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IN VITRO ANTIOXIDANT ACTIVITY OF 9-SUBSTITUTED DERIVATIVES OF 9-PHENYL-5,9-DIHYDROPYRIMIDO[4,5-*d*][1,2,4] TRIAZOLO[1,5-*a*]PYRIMIDINE-6,8(1*H*,7*H*)-DIONE

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ABSTRACT

A green, efficient procedure has been developed for the synthesis of 9-substituted derivatives of 9-phenyl-5,9-dihydropyrimido[4,5-*d*] [1,2,4] triazolo[1,5-*a*] pyrimidine-6,8(1*H*,7*H*)-dione from a multicomponent one pot three molecule condensation of 3-amino-1*H*-1,2-4-triazole, Barbituric acid and tetrabutyl ammonium hydrogen sulphate (TBAHS) in water-ethanol with different substituted aromatic aldehydes were screened for their *In Vitro* Antioxidant activity.

KEYWORDS: 3-amino-1*H*-1,2-4-triazole, Barbituric acid, Aromatic Aldehyde , TBAHS, MCR's.

INTRODUCTION

Triazolo [1,5-*a*] pyrimidinedione occupies an important position in chemistry and biology. The most of chemist widespread as growing interest to the development of triazolo [1,5-*a*] pyrimidinedione because of the diverse pharmacological as well as biological properties such as antimicrobial[1], antitumor[2], analgesic[3], anticancer[4] antifungal activity[5], anti HIV activitiy[6], antibiotics[7], antileishmamial activity against Leishmani donovani promastigotes.[8] Moreover, triazolo [1,5-*a*] pyrimidine having binding capacity with metal ions to form a stable complex of coordination chemistry.[9] In addition, it is used for the

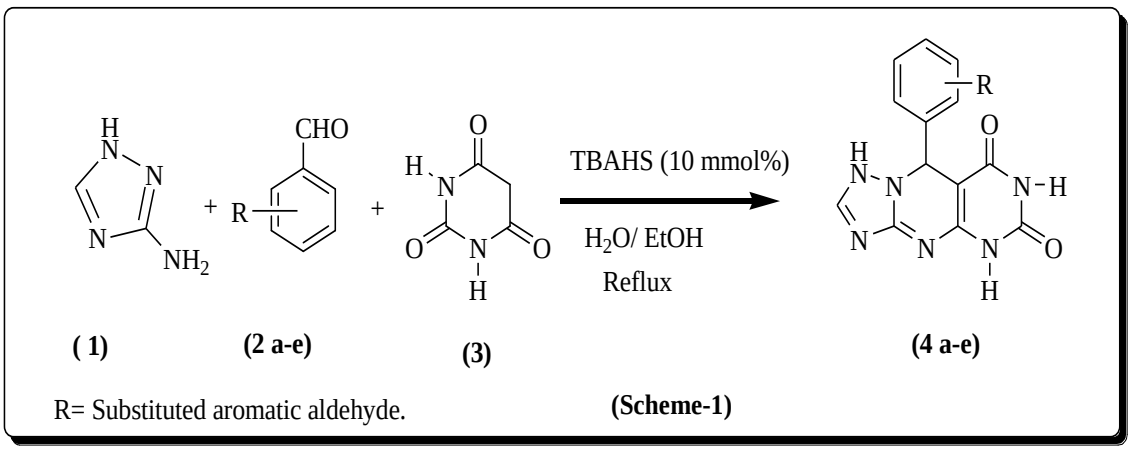
formation of the open chain analogs of crown ether, which having medicinal role for transportation function in living cells.[10]

Triazolo [1,5-*a*] pyrimidine synthesis by using reported catalyst like Lewis acid such as Zinc dichloride[11], piperidine[12], dioxane in methanol[13], methanol in hydrochloric acid.[14] Some of the method reported above use expensive catalysts, strong acidic conditions, higher temperature, require long reaction time, resulting cumbersome product isolation procedure.

Acidic TBAHS act as a phase transfer catalyst (PTC) and it perform much organic transformation under mild condition. Thus new route utilizing a MCR protocol, for the synthesis of triazolo [1,5-*a*] pyrimidinedione can attacks considerable attention in the search of method for rapid entry of these heterocycles. Consequently, we thought that there is scope for further innovation towards milder reaction condition, short reaction time and better yield in choosing TBAHS for this multicomponent reaction (MCRs).

RESULT AND DISCUSSION

A mixture of different substituted benzaldehydes (10 mmol) **(2a-e)**, 3-amino-1*H*-1,2-4-triazole (10 mmol) **(1)** barbituric acid (10 mmol) **(3)** and tetrabutyl ammonium hydrogen sulphate (TBAHS) (10 mmol %) in (10 ml) water and ethanol was refluxed for 2-4 hrs. The reaction mixture was cooled to room temperature and poured in to ice cold water. The precipitate obtained was filtered and washed with water, recrystallized from ethanol to afford the 9-substituted derivatives of 9-phenyl-5,9-dihydropyrimido[4,5-*d*] [1,2,4] triazolo[1,5-*a*] pyrimidine-6,8(1*H*,7*H*)-dione **(4a-e)**. The progress of the reaction was monitored by TLC. These synthesized products **(4a-e)** were completely characterized from IR, ^{1}H-NMR, Mass and ^{13}C-NMR spectroscopic technique and also elemental analysis.

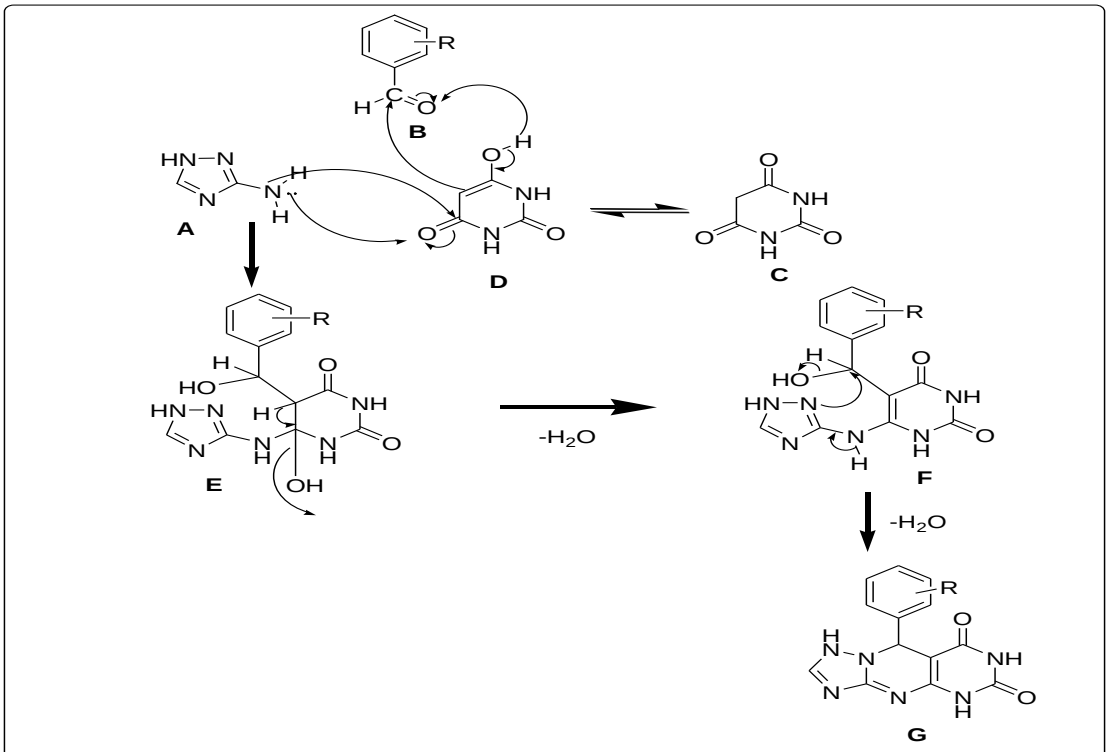


(1) **(2 a-e)** **(3)** **(4 a-e)**

R= Substituted aromatic aldehyde. **(Scheme-1)**

The formation of compounds **(4b)** shows IR spectrum in KBr exhibited stretching band at 3294 cm^{-I} cm^{-I} for the –NH, medium intensity band at 1689 cm^{-I},1604 cm^{-I} for conjugated C=O stretching. The ^{1}H-NMR (400 MH_Z, DMSO-d_6) was recorded in DMSO, it shows characteristic singlet peak at δ 6.80 ppm and mass spectra(ESI) shows molecular ion peak at m/z 317 ($M^{+\cdot}$, 100%), 318 ($M^{+\cdot}$,+1) and ^{13}C-NMR (400 MH_Z) in DMSO shows signal at δ 163, 160 ppm for C=O.

The plausible mechanism involves TBAHS catalyzed Knovenagel-Michael addition condensation between an aldehyde **(B)**, barbituric acid **(C**) and 3-amino-1*H*-1,2-4-triazole **(A)**, followed dehydration by cyclization to triazolo[1,5-*a*] pyrimidinedione **(G).**



R- different sustituted aromatic aldehydes

Scheme : Plausible mechanism for the formation of triazolo[1,5-*a*]pyrimidinedione

Table 1: Multicomponent reaction of 3-amino-1*H*-1,2-4-triazole (1), barbituric acid (2), and aromatic aldehyde (2a-e), for the synthesis of 4a-4e.

Entry	Subst. Aldehyde (Ar)	Products	Time (Hrs)	Yield%	M.P.^{0}C
1	-C_6H_4	**4a**	3.0	71	233-236
2	4-Cl -C_6H_4	**4b**	3.5	73	240-242
3	2- Cl -C_6H_4	**4c**	4.0	62	231-233
4	4-OCH_3-C_6H_3	**4d**	3.5	76	189-192
5	3,4-diOCH_3-C_6H_3	**4e**	3.0	72	220-222

EXPERIMENTAL

Open capillary tubes were used for melting points of isolated synthesized compounds and are uncorrected. Perkin-Elmer FTIR spectrophotometer was used for IR (KBr) spectra of compounds. Mass spectral data were recorded on liquid chromatography mass spectrometer (Shimadzu 2010Ev) using ESI probe. The ^{1}H and ^{13}C NMR spectra were recorded on various spectrometers at 400MHz using TMS as an internal standard.

General procedure for the synthesis of 9-substituted derivatives of 9-(4'-phenyl)-5,9-dihydropyrimido[4,5-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-6,8(1*H*,7*H*)-dione (4a-e)

A mixture of different substituted aromatic aldehydes (10 mmol) **(2a-e)**, 3-amino-1*H*-1,2-4-triazole, barbituric acid (10 mmol) **(3)** and tetrabutyl ammonium hydrogen sulphate (TBAHS) in (10 ml) water-ethanol was refluxed for 3-4 hrs, The progress of reaction was monitored by TLC. The reaction mixture was cooled to room temperature and poured into ice cold water. The solid obtained was filtered, washed with water and recrystallized by ethanol to give **(4a-e)**. The reaction was monitored by TLC.

Spectral Analysis

9-phenyl-5,9-dihydropyrimido[4,5-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-6,8(1*H*,7*H*)-dione (4a)

M.P. 233-236^{0}C, Yield 71%. IR (KBr/ cm^{-1}) 3228 (-NH), 1701,1623 (2 C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 2.96 (s, 1H, =NH), δ 5.50 (s, 1H, =CH), δ 6.3 (s, 1H, -CH), δ 6.7-7.9(m, 5H, Ar-H), δ 11.3 and δ 11.4 (2 bs,2H,-NH); EI-MS (m/z: RA %): 282 ($M^{+\cdot}$, 100%),. Elemental analysis calculated data for $C_{13}H_{10}N_6O_2$; C, 55.32; N, 29.77. Found: C, 55.30; N, 29.75.

9-(4'-chlorophenyl)-5,9-dihydropyrimido[4,5-*d*] [1,2,4] triazolo[1,5-*a*]pyrimidine-6,8(1*H*,7*H*)- dione (4b)

M.P. 232-234^{0}C, Yield 73%. IR (KBr/ cm^{-1}) 3294 (-NH), 1689,1604 (2 C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 2.91 (s, 1H, =NH), δ 5.75 (s, 1H, =CH), δ 6.8 (s, 1H, -CH), δ 6.8-7.3 (m, 4H, Ar-H), δ 11.1 and δ 11.2 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 317 ($M^{+\cdot}$, 100%),318 ($M^{+\cdot}$, +1),. ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 163,160, 151, 150, 155, 143, 140, 128, 127,90,57, 51, 40, 39, 38. Elemental analysis calculated data for $C_{13}H_9ClN_6O_2$; C, 49.30; N, 26.54. Found: C, 49.28; N, 26.52.

9-(2'-chlorophenyl)-5,9-dihydropyrimido[4,5-*d*] [1,2,4] triazolo[1,5-*a*]pyrimidine-6,8(1*H*,7*H*)-dione (4c)

M.P. 231-233^{0}C, Yield 62% .IR (KBr/ cm^{-1}) 3254 (-NH), 1702, 1695 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 2.83 (s, 1H, =NH), δ 5.60 (s, 1H, =CH), δ 6.7 (s, 1H, -CH), δ 6.4-7.8 (m, 4H, Ar-H), δ 11.2 and δ 11.3 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 317 ($M^{+\cdot}$, 100%), 318 ($M^{+\cdot}$, +1). Elemental analysis calculated data for $C_{13}H_9ClN_6O_2$; C, 49.30; N, 26.54. Found: C, 49.27; N, 26.53.

9-(4'-methoxyphenyl)-5,9-dihydropyrimido[4,5-*d*][1,2,4]triazolo[1,5-*a*]pyrimidine-6,8 (1*H*,7*H*) -dione (4d)

M.P. 195-197^{0}C, Yield 76%. IR (KBr/ cm^{-1}) 3374 (-NH), 1697,1612 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 3.67 (s, 1H, =NH), δ 3.85(s, 3H, -Ar-OCH$_3$), δ 5.34 (s, 1H, =CH), δ 5.9 (s, 1H, -CH), δ 6.8-7.5 (m, 4H, Ar-H), δ 10.3 and δ 11.1 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 312 ($M^{+\cdot}$, 100),. ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 165, 163,162, 159, 156, 155,150, 151, 148, 139,137, 130, 128,125, 115, 113,112, 90, 40, 39, 38. Elemental analysis calculated data for $C_{14}H_{12}N_6O_3$; C, 53.85; N, 26.97. Found: C, 53.83; N, 26.96.

9-(3',4'-dimethoxyphenyl)-5,9–dihydropyrimido[4,5-*d*] [1,2,4] triazolo [1,5-*a*] pyrimidine-6,8 (1*H*,7*H*)-dione (4e)

M.P. 220-222^{0}C, Yield 72%. IR (KBr/cm^{-1}) 3224 (-NH), 1739,1693 (2C=O); ^{1}H NMR (400MHz, DMSO-d$_6$ / ppm) δ 3.35 (s, 1H, =NH), δ 3.6 and δ 3.8 (2s, 6H, -2Ar-OCH$_3$), δ 6.62 (s, 1H, -CH), δ 7.89-8.41 (m, 3H, Ar-H), δ 11.17 and δ 11.29 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 342 ($M^{+\cdot}$ +1, 100%),. ^{13}C NMR (400 MHz, DMSO–d$_6$/ppm) δ: 163, 162 (C=O), 155, 153 (C-4b), 150,147, 131, 128, (Ar-C), 125, (C-9a),116, 115, 111, 55, 40, 39, 38. Elemental analysis calculated data for $C_{15}H_{14}N_6O_4$; C, 56.75; N, 28.36. Found: C, 56.73; N, 28.34.

BIOLOGICAL ACTIVITY

Antioxidant Activity

A) DPPH (1, 1-diphenyl-2-picrylhydrazyl) radical scavenging assay

DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging assay was performed as per earlier reported method.[15] The reaction cocktail was prepared by mixing individual newly synthesized organic compounds is added to equal volume of 0.1 mM solution of DPPH radical in absolute ethanol. After 20 minutes of incubation at room temperature, the DPPH

reduction was calculated by reading the absorbance at 517 nm using UV-Visible spectrophotometer. Ascorbic acid (1mM) was used as reference compound.

The compound **(4d, 4b and 4e)** shows remarkable antioxidant activity against DDPH radical scavenging activity with reference of ascorbic acid (91.4 ± 0.021).

B) OH radical scavenging assay

Hydroxy radicals scavenging activity was measured with Fenton's reaction (Rollet –Labelle et al., 1998). The reaction mixture contained 60 µl of $FeCl_2$ (1mM), 90 µl of 1,10-phenanthroline(1mM), 2.4 ml of phosphate buffer (pH 7.8),150 µl of 0.17M H_2O_2 and 1.5 ml of individual newly synthesized organic compounds (1mM). The reaction mixture was kept at room temperature for 5 minutes incubation and the absorbance was recorded at 560 nm using UV-Visible spectrophotometer. Ascorbic acid (1mM) was used as the reference compound.

The compound **(4c, 4b and 4e)** shows good OH radical scavenging activity as compared with Ascorbic acid (89.5 ± 0.021).

Table 2: Antioxidant activity of tested compounds (4a-4e)

Entry	Compound Code	R-	% Radical scavenging activity	
			DPPH radical scavenging	OH radical scavenging
01	**4a**	Ar-H	30.2 ± 0.65	41.1 ± 1.26
02	**4b**	4-Cl	**65.6 ± 0.84**	**67.2 ± 1.74**
03	**4c**	2-Cl	60.9 ± 1.78	**71.2 ± 1.32**
04	**4d**	4-OCH_3	**84.3 ± 1.32**	56.9 ± 0.42
05	**4e**	4,3-di-OCH_3	**63.4 ± 0.72**	**60.2 ± 1.31**
06		**Ascorbic Acid (Standard)**	91.4 ± 0.021	89.5 ± 0.021

CONCLUSION

In conclusion, we have synthesized an efficient and facile method have *In Vitro* Antioxidant activity of 9-substituted derivatives of 9-(4'-phenyl)-5,9-dihydropyrimido [4,5-*d*] [1,2,4] triazolo [1,5-*a*] pyrimidine-6,8(1*H*,7*H*)-dione by reaction of corresponding substituted benzaldehydes, 3-amino-1*H*-1,2-4-triazole and barbituric acid in presence of tetrabutyl ammonium hydrogen sulphate (TBAHS) in water and ethanol. The product can be easily isolated by simple workup technique, requires ambient reaction condition, less expensive, short time and give excellent yield. Among these synthesized compounds shows *In Vitro* Antioxidant activity.

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