Advanced Pharmaceutical Bulletin, **2014**, 4(2), 105-112
doi: http://dx.doi.org/10.5681/apb.2014.017
http://apb.tbzmed.ac.ir/

Microwave Assisted Synthesis of 1-[5-(Substituted Aryl)-1*H*-Pyrazol-3-yl]-3,5-Diphenyl-1*H*-1,2,4-Triazole as Antinociceptive and Antimicrobial Agents

Shantaram Gajanan Khanage[1]*, Popat Baban Mohite[1], Ramdas Bhanudas Pandhare[2], S. Appala Raju[3]

[1] *Department of Pharmaceutical Chemistry and PG studies, M.E.S. College of Pharmacy, Sonai, Ahmednagar, Maharashtra, India-414105.*

[2] *Department of Pharmacology, M.E.S. College of Pharmacy, Sonai, Tq-Newasa, Dist.-Ahmednagar, Maharashtra, India-414105.*

[3] *Department of Pharmaceutical chemistry, H.K.E.'S College of Pharmacy, Sedam road, Gulbarga, Karnataka, India-585105.*

ARTICLEINFO

Article Type:
Research Article

Article History:
Received: 31 March 2013
Revised: 8 June 2013
Accepted: 8 June 2013
ePublished: 24 December 2013

Keywords:
Microwave
Antinociceptive
Antimicrobial
Hot plate method
MIC
Chalcones

ABSTRACT

Purpose: An efficient technique has been developed for microwave assisted synthesis of 1-[5-(substituted aryl)-1H-pyrazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole as antinociceptive and antimicrobial agents.

Methods: The desired compounds (S_1-S_{10}) were synthesized by the microwave irradiation via cyclization of formerly synthesized chalcones of 3,5-diphenyl-1*H*-1,2,4-triazole and hydrazine hydrate in mild acidic condition. All newly synthesized compounds were subjected to study their antinociceptive and antimicrobial activity. The analgesic potential of compounds was tested by acetic acid induced writhing response and hot plate method. The MIC values for antimicrobial activity were premeditated by liquid broth method.

Results: The compounds S_1, S_2, S_4, S_6 and S_{10} were found to be excellent peripherally acting analgesic agents when tested on mice by acetic acid induced writhing method and compounds S_3, S_6 and S_1 at dose level of 100 mg/kg were exhibited superior centrally acting antinociceptive activity when tested by Eddy's hot plate method. In antimicrobial activity compound S_{10} found to be broad spectrum antibacterial agent at MIC value of 15.62 µg/ml and compound S_6 was exhibited antifungal potential at 15.62 µg/mL on both fungal strains.

Conclusion: Some novel pyrazoles clubbed with 1,2,4-triazole derivatives were synthesized and evaluated as possible antimicrobial, centrally and peripherally acting analgesics.

Introduction

Triazole derivatives have always attracted the attention of medicinal chemists because of their many therapeutic applications. 1,2,4-triazoles and related fused heterocyclic derivatives are of great biological interest such as anticancer,[1,2] antimicrobial,[3-6] anticonvulsant,[7] anti-inflammatory, analgesic,[8,9] antidepressant,[10] antitubercular,[11,12] antimalarial[13] and hypoglycemic[14] activities.

The pyrazole ring system is a five membered heterocyclic ring structure composed of two nitrogen atoms and used in the synthesis of pharmaceuticals. The pyrazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities. In the past few years, the therapeutic interest of pyrazole derivatives in pharmaceutical and medicinal field has been given a great attention to the medicinal chemist. Literature survey reveals that pyrazole derivatives are well known to have anti-inflammatory, analgesic, antipyretic,[15] antibacterial,[16,17] anticancer,[18,19] antituberculine,[20] antimalarial[21] activities. This stimulated our interest to synthesize some novel pyrazoles derivatives containing triazole moiety of biological importance.

In addition to previously synthesized compounds 1-(3,5-diphenyl-1H-1,2,4-triazol-1-yl)-3-(substituted aryl) prop-2-en-1-one[22,23] some new analogues of titled nucleus were synthesized and evaluated for antinociceptive and antimicrobial activity. The structures of the compounds were confirmed by FTIR, NMR and mass spectroscopy studies, their antinociceptive activity was evaluated by chemical nociception model of acetic acid induced writhing response and hot plate method on mice. The antimicrobial activity was studied by (Minimum Inhibitory Concentration) MIC.

In continuation of our previous work on 3,5-disubstituted 1,2,4-triazole and related clubbed heterocycles,[22,23] in

***Corresponding author:** Shantaram Gajanan Khanage, M.E.S. College of Pharmacy, Sonai, At post-Sonai, Tq-ewasa, Dist.-Ahmednagar, Maharashtra-414105, India. Tel: +91 9822652921, Fax: 02427 230948, Email: 1982@gmail.com

this article the efforts have been made to synthesize and explore the antinociceptive and antimicrobial activity of some novel 1-[5-(substituted aryl)-1*H*-pyrazol-3-yl]-3,5-diphenyl-1*H*-1,2,4-triazole derivatives.

Materials and Methods

Chemicals

The melting points were determined in open tube capillary using Thermonik precision apparatus and are uncorrected. The purity of compounds was checked by TLC on silica gel G plates. IR spectra were recorded on PERKIN ELMER 8201 PC IR spectrophotometer. ^{1}HNMR spectra were recorded in DMSO on BRUKER DRX NMR spectrometer (400 MHz). Mass spectra (FAB-MS) of the compounds were recorded on 70V on JEOL D-300 spectrophotometer (Jeol Ltd., Tokyo, Japan). Microwave assisted reactions were carried out in a Catalysts Microwave synthesizer. Elemental analysis for C, H and N were performed on a PERKIN ELMER 240 elemental analyzer. The reaction time of animals on hot plate were studied on digital analgesiometer. The standard drugs Ibuprofen, Pentazocine, Ampicilline, Ciprofloxacine and Fluconazole were obtained as gift sample from Wockhardt Ltd., Aurangabad, India. Sabouraud Dextrose broth (SDB), Sabouraud Dextrose Agar (SDA), Peptone water and solvents used for the experimental work were commercially procured from E. Merck Ltd., Mumbai, India and Qualigens Ltd., Mumbai, India.

Animals and microbial cultures

Adult mice (Swiss strain 25-30 g) were used for evaluation of antinociceptive activity. The animals were housed under standard environmental conditions (light period of 12 h/day, temperature 25-27 °C and relative humidity 30-70%) with access to food and water *ad libitum*. The experiment was performed according to Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and the experimental protocol was approved by the Institutional Local Animal Ethical Committee. The microbial cultures of gram positive bacteria *B. subtillis* (NCIM 2063), gram negative bacteria *E. coli* (NCIM 2065), yeast *C. albicans* (NCIM 3471) and mold *A. niger* (NCIM 1196) were procured from National Centre for Industrial Microorganisms (NCIM), Pune, India.

Synthesis of 1-[5-(substituted aryl)-1H-pyrazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole derivatives (S_1-S_{10})

The desired compounds (S_1-S_{10}) were synthesized by the cyclization of 1-(3,5-diphenyl-1*H*-1,2,4-triazol-1-yl)-3-(substituted aryl) prop-2-en-1-one (Chalcones, 0.01 mol) and hydrazine hydrate (0.01 mol) in presence of small amount of glacial acetic acid. The reaction mixture was subjected to microwave irradiation for 10 minutes at 280 w. After the completion of reaction, the precipitate of product obtained was washed with cold water and dried. The crude product was recrystallized from ethanol water mixture (1:1). All the compounds were obtained in good yield. Physical and spectral data of the compounds S_1-S_{10} are mentioned in Figure 1 and Table 1.

Figure 1. Physical and analytical data of compounds S_1- S_{10}.

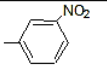
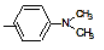
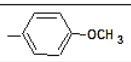
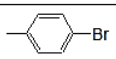
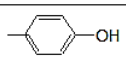
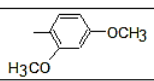
Compound	Ar	Molecular Formula	M.P. (°C)	Yield (%)	Elemental Analysis (found)		
					%C	%H	%N
S_1		$C_{23}H_{16}ClN_5$	155-157	89	69.43 (69.23)	4.05 (4.13)	17.60 (17.36)
S_2		$C_{23}H_{16}N_6O_2$	150-152	79	67.64 (67.77)	3.95 (3.99)	20.58 (20.66)
S_3		$C_{25}H_{22}N_6$	149-151	83	73.87 (73.89)	5.46 (5.19)	20.67 (20.47)
S_4		$C_{24}H_{19}N_5O$	158-159	91	73.27 (73.12)	4.87 (4.86)	17.80 (17.89)
S_5		$C_{21}H_{15}N_5O$	142-144	93	71.38 (71.30)	4.28 (4.28)	19.82 (19.70)
S_6		$C_{23}H_{16}ClN_5$	160-162	87	69.43 (69.19)	4.05 (4.09)	17.60 (17.63)
S_7		$C_{23}H_{17}N_5$	155-157	76	76.01 (76.22)	4.71 (4.77)	19.27 (19.45)
S_8		$C_{23}H_{16}BrN_5$	179-181	72	62.46 (62.32)	3.65 (3.69)	15.83 (15.77)
S_9		$C_{23}H_{17}N_5O$	165-167	74	72.81 (72.77)	4.52 (4.60)	18.46 (18.74)
S_{10}		$C_{25}H_{21}N_5O_2$	170-172	85	70.91 (70.43)	5.00 (4.92)	16.54 (16.53)

Table 1. Spectral data of compound S_1-S_{10}.

Compound	IR(KBr) cm^{-1}	^{1}H-NMR (δ ppm)	MS (FAB, positive ion mode) *m/z* $[M+1]^+$
S_1	3071 (Ar-CH), 1620 (C=N, triazole),785(-Cl),3291 (NH, pyrazole)	7.45-8.49 (14H, m, Ar-H), 6.12 (1H, s, NH of pyrazole), 3.23 (1H, s, CH of pyrazole)	398
S_2	3077 (Ar-CH), 1623 (C=N, triazole),1557 (-NO2), 3285 (NH, pyrazole)	7.48-8.20 (14H, m, Ar-H), 6.27 (1H, s, NH of pyrazole), 3.30 (1H, s, CH of pyrazole)	408
S_3	3070 (Ar-CH), 1628 (C=N, triazole), 3153, 3149 (-NCH3), 3273 (NH, pyrazole)	7.40-8.41 (14H, m, Ar-H), 6.31 (1H, s, NH of pyrazole), 3.29 (1H, s, CH of pyrazole), 3.16 (6H, s, -N(CH_3)2)	406
S_4	3074 (Ar-CH), 1626 (C=N, triazole), 1157 (-OCH3), 3246(NH, pyrazole)	7.36-8.31 (14H, m, Ar-H), 6.25 (1H, s, NH of pyrazole), 3.33 (1H, s, CH of pyrazole), 3.82 (3H, s, OCH_3),	393
S_5	3068 (Ar-CH), 1625 (C=N, triazole), 1226 (C-O-C), 3259 (NH, pyrazole)	6.89-8.48 (13H, m, Ar-H), 6.16 (1H, s, NH of pyrazole), 3.35 (1H, s, CH of pyrazole)	353
S_6	3075 (Ar-CH), 1627 (C=N, triazole), 779 (-Cl), 3297 (NH, pyrazole)	7.22-8.53 (14H, m, Ar-H), 6.26 (1H, s, NH of pyrazole), 3.24 (1H, s, CH of pyrazole)	398
S_7	3079, 3072 (Ar-CH), 1623 (C=N, triazole),3267 (NH, pyrazole)	6.45 -8.59 (15H, m, Ar-H), 6.33 (1H, s, NH of pyrazole), 3.37 (1H, s, CH of pyrazole)	363
S_8	3069 (Ar-CH), 1621 (C=N, triazole),696 (-Br), 3270 (NH, pyrazole)	7.35-8.44 (14H, m, Ar-H), 6.28 (1H, s, NH of pyrazole), 3.31 (1H, s, CH of pyrazole)	442
S_9	3077 (Ar-CH), 1625(C=N, triazole), 3359(-OH).	7.43-8.29 (14H, m, Ar-H), 6.13 (1H, s, NH of pyrazole), 3.35 (1H, s, CH of pyrazole), 10.27 (s, 1H, Ar-OH)	379
S_{10}	3081 (Ar-CH), 1628 (C=N, triazole,1153 (-OCH3), 3278 (NH, pyrazole)	7.41-8.47 (13H, m, Ar-H), 6.31 (1H, s, NH of pyrazole), 3.32 (1H, s, CH of pyrazole), 3.79 (6H, s, OCH_3)	423

Evaluation of antinociceptive activity

Study protocol was approved by the Institutional Animal Ethics Committee for the purpose of control and supervision of experiments on animals (IAEC, Approval No.1211/ac/08/CPCSEA) before experiment. Swiss strain albino mice of either sex weighing 25–30 g were used for this study. The test compounds were administered intraperitoneally in 10% v/v Tween 80 suspension. The antinociceptive activity was evaluated using acetic acid induced writhing (abdominal constriction test) test and Hot plate method.

Acute toxicity study

The acute toxicity for the test compounds was determined by the Miller and Tainter method administering the compounds intraperitoneally. LD_{50} of the test compounds calculated by Miller and Tainter (1944) method,[24] initially least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) were determined by hit and trial method. For the antinociceptive activity the LD_{50} of the test compounds was measured at 100 mg/kg (Table 2).

Table 2. Determination of LD_{50} mg/kg values of test compounds by Miller and Tainter method.

compound	Least tolerated Dose with 100% mortality	Most tolerated Dose with 0% mortality	LD_{50} mg/kg with 50% mortality
S_1-S_{10}	25	200	100

Acetic acid induced writhing method (Abdominal Constriction Test)[25]

The animals were divided into 12 groups of six mice each. The control group of animals was administered with 10% v/v Tween 80 (0.5 ml) suspension. The animals of another group were injected intraperitoneally with standard drug Ibuprofen (10 mg/kg). After 20 min of the administration the test compounds, all the groups of mice were given with the writhing agent 3% v/v aqueous acetic acid in a dose of 2 ml/kg intraperitoneally. The writhing produced in these animals was counted visually for 15 min and the numbers of writhings produced in treated groups were compared with control group. The results of analgesic activity are recorded in Table 3. Analgesic activity in percent was calculated by using following formula.

$$\text{Protection} = 100 - [\{(\text{No. of writhes in treated mice})/(\text{No. of writhes in untreated mice})\} \times 100]$$

Table 3. Evaluation of analgesic activity by acetic acid induced writhing method.

Sr. No.	Treatment	Dose (mg/kg)	Writhing episodes in 15 min (Mean ± S.E.M.)	Percent protection
1	Control	-	39.42±0.4247	-
2	Ibuprofen	10	11.56±0.3458**	71
3	S_1	100	13.23±0.5647**	66
4	S_2	100	15.56±0.4475**	61
5	S_3	100	16.14±0.5895**	59
6	S_4	100	13.09±0.7858**	67
7	S_5	100	18.36±0.3256**	53
8	S_6	100	14.89±0.5358**	62
9	S_7	100	22.68±0.3874**	42
10	S_8	100	20.47±0.4578**	48
11	S_9	100	16.78±0.6544**	57
12	S_{10}	100	14.47±0.7478**	63

** P < 0.01 represent significant difference when compared with control groups.

Hot plate method

The analgesic activity measured by central analgesia of hot plate method.[26] The temperature of a metal surface in the hot plate test was set at 55±1.0 °C. The time taken by the animals to lick the fore or hind paw or jump out of the place was taken as the reaction time. Latency to the licking paws or jumping from plate was determined before and after treatment. The latency was recorded at the time of 0 (just before any treatment) and 15, 30 and 60 min after intraperitoneal administration of test compounds. A latency period of 15 sec was defined as complete analgesia as cut off time to prevent damage to mice. The reference compound Pentazocine was administered in a dose of 5 mg/kg. The time course of hot plate latency was expressed as the percentage of the maximum possible effect (%MPE) according to the following formula:

$$\%\text{MPE} = \frac{(\text{post drug latency}) - (\text{pre drug latency})}{(\text{cut off time}) - (\text{pre drug latency})} \times 100$$

After the treatment of test and reference compounds, the pain thresholds of the animals were observed and presented in Table 4.

Table 4. Evaluation of analgesic activity by Hot plate method.

Treatment	Average Reaction Time in seconds before treatment (Mean ± S.E.M.)	Reaction time in seconds after treatment (Mean ± S.E.M.)			%MPE
		15 min	30 min	60 min	
Control	4.75±0.1547	4.75±0.1683	4.75±0.2663	4.75±0.4541	-
Pentazocine	4.70±0.5012**	7.70±0.5611**	9.67±0.4602**	11.81±0.5254**	69.02
S_1	4.66±0.5521**	7.65±0.6346**	9.83±0.5645**	11.67±0.4369**	67.79
S_2	4.63±0.4985**	7.60±0.5234**	9.87±0.4865**	11.54±0.4356**	66.44
S_3	4.67±0.4123**	7.52±0.6532**	9.92±0.5433**	11.79±0.4156**	68.92
S_4	4.61±0.5374**	7.55±0.5585**	9.72±0.5658**	11.47±0.5541**	66.02
S_5	4.69±0.4374**	6.45±0.5418**	8.36±0.6256**	9.27 ±0.5454**	44.42
S_6	4.65±0.4969**	7.42±0.4769**	9.79±0.5169**	11.71±0.5075**	68.21
S_7	4.56±0.5154**	6.70±0.4559**	8.49±0.5474**	9.37±0.5525**	46.07
S_8	4.43±0.4541**	7.52±0.5585**	9.77±0.5456**	10.82±0.6084**	60.65
S_9	4.56±0.46735**	7.39±0.4646**	9.53±0.5136**	10.53±0.6359**	57.18
S_{10}	4.68±0.5548**	7.56±0.6636**	9.59±0.5552**	10.37±0.4427**	55.13

** p<0.01 represent the significant difference when compared with control group.

Statistical analysis

Data were presented as arithmetic mean±SEM. Statistical analysis was performed by one way variance (ANOVA) followed by Dunnett's test. ''p'' value of less than 0.05 was considered as statistically significant.

Antimicrobial Activity

Determination of Minimum Inhibitory Concentration (MIC): The Minimum Inhibitory Concentration (MIC) of the test compounds against *Bacillus subtillis* (NCIM 2063), *Escherichia coli* (NCIM 2065), *Candida albicans* (NCIM 3471) and *Aspergillus niger* (NCIM 1196) was determined by liquid broth method of two fold serial dilution technique.[27] In this assay, the minimum concentration of each test compound required to inhibit the growth of microorganism was determined. The final concentration of test compounds ranged from 250 to 7.81µg/ml. Ampiciline and Ciprofloxacine were used as a standard antibacterial drug and Fluconazole was used as a standard antifungal drug. All the standard drugs were tested at concentrations ranging from 100 to 3.12 µg/ml respectively. The tubes were inspected visually to determine the growth of the organism as indicated by turbidity. MIC values of each tested compound recorded in Table 5 and 6.

Table 5. Antibacterial activity data of 1-[5-(substituted aryl)-1H-pyrazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole derivatives.

Comp	Concentration in µg/ml against B. Subtillis						MIC µg/ml	Concentration in µg/ml against E. Coli						MIC µg/ml
	250	125	62.5	31.25	15.62	7.81		250	125	62.5	31.25	15.62	7.81	
S_1	-	-	-	-	+	+	31.25	-	-	-	-	+	+	31.25
S_2	-	-	-	-	+	+	31.25	-	-	-	-	+	+	31.25
S_3	-	-	-	+	+	+	62.5	-	-	+	+	+	+	125
S_4	-	-	-	-	+	+	31.25	-	-	-	-	-	+	15.62
S_5	-	-	+	+	+	+	125	-	-	-	+	+	+	62.5
S_6	-	-	-	+	+	+	62.5	-	-	-	-	+	+	31.25
S_7	-	+	+	+	+	+	250	-	+	+	+	+	+	250
S_8	-	-	-	+	+	+	62.5	-	-	-	+	+	+	62.5
S_9	-	-	-	-	+	+	31.25	-	-	-	-	+	+	31.25
S_{10}	-	-	-	-	-	+	15.62	-	-	-	-	-	+	15.62

Standard drug Ampiciline showed MIC at 6.25 µg/ml and Ciprofloxacin showed MIC at 6.25 µg/ml.

"-" growth of the organism in test tube not observed, "+" growth of the organism in test tube observed

Table 6. Antifungal activity data of 1-[5-(substituted aryl)-1H-pyrazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole derivatives.

Comp	Concentration in µg/ml against C. albicans						MIC µg/ml	Concentration in µg/ml against A. niger						MIC µg/ml
	250	125	62.5	31.25	15.62	7.81		250	125	62.5	31.25	15.62	7.81	
S_1	-	-	-	-	+	+	31.25	-	-	-	-	+	+	31.25
S_2	-	-	-	-	-	+	15.62	-	-	-	-	+	+	31.25
S_3	-	-	-	+	+	+	62.5	-	-	+	+	+	+	125
S_4	-	-	-	-	+	+	31.25	-	-	-	-	-	+	15.62
S_5	-	-	+	+	+	+	125	-	-	+	+	+	+	125
S_6	-	-	-	-	-	+	15.62	-	-	-	-	-	+	15.25
S_7	-	-	+	+	+	+	125	-	-	-	+	+	+	62.5
S_8	-	-	-	-	+	+	31.25	-	-	-	-	-	+	15.25
S_9	-	-	-	+	+	+	62.5	-	-	-	+	+	+	62.5
S_{10}	-	-	-	-	+	+	31.25	-	-	-	-	+	+	31.25

Standard drug Fluconazole showed MIC at 6.25 µg/ml

"-" growth of the organism in test tube not observed, "+" growth of the organism in test tube observed

Results and Discussion

The synthesis of 1-[5-(substituted aryl)-1H-pyrazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole derivatives (S_1-S_{10}) depicted in Figure 2. The previously synthesized chalcones were cyclized with hydrazine hydrate in acidic medium to get various pyrazoles clubbed with 1,2,4-triazole. Infra-red spectrum of compounds S_1-S_{10} showed a sharp absorption at 1557, 779-785, 1157, 3149, 696, 3359 and 3068-3081 cm^{-1} which is accredited to -NO_2, -Cl, -OCH_3, -N-$(CH_3)_2$, Br, OH and aromatic region. Synthesized compounds showed appropriate ^{1}H-NMR signals aromatic protons showed multiplets in the range of δ 6.45-8.59, the anticipated signals with proper multiplicities for different types of protons were observed for the compounds. Mass spectra of the compounds showed molecular ion peaks with high abundance at m/z in concurrence with their molecular formula.

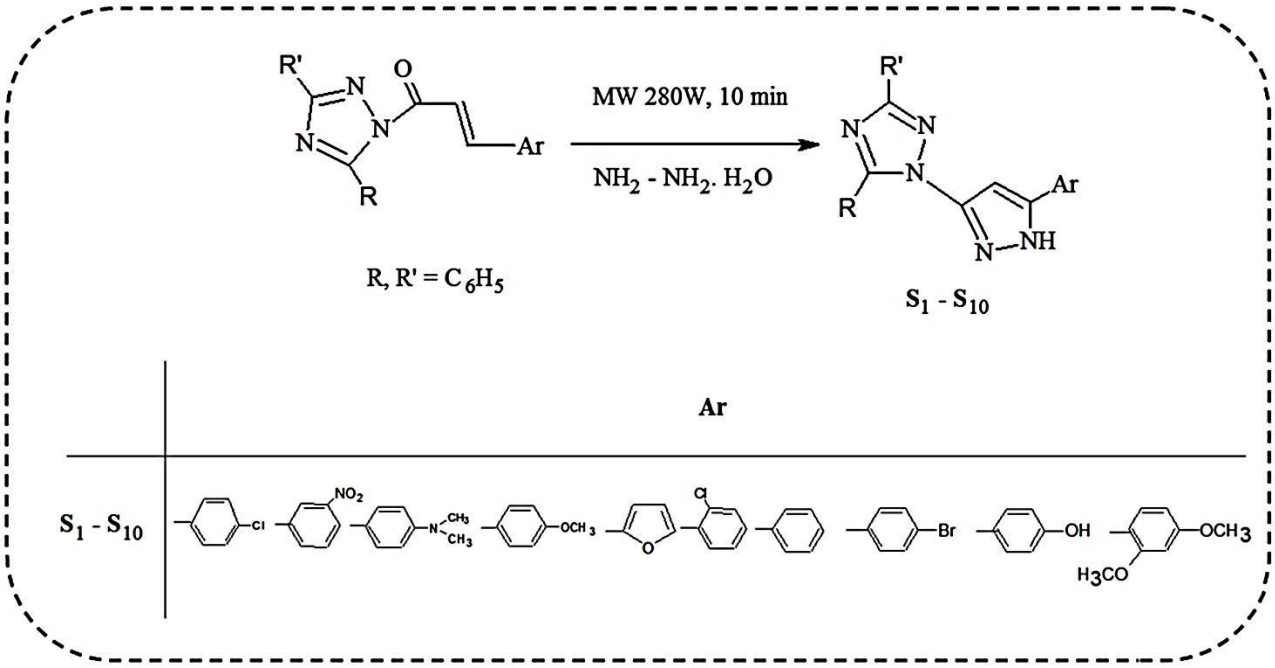


Figure 2. Microwave assisted synthesis of compounds S_1-S_{10}

All compounds are tested for their central and peripheral antinociceptive activity, in acetic acid induced writhing method compounds S_1, S_2, S_4, S_6 and S_{10} were found to be excellent analgesic agents with 66, 61, 67, 62 and 63 percentage of protection respectively. SAR study for peripherally acting analgesics stated that electron withdrawing groups (EWG) such as chloro and nitro on para, meta and ortho positions and electron releasing group (ERG) like methoxy on ortho and para position of phenyl ring present on pyrazole nucleus exhibited potential activity. Bromo, hydroxy and dimethylamino substituted analogues showed moderate antinociceptive activity. The ortho, meta and para positions of phenyl ring substituted on 5th position of pyrazole ring are found to be crucial and important site for lead modification.

Hot plate method was studied to perceive the centrally acting analgesic effect of newly synthesized compounds, Pentazocine 5 mg/kg significantly increased the hot plate latency producing a highest %MPE at 69.02. Compounds S_1, S_2, S_3, S_4 and S_6 significantly increased the hot plate latency when compared to the control group. Compounds S_8, S_9 and S_{10} showed moderate activity. The highest antinociception induced by compounds S_3, S_6 and S_1 at dose of 100 mg/kg were observed with 68.92, 68.21 and 67.79% MPE respectively. Compounds substituted with 3-nitro, phenyl, 4-chloro, 2-chloro, 4-methoxy, 4-bromo, 4-hydroxy and 2,4-dimethoxy groups demonstrated dynamic analgesic activity. Dimethyl aminophenyl and 2-furyl substituted analogues found to be weak analgesic agents from the tested series.

Newly synthesized compounds were furthermore tested for their *in vitro* antimicrobial activity against various NCIM reference bacterial strains of *B. subtillis*, *E. coli* and fungal strains of *C. albicans* and *A. niger*. The MIC values were calculated using liquid broth method of two fold serial dilution technique. All compounds showed a reasonable level of antibacterial and antifungal activity at the µg/ml level, antibacterial activity ranging from the lowest MIC value of 15.62 µg/ml for compound S_{10} against *B. subtillis* and *E. coli*, compound S_4 against *E. coli* to the highest of 250 µg/ml for compound S_7 against *B. subtillis* and *E. coli*. The MIC values of antifungal activity ranging from the lowest MIC value of 15.62 µg/ml for compound S_2 and S_6 against *C. albicans*, compounds S_4, S_6 and S_8 against *A. niger* to the highest of 125 µg/ml for compound S_5 against *C. albicans* and *A. niger*. The order of the antimicrobial activity in tested compounds seems to depend on the nature of the EWG in position 2, 3 and 4 of phenyl ring of pyrazole nucleus varies in the order 2-

chloro > 3-nitro > 4-chloro > 4-bromo, moreover the ERG like methoxy in position 2 and 4 of phenyl ring of pyrazole nucleus. The screening results also suggested that 3, 5 disubstituted triazole ring might be performing vital role in the antimicrobial activity.

Conclusion

A new class of pyrazole containing 3,5-disubstituted triazole was synthesized and evaluated as antimicrobial, centrally and peripherally acting analgesic agents and found to be potential therapeutic agent. The increase in biological activity is attributed to the presence of 3-nitro, 2-chloro, 4-chloro and 4-methoxy groups on phenyl ring of pyrazole ring. These results suggest that novel series of pyrazoles clubbed with triazole moiety are interesting lead molecules for further synthetic and biological evaluation.

Acknowledgments

Authors are highly thankful to *BCUD, University of Pune*, Pune, India for providing financial assistance for entire course of investigation. Authors are also grateful to Principal M.E.S. College Pharmacy, Sonai and *Prashant Patil Gadakh*, Secreatary, Mula Education Society for providing excellent research facilities for this work.

Conflict of interest

All the authors report no conflicts of interest.

References

1. Al-Soud YA, Al-Masoudi NA, Ferwanah Ael R. Synthesis and properties of new substituted 1,2,4-triazoles: potential antitumor agents. *Bioorg Med Chem* 2003;11(8):1701-8.
2. Khanage SG, Mohite PB, Raju SA. Synthesis, anticancer and antibacterial activity of some novel 1,2,4-triazole derivatives containing pyrazole and tetrazole rings. *Asian J Res Chem* 2011;4(4):567-73.
3. Lingappa B, Girisha KS, Kalluraya BN, Rai S, Kumari NS. Regioselective reaction: Novel Mannich bases derived from 3-(4,6-disubstituted-2-thiomethyl)3-amino-5-mercapto-1,2,4-triazoles and their antimicrobial properties. *Indian J Chem* 2008;47B:1858-64.
4. Rao G, Rajasekran S, Attimarad M. Synthesis and Antimicrobial activity of Some 5-phenyl-4-substituted amino-3-mercapto (4*H*) 1,2,4-triazoles. *Indian J Pharm Sci* 2000;62(6):475-7.
5. Jalilian AR, Sattari S, Bineshmarvasti M, Shafiee A, Daneshtalab M. Synthesis and in vitro antifungal and cytotoxicity evaluation of thiazolo-4H-1,2,4-triazoles and 1,2,3-thiadiazolo-4H-1,2,4-triazoles. *Arch Pharm (Weinheim)* 2000;333(10):347-54.
6. Lazarevic M, Dimova V, Molnar GD, Kakurinov V, Colanceska RK. Synthesis of some N1-aryl/heteroarylaminomethyl/ethyl-1,2,4-triazoles and their antibacterial and antifungal activities. *Heterocycl Commun* 2001;7(6):577-82.
7. Chimirri A, Bevacqua F, Gitto R, Quartarone S, Zappala MD, Sarro A, et al. Synthesis and anticonvulsant activity of new 1-*H*-triazolo[4,5-c][2,3]benzodiaze-pines. *Med Chem Res* 1999;9:203-12.
8. Hunashal RD, Ronad PM, Maddi VS, Satyanarayana D, Kamadod MA. Synthesis, anti-inflammatory and analgesic activity of 2-[4-(substituted benzylideneamino)-5-(substitutedphenoxymethyl)-4*H*-1,2,4-triazol-3-yl-thio] acetic acid derivatives. *Arab J Chem* 2011;1-9.
9. Khanage SG, Mohite PB, Pandhare RB, Raju SA. Study of analgesic activity of novel 1,2,4-triazole derivatives bearing pyrazole and tetrazole moiety. *J Pharm Res* 2011; 4(10):3609-11.
10. Kane JM, Dudley MW, Sorensen SM, Miller FP. Synthesis of 1,2,4-Dihydro-3*H*-1,2,4-triazole-3-thiones as potential antidepressant agents. *J Med Chem* 1988;31(6):1253-8.
11. Husain MI, Amir M, Singh E. Synthesis and antitubercular activities of [5-(2furyl)-1,2,4-triazoles-3yl thio] acehydrazide derivatives. *Indian J Chem* 1987;26B:2512-54.
12. Khanage SG, Mohite PB, Pandhare RB, Raju SA. Investigation of pyrazole and tetrazole derivatives containing 3,5 disubstituted-4*H* 1,2,4-triazole as a potential antitubercular and antifungal agent. *Bioint Res Appl Chem* 2012; 2(2):277-83.
13. Xiao Z, Waters NC, Woodard CL, Li Z, Li PK. Design and synthesis of Pfmrk inhibitors as potential antimalarial agents. *Bioorg Med Chem Lett* 2001;11(21):2875-8.
14. Mhasalkar MY, Shah MH, Pilankar PD, Nikam ST, Anantanarayanan KG, Deliwala CV. Synthesis and hypoglycaemic activity of 3-aryl(or pyridyl)-5-alkyl amino-1,3,4, Thiadiazole and some sulfonyl ureas derivatives of 4*H*-1,2,4 triazoles. *J Med Chem* 1971;14(10):1000-3.
15. Badawey E, El-Ashmawey IM. Anti-inflammatory, analgesic and antipyretic activity of some new 1-(pyrimidin-2-yl)-3-pyrazoline-5-ones and 2-(pyrimidin-2- yl)-1,2,4,5, 6,7-hexahydro-3H-indazol-3-ones. *Eur J Med Chem* 1998;33:349-61.
16. Tanitame A, Oyamada Y, Ofuji K, Terauchi H, Kawasaki M, Wachi M, et al. Synthesis and antibacterial activity of a novel series of DNA gyrase inhibitors: 5-[(E)-2-arylvinyl]pyrazoles. *Bioorg Med Chem Lett* 2005;15(19):4299-303.
17. Sahu SK, Banerjee M, Samantray A, Behera C, Azam MA. Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel pyrazoline derivatives. *Trop J Pharm Res* 2008;7(2):961-8.
18. Bouabdallah I, M'barek LA, Zyad A, Ramdani A, Zidane I, Melhaoui A. Anticancer effect of three pyrazole derivatives. *Nat Prod Res* 2006;20(11):1024-30.

19. Lv PC, Li HQ, Sun J, Zhou Y, Zhu HL. Synthesis and biological evaluation of pyrazole derivatives containing thiourea skeleton as anticancer agents. *Bioorg Med Chem* 2010;18(13):4606-14.
20. Castagnolo D, De Logu A, Radi M, Bechi B, Manetti F, Magnani M, et al. Synthesis, biological evaluation and SAR study of novel pyrazole analogues as inhibitors of Mycobacterium tuberculosis. *Bioorg Med Chem* 2008;16(18):8587-91.
21. Sanjay K, Gyanendra K, Mili K, Avadhesha S, Namita S. Synthesis and evaluation of substituted pyrazoles: potential antimalarials targeting the enoyl-acp reductase of plasmodium falciparum. *Synth Commun* 2006;36(2):215-26.
22. Khanage SG, Mohite PB, Pandhare RB, Raju SA. Synthesis, characterization and antimicrobial evaluation of 3,5 diphenyl-1*H*-1,2,4-triazole containing pyrazole function. *Bioint Res Appl Chem* 2012;2(3):313-9.
23. Khanage SG, Raju SA, Mohite PB, Pandhare RB. Analgesic Activity of Some 1,2,4-Triazole Heterocycles Clubbed with Pyrazole, Tetrazole, Isoxazole and Pyrimidine. *Adv Pharm Bull* 2013;3(1):13-8.
24. Miller LC, Tainter ML. Estimation of LD_{50} and its error by means of log-probit graph paper. *Proc Soc Exp Bio Med* 1944;57:261-4.
25. Siegmund E, Cadmus R, Lu G. A method for evaluating both non-narcotic and narcotic analgesics. *Proc Soc Exp Biol Med* 1957;95(4):729-31.
26. Eddy NB, Leimbach D. Synthetic analgesics. II. Dithienylbutenyl- and dithienylbutylamines. *J Pharmacol Exp Ther* 1953;107(3):385-93.
27. Gibbons S, Ohlendorf B, Johnsen I. The genus Hypericum--a valuable resource of anti-Staphylococcal leads. *Fitoterapia* 2002;73(4):300-4.

