Acta Pharmaceutica Sciencia
50: 23-34 (2008)

Synthesis and Pharmacological Evaluation of Heterocyclic Indole Derivatives

Mohammad Amir*, Harish Kumar, Suroor A. Khan and Sadique A. Javed

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi - 110 062, India.

Abstract

Two series of the compounds having indole and imidazole moeity in the same molecule have been synthesized. The structure of the newly synthesized compounds was supported by IR, ^{1}H NMR and Mass spectral data. These compounds were investigated for their anti-inflammatory, analgesic, ulcerogenic, lipid peroxidation and antimicrobial activities. Some of the synthesized compounds showed potent anti-inflammatory activity along with minimal ulcerogenic effect and lipid peroxidation, compared to indomethacin. Some of tested compounds also showed significant antimicrobial activity against tested bacterial and fungal strains. The most potent compound in both series, 4-(1*H*-Indol-3-yl-methylidene)-2-phenyl-1-[2-(6-methoxy-2-naphthyl) propanamido]-5-oxo-imidazoline **(5c)** was further tested for its hepatotoxicity.

Key words: Indole/imidazoline, Anti-inflammatory, Analgesic, Ulcerogenicity, Lipid peroxidation, Antimicrobial

Introduction

Imidazole and indole residues are probably the most well known heterocycles, which are common and important feature of a variety of natural products and medicinal agents. Compounds carrying indole residue e.g. indomethacin (Roberts *et al.*, 2001), tenidap (Moore *et al.*, 1996) are NSAIDs and have shown to exert anti-inflammatory effects. Indole, the potent basic pharmacodynamic nucleus, has been reported to possess a wide variety of biological properties viz, anti-inflammatory (Radwan *et al.*, 2007; Amir *et al.*, 1997; Misra *et al.*, 1996), anticonvulsant (Altintas *et al.*, 2006) and antimicrobial (Ryu *et al.*, 2007). Mitomycines are natural antibiotics bearing indole residue that are also anticancer compounds as DNA cross-linking agents (Scott and Williams, 1998). Furthermore, imidazole nucleus forms the main structure of some well-known components of human organisms i.e. the amino acid histidine, vit-B_{12}, histamine and biotin. It is also present in the structure of many natural or synthetic drug molecules i.e. cimetidine, azomycin and metronidazole. Besides these, imidazole/imidazoline nucleus containing compounds exhibit a wide spectrum of biological activities such as anti-inflammatory and analgesic (Almansa *et al.*, 2003; Barta *et al.*, 1998), anticonvulsant (Puratchikody *et al.*, 2004), antimicrobial (Khabnadideh *et al.*, 2003; Matysiak *et al.*, 2003) and antitubarcular (Gupta *et al.*, 2004).

Encouraged by these observations and in continuation of our research work on the synthesis of heterocyclic compounds (Amir *et al.*, 2006; Amir and Kumar, 2005), we report herein, the synthesis of a hybrid molecule containing both the indole and imidazole residues. The newly synthesized compounds were tested for their anti-inflammatory, analgesic and antimicrobial activities.

* Corresponding author: mamir_s2003@yahoo.co.in

Material and Methods:

Melting points were determined in open capillary tubes and are uncorrected. IR (KBr) spectra were recorded on a Nicolet, 5PC FTIR spectrometer and ^{1}H NMR spectra were recorded in $CDCl_3$/DMSO-d_6 on a Bruker DRX-300 (300 MHz FT NMR) spectrometer using TMS as internal reference (Chemical Shift in δ ppm). Mass spectra were recorded at Jeol SX-102 spectrometer. Chemicals were purchased from Merck Chemical Company, S. D. Fine (India) and Qualigens (India). The purity of various synthesized compounds was checked by TLC and elemental analysis. Spectral data (^{1}H NMR, IR and mass) of the synthesized compounds were in full agreement with the proposed structures. Acid hydrazides were prepared by the procedure given in literature (Amir and Kumar, 2004).

Chemistry:

4-(1H-indol-3-ylmethylidine)-2-phenyl-1,3-oxazolin-5-one (Table 1)

A mixture of indole-3-carboxyldehyde (0.01 mol), benzoyl glycine (0.01 mol), fused sodium acetate (0.015 mol) and acetic anhydride (7.15 mL) was heated on a hot plate with constant shaking. When the mixture liquefied completely, it was transferred on water bath and refluxed for another 4 h. Ethanol (10 mL) was added slowly to the cooled mixture and the contents were allowed to stand overnight. Solid filtered, washed with boiling water and recrystalised with acetone.

IR (KBr ν cm^{-1}) = 3174 (NH), 2927 (C-H), 1685 (C=O), 1637 (C=N). ^{1}H NMR ($CDCl_3$): δ 7.38-8.40 (m, 10 Ar-H & 1CH, 11H), 8.75 (bs, NH, 1H). MS *m/z*: 288 (M^+).

Aryl thiosemicarbazides (2a-h)

A mixture of substituted aniline (0.02 mol), ethanol (95%, 10 mL) and ammonia (5 mL) was cooled to below 30°C and CS_2 (2 mL) was added slowly with in 15 min with shaking. After complete addition of CS_2, the solution was allowed to stand for 1 h, followed by addition of sodium chloroacetate (0.02 mol). The reaction was exothermic with change in colour from red to yellowish green. Hydrazine hydrate (99%, 2 mL) was added to the solution. The mixture was warmed gently, filtered, boiled to half of its volume and kept overnight. Solid thiosemicarbazides thus separated out were filtered, dried and recrystallised with suitable solvent.

2e: IR (KBr ν cm^{-1}) = 3278 (NH), 1132 (C=S). ^{1}H NMR (DMSO-d_6,): δ ppm 2.14 (s, *o*-CH_3, 3H), 2.25 (s, 3H, *p*-CH_3, 3H), 4.70 (bs, NH_2, 2H), 6.94 (d, *J* = 7.1 Hz, Ar-H, 1H), 7.01 (s, Ar-H, 1H), 7.34 (d, *J* = 7.1 Hz, Ar-H, 1H), 9.00 (bs, Ar-NH, 1H), 9.29 (bs, CS-NH, 1H). MS *m/z*: 195 (M^+).

2g: IR (KBr ν cm^{-1}) = 3199 (NH), 1103 (C=S). ^{1}H NMR (DMSO-d_6): δ ppm 5.10 (bs, 2H, NH_2), 7.36-7.70 (m, 3Ar-H & 1NH, 4H), 8.30 (bs, CS-NH, 1H). MS *m/z*: 219 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-substituted phenyl thiourido)-5-oxo-imidazoline (4a-h)

Compound **1** (0.01 mol) and aryl thiosemicarbazides **2a-h** (0.01 mol) were dissolved in pyridine (15 mL) and refluxed for 14-18 h. Resulting mass thus obtained was allowed to cool to room temperature and poured over crushed ice and neutralized with dilute hydrochloric acid. Solid obtained was filtered, washed with water and recrystalised with suitable solvent.

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-methoxyphenyl thiourido)-5-oxo-imidazoline (4a)

IR (KBr ν cm^{-1}) = 3250 (NH), 2927 (C-H), 1680 (C=O), 1614 (C=N), 1244 (C-O-C), 1124 (C=S). ^{1}H NMR (DMSO-d_6): ppm δ 3.77 (s, OCH_3, 3H), 7.20-8.91 (m, 14Ar-H, 1CH & 1NH, 16H), 11.69 (bs, NH-CS-NH, 2H). MS *m/z*: 467 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(2-methylphenyl thiourido)-5-oxo-imidazoline (4b)

IR (KBr ν cm^{-1}) = 3256 (NH), 2928 (C-H), 1689 (C=O), 1639 (C=N), 1127 (C=S). ^{1}H NMR (DMSO-d_6): δ 2.18 (s, CH_3, 3H), 7.24-8.90 (m, 14Ar-H & 1CH, 15H), 9.93 (bs, NH, 1H), 10.38 (bs, Ar-NH, 1H), 10.74 (bs, CS-NH, 1H). MS *m/z*: 451 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(3-methylphenyl thiourido)-5-oxo-imidazoline (4c)

IR (KBr ν cm^{-1}) = 3230 (NH), 2938 (C-H), 1675 (C=O), 1623 (C=N), 1131 (C=S). ^{1}H NMR ($CDCl_3$): δ 2.17 (s, CH_3, 3H), 7.10-8.42 (m, 14Ar-H & 1CH, 15H), 9.44 (bs, NH, 1H), 11.13 (bs, NH-CS-NH, 2H). MS *m/z*: 451 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-methylphenyl thiourido)-5-oxo-imidazoline (4d)

IR (KBr ν cm^{-1}) = 3220 (NH), 2927 (C-H), 1679 (C=O), 1633 (C=N), 1123 (C=S). ^{1}H NMR ($CDCl_3$): δ 2.31 (s, CH_3, 3H), 7.21-8.09 (m, 14Ar-H & 1CH, 15H), 9.49 (bs, NH, 1H), 11.19 (bs, NH-CS-NH, 2H). MS *m/z*: 451 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(2,4-dimethylphenyl thiourido)-5-oxo-imidazoline (4e)

IR (KBr ν cm^{-1}) = 3229 (NH), 2937 (C-H), 1670 (C=O), 1639 (C=N), 1143 (C=S). ^{1}H NMR ($CDCl_3$): δ 1.83 (s, *o*-CH_3, 3H), 2.11 (s, *p*-CH_3, 3H), 6.74-7.76 (m, 13 Ar-H & 1CH, 14H), 9.52 (bs, NH, 1H), 10.54 (bs, Ar-NH, 1H), 11.16 (bs, CS-NH, 1H). MS *m/z*: 465 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-fluorophenyl thiourido)-5-oxo-imidazoline (4f)

IR (KBr ν cm^{-1}) = 3298 (NH), 2936 (C-H), 1680 (C=O), 1611 (C=N), 1098 (C=S). ^{1}H NMR ($CDCl_3$): δ 7.24-8.19 (m, 14Ar-H & 1CH, 15H), 9.38 (bs, NH, 1H), 11.35 (bs, NH-CS-NH, 2H). MS *m/z*: 490 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(3-chloro-4-fluorophenyl thiourido)-5-oxo-imidazoline (4g)

IR (KBr ν cm^{-1}) = 3180 (NH), 2932 (C-H), 1690 (C=O), 1636 (C=N), 1127 (C=S). ^{1}H NMR ($CDCl_3$): δ 7.13-8.57 (m, 13Ar-H & 1CH, 14H), 9.03 (bs, NH, 1H), 10.31 (bs, Ar-NH, 1H), 11.29 (bs, CS-NH, 1H). MS *m/z*: 490 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-bromophenyl thiourido)-5-oxo-imidazoline (4h)

IR (KBr ν cm^{-1}) = 3218 (NH), 2990 (C-H), 1679 (C=O), 1623 (C=N), 1122 (C=S). ^{1}H NMR (DMSO-d_6): δ 7.20-8.90 (m, 14Ar-H, 1CH & 1NH, 16H), 11.68 (bs, NH-CS-NH, 2H). MS *m/z*: 516 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-[2-substituted phenyl acetamido/propanamido] -5-oxo-imidazoline (5a-f)

Compound **1** (0.01 mol) and appropriate acid hydrazide **3a-f** (0.01 mol) was dissolved in pyridine (15 mL) and refluxed for 19-22 h. Resulting mass thus obtained was allowed to cool to room temperature and poured over crushed ice and neutralized with dilute hydrochloric acid. Solid thus separated out was filtered, washed with water and recrystallised with suitable solvent.

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-[2-(2,6-dichloroanilino) phenyl acetamido] -5-oxo-imidazoline (5a)

IR (KBr ν cm^{-1}) = 3221 (NH), 2930 (C-H), 1701 (C=O), 1630 (C=N). ^{1}H NMR (DMSO-d_6): δ 4.19 (s, CH_2, 2H), 6.80-8.40 (m, 17Ar-H & 1CH, 18H), 11.33 (bs, NH, 1H), 11.48 (bs, Ar-NH, 1H), 11.60 (bs, CO-NH, 1H). MS *m/z*: 580 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-[2-(4-isobutylphenyl) propanamido]-5-oxo-imidazoline (5b)

IR (KBr ν cm^{-1}) = 3234 (NH), 2927 (C-H), 1695 (C=O), 1639 (C=N). ^{1}H NMR (DMSO-d_6): δ 0.83 [d, J = 6.6 Hz, $(CH_3)_2$, 6H], 1.41 (d, J = 6.9 Hz, CH_3, 3H), 1.76-1.82 (m, $CHCH_2$, 1H), 2.38 (d, J = 7.0 Hz, CH_2, 2H), 4.73 (q, J = 6.9 Hz, $CHCH_3$, 1H), 7.04-8.58 (m, 14Ar-H & 1CH, 15H), 10.95 (bs, NH, 1H), 11.53 (bs, CO-NH, 1H). MS *m/z*: 490 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-[2-(6-methoxy-2-naphthyl) propanamido]-5-oxo-imidazoline (5c)

IR (KBr ν cm^{-1}) = 3192 (NH), 2927 (C-H), 1698 (C=O), 1605 (C=N), 1225 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 1.49 (d, J = 6.9 Hz, CH_3, 3H), 3.82 (s, OCH_3, 3H), 4.84 (q, J = 6.9 Hz, $CHCH_3$, 1H), 7.09-8.39 (m, 16 Ar-H & 1CH, 17H), 11.00 (bs, NH, 1H), 11.57 (bs, CO-NH, 1H). MS *m/z*: 514 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(4-hydroxyphenyl acetamido)-5-oxo-imidazoline (5d)

IR (KBr ν cm^{-1}) = 3305 (OH), 3196 (NH), 2958 (C-H), 1690 (C=O), 1631 (C=N). ^{1}H NMR (DMSO-d_6): δ 3.90 (s, CH_2, 2H), 6.66-8.36 (m, 14 Ar-H & 1CH, 15H), 9.27 (bs, NH, 1H), 11.00 (bs, CO-NH, 1H), 11.54 (bs, OH, 1H). MS *m/z*: 436 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(2,4-dichlorophenoxy acetamido)-5-oxo-imidazoline (5e)

IR (KBr ν cm^{-1}) = 3173 (NH), 2936 (C-H), 1700 (C=O), 1638 (C=N), 1248 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 5.16 (s, OCH_2, 2H), 7.09-7.82 (m, 13 Ar-H & 1CH, 14H), 9.15 (bs, NH, 1H), 11.08 (bs, CO-NH, 1H). MS *m/z*: 505 (M^+).

4-(1H-Indol-3-yl-methylidene)-2-phenyl-1-(2,4,6-trichlorophenoxy acetamido)-5-oxo-imidazoline (5f)

IR (KBr ν cm^{-1}) = 3282 (NH), 2930 (C-H), 1677 (C=O), 1615 (C=N), 1248 (C-O-C). ^{1}H NMR (DMSO-d_6): δ 5.31(s, OCH_2, 2H), 7.13-7.79 (m, 12Ar-H & 1CH, 13H), 9.24 (bs, NH, 1H), 11.02 (bs, CO-NH, 1H). MS *m/z*: 540 (M^+).

Biological evaluation:

Adult male Wistar strain rats of either sex, weighing 150-200 g were used for anti-inflammatory, ulcerogenic and lipid peroxidation activities, whereas Swiss albino mice weighing 25-30 g were used for analgesic and anticonvulsant activities. The animals were allowed food and water *ad libitum* except during the experiments. They were housed in room at 25 ± 2 °C, and 50 ± 5% relative humidity with 12 h light/dark cycle. The animals were randomly allocated into groups at the beginning of all the experiments. The experimental protocol was approved by the animal ethics committee of Hamdard University. All the test compounds and the reference drugs were administered orally, suspended in 0.5% carboxymethyl cellulose (CMC) solution except for anticonvulsant activity for which test compounds and standard drug were administered intraperitoneally (i.p.) suspended in Tween 80 (1%) solution.

Anti-inflammatory activity

The synthesized compounds were evaluated for their anti-inflammatory activity using carrageenan induced hind paw edema method (Winter *et al.*, 1962). The animals were randomly allocated into groups of six animals each and were fasted for 24 h before the experiment with free access to water. Control group received only 0.5% carboxymethyl cellulose solution. Standard drug indomethacin was administered orally at a dose of 10 mg/kg. The test compounds were administered orally at an equimolar oral dose relative to 10 mg/kg indomethacin. 0.1 mL of 1.0% carrageenan solution in saline was injected subcutaneously into the sub plantar region of the right hind paw of each rat, 1 h after the administration of the test compounds and standard drug. The right hind paw volume was measured before and after 3h and 4h of carrageenan treatment by means of a plethysmometer. The percent anti-inflammatory activity was calculated according to the following formula:

Percent anti-inflammatory activity (%) = $(V_c\text{-}V_t/V_c) \times 100$

where, V_t represents the mean increase in paw volume in rats treated with test compounds and V_c represents the mean increase in paw volume in control group of rats.

Analgesic activity

Analgesic activity was evaluated by tail immersion method (Adeyemi *et al.*, 2004). Swiss albino mice allocated into different groups consisting of six animals in each, of either sex, weighing 25-30 g were used for the experiment. Analgesic activity was evaluated after oral administration of the test compounds at an equimolar dose relative to 10 mg/kg indomethacin. Test compounds and standard drugs were administered orally as suspension in carboxymethyl cellulose solution in water (0.5 % w/v). The analgesic activity was assessed before and after 4 h interval of the administration of test compounds and standard drugs. The lower 5 cm portion of the tail was gently immersed into thermostatically controlled water at 55 ± 0.5°C. The time in second for tail recovering from water was taken as the reaction time with a cut of time of immersion, set at 10 seconds for both control as well as treated groups of animals.

Acute ulcerogenicity

Ulcerogenic activity (Cioli *et al.*, 1979) was evaluated after oral administration of the test compounds at an equimolar dose relative to 30 mg/kg indomethacin. The animals were allocated into different groups consisting of six animals in each group. Control group received only 0.5% carboxymethylcellulose solution. Food but not water was removed 24 h before administration of the test compounds. After the drug treatment, the rats were fed with normal diet for 17 h and then sacrificed. The stomach was removed and open along the greater curvature, washed with distilled water and cleaned gently by dipping in normal saline. The mucosal damage was examined by means of a magnifying glass. For each stomach the mucosal damage was assessed according to the following scoring system:

0.5: redness, 1.0: spot ulcers, 1.5: hemorrhagic streaks, 2.0: ulcers > 3 but ≤ 5, 3.0: ulcers >5. The mean score of each treated group minus the mean score of control group was regarded as severity index of gastric mucosal damage.

Lipid peroxidation

Lipid peroxidation (Ohkawa *et al.*, 1979) in the gastric mucosa was determined on the same animals used for the ulcerogenic activity. After screening for ulcerogenic activity, the gastric mucosa was scraped with two glass slides, weighed (100 mg) and homogenized in 1.8 mL of 1.15% ice cold KCl solution. The homogenate was supplemented with 0.2 mL of 8.1% sodium dodecyl sulphate (SDS), 1.5 mL of acetate buffer (pH 3.5) and 1.5 mL of 0.8% thiobarbituric acid (TBA). The mixture was heated at 95°C for 60 min. After cooling the reactants were supplemented with 5 mL of the mixture of *n*-butanol and pyridine (15:1 v/v), shaken vigorously for 1 min and centrifuged for 10 min at 4000 rpm. The supernatant organic layer was taken out and absorbance was measured at 532 nm on UV spectrophotometer. The results were expressed as nmol MDA/100 mg tissue, using an extinction coefficient of 1.56×10^5 cm^{-1} M^{-1}.

Hepatotoxic studies

The study was carried out on Wistar albino rats of either sex weighing 150-200 g Animal were devided into three groups, six rats in each. Group 1 was kept as control and received only vehicle (0.5% (w/v) solution of carboxymethylcellulose in water), rest of the groups received test compounds, at an equimolar oral dose relative to 10 mg/kg flurbiprofen in 0.5 % w/v solution of carboxymethylcellulose in water once in a day for 15 days. After the treatment (15 days) blood was obtained from all the groups of rats by puncturing the retro-orbital plexus. Blood samples were allowed to clot for 45 min at room temperature and serum was separated by centrifugation at 2500 rpm for 15 min and analyzed for various biochemical parameters.

Assessment of liver function

Assessment of liver function such as serum glutamate oxaloacetate transaminase (SGOT) and serum glutamate pyruvate transaminase (SGPT) were estimated by a reported method (Reitman and Frankel *et al.*, 1957). The alkaline phosphatase, total protein and total albumin were measured according to the reported procedures (King and Armstrong *et al.*, 1934; Varley *et al.*, 1988). All the data are recorded in Table 4.

Histopathological studies of liver

The histopathological studies were carried out by reported method (Luna *et al.*, 1968). The rats were sacrificed under light ether anesthesia after 24 h of the last dosage, the liver were removed and washed with normal saline and stored in formalin solution. Section of 5-6 microns in thickness were cut, stained with haematoxylin and eosin and then studied under an electron microscope (Figures 1 and 2).

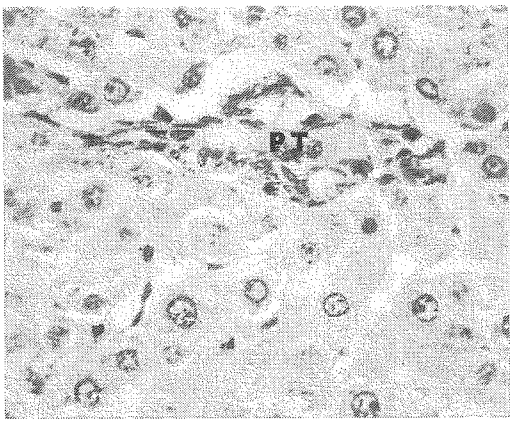


Figure 1. Control: Section of liver showing normal hepatic parenchyma with portal triad (400x)

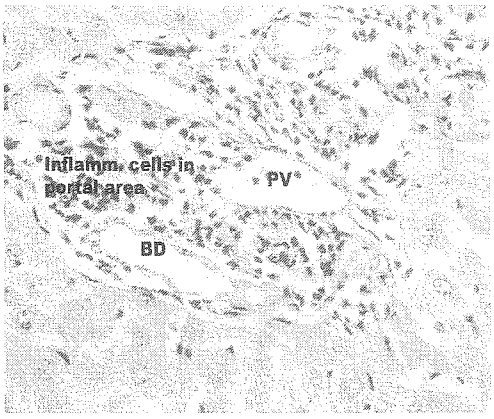


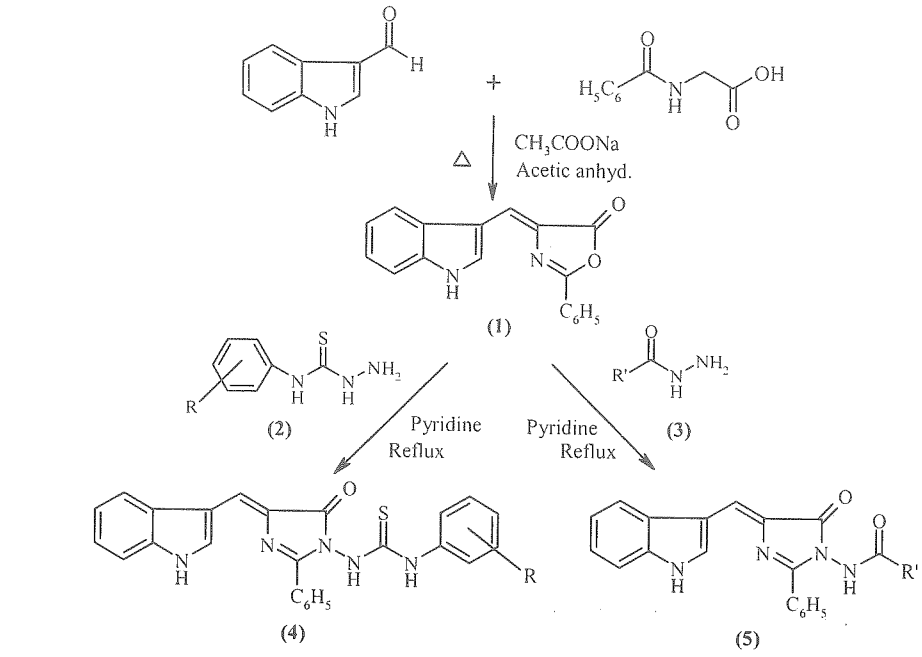
Figure 2. 5c: section of liver showing hepatic parenchyma with inflammatory cells structures infiltrating the portal triad structures (400x).

Antibacterial and antifungal activities

Antibacterial activity of the synthesized compounds was determined *in vitro* by using cup plate method (Murray *et al.*, 1995) against *Staphylococcus aureus* (gram-positive), *Escherichia coli* (gram negative) at 200, 100, 50, 25, 12.5 and 6.25 μg mL^{-1} concentration respectively, in the nutrient agar media by measuring the zone of inhibition in mm. Standard antibiotic ofloxacin was used as reference drug at 50, 25, 12.5 and 6.25 μg mL^{-1} concentrations.

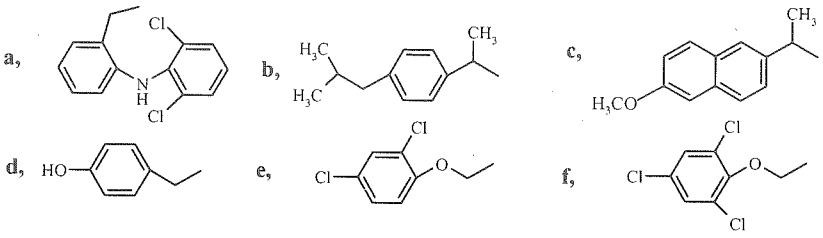
Similarly, the antifungal activity of the synthesized compounds were determined *in vitro* by cup plate method against fungal strain *Candida albicans* at 200, 100, 50, 25, 12.5 and 6.25 μg mL^{-1} concentrations in sudroad dextrose medium by using ketoconazole as standarad drug at 50, 25, 12.5 and 6.25 μg mL^{-1} concentrations. The zone of inhibition was measured in mm. The compounds which showed inhibition at 25 μg mL^{-1} concentration were further tested at 12.5 and 6.25 μg mL^{-1} concentrations. Dimethyl formamide (DMF) was used as solvent to prepare the desired concentration of the synthesized compounds.

Scheme 1



R = a: 4-OCH_3; b: 2-CH_3; c: 3-CH_3; d: 4-CH_3; e: 2,4-$(CH_3)_2$; f: 4-F; g: 3-Cl-4-F; h: 4-Br

R' =



Results and discussion

The anti-inflammatory activity of the synthesized compounds **4a-h,** and **5a-f** was evaluated by carrageenan induce paw edema method. Since the percentage of inhibition was found to be higher after 4 h, this was used as a basis of discussion. The tested compounds showed anti-inflammatory activity ranging from 22.72 - 83.32%, whereas standard drug indomethacin showed 82.57% inhibition after 4 h (Table 2). The compound **5c** having 2-(6-methoxy-2-naphthyl)propanamido group at 1st position of imidazole ring showed an anti-inflammatory activity (83.32%) higher than the standard drug indomethacin (82.57%). When this group was replaced by 2-(2,6-dichloroanilino)phenyl acetamido **(5a)** and 2,4-dichloro phenoxy acetamido **(5e)** the activity decreased (73.48 and 70.45% respectively). The activity was further decreased (65.90%) when *p*-hydroxyphenyl acetamido group **(5d)** was introduced in the imidazole ring. Rest of the compounds **(5b** and **5f)** showed weak activity. It was noticed that the replacement of propanamido/acetamido group by thiourido group also resulted in significant anti-inflammatory activity. The compounds **4e, 4d** and **4b** having 2,4-dimethylphenylthiourido, 4-methylphenyl thiourido and 2-methylphenyl thiourido group showed 79.54, 76.50 and 70.45% anti-inflammatory activity. The other compounds having thiourido moiety showed moderate to weak activity. Thus it was concluded that imidazole

derivatives having both propanamido/acetamido and thiourido group showed significant anti-inflammatory activity.

The compounds **4b, 4d, 4e, 5a, 5c** and **5e**, which showed significant anti-inflammatory activity were further tested for their analgesic effect at the same oral dose as used for the anti-inflammatory activity. The results for analgesic activity are presented as percentage analgesia in Table 3. The tested compounds showed % analgesia ranging from 29.8 to 94.7% whereas, standard drug indomethacin showed 70.7% analgesia at the same oral dose. The compounds **(5c)** which showed the maximum anti-inflammatory activity, also showed maximum (94.7%) analgesic effect. All the tested compounds having thiourido moiety i.e. **4d** (4-methylphenyl thiourido), **4e** (2,4-dimethylphenyl thiourido) and **4b** (2-methylphenyl thiourido) at 1st position of imidazoline ring showed more analgesic effect 91.8%, 85.3% and 72.2% respectively than that of standard drug.

The compounds **(4b, 4d, 4e, 5a, 5c** and **5e)** which were tested for their analgesic effect were further tested for their acute ulcerogenicity and lipid peroxidation (Table 3). The tested compounds showed severity index ranging from 0.500 to 1.000 whereas the standard drug indomethacin showed a high severity index of 2.333. The compound **5c** which showed maximum anti-inflammatory and analgesic activity also showed minimum ulcerogenicity (severity index 0.500), whereas compound having 2-methylphenyl thiourido **(4b)** and 2,4-dichlorophenoxy acetamido **(5e)** group at 1st position of imidazoline nucleus showed maximum ulcerogenicity (severity index 1.000). The result showed that all the tested compounds of the series showed less ulcerogenicity than that of standard drug. It has been reported in the literature that compounds showing less ulcerogenic activity also showed reduced malondialdehyde (MDA) content, a byproduct of lipid peroxidation. Therefore an attempt was made to correlate the decrease in ulcerogenicity of the compounds with that of lipid peroxidation. All the compounds screened for ulcerogenic activity were also analyzed for lipid peroxidation. The lipid peroxidation was measured as nmol MDA/100mg tissue. The tested indole and imidazole, derivatives showed significant decrease in lipid peroxidation (reduction in MDA content) ranging from 4.98 to 5.66 MDA/100mg in comparison to their reference drugs indomethacin (9.29 MDA/100mg). A control group was also used which showed 3.25 nmol MDA/100mg tissue.

The compound 4-(1*H*-Indol-3-yl-methylidene)-2-phenyl-1-[2-(6-methoxy-2-naphthyl) propanamido]-5-oxo-imidazoline **(5c)** showing potent anti-inflammatory and analgesic activities with minimum ulcerogenicity and lipid peroxidation were further studied for their hepatotoxic effect. The compound was studied for its effect on biochemical parameters (serum enzymes, total protein and total albumin) and histopathology of liver. As shown in Table 4, activities of liver enzymes SGOT, SGPT, alkaline phosphatase and total protein, total albumin were significantly changed with respect to control values. These changes were further confirmed by histopathological studies of the liver sample (Figure 2), which showed hepatic parenchyma with inflammatory cells infiltrating the Portal Triad in comparison to control group (Figure 1). No hepatocyte necrosis or degeneration was seen in the sample.

The results of the antimicrobial activities of the newly synthesized compounds were reported as minimum inhibition concentration (MIC) against *Staphylococcus aureus*, *Escherichia coli* and *Candida albicans* (Table 2). None of the synthesized compounds have shown significant antibacterial activity against *Staphylococcus aureus*. Compounds **4b, 4g** and **5c** exhibited MIC of 12.5 μg mL^{-1} against *Escherichia coli*. Compounds **5d** and **5f** were found to have moderate inhibitory activity (MIC of 25 μg mL^{-1}) against *Candida albicans*. The other compounds have not exhibited any significant antimicrobial activity against any of the strain used. The standard

drug ofloxacin showed MIC of 6.25 μg mL^{-1} against *Escherichia coli & Staphylococcus aureus* and ketoconazole showed MIC of 6.25 μg mL^{-1} against *Candida albicans*.

In conclusion, promising results have been obtained from these two series of compounds. These results clearly indicate that few of the hybrid molecules having both indole and imidazole residues showed significant anti-inflammatory and analgesic activities. Among these compounds 4-(1*H*-Indol-3-yl-methylidene)-2-phenyl-1-[2-(6-methoxy-2-naphthyl) propan-amido]-5 -oxo-imidazoline **(5c)** showed maximum anti-inflammatory and analgesic activity with minimum ulcerogenicity and lipid peroxidation and has emerged as a lead compound. These compounds were also tested for their antimicrobial activity and were found to be moderately active.

Table 1. Physical data of the synthesized compounds.

Compd.	Recrystallising Solvent	Yield %	M. P. (°C)	Mol. Formula	Mol. Wt.	% Nitrogen Calcd. (Found)
1	Acetone	41	154	$C_{18}H_{12}N_2O_2$	288	9.72 (9.79)
2a	Ethanol	75	148	$C_8H_{11}N_3OS$	197	21.30 (21.38)
2b	Ethanol	66	138	$C_8H_{11}N_3S$	181	23.18 (23.26)
2c	Acetone	73	92	$C_8H_{11}N_3S$	181	23.18 (23.11)
2d	Ethanol	65	110	$C_8H_{11}N_3S$	181	23.18 (23.10)
2e	Ethanol	55	116	$C_9H_{13}N_3S$	195	21.52 (21.46)
2f	Ethanol	64	152	$C_7H_8FN_3S$	185	22.69 (22.60)
2g	Ethanol	71	146	$C_7H_7ClFN_3S$	219	19.13 (19.20)
2h	Methanol	79	160	$C_7H_8BrN_3S$	246	17.07 (17.00)
4a	Ethanol	55	>300	$C_{26}H_{21}N_5O_2S$	467	14.98 (15.04)
4b	Ethanol	67	174	$C_{26}H_{21}N_5OS$	451	15.51 (15.60)
4c	Ethanol	61	294	$C_{26}H_{21}N_5OS$	451	15.51 (15.59)
4d	Ethanol	57	290	$C_{26}H_{21}N_5OS$	451	15.51 (15.46)
4e	Ethanol	52	178	$C_{27}H_{23}N_5OS$	465	15.04 (15.08)
4f	Ethanol	49	182-184	$C_{25}H_{18}FN_5OS$	455	15.37 (15.32)
4g	Ethanol	69	178	$C_{25}H_{17}ClFN_5OS$	490	14.29 (14.33)
4h	Ethanol	70	>300	$C_{25}H_{18}BrN_5OS$	516	13.56 (13.62)
5a	Acetone	63	274	$C_{32}H_{23}Cl_2N_5O_2$	580	12.06 (12.12)
5b	Methanol	56	130	$C_{31}H_{30}N_4O_2$	490	11.42 (11.49)
5c	Ethanol	61	240	$C_{32}H_{26}N_4O_3$	514	10.89 (10.81)
5d	Ethanol	58	232-234	$C_{26}H_{20}N_4O_3$	436	12.84 (12.93)
5e	Ethanol	64	168	$C_{26}H_{18}Cl_2N_4O_3$	505	11.09 (11.13)
5f	Ethanol	73	126	$C_{26}H_{17}Cl_3N_4O_3$	540	10.38 (10.43)

Table 2. Anti-inflammatory and antimicrobial activity of the synthesized compounds

Compound	Anti-inflammatory activity[#] % inhibition ± SEM		Antimicrobial activity MIC[##]		
	After 3 h	After 4 h	*S. aureus*	*E. coli*	*C. albicans*
4a	32.56 ± 3.79	34.08 ± 3.47**	- -	200	200
4b	68.93 ± 3.60	70.45 ± 3.26**	- -	12.5	50
4c	65.15 ± 1.51	66.66 ± 1.92**	50	200	100
4d	72.72 ± 1.66	76.50 ± 1.82	50	200	- -
4e	74.24 ± 0.95	79.54 ± 1.94	200	50	200
4f	30.29 ± 3.03	31.05 ± 3.60**	- -	50	- -
4g	49.24 ± 1.39	52.27 ± 1.01**	- -	12.5	50
4h	61.35 ± 2.27	64.38 ± 2.47**	- -	200	200
5a	71.21 ± 1.51	73.48 ± 1.82	200	200	100
5b	21.96 ± 2.17	22.72 ± 2.03**	200	200	- -
5c	78.02 ± 2.17	83.32 ± 0.95	200	12.5	100
5d	65.15 ± 1.51	65.90 ± 1.02**	- -	200	25
5e	68.18 ± 1.53	70.45 ± 1.55**	- -	200	50
5f	29.54 ± 3.85	30.29 ± 3.45**	- -	100	25
Indomethacin	72.72 ± 2.03	82.57 ± 1.82	xx	xx	Xx
Ketoconazole	Xx	xx	xx	xx	6.25
Ofloxacin	Xx	xx	6.25	6.25	Xx

[- -] Did not show any activity

[xx] Not tested

[#]Relative to standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n=6; **p<0.01.

[##]μg mL^{-1}

Table 3. Analgesic, ulcerogenic and lipid peroxidation activity of selected compounds.

Compound	Analgesic Activity[#]			Ulcerogenic activity (Severity index ± SEM)[##]	nmol MDA content ± SEM / 100 mg tissue[##]
	Pre-treatment/ normal 0 h (s)	Post-treatment/ after 4 h (s)	% Inhibition		
4b	1.22 ± 0.113	2.11 ± 0.145	72.2***	1.000 ± 0.22**	5.66 ± 0.15**
4d	1.26 ± 0.108	2.43 ± 0.188	91.8***	0.666 ± 0.10**	5.42 ± 0.33**
4e	1.59 ± 0.121	2.95 ± 0.278	85.3*	0.667 ± 0.11**	5.09 ± 0.29**
5a	1.45 ± 0.124	1.88 ± 0.115	29.8	0.750 ± 0.11**	5.58 ± 0.19**
5c	1.48 ± 0.122	2.85 ± 0.197	94.7***	0.500 ± 0.00**	4.98 ± 0.10**
5e	1.40 ± 0.121	1.91 ± 0.120	37.1***	1.000 ± 0.18**	5.60 ± 0.32**
Indomethacin	1.16 ± 0.064	1.98 ± 0.071	70.7***	2.333 ± 0.17	9.29 ± 0.33
Control				0.00	3.25 ± 0.05

[#]Relative to normal and data were analyzed by paired student's *t* test for n=6; ***p<0.0001, **p<0.001, *p<0.01

[##]Relative to their respective standard and data were analyzed by ANOVA followed by Dunnett's multiple comparison test for n=6; **p<0.01, *p<0.05.

Table 4. Effect of compounds on serum enzymes, total proteins and total albumin.

Compound	SGOT I Units/mL[#]	SGPT I Units/mL[#]	Alkaline Phophatase[#]	Total protein g/dL[#]	Total albumin g/dL[#]
Control	148.67 ± 1.50	27.67 ± 0.84	13.06 ± 0.25	1.80 ± 0.01	1.67 ± 0.01
5c	165.67 ± 0.42**	35.5 ± 1.59**	28.58 ± 0.11**	0.95 ± 0.04**	1.14 ± 0.05**

[#]Relative to control and data were analyzed by ANOVA followed by Dunnett's multiple comparison test, for n=6; **p<0.01

Acknowledgements

The authors are thankful to Head of the Department, Pharmaceutical Chemistry for providing laboratory facilities, Central Drug Research Institute (CDRI) for spectral analysis of the compounds and Majeedia Hospital, Hamdard University for providing necessary strains of bacteria and fungus. Authors are also thankful to Mrs. Shaukat Shah, in-charge of animal house for providing Wistar rats and Dr. A. Mukherjee, MD, Department of Pathology, All India Institute of Medical Sciences (AIIMS), New Delhi, for carrying out histopathological studies.

References

Adeyemi, O.O., Okpo, O.S. and Okpaka, O. (2004). The analgesic effect of the methanolic extract of *Acanthus montanus*. *J. Ethnopharmacol.* 90: 45-48.

Amir, M., Dhar, N. and Tiwari, S.K. (1997). Synthesis and anti-inflammatory activity of some new indole and indazole derivatives. *Indian J. Chem.* 36B: 96-98.

Amir, M., Agarwal, H.K., Javed S. and Kumar, H. (2006). Synthesis and anti-inflammatory activity of some new pyrazole and indazole derivatives. *Indian J. Heterocyclic Chem.* 16: 175-178.

Amir, M., and Kumar, S. (2004). Synthesis of some new 2,5-disubstituted 1,3,4-oxadiazole derivatives and their anti-inflammatory activity. *Indian J. Heterocyclic Chem.* 14: 51-54.

Amir, M. and Kumar, S. (2005). Anti-inflammatory and gastro sparing activity of some new indomethacin derivatives. *Arch. Pharm. Chem. Life. Sci.* 338: 24-31.

Amir, M. and Kumar, S. (2005). Synthesis and anti-inflammmatory, analgesic ulcerogenic and lipid peroxidation activities of 3,5-dimethyl pyrazoles, 3-methyl pyrazole-5-ones and 3,5-dimethyl pyrazolines. *Indian J. Chem.* 44B: 2532-2537.

Almansa, C., Alfon, J., Arriba, A.F.D., Cavalcanti, F.L., Escamilla, I., Gomez, L.A., Miralles, A., Soliva, R., Bartroli, J., Carceller, E., Merlos, M. and Rafanell, J.G. (2003). Synthesis and structure activity relationship of a new series of COX-2 selective inhibitor: 1,5-diarylimidazoles. *J. Med. Chem.* 46: 3463-3475.

Altintas, H., Ates, O., Uyde-Dogan, B.S., Alp, F.I., Kaleli, D., Ozdemir, O., Birteksoz, S., Otuk, G., Atana, D. and Uzun, M. (2006). Synthesis and evaluation of antimicrobial and anticonvulsant activities of some new 3-[2-(5-aryl-1,3,4-oxadiazol-2-yl/4-carbethoxy methylthiazol-2-yl)imino-4-thiazolidinon-5-ylidene]-5-substituted/ non substituted-1*H*-indole-2-ones and investigation of their structure-activity relationships. *Arzeimittelforschung*. 56: 239-248.

Barta, T.E., Stealey, M.A., Collins, P.W. and Weier, R.M. (1998). Anti-inflammatory 4,5-diarylimidazoles as selective cyclooxygenase inhibitors. *Bioorg. Med. Chem. Lett.* 8: 3443-3448.

Cioli, V., Putzolu, S., Rossi, V., Barcellona, S. P. and Corradino, C. (1979). The role of direct tissue contact in the production of gastro-intestinal ulcers by anti-inflammatory drugs in rats, *Toxicol. Appl. Pharmacol.* 50: 283-289.

Gupta, P., Hameed S. and Jain, R. (2004). Ring substituted imidazoles as a new class of anti-tuberculosis agents. *Eur. J. Med Chem.* 39: 805-814.

Khabnadideh, S., Rezaei, Z., Nezhad, A.K., Bahrinajafi, R., Mohamadi, R. and Farrokhroz, A.A. (2003). Synthsis of *N*-alkylated derivatives of imidazole as antibacterial agents. *Bioorg. Med. Chem. Lett.* 13: 2863-2865.

King, E. J. and Armstrong, A.R. (1934). A convenient method for determining serum and bile phosphatase activity. *Can. Med. Assoc. J.* 31: 376-381.

Luna, L.G. (1968). Manual of histological staining methods of the armed forces Institute of pathology, Eds. 3rd ed.; Mc-Graw-Hill: New York, 567-568.

Matysiak, J., Niewiadomy, A., Kulak, E.K. and Niewiadomy, G.M. (2003). Synthesis of some 1-(2,4-dihydroxythiobenzoyl)imidazoles, imidazolines and tetrazoles and their potent activity against Candida species. *Il Farmaco* 13: 455-461.

Misra, U., Hitkari, A., Saxena, A.K., Gurtu, S. and Shanker, K. (1996). Biologically active indolylmethyl-1,3,4-oxadiazoles, 1,3,4-thiadiazoles, 4*H*-1,3,4-triazoles and 1,2,4-triazines. *Eur. J. Med. Chem.* 29: 629-634.

Moore, P.F., Larson, D.L., Otterness, I.G., Weissman, A., Kadin, S.B., Sweeney, F.J., Eskara, J.D., Nagahisha, A., Sakakibara, M. and Carty, T.J. (1996). Tenidap, a structurally novel drug for the treatment of arthritis: anti-inflammatory and analgesic properties. *Inflamm. Res.* 45: 40-48.

Murray, P.R., Baron, E.J., Pfallar, M.A., Tenover, F.C. and Yolken, R.H. (1995). *Manual of clinical microbiology*, in: G.L. Woods, J.A. Washington (Eds.), Antimicrobial agents and susceptibility testing, *Am. Soc. Microbiol.* Washington, DC.

Ohkawa, H., Ohishi, M. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal. Biochem.* 95: 351-358.

Puratchikody, A. Nallu, M. and Gopalakrishnan, S. (2004). Synthesis of some new triphenylimidazoles of biological interest. *Indian J. Hetero. Chem.* 14: 149-150.

Radwan, M.A.A., Ragab, E.A., Sabry, N.M. and El-Shenawy, S.M. (2007). Synthesis and biological evaluation of new 3-substituted indole derivatives as potential anti-inflammatory and analgesic agents. *Bioorg. Med. Chem.*15: 3832-3841.

Reitman, S. and Frankel, S. (1957). A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. *Am. J. Clin. Pathol.* 28: 56.

Roberts, I.I.L.J., Morrow, J.D. (2001) *Goodman and Gilman,s Pharmacological Basis of Therapeutics,* McGraw-Hill, New York. 705-706.

Ryu, C.K., Lee, J.Y., Park, R.E, Ma, M.Y. and Nho, J.H. (2007). Synthesis and antifungal activity of 1*H*-indole-4,7-diones. *Bioorg. Med. Chem. Lett.*17: 127-131.

Scott, R.R. and Williiams, R.M. (1998). DNA cross-linking agents as antitumor drugs. *Chem. Rev.* 98: 2723-2796.

Varley, H. (1988). Practical clinical biochemistry, Eds. 1st ed.; CBS Publishers and Distributors: New Delhi, 236-238.

Winter, C.A., Risley, E. A. and Nuss, G. W. (1962). Carrageenan induced edema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.* 111: 544-547.

Received: 07.05.2007
Accepted: 05.11.2007