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Certain 4-Iminoflavones Derivatives: Synthesis, Docking Studies, Antiasthmatic and Antimicrobial Agents

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In this present study, certain substituted 4-iminoflavone derivatives **5(a-j)** were synthesized. All the synthesized compounds were evaluated for antiasthmatic and antimicrobial activity. Among the synthesized compounds, **5f** was found to be the most promising against asthmatic models, whilst, **5h** was found active against all the microbial strains. Antiasthmatic activity was correlated and evaluated with various models, *e.g.*, citric acid induced cough model, OVA induced asthma model (biochemical estimation for cell infiltrations, estimation of lipid peroxidation and glutathione and estimation of TNF-α and IL-6 and histamine induced response). Compound **5f** was found to show 2.20 ± 0.047 number of cough whilst codeine showed 1.40 ± 0.548; compound **5f** produced considerable reduction in neutrophils, lymphocytes, eosinophils and leukocytes; decrease in LPO level (lung and BALF) and increment in GSH level (lungand BALF); and decreased the level of TNF-α and IL-6, respectively. On the other hand, **5h** showed MIC 6.25 µg/mL against *B. subtilis* and *S. aureus*, 3.1 µg/mL against *E. coli*, 1.55 µg/mL against *S. typhi* and 6.25 µg/mL against *C. albicans,* respectively. In addition to gain better understanding on the biological activities of synthesized compounds, molecular docking study was performed within the binding site of human histamine H_1 receptor and Glc-6-P synthase revealing compound **5f** and **5h** as best fit within the respective receptor pockets.

Keywords: 4-Iminoflavone, Antiasthmatic, Antimicrobial activity.

INTRODUCTION

Flavonoids are a group of chemical moieties of the compounds whose structure is based on C_6-C_3-C_6 *i.e.* two phenyl rings are attached through a propane bridge. Flavonoids are a family of plant compounds with a similar flavone backbone composed of two aromatic rings and an oxygen heterocycle attached [1]. Flavonoids can be classified into various classes *i.e.* flavonols (quercetin, kaempferol), flavones (luteolin, apigenin), flavanones (hesperetin), flavonoid glycosides (rutin), flavonolignans (silibinin), flavans (catechin, epicatechin), isoflavones (genistein, daidzein), anthocyanidins (cyanidin, delphinidin), aurones (leptosidin, aureusidin), leucoanthocyanidins (teracacidin), neoflavonoids (coutareagenin, dalbergin), chalcones [2]. All classes of flavonoids exhibits variety of biological activities, but among them, the flavones have been considerably explored. In recent years, substituted synthetic flavonoids have been dragging continuous attention due to their broad range of biological activities. Flavonoids are very well know and documented to possess antioxidant effects, antiviral and leishmanicidal activity, ovipositor stimulant of phytoalexins, anti-HIV, vasodilator, bactericidal, DNA cleavage, anti-inflammatory, antimutagenic, anti-asthmatic and anticancer. Especially, flavones (2-phenyl-chromones) exhibit a wide variety of activities [3-6]. Therefore, they exhibit diverse type of properties that are beneficial for human health *via* interacting with a number of cellular targets involved in critical cell signaling pathways in the body. Especially, flavone derivatives have been reported to possess anti-asthmatic activity *via* targeting FcεRI receptors as well as exhibiting leukotriene antagonism [7]. Histamine is implicated as a mediator of some of the symptoms of allergic rhinitis and other allergic diseases, but its importance in asthma is much less well understood. However, some studies also suggests the potential value of H_1 antihistamines in the treatment of asthma and number of groups have been reported compounds of potential therapeutic benefit that were claimed to possess the combined properties of both histamine H_1 antagonism and mast cell stabilization, likely, BM 15100 [8,9].

Substituted flavones are also known to show potential pharmacological profile but no natural flavonoids have been

reported with halogens as substituent. Substitution of the B ring is known to enhance antibacterial activity, with 3′-chloro, 4′-chloro and 4′-bromo analogues each being approximately twice as effective as their parent compound against *S. aureus* and four times more active against *Enterococcus faecalis*. Also, the 2′,4′-dichloro derivative exhibited a 4-8 folds improvement in activity against *S. aureus* [10]. However, a little work has been carried out on iminoavones revealing antiasthmatic and antimicrobial activities. Additionally, we were also interested to evaluate the antiasthmatic and antimicrobial activity of flavones derivatives possessing nitrogen atom at 4-position. Therefore, in order to search for new compounds that have potential activity for asthma and microbial infections, this paper describes the synthesis of variably substituted 4-iminoavones. Moreover, to gain much understanding, the docking studies were performed on specific targets in order to examine the binding affinity of respective molecules within the pocket.

EXPERIMENTAL

All chemicals and solvents were supplied by Merck and S.D. Fine Chemical Limited, Mumbai. The reactions were monitored with the help of thin-layer chromatography using pre-coated aluminum sheets with GF_{254} silica gel, 0.2 mm layer thickness (E. Merck). Solvent systems of benzene-acetone (9:1) and chloroform-methanol (9:1), (9.5:0.5) were employed. Melting points of the synthesized compounds were recorded on the Veego (VMP-MP) melting point apparatus. IR spectrum was obtained on Agilent technologies, Microlab-PC, Cary 630 Infrared spectrometer, (model FTIR-630). Both ^{1}H NMR ($CDCl_3$/DMSO-d_6) and ^{13}C NMR ($CDCl_3$/DMSO-d_6) spectra of the synthesized compounds were performed with Bruker Avance-II 400 NMR Spectrometer operating at 400 MHz in SAIF, Panjab University, Chandigarh, India. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Mass spectra of the synthesized compounds were recorded at MAT 120 in SAIF, Panjab University, Chandigarh, India.

In view of the importance of pharmacological active flavones, 4-iminoflavones were aimed to synthesize. The replacement of oxygen atom of the keto group was carried out with an amino group containing phenyl hydrazine. Our synthetic approach was initiated with the synthesis of various intermediate chalcones **3(a-j)** which was achieved through Claisen-Schmidt condensation. Targets compounds were synthesized *via* initial preparation of chalcones **3(a-j)** using easily accessible starting materials, 2-hydroxy-4-methoxy-acetophenone and substituted aldehydes in presence of 10 % KOH and ethanol (**Scheme-I**). Thereafter the chalcones were then oxidative cyclized in the presence of iodine to furnish the flavone derivatives **4(a-j)** in good yields. Notably, the reaction was conducted under 1,4-dioxane solvent system. It has been reported that the method followed for preparation of flavones corresponds to final products with satisfactory yields with both electron withdrawing and electron donating moieties. The plausible mechanism for the formation of flavone ring system **4(a-j)**, using iodine mediated oxidative cyclization is presented in **Scheme-I**. The mechanism involves isomerization of the initial adduct followed by intramolecular cyclization resulting in its hemiacetal species (a) which gets converted to more reactive flavylium ion, (b) furthermore, water molecules attach on the more reactive position to form adduct and finally, (c) on oxidation in presence of iodine yields final products *i.e.* flavones **4(a-j)**. The final product series **5(a-j)**, 4-iminoflavones was synthesized by refluxing the flavone **4(a-j)** with phenyl-hydrazine in presence of concentrated H_2SO_4 in ethanol absolute.

Antiasthmatic evaluation: Male Hartley Guinea pigs (500-600 g of either sex) were procured from the animal house Shoolini University Solan, India. Animals were maintained in the animal house within ordinary cages and exposed to normal day-night cycles under standard conditions with the temperature at 25 ± 5 °C and the relative humidity of 55-60 %, moreover, food and water *ad libitum*. All the protocols were in accordance with guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA) in India, in addition, approved by Institutional Animal Ethics Committee (IAEC).

Spectral characterization of synthesized compounds

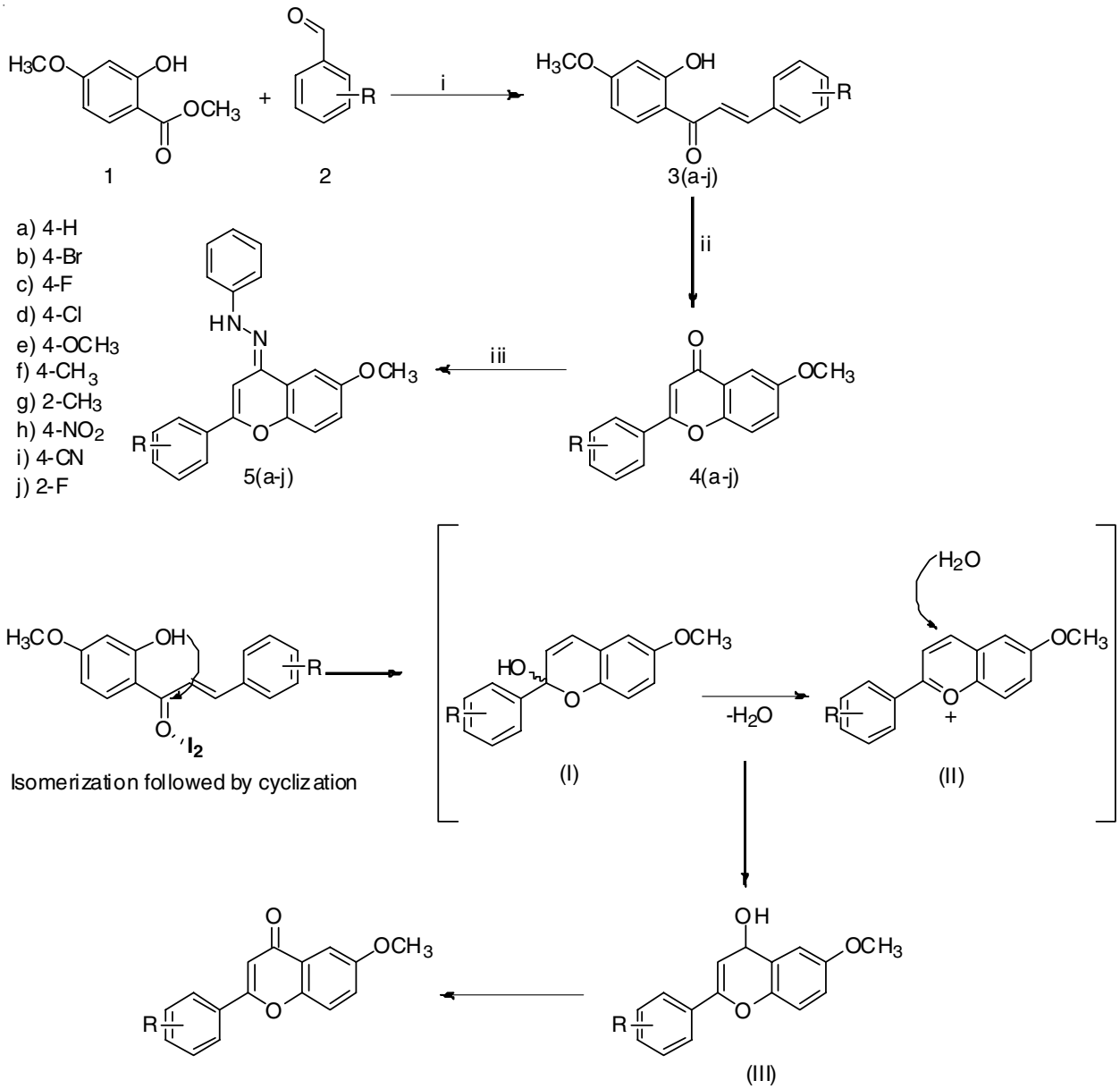
1-(2-Hydroxy-4-methoxyphenyl)-3-phenylprop-2-en-1-one (3a): Yield 60 %; m.p.: 148-150 °C; IR (KBr, ν_{max}, cm^{-1}): 3396.32, 2989.54, 2978.74, 1687.35; ^{1}H NMR ($CDCl_3$) δ ppm: 10.1 (bs, 1H, OH), 8.43 (d, 1H, Ar-H), 7.93 (s, 1H, Ar-H), 7.16 -6.97 (m, 4H, Ar-H), 6.89 (s, 2H, CH=CH), 6.66 (s, 1H, Ar-H), 3.78 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 180.3, 156.2, 154.5, 144.7, 135.6, 128.9, 127.6, 127.2, 124.3, 122.1, 120.2, 118.8, 116.4, 55.3, 23.6; MS (*m/z* %) $C_{16}H_{14}O_3$: 254.09 [M+].

3-(4-Bromophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (3b): Yield 54 %; m.p.: 152-156 °C; IR (KBr, ν_{max}, cm^{-1}): 3402.28, 2999.04, 2984.25, 1695.59, 634.48; ^{1}H NMR ($CDCl_3$) δ ppm: 10.7 (bs, 1H, OH), 8.59 (d, 1H, Ar-H), 7.98 (s, 1H, Ar-H), 7.48-7.02 (m, 4H, Ar-H), 7.12 (s, 2H, CH=CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 184.6, 158.5, 156.9, 149.4, 132.5, 129.3, 128.8, 126.5, 120.9, 117.2, 52.1, 23.6, 15.6, 11.2; MS (*m/z* %) $C_{16}H_{13}O_3Br$: 332.2 [M+].

3-(4-Fluorophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (3c): Yield 54 %; m.p.: 158-160 °C; IR (KBr, ν_{max}, cm^{-1}): 3413.51, 3002.35, 2991.56, 1697.48, 1035.20; ^{1}H NMR ($CDCl_3$) δ ppm: 12.01 (bs, 1H, OH), 9.11 (d, 1H, Ar-H), 8.12 (s, 1H, Ar-H), 7.76-7.34 (m, 4H, Ar-H), 7.32 (s, 2H, CH=CH), 3.98 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 188.7, 168.3, 160.5, 153.8, 145.2, 136.1, 130.5, 129.5, 128.2, 126.6, 125.4, 120.1, 118.5, 55.3; MS (*m/z* %) $C_{16}H_{13}O_3F$: 272.12 [M+].

3-(4-Chlorophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (3d): Yield 48 %; m.p.: 152-154 °C; IR (KBr, ν_{max}, cm^{-1}): 3405.87, 2998.18, 2956.84, 1718.04, 640.49; ^{1}H NMR ($CDCl_3$) δ ppm: 11.78 (bs, 1H, OH), 8.85 (d, 1H, Ar-H), 8.58-7.53 (m, 5H, Ar-H), 7.22 (s, 2H, CH=CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 186.3, 165.6, 159.2, 150.5, 144.1, 134.6, 128.2, 126.9, 125.4, 123.8, 120.4, 118.7, 114.6, 54.6; MS (*m/z* %) $C_{16}H_{13}O_3Cl$: 288.70 [M+].

1-(2-Hydroxy-4-methoxyphenyl)-3-(4-methoxyphenyl)-prop-2-en-1-one (3e): Yield 57 %; m.p.: 150-152 °C; IR (KBr, ν_{max}, cm^{-1}): 3388.92, 2984.42, 2943.17, 1710.37; ^{1}H NMR ($CDCl_3$) δ ppm: 10.12 (bs, 1H, OH), 8.30-7.22 (m, 6H, Ar-H),



Scheme-I: Synthesis of substituted targeted compounds **5(a-j)**. Reactants used; (i) aldehydes, 10 % KOH, ethanol, (ii) I_2, 1,4-dioxane, (iii) reflux, phenyhydrazine, ethanol, conc. H_2SO_4 and, plausible mechanism of synthesized flavone using iodine

7.02 (s, 2H, CH=CH), 3.75 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 183.8, 162.4, 155.8, 148.6, 142.5, 132.5, 126.1, 125.7, 123.5, 122.5, 120.8, 117.1, 115.5, 55.2; MS (*m/z* %) $C_{17}H_{16}O_4$: 284.38 [M+].

1-(2-Hydroxy-4-methoxyphenyl)-3-*p*-tolylprop-2-en-1-one (3f): Yield 61 %; m.p.: 144-146 °C; IR (KBr, ν_{max}, cm^{-1}): 3382.65, 2988.74, 2968.40, 1701.02; ^1H NMR ($CDCl_3$) δ ppm: 9.8 (bs, 1H, OH), 8.40 (s, 1H, Ar-H), 7.90 (s, 1H, Ar-H), 7.19 -6.86 (m, 4H, Ar-H), 6.94 (s, 2H, CH=CH), 6.72 (s, 1H, Ar-H), 3.74 (s, 3H, OCH_3), 2.67 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 178.4, 159.7, 152.6, 142.1, 136.4, 132.9, 128.2, 127.3, 126.8, 122.6, 120.7, 117.3, 112.7, 55.7, 23.3; MS (*m/z* %) $C_{17}H_{16}O_3$: 268.33 [M+].

1-(2-Hydroxy-4-methoxyphenyl)-3-*o*-tolylprop-2-en-1-one (3g): Yield 54 %; m.p.: 140-142 °C; IR (KBr, ν_{max}, cm^{-1}): 3395.29, 2993.19, 2971.31, 1698.85; ^1H NMR ($CDCl_3$) δ ppm: 9.3 (bs, 1H, OH), 8.21 (s, 1H, Ar-H), 7.32 -6.70 (m, 5H, Ar-H), 6.97 (s, 2H, CH=CH), 6.76 (s, 1H, Ar-H), 3.84 (s, 3H, OCH_3), 2.73 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 175.3, 157.5, 150.8, 144.6, 136.7, 136.2, 130.5, 129.6, 127.8, 126.4, 124.2, 122.7, 120.5, 118.5, 114.7, 55.4, 23.6; MS (*m/z* %) $C_{17}H_{16}O_3$: 269.40 [M+1].

1-(2-Hydroxy-4-methoxyphenyl)-3-(4-nitrophenyl)-prop-2-en-1-one (3h): Yield 63 %; m.p.: 162-164 °C; IR (KBr, ν_{max}, cm^{-1}): 3380.84, 3002.44, 2992.71, 2964.25, 1714.64; ^1H NMR ($CDCl_3$) δ ppm: 10.06 (bs, 1H, OH), 8.28-7.15 (m, 6H, Ar-H), 7.07 (s, 2H, CH=CH), 3.78 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 185.7, 160.6, 154.1, 140.2, 136.6, 126.8, 126.2, 124.5, 123.3, 122.9, 120.1, 116.6, 114.3, 55.1; MS (*m/z* %) $C_{16}H_{13}NO_5$: 299.29 [M+].

4-[3-(2-Hydroxy-4-methoxyphenyl)-3-oxoprop-1-enyl]-benzonitrile (3i): Yield 50 %; m.p.: 164-166 °C; IR (KBr, ν_{max}, cm^{-1}): 3392.18, 2974.69, 2857.83, 1718.73; ^1H NMR ($CDCl_3$) δ ppm: 10.25 (bs, 1H, OH), 8.42 (s, 1H, Ar-H), 8.36-

7.29 (m, 5H, Ar-H), 7.19 (s, 2H, CH=CH), 3.81 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 179.2, 159.8, 152.1, 145.6, 142.9, 136.7, 132.5, 126.7, 125.2, 124.8, 122.0, 120.5, 115.2, 55.6, 23.2; MS (*m/z* %) $C_{17}H_{13}NO_3$: 279.30 [M+].

3-(2-Fluorophenyl)-1-(2-hydroxy-4-methoxyphenyl)-prop-2-en-1-one (3j): Yield 48 %; m.p.: 152-156 °C; IR (KBr, ν_{max}, cm^{-1}): 3410.04, 3000.23, 2988.63, 1704.61, 1030.15; ^1H NMR ($CDCl_3$) δ ppm: 11.78 (bs, 1H, OH), 9.04 (d, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 7.72-7.30 (m, 4H, Ar-H), 7.26 (s, 2H, CH=CH), 3.88 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 189.4, 160.4, 158.7, 152.6, 144.6, 135.5, 130.5, 129.2, 128.8, 125.9, 125.2, 124.2, 121.6, 120.5, 117.9, 114.7, 55.8; MS (*m/z* %) $C_{16}H_{13}O_3F$: 272.25 [M+].

6-Methoxy-2-phenyl-4H-chromen-4-one (4a): Yield 70 %; m.p.: 168-170 °C; IR (KBr, ν_{max}, cm^{-1}): 2978.49, 1652.16, 1601.03; ^1H NMR ($CDCl_3$) δ ppm: 7.30 (d, 2H, Ar-H), 7.21 (d, 2H, Ar-H), 7.15 (s, 1H, Ar-H), 6.71-7.02 (m, 4H, Ar-H), 3.71 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 180.5, 163.7, 155.2, 148.1, 130.6, 128.3, 128.0, 126.9, 124.8, 120.4, 118.2, 115.6, 105.3, 56.2; MS (*m/z* %) $C_{16}H_{12}O_3$: 252.25 [M+].

2-(4-Bromophenyl)-6-methoxy-4H-chromen-4-one (4b): Yield 71 %; m.p.: 164-166 °C; IR (KBr, ν_{max}, cm^{-1}): 2980.69, 1657.25, 1610.44, 630.28; ^1H NMR ($CDCl_3$) δ ppm: 7.43 (d, 2H, Ar-H), 7.30 (d, 2H, Ar-H), 7.23 (s, 1H, Ar-H), 6.82-7.14 (m, 4H, Ar-H), 3.82 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 183.8, 165.1, 156.1, 149.2, 130.9, 129.3, 128.1, 126.3, 125.0, 120.8, 117.5, 114.8, 104.6, 55.8; MS (*m/z* %) $C_{16}H_{11}O_3Br$: 331.18 [M+].

2-(4-Fluorophenyl)-6-methoxy-4H-chromen-4-one (4c): Yield 68 %; m.p.: 172-174 °C; IR (KBr, ν_{max}, cm^{-1}): 2996.35, 1660.28, 1616.12, 1015.54; ^1H NMR ($CDCl_3$) δ ppm: 7.54 (d, 2H, Ar-H), 7.41 (d, 2H, Ar-H), 7.28 (s, 1H, Ar-H), 6.87-7.22 (m, 4H, Ar-H), 3.91 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 185.1, 166.6, 155.7, 149.6, 131.4, 129.5, 128.4, 126.7, 125.3, 121.1, 118.8, 114.9, 106.2, 56.4; MS (*m/z* %) $C_{16}H_{11}O_3F$: 270.26 [M+].

2-(4-Chlorophenyl)-6-methoxy-4H-chromen-4-one (4d): Yield 65 %; m.p.: 166-168 °C; IR (KBr, ν_{max}, cm^{-1}): 2985.03, 1658.75, 1614.33, 654.15; ^1H NMR ($CDCl_3$) δ ppm: 7.47 (d, 2H, Ar-H), 7.36 (d, 2H, Ar-H), 7.25 (s, 1H, Ar-H), 6.84-7.15 (m, 4H, Ar-H), 3.84 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 184.6, 165.4, 156.2, 149.8, 131.1, 129.4, 128.2, 126.6, 125.2, 120.9, 117.8, 114.8, 105.5, 56.1; MS (*m/z* %) $C_{16}H_{11}O_3Cl$: 286.72 [M+].

6-Methoxy-2-(4-methoxyphenyl)-4H-chromen-4-one (4e): Yield 74 %; m.p.: 158-160 °C; IR (KBr, ν_{max}, cm^{-1}): 2971.47, 1648.35, 1605.24; ^1H NMR ($CDCl_3$) δ ppm: 7.26 (d, 2H, Ar-H), 7.15 (d, 2H, Ar-H), 7.04 (s, 1H, Ar-H), 6.66-6.95 (m, 4H, Ar-H), 3.74 (s, 6H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 182.5, 162.5, 154.6, 148.6, 130.2, 128.5, 128.1, 126.6, 124.7, 120.8, 118.5, 114.9, 105.5, 56.6; MS (*m/z* %) $C_{17}H_{14}O_4$: 282.29 [M+].

6-Methoxy-2-*p*-tolyl-4H-chromen-4-one (4f): Yield 71 %; m.p.: 164-166 °C; IR (KBr, ν_{max}, cm^{-1}): 2976.24, 1644.06, 1602.45; ^1H NMR ($CDCl_3$) δ ppm: 7.25 (d, 2H, Ar-H), 7.19 (d, 2H, Ar-H), 7.11 (s, 1H, Ar-H), 6.69-6.98 (m, 4H, Ar-H), 3.78 (s, 3H, OCH_3), 2.85 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 182.8, 163.6, 155.4, 149.7, 136.2, 129.5, 128.3, 126.6, 125.1, 120.8, 118.5, 115.0, 104.5, 55.6, 23.8; MS (*m/z* %) $C_{17}H_{14}O_3$: 266.30 [M+].

6-Methoxy-2-*o*-tolyl-4H-chromen-4-one (4g): Yield 75 %; m.p.: 160-162 °C; IR (KBr, ν_{max}, cm^{-1}): 2971.89, 1640.75, 1601.58; ^1H NMR ($CDCl_3$) δ ppm: 7.29 (d, 2H, Ar-H), 7.23 (d, 2H, Ar-H), 7.16 (s, 1H, Ar-H), 6.76-7.08 (m, 4H, Ar-H), 3.73 (s, 3H, OCH_3), 2.81 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 182.2, 163.2, 155.8, 149.1, 137.4, 130.7, 128.9, 127.7, 126.4, 125.9, 125.3, 120.2, 118.2, 115.5, 105.7, 56.2, 23.5; MS (*m/z* %) $C_{17}H_{14}O_3$: 266.29 [M+].

6-Methoxy-2-(4-nitrophenyl)-4H-chromen-4-one (4h): Yield 77 %; m.p.: 174-176 °C; IR (KBr, ν_{max}, cm^{-1}): 3002.53, 2986.71, 1654.03, 1612.65; ^1H NMR ($CDCl_3$) δ ppm: 7.28 (d, 2H, Ar-H), 7.19 (d, 2H, Ar-H), 7.09 (s, 1H, Ar-H), 6.71-7.06 (m, 4H, Ar-H), 3.77 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 183.8, 163.8, 155.7, 149.2, 147.5, 136.6, 127.3, 125.8, 121.5, 120.6, 118.9, 114.3, 104.7, 55.5; MS (*m/z* %) $C_{16}H_{11}NO_5$: 297.26 [M+].

4-(6-Methoxy-4-oxo-4H-chromen-2-yl)benzonitrile (4i): Yield 58 %; m.p.: 178-180 °C; IR (KBr, ν_{max}, cm^{-1}): 2974.29, 2848.52, 2215.35, 1658.76; ^1H NMR ($CDCl_3$) δ ppm: 7.32-6.96 (m, 8H, Ar-H), 3.78 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 182.6, 162.5, 155.1, 149.6, 146.8, 136.9, 126.8, 125.3, 122.6, 120.2, 118.4, 115.7, 114.9, 104.5, 56.3; MS (*m/z* %) $C_{17}H_{11}NO_3$: 277.28 [M+].

2-(2-Fluorophenyl)-6-methoxy-4H-chromen-4-one (4j): Yield 69 %; m.p.: 184-186 °C; IR (KBr, ν_{max}, cm^{-1}): 2989.06, 1659.38, 1612.33; ^1H NMR ($CDCl_3$) δ ppm: 7.42 (d, 2H, Ar-H), 7.35 (d, 2H, Ar-H), 7.20 (s, 1H, Ar-H), 6.84-7.23 (m, 4H, Ar-H), 3.93 (s, 3H, OCH_3); ^{13}C NMR ($CDCl_3$) δ ppm: 182.4, 163.8, 156.4, 149.7, 137.8, 131.3, 128.5, 126.8, 125.6, 124.2, 120.5, 118.6, 114.1, 104.0, 56.5, 23.8; MS (*m/z* %) $C_{16}H_{11}O_3F$: 270.27 [M+].

1-(6-Methoxy-2-phenyl-4H-chromen-4-ylidene)-2-phenylhyrazine (5a): Yield 54 %; m.p.: 254-256 °C; IR (KBr, ν_{max}, cm^{-1}): 3312.65, 2993.58, 1672.67, 1624.42; ^1H NMR (DMSO-d_6) δ ppm: 10.0 (s, 1H, NH), 7.72 (d, 1H, Ar-H), 7.58 (d, 1H, Ar-H), 7.48-7.02 (m, 5H, Ar-H), 6.84 (s, 1H, Ar-H), 6.74-6.42 (m, 7H, Ar-H), 3.62 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 157.5, 155.2, 154.6, 148.4, 143.2, 130.7, 129.6, 128.5, 127.9, 126.3, 121.1, 120.7, 120.5, 118.4, 114.0, 113.8, 90.6, 55.8; MS (*m/z* %) $C_{22}H_{18}N_2O_2$: 343.39 [M+1].

1-(2-(4-Bromophenyl)-6-methoxy-4H-chromen-4-yidene)-2-phenylhydrazine (5b): Yield 62 %; m.p.: 258-260 °C; IR (KBr, ν_{max}, cm^{-1}): 3322.49, 2998.62, 1684.37, 1620.08, 664; ^1H NMR (DMSO-d_6) δ ppm: 10.2 (s, 1H, NH), 7.79 (d, 1H, Ar-H), 7.68-7.09 (m, 5H, Ar-H), 6.89 (s, 1H, Ar-H), 6.74-6.41 (m, 7H, Ar-H), 3.72 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 157.8, 154.3, 153.9, 148.7, 143.5, 130.4, 129.5, 128.7, 127.2, 126.5, 121.1, 120.7, 118.7, 116.1, 114.9, 113.2, 90.8, 55.2; MS (*m/z* %) $C_{22}H_{17}N_2O_2Br$: 421.29 [M+].

1-[2-(4-Fluorophenyl)-6-methoxy-4H-chromen-4-ylidene]-2-phenylhydrazine (5c): Yield 58 %; m.p.: 266-268 °C; IR (KBr, ν_{max}, cm^{-1}): 3328.60, 2999.84, 1685.42, 1624.24, 1010.51; ^1H NMR (DMSO-d_6) δ ppm: 10.32 (s, 1H, NH), 7.86 (d, 1H, Ar-H), 7.80-7.15 (m, 5H, Ar-H), 6.91 (s, 1H, Ar-H), 6.79-6.50 (m, 7H, Ar-H), 3.84 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 158.5, 155.9, 153.5, 148.5, 142.8, 130.8,

129.2, 128.8, 127.5, 125.3, 121.8, 120.6, 118.6, 115.8, 114.1, 113.7, 91.6, 55.7; MS (*m/z* %) $C_{22}H_{17}N_2O_2F$: 360.4 [M+].

1-[2-(4-Chlorophenyl)-6-methoxy-4H-chromen-4-ylidene]-2-phenylhydrazine (5d): Yield 63 %; m.p.: 262-264 °C; IR (KBr, ν_{max}, cm^{-1}): 3325.56, 2986.18, 1680.38, 1619.64, 590.28; ^{1}H NMR (DMSO-d_6) δ ppm: 10.22 (s, 1H, NH), 7.86-7.12 (m, 6H, Ar-H), 6.86 (s, 1H, Ar-H), 6.73-6.45 (m, 7H, Ar-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 157.6, 155.4, 153.3, 148.8, 143.5, 130.4, 129.6, 128.7, 127.2, 125.5, 121.4, 120.2, 118.5, 116.8, 114.8, 113.2, 90.9, 55.5; MS (*m/z* %) $C_{22}H_{17}N_2O_2Cl$: 377.8 [M+1].

1-[6-Methoxy-2-(4-methoxyphenyl)-4H-chromen-4-ylidene]-2-phenylhydrazine (5e): Yield 60 %; m.p.: 258-260 °C; IR (KBr, ν_{max}, cm^{-1}): 3319.38, 2980.54, 1676.61, 1616.09; ^{1}H NMR (DMSO-d_6) δ ppm: 10.08 (s, 1H, NH), 7.75-6.92 (m, 6H, Ar-H), 6.78 (s, 1H, Ar-H), 6.64-6.28 (m, 7H, Ar-H), 3.71 (s, 6H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 156.9, 155.3, 153.5, 147.7, 143.9, 130.4, 129.4, 128.2, 127.6, 125.5, 121.9, 120.6, 118.9, 115.6, 114.5, 113.0, 91.2, 55.9; MS (*m/z* %) $C_{23}H_{20}N_2O_2$: 372.4 [M+].

1-[6-Methoxy-2-(*p*-tolyl)-4H-chromen-4-ylidene]-2-phenylhydrazine (5f): Yield 55 %; m.p.: 250-252 °C; IR (KBr, ν_{max}, cm^{-1}): 3318.09, 2991.39, 1679.56, 1618.35; ^{1}H NMR (DMSO-d_6) δ ppm: 10.03 (s, 1H, NH), 7.68 (d, 1H, Ar-H), 7.54-6.98 (m, 5H, Ar-H), 6.82 (s, 1H, Ar-H), 6.66-6.34 (m, 7H, Ar-H), 3.74 (s, 3H, OCH_3), 2.75 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 158.2, 155.6, 153.6, 148.9, 143.2, 130.8, 129.1, 128.3, 127.8, 126.8, 121.9, 120.4, 118.6, 116.5, 114.5, 113.8, 91.2, 56.4, 23.8; MS (*m/z* %) $C_{23}H_{20}N_2O_2$: 357.2 [M+1].

1-[6-Methoxy-2-(*o*-tolyl)-4H-chromen-4-ylidene]-2-phenylhydrazine (5g): Yield 58 %; m.p.: 248-250 °C; IR (KBr, ν_{max}, cm^{-1}): 3316.39, 2994.37, 1680.07, 1621.46; ^{1}H NMR (DMSO-d_6) δ ppm: 10.05 (s, 1H, NH), 7.64-6.94 (m, 6H, Ar-H), 6.78 (s, 1H, Ar-H), 6.62-6.30 (m, 7H, Ar-H), 3.76 (s, 3H, OCH_3), 2.72 (s, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 157.5, 155.6, 153.9, 148.3, 143.8, 130.5, 129.8, 128.2, 127.6, 126.4, 121.3, 120.7, 118.5, 116.4, 114.8, 113.2, 90.6, 55.8, 23.9; MS (*m/z* %) $C_{23}H_{20}N_2O_2$: 356.2 [M+].

1-[6-Methoxy-2-(4-nitrophenyl)-4H-chromen-4-ylidene]-2-phenylhydrazine (5h): Yield 60 %; m.p.: 272-274 °C; IR (KBr, ν_{max}, cm^{-1}): 3332.29, 3003.85, 2986.59, 1680.19, 1622.24; ^{1}H NMR (DMSO-d_6) δ ppm: 10.16 (s, 1H, NH), 7.92 (s, 1H, Ar-H), 7.86-6.98 (m, 5H, Ar-H), 6.76 (s, 1H, Ar-H), 6.70-6.30 (m, 7H, Ar-H), 3.77 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 156.5, 155.7, 154.0, 147.6, 143.5, 130.6, 129.8, 128.7, 127.2, 126.1, 121.7, 120.6, 118.0, 115.8, 114.6, 113.8, 91.6, 55.2; MS (*m/z* %) $C_{22}H_{17}N_3O_4$: 387.4 [M+]

4-[6-Methoxy-4-(2-phenylhydrazono)-4H-chromen-2-yl]benzonitrile (5i): Yield 48 %; m.p.: 268-270 °C; IR (KBr, ν_{max}, cm^{-1}): 3305.95, 2986.40, 2218.53, 1679.62, 1620.18; ^{1}H NMR (DMSO-d_6) δ ppm: 10.06 (s, 1H, NH), 7.79-6.88 (m, 6H, Ar-H), 6.81 (s, 1H, Ar-H), 6.72-6.30 (m, 7H, Ar-H), 3.74 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 157.2, 155.6, 153.2, 146.6, 143.9, 130.8, 129.2, 128.9, 127.0, 125.2, 121.5, 120.4, 118.8, 116.4, 114.1, 113.8, 90.6, 55.6; MS (*m/z* %) $C_{23}H_{17}N_3O_2$: 367.4 [M+].

1-[2-(2-Fluorophenyl)-6-methoxy-4H-chromen-4-ylidene]-2-phenylhydrazine (5j): Yield 66 %; m.p.: 268-270 °C; IR (KBr, ν_{max}, cm^{-1}): 3326.27, 2998.09, 1683.48, 1622.46, 1006.76; ^{1}H NMR (DMSO-d_6) δ ppm: 10.24 (s, 1H, NH), 7.82 (d, 1H, Ar-H), 7.76-7.10 (m, 5H, Ar-H), 6.88 (s, 1H, Ar-H), 6.72-6.42 (m, 7H, Ar-H), 3.80 (s, 3H, OCH_3); ^{13}C NMR (DMSO-d_6) δ ppm: 158.9, 155.5, 153.3, 148.4, 143.6, 130.5, 129.2, 128.5, 127.6, 126.8, 125.8, 121.6, 120.7, 118.8, 116.6, 114.0, 113.1, 90.3, 52.2; MS (*m/z* %) $C_{22}H_{17}N_2O_2F$: 361.4 [M+1].

Citric acid-induced cough model: Animals were divided into different groups. Each group included 5 animals (n = 5). Citric acid induced cough model was used to perform the experiment as reported by Laude *et al*. [11]. On 1st day, after a 3 min acclimatization period, the animals were initially exposed to normal saline and 5 min later to aerosolized 7.5 % w/v citric acid for a period of 10 min. Animal selection was conducted on the basis of number of coughs (< 7 or15 >) on exposure to aerosolized 7.5 % citric acid or their tendency to cough on exposure to aerosolized saline was excluded as this taken as an indication of infection or hyper-reaction. Whilst on the same day, cough challenge was given for each animal and a minimum interval of 24 h was allowed between challenges to eliminate any short-term prophylaxis. Each animal will be placed in a perspex chamber (30 cm × 20 cm × 20 cm) and exposed to an aerosolized aqueous solution of 7.5 % w/v citric acid for a period of 10 min. The animals were observed continuously and number of coughs and latency time to initial cough response was documented. The samples were intraperitoneally administered at 30 min before the second challenge of aerosolized citric acid (75 % w/v) solution for 10 min. All the synthesized compounds **5(a-j)** were administered (i.p.) at dose of 30 mg/kg, dissolved in normal saline. Codeine 10 mg/kg was administered as standard.

OVA induced asthma model: According to the method of Andersson and Bergstrand [12] animals were actively sensitized. The intraperitoneal injection of 20 µg ovalbumin and 100 mg $Al(OH)_3$ dissolved in normal saline (0.2 mL) was given twice in a gap of 7 days. After 2 weeks, the animals were placed in perspex chamber equipped with an ultrasonic nebulizer and challenged with 0.5 % ovalbumin inhalation in order to verify the occurrence of sensitization. The animals showing airway hyper-responsiveness, to the inhaled antigen, were referred to as sensitized animals. Afterward, the animals were divided into different groups, each comprising 5 animals (n = 5): SC (sensitized control), AMP (aminophylline 50 mg/kg) and all the test compounds **5(a-j)** 30 mg/kg were given intraperitoneally for 7 days dissolved in pyrogen free water.

Biochemical estimation for cell infiltrations: After final ovalbumin challenge of 24 h, animals were sacrificed using sodium pentobarbitone (200 mg/kg, i.p.). The trachea was immediately cannulated and the lungs were lavaged with 5 × 4 mL aliquots of Ca^{2+} and Mg^{2+} free 0.1 M phosphate buffered saline solution of pH 7.4. The collected BAL fluid was centrifuges and used for further biochemical estimations. The obtained lung tissue was dissected out, washed in cold saline, dried and homogenated (10 %) in 10 mL phosphate buffer saline pH 7.4. The homogenate was centrifuged and supernatant was used for biochemical estimation [13].

Estimation of lipid peroxidation (LPO) and glutathione (GSH): The experiment was conducted according to the method given by Ohkawa *et al.* [13]. Briefly, at first the organ

was rinsed with ice cold normal saline followed by 0.15 M HCl (pH 7.4) for LPO estimation and 10 % w/v in 0.1 M phosphate buffer (pH 7.4) for GSH estimation. In case of LPO, the conditions maintained were temperature (95 °C), pH (acidic), absorbance (532 nm) and light path (1 cm) respectively. Afterward, 0.2 mL of tissue homogenate was mixed with 0.2 mL of 8.1 % SDS, 1.5 mL of 20 % acetic acid, 1.5 mL of 8 % TBA and the volume was made up to 4 mL with double distilled water. The prepared sample was heated on water 95 °C for 60 min and allowed to cool. Afterward 5 mL of butanol:pyridine (15:1) was added and vortexed for 2 min, followed by centrifugation at 3000 rpm for 10 min. Upper organic layer was collected and estimation was carried out spectrophotometrically. Whilst, in case the conditions were maintained as temperature (25 °C), pH (acidic), absorbance (412 nm) and light path (1 cm) respectively. Homogenate sample was taken and 20 % TCA and 1 μM EDTA was added. The sample was centrifuged for 10 min at 2000 rpm. Supernatant (200 mL) was taken and transferred to new tube followed by addition of 1.8 mL of Ellman/s reagent with volume make up to 2 mL using double distilled water. The absorbance was recorded at 412 nm [14].

Estimation of TNF-α and IL-6 and histamine induced response: For estimation of TNF-α and IL-6, RayBio® TNF-α ELISA (Enzyme-linked immunosorbent assay) kit was used and accordingly the assay was carried out. All standards and samples were run using 96-well microliter plates. In addition, response of synthesized best candidate was examined for histamine induced response *via* goat tracheal method [15]. Briefly, goat trachea was procured and cut into individual ring. Thereafter, it was suspended in bath containing kreb's solution maintained at 37 ± 5 °C stream of CO_2 and O_2 was bubbled through organ tube. As a result dose response effect was examined.

Antimicrobial activity: All the synthesized derivatives **5(a-j)** were screened for *in vitro* antibacterial and antifungal activity. Compounds were tested against the *Bacillus subtilis* (ATCC-6633), *Stapylococcus aureus* (ATCC-25923), *Escherichia coli* (ATCC-25922) and *S. typhi* (ATCC-6539). However, to evaluate the antifungal activity of iminoflavones *Candida albicans* (ATCC-24433) was considered [16]. Imipenem and amphotericin B were used as the standard drugs. The antimicrobial testing was performed by the methods of National Committee for Clinical Laboratory Standards [17]. Mirodilution method was employed and respective MICs were obtained against specific strains. The solution of the newly synthesized compounds and standard drugs was prepared at 200, 100, 50, 25, 12.5, 6.25, 3.1, 1.55, 0.78, 0.39, 0.19 μg/mL concentrations in the wells of microplates by diluting in the liquid double stranded nutrient broth. The bacterial suspensions used for inoculation were prepared of 10^5 cfu/mL by diluting fresh cultures at MacFarland 0.5 density (10^7 cfu/mL). Suspensions of the bacteria at 10^5 cfu/mL concentration were inoculated. There were 10^4 cfu/mL bacteria in the wells after inoculations. Nutrient broth was used for diluting the bacterial suspension. DMSO, pure microorganisms and pure media were used as control wells. The trays were incubated at 37 °C in a humid chamber and MIC endpoints were read after 24 h of incubation. The same procedure was used for antifungal activity. The lowest concentration of the compound that completely inhibits macroscopic growth was determined and minimum inhibitory concentrations (MICs) were reported.

Docking study: All the synthesized derivatives were examined for their binding affinity towards antiasthmatic and antimicrobial activity. Crystal structure of human histamine H_1 receptor (PDB code 3RZE) [18] and Glc-6-P synthase (PDB code 1MOQ) were obtained from RCSB protein data bank and docking study was performed on each target. AutoDock 4.2 package software was used to examine the binding affinity of newly synthesized derivatives within the binding pockets [19]. Initially, ChemDraw Ultra 12.0 was used to draw chemical structures and energy minimization was performed. Autogrid program of AutoDock suit was utilized for generation of grid around binding pocket within target protein. Finally, docking simulation was carried out with AutoDock 4.2. Ligplot and UCSF Chimera version 1.8.1 were used for analysis of docking results *i.e.* protein ligand interaction and visualization of docked proteins ligand complexes [20].

RESULTS AND DISCUSSION

Antiasthmatic activity

Citric acid-induced cough model: It has been documented that the guinea pigs and humans respond to similar concentration of citric acid and responses are well correlated with concentration-response relationship. Citric acid induction resulted significant cough in Guinea pigs as evidenced by decreased cough latency time and increased cough frequencies. Pretreatment with codeine (standard) and compounds **5(a-j)** showed notable increase in cough latency as well as decrease in cough frequencies per 10 min in comparison to control (Table-1 and Fig. 1). Among the synthesized compounds, **5f** showed the highest antitussive effect at a dose of 30 mg/kg, i.p in comparison to codeine (10 mg/kg). Compound **5f** was found to show 2.20 ± 0.047 number of cough whilst codeine showed 1.40 ± 0.548. However, compounds **5j**, **5h** and **5c** also showed substantial and considerable activity, likely, 3.60 ± 0.548, 4.72 ± 0.421 and 4.89 ± 0.521, respectively. Interestingly, compound having nitro at 4th position and methyl substitutions (position 4) were highly active. In addition, fluoro containing moieties at 2nd and 4th position also showed prominent activity in comparison to other halogen substituted compounds. However, most electron withdrawing substituted groups showed promising results among the synthesized derivatives.

OVA induced asthma model: This study presents the respiratory hyper-reactivity induced by an exposure to ovalbumin aerosol in sensitized guinea pigs. The interaperitoneal injection

TABLE-1
COUGH RESPONSE OF SYNTHESIZED COMPOUNDS **5(a-j)**

Treatment	No. of cough	Treatment	No. of cough
Control	18.80 ± 0.447	**5e**	7.60 ± 1.140
Codeine	1.40 ± 0.548	**5f**	2.20 ± 0.047
5a	10.80 ± 0.837	**5g**	11.40 ± 0.528
5b	10.92 ± 0.721	**5h**	4.72 ± 0.421
5c	4.89 ± 0.521	**5i**	6.71 ± 1.530
5d	13.40 ± 1.517	**5j**	3.60 ± 0.548

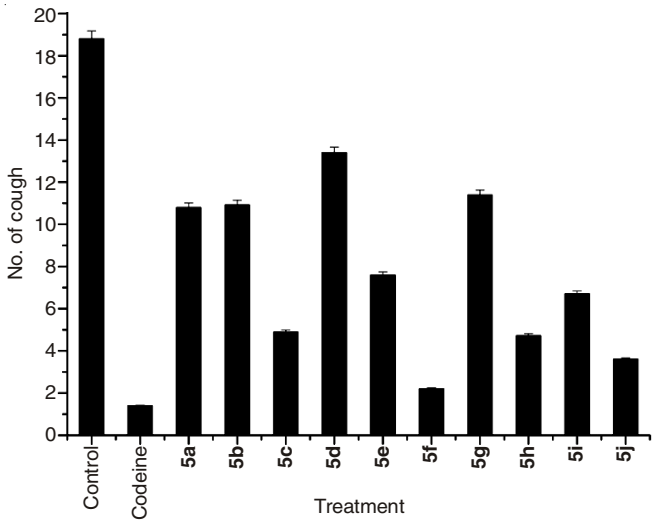


Fig. 1. Plot representing number of cough obtained with respect to the treatment

of 20 µg ovalbumin and 100 mg $Al(OH)_3$ twice in a gap of 7 days caused allergic reactions in male Hartley guinea pigs and the success rate of sensitization method was 89.79 %, which showed allergic reactions to inhaled antigen. This exposure resulted pulmonary asthma like symptoms in antigen induced sensitized animals which was supported by the increase in eosinophilic accumulation in bronchial tissue and BAL fluid associated with chronic airway inflammation.

Eosinophilic inflammation is known to be a paramount parameter of bronchial asthma. In addition, other cell infiltrates were also estimated, likely, neutrophils, lymphocytes and leukocytes. It was found that the % count was quite high in control animals, whereas, the synthesized compounds showed promising results in comparison to standard drug *i.e.* codeine. Control group did not receive any treatment and the cell infiltration was estimated at a higher side. Among all the synthesized compounds, **5f** and **5j** showed significant effect (Table-2). Compound **5f** produced considerable reduction in neutrophils (7.68 ± 0.811), lymphocytes (2.58 ± 0.432), eosinophils (16.80 ± 1.332) and leukocytes (10.38 ± 0.870) whereas, compound **5j** also showed notable effect on neutrophils (8.76 ± 0.69), lymphocytes (2.79 ± 0.461), eosinophils (16.93 ± 1.231) and leukocytes (10.49 ± 0.922) in comparison to codeine; neutrophils (6.66 ± 1.216), lymphocytes (2.40 ± 0.412), eosinophils (15.82 ± 1.366) and leukocytes (9.96 ± 0.602), respectively. Therefore, the present study showed that the compounds at an intended dose of 30 mg/kg were active in comparison to codeine at a dose of 10 mg/kg. This study also supports the previous experiment, as compound **5f** was found to be highly active among the synthesized ones and significantly reduced the eosinophil accumulation in the sensitized guinea pigs lungs and BALF.

Oxidative stress plays an important role in pathophysiology of asthma leading various detrimental effects on airway function, including airway smooth muscle contraction, mucus hypersecretion and vascular exudation as evidenced by LPO reaction and decrease in GSH levels in BALF and lung tissue, as compared to control. Table-3 presents the LPO and GSH

TABLE-2
EFFECT OF TEST DRUGS ON ANTIGEN INDUCES CELL INFILTRATION

Treatment	Neutrophils (%)	Lymphocytes (%)	Eosinophils (%)	Leukocytes (%)
Control	14.40 ± 1.840	5.64 ± 0.9840	40.70 ± 0.401	18.18 ± 3.356
AMN	6.66 ± 1.216	2.40 ± 0.4120	15.82 ± 1.366	9.96 ± 0.602
5a	10.22 ± 0.210	4.42 ± 0.3770	19.23 ± 2.060	11.54 ± 0.970
5b	11.61 ± 0.761	4.67 ± 0.4219	22.60 ± 2.493	12.86 ± 0.527
5c	9.99 ± 0.731	3.06 ± 0.5320	17.99 ± 1.071	10.99 ± 0.960
5d	10.21 ± 1.230	4.20 ± 0.6600	18.94 ± 1.011	11.26 ± 0.986
5e	12.08 ± 1.076	4.89 ± 0.4810	30.76 ± 2.640	16.18 ± 1.001
5f	7.68 ± 0.811	2.58 ± 0.4320	16.80 ± 1.332	10.38 ± 0.870
5g	11.82 ± 0.841	4.78 ± 0.4970	27.04 ± 2.131	14.88 ± 0.482
5h	9.96 ± 0.532	2.99 ± 0.5220	17.26 ± 1.128	10.63 ± 0.995
5i	10 ± 0.231	3.27 ± 0.5620	18.82 ± 1.016	11.01 ± 0.931
5j	8.76 ± 0.690	2.79 ± 0.4610	16.93 ± 1.231	10.49 ± 0.922

TABLE-3
FFECT OF COMPOUNDS **5(a-j)** TREATMENT ON OXIDATIVE STRESS IN LUNG AND BALF HOMOGENATES

Treatment	Oxidative stress in lung homogenates		Oxidative stress in BALF homogenates	
	LPO (nM MDA/g wet tissue)	GSH (nmol/g wet tissue)	LPO (nM MDA/g wet tissue)	GSH (nmol/g wet tissue)
Control	51.16 ± 6.811	0.35 ± 0.054	54.50 ± 10.501	0.27 ± 0.063
AMN	17.17 ± 5.349	0.71 ± 0.113	18.50 ± 7.583	0.67 ± 0.144
5a	25.50 ± 8.131	0.52 ± 0.072	25.23 ± 6.79	0.51 ± 0.029
5b	32.23 ± 8.231	0.46 ± 0.064	31.17 ± 8.772	0.45 ± 0.018
5c	19.26 ± 2.23	0.62 ± 0.123	22.79 ± 5.233	0.55 ± 0.231
5d	23.29 ± 6.275	0.56 ± 0.079	24.50 ± 6.604	0.53 ± 0.035
5e	39.17 ± 6.625	0.39 ± 0.065	41.83 ± 8.028	0.38 ± 0.071
5f	18.83 ± 2.236	0.7 ± 0.123	19.61 ± 7.831	0.66 ± 0.113
5g	33.17 ± 11.844	0.43 ± 0.081	39.17 ± 10.448	0.41 ± 0.101
5h	19.01 ± 2.261	0.65 ± 0.086	21.27 ± 5.431	0.59 ± 0.129
5i	20.61 ± 6.121	0.59 ± 0.089	23.98 ± 5.692	0.51 ± 0.230
5j	18.99 ± 2.241	0.69 ± 0.121	20.16 ± 5.578	0.63 ± 0.105

levels expressed in BALF and lung tissue homogenate samples. The control showed significant increase in LPO (lung; 51.16 ± 6.811 and BALF; 54.50 ± 10.501) and decrease in GSH (lung; 0.35 ± 0.054 and BALF; 0.27 ± 0.063). The synthesized compounds **5(a-j)** were tested (30 mg/kg) in order to analyze their impact over LPO and GSH. Compounds **5f**, **5j**, **5h**, **5c** and **5i** showed substantial and considerable effects over LPO as well as GSH. Among these, **5f** having methyl substitution at *para* position was found to be the most active one. Compound **5f** showed significant decrease in LPO level (lung; 18.83 ± 2.236 and BALF; 19.61 ± 7.831) and increment in GSH level (lung; 0.7 ± 0.123 and BALF; 0.66 ± 0.113), respectively. The present experiment showed that iminoflavones have a significant impact on LPO and GSH levels.

TNF-α and IL-6 have been identified as important pathogenic mediators in asthma/inflammation associated asthma. TNF-α and IL-6 were estimated in comparison to standard *i.e.* codeine (TNF-α 4.05 ± 0.896 and IL-6 10.97 ± 1.034), whilst, in control the level was found at higher side (TNF-α 19.98 ± 1.764 and IL-6 10.97 ± 1.034) as shown in Table-4 and Fig. 2. All the compounds were tested and **5f** showed significant activity *via* decreasing the level of TNF-α (4.33 ± 0.520) and IL-6 (3.03 ± 0.281). All the experiments were found to support each other as compound **5f** was found to be the most active one. Therefore, the compound **5f** was examined for response against histaminic induced contraction. The compound at optimized doses showed significant relaxation. Interestingly, the relaxation was found to increase with increment in dose, whilst, control (at different dose) were found to show contraction due to induction of histamine. This revealed that **5f** considerably inhibited the contractile effect of histamine thus produces bronchodilation. Therefore, compound **5f** was found active in all the experiments, supporting each other.

Antimicrobial activity: The antimicrobial activity was determined in terms of minimum inhibitory concentration (MIC) of the synthesized compounds **5(a-j)**. The activity was determined against the Gram-positive *Bacillus subtilis, Stapylococcus aureus*, the Gram-negative *Escherichia coli*, *Salmonella typhi* and the fungi *Candida albicans*. The obtained

TABLE-4
ESTIMATION OF TNF-α AND IL-6 AFTER TREATMENT WITH COMPOUND **5(a-j)**

Treatment	TNF-α (ng/mL)	IL-6 (ng/mL)
Control	19.98 ± 1.764	10.97 ± 1.034
AMN	4.05 ± 0.896	2.82 ± 0.214
5a	6.86 ± 1.102	5.62 ± 0.421
5b	8.31 ± 1.118	6.79 ± 0.409
5c	5.14 ± 0.901	3.76 ± 0.577
5d	5.51 ± 0.931	4.69 ± 0.171
5e	13.49 ± 0.504	8.99 ± 0.610
5f	4.33 ± 0.520	3.03 ± 0.281
5g	12.81 ± 0.739	8.23 ± 0.521
5h	4.00 ± 0.731	3.59 ± 0.461
5i	5.20 ± 0.921	4.52 ± 0.161
5j	4.10 ± 0.611	3.23 ± 0.321

data revealed that compounds inhibited the growth of the selected microorganisms *in vitro* showing MIC values between 3.1 to 100 µg/mL as shown in Table-5. Newly synthesized derivatives **5(a-k)** showed satisfactory to good potential against gram negative bacteria (*E. coli* and *S. typhi*) and *C. albicans*. Compounds **5h** was found to the most active candidate among the synthesized derivatives showing significant activity against all the strains. Compound **5h** showed MIC 6.25 µg/mL against *B. subtilis* and *S. aureus*, 3.1 µg/mL against *E. coli*, 1.55 µg/mL against *S. typhi* and 6.25 µg/mL against *C. albicans,* respectively. Moreover, other compounds of the series likely, **5c**, **5d**, **5f** and **5j** were also found active against all strains. All the compounds against *C. albicans* showed inhibition within a range of MIC 3.1-50 µg/mL. Compounds **5c**, **5d** and **5h** were found to be the most active candidate against *C. albicans* with MIC 3.1, 6.25 and 6.25 µg/mL, respectively (Table-6). Interestingly, compound having halogen substitution were found to show better inhibition against *C. albicans* in comparison to other compounds. However, very few of the synthesized compounds were found inactive against specific strains.

Docking study: Most employed and popular software was used in the present study in order to visualize and optimize the binding affinity of synthesized new molecules on specific

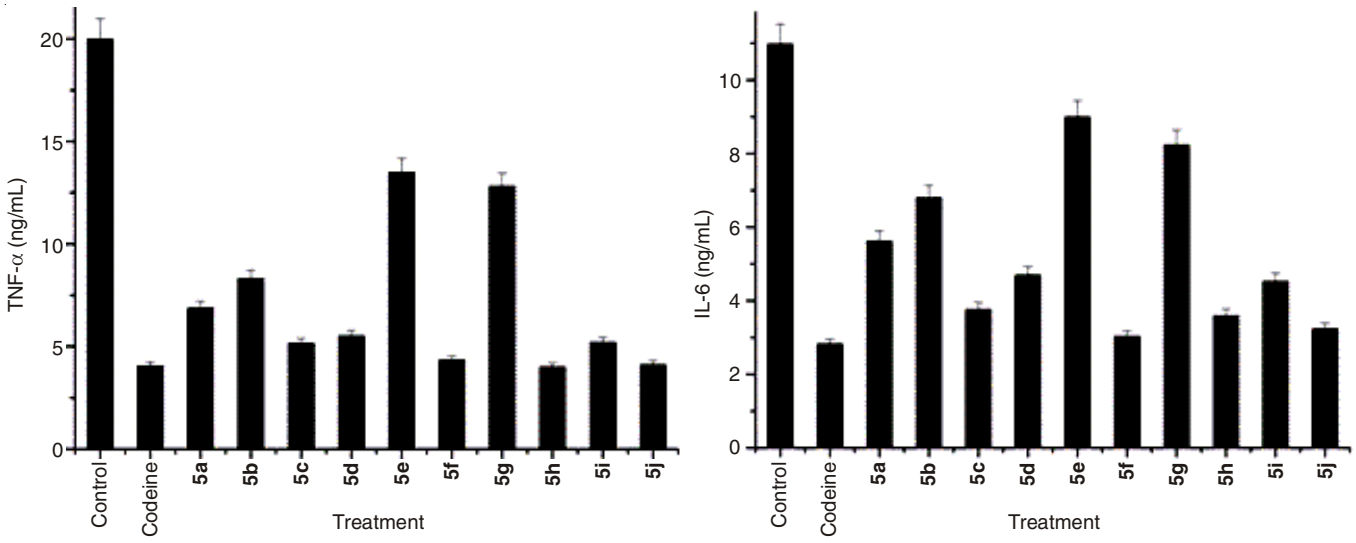


Fig. 2. Plots presenting estimated TNF-α (ng/mL) and IL-6 (ng/mL) with respect to the treatment

TABLE-5
RESPONSE OF **5f** TO HISTAMINE (10 µg/mL) INDUCED CONTRACTION AT DIFFERENT DOSES

Dose (mL)	Response (mm)	Response	Relaxation (%)
Histamine (0.05)	13	Contraction	–
Histamine (0.1)	20	Contraction	–
Histamine (0.2)	28	Contraction	–
Histamine (0.4)	37	Contraction	–
Histamine (0.8)	46	Contraction	–
Histamine (1.6)	51	Contraction	–
Histamine (3.2)	56	Contraction	–
Histamine (6.4)	48	Contraction	–
5f (5 mg/mL) + Histamine (3.2)	22	Relaxation	60.71
5f (10 mg/mL) + Histamine (3.2)	18	Relaxation	67.85
5f (20 mg/mL) + Histamine (3.2)	15	Relaxation	73.21

drug targets. The significant result of antiasthmatic and antimicrobial activity prompted is to investigate the docking of molecules on well-known targets. Active site residues of human histamine H1 receptor according to co-crystal structure with inhibitors; Trp 428(A), Phe 432(A), Asp 107(A), Tyr 108(A), Ser 111(A), Tyr 431(A), Trp 158(A), Tyr 458(A), Asn 198(A), Thr 112(A), Thr 194(A), Phe 424(A), Phe 435(A) were taken to define binding pocket within target protein, whereas, active site residues of glucosamine-6-phosphate synthase according to co-crystal structure with inhibitors (Cys 300, Gly 301, Thr 302, Ser 303, Ser 347, Gln 348, Ser 349, Thr 352, Val 399, Ser 401, Ala 602 and Lys 603) were taken to define binding pocket within target protein. Ligands were ranked according to docking score *i.e.* estimated free energy of binding and free energy of binding of ligand was in the range -8.63 to -10.48 (Histamine H1 receptor, Fig. 3) and -7.53 to -8.45 (glucosamine-6-phosphate synthase, Fig. 4). Free energy of binding of ligands was in good agreement with wet lab biological activities. Top ranked compounds were also found active in lab experiments. Protein ligand analysis also showed strong interactions with target protein and had four hydrogen bond interaction with different residues (Tyr 108, Ser 111, Tyr 431 and Tyr 458) in H1 receptor site, whereas, six hydrogen bond interaction with different residues (Ser 303, Ser 347, Thr 352, Thr 355 and Glu 488) in active site of antimicrobial and antifungal activity.

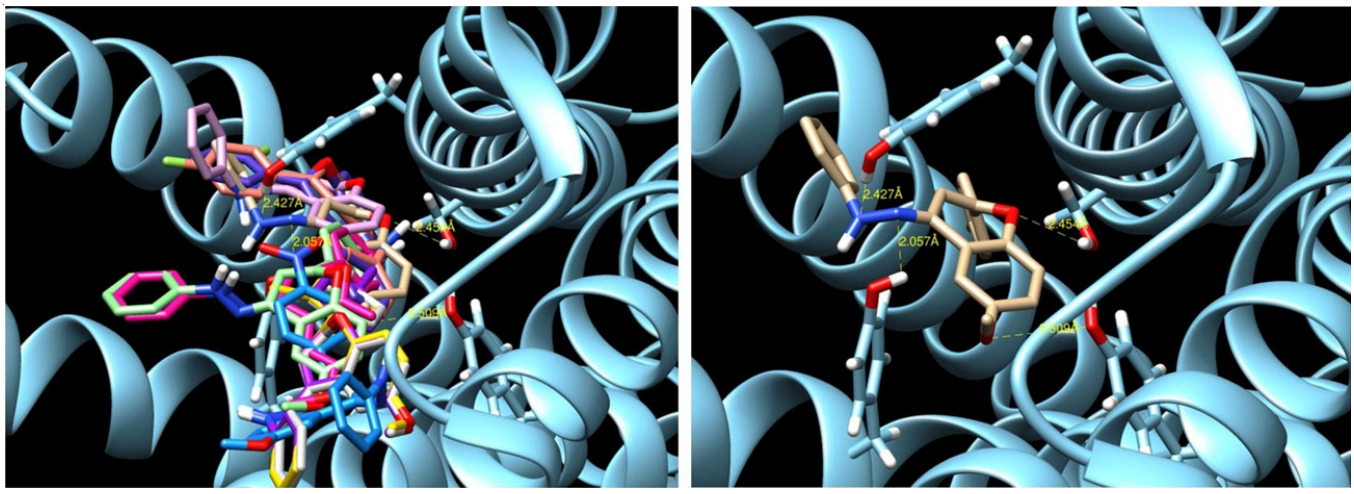
Conclusion

It is concluded that synthesized 4-iminoflavones **5(a-j)** showed good potential against asthma and microbial strains.

TABLE-6
ANTIMICROBIAL ACTIVITY AND DOCK SCORE OF THE SYNTHESIZED COMPOUNDS **5(a-j)**

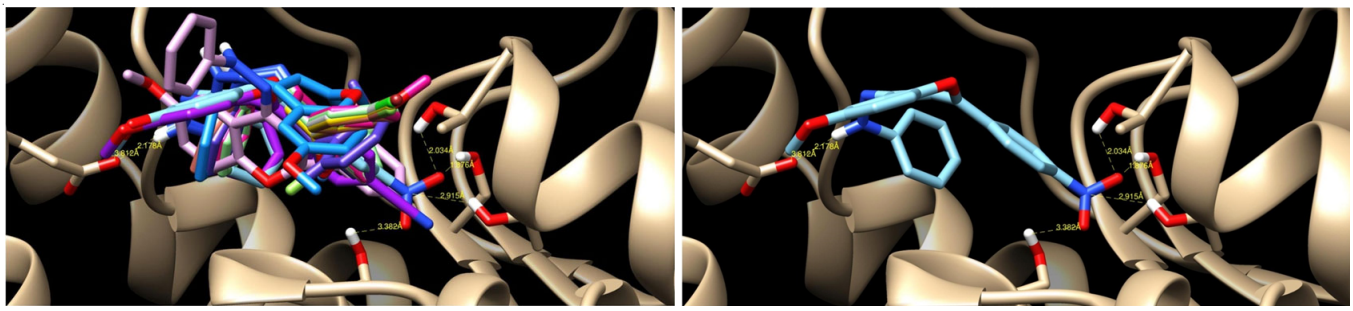
Compound	Dock score	*B. subtilis*	*S. aureus*	*E. coli*	*S. typhi*	*C. albicans*
5a	-7.57	–	–	100.00	50.00	–
5b	-7.86	50.00	–	25.00	50.00	–
5c	-7.53	6.25	12.50	12.50	6.25	3.10
5d	-7.81	25.00	25.00	50.00	25.00	6.25
5e	-7.62	50.00	–	50.00	50.00	25.00
5f	-7.86	25.00	25.00	12.50	25.00	50.00
5g	-7.90	25.00	–	6.25	12.50	–
5h	-8.47	6.25	6.25	3.10	1.55	6.25
5i	-7.84	–	–	50.00	100.00	–
5j	-7.96	12.50	6.25	3.10	6.25	12.50
Imipenem	–	3.10	1.55	0.19	0.78	–
Amphoterecin B	–	–	–	–	–	1.55

(–) denotes no activity



(a) (b)

Fig. 3. Docking images (a) overlapping of all compounds **5(a-j)** within binding pocket of histamine receptor and (b) binding of the high ranking generated conformers for compound **5f**



(a) (b)

Fig. 4. Docking images (a) overlapping of all compounds **5(a-j)** within 3D structure of GlcN-6-P synthase and (b) binding of the high ranking generated conformers for compound **5h**

Different substitutions on the flavones' skeleton are responsible for the respective biological activity. Similarly, *p*-substituted compounds were found to be the active one with methyl (**5f**) and nitro (**5h**) groups. Docking study revealed the best fit compounds to be the most active compounds in wet laboratory experiments. The presented study exhibited that 4-imino-flavones could be due to the combined effect of the heteroatom with respect to the substitutions present in the ring system.

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