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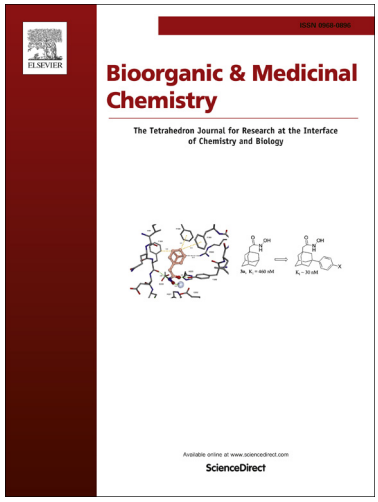

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Graphical Abstract

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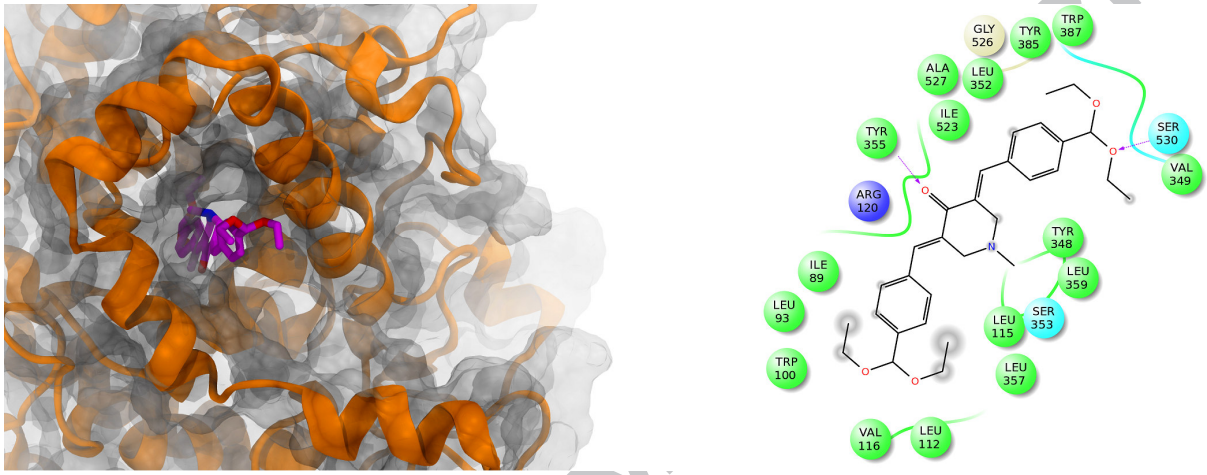

Pharmacological Evaluation and Docking Studies of α, β-Unsaturated Carbonyl Based Synthetic Compounds as Inhibitors of Secretory Phospholipase A_2, Cyclooxygenases, Lipoxygenase and Proinflammatory Cytokines

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ABSTRACT

Arachidonic acid and its metabolites have generated high level of interest among researchers due to their vital role in inflammation. The inhibition of enzymes involved in arachidonic acid metabolism has been considered as synergistic anti-inflammatory effect. A series of novel α, β-unsaturated carbonyl based compounds were synthesized and evaluated for their inhibitory activity on secretory phospholipase A_2 ($sPLA_2$), cyclooxygenases (COX), soyabean lipoxygenase (LOX) in addition to proinflammatory cytokines comprising IL-6 and TNF-α. Six α, β-unsaturated carbonyl based compounds (**2**, **3**, **4**, **12**, **13** and **14**) exhibited strong inhibition of $sPLA_2$ activity, with IC_{50} values in the range of 2.19 to 8.76 μM. Nine compounds **1-4** and **10-14** displayed inhibition of COX-1 with IC_{50} values ranging from 0.37 to 1.77 μM (lower than that of reference compound), whereas compounds **2**, **10**, **13** and **14** strongly inhibited the COX-2. The compounds **10**-**14** exhibited strong inhibitory activity against LOX enzyme. All compounds were evaluated for the inhibitory activities against LPS-induced TNF-α and IL-6 release in the macrophages. On the basis of screening results, five active compounds **3**, **4**, **12**, **13** and **14** were found strong inhibitors of TNF-α and IL-6 release in a dose-dependent manner. Molecular docking experiments were performed to clarify the molecular aspects of the observed COX and LOX inhibitory activities of the investigated compounds. Present findings increases the possibility that these α, β-unsaturated carbonyl based compounds might serve as beneficial starting point for the design and development of improved anti-inflammatory agents.

KEYWORDS: Tumor necrosis factor alpha; lipopolysaccharides; curcumin; Claisen-Schmidt condensation.

1. INTRODUCTION

Arachidonic acid and its metabolites have generated great interest due to their important role in inflammation. The important targets for the design of novel and safe anti-inflammatory agents include the inhibition of enzymes involved in arachidonic acid metabolism as secretory phospholipase A_2 ($sPLA_2$); cyclooxygenase (COX) and lipoxygenase (LOX) and also pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6). The metabolism of arachidonic acid leads to the formation of lipid mediators. Phospholipases A_2 (PLA_2s) are abundant enzymes that catalyze the hydrolysis of ester bond at the sn-2 position of glycerophospholipids. The released fatty-acid, such asarachidonic acid (AA), may be enzymatically metabolized into strong pro-inflammatory mediators known as eicosanoids (prostaglandins, leucotrienes and thromboxanes), though the lyso-phospholipid, other product of the PLA_2 catalyzed reaction in the case of lyso-platelet-activating-factor (lyso-PAF), might be converted into PAF (another renowned pro-inflammatory mediator) by the enzyme PAF-acetyltransferase. The suppression of production of lipid mediators has long been considered as therapeutical strategy due to their involvement in various pathological processes[1].

The first two steps in the biosynthesis of prostaglandins (PGs) are catalyzed by cyclooxygenase (COX) from the substrate arachidonic acid. At least two forms of this enzyme are known to exist[2-3]. Among them, COX-1 is constitutively expressed and maintains normal physiologic function and the PGs produced by this enzyme play a protective role. The other form cyclooxygenase-2 (COX-2), is an inducible form and its expression is affected by several stimuli including mitogens, oncogenes, tumorpromoters, and growth factors[3]. LOX enzymes catalyze arachidonic acid into leukotrienes and lipoxins. These mediators play a vital role during inflammation. Leukotrienes and prostaglandins are known to enhance the inflammatory reaction

whereas; the lipoxins have primarily anti-inflammatory effect. The important role of arachidonic acid and its metabolites in cancer biology and various types of cardiovascular diseases has developed interest amongst researchers. Latest research in the field of inflammation has given valuable information for the better understanding of modulation of arachidonic acid metabolism. The inhibition of enzymes involved in arachidonic acid metabolism has been deliberated as the synergistic anti-inflammatory effect with enhanced spectrum of activity[4].

Inflammation is a common phenomenon that is related to several diseases including cardiovascular diseases and cancer[5-7]. The pro-inflammatory cytokines, IL-6 and TNF-α are involved in the pathogenesis of numerous inflammatory disorders including rheumatoid arthritis (RA), inflammatory bowel disease, osteoarthritis, psoriasis, endotoxemia and/or toxic shock syndrome[8-16]. Regardless of pro-inflammatory attributes, these cytokines have widespread functions for retaining the normal cellular physiology. TNF-α can induce apoptosis and secretion of cytokines such as IL-1, IL-6 and IL-10 and can also activate T cells and other inflammatory cells. Conversely, an excess of TNF-α and IL-6 is attributed to the development of several human diseases as well as inflammatory disorders. The inhibition of cytokines, particularly TNF-α, has been successful in several clinical trials for the treatment of RA. Hence, the inhibition of TNF-α, pro-inflammatory cytokines, and over-expressions of cytokines has been recognized as an attractive target for the design and development of novel anti-inflammatory agents[17-20].

According to literature, curcumin and related α, β-unsaturated carbonyl based compounds exhibit a variety of pharmacological activities including potent antioxidant[21], anti-inflammatory[22-24], antiviral[24], antibacterial[25], antifungal[26], antitubercular[27] and immunomodulatory properties[28]. Recently, we have reported the synthesis and effects of curcumin-like α, β-unsaturated carbonyl based compounds on secretory phospholipase A_2,

cyclooxygenases, lipoxygenase, microsomal prostaglandin E Synthase-1 and immune system respectively[28-29]. Some compounds were found to be potent anti-inflammatory agents. These interesting findings encouraged us to synthesize a novel series of α, β-unsaturated carbonyl based compounds and evaluate their effects on enzymes involved in arachidonic acid metabolism and pro-inflammatory cytokines. Molecular docking experiments were also performed to clarify the molecular basis of the observed COX and LOX inhibitory activities of the investigated α, β-unsaturated carbonyl based compounds.

2. RESULTS AND DISCUSSION

2.1. Chemistry

Thirty novel curcumin related compounds (ten different types) were synthesized as reported before[28-29]. The α, β-unsaturated carbonyl based compounds (**1-7**; **11-17**; **21-27**) were synthesized by a Claisen-Schmidt condensation[28] between various ketones and the appropriate aryl aldehyde ata molar ratio of 1:2 in ethanol in the presence of NaOH. Moreover, some compounds were synthesized in acetic acid by passing through the mixture of dry HCl gas. Nine compounds (**8-10**; **18-20**; **28-30**) related to chalcone analogues were synthesized by similar Claisen-Schmidt condensation at a molar ratio 1:1 of ketone and aldehyde (Scheme 1). Eight compounds (**7**,**8**, **10**, **17**, **18**, **20**, **27**,**28**) were synthesized by using both NaOH and dry HCl gas mixture. The catalytic system using HCl gas mixture gave all compounds more efficiently, with high percentage yield as compared to the catalytic system involving NaOH. A mixture ofseveral unidentified products was also formed when NaOH was used and extensive purification was required (column chromatography). Synthesized compounds were purified by recrystallization and column chromatography using appropriate solvent systems. All thirty synthesized

compounds were characterized by using different spectroscopic techniques. Elemental analysis of C, H, N, and melting points of the compounds were also determined.

2.2. *Inhibition of secretory phospholipase A_2-V*

The inhibition of PLA_2 represents a key strategy for the prevention of inflammation. The inhibitory potency of all compounds (at a concentration of 1.25 to 20 µg/mL) against $sPLA_2$ was evaluated by an *in vitro* enzymatic photometric assay based on Ellman's method (Table 1). Curcumin and dexamethasone were positive controls. Curcumin which was used as reference compound inhibited the $sPLA_2$ activity by 81.57% (IC_{50} 11.10 µM). The previous studies on other isoforms of PLA_2 reported that curcumin inhibited the activity of PLA_2, and the isoforms of PLA_2 enzymes showed more than 70% homology[30-31]. Compounds **2**, **3**, **4**, **12**, **13** and **14** strongly inhibited $sPLA_2$-V in a dose dependent manner (Figure 1), with IC_{50} 2.19 to 8.76µM, which were lower or comparable to curcumin (10.12 µM).

2.3. *Inhibition of COX-1 and COX-2*

Modulation of arachidonic acid metabolism by inhibiting COX-1 and COX-2 is considered as one of the anti-inflammatory mechanisms. The cyclooxygenase (COX-1 and COX-2) inhibitory activity of α, β-unsaturated carbonyl based compounds was determined by *in vitro* COX-inhibitor screening assay at a concentration of 40 µg/mL[31]. The IC_{50} values were calculated and presented in Table 1. The COX-1 and COX-2 inhibitory effect of these α, β-unsaturated carbonyl based compounds was compared with pure curcumin. The α, β-unsaturated carbonyl based compounds showed affinity towards COX-1, with compounds (**1, 2, 3, 4, 10, 11, 12, 13** and **14**) exhibiting strong inhibitory effect (81.79 - 92.22%) on the activity of COX-1. Curcumin was used as a reference compound while Indomethacin served as positive control. Most of the compounds

exhibited better COX-1 inhibitory activity than that of curcumin with IC_{50} 0.37 to 21.54 µM, which was lower than that of curcumin (29.55 µM); however the inhibitory activity of compound **20** was comparable with curcumin with IC_{50}29.33 µM. The compound **4** showed highest COX-1 inhibition (IC_{50} 0.37 µM) followed by compound **11** (IC_{50} 0.42 µM). The compounds were less efficient in suppressing the activity of COX-2, as compared to COX-1. Six compounds (**2, 7, 10, 13, 14** and **18**) exhibited COX-2 inhibitory activity with IC_{50}<15 µM. Compound **13** showed highest COX-2 inhibition with IC_{50} 7.88 µM. Majority of compounds displayed better COX-2 inhibitory activity with IC_{50}<100 µM. It was observed that the compounds inhibited COX activity in a dose-dependent manner. These results suggest that α, β-unsaturated carbonyl based compounds act as inhibitors of COX enzymes and exhibited anti-inflammatory effects.

2.4. In vitro inhibition of soybean lipoxygenase

We evaluated the ability of our synthesized compounds to inhibit soybean lipoxygenase. Curcumin was used as reference. Curcumin has been reported to inhibit 5-LOX activity both in *in vitro* and *in vivo* models and was used as reference in this assay[32-33]. Katsori et al. reported the inhibitory effect of curcumin on soybean LOX [34]. The inhibitory effect of our newly synthesized compounds (at concentration of 2.5 to 40 µg/mL) on LOX was determined (Table 1). The preliminary screening revealed that compounds **4**, **10**, **11**, **12**, **13** and **14** strong inhibitors of LOX with percentage inhibitions ranging from 65 - 85 %. DMSO was used as negative control and did not affect the activity of enzyme. At 40 µg/mL, compounds **4** and **10** (67.57 and 69.52 %) were equipotent to curcumin (68.28%). The compounds **10**, **11**, **12**, **13** and **14** had IC_{50} values ranging from 29.28 to 52.22 µM, which were lower or comparable to that of curcumin (59.52 µM). The compound **11** exhibited strongest LOX inhibitory activity (82.72%) among all the compounds,

with IC_{50}29.28 µM. Hence, it can be concluded that the compounds inhibited LOX activity in a dose-dependent manner (Figure 2).

2.5. Inhibition of TNF-α and IL-6 release in LPS-stimulated macrophages

Lipopolysaccharide (LPS) is a well-studied immune stimulator that induces a systemic inflammation response [35], particularly, the expression of proinflammatory cytokines, such as TNF-α and IL-6. The inhibitory effect of new compounds against LPS-induced TNF-α and IL-6 release in mouse macrophages (RAW 264.7) was evaluated. The macrophages were pre-treated with 10 µM compounds for 2 h followed by the incubation with 0.5 µg/ml LPS for 22 h. Enzyme-linked immunosorbant assays (ELISA) was performed to detect the amount of TNF-α and IL-6 in media.

The results of the enzyme-linked immunosorbant assay of α, β-unsaturated carbonyl based compounds are presented in Figure 3. Compounds **2**, **3**, **4**, **7**, **12**, **13** and **14** exhibited strongest percentage inhibitory activity (73 to 91 %) against LPS-induced TNF-α expression, while compounds **3**, **4**, **6**, **12**, **13**, **14** and **16** showed strong inhibitory effects (76 - 91 %) against IL-6 release as compared to the LPS-control. Compounds **3**, **4**, **13** and **14** were more potent than curcumin at the same concentration in inhibiting LPS-induced TNF-α and IL-6 expression. Compounds **3** and **14**, displayed the strongest inhibitory effect (91%) on LPS-induced TNF-α and IL-6 release among all compounds, compared to the LPS-control. On the other hand, three compounds (**9**, **27** and **28**) showed immunostimulatory effects and increased the release of TNF-α from stimulated macrophages and similarly three compounds (**19**, **28** and **30**) showed increase in release of IL-6.

2.6. Structure-activity relationship analysis of α, β-unsaturated carbonyl based compounds

All α, β-unsaturated carbonyl based compounds were evaluated for their inhibitory effects on $sPLA_2$, COX and LOX enzymes (Table 1) and on release of TNF-α and IL-6 (Figure 3). The analysis of results and structure-activity relationship (SAR) revealed that compounds **3**, **4**, **13** and **14** exhibited strong inhibitory effects on four enzymes of arichidonic acid metabolism and on release of TNF-α and IL-6 from macrophages.

After analyzing SAR, it can be observed that that the linkers derived from ketones during synthesis play important roles in terms of inhibitory activity. Variable activity trends were seen amongst different series of α, β-unsaturated carbonyl based compounds when either linear or cyclic linkers were used between the two aromatic rings of compounds. Generally, *N*-methyl-4-piperidone linker containing compounds (**4**, **14**, **24**) showed potent anti-inflammatory activities, moderate activity was exhibited by compounds (**3**, **13**, **23**) with 4-piperidone linker, whereas three compounds (**2**, **12**, **22**) with tetrahydropyran-4-one displayed good inhibition power, yet it was lesser as compared to compounds having 4-piperidone linker. Bis-derivatives, possessing either1-Benzyl-4-piperidone (**5**, **15**, **25**), acetone (**6**, **16**, **26**) or cyclopentanone (**7**, **17**, **27**) linker, were less active as compared to the others, however compound **7** exhibited good inhibition of IL-6 and COX-1. Compounds **4** and **14** containing *N*-methyl-4-piperidone linker were observed to be strong inhibitors of $sPLA_2$, COX-1, LOX and cytokines. Synthetic α, β-unsaturated carbonyl based compounds possessing the 4-piperidone moiety (**3**and **13**) also showed good inhibitory activity but comparatively weaker as compared to *N*-methyl-4-piperidone linker, except compound **3** that exhibited more inhibition of TNF-α release in contrast to compound **4**. Compounds **23** and **24** containing 4-piperidone and *N*-methyl-4-piperidone linker did not show strong anti-inflammatory activities in any assay that can be result of pyrrolidine introduction at

position 4 of the aromatic rings. It can be further concluded that the presence of pyrolidine at position 4 of compounds (**21**-**30**) rendered the compounds only slightly active.

Effects of substitution of various groups on aromatic rings of compounds were also compared. It was seen that introduction of 2-nitro-4-dimethylamine combination on the aromatic rings enhanced the anti-inflammatory activity by increased inhibition of $sPLA_2$, COX-2, LOX, IL-6, and TNF-α. In all the three groups (based on substitution pattern **1-10**, **11-20**,**21-30**), compounds bearing diethoxymethyl group at position 4 of rings showed significantly reduced anti-inflammatory activity except **3** and **4** which showed more COX-1 inhibition in comparison to compound **13** and **14**. Moreover, the presence of pyrolidine at position 4 of compounds (**21-30**) resulted in compounds which were not highly active.

2.7. Docking studies

2.7.1. COX-1/COX-2

Molecular docking experiments were employed in order to clarify the molecular basis of the observed COX inhibitory activities of the investigated curcumin-like α, β-unsaturated carbonyl based compounds. All the compounds were docked into the 3-D COX-1 and COX-2 structures complexed with indometacin (PDB codes: 1PGG[36] for COX-1, and 4COX[37] for COX-2, focusing the conformational search in the related ligand binding sites (LBDs).

Regarding COX-1 predicted complexes, we generally observed a suitable accommodation of the compounds in the related LBD. All the active compounds are able to deeply occupy the binding cavity, establishing at the same time both polar and hydrophobic interactions. In these cases, we found the presence of H-bonds between the investigated compounds and Ser530, and/or Tyr355, and/or Arg120, thus respecting the binding mode

reported by Selvam et al. for other curcumin analogues[38]. The remaining hydrophobic parts of the investigated compounds are involved in vdW interactions with the other residues belonging to the COX-1 LBD, namely Leu93, Tyr355, Leu357, Tyr385, Leu384, Phe518, Ile523. In the binding pose of the most active compound **4** is represented (Figure 4).

Compound **4** establishes several hydrophobic interactions along the whole COX-1 LBD. In the most external part of the binding site, we found vdW contacts between the terminal (diethoxymethyl)-benzyl part of **4** and Ile89, Leu93, Trp100, Leu112, Leu115, Val116, and Leu357. The central core of the molecule is involved in a hydrogen bond between the lactone oxygen and terminal -OH in the side chain of Tyr355 and in polar contacts with Arg120, while other hydrophobic interactions are detectable with Tyr348 and Leu359. The other terminal (diethoxymethyl)-benzyl substituent, located in the internal part of the COX-1 LBD, establishes a H-bond with Ser530 and vdW contacts with Val349, Leu352, Tyr385, Trp387, Ile523, Gly526 and Ala527 (Figure 4).

Concerning inactive compounds, docking models highlight the lack of fundamental interactions with the protein counterpart (Supporting Information)

As known, COX-2 differs from COX-1 for several amino acidic substitutions in the related LBD (V523I, V434I, R513H, and A516S). The main structural differences between the two isoforms are related to the first two modifications, and in particular the presence of two valins instead of isoleucins determines a larger space in the COX-2 LBD that can be exploit for the design of selective inhibitors. We generally observed a decrement of the experimental inhibitory activity of the investigated compounds that are generally less fitted in the larger COX-2 binding site. For example, compound **4** is in this case placed in the LBD with a reduced shape

complementarity, establishing at the same time less contacts with the receptor counterpart. Moreover, an inversion of the central core is also observable, determining the loss of the H-bond with Tyr355 (Figure 5).

Compounds **5**, **9**, **11**, **22**, **24** and **29** showed a selective inhibition for COX-1, being inactive to COX-2. Once again, this can be ascribed to the larger COX-2 LBD, that determines the occupation of the additional space behind the channel generated by the V523I substitution instead of the deeper part of the binding groove. For example, compound **11** oriented one of its 4-(dimethylamino)-2-nitro-benzyl group in this additional cavityin the binding site, losing the interactions with Ser530 and the related close residues (Figure-supporting information).

2.7.2. Docking on Soybean Lipoxygenase (LOX)

As reported by Jankun et al.,[39-40] curcumin is able to interact with soybean LOX binding its central cavity, close to the residues directly contacting with Iron cofactor. The different crystal structures of this protein are characterized by a restricted internal space, that makes necessary the use of flexible docking procedures to rightly dock the investigated compounds[41]. In particular, starting from crystal structure of soybean LOX (PDB code1HU9), five key residues were chosen to be flexible during docking experiments: Ser510, Gln514, Trp519, Arg562, and Phe576.

Compound **11**, showing the better inhibitory activity on the investigated target, occupies this binding cavity establishing several polar (Ser510, His513, His523, Glu527, Arg562, Thr575, Gln716, Arg726, Asp766) and hydrophobic interactions (Tyr207, Leu565, Ile770, Leu773), while one of the nitro groups of the molecule points to the iron cofactor (Figure 6).

Active compounds not showing nitro groups are able to bind iron in different ways; for example, compound **10** can interact with the cofactor or with the terminal -(dietoxymehyl)phenil

group, or thanks to the diketo-moiety on the opposite part of the molecule (Figure 7). Also in this case, compounds showing the pyrrolidine terminal groups are inactive, and this is mainly due to their minor flexibility preventing the productive interaction with the metal in the LBD (e.g., compound **27**, Figure in supporting information).

3. CONCLUSIONS

Herein, we report the pharmacological evaluation and molecular modeling of thirty novel α, β-unsaturated carbonyl based synthetic compounds. We evaluated their inhibitory activities against the enzymes involved in biosynthesis of arachidonic acid and its metabolites. Among these compounds, numerous derivatives were able to inhibit the activity of PLA_2, COX, LOX, IL-6 and TNF-α. The most potent enzyme and cytokine inhibitors in this series correspond to *N*-methyl-4-piperidone and 4-piperidone moieties. Inhibition of these enzymes and cytokines by α, β-unsaturated carbonyl based compounds highlights the anti-inflammatory property of these compounds. It increases the possibility that curcumin related α, β-unsaturated carbonyl based might serve as a starting point for the design and development of new and improved anti-inflammatory agents.

4. EXPERIMENTAL SECTION

4.1. Materials

All reagents and chemicals were purchased from Sigma-Aldrich, Merck and Acros Organics (above 98% purity) and were used without further purification. Chemicals used in $sPLA_2$ assay include 1,2-bis(heptanoylthio)-phosphatidylcholine, 5,5-dithiobis (2-nitrobenzoic acid) (DTNB), and recombinant human PLA_2-V from Cayman Chemicals U.S.A. $CaCl_2$, KCl, and HCl were purchased from Merck, Germany. Curcumin, dimethyl sulphoxide (DMSO), ethylene diamine

tetra acetic acid (EDTA), reduced glutathione (GSH) and $SnCl_2$ were obtained from Sigma-Aldrich Steinheim, Germany. Cyclooxygensae activity was determined by using a COX Inhibitor Screening Kit from Cayman Chemicals U.S.A. Soyabean lipooxygenase, arachidonic acid and potassium hydroxide were also obtained from Cayman Chemicals U.S.A.TNF-α and IL-6 in the media were determined by ELISA using mouse TNF-α (Catalogue No. 500850) and mouse IL-6 (Catalogue No. 583371) ELISA Kits (Cayman, USA). Mouse RAW 264.7 macrophages were obtained from Abcam, UK (Cambridge, ENG). Cell culture reagents and fetal bovine serum (FBS) were obtained from Sigma Aldrich and FBS was heat-inactivated for 30 min at 65°C. LPS purchased from Sigma was dissolved in PBS. Synthetic compounds were dissolved in DMSO.

4.2. General Procedures

JEOL ECP spectrometer operating at 500 MHz was used to record ^{1}H and ^{13}C NMR spectra, with Me_4Si as internal standard and $CDCl_3$ or DMSO-d^6 as the solvents. Electrospray ionization mass spectrometry (ESI-MS) on MicroTOF-Q mass spectrometer (Bruker) recorded high resolution mass spectra (HRMS) of the compounds. Microanalyses of the compounds were carried out on Fison EA 1108 elemental analyzer. KBr disc method was used to record infrared spectra on a Perkin Elmer 400 (FTIR) spectrometer. Silica gel 60 (230-400mesh) (Merck) was used for flash column chromatography, whereas thin layer chromatography (TLC) was carried out on pre-coated silica plates (kiesel gel 60 F_{254}, BDH). Melting points of the compounds were determined on an electrothermal instrument.

4.3. Synthesis of α, β-Unsaturated Carbonyl based Compounds

Twenty one α, β-unsaturated carbonyl based compounds (**1-7**; **11-17**; **21-27**) were synthesized by coupling of suitable aromatic aldehyde with the ten types of ketones at a molar ratio of 1:2,

whereas nine (**8-10**; **18-20**; **28-30**) were synthesized at a ratio of 1:1, under base catalyzed Claisen–Schmidt condensation reaction. Scheme 1 demonstrates the synthesis of α, β-unsaturated carbonyl based compounds. The appropriate aromatic aldehyde (20 mmol, 2equivalant) and suitable ketone (10 mmol, 1 equivalent) were mixed and dissolved in ethanol in a round bottomed flask and stirred at 5°C for a few minutes. A 40% NaOH solution in ethanol was added drop wise into this solution and the resulting mixture was allowed to stir at room temperature (27 °C) for 1-24 h. The appearance of precipitate and color changes of the reaction mixture acted as indicative markers of product formation. The reaction was monitored by TLC and on completion; the reaction was stopped by adding acidified ice. The α, β-unsaturated carbonyl based compounds were isolated by either column chromatography or recrystallization.

4.3.1. 2,6-Bis[4-(diethoxymethyl)benzylidene]cyclohexanone **(1)**

Yellow crystals (2.59 g, 54%). mp: 112-114 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.89 (s, 2H), 7.29 (d, J=8Hz, 4H), 6.82 (d, J=8Hz, 4H), 5.89 (s, 2H), 3.42 (q, J=7.5, 8H), 2.32 (t, J=12.0 Hz, 4H), 1.82 (m, 2H), 1.24 (t, J=7.5, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 186.4, 152.9, 144.4, 136.5, 132.5, 127.3, 126.5, 101.1, 55.0, 28.3, 27.8, 16.2;HRMS (ESI) m/z: 479.68 $[M+H]^+$, Microanalysis calculated for $C_{30}H_{38}O_5$ (478.62), C: 75.28%, H: 8.00%. Found C: 75.42%, H: 8.12%.

4.3.2. 3,5-Bis-(4-diethoxymethyl-benzylidene)-tetrahydro-pyran-4-one ***(2)***

Pale Yellow crystals (3.06 g, 64%). mp: 106-108 ˚C;^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.85 (s, 2H), 7.32 (d, J=8Hz, 4H), 6.95 (d, J=8Hz, 4H), 5.52 (s, 2H), 3.62 (q, J=7.5, 8H), 2.69 (s, 4H), 1.18 (t, J=8, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 187.2, 152.7, 143.8, 136.7, 131.9, 126.2, 125.2,

101.5, 62.4, 55.2, 16.1; HRMS (ESI) m/z: 481.65 $[M+H]^+$, Microanalysis calculated for $C_{29}H_{36}O_6$ (480.59), C: 72.48%, H: 7.55%. Found C: 72.62%, H: 7.72%.

4.3.3. 3,5-Bis[4-(diethoxymethyl)benzylidene]piperidin-4-one **(3)**

Yellow solid (2.89 g, 60%). mp: 101-102 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.68 (s, 2H), 7.12 (d, J=7.5Hz, 4H), 6.93 (d, J=7.5Hz, 4H), 5.65 (s, 2H), 3.36 (q, J=7.5, 8H), 2.65 (s, 4H), 1.27 (t, J=7.0, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 185.2, 145.1, 144.5, 138.5, 136.2, 128.1, 127.8, 102.1, 55.1, 48.7, 16.4 ;HRMS (ESI) m/z: 480.71 $[M+H]^+$, Microanalysis calculated for $C_{29}H_{37}NO_5$ (479.61), C: 72.62%, H: 7.78%, N: 2.92%. Found C: 72.71%, H: 7.86%, N: 3.10%.

4.3.4. 3,5-Bis[4-(diethoxymethyl)benzylidene]-1-methyl-piperidin-4-one **(4)**

Light Yellow solid (2.12 g, 43%).mp: 142-144 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.63 (s, 2H), 7.32 (d, J=8Hz, 4H), 7.15 (d, J=8Hz, 4H), 5.59 (s, 2H), 3.29 (q, J=7.5, 8H), 2.71 (s, 4H), 2.12 (s, 3H), 1.20 (t, J=7.0, 12H);^{13}C NMR (500 MHz, $CDCl_3$) δ: 189.7, 148.2, 147.5, 139.8, 135.1, 128.4, 127.1, 101.4, 56.6, 51.2, 40.1, 16.7 ;HRMS (ESI) m/z: 494.65 $[M+H]^+$, Microanalysis calculated for $C_{30}H_{39}NO_5$ (493.63), C: 72.99%, H: 7.96%, N: 2.84%. Found C: 72.95%, H: 7.99%, N: 2.87%.

4.3.5. 1,5-Bis-(4-diethoxymethyl-phenyl)-penta-1,4-dien-3-one **(6)**

Pale brownish solid (1.96 g, 45%). mp: 98-100 °C;^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.82 (d, J=6.5Hz, 4H),7.71 (d, J=8Hz, 2H) , 7.49 (d, J=6.5Hz, 2H), 7.16 (d, J=7Hz, 4H), 5.25 (s, 2H), 3.52 (q, J=7Hz, 8H), 1.17 (t, J=7.0, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.5, 152.2, 148.6, 139.7, 136.4, 135.2, 132.8, 102.2, 55.5, 16.9 ;HRMS (ESI) m/z: 439.62 $[M+H]^+$, Microanalysis calculated for $C_{27}H_{34}O_5$ (438.56), C: 73.94%, H: 7.81%. Found C: 74.12%, H: 7.89%.

4.3.6. 2,6-Bis-(4-dimethylamino-2-nitro-benzylidene)-cyclohexanone **(11)**

White powder (2.78 g, 62%). mp: 139-140 ˚C;δ: 7.94 (s, 2H), 7.55 (d, J=8Hz, 2H), 7.41 (d, J=8Hz, 2H), 7.13 (s, 2H), 3.15 (s, 12H), 2.35 (t, J=12.0 Hz, 4H), 1.87 (m, 2H) ; ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.7, 149.5, 145.7, 145.6, 140.1, 128.2, 118.6, 117.6, 106.5, 46.8, 29.1, 27.5;HRMS (ESI) m/z: 451.64 $[M+H]^+$, Microanalysis calculated for $C_{24}H_{26}N_4O_5$ (450.49), C: 63.99%, H: 5.82%, N: 12.44%. Found C: 64.12%, H: 5.72%, N: 12.42%.

4.3.7. 3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]tetrahydro-pyran-4-one **(12)**

White powder (2.92 g, 65%). mp: 188- 190 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.89 (s, 2H), 7.46 (d, J=8Hz, 2H), 7.14 (d, J=8Hz, 2H), 7.05 (s, 2H), 3.13 (s, 12H), 2.93 (s, 4H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 190.5, 149.2, 146.9, 145.1, 139.9, 128.4, 118.9, 118.1, 107.8, 65.5, 46.2;HRMS (ESI) m/z: 475.52 $[M+Na]^+$, Microanalysis calculated for $C_{23}H_{24}N_4O_6$ (452.46), C: 61.05%, H: 5.35%, N: 12.38%. Found C: 61.24%, H: 5.59%, N: 12.42%.

4.3.8. 3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]piperidin-4-one **(13)**

Light yellow solid (2.28 g, 51%). mp: 192- 194 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.82 (s, 2H), 7.72 (d, J=8Hz, 2H), 7.21 (d, J=8Hz, 2H), 6.92 (s, 2H), 3.17 (s, 12H), 3.13 (s, 4H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 189.4, 149.7, 148.6, 142.5, 140.7, 127.3, 118.8, 118.1, 106.5, 49.4, 46.9;HRMS (ESI) m/z: 452.52 $[M+H]^+$, Microanalysis calculated for $C_{23}H_{25}N_5O_5$ (451.48), C: 61.19%, H: 5.58%, N: 15.51%. Found C: 61.42%, H: 5.62%, N: 15.89%.

4.3.9. 3,5-Bis[4-(dimethylamino)2-nitro-benzylidene]-1-methyl-piperidin-4-one **(14)**

White powder (2.56 g, 55%). mp: 181- 182 ˚C; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.76 (s, 2H), 7.45 (d, J=8Hz, 2H), 7.23 (d, J=8Hz, 2H), 7.13 (s, 2H), 3.21 (s, 12H), 3.03 (s, 4H), 2.19 (s, 3H); ^{13}C

NMR (500 MHz, $CDCl_3$) δ: 187.5, 148.9, 148.2, 143.2, 141.8, 126.7, 119.6, 118.9, 106.2, 44.5, 46.1, 38.8;HRMS (ESI) m/z: 466.72 $[M+H]^+$, Microanalysis calculated for $C_{24}H_{27}N_5O_5$ (465.50), C: 61.92%, H: 5.85%, N: 15.04%. Found C: 61.99%, H: 5.91%, N: 15.19%.

4.3.10. 1,5-Bis-(4-dimethylamino-2-nitro-phenyl)-penta-1,4-dien-3-one **(16)**

Pale yellow solid (2.97 g, 68%). mp: 177-179 ˚C;^{1}H NMR (500 MHz, $CDCl_3$) δ: 7.70 (d, J=6Hz, 2H), 7.68 (d, J=8Hz, 2H), 7.44 (d, J=6Hz, 2H),7.25 (d, J=8Hz, 2H), 7.12 (s, 2H), 3.15 (s, 12H); ^{13}C NMR (500 MHz, $CDCl_3$) δ: 187.5, 150.9, 148.6, 144.4, 132.8, 128.1, 117.5, 113.8, 107.9, 46.7; HRMS (ESI) m/z: 409.45 $[M-H]^+$, Microanalysis calculated for $C_{21}H_{22}N_4O_5$ (410.42), C: 61.45%, H: 5.40%, N: 13.65%. Found C: 61.48%, H: 5.62%, N: 13.77%.

4.4. Secretory Phospholipase A_2 -V ($sPLA_2$-V) Activity Assay

The effect of α, β-unsaturated carbonyl based compounds on the activity of $sPLA_2$ enzyme was investigated by a photometric assay based on Ellman's method[42]. Briefly, the hydrolysis of sn-2ester bond of the substrate 1,2-bis(heptanoylthio)-glycerophosphocholine by PLA_2-V resulted in exposure of free thiols. These thiols triggered the alteration of DTNB (5,5–dithio-bis-(2-nitrobenzoic acid) to 2-nitro-5-thiobenzoic acid, which was detected photometrically at 405 nm. Afterwards, the assay was proceeded in an aqueous buffer solution (pH 7.5) containing KCl(94 mM), $CaCl_2$ (9mM), Tris (24 mM) and Triton-X 100 (280 BM). Prior to the start of assay, substrate and PLA_2-V were re- suspended in assay buffer, and DTNB was dissolved in a solution of Tris-HCl (pH 8).The enzyme and DTNB yielded final concentrations of 100 ng/mL and 87 BM, respectively. The assay was performed in 96-well microliter plates, containing DTNB, substrate solution and the respective test substance. The maximum (100%) activity of the

enzyme was calculated by adding substrate and enzyme only. DMSO was used as negative control and was inactive at the concentration used in the assay (1.7% v/v).

4.5. Cyclooxygenase Assay

The effect of α, β-unsaturated carbonyl based compounds on COX-1and COX-2 was determined by using a COX Inhibitor Screening Kit (Catalog No 560131) from Cayman Chemicals, Ann Arbor, Michigan, U.S.A. The reaction mixture was prepared with 1µM heme and COX-1 (ovine) or COX-2 (human recombinant) enzymes in 100mM Tris-HCl buffer, and then pre-incubated for 10 min in a water bath (37 °C) at pH 8.0. The reaction was started by adding 10µl arachidonic acid (final concentration in reaction mixture 100µM). After 2 min, the reaction was terminated by adding 1M HCl and lastly PGE_2 was quantified by ELISA. The compounds were dissolved in DMSO and diluted to desired concentration with potassium phosphate buffer. After transferring the compounds to 96 well plate coated with a mouse anti-rabbit IgG, tracer prostaglandin acetylcholine esterase and primary antibody (mouse anti PGE_2) were added and the plates were kept for overnight. Thereafter, the reaction mixtures were removed and the wells were washed with 10mM potassium phosphate buffer containing 0.05% Tween 20. Ellman's reagent (200µl) was then added to all the wells and were incubated at room temperature for 60 min, until the control wells gave an OD=0.3-0.8 at 412 nm. A standard curve with PGE_2 was generated from the same plate, which was used to calculate the PGE_2 levels produced in the presence of test compounds. The results were expressed as percentage relative to control (solvent treated samples). All these experiments were performed in triplicate and values normally agreed within 10%.

4.6. Lipoxygenase Activity Assay

The effect of α, β-unsaturated carbonyl based compounds on purified LOX enzyme was investigated by a standard colorimetric assay[43]. Soybean lipoxygenase was used as enzyme source. The assay was performed in 0.1 M Tris-HCl buffer solution (pH 7). Before the start of the assay, LOX enzyme was re-suspended in buffer. The substrate was dissolved in equal volume of potassium hydroxide, vortexed and diluted to get the concentration of 1 mM. The assay was performed in 96-well microliter plates at room temperature containing substrate, enzyme and test substance. The maximum (100%) activity of enzyme was calculated by adding the substrate and enzyme. To determine the activity, 90 BL of LOX and 10 BL of respective test sample was added. The reaction was initiated by adding substrate solution to all wells for 5 min. After that, 100 BL of chromogen solution was added to all wells for 5 min to stop enzyme catalysis. In the next step,the absorbance was measured at 490 nm by using Tecan® Infinite Pro 200microplate reader.

4.7. Cell treatment and ELISA assay for TNF-α and IL-6

Mouse RAW 264.7 macrophages were incubated in DMEM supplemented with 10% FBS, 1% Pen Strep at 37°C with 5% CO_2. The cells were pre-treated with 10 µM α, β-unsaturated carbonyl based compounds or vehicle control for 2 h, and then treated with LPS (0.5 µg/ml) for 22 h. The culture medium and cells were collected separately after the treatment. The medium collected was centrifuged at 1000 rpm for 10 min. The levels of TNF-α and IL-6 in the medium were measured by mouse TNF-α and mouse IL-6 ELISA Kits (Cayman, USA). The supernatant obtained after centrifugation was separated and stored at -80°C until use. The cells were washed with PBS and harvested with cell lysis buffer (Tris-HCl 20 mM, NP40 1%, NaCl 150 mM, EDTA 2 mM,Na3VO4 200 mM, SDS 0.1%, NaF 20 mM). The mixed liquor was shaken vigorously for 10 min in lysis buffer at 0°C. After centrifugation at 12,000 rpm for 30

min at 4°C, the total protein was collected and the concentrations were calculated using Bio-Rad protein assay reagents. The total amount of inflammatory factor in the medium was normalized to the total protein amount of the viable cell pellets.

4.8. Computational details

The chemical structures of investigated compounds were built with Maestro (version 9.6)[44] Build Panel and then prepared with LigPrep software. We applied an optimization (Conjugate Gradient, 0.05 Å convergence threshold) of the structures to identify possible three-dimensional starting models of each compounds for the subsequent docking calculations. Then, all the structures were converted in the .pdbqt format using OpenBabel software (version 2.3.2)[45], adding Gasteiger charges.

Protein 3D models of COX-1, COX-2, and Soybean LOX were prepared starting from the X-ray structures in the Protein Data Bank database (PDB codes: 1PGG for COX-1, 4COX for COX-2, and 1HU9 for Soybean LOX).Water molecules were removed, and .pdb files obtained were then processed with Autodock Tools 1.5.6 and converted in .pdbqt format, merging non polar hydrogens and adding Gasteiger charges. Charge deficit was spread over all atoms of related residues.

Docking calculations were performed using the Autodock-Vina software[46]. In the configuration files linked to 3D structures of the proteins, we specified coordinates and dimensions along x,y,z axes of the grid related to the site of presumable pharmacological interest, with spacing of 1.0 Å between the grid points. In particular, for COX-1 target, we set a grid of 24×20×24 and centered at 27.631 (x), 33.575 (y), and 210.355 (z). Regarding COX-2, the grid was centered at 25.453 (x), 17.467 (y), 17.793 (z), and with dimensions of 20×22×22.The exhaustiveness value was set

to 64, saving 30 conformations as maximum number of binding modes. Regarding Soybean LOX target, we employed Autodock-Vina flexible docking procedure, choosing residues Ser510, Gln514, Trp519, Arg562, and Phe576 as flexible and the rest of the protein as rigid. We set a grid of 224×28×20 and centered at 21.156 (x), 3.403 (y), and 20.652 (z), setting the exhaustiveness to 64, and saving 30 conformations as maximum number of binding modes.

For all the investigated compounds, all open-chain bonds were treated as active torsional bonds. Docking results were analyzed with Autodock Tools 1.5.6. Illustrations of the 3D models were generated using Autodock Tools 1.5.6[47],VMD[48] and Maestro software.

4.9. Statistical analysis

All the experiments were performed in triplicates and data presented as the mean ± standard error of mean (S.E.M.). The IC_{50} values were calculated by using Graph Pad Prism 5 software. Data was analyzed using a one-way analysis of variance (ANOVA) for multiple comparisons. $P < 0.05$ was considered to be statistically significant.

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Notes

The authors declare no competing financial interest.

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Captions

Scheme 1: Structures and synthesis scheme of α, β-unsaturated carbonyl based compounds. Reagents and conditions: (i) NaOH, EtOH, Room temperature.

Table 1: Inhibition of secretory Phospholipase A_2-V ($sPLA_2$-V), COX-1, COX-2 and lipooxygenase activities by synthetic compounds.

Figure 1: Concentration dependent inhibitory effects of diarylpentanoid analogues on activity of secretory phospholipase A_2-V. Graph represents the % inhibition by most active compounds along with curcumin. *$p<0.05$, **$p<0.01$ are significant difference with respect to curcumin. The data shown are an average of three independent experiment and values are mean ± S.D.

Figure 2: Concentration dependent inhibitory effects of diarylpentanoid analogues on activity of lipoxygenase. Graph represents the % inhibition by most active compounds along with curcumin. *$p<0.05$, **$p<0.01$ are significant difference with respect to curcumin. The data shown are an average of three independent experiment and values are mean ± S.D.

Figure 3: Curcumin and related synthetic compounds inhibited LPS-induced TNF-α and IL-6 secretion in RAW 264.7 macrophages. Cells were pretreated with synthetic compounds (10μM) for 2 hrs, then treated with LPS (0.5 μg/ml) for 22 hrs. The results are expressed as percent of LPS control.

Figure 4: a) 3-D docking model of **4**(colored by atom type: C magenta, O red, N blue) in the ligand binding site (LBD) of COX-1 (secondary structure colored in orange, residues interacting with **4** highlighted in licorice and colored by atom type: C grey O red, N blue, polar H light grey); b) 2D panel representing the interactions between **4** and the residues of the receptor counterpart.

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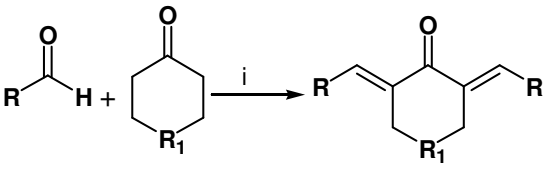
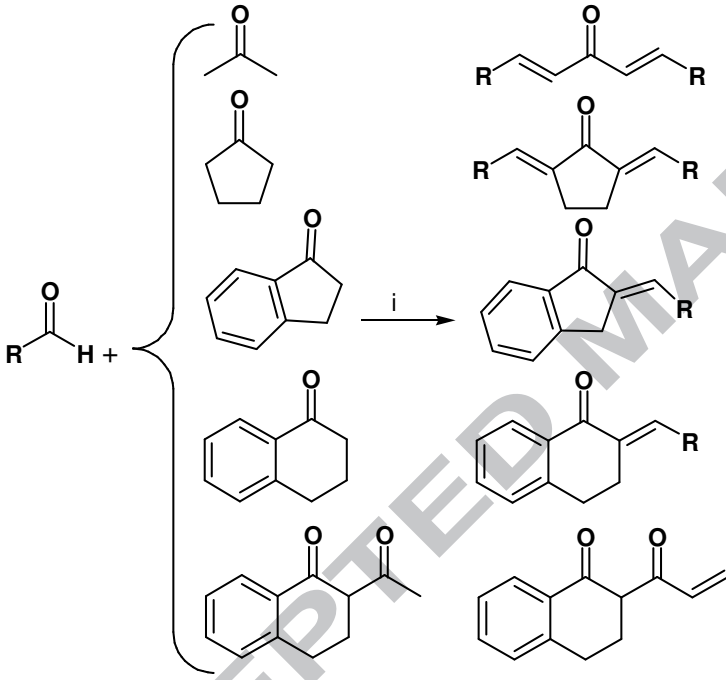
Figure 6: a) 3-D docking model of **11**(colored by atom type: C ochre, O red, N blue) in the LBD of soybean LOX (secondary structure colored in grey, flexible residues interacting with **11** highlighted in licorice and colored by atom type: C grey from the original PDB coordinates; C light blue after movements due to the flexible docking); b) 2D panel representing the interactions between **11** and the residues of the receptor counterpart.

Figure 7: 3-D docking model of **10**(colored by atom type: C green, O red, N blue) in the LBD of soybean LOX (secondary structure colored in grey, flexible residues interacting with **10** highlighted in licorice and colored by atom type: C grey from the original PDB coordinates; C light blue after movements due to the flexible docking).



	R	R_1
1	4-Diethoxymethyl-benzene	CH_2
2	4-Diethoxymethyl-benzene	O
3	4-Diethoxymethyl-benzene	NH
4	4-Diethoxymethyl-benzene	NCH_3
5	4-Diethoxymethyl-benzene	NCH_2-benzene
11	Phenyl-2-nitro-4-dimethylamine	CH_2
12	Phenyl-2-nitro-4-dimethylamine	O
13	Phenyl-2-nitro-4-dimethylamine	NH
14	Phenyl-2-nitro-4-dimethylamine	NCH_3
15	Phenyl-2-nitro-4-dimethylamine	NCH_2-benzene
21	Phenyl-4-pyrrolidine	CH_2
22	Phenyl-4-pyrrolidine	O
23	Phenyl-4-pyrrolidine	NH
24	Phenyl-4-pyrrolidine	NCH_3
25	Phenyl-4-pyrrolidine	NCH_2-benzene



	R
6	4-Diethoxymethyl-benzene
16	Phenyl-2-nitro-4-dimethylamine
26	Phenyl-4-pyrrolidine
7	4-Diethoxymethyl-benzene
17	Phenyl-2-nitro-4-dimethylamine
27	Phenyl-4-pyrrolidine
8	4-Diethoxymethyl-benzene
18	Phenyl-2-nitro-4-dimethylamine
28	Phenyl-4-pyrrolidine
9	4-Diethoxymethyl-benzene
19	Phenyl-2-nitro-4-dimethylamine
29	Phenyl-4-pyrrolidine
10	4-Diethoxymethyl-benzene
20	Phenyl-2-nitro-4-dimethylamine
30	Phenyl-4-pyrrolidine

Scheme 1: Structures and synthesis scheme of α, β-unsaturated carbonyl based compounds. Reagents and conditions: (i) NaOH, EtOH, Room temperature.

Table 1: Inhibition of secretory Phospholipase A_2-V (sPLA$_2$-V), COX-1, COX-2 and lipooxygenase activities by synthetic compounds.

Compound	sPLA2-V	COX-1	COX-2	LOX
	IC_{50} (µM)	IC_{50} (µM)	IC_{50} (µM)	IC_{50} (µM)
1	12.57±1.89	1.77±0.25	17.41±1.83	83.12±1.89
2	8.76±2.35	0.99±0.17	11.21±0.69	91.03±2.56
3	7.14±1.85	0.46±0.05	27.65±2.56	88.42±1.47
4	4.21±1.74	0.37±0.21	38.21±4.74	65.61±4.31
5	17.78±2.56	3.17±0.45	>100	ND
6	19.22±4.10	3.22±0.89	31.45±2.31	210.28±5.42
7	45.32±1.67	2.73±0.72	14.76±1.28	ND
8	37.29±2.75	2.22±1.02	19.32±1.89	ND
9	58.19±4.19	9.06±1.27	>100	ND
10	27.66±1.55	1.12±0.65	13.32±1.86	52.22±1.22
11	11.25±1.20	0.42±0.32	>100	29.28±3.20
12	7.79±2.91	0.72±0.42	18.55±0.98	44.67±1.79
13	4.19±0.52	0.67±0.21	7.88±1.21	39.54±0.55
14	2.19±1.42	1.22±0.54	9.12±1.67	38.26±1.84
15	15.22±1.20	14.56±0.98	19.22±1.92	210.82±3.29
16	18.16±1.20	3.96±1.65	15.00±2.45	103.47±2.98
17	38.42±0.59	ND	>100	ND
18	27.99±0.89	9.91±2.15	14.85±1.11	ND
19	37.55±1.22	21.54±0.89	27.96±1.52	ND
20	22.21±2.70	29.33±1.27	37.66±1.78	156.82±1.67

21	28.32±1.68	33.54±2.31	54.32±3.25	ND
22	15.18±1.62	39.42±1.29	>100	ND
23	12.14±1.10	ND	>100	ND
24	11.18±0.92	69.22±0.88	>100	ND
25	19.22±1.34	51.32±1.27	57.22±1.28	ND
26	29.32±2.20	49.33±1.94	69.82±2.20	ND
27	55.72±2.23	37.75±1.49	46.22±1.99	ND
28	67.32±1.84	ND	54.64±2.34	ND
29	54.18±0.89	33.45±1.73	>100	ND
30	44.12±1.65	ND	61.23±1.65	ND
Curcumin	10.12±0.8	29.65±1.91	>100	59.52±2.27
Dexamethasone	0.61±0.01	-	-	-
Indomethacin*	-	0.21±0.03	3.24±0.01	-
NDGA**	-	-	-	9.23±0.33

*30 µM concentration
**16 µM concentration, NDGA: Nordihydroguaiaretic acid
Values are the mean ± SD; n = 3. ND, not determined

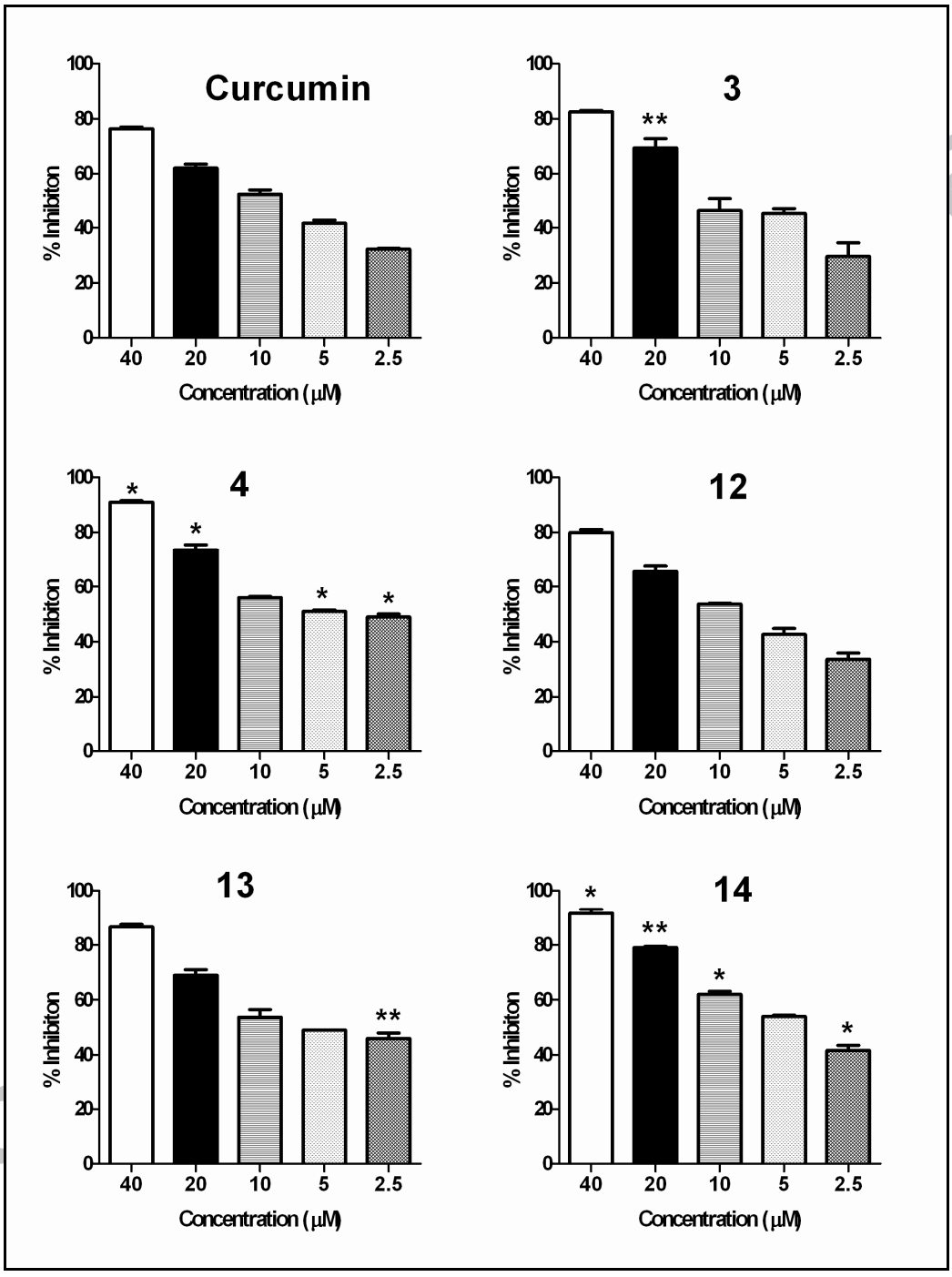


Figure 1: Concentration dependent inhibitory effects of curcumin related compounds on activity of secretory phospholipase A_2-V. Graph represents the % inhibition by most active compounds along with curcumin. *p<0.05, **p<0.01 are significant difference with respect to curcumin. The data shown are an average of three independent experiment and values are mean ± S.D.

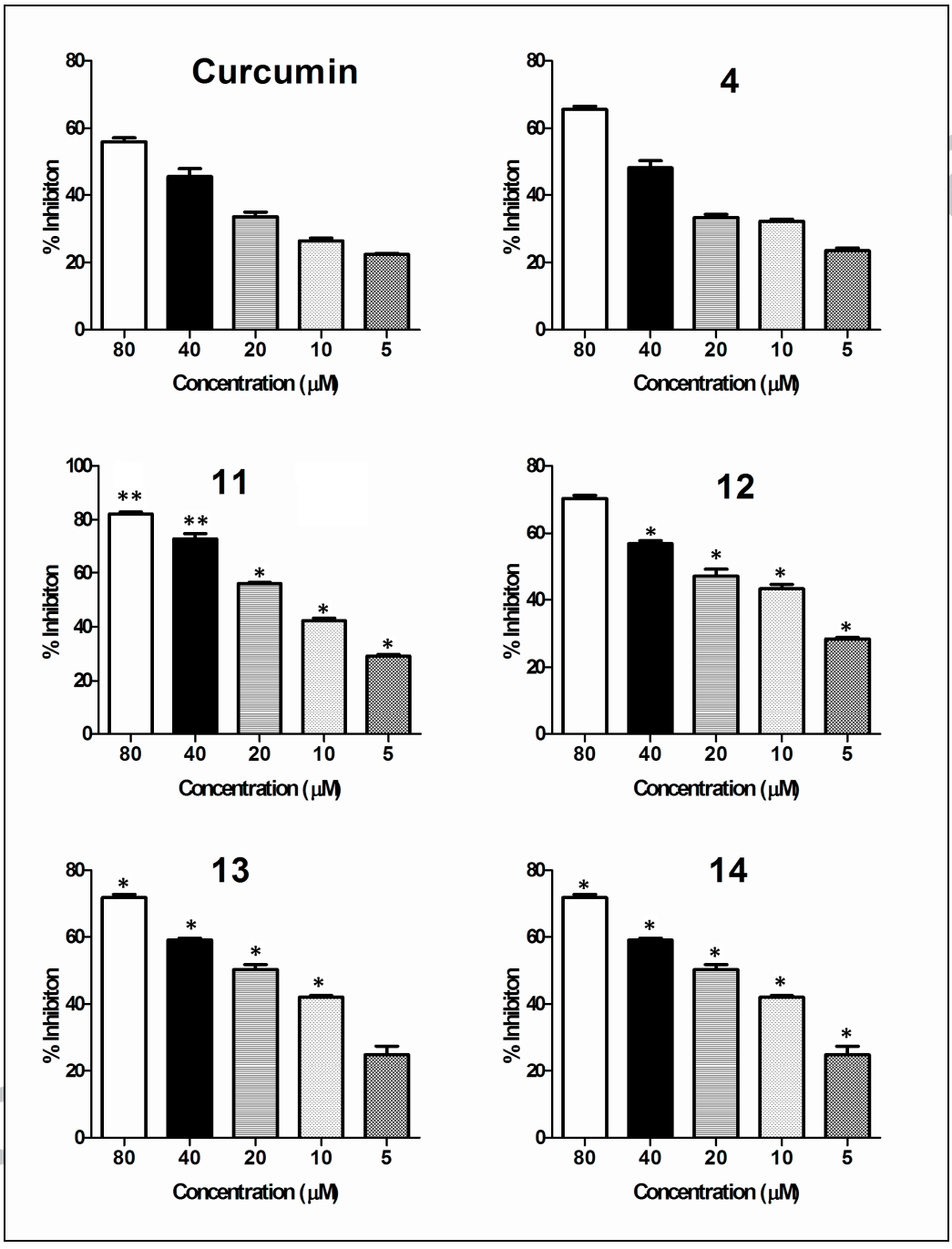
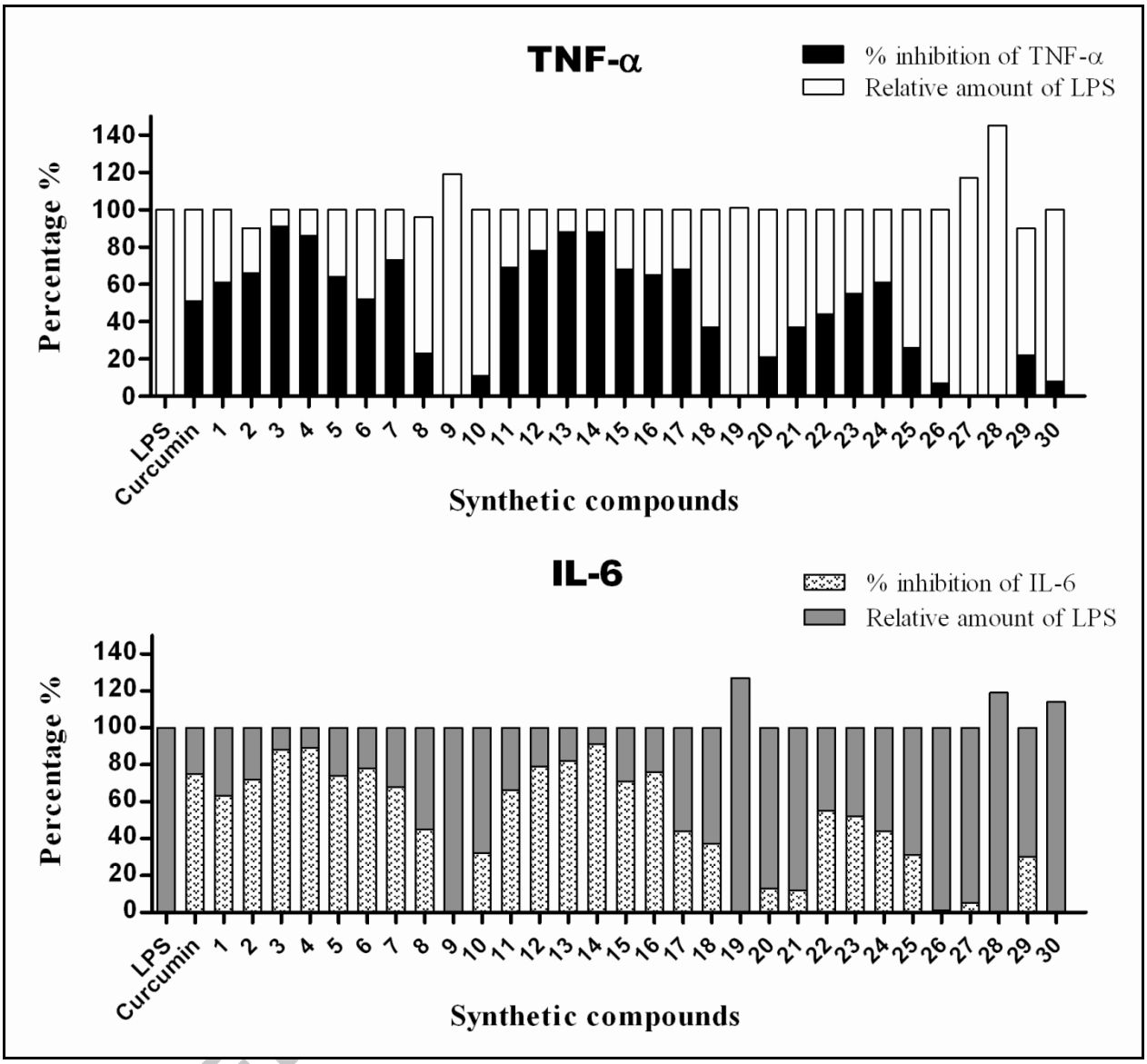


Figure 2: Concentration dependent inhibitory effects of synthetic compounds on activity of lipoxygenase. Graph represents the % inhibition by most active compounds along with curcumin.*p<0.05, **p<0.01 are significant difference with respect to curcumin. The data shown are an average of three independent experiment and values are mean ± S.D.



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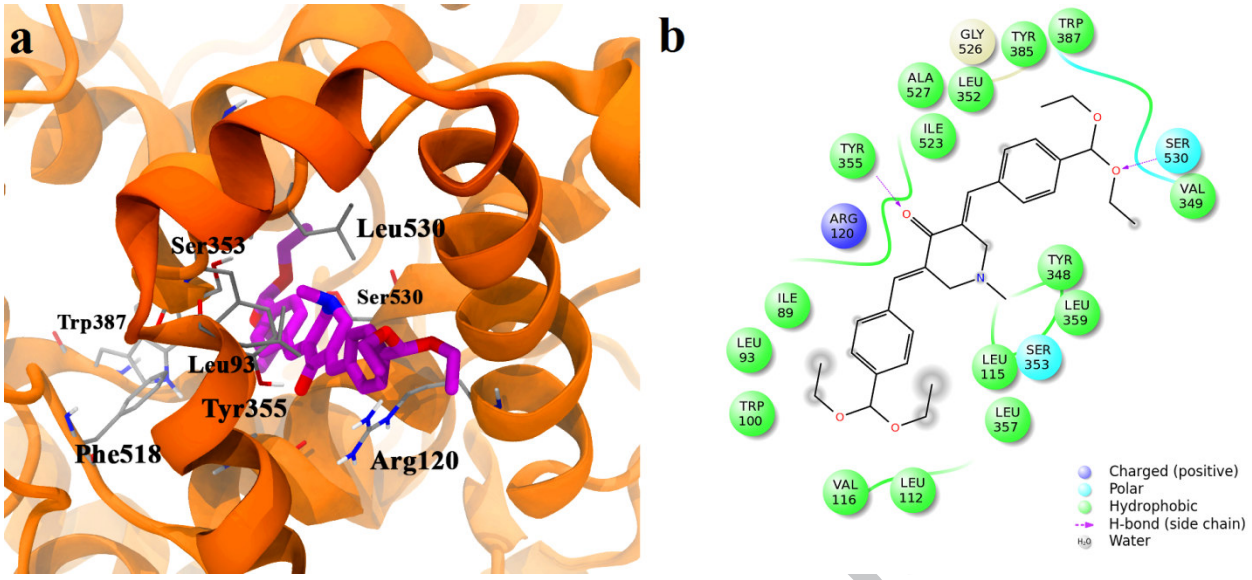

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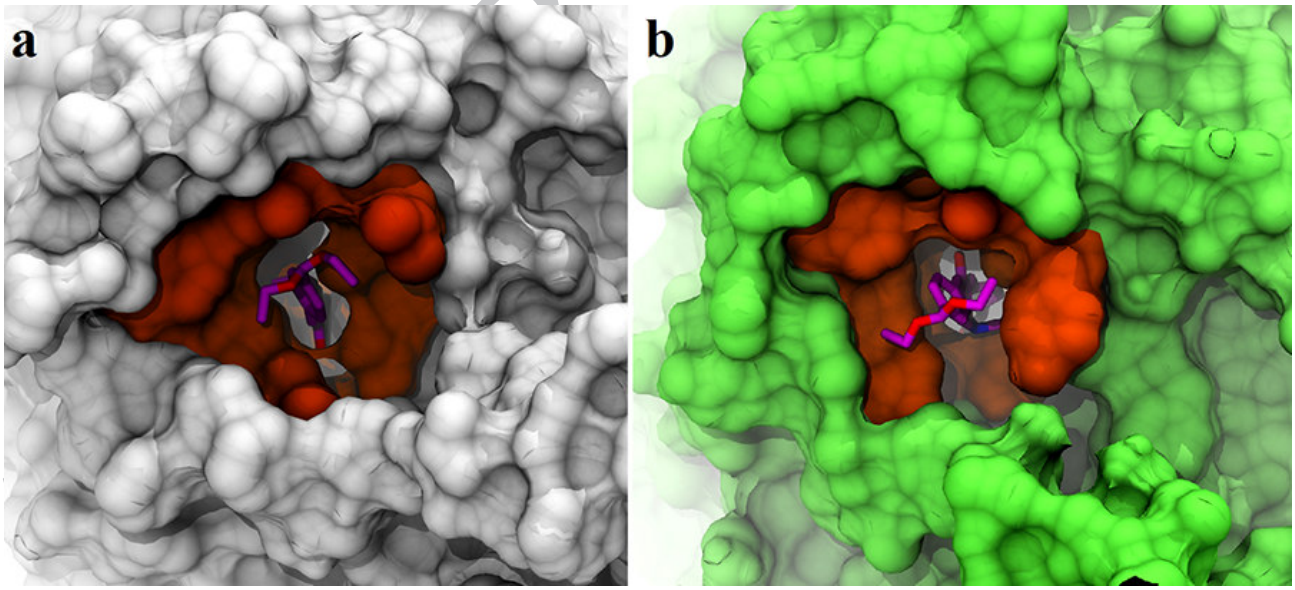

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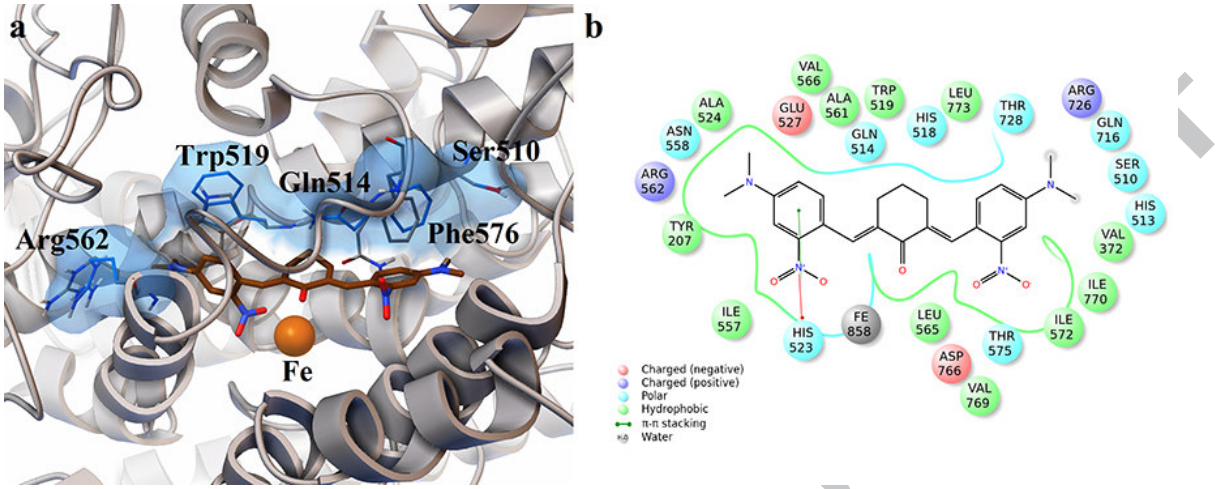

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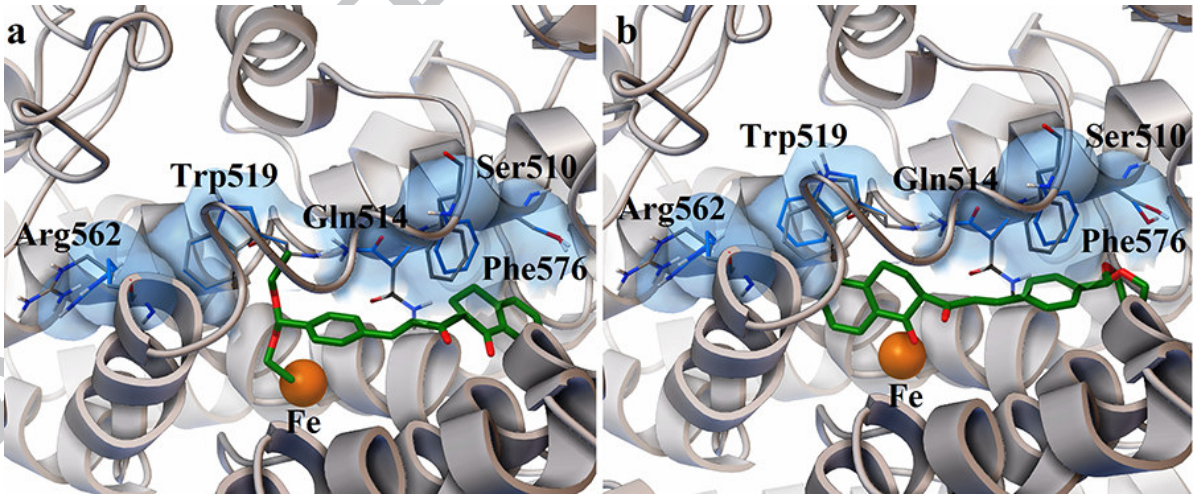

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