SYNTHESIS AND ANTIMICROBIAL ACTIVITY OF SOME NEW 2,5 DISUBSTITUTED 1,3,4-THIADIAZOLES

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Abstract

Aniline, 4-Chloro aniline, 3,4-dichloro aniline were treated with carbon disulphide and concentrated ammonia in the presence of lead nitrate and methanol to get 1-Phenyl, 4-Chlorophenyl, 3,4-dichlorophenylisothiocynates respectively. Ethyl benzoate, Methyl salicylate, 4-hydroxy methyl benzoate, 4-amino ethyl benzoate, 2-bromo ethyl benzoate, 4-bromo ethyl benzoate, 3,4-dimethoxy benzoates were treated with hydrazine hydrate(98%) in presence of absolute ethanol to get their respective substituted benzo hydrazides. which are further treated with 1-phenyl, 4-chlorophenyl, 3,4-dichlorophenyl isothiocynates in presence of absolute ethanol to get their respective substituted Thiosemicarbzides.which are undergo dehydrative cyclization with concentrated sulphuric acid to furnished with corresponding substituted 1,3,4-thiadiazoles.the newly synthesized compounds were characterized by spectral and elemental analysis and the compounds were tested for antimicrobial activity.

Key words: Thiadiazole,phenyl isothionates,benzohydrazide.

Introduction

The chemistry of 1,3,4-thiadiazoles and its derivatives have been studied due to their close association with diverse pharmacological properties. Owing to the importance and established physiological activity of these compounds, it was thought to synthesize and investigate compounds with comparable structures. Thus the basis of the present investigation was centered around the fact that certain structural units present in biological active compounds are also found in other compounds of similar properties. Affecting structural variation and modifying molecular structure could better explore biological activity. It is well established that slight alterations in the structure of certain compounds are able to bring drastic changes in biological activity.1,3,4-thiadiazole derivatives were among the various heterocycles that have received a great deal of attention during last decades especially as antimicrobial agents most of the substitution at 2nd and 5th position of thiadiazole ring.Owing to the importance and established pharmacological activity of these compounds we are directed our attention towards synthesis of some new 2,5,disustituted 1,3,4-thiadiazole derivatives with object of screening them for antimicrobial activity.

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Materials and methods

All the chemicals required for the present study were obtained from SD Fine chemicals, Mumbai. Melting points were determined by open capillary tube method and using melting point apparatus were uncorrected. TLC was run on silica gel-g plates using benzene: acetone (8:2) as irritants; the spot were located by exposure to iodine vapors as visualizing agent. The IR of the compounds were recorded on Thermo Nicolet FTIR 200 spectrophotometer by using KBr pellet technique and ^{1}H NMR of the title compounds was recorded on BRUKER ADVANCE II 400 NMR spectrometer. DMSO and $CDCl_3$ were used as solvents. The mass spectra of the compounds were recorded using FAB+.

During the present investigation Analine, 4-Chlro aniline,3,4-dichlroanaline is condensed with carbon disulphide and concentrated ammonia in presence of methanol to get respective phenyl isothocynates (I).Ethyl benzoate, Methyl salicylate, 4-hydroxy methyl benzoate, 4-amino ethyl benzoate, 2-bromo ethyl benzoate, 4-bromo ethyl benzoate, 3,4-dimethoxy benzoates were

treated with hydrazine hydrate(98%) in presence of absolute ethanol to get their respective substituted benzohydrazides (II).Treatment of benzohydrazides (II) with phenyl isothiocynates (I) in presence of absolute ethanol to get respective substituted Thiosemicarbazides (III) which are treated with concentrated sulphuric acid gave the title compounds (Scheme). The structure of all new compounds were established by spectral and analytical data.

SCHEME

R=H, 4-Cl, 3,4-Dichloro
R_1=CH_3, C_2H_5
R_2= H, 2-OH, 4-OH, 4-NH_2, 2-Br, 4-Br, 3,4-Dimethoxy

2.1 General procedure for the Synthesis of phenyl isothiocynates

A mixture of analine (0.06 mole,7.68gm), carbon disulphide (0.09mole,5.8ml) and methanol (15 ml) were cooled at about 10^0C.concentrated ammonia (33%,0.32 mole, 5 ml) was added drop wise to the reaction mixture with continues stirring. The mixture was allowed to stand overnight. water was added to the reaction mixture (100 ml). An aqueous solution of lead nitrate (0.06 mole, 20.6 gm) was slowly added to the solution the mixture was then steam distilled to yield phenyl isothiocynates.

The IR spectrum: The phenylisothiocynates exhibits the prominent characteristic absorption band at 2089 cm^{-1} attributed to N=C=S group

2.2 General procedure for the Synthesis of 1-Benzohydrazides

Dissolved the Aromatic esters (0.1mole) in 50 ml of ethanol and hydrazine hydrate (0.15 mole, 7.3 ml 98%) was added drop wise to the reaction mixture with stirring. The resulting mixture was refluxed for 6hrs.excess ethanol was distilled out and the contents were allowed to cool. Then mixture was added to crushed ice. The resulting solid product was filtered and recrystalised from ethanol.

The IR spectrum:

The 1-benzohydrazides exhibiting the characteristic band in the region 3220-3299 cm^{-1} NH-NH_2(NH stretching), 3018 cm^{-1}(Aryl, CH Stretching), 1660cm^{-1}(C=O Stretching) 1614 cm^{-1} (C=N Stretching) and 1567 cm^{-1}

2.3 General procedure for the Synthesis of 2-benzoyl-N-(Phenyl)-thiosemicarbzides.

1-benzohydrazides (0.01mole)was dissolved in absolute ethanol(30-40ml) depending upon the solubility and phenyl isothiocynates (0.01 mole,) was separately dissolved in absolute ethanol (30 ml).Then the solution of phenyl isothiocynate was poured in to the solution of hydrazides with continuous stirring. The reaction mixture was refluxed for 1hr. then concentrated to 1/3rd of its volume and cooled to room temperature. As a result white solid crystals appeared. The solid was then filtered and recrystalised from ethanol.

The IR spectrum: The 2-benzoyl-N-(4-chlorophenyl) thiosemicarbazides exhibits the characteristic band in the region 3177 cm^{-1} (NH stretching),1665 cm^{-1}(C=O Stretching), 1548 cm^{-1} (C=C Stretching),1605 cm^{-1} (C=N Stretching), 1254 cm^{-1} C=S (Stretching).

2.4 General procedurer for the Synthesis of 2-(4-chlorophenyl, 1-phenyl, 3,4dichlorophenyl amino)-5-(1-phenyl, 2-hydroxyphenyl 4-hydroxyphenyl, 4-aminophenyl, 2-bromophenyl, 4-bromophenyl and 3,4,-dimethoxyphenyl)-1,3,4-thiadiazoles (A_1-A_{15}).

Each thiosemicarbzide (0.0007 mole, 0.2gm) was added portion wise to 25 ml of concentrated sulphuric acid at 0^{o}C with continuous stirring. The reaction mixture was further stirred for 3hrs.at room

temperature. Then it was poured in to ice-water mixture to precipitate a crude solid. The crude solid was filtered, dried and recrystalised from a mixture of acetic acid and water (1:1) to furnish 2,5,-disustituted 1,3,4-thiadiazoles and characterization of these compounds are given in table-1.

Spectral Datas of final compounds IR Spectrum:

The compound A_1 N-(4-chlorophenyl)-5-phenyl 1,3,4-thiadiazol-2-amine exhibits the characteristic bands in the region 3236 cm^{-1} (NH stretching),3049 cm^{-1} (Ar CH Stretching),1544, 1488 cm^{-1}(C=C ring Stretch),1599 cm^{-1} (C=N Stretching), 721 cm^{-1} (C-Cl Stretching).

^{1}H NMR spectrum: The compound A_1 shows δ 10.14 (s,1H, NH), δ 7.25-7.85 (m,9H, Ar-H),

Mass spectrum: The compound A_1 shows M+1 peak at 289

IR Spectrum: The compound A_6 5-(4-bromophenyl)-N-(4-chlorophenyl) 1,3,4-thiadiazol-2-amine exhibits the characteristic bands in the region 3406 cm^{-1} (NH Stretching), 2923 cm^{-1} (Ar CH Stretching), 1606 cm^{-1} (C=N Stretching),1566,1495,1439 cm^{-1} (C=C ring Stretch), 756 cm^{-1} (C-Cl Stretching).

^{1}H NMR spectrum: The compound A_6 shows δ 10.30 (s,1H, NH), δ 7.25-7.75 (m, 8H, Ar-H).

Mass spectrum: The compound A_1 shows M+1 peak at 366.

IR absorption bands of remaining similar compounds illustrated in table no-2 and all remaining similar compounds shows NMR signals at δ 10.00-10.5 ppm singlet for NH hydrogen and δ 7.25-8.35 ppm multiplate for aromatic hydrogens.

Antimicrobial activity:

The synthesized compounds are screened against bacteria and fungi to know there antimicrobial activity. To screen these compounds for antibacterial activity bacteria's like Staphylococcus aureus (Gram+ve) and Escherichia coli (Gram-ve) and for he antifungal activity fungi like Candida albicans were used and this is done by broad dilution method.

The compounds shows considerable activity against all species tested at 100 µgm/disc and 200µgm/disc of the compounds and results are illustrated in table-3 and Ciprofloxacin were used as std drug. The compounds shows significant antifungal activity against Candida albicans and result are tabulated in table-4 and Fluconazole were used as std drug.

Table-1: Characterisation and physical data of synthesized compounds

Compound Code	R	R_2	Molecular formula	MP(°C)	%YIELD	%Analysis,Found(Calcd) C	H	N
A_1	p-Cl	H	$C_{14}H_{10}ClN_3S$	226-228	74.12	58.53	3.48	14.63
A_2	p-Cl	o-OH	$C_{14}H_{10}ClN_3OS$	238-240	70.36	55.44	3.30	13.08
A_3	p-Cl	p-OH	$C_{14}H_{10}ClN_3OS$	276-277	55.29	55.44	3.30	13.08
A_4	p-Cl	p-NH_2	$C_{14}H_{11}ClN_4S$	188-190	79.52	55.62	3.64	18.50
A_5	p-Cl	o-Br	$C_{14}H_9BrClN_3S$	248-250	45.24	46.02	2.46	11.50
A_6	p-Cl	p-Br	$C_{14}H_9BrClN_3S$	262-264	55.69	46.02	2.46	11.50
A_7	p-Cl	Om di-OCH_3	$C_{16}H_{14}N_3SCl$	222-224	30.47	60.95	4.44	13.33
A_8	H	H	$C_{14}H_{11}N_3S$	296-298	82.01	66.40	4.34	16.6
A_9	H	o-OH	$C_{14}H_{11}N_3OS$	268-270	75.34	62.45	4.09	15.60
A_{10}	H	o-Br	$C_{14}H_{10}BrN_3S$	265-266	63.69	50.75	3.02	12.68
A_{11}	H	p-Br	$C_{14}H_{10}BrN_3S$	276-278	73.49	50.75	3.02	12.68
A_{12}	o.m, di-Cl	H	$C_{14}H_9Cl_2N_3S$	236-238	48.75	52.33	2.08	13.08
A_{13}	o,m di-Cl	o-OH	$C_{14}H_9Cl_2N_3OS$	228-230	28.87	49.85	2.37	12.46
A_{14}	o.m, di-Cl	o-Br	$C_{14}H_8BrCl_2N_3S$	250-252	42.29	42.10	2.00	10.52

All compounds gave correct elemental data.

Table-2 Characteristic IR absorption bands of remaining similar compounds

Compound code	NH cm^{-1}	OH cm^{-1}	Ar-CH cm^{-1}	C–N cm^{-1}	C–C cm^{-1}	C-Cl cm^{-1}	C-Br cm^{-1}
A_2	3254	3437	2992	1619	1563,1498	760	
A_3	3250	3431	3001	1607	1552,1482	757	
A_4	3211	-	3001	1601	1546,1491	773	
A_5	3303	-	2998	1601	1566,1495	755	
A_7	3220	-	2995	1600	1556,1499	753	
A_8	3224	-	3054	1598	1548,1492	722	
A_9	3302	3402	3003	1603	1547,1478	722	
A_{10}	3300	-	3000	1603	1547,1478	-	650
A_{11}	3305	-	3006	1606	1547,1475	-	652
A_{12}	3324	-	2998	1617	1534,1493	747	
A_{13}	3252	3452	3005	1600	1538,1493	747	
A_{14}	3348	-	3009	1600	1534,1493	750	
A_{15}	3350	-	3008	1603	1534,1495	750	

Table-3: Antibacterial screening result of compounds A_1-A_{15} diameter of Inhibition zone (in mm)

Compound code	S.aureus		E.coli	
	100 µgm	200 µgm	100 µgm	200 µgm
A_1	03mm	05mm	05mm	06mm
A_2	28mm	29mm	28mm	29mm
A_3	02mm	03mm	06mm	04mm
A_4	26mm	27mm	27mm	28mm
A_5	04mm	05mm	05mm	06mm
A_6	03mm	04mm	05mm	06mm
A_7	03mm	05mm	05mm	06mm
A_8	05mm	05mm	05mm	06mm
A_9	06mm	05mm	05mm	06mm
A_{10}	06mm	05mm	05mm	06mm
A_{11}	28mm	29mm	28mm	29mm
A_{12}	27mm	28mm	25mm	26mm
A_{13}	08mm	06mm	05mm	06mm

NOTE: Ciprofloxacin was used as a standard. Resistant=5-10mm Sensitive = 25 - 35mm

Table-4: Evaluation of antifungal activity of the synthesized compounds against Candida albicans.

SL. NO.	Compound	Observation	Interpretation
1	A_1	No colonies	Sensitive
2	A_2	No colonies	Sensitive
3	A_3	Few colonies, around 50 CFU	Intermediate
4	A_4	No colonies	Sensitive
5	A_5	Few colonies, around 50 CFU	Intermediate
6	A_6	Abundant colonies	Resistance
7	A_7	No colonies	Sensitive
8	A_8	Few colonies, around 50 CFU	Intermediate
9	A_9	No colonies	Sensitive
10	A_{10}	No colonies	Sensitive
11	A_{11}	No colonies	Sensitive
12	A_{12}	No colonies	Sensitive

Positive control (Fluconazole: 64 µg/ml):- Sensitive (No colonies were observed). Negative control (plane SDA):- Resistant (Abundant colonies). CFU = Colony Forming Unit.

Results and discussion

The synthesized compounds illustrated in scheme. The characterization of synthesized compounds is based on the IR, NMR and Mass spectra.

The IR spectra of phenyl isothiocynates showed characteristic absorption at 2089 cm^{-1} for N=C=S group and the IR spectra of substituted benzohydrazides showed the characteristic absorption at 3299-3220 cm^{-1} for NH-NH_2 and 1660 cm^{-1} for C=O-NH. The IR spectra of substituted thiosemicarbzides showed characteristic absorption at 1548-1605 cm^{-1} for C=C/ C=N and showed the peak in the region 1665-1680 cm^{-1} due to the Carbonyl absorption and characteristic absorption at 1240-1258 cm^{-1} attributed to the C=S thiourea residue. The dehydrative cyclization of these thiosemicarbzide in concentrated H_2SO_4 afford corresponding substituted thiadiazoles.in IR spectra of these compounds shows the absence of signals in the

region 1655-1682 cm^{-1} established the lack of C=O group however they exhibited the absorption of N-H in the region 3200-3400 cm^{-1} In the NMR spectra of these compounds were observed in expected region. The proton signal due to the NH group was recorded between 10.00 to 10.50 ppm and the aromatic protons signals appear at 6.80 to 8.35 ppm.

Anti-bacterial activity

Synthesized of 2-(1-phenyl, 4-chlorophenyl, 3,4-dichlorophenylamino)-5-(1-phenyl, 2-hydroxyphenyl, 4-hydroxyphenyl 4-aminophenyl, 2-bromophenyl,4-bromophenyl, 3,4-dimethoxyphenyl)-1,3,4-Thiadiazoles were tested for the antibacterial activity against gram +ve (Staphylococcus aureus) and gram –ve (*Escheria coli*), the tested compounds A_2, A_4, A_{11} and A_{12} shows considarable antibacterial activity compared to standard drug Ciprofloxacin.

Anti-fungal activity

Synthesized of 2-(1-phenyl, 4-chlorophenyl, 3,4-dichlorophenyl amino)-5-(1-phenyl, 2-hydroxyphenyl, 4-hydroxyphenyl 4-aminophenyl, 2-bromophenyl, 4-bromophenyl, 3,4-dimethoxyphenyl)-1,3,4-Thiadiazoles were tested for antifungal activity against *Candida albicans* Among the compounds tested; A_1, A_2, A_4, A_7, A_9, A_{10}, A_{11}, A_{12}, A_{14}, showed significant antifungal activity and A_3, A_5, A_8 showed less antifungal activity against *Candida albicans* compared to activity shown by standard Fluconazole.

Conclusion:

Antimicrobial activity of all 15 compounds were determined using Ciprofloxacin and Flucanzole as standard drugs.Amongst all compounds only A_2, A_4, A_{11},and A_{12} showed antibacterial activity against *S.aureus*(gram+ve) and *E.coli* (gram-ve) compared to standard drug and A_1, A_2, A_4, A_7, A_9, A_{10}, A_{11}, A_{12}, A_{14} showed significant antifungal activity and A_3, A_5, A_8 showed less antifungal activity against *Candida albicans* compared to activity shown by standard Fluconazole still further study is required.

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References

Biological assay, *Indian Pharmacopoeia* published by Govt. of India., 1996; (2): A-88

Hatice N D, Arzu D, Sevim R, Goksel S, Mearl K U, Damarul G. *Biorg. Med. Chem.* 2002; 10 (5): 2893-2898.

Khosrow Z, Khalil F, Taranch T, Mohammad R, Shariatzadeh. *Turk. J. Chem.* 2002; 27 (3): 119-125.

Khosrow Z, Khalil F, Taranch T, Mohammad R, Shariatzadeh. *Turk. J. Chem*. 2003; 28 (6): 95-100,

Mathew V, Keshavayya J, Vaidya V P. *Eur. J. Med. Chem*. 2006; 41(3):1048-1058,

Stahle H.Med. Chem. Proc. Int. Symp Main Lect, 1974; 75.

Talawar MB, Desai S R, Somannavar Y S, Marihal S C, Bennur S C.

Zuhair Muhi-Eledeen Fahad Hussain, Salman Rasheed. *Eur. J. Med. Chem.* 1998; 23: 133-137.