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Original article

Synthesis and anti-inflammatory activity of novel (substituted)benzylidene acetone oxime ether derivatives: Molecular modeling study

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ABSTRACT

Herein, we report the design, synthesis, and pharmacological properties of a series of substituted benzylidene acetone oxime ether derivatives from the corresponding oxime derivatives. All the newly synthesized compounds were investigated *in vivo* for their anti-inflammatory activities using carrageenin-induced rat paw oedema model. Among the compounds examined, compounds **5b** and **7a** showed the highest activity, nearly equivalent to that of the standard drug diclofenac sodium. Hence, they were screened for their analgesic activities using acetic acid-induced writhing model in mice and also, their ulcerogenic effects were studied. Compound **7a** was found to possess significant anti-inflammatory and analgesic activities with negligible ulcerogenic effect. Docking study of the synthesized compound **7a** into the active site of COX-1 and COX-2 revealed a similar binding mode to SC-558, a selective COX-2 inhibitor.

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1. Introduction

Inflammation is a normal response to any noxious stimulus that threatens the host and may vary from a localized response to a generalized one [1]. The inflammatory process protects our body from diseases by releasing cells and mediators that combat foreign substances and prevent infection [2]. In 1971 [3], the role of prostaglandins (PGs) in the inflammatory process was observed. PGs are synthesized from arachidonic acid which is released by the action of phospholipase A_2 on damaged tissues. Arachidonic acid is converted by cyclooxygenase (COX) enzymes to cyclic PGG_2 and PGH_2 which cause vasoconstriction and pain. They, in turn, are converted to PGE_2 and $PGF_{2\alpha}$ which cause vasodilatation and pain [4].

Two isoenzymes of cyclooxygenase were postulated, cyclooxygenase-1 (COX-1) and cyclooxygenase-2 (COX-2). COX-1 is a constitutive enzyme and is responsible for the production of the basic level of PGs. Inhibition of this enzyme by all older, non-selective non-steroidal anti-inflammatory drugs is primarily responsible for a number of their side effects [5]. However, the existence of COX-2 enzyme was confirmed [5]. It is an inducible enzyme which is induced in response to the release of several proinflammatory mediators, leading to the inflammatory non-selective NSAID response and pain [6]. To our knowledge, several attempts to derive COX selective inhibitors from flurbiprofen (**A**) [7] and indomethacin (**B**) [8] have been published (Fig. 1). These strategies introduced the desired selectivity by systematic structural modification of the lead NSAIDs. Alternatively, selectivity may be introduced by using the available information on the tricyclic COX-2 selective inhibitors (Fig. 1) structurally related to SC-558 (**C**) [9]. Thus, there was an active search for the development of specific inhibitors of COX-2 enzyme.

In this context, the present work describes the synthesis and the investigation of the analgesic and anti-inflammatory properties of new substituted benzylidene acetone oxime ether derivative (**D**) (Fig. 1). Our strategy for the synthesis of such derivatives is based on the modification of the structure of the known potent non-selective NSAID inhibitor. The strategy is intended to obtain potent anti-inflammatory activity without ulcerogenic effects using traditional medicinal chemistry techniques motivated by the comparative modeling of COX-1 and -2 complexed with **A** and **C** together with the available pharmacophore.

2. Results and discussion

2.1. Chemistry

2.1.1. Synthesis of compounds 1–11 (Scheme 1)

Aldol condensation of benzaldehyde or its derivatives with acetone was performed in the presence of aqueous ethanolic

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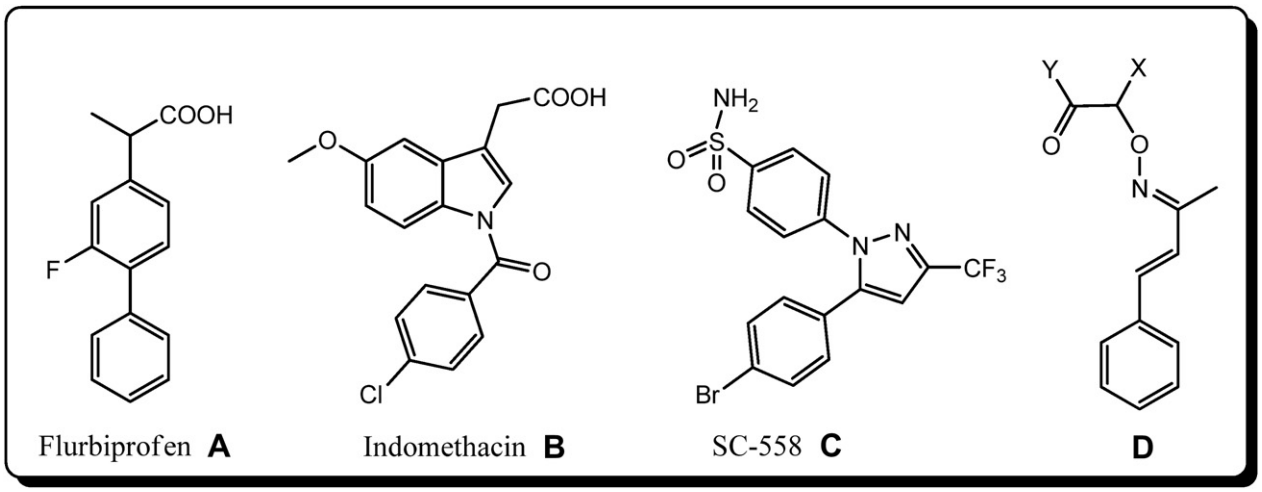
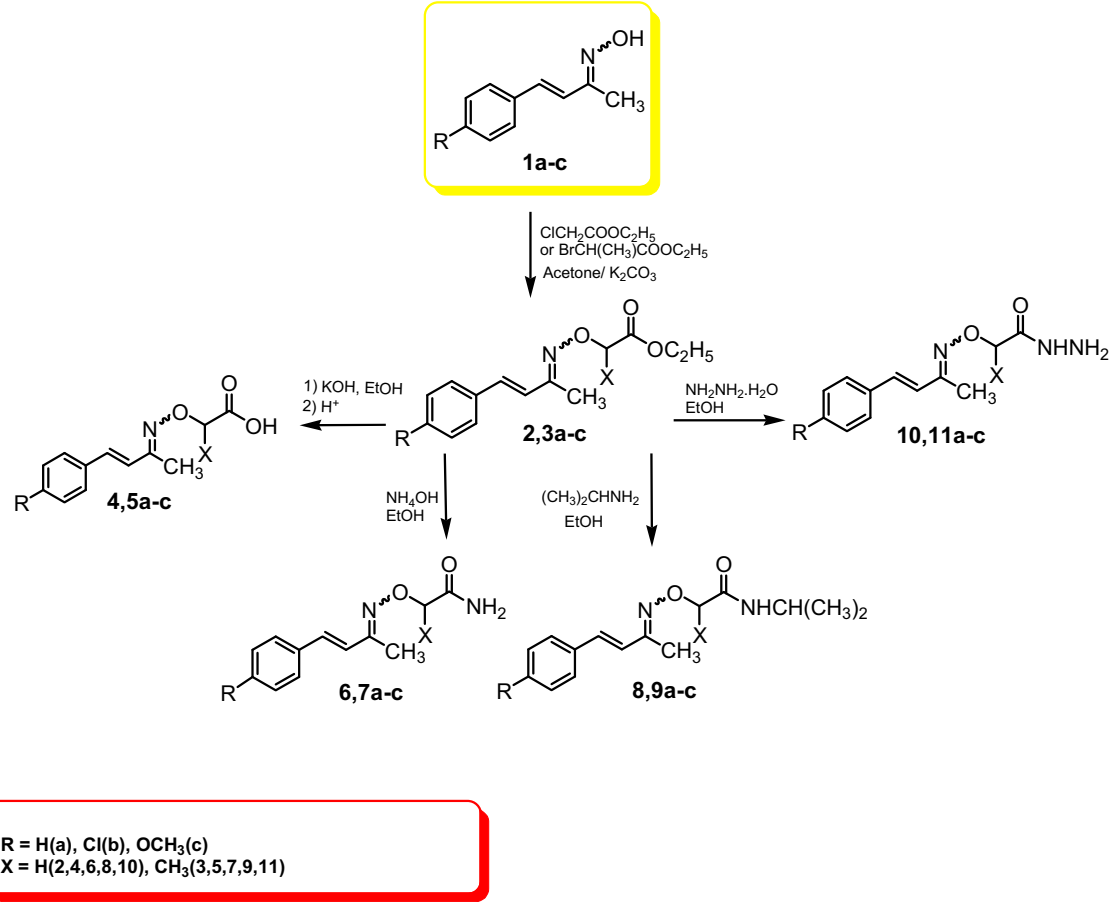


Fig. 1. Representative examples of non-selective (**A**, **B**) and selective (**C**) COX inhibitors and the general structure of the synthesized substituted benzylidene acetone oxime ether derivatives (**D**).

sodium hydroxide to give the corresponding *E*-4-arylbut-3-en-2-ones [10–14]. The latter chalcone derivatives reacted with hydroxylamine hydrochloride in ethanol in the presence of sodium hydroxide at room temperature to afford the corresponding oxime derivatives **1a–c** [15].

Refluxing the appropriate oxime derivatives **1a–c** with ethyl chloroacetate or ethyl 2-bromopropionate in dry acetone in the presence of anhydrous potassium carbonate gave ethyl {[((*E*)-4-arylbut-3-en-2-ylidene)amino]oxy}acetates **2a–c** and ethyl 2-{[((*E*)-4-arylbut-3-en-2-ylidene)amino]oxy}propanoates **3a–c**, respectively.

Conversion of the ester derivatives **2** and **3** into different carboxylic acid derivatives is outlined in Scheme 1 for example; hydrolysis of compounds **2** and **3** using potassium hydroxide



Scheme 1. Synthesis of aminooxy (acetic acid, propanoic acid, acetamide, propanamide, acetohydrazide and propanoic acid hydrazide) derivatives.

followed by neutralization with 10% hydrochloric acid afforded the corresponding carboxylic acids **4**,**5a–c**. Reaction of the ester compounds **2** and **3** with ammonia afforded primary amide derivatives **6**,**7a–c** while reacting with isopropylamine afforded the corresponding secondary aliphatic amide derivatives **8**,**9a–c**. Synthesis of the hydrazide derivatives **10**,**11a–c** was carried out by refluxing compounds **2** and **3**, respectively with hydrazine hydrate in absolute ethanol. The spectral and microanalytical data for compounds **2–11a**,**b**,**c** were consistent with their chemical structures.

*2.1.2. Synthesis of compounds **13–15** (Scheme 2)*

The appropriate aniline derivatives reacted with chloroacetyl chloride in glacial acetic acid in the presence of anhydrous sodium acetate to afford 2-chloro-*N*-arylacetamides **12a–c** [16–18]. Treatment of the oxime derivatives **1a–c** with sodium methoxide in anhydrous methanol afforded oximate sodium salt intermediates which were converted into *N*-aryl-{[((*E*)-4-arylbut-3-en-2-ylidene)amino]oxy}acetamides **13–15a**,**b**,**c** upon reacting with compounds **12a–c** in dry DMF. The structures of the isolated products **13–15a**,**b**,**c** were established on the basis of their elemental and spectral analyses.

2.2. Biological activity

2.2.1. In vivo anti-inflammatory studies

All the newly synthesized compounds and diclofenac sodium, as a reference drug, were subjected to *in vivo* anti-inflammatory studies using carrageenin-induced rat paw oedema model. All the tested compounds showed reasonable percentages of oedema reduction of about 21.66% for compound **3c** and 66.70% for compound **5b** (Table 1). The major exception proved to be compound **4c** being almost inactive, with 7.35% oedema reduction. Generally speaking, carboxylic acids and primary amide derivatives showed the highest anti-inflammatory activities among the tested compounds especially propanoic acid derivatives **5** and propanamides **7**. Compounds **5b** and **7a** showed the highest anti-inflammatory activity with 66.70% and 63.23% oedema reduction, respectively. Esterification of the carboxylic acids **4** and **5** resulted in a notable decrease in the activity. In most cases, the propanoic acid derivatives were more active than the acetic acid derivatives.

Compounds **5b** and **7a** were studied in detail for their anti-inflammatory activities in comparison with diclofenac sodium using three graded doses (Table 2) and the ED_{50} was calculated, 29.92 mg/kg for compound **5b** and 34.30 mg/kg for compound **7a**. On the other hand the ED_{50} for diclofenac sodium was 30.65 mg/kg.

2.2.2. Analgesic activity

The analgesic activity of compounds **5b** and **7a** was studied by using acetic acid-induced writhing test in mice. Compound **5b** showed lower analgesic activity than diclofenac sodium while compound **7a** showed analgesic potency about half the analgesic effect of diclofenac sodium (Table 3).

2.2.3. Potential ulcerogenicity

The potential ulcerogenic effects of compounds **5b** and **7a** were studied in comparison with diclofenac sodium using the method of Adami et al. Compound **5b** showed ulcerogenic effect as diclofenac sodium and this may be attributed to the presence of free carboxylic group. On the other hand the amide derivative **7a** showed negligible ulcerogenic effect (Table 4).

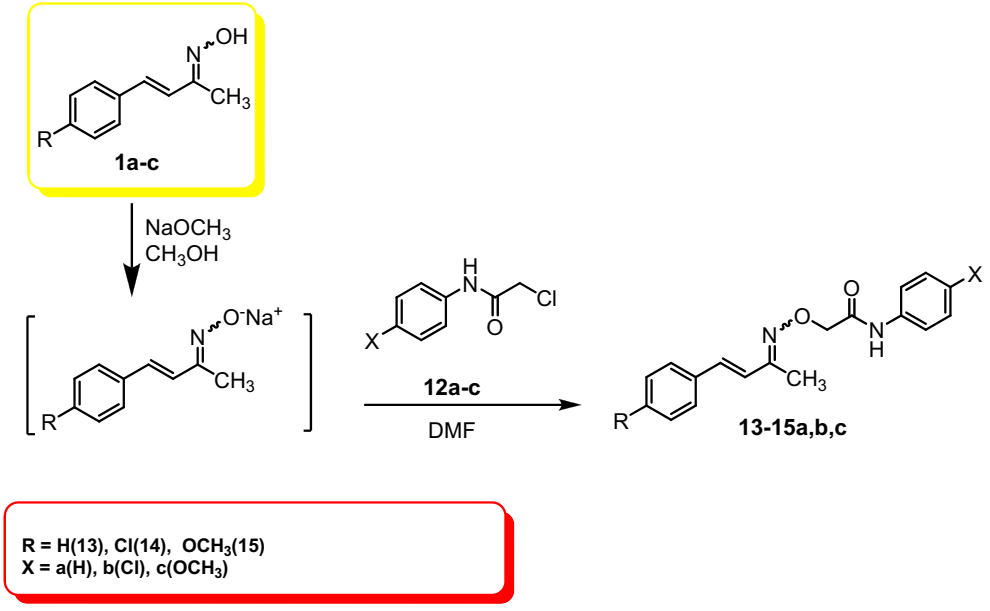
2.2.4. Determination of acute toxicity (LD_{50})

The LD_{50} for diclofenac sodium and compounds **5b** and **7a** was calculated following the method of Litchfield and Wilcoxon. The LD_{50} was 744.6 mg/kg with 95% confidence limits 536.7–1033.1 mg/kg and 146.178 with 95% confidence limits 106.95–199.79 mg/kg for the tested compounds and diclofenac sodium, respectively. Therapeutic index was calculated by dividing LD_{50} over ED_{50}, it was 24.89 for compound **5b**, 20.10 for compound **7a** and 4.76 for diclofenac sodium. Hence, compounds **5b** and **7a** have higher therapeutic index than diclofenac sodium and appear to be relatively less toxic anti-inflammatory agents.

2.3. Molecular calculation and results

2.3.1. Conformational analysis

In an attempt to gain a better insight on the molecular structures of the active compound **5b** as a representative example, conformational analysis of the target compound has been



Scheme 2. Synthesis of *N*-aryl-{[((*E*)-4-arylbut-3-en-2-ylidene)amino]oxy}acetamide derivatives.

Table 1
Results of anti-inflammatory activity of the tested compounds against carrageenin-induced rat paw oedema in rats.

Compounds	Mean % increase in paw weight ± SEM	% Inhibition of paw oedema from control group
Control	29.13 ± 1.03$^{\delta}$	–
Diclofenac	8.12 ± 0.49*	72.13
2a	20.46 ± 1.05*$^{\delta}$	29.76
2b	21.49 ± 1.07*$^{\delta}$	26.23
2c	22.68 ± 1.03*$^{\delta}$	22.14
3a	19.46 ± 1.13*$^{\delta}$	33.20
3b	16.16 ± 0.83*$^{\delta}$	44.52
3c	22.82 ± 0.56*$^{\delta}$	21.66
4a	15.84 ± 0.79*$^{\delta}$	45.62
4b	14.40 ± 0.63*$^{\delta}$	50.57
4c	26.99 ± 1.31$^{\delta}$	7.35
5a	17.23 ± 0.96*$^{\delta}$	40.85
5b	9.70 ± 0.94*	66.70
5c	15.91 ± 1.15*$^{\delta}$	45.38
6a	14.65 ± 0.83*$^{\delta}$	49.71
6b	14.34 ± 1.05*$^{\delta}$	50.77
6c	18.00 ± 1.10*$^{\delta}$	38.21
7a	10.71 ± 0.87*	63.23
7b	12.37 ± 0.78*$^{\delta}$	57.54
7c	12.08 ± 0.74*$^{\delta}$	58.53
8a	20.12 ± 1.04*$^{\delta}$	30.93
8b	20.83 ± 0.73*$^{\delta}$	28.49
8c	16.99 ± 0.98*$^{\delta}$	41.68
9a	18.24 ± 0.65*$^{\delta}$	37.38
9b	16.65 ± 0.73*$^{\delta}$	42.84
9c	21.11 ± 0.83*$^{\delta}$	27.53
10a	20.68 ± 0.95*$^{\delta}$	29.01
10b	22.79 ± 1.14*$^{\delta}$	21.76
10c	22.01 ± 1.07*$^{\delta}$	24.44
11a	12.74 ± 1.12*$^{\delta}$	56.27
11b	16.46 ± 0.95*$^{\delta}$	43.49
11c	17.84 ± 1.37*$^{\delta}$	38.76
13a	18.81 ± 0.88*$^{\delta}$	35.43
13b	20.78 ± 0.84*$^{\delta}$	28.66
13c	14.67 ± 1.37*$^{\delta}$	49.64
14a	20.55 ± 0.94*$^{\delta}$	29.45
14b	17.22 ± 1.29*$^{\delta}$	40.89
14c	15.10 ± 0.98*$^{\delta}$	48.16
15a	16.63 ± 0.42*$^{\delta}$	42.91
15b	16.51 ± 1.02*$^{\delta}$	43.32
15c	18.28 ± 1.22*$^{\delta}$	37.25

* Significant difference from control group using unpaired Student's "*t*" test $p < 0.05$.
$^{\delta}$ Significant difference from diclofenac-treated group using unpaired Student's "*t*" test $p < 0.05$.

performed by use of the MM+ force field [19] (calculations in vacuo, bond dipole option for electrostatics, Polak–Ribiere algorithm, RMS gradient of 0.01 kcal/Å mol) as implemented in HyperChem 5.1 [20]. The most stable conformer was fully optimized with by AM1 semi-empirical molecular orbital calculation [21]. The global minimum was confirmed as true minimum and not saddle point by the absence of negative eigen value of the Hessian through frequency calculation. At this stage, calculations at the AM1 level were considered in order to determine relative energies of the *syn*- and *anti*-isomers of both *trans*- and *cis*-isomers of arylideneoxime derivatives. The results show that the occurrence of four isomeric conformations for **5b**, each pair **5b**-*trans*-*Anti*, **5b**-*cis*-*Anti* and **5b**-*trans*-*Syn*, **5b**-*cis*-*Syn* (Fig. 2) have approximately similar heat of formation and *anti*-conformers was more stable than *syn*-conformers by 2 kcal/mol.

Table 2
Results of anti-inflammatory activity of compounds **5b** and **7a** against carrageenin-induced rat paw oedema in rats at three graded doses.

Compounds	Dose mg/kg	% Inhibition of paw oedema from control group	ED_{50} mg/kg
Control	–		
Diclofenac	30	46.00	30.65
	50	72.13	
	75	80.20	
5b	30	45.12	29.92
	45	66.70	
	75	75.25	
7a	30	37.73	34.30
	37	63.23	
	75	69.10	

Table 3
Results of analgesic activity of compounds **5b** and **7a** against acetic acid-induced writhing in mice.

Compounds	Dose (mg/kg)	No. of writhes in 20 min after acetic acid treatment (mean ± S.E.M.)	% Inhibition of number of writhes from control group
Control		45.67 ± 0.37	–
Diclofenac	50	13.20 ± 0.57	71.10
5b	45	36.40 ± 0.66	20.30
	100	27.80 ± 0.47	39.13
7a	37	21.20 ± 0.98	53.58
	100	8.00 ± 0.40	82.48

2.3.2. ADME profiling

The bioavailability of the most active compounds **4b**, **5b**, **6b, 7a–c** and **11b** and the reference drug diclofenac was assessed using ADME (absorption, distribution, metabolism, elimination) prediction methods. In particular, we calculated the compliance of compounds to the Lipinski's rule of five [22]. This approach has been widely used as a filter for substances that would likely be further developed in drug design programs. Briefly, this simple rule is based on the observation that most orally administered drugs have a molecular weight $\leq$500, a log $P \leq 5$, hydrogen bond donor sites $\leq$5 and hydrogen bond acceptor sites (N and O atoms) $\leq$10. In addition, we calculated the total polar surface area (TPSA) since it is another key property that has been linked to drug bioavailability. Thus, passively absorbed molecules with a TPSA > 140 are thought to have low oral bioavailability [23]. Molecules violating more than one of these rules may have problems with bioavailability. Predictions of ADME properties for studied compounds are given in Table 5. The results show that all synthesized compounds comply with these rules and even diclofenac shows no violation. Theoretically, these compounds should present good passive oral absorption and differences in their bioactivity cannot be attributed to this property.

2.3.3. Docking studies

The level of ulcerogenic potential and anti-inflammatory activities of compounds **5b** and **7a** prompted us to perform molecular docking studies to understand the ligand–protein interactions in detail. All the calculations were performed using

Table 4
Ulcerogenic potential of compounds **5b**, **7a** and diclofenac sodium in mice.

Compounds	Average number of ulcers	Mean sum of lengths of elongated ulcer mm ± SEM
Control	0.0	0.0
Diclofenac	6	7.9 ± 0.81
5b	7	8.1 ± 0.87
7a	2	0.3 ± 0.05*

*Significantly less than the diclofenac sodium treated group using unpaired Student's "*t*" test $p < 0.05$.

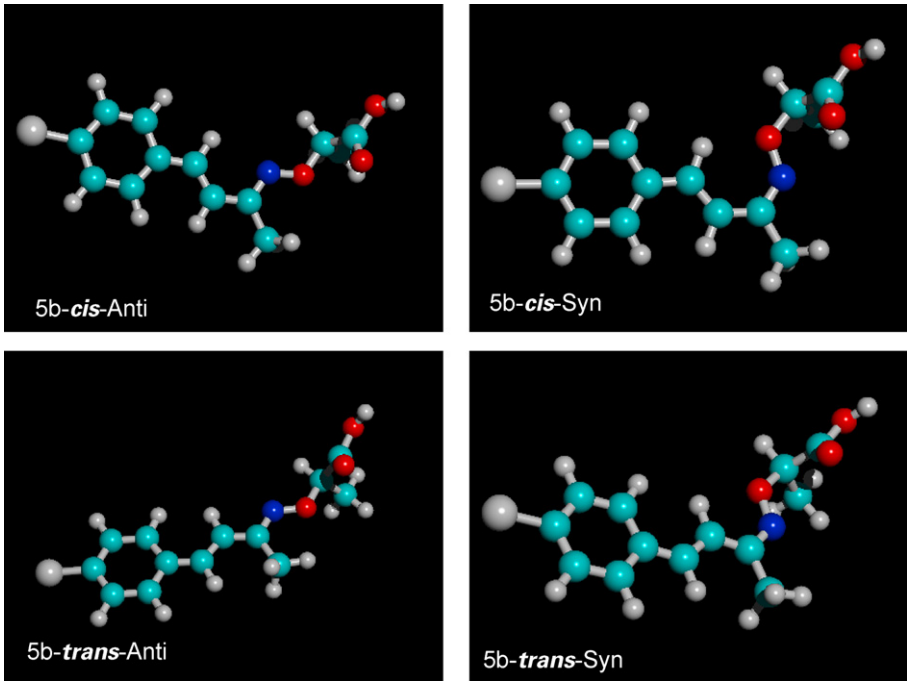


Fig. 2. Most stable conformers of the active compound **5b** as a representative example with ball and cylinder rendering.

MOE 2008.10 software [24] installed on 2.0G Core 2 Duo. The crystal structures of COX-1 and COX-2 enzymes complexed with flurbiprofen [25] and SC-558 [26] [1EQH, 1CX2] were used for the docking. The active site of the enzyme was defined to include residues within a 10.0 Å radius to any of the inhibitor atoms. The automated docking program of MOE was used to dock compounds **5b** and **7a** on the active sites of both COX-1 and COX-2 enzymes. For each compound the most stable docking model was selected according to the best scored conformation predicted by the MOE scoring function. The complexes were energy-minimized with an MMFF94 force field [27] till the gradient convergence 0.05 kcal/mol was reached. The two compounds could dock into the active site of COX-1 successfully. The binding energies of −40.17 and −7.99 kcal/mol were obtained for **5b** and **7a**, respectively (Table 6, Fig. 3). Compound **5b** complexes with COX-1 showed the occurrence of two strong hydrogen bonds with Arg 120 and Tyr 355 with distances 2.63 Å and 2.40 Å, respectively. The lower interaction energy observed for **7a** rationalizes the insufficient binding of amide fragment into the COX-1 active site than that of the carboxylate fragment of **5b** (Fig. 3). The insufficient binding can be explained in terms of the occurrence of only one weak hydrogen bond between the amide group and Arg 120 (3.28 Å) and the absence of hydrogen bonding with Tyr 355. The hydrophobic phenyl ring and conjugated pi-system of the two double bonds of **5b** was surrounded by active site amino acid residues Tyr 385, Leu 352, Trp 387, Gly 526, Ala 527, Val 349, Ile 523, Met 522 and Ser 530. A similar hydrophobic interaction trend was observed for **7a** complexes with COX-1.

On the other hand, compounds **5b** and **7a** were modeled in the active site of COX-2 enzyme (Table 6, Fig. 4). The binding energies of −10.57 and −55.09 kcal/mol were obtained for **5b** and **7a**, respectively. The carboxylate fragment of **5b** formed a weak hydrogen bonding interaction with Arg 120 (3.05 Å) which may be attributed to the deep entrance of hydrophobic aromatic part into the secondary pocket of COX-2 with hydrophobic interaction with Ala 516, Val 523, Val 349 and Gly 354 and lacking the hydrogen

Table 5
Calculated Lipinski's rule of five for the most active compounds.

Compd. no.	% Inhibition of paw oedema[a]	Parameter					Nviolations[g]
		Log *p*[b]	TPSA[c]	MW[d]	nON[e]	nOHNH[f]	
Diclofenac	72.13	4.56	49.32	292.15	3	2	0
5b	66.70	3.22	58.89	267.71	4	1	0
7a	63.23	2.03	64.69	232.28	4	2	0
7c	58.53	2.08	73.92	262.30	5	2	0
7b	57.54	2.70	64.69	266.72	4	2	0
11a	56.27	0.955	76.71	247.29	5	3	0
6b	50.77	2.34	64.69	252.70	4	2	0
4b	50.57	2.85	58.89	253.68	4	1	0

[a] Data taken from Table 1.
[b] Calculated lipophilicity.
[c] Total polar surface area.
[d] Molecular weight.
[e] Number of hydrogen bond acceptors.
[f] Number of hydrogen bond donors.
[g] Number of violations from Lipinski's rule of five.

Table 6
Docking results of compounds **5b** and **7a** into the active sites of COX-1 and COX-2.

Compd. no.	Against COX-1[a]		Against COX-2 [a]	
	H-bond (distance Å, strength)	$E_{int.}$ (kcal mol^{-1}) [b]	H-bond (distance Å, strength)	$E_{int.}$ (kcal mol^{-1}) [b]
5b	Arg 120 (2.63, strong) Tyr 355 (2.40, strong)	−40.17	Arg 120 (3.05, weak)	−10.57
7a	Arg 120 (3.28, weak)	−7.99	His 90 (2.70, strong) Gln 192 (2.47, strong) Phe 518 (3.42, weak)	−55.09

[a] Details of hydrogen bonding are explained in Figs. 3–5.
[b] Interaction energy between the ligand and receptor.

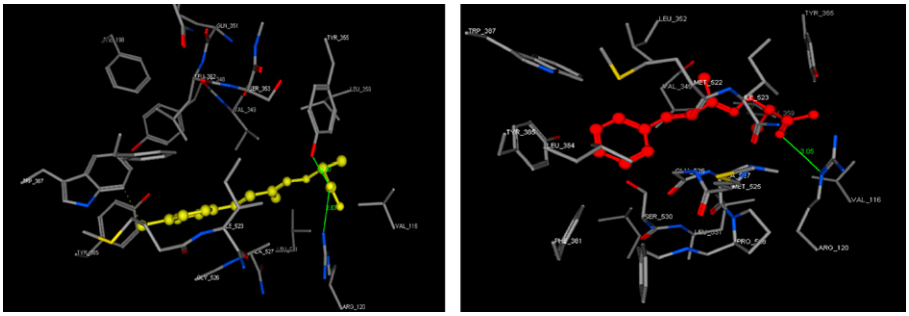


Fig. 3. Docking of compounds **5b** (left panel) and **7a** (right panel) into the active site of COX-1. Hydrogen bonds are shown in green.

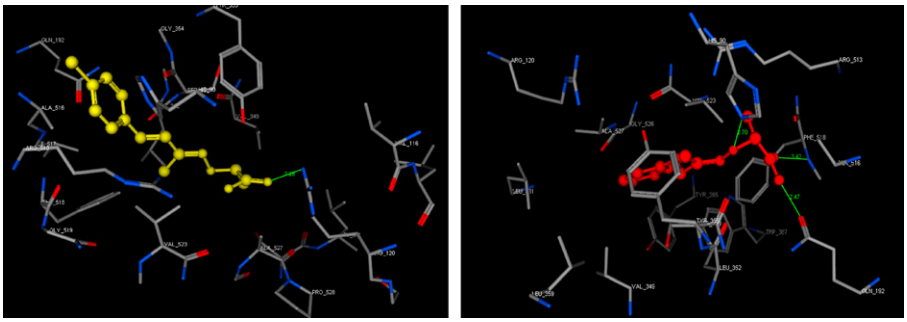


Fig. 4. Docking of compounds **5b** (left panel) and **7a** (right panel) into the active site of COX-2. Hydrogen bonds are shown in green.

bond pattern which was observed with COX-1. On the other hand compounds **7a** produce an inverted mode of binding with COX-2 with a deep movement into the hydrophilic pocket. Unlike **5b**, the amide group of **7a** is able to reach the hydrophilic pocket and is involved in strong hydrogen bonding with His 90 (2.7 Å) and Gln 192 (2.47 Å) and weak hydrogen bonding with Phe 518 (3.42 Å). Such interactions are almost essential for COX-2 inhibitory activity, as exemplified by the binding interaction of SC-558, an analogue of celecoxib co-crystallized in the COX-2 active site [26]. In addition, such interaction forces the phenyl ring to adopt a specific orientation at the top of the channel. This moiety is involved in hydrophobic interaction with Trp 387, Tyr 385, Val 523, Leu 352, Ser 353, Val 349, Phe 518, and Ala 527. The lateral pocket of COX-2 would therefore be responsible for the COX-2 selectivity of **7a** and contributed to stabilize the ligand–enzyme complexes (Figs. 4 and 5).

The complex generated by docking studies of **7a** with COX-2 and superimposition with the structure of the selective inhibitor, SC-558, co-crystallized with COX-2 [26], illustrated in Fig. 5, shows that compound **7a** can bind in the active site of this enzyme in approximately similar fashion as the pyrazolic prototype (SC-558). Comparison of the interactions performed by SC-558 in the crystal and the docked structure of **7a** with COX-2 (Fig. 4) shows that the amide group of **7a** hydrogen bonds to the amino acid residue Gln 192 and Phe 518, similar to the sulfonyl moiety of the pharmacophoric sulfonamide group pertaining to SC-558. Moreover, an additional hydrogen bond was observed between the oxime oxygen and His 90, while NH_2 moiety of sulfonamide fragment hydrogen bonded with Arg 513. Additionally, the two double bond systems are positioned in the same region as *p*-sulfonamido-phenyl ring of SC-558, while, the aromatic ring of **7a** is close to the *p*-Br-phenyl ring of SC-558 (**C**), in the aromatic region of the active site lined by aromatic amino acid residues such as Phe 518, Tyr 385 and Trp 387, among others. This result suggests extra interactions of **7a** in this hydrophobic pocket and indicates an inverted pharmacophore compared to what has been described for **5b** COX-1 inhibitors. In short, the described interactions are typical of selective inhibitors of COX-2, confirming the molecular design of the reported class of analgesic and anti-inflammatory amide derivatives [28].

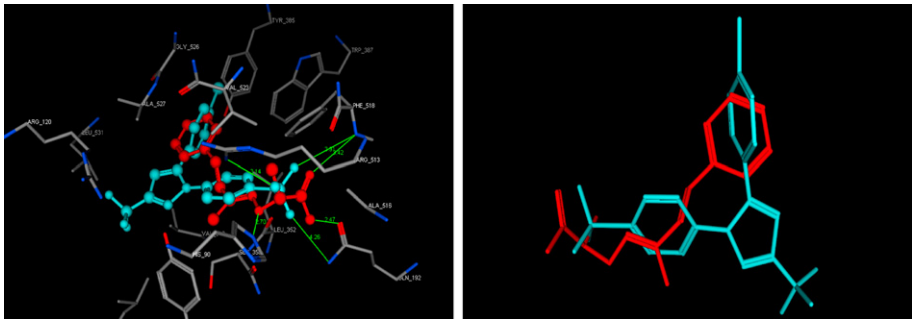


Fig. 5. Alignment of **7a** (red) and selective inhibitor SC-558 (cyan) in the active site of COX-2. Hydrogen bonds are shown in green.

3. Conclusion

Different (substituted) benzylidene acetone oxime ether derivatives were synthesized and screened for anti-inflammatory activity. Compounds **5b** and **7a** with the highest anti-inflammatory activities were subjected to analgesic and ulcerogenic studies. Compound **7a** showed negligible ulcerogenic effect with higher safety and better therapeutic index than diclofenac sodium, so it appears promising. Molecular docking studies further help in understanding the various interactions between the ligands and enzyme active sites in detail and thereby help to design novel potent inhibitors. This result was corroborated by molecular docking studies with ulcerogenic potential, which showed that this compound presents the pharmacophoric requisites for COX-2 inhibition. Molecular docking studies further supported the strong ulcerogenic inhibitory activity of **7a** compared to **5b** and further help in understanding the various interactions between the ligands and enzyme active sites in detail and thereby help to design novel potent inhibitors.

4. Experimental

4.1. General

Melting points were measured with a *Fisher–Johns* melting point apparatus and are uncorrected. IR spectra were recorded on Mattson 5000 FT-IR spectrometer. ^{1}H NMR spectra were determined in $CDCl_3$ or DMSO-d_6 on FT-NMR spectrometer (200 MHz) Gemini Varian using TMS as internal standard. Mass spectra were measured on JEOL JMS-600H spectrometer. Elemental analysis was carried out at the Microanalytical Center of Cairo University. (*E*)-4-Phenylbut-3-en-2-one [13], (*E*)-4-(4-chlorophenyl)but-3-en-2-one [10,14], (*E*)-4-(4-methoxyphenyl) but-3-en-2-one [10], [((*E*)-4-phenylbut-3-en-2-ylidene)amino]hydroxide **1a** [15], [((*E*)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]hydroxide **1b** [15], [((*E*)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino] hydroxide **1c** [15], {[((*E*)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetic acid **4a** [29], 2-chloro-*N*-phenylacetamide **12a** [16,30], 2-chloro-*N*-(4-chlorophenyl)acetamide **12b** [16,18] and 2-chloro-*N*-(4-methoxyphenyl) acetamide **12c** [16,18]were prepared following the procedures reported in the literature.

4.2. General method for synthesis of ethyl {[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetates (**2a–c**) and ethyl 2-{[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}propanoates (**3a–c**) (Scheme 1)

A mixture of the appropriate oxime derivative **1a–c** (0.05 mol), ethyl chloroacetate (7.35 g, 0.06 mol) or ethyl 2-bromopropionate (10.86 g, 0.06 mol) and anhydrous potassium carbonate (13.8 g, 0.10 mol) in dry acetone (25 mL) was heated under reflux for 24 h. The reaction mixture was then filtered and the filtrate was evaporated under reduced pressure. The residues of compounds **2a**,**b** and **3c** were crystallized from ethanol while those of compounds **2c** and **3a**,**b** were dissolved in methanol and then purified by preparative TLC using benzene:ethyl acetate (5:1) as an eluting system.

4.2.1. Ethyl {[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetate (**2a**)

Yield, 73%; mp 55–56 °C; IR (KBr) ν_{max}/cm^{-1} 1739 (C=O), 1603 (C=N), 1485 (C=C). ^{1}H NMR (CD_3OD); δ 1.33 (t, 3H, $COOCH_2CH_3$), 2.19 (s, 3H, $CNCH_3$), 4.26 (q, 2H, $COOCH_2CH_3$), 4.72 (s, 2H, OCH_2COO), 6.83 (d, 1H, Ph-CH=CH), 7.08 (d, 1H, Ph-CH=CH), 7.32–7.55 (m, 5H, Ar-H). ^{13}C NMR (CD_3OD); δ 9.15 (CN–CH_3), 13.15 ($COOCH_2CH_3$), 60.72 ($COOCH_2CH_3$), 70.31 (OCH_2COO), 124.67 (Ar-CH=CH), 126.63 (4′Ar-C), 129.48 (3′,5′ Ar-C), 129.82 (2′,6′ Ar-C), 132.81 (1′ Ar-C), 136.72 (Ar-CH=CH), 157.53 (C=N), 170.31 ($COOC_2H_5$). MS *m*/*z* (%) 249.00 (1.6, $M^+ + 2$), 248.00 (9.90, $M^+ + 1$), 247.00 (26.00, M^+), 246.00 (32.90), 232.00 (0.30), 218.00 (2.10), 202.00 (0.30), 174.00 (0.30), 160.00 (6.90), 144.00 (100.00), 129.00 (5.70), 103.00 (46.90), 90.00 (0.30), 77.00 (28.60). Anal. calcd. for $C_{14}H_{17}NO_3$ (%): C, 68.00; H, 6.93; N, 5.66. Found: C, 67.92; H, 6.81; N, 5.94.

4.2.2. Ethyl {[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetate (**2b**)

Yield, 70%; mp 59–62 °C; IR (KBr) ν_{max}/cm^{-1} 1741 (C=O), 1600 (C=N), 1488 (C=C). ^{1}H NMR (CD_3OD); δ 1.33 (t, 3H, $COOCH_2CH_3$), 2.18 (s, 3H, $CNCH_3$), 4.27 (q, 2H, $COOCH_2CH_3$), 4.63 (s, 2H, OCH_2COO), 6.83 (d, 1H, Ar-CH=CH), 7.07 (d, 1H, Ar-CH=CH), 7.40 (d, 2H, 2′,6′ Ar-H), 7.55 (d, 2H, 3′,5′ Ar-H). ^{13}C NMR ($CDCl_3$); δ 11.21 ($CNCH_3$), 14.95 ($COOCH_2CH_3$), 61.21 ($COOCH_2CH_3$), 70.98 (OCH_2COO), 123.36 (Ar-CH=CH), 126.76 (4′ Ar-C), 129.48 (3′,5′ Ar-C), 129.82 (2′,6′ Ar-C), 132.81 (1′ Ar-C), 136.72 (Ar-CH=CH), 157.53 (C=N), 170.31 ($COOC_2H_5$). Anal. calcd. for $C_{14}H_{16}ClNO_3$ (%): C, 59.68; H, 5.72; N, 4.97. Found: C, 59.38; H, 6.10; N, 5.33.

4.2.3. Ethyl {[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetate (**2c**)

Yield, 65%; oil; IR (KBr) ν_{max}/cm^{-1} 1742 (C=O), 1599 (C=N), 1491 (C=C). ^{1}H NMR ($CDCl_3$); δ 1.17 (t, 3H, $COOCH_2CH_3$), 2.01 (s, 3H, $CNCH_3$), 3.69 (s, 3H, OCH_3), 4.10 (q, 2H, $COOCH_2CH_3$), 4.53 (s, 2H, OCH_2COO), 6.68 (d, 1H, Ar-CH=CH), 6.75 (d, 2H, 3′,5′ Ar-H), 7.06 (d, 1H, Ar-CH=CH), 7.27 (d, 2H, 2′,6′ Ar-H). Anal. calcd. for $C_{15}H_{19}NO_4$ (%): C, 64.97; H, 6.91; N, 5.05. Found: C, 65.29; H, 6.55; N, 5.16.

4.2.4. Ethyl 2-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}propanoate (**3a**)

Yield, 40%; oil; IR (KBr) ν_{max}/cm^{-1} 1739 (C=O), 1603 (C=N), 1488 (C=C). ^{1}H NMR ($CDCl_3$); δ 1.25 (t, 3H, $COOCH_2CH_3$), 1.48 (d, 3H, $CHCH_3$), 2.10 (s, 3H, $CNCH_3$), 4.20 (q, 2H, $COOCH_2CH_3$), 4.71 (q, 1H, $CHCH_3$), 6.79 (d, 1H, Ph-CH=CH), 6.98 (d, 1H, Ph-CH=CH), 7.26–7.49 (m, 5H, Ar-H). Anal. calcd. For $C_{15}H_{19}NO_3$ (%): C, 68.94; H, 7.33; N, 5.36. Found: C, 68.87; H, 7.65; N, 5.16.

4.2.5. Ethyl 2-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}propanoate (**3b**)

Yield, 40%; oil; IR (Nujol Mull) ν_{max}/cm^{-1} 1741 (C=O), 1600 (C=N), 1490 (C=C). ^{1}H NMR ($CDCl_3$); δ 1.25 (t, 3H, $COOCH_2CH_3$), 1.50 (d, 3H, $CHCH_3$), 2.10 (s, 3H, $CNCH_3$), 4.22 (q, 2H, $COOCH_2CH_3$), 4.74 (q, 1H, $CHCH_3$), 6.80 (d, 1H, Ar-CH=CH), 7.04 (d, 1H, Ar-CH=CH), 7.28 (d, 2H, 3′,5′ Ar-H), 7.50 (d, 2H, 2′,6′ Ar-H). Anal. calcd. for $C_{15}H_{18}ClNO_3$ (%): C, 60.91; H, 6.13; N, 4.74. Found: C, 60.67; H, 6.40; N, 4.65.

4.2.6. Ethyl 2-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}propanoate (**3c**)

Yield, 45%; mp 44–46 °C; IR (KBr) ν_{max}/cm^{-1} 1743 (C=O), 1601 (C=N), 1493 (C=C). ^{1}H NMR ($CDCl_3$); δ 1.25 (t, 3H, $COOCH_2CH_3$), 1.45 (d, 3H, $CHCH_3$), 2.09 (s, 3H, $CNCH_3$), 3.79 (s, 3H, OCH_3), 4.18 (q, 2H, $COOCH_2CH_3$), 4.68 (q, 1H, $CHCH_3$), 6.60 (d, 1H, Ar-CH=CH), 6.87 (d, 2H, 3′,5′ Ar-H), 6.93 (d, 1H, Ar-CH=CH), 7.40 (d, 2H, 2′,6′ Ar-H). ^{13}C NMR ($CDCl_3$); δ 11.35 (CN-CH_3), 15.29 ($COOCH_2CH_3$), 17.97 ($CHCH_3$), 56.59 (OCH_3), 62.93 ($COOCH_2CH_3$), 79.47 ($CHCH_3$), 116.10 (3′,5′ Ar-C), 124.55 (Ar-CH=CH), 130.15 (1′ Ar-C), 131.15 (2′,6′ Ar-C), 135.88 (Ar-CH=CH), 159.48 (C=N), 162.33 (4′ Ar-C), 175.54 ($COOC_2H_5$). Anal. calcd. for $C_{16}H_{21}NO_4$ (%): C, 65.96; H, 7.27; N, 4.81. Found: C, 65.84; H, 7.54; N, 4.73.

4.3. General method for synthesis of {[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetic acids (**4b**,**c**) and 2-{[((E)-4-arylbut-3-en-2-ylidene) amino]oxy}propanoic acids (**5a–c**) (Scheme 1)

A mixture of compound **2**,**3a–c** (0.05 mol) and potassium hydroxide (3.36 g, 0.06 mol) in ethanol (30 mL) was stirred at room temperature for 48 h. The solvent was evaporated under reduced

pressure and the residue was triturated with water and then filtered. The filtrate was neutralized with 10% HCl and extracted with diethyl ether (3 × 15 mL). The extract was evaporated under reduced pressure and the obtained residues for compounds **4b**,**c** and **5a**,**c** were crystallized from ethanol while compound **5b** was purified by preparative TLC using benzene:ethyl acetate (5:1) as an eluting system.

4.3.1. {[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene) amino]oxy}acetic acid (**4b**)

Yield, 45%; mp 109–110 °C; IR (Nujol Mull) ν_{max}/cm^{-1} 3031 (OH), 1709 (C=O), 1583 (C=N), 1480 (C=C). ^1H NMR (CD_3OD); δ 2.18 (s, 3H, $CNCH_3$), 4.70 (s, 2H, OCH_2COO), 6.84 (d, 1H, Ar-CH=CH), 7.05 (d, 1H, Ar-CH=CH), 7.40 (d, 2H, 2′,6′ Ar-H), 7.55 (d, 2H, 3′,5′ Ar-H), 11.00 (s, 1H, COOH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.26 ($CNCH_3$), 71.35 (OCH_2COOH), 122.44 (Ar-CH=CH), 126.79 (3′,5′Ar-C), 129.85 (2′,6′ Ar-C), 132.60 (1′ Ar-C), 133.51 (4′ Ar-C), 136.31 (Ar-CH=CH), 157.10 (C=N), 171.92 (COOH). Anal. calcd. for $C_{12}H_{12}ClNO_3$ (%): C, 56.81; H, 4.77; N, 5.52. Found: C, 56.64; H, 5.00; N, 5.36.

4.3.2. {[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene) amino]oxy}acetic acid (**4c**)

Yield, 55%; mp 124–126 °C; IR (Nujol Mull) ν_{max}/cm^{-1} 3038 (OH), 1718 (C=O), 1586 (C=N), 1486 (C=C). ^1H NMR (CD_3OD); 2.17 (s, 3H, $CNCH_3$), 3.85 (s, 3H, OCH_3), 4.65 (s, 2H, OCH_2COOH), 6.71 (d, 1H, Ar-CH=CH), 6.95 (d, 2H, 3′,5′ Ar-H), 7.02 (d, 1H, Ar-CH=CH), 7.49 (d, 2H, 2′,6′ Ar-H), 11.00 (s, 1H, COOH, D_2O-exchang.). MS *m*/*z* (%); 251.00 (0.69, $M^+ + 2$), 250.00 (5.08, $M^+ + 1$), 249.00 (35.62, M^+), 248.00 (37.47), 174.00 (100.00), 160.00 (13.54), 144.00 (9.30), 131.00 (35.33), 130.00 (10.73), 107.00 (2.08). Anal. calcd. for $C_{13}H_{15}NO_4$ (%): C, 62.64; H, 6.07; N, 5.62. Found: C, 62.33; H, 6.02; N, 5.33.

4.3.3. 2-{[((E)-4-Phenylbut-3-en-2-ylidene)amino]oxy}propanoic acid (**5a**)

Yield, 35%; mp 110–111 °C; IR (Nujol Mull) ν_{max}/cm^{-1} 3039 (OH), 1720 (C=O), 1587 (C=N), 1486 (C=C). ^1H NMR ($CDCl_3$); δ 1.53 (d, 3H, $CHCH_3$), 2.17 (s, 3H, $CNCH_3$), 4.73 (q, 1H, $CHCH_3$), 6.82 (d, 1H, Ph-CH=CH), 7.05 (d, 1H, Ph-CH=CH), 7.30–7.54 (m, 5H, Ar-H), 12.86 (s, 1H, COOH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.39 (CN–CH_3), 18.11 ($CHCH_3$), 79.49 ($CHCH_3$), 127.02 (Ar-CH=CH), 128.78 (4′ Ar-C), 130.42 (2′,6′ Ar-C), 130.67 (3′,5′ Ar-C), 136.13 (1′ Ar-C), 138.58 (Ar-CH=CH), 159.20 (C=N), 177.60 (COOH). Anal. calcd. for $C_{13}H_{15}NO_3$ (%): C, 66.94; H, 6.48; N, 6.00. Found: C, 66.78; H, 6.65; N, 5.85.

4.3.4. 2-{[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene) amino]oxy}propanoic acid (**5b**)

Yield, 35%; oil; IR (Nujol Mull) ν_{max}/cm^{-1} 3042 (OH), 1723 (C=O), 1589 (C=N), 1488 (C=C). ^1H NMR (DMSO-d_6); δ 1.55 (d, 3H, $CHCH_3$), 2.08 (s, 3H, $CNCH_3$), 4.64 (q, 1H, $CHCH_3$), 6.82 (d, 1H, Ar-CH=CH), 7.10 (d, 1H, Ar-CH=CH), 7.40 (d, 2H, 2′,6′ Ar-H), 7.62 (d, 2H, 3′,5′ Ar-H), 12.85 (s, 1H, COOH, D_2O-exchang.).

Anal. calcd. for $C_{13}H_{14}ClNO_3$: C, 58.32; H, 5.27; N, 5.23. Found: C, 58.12; H, 5.40; N, 5.14.

4.3.5. 2-{[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene) amino]oxy}propanoic acid (**5c**)

Yield, 40%; mp 55–58 °C; IR (Nujol Mull) ν_{max}/cm^{-1} 3040 (OH), 1719 (C=O), 1585 (C=N), 1485 (C=C). ^1H NMR ($CDCl_3$); δ 1.51 (d, 3H, $CHCH_3$), 2.15 (s, 3H, $CNCH_3$), 3.84 (s, 3H, OCH_3), 4.71 (q, 1H, $CHCH_3$), 6.68 (d, 1H, Ar-CH=CH), 6.93 (d, 2H, 3′,5′ Ar-H), 6.98 (d, 1H, Ar-CH=CH), 7.46 (d, 2H, 2′,6′ Ar-H), 12.83 (s, 1H, COOH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.43 (CN–CH_3), 17.66 ($CHCH_3$), 56.67 (OCH_3), 79.43 ($CHCH_3$), 116.09 (3′,5′ Ar-C), 124.73 (Ar-CH=CH), 130.21 (1′ Ar-C), 130.96 (2′,6′ Ar-C), 135.87 (Ar-CH=CH), 159.49 (C=N), 162.40 (4′ Ar-C), 177.61 (COOH). Anal. calcd. for $C_{14}H_{17}NO_4$ (%): C, 63.87; H, 6.51; N, 5.32. Found: C, 63.73; H, 6.80; N, 5.17.

4.4. General method for synthesis of {[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetamides (**6a**–**c**), 2-{[((E)-4-arylbut-3-en-2-ylidene)amino] oxy}propanamides (**7a**–**c**) (Scheme 1)

A mixture of compound **2**,**3a**–**c** (0.05 mol) and ammonia solution (33%, 30 mL) in ethanol (50 mL) was stirred at room temperature for 48 h. The solvent was evaporated under reduced pressure and the residue was triturated with water, filtered, dried and crystallized from ethanol.

4.4.1. {[((E)-4-Phenylbut-3-en-2-ylidene)amino]oxy}acetamide (**6a**)

Yield, 40%; mp 128–129 °C; IR (KBr) ν_{max}/cm^{-1} 3463 (NH_2), 1683 (C=O), 1606 (C=N), 1513 (C=C). ^1H NMR (CD_3OD); δ 2.22 (s, 3H, $CNCH_3$), 4.60 (s, 2H, OCH_2CO), 6.86 (d, 1H, Ph-CH=CH), 7.10 (d, 1H, Ph-CH=CH), 7.32–7.57 (m, 5H, Ar-H), 7.63 (s, 2H, NH_2, D_2O-exchang.). Anal. calcd. for $C_{12}H_{14}N_2O_2$ (%): C, 66.04; H, 6.47; N, 12.84. Found: C, 66.24; H, 6.59; N, 12.53.

4.4.2. {[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene) amino]oxy}acetamide (**6b**)

Yield, 42%; mp 131–133 °C; IR (KBr) ν_{max}/cm^{-1} 3461 (NH_2), 1682 (C=O), 1603 (C=N), 1512 (C=C). ^1H NMR ($CDCl_3$); δ 2.14 (s, 3H, $CNCH_3$), 4.48 (s, 2H, OCH_2CO), 6.89 (d, 1H, Ar-CH=CH), 7.11 (d, 1H, Ar-CH=CH), 7.44 (d, 2H, 2′,6′ Ar-H), 7.66 (d, 2H, 3′,5′ Ar-H), 7.68 (s, 2H, NH_2, D_2O-exchang.).^{13}C NMR ($CDCl_3$); δ 10.34 ($CNCH_3$), 73.47 (OCH_2CO), 126.77 (Ar-CH=CH), 129.21 (3′,5′Ar-C), 129.60 (2′,6′ Ar-C), 133.39 (1′Ar-C), 133.76 (4′ Ar-C), 157.56 (C=N), 171.87 ($CONH_2$). Anal. calcd. for $C_{12}H_{13}ClN_2O_2$ (%): C, 57.04; H, 5.19; N, 11.09. Found: C, 57.31; H, 4.89; N, 11.30.

4.4.3. {[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene) amino]oxy}acetamide (**6c**)

Yield, 43%; mp 160–162 °C; IR (KBr) ν_{max}/cm^{-1} 3460 (NH_2), 1684 (C=O), 1600 (C=N), 1510 (C=C). ^1H NMR (CD_3OD); δ 2.20 (s, 3H, $CNCH_3$), 3.85 (s, 3H, OCH_3), 4.58 (s, 2H, OCH_2CO), 6.72 (d, 1H, Ar-CH=CH), 6.96 (d, 2H, 3′,5′ Ar-H), 7.04 (d, 1H, Ar-CH=CH), 7.49 (d, 2H, 2′,6′ Ar-H), 7.62 (s, 2H, NH_2, D_2O-exchang.). Anal. calcd. for $C_{13}H_{16}N_2O_3$ (%): C, 62.89; H, 6.50; N, 11.28. Found: C, 62.78; H, 6.67; N, 10.92.

4.4.4. 2-{[((E)-4-Phenylbut-3-en-2-ylidene) amino]oxy}propanamide (**7a**)

Yield, 47%; mp 104–107 °C; IR (KBr) ν_{max}/cm^{-1} 3322 (NH_2), 1668 (C=O), 1604 (C=N), 1511 (C=C). ^1H NMR ($CDCl_3$); δ 1.55 (d, 3H, $CHCH_3$), 2.19 (s, 3H, $CNCH_3$), 4.64 (q, 1H, $CHCH_3$), 6.83 (d, 1H, Ph-CH=CH), 7.07 (d, 1H, Ph-CH=CH), 7.19 (s, 2H, NH_2, D_2O-exchang.), 7.40–7.65 (m, 5H, Ar-H).^{13}C NMR ($CDCl_3$); δ 10.96 (CN–CH_3), 18.81 ($CHCH_3$), 79.34 ($CHCH_3$), 125.71 (Ph-CH=CH), 127.37 (4′ Ar-C), 128.90 (2′,6′ Ar-C), 129.23 (3′,5′ Ar-C), 134.09 (1′ Ar-C), 136.57 (Ph-CH=CH), 156.77 (C=N), 174.40 ($CONH_2$). Anal. calcd. for $C_{13}H_{16}N_2O_2$ (%): C, 67.22; H, 6.94; N, 12.06. Found: C, 67.09; H, 7.10; N, 11.95.

4.4.5. 2-{[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene) amino]oxy}propanamide (**7b**)

Yield, 35%; mp 78–80 °C; IR (KBr) ν_{max}/cm^{-1} 3320 (NH_2), 1667 (C=O), 1601 (C=N), 1510 (C=C). ^1H NMR ($CDCl_3$); δ 1.51 (d, 3H, $CHCH_3$), 2.10 (s, 3H, $CNCH_3$), 4.61 (q, 1H, $CHCH_3$), 6.84 (d, 1H, Ar-CH=CH), 7.06 (d, 1H, Ar-CH=CH), 7.21 (s, 2H, NH_2, D_2O-exchang.), 7.42 (d, 2H, 2′,6′ Ar-H), 7.63 (d, 2H, 3′,5′ Ar-H). ^{13}C NMR ($CDCl_3$); δ 10.95 (CN–CH_3), 17.80 ($CHCH_3$), 79.37 ($CHCH_3$), 126.58 (Ar-CH=CH), 129.08

(3′,5′ Ar-C), 132.78 (2′,6′ Ar-C), 133.27 (1′ Ar-C), 135.57 (4′ Ar-C), 137.90 (Ar-CH=CH), 156.69 (C=N), 174.34 ($CONH_2$). Anal. calcd. for $C_{13}H_{15}ClN_2O_2$ (%): C, 58.54; H, 5.67; N, 10.50. Found: C, 58.42; H, 5.90; N, 10.68.

4.4.6. 2-{[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene)amino]oxy}propanamide (**7c**)

Yield, 42%; mp 100–102 °C; IR (KBr) ν_{max}/cm^{-1} 3323 (NH_2), 1669 (C=O), 1605 (C=N), 1514 (C=C). ^1H NMR ($CDCl_3$); δ 1.50 (d, 3H, $CHCH_3$), 2.17 (s, 3H, $CNCH_3$), 3.83 (s, 3H, OCH_3), 4.61 (q, 1H, $CHCH_3$), 6.68 (d, 1H, Ar-CH=CH), 6.72 (d, 2H, 3′,5′ Ar-H), 6.93 (d, 1H, Ar-CH=CH), 7.20 (s, 2H, NH_2, D_2O-exchang.), 7.47 (d, 2H, 2′,6′ Ar-H). ^{13}C NMR ($CDCl_3$); δ 11.48 (CN–CH_3), 18.72 ($CHCH_3$), 56.65 (OCH_3), 81.23 ($CHCH_3$), 116.09 (3′,5′ Ar-C), 124.63 (Ar-CH=CH), 130.21 (1′ Ar-C), 131.22 (2′,6′ Ar-C), 135.99 (Ar-CH=CH), 159.88 (C=N), 162.41 (4′ Ar-C), 179.55 ($CONH_2$). Anal. calcd. for $C_{14}H_{18}N_2O_3$ (%): C, 64.10; H, 6.92; N, 10.68. Found: C, 63.87; H, 7.21; N, 10.56.

4.5. General method for synthesis of N-(prop-2-yl)-{[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetamides (**8a–c**) and N-(prop-2-yl)-2-{[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}propanamides (**9a–c**) (Scheme 1)

A mixture of compound **2**,**3a–c** (0.05 mol) and isopropylamine (30 mL) in ethanol (50 mL) was stirred at room temperature for 48 h. The reaction mixture was evaporated under reduced pressure; the residue was triturated with water, filtered, dried and crystallized from ethanol.

4.5.1. N-(Prop-2-yl)-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetamide (**8a**)

Yield, 75%; mp 102–104 °C; IR (KBr) ν_{max}/cm^{-1} 3290 (NH), 1651 (C=O), 1550 (C=N), 1490 (C=C). ^1H NMR (CD_3OD); δ 1.22 (d, 6H, $CH(CH_3)_2$), 2.22 (s, 3H, $CNCH_3$), 4.09–4.13 (m, 1H, $CH(CH_3)_2$), 4.57 (s, 2H, OCH_2CO), 6.85 (d, 1H, Ph-CH=CH), 7.09 (d, 1H, Ph-CH=CH), 7.33–7.57 (m, 6H, Ar-H and NH (D_2O-exchang.)). Anal. calcd. for $C_{15}H_{20}N_2O_2$ (%): C, 69.20; H, 7.74; N, 10.76. Found: C, 69.33; H, 7.49; N, 10.86.

4.5.2. N-(Prop-2-yl)-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**8b**)

Yield, 50%; mp 79–80 °C; IR (KBr) ν_{max}/cm^{-1} 3289 (NH), 1645 (C=O), 1552 (C=N), 1488 (C=C). ^1H NMR (DMSO-d_6); δ 1.22 (d, 6H, $CH(CH_3)_2$), 2.11 (s, 3H, $CNCH_3$), 4.37–4.41 (m, 1H, $CH(CH_3)_2$), 4.46 (s, 2H, OCH_2CO), 6.85 (d, 1H, Ar-CH=CH), 7.09 (d, 1H, Ar-CH=CH), 7.42 (d, 2H, 2′,6′ Ar-H), 7.47 (d, 2H, 3′,5′ Ar-H), 7.64 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR (DMSO-d_6); δ 10.97 ($CNCH_3$), 22.74 ($CH(CH_3)_2$), 40.40 ($CH(CH_3)_2$), 73.16 (OCH_2CO), 124.41 (4′ Ar-C), 128.60 (Ar-CH=CH), 129.22 (3′,5′ Ar-C), 133.33 (2′,6′ Ar-C), 134.13 (1′ Ar-C), 135.88 (Ar-CH=CH), 156.97 (C=N), 168.06 (CONH). Anal. calcd. for $C_{15}H_{19}ClN_2O_2$ (%): C, 61.12; H, 6.50; N, 9.50. Found: C, 60.98; H, 6.78; N, 9.35.

4.5.3. N-(Prop-2-yl)-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**8c**)

Yield, 70%; mp 128–130 °C; IR (KBr) ν_{max}/cm^{-1} 3292 (NH), 1652 (C=O), 1551 (C=N), 1493 (C=C). ^1H NMR (CD_3OD); δ 1.22 (d, 6H, $CH(CH_3)_2$), 2.20 (s, 3H, $CNCH_3$), 3.85 (s, 3H, OCH_3), 4.10–4.14 (m, 1H, $CH(CH_3)_2$), 4.55 (s, 2H, OCH_2CO), 6.71 (d, 1H, Ar-CH=CH), 6.95 (d, 2H, 3′,5′ Ar-H), 7.04 (d, 1H, Ar-CH=CH), 7.50 (d, 2H, 2′,6′ Ar-H), 7.64 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{16}H_{22}N_2O_3$ (%): C, 66.18; H, 7.64; N, 9.65. Found: C, 65.94; H, 7.89; N, 9.54.

4.5.4. N-(Prop-2-yl)-2-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}propanamide (**9a**)

Yield, 45%; mp 111–112 °C; IR (KBr) ν_{max}/cm^{-1} 3285 (NH), 1657 (C=O), 1551 (C=N), 1496 (C=C). ^1H NMR (DMSO-d_6); δ 1.08 (d, 6H, $CH(CH_3)_2$), 1.35 (d, 3H, $CHCH_3$), 2.12 (s, 3H, $CNCH_3$), 3.80–3.94 (m, 1H, $CH(CH_3)_2$), 4.53 (q, 1H, $CHCH_3$), 6.80 (d, 1H, Ph-CH=CH), 7.08 (d, 1H, Ph-CH=CH), 7.27–7.61 (m, 6H, Ar-H and NH (D_2O-exchang.)). Anal. calcd. for $C_{16}H_{22}N_2O_2$ (%): C, 70.04; H, 8.08; N, 10.21. Found: C, 69.84; H, 8.35; N, 10.12.

4.5.5. N-(Prop-2-yl)-2-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}propanamide (**9b**)

Yield, 40%; mp 75–78 °C; IR (KBr) ν_{max}/cm^{-1} 3284 (NH), 1656 (C=O), 1550 (C=N), 1494 (C=C). ^1H NMR (DMSO-d_6); δ 1.11 (d, 6H, $CH(CH_3)_2$), 1.36 (d, 3H, $CHCH_3$), 2.10 (s, 3H, $CNCH_3$), 3.83–3.97 (m, 1H, $CH(CH_3)_2$), 4.54 (q, 1H, $CHCH_3$), 6.83 (d, 1H, Ar-CH=CH), 7.08 (d, 1H, Ar-CH=CH), 7.29 (d, 2H, 2′,6′ Ar-H), 7.56 (d, 2H, 3′,5′ Ar-H), 8.16 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{16}H_{21}ClN_2O_2$ (%): C, 62.23; H, 6.85; N, 9.07. Found: C, 62.40; H, 6.69; N, 9.18.

4.5.6. N-(Prop-2-yl)-2-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}propanamide (**9c**)

Yield, 40%; mp 157–158 °C; IR (KBr) ν_{max}/cm^{-1} 3283 (NH), 1655 (C=O), 1549 (C=N), 1493 (C=C). ^1H NMR ($CDCl_3$); δ 1.13 (d, 6H, $CH(CH_3)_2$), 1.42 (d, 3H, $CHCH_3$), 2.13 (s, 3H, $CNCH_3$), 3.79 (s, 3H, OCH_3), 3.96–4.01 (m, 1H, $CH(CH_3)_2$), 4.62 (q, 1H, $CHCH_3$), 6.67 (d, 1H, Ar-CH=CH), 6.90 (d, 2H, 3′,5′ Ar-H), 6.96 (d, 1H, Ar-CH=CH), 7.43 (d, 2H, 2′,6′ Ar-H), 8.14 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.47 (CN–CH_3), 18.61 ($CHCH_3$), 23.32 ($CH(CH_3)_2$), 43.26 ($CH(CH_3)_2$), 56.64 (OCH_3), 81.27 ($CHCH_3$), 116.09 (3′,5′ Ar-C), 124.65 (Ar-CH=CH), 130.20 (1′ Ar-C), 131.25 (2′,6′ Ar-C), 135.96 (Ar-CH=CH), 159.88 (C=N), 162.41 (4′ Ar-C), 179.54 (CONH). Anal. calcd. for $C_{17}H_{24}N_2O_3$ (%): C, 67.08; H, 7.95; N, 9.20. Found: C, 66.88; H, 8.09; N, 9.08.

4.6. General method for synthesis of {[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetohydrazides (**10a–c**) and 2-{[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}propanoic acid hydrazides (**11a–c**) (Scheme 1)

A mixture of compound **2**,**3a–c** (0.05 mol) and hydrazine hydrate (99%, 0.10 mol) in absolute ethanol (20 mL) was heated under reflux for 24 h. The solvent was evaporated under reduced pressure, the residue was triturated with water, filtered, dried and crystallized from ethanol.

4.6.1. {[((E)-4-Phenylbut-3-en-2-ylidene)amino]oxy}acetohydrazide (**10a**)

Yield, 30%; mp 125–126 °C; IR (KBr) ν_{max}/cm^{-1} 3373 (NH_2), 3327 (NH), 1646 (C=O), 1606 (C=N), 1511 (C=C). ^1H NMR (CD_3OD); δ 2.20 (s, 3H, $CNCH_3$), 3.36 (s, 2H, NH_2, D_2O-exchang.), 4.60 (s, 2H, OCH_2CO), 6.83 (d, 1H, Ph-CH=CH), 7.09 (d, 1H, Ph-CH=CH), 7.24–7.56 (m, 6H, Ar-H and NH (D_2O-exchang.)). Anal. calcd. for $C_{12}H_{15}N_3O_2$ (%): C, 61.79; H, 6.48; N, 18.01. Found: C, 61.87; H, 6.78; N, 17.74.

4.6.2. {[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetohydrazide (**10b**)

Yield, 30%; mp 145–147 °C; IR (KBr) ν_{max}/cm^{-1} 3370 (NH_2), 3325 (NH), 1646 (C=O), 1604 (C=N), 1510 (C=C). ^1H NMR (CD_3OD); δ 2.19 (s, 3H, $CNCH_3$), 3.75 (s, 2H, NH_2, D_2O-exchang.), 4.64 (s, 2H, OCH_2CO), 6.83 (d, 1H, Ar-CH=CH), 7.07 (d, 1H, Ar-CH=CH), 7.35 (s, 1H, NH, D_2O-exchang.), 7.40 (d, 2H, 2′,6′ Ar-H), 7.55 (d, 2H, 3′,5′ Ar-H). Anal. calcd. for $C_{12}H_{14}ClN_3O_2$ (%): C, 53.84; H, 5.27; N, 15.70. Found: C, 53.61; H, 5.52; N, 15.58.

4.6.3. {[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetohydrazide (**10c**)

Yield, 30%; mp 148–150 °C; IR (KBr) ν_{max}/cm^{-1} 3372 (NH_2), 3326 (NH), 1644 (C=O), 1606 (C=N), 1511 (C=C). ^1H NMR (CD_3OD);

δ 2.19 (s, 3H, $CNCH_3$), 3.80 (s, 2H, NH_2, D_2O-exchang.), 3.85 (s, 3H, OCH_3), 4.59 (s, 2H, OCH_2CO), 6.70 (d, 1H, Ar-CH=CH), 6.86 (d, 2H, 3′,5′ Ar-H), 6.94 (s, 1H, NH, D_2O-exchang.), 7.03 (d, 1H, Ar-CH=CH), 7.49 (d, 2H, 2′,6′ Ar-H). Anal. calcd. for $C_{13}H_{17}N_3O_3$ (%): C, 59.30; H, 6.51; N, 15.96. Found: C, 59.40; H, 6.68; N, 15.72.

4.6.4. 2-{[((E)-4-Phenylbut-3-en-2-ylidene)amino]oxy}propanoic acid hydrazide (**11a**)

Yield, 30%; mp 125–126 °C; IR (KBr) ν_{max}/cm^{-1} 3401 (NH_2), 3310 (NH), 1652 (C=O), 1585 (C=N), 1530 (C=C). ^1H NMR ($CDCl_3$); δ 1.49 (d, 3H, $CHCH_3$), 2.18 (s, 3H, $CNCH_3$), 4.22 (s, 2H, NH_2, D_2O-exchang.), 4.67 (q, 1H, $CHCH_3$), 6.81 (d, 1H, Ph-CH=CH), 7.06 (d, 1H, Ph-CH=CH), 7.31–7.53 (m, 5H, Ar-H), 9.08 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.47 (CN–CH_3), 18.56 ($CHCH_3$), 80.59 ($CHCH_3$), 126.94 (Ph-CH=CH), 128.78 (4′ Ar-C), 130.48 (2′,6′ Ar-C), 130.69 (3′,5′ Ar-C), 136.28 (1′ Ar-C), 138.51 (Ph-CH=CH), 159.65 (C=N), 175.11 ($CONHNH_2$). Anal. calcd. for $C_{13}H_{17}N_3O_2$ (%): C, 63.14; H, 6.93; N, 16.99. Found: C, 63.03; H, 7.21; N, 16.86.

4.6.5. 2-{[((E)-4-(4-Chlorophenyl)but-3-en-2-ylidene)amino]oxy}propanoic acid hydrazide (**11b**)

Yield, 25%; mp 133–134 °C; IR (KBr) ν_{max}/cm^{-1} 3399 (NH_2), 3309 (NH), 1652 (C=O), 1583 (C=N), 1529 (C=C). ^1H NMR ($CDCl_3$); δ 1.36 (d, 3H, $CHCH_3$), 2.11 (s, 3H, $CNCH_3$), 4.21 (s, 2H, NH_2, D_2O-exchang.), 4.49 (q, 1H, $CHCH_3$), 6.85 (d, 1H, Ar-CH=CH), 7.10 (d, 1H, Ar-CH=CH), 7.46 (d, 2H, 2′,6′ Ar-H), 7.67 (d, 2H, 3′,5′ Ar-H), 9.06 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{13}H_{16}ClN_3O_2$ (%): C, 55.42; H, 5.72; N, 14.91. Found: C, 55.62; H, 5.56; N, 14.83.

4.6.6. 2-{[((E)-4-(4-Methoxyphenyl)but-3-en-2-ylidene)amino]oxy}propanoic acid hydrazide (**11c**)

Yield, 30%; mp 140–141 °C; IR (KBr) ν_{max}/cm^{-1} 3395 (NH_2), 3305 (NH), 1651 (C=O), 1580 (C=N), 1525 (C=C). ^1H NMR ($CDCl_3$); δ 1.48 (d, 3H, $CHCH_3$), 2.16 (s, 3H, $CNCH_3$), 3.83 (s, 3H, OCH_3), 4.20 (s, 2H, NH_2, D_2O-exchang.), 4.64 (q, 1H, $CHCH_3$), 6.67 (d, 1H, Ar-CH=CH), 6.85 (d, 2H, 3′,5′ Ar-H), 6.97 (d, 1H, Ar-CH=CH), 7.46 (d, 2H, 2′,6′ Ar-H), 9.06 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.44 (CN–CH_3), 15.52 ($CHCH_3$), 56.52 (OCH_3), 80.50 ($CHCH_3$), 115.66 (3′,5′ Ar-C), 124.66 (Ar-CH=CH), 130.18 (1′ Ar-C), 131.22 (2′,6′ Ar-C), 135.96 (Ar-CH=CH), 159.84 (C=N), 161.50 (4′ Ar-C), 175.19 ($CONHNH_2$). Anal. calcd. for $C_{14}H_{19}N_3O_3$ (%): C, 60.63; H, 6.91; N, 15.15. Found: C, 60.46; H, 6.65; N, 15.32.

4.7. General method for synthesis of N-aryl-{[((E)-4-arylbut-3-en-2-ylidene)amino]oxy}acetamides (**13**–**15a**,**b**,**c**) (Scheme 2)

A mixture of the oxime derivative **1a**–**c** (0.05 mol) and sodium methoxide (2.70 g, 0.05 mol) in anhydrous methanol (50 mL) was heated under reflux for 2 h. The solvent was evaporated under reduced pressure and the residual oximate salt was dissolved in dry DMF (5 mL). A solution of the appropriate 2-chloro-*N*-arylacetamide derivative (**12a**–**c**) (0.05 mol) in dry DMF (10 mL) was added to the previous oximate sodium salt solution and the reaction mixture was heated at 80 °C for 48 h. After cooling, the reaction mixture was poured over crushed ice (10 g) and stirred for 5 min. The separated solid was filtered, washed with water, dried and crystallized from ethanol.

4.7.1. N-Phenyl-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetamide (**13a**)

Yield, 30% (a), 45% (b); mp 97–99 °C; IR (KBr) ν_{max}/cm^{-1} 3422 (NH), 1653 (C=O), 1603 (C=N), 1511 (C=C). ^1H NMR (DMSO-d_6); δ 2.10 (s, 3H, $CNCH_3$), 4.85 (s, 2H, OCH_2CO), 6.58 (d, 1H, Ph-CH=CH), 7.02 (d, 1H, Ph-CH=CH), 7.15–7.62 (m, 10H, Ar-H), 10.02 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 10.32 ($CNCH_3$), 71.34 (OCH_2CO), 122.14 (2″, 6″ Ar-C), 124.34 (Ph-CH=CH), 128.49 (4′ Ar-C), 130.13 (4″ Ar-C), 130.51 (2′,6′ Ar-C), 131.06 (3′,5′ Ar-C), 131.18 (3″,5″ Ar-C), 135.81 (1′ Ar-C), 137.80 (Ph-CH=CH), 138.85 (1″ Ar-C), 157.43 (C=N), 170.30 (CONH). Anal. calcd. for $C_{18}H_{18}N_2O_2$ (%): C, 73.45; H, 6.16; N, 9.52. Found: C, 73.29; H, 6.35; N, 9.43.

4.7.2. N-(4-Chlorophenyl)-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetamide (**13b**)

Yield, 35% (a), 50% (b); mp 107–109 °C; IR (KBr) ν_{max}/cm^{-1} 3395 (NH), 1664 (C=O), 1600 (C=N), 1511 (C=C). ^1H NMR (DMSO-d_6); δ 2.11 (s, 3H, $CNCH_3$), 4.84 (s, 2H, OCH_2CO), 6.56 (d, 1H, Ph-CH=CH), 7.01 (d, 1H, Ph-CH=CH), 7.14–7.63 (m, 9H, Ar-H), 10.03 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{18}H_{17}ClN_2O_2$ (%): C, 65.75; H, 5.21; N, 8.52. Found: C, 65.52; H, 5.39; N, 8.64.

4.7.3. N-(4-Methoxyphenyl)-{[((E)-4-phenylbut-3-en-2-ylidene)amino]oxy}acetamide (**13c**)

Yield, 30% (a), 40% (b); mp 100–103 °C; IR (KBr) ν_{max}/cm^{-1} 3392 (NH), 1653 (C=O), 1601 (C=N), 1512 (C=C). ^1H NMR (DMSO-d_6); δ 2.11 (s, 3H, $CNCH_3$), 3.73 (s, 3H, OCH_3), 4.80 (s, 2H, OCH_2CO), 6.59 (d, 1H, Ph-CH=CH), 7.05 (d, 1H, Ph-CH=CH), 7.14–7.62 (m, 9H, Ar-H), 10.01 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{19}H_{20}N_2O_3$ (%): C, 70.35; H, 6.21; N, 8.64. Found: C, 70.52; H, 6.09; N, 8.84.

4.7.4. N-Phenyl-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**14a**)

Yield, 45% (a), 60% (b); mp 137–138 °C; IR (KBr) ν_{max}/cm^{-1} 3399 (NH), 1650 (C=O), 1600 (C=N), 1509 (C=C). ^1H NMR (DMSO-d_6); δ 2.11 (s, 3H, $CNCH_3$), 4.85 (s, 2H, OCH_2CO), 6.57 (d, 1H, Ar-CH=CH), 7.03 (d, 1H, Ar-CH=CH), 7.14–7.64 (m, 9H, Ar-H), 10.05 (s, 1H, NH, D_2O-exchang.). ^{13}C NMR ($CDCl_3$); δ 11.29 ($CNCH_3$), 71.34 (OCH_2CO), 122.76 (2″, 6″ Ar-C), 123.73 (Ar-CH=CH), 128.71 (4′ Ar-C), 130.13 (4″ Ar-C), 130.51 (2′,6′ Ar-C), 131.14 (3′,5′ Ar-C), 132.71 (3″,5″ Ar-C), 135.81(1′ Ar-C), 137.80 (Ar-CH=CH), 138.94 (1″ Ar-C), 157.08 (C=N), 171.87 (CONH).Anal. calcd. for $C_{18}H_{17}ClN_2O_2$ (%): C, 65.75; H, 5.21; N, 8.52. Found: C, 65.52; H, 5.39; N, 8.64.

4.7.5. N-(4-Chlorophenyl)-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**14b**)

Yield, 35% (a), 45% (b); mp 100–102 °C; IR (KBr) ν_{max}/cm^{-1} 3420 (NH), 1649 (C=O), 1601 (C=N), 1505 (C=C). ^1H NMR (DMSO-d_6); δ 2.11 (s, 3H, $CNCH_3$), 4.83 (s, 2H, OCH_2CO), 6.57 (d, 1H, Ar-CH=CH), 7.00 (d, 1H, Ar-CH=CH), 7.14–7.63 (m, 8H, Ar-H), 10.04 (s, 1H, NH, D_2O-exchang.).

Anal. calcd. for $C_{18}H_{16}Cl_2N_2O_2$ (%): C, 59.52; H, 4.44; N, 7.71. Found: C, 59.40; H, 4.68; N, 7.62.

4.7.6. N-(4-Methoxyphenyl)-{[((E)-4-(4-chlorophenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**14c**)

Yield, 30% (a), 50% (b); mp 140–142 °C; IR (KBr) ν_{max}/cm^{-1} 3422 (NH), 1651 (C=O), 1602 (C=N), 1508 (C=C). ^1H NMR (DMSO-d_6); δ 2.09 (s, 3H, $CNCH_3$), 3.75 (s, 3H, OCH_3), 4.78 (s, 2H, OCH_2CO), 6.94 (d, 1H, Ar-CH=CH), 7.30 (d, 1H, Ar-CH=CH), 7.49–7.78 (m, 8H, Ar-H), 10.01 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{19}H_{19}ClN_2O_3$ (%): C, 63.60; H, 5.34; N, 7.81. Found: C, 63.41; H, 5.60; N, 7.64.

4.7.7. N-Phenyl-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**15a**)

Yield, 45% (a), 55% (b); mp 107–108 °C; IR (KBr) ν_{max}/cm^{-1} 3400 (NH), 1640 (C=O), 1605 (C=N), 1501 (C=C). ^1H NMR (DMSO-d_6); δ 2.11 (s, 3H, $CNCH_3$), 3.73 (s, 3H, OCH_3), 4.80 (s, 2H, OCH_2CO), 6.59 (d, 1H, Ar-CH=CH), 6.83 (d, 1H, Ar-CH=CH), 7.14–7.64 (m, 9H, Ar-H), 10.01 (s, 1H, NH, D_2O-exchang.).Anal. calcd. for $C_{19}H_{20}N_2O_3$ (%): C, 70.35; H, 6.21; N, 8.64. Found: C, 70.12; H, 6.40; N, 8.34.

*4.7.8. N-(4-Chlorophenyl)-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**15b**)*

Yield, 45% (a), 65% (b); mp 137–139 °C; IR (KBr) ν_{max}/cm^{-1} 3421 (NH), 1649 (C=O), 1600 (C=N), 1500 (C=C). ^1H NMR (DMSO-d_6); δ 2.09 (s, 3H, $CNCH_3$), 3.73 (s, 3H, OCH_3), 4.81 (s, 2H, OCH_2CO), 6.59 (d, 1H, Ar-CH=CH), 6.97 (d, 1H, Ar-CH=CH), 7.00–7.54 (m, 8H, Ar-H), 10.05 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{19}H_{19}ClN_2O_3$ (%): C, 63.60; H, 5.34; N, 7.81. Found: C, 63.43; H, 5.63; N, 7.70.

*4.7.9. N-(4-Methoxyphenyl)-{[((E)-4-(4-methoxyphenyl)but-3-en-2-ylidene)amino]oxy}acetamide (**15c**)*

Yield, 30% (a), 45% (b); mp 124–125 °C; IR (KBr) ν_{max}/cm^{-1} 3421 (NH), 1652 (C=O), 1600 (C=N), 1511 (C=C). ^1H NMR (DMSO-d_6); δ 2.09 (s, 3H, $CNCH_3$), 3.79 (s, 6H, 2° CH_3), 4.81 (s, 2H, OCH_2CO), 6.59 (d, 1H, Ar-CH=CH), 6.97 (d, 1H, Ar-CH=CH), 7.01–7.54 (m, 8H, Ar-H), 10.06 (s, 1H, NH, D_2O-exchang.). Anal. calcd. for $C_{20}H_{22}N_2O_4$ (%): C, 67.78; H, 6.26; N, 7.90. Found: C, 67.54; H, 6.50; N, 7.74.

5. Pharmacology

5.1. Materials and animals

Wister rats weighing in the range 100–120 g and Swiss albino mice of either sex weighing 20–25 g were purchased from local source and kept at room temperature (22 ± 2 °C) in a light-controlled room with an alternating 12 h light/dark cycle. Animals were allowed to become acclimatized to laboratory conditions before experimentation and allowed free access to standard food and water. The tested compounds were prepared as suspension in 2% gum acacia in sterile distilled water. Diclofenac sodium was used as a standard drug, dissolved in sterile distilled water. The positive control group animals received the reference drug while the negative control received only the vehicle.

5.2. Anti-inflammatory activity

The anti-inflammatory activity was evaluated using *in vivo* carrageenin-induced rat paw oedema model [30] which is considered the most conventional one for acute inflammation. The rats were divided into 41 groups, 5 rats each, and were injected intraperitoneally with equimolar doses (equivalent to 0.169 M/kg) of the tested compounds and reference drug (diclofenac sodium). One hour after drug administration, rats were subcutaneously injected with 0.05 mL carrageenin (1% solution in saline prepared 24 h before use) into the subplantar tissue of the right hind paw. The left hind paw of each rat received a subplantar injection of equal volume of normal saline. Three hours after carrageenin injection, rats were killed by cervical dislocation, then the right and the left hind paws of each rat were cut at the tibiotarsic articulation and weighed. The difference in weight between right and left paws was recorded for each rat. The percentage increase in weight of the carrageenin-injected paw over the other paw was calculated and percentage reduction of oedema from the control group was used as a measure of the activity. Compounds **5b** and **7a** as well as diclofenac sodium were tried in three graded doses to determine the ED_{50}.

5.3. Analgesic activity

Swiss albino mice were divided into 6 groups, each comprised 6 mice, and received orally the tested compounds and diclofenac sodium. After 2 h, 0.2 mL of acetic acid 3% was injected intraperitoneally into each mouse. The number of writhing in 20 min after treatment was calculated and the percentage reduction in number of writhes from the control group was calculated and used as a measure of the analgesic activity [31].

5.4. Potential ulcerogenicity

Ulcerogenic liability was determined following the method of Adami et al. [32]. The rats were divided into 4 groups, 5 rats each, the tested compounds (**5b** and **7a**) and diclofenac sodium were given orally to the rats in equimolar doses (equivalent to 0.169 M/kg). The doses were repeated daily for 5 days. The animals were sacrificed 10 h after the last treatment, by over-dose of ether. The stomach was removed, rinsed with saline, opened along the greater curvature, studied with hand lens (×10 magnification). The number of ulcers and the total sum of lengths of ulcers for each animal were recorded and the mean was calculated and taken as ulcer index.

5.5. Determination of acute toxicity

Twelve groups of wister rats each comprised of 5 animals were used in this study (4 groups for each compound). Compounds **5b** and **7a** were given intraperitoneally at doses of 250, 500, 750 and 1000 mg/kg to rat groups while diclofenac sodium was given at doses of 100, 150, 200 and 300 mg/kg. The percentage mortality in each group was determined 24 h after administration. Calculation of LD_{50} and 95% confidence limits was done according to the method of Litchfield and Wilcoxon [33].

6. Molecular modeling methods

6.1. Conformational search

Initial structures for the molecules **5b** and **7a** were constructed using the HyperChem program version 5.11. The MM+ (calculations in vacuo, bond dipole option for electrostatics, Polake–Ribiere algorithm, and RMS gradient of 0.01 kcal/mol) conformational searching in torsional space was performed using the multiconformer method [34]. Energy minima for compounds **5b** and **7a** were determined by a semi-empirical method AM1 (as implemented in HyperChem 5.11). The conformations thus obtained were confirmed as minima by vibrational analysis.

6.2. Docking methodology

Docking studies have been performed using MOE 2008.10. With this purpose, crystal structure of both COX-1/flurbiprofen (a non-selective inhibitor) and COX-2/SC-558 (a selective inhibitor) complexes (PDB codes: 1EQH and 1CX2, respectively) were obtained from the Protein Data Bank in order to prepare both proteins for docking studies. Docking procedure was followed using the standard protocol implemented in MOE 2009.10 and the geometry of resulting complexes was studied using the MOE's Pose Viewer utility.

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