Research Article

# Carprofen: a theoretical mechanistic study to investigate the impact of hydrophobic interactions of alkyl groups on modulation of COX-1/2 binding selectivity

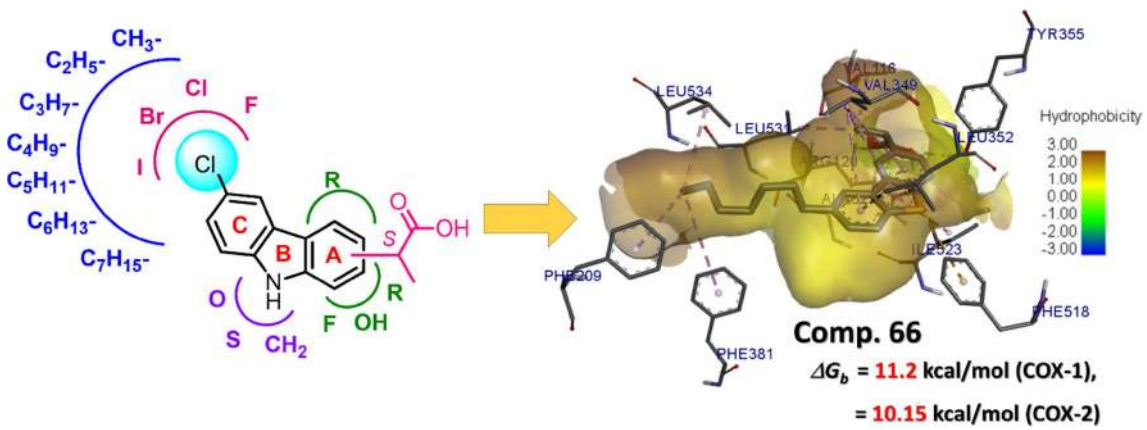
**Ahmed M. Gouda**[1] · **Faisal A. Almalki**[2]



**Abstract**

Development of selective COX-1 and COX-2 was successfully used to overcome GIT side effects of the classical NSAIDs. Currently, virtual screening and docking study were extensively used to design of new potent and safe drug candidates. In this study, four series of carprofen derivatives were designed by isosteric replacement of the –NH– with –O–, –S– and $–CH_2–$ groups. More than 90 derivatives bearing different alkyl substituents were designed in this study. AutoDock software was used to explore the binding mode, affinity and selectivity of the designed analogs to COX1/2. The results revealed that position and length of alkyl substituents have remarkable effect on the binding mode. Substitution with alkyl groups at C1 and C6 of the four scaffolds improved binding to COX-1, while at C4 enhanced COX-2 binding affinity. Compound **66** displayed the highest binding affinity for COX-1 and COX-2 with $\Delta G_b$ of 11.2 and 10.15 kcal/mol, respectively. Compound **56** and **99** displayed the highest potential selectivity to COX-1 and COX-2, respectively. Drug-likeness study and synthetic accessibility were evaluated for the most promising analogs. Compound **29** displayed drug-likeness score (DLS) of 0.96 compared to 0.3 for carprofen. Taken together, these results highlighted the importance of hydrophobic interactions in modulation of COX-1/2 binding selectivity of new potential NSAIDs.

**Graphical abstract**

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## 1 Introduction

Development of selective cyclooxygenase-2 (COX-2) inhibitors was suggested as an attractive strategy to overcome GIT side effects of traditional NSAIDs [1–5]. Diverse scaffolds with potential COX inhibitory activity were reported with the great advances in heterocyclic synthesis [6, 7]. Valdecoxib **1** (Fig. 1) displayed high selectivity for COX-2 with selectivity index higher than 3930 [8]. Although coxibs displayed safe GIT profile, but their use was associated with cardiovascular and renal side effects [9]. However, selective COX-2 inhibitors still attract the attention due to their antiproliferative activity [10–12].

On the other hand, mofezolac **2** (Fig. 1) was recently developed as selective COX-1 inhibitor with analgesic anti-inflammatory potential and low ulcerogenic liability [13]. Mofezolac has also displayed promising anticancer activity in intestinal cancer [14, 15].

Nonselective NSAIDs such as profens still represent one of the most widely used drugs. Of these NSAIDs, carprofen was used in humans for more than 10 years before withdrawal from the market on commercial grounds. Later, it was used in animals to treat pain and inflammation. The incidence of GIT side effects with carprofen resembles those of other nonselective COX inhibitors [16, 17].

Mechanistic studies of carprofen were reported in several reports. In whole blood assay, carprofen displayed higher selectivity for COX-1 over COX-2 [18]. However, in vitro evaluation of COX inhibitory activity revealed selective inhibition of COX-2 [19, 20]. Moreover, carprofen displayed fatty acid amide hydrolase (FAAH) inhibitory activity which could contribute to its analgesic and anti-inflammatory activity [20, 21].

### 1.1 Rational design

In the last few years, virtual screening and molecular docking were used successfully in the identification of many potent and selective COX inhibitor [22, 23]. Extensive work in this field have focused on polar substituents to modulate COX selectivity. On the other hand, hydrophobic interactions of the alkyl group gained little attention in this field which may be attributed to weakness the hydrophobic interactions as compared by ionic and hydrogen bonding.

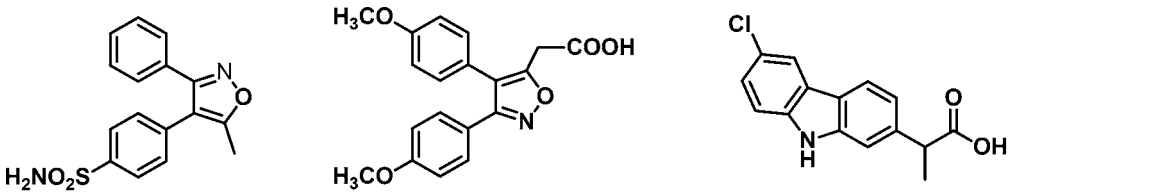
However, the hydrophobic interactions (Fig. 2) of the aliphatic chain in arachidonic acid play an important role in the binding of this natural substrate to COX-1/2 [24]. Accordingly, it was of interest to design new carprofen analogs bearing different aliphatic substitutions to evaluate their impact on the binding affinity to COX-1/2.

In this study, three scaffolds were generated from S-carprofen by isosteric replacement of the -NH in carprofen with –S–, –O– and $–CH_2–$ groups. The four scaffolds were derivatized by different alkyl/halo substituents (Fig. 3). The impact of these alkyl substitutions on binding affinity to COX-1/2 was evaluated in a docking study. Moreover, the effect of variation of the position of the propionic acid side chain on binding affinity to COX-1/2 was also investigated.

## 2 Experimental

### 2.1 Docking study

In this work, the molecular docking study was performed to evaluate affinity and selectivity of the newly designed




**1, Valdecoxib**
$IC_{50}$ = 157.2 μM (COX-1)
= 0.04 μM (COX-2)
SI (COX-2) = **3930**

**2, Mofezolac**
$IC_{50}$ = 0.0079 μM (COX-1)
= > 50 μM (COX-2)
SI (COX-1) = **6329**

**3, Carprofen**
$IC_{50}$ = 0.087 μM (COX-1)
= 4.3 μM (COX-2)
SI (COX-1) = **49.43**

$IC_{50}$ = 74 μM (FAAH)
$IC_{50}$ = 22.3 μM (COX-1)
= 3.9 μM (COX-2)
SI (COX-2) = **5.71**

**Fig. 1** Rofecoxib, mofezolac and carprofen and their $IC_{50}$ against COXs

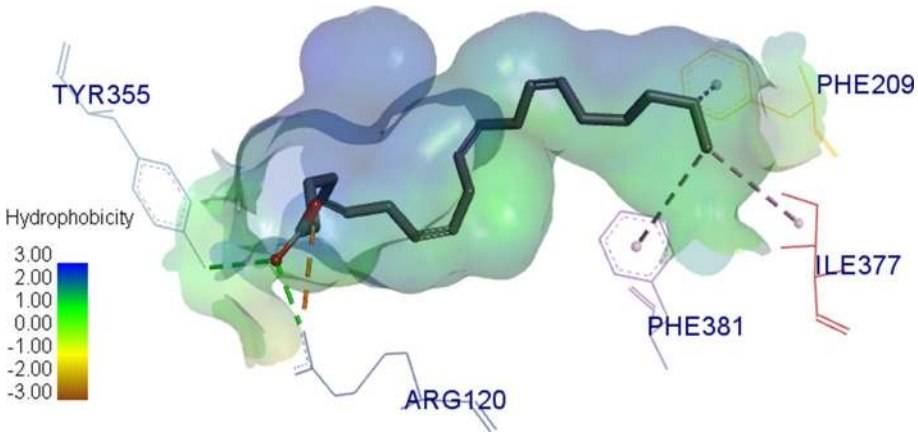



**Fig. 2** Arachidonic acid into the active site of COX-1 (pdb code: 1diy) showing hydrophobic interactions with PHE209 and PHE381 amino acids

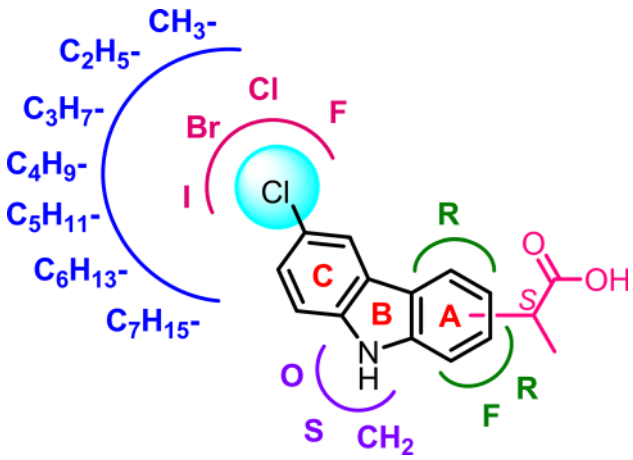



**Fig. 3** Structural modification of carprofen scaffold

carprofen analogs to COX-1/2. The binding modes of the analogs with the promising affinity were also investigated.

#### 2.1.1 Preparation of ligands and the protein files

AutoDock 4.2 was used to perform the docking study of the designed carprofen analogs into COX-1 (PDB code: 1EQG) [25] and COX-2 (PDB code: 1CX2) [26]. The crystal structure of the two enzymes were obtained from protein data bank (http://www.rcsb.org/pdb). The preparation of the ligands was done according to our previous reports [4, 12]. The study was performed after validation of the docking scenario by re-docking the native ligands into their corresponding COX enzymes. The binding modes and interactions of the native ligands with the key amino acids in the active site were identified and compared with the reported data. The re-docked ligands superimposed onto the position of the native ligand.

#### 2.1.2 Preparation of grid and docking parameters

AutoGrid tool in AutoDock was used to create the grid parameter files. 3D grid with final size of 60 × 60 × 60 Å with 0.375 Å spacing was created. Genetic algorithm was used as the searching parameters in the docking study while docking parameters were set to the default values. The ligands were docked as flexible molecules and the proteins were used as rigid molecules. The best in the top ten conformations with the highest best binding-free energy values were determined.

#### 2.1.3 Analysis and visualization of the docking results

The results of the docking study including binding-free energy ($\Delta G_b$), and inhibition constants ($K_i$) were analyzed by AutoDock 4.2 while the binding modes were visualized by Discovery Studio Visualizer (v16.1.0.15350).

### 2.2 Drug-likeness, ADME and toxicity study

The molecular weight (MW), molar refractivity (MR), lipophilicity (MlogP), number of hydrogen bond donor ($H_D$), number of hydrogen bond acceptor ($H_A$), Lipinski's violations, number of rotatable bonds and topological polar surface area (TPSA) of the new compounds and carprofen **3** were calculated using SwissADME webserver (http://www.swissadme.ch/) [27]. Marvin JS sketcher (version 16.4.18, 2016, www.chemaxon.com) was used to draw the chemical structures of the designed analogs, converted to smiles by JChem web service which undergo a series of calculations to compute different physicochemical descriptors related to drug-likeness. The results were obtained as Excel output file. The molecular volume and drug-likeness score (DLS) were calculated using Molsoft webserver (http://molsoft.com/mprop/). The results were presented in Table 7.

## 3 Results and discussion

### 3.1 Docking study

#### 3.1.1 Docking study of S-carprofen into COX-1/2

The S-Carprofen was docked into the active site of COX-1/2 using AutoDock 4.2. Both ovine COX-1 (pdb code: 1EQG) [25] and COX-2 (pdb code: 1CX2) [26] were used in the docking study. A rigid docking scenario of the ligands into COXs was applied. Validation of the docking study was done by redocking the native ligands into their corresponding COX. The results of the study including binding affinity, inhibition constants and hydrogen bonds were represented in Table 1.

The S-carprofen displayed binding-free energy of 10.02 kcal/mol and inhibition constant of 45.36 nM with COX-1 indicating higher affinity for the enzyme than ibuprofen. Three hydrogen bonds were formed between the carboxylic group oxygen in carprofen with ARG120 and TYR355 in COX-1 with bond length (BL) in the range of 1.72–2.01 Å (Fig. 4).

S-Carprofen showed 16 hydrophobic interactions of the amide-pi stacked, pi-sigma, pi-sulfur, alkyl, and pi-alkyl types (BL = 3.27–5.35 Å) with VAL116, VAL349, LEU359, PHE381, LEU384, TYR385, TRP387, ILE523, GLY526, ALA527, and LEU531 amino acids in COX-1. In addition, one pi-sulfur interaction was formed with MET522 (BL = 5.94 Å) (Fig. 4).

On the other hand, the S-carprofen displayed binding free energy of 8.66 kcal/mol and inhibition constant of 450.2 nM with COX-2. One conventional hydrogen bond was observed between carbonyl oxygen in carprofen with the hydroxyl group in TYR355 with BL of 2.01 Å (Table 1). Moreover, an additional carbon hydrogen bond was observed between oxygen in OH group of carprofen and the hydrogen of the δ-carbon in ARG120 (BL = 2.17 Å).

S-carprofen formed fifteen hydrophobic interactions of the pi-sigma, alkyl and pi-alkyl types with VAL349, LEU352, LEU359, PHE381, LEU384, TYR385, TRP387, VAL523, ALA527, and LEU531 amino acids in COX-2 (BL = 3.70–5.49 Å) (Fig. 4).

Generally, the S-carprofen **3** displayed high binding-free energy to COX-1 ($\Delta G_b$ = 10.02 kcal/mol) over COX-2 ($\Delta G_b$ = 8.66 kcal/mol). These results indicate that S-carprofen **3** has higher potential selectivity for COX-1 than COX-2.

The relationship between the half maximal inhibitory concentration ($IC_{50}$) and inhibition constant ($K_i$) was described in several reports [28, 29]. Both $IC_{50}$ and $K_i$ are used to express the relative potency of an inhibitor. The smaller the value of $IC_{50}$ and $K_i$, the stronger the inhibitory activity. The relationship between $IC_{50}$ and $K_i$, of a competitive inhibitor was described by Cheng– Prusoff according to the Eq. 1 where [S] is the substrate concentration and $K_M$ is Michaelis constant of the substrate [28].

$$IC_{50} = K_i\left(1 + \frac{[S]}{K_M}\right) \quad (1)$$

In this study, the binding free energy against the two COXs and $K_i$ ratio (ratio of $K_i$ of COX-2 to $K_i$ of COX-1) of the designed compounds will be used to compare their affinities and potential selectivity. S-carprofen displayed $K_i$ ratio of 9.9 which indicate that carprofen has 9.9 times higher potential selectivity to inhibit COX-1 over COX-2. This result

**Table 1** Results of the docking study of S-carprofen into COX-1 (pdb: 1EQG) [25] and COX-2 (pdb code: 1CX2) [26] in comparison to the redocked native ligands (ibuprofen and SC-588)

| COX I/Comp. | $\Delta G_b^a$ | $K_i^b$ (nM) | No of H-bonds | Atoms in H-bonding | | Length[c] (Å) |
|---|---|---|---|---|---|---|
| | | | | In the ligand | In the enzyme | |
| **COX-1** | | | | | | |
| (*S*)-Carprofen | −10.02 | 45.36 | 3 | OH | NH of ARG120 | 1.72 |
| | | | | C=O | OH of TYR355 | 1.92 |
| | | | | C=O | $NH_2$ of ARG120 | 2.01 |
| (*S*)-Ibuprofen | −7.96 | 1470 | 3 | OH | NH of ARG120 | 1.65 |
| | | | | C=O | $NH_2$ of ARG120 | 1.78 |
| | | | | C=O | OH of Tyr355 | 1.83 |
| **COX-2** | | | | | | |
| (*S*)-Carprofen | −8.66 | 450.2 | 2 | C=O | OH of TYR355 | 2.01 |
| | | | | OH | δ-C of ARG120 | 2.17 |
| Sc-588 | −10.78 | 12.59 | 4 | O of $SO_2$ | NH of HIS90 | 2.03 |
| | | | | $H^1$ of $NH_2$ | CO of LEU352 | 2.08 |
| | | | | $H^2$ of $NH_2$ | CO of GLN192 | 1.97 |
| | | | | $CF_3$ | NH of ARG120 | 2.24 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]Length of in angstrom (Å)

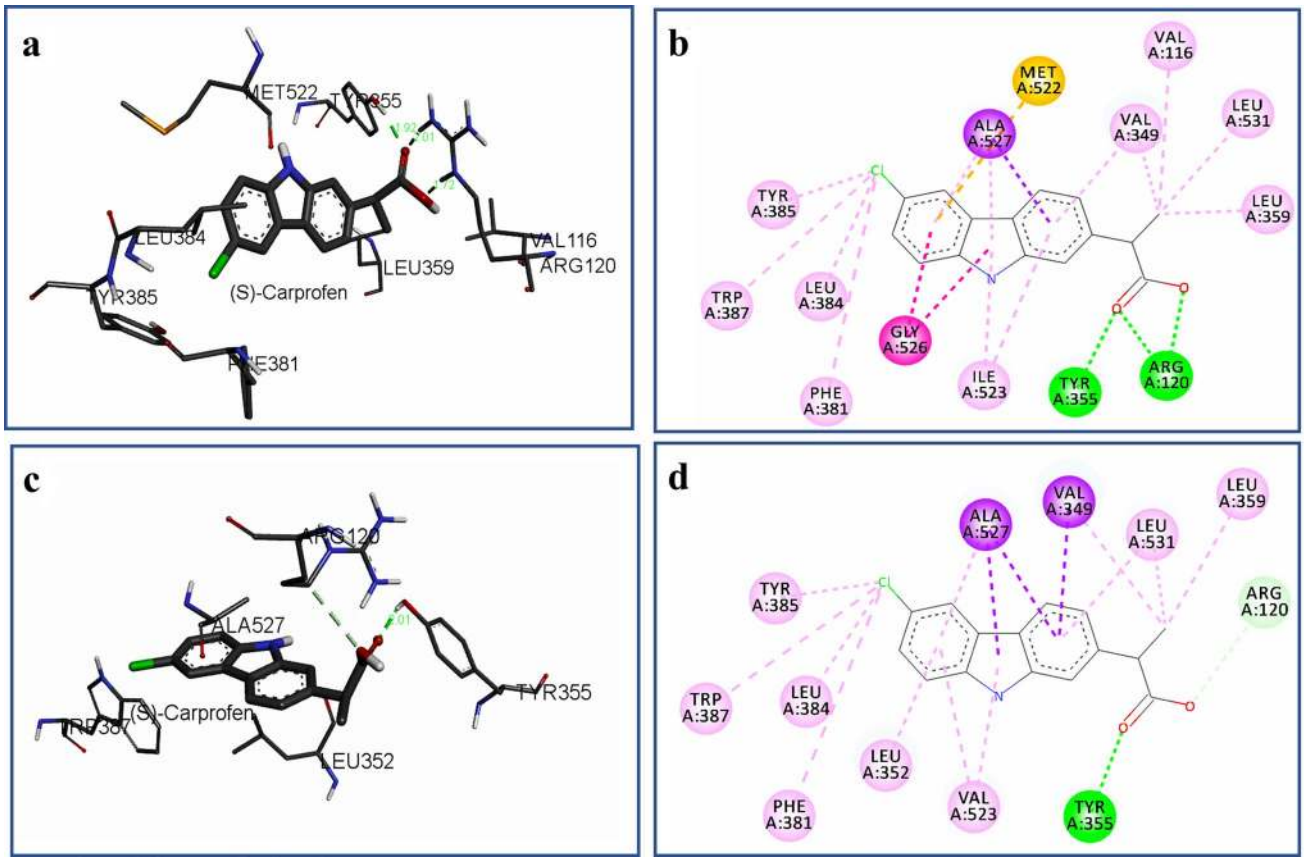



**Fig. 4** Binging modes of S-carprofen **3**: **a** 3D binding mode into COX-1 (pdb code: 1EQG); **b** 2D binding mode into COX-1, **c** 3D binding mode into COX-2 (pdb code: 1CX2); **d** 2D binding mode into COX-2 showing hydrogen bonds (▬), carbon hydrogen bonds (▬), hydrophobic interactions of the amide-pi stacked (▬), pi-sulfur (▬), pi-sigma (▬), pi–pi T-shaped (▬), pi-alkyl (▬) and alkyl (▬) types

is matched with the results of carprofen inhibitory activity of COX in the whole blood assay [18].

### 3.1.2 Docking Study of carprofen analogs into COX-1/2

**3.1.2.1 (S)-2-(9H-carbazol-2-yl)propanoic acid derivatives** To investigate the effect of the position of the carboxylic acid side chain on the binding affinity of S-carprofen to COXs, three analogs **4**–**6** were designed (Fig. 5). The results of the docking study revealed a decrease in binding affinity of the three analogs to COX-1, as compared to S-carprofen. Moreover, compound **5** and **6** showed a slight and marked decrease in their binding affinity to COX-2, respectively. On the other hand, compound **4** showed slightly higher affinity for COX-2 as compared to S-carprofen (Table 2).

The values of the $K_i$ ratio showed that compound **5** has higher potential selectivity to COX-1 than S-carprofen (Table 2). Based on these results, the position of propionic acid chain is important for directing the binding selectivity for COX-1 or COX-2. Accordingly, compound **5** was selected for further investigation as a potential scaffold with high binding selectivity to COX-1.

Investigation of the impact of variation of the 6-chloro group in S-carprofen with other halogens (F, Br or I) on binding affinity was investigated. A new series of carbazole derivatives **7**–**12** was designed bearing different halogens at C6 was designed (Fig. 5). Replacement of the 6-chloro in S-carprofen with fluoro, bromo, and iodo resulted in a marked decreased selectivity ratio to COX-1. The 6-iodo analog **9** displayed the highest binding affinity for COX-2 (Table 2).

It was clear that the binding affinity to COX-2 increases as the atomic size increases. These results are matched with the fact that the active site of COX-2 (394Å) is larger than that of COX-1 (316Å) [30].

The carbazole derivatives **13**–**26** was modelled after compound **5** to investigate the effect of 6-alkyl/1-fluoro substitutions on the binding affinity to COX-1/2 (Fig. 5). Among the designed analogs **13**–**26**, compounds **15** with 6-propyl substituent displayed highest binding affinity for

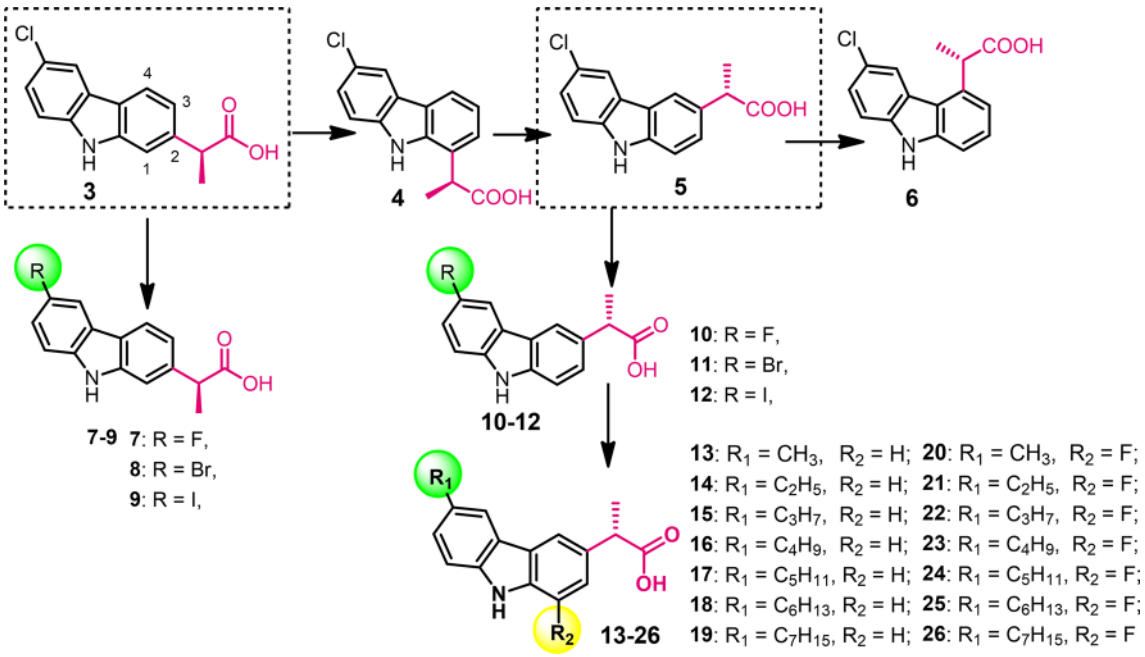



**Fig. 5** Chemical structures of (S)-enantiomers of compounds **3** and **5**–**12**

**Table 2** Results of the docking study of compounds **3**–**26** into the COX-1/2

| Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] | Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | | | COX-1 | COX-2 | COX-1 | COX-2 | |
| **3** | −10.02 | −8.66 | 45.36 | 450.2 | 9.9 | **15** | −10.29 | −8.59 | 28.69 | 501.29 | 17.47 |
| **4** | −9.95 | −8.77 | 50.77 | 374.47 | 7.38 | **16** | −10.39 | −8.86 | 24.36 | 321.07 | 13.18 |
| **5** | −9.96 | −8.53 | 50.33 | 559.54 | 11.12 | **17** | −10.53 | −9.33 | 18.97 | 144.99 | 7.64 |
| **6** | −8.66 | −7.70 | 448.8 | 2280 | 5.08 | **18** | −10.78 | −9.44 | 12.64 | 119.64 | 9.47 |
| **7** | −9.54 | −8.11 | 102.16 | 1140 | 11.16 | **19** | −9.34 | −10.07 | 142.48 | 41.29 | 0.29 |
| **8** | −10.06 | −8.84 | 42.17 | 332.19 | 7.88 | **20** | −9.75 | −8.51 | 71.23 | 583.06 | 8.19 |
| **9** | −9.97 | −8.93 | 48.9 | 286.82 | 5.87 | **21** | −10.11 | −8.50 | 38.9 | 592.21 | 15.22 |
| **10** | −9.27 | −8.12 | 161.19 | 1120 | 6.95 | **22** | −10.17 | −8.54 | 35.17 | 545.35 | 15.51 |
| **11** | −10.13 | −8.55 | 37.72 | 542.19 | 14.37 | **23** | −10.33 | −8.43 | 26.57 | 666.14 | 25.07 |
| **12** | −10.00 | −8.61 | 47.16 | 487.71 | 10.34 | **24** | −10.46 | −8.85 | 21.5 | 324.56 | 15.09 |
| **13** | −9.79 | −8.55 | 66.87 | 537.33 | 8.04 | **25** | −10.64 | −9.24 | 15.81 | 167.93 | 10.6 |
| **14** | −10.18 | −8.55 | 34.52 | 539.6 | 15.63 | **26** | −9.87 | −9.80 | 58.15 | 65.82 | 1.13 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]The ratio of Ki of COX-2 to Ki of COX-1

COX-1 while compound **19** showed the highest affinity for COX-2.

On comparing the $K_i$ ratio of compounds **13**–**19** with their corresponding fluorinated analogs **20**–**26**, a noticeable increase in the $K_i$ ratio indicating higher potential of COX-1 selectivity. Among the carbazole derivative **3**–**26**, compound **23** with the 6-butyl group displayed the highest potential selectivity for COX-1 ($K_i$ ratio = 25.07) (Table 2).

Different types of interaction between compound **23** and amino acids in the active site of COX-1 were represented in Fig. 6. Compound **23** formed 2 classical hydrogen bonds with ARG120 in COX-1 with BL of 1.64 and 1.96 Å. A third carbon hydrogen bond was observed between the 1-fluoro with α-CH of SER523 (BL = 1.84 Å). Additionally, compound **23** displayed 16 hydrophobic interactions of the pi-sigma, pi-alkyl and alkyl types with amino acids in the active site of COX-1 (BL = 3.69–5.38 Å) (Fig. 6).

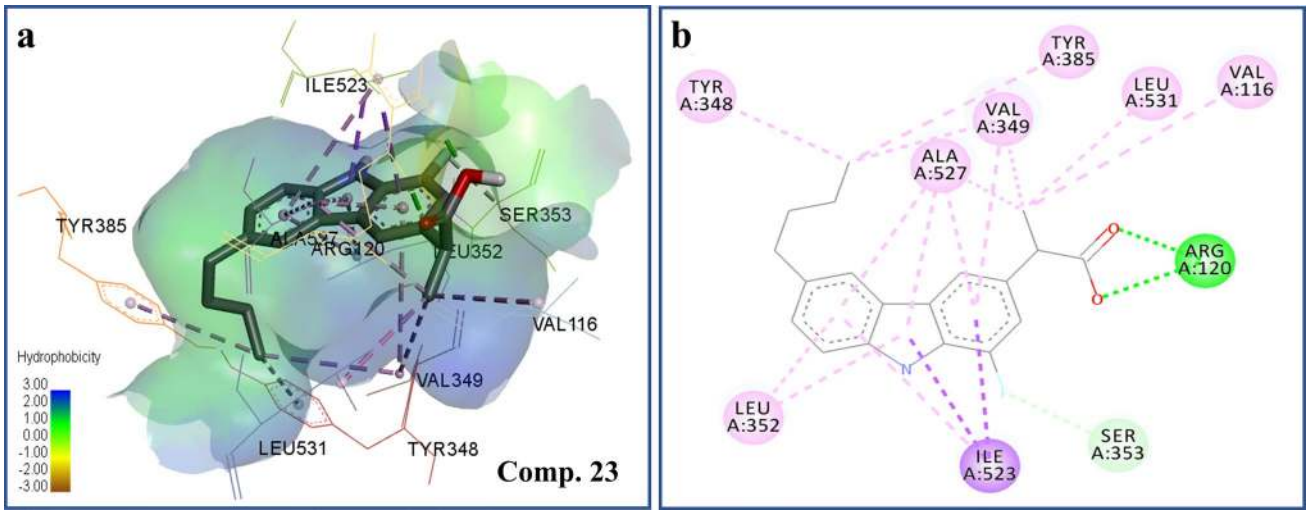



**Fig. 6** Binging modes of compound **23** into COX-1 (pdb code: 1EQG): **a** 3D binding mode, receptor surface hydrophobicity visualized; **b** 2D binding mode showing hydrogen bonds (▬▬), carbon hydrogen bonds (▬▬) and hydrophobic interactions of the pi-sigma (▬▬), pi-alkyl (▬▬) and alkyl (▬▬) types

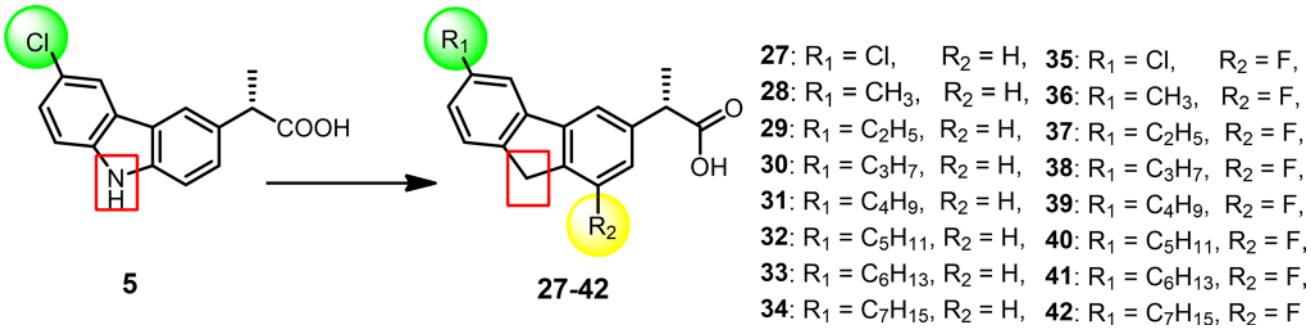



**Fig. 7** Chemical structures of (S)-enantiomers of compounds **27**–**42**

**Table 3** Results of the docking study of compounds **27**–**42** into the COX-1/2

| Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] | Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | | | COX-1 | COX-2 | COX-1 | COX-2 | |
| **27** | −10.16 | −8.68 | 36.0 | 431.94 | 12 | **35** | −10.09 | −8.74 | 39.9 | 394.87 | 9.9 |
| **28** | −10.01 | −8.66 | 45.77 | 451.81 | 9.87 | **36** | −9.99 | −8.66 | 47.44 | 449.65 | 9.48 |
| **29** | −10.42 | −8.85 | 22.94 | 325.14 | 14.17 | **37** | −10.32 | −8.90 | 27.04 | 297.22 | 10.99 |
| **30** | −10.47 | −8.98 | 21.04 | 263.12 | 12.51 | **38** | −10.36 | −8.91 | 25.43 | 294.94 | 11.59 |
| **31** | −10.62 | −9.19 | 16.49 | 184.35 | 11.18 | **39** | −10.54 | −8.91 | 18.68 | 293.27 | 15.69 |
| **32** | −10.76 | −9.65 | 12.89 | 97.89 | 7.59 | **40** | −10.82 | −9.05 | 11.78 | 234.03 | 19.87 |
| **33** | −10.63 | −10.84 | 16.19 | 11.29 | 0.69 | **41** | −10.75 | −9.30 | 13.1 | 151.96 | 11.6 |
| **34** | −10.82 | −9.84 | 11.67 | 61.11 | 5.24 | **42** | −10.82 | −9.27 | 11.8 | 159.01 | 13.47 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]The ratio of Ki of COX-2 to Ki of COX-1

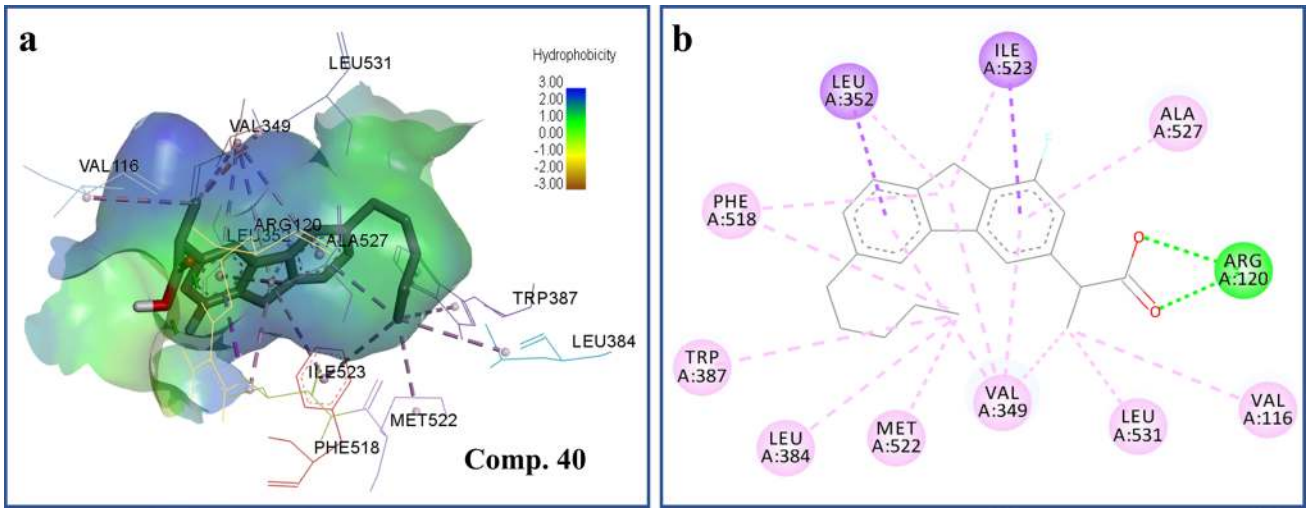



**Fig. 8** Binging modes of compound **40** into COX-1 (pdb code: 1EQG): **a** 3D binding mode, receptor surface hydrophobicity visualized; **b** 2D binding mode showing hydrogen bonds (▬▬), and hydrophobic interactions of the pi-sigma (▬▬), pi-alkyl (▬▬) and alkyl (▬▬) types

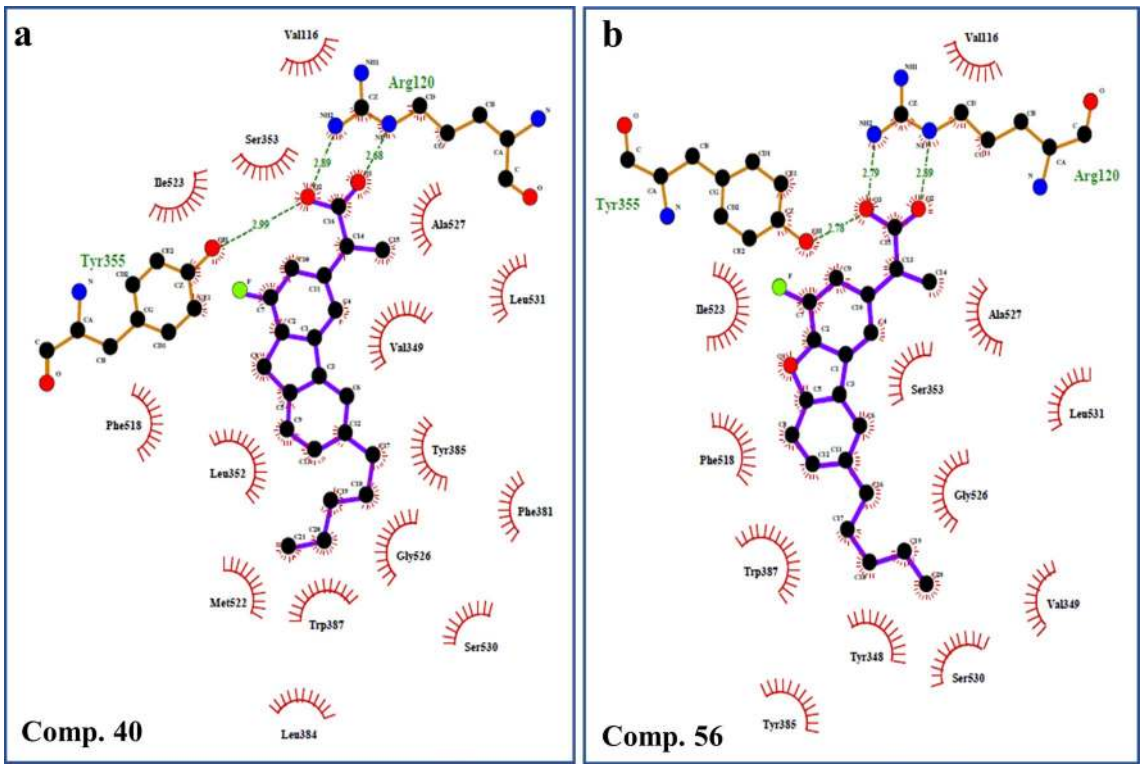



**Fig. 9** **a** LigPlot view of compound **40** into the active site of COX-1 showing three hydrogen bonds with ARG120 and TYR355 with bond length in the range of 2.68–2.99 Å; **b** LigPlot view of compound **56** into the active site of COX-1 showing three hydrogen bonds with ARG120 and TYR355 with bond length in the range of 2.78–2.89 Å

**3.1.2.2 (S)-2-(9H-fluoren-3-yl)propanoic acid derivatives** To design new analogs with enhanced COX-1 selectivity, a new series of fluorene derivatives **27**–**42** were obtained by isosteric replacement of NH group in compound **5** with $-CH_2-$ group (Fig. 7). The effect of alkyl/fluoro substitutions groups on the binding affinity to COXs was investigated.

Among the alkyl derivatives **27**–**42**, compound **34**, **40** and **42** displayed the highest binding affinity ($\Delta G_b$= -10.82)

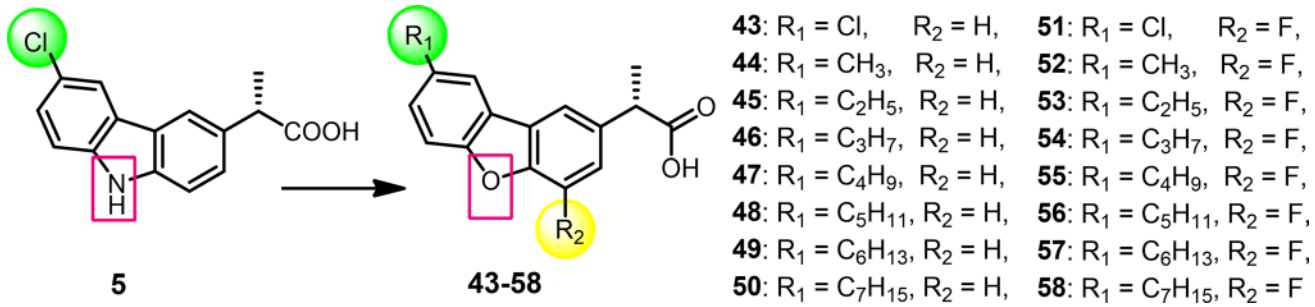



**Fig. 10** Chemical structures of the (S)-enantiomers of compounds **43**–**58**

**Table 4** Results of the docking study of compounds **43**–**58** into the COX-1/2

| Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] | Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | | | COX-1 | COX-2 | COX-1 | COX-2 | |
| **43** | −10.14 | −8.77 | 37.12 | 375.24 | 10.11 | **51** | −10.07 | −8.73 | 41.23 | 400.25 | 9.71 |
| **44** | −9.98 | −8.78 | 48.23 | 367.73 | 7.63 | **52** | −9.96 | −8.73 | 50.34 | 400.08 | 7.95 |
| **45** | −10.38 | −8.70 | 24.69 | 416.23 | 16.86 | **53** | −10.31 | −8.69 | 27.85 | 424.52 | 15.24 |
| **46** | −10.48 | −8.65 | 20.66 | 459.4 | 22.24 | **54** | −10.42 | −8.33 | 23.17 | 781.08 | 33.71 |
| **47** | −10.56 | −9.02 | 18.23 | 242.65 | 13.31 | **55** | −10.45 | −8.93 | 21.95 | 282.7 | 12.87 |
| **48** | −10.82 | −9.51 | 11.72 | 107.15 | 9.14 | **56** | −10.70 | −8.47 | 14.44 | 622.28 | 43.09 |
| **49** | −11.01 | −9.72 | 8.44 | 75.17 | 8.91 | **57** | −10.8 | −9.17 | 12.09 | 190.19 | 15.73 |
| **50** | −11.13 | −10.0 | 6.98 | 46.39 | 6.65 | **58** | −11.12 | −9.20 | 7.04 | 179.4 | 25.48 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]The ratio of Ki of COX-2 to Ki of COX-1

for COX-1, while compound **33** showed the highest affinity ($\Delta G_b$=-10.84) for COX-2 (Table 3).

The $K_i$ ratio of compound **27**–**42** was presented in Table 3. Except for compound **33** which showed weak potential selectivity for COX-2 ($K_i$ ratio = 1.43 for COX-2), all other compounds displayed higher binding affinity for COX-1 over COX-2 indicating potential selectivity ($K_i$ ratio = 7.59–19.87).

In comparison to S-carprofen which formed 3 hydrogen bonds with COX-1, compound **40** formed two hydrogen bonds only with ARG120, with BL of 1.68 and 1.89 Å (Fig. 8). Moreover, LigPlot view showed a third hydrogen bonds between compound **40** and TYR355 with BL of 2.99 Å (Fig. 9). Compound **40** formed also 16 hydrophobic interactions with COX-1 with BL in the range of 3.52–5.42 Å. Five of these hydrophobic interactions were due to the pentyl group.

**3.1.2.3 (S)-2-(dibenzo[b,d]furan-3-yl)propanoic acid derivatives** Replacement of the NH group in compound **5** with the isosteric oxygen yielded 2-(dibenzo[b,d]furan-2-yl) propanoic acid **43**. The modification was done to design new analogs with high binding selectivity to COX-1. Substitution with alkyl/fluoro groups was done and the designed analogs were evaluated for their binding affinity for COX-1/2 (Fig. 10).

Among compounds **43**–**58**, compound **50** with the heptyl chain displayed the highest binding affinity for COX-1 ($\Delta G_b$=-11.13) and COX-2 ($\Delta G_b$=-10.0) (Table 4).

The values of the $K_i$ ratio of compound **51**–**58** showed the fluorinated analogs have relatively higher $K_i$ ratio than their corresponding derivatives **43**–**50**. Compound **56** with the pentyl side chain has the highest potential selectivity for COX-1 ($K_i$ ratio = 43.09) (Table 4).

Compound **56** with the pentyl side chain displayed the highest potential selectivity for COX-1 ($K_i$ ratio = 43.09) (Table 4). Compound **56** formed two hydrogen bonds only with ARG120 (classical) and SER353 (nonclassical) amino acids in COX-1, with BL of 1.90 and 1.89 Å, respectively (Fig. 11). LigPlot view of compound **56** showed 3 hydrogen bonds with ARG120 and TYR355 with BL in the range of 2.78–2.89 Å (Fig. 9). Moreover, compound **56** formed also 14 hydrophobic interactions with amino acids in the active site of COX-1 with bond distances in the range of 3.67–5.32 Å (Fig. 11).

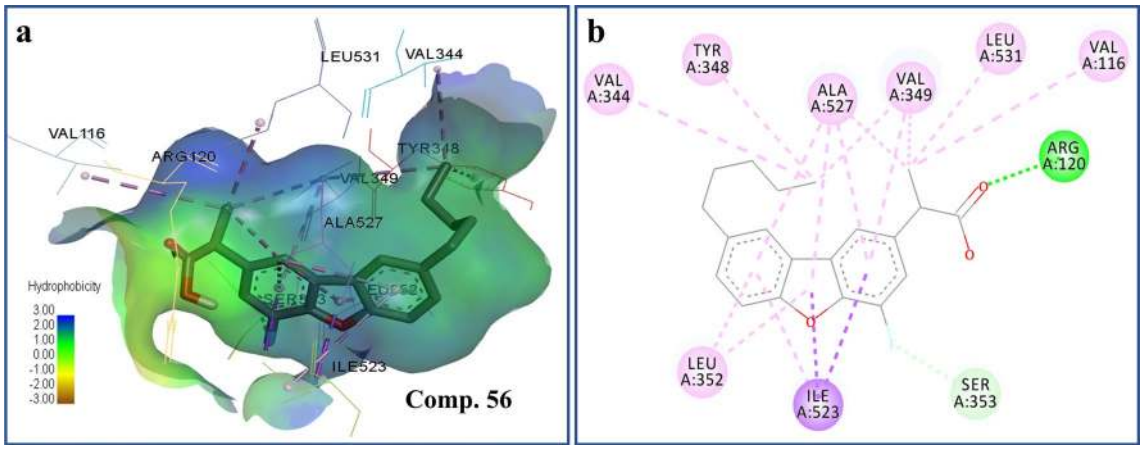



**Fig. 11** Binging modes of compound **56** into COX-1 (pdb code: 1EQG): **a** 3D binding mode; **b** 2D binding mode, showing hydrogen bonds (▬▬), carbon hydrogen bonds (▬▬), hydrophobic interactions of the pi-sigma (▬▬), pi-alkyl (▬▬) and alkyl (▬▬) types

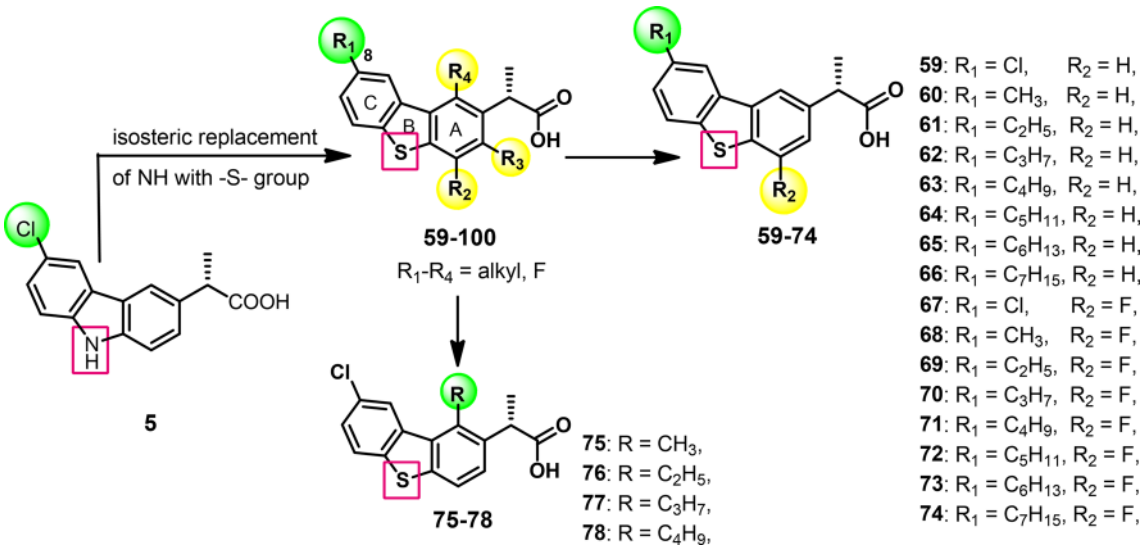



**Fig. 12** Design strategy and chemical structural of the (S)-enantiomers of compounds **59**–**78**

**3.1.2.4 (S)-2-(dibenzo[b,d]thiophen-3-yl)propanoic acid derivatives** The dibenzo[b,d]thiophene scaffold was obtained from compound **5** by isosteric replacement of NH with -S- group. Derivatization was obtained by substitution with fluoro/alkyl to evaluate their effect on binding affinity to COXs (Fig. 12).

The results of the docking study of the dibenzo[b,d] thiophene analogs **59**–**78** into COX-1/2 revealed that compound **66** has the highest binding affinity to both COX-1/2 (Table 5).

Like S-carprofen, the dibenzo[b,d]thiophene **59**–**78** displayed higher binding affinities for COX-1 over COX-2 indicating potential selectivity for COX-1 (Table 5).

The second series of the thiophen analogs **79**–**100** were designed bearing alkyl groups at C3, C4 and C8 (Fig. 13). Compounds **79**–**100** were docked into the active site of COX-1/2. The results of the study revealed sharp decrease in the binding affinity to COX-1 with a simultaneous increase in the affinity to COX-2. Accordingly, the potential selectivity to COX-1 was reversed with most of the derivatives in this series (Table 6).

Among the tested analogs **79**–**100**, compound **88** displayed the highest binding affinity to COX-1 while compound **99** displayed the highest affinity to COX-2 (Table 6). The values of the $K_i$ ratio of compound **79**–**100** showed that only compound **80**, **83**, **88**, **89** and **94** have potential selectivity to COX-1 with lower potential selectivity compared to S-carprofen. On the other hand, the remaining analogs showed potential selectivity to COX-2 where compound **92** and **99** showed the lowest ratio with $K_i$ ratio of

**Table 5** Results of the docking study of compounds **59**–**78** into the COX-1/2

| Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] | Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | | | COX-1 | COX-2 | COX-1 | COX-2 | |
| **59** | −9.93 | −8.69 | 52.48 | 423.24 | 8.07 | **69** | −10.08 | −8.87 | 40.7 | 314.3 | 7.72 |
| **60** | −9.88 | −8.65 | 56.86 | 460.17 | 8.09 | **70** | −10.19 | −9.08 | 33.99 | 220.57 | 6.49 |
| **61** | −10.30 | −8.91 | 28.14 | 296.63 | 10.54 | **71** | −10.36 | −9.03 | 25.36 | 238.65 | 9.41 |
| **62** | −10.34 | −9.14 | 26.42 | 199.76 | 7.56 | **72** | −10.67 | −9.28 | 15.04 | 157.68 | 10.48 |
| **63** | −10.40 | −9.36 | 23.87 | 137.44 | 5.76 | **73** | −10.45 | −9.52 | 21.75 | 105.42 | 4.85 |
| **64** | −10.73 | −9.67 | 13.67 | 81.7 | 5.98 | **74** | −10.23 | −9.96 | 31.62 | 50.29 | 1.59 |
| **65** | −10.53 | −9.91 | 19.04 | 54.8 | 2.88 | **75** | −9.68 | −8.30 | 80.31 | 829.27 | 10.33 |
| **66** | −11.2 | −10.15 | 6.15 | 36.5 | 5.94 | **76** | −9.84 | −8.67 | 61.15 | 442.66 | 7.2 |
| **67** | −9.74 | −8.67 | 72.95 | 440.98 | 6.04 | **77** | −10.0 | −8.64 | 46.83 | 465.09 | 9.93 |
| **68** | −9.64 | −8.65 | 85.86 | 459.2 | 5.35 | **78** | −10.31 | −8.83 | 27.55 | 338.68 | 12.3 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]The ratio of Ki of COX-2 to Ki of COX-1

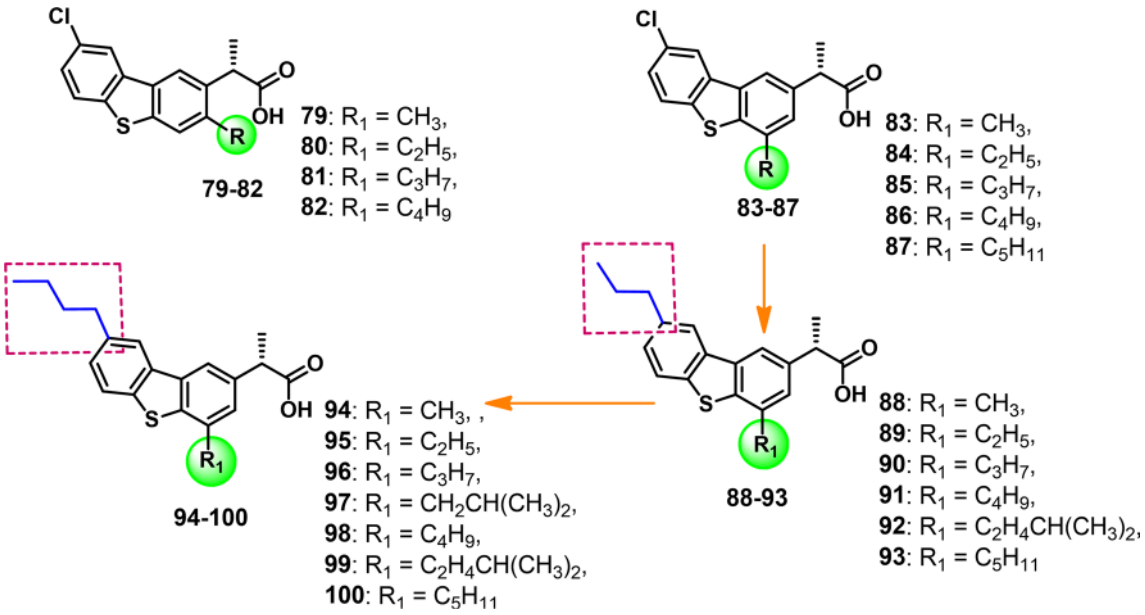



**Fig. 13** Design strategy and chemical structures of S-enantiomers of compounds **79**–**82**

0.032 and 0.025, respectively. The binding mode of these two compounds were presented in Table 6.

Compound **92** displayed higher binding affinity ($\Delta G_b$ = 9.96 kcal/mol) for COX-2 than S-carprofen ($\Delta G_b$ = 8.66 kcal/mol). Although compound **92** and S-carprofen formed 2 hydrogen bonds with COX-2, but compound **92** displayed higher number of hydrophobic interactions (18) compared to 15 only for S-carprofen (Fig. 14).

Moreover, compound **92** showed sharp decrease in the binding affinity to COX-1 compared to S-carprofen. As a result, compound **92** displayed high potential selectivity for COX-2 over COX-1 ($K_i$ ratio = 0.032).

Compound **99** with the isopentyl side chain displayed binding affinity for COX-2 ($\Delta G_b$ = 10.33 kcal/mol) higher than S-carprofen, with the highest potential selectivity for COX-2 ($K_i$ ratio = 0.025) (Table 6). The high affinity and potential selectivity to COX-2 of compound **99** is attributed to the formation of 3 hydrogen bonds with HIS90, LEU352 and ARG513 amino acids in COX-2 (BL = 2.06–3.07 Å) compared to 2 hydrogen bonds only for S-carprofen (Fig. 15).

Moreover compound **99** formed also one electrostatic interaction with of the pi-cation type with ARG120 and 21 hydrophobic interactions (BL = 3.62–5.42 Å) with COX-2 which contribute to this high selectivity. Ten of these

**Table 6** Results of the docking study of compounds **79**–**100** into the COX-1/2

| Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] | Comp. | $\Delta G_b^a$ | | $K_i^b$ | | $K_i$ ratio[c] |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | COX-1 | COX-2 | COX-1 | COX-2 | | | COX-1 | COX-2 | COX-1 | COX-2 | |
| **79** | −8.08 | −8.93 | 1200 | 286.12 | 0.238 | **90** | −7.41 | −9.21 | 3710 | 178.02 | 0.048 |
| **80** | −9.46 | −8.86 | 116.09 | 319.77 | 2.76 | **91** | −7.53 | −9.51 | 3000 | 106.99 | 0.036 |
| **81** | −8.35 | −9.16 | 758.23 | 193.89 | 0.226 | **92** | −7.92 | −9.96 | 1560 | 50.42 | 0.032 |
| **82** | −8.28 | −9.40 | 857.81 | 128.03 | 0.149 | **93** | −10.05 | −10.06 | 42.76 | 42.21 | 0.987 |
| **83** | −9.58 | −9.20 | 95.48 | 179.28 | 1.88 | **94** | −10.21 | −9.47 | 32.73 | 114.99 | 3.51 |
| **84** | −9.10 | −9.37 | 213.72 | 135.77 | 0.635 | **95** | −7.75 | −9.38 | 2100 | 133.32 | 0.063 |
| **85** | −8.98 | −9.58 | 260.01 | 94.62 | 0.364 | **96** | −7.69 | −9.6 | 2320 | 92.2 | 0.04 |
| **86** | −8.49 | −9.87 | 601.9 | 58.73 | 0.098 | **97** | −8.07 | −9.84 | 1220 | 61.74 | 0.051 |
| **87** | −9.74 | −9.81 | 72.16 | 64.39 | 0.892 | **98** | −8.56 | −10.11 | 533.67 | 38.61 | 0.072 |
| **88** | −10.24 | −9.08 | 31.11 | 221.44 | 7.12 | **99** | −8.14 | −10.33 | 1070 | 26.82 | 0.025 |
| **89** | −9.38 | −9.29 | 132.1 | 154.57 | 1.17 | **100** | −9.39 | −9.95 | 130.04 | 51.11 | 0.393 |

[a]Binding free energy (kcal/mol)

[b]Inhibition constant (nM)

[c]The ratio of Ki of COX-2 to Ki of COX-1

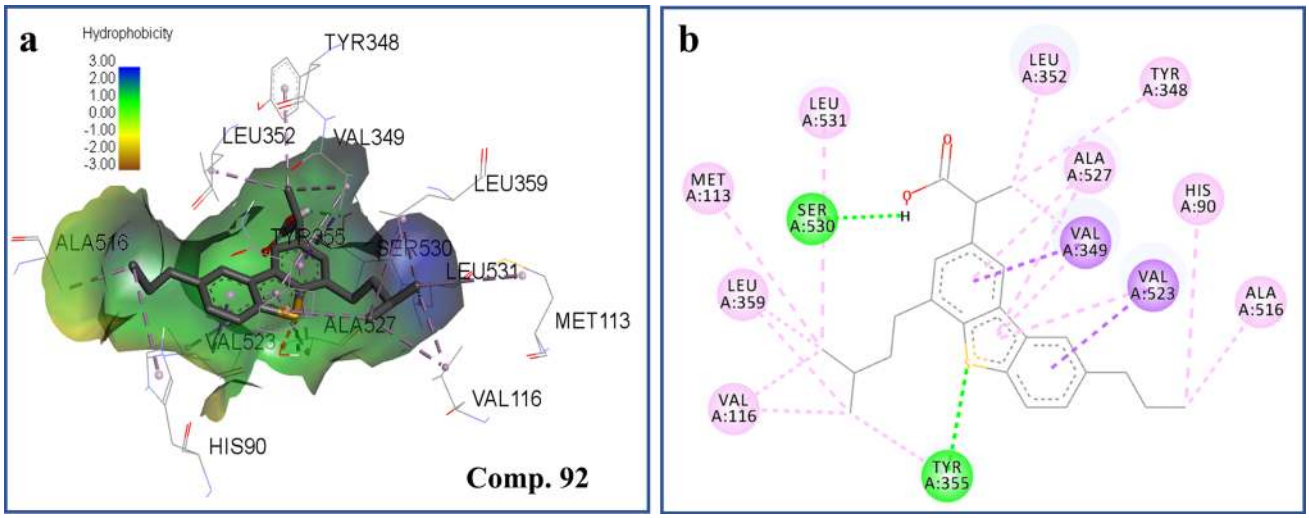



**Fig. 14** Binging modes of compound **92** into COX-2 (pdb code: 1CX2): **a** 3D binding mode; **b** 2D binding mode, showing hydrogen bonds (▬▬), carbon hydrogen bonds (▬▬), sulfur-X (▬▬), hydrophobic interactions of the pi-sigma (▬▬), pi-alkyl (▬▬) and alkyl (▬▬) types

interactions were due to the aliphatic butyl/isopentyl side chains (Fig. 15).

LigPlot view of compound **99** showed two hydrogen bonds with ARG513 and LEU352 with BL of 2.96 and 3.31 Å, respectively, while LigPlot view of compound **92** showed one hydrogen bond only with TYR385 with BL of 2.85 Å (Fig. 16).

### 3.2 Drug-likeness and synthetic feasibility study

During the last 3 decades huge number of compounds were reported with in vitro analgesic and anti-inflammatory activities, but only few numbers of these compounds were passed to clinical trials. This problem is mainly attributed to pharmacokinetic problems. As a result, the most promising analogs (**5**, **7**, **11**, **15**, **23**, **29**, **38**, **40**, **46**, **54**, **56**, **61**, **92**, **99**) in this study were selected for drug-likeness study. The study was performed using both SwissADME (http://www.swissadme.ch/), developed by the Molecular Modeling Group of the Swiss Institute of Bioinformatics [27], and Molsoft (http://molsoft.com/mprop/) which was developed by Molsoft LLC according to our previous reports [12, 31].

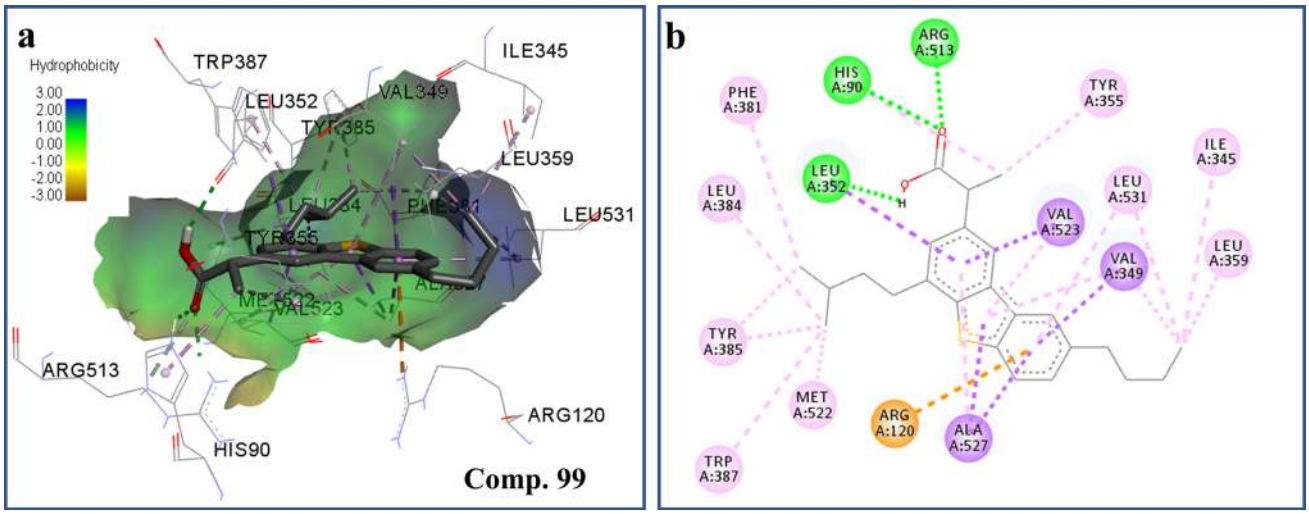



**Fig. 15** Binging modes of compound **99** into COX-2 (pdb code: 1CX2): **a** 3D binding mode; **b** 2D binding mode, showing hydrogen bonds (▬), carbon hydrogen bonds (▬), electrostatic interaction of the pi-cation (▬), hydrophobic interactions of the pi-sigma (▬), pi-alkyl (▬) and alkyl (▬) types

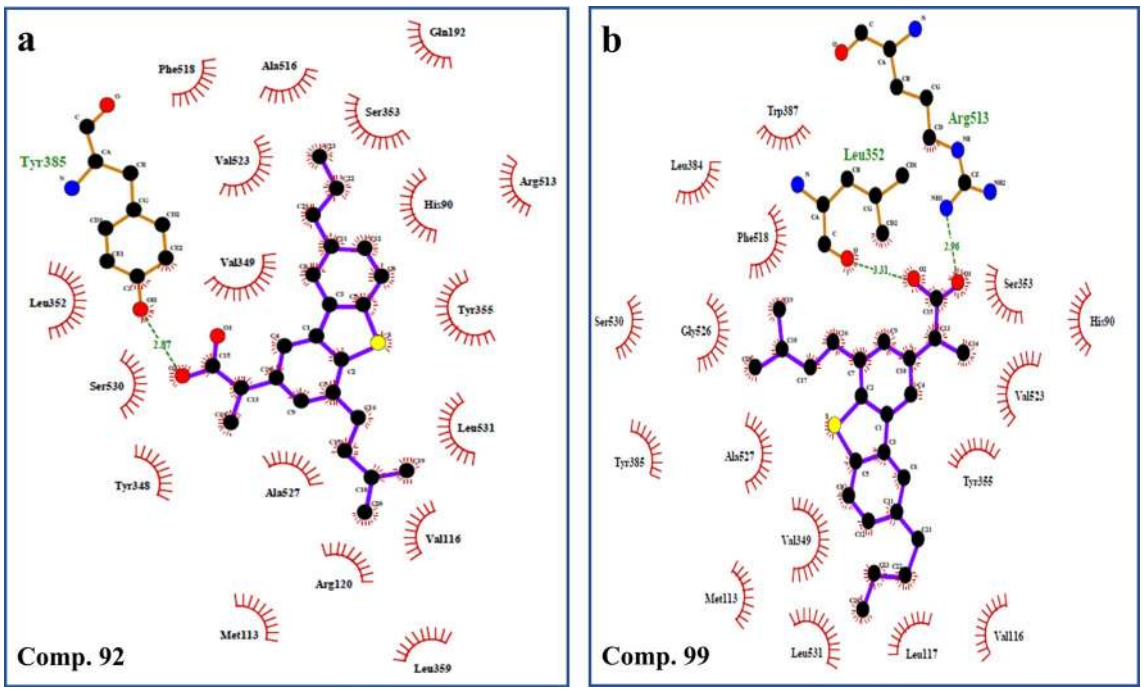



**Fig. 16** **a** LigPlot view of compound **92** into the active site of COX-2 (pdb code: 1CX2) showing one hydrogen bond with TYR355 with bond length of 2.87 Å; **b** LigPlot view of compound **99** into the active site of COX-1 showing two hydrogen bonds with ARG120 and LEU352 with bond length of 2.96 and 3.33 Å, respectively

The study revealed that all the selected compounds displayed molecular weight, molecular volume in the range of 257.26–382.56 (< 500), log P values (MlogP) in the range of 2.94 -5.49, hydrogen bond donors range ($H_D$) ≤ 5 and hydrogen bond acceptors ($H_A$) ≤ 10 (Table 7).

Except for compound **56**, all the selected compounds showed drug likeness score (DLS) in the range of 0.1–0.96, as compared to 0.30 for S-carprofen. The

**Table 7** Molecular properties related to drug-likeness

| Comp. | MW | MV[a] | TPSA | MlogP | RBs | $H_A$ | $H_D$ | LVs | DLS[a] | SA |
|---|---|---|---|---|---|---|---|---|---|---|
| **3** | 273.71 | 249.92 | 53.09 | 3.07 | 2 | 2 | 2 | No | 0.30 | 2.2 |
| **5** | 273.71 | 249.92 | 53.09 | 3.07 | 2 | 2 | 2 | No | 0.42 | 2.18 |
| **7** | 257.26 | 238.64 | 53.09 | 2.94 | 2 | 3 | 2 | No | 0.27 | 2.28 |
| **11** | 318.17 | 254.58 | 53.09 | 3.19 | 2 | 3 | 2 | No | 0.18 | 2.23 |
| **15** | 281.35 | 289.35 | 53.09 | 3.28 | 4 | 2 | 2 | No | 0.95 | 2.37 |
| **23** | 313.37 | 313.01 | 53.09 | 3.89 | 5 | 3 | 2 | No | 0.32 | 2.81 |
| **29** | 266.3 | 273.43 | 37.30 | 3.74 | 3 | 2 | 1 | No | 0.96 | 3.09 |
| **38** | 298.35 | 298.12 | 37.30 | 4.35 | 4 | 3 | 1 | 1[b] | 0.37 | 3.37 |
| **40** | 326.40 | 333.93 | 37.30 | 4.80 | 6 | 3 | 1 | 1[b] | 0.10 | 3.61 |
| **46** | 282.33 | 280.88 | 50.44 | 3.28 | 4 | 3 | 1 | No | 0.75 | 3.02 |
| **54** | 300.32 | 288.37 | 50.44 | 3.66 | 4 | 4 | 1 | No | 0.26 | 3.17 |
| **56** | 328.38 | 324.18 | 50.44 | 4.12 | 6 | 4 | 1 | No | −0.02 | 3.38 |
| **61** | 284.37 | 282.19 | 65.54 | 3.92 | 3 | 2 | 1 | No | 0.81 | 2.67 |
| **92** | 368.53 | 387.39 | 65.54 | 5.28 | 7 | 2 | 1 | 1[b] | 0.69 | 3.62 |
| **99** | 382.56 | 405.30 | 65.54 | 5.49 | 8 | 2 | 1 | 1[b] | 0.51 | 3.74 |

MW, molecule weight; MV, molecular volume ($A^3$); TPSA, topological polar surface area ($A^2$); MlogP, Moriguchi's logP; RBs, rotatable bonds; $H_A$, hydrogen bond acceptors; $H_D$, hydrogen bond donors; SA, Synthetic accessibility; DLS, Drug-likeness score

[a]Parameters calculated using Molsoft (http://www.swissadme.ch/), other parameters were calculated using (http://molsoft.com/mprop/); 1[b], one violation, MlogP > 4.15

synthetic accessibility of the selected compounds was in the range of 2.18–3.74 compared to 2.2 for S-carprofen.

## 4 Conclusion

In this study, four series of carprofen analogs were designed by isosteric replacement of the -NH- with -O-, -S- and -$CH_2$- groups. The impact of fluoro/alkyl substitutions on binding affinity and inhibition constants of the designed analogs was evaluated using molecular docking study. The results revealed that the binding affinity to COX-1/2 was dependent on position and length of the alkyl group. Compound **66** displayed the highest binding affinity for COX-1 and COX-2 with $\Delta G_b$ = 11.2 and 10.15 kcal/mol, respectively. Compound **56** displayed the highest potential selectivity for COX-1 ($K_i$ ratio = 43.09), while compound **99** was the most selective for COX-2 ($K_i$ ratio = 39.9). Compound **29** showed drug-likeness score of 0.96, as compared to 0.30 for S-carprofen, while compound **5**, **7** and **11** showed synthetic accessibility score comparable to the parent carprofen. Taken together, these results highlighted the impact of hydrophobic interactions of alkyl groups in modifying affinity and selectivity to COX-1 or COX-2.

## Compliance with ethical standards

**Conflict of interest** The authors declare that they have no conflict of interest.

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