Asian Journal of Chemistry; Vol. 29, No. 11 (2017), 2559-2564
ASIAN JOURNAL OF CHEMISTRY
https://doi.org/10.14233/ajchem.2017.20865

Design, Synthesis, Molecular Docking and Biological Evaluation of Novel Coumarin-Oxime Ether Derivatives as COX-2 Inhibitors

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Received: 15 June 2017; *Accepted*: 28 August 2017; *Published online*: 29 September 2017; AJC-18591

Coumarin-oxime ether derivatives **(14-25)** were synthesized by an efficient and straight forward procedure from the reaction of 3-acetyl coumarin **(1)** and *o*-substituted benzyl hydroxyl amines **(2-13)** in pyridinium *p*-toluenesulfonate/dichloromethane (PPTS/DCM) at reflux temperature. High yields and simple operations are important features of this methodology. The method is very useful for the construction of many biologically active oxime ether derivatives. The structures of the synthesized compounds are established based on IR, NMR and MASS spectrometry. Molecular docking studies were performed against selective COX-2 enzyme using Discovery Studio v3.5. The compounds with good LibDock score were screened for their *in vivo* anti-inflammatory activity by paw edema method, employing indomethacin as a reference standard.

Keywords: 3-Acetyl coumarin, Benzyl hydroxylamines, Oxime ethers, Docking, COX-2.

INTRODUCTION

Coumarin and its derivatives are some of the most important oxygen heterocycles and are extensively found in various natural and synthetic products [1]. These compounds are broadly classified as, simple coumarins, furanocoumarins, pyranocoumarins, biscoumarins, triscoumarins and coumarinolignans. They are effective pharmacophores, widely used in the drug design and synthesis of novel bioactive compounds [2]. They possess different biological activities such as anticoagulant and cardiovascular activities [3], antimicrobial activities [4], anticancer [5], anti-inflammatory and antioxidant [6], antiviral [7] and enzyme-inhibition effect [8]. At this juncture, fused coumarin derivatives have attracted attention because of their biological properties [9], like antiproliferative [10], anti-inflammatory [11] activities.

The oxime ether name is an abbreviation of oxy-imine ether. The oxime ether moiety (Fig. 1) is a privileged group in chemistry due to its presence in a large number of medicinal scaffolds that exhibit a broad range of biological and pharmaceutical properties, such as antifungal [12] antibacterial [13], anti-enteroviral [14], antiprotozoal [15], anti-inflammatory [16,17], anticonvulsant [18], anticancer [19], antitumor [20] activities. Therefore, oxime-ether moiety is usually chosen as an efficiently active group in the drug discovery.

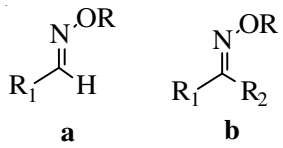
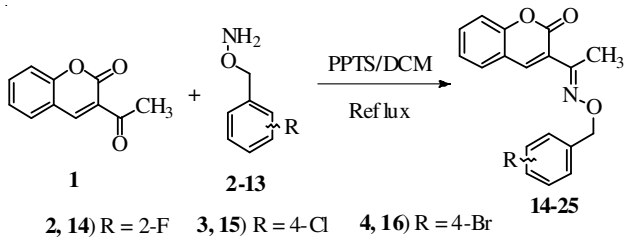


Fig. 1. General structure of aldoximes (a) and ketoxime *o*-ethers (b)

However, up to date, oxime-ethers containing coumarin moiety has not been reported much. With the aim to seek new oxime-ether with high anti-inflammatory activity, here we introduced oxime-ether moiety into coumarin structure according to the sub-structure link way and synthesized twelve novel coumarin oxime-ethers. The structures of the synthesized compounds were assigned on the basis of spectral analysis and elemental analysis. The compounds were evaluated for their anti-inflammatory activity.

EXPERIMENTAL

There are two methods for the synthesis of oxime-ethers [21,22]. In the present study, we have synthesized a novel series of coumarin-oxime ethers **(14-25)** *via* condensation reaction of 3-acetyl coumarin **(1)** and *o*-substituted benzylhydroxylamines **(2-13)** in PPTS/DCM (pyridinium *p*-toluenesulfonate/dichloromethane) at reflux temperature in a single step shown in **Scheme-I**.



2, 14) R = 2-F **3, 15**) R = 4-Cl **4, 16**) R = 4-Br
5, 17) R = 2-Cl **6, 18**) R = 4-F **7, 19**) R = 2,4-Dichloro
8, 20) R = 2-OCH_3 **9, 21**) R = 4-OCH_3 **10, 22**) R = 4-CN
11, 23) R = 2-CN **12, 24**) R = 3-CN **13, 25**) R = 3-OCH_3

Scheme-I: Synthesis of coumarin oxime ethers (**14-25**)

Initially, 3-acetyl coumarin (**1**) was synthesized from the reaction of salicyaldehyde and ethyl acetoacetate in the presence of pyridine and ethyl alcohol. N-Hydroxy phthalimide was alkylated with substituted benzyl halides in the presence of K_2CO_3 and DMF at room temperature. These intermediates are reacted with hydrazine hydrate in ethanol at reflux temperature to give *o*-(substituted benzyl)hydroxylamines (**2-13**) [23]. Coumarin oxime ethers (**14-25**) were synthesized from the reaction of 3-acetyl coumarin (**1**) and *o*-substituted benzylhydroxylamines (**2-13**) in PPTS/DCM at reflux temperature.

General procedure for the synthesis of compounds (14-25): To a stirred solution of 3-acetyl coumarin (**1**) (150 mg, 0.79 mmol) in DCM (15 mL) was added to PPTS (240 mg, 0.95 mmol) along with O-(2-fluorobenzyl)hydroxylamine **(2)** (134 mg, 0.95 mmol) and refluxed continued for 3 h. After completion of the reaction by TLC detection, Water (30 mL) and DCM (15 mL) were added and layers separated, dried over Na_2SO_4 and concentrated to yield the crude oxime ether, which was further purified by column chromatography using silca gel (60 × 120) by eluting with pet. ether:ethyl acetate (9:1) to yield pure compound.

3-(1-(2-Fluorobenzyloxyimino)ethyl)coumarin (14): Yield: 90 %; white solid; m.p.: 137-138 °C; IR (KBr, ν_{max}, cm^{-1}): 2890-2910, 1721 (-C=O), 1609 (-C=N), 1039 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 2.28 (s, $-CH_3$), 5.30 (s, $-OCH_2$), 7.16-7.17 (m, H-5' & H-6'), 7.28-7.30 (m, H-6, H-3' & H-8), 7.42-7.46 (m, H-7), 7.52-7.54 (m, H-5 & H-4'), 7.87 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 ($-CH_3$), 70.2 ($-OCH_2$), 115.2 (C-3'), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 124.5 (C-5'), 125.2 (C-3), 125.4 (C-6), 127.9 (C-5), 128.4 (C-7), 128.7 (C-6'), 128.9 (C-1'), 129.2 (C-4'), 137.0 (C-4), 159.5 (C-2'), 163.2 (-C=N), 166.0 (C-2), DIPMS: *m/z* at 312.3 (M+1), Anal. calcd. (%) for $C_{18}H_{14}NO_3F$: C, 69.45; H, 4.53; N, 4.50. Found: C, 69.54; H, 4.51; N, 4.56.

3-(1-(4-Chlorobenzyloxyimino)ethyl)coumarin (15): Yield: 95 %; white solid; m.p.: 173-174 °C; IR (KBr, ν_{max}, cm^{-1}): 2892-2912, 1723 (-C=O), 1607 (-C=N), 1041 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.31 (s, $-CH_3$), 5.24 (s, $-OCH_2$), 7.28-7.41 (m, H-2' to H-6', H-6 & H-8), 7.52-7.54 (m, H-5 & H-7), 7.87 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 ($-CH_3$), 77.0 ($-OCH_2$), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 125.4 (C-6), 125.5 (C-3), 127.9 (C-5), 128.3 (C-7), 129.0 (C-6'), 128.9 (C-3' & C-5'), 129.7 (C-2' & C-6'), 133.2 (C-4'), 135.4 (C-1'), 137.0 (C-4), 163.2 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 328.7 (M+1), Anal. calcd. (%) for $C_{18}H_{14}NO_3Cl$: C, 65.96; H, 4.31; N, 4.27. Found: C, 65.95; H, 4.33; N, 4.26.

3-(1-(4-Bromobenzyloxyimino)ethyl)coumarin (16): Yield: 95 %; white solid; m.p.: 213-214 °C; IR (KBr, ν_{max}, cm^{-1}): 2884-2892, 1725 (-C=O), 1604 (-C=N), 1042 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.27 (s, $-CH_3$), 5.17 (s, $-OCH_2$), 7.26-7.34 (m, H-2', H-6' & H-6, H-8), 7.48-7.56 (m, H-5, H-3' & H-7, H-5'), 7.83 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.6 ($-CH_3$), 77.2 ($-OCH_2$), 115.1 (C-4a), 116.1 (C-8), 118.1 (C-8a), 122.0 (C-4'), 125.4 (C-6), 125.5 (C-3), 127.9 (C-5), 128.3 (C-7), 129.0 (C-6'), 129.3 (C-2' & C-6'), 131.8 (C-3' & C-5'), 136.3 (C-1'), 137.0 (C-4), 162.8 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 373.2 (M+1), 375.2 (M+3); Anal. calcd. (%) for $C_{18}H_{14}NO_3Br$: C, 58.08; H, 3.79; N, 3.76. Found: C, 58.11; H, 3.83; N, 3.79.

3-(1-(2-Chlorobenzyloxyimino)ethyl)coumarin (17): Yield: 90 %; white solid; m.p.: 175-176 °C; IR (KBr, ν_{max}, cm^{-1}): 2892-2912, 1720 (-C=O), 1610 (-C=N), 1038 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.28 (s, $-CH_3$), 5.30 (s, $-OCH_2$), 7.16-7.17 (m, H-5' & H-6'), 7.28-7.30 (m, H-6, H-3' & H-8), 7.42-7.46 (m, H-7), 7.52-7.54 (m, H-5 & H-4'), 7.87 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 ($-CH_3$), 71.9 ($-OCH_2$), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 125.2 (C-3), 125.4 (C-6), 127.0 (C-5'), 127.9 (C-5), 128.3 (C-7), 128.5 (C-6'), 129.0 (C-3'), 128.9 (C-4'), 130.7 (C-2'), 137.0 (C-4), 138.2 (C-1'), 163.2 (-C=N), 166.0 (C-2), DIPMS: *m/z* at 328.7 (M+1), Anal. calcd. (%) for $C_{18}H_{14}NO_3ClF$: C, 65.96; H, 4.31; N, 4.27. Found: C, 65.54; H, 4.41; N, 4.26.

3-(1-(4-Fluorobenzyloxyimino)ethyl)coumarin (18): Yield: 95 %; white solid; m.p.: 191-192 °C; IR (KBr, ν_{max}, cm^{-1}): 2896-2918, 1725 (-C=O), 1609 (-C=N), 1045 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.26 (s, $-CH_3$), 5.18 (s, $-OCH_2$), 7.04-7.08 (m, H-5' & H-6'), 7.33-7.39 (m, H-6, H-3' & H-8), 7.52-7.54 (H-7 & H-2'), 7.64-7.69 (m, H-5), 7.86 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 ($-CH_3$), 77.0 ($-OCH_2$), 115.3 (C-4a), 115.7 (C-3' & C-5'), 116.1 (C-8), 118.1 (C-8a), 125.4 (C-6), 125.2 (C-3), 127.9 (C-5), 128.3 (C-7), 128.7 (C-6'), 128.7 (C-2' & C-6'), 132.9 (C-1'), 137.0 (C-4), 161.8 (C-4'), 163.2 (-C=N), 166.1 (C-2); DIPMS: *m/z* at 328.7 (M+1), Anal. calcd. (%) for $C_{18}H_{14}NO_3F$: C, 69.45; H, 4.53; N, 4.50. Found: C, 69.55; H, 4.43; N, 4.46.

3-(1-(2,4-Dichlorobenzyloxyimino)ethyl)coumarin (19): Yield: 90 %; white solid; m.p.: 215-216 °C; IR (KBr, ν_{max}, cm^{-1}): 2890-2910, 1729 (-C=O), 1611 (-C=N), 1031 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.29 (s, $-CH_3$), 5.31 (s, $-OCH_2$), 7.26-7.29 (m, H-8, H-5' & H-6'), 7.35-7.41 (m, H-3' & H-7), 7.52-7.57 (m, H-5 & H-6), 7.86 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 ($-CH_3$), 71.9 ($-OCH_2$), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 125.2 (C-3), 125.4 (C-6), 127.1 (C-5'), 127.9 (C-5), 128.3 (C-7), 129.9 (C-6'), 130.6 (C-3'), 133.8 (C-2'), 134.6 (C-4'), 136.3 (C-1'), 137.0 (C-4), 163.2 (-C=N), 166.0 (C-2), DIPMS: *m/z* at 363.2 (M+1), Anal. calcd. (%) for $C_{18}H_{13}NO_3Cl_2$: C, 59.69; H, 3.62; N, 3.87. Found: C, 59.54; H, 3.61; N, 3.96.

3-(1-(2-Methoxybenzyloxyimino)ethyl)coumarin (20): Yield: 85 %; white solid; m.p.: 142-144 °C; IR (KBr, ν_{max}, cm^{-1}): 2892-2912, 1723 (-C=O), 1610 (-C=N), 1041 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.38 (s, $-CH_3$), 3.83 (s, $-OCH_3$), 5.30 (s, $-OCH_2$), 6.92-6.96 (m, H-3', H-4' & H-5'), 7.25 (m, H-6'), 7.40-7.44 (m, H-6), 7.51 (dd, *J* = 1.09 Hz, 8.05Hz, H-8), 7.65 (m, H-7), 7.54 (s, H-4), 7.84 (dd, *J* = 1.61 Hz, 7.72 Hz,

H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 (-CH_3), 71.1 (-OCH_2), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 124.0 (C-5'), 125.2 (C-3), 125.4 (C-6), 127.2 (C-1'), 127.9 (C-5), 128.3 (C-7), 128.6 (C-4'), 129.0 (C-3'), 129.7 (C-6'), 137.0 (C-4), 156.7 (C-2'), 163.2 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 324.3 (M+1), Anal. calcd. (%) for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.54; H, 5.41; N, 4.36.

3-(1-(4-Methoxybenzyloxyimino)ethyl)coumarin (21): Yield: 95 %; white solid; m.p.: 213-214 °C; IR (KBr, ν_{max}, cm^{-1}): 2888-2908, 1721 (-C=O), 1609 (-C=N), 1042 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.34 (s, -CH_3), 5.21 (s, -OCH_2), 6.96-7.01 (m, H-2', H-3' & H-5', H-6'), 7.42-7.46 (m, H-6 & H-8), 7.54 (s, H-4), 7.65-7.69 (m, H-7), 7.84 (dd, *J* = 1.61 Hz, 7.72 Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 (-CH_3), 77.0 (-OCH_2), 114.5 (C-3' & C-5'), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 125.2 (C-3), 125.4 (C-6), 127.9 (C-5), 128.3 (C-7), 129.3 (C-2' & C-6'), 129.6 (C-1'), 137.0 (C-4), 159.5 (C-4'), 163.2 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 324.3 (M+1), Anal. calcd. (%) for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.31; N, 4.33. Found: C, 70.65; H, 5.33; N, 4.26.

3-(1-(4-Cyanobenzyloxyimino)ethyl)coumarin (22): Yield: 85 %; white solid; m.p.: 223-224 °C; IR (KBr, ν_{max}, cm^{-1}): 2892-2912, 1723 (-C=O), 1607 (-C=N), 1041 (N-O), 2230 (-CN); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.37 (s, -CH_3), 5.22 (s, -OCH_2), 7.32-7.39 (m, H-2', H-6', & H-6), 7.44 (dd, *J* = 1.09 Hz, *J* = 8.05 Hz, H-5 & H-7), 7.65-7.71 (m, H-7), 7.82 (dd, *J* = 2.23 Hz, *J* = 8.29 Hz, H-3' & H-5'), 7.87 (s, H-4), 8.03 (dd, *J* = 1.61 Hz, 7.72Hz, H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 (-CH_3), 77.0 (-OCH_2), 111.5 (C-4'), 115.3 (C-4a), 116.1 (C-8), 118.1 (C-8a), 118.6 (4'-CN), 125.4 (C-6), 125.5 (C-3), 127.9 (C-5), 128.3 (C-7), 127.9 (C-2' & C-6'), 132.4 (C-3' & C-5'), 141.6 (C-1'), 137.0 (C-4), 163.2 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 319.3 (M+1), Anal. calcd. (%) for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.95; H, 4.41; N, 8.82.

3-(1-(2-Cyanobenzyloxyimino)ethyl)coumarin (23): Yield: 80 %; white solid; m.p.: 153-155 °C; IR (KBr, ν_{max}, cm^{-1}): 2895-2915, 1727 (-C=O), 1607 (-C=N), 1041 (N-O), 2233 (-CN); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.30 (s, -CH_3), 5.31 (s, -OCH_2), 7.16-7.17 (m, H-5' & H-6'), 7.28-7.31 (m, H-6, H-3' & H-8), 7.42-7.46 (m, H-7), 7.51-7.56 (m, H-5 & H-4'), 7.89 (s, H-4); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.9 (-CH_3), 70.6 (-OCH_2), 115.3 (C-3'), 115.7 (C-4a), 116.1 (C-8), 119.8 (C-8a), 124.7 (C-5'), 125.3 (C-3), 125.8 (C-6), 127.9 (C-5), 128.3 (C-7), 128.7 (C-6'), 128.9 (C-1'), 129.6 (C-4'), 137.3 (C-4), 159.6 (C-2'), 163.4 (-C=N), 166.0 (C-2), DIPMS: *m/z* at 319.3 (M+1), Anal. calcd. (%) for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.54; H, 4.49; N, 8.76.

3-(1-(3-Cyanobenzyloxyimino)ethyl)coumarin (24): Yield: 75 %; white solid; m.p.: 192-193 °C; IR (KBr, ν_{max}, cm^{-1}): 2891-2911, 1729 (-C=O), 1608 (-C=N), 1049 (N-O), 2236 (-CN); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.31 (s, -CH_3), 5.33 (s, -OCH_2), 7.33-7.42 (m, H-6 & H-8), 7.50-7.55 (m, H-5' & H-6'), 7.68 (td, H-7, *J* = 8.05 Hz, *J* = 1.61Hz, *J* = 7.52 Hz), 7.97 (m, H-4'), 8.01-8.03 (m, H-4 & H-5), 8.07 (d, H-2', *J* = 1.55 Hz, *J* = 1.55 Hz); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.5 (-CH_3), 76.5 (-OCH_2), 112.8 (C-3'), 116.1 (C-8), 115.7 (C-4a), 118.6 (3-CN), 119.8 (C-8a), 125.3 (C-3), 125.4 (C-6), 127.7 (C-5), 128.3 (C-7), 129.7 (C-5'), 131.1 (C-4'), 131.3 (C-2'), 131.4 (C-6'), 137.0 (C-4), 141.9 (C-1'), 163.4 (-C=N), 166.0 (C-2); DIPMS: *m/z* at 319.3 (M+1), Anal. calcd. (%) for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.53; H, 4.51; N, 8.74.

3-(1-(3-Methoxybenzyloxyimino)ethyl)coumarin (25): Yield: 85 %; white solid; m.p.: 211-212 °C; IR (KBr, ν_{max}, cm^{-1}): 2895-2915, 1728 (-C=O), 1610 (-C=N), 1041 (N-O); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 2.32 (s, -CH_3), 3.83 (s, -OCH_3), 5.31 (s, -OCH_2), 6.87-6.97 (m, H-2' & H-4'), 7.05-7.23 (m, H-5' & H-6'), 7.33-7.42 (m, H-6 & H-8), 7.68 (td, H-7, *J* = 8.05 Hz, *J* = 1.61Hz, *J* = 7.52 Hz), 8.01-8.03 (m, H-4 & H-5); ^{13}C NMR ($CDCl_3$, 100.6 MHz): δ 11.6 (-CH_3), 55.8 (-OCH_3), 77.3 (-OCH_2), 113.2 (C-4'), 113.5 (C-2'), 115.7 (C-4a), 116.1 (C-8), 119.4 (C-6'), 119.8 (C-8a), 125.3 (C-3), 125.4 (C-6), 127.9 (C-5), 128.3 (C-7), 129.9 (C-5'), 137.0 (C-4), 142.2 (C-1'), 160.8 (C-3'), 163.2 (-C=N), 166.5 (C-2); DIPMS: *m/z* at 324.3 (M+1), Anal. calcd. (%) for $C_{19}H_{17}NO_4$: C, 70.58; H, 5.30; N, 4.33. Found: C, 70.53; H, 45.31; N, 4.43.

Molecular docking studies: Computational studies of the novel coumarin oxime ether derivatives were done using Discovery studio v 3.5. The three dimensional structure of celecoxib bound at the COX-2 active site (PDB ID: 3LN1) was retrieved from the Brookhaven Protein Data Bank (PDB), USA (http://www.rcsb.org/pdb). Protein preparation and ligand preparation wizard were used for protein and ligand preparation respectively. Initially, ions, water molecules, all the internal ligands were removed and missing atoms were inserted before minimization of the target protein. Alternate conformations (disorder) were removed.

The bioactive binding poses of inhibitors in the active site of the enzyme was generated by using a LibDock program of Discovery Studio. The ligand poses were placed in to polar and apolar interactions site. MMFF force field was used for energy minimization of the ligands. 'Hotspots' are the binding site features. The hotspot map counts for polar and apolar cluster in the active site of the protein which is further used for the alignment of the ligand conformations to the interaction sites of the protein. All the minimized ligand poses and their rankings are based on the ligands score. In accordance with the LibDock score, each pose is assessed for binding energy, by a simple pairwise method. The ligands with high LibDock scores are considered for estimating binding energies of the protein-ligand complex. The complex pose with the best binding energy is used for further binding mode analysis. For the docking validation, the co-crystallized ligand CEL in the *Musmusculus* COX-2 binding site is redocked. The binding affinities of the synthesized compounds were compared and analyzed in reference to celecoxib to identify structural characteristics of the complexes formed by these compounds and the protein.

Anti-inflammatory activity-carrageenan-induced rat paw edma assay: Anti-inflammatory activity of synthesized compounds was assessed by carrageenan-induced rat paw edema method which was described by Winter *et al*. [24]. Carrageenan (0.1 mL, 1 %) was administered into the plantar surface of the right hind paw of the animals to induce edema. Wistar rats weighing between 190 and 260 g, were used in the current study.

Indomethacin was used as a reference drug. The rats were divided into eight groups, each group consist of five animals.

Group-1 referred as control (animals received carrageenan along with the vehicle [0.5 % carboxy methyl cellulose (CMC)] and Group-2 received standard reference (indomethacin) along with the vehicle prior to the administration of carrageenan. Group 3-8 received the test compounds. All the test compounds were suspended in 0.5 % of CMC and administered orally (10 mg/kg) 60 min prior to the injection of 0.1 mL of freshly prepared carrageenan (1 %) in physiological solution (154 mM NaCl) into the sub-planter tissue of hind paw of each rat. Plethysmometer apparatus was used to measure the volume prior to the administration of carrageenan, 2 and 4 h after the injection. The increase in volume of the rat paw was adopted as a measure of edema. The antiedematous effects of the compounds were estimated as percentage inhibition of the induced inflammation in comparision with control. Statistical analysis was also carried out using a one-way analysis of variance (ANOVA). A significance level of $p < 0.001$ denoted significance in all cases.

The percentage inhibition of edema was calculated using the formula given below:

$$(V_c - V_t/V_c) \times 100$$

V_c is the increase in paw volume of control; V_t is the increase in paw volume after administration of the test compound.

RESULTS AND DISCUSSION

Docking: Molecular docking studies are used to study the binding modes and affinities of our synthesized compounds with *Musmusculus* COX-2 (PDB ID: 3LN1). All the ligands were targeted to celecoxib bound at the COX-2 site. The best ligand conformation is chosen on the base of LibDock score and highly interacting amino acid residues. Of the ten conformations generated for each compound, the compound with the highest LibDock score is taken for interaction analysis of the hydrogen bonding. LibDock scores of all the compounds along with their hydrogen bond interactions and bond lengths are depicted in Table-1. The interaction of amino acid residues with bound ligands as represented by different colours *e.g.*, pink indicates electrostatic interaction, purple indicates covalent bond and green colour indicates vander waals molecular interaction (Fig. 2). From the overall docking and interaction analysis, the best conformation of the compound 23 docked complex shows high LibDock score of 141.261 kcal/mol and forms hydrogen bonds with the protein COX-2. Hydrogen bonds are formed between the H12 of arginine 106 with the N24 atom of compound 23 with a hydrogen bond distance of 2.303000 Å and the second hydrogen bond is formed with the same amino acid arginine 106 between H22 with the N24 atom of compound 23 with a hydrogen bond distance of 1.976000.

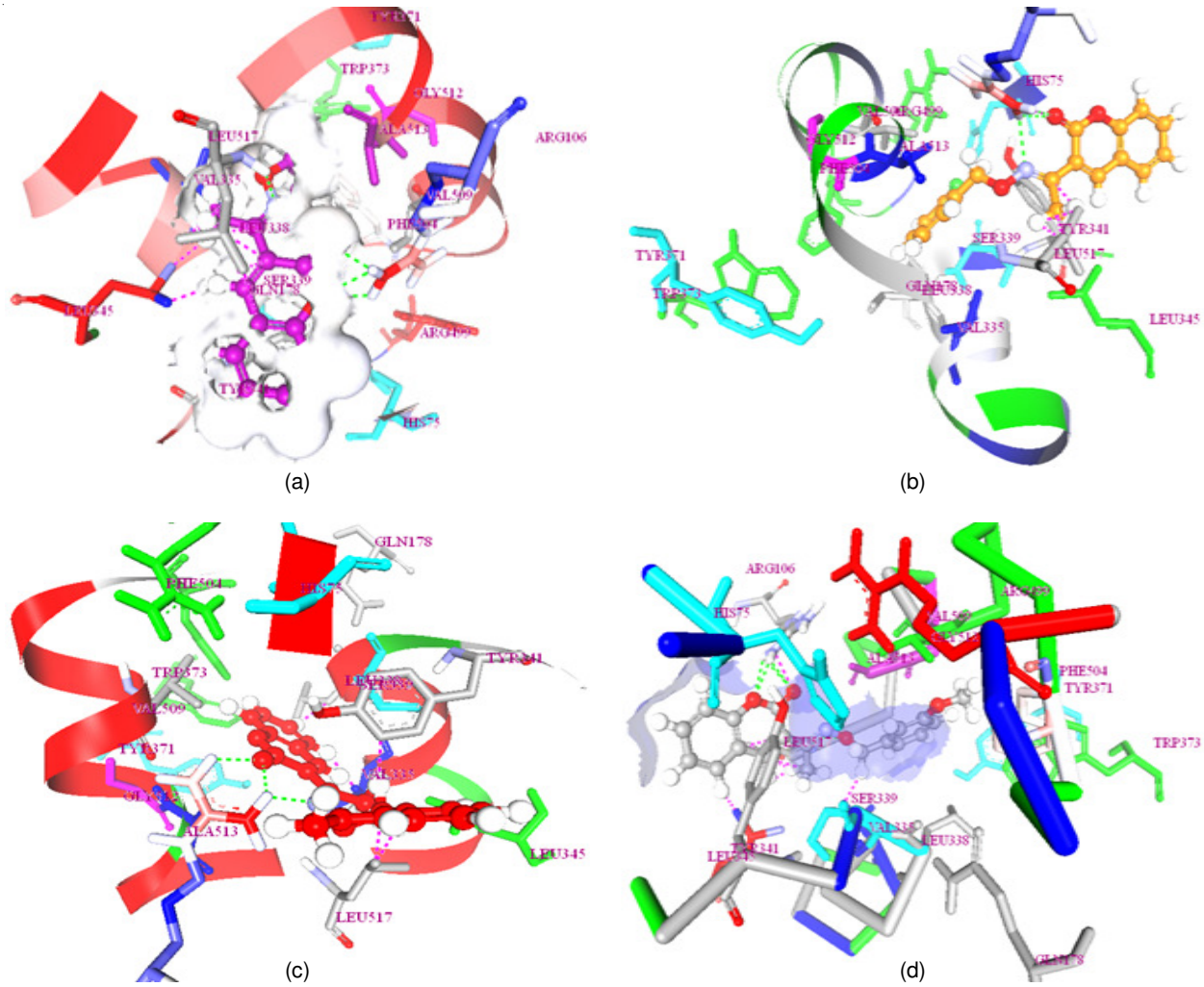


Fig. 2. Molecular interaction of 3LN1 with (a) compound **14**, (b) compound **17**, (c) compound **20** and (d) compound **23**

TABLE-1
INTERACTION OF LIGAND AND AMINO ACIDS

Compound No.	Interacting amino acids	Interacting atoms	H-Distance
14	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH11- Comp14:O9 A:ARG106:HH11 -Comp14:O7 A:ARG106:HH12 -Comp14:O7 A:LEU517:HN - Comp14:N13 Comp14:H28 - A:LEU345:CD2 Comp14:H30 - A:LEU345:CD1	2.490000 2.467000 2.256000 1.836000 1.996000 2.133000
15	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH11 - Comp15:O9 A:ARG106:HH11 - Comp15:N13 A:ARG106:HH12 - Comp15:O9 A:ARG106:HH12 - Comp15:N13 Comp15:H30 - A:TYR341:CE2 Comp15:H36 - A:VAL509:CG1	2.299000 2.315000 1.951000 2.099000 2.182000 2.028000
16	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH12 - Comp16:N13 A:ARG106:HH12 - Comp16:O9 A:ARG106:HH11 - Comp16:O9 A:ARG106:NH1 - Comp16:O9	2.289000 1.722000 2.214000 2.211000
17	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH12 - Comp17:N13 A:ARG106:HH11 - Comp17:O9 A:ARG106:HH12 - Comp17:O9 Comp17:C12 - A:LEU517:CD1 Comp17:C14 - A:LEU517:CG	2.458000 2.195000 1.637000 2.572000 2.388000
18	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH11 - Comp18:O7 A:ARG106:HH12 - Comp18:O7 A:ARG106:HH12 - Comp18:O9 Comp18:H25 - A:LEU345:CD2 Comp18:H35 - A:LEU338:CD2	2.418000 2.030000 2.457000 1.942000 1.935000
19	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:TYR371:HH - Sc1_Comp19:Cl23 Comp19:H25 - A:TYR341:CE2	2.342000 2.158000
20	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH22 - Sc1_Comp20:O9 A:ARG106:HH12 - Sc1_Comp20:O9 A:ARG106:HH12 - Sc1_Comp20:N13 A:TYR341:CE2 - Comp20:H34 A:LEU338:CB - Comp20:H28	2.393000 2.391000 2.322000 1.959000 2.186000
21	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH12 - Comp21:N13 A:ARG106:HH11 - Comp21:N13 A:TYR341:CE2 - Comp21:H31	2.378000 2.018000 1.999000
22	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:TYR371:HH - Comp22:N24 A:ARG106:HH11 - Comp22:O9 A:ARG106:HH12 - Comp22:O9 Comp22:H30 - A:LEU345:CD2	2.344000 2.003000 1.508000 2.141000
23	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:TYR341:HH - Comp23:N24 A:ARG106:HH22 - Comp23:N24 A:ARG106:HH12 - Comp23:N24 Comp23:C23 - A:ARG106:HH12	2.366000 1.976000 2.303000 1.977000
24	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:ARG106:HH12 - Comp24:N13 A:ARG106:HH12 - Comp24:O15 A:ARG499:HH22 - Comp24:N24 A:LEU517:HN - Comp24:O9 Comp24:H38 - A:TYR341:OH	2.158000 2.341000 2.018000 1.875000 1.733000
25	Tyr371, Trp373, Phe504, Gly512 Gln178, Arg499, Arg106, Leu517 Leu338, Phe504His75, Val509 Val335, Ala513, Leu345, Leu517	A:LEU517:HN - Comp25:O15 A:LEU517:HN - Comp25:N13 A:ARG106:HH12 - Comp25:O7 A:LEU345:CD2 - Comp25:H26 A:LEU517:CD1 - Comp25:C11	2.279000 2.270000 2.148000 1.984000 1.977000
Celecoxib	Leu345, Leu157, Arg106, Val335, Tyr341, Leu338 Ser339, Gln178, Ala513 Gly512, Tyr371, Val509 His75, Trp373, Phe504 Gln178	Celecoxib:H28 - A:HIS75:NE2 Celecoxib:H28 - A:SER339:O A:ARG499:HH11 - Celecoxib:O6 Celecoxib:H28 - A:HIS75:NE2 A:PHE504:HN - Celecoxib:N9 Celecoxib:H28 - A:PHE504:HN	2.387000 2.413000 2.308000 2.387000 2.081000 1.396000

Antiinflammatory activity: The compounds with good Libdock scores (Table-2) were selected for antiinflammatory activity using carrageenan-induced rat paw edema model and exhibited protection against carrageenan-induced edema. The protection ranged up to 70 %, while the reference drug (indomethacin) showed 76 % at an equivalent dose. Among all the tested compounds, 3-(1-(2-cyanobenzyloxyimino)ethyl)coumarin showed remarkable antiinflammatory activity compared to other derivatives. This compound has cyano group at the *ortho*-position of aromatic ring showed enhanced activity compared to other aromatic analogues (Table-3).

TABLE-2
LibDock SCORES OF THE COMPOUNDS

Compd. No.	Electrostatic energy	van der Waals energy	LibDock score
14	3.562	3.861	137.534
15	-2.752	3.359	130.704
16	-1.316	3.297	130.123
17	2.997	4.745	135.833
18	-4.282	3.059	135.833
19	-1.752	5.172	133.259
20	4.079	4.555	139.171
21	-5.834	3.847	120.812
22	3.938	3.836	124.802
23	2.859	4.81	141.261
24	3.635	3.647	133.009
25	-6.087	3.572	138.151
Celecoxib	4.465	4.762	145.691

TABLE-3
ANTI-INFLAMMATORY ACTIVITY
OF THE TESTED COMPOUNDS*

Treatment	Volume of Paw edema (mL)	
	2 h	4 h
Control	0.93 ± 0.01	0.89 ± 0.02
Indomethacin	0.22 ± 0.01 (76.06)	0.19 ± 0.02 (78.64)
14	0.36 ± 0.03 (60.87)	0.35 ± 0.03 (60.67)
17	0.37 ± 0.03 (59.78)	0.36 ± 0.02 (58.01)
18	0.39 ± 0.02 (57.61)	0.38 ± 0.02 (57.29)
20	0.35 ± 0.01 (61.96)	0.33 ± 0.03 (62.30)
23	0.28 ± 0.01 (68.30)	0.26 ± 0.02 (70.40)
25	0.38 ± 0.02 (59.76)	0.38 ± 0.02 (57.10)

*Values are expressed as mean ± SEM, (n = 5) and analyzed by ANOVA. Values in parenthesis (percentage inhibition of edema).

Conclusion

Herewith, we report the simple and efficient method for synthesis of novel coumarin-oxime ether derivatives (**14-25**). The present methodology used to synthesize oxime ether derivatives which is the first example in coumarin chemistry can be further explored for the synthesis of various heterocycle-coumarin oxime ether derivatives. All the synthesized compounds were docked with 3LN1 and Libdock scores are measured. The compounds which got the good libDock score evaluated for their *in vivo* anti-inflammatory activity. The compound 3-(1-(2-cyanobenzyloxyimino)ethyl)coumarin (**23**) exhibited good antiinflammatory activity.

ACKNOWLEDGEMENTS

The authors express their thanks to The Head, Department of University College of Technology, Osmania University and Management of RBVRR Women's College of Pharmacy for their constant support and for providing necessary facilities.

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