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Design, Synthesis, Antimicrobial and Anti-inflammatory Activity of N-Pyrazolyl Benzamide Derivatives

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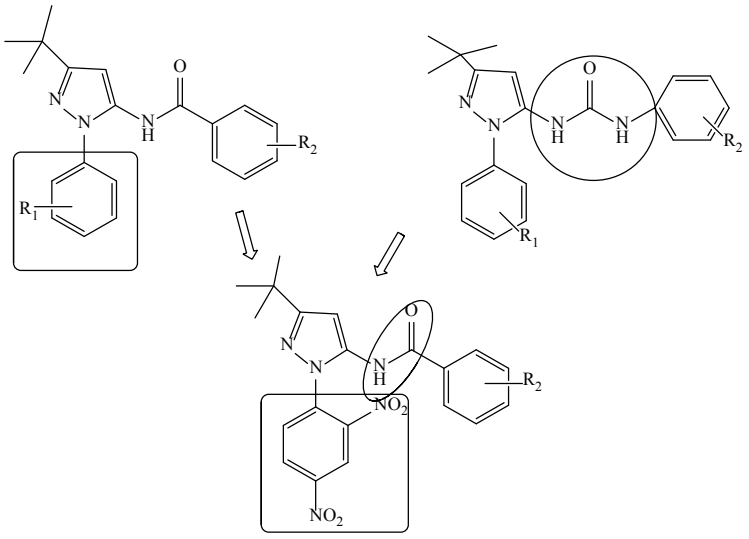
Abstract

New N-Pyrazolylbenzamide derivatives which possess 2, 4-dinitrophenyl group were synthesized using 4, 4-dimethyl-3-oxo-pentanenitrile and 2, 4-dinitrophenyl hydrazine hydrochloride to afford an intermediate compound. The intermediate on auxiliary aroylation with substituted benzoyl chlorides in the presence of base yielded the subsequent N-Pyrazolylbenzamide derivatives (5a-l). The structures of newly synthesized compounds were elucidated by ^{1}H NMR, FT IR and Mass spectral analysis. The anti-inflammatory activity of all newly synthesized was evaluated using carrageenan induced paw edema model and antimicrobial activity by serial dilution method. Six compounds (5d, 5f and 5h-k) showed consistently good anti-inflammatory activity in particular 4-trifluoromethyl-. N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide (5h) was found to be the most effective among the other derivatives. The antimicrobial screening of all synthesized molecules showed that compounds 5f and 5h possess superior and encouraging activity against tested organisms.



New N-Pyrazolylbenzamide derivatives containing 2, 4-dinitrophenyl group were designed from diarylureas which resulted in N-(3-tert-butyl-2, 4-dinitro-1-phenyl-1H-pyrazol-5-yl)benzamide derivatives. All the compounds were screened for antimicrobial and anti-inflammatory activity.

Keywords: Pyrazole; Benzamides; Anti-inflammatory; Antibacterial; Antifungal

Abbreviations: DMSO: Dimethyl sulfoxide; h: Hour; s: Singlet; d: Doublet; t: Triplet; bs: Broad singlet; m: Multiplet; IR: Infra-red; ^{1}H NMR: Proton nuclear magnetic resonance; Mol: Moles; M. Wt.: Molecular weight; mL: Milli liters; m.p.: Melting point; min: Minutes; mm: Millimetres; °C: Degree Centigrade; $CDCl_3$: Chloroform (Deuteriated); DCM: Dichloromethane; TLC: Thin layer chromatography; str: Stretching; Bnd: Bending; spp: Species; µg: Micro gram

Introduction

Inflammation is a key area of research for many pharmaceutical companies. Patients suffering from inflammatory disorders including rheumatoid arthritis (RA) require therapeutic agents that not only to demonstrate anti-inflammatory properties but also to protect against cartilage degradation [1]. TNF-α and IL-1β which are also known as proinflammatory cytokines are frequently found in numerous inflammatory diseases at elevated levels [2,3]. Clinically to alleviate inflammation steroidal and nonsteroidal anti-inflammatory agents are employed [4] and maximum of such agents have shown severe side effects which aspire need of safe antiinflammatory agents [5].

Twentieth century is known for its contribution in medical

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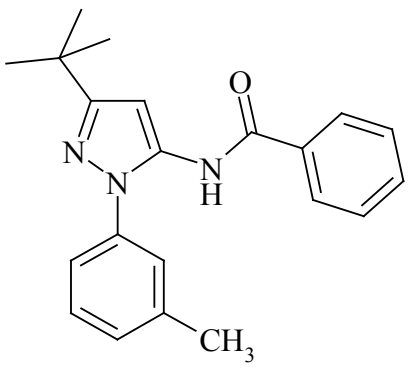
fraternity for controlling various microbial diseases including tuberculosis [6]. Many diseases which were caused by microbes were effectively contained and this was possible because of search of new and potent antimicrobial agents either from natural sources or synthetic routes [7-9]. Last decade of 20th century and early part of 21st century is contradictory to the remarkable success of medical sciences for the treatment of infectious diseases. This is mainly due to emergence of resistant strains of organisms which resulted in ineffectiveness of many antibiotics in the clinical treatment and also lackluster approach of giant pharmaceutical company towards new antimicrobial drug research [10-12]. Most of the available antibacterial agents have now become least effective or ineffective against methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Staphylococcus aureus*, resistant strains of *Escherichia coli* and new bacteria which express genes encoded for carbepenamase etc. [13]. Apart from this, not a single antibiotic with different chemical structure and mechanism of action has reached to clinical stage. Thus infectious diseases are still known to be the cause of mortality among the third world countries including India and the need of potent and safe clinical agents to contain infectious disease still persists [14].

Pyrazole and its derivatives occupy an important position in medicinal and pesticide chemistry due to a wide range of bioactivities such as antimicrobial [15], anticancer [16], anti-inflammatory [17], antidepressant, anticonvulsant, antihyperglycemic, antipyretic, antibacterial, antifungal, antiviral [18] and selective enzyme inhibitory activities [19]. Substituted pyrazole and its analogs have been used as precursors for synthesis of various biologically active molecules [20].

Our group is extensively working on 5-aminopyrazole derivatives as antiinflammatory agent and are first to report that pyrazole compounds with a tertiary butyl groups also demonstrate important antimicrobial activity [21]. In the previous publication, a simple monosubstituted aromatic group was inserted at the 1st position of pyrazole and one such molecule VHw (Figure 1) exhibited antibacterial activity in nano gram/mL range which encouraged us to explore chemical space in the substitutions on pyrazole scaffold and synthesize new generation with disubstituted aromatic group at the same position. This manuscript is aimed to report the synthesis and pharmacological activities of pyrazole containing benzamide compounds bearing a 2, 4-dinitrophenyl group at the 1st position of pyrazole.

Materials and Methods

Six stage reaction station Radleys discovery Technologies, Germany was used for important reactions. The course of reaction and purity were ascertained by performing TLC. Chemicals and solvents were obtained from S. D. Fine chemicals, Merck and Sigma Aldrich; before use solvents were purified. The melting points of the synthesized compounds were determined by open glass capillaries using a Polmon melting point apparatus and are uncorrected. Infrared spectra were recorded on Shimadzu FT IR spectrophotometer with KBr. Mass spectra were obtained on VG-7070H mass spectrometer and ^{1}H NMR were recorded at 300 MHz on a Bruker Avance NMR spectrometer in $CDCl_3$ or DMSO- d_6 using tetramethyl silane (TMS) as internal standard. Thin layer chromatography was performed on pre-coated silica gel F254 (Merck) and column chromatography was performed using silica gel 60-120 mesh.



VHw, 400 ng/mL

Figure 1: Structure of VHw along with antibacterial activity against *Bacillus subtilis* (MTCC 619).

Synthesis of 5-amino-3-t-butyl-1-(2', 4'-dinitro)phenyl-1H-pyrazole (3)

A mixture of 4, 4-dimethyl-3-oxo-pentanenitrile (1, 34 mM), 2, 4-dinitrophenyl hydrazine hydrochloride (2**,** 35 mM) and 50 mL absolute ethanol along with few drops of AcOH were heated at the reflux temperature for overnight and cooled to room temperature. The mixture was evaporated under vacuum and the residue thus obtained was washed with ether, suspended in EtOAc, and treated with 1 M NaOH solution. The organic layer then separated, washed with brine, dried over anhydrous magnesium sulphate and concentrated. The solid which separated was collected, then washed with a mixture of ether and hexane to give 5-amino-3-t-butyl-1-(2', 4'-dinitro)phenyl-1H-pyrazole (3) [22].

General procedure for N-[3'-*t*-butyl-1'-(2", 4"-dinitro) phenylpyrazol-5'-yl] benzamide (5a-l)

To a solution of 5-amino-3-*t*-butyl-1-(2', 4'-dinitro)phenyl-1H-pyrazole (3, 9.3 mM) in dichloromethane (5 mL), triethyl amine (23 mM) was added drop wise. The appropriate benzoylchlorides (4a-k, 12 mM) in dichloromethane were added to the above reaction mixture drop wise and stirred for 3 hours at room temperature. The mixture was further diluted with dichloromethane and washed with water, brine followed by once again with water. Sodium sulfate was used to dry the organic layer; solvent was removed under vacuum to get the final derivatives (5a-l). All the compounds were purified over silica to get pure N-(3-tert-butyl-2, 4-dinitro-1-phenyl-1H-pyrazol-5-yl) benzamides (5a-l).

Compound characterization

N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5a: Yield: 85%, m.p.: 190-192°C, IR (KBr) ν cm^{-1}: 3362 cm^{-1} (NH str.), 3020 cm^{-1} (CH str. aromatic), 2950 cm^{-1} (CH str. methyl), 1643 cm^{-1} (NH bnd) ^{1}H NMR ($CDCl_3$) δ (ppm): 1.23 (s, 9H, *t*-butyl), 6.18 (s, 1H, C_4, pyrazole), 7.15-7.55 (m, 3H, C_3', C_4', C_5' aromatic), 7.7-7.8 (m, 2H, C_5, C_6, aromatic), 7.9-8.1 (d, 2H, C_2', C_6', aromatic), 8.2 (s, 1H, C_3, aromatic), 9.61 (bs, 1H, NH amide). ESI-MS (m/z)**:** 410, 100% $[M+H]^+$.

4-Fluoro-N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5b: Yield: 82%, m.p.: 195-197°C, IR (KBr) ν cm^{-1}: 3200 cm^{-1} (NH str.), 3018 cm^{-1} (CH str. aromatic), 2950 cm^{-1} (CH str. methyl), 1686 cm^{-1} (CO str.), 1620 cm^{-1} (NH bnd). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.35 (s, 9H, *t*-butyl), 6.21 (s, 1H, C_4, pyrazole), 7.3-7.4 (d, 2H, C_3', C_5' aromatic), 7.55 -7.64 (d, 1H, C_6, aromatic), 7.7 -8.2 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 9.79 (bs, 1H, NH amide). ESI-MS (m/z): 428, 100% $[M+H]^+$.

3-Fluoro- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5c: Yield: 80%, m.p.: 191-192°C, IR (KBr) ν cm^{-1}: 3240 cm^{-1}

(NH str.), 3060 cm^{-1} (CH str. aromatic), 2965 cm^{-1} (CH str. methyl), 1651 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.34 (s, 9H, t-butyl), 6.32 (s, 1H, C_4, pyrazole), 7.12-7.2 (t, 1H, C_5' aromatic), 7.45-7.57 (d, 1H, C_4' aromatic), 7.62 -7.74 (d, 1H, C_6 aromatic), 7.85 -8.3 (m, 4H, C_3, C_5, C_2', C_6' aromatic), 9.36 (bs, 1H, NH amide). ESI-MS (m/z)**:** 428, 100% $[M+H]^+$.

4-Chloro- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5d: Yield: 70%, m.p.: 198-200°C, IR (KBr) ν cm^{-1}: 3220 cm^{-1} (NH str.), 3017 cm^{-1} (CH str. aromatic), 2969 cm^{-1} (CH str. methyl), 1640 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.32 (s, 9H, *t*-butyl), 6.18 (s, 1H, C_4, pyrazole), 7.2-7.33 (d, 2H, C_3', C_5' aromatic), 7.46 -7.55 (d, 1H, C_6, aromatic), 7.60 -8.3 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 9.77 (bs, 1H, NH amide). ESI-MS (m/z): 444, 100% $[M+H]^+$.

3-Chloro- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5e: Yield: 78%, m.p.: 191-192°C, IR (KBr) ν cm^{-1}: 3215 cm^{-1} (NH str.), 3016 cm^{-1} (CH str. aromatic), 2975 cm^{-1} (CH str. methyl), 1653 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.31 (s, 9H, *t*-butyl), 6.18 (s, 1H, C_4, pyrazole), 7.04-7.1 (t, 1H, C_5' aromatic), 7.33-7.41(d, 1H, C_4' aromatic), 7.6 -7.67 (d, 1H, C_6 aromatic), 7.78 -8.1 (m, 4H, C_3, C_5, C_2', C_6' aromatic), 9.39 (bs, 1H, NH amide). ESI-MS (m/z)**:** 444, 100% $[M+H]^+$.

4-Methoxy- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5f: Yield: 73%, m.p.: 197-199°C, IR (KBr) ν cm^{-1}: 3204 cm^{-1} (NH str.), 3025 cm^{-1} (CH str. aromatic), 2968 cm^{-1} (CH str. methyl), 1635 cm^{-1} (NH bnd). ^1H NMR (CDCl3) δ (ppm): 1.25 (s, 9H, *t*-butyl), 3.4 (s, 3H, methoxy), 6.20 (s, 1H, C_4, pyrazole), 6.9-7.03 (d, 2H, C_3', C_5' aromatic), 7.39 -7.47 (d, 2H, C_6, aromatic), 7.8 -7.99 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 9.36 (bs, 1H, NH amide). ESI-MS (m/z)**:** 440, 100% $[M+H]^+$.

3-Methoxy- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5g: Yield: 78%, m.p.: 197-199°C, IR (KBr) ν cm^{-1}: 3227 cm^{-1} (NH str.), 3006 cm^{-1} (CH str. aromatic), 2972 cm^{-1} (CH str. methyl), 1615 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.28 (s, 9H, *t*-butyl), 3.7 (s, 3H, methoxy), 6.32 (s, 1H, C_4, pyrazole), 6.87-6.98 (m, 2H, C_4', C_5' aromatic), 7.7-7.8 (d, 1H, C_6 aromatic), 7.82 -8.05 (m, 4H, C_3, C_5, C_2', C_6' aromatic), 9.2 (bs, 1H, NH amide). ESI-MS (m/z)**:** 440, 100% $[M+H]^+$.

4-Trifluoromethyl- N-[3'-*t*-butyl-1'-(2", 4"-dinitro) phenylpyrazol-5'-yl] benzamide 5h: Yield: 75%, m.p.: 194-196°C, IR (KBr) ν cm^{-1}: 3210 cm^{-1} (NH str.), 3005 cm^{-1} (CH str. aromatic), 2960 cm^{-1} (CH str. methyl), 1648 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.36 (s, 9H, *t*-butyl), 6.25 (s, 1H, C_4, pyrazole), 7.5-7.68 (m, 3H, C_6, C_3', C_5' aromatic), 7.87 -8.2 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 10.1 (bs, 1H, NH amide). ESI-MS (m/z)**:** 478, 100% $[M+H]^+$.

4-Methyl- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5i: Yield: 74%, m.p.: 190-192°C, IR (KBr) ν cm^{-1}: 3200 cm^{-1} (NH str.), 3040 cm^{-1} (CH str. aromatic), 2945 cm^{-1} (CH str. methyl), 1645 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.34 (s, 9H, *t*-butyl), 2.30 (s, 3H, CH_3), 6.41(s, 1H, C_4, pyrazole), 6.74-6.9 (d, 2H, C_3', C_5' aromatic), 7.45 -7.54 (d, 1H, C_6, aromatic), 7.76 -7.99 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 9.68 (bs, 1H, NH amide). ESI-MS (m/z)**:** 424, 100% $[M+H]^+$.

4-Nitro- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5j: Yield: 81%, m.p.: 195-197°C, IR (KBr) ν cm^{-1}: 3243 cm^{-1} (NH str.), 3028 cm^{-1} (CH str. aromatic), 2920 cm^{-1} (CH str. methyl), 1644 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.27 (s, 9H, *t*-butyl), 6.53 (s, 1H, C_4, pyrazole), 7.6-7.7 (md, 1H, C_6, aromatic), 7.92-8.1 (m, 6H, C_3, C_5, C_2', C_3', C_5', C_6' aromatic), 10.2 (bs, 1H, NH amide). ESI-MS (m/z)**:** 455, 100% $[M+H]^+$.

3-Nitro-N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5k: Yield: 83%, m.p.: 192-195°C, IR (KBr) ν cm^{-1}: 3240 cm^{-1} (NH str.), 3028 cm^{-1} (CH str. aromatic), 2960 cm^{-1} (CH str. methyl), 1650 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.42 (s, 9H, *t*-butyl), 6.23 (s, 1H, C_4, pyrazole), 7.31-7.4 (t, 1H, C_5'aromatic), 7.5-7.64 (d, 1H, C_6aromatic), 7.9-8.4 (m, 5H, C_3, C_5, C_2', C_4', C_6', aromatic), 10.3 (bs, 1H, NH amide). ESI-MS (m/z)**:** 455, 100% $[M+H]^+$.

4-Amino- N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamide 5l: Yield: 60%, m.p.: 191-193°C, IR (KBr) ν cm^{-1}: 3410 and 3300 cm^{-1} (NH antisym and sym str.), 3010 cm^{-1} (CH str. aromatic), 2932 cm^{-1} (CH str. methyl), 1645 cm^{-1} (NH bnd). ^1H NMR ($CDCl_3$) δ (ppm): 1.30 (s, 9H, *t*-butyl), 4.1 (s, 2H, NH_2), 6.45 (s, 1H, C_4, pyrazole), 6.8-6.94 (d, 2H, C_3', C_5' aromatic), 7.5-7.58 (d, 1H, C_6, aromatic), 7.8-8.05 (m, 4H, C_3, C_5, C_2', C_6', aromatic), 9.57 (bs, 1H, NH amide). ESI-MS (m/z)**:** 425, 100% $[M+H]^+$.

Antibacterial and antifungal activity

Cultures of *Bacillus subtilis* (MTCC 441), *Staphylococcus aureus* (MTCC 96), *Escherichia coli* (MTCC 1687), *Proteus vulgaris* (MTCC 742), *Aspergillus flavus* (MTCC 870) and *Candida albicans* (MTCC 854) were used to investigate the antimicrobial activities of compounds 5a-l. The *in vitro* antimicrobial activity was performed by the serial dilution method to find out minimum inhibitory concentration in Muller Hinton broth. MIC of all the compounds was established according to the guidelines mentioned in National Committee for Clinical Laboratory Standards (NCCLS) document M27-A [23]. DMSO was served as solvent to prepare stock solutions (100 µg/ml) of test compounds and standard drugs, serial dilutions of the compounds (100, 50, 25, 3.12, 1.6, 0.8 and 0.4 µg/ml) were prepared from the respective stock solution to determine the MIC employing overnight cultures of all four microbes [24]. MC Farland standards were adopted and Streptomycin and Fluconazole were used as standards. MIC was interpreted as the lowest concentration of the antimicrobial compound which will inhibit the visible growth of the microorganisms after certain period of incubation.

Carrageenan induced rat hind paw edema

All the newly synthesized compounds 5a-l were screened for anti-inflammatory activity in carrageenan induced rat hind paw edema Winter assay method [25]. All tested compounds were suspended in water with few drops of Tween-80. Fourteen groups of 6 wistar rats weighing 150-200 g were grouped for test, standard and control. The animals were housed in standard conditions with food and water *ad libitum*. A single dose of 10 mg/Kg of compounds 5a-l was administered orally to the respective groups and after 30 minutes carrageenan (1%, 1 mL was injected intradermally into the intraplantar region of the right hind paw) was injected in plantar region of hind paw for all groups. The control group received 1 mL vehicle and Diclofenac 50 mg/Kg dose was administered in control and standard group respectively. Rat edema was evaluated by measuring the rat paw volume at intervals 1, 2, 3 and 4 hour using digital plethysmometer (Ugo Basile) [26]. The inhibition percentage was calculated as:

$$\% \text{ Inhibition of edema} = (1 - Et/Em) \times 100$$

Where Et (mL) represents the average value of the edema in ml in treated groups in 1-4 h after carrageenan injection, while Em (mL) represents the average value of the edema in ml in control group in 1-4 h after carrageenan injection.

Results and Discussion

Chemistry

In our previous studies, we synthesized several amide derivatives and tested them for their anti-inflammatory activity [27]. As part of our continuous efforts in this area, a series of some new pyrazole derivatives containing substituent's at 1, 3, 5-positions were synthesized according to Scheme 1. The intermediate compound 5-amino-3-t-butyl-1-(2', 4'-dinitro)phenyl-1H-pyrazole 3 was prepared in good yields by refluxing 4, 4-dimethyl-3-oxo-pentanenitrile 1 and 2, 4-dinitrophenyl hydrazine hydrochloride 2. The FT IR spectrum of 3 showed the presence of bands characteristic for primary amine at 3423.42 cm^{-1} and 3264.21 cm^{-1} which are attributed to the asymmetric and symmetric stretching respectively and aromatic hydrogen stretching band located at 3056.75 cm^{-1}. The CH_3 groups of t-butyl vibrations were observed at 2961.33 cm^{-1} and 2828.42 cm^{-1} and peak assigned for bending vibration of NH group was observed at 1625.94 cm^{-1}. The ^1H NMR of 3 revealed a broad singlet at δ 3.77 ppm characteristic for primary amine group, multiplet at delta value of 8.05, 7.35 and 7.63 for C_3, C_5 and C_6 aromatic protons and a pyrazolyl-C_4-H as a singlet at 6.1 ppm. The nine *t*-butyl protons were found as singlet at δ 1.23 ppm. The EI Mass spectrum of 3 showed molecular ion peak at m/z 215.

When 5-amino-3-t-butyl-1-(2', 4'-dinitro)phenyl-1H-pyrazole 3 was stirred with substituted benzoylchlorides 4a-k in dichloromethane and triethylamine, pyrazolylbenzamides 5a-l were obtained in moderate to good yields (5j was reduced with stannous chloride to form 5l). The structures of the isolated compounds were determined by spectral methods.

The FT IR spectrum of 5a revealed characteristic NH band at 3362 cm^{-1}, the aromatic hydrogen stretching was found at 3020 cm^{-1} and stretching vibrations of CH_3 group of *t*-butyl band was noticed at 2950 cm^{-1}. A band at 1595 cm^{-1} was assigned for the C=C stretching. The ^1H NMR spectra displayed a broad absorption peak at δ 9.61 which was due to resonance of NH proton of amide while the pyrazol-C_4-H and nine t-butyl protons appears as singlet's at δ 6.18 and 1.23 ppm respectively. The aromatic protons appeared as doublet at δ 7.9-8.1 which indicated the resonance of C_2', C_6' protons. Three aromatic protons C_3', C_4', C_5' resonated as multiplet at δ 7.15-7.55 and another multiplet at δ 7.7-7.8 integrated for two protons C_5 and C_6. C_3 proton was observed as singlet at δ 8.2.

Pharmacology

Antimicrobial activity: All synthesized compounds 5 (a-l) were screened for *in vitro* activity against *Bacillus subtilis*, *Staphylococcus aureus* representing Gram positive bacteria, *E. coli*, *Proteus vulgaris* representing Gram negative organism, *Aspergillus flavus* and *Candida albicans* representing fungal strains. Streptomycin and Fluconazole were used as antibacterial and antifungal standard compound respectively during the studies. The antimicrobial activity of the synthesized compound was evaluated by measuring the MIC value and their results were compared with those of standards and are represented in Tables 1 and 2.

All the N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamides (5a-l) displayed antibacterial activity in the range of 3.12 - 100 μg/mL concentrations. Among the tested compounds, it was noticed that compounds 5a demonstrated MIC value of 50 μg/mL against Gram positive bacteria and 100 μg/mL against Gram negative

Comp No	Mol. For.	Mol. Wt.	Yield (%)	m.p. (°C)	R_f	cLogP
5a	$C_{20}H_{19}N_5O_5$	409.40	85	190-192	0.49	4.44
5b	$C_{20}H_{18}FN_5O_5$	427.39	82	195-197	0.48	4.60
5c	$C_{20}H_{18}FN_5O_5$	427.39	80	191-192	0.50	4.60
5d	$C_{20}H_{18}ClN_5O_5$	443.84	70	198-200	0.52	5.17
5e	$C_{20}H_{18}ClN_5O_5$	443.84	78	191-192	0.55	5.17
5f	$C_{21}H_{21}N_5O_6$	439.42	73	197-199	0.53	4.53
5g	$C_{21}H_{21}N_5O_6$	439.42	78	197-199	0.54	4.53
5h	$C_{21}H_{18}F_3N_5O_5$	477.39	75	194-196	0.51	5.36
5i	$C_{21}H_{21}N_5O_5$	423.42	59	233-236	0.56	4.25
5j	$C_{20}H_{18}N_6O_7$	454.39	81	195-197	0.45	4.34
5k	$C_{20}H_{18}N_6O_7$	454.39	83	192-195	0.61	4.34
5l	$C_{20}H_{20}N_6O_5$	424.41	60	191-193	0.65	3.53

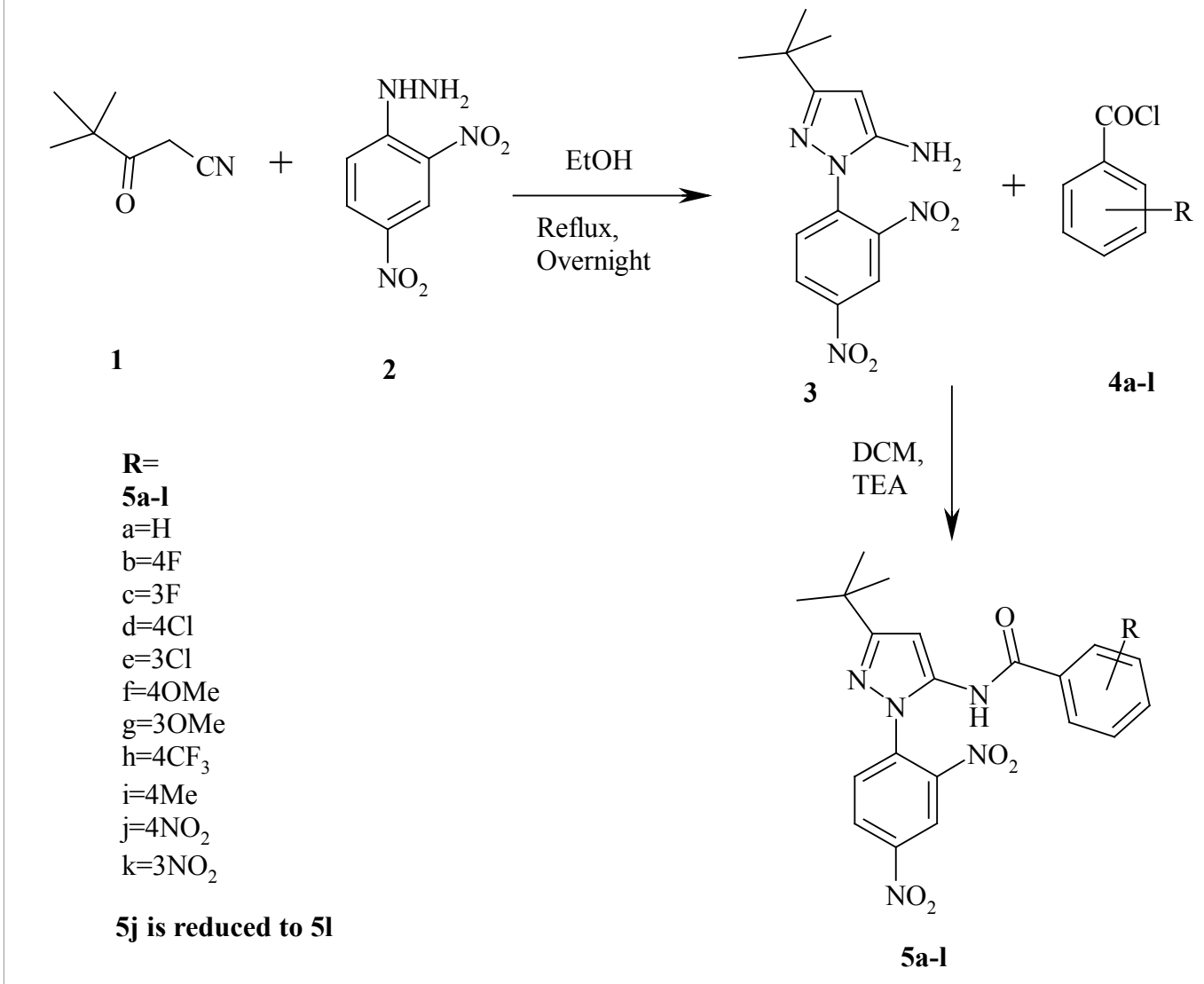
Table 1: Physical properties of synthesized compounds.

S No	Comp Code	R	Anti-bacterial				Anti-fungal	
			BS	SA	EC	PV	AF	CA
	5a	H	50	50	100	100	12.5	50
	5b	4-F	6.25	12.5	6.25	6.25	3.12	6.25
	5c	3-F	100	100	50	50	100	100
	5d	4-Cl	12.5	12.5	12.5	12.5	3.12	6.25
	5e	3-Cl	100	100	50	50	12.5	50
	5f	4-OCH_3	3.12	3.12	12.5	6.25	3.12	3.12
	5g	3-OCH_3	100	100	50	50	50	12.5
	5h	4-CF_3	3.12	3.12	6.25	6.25	3.12	3.12
	5i	4-CH_3	6.25	6.25	12.5	12.5	6.25	6.25
	5j	4-NO_2	3.12	3.12	12.5	12.5	6.25	6.25
	5k	3-NO_2	100	100	50	50	12.5	12.5
	5l	4-NH_2	50	50	50	50	100	100
Streptomycin			50	50	50	50		
Fluconazole							50	50

Table 2: Antimicrobial activity (MIC in microgram/mL) of compounds 5a-l.

S No	Comp Code	R	% inhibition at different intervals			
			1 hr	2 hr	3 hr	4 hr
	5a	H	51.4	55.7	71.3	75.6
	5b	4-F	49.6	51.4	63.1	61.9
	5c	3-F	40.3	54.7	60.2	56.1
	5d	4- Cl	57.6	72.5	77.9	87.6
	5e	3-Cl	66.3	74.1	77.8	53.4
	5f	4-OCH_3	64.9	73.8	78.9	83.6
	5g	3-OCH_3	51.3	60.1	65.2	51.7
	5h	4-CF_3	65.4	86.5	87.9	89.4
	5i	4-CH_3	58.3	70.1	75.2	82.2
	5j	4-NO_2	69.4	78.3	81.1	83.2
	5k	3-NO_2	62.5	76.8	79.1	80.3
	5l	4-NH_2	43.6	55.1	60.2	62.2
Diclofenac (50 mg/Kg)			54	77.9	82.1	92.3

Table 3: Anti-inflammatory activity (% inhibition) of compounds 5a-l.



Scheme 1: New pyrazole derivatives containing substituent's at 1, 3, 5-positions.

bacteria. Compounds 5f, 5h, 5j have shown higher activity than standard, while the compounds 5c, 5e, 5g and 5k have shown lesser activity against both Gram positive and Gram negative bacteria. Rests of the molecules were active in the range of 6.25-50 µg/mL. The most effective compounds 5f, 5h, 5j with MIC value of 3.12 µg/mL were found to be potent against both the gram Gram positive organisms.

All the compounds were also evaluated for their antifungal activity against *Aspergillus flavus* and *Candida albicans* and results were compared against Fluconazole as standard. Compounds 5a-l demonstrated antifungal activity against both the tested organisms with MIC values in the range of 3.12-50 µg/mL. Among the compounds, 5f and 5h were most potent against fungal strains with MIC value of 3.12 µg/mL followed by compounds 5i and 5j with 6.25 µg/mL against both *Aspergillus flavus* and *Candida albicans* as comparable to that of Fluconazole. Compounds 5b and 5d were highly active against only one strain and displayed promising antifungal activity at MIC 3.12 µg/mL. In general, the target compounds 5f, 5h and 5j with substitution like methoxy, trifluoromethyl, nitro at para position respectively showed more significant antimicrobial activity than standard. Two compounds 5c and 5l were least potent with MIC 100 µg/mL.

Anti-inflammatory activity: All the twelve synthesized molecules 5a-l were further explored for anti-inflammatory activity by rat paw induced edema model and the results were compared against blank and reference as Diclofenac, the results are listed down in the Table 3. A dose of 10 mg/Kg was set for all test compounds which were administered orally as suspension. Almost all of the tested compounds showed promising anti-inflammatory activity at 4th hour of carrageenan administration however four compounds 5b, 5c, 5e and 5g showed decrease in anti-inflammatory activity at the 4th hour. Compounds 5c, 5e and 5g demonstrated 50% anti-inflammatory activity at 4th hour and this may be attributed to the substitution at meta or 3rd position with chloro, methoxy and nitro groups. Unsubstituted compound 5a displayed significant activity of 75.6% at the 4th hour.

Among the synthesized compounds highest potency was exhibited by compound 5h in comparison to Diclofenac with the activity of 89.4% inhibition followed by compound 5d, 5f, 5j, 5i and 5k which demonstrated over 80% activity at 4th hour. The activity was found to be increasing when electron withdrawing groups were inserted at R_4 at para position among them only 5b and 5l with fluoro and amino substituent showed less activity.

Conclusion

Various N-[3'-*t*-butyl-1'-(2", 4"-dinitro)phenylpyrazol-5'-yl] benzamides (5a-l) were synthesized by following reported methods and the structure of the compounds 5a-l were confirmed by various analytical techniques. The target compounds (5a-l) were evaluated for antimicrobial and anti-inflammatory activities and showed potent antimicrobial, anti-inflammatory activity.

Amongst the compounds screened, the highest potent activity was observed for compound 5h for both anti-inflammatory activity and antimicrobial activity. Several compounds exhibited peak anti-inflammatory activity in the 3rd and 4th hour of carrageenan administration. Compounds 5d, 5f, 5h, 5i, 5j and 5k showed over 80% anti-inflammatory activity even in the 4th hour which may be due to presence of substitution at para position and among the entire compounds screened compound 5h was found to be the most potent compound.

Antimicrobial activity of compounds for revealed that maximum compounds showed MIC value lesser than 50 µg/mL and compounds 5f, 5h and 5j showed MIC value 3.12 µg/mL against numerous strains. Compounds 5f and 5 h also exhibited MIC value 3.12 µg/mL against both fungal strains and compound 5d with para chloro substitution has MIC value 3.12 µg/mL against one fungal strain. This study will definitely beneficial to develop future anti-inflammatory and antimicrobial compounds with potent activity.

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