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ORIGINAL ARTICLE

New ursolic acid derivatives bearing 1,2,3-triazole moieties: design, synthesis and anti-inflammatory activity in vitro and in vivo

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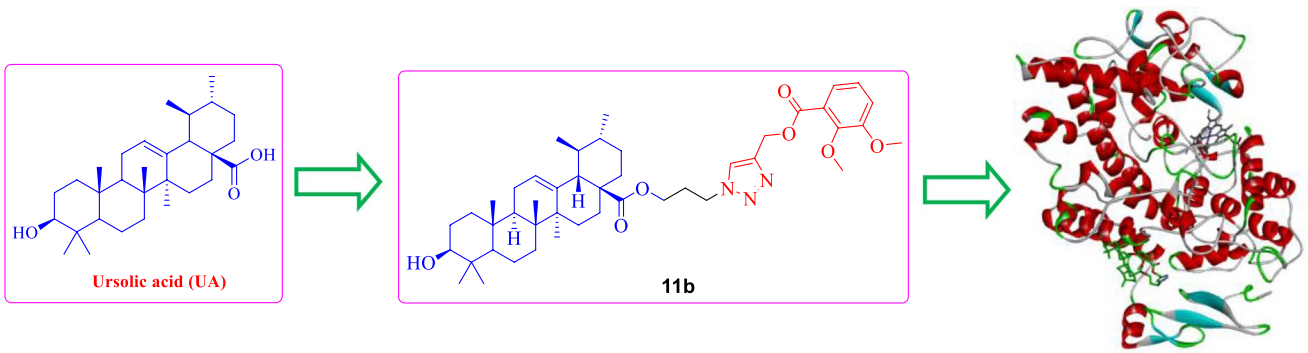
Abstract

In order to discover novel anti-inflammatory agents, three series of compounds obtained by appending 1,2,3-triazole moieties on ursolic acid were designed and synthesized. All compounds have been screened for their anti-inflammatory activity by using an ear edema model. The potent anti-inflammatory compound was subjected to in vitro cyclooxygenase COX-1/COX-2 inhibition assays. In general, the derivatives were found to be potent anti-inflammatory activity. Especially, the compound **11b** exhibited the strongest activity of all of the compounds prepared, with 82.81% inhibition after intraperitoneal administration, which was better than celecoxib as a positive control. Molecular docking results unclose the rationale for the interaction of the compound **11b** with COX-2 enzyme. Further studies revealed that compound **11b** exhibited effective COX-2 inhibitory activity, with half-maximal inhibitor concentration (IC_{50}) value of 1.16 μM and selectivity index (SI = 64.66) value close to that of celecoxib (IC_{50} = 0.93 μM, SI = 65.47). Taken together, these results could suggest a promising chemotype for development of new COX-2-targeting anti-inflammatory agent.

Graphic abstract



Keywords Ursolic acid · Anti-inflammatory activity · Molecular docking · COX-1/COX-2

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Introduction

Inflammation is a fundamental protective response of the immune system to multiple injuries and different pathogens, but the uncontrolled inflammation is responsible for serious and chronic problems [1, 2]. Nonsteroidal anti-inflammatory drugs demonstrated useful inhibition properties against α-glycosidase, AR and SDH enzymes [3]. NSAIDs as indomethacin and ibuprofen work by inhibiting cyclooxygenase (COX) enzymes, which catalyze prostaglandin biosynthesis through arachidonic acid. Cyclooxygenase enzymes (COXs) exist in two isoforms: constitutive form (COX-1) and inducible form (COX-2) [4, 5]. Representing this category of drugs celecoxib, rofecoxib and etoricoxib are/were some of the highly effective COX-2 inhibitors [6, 7]. However, the cardiovascular effects led to the withdrawal of rofecoxib and etoricoxib from the market. Thus, new anti-inflammatory agent with high therapeutic efficacy and fewer side effects are needed.

Ursolic acid (UA), a pentacyclic triterpene, exists abundantly in the plant kingdom and as constituents of medicinal herbs. UA and their derivatives (compounds **A-C**) (Fig. 1) have been investigated in detail by several groups, and many interesting biological properties have been detected, among them antitumor [8], antimicrobial [9] and anti-inflammatory [10]. Triazole-containing compounds (**D-F**) (Fig. 1) are able to bind to a variety of enzymes and receptors by non-covalent interactions, hence displaying a large array of biological activities [11]. Among these triazole, 1,2,3-triazoles are imperative class of heterocycles and play a major role in medicinal chemistry with anticancer [12], anti-inflammation [13, 14] and antiobesity [15]. We hypothesized that several natural sources derivatives (compounds **G-I**) (Fig. 1) have beneficial effect on the anticancer [16, 17] and anti-inflammation effects [9, 18]. In continuity to our previous studies and keeping in mind the medicinal potential of ursolic acid and 1,2,3-triazoles fragments, it was planned to hybridize these two fragments along with the incorporation of another potent UA-based anti-inflammation agents. Thence, three novel series of UA derivatives (Fig. 2) were synthesized and evaluated through biological experiments for their selective target of COX-2 and for the anti-inflammation activity.

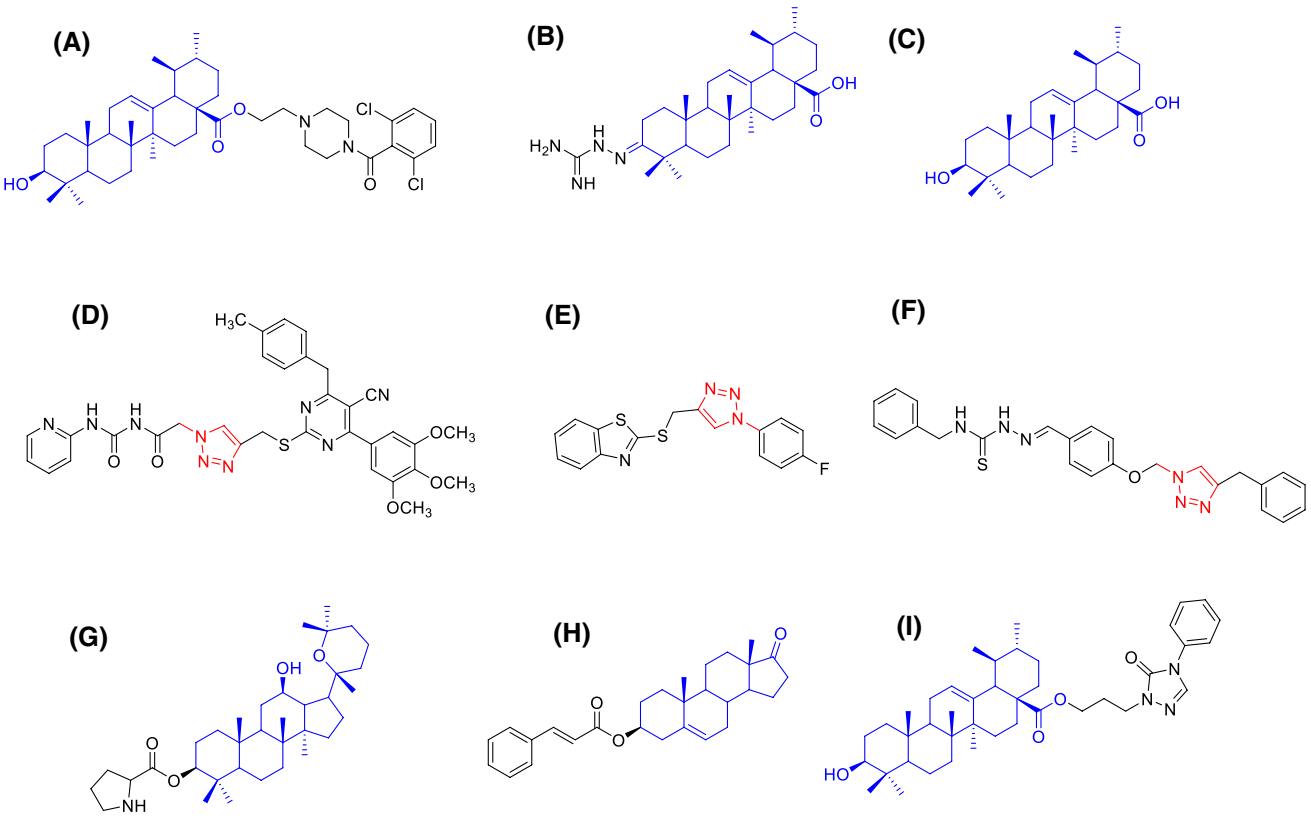
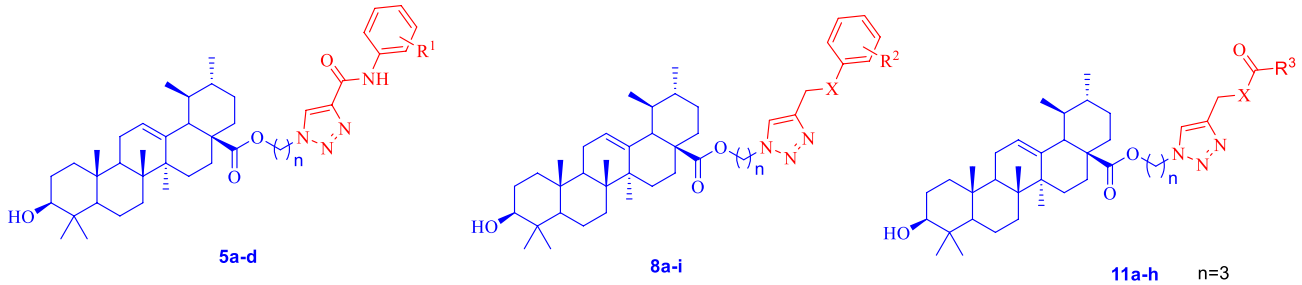


Fig. 1 Chemical structures of reported triterpenes and 1,2,3-triazoles derivatives



5a R^1=3,4-di-CH_3
5b R^1=4-CH_3
5c R^1=2-F
5d R^1=H
8a R^2=3-OCH_3 X= O
8b R^2=2,4-di-CH_3 X= O
8c R^2=3-CF_3 X= O
8d R^2=2,4-di-Cl X= O
8e R^2=H X= O
8f R^2=H X= NH
8g R^2=2-F X= NH
8h R^2=4-CH_3 X= NH
8i R^2=3,4-di-CH_3 X= NH
11a R^3=Phenyl(H) X= O
11b R^3=Phenyl(2,3-di-OCH_3) X= O
11c R^3=Phenyl(4-OCH_3) X= O
11d R^3=Phenyl X= NH
11e R^3=Phenyl(2,3-di-OCH_3) X= NH
11f R^3=Phenyl(4-CH_3) X= NH
11g R^3=Phenyl(4-OCH_3) X= NH
11h R^3=Furyl X= NH

Fig. 2 Structures of target compounds **5a-d**, **8a-i** and **11a-h**

Results and discussion

Chemistry

The synthetic route of all compounds is depicted in Scheme 1. UA was treated with 1,3-dibromopropane in DMF for 24 h to obtain intermediate **1**, which was introduced with sodium azide in DMF to obtain intermediate **2**. The intermediate **4** was synthesized by reacting propiolic acid with substituted aniline in the presence of DCC and DMAP in dry dichloromethane. Intermediate **7** was prepared by the reaction of their respective aniline or phenol with propargyl bromides in the presence of anhydrous potassium carbonate in acetone for 12 h [19]. Intermediate **10** was synthesized in the same way as intermediate **4** or intermediate **7**. In the final step, the compounds (**4**, **7** or **10**) were further reacted with intermediate **2** via copper-catalyzed azidealkyne (3 + 2) cycloaddition reaction afforded desired products (**5a-d**, **8a-i** and **11a-h**) [20]. All the synthesized compounds were thoroughly characterized by their spectral data.

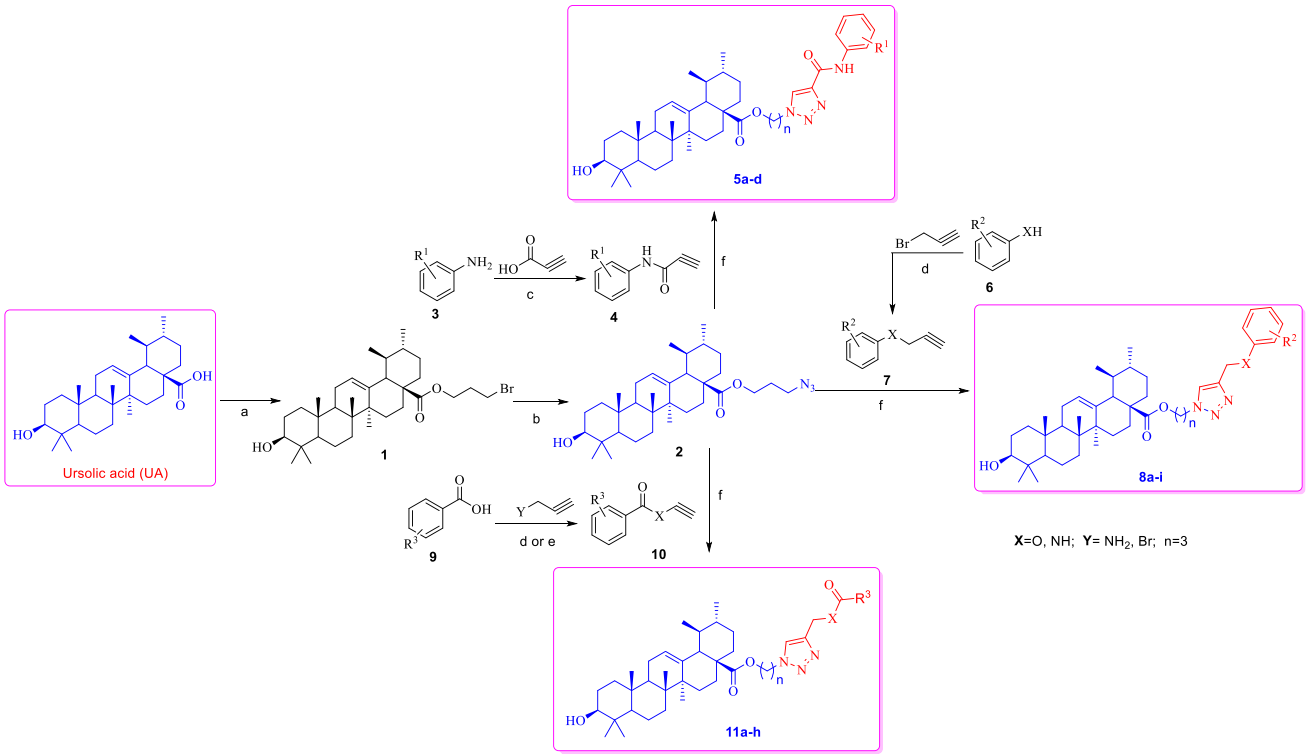
Anti-inflammatory activity

Twenty-one of the UA derivatives and the reference drug celecoxib were examined for their anti-inflammatory activity using an in vivo xylene-induced ear edema model [21]. Most of the synthesized compounds exhibited potent anti-inflammatory activity at a dose of 100 mg/kg with inhibition rates ranging from 22.50% to 82.81% (Table 1). Six compounds, namely **8d**, **8e**, **8 h**, **8i**, **11d** and **11 g**, showed anti-inflammatory activity with more than 65.00% inhibition, which was nearly equivalent to that of the inhibition shown by celecoxib (65.00%). It is noteworthy that compound **11b**, with 82.81% inhibition, exhibited the strongest anti-inflammatory activity. Moreover, the activity of **11b** was higher than that of celecoxib. The position or physicochemical properties of the different substituents on the phenyl ring had minimal impact on the anti-inflammatory properties of these compounds, indicating that the electronic effect of the substituent on the benzene ring is not critical. Furthermore, a comparison of the compounds in series **5** and **8** revealed that the introduction of a ketone moiety reduced the anti-inflammatory activity. To confirm the need for further evaluation, we estimated the cytotoxicity of selected compounds using the MTT assay on SGC-7901, BEL-7402 and LO2 cells. As shown in Table 2, compounds **8d**, **8e**, **11b** and **11e** exhibited weak cytotoxicity.

Compound **11b** was further assessment of its anti-inflammatory activity. A dose of 100 mg/kg was administered via the oral route at the time intervals of 1, 2, 3, 4, 5 and 24 h after xylene application, and the inhibition rates were found (Table 3). The activity of compound **11b** (54.70%) was found to the maximum after 2 h and was slightly higher to that of celecoxib (51.01%) at this time point. Compound **11b** was also screened at concentrations of 100, 50 and 25 mg/kg after 2-h oral administration. As shown in Table 4, compound **11b** exhibited a significant inhibitory effect toward ear inflammation inhibition (58.83%) at a dose of 100 mg/kg, which was almost similar to that of celecoxib (58.77%).

In vitro cyclooxygenase (COX-1/2) inhibition assay

The ability of compound **11b** to inhibit COX-1 and COX-2 enzymes was determined via measuring their peroxidase activity using a colorimetric enzyme immune assay (EIA) kit [22–24]. Furthermore, the COX-2 selectivity index (COX-2 SI) value was calculated as [IC_{50} (COX-1)/IC_{50} (COX-2)] and compared to the reference drug (celecoxib as a selective COX-2 inhibitor) [25]. Compound **11b** displayed a potency value (IC_{50} = 1.16 μM) close to the value for



5a R^1=3,4-di-CH_3 | **8b** R^2=2,4-di-CH_3 X= O | **8g** R^2=2-F X= NH | **11c** R^3=Phenyl(4-OCH_3) X= O
5b R^1=4-CH_3 | **8c** R^2=3-CF_3 X= O | **8h** R^2=4-CH_3 X= NH | **11d** R^3=Phenyl X= NH
5c R^1=2-F | **8d** R^2=2,4-di-Cl X= O | **8i** R^2=3,4-di-CH_3 X= NH | **11e** R^3=Phenyl(2,3-di-OCH_3) X= NH
5d R^1=H | **8e** R^2=H X= O | **11a** R^3=Phenyl(H) X= O | **11f** R^3=Phenyl(4-CH_3) X= NH
8a R^2=3-OCH_3 X= O | **8f** R^2=H X= NH | **11b** R^3=Phenyl(2,3-di-OCH_3) X= O | **11g** R^3=Phenyl(4-OCH_3) X= NH
11h R^3=Furyl X= NH

Scheme 1 Synthetic scheme for target compounds. Reagents and conditions: **a** $Br(CH_2)_3Br$, K_2CO_3, KI, DMF, rt, 12 h; **b** NaN_3, DMF, rt, 2 h; **c** propiolic acid, DCC, DMAP, rt, 12 h; **d** propargyl bromide, K_2CO_3, KI, acetone, reflux, 6 h; **e** propargyl amine, DCC, DMAP, rt, 12 h; **f** sodium ascorbate, $CuSO_4{\cdot}5H_2O$, DMF, H_2O, rt, 12 h

celecoxib (IC_{50} = 0.93 μM). Considering COX-2 selectivity index values, compound **11b** showed selectivity index value (SI = 64.66) close to that of celecoxib (SI = 65.47) (Table 5).

Molecular docking

To validate the data obtained by the in vitro COX-2 assay, molecular docking studies of **11b** were performed in comparison with celecoxib using COX-2 protein (PDB entry 4COX). AutoDock Vina was used to execute molecular docking. Discovery Studio was used for interaction analysis, binding conformation and making diagrams. The result shows that compound **11b** may bind well to the COX-2 protein and while interacting with Arg44 (5.44 Å and 2.57 Å), Tyr122 (5.03 Å and 4.39 Å), and Gln372 (2.01 Å). It is apparent that compound **11b** (− 8.1 kcal/Mol) may bind to 4COX in a way similar to the binding of celecoxib (− 8.0 kcal/Mol) (Fig. 3). These molecular docking data provide certain theoretical support for experimental results and the subsequent structural optimization efforts.

Conclusions

Three structurally different series of compounds consisting of ursolic acid and 1,2,3-triazole hybrids were screened for the anti-inflammatory activity. The results clearly provided confidence for further development of new analogues based on this chemical scaffold. In preliminary tests, compound **11b** showed high edema inhibition percentage (82.81%) compared to celecoxib (65.00%). Also, compound **11b** was found to have potent COX-2 inhibitory activity (IC_{50} = 1.16 μM, SI = 64.66) close to the COX-2 selective reference drug celecoxib (IC_{50} = 0.93 μM, SI = 65.47). Moreover, molecular modeling studies also indicated toward the most probable binding site interactions of compound **11b** in the active site. In view of the above, it may be concluded that compound **11b** is an efficient lead compound to development of better COX-2 inhibitors and anti-inflammatory agents.

Table 1 Anti-inflammatory activities of compounds **5a-d**, **8a-i**, **11a-h** and celecoxib following i.p. administration

	Dose (mg/kg)	Number of mice	Edema mean ± S.D. (mg)	Inhibition rate (%)
DMSO	100	5	8.00 ± 0.90	–
Celecoxib	100	5	2.80 ± 0.65**	65.00
UA	100	5	4.80 ± 0.58**	55.34
5a	100	5	6.20 ± 0.60**	22.50
5b	100	5	5.83 ± 0.35	27.19
5c	100	5	6.90 ± 0.80*	13.75
5d	100	5	4.95 ± 1.15	38.13
8a	100	5	3.10 ± 1.00	61.25
8b	100	5	5.30 ± 1.65	33.75
8c	100	5	3.48 ± 1.15**	56.56
8d	100	5	2.80 ± 0.45**	65.00
8e	100	5	2.50 ± 0.70***	68.75
8f	100	5	3.80 ± 0.80*	52.50
8 g	100	5	3.40 ± 0.45**	57.50
8 h	100	5	2.75 ± 0.45**	65.63
8i	100	5	2.68 ± 0.65***	66.56
11a	100	5	4.17 ± 0.70**	55.31
11b	100	5	1.38 ± 0.65***	82.81
11c	100	5	4.03 ± 0.25*	49.69
11d	100	5	2.35 ± 0.85***	70.63
11e	100	5	3.20 ± 0.59*	60.00
11f	100	5	5.95 ± 0.45	25.63
11 g	100	5	2.75 ± 0.65**	65.63
11 h	100	5	2.88 ± 1.00**	64.06

*$0.01 < p < 0.05$ compared with vehicle group, **$p < 0.01$, *** $p < 0.001$ compared with a vehicle group

–No anti-inflammatory activity

Table 2 Cytotoxicity data (IC_{50}, μmol/L) of compounds **8d**, **8e**, **11b** and **11e** in SGC-7901, BEL-7402 and LO2 cells

Compound	IC_{50} (μmol/L)[a]		
	SGC-7901[b]	BEL7402[c]	LO2[d]
8d	> 100	> 100	> 100
8e	> 100	> 100	> 100
11b	> 100	> 100	> 100
11e	> 100	> 100	> 100

[a]Values are the average of three independent experiments running in triplicate. Variation was generally between 5 and 10%; [b] human gastric cancer cells; [c] human liver cancer cells; [d] human normal hepatic cells

Materials and methods

General procedures

Melting points were determined in open capillaries and were uncorrected. The reactions were monitored by thin layer chromatography (TLC). ^{1}H and ^{13}C-NMR spectra were performed on an AV-300 or AV-400 spectrometer (Bruker, Zurich, Switzerland) operating at 300 MHz for ^{1}H and 101 MHz for ^{13}C NMR while using TMS as the internal standard. *J* values are given in Hertz. Mass spectra were measured on an MALDI-TOF (Shimadzu, Japan). High-resolution mass spectra were measured on a Thermo Scientific LTQ Orbitrap XL spectrometer.

Table 3 Anti-inflammatory activity of compound **11b** administered orally at different times before xylene application

Time (h)	Dose (mg/kg)	Number of mice	Inhibition rate (%)	
			11b	Celecoxib
1	100	5	40.97**	30.62*
2	100	5	54.70***	51.01***
3	100	5	47.29***	43.39**
4	100	5	31.27*	17.05
5	100	5	24.10*	11.79
24	100	5	3.08	4.17

*$0.01 < p < 0.05$ compared with vehicle group, **$p < 0.01$, *** $p < 0.001$ compared with a vehicle group

Table 4 Anti-inflammatory activity of compound **11b** administered orally at different doses

Time (h)	Dose (mg/kg)	Number of mice	Inhibition rate (%)	
			11b	Celecoxib
2	100	5	58.83***	58.77***
2	50	5	30.88*	30.74*
2	25	5	16.81*	17.11

*$p < 0.05$, *** $p < 0.001$ compared with a vehicle group

Table 5 In vitro COX-1/COX-2 enzyme inhibition assays[a]

Cmpd	COX-1, IC_{50} (μM)[a]	COX-2, IC_{50} (μM)[a]	COX-2, SI[b]
11b	75.01	1.16	64.66
Celecoxib	60.89	0.93	65.47

[a]Data are shown as IC_{50} (μM)

[b]The in vitro COX-2 selectivity index (COX-1/COX-2)

General procedure for the preparation of compound 1

UA (1 mmol) was dissolved in DMF (15 mL), and K_2CO_3 (2 mmol), KI (0.5 mmol) and dibromoalkane (4 mmol) were added. After being stirred for another 12 h, the reaction mixture was poured onto 100 mL of distilled water

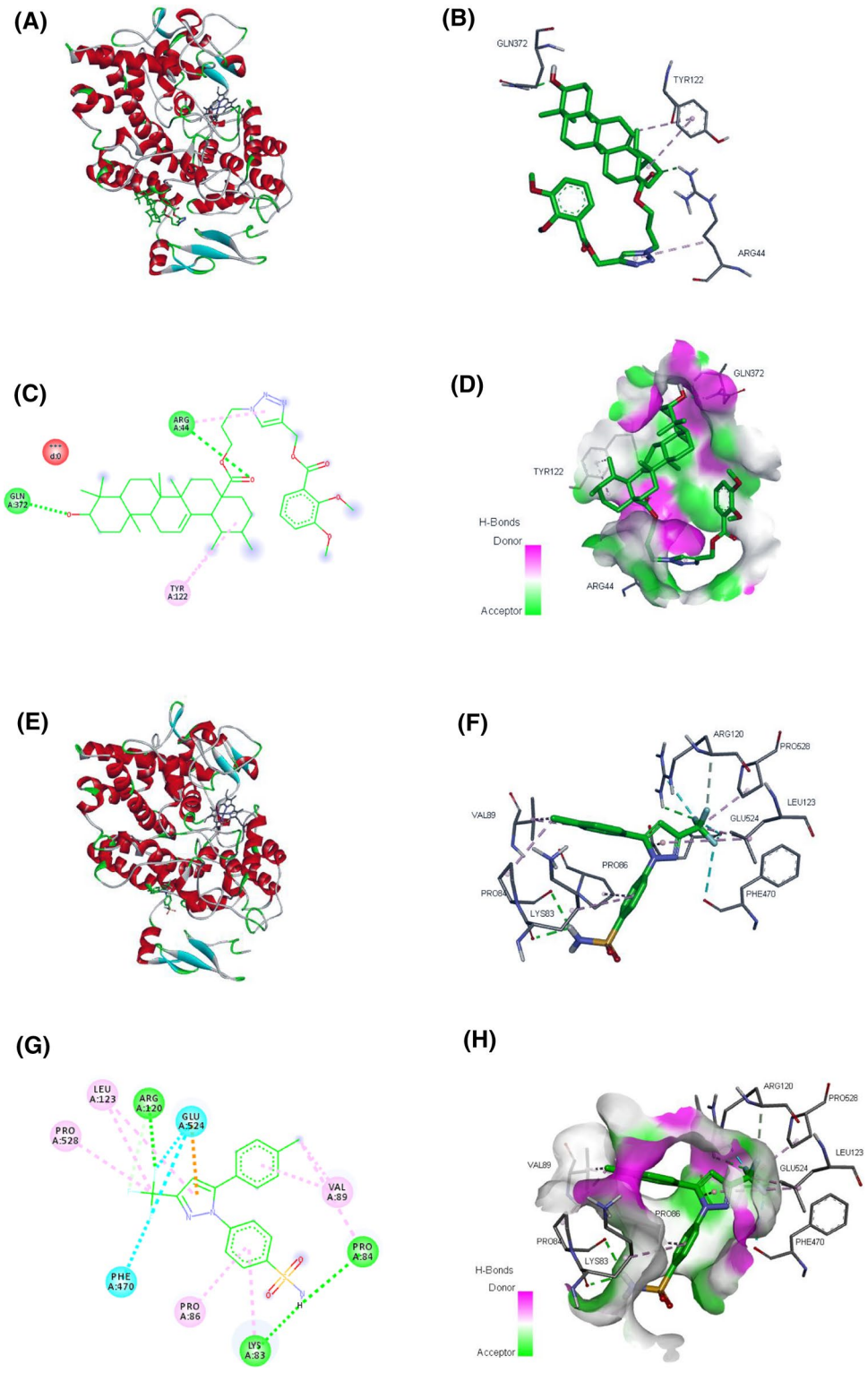


Fig. 3 Binding modes of compounds **11b** and **celecoxib** with 4COX. **a** Compound **11b** in binding site of 4COX. **b** Docking study of compound **11b** with 4COX. **c** Docking study of compound **11b** with 4COX. **d** Docked conformation of the most active compound **11b** in COX-2. **e Celecoxib** in binding site of 4COX. **f** Docking study of **celecoxib** with 4COX. **g** Docking study of **celecoxib** with 4COX. **h** Docked conformation of the most active **celecoxib** in COX-2

and partitioned with ethyl acetate. The organic layer was washed with brine (3 × 10 mL), dried over anhydrous Na_2SO_4 and purified via silica gel column chromatography with petroleum ether/ethyl acetate to yield the target compound **1**.

General procedure for the preparation of compound 2

Compound **1** (1 mmol) and sodium azide (2 mmol) were weighed out and transferred to a vial to which DMF/H_2O

(1:1, 50 mL) was subsequently added. The reaction was stirred at 80 °C for 24 h and then cooled to room temperature. The reaction mixture was poured into water and extracted with ethyl acetate. The combined organic layers were dried with Na_2SO_4, filtered. After the removal of the solvent under vacuum, compound **2** was obtained.

General procedure for the preparation of compounds 5a-d, 8a-i and 11a-h

Compound **2** (0.15 mmol) and the appropriate azide (0.30 mmol) in CH_2Cl_2 (5 mL), H_2O (5 mL), $CuSO_4{\cdot}5H_2O$ (0.25 mmol) and Na-L-ascorbate (0.5 mmol) were added. The reaction was stirred at room temperature overnight where upon a saturated sodium chloride solution was added and the mixture was extracted with dichloromethane (3×). The combined organic extracts wsere washed with brine (50 mL), dried over Na_2SO_4, filtered, and the solvent was removed in a rotary evaporator under reduced pressure to afford the crude product. The residue was purified by flash column chromatography to afford the target compounds **5a-d**, **8a-i** and **11a-h** each as a white solid.

3-(4-((3,4-Dimethylphenyl)carbamoyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate(5a) White solid. Yield: 75%; m.p. 179–181 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.88 (s, 1H, NH), 8.17 (s, 1H, CH), 7.49 (s, 2H, Ar–H), 7.15 (d, J = 8.2 Hz, 1H, Ar–H), 5.28 (s, 1H, CH), 4.53 (s, 2H, CH_2), 4.33 (t, J = 6.7 Hz, 1H, CH), 4.18–3.99 (m, 2H, CH_2), 3.27–3.17 (m, 1H, CH), 2.29 (d, J = 10.5 Hz, 8H, CH and CH_2), 2.19–0.74 (m, 43H, protons in UA skeleton and CH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 176.23, 137.28, 136.31, 131.93, 129.07, 124.68, 120.18, 116.38, 78.00, 59.39, 54.21, 51.95, 47.26, 46.85, 46.51, 41.11, 38.55, 38.07, 37.85, 37.73, 37.61, 35.97, 35.79, 32.01, 30.92, 29.58, 27.12, 26.98, 26.21, 23.28, 22.54, 22.30, 20.12, 18.91, 18.19, 17.29, 16.16, 15.97, 14.58, 14.43, 13.09. HRMS (ESI) *m/z* calcd for $C_{44}H_{64}N_4NaO_4$ $[M+Na]^+$: 735.4830, found: 735.4830.

3-(4-(p-Tolylcarbamoyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (5b)White solid. Yield: 70%; m.p. 95–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.91 (s, 1H, NH), 8.18 (s, 1H, CH), 7.60 (d, J = 8.3 Hz, 2H, Ar–H), 7.20 (d, J = 8.2 Hz, 2H, Ar–H), 5.28 (s, 1H, CH), 4.53 (s, 2H, CH_2), 4.33 (t, J = 6.7 Hz, 1H, CH), 4.10 (s, 2H, CH_2), 3.27–3.18 (m, 1H, CH), 2.37 (s, 3H, CH_3), 2.31 (d, J = 6.5 Hz, 2H, CH_2), 2.22–0.74 (m, 43H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 134.11, 129.56, 125.90, 119.91, 78.99, 60.44, 55.24, 52.95, 48.42, 47.36, 42.26, 39.58, 39.07, 38.95, 37.07, 33.04, 31.90, 30.62, 29.50, 28.03, 27.24, 24.30, 23.52, 23.32, 22.70, 21.14, 20.92, 18.28, 17.15, 17.00, 15.54, 14.12. MS (MALDI-TOF) *m/z* 699 $[M+H]^+$.

3-(4-((2-Fluorophenyl)carbamoyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (5c)White solid. Yield: 68%; m.p. 167–169 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.20 (s, 1H, NH), 8.45 (s, 1H, CH), 8.20 (s, 1H, Ar–H), 7.23–7.07 (m, 3H, Ar–H), 5.28 (s, 1H, CH), 4.54 (s, 2H, CH_2), 4.10 (s, 2H, CH_2), 3.22 (s, 1H, CH), 2.32 (s, 2H, CH_2), 2.21–0.73 (m, 44H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 125.97, 125.71, 124.85, 121.64, 115.26, 79.04, 60.44, 55.24, 53.00, 48.35, 42.09, 39.69, 39.18, 36.86, 32.97, 30.71, 29.66, 29.45, 28.09, 27.34, 24.27, 23.64, 21.08, 18.31, 17.29, 15.69. MS (MALDI-TOF) *m/z* 703 $[M+H]^+$.

3-(4-(Phenylcarbamoyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (5d)White solid. Yield: 72%; m.p. 89–91 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.00 (s, 1H, NH), 8.21 (s, 1H, CH), 7.72 (d, J = 8.1 Hz, 2H, Ar–H), 7.39 (d, J = 7.8 Hz, 2H, Ar–H), 7.17 (d, J = 7.4 Hz, 1H, Ar–H), 5.27 (s, 1H, CH), 4.52 (d, J = 5.7 Hz, 2H, CH_2), 4.21–3.98 (m, 2H, CH_2), 3.27–3.17 (m, 1H, CH), 2.36–2.26 (m, 2H, CH_2), 2.20–0.73 (m, 44H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 685 $[M+H]^+$.

3-(4-((3-Methoxyphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8a)White solid. Yield: 80%; m.p. 125–127 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H, CH), 7.21 (d, J = 8.4 Hz, 1H, Ar–H), 6.59 (d, J = 7.7 Hz, 3H, Ar–H), 5.26 (d, J = 13.6 Hz, 3H, CH and CH_2), 4.47 (s, 2H, CH_2), 4.20–3.96 (m, 2H, CH_2), 3.81 (s, 3H, OCH_3), 3.28–3.17 (m, 1H, CH), 2.26 (s, 2H, CH_2), 1.92–0.75 (m, 44H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 130.14, 125.88, 123.03, 107.08, 101.45, 78.93, 60.73, 55.44, 53.07, 48.22, 47.64, 42.09, 39.52, 39.16, 37.07, 33.20, 31.01, 29.82, 28.25, 27.28, 24.40, 23.44, 21.25, 18.35, 17.35, 15.40. MS (MALDI-TOF) *m/z* 702 $[M+H]^+$.

3-(4-((2,4-Dimethylphenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8b)White solid. Yield: 75%; m.p. 62–64 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.62 (s, 1H, CH), 6.98 (s, 2H, Ar–H), 6.87 (s, 1H, Ar–H), 5.25 (d, J = 15 Hz, 3H, CH and CH_2), 4.46 (s, 2H, CH_2), 4.08 (d, J = 5.7 Hz,

2H, CH_2), 3.28–3.16 (m, 1H, CH), 2.25 (m, 9H, CH, CH_2 and CH_3), 1.96–0.75 (m, 43H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.31, 154.32, 138.30, 131.65, 130.24, 127.07, 126.69, 125.66, 111.63, 78.98, 62.63, 60.62, 55.21, 52.96, 48.26, 47.43, 42.13, 39.56, 39.09, 38.89, 38.75, 38.61, 36.90, 33.05, 30.63, 29.71, 29.49, 28.07, 27.23, 24.28, 23.56, 23.32, 21.17, 20.48, 18.29, 17.21, 17.02, 16.25, 15.63, 15.44. HRMS (ESI) *m/z* calcd for $C_{44}H_{66}N_3O_4$ $[M+H]^+$ 700.50478, found 700.50458.

3-(4-((3-(Trifluoromethyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8c)White solid. Yield: 75%; m.p. 151–153 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (s, 1H, CH), 7.43 (s, 1H, Ar–H), 7.25 (d, $J=8.6$ Hz, 3H, Ar–H), 5.30 (s, 3H, CH and CH_2), 4.50 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 3.26 (s, 3H, CH and CH_2), 2.25–0.75 (m, 44H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 762 $[M+Na]^+$.

3-(4-((2,4-Dichlorophenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8d)White solid. Yield: 70%; m.p. 59–61 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.75 (d, $J=6.1$ Hz, 1H, CH), 7.39 (s, 1H, Ar–H), 7.21 (s, 1H, Ar–H), 7.10 (s, 1H, Ar–H), 5.31 (m, 3H, CH and CH_2), 4.50 (s, 2H, CH_2), 4.11 (s, 2H, CH_2), 3.22 (s, 1H, CH), 2.81 (s, 2H, CH_2), 2.25–0.74 (m, 44H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 762 $[M+Na]^+$.

3-(4-(Phenoxymethyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8e)White solid. Yield: 70%; m.p. 131–133 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.76 (s, 1H, CH), 7.37–7.29 (m, 2H, Ar–H), 7.06–6.96 (m, 3H, Ar–H), 5.30 (m, 3H, CH and CH_2), 4.50 (s, 2H, CH_2), 4.08 (m, 2H, CH_2), 3.53 (s, 2H, CH_2), 3.21 (s, 1H, CH), 2.37–0.76 (m, 44H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.33, 158.21, 138.32, 129.57, 125.65, 122.74, 121.30, 114.75, 78.99, 62.04, 60.61, 55.21, 52.96, 48.27, 47.44, 42.13, 39.56, 39.09, 38.88, 38.75, 38.61, 36.97, 36.83, 33.04, 30.62, 29.71, 29.50, 28.15, 27.99, 27.22, 24.27, 23.56, 23.32, 21.17, 18.30, 17.22, 17.02, 15.64, 15.45. MS (MALDI-TOF) *m/z* 672 $[M+H]^+$.

3-(4-((Phenylamino)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8f)White solid. Yield: 68%; m.p. 102–104 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.57 (s, 1H, CH), 7.23 (s, 2H, Ar–H), 6.81 (s, 3H, Ar–H), 5.28 (s, 1H, CH), 4.51 (s, 2H, CH_2), 4.41 (s, 2H, CH_2), 4.33 (t, $J=6.7$ Hz, 1H, CH), 4.15–3.93 (m, 2H, CH_2), 3.23 (d, $J=4.5$ Hz, 1H, CH), 2.25–0.74 (m, 45H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 671 $[M+H]^+$.

3-(4-(((2-Fluorophenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8 g)White solid. Yield: 65%; m.p. 145–147 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.78 (s, 1H, CH), 7.37 (s, 2H, Ar–H), 7.18 (d, $J=9.4$ Hz, 2H, Ar–H), 5.31 (s, 1H, CH), 4.53 (s, 2H, CH_2), 4.07 (m, 2H, CH_2), 3.73 (d, $J=7.0$ Hz, 1H, CH), 3.48 (s, 1H, CH), 3.25 (d, $J=6.3$ Hz, 1H, CH), 1.92–0.75 (m, 46H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.31, 138.29, 125.63, 124.60, 121.72, 117.54, 114.66, 114.48, 112.60, 78.98, 60.58, 55.19, 52.94, 48.24, 47.49, 47.30, 42.11, 39.70, 39.54, 39.07, 38.86, 38.74, 38.59, 36.95, 36.80, 33.03, 30.60, 29.69, 29.48, 28.14, 27.96, 27.20, 24.25, 23.54, 23.30, 21.15, 18.28, 17.19, 17.00, 15.62, 15.42. HRMS (ESI) *m/z* calcd for $C_{42}H_{62}FN_4O_3$ $[M+H]^+$ 689.48005, found 689.48004.

3-(4-((p-Tolylamino)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8 h)White solid. Yield: 70%; m.p. 207–208 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.97 (s, 1H, CH), 7.78 (s, 1H, NH), 7.15 (m, 4H, Ar–H), 5.28 (s, 1H, CH), 5.07 (s, 2H, CH_2), 4.53 (s, 2H, CH_2), 4.07 (m, 2H, CH_2), 3.74 (d, $J=7.0$ Hz, 1H, CH), 3.49 (s, 1H, CH), 3.24 (s, 1H, CH), 2.38 (s, 3H, CH_3), 1.93–0.75 (m, 44H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.31, 146.01, 138.28, 129.83, 125.64, 121.89, 113.97, 78.98, 60.59, 55.19, 52.93, 48.24, 47.50, 47.26, 42.11, 40.66, 39.54, 39.07, 38.86, 38.74, 38.60, 36.96, 36.80, 33.03, 30.59, 29.68, 29.48, 28.14, 27.97, 27.21, 24.25, 23.54, 23.30, 21.15, 20.45, 18.29, 17.19, 17.00, 15.63, 15.45. MS (MALDI-TOF) *m/z* 684 $[M+H]^+$.

3-(4-(((3,4-Dimethylphenyl)amino)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (8i).

White solid. Yield: 75%; m.p. 171–173 °C. ^1H NMR (300 MHz, $CDCl_3$) δ 7.84–7.70 (m, 1H, CH), 7.56 (s, 1H, Ar–H), 7.14 (s, 1H, Ar–H), 6.99 (s, 1H, Ar–H), 5.29 (s, 1H, CH), 5.00 (s, 1H, CH), 4.49 (s, 2H, CH_2), 4.33 (s, 1H, CH), 4.11 (m, 2H, CH_2), 3.30–3.18 (m, 1H, CH), 2.28 (m, 9H, CH, CH_2 and CH_3), 1.93–0.78 (m, 43H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.31, 138.28, 137.46, 130.33, 125.64, 121.78, 115.46, 111.00, 79.00, 60.61, 55.20, 52.94, 48.24, 47.51, 47.25, 42.11, 40.55,

39.55, 39.07, 38.86, 38.74, 38.60, 36.97, 36.80, 33.03, 30.59, 29.68, 29.48, 28.14, 27.98, 27.21, 24.25, 23.54, 23.31, 21.15, 20.04, 18.75, 18.29, 17.20, 17.00, 15.62, 15.45. MS (MALDI-TOF) *m/z* 699 $[M+H]^+$.

3-(4-((Benzoyloxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11a)White solid. Yield: 80%; m.p. 160–162 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (d, $J=7.1$ Hz, 2H, Ar–H), 7.73 (s, 1H, CH), 7.56 (d, $J=7.3$ Hz, 1H, Ar–H), 7.44 (d, $J=7.5$ Hz, 2H, Ar–H), 5.49 (s, 2H, CH_2), 5.27 (s, 1H, CH), 4.46 (s, 2H, CH_2), 4.07 (m, 2H, CH_2), 3.21 (s, 1H, CH), 2.26 (d, $J=6.7$ Hz, 2H, CH_2), 2.18–0.73 (m, 44H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 700 $[M+H]^+$.

3-(4-(((2,3-Dimethoxybenzoyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11b)White solid. Yield: 80%; m.p. 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.82 (s, 1H, Ar–H), 7.38–7.32 (m, 1H, CH), 7.11–7.06 (m, 2H, Ar–H), 5.52 (s, 2H, CH_2), 5.27 (s, 1H, CH), 4.48 (s, 2H, CH_2), 4.07 (m, 2H, CH_2), 3.88 (d, $J=1.5$ Hz, 6H, OCH_3), 3.21 (s, 1H, CH), 3.00 (s, 1H, CH), 2.91 (s, 1H, CH), 2.24 (m, 3H, CH and CH_2), 2.05–0.73 (m, 41H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 760 $[M+H]^+$.

3-(4-(((4-Methoxybenzoyl)oxy)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11c)White solid. Yield: 65%; m.p. 63–65 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (d, $J=8.9$ Hz, 2H, Ar–H), 7.70 (s, 1H, CH), 6.91 (d, $J=8.9$ Hz, 2H, Ar–H), 5.45 (s, 2H, CH_2), 5.27 (s, 1H, CH), 4.44 (d, $J=4.7$ Hz, 2H, CH_2), 4.07 (m, 2H, CH_2), 3.86 (s, 3H, OCH_3), 3.21 (s, 1H, CH), 2.25 (m, 3H, CH and CH_2), 2.08–0.73 (m, 43H, protons in UA skeleton). HRMS (ESI) *m/z* calcd for $C_{44}H_{64}N_3O_6$ $[M+H]^+$ 730.47896, found 730.47919.

3-(4-(Benzamidomethyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11d)White solid. Yield: 72%; m.p. 95–97 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.55 (s, 1H, NH), 8.16 (s, 1H, CH), 7.92 (d, $J=7.6$ Hz, 2H, Ar–H), 7.50–7.43 (m, 1H, Ar–H), 7.39 (d, $J=7.3$ Hz, 2H, Ar–H), 5.24 (s, 1H, CH), 4.84 (s, 2H, CH_2), 4.51 (s, 2H, CH_2), 4.17–3.98 (m, 2H, CH_2), 3.24 (d, $J=5.1$ Hz, 1H, CH), 2.34–2.25 (m, 2H, CH_2), 2.17–0.71 (m, 44H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 699 $[M+H]^+$.

3-(4-((2,3-Dimethoxybenzamido)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11e).

White solid. Yield: 68%; m.p. 61–63 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.77 (d, $J=5.4$ Hz, 1H, NH), 7.80 (s, 1H, CH), 7.67 (d, $J=1.6$ Hz, 1H, Ar–H), 7.14 (t, $J=8.0$ Hz, 1H, Ar–H), 7.05 (d, $J=1.5$ Hz, 1H, Ar–H), 5.25 (s, 1H, CH), 4.79 (d, $J=5.7$ Hz, 2H, CH_2), 4.44 (d, $J=4.6$ Hz, 2H, CH_2), 4.31 (s, 1H, CH), 4.10 (m, 2H, CH_2), 3.90 (d, $J=9.0$ Hz, 6H, OCH_3), 3.21 (d, $J=4.6$ Hz, 1H, CH), 2.24 (m, 3H, CH and CH_2), 2.34–2.25 (m, 2H, CH_2), 2.04–0.72 (m, 42H, protons in UA skeleton). HRMS (ESI) *m/z* calcd for $C_{45}H_{67}N_4O_6$ $[M+H]^+$ 759.50551, found 759.50586.

3-(4-((4-Methylbenzamido)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11f)White solid. Yield: 76%; m.p. 112–114 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (d, $J=7.7$ Hz, 2H, Ar–H), 7.66 (s, 1H, CH), 7.21 (d, $J=8.0$ Hz, 2H, Ar–H), 5.28 (d, $J=14.2$ Hz, 1H, CH), 4.69 (d, $J=5.5$ Hz, 2H, CH_2), 4.41 (s, 2H, CH_2), 4.05 (m, 2H, CH_2), 3.20 (s, 1H, CH), 2.38 (s, 3H, CH_3), 2.17–0.72 (m, 46H, protons in UA skeleton). MS (MALDI-TOF) *m/z* 713 $[M+H]^+$.

3-(4-((4-Methoxybenzamido)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11 g)White solid. Yield: 82%; m.p. 118–120 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.80 (d, $J=8.8$ Hz, 2H, Ar–H), 7.68 (s, 1H, CH), 6.86 (d, $J=7.4$ Hz, 2H, Ar–H), 5.23 (s, 1H, CH), 4.64 (d, $J=5.3$ Hz, 2H, CH_2), 4.38 (s, 2H, CH_2), 4.06 (m, 2H, CH_2), 3.80 (s, 3H, OCH_3), 3.18 (s, 1H, CH), 2.16 (m, 5H, CH and CH_2), 2.02–0.70 (m, 41H, protons in UA skeleton). ^{13}C NMR (101 MHz, $CDCl_3$) δ 177.34, 162.34, 138.27, 128.90, 125.67, 113.78, 78.99, 60.73, 55.44, 55.21, 52.94, 48.24, 47.51, 42.11, 39.55, 39.07, 38.86, 38.75, 38.62, 36.97, 36.83, 35.35, 33.04, 30.61, 29.70, 29.50, 28.17, 27.98, 27.22, 24.26, 23.55, 23.31, 21.16, 18.30, 17.20, 17.01, 15.67, 15.46. MS (MALDI-TOF) *m/z* 751 $[M+Na]^+$.

3-(4-((Furan-2-carboxamido)methyl)-1H-1,2,3-triazol-1-yl)propyl(1S,2R,4aS,6aS,6bR,10S,12aR,12bR,14bS)-10-hydroxy-1,2,6a,6b,9,9,12a-heptamethyl-1,3,4,5,6,6a,6b,7,8,8a,9,10,11,12,12a,12b,13,14b-octadecahydropicene-4a(2H)-carboxylate (11 h)White solid. Yield: 78%; m.p. 107–109 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.63 (s, 1H, NH), 7.45 (s, 1H, CH), 7.13 (d, $J=3.4$ Hz, 2H, Fura-H), 6.50 (s, 1H, Fura-H), 5.27 (s, 1H, CH), 4.70 (d, $J=5.9$ Hz, 2H, CH_2), 4.42 (s, 2H, CH_2), 4.06 (m, 2H, CH_2), 3.22 (s, 1H, CH), 2.24 (s, 3H, CH and CH_2), 2.06–0.74 (m, 44H, protons

in UA skeleton). HRMS (ESI) *m/z* calcd for $C_{41}H_{61}N_4O_5$ $[M+H]^+$ 689.46365, found 689.46356.

Evaluation of anti-inflammatory activity in vivo

In the primary screening, all tested compounds, celecoxib was freshly prepared prior to administered i.p. at a dose of 100 mg/kg to mice and at a concentration of 0.2 mL/20 g body weight. In the latter evaluation, compound **11b** and celecoxib were homogenized with 0.5% sodium carboxymethylcellulose (CMC-Na) and administered via the oral route to mice at a concentration of 0.2 mL/20 g mice weight. To explore the peak activity of the compound, edema was quantified at different intervals. Compound **11b** and celecoxib homogenized with 0.5% CMC-Na were administered orally to mice (lower doses of 50 and 25 mg/kg and 0.2 mL/20 g mice body weight).

In vitro cyclooxygenase (COX-1/2) inhibition assay

EIA kit was used to detect the in vitro inhibitory activity of human COX-1/COX-2 for the standard drug and compound **11b** following the instruction of manufacture as mentioned before. Compound **11b** was incubated with the enzyme for 5 min at 25ºC before addition of arachidonic acid and the colorimetric substrate; plate reader was used to measure the absorbance at 590 nm. IC_{50} was performed by using five different concentrations of each compound according to their molecular weight and measuring percentage of enzyme inhibition either for COX-1 or COX-2 enzymes at each concentration.

Molecular docking study

To validate the data obtained by the in vitro COX-2 assay, molecular docking study of **11b** was performed in comparison with celecoxib using COX-2 protein (PDB entry 4COX). AutoDock Vina was used to execute molecular docking. Discovery Studio was used for interaction analysis, binding conformation and making diagrams. Then, the final step was to verify the binding of the UA derivatives with several amino acids associated with observed with the hydrogen bonds and their steric interactions [26, 27].

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References

1. Coussens LM, Werb Z (2002) Inflammation and cancer. Nature 420:860–867. https://doi.org/10.1038/nature01322
2. Lucas SM, Rothwell NJ, Gibson RM (2006) The role of inflammation in CNS injury and disease. Br J Pharmacol 147:S232-240. https://doi.org/10.1038/sj.bjp.0706400
3. Demir Y, Duran HE, Durmaz L, Taslimi P, Beydemir S, Gulçin I (2020) The influence of some nonsteroidal anti-inflammatory drugs on metabolic enzymes of aldose reductase, sorbitol dehydrogenase, and α-glycosidase: a perspective for metabolic disorders. Appl BiochemI Biotech 190:437–447. https://doi.org/10.1007/s12010-019-03099-7
4. Funk CD (2001) Prostaglandins and leukotrienes: advances in eicosanoid biology. Science 294:1871–1875. https://doi.org/10.1126/science.294.5548.1871
5. Pairet M, Engelhardt G (1996) Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. Fundam Clin Pharmacol 10:1–17. https://doi.org/10.1111/j.1472-8206.1996.tb00144.x
6. Penning TD, Tally JJ, Bertenshaw SR, Carter JS, Collins PW, Docter S, Graneto MJ, Lee LF, Malecha JW, Miyashiro JM, Rogers RS, Rogier DJ, Yu SS, AndersonGD BEG, Cogburn JN, Gregory SA, Koboldt CM, Perkins WE, Seibert K, Veenhuizen AW, Zhang YY, Isakson PC (1997) Synthesis and biological evaluation of the 1,5-diarylpyrazole class of cyclooxygenase-2 inhibitors: identification of 4-[5-(4-methylphenyl)-3-(trifluoromethyl)-1H-pyrazol-1-yl]benze nesulfonamide (SC-58635, celecoxib). J Med Chem 40:1347–1365. https://doi.org/10.1021/jm960803q
7. Tally JJ, Bertenshaw SR, Brown DL, Carter JS, Kellogg MS, Koboldt CM, Yuan J, Zhang YY, Seibert K (2000) N-[[(5-methyl-3-phenylisoxazol-4-yl)-phenyl]sulfonyl]propanamide, sodium salt, parecoxib sodium: a potent and selective inhibitor of COX-2 for parenteral administration. J Med Chem 43:1661–1663. https://doi.org/10.1021/jm000069h
8. Liu MC, Yang SJ, Jin LH, Hu DY, Xue W, Song BA, Yang S (2012) Synthesis and cytotoxicity of novel ursolic acid derivatives containing an acyl piperazine moiety. Eur J Med Chem 58:128–135. https://doi.org/10.1016/j.ejmech.2012.08.048
9. Wu J, Ma S, Zhang TY, Wei ZY, Wang HM, Guo FY, Zheng CJ, Piao HR (2019) Synthesis and biological evaluation of ursolic acid derivatives containing an aminoguanidine moiety. Med Chem Res 28:959–973. https://doi.org/10.1007/s00044-019-02349-x
10. Checker R, Sandur SK, Sharma D, Patwardhan RS, Jayakumar S, Kohli V, Sethi G, Aggarwal BB, Sainis KB (2012) Potent anti-inflammatory activity of ursolic acid, a triterpenoid antioxidant, is mediated through suppression of NF-κB, AP-1 and NF-AT. PLoS ONE 7:e31318. https://doi.org/10.1371/journal.pone.0031318
11. Csuk R, Niesen-Barthel A, Schafer R, Barthel A, Al-Harrasi A (2015) Synthesis and antitumor activity of ring a modified 11-keto-β-boswellic acid derivatives. Eur J Med Chem 92:700–711. https://doi.org/10.1016/j.ejmech.2015.01.039
12. Ma LY, Wang B, Pang LP, Zhang M, Wang SQ, Zheng YC, Shao KP, Xue DQ, Liu HM (2015) Design and synthesis of novel 1,2,3-triazole-pyrimidine-urea hybrids as potential anticancer agents. Bioorg Med Chem Lett 25:1124–1128. https://doi.org/10.1016/j.bmcl.2014.12.087
13. Shafi S, Alam MM, Mulakayala N, Mulakayala C, Vanaja J, Kalle AM, Pallu R, Alam MS (2012) Synthesis of novel 2-mercapto benzothiazole and 1,2,3-triazole based bis-heterocycles: their anti-inflammatory and anti-nociceptive activities. Eur J Med Chem 49:324–333. https://doi.org/10.1016/j.ejmech.2012.01.032
14. Vanga NR, Kota A, Sistla R, Uppuluri M (2017) Synthesis and anti-inflammatory activity of novel triazole hybrids of (+)-usnic acid, the major dibenzofuran metabolite of the lichen Usnea

longissima. Mol Divers 21:273–282. https://doi.org/10.1007/s11030-016-9716-5
15. Kinfe HH, Belay YH, Joseph JS, Mukwevho E (2013) Evaluation of the Influence of thiosemicarbazone-triazole hybrids on genes implicated in lipid oxidation and accumulation as potential anti-obesity agents. Bioorg Med Chem Lett 23:5275–5278. https://doi.org/10.1016/j.bmcl.2013.08.028
16. Liu XK, Ye BJ, Wu Y, Lin ZH, Zhao YQ, Piao HR (2011) Synthesis and anti-tumor evaluation of panaxadiol derivatives. Eur J Med Chem 46:1997–2002. https://doi.org/10.1016/j.ejmech.2011.02.022
17. Liu XK, Ye BJ, Wu Y, Nan JX, Lin ZH, Piao HR (2012) Synthesis and antitumor activity of dehydroepiandrosterone derivatives on Es-2, A549, and HepG2 cells in vitro. Chem Biol Drug Des 79:523–529. https://doi.org/10.1111/j.1747-0285.2011.01311.x
18. Wei ZY, Chi KQ, Wang KS, Wu J, Liu LP, Piao HR (2018) Design, synthesis, evaluation, and molecular docking of ursolic acid derivatives containing a nitrogen heterocycle as anti-inflammatory agents. Bioorg Med Chem Lett 28:1797–1803. https://doi.org/10.1016/j.bmcl.2018.04.021
19. Yu B, Qi PP, Shi XJ, Huang RL, Guo H, Zheng YC, Yu DQ, Liu HM (2016) Efficient synthesis of new antiproliferative steroidal hybrids using the molecular hybridization approach. Eur J Med Chem 117:241–255. https://doi.org/10.1016/j.ejmech.2016.04.024
20. Kant R, Singh V, Nath G, Awasthi SK, Agarwal A (2016) Design, synthesis and biological evaluation of ciprofloxacin tethered bis-1,2,3-triazole conjugates as potent antibacterial agents. Eur J Med Chem 124:218–228. https://doi.org/10.1016/j.ejmech.2016.08.031
21. Zheng XJ, Li CS, Cui MY, Song ZW, Bai XQ, Liang CW, Wang HY, Zhang TY (2020) Synthesis, biological evaluation of benzothiazole derivatives bearing a 1,3,4-oxadiazole moiety as potential anti-oxidant and anti-inflammatory agents. Bioorg Med Chem Lett 30:127237. https://doi.org/10.1016/j.bmcl.2020.127237
22. Rao PNP, Amini M, Li HY, Habeeb AG, Knaus EE (2003) Design, synthesis, and biological evaluation of 6-substituted-3-(4-methanesulfonylphenyl)-4-phenylpyran-2-ones: a novel class of diarylheterocyclic selective cyclooxygenase-2 inhibitors. J Med Chem 46:4872–4882. https://doi.org/10.1021/jm0302391
23. Jr BR, Fink RC, Li D, McMichael M, Tower CM, Smith RD, Alberte RS (2009) Pro-inflammatory enzymes, cyclooxygenase 1, cyclooxygenase 2, and 5-lipooxygenase, inhibited by stabilized rice bran extracts. J Med Food 12:615–623. https://doi.org/10.1089/jmf.2008.0133
24. Kandemir FM, Kucukler S, Caglayan C, Gur C, Batil AA, Gulçin I (2017) Therapeutic effects of silymarin and naringin on methotrexateinduced nephrotoxicity in rats: biochemical evaluation of anti-inflammatory, antiapoptotic, and antiautophagic properties. J Food Biochem 41:e12398. https://doi.org/10.1111/jfbc.12398
25. Abdellatif KRA, Abdelall EKA, Lamie PF, Labib MB, El-Nahaas E, Abdelhakeem MM (2020) New pyrazole derivatives possessing amino/methanesulphonyl pharmacophore with good gastric safety profile: design, synthesis, cyclooxygenase inhibition, anti-inflammatory activity and histopathological studies. Bioorg Chem 95:103540. https://doi.org/10.1016/j.bioorg.2019.103540
26. Khan A, Diwan A, Thabet HK, Imran M (2020) Synthesis of novel N-substitutedphenyl-6-oxo-3-phenylpyridazine derivatives as cyclooxygenase-2 inhibitors. Drug Dev Res 81:573–584. https://doi.org/10.1002/ddr.21655
27. Dawood DH, Batran RZ, Farghaly TA, Khedr MA, Abdulla MM (2015) New coumarin derivatives as potent selective COX-2 inhibitors: synthesis, anti-inflammatory, QSAR, and molecular modeling studies. Arch Pharm 348:875–888. https://doi.org/10.1002/ardp.201500274