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SYNTHESIS, BIOLOGICAL EVALUATION AND MOLECULAR DOCKING OF DIARYLIMIDAZOLE DERIVATIVES AS NEW POTENTIAL ANTI-INFLAMMATORY AGENTS TARGETING COX-2 ENZYME

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*A series of diaryl imidazole derivatives **(5a-h)** was designed as novel selective COX-2 anti-inflammatory drug candidates. The designed compounds were synthesised, and their structures were characterized by spectroscopic analysis. The anticipated anti-inflammatory activity for the synthesized compounds was assessed by in-vitro cyclooxygenases (COX-1/COX-2) inhibition assay. Most of tested compounds demonstrated moderate COX-2 inhibitory activity and selectivity (IC_{50} = 0.068 – 0.80 µm, SI = 7.5 - 175) in comparison to indomethacin (IC_{50} = 0.079 µm, SI = 12.5) and celecoxib (IC_{50} = 0.046 µm, SI = 315.22). Molecular modelling study of the synthesized compounds into the active site of COX-2 enzyme was performed to rationalize their preferred binding affinity; except for compound **5f**, all compounds showed binding with amino acids at the selectivity pocket Additionally, In-silico simulation studies explored the drug drug-likeness parameters of the synthesized compounds, all synthesized compounds exhibited promising physicochemical parameters and pharmacokinetics according to the in-silico simulation results.*

Keywords: *COX-1/ COX-2 inhibitors; imidazole; anti-inflammatory; docking*

INTRODUCTION

Multiple diseases such as cancer[1], metabolic syndromes[2], neurodegenerative disorder[3] and autoimmune disorders[4] are exacerbated by inflammation. The most common strategy to treat inflammatory symptoms is to inhibit the arachidonic acid cascade that generate the inflammatory prostaglandins (PGs) *via* interrupting cyclooxygenase (COX-1 and COX-2) pathways as in non-steroidal anti-inflammatory drugs (NSAIDs)[5]. The extensively used non-selective NSAIDs, such as aspirin and indomethacin, are combined with gastrointestinal complications[6]. On the other hand, highly selective COX-2 inhibitors such as valdecoxib and rofecoxib, were innately associated with several cardiovascular complaints[7]. Thus, there is still a great need for innovative anti-inflammatory agents with better therapeutic profile. Imidazole scaffold was reported as promising core for developing effective anti-inflammatory agents such as compounds I-III (Fig.1)[8-10]. Likewise, ketoconazole[11] and flumizole[12] are antifungal drugs in the market, however they were proved to exhibit anti-inflammatory activity (Fig.1)

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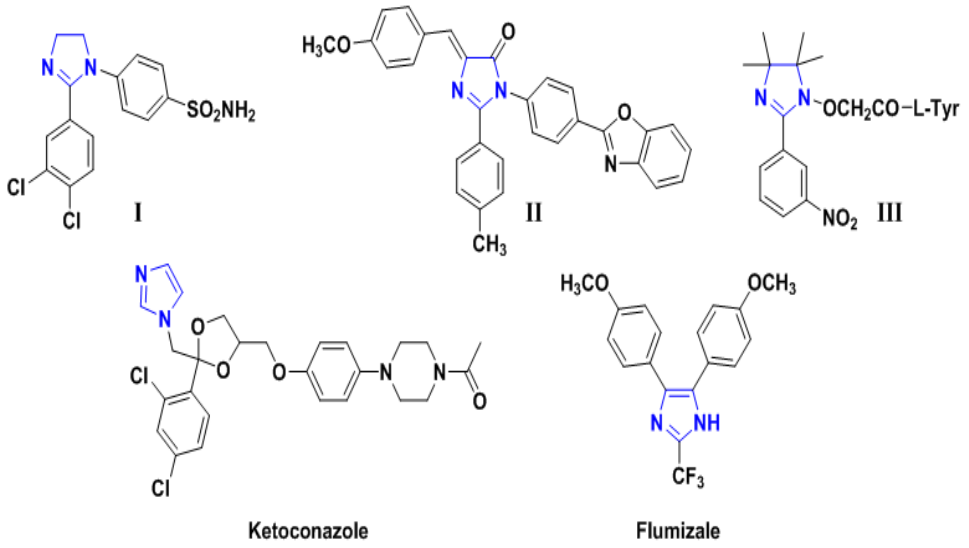


Fig.1: Structures of previously reported anti-inflammatory imidazole derivatives**.**

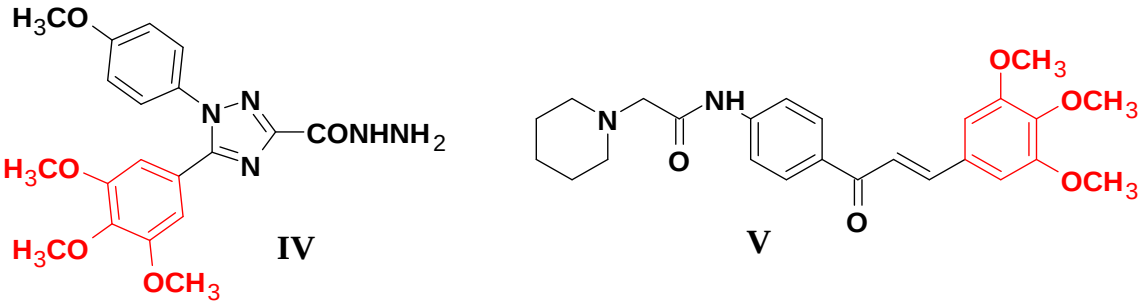


Fig.2: Representative examples for trimethoxyphenyl derivatives with selective COX-2 inhibitory activity**.**

The current study aimed to design new anti-inflammatory agents with selective COX-2 inhibitory activity compared to celecoxib. The design is set to satisfy the pharmacophoric features of selective COX-2 inhibitors containing two aromatic groups attached to a heterocyclic ring. A bulky arylidene trimethoxyphenyl moiety is attached with the imidazole scaffold replacing the celecoxib CF_3 group and two phenyl groups are attached to the imidazole ring representing the aromatic moieties. Modifying the substituents on one of the phenyl residues to modulate the electronic and/or physicochemical characteristics of the molecules for better receptor interaction. (Fig. 3) The synthesized compounds were evaluated for their *in vitro* COX-1 and COX-2 inhibitory activity, and molecular docking study was carried out to explore their binding mode to enzyme active sites. Finally, the pharmacokinetic profile and physicochemical properties of the tested compounds were predicted by utilizing *in silico* simulation study.

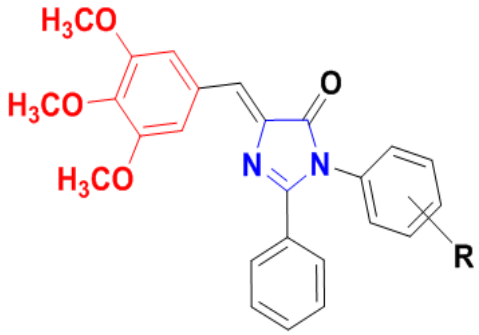


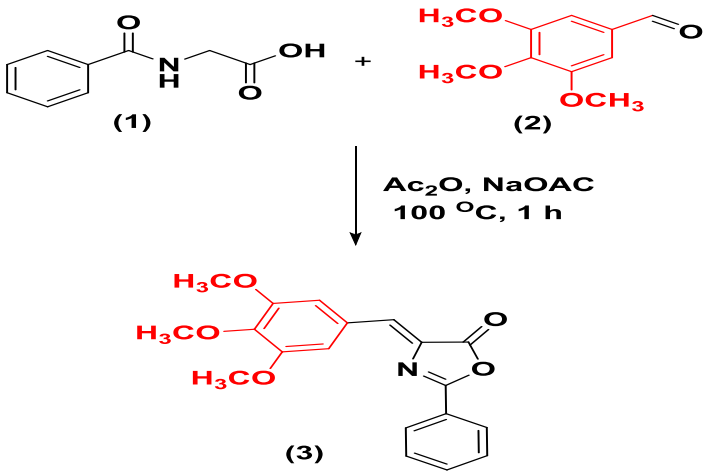
Fig.3: Design of the newly synthesized compounds.

RESULTS AND DISCUSSION

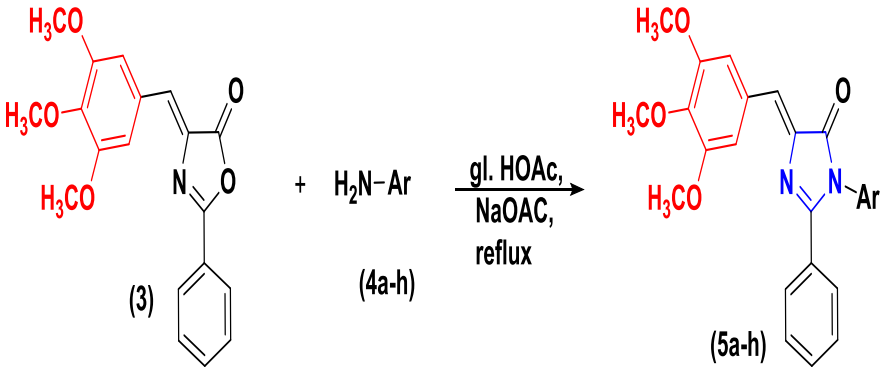
Chemistry

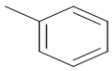
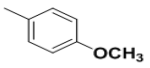
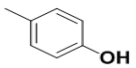
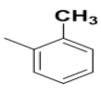
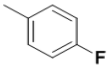
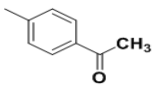
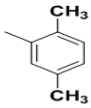
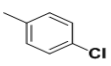
Scheme (1) and (2) sketched the synthetic pathway needed to deliver the target compounds **5a-h**. The Erlenmeyer– Plochl azlactone synthesis[15] was utilized to prepare the precursor **3**; where *N*-benzoylglycine (**1**) was reacted with 3,4,5-trimethoxy benzaldehyde (**2**) in acetic anhydride in presence of sodium acetate (scheme 1). The oxazolone **3** was allowed to react with a number of aniline derivatives **4a-h** to furnish the target imidazolones **5a-h** (scheme 2). The proposed structures of the prepared imidazolone derivatives **5a-h** were evidenced by their IR, ^{1}H NMR , ^{13}C NMR and elemental analysis.

Scheme (1): Synthesis of oxazolone (**3**).



Scheme (2): Synthesis of imidazole-4-one derivatives (**5a-h**)



Cpd	Ar	Cpd	Ar	Cpd	Ar
5a		5d		5g	
5b		5e		5h	
5c		5f			

Biological Evaluation

***In vitro* COX-1 and COX-2 inhibition assays**

The efficiency of the target compounds **5a-h** against COX enzymes was determined by screening their *in vitro* inhibition of human COX-1 and COX-2 compared to celecoxib and indomethacin as standard drugs. Results were expressed as IC_{50} and COX-2 selectivity indices (SI) were calculated as COX-1 IC_{50} / COX-2 IC_{50} (Table 1). The results showed that all tested compounds had weak inhibitory activity against COX-1 (IC_{50} = 6.0 – 12.5 µm) when compared to indomethacin (IC_{50} = 0.99 µm), however have more COX-1 inhibitory activity than celecoxib (IC_{50} = 14.5 µm). On the other hand, the synthesized compounds exhibited COX-2 inhibitory activity (IC_{50} = 0.068 – 0.80 µm) comparable to that of indomethacin (IC_{50} = 0.079 µm) but lower than celecoxib (IC_{50} = 0.046 µm). Accordingly, all compounds displayed more selectivity towards COX-2 than COX-1 with different efficacies (SI = 7.5 - 175) showing higher selectivity – except **5f** – than indomethacin (SI = 12.5) assuming that the target compounds are supposed to posses safer gastric profile. Among the tested compounds, 4-flurophenyl-imidazole derivative **(5e)** was the most selective and have high potency (IC_{50} = 0.069, SI = 181.16), However, 4-methoxyphenyl-imidazole derivative (**5d)** was the most potent and with high selectivity towards COX-2 enzyme (IC_{50} = 0.060, SI = 175).

Table 1: COX-1 and COX-2 *in vitro* enzyme inhibitory activities illustrating COX-2 selectivity indices of synthesized compounds and references.

Compound	[a] COX-1 IC_{50} (µm)	[a] COX-2 IC_{50} (µm)	[b] SI COX-1/COX-2
Celecoxib	14.5	0.046	315.22
Indomethacin	0.99	0.079	12.53
5a	7.5	0.135	55.56
5b	8.5	0.103	82.52
5c	11.5	0.089	129.21
5d	10.5	0.060	175
5e	12.5	0.069	181.16
5f	6.0	0.80	7.5
5g	8.0	0.099	80.81
5h	10.5	0.068	154.41

*$p < 0.05$ vs celecoxib. #$p < 0.05$ vs indomethacin

[a] IC_{50} in (µM) concentration is expressed as mean ± SEM, for 3 replications.

[b] Selectivity index (SI) = $\frac{\text{COX-1 IC50}}{\text{COX-2 IC50}}$

Molecular Docking

The orientation of designed compounds **(5a-5h)** was investigated through docking of the designed compounds within the active site of cyclooxygenase-2 enzyme (COX-2).

The protein data bank (PDB: 1CX2) was used to download (COX-2) co-crystallized with SC-558[16]. Molecular Operating Environment (MOE) version MOE 2019.0102 was used for the docking studies. Redocking of the co-crystallized ligand SC-558 within the active site was performed to validate the used docking parameters. All the fundamental interactions of co-crystallized ligand SC-558 at the active binding site is reproducible using the performed docking setup with redocking RMSD 0.937 Å. COX-1 and COX-2 differ in their binding site; the major difference is the bigger size of the COX-2 binding site compared to COX-1 because of the presence of the extra side pocket. This side pocket includes Arg 513 (replaced by His 513 in Cox-1), Val 523 (replaced by Ileu 523 in COX-1), Tyr 355 and His 90[17&18]. This side pocket is responsible for selectivity. Compounds **(5a-5d)** fitted into COX-2 active site, except for compound **5f**, all compounds showed binding with amino acids at the selectivity pocket (Fig 4). Docking scores extended from −6.855 to −4.235 kcal/mol. (Table 2). The docking score for the most potent compound **5d** within COX-2 active site is -5.264 Kcal/mol. forming hydrogen bond interaction with His 90 and Arg 513 *via* imidazole carbonyl and Pi-H interaction with Ser353 (Fig.5).

Table 2: Molecular docking details of target compounds with COX-2 (PBD:1CX2)

Code	Docking scores (Kcal/mol)	Amino acids interactions	Binding groups	Type of bond	Bond length (Å)
5a	-6.565	Arg120	Unsubstituted phenyl ring	Π-cataion	4.2
5b	-6.082	Tyr355 Ser353 Arg513	Carbonyl group Unsubstituted phenyl ring 4-methylphenyl ring	H-bond Pi-H Pi-Cation	2.70 4.33 3.70
5c	-4.235	His 90 Arg513	Carbonyl group Carbonyl group	H-bond H-bond	3.36 2.75
5d	-5.061	His90 Arg513 Ser353	Carbonyl group Carbonyl group OCH_3-phenylring	H-bond H-bond Pi-H bond	3.25 2.68 3.85
5e	-6.855	Tyr355 Ser353	Carbonyl group Unsubstituted phenyl ring	H-bond Pi-H	2.88 4.30
5f	-5.674	Glu524 Lys83	Cl-phenyl group Carbonyl group	H-bond H-bond	3.30 3.37
5g	-5.626	Tyr355	Carbonyl group	H-bond	2.82
5h	-6.553	His90	OCH_3	Pi-H	4.29

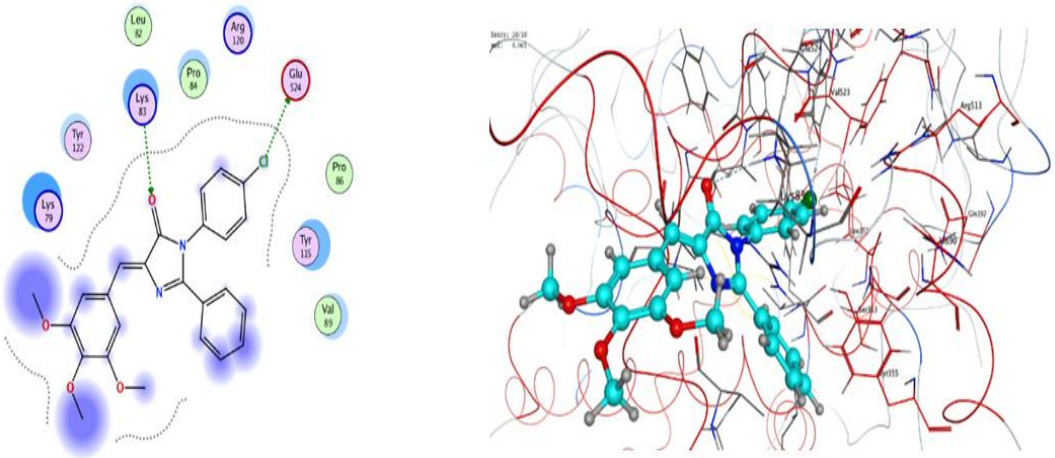


Fig. 4: Two/Three-dimensional (2D, 3D) Docking and binding pattern of compound **5f** (blue) into COX-2 active site (PBD: 1CX2).

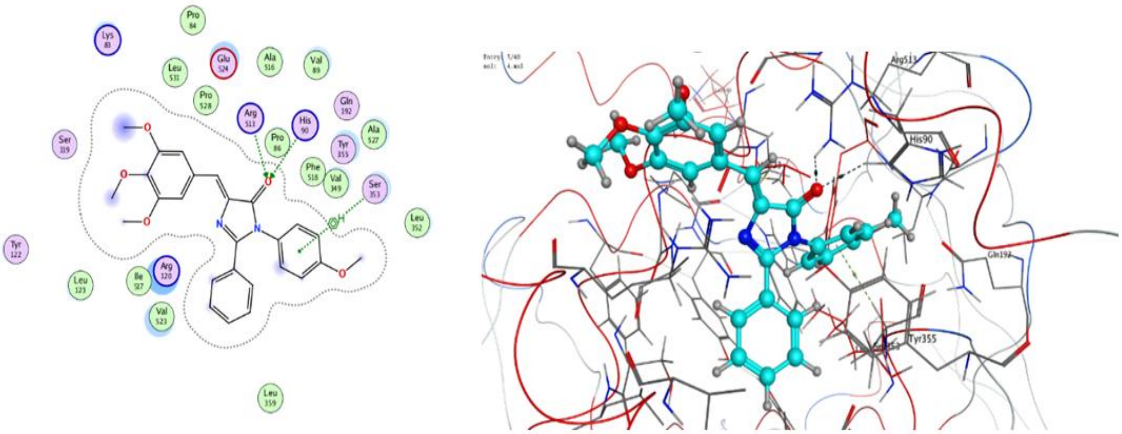


Fig. 5: Two/Three-dimensional (2D, 3D) Docking and binding pattern of compound **5d** (blue) into COX-2 active site (PBD: 1CX2).

In-Silico Study

Molinspiration Chemoinformatics server[19] was employed for virtual screening of the tested compounds to predict their bioactivity (Table 3). The software depends on determining a number of molecular properties describing hydrophobicity, hydrogen bonding, molecule size and flexibility. The obtained data suggested that all synthesized imidazole derivatives – except **5c** – did not violate Lipinski's rule, hence indicate clearly their relevant oral bioavailability. Additionally, the extrapolation of absorption, distribution, metabolism, excretion, and toxicity of the target compounds was carried out using Pre-ADMET software[20]. It applied Caco2 and MDCK cell-based studies to estimate HIA (the degree of human intestinal absorption). Furthermore, it estimated details on penetration of blood brain barrier and proportion of drug to plasma protein binding. It also gives information about the probability of drug-drug interaction based on their simulated inhibition to CYP2D6 enzyme that is elaborate in several medications metabolism (Table 4). The found parameters showed excellent human intestinal absorption (96.51 % – 97.81 %), low affinity to cross the blood brain barrier (BBB) (0.067 – 0.589) and very good plasma protein binding affinity (89.68 % - 92.16 %). all compounds were found to have no inhibitory effect on CYP2D6 enzyme, indicating minimal chance of medication interactions. Based on the *in-silico* simulation investigations it is revealed that imidazole derivatives **5a-h** have adequate pharmacokinetic parameters

Table 3: Molinspiration server data for synthesized compounds illustrating lipinisk's rules of fives.

Comp.	MLogp	M.WT	nON HBA	nOHNH HBD	Lipinsk's violation	TPSA	%ABS	Volume	nRotB
5a	4.18	414.46	6	0	0	62.60	87.403	375.10	0
5b	4.79	428.49	6	0	0	62.60	87.403	391.66	6
5c	5.43	442.51	6	0	1	62.60	87.403	408.22	6
5d	4.24	444.49	7	0	0	71.83	84.21	400.65	7
5e	4.35	432.45	6	0	0	62.60	87.403	380.03	6
5f	4.86	448.91	6	0	0	62.60	87.403	388.64	6
5g	3.70	430.46	7	1	0	82.83	80.42	383.12	6
5h	4.08	456.50	7	0	0	79.67	81.51	410	7
Celecoxib	2.97	٤٧٩.٩٥	9	4	0	127.83	64.90	393.69	7

Table 4: Pre-ADME programme results, demonstrating cell permeability, absorption and bioavailability:

Compound	Caco2	MDCK	HIA	BBB	PPB	CYP-2D6 inhibition
5a	54.8298	2.73836	97.415721	0.285023	89.936864	None
5b	54.8758	0.0807731	97.429806	0.208447	90.186639	None
5c	54.9149	0.0519867	97.455553	0.212252	90.373215	None
5d	55.0356	0.117364	97.566638	0.559412	89.678652	None
5e	54.684	0.123024	97.416340	0.589357	91.281107	None
5f	51.6273	0.729315	97.567968	0.40891	92.159973	None
5g	42.4907	0.101508	96.507428	0.06741	89.834744	None
5h	48.373	0.0508198	97.809805	0.52715	89.766918	None
Celecoxib	0.499443	45.0486	96.687062	0.0272635	91.077216	None

Conclusion

In summary, a series of diaryl imidazole derivatives **5a-h** was synthesized. The synthesized compounds were evaluated *in vitro* as COX-1/COX-2 inhibitors. Most of the tested compounds exhibited comparable COX-2 inhibitory activity to the reference indomethacin but with better selectivity. Compound **5d** was the most potent and exhibited high selective COX-2 inhibitory activity (IC_{50} = 0.060, SI = 175). Molecular modelling study explored affinity of synthesized compounds to fit into COX-2 active site. Moreover, all synthesized compounds exhibited promising physicochemical parameters and pharmacokinetics according to the *in-silico* simulation results.

EXPERIMENTAL

Chemistry

Stuart Melting Point apparatus was used to determine melting points (°C) of the obtained compound. ^{1}H-NMR spectra were recorded on AVANCE-III High Performance FT-NMR spectrum 400 MHz and ^{13}C-NMR spectra were carried on AVANCE-III High Performance FT-NMR spectrum (100 MHz) at ANAR Center, Zagazig University, Egypt where dimethyl sulfoxide DMS0-d_6 was used as a solvent..

2-Phenyl-4-(3,4,5-trimeth0xybenzylidene) 0xazol-5(4*H*)-0ne (3)

Bright yellow crystals (96 % yield). M.p 202 °C; Lit 203-204 [21]. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 8.10 (d, *J* = 7.4 Hz, 2 H, Ar-H), 7.74 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.71 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.64 (t, *J* = 7.4 Hz, 2 H, Ar-H), 7.30 (s, 1H, -CH=), 3.88 (s, 6H, 2 X 0CH_3), 3.77 (s, 3H, 0CH_3). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 166.9 (CO), 162.5 (C2 oxazolone), 152.9 (C3 & C5 trimethoxyphenyl), 140.5 (C4 trimethoxyphenyl), 133.6 (C4 oxazolone), 132.0 (Ar-CH), 131.0 (Ar-C), 129.4 (Ar-CH), 128.7 (Ar-CH), 127.8 (Ar-C) , 125.1 (-CH=), 109.9 (CH-2 & CH-6 trimethoxyphenyl), 60.3 (OCH_3), 55.9 (2 X OCH_3).

2,3-diphenyl-5-(3,4,5-trimeth0xybenzylidene) -3,5-dihydr0-4*H*-imidaz0l-4-0ne (5a)

Yellow crystals (96 % yield). M.p. 193 °C. IR (KBr, Cm^{-1}): 3097, 3059, 3001, 2960, 2905, 1712, 1574, 1123. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.86 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.54 (d, *J* = 7.4 Hz, 2 H, Ar-H), 7.51- 7.45 (m, ٤H, Ar-H), 7.40 (t, *J* = 7.4 Hz, 2 H, Ar-H), 7.31 (d, *J* = 7.4 Hz, 2 H, Ar-H), 7.25 (s, 1H, -CH=), 3.87 (s, 6H, 2 X 0CH_3), 3.77 (s, 3H, 0CH_3). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 166.7 (CO), 160.0 (C2 oxazolone), 152.8 (C3 & C5 trimethoxyphenyl), 139.9 (C4 trimethoxyphenyl), 137.6 (C4 oxazolone), 134.7(Ar-CH), 131.5 (Ar-C), 129.6 (Ar-CH), 129.4 (Ar-CH), 128.8 (Ar-CH), 128.5 (Ar-C), 127.9 (-CH=), 110.0 (CH-2 & CH-6 trimethoxyphenyl), 60.2 (0CH_3), 55.9 (2 X 0CH_3). Elemental analysis Calculated/Found for $C_{25}H_{22}N_2O_4$; C,72.45 /72.13 ; H,5.35 /5.16; N, 6.76/6.89.

2-Phenyl-3-(2-methylphenyl)-5-(3, 4, 5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5b)

Bright yellow crystals (86 % yield). M.p. 220 °C. IR (KBr, Cm^{-1}): 3070, 2972, 2937, 1704, 1574, 1362, 1175. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.86 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.52 (d, *J* = 7.4 Hz, 2 H, Ar-H), 7.48 (d, *J* = 7.4 Hz, 1H), 7.42 - 7.36 (m,

4H, Ar-H), 7.33 - 7.29 (m, 1H, Ar-H), 7.26-7.24 (m, 2H, Ar-H and -CH=), 3.87 (s, 6H, 2 X $0CH_3$), 3.77 (s, 3H, $0CH_3$), 2.09 (s, 3 H, CH_3). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 170.1 (CO), 160.2 (C2 oxazolone), 153.3 (C3 & C5 trimethoxyphenyl), 140.4 (C4 trimethoxyphenyl), 137.8 (Ar-C), 136.2 (Ar-C), 134.4 (C4 oxazolone), 132.2 (Ar-CH), 131.6 (Ar-C), 130.0 (Ar-CH), 129.8 (Ar-C), 129.4 (Ar-CH), 129.1 (Ar-CH), 128.6 (Ar-CH), 128.5 (Ar-CH), 128.3 (Ar-CH), 127.7 (=CH-), 110.5 (CH-2 & CH-6 trimethoxyphenyl), 60.7 ($0CH_3$), 56.4 (2 X $0CH_3$), 17.8 (CH_3). Elemental analysis Calculated/Found for $C_{26}H_{24}N_2O_4$; C, 72.88 /73.15; H, 5.65 /5.79; N, 6.54/6.73.

3-(2,5-Dimethylphenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5c)

Yellow crystals (78 % yield). M.p. 190 °C. IR (KBr, Cm^{-1}): 3094, 3001, 2937, 1710, 1573, 1155. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.85 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.55 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.50 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.4 (t, *J* = 7.6 Hz, 2H, Ar-H), 7.28 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.24 (s, 1H, =CH-), 7.22 (d, *J* = 7.8 Hz, 1H, Ar-H), 7.10 (s, 1H, Ar-H), 3.87 (s, 6H, 2 X $0CH_3$), 3.77 (s, 3H, $0CH_3$), 2.27 (s, 3H, CH_3), 2.00 (s, 3H, CH_3). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 169.7 (CO), 159.7 (C2 oxazolone), 152.8 (C3 & C5 trimethoxyphenyl), 139.9 (C4 trimethoxyphenyl), 137.4 (Ar-C), 136.6 (Ar-C), 133.7 (C4 oxazolone), 132.4 (Ar-CH), 131.7 (Ar-C), 130.9 (Ar-CH), 130.0 (Ar-C), 129.5 (Ar-CH), 129.1 (Ar-CH), 128.7 (Ar-CH), 128.6 (Ar-C), 128.1 (Ar-CH), 127.9 (=CH-), 110.0 (CH-2 & CH-6 trimethoxyphenyl), 60.2 ($0CH_3$), 55.9 (2 X $0CH_3$), 20.3 (CH_3), 16.9 (CH_3). Elemental analysis Calculated/Found for $C_{27}H_{26}N_2O_4$; C,73.28 /73.50 ; H,5.92 /6.11; N, 6.33/6.49.

3-(4-methoxyphenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5d).

Yellow crystals (89 % yield). M.p. 185 °C. IR (KBr, Cm^{-1}): 3009, 2972, 2938, 1714, 1574, 1252, 1122.^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.84 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.56 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.48 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.40 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.26-7.18 (m, 3H, 2 Ar-H and =CH-),), 7.02 (d, *J* = 8.7 Hz, 2H, Ar-H), 3.85 (s, 6H, 2 X $0CH_3$), 3.80 (s, 3H, $0CH_3$), 3.76 (s, 3H, $0CH_3$). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 170 (CO), 160.4 (C2 oxazolone), 159.2 (Ar C), 152.9 (C3 & C5 trimethoxyphenyl), 137.7 (C4 trimethoxyphenyl), 131.6 (C4 oxazolone), 129.8 (Ar-CH), 129.6 (Ar-C), 129.3 (Ar-CH), 128.9 (Ar-CH), 128.6 (Ar-C), 128.0 (Ar-CH), 127.8 (Ar-C), 127.3 (-CH=), 114.7 (Ar-CH), 110.1 (CH-2 & CH-6 trimethoxyphenyl), 60.4 ($0CH_3$), 56.0 (2 X $0CH_3$), 55.5 ($0CH_3$). Elemental analysis Calculated/Found for $C_{26}H_{24}N_2O_5$; C,70.26 /70.43 ; H,5.44 /5.61; N, 6.30/6.46. (TIC-MS) calculated for $C_{26}H_{24}N_2O_5$ 444.16 [M]+ Found: 444.10

3-(4-Flourophenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5e)

Yellow crystals (89 % yield). M.p. 197 °C. IR (KBr, Cm^{-1}): 3100, 2998, 2958, 1721, 1573, 1505, 1123. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.84 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.53 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.49 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.42 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.39-7.35 (m, 3H, Ar-H), 7.33-7.31 (m, 1H, Ar-H), 7.24 (s, 1H, =CH-), 3.85 (s, 6H, 2 X $0CH_3$), 3.76 (s, 3H, $0CH_3$). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 169.7 (CO), 162.8 (C2 oxazolone), 161.6 (d, *J*= 245.5 Hz, C-F), 152.8 (C3 & C5 trimethoxyphenyl), 139.9 (C4 trimethoxyphenyl), 137.5 (Ar-C), 131.5 (C4 oxazolone), 131.0 (Ar-CH), 130.1 (d, *J*= 8.9 Hz, Ar-CH), 129.6 (Ar-C), 129.4 (Ar-CH), 128.8 (Ar-CH), 128.5 (Ar-C), 127.9 (-CH=), 116.3 (d, *J*= 22.9 Hz, Ar-CH), 110.0 (CH-2 & CH-6 trimethoxyphenyl), 60.2 ($0CH_3$), 55.9 (2 X $0CH_3$). Elemental analysis Calculated/Found for $C_{25}H_{21}FN_2O_4$; C,69.44 /69.71 ; H,4.89 /4.95; N,6.48 /6.72.

3-(4-Chlorophenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5f)

Yellow crystals (74 % yield). M.p. 194 °C. IR (KBr, Cm^{-1}): 3090, 3068, 3056, 2991, 2960, 1720, 1573, 1284, 1121. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 7.84 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.56 - 7.52 (m, 4H, Ar-H), 7.50 (d, *J* = 7.4 Hz, 1H, Ar-H), 7.43 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.34 (d, *J* = 8.6 Hz, 2H, Ar-H), 7.25 (s, 1H, =CH-), 3.85 (s, 6H, 2 X $0CH_3$), 3.76 (s, 3H, $0CH_3$). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 169.9 (CO), 160.2 (C2 oxazolone), 153.3 (C3 & C5

trimethoxyphenyl), 140.5 (C4 trimethoxyphenyl), 137.9 (Ar-C), 134.0 (Ar-C), 133.4 (C4 oxazolone), 132.0 (Ar-CH), 130.1 (Ar-C), 130.0 (Ar-C), 129.8 (Ar-CH), 129.3 (Ar-CH), 129.1 (Ar-CH), 129.0 (Ar-CH), 128.5 (=CH-), 110.6 (CH-2 & CH-6 trimethoxyphenyl), 60.7 ($0CH_3$), 56.4 (2 X $0CH_3$). Elemental analysis Calculated/Found for $C_{25}H_{21}ClN_2O_4$; C,66.89 /67.16 ; H,4.72 /4.86; N,6.24 /6.42.

3-(4-hydroxyphenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5g)

Pale yellow crystals (83 % yield). M.p. 310 °C. IR (KBr, Cm^{-1}): 3103, 3007, 2981, 2966, 1678, 1572, 1321, 1161. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 9.81 (s, 1H, OH, exchangeable with D_2O), 7.83 (s, 2H, C2-H & C6-H trimethoxyphenyl), 7.57 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.49 (t, *J* = 7.4 Hz, 1H, Ar-H), 7.40 (t, *J* = 7.4 Hz, 2H, Ar-H), 7.20 (s, 1H, =CH-), 7.09 (d, *J* = 8.6 Hz, 2H, Ar-H), 6.83 (d, *J* = 8.6 Hz, 2H, Ar-H), 3.85 (s, 6H, 2 X $0CH_3$), 3.76 (s, 3H, $0CH_3$). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 170 (CO), 160.8 (C2 oxazolone), 160.6 (Ar C), 158.0 (Ar C), 153.3 (C3 & C5 trimethoxyphenyl), 140.3 (C4 trimethoxyphenyl), 138.1 (Ar-CH), 131.9 (C4 oxazolone), 130.2 (Ar-CH), 129.6 (Ar-C), 129.3 (Ar-CH), 128.9 (Ar-CH), 127.9 (Ar-C), 126.2 (-CH=), 116.4 (Ar-CH), 110.5 (CH-2 & CH-6 trimethoxyphenyl), 60.7 ($0CH_3$), 56.4 (2 X $0CH_3$). Elemental analysis Calculated/Found for $C_{25}H_{22}N_2O_5$; C,69.76 /69.94 ; H,5.15 /5.39; N,6.51/6.79. (TIC-MS) calculated for $C_{25}H_{22}N_2O_5$ 430.15 [M]+ Found: 430.64

3-(4-Acetylphenyl)-2-phenyl-5-(3,4,5-trimeth0xybenzylidene)-3,5-dihydr0-4*H*-imidaz0l-4-0ne (5h)

Pale yellow crystals (83 % yield). M.p. 243 °C. IR (KBr, Cm^{-1}): 3119, 2940, 1713, 1683, 1571, 1369, 1121. ^{1}H NMR (400 MHz, DMS0-d_6) δ ppm: 8.12 (d, *J* = 7.2 Hz, 1H, Ar-H), 8.05 (d, *J* = 7.2 Hz, 1H, Ar-H), 7.85 (s, 1H, C2-H & C6-H trimethoxyphenyl), 7.80 – 7.69 (m, 2H, Ar-H), 7.69 – 7.60 (m, 1H, Ar-H), 7.55 - 7.50 (m, 2H, Ar-H), 7.43 (d, *J* = 7.4 Hz, 2H, Ar-H), 7.32- 7.28 (m, 1H, =CH-), 3.87 (s, 6H, 2 X $0CH_3$), 3.77 (s, 3H, $0CH_3$), 2.51 (s, 3H, $CO\underline{CH_3}$). ^{13}C NMR (100 MHz, DMS0-d_6) δ ppm: 197.3 (CO), 169.2 (CO), 159.6 (C2 oxazolone), 152.8 (C3 & C5 trimethoxyphenyl), 140.0 (C4 trimethoxyphenyl), 138.6 (Ar-C), 137.4 (Ar-C), 136.2 (Ar-CH), 131.6 (C4 oxazolone), 129.5 (Ar-CH), 129.2 (Ar-C), 128.8 (Ar-CH), 128.6 (Ar-CH), 128.5 (Ar-CH), 128.3 (Ar-C), 127.8 (-CH=), 110.1 (CH-2 & CH-6 trimethoxyphenyl), 60.2 ($0CH_3$), 55.9 (2 X $0CH_3$), 26.8 (CH_3). Elemental analysis Calculated/Found for $C_{27}H_{24}N_2O_5$; C,71.04 /70.89 ; H,5.30 /5.47; N,6.14 /6.30.

Biological Evaluation

The *in vitro* COX-1 and COX-2 inhibition assay for the synthesized imidazolone derivatives was investigated utilising the Cayman colorimetric COX (ovine) inhibitor screening assay kit (Catalog No. 560131) according to the manufacturer's instructions[22].

Molecular Docking

Docking studies were carried out on T3600 workstation Dell precision with Intel Xeon® CPU-1650.0 @ 3.20 GHz using the computational Molecular Operating Environment software (MOE 2019.09; Chemical Computing Group, Canada).

COX-2 enzyme co-crystallized with ligand SC-558 was downloaded from protein data base (PDB ID: 1CX2)[16]. MOE quick preparation tool was utilized for structural preparation as wel as protonation of the downloaded co-crystallized enzyme. Water molecules and other chains were removed. Mdb file was created for the targeted compounds. The active site was chosen using atom selector at the toolbar. Alph Triangle placement methodology was used as docking parameter. London dG scoring function was used to calculate each pose free binding energy as the initial scoring methodology and GBVI/WSA dG as the final scoring methodology. The methodology described above was adopted to predict ligand-enzyme interactions at the active site.. The best docking pose for each compound was selected according to the scoring function, binding interactions with amino acids residues along with the preferred orientation at the active site showing best alignment with the co-crystallized ligand.

In-Silico Study

Both Molinspiration software[19] and PreADMET[20] were utilized to demonstrate the predicted drug-likeness and pharmacokinetics for the synthesized compounds.

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نشرة العلوم الصيدليـــــة
جامعة أسيوط

تشييد والتقييم البيولوجي والالتحام الجزيئي لمشتقات دياريليميدازول كعوامل محتملة جديدة مضادة للالتهابات تستهدف إنزيم COX-2

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تم تصميم وتشييد سلسلة من مشتقات diaryl imidazole(5a-h) كعقار انتقائي جديد لمضادات الالتهابCOX-2 ، وتاكدت هياكلها باستخدام التحليل الطيفي. تم تقييم النشاط المضاد للالتهابات المتوقع للمركبات المركبة بواسطة مقايسة تثبيط إنزيمات الأكسدة الحلقية في المختبر .(COX-1 / COX-2) أظهرت معظم المركبات المختبرة نشاطًا وانتقائية مثبطة لـــ COX-2 (IC50 = 0.068 - 0.80 ميكرومتر ، (SI = 7.5 - 175 مقارنة بالإندوميتاسين (IC50 = 0.079 ميكرومتر ، (SI = 12.5 وسيليكوكسيب (IC50 0.046 =ميكرومتر ، (SI = 315.22 و قد تم إجراء دراسة النمذجة الجزيئية لتوضيح وضع الارتباط للمركبات المصممة في الموقع النشط لإنزيم .COX-2 بالإضافة إلى ذلك ، اجريت دراسات المحاكاة لتشابه الدواء باستخدام الكمبيوتر للمركبات المصنعة.