Asian Journal of Chemistry Vol. 22, No. 4 (2010), 2659-2663

Syntheses, Characterization and Antimicrobial Activity of Some Substituted 1,2,4-Triazole Derivatives

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Syntheses of a series of 4-amino-3,5-dialkyl-1,2,4-triazoles are described. In present work, iminoester hydrochlorides (**1**) was treated with acyl hydrazines to obtain acylhydrazones (**2**). The compound acylhydrazones (**2**) converted to the 4-amino-3,5-dialkyl-1,2,4-triazoles (**3**) in good yields by treatment with hydrazine hydrate. The newly compounds were characterized by spectral and elemental analyses. Some compounds were screened for their antibacterial activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. All compounds carring 1,2,4-triazole moiety showed significant antimicrobial activity.

Key Words: 1,2,4-Triazole, Acyl hydrazone, Antimicrobial activity.

INTRODUCTION

The chemistry of heterocyclic compounds continuous to be an explore field in the organic chemistry. The importance of 1,2,4-triazole derivatives lies in the field that these have occupied an unique position in heterocyclic chemistry due to their biological activities[1-3]. 4-Amino-3,5-dialkyl-1,2,4-triazoles (**3**) have been obtained by treating acylhydrazones (**2**) and hydrazine hydrate through the cyclization reaction. In views of these observations and in continuation of our earlier work[4-9] on the syntheses of some 1,2,4- and 1,2,3-triazole derivatives, we now report the syntheses of some more triazoles derived from acylhydrazones and their antibacterial activities.

EXPERIMENTAL

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 PMR spectra (DMSO-d_6) on an EM 390 spectrometer using TMS as an internal standard (chemical shift in δ ppm). Purity of the compounds was checked by TLC using silica gel G. All compounds showed satisfactory elemental analyses.

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Iminoester hydrochlorides (1a-c): A mixture of corresponding aromatic nitrile (0.01 mol), absolute alcohol (0.01 mol) and dry hydrogen chloride (0.01 mol) was ice-cooled. The resulting solution was then allowed to stand at 0 ºC in the refrigerator for 15 h, after which cold absolute ether was added and the obtained compound was filtered off immediately, washed with cold absolute ether and dried in a dessicator to obtained iminoester hydrochlorides (**1**). The IR (KBr) of the isolated iminoester hydrochlorides indicates a prominent characteristic band at 2982 cm^{-1} attributed to an NH_2^+ group (yield 52 %).

Ethyl carboxylate acylhydrazones (2a-c): General procedure: Iminoester hydrochlorides (**1a-c**) (0.01 mol) were reacted with acyl hydrazine (0.01 mol) in the presence of absolute ethanol (50 mL) was refluxed for 5 h at 05 ºC. Then, the precipitated ammonium chloride was filtered off. After evaporating the solvent at 40 ºC under reduced pressure, a white solid appeared. The crude solid was filtered, washed with water and recrystallized from appropriate solvent to give compounds (**2a-c**) yield (55-75 %).

Ethyl N-acetyl-4-methylbenzenecabohydrazonoate (2a): It was prepared by using the above method, (yield 55 %) m.p. 105 ºC, Anal. calcd. for $C_{12}H_{16}N_2O_2$: C, 50.38; H, 6.38; N, 29.39 %; Found: C, 50.32; H, 6.48; N, 29.79 %. IR (KBr): 3202 (NH), 1670 (C=O) and 1620 cm^{-1} (C=N); PMR: δ 1.35 (3H, t, CH_3), 2.01 (3H, s, $COCH_3$), 2.1 (3H, s, CH_3), 4.16 (2H, q, OCH_2), 7.52 (2H, d, aromatic-H), 7.88 (2H, d, aromatic-H) and 10.58 ppm (1H, s, NH); MS: m/z 195 (M^+) other peaks were observed at 162, 135, 105, 78 and 55.

Ethyl N-(4-hydroxybenzoyl)-4-methylbenzenecabohydrazonoate (2b): It was prepared by using the above method, (yield 62 %) m.p. 150 ºC, Anal. calcd. for $C_{17}H_{18}N_2O_3$: C, 50.38; H, 6.28; N, 28.95 %; Found: C, 50.88; H, 6.35; N, 29.02 %. IR (KBr, ν_{max}, cm^{-1}): 3222 (NH), 3198(OH), 1665 (C=O) and 1610 (C=N); PMR: δ 1.22 (3H, t, CH_3), 2.36 (3H, s, CH_3), 4.31 (2H, q, OCH_2), 6.92 (2H, d, aromatic-H), 7.35 (2H, d, aromatic-H), 7.84 (2H, d, aromatic-H), 7.97 (2H, d, aromatic-H), 9.93 (1H, s, NH) and 10.18 ppm (1H, s, OH); MS: m/z 198 (M^+) other peaks were observed at 178, 141, 108, 88, 68 and 54.

Ethyl N-isonicotinoyl-4-methylbenzenecabohydrazonoate (2c): It was prepared by using the above method, (yield 55 %) m.p. 100 ºC, Anal. calcd. for $C_{16}H_{17}N_3O_2$: C, 61.20; H, 6.22; N, 22.31 %; Found: C, 61.18; H, 6.31; N, 22.52 %. IR (KBr, ν_{max}, cm^{-1}): 3345 (NH), 1666 (C=O) and 1622 (C=N); PMR: δ 1.31 (3H, t, CH_3), 2.38 (3H, s, CH_3), 4.30 (2H, q, OCH_2), 7.35 (2H, d, aromatic-H), 7.92 (2H, d, aromatic-H), 8.10 (2H, bs, aromatic-H), 8.78 (2H, bs, aromatic-H) and 10.86 ppm (1H, s, NH); MS: m/z 188 (M^+) other peaks were observed at 158, 138, 118, 103, 86 and 54.

4-Amino-3,5-dialkyl-1,2,4-triazoles (3a-c): General procedure: Ethyl carboxylate acylhydrazone (**2**) (0.01 mol) was refluxed with hydrazine hydrate (0.01 mol) in 1-propanol (50 mL) for 24 h. Then, the reaction mixture was cooled to room temperature and a white solid appeared. The crude solid was filtered, washed with benzene and recrystallized from appropriate solvent to give compounds (**3a-c**) yield (85-90 %).

4-Amino-3-methyl-5-(4-tolyl)-4*H*-1,2,4-triazole (3a): It was prepared by using the above method, (yield 88 %) m.p. 220 ºC, Anal. calcd. for $C_{10}H_{12}N_4$: C, 48.19; H, 5.12; N, 37.62 %; Found: C, 47.99; H, 5.14; N, 37.70 %. IR (KBr, ν_{max}, cm^{-1}): 3244-3142 (NH_2) and 1652 (C=N); PMR: δ 2.39 ($2CH_3$), 6.06 (2H, s, NH), 7.31 (2H, d, aromatic-H) and 7.90 ppm (2H, d, aromatic-H); MS: m/z 201 (M^+) other peaks were observed at 189, 167, 137, 101, 78 and 52.

4-Amino-3-(4-hydroxyphenyl)-5-(4-tolyl)-4*H*-1,2,4-triazole (3b): It was prepared by using the above method, (yield 90 %) m.p. 285 ºC, Anal. calcd. for $C_{15}H_{14}N_4O$: C, 46.58; H, 5.65; N, 38.05 %; Found: C, 47.06; H, 5.68; N, 39.18 %. IR (KBr, ν_{max}, cm^{-1}): 3318-3274 (NH_2), 3201 (OH) and 1652 (C=N); PMR: δ 2.39 (3H, s, aromatic-CH_3), 6.15 (2H, s, NH_2), 6.95 (2H, d, aromatic-H), 7.42 (2H, d, aromatic-H), 7.86 (2H, s, aromatic-H), 8.02 (2H, d, aromatic-H) and 9.91 ppm (1H, s, OH); MS: m/z 205 (M^+) other peaks were observed at 183, 172, 106, 69 and 49.

4-Amino-3-(pyridine-4-yl)-5-(4-tolyl)-4*H*-1,2,4-triazole (3c): It was prepared by using the above method, (yield 85 %) m.p. 265 ºC, Anal. calcd. for $C_{14}H_{13}N_5$: C, 45.32; H, 5.61; N, 37.67 %; Found: C, 45.12; H, 5.14; N, 38.05 %. IR (KBr, ν_{max}, cm^{-1}): 3320-3190 (NH_2) and 1605 (C=N); PMR: δ 2.40 (3H, s, aromatic-CH_3), 6.38 (2H, s, NH_2), 7.41 (2H, d, aromatic-H), 7.90 (2H, d, aromatic-H), 8.05 (2H, d, aromatic-H) and 8.74 ppm (2H, bs, aromatic-H); MS: m/z 207 (M^+) other peaks were observed at 181, 178, 169, 107, 83 and 56.

Antibacterial activity: The antibacterial activity of three compounds were investigated by employing filter paper disc method[10-13]. Representative organisms selected for evaluation of antibacterial activity were *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. The antibacterial activity of each compound was evaluated at 100 and 10 μg mL^{-1} concentrations. The compounds were tested as a solution or suspension in DMF. An important and useful drug Ampicillin was also tested under similar conditions, with view to compare the results.

Ampicillin is a β-lactum antibiotic[14] that has been used extensively to treat bacterial infections since 1961. Ampicillin is designated chemically as (2S,5R,6R)-6-([(2R)-2-amino-2-phenylacetyl]amino)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo-[3.2.0]heptane-2-carboxylic acid. Ampicillin is able to penetrate Gram-positive and some Gram-negative bacteria[15]. Ampicillin acts as a competitive inhibitor of enzyme transpentidase. As a powder ampicillin is white with slight yellow cast and is soluble in water.

The result indicates that all three compounds showed good activity (Table-1). All three compounds showed very good activity against *S. aureus, E. coli, B. subtilis* and *P. aeruginosa*. From the above observation it is clear that the 1,2,4-triazole derivatives are more active and play a prominent role in the antimicrobial activity.

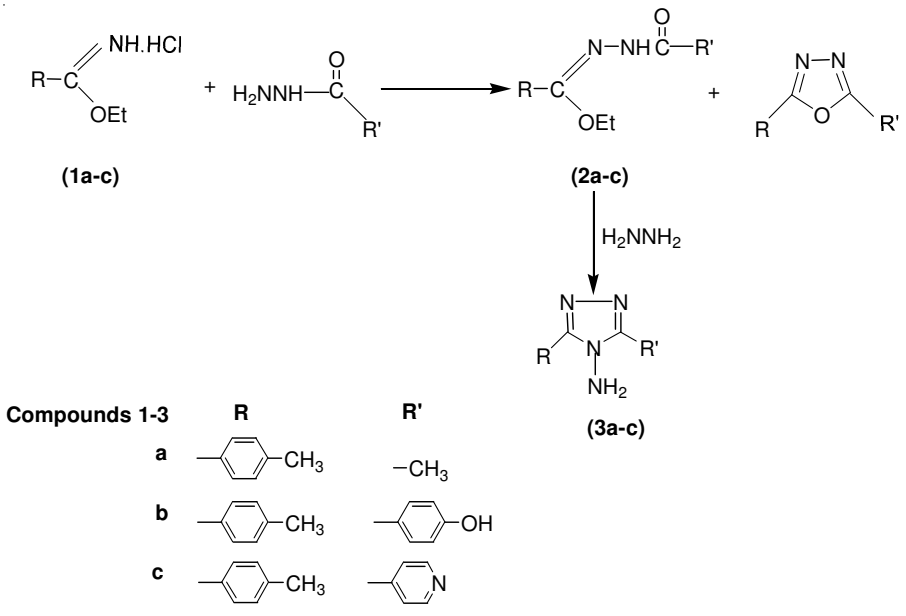
RESULTS AND DISCUSSION

In the present work, compound iminoester hydrochlorides (**1a-c**) required as starting material was obtain in one-pot reaction by passing HCl gas through solution of aryl cyanide with absolute ethanol, followed by precipitation with ether. Ethyl

TABLE-1
EVALUATION OF ANTIBACTERIAL ACTIVITY OF THE COMPOUNDS

Compd.	Zone of inhibition (mm)							
	S. aureus		*E. coli*		*B. subtilis*		*P. aeruginosa*	
	100 μg mL^{-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}
3a	16	15	16	14	15	14	17	13
3b	17	14	15	13	16	14	16	14
3c	17	15	14	14	17	13	16	13
Standard (Ampicillin)	25	21	24	20	24	20	24	21
Control	00	00	00	00	00	00	00	00

carboxylate acylhydrazones (**2a-c**) were obtained by the condensation of iminoester hydrochlorides (**1**) with acyl hydrazines. Compounds 4-amino-3,5-dialkyl-1,2,4-triazoles (**3a-c**) were obtained by treatment of compounds ethyl carboxylate acyl hydrazones (**2a-c**) with hydrazine hydrate. The reaction was carried out in 1-propanol at refluxing temperature for 24 h and the desired 4-amino-3,5-dialkyl-1,2,4-triazoles (**3a-c**) were yielded (**Scheme-I**).



Scheme-I

The compound 4-amino-3,5-dialkyl-1,2,4-triazoles (**3a-c**) containing 1,2,4-triazole moiety is more active and plays a prominent role in antimicrobial activity. The structure of all the compounds are confirmed by IR, PMR and MS spectral data.

Conclusion

In conclusion, 4-amino-3,5-dialkyl-1,2,4-triazoles (**3a-c**) were synthesized and characterized. All these compounds containing 1,2,4-triazole moiety is more active and plays a prominent role in antimicrobial activity. The structure of all the compounds are confirmed by IR, PMR and MS spectral data and are further supported by correct elemental analysis.

ACKNOWLEDGEMENTS

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

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(*Received*: 18 March 2009; *Accepted*: 14 December 2009) AJC-8181