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

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Abstract: Syntheses of a series of 3-alkyl-4- amino-5- cyanomethyl-4*H*-1, 2, 4-triazoles with active methylene groups are described. The newly synthesized 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 the compounds carrying 1, 2, 4- triazole moiety showed significant biological activity.

Keywords: 1, 2, 4-Triazole, Cyanoacetic acid hydrazide, Acylhydrazines, Cyclization, Antibacterial activity.

Introduction

The chemistry of heterocyclic compounds continuous to be an explore field in the organic chemistry. The importance of triazole derivative lies in the field that these have occupied a unique position in heterocyclic chemistry due to their antimicrobial activity¹⁻³.

3-Alkyl-5-cyanomethyl-4*H*-1, 2, 4-triazole derivatives have been obtained by treating alkylimidate hydrochlorides and ester ethoxycarbonyl hydrazones with cyanoacetic acid hydrazide⁴. It was reported that *tert*-butylcarbazates can be hydrolyzed in acidic solutions to give hydrazinium salts^{5,6}. Indeed, some 3, 5-dialkyl-4-*tert*-butoxycarbonylamino-4*H*-1,2,4-triazole derivatives have also been hydrolyzed to their corresponding salts by treatment with 6 N HCl. These hydrochlorides could be converted to 3,5-dialkyl-4-amino-4*H*-1,2,4-triazoles by using equivalent amounts of ethoxide anion or ethanol⁷. In views of these observations and in continuation of our earlier work⁸⁻¹⁰ on the syntheses of some 1, 2, 4- & 1, 2, 3- triazole derivatives, we now report the syntheses of some more triazole derivatives derived from ethyl carboxylate *tert*-butoxycarbonyl hydrazones and their biological 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-d₆) 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.

*Ethyl carboxylate tert-butoxycarbonyl hydrazone(1)*¹¹

Alkylimidate hydrochlorides (0.01 mol) were reacted with *tert*-butylcarbазate (0.01 mol) at 120 °C for the period of 3.5 hours. After cooling the separated ethyl carboxylate *tert*-butoxy carbonyl hydrazone was taken out, dried and used directly for the next step without further purification (yield 78%).

3-Alkyl(alkylaryl)-4-*tert*-butoxycarbonylamino-5-cyanomethyl-4H-1, 2,4-triazoles(2)

General procedure

A suspension of ethylcarboxylate *tert*-butoxycarbonylhydrazones (0.01 mol) and 1:2 mixture of ethylacetate-petroleum ether water (5 mL) were heated at 120 °C for the period of 2.0 hours. On cooling the mixture in a deep freezer, a white solid appeared. It was filtered, washed with water and recrystallized from ethyl acetate-petroleum ether (1:2), (**2**) yield (70-85%).

2a: Yield 78%, m.p. 208 °C, Anal.Calc. for C₁₀H₁₅N₅O₂: C, 50.68; H, 6.34; N, 29.49%; Found: C, 50.57; H, 6.49; N, 29.76%. IR (KBr) : 3213 (N-H), 3041 (aromatic C–H stretching), 1680 (C=O), 1641 (C=N), and 1515 cm⁻¹ (C–N) ; PMR: δ 1.38 (9H,s,(CH₃)₃); 2.38-2.40 (3H,s, CH₃);3.85(2H,s,CH₂) and 10.22 ppm (1H of NH,s); MS : *m/z* 192 (M⁺) other peaks were observed at 168,132,128,107,89, and 54.

2b: Yield 75%, m.p. 102 °C, Anal.Calc. for C₁₁H₁₂N₅O₂: C, 50.68; H, 6.14; N, 28.98%; Found: C, 50.98; H, 6.24; N, 29.01%. IR (KBr) :3135 (N-H), 3044 (aromatic C–H stretching), 1655 (C=O), 1566,1498 (C=N), and 1478 cm⁻¹ (C–N) ; PMR: δ 1.25 (3H,t, CH₃);1.45 (9H,s,(CH₃)₃); 4.25(2H,s,CH₂) and 9.88 ppm (1H of NH,s); MS : *m/z* 201 (M⁺) other peaks were observed at 179,139,109,88,69 and 54.

2c: Yield 72-75%, m.p. 95 °C, Anal.Calc. for C₁₆H₁₉N₅O₂: C, 61.21; H, 6.11; N, 22.34%; Found: C, 61.19; H, 6.21; N, 22.54%. IR (KBr) : 3150 (N-H), 3006 (aromatic C–H stretching), 1688 (C=O), 1567,1492(C=N), and 1463cm⁻¹ (C–N); PMR: δ 1.39 (9H,s,(CH₃)₃); 2.30-2.37 (3H,s,CH₃); 4.15(2H,s,CH₂);7.2- 7.35(5H,m,Ar-H)and 10.12 ppm (1H of NH,s); MS : *m/z* 187 (M⁺) other peaks were observed at 159,139,119,104,87 and 54.

2d: Yield 70-75%, m.p. 132 °C, Anal.Calc. for C₁₆H₁₈ClN₅O₂: C, 55.28; H, 5.21; N, 20.27%; Found: C, 55.28; H, 5.19; N, 20.63%. IR (KBr) : 3142 (N-H), 3106 (aromatic C–H stretching), 1651 (C=O), 1539,1492 (C=N), and 1433 cm⁻¹ (C–N) ; PMR: δ 1.42 (9H,s,(CH₃)₃), 2.39 (3H,s, CH₃); 4.15(2H,s,CH₂); 7.8(5H,m,Ar-H)and 10.21 ppm (1H of NH,s); MS : *m/z* 186 (M⁺) other peaks were observed at 159,137,119,107,89 and 55.

3-Alkyl (alkyl aryl)-4-amino-5-cyanomethyl-4H-1, 2, 4-triazole hydrochloride(3)

General procedure

A mixture of compound (**2**) (0.01mol), hydrochloric acid (15 mL) and tetrahydrofuran (5 mL) was refluxed for the period of 35 minutes. The resulting solution was evaporated at 38 °C under reduced pressure, cooled and poured on crushed ice. The solid separated was filtered, washed with water and recrystallized from ethanol-diethyl ether (1:2), (**3**) yield (80-85%).

3a: Yield 82%, m.p. 167 °C, Anal.Calc. for C₅H₈ClN₅: C, 34.56; H, 4.64; N, 40.33%; Found: C, 34.7; H, 4.56; N, 40.50%. IR (KBr): 3161, 3121 (NH₃⁺), 3022 (aromatic C–H stretching), absence of band for C=O of CONH at 1651, 1621 (C=N), and 1487 cm⁻¹ (C–N);

PMR: δ 2.68 (3H,s, CH₃), 4.62(2H,s,CH₂) and 7.2 ppm (3H,s, NH₂); MS : m/z 198 (M⁺) other peaks were observed at 177,167,138,109,88 and 55.

3d: Yield 83%, m.p. 165 °C, Anal.Calc. for C₁₁H₁₁Cl₂N₅: C, 46.52; H, 3.89; N, 24.66%; Found: C, 46.56; H, 4.16; N, 24.62%. IR (KBr) : 3130,3117 (NH₃⁺), 3010 (aromatic C–H stretching), absence of band for C=O of CONH at 1662, 1632,1495 (C=N), and 1488 cm⁻¹ (C–N) ; PMR: δ 2.71 (3H,s, CH₃), 4.38,4.56(2H,s,CH₂) and 7.32 ppm (3H,s, NH₂); MS : m/z 197 (M⁺) other peaks were observed at 179,162,139,106,78 and 44.

3-Alkyl (alkyl aryl)-4-amino-5-cyanomethyl-4H-1, 2, 4-triazoles(4)

General procedure

Compound (**3**) (0.01 mol), was treated with an equivalent amount of 2N KOH in ethanol and refluxed for the period of 6 hours. The resulting solution was evaporated at 38 °C under reduced pressure, cooled and poured on crushed ice, a white solid appeared. The solid separated was filtered, washed with water and recrystallized from ethyl acetate-petroleum ether (1:2), (**4**) yield (75-85%).

4a: Yield 78%, m.p. 130 °C, Anal.Calc. for C₅H₇N₅: C, 43.78; H, 5.20; N, 51.05%; Found: C, 43.68; H, 5.07; N, 50.85%. IR (KBr) : 3268,3138 (NH₂), 3048 (aromatic C–H stretching), absence of band for C=O of CONH at 1658, 1650,1639 (C=N), and 1491 cm⁻¹ (C–N) ; PMR: δ 2.36 (3H,s, CH₃), 4.21(2H,s,CH₂) and 5.90 ppm (2H,s, NH₂); MS : m/z 205 (M⁺) other peaks were observed at 201,189,159,138,121,106 and 44.

4d: Yield 82%, m.p. 128 °C, Anal.Calc. for C₁₁H₁₀ClN₅: C, 53.31; H, 4.06; N, 28.38%; Found: C, 53.32; H, 4.11; N, 28.68%. IR (KBr) : 3326,3205 (NH₂), 3016 (aromatic C–H stretching), absence of band for C=O of CONH at 1662, 1527,1499 (C=N), and 1485 cm⁻¹ (C–N); PMR: δ 2.41 (3H,s, CH₃), 4.10,4.18 (2H,s,CH₂), 5.92 (2H,s,NH₂) and 7.38 ppm (5H,m, Ar-H); MS : m/z 199 (M⁺) other peaks were observed at 187,185,167,135,119,104,76 and 44.

Biological activity

The antibacterial activity of twelve compounds was studied by employing filter paper disc method¹²⁻¹⁵. 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 μ g mL⁻¹ and 10 μ g 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 *beta*-lactum antibiotic¹⁶ 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 bacteria¹⁷. Ampicillin acts as a competitive inhibitor of enzyme transpeptidase. As a powder ampicillin is white with slight yellow cast and is soluble in water. Ampicillin is often used as a selective agent in molecular biology to confirm the uptake of genes by bacteria. Ampicillin applied for the treatment of pneumonia patients, bronhopneumonijami with abscesses light, angina, peritonitis, cholecystitis, sepsis, intestinal infections and postoperative infection in the soft tissue and other infections caused by susceptible to the microorganism¹⁸.

The results indicate that all these compounds showed moderate to strong activity (Table 1). Compounds **2a**, **2c**, **2d**, **3a**, **3d**, **4a** and **4d** showed very good activity against *S. aureus*, *E. coli*, *B. subtilis* and *P. aeruginosa*. Similarly, compounds **2b**, **3b**, **3c**, **4b**, and **4c** showed moderate to mild activity against all four bacteria. From the above observation it is clear that the 1, 2, 4-triazole derivatives are more active and play a prominent role in the biological activity.

Table 1. Evaluation of antibacterial activity of the compounds.

Compd.	Zone of Inhibition/mm							
	<i>S. aureus</i>		<i>E. coli</i>		<i>B. subtilis</i>		<i>P.aeruginosa</i>	
	100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$	100 $\mu\text{g mL}^{-1}$	10 $\mu\text{g mL}^{-1}$
2a	17	13	13	12	16	13	16	15
2b	-	-	10	09	09	07	10	08
2c	15	14	16	14	15	13	16	13
2d	17	14	14	13	15	13	16	14
3a	16	15	13	13	17	13	15	13
3b	-	-	11	08	09	08	-	-
3c	10	09	09	07	09	08	-	-
3d	16	15	16	13	15	12	15	14
4a	17	14	16	14	15	13	16	13
4b	10	08	-	-	-	-	09	08
4c	09	07	-	-	09	08	09	07
4d	17	14	16	15	17	13	15	14
Standard (Ampicillin)	27	21	24	20	24	20	24	20
Control	00	00	00	00	00	00	00	00

(-) No inhibition zone

Results and Discussion

In the present work, compound ethyl carboxylate *tert*-butoxycarbonyl hydrazone(**1**) required as starting material was obtain in one-pot reaction by heating alkylimidate hydrochlorides and *tert*-butylcarbazate at 120 °C for the period of 3.5 hours. Compound (**1**) was treated with cyanoacetic acid hydrazide to obtain the corresponding 3-alkyl-4-*tert*-butoxycarbonylamino-5-cyanomethyl-4*H*-1, 2, 4-triazoles (**2**). Compounds (**2a**) and (**2d**) were converted to the corresponding 3-alkyl-4-amino-5-cyanomethyl-4*H*-1,2,4-triazole hydrochlorides (**3a** & **3d**) in good yields by treatment with 6*N* HCl. Formation of 3-alkyl-4-amino-5-cyanomethyl-4*H*-1,2,4-triazoles (**4a** & **4d**) was achieved when compounds **3a** and **3d** were treated with an equivalent amount of 2*N* KOH. (Scheme 1).

Scheme 1.

Conclusion

In conclusion, a group of 1, 2, 4-triazole derivatives incorporating active methylene groups were synthesized and characterized. All these compounds containing 1, 2, 4-triazole moiety

is more active and plays a prominent role in biological activity. The structure of all the compounds are confirmed by IR, PMR & MS spectral data, and are further supported by correct elemental analysis (experimental part).

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References

- 1 Zeeh B and Goetz H, *J Indian Chem Soc.*, 1981, **280**, 2923-2926.
- 2 Gamala A and Ahmed M, *J Indian Chem Soc.*, 1997, **74**, 624-625.
- 3 Parmar S S, Choudhary M, Choudhary S K, Kumar S and Sapiro H R, *J Parm Sci.*, 1997, **66**, 971-973.
- 4 İkizler A A, İkizler A and Uzunismail N, *OPPI Briefs*, 1994, **24**, 865-868.
- 5 Ghalil N I, Venton D L, Hung S C and Le Breton G C, *J Org Chem.*, 1981, **46**, 5413-5414.
- 6 Krause J G, Kwan S and George B, *J Org Chem.*, 1972, **37**, 2040-2042.
- 7 İkizler A, Demirbas N and İkizler A A, *J Heterocyclic Chem.*, 1996, **33**, 1765-1769.
- 8 Singh D K, Singh R J, Mahadevan K M, Vagdevi H M and Vaidha V P, *Indian J Chem.*, 2003, **42B**, 1931-1932.
- 9 Mahadevan K M, Singh D K and Singh R J, *Indian J Heterocycl Chem.*, 2003, **13**, 151-155.
- 10 Singh R J, *J Purvanchal Academy of Sciences*, 2006, **12** (Ser.B), ISSN0972-3498, 21-24.
- 11 İkizler A.A, İkizler A, Yüksek H, Bahceci S and Sancak K, *Turk J Chem.*, 1994, **18**, 51-56.
- 12 Cruickshank R, Duguid J P, Marmoin B P and Swam H A, Eds., *The practice of Medical Microbiology*, Vol.3, 12th Ed., Churchill Living stone, London, 1975, p.544.
- 13 Bradshaw L J, Ed, *A Text book of Microbiology*, 1979, pp.1151-1162.
- 14 Rich S and Horsfall J G, *Phytopathology*, 1952, **42**, 477-480.
- 15 Seeley H W and Vandenmark P J, *Microbes in action: A Laboratory Mannual of Microbiology*, D B Taraporevala & Sons Pvt. Ltd. Bombay, 1975, pp.55-88.
- 16 Elene J B and Sydney M F, *Methods for testing Antimicrobial Effectiveness, Diagnostic Microbiology*, Mosiy Company, USA, pp.171-194.
- 17 Ersan S, Nacak S and Berken R, *Farmaco*, 1998, **53**, 773-776.
- 18 Ahmad I, Mehmood Z and Mohammad F, *J Ethnopharmacol.*, 1998, **62**, 183-193.