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CHEMICAL MODIFICATION OF PARACETAMOL AND THEIR ANTIMICROBIAL AND PHARMACOLOGICAL EVALUATION

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Abstract: Three different paracetamol derivatives (II, III and IV) were synthesized and characterized by FTIR, ^{1}H NMR, LC-MS and elemental analysis. The synthesized compounds (II, III and IV) were examined for their acute oral toxicity using OECD guideline 423. The experimental studies revealed that the synthesized compounds II, III and IV showed moderate to excellent analgesic, antipyretic and anti-inflammatory activities as compared to the standard drug. The synthesized compounds were evaluated for their antibacterial (*Staphylococcus aureus, Bacillus subtillis, Escherichia coli, Enterobacter aerogenes*) and antifungal (*Penicillium chryosogenum, Aspergillus niger, Candida albicans*) activities using well plate method. The synthesized compounds II, III and IV exhibited moderate to excellent antimicrobial activity at minimum inhibitory concentrations (MIC) of 150, 30 and 100 µg/mL respectively.

Keywords: Paracetamol derivative, acute oral toxicity, pharmacological activities, antimicrobial activities.

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INTRODUCTION

In the recent society long-life becomes a global trend and humans expect to spend their lives without misgivings about their health. Nevertheless, global come-and-go of people throughout the world brings epidemic of serious sickness like current going-around influenza. Therefore, scientists are requested the development of effective medicines, appropriate therapy and rapid inspection. Then one of focusing in life science is the investigation concerning the chemical modification of standard drugs and their pharmacological evaluation. Paracetamol (N-acetyl p-aminophenol) is widely used as an analgesic[1] and antipyretic drug[2]. Paracetamol classified as a member of the non-sterioidal anti-inflammatory drug (NSAIDs). Paracetamol and various paracetamol derivatives are very important compounds which have been found to maintain significant pharmacological activity are represent important moiety in creation of novel medical materials, adversity of pharmacological activities have been attributed to them such as, antioxidant[3], anti-inflammatory[4-6], analgesic[5-7] and immunomodulatory[8] activities. Paracetamol and its derivatives are of great significance due to their important roles in pharmacological systems. Inspite of numbers of repotrs on the derivatization of the basic structure of paracetamol available in the literature, efforts are continuing to find to enhance the pharmacological activity of basic paracetamol for combining and diverse applications.

The basic structure of paracetamol exhibits little or no anti-inflammatory activity in animals[9,10] and diminutive anti-inflammatory activity in man[11]. The overdoses of unmodified paracetamol can lead to the accumulation of toxic metabolites, causing severe and sometimes fatal hepatotoxicity and nephrotoxicity[12,13]. Therefore, researchers are interested to synthesize new paracetamol derivatives having less toxic and more effective pharmacological activities. Thus, intense efforts are being undertaken to expand more promising and effective paracetamol derivatives for use in the clinical arena.

The synthesis of sulphonamide, diclofenac sodium and norfloxacin remains of great interest due to the wide applications in the pharmaceutical arena. Sulphonamide derivatives have exposed significant biological activities such as, antimicrobial[14], anticonvulsant[15], analgesic[15], anti-inflammatory[16] and antiviral[17] activities. Diclofenac sodium is pharmacological active drug[18,19]. Diclofenac sodium derivatives have shown some potential pharmacological activities such as, antifungal[20], anti-inflammatory[21], analgesic and antipyretic. Norfloxacin is a chemically active substitute and its various derivatives have exposed antibacterial[22], antituberculous[23] and anti-HIV[24] activities. This gave a great impetus to the search for potential pharmacologically active drugs carrying sulfonamide, diclofenac sodium and norfloxacin substituents. Prompted by these observations and our research on pharmacologically important paracetamol derivatives. We hereby report the synthesis and their pharmacological studies of some new substituted paracetamol carrying sulfonamide, diclofenac sodium and norfloxacin moieties.

MATERIALS AND METHODS

[4-amino N-acetyl] phenol (Paracetamol) and Sodium 2-[(2, 6-dichlorophenyl)-amino] phenyl acetate (Diclofenac sodium) were generous gift samples from Cadila pharmaceutics, Ahmedabad and Unique pharmaceutics, Ankleshwar, respectively. 1-Ethyl-6-fluoro-1, 4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid (Norfloxacin) and 4-amino phenyl sulphonamide was purchased from Sigma-Aldrich, Mumbai. All other chemicals and solvents were obtained from various commercial sources and used without further purification.

Melting points were determined by the open capillary method and were uncorrected. FTIR spectra of the synthesized compounds were recorded on a Shimadzu-8400S, using KBr pellets in 10^{-4} resolution and 30 scans. ^{1}H NMR spectra were recorded on a Varian spectrometer, USA at 400 MHz and Mercury Plus 300 MHz at room temperature. Samples were prepared in CD_3COCD_3, CD_3OH and DMSO-d_6 containing TMS as an internal standard. Splitting patterns were designated as follows: s, singlet; d, doublet; t, triplet; m, multiplet. Chemical shift values were given in parts per million (ppm). The Liquid Chromatography Mass Spectra (LC-MS) were recorded on a Varian Inc, USA, 410 Prostar Binary LC with 500 MS IT PDA detectors. Elemental analyses were performed on Thermo finnigan, Italy, Flash EA 1112 series CHNS-O Analyzer. Analgesic activity was performed on Medicraft heated plate analgesiometer, INCO, India and on electric water bath, MAC scientific, India. Anti-inflammatory activity was carried out by using plethysmometer, 7140-UGO Basile, Biological Research Apparatus, Italy.

Synthesis of (4-amino N-acetyl) phenyl 2-chloroacetate (I)

A mixture of (4-amino N-acetyl) phenol (0.08 mol) and 2-chloro acetyl chloride (0.08 mol) in 400 mL dry benzene were refluxed for 3 h with constant stirring. The completion of the reaction was monitored through TLC. The crude product was obtained after cooling the reaction mixture and removing excess of solvent under reduced pressure. The white crude product was recrystallized using acetone. Yield: 62%, m.p. 138-142°C. FTIR (KBr), ν (cm^{-1}): 3340(m) (N-H, amide), 3120(vw) (C-H, of Ar & $COCH_3$), 2900 (C-H, CH_2), 1780 (C=O, ester), 1680 (C=O, amide), 1610 (C=C, Ar), 1580 (N-H, amide), 1430 (C-H, CH_2), 1375(s) (C-H, CH_3), 1260 (Ar-O-CO), 800 (C-H, p-substituted Ar), 700 (C-Cl). ^{1}H NMR (DMSO-d_6, 400 MHz), δ (ppm): 9.62 (s, 1H, NH amide), 7.30-7.28 (d, 1H, Ar-3H, Ar-5H), 6.61-6.41 (d, 1H, Ar-2H, Ar-6H), 3.44 (s, 2H, CH_2), 2.24 (s, 3H, CH_3 amide). MS (m/z): (M^+) 227.02, (M+1) 228.02. Calcd. for $C_{10}H_{10}O_3NCl$: C, 52.76; H, 4.43; O, 21.08; N, 6.15. Found: C, 52.73; H, 4.42; O, 21.05; N, 6.17.

General procedure of synthesis of 4-acetamidophenyl N'-(sulphanilamide) acetate (II) and Sodium 2-[(2, 6-dichlorophenyl) [4-amino N-acetyl) phenoxy acetyl]-amino] phenyl acetate (III)

A mixture of compound I (0.006 mol) and 4- amino phenyl sulphonamide (0.04 mol)/Sodium 2-[(2, 6-dichlorophenyl)-amino] phenyl acetate (0.012 mol) were refluxed with a solution of 70 mL, 1% sodium bicarbonate for 3 h with constant stirring. The completion of the reaction was checked by TLC. The reaction mixture was allowed to cool at room temperature. The crude product was separated by filtration. The white crude product was recrystallized from methanol.

4-acetamidophenyl N'-(sulphonilamide) acetate (II)

Yield: 82%, m.p. 158-160°C. FTIR (KBr), ν (cm^{-1}): 3500 (N-H, 1º amine), 3280 (N-H, 2º amide), 3360 (N-H, 2º amine), 3090 (vw) (C-H, of Ar & $COCH_3$), 2890 (ms) (C-H, CH_2), 1785 (s) (C=O, ester), 1620 (C=O, 2º amide), 1600 (wm) (C=C, Ar), 1500 (ms) (N-H bending, 2º amide), 1470 (ms) (C-H bending, CH_2), 1330 (s) (S=O str. & C-N stretching of 3º amine), 1190 (s) (Ar-O-CO), 1160 (s) (S=O str), 820 (C-H bending, p-substituted Ar). ^{1}H NMR: (CD_3COCD_3, 300 MHz, δ ppm): 8.24 (s, 1H, NH amide), 7.58-7.53 (d, 1H, Ar1-2H, Ar1-6H, Ar2-2H, Ar2-6H), 6.73-6.69 (d, 1H, Ar1-3H, Ar1-5H, Ar2-3H, Ar2-5H), 5.35 (d, 2H, CH_2), 5.33 (t,1H, NH), 2.02 (s, 3H, CH_3, s, 2H, NH-SO_2). MS (m/z): (M^+) 363.09. Anal. Calcd. for $C_{16}H_{17}O_5N_3S$: C, 52.84; H, 4.60; O, 21.66; N, 9.48. Found: C, 52.91; H, 4.48; O, 21.50; N, 9.55.

Sodium 2-[(2, 6-dichlorophenyl) [4-amino N-acetyl)phenyl acetate]-amino]phenyl acetate (III)

Yield: 83%, m.p. 282-286°C. FTIR (KBr), ν (cm^{-1}): 3240 (N-H, amide),3180 (vw) (C-H, Ar), 2980 (ms) (C-H, CH_2), 1790 (s) (C=O, of ester & R-CO-ONa), 1650 (s) (C=O, amide), 1580 (wm) (C=C, Ar), 1510 (ms) (N-H bending, 2° amide), 1420 (ms) (C-H, CH_2), 1360 (ms) (C-N, 3° amine), 1300 (C-H bending, CH_2), 1180 (s) (Ar-O-CO), 820 (s) (C-H bending, p-substituted Ar), 790 (C-H bending, 1,2,6 substituted Ar), 755 (C-H bending, 1,2 substituted Ar), 710 (C-Cl). ^{1}H NMR (CD_3OD, 300 MHz) δ (ppm): 8.03 (s, 1H, NH amide), 7.37-7.34 (d, 1H, Ar1-2H, Ar1-6H), 7.21-7.18 (d, 1H, Ar1-3H, Ar1-5H), 7.03-6.99 (d, 1H, Ar3-3H, Ar3-5H, Ar2-4H, Ar2-6H), 6.85-6.82 (d, 1H, Ar3-4H, Ar2-5H), 6.37-6.35 (d, 1H, Ar2-3H), 4.91 (s, 2H, CH_2), 3.63 (s, 2H, CH_2COONa), 3.30 (s, 3H, CH_3 amide). MS (m/z): (M^+) 509.29. Anal. Calcd. for $C_{24}H_{19}Cl_5N_2NaO_5$: C, 56.60, H, 3.76, O, 15.71, N, 5.50. Found: C, 56.73, H, 3.58, O, 15.62, N, 5.68.

Synthesis of 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid (IV)

A mixture of compound I (1.0 mmol) and 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid (1.0 mmol) in DMF (15 mL), $NaHCO_3$ (0.21 mmol) was added and the resulted mixture was stirred at room temperature overnight. After completion of the reaction, water was added, and the resulted precipitate was filtered off, washed with water, and crystallized from ethanol. Yield: 90%, m.p. 185-189°C. FTIR (KBr), ν (cm^{-1}): 3320 (N-H, amide), 3120 (C-H, Ar), 2890 (C-H, of ethyl chain), 2560 (O-H, of Ar-COOH), 1785 (C=O, ester), 1720

(C=O, of 2° amide and quinoline), 1610 (C=C, Ar), 1590 (N-H bending, 2° amide), 1480 (C-H str., of CH_2), 1390(s) (C-H bending, of amide and ethyl chain), 1375 (C-N, 3° amine), 1190 (Ar-O-CO), 1060 (C-F), 810 (C-H bending, p-substituted Ar). ^{1}H NMR (DMSO, 400 MHz) δ (ppm): 9.65 (s, 1H, COOH), 8.89 (s, 1H, -NH of amide), 7.85-7.82 (d, 1H, quinoline-2H), 7.30-7.27 (d, 2H, Ar-3H, Ar-5H), 7.16-7.11 (d, 1H, quinoline-6H), 7.12-7.03 (d, 2H, Ar-6H, Ar-2H), 6.63 (d, 1H, quinoline-9H), 4.57-4.51 (m, 4H, piperazin), 3.22 (s, 2H, CH_2), 2.90 (q, 2H, CH_2 of ethyl), 2.46-2.39 (m, 4H, piperazin), 1.86 (s, 3H, CH_3 of amide), 1.35 (t, 3H, CH_3 of ethyl). MS (m/z): (M^+) 510.16. Anal. Calcd. for C_{26} H_{27} O_6N_4 F: C, 61.17, H, 5.31, O, 18.80, N, 10.97. Found: C, 61.29, H, 5.45, O, 18.69, N, 10.86.

Pharmacological evaluation Animals used

Adult Wistar albino rats weighing between 150–200 g were used for the pharmacological evaluation studies. The animals were maintained under normal laboratory condition and kept in standard polypropylene cages at 30 ± 2°C temperature and 60 to 65% relative humidity. These rats were provided standard diet and water *ad libitum.* The set of rules followed for animal experiment were approved by the institutional animal ethical committee (VBT/IAEC/10/12/40).

Acute oral toxicity study

Acute oral toxicity[25] of synthesized compounds (II, III and IV) were performed by following the OECD guideline 423 using Wistar albino rat animals. Different drug doses (50-6000 mg/kg as per body weight of the animal) were prepared in aqueous suspension of acacia gum and administered orally. The dose at which 50% animals were dying, that dose was selected as the lethal dose (LD_{50}). 1/10th part of lethal dose (LD_{50}) was selected as an effective dose (ED_{50}). The screenings of pharmacological activities of the synthesized compounds were carried out at ED_{50} dose.

Analgesic activity

Analgesic activity[26] was carried out by hot plate and tail immersion methods. In both methods, rats were taken in five groups. Each group consists of six rats. All the animals were fasted for 18 hrs before the beginning of the experiment and water given *ad libitum*. The animals of group I were treated with 2% acacia suspension prepared in distilled water served as a control. The animals of group II were given paracetamol (562 mg/kg, orally) served as a reference standard[27]. The animals of group III, IV and V were orally administered with compound II (600 mg/kg), III (100 mg/kg) and IV (600 mg/kg) respectively.

Hot plate and tail immersion method

In case of hot plate method, rat was placed on the hot plate maintained at a temperature of 55 ± 0.5ºC. The latency to flick the paw or lick or jump from the hot plate was noted as the reaction time. The reaction time was noted in triplicate at the time interval of 0, 15, 30, 45, 60, 90 and 120 min. The cut off time was considered as 30 sec for each measurement.

In case of tail immersion method, the distal 2-3 cm portion of rat tail was immersed in hot water maintained at 55 ± 1ºC. The time taken by the rat to withdraw the tail from hot water bath was noted as reaction time. This experiment was repeated three times at a time interval of 0, 15, 30, 45, 60, 90 and 120 min. The percent analgesic activity (PAA) was calculated by the following formula,

$$PAA = \left[\frac{T_2 - T_1}{T_2}\right] \times 100$$

Where, T_1 is the reaction time (second) before treatment, T_2 is the reaction time (second) after treatment.

Antipyretic activity

An antipyretic activity[28,29] was screened by the Yeast induced pyrexia method. Animals were fasted for 24 hrs before inducing pyrexia. Pyrexia was induced by administration of 15% w/v aqueous suspension of Brewer's yeast subcutaneously below the nape of the neck at the dose of 20 mL/kg of body weight. Immediately after yeast administration, food was withdrawn. After 18 hrs of yeast injection, the doses of paracetamol as a standard drug (562 mg/kg body weight), compound II (600 mg/kg), III (100 mg/kg) and IV (600 mg/kg) were given orally. The compound II, III, IV and standard drug were dissolved in aqueous suspension of 2% gum acacia. The control group received only an aqueous suspension of 2% gum acacia at the dose of 100 mg/kg body weight. Rectal temperature was determined by introducing a clinical thermometer 1 inch into the rectum and keeping it inside for 1 minute before and 18 hrs after Brewer's yeast injection at time interval of 30, 60, 90, 120 and 180 minutes. Percentage reduction in rectal temperature was calculated by following formula,

$$\% \text{ reduction} = \frac{\text{Temp. 18 hrs after yeast - Temp. after drug/compound II at different hours}}{\text{Temp. 18 hrs after yeast - normal rectal temp. prior to yeast administration}} \times 100$$

Anti-inflammatory activity

Anti-inflammatory activity[30] of compound II, III and IV was evaluated by carrageenan induced rat hind paw edema method. Animals were fasted overnight with free access to water before

the experiment. In control, test and standard groups, acute inflammation were produced by sub-planter injection of 0.1 mL of freshly prepared 1% suspension of Carrageenan (in normal saline) in the right hind paw of the rats. The solutions of test compound II (600 mg/kg), III (100 mg/kg) and IV (600 mg/kg) and standard drug diclofenac sodium (100 mg/kg) were prepared in 2% aqueous suspension of gum acacia and administered orally, 1 h before carrageenan injection. The control group received only vehicle (2 mL/kg). Paw volume was measured plethysmometrically at 0 to 4 hrs after carrageenan injection. Percentage inhibition of paw volume was calculated by following formula,

$$\%\,\text{inhibition of edema} = \left[1 - \frac{Vt}{Vc}\right] \times 100$$

Where, Vt = mean paw volume of the test group, Vc = mean paw volume of the control group.

All the data for the pharmacological activities were expressed as Mean ± SEM (n=6). Statistical analysis was performed by using one way (ANOVA) followed by Student's t-test. (*) for $P < 0.05$, (**) for $P < 0.01$ and (***) for $P < 0.001$ were considered as significant relative to control values.

Antibacterial and antifungal activities

The antibacterial[31,32] and antifungal[33,34] activities of synthesized compounds (II, III and IV) were determined by well plate method. The synthesized compounds II, III and IV were dissolved in dimethyl sulphoxide (DMSO) at a concentration range of 10-150 µg/mL and stored at -20°C. DMSO was used as a control. The primary literature[35] survey revealed that DMSO solvent does not inhibit the growth of bacteria.

In vitro antibacterial activity was carried out against 24 h old cultures of bacterial strains. In the present work, *Escherichia coli (Ec), Staphylococcus aureus (Sa), Bacillus subtillis (Bs)* and *Enterobacter aerogenes (Ea)* were used to investigate the activity. 20 mL of sterilized agar media was poured into each pre-sterilized petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37°C for an hour. About 60 µL of 24 h old culture suspensions was poured and neatly swabbed with the pre-sterilized cotton swabs. 6 mm diameter sterile cork borer was used to punch carefully in well and 30 µL of test solutions (compound II, III and IV) were added into each labeled well. The plates were incubated for 24 h at 37°C. Each inhibition zone that appeared after 24 h, around the well in each plate was measured as a zone of inhibition. Experiments were carried out in triplicates and standard deviation was calculated.

Antifungal activity of newly synthesized compounds II, III and IV were carried out against *Penicillium chrysogenum (Pc), Aspergillus niger (An)* and *Candida albicans (Ca).* Sabourands agar

media were prepared by dissolving peptone (10 g), D-glucose (40 g) and agar (20 g) in double distilled water (1000 mL) and pH was adjusted to 5.7. Normal saline (0.9% w/v NaCl) was used to make a suspension of spore of fungal strains for lawning. A loopful of particular fungal strain was transferred to 3 mL saline to get a suspension of corresponding species. 20 mL of agar media was poured into each petri dish. Excess of suspension was decanted and plates were dried by placing in an incubator at 37 °C for 1 h. 6 mm diameter sterile cork borer was used to punch carefully in well and 30 µL test solution of compounds II, III and IV were added in each labeled well. DMSO solvent was used as a control. The petri dishes were prepared in triplicate and maintained at 25°C for 72 h. Antifungal activity was determined by measuring the diameter of inhibition zone.

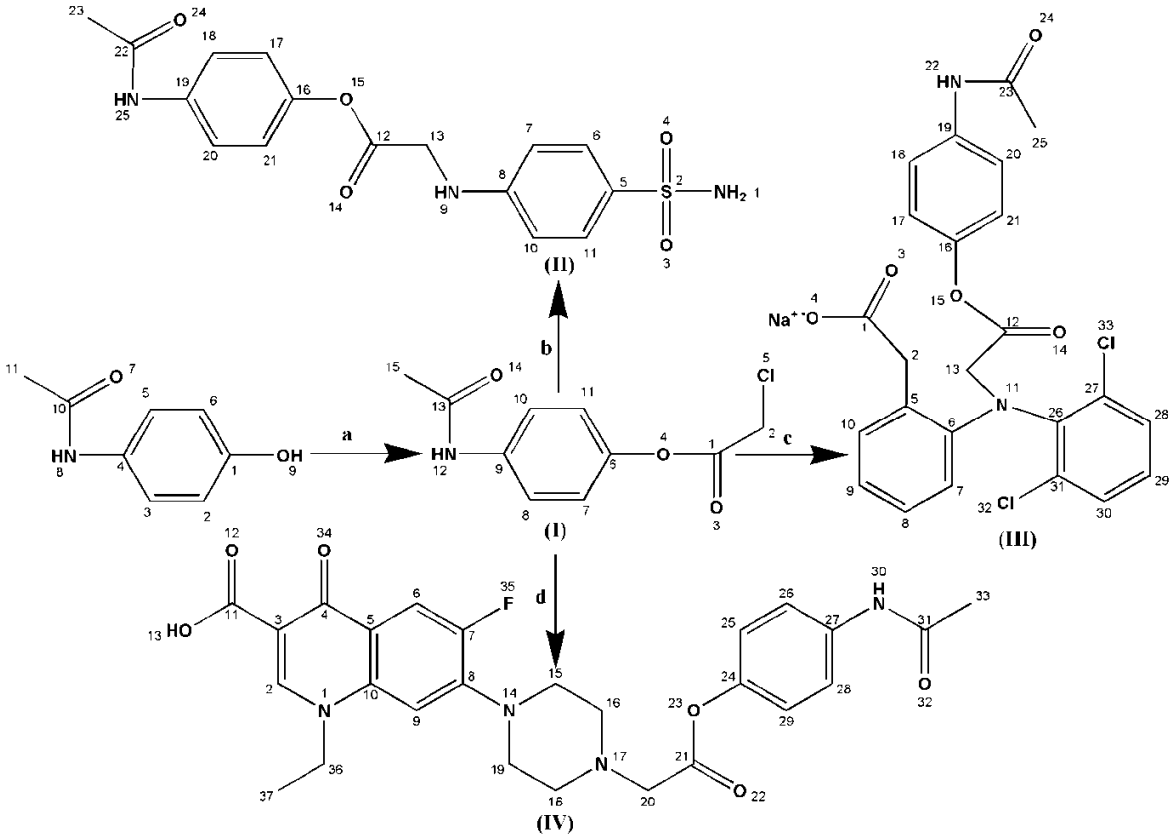
Antibacterial and antifungal activity of each compound were compared with streptomycin and flucanazole as a standard drug, respectively. Zones of inhibition and percentage of relative inhibition zone diameter (% RIZD) were determined for compounds II, III and IV. The percentage of relative inhibition zone diameter (% RIZD) was calculated in term of inhibition zone obtained for control as compared to zone of inhibition obtained from the standard at the same concentration. The antimicrobial activity was calculated by applying the following formula,

$$\% \text{ RIZD} = \left[\frac{\text{IZD sample - IZD control}}{\text{IZD standard}} \right] \times 100 \%$$

Where, RIZD is the percentage of relative inhibition zone diameter. IZD is the inhibition zone diameter (nm).

RESULTS

The synthesis of paracetamol derivatives **(II-IV)** are described in scheme 1. (4-amino N-acetyl) phenyl 2-chloroacetate **(I)** (62%) was prepared by acetylation[36] of (4-amino N-acetyl) phenol with 2-chloro acetyl chloride. 4-acetamidophenyl N'-(sulphanilamide) acetate **(II)** (82%) and Sodium 2-[(2, 6-dichlorophenyl) [4-amino N-acetyl) phenyl acetate]-amino] phenyl acetate **(III)** (83%) were obtained in good yield by the condensation reaction[37] of compound I with 4- amino phenyl sulphonamide/Sodium 2-[(2, 6-dichlorophenyl)-amino] phenyl acetate. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid **(IV)** (90%) was acquired in excellent yield by the condensation reaction[38,39] of compound I and 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid.



Synthetic scheme: Synthetic route for the compounds I-IV

Reagent and condition: (a) $ClCH_2COCl$, dry C6H6, reflux, 3 h, (b) 4- amino phenyl sulphonamide, $NaHCO_3$, reflux, 3 h, (c) Sodium 2-[(2,6-dichlorophenyl)-amino] phenyl acetate, $NaHCO_3$, reflux, 3 h and (d) 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[1-piperazinyl]-3-quinoline carboxylic acid, DMF, $NaHCO_3$, RT.

The structures of synthesized compounds (I-IV) were characterized by FTIR, ^{1}H NMR, LC-MS and elemental analysis. The physical parameters of synthesized compounds **(II-IV)** are summarized in Table 1. FTIR spectrum of compound I showed absorption band at 1780 cm^{-1},

1430 cm^{-1} and 1260 cm^{-1} attributed to the presence of C=O, CH_2 and Ar-O-CO (acetate) groups, respectively. ^{1}H NMR spectrum of the compound I showed the singlet at δ 3.44 for CH_2 proton, doublet at δ 7.30-7.28, 6.61-6.41 for aromatic protons and singlet at δ 9.622 for NH protons. The mass spectrum of compound I showed a molecular ion peak at m/z = 227.02 (M^+), 228.02 (M+1), which is in agreement with the molecular formula $C_{10}H_{10}O_3NCl$. The FTIR spectra of compounds (II-IV) exhibited characteristic bands around 1790-1780 cm^{-1} 1260-1060 cm^{-1} and 1480-1300 cm^{-1} which are showed presence of C=O, Ar-O-CO and CH_2 groups in compounds (II-IV), respectively. The compound II showed C-N stretching of secondary amine at 1330 cm^{-1}

while C-N stretching of tertiary amine of compound III and IV showed absorption at 1360 cm^{-1} and 1375 cm^{-1} respectively. The structures of compounds II, III and IV were confirmed by ^1H NMR spectra, doublet obtained for the CH_2 proton of compound II at δ 5.35 whereas, singlet obtained for the CH_2 proton of compound III and IV at δ 4.91 and 3.22. FTIR spectra and ^1H NMR signals showed that the CH_2 presence in each synthesized compound and formed a bridge between compound I and various substituted moieties (Sulphanilamide, Diclofenac Sodium and Norfloxacin). The mass spectra of compounds (II, III and IV) showed a molecular ion peak at m/z = 363.09 (M^+), 509.29 (M^+), 510.16 (M^+) and which are in agreement with the molecular formulas $C_{16}H_{17}O_5N_3S$, $C_{24}H_{19}Cl_5N_2NaO_5$ and $C_{26}H_{27}O_6N_4F$, respectively. The elemental analyses of synthesized compounds are characterized in the experimental part.

Acute oral toxicity of compounds (II, III and IV) was carried out by following the OECD guideline 423 using Wistar albino rat animals. The experimental studies revealed that the lethal dose (LD_{50}) of the synthesized compounds (II, III and IV) was found to be 6000, 1000 and 6000 mg/kg, respectively. $1/10^{th}$ dose of LD_{50} was taken as an effective dose (ED_{50}) for pharmacological study. Therefore, ED_{50} for synthesized compound II, III and IV were 600, 100 and 600 mg/kg body weight of animal, respectively.

The analgesic activity was carried out on rat by hot plate and tail immersion methods using paracetamol as a standard drug (Fig. 1 and 2). The screening results revealed that all the synthesized compounds (II, III and IV) showed moderate to excellent analgesic potential than that of the standard paracetamol (Table 2 and 3). The compounds II and III showed the highest percentage of analgesia (62-66% and 66-69%) at 45 minutes as compared to the standard paracetamol drug (62-63%). Compound IV showed a moderate percentage of analgesia (58-60%) at 90 minutes as compared to the standard paracetamol drug (62-63%).

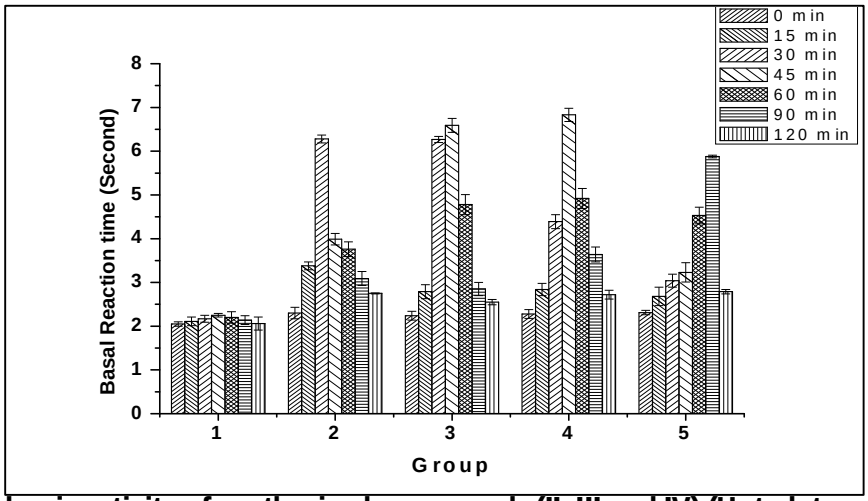


Figure 1. Analgesic activity of synthesized compounds (II, III and IV) (Hot plate method)

Groups: 1 = Control, 2 = paracetamol (562 mg/kg), 3 = Compound II (600 mg/kg), 4 = Compound III (100 mg/kg), 5 = Compound IV (600 mg/kg). The observations are mean ± SEM (n=6).

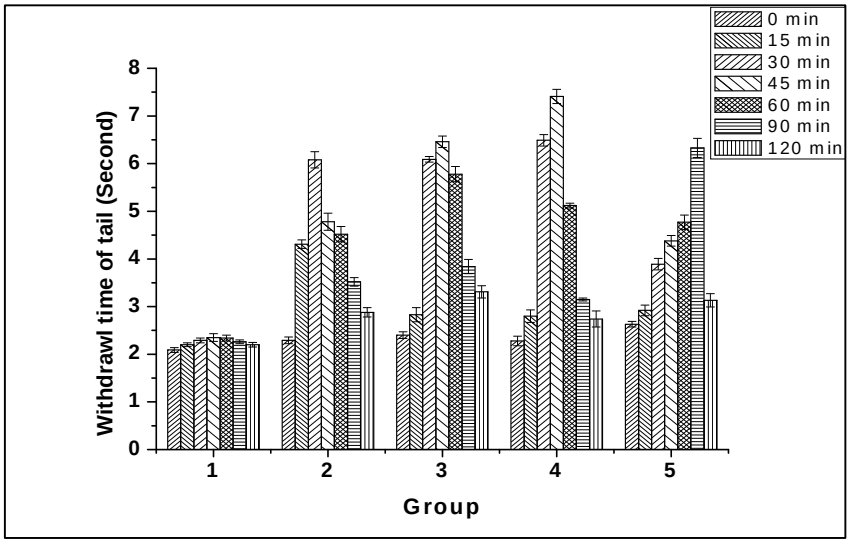


Figure 2. Analgesic activity of synthesized compounds (II, III and IV) (Tail immersion method)

Groups: 1 = Control, 2 = paracetamol (562 mg/kg), 3 = Compound II (600 mg/kg), 4 = Compound III (100 mg/kg), 5 = Compound IV (600 mg/kg). The observations are mean ± SEM (n=6).

The synthesized compounds II, III and IV showed excellent antipyretic potency than standard drug paracetamol which is presented in Fig. 3. The synthesized compounds II, III and IV showed significant decreases in elevated body temperature of rats. Compounds II, III and IV showed 82%, 97% and 83% antipyretic activity at 180, 120 and 180 minutes respectively, whereas, the standard paracetamol drug exhibited 80% antipyretic activity. The percentage of analgesic activity is elicited in Table 4.

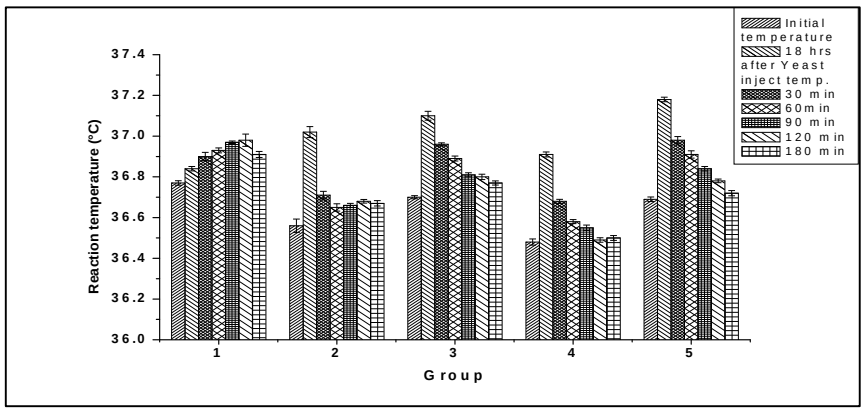


Figure 3. Antipyretic activity of synthesized compounds (II, III and IV)

Groups: 1 = Control (100 mg/kg), 2 = paracetamol (562 mg/kg), 3 = Compound II (600 mg/kg), 4 = Compound III (100 mg/kg), 5 = Compound IV (600 mg/kg). The observations are mean ± SEM (n=6).

The anti-inflammatory effect of the compound II, III and IV on carrageenan-induced oedema in the rat right hind paw are presented in Fig. 4. Compounds II, III and IV were produced a significant reduction in oedema caused by carrageenan. The synthesized compounds II, III and IV were found to be 58%, 66% and 51% anti-inflammatory potencies as compared to the standard diclofenac sodium (50%) drug. The percentage of anti-inflammatory activities of compound II, III and IV are summarized in table 5.

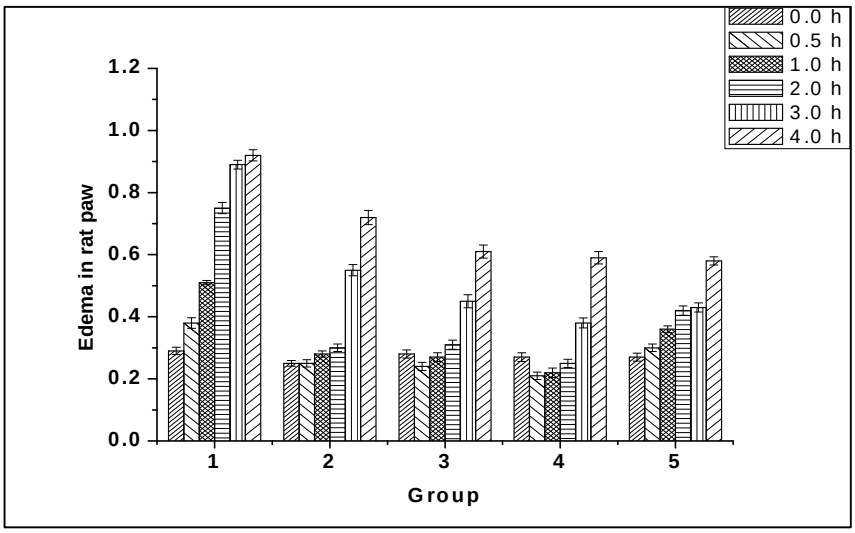


Figure 4. Anti-inflammatory activity of synthesized compounds (II, III and IV)

Groups: 1 = Control (2 mL/kg), 2 = Diclofenac sodium (100 mg/kg), 3 = Compound II (600 mg/kg), 4 = Compound III (100 mg/kg), 5 = Compound IV (600 mg/kg). The observations are mean ± SEM (n=6).

In vitro antibacterial and antifungal activities of synthesized compounds II, III and IV were determined by well plate method. The synthesized compounds (II, III and IV) were evaluated for their antibacterial and antifungal activities against gram positive *S. aureus, B. subtillis* and gram negative *E. coli , E. aerogenes* bacteria and the fungi *P. chrysogenum, A. niger* and *C. albicans*. Table 6 and 7 comprise the *in vitro* results of antibacterial and fungal activities of the synthesized compounds under study expressed as minimum inhibitory concentrations (MICs) ranging from 30 to 150 µg/mL.

DISCUSSION

The spectral data of the three synthesized compounds are in conformity with the structures suggested for the products: 4-acetamidophenyl N'-(sulphanilamide) acetate **(II)**; Sodium 2-[(2, 6-dichlorophenyl) [4-amino N-acetyl) phenyl acetate]-amino] phenyl acetate **(III)**; 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7-[4-[(4-amino N-acetyl) phenoxy carbonyl methyl]-1-piperazinyl]-3-quinoline carboxylic acid **(IV)**. The structure modification in paracetamol tends to new doses level for the synthesized compounds. It was obtained by using OECD 423 guideline and all the pharmacological activities were carried out at that dose level.

In synthesized compounds, a phenolic oxygen atom of paracetamol is bind with carbonyl carbon atom and formed ester, so basicity decreases and reactivity of ring increases. This leads to enhance the pharmacological activities of synthesized compounds. The synthesized compounds II and III having a such ester linkage at C_{16} position and in compound IV at C_{24} position. Further, an enrichment of analgesic and antipyretic activities of compound II, III and IV also attributed to the presence of the acetamide group at C_{19} position in compound II and III, and at C_{27} position in compound IV. The improvement in pharmacological activities of compound II showed due to the presence of the sulfonamide at C_{13} position. The sulfonamide increase COX-2 selectivity, because of the presence of a side pocket in the structure of COX-2 enzymes where the sulfonamide group can easily fit[40]. This increase in COX-2 selectivity improves the anti-inflammatory as well as analgesic activities. The compound III exhibited inhibition of edema due to the presence of carboxylic acid group at C_2 position in diclofenac sodium moiety. The diclofenac sodium substituent enhance the activity of compound III, due to the inhibition of both leukocyte migration and the enzyme cyclooxygenase (COX-1 and COX-2), leading to the peripheral inhibition of prostaglandin synthesis and action on the hypothalamus resulting in peripheral dilation, which increased cutaneous blood flow and reduce the heat. Therefore, the compound III showed good analgesic, antipyretic and anti-inflammatory activities than that of the standard drugs. The substitution of ester linker methylene group with piperazinyl ring of norfloxacin, generate noticeable effects in decreasing the inflammation. Thus, compound IV showed moderate anti-inflammatory activity.

The sulfonamide are very good antimicrobial agents as per traditional drug review. Thus, the substitution of a sulfonamide group at C_5 position in aromatic ring increase the antimicrobial activity of compound II. The compound II showed excellent antibacterial activity at MIC value of 150 µg/mL against *S. aureus, E. coli* and good potency against *B. subtilis* as compared to the standard streptomycin drug. The compound II showed moderate antifungal activity against *A. niger* and *C. albicans* as compared with standard flucanazole drug. Compound III has chlorine atoms at C_{27} and C_{31} positions in an aromatic ring exhibited excellent antibacterial activity against *E. coli, S. aureus, B. subtilis* and good activity against *E. aerogenes* at MIC value of 30

µg/mL as compared to the standard streptomycin drug. Moreover, compound III showed excellent antifungal activity against *C. albicans* and good potency against A. niger, P. chrysogenum as compared to the standard flucanazole drug. The presence of fluorine atom at C_7 in a quinoline ring of compound IV exhibited excellent to good antibacterial activity at MIC value of 100 µg/mL. The experimental results revealed that compound IV showed excellent antibacterial activity against *E. aerogenes, S. aureus* and good activity against *E. coli., B. subtilis.* The compound IV did not show potency against any fungal strains.

CONCLUSION

A straightforward synthesis of paracetamol derivatives (II, III and IV), acute oral toxicity, analgesic, antipyretic, anti-inflammatory and antimicrobial studies are described in this paper. During the experimental work, it was observed that the results of the analgesic, antipyretic, anti-inflammatory activities of synthesized compounds (II, III and IV) have been shown moderate to excellent potencies as compared to the standard drug. The improvement in pharmacological activities exhibited by synthesized compounds clearly reveals that the presence of phenolic oxygen atom linkage with carbonyl carbon atom and also the presence of various substitutes (sulfanilamide, diclofenac, norfloxacin). The synthesized compounds II, III and IV showed good to excellent antibacterial activity as compared to the standard streptomycin drug. Moreover, antifungal studies revealed that the synthesized compounds II and III exhibited moderate to excellent antifungal potencies as compared to the standard flucanazole drug. The presence of various substitutes like sulfanilamide, diclofenac, norfloxacin are important for antimicrobial activities.

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Table 1. The physical parameters of synthesized compounds (I-IV)

Compounds	Molecular Formula	Yield (%)	Molecular Weight	M.P. (°C)
I	$C_{10}H_{10}O_3NCl$	62	227	138-142
II	$C_{16}H_{17}O_5\ N_3S$	82	363	158-160
III	$C_{24}H_{19}Cl_5N_2NaO_5$	83	509	282-286
IV	$C_{26}H_{27}O_6N_4F$	90	510	185-189

Table 2. Percentage analgesia of synthesized compounds (II, III and IV) by hot plate method

Compounds	Percentage of Analgesia					
	15	30	45	60	90	120
Std. pcm drug	31.95**	63.38***	42.36***	38.83***	25.57***	16.36**
II	19.71***	64.27**	66.01***	53.14***	21.40***	12.16*
III	19.72***	48.06***	66.62***	53.66***	37.36***	16.18**
IV	13.81**	24.01**	28.48***	49.01***	60.71***	17.20***

Std.: Standard; pcm: Paracetamol; Significance relative to control values: (*) for P < 0.5, (**) for P < 0.01 and (***) for P < 0.001. (ANOVA followed by Student's t-test).

Table 3. Percentage analgesia of synthesized compounds (II, III and IV) by tail immersion method

Compounds	Percentage of Analgesia					
	15	30	45	60	90	120
Std. pcm drug	46.87***	62.34***	52.09***	49.34***	34.94***	20.49***
II	15.19**	60.59***	62.58***	58.48***	37.50***	27.49***
III	18.57**	64.87***	69.23***	55.47***	27.62***	16.79*
IV	09.93***	32.39***	39.95***	44.86***	58.45***	15.97***

Std.: Standard; pcm: Paracetamol; Significance relative to control values: (*) for P < 0.5, (**) for P < 0.01 and (***) for P < 0.001. (ANOVA followed by Student's t-test).

Table 4. The percentage of antipyretic activity of test compounds (II, III and IV) on Yeast induced pyrexia in rats

Group	Time after administration				
	30 min	60 min	90 min	120 min	180 min
Std. pcm drug	67.39***	80.43***	78.26***	73.91***	76.09***
II	35.00*	52.50*	72.50***	75.00***	82.50***
III	53.49***	76.74***	83.72***	97.67***	95.35***
IV	40.82*	55.10	69.39***	81.63***	83.67***

Std.: Standard; pcm: Paracetamol; Significance relative to control values: (*) for P < 0.5, (**) for P < 0.01 and (***) for P < 0.001. (ANOVA followed by Student's t-test).

Table 5. The percentage of anti-inflammatory activity of test compounds (II, III and IV) against the carrageenan-induced paw edema in rats

Group	Time (h)				
	0.5 h	1 h	2 h	3 h	4 h
Std. drug	34.21***	39.22***	50.67***	38.20***	21.74***
II	36.84***	47.06***	58.67***	49.44***	33.70***
III	44.74***	56.86***	66.67***	57.30***	35.87***
IV	21.05**	29.41***	44.00***	51.69***	36.96***

Std.: Standard drug; diclofenac sodium (100 mg/kg); Significance relative to control values: (*) for $P < 0.5$, (**) for $P < 0.01$ and (***) for $P < 0.001$. (ANOVA followed by Student's t-test).

Table 6. Antimicrobial activity of synthesized compounds (II, III and IV) in term of zone of inhibition

Compounds	Zone of inhibition in mm						
	Bacterial species				Fungal species		
	Sa	Bs	Ec	Ea	Pc	An	Ca
II	18 ± 0.04	15± 0.05	21 ± 0.03	-	-	10 ± 0.05	11 ± 0.02
III	18 ± 0.03	18 ± 0.03	23 ± 0.01	17 ± 0.02	13 ± 0.02	15 ± 0.01	18 ± 0.01
IV	17 ± 0.02	17 ± 0.01	18 ± 0.01	19 ± 0.02	-	-	-
Std. Strept.	24 ± 0.02	25 ± 0.01	26 ± 0.02	25 ± 0.02	-	-	-
Std. Fluc.	-	-	-	-	24 ± 0.01	25 ± 0.02	24 ± 0.02
Control	-	-	-	-	-	-	-

Std.: Standard; Strept. Streptomycin; Fluc.: Flucanazole; Cup diameter: 6 mm; Volume of the solution: 30 µL per cup. Sa: *Staphylococcus aureus* (gm +ve); Bs: *Bacillus subtilis* (gm +ve); Ec: *Escherichia coli* (gm -ve); Ea: *Enterobacter aerogenes* (gm -ve); Pc: *Penicillium chrysogenum*; An: *Aspergillus niger*; Ca: *Candida albicans*. The compounds II, III and IV were tested at concentrations of 150, 30, 100 µg/mL, respectively. DMSO is used as a control.

Table 7. Antimicrobial activity of synthesized compounds (II, III and IV) in term of %RIZD

Compounds	Percentage relative inhibition zone diameter (% RIZD)						
	Bacterial species				Fungal species		
	Sa	Bs	Ec	Ea	Pc	An	Ca
II	75.00	60.00	80.77	-	-	40.00	45.83
III	75.00	72.00	88.46	68.00	54.17	60.00	75.00
IV	70.83	68.00	69.23	76.00	-	-	-

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