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Synthesis and Evaluation of Some Umbelliferone Derivatives as Anti-inflammatory Agents

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ABSTRACT

Seventeen 7-hydroxy coumarin (umbelliferone) derivatives were synthesized in an almost quantitative yield via reaction with the corresponding ethylchloroacetate, p-substituted benzyl chloride, 2-bromoacetophenone or 2-chloroacetanilide in the presence of anhydrous potassium carbonate in dry acetone. The compounds were tested for their anti-inflammatory activity using cotton pellet implantation test and carrageenan-induced rat paw edema. The ulcerogenic effect of the compounds was also studied comparing with the NSAID indomethacin.

Keywords : *7-Hydroxy coumarin (umbelliferone); synthesis; anti-inflammatory; ulcerogenic effects.*

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INTRODUCTION

Coumarins are a group of naturally occurring plant phenols derived biosynthetically from hydroxy cinnamic acids [1]. Coumarin derivatives were found to have the ability to modulate the functional activity of several types of cells. The reported biological activities of coumarins include: anti-inflammatory, anticoagulant, antioxidant and
\analgesic effects [2-6].

Coumarin, the parent compound first isolated from the tonka tree *Coumarouna odorata* [7] has long-established efficacy in reduction of lymphoedema in man [8]. Scopoletin, a 6-hydroxy-7-methoxycoumarin, was found to suppress PGE2 production in LPS-stimulated RAW 264.7 macrophages by inhibiting COX-2 but not COX-1 protein expression[9]. Selective COX-2 inhibitors, which lack gastro-intestinal side effects, might be more beneficial in the treatment of inflammatory diseases [10]. The anti-inflammatory activity of several medicinal plants were attributed to their coumarin contents [6, 11-17]. In the recent years, synthesis and evaluation of the anti-inflammatory activities of coumarin derivatives were reported by different groups of medicinal chemists [8-21].

In the present work synthesis and anti-inflammatory activity of some 7-hydroxy coumarin (umbelliferone) derivatives are presented. The ulcerogenic effect of the synthesized compounds was studied comparing with the NSAID indomethacin. Moreover, the acute toxicity for the most active compounds was evaluated via both oral and parenteral routes of administration.

MATERIAL AND METHODS

Chemistry

Melting points were determined in open glass capillaries using a Thomas capillary melting point apparatus and are uncorrected. Umbelliferone (7-hydroxy coumarin) was purchased from Aldrich company, other chemical from E. Merck, Fluka AG. IR spectra were obtained as KBr pellets on a PYE UNICAM FT-IR spectrometer (υ cm^{-1}). ^{1}H-NMR spectra were recorded on a Bruker DRX500 MHz instrument at 500 MHz for ^{1}H and 125 MHz for ^{13}C using DMSO as solvent. Proton and carbon chemical shifts are reported in parts per million (ppm) relative to residual deteriorated solvent peaks. Standard Bruker pulse programs were used for APT, DEPT, 2D NMR spectra. EI-MS were obtained using Finnegan MAT 300 mass spectrometer. Micro analytical data (C, H, N) agreed with the proposed structures within ± 0.4 % of the theoretical values. Follow up of the reactions and checking the purity of the compounds were made by TLC on silica gel-protected aluminium sheets (Type 60 GF254, Merck) and the spots were detected by exposure to UV-lamp at λ 254 nm for few seconds.

General procedure for synthesis:

A mixture of equimolar amounts of umbelliferone (7-hydroxy coumarin), the selected ethylchloroacetate, *p*-substituted benzyl chloride, 2-bromoacetophenone or 2-chloroacetanilid derivatives and anhydrous potassium carbonate (1mmol) in dry acetone (30 ml) were stirred over night at room temperature. The reaction mixtures were filtered and the filtrates were evaporated under reduced pressure. The residues obtained were crystallized from ethanol to obtain the corresponding products.

1. ethyl 2-(2-oxo-2H-chromen-7-yloxy)acetate

Yield: 73%, mp: 110-112 °C ^{1}H NMR: 7.65 (d ,1H, *J*= 8.9 Hz), 7.41 (d, 1H, *J*= 8.3 Hz), 6.90 (dd, 1H, *J*= 8.3 , 1.7 Hz), 6.79 (d, 1H, *J*= 1.7 Hz), 6.28 (d, 1H , *J*= 8.9 Hz), 4.69 (s, 2H), 4.30 (q, 2H , *J*= 7.0 Hz), 1.33 (t, 3H , *J*= 7.0 Hz). ^{13}C NMR:167.9, 160.9, 160.8, 155.7, 143.1, 128.9, 113.8, 113.3, 112.8, 101.7, 65.4, 61.7, 14.1. MS *m/z*: 248 (M^+ 71 %). Anal. For: ($C_{13}H_{12}O_5$) Calcd. /Found (%): C, 62.90 (62.96); H, 4.87 (4.85); N, 32.23 (32.19).

2. 7-benzyloxy-2H-chromen-2-one

Yield: 69%, mp: 167-169 °C ^{1}H NMR: 7.66 (d, 1H, *J*= 9.0 Hz), 7.49-7.29 (m, 6H), 6.95 (dd, 1H, *J*= 8.5, 1.5 Hz), 6.92 (d, 1H, *J*= 1.5 Hz), 6.28 (d, 1H , *J*= 9.0 Hz), 5.16 (s, 2H). ^{13}C NMR: 161.9, 161.2, 155.8, 143.4, 135.8, 128.8, 128.7, 127.5, 113.3, 113.2, 112.7, 101.9, 70.5.MS *m/z*: 252 (M^+ 100 %). Anal. For: ($C_{16}H_{12}O_3$) Calcd. /Found (%): C, 76.18 (76.20); H, 4.79 (4.81); N, 19.03 (18.99).

3. 7-(4-Nitrobenzyloxy)-2H-chromen-2-one

Yield: 65%, mp: 176-178 °C ^{1}H NMR: 8.27 (d ,2H, *J*= 8.5 Hz), 8.0 (d, 1H, *J*= 9.0 Hz), 7.75 (d, 2H, *J*= 8.5 Hz), 7.66 (d, 1H, *J*= 8.5 Hz), 7.06 (dd, 1H, *J*= 8.5,1.5 Hz), 7.09 (d , 1H , *J*= 1.5 Hz), 6.30 (d, 1H, *J* = 9.0 Hz), 5.41 (s, 2H). ^{13}C NMR:160.9, 160.1, 155.2, 147.1, 144.1, 129.6, 128.4, 128.4, 123.6, 112.9, 112.8, 112.7, 101.7, 68.6.MS *m/z*: 297 (M^+ 90 %). Anal. For: ($C_{16}H_{11}NO_5$) Calcd. /Found (%): C, 64.65 (64.55); H, 3.73 (3.74); N, 4.71 (4.72); O, 26.91 (26.99).

4.: Yield: 70%, mp: 168-170 °C ^{1}H NMR: 8.05 (bd ,2H, *J*= 7.0 Hz), 7.95 (d, 1H, *J*= 9.0 Hz), 7.64 (d, 1H, *J*= 8.4 Hz), 7.59 (t, 3H, *J*= 7.6 Hz) 7.03 (dd, 1H, *J*= 8.5, 1.5 Hz), 7.09 (d, 1H, *J*= 1.5 Hz), 6.30 (d ,1H , *J*= 9.0Hz), 5.75 (s, 2H). ^{13}C NMR: 194.2, 161.7, 160.9, 155.8, 144.7, 134.7, 134.4, 129.9, 129.3, 128.4, 113.3, 113.2, 113.1, 102.1, 71.1. MS *m/z*: 280 (M^+ 22 %). Anal. For: ($C_{17}H_{12}O_4$) Calcd. /Found (%): C, 72.85 (72.75); H, 4.32 (4.33); O, 22.83 (22.92).

5.: Yield: 61%, mp: 156-158 °C ^{1}H NMR: 7.91 (d ,2H, *J*= 8.0 Hz), 7.34 (d, 2H, *J*= 8.0 Hz), 7.66 (d, 1H, *J*= 8.9 Hz), 7.42 (d, 1H, *J*= 8.4 Hz), 6.95 (dd, 1H, *J*= 8.4, 2.0 Hz), 6.81 (d , 1H , *J*= 2.0 Hz), 6.28 (d, 1H, *J*= 8.9 Hz), 5.83 (s, 2H), 2.47 (s, 3H). ^{13}C NMR: 192.7, 161.2, 161.1, 155.9, 145.4, 143.3, 131.6, 129.7, 129.1, 128.1, 113.6, 113.2, 112.9, 101.9, 70.5, 31.0. MS *m/z*: 294 (M^+ 5 %). Anal. for: ($C_{18}H_{14}O_4$) Calcd. /Found (%): C, 73.46 (73.38); H, 4.79 (4.78); O, 21.75 (21.84).

6.: Yield: 74%, mp: 192-194 °C ^{1}H NMR: 8.04 (d ,2H, *J*= 8.5 Hz), 7.99 (d, 1H, *J*= 9.1 Hz), 7.65 (d, 2H, *J*= 8.5 Hz) 7.61 (d, 1H, *J*= 8.4 Hz), 7.09 (d ,1H, *J*= 2.0 Hz), 6.95 (dd, 1H, *J*= 8.4, 2.0 Hz), 6.29 (d , 1H , *J*= 9.2 Hz), 5.73 (s, 2H). ^{13}C NMR: 192.8, 161.1, 160.2, 155.2, 144.2, 138.7, 132.9, 129.8, 129.8, 128.9, 112.9, 112.8, 112.7, 101.6, 70.6. MS *m/z*: 314 -316 (M^+ 12 %, M^+ 2 4 %). Anal. for ($C_{17}H_{11}ClO_4$) Calcd. /Found (%): C, 64.88 (64.94); H, 3.52 (3.51); Cl, 11.26 (11.27); O, 20.34 (20.28).

7.: Yield: 67%, mp: 183-185 °C ^{1}H NMR: 7.91 (d ,2H, *J*= 8.0 Hz) ,7.66 (d, 1H, *J*= 8.9 Hz) , 7.42 (d, 1H, *J*= 8.4 Hz), 7.34 (d, 2H, *J*= 8.0 Hz) , 6.95 (dd, 1H, *J*= 8.4, 2.0 Hz) , 6.81 (d, 1H, *J*= 2.0 Hz), 6.28 (d, 1H, *J*= 8.9) , 5.38 (s, 2H) . ^{13}C NMR: 193.0, 161.1, 160.2, 155.2, 144.2, 133.2, 131.8, 129.9, 129.4, 127.9, 112.8, 112.8, 112.7, 101.6, 70.5. MS *m/z*: 358-360 (M^+ 13 % , M^+ + 2 10 %,). Anal. For: ($C_{17}H_{11}BrO_4$) Calcd. /Found (%): C, 56.85 (56.77); H, 3.09 (3.10); Br, 22.25 (22.29); O, 17.81 (17.84).

8.: Yield: 72%, mp: 292-293 °C ^{1}H NMR: 7.99 (d, 1H, *J*= 9.0 Hz), 7.93 (d, 2H, *J*= 8.4 Hz), 7.78 (d, 2H, *J*= 8.4 Hz), 7.66 (d, 1H, *J*= 8.5 Hz), 7.04 (m, 2H), 6.30 (d, 1H, *J*= 9.0), 4.91 (s, 2H), 2.53 (s, 3H). ^{13}C NMR: 169.9, 167.1, 161.5, 160.7, 155.7, 144.8, 144.2, 132.3, 129.9, 129.8, 113.3, 113.3, 113.2, 102.2, 68.0, 17.6. MS *m/z*:

337 (M^+ 21 %). Anal. For: ($C_{19}H_{15}NO_5$) Calcd. /Found (%): C, 67.67 (67.65); H, 4.49 (4.48); N, 4.12 (4.15); O, 23.72 (23.72).
9.: Yield: 68%, mp: 212-214 °C ^{1}H NMR: 7.95 (d, 1H, J= 9.0 Hz), 7.54 (d, 1H, J= 8.4 Hz), 6.82 (d, 2H, J= 8.4 Hz), 6.85 (m, 2H), 6.32 (d , 1H , J = 9.0 Hz), 6.23 (d, 2H, J= 9.0), 4.60 (s, 2H). ^{13}C NMR: 169.3, 161.2, 160.8, 156.2, 155.8, 144.9, 131.3, 129.4, 113.4, 113.3, 113.2, 112.1, 101.7, 69.1. MS *m/z*: 311 (M^+ 24 %). Anal. For: $C_{17}H_{13}NO_5$) Calcd. /Found (%): C, 65.58 (65.59); H, 4.20 (4.21); N, 4.51 (4.50); O, 25.71 (25.70).
10.: Yield: 63%, mp: 160-162 °C ^{1}H NMR: 10.01 (s,1H, NH), 7.99 (d,1H, *J*= 9.0 Hz), 7.66 (d, 1H, *J*= 8.4 Hz), 7.53 (d, 2H, *J*= 8.7 Hz), 7.04 (m, 2H), 6.89 (d , 2H, *J*= 8.4 Hz), 6.32 (d, 1H, *J*= 9.0), 4.81 (s , 2H), 3.99 (q , 2H, *J*= 6.5 Hz), 1.31 (t, 3H, *J*= 1.3 Hz). ^{13}C NMR: 165.8, 161.4, 160.6, 155.7, 155.4, 144.6, 131.7, 130.0, 121.8, 114.9, 113.3, 113.3, 113.2, 102.2, 67.9, 63.6, 15.2. MS *m/z*: 339 (M^+ 12 %). Anal. For: ($C_{19}H_{17}NO_5$) Calcd. /Found (%): C, 67.27 (67.25); H, 5.02 (5.05); N, 4.12 (4.13); O, 23.59 (23.57).
11.: Yield: 60%, mp: 230-231 °C ^{1}H NMR: 10.31 (s, 1H, NH) 7.99 (d, 1H, *J*= 9.0 Hz), 7.68–7.66 (m, 3H), 7.39 (d, 2H, *J*= 8.7 Hz), 7.05 (m, 2H), 6.31 (d, 1H, *J*= 9.0), 4.86 (s, 2H). ^{13}C NMR: 166.5, 161.4, 160.6, 155.6, 144.6, 137.7, 130.0, 129.1, 127.9, 121.8, 113.4, 113.4, 113.2, 102.2, 67.8. MS *m/z*: 329 – 331 (M^+ 18 %, M^+ +2 6 %,). Anal. For: ($C_{17}H_{12}ClNO_4$) Calcd. /Found (%): C, 61.90 (61.92); H, 3.70 (3.67); Cl, 10.77(10.75); N, 4.24 (4.25); O, 19.39 (19.41).
12.: Yield: 69%, mp: 224-225 °C ^{1}H NMR: 10.31 (s, 1H, NH), 7.99 (d, 1H, *J*= 9.0 Hz), 7.66 (d, 1H, *J*= 8.9Hz), 7.62 (d, 2H, *J*= 8.9 Hz), 7.51 (d, 2H, *J*= 8.9 Hz), 7.03 (m, 2H), 6.30 (d ,1H, *J*= 9.0), 4.85 (s, 2H). ^{13}C NMR: 166.5, 161.4, 160.6, 155.6, 144.6, 138.2, 132.0, 130.0, 122.1, 115.9, 113.4, 113.4, 113.2, 102.2, 67.8. MS *m/z*: 372-374 (M^+ 11 %, M^+ +2 8 %,). Anal. For: ($C_{17}H_{12}BrNO_4$) Calcd. /Found (%): C, 54.59 (54.57); H, 3.25 (3.23); Br, 21.32 (21.35); N, 3.70 (3.74); O, 17.11 (17.10).
13.: Yield: 78%, mp: 190-191 °C ^{1}H NMR: 10.35 (s,1H, NH), 7.99 (d,1H, *J*= 9.2 Hz), 7.66 (d,1H, *J*= 8.8 Hz), 7.64 (d, 2H, *J*= 7.5 Hz), 7.33 (d, 2H, *J*= 8.0 Hz), 7.09 (t ,1H , *J*= 7.5 Hz), 7.04 (m, 2H), 6.31 (d , 1H, *J*= 9.2), 4.85 (s, 2H). ^{13}C NMR: 166.3, 161.4, 160.6, 155.7, 144.7, 138.8, 130.0, 129.2, 124.2, 120.2, 113.4, 113.4, 113.2, 102.2, 67.9. MS *m/z*: 295 (M^+ 17 %). Anal. For: $C_{17}H_{13}NO_4$) Calcd. /Found (%): C, 67.15 (69.15); H, 4.45 (4.44); N, 4.72 (4.74); O, 21.70 (21.67).
14.: Yield: 70%, mp: 194-196 °C ^{1}H NMR: 10.36 (s, 1H, NH), 7.99 (d ,1H, *J*= 8.9 Hz),7.67 (d, 1H, *J*= 8.8 Hz), 7.54 (d, 2H, *J*= 8.8 Hz), 7.04 (m, 2H), 6.90 (d , 2H, *J*= 8.9), 6.32 (d, 1H , *J*= 8.9 Hz), 4.81 (s, 2H), 3.73 (s , 3H). ^{13}C NMR: 165.8, 161.4, 160.6, 156.1, 155.7, 144.7, 131.8, 130.0, 121.9, 114.4, 113.4, 113.4, 113.2, 102.2, 67.9, 55.7. MS *m/z*: 325 (M^+ 14 %). Anal. For: ($C_{18}H_{15}NO_5$) Calcd. /Found (%): C, 68.46 (66.46); H, 4.64 (4.65); N, 4.32 (4.31); O, 24.57 (24.59).
15.: Yield: 61%, mp: 207-209 °C ^{1}H NMR: 10.38 (s,1H, NH), 7.99 (d,1H, *J*= 9.0 Hz), 7.66 (d, 1H, *J*= 9.0 Hz), 7.66 (d, 2H, *J*= 8.7 Hz), 7.16 (d, 2H , *J*= 8.7 Hz), 7.05 (m , 2H), 6.31(d, 1H, *J*= 9.0 Hz), 4.85 (s, 2H). ^{13}C NMR: 166.3, 161.4, 160.6, 157.8, 155.7, 144.7, 135.2, 130.0, 122.1, 115.9, 115.7, 113.4, 113.2, 102.2, 67.2. MS *m/z*: 313 (M^+ 18 %). Anal. For: ($C_{17}H_{12}FNO_4$) Calcd. /Found (%): C, 66.18 (65.18); H, 3.87 (3.86); F, 6.05 (6.06); N, 4.44 (4.47); O, 20.45 (20.43).
16.: Yield: 62%, mp: 206-207 °C ^{1}H NMR: 10.12 (s, 1H, NH), 7.99 (d,1H, *J*= 9.0 Hz), 7.67 (d, 1H, *J*= 8.5 Hz), 7.52 (d, 2H, *J*= 8.3 Hz), 7.13 (d, 2H , *J*= 8.3 Hz), 7.04 (m , 2H), 6.31 (d, 1H, *J*= 9.0 Hz), 4.83 (s , 2H), 2.26 (s , 3H). ^{13}C NMR: 166.0, 161.5, 160.6, 155.7, 144.7, 136.3, 133.2, 130.0, 129.6, 120.2, 113.3, 113.2, 102.2, 67.9, 20.91. MS *m/z*: 309 (M^+ 25 %). Anal. For: ($C_{18}H_{15}NO_4$) Calcd. /Found (%): C, 69.88 (69.89); H, 4.87 (4.89); N, 4.55 (4.53); O, 20.70 (20.69).
17.: Yield: 64%, mp: 202-204 °C ^{1}H NMR: 10.36 (s, 1H, NH) 8.05–8.01 (m, 2H), 7.97–7.95 (m, 1H), 7.82 (d, 1H, *J*= 8.0 Hz), 7.70–7.66 (m, 2H), 7.56–7.50 (m, 3H), 7.12–7.10 (m, 2H), 6.33 (d, 1H, *J*= 9.2 Hz), 5.03 (s, 2H). ^{13}C NMR: 167.2, 161.5, 160.7, 155.7, 144.7, 134.2, 133.5, 130.0, 128.7, 128.6, 126.6, 126.4, 126.3, 126.0, 123.3, 122.8, 113.3, 113.3, 102.2, 67.9, 17.6. MS *m/z*: 349 (M^+ 28 %). Anal. For: ($C_{21}H_{15}NO_4$) Calcd. /Found (%): C, 73.03 (73.03); H, 4.36 (4.38); N, 4.07 (4.06); O, 18.54 (18.53).

Biological evaluation:

Anti-inflammatory testing:

Cotton pellet implantation test* [23]*:

Adult male Sprague-Dawley rats (120-140 g) were used. They were acclimated 1 week prior to use and allowed unlimited access to standard rat chow and water. Prior to the start of experiment, the animals were randomly divided into groups (6 rats each). Cotton pellet (35 ± 1 mg) cut from dental rolls were impregnated with 0.2 ml (containing 0.01mmol) of a solution of the test compound in chloroform or acetone and the solvent was allowed to evaporate. Each cotton pellet was subsequently injected with 0.2 ml of an aqueous solution of antibiotics (1 mg penicillin G and 1.3 mg dihydrostreptomycin / ml). Two pellets were implanted subcutaneously, one in each axilla of the rat, under mild general anesthesia. One group of animals received the standard reference indomethacin and the antibiotics at the same level. Pellets containing only the antibiotics were similarly implanted in the control rats. Seven days later, the animals were sacrificed and the two cotton pellets, with adhering granulomas, were removed, dried for

48 h at 60°C and weighed. The increment in dry weight (difference between the initial and final weights) was taken as a measure of granuloma ± S. E. This was calculated for each group and the percentage reduction in dry weight of granuloma from control value was also calculated. The ED50 values were determined through dose-response curves, using doses of 4, 7, 10 and 15µmol for each compound.

Carrageenan-induced rat paw edema (24):

Male albino rats weighing 120-150g were used throughout the work. They were kept in the animal house under standard conditions of light and temperature with free access to food and water. The animals were randomly divided into groups of six rats each. The paw edema was induced by subplantar injection of 50 µL of 2% carrageenan solution in saline (0.9 %). Indomethacin and the test compounds were dissolved in DMSO and were injected subcutaneously in a dose of 10 µmol/ kg body weight, 1h prior to carrageenan injection. DMSO was injected to the control group. The volume of paw edema (mL) was determined by means of water plethysmometer immediately after injection of carrageenan and 4 h later. The increase in paw volume between time 0 and +4h was measured. The percentage protection against inflammation was calculated as follows:

$$Vc\text{-}Vd \,/\, Vc \times 100$$

Where Vc is the increase in paw volume in the absence of test compound (control) and Vd is the increase of paw volume after injection of the test compound. Data were expressed as the mean ± SEM. Significant difference between the control and the treated groups was performed using Student's t-test and P values. The differences in results were considered significant when $P < 0.001$. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also calculated. The anti-inflammatory activity of the test compounds relative to that of indomethacin was also determined.

Ulcerogenic effects:

All synthesized compounds were evaluated for their ulcerogenic potential in rats [25]. Indomethacin was used as reference standard. Male albino rats (100-120 g) were fasted for 12 h prior to the administration of the compounds. Water was given *ad libitum*. The animals were divided into groups, each of six animals. Control group received 1% gum acacia orally. Other groups received indomethacin or the test compounds orally in two equal doses at 0 and 12 h for three successive days at a dose of 30 µM / Kg per day. Animals were sacrificed by diethyl ether 6 h after the last dose and the stomach was removed. An opening at the greater curvature was made and the stomach was cleaned by washing with cold saline and inspected with a 3X magnifying lens for any evidence of hyperemia, hemorrhage, definite hemorrhagic erosion or ulcer. An arbitrary scale was used to calculate the ulcer index which indicates the severity of the stomach lesions[24]. The percentage ulceration for each group was calculated as follows:

$$\% \text{ Ulceration} = \frac{\text{Number of animals bearing ulcer in a group}}{\text{Total number of animals in the same group}} \times 100$$

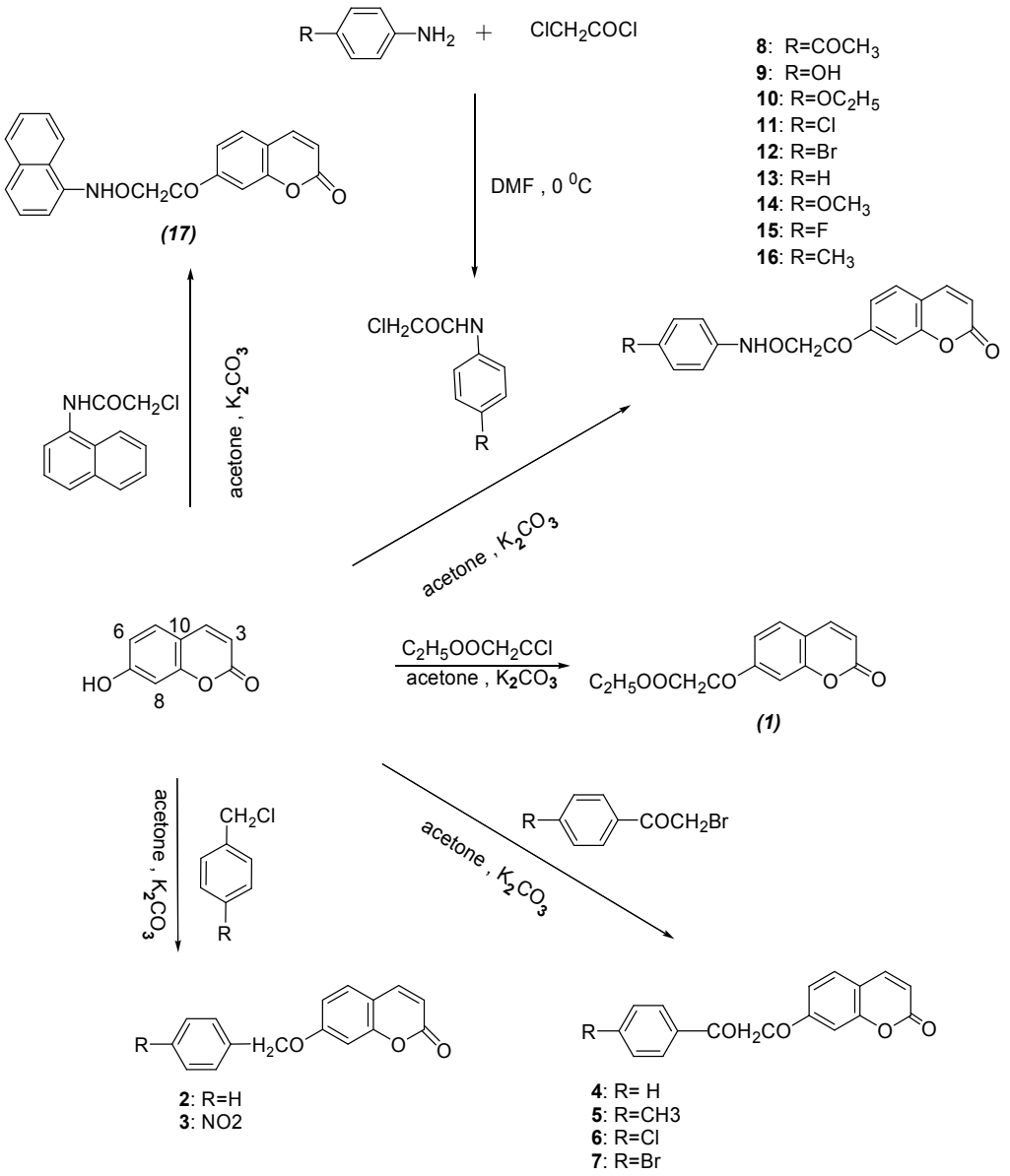
Acute toxicity:

Compounds 2, 3, 4, 9, 10, 15 and 17 were further investigated for their oral acute toxicity in male mice[26,27] (each 20 g, supplied by Medical Research Institute, Alexandria University). Groups of mice each consisting of six animals were used. The compounds were given orally suspended in 1% gum acacia, in doses of 1, 10, 100, 200, 250, 300 mg / kg, respectively. Twenty four hours later, the % mortality in each group and for each compound was recorded. Moreover, these compounds were tested for their parenteral acute toxicity, groups of mice each consisting of six animals were used. The compounds or their vehicle, propylene glycol (control) was given by intraperitoneal injection in doses of 10, 25, 50, 75, 100 mg / kg, respectively. Survival was followed up to 7 days [28].

RESULTS AND DISCUSSION

Chemistry

The strategies adopted for the synthesis of the intermediates and target compounds are depicted in the following scheme.



Alkylation of 7-hydroxycoumarin with ethylchloroacetate afforded 7-oxymethylcarbonylethoxy coumarine **(1).**

Interaction of 7-hydroxycoumarine with p-substituted benzyl chloride afforded 7-benzyloxycoumarine **(2-3)**.

Reaction of 7-hydroxycoumarine with appropriate *p*-substituted 2-bromoacetophenone gave the corresponding 7-benzoxymethyloxycoumarine (4-7).

The reaction of substituted primary aromatic amine with chloroacetylchloride in DMF at 0°c afforded the corresponding 2-chloroacetanilides [29].

The interaction of the appropriate *p*-substituted chloroacetanilides with 7-hydroxycoumarine produce the corresponding 7-*N*-substituted phenylcarbamoyloxycoumarine [8-16] or 7-naphthylcarbamoyloxycoumarine [17].

The selected agents were performed in the presence of anhydrous potassium carbonate in dry acetone[29] to produce the target compound in almost quantitative yields.

The structures were determined from 1D- and 2D-NMR experiments. ^{1}H- and ^{13}C-NMR spectra showed the characteristic signals for the 7-hydroxy coumarin skeleton (positions 2- 10) in all compounds. Both IR and ^{13}C-NMR of compounds **2**, **3** showed only the coumarin carbonyl at 1720 cm^{-1}, 161.9 ppm and 1728 cm^{-1}, 160.9 ppm respectively. The rest of the compounds showed IR bands and ^{13}C-NMR signals for an additional ester (compound **1**) or ketone carbonyl. All the other carbon signals for the 7-O substituents were clear in the ^{13}C-NMR spectra and the EI-MS showed the M^{+} in all cases.

Biological evaluation:

The anti-inflammatory activity of the synthesized compounds **1-17** was evaluated applying the cotton-pellet granuloma bioassay in rats[23] using indomethacin as a reference standard. The ED50 values were determined for each compound. Data were expressed as the mean ± SEM. Significant difference between the control and the treated groups was performed using Student's *t*-test and *P* values **(Table 1).**

Table 1: The anti-inflammatory activity (ED_{50}, µmol)[a] and ulcerogenic activity[a]

Test Compound	ED_{50} (µmol)	% Ulceration
Indomethacin	**9.58**	**100**
1	**14.34**	**20**
2	**10.56**	**30**
3	**9.22**	**10**
4	**9.86**	**10**
5	**12.64**	**10**
6	**10.10**	**40**
7	**13.86**	**40**
8	**13.52**	**10**
9	**9.36**	**10**
10	**9.32**	**30**
11	**11.24**	**20**
12	**18.12**	**30**
13	**12.64**	**10**
14	**12.28**	**20**
15	**9.58**	**30**
16	**11.96**	**10**
17	**9.28**	**10**

Table 2: Effect of compounds 2, 3, 4, 6, 9, 10, 15 and 17 on carregeenan-induced rat paw edema (ml), % protection and activity.

Test compound	Increase in paw edema (ml) ± SEM[a,b]	% Protection	Activity relative to Indomethacin
Control	0.95 ± 0.025	0.0	0.0
Indomethacin	0.26± 0.027	72.6	100
2	0.39 ± 0.031	58.9	81.12
3	0.28± 0.022	70.5	97.10
4	0.32 ± 0.015	66.3	91.32
6	0.35± 0.028	63.1	86.91
9	0.32 ± 0.022	66.3	95.59
10	0.29± 0.034	69.4	97.10
15	0.31 ± 0.038	64.2	88.42
17	0.34± 0.026	63.1	86.91

[a]SEM denotes the standard error of the mean.

[b]All data are significantly different from control ($P < 0.001$).

The difference in results was considered significant when $P < 0.001$ **(Table 1).** Compounds **3, 4, 6, 9, 10, 15** and **17** (ED50 = 9.22-10.56 µmol) have anti-inflammatory activity comparable to that of indomethacin (ED50 = 9.58 µmol). Compounds **3** and **17** proved to be the most active anti-inflammatory agents in the present study (ED50 = 9.22 and 9.28 µmol).

Compounds **3, 4, 6, 9, 10, 15** and **17** that showed anti-inflammatory activity in the cotton pellet-induced granuloma bioassay, were further evaluated for their in vivo systemic effect using the carrageenan-induced paw edema bioassay in rats[24]. The results **(Table 1)** revealed that all test compounds were active. Compounds **3** and **10** exhibited the most effective systemic anti-inflammatory activities (70.5 and 69.4 % protection respectively) comparable to that of indomethacin (74.1 % protection). In this experiment compound **17** was not as active as in the cotton pellet-induced granuloma model (63.1% protection).

The seventeen compounds were evaluated for their ulcerogenic potential in rats. All of them proved to have a superior GI safety profiles (10– 40% ulceration) in the population of the test animals at the oral doses of 30 µmol/Kg /day, when compared with indomethacin; the reference drug; which was found to cause 100 % ulceration under the same experimental conditions **(Table 2)**.

In this experiment compound **3** retained its superiority as it showed the minimal ulcerogenic effect (10%) among the other compounds. Compound **17** showed the same ulcerogenic effect (10%), though is it less active as anti-inflammatory. The ulcerogenic effect of compound **10** showed higher ulcerogenic effect (30%) comparing with **3** and **17**. However, all of them are much safer to stomach than indomethacin. Compounds **2, 3, 4, 6, 9, 10, 15** and **17** with the most promising anti-inflammatory effect were further evaluated for their oral acute toxicity in male mice using the method described in the literature[26 27]. The obtained results indicated that most of the tested compounds proved to be non-toxic and well tolerated by the experimental animals up to 200 mg / Kg. Moreover, these compounds were tested for their toxicity through parenteral route [28]. The results revealed that all the test compounds were non-toxic up to 100 mg /Kg. It can be safely concluded that compounds **3 and 10** proved to be the most active anti-inflammatory agent in the present study with minimum ulcerogenic effect (10% compared to 100% of indomethacin) and good safety margin. Its anti-inflammatory activity is comparable to that of indomethacin (ED50 = 9.22 µmol) and non toxic up to 200 mg/kg orally.

Preliminary molecular modeling and docking studies:

For molecular modeling and structure-based drug design, knowledge of the 3D structure of the target protein is inevitably required. The approach followed here starts from a known binding mechanism. A lead structure is designed rationally and experimentally tested. The obtained results are fed back into a design cycle as new information. So a computational study was performed to elucidate the hypothetical binding mode of the ligands which show a different activity profiles and to interpret our experimental results. Docking as a very useful strategy used to predict the binding site, conformation and affinity of the drug into its biological target by virtual screening of databases to select the most promising molecules to target a biomolecule, and to study the mechanism of an enzymatic reaction, as well as to identify possible binding modes for a ligand. In the present work, we used Molegro, and DS Viewer programs. Molegro is a suit of automated docking tools, which allows flexible ligand docking. Molegro predicts how small molecules, such as substrates or drug candidates bind to a receptor of known 3D structure by docking of the ligand to the target protein.The scoring functions used are empirically derived, that allows the prediction of the binding energies of docked ligands.

The protein target needs to be prepared and modeled according to the format requirements of the docking algorithms used. Thus the crystal structure of (COX2) cycloxygenase II was downloaded from the Brookhaven Protein Databank (www. Rcsb. Org PDB ID 4COX) [22] for using with Molegro software (**Figure 1**).

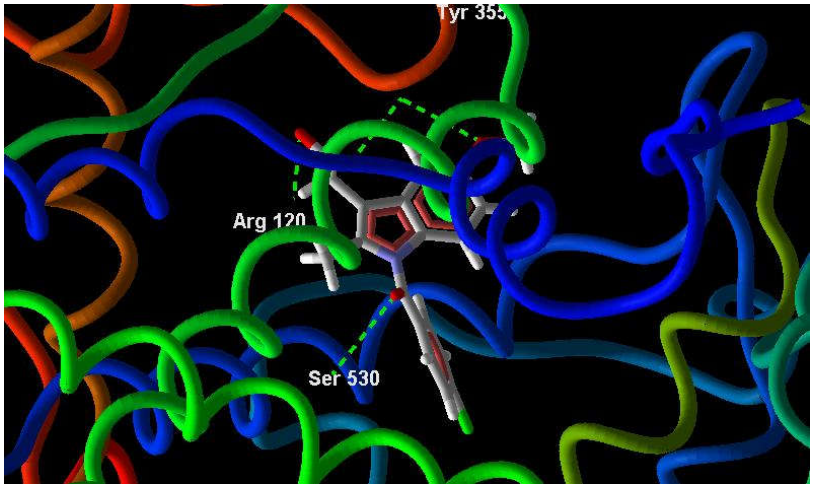


Figure 1: X-ray structure of COX2 with co-crystalliyed ligand. Interactions between H-bonded atoms are indicated by dotted lines. Hydrogen (white), nitrogen (blue), and oxygen (red)

The water molecules and co-crystalliyed ligand are removed. Molegro provides a nice graphical user interface for working. The protein for docking with molegro is prepared using by adding polar hydrogens to the protein atoms and assigning charges afterwards, For the ligand, all hydrogen atoms must be present on it to calculate partial atomic charges on the ligand. The protein active site **(Figure2)** is defined by placing a grid over the center of co-crystallized ligand. Hydrogens are also added to the ligand and charges are assigned. Before a protein is ready for docking simulations, all the necessary grid maps are calculated prior to docking.

dotted lines. Hydrogen (white), nitrogen (blue), and oxygen (red)

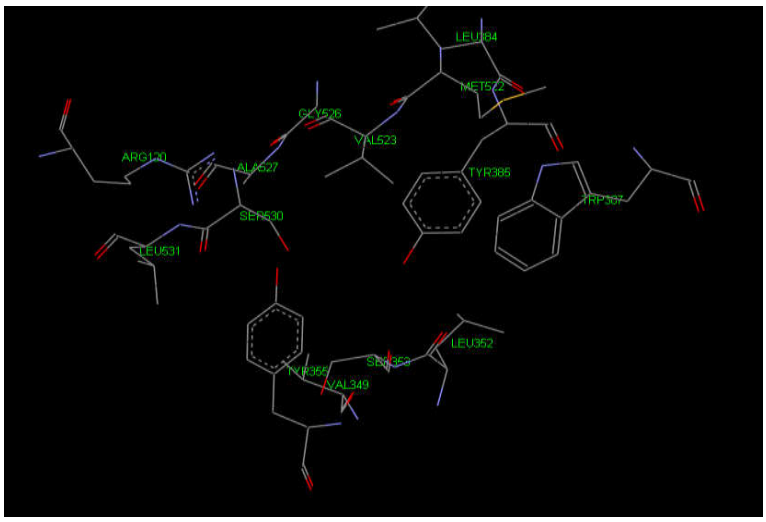


Figure 2: The active site of COX2 showing the most important Residues.

From molecular modeling studies we can conclude that, the proposed binding mode of compound **3** is that the oxygen atom of the hydroxyl at position 7 of the coumarin core of the ligand is involved in a hydrogen bonding interaction with Ser530 and one oxygen of the nitro group on the phenyl ring is involved in another hydrogen bonding with Gly526. Furthermore, the affinity of the ligand to receptor is potentiated by aromatic stacking interactions with Tyr385 which contributes to an increase in the affinity of the ligand (**Figure 3**).

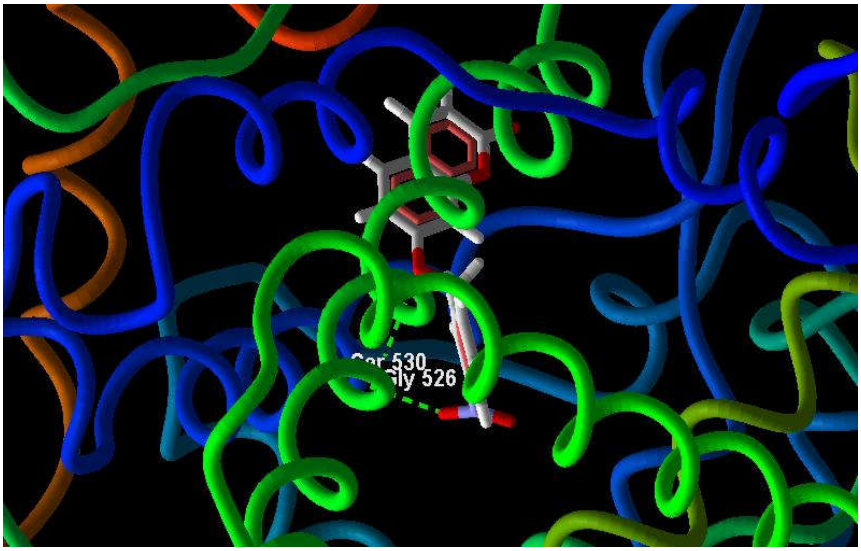


Figure 3: Predicted binding mode for compound **3** with COX2. Interactions between H-bonded atoms are indicated by dotted lines. Hydrogen (white), nitrogen (blue), and oxygen (red)

The obtained binding modes of compounds (**9- 17**) (**Figure 4**) with COX2 follow the general pattern observed for compound **3**. As before, the hydrogen bonding with Ser530 and aromatic stacking interactions with the lipophilic pocket are maintained .

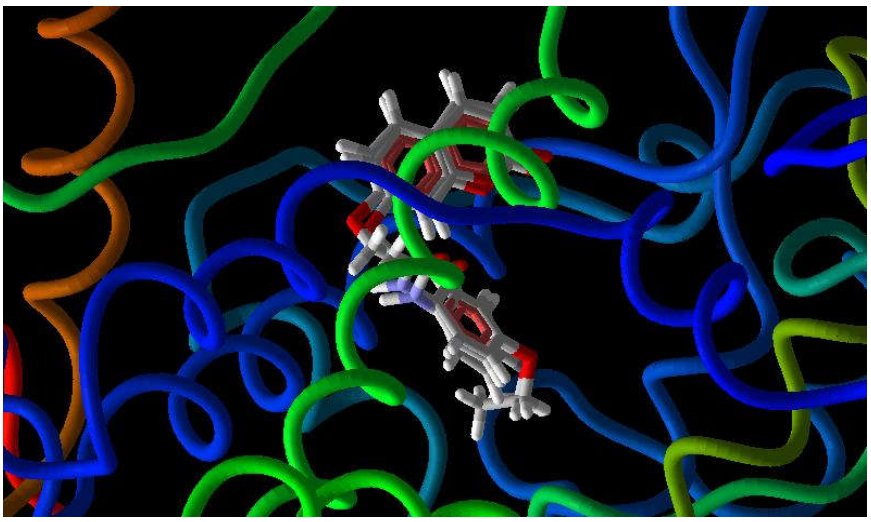


Figure 4: predicted binding modes for compounds (**9-16**) with COX2.

In Addition, the hydrophobic substitution of the aromatic of the ligands are located in the hydrophobic pocket formed by Phe518, Val349, Leu354, Tyr385 and Trp387 and the π staking are a possible reason for increasing the affinity of the ligands (**15-17**) where the strength of the interaction increases with increased hydrophobic interaction which would improve the binding affinity with the ligands (**Table 3**).

Table 3: The calculated energy and binding affinities for the ligands.

Ligand	MolDockScore	Rerank Score	HBond	Docking Score	Similarity Score
Indomethacin	-140.777	-114.814	-3.65375	-638.51	-491.067
1	-96.1696	-83.4048	-2.4586	-386.191	-291.536
2	-96.9266	-78.08	-0.0742989	-473.959	-382.597
3	-119.172	-100.139	-3.6009565	-504.005	-393.685
4	-100.259	-81.1359	-3.05194	-487.74	-386.693
5	-105.732	-75.9514	-3.73854	-498.144	-391.502
6	-116.281	-96.7158	-0.496319	-505.125	-388.646
7	-111.527	-87.1293	-1.55368	-499.606	-386.056
8	-105.732	-75.9514	-3.73854	-498.144	-391.502
9	-118.54	-86.5487	-0.205386	-524.753	-415.326
10	-119.081	-80.2888	-0.799372	-539.958	-428.132
11	-107.816	-80.2819	-2.5	-470.644	-364.706
12	-105.575	-82.7463	-0.746093	-521.918	-415.327
13	-104.67	-52.1999	-0.718581	-492.563	-389.311
14	-113.218	-78.8258	-0.814107	-535.52	-419.493
15	-118.868	-85.2459	-0.836493	-526.24	-415.829
16	-112.157	-80.7626	-0.475554	-543.866	-420.185
17	-118.991	-63.4706	-0.470861	-544.447	-427.867

Lengthening the space group by introduction of amide to the ligands (**9-17**) allows entrance of the phenyl (aromatic side chain) to the additional hydrophobic pocket in COX2 through the molecular gate formed by Phe518 and Val523 which is very important for increasing the affinity of the ligands for the receptor, also the nitrogen of the amide hydrogen bonding with Ser530 help anchoring the ligands and fix the substituted phenyl in the lipophilic pocket containing Phe518, Val523, Leu384, Tyr385 and Trp387(**Figure 5**).

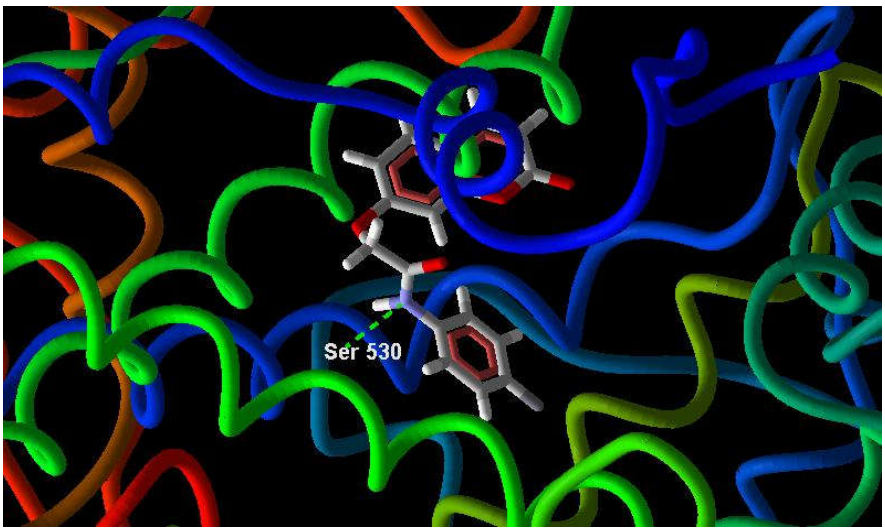


Figure 5: Predicted binding mode for compound **15** with COX2.
Interactions between H-bonded atoms are indicated by dotted lines.

Furthermore, the oxygen atom as substituents on the aromatic ring of the ligands serve as hydrogen bond acceptor (**9 and 10**) and increase the affinity for the COX2 receptor for example, the obtained result for the compound **10** is virtually almost the same as that of compound **3**, where the coumarin moiety of the ligand reside in a similar position. Additionally, the oxygen of the ethoxy group of the ligand is stabilized by hydrogen bonding interactions with Trp387 that mediate the hydrophobic interaction of the aromatic moiety(**Figure 6**).

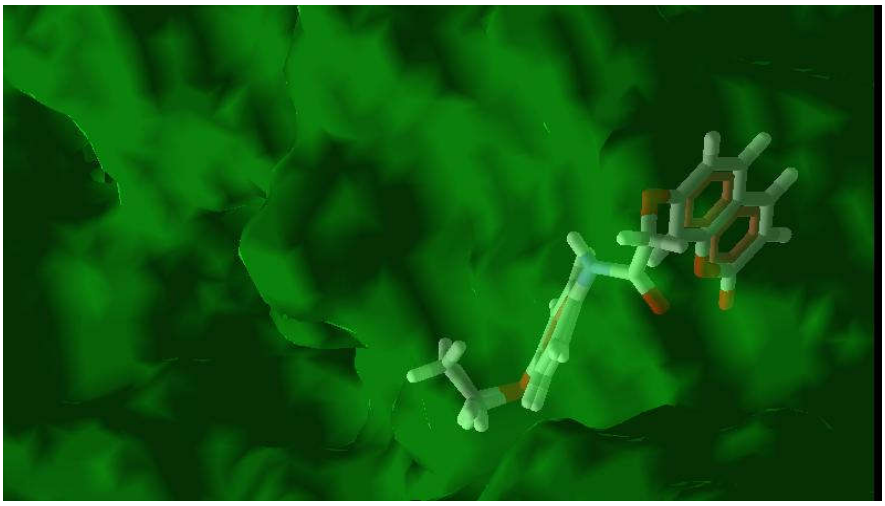


Figure 6: Surface showing binding mode for 9 and **10** with COX2

However, the substituents on the nitrogen atom of the space group of the ligands with aliphatic groups can decrease the affinity for the COX2 (**Table 3**). Furthermore, increasing the hydrogen bond acceptors or donars on the aromatic substituents (**3, 9** and **10**) can increase the affinity for the COX2 where these moieties are located inside the polar groups formed by Gln526, Ala527 and Ser530 . Also, because of the existence of additional hydrogen bonding and desirable interactions, compounds **3** and **10** have higher affinity towards the receptor than the other ligands.
The obtained binding mode of compound **10** with COX2 is virtually the same as that of compound **3**, In Addition, the aromatic ring of the ligand is located in the hydrophobic pocket formed by Leu384, Trp387, Val523, Phe518 and Tyr385. Besides, the hydrogen bonding interaction formed by interaction of oxygen atom of ethoxy side chain and amino acid Trp387 help locking the confirmation in the hydrophobic pocket which In particular could be suggested to provide significant stability of the complex. Moreover, the supposed orientation of the ligand inside the binding site of COX2 has the same volume of the putative pocket and that gives a possible explanation for the increased affinity of this ligand in comparison with other ligands (**Figure 7**).

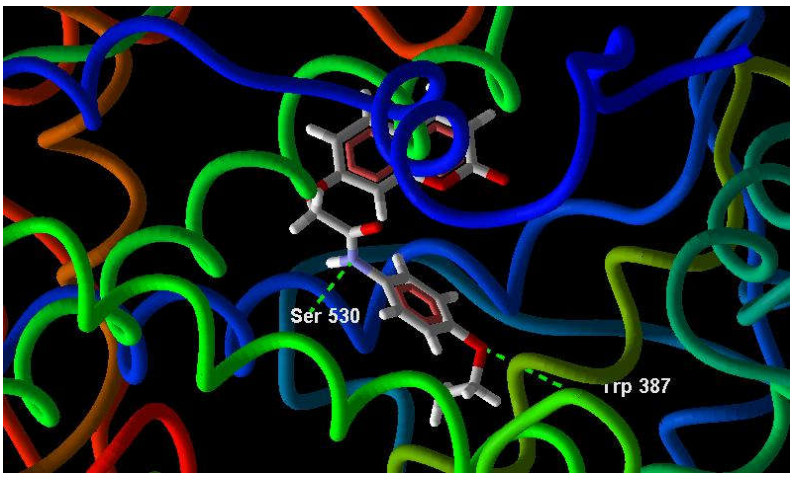


Figure 7. Predicted binding mode for **10** with COX2. Interactions between H-bonded atoms are indicated by dotted lines.

In summary, the obtained results indicated that all studied compounds have a similar position and orientation inside the putative binding site of COX2 (**Figure 8**). In addition, the results of the docking explain that some of these compounds have good binding affinity to the receptor and the computed values reflect the overall trend (**Table 3**).

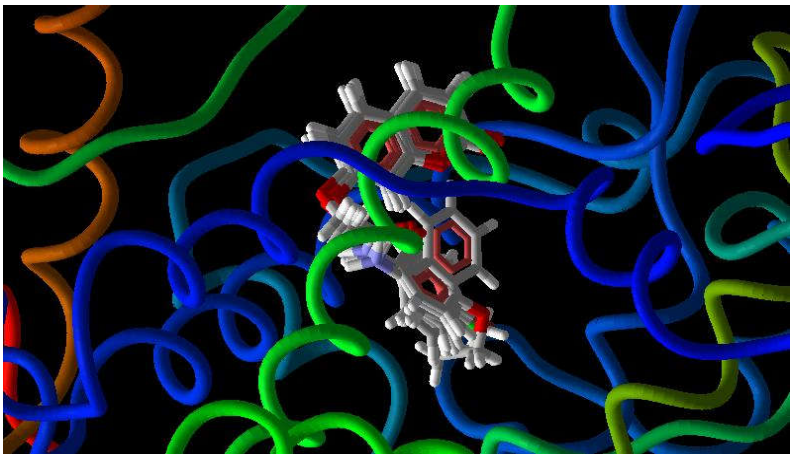


Figure 8. Superposition of the final ligand placements

Furthermore, the present study has highlighted that the hydrophobic moiety of the ligand is an attractive scaffold for obtaining potent COX2 inhibition where the amino acids Leu384, Trp387, Val523, Phe518 and Tyr385 within the binding pocket form the hydrophobic cavity.
Furthermore, the affinity of the ligands would increase if the ligands have the hydrogen bond acceptors or donars on the space group between coumarin moiety and aromatic group where these moieties are located inside the polar groups formed by Gl526, Ala527and Ser530 so that the introduction of hydrogen bond acceptor or donor moiety between the aromatic ring and the coumarin moiety leads to an increase in the COX2 affinity and could provide a very potent compound for the COX2 receptor.
For a ligand with high affinity for the COX2 receptor, the presence of the hydrogen bond acceptor and the aryl substituted with hydrophobic groups are an essential, where the introduction of a phenyl ring increased COX2 affinity. And a lipophilic residue is required within close proximity for hydrophobic interactions. The mainly polar amino acids of the hydrophilic part of the pocket seem to be responsible for anchoring the compound and locking the confirmation in position that increase hydrophobic contact area which in turn leads to increase the affinity of ligands to the COX2 receptor.

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