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Synthesis and biological evaluation of N^3-(4-substituted phenyl)-N^5-phenyl-4H-1, 2, 4-triazole-3,5-diamine derivatives

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ABSTRACT

A series of some new N^3-(4-Substituted phenyl)-N^5-Phenyl-4H-1, 2, 4-Triazole-3,5-diamine derivatives (6a-f) were synthesized from different substituted aniline(1a-f) and tested for antibacterial, antifungal activities against human pathogenic bacteria and fungi. The structure of the synthesized compounds was elucidated by IR and ^{1}HNMR spectroscopic techniques. The antibacterial and antifungal screening of the synthesized compound were performed *in-vitro* by the disc diffusion method.

Key words: Triazole, Anti-bacterial, Anti-fungal, disc diffusion.

INTRODUCTION

1, 2, 4 – Triazole nucleus is reported to exhibit antibacterial,[1] antifungal,[2] pesticidal[3], hypoglycaemic[4], anti-inflammatory[5], anticonvulsant[6], antitumour[7], anthelmintic[8], anti-tubercular[9], diuretic[10], antioxidant[11] and molluscicidal[12] activity. Keeping in view of the above observation, it is thought of interest to synthesize some new N^3-(4-substituted phenyl)-N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine derivatives.

Different substituted aniline (1a-f) is reflux with ammonium thiocyanate in the presence hydrochloric acid to give respective thiourea (2a-f). Then respective thiourea (2a-f) is further reacted with benzyl chloride and ethanol to get substituted 2-benzyl-1-(4-substituted phenyl) isothiourea (3a-f).The compound (3a-f) treated with 1-isothiocyanato benzene to get the (E)-1-((4-substituted phenylamino) (benzylthio) methylene)-3-phenylthiourea (4a-f). Then compounds (4a-f) undergo bromine cyclization to yield different (12E)-4-substituted-N ((E)-5-(phenylimino) -1, 2, 4-dithiazolidin-3-ylidene) benzenamine (5a-f). Finally, (12E)-4-substituted-N ((E)-5-(phenylimino) -1, 2, 4-dithiazolidin-3-ylidene) benzene amine and hydrazine hydrate and ethanol to get N^3-(4-substituted phenyl)-N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6a-f).

The structures of newly synthesized compounds were confirmed by their spectral analysis. The I.R. spectrum and ^{1}HNMR. I.R. spectra of the compounds showed characteristic peaks at 3297-3612 cm^{-1}, 2916-3293 cm^{-1} , 2357-2361 cm^{-1} and 1619-1642 cm^{-1} due to presence of C= N, N-H, C-H, C= N, and C=C functional groups. In ^{1}HNMR spectra of representative compounds; chemical shifts (δ) were observed due to aromatic proton at δ: 6.35 to 7.94(Ar-CH) and 4.00- 4.38 (Ar-NH) 8.067-9.538 (NH).

Antibacterial and antifungal activity

Five compounds (6a-f) were screened for their antibacterial and antifungal activity. Antibacterial activity was carried out against Gram positive - *Staphylococcus aureus* (MTCC-737), Gram negative - *Escherichia coli* (MTCC-1687) and *Fungus Aspergillus Niger* (MTCC-228) and Candida *albicans* (MTCC-183) by disc diffusion method using 200 µg of test sample in each cup. Ciprofloxacin was used as control. Similarly the Antifungal activity was carried out against fungus C*andida albicans* by cup plate method using 200 µg/ml of test sample in DMSO. The drug Ampicillin and Clotrimazole used as standard drug for antibacterial and antifungal activities. Most of the compounds showed moderate to good antimicrobial activity.

EXPERIMENTAL

All the melting points were determined in open capillaries. I.R. Spectra (KBr) were recorded on FTIR Spectrophotometer (Shimadzu PC, 4000-400cm-1). The NMR spectra were recorded on dpx-300 spectrophotometer in DMSO, TMS was the internal reference, chemical shift are express in δ ppm.

Preparation of different 1-(4-substituted Phenyl) thiourea (2)

The ammonium thiocyanate **(**0.1 M) was dissolved in minimum quantity of water, added with continuous stirring into a mixture of Para- substituted aniline (0.1 M) and few ml. of conc. HCl. The content were refluxed on water bath for few hour, then poured down into the cold water with vigorous stirring. The product which separated out was collected. (2a-f)

Preparation of 2-Benzyl-1-(4-substituted phenyl) isothiourea (3)

1-(4-Substituted phenyl) thiourea (0.1M) and benzyl chloride (0.1M) were refluxed in ethanol for 2 hour. The solvent was evaporated off to give solid mass which was extracted with solvent benzene after basification with ammonia solution. The organic extract was filtered was evaporated to get a 2-Benzyl-1-(4-substituted phenyl) isothiourea as a thick liquid.

Preparation of (E)-1-((4-substituted phenylamino) (benzylthio) methylene)-3-phenylthiourea (4)

In the thick liquid of 2-benzyl-1-(4-substituted phenyl) isothiourea, added 1-isothiocyanatobenzene (3 ml) and content were refluxed for 3 hours. After completion of reaction the solvent was evaporated off to get the viscous compound which was (E)-1-((4-substituted phenylamino) (benzylthio) methylene)-3-phenylthiourea. It was washed with petroleum ether to remove unreacted isothiocynate.

Preparation of (12E)-4-substituted-N ((E)-5-(phenylimino)-1, 2, 4-dithiazolidin-3-ylidene) benzenamine (5)

(E)-1-((4-substituted phenylamino) (benzylthio) methylene)-3-phenylthiourea made into a thin paste with chloroform and oxidized with drop wise addition of bromine solution (3ml). The contents were stirred at room temperature for half an hour. The viscous mass, which separated out was washed with petroleum ether and crystallized with alcohol to get (12E)-4-substituted-N ((E)-5-(phenylimino)-1, 2, 4-dithiazolidin-3-ylidene) benzenamine.

Preparation of N^3-(4-substituted phenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6)

Equimolar quantities of (12E)-4-substituted-N ((E)-5-(phenylimino)-1, 2, 4-dithiazolidin-3-ylidene) benzene amine and hydrazine were refluxed with ethanol for 8 hours. The content were concentrated and cooled to get N^3-(4-substituted phenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine. The crude product was finally crystallized from dilute alcohol.

N^3, N^5-diphenyl-4H-1, 2, 4-triazole-3, 5-diamine (6a)

I.R. (KBr): 3448 (N-H stretching) 3268 (ArC-H) 2357 (C=N) 1634 (C=C) 1487 (C-N) 1298(C-H), ^{1}HNMR (DMSO) δ-4.0 (2H, ArC-NH) 6.46-7.01 (10H, ArC-NH) 6.11(1H, N-H).

N^3-(4-fluorophenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6b)

I.R. (KBr): 3297 (N-H stretching) 3074 (Ar C-H) 2357 (C=N) 1634 (C=C) 1487 (C-N) 1238(C-F), ^{1}HNMR (DMSO) δ-4.0 (2H, Ar-C-NH) 6.46-7.01 (9H, Ar-C-NH) 6.02(1H, N-H).

N^3-(4-chlorophenyl) - N^5-phenyl-4H-1, 2, 4-

triazole-3, 5-diamine (6c)

I.R. (KBr): 3418 (N-H stretching) 2917 (Ar C-H) 2357 (C=N) 1619 (C=C) 1691 (C-N) 826 (C-Cl), ^{1}HNMR (DMSO) δ-4.0 (2H, Ar-C-NH) 6.46-7.02 (9H, Ar-H) 5.98(1H, N-H).

N^3-(4-bromophenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6d)

I.R. (KBr): 3442 (N-H stretching) 2916 (Ar C-H) 2360 (C=N) 1630 (C=C) 1384 (C-N) 1110(C-H) 719 (C-Br), ^{1}HNMR (DMSO) δ-4.0 (2H, Ar-C-NH) 6.35-7.18 (9H, Ar-H) 5.87(1H, N-H).

N^3-(4-iodophenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6e)

I.R (KBr): 3417 (N-H stretching) 2974 (Ar C-H) 2346 (C=N) 1642 (C=C) 1371 (C-N) 1233 (C-H) 545 (C-I), ^{1}HNMR (DMSO) δ-4.0 (2H, Ar-C-NH) 6.23-7.39 (9H, Ar-H) 5.71(1H, N-H).

N^3-(4-nitrophenyl) - N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine (6f)

I.R (KBr): 3442 (N-H stretching) 2929 (Ar C-H) 2361 (C=N) 1633 (C=C) 1354 (C-N) 1274 (C-H) 1384 (C-NO_2), ^{1}HNMR (DMSO) δ-4.0 (2H, Ar-C-NH) 6.23-7.39 (9H, Ar-H) 5.79(1H, N-H).

RESULTS AND DISCUSSION

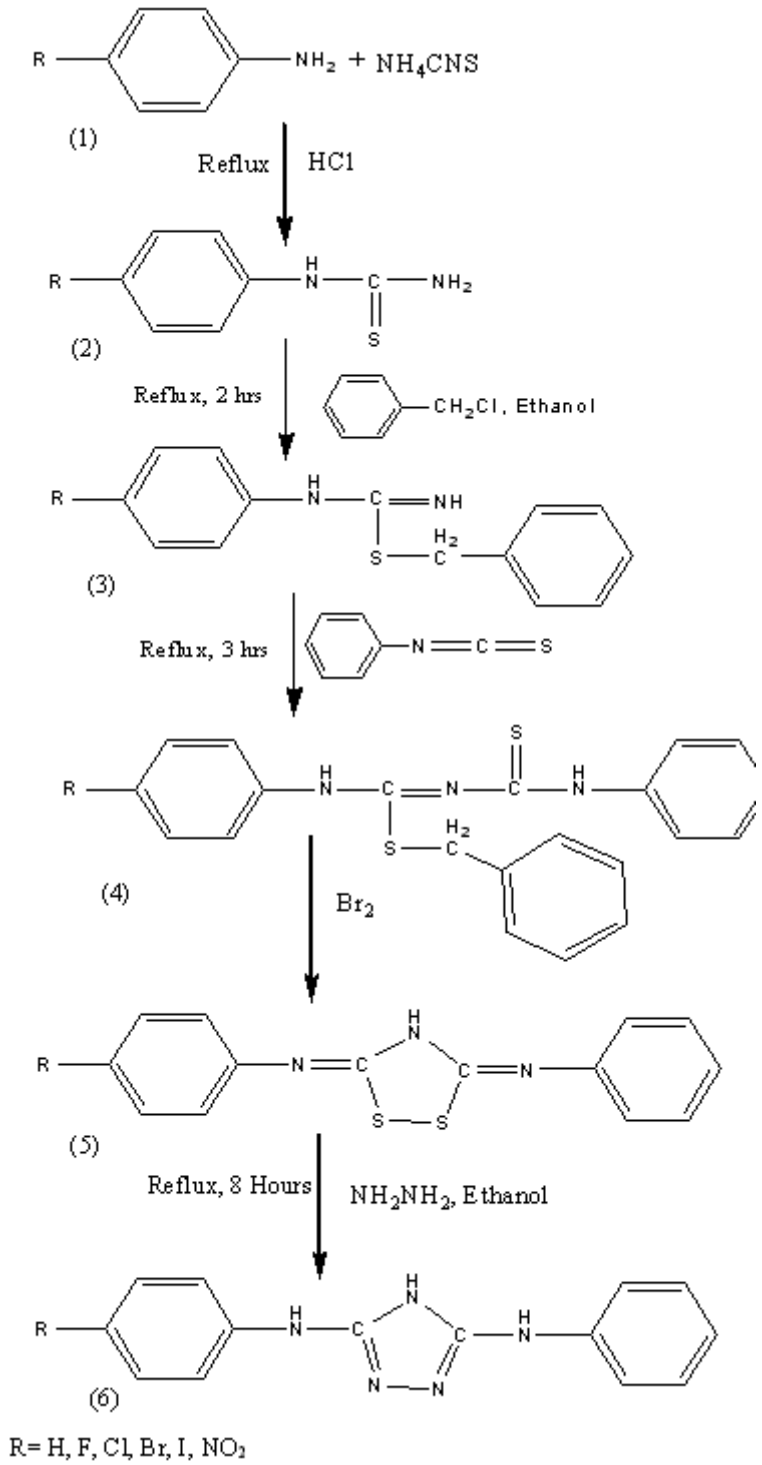
All the synthesized compounds were confirmed by their spectral data (Table 1) and the screened for anti-bacterial against *Staphylococcus aureus*, *Escherichia coli* and anti-fungal activity against *Candida albicans* and *Aspergillus niger* compounds (6a-f) showed moderate to good antibacterial and antifungal activity (Table-2) when compared to that of standard Ampicillin and Clotrimazole respectively.

Table 1: Physical data of the final compounds

S. No	Compound	R	M.P. (O°C)	Yield (%)	Molecular Formula
1	6a	H	245	43.8	$C_{14}H_{13}N_5$
2	6b	F	247	44.6	$C_{14}H_{12}N_5F$
3	6c	Cl	261	50.1	$C_{14}H_{12}N_5Cl$
4	6d	Br	258	45.4	$C_{14}H_{12}N_5Br$
5	6e	I	236	50.9	$C_{14}H_{12}N_5I$
6	6f	NO_2	272	58.4	$C_{14}H_{12}N_6O_2$

Table 2: Antibacterial and Antifungal Activities of Compounds

S. No	Compound	R	Antibacterial activity		Antifungal activity	
			***Staphylococcus* aureus (MTCC-737)**	***Escherichia* coli (MTCC-1687)**	***Candida* albicans (MTCC-183)**	***Aspergillus* niger (MTCC-228)**
1.	6a	H	++	+	++	++
2.	6b	F	++	++	++	+++
3.	6c	Cl	+	++	++	++
4.	6d	Br	++	++	++	++
5.	6e	I	+	++	+	++
6.	6f	NO_2	++	++	+++	++
7.	Ampicillin	-	+++	+++	-	-
8.	Clotrimazole	-	-	-	+++	+++
9.	DMSO	-	-	-	-	-



Scheme 1: Preparation of different N^3-(4-substituted phenyl) -N^5-phenyl-4H-1, 2, 4-triazole-3, 5-diamine

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