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Synthesis and antimicrobial activity of some new 2,5 disubstituted 1,2,4-triazoles

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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 Thiosemicarbazides.which are undergo dehydrative cyclization with concentrated sodium hydroxide to furnished with corresponding substituted 1,2,4-triazoles.the newly synthesized compounds were characterized by spectral and elemental analysis and the compounds were tested for antimicrobial activity.

Key words: Triazole, phenylisothiocynates, benzohydrazide.

INTRODUCTION

Antimicrobials reduce or completely block the growth and multiplication of bacteria. This has made unique for the control of deadly infectious diseases such as Pneumonia, meningitis, tuberculosis, malaria and aids. Derivatives of 1,2,4-triazole condensed nucleus system found to have diverse pharmacological activities. Such as fungicidal, insecticidal, bactericidal, herbicidal, antitumor, anti-inflammatory, CNS stimulant properties and antiviral agents. Examples of such compounds bearing the 1,2,4-triazole moieties are flucanazole, a powerful azole antifungal agent[1].

The chemistry of 1,2,4-triazoles 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.[2] Owing to the importance and established pharmacological activity of these compounds we are directed our attention towards synthesis of some new 2,5,disustituted 1,2,4-triazole derivatives with object of screening them for antimicrobial activity.

MATERIAL 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

General procedure for the Synthesis of phenyl isothiocynates (I)

A mixture of aniline (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.(I)

General procedure for the Synthesis of 1-Benzohydrazides (II)

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.exess 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. (II)

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. (III)

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)-1,2,4-triazoles IV (A_1-A_{15})

Each thiosemicarbazides (0.0004 mole, 0.2gm) was added portion wise to 15 ml of 2M sodium hydroxide solution. The reaction mixture was refluxed and completion of reaction is checked by using TLC after the completion of reaction, the mixture was allowed to cool and then filtered and filtrate was acidified with 2M hydrochloric acid the precipited solid was filtered, washed thoroughly with water, dried and recrystalized from ethanol and water to furnish 2,5,-disustituted 1,3,4-thiadiazoles (IV) and characterization of these compounds are given in Table-I.

Spectral Data's of final compounds

IR Spectrum

The compound **A_1 N-(4-chlorophenyl)-5-phenyl 1,2,4-triazol-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 ä 10.14 (s,1H, NH), δ 7.25-7.85 (m,9H, Ar-H),

IR Spectrum

The compound A_6 5-(4-bromophenyl)-N-(4-chlorophenyl) 1,2,4-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).

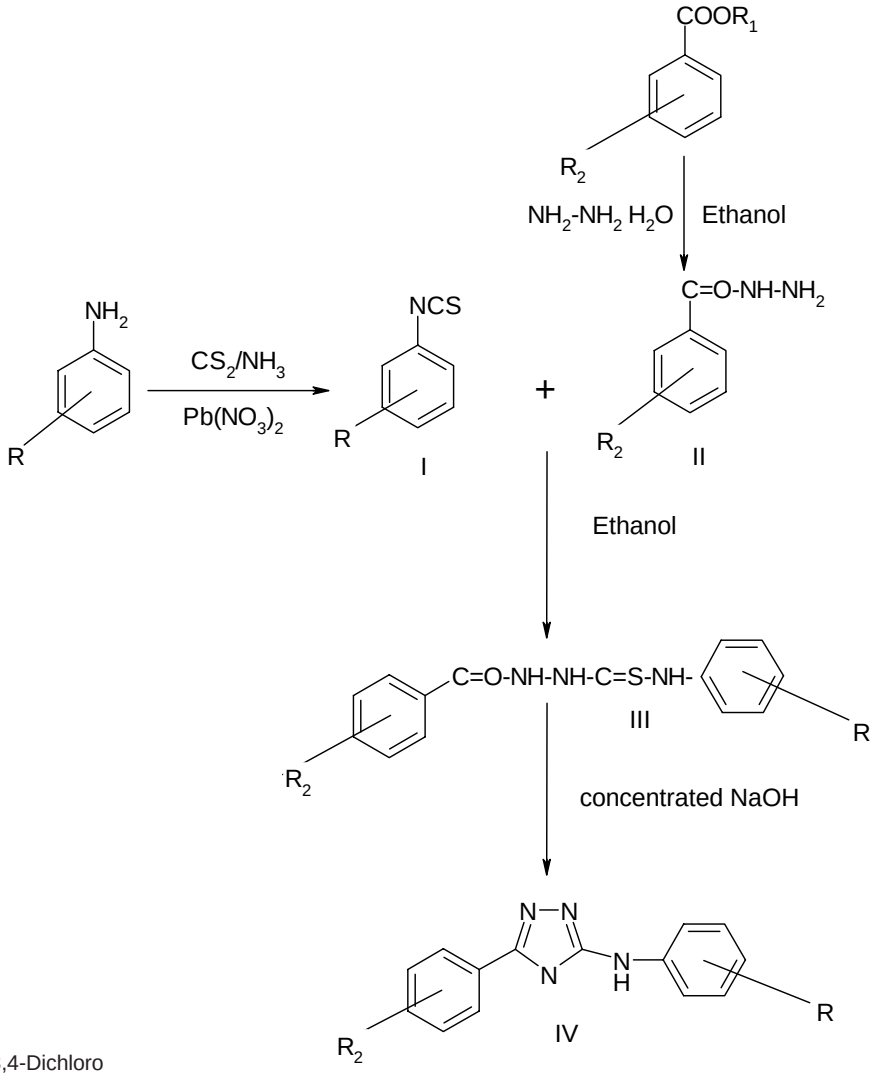
^{1}H NMR spectrum

The compound A_6 shows δ 10.30 (s,1H, NH), δ 7.25-7.75 (m, 8H, Ar-H).

IR absorption bands of remaining similar compounds illustrated in Table-II 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 multiplates for aromatic hydrogens. DMSO and $CDCl_3$ were used as solvents.

RESULTS AND DISCUSSION

Synthesis 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)-1,2,4-Triazoles.were carried out by literature methods.[3-5] The phenyl isothiocynates (I) were reacted with benzohydrazides (II) in presence of ethanol to get thiosemicarbazides (III) and which is treated with concentrated Sodium Hydroxide to furnished with corresponding substituted 1,2,4-triazoles (IV) (Scheme). Synthesized compounds were characterized by analytical and IR, NMR spectral data.



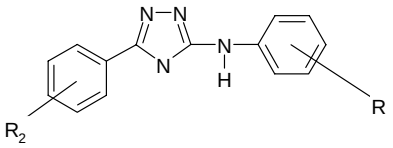
R=H, 4-Cl, 3,4-Dichloro

R_1=CH_3, C_2H_5

R_2= H, 2-OH, 4-OH, 4-NH_2, 2-Br, 4-Br, 3,4-Dimethoxy

Scheme

Table 1: Characterization and physical data of synthesized compounds



Compound code	R	R_2	Molecular formula	m.p. (°C)	% Yield	%Analysis, Found(Calcd) C	H	N
A_1	p-Cl	H	$C_{14}H_{10}ClN_4$	220-222	74.12	61.99	3.69	20.66
A_2	p-Cl	o-OH	$C_{14}H_{10}ClN_4O$	230-232	70.36	58.44	3.48	19.51
A_3	p-Cl	p-OH	$C_{14}H_{10}ClN_4O$	272-274	55.29	58.44	3.48	19.51
A_4	p-Cl	p-NH_2	$C_{14}H_{11}ClN_5$	178-180	79.52	58.74	3.84	24.47
A_5	p-Cl	o-Br	$C_{14}H_9BrClN_4$	245-246	45.24	48.13	2.57	16.04
A_6	p-Cl	p-Br	$C_{14}H_9BrClN_4$	252-254	55.69	48.13	2.57	16.04
A_7	p-Cl	o,m,di-OCH_3	$C_{16}H_{14}N_4Cl$	212-214	30.47	60.21	4.68	18.72
A_8	H	H	$C_{14}H_{11}N_4$	286-288	82.01	71.48	4.68	23.82
A_9	H	o-OH	$C_{14}H_{11}N_4O$	258-260	75.34	69.93	4.38	22.31
A_{10}	H	o-Br	$C_{14}H_{10}BrN_4$	255-256	63.69	53.67	3.19	17.89
A_{11}	H	p-Br	$C_{14}H_{10}BrN_3$	266-268	73.49	53.67	3.19	17.89
A_{12}	o.m,di-Cl	H	$C_{14}H_9Cl_2N_4$	226-228	48.75	54.72	2.93	18.24
A_{13}	o,m di-Cl	o-OH	$C_{14}H_9Cl_2N_3O$	218-220	28.87	52.01	2.78	17.33
A_{14}	o.m,di-Cl	o-Br	$C_{14}H_8BrCl_2N_4$	230-232	42.29	43.63	2.07	14.54
A_{15}	o,m di-Cl	p-Br	$C_{14}H_8BrCl_2N_4$	268-270	50.36	43.63	2.07	14.54

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 activity data of synthesized compounds

S No.	Name of the compounds	Mean zone of inhibition (in mm)			
		Staphylococcus aureus (+ve)		*Escherichia coli(-ve)*	
		100µg	200µg	100µg	200µg
01	Ciprofloxacin	31	34	32	35
02	A_1	03	05	06	09
03	A_2	28	29	29	32
04	A_3	09	12	10	12
05	A_4	29	31	28	31
06	A_5	11	14	12	13
07	A_6	10	11	11	11
08	A_7	10	12	09	10
09	A_8	09	10	11	11
10	A_9	10	12	11	13
11	A_{10}	12	13	13	12
12	A_{11}	28	31	30	32
13	A_{12}	11	13	13	11
14	A_{13}	11	11	11	10
15	A_{14}	09	11	12	11
16	A_{15}	09	13	10	12

Table 4: Antifungal activity data of synthesized compounds

S No.	Name of the compounds	Mean zone of inhibition (in mm)			
		Candida albicans		*Aspergillus flavus*	
		100µg	200µg	100µg	200µg
1	Fluconazole	21	24	22	24
2	A_1	20	23	20	22
3	A_2	20	22	21	23
4	A_3	10	09	09	11
5	A_4	21	23	21	22
6	A_5	12	09	11	10
7	A_6	09	09	11	14
8	A_7	19	21	21	23
9	A_8	11	11	13	16
10	A_9	20	22	21	23
11	A_{10}	20	22	21	23
12	A_{11}	20	22	21	23
13	A_{12}	20	22	21	23
14	A_{13}	10	11	11	12
15	A_{14}	20	22	21	23
16	A_{15}	11	10	12	10

Anti-bacterial activity

The newly synthesized compounds were screened for their antibacterial activity against *Escherichia coli* (ATTC-25922) and *Staphylococcus aureus* (ATTC-5278523) bacterial strains by the disk diffusion method [6-8]. Ciprofloxacin was used as a standard drug at a concentration of 100and 200 mcg/ml and results are given in Table-III.

Anti-fungal activity

Newly prepared compounds were screened for their antifungal activity against *Aspergillus flavus* (NICM No.524) and *Candida albicans* (NICM No.3100) in DMSO by the serial plate dilution method [9,10]. Fluconazole was used as a standard drug at a concentration of 100and 200 mcg/ml and results are given in Table-IV.

CONCLUSION

Antimicrobial activity of all 15 compounds were determined using Ciprofloxacin and Fluconazole 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* and *Aspergillus flavus* compared to activity shown by standard Fluconazole.

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