www.sphinxsai.com

International Journal of ChemTech Research
CODEN(USA): IJCRGG ISSN : 0974-4290
Vol.2, No.4, pp 1992-1997, Oct-Dec 2010

Synthesis, Characterization and Analgesic Activity of some 4H-1, 2, 4-Triazole Derivatives

P. K. Goyal*[1], A. Bhandari[2], A. C. Rana[3] ,C.B.Jain

[1]B N Girls' College of Pharmacy, Udaipur (Raj.) , India

[2]Jodhpur Pharmacy College, Jodhpur (Raj.) ,India

[3]Rayat Bahara College of Pharmacy, Ropad Campus, Chandigarh , India

[4]Rajasthan Univ. of Health Sciences, Jaipur , India

**Corres.author: goel.pradeep@rediffmail.com*
Mobile: 09414871652

ABSTRACT: The present work is concern with the synthesis of different 3-substituted -4*H*-1, 2, 4- triazoles **4 (a-j)** with the objective of discovering novel and potent analgesic agents. Structures of the synthesized compounds were elucidated by spectral and elemental analysis. The obtained compounds were evaluated for their analgesic activity using hot plate method. Result showed that among the series of tested compounds, the compound 4d & 4e exhibit higher activity than the standard drug (aspirin). The over all study reveals that the titled compounds substituted either with phenyl ring or phenyl rings substituted with strong electron releasing groups showed moderate to excellent analgesic activity. The LD_{50} was determined on mice by injecting increasing dose of test compounds (> 500mg / Kg)
KEY WORDS: Synthesis, 4H-1, 2, 4-triazole, analgesic, acute toxicity.

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs), which are widely used for reducing pain and swelling associated with inflammation, represent area of continuous and ever growing development. Therefore 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 shows broad spectrum of biological activities, possibly due to the presence of >N-C-S moiety [11,12]. Therefore 5-mercapto-[1, 2, 4] - triazole 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 [14-16]. On the basis of above observations and as a part of our program aimed at developing new biologically active compounds, in this work we report the synthesis of 3-substituted -4*H*-1, 2, 4- triazoles (as shown in the scheme) having potent analgesic activity.

MATERIAL AND METHOD: The purity and homogeneity of the synthesized compounds was routinely ascertained by the thin layer chromatography (TLC), carried out on silica gel (MN-Kieselger G., 0.2 mm thickness) and spots were located in iodine chamber. 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 IR

spectra (KBr in cm^{-1}) were recorded on FTIR spectrophotometer (Shimadzu 8400 S Japan). The ^{1}H-NMR spectra were recorded on Bruker Avance II 400 NMR spectrometer using DMSO- *d*6 as solvent and TMS as an internal standard (chemical shift in δ ppm). The Mass spectra of compounds were determined with Waters Q –Toff-micro spectrometer. The alcoholic solution of the synthesized compounds (analytical grade, 1mg/100mL) was scanned on UV spectrophotometer (Shimadzu 1700, Kyoto, Japan) in the region of 200-400 nm. The elemental analyses were carried out on model Vario H- III C, H, N analyzer. The analgesic activity of all synthesized compounds **4(a-j)** was evaluated using hot plate method and compared with the standard drug Aspirin.

EXPERIMENTAL WORK:

SYNTHESIS[17- 19] : The carboxylic acid hydrazides **1(a-b)** were synthesized by the reaction of acetic acid or benzoic acid with hydrazine hydrate (99%) in presence of ethanol. These acid hydrazides were condensed with carbondisulphide in ethanolic potassium hydroxide for 12-16 hours at room temprature to yield the potassium -3-acyl-dithiocarbazates salt **2(a-b).** The k- salts are cyclized with hydrazine to 3-substituted -4-amino-4*H*-1, 2, 4-triazoles-5-thiol **3 (a-b).** The compounds 3 (a-b) were diazotized with sodium nitrite as per the standard procedure. The corresponding solutions of diazotized product in 10% NaOH were prepared and cooled by addition of crushed ice. To this cooled preparations, the cold solution of different amines in. 10% aq. NaOH were added and 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 yield, m.p., mol.formula, R_f-value and elemental analysis of all the synthesized compounds are given in **Table 1.**

The **characterization** data of all the synthesized compounds are as 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^+].

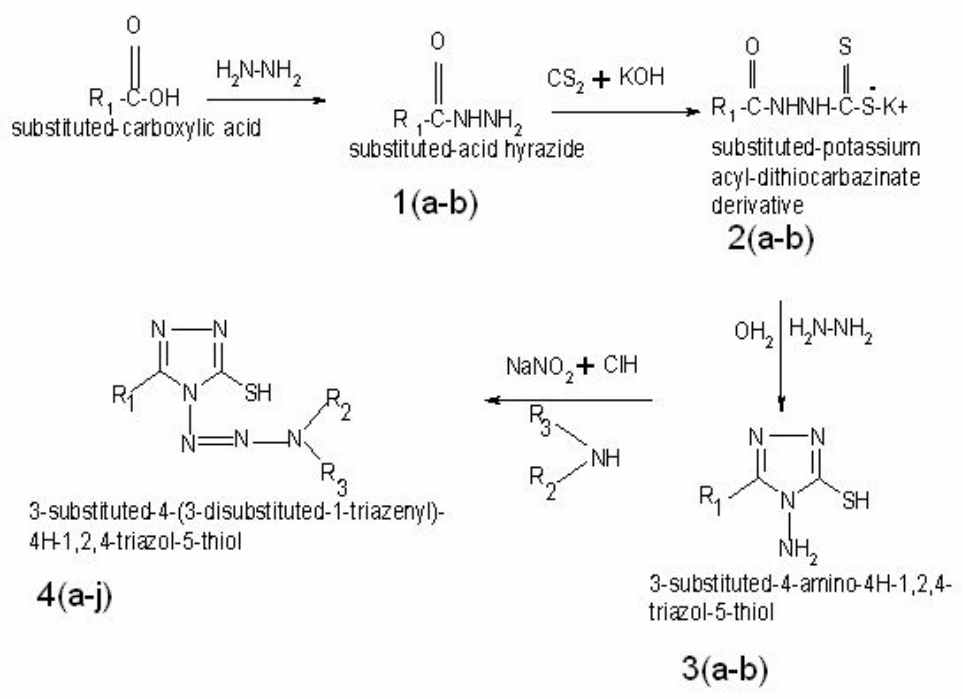
ANALGESIC ACTIVITY[20-22]:

The analgesic activity of all the test compounds was evaluated by using hot plate method and the instrument used for this purpose was Eddy's hot plate.

The albino mice (55 no., weighing 20-25 g) were divided into eleven groups with five animals per cage. The first group was for standard drug (Aspirin) and rest of ten groups were for the synthesized compounds. The solutions of the test compounds were prepared in carboxy methyl cellulose (CMC) (2% w/v).

The test and standard compound were administered orally at dose of 50 mg/kg. The basal reaction time, for jump response, when animals placed on hot plate (maintained at constant temperature of 55^0C) was observed and reaction time of animals on hot plate at 0, 0.5, 1.0 and 2.0 hour, after administration of the test and standard compounds, was also noted. The percent increase in reaction time (as an index of analgesia) at each time interval was calculated and reported in **Table 2**. Comparison of the analgesic activities exhibited by the test and standard drug is shown in **graph 1**.

SYNTHETIC SCHEME:



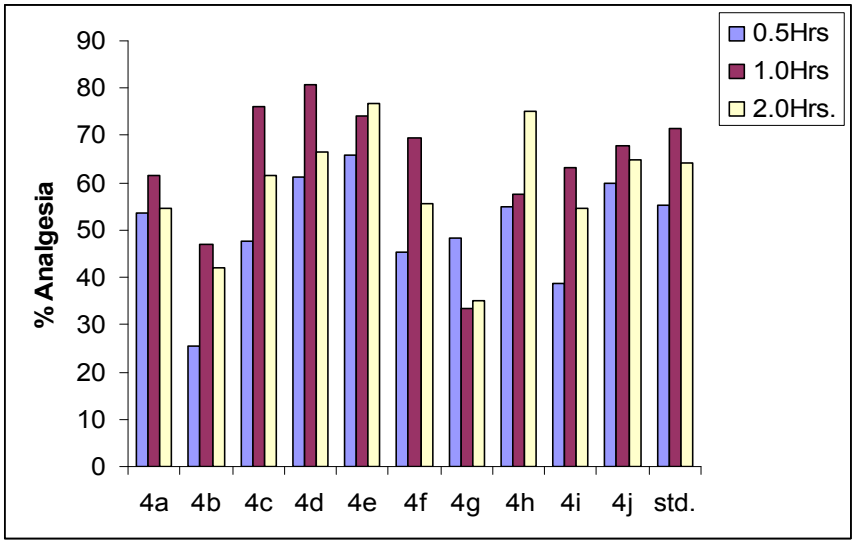
Comp.	R_1	R_2	R_3	Comp.	R_1	R_2	R_3
4a	CH_3	H	C_6H_5	**4f--**	C_6H_5	H	C_6H_5
4b	CH_3	H	C_6H_4-NO_2-*p*	**4g**	C_6H_5	H	C_6H_4-NO_2-*p*
4c	CH_3	H	C_6H_4- CH_3-*o*	**4h**	C_6H_5	H	C_6H_4- CH_3-*o*
4d	CH_3	H	C_6H_4-OCH_3-*p*	**4i**	C_6H_5	H	C_6H_4-OCH_3-*p*
4e	CH_3	C_6H_5	C_6H_5	**4j**	C_6H_5	C_6H_5	C_6H_5

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

Comp.	Mol.For. [Wt.]	Yield [%]	M.P. (^0C)	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	$C_9H_9\ N_7\ O_2S$ [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	$C_{14}H_{11}\ N_7\ S\ O_2$ [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]

Table 2: Percent analgesia of the test and standard drug with mean and sem Values

Compound no.	Dose (Mg/kg, *p.o.*)	% Analgesia (Mean ± SEM) after		
		0.5 h	1.0 h	2.0 h
4a	50	53.44 ± 1.80	61.68 ± 2.17	54.58 ± 2.76
4b	50	25.42 ± 2.60	47.04 ± 3.00	42.0 ± 2.96
4c	50	47.58 ± 2.74	75.94 ± 1.08	61.70 ± 3.17
4d	50	61.10 ± 1.12	80.74 ± 1.01	66.46 ± 1.00
4e	50	65.86 ± 2.53	74.26 ± 1.99	76.90 ± 4.61
4f	50	45.24 ± 3.20	69.60 ± 2.00	55.5± 4.63
4g	50	48.3 ± 2.65	33.36 ± 1.32	35.14 ± 1.42
4h	50	54.78 ± 1.70	57.56 ± 2.45	74.96 ± 3.00
4i	50	38.76 ± 2.12	63.14 ± 2.98	54.74± 3.82
4j	50	59.84 ± 2.24	67.96 ± 2.32	64.70 ± 2.52
Std. (Aspirin)	50	55.40 ± 1.65	71.36 ± 1.08	64.04 ± 1.93



Graph 1: Comparison of the analgesic activity exhibited by the test and standard compounds at time interval of 0.5, 1.0 and 2.0 hrs.

RESULT AND DISCUSSION:

All the synthesized compounds **4(a-j)** were evaluated for analgesic activity to observe the effects of electron releasing/ electron withdrawing groups present on C_4 and C_5 of triazole ring. The analgesic activity of the tested compounds was evaluated at 0.5, 1 .0 and 2.0 hour of time interval.

At 0.5h of time interval, the compounds 4a, 4h and 4j showed short onset of action and the activity is comparable with the standard drug (aspirin). At 1.0h of time interval the compounds 4c, 4f and 4j exhibit 75.94, 69.60 and 67.96 % analgesia respectively as compared to 71.36% analgesia exhibited by the standard drug. Observation of the structures of 4f and 4j revealed the presence of phenyl ring at C_5 and triazenyl ring (substituted with $-C_6H_5$) at C_4 of triazole nucleus where as the compound 4c has $-CH_3$ group at C_5 and substituted phenyl ring at triazenyl nucleus of C_4. At 2.0 h of time interval the compounds 4h and 4j showed 74.96 and 64.70 % analgesia in comparison to 64.04 % analgesia shown by the standard compound.

In overall, the compounds 4d and 4e exhibit highest analgesic activity i.e. more than the standard drug (Aspirin) where as compounds 4b and 4g have lower activity than Aspirin. On the basis of above observations it is assumed that, among the series of tested derivatives, the analgesic activity was exhibited excellently by the compounds substituted either with phenyl ring or phenyl rings substituted with strong electron releasing groups where as the compounds substituted with electron withdrawing groups have moderate analgesic activity.

Acute toxicity: The median lethal dose (LD_{50}) for each test compound when given orally, was (determined in mice) found to be > 500 mg/kg.

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 B.S. 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. Eddy N.B. and Leimbach D.J., J. Pharmacol. Expt. Therap., 1953, 107, 385.
21. Kulkarni S.K., Handbook of Experimental Pharmacology, 3rd Edn., Vallabh Prakashan, 1999, 125.
22. Ghosh, M.N., Evaluation of Analgesic Agents, Fundamentals of Experimental Pharmacology, 2nd Edn., Scientific Book Agency, Calcutta, 1984.

*****