IJPSR (2017), Volume 8, Issue 12 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 10 April, 2017; received in revised form, 27 June, 2017; accepted, 17 September, 2017; published 01 December, 2017

STRUCTURAL DOCKING STUDIES AND BIOLOGICAL EVALUATION OF SOME NEWLY SYNTHESIZED SCHIFF BASE DERIVED QUINAZOLINONE DERIVATIVES

Poonam Arora [1], Kuljit Kaur [1], Rajanpreet Kaur [1] and Neelima Dhingra[*2]

GHG Khalsa College of Pharmacy [1], Gurusar Sadhar, Ludhiana - 141104, Punjab, India.
University Institute of Pharmaceutical Sciences [2], Panjab University, Chandigarh - 160014, Punjab, India.

Keywords:

Quinazolinone, COX-2, Docking, Anti-inflammatory, GLIDE

Correspondence to Author:
Dr. Neelima Dhingra

Assistant Professor, Department of Pharmaceutical Chemistry, University Institute of Pharmaceutical Sciences, Panjab University, Chandigarh - 160014, Punjab, India.

E-mail: neelimad08@gmail.com

ABSTRACT: Present studies describe the synthesis of a series of Schiff base derived quinazolinone derivatives (6-14) with the aim to have better anti inflammatory agents. The prepared compounds were characterized using chemical and spectral (IR, NMR and Mass) studies. Pharmacological evaluation of the newly synthesized derivatives indicated that compounds 8, 11 and 14 exhibit significant anti-inflammatory activity in carrageenan-induced rat paw oedema assay, and their effects are found to be comparable that of standard drug Indomethacin. Molecular docking studies were further employed to investigate the binding interactions to COX-2 active site residues and to study the stability of docked conformation in detail. The experimentally determined COX-2 inhibitory activity was found to be well correlating with binding modes predicted for compounds by GLIDE dock scoring function. The 2,3-diaryl-2,3-dihydro-1H-quinazolin-4-one pharmacophore reported herein represents a new lead for further development of novel non-steroidal anti-inflammatory agents.

INTRODUCTION: The molecular modifications of a promising lead compound are still a major line of approach for the discovery of new drugs. Quinazolin-4(3*H*)-one represents a versatile lead molecule for the design of potential bioactive agents [1-7] and the pharmaceuticals containing quinazolinone nucleus show varied biological activities as different substitution patterns results in diverse biological properties [8-10]. Number of quinazoline derivatives with different substitutions at 2,3,4,6 positions have been reported and studied as antitumor [11], antibacterial [12], anticonvulsant [13], antimalarials [14] and antiviral agents [15] as shown in **Table 1**.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.8(12).5088-99

Article can be accessed online on: www.ijpsr.com

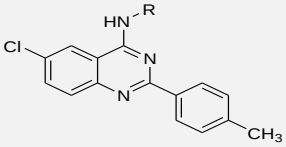
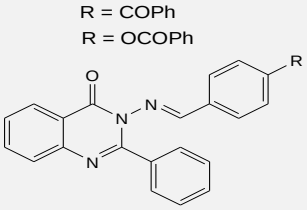
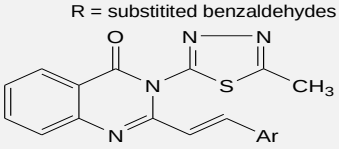
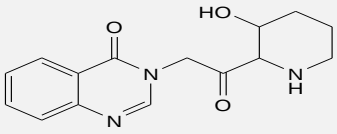
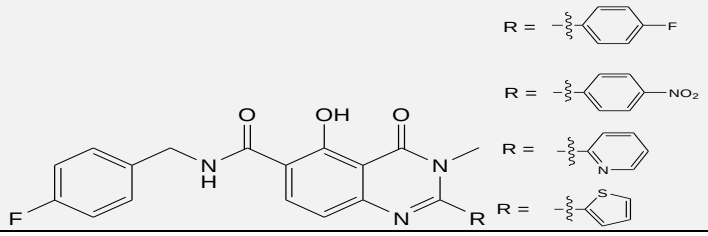
DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.8 (12).5088-99

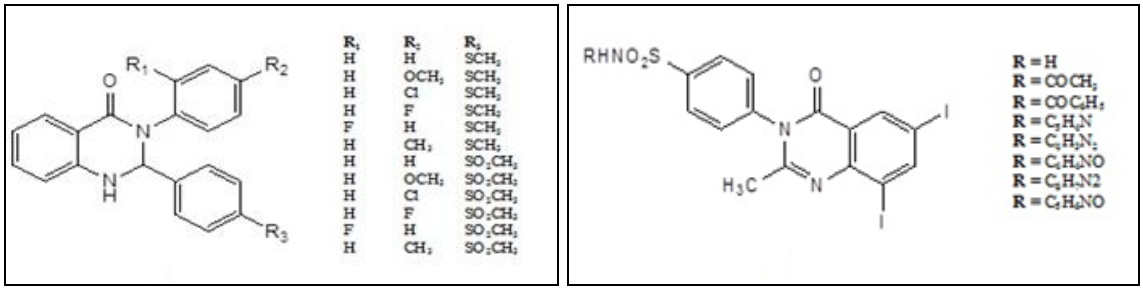
Further, it has been reported that substitution pattern by different moieties at 2/3 position of quinazoline nucleus markedly influences the anti-inflammatory activity [16, 17] (**Fig. 1**).

The commonly proposed mechanism of action of compounds used in inflammation is by lowering prostaglandin production through inhibition of cyclo-oxygenase (COX) enzyme [18, 19]. Two different forms of COX as COX-1 and COX-2 has been isolated and identified. This enzyme plays a crucial role in both pathological and physiological actions during inflammation. COX-1 is known as housekeeping enzyme, while COX-2 has been found to be expressed in pathological conditions, thus triggers inflammatory signals. Potent and selective COX-2 inhibitors by blocking the production of prostaglandin in inflammatory cells, without affecting in the homeostatic and gastro-protective actions mediated by COX-1, would be a logical approach towards treatment of inflammation [20].

Thus aiming at the discovery of newer, safer potent and selective COX-2 inhibitors with anti-inflammatory activity, and to investigate the influence of structural variation, quinazolinone nucleus was used as a template. Further given the significance of different substitutions at varied position in reported compounds (**1** and **2**), here present study describes the synthesis and evaluation of a series of novel compounds or derivatives of 4(3*H*)-quinazolinone, with structure modifications involving phenyl moiety at position 2 and formation of schiff base derived from acetophenones at position 3 [21, 22]. The structures of the compounds synthesized were assigned on the basis of ^{1}H NMR, IR, and mass spectral data. Further, the selectivity of the target molecules towards COX-2 has been predicted by docking studies [21-23]. The molecular interactions and binding mode of the compounds was proposed using GLIDE program. Moreover, it was considered of interest to investigate the influence of structural variation on the anticipated biological activities, through anti-inflammatory activity and is compared with indomethacin activity.

TABLE 1: DIFFERENTLY SUBSTITUTED QUINAZOLINONE DERIVATIVES WITH VARIED ACTIVITIES

Position	Structure	Activity	Reference
2,4		Anti-tumor	11
2,3	R = COPh R = OCOPh 	Antibacterial	12
2,3	R = substitited benzaldehydes 	Anti-convulsant and CNS depressant	13
3		Antimalarial	14
2,3,6		Antiviral	15



A **B**

FIG. 1: COMPOUNDS WITH DIFFERENT SUBSTITUTIONS AT 2/3 POSITION OF QUINAZOLINONE NUCLEUS SHOWING ANTI-INFLAMMATORY ACTIVITY

MATERIALS AND METHODS:

Chemistry:

Materials and Equipment: Melting points (mp) of products were determined by open capillary method using digital melting point apparatus and were uncorrected. FTIR spectra were recorded on a Perkin Elmer FT-IR RXI spectrophotometer using potassium bromide discs. Proton Nuclear Magnetic Resonance (^{1}H NMR) spectra were recorded on Bruker Advance II 400MHz spectrometer in dimethyl sulphoxide (DMSO-d_6) using trimethyl-silane (TMS) as internal standard. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F_{254} (E. Merck, Mumbai) and spots were visualized under UV light (254nm).

Electron spray ionization mass spectrometry (ESI-MS) study was performed using a Q-ToF quadrupole time of flight mass spectrometer (Waters) equipped with an electronspray source. Silica gel from E. Merck, Mumbai (60-120 mesh) was used for column chromatography. Anthranilic acid, benzoyl chlorides and acetophenones were purchased from Sigma Chemical (Berlin, Germany). All chemicals used were of analytical grade and commercially available.

General Procedure for the Synthesis of 2-phenyl benzoxazin-4(3H)-one (4a-4c): Anthranilic acid (0.01mol) was dissolved in 30ml of anhydrous pyridine with stirring at room temperature. The mixture was cooled to 0 °C and a solution of different substituted benzoyl chlorides (3a-3c) (0.01mol) in anhydrous pyridine (30ml) was slowly added to this solution with constant stirring for 3 h. The pasty mass obtained was diluted with water and the resulting mixture was treated with 5% sodium bicarbonate solution to remove the unreacted acid, when the effervescence ceased. The precipitate were filtered and washed with water to remove adhered pyridine. The 2-phenyl benzoxazine (4a-4c) thus obtained was dried and recrystallized from ethanol.

2-(4-Nitrophenyl)-benzoxazin-4(3H)-one (4a): Yellow crystalline solid, Yield: 69%, M.p. 157-159 °C. IR (KBr) cm^{-1}: 1768, 1682, 1064, 1595, 3078, 1352, 1519. ^{1}H NMR (400MHz, DMSO-d_6): δ 8.68-8.64 (1H, m, quinazolinone), 8.39-8.35 (2H, m, Ar-NO_2), 8.19-8.12 (2H, m, Ar-NO_2), 7.83-7.79 (1H, m, quinazolinone), 7.54-7.50 (1H, m, quinazolinone), 7.19-7.15 (1H, m, quinazolinone). ESI-MS (m/z): 268 ($C_{14}H_8N_2O_4$) (100%), 146 ($C_8H_4NO_2$), 122 ($C_6H_4NO_2$).

2-(2-Chlorophenyl)-benzoxazin-4(3H)-one (4b): Yellow crystalline solid, Yield 66%, M.p. 155-157 °C. IR (KBr) cm^{-1}: 1768, 1620, 1090, 3075, 764. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.20-8.18 (1H, m, quinazolinone), 8.01-7.97 (1H, m, quinazolinone) 7.94-7.92 (1H, m, quinazolinone), 7.75-7.61 (4H, m, Ar-H), 7.57-7.54 (1H, m, quinazolinone). ESI-MS (m/z): 258 (100%) ($C_{14}H_8NO_2Cl$), 146 ($C_8H_4NO_2$).

2-(4-Methylphenyl)-benzoxazin-4(3H)-one (4c): White crystalline solid, Yield 70%, M.p. 152-153 °C. IR (KBr) cm^{-1}: 1757, 1607, 1009, 1569, 3031. ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.15-8.15 (1H, d, quinazolinone), 8.10-8.08 (2H, d, Ar-H), 7.96-7.92 (1H, m, quinazolinone), 7.71-7.69 (1H, m, quinazolinone), 7.63-7.59 (1H, m, quinazolinone), 7.41-7.39 (2H, d, Ar-H), 2.41 (3H, s, CH_3). ESI-MS (m/z): 238 (100%) ($C_{15}H_{11}NO_2$), 146 ($C_8H_4NO_2$), 119 ($C_7H_4O_2$).

General Procedure for the Synthesis of 2-phenyl-3-amino quinazolin-4-one (5a- 5c): For the synthesis of the compounds (**5a- 5c**), various 2-phenyl benzoxazine derivatives (**4a- 4c**) (0.005mol) were treated with hydrazine hydrate (0.01mol). Anhydrous pyridine (25ml) was added drop wise, and the mixture was stirred for 30 min at room temperature, followed by refluxing for 6-9 h. The reaction mixture was allowed to cool at room temperature and poured into ice cold water containing dilute hydrochloric acid. On standing for 1 h, solidification occur, the product was filtered off, washed with water and dried.

3-Amino- 2- (4-nitrophenyl)- quinazolin- 4(3H)-one (5a): Yellow crystalline solid, Yield 62%, M.p. 157-160 °C. IR (KBr) cm^{-1}: 3302, 3220, 1449, 1672, 1598, 1256, 1524, 3076, 1346, 1524. ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ 3.58 (2H, s, NH_2), 8.67-8.65 (1H, m, quinazolinone), 8.38-8.34 (2H, m, Ar-H), 8.20-8.13 (2H, m, quinazolinone), 7.82-7.80 (1H, m, quinazolinone), 7.55-7.51 (1H, m, quinazolinone), 7.19-7.15 (1H, m, quinazolinone). ESI-MS (m/z): 283 ($C_{14}H_{10}N_4O_3$) (100%), 122 ($C_6H_4NO_2$), 160 ($C_8H_6N_3O$).

3-Amino-2- (2-chlorophenyl)- quinazolin- 4(3H)-one (5b): White crystalline solid; Yield 67%, M.p. 157-159 °C. IR (KBr) cm^{-1}: 1676, 1598, 1256, 1598, 2920, 764, 3484. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.58 (2H, s, NH_2), 8.65-8.64 (1H, m, quinazolinone), 8.36-8.32 (2H, m, Ar-H), 8.10-8.18 (2H, m, quinazolinone), 7.80-7.78 (1H, m, quinazolinone), 7.53-7.49 (1H, m, quinazoli none), 7.17-7.13 (1H, m, quinazolinone). ESI-MS (m/z): 146 ($C_8H_4NO_2$), 272 (100%) ($C_{14}H_{10}N_3OCl$), 236 ($C_{14}H_{10}N_3O$), 255 ($C_{14}H_9N_2O$), 160 ($C_8H_6N_3O$), 112 (C_6H_4Cl).

3-Amino-2-(4-methylphenyl)- quinazolin- 4(3H)-one (5c): Yellow crystalline solid, Yield 65%, M.p. 155-157 °C. IR (KBr) cm^{-1}: 1668, 1598, 1256, 2920, 3488. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 3.55 (2H, s, NH_2), 8.15-8.15 (1H, d, quinazolinone), 8.10-8.08 (2H, d, Ar-H), 7.96-7.92 (1H, m, quinazolinone), 7.71-7.68 (1H, m, Ar-H), 7.62-7.55 (1H, m, Ar-H), 7.40-7.38 (2H, d, quinazolinone) 2.45 (3H, s, CH_3). ESI-MS (m/z): 251 (base peak), 236 ($C_{14}H_{10}N_3O$), 144 ($C_{15}H_{13}N_3O$), 160 ($C_8H_6N_3O$) (fragmentation).

General Procedure for the Synthesis of Quinazolinone Fused Schiff Bases (6-14): To a solution of the appropriate acetophenones (0.01mol) dissolved in ethanol (20ml), was added different substituted 3-amino-2-phenylquinazolin-4-one derivative (**5a-5c**) (0.01mol) and pH of the resultant solution was adjusted to 4.0-4.5 using glacial acetic acid. The resulting mixture was refluxed for 2-3 h. The solid thus obtained was filtered and purified by column chromatographic method using n-hexane/ethyl acetate (75:25) as eluents.

3- [2- (4- Chlorophenyl)- ethylimino]- 2- (4-nitrophenyl)- quinazolin- 4(3H)-one (6): Yellow crystalline solid, Yield 65%, M.p. 162-164 °C. IR (KBr) cm^{-1}: 1765, 1351, 1520, 1685, 1253, 775, 2870, 1598, 3075. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.36-8.34 (1H, m, quinazolinone), 8.30-8.26 (2H, m, Ar-H NO_2 containing), 8.24-8.18 (2H, d, Ar-NO_2), 8.03-7.91 (2H, m, Ar-Cl), 7.87-7.84 (2H, m, Ar-Cl), 7.83-7.79 (1H, m, quinazolinone), 7.77-7.71 (1H, m, quinazolinone), 7.44-7.39 (1H, m, quinazolinone), 2.12 (3H, s, CH_3). ESI-MS (m/z): 283 (100%) ($C_{14}H_{10}N_4O_3$), 126 ($C_6H_4NO_2$), 237 ($C_{14}H_{10}N_3O$). Anal. Calculated for $C_{22}H_{15}N_4O_3Cl$: N, 13.38%. Found: N, 16.53%.

3- [2- (3- Nitrophenyl)- ethylimino]- 2- (4-nitro phenyl)- quinazolin- 4(3H)-one (7): Yellow crystalline solid, Yield 62%, M.p. 158-160 °C. IR (KBr) cm^{-1}: 1679, 1343, 1520, 1641, 1251, 2923, 1581, 3088. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.68-8.69 (1H, m, quinazolinone), 8.44-8.41 (2H, m, Ar-H), 8.35-8.33 (2H, m, Ar-H), 8.30-8.28 (1H, m, Ar-H), 8.24-8.22 (1H, m, Ar-H), 8.11-8.08 (1H, m, quinazolinone), 7.83-7.76 (2H, m, Ar-H), 7.73-7.71 (1H, m, quinazolinone), 7.57-7.53 (1H, m, quinazolinone), 2.69 (3H, s, CH_3). ESI-MS (m/z): 283 (100%) ($C_{14}H_{10}N_4O_3$), 254 ($C_{14}H_{10}N_3O_2$), 237 ($C_{14}H_{11}N_3O$), 126 ($C_6H_4NO_2$). Anal. Calculated for $C_{22}H_{15}N_5O_5$: N, 16.31%. Found: N, 16.85%.

3- [2- (3- Hydroxyphenyl)- ethylimino]- 2-(4-nitrophenyl)-quinazolin- 4(3H)- one (8): Yellow crystalline solid, Yield 67%, M.p. 140-141 °C. IR (KBr) cm^{-1}: 1684, 1343, 1524, 1638, 1275, 1594, 3115, 3291. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.35-8.32 (1H, d, quinazolinone), 8.23-8.20 (2H, m, Ar-H), 8.07-8.04 (1H, m, Ar-H), 7.90-7.85 (2H, m, Ar-H), 7.76-7.74 (2H, m, Ar-H), 7.63-7.59 (1H, m, Ar-H), 7.58-7.55 (2H, m, quinazolinone), 7.54-7.53 (1H, m, quinazolinone), 2.42 (3H, s, CH_3). ESI-MS (m/z): 283 (100%), 254 ($C_{14}H_{10}N_3O_2$), 237 ($C_{14}H_{11}N_3O$), 126 ($C_6H_4NO_2$). Anal. Calculated for $C_{22}H_{16}N_4O_4$: N, 13.99%. Found: N, 17.57%.

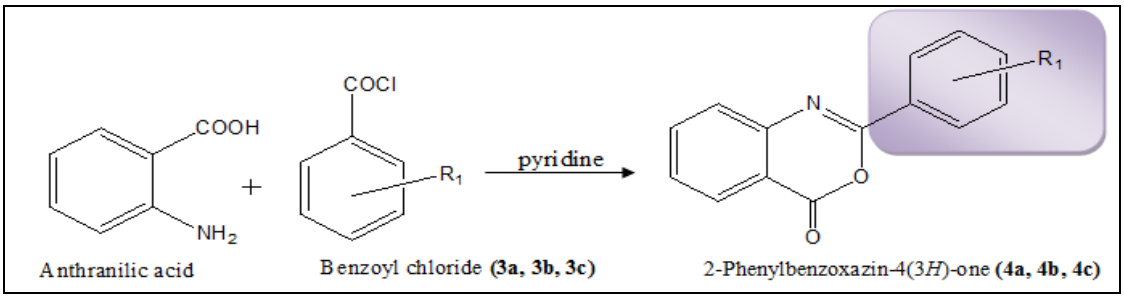
3- [2- (4- Chlorophenyl)- ethylimino]- 2- (2-chlorophenyl)- quinazolin-4(3H)-one (9): Yellow compound, Yield: 70%, M.p. 198-200 °C. IR (KBr) cm^{-1}: 1663, 748, 1590, 1272, 3055, 1521, 3245. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.35-8.33 (1H, m, quinazolinone), 7.97-7.95 (1H, d, Ar-H), 7.87-7.86 (2H, m, quinazolinone), 7.76-7.74 (1H, m, quinazolinone), 7.67-7.47 (6H, m, Ar-H), 7.35-7.29 (1H, m, Ar-H), 2.36 (3H, s, CH_3). ESI-MS (m/z): 258 ($C_{14}H_9ClN_2O$) (100%), 146 ($C_8H_5N_2O$), 272 ($C_{14}H_{10}ClN_3O$), 236 ($C_{14}H_9N_3O$), 312 ($C_{16}H_{11}N_4O_3$). Anal. Calculated for $C_{22}H_{15}N_3OCl_2$: N, 10.39%. Found: N, 10.29%.

3- [2- (3- Nitrophenyl)- ethylimino]- 2- (2-chloro phenyl)- quinazolin- 4(3H)- one (10): Lemon coloured solid, Yield 67%, M.p. 178-180 °C. IR (KBr) cm^{-1}: 1645, 1348, 1524, 1524, 1348, 755, 2962, 3093. ^1H NMR (400 MHz, DMSO-d_6, ppm):

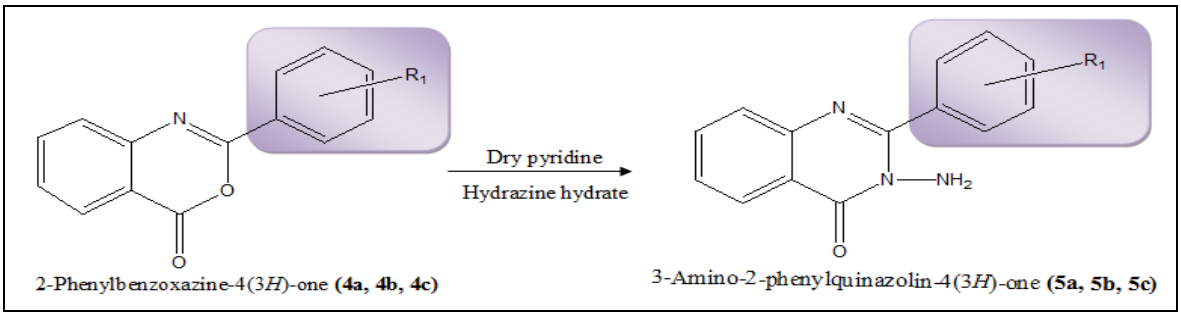
δ 8.59-8.58 (2H, m, Ar-NO_2), 8.36-8.34 (2H, m, Ar-Cl), 8.44-8.41 (2H, m, Ar-Cl-NO_2), 8.24-8.22 (2H, m, Ar-H quinazolinone), 8.21-8.20 (2H, m, quinazolinone), 7.32-7.30 (1H, m, Ar-Cl) 7.82-7.78 (2H, m, Ar-H) 7.87-7.85 (1H, m, Ar-Cl), δ 2.44 (3H, s, CH_3). ESI-MS (m/z): 258 ($C_{14}H_9ClN_2O$), 272 ($C_{14}H_{10}ClN_3O$) (100%), 236 ($C_{14}H_9N_3O$), 312 ($C_{16}H_{11}N_4O_3$). Anal. Calculated for $C_{22}H_{15}N_4O_3Cl$: N, 13.38%. Found: N, 11.94%.

3- [2- (3- Hydroxyphenyl)- ethylimino]- 2-(2-chlorophenyl)- quinazolin- 4(3H)- one (11): Whitish yellow solid, Yield 67%, M.p. 218-220 °C. IR (KBr) cm^{-1}: 1647, 1599, 1270, 753, 2924, 3060, 3289. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.54-8.52 (1H, m, quinazolinone), 7.87-7.86 (1H, m, Ar-OH), 7.81-7.43 (8H, m, Ar-H and quinazolinone), 7.26-7.22 (1H, m, Ar-Cl), 6.84-6.78 (1H, m, Ar-Cl), 2.31 (3H, s, CH_3), 11.35 (1H, s, OH). ESI-MS (m/z): 258 (100%) ($C_{14}H_9ClN_2O$), 146 ($C_8H_6N_2O$), 272 ($C_{14}H_{10}N_3ClO$). Anal. Calculated for $C_{22}H_{15}N_4O_3Cl$: N, 10.78 %. Found: N, 11.33 %.

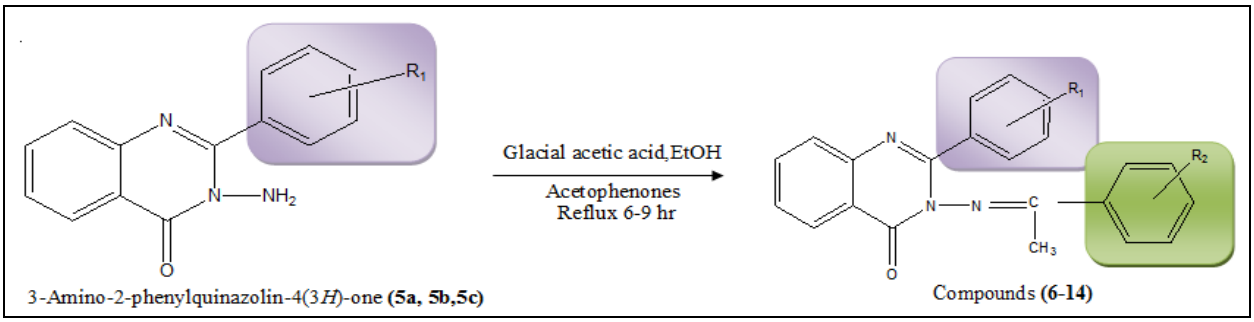
Step 1:



Step 2:



Step 3:



6. R_1=4-NO_2, R_2 =4-Cl, **7.** R_1=4-NO_2, R_2 =3-NO_2, **8.** R_1=4-NO_2, R_2 = 3-OH, **9.** R_1= 2-Cl, R_2 =4-Cl, **10.** R_1= 2-Cl, R_2 =3-NO_2, **11.** R_1= 2-Cl, R_2 = 3-OH, **12.** R_1= 4-CH_3, R_2 =4-Cl, **13.** R_1= 4-CH_3, R_2 =3-NO_2, **14.** R_1= 4-CH_3, R_2 = 3-OH

SCHEME 1S: STEPS INVOLVED IN THE SYNTHESIS OF SCHIFF BASE DERIVED QUINAZOLINONE DERIVATIVE

3- [2- (4- Chlorophenyl)- ethylimino]- 2- (4-methylphenyl)-quinazolin-4(3H)-one (12): White crystalline solid, Yield 70%, M.p. 203-205 °C. IR (KBr) cm^{-1}: 1653, 1529, 1312, 760, 3054, 1603, 3235. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.60-8.58 (1H, m, quinazolinone), 7.90-7.84 (5H, m, Ar-H and quinazolinone), 7.60-7.55 (1H, m, Ar-H), 7.46-7.44 (2H, m, quinazolinone), 7.36-7.34 (2H, m, Ar-H), 7.25-7.21 (1H, m, Ar-H), 2.41-2.39 (6H, s, CH_3). ESI-MS (m/z): 238 (100%)

($C_{15}H_{12}N_2O$), 252 ($C_{15}H_{13}N_3O$), 126 (C_7H_7Cl), 292 ($C_{16}H_{11}ClN_3O$). Anal. Calculated for $C_{23}H_{18}N_3OCl$: N, 10.83 %. Found: N, 11.36 %.

3- [2- (3- Nitrophenyl)- ethylimino]- 2- (4- methylphenyl)- quinazoline- 4(3H)- one (13): Yellow crystalline solid, Yield 70%, M.p. 216-218 °C. IR (KBr) cm^{-1}: 1644, 1344, 1526, 1606, 1102, 2921, 3093. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.63-8.62 (1H, m, quinazolinone), 8.48-8.45 (1H, d, Ar-NO_2), 8.39-8.37 (1H, d, Ar-NO_2), 8.29-8.27 (1H, m, quinazolinone), 7.85-7.81 (2H, m, Ar-NO_2), 7.76-7.75 (2H, m, quinazolinone), 7.64-7.61 (2H, m, Ar-CH_3), 7.39-7.37 (2H, m, Ar-CH_3), 2.68-2.36 (6H, s, $(CH_3)_2$). ESI-MS (m/z): 238 (100%) ($C_{15}H_{11}N_2O$), 252 ($C_{15}H_{11}N_3O$), 337 ($C_{22}H_{15}N_3O$), 119 (C_8H_8N). Anal. Calculated for $C_{23}H_{18}N_4O_3$: N, 14.06%. Found: N, 12.67%.

3- [2- (3- Hydroxyphenyl)- ethylimino]- 2- (4- methylphenyl)- quinazolin- 4(3H)- one (14): Whitish yellow solid, Yield 68%, M.p. 208-210 °C. IR (KBr) cm^{-1}: 1646, 1599, 1101, 2949, 1513, 3060, 3289. ^1H NMR (400 MHz, DMSO-d_6, ppm): δ 8.58-8.56 (1H, d, quinazolinone), 7.93-7.92 (1H, d, Ar-OH), 7.86-7.80 (2H, d, Ar-OH), 7.80-7.75 (2H, d, Ar-CH_3), 7.63-7.57 (1H, m, Ar-H), 7.40-7.38 (2H, d, Ar-H), 7.27-7.22 (1H, m, Ar-H), 6.87-6.83 (2H, d, Ar-H), 2.38 (3H, s, CH_3), 2.33 (3H, s, CH_3), 11.91 (1H, s, OH). ESI-MS (m/z): 238 (100%) ($C_{15}H_{12}N_2O$), 119 (C_8H_8N), 276 ($C_{15}H_{12}N_2O$). Anal. Calculated for $C_{22}H_{15}N_4O_3Cl$: N, 12.03%. Found: N, 12.03%.

Pharmacology: The pharmacological evaluation or anti-inflammatory activity of the prepared compounds was performed by carrageenan-induced rat paw edema assay [24].

Animals: Wistar rats (8-10 weeks old; 200-250g) of both sexes were housed in plastic cages under standard laboratory conditions and maintained on rat chow and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) and conducted according to the guidelines of Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), New Delhi, India.

Anti-inflammatory Activity: Anti-inflammatory activity of the newly synthesized compounds was determined using carrageenan-induced foot paw edema assay method in rats and indomethacin was used as a standard drug [9]. Unless otherwise stated, the following conditions were employed in all experiments. Target compounds (6-14) were suspended in aqueous solution of carboxymethyl cellulose (CMC, 0.5% w/v) and administered orally at a dose level of 10mg/kg of synthesized compounds (6-14), respectively. Control animals were similarly treated with aqueous solution of carboxymethyl cellulose (CMC, 0.5% w/v). After 30 min, 0.1ml of freshly prepared 1% carrageenan solution in normal saline was injected into the subplantar region of the right hind paw of each rat. The increase in paw volume was measured by using plethysmometer (water displacement, UGO BASILE, Italy) at 2, 3 and 4 h after carrageenan challenge. The percentage inhibition of inflammation was calculated in comparison to the control group by the given formula:

$$\frac{V_c - V_t}{V_c} \times 100$$

Where V_c is the increase in paw volume of control (without drug) and V_t is the increase in paw volume after administration of the test compound.

Molecular Docking Study: Docking studies were performed to find the hypothetical binding of quinazolinone containing Schiff bases with the target protein COX-2 by using the program GLIDE (Grid-based Ligand Docking with Energetic) module version Schrodinger 9.2, Schrodinger, LLC, New York, NY (Schrodinger Inc.).

Preparation of Protein: The crystal structure of COX-2 enzyme (PDB ID: 1CX2) was obtained from the RCSB Protein Data Bank, where the selective COX-2 inhibitor SC-558 houses in its active site. This PDB structures was downloaded, refined and prepared using Schrodinger protein preparation wizard tool (GLIDE), which performs the following steps: assigning of bond orders, addition of hydrogen, and optimization of hydrogen bonds by flipping amino side chains, correction of charges, and minimization of the protein complex. All the bound water molecules, ligands and cofactors were removed (pre-process) from the protein and were taken in mae format. The tool neutralized the side chains that are not close to the binding cavity and do not participate in salt bridges.

Preparation of Ligands: Structures of the ligands 2,3-disubstituted quinazolinone derivatives containing Schiff bases (6-14) were drawn by using chemdraw ultra 8.0 and the three-dimensional structures of the compounds were constructed using the Maestro interface. All 3D ligands were subjected to energy minimization and optimization using Ligprep module. LigPrep is a utility of Schrodinger software suit that combines tools for generating 3D structures from 1D (Smiles) and 2D (SDF) representation, searching for tautomers, steric isomers and perform a geometry minimization of the ligands.

Validation of the Docking Protocol in Glide: The most suitable method of evaluating the accuracy of a docking procedure is to determine how closely the lowest energy pose predicted by the scoring function resembles an experimental binding mode as determined by X-ray crystallography. In the present study, the docking of protein with its already present ligand was performed to test the reliability and reproducibility of the docking protocol for our study. The root mean square deviation (RMSD) between the predicted conformation and the observed X-ray crystallographic conformation of the ligand by Glide (3A) was analyzed. This indicates the reliability of the docking method in reproducing the experimentally observed binding mode for target proteins.

GLIDE Docking: The impref script runs a series of restrained impact energy minimizations using the Impact utility. Minimizations were run until the average root mean square deviation (rmsd) of the non-hydrogen atoms reached 0.3 Å. Glide uses two boxes that share a common centre to organize its calculations: a larger enclosing box and a smaller binding box. The grids themselves are calculated within the space defined by the enclosing box. The binding box defines the space through which the centre of the defined ligand will be allowed to move during docking calculations. It provides a measure of the effective size of the search space.

The only requirement on the enclosing box is that it should be large enough to contain all ligand atoms, even when the ligand centre is placed at an edge or vertex of the binding box. Grid files were generated using the cocrystallized ligand at the centre of the two boxes. The size of the binding box was set at 20 Å in order to explore a large region of the protein. The initial geometry of the structures was optimized using the OPLS-2005 force field performing 1000 steps of conjugate gradient minimization. The compounds were subjected to flexible docking using the pre-computed grid files. For each compound the 100 top-scored poses were saved and analyzed and only the best scoring poses were selected for the study.

Prediction of Pharmacokinetic Parameter (*in silico*): The *in silico* pharmacokinetic properties of the designed quinazoline analogues (6-14) were estimated by means of molecular weight, dipole moment, H-bond donors, H-bond acceptors, QPlogP o/w, QPlogS, QlogBB, human oral absorption, and polar surface area (PSA), molecular surface descriptors (solvent accessible surface area) and other parameters such as wiener index (WI) were also determined using QikProp (version9.2) module of Schrödinger. As well as the Lipinski' rule of five was to filter molecules with filter for drug-like properties has also been estimated.

RESULTS AND DISCUSSIONS:

Chemistry: Present work described the synthesis of nine analogous of 2,3-diaryl-3*H*-quinazolin-4-ones (6-14) and the synthetic strategy adopted for the synthesis of target compounds is outlined in **Scheme 1**. Benzoxazinones (4a-4c) were prepared from commercially available anthranilic acid and its treatment with differently substituted benzoyl chloride (3a, 3b, 3c). 3-Amino-2-phenyl-quinazolinones (5a-5c) were synthesized from various benzoxazinones by treatment with hydrazine hydrate in anhydrous pyridine. Final target compounds (6-14) were prepared from 3-amino-2-phenylquinazolinones (5a-5c), by adding different substituted acetophenones in acidic medium. Progress of the reaction was monitored by TLC using chloroform: methanol (9:1) as mobile phase. The structures of the newly synthesized compounds were confirmed by IR, NMR and mass spectral analysis followed by their physicochemical properties determination.

Anti-inflammatory Activity: The antiinflammatory activity of the newly synthesized compounds (6-14) was evaluated by applying carrageenan-induced paw edema bioassay in rats [1, 22] and indomethacin

was used as standard drug. Inflammation was produced by injecting 0.1ml of 1% w/v of carrageenan into the subplanatar region, and reproducible and substantial inflammation was observed in all the groups. Plethysmometer was used to measure to paw volume before and after carrageenan treatment at 1 hour time interval upto 4 hours after carrageenan challenge and percentage inhibition of inflammation was calculated in comparison to the control. Rats of either sex were randomized into control, standard and test groups of five rats each. Indomethacin (10mg/kg body weight) was administered orally as suspension (0.5% w/v carboxymethyl cellulose) to standard group whereas 1% w/v carboxymethyl cellulose (vehicle alone) was given to control group. The results were expressed as mean ± S.E (n = 3). Statistically significant difference between vehicle control group and treatment group data was analyzed by one way ANOVA test followed by the least significant difference (L.S.D.). The observations for the paw volume are given in **Table 2**.

TABLE 2: ANTI-INFLAMMATORY DATA OF 2, 3-DIARYL-3*H*-QUINAZOLIN-4-ONES DERIVATIVES (6-14) ON CARRAGEENAN-INDUCED PAW EDEMA IN RATS

Treatment	Volume of paw edema in ml (% edema inhibition)		
	After 2-hour	After 3-hour	After 4-hour
Control	0.87± 0.035	0.84±0.022	0.84±0.027
Indomethacin (Standard)	0.17±0.019(79)	0.22± 0.018(75)	0.22±0.022(75)
6	0.39±0.019(35)	0.39±0.023(30)	0.38±0.019(40)
7	0.62±0.037(29)	0.60±0.026(29)	0.62±0.010(26)
8	0.35±0.021(60)	0.33±0.023(61)	0.36±0.022(60)
9	0.47±0.030(46)	0.45±0.007(46)	0.45±0.080(46)
10	0.63±0.016(28)	0.63±0.015(25)	0.62±0.014(26)
11	0.36±0.029(59)	0.34±0.015(60)	0.36±0.022(57)
12	0.46±0.016(47)	0.44±0.015(48)	0.47±0.027(44)
13	0.47±0.018(46)	0.46±0.035(45)	0.47±0.018(44)
14	0.39±0.030(55)	0.37±0.020(59)	0.34±0.029(57)

All values are expressed as mean ± S.E.M

Compounds were found to exhibit anti-inflammatory activity in the range of 25 to 61%, however, the significant decrease in the paw volume was observed at third hour when administered at doses of 10mg/kg body weight (**Table 2**). Among the newly synthesized series of 2,3- diaryl- 3*H*- quinazolin- 4- ones, the tested compounds 8, 11, 14 showed more than 50% edema inhibition and the difference in paw thickness was comparable to the standard drug indomethacin (**Fig. 2**). The (OH) hydroxyl group at R_2 position of 2, 3-diaryl-3*H*-quinazolin-4-ones in compounds 8, 11 and 14 appears to have a positive effect on the anti-inflammatory potency of these compounds.

On the other hand, compounds 7 and 10 demonstrated 29% and 25% inhibition (after 3 h) and were found to be the least active towards carrageenan-induced edema in comparison to the standard drug indomethacin. Further, anti-inflammatory activity was not only found to be comparable with standard drug, but with a longer duration as activity was retained upto 24 hr.

This phenomenon may be partly be due to the low systemic bioavailability of test compounds following oral dosing, efficient first pass metabolism and some degree of intestinal metabolism [10].

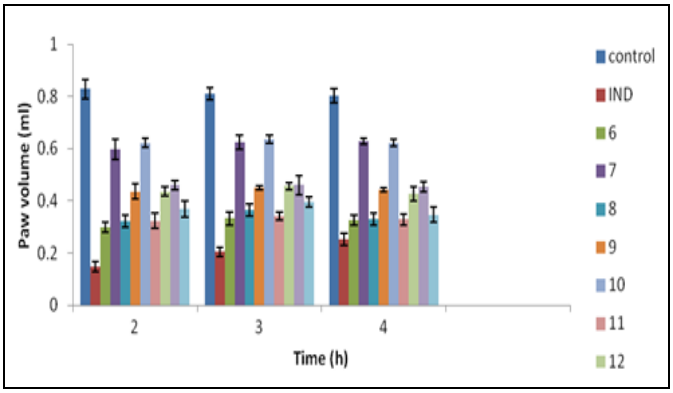


FIG. 2: VOLUME OF PAW EDEMA AFTER 2, 3, 4 hr

Molecular Docking Study: The final compounds (6-14) were evaluated *in silico* (docking) to recognize their hypothetical binding mode using the X-ray crystal structure of COX-2 (PDB ID: 2OYE) and also to rationalize its structure activity relationship.

To investigate the ability of molecular docking to reproduce an experimentally observed ligand-binding mode, the co-crystallized ligand IM8-700 (a selective COX-2 inhibitor) has been used as reference ligand. Hundreds of generated conformations of the compounds 6-14 were docked back into its binding site of the crystal structure of COX-2 using the program GLIDE (Grid-based Ligand Docking with Energetic) module version 9.2, Schrödinger. As many as top-scored poses were saved for different conformers and analyzed, but the top docked conformations (poses) closely resembled the co-crystallized conformation of non-hydrogen atomic positions of the ligand IM8-700 was selected for docking studies to recognize their hypothetical binding mode using the X-ray crystal structure of COX-2 (PDB ID:1CX2). **Table 3** summarizes the result of the docking study presented as Glide energies and Glide score.

Docking studies of the indomethacin, a nonselective COX-2 inhibitor, indicates the strong salt bridge interaction of carboxylate ion with the Arg120 in COX-2 isoenzymes (**Fig. 3a**) that gives a more generalized anchoring point for all the classical NSAIDs thus limiting their selectivity due to curtailed freedom of movement of the ligand [25]. Further, acetyl CH_2 of indomethacin formed a strong hydrogen bond with the phenolic OH group of Tyr355 at a distance of 1.5829 Å, whereas the chlorophenyl group showed significant pep stacking interaction with the phenyl ring of Phe518.

In comparison to indomethacin, all the newly synthesized compounds have been found to interacted and fitted into the known classical sites with best scores configurations at COX- 2. Further present studies revealed compounds 8, 11 and 14 posses better docking score because of their strong direct interactions with amino acids HIS 90 of COX-2 (**Fig 3b, 3c, 3d**) (**Table 3**). 4-hydroxyl group at R_2 of quinazolinone ring of all these compounds showed strong H-bond with HIS -90 at a distance of 1.788, 1.843 and 2.000 Å (**Fig. 3b, 3c and 3d**) respectively. Compound 11 was found to be more active than the compound 8 and 14. The favourable binding conformation and higher interaction energy of 8, 11 and 14 (**Table 3**) suggest their prominent anti-inflammatory activity and are in agreement with the biological data. The **Table 3** also suggests that these compounds require less energy for their interaction with protein molecule. Overall all the docking results are in agreement with the biological data. The G-score is also indicative of their higher anti-inflammatory activity in comparison to other compounds.

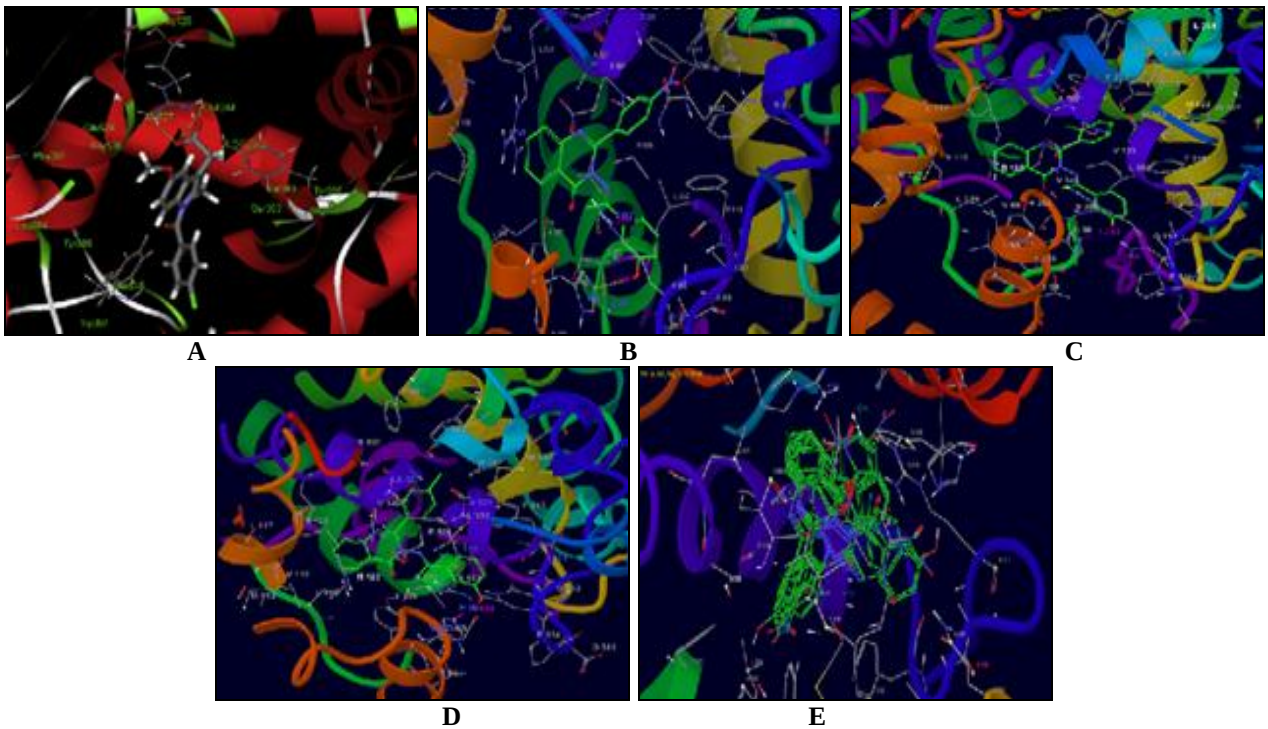


FIG. 3: COMPOUNDS SHOWING INTERACTION WITH HIS-90; 3A- INTERACTION OF INDOMETHACIN WITH ARG-120; 3B, 3C AND 3D- COMPOUNDS 8, 11 AND 14 SHOWING INTERACTIONS WITH HIS-90 AT THE H-BOND DISTANCE OF 1.788, 1.843 AND 2.000 Å RESPECTIVELY

TABLE 3: ENERGY CONTRIBUTIONS FOR COMPOUND 6-14

Compounds	Total interaction energy (a.u)	Protein-ligand interaction energy (a.u)	G score (a.u)
6	-21513.09	43.07	-9.09
7	-21504.99	46.34	-6.13
8	-21519.42	38.28	-9.76
9	-21508.06	35.55	-8.77
10	-21507.38	38.78	-4.44
11	-21521.20	30.96	-9.41
12	-21518.67	37.44	-7.62
13	-21510.83	40.67	-7.35
14	-21525.18	32.70	-9.35

Prediction of Pharmacokinetic Parameter (*in silico*): High oral bioavailability is an important factor for the development of bioactive molecules as therapeutic agents and the significant descriptors and pharmaceutically relevant properties which could influence the cell permeability and bioavailability for the synthesized compounds (6-14) were evaluated using QikProp (version9.2) module of Schrödinger and are given in **Table 4**. These parameters include molecular weight, H-bond donors, and H-bond acceptors, log P (octanol/water), log Kp (skin permeability) and humoral absorption. Some of the recognized parameters important for prediction of drug transport properties such as QPlogP o/w, QPlogS, and polar surface area (PSA) [25] were also determined. Further, steric and molecular surface descriptors, *e.g.* solvent accessible surface area (SASA) and other parameters such as wiener index (WI) were calculated. ADME prediction methods were used to assess the bioavailability of the test compounds (6-14). Herein, we also calculated the compliance of all the compounds to the Lipinski's 'rule of five'[26] which has been widely used to determine if a chemical compound with a certain pharmacological or biological activity has properties that would make it a likely orally active drug in humans. Lipinski *et al.,* [26] used these molecular properties in formulating his 'Rule of Five' and as a filter for drug-like properties. The rule states that most molecules with good membrane permeability have $\log P \leq 5$, $MW \leq 500$, the number of hydrogen bond acceptors ≤ 10, and the number of hydrogen bond donors ≤ 5. A compound that fulfils at least three out of the four criteria is said to adhere to 'Lipinski's Rule of Five'.

A poor permeation or absorption is more likely when there are more than five H-bond donors and ten H-bond acceptors. The series (6-14) under investigation has not only the most of the compounds possessing less number of hydrogen bond donors (<5) but also does possess considerable number of acceptors (<10). **Table 4** enlists the values of these properties for the newly synthesized molecules. None of the molecules has been found to violating Lipinski rule thus not only associated with good bioavailability but also suggesting that the active compounds can be used as templates for further drug discovery effort.

TABLE 4: MOLECULAR PROPERTIES OF THE SYNTHESIZED COMPOUNDS (6-14) USING QIKPROP SOFTWARE

Compound	Molecular weight (MW)[a]	Dipole[b]	Solvent accessible surface area (SASA)[c]	Donor HB[d]	Acceptor HB[e]	QPlogP$_{0W}$[f]/ QPlogS[g]	Human oral absorption[h] (%)	No.of violation LR[i]	QlogBB[j]	PSA[k]	Rule of Five
6	418.38	6.08	673.24	0	4	4.96/-6.37	100	0	1.00	86.81	0
7	429.39	6.61	687.68	0	5	3.70/-5.65	79.3	0	-2.25	131.99	0
8	400.39	6.79	672.57	1	4.75	3.99/-5.80	90.1	0	-1.70	107.88	1
9	408.28	5.60	626.93	0	3	5.98/-6.42	100	0	-0.17	44.47	0
10	418.83	6.29	671.09	0	5	4.95/-6.25	100	0	-0.99	85.88	1
11	389.84	3.43	644.09	1	3.75	5.06/-6.06	100	0	-0.51	62.23	1
12	387.87	3.88	665.85	0	3	6.020/-6.92	100	0	-0.01	43.70	0
13	398.42	7.56	682.36	0	3	4.78/-6.24	100	0	-1.19	86.79	0
14	369.42	2.14	657.28	1	3.75	4.94/ -6.06	100	0	0.64	62.85	0

CONCLUSION: Molecular modelling studies confirm biological data. All our compounds 6-14 were docked into the active site of COX-2 protein and the result showed that the compounds 8, 11, 14 were more active than other compounds. Compound 8, 11 and 14 showed hydrogen bond interaction with the HIS-90 which is required to show COX-2 selectivity.

Further, the compounds comply with Lipinski's rule of five which signifies a good absorption and hence, good bioavailability. Compound 11 was found to be most active quinazolinone derivative.

ACKNOWLEDGEMENT: We thank UGC for providing financial help through UGC networking Resource centre A1 module programme at Panjab University, Chandigarh, India.

CONFLICT OF INTEREST: The authors declared that there are no any conflicts of interest.

REFERENCES:

1. Hu J, Zhang Y, Dong L, Wang Z, Chen L, Liang D, Shi D, Shan X and Liang G: Design, Synthesis, and Biological Evaluation of Novel Quinazoline Derivatives as Anti-inflammatory Agents against Lipopolysaccharide-induced Acute Lung Injury in Rats. Chemical Biology and Drug Design 2015; 85(6): 672–68
2. Aboraia, AS, Mohammed A, Mostafa HA, Mohamed MA and Osama IE-S: Design, synthesis and COX1/2 inhibitory activity of new quinazoline-5-one derivatives. Journal of Advances in Chemistry 2017; 13(12): 6051-6059.
3. Chaitanya P, Reddy GD, Varun G, Srikanth LM, Prasad VV and Ravindernath A: Design and synthesis of quinazolinone derivatives as anti-inflammatory agents: pharmacophore modelling and 3D QSAR studies. Medicinal Chemistry 2014; 10(7): 711-23.
4. Zayed MF and Hassan MH: Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents Saudi Pharmaceutical Journal 2014; 22(2): 157-16.
5. Al-Shamary DS, Al-Alshaikh MA, Kheder NA, Mabkhot YN and Badshah SL: Molecular docking and biological evaluation of some thioxoquinazolin-4(*3H*)-one derivatives as anticancer, antioxidant and anticonvulsant agents. Chemistry Central Journal 2017; 11: 48.
6. Ayyad RA, Sakr HM and El-Gamal KM: Design, Synthesis, Computer Modelling and Analgesic Activity of Some New Disubstituted Quinazolin-4(3H)-ones Medicinal Chemistry 2016; 6: 5.
7. Asif M: Chemical Characteristics, Synthetic Methods, and Biological Potential of Quinazoline and Quinazolinone Derivatives. International Journal of Medicinal Chemistry 2014; 2014: 27.
8. Jafari E, Khajouei MR, Hassanzadeh F, Hakimelahi GH and Khodarahmi GA: Quinazolinone and quinazoline derivatives: recent structures with potent antimicrobial and cytotoxic activities. Research in Pharmaceutical Sciences 2016; 11(1): 1–14.
9. Rajput CS and Singhal S: Synthesis, Characterization, and Anti-Inflammatory Activity of Newer Quinazolinone Analogs. Journal of Pharmaceutics 2013; 7.
10. Manivannan E and Chaturvedi SC: Analogue-based design, synthesis and docking of non-steroidal anti-inflammatory agents. Part 2: Methyl sulfanyl/methyl sulfonyl substituted 2, 3-diaryl-2, 3-dihydro-1*H*-quinazolin-4-ones. Bioorganic and Medicinal Chemistry 2012; 20: 7119.
11. El-Azab AS, Al-Omar MA, Abdel-Aziz AA, Abdel-Aziz NI, el-Sayed MA, Aleisa AM, Sayed-Ahmed MM and Abdel-Hamide SG: Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: Molecular docking study. European Journal of Medicinal Chemistry 2010; 45: 4188.
12. Nanda AK, Ganguli S and Chakraborty R: Antibacterial activity of some 3-(arylideneamino)-2-phenylquinazoline-4(3H)-ones: Synthesis and preliminary QSAR studies. Molecules 2007; 12: 2413
13. Jatav V, Mishra P, Kashaw S and Stables JP: CNS depressant and anticonvulsant activities of some novel 3-[5-substituted 1,3,4-thiadiazole-2-yl]-2-styryl quinazoline-4(3*H*)-ones. European Journal of Medicinal Chemistry 2008; 43: 1945.
14. Zhu S, Wang J, Chandrashekar G, Smith E, Liu X and Zhang Y: Synthesis and evaluation of 4-quinazolinone compounds as potential antimalarial agents. European Journal of Medicinal Chemistry 2010; 45: 3864.
15. Wang Z, Wang M, Yao X, Li Y, Tan J, Wang L, Qiao W, Geng Y, Liu Y and Wang Q: Design, synthesis and antiviral activity of novel quinazolinones. European Journal of Medicinal Chemistry 2012; 53: 275.
16. Tiwary BK, Pradhan K, Nanda AK and Chakraborty R: Implication of Quinazoline-4(3H)-ones in Medicinal Chemistry: A Brief Review. Journal of Chemical Biology & Therapeutics 2015; 1: 104.
17. Chatterjee N, Das S, Bose D, Banerjee S, Das S, Chattopadhyay D and Saha KD: Synthesis and biological evaluation studies of novel quinazolinone derivatives as antibacterial and anti-inflammatory agents. Toxicology Applied Pharmacology 2012; 264: 182.
18. Hussein MA: Synthesis, anti-inflammatory, and structure antioxidant activity relationship of novel 4-quinazoline. Medicinal Chemistry Research 2013; 22(10): 4641–4653.
19. Singh S, Pandey VP, Naaz H, Singh P and Dwivedi UN: Structural modelling and simulation studies of human cyclooxygenase (COX) isozymes with selected terpenes: Implications in drug designing and development. Computers in Biology and Medicine 2013; 43: 744.
20. Ekins S, Mestres J and Testa B: *In silico* pharmacology for drug discovery: applications to targets and beyond. British Journal of Pharmacology 2007; 152: 21.
21. Alafeefy AM, Kadi AA, Al-Deeb OA, El-Tahir KEH and Al-jaber NA: Synthesis, analgesic and anti-inflammatory evaluation of some novel quinazoline derivatives. European Journal of Medicinal Chemistry 2010; 45: 4947.
22. Farag AA, Khalifa ME, Sadik AN, AY Samir, Al-Sehemi GA and Ammar AY: Synthesis, characterization, and evaluation of some novel 4(3*H*)-quinazolinone derivatives as anti-inflammatory and analgesic agents. Medicinal Chemistry Research 2013; 22: 440.
23. Mohamed MS, Kamel MM, Kassem EM, Abotaleb N, Khedr M and Ahmed MF: Synthesis, biological evaluation and molecular docking of quinazoline-4(1*H*)-one derivatives as anti-inflammatory and analgesic agents. Acta Poloniae Pharmaceutica 2011; 68: 665.

24. El-Hashash MA, Azab ME, Faty RA and Amr Ael-G: Synthesis, antimicrobial and anti-inflammatory activity of some new benzoxazinone and quinazolinone candidates. Chemical and Pharmaceutical Bulletein (Tokyo) 2016; 64(3): 263-71.
25. Dhingra MS, Kishore P, Chadha R, Sing T and Karan M: Synthesis, evaluation, and molecular docking studies of cycloalkyl/aryl-3,4,5-trimethylgallates as potent non-ulcerogenic and gastroprotective anti-inflammatory agents. Medicinal Chemistry Research 2014; 23: 87.
26. Lipinski CA, Lombardo F, Dominy BW and Feeney PJ: Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Advanced Drug Delivery Reviews 2001; 46: 3.

How to cite this article:
Arora P, Kaur K, Kaur R and Dhingra N: Structural docking studies and biological evaluation of some newly synthesized schiff base derived quinazolinone derivatives. Int J Pharm Sci Res 2017; 8(12): 5088-99.doi: 10.13040/IJPSR.0975-8232.8(12).5088-99.