Syntheses, characterization and antimicrobial screening of some novel 3, 5-diaryl-4H-1, 2, 4-triazole derivatives

Ram Janam Singh[a*]
[*]*Quality Control Laboratory, Indian Oil Corporation Limited, Panipat Refinery Panipat, Haryana (India)*
[a]*Synthetic Research Laboratory, Department of Chemistry, Faculty of Science, Tilak Dhari Post Graduate College, Jaunpur (India)*

Abstract

The syntheses of a series of 3, 5-diaryl-4H-1, 2, 4-triazole derivatives are described. A total of six new compounds were synthesized and characterized by IR, PMR, MS spectral data and elemental analyses. All newly synthesized compounds were screened for their antibacterial activity against *S. aureus, E. coli, B. subtilis and P. aeruginosa*. Compounds containing aryl substituents at position six and the 1, 2, 4- triazole moiety at position one or two showed reasonable antibacterial activity.

Keywords: 1, 2, 4-triazole, ethylbenzoatebenzoylhydrazone, hydrazine hydrate, antibacterial activity.

Introduction

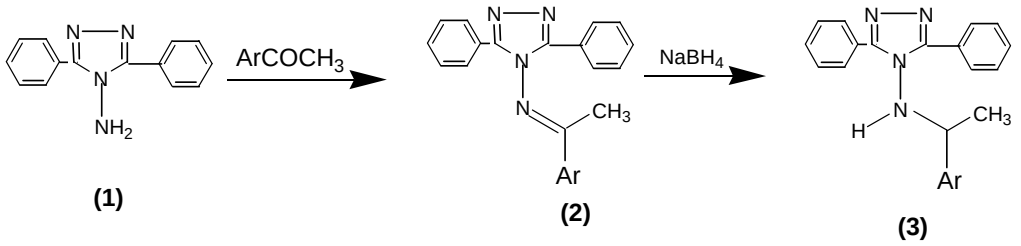
The chemistry of heterocyclic compounds continues to be an active field in the organic chemistry. Triazole- derivatives have occupied a unique position in heterocyclic chemistry due to their antimicrobial activities [1-2].

1, 2, 4- Triazoles exhibit a wide range of therapeutical properties [3]. The syntheses of 1,2,4-triazoles has also attracted wide spread attention due to the diverse agricultural, industrial and biological activities, including anti-inflammatory, analgesic, antitumoral, anticonvulsant and tranquilizing activities shown by these compounds. In view of these observations and in continuation of our earlier work [4-17] on the syntheses of some 1,2,4- and 1,2,3- triazole derivatives, we now report the synthesis of some novel 1,2,4-triazole derivatives derived from 4-amino-3,5-diphenyl-4H-1,2,4-triazole.

Results & Discussion

4-amino-3,5-diphenyl-4H-1,2,4-triazole **1** which is required as starting material were obtained in an one-pot reaction by heating ethylbenzoatebenzoylhydrazone, hydrazine hydrate and 1-propanol, under reflux conditions for 30 hours. The reaction mixture is cooled, washed with benzene, insoluble part in benzene was crystallized from 1-propanol to afford the compound **1** (88%). Compounds **2a-c** were prepared by the condensation of compound **1** with various ketones in acetic acid. During the reduction of compounds **2a-c**, the formation of multiple products was possible due to the possibility of the reduction of hetero ring.

However, the reduction was performed on the imino group of **3a-c** without affecting the hetero ring by using $NaBH_4$ as selective reducing agent. **Scheme 1**



(1) **(2)** **(3)**

Compound		Ar		
2, 3	a	Phenyl	b	4-Methoxyphenyl
	c	4 - Nitrophenyl		

Scheme 1

The IR spectrum of the compound **2a-c** showed C=N characteristic absorption bands between 1608-1566 region. The PMR spectrum of **2a-c** exhibits a singlet characteristic signals at δ 1.85-1.94 (3H, s, CH_3) and mass spectra of **2a-c** showed molecular ion peaks at m/z 294 (M^+) in confirmity with the assigned molecular formulae.

The IR spectrum of the compound **3a-c** showed NH characteristic absorption bands between 3295-3212 region. The PMR spectrum of **3a-c** exhibits doublet characteristic signals at δ 7.22-7.42 (1H, d, NH) and a multiplet at 3.75-3.85 (1H, m, CH) and mass spectra of **3a-c** showed molecular ion peaks at m/z 296 (M^+) in confirmity with the assigned molecular formulae.

These compounds were tested against various bacterial strains and the details are provided in the experimental section. All synthesized compounds showed reasonable activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*.

Materials and Methods

Melting and boiling points were determined on a Gallen Kamp apparatus in open capillaries and are uncorrected. IR spectra (KBr in cm^{-1}) were recorded on a Jasco FT-IR 5300 spectrophotometer and proton magnetic resonance (PMR) spectra (DMSO-d6) on a Varian EM-390 spectrometer using TMS as an internal standard (chemical shift in δ ppm). Mass spectra were recorded on a Jeol JMS-D 300Mass spectrometer operating at 70eV.The purity of the compounds was confirmed by TLC using silica gel G and purified by column chromatography. For TLC, Merck silica gel 60G plate was used. For column chromatography, Merck silica gel 60 (0.063-0.200mm) was used. The necessary chemicals were obtained from Merck and Fluka. All compounds showed satisfactory elemental analyses.

4-Amino-3, 5-diphenyl-4H-1, 2, 4-triazole (1)

Ethylbenzoatebenzoylhydrazone (0.01 mol) was reacted with hydrazine hydrate (0.01 mol) in the presence of 1-propanol (50 mL); the reaction mixture was refluxed for a period of 30 hours. After cooling, the precipitate was filtered and dried. The dried product was washed with 30 mL of benzene. The insoluble part in benzene was recrystallized from 1-propanol to afford the compound 4-amino-3, 5-diphenyl-4H-1, 2, 4-triazole **(1)** and was used directly for the next step without further purification (yield 88%).

General procedure for compounds (2a-c)
A suspension of compound **1** (0.01 mol), corresponding ketones (0.01 mol) and glacial acetic acid (30mL) was heated under reflux for 30 hours. After cooling, the mixture was poured in to a beaker containing 100mL of ice-water and the precipitate was filtered and dried. The dried product was recrystallized from an appropriate solvent to give the desired compounds (yield 75%).

3, 5-Diphenyl-4-[1-phenylethylidenamino)-4H-1, 2, 4-triazole (2a)
Recrystallized from ethyl acetate, (yield 75%). Mp 205 ^{0}C. IR (KBr): 1608, 1566 (C=N) and 759 cm^{-1} (monosubstituted benzene). PMR: δ 1.87 (3H, s, CH_3), 7.45-7.68 (9H, m, Ar-H), 7.77-7.91(4H, m, Ar-H), and 8.05 ppm (2H, d, Ar-H). MS: m/z 294 (M^+) other peaks observed at 159, 143, 131, 114, 97, 87, 53, 51 and 48. Anal.Calc. for $C_{22}H_{18}N_4$, C, 62.22; H, 2.33; N, 26.09 %; Found C, 61.69; H, 3.22; N, 25.98%.

3, 5-Diphenyl-4-[1-(4-methoxyphenyl) ethylidenamino]-4H-1, 2, 4-triazole (2b)
Recrystallized from ethanol, (yield 75%). Mp 192 ^{0}C. IR (KBr): 1605, 1598 (C=N) and 761 cm^{-1} (monosubstituted benzene). PMR: δ 1.85 (3H, s, CH_3), 3.86 (3H, s, OCH_3), 7.09 (2H, d, Ar-H), 7.31-7.62 (6H, m, Ar-H), 7.81-8.02 (4H, m, Ar-H) and 8.05 ppm (2H, d, Ar-H). MS: m/z 294 (M^+) other peaks observed at 169, 149, 133, 114, 96, 88, 55, 52 and 47. Anal.Calc. for $C_{23}H_{20}N_4O$, C, 60.36; H, 2.45; N, 24.89 %; Found C, 60.19; H, 2.96; N, 24.49%.

3, 5-Diphenyl-4-[1-(4-nitrophenyl) ethylidenamino]-4H-1, 2, 4-triazole (2c)
Recrystallized from ethanol, (yield 75%). Mp 220 ^{0}C. IR (KBr): 1603, 1582 (C=N) and 763 cm^{-1} (monosubstituted benzene). PMR: δ 1.94 (3H, s, CH_3), 7.41-7.62 (6H, m, Ar-H), 7.71-7.92 (4H, m, Ar-H), 8.26 (2H, d, Ar-H) and 8.41ppm (2H, d, Ar-H). MS: m/z 294 (M^+) other peaks observed at 167, 141, 136, 120, 89, 77, 55, 49 and 45. Anal.Calc. for $C_{22}H_{17}N_5O_2$, C, 60.36; H, 2.45; N, 24.89 %; Found C, 60.19; H, 2.96; N, 24.49%.

General procedure for compounds (3a-c)
A suspension of compound **2a-c** (0.01 mol), methanol (50 mL) and $NaBH_4$ (0.01 mol) was heated under reflux for 1.5 hours and then allowed to cool. After concentration at 25 ^{0}C under reduced pressure, the crude solid was washed with water, filtered and dried. The dried product was recrystallized from an appropriate solvent to give the desired compounds (yield 70-85%).

3, 5-Diphenyl-4-(1-phenylethylamino)-4H-1, 2, 4-triazole (3a)
Recrystallized from ethyl acetate, (yield 85%). Mp 215^{0}C. IR (KBr): 3295 (NH), 1534 (C=N) and 760 cm^{-1} (monosubstituted benzene). PMR: δ 1.09 (3H, d, CH_3), 3.76 (1H, m, CH), 7.25 (1H, d, NH), 6.71(2H, d, Ar-H), 7.01-7.21(3H, m, Ar-H), 7.51-7.71 (6H, m, Ar-H) and 7.81-8.16 ppm (4H, m, Ar-H). MS: m/z 296 (M^+) other peaks observed at 144, 129, 122, 123, 88, 77, 66, 53 and 44. Anal.Calc. for $C_{22}H_{20}N_4$, C, 60.22; H, 2.23; N, 25.09 %; Found C, 60.19; H, 2.60; N, 24.48%.

3, 5-Diphenyl-4-[1-(4-methoxyphenyl) ethylamino)-4H-1, 2, 4-triazole (3b)
Recrystallized from ethyl acetate, (yield 80%). Mp 162^{0}C. IR (KBr): 3212 (NH), 1612(C=N) and 761 cm^{-1} (monosubstituted benzene). PMR: δ 1.09 (3H, d, CH_3), 3.51 (3H, s, OCH_3), 3.76 (1H, m, CH), 7.22(1H, d, NH), 6.71(2H, d, Ar-H), 7.04(2H, d, Ar-H), 7.41-7.62 (6H, m, Ar-H) and 7.81-8.02 ppm (4H, m, Ar-H). MS: m/z 296 (M^+) other peaks observed at 145,

132, 124, 121, 87, 77, 67, 55 and 42. Anal.Calc. for $C_{23}H_{22}N_4O$, C, 60.02; H, 2.92; N, 24.72 %; Found C, 59.96; H, 2.82; N, 24.82%.

3, 5-Diphenyl-4-[1-(4-nitrophenyl) ethylamino)-4H-1, 2, 4-triazole (3c)
Recrystallized from ethyl acetate, (yield 70%).Mp 222^0C. IR (KBr): 3222 (NH), 1605 (C=N) and 762 cm^{-1} (monosubstituted benzene). PMR: δ 1.06 (3H,d, CH_3), 3.85 (1H, m, CH), 7.42 (1H, d, NH), 6.79-7.05 (2H, m, Ar-H), 7.49 (2H, m, Ar-H), 7.49-7.71 (5H, m, Ar-H), 7.71-7.92 (3H, m, Ar-H) and 7.92-8.10 ppm (2H, m, Ar-H). MS: m/z 296 (M^+) other peaks observed at 147, 133, 126, 119, 85, 75, 66, 54 and 44. Anal.Calc. for $C_{22}H_{19}N_5O_2$, C, 60.42; H, 2.83; N, 25.39 %; Found C, 59.89; H, 2.70; N, 24.54%.

Antibacterial activity
The antibacterial activity of six compounds **(2a-c & 3a-c)**, was investigated by employing the filter paper disc method [18-20]. Representative organisms selected for evaluation of antibacterial activity were *S. aureus, E. coli, B. subtilis* and *P. aeruginosa.* The antibacterial activity of each of the compounds was evaluated in triplicate at 100 µg $mL^{-1}$and 10 µg mL^{-1} concentrations. The compounds were tested as a solution or suspension in DMF (99.80 % anhydrous). An important and useful control drug Ampicillin was also tested under similar conditions, with view to compare the results.
The result indicates that all of the synthesized compounds showed moderate to strong activity against these bacterial strains. All compounds showed good activity against *S. aureus, E. coli, B. subtilis and P. aeruginosa*. From the above observation it is clear that the 3,5-driaryl-4H-1,2,4-triazole derivatives are more active and also the substituents phenyl, 4-methoxyphenyl and 4-nitrophenyl in 3,5-driaryl-4H-1,2,4-triazole derivatives plays a prominent role in the antimicrobial screening. The evaluations of antibacterial activity of the synthesized compounds are shown in Table 1.

Table 1 Evaluation of antibacterial activity of the compounds

Average zone of Inhibition/mm								
Compd.	***S. aureus***		***E. coli***		***B. subtilis***		***P.aeruginosa***	
	100 µg L^{-1}	10 µg mL^{-1}	100 µg mL^{-1}	10 µg mL^{-1}	100 µg mL^{-1}	10 µg mL^{-1}	100 µg mL^{-1}	10 µg mL^{-1}
2a	17	15	15	14	16	14	17	15
2b	16	15	15	14	15	13	20	18
2c	21	19	16	14	14	13	14	14
3a	17	15	16	13	15	14	15	13
3b	14	13	13	13	14	12	14	12
3c	18	16	15	15	14	13	15	14
Standard (Ampicillin)	27	21	25	19	23	19	23	19
Control	00	00	00	00	00	00	00	00

Conclusion
We have described an easy procedure for the preparation of some 3, 5-Driaryl-4H-1, 2, 4-triazole derivatives from 4-amino-3, 5-diphenyl-4H-1, 2, 4-triazole and its antibacterial activity. All these compounds containing 1, 2, 4-triazole moiety is more active and plays a

prominent role in antimicrobial activity. The structures of all the compounds were confirmed by IR, PMR & MS spectral data, and were further supported by correct elemental analysis (experimental part).

Acknowledgements
The author thanks Head, RSIC, CDRI, Lucknow for the analytical and spectral data. Head of the Department of Microbiology, I.M.S. B.H.U for the biological screening and to the Head of the Chemistry Department of Tilak Dhari Post Graduate College, Jaunpur for providing Laboratory facilities.

References

[1] S. Kubota, M. Uda, K. Sato, *Chem.Pharm.Bull.*, **1978**, 26(3), 893-897.
[2] S. Jantova, G. Greif, R. Pavlovicova, L. Cipak, *Folia Microbial.*, **1998**, 43(1), 75-78.
[3] S. Ersan, S. Nacak, R. Berkem, II *farmaco*, **1999**, 53(12), 773-776.
[4] R. J. Singh, PhD thesis, Tilak Dhari Post Graduate College, Veer Bahadur Singh Purvanchal University (Jaunpur, Uttar Pradesh, India, **2002**).
[5] R. J. Singh, D. K. Singh, *J. Purvanchal Acad. Sci. Ser.B*, **2004**, 10, 31-36.
[6] R. J. Singh, *J. Purvanchal Acad. Sci. Ser.B*, **2006**, 12, 21-24.
[7] R. J. Singh, A. N. Singh, N. Singh, *J. U. Chem.*, **2007**, 3(1).
[8] R. J. Singh, D. K. Singh, 2nd National Symposium on Analytical Sciences (NSAS): Analytical Innovations for Process and Technology Development, 23-25 Nov. **2008**, 118.
[9] R. J. Singh, D. K. Singh, *E-J.Chem.*, **2009**, 6(3), 796-800.
[10] R. J. Singh, D. K. Singh, *S. Afr. J. Chem.*, **2009**, 62, 105-108.
[11] R. J. Singh, D. K. Singh, *J. Purvanchal Acad. Sci. Ser.B*, **2009**, 15, 81-86.
[12] R. J. Singh, D. K. Singh, *J. Purvanchal Acad. Sci. Ser.B*, **2009**, 15, 87-94.
[11] R. J. Singh, D. K. Singh, *E-J.Chem.*, **2009**, 7 (In press).
[14] R. J. Singh, D. K. Singh, *Asian J. Chem.*, **2009**, 22(In press).
[15] R. J. Singh, *RASAYAN J.Chem.*, **2009**, 2(3) (In press).
[16] R. J. Singh, *Int. J. Appl.Chem.*, **2009**, 5(2), 81-84.
[17] R. J. Singh, D. K. Singh, *Int. J. Chem. Tech. Res.*, **2009**, 1(4) (In press).
[18] R. Cruickshank, J. P. Duguid, B.P. Marmoin, H.A. Swam, Ed.; The practice of Medical Microbiology, 12th edn., Churchill Living stone, London, Vol.3, **1975**.
[19] H.W. Seeley, P.J. Vandenmark; Microbes in action: A Laboratory Mannual of Microbiology, D.B.Taraporevala & Sons Pvt. Ltd. Bombay, **1975**.
[20] L.J. Bradshaw, Ed.; A Text book of Microbiology, W.P. Sounders Co., Philadelphia, London, **1979**.