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4,8-Disubstituted 6-Methyl-3,4-Dihydroimidazo[1,5-B][1,2,4]Triazin-8H-One As Novel Mast Cell Degranulation Inhibitors

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ABSTRACT

The present research investigation was undertaken to evaluate the antiasthmatic activity of newly synthesized 4,8-disubstituted 6-methyl-3,4-dihydroimidazo[1,5-b][1,2,4]triazin-8H-one in experimental animals. The presented chemical compounds were synthesized using Erlenmeyer-Azlactone synthesis and condensation reactions starting from various aromatic aldehydes. Synthesis of compounds was confirmed by melting point, HPTLC, FT-IR, ^{1}H NMR, ^{13}C NMR and LC-MS. Synthesized compounds were subjected to *in vivo* acute toxicity studies and *in vitro* mast cell degranulation studies using 48/80 as degranulating agent. Out of seven tested compounds, three compounds viz 5e, 5f and 5g were significantly inhibited mast cell degranulation and are found to be potent antiasthmatic agents. Out of these three compounds, most promising compound was 5e with average % mast cell degranulation of 11.33±0.2 ($p<0.01$).

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INTRODUCTION

Asthma is the chronic disorder which tends to increase in both prevalence and severity. It is a disease of airway characterized by variable and recurring symptoms, reverse airflow obstruction and bronchospasm. Symptoms include wheezing, coughing and chest tightness [1]. Mast cells are activated by allergens and release mediators (histamine, leukotrienes, prostaglandins) that result in bronchoconstriction of airway [2]. Common laboratory evaluation of antiasthmatic activity includes histamine induced contraction of smooth muscle, histamine release from mast cell, antigen-antibody reaction and mast cell degranulation method [3]. Degranulation is a cellular process that releases antimicrobial cytotoxic molecules from secretory vesicles called granules found inside some cells. It is used by several different cells involved in the immune system, including granulocytes (neutrophils, basophils and eosinophils) and mast cells. It is also used by certain lymphocytes such as natural killer (NK) cells and cytotoxic T cells, whose main purpose is to destroy invading microorganisms. Antigens interact with IgE molecules already bound to high affinity Fc receptors on the surface of mast cells to induce degranulation. The mast cell releases a mixture of compounds, including histamine, proteoglycans, serotonin, and serine proteases from its cytoplasmic granules and that mainly provokes narrowing and inflammation of bronchial airways and subsequently asthma [4]. So, in that direction it was planned to inhibit the mast cell degranulation using newly reported compounds which will prevent further consequences and development of asthma.

MATERIALS AND METHODS

All reagents were used as purchased from E. Merck and used without further purification. Melting points were determined by using a Remi digital melting point determination apparatus and are uncorrected. Purity of compounds were checked by High Performance Thin Layer Chromatography (HPTLC) and was performed on CAMAG twin with applicator Linomat-IV and plate specifications are Merck precoated silica gel 60 F_{254} with 0.2 mm thickness. Spectroscopic data were recorded by using FT-IR (Shimadzu spectrophotometer 8400 using KBr), ^{1}H NMR (Varian Mercury 400, Model- Unity AS400, serial-S0121719, frequency 400 MHz using DMSO as a solvent and tetramethylsilane (TMS) as an internal standard and chemical shifts were expressed as δ values in ppm), ^{13}C NMR (INOVA-300 with 75 MHz frequency DMSO as a solvent and tetramethylsilane (TMS) as an internal standard), LC-MS

(Bench top Agilent 1100 series LC–MSD (Agilent Technologies, Waldbronn, Germany), Column: C18, preparation on ODS (octadecylsilica) Hypersil column (Agilent Technologies), Flow-rate was 0.25 mL min^{-1} to 0.50 mL min^{-1}). Antiasthmatic activity was evaluated using mast cell stabilization method.

Route of synthesis

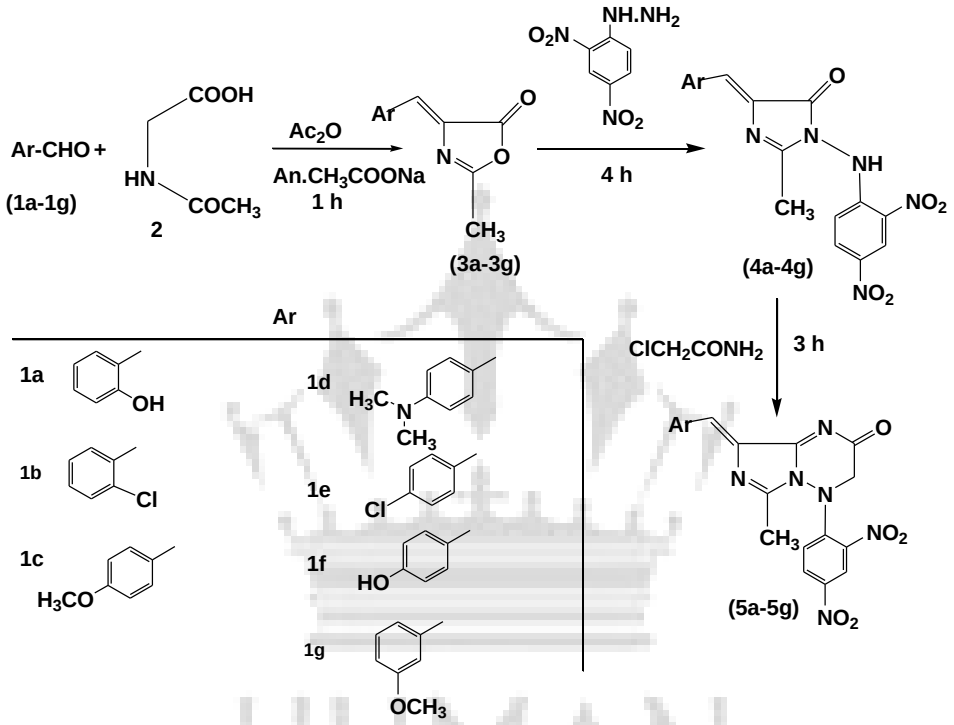


Figure 1: Route of synthesis

General procedure for synthesis of compounds [3a-3g] by Erlenmeyer-Azlactone synthesis[5]

Warm a mixture of 29 g (0.25 mol) of *N*-acetylglycine, 37.5 ml (0.37 mol) of aromatic aldehydes, 1a-1g, 15 g (0.183 mol) of anhydrous sodium acetate and 59 mL (0.62 mol) of acetic anhydride in 500 mL flask equipped with a reflux condenser, on water bath with occasional shaking until solution is complete (10-20 min). Boil the resulting solution for 1 h, cool and leave in a refrigerator overnight. Stir the solid mass of yellow crystals with 60 mL of cold water, transfer to a Buchner funnel and wash well with cold water. Wash with a little ether. Crystallized from carbon tetrachloride and used for next step of synthesis. The properties of compounds are given below.

4-(2-hydroxybenzylidene)-2-methyloxazol-5(4H)-one [3a]

Mol. Form. $C_{11}H_9NO_3$ (203.19); Ar = (-C_6H_5-*o*-OH); mp 305-307 ºC; yield: 89%; IR (KBr, Vmax, cm $^{-1}$): 855 (C-H bend), 1294 (C-O str), 1355 (C-N str), 1520 (C=C str), 1605 (C=N str), 1671 (C=O str), 2970 (CH_3 str), 3041 (C-H str), 3324 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 1.42 (s, 1H, CH_3), 6.68 (s, 1H, CH), 6.71 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.97 (t, 1H, ArH), 7.13 (d, 1H, ArH), 11.32 (s, 1H, OH).

4-(2-chlorobenzylidene)-2-methyloxazol-5(4H)-one [3b]

Mol. Form. $C_{11}H_8ClNO_2$ (221.63); Ar = (-C_6H_5-*o*-Cl); mp 85-88 ºC; yield: 95%; IR (KBr, Vmax, cm $^{-1}$): 736 (C-Cl str), 888 (C-H bend), 1296 (C-N str), 1519 (C=C str), 1632 (C=N str), 1706 (C=O str), 2951 (CH_3 str), 3057 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 7.08-7.25 (m, 4H, ArH), 7.91 (s, 1H, CH).

4-(4-methoxybenzylidene)-2-methyloxazol-5(4H)-one [3c]

Mol. Form. $C_{12}H_{11}NO_3$ (217.22); Ar = (-C_6H_5-*p*-OCH_3); mp 220-222 ºC; yield: 77%; IR (KBr, Vmax, cm $^{-1}$): 867 (C-H bend), 1298 (C-O str), 1305 (C-N str), 1547 (C=C str), 1625 (C=N str), 1667 (C=O str), 2979 (CH_3 str), 3067 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 1.87 (s, 3H, CH_3), 6.42 (s, 1H, CH), 6.77 (d, 2H, ArH), 6.84 (t, 1H, ArH), 7.14 (t, 2H, ArH).

4-[4-(dimethylamino)benzylidene]-2-methyloxazol-5(4H)-one [3d]

Mol. Form. $C_{13}H_{14}N_2O_2$ (230.26); Ar = [-C_6H_5-*p*-*N*-$(CH_3)_2$]; m.p 82-85 ºC; yield: 82%; IR (KBr, Vmax, cm $^{-1}$): 802 (C-H bend), 1263 (C=C str), 1544 (C=N str), 1637 (C=N str), 1759 (C=O str), 2967 (CH_3 str), 3077 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.25 (s, 1H, CH_3), 2.85 (s, 6H, 2 × CH_3), 6.58 (s, 1H, CH), 7.14 (d, 2H, ArH), 7.65 (d, 2H, ArH).

4-(4-chlorobenzylidene)-2-methyloxazol-5(4H)-one [3e]

Mol. Form. $C_{11}H_8ClNO_2$ (221.63); Ar = (-C_6H_5-*p*-Cl); mp175-176 ºC; yield: 76%; IR (KBr, Vmax, cm $^{-1}$): 763 (C-Cl str), 824 (C-H bend), 1313 (C-N str), 1585 (C=C str), 1650 (C=N str), 1790 (C=O str), 2979 (CH_3 str), 3086 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 7.22 (d, 2H, ArH), 7.23 (d, 2H, ArH), 7.63 (s, 1H, CH).

4-(4-hydroxybenzylidene)-2-methyloxazol-5(4H)-one [3f]

Mol. Form. $C_{11}H_9NO_3$ (203.19); Ar = (-C_6H_5-*p*-OH); mp 140-142 ºC; yield: 92%; IR (KBr,

Vmax, cm $^{-1}$): 863 (C-H bend), 1197 (C-O str), 1296 (C-N str), 1635 (C=C str), 1685 (C=N str), 1757 (C=O str), 2974 (CH_3 str), 3036 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.35 (s, 1H, CH_3), 5.01 (s, 1H, CH), 6.69 (d, 2H, ArH), 7.14 (d, 2H, ArH), 7.64 (s, 1H, OH).

4-(3-methoxybenzylidene)-2-methyloxazol-5(4H)-one [3g]

Mol. Form. $C_{12}H_{11}NO_3$ (221.63); Ar = (-C_6H_5-*m*-OCH_3); mp 195-197 ºC; yield: 88%; IR (KBr, Vmax, cm $^{-1}$): 881 (C-H bend), 1170 (C-O str), 1300 (C-N str), 1509 (C=C str), 1680 (C=N str), 1772 (C=O str), 2902 (CH_3 str), 3068 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz): 2.34 (s, 1H, CH_3), 3.73 (s, 1H, OCH_3), 6.65 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.10 (t, 1H, ArH), 7.64 (s, 1H, CH).

Typical procedure for synthesis of compounds [4a-4g]: A solution of **3a-3g** (6 mmole) in dry benzene (30 mL) and 2,4-dinitro phenylhydrazine (5 mmole) was heated under reflux for 4 h. Then the mixture was poured upon water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

1-(2,4-dinitrophenylamino)-4-(2-hydroxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4a]

Mol. Form. $C_{17}H_{13}N_5O_6$ (383.31); Ar = (-C_6H_5-*o*-OH); mp 190-193 ºC, yield: 67%; HPTLC: R_f 0.58, Toluene: ethyl acetate (8:2); IR (KBr, Vmax, cm $^{-1}$): 849 (C-H bend), 1261 (C-O str), 1324 (C-N str), 1672 (C=C str), 1672 (C=N str), 1763 (C=O str), 2966 (CH_3 str), 3041 (C-H str), 3476 (N-H str), 3623 (OH str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.54 (s, 3H, CH_3), 6.43 (s, 1H, CH), 6.64 (s, 2H, NH), 6.69 (d, 1H, ArH), 6.74 (t, 1H, ArH), 6.94 (t, 1H, ArH), 7.18 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.03 (s, 1H, ArH), 11.72 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.93, 40.87, 108.98, 113.93, 114.65, 119.45, 124.65, 127.35, 129.46, 130.76, 144.24, 149.67, 151.87, 166.13; LC-MS (m/z): 383.31 [M^++1].

1-(2,4-dinitrophenylamino)-4-(2-chlorobenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4b]

Mol. Form. $C_{17}H_{12}ClN_5O_5$ (401.76); Ar = (-C_6H_5-*o*-OH); mp 188-189 ºC, yield: 79%; HPTLC: R_f 0.76, Toluene: ethyl acetate (8:2); IR (KBr, Vmax, cm $^{-1}$): 624 (C-Cl str), 824 (C-H bend), 1345 (C-N str), 1525 (C=C str), 1645 (C=N str), 1749 (C=O str), 2969 (CH_3 str), 3067 (C-H str), 3398 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.32 (s, 3H, CH_3), 7.08 (t, 1H, ArH), 7.09 (t, 1H, ArH), 7.18 (d, 1H, ArH), 7.22 (d, 1H, ArH), 7.24 (d, 1H, ArH), 7.82 (s, 1H, CH),

8.50 (d, 1H, ArH), 9.04 (s, 1H, ArH), 9.14 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.97, 56.29, 108.47, 122.45, 113.78, 116.87, 119.15, 120.87, 128.45, 129.93, 131.47, 144.72, 145.83, 151.54, 152.09, 166.34; LC-MS (m/z): 401.76 [M^++1].

1-(2,4-dinitrophenylamino)-4-(4-methoxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4c]

Mol. Form. $C_{18}H_{15}N_5O_6$(397.34); **Ar** = (-C_6H_5-*p*-OCH_3); mp 223-224 ºC, **yield:** 53%; **HPTLC:** R_f0.62,Toluene: ethyl acetate (7:3); **IR (KBr, Vmax, cm^{-1}):** 887 (C-H bend), 1239 (C-O str), 1387 (C-N str),1612 (C=C str), 1627 (C=N str), 1745 (C=O str), 2971 (CH_3 str), 3067 (C-H str), 3410 (N-H str); ^{1}H **NMR(δ, ppm, DMSO-*d*6, 400 MHz) :** 2.36 (s, 3H, CH_3), 6.31 (s, 1H, CH), 6.76 (d, 2H, ArH), 6.81 (s, 1H,NH), 6.82 (t, 1H, ArH), 7.14 (t, 2H, ArH), 7.23 (d, 1H, ArH), 8.52 (d, 1H, ArH), 9.04 (s, 1H, ArH); **^{13}CNMR (δ, ppm, DMSO-*d*6, 75 MHz) :** 21.31, 108.55, 113.31, 115.86, 116.12, 119.21, 121.23, 127.81,129.65, 130.61, 144.72, 151.81, 158.91, 166.13; **LC-MS (m/z):** 397.34 [M^++1].

1-(2,4-dinitrophenylamino)-4-(4-(dimethylamino)benzylidene)-2-methyl-1H-imidazol-5(4H)-one [4d]

Mol. Form. $C_{19}H_{18}N_6O_5$ (410.38); Ar = [-C_6H_5-*p*-*N*-$(CH_3)_2$]; mp 87-89 ºC, yield: 59%; HPTLC: R_f 0.72, Toluene: ethyl acetate (9:1); IR (KBr, Vmax, cm $^{-1}$): 861 (C-H bend), 1306 (C-N str), 1548 (C=C str), 1635 (C=N str), 1764 (C=O str), 2979 (CH_3 str), 3031 (C-H str), 3410 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.39 (s, 3H, CH_3), 2.88 (s, 6H, 2 × CH_3), 6.19 (s, 1H, NH), 6.34 (s, 1H, CH), 6.55 (d, 2H, ArH), 7.11 (d, 2H, ArH), 7.18 (d, 1H, ArH), 8.64 (d, 1H, ArH), 9.02 (d, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.76, 108.87, 113.24, 119.35, 127.89, 128.78, 129.65, 130.76, 133.23, 134.21, 144.87, 151.09, 166.12; LC-MS (m/z): 410.38 [M^++1].

1-(2,4-dinitrophenylamino)-4-(4-chlorobenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4e]

Mol. Form. $C_{17}H_{12}ClN_5O_5$ (401.76); Ar = (-C_6H_5-*o*-OH); mp 140-141 ºC, yield: 64%; HPTLC: R_f 0.73, Toluene: ethyl acetate (8:2); IR (KBr, Vmax, cm $^{-1}$): 768 (C-Cl str), 813 (C-H bend), 1276 (C-N str), 1517 (C=C str), 1653 (C=N str), 1760 (C=O str), 2961 (CH_3 str), 3067 (C-H str), 3491 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 7.18 (d, 1H, ArH), 7.23 (d, 1H, ArH), 7.24 (d, 2H, ArH), 7.56 (d, 1H, CH), 8.21 (s, 1H, NH), 8.53 (s, 1H, ArH),

9.03 (d, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.11, 108.27, 113.24, 115.54, 119.87, 127.83, 129.32, 130.27, 144.97, 152.09, 157.98, 166.85; LC-MS (m/z): 401.76 [M^++1].

1-(2,4-dinitrophenylamino)-4-(4-hydroxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4f]

Mol. Form. $C_{17}H_{13}N_5O_6$ (383.51); Ar = (-C_6H_5-*o*-OH); mp 145-147 ºC, yield: 69%; HPTLC: R_f 0.65, Toluene: ethyl acetate (6:4); IR (KBr, Vmax, cm $^{-1}$): 855 (C-H bend), 1234 (C-O str), 1323 (C-N str), 1561 (C=C str), 1654 (C=N str), 1731 (C=O str), 2962 (CH_3 str), 3047 (C-H str), 3431 (N-H str), 3626 (OH str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 5.02 (s, 1H, OH), 6.66 (d, 2H, ArH), 7.13 (d, 2H, ArH), 7.18 (d, 1H, ArH), 7.56 (s, 1H, CH), 8.51 (d, 1H, ArH), 9.04 (s, 1H, ArH), 9.21 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.38, 108.76, 113.54, 119.54, 126.87, 127.34, 128.65, 129.65, 130.54, 131.24, 133.76, 144.87, 151.14, 164.20; LC-MS (m/z): 383.51 [M^++1].

1-(2,4-dinitrophenylamino)-4-(3-methoxybenzylidene)-2-methyl-1H-imidazol-5(4H)-one [4g]

Mol. Form. $C_{18}H_{15}N_5O_6$ (397.34); Ar = (-C_6H_5-*o*-OH); mp 90-91ºC, yield: 57%; HPTLC: R_f 0.73, Toluene: ethyl acetate (7:3); IR (KBr, Vmax, cm $^{-1}$): 842 (C-H bend), 1167 (C-O str), 1323 (C-N str), 1534 (C=C str), 1624 (C=N str), 1742 (C=O str), 2965 (CH_3 str), 3097 (C-H str), 3454 (N-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 6.65 (d, 1H, ArH), 6.81 (s, 1H, ArH), 6.86 (d, 1H, ArH), 7.11 (t, 1H, ArH), 7.19 (d, 1H, ArH), 7.45 (s, 1H, CH), 8.50 (d, 1H, ArH), 9.05 (s, 1H, ArH), 9.12 (s, 1H, NH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.87, 55.09, 108.04, 112.67, 113.02, 113.85, 118.45, 119.54, 129.63, 129.73, 130.40, 137.54, 144.76, 161.89, 166.43; LC-MS (m/z): 397.34 [M^++1].

Typical procedure for synthesis of compounds [5a-5g]: A solution of **4a-4g** (8 mmole) and chloroacetamide (8 mmole) was refluxed for 3 h in boiling *N*, *N*-dimethylformamide (30 mL). Then the mixture was poured into water. The precipitated solid was filtered off, dried and crystallized from ethanol to get the desired compounds.

8-(2-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5a]

Mol. Form. $C_{19}H_{14}N_6O_6$(422.35); Ar = (-C_6H_5-*o*-OH); mp 82-83 ºC, yield: 89%; HPTLC:R_f 0.77, Toluene: ethyl acetate (9:1); IR (KBr, Vmax, cm $^{-1}$): 861 (C-H bend), 1274 (C-O str), 1372 (C-N

str), 1561 (C=C str), 1692 (C=N str), 1762 (C=O str), 2837 (=CH_2 str, sym), 2915 (=CH_2 str, asym), 2972 (CH_3 str), 3061 (C-H str), 3634 (OH str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.18 (s, 2H, CH_2), 6.67 (d, 1H, ArH), 6.77 (t, 1H, ArH), 6.81 (s, 1H, CH), 6.92 (t, 1H, , ArH), 7.17 (d, 1H, ArH), 7.21 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.04 (s, 1H, ArH), 11.79 (s, 1H, OH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.65, 61.44, 102.75, 116.98, 117.54, 119.21, 121.24, 127.11, 127.89, 129.88, 132.65, 139.98, 143.23, 144.28, 158.87, 166.43, 164.56, 200.45; LC-MS (m/z): 422.35 [M^++1].

8-(2-chlorobenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5b]

Mol. Form. $C_{19}H_{13}ClN_6O_5$(440.79); Ar = (-C_6H_5-*o*-OH); mp 199-201 ºC, yield: 62%;HPTLC: R_f 0.79, Toluene: ethyl acetate (6:4); IR (KBr, Vmax, cm $^{-1}$): 624 (C-Cl str), 823 (C-H bend), 1323 (C-N str), 1545 (C=C str), 1623 (C=N str), 1751 (C=O str), 2848 (=CH_2 str, sym), 2915 (=CH_2 str, asym), 2974 (CH_3 str), 3088 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.86 (s, 1H, CH), 7.08 (t, 1H, ArH), 7.09 (t, 1H, ArH), 7.18 (d, 1H, ArH), 7.22 (d, 1H, ArH), 7.24 (d, 1H, ArH), 8.50 (d, 1H, ArH), 9.04 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 20.16, 61.46, 102.62, 115.98, 119.62, 125.27, 126.87, 127.65, 128.45, 129.76, 130.00, 131.26, 133.27, 139.45, 143.54, 144.87, 164.28, 200.18; LC-MS (m/z): 440.79 [M^++1].

8-(4-methoxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5c]

Mol. Form. $C_{20}H_{16}N_6O_6$(436.37); Ar = (-C_6H_5-*p*-OCH_3); mp 188-189 ºC, yield: 61%;HPTLC: R_f 0.81, Toluene: ethyl acetate (9:1); IR (KBr, Vmax, cm $^{-1}$): 884 (C-H bend), 1231 (C-O str), 1376 (C-N str), 1616 (C=C str), 1644 (C=N str), 1753 (C=O str), 2915 (=CH_2 str, sym), 2951 (=CH_2 str, asym), 2979 (CH_3 str), 3057 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.57 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.66 (s, 1H, CH), 6.73 (d, 2H, ArH), 6.82 (t, 1H, ArH), 7.11 (t, 2H, ArH), 7.22 (d, 1H, ArH), 8.51 (d, 1H, ArH), 9.03 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.03, 55.00, 62.04, 110.00, 117.98, 118.95, 119.98, 123.00, 129.05, 130.98, 131.00, 144.81, 151.87, 155.98, 164.92, 200.09; LC-MS (m/z): 436.37 [M^++1].

8-(4-(dimethylamino)benzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4] triazin-2(8H)-one [5d]

Mol. Form. $C_{21}H_{19}N_7O_5$(449.41); Ar = (-C_6H_5-*o*-OH); mp 210-211 ºC, yield:56%; HPTLC: R_f 0.58, Toluene: ethyl acetate (6:4); IR (KBr, Vmax, cm $^{-1}$): 856 (C-H bend), 1301 (C-N str), 1542 (C=C str), 1628 (C=N str), 1719 (C=O str), 2850 (=CH_2 str, sym), 2917 (=CH_2 str, asym), 2959 (CH_3 str), 3090 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.74 (s, 3H, CH_3), 2.85 (s, 6H, 2 × CH_3), 4.17 (s, 2H, CH_2), 6.20 (d, 2H, ArH), 6.56 (s, 1H, CH), 7.16 (d, 2H, ArH), 7.19 (d, 2H, ArH), 8.52 (d, 1H, ArH), 9.05 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.98, 40.78, 61.93, 102.65, 114.34, 115.12, 119.24, 124.71, 127.35, 127.65, 127.70, 132.89, 139.13, 143.68, 144.87, 148.95, 164.23, 200.14; LC-MS (m/z): 449.41 [M^++1].

8-(4-chlorobenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5e]

Mol. Form. $C_{19}H_{13}ClN_6O_5$(440.79); Ar = (-C_6H_5-*o*-OH); mp 120-122 ºC, yield: 66%;HPTLC: R_f 0.61, Toluene: ethyl acetate (8:2); IR (KBr, Vmax, cm $^{-1}$): 756 (C-Cl str), 816 (C-H bend), 1265 (C-N str), 1529 (C=C str), 1662 (C=N str), 1765 (C=O str), 2845 (=CH_2 str, sym), 2931 (=CH_2 str, asym), 2967 (CH_3 str), 3087 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.34 (s, 3H, CH_3), 4.19 (s, 2H, CH_2), 6.63 (s, 1H, CH_3), 7.18 (d, 1H, ArH), 7.22 (d, 2H, ArH), 7.23 (d, 2H, ArH), 8.52 (d, 1H, ArH), 9.03 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.39, 61.98, 102.76, 115.14, 119.23, 127.00, 127.89, 128.12, 129.23, 132.87, 133.76, 134.23, 139.27, 143.98, 144.98, 164.23, 200.12; LC-MS (m/z): 440.79 [M^++1].

8-(4-hydroxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5f]

Mol. Form. $C_{18}H_{14}N_6O_6$(422.35); Ar = (-C_6H_5-*o*-OH); mp 155-157 ºC, yield: 88%;HPTLC: R_f 0.66, Toluene: ethyl acetate (9:1); IR (KBr, Vmax, cm $^{-1}$): 845 (C-H bend), 1245 (C-O str), 1334 (C-N str), 1554 (C=C str), 1632 (C=N str), 1747 (C=O str), 2839 (=CH_2 str, sym), 2928 (=CH_2 str, asym), 2977 (CH_3 str), 3141 (C-H str), 3625 (O-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 4.17 (s, 2H, CH_2), 5.02 (s, 1H, OH), 6.60 (s, 1H, CH), 6.66 (d, 2H, ArH), 7.13 (d, 2H, ArH), 7.19 (d, 1H, ArH), 8.50 (d, 1H, ArH), 9.06 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.47, 61.23, 102.76, 115.28, 116.23, 119.60, 127.00, 127.98, 128.12, 132.76, 139.98, 143.23, 144.26, 156.98, 164.92, 200.16; LC-MS (m/z): 422.35 [M^++1].

8-(3-methoxybenzylidene)-3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-b][1,2,4]triazin-2(8H)-one [5g]

Mol. Form. $C_{20}H_{16}N_6O_6$(436.37); Ar = (-C_6H_5-*o*-OH); mp 105-108 ºC, yield: 77%;HPTLC: R_f 0.73, Toluene: ethyl acetate (7:3); IR (KBr, Vmax, cm $^{-1}$): 843 (C-H bend), 1133 (C-O str), 1375 (C-N str), 1512 (C=C str), 1645 (C=N str), 1734 (C=O str), 2848 (=CH_2 str, sym), 2925 (=CH_2 str, asym), 2971 (CH_3 str), 3069 (C-H str); ^{1}H NMR (δ, ppm, DMSO-*d*6, 400 MHz) : 2.35 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.17 (s, 2H, CH_2), 6.60 (s, 1H, CH), 6.66 (s, 1H, ArH), 6.81 (d, 1H, ArH), 6.86 (d, 1H, ArH), 7.11 (t, 1H, ArH), 7.19 (d, 1H, ArH), 8.51 (d,1H, ArH), 9.05 (s, 1H, ArH); ^{13}C NMR (δ, ppm, DMSO-*d*6, 75 MHz) : 21.98, 55.90, 61.86, 102.56, 110.85, 113.56, 115.76, 118.76, 119.54, 127.65, 128.05, 131.56, 134.87, 138.87, 141.65, 143.54, 144.12, 161.45, 165.25, 200.65; LC-MS (m/z): 436.37 [M^++1].

Acute Oral Toxicity Studies

These studies were carried out according to the guidelines number 423 prescribed by Organization for Economic Co-operation and Development (OECD). Protocols were sanctioned by IAEC with number CPCSEA/IAEC/INV/31/2013. A dose of 2000 mg/kg will be given orally to overnight fasted animals and observed for signs of toxicity such as hyperactivity, grooming, convulsions, sedation and hyperthermia continuously for 2 h and for mortality up to 24 h after administration of the doses [6].

Biological Evaluation by Mast Cell Stabilization [7]

Healthy albino Wistar rats (150-250 g) of either sex were divided into 7 groups each containing 06 animals. On first day of sensitization, all animals from each group were injected with compound 48/80 (1 mg/kg s.c). Standard (Ketotifen 10 µg/mL) and test compounds (solution of synthesized compounds 10 µg/mL) were given for 15 days. On 15th day; 2 hours after the assigned treatment; mast cells were collected from peritoneal cavity. 10 ml of normal saline solution was injected into peritoneal cavity and abdomen was gently messaged for 90 sec. The peritoneal cavity was carefully opened and the fluid containing mast cell was aspirated and collected in siliconised test tube containing 7 to 10 ml of RPMI-1640 medium pH 7.2 -7.4 . The mast cells were then washed thrice by centrifugation and pellets of mast cell were collected in the RPMI-1640 medium. The mast cell suspension stained with 1 % toludine blue and observed under microscope 45x. Total 100 cells were counted from different visual areas. The numbers of

intact and degranulated cells were counted and % protection was calculated. =1 % carboxymethyl cellulose (1 ml/kg p.o.) was used as control.

Statistical Analysis

Data was expressed as Mean± S.E.M. compared with Control (ANOVA followed by Dunnett's test) and $^{**}p<0.01$ were considered as statistically significant (n= 6).

RESULTS

Acute Oral Toxicity Studies

No mortality and the sign of toxicity were observed at the dose of 2000 mg/kg. Hence, 1/10th (200 mg /kg) of this dose was selected for pharmacological evaluation.

Biological evaluation by mast cell stabilization

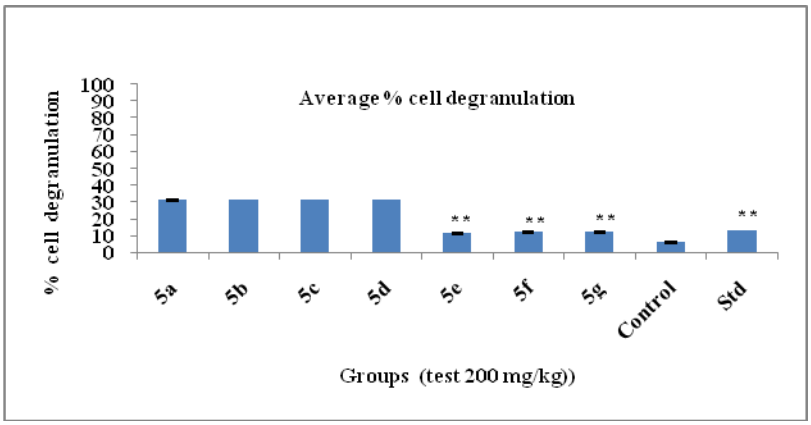
The results for % mast cell and average % mast cell degranulation with SEM are indicated in Table 1 and 2 respectively.

Table 1: % mast cell degranulation (n=6)

Compound code	5a	5b	5c	5d	5e	5f	5g	Control	Std
% mast cell degranulation	31.12	31.02	31.04	31.23	10.65	11.09	13	33.25	13.28
	31.06	31.02	31.1	31.04	11.32	14.65	12.87	33.1	13.43
	31.02	31.06	31.18	31.09	12.34	13.43	14.84	33.28	13.08
	31.02	31.08	31.12	31.31	11.61	12.34	13.34	32.75	13.24
	32.01	31.06	32.01	32.03	10.98	13.12	12.45	32.6	13.92
	31.08	31.48	32.02	31.04	11.23	10.32	11.9	33.01	13.63

Table 2: Average % mast cell degranulation

	5a	5b	5c	5d	5e	5f	5g	Control	Std
Mean	31.19	31.17	31.49	31.25	11.33	12.09	12.28	6	13.45
SEM	0.13	0.08	0.18	0.13	0.2	0.63	0.38	0.33	0.13



Graphical presentation for Table 2

DISCUSSION

All compounds including intermediate were characterized by physicochemical parameters such as MP, HPTLC (Rf value) and spectroscopic methods like IR, HNMR, C-NMR, LC-MS etc and all compounds showed best correlation with the same. From Table 2, it is clear that compounds 5e and 5f were found to be most active antiasthmatic compounds as their average % mast cell degranulation was 11.33±0.2 and 12.09±0.63 and this may be may be because of presence of strong electron withdrawing group like chloro and hydroxy at para positions.

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