Focused On In-Silico, Design, Synthesis & Evaluation Of 8-Hydroxyquinoline Based Nucleus Covering Prominent Target PDE4 As Anti-Asthmatic Agent

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Abstract : Starting with 8-hydroxy quinoline, ten (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 derivatives were created via aldol condensation of substituted benzaldehydes with quinoline chalcones. For anti-asthmatic activity, molecular docking studies were performed on PDE 4 enzyme. Docking studies for anti-asthmatic activity show that compounds 2, 5, and 9 have significant binding interactions with the PDE 4 enzyme. According to studies, replacing the electron withdrawing and electron donating substitutions at the phenyl ring has good anti-asthmatic efficacy. Hence, it is concluded that the synthesized compounds have potent analgesic and anti-asthmatic properties.

Introduction

As indicated by the huge number of commercially available drugs that contain this heterocycle, a variety of substituted quinolone chalcones derivatives are well known for their vital role in the design of novel pharmacological moieties for therapeutic application. Due to their diverse spectrum of biological activities, quinoline chalcones compounds are of pharmacological significance and have caught the attention of both chemists and biologists. Particularly, the basic building blocks for many naturally occurring compounds are quinoline chalcones substances. The broad variety of biological effects of quinoline compounds have been covered in great detail by numerous authors. (1) Analgesic, anti-inflammatory, insecticidal and antipyretic (2), antimicrobial(3), anticancer(4), anticonvulsant (5), immuno modulatory and antitumor (6), anti mutogenic (7), nutrition enhancing property (8)(9), trypanocidal (10), bio enhancing property (11)

Asthma is a chronic inflammatory disease of the airways that causes variable airflow limitation and airway hyperresponsiveness (AHR) to a variety of stimuli.(12) Asthma is associated with genetic, allergic, environmental, Infectious, emotional, and nutritional components. Because of their unique tissue distribution, structural properties, and functional properties, phosphodiesterase (PDE) enzymes are frequently targets for pharmacological inhibition. Pharmacological inhibition of specific PDE isoforms has been shown to be therapeutically beneficial for a variety of indications.(13) The development of PDE 4 inhibitors represents a promising approach to the treatment of chronic inflammatory diseases in asthma. As a result, the PDE 4 enzyme (PDB Code: 3FRG) was chosen as the target for docking studies.

The molecular docking method may be used to describe the atomic level interaction between a small molecule and a protein, allowing characterization of small-molecule behavior in target protein binding sites and elucidating critical biochemical processes. Predicting the ligand structure as well as its position and orientation within these sites (known as pose) and determining the binding affinity are the two primary processes in the docking procedure. Knowing where the binding site is, before starting the docking process dramatically improves docking efficiency. Cavity detection tools or web servers can be used to find putative active sites within proteins if the binding sites are unknown. A thorough understanding of the general principles governing the nature of interactions between ligands and their protein or nucleic acid targets (van der Waals, hydrogen bonding, and electrostatic interactions) may provide a framework for designing potential drug leads with the desired potency and specificity for a given therapeutic target.(14)(15)

Several well-known compounds with (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 derivatives can be synthesised in a highly efficient manner, most likely via a Friedel-Craft acylation, Fries rearrangement, and Aldol condensation, and their interaction with PDE 4 enzymes for anti-asthmatic activity was studied.(16) Combining pharmacophore units with different biological activities results in a new hybrid entity with greater biological processes and efficacy than the parent medications. Today, unique bioactive hybrid compounds based on quinoline with improved antiasthmatic efficacy have been developed based on previous discoveries.

Materials and Methods

Rationale of molecular design

The present work addresses the designing and synthesis of new structural analogues of quinoline derivatives as anti-asthmatic drugs for the inhibition of PDE4 enzyme. Literature suggested that PDE4 is a potent anti-asthmatic target, because PDE4 is an enzyme that helps to regulate airway inflammation so, PDE4 inhibition can supress a variety of inflammatory cell function that contribute to their anti-inflammatory actions in asthma. From the reported literature it was found that substitution of methoxy group at R4 &

R5 position will be able to show most potent anti-asthmatic activity. Various electron withdrawing & donating substitutions at the phenyl ring will be designed & substituted derivatives will be docked against the PDE4 & the highest scoring docked ligands will be further synthesized, characterized and evaluated for anti-asthmatic activity.

***In-silico* studies**

***In-silico* docking Studies**

Docking studies were carried out for the designed compounds against the PDE4 protein (PDB: 3FRG)(16) to examine the mode of binding with the proposed target. The results of docking studies revealed that the docked compounds have good binding affinities against 3FRG with binding free energies ranging from -9.4 to -10.5 kcal/mol (Table 2).

A molecular docking protocol was implemented using the Autodock Vina software. The target proteins were prepared and the active binding sites identified based on the positional coordinates of the co-crystallized inhibitors. The results of the docking protocol were validated by re-docking of the co-crystallized ligand (SK4) inside the active sites of 3FRG. The root mean square deviations (RMSD) between the re-docked conformers and the co-crystallized conformers of SK4 was 1.5, which confirms the validity of the docking protocol.

The crystal structure of the target protein PDE4 (PDB ID: 3FRG, resolution 1.70 Å was downloaded from Protein Data Bank (http://www.pdb.org). Autodock Vina software was used for the docking analysis. In these studies, the free energies and binding modes of the designed molecules against PDE4 protein was determined. At first, the water molecules were removed from the crystal structures of protein, retaining only one chain in enzyme. SK4 (The co-crystallized ligand) were utilized as references in the docking processes against 3FRG protein. After that, in order to prepare the target molecule for binding with the designed compounds, the target protein were subjected to protonation step. Then, the hydrogen atoms were hidden to make the areas of interaction clearer. Next, the energy of all systems were minimized followed by identification the binding pockets of the target protein. The structures of the designed compounds and the co-crystallized ligand, SK4 were drawn using ChemDraw Professional 12 and saved as .cdx format. For proper geometrical arrangements in space, 2D structures were exported in Chem 3D and energy minimization was performed. The ligand structures were saved in **.pdb** format.

Validation process was performed for each target by running the docking process for only the co-crystallized ligand. Low RMSD values between docked and crystal conformations indicate valid performance. The docking procedures were carried out utilizing a default protocol. The output from of autodock vina was further analyzed and visualized using Discovery Studio Visualizer (BIOVIA) software.

***In-silico* ADMET Prediction**

ADMET properties of compounds were predicted using ADMET lab 2.0 and are compiled in table no. 8 and 9. The structure of all the compounds was drawn using ChemDraw Ultra 2.0 and SMILES format of all the structures were pasted into the dialog box of ADMET lab 2.0. Then ADMET lab 2.0 protocol was applied to calculate the different descriptors. (https://admetmesh.scbdd.com/)

We designed and synthesised ten (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 derivatives to be tested as anti-asthmatic agents. Our targeted compounds were synthesised in the manner depicted in Scheme 1. All chemicals used were of analytical grade from, BLD Pharm. Melting points (MP) of all the synthesized compounds were determined by the 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 Hexane: Acetone (6:4). Spots were visualized using UV chamber. IR spectra were recorded on Shimadzu IR spectrophotometer by using KBr pellets technique. 1 H NMR was recorded on Burke AMX 60 MHz spectrophotometer by using DMSO as solvent.

Chemistry

General procedure for the synthesis of quinoline chalcone derivatives

Step I: (A) Synthesis of quinolin-8-ylacetate from 8-hydroxy quinoline (Friedel–Craft acylation).

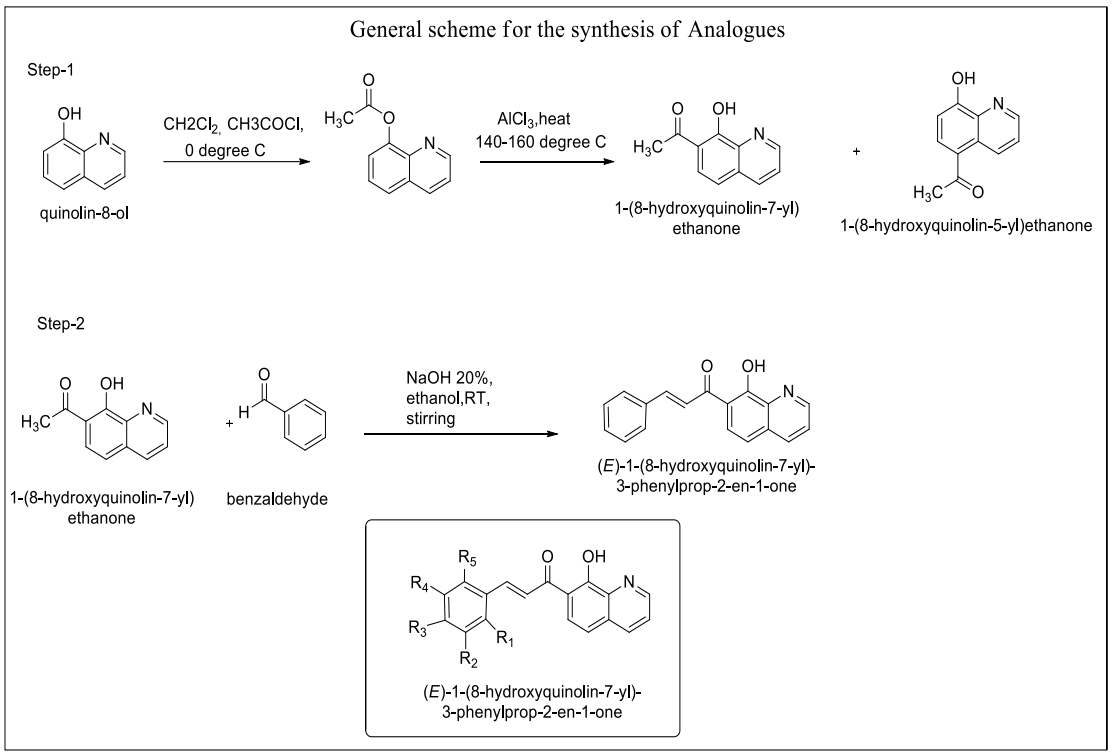
8-hydroxyquinoline 7.250gm (0.05mol) and 25 ml of methylene dichloride were placed in a 250ml round bottom flask and agitated on an ice bath for 30 minutes. To this chilled solution, 4.42ml (0.05mol) of acetyl chloride was added drop by drop, and the reaction mixture was stirred for 2 hours, reaction was monitored by TLC using (hexane: Acetone 6:4) as a mobile phase. After the reaction was finished, a light yellow-white precipitate was produced, which was filtered, washed with methylene dichloride, dried, and then recrystallized from hot water.(1)

(B) Synthesis of 7-acetyl-8-hydroxy quinoline from quinolin-8-yl acetate (Fries rearrangement).

Quinolin-8-yl acetate, 6.54 g (1 mol), and aluminium tri chloride, 16.65 g (2.5 mol) were placed in a 250 ml round bottom flask. The reaction mixture was heated at 160–175 °C for 1.5 hours, yielding an orange colour mass. Dilute hydrochloric acid was then added to the mixture to dissolve the aluminum–quinoline complex. After that, the para and ortho isomers that had produced were separated by steam distillation, and the process was seen using a TLC method with a mobile phase of (hexane: ethyl acetate 6:4).(1)

Step II: Synthesis of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 ones (Aldol condensation).

7-acetal-8-hydroxyquinoline (1 mol) was dissolved in 5 ml of 20% sodium hydroxide, added to this reaction mixture, and benzaldehyde derivative (1 mol) was added. After this reaction mixture was stirred for 6–8 hours at room temperature, the product was poured into ice cold water, its pH was adjusted to 4 with 10% HCl, and it was then filtered, washed, and dried. TLC, IR, NMR, Mass, and elemental analyses were used to characterise all the produced compounds. (Table 1)(1)



Scheme 1 General scheme for synthesis of compounds.

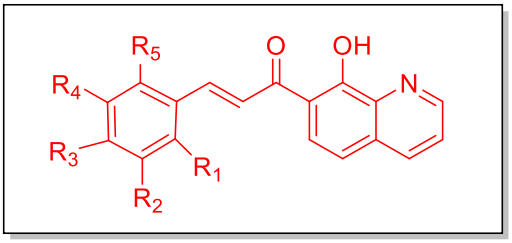


Table 1 Synthesized derivatives of quinoline chalcones.

Compounds	R1	R2	R3	R4	R5
PQ-01	OH	OCH_3	H	H	H
PQ-02	H	H	OCH_3	H	H
PQ-03	H	CH_3	H	OCH_3	H
PQ-04	H	CH_3	H	H	OCH_3
PQ-05	H	H	H	OCH_3	Cl
PQ-06	H	H	H	Cl	OCH_3
PQ-07	H	OCH_3	H	Br	H
PQ-08	H	H	OH	H	H
PQ-09	H	H	Cl	H	H
PQ-10	H	H	CH_3	H	H

Results and Discussions

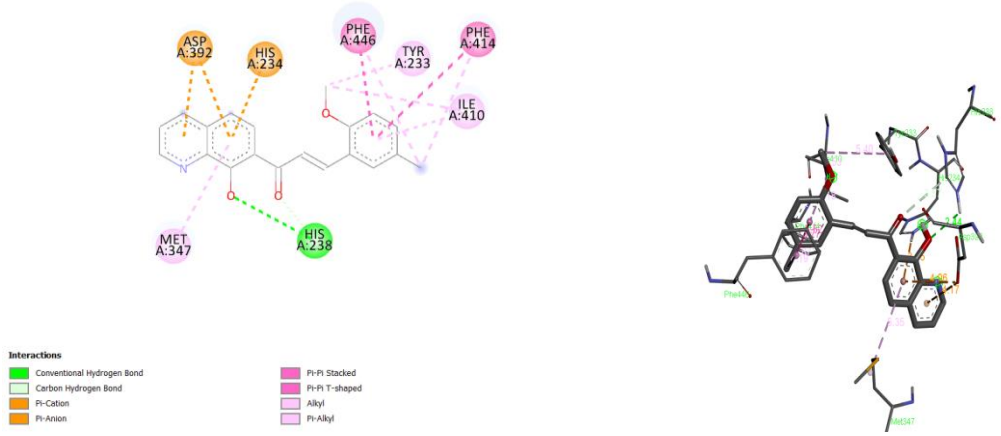
***In-silico* docking**

On the phosphodiesterase enzyme (PBD Code: 3FRG), preliminary docking studies of SK4 and (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 derivatives were conducted. The positive binding of these ligands to the Phospho-diesterase enzymes is indicated by the docking score and binding energies acquired. The interactions between the ligand molecules and the protein were investigated. Compound 2 displayed seven hydrophobic connections between amino acids MET 347, ASP 392, HIS 234, PHE 446, TYR 233, PHE 414, and ILE 410 in addition to a hydrogen bond with amino acid HIS 238 as shown in Fig. 1. Compound 2 has demonstrated anti-asthmatic activity with a docking score of -10.5, which is equivalent to reference ligand (-8.7).

Table 2: The docking binding free energies of the synthesized compounds against PDE-4 enzymes

Compounds	Docking Scores
PQ-01	-10.0
PQ-02	-10.5

PQ-03	-9.9
PQ-04	-9.8
PQ-05	-9.4
PQ-06	-10.3
PQ-07	-10.3
PQ-08	-9.9
PQ-09	-9.8
PQ-10	10.2
CO-CRYSTAL (SK4)	-8.7



(a) **(b)**

Figure 1: (a) 2D and (b) 3D interaction of compound 2 with PDE-4 enzyme (PDB: 3FRG)

***In-silico* ADMET studies**

ADMET properties of compounds were predicted using ADMET lab 2.0. and the results are compiled in table no. 6. As predicted by ADMET Lab 2.0, all the compounds follow Lipinski rule of five suggesting that they have drug likeliness. The HIA value of most of the compounds lies below 0.3 suggesting that can be absorbed in the intestine very well.

Table No. 3: ADME Prediction results of all synthesized compounds

Compound Code	Lipinski Rule	Log P[a]	Caco-2[b]	PPB[c]	BBB Penetration	Clearance[e]
PQ-01	Accepted	3.865	-4.794	100.7%	0.333	3.47
PQ-02	Accepted	3.598	-4.887	100.9%	0.092	4.103
PQ-03	Accepted	4.301	-4.909	101.0%	0.077	3.908
PQ-04	Accepted	4.242	-4.879	100.6%	0.131	5.07
PQ-05	Accepted	4.211	-4.814	101.2%	0.09	4.781
PQ-06	Accepted	4.374	-4.849	101.2%	0.076	2.954
PQ-07	Accepted	4.652	-4.906	100.6%	0.098	0.957
PQ-08	Accepted	3.516	-4.846	100.2%	0.257	6.815
PQ-09	Accepted	4.504	-4.817	101.0%	0.154	2.175
PQ-10	Accepted	4.276	-4.821	100.7%	0.257	3.222

a) Log of the octanol/water partition coefficient. Optimal: 0~3,
Optimal: higher than -5.15 Log unit,

b) Blood-Brain Barrier Penetration, Category 1: BBB+; Category 0: BBB-; The output value is the probability of being BBB+,

c) Plasma Protein Binding, Optimal: < 90%. Drugs with high protein bound may have a low therapeutic index.

d) Lipinski rule: MW ≤ 500; logP ≤ 5; Hacc ≤ 10; Hdon ≤ 5, If two properties are out of range, poor absorption or permeability is possible, one is acceptable.

Table 4: Toxicity prediction results of all synthesized compounds

Compound Code	HERG Blockersa	H-HT (Human Hepatotoxicity)[b]	Carcinogenicity	Respiratory Toxicity[d]
PQ-01	0.121	0.052	0.866	0.851
PQ-02	0.04	0.386	0.754	0.845
PQ-03	0.113	0.113	0.697	0.933
PQ-04	0.077	0.055	0.836	0.894
PQ-05	0.066	0.048	0.747	0.913

PQ-06	0.088	0.053	0.752	0.925
PQ-07	0.138	0.106	0.538	0.922
PQ-08	0.068	0.049	0.586	0.773
PQ-09	0.1	0.049	0.595	0.738
PQ-10	0.082	0.034	0.727	0.829

As predicted by ADMET Lab 2.0, toxicity profile of all compounds is within limits. All the compounds belong to category 0 of carcinogenicity, respiratory toxicants and HERG blockers suggesting that these compounds might not be carcinogenic or respiratory toxicants.

Spectral data

PQ-01 (E)-3-(2-hydroxy-3methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop-2-en1-one

This compound was obtained as blackish greenish powder m.p 500-520°C, I.R (KBr): υ 3377, 3282, (O-H peak), 3165 (O-CH3 Stretching), 1734 (C=O), 1591 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 10.36 (s, 1H,), 9.54 (s, 1H), 8.26 (s, 1H), 7.93 (m, 2H), 7.53 (d, 1H, J=5.4 Hz), 7.29 (m, 3H), 7.21 (m, 2 H), 3.86 (s, 3H) MS m/z=321 M+

PQ-02 (E)-1-(8-hydroxyquinolin-7-yl)-3-(4-methoxyphenyl)prop-2-en-1-one

This compound was obtained as greenish brown powder m.p 375-395°C, IR (KBr): υ 3305, (O-H peak), 3091 (O-CH3 Stretching), 1755 (C=O), 1600 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 9.28 (s, 1H,), 8.42 (s, 1H), 8.21 (d, 1H), 7.70 (d, 1H), 7.40 (d, 1H, J=5.4 Hz), 7.18 (m, 3H), 6.96 (m, 2 H), 3.90 (s, 3H) MS m/z=305 M+

PQ-03 (E)-1-(8-hydroxyquinolin-7-yl)-3-(3-methoxy-5methylphenyl) prop-2-en-1- one

This compound was obtained as green crystal m.p 410-425°C, IR (KBr): υ 3379 (O-H peak), 3159 (O-CH3 Stretching), 3111 (C-H Stretching), 1732 (C=O bonding), 1591 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 9.38 (s, 1H,), 8.33 (s, 1H), 7.93 (d, 1H), 7.77 (d, 1H), 7.39 (m, 5H), 6.92 (s, 1H), 3.97 (s, 3H), 2.56 (s, 3H) MS m/z=319 M+

PQ-04 (E)-1-(8-hydroxyquinolin-7-yl)-3-(2-methoxy-5methylphenyl) prop-2-en-1- one

This compound was obtained as chocolate brown powder m.p 410-430°C, IR (KBr): υ 3383 (O-H peak), 3136 (O-CH3 Stretching), 3089 (C-H Stretching), 1683 (C=O bonding), 1614 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 9.17 (s, 1H,), 8.34 (s, 1H), 7.76 (d, 1H), 7.34 (m, 5H), 7.25 (s, 1H), 3.97 (s, 3H), 2.37 (s, 3H) MS m/z=319 M+

PQ-05 (E)-3-(2-chloro-3methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop-2-en-1- one

This compound was obtained as greenish brown powder, m.p 420-440°C, IR (KBr): υ 3390 (O-H peak), 3091 (O-CH3 Stretching), 3028 (C-H Stretching), 1772 (C=O bonding), 1598 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 9.19 (s, 1H,), 8.35 (d, 1H, J=5.4 Hz), 7.84 (d, 1H, J=8.4 Hz), 7.71 (d, 1H, J=6.2), 7.58 (s, 1H), 7.34 (s, 1H) 7.15 (s, 1H), 3.97 (s, 3H) MS m/z=340 M+

PQ-06 (E)-3-(3-chloro-2-methoxyphenyl)-1-(8-hydroxyquinolin-7-yl) prop-2-en-1- one

This compound was obtained as grayish powder, m.p 420-440°C, IR (KBr): υ 3338 (O-H peak), 3091 (O-CH3 Stretching), 3064 (C-H Stretching), 1741 (C=O bonding), 1600 (C=C bonding) cm-1 . 1H NMR (400 MHz, DMSO-d6) δ 9.23 (s, 1H,), 8.44 (d, 1H, J=5.2 Hz), 7.95 (d, 1H, J=8.8 Hz), 7.81 (d, 1H, J=6.2), 7.54 (s, 1H), 7.35 (s, 1H) 7.18 (s, 1H), 4.07 (s, 3H) MS m/z=340 M+

PQ-07 (E)-3-(3-bromo-5-methoxyphenyl)- 1-(8-hydroxyquinolin-7-yl) prop-2-en1-one

This compound was obtained as brown powder, m.p 450-470°C, IR (KBr): υ 3383 (O-H peak), 3134 (O-CH3 Stretching), 3089 (C-H Stretching), 1705 (C=O bonding), 1664 (C=C bonding) cm-1 .

PQ-08 (E)-3-(4-hydroxyphenyl)-1-(8-hydroxyquinolin-7-yl)prop-2-en-1-one

This compound was obtained as light cream crystal, m.p 455-470°C, IR (KBr): υ 3408, 3126 (O-H peak), 1714 (C=O bonding), 1579 (C=C bonding) cm-1 .

PQ-09 (E)-3-(4-chlorophenyl)-1-(8-hydroxyquinolin-7-yl) prop-2-en-1-one

This compound was obtained as brown crystal, m.p 385-400°C, IR (KBr): υ 3097 (O-H peak), 1755 (C=O bonding), 1666 (C=C bonding) cm-1 .

PQ-10 (E)- 1-(8-hydroxyquinolin-7-yl)-3-(p-tolyl) prop-2-en-1-one

This compound was obtained as light brown powder, m.p 365-380°C, IR (KBr): υ 3408 (O-H peak), 1714 (C=O bonding), 1579 (C=C bonding) cm-1.

Conclusion

50 derivatives of (E)-1-(8-hydroxyquinolin-7-yl)-3-phenylprop-2-en-1 were designed in total; all 50 derivatives demonstrated favourable interactions with the amino acid residues in the active site of phosphodiesterase-4 (pdb: 3FRG) and had higher docking scores than the co-crystallized ligands (SK4). Among them, total 10 most potent compounds were synthesized and characterized. Compounds 2, 5 and 9 have been found to exert better binding interactions with PDE 4 and compound 2 have been found to show good percentage inhibition. Electron withdrawing & donating substitutions at the phenyl ring results in good anti-asthmatic activity.

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