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Molecular docking and synthesis of 8-substituted 3,4-dihydro-6-methyl-4-(2,4-dinitrophenyl)imidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one derivatives as novel antiasthmatic agents

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ABSTRACT

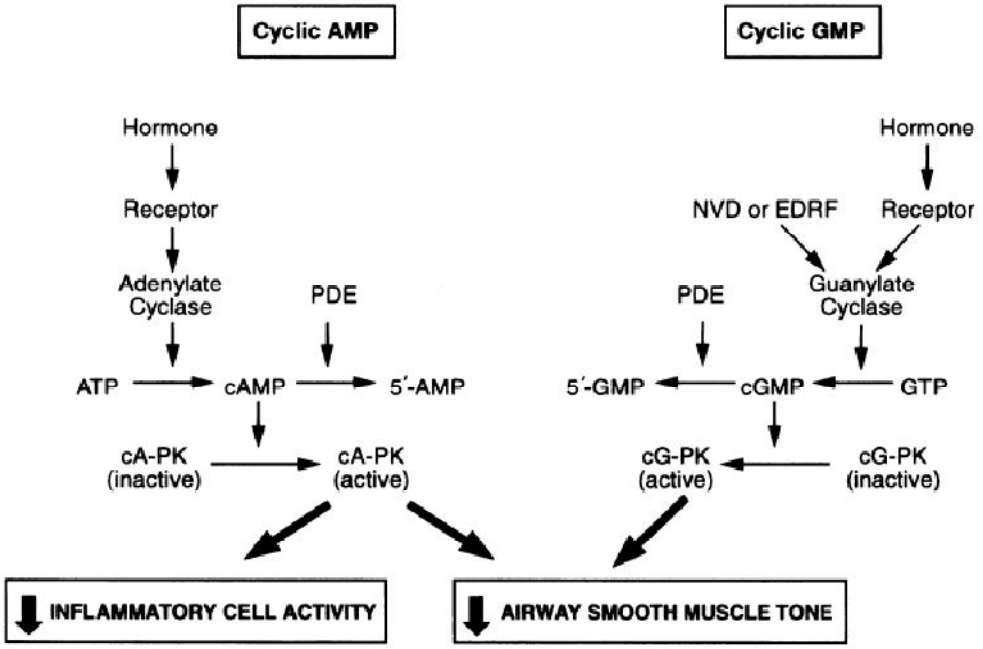
In the last few years there has been a growing interest for the therapeutic applications of phosphodiesterase4 (PDE4) inhibitors. PDE4 inhibitors are currently under development for the treatment of respiratory diseases including asthma and COPD. The rationale for the development of this drug class stems from our understanding of the role of PDE4 in suppressing the function of a range of inflammatory and resident cells thought to contribute toward the pathogenesis of these diseases. Similarly, numerous preclinical in vivo studies have shown that PDE4 inhibitors suppress characteristic features of these diseases, namely, cell recruitment, activation of inflammatory cells and physiological changes in lung function in response to a range of insults to the airways. The ability of the PDE4 inhibitors to relax airway smooth muscle on one hand and to suppress the function of a range of inflammatory cells on the other hand, led to concentration of the research efforts on drugs of this class. We synthesized new class of PDE4 inhibitors, 6-methyl-3,4-dihydroimidazo[1,5-b][1,2,4]triazin-2(8H)-one structurally unrelated to rolipram as promising agents for treatment of asthma and investigated antiasthmatic activity using guinea pig tracheal chain method. Results shows that synthesized compounds have prominent antiasthmatic activity.

Keywords: Phosphodiesterase4, Asthma, Imidazole[1,5-*b*][1,2,4]triazine, Guinea pig trachea.

INTRODUCTION

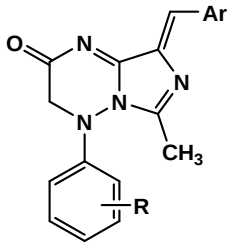
PDE4 catalyses the hydrolysis of cyclic 3', 5' adenosine monophosphate (cAMP) to adenosine monophosphate (AMP), which terminates the downstream signaling of this second messenger. For PDE4, there are four gene families (A–D), although there is added complexity with over 20 splice variants. Hydrolysis of cyclic AMP is a common feature of this family, and it is clear that these isoforms can be targeted to different domains within the extracellular compartment and their activity differentially

regulated by kinases, suggesting that these isoforms have specific functions in the control of cellular activity. [1]

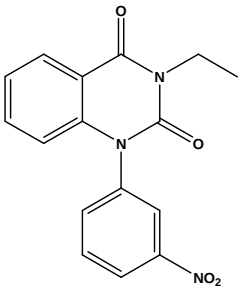


Role of cyclic nucleotides in the function of inflammatory cells and airway smooth muscle. See text for details. PDE—phosphodiesterase; cA-PK—cAMP dependent protein kinase; cG-PK—cGMP dependent protein kinase; NVD—nitrovasodilators; EDRF—endothelium derived relaxant factor.

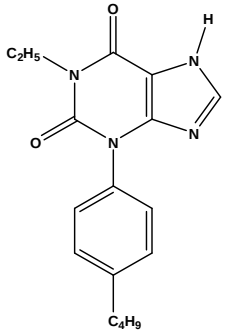
PDE-4 is termed the cAMP specific phosphodiesterase because its affinity for cAMP (K_m = 2 µmol L^{-1}) is much greater than its affinity for cGMP (K_m= 100, µmol L^{-1})[2]. Two main directions of research were followed in the field of PDE4 inhibitors: that of the manipulation of the rolipram structure, aimed to dissociate catalytic site inhibition and binding site affinity and the search for structurally different inhibitors. [3] Unfortunately, the development of pioneer PDE4 inhibitors, such as the archetypal Rolipram and Ariflo has been hampered by their propensity to induce various side effects, such as nausea, emesis, gastric acid secretion, or central nervous system activation. As a result, these compounds suffered from a limited therapeutic index. Thus, the design of novel, potent and selective second-generation PDE4 inhibitors with reduced emetogenic properties represents a critical need and is still a challenge in the pharmaceutical industry. It was initially proposed that rolipram and similar compounds produced side effects via the binding to a high affinity rolipram binding site (HARBS) distinct from the catalytic site of PDE4. It has now been clarified that the HARBS corresponds to the holoenzyme conformer of the PDE4, and that the emetic response is a consequence of inhibition of PDE4, especially the PDE4D, in non-target tissues. Therefore, one way to obtain potent selective PDE4 inhibitors with an improvement in the therapeutic index is to develop new compounds which is completely unrelated to catechol ether derivatives (e.g. rolipram) and the utility of the this approach can be utilized successfully. Many rolipram analogues, as well as other PDE4 inhibitors bind with HARBS [4]. These side effects are mainly due to stimulation of locus coeruleus (LC) that plays an important role in mediating neuronal emesis by firing action potential spontaneously, which leads to persistent inward of Ca^{2+} current [5]. But studies also support the proposal that, low affinity rolipram binding site (LARBS) also exist in and so rolipram unrelated compounds will binds to LARBS and it is noteworthy that the therapeutic effects appear to be related to LARBS binding, whereas the side effects are more likely related to HARBS binding [6,7]. Keeping in mind all above facts here we reported Molecular Docking, synthesis and in vitro evaluation of 6-methyl-3,4-dihydroimidazo[1,5-*b*][1,2,4]triazin-2(8*H*)-one derivatives as novel PDE4 inhibitors having following general structures which are structurally unrelated to rolipram and tested them for antiasthmatic activity.



Our novel compounds are related to quinazolinediones (e.g. nitraquazone, IC_{50}= 1.9 µM) [8] and xantines (e.g. arofylline, IC_{50}= 5.5 µM, significant improvement in pulmonary function with safe profile) [9] which are orally active selective PDE4 inhibitors, well absorbed and non-emetic / less emetic compounds [10].

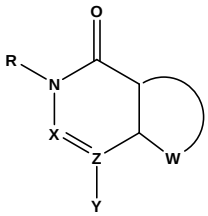


Nitroquazone

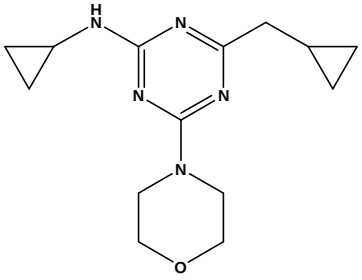


Arophylline

General structure of quinazolinediones is shown below.



According to pharmacophoric model proposed by Polymeropoulos et al. for PDE4 inhibitors, the unique structure is one in which X=N, CO, CH, Y= bulky alkyl or aryl, Z= N, C and W= N, CH. On this basis, our structure satisfies these SAR conditions as shown in above structure[11]. 2,4,6-trisubstituted triazines represent a structurally novel class of PDE4 inhibitors (IC_{50}= 150 nM) endowed with potent bronchodilatory effect, synthesized at UCB Pharma. This shows that triazine compounds can also produce PDE4 inhibition, we tried substituted 1,2,4-triazines [12].



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}). Molecular docking studies were done by using V Life MDS docking software.

Molecular docking studies : In order to rationalize the biological results, molecular docking studies was performed on the various compounds. The compounds were docked into PDE4D catalytic domain (PDB entry 3IAK). Molecular docking studies were carried out by using Grip method of V Life MDS docking software. V Life MDS consists of following modules: Engine, QSAR Plus, 3D QSAR, Chem. DBS, Lead Grow, Pro Viz, BioPredicta, and Mol Sign. Grip methods is used because it explores binding mode of ligand exhaustively over a grid in the cavity and hence can be used mainly when the cavity size is small. Stochastic method is used as Genetic Algorithm (GA) based method. V Life MDS provides an array of Scoring functions used are PLP score, XC score and Steric + Electrostatic score for evaluation of docked poses. The molecules were prepared using CHEM DRAW ULTRA 8.0. Molecules were then converted to 3D (i.e. mol format). Further optimization of molecules was done. Various parameters were set to perform optimization of batch of ligands are: Maximum no. of cycles: 1000, Convergence criteria (rams gradient): 0.1, Medium's dielectric constant: 1 (vacuo), Force Field: MMFF, Gradients Type: Analytical option and optimize the batch of ligands. Optimization of protein was done by using Phosphodiesterase4 (PBD Code: 3IAK) bound with rolipram, as reference ligand, obtained from PDB data bank was selected for the study. Rolipram was extracted from protein and used as reference ligand for docking. According to docking calculations, compounds could bind to the PDE4D catalytic site occupying the part of pocket where rolipram, the co-crystallized ligand, binds.

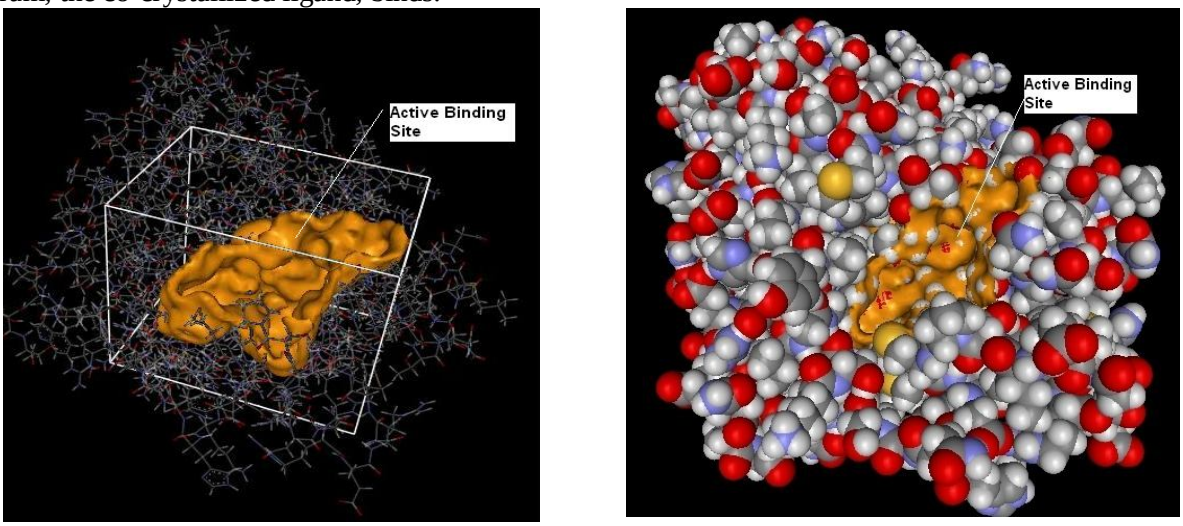


Figure 1. Molecular structure of the Phosphodiesterase4 (PDB: 3IAK) in line form and space fill form showing active binding site.

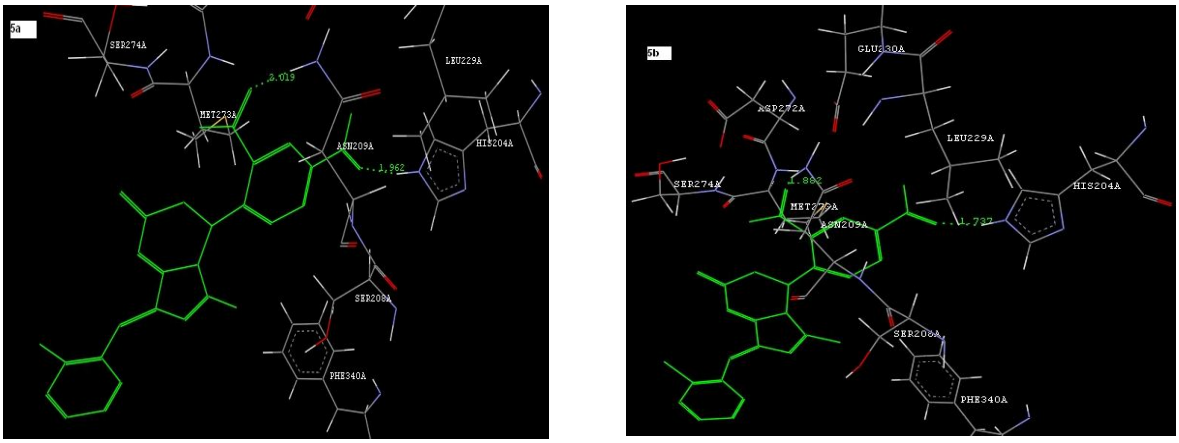


Figure 2. 3D image showing hydrogen bond interaction of compound 5a and 5b with 3IAK has docking score of -66.457858 and -59.973595 respectively.

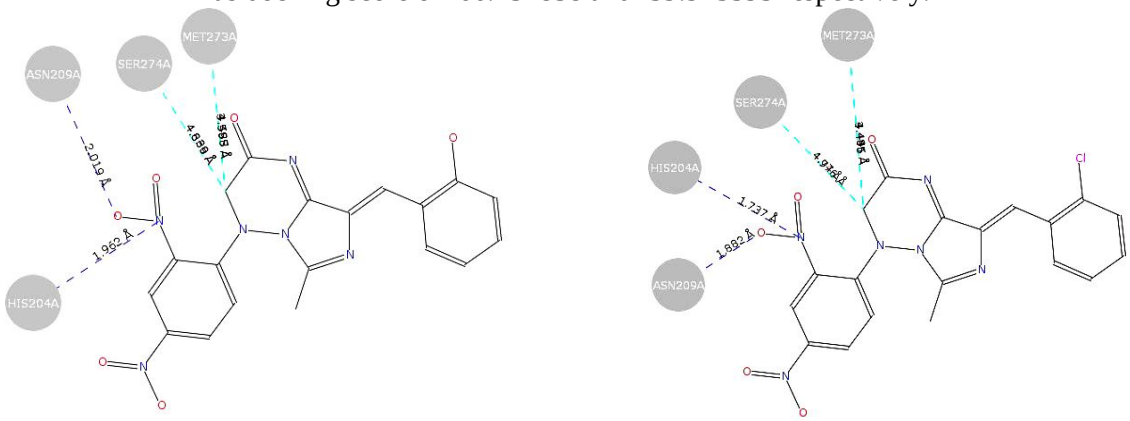


Figure 3. 2D image showing hydrogen bond interaction (blue dotted line) and hydrophobic interactions (green dotted line) of compound 5a and 5b with amino acid residues of PDE4, respectively.

Docking scores of synthesized compounds (PDB: 3IAK)

Placement	Score
5a_opt_P26	-64.158955
5b_opt_P16	-59.973595
5c_opt_P23	-58.757982
5d_opt_P17	-57.998927
5e_opt_P1	-57.937280
5f_opt_P10	-57.789557
Rolipram_opt_P29	-57.782489
5g_opt_LP2	-51.631286

Synthesis of Compounds: The scheme of synthesis is shown in fig4.

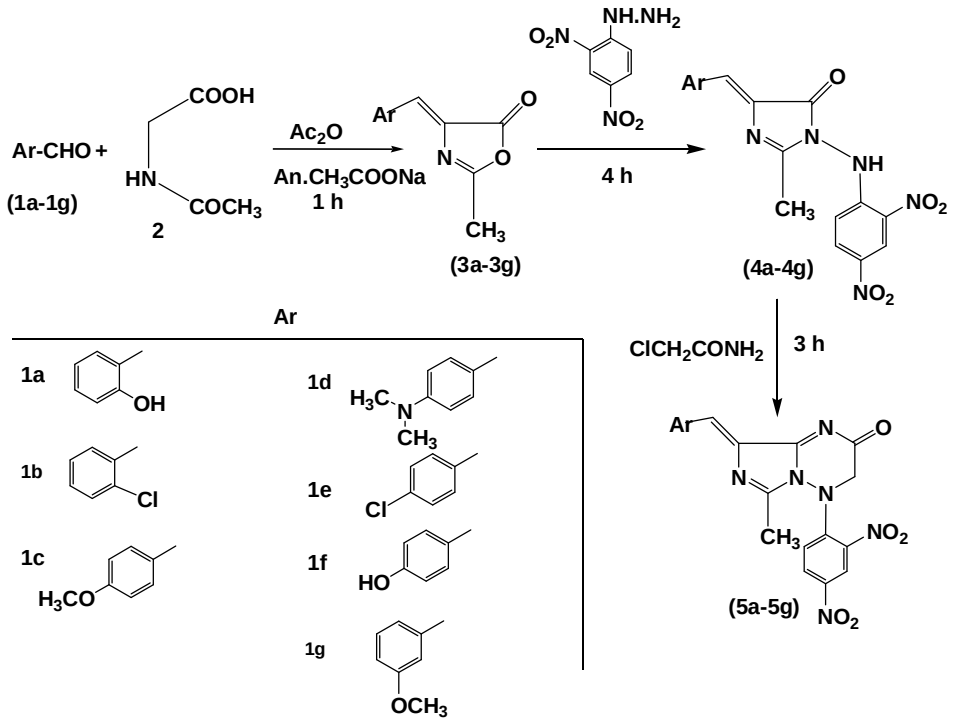


Figure 4: Scheme of synthesis.

General procedure for synthesis of compounds, 3a-3g by Erlenmeyer-Azlactone synthesis [13] : 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_f 0.62, 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}C NMR (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 chloro acetamide (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].

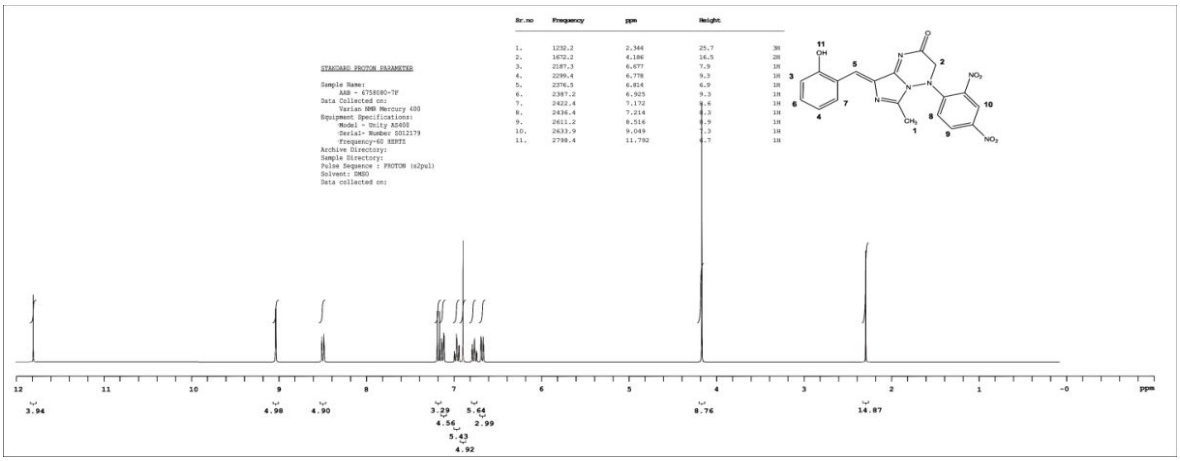


Figure 5: ^{1}H NMR of compound 5a

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].

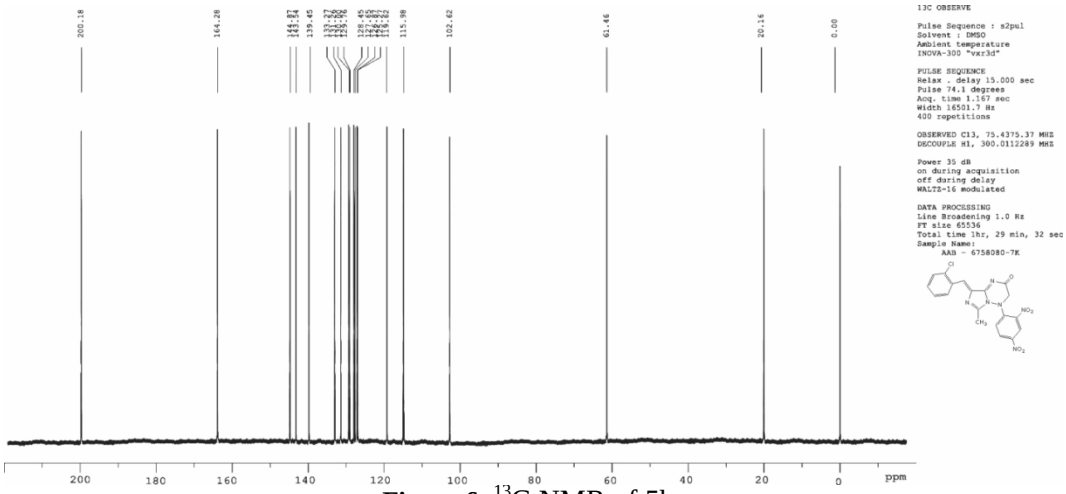


Figure 6: ^{13}C NMR of 5b.

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].

Antiasthmatic activity [14] : Guinea pig tracheal chain method was used to evaluate anti-asthmatic activity of synthesized compounds. The guinea pig tracheal tissue was obtained immediately after slaughter of animals. Pieces of trachea were collected in ice cold oxygenated Kreb's solution. Guinea pig 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, KH_2PO_4:1.2, $MgCl_2$:1.2, Glucose: 11.5) and maintained at 37±5° C. A stream of air was bubbled through the organ tube (1 bubble sec^{-1}). 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 g. 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 of 100 µg ml^{-1} of each compound was added to reservoir separately 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. Graph of percentage of maximum contractile response on ordinate and molar concentration of histamine abscissa was plotted to record dose response curve of histamine, in absence and in presence of synthesized derivatives.

Table 1. Effect of synthesized compounds (100 µg ml^{-1}) on histamine induced contraction of isolated guinea pig tracheal chain preparation. A=height in cm, B=% response.

Histamine		**Compound**		**Compound code and response of histamine on isolated tissue of guinea pig tracheal chain**									
ml	µg	ml	µg	**5a**		**5b**		**5c**		**5d**		**5e**	
				A	B	A	B	A	B	A	B	A	B
0.1	10	--	--	0.5	41.66	0.5	31.50	0.8	44.44	0.4	57.14	0.4	33.33
0.2	20	--	--	0.6	55.55	1.0	62.50	1.0	55.55	0.5	71.42	0.6	50.00
0.4	40	--	--	0.8	66.66	1.2	75.00	1.2	66.66	0.6	85.71	0.7	58.33
0.8	60	--	--	1.0	83.33	1.5	93.75	1.6	88.88	0.7	100.00	0.1	83.33
1.6	120	--	--	1.2	100.00	1.6	100.00	1.8	100.00	0.7	100.00	1.2	100.00
Dose 0.8 µg was selected for next set of experiment.													
0.8	80	0	0	1.0	100.00	1.5	100.00	1.0	0	0.7	0	1.6	100
0.8	80	0.1	10	0.5	44.44	1.0	66.66	0.5	50	0.4	50	0.9	56.25
0.8	80	0.2	20	0.4	33.33	0.7	46.66	0.4	40	0.38	40	0.8	50
0.8	80	0.4	40	0.3	22.22	0.5	33.30	0.39	39	0.3	39	0.7	43.75

0.8	80	0.8	60	0.2	11.11	0.1	6.66	0.35	35	0.2	35	0.5	31.25
0.8	80	1.6	120	0	0	0	0	0	0	0	0	0	0

Out of the seven synthesized compounds, compounds **5a-5e** were tested for their antiasthmatic activity.

Table 2. Percent inhibition of compounds at increasing doses.

Dose (µg ml$^{-1)}$	Compound code and % Inhibition				
	5a	**5b**	**5c**	**5d**	**5e**
10	55.56	33.34	50	42.86	43.75
20	66.67	53.34	60	45.72	50
40	77.78	66.67	61	57.15	56.25
80	88.89	93.34	65	71.43	68.75
100	100	100	100	100	100

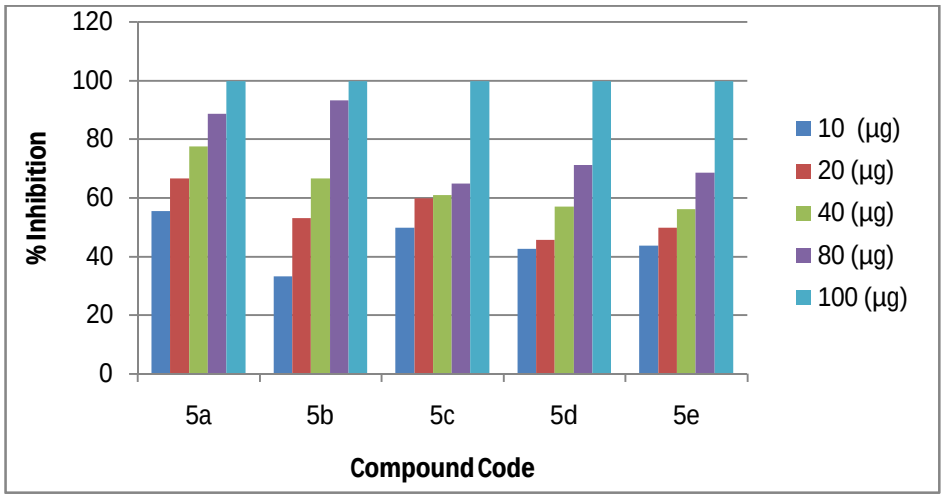


Figure 7: Graphical representation of % inhibition against concentration.
Final concentration of compound was 100 µg ml^{-1}**.**

RESULTS AND DISCUSSION

All synthesized compounds were evaluated using physical data such as melting point and R_f values as well as spectroscopic methods IR, ^{1}H NMR, ^{13}C NMR, LC-MS etc and showed best correlation with the same. These compounds were also evaluated for their antiasthmatic activity using guinea pig tracheal chain method (table 1 and 2). All the synthesized imidazo[1,5-b][1,2,4]triazin-2(8H)-one analogues exhibited interaction with the amino acid residues in active site of PDE4 (PDB Code: 3IAK). Mainly nitro group of the all compounds is responsible for showing the activity. For compound **5a**, docking studies shows that histidine at position 204 interacts with N atom of the *ortho* the nitro group with a distance of 3.019 A$^{\circ}$ and asparagine at position 209 interacts with O atom of *ortho* nitro group with a distance of 1.962 A$^{\circ}$. Compound **5a** shows docking score of -64.158955. Same docking results were obtained for compound **5b**, which interacts with same amino acid residues but the distances are 1.73A^{o} and 1.882 A^{o} respectively while docking score is -59.973595. This indicates that docking studies and pharmacological screening gives structure activity relationship. In an average compound **5a** is most active. But in remaining

compounds, dose dependent effect is observed. Out of compound **5a**, at 80 & 60 µg, compound **5b** is most active and at 40, 20 & 10 µg, compound **5c** is most active.

APPLICATIONS

The overall study indicates that imidazo[1,5-b][1,2,4]triazin-2(8H)-one analogues may be developed and explored as effective novel antiasthmatic drugs.

CONCLUSIONS

From docking studies, it is clear that nitro group is mainly responsible for giving binding interactions with amino acid residues mentioned above. But out of *para* and *ortho* nitro group, later is mainly involved in binding interactions. Secondly, compounds containing o-hydroxybenzylidene and o-chlorobenzylidene at **8th** position of imidazo[1,5-b][1,2,4]triazin-2(8H)-one shows higher docking scores and hence highest antiasthmatic activity as compared to other groups, which reveals that *ortho* substitution is important.

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