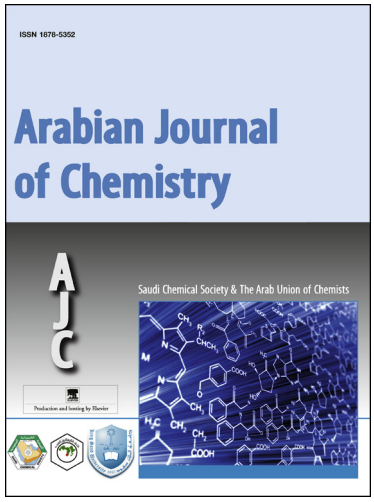

Original article

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Synthesis and evaluation of Analgesic, anti-asthmatic activity of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones

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Abstract

Seventeen (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones derivatives were synthesized via aldol condensation of substituted benzaldehydes with quinoline chalcones starting from 8-hydroxy quinoline. Molecular docking studies were performed on COX -2 protein for analgesic activity and PDE 4 enzyme for anti-asthmatic activity. Docking studies for analgesic activity reveals that the compounds **2**, **4**, **12**, **14**, and **15** showed significant interaction in terms of hydrogen bonding, hydrophobic attachment and vanderwaal interaction with COX-2. The docking studies and pharmacological screening indicate that substitution of hydroxyl and conjugated ketone groups on aldehyde ring and quinoline ring accelerates analgesia with better binding to active site. Eddy's hot plate method was used to evaluate analgesic activity of the synthesized compounds. Compounds showed substantial increase in reaction time when compared with standard pentazocin. Compounds **2**, **4**, **7**, **9** and **13** showed significant binding interactions with PDE 4 enzyme and hence were selected for evaluation of anti-asthmatic activity using goat tracheal chain method. Studies reveals that substitution of methoxy group at 4^{th}& 5^{th} positions for compounds **2**, **4 & 7** leads to significant percentage inhibition of histamine induced contraction. The synthesized compounds are thus found to be potent as analgesic and anti-asthmatic agents.

Key words

Analgesic, anti-asthmatic, cyclooxygenase, docking, phosphodiesterase, quinoline

Synthesis and evaluation of Analgesic, anti-asthmatic activity of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones

1. Introduction

Quinoline chalcones and its derivatives have been found to exert wide range of pharmacological activities like antibacterial (Khan et al., 2006; Vibhute et al., 2010), anticancer (Selvendiran et al.,2004), anti HIV (Gupta et al., 2010), anticonvulsant (Aukunuru et al., 2009), immuno modulatory and antitumor (Sunila,Kuttan,2004), analgesic, anti-inflammatory, insecticidal and antipyretic (Kumar et al., 2007),anti-mutagenic(Shenoy et al.,1992), nutrition enhancing property (Han et al., 2008; Wattanathorn et al 2008), trypanocidal (Welisson et al.,2008) etc. Quinoline chalcones have also been found to show good bio enhancing property (Ayal, Badi, 2010). Due to this wide range of pharmacological activity many attempts have been made to prepare various novel substituted derivatives of quinoline chalcone to explore their potential biological activities.

Molecular Docking is a computational method to find out binding modes of ligand to their receptors rapidly. Most of the biological reactions get triggered by binding of a small molecular ligand to protein. Drugs exert their pharmacological reactions depend only upon their successful binding to their receptor's active site. Binding mode of ligands with their receptors is a crucial in successful design of more efficient drugs. V Life MDS facilitates comprehensive in-silico approach to design, visualize, predict and analyze the small molecules as well as proteins and study their interaction. In the present work we have used docking techniques to evaluate the interaction of compounds with the appropriate target protein.The compounds with better binding interactions with protein were synthesized and activity studies are performed to correlate structure and biological activity of the selected compounds.

1.1 Analgesic activity:

Inflammation is one of the severe complications for many patients and non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly used analgesics worldwide. Cyclooxygenases (COX) is one of the key enzyme in the synthesis of prostaglandins, the main mediators of inflammation, pain and increased body temperature (hyperpyrexia). (Kurumbail et al., 1996; Michaelidou & Litina et al., 2003).

Cyclooxygenase-2 (COX-2) is responsible for elevated production of prostaglandins in inflamed joint tissues and is involved in the mediation of pain. In contrast, COX-1 is involved in the synthesis of eicosanoids that have important homeostatic functions, for example, in the gastric mucosa and platelets. Conventional NSAIDs non selectively inhibit both COX isoforms (Kujubu et al., 1991). Development of selective COX-2 inhibitors will be thus useful to establish good therapeutic activity for analgesia. Hence, COX-2 was selected as target for docking studies. Docking studies are useful to predict the binding site and binding affinity of compounds with COX-2 enzyme.

1.2 Anti-asthmatic activity:

Asthma is a chronic inflammatory disease of the airways which is characterized by variable airflow limitation and airway hyper-responsiveness (AHR) to various stimuli. (Barnes, 2008). Asthma is associated with genetic, allergic, environmental, Infectious, emotional, and nutritional components. Phosphodiesterase (PDE) enzymes are often targets for pharmacological inhibition due to their unique tissue distribution, structural properties and functional properties. Pharmacological inhibition of select PDE isoforms has proven therapeutically beneficial for several indications (De Visser et al., 2008). PDE4 inhibitor development presents a promising approach to the treatment of chronic inflammatory diseases in asthma. Hence, PDE 4 enzyme (PDB Code: 3FRG), was selected as target for docking studies.
We have designed various structural derivatives of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones and studied their interaction with COX-2 enzyme for analgesic activity. Anti-asthmatic activity was performed by docking studies with PDE 4 enzymes (Oliveira et. al., 2006). The compounds were subjected for evaluation of their analgesic activity in mice and goat tracheal method was used for evaluation of anti-asthmatic activity.

2. Material and Methods

We have synthesized seventeen derivatives of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones to be tested as analgesic and anti-asthmatic agents. The synthesis of our targeted compounds **1**–**17** was done as shown in scheme 1. All chemicals used were of analytical grade from, SD Fine. Melting point (MP) of all the synthesized compounds were determined by open capillary tube method and are uncorrected. The purity of all compounds was checked by TLC. TLC was run on Silica Gel G plates using chloroform and methanol (9:1). Spots were visualized using iodine vapor chamber. IR spectra were recorded on Shimadzu IR spectrophotometer by using KBr pellets technique.1H-NMR was recorded on Burke AMX 60 MHz spectrophotometer by using DMSO as solvent. Mvtex Analgesiometer (Eddy's hot plate) was used for estimation of analgesic activity by Eddy's hot plate method. Animal experimental protocol was approved by Institutional Animal Ethics Committee (Reg. No. 941/Po/c/06/CPCSEA)

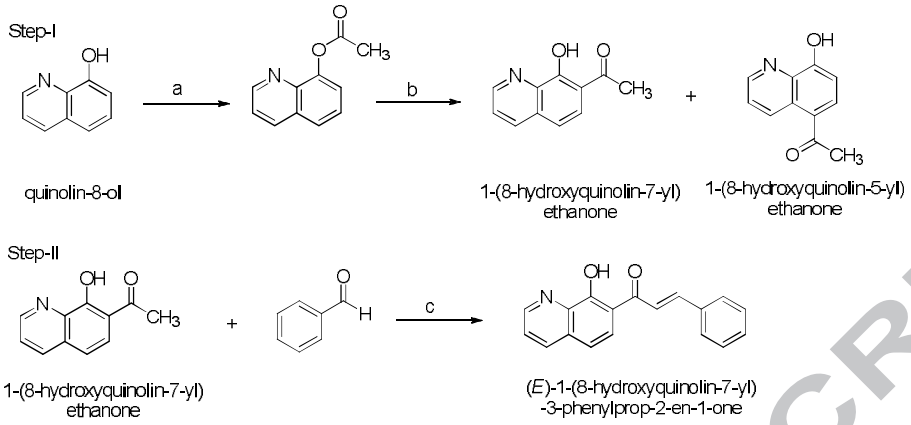
2.1 General procedure for the synthesis of quinoline chalcones *derivatives.*

Step I: A) Synthesis of quinolin-8-ylacetate from 8-hydroxy quinoline (Friedel-Craft acylation).
In a 250 ml round bottom flask, 8-hydroxy quinoline 7.250 gm (0.05mol) and 25ml of methylene dichloride were taken and stirred on ice bath for 30 min, to this chilled solution acetyl chloride 4.42 ml (0.05mol) was added drop wise and reaction mixture was stirred for 2 hr, reaction was monitored by TLC using hexane: acetone (3:2) as a mobile phase. After completion of reaction, pale yellow-white colored precipitate formed was filtered, washed with methylene dichloride, dried and recrystallized from hot water.

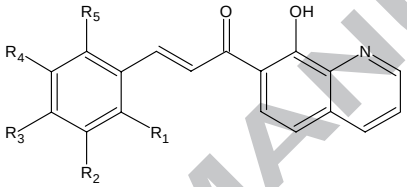
B) Synthesis of 7-acetyl-8-hydroxy quinoline from quinolin-8-yl acetate (Fries rearrangement).
In a 250 ml round bottom flask, quinolin-8-yl acetate 6.54 gm(1 mol) and aluminum tri chloride 16.65 gm (2.5 mol) were taken and reaction mixture was heated at 160^0C for 1.5 hours, orange colored mass was obtained to this dilute hydrochloric acid was added to break the aluminium–quinoline complex. Para & Ortho isomers formed were separated by steam distillation, separation of isomer was monitored by TLC method using hexane : ethyl acetate as mobile phase.

Step II: Synthesis of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones. (Aldol condensation).
In a round bottom flask 7-acetal-8-hydroxyl Quinoline (1 mol) dissolved in 5ml of 20% sodium hydroxide was taken and to this reaction mixture benzaldehyde derivative (1 mol) was added and kept at room temperature for 5 min after this 20 ml of ethanol was added as solvent and reaction mixture was stirred for 6 - 8 hr, product formed was poured in ice cold water, P^H was adjusted to 4 with 10% of HCl, product was filtered, washed and dried. All the synthesized compounds were characterized by TLC, IR, NMR and elemental analysis (Table 1).

Scheme 1 : General scheme for synthesis of compounds



a)CH_2Cl_2,CH_3COCl, 0°C; b) $AlCl_3$,heat 140-160°C;c) NaOH 20%, ethanol, RT, stirring.



(E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones

Table 1 Synthesized derivatives of Quinoline chalcones

Prod. Code	R1	R2	R3	R4	R5	% Yield	R.F. Value
1	OH	Cl	H	Cl	H	71	.54
2	OH	H	OCH_3	H	H	81	.76
3	OH	H	H	OH	H	60	.68
4	OH	H	H	OCH_3	H	75	.74
5	OH	OC_2H_5	H	H	H	68	.81
6	H	OH	OH	H	H	65	.59
7	H	H	OCH_3	H	H	45	.63
8	OH	Br	H	Br	H	70	.54
9	OH	H	NC_2H_5	H	H	40	.74
10	H	H	H	H	H	55	.65
11	OH	OH	H	H	H	42	.75
12	OH	H	OH	H	H	58	.69
13	H	H	OH	H	H	74	.72
14	H	OCH_3	H	Br	H	55	.55
15	OH	H	OH	H	OH	67	.34
16	OH	H	H	NO_2	H	78	.67
17	OH	H	H	H	H	77	.45

2.2 Pharmacological evaluation

All compounds were evaluated for Analgesic and anti-asthmatic activity

2.2.1. Acute toxicity study

The acute oral toxicity of synthesized compounds was determined in albino mice using revised OECD guideline No. 425. From the acute toxicity studies the doses of 10 mg/kg was selected for further evaluation of analgesic activity.

2.2.2. Analgesic activity

Male swiss albino mice (20-30 g) were used to assess analgesic activity of reference and test compounds. The animals were housed in cages at 18-20°C for 12 hr. Light/dark cycle and relative humidity of 55-60%, allowing food (Purina Chow) and water *ad libitum*. All test compounds were administered orally, by suspending in 0.5% carboxy methylcellulose (CMC) solution. Pentazocine (0.1 ml) 10 mg/kg was administered intra-peritonealy as standard. The analgesic activity of the test compounds was assessed by means of their ability to increase reaction time in swiss albino mice.

2.2.3. Eddy's hot plate method
Analgesic activity was studied using Eddy's hot plate method in swiss albino mice (Kulkarni,1999). Mice were divided into six different groups; food was withdrawn 12 hours prior to drug administration till completion of experiment. The animals were weighed and numbered appropriately. Basal reaction time was recorded by placing the animals on hot plate and recording the time until either licking or jumping. To standard group Pentazocine (0.1 ml) 10 mg/kg was administered intra-peritonealy. Synthesized compounds were given orally to the test groups (0.1 ml, 10mg/kg). Analgesic activity of synthesized compounds was studied at equimolar doses. The response time is recorded 30, 60, and 90, 120 and 180 min following oral administration of the standard or the test compound. A cut off period of 15 sec was observed to prevent tissue damage of the tail of animals.

2.2.4.Anti-asthmatic activity
Goat tracheal chain method was used to evaluate anti-asthmatic activity of synthesized compounds. The goat tracheal tissues were obtained immediately after slaughter of animals. Pieces of trachea were collected in ice-cold oxygenated Kreb's solution. Goat trachea was cut into individual rings and tied together in series to form a chain.It was suspended in bath containing Krebs Henseleit (composition (mM) NaCl:115, KCl:4.7, $CaCl_2$:2, $NaHCO_3$:25, $KH2PO_4$:1.2, $MgCl_2$:1.2, Glucose:11.5) and maintained at 37±1°C.A stream of air was bubbled through the organ tube (1 bubble/sec). One end of the tracheal muscle was attached to S-shaped aerator and the other was attached to isotonic frontal writing lever to a drum.The tissue was allowed to equilibrate for 45 min. under a load of 400 gm. A dose response curve for histamine was recorded at various molar concentrations by maintaining 15 min time cycle. After obtaining dose response curve of histamine on trachea 0.1 ml and100 µg/mlof synthesized compounds were added to reservoir and dose of histamine which shows measurable response is added and response recorded. Increasing doses of drug were given while histamine dose was kept constant and responses were recorded.

2.3. *Spectral data of synthesized compounds*

2.3.1. (E)-3-(3, 5-dichloro -2-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop- 2-en-1-one:
This compound was obtained as greenish brown powder (ethanol), m.p.: 132-136°C, FT-IR 1655 Conjugated ketone, 1602 C=N, 1204 CH=CH, 1 H NMR δ (deutorated Chloroform) 7.2,7.9 (1H,dd,), 7.4 (1H,dd), 6.8,7.0 (d, 1H), 4.9 (s,1H), 7.5, 7.8 (d,1H), 6.6, 6.8 (s, 1H)); ms: m/z 344(3.0), 314 (6.4), 173 (54), 137 (95), 104(100), 79 (84), *Anal*. Calcd. for $C_{18}H_{11}O_3NCl_2$: C, 60; H3.08; N, 3.89; O,3.33; Cl,19.69; Found: C, 61.33; H,2.67; N,3.89; O,3.33;Cl, 20%.

2.3.2. (E)-3-(2-hydroxy-4-methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one:
This compound was obtained as greenish powder (ethanol),m.p.:178-183^{0}C, FT-IR 1654 conjugated ketone, 1349 C=N, 1201 CH=CH, 2955 –CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.8 (1H,d), 7.3 (1H,dd), 7.8 , 7.51 (d, 1H), 4.9 (s,1H), 7.2,7.5 (d,1H), 6.0, 5.8 (d, 1H) , 6.9(s, 1H), 3.9 (s, 3H); *Anal*. Calcd. For$C_{19}H_{15}NO_4$ C, 71; H, 4.71; N, 4.36; O, 19.92; Found: C, 70.10; H, 5.61; N, 5; O, 19.92%.

2.3.3. (E)-3-(2, 5-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one:
This compound was obtained as Blackish greenish powder (ethanol),m.p.:122-124°C, FT-IR 1665 conjugated ketone, 1501 C=N, 1228 CH=CH, 2955 –CH, 1 H NMR δ (deutorated Chloroform) 8.0, 8.8(1H,d), 8.0 (1H,dd), 7.4 , 7.6 (d, 1H), 4.6 (s,1H), 7.2,7.5 (d,1H), 6.2, 6.0 (d, 1H); *Anal*. Calcd. for $C_{18}H_{13}NO_4$: C,70.35; H, 4.71; N, 4.36, O: 19.92, Found: C, 71; H, 5.67; N, 4.56; O ,20%

2.3.4. (E)-3-(2-hydroxy-5-methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one : This compound was obtained as green powder (ethanol),m.p.:109-113°C, FT-IR 1656 conjugated ketone, 1495 C=N, 1228 CH=CH, 2911- 3034 –CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.9 (1H,d), 7.3 (1H,dd), 7.51 , 7.8 (d, 1H), 4.9 (s,1H), 7.2,7.5 (d,1H), 6.2,6.0 (d, 1H

) , 6.9(s, 1H), 3.9 (s, 3H); *Anal.* Calcd. for $C_{19}H_{15}NO_4$: C, 71.02; H, 4.71; N,4.36; O,19.92, Found: C,73; H, 3.29; N ,4.36; O, 21.36%

2.3.5. (E)-3-(3 –ethoxy-2-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one : This compound was obtained as green powder (ethanol),m.p.:145-148°C, FT-IR 1637 conjugated ketone, 1387 C=N, 1211 CH=CH, 2979- 3353 –CH, 1 H NMR δ (deutorated Chloroform) 8.1,8.8 (1H,d), 7.2 (1H,dd), 7.8 , 7.6 (d, 1H), 4.8 (s,1H), 6.6 ,6.8 (s,1H), 3.98 (q, 2H) , 1.39 (dd, 3H) ;*Anal*. Calcd. for $C_{20}H_{17}NO_4$: C,71.63; H,4.26; N ,4.56; O,20.83; Found: C,72.28; H,4.01; N ,5.23; O, 21.83%

2.3.6. Synthesis of (E)-3-(3, 4-dihydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one: This compound was obtained as green powder (ethanol), m.p.:137-140°C, FT-IR 1647 conjugated ketone, 1497 C=N, 1295 CH=CH, , 1 H NMR δ (deutorated Chloroform) 8.1, 8.9 (1H,d), 7.3 (1H,dd), 7.3 ,7.6 (d, 1H), 4.9 (s,1H), 7.2,7.5 (d,1H), 6.2,6.6 (d 1H), 6.4 (s, 1H) ;*Anal*. Calcd. for$C_{18}H_{13}NO_4$: C,70.35; H, 4.26; N,4.56; O,20.83; Found: C, 71.13; H, 5.04; N ,5.00; O,19.78%

2.3.7. Synthesis of (E)-1- (8-hydroxyquinolin-7-yl)-3-(4-methoxyphenyl) prop- 2-en-1-one:
This compound was obtained as green powder (ethanol),m.p.:134-137°C, FT-IR 1679 conjugated ketone, 1501 C=N, 1253 CH=CH 2951-3043 ,1 H NMR δ (deutorated Chloroform) 8.2,8.6 (1H,d), 7.2 (1H,dd), 7.8,7.5 (d,1H), 4.9 (s,1H), 7.4,7.6 (d,1H), 6.5, 6.8 (d,1H), 3.7 (s,3H) ; *Anal.* Calcd. for $C_{19}H_{13}NO_3$: C,74.74; H,4.95;N,4.59;O,15.72;Found: C, 76.74; H, 3.95; N, 3.59; O,15.95%

2.3.8. (E)-3-(3, 5-dibromo-2-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop- 2-en-1-one: This compound was obtained as brown powder (ethanol),m.p.:168-170°C, FT-IR 1635 conjugated ketone, 1456 C=N, 1235 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.8, 8.1 (1H,d), 7.2 (1H,dd), 7.8, 7.5 (d,1H), 8.2,8.7 (d,1H), 4.8 (s,1H), 7.2,7.4 (d,1H) ;*Anal.* Calcd. for$C_{18}H_{11}NO_3Br_2$: C,48.14; H,2.47;Br ,35.58;O,10.69;N,3.12;Found: C,50; H,3.27; N,2.36 ; O,10.81; Br ,33.56%

2.3.9. (E)-3-(4-(diethylamino)-2-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop -2-en-1-one:
This compound was obtained as grayish crystals (ethanol),m.p.:176-179°C, FT-IR 1640 conjugated ketone, 1497 C=N, 1234,1317 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.1,8.6 (1H,d), 7.2 (1H,dd), 7.9,7.7 (d,1H), 7.8,8.0 (d,1H),4.9 (s,1H), 5.7 (s,1H), 4.2 (s,1H), 6.8,5.6 (d,1H), 3.2 (q,2H), 1.13 (dd,3H);, *Anal*. Calcd. for$C_{22}H_{22}N_2O_2$: C, 72.91; H, 6.12;N ,7.73; O ,13.24; Found: C, 73.13; H, 7.42; N,7; O,11.36%

2.3.10. (2E, 4E)-1-(8-hydroxyquinolin-7-yl)-5-phenylpenta-2,4-dien-1-one:
This compound was obtained as chocolate brown crystals (ethanol),m.p.:165-170°C, FT-IR 1671 conjugated ketone, 1499 C=N, 1232 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.4 (1H,d), 7.2 (1H,dd), 7.4,7.6 (d,1H), 7.6,7.9 (d,1H), 7.2 (dd,1H), 7.3 (dd,1H),7.4 (dd,1H); *Anal*. Calcd. for $C_{20}H_{15}NO_2$: C, 79.72; H, 6.12; N,7.73; O ,13.24;Found: C, 81.12; H ,4.37; N, 7.97; O ,14.13 %

2.3.11. (E)-3-(2, 3-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one:
This compound was obtained as chocolate brown crystals (ethanol),m.p.:189-193°C, FT-IR 1604 conjugated ketone, 1495 C=N, 1119 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.8 (1H,d), 7.3 (1H,dd), 7.9,7.7 (d,1H), 7.4,7.8 (d,1H), 4.9 (s,1H), 6.7 (dd,1H), 6.6,6.4 (d,1H); *Anal*. Calcd. for $C_{18}H_{13}NO_4$ C,70.35; H, 4.26; N ,4.56; O ,20.83; Found: C,71.11; H ,3.15; N, 4.78;O ,20.22%

2.3.12. (E)-3-(2, 4-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one:
This compound was obtained as chocolate brown crystals (ethanol),m.p.:129-132°C, FT-IR 1720 conjugated ketone, 1466 C=N, 1111 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.1,8.7 (1H,d), 7.2 (1H,dd), 7.9,7.6 (d,1H), 7.4, 7.6 (d,1H), 6.0 (s,1H), 6.5,6.9 (d,1H); *Anal*. Calcd. for $C_{18}H_{13}NO_4$: C, 70.35; H ,4.26; N,4.56; O, 20.83;Found: C ,71.11; H ,3.15; N, 4.78,;O ,20.22%

2.3.13. (E)-3-(4-hydroxyphenyl-1-(8-hydroxyquinolin-7-yl) prop -2-en-1-one : This compound was obtained as chocolate brown crystals (ethanol),m.p.:174-178°C, FT-IR 1720 conjugated ketone, 1466 C=N, 1111 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.1,8.7 (1H,d), 7.2 (1H,dd), 7.6,7.9 (d,1H), 7.4,7.9 (d,1H), 4.8 (s,1H), 6.7,6.9 (d,1H); *Anal*. Calcd. for $C_{18}H_{13}NO_3$: C,74.22;H,4.50; N,4.81 ;O,16.48; Found:C,73;H,4.72; N,5.2;O,16.24%

2.3.14. (E)-3-(5-bromo-2-hydroxy-3-methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop-2-en-1-one: This compound was obtained as chocolate brown crystals (ethanol),m.p.:154-158°C, FT-IR 1662 conjugated ketone, 1450 C=N, 1247 CH=CH,2938-3066 – CH: 1 H NMR δ (deutorated Chloroform) 8.1, 8.8 (1H,d), 7.3 (1H,d), 7.4,7.8 (d,1H), 7.4,7.8 (d,1H),4.9 (s,1H),6.9 (s,1H), 6.4 (s,1H), 6.6 (s,1H), 3.6 (s,3H) ; *Anal*. Calcd. for $C_{19}H_{12}NO_4Br$: C, 57.02; H, 5.35;N,4.56; O ,10.62 ; Br,19.96 ;Found: C,58; H,5.32; N,5.12; O ,11.12;B, 20.67%

2.3.15. (E) -3- (2, 4, 6-trihydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop- 2-en-1 –one: This compound was obtained as chocolate brown crystals (ethanol),m.p.:145-148°C, FT-IR 1662 conjugated ketone, 1450 C=N, 1247 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.1,8.6 (1H,d), 7.2 (1H,s),7.4,7.8(d,1H), 7.4,7.8 (d,1H), 4.8 (s,1H), 5.7 (s,1H); *Anal.* Calcd.for$C_{18}H_{13}NO_5$: C, 66.87; H, 4.05; N ,4.56; O ,24.74; Found: C, 68.12; H, 3.57; N, 3.1;O ,22.24%

2.3.16. (E)-3-(2-hydroxy-5-nitro phenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en:
This compound was obtained as chocolate brown crystals (ethanol),m.p.:147-151°C, FT-IR 1576 conjugated ketone, 1374 C=N, 1228 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.4 (1H,d) 7.3 (s,1H), 7.2,7.6 (d,1H), 7.9,7.7 (d,1H), 4.9 (s,1H), 6.9,7.7 (dd,1H), 8.0 (s,1H) ; *Anal*. Calcd. for $C_{18}H_{14}N_2O_5$: C, 64.29; H, 3.60; N, 8.33; O ,23.79; Found: C ,65; H,4.1; N,6.22;O ,22.10 %

2.3.17. (E)-3-(2-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl) -prop-2-en- 1-one:
This compound was obtained as chocolate brown crystals (ethanol), m.p.:167-170°C, FT-IR 1633 conjugated ketone, 1465 C=N, 1229 CH=CH, 1 H NMR δ (deutorated Chloroform) 8.0,8.6 (1H,d) 7.3 (s,1H), 7.2, 7.6 (d,1H), 7.6,7.8 (d,1H), 4.9 (s,1H), 6.9,6.7 (dd,1H), 6.6,6.9 (d,1H) ; 344(3.0), *Anal*. Calcd. for $C_{18}H_{13}NO_3$: C,74.22; H ,4.50; N ,4.81; O ,16.48;Found: C,73.56; H ,5.53; N ,4.62; O ,15.59 %.

2.4 Molecular modeling studies

To pre-asses the analgesic and anti asthmatic behavior of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones derivatives **1**–**17** on a structural basis, automated docking studies were carried out using V-LIFE Docking Software. The scoring functions and hydrogen bonds formed with the surrounding amino acids are used to predict their binding modes, their binding affinities and orientation of these compounds at the active site of the enzyme.
Analgesic activity was performed on COX-2 protein (1CX2) co-crystallized with S58 as reference ligand and for Anti-asthmatic activity Phosphodiesterase enzyme (PBD Code: 3FRG) bound with SK4 as reference ligand was obtained from PDB data bank.

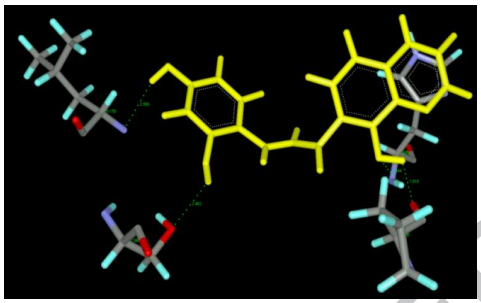


Figure 1: Compound **12** docking with 1CX 2 protein

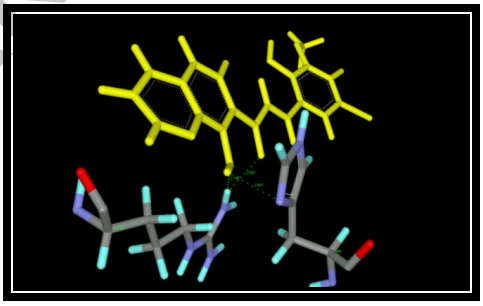


Figure 2: Compound **14** docking with 1CX 2 protein

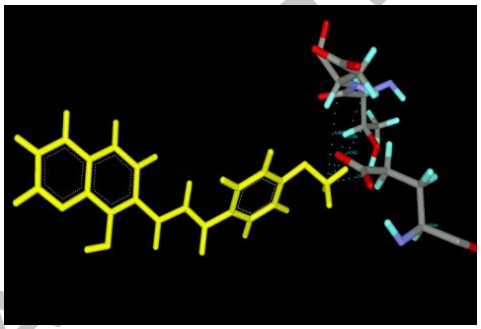


Figure 3: Compound **2** docking with 3FRG

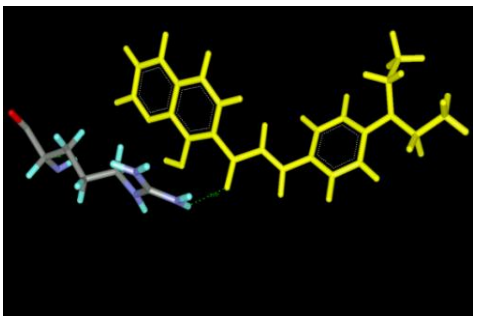


Figure 4: Compound **9** docking with 3FRG

3. Result and discussion

3.1 Chemistry:
Starting from 8-hydroxy quinoline, quinoline-8-ylacetate was prepared by Friedel-craft acylation using acetyl chloride and methylene dichloride. Acetate formed was subjected for fries rearrangement to afford *O* and *P* form of 1-(8-hydroxyquinolinyl) ethanone. Stem distillation was used to separate these two forms and *O* form 1-(8-hydroxyquinolin-7-yl) has been further subjected to aldol condensation by using various substituted benzaldehydes as shown in **Scheme 1.** Total 17 compounds have been synthesized (**Table 1**). The structures of the compounds have been established on the basis of spectral data. IR, 1H NMR, and elemental analysis.
3.2Molecular docking studies:
For analgesic activity COX-2 was selected as target protein which consists of three important regions. First Hydrophobic pockets consist of Tyr^{385}, Try^{387}, Phe^{518}, Ala^{201}, Tyr^{248}, Leu^{352}. Second is hydrophilic amino acid consist of

Arg^{120}, Glu^{524}, Tyr^{355} amino acids. Third region is side pockets containing His^{90}, Arg^{513}, Val^{523} amino acids. From analgesic docking studies it is found that compounds **2**, **4**, **12**, **14**, **15** are able to bind with amino acids like His^{90} Arg^{513} Tyr^{355}, Arg^{120} which are present in active site of COX-2 enzyme with hydrogen bond distance ranging from 1.689A°-2.540A°. Reference molecule showed three hydrogen bond interactions with His^{90}, Val^{116}, Arg^{120}. Ligand compound **15** has shown most interesting result by forming five hydrogen bond with active amino acids like Arg^{120}, Tyr^{355}, Arg^{513} in enzyme cavity. The docking score for compound was found to be -53.57 which is comparable with reference ligand score (-52.64). Similarly compounds **4**, **12**, **14** have formed three hydrogen bond and compound **2** has formed 2 hydrogen bonds. Compound **2** has docking score of -65.19. Compound **4** showed docking score of -61.58. Rest of the compounds showed hydrophobic and van der wall interaction with protein molecule.
Compounds **12** and **14** have shown good binding energy values and interaction of compound **12** with 1CX2 has docking score of -66.457858, and forms 4 hydrogen bonds as shown in Figure 1**.** Interaction of compound **14** with 1CX2 has docking score of -63.216157, and forms 3 hydrogen bonds as shown in Figure 2**.** Overall compounds **2**, **4**, **12**, **14**, **15** have shown good docking and binding interaction with the selected target protein and the binding energies and docking scores are favorable in designing of the compounds for evaluation of their analgesic activities.

In case of anti-asthmatic activity, the preliminary docking studies of SK4 and (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones derivatives on the Phospho-diesterase Enzyme (PBD Code: 3FRG) were carried out. The docking score and binding energies obtained shows favourable binding of these ligands to the Phospho-diesterase enzymes. The interactions between the ligand molecules and protein were studied. Compound **2** showed four hydrophobic bonds between amino acid THR345, distance 4.790 and 4.162, GLU304 at a distance of 3.760, ASP346 at a distance of 4.483, as shown in Figure 3. Compound **2** has shown docking score of -73.77 which is comparable with reference ligand (-74.72) Compound **9** showed hydrophobic bond interactions with HIS306 residues and docking score of -71.16 as shown in Figure 4. Compounds **13** form two hydrogen bond interactions with HIS278A, GLU304A & ASP346A with docking score of -70.85. Compound **4** has docking score of -83.129846, and form one hydrogen bond between amino acid CYS432A 4465H with 5346N at distance 2.461. Compound **7** has docking score of -73.462163 and form 2 hydrophobic bonds between amino acid SER282A 2007C, 2010C with 5363C at distance 4.731 and 4.162. Compounds **2**, **4**, **7**, **9**, **13** thus have shown good hydrogen bond interactions and docking energies are comparable with reference ligand for anti asthmatic activity.

3.3. Analgesic activity

Compounds were tested for their in vivo analgesic activity using Eddy's hot plate method. Analgesic activity of the test compounds were compared with respect to control. Data are expressed as Mean reaction time ± S.E.M**.** analyzed by Two-way ANOVA followed by Dunnett test. Pentazocine at the dose of 10 mg/kg showed good analgesic activity ($P< 0.01$) at all time intervals as compared to control group. Compounds **2**, **4**, **12**, **14** and **15** at 10mg/kg showed good analgesic activity at all time intervals which is comparable with standard (Figure 5). The synthesized chalcone compounds might exert their analgesic activity by inhibiting COX-2 enzyme which is more specific for prostaglandin synthesis. Currently available COX-2 inhibitors are having adverse effects like flu-like symptoms, fatigue, back pain, hypertension, edema, heart pain etc. These newly synthesized chalcones might be useful in lowering the adverse effects of current drugs and might exert more potent analgesic activity. The compounds have been found to show better binding interactions with COX-2 enzymes and their analgesic responses are significantly comparable with standard. Compounds **1**, **3**, **6**, **7**, **8**, **9** and **11** shown moderate analgesic activity. Almost all the derivatives showed good analgesic activity at 2 hr interval except **5**, **10**, **13**, **16** and **17** (Table 2).

Table 2: Analgesic activity data of compounds using Eddy's hot plate method, N = 6 Two way ANOVA followed by Dunnett's test is applied for statistical analysis **p<0.01 when compared with vehicle treated control group.

Comp /Dose (mg/kg)	Basal reaction time(sec)	Reaction time(sec)					
		15 min	30 min	60 min	90 min	120 min	180 min
Control	5.115± 0.2465	5.073± 0.3404	5.427± 0.0799	4.98± 0.1577	5.44± 0.1407	5.61± 0.1349	5.16± 0.2383
Standard (10)	5.180± 0.1233	10.065± 0.1780**	11.712± 0.444**	12.910± 0.188**	13.617± 0.338**	14.032± 0.053**	10.470± 0.401**
2 (10)	5.064± 0.3123	8.510± 0.3215**	9.618± 0.3190**	11.078± 0.3906**	11.967± 0.3551**	7.980± 0.2859**	7.713± 0.2726**
4 (10)	5.342± 0.4231	7.217± 0.098**	9.010± 0.1041**	10.728± 0.7188**	12.720± 0.5656**	8.363± 0.2279**	7.107± 0.2305**
12 (10)	5.012± 0.3654	8.1834± 0.4144**	10.875± 0.7989**	11.102± 0.6577**	11.884± 0.3220**	9.203± 0.2306**	9.467± 0.259**
14 (10)	4.978± 0.2454	8.468± 0.3361**	9.183± 0.3223**	12.040± 0.6282**	11.513± 0.3306**	8.188± 0.2801**	7.708± 0.1741**
15 (10)	4.892± 0.2342	7.930± 0.3964**	9.465± 0.3255**	10.507± 0.3608**	9.762± 0.4983**	7.895± 0.3734**	7.295± 0.2905**

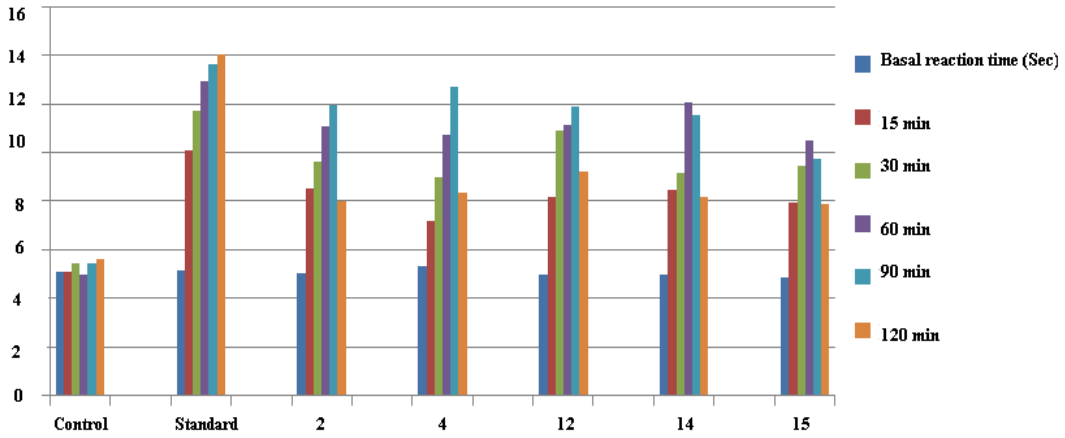


Figure 5: Analgesic activity of compounds

3.4. Anti-asthmatic activity

Mortality rate due to asthma has been increased substantially since past few years. Currently broncho dilators are used for the treatment of acute symptoms and anti-inflammatory drugs have also been used for treating chronic inflammation induced exacerbations. Chalcones have also been found to exert significant analgesic activity. Chalcones might exert their significant anti-asthmatic activity by blocking broncho constriction, mucus production and inflammatory reactions which occur due to asthma. Present drugs like zafirlukast and montelukast have adverse effects like liver toxicity, dyspepsia, headaches and GI symptoms which might be lowered by the novel synthesized chalcones in the present research work. Compounds **2**, **4**, **7**, **9** and **13** have been found to show significant percentage inhibition against histamine induced contractility (Table 3). Rest of the compounds did not produce any significant activity (Figure 6).

Table 3 Percent inhibition (%) of test compounds at increasing doses of compounds

Dose (µ gm/ml)	% Inhibition				
	2	**4**	**7**	**9**	**13**
10	50	42.86	43.75	55.56	33.34
20	60	45.72	50	66.67	53.34
40	61	57.15	56.25	77.78	66.67
80	65	71.43	68.75	88.89	93.34
100	100	100	100	100	100

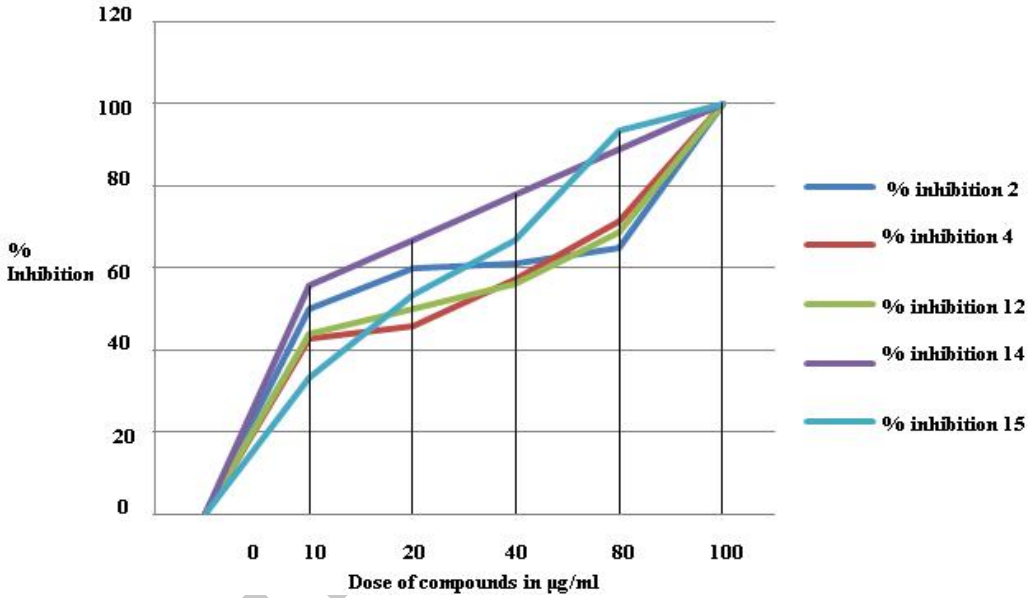


Figure 6: Percentage **(**%) inhibition against concentration of test solution.

4. Conclusion

(E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones derivatives exhibited favourable interaction with the amino acid residues in active site of cycloxygenase-2 enzyme (PDB Code: 1 CX2), Compounds substituted with hydroxyl and conjugated ketone group (**2, 12**, **14**,**& 15)** showed increase in reaction time. Methoxy group in compound **(4)** also indicated better binding in docking studies and activity. The reaction time for the compounds is comparable with the standard pentazocine. Compounds **2**, **4**, **12**, **14** and **15** thus found to exhibit better interaction with the COX-2 proteins and have also shown significant analgesic activity.

(E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones derivative have also showed PDE 4 inhibitory activity. Compounds **2**, **4**, **7**, **9** and **13** have been found to exert better binding interactions with PDE 4 and compound **9** and **13** have been found to show good percentage inhibition. Substitution of methoxy group for compounds **2**, **4 & 7** leads to significant percentage inhibition of histamine induced contraction.Substitution of N- methyl group at aldehyde and conjugated ketone group in compounds **9 & 13** also shown better binding in docking studies and inhibitory potency in further studies. The compounds thus can become potential molecule as analgesic and anti-asthmatic agents.

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