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COMMUNICATIONS

Semisynthesis of ursolic acid-2-(2-thienylidene)-oxadiazole hybrid molecule and an evaluation of its COX inhibition property

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Abstract

A new derivative of ursolic acid containing dual functionality was synthesized. Molecular docking studies and in vitro analysis indicated promising COX inhibition potential for the molecule. The synthesis, characterization, in silico and in vitro studies of the molecule are described here.

1 | INTRODUCTION

Natural products and their semisynthetic derivatives–based drugs constitute a large percentage of marketed drugs that are routinely used today for the treatment of various diseases.[1] The development of synthetic biology and genetically engineered biosynthesis[2] are increasingly contributing to the development of natural product–based drug discovery *hand in hand* with new methods for isolation and advanced characterizations.[3] Pentacyclic triterpenoids constitute a class of secondary metabolites that could be potential candidates, which can be used for the treatment of various diseases. For instance, the pharmacology of ursolic acid (UA, **1**) and oleanolic acid (OA, **2**) are well studied in the literature (Figure 1),[4] and several of their semisynthetic derivatives have been developed and screened.[5]

UA (3-hydroxy-urs-12-en-28-oic acid) is the main active component in many traditional Chinese medicines.[6] The biological effects of UA include anti-inflammatory,[7] anticancer,[8] antidiabetic,[9] antihepatodamage,[10] and antibacterial[11] effects. The main concerns regarding UA is its low water solubility, which leads to limited bioavailability in human body. Several chemical modifications of UA at C-3, C-12, and C-28 positions have been done in order to tackle this problem;[5b] however, there have not been much reports on the synthesis and evaluation of hybrid molecules from UA. Hybrid molecules refer to structures having two (or more than two) structural domains having different biological functions.[12] It has been reported that Claisen-Schmidt condensation of 3-oxo-UA with various aldehydes led to the synthesis of 2-arylidene derivatives, which showed increased α-glucosidase inhibitory activity.[13] Very recently, it has been reported that di(2-thienylidene) cyclohexanones exhibited increased anti-inflammatory activity via COX and LOX inhibition pathways.[14] Inspired by the above literature reports and due to our continued interest in the synthesis and screening of OA and UA derivatives,[15] we have done the synthesis and evaluation of a novel UA hybrid molecule as discussed below.

2 | RESULTS AND DISCUSSION

UA was isolated from leaves of *Ocimum sanctum* Linn by using standard protocol.[15,16] The crude powder obtained after extraction was column chromatographed using silica gel to yield pure UA as a white powder, and the

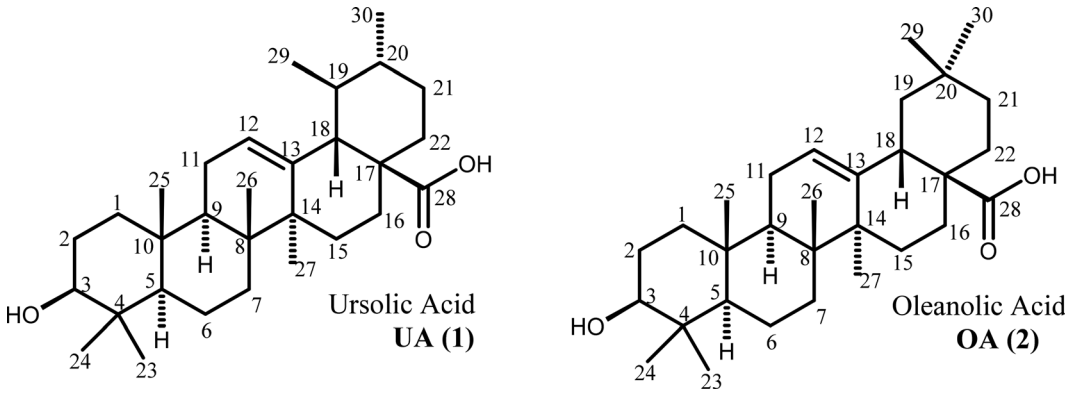
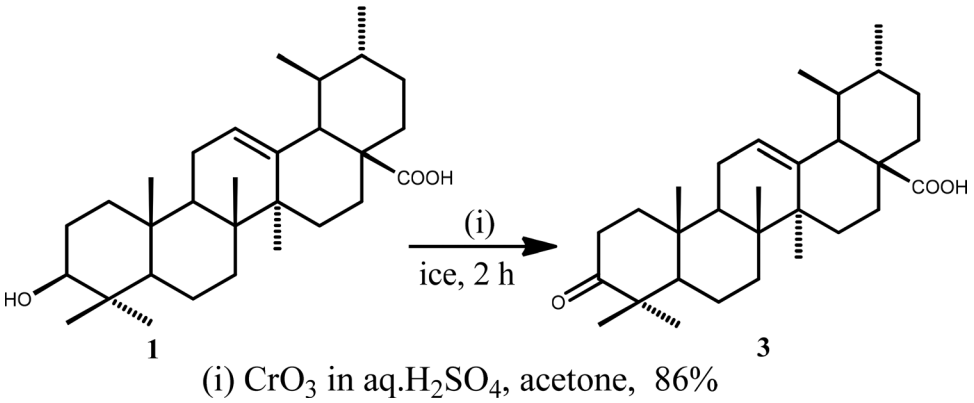


FIGURE 1 Ursolic acid (UA) and oleanolic acid (OA)



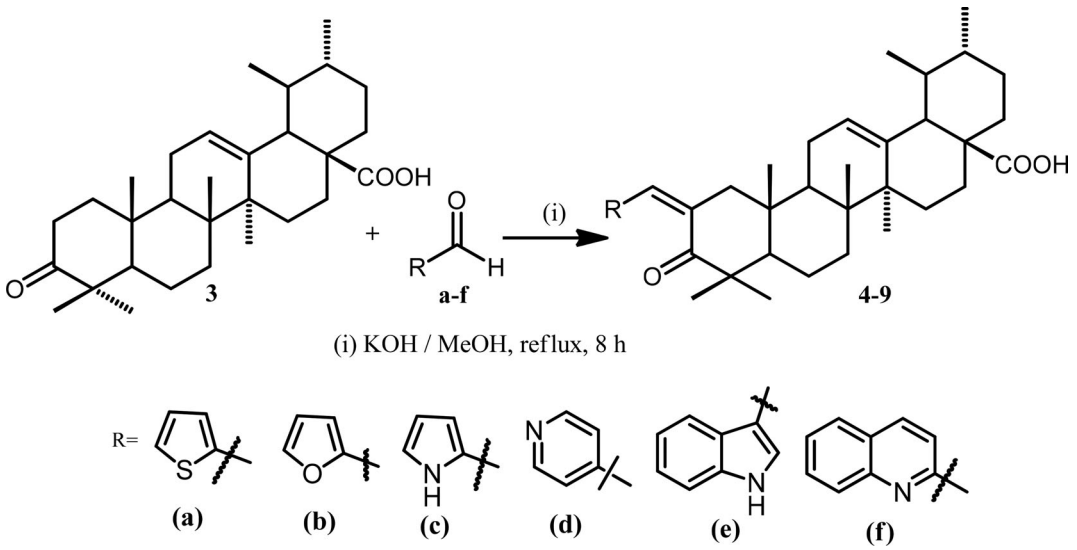
SCHEME 1 Oxidation of ursolic acid (UA) using Jones reagent (CrO_3 in aq.H_2SO_4)

structure was confirmed based on spectral values, which was found to be comparable with the literature.[17] Quantification of UA present in *O Sanctum* Linn was also done using high-performance thin-layer chromatography (HPTLC), and the results are presented in the Supporting Information. The purified UA was then converted to its 3-oxo-derivative (**3**) by reaction with Jones reagent (CrO_3 in aq.H_2SO_4) in acetone for 2 hours following literature procedure (Scheme 1).[18] The resulting product was purified by column chromatography and was characterized by spectroscopic analysis. From the infrared (IR) spectrum, the disappearance of the alcoholic group attached to the C-3 position of UA at 3423 cm^{-1} and the appearance of the carbonyl group at 1687 cm^{-1} indicated the conversion of UA to 3-oxo-urs-12-en-28-oic acid (**3**). In the ^{13}C NMR spectrum, the peak at δ 217.7 ppm indicate the presence of carbonyl carbon at C-3 of **3** while the carbonyl carbon of the acid group was found at δ 184 ppm.

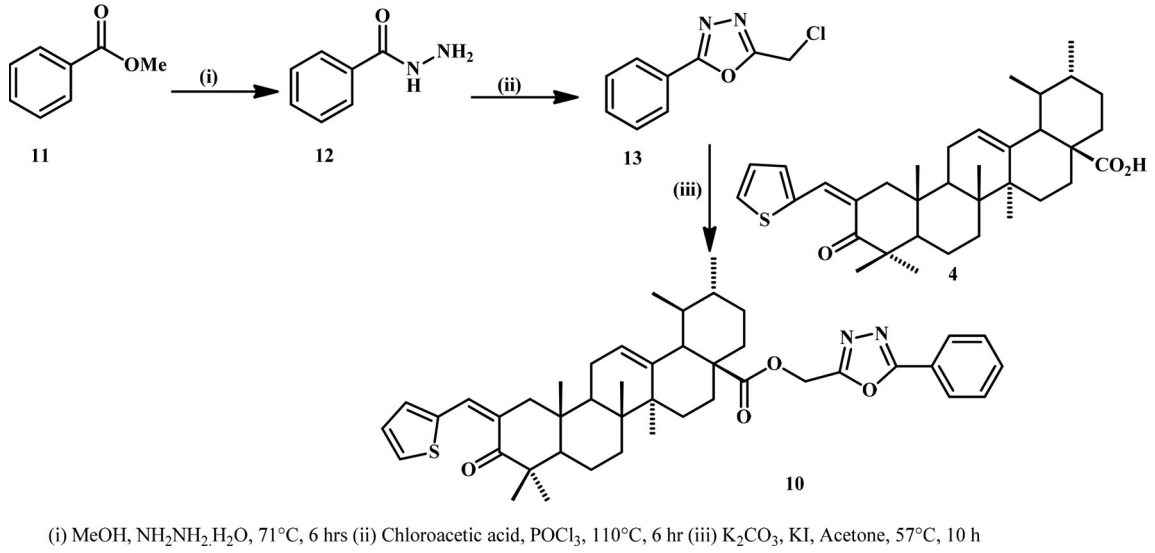
Compound **3** obtained was then subjected to Claisen-Schmidt condensation with various heterocyclic aldehydes **a** to **f**. The reaction was conducted in the presence of methanolic potassium hydroxide by refluxing the mixture for 8 hours[13] as shown in Scheme 2.

It was observed that thiophene-2-aldehyde (**a**) underwent Claisen-Schmidt condensation with **3** yielding the product **4** in 68% yield while only lower yields of the condensation products **5** to **9** were obtained with the other aldehydes **b** to **f** and therefore **4** was taken up for further studies. In the IR spectrum of **4**, the peak at 1684 cm^{-1} was attributed to the carbonyl at C-3 conjugated to the thiophene ring. In the ^1H NMR spectrum, the signals between δ 7.0 and 7.3 ppm indicated the aromatic protons of the thiophene ring and that at δ 6.8 ppm confirmed the presence of the olefin hydrogen in the conjugated system. The signal at δ 5.7 ppm indicated the H attached to C-12 of UA. In ^{13}C NMR spectrum, the aromatic carbons were observed between δ 137.5 and 126.9 ppm. LC-MS analysis for compound **4** revealed a peak at *m/z* 550.350, which confirmed the [M + 2] peak of the thienylidene derivative **4**.

Incorporation of *N*-heterocycles in the C-28 position of UA has been proved to be advantageous in generating anticancer[19] and anti-inflammatory agents.[20] Introduction of the N atom increases the interaction between the molecule and the target as N atom can act as an H-bond donor or acceptor. In particular, studies have shown that 1,3,4-oxadiazoles can facilitate transmembrane diffusion



SCHEME 2 Claisen-Schmidt condensation of **3** with various heterocyclic aldehydes



SCHEME 3 Synthesis of compound **10**

by increasing the lipophilicity of the drug.[21] Cyclooxygenase (COX) enzymes COX-1 and COX-2 produce prostaglandins that induce inflammation, pain, and fever in which prostaglandins from COX-1 is responsible for the protection of stomach and intestinal lining. In addition, COX-1 also activates platelets and is involved in proper kidney function.[22] COX-2 is an inducible enzyme, found only in certain conditions, and is responsible for inflammatory diseases and carcinogenesis.[23] Inhibition of COX-2 is necessary while inhibition of COX-1 causes side effects. Nonselective non-steroidal anti-inflammatory drugs (NSAID) inhibit the platelet aggregation and increase the risk of gastrointestinal ulcers as they inhibit COX-1 also. In this context, it is important to develop anti-inflammatory drugs that exhibit increased COX-2 inhibition. In silico analysis described below has shown that compound **10**, which incorporates a 1,3,4-oxadiazole in the C-28 position of UA, shows increased COX-2 inhibition than compound **4**.

Compound **10** was obtained by reacting **4** with 2-chloromethyl-5-phenyl-1,3,4-oxadiazole **13** in the presence of KI/K_2CO_3 in acetone under reflux following a reported procedure.[20] Solvent was removed by evaporation, and residue was subjected to column chromatography to obtain the desired product. Scheme 3 outlines the synthetic strategy adopted for synthesis of compound **10**.

The IR spectrum of compound **10** showed peaks at 1732 and 1654 cm^{-1} corresponding to the ester and

TABLE 1 Docking results of compounds **4**-**10**, UA and celecoxib with COX-2 (PDB: 3NTG)

Compound	Libdock Score	H bond Forming Residues	No. of Hydrogen Bonds	Hydrogen Bond Distance
4	107.42	THR198	1	2.40
5	93.20	THR198	1	1.84
6	100.99	THR198	1	2.66
7	105.32	THR198	1	2.48
8	118.39	ASN368	1	2.35
9	82.716	GLN440	1	1.64
10	127.48	HIS372 THR198	2	2.06 1.91
UA	89.54	TYR134 THR198 ASN368	3	2.78 2.45 1.79
Celecoxib	82.85	TRP373	1	2.69

conjugated ketone carbonyls, respectively. In the proton NMR, aromatic protons of the phenyl ring and thiophene were observed between δ 8.06 and 7.31 ppm. Olefinic proton adjacent to the thiophene ring was observed at δ 7.1 ppm. The methylene protons adjacent to the ester carbonyl carbon were found at δ 5.40 to 5.23 ppm. The absence of peak at 181.8 ppm in the ^{13}C NMR and formation of a new peak at 175.6 ppm indicated the ester carbonyl formation. Alkene carbons in the diazole moiety was found at δ 165.7 and δ 162.6 ppm. The bridged methylene carbon between the ester and oxadiazole moiety is found at δ 59.5 ppm. Mass spectral analysis also confirmed the structure of the compound.

Concurrently, an in silico analysis of the COX-2 inhibition by the heteroarylidene products were studied using BIOVIA, Discovery studio, 2018. The X-ray crystallographic structure file of COX-2 (PDB Id: 3NTG) with resolution 2.19 Å was retrieved from the protein data bank. Prior to docking, all crystallographic water molecules and bound ligands were removed manually.[24a] Protonation, optimization of side chain conformation, and missing loop modelling were done based on the protein preparation protocol of Discovery studio.[24b] Compounds **4** to **9** and **10**, UA, and standard anti-inflammatory drug celecoxib were prepared and were docked with target protein and binding interaction of ligands, and protein were studied using Ligplot. Table 1 shows the docking results obtained.

UA interacts with COX-2 by forming three hydrogen bonds with ASN 368, THR198, and TYR134 and was found to have a libdock score of 89.54. The amino acid residue THR198 in COX-2 interacts with compound **4** via a hydrogen bond with 107.42 as the docking score. Compound **5** shows libdock score of 93.20 by forming hydrogen bond with THR198, and hydrogen bond distance was 1.84 Å. Compound **6** shows a libdock score of 100.99, and it was observed that amino acid residue is THR198 with a hydrogen bond distance of 2.66 Å. The interacting amino acid residue THR198 for compound **7** with a hydrogen bond 2.48 Å produces a libdock score of 105.32. For compound **8**, docking score observed was 118.39 with a hydrogen bond (2.35 Å) formed with residue ASN 368. For compound **9**, one direct interaction with the residue of the binding cavity of COX-2 through GLN440 having a hydrogen bond with a distance of 1.64 Å was observed. Celecoxib, which was used as reference drug, showed libdock score of 82.85, and the hydrogen bond forming amino acid residue was TRP 373 with a distance of 2.69 Å. The most important finding from the docking study was that compound **10** shows highest libdock score (127.48) having two hydrogen bond amino acid residues (HIS372 and THR198). From the in silico studies, it was found that most of the molecules have better affinity towards COX-2 than that of UA and Celecoxib. Interaction of these ligands and proteins is given in Figure 2 in which A to D show the binding poses of compounds **4** and **10**, UA, and celecoxib and E to H show 2-D molecular docking models of compounds **4** and **10**, UA, and celecoxib with COX-2, respectively.

The compounds **4** and **10** along with UA were subjected to in vitro anti-inflammatory analysis.[25] Lipopolysaccharide (LPS)-stimulated RAW cells were used for the study, which were exposed to different concentrations (25, 50, and 100 μg/mL) of sample solutions and incubated for 24 hours. After incubation, the COX inhibitory assays were performed using the cell lysate. COX activity was determined by reading absorbance at 632 nm, and the results are summarized in Table 2.

It was also found that the percentage inhibition increases with increase in concentration of the samples,

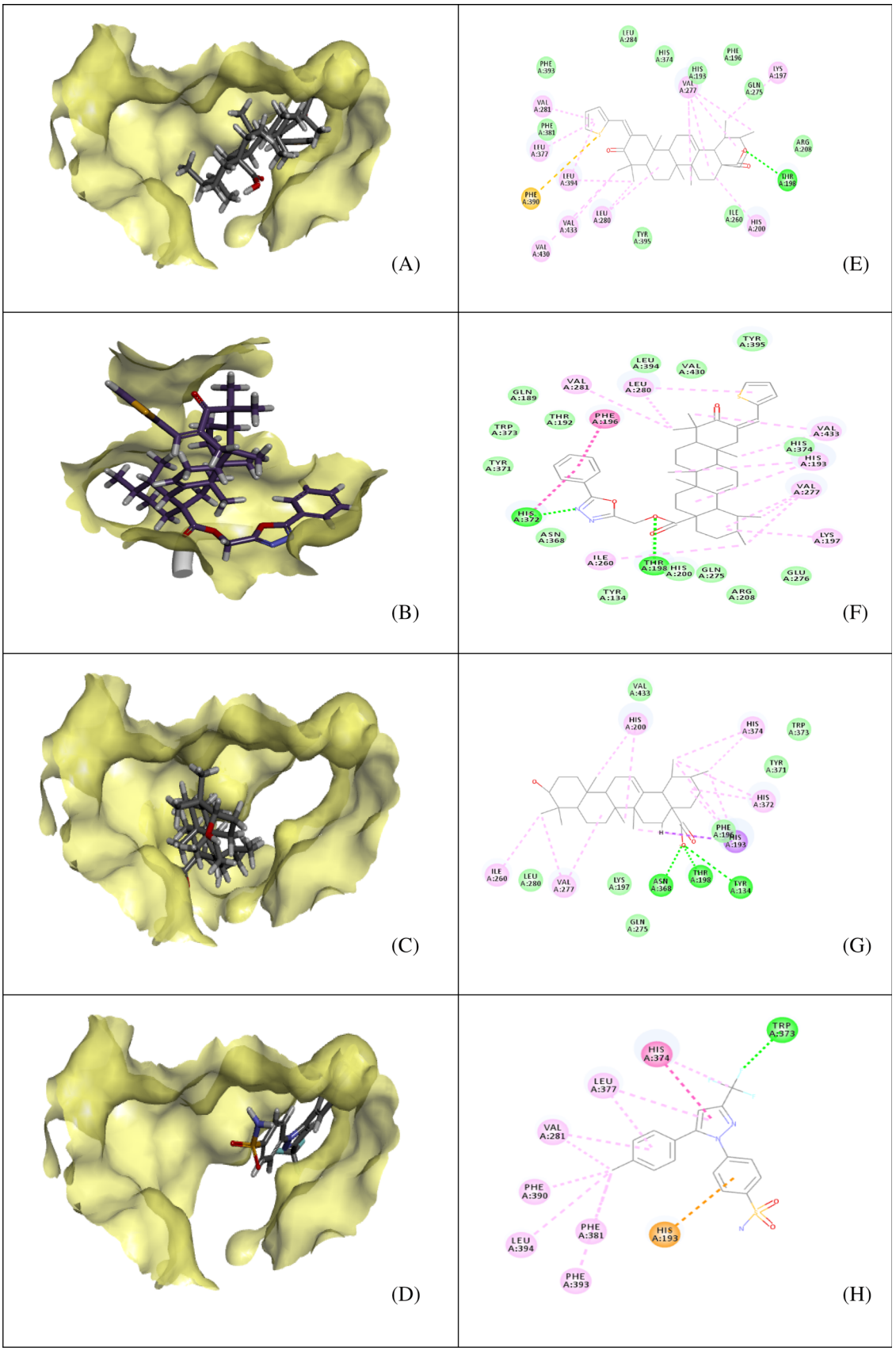


FIGURE 2 Docking of compounds **4** and **10**, ursolic acid (UA), and celecoxib with COX-2

TABLE 2 COX inhibitory assay of UA, **4**, and **10**

Compound	Concentration (μg/mL)	OD at 632 nm	Inhibition (%)
UA	Control	*0.2135*	*0.00*
	25	0.1537	28.01
	50	0.0411	33.91
	100	0.1309	38.69
4	Control	*0.1946*	*0.00*
	25	0.1531	21.33
	50	0.1356	30.32
	100	0.1216	37.51
10	Control	*0.1834*	*0.00*
	25	0.1397	23.83
	50	0.1267	30.92
	100	0.1020	44.38

Note: Control (italic values) refers to unstimulated RAW cells.

which proves that the compounds acted in a dose-dependent manner. in vitro studies have showed that 44.38% COX inhibition could be achieved using compound **10** at a concentration of 100 ppm.

3 | CONCLUSION

Semisynthetic hybrid derivative of UA containing thiophene and 1,3,4-oxadiazole was synthesized in the present study. From the in silico and in vitro studies, it is found that the hybrid molecule **10** showed highest docking score and COX inhibition activity. COX-2 has long been considered as a target for anticancer drug development, and combination treatments of chemotherapy with COX-2 inhibitors have shown promising results.[26] Taking into account the tremendous potential for developing UA as an anticancer agent,[8b] the current study has a significance in bringing out a dual role for the semisynthetic derivative, as a COX inhibitor possessing anticancer properties. Further studies to find the in vitro selectivity associated with COX 2 inhibition and in vivo studies are underway.

4 | EXPERIMENTAL

4.1 | Instrumentation

Heterocyclic aldehydes were purchased from Spectrochem Pvt. Ltd Mumbai and was used without further purification. Solvents and other reagents were obtained from local suppliers. Detailed synthetic procedure and characterization data of compounds are given below. Analytical thin-layer chromatography (TLC) was performed on silica gel coated on aluminum sheets and was monitored using UV light of wave length 254 nm. Column chromatography was done using silica gel (60-120 and 100-200 mesh). Fourier transform infrared (FTIR) was done using Agilient CARY 630 FTIR instrument. ^{1}H NMR and $^{13-}$C NMR of the compounds were recorded with Bruker 400-MHz spectrometer using $CDCl_3$ or DMSO-d_6 as solvents. LC-MS analysis was performed by SHIMADZU Triple quadrupole LCMS/MS-8045 coupled with LC-30AD liquid chromatogram instrument equipped with electrospray ionization (ESI) mode. Melting points were recorded on an electrochemical digital melting point apparatus, Analab Scientific instruments Pvt. Ltd, and are uncorrected. HPTLC profile was recorded using DESKTOP-60R1I2G, version 2.5.18053.1. For molecular docking study, the software used was BIOVIA Discovery studio, 2018. Anti-inflammatory assay was carried out at Biogenix Research Center.

4.2 | Plant material

UA was extracted from Tulsi leaf (*O Sanctum* L) powder that is purchased from Krishna Ayurvedic Stores, Trivandrum.

4.3 | Extraction and isolation of UA

About 500 g of Tulsi leaf powder was subjected to soxhlet extraction using hexane and ethyl acetate as solvents successively. The ethyl acetate extract was collected, and the solvent was removed by rotary evaporator. The residue was purified by column chromatography on silica gel (60-120 mesh). On elution with a mixture of hexane and ethyl acetate (80:20), UA was obtained as a white powder (250 mg of UA was obtained after column chromatography of 2-g ethyl acetate extract). The peak at 3432 cm^{-1} in the IR spectrum of UA corresponded to the OH stretching vibration while that at 1691 cm^{-1} was attributed to the carbonyl stretch of the COOH group.

4.4 | Molecular docking study

4.4.1 | Protein preparation

The X-ray crystallographic structure file of COX-2 (3NTG) with resolution 2.19 Å was retrieved from Protein Data Bank (PDB). Prior to docking, all crystallographic water molecules and bound ligands were removed

manually. Protonation, optimization of side chain conformation, and missing loop modelling were done based on protein preparation protocol of discovery studio.

4.4.2 | Molecular docking

Binding site for target protein was defined using PDB site records, and site sphere coordinates were set as 24.9159, 34.8601, 40.3226, and 12.6 Å. UA, seven of its derivatives (**4-10)**, UA, and standard drug celecoxib were prepared and allowed to dock with target protein. Number of hotspots were set as 100, docking tolerance as 0.25. All other parameters were set as default, and FAST was selected as conformation method with maximum conformation of 255. Docking results of UA and its seven derivatives were compared with the standard drug celecoxib and are reported in the manuscript.

4.5 | LC-MS analysis

The compound **4** was analyzed by SHIMADZU Triple quadrupole LCMS/MS-8045 coupled with LC-30AD liquid chromatogram instrument. Chromatographic separation performed on Shim-pack GIST C_8 column (150 × 2.1 mm, I.D., 2 μm) using methanol as mobile phase system at a flow rate of 0.2 mL/min. Column temperature was set at 40°C. Mass spectrometric analysis was performed with an ESI source. The Auto MS operation parameters were described as follows: Nebulizing gas (N_2) flow, drying gas flow, and heating gas flow were set as 3.0, 10.0, and 10.0 L/min, respectively. Interface voltage set as 3.0 kV having an interface current 6.2 μA and interface temperature maintained at 300°C. Detector voltage is 1.80 kV. Both full scan mode and single ion monitoring of compound **4** were done at the range of *m/z* 50 to 1000 in positive ion mode. A peak at *m/z* 550.350 confirmed the [M + 2] peak of the thienylidene derivative **4**.

4.6 | Anti-inflammatory assay

RAW 264.7 cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India, and maintained Dulbecco's modified Eagle's medium (DMEM) (Sigma-Aldrich, USA).

The cell line was cultured in 25-cm^3 tissue culture flask with DMEM supplemented with 10% Fetal bovine serum (FBS), L-glutamine, sodium bicarbonate (Merck, Germany), and antibiotic solution containing penicillin (100 U/mL), streptomycin (100 μg/mL), and Amphoteracin B (2.5 μg/mL). Cultured cell lines were kept at 37°C in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany). The cells were grown to 60% confluency followed by activation with 1-μL LPS (1 μg/mL). LPS-stimulated RAW cells were exposed with different concentrations (25, 50, and 100 μg/mL) of sample solution and incubated for 24 hours. After incubation, the anti-inflammatory assays were performed using the cell lysate.

The COX activity was assayed by the method of Walker and Gierse; 100-μL cell lysate was incubated with Tris-HCl buffer (pH 8), glutathione 5mM/L, and hemoglobin 5mM/L for 1 minute at 25°C. The reaction was initiated by the addition of arachidonic acid 200mM/L and terminated after 20-minute incubation at 37°C, by the addition 200 μL of 10% trichloroacetic acid in 1 N hydrochloric acid. After the centrifugal separation and the addition of 200 μL of 1% thiobarbiturate, the tubes were boiled for 20 minutes. After cooling, the tubes were centrifuged for 3 minutes. COX activity was determined by reading absorbance at 632 nm.

Percentage inhibition of the enzyme was calculated as

$$\%\text{inhibition} = \frac{\text{Absorbance of control} - \text{Absorbance of test}}{\text{Absorbance of control}} \times 100.$$

4.6.1 | Procedure for synthesis of compound 4

To a stirring solution of potassium hydroxide (2 equiv., 0.35 mmol, 19 mg) in methanol (5 mL), compound **3** (1 equiv., 0.175 mmol, 80 mg) dissolved in methanol (5 mL) was added drop wise using a pressure equalizer. Then thiophene-2-aldehyde **a** (1 equiv., 0.175 mmol, 19 mg) was added to the above solution. The reaction mixture was stirred for 8 hours at 65°C. The completion of the reaction was tested by TLC analysis. Solvent was removed, and reaction mixture was worked up using methylene dichloride/water; the combined organic layers were dried over anhydrous sodium sulfate, and the residue was dried in vacuo, and the product **4** obtained was further purified by column chromatography using silica gel (100-200 mesh) as stationary phase and mixture of 10% EtOAc-Hexane as mobile phase to obtain amorphous powder.[6] Yield 68%. Melting point 167-169°C. FTIR (neat, ν cm^{-1}): 2917, 2850, 1684, 1650, 1456, 1374. ^{1}H NMR (400 MHz, $CDCl_3$): 7.31 (d, 1H), 7.02-7.00 (m, 2H), 6.83 (s, 1H), 5.70 (s, 1H), 3.77 (s, 1H), 3.73 (s, 1H), 2.48 (s, 1H), 2.32 (t, 2H), 2.07 (t, 4H), 1.64 (s, 1H), 1.51-1.41 (m, 10H), 1.39-1.32 (t, 5H), 1.29-1.06 (m, 5H), 1.00 (s, 6H), 0.98 (s, 1H), 0.94 (s, 3H), 0.86 (s, 1H), 0.84 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): 206.0, 181.8, 137.5, 133.0, 130.8, 128.6, 128.5, 128.0, 126.9, 49.7, 45.5, 44.9,

42.6, 38.2, 37.7, 37.5, 36.9, 36.7, 32.6, 30.9, 29.6, 28.4, 27.0, 26.3, 24.9, 22.4, 21.6, 20.8, 18.1, 17.7, 16.0, 15.9, 14.4, 13.1. LC/MS-ESI: m/z calculated for $[C_{35}H_{48}O_3S + 2]$: 550.332; found: 550.330.

4.6.2 | Procedure for synthesis of compound 10

A mixture of compounds **4** (1 equiv., 0.182 mmol, 100 mg) and **13** (1 equiv., 0.182 mmol, 0.035 mg) was refluxed in 10-mL acetone at 57°C for 10 hours in the presence of KI (2 equiv., 0.364 mmol, 60.42 mg)/K_2CO_3 (2 equiv., 0.364 mmol, 49.14 mg). Solvent was removed under reduced pressure, and reaction mixture was worked up using DCM/brine solution and was dried over Na_2SO_4. Residue was purified by silica gel column chromatography, and product was eluted with 10% EtOAc: hexane solvent in 64% yield. Melting point 204-207°C. FTIR (neat, ν cm^{-1}): 2928, 2865, 1732, 1654, 1575, 1382. $^{1-}$H NMR (400 MHz, $CDCl_3$): 8.06-8.02 (m, 2H), 7.71 (s, 1H), 7.55-7.48 (m, 3H), 7.33-7.31 (m, 1H), 5.74 (s, 1H), 5.40-5.23 (m, 2H), 2.52-2.49 (m, 3H), 2.2381-2.23 (m, 6H), 1.74-1.6 (m, 7H), 1.58-1.40 (m, 5H), 1.39-1.30 (m, 3H), 1.25 (s, 1H), 1.17 (s, 2H), 1.14-1.10 (m, 6H), 1.09-1.06 (m, 4H), 1.01-0.97 (m, 4H), 0.96-0.88 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): 206.5, 175.6, 165.7, 162.6, 139.4, 133.9, 132.5, 132.0, 131.7, 130.8, 129.8, 129.1, 128.9, 127.4, 126.9, 126.8, 123.9, 123.2, 59.5, 55.2, 54.8, 52.6, 47.9, 47.3, 44.9, 43.8, 38.7, 38.6, 37.7, 35.8, 31.6, 29.7, 29.6, 28.4, 27.3, 23.7, 21.7, 21.0, 20.8, 20.4, 19.0, 18.5, 17.1, 16.9. HRMS-ESI (m/z) calculated for $[C_{44}H_{54}N_2O_4S$ -1]: 706.3804; found: [M-1]: 705.3800.

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REFERENCES

[1] D. J. Newman, G. M. Cragg, *J. Nat. Prod.* **2007**, *70*, 461.
[2] M. Q. Zhang, *Curr. Op. Biotech.* **2007**, *18*, 478.
[3] Q. W. Zhang, L. -G. Lin, W. -C. Ye, *Chin. Med.* **2018**, *13*, 20.
[4] J. Liu, *J. Ethnopharmacol.* **1995**, *49*(2), 57.
[5] (a) T. B. Ayeleso, M. G. Matumba, E. Mukwevho, *Molecules* **2017**, *22*, 1915. (b) S. Mlala, A. O. Oyedeji, M. Gondwe, O. O. Oyedeji, *Molecules* **2019**, *24*, 2751.
[6] M. C. Liu, S. J. Yang, L. H. Jin, D. Y. Hu, W. Xue, B. An Song, S. Yang, *Eur. J. Med. Chem.* **2012**, *58*, 128.
[7] M. K. Shanmugam, X. Dai, A. P. Kumar, B. K. Tan, G. Sethi, A. Bishayee, *Biochem. Pharmacol.* **2013**, *85*(11), 1579.
[8] (a) D. K. Kim, J. H. Baek, C. M. Kang, M. A. Yoo, J. W. Sung, D. K. Kim, K. W. Kim, *Int. J. Cancer.* **2000**, *87*(5), 629. (b) P. Liu, R. Du, X. Yu, *Med. Sci. Monit.* **2019**, *25*, 1283.
[9] P. P. Wu, K. Zhang, Y. J. Lu, P. He, S. Q. Zhao, *Eur. J. Med. Chem.* **2014**, *80*, 502.
[10] B. Saraswat, P. K. Visen, D. P. Agarwal, *Phytother. Res.* **2000**, *14*(3), 163.
[11] D. Chattopadhyay, G. Arunachalam, A. B. Mandal, T. K. Sur, S. C. Mandal, S. K. J. Bhattacharya, *Ethnopharmacol.* **2002**, *82* (2–3), 229.
[12] B. Meunier, *Acc. Chem. Res.* **2008**, *41*, 69.
[13] P. -P. Wu, B. -J. Zhang, X.-P. Cui, Y. Yang, Z.-Y. Jiang, Z.-H. Zhou, Y. Y. Zhong, Y.-Y. Mai, Z. Ouyang, H.-S. Chen, J. Zheng, S.-Q. Zhao, K. Zhang, *Sci. Rep.* **2017**, *7*, 45578. https://doi.org/10.1038/srep45578.
[14] S. Kar, G. Ramamoorthy, S. Sinha, M. Ramanan, J. K. Pola, N. R. Golakoti, J. B. Nanubolu, S. K. Sahoo, R. B. Dandamudi, M. Doble, *New J. Chem.* **2019**, *43*, 9012.
[15] N. S. Ratheesh, A. Deepthi, K. Adarsh, *S. Ind. J. Chem. Sec B* **2016**, *55B*(5), 598.
[16] J. B. Harborne, *Phytochemical Methods - A Guide to Modern Technique of Plant Analysis*, 2nd ed., Dordrecht: Springer Science & Business Media, **1984**.
[17] S. C. Gnoatto, A. Dassonville-Klimpt, S. Da Nascimento, *K.; Gosmann, G.; Moslemi, S. Eur. J. Med. Chem.* **2008**, *43*(9), 1865.
[18] T. Honda, H. J. Finlay, G. W. Gribble, N. Suh, M. B. Sporn, *Chem. Lett.* **1997**, *7*(13), 1623.
[19] S. -X. Hua, R. -Z. Huang, M. -Y. Ye, Y. -M. Pan, G. -Y. Yao, Y. Zhang, H. -S. Wang, *Eur. J. Med. Chem.* **2015**, *95*, 435.
[20] Z. -Y. Wei, K. -Q. Chi, K. -S. Wang, J. Wu, L. -P. Liu, H. -R. Piao, *Bioorg. Med. Chem. Lett.* **2018**, *28*, 1797.
[21] S. Bajaj, V. Asati, J. Singh, P. P. Roy, *Eur. J. Med. Chem.* **2015**, *97*, 124.
[22] K. D. Rainsford, S. Tsang, R. H. Hunt, N. Al-Jehani, *Inflammopharmacology.* **1995**, *3*(4), 299.
[23] Y. Li, W. He, T. Liu, Q. Zhang, *Mol. Diagn. Ther.* **2010**, *14*(6), 351.
[24] (a)F. C. Bernstein, T. F. Koetzle, G. J. Williams, E. F. Meyer Jr.., M. D. Brice, J. R. Rodgers, M. J. Tasumi, *Mol. Bio.* **1977**, *112*(3), 535; (b)B. I. O. V. I. A. Dassault Systèmes, *Discovery Studio Client v 18.01.100.18065*, San Diego, Dassault Systèmes **2018**.
[25] M. C. Walker, J. K. Gierse, *Mol. Biol.* **2010**, *644*, 131.
[26] J. -B. Méric, S. Rottey, K. Olaussen, J. -C. Soria, D. Khayat, O. Rixe, J. -P. C. Spano, *Rev. Oncol.* **2006**, *59*, 51.

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