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Fruit juice catalyzed synthesis of 5- substituted 1,3,4-thiadiazole 2-amine and their antimicrobial activity

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Abstract

The present study involves the reaction of various substituted aldehyde with thiosemicarbazide in presence of ethanol and 10 ml crane berry fruit juice. With this method we have synthesized 5-(substituted)- 1,3,4-thiadiazole-2-amines (1-7). All the prepared compounds were characterized by elemental analysis, M.P., Co-. TLC, and spectroscopic method. All the compounds were screened in vitro for their antibacterial activity against a variety of microbial strains such as Proteus mirabilis, Pseudomonas aeruginos, staphylococcus aureus and bacillus subtilis. Most of the compounds has shown antibacterial activity when compared with the standard drugs.

Key words- 1,3,4-Thiadiazole, antibacterial screening,Crane berry

Introduction

Heterocycles containing nitrogen, oxygen, sulphur and selenium possess varied pharmacological activities[1]. Thiadiazole is a 5- membered heterocyclic ring system containing two nitrogen and one sulphur atom. Among the three isomers of thiadiazole, 1,3,4-thiadiazole exhibits wide spectrum of biological activities which could be due to the presence of –N=C=S moiety[2]. The detailed literature survey revealed that thiadiazole are widely exposed to therapeutic world, because of their known anti-HIV[3], anti-inflammatory[4], anticancer[5], antitubercular[6], antihypertensive[7], antibacterial[8], antifungal[9], antioxidant, antiprotozoal[10], anticonvulsant[10], diuretic[10], radio protective[11], sedative-hypnotic[8] and CNS neurotoxicity[11] activities. Sulphonamide drugs containing 1,3,4-thiadiazole were reported as antibacterial agents in the market. Further the drugs such as, acetazolamide and methazolamide are the 1,3,4-thiadiazole derivatives and were reported for their diuretic property. Another drug worthwhile to be mentioned here is sulfamethazole, which was referred for their antimicrobial activity[12].

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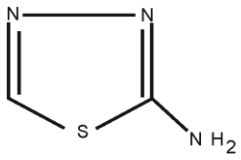
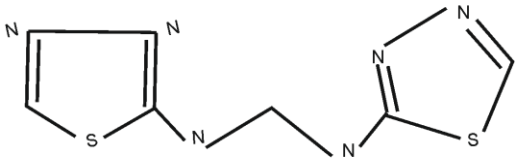
Furthermore, identification of new compounds for the treatment of cancer is an important undertaking in pharmaceutical research. The unusual ring structure of 2-amino-1,3,4-thiadiazoles and their related compounds with their effect in a number of experimental tumor systems have attracted considerable interest in cancer chemotherapy[13]. For example, 2-amino-1,3,4-thiadiazole [compound 1a] for advanced non squamous cervical carcinoma[14] and 2.2-(methylene dimino) bis- 1,3,4-thiadiazole (compound 1b) for antitumor activity[15].



[Compound 1a]

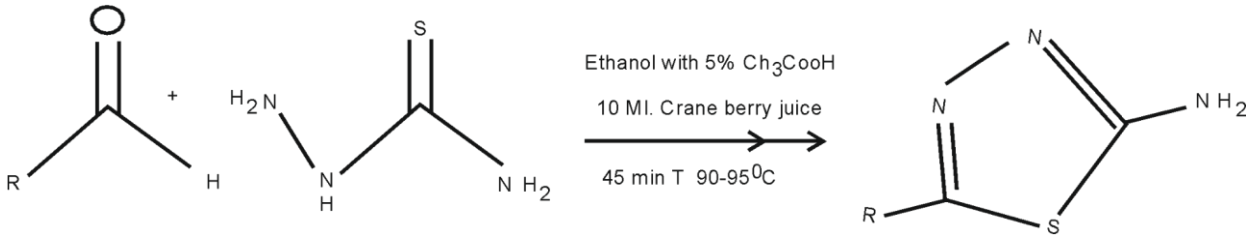


[Compound 1b]

In the view of the above findings, it was considered worthwhile to synthesis new analogues of 1,3,4-thiadiazole with further modification on 2nd and 5th position in order to optimize the structure activity relationship (SAR) and their potency against inflammation and cancer.

Present work

We have developed highly efficient procedure for the synthesis of 5-substituted 1,3,4-thiadiazole-2-amines derivatives using crane berry juice as catalyst. When by catalytic reaction of thiosemicarbazole with crane berry juice we found 2-amino-5-aryl 1,3,4-thiadiazole in high yield.



Chemical and Apparatus:

All the reaction and solvents obtained from local suppliers and used without further purification M.P. was determined on a to shniwal apparatus. IR, ^{1}H NMR spectra of synthesized compounds were recorded on using Shimadzu. FT-IR 8310 in KBR, Proton and ^{13}C recorded on Bruker Biospin Av ance-300MHz.

General procedure of synthesis:

Intermediate compound thiosemicarbazones was prepared by refluxing equimolar quantity of alcoholic solution of aldehyde thiosemicarbazide (5% glacial acetic acid solution).[16]Reaction mixture was cooled at room temperature and recrystallized with alcohol. Suspended aqueous solution of thiosemicarbazone (0.05ml) warm with aqueous solution of $FeCl_3$ (0.015mol.) for 10 min then add crane berry juice (10ml) with constant stirring. Reaction mixture was neutralize with 10% ammonia solution and precipitated amine was filter and recrystallise with alcohol[17].

Preparation of Cranberry juice

Ripe and unripe cranberry was purchased from local market Jaunpur (U.P.) wash thoroughly with running tap water followed by distilled water. After removal of surface water it was grinded into grinder mixer for slurry. Slurry filtered with muslin cotton cloth and juice stored at 10°C for reaction.

Composition of Cranberry juice

Cranberry juice contains citric acid (s) 2.1-4.9% and citric acid (L). Quinic acid 0.3-1.3%, malic acid 1.4-4.3%. Other constituents glucose 29.6-39.6%, Fuctose 58.9-68.7%, sucrose 1.7-1.9%^ and water 87%. In the beginning reaction was performed without catalyst in presence of solvent but no product obtained in visible light assisted reaction. It is interesting fact that visible light irradiation reactions are slower than microwave assisted reaction. The rat of reaction was dependent on the acidity of medium. When we compare effectiveness of fruit juices, it was found that pH valve of cranberry juice (2.9-3.4) is higher than the pH value of apple juice (3.3-3.9), grape juice (3.0-3.7) and pomegranate juice (2.9-3.2) we know as per literature study that biginelli reactions acid catalyst reaction and as acidity increase (decrease in pH value) rate of reaction increases.

Anti –bacterial screening

A. Method :- Cup-plate agar diffusion method usinmg nutrient agar was used.

Petri dishes of agar are prepared by pouring melted agar media prior inoculated of selected microorganism. After the solidification of agar cups are made of the help of borer and cups are filled with solution of suitable concentration of sample and standard respectively and are inoculated at 35°C for 24 hrs. the antimicrobial agents diffuses

using the agar around its cup and produces a characteristic zone of inhibition of the microorganism sensitive to the sample, the diameter of which was measured.

B. Material used:-

1) **Culture:-** Two gram positive (G^+ve) and one gram negative (G^-ve) were preferred for screening.
   - **a)** Gram positive organism: *Staphylococcus aureus* (ATCC29737) and *Bacillus subtilis* (ATCC6633).
   - **b)** Gram negative organism: Escherichia coli (NCTC10418).
2) **Apparatus:-** sterile Petri plates, sterile cotton swabs, sterile cork borer, sterile test tubes, 1ml syringes, micropipette, inoculating loop and spirit lamp.
3) **Media:-** Nutrient agar media from Hi-media was utilized with composition:

Peptic digest of animal tissue	5.00g/ml
Sodium chloride	5.00g/ml
Beef extract	1.50g/ml
Yeast extract	1.50g/ml
Agar	15.00g/ml

Dissolve 28 gm of media in 100ml of distilled water by heating, sterilized utilizing autoclave at 121°C temperature and 151b/Inch^2 pressure for 15 minutes.

C. Preparation of inoculums:-

One day before to these screening, inoculation of the above bacterial was made in the nutrient agar media and incubated at 36°C for 18-24 hrs.

D. Preparation of test solution:-

Any test compound (2mg) was dissolved in *dimethylformamide* (5ml) to provide stock solution of concentration 200 µg/ml. Then 0.1 ml of this solution was used for testing.

E. Preparation of standard solution:-

Standard drug *ciprofloxacin* was used at the concentration of 200 µg/ml.

F. Method of testing:-

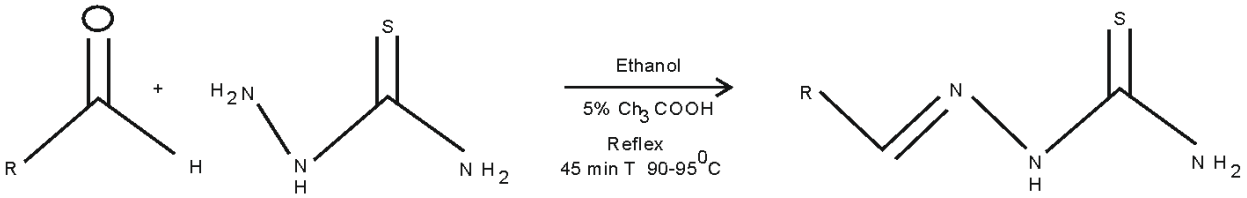
Nutrient agar plates were provide by pouring 15ml of the medium into each sterilized Petri dish and were allowed to set at room temperature. The cell suspension was standardized to the density of 5360 nm using spectrophotometer and was inoculation over the surface of agar medium using sterile cotton swab. The cups were scooped in every plate a sterile cork borer of 6mm diameter[17]

Then the solution of test compounds (0.1ml) were added in cups by using micropipettes and these plates were incubated at 37°C for 48 hrs. The zone of inhibition was measured in mm for every organism.

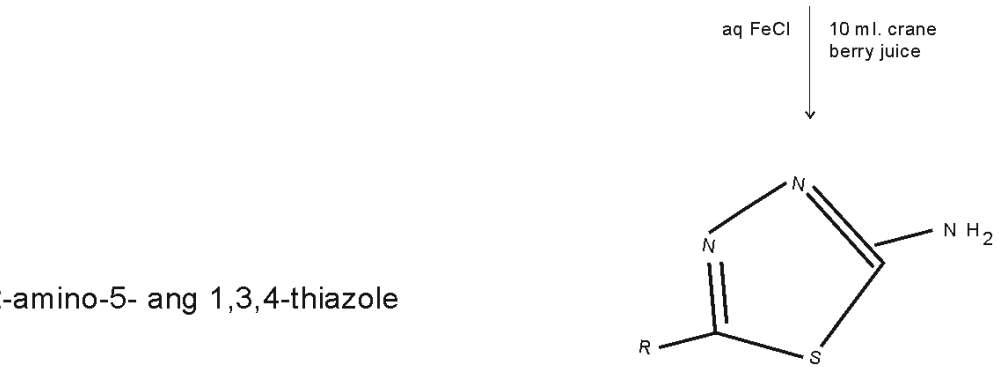
Result and discussions

Synthesis of 5 substituted 1,3,4-thiadiazole-2-amino

As shown is projected scheme of synthesis we prepared through cyclization of thiosemicarbazones. Elemental analysis and M.P. of synthesized compounds are in good agreement with the proposed formulae of the ligands[18].



Aldehgde Thiosemi carbazide Thiosemicarbazole



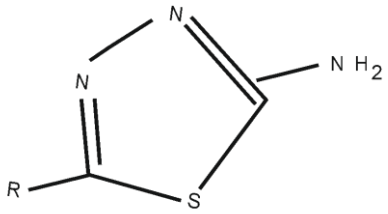
2-amino-5- ang 1,3,4-thiazole

Optimization of reaction

Optimization of reaction with respect to fruit juice as catalyst and reaction time was investigated on selected model of reaction taking aryl aldehyde thiosemicarbazide. All reaction was performed in constant reaction condition except catalyst concentration and reflux time. Completion of reaction was checked on TLC plate.

S. No.	Juice cone(ml)	Reflux time (min)	Yield (%)
1	0.5	30	Trace
2	0.5	45	Trace
3	2	30	Trace
4	2	45	24
5	6	45	50
6	8	45	69
7	10	45	88

Table:-1 Characterization of 2-amino-5 substituted thiadiazoles.



2-amino-5- aryl 1,3,4- thiazoles

S. No.	R	M.P. (°C) Observed		Molecular formula	m/z	
		OBS.	Reported		Found	Reported
1	4-Isopropyl-benzaldehyde	174.5[19]	173.0	$C_{11}H_{13}N_3S$	219.30	220.05[19]
2	4-Dimethyl-aminobenzaldehyde	136.8[19,20]	135.0	$C_{10}H_{12}N_4S$	220.28	221.1[19,20]
3	Vertraldehyde or 3,4,-dimethoxybenzaldehyde	150.0[20]	152.0	$C_{10}H_{11}N_3O_2S$	237.27	238.0[20]
4	4-Fluorobezaldehyde	216.0[21,22]	214-218	$C_8H_6N_3SF$	195.21	196.01[21,22]
5	4-Chlorobezaldehyde	208.5[22]	206-210	$C_8H_6N_3SCl$	211.66	212.5[22]
6	4-Chloro-1-mthyle-pyrazol carboxaldehyde	268.4[23]	265-270	$C_6H_6N_5SCl$	215.56	215.66[23]
7	3-Nitrobenzaldehyde	207.5[23,24]	206-208	$C_8H_6N_4O_2S$	222.9	222.22[23,24]

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Table:-2 Spectral analysis of synthesized 2-amino-5-substituted thiadiazole.

S. No.	I.U.P.A.C name	IR	^{1}H NMR	^{13}C-NMR
1	5-[4-(propan-2yl)phenyl]-1,3,4-thiadiazole-2-amine	3091-3275 (NH_2) 1628 (C=N), 1507 (C=Car), 2957 (-CH aromatic) 823 (C-S-Cstr)	1-16-1.21 (d,6H), 7.64 (d,2H, J=7.6Hz, aromatic), 7.31 (d, 2H, J=7.6Hz, aromatic), 7.33 (2H(S) NH_2), 2.87 (m, 1H, -CH)	23ppm, 32ppm, 128ppm, 126ppm, 129ppm, 127ppm, 151ppm, 155ppm & 169ppm
2	5-[4-(Dimethylamine)phenyl]-1,3,4-thiadiazole-2-amine	3145-3249 (NH_2) 2947 (-CH aromatic), 2894(-CH), 1595 (C=N), 1510 (C=C), 813 (C-S-C)	2.7-2.9 (m, 6H), 6.6-7.09 (m, 4H, aromatic), 7.7-7.8 (d,2H, NH_2)	44ppm, 112ppm, 111ppm, 126ppm, 126ppm, 128ppm, 131ppm &132ppm
3	5-(3,4-dimethoxyphenyl)-1,3,4-thiadiazole-2-amine	3254-3347 (NH_2) 3041 (-CH aromatic), 2955-2824(-CH),1615 (C=N), 1505 (=N), 854 (C-S-C)	3.75 (S,6H, dimethoxy), 6.8-7.2 (m, 3H, aromatic), 7.25 (S, 2H, NH_2)	56ppm, 57ppm, 108ppm, 110ppm, 121ppm, 125ppm, 150ppm, 151ppm, 157ppm &169ppm

4	5-(4-Fluorophenyl)-1,3,4-thiadiazole-2-amine	3000-3401 (NH_2) 2975 (-CH aromatic), 1592(C=N),1507 (C=C), 1000 (C=F), 828 (C-S-C)	7.25-7.37 (d, 4H, aromatic), 7.59 (S, 2H, NH_2)	117ppm, 129ppm, 156ppm, 161ppm, 165ppm&169ppm
5	5-(4-chlorophenyl)-1,3,4-thiadiazole-2-amine	3071-3244 (NH_2) 1592 (C=N), 1509 (C=Car), 828 (C-S-Cstr), 682 (C-Cl)	7.76 (d,2H, aromatic, J=8Hz), 7.52 (d, 2H, aromatic, J=7.6Hz), 7.46 (S, 2H, NH_2)	128.4ppm, 129.7ppm, 130.4ppm, 134.5ppm, 155.7ppm & 169.5ppm
6	5-(4-Chloro-1-methyl-1H-pyrazol3-yl)-1,3,4-thiadiazole-2-amine	3093-3257 (NH_2) 1637 (C=N), 1499 (C=Car), 2937 (-CH aromatic) 829 (C-S-Cstr), 682 (C-Cl)	3.82 (S, 3H, CH_3), 7.38 (S, 1H, aromatic), 8.05 (S, 2H, NH_2)	107ppm, 133ppm, 139ppm, 148ppm, & 169ppm
7	5-(3-Nitrophenyl)-1,3,4-thiadiazole-2-amine	3138-3269 (NH_2) 1623(C=N),1471(C=Cstr), 775 (C-S)	8.294 (S, 1H, NH_2), 8.07 (d, 1H, 7.2Hz), 7.93 (d, 1H, 7.2Hz), 7.59 (1H(t),8Hz,7.6HZ)	120.5ppm, 124.1ppm, 131.4ppm, 132,9ppm, 132.7ppm, 148.7ppm, 154.6ppm & 169.7ppm

Antibacterial study:-

Agar plate were perceived with 16- 20 hrs and may be continued to incubate for 48 hrs. The zone of prohibition of the synthesized compounds were measured and the compound with the standard compound disc.

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Compounds	**M I C (µg/ml)**			
	Proteus mirabilis	Pseudomonas aeruginos	Staphylococcus aureus	Bacillus subtilis
1	98	97	42	52
2	85	114	70	85
3	70	104	62	73
4	75	90	55	72
5	66	87	48	67
6	73	75	46	69
7	84	82	45	66
Ciprofloxacin	29	27	28	25

All synthesized compounds were evaluated for antibacterial activity by serial dilution method. All the synthesized compounds possessed antibacterial activity. MIC value of the compound was deduced from the antibacterial assay method employed.

Conclusion

On behalf of above finds worthwhile synthesis of new analogues 1,3,4-thiadiazole with further modification on 2^{nd} and 5^{th} position of heterocyclic rings. The optimization of structure activity relationship (SAR) it concluded that its potency enhance against inflammation and cancer.

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