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Synthesis and Antimicrobial, Antiinflammatory and Antibacterial Activity of New Triazolothiadiazole Derivatives

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ABSTRACT

The aim of present research work is to synthesize certain substituted triazolothiadiazole derivatives of biological interest. Among all the synthesized compounds some of them showed good anti-inflammatory, antimicrobial and antifungal activity when compared with standard drug. From the findings obtained, it can be concluded that triazolothiadiazole moiety linked to various substituted aryl carboxylic acid and substituted aromatic aldehyde compounds could be synthesized as an approach to obtain potential anti-inflammatory, antimicrobial agents and antifungal agents. Mefenamic acid, used as a starting material and it was identified by their physical, chemical and spectral data and all necessary solvents were purified by double distillation prior to use for synthesis.

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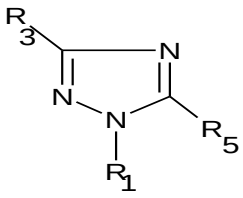
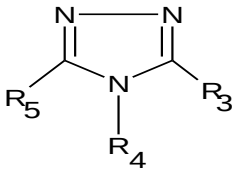
INTRODUCTION:

1,2,4-triazoles can be considered as cyclic hydrazidines with hydrogen or some other substituent on either hydrazine nitrogen as in **(1)** or amide nitrogen as in **(2)**. The prefixes 1H and 4H are used to distinguish **(1)** and **(2)** respectively. The name 1,3,4-triazole for **(2)** appears in some theoretical papers but it is deceptive when **(1)** and **(2)** are tautomers (with R^1= R^4 =H) In order to list the substituent in alphabetical order or deal with rearrangements between annular nitrogen some authors have used the name '1,3,5-triazoles' thus breaking one general rule of nomenclature in order to comply with another.

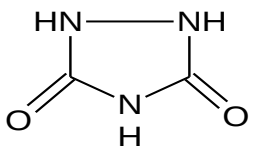


(1)

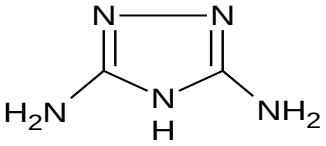


(2)

The hetero aromatic triazole ring system is composed of five atoms, two carbons, and the three nitrogens, which can be arranged in two combinations to give either v-triazole (v-vicinal) 1,2,3-triazole or s-triazole (s-symmetrical) or 1,2,4-triazole. A few trivial names of 1,2,4-triazoles are in common use: 3,5-dioxo-1,2,4-triazolidines **(3)** are called urazoles and the corresponding 3,5-diamino compounds **(4)** guanazoles.



(3)



(4)

Partially or fully reduced triazoles (triazolines and triazolidines, respectively) with monovalent substituents are relatively unstable and of little current interest. Like their aliphatic analogues, non-aromatic triazolines and triazolidines are readily hydrolyzed in acidic medium; other electrophilic reagents also open the ring.

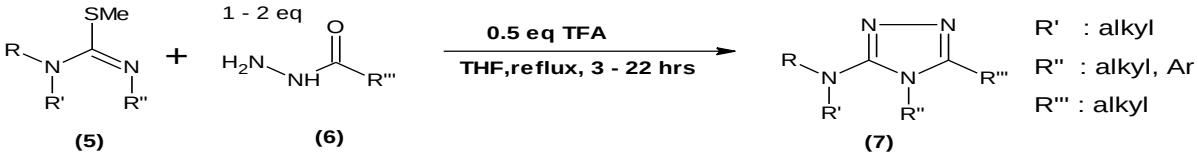
1,2,4-triazole:

During recent year there has been intense investigation of different classes of triazole compounds, many of which known to possess interesting biological properties such as antibactarial[1-3], anti-inflammatory[4-6], and antifungal activity[7].

Recent strategies in the synthesis of 1,2,4-triazole:

Method 1:

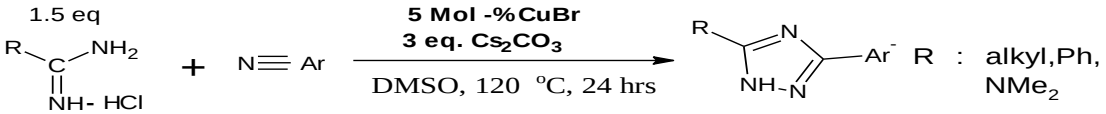
Batchelor. *et al.*[8] 3-*N*,*N*-Dialkylamino-1,2,4-triazoles can be prepared from *S*-methylisothioureas and acyl hydrazides in good yields. The reaction conditions are relatively mild and tolerate a broad range of functional groups.



(5) (6) (7)

Method 2:

Nagasawa. *et al.* [9] a copper-catalyzed reaction under an atmosphere of air provides 1,2,4-triazole derivatives by sequential N-C and N-N bond-forming oxidative coupling reactions. Starting materials and the copper catalyst are readily available and inexpensive. A wide range of functional groups are tolerated.



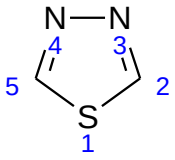
(8) (9)

There are several isomers of thiadiazole, that is 1, 2, 3-Thiadiazole **(10)**, 1, 2, 5-thiadiazole **(11)**, 1, 2, 4-thiadiazole **(12)** 1, 3, 4-thiadiazole **(13)**

1,2,3-thiadiazole **(10)** | 1,2,5-thiadiazole **(11)** | 1,2,4-thiadiazole **(12)** | 1,3,4-thiadiazole **(13)**

1, 3, 4-thiadiazole is the isomers of thiadiazole series. A glance at the standard reference works shows that more studies have been carried out on the 1,3,4-thiadiazole than all the other isomers combined member of this ring system found their way in to such diverse applications as pharmaceuticals, oxidation inhibitor, cyanide dyes, metal complexing agents.

The ending *–azole* designates a five membered ring system with two or more heteroatoms, one of which is nitrogen, the ending *–ole* is used for other five membered heterocyclic ring without nitrogen. The numbering of monocyclic azole system begins with the heteroatom that is in the highest group in the periodic table and with the element of lowest atomic weight in that group. Hence the numbering of 1, 3, 4-thiadiazole **(13)** is done in following manner. This designate that one sulphur group is present in the ring.



(13)

1, 3, 4-Thiadiazole:

During recent year there has been intense investigation of different classes of thiadiazole compounds, many of which known to possess interesting biological properties such as antimicrobial[10-12], anti-inflammatory[13-14], and antifungal activity[15].

Recent strategies in the synthesis of 1, 3, 4-Thiadiazole:

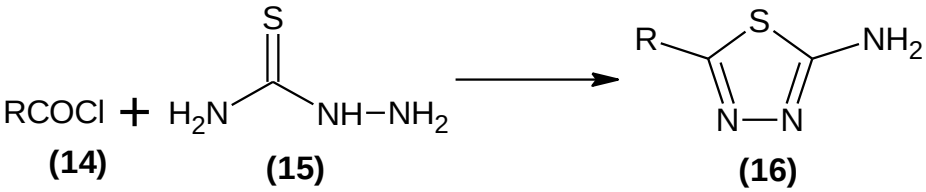
Recent strategies on the synthesis of 1, 3, 4-Thiadiazole derivatives can be summarized in to following points:

(a) From thiosemicarbazides:

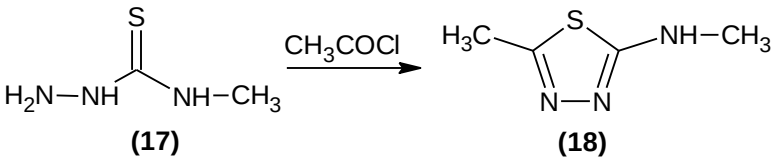
Many synthesis of the 1, 3, 4-thiadiazole proceed from thiosemicarbazide or substituted thiosemicarbazide.

Method 1:

Freund. *et al.*[16] have shown that thiosemicarbazide **(15)** cyclizes directly to 2-amino-5-methyl-1, 3, 4-thiadiazole **(16)** with acetyl chloride **(14)**. This simple route to 2-amino-5-substituted -1, 3, 4-thiadiazole seems to be quite general. In example shown **R** may be methyl, norhydnocarpyl, benzyl, cyclopropyl and many others,



Pulvermacher. *et al*.[17] had earlier showed that acetyl chloride **(14)** could bring about the cyclization of alkyl-or aryl- substituted thiosemicarbazide. For example, the action of acetyl chloride on 4-methylthiosemicarbazide **(17)** produces 5-methyl-2-methylamino-1, 3, 4-thiadiazole **(18).**



Method 2:

Pulvermacher. *et al*.[17] observed that formic acid could cyclize the alkanoyl halides by acylation. He found that by heating 4-phenyl thiosemicarbazide **(19)** with formic acid, 2-anilino-1, 3, 4-thiadiazole **(20)** was formed.

(b) From Thiocarbazides:

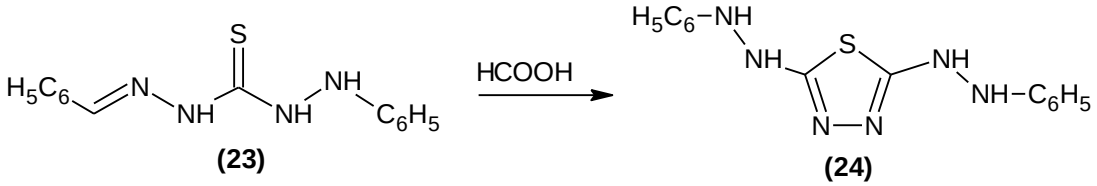
There are two methods by which 1, 3, 4-thiadiazole can be prepared from Thiocarbazides.

Method 1:

If 1-phenylthiocarbazide **(21)** is heated with formic acid, it is converted to 2-phenylhydrazino-1, 3, 4-thiadiazole **(22)**[18]**.**

Method 2:

This method is related to the oxidation of 1-phenylbenzalthiocarbazone **(23)** 2-phenylhydrazino-1, 3, 4-thiadiazole **(24)**[19]**.**



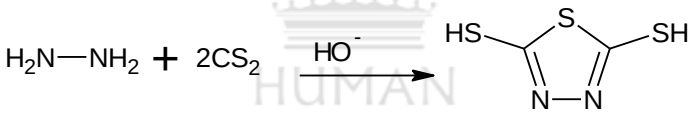
(23) **(24)**

(c) From Dithiocabazates:

Following methods have been reported for the preparation of 1, 3, 4-thiadiazole from Dithiocabazates

Method 1.

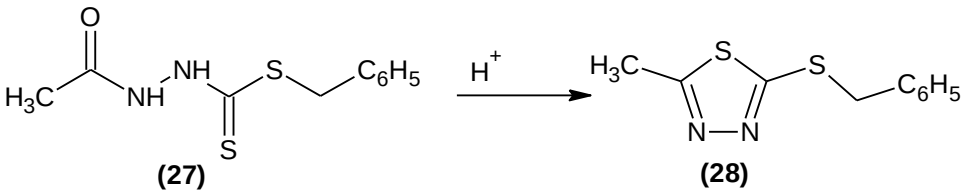
Another route to 1, 3, 4-thiadiazole is via substituted dithiocarbazic acid and their esters. A reaction which belongs in this group is the formation of 2,5-dimercapto-1, 3, 4-thiadiazole **(25)** by action of carbon disulphide on hydrazine **(26)** in basic medium[20-21].



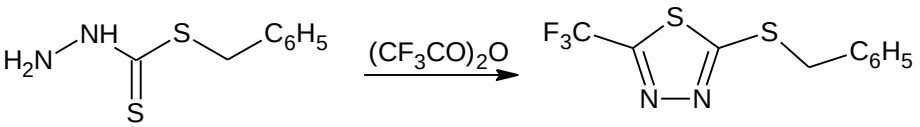
(25) **(26)**

Method 2.

When 3-acyldithiocarbazic esters **(27, 28)** are treated with acids, they cyclize to form substituted thiadiazole **(29, 30)**[22, 23.] This is a quite general reaction. Both benzyl and methyl 3-acyldithiocarbazates have been employed.



(27) **(28)**

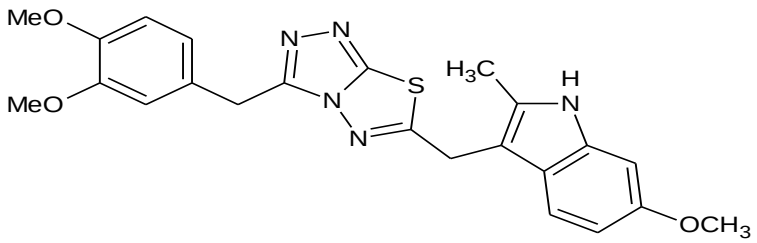


(29) **(30)**

Recent Advancement in the Therapeutic Potential of 1,3,4 Thiadiazole Derivatives:

Analgesic and Anti-inflammatory Activity:

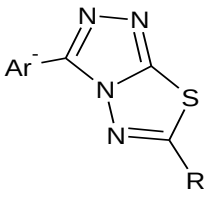
Vinod Mathew. *et al.*[24]. Have synthesized several 3, 6-disubstituted-1, 2, 4-triazolo [3, 4-b]-1, 3, 4-thiadiazole and their dihydro analogues. They found that anti-inflammatory and analgesic activity screening of the tested compounds **(31)** showed good anti-inflammatory and analgesic activities.



Fig; Compound 31

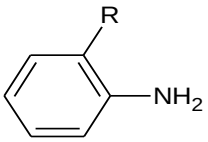
Currently available non-steroidal anti-inflammatory drugs (NSAIDs) like ibuprofen, flurbiprofen, fenbufen and naproxen exhibit gastric toxicity. Literature survey revealed that modification of the carboxyl function of representative NSAIDs resulted in increased anti-inflammatory activity with reduced ulcerogenic effect[25,26].

Certain compounds bearing 1, 2, 4-triazole and 1, 3, 4-thiadiazole nuclei possess significant anti-inflammatory activity with reduced GI toxicity. Mohd. Amir et.al[27]. Have replaced the carboxylic acid group of 2-(4-isobutylphenyl) propanoic acid and biphenyl-4-yloxy acetic acid by a composite system, which combines both the triazole and the thiadiazole nucleus in a ring to give a compact and planar structure. It was interesting to note that seven cyclized compounds **32a, 32b, 33a, 33b, 34a, 34b** and **34c** were found to have anti-inflammatory properties comparable to their standard reference drugs ibuprofen and flurbiprofen.

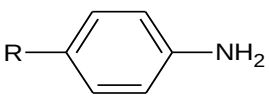


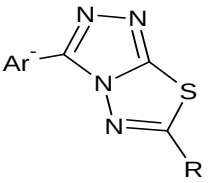
COMPOUND 32

COMPOUND 32a, R=

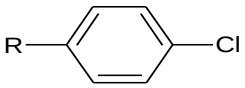


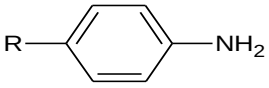
COMPOUND 32b, R=

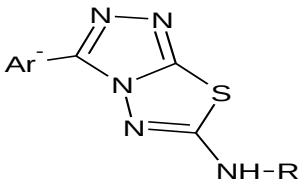




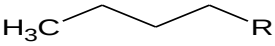
COMPOUND 33

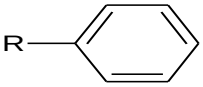
COMPOUND 33a, R= 

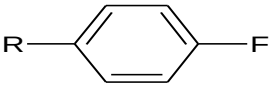
COMPOUND 33b, R= 



COMPOUND 34

COMPOUND 34a, R= 

COMPOUND 34b, R= 

COMPOUND 34c, R= 

When these compounds were subjected to analgesic activity by tail immersion method in mice, all compounds exhibited moderate to good activity. These compounds were also tested for ulcerogenic activity and lipid peroxidation, and showed superior GI safety profile along with reduction in lipid peroxidation as compared with ibuprofen and flurbiprofen.

Antimicrobial and Antiinflammatory Activity:

Mohd. Amir. *et al.* [27] have also found that Compound **(32b)** demonstrated about half the activity of ofloxacin against E. coli. The other compounds showed moderate to weak antibacterial activity against S. aureus and E. coli. The synthesized compounds showed weak antifungal activity against C. albican, except for compound **(34c)** that showed half of the activity of the antifungal drug (ketoconazole). Thus the triazolothiadiazole derivatives were found having dual functional properties (anti-inflammatory-analgesic and antimicrobial), and represent a promising class of compounds with an interesting pharmacological profile .

Present Work

In view of varied biological and pharmacological importance of triazolothiadiazole derivatives, it is felt worthwhile to evaluate them for possible activities. The compounds synthesized were screened for their anti-inflammatory, antibacterial and antifungal activities.

The details of each of the methods are presented in the experimental section along with the observations recorded in tables.

1. ANTI-INFLAMMATORY ACTIVITY

Inflammation is the body's response to noxious or injurious stimuli, characterized by warmth, redness of the skin, pain, swelling and loss of function. Inflammation is a part of host defense mechanism. There are several tissue factors that are known to be involved in the inflammatory reactions such as releases of histamines, bradykinin and prostaglandins.

Essentially there are acute and chronic of inflammation. The classical signs of acute inflammation are warmth, redness, pain, swelling and loss of function. Chronic inflammation is also characterized by long lasting pain, redness and swelling and is caused by the persistence of an irritant, which may be biological, physical or chemical in nature.

Experimental models for testing anti-inflammatory activity

Acute inflammatory condition is produced in the animals by adapting the following methods[81].

a) Carrageenan-induced pedal inflammation

b) Egg-white induced pedal inflammation

c) Dextrin-induced pedal inflammation

Chronic inflammatory condition is produced in the animals by adapting the following methods

a) Formaldehyde-induced pedal inflammation

b) Implantation of cotton pellets

c) Granular pouch

d) Tuberculin sensitivity

e) Fred's adjuvant

Instruments used to measure the paw oedema are,

a) Plethysmograph

b) Setlines apparatus

Procedure:

The compounds were tested for anti-inflammatory activity by carrageenan induced rat paw oedema model[82] employing Zeitlin apparatus to measure the paw thickness.

Materials:

All the materials used for this experiment are of analytical grade. Carrageenan was procured from Hi-media. Sodium CMC (E. Merck), Saline (Core health care), Mefanemic acid (IP) was purchased from the local supplier.

Preparation of sodium CMC suspension:

Stock suspension of sodium CMC was prepared by triturating 1g of sodium-CMC in 100 ml of distilled water and used for suspending the test compounds and standard drug.

Preparation of carrageenan suspension:

1% suspension of carrageenan sodium salt was prepared by sprinkling 100mg of carrageenan powder in 10 ml of saline (0.9% NaCl) solution and set aside to soak for 1 hr. A homogenous suspension was then obtained by through mixing with a magnetic stirrer.

Experimental Procedure:

Albino rats of either sex, weighing between 200-250 g were used in experiment. They were divided into seventeen groups of six animals each. All groups were fasted for overnight and allowed water and *libitium.* The animals were given following treatment.

Inflammation was induced by injecting 0.05 ml of 1% carrageenan suspension subcutaneously into the sub plantar region of the right hind paw and 0.05 ml of saline was injected into the sub plantar region of the left hind paw[83] for all groups. One hour prior to carrageenan injection, the groups III to XVIII treated with test compounds (10 mg/kg).

1% sodium CMC gel (1 ml/kg), was given to group-I used as carrageenan treated control and the standard drug aceclofenac (2mg/kg) was administered to group-II. All the doses were administered orally. Anti-inflammatory activity was evaluated by measuring carrageenan induced paw oedema[84].

Measurement of paw thickness:

The thickness of the both paws of each rat was measured before carrageenan injection and after carrageenan injection at time intervals 2 and 4 hours using Zeitlin`s constant load lever method[85,86] consisting of a graduated micrometer combined with a constant loaded lever system to magnify the small changes in paw thickness during the course of the experiment. The percent increase of paw oedema thickness[85] was determined at 2 and 4 hours after induction of inflammation.

$$\text{Percentage increase in paw thickness} = (Yt - Yo / Yo) \times 100$$

Where Yt = paw thickness at the time `t` hours (after injection)

Yo = paw thickness at the time `0` hours (before injection)

The percent inhibition of paw oedema thickness is calculated using the formula,

$$\text{Percentage inhibition} = (1 - Yt / Yo) \times 100$$

Where Yt = Average increase in paw thickness in groups tested with test Compounds.

Yo = Average increase in paw thickness in control

The results and statistical analysis of anti-inflammatory activity of mefenamic acid and the compounds tested are shown in **Table-6** and **Fig -XIII**.

Table-1

Anti-inflammatory activity of synthesized compounds

Compd. No	***Ar***	Dose (µmol/kg body weight)	Mean value (± SEM) of oedema volume at different intervals		Percentage of anti-inflammation at different intervals	
			2h	**4h**	**2h**	**4h**
Control (2%gum acacia)	--	31	0.254 (±0.009)	0.225 (±0.007)	--	--
Standard Mefanemic acid	--	31	0.117 (±0.018)	0.031 (±0.003)	53.68	86.22
5a	C_6H_5	31	0.189 (±0.002)	0.135 (±0.003)	45.59	60.76
5b	C_8H_9O(*m*)	31	0.157 (±0.003)	0.131 (±0.001)	38.18	41.77
5c	C_6H_4Cl(*m*)	31	0.183 (±0.006)	0.147 (±0.005)	47.95	64.66
5d	$C_6H_5O_2$(*p*)	31	0.197 (±0.001)	0.160 (±0.001)	42.44	48.88
5e	C_6H_6N(*m*)	31	0.148 (±0.005)	0.088 (±0.001)	41.73	60.88
6a	C_6H_5	31	0.191 (±0.003)	0.138 (±0.005)	44.80	68.66
6b	C_6H_5O (*p*)	31	0.127 (±0.010)	0.040 (±0.003)	50.00	82.22
6c	C_7H_7O(*p*)	31	0.125 (±0.002)	0.032 (±0.003)	50.78	84.89
6d	C_6H_4Cl(*p*)	31	0.126 (±0.003)	0.042 (±0.004)	50.39	81.33
6e	$C_8H_{10}N$(*p*)	31	0.136 (±0.011)	0.056 (±0.006)	46.45	75.11
6f	C_4H_3O	31	0.171 (±0.003)	0.138 (±0.005)	42.67	68.66

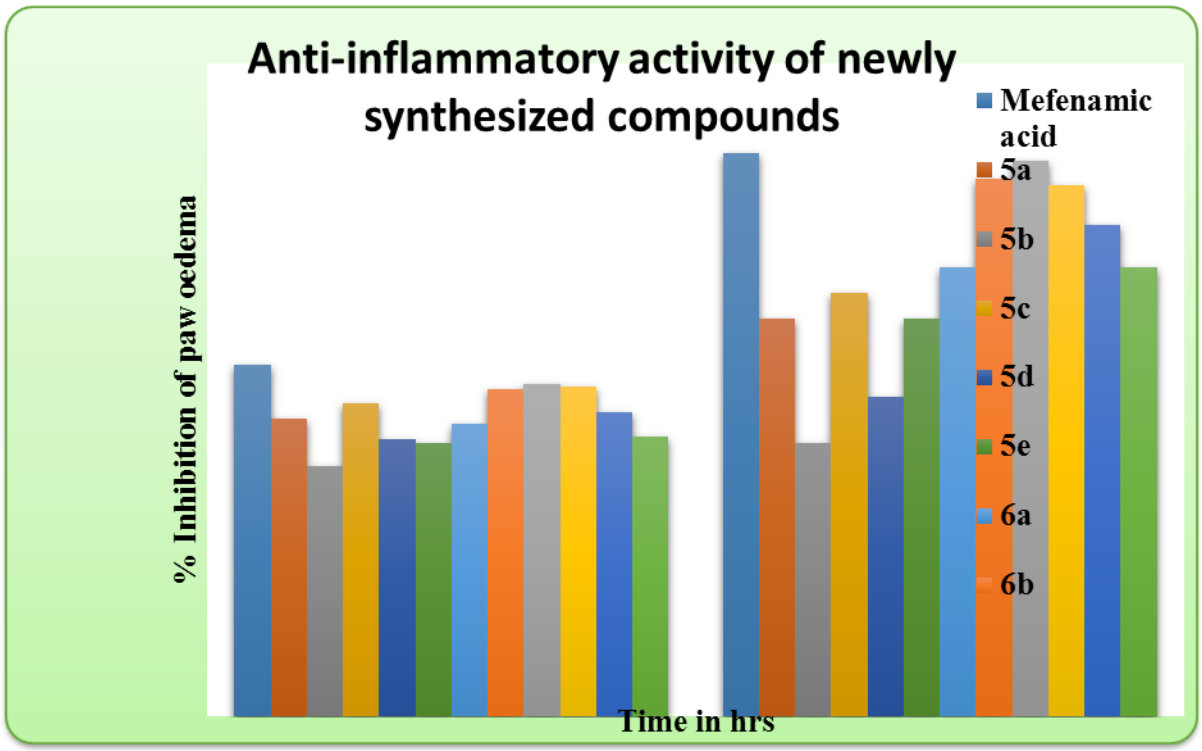


Fig.1. Anti-inflammatory activity of Triazolothiadiazole derivatives, (5a-e) and (6a-f).

2. ANTIBACTERIAL ACTIVITY[86, 87]

The antibacterial activity of synthesized compounds where evaluated against gram positive organism namely *E. coli* and gram negative organism *S. aureus* by using cup-plate method. Ciprofloxacin (100µg/0.1ml) were employed as reference standard to compare the result.

Material and Method

All the synthesized compounds were screened for antibacterial activity against the above mentioned strains by cup-plate diffusion method. The following materials were used for the testing.

Sterilized Petri dish, pipettes and beakers.

8 to 24 hrs old growth cultures in nutrient broth

Sterilized test tubes

Sterile 6mm cork borer

Sterile inoculation loops

Sterilized fine pointed forceps

Nutrients Broth

Mueller Hinton agar

Preparation of Media

Mueller Hinton agar was prepared by dissolving beef influsion (300.0g), agar (17.0g), casein acid hydrolysate (17.50g) and starch (1.50g) in distilled water to produce 1 liter of medium. The pH of the solution was adjusted to 7.3 ± 0.2. Then it was sterilized for 30 min. at 15 lbs Pressure in an autoclave.

Preparation of Sub Cultures

One day prior to test the microorganisms were inoculated into the sterilized nutrient broth and incubated at 37^0C for 24 hrs. On the day of testing, the organisms were sub cultured into sterile nutrient broth. After incubating the same for 3 hrs, the growth thus obtained was used as inoculums for the test.

Sterilization of Media and Glass Wares

The media used in the presents study, Mueller Hinton agar and nutrient broth, were sterilized in the conical flasks of suitable capacity by autoclaving at 15 lbs pressure for about 20 mins. The cork borer, Petri dish, test tubes, micropipette's tips and pipettes were sterilized in hot air oven at 160°C for one hour.

Preparation of Solutions

Ciprofloxacin: 10mg of ciprofloxacin was dissolved in 10ml of DMF to get a concentration of 100µg/0.1ml.

Compound: 10mg each test compound was dissolved in 10ml of DMF in serial and suitably labeled sterile test tubes, thus giving a final concentration of 100µg/0.1ml.

METHOD OF TESTING (Cup plate method) [88]

This method depends on the diffusion of an antibiotic from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added microorganism is

prevented entirely in a circular area or zone around the cavity containing the added solution of test compounds.

A previously liquefied medium was inoculated appropriated to the assay with the requisite quantity of the suspension of the microorganisms between 40-50°C and the inoculated medium was poured into Petri dish to give a depth of 3-4 mm. ensured that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.

The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organisms occurs before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, three cups of each 6mm diameter were punched and scooped out the set agar in each Petri dish (cups were numbered for the particular compound and standards). Using sterile pipettes, the standard and the sample solutions (0.1ml) of known concentration were fed into the borer cups. The order of the solutions was as follows.

Cup-1: Standard (ciprofloxacin)

Cup-2: Solvent control (DMF)

Cup-3: Test compound

The dishes were left standing for two hrs at room temperature as a period of pre -incubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24hrs at 37°C. The zone of inhibition developed, if any, was then accurately and recorded. Each zone of inhibition recorded was average of three measurements. Zone of inhibition for DMF was done separately.

The antibacterial activities of test compounds are discussed with comparison to standard drug ciprofloxacin. The data of antibacterial activity of standard, control and test compounds is given in the Table.

INDEX

Antibacterial activity of standard and control

Treatment	**Zone of inhibition in mm***	
	E. coli	***S. aureus***
Standard Ciprofloxacin Control DMF	26 6	28 6

*Diameter of borer 6mm

Key for interpretation

<9mm : inactive

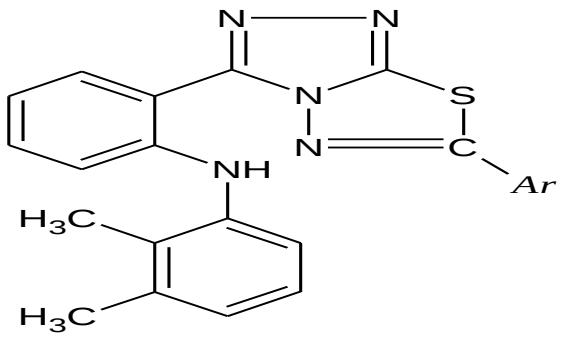
9-12mm : weakly active

13-16mm : moderately activity

>16mm : highly active

Table – 2

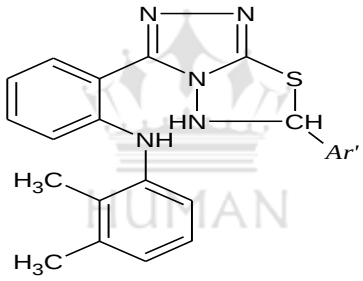
Antibacterial activity of *N*-{2-[6-aryl [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]phenyl}-2,3-dimethylaniline, (5a-e).



Compound No.	*Ar*	Zone of inhibition in mm	
		E. coli	***S. aureus***
5a	C_6H_5	18	18
5b	C_8H_9O(*m*)	17	16
5c	C_6H_4Cl(*m*)	20	12
5d	$C_6H_5O_2$(*p*)	21	15
5e	C_6H_6N(*m*)	16	12

Table-3

Antibacterial activity of *N*-{2-[6-aryl-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]phenyl}-2,3-dimethylaniline, (6a-f).



Compound No.	*Ar'*	Zone of inhibition in mm	
		E. coli	***S. aureus***
6a	C_6H_5	12	20
6b	C_6H_5O (*p*)	14	16
6c	C_7H_7O(*p*)	15	18
6d	C_6H_4Cl(*p*)	12	17
6e	$C_8H_{10}N$(*p*)	12	15
6f	C_4H_3O	11	15

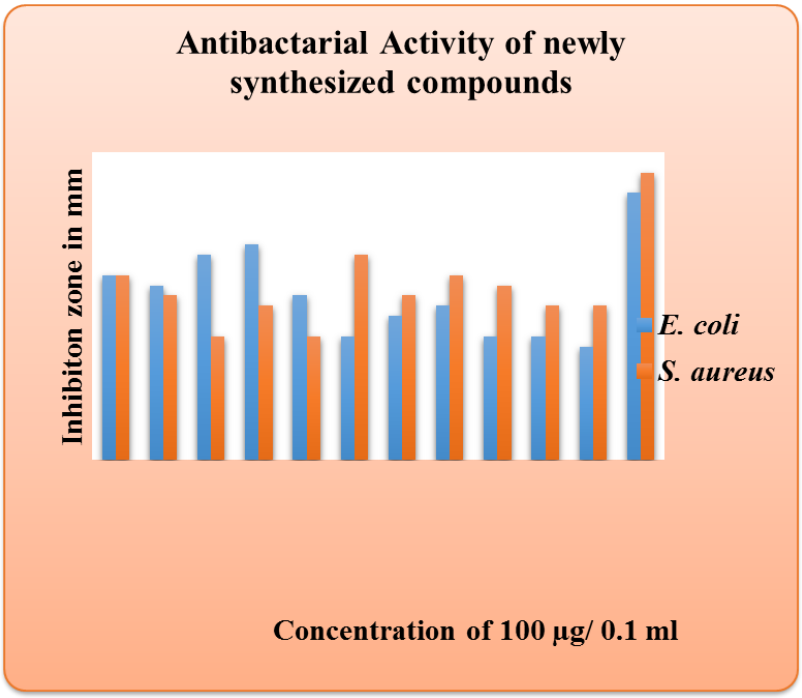


Fig.2.Antimicrobial activity of triazolothiadiazole derivatives (5a-e) and (6a-f).

3. ANTIFUNGAL ACTIVITY[89]

The compounds were synthesized during present course of study were screened for their antifungal activity against *Microsporum canis* using the standard antifungal drug Griseofulvin by cup plate diffusion method.

Materials and method

The following materials were used:

1) Sabouraud Dextrose Agar

2) Sterile Petri Dish

3) 16-18 hrs old growth culture in Sabouraud Dextrose Broth

4) Micropipette with Tips

5) Sterile test tubes for preparation of solutions of the test compounds in desired concentration.

Preparation of Media

The media used for antifungal activity is also known as Sabourauds-Dextrose agar, was prepared by dissolving Dextrose (40.00 g), mycological peptone (10.00 g) and agar (15.00 g) in 1000 ml distilled water. This solution was sterilized for 30minutes and adjusted pH 5.6 ± 0.2.

Preparation of Sub-culture

The one microorganisms used for testing of antifungal activity of various compounds were procured from the Veternary College, Nandi Nagar, Bidar. Two days prior to the testing the organisms were sub-cultured into sterile nutrient broth. After incubating the same for two days the growth obtained was used as inoculum for the test.

Sterilization of Media and Glass Wares

The media used in the present study, was sterilized in the conical flask of suitable capacity by autoclaving at 15 lbs pressure for about 45 min. The cork borer, Petri dish, test tubes and pipettes were sterilized in hot air oven at 160°C for one hour.

Preparation of Solutions

1. Standard: 10mg of gresiofluvin was dissolved in 10ml of DMF to get a concentration 100µg/0.1ml.

2. Compounds: 10mg of each test compounds was dissolved in 10ml of DMF in serially and suitably labeled in sterile test tube thus giving final concentration of 100µg/0.1ml.

Method of Testing (Cup plate method)

This method depends on the diffusion of an antifungal agent from a cavity through the solidified agar layer in a Petri dish to an extent such that growth of the added micro-organism is prevented entirely in a circular area or zone around the cavity containing a solution of antifungal agent.

A previously liquefied medium was inoculated appropriate to the assay with the requisite quantity of the suspension of the microorganism between 40-50°C and the inoculated medium was poured into Petri dish to give a depth of 3-4 mm, ensured that the layers of medium were uniform in thickness by placing the dishes on a leveled surface.

The dishes thus prepared were stored in a manner so as to ensure that no significant growth or death of the test organisms occur before the dishes were used and the surface or the agar layer was dry at the time of use. With the help of a sterile cork borer, four cups of each 6mm diameter were punched and scooped out the set agar in each Petri dish (cups were numbered for the particular compound and standards). Using sterile pipettes, the standard and the sample solutions (0.1ml) of known concentration were fed into the borer cups. The dishes were left standing for 2 hrs at room temperature as a period of preincubation diffusion to minimize the effects of variation in time among the application of different solutions. These were then incubated for 24hrs at 37°C. The zone of inhibition developed, if any, was then accurately measured, and recorded. Each zone of inhibition recorded was average of three measurements.

INDEX Antifungal activity of standard and control

Treatment	**Zone of inhibition in mm***	
	M. canis	***C. albicans***
Standard Griseofulvin Control DMF	25 6	25 6

*Diameter of borer 6mm

Key for interpretation

<13mm : inactive

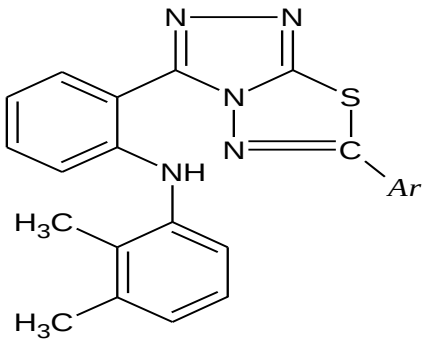
13-15mm : weakly active

16-20mm : moderately activity

>20mm : highly active

Table – 4

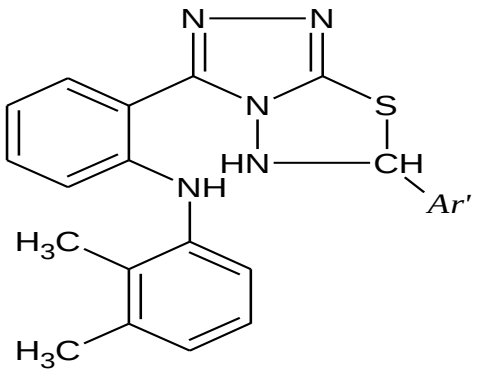
Antifungal activity of *N*-{2-[6-aryl [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]phenyl}-2,3-dimethylaniline, (5a-e).



Compound No.	*Ar*	Zone of inhibition in mm	
		M. canis	***C. albicans***
5a	C_6H_5	20	18
5b	C_8H_9O(*m*)	16	14
5c	C_6H_4Cl(*m*)	17	15
5d	$C_6H_5O_2$(*p*)	15	15
5e	C_6H_6N(*m*)	14	12

Table- 5

Antifungal activity of *N*-{2-[6-phenyl-5,6-dihydro [1,2,4]triazolo[3,4-*b*][1,3,4]thiadiazol-3-yl]phenyl}-2,3-dimethylaniline, (6a-e).



Compound No.	*Ar'*	Zone of inhibition in mm	
		M. canis	***C. albicans***
6a	C_6H_5	18	17
6b	C_6H_5O (*p*)	17	16
6c	C_7H_7O(*p*)	17	12
6d	C_6H_4Cl(*p*)	18	14
6e	$C_8H_{10}N$(*p*)	16	13
6f	C_4H_3O	20	16

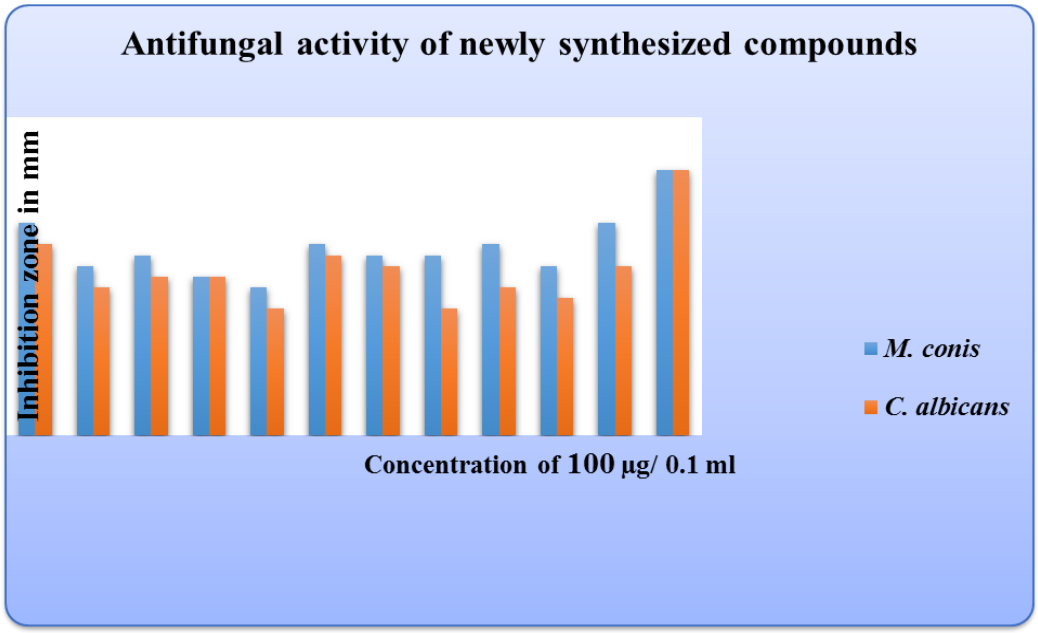


Fig.3.Antifungal activity of triazolothiadiazole derivatives (5a-e) and (6a-e).

RESULT AND DISCUSSION

1. ANTI-INFLAMMATORY ACTIVITY

The anti-inflammatory activity of triazolothiadiazine derivatives are evaluated by using carrgeenan induced rat paw oedema method. The results and statistical analysis of this activity shown in **Table-1, Fig. 1.**

Compounds **(5a-e** and **6a-f)** showed significant anti-inflammatory activity when compared with the standard drug mefenamic acid. In addition, it was found that compounds **6b, 6c, 6d**

and 6e possessed maximum activity and compounds **5a, 5c, 5e,** were had moderate activity and this may be due to the presence of phenyl, 2-chlorophenyl,4-aminophenyl, hydroxyl phenyl, 4-methoxyphenyl, 4-chlorophenyl and 4-(dimethylamino) phenyl pharmacophore at triazolothiadiazole ring system.

2. ANTIBACTERIAL ACTIVITY

All the triazolothiadiazole derivatives **(5a-e and 6a-f)** have been evaluated for their antimicrobial activity against *E. coli* (gram positive) and *S.aureus* (gram negative) using agar cup-plate diffusion method. The results of this evaluation compared with ciprofloxacin as reference standard. The antimicrobial activity results and statistical analysis were presented in **Table 2,3 Fig. .** *Compounds* **(5a-e and 6a-f)** showed significant antibacterial activity at 100µg Concentration level when compared with standard drug. Among all the compounds tested, compounds **5a-e** highly active against *E.coli and* **6a-d** highly active against *S.aureus* and remaining compounds shows moderate activity.

3. ANTIFUNGAL ACTIVITY

The antifungal activity of triazolothiadiazole derivatives **(5a-e and 6a-f)** have been evaluated against *Microsporum canis* and *Candida albicans,* Griseofulvin employed as reference standard and using cup-plate method.

A close examination of **Table 4 and5 Fig.3** pertaining to the antifungal data of triazolothiadiazole derivatives revealed that all the compounds in this series have been found effective against both fungi at 100µg/0.1ml Concentration level when compared with reference standard griseofulvin. Compounds **5a, 5c, 6a, 6b, 6c, 6d and 6f** possessed maximum activity and rest of compounds shows moderate activity.

CONCLUSION

The aim of present research work is to synthesize certain substituted triazolothiadiazole derivatives of biological interest. Among all the synthesized compounds some of them showed good anti-inflammatory, antimicrobial and antifungal activity when compared with standard drug. From the findings obtained, it can be concluded that:

- triazolothiadiazole moiety linked to various substituted aryl carboxylic acid and substituted aromatic aldehyde compounds could be synthesized as an approach to obtain potential anti-inflammatory, antimicrobial agents and antifungal agents.

- Mefenamic acid, used as a starting material and it was identified by their physical, chemical and spectral data and all necessary solvents were purified by double distillation prior to use for synthesis.

- Synthesized triazolothiadiazole derivatives containing mefenamic acid nucleus gave satisfactory results for various evaluations like TLC, physical data, elemental analysis, spectral data and biological activities.

- Completion of reactions was confirmed by TLC by using suitable solvent systems. Compounds were crystallized by suitable solvents and dried properly. Elemental analysis found was approximately correct with the calculated values.

- Characterization of the synthesized compounds was done by FT-IR, ^{1}HNMR and LC-MS spectral data. The structures, functional groups and molecular weights of the compounds were confirmed.

- The compounds synthesized were screened for their antibacterial, antifungal and some selected compounds evaluated for anti-inflammatory activity the results were compared with standard drugs Ciprofloxacin, Griseofulvin and Mefanemic acid respectively.

- Most of the compounds with few exceptions could exhibit a significant activity, against bacteria *B.subtilis, P.aeruginosa,* fungi such as *Mycosporum canis, Candida abican.*

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