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Rapid and efficient synthesis of microwave assisted some bis-1, 2, 4-triazole derivatives and their antioxidant and anti-inflammatory evaluation.

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ABSTRACT

A series of bis-triazole derivatives, 5, 5/-methylenebis (4-substituted phenyl/alkyl-4H-1, 2, 4-triazole-3-thiol) 4(a-f) have been synthesized both under conventional and Microwave irradiation (MW) technique. The reaction time decreases from hours to minute's along with yield enhancement. All the synthesized compounds have been confirmed by I.R. and ^{1}H NMR technique. Representative compounds have been screened for their antioxidant by DPPH method and anti-inflammatory activities by carrageenin induced paw oedema method. One of the compound 4(c) was found to have potent antioxidant and anti-inflammatory activity.

Keywords: Bis-triazole, microwave irradiation, anti-oxidant, anti-inflammatory activity, Elisa reader.

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INTRODUCTION

A literature survey revealed that 1, 2, 4 triazole posses broad spectrum of biological importance. Triazole derivative are currently taking primary focus on the drug discovery research because of the antimicrobial [1], antinflammatory and analgesic [2,3], anticancer [4], anticonvulsant [5] , antitubercular[6] activities. Survey of literature also revealed that bis- 1, 2, 4- triazole and their Schiff bases enhanced antibacterial and antifungal activity[7]. Encouraged by these observations, we report in the paper the synthesis of some bis- 1, 2, 4- triazole derivatives by microwave irradiation technique and their antioxidant and anti-inflammatory evaluation. Microwave mediated reaction have emerged as a powerful technique to promote a variety of chemical reactions. The microwave irradiation is used for carrying out chemical transformation which are pollution free and eco-friendly [8, 9]. Application of microwave methodology in heterocyclic chemistry in terms of enhancements in the rate of reaction and in yield is striking [10, 11]. Undoubtedly, microwaves are going to be highly important in future synthesis of heterocyclic compounds.

MATERIALS AND METHODS

All chemicals were obtained from sdfine chemicals Chennai. All chemicals and solvents used were of analytical grade.

Experimental Section

The melting points were determined in open capillaries using LABINDIA digital melting point apparatus expressed in ºC and are uncorrected. The reactions were monitored by TLC and spots are detected by UV chamber and also using iodine as visualizing agent. The IR spectra of the compounds were recorded on Perkin-Elmer Infrared-283 Spectrophotometer using KBr pellets and are expressed in cm^{-1}. Nuclear magnetic spectra (^{1} H-NMR) were obtained from Brucker DRX– 300 MHz spectrophotometer using DMSO and $CDCl_3$ as solvent. Chemical shift are reported in (ppm) using TMS as internal standard. Microwave assisted reactions were carried out in a “QPro-M Modified Microwave System” made in Canada. All the solvents (AR grade) and reagents were purified and dried according to the procedures given in Vogel’s Textbook of Practical Organic Chemistry.

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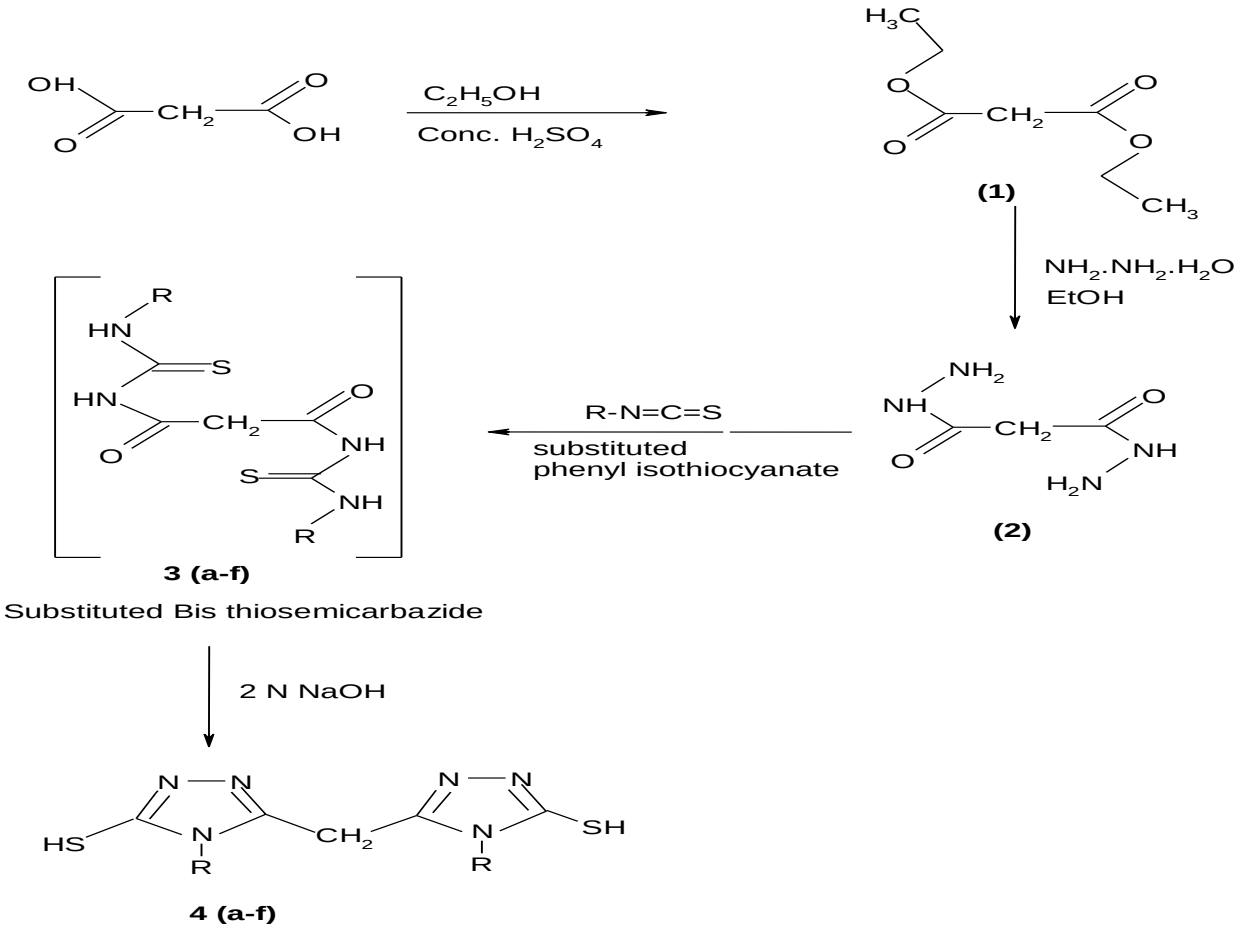

Scheme-1

Synthesis of malonic acid Bis ester

(i) By conventional Method

Malonic acid (0.01moles) was dissolved in 50ml of ethanol and to this few drops of concentrated sulphuric acid were added and reaction mixture was refluxed for 6-8 h. The progress of the reaction was monitored with the help of TLC. After completion, excess of ethanol was distilled off under reduced pressure and residue poured into ice cold water. The solid obtained was filtered and recrystallized from ethanol. The yield was found to be 62 %

(ii) By Microwave Method

Malonic acid (0.01 moles) was dissolved in 10ml of ethanol and to this few drops of concentrated Sulphuric acid were added. The reaction mixture was covered with petridish and kept in microwave at 450 W for 2-5 min. It was then poured into ice cold water and the solid obtained was filtered and recrystallized from ethanol. The m.p., IR and ^{1}H NMR data are in

agreement with those obtained for the products synthesized by other reported method [12]. The yield was found to be73 %

Synthesis of malonic acid Bis Hydrazide

(i) By conventional Method

Malonic acid Bisesters (0.01moles) and hydrazine hydrate (0.04 moles) was dissolved in 50ml of ethanol and the reaction mixture was refluxed for 6-8 h. After completion, excess of ethanol was distilled off under reduced pressure. Residue obtained was poured into ice cold water and the solid obtained was filtered. The filtrate was recrystallized from ethanol. The yield was found to be 65%.

(ii) By Microwave Method

Malonic acid Bisester (0.001 moles) was dissolved in 10ml of ethanol. To this hydrazine hydrate (0.004 moles) was added. The reaction mixture was covered with petridish and kept in microwave at 400 W for 2-5 mints. After completion, excess of ethanol was distilled off under reduced pressure and residue poured into ice cold water. The solid obtained was filtered and recrystallized from ethanol. The yield was found to be 79%.The m.p., IR and ^{1}H NMR data are in agreement with those obtained for the products synthesized by other reported method.

General method for the synthesis of 5,5$^{/}$-methylene-bis (4-Substituted phenyl/ /alkyl – 4H – 1,2,4 – triazoles -3-thiols 4(a-f)

(i) By conventional Method

The substituted phenyl isothiocyanate (0.004 moles) in 30 ml of absolute ethanol was poured with stirring into the solution of malonic acid bis hydrazide (0.004 moles) in 50 ml ethanol. Reaction mixture was refluxed for 6-8 hr. after completion of reaction confirmed by TLC, the reaction mixture was cooled at room temperature. A white solid crystal of substituted bis thiosemicarbazide 3(a-f) formed which was added without any further purification to 15 ml of 2M sodium hydroxide solution. The reaction mixture was refluxed for 6-8 hrs. After completion, reaction mixture was allowed to cool, filtered and acidified with 2 M hydrochloric acid. The precipitate obtained was filtered washed with water and dried and recrystallized from acetonitrile to get the desired substituted bis-triazole. The yield was found to be 65%.

(ii) By Microwave Method

The substituted phenyl isothiocyanate (0.004 moles) in 30 ml of absolute ethanol was poured with stirring into the solution of malonic acid bis hydrazide (0.004 moles) in 50 ml

ethanol. The reaction mixture was covered with Petridish and kept in microwave at 500 W for 2-5 minutes. After completion of reaction confirmed by TLC, the reaction mixture was cooled at room temperature. A white solid crystal of substituted bis thiosemicarbazide **3(a-f)** formed which was added without any further purification to 15 ml of 2M sodium hydroxide solution. The reaction mixture was again covered with petridish and kept in microwave at 500 W for 2-5 minutes. After completion, reaction mixture was allowed to cool, filtered and acidified with 2 M hydrochloric acid. The precipitate obtained was filtered washed with water and dried and recrystallized from acetonitrile to get the desired substituted bis-triazole **4(a-f).**

5, 5^{l}-methylenebis (4- Phenyl – 4H – 1,2,4 – triazoles -3-thiols, (4a)
IR (KBr): 2900 (C-H), 2500 (S-H), 1450(*Ar* C=C), 1360 (C-N)
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 2.5 (s, 2H, -CH_2), 3.5(s, 2H, -S*H*), 6.90-7.30 (m, 10H, *ArH)*

5, 5^{l}-methylenebis [4-(4-methoxyphenyl)-4*H*-1, 2, 4-triazole-3-thiol, (4 b)
IR (KBr): 2900 (C-H), 2550 (S-H), 1460(*Ar* C=C), 1360 (C-N)
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 2.3 (s, 2H, -CH_2), 3.4 (s, 2H, -S*H*), 3.8 (s, 6H, -OCH_3 x 2), 6.70-7.20 (m, 10H, *ArH)*

5, 5^{l}-methylenebis [4-(4-methylphenyl)-4*H*-1, 2, 4-triazole-3-thiol, (4 c)
IR (KBr): 2950 (C-H), 2550 (S-H), 1460-1600(Ar C=C), 1360 (C-N)
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 2.2 (s, 6H, -CH_3 x 2), 2.4 (s, 2H, -CH_2), 3.5 (s, 2H, -S*H*), 6.80-7.20 (m, 8H, *ArH)*

5, 5^{l}-methylenebis [4-(3-methylyphenyl)-4*H*-1, 2, 4-triazole-3-thiol, (4d)
IR (KBr): 2900(C-H), 2350 (S-H), 1460-1600(Ar C=C), 1340 (C-N)
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 2.3 (s, 6H, -CH_3 x 2), 2.4 (s, 2H, -CH_2), 3.4 (s, 2H, -S*H*), 6.50-7.50 (m, 8H, *ArH*)

5, 5^{l}- methylene bis (4-cyclohexyl) – 4H- 1, 2, 4-triazole-3-thiol, (4e)
The compound 4 (e) could not be obtained in pure state
IR (KBr): 2950(C-H), 2350 (S-H), 1340 (C-N). No Ar C=C stretching was observed at 1450-1600 cm^{-1}
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 1.3-1.9 (m, 22H, -Cyclo*H*), 2.2 (s, 2H, -CH_2), 4.5 (s, 2H, -S*H*),

5, 5^{l}- methylene bis (4-butyl) – 4H- 1, 2, 4-triazole-3-thiol, 4(f)
IR (KBr): 3000(C-H), 2365 (S-H), 1370 (C-N)
^{1}HNMR ($CDCl_3$ + DMSO-d_6): δ 0.9 (t, 6H, -CH_3 x 2), 1.2 (m, 4H, -C$H_2$$CH_3$x2), 1.4 (m, 4H, -$CH_2CH_2$ x 2), 1.5 (s, 2H, -CH_2), 3.9 (t, 4H, -C$H_2$$CH_2$ x 2), 4.1 (s, 2H, -S*H*),

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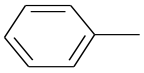
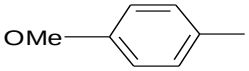
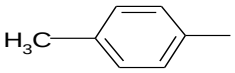
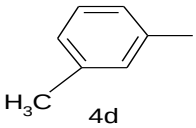
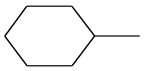
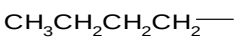
RESULTS AND DISCUSSION

The starting compound namely the bis hydrazide (2) was synthesized from the reaction of hydrazine hydrate with bis ester. The reaction of (2) with substituted phenyl isothiocyanate [13] was carried out by both conventional and microwave method to get substituted bis thiosemicarbazide 3(a-f). In conventional method the reaction was carried out in ethanol, it took 6-8 hr, but it took only 2-5 min under microwave irradiation.

The cyclization reaction of substituted bis thiosemicarbazide 3(a-f) without any purification and isolation with 2 N NaOH was carried out by conventional method to yield bis-1, 2, 4 triazole 4(a-f). It took 6-8 hr, while under microwave irradiation, the reaction was completed in 2-5 min. Compound 4 (e) was not obtained in pure form.

(Scheme I)

Compd (R)	m.p. (°C)	Conventional method		Microwave method	
		Yield (%)	Time (hr)	Yield (%)	Time (min)
 4a	309-311	64	7	76	2.5
 4b	298-301	62	6	74	3
 4c	314-316	60	7	76	2
 4d	300-302	63	8	75	5
 4e	316-324	69	8	72	4.5
 4f	253-255	65	7	70	4

A comparative study in term of yield and reaction period for the final compound 4(a-f) is shown in (Table **I)**

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Antioxidant Activity Study

All the six synthesized bis-1, 2; 4-triazoles were screened for their antioxidant activity.

Samples are added to a free radical-generating system, inhibition of the free radical action is measured and this inhibition is related to antioxidant activity of the sample. Methods vary greatly as to the generated radical, the reproducibility of the generation process, and the endpoint that is used for the determination.

All the compounds were tested for *in vitro* antioxidant activity using several standard methods. The final concentration of the samples and standard solutions used is 100 μg/ml. The absorbance was measured spectrophotometrically against the corresponding blank solution. The percentage inhibition was calculated by using the following formula.

$$(\%)\ \text{Inhibition} = \frac{\text{OD control - OD sample}}{\text{OD control}} \times 100$$

The IC_{50} value was calculated by interpolating % inhibition and concentration tested. IC_{50} value is the concentration of the sample required to scavenge50% of free radicals was calculated.

Reagents

2, 2-Diphenly 1-picryl hydrazyl solution (DPPH, 100 μM): Accurately weighed 22 mg of DPPH and dissolved in 100 ml of methanol. From this stock solution, 18 ml was diluted to 100 ml with methanol to obtain 100 μM DPPH solution.

Preparation of sample solutions

Accurately weighed 2.1 mg of each of the compounds and dissolved in 1 ml of freshly distilled DMSO separately to obtain solutions of 2.1 mg/ml concentration.

Preparation of standard solutions

Accurately weighed 10.5 mg each of ascorbic acid and rutin and dissolved in 0.95 ml of freshly distilled DMSO to get 10.5 mg/ml concentration. These solutions were serially diluted with DMSO to get the lower concentrations.

Procedure

The assay was carried out in a 96 well microtitre plate. To 200 µl of DPPH solution, 10 µl of each of the extract or standard solution was added separately in wells of the microtitre plate. The plates were incubated at 37 ^{o}C for 30 min and the absorbance of each solution was measured at 490 nm using ELISA reader.

Table 2- Antioxidant activity by DPPH method

S.No	Test Compound	IC_{50} values (µg/ml)
1.	**4(a)**	**9.4**
2.	**4(b)**	**3.9**
3.	**4(c)**	**6.1**
4.	**4(d)**	**10.3**
5.	**4(e)**	**30.3**
6.	**4(f)**	**11.3**
7.	**STANDARD (Ascorbic acid)**	**5.2**

The antioxidant activity for all six compounds was screened by DPPH method. In DPPH method, the compound 4(c) exhibited good radical scavenging activity, where the compound 4(a), 4(b), 4(d) and 4(f) exhibited moderate activity. Ascorbic acid was used as standard. The compound 4(e) did not show any significant activity. The IC_{50} values of all the test compounds were recorded in (**Table 2.)**

Anti-inflammatory study

Method: Carrageenin induced paw oedema model

Materials

Animals

Male wister rats (150-175g)

Drug

Indomethacin

Chemical

Corrageenin (1% w/v)

Compounds: **4 (a-f)**

Method

The animals were dividend into eight groups.

Group I Solvent control: 0.3% CMC (2ml/kg body weight; p.o)
Group II Positive control; Indomethacin (10 mg/kg; p.o)
Group III 5, 5/-methylenebis (4-phenyl-4*H*-1, 2, 4-triazole-3-thiol), **4(a)**
Group IV 5, 5/-methylenebis [4-(4-methoxyphenyl)-4*H*-1, 2, 4-triazole-3-thiol] **4(b)**
Group V 5, 5/-methylenebis [4-(4-methylyphenyl)-4*H*-1, 2, 4-triazole-3-thiol] **4(c)**
Group VI 5, 5/-methylenebis [4-(3-methylyphenyl)-4*H*-1, 2, 4-triazole-3-thiol] **4(d)**
Group VII 5, 5/- methylene bis (4-cyclohexyl) – 4H- 1, 2, 4-triazole-3-thiol] **4(e)**
Group VIII 5, 5/- methylene bis (4-butyl) – 4H- 1, 2, 4-triazole-3-thiol], **4(f)**

Overnight starved male Wister albino rats were injected with 0.1 ml of 1% carrageenin to the right hind paw (sub plantar region) by subcutaneous route one hour after the administration of the test compounds. The paw volume was measured using Plethysmometer (UGO Basle, Italy) before and after the injection of pholigistic agent at 0 and 3 hour. The difference between the initial paw volume to that of the paw volume taken at different time intervals was considered as increase in paw volume. The percentage inhibition of oedema of each group was calculated[62] as follows.

$$\% \text{ inhibition rate} = \frac{V_t - V_c}{V_c} \times 100$$

Where Vc is the change in paw volume of control group and V_t is the change in paw volume of treated group.

Data Analysis

Mean change in paw volume of animals treated with synthesized compounds were compared with CMC treated control animals by One Way Analysis of Variance (ANOVA), followed by Dunnett's Multiple Comparison test. $P < 0.01$ was considered significant statistically. The result obtained is given in (**Table 3).** The results show that all the test compounds especially 4(c) showed significant ($p < 0.0001$) reduction in paw edema except compound 4 (e) when compared to control. The % inhibition ranged from 53 – 62 for the first two hours when compared to indomethacin. However, further investigation is required to find out whether the test compounds exhibit anti-inflammatory potential in sub acute and chronic models of inflammation and also to further evaluates the role of opioid receptor and other central neurotransmitter such as 5 – HT, acetylcholine, and neuropeptide in the role of analgesia produced by these compounds.

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Table 3- Effect of some New Bis-1, 2, 4 Triazole Derivatives on Carrageenin-Induced Paw Oedema in Rats

Treatment	Dose mg/kg (p.o.)	N	Mean change in paw volume (ml)				
			30 min	1h	2h	3h	5h
Control	0.3	8	0.38±0.06	0.52±0.08	0.57±0.09	0.76±0.11	1.10±0.07
Indomethacin	10	8	0.35±0.07	0.35±0.07	0.36±0.11	0..34±0.09	0.32±0.08**
4(a)	100	5	0.24±0.05	0.34±0.05	0.39±0.08	0.52±0.10*	0.60±0.02**
4(b)	100	5	0.38±0.14	0.41±0.07	0.56±0.04	0.77±0.16	0.89±0.10**
4(c)	100	5	0.31±0.03	0.33±0.05	0.43±0.05	0.44±0.14	0.46±0.16
4(d)	100	5	0.35±0.06	0.59±0.09	0.62±0.11	0.79±0.09*	0.99±0.05**
4(e)	100	6	0.37±0.05	0.52±0.06	0.72±0.08	0.80±0.08	0.92±0.72**
4(f)	100	6	0.25±0.05	0.29±0.05	0.39±0.07	0.48±0.04**	0.55±0.13**

*P < 0.05, *P < 0.01, *P < 0.001

SUMMARY AND CONCLUSION

Bis-1, 2, 4-Triazole derivative were synthesized by conventional and microwave method. The percentage yield was more by microwave method than conventional method because the microwave method provided rapid chemical transformation in liquid / solution / solids and required only 2-5 minutes to complete the reaction whereas it was required 6-8 hours to complete the reaction by conventional method and the compounds formed were more pure than conventional method.

All the derivatives exhibited significant anti-oxidant and anti-inflammatory potential except compound 4(e). One of the compound 4(c) was found to have potent antioxidant and anti-inflammatory activity. However, further investigation is required to find out whether the test compounds exhibit anti-inflammatory potential in sub acute and chronic models of inflammation and also to further evaluates the role of opioid receptor and other central neurotransmitter such as 5 – HT, acetylcholine, and neuropeptide in the role of analgesia produced by these compounds.

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