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SYNTHESIS OF 2-AMINO-5-ARYL-1,3,4-THIADIAZOLE CATALYZED BY ORANGE JUICE AND ANTIMICROBIAL ACTIVITY

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Abstract

Amongst sulfur and nitrogen containing heterocyclic compounds the 2-aminothiadizole scaffold is one of the characteristic structures in drug development as this essential revelation has several biological activities abiding it to act as an anticancer, antioxidant, antimicrobial and anti-inflammatory agent, among other things. Additionally, various 2-aminothiadizole-based derivatives as medical drugs have been broadly used to remedy different kinds of diseases with high therapeutic influence, which has led to their wide innovations. Owing to their wide scale of biological activities their structural variations have produced attention amongst medicinal chemists. This compound was synthesized using biocatalyst orange juice which minimize reaction duration and enhance yield. Maximum compounds shows antibacterial and anti-fungal activities.

Keywords: *2-amino thiadiazole, antibacterial, antifungal, orange juice*

Intoduction:

Humans have faced microbial diseases for centuries. Over time, numerous natural extracts and antimicrobial drugs have been explored by both Ayurvedic practitioners and scientific researchers. However, the rise of multidrug-resistant microorganisms has become a significant global concern, creating an urgent need to discover novel drugs for treating microbial infections. In this context, heterocyclic compounds have emerged as important therapeutic agents due to their versatile applications. Notably, heterocyclic compounds containing nitrogen (N) and sulfur (S) atoms are widely utilized in medicine, industry, and agriculture[1]. Among these, small-ring heterocyclic compounds such as thiazoles and thiadiazoles showed a wide range of pharmacological properties. These include anti-cancer[2], anti-bacterial[3], schistosomicidal[4], antipyretic[5], hypoglycemic[6], anti-HIV[7,8], anti-hypertensive[8], and anti-inflammatory[9] properties. Currently, many thiadiazole-substituted medicine are available. For example: methazolamide and Acetazolamide**:** act as carbonic anhydrase blockers are applied as diuretics.

These compound shows a broad spectrum of medical potential because the nucleophilic 2-amino group can form hydrogen bonds and Schiff bases, the electron-rich and metabolically robust 1,3,4-thiadiazole ring supplies an N=C–S pharmacophore that engages enzyme pockets[10], and the tunable 5-aryl substituent modulates lipophilicity and electronics to target specific pathogens[11] or disease pathways attributes that together underpin its reported antibacterial[12], antifungal[13], antipyretic[14], anticancer[15], hypoglycaemic[16], schistosomicidal[17], antihypertensive[18], anti-HIV[19] and anti-inflammatory[20] activities.

The thiadiazole ring serves as a pharmacophore, meaning it is a functional group or structural component crucial for the biological activity of many drugs. Additionally, the thiadiazole ring is considered a bioisostere of the thiazole ring, which is notably present in the structures of third and fourth-generation cephalosporin antibiotics. This structural similarity enables medicinal chemists to incorporate the thiadiazole moiety in place of thiazole during drug design, thereby expanding opportunities for the synthesis and development of new antimicrobial agents. This substitution can help optimize drug properties and enhance antimicrobial efficacy, making the thiadiazole scaffold valuable in pharmaceutical research and development.[21]

Upadhyay and Mishra[22] prepared derivatives of 5-(4-substituted phenyl)-1,3,4-thiadiazol-2-amine and assessed their antibacterial effects against Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa, along with antifungal activity against Aspergillus niger and Candida albicans using the disk diffusion method; compounds with fluorine and chlorine substituents exhibited strong inhibition ranging from 81% to 91% and MIC values between 20 and 28 μg/mL, similar to ciprofloxacin's. Alok Pandey & team[23] had prepared Schiff bases of 2-amino-5-aryl-1,3,4-thiadiazole where as Mohammad Soleiman-Beigi[24] had synthesized of 2-amino-5-aryl-1,3,4-thia-diazole compounds and studied their biological properties. Karigar asif[25] had synthesized of 2-amin-5-Ar-1,3,4-thiadiazole derivative and screened their antimicrobial properties.

Materials & Methods:

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.

Preparation of the Thiosemicarbazone Intermediate (Panchangan 2018)[13].An equimolar mixture of the target aldehyde (hot ethanolic solution) and thiosemicarbazide (aqueous, glacial acetic-acid–buffered) is placed in a 250 mL conical flask and irradiated at 200 W in a domestic microwave oven for 12 min. Thin-layer chromatography (silica gel G, benzene:methanol 8:2) confirms complete conversion. The hot reaction mass is allowed to cool, the solid thiosemicarbazone is collected by filtration, washed with cold ethanol–water, and recrystallised from absolute ethanol to give analytically pure crystals (m.p. 176 °C).

Preparation of fruit Juice:-

Preparation of fruit juice: Mature oranges from the Jaunpur fruit market (U.P.) were rinsed with tap water, peeled with a clean knife to remove the thick rind, juiced in a citrus electric grinder, the juice was filtered through muslin cloth into a glass beaker, and the clarified extract was stored at 10 °C until use in subsequent syntheses.

Selection of catalyst:-

Different organic and inorganic catalyst used for synthesis of 2-amino-5aryl-1,3,4-thiodiazole synthesis. Those are toxic and hazardous. We had try different biocatalyst for above synthesis but found orange juice is stable for this synthesis. Different quantity of orange juice employed for synthesis.

Optimization of catalyst:

Biocatalyst (orange juice) had been used having different quantities for optimization of reaction. Optimization of reaction are presented in table 1.

Table 1: Optimization of catalyst (orange juice)

Catylest	Quantity used	Time	Yield (%)
Orange juice	2ml	15min	-
Orange juice	4ml	15-20min	-
Orange juice	6ml	16-20min	30%
Orange juice	8ml	16 min	49%
Orange juice	10ml	14 min	71%
Orange juice	12ml	12 min	83%
Orange juice	14ml	12 min	79%
Orange juice	16ml	12 min	81%

Preparation of 5-substituted 1,3,4-Thiadiazole-2-amino derivatives.

Preparation of above compounds are conducted in two steps as under.

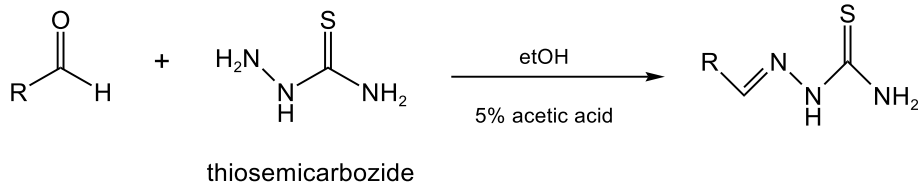
(i) Microwave-assisted synthesis of thiosemicarbazone intermediate:

Equimolar hot alcoholic solutions of the chosen aldehyde and thiosemicarbazide were combined with a catalytic amount of glacial acetic acid in a 100 mL conical flask. The mixture was exposed to 200 W irradiation in a domestic microwave oven for 12 minutes, following the procedure of Panchangan (2018)[11] Development of reaction observed by TLC. On termination of reaction flask was permitted to chill and dense product was getheard by vacuum categorization & recrystallised from ethenol to produce the pure thiosemicarbazone (m.p. 176 °C).

(ii) Synthesis of thiadiazoles derivative:

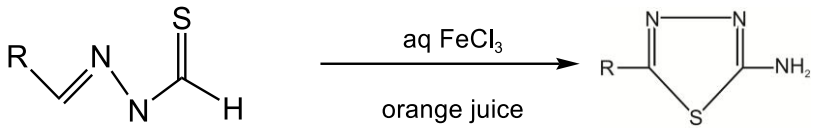
To an aq. solution of 0.05mol. the thiosemicarbazone, a hot aqueous liquid of ferric chloride ($FeCl_3$, 0.015 mol) was added slowly with constant stirring. After an initial 10-minute stirring period, the reaction mixture was brought to reflux at 85–95°C and maintained for 50 minutes to ensure activation. Following this, 12 mL of orange juice was introduced gradually as a biocatalyst.[12] The reaction was completed by neutralizing the mixture with an ammonium hydroxide (NH_4OH) solution, which precipitated the product. The precipitate was then coagulated by filtration, dried and purified by recrystallization from alcohol.

Step-I



thiosemicarbozide

Step-II

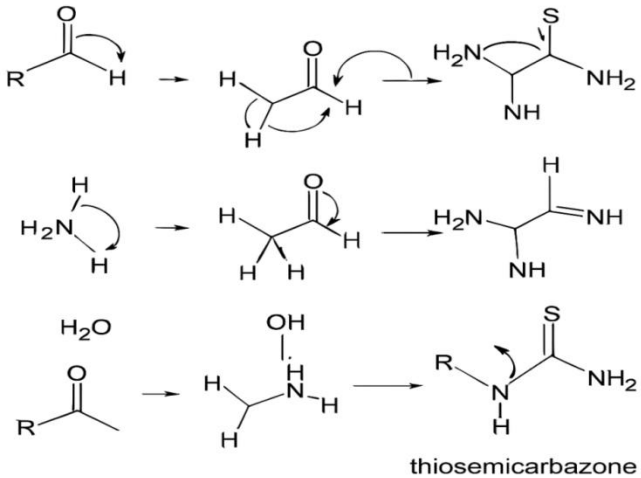


R = 4-Isopropylbenzaldehyde, 4-dimethylaminobenzaldehyde, 3,4-dimethoxybenzaldehyde (Veratraldehyde), 4-fluorobenzaldehyde,
4-chlorobenzaldehyde, 4-chloro-1-methyl-pyrazole-carboxaldehyde
4-methoxybenzaldehyde

Scheme 1: preparation of 2-NH_2-5-Ar-1,3,4-thiadiazole catalyzed with orange juice

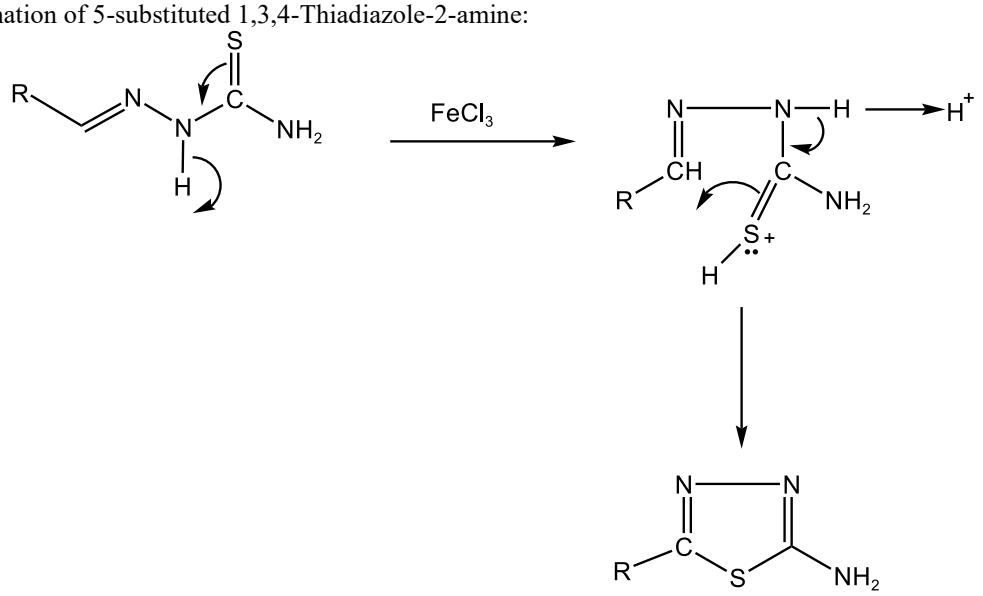
Mechanism for Reaction:

1. Formation of thiosemicarbazone:



thiosemicarbazone

2. Formation of 5-substituted 1,3,4-Thiadiazole-2-amine:



Scheme 2: Mechanism for Reaction

Coding of synthesized compounds:-

As per various aldehyde/acids seven compounds are synthesized. Name of synthesized compounds are R(substituents) are presented in table 2.

Table 2: Coding and name of synthesized derivatives

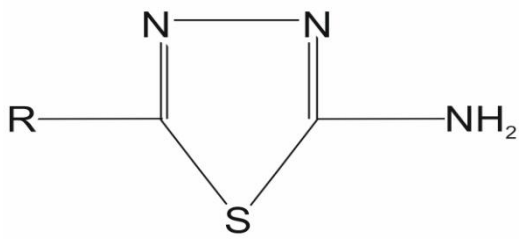
Code of compounds	R	Name of compounds
TZ1	4-isopropylbenzaldehyde	5-[4-(propen-2yl)phyenl]-1,3,4-thiadiazol-2amine
TZ2	4-dimethylaminobenzaldehyde	5-[4-Me_2-amino)ph]-1,3,4-thiadiazol-2amine
TZ3	3,4-dimethoxybenzaldehyde (vertraldehyde)	5-(3,4-Me-o-ph)-1,3,4-thiadiazol-2amine
TZ4	4-fluoro benzaldehyde	5-(4-flurophenyl) -1,3,4-thiadiazol-2amine
TZ5	4-Cl-benzaldehyde	5-(4-Cl-Ph) -1,3,4-thia-diazol-2amine
TZ6	4-chloro-1-methyl-pyrazole carboxaldehyde	5-(4-chloro-1methyl1H-pyrazole3-yl) -1,3,4-thiadiazol-2amine
TZ7	3-nitro benzaldehyde	5-(3-NO_2-Ph)- 1,3,4-thia-diazol-2-NH_2

Physical data of synthesized compounds

Table 3: Physical data of synthesized compounds

S. N.	Name of compounds	Code	Yield %	M.P.		Mol. Formula	Elemental analysis	
				Report.	Obs.		N % found/ cal.	S % found/ cal.
1	5-[4-(propen-2yl)phyenl]-1,3,4-thiadiazol-2amine	TZ1	83	172-176[25]	177	$C_{11}H_{13}N_3S$	19.13/ 19.09	14.53/ 14.55
2	5-[4-dimethylamino)phenyl] -1,3,4-thiadiazol-2amine	TZ2	80.5	135-140[25]	139	$C_{10}H_{12}N_4S$	25.45/ 25.41	14.56/ 14.53
3	5-(3,4-dimethoxyphenyl) -1,3,4-thiadiazol-2amine	TZ3	81.5	148-152[25]	151	$C_{10}H_{11}N_3O_2S$	17.07/ 17.09	13.51/ 13.48
4	5-(4-flurophenyl) -1,3,4-thiadiazol-2amine	TZ4	82.2	214-218[25]	219	$C_8H_6N_3SF$	21.52/ 21.47	16.42/ 14.43
5	5-(4-chlorophenyl) -1,3,4-thiadiazol-2amine	TZ5	81.8	206-210[25]	211	$C_8H_6N_3SCl$	20.13/ 20.16	15.15/ 15.14
6	5-(4-chloro-1methyl1H-pyrazole3-yl) -1,3,4-thiadiazol-2amine	TZ6	78.6	265-270[25]	271	$C_6H_6N_5SCl$	32.92/ 32.89	14.87/ 14.83
7	5-(3-nitrophenyl)- 1,3,4-thiadiazol-2amine	TZ7	79.5	206-208[25]	209.1	$C_8H_6N_4O_2S$	25.31/ 25.33	14.43/ 14.40

Characterization of compounds:



Derivatives of synthesized compounds are characterized by IR and ^{1}H NMR spectroscopy. The spectroscopy data are presented in table 4.

Table 4: 2-amino-5-Ar-1,3,4-thiadiazole compounds

S. N.	R (Aldehydes)	Compounds name	IR ($KBrm^{-1}$)	1HNMRC
1	4-isopropyl benzoldehyde	TZ-1	3095-3270-NH_2 1620(C=N), 1510C (Ar C=C), 2953 (-CH aromatic), 821 (C-S-Cs/r)	1.15-1.20(d,6H) 7.65(d,2H, aromatic), 7.23(d,2H aromamtic), 7.35(2H,S)NH_2, 2.90(m, 1H,-CH)
2	4-dimethylamino benzaldehyde	TZ-2	3140-3250(NH_2) 29644(-CH-Ar) 2895(-CH), 1592(C=N) 1503(C=C, 8.11(C-S-C)	2.71-2.89 (mul, 6H), 5.99-6.71 (mul, 4HJaromt), 6.94-6.99 (db, 2H NH_2)
3	3,4.dimethoxy benzaldehyde	TZ-3	3254-3341(-NH2) 3035 (-CH aromatic), 2951-2825(-CH) 1615(C=N, 1506(C=N, 856(C-S-C)	3.72(s,6H, $(MeO)_2$), 6.85-7.12(m,3H, Ar), 7.23(S,.3H, NH_2)
4	4-Fluoro benzaldehyde	TZ-4	3005-3385(-NH_2), 2975(-CH aromatic), 1582 (N=C), 1509 (C=C), 1006 (F=C). 832 (C-S-C)	7.24-7.35(d, 4H, aromatic 7.54 (s, 2H, NH_2)
5	4.chloro benzoldehyde	TZ-5	3070-3241 (NH_2) 1595 (C=N) 1510 (C=Cor) 825 (C-S-Sc/r) 682 (C-Cl)	7.73 (d,2H, aromatic) 7.50 (d, 2H aromatic) 746 (S,2H, NH_2)
6	4-chloro-1-mehyl-pyrozole carboxeldehyde	TZ-6	3090-3251(NH2) 1632 (C=N) 1501 (C=Car) 2931 (-CH Ar) 832 (c-S-Sc/r) 685 (C-Cl)	3.84 (S,3H, CH_3) 7.39 (S,1H, Ar) 8.06 (S,2H, NH_2)
7	Nitro benzaldehyde	TZ-7	43142-3270(NH_2) 1628 (C+N) 1474 (C=Cs+r) 776 (C-S)	8.296 (S,1H, NH_2) 8.08 (d,1H) 7.92 (d,1H) 7.56 (d,1H) 7.62 (S,2H, NH_2)

Antimicrobial study:

Antibacterial screening was carried out at 10, 50 and 100 mg mL^{-1}. Nutrient-agar medium was melted, poured into Petri dishes and allowed to solidify in an inverted position inside the incubator for about 2.5hrs. Stock liquids of each examinee compound were made at the same three conc. Fresh sub-cultures of the test microorganisms were then evenly swabbed over the agar surface. Sterile paper discs, previously soaked in the respective drug solutions, were placed on the immunized plates & it was incubated at 37°C for 19–23hrs. area of inhibition were observed to assess antibacterial potency.

Observation:

The zones of inhibition were measured in mm by scale. Area of reticence is the diameter of circular area. An important drug ciprofloxacin was also tested under similar condition for comparing the results.

The antibacterial activity of each compounds TZ_1-TZ_7 were evaluated at 10, 50, 100µgmL^{-1} concentrations. The antibacterial property in respect to zone of reserve has been listed in Table-5.

Antibacterial property of 2-amino-5-Ar, 1,3,4-thiadiazole:
The antibacterial observation are presented in table 5.

Table 5: Antibacterial activity of synthesized 2-amino-5-aryl, 1,3,4-thiadiazole 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
TZ1	11	8	3	10	9	7
TZ2	9	5	5	5	4	2
TZ3	10	9	6	9	8	5
TZ4	12	10	7	12	10	5
TZ5	18	16	9	16	12	8
TZ6	13	11	9	11	9	7
TZ7	17	13	10	19	12	8
Ciprofloxacin (Standard)	**29**	**21**	**17**	**21**	**17**	**11**

Analysis of table reveals that compound TZ_4, TZ_6 are more toxic against both the organism *S. aureus* and *E. coli* in order of ciprofloxacin at 100 □g mL^{-1}. It shows that antibacterial activity decreases upon dilution.

Antifungal activity of 2-amino-5-aryl, 1,3,4-thiadiazole:
The antifungal activities of synthesized compounds are presented in table 6.

Table 6: Anti-Fungal property of 2-amino-5-aryl, 1,3,4-thiadiazole

Code of Compound	Area of reticence (m.m.)					
	A. niger			***C. albicans***		
	Concentration used in μgmL^{-1}			**Concentration used in μgmL^{-1}**		
	100	50	10	100	50	10
TZ1	35	24	13	30	19	13
TZ2	32	25	15	31	20	19
TZ3	30	21	14	29	15	13
TZ4	29	22	16	27	17	14
TZ5	35	25	14	30	18	13
TZ6	31	26	15	32	25	24
TZ7	30	23	15	29	14	14
Griseofulvin (Standard)	**70**	**58**	**40**	**62**	**53**	**35**

Above table explained that most of the derivatives showed significant antifungal activity at 100□g/mL against both organism but their toxicity decreased considerably upon dilution. Compound TZ_1, TZ_5 and TZ_8 are more toxic against both fungus at higher concentration.

Conclusion: 5-aryl 2-amino 1,3,4-thiodiazole compounds are prepared with biocatalyst in MW irradiation is avoid catalytic hazardousness. The yield of synthesized derivative enhanced due to employing MW irradiation.

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