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SYNTHESIS OF 2-AMINO-5ALKYL-1,3,4-THIODIAZOLE CATALYZED BY PHALSA JUICE AND ITS ANTIBACTERIAL STUDY

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Abstract

In contrast of an alkyl chain at the same site mainly adds lipophilicity: 2-amino-5-alkyl-1,3,4-thiadiazoles display solid antibacterial, antifungal and anti-inflammatory effects, yet aryl analogues surpass them in potency across several endpoints. The antibacterial, antitumour, antifungal and antiparasitic assays, because aromatic substitution simultaneously enhances target affinity and cellular uptake. The preparation of 2-amino-5-alkyl-1,3,4-thiadiazoles was accomplished following the established procedure of Arun et. al. Preparation of N-(p-exchanged benzylidene)-5-alkyl-1, 3, 4-thiadiazole-2-amine. N-(p- exchanged benzylidene)-5-alkyl-1,3,4-thiadiazole -2-amine were prepared under the procedure of Bijo Mathew et.al. Antibacterial screening results of 2-Amino-5alkyl-1,3,4-thiodaizole derivatives was screened on bacteria S. aureus & E. coli.

Keywords: *Lipophilicity, Thiadiazoles, Antibacterial, S. aureus & E. coli.*

INTRODUCTION:

2-amino-5-alkyl-1,3,4-thiodiazole is biologically active hetrocyclic compound. Replacing the alkyl attached at the 5th-place of the thiadiazole structure with an aryl group markedly boosts the scaffold's pharmacological breadth. Structure–activity analyses show that the electronic richness and planarity of the aryl ring extend conjugation through the 1,3,4-thiadiazole core, strengthening π-stacking and hydrogen-bond interactions with enzyme pockets and nucleic-acid grooves.[1] By contrast, an alkyl chain at the same site mainly adds lipophilicity: 2-amino-5-alkyl-1,3,4-thiadiazoles display solid antibacterial, antifungal and anti-inflammatory effects[2,3], yet aryl analogues surpass them in potency across several endpoints—antibacterial[4,5], antitumour[6,7], antifungal[5] and antiparasitic[8,9] assays—because aromatic substitution simultaneously enhances target affinity and cellular uptake. The generic framework is depicted with its structure highlighting the modulable 5-aryl position that underpins this broad bio-activity profile.Tatiana S. Kokovina et.al,[10] had synthesized novel procedure for preparation of 1,3,4-thiadiazol-2-amine compounds in a 1-pot manner in presence of reaction between a thio-semicarbazide and acetic acid without toxic additives such as phosphorous chloride or $SOCl_2$. The reaction was investigated using polyphosphate ester. It was found that, using polyphosphate ester, the reaction between the thiosemicarbazide and acetic acid proceeds in one-pot through 3-steps with the formation of corresponding 2-amino-1,3,4-thia-diazole. Bijo Mathew studied[11] involves the reaction of several substituted aromatic acid with thiosemicarbazide in existence of conc. sulphuric acid to give rise 2-amino(5-phenyl-substituted) 1,3,4-thiadiazole compounds through ring formation. These compounds on reaction with other aryl aldehyde in presence of conc. sulphuric acid formed Schiff's bases.

Arun KP et.al.[12] had study synthesis of N-(4-methylbenzylidene)-5-methyl-1,3, 4-thiadiazole-2-amine.

Kaboudin, B et.al[13] had prepared N-(4-F-benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine and N-(4-NO-benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine which possess antimicrobial activity.

Material and Method:

Chemical and Apparatus:-

All necessary, chemical and solvents of AR grade were obtained from local suppliers in Jaunpur (U.P.) representing Sigma & Aldrich Company. These chemicals were used as received, without further purification. Standard, established techniques were employed for the synthesis and identification of the target compounds.

Synthesis: This compound synthesized in three steps:

Step 1- The preparation of 2-amino-5-alkyl-1,3,4-thiadiazoles was accomplished following the established procedure of Arun et al.[14] Equimolar amounts of the appropriate aromatic acid (0.1 mol) and thiosemicarbazide (0.1 mol) were dissolved in 30 mL of concentrated sulfuric acid and heated at 80–90°C in a water bath for 7–8 hours. Upon completion, the reaction mixture was cooled to room temperature, carefully poured onto crushed ice, and neutralized with aqueous ammonia solution. The resulting crude precipitate was collected by filtration, washed thoroughly with distilled water, dried under vacuum, and purified by recrystallization from hot water-ethanol to afford the desired 2-amino-5-alkyl-1,3,4-thiadiazole derivatives in good yields.

Step 2- Preparation of N-(*p*-substituted benzylidene)-5-alkyl-1, 3, 4-thiadiazole-2-amines were prepared under the procedure of Bijo Mathew et al.[15] Equimolar amounts of 2-amino-5-alkyl-1,3,4-thiadiazole (0.06 mol) and the corresponding aryl aldehyde (0.06 mol.) were dissolved in 30ml methanol, treated with a few drops of glacial acetic acid, and heated on a waterbath at 60–70 °C for 4hrs. After methanol was detached below compact force, the crude condensed product were collected and purified by recrystallisation from methanol, affording the desired Schiff bases in good yield.

Step 3- Preparation of 2-Ar-3-(5-alkyl-1, 3, 4-thiadiazol-2-yl)-thiazolidin-4-one with method of Bijo Mathew et al., [15] A series of thiazolidinones was obtained by reacting thio-glycolic acid (0.03 mol) with the corresponding Schiff bases, N-(p-attached benzylidene)-5-alkyl-1,3,4-thia-diazole-2-amine (0.03 mol), in 30 mL of 1,4-dioxane containing a catalyst 4 ml phalsa juice . The mixture was heated on a water bath at 70–80 °C for 7 h, then cooled, poured into cold water, and the resulting precipitate was collected by filtration. Final purification by recrystallisation from rectified spirit afforded the desired thiazolidinone derivatives in good yield.

Preparation of Phalsa Juice:

Fresh unripe awalawas procured from local market Jaunpur. Phalsa pressed in mixer and termed juice was filter using filter paper for clear Phalsa juice.

Composition of phalsa juice:

It is complex mixture of organic acids, vitamins mineral and other inorganic content. It contains 85-90% water, 5-8% citric acid, 0.3-1% malic acid, 1-3% sugar, 30-50 mg/100ml vitamin B_1, B_2& B_3 and pH K, Co, Mg & P minerals. It is highly acidic having pH 2.0-2.6.

Antibacterial Study:

Cup-plate Agar diffusion procedure (Agar as nutrient):-

Organism - Gram positive organism- Staphylococcus aureus and Gram negative organism- Escherichia coli.

Equipments- Sterile cotton swabs, Sterile Petri plates, Sterile cork borer, Sterile test tubes, micropipette, 1ml syringes, inoculating loop and spirit lamp.

Media- Nutrient Agar media from Hi-media employed having following concentrations.

Table 1: Composition of Agar media

S.No.	Component	Amount (gm/L)
1.	Peptic digest of animal tissue	4.8
2.	Sodium chloride	1.60
3.	Yeast extract	1.45
4.	Beef extract	1.45
5.	Agar	14.0

Dissolve 28gm of media in 1 litre DDW by heating sterilized using autoclave at 120ºC temp., 15 lb/inch pressure for 15 min. pH of media is 7.4.

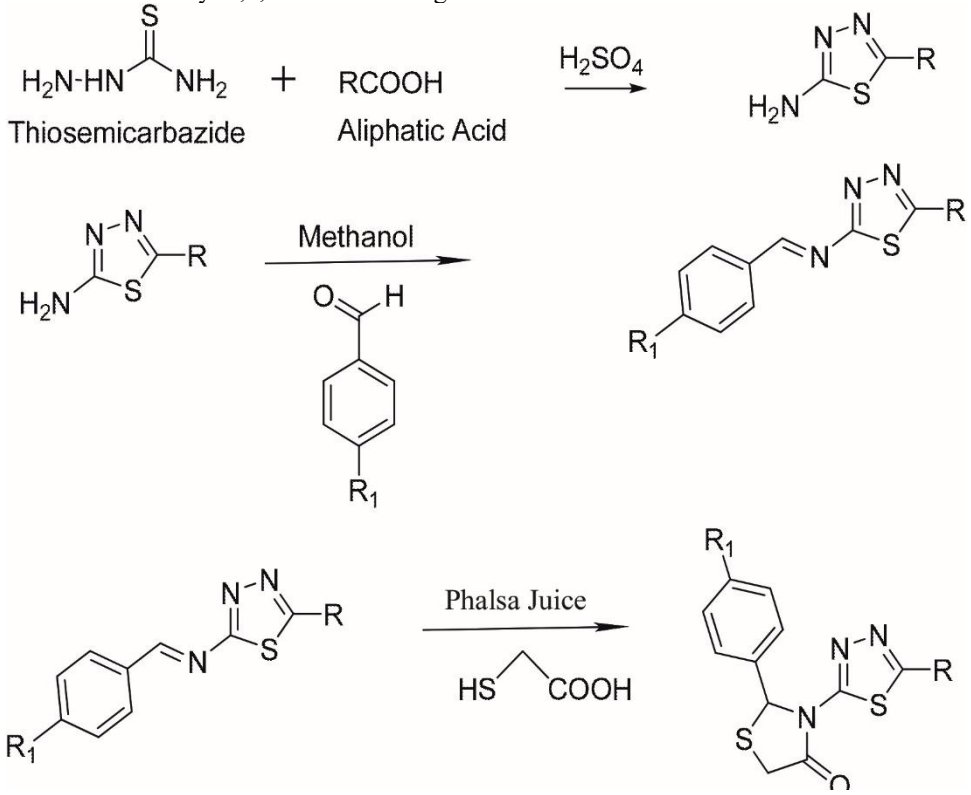
Result & discussion:

Synthesized compound are presented in table 2 along with their code.

Table 2: Coding of compound Synthesized compound

Compound Code	Name of Compound
TD-1	N-(4-chlorobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine
TD-2	N-(4-hydroxybenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine
TD-3	N-(4-Bromo-benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine
TD-4	N-(4-hydroxy-benzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine
TD-5	N-(4-chlorobenzylidene)-5-propyl-1, 3, 4-thiadiazole-2-amine
TD-6	N-(4-hydroxy-benzylidene)-5-propyl-1, 3, 4-thiadiazole-2-amine
TD-7	N-(4-chlorobenzylidene)-5- butyl -1, 3, 4-thiadiazole-2-amine
TD-8	N-(4-Bromo-benzylidene)-5- butyl -1, 3, 4-thiadiazole-2-amine
TD-9	N-(4-hydroxy-benzylidene)-5-butyl-1, 3, 4-thiadiazole-2-amine
TD-10	N-(4-methoxylbenzylidene)-5-butyl-1, 3, 4-thiadiazole-2-amine
TD-11	N-(4-chlorobenzylidene)-5-pentyl -1, 3,4-thiadiazole-2-amine
TD-12	N-(4-hydroxy-benzylidene)-5-pentyl-1,3,4-thiadiazole-2-amine

Synthesis result of 2-amino-5-alkyl 1,3,4 thiadiazole is given below:



R_1 = Cl, Br, OH, OCH_3

R = CH_3, CH_2CH_5, C_3H_7, C_4H_9, C_5H_{11}

Scheme 1: Synthesis of 2-Amino-5alkyl-1,3,4-thiodaizole (1 to 12)

Optimization of Reaction:

Phalsa juice facilitate formation of 2-Amino-5alkyl-1,3,4-thiodaizole. Minimum conc. of awala juice does not affect reaction time and yield. Optimization of juice which alter addition carboxylic acid are present in table 3.

Table 3: Optimization of catalyst (Phalsa juice)

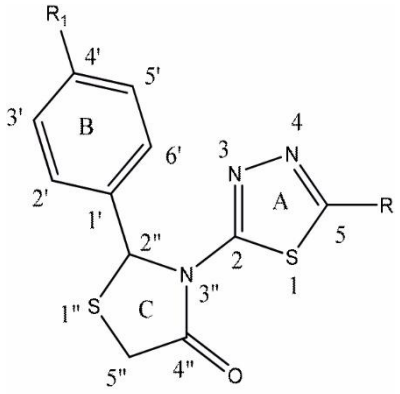
S.No.	Quantity of thiosemicarbozide	Volume of Phalsa juice	Reaction time (min)	Yield %
1	0.01 mol.	0.5ml	120	Nil
2	0.01 mol.	1.0ml	130	Nil
3	0.01 mol.	1.5ml	140	28
4	0.01 mol.	2.0ml	150	46
5	0.01 mol.	2.5ml	150	56
6	0.01 mol.	3.0ml	150	92
7	0.01 mol.	3.5ml	180	83
8	0.01 mol.	4.0ml	180	91

For characterization of synthesis compounds physical data are given in table 4.

Table 4: Physical Data of synthesized compounds

Compd. Code	R	R_1	Yield %	Mol. F.	**Elemental analysis**		M.P.(^{0}C)	
					N	**S**	Rptd.	Fnd
AT-1	CH_3	Cl	71.7	$C_{10}H_8ClN_3S$	17.68/ 17.65	1	135-137[15]	137.5
AT-2	CH_3	OH	72.5	$C_{10}H_9N_3OS$	19.16/ 19.17	2	143-145[15]	146
AT-3	CH_2CH_3	Br	74.5	$C_{11}H_{10}BrN_3S$	14.19/ 14.21	3	142-144[15]	143.5
AT-4	CH_2CH_3	OH	76.07	$C_{11}H_{11}N_3OS$	18.01/ 18.06	4	143-146[15]	145.5
AT-5	$CH_2CH_2CH_3$	Cl	78.6	$C_{12}H_{12}ClN_3S$	15.81/ 15.83	5	170-173[15]	174.7
AT-6	(CH2)2CH3	OH	69.2	C12H13N3OS	16.99/ 17.01	6	148-151[15]	152.2
AT-7	C_4H9	Cl	81.6	C13H14ClN3S	15.02/ 15.06	7	175-178[15]	177.8
AT-8	C_4H9	Br	83.3	C13H14BrN3S	12.96/ 12.74	8	147-150[15]	151
AT-9	C_4H9	OH	74.6	C13H15N3OS	16.08/ 16.12	9	150-153[15]	154.6
AT-10	C_4H9	OCH_3	85.6	C14H17N3OS	15.26/ 15.31	10	140-144[15]	145.2
AT-11	C_5H11	Cl	72.5	C14H16ClN3S	14.30/ 14.36	11	176-179[15]	180.2
AT-12	C_5H11	OH	76.7	C14H17N3OS	15.26/ 15.31	12	154-156[15]	156.2

Characterization:



R_1= Cl, Br, OH, OCH_3
R= CH_3, CH_2CH_5, C_3H_7, C_4H_9, C_5H_{11}

Fig. 1: 2-Amino,5-(Phenylsubstituted)1,3,4-Thia-diazole compounds

Table 5: Classification of compd. derivatives

Compd. Name	**IR KBr(cm-1)**	**1H NMR DMSOd_6 (δppm)**
N-(4-chlorobenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine	1560, 1435, 1392 (C=C structure stre) 1650(C=N), 1092(Ar-Cl), 1045 (N-N), 826 (p-di-exchanged C_6H_6), 656(C-S-C)	8.11(si,1H, CH), 7.02-7.04(4H,m, ArH), 2.43 (3H, s,CH_3)
N-(4-hydroxybenzylidene)-5-methyl-1, 3, 4-thiadiazole-2-amine	3305(Ar-OH), 3041(Ar-C-H stre), 1675 (C=N),1530, 1428, 1329 (C=C structure stre), 642 (C-S-C), 1031(N-N), 821 (p-di-substituted benzene)	8.08(si,1H, CH), 7.02-7.19(4H, mul, ArH), 4.82(1H, s,OH), 2.49(3H, s,CH_3)
N-(4-bromobenzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine	3066(aromatic CH str), 1656(C=N), 1580, 1491, 1305(C=C str), 639(C-S-C), 1070(Ar-Br), 1026(N-N), 809 (p-di-substituted benzene)	8.26(s,1H, CH), 7.04-7.14(mul,4H, Ar-H), 2.22-2.26(q,2H,CH_2), 1.11-1.49(tr,3H,CH_3)

N-(4-hydroxybenzylidene)-5-ethyl-1, 3, 4-thiadiazole-2-amine	3305(ArOH), 3092(Ar C-H str), 1670(C=N), 1592, 1421, 1369(C=C structure stre), 1031(N-N), 813(p-di-exchanged C_6H_6), 648(C-S-C)	8.08(si,1H, CH), 7.04-7.16 (mul,4H, Ar-H), 4.65(si,1H,OH), 2.44-2.48(q,2H,CH_2), 0.91-0.95(tr,3H,CH_3)
N-(4-chlorobenzylidene)-5-propyl-1, 3, 4-thiadiazole-2-amine	3051(Ar C-H stre), 1675(C=N, 1572, 1448, 1370(C=C structure stre), 1082(Ar-Cl), 1032(N-N),814(p-di- exchanged C_6H_6), 651(C-S-C)	8.18 (1H, si, CH), 7.02-7.86(4H, mul, Ar-H), 2.45-2.87 (2H, tr, CH_2), 1.55-1.76(2H, mul, CH_2), 0.93-0.95 (3H, tr, CH_3)
N-(4-hydroxybenzylidene)-5-propyl-1, 3, 4-thiadiazole-2-amine	3304(ArOH), 3092(Ar C-H stre), 1671(C=N), 1551, 1461, 1348(C=C structure stre), 1032(N-N), 812 (p-di-exchanged benz), 652(C-S-C)	8.11(si,1H, CH), 7.0-7.21(4H, mul, ArH), 2.43-2.52(2H, tr, CH_2), 1.58-1.67(2H, mul, CH_2), 0.96-1.02 (3H, tr, CH_3), 4.94-5.02 (si,1H,OH)
N-(4-chlorobenzylidene)-5-butyl -1, 3, 4-thiadiazole-2-amine	3091(aromatic C-H str), 1652(C=N), 1575, 1449, 1362(C=C structure stre), 1088, (Ar-Cl), 1032(N-N), 821 (p-di- exchanged benz), 652(C-S-C)	8.16 (1H,si, CH), 7.14-7.56 (4H,mul, ArH), 2.48-2.67 (2H, tr, CH_2), 1.73-1.38 (2H, mul, CH_2), 1.53-1.55 (2H,mul, CH_2), 0.86-0.96 (3H, tr, CH_3)
N-(4-bromobenzylidene)-5-butyl -1, 3, 4-thiadiazole-2-amine	3072(aromatic C-H str), 1651(C=N), 1572, 1455, 1406(C=C ring str), 1066, (Ar-Br), 1018(N-N), 806 (p-di- exchanged benz), 643(C-S-C)	8.18 (1H, si, CH), 7.13-7.19 (4H, mul, ArH), 2.21-2.23 (2H, tr, CH_2), 1.32-1.37 (2H, mul, CH_2), 1.68-1.73 (2H, mul, CH_2), 0.87-0.93 (3H,tr, CH_3)
N-(4-hydroxybenzylidene)-5-butyl-1, 3, 4-thiadiazole-2-amine	3306(ArOH), 3062(Ar C-H stre), 1675(C=N), 1583, 1465, 1361(C=C structure stre), 1033(N-N), 811 (p-di-exchanged benz), 648(C-S-C)	8.11 (1H, si, CH), 7.03-7.29 (4H, mul, ArH), 5.02 (1H, si, OH), 2.43-2.57 (2H, tr,CH_2), 1.37-1.40 (2H, mul,CH_2), 0.97-0.98 (3H, tr,CH_3)
N-(4-methoxylbenzylidene)-5-butyl-1, 3, 4-thiadiazole-2-amine	3082(aromatic C-H str), 1661(C=N), 1552, 1463, 1391(C=C structure stre), 1334(Ar-OCH_3), 1029(N-N), 813(p-di- exchanged benz), 642(C-S-C)	8.14 (1H, si, CH), 7.06-7.28 (4H, mul, ArH), 3.66-3.69 (3H, si,OCH_3), 2.14-2.18 (2H, tr, CH_2), 1.31-1.54 (2H, mul, CH_2), 1.61-1.70 (2H,mul, CH_2), 0.92-0.98 (3H, tr, CH_3)
N-(4-chlorobenzylidene)-5-pentyl -1, 3, 4-thiadiazole-2-amine	3072 (aromatic C-H stre), 1071(C=N), 1542, 1484, 1381(C=C structure stre), 1081(Ar-Cl), 1036(N-N), 814(p-di-substituted benzene), 641(C-S-C)	8.14(s,1H, CH), 7.13-7.56(mul, 4H, ArH), 2.50-2.76 (tr,2H,CH_2), 1.21-1.24 (mul,2H,CH_2), 1.32-1.38 (mul,2H,CH_2), 1.54-1.62 (2H,mul, CH_2), 0.87-0.94 (3H, tr, CH_3)
N-(4-hydroxybenzylidene)-5-pentyl-1, 3, 4-thiadiazole-2-amine	3311(ArOH), 3062 (Ar C-H stre), 1681 (C=N), 1555, 1466, 1335(C=C structure stre), 1041(N-N), 811(p-di- exchanged benz), 649(C-S-C)	8.08 (si,1H, CH), 7.03-7.28 (4H, mul, ArH), 5.12 (1H, si, OH), 2.44-2.59 (2H, tr, CH_2), 1.37-1.43 (2H, mul,CH_2), 1.47-1.63 (2H, mul, CH_2), 1.66-1.73 (2H, mul, CH_2), 0.94-0.98 (3H, tr, CH_3)

Antibacterial property of 2-Amino-5alkyl-1,3,4-thiodaizole compounds

Antibacterial screening results of 2-Amino-5alkyl-1,3,4-thiodaizole derivatives are given in Table 6.

Table 6: Antibacterial observation of 2-Amino-5alkyl-1,3,4-thiodaizole compounds

Code of Compound	**Area of reticence (mm)**					
	S. aureus			***E. coli***		
	Concentration used in μgml^{-1}			Concentration used in μgml^{-1}		
	100	50	10	100	50	10
TD-1	15	12	10	15	13	10
TD-2	19	15	11	17	15	9
TD-3	17	13	10	15	11	8
TD-4	18	15	11	16	13	10
TD-5	13	8	6	12	8	6
TD-6	12	9	7	11	9	7
TD-7	18	14	11	17	13	9
TD-8	15	11	7	13	11	8
TD-9	11	9	6	12	9	6
TD-10	14	10	8	17	13	10
TD-11	18	14	9	13	10	7
TD-12	10	8	5	15	11	5
Ciprofloxacin (Standard)	**28.5**	**20**	**17**	**21**	**17**	**11.5**

Compounds TD_2, TD_3, TD_4, TD_7 and TD_{11} are more toxic against both bacterial and fungal species at all concentrations.

Conclusion:

More than hundred derivative can be synthesized from this compound. Although synthesis of this compound is multistep but eco-friendly method of synthesis avoids hazardous condition which produced better yield.

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