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Synthesis of Novel 3-Mercapto 1,2,4- Triazole Derived Mannich Basses and their Biological Activities

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ABSTRACT:

Triazole, a heterocyclic nucleus has attracted a wide consideration of the medicinal chemist in search for the new therapeutic molecules. The main of the study is to synthesize novel 3-mercapto 1,2,4- triazole derived mannich basses and their biological activities. All the compounds exhibited anti-inflammatory activity when compared with ASA as well as ibuprofen. The compounds 3a and 3b reduced carrageenan-induced edema in Swiss white mice by 33.6% and 69.1%, respectively. This result is significant ($p<0.001$) when compared with the control group treated with the saline solution (Table 1).The compounds 4 and, 5 were able to reduce the edema by 17.2% and 56.1%, respectively, as shown in Table 1. Therefore, substances 3b and 5 showed the best anti-inflammatory activity in terms of edema inhibition. In this study, new series of 3-mercapto-1,2,4-triazoles 3a, 3b, 4 and 5, the were synthesized and their anti-inflammatory activity was determined. Several of the newly synthesized derivatives displayed promising antimicrobial and anti-inflammatory activities compared to known antibacterial, antifungal and anti-inflammatory drugs. Though, the mechanism of the biological activity needs further investigations, which are in progress.

Keywords: Heterocyclic, 3-mercapto 1,2,4- triazole, antimicrobial, and anti-inflammatory.

INTRODUCTION

Triazole

Triazole, a heterocyclic nucleus has attracted a wide consideration of the medicinal chemists in search for new therapeutic molecules. Now a day's research is concentrated on the beginning of new and safe therapeutic agents of clinical importance. The heterocycles are gaining their importance as being the center of activity. The nitrogen-containing heterocycles are found in abundance in most of the medicinal compounds. The synthesis of high nitrogen-containing heterocyclic systems has been increasing interest over the past decade. Triazoles are well known five membered heterocyclic compounds belong to one of the most widely used class of antifungal drugs known as azoles.

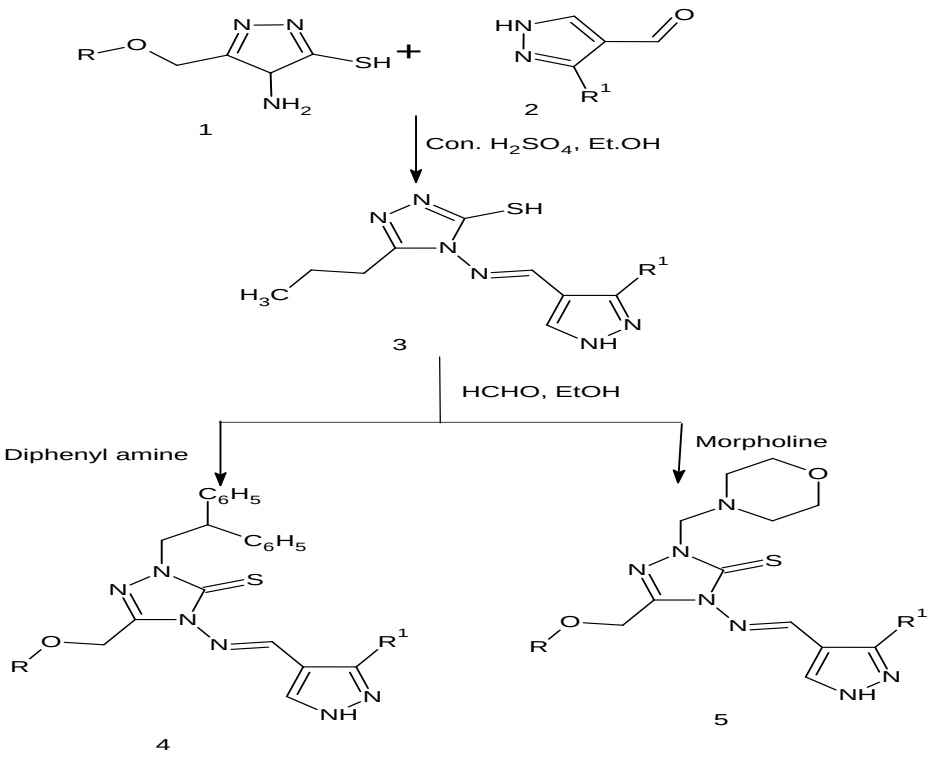
MATERIAL AND METHOD

Experimental Procedure:

Melting points were determined using Buchi-510 instrument. IR spectra were recorded on Perkin-Elmer-683 series spectrometer with KBr optics, and 1H NMR (300 MHz) were recorded on BrukerAvance 400 spectrometer using TMS as internal standard (chemical shifts and ppm). Mass spectra were recorded on a VG micromass70-70 H instrument. CHN analysis was carried out using Vario Micro Cube Elementar instrument.

Present work

Synthesis



Compound	R	R^1
3a	2-tolyl	4-fluorophenyl
3b	4-tolyl	4-fluorophenyl
4	4-tolyl	4-fluorophenyl
5	4-tolyl	4-fluorophenyl

4.2.1.1 General method for the preparation of 4-({[3-substituted-1H-pyrazol-4-yl]methylidene}amino)-5-[(substituted)methyl]-1,2,4-triazole-3-thiols (Schiff bases)

0.01 mole of 5-[(substituted)methyl]-4-amino-3-mercapto-1,2,4-triazole **1**, was dissolved in minimum amount of ethanol (5 mL). To this, was added 0.01 mole of 3-substituted-1*H*-pyrazole-4-carbaldehyde **2,** in hot ethanol (4 mL). Catalytic amount of concentrated sulphuric acid was added to the above mixture and the contents were refluxed on water bath for 10 h. The resulted solution was allowed to stand overnight and the precipitated solid was filtered, washed, dried and recrystallized from alcohol to yield the Schiff bases (**3)**.

4.2.1.2 General method for the preparation of 4-({[3-substituted-1H-pyrazol-4-yl]methylidene}amino)-2-substituted-5-[(substituted)methyl]-1,2,4-triazole-3-thiones (Mannich bases) (4 and 5)

6 millimole of Schiff base **3** was dissolved in minimum amount of hot ethanol (4 mL). To this, 1 mL of 40 % formaldehyde was added. To the above solution, 6 millimole of secondary amine (diphenylamine / morpholine) dissolved in minimum ethanol (1 mL) was introduced. The mixture was stirred for 24 h at room temperature. The solid separated was filtered, dried and recrystallized from ethanol-dioxane mixture, to yield the Mannich bases **4** and **5**.

4.3 BIOLOGICAL ACTIVITY

4.3.1 Anti-inflammatory activity

Animals

Swiss albino (male and female) mice weighing 20-30 g (10-24 weeks female), (5-14 weeks male) and *Wister Kyoto* rats (male and female) weighing 200-300 g (8-10 weeks old) were used for the present study. Animals were given a week time to get acclimatized with laboratory conditions of the Department of Pharmacy, University of Sargodha, Sargodha, Pakistan. Animals were kept under standard laboratory conditions with a controlled environment of

temperature 23 ± 3 C, humidity (60% ± 10%) an 12 h light/dark cycle. Animals were kept in polypropylene rat/mice cages in a group consisting of not more than six rats/mice per cage. They were given free access to food with a standard rodent pellet diet and drinking water. Animal handling was according to the National research guidelines[14]. Prior the animal experiment, our pharmacological protocols were approved by institutional ethical committee, College of Pharmacy, University of Sargodha, Pakistan with approval No (67B18 IAEC/UOS). All the animals (mice and rats) used in current study were released after completion of the specified studies.

Drug and chemicals

Compounds**,** acetylsalicylic acid (ASA), and ibuprofen (obtained from Bristol-Myers Squibb, Brazil) were dissolved individually in 1% CMC and administrated as described above at doses of 50, 100, 150, 200, 250, and 350 mg/Kg.

Experimental design

The drugs used for comparison purposes were **3a–c**, **5a–c**, ibuprofen, and ASA. All compounds were suspended in 1% carboxymethylcellulose (CMC) and single dose of 250 mg/Kg was administered intraperitoneally, in the morning [15]. Other animal group received 1% CMC. Two positive and one negative anti-inflammatory control tests were done in three animal groups by intraperitoneal administration of 250 mg/Kg of acetylsalicylic acid (ASA), a standard dose for pharmacological comparative tests, 250 mg/Kg of ibuprofen (Laboratory Teuto Brazilian Ltd., Brazil), and 0.9% of aqueous saline solution, respectively. The anti-inflammatory activity was determined by Levy's method [16]. Carrageenan (Sigma, St. Louis, USA), 0.1 mL of a 1% solution in 0.9% NaCl, was injected through the plantar tissue of the right hind paw of each mouse to produce inflammation. After four hours, the animals were sacrificed under anesthesia and their paws were cut and weighed. The results were analyzed according to the percentage of inflammation reduction as described earlier [16].

The following formula was used to determine the percentage % inhibition in edema.

$$\text{Inhibition}(\%) = \frac{(V_t - V_0)^{\text{control}} - (V_t - V_0)^{\text{tested}}}{(V_t - V_0)^{\text{control}}} \times 100$$

Vt represents mice paw volume at time interval't', Vo is the initial mice paw volume (basal value), $(Vt- Vo)^{control}$ is edema produced in the control group and $(Vt- Vo)^{treated}$ is edema produced in the treated group.

4.4 Statistical analysis

All results are expressed as mean ± SEM for experiments. Statistical evaluation was undertaken by analysis of variance (ANOVA) followed by Turkey test for multiple comparisons. $p < 0.05$ was used as the criterion of statistical significance.

5. RESULT AND DISCUSSION

5.1 Characterization

***4-({[3-(4-fluorophenyl)-1H-pyrazol-4-yl]methylidene}amino)-5-[(2-methylphenoxy)methyl]-1, 2, 4-triazole-3-thiol* (3a)[17]**

Creamy solid (77 %); m.p. 229-231 °C; IR (KBr) [cm-1]: 3131 (Ar. C-H *str*.), 2938, 2853 (methyl C-H *str*.), 1604 (C=N *str*.), 1510 (Ar. C=C *str*.), 1090 (Ar. C-O-C *sym. str*.), 1235 (Ar. C-O-C *asym. str*.); 1H-NMR (400 MHz, acetone-d6) [ppm]: δ 2.35 (s, 3H, CH3), δ 5.23 (s, 2H, OCH2), δ 6.9-7.9 (m, 8H, Ar.H), δ 7.3 (s, 1H, pyrazole 5-H), δ 8.18 (brs, pyrazole N-H), δ 9.95 (s, 1H, N=CH), δ 11.8 (brs, 1H, S-H) ; MS (m/z): 408 (M+); Anal. calcd. for C20H17FN6OS; C, 58.83; H, 4.17; N, 20.59. Found: C, 58.91; H, 4.20; N, 20.63.

***4-({[3-(4-fluorophenyl)-1H-pyrazol-4-yl]methylidene}amino)-5-[(4-methylphenoxy)methyl]-1,2,4-triazole-3-thiol* (3b)**

Creamy solid (85 %); m.p. 240-242 °C; IR (KBr) [cm-1]: 3138 (Ar. C-H *str*.), 2940, 2860 (methyl C-H *str*.), 1604 (C=N *str*.), 1508 (Ar. C=C *str*.), 1090 (Ar. C-O-C *sym. str*.), 1235 (Ar. C-O-C *asym. str*.); 1H-NMR (400 MHz, acetone-d6) [ppm]: δ 2.24 (s, 3H, Ar.CH3), δ 5.22 (s, 2H, OCH2), δ 7.32 (s, 1H, pyrazole 5-H), δ 8.2 (brs, pyrazole N-H), δ 6.9-7.9 (m, 8H, Ar.H), δ 10.14 (s, 1H, N=CH), δ 12.82 (brs, 1H, S-H); MS (m/z): 408 (M+); Anal. calcd. for C20H17FN6OS; C, 58.83; H, 4.17; N, 20.59. Found: C, 58.91; H, 4.19; N, 20.64.

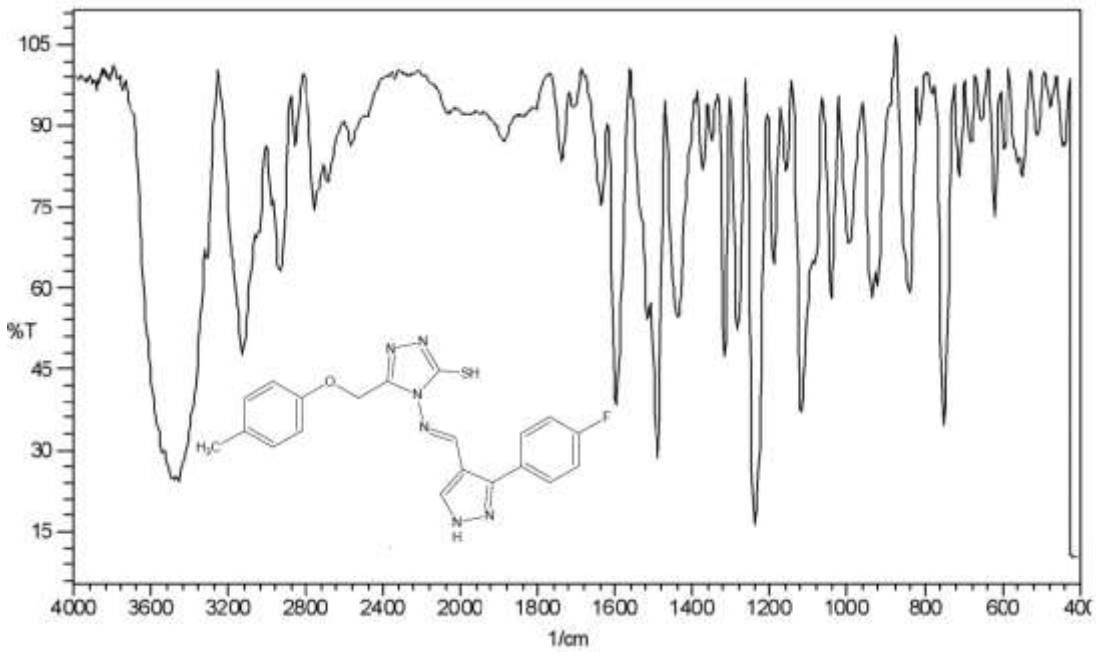


Fig.1 : IR spectrum of compound 3b

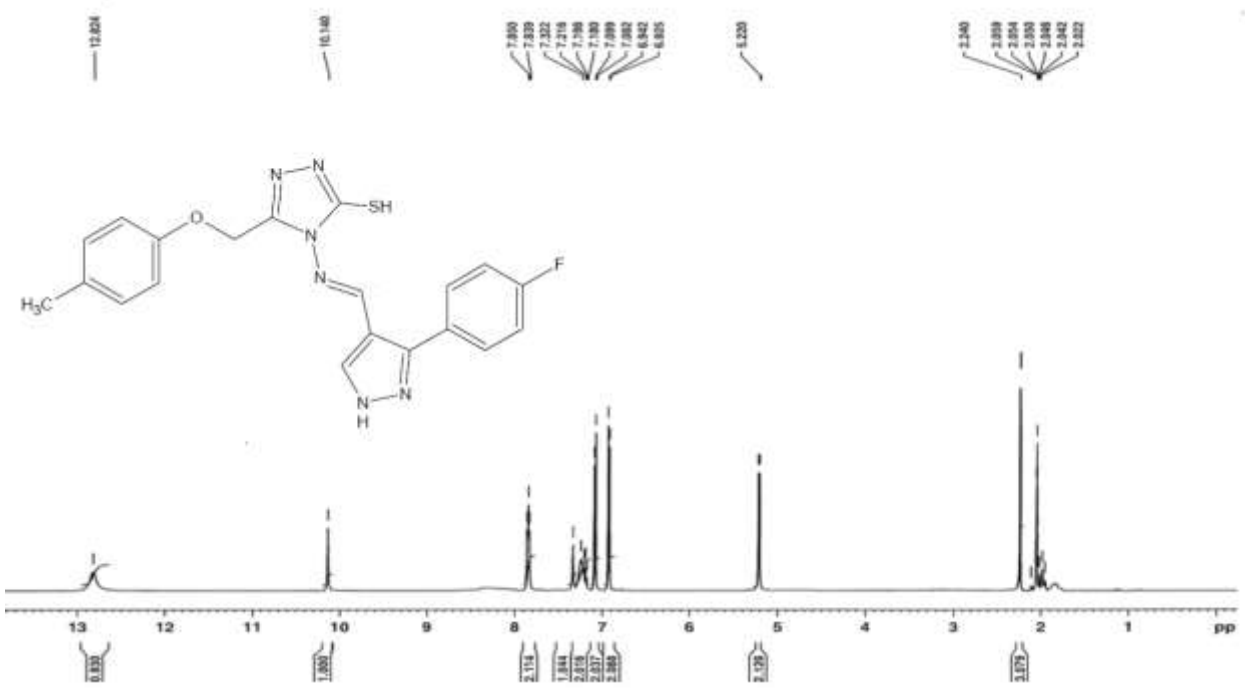


Fig.2: 1H-NMR spectrum of compound (**3b**)

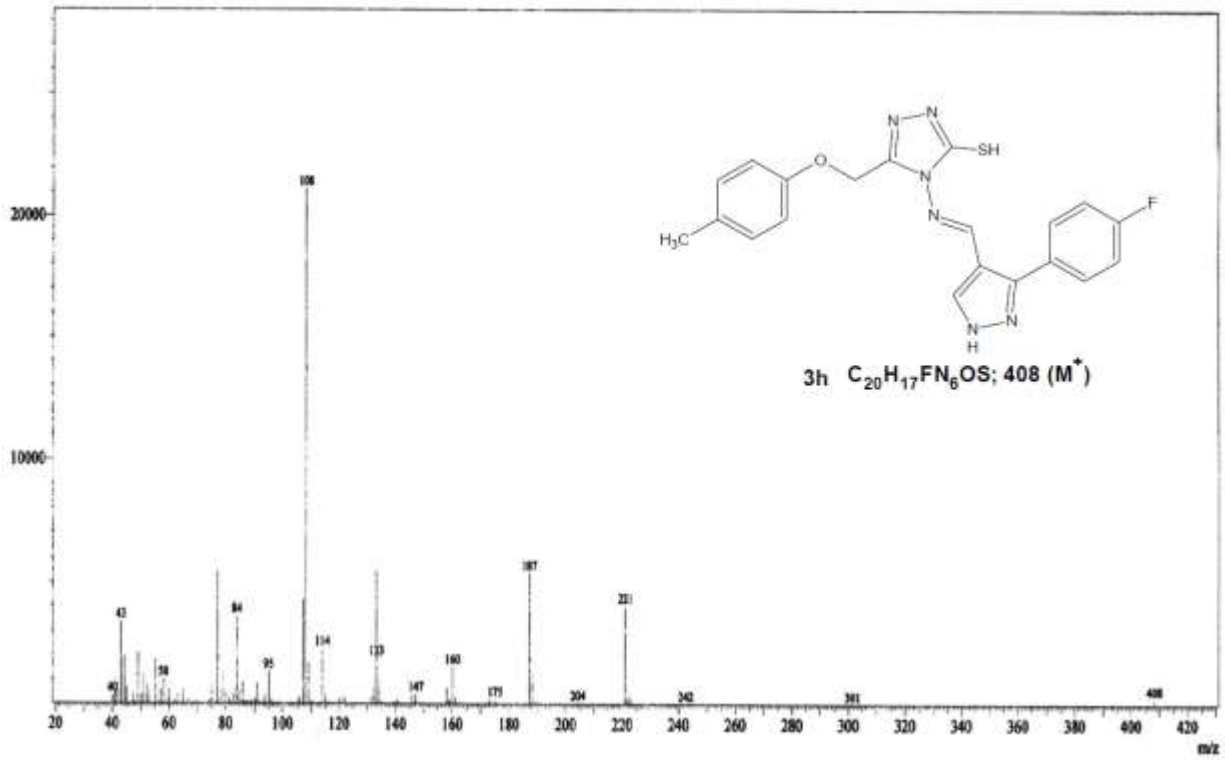


Fig. 3: Mass spectrum compound (**3b**)

4-({[3-(4-fluorophenyl)-1H-pyrazol-4-yl]methylidene}amino)-2-[(diphenylamino)methyl]-5-{(4-methylphenoxy)methyl}-1,2,4-triazole-3-thione **(4)**

Creamy white solid (72 %); m.p. 220-222 °C; IR (KBr) [cm-1]: 3140 (Ar. C-H *str*.), 2950, 2860 (methyl C-H *str*.), 1610 (C=N *str*.), 1508 (Ar. C=C *str*.), 1340 (C=S *str*.), 1090 (Ar. C-O-C *sym. str*.), 1233 (Ar. C-O-C *asym. str*.); 1H-NMR (400 MHz, acetone-d6) [ppm]: δ 2.32 (s, 3H, Ar. CH3), δ 5.21 (s, 2H, OCH2), δ 5.65 (s, 2H, >N-CH2-N<), δ 7.12 (s, 1H, pyrazole 5-H), δ 8.34 (brs, pyrazole N-H), δ 6.9-7.9 (m, 18H, Ar.H), δ 9.93 (s, 1H, N=CH); MS (m/z): 182 (N-methyl-N,N-diphenylamine cation); Anal. calcd. for C33H28FN7OS; C, 67.23; H, 4.75; N, 16.64. Found: C, 67.14; H, 4.73; N, 16.59.

4-({[3-(4-fluorophenyl)-1H-pyrazol-4-yl] methylidene} amino)-2-(morpholin-4-ylmethyl)-5-{(4-methylphenoxy) methyl}-1,2,4-triazole-3-thione **(5)**

Creamy solid (64 %); m.p. 162-164 °C; IR (KBr) [cm-1]: 3130 (Ar. C-H *str*.), 2950, 2860 (methyl C-H *str*.), 1607 (C=N *str*.), 1508 (Ar. C=C *str*.), 1325 (C=S *str*.), 1095 (Ar. C-O-C *sym. str*.), 1230 (Ar. C-O-C *asym. str*.); 1H-NMR (400 MHz, CDCl3) [ppm]: δ 2.30 (s, 3H, Ar. CH3), δ 2.69 (4H, CH2-N-CH2), δ 3.73 (4H, CH2-O-CH2), δ 5.13 (s, 2H, OCH2), δ 5.64 (s, 2H, >N-CH2-N<), δ 7.16 (1H, pyrazole 5-H), δ 8.22 (brs, pyrazole N-H), δ 6.9-8 (m, 8H, Ar.H), δ 9.95 (s, 1H, N=CH); MS (m/z): 100 (morpholinomethyl cation); Anal. calcd. for C25H26FN7O2S; C, 59.17; H, 5.13; N, 19.33. Found: C, 59.24; H, 5.16; N, 19.38.

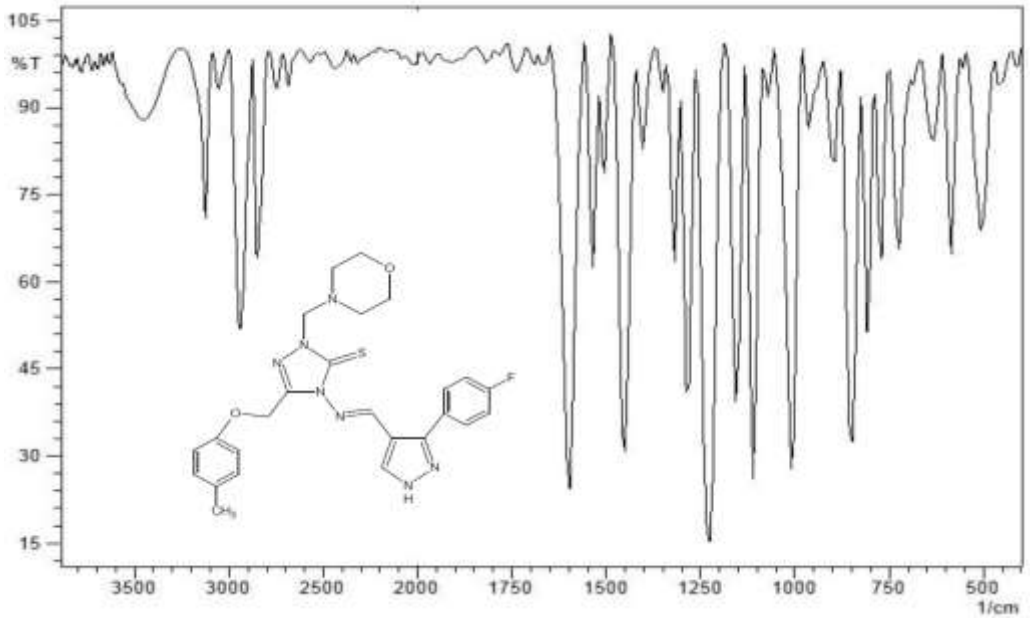


Fig.4: IR spectrum of compound (**5**)

Fig.5: 1H**NMR** spectrum of 4-({[3-(4-fluorophenyl)-1*H*-pyrazol-4- yl]methylidene}amino)-2-(morpholin-4-ylmethyl)-5-{(4-methylphenoxy)methyl}-1,2,4- triazole-3-thione (**5**)

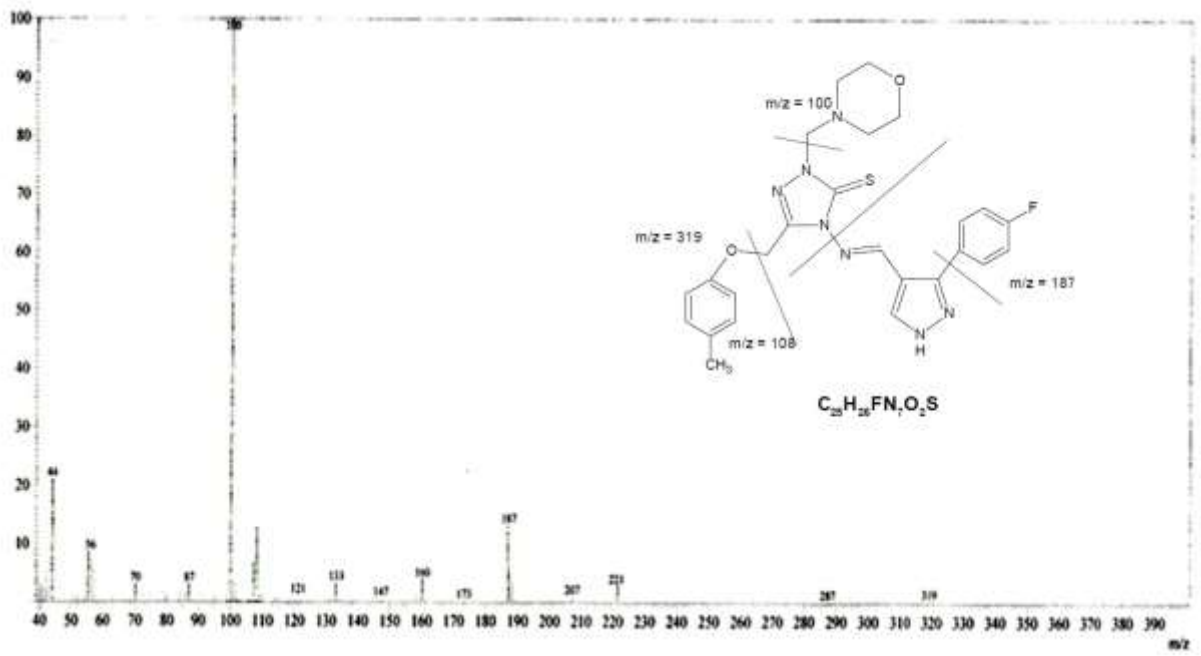


Fig.6: Mass spectrum of (**5**)

Biological evaluation

Anti-inflammatory activity

Acute anti-inflammatory activity of 3-mercapto-1,2,4-triazole after carageenin-induced edema at dose 250mg/kg.

Compounds	Difference in paw weight (**g**) ± SEM	Edema inhibition (%)
3a	0.1032 ± 0.0129**	33.6
3b	0.0484 ± 0.0160**	69.1
4	0.1245 ± 0.0119**	17.2
5	0.0681 ± 0.0128**	56.1
1% Carboxymethylcellulose	0.1460 ± 0.0168^{ns}	6.1
0.9% saline solution	0.1552 ± 0.0172^{ns}	-------
Ibuprofen	0.0420 ± 0.0134**	73.2
ASA	0.0501 ± 0.0237**	68.1

Significant differences: *$P < 0.05$; **$P < 0.001$; ns: not significant

All the compounds exhibited anti-inflammatory activity when compared with ASA as well as ibuprofen. The compounds **3a** and **3b** reduced carrageenan-induced edema in Swiss white mice by 33.6% and 69.1%, respectively. This result is significant ($p<0.001$) when compared with the control group treated with the saline solution (Table 1).

The compounds **4 and**, **5** were able to reduce the edema by 17.2% and 56.1%, respectively, as shown in Table 1. Therefore, substances **3b** and **5** showed the best anti-inflammatory activity in terms of edema inhibition.

CONCLUSION

In this study, new series of 3-mercapto-1,2,4-triazoles **3a, 3b, 4 and 5**, the were synthesized and their anti-inflammatory activity was determined. Several of the newly synthesized derivatives displayed promising antimicrobial and anti-inflammatory activities compared to known antibacterial, antifungal and anti-inflammatory drugs. Though, the mechanism of the biological activity needs further investigations, which are in progress.

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