Synthesis and Antioxidant Activity of Pyrimido [4,5-*d*] [1,3,4]Thiadiazolo[3,2-*a*]Pyrimidinedione

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Abstract: A green, simple, efficient and environmentally bengin procedure has been developed for the synthesis of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione derivatives from one-pot three component condensation reactions of barbituric acid, substituted aromatic aldehydes, and 2-amino 5-(4-chlorophenyl)-1,3,4-thiadiazole using Tetrabutyl ammonium hydrogen sulphate (TBAHS) as a green catalyst in ethanol-water. The newly synthesized compounds evaluated by their spectral techniques and screened for their Antioxidant activity.

***Index Terms*: Barbituric acid, 2-amino 5-(4-chlorophenyl)-1,3,4-thiadiazole, Aromatic Aldehyde , MCRs, TBAHS.**

I. INTRODUCTION

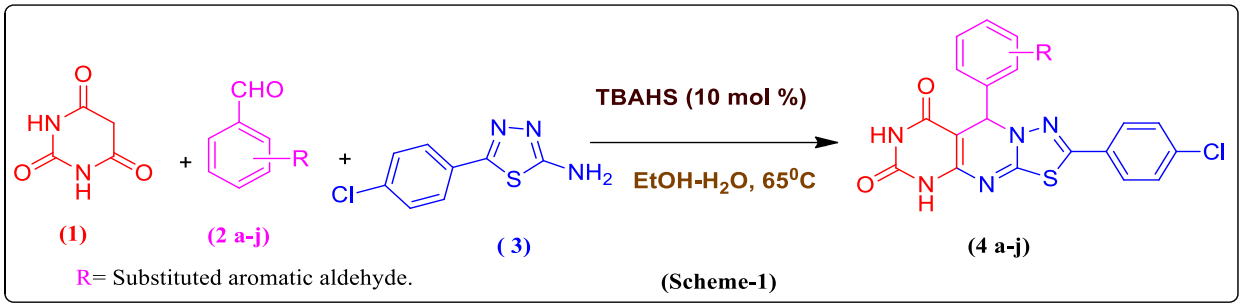
Heterocyclic compounds are vital class of organic chemistry include pharmaceutical and biological properties [1]. In a multicomponent reaction (MCRs) three or more reactant are converted into a higher molecular weight compound in a one pot method. Today's, MCR protocol has become very popular in the field of Chemistry. The thiadiazoles exhibit a wide range of biological properties and pharmacological properties such as antimicrobial [2], anti-inflammatory [3] and anticancer [4].

Thiadiazolo [3,2-*a*] pyrimidine dione derivatives occupies an important role in organic chemistry and medicinal field, because of its has diverse application such as antifungal activity [5], antitumor [6], antioxidant [7], anticonvulsant [8], antihypertensive [9], analgesic [10], anti HIV activity [11], and antibiotics[12]. In addition to these derivatives, its exhibit agrochemical properties.

Derivatives of thiadiazolo [3,2-*a*] pyrimidine was reported through the various catalyst like NaOH in ethanol [13] , molecular iodine[14], 2-[5-(4-methoxyphenyl) 4-*H*-1,2,4-triazole] acetic acid [15], and SBA-15 [16]. Some of the reported methods were also reported expensive catalysts, strong acidic conditions, higher temperature, require longer 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. Therefore, the new path utilizing a MCR protocol, for the synthesis of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione derivatives can attract considerable attention in the field of 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).

II. RESULT AND DISCUSSION



(Scheme-1)

We have performed the model reaction using different stoichiometric amount of catalyst. The catalyst screening result are summarized in (Table 2). It was observed that the excellent yield was gained by using Tetrabutyl ammonium hydrogen sulphate (TBAHS) (Table 2, entry 5). The use of aqueous ethanol as solvent in reaction protocol exhibit remarkable benefits like environmentally safe, comparatively cheaper to operate and easy work up.

Analyzed the influence of different parameters on the model reaction, we revolved our focus towards the 9-substituted derivatives of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione **(4a-j)** using one pot three component reaction of barbituric acid **(1),** different substituted aldehydes **(2a-j)**, and 5-(4-chlorophenyl)-1,3,4-thiadiazole **(3)** were refluxed in the presence of Tetrabutyl ammonium hydrogen sulphate (TBAHS) (10 mol%) in aqueous ethanol **(Scheme 1)**, the result are summarized in Table **3**. The desired products (**4a-j**) were obtained to excellent yields. These synthesized products **(4a-j)** were completely characterized from IR, ^{1}H-NMR, Mass and ^{13}C-NMR spectroscopic technique and also elemental analysis. We proposed tentative plausible mechanism for the formation of **(4a-j)** in the presence of Tetrabutyl ammonium hydrogen sulphate (TBAHS) The overall; mechanism takes place according to Knoevenagels-Micheal reaction **(Scheme-II).**

Table 1: Optimization of the reaction conditions using different solvents.[a]

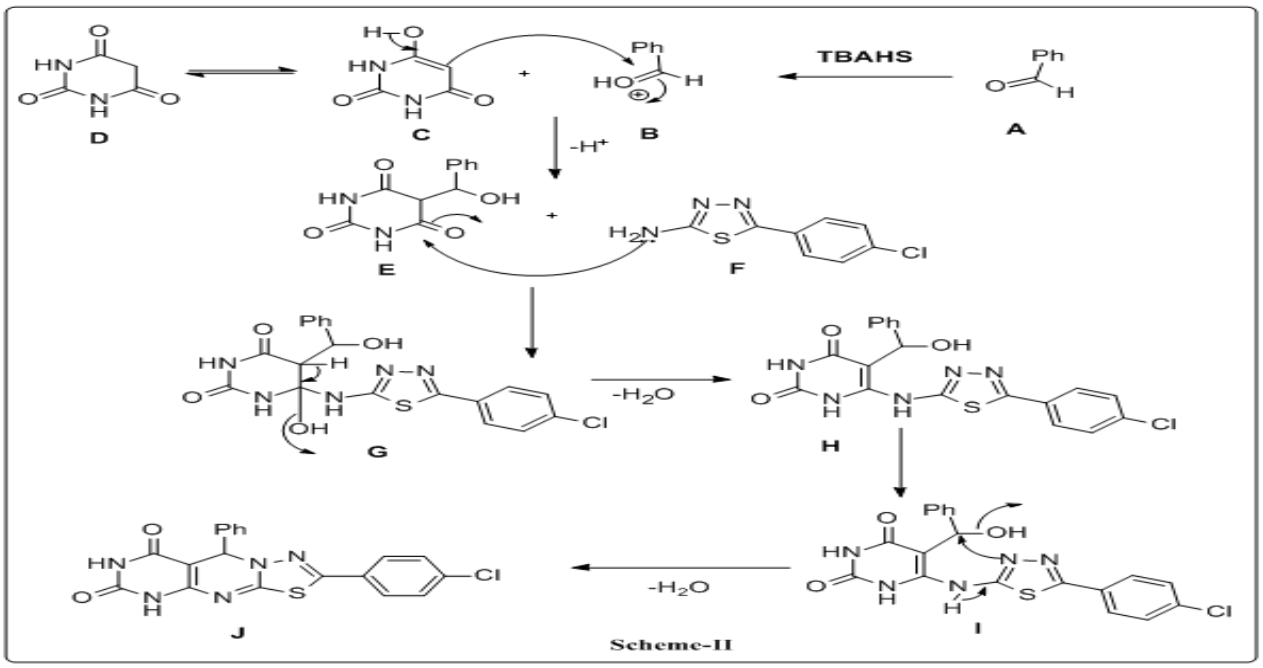
Entry	Solvent	Reaction Time (h)	Yield (%)[b]
1	Toluene	8.0	32
2	DCM	8.0	40
3	DMF	7.0	45
4	Water	6.0	55
5	Ethanol	5.5	65
6	Ethanol-Water	4.0	84

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and Tetrabutyl ammonium hydrogen sulphate were refluxed at 65 ^{0}C.
[b] Isolated yields.

Table 2: Optimization Study for the amount of Tetrabutyl ammonium hydrogen sulphate.[a]

Entry	Catalyst (mole %)	Temperature (^{0}C)	Reaction Time (h)	Yield %[b]
1	01	65	3.0	35
2	02	65	3.0	45
3	05	65	3.0	65
4	08	65	3.0	75
5	10	65	3.0	84
6	15	65	3.0	84
7	20	65	3.0	84

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and Tetrabutyl ammonium hydrogen sulphate were refluxed at 65 ^{0}C.
[b] Isolated yields.



Scheme-II

Table 3: Three component reaction of barbituric acid **(1),** aromatic aldehydes **(2a-j)** and 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole **(3),** for the synthesis of **(4a-4j).**[a]

Entry	Aldehydes	Time (Hrs)	Yield (%)[b]	Mp (^{0}C)
4a	-C_6H_5	3.5	65	204-206
4b	4'-OCH_3 -C_6H_4	3.0	84	210-212
4c	4'-CH_3 -C_6H_4	3.5	70	240-242
4d	4'-Br -C_6H_4	3.5	68	268-270
4e	3'- Br -C_6H_4	3.0	75	220-222
4f	4'-Cl -C_6H_4	3.5	69	278-280
4g	4', 3'-di Cl -C_6H_3	2.5	78	230-232
4h	4'-OH -C_6H_4	3.0	76	240-242
4i	4'-F -C_6H_4	3.5	66	245-247
4j	4'-NO_2 -C_6H_4	4.0	60	286-288

[a] ***Reaction conditions:*** Barbituric acid (1 mmol), 2-amino-5-(4-chlorophenyl)1,3,4-thiadiazole (1 mmol), with substituted benzaldehydes (1 mmol) in EtOH-H_2O and Tetrabutyl ammonium hydrogen sulphate were refluxed at 65 ^{0}C. [b] Isolated yields.

III. 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 300 & 400MHz using TMS as an internal standard.

General procedure for the synthesis of 9-substituted derivatives of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione(4a-j) :

A mixture of barbituric acid **(1)**, different substituted aromatic aldehydes **(2a-j)** and 2-amino-5-(4-chlorophenyl) 1,3,4-thiadiazole **(3)** was refluxed in presence of Tetrabutyl ammonium hydrogen sulphate (TBAHS) (10 mol %) in ethanol-water (10 ml ethanol and 10 ml) for 4hrs. The progress of reaction was monitored by TLC. The reaction mixture was cooled to room temperature and separated solid product was filtered, washed with water and recrystallized from ethanol to afford **(4a-j).** These synthesized products **(4a-j)** were completely characterized from spectroscopic technique and also elemental analysis.

IV. SPECTRAL ANALYSIS

2-(4-chlorophenyl)-9-phenyl-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione (4a) :

M.P. 204-206 ^{0}C, Yield 65%. IR (KBr/ cm^{-1}) 3230 (-NH), 1712,1630 (2 C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 6.6 (s, 1H, -CH), 6.5-8.2 (m, 09 H, Ar-H), 10.2 and 11.2 (2 bs,2H,-NH); EI-MS (m/z: RA %): 409 ($M^{+\cdot}$, 100%), 409 ($M^{+\cdot}$, 37%). Elemental analysis calculated data for $C_{19}H_{12}ClN_5O_2S$; C, 55.68; N, 17.09. Found: C, 5570; N, 17.10.

2-(4-chlorophenyl)-9-(4-methoxyphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione (4b) :

M.P. 210-212 ^{0}C , Yield 84 %. IR (KBr/ cm^{-1}) 3210 (-NH), 1725, 1670 (2 C=O),1268 (-O-R); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 3.12 (s, 3H, -Ar-OCH_3), 6.45 (s, 1H, -CH), 7.0-8.0 (m, 8H, Ar-H), 11.2 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 439 ($M^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d_6/ ppm) δ: 164, 162, 155, 148, 142, 129, 126, 90 ,55, 50, 42, 30. Elemental analysis calculated data for $C_{20}H_{14}ClN_5O_3S$; C, 54.61; N, 15.52. Found: C, 54.62; N, 15.94.

2-(4-chlorophenyl)-9-(4-methylphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*] pyrimidine-6,8 (7*H*,9*H*)-dione (4c) :

M.P. 240-242 ^{0}C, Yield 70 %. IR (KBr/ cm^{-1}) 3205 (-NH), 1710, 1660 (2 C=O) ; ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 2.03 (s, 3H, -Ar-CH_3), 6.20 (s, 1H, -CH), 7.5-8.6 (m, 8H, Ar-H), 11.1 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 423 ($M^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d_6/ ppm) δ: 165, 163, 152, 145, 140, 130, 125, 92 ,52, 48, 40, 32. Elemental analysis calculated data for $C_{20}H_{14}ClN_5O_2S$; C, 56.67; N, 16.52. Found: C, 56.69; N, 16.94.

9-(4-bromophenyl)-2-(4-chlorophenyl)-5*H*-pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidine-6,8 (7*H*,9*H*)-dione (4d) :

M.P. 268-270 ^{0}C, Yield 68%. IR (KBr/ cm^{-1}) 3140 (-NH), 1680,1610 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.9 (s, 1H, -CH), 7.2-8.5 (m, 8H, Ar-H), 10.2 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 486, ($M^{+\cdot}$+3, 100) ; ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 166, 165, 164, 160, 150, 148, 130,132, 130, 126, 120, 90 ,50, 45, 40,30. Elemental analysis calculated data for $C_{19}H_{11}BrClN_5O_2S$; C, 46.69; N, 14.33. Found: C, 46.72; N, 14.35.

9-(3-bromophenyl)-2-(4-chlorophenyl)-5*H*-pyrimido[4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidine-6,8(7*H*,9*H*)-dione (4e):

M.P. 220-222 ^{0}C, Yield 75%. IR (KBr/ cm^{-1}) 3130 (-NH), 1692,1610 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.8 (s, 1H, -CH), 7.2-8.4(m, 8H, Ar-H), 10.2 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 486 ($M^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 169, 168,167, 164, 161,156, 152, 150, 146, 135,134, 130, 129,126, 121, 120, 95 ,40, 40,32. Elemental analysis calculated data for $C_{19}H_{11}BrClN_5O_2S$; C, 46.69; N, 14.33. Found: C, 46.71; N, 14.34.

2,9-bis(4-chlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione (4f) :

M.P. 278-280 ^{0}C, Yield 69% . IR (KBr/ cm^{-1}) 3264(-NH) 1728, 1684 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.9 (s, 1H, -CH), 7.1-8.2 (m, 8H, Ar-H), 10.9 and 11.3 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 443 ($M^{+\cdot}$, 100%); ^{13}C NMR (400 MHz, DMSO–d_6/ ppm) δ: 169,166,162,152,150,140, 135, 130, 125,117,112, 92 ,58, 42, 30.Elemental analysis calculated data for $C_{19}H_{11}Cl_2N_5O_2S$; C, 51.36; N, 15.76. Found: C, 51.38; N, 15.78.

2-(4-chlorophenyl)-9-(2,4-dichlorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione(4g):

M.P. 230-232 ^{0}C, Yield 78%.IR (KBr/cm^{-1}) 3142 (-NH), 1690,1640 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.8 (s, 1H, -CH), 7.10-8.22 (m, 7H, Ar-H),10.30 and 11.22 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 476 ($M^{+\cdot}$ +1, 100%); ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 168, 166 164,150, 140, 135,130, 128,124, 120, 80,40,32,30. Elemental analysis calculated data for $C_{19}H_{10}Cl_3N_5O_2S$; C, 47.67; N, 14.63. Found: C, 47.69; N, 14.65.

2-(4-chlorophenyl)-9-(4-hydroxyphenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione(4h):

M.P. 240-242 ^{0}C , Yield 78 %. IR (KBr/ cm^{-1}) 3510(-OH), 3225 (-NH), 1730, 1680 (2 C=O) ; ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 4.5 (s, 1H, -Ar-OH), 6.05 (s, 1H, -CH), 7.2-8.4(m, 8H, Ar-H), 11.2 and 11.2 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 425 ($M^{+\cdot}$, 100%). ^{13}C NMR (400 MHz, DMSO–d_6/ ppm) δ: 168, 164,150, 142, 135,125,50, 45, 35, 30. Elemental analysis calculated data for $C_{19}H_{12}ClN_5O_3S$; C, 53.59; N, 16.45. Found: C, 53.61; N, 16.46.

2-(4-chlorophenyl)-9-(4-fluorophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione(4i):

M.P. 245-266 ^{0}C, Yield 66%. IR (KBr/ cm^{-1}) 3150 (-NH), 1670,1620 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.6 (s, 1H, -CH), 7.1-8.4 (m, 8H, Ar-H), 10.1 and 11.2 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 427, ($M^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 167, 166, 162, 160, 155, 145, 135,130, 120, 90 ,55, 40,32. Elemental analysis calculated data for $C_{19}H_{11}ClFN_5O_2S$; C, 53.34; N, 16.37. Found: C, 53.36; N, 16.39.

2-(4-chlorophenyl)-9-(4-nitrophenyl)-5*H*-pyrimido[4,5-*d*][1,3,4]thiadiazolo[3,2-*a*]pyrimidine-6,8(7*H*,9*H*)-dione(4j):

M.P. 286-288 ^{0}C, Yield 60%. IR (KBr/ cm^{-1}) 3240 (-NH), 1690,1640 (2C=O); ^{1}H NMR (400MHz, DMSO-d_6 / ppm) δ 5.9 (s, 1H, -CH), 7.2-8.5 (m, 8H, Ar-H), 10.2 and 11.4 (2 bs, 2H,-NH); EI-MS (m/z: RA %): 454, ($M^{+\cdot}$+3, 100); ^{13}C NMR (400 MHz, DMSO–d_6/ppm) δ: 168, 165, 163, 160, 158, 146, 130, 125, 95 ,50, 45,29. Elemental analysis calculated data for $C_{19}H_{11}ClN_6O_4S$; C, 50.17; N, 18.48. Found: C, 50.19; N, 18.50.

V. 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[17]. 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 and 4j)** shows remarkable antioxidant activity against DDPH radical scavenging activity with reference of ascorbic acid (91.4 ± 0.021), (Table **4**).

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 **(4d, 4g and 4j)** shows good OH radical scavenging activity as compared with Ascorbic acid (89.5 ± 0.021), (Table **4**).

Table 4: Antioxidant activity of tested compounds **(4a-4j)**.

Entry	Compound Code	% Radical scavenging activity	
		DPPH radical scavenging	OH radical scavenging
1	4a	45.4 ± 0.65	52.0 ± 1.01
2	4b	62.6 ± 0.66	64.2 ± 1.42
3	4c	60.9 ± 1.46	62.0 ± 1.00
4	4d	86.2 ± 1.06	84.1 ± 0.44
5	4e	80.6 ± 1.40	83.6 ± 1.55
6	4f	78.0 ± 1.35	76.0 ± 1.68
7	4g	80.8 ± 1.20	85.2 ± 1.06
8	4h	62.4 ± 1.60	55.0 ± 1.04
9	4i	66.0 ± 1.01	58.1 ± 1.49
10	4j	84.6 ± 1.26	85.0 ± 1.98
11	**Ascorbic Acid (Standard)**	**91.4 ± 0.021**	**89.5 ± 0.021**

VI. CONCLUSION

In conclusion, We have developed a green, efficient and eco-friendly synthesis for the preparation of pyrimido [4,5-*d*] [1,3,4] thiadiazolo [3,2-*a*] pyrimidinedione **(4a-j)** derivatives by one-pot three component condensation reactions of barbituric acid **(1)**, substituted aromatic aldehyde **(2a-j)**, and 2-amino-5-(4-chlorophenyl) 1,3,4-thiadiazole **(3)** in presence of Tetrabutyl ammonium hydrogen sulphate (TBAHS) in aqueous ethanol medium. The product can be easily isolated by simple workup technique, short time, less expensive, ambient reaction condition, and give excellent isolated yields. These synthesized compounds screened Antioxidant activity.

VII. ACKNOWLEDGMENTS

Authors are grateful to Principal, D.A.B.N. Arts and Science College, Chikhali for providing laboratory facilities, and The Director, Panjab University, Chandigarh for providing spectra.

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