Synthesis and Biological Activities of Novel 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines

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Abstract
A straightforward method has been developed for the synthesis of new anti-inflammatory 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidine 5 from aminocyanopyrazole. These compounds were screened for their anti-inflammatory, gastroprotective, analgesic, antioxidant and anticaudial activities. Studies of structure-activity relationships have led to selection of compound 6-(4-methoxyphenyl)-3-methyl-1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidine, 5a which exhibited the most potent activities. The structures of all new compounds were elucidated using IR, 1H NMR, 13C NMR and HRMS.

Keywords: Anti-inflammatory; Gastroprotective; Antioxidant; Anticandidal; Analgesic; Pyrazolo[3,4-d]pyrimidine, 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines

Introduction
The growing incidence of drug-resistant infection diseases has stimulated the need for the development of new drugs. Since the few last decades, synthetic chemistry has been recognized to be rich source of bioactive metabolites with varied biological and pharmacological activities. Currently, Non-steroidal anti-inflammatory drugs (NSAIDs) are used throughout the world for the treatment of inflammation, pain and fever; however most of these produce several adverse reactions such as ulcers and hemorrhage [1]. In addition, reactive oxygen species (ROS) and free radicals play important roles in degenerative or pathological processes leading to many health disorders including inflammatory and cancer diseases [2]. The harmful effect of the free radicals can however, be blocked by synthetic antioxidants such as butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA), tert-butylhydroquinone (TBHQ) and propyl gallate (PG) [3]. However, due to their adverse side effects, search for more effective antioxidants has become crucial. Pyrazolopyrimidines, class of sedative and anxiolytic drugs [4], and its derivatives constitute a rich source of a wide variety of structurally unusual metabolites and seem to be an endless source of new chemical constituents.

In order to determine the role of methyl group and methoxy group of pyrazolopyrimidines in the antioxidant, anti-inflammatory, gastroprotective, analgesic and anticaudial activities, we have synthesized new 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines 5 and evaluate their pharmacological activities. Interestingly, we found that the activity of new compounds is dependent on the location of the methyl and methoxy group. Our results provided new evidence for the relationship between chemical structure and pharmacological activities as the case of 5a and 5b.

Materials and Methods
Chemistry
Phenyl hydrazine, malononitrile, triethylthioether, ammoniac, α-Bromoacetophenone, PTSA, CH₃COOH and solvents used in this work were obtained from Aldrich and Fluka and were used without further purification.

Melting points were measured on an Electrothermal apparatus. Progress of their actions was monitored with TLC using aluminium sheets with silica gel 60 F254 from Merck. Spectra IR were recorded on a Perkin-Elmer PARAGON FT-IR spectrometer covering field 400–4000 cm⁻¹. The spectra of 1H NMR and 13C NMR was recorded on a Varian Mercury 400 instrument, 1H at 400 MHz and 13C at 100 MHz. Mass spectrometry (HRMS). The chemical shifts are expressed in parts per million (ppm) by using tetramethylsilane (TMS) as internal reference. The multiplicities of the signals were indicated by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and coupling constants are expressed in Hertz.

Keywords: Anti-inflammatory; Gastroprotective; Antioxidant; Anticandidal; Analgesic; Pyrazolo[3,4-d]pyrimidine, 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines

Keywords: Anti-inflammatory; Gastroprotective; Antioxidant; Anticandidal; Analgesic; Pyrazolo[3,4-d]pyrimidine, 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines

Synthesis and spectral data of compounds 2-5: The general synthetic procedure employed for 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines 5 are outlined in Scheme 1.

5-amino-4-cyano N1-phenyl pyrazoles (2): 5-Amino-4-cyano-1-N1-phenyl pyrazoles prepared via a standard addition of hydrazine derivatives to ketene ethoxymethylene compounds following the reported procedure. Recrystallization from ethanol afforded pure 2 in good yields [5,6].

4-cyano N1-phenyl pyrazolo[5-imidates (3): The required 5-amino-4-cyano N1-phenyl pyrazoline (1.0 mmol) was treated with triethylthioether (6.0 mmol) and a catalytic amount of acetic acid and the mixture was refluxed for 24 h. After cooling, the reaction mixture was evaporated. The product was filtered, washed with diethyl ether then purified by recrystallization (ethanol).

4-amino N1-phenyl pyrazolo[3,4-d] pyrimidine (4): A solution of imidate 3 (1.0 mmol) in dry ethanol (5 mL) was treated with ammoniac (2.0 mmol) and a catalytic amount of acetic acid. The reaction mixture

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was refluxed for 6 h, and the formed solid was collected by filtration, dried and recrystallized from ethanol to give compound 4.

\[ \text{a) 4-amino-N'-phenyl-1H-pyrazolo[3,4-d]pyrimidine 4a:} \]

Yield 83%; mp 228 °C; IR (cm\(^{-1}\)) \(\nu\text{C} = \text{N} 1597, 1638, 1665; \text{RMN } \delta \text{ppm, DMSO-d}_6: 14.37 (\text{CH}_3); 14.44 (\text{CH}_3)

\[ \text{b) 4-amino-3-methyl-N'-phenyl-1H-pyrazolo[3,4-d]pyrimidine 4b:} \]

Yield 68%; mp 192 °C; IR (cm\(^{-1}\)) \(\nu\text{C} = \text{N} 1597, 1638, 1663; \text{RMN } \delta \text{ppm, DMSO-d}_6: 2.65 (3\text{H}, \text{s, CH}_3); 4.28 (2\text{H}, \text{s, NH}), 7.28 (1\text{H}, \text{J=7.3Hz, ArH}); 7.56 (2\text{H}, \text{J=7.3Hz, ArH}

\[ \text{c) 4-amino-6-methyl-N'-phenyl-1H-pyrazolo[3,4-d]pyrimidine 4c:} \]

Yield 70%; mp 160 °C; IR (cm\(^{-1}\)) \(\nu\text{C} = \text{N} 1597, 1638, 1663; \text{RMN } \delta \text{ppm, DMSO-d}_6: 2.65 (3\text{H}, \text{s, CH}_3); 4.28 (2\text{H}, \text{s, NH}), 7.28 (1\text{H}, \text{J=7.3Hz, ArH}); 7.56 (2\text{H}, \text{J=7.3Hz, ArH}

\[ \text{d) 6-(4-methoxyphenyl)-3-methyl-N'-phenyl-1H-pyrazolo[3,4-d]pyrimidine 5a:} \]

Yield 74%; mp 210 °C; IR (cm\(^{-1}\)) \(\nu\text{C} = \text{N} 1505, 1542, 1593; \text{RMN } \delta \text{ppm, DMSO-d}_6: 3.84 (3\text{H}, \text{s, CH}_3); 7.07 (2\text{H}, \text{J=7.3Hz, ArH}^1 \text{ and ArH}^2); 7.39 (1\text{H}, \text{J=7.2Hz, ArH}); 7.60 (2\text{H}, \text{J=5.4Hz, ArH}

\[ \text{e) 6-(4-methoxyphenyl)-3-methyl-N'-phenyl-1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines 5a-i:} \]

to a mixture of 4-amino-N'-phenyl-1H-pyrazolo[3,4-d]pyrimidine 4 (1mmol) and α-bromoacetophenone (1mmol) in 3 mL of ethanol, was added p-TsOH (5%) then refluxed for 12 h. After completion of the reaction, as indicated by TLC (EtOAc–hexane, 90:10), the precipitate product was separated by filtration and washed with ethanol and was crystallized from a suitable solvent to obtain pure product.
6-p-toly-1-N'-phenyl-1,7-dihydropyrazolo[3,4-d]
imidazo[1,2-f]pyrimidine 5c: Yield 81%; mp 166 °C; IR (cm−1): ν(C=O) 1519, 1550, 1594; RMN 'H (6 ppm, DMSO-d6): 2.37 (3H, s, CH3); 7.04 (2H, d, J=8.4Hz, ArH and Ar'H); 7.34 (1H, t, J=7.5Hz, ArH); 7.61 (2H, t, J=5.9Hz, ArH and Ar'H); 7.92 (2H, d, J=6.0Hz, ArH and Ar'H); 8.13 (2H, d, J=7.5Hz, ArH and Ar'H); 8.56 (1H, s, H); 8.71 (1H, s, H); 9.42 (1H, s, ArH); 10.41 (1H, s, NH); 14.21 (CH3); 14.42 (CH); 14.64 (CH); 14.85 (CH3); 150.58 (C-2' and C-6'), 152.87 (C-5'), 157.24 (C-3'), 159.36 (C-1'), 159.79 (C-4'); HRMS Calculated for C28H23N5O: 391.1689; found: 391.1684.

6-(4-Chlorophenyl)-3-methyl-N'-phenyl-1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidine 5h: Yield 58%; mp 192 °C; IR (cm−1): ν(C=O) 1504, 1550, 1594; RMN 'H (6 ppm, DMSO-d6): 2.28 (3H, s, CH3); 2.36 (3H, s, CH3); 7.05 (2H, d, J=7.5Hz, ArH and Ar'H); 7.40 (1H, t, J=8.5Hz, ArH); 7.59 (2H, t, J=5.4Hz, ArH and Ar'H); 7.92 (2H, d, J=6.3Hz, ArH and Ar'H); 8.01 (2H, d, J=5.1Hz, ArH and Ar'H); 8.51 (1H, s, H); 9.34 (1H, s, ArH); 10.40 (1H, s, NH); 14.21 (CH3); 14.42 (CH); 14.64 (CH); 14.85 (CH3); 150.58 (C-2' and C-6'), 152.87 (C-5'), 157.24 (C-3'), 159.36 (C-1'), 159.79 (C-4'); 162.17 (C-3); 147.77 (C-2'); 145.48 (C-4a); 149.10 (C-9a); HRMS Calculated for C28H20Cl2N5O: 509.1144; found: 509.1092.

Pharmacology

Chemicals and drugs: Carrageenan (BDH Chemicals Ltd., Poole, England), ranitidine (Medis, Tunis, Tunisia) and diclofenac (Medis, Tunis, Tunisia) were purchased from Central pharmacy of Tunisia.

Animals: Westar rats of either sex, weighing 150-180 g of both sexes were obtained from Pasteur Institute (Tunis, Tunisia). Housing conditions and in vivo experiments were approved according to the guidelines established by the European Union on Animal Care (CCE Council 86/690).

Antioxidant activities:

DPPH radical-scavenging activity: The free radical-scavenging activity of compounds (5a, b, c, d) were evaluated using the stable radical DPPH, according to the method of Kim et al. [7]. One millilitre of diluted sample (1 mg/mL) was added to 1 mL of the ethanolic DPPH solution. The mixture was then shaken and allowed to stand at room temperature in the dark. After 30 min, the decrease in absorbance at 517 nm was measured against a blank (ethanol solution) by using a UV–Vis spectrophotometer. A mixture consisting of 1 mL of ethanol and 1 mL of DPPH solution was used as the control. The radical-scavenging activity of test samples, expressed as percentage inhibition of DPPH, was calculated according to the formula:

\[
\% \text{ inhibition} = \frac{\text{A}_{0} - \text{A}_{t}}{\text{A}_{0}} \times 100
\]

where \(\text{A}_{0}\) and \(\text{A}_{t}\) are the absorbance values of the control and of the test sample, respectively. The compound concentration providing 50% inhibition (IC50) was calculated from the graph of inhibition percentage plotted against

6-(1-Chlorophenyl)-N'-phenyl-1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidine 5g: Yield 79%; mp 168 °C; IR (cm−1): ν(C=O) 1501, 1556, 1595; RMN 'H (6 ppm, DMSO-d6): 7.45 (1H, t, J=7.3Hz, ArH'); 7.54 (2H, d, J=8.4Hz, ArH' and ArH'); 7.62 (2H, t, J=8.1Hz, ArH' and ArH'); 8.05 (2H, d, J=8.4Hz, ArH' and ArH'); 8.14 (2H, d, J=7.8Hz, ArH' and ArH'); 8.58 (1H, s, H); 8.66 (1H, s, H); 9.35 (1H, s, H); RMN 'C (6 ppm, DMSO-d6): 108.66 (3C-3a); 120.25 (C-5); 121.77 (C-3' and C-5'), 122.68 (C-6), 127.12 (C-4'), 127.40 (C-2' and C-6'), 128.85 (C-3' and C-5'), 129.27 (C-2' and C-6'), 131.83 (C-1'), 132.55 (C-4'), 133.73 (C-3'), 140.08 (C-8), 142.98 (C-4a), 144.46 (C-9a); HRMS Calculated for C26H20Cl2N6O: 435.0781; found: 435.0789.

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The antifungal effect of compounds 5a, b, c, d was tested against Candida albicans (ATCC 10231) and Candida glabrata (ATCC 10231) by the broth dilution method (microdilution using 96-well microplates), following the procedure of visible fungal growth was measured by the broth dilution method (22019). The minimal inhibitory concentration (MIC) preventing growth of at least one hind limb. The compound was injected i.p. of 1% acetic acid 30 min after treatment. The number of writhing was recorded during 30 min commencing 5 min after the acetic acid injection. A writhe is indicated by abdominal constriction and was recorded during 30 min commencing 5 min after the acetic acid injection. The remaining group was treated with compounds 5a, b, c, d (50 and 100 mg/kg, s/c). All animals received 10 ml/kg body weight of vehicle (Tween 80/absolute ethanol/saline solution (0.9 %) in the ratio 1:1:18) (2.5 mL/kg, i.p). The control group received the acetylsalicylate of lysine (ASL) (200 mg/kg) by the same route, as a reference drug. The reference group received ranitidine (60 mg/kg, i.p). One hour later, the animals were sacrificed and their stomachs were removed and treated with 150 mM HCl/EtOH 1:1:18 (2.5 mL/kg, i.p). The ferric reducing antioxidant power (FRAP) of compounds (5a, b, c, d) was compared with ascorbic acid on DPPH radicals increased the DPPH radical-scavenging activity and ferric reducing antioxidant power (FRAP). Figure 1 show that the radical-scavenging activity of compounds (5a, b, c, d) were compared with ascorbic acid used as standard.

Carrageenan-induced rat paw oedema: Wistar rats were divided into groups of six animals. Oedema was induced by injecting 0.05 mL of 1% carrageenan subcutaneously into the sub-plantar region of the left hind paw. Compounds 5a, b, c and d (50 and 100 mg/ kg) were administered intraperitoneally (i.p.). The control group received the vehicle (Tween 80/absolute ethanol/saline solution (0.9 %) in the ratio 1:1:18) (2.5 mL/kg, i.p.). The reference group received diclofenac (25 mg/kg, i.p.). All drugs were administered 30 min before the injection of carrageenan. Measurement of paw size was done by means of volume placement technique using plethysmometer (Ugo Basile no.7140) immediately before carrageenan injection and 1, 2, 3, 4 and 5 h after carrageenan injection. Percentages of inhibition in our anti-inflammatory tests were obtained for each group using the following ratio: [(Vt-Vc) control-(Vt-Vc)treated] × 100/(Vt-Vc)Control

Where, Vt is the average volume for each group and Vc is the average volume obtained for each group before any treatment.

Acetic acid writhing test in mice: The analgesic activity was performed according to the method of Ayed et al. [10]. Swiss mice (20–30 g) were selected one day prior to each test and were divided into six groups of six mice each. One group served as control (vehicle 10 ml/kg) by subcutaneous injection (s.c). The second group was given the acetyl salicylate of lysine (ASL) (200 mg/kg), by the same route, as a reference drug. The remaining group was treated with compounds 5a, b, c, d (50 and 100 mg/kg, i.p). All animals received 10 ml/kg (i.p.) of 1% acetic acid 30 min after treatment. The number of writhing was recorded during 30 min commencing after the acetic acid injection. A writh is indicated by abdominal constriction and stretching of at least one hind limb.

Anticandidal activity: The antifungal effect of compounds 5a, b, c, d was tested against three Candida strains (Candida albicans ATCC 90028, Candida glabrata ATCC 90030 and Candida parapsilosis ATCC 22019). The minimal inhibitory concentration (MIC) preventing visible fungal growth was measured by the broth dilution method (microdilution using 96-well microplates), following the procedure of Hammer et al. [11]. Compounds 5a, b, c, d solutions were prepared by dissolution in 10% dimethyl sulfoxide (DMSO). The compounds concentrations tested ranged from 0.1 to 10 mg/ml. The MIC of each compound was defined as the lowest concentration which inhibited candidal growth after incubation at 37°C between 18 and 24 h. The minimal fungidical concentration (MFC) was determined by subculture on blood agar at 37°C between 18 and 24 h. Amphotericin B was used as antifungal positive control.

Statistical analysis
Data are presented as the mean ± standard error of the mean (s.e.m). Statistical analysis was performed using Student’s t-test. The significance of difference was considered to include values of P<0.05.

Results and Discussion

Chemistry
The 5-amino-4-cyano-N1-phenylpyrazole 2, used as a starting material, was prepared in two steps following a similar method reported by Petrie and al [12-14]. The reaction of compound 4 with a-bromoacetophenone in the presence of catalytic amount of acid furnished the corresponding cyano-pyrazolimidates 3 which subsequently were transformed to the corresponding amine pyrazolopyrimidines 4 upon treatment with ammoniac [5,15-17]. Reaction of compound 4 with a-bromoacetophenone in the presence of a catalytic amount of PTSA (5 mol%) in refluxing ethanol furnished the 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines derivatives 5. 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines derivatives 5 were isolated as stable compounds in good yields.

The reaction occurs by a primary amino group intercepts a bromine atom liberating HBr, followed by an intracyclisation and elimination of a water molecule to give 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines 5a-i. It is interesting to note that time reaction and yield of products are directly related to the nature of substituent (Rf, Rs, Rg). The yields of compounds 5a and 5c are 74 and 81 %, respectively. When R is a hydrogen substituent, the product is obtained with superior yield in short time (e.g., Compounds 5b and 5c and 5g). From these results, we conclude that the electronic nature of the substituent on the bromocarotenophene has a significant role on the reaction outcome. The correct identity of compound 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines was confirmed by 'H NMR, 13C NMR and HRMS (Scheme 1; Table 1).

Pharmacology
Antioxidant activities: Free radicals have an important role in pathogenesis of a wide range of diseases including inflammation. Antioxidants can prevent biological and chemical substances from free radical induced oxidative damage and stress. Consequently, multipotent antioxidants have gained a great attention from scientists for their potential in treatment of many diseases [18]. In this study, two methods were used to evaluate the antioxidant activity of compounds 5a, b, c, d, the DPPH radical-scavenging activity and ferric reducing antioxidant power (FRAP). Figure 1 show that the radical-scavenging activity of compounds (5a, b, c, d) and ascorbic acid on DPPH radicals increased in dose-dependent manner. The IC50 values calculated from the graph (Figure 1) show that compound 5a exhibited significant antioxidant activity with IC50 values of 0.037 mg/ml, whereas, compound 5b exhibited moderate activity (IC50 value of 0.049 mg/mL). Compounds...
5c, d don’t have any activity. So, compound 5a, b have the highest free radical scavenging activity, which was found to be comparable with that of ascorbic acid (IC50=0.013 mg/mL) (Table 2).

In addition, Figure 2 shows the ability of compounds (5a, b, c, d) and ascorbic acid to reduce Fe2+ to Fe3+ at different concentration ranges. The reducing potential of compounds (5a, b, c, d) and ascorbic acid increased with increase of concentrations. Compound 5a had the highest reducing power, with IC50 = 0.042 mg/mL followed by compound 5b (IC50 = 0.065 mg/mL) (Table 2). The FRAP values of compounds 5a, b were comparable with that of ascorbic acid (IC50 = 0.025 mg/mL), however compounds 5c, d showed a lower reducing power (Table 2). These different values of IC50 indicated that the order of increasing reductive potential of ferric iron was 5b < 5a < ascorbic acid.

The reducing power of various compounds might be due to their hydrogen-donating ability as described by Shimada et al. [19]. So, compounds 5a, b is good electron donors and could terminate the free-radical chain reactions by converting free radicals to more stable radicals (Figure 2).

**Anti-inflammatory activity:** The anti-inflammatory activity of compounds 5a, b, c, d against acute pedal edema (induced by carrageenan) is shown in Table 3 and the results are comparable to that of the standard drug diclofenac, a potent inhibitor of cyclooxygenase 2 (Cox-2). Carrageenan induced paw edema remained even 6 h after its injection into the sub plantar region of rat paw. Diclofenac as a reference standard drug inhibited the edema formation due to carrageenan to an extent of 58.22% (at 3 h) at the dose of 25 mg/kg. The compounds 5a, b, c, d inhibited, significantly edema formation in rats (p<0.01) in a dose-dependent manner. Compound 5a at the dose of 50 mg/kg inhibited edema formation to the extent of 74.6% (at 3 h) and the edema was found to be reduced to 5.25 ± 2 10^-2 mL (Figure 3). The compound 5b has also a height activity at a dose of 50 mg/kg with a percentage of inhibition of edema of 65.54% (at 3 h), while compounds 5c and 5d at a dose of 50 mg/kg reduced edema with a percentage of 48.66 and 44.21%, respectively. The presence of edema is one of the prime signs of inflammation [20]. It has been documented that carrageenan induced rat paw edema is suitable in vivo model to study anti-inflammatory drugs both steroidal and non-steroidal since it involves several mediators [21]. This method was chosen for the present study since edema induced by carrageenan is the most prominent acute experimental model in search for new anti-inflammatory drugs [20]. Carrageenan induced edema has been commonly used as an experimental animal model for acute inflammation and is believed to be biphasic [22]. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the damaged tissue surroundings. The late phase (2.5-5 h) is due to the over production of prostaglandin and nitric oxide with peak at 5 h, produced by inducible isofoms of Cox (cox-2) and nitric oxide synthase (iNOS) [23].

However, treatment with compounds 5a, b, c, d significantly reduced carrageenan on induced inflammation in both the phases (1-5 h) of the experiment. Based on this, it may be that compounds 5a, b, c, d have a non-selective inhibiting effect on the release or actions of these mediators of inflammation and the suppression of the 1st phase may be due to inhibition of the release of early mediators, such as histamine and serotonin and the action in the 2nd phase may be explained by an inhibition of cyclooxygenase 2.

**Gastroprotective activity:** The results of gastroprotective activity of compounds 5a, b, c, d on gastric ulcer induced by HCl/ethanol solution are shown in Table 4. Oral administration of the damaging agent to the control group clearly produced a mucosal damage characterized by

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**Table 1:** Synthesis of 1,7-dihydropyrazolo[3,4-d]imidazo[1,2-f]pyrimidines 5a–l.

| Compounds | R1  | R2  | R3  | Yields (%) | Mp (°C) | Reaction time (h) |
|-----------|-----|-----|-----|------------|--------|-------------------|
| 5a        | CH3 | H   | 4-OCH3 | 74        | 210    | 12                |
| 5b        | H   | H   | 4-OCH3 | 78        | 171    | 7                 |
| 5c        | CH3 | H   | 4-CH   | 81        | 166    | 5                 |
| 5d        | CH3 | H   | 4-CH   | 89        | 206    | 12                |
| 5e        | H   | CH3 | 4-OCH3 | 62        | 212    | 24                |
| 5f        | H   | CH3 | 4-CH   | 58        | 205    | 18                |
| 5g        | H   | H   | 4-Cl   | 79        | 168    | 5                 |
| 5h        | CH3 | H   | 4-Cl   | 69        | 192    | 10                |
| 5i        | CH3 | H   | 4-Cl   | 67        | 189    | 17                |

Values are expressed as mean ± SEM of triplicate measurement.

**Table 2:** IC50 values of DPPH radical scavenging activity and reducing power of compound 5a, 5b, 5c and 5d.

| Sample | IC50a of DPPH radical-scavenging activity (mg/ml) | IC50b of reducing power (mg/ml) |
|--------|-----------------------------------------------|---------------------------------|
| 5a     | 0.037 ± 0.01                                  | 0.042 ± 0.02                    |
| 5b     | 0.049 ± 0.03                                  | 0.065 ± 0.03                    |
| 5c     | -                                             | -                               |
| 5d     | -                                             | -                               |
| Ascorbic acid | 0.013 ± 0.02                                      | 0.025 ± 0.01                     |

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**Figure 1:** Free radical-scavenging activity of compounds 5a, 5b, 5c and 5d and ascorbic acid on DPPH.

**Figure 2:** Reductive potential of compounds 5a, 5b, 5c and 5d and ascorbic acid using spectrophotometric detection of Fe2+-Fe3+ transformations.
multiple haemorrhage red bands of different sizes along the long axis of the glandular stomach [9].

As shown in Table 4, the compound 5a significantly exhibited, at the dose of 100 mg/kg, the higher inhibition of gastric lesions (76.53%) than compound 5b at the same dose (65.48%). The gastroprotective effect of the two compounds 5a and 5b were similar to the effect produced by the reference drug, ranitidine, which exhibited 65.24% of inhibition. However, 5c and 5d were less effective than the reference drug; the percentage of inhibition of gastric lesions was 46.11% and 39.36%, respectively, at the dose of 100 mg/kg (Figure 4). As described by Jonsson et al. ethanol administration resulted in severe mucosal damage through an increase in reactive oxygen species generation and a decrease in the endogenous antioxidant defense mechanisms. So, oxygen-derived free radicals (ROS) may contribute to ethanol induced gastric mucosal lesions [24]. Thus, compounds 5a and 5b may function by decreasing the redox state in HCl/ethanol induced gastropathy (Figure 4).

**Algesic activity:** In addition to the anti-inflammatory activity, the study of analgesic properties of compounds 5a, b, c, d were also evaluated. Among the several models of visceral pain, we use the acetic acid writhing test in mice. Acetic acid acts indirectly by inducing the release of endogenous mediator, which stimulates the nociceptive neurons sensitive to NSAIDs (nonsteroidal anti-inflammatory drugs) and/or opioids [25]. Injection of acetic acid into the control mice resulted in 72.44 writhes. Pretreatment with compounds 5a, b, c, d at doses 50 and 100 mg/kg reduced the number of writhes in a dose dependent manner. Interestingly, compounds 5a (16.82 writhes, 76.78% of inhibition) and 5b (20.84 writhes, 71.23% of inhibition) at a dose of 100 mg/kg registered higher levels of analgesic activity than 5c (39.22 writhes, 45.85% of inhibition) and 5d (32.56 writhes, 55% of inhibition) and approaches the activity of the standard drug ASL (20.24 writhes, 72% of inhibition) (Table 5).

**Anticandidal activity:** Anticandidal activity is reported as MIC and MFC (Table 6). All compounds showed significant antifungal activity against *Candida* strains. The best activity was observed with compounds 5a, b against *Candida albicans*. 5a had the highest anticandidal effect in all yeast strains with MIC ranged from 0.62 to 1.22 mg/ml, followed by 5b, d and c which demonstrated antifungal properties depending from the candidal strains. The MICs ranged from 0.84 to 3.42 mg/ml.
In conclusion, our results indicate that compounds 5a and 5b have potential anti-inflammatory and analgesic effects associated with antifungal and gastroprotective properties against HCl/ethanol induced gastric ulcer. The mechanism of 5a and 5b mediated protection may be related to decreases in free radical production. These observations raise the possibility of compounds 5a and 5b being used to treat inflammation and to improve resistance to gastric mucosal injury.

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