Advanced Pharmaceutical Bulletin, **2013**, 3(1), 13-18
doi: http://dx.doi.org/10.5681/apb.2013.003
http://apb.tbzmed.ac.ir/

Analgesic Activity of Some 1,2,4-Triazole Heterocycles Clubbed with Pyrazole, Tetrazole, Isoxazole and Pyrimidine

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ARTICLE INFO

Article Type:
Research Article

Article History:
Received: 26 August 2012
Revised: 1 September 2012
Accepted: 4 September 2012
ePublished: 7 February 2013

Keywords:
Triazole
Analgesic Activity
Pyrazole
Tetrazole
Isoxazole
Pyrimidine

ABSTRACT

***Purpose*:** In the present study *in vivo* analgesic activity of some previously synthesized 1,2,4-triazole derivatives containing pyrazole, tetrazole, isoxazole and pyrimidine ring have been evaluated. ***Methods*:** Acetic acid induced writhing method and Hot plate method has been described to study analgesic activity of some 1,2,4-triazole derivatives containing pyrazole, tetrazole, isoxazole and pyrimidine as a pharmacological active lead. ***Results*:** Thirty six different derivatives containing 1,2,4-triazole ring were subjected to study their *in vivo* analgesic activity. Chloro, nitro and methoxy, hydroxy and bromo substituted derivatives showed excellent analgesic activity and dimethylamino, furan and phenyl substituted derivatives showed moderate analgesic activity in both of the methods. Compounds IIIa, IIId, IIIf, IIIi, IIIj, IVa, IVb, IVd, IVf, IVh, IVj IV3a and IIj were found to be superior analgesic agents after screening by Acetic acid induced writhing method. Compounds IIIb, IIId, IIIf, IIIh, IIIj, IVa, IVb, IVd, IVf, IVh, IVi, IV3c, IV3e and IIj were showed analgesic potential after screening of Hot plate method. ***Conclusion*:** All tested compounds containing 1,2,4-triazole were found to be promising analgesic agents, for this activity pyrazole, tetrazole, isoxazole and pyrimidine leads might be supported.

Introduction

Analgesic and anti-inflammatory drugs are one of the most valuable medicaments that used in many of disease for relief of pain and inflammation. Most analgesic and anti-inflammatory drugs available in the market, still present a wide range of many problems such as efficacy and undesired effects including GIT disorders and other unwanted effects,[1] that limit their clinical usefulness and remain to be solved and leaving an open door for new and better compounds.[2] This situation highlights the need for advent of safe, novel and effective analgesic and anti-inflammatory compounds.[3] 1,2,4-triazole received sheer attention of medicinal chemists because of their many therapeutic applications like anticancer,[4,5] antimicrobial,[6-9] anticonvulsant,[10] anti-inflammatory, analgesic,[11] antidepressant,[12] antitubercular,[13] antimalarial[14] and hypoglycemic[15] activities.

We have reported that 5-methyl-2-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-2,4-dihydro-3*H*-pyrazol-3-one and 5-phenyl-1-[(5-substituted aryl-4*H*-1,2,4-triazol-3-yl)methyl]-1*H*-tetrazole had significant anticancer activity specially on renal cancer cell lines (UO-31) as well *in vitro* antibacterial activity against gram positive bacterial *S. aureus* NCIM 2079, *B. subtillis* NCIM 2063 and gram negative bacterial *E. coli* NCIM 2065, *P. aeruginosa* NCIM 2863 strains.[16] More recently we have reported antimicrobial, antitubercular and anticancer activity of 1-[5-(substituted aryl)-1,2-oxazol-3-yl]-3,5-diphenyl-1H-1,2,4-triazole (4a-g).[17] In continuation of our previous work[18-20] in this article the attempts have been made to explore the analgesic potential of some formerly synthesized 1,2,4-triazoles clubbed with pyrazole, tetrazole, isoxazole and pyrimidine heterocycles.

Materials and Methods

The standard analgesic drugs Ibuprofen and Pentazocine, solvents used for the experimental work were commercially procured from E. Merck India and Qualigens India. Swiss strain albino mice for study were procured from National Toxicology Center, Pune.

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Evaluation of analgesic 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.

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,[21] initially least tolerated (smallest) dose (100% mortality) and most tolerated (highest) dose (0% mortality) were determined by hit and trial method. After determination of two doses we have selected five doses in between the least tolerated and most tolerated doses were given intraperitoneally to 5 groups of mice, 10 animals in each group. The animals were observed for first 2 hours and then at 6^{th} and 24^{th} hour for any toxic symptoms. After 24 hours, the number of deceased animals was counted in each group and percentage of mortality calculated. From the obtained data determined the LD_{50} of the test compounds by using probit value transformations.

Acetic acid induced writhing method (Abdominal Constriction Test)

The animals were divided into 38 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 1. Analgesic activity in percent was calculated by using following formula.

Protection = 100-[{(No. of writhes in treated mice)/(No. of writhes in untreated mice)}×100]

Hot plate method

The method of Eddy and Leimbach was adopted for the study. The temperature of a metal surface in the hot plate test was set at 55±1.0ºC. 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:

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

After the treatment of test and reference compounds, the pain thresholds of the animals were observed and presented in Table 2.

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.

Results and Discussion

Acetic acid induced writhing method

Abdominal construction responses induced by acetic acid is a sensitive procedure to establish efficacy of peripherally acting analgesics, it may causes increase in the level of PGE_2 and PGF2a by intraperitoneally administration of acetic acid. The analgesic activity was expressed as percentage of protection. All tested compounds exhibited activity in a dose range of 25-100 mg/kg. Writhing episodes and percent protection of tested compounds for analgesic activity are summarized in Table 1. Compound IIIa, IIId, IIIf, IIIi, IIIj, IVa, IVb, IVd, IVf, IVh, IVj IV3a and IIj (Figure 1 and 2) were found to be superior analgesic agents with 55, 60, 57, 57, 57, 57, 55, 60, 60, 56, 63, 60 and 60% analgesic activity respectively as compared to other tested compounds. Chloro, nitro, methoxy, hydroxy and bromo substituted derivatives exhibited excellent analgesic activity where as dimethylamino, furan and phenyl substituted derivatives showed moderate analgesic activity. Substituted phenyl ring present on isoxazole, pyrimidine and 1,2,4-triazole nucleus might have attributed crucial role to show analgesic activity. Compounds with substitution of chloro, methoxy, bromo on 4^{th} position of phenyl ring present on isoxazole, pyrimidine and triazole nucleus showed maximum analgesic activity. Compounds IIId, IVd, IVf, IVj, IV3a and IIj were exhibited comparative analgesic property (up to 60% protection) with standard drug Ibuprofen (66%) as illustrated in Table 1. because of 4-methoxy, 4-chloro, 2-chloro, 2,4-dimethoxy groups present on phenyl ring of pharmacologically active isoxazole, pyrimidine, and triazole chalcone heterocycles.

Hot plate method

All compounds tested by Eddy's hot plate method exhibited activity in a dose range of 25-100 mg/kg. The analgesic activity measured by central analgesia.

Pentazocine 5 mg/kg significantly increased the hot plate latency producing a highest %MPE at 69.02. Compounds IIIb, IIIj, IVa, IVd, IVf, IVh, IVi, and IIj significantly increased the hot plate latency when compared to the control group. The highest antinociception induced by compounds IIIj, IVd and IVf at dose of 50 mg/kg were observed with 64.82, 66.98 and 67.21 %MPE respectively. The analgesic activity of compounds IIIb, IIId, IIIf, IIIh, IIIj, IVa, IVb, IVd, IVf, IVh, IVi, IV3c, IV3e and IIj were comparable to Pentazocine after 15, 30 and 60 min. 2-chloro, 3-nitro, 4-chloro, 4-methoxy, 4-bromo, 4-hydroxy and 2,4-dimethoxy substituted analogs exhibited dynamic analgesic activity. 4-dimethyl amino and 2-furyl substituted compound of tested series of 1-(3,5-diphenyl-1H-1,2,4-triazol-1-yl)-3-(substituted aryl) prop-2-en-1-one (Chalcones) were also acquired higher hot plate latency with 10.76 and 10.74 sec respectively after 60 min. Compounds from series of 1-[5-(substituted aryl)-1,2-oxazol-3-yl]- 3,5-diphenyl-1H-1,2,4-triazoles and 6-(substituted aryl)-4-(3,5-diphenyl-1H-1,2,4-triazol-1-yl)-1,6-dihydropyrimidine-2-thiol obtained excellent analgesic potential.

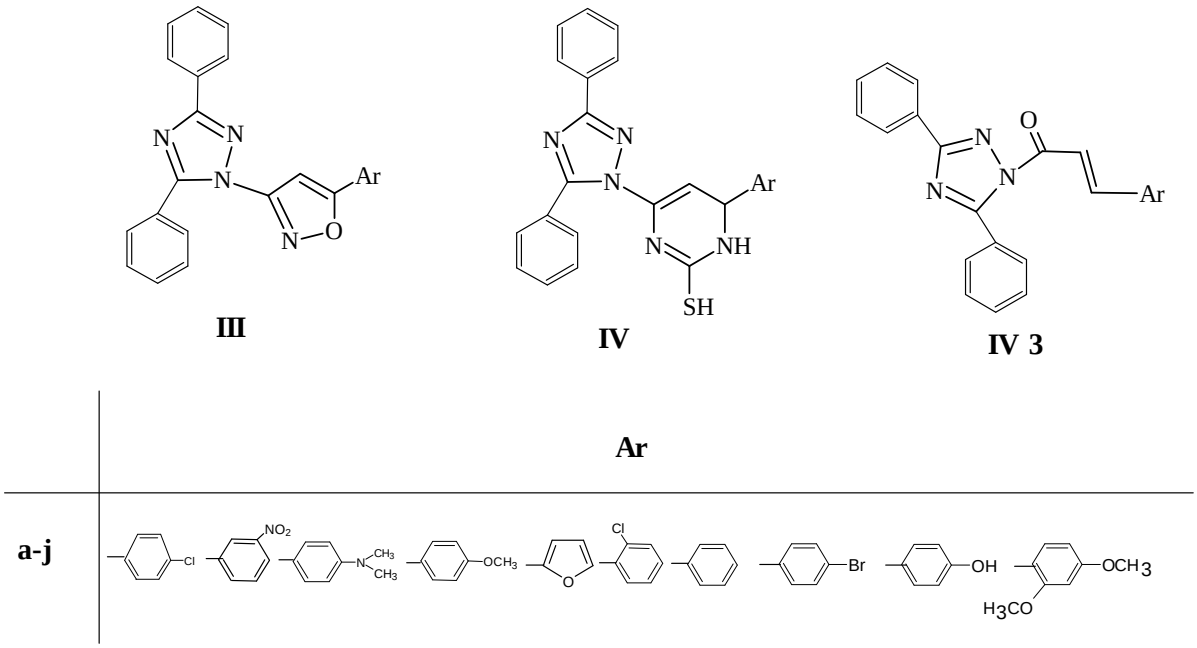

Figure 1. Structures of compounds of scheme III, IV and IV3.

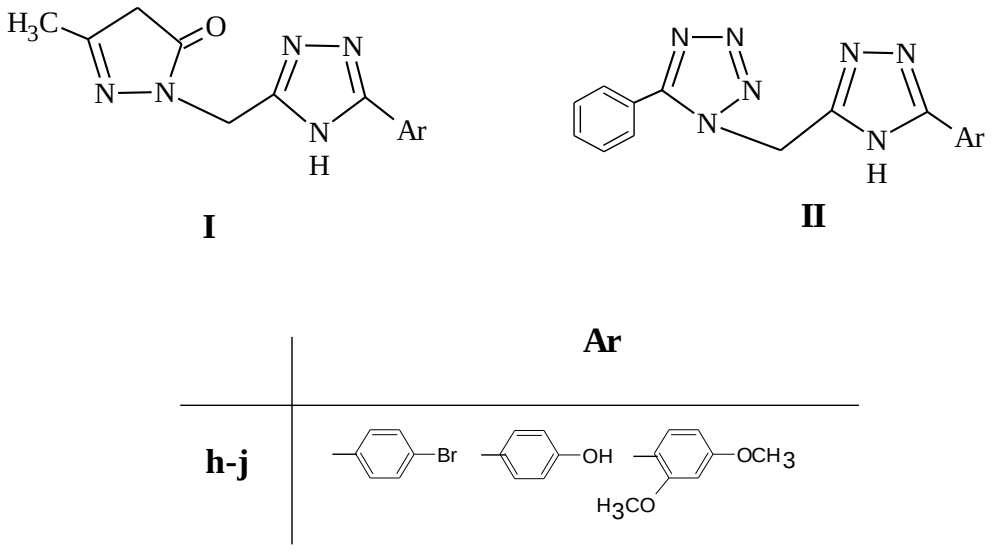

Figure 2. Structures of compounds of scheme I and II.

Table 1. 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	--	38.70±0.5547	-
2	Ibuprofen	10	13.19±0.6921**	66
3	IIIa	50	17.28±0.6647**	55
4	IIIb	50	18.24±0.6425**	53
5	IIIc	50	24.89±0.5354**	36
6	IIId	50	15.65±0.7845**	60
7	IIIe	50	26.45±0.8121**	32
8	IIIf	50	16.46±0.5744**	57
9	IIIg	50	27.65±0.5478**	29
10	IIIh	50	18.57±0.4545**	52
11	IIIi	50	16.49±0.6545**	57
12	IIIj	50	16.47±0.2254**	57
13	IVa	50	16.45±0.5456**	57
14	IVb	50	17.49±0.5641**	55
15	IVc	50	21.73±0.5974**	44
16	IVd	50	15.44±0.6133**	60
17	IVe	50	22.45±0.5525**	42
18	IVf	50	15.36±0.6455**	60
19	IVg	50	19.65±0.7322**	49
20	IVh	50	17.22±0.4855**	56
21	IVi	50	19.47±0.6513**	50
22	IVj	50	14.24±0.4745**	63
23	IV3a	100	15.44±0.4454**	60
24	IV3b	100	22.65±0.5546**	42
25	IV3c	100	19.47±0.5941**	50
26	IV3d	100	21.77±0.4423**	44
27	IV3e	100	25.49±0.4473**	34
28	IV3f	100	19.42±0.6651**	50
29	IV3g	100	26.56±0.4329**	31
30	IV3h	100	28.56±0.7325**	26
31	IV3i	100	23.58±0.6423**	39
32	IV3j	100	19.26±0.4523**	50
33	Ih	25	20.54±0.6425**	47
34	Ii	25	22.89±0.5214**	41
35	Ij	25	18.34±0.4527**	53
36	IIh	25	20.57±0.8592**	47
37	IIi	25	22.44±0.6854**	42
38	IIj	25	15.47±0.8957**	60

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

Table 2. 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	60min	
Control	4.75±0.1547	4.75±0.1583	4.75±0.2563	4.75±0.4941	-
Pentazocine	4.70±0.4012**	7.70±0.5211**	9.67±0.4302**	11.81±0.3254**	69.02
IIIa	4.56±0.5221**	7.23±0.6326**	9.23±0.5745**	10.76±0.4369**	59.38
IIIb	4.63±0.4785**	7.49±0.2234**	9.87±0.3865**	11.15±0.5356**	62.87
IIIc	4.67±0.4523**	6.52±0.6542**	9.34±0.5413**	9.53±0.4356**	47.04
IIId	4.63±0.6374**	7.46±0.7585**	9.52±0.2658**	10.47±0.7541**	56.31
IIIe	4.69±0.4474**	6.45±0.5428**	8.36±0.6756**	9.27 ±0.5854**	44.42
IIIf	4.65±0.4469**	7.42±0.3769**	9.49±0.5369**	10.71±0.5775**	58.55
IIIg	4.56±0.5854**	6.70±0.4459**	8.49±0.7474**	9.27±0.6525**	45.11
IIIh	4.43±0.3544**	7.50±0.7585**	9.57±0.6456**	10.52 ±0.6785**	57.61
IIIi	4.71±0.4675**	6.39±0.3646**	9.03±0.5236**	9.46±0.5359**	46.16
IIIj	4.68±0.7548**	7.56±0.5636**	9.52±0.6552**	11.37±0.6426**	64.82
IVa	4.72±0.5957**	7.68±0.4523**	9.56±0.3356**	11.09±0.5517**	61.96
IVb	4.67±0.4358**	7.45±0.6984**	9.16±0.3548**	10.59±0.5478**	57.30
IVc	4.55±0.4785**	6.59±0.4578**	9.44±0.5349**	9.69±0.8689**	49.18
IVd	4.58±0.5878**	7.66±0.6689**	9.50±0.4245**	11.56±0.6741**	66.98
IVe	4.39±0.4589**	6.89±0.7481**	8.59±0.6478**	9.75 ±0.4589**	50.51
IVf	4.66±0.5958**	7.53±0.3549**	9.80±0.3358**	11.61±0.6358**	67.21
IVg	4.45±0.5869**	7.72±0.5269**	9.58±0.5895**	10.46±0.5692**	56.96
IVh	4.41±0.5321**	7.80±0.4781**	9.57±0.5696**	10.82 ±0.3324**	60.52
IVi	4.64±0.2966**	7.56±0.3826**	9.45±0.7851**	10.89±0.5369**	60.32
IVj	4.52±0.2853**	7.70±0.5239**	9.43±0.4665**	10.44±0.6745 **	56.48
IV3a	4.64±0.4789**	7.34±0.3545**	9.12±0.3456**	10.12±0.2985**	52.89
IV3b	4.56±0.5884**	7.11±0.5789**	8.36±0.6398**	9.47±0.6489**	47.03
IV3c	4.67±0.3956**	7.49±0.5212**	9.27±0.3369**	10.76±0.6245**	58.95
IV3d	4.73±0.4525**	7.13±0.2845**	9.10±0.3235**	9.46±0.5469**	46.05
IV3e	4.59±0.5823**	6.93±0.5861**	9.14±0.6478**	10.74 ±0.4369**	59.07
IV3f	4.54±0.4969**	7.23±0.5326**	9.50±0.3756**	10.32±0.6542**	55.25
IV3g	4.49±0.5478**	7.02±0.4689**	8.48±0.5225**	8.93±0.5692**	42.24
IV3h	4.61±0.4526**	6.80±0.4369**	8.55±0.5236**	9.51 ±0.5236**	47.16
IV3i	4.67±0.6325**	6.56±0.3989**	8.47±0.5364**	9.37±0.6346**	45.49
IV3j	4.71±0.5963**	7.31±0.2845**	9.46±0.3623**	10.20±0.4126 **	53.35
Ih	4.46±0.6522**	7.25±0.4545**	8.55±0.2625**	9.88±0.4121**	51.18
Ii	4.29±0.3532**	7.45±0.4666**	9.12±0.3644**	9.83±0.5481**	51.72
Ij	4.34±0.5418**	7.75±0.6641**	9.85±0.4236**	10.24±0.3678**	55.34
IIh	4.54±0.6645**	6.96±0.4425**	8.86±0.3356**	9.87±0.4356**	50.95
Iii	4.59±0.5414**	6.45±0.3784**	8.22±0.3541**	9.45±0.4678**	46.68
IIj	4.74±0.4775**	7.35±0.4125**	9.45±0.5925**	10.96±0.6486**	60.62

Dose: 25 mg/kg for compounds Ih-Ij, 50mg/kg for IIIa-j and IVa-j, 100mg/kg for IV3a-j and 5mg/Kg for Pentazocine.
** p<0.01 represent the significant difference when compared control group.

Conclusion

The results of the present investigation reveals that the increase in analgesic activity is attributed to the presence of 2-chloro, 3-nitro, 4-chloro, 4-methoxy, 4-bromo, 4-hydroxy and 2,4-dimethoxy groups on phenyl ring of isoxazole, pyrimidine, pyrazole, tetrazole and triazole. All tested heterocycles posses central and peripheral analgesic property. Perceptibly the comparative evaluation of active compounds will required in the further studies, the data reported in this article may be helpful guide for the medicinal chemist who is working in this area.

Acknowledgments

Authors are highly thankful to National Toxicology Center, Pune, for providing animals to caring out analgesic activity and acute toxicity study. We are also grateful of 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.

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