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ANTI-INFLAMMATORY, ANALGESIC AND ANTIMICROBIAL ACTIVITIES OF SOME SYNTHETIC FURANONES AND THEIR PYRROLONE DERIVATIVES

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ABSTRACT: In the present study, 3-naphthoyl propionic acid and various appropriate reagents were used to synthesize, ten new synthetic furanone derivatives, *i.e.* 3-arylidene-5-(naphthalen-2-yl) furan-2(3H)-one (2-11). Selected furanones were made to react with ammonia and benzylamine to yield analogous pyrrolones (12-16) and N-benzyl pyrrolones (17-21), respectively, which were characterized on the basis of ^{1}H NMR, Mass spectroscopic data. Synthesized compounds were tested for anti-inflammatory activity by the carrageenan-induced rat paw edema method, compounds displaying compelling anti-inflammatory activity were screened for analgesic activity by employing the acetic-acid induced writhing method, and selected compounds were tested for ulcerogenic activity. Screening of the newly synthesized compounds was also done for their antibacterial profile against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa,* and antifungal activity against *Aspergillus flavus, Aspergillus niger.* One of the derivatives numbered (9) displayed strong anti-inflammatory, analgesic, and antimicrobial activities, and it could prove as a promising candidate for the development of new and safer leads.

INTRODUCTION: Butenolides derivatives are a set of essential compounds containing distinctive carbon skeleton of 2(5*H*) and 2(3*H*) furanone and can be considered as furan derivatives *i.e.*, 2, 3 and 2, 5 dihydro furan-2-one [1-3,] and the name "furanone" was initially used by Klobb [4]. They are also acknowledged as butyrolactones / crotono-lactones / (α, β)-angelica lactones [5, 6].

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They are also represented by the term "Furanones" [7] hence $\Delta^{\beta,\gamma}$–furanones are 2(3*H*)-furanones and $\Delta^{\alpha,\beta}$-furanones are 2(5*H*)-furanones. There are a bunch of steroids that contain α, β unsaturated furanone ring in the side chain; such steroids are named cardenolides [8-10]. Furanones consist of unsaturated γ- lactone, which is widespread in many natural products [11-12]. Some of them are present in plants as Glycosides. Furanones are reported to have an important biological profile of activities like anti-inflammatory, analgesic, anti-bacterial, antiviral, anticonvulsant, and anticancer [13-15]. Hydrolysis of these compounds and hydrogenation of the double bond in their structure results in loss of activity of these compounds.

The lactone ring present in furanones is very reactive and used for synthesis of pyrrolones (nitrogen heterocycles) with significant biological activities [16-17]. Pyrrolones are five-membered heterocyclic lactams, either Δ^1 or Δ^2 derivatives have been described by several authors [18-19], also well known as pyrrolin-2-ones. Pyrrolinones have been used effectively in the synthesis of numerous alkaloids [20-24] and are suitable predecessor for unusual γ-amino acids such as statins and its analogs [25]. There are so several instances where pyrrolone containing moiety reported with various pharmacological activities [26-28]. A novel series containing furanone derivatives displaying good to significant results of antimicrobial, analgesic, and anti-inflammatory activities with reduced gastric ulceration has been informed in literature [29-31]. So it is thought worthwhile in order to explore further the diversity of furanone nucleus, to synthesize new derivatives of pyrrolones, N-benzyl-pyrrolone derivatives based on furanone moiety. All the prepared compounds were also evaluated for their bactericidal activity against *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* and anti-fungal activity against *Aspergillus flavus* and *Aspergillus niger*.

MATERIALS AND METHODS:

Chemistry: Labindia MR-VIS visual melting point apparatus was used to get melting points of the compounds and were uncorrected. Thin-layer chromatography was utilized to identify the purity of newly synthesized compounds. The solvents systems used for the same was - Pet Ether: Toluene: Ethyl acetate (5:4:1) (PTE). Iodine chamber and UV Cabinets were the techniques used for visualization of TLC plates. The proton magnetic resonance spectra were obtained on Bruker 300 MHz instruments in $CDCl_3$, DMSO using Tetra Methyl Silane (TMS) as internal standard. The IR spectra were obtained on potassium bromide pellets using Hitachi spectrophotometer 150-20. Mass spectra were obtained on a Jeol JMS-D 300 instrument fitted with a JMS 2000 data system at 70 eV.

Procedure for the Synthesis of Aroyl Propionic Acid: Freshly prepared succinic anhydride (1mol) was condensed with sublimated naphthalene (1mol) in the presence of anhydrous aluminium chloride (2.1mol) and dry nitrobenzene as a solvent to yield 3-(naphthoyl) propionic acid (1). Refluxed the reaction mixture continuously with stirring for four h. Addition of anhydrous $AlCl_3$ was accomplished after 30 min of reaction commencement. As the reaction was done, excessive nitrobenzene was distilled off. The product was then purified and dissolved in sodium hydroxide solution and filtered after the addition of dilute hydrochloric acid. The obtained solid mass was washed with cold water, dried and recrystallized using methanol, and used further for synthesis of furanone derivatives.

Procedure of Synthesis of 3-arylidene-5-(naphthalen-2-yl) furan-2(3H)-one (2-11): A mixture containing 3-(naphthoyl) propionic acid (1 mol) in acetic anhydride (10-15 ml), triethylamine, and an aromatic aldehyde (1mol) was refluxed under anhydrous conditions for four hours. The reaction completion was detected with the help of TLC using (PTE) (5:4:1) as a mobile phase. As the reaction ends, the whole content was poured on ice successively with stirring. A colored solid mass gets separated out, which was filtered, washed, and dried, further crystallized by using methanol to give the desired product.

3- (4-bromobenzylidene)-5-(naphthalen-2-yl)furan-2(3H)-one (2) yellow colored compound, Yield 45%, mp 177-179 °C; IR: 1770, 1602; ^{1}H-NMR: δ 6.95, s, 1H(βH), 7.46,s,1H (Olefinic H), 7.56-8.46,m,11H (Aromatic proton); MS: *m/z* 378 (M^+), 155, 127.

3- (4-chlorobenzylidene)-5-(naphthalen-2-yl)furan-2(3H)-one (3) yellow colored compound, Yield 47%, mp 180°C; IR: 1762, 1450-1600;): ^{1}H-NMR δ 7.04, s, 1H (βH), 7.47, s, 1H (Olefinic H), 7.53-8.31, m, 11H (Aromatic proton); MS: *m/z* 332 (M^+), 155, 127.

3- (4- (trifluoromethyl)benzylidene)-5-(naphthalen-2-yl)furan-2(3H)-one (4) *brown colored compound*, Yield 25%, mp 188°C; IR: 1770, 1450-1600; ^{1}H-NMR δ 6.90, s, 1H (βH), 7.45, s, 1H (Olefinic H), 7.54-8.4,m,11H (Aromatic proton); MS: *m/z* 366 (M^+), 155, 127.

3- (2-nitrobenzylidene)- 5-(naphthalen-2-yl)furan-2 (3H)-one (5) *brown Red colored compound*, Yield 30%, mp 170°C; IR:1774, 1450-1600; ^{1}H-NMR δ 6.90, s, 1H, 7.47, s, 1H (Olefinic H), 7.55-8.30, m, 11H (Aromatic proton); MS: *m/z* 343 (M^+), 155, 127.

3-(4-methylbenzylidene)-5-(naphthalen-2-yl)furan-2(3H)-one (6) yellow colored compound, Yield 40%, mp 140°C; IR: 1759, 1450-1600; ^{1}H-NMR δ 6.9, s, 1H, 7.48, s, 1H (Olefinic H), 7.54-7.98, m, 11H; 2.23, s, 3H,-CH_3 (Aromatic proton); MS: *m/z* 312 (M^+), 155, 127.

3- (4-fluorobenzylidene)-5-(naphthalen-2-yl)furan-2(3H)-one (7) yellow colored compound, Yield 32%, mp 176°C; IR: 1760, 1450-1600; ^{1}H-NMR δ 6.93, s,1H (βH), 7.45, s,1H (Olefinic H), 7.54-8.20,m,11H (Aromatic proton); MS: *m/z* 316 (M^+), 155, 127.

3- (3- nitrobenzylidene)-5-(naphthalen-2-yl)furan-2 (3H)-one (8) yellow colored compound, Yield 35%, mp 171 °C; IR: 1774, 1450-1600; ^{1}H-NMR δ 6.90, s,1H (βH), 7.47, s,1H (Olefinic H), 7.54-8.38, m, 11H (Aromatic proton); MS: *m/z* 343 (M^+), 155, 127.

3- (4 -methoxybenzylidene)- 5- (naphthalen-2-yl) furan- 2(3H)- one (9) brown colored compound, Yield 25%, mp 122°C; IR: 1755, 1450-1600; ^{1}H-NMR δ 6.9, s, 1H (βH), 7.48, s, 1H (Olefinic H), 7.54-7.98, m, 11H (Aromatic proton); MS: *m/z* 328 (M^+), 155, 127.

3-benzylidene-5-(naphthalen-2-yl)furan-2(3H)-one (10) yellow colored compound, Yield 30%, mp 171°C; IR: 1759, 1450-1600; ^{1}H-NMR δ 6.90,s,1H (βH), 7.46, s, 1H (Olefinic H), 7.5-8.0, m, 12H (Aromatic proton); MS: *m/z* 328 (M^+), 155, 127.

3- (4- (dimethylamino)benzylidene)-5-(naphthalen-2-yl) furan- 2(3H)- one (11) yellow colored compound, Yield 30%, mp 171 °C; IR: 1751, 1450-1600; ^{1}H-NMR δ 7.0, s, 1H (βH), 7.41, s, 1H (Olefinic H), 6.72-7.93, 11H; 3.1, s, 6H, 2x (CH_3) (Aromatic proton); MS: *m/z* 341 (M^+), 155, 127.

Procedure for the Synthesis of 3-arylidene-5-(naphthalen-2-yl)-2(3H)-pyrrolone (12-16): To the solution of 3-benzylidene-5-(naphthalen-2-yl) furan-2(3H)-one (1mol) in water (12.5ml) and ethanol (25ml), concentrated ammonia solution was added then refluxed for 30 min. The concentrated ammonia solution (25ml) and anhydrous K_2CO_3 (1mol) was added after one hour during refluxing. More ammonia solution was added from time to time. The reaction completion was detected gradually with the help of TLC using (PTE) (5:4:1). Orange-red product was obtained. Washed the product using hot water and recrystallization was accomplished with methanol to obtain products from 12 to 16.

3-(4-bromobenzylidene)-5-(naphthalen-2-yl)-2(3H)-pyrrolone (12) yellow colored compound, Yield 20%, mp 104°C; IR: NH-3418 CO-1674, 1602; ^{1}H-NMR δ 7.10, s, 1H (βH), 7.41, s, 1H (Olefinic H), 7.45-8.23, m, 12H (Aromatic proton); MS: *m/z* 375 (M^+), 152, 127.

3-(4-chlorobenzylidene)-5-(naphthalen-2-yl)-2(3H)-pyrrolone (13) yellow colored compound, Yield 25%, mp 118°C; IR: NH-3421 CO-1670, 1602; ^{1}H-NMR δ 6.95, s, 1H (βH), 7.47, s, 1H (Olefinic H), 7.53-8.3, m, 12H (Aromatic proton); MS: *m/z* 331 (M^+), 153, 127.

3-(4-flourobenzylidene)-5-(naphthalen-2-yl)-2(3H)-pyrrolone (14**)** yellow colored compound, Yield 30%, mp 121 °C; IR: NH-3441 CO-1674, 1602; ^{1}H-NMR δ 6.93,s,1H (βH), 7.45, s, 1H (Olefinic H), 7.54-8.20, m, 12H (Aromatic proton); MS: *m/z* 315 (M^+), 153, 127.

3- (2-nitrobenzylidene)-5-(naphthalen-2-yl)-2(3H)-pyrrolone (15) yellow colored compound, Yield 25%, mp 107°C; IR: NH-3420 CO-1695, 1602; ^{1}H-NMR δ 6.99,s,1H (βH), 7.46, s, 1H (Olefinic H), 7.5-8.38, m, 11H (Aromatic proton); MS: *m/z* 342 (M^+), 152, 127.

3-(4-methylbenzylidene)-5-(naphthalen-2-yl)-2(3H)-pyrrolone (16) yellow colored compound, Yield 28%, mp 101°C; IR: NH-3410 CO-1690, 1602; ^{1}H-NMR δ 6.9, s, 1H (βH), 7.48, s, 1H (Olefinic H), 2.23, s, 3H, -CH_3, 7.54-7.98, m, 11H (Aromatic proton); MS: *m/z* 311 (M^+), 152, 127.

General Procedure for the Synthesis of 3-arylidene- 5- (naphathyl)-1-benzyl-2(3H)-pyrrolone (17-21): Synthesis of the titled compounds was done by adopting the following two steps consecutively.

Synthesis of γ-Ketobenzylamide: Furanones (3 mol) and benzylamine (4 mol) were refluxed for two hours in the existence of dry benzene. The reaction completion was detected from time to time with the help of TLC using (PTE) (5:4:1) as a mobile phase. As the reaction proceeds towards

end, the solvent remaining was distilled off. Washed the product so obtained with petroleum ether and air-dried. The crude product was used for further steps of synthesis.

Lactamization of γ-Ketobenzylamide: Recovered product was refluxed with 20 ml HCl (6 N) for one hour. The reaction completion was detected from time to time with the help of TLC using (PTE) (5:4:1) as a mobile phase. The content was cooled, and product was separated, washed thoroughly with water, and purified from methanol to give 17-21.

3- (4- chlorobenzylidene)-5-(naphthyl)-1-benzyl-2 (3H)-pyrrolone (17) yellow colored compound, Yield 30%, mp 146°C; IR: CO-1697, 1602; ^{1}H-NMR δ 6.67, s, 1H (βH), 7.46, s, 1H (Olefinic H), 7.52-8.34, m, 16H (Aromatic proton); 4.98, s, 2H **(**CH_2 proton); MS: *m/z* 421(M^+), 330, 155, 127,91.

3- (4-methylbenzylidene)- 5-(naphthyl)-1-benzyl-2 (3H)-pyrrolone (18) yellow crystal, Yield 28%, mp 146°C; IR: CO-1690, 1602; ^{1}H-NMR δ 6.46, s, 1H (βH), 7.41, s, 1H (Olefinic H), 7.52-7.9, m, 16H (Aromatic proton); 4.92, s, 2H **(**CH_2 proton); MS: *m/z* 401(M^+), 310, 155, 127, 91.

3- (4- bromobenzylidene)- 5-(naphthyl)-1-benzyl-2 (3H)-pyrrolone (19) yellow crystal, Yield 28%, mp 125°C; IR: CO-1690, 1602; ^{1}H-NMR δ 6.71, s, 1H (βH), 7.46, s, 1H (Olefinic H), 7.5-8.3, m, 16H (Aromatic proton); 4.92, s, 2H **(**CH_2 proton); MS: *m/z* 466 (M^+), not observed, 155, 127, 91.

3- (4-N, N Dimethyl amino benzylidene)-5-(naphthyl)-1-benzyl-2(3H)-pyrrolone (20) yellow crystal, Yield 30%, mp 130°C; IR: CO-1688, 1602; ^{1}H-NMR δ 6.50, s, 1H (βH), 7.45, s, 1H (Olefinic H), 7.53-8.01,m,16H (Aromatic proton); 4.92, s, 2H **(**CH_2 proton); MS: *m/z* 430 (M^+), 309, 155, 127, 91.

3-(4-methoxybenzylidene)-5-(naphthyl)-1-benzyl-2 (3H)-pyrrolone (21) yellow crystal, Yield 22%, mp 152°C; IR: CO-1680, 1602; ^{1}H-NMR δ 6.76,s,1H (βH), 7.43, s, 1H (Olefinic H), 7.52-7.92, m.16H (Aromatic proton); 4.96, s, 2H **(**CH_2 proton); MS: *m/z* 417 (M^+), 326, 155, 127, 91.

Biological Evaluation:

Experimental Animals: For conducting the experiment, the animals were maintained under standard laboratory conditions of temperature (25 ± 2 °C) and humidity (55 ± 5%). All the animals were fed with a commercial pellet diet, and water was provided *ad libitum* throughout the course of study. Permission for conducting the experiment was obtained from the Institutional Animal Ethical Committee (IAEC) Kurukshetra University, Kurukshetra (Regd No. 562/GO/Re/S/02/CPCSEA) and was in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Anti-inflammatory Activity: The anti-inflammatory biological profile of the discovered derivatives was carried out by the reported method [32]. Nimesulide was used at a dose of 4mg/kg as a standard drug. The test (50mg/kg) and standard compounds were administered orally. All the doses of standard and test drugs were prepared in 0.5% w/v Tween 80 which served as drug vehicle. The control group was given only 0.2 ml (10ml/kg) of Tween. After then, 1% w/v carrageenan suspension (0.1 ml) was injected subcutaneously into hind paw of each rat. The paw volume was determined by the mercury displacement method in plethysmograph at 0, 30 60, 90, 120 min intervals. The percent inflammation of paw was computed with the help of the formula given below.

$$\Delta T = T_t - T_0$$
$$\% \text{ inflammation} = \Delta T/T_0 \times 100$$
$$\% I = 100 - \% \text{ inflammation}$$

Where, T_t = the right hind paw thickness at time t, T_0= the right hind paw thickness before sub-planter injection of carrageenan.

Analgesic Activity: Acetic acid-induced writhing test was carried out in Swiss albino mice using the previously reported method [33]. Swiss albino mice of either sex weight between 20-25g were used. Animals were divided into a group of 6 animals, each for control, standard, and test derivatives. Control group of 6 animals received 0.2 ml (10ml/kg) of Tween alone. Standard group received diclofenac sodium at dose of 50mg/kg body weight and rest of the groups were administered all synthesized compounds orally at a 50mg/kg. Post 30 min administration of test samples, 1% v/v acetic acid (inject 1ml/100g i.p. of body weight of the animal) was given to mice intraperitoneally. Mice were observed for ten minutes and counted the number of writhes for each animal.

The percentage protection was calculated by following formula:

% Protection = 100 – No. of writhes in test × 100 / No. of writhes in control

Ulcerogenic Activity: Rats weighing 180-200 grams were used for the evaluation of ulcerogenic activity, which was carried out by reported method [34]. Test samples and standard drug (diclofenac) were administered by oral route at 50 mg/kg. All the doses of standard and test drugs were prepared in 0.5% w/v tween 80, which served as drug vehicle. Control rats received vehicle (0.2 ml (10 ml/kg) alone orally. Animals were sacrificed after 4 hours of dose administration. The gastric mucosa of the rat was examined by means of magnifying lens for ulcer score.

***In-vitro* Antibacterial Activity:** Newly synthesized furanones and pyrrolones were analyzed for their bactericidal activity against *Escherichia coli* (ATCC-8739), *Staphylococcus Aureus* (ATCC-29737), and *Pseudomonas Aeruginosa* (NCLM-2035) at 100 µl mL^{-1} by Agar well diffusion method [35, 36]. By virtue of the zone reader (Hi Antibiotic zone scale), Zone of inhibition was measured for each compound. Ciprofloxacin and DMSO were employed as positive control and negative control. Minimum inhibitory concentration (MIC) of various compounds was tested through macro dilution tube method as suggested by NCCLS [35]. In this, the test compounds series were prepared in the concentration range from 128 to 25µg/ml.

***In-vitro* Antifungal Activity:** The antifungal activity of prepared compounds was tested by poison food technique [37]. Sabouraud Dextrose Agar (SDA), was used for mold growth at 25 ºC for 7 days to be used for inocula. The 15ml melted SDA (45 ºC) was poisoned by the addition of 100µl of test compounds in the concentration of 4.0mg/ml each dissolved in DMSO and solidified at room temperature. In this technique, DMSO was taken as the negative control, whereas Fluconazole was a positive control. All these experiments were carried out in triplicates.

RESULTS AND DISCUSSION:

Chemistry: Novel 3-arylidene-5-(naphthalen-2-yl) furan-2(3H)-ones (2-11) were prepared by using 3-(naphthoyl) propionic acid (1) with various aromatic aldehydes in presence of triethylamine in acetic anhydride as solvent. 3-arylidene-5-(naphthalen-2-yl)- 2(3H)- pyrrolone derivatives (12-16) were obtained by reacting the appropriate furanones utilizing dry ammonia in absolute ethanol. The 3-arylidene- 5- (naphathyl)-1-benzyl-2(3H)-pyrrolone (17-21) were synthesized stepwise, firstly by reacting the appropriate furanone derivatives and benzylamine with the help of dry benzene as solvent resulting from giving γ-ketobenzylamides, which were further lactamized to give the corresponding benzylpyrrolones. Spectral data of IR, NMR and MS were in accordance with the structures assigned to the compounds. ^{1}H-NMR spectral data of furanones clearly exhibited two singlets around δ 6.9 and δ7.4 ppm for one proton each of the ring βH and another can be allocated for the olefinic hydrogen of the arylidene substituent. While for N-benzyl pyrrolone, in addition to the above-obtained peaks, there appears one characteristic singlet which can be attributed to two methylene hydrogens at δ4.9. The mass spectra of the title compounds was also according to mass to charge ratio. The major fragment came out to be $C_{10}H_7$-C≡O^+ arising from heterocyclic oxygen which loses CO to give $C_{10}H_7^+$. Again there a peak appeared at *m/z* 127 that also corresponds to $C_{10}H_7^+$ proved the product formation. Now about spectral results of pyrrolones (12-16), the major fragmentation is obtained as R-C_6H_4-C≡N^+H, which is followed by loss of HCN to provide $C_{10}H_7^+$. For the compounds (17-21), decrease of 91 mass units was observed that can be correlated to the benzyl moiety from the molecular ion along with peaks at *m/z* 91.

Anti-inflammatory Activity: Carrageenan induced rat paw edema method was used to assess the *in-vivo* anti-inflammatory activity of all synthesized compounds. Two of the derivatives (5) and (9) exhibited maximum anti-inflammatory activity of 48% inhibition at 120 min as compared to a standard drug that displayed inhibition of 46% at 120 min. Compound (1), (4), and (7) displayed inhibition of 47% each at 120 min. Among the list of prepared compounds it was found that furanones exhibited significant anti-inflammatory activity as compared to pyrrolones and N- benzyl pyrrolones. Results are tabulated in **Table 1**.

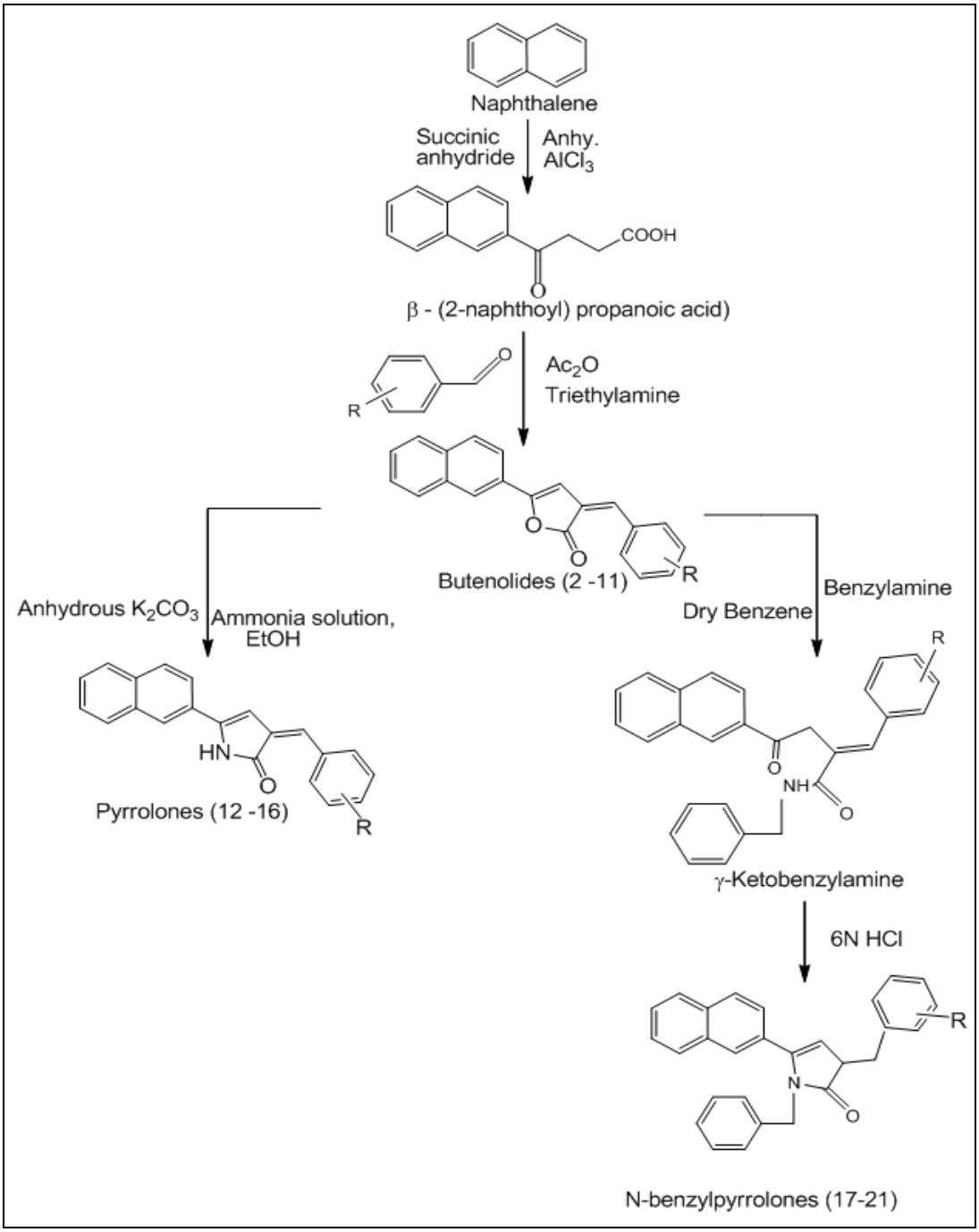

FIG. 1: SYNTHETIC SCHEME FOR TITLE COMPOUNDS

Analgesic Activity: The synthesized compounds displaying compelling anti-inflammatory activity were screened for analgesic activity by employing acetic-acid induced writhing method. The percentage inhibition was obtained in the range of 59 to 74% **Table 2**. Maximum of the compounds that were screened for analgesic activity showed better results as compared to standard. Compound (9) exhibited maximum analgesic activity (74%), which was found to be comparable with the standard displaying percentage inhibition 76.26% at 50 mg/kg.

Ulcerogenic Activity: Few compounds were analyzed for ulcerogenic activity by pylorus ligated method. The average score of ulcers in the gastric mucosa after oral administration of title compounds are reported in **Table 3**. The furanones (3) and pyrrolones (12) derivatives were significantly less ulcerogenic than the standard and aroyl propionic acid.

Antimicrobial Activity: In the present study, twelve chemically synthesized compounds were evaluated against various strains of gram-positive,

gram-negative bacteria and fungus. MIC (Minimum Inhibitory concentration) of those compounds was determined, which were found active in primary screening. Ciprofloxacin and Fluconazole were used as standard drugs. Tested compounds of the series exhibited good antimicrobial properties against both bacteria and fungi. Compounds (12) and (14) were observed to be the most dominant of the series against *S. aureus* with zone of inhibition stretching from 20.6 to 21.6 mm, and derivative (2) displayed significant activity against *B. subtilis*, with zone of inhibition of 18.6. However, none of the examined compounds showed any activity against Gram-negative bacteria **Table 4**.

Minimum Inhibitory Concentration (MIC) of all the synthesized derivatives fall in the range of 32 to 128 µg/ml against S. *aureus*, with compounds (12) and (14) with highest of the range MIC of 32µg/ml, whereas in the case of *B. subtilis*, MIC of compounds ranged between 64 and 128 µg/ml with compounds (2) and (12) displaying highest MIC of 64 µg/ml **Table 5**.

Of the tested products, compounds (1), (9), (13), (14), and (18) illustrated 50% inhibition of mycelial growth against *Aspergillus niger* fungus strain, whereas compound (12), (13), (14) and (19) demonstrated 50% inhibition of mycelial growth against *Aspergillus flavus* **Table 6**.

Statistical Analysis: All the results of Anti-inflammatory activity **Table 1**, Analgesic activity **Table 2**, Ulcerogenic activity **Table 3**, are articulated as Mean ± Standard error (SEM). Obtained data was investigated on one-way ANOVA pursued by Dunnett: Compare all vs. control. p-value <0.05 are considered as statistically significant and p-value < 0.01 are measured as extremely significant.

TABLE 1: EFFECT OF COMPOUNDS ON CARRAGEENAN INDUCED PAW EDEMA VOLUME IN RATS

Treatment (Compound No.)	Paw edema volume Mean ± SEM (% inhibition)			
	30 min.	60 min	90 min.	120 min.
Control	0.40±0.02	0.65±0.02	0.74±0.0*	0.93±0.01*
Standard	0.31±0.02(10%)	0.41±0.02*(36%)	0.47±0.0*(36%)	0.50±0.0(46%)
(1)	0.36±0.03(15%)	0.47±0.03*(27%)	0.50±0.0*(20%)	0.49±0.01*(47%)
(2)	0.46±0.01(17%)	0.43±0.05*(33%)	0.47±0.0*(36%)	0.53±0.02*(43%)
(3)	0.47±0.09(15%)	0.47±0.04*(27%)	0.47±0.07*(36%)	0.53±0.02*(43%)
(4)	0.34±0.02(15%)	0.36±0.01*(44%)	0.40±0.08*(45%)	0.49±0.03*(47%)
(5)	0.46±0.019(12%)	0.45±0.03*(30%)	0.53±0.01*(28%)	0.55±0.03*(48%)
(6)	0.39±0.04(12%)	0.42±0.02*(35%)	0.49±0.03*(33%)	0.52±0.02*(44%)
(7)	0.34±0.02(13%)	0.36±0.01*(44%)	0.40±0.08*(45%)	0.49±0.03*(47%)
(8)	0.45±0.04(12%)	0.47±0.02*(27%)	0.53±0.05*(28%)	0.60±0.03*(35%)
(9)	0.47±0.05(17%)	0.43±0.05*(33%)	0.47±0.07*(36%)	0.55±0.03*(48%)
(10)	0.45±0.06(12%)	0.47±0.04*(27%)	0.47±0.07*(36%)	0.53±0.02*(43%)
(11)	0.47±0.02(17%)	0.58±0.02(10%)	0.58±0.02(21%)	0.60±0.03*(35%)
(12)	0.35±0.08(12%)	0.56±0.02**(13%)	0.57±0.02*(22%)	0.59±0.03*(36%)
(13)	0.35±0.03(12%)	0.45±0.02*(30%)	0.48±0.01*(35%)	0.66±0.01*(29%)
(14)	0.35±0.02(12%)	0.45±0.02**(30%)	0.46±04*(37%)	0.66±0.01*(29%)
(15)	0.40±0.02(0%)	0.47±0.04*(27%)	0.48±0.04*(35%)	0.57±0.02*(38%)
(16)	0.35±0.01(12%)	0.47±0.03(27%)	0.64±0.01(13%)	0.65±0.01*(30%)
(17)	0.45±0.06(12%)	0.56±0.02(13%)	0.60±0.02(18%)	0.54±0.05*(41%)
(18)	0.45±0.03(12%)	0.50±0.01**(23%)	0.55±0.03*(25%)	0.60±0.03*(35%)
(19)	0.46±0.02(15%)	0.49±0.03*(24%)	0.49±0.0*(33%)	0.56±0.02*(39%)
(20)	0.47±0.04(17%)	0.58±0.02(10%)	0.58±0.02(21%)	0.59±0.03*(36%)
(21)	0.43±0.02(17%)	0.44±0.01*(32%)	0.45±0.0*(39%)	0.51±0.03*(45%)

Paw edema volume expressed as Mean ± SEM; n = 6 animals; *p< 0.01, **p < 0.05 compared to control

TABLE 2: EFFECT OF COMPOUNDS ON ACETIC-ACID INDUCED WRITHING IN MICE

Treatment (Compound No.)	Dose (mg/kg)	Number of Writhes Mean ± SEM	% inhibition
Control	-------	39.60 ± 2.135**	--------
Standard	50	9.40 ± 0.50**	76.26%
(I)	50	13.00 ± 1.37**	67%
(2)	-do-	13.20 ± 1.37**	66%
(3)	-do-	15.00 ± 0.70**	62.12%
(4)	-do-	16.20 ± 0.70*	59%
(5)	-do-	15.90 ± 1.00*	59.80%

(7)	-do-	14.60 ± 1.74**	64.50%
(8)	-do-	12.40 ± 1.36**	68%
(9)	-do-	10.00 ± 0.97**	74%
(12)	-do-	11.50 ± 0.70**	70%
(13)	-do-	12.80 ± 1.02**	67.60%
(15)	-do-	11.65 ± 1.22**	70.50%
(17)	-do-	14.60 ± 1.74*	63%
(21)	-do-	12.80 ± 1.49**	67.67%

Number of wriths expressed as Mean ± SEM; n = 6 animals; *p< 0.01, **p < 0.05 compared to control

TABLE 3: ULCEROGENIC POTENTIAL OF SYNTHETIC COMPOUNDS

Treatment	Dose (mg/Kg)	Average number of ulcer score ± SEM
Control	-	0.00±0.00
Diclofenac	50	2.6±0.26*
(1)	-do-	2.5±0.30*
(3)	-do-	1.3±0.16 [a]
(12)	-do-	1.5±0.12 [a]

Ulcerogenic potential is expressed as average number of ulcer score ± SEM ; n = 6 animals; *p < 0.01 as compared to control; [a]p < 0.01 as compared to Diclofenac

TABLE 4: *IN-VITRO* ANTIMICROBIAL ACTIVITY OF CHEMICAL COMPOUNDS THROUGH AGAR WELL DIFFUSION METHOD

Compound No.	Diameter of growth of inhibition zone (mm)[a]			
	S. aureus	***B. subtilis***	***E. coli***	***P. aeruginosa***
(1)	19.6	-	-	-
(2)	19.6	18.6	-	-
(3)	18.3	16.3	-	-
(6)	17.3	15.3	-	-
(7)	16.3	15.6	-	-
(9)	16.6	17.3	-	-
(11)	17.3	-	-	-
(12)	21.6	17.6	-	-
(13)	16.3	-	-	-
(14)	20.6	-	-	-
(15)	16.3	-	-	-
(19)	15	-	-	-
Ciprofloxacin	26.0	24.0	25.0	22.0

TABLE 5: MINIMUM INHIBITORY CONCENTRATION (MIC) (in µg/ml) OF COMPOUNDS BY USING MACRO-DILUTION METHOD

Compound no.	***Staphylococcus aureus***	***Bacillus Subtilis***
(1)	64	Nt
(2)	64	64
(3)	64	128
(4)	128	>128
(7)	>128	>128
(9)	>128	128
(11)	128	Nt
(12)	32	64
(13)	>128	Nt
(14)	32	Nt
(18)	>128	Nt
(19)	>128	Nt
Ciprofloxacin	5	5

nt- Not tested

TABLE 6: *IN-VITRO* ANTIMICROBIAL ACTIVITY OF SYNTHETIC CHEMICAL COMPOUNDS THROUGH POISONED FOOD METHOD

Compound no.	Mycelial growth inhibition (%)	
	Aspergillus niger	***Aspergillus flavus***
(1)	50	44.4
(2)	38.8	44.4
(3)	42.2	42.2
(6)	42.2	48.8
(7)	44.4	42.2
(9)	55.5	48.8
(11)	42.2	48.8
(12)	40	55.5
(13)	53.3	55.5
(14)	61.1	53.3
(18)	55.5	48.8
(19)	42.2	50
Fluconazole	81.1	77.7

Percentage inhibition of myelial growth = average diameter of fungal colony in negative control sets (dc) - average diameter fungal colony in experimental sets (dt) / dc × 100

Structure-Activity Relationship: Although any straightforward structure-activity correlation cannot be drawn within substituted series as there is a difference in activity between each member still, the structure-activity relationship of fused resultant compounds can be proposed on the grounds of their chemical nature and the position of the substitutes attached on the heterocyclic ring, *i.e.*, furanone, pyrrolone, and N-benzyl pyrrolone. Substituted derivatives showed more activity than unsubstituted ones. Electron releasing and withdrawing groups have shown variable effects on both the series of heterocyclic rings like compounds having electron releasing methoxy group at para position of arylidene moiety in furanone derivatives was found to have better anti-inflammatory as well as analgesic activities as compared to methoxy group in N-benzyl pyrrolone ring. Similarly, electro-negative groups like halogens, when substituted on arylidene rings in furanones, displayed better pharmacological activity than no substitution. It seems that the electron releasing and withdrawing effect is not playing a role in activity.

Moreover, from the data obtained, it was attractive to note that surrogating oxygen atom with nitrogen *i.e.* transforming furanones to pyrrolones, caused a significant decrease in the level of anti-inflammatory and analgesic activities. This transformation in activity may be attributed to proton donor capacity of nitrogen atom as compared to oxygen. It culminates from the overall comparison of biological activities that the presence of an electron releasing group on arylidene ring of furanones potentially increases biological profile of the series.

CONCLUSION: In the present study, furanones and pyrrolone compounds have been synthesized, out of which compound (9) exhibited significant anti-inflammatory and analgesic activities compared to standard drug. An ulcerogenic study revealed that compounds having furanones ring did not originate any damage in the stomach and liver. Furthermore, in furanones compound (9) and in pyrrolones compound (12) showed good inhibitory activity against Gram-positive bacteria and fungi. Therefore, after analyzing the results, compound (9) shows significant anti-inflammatory and antimicrobial activities, and it can be a promising candidate for developing new and safer agents.

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