Abstract

Purpose: Microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice. The compound possessing all three activities is not common. The purpose of the present study was to examine whether molecular modification might result in detection of new potential antirheumatic drugs having antimicrobial activities.

Method: A series of novel 4-(5′-substituted aryl-4′, 5′-dihydropyrazole-3′-yl-amino) phenols 2a-f have been synthesized by treating substituted aryl-N-chalconyl amino phenols 1a-f with hydrazine hydrate. The starting materials were synthesized from p-aminoacetophenone. Their structures were confirmed by IR, 1H NMR spectral data. The synthesized compounds were investigated for analgesic, anti-inflammatory and antimicrobial activities.

Result: The data reported in Tables 2, 3 & 4 shows that effect of variation in chemical structure on activity was rather unpredictable. Seldom did a particular structural modification lead to uniform alteration in activity in all tests. The substitution which appeared to be most important for high order of activity in the greatest number of test was the p-chloroaryl group. The introduction of p-nitro and p-hydroxy group in aryl moiety of the pyrazole analogs 2c and 2e produce compounds with potent analgesic, anti-inflammatory and, in a few cases, antimicrobial properties.

Conclusion: The observed increase in analgesic, anti-inflammatory and antimicrobial activities are attributed to the presence of 4-NO₂, 2-OH and 4-Cl in phenyl ring at 5-position of pyrazoline ring of synthesized compounds. In some cases their activities are equal or more potent than the standard drugs.

Keywords: Pyrazole, Analgesic, Anti-inflammatory, Antibacterial activity.

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INTRODUCTION
Pyrazole derivatives have a long history of application in agrochemicals and pharmaceutical industry as herbicides and active pharmaceuticals. The recent success of pyrazole COX-2 inhibitor has further highlighted the importance of these heterocycles in medicinal chemistry. A systematic investigation of this class of heterocyclic lead revealed that pyrazole containing pharmacoactive agents play important role in medicinal chemistry. The prevalence of pyrazole cores in biologically active molecules has stimulated the need for elegant and efficient ways to make these heterocyclic lead. The treatment of pain continues to be the subject of considerable pharmaceutical and clinical research. Microbial infections often produce pain and inflammation. Chemotherapeutic, analgesic and anti-inflammatory drugs are prescribed simultaneously in normal practice. The compound possessing all three activities is not common. It has been reported that pyrazoline possess analgesic, anti-inflammatory and antimicrobial activities. In view of these above, an attempt has been undertaken for the synthesis of the some novel 4-(5′-substituted aryl-4′, 5′-dihydropyrazole-3′-yl-amino) phenols possessing potent biological activities. The synthesized compounds were tested for their possible analgesic, anti-inflammatory and anti-microbial activities.

EXPERIMENTAL
Equipment
Melting points were determined in open capillaries and were uncorrected by melting point determining apparatus (SISCO). Purity of the compounds were checked by TLC. IR spectra (KBr, cm⁻¹) were recorded on a JASCO FT/IR 410 spectrophotometer. ¹H NMR (CDCl₃) on a Bruker DPX 300-MHz spectrometer using TMS as an internal reference (chemical shifts in δ ppm). C, H and N analysis were carried out on a Euro EA (Italy) analyzer.

Materials
Pure paracetamol (ODCL, India), ciprofloxacin (Alkem, India), clotrimazole (Glennmark, India). Methanol, Hydrazine hydrate, ethanol (all from (SD-Fine Chemical, India), Sodium Hydroxide benzaldehyde, furfuraldehyde, 4-nitrobenzaldehyde, p-anisaldehyde, salicylaldehyde, 4-chloro benzaldehyde, Carrageenan (all from Merck, Germany), carboxymethylcellulose (Sigma, India), Dimethylformamide (Aldrich), Mullar hinton agar and Sabouraud dextrose agar (Hi-Media, India).

Preparation of N-(4-hydroxyphenyl)-3-phenylacrylamide (1a)
To a mixture of p-hydroxyacetoaminophene (0.01 mol) and benzaldehyde (0.01 mol) in ethanol, 2 % sodium hydroxide solution (1 ml) was added drop wise with constant stirring over a period of 30 min. and the reaction mixture was stirred for another 10 h at room temperature and then refluxed for 6 h. The excess solvent was distilled off and the solid obtained was poured into ice-cold water. The solid thus obtained was filtered, dried and recrystallised from ethanol. Compounds 1b-f were prepared similarly by using different arylaldehydes. Their melting points, % yields and molecular formula are given in Table-1.

1a (R = -C₆H₅): m.p. 150°C, yield: 72%, IR(KBr in cm⁻¹) : 3452 (Ar–OH str.), 3301 (NH str.), 3016 (C-H str.), 1610 (C=C str.), ¹H- NMR (δ ppm) (CDCl₃), 7.1-7.8 (2H,d,CH), 6.11 (1H,s,N-H), 7.70 (1H,s,N-H), 5.35 (1H, s, Ar-OH), 6.76 – 8.00 (m, Ar-H ). Analysis (C₁₅H₁₃O₂N ) cal(found)%: C 75.30(75.52) H 5.48(4.98) N 5.85(6.21).,MS:(m/z) : 239(M⁺).

1b (R = - Furyl): IR(KBr in cm⁻¹) : 3300 (Ar–OH str.), 3253 (NH str.), 2922 (CH₂ str.), 1476(C=C str.), 1137 (C-O-C str.); ¹H- NMR (δ ppm) (CDCl₃), 7.13-7.21 (2H,d,CH), 7.11-7.21 (2H,d,CH), 6.11 (1H,s,N-H), 7.00 (1H,s,N-H), 5.35 (1H, s, Ar-OH), 6.76 – 8.00 (m, Ar-H ). Analysis (C₁₃H₁₁O₃N ) cal(found)%: C 68.11(68.43) H 4.84(4.49) N 5.85(6.21).,MS:(m/z) : 229(M⁺).

1c (R = - p-NO₂-C₆H₄):- IR(KBr in cm⁻¹) : 3490 (Ar-OH str.), 3127 (NH str.), 2927 (CH₂ str.), 1730 (C=O str.), 1560 (C-NO₂ str.), 1476 (C=C str.), 7.13-7.21 (2H,d,CH), 6.21 (1H,s,N-H), 7.38 (1H, s, N-H), 6.55 (1H, s, Ar-OH), 6.76 – 8.00 (m, Ar-H ). Analysis (C₁₅H₁₃O₄N₂ ) cal(found)%: C 68.11(68.43) H 5.79(5.89).,MS:(m/z) : 317(M⁺).
Preparation of 4-[(5'-phenyl-4', 5'-dihydropyrazol-3'-yl) amino] phenol (2a)

A mixture of compound 1a (0.01mol) and hydrazine hydrate (0.01mol) in ethanol (30ml), were refluxed for 6 h on a water bath. The reaction mixture was concentrated, cooled and poured into ice-cold water. The resulting solid 2a was filtered, dried and recrystallised from ethanol. Compounds 2b-f were prepared similarly. Their melting points, % yields and molecular formula are given in Table-1. 2a(R = -C6H5): IR (KBr in cm⁻¹): 3462 (Ar-0H), 3278 (NH), 2378 (NH), 1749 (C=O), 1665 (C=C), 1614 (C=C), 1510 (C=O), 1405 (C-O-C); MS (m/z): 253(M⁺), 2b (R=Furyl): IR (KBr in cm⁻¹): 3312 (Ar-0H), 3261 (NH), 3264 (NH), 3065 (O-H), 2927 (CH₂), 1630 (C=N), 1464 (C-C), 1137 (C-O-C); 1H- NMR (6 ppm) (CDCl₃): 2.13-2.22 (1H, d, CH₂), 6.24 (1H, s, N-H), 7.38 (1H, d, N-H), 4.54- 4.61 (2H, d, CH₂), 5.63 (1H, s, Ar-0H), 6.63 – 7.87 (m, Ar -H). Analysis (C₁₃H₁₇N₃O₂) cal (found) %: C 65.82(66.12) H 4.90(4.92) N 14.60(14.29). MS (m/z): 298(M⁺).

Animals

Wistar albino mice (20-30 g) and Swiss albino rats (100 – 140 g) of either sex were selected
for the experiments. Animals were allowed to be acclimatise for a period of 2 weeks in our laboratory environment prior to the study. Animals were housed in polypropylene cages (4 animals per cage), maintained under standard laboratory conditions (i.e. 12:12 hour light and dark sequence; at an ambient temperature of 25±2°C; 35-60% humidity); the animals were fed with standard rat pellet diet (Hindustan Liver Ltd.Mumbai) and water ad libitum. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

**Analgesic activity**
The analgesic activity was determined by tail flick method. Wistar albino mice of either sex (20-30g) in the groups of six animals each were selected by random sampling technique. Paracetamol at a dose level of 100 mg/kg was administered as a reference drug for comparison. The test compounds at dose level of 100mg/kg were administered orally by intragastric tube. The animals were held in position by a suitable restrained with the tail extending out and the tail (up to 5 cm) was then dipped in a beaker of water maintained at 55 ± 5°C. The time in seconds taken to withdraw the tail clearly out of water was taken as the reaction time. The reading was recorded at 30, 60, 120 and 180 min. after administration of compounds. A cut off point of 10 sec. was observed to prevent the tail damage. The results are presented in Table-2.

**Anti-inflammatory activity**
The anti-inflammatory activity was determined by carrageenan-induced rat paw oedema method in abino rats (n=6) of either sex (100-140 g). Rats were selected by random sampling technique. Paracetamol (100mg/kg) was administered as a reference drug. The test compounds were administered at dose level of 100 mg/kg orally 30 min. prior to the administration of carrageenan in the right hind paw of the rats. The paw thickness was measured using vernier callipers at 30, 60, 120 and 180 min. after carrageenan administration. The results are presented in Table-3.

**Antimicrobial activity**
*In vitro* antimicrobial study was carried on Muller hinton agar (Hi-media) plates (37°C, 24 h) by agar diffusion cup plate method. All the compounds were screened for antimicrobial activity at 100 µg/ml concentration against the following bacterial strains: *Staphylococcus aureus*, *Staphylococcus faecalis*, *Escherichia coli*, and *Salmonella typhi*. Antifungal activity was tested on Sabouraud dextrose agar (Hi-media) plates (26°C, 48-72 h) by cup plate method against *Candida albicans* and *Aspergillus niger* at the concentration level of 100 µg/ml, Ciprofloxacin and Clotrimazole were used as a standards for comparison of antibacterial and antifungal activity under the similar conditions. DMF was used as a solvent control for both antibacterial and antifungal activities. The results are presented in Table-4.

**Statistical analysis**
Data were analyzed by one –way ANOVA followed by Dunnett’s t-test using computerized Graph Pad Instat version 3.05 (Graph Pad software, U.S.A.).

**RESULTS**
Biological results are reported in Table 2, 3 and 4, which also records the effects of the standard drug included for comparison. Series of compound are prepared in this study exhibited significant pharmacological properties in different biological models. The general pattern of pharmacological activity encountered in this synthesized compounds was seen mainly in their effect on pain perception and local inflammation. However, there was a small, well defined antimicrobial activity range associated with many of these compounds. Considerable variation of these effects were seen with each structural change, varying from agents that had less activity to those with high potency, and significant changes in potency resulted even from minor change in chemical structure as shown in Table2,3 and 4.
Analgesic Activity: Some of the compounds in this series exhibited activity in experimental models used. The particular interests are the results obtained in the Glassman’s procedure which utilizes selective inhibition of inflammatory pain as a creation for anti-inflammatory drugs. When the structure of this synthesized compound is compared, it would appear that replacement in R with a p-nitro, p-methoxy and p-chloro aryl groups (2c, 2e &2f) showed promising analgesic activity.

Anti-inflammatory Activity: A number of agents caused marked reduction of the carrageenan induced edema of the rat foot, however, with exception of compounds 2f (R = p-Nitro phenyl). In this test also only analogs with a p-Methoxy phenyl group in R (2d) showed equal to that exhibited by the standard paracetamol . Compounds 2f, in

**Table 1:** Characterization data of compounds 1a-f and 2a-f

| Compound | (R)     | Mol. Form.     | M.P. (°C) | Yield (%) |
|----------|---------|----------------|-----------|-----------|
| 1a       | -C₆H₅   | C₁₃H₁₃O₂N      | 148-150   | 72        |
| 1b       | -2-furyl | C₁₃H₁₃O₂N      | 160-162   | 63        |
| 1c       | -4-NO₂-C₆H₄ | C₁₅H₁₂O₂N₂ | 108-110   | 81        |
| 1d       | -4-OCH₃-C₆H₄ | C₁₆H₁₅O₂N | 152-154   | 74        |
| 1e       | -2-OH-C₆H₄ | C₁₅H₁₃O₂N      | 142-144   | 72        |
| 1f       | -4-Cl-C₆H₄ | C₁₅H₁₄O₂Cl    | 150-152   | 75        |
| 2a       | -C₆H₅   | C₁₃H₁₃N₂O      | 161-163   | 62        |
| 2b       | -2-furyl | C₁₃H₁₃N₂O₂     | 159-161   | 73        |
| 2c       | -4-NO₂-C₆H₄ | C₁₅H₁₄N₂O₃ | 182-184   | 69        |
| 2d       | -4-OCH₃-C₆H₄ | C₁₆H₁₇N₂O₂ | 143-145   | 72        |
| 2e       | -2-OH-C₆H₄ | C₁₅H₁₅N₂O₂     | 164-166   | 66        |
| 2f       | -4-Cl-C₆H₄ | C₁₅H₁₄N₂OCl   | 190-192   | 78        |

**Table 2:** Analgesic activity (tail flick method) of compounds 2a-f

| Compd. | Dose | Dose | Percentage of analgesic activity | Percentage of analgesic activity | Percentage of analgesic activity | Percentage of analgesic activity |
|--------|------|------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|        | mg/kg| 30 min. | 1 hour | 2 hour | 3 hour | 1 hour | 2 hour | 3 hour | 1 hour | 2 hour | 3 hour |
| 2a     | 100  | 27 ± 0.12 | 30 ± 0.23 | 35 ± 0.43 | 30±0.11 |         |       |       |       |       |       |
| 2b     | 100  | 33± 0.25 | 42± 0.09 | 44± 0.40 | 38± 0.31 |         |       |       |       |       |       |
| 2c     | 100  | 38± 0.54 | 43± 0.23 | 47± 0.43 | 38± 0.29 |         |       |       |       |       |       |
| 2d     | 100  | 44± 0.23 | 53± 0.29 | 58± 0.33 | 45± 0.36 |         |       |       |       |       |       |
| 2e     | 100  | 36± 0.32 | 45± 0.16 | 47± 0.36 | 38± 0.42 |         |       |       |       |       |       |
| 2f     | 100  | 42± 0.23 | 50± 0.73 | 50± 0.87 | 38± 0.65 |         |       |       |       |       |       |
| Paracetamol | 100 | 38± 0.42 | 47± 0.82 | 52± 0.71 | 33± 0.31 |         |       |       |       |       |       |
| Control | _    | 3 ± 0.26 | 6 ± 0.44 | 4 ± 0.57 | 4 ± 0.91 |         |       |       |       |       |       |

Results are expressed in mean ± SEM (n=6) significance levels * P<0.05, ** P < 0.01 and *** P < 0.001 as compared with the respective control.
addition to being the most potent agents of this series against rat-foot inflammation, were also found to be among the most active analgesic when assayed in Glassman's analgesic model.

**Antimicrobial Activity:** The in-vitro antimicrobial activity of compounds (2a-f) were determined by agar cup plate method, The results of which are summarized in Table 4. The antimicrobial data in Table 4 clearly showed that the halogen, nitro & hydroxyl phenyl groups is by far the most active substituted R group. The methoxy group generally confers week antimicrobial activity. Phenyl and Furly substitution are weakly active to inactive among the synthesized compounds. Compounds 2c, 2e and 2f showed good activity against *S. aureous* and *S. typhi*. The compound 2c & 2f exhibit promising activity against *C. albicans* and *A. niger*. However, the tested compounds were less active in comparison to Ciprofloxacin and Clotrimazole (standard Drugs).

### Table 3: Anti-inflammatory activity (carrageenan induced rat paw oedema method) of compounds 2a-f.

| Compd. | Dose (mg/kg) | Percentage inhibition (%) | 30 min. | 1 hour | 2 hour | 3 hour |
|--------|-------------|---------------------------|---------|--------|--------|--------|
| 2a     | 100         | 26 ± 0.10                 | 32 ± 0.62 | 39 ± 0.10 | 33 ± 0.07 |
| 2b     | 100         | 28 ± 0.19                 | 37 ± 0.17 | 43 ± 0.78 | 36 ± 0.17 |
| 2c     | 100         | 27 ± 0.41                 | 33 ± 0.81 | 38 ± 0.67 | 29 ± 0.24 |
| 2d     | 100         | 26 ± 0.40                 | 32 ± 0.36 | 35 ± 0.96 | 27 ± 0.66 |
| 2e     | 100         | 28 ± 0.27                 | 35 ± 0.49 | 41 ± 0.11 | 32 ± 0.53 |
| 2f     | 100         | 29 ± 0.78                 | 33 ± 0.27 | 34 ± 0.42 | 27 ± 0.62 |
| Control | -          | 5.11 ± 0.28               | 6.13 ± 0.26 | 5.68 ± 0.36 | 3.30 ± 0.91 |
| Paracetamol | 100           | 26 ± 0.29                 | 30 ± 0.22 | 34 ± 0.91 | 28 ± 0.62 |

Results are expressed in mean ± SEM. (n=6) significance levels *P < 0.05, **P < 0.01 and ***P < 0.001 as compared with the respective control.

### Table 4: Antibacterial and antifungal activity of compounds 2(a-f)

| Compd. | Conc. (µg/ml) | Zone of inhibition (mm) |
|--------|---------------|-------------------------|
|        | S. a | S. f | E. c | S. t | C. a | A. n |
| 2a     | 100  | 14   | 16   | 16   | 12   | 13   |
| 2b     | 100  | 13   | 12   | 15   | 11   | 12   |
| 2c     | 100  | 18   | 20   | 21   | 19   | 17   |
| 2d     | 100  | 16   | 15   | 17   | 14   | 14   |
| 2e     | 100  | 21   | 16   | 17   | 19   | 20   |
| 2f     | 100  | 18   | 18   | 19   | 18   | 21   |
| Ciprofloxacin | 10       | 29   | 31   | 32   | 26   | -    |
| Clotrimazole  | 20       | -    | -    | -    | 28   | 27   |

*Average of three readings
S. a = *Staphylococcus aureus*; S.f = *Staphylococcus faecalis*; E. c = *Escherichia coli*; S. t = *Salmonella typhi*; C. a = *Candida albicans*; A. n = *Aspergillus niger*
The purpose of the present study was to examine whether molecular modification might result in detection of new potential antirheumatic drugs. A series of compounds were prepared and assayed in a variety of biological test for analgesic, anti-inflammatory and antimicrobial activity. The data reported in Table 2, 3 & 4 shows that effect of variation in chemical structure on activity was rather unpredictable. Seldom did a particular structural modification lead to uniform alteration in activity in all tests. However some point of interest did emerge and a few generalizations can be made. The substitution which appeared to be most important for high order of activity in the greatest number of test was the p-choloroaryl group. The introduction of Para nitro and p-hydroxy group in aryl moiety of the pyrazole analogs 2c and 2e produce compounds with potent analgesic, anti-inflammatory and, in a few cases, antimicrobial properties.

**CONCLUSION**

In conclusion, the results of this investigation revealed that the observed increase in analgesic, anti-inflammatory and antimicrobial activities are attributed to the presence of 4-NO₂, 2-OH and 4-Cl in phenyl ring at 5-position of pyrazoline ring of synthesized compounds. Obviously, the comparative evaluation of active compounds will required further studies; the data reported in this article may be helpful guide for the medicinal chemist who are working in this area.
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