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Design, synthesis and anti-inflammatory/analgesic evaluation of novel di-substituted urea derivatives bearing diaryl-1,2,4-triazole with dual COX-2/sEH inhibitory activities

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ABSTRACT

Herein, two novel series of diaryl-1,2,4-triazole hybrid to amide conjugates (**5a-e**) or urea conjugates (**10a-f**) have been synthesized followed by *in vitro* evaluation against cyclooxygenase-2/soluble epoxide hydrolase (COX-2/sEH) enzymes using ELISA enzyme assays. *In vivo* analgesic and anti-inflammatory activities for the new compounds have been carried out using the reported animal protocols. The preliminary results revealed that compounds **10e** and **10c** were the most active compounds against both COX-2/sEH enzymes (COX-2 IC_{50} = 1.98 μM and 2.13 μM; sEH = 1.09 and 1.23 nM, respectively). Moreover, the *in vivo* screening assays confirmed their superiority compared to the other derivatives by exhibiting higher anti-inflammatory and analgesic activity (91.27 and 89.32% edema inhibition; 55.97–50.00% writhing inhibition, respectively) than celecoxib (88.30% edema inhibition; 13.43% writhing inhibition). Collectively, compounds **10e** and **10c** can be considered as promising dual COX-2/sEH inhibitors with expected less cardiovascular adverse effects affording good anti-inflammatory and analgesic leads for further optimization.

1. Introduction

Inflammation is considered as the leading and permanent sign in several diseases, including atherosclerosis, auto-immune and several other infectious cases [1] as well as age-related diseases that were known as inflame-aging attributed tissue damage or degeneration [2]. The current used treatment protocols for the inflammatory conditions are based on the different mediators involved in the inflammation cascade such as vasoactive amines, arachidonic acid metabolites, cytokines, chemokines. Arachidonic acid is considered as the primary and important target in the inflammatory treatment due to its role as a foundation stone in the phospholipid membrane in all mammalian cells. Moreover, arachidonic acid acts as the precursor for the synthesis of numerous inflammatory mediators mainly eicosanoids through mainly three pathways: COX pathway, LOX pathway and CYP450 pathway [3,4]. Pharmaceutical researchers focused on those enzymes in the treatment ways of the inflammation. Consequently, a lot of drugs have been produced with diverse biological effect including celecoxib, rofecoxib and valdecoxib [5–7] as anti-inflammatory agents through COX pathway; Zileuton as the primary chosen drug against allergy [6–8] through LOX pathway and AUDA that regulate the inflammation in the atherosclerotic lesions by CYP^{450} pathway [9].

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most broadly consumed drugs for relief of inflammation and pain through non-selective inhibition of both the “housekeeping” COX-1 and the “inducible” COX-2 enzymes. Unfortunately, many adverse effects have been emerged including mainly gastro-intestinal toxicity bleeding and renal dysfunction accompanying with their administration [10]. Thus, subsequent attempts have been focused on the selective inhibition of COX-2 which lighten the research way to reserve COX-2-dependent therapeutic effects of NSAIDs with avoid-

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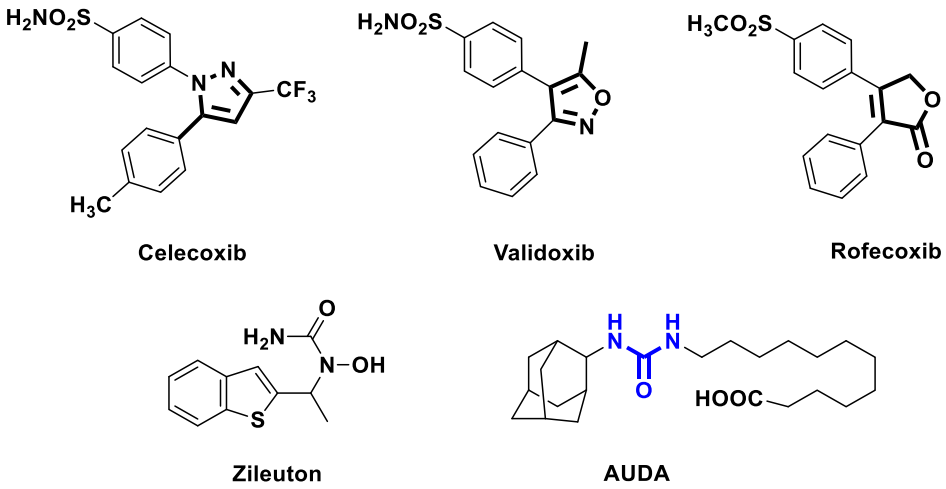


Fig. 1. Examples of some arachidonic acid pathways inhibitors.

ing COX-1-dependant GI adverse effects. This approach received a lot of plaudits among both researchers and patients until the explosion of their adverse effect bombshell; the cardiovascular toxicity. That cardiovascular toxicity was tantamount the storm that turned the scales upside down, which leads to the withdrawal of some coxibs from the market, *e.g.* rofecoxib in 2004 and valdecoxib in 2005 [11,12]. This defect was explained as a result of the imbalance between prostacyclin (antithrombotic): thromboxane (prothrombotic) ratio in the vascular wall which in turn promoted platelet aggregation and atherosclerosis [13–15].

Much efforts and research have been exerted to overcome those adverse effects by many trials, including the use of NO–NSAIDs and use the combination with a low dose of aspirin to decrease the biosynthesis of platelets [16,17]. However, enthusiasm for targeting the COX pathway was minimized by their adverse effects.

Until 1980, the flame of the challenge was lit once again by discovering the third arachidonic acid metabolite pathway CYP_{450} which released epoxyeicosatrienoic acid (EET) under the effect of epoxygenase enzyme CYP2J or CYP2C [18,19]. EET has many biological effects, including vasodilation, anti-inflammatory, vascular smooth muscle relaxation, platelets anti-aggregation, pro-angiogenic and protection of the cardiovascular disorders [20]. On the other hand, the hydrolase CYP4A enzyme generates 20-HETE that known for its vasoconstriction effect [21,22]. Therefore, EET has been considered as a key target for treating cardiovascular diseases including stroke and hypertension [23,24]. Unfortunately, EET level decreases under the effect of soluble epoxide hydrolase (sEH) due to their conversion into corresponding inactive dihydroxyeicosatrienoic acid (DHET) which in turn diminish their cardioprotective effects. Constantly, to preserve the EET level, the sEH enzyme should be inhibited.

According to the aforementioned studies, hybridization of both COX-2/sEH inhibitors benefits could a valid approach for developing novel drugs that preserve the anti-inflammatory activity of selective COX-2 without their cardiovascular side effects [25]. Consequently, the present work has been concerned with designing and synthesis of novel therapeutic agents with more efficacy and less toxicity (Fig. 1).

Therefore, a panel of dual COX/sEH inhibitors has been discovered as showed in **Fig. 2**. PTUPB, (4-(5-phenyl-3-{3-[3-(4-*tri*-fl*uoromethyl-phenyl*)-*ureido*]-propyl}-pyrazol-1-yl)-benzenesulfonamide, is one of the superior sEH/COX-2 dual inhibitors [26]. It is effective against many pathological conditions including inflammation [26], tumor growth/ metastasis in murine lung cancer [27], cardiovascular disorders [28], metabolic syndrome and type-2 diabetes [29]. Recently, our research team has reported, for the first time, PTPUP, 4-(1-phenyl-3-(3-(4-(trifluoromethyl)phenyl)ureido)−1H-pyrazol-5-yl)benzenesulfonamide as a potent dual COX-2/sEH inhibitor with good anti-inflammatory/analgesic activity attending with significant safe cardiovascular profile [30].

2. Rational design

Various diaryl 1,2,4-triazole derivatives were recently exhibited good selective COX-2 inhibitory activity [31–34]. For example lead compound I, 4-(1-(4-Chlorophenyl)-3-(methylthio)-1*H* -1,2,4-triazole-5-yl)benzenesulfonamide, has elucidated equal potency in selective inhibition of COX-2 (IC_{50} = 0.37 μM, SI = 0.018) referring to celecoxib [35]. Furthermore, recent studies reported that the presence of urea/amide linker, which linked to secondary different pharmacophore, have been considered as essential groups

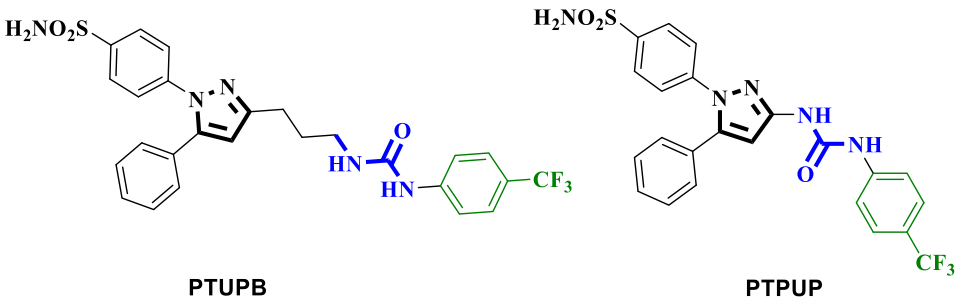


Fig. 2. Examples of some dual COX/sEH inhibitors.

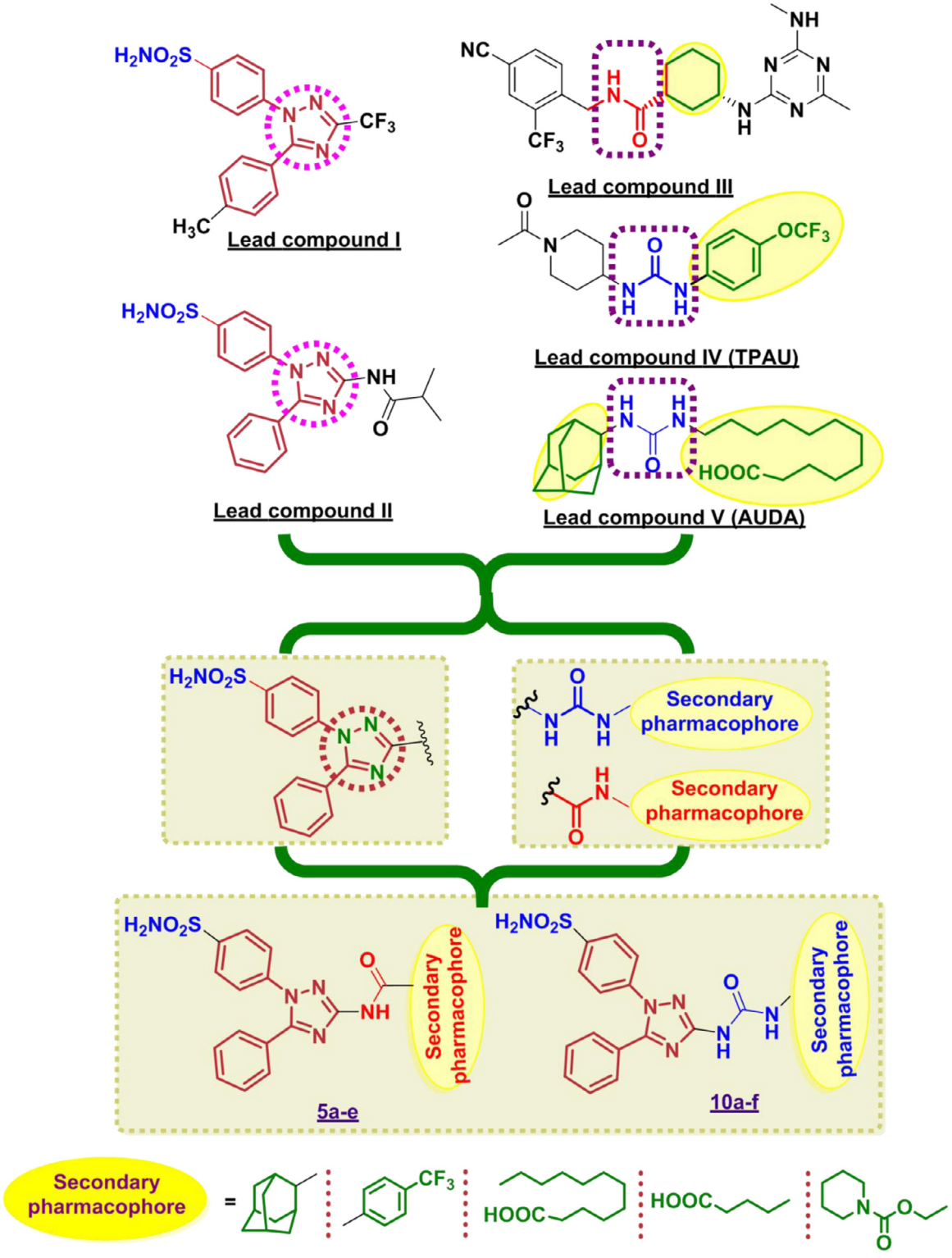


Fig. 3. Strategy for design of target dual COX-2/sEH inhibitor.

for sEH inhibition [36]. In the present work, the pyrazole nucleus was replaced with as 1,2,4-triazole bioisostere nucleus linked to urea/amide moieties. In doing so, a set of novel 1,5-diaryl-1,2,4-triazole derivatives linked to urea/amide moieties have been synthesized and estimated biologically as dual COX/sEH inhibitors as depicted in **Fig. 3**.

3. Materials and methods

3.1. Chemistry

All chemical reagents were of analytical grade and were consumed without further purifications. Drying of the solvents was

carried out as followed in the literature when necessary. Following up the reactions and ensuring the purity of the compounds have been checked using thin-layer chromatography (TLC). TLC analysis was proceeded using Macherey-Nagel Alugram Sil G/UV254 silica gel plats in which their eluting system is Hexane-Ethyl acetate (6:4). Determination of the melting points are carried out using IA 9100MK-Digital melting point apparatus where the obtained values are uncorrected. Elemental analyses for C, H, and N were achieved using Perkin- Elmer 2400 analyzer (Perkin-Elmer, Norwalk, CT) at the mycology and biotechnology regional center at Al Azhar University, Egypt. Infrared spectra (IR) were recorded using a Bruker FT-IR spectrophotometer Vector 22 in wavenumber (cm^{-1}) from KBr discs at the micro-analytical center, Faculty of Science, Cairo University. Chemical shifts of ^1H NMR and ^{13}C NMR spectra were recorded and J values were given in Hz using Bruker APX400 spectrometer at 400 MHz and 101 MHz, respectively in the DMSO–d_6 at the Faculty of Pharmacy, Beni-Suef University. Mass spectra were verified using Finnegan MAT, SSQ 7000, Mass spectrometer, at 70 eV (EI) at the micro-analytical center, Faculty of Science, Cairo University.

General procedure for the synthesis of compounds **(5a-e)**:

Compound **4** (3.44 g, 10 mmol) was dissolved in acetic acid (50 mL), followed by addition of the appropriate amine (10 mmol) and heated under reflux for 3 h in presence of anhydrous sodium acetate. The reaction mixture was poured onto ice water (50 mL) after cooling to room temperature affording precipitate which was filtered off. Recrystallized of the precipitate was carried out using ethanol after washing with water giving the pure desired compound **5**.

4-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazole-3-carboxamido)butanoic acid **(5a)**: Off-white solid (51%); m.p. 232–236 °C. IR (cm^{-1}): 3500–2800 (COOH), 3333 (NH), 2934 (CH aliphatic), 1710 (COOH), 1684 (CONH-), 1501 (*C = N*), 1325 and 1162 (SO_2NH_2).^1H NMR (400 MHz, DMSO–d_6): δ 10.92 (s, 1H, COO<u>H</u> exchangeable with D_2O), 8.79 (s, 1H, <u>NH</u> exchangeable with D_2O), 7.94–7.96 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.66–7.68 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.57 (m, 2H, Ar-<u>H</u>), 7.51–7.53 (m, 3H, Ar-<u>H</u>), 7.47–7.49 (d, J = 8 Hz, 2H, SO_2<u>NH</u>$_2$ exchangeable with D_2O), 2.27–2.31 (t, J = 8 Hz, 2H, -NHC<u>H</u>$_2$-), 1.91(m, 1H, -C<u>H</u>(H)COOH-), 1.78–1.81 (t, J = 8 Hz, 2H,-CH$_2$C<u>H</u>$_2$CH$_2$-), 1.24 (m, 1H,-C<u>H</u>(H)COOH-). ^{13}C NMR (101 MHz, DMSO–d_6): δ 174.71, 159.07, 157.20, 155.25, 145.10, 140.23, 131.14, 129.47, 129.25, 127.58, 126.69, 120.83, 31.58, 24.97, 21.51. MS (EI): *m/z* 429 (M^+). Anal. Calcd. For $C_{19}H_{19}N_5O_5S$: C, 53.14; H, 4.46; N, 16.31. Found: C, 53.50; H, 4.69; N, 16.52.

N-(adamantan-1-yl)−5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazole-3-carboxamide **(5b)**:

Off-white solid (53%); m.p. 263–267 °C. IR (cm^{-1}): 3320 (NH), 3238 (CH aromatic), 2921 (CH aliphatic), 1663 (CONH), 1501 (*C = N*), 1327, 1159 (SO_2NH_2). ^1H NMR (400 MHz, DMSO–d_6): δ 8.33 (s, 1H, <u>NH</u> exchangeable with D_2O), 7.94–7.96 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.67–7.52 (m, 3H, Ar-<u>H</u>), 7.44–7.49 (m, 6H, 4H of Ar-<u>H</u> and 2H of SO_2<u>NH</u>$_2$ exchangeable with D_2O), 2.10–1.99 (m, 6H, -NHCC<u>H</u>$_2$-), 1.75–1.91 (m, 3H, -C<u>H</u>(CH$_2$)$_2$-), 1.60–1.67 (m, 6H, -CHC<u>H</u>$_2$CH-). ^{13}C NMR (101 MHz, DMSO–d_6): δ 164.93, 158.05, 154.28, 152.37, 144.69, 140.59, 133.96, 130.81, 129.27, 127.48, 126.36, 52.10, 36.36, 29.29, 24.21. MS (EI): *m/z* 477 (M^+). Anal. Calcd. For $C_{25}H_{27}N_5O_3S$: C, 62.87; H, 5.70; N, 14.66. Found: C, 62.71; H, 5.86; N, 14.85.

Ethyl 1-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazole-3-carbonyl)piperidine-4-carboxylate **(5c)**: White solid (57%); m.p. 144–146 °C. IR (cm^{-1}): 3442 (NH), 3076 (CH aromatic), 2972 (CH aliphatic), 1729 (*C = O*), 1494 (*C = N*), 1350, 1166 (SO_2NH_2). ^1H NMR (400 MHz, DMSO–d_6): δ 7.95–7.97 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.67–7.69 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.57 (m, 2H, Ar-<u>H</u>), 7.49–7.51 (m, 5H, 3H of Ar-<u>H</u> and 2H of SO_2<u>NH</u>$_2$ exchangeable with D_2O), 4.38–4.41 (m, 1H, -NC<u>H</u>(H)CH$_2$-), 4.09–4.15 (m, 3H, 2H of –C<u>H</u>$_2$CH$_3$ and 1H of -NCH(<u>H</u>)CH$_2$-), 3.31 (m, 1H, -NC<u>H</u>(H)CH$_2$-), 3.02–3.08 (m, 1H, -NC<u>H</u>(H)CH$_2$), 2.69–2.75 (m, 1H, -C<u>H</u>COO-), 1.89–1.99 (m, 2H, -CHC<u>H</u>(H)-), 1.55–1.61 (m, 2H, -CHCH(<u>H</u>)-), 1.19–1.21 (m, 3H, –CH$_2$C<u>H</u>$_3$). ^{13}C NMR (101 MHz, DMSO–d_6): δ 174.22, 160.35, 157.34, 154.60, 145.03, 140.21, 131.11, 129.44, 129.29, 127.35, 127.50, 126.63, 60.53, 46.20, 29.08, 28.10, 14.53. MS (EI): *m/z* 483 (M^+). Anal. Calcd. For $C_{23}H_{25}N_5O_5S$: C, 57.13; H, 5.21; N, 14.48. Found: C, 57.50; H, 5.38; N, 14.72.

5-phenyl-1-(4-sulfamoylphenyl)-N-(4-(trifluoromethyl)phenyl)−1H-1,2,4-triazole-3-carboxamide **(5d)**: Brown solid (63%); m.p. 277–279 °C. IR (cm^{-1}): 3325 (NH), 3112 (CH aromatic), 1701 (CONH), 1530 (*C = N*), 1331, 1166 (SO_2NH_2). ^1H NMR (400 MHz, DMSO–d_6): δ 10.97 (s, 1H, <u>NH</u> exchangeable with D_2O), 8.13–8.15 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.98–8.00 (d, J = 8 Hz, 3H, Ar-<u>H</u>), 7.67 (m, 4H, Ar-<u>H</u>)), 7.54–7.60 (m, 4H, Ar-<u>H</u>), 7.49–7.51 (d, J = 8 Hz, 2H, SO_2NH_2 exchangeable with D_2O). ^{13}C NMR (101 MHz, DMSO–d_6): δ 158.01, 156.81, 155.70, 145.32, 142.33, 140.12, 131.29, 129.58, 129.29, 127.52, 127.15, 126.84, 126.42, 126.13, 123.43, 121.09. MS (EI): *m/z* 487 (M^+). Anal. Calcd. For $C_{22}H_{16}F_3N_5O_3S$: C, 54.21; H, 3.31; N, 14.37. Found: C, 54.43; H, 3.59; N, 14.50.

11-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazole-3-carboxamido)undecanoic acid **(5e)**: White solid (63%); m.p. 244–246 °C. IR (cm^{-1}): 3400–2500 (CO<u>OH</u>), 3036 (CH aromatic), 2922 (CH aliphatic), 1723 (<u>CO</u>OH), 1560 (*C = N*), 1322, 1164 (SO_2NH_2). ^1H NMR (400 MHz, DMSO–d_6): δ 12.02 (s, 1H, CO<u>OH</u>), 8.69 (s, 1H, <u>NH</u> exchangeable with D_2O), 7.94–7.96 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.66–7.68 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.51–7.65 (m, 7H, 5H of Ar-<u>H</u> and 2H of SO_2<u>NH</u>$_2$ exchangeable with D_2O), 3.29 (m, 2H, -NHC<u>H</u>$_2$-), 2.19 (m, 2H, -C<u>H</u>$_2$COOH-), 1.50 (m, 4H, 2H of -CH$_2$C<u>H</u>$_2$CH$_2$COOH and 2H of –NHCH$_2$C<u>H</u>$_2$CH$_2$-), 1.26 (m, 12H, -CH$_2$C<u>H</u>$_2$CH$_2$-). ^{13}C NMR (101 MHz, DMSO–d_6): δ 174.99, 158.85, 157.32, 155.19, 145.11, 140.23, 131.12, 129.47, 127.24, 127.45, 127.38, 126.66, 34.13, 29.53, 29.43, 29.36, 29.22, 29.02, 26.88, 24.97. MS (EI): *m/z* 527 (M^+). Anal. Calcd. For $C_{26}H_{33}N_5O_5S$: C, 59.18; H, 6.30; N, 13.27. Found: C, 59.46; H, 6.57; N, 13.56.

5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazole-3-carbonyl azide **(8)**: A cold solution of hydrazide intermediate **7** (0.358 g, 1.0 mmol) was prepared by dissolving in mixture of acetic acid (6 mL): 1 N HCl (3 ml): water (25 mL), followed by addition of a solution of $NaNO_2$ (0.87 g, 1.0 mmol) in 3 mL cold water. During stirring, a yellow syrup was formed, extracted with ethyl acetated, washed with cold 3% $NaHCO_3$, H_2O. The extract was dried over Na_2SO_4 followed by the next procedure without purification.

General procedure for synthesis of compounds **(10a-f)**:

The acyl azide **8** (0.369 g, 1.0 mmol) was heated for 1 hour in dry toluene affording the isocyanate **9.** The reaction mixture was allowed to be cooled, where the isocyanate intermediate **9** was dissolved in anhydrous pyridine then the appropriate amine (0.01 mmol) was added. The mixture was undergone to reflux for 24 h followed by pouring onto ice/H_2O containing drops of concentrated HCl. Extraction of the compound was carried out using ethyl acetate followed by drying over anhydrous $MgSO_4$. The purification was performed using column chromatography which elutes the final compounds **10a-f** using petroleum ether:ethyl acetate gradient.

4-(3-(3-cyclohexylureido)−5-phenyl-1H-1,2,4-triazol-1-yl)benzenesulfonamide **(10a)**: White solid (54%); m.p. 250–252 °C. IR (cm^{-1}): 3361 (NH), 3159 (CH aromatic), 2928 (CH aliphatic), 1669 (NHCONH), 1563 (*C = N*), 1322, 1160 (SO_2NH_2).^1H NMR (400 MHz, DMSO–d_6): δ 7.82–7.84 (d, J = 8 Hz, 2H, Ar-<u>H</u>), 7.72 (m, 2H, Ar-<u>H</u>), 7.47 (m, 3H, Ar-<u>H</u>), 7.38–7.40 (m, 4H, 2H of Ar-<u>H</u> and 2H of SO_2<u>NH</u>$_2$ exchangeable with D_2O), 5.74 (s, 1H, NH exchangeable with D_2O), 4.14 (s, 1H, NH exchangeable with D_2O), 2.98 (m, 1H, -NHC<u>H</u>CH$_2$-), 1.55–1.59 (m, 4H, -NHCHC<u>H</u>$_2$CH$_2$-), 1.29 (m, 2H, -(CH$_2$)$_2$C<u>H</u>$_2$(CH$_2$)$_2$-), 1.12–1.14 (m, 2H, -CHCH$_2$C<u>H</u>(H)CH$_2$-), 0.87–0.88 (m, 2H,-CHCH$_2$CH(<u>H</u>)CH$_2$-).^{13}C NMR (101 MHz, DMSO–d_6):

δ 164.21(-NHC(N)$_2$-of triazol), 152.97 (-NHCONH-), 141.65 (-CHC(N)$_2$-of triazol), 141.15, 132.08, 130.52, 129.09, 128.99, 128.39, 127.92, 125.77, 52.64 (-NHCHCH$_2$-), 33.59 (-CHCH$_2$CH$_2$-), 24.80 (-(CH$_2$)$_2$CH$_2$(CH$_2$)$_2$-), 22.99 (-CHCH$_2$CH$_2$CH$_2$-). MS (EI): *m/z* 440 (M^+). Anal. Calcd. For $C_{21}H_{24}N_6O_3S$: C, 57.26; H, 5.49; N, 19.08. Found: C, 57.43; H, 5.68; N, 18.97.

4-(3-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazol-3-yl)ureido)butanoic acid **(10b)**: White solid (51%); m.p. 210–212 °C. IR (cm^{-1}): 3403–2800 (COOH), 2929 (CH aliphatic), 1721 (COOH), 1658 (NHCONH), 1551 (*C = N*), 1330, 1161 (SO_2NH_2). ^{1}H NMR (400 MHz, DMSO–d_6): δ 7.81–7.94 (m, 4H, Ar-H), 7.47–7.49 (m, 3H, Ar-H), 7.42–7.45 (m, 5H, 2H of Ar-H, 2H of SO_2NH_2 exchangeable with D_2O and 1H of NH exchangeable with D_2O), 5.78 (s, 1H, NH exchangeable with D_2O), 3.21 (m, 2H, -NHCH$_2$CH$_2$-), 1.97–2.02 (m, 2H, -CH$_2$CH$_2$COOH), 1.19–1.24 (m, 2H, -CH$_2$CH$_2$CH$_2$-). ^{13}C NMR (101 MHz, DMSO–d_6): δ 172.6, 159.1, 157.2, 155.3, 145.1, 140.2, 131.1, 129.5, 129.3, 127.6, 126.7, 120.8, 31.6, 24.9, 21.5. MS (EI): *m/z* 444 (M^+). Anal. Calcd. For $C_{19}H_{20}N_6O_5S$: C, 51.34; H, 4.54; N, 18.91. Found: C, 51.60; H, 4.63; N, 19.12.

4-(3-(3-(adamantan-1-yl)ureido)−5-phenyl-1H-1,2,4-triazol-1-yl)benzenesulfonamide **(10c)**:

White solid (53%); m.p. 176–177 °C. IR (cm^{-1}): 3396 (NH), 3067 (CH aromatic), 2911 (CH aliphatic), 1671 (NHCONH), 1548 (*C = N*), 1323, 1165 (SO_2NH_2). ^{1}H NMR (400 MHz, DMSO–d_6): δ 9.53 (s, 1H, NH exchangeable with D_2O), 7.90–7.88 (d, *J* = 8 Hz, 2H, Ar-H), 7.78 (s, 1H, NH exchangeable with D_2O), 7.71–7.73 (m, 2H, Ar-H), 7.67–7.70 (m, 2H, Ar-H), 7.54–7.58 (m, 3H, Ar-H), 7.45–7.47 (d, *J* = 8 Hz, 2H of SO_2NH_2 exchangeable with D_2O), 1.99–2.05 (m, 6H, -NHCCH$_2$), 1.65 (m, 3H, -CH$_2$CHCH$_2$-), 1.28 (m, 6H, -CHCH$_2$CH). ^{13}C NMR (101 MHz, DMSO–d_6): δ 164.93, 157.57, 154.28, 152.37, 145.12, 140.19, 133.96, 130.81, 129.27, 127.48, 126.36, 52.10, 36.51, 29.29, 24.21. MS (EI): *m/z* 492 (M^+). Anal. Calcd. For $C_{25}H_{28}N_6O_3S$: C, 60.96; H, 5.73; N, 17.06. Found: C, 60.89; H, 5.86; N, 17.34.

Ethyl-1-((5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazol-3-yl)carbamoyl)piperidine-4-carboxylate **(10d)**: Off-white solid (57%); m.p. 184–187 °C. IR (cm^{-1}): 3376 (NH), 3053 (CH aromatic), 2939 (CH aliphatic), 1726 (COOCH$_2$CH$_3$), 1647 (NHCON), 1552 (*C = N*) 1307, 1170 (SO_2NH_2). ^{1}H NMR (400 MHz, DMSO–d_6): 9.46 (s, 1H, NH exchangeable with D_2O), 7.83–7.85 (d, *J* = 8 Hz, 2H, Ar-H), 7.61–7.63 (d, *J* = 8 Hz, 2H, Ar-H), 7.42–7.49 (m, 7 H, 5H of Ar-H and 2H of SO_2NH_2 exchangeable with D_2O), 4.01–4.09 (m, 4H, 2H of -NCH(H)CH$_2$- and 2H of –CH$_2$CH$_3$), 3.52–3.54 (m, 1H, -NCH(H)CH$_2$-), 2.91–2.97 (m, 1H, –NCH(H)CH$_2$-), 2.57–2.61 (m, 1H, -CH$_2$CHCOO-), 1.82–1.90 (m, 2H, -CH$_2$CHCOO-), 1.45–1.58 (m, 2H, -CH$_2$CHCOO-), 1.18–1.21(m, 3H, –CH$_2$CH$_3$). ^{13}C NMR (101 MHz, DMSO–d_6): δ 174.22, 160.35, 157.34, 154.60, 145.03, 140.21, 131.11, 129.44, 129.29, 127.50, 127.35, 126.63, 60.53, 46.20, 29.08, 28.10, 14.53. MS (EI): *m/z* 498 (M^+). Anal. Calcd. For $C_{23}H_{26}N_6O_5S$: C, 56.24; H, 5.51; N, 16.40. Found: C, 56.49; H, 5.39; N, 16.72.

N-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazol-3-yl)−2-(4(trifluoromethyl)phenyl)acetamide **(10e)**: Brown solid (63%); m.p. 257–259 °C. IR (cm^{-1}): 3395 (NH), 3186 (CH aromatic), 1698 (NHCONH), 1557 (*C = N*), 1324, 1170 (SO_2NH_2). ^{1}H NMR (400 MHz, DMSO–d_6): δ 10.31 (s, 1H, NH exchangeable with D_2O), 10.25 (s, 1H, NH exchangeable with D_2O), 7.73–7.75 (d, *J* = 8, 2H, Ar-H), 7.67–7.71 (m, 5H, Ar-H), 7.46–7.52 (m, 6H, Ar-H), 7.40–7.42 (d, *J* = 8 Hz, 2H, SO_2NH_2 exchangeable with D_2O). ^{13}C NMR (101 MHz, DMSO–d_6): δ 158.01, 156.81, 155.70, 145.32, 142.33, 140.12, 131.29, 129.58, 129.29, 127.52, 127.15, 126.84, 126.42, 126.13, 123.43, 121.09. MS (EI): *m/z* 503 (M^{+1}). Anal. Calcd. For $C_{22}H_{17}F_3N_6O_3S$: C, 52.59; H, 3.41; N, 16.73. Found: C, 52.73; H, 3.68; N, 16.98.

11-(3-(5-phenyl-1-(4-sulfamoylphenyl)−1H-1,2,4-triazol-3-yl)ureido)undecanoic acid **(10f)**:

White solid (63%); m.p. 198–202 °C. IR (cm^{-1}): 3393–2500 (COOH), 3035 (CH aromatic), 2925 (CH aliphatic), 1725 (COO), 1645 (NHCONH), 1549 (*C = N*), 1326, 1161 (SO_2NH_2). ^{1}H NMR (400 MHz, DMSO–d_6): δ 9.75 (s, 1H, NH exchangeable with D_2O), 9.70 (s, 1H, NH exchangeable with D_2O), 7.88–7.86 (m, 2H, Ar-H), 7.68–7.81(m, 2H, Ar-H), 7.75 (m, 1H, Ar-H), 7.46–7.56 (m, 4H, Ar-H), 7.34–7.36 (d, *J* = 8 Hz, 2H, SO_2NH_2 exchangeable with D_2O), 2.74–2.99 (m, 2H, -NHCH$_2$-), 2.02–2.17 (m, 2H, -CH$_2$COOH-), 1.46 (m, 4H, 2H of -CH$_2$CH$_2$CH$_2$COOH and 2H of –NHCH$_2$CH$_2$CH$_2$-), 1.23 (m, 12H, 6*-CH$_2$CH$_2$CH$_2$-). ^{13}C NMR (101 MHz, DMSO–d_6): δ 174.94, 158.83, 157.32, 155.17, 145.14, 140.28, 129.49, 131.14, 129.27, 127.40, 127.31, 126.62, 34.10, 29.57, 29.43, 29.37, 29.29, 29.05, 26.83, 24.98. MS (EI): *m/z* 542 (M^+). Anal. Calcd. For $C_{26}H_{34}N_6O_5S$: C, 57.55; H, 6.32; N, 15.49. Found: C, 57.79; H, 6.45; N, 15.70.

Table 1
The *in vitro* COX-1 and COX-2 inhibitory activity of the new diaryl-1,2,4-triazole-based derivatives **5a-e** and **10a-f**, *versus* celecoxib as a reference.

Compound	COX Inhibition (IC_{50} μM)[a]		Selectivity Index[b]	sEH inhibition (IC_{50} nM)[c]
	COX-1	COX-2		
5a	12.98	4.38	2.96	3.12±0.09
5b	9.15	2.34	3.91	2.23±0.04
5c	10.33	4.45	2.32	3.45±0.016
5d	11.58	4.02	2.88	1.95±0.011
5e	13.02	5.03	2.58	4.02±0.05
10a	10.23	3.05	3.35	1.78±0.012
10b	11.23	3.69	3.02	3.21±0.031
10c	8.85	2.13	4.15	1.23±0.07
10d	11.89	4.35	2.73	2.34±0.05
10e	9.15	1.98	4.62	1.09±0.09
10f	9.12	3.13	2.91	2.98±0.013
Celecoxib	6.12	0.95	6.44	261.14±15.1
AUDA	–-	–-	–-	0.49±0.009

[a] The IC_{50} values for the test compound denote the concentration of the drug that elicits 50% inhibition of COX-1 and COX-2 enzymes. The data were generated with the aid of specific COX-1 and COX-2 enzyme activity assay (Cayman Chemicals Inc., Ann Arbor, MI, USA). The table outlines the mean IC^{50} values for COX-1 and COX-2 where the deviation from the mean is < 10% of the mean value.

[b] The selectivity index for each tested compound was determined by the ratio of COX-1 IC_{50}/COX-2 IC_{50}.

[c] The IC_{50} values for the test compound denote the concentration of the drug that elicits 50% inhibition of sEH enzyme.

3.2. Biological evaluation

3.2.1. In vitro *COX-1/COX-2 inhibitory activities*

The *in vitro* inhibitory activity of the new compounds against both COX subtypes was tested using enzyme immunoassay (EIA) kits from Cayman Chemical Company (Cat. no.701070 and 701080, Cayman Chemical, Ann Arbor, MI). The assay principle is to reduce COX-derived PGH_2 into $PGF_{2\alpha}$ via enzyme immune-sorbent assay (ELISA) followed by measuring the $PGF_{2\alpha}$ concentration spectrophotometrically at 412 nm [37,38]. The COX-1/COX-2 inhibitory activity and IC_{50} of the tested compounds were determined, compared with the various control incubations and written down in **Table 1**.

3.2.2. In vitro *sEH inhibitory activity*

The IC_{50} of the novel compounds against sEH enzyme was determined using soluble epoxide hydrolase cell-based assay kit (Cat. no. 600090, Cayman Chemical, Ann Arbor, MI) through the fluorescence-based method. The fluorescent intensity of 6–methoxy-2-Naphthaldehyde (highly fluorescent product at λ_{ex} = 330 nm, λ_{em} = 465 nm) that produced from the hydrolysis of the sEH-substrate Epoxy Fluor 7 [39] was revealed for the tested compounds wells, compared with the control wells and recorded as shown in **Table 1**.

Table 2
The *in vivo* anti-inflammatory of the new diaryl-1,2,4-triazole-based derivatives **5a-e** and **10a-f**, in carrageenan paw edema model.

Compound	Change in paw volume in (ml) after drug digestion (±SEM)[a]			Anti-inflammatory activity (% inhibition)[b]		
	1h	3h	5h	1h	3h	5h
5a	10.68±0.08	7.92±0.15	6.84±0.05	34.72	53.41	64.89
5b	6.64±0.44	4.81±0.36	3.79±0.40	58.80	71.71	80.54
5c	8.84±0.08	7.16±0.15	7.28±0.20	45.97	57.88	62.63
5d	8.84±0.16	4.92±0.20	3.58±0.41	45.97	71.06	81.62
5e	9.87±0.42	6.40±0.14	5.87±0.14	39.67	62.35	69.87
10a	2.97±0.33	2.28±0.27	2.35±0.38	81.85	86.59	87.94
10b	9.30±0.65	5.44±0.72	5.01±0.47	43.15	68.00	74.28
10c	5.87±0.25	3.99±0.27	2.08±0.21	64.12	76.53	89.32
10d	7.92±0.22	6.40±0.44	5.44±0.30	51.59	62.35	72.07
10e	3.22±0.29	3.17±0.31	1.70±0.27	80.32	81.35	91.27
10f	7.28±0.20	4.40±0.27	3.88±0.22	55.50	74.12	80.08
Celecoxib	3.08±0.15	2.94±0.08	2.28±0.14	81.17	82.71	88.30
Control	16.36±0.24	17.00±0.28	19.48±0.26	–	–	–

[a] Values are mean ± SEM (Standard error of mean).
[b] % edema inhibition= (Tc-Tt/Tc) × 100 where Tc is the mean increase of paw diameter thickness in the control group. Tt denotes the mean increase of paw diameter thickness in response to the tested compound in rats.

Table 3
The *in vivo* analgesic activity of the new diaryl-1,2,4-triazole-based derivatives **5a-e** and **10a-f**, in acetic acid-induced writhing test.

Compound	No. of Writhes in 5–15 min after treatment (Mean ± SE)[a]	% Inhibition
5a	21.50±0.5	35.82
5b	24.25±0.6	27.61
5c	24.75±0.7	26.11
5d	21.00±0.6	37.31
5e	26.00±0.6	22.38
10a	19.75±0.4	41.04
10b	25.25±0.7	24.62
10c	16.75±0.5	50.00
10d	24.50±0.7	26.86
10e	14.75±0.5	55.97
10f	23.00±0.4	31.34
Celecoxib	29.00±0.6	13.43
Control	33.50±0.8	–

[b]% Analgesic activity = $\frac{(Wc - Wt)\ x\ 100}{Wc}$ where Wc is mean writhing of the control group, Wt is mean writhing of the tested group.
[a] Values are mean ± SE (Standard error).

3.2.3. Determining the anti-inflammatory activity using carrageenan-induced paw edema assay

To examine the anti-inflammatory potential of the tested compounds, the carrageenan-induced paw edema model was used, as established by Winter et al. [40]. The current study employed male Albino Wister rats (120–150 g body weight) that were procured from the animal house, Nahda University, Beni-Suef. The rats were housed in stainless steel cages (4 animals per cage) and they were fasted for 24 h with *ad libitum* access to the drinking water before the start of the experiment. All animal handling was carried out in strict accordance with the protocol approved by the Institutional Research Ethical Committee of Faculty of Pharmacy, Beni-Suef University. The experimental animals received the test compounds **10a-f** and celecoxib (dissolved in 10% DMSO aqueous solution v/v) at the dose of 50 mg/kg body weight. Additionally, the control group received the 10% DMSO aqueous solution (v/v) vehicle. The paw edema was induced by the injection of 100 µL of 1% carrageenan-sodium gel (Sigma-Aldrich, USA) into the sub-planter region of the right hind paw of rats. The paw edema was monitored by measuring the rat's hind paw thickness with the aid of Vernier calliper (SMIEC) immediately after carrageenan injection and 1, 3, and 5 h post the inflammagen injection. The decrease of paw edema was regarded as an index for the anti-inflammatory activity. **Table 2** depicts the% edema inhibition data.

3.2.4. Determining the analgesic activity using acetic acid-induced writhing test

Four mice in each group were used for performing the acetic-acid induced writhing test [41]. The tested compounds and celecoxib were orally administered by gavage (10 mg/kg) to animals, whereas the control group received the vehicle only. One hour later, 0.01 mL/g of 0.6%v/v acetic acid solution was injected intraperitoneally to trigger the pain. 1 hour later. The animals were observed after 5 min of pain induction and for 20 min to record and count the writhing episodes. The analgesic activity was determined through the calculation of the% analgesic activity that were outlined as shown in **Table 3**.

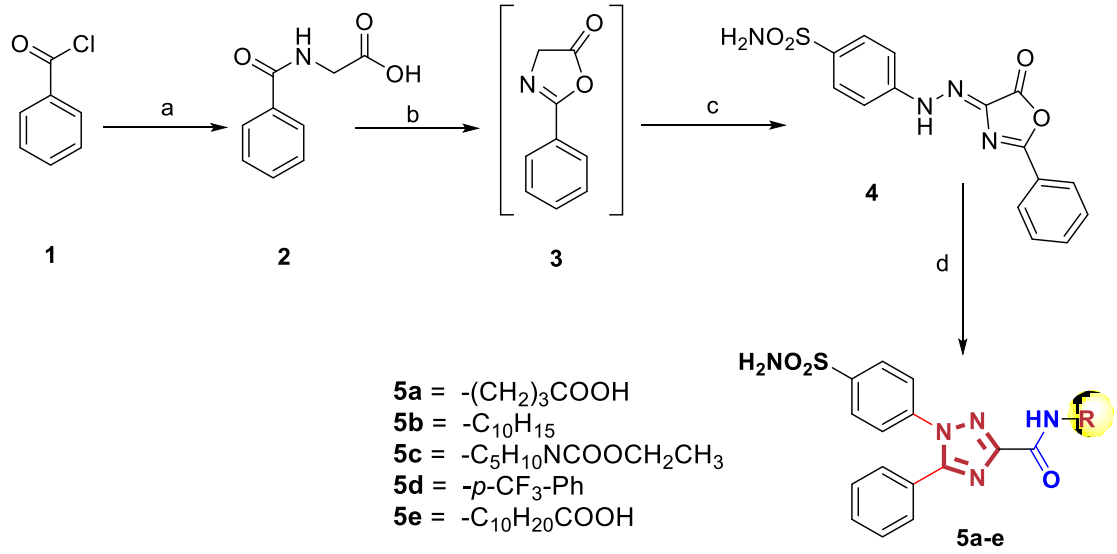
3.3. Physicochemical parameters

The physicochemical parameters of newly synthesized compounds were determined using the Computational prediction approach. Molinspiration online property calculation toolkit was used to calculate the number of rotatable bonds and molecular polar surface area. To express the degree of absorption, the absorption

Table 4
Lipinski parameters and calculated absorption of **5a-e** and **10a-f** compounds.

Compound	%ABS[a]	tPSA[b]	Nrotb[c]	nON ≤10[d]	nOHNH ≤5[e]	miLog*P* ≤5	MW ≤500	n violations ≤1
5a	54.73	157.28	8	10	4	0.63	429.46	1
5b	67.60	119.98	5	8	3	3.77	477.59	0
5c	61.56	137.50	7	10	2	2.10	483.55	0
5d	67.60	119.98	6	8	3	3.44	487.46	0
5e	54.73	157.28	15	10	4	4.17	527.65	2
10a	63.45	132.01	5	9	4	3.28	440.53	0
10b	50.58	169.31	8	11	5	1.16	444.47	2
10c	63.45	132.01	5	9	4	4.30	492.61	0
10d	57.41	149.52	7	11	3	2.63	498.56	2
10e	63.45	132.01	6	9	4	3.96	502.48	1
10f	50.58	169.31	15	11	5	4.70	542.66	4

[a] % ABS denotes the calculated absorption for each compound;.
[b] tPSA denotes the topological polar surface area for each compound;.
[c] nrotb denotes the number of rotatable bonds;.
[d] nON≤10 denotes the number of hydrogen bond acceptors that are equal to or less than ten;.
[e] nOHNH≤5 denotes the number of hydrogen bond donors that are equal to or less than five.



Scheme 1. The synthesis of compounds 5a-e. Reagents and conditions: a) Glycine, NaOH, rt; b) Ac_2O, reflux, 40 min; c) Freshly prepared solution of diazonium salt of the sulfanilamide, NaOAc, rt; d) NaOAc, Acetic acid, reflux at 89 °C (yield: 51–63%).

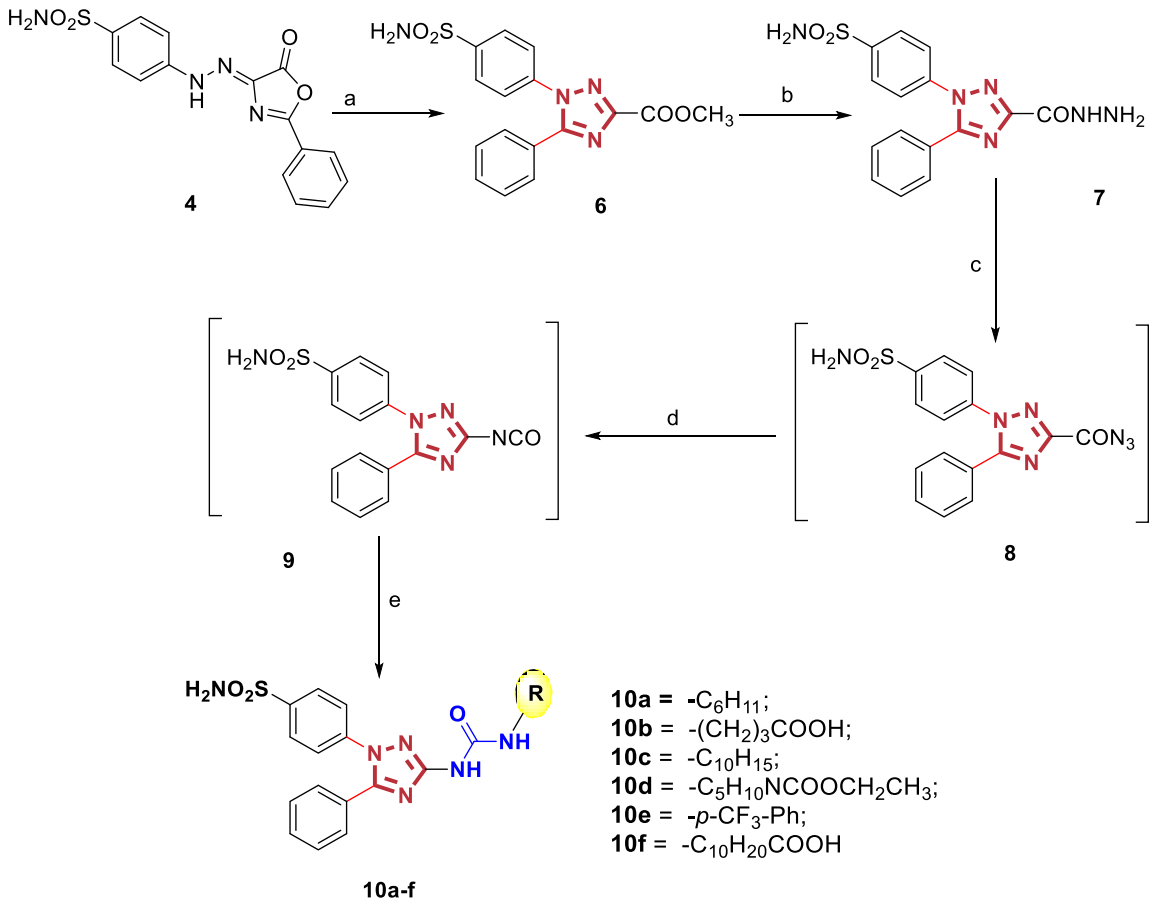
percentage was calculated from the following formula:%ABS = 109 - (0.345 × tPSA). All the obtained molecular properties were recorded in **Table 4** [42–44].

4. Results and discussion

4.1. Chemistry

The final novel compounds derivatives **5a-e** and **10a-f** were synthesized by the illustrated reaction sequence as shown in **Schemes 1** and **2**. The starting compound, hippuric acid **(2)**, had been prepared in high yields through the reported method by the reaction of glycine with benzoyl chloride **(1)** in presence of 10% NaOH [45]. The carboxylic group of hippuric acid **(2)** was activated through heating with acetic anhydride affording the mixed anhydride which underwent intra-molecular cyclization affording 2-phenyloxazol-5(4H)-one **(3)** [46,47]. The furnished phenyloxazol-5(4H)-one **(3)** was subsequently coupled with hydrazine salt of sulfanilamide, which was prepared by diazotization of sulfanilamide primary amine using sodium nitrite in 5 M HCl, affording intermediate **(4)** according to Kuskov-like reaction [48]. The final targeted 1,2,4-triazole-3-carboxamide **5a-e** were obtained by heating compound **(4)** in glacial acetic acid in the presence of anhydrous CH_3COONa. The postulated structures were confirmed by their physical, analytical and spectral data. ^{1}H NMR spectrum of (**5b**) showed a single peak of NH at 8.33 ppm, along with the typical peaks of adamantyl protons in rang 1.60–2.10 ppm. ^{13}C NMR spectrum showed peaks at 24.2, 29.2, 36.3, and 52.1 refereeing to the characteristic adamantyl carbons.

On the other hand, the targeted urea derivatives compounds **10a-f** were synthesized as inspected in **Scheme 2**. The hydrazide compound **(7)** was synthesized by with treating of compound **(4)** with methanolic solution of KOH at room temperature according to the reported methods (Sawdey rearrangement) [48] affording ethyl ester of 1,2,4-triazole **(6)**. Subsequently, heating of ethyl ester of 1,2,4-triazole **(6)** with hydrazine hydrate providing the key starting hydrazide **(7)** as reported [49]. The targeted urea compounds were prepared through treating of the hydrazide compound **(7)** with *in situ* generated nitrous acid affording the acyl azide intermediate **(8)**, which simultaneously heated under reflux in toluene without further purification due to its instability affording isocyanate intermediate **(9)** via Curtius Rearrangement. Respectively, the isocyanate intermediate **(9)** was condensed with different amine in presence of pyridine releasing urea compounds **10a-f**. The proposed structures were proven by their analytical and spectral analysis. ^{1}H NMR of compound **10e** indicated appearance of the characteristic peaks of two NH groups of di-substituted urea at δ 10.31 and 10.25 ppm that hide upon deuteration, as well as the trifluo-



Scheme 2. The synthesis of compounds 10a-f. Reagents and conditions: a) 5% potassium hydroxide, CH_3OH, rt; b) $NH_2NH_2.H_2O$, EtOH, reflux, 6 h; c) Sodium nitrite/ HCl, 0–5 °C; d) CH_3-pH, reflux at 110 °C, 0.5 h; e) Applicable amine, pyridine, reflux, reflux at 101 °C (yield: 51–63%), 24 h.

romethyl aniline peaks at δ 7.42–7.73 ppm. ^{13}C NMR revealed appearance of carbonyl group of di-substituted urea at δ 156.81 ppm in addition to the peak of $-CF_3$ at 126.42 ppm. Mass spectrum of **10e** revealed M^{+1} peak at *m/z* 503 constituting the last peak of the spectrum.

4.2. Biological evaluation

4.2.1. *The* in vitro *assay*

With the aid of the corresponding/specific enzyme immunoassay (EIA) kits that target COX-1 and COX-2 enzymes, the anti-inflammatory activity of the new derivatives **5a-e** and **10a-f** were determined (Cat. no. 701070 and 701080, respectively, Cayman Chemical Company, Ann Arbor, MI), as formerly established [38,50]. On the other hand, the *in vitro* sEH enzyme inhibition ability was measured using soluble epoxide hydrolase cell-based assay kit (sEH; cat. no. 600090, Cayman Chemical, Ann Arbor, MI), as instructed by the provider and reported before [30]**.** The results were outlined in **Table 1** and were presented as the IC_{50} values, where celecoxib and AUDA were used as positive controls. Moreover, the selectivity index to the COX isoenzymes was calculated for the test compounds via determining the IC_{50} ratio for COX-1 to COX-2, as demonstrated in **Table 1**. Variable inhibitory activities against COX-1/COX-2 and sEH for the tested compounds were observed. Concerning COX-1/COX-2 inhibitory activity for the tested compounds, although they demonstrated relatively low COX-1 isoenzyme inhibition (IC_{50} range 8.85–13.02 μM), they displayed a slightly high COX-2 isoenzyme inhibition (IC_{50} range1.98–5.03 μM). Derivatives of urea compounds **10a-f** were noticed to be the most common against COX-2 among the rest of the compounds (COX-2 IC_{50} range 1.98–4.35 μM; SI range 2.91–4.62) when compared *versus* celecoxib (COX-2 IC_{50} = 0.95 μM; SI = 6.44). Noticeably, the compounds with the urea linker **(10e** and **10c)** were the most active against COX-2 among the rest of compounds (COX-2 IC_{50} = 1.98 μM and 2.13 μM; SI = 4.62 and 4.15, respectively). On the other hand, compounds **5d** and **5e,** with amide linker, showed the least active against COX-2 (COX-2 IC_{50} = 4.02 and 5.03 μM; SI = 2.88 and 2.58, respectively). Regarding sEH inhibition, all the compounds were compared *versus* the reference sEH inhibitor AUDA. In this regard, the tested compounds demonstrated a weak inhibitory activity against sEH with IC_{50} range 1.78 – 4.02 nM compared to AUDA (IC_{50} = 0.49 nM), with the exception of for two compounds **10e** and **10c** that afforded good sEH inhibition, as evidenced with IC_{50} values of 1.09 and 1.23 nM, respectively. Moreover, urea linked compounds **10a-f** showed sEH inhibitory activity (IC_{50} = 1.09 – 3.21 nM) better than that achieved by the amide-linked derivatives **5a-e** (IC_{50} = 1.95 – 4.02 nM). Together, these findings elucidate that the evaluated compounds with the urea linker **10a-f** demonstrated higher potency as selective dual inhibitors of COX-2/sEH enzymes than their counterparts that contained the amide linker **5a-e**.

4.2.2. In vivo *screening activity*

4.2.2.1. The anti-inflammatory activity determined by the carrageenan-induced paw edema assay. The carrageenan-induced rat paw edema model was used to assess the anti-inflammatory activity of the newly synthesized compounds **5a-e** and **10a-f**, as described [40]. The anti-inflammatory activity was presented as the inhibition% of the paw diameter thickness at 1, 3 and 5 h, following the inoculation of carrageenan to the paw. The data is outlined in **Table 2** and the obtained results were compared *versus*

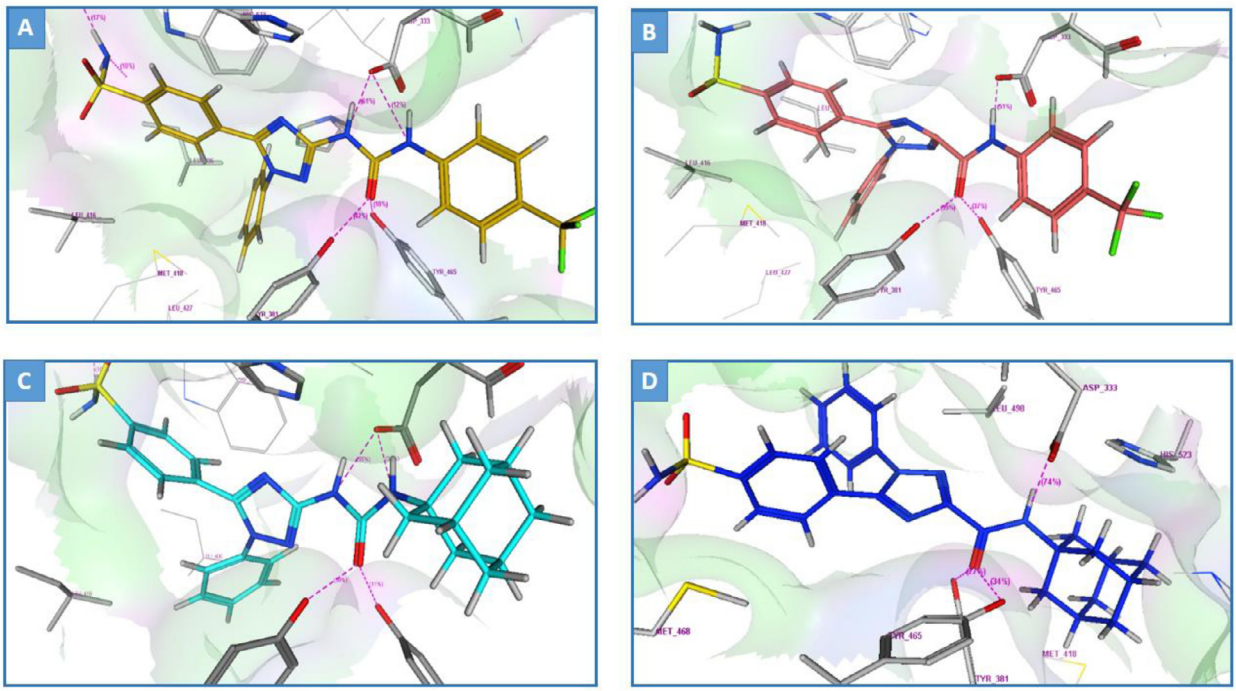


Fig. 4. Docking and binding pattern of some selected compounds inside sEH enzyme active pocket (PDB code: 1VJ5); (A) Binding interactions and mode of compound **10e** (yellow) within sEH enzyme active site; (B) Binding interactions and mode of compound **5e** (red) within sEH enzyme active site; (C) Binding interactions and mode of compound **10c** (cyan) within sEH enzyme active site; (D) Binding interactions and mode of compound **5c** (blue) within sEH enzyme active site. The ligands are presented in stick model. Hydrogen bonds were represented as dashed pink lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

celecoxib as a reference anti-inflammatory drug at the same time points. In this regard, 1 h after carrageenan paw injection, all the tested compounds showed significant anti-inflammatory activity (34.72–81.85% edema inhibition) in comparison to celecoxib that elicited 81.17% edema inhibition at the same intervals. Among the synthesized compounds, **10a** and **10e** demonstrated marked edema inhibition (81.85 and 80.32%, respectively) which is very close to that afforded by the reference celecoxib (81.17%) at the same time point. After 3 h, the anti-inflammatory activity of all the tested compounds increased to reach (53.41–86.95% edema inhibition) compared to celecoxib that showed a slight increase in its activity (82.71% edema inhibition). Interestingly, the activity of **10a** and **10e** also slightly increased to reach (86.59 and 81.35%, respectively). After 5 h, all the evaluated compounds showed an increase in edema inhibition percentage activities (62.36–91.27%) compared to celecoxib (88.30% edema inhibition). Compounds **10a, 10c, 10e, 5b** and **5d** elicited the highest anti-inflammatory activity (80.45–91.27% edema inhibition) among all the synthesized compounds. It is noteworthy to mention that two compounds, namely, **10e** followed by **10c,** showed higher anti-inflammatory activity (91.27 and 89.32% edema inhibition, respectively) than celecoxib (88.30% edema inhibition) at the 5-hour time point. Together, these data reveal that the tested compounds with the urea linker **10a-f** compounds demonstrated a higher anti-inflammatory activity, *versus* the compared to the amide-containing **5a-e** compounds. Notably, these findings are in harmony with the observed COX-1/COX-2 inhibitory activities *in vitro,* confirming the role of addition of trifluoromethyl aniline/adamantyl moieties in the improvement of the anti-inflammatory activity.

4.2.2.2. Acetic acid induced writhing test for analgesic activity. The acetic-acid induced writhing model was used to examine the analgesic activity of the newly synthesized compounds. The analgesic activity was compared *versus* the reference anti-inflammatory celecoxib [41]. The results were expressed as percentage inhibition of the number of writhing measured for 15–20 min after acetic-acid injection as shown in **Table 3**. The results obtained showed that compounds **5a-e** and **10a-f** showed mild to moderate analgesic activity (22.38–55.97% writhing inhibition) compared to celecoxib (13.43% writhing inhibition). Noticeably, compounds **10c** and **10e** showed the highest analgesic activity (50.00–55.97% writhing inhibition) among the rest of the compounds. From the aforementioned results, the following was concluded that the compounds that have a urea linker showed higher analgesic activity (24.62–55.97% writhing inhibition) than the corresponding compounds associated with an amide linker (22.38–37.31% writhing inhibition).

4.3. Molecular docking

Understanding the binding interactions and selectivity difference of the novel 1,2,4-triazole derivatives towards sEH enzyme was achieved by comparing the docking simulation study of the active urea-linked derivatives **(10c** and **10e)** with other the amide-linked analogues (**5c** and **5e)** into the active sites of sEH using Molecular operating environment (MOE) version 2008.10 [26,30]. This study was carried out utilizing 3D protein structure data of sEH retrieved from the protein data bank (PDB code: 1VJ5) [51]. Accordingly, The 3D crystal structure of sEH complexed (PDB code: 1VJ5) with a co-crystallized inhibitor, **CIU** (*N*-cyclohexyl-*N`-(4*-iodophenyl)urea), was chosen for this study where it showed four main hydrogen bonding interactions; two bonds between 2NHs of urea pharmacophore and Asp333 (1.52 A^o and 1.94 A^o), two bonds between Carbonyl and Tyr 381 (2.54 A^o) and Tyr465 residue (2.67 A^o) [26]. Interestingly enough, our most potent compounds associated with urea linker (**10c** and **10e)** showed higher activity and better analogism to CIU by achieving the same four main hydrogen bonding interactions with Asp333, Tyr381 and Tyr465 amino acid residues. Moreover, **10c** and **10e** formed an additional hydro-

gen bond that strengthens the interaction with sEH enzyme, and it might be the reason behind their superior activity as shown in Fig. 4 (**A** and **B**). On the other hand, the parallel amide linked compounds (**5c** and **5e**) elicited lesser activity in comparison to the urea linked compounds because they formed only three main hydrogen bonding interactions with Asp333, Tyr381 and Tyr465 residues in comparison to ClU due to the absence of second NH group which proved the essentiality of urea linker for high sEH inhibitory activity Fig. 4 (**C** and **D**). By focusing and comparing of urea and amide linked compounds, it was clear that urea linked compounds (**10e** and **10c**) were more potent against sEH inhibition than their amide linked compounds analogs (**5e** and **5c**; respectively). Interestingly, the aforementioned results were harmonious with both *in vivo* and *in vitro* values that affirmed compounds possessing urea linker (**10c** and **10e**) interact strongly to sEH than the corresponding compounds with amide linkers.

4.4. Physicochemical parameters

The physicochemical parameters of all the target compounds was predicted with the aid of computational study using Molinspiration tool [42–44]. The rule of five adopted by Lipinski's (RO5) has applied to all the newly synthesized compounds, except for one parameter (MW). Moreover, all tested compounds are expected to have good absorption after computation of calculated absorption%ABS (%ABS range 50.58–67.60%). Consequently, the aforementioned data revealed that the newly synthesized compounds possessed promising pharmacokinetic properties as recorded in Table 4.

5. Conclusion

In summary, two novel series of diaryl-1,2,4-triazole linked to amide conjugates (**5a-e**) or urea conjugates (**10a-f**) have been synthesized and evaluated for activity against both COX-2 and sEH. The *in vitro* and *in vivo* results were consistent with each other especially for the compounds **10e** that exhibited the highest *in vitro/in vivo* activities (COX-2 IC_{50} = 1.98 μM; sEH = 1.09 nM; edema inhibition = 91.27%; writhing inhibition = 55.97%) followed by **10c** (COX-2 IC_{50} = 2.13 μM; sEH = 1.23 nM,; edema inhibition = 89.32%; writhing inhibition = 50.00%) in comparing to celecoxib (88.30% edema inhibition; 13.43% writhing inhibition). From the aforementioned results, compounds **10e** and **10c** could be characterized as promising leads that merit further consideration and optimization for development of more potent and safer anti-inflammatory and analgesic agents.

Credit author statement

Ahmed H. Abdelazeem: Conceptualization, Methodology, Reviewing and Editing, Software. **Asmaa G. Safi El-Din**: Methodology, Software, Writing-Original draft. **Hany H. Arab**: Funding acquisition, Reviewing and Editing. Mohammed T. El-Saadi: Supervision, Data curation, Reviewing and Editing. **Samir M. El-Moghazy**: Conceptualization, Supervision, Data curation, Validation. **Noha H. Amin:** Supervision, Data curation, Writing- Reviewing and Editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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