EMPRO

Current Research in Pharmaceutical Sciences

Available online at www.crpsonline.com

ISSN 2250 – 2688

Received: 20/08/2012
Revised: 15/09/2012
Accepted: 22/09/2012

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Synthesis, Characterization and Anti-inflammatory Activity of 1, 2, 4 Triazole Derivatives

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ABSTRACT

A series of 1, 2, 4 triazole derivatives were synthesized and evaluated for their anti-inflammatory activity by paw edema test. All the synthesized compounds were in good agreement with spectral and physical data. Most of the compounds synthesized were active and the most potent synthesized compounds showed greater % inhibition than the standard drug ibuprofen. Conclusions could be drawn from the synthesized compounds that sulfonyl group plays an important role in anti-inflammatory activity. The Cut off LD50 was found to be 500mg/kg for all synthesized compounds when given orally.

Key words: 1,2,4 Triazole, Synthesis, Anti-inflammatory activity and LD50

1. INTRODUCTION

The enormous after side effect of steroid therapy necessitated an accelerated research towards the development of NSAIDs since the past few decades. Large number of these agents have been put into clinical usage widely thereby exhibiting positive therapeutic efficacy with fewer untoward reactions. NSAIDs acts by inhibiting prostaglandin synthesis by causing complete blockade of precursor enzyme Cyclooxygenase [1].Two isoforms have been duly recognized for cyclooxgenase COX-1 and COX-2. COX-1, a constitutively expressed isoform, is found in platelets, kidneys, GI tract and is responsible for the homeostatic maintenance of the kidneys and GI tract. The COX-2 enzyme is the inducible isoform, produced by various cell types during injury [2]. However, Administration of NSAIDs on long term basis may lead to development of gastric ulcers, bleeding, and renal disorders [3-6]. Thus synthesis of more selective COX-2 inhibitor result in better tolerated and safer drugs. Synthetic approaches based upon NSAIDs' chemical modification have been taken with the aim of improving their safety profile.

A number of 1,2,4 triazole derivatives has been reported in literature possessing significant anti-microbial[7], anti-inflammatory activity[8-11], analgesic[11,12], antitumor[13], antihypertensive activity[14]. In continuation with the above researches we proposed to synthesized some triazole derivatives to design and synthesize some novel compounds like 4-[(4-methylphenyl) sulfonyl]-5-phenyl-4H-1,2,4-triazole-3-thiol and their corresponding condensed 3,6-disubstituted 7H-1,2,4-triazolo[3,4-b]-1,3,4-thiadiazine, 3-phenyl[1,2,4]triazole[3,4 b][1,3,4]thiadiazol-6(5H)-one which were expected to show anti-inflammatory properties. This paper discusses the most common and useful procedure for synthesizing 4-amino-3-mercapto-1, 2, 4-triazoles, the utility of triazoles in the synthesis of triazole diazines, and the evaluation of anti-inflammatory/analgesic activities of both derivatives.

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2. MATERIALS AND METHODS

All chemicals obtained were of analytical grade. Melting points were determined by open capillary method. The purity and homogeneity of the plates was checked routinely by thin layer chromatography on precoated silica gel plates using solvent system methanol, carbon tetrachloride, acetone in the ratio of 50:40:10. The IR spectra were recorded on a PERKIN-ELMER FTIR spectrophotometer and 1H NMR on BRUKER AVANCE II 400 NMR spectrometer in $CDCl_3$/ DMSO-d_6 using tetramethylsilane as internal standard and chemical shift expressed in δ ppm. The ^1H NMR spectroscopy was done at Punjab University. Chemical shifts are reported in values (ppm) relative to Me_4Si line as internal standard and J values are reported in Hertz.

General Procedure for Synthesis of Esters of Aromatic acid

The reaction of benzoic acid ester upon reaction with hydrazine hydrate (99%) and carbon di sulfide in the presence of ethanolic potassium hydroxide gave potassium salt of dithiocarbazinate (1) in quantitative yield. Further, the potassium salt upon reaction with hydrazine hydrate (99%) gave 5-Aryl-4-Amino-3-Mercapto-1,2,4-Triazole (2) which was used as the starting material. The triazole when treated with 4-methyl benzene sulfonyl chloride in dry pyridine afforded 4-[(4-methylphenyl) sulfonyl]-5-phenyl-4H-1, 2, 4-triazole-3-thiol (3).

Condensation of triazole (2) with aromatic aldehyde (p-bromo benzaldehyde, p-chlorobenzaldehyde and p-hydroxybenzaldehyde) in refluxing ethanol in the presence of catalytic amount of piperidine furnished Schiffs base (4a-4c). Fusion of triazole (2) with urea gave compound (5) in about 80% yield. The reaction of triazole (2) with equivalent amount of chloracetaldehyde in refluxing ethanol gave 3-phenyl-7H-[1,2,4]triazolo[3,4-b][1,3,4]thiadiazine. On the other hand, reaction of triazole (2) in the presence of carbondisulfide and dry pyridine gave 3-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione (Figure 1).

(1) Synthesis of Potassium Dithiocarbazinate

Aromatic esters (0.1 mol) and 80 % hydrazine hydrate (0.1 mol) was refluxed on a water bath for 15 min. Enough absolute methanol was added to obtain a clear solution. Again contents were refluxed for 4hrs. Excess alcohol was evaporated and solution was cooled down. The solid obtained was separated and recrystallised from ethanol to get the needle shaped crystals. M.P-126-128^oC;IR(KBr): 3319 cm^{-1}, 3263 cm^{-1} (N-H stretch), 3046 cm^{-1} (aromatic C-H stretch),1654 cm^{-1} (C=O stretch), 1579 cm^{-1} (N-H bend), 1496 cm^{-1} (C-N stretch); NMR: δ8(t,2H,-NH), δ7.2-7.3(m,5H,-C_6H_5) δ2.8(d,1H,-NH).

Produced aromatic hydrazides (0.02 mol), KOH (0.012 mol) and CS_2 (0.015 mol) in absolute ethanol (350 ml) were stirred for 10 hrs. After the completion of reaction, ether (200 ml) was added. The obtained precipitate was filtered, washed and dried. The synthesized dithiocarbazinate was used for the next step without further purification.M.P-286-288 ^oC ; IR(KBr): 3310 cm^{-1} (N-H stretch), 3046 cm^{-1} (aromatic C-H stretch), 1576 cm^{-1} (N-H bend), 1164 cm^{-1} (C-N stretch),1244 cm^{-1} (C=S stretch), 661 cm^{-1} (C-S stretch);NMR: δ 8(d,1H,-NH), δ7.4-7.9(m,5H,-C_6H_5), δ 2(d,1H,-NH).

(2) Synthesis of 5-Aryl-4-Amino-3-Mercapto-1, 2, 4-Triazole

Substituted produced dithocarbazinate (0.1 mol), hydrazine hydrate (0.3 mol) and water (30 ml) was refluxed for 3 hrs, H_2S evolved during the reaction and clear solution resulted, enough cold water was added and cooled to 5^0C. Acidified the cooled solution with dil. HCl. Obtained precipitate was filtered, washed and recrystallized from 95% ethanol. M.P-172-174^oC; IR (KBr):3465 cm^{-1}, 3406 cm^{-1} (NH stretch), 3116 cm^{-1} (aromatic C-H stretch), 2584 cm^{-1} (S-H stretch), 1614 cm^{-1} (C=C stretch),1580 cm^{-1} (C=N stretch),1294 cm^{-1} (C=S stretch); NMR: δ7.2-7.6(m,5H,-C_6H_5) δ3(s,1H,-SH), δ2(s,2H,-NH_2)

(3) Synthesis of 4-[(4-methylphenyl) sulfonyl]-5-phenyl-4H-1,2,4-triazole-3-thiol

A mixture of triazole (2) (0.01mol) and 4-methyl benzene sulfonyl chloride (0.01mol) in dry pyridine (20ml) was refluxed for 3 hr. It was than cooled and poured on ice. A solid product was obtained by filtration which was recrystallised from ethanol.M.P-80^0C ; IR(KBr):3260 cm^{-1} (NH stretch),3116 cm^{-1} (aromatic C-H stretch), 2454 cm^{-1} (S-H stretch), 1614 cm^{-1} (C=C stretch),1620 cm^{-1} (C=N stretch),1160 cm^{-1} & 950 cm^{-1} (SO2) ; NMR: δ 2 (s,1H,NH exchangeable), δ 2.35 (s,3H,Phen-CH3), δ 3.2 (s,1H,SH), δ 7.34-7.8 (m,4H,ArH)

(4) Synthesis of Schiffs bases

A mixture of triazole (2) (0.01mol) and the corresponding aldehyde (0.01mol) in ethanol (25ml) was treated with conc. HCl (0.5ml) and mixture was refluxed for 2 hr. The reaction mixture on cooling was filtered and purified by recrystallisation

4a) Prepared by p-chlorobenzaldehyde (4-{[(4 chlorophenyl)methylene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-

thiol): M.P-110-112^{0}C ; IR (KBr):1603 cm^{-1} (C=N), 2530 cm^{-1} (SH), 3010 cm^{-1} (aromatic C-H stretch) NMR: δ 6.9-7.5(m., 4H, ArH) & NMR: δ 8.3(s,1H,-N=CH)

4b) Prepared by p-methoxy benzaldehyde(4-{[(4-methoxyphenyl)methylene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol): M.P-105-108^{0}C IR(KBr): 1605 cm^{-1} (C=N), 2557 cm^{-1}(SH),3120 cm^{-1} (aromatic C-H group).NMR: δ 3.72 (s,3H,OCH3), δ 6.9-7.4(m,4H,-ArH).

4c) Prepared by p-bromo benzaldehyde(4-{[(4-bromophenyl)methylene]amino}-5-phenyl-4*H*-1,2,4-triazole-3-thiol) :M.P- 115-119^{0}C; IR(KBr): 1605 cm^{-1} (C=N), 2527 cm^{-1} (SH), 3020 cm^{-1} (aromatic C-H group); H^1 NMR: δ 6.9-7.5 (m.,4H,ArH) & δ 8.1(s,1H,N=CH).

(5) Synthesis of 3-phenyl[1,2,4]triazole[3,4-b][1,3,4]thiadiazol-6(5H)-one

A mixture of triazole (2) and urea was heated at 180-190^{0}C for 6 hr. The mixture was cooled and was added to the sodium hydroxide solution (5%, 20ml), then filtered and the filtrate was acidified with dilute HCl. The solid product was than recrystallized from ethanol to give 5. M.P-80-82^{0}C; IR (KBr):3218 cm^{-1} (NH stretch), 3116 cm^{-1} (aromatic C-H stretch), 2454 cm^{-1} (S-H stretch), 1670 cm^{-1} (CO stretch), 1590 cm^{-1} (C=N stretch); NMR: δ 8 (s, 1H, NH exchangeable).

(6) Synthesis of 3-phenyl-7H-[1, 2, 4] triazole [3, 4-b][1,3,4]thiadiazine

A mixture of triazole (2) (0.01mol), chloroacetaldehyde (0.02mol) and concentrated hydrochloric acid (2ml) in ethanol (150ml) was refluxed for 3 hrs. After removal of ethanol, the resulting solid was filtered and washed with water. The crude was than purified by recrystallization from ethanol. M.P-75-77^{0}C; I.R (KBr):1620 cm^{-1} (C=N) ; NMR δ 7.5(t, =CH), δ 3.1(CH_2 of ring).

(7) Synthesis of 3-phenyl[1,2,4]triazolo[3,4-b][1,3,4]thiadiazole-6(5H)-thione

A mixture of triazole (2) (0.01mol), carbon disulfide (0.01mole) and dry pyridine (20 ml) was heated under reflux for 2.5 hrs. It was cooled and poured on ice water. A solid product was obtained by filtration and was recrystallized from ethanol. M.P-70-72^{0}C; I.R (KBr):1620 cm^{-1} (C=N), 1300 cm^{-1} (C=S), 3220 cm^{-1} (NH); NMR δ 2(s, 1H, NH exchangeable)

3. RESULTS AND DISCUSSION

All the newly synthesized compounds were screened for anti-inflammatory activity against Albino Wistar rats by paw edema test using standard drug Ibuprofen and synthesized compounds 3-7 (54 in number weighing 200-225g). The dose, of standard drug as well as synthesized compounds administered in animals, was 50 mg/ kg and was given through oral feeding tube with the help of tuberculin syringe. The control group was administered with normal saline (0.9% w/v NaCl) (2.5ml/kg) orally and other groups with drugs suspended in CMC as per body weight. After 30 minutes, 0.1ml of 1% w/v solution of carrageenan in normal saline was injected into the planter region of left paw of the rats. The right paw served as a referenced inflamed paw for comparison. The paw volume of both the legs of rats treated with control, standard and test compounds were recorded using plethysmometer, after 1, 2, 3 and 4Hr after carrageenan challenge. The percentage inhibition of the inflammation in the drug treated animals were recorded and calculated using the formula

$$\% \text{ Inhibition} = 100^* [1-(Vt/Vc)]$$

Percent inhibition of the edema between the control group and compound treated groups was calculated and were compared with the group receiving a standard drug Ibuprofen. Anti-inflammatory activity of all synthesized compounds screened is reported under results and discussion (Table 2). Acute toxicity studies were conducted with the use of minimum number of animals per step with an oral dose of 500mg/Kg.

Table No 1: Physical constants of all synthesized derivatives

Comp	Mol. Formula (Mol. Wt)	Yield	Melting Point	Rf
3	$C_{14}H_{12}N_4O_2S_2$(332.4)	73	80^{0}C	0.58
4a	$C_{15}H_{11}N_4SCl$(314.3)	75	110-112^{0}C	0.69
4b	$C_{16}H_{12}N_4SO$(311.3)	80	105-108^{0}C	0.64
4c	$C_{15}H_{11}N_4SBr$(358)	80	115-117^{0}C	0.74
5	$C_9H_6N_4OS$(218.2)	71	80-82^{0}C	0.63
6	$C_{10}H_8N_4S$(216.2)	78	75-77^{0}C	0.70

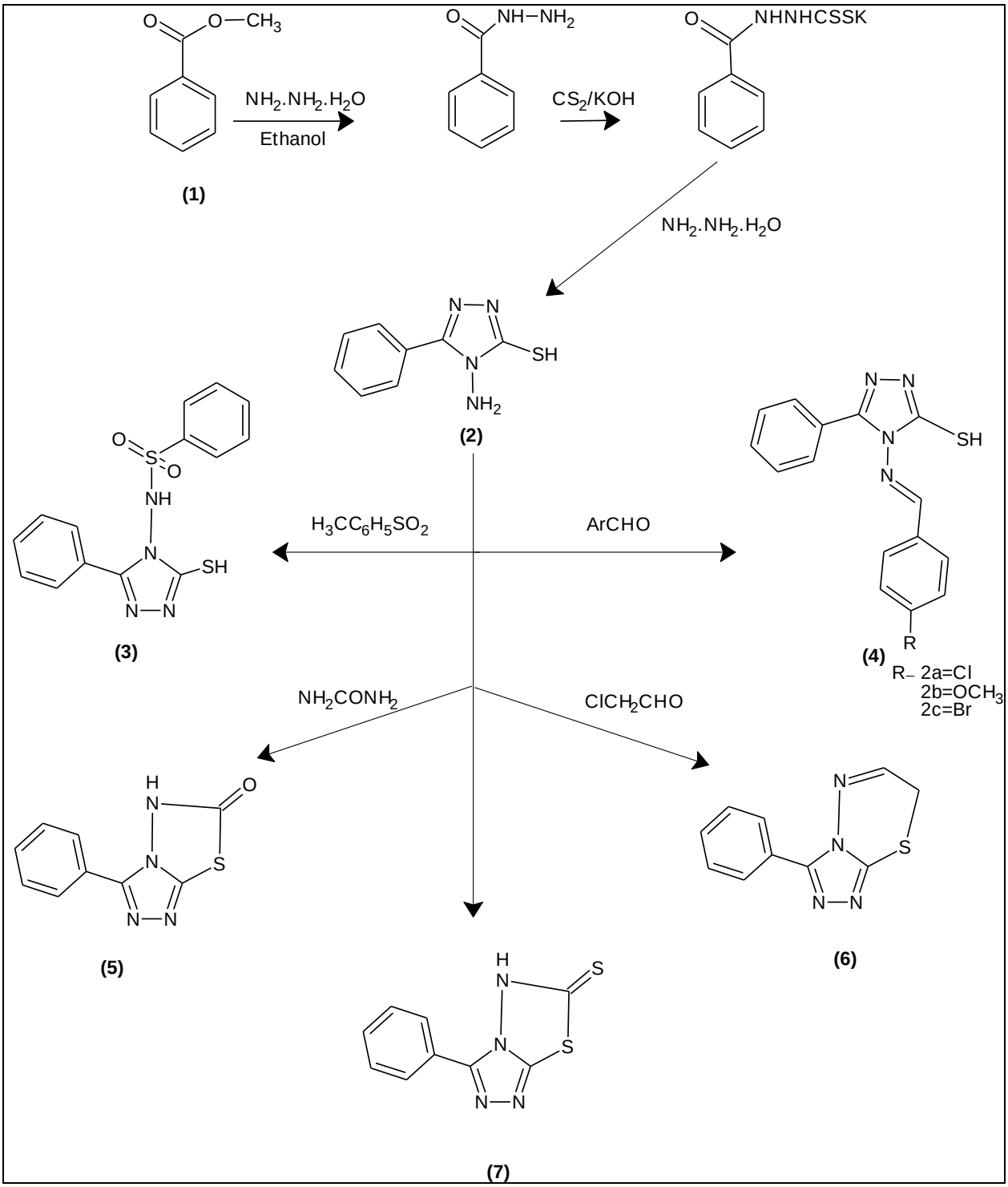

Figure 1: Synthetic scheme for triazole derivatives

Table No 2: Percentage inhibition of carrageenan induced rat paw edema exhibited by test and standard compounds

Group	**Dose (mg/kg Body wt)**	**1 HR Mean ml ± SE**	**2 HR Mean ml ± SE**	**3 HR Mean ml ± SE**	**4 HR Mean ml ± SE**	**% Inhibition (4^{th} Hr)**
Control	50mg/kg	1.31±0.021	1.41±0.0141	1.42±0.0353	1.43±0.0070	
Standard Ibuprofen	50mg/kg	0.71±0.0141	0.83±0.0083	0.91±0.0141	0.7±0.0053	46.1%
3 drug	50mg/kg	0.62±0.0353	0.62±0.0353	0.67±0.0353	0.67±0.0071	53%
4a drug	50mg/kg	0.72±0.0075	0.75±0.0063	0.74±0.0105	0.71±0.0082	50.3%
4b drug	50mg/kg	0.87±0.0707	0.91±0.0141	0.92±0.035	0.92±0.0093	35.6%
4c drug	50mg/kg	1.15±0.0087	1.25±0.0091	1.23±0.0078	1.1±0.014	23%
5 drug	50mg/kg	0.75±0.0082	0.80±0.0075	0.81±0.0072	0.72±0.0075	49.6%
6 drug	50mg/kg	0.85±0.0089	0.86±0.012	0.90±0.0105	0.86±0.0052	39.8%
7 drug	50mg/kg	0.92±0.0052	0.96±0.0061	0.97±0.0072	0.92±0.0081	35.6%

4. CONCLUSION

Physical data (M.P) and spectroscopic data (I.R, NMR) of the above synthesized compounds revealed their successful synthesis .Anti-inflammatory activity was screened using carrageenan induced paw edema method. The drugs at the oral dose of 50mg/kg showed good results and caused a significant inhibition in the carrageenan induced rat paw edema. Among the synthesized compounds, compound 3 was found to be most active and its inhibition in edema volume was noted to be 53% as compared to the standard drug, ibuprofen, which caused maximum inhibition of 46%.The second drug which showed a little lesser inhibition of 50.3% as compared to the first drug and the least inhibition was caused by 4c which showed only 23% inhibition. Thus from the overall findings it can be concluded that sulfonyl group plays an important role in anti-inflammatory activity as well as it can be seen that free sulfur groups display more activity than sulfur in a ring.

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