Asian Journal of Pharmaceutical and Clinical Research
Vol. 3, Issue 3, 2010 ISSN - 0974-2441

Research Article

SYNTHESIS OF SOME 3-SUBSTITUTED -4H-1, 2, 4-TRIAZOLE DERIVATIVES WITH POTENT ANTI-INFLAMMATORY ACTIVITY

PRADEEP K GOYAL*[1], ANIL BHANDARI[2], AC RANA[3], CB JAIN[4]

[1]B N Girls' College of Pharmacy, Udaipur, Rajasthan, India. E-mail: goel.pradeep@rediffmail.com
[2]Jodhpur Pharmacy College, Jodhpur, Rajasthan, India.
[3]Rayat Bahara College of Pharmacy, Ropad Campus, Chandigarh. Punjab, India.
[4]Rajasthan.Univ.of Health.Sci. , Jaipur

ABSTRACT

Some new derivatives of 3-substituted -4*H*-1, 2, 4- triazoles 4 (a-j) were synthesized through the reaction of 5-alkyl / aryl diazo substituted 4*H*-1, 2,4- triazoles- 3-thiole with different aliphatic and aromatic amines to yield the titled compounds. Structure of new compounds were verified on the basis of spectral (UV, IR, ^{1}H-NMR, MS) and elemental (C, H, N, S) analysis. All the synthesized compounds were evaluated for anti-inflammatory activities and acute toxicity. Most of the compounds showed potent and significant results compared to standard Ibuprofen. The cut off LD_{50} was > 500mg / Kg for each test compound when given orally.

Key words: Synthesis, 4H-1, 2, 4-triazole, anti-inflammatory, acute toxicity

INTRODUCTION

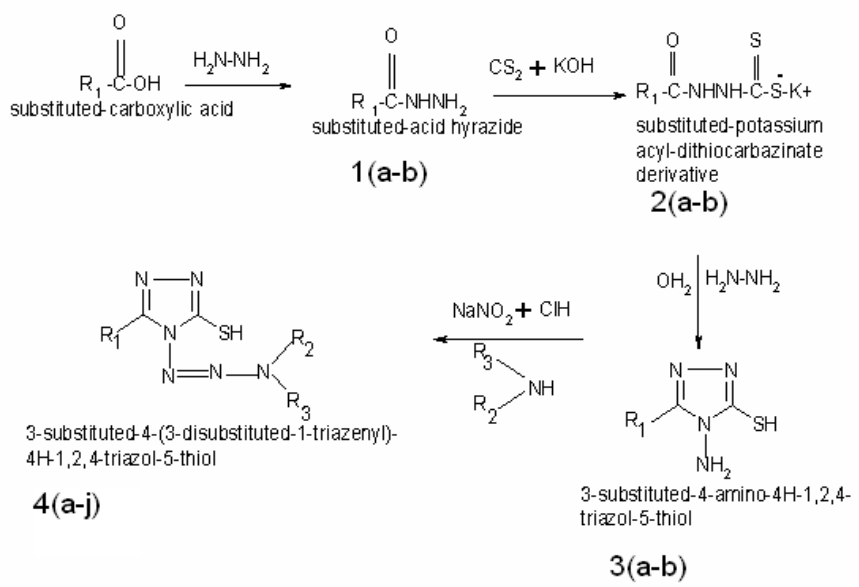
During the last few decades, a considerable attention has been devoted to synthesis of 1,2,4 – triazole derivatives possessing comprehensive bioactivities such as antimicrobial[1-3], anti-inflammatory[4], analgesic[5], antitumorial[6], antihypertensive[7], anticonvulsant[8,9] and antiviral activities[10]. The 1, 2, 4 – triazoles show such broad spectrum of biological activities, possibly due to the presence of >N-C-S moiety [11,12]. Therefore 5-mercapto-[1, 2, 4] -trizoles derivatives have found applications as antibacterials, antitumour and anti-inflammatory agents, pesticides and herbicides[13].

It was reported that the incorporation of various substituents and the halogen atom into the heterocyclic ring system augment the such biological activities considerably[14-16]. The imidazole derivative i.e. dacarbazine which contains amino-diazo chain exhibit antineoplastic and antibacterial activity. On the basis of above observations it was planned to prepare 4*H* – 1, 2, 4 – triazole derivatives substituted at position 1 by amino-diazo chain. The presence of this chain on triazole moiety was assumed to possess very potent antimicrobial, analgesic and anti-inflammatory activity.

MATERIALS AND METHODS

All the chemicals required were purchased from the local suppliers and were purified by established methods. The melting points were recorded by open capillary method and are uncorrected. The purity and homogeneity of the synthesized compounds was routinely ascertained by the thin layer chromatography, performed on plates coated with silica gel- G using the solvent system methanol: carbon tetrachloride: acetone in the ratio of 50: 40: 10. The absorption maxima of the synthesized compounds were recorded in methanol / ethanol (analytical grade, 1mg/100mL). The methanolic / ethanolic solutions of the synthesized compounds were scanned on Shimadzu UV 1700 spectrophotometer, Kyoto, Japan; in the region 200-400 nm. The IR spectra were recorded in KBr disc on FTIR Shimadzu 8400 S Japan. The ^{1}H-NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using *d*6-DMSO as solvent and TMS as an internal solvent. The Mass spectra were recorded on Waters Q –Toff-micro spectrometer. The C, H, N, S analyses were carried out on Vario H- III elemental analyzer. Anti-inflammatory activity evalution was carried out using carrageenan- induced paw edema in albino rats and compared with the group receiving a standard drug Ibuprofen.

SYNTHETIC SCHEME



Comp.	R_1	R_2	R_3	Comp.	R_1	R_2	R_3
4a	CH_3	H	C_6H_5	**4f--**	C_6H_5	H	C_6H_5
4b	CH_3	H	C_6H_4-NO_2-*p*	**4g**	C_6H_5	H	C_6H_4-NO_2-*p*
4c	CH_3	H	C_6H_4- CH_3-*o*	**4h**	C_6H_5	H	C_6H_4- CH_3-*o*
4d	CH_3	H	C_6H_4-OCH_3-*p*	**4i**	C_6H_5	H	C_6H_4-OCH_3-*p*
4e	CH_3	C_6H_5	C_6H_5	**4j**	C_6H_5	C_6H_5	C_6H_5

EXPERIMENTAL WORK

Synthesis [17-19]

Compounds 3(a-b) were synthesized by established methods and were obtained as per the reported yield. The compounds 3 (a-b) were diazotized as per the standard procedure. The corresponding solutions of diazotized product in 10% NaOH were prepared and cooled by addition of crushed ice. To the cooled preparations, cold solution of different amines in. 10% aq. NaOH were added and were stirred vigorously to obtain the titled compound 4 (a-j). All the synthesized compounds were recrystallized with rectified spirit. Good results (highest yields) were obtained when the diazotized compound and amines were taken in equimolar concentration. The physical constants of all the synthesized compounds are given in Table 1. The characterization data of all the synthesized compounds are follows:

4a : 3-methyl -4-[3-phenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol : λ_{max} 355nm (methanol) ; IR (KBr, V max, cm^{-1}): 3382.91 (-NH), 3203.54 (-CH, Ar), 1602.74 (-N=N), 1199.64 (-C=S,triazole), 750.26 (-CH),763.76 (out of plane, Ar, C-H), ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.03 [s, 3H, CH_3] ,4.67 [s, 1H, NH], 6.61-6.99 [m, 5H, Ar-ring], 11.41 [s, SH], MS: m/z 234 [M^+] ,

4b: 3- methyl -4-[3-(4-nitro phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol : λ_{max} 385nm (methanol) ; IR (KBr, V max, cm^{-1}): 3377.12 (-NH), 3271.05 (-CH, Ar), 1596.95 (-N=N), 1245.93 (-C=S,triazole), 754.12 (out of plane, Ar, C-H), ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.08 [s, 3H, CH_3] ,4.67 [s, 1H, NH], 6.54-6.57 [m, 4H, C_6H_4], 11.41 [s, SH], MS: m/z 278 [M^+]

4c: 3-methyl -4-[3-(2-methyl phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol : λ_{max} 356nm (ethanol) ;: IR (KBr, V max, cm^{-1}): 3386.77 (-NH), 2916.17 (-CH, Ar), 1600.81 (-N=N), 1147.57 (-C=S,triazole), 752.19 (out of plane, Ar, C-H), ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.06 [s, 6H, $2CH_3$] ,4.64 [s, 1H, NH], 6.58-6.62 [m, 4H, C_6H_4], 11.39 [s, SH], MS: m/z 248 [M^+]

4d: 3-methyl -4-[3-(4-methoxy phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol: λ_{max} 338nm (ethanol) ;: IR (KBr, V max, cm^{-1}): 3168.83 (-NH), 2995.25 (-CH, Ar), 1602.74 (-N=N), 1159.14 (-C=S,triazole), 754.12, 730.97 (out of plane, Ar, C-H), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 1.82 [s, 3H, -CH_3] ,2.02 [s, 3H, - OCH_3], 4.62[s,NH], 6.52-6.58 [m, 4H, -C_6H_4], ; MS: m/z 263 [M^+]

4e: 3-methyl -4-[3,3-diphenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol: λ_{max} 354nm (ethanol) ;: IR (KBr, V max, cm^{-1}): 3382.91 (-NH), 3041.53 (-CH, Ar), 1596.95 (-N=N), 1172.64 (-C=S,triazole), 744.47 (out of plane, Ar, C-H), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.11 [s, 3H, CH_3] ,6.62-6.98 [m, 10H, Ar-ring], MS: m/z 310 [M^+].

4f- : 3-phenyl -4-[3-phenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol ; λ_{max} 342nm (methanol) ;: IR (KBr, V max, cm^{-1}): 3438.84 (-NH), 3290.33 (-CH, Ar), 1639.38 (-N=N), 1161.07 (-C=S,triazole), 642.25,669.25 (out of plane, Ar, C-H,), ^{1}H-NMR (DMSO-*d*6, δ ppm): 4.52 [s, 1H, NH], 6.99-7.48 [m, 10H, Ar-ring], 11.60 [s, 1H,SH], ' MS: m/z 296 [M^+]

4g : 3-phenyl -4-[3-(4-nitro phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol ; λ_{max} 310nm (methanol) ;: IR (KBr, V max, cm^{-1}): 3465.84 (-NH), 2918.10 (-CH, Ar), 1589.23 (-N=N), 1164.92 (-C=S,triazole), 754.12 (out of plane, Ar, C-H,), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 4.54 [s, 1H, NH], 6.96-7.36 [m, 9H, Ar-ring], 11.61 [s, 1H,SH], ;' MS: m/z 341 [M^+].

4h : 3-phenyl -4-[3-(2-methyl phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol ; λ_{max} 328nm (ethanol) ;: IR (KBr, V max, cm^{-1}): 3363.62 (-NH), 3060.82 (-CH, Ar), 1596.95 (-N=N), 1157.21 (-C=S,triazole), 750.26,761.83 (out of plane, Ar, C-H,), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.06 [s, 3H, CH_3], 4.31 [S,1H, NH] , 6.74-7.12 [m, 9H, Ar-ring], 10.98 [s, 1H,SH], ;' MS: m/z 310 [M^+]

4i : 3-phenyl -4-[3-(4-methoxy phenyl)-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol ; λ_{max} 366nm (methanol) ;: IR (KBr, V max, cm^{-1}): 3421.48 (-NH), 2921.96 (-CH, Ar), 1602.74 (-N=N), 1176.5 (-C=S,triazole), 669.25 (out of plane, Ar, C-H,), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 2.16 [s, 3H, OCH_3], 4.07 [S,1H, NH] , 6.70-6.99 [m, 9H, Ar-ring], , ;' MS: m/z 326 [M^+].

4j : 3-phenyl -4-[3,3-diphenyl-1-triazenyl]-4*H*-1,2,4-triazole-5-thiol ; λ_{max} 356nm (ethanol) ;: IR (KBr, V max, cm^{-1}): 3382.91 (-NH), 3101.32 (-CH, Ar), 1596.95 (-N=N), 1172.64 (-C=S,triazole), 690.47,744.47 (out of plane, Ar, C-H,), ; ^{1}H-NMR (DMSO-*d*6, δ ppm): 6.77-7.14 , [m, 15H, Ar-ring] , 10.86 [S,1H, SH] , ;' MS: m/z 372 [M^+].

Table1: Physical Constants of all the synthesized derivatives 4 [a-j]

Comp	Mol. For [Wt.]	Yield [%]	M.P.(^{0}C)	R_f	C. %Cal. [%found]	H. %Cal.[%found]	N. %Cal.[%found]	S. %Cal.[%found]
4a	$C_9H_{10}N_6S$ [234]	31.11	111-14	0.58	46.15 [46.14]	4.27 [4.28]	35.89 [35.88]	13.67 [13.68]
4b	$C_9H_9N_7O_2S$ [279]	65.23	134-36	0.69	38.70 [38.68]	3.52 [3.56]	35.12 [35.14]	11.46 [11.48]
4c	$C_{10}H_{12}N_6S$ [248]	31.47	152-54	0.64	48.38 [48.34]	4.83 [4.85]	33.87 [33.85]	12.90 [12.94]
4d	$C_{10}H_{12}N_6OS$ [264]	70.93	212-15	0.62	45.45 [45.46]	4.54 [4.56]	31.81 [31.85]	12.12 [12.08]
4e	$C_{15}H_{14}N_6S$ [310]	73.82	256-58	0.74	58.06 [58.06]	4.51[4.53]	27.09 [27.07]	10.32 [10.33]
4f	$C_{14}H_{12}N_6S$ [296]	69.42	208-11	0.56	56.75[56.74]	4.05[4.03]	28.73[28.72]	10.81[10.83]
4g	$C_{14}H_{11}N_7SO_2$ [341]	28.98	274 -76	0.72	49.26[49.24]	3.22[3.24]	28.73[28.72]	9.38[9.36]
4h	$C_{15}H_{14}N_6S$ [310]	23.99	253-55	0.61	58.06[58.07]	4.57[4.50]	27.09[27.08]	10.32[10.34]
4i	$C_{15}H_{14}N_6SO$ [326]	19.15	284-86	0.74	55.21[55.23]	4.29[4.27]	25.76[25.72]	9.83[9.69]
4j	$C_{20}H_{16}N_6S$ [372]	57.34	277-79	0.48	64.57[64.54]	4.30[4.31]	22.58[22.56]	8.60[8.64]

ANTI-INFLAMMATORY ACTIVITY [20-22]

The anti-inflammatory activity of the standard drug Ibuprofen and synthesized compounds 4 (a-j) was determined against carrageenan induced acute paw edema in albino rats (72 no. weighing 200-225g). The dose, of standard drug and synthesized compounds administered in animals, was 50 mg/ kg by oral route using oral feeding tube through tuberculin syringe. The stock suspensions of standard and synthesized compound were prepared in concentration of 10 mg/ml of 2% w/v CMC in distilled water. The control group was administered with normal saline (0.9% w/v NaCl) (2.5ml/kg) orally and other groups with respective drug suspension in CMC as per body weight. After 30 minute, 0.1 ml of 1% w/v solution of carrageenan in normal saline was injected in the planter region of left paw of the rats. The right paw served as reference non-

inflammed paw for comparison.

The paw volume of both the legs of rats treated with control, standard and test compounds were recorded by mercury displacement method using plethysmograph, at 30, 60 and 120 min after carrageenan challenge. Percent inhibition of the edema between the control group and compound treated groups was calculated and 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.

Table 2: Percentage inhibition of carrageenan- induced rat paw edema after , exhibited by the Test and Standard compounds

Comp. No.	Dose (mg/kg, p.o)	% Inhibition of carrageenan-induced rat paw edema at		
		0.5 hr	1.0 hr	2.0 hr
Control (N/saline)	2.5 ml/kg	-	-	-
4a	50 mg/kg	93.82	84.42	90.69
4b	50 mg/kg	19.75	38.52	19.18
4c	50 mg/kg	65.43	78.68	92.44
4d	50 mg/kg	79.01	80.32	74.16
4e	50 mg/kg	80.02	63.11	38.37
4f	50 mg/kg	98.76	86.88	94.18
4g	50 mg/kg	38.27	36.06	65.69
4h	50 mg/kg	76.54	83.60	100.0
4i	50 mg/kg	81.48	65.57	87.20
4j	50 mg/kg	25.92	88.52	84.88
Std. (Ibuprofen)	50 mg/kg	62.96	81.96	88.37

Acute toxicity studies (for all synthesized compounds) were conducted using OECD guidelines No. 423 published in the year 2000. The method is based upon a stepwise procedure with the use of minimum number of animals per step. The animals (albino mice 20-25g) were randomly group housed with three per cage and the test drugs administered orally at a dose level of 500 mg/kg.

RESULTS AND DISCUSSION

The physical constants and characterization data of all the synthesized compounds reveals their successful synthesis. Anti-inflammatory activity of all the synthesized compounds 4(a-j) was screened by carrageenan- induced rat paw edema method against the standard drug Ibuprofen. This activity was evaluated for the period of 2h, after the carrageenan challenge, in the time intervals of 0.5, 1.0 and 2.0h.

After 0.5h, the compounds 4c, 4d, 4e and 4i showed 65.43, 79.01, 80.02 and 81.48% edema inhibition in comparison to 62.96% edema inhibition shown by the standard drug Ibuprofen. The data indicate the less onset of action and comparable activity of these compounds. At 1.0h of time interval the compounds 4c, 4d, 4e, 4i and 4j exhibited 78.68, 80.32, 63.11, 65.57 and 88.52 % edema inhibition respectively in comparison to 81.96% edema inhibition shown by the standard drug.

The standard drug Ibuprofen showed the maximum anti-inflammatory activity (88.37 % edema inhibition) after 2.0h. Comparable activity at this time interval was exhibited by the compounds 4c- 92.44%, 4d- 74.16%, 4i -87.20% and 4j- 84.88% edema inhibition.

The result showed that in the time intervals of 0.5, 1.0 and 2.0h, the compounds 4a, 4f and 4h have highest anti-inflammatory activity i.e. more than the standard drug . Therefore from the overall findings it can be concluded that the titled compounds substituted either with phenyl ring or phenyl rings substituted with strong electron releasing groups (e.g. C_6H_5, C_6H_4- CH_3-*o*) showed excellent anti-inflammatory activity.

Acute Toxicity

All the synthesized derivatives ten compounds viz. 4(a-j) were tested for acute toxicity study. None of the test compound, at a limit test one dose level for a dose 500 mg/kg, showed any mortality. No mortality, no body weight changes, no toxic signs were noticed during the 14 days period of observation. Thus the cut off LD_{50} was > 500 mg/kg for each test compound when given orally.

ACKNOWLEDGEMENT

Authors are grateful to the Director, SAIF, CIL, Punjab University, Chandigarh for providing NMR, MASS spectral facilities and to the Head, SAIF, CDRI, Lucknow for C, H, N, S analysis of the synthesized compounds.

REFERENCES

1. Holla B S, Gonsalves R & Shenoy S, Farmaco, 1998; 53: 574.
2. Ersan S, Nacak S & Bercem R, Farmaco, 1998; 53: 773.
3. Gulerman N N, Dogan H N, Rollas S & Johansson C, Elik C C, Farmaco, 2001; 56: 953.
4. Maxwell J R, Wasdahl D A & Wolfson A C, J Med Chem. 1994; 27(12): 1565.
5. Turan-Zitouni G, Kapalancikli Z A, Erol K & Kilic F S, Farmaco, 1999; 54: 218.
6. Demirbas N & Demirbas U A, Bioorg, Med Chem, 2002; 10: 3717.
7. Paulvannan K, Chen T & Hale R, Tetrahedron, 2000; 56: 8071.
8. Saksena R S & Yasmeen R, Indian J Chem., 1987; 26B: 290.
9. Husan M I, Amir M & Singh E, Indian J Chem, 1987; 26B: 290.
10. Kritsanida M, Mouroutsou A, Marakos P, Pouli, Npapakonstantinou-Garoufalias S, Pannecouque C, Witvrouw M & Clercq D B, II Farmaco, 2002; 57: 253.
11. Holla B S, Poorjary K N, Rao B S & Shivananda M K, Eur J Med Chem, 2002; 37: 511.
12. Omar A, Mohsen M E & Wafa O M, J Heterocycl Chem, 1986; 23: 1339.
13. Li X, Si Z X, Pesticides 2003; 42(1): 4.
14. Mohan R R, Agarwal R & Mirsa V S, Indian J Chem 1985; 24B: 78.
15. Dubey V S & Ingle V S, Indian J Chem Soc 1989; 66: 174.
16. Bayer H O, Cook R S & Von Meyer W C, S Afr Patent, 7 004 373 (1971), Chemistry Abstract 1972; 76: 113224.
17. Reid J R and Heindel N D, j. heterocyclic chem 1976; vol. 13 issue 4: 925.
18. Furniss BS et al, Vogel's, Textbook of Practical Organic Chemistry, V Edition, Pearson Education, 952.
19. Aceites G Y, Synthesis, Antibacterial and Surface Activity of 1, 2, 4-triazole Derivatives, Abril-Junio 2006; 57 (2): 180-188.
20. Winter CA et al., Proc. Soc. Exp. Biol. 1962; 111: 544.
21. Kulkarni SK et al., Arch. Int. Pharmacodyn. 1986; 279: 324.
22. Chakraborty A. et al., Indian J. Pharmacol. 2004; 36: 148.