Synthesis of a New Series of Substituted Pyrimidines and Its Evaluation for Antibacterial and Antinociceptive Effects

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Abstract:

Background: Pyrimidines are a well-known group of compounds reported to have different biological activities. Prompted from the diversity of its wider use and being an integral part of genetic material, an effort was made to synthesize a novel series of amino-pyrimidine derivatives of pharmaceutical interest by condensing the guanidinyl derivative of nalidixic acid with different chalcones.

Methods: The structures of all synthesized compounds were established on the basis of IR and 1HNMR spectral studies. All of the new compounds in this series were screened for antimicrobial activity. Gram +ve and Gram -ve strains were used to ascertain the spectrum of activity. ED50 values in the tail flick test were determined and recorded. Analgesic potential of compounds by using tail flick test in SWR male mice have also revealed promising results.

Results: All of the derivatives were effective in Gram -ve test against E. coli. None of the compounds show any inhibition of Gram +ve strain S. aureus. m-Bromo substitution derivative of amino-pyrimidines showed appreciable activity against E. coli, while 2,4 dichloro and p-chloro substitution derivatives also demonstrated improved activity. Compound 4 was most potent. The order of potency for these derivatives was 4>5>6>1>2>7>3. Parallel to antimicrobial activity, m-bromo substitution derivative showed significant (P<0.01) antinociceptive response in comparison to control, and this effect was comparable to aspirin group. Trimethoxy substitution of benzene ring demonstrated moderate activity, whereas p-bromo substitution essentially had no antinociceptive effects in mice.

Conclusion: Comparing meta- and para- bromo substitutions, there had been significant (P<0.01) difference in the antinociceptive response of both the bromo-substituted derivatives. It was observed that bromo-substitution at meta- position demonstrated comparatively higher potential for its antibacterial as well as antinociceptive properties.

Keywords: Synthesis, Chalcones, Pyrimidine derivatives, Antinociception, Antimicrobial activity.

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Introduction

Pyrimidine, being an integral part of DNA and RNA, imparts to diverse pharmacological properties, as effective bactericide, fungicide, vermicide, insecticide, anticancer and antiviral agents [1]. Certain pyrimidine derivatives are also known to display antimalarial, antifilarial and antileishmanial activity [2]. The finding that 2,4 diamino pyrimidines inhibit the growth of microorganisms by interfering with their utilization of folic acid led to the synthesis and evaluation of a variety of substituted analogs. Further research led to the development of two successful antimalarial drugs pyrimethamine [3-4] and trimethoprim [5-6]. Supplementary modifications have yielded useful pyrimidine derivatives like oxythiamine [7, 8] and neopyrithiamine [9, 10], which are vitamin B1 antagonist and antiviral agent cytosine arabinoside [11], respectively.

Literature has suggested that the aryl azo coupling occurs at the 5th position in the pyrimidine ring and various substituted 5-aryl azo pyrimidines have been synthesized and characterized as antagonists to folic acid. A series of 1-benzylbenzimidazole and 3-benzylimidazo[4,5-b]pyridine substituted in the 2-position by an alkanoic or mercaptoalkanoic acid chain was synthesized for evaluation as potential thromboxane A2/prostaglandin H2 (TXA2/PGH2) receptor antagonists [12]. The affinity of each compound for washed human plateleTXA2/PGH2 receptors was determined by radioligand binding studies using [125I]PTA-OH. Structure-activity relationships led to the conclusions that 2-alkanoic acid derivatives were slightly more potent than 2-mercaptoalkanoic acids. Gupta and Prakash [13] synthesized nucleosides of 5,7-disubstituted pyrido [2,3-d] pyrimidines having antibacterial activity. Khan and Gupta [14] described certain hexahydropyrimidine derivatives possessing antiinflammatory and analgesic activity. Chandra et al. [15] demonstrated synthesis of novel terpenyl pyrimidines having antileishmanial activity.

Observations in the literature on the wide spectrum of pharmacological effects of various pyrimidine derivatives prompted us to plan the synthesis of pyrimidines by treating nalidixic acid ester with guanidine carbonate in the presence of DMF and subsequently the resulting product was condensed with various chalcones to obtain a series of substituted pyrimidine derivatives. The presence of nitrogen in the derived compounds was anticipated to impart towards their antinociceptive and antimicrobial activity.

Experimental Methods

Animals and strains

Swissalbino(SwR) male mice, Staphylococcus aureus (S. aureus, NCTC 10418) and Escherichia coli (E. coli, NCTC6571) were used.

Chemicals and reagents

Nalidixic acid, conc. HCl, CCI4, guanidine carbonate, ethanol, dimethformamide, (DMF), methanol (MeOH), benzene, petroleum ether, dioxane, piperidine, dichloromethane (DCM) (BDH Chemicals Ltd., Poole, UK), 3-acetyl coumarin, carboxymethyl-cellulose sodium salt (CMC), and aspirin (E. Merck AG, Darmstadt, Germany) were used. Sparfloxacin, peptone, yeast extract, meat extract, dextrose, agar, nutrient broth, aldehydes (3,4,5 trimethoxy benzaldehyde, O-hydroxybenzaldehyde, p-bromobenzaldehyde, m-bromobenzaldehyde, 2,4-dichlorobenzaldehyde, p-chlorobenzaldehyde, 2,6-dichlorobenzaldehyde) and other chemicals and reagents used in this study were of analytical reagent grade procured from commercial sources.

Synthesis of 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1,4, dihydro-1,8, naphthyridine-4-one from nalidixic acid (Scheme 1, structure 1):

Almost 6 g (5 ml) of conc. HCl and 30 g (0.2 moles) of nalidixic acid was added to 145 ml (12.5 moles) of absolute ethanol that was chilled in an ice bath and the mixture was refluxed for 8 hours. The pure ester was isolated by extracting with CCl4 and dried. The purity of the ester was established by single spot on TLC plate using benzene: methanol (8:2) solvent system.

Preparation of 3-guanidinyl carboxy-7-methyl 1-ethyl, 1,4 dihydro, 1,8 naphthyridine 4-one (Scheme 1, structure 2):

A mixture of nalidixic acid ester (10 mmoles) and guanidine carbonate (40 mmoles) were added to 30 ml of DMF. The reaction mixture was refluxed for 4 hours and kept overnight for the completion of the reaction, and then poured on crushed ice. The resultant solid was washed, filtered and dried. The compound was recrystallized from methanol, benzene mixture; dried compound was then washed with petroleum ether.
Synthesis of substituted pyrimidines

Scheme 1

![Synthesis of substituted pyrimidines](image)

**Synthesis of chalcones (Scheme 2)**

![Synthesis of chalcones](image)

Novel chalcones were synthesized from 3-acetyl coumarin and a variety of aldehydes, and subsequently these chalcones were used to synthesize a series of condensation products having pyrimidine ring of pharmaceutical interest.

In general, 8 mmoles of 3-acetyl coumarin and 9 mmoles of various aldehydes were refluxed in the presence of a catalyst for a suitable time. The contents were concentrated and cooled down to about 10°C. The products were filtered under reduced pressure and recrystallized. The purity of the compounds was confirmed by TLC, using silica gel G as stationary phase and benzene-acetone (8:2) solvent system. All the synthesized chalcones were characterized by spectral data.

**Synthesis of substituted pyrimidines (structure 6):**

Chalcones were condensed with 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1,4 dihydro, 1,8 naphthyridine-4-one to produce a series of pyrimidine derivatives.

An aliquot of 1 mmole of different chalcones synthesized in Scheme 2 (structure 5) were mixed with equal quantity of 3-guanidinyl carboxy-7-methyl 1-ethyl, 1,4 dihydro, 1,8 naphthyridine 4-one (Scheme 1, structure 2) using 25 ml of dioxane as solvent, 5-6 drops of piperidine as catalyst and refluxed for 11 hours. The reaction mixture was concentrated to half of its original volume and cooled down to normal temperature. The cold reaction mixture was put on crushed ice. The product was filtered under reduced pressure and recrystallized from the mixture of dichloromethane and methanol. Melting points and yield were recorded. The purity of compound was checked by TLC using silica gel G as stationary phase and benzene: methanol (8:2) as solvent system. The compounds were also identified on the basis of spectral data.

**Biological Evaluation**

**Animals and treatments**

Swiss albino (SWR) male mice, weighing 23-26 g, 8-10 weeks old were used. The animals were housed in groups to acclimatize to laboratory conditions for three days before the start of the experiment as for diet, water, temperature, relative humidity and light cycle (7:0 am to 7:0 p.m.). Food and water were made freely accessible.

As a criterion for the dose selection, different doses/concentrations of the synthesized...
pyrimidines were employed to see their individual responses in both the Tail-flick and antimicrobial activity tests. The doses of test compounds versus their response were recorded and the dose/concentration of any compound in the series having equivalent response to the standard drug was selected to be used for all the compounds in the series in both the tests.

**Determination of ED50 using Tail-flick model**

Several doses of each compound were used to construct a dose-response curve. A minimum of 5 animals per dose level were used. Groups of male mice were given synthesized derivatives by intraperitoneal (i.p.) route and tested for response 30 minutes post-treatment using Tail-flick model. From dose/response curve analysis of antinociceptive activities, median antinociceptive dose (ED50) was calculated for each compound by using Litchfield and Wilcoxon test. A computer program (Pharmacological Calculation System, version 3.2, Medical College of Georgia, Augusta, Georgia, USA) was used to calculate this parameter.

**Antinociceptive Evaluation**

In the preliminary experiments on antinociception, it was found that a dose of 100 mg/kg was effective after 30 minutes of administration of some test compounds. On the day of the experiment, the groups of mice were given test compounds (compounds 1-7) and tested for antinociceptive activity 30 minutes post-treatment using Tail-flick model. A minimum of 6 animals per group were used. The suspension of test compounds (in aqueous CMC) was administered i.p. at a constant dose of 100 mg/kg body weight. The control group of animals was given 0.5% CMC solution, only. Another group of animals was administered with aspirin (25 mg/kg) as a standard drug by i.p. route and used for comparison. The pain threshold was recorded by using Columbus Instruments' Tail-Flick Analgesia Meter (Columbus Instruments, Hague Ave, Columbus, OH, USA) as described originally by D'Amour and Smith. Each animal was put in a restrainer and confined so that it was relatively immobile. Stress and suffocation could be avoided by using a cloth over the animal. The tail of the animal was marked 3 cm from the tip and placed on a sensing groove on top of the instrument. The tail of the mice was placed on the light source that produces heat by radiation and apparatus was switched on. As soon as the rodent flick its tail, the instrument turns off the timer using the automatic detection circuitry. Latency to flick tail from the heat source was recorded on the timer of the instrument automatically for individual animals in each group. Treatment cases where the animal failed to respond the test was stopped maximum at 20 seconds to prevent tissue injury.

**Antimicrobial testing procedure**

Staphylococcus aureus (S. aureus, NcTc 10418) and Escherichia coli (E. coli, NcTc6571) test strains were used. Antimicrobial testing of the synthesized compounds was done by agar diffusion method (cup and plate method). A loopful of culture from frozen agar slants was introduced into 10 ml of sterilized nutrient broth and incubated for 24 hours. To obtain working culture, the stock culture was diluted by serial dilution method and
the incubation size of 1:100 was used for further studies. Meat Peptone agar medium (peptone 6.9 g, yeast extract 3.0 g, meat extract 1.5 g, dextrose 1 g, agar 15 g, distilled water q.s. 1L, pH 7.4±0.2) was used in this test. Heterocyclic test compounds were screened for antibacterial activity using DMSO as a solvent. Based on the preliminary experiments, the concentration of test compounds was set to 100 µg/ml and that of standard was set to 20 µg/ml. Washed spores of these organisms were added into sterile and cooled media at 45°C and these seeded media were poured into Petri dishes and allowed to solidify. After the setting of the medium 8 mm diameter, the cavities were bored. Test compounds (100 µg/ml) were placed serially in the cavities using micropipettes and allowed to diffuse for one hour and incubated at 37°C for 24 hours. Sparfloxacin was used as a reference drug.

Statistical Evaluation
The data are expressed as mean ± SEM. The analysis of variance (ANOVA one way) was used and critical values for P<0.01 were considered significant. In post-hoc analysis, Tukey-Kramer multiple comparison test was employed and is stated at the appropriate place.

Results and Discussion
The results of the present studies are given in Tables 1-5.

Chemical synthesis
Synthesis of 3-(3′-(7′-methyl-1′-ethyl-1′, 4′ dihydro-4′-oxo 1′, 8′ naphthryidine) carboxy)-6-coumaryl-4 (3′′, 4′′, 5′′ trimethoxyphenyl)-2-aminopyrimidine (Compound 1):

The condensation of the chalcone C1 with 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1, 4 dihydro 1, 8 naphthyridine-4-one (structure 2) yielded this compound which was recrystallized from DCM and MeOH. M.P. = 250°C. IR spectra of the compound show absorption bands at 1740, 1700, 1628, 1650 and 1590, 1540 cm-1 due to the presence of coumaryl and unsaturated carbonyl groups C=O and C=N, respectively. Other bands appear at 1470, 1440, 1308, 1290, 1210, 1128, 1118, 1100, 950, 800 and 770 cm-1. 1H NMR of the compound revealed that three protons of -CH3 of the ethyl group appears as broad triplet at δ 1.25 (3H, br-t, CH2 CH3). Two protons of the ethyl group appear as multiplet at δ 4.45 (2H, t, CH2 CH3). Olefinic –CH3 group appear as singlet at δ 2.525 (3H, 5, CH3). Nine methoxy proton appear as a two-singlet at δ 3.38 (6H, s, 2-OCH3) and 3.49 (3H, s, OCH3). O-coupled aromatic protons appear as a doublet at δ 7.43 (2H, d, J = 10Hz, Ar-H), δ 7.61 (1H, d, J = 10Hz, Ar-H), δ 7.67 (1H, d, J = 10Hz, Ar-H), δ 7.85 (1H, d, J = 10Hz, Ar-H) and δ 8.41 (1H, d, J = 10Hz, Ar-H). Meta coupled aromatic proton appear as a singlet at δ 7.4 (1H, s, Ar-H), δ 7.51 (1H, s, Ar-H), δ 8.22 (1H, s, Ar-H) and δ 8.59 (1H, s, Ar-H).

The condensation of chalcone C2 with 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1, 4 dihydro 1, 8 naphthyridine-4-one (structure 2) in the presence of piperidine yielded the required compound and recrystallized from DCM and MeOH. M.P. = 245°C. The IR spectra of the compound revealed that absorption bands shown absorption bands appear at 1730, 1608 cm-1 due to the presence of coumaryl and unsaturated carbonyl groups C=O, C=N. Other bands appear at 1572, 1557, 1452, 1421, 1374, 1290, 1271, 1199, 1101, 1073, 1024, 936, 756, 688,
452 cm\(^{-1}\). Mass spectra of the compound shows that the molecular ion appear at m/z 546 (C\(_{31}\) H\(_{24}\) O5 N5\(^{+}\)). The base fragment appears at m/z 356 (C\(_{20}\)H\(_{11}\)O4N\(_{3}\)) [M \pm C\(_{11}\)H\(_{11}\)ON\(_{2}\)]. Other fragments appear at m/z 329, m/z, 327, m/z 299, m/z 270, m/z 243, m/z 215 (C\(_{12}\) H\(_{11}\)O2N\(_{2}\)), m/z 156, m/z 142, m/z 113, m/z 94, m/z 58.

**Synthesis of 3-(3''-(7''-methyl-1''-ethyl-1'', 4'' dihydro-4''-oxo 1'', 8'' naphthyridine) carboxy)-6-coumaryl-4-(O-hydroxy phenyl)-2''-aminopyrimidine (Compound 2):**

The coumaryl chalcon C\(_{3}\) was condensed with 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1', 4 dihydro, 1, 8 naphthyridine-4-one (structure 2) in the presence of piperidine which yielded this compound and recrystallized from DCM and MeOH. The IR spectra of the compound shows absorption bands at 1751, 1699, 1635 cm\(^{-1}\) due to the presence of coumaryl and unsaturated carbonyl groups and C=\(\text{C}\) and C=\(\text{N}\). Other bands appear at 1484, 1250, 1176, 1085, 1039, 857 and 713 cm\(^{-1}\). M.P. = 255\(^{\circ}\)C.

**Synthesis of 3-(3''-(7''-methyl-1''-ethyl-1'', 4'' dihydro-4''-oxo 1'', 8'' naphthyridine) carboxy)-6-coumaryl-4-(p-bromophenyl)-2''-aminopyrimidine (Compound 3):**

The coumaryl chalcon C\(_{4}\) was condensed with 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1', 4 dihydro, 1, 8 naphthyridine-4-one (structure 2) which yielded this compound that was recrystallized from DCM and MeOH. M.P. = 252\(^{\circ}\)C. The IR spectra of the compound shows that absorption bands appear at 1747, 1734, 1717, 1699, 1684, 1672 and 1650 cm\(^{-1}\) due to the presence of coumaryl and unsaturated carbonyl groups and C=\(\text{C}\) and C=\(\text{N}\), respectively. Other bands appear at 1558, 1541, 1521, 1508, 1490, 1472, 1457, 1419 and 1396 cm\(^{-1}\). 1H NMR spectra of the compound shows that three protons of -CH3 of the -C2H5 group appear as a broad triplet at \(\delta\) 1.41 (3H, br-t, CH2 CH3). Two protons of the -C2H5 group appear as a multiplet at \(\delta\) 4.65 (2H, m, CH2 CH3). Six aromatic protons appear as a multiplet at \(\delta\) 7.48 (6H, m, Ar-H). Two protons also appear as a multiplet at \(\delta\) 7.65 (2H, m, Ar-H). Two ortho-coupled protons appear as two doublets at \(\delta\) 7.84 and \(\delta\) 8.40, one proton each. Two m-coupled protons appear as a singlet at \(\delta\) 8.23 and \(\delta\) 8.59 for one each proton. Pyrimidine proton appears as singlet at \(\delta\) 7.61 (1H, 5, Ar-H).

**Synthesis of 3-(3''-(7''-methyl-1''-ethyl-1'', 4'' dihydro-4''-oxo 1'', 8'' naphthyridine) carboxy)-6-coumaryl (2, 4-dichlorophenyl)-2''-aminopyrimidine (Compound 5):**

The coumaryl chalcon C\(_{5}\) was condensed with compound 3-guanidinyl carboxy-7-methyl, 1-ethyl, 1, 4 dihydro 1, 8 naphthyridine-4-one (structure 2) in the presence of piperidine yielding this compound which was recrystallized from DCM and MeOH. M.P. = 160\(^{\circ}\)C. The IR spectra of the compound shows absorption bands at 1648, 1663, 1584, 1528 cm\(^{-1}\) due to the presence of coumaryl and unsaturated carbonyl groups and C=\(\text{C}\), C=\(\text{N}\). Other bands appear at 1471, 1442, 1409, 1215, 1176, 1133, 1088, 1051, 872 and

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<th>C Calculated</th>
<th>H Found</th>
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*Synthesis of 3-(3''-(7''-methyl-1''-ethyl-1'', 4'' dihydro-4''-oxo 1'', 8'' naphthyridine) carboxy)-6-coumaryl-4-(O-hydroxy phenyl)-2''-aminopyrimidine (Compound 2):*
743 cm⁻¹. 1H NMR spectra of the compound shows three protons of the ethyl group appear as a broad triplet at δ 1.54 (3H, br-t, CH₂ CH₃). Two protons of ethyl group appear as multiplet at δ 6.95 (1H, m, CH₂). Two aromatic meta-coupled protons appear as singlet at δ 7.25 (2H, s, Ar-H).

Four aromatic protons appear as a multiplet at δ 7.403 (4H, m, Ar-H). Three O-coupled proton appear as three doublets at δ 7.52 (1H, d, J = 12 Hz, Ar-H), 7.63 (1H, d, J = 12 Hz, Ar-H) and 8.13 (1H, d, J = 12 Hz, Ar-H). Two more aromatic protons appear as singlet at δ 8.02 (1H, s, Ar-H) and δ 8.06 (1H, s, Ar-H). Pyrimidine proton appears as a singlet at δ 7.61 (1H, s, Ar-H).

**Synthesis of 3-(3′-(7′-methyl-1′-ethyl-1′, 4′ dihydro-4′-oxo-1′, 8′ napththyridine) carboxy)-6-coumaryl-4-(p-chlorophenyl phenyl) 2-aminopyrimidine (Compound 6):**

The condensation of chalcone C6 with 3-guanidinyl carboxy-7-methyl 1-ethyl, 1, 4 dihydro, 1, 8 napththyridine-4-one (structure 2) yielded the required compound which was recrystallized from DCM and MeOH. M.P. = 55°C. The IR spectra of the compound revealed that absorption bands appear at 1722.7, 1685, 1607, 1544 cm⁻¹ due to the presence of coumaryl ketone, saturated ketone, C=C and C=N, respectively. Other bands appear at 1454, 1435, 1385, 1268, 1178, 1122, 854, 757, 668 cm⁻¹. 1H NMR of the compound revealed that three protons of methyl group of ethyl group appear as broad triplet at δ 1.25 (3H, br-t, CH₂ CH₃). Two protons of the -C₂H₅ group appear at δ 4.72 (2H, q, CH₂ CH₃). Olefinic methyl appears as a singlet at δ 2.42 (3H, s, -CH₃). One aromatic proton appears as a broad singlet at δ 6.82. Another proton appears at δ 6.97 as broad singlet (1H, br-s, Ar-H). Three aromatic protons appear as multiplet at δ 7.15 (3H, m, Ar-H). Other three aromatic protons appear as multiplet at δ 7.35 (3H, m, Ar-H). Two protons appear as a multiplet at δ 7.53 (2H, m, Ar-H). One proton of pyrimidine ring appears as singlet at δ 7.8 (1H, s, NH). Other two protons appear as a multiplet at δ 8.04 (2H, m, Ar-H).

**Biological evaluation**

Chalcone substituted pyrimidines showed appreciable activity against Gram -ve strains, but failed to demonstrate any activity against Gram +ve strains. In the case of Gram -ve strain (E. coli) bromo substitution derivative, p-chloro substitution and 2.4 dichloro substitution derivatives showed improved antibacterial activity. However, the antimicrobial activity of m-bromo derivative was high as compared to control. Trimethoxy derivatives had weak inhibitory properties (Table 3).

The structures shown against compound number represent substitution at X-position in a pyrimidine.

In the determination of ED₅₀ values using Tail-flick test, meta bromo-substituted derivative was most potent, while p-bromo substitution demonstrated ED₅₀ values more than twofold of m-bromo-substituted derivative. The increasing order of the ED₅₀ values for these derivatives is 4<5<6<1<2<3<7<8 (Table 4).

Five male mice were used at each dose level for Litchfield and Wilcoxon test. Similarly, pyrimidine derivatives like m-bromo derivative showed significantly (P<.001) higher
antinociceptive activity than chloro substituted derivatives; trimethoxy substitution of benzene ring demonstrated moderate activity, and the antinociceptive response of m-bromo derivative was comparable to that of the standard drug. However, comparing m- and p-bromo substitutions, it was observed that p-bromo substitution had least antinociceptive effect and it was close to the control. There had been significant ($P < 0.01$) difference in the antinociceptive response of both the bromo-substituted derivatives. Evidence in the literature for such effects is lacking; hence a comparison is difficult. In the present studies, it was observed that bromo-substitution at

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<th>Substitution At X- position</th>
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<td>Nalidixic acid</td>
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Table 3. Antimicrobial activity of the synthesized compounds against Gram +ve and Gram –ve microorganisms

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<td>Substitution at X</td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
<td><img src="image" alt="Structure" /></td>
</tr>
<tr>
<td>ED50 mg/kg (95% CL)</td>
<td>75.00</td>
<td>85.89</td>
<td>125.00</td>
<td>50.00</td>
<td>71.42</td>
<td>71.42</td>
<td>97.82</td>
</tr>
</tbody>
</table>

Table 4. Determination of ED50 of synthesized pyrimidine derivatives in the induction of analgesia by using Tail-flick test in mice
meta-position demonstrated comparatively higher potential for its antibacterial as well as antinociceptive properties (Table 5).

The structures shown against compound number represent substitution at X-position in a chalcone substituted pyrimidine. Six animals were used in each group. Latency in Tail-flick response was observed 30 minutes after the treatments. Treatment groups were compared by using ANOVA. The level of significance was accepted at $P<0.01$. In post hoc analysis, Tukey-Kramer multiple comparison test was used. $P<0.001$, compared to Naïve control; @ $= P<0.01$, compared to aspirin group; and # = $P<0.01$, compared to compound 3.

References


Table 5. Effect of synthesized pyrimidines on the nociceptive response of mice in Tail-flick test

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Treatment</th>
<th>Substitution at X-Position</th>
<th>Tail-flick Latency Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Naïve Control (0.5% CMC)</td>
<td>--</td>
<td>2.3 ± 0.204</td>
</tr>
<tr>
<td>2</td>
<td>Aspirin (25 mg/kg, i.p.)</td>
<td>--</td>
<td>9.0 ± 1.061***</td>
</tr>
<tr>
<td>3</td>
<td>Compound 1 (100 mg/kg, i.p.)</td>
<td></td>
<td>6.0 ± 0.694</td>
</tr>
<tr>
<td>4</td>
<td>Compound 2 (100 mg/kg, i.p.)</td>
<td></td>
<td>5.3 ± 0.122</td>
</tr>
<tr>
<td>5</td>
<td>Compound 3 (100 mg/kg, i.p.)</td>
<td></td>
<td>3.6 ± 2.368@</td>
</tr>
<tr>
<td>6</td>
<td>Compound 4 (100 mg/kg, i.p.)</td>
<td></td>
<td>9.0 ± 1.061***#</td>
</tr>
<tr>
<td>7</td>
<td>Compound 5 (100 mg/kg, i.p.)</td>
<td></td>
<td>6.3 ± 0.216</td>
</tr>
<tr>
<td>8</td>
<td>Compound 6 (100 mg/kg, i.p.)</td>
<td></td>
<td>6.3 ± 0.216</td>
</tr>
<tr>
<td>9</td>
<td>Compound 7 (100 mg/kg, i.p.)</td>
<td></td>
<td>4.6 ± 0.236</td>
</tr>
</tbody>
</table>


