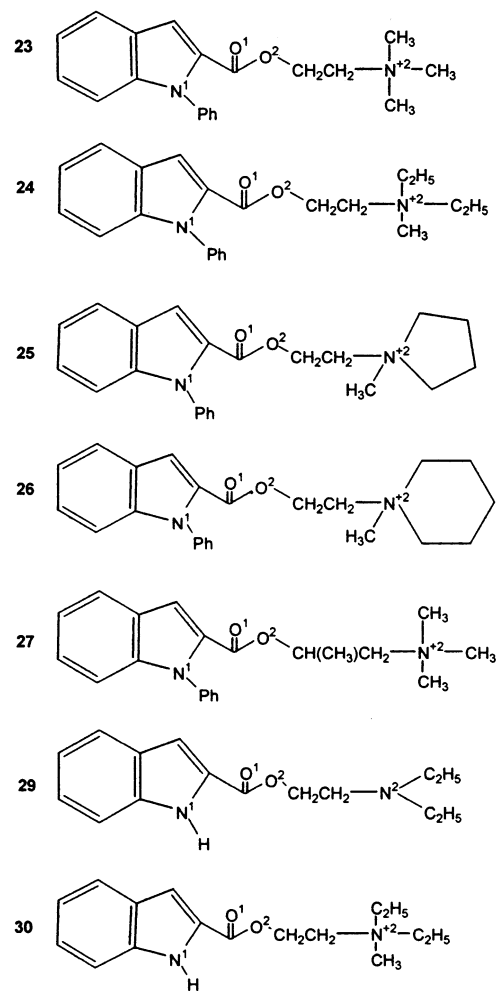


Figure 10. Indomethacin derivatives (compounds **23-27** and **29**, **30**) studied for docking mode and atoms responsible for H-bonding with COX. Note: Details of atoms with superscripts 1 and 2 are indicated in Table III.

*4.1.7. 1-Phenyl indole-2-dialkylaminoalkyl carboxylate hydrogen chloride salts (**17–22**)*

Compounds **17–22** were synthesised as described for compounds **5–9**. Compound **15** (0.4 g, 1.69 mmol) and 0.274 g (1.69 mmol) 1,1′-carbonyldiimidazole were used.

17: Yield 0.3229 g, 55.53%; m.p. 178–180 °C; MS; *m/z*: 308 [M^+], ^{1}H-NMR (DMSO-d_6): δ 2.80 (s, 6H, $N(CH_3)_2$), 3.30 (t, 2H, $CH_2N(CH_3)_2$), 4.50 (t, 2H, $COOCH_2$), 7.05–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1725. Anal. ($C_{19}H_{20}N_2O_2 \cdot HCl$) C, H, N, O.

18: Yield 0.2403 g, 38.22%; m.p. 167–168 °C; MS; *m/z*: 336 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 1.45 (t, 6H, N(CH_2CH_3)$_2$), 3.20 (q, 4H, N(CH_2CH_3)$_2$), 3.35 (t, 2H, CH_2N(CH_2CH_3)$_2$), 4.75 (t, 2H, COOCH_2), 7.05–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{21}H_{24}N_2O_2$·HCl) C, H, N, O.

19: Yield 0.2351 g, 37.59%; m.p. 175 °C; MS; *m/z*: 334 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 2.10–2.20 (t, 4H, pyrrolidine protons), 2.80 and 3.40 (t, 4H, pyrrolidine protons), 3.85 (t, 2H, CH_2-pyrrolidine), 4.75 (t, 2H, COOCH_2), 6.90–7.80 (m, 10H, aromatic protons), 12.70 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{21}H_{22}N_2O_2$·HCl) C, H, N, O.

20: Yield 0.2664 g, 41.05%; m.p. 182–184 °C; MS; *m/z*: 348 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 1.20–3.63 (m, 10H, piperidine protons), 3.45 (t, 2H, CH_2-piperidine), 4.75 (t, 2H, COOCH_2), 7.00–7.80 (m, 10H, aromatic protons), 12.60 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{22}H_{24}N_2O_2$·HCl·0.1H_2O) C, H, N, O.

21: Yield 0.2155 g, 35.61%; m.p. 185–187 °C; MS; *m/z*: 322 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 1.20 (d, 3H, COOCH(CH_3), 2.75 (s, 6H, N(CH_3)$_2$), 3.35 (t, 2H, CH_2N(CH_3)$_2$), 5.30 (m, 1H, COOCH(CH_3), 7.05–7.80 (m, 10H, aromatic protons), 10.40 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{20}H_{22}N_2O_2$·HCl) C, H, N, O.

22: Yield 0.2802 g, 39.35%; m.p. 171–172 °C; MS; *m/z*: 349 [M$^+$], ^{1}H-NMR (DMSO-d_6): δ 2.10 (t, 4H, piperazine potons), 3.00–3.30 (t, 4H, piperazine po-

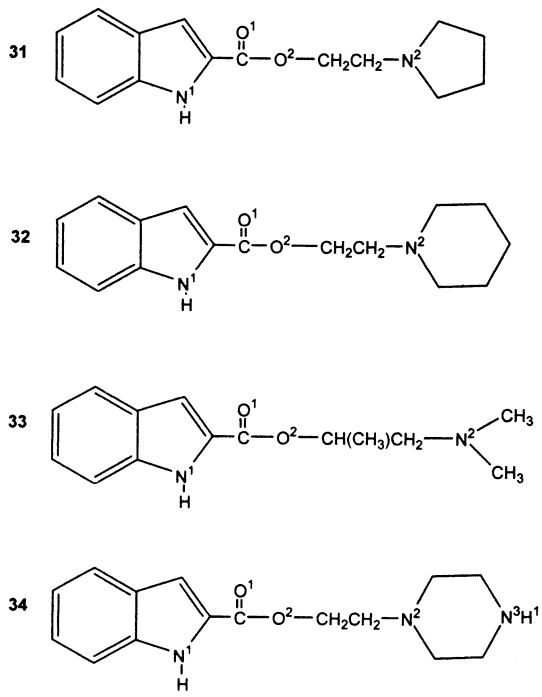


Figure 11. Indomethacin derivatives (compounds **31-34**) studied for docking mode and atoms responsible for H-bonding with COX. Note: Details of atoms with superscripts 1 and 2 are indicated in Table V.

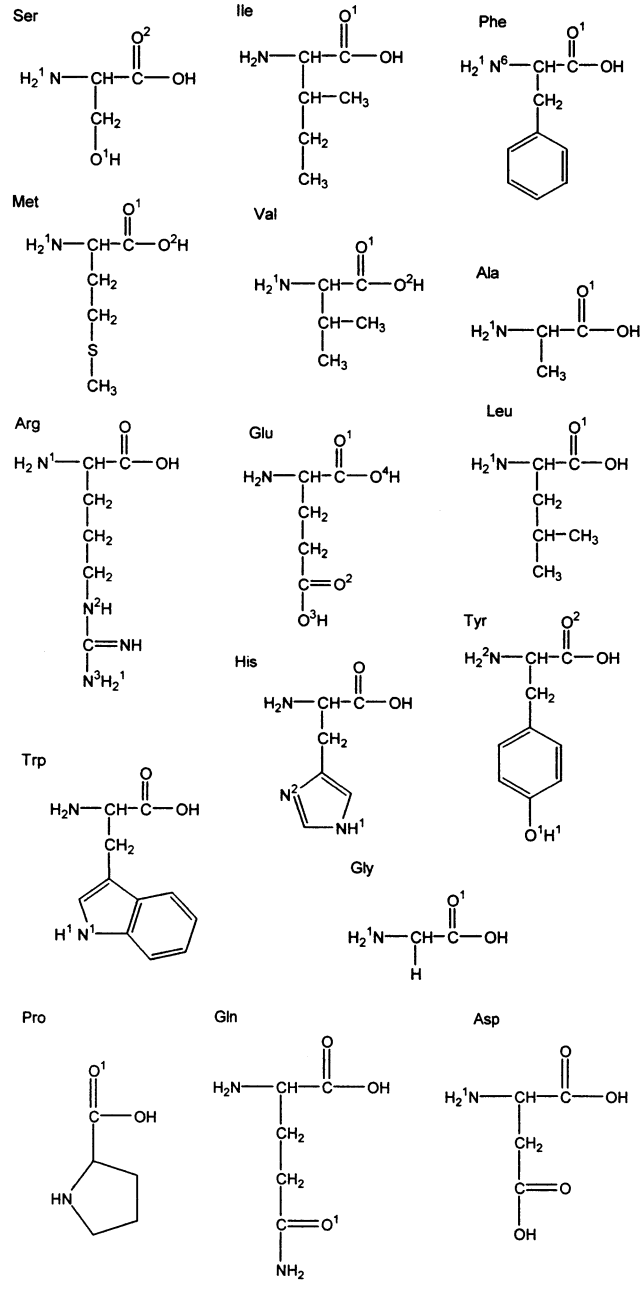


Figure 12. Amino acid residues involved in hydrogen bonding: superscripts indicate the atom responsible for hydrogen bonding with ligand molecules.

tons), 3.80 (t, 4H, CH_2-piperazine), 4.65 (t, 2H, COOCH_2), 7.05–7.85 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{21}H_{23}N_3O_2 \cdot 2HCl$) C, H, N, O.

*4.1.8. 1-Benzyl indole-2-dialkylaminoalkyl carboxylate methyl iodide salts (**23**–**27**)*

Compounds **23**–**27** were synthesised as described for compounds **10**–**14**.

23: Yield 0.2688 g, 35.40%; m.p. 208 °C; MS; *m*/*z*: 308 [M^+], ^{1}H-NMR (DMSO-d_6): δ 3.10 (s, 9H, N(CH_3)$_3$), 3.70 (t, 2H, CH_2N(CH_3)$_2$), 4.60 (t, 2H, COOCH_2), 7.05–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{19}H_{20}N_2O_2 \cdot CH_3I$) C, H, N, O.

24: Yield 0.374 g, 46.37%; m.p. 197–198 °C; MS; *m*/*z*: 336 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.20 (t, 6H, N(CH_2CH_3)$_2$), 3.00 (s, 3H, N^+–CH_3), 3.35 (q, 4H, N(CH_2CH_3)$_2$), 3.65 (t, 2H, CH_2N(CH_2CH_3)$_2$), 4.60 (t, 2H, COOCH_2), 7.15–7.85 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1725. Anal. ($C_{21}H_{24}N_2O_2 \cdot CH_3I$) C, H, N, O.

25: Yield 0.314 g, 39.10%; m.p. 202–203 °C; MS; *m*/*z*: 334 [M^+], ^{1}H-NMR (DMSO-d_6): δ 2.20 (m, 4H, pyrrolidine protons), 3.20 (s, 3H, N^+–CH_3), 3.65 (t, 4H, pyrrolidine protons), 3.80 (t, 2H, CH_2-pyrrolidine), 4.70 (t, 2H, COOCH_2), 7.05–7.85 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{21}H_{22}N_2O_2 \cdot CH_3I$) C, H, N, O.

26: Yield 0.3142 g, 38.00%; m.p. 213–215 °C; MS; *m*/*z*: 348 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.65–1.78 (m,

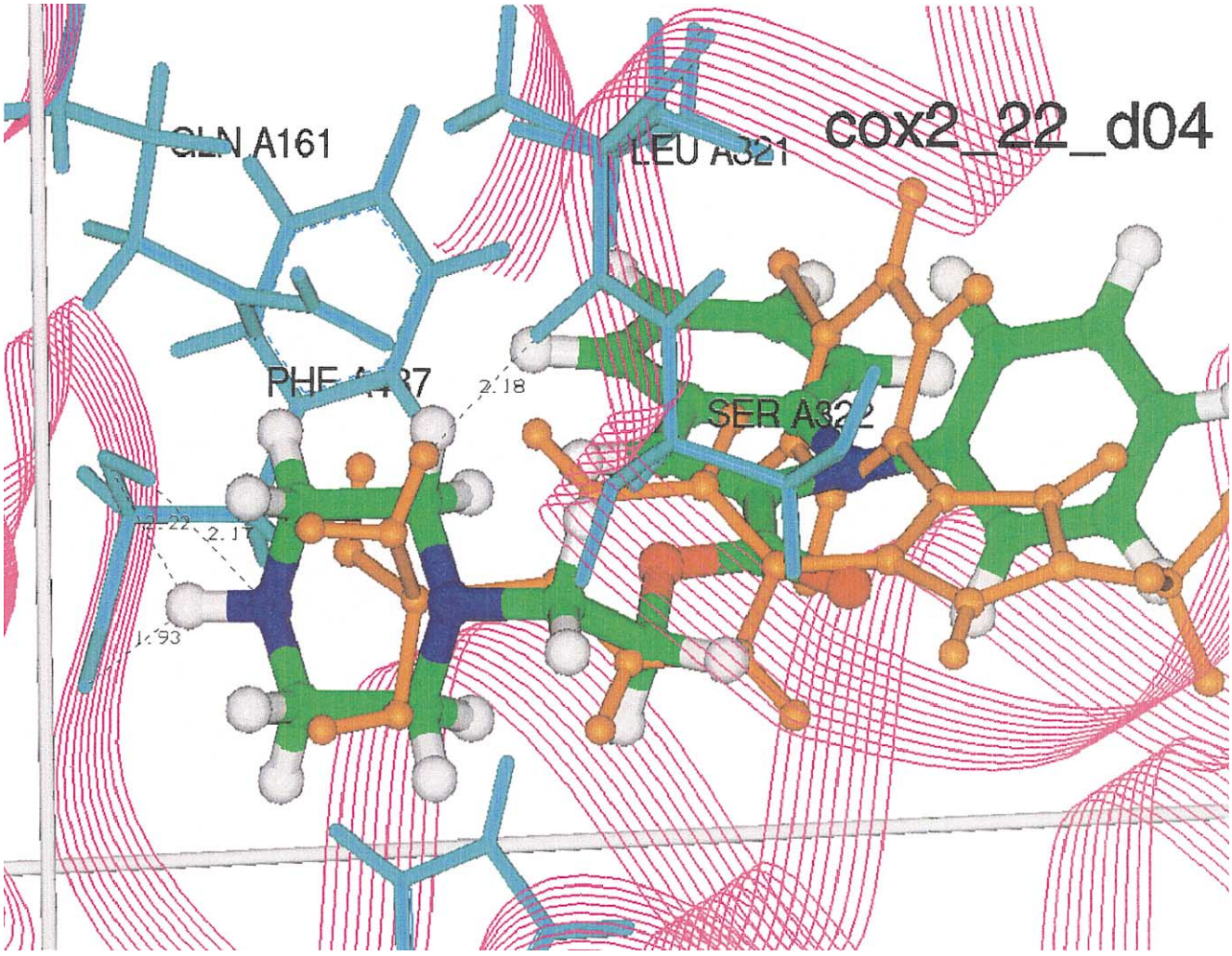


Figure 13.

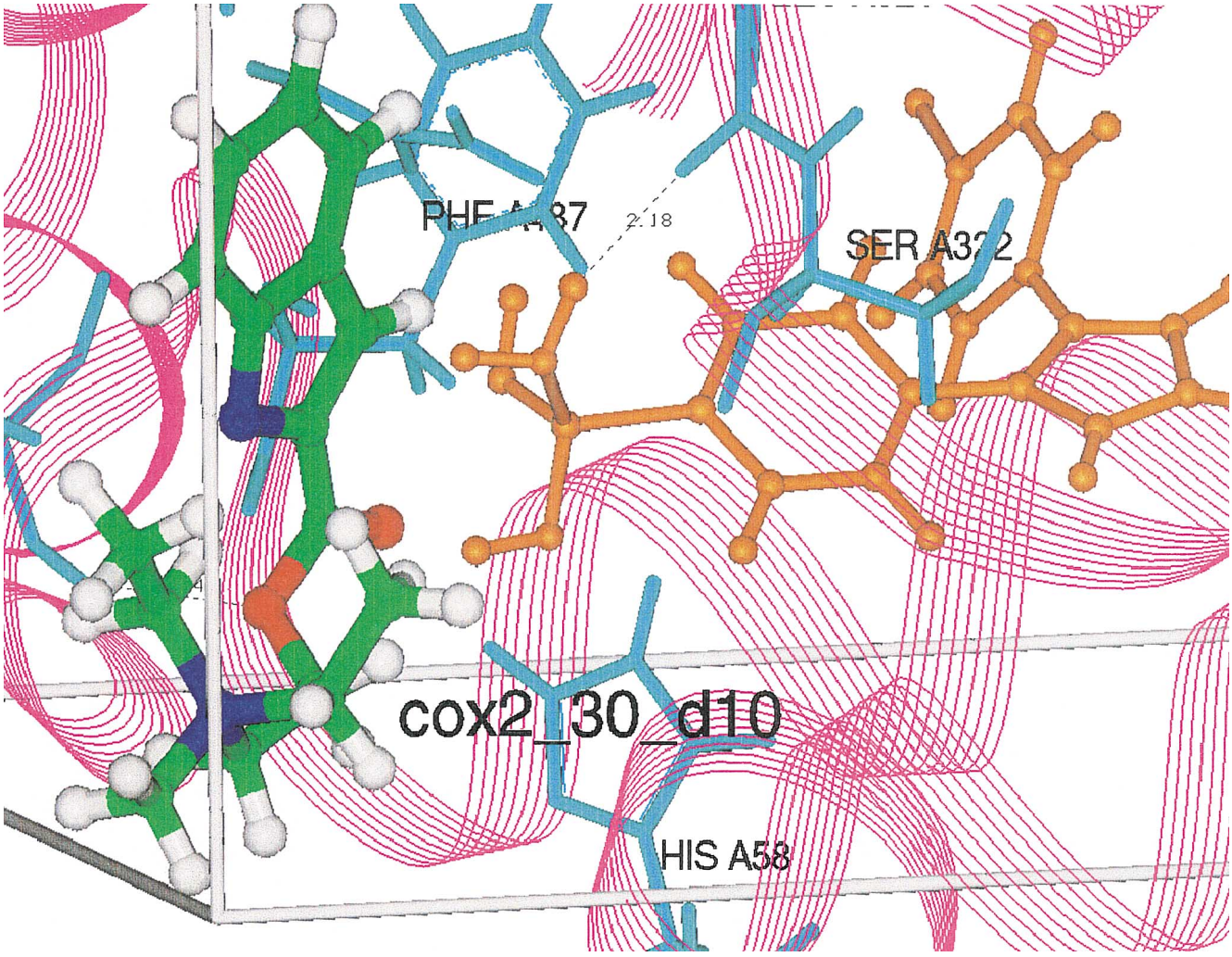


Figure 14.

6H, piperidine protons), 1.85–1.98 (m, 4H, piperidine protons), 3.25 (s, 3H, N^+–CH_3), 3.40 (t, 2H, CH_2–piperidine), 4.65 (t, 2H, COOCH_2), 7.00–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{22}H_{24}N_2O_2 \cdot CH_3I$) C, H, N, O.

27: Yield 0.3699 g, 47.25%; m.p. 223–225 °C; MS; m/z: 322 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.30 (d, 3H, COOCH(CH_3), 3.15 (s, 9H, N(CH_3)$_3$), 3.50 (t, 2H, CH_2N(CH_3)$_3$), 5.40 (m, 1H, COOCH(CH_3), 7.05–7.80 (m, 10H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{20}H_{23}N_2O_2 \cdot CH_3I$) C, H, N, O.

*4.1.9. N–H Indole-2-dialkylaminoalkyl carboxylate hydrogen chloride salts (**29**–**33**)*

Compound **29** was synthesised as described for compounds **5**–**9**. Indole-2-carboxylic acid (1.0 g, 6.2 mmol) and 1.1 g (6.2 mmol) 1,1'-carbonyldiimidazole were used.

29: Yield 1.1631 g, 56.25%; m.p. 178 °C; MS; m/z: 260 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.35 (t, 6H, N(CH_2CH_3)$_2$), 3.20 (q, 4H, CH_2N(CH_2CH_3)$_2$), 3.35 (t, 2H, CH_2N(CH_2CH_3)$_2$), 4.55 (t, 2H, COOCH_2), 7.05–7.70 (m, 5H, aromatic protons), 12.00 (s, 1H, NH). IR

ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{15}H_{20}N_2O_2 \cdot 2HCl \cdot 0.1H_2O$) C, H, N, O.

31: Yield 1.3781 g, 67.05%; MS; *m/z*: 258 [M^+], ^{1}H-NMR (DMSO-d_6): δ 0.70–5.10 (m, 10H, pyrrolidine protons and CH_2-pyrrolidine), 4.65 (t, 2H, COOCH_2), 6.90–7.80 (m, 5H, aromatic protons), 12.45 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1720. Anal. ($C_{15}H_{18}N_2O_2 \cdot 2HCl$) C, H, N, O.

32: Yield 1.4077 g, 65.71%; MS; *m/z*: 272 [M^+], ^{1}H-NMR (DMSO-d_6): δ 0.70–3.60 (m, 12H, piperidine protons and CH_2-piperidine), 4.90 (t, 2H, COOCH_2), 7.05–7.75 (m, 5H, aromatic protons), 12.20 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{16}H_{20}N_2O_2 \cdot 2HCl$) C, H, N, O.

33: Yield 1.2890 g, 65.07%; MS; *m/z*: 246 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.30 (d, 3H, COOCH(CH_3), 2.80 (s, 6H, N(CH_3)$_2$), 3.40 (t, 2H, CH_2N(CH_3)$_2$), 5.40 (m, 1H, COOCH(CH_3), 7.05–7.80 (m, 5H, aromatic protons), 9.12 (s, 1H, NH). IR ν_{max} (KBr, cm^{-1}) 1715. Anal. ($C_{14}H_{18}N_2O_2 \cdot 2HCl \cdot 0.2H_2O$) C, H, N, O.

34: Yield 1.4925 g, 62.85%; MS; *m/z*: 273 [M^+], ^{1}H-NMR (DMSO-d_6): δ 2.10 (t, 4H, piperazine protons), 3.00–3.30 (t, 4H, piperazine protons), 3.80 (t, 2H, CH_2-piperazine), 4.65 (t, 2H, COOCH_2), 7.05–7.85 (m, 5H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{15}H_{19}N_3O_2 \cdot 3HCl$) C, H, N, O.

*4.1.10. N–H Indole-2-diethylaminoethyl carboxylate methyl iodide salt (**30**)*

Compound **30** was synthesised as described for compounds **10**–**14**.

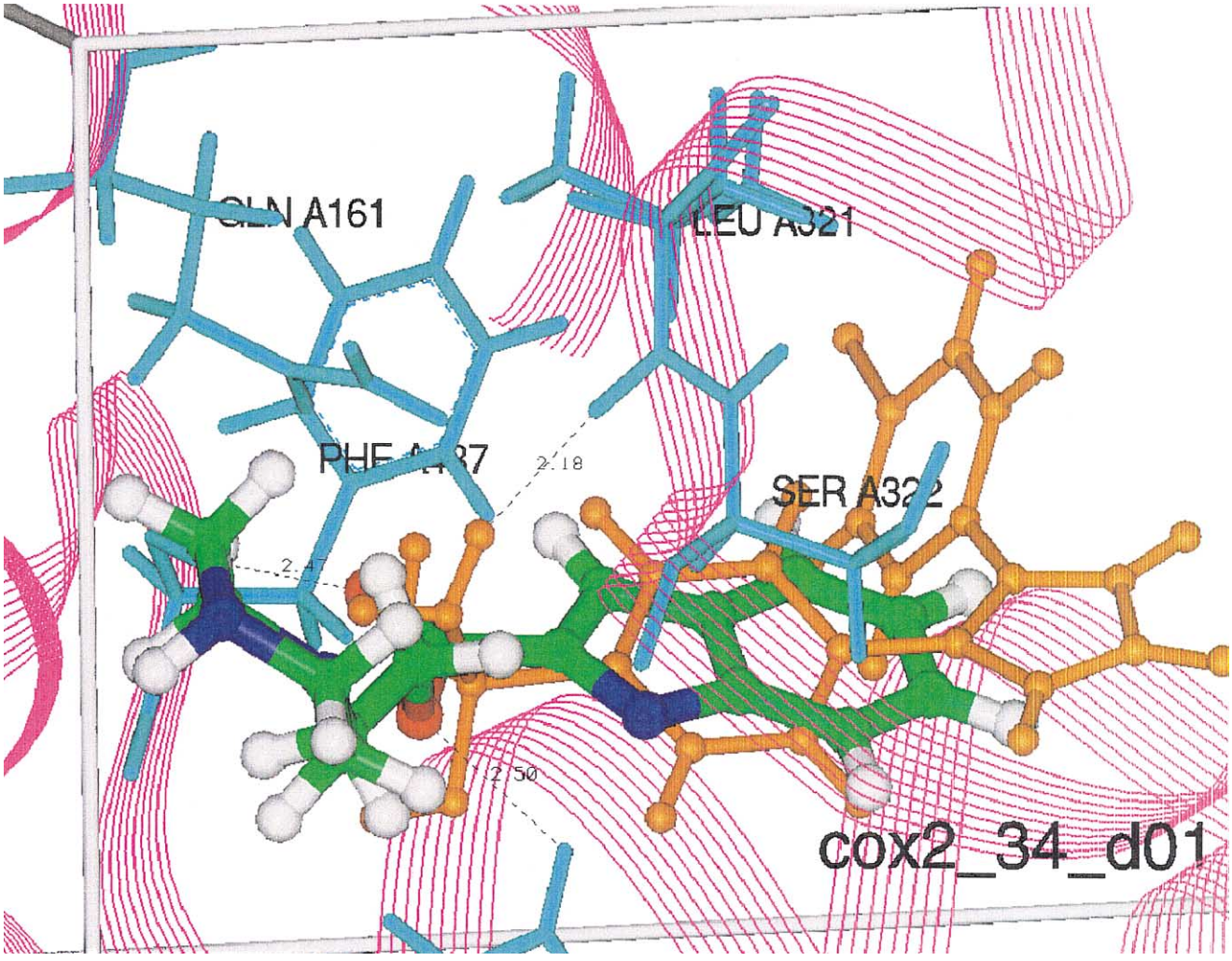
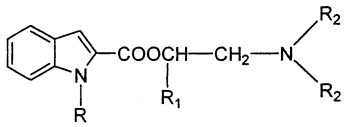


Figure 15.

Table VI. Structural formulas of compounds **5**–**34**.



Compound	IC_{50} (μM)	
	COX-1	COX-2
NS-398	0.12	>66
SC-299	0.060	>66
Indomethacin	0.75	0.05
22	50.0	ND
30	50.0	ND
34	50.0	ND

ND = Not determined.

30: Yield 1.1544 g, 46.25%; m.p. 204–205 °C; MS; *m/z*: 260 [M^+], ^{1}H-NMR (DMSO-d_6): δ 1.10 (t, 6H, $N(CH_2CH_3)_2$), 3.14 (s, 3H, N^+–CH_3), 3.50 (q, 4H, $CH_2N(CH_2CH_3)_2$), 3.75 (t, 2H, $CH_2N(CH_2CH_3)_2$), 4.70 (t, 2H, $COOCH_2$), 7.05–7.30 (m, 5H, aromatic protons). IR ν_{max} (KBr, cm^{-1}) 1710. Anal. ($C_{15}H_{20}N_2O_2 \cdot CH_3I$) C, H, N, O.

4.2. Enzyme assays

For enzymologic method and chemicals see Ref. [23].

Acknowledgements

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