Research Article

SYNTHESIS AND EVALUATION OF SOME NEWLY TRIAZOLYLINDOLE DERIVATIVES FOR ANTIFUNGAL ACTIVITY

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ABSTRACT

A series of novel 2-((2-(phenyl/4-chlorophenyl)-1-((4-((Z)-1-(substitutedphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-1H-indol-3-yl)methyleneamino)benzoic acid (9-16) were synthesized by treating 2-((1-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-(phenyl/4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (7-8) with various substituted acetophenone. The structure of the compounds was characterized by IR and ¹HNMR spectral study and elemental analysis. All the newly synthesized compounds were screened for their antifungal activity against *C. albicans*, *C. krusei* G03, *C. glabrata* H05 and *C. parapsiolsis* 22019 and compared with standard drug Griseofulvin.

Keywords: Indole, Triazole, Antifungal activity, Griseofulvin

INTRODUCTION

The chemistry of heterocyclic compounds has been an interesting field of study of long time. The synthesis of novel indole derivatives and investigation of their chemical and biological behavior has gained more importance in recent decades for biological and pharmaceutical reasons. Indole represents an important class of heterocyclic compounds. Indole and their derivatives possess broad spectrum of biological activity in both medicinal and pharmaceutical, such as antifungal¹⁻³, anticancer⁴, antimicrobial⁵, antipsychotic⁶ and anticonvulsant⁷ etc. The triazole moiety is a versatile lead molecule in pharmaceutical development and has a wide range of biological activities like antifungal⁸⁻¹⁰, antimicrobial¹¹, anticonvulsant¹², anti-inflammatory¹³ and antihelmintic¹⁴ etc. As these two heterocycles are potent as biologically active compound, in the present study we are planned to prepared new compounds containing both indole derivatives fused with triazole ring and hope to possess better antifungal activity.

MATERIAL AND METHODS

Chemistry

All reagents and solvents were of analytical grade and used directly. Reactions were routinely performed in oven-dried borosil glassware. The melting points of compounds were determined in open capillaries with the help of thermionic melting point apparatus and were uncorrected. The homogeneity of all newly synthesized compounds was routinely checked by thin layer chromatography (TLC) on silica gel G plates and spots were located by using iodine chamber. Elemental analysis (C, H, N) of all the synthesized compounds were determined by perkin-Elmer 2400 elemental analyzer, and results were found within the ± 0.4 % of theoretical values. The IR spectra were recorded on a Beckman Acculab-10 Spectrometer ($\nu_{\max}$ in cm^{-1}) and the ¹HNMR spectra were recorded by Bruker DPX-300MHz using CDCl₃ as solvent. The synthesis of the target compounds was accomplished according to the reaction sequence illustrated in Scheme 1.

General procedure for preparation of 2-((2-(phenyl/4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (1-2)

A mixture of 2-phenyl/4-chlorophenyl-1H-indole-3-carbaldehydes (0.1 mol) and anthranilic acid (0.1 mol) in absolute ethanol (90 ml) containing few drops of glacial acetic acid was refluxed for 28 h and excess of solvent was distilled off. The solids thus obtained were filtered, dried and re crystallized from ethyl acetate to yield compounds 1-2

2-((1-(2-ethoxy-2-oxoethyl)-2-(phenyl/4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (3-4)

A mixture of compounds 1-2 (0.1 mol), ethyl chloroacetate (0.1 mol), anhydrous acetone (90 ml) and NaOH (16 g) was heated under reflux of 22 h. The reaction mixtures were cooled, wash with excess of water, filtered and dried, again washed with ethanol and re crystallized from appropriate solvent to yield compounds 3-4.

2-((1-(2-hydrazinyl-2-oxoethyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (5-6)

A mixture of compounds 3-4 (0.1 mol) and hydrazine hydrate (0.2 mol) in absolute ethanol (80 ml) was refluxed for 18 h then cooled and poured on crushed ice and separated. The solids were filtered, washed with cold water, dried and re crystallized from appropriate solvent to yield compounds 5-6.

2-((1-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-(phenyl/4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (7-8)

To the ice cold mixture of compounds 5-6 (0.1 mol) and KOH (0.4 mol) in dry ethanol (90 ml), carbon disulphide (0.1 mol) was added with magnetic stirring. The reaction mixtures were stirred further at room temperature for 24 h. The separated solids were filtered washed with dry ether and dried. These were further magnetically stirred with hydrazine hydrate (0.15 mol) on boiling water bath for 4 h till the evolution of H₂S ceased. The reaction mixtures were poured into ice-cold water then filtered, washed with water, dried and re crystallized from acetic acid to yield compounds 7-8.

2-((1-((4-((Z)-1-(substitutedphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-(phenyl/4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (9-16)

A solution of compounds 7-8 (0.1 mol) in methanol (50 ml) was refluxed with substituted acetophenone (0.1 mol) in the presence of glacial acetic acid (4 ml) for 10 h. The reaction mixtures were concentrated, cooled and then poured into ice water. The separated solids were filtered and re crystallized from ethanol to yield compounds 9-16.

Physical and elemental data of compounds 1-16 are given in Table 1 and IR and ¹HNMR data of these compounds are giving below.

2-((2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (1)

IR (KBr) in Cm^{-1} : 3345 (NH), 3032 (C-H Ar), 1680 (C=N), 1530 (C-C of Ar); ¹H-NMR (CDCl_3) δ in ppm: 9.76 (s, 1H, (COOH), 8.57 (s, 1H, NH), 8.40 (s, 1H, N=CH), 7.13 -8.15 (m, 13H, Ar-H).

2-((2-(4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (2)

IR (KBr) in Cm^{-1} : 3345 (NH), 3038 (C-H aromatic), 1682 (C=N), 1535 (C-C of Ar), 766 (C-Cl); ¹H-NMR (CDCl_3) δ in ppm: 9.74 (s, 1H, (COOH), 8.59 (s, 1H, NH), 8.42 (s, 1H, N=CH), 7.15 -8.16 (m, 12H, Ar-H).

2-((1-(2-ethoxy-2-oxoethyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (3)

IR (KBr) in Cm^{-1} : 3037 (C-H Ar), 1686 (C=N), 1534 (C-C of Ar); ¹HNMR (CDCl_3) δ in ppm: 9.78 (s, 1H, (COOH), 8.44 (s, 1H, N=CH), 7.13 -8.12 (m, 13H, Ar-H), 4.67 (s, 2H, N-CH₂), 4.39 (q, J=7 Hz, 2H ester CH₂), 1.67 (t, J=7 Hz, 3H, ester CH₃).

2-((2-(4-chlorophenyl)-1-(2-ethoxy-2-oxoethyl)-1H-indol-3-yl)methyleneamino)benzoic acid (4)

IR (KBr) in Cm^{-1} : 3038 (C-H Ar), 1680 (C=N), 1533 (C-C of Ar), 754 (C-Cl); ¹H-NMR (CDCl_3) δ in ppm: 9.75 (s, 1H, (COOH), 8.47 (s, 1H, N=CH), 7.12 -8.16 (m, 12H, Ar-H), 4.64 (s, 2H, N-CH₂), 4.36 (q, J=7 Hz, 2H ester CH₂), 1.65 (t, J=7 Hz, 3H, ester CH₃).

2-((1-(2-hydrazinyl-2-oxoethyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (5)

IR (KBr) in Cm^{-1} : 3345 (NH), 3030 (C-H Ar), 1687 (C=N), 1539 (C-C of Ar); ¹H-NMR (CDCl_3) δ in ppm: 9.76 (s, 1H, (COOH), 8.99 (s, 1H, NH), 8.57 (s, 2H, NH₂), 8.40 (s, 1H, N=CH), 7.13 -8.15 (m, 13H, Ar-H), 4.65 (s, 2H, N-CH₂).

2-((2-(4-chlorophenyl)-1-(2-hydrazinyl-2-oxoethyl)-1H-indol-3-yl)methyleneamino)benzoic acid (6)

IR (KBr) in Cm^{-1} : 3349 (NH), 3036 (C-H Ar), 1688 (C=N), 1536 (C-C of Ar), 763 (C-Cl); ¹HNMR (CDCl_3) δ in ppm: 9.78 (s, 1H, (COOH), 8.97 (s, 1H, NH), 8.59 (s, 2H, NH₂), 8.43 (s, 1H, N=CH), 7.14 -8.14 (m, 13H, Ar-H), 4.68 (s, 2H, N-CH₂).

2-((1-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (7)

IR (KBr) in Cm^{-1} : 3249 (NH₂), 3031 (C-H Ar), 1682 (C=N), 1537 (C-C of Ar), 1020 (N-N); ¹HNMR (CDCl_3) δ in ppm: 11.43 (s, 1H, SH), 9.78 (s, 1H, (COOH), 8.53 (s, 2H, NH₂),

8.45 (s, 1H, N=CH), 7.11 -8.13 (m, 13H, Ar-H), 4.63 (s, 2H, N-CH₂).

2-((1-((4-amino-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-(4-chlorophenyl)-1H-indol-3-yl)methyleneamino)benzoic acid (8)

IR (KBr) in Cm^{-1} : 3240 (NH₂), 3038 (C-H Ar), 1687 (C=N), 1535 (C-C of Ar), 1026 (N-N), 762 (C-Cl); ¹HNMR (CDCl_3) δ in ppm: 11.49 (s, 1H, SH), 9.76 (s, 1H, (COOH), 8.58 (s, 2H, NH₂), 8.48 (s, 1H, N=CH), 7.13 -8.15 (m, 12H, Ar-H), 4.66 (s, 2H, N-CH₂).

2-((1-((5-mercapto-4-((Z)-1-phenylethylideneamino)-4H-1,2,4-triazol-3-yl)methyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (9)

IR (KBr) in Cm^{-1} : 3343 (NH), 3033 (C-H Ar), 1683 (C=N), 1537 (C-C of Ar), 1029 (N-N); ¹HNMR (CDCl_3) δ in ppm: 11.40 (s, 1H, SH), 9.79 (s, 1H, (COOH), 8.45 (s, 1H, N=CH), 7.14 -8.13 (m, 18H, Ar-H), 4.60 (s, 2H, N-CH₂), 3.56 (s, 3H, CH₃).

2-((2-(4-chlorophenyl)-1-((5-mercapto-4-((Z)-1-phenylethylideneamino)-4H-1,2,4-triazol-3-yl)methyl)-1H-indol-3-yl)methyleneamino)benzoic acid (10)

IR (KBr) in Cm^{-1} : 3348 (NH), 3036 (C-H Ar), 1685 (C=N), 1531 (C-C of Ar), 1027 (N-N), 765 (C-Cl); ¹HNMR (CDCl_3) δ in ppm: 11.43 (s, 1H, SH), 9.79 (s, 1H, (COOH), 8.49 (s, 1H, N=CH), 7.13 -8.15 (m, 17H, Ar-H), 4.65 (s, 2H, N-CH₂), 3.52 (s, 3H, CH₃).

2-((1-((4-((Z)-1-(2-hydroxyphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (11)

IR (KBr) in Cm^{-1} : 3440 (OH), 3346 (NH), 3037 (C-H Ar), 1686 (C=N), 1534 (C-C of Ar), 1025 (N-N); ¹HNMR (CDCl_3) δ in ppm: 12.24 (s, 1H, OH), 11.48 (s, 1H, SH), 9.78 (s, 1H, (COOH), 8.42 (s, 1H, N=CH), 7.11 -8.14 (m, 17H, Ar-H), 4.67 (s, 2H, N-CH₂), 3.59 (s, 3H, CH₃).

2-((2-(4-chlorophenyl)-1-((4-((Z)-1-(2-hydroxyphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-1H-indol-3-yl)methyleneamino)benzoic acid (12)

IR (KBr) in Cm^{-1} : 3434 (OH), 3345 (NH), 3032 (C-H Ar), 1680 (C=N), 1530 (C-C of Ar), 1021 (N-N), 768 (C-Cl); ¹HNMR (CDCl_3) δ in ppm: 12.25 (s, 1H, OH), 11.43 (s, 1H, SH), 9.76 (s, 1H, (COOH), 8.40 (s, 1H, N=CH), 7.13 -8.15 (m, 16H, Ar-H), 4.65 (s, 2H, N-CH₂), 3.52 (s, 3H, CH₃).

2-((1-((5-mercapto-4-((Z)-1-(4-methoxyphenyl)ethylideneamino)-4H-1,2,4-triazol-3-yl)methyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (13)

IR (KBr) in Cm^{-1} : 3349 (NH), 3030 (C-H Ar), 1685 (C=N), 1533 (C-C of Ar), 1025 (N-N); ¹HNMR (CDCl_3) δ in ppm: 11.40 (s, 1H, SH), 9.78 (s, 1H, (COOH), 8.44 (s, 1H, N=CH), 7.12 -8.15 (m, 17H, Ar-H), 4.68 (s, 2H, N-CH₂), 3.81 (s, 3H, OCH₃), 3.54 (s, 3H, CH₃).

2-((2-(4-chlorophenyl)-1-((5-mercapto-4-((Z)-1-(4-methoxyphenyl)ethylideneamino)-4H-1,2,4-triazol-3-yl)methyl)-1H-indol-3-yl)methyleneamino)benzoic acid (14)

IR (KBr) in Cm^{-1} : 3347 (NH), 3035 (C-H Ar), 1687 (C=N), 1536 (C-C of Ar), 1021 (N-N), 761 (C-Cl); ¹HNMR

(CDCl₃) δ in ppm: 11.42 (s, 1H, SH), 9.78 (s, 1H, (COOH)), 8.43 (s, 1H, N=CH), 7.10 -8.13 (m, 16H, Ar-H), 4.63 (s, 2H, N-CH₂), 3.84 (s, 3H, OCH₃), 3.58 (s, 3H, CH₃)

2-((1-((4-((Z)-1-(4-hydroxy-3-methoxyphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-2-phenyl-1H-indol-3-yl)methyleneamino)benzoic acid (15)

IR (KBr) in Cm⁻¹: 3429 (OH), 3349 (NH), 3033 (C-H Ar), 1685 (C=N), 1533 (C-C of Ar), 1020 (N-N); ¹HNMR (CDCl₃) δ in ppm: 12.24 (s, 1H, OH), 11.45 (s, 1H, SH), 9.78 (s, 1H, (COOH)), 8.45 (s, 1H, N=CH), 7.14 -8.16 (m, 16H, Ar-H), 4.65 (s, 2H, N-CH₂), 3.82 (s, 3H, OCH₃), 3.52 (s, 3H, CH₃)

2-((2-(4-chlorophenyl)-1-((4-((Z)-1-(4-hydroxy-3-methoxyphenyl)ethylideneamino)-5-mercapto-4H-1,2,4-triazol-3-yl)methyl)-1H-indol-3-yl)methyleneamino)benzoic acid (16)

IR (KBr) in Cm⁻¹: 3426 (OH), 3345 (NH), 3032 (C-H Ar), 1680 (C=N), 1530 (C-C of Ar), 1021 (N-N), 765 (C-Cl); ¹HNMR (CDCl₃) δ in ppm: 12.25 (s, 1H, OH), 11.43 (s, 1H, SH), 9.76 (s, 1H, (COOH)), 8.40 (s, 1H, N=CH), 7.13 -8.15 (m, 16H, Ar-H), 4.65 (s, 2H, N-CH₂), 3.82 (s, 3H, OCH₃), 3.52 (s, 3H, CH₃)

Table 1: Physical and analytical data of the compounds 1-16

Comp.	R	R'	Mol. Wt.	Yield%	M.P. ^(°C)	Mol. Formula	Analysis % found (calculated)		
							C	H	N
1	C ₆ H ₅	H	340.37	87	178	C ₂₂ H ₁₆ N ₂ O ₂	77.64 (77.63)	4.75 4.74	8.20 8.23
2	4-ClC ₆ H ₄	2-OH	374.82	85	185	C ₂₂ H ₁₃ ClN ₂ O ₂	70.52 (70.50)	4.06 4.03	7.45 7.47
3	C ₆ H ₅	4-OCH ₃	426.46	80	169	C ₂₆ H ₂₂ N ₂ O ₄	73.22 (73.23)	5.21 5.20	6.55 6.57
4	4-ClC ₆ H ₄	4-OH and 3-OCH ₃	460.91	82	192	C ₂₆ H ₂₁ ClN ₂ O ₄	67.73 (67.75)	4.56 4.59	6.06 6.08
5	C ₆ H ₅	H	412.44	79	198	C ₂₄ H ₂₀ N ₄ O ₃	69.87 (69.89)	4.86 4.89	13.59 13.58
6	4-ClC ₆ H ₄	2-OH	446.89	78	191	C ₂₄ H ₁₉ ClN ₄ O ₃	64.52 (64.50)	4.27 4.29	12.56 12.54
7	C ₆ H ₅	4-OCH ₃	468.53	83	210	C ₂₅ H ₂₀ N ₆ O ₂ S	64.10 (64.09)	4.34 4.30	17.91 17.94
8	4-ClC ₆ H ₄	4-OH and 3-OCH ₃	502.98	77	236	C ₂₅ H ₁₉ ClN ₆ O ₂ S	59.68 (59.70)	3.83 3.81	16.70 16.71
9	C ₆ H ₅	H	570.66	75	224	C ₃₃ H ₂₆ N ₆ O ₂ S	69.47 (69.45)	4.58 4.59	14.76 14.73
10	4-ClC ₆ H ₄	2-OH	605.11	78	243	C ₃₃ H ₂₅ ClN ₆ O ₂ S	65.54 (65.50)	4.18 4.16	13.88 13.89
11	C ₆ H ₅	4-OCH ₃	586.66	76	256	C ₃₃ H ₂₆ N ₆ O ₃ S	67.54 (67.56)	4.46 4.47	14.35 14.33
12	4-ClC ₆ H ₄	4-OH and 3-OCH ₃	621.11	70	274	C ₃₃ H ₂₅ ClN ₆ O ₃ S	63.83 (63.81)	4.07 4.06	13.50 13.53
13	C ₆ H ₅	H	600.69	65	268	C ₃₄ H ₂₈ N ₆ O ₃ S	67.99 (67.98)	4.72 4.70	13.97 13.99
14	4-ClC ₆ H ₄	2-OH	635.13	63	285	C ₃₄ H ₂₇ ClN ₆ O ₃ S	64.33 (64.30)	4.26 4.28	13.25 13.23
15	C ₆ H ₅	4-OCH ₃	616.69	61	266	C ₃₄ H ₂₈ N ₆ O ₄ S	66.24 (66.22)	4.56 4.58	13.67 13.63
16	4-ClC ₆ H ₄	4-OH and 3-OCH ₃	651.13	57	279	C ₃₄ H ₂₇ ClN ₆ O ₄ S	62.75 (62.72)	4.17 4.18	12.93 12.91

Table 2: Antifungal activity of compounds 1-16

Comp. No	R	R ^I	Fungal growth inhibition (diameter in mm)			
			<i>Candida albicans</i>	<i>Candida krusei</i> G03	<i>Candida glabrata</i> H05	<i>Candida parapsilosis</i>
1	C ₆ H ₅		7	-	-	9
2	4-ClC ₆ H ₄		9	6	-	8
3	C ₆ H ₅		-	8	7	-
4	4-ClC ₆ H ₄		11	-	9	12
5	C ₆ H ₅		14	12	-	11
6	4-ClC ₆ H ₄		11	10	11	14
7	C ₆ H ₅		15	12	10	-
8	4-ClC ₆ H ₄		17	14	11	14
9	C ₆ H ₅	H	20	-	-	16
10	4-ClC ₆ H ₄	H	21	15	13	18
11	C ₆ H ₅	2-OH	19	13	10	15
12	4-ClC ₆ H ₄	2-OH	23	14	12	17
13	C ₆ H ₅	4-OCH ₃	25	16	14	20
14	4-ClC ₆ H ₄	4-OCH ₃	24	18	16	21
15	C ₆ H ₅	4-OH and 3-OCH ₃	27	20	18	23
16	4-ClC ₆ H ₄	4-OH and 3-OCH ₃	29	22	17	25
Griseofulvin			25	18	16	22

Antifungal Activity

All the newly synthesized compounds and the standard drug, griseofulvin were tested for their antifungal activity by employing the standard agar disc diffusion method¹⁵. The following strains of fungi have been used in this study: *Candida albicans*, *Candida krusei* G03, *Candida glabrata* H05 and *Candida parapsiolsis* 22019. All cultures were maintained on [Sabouraud-dextrose agar] SDA and incubated at 30°C. To prepare homogeneous suspensions of the above mentioned fungi for the disc assays, they were grown in Sabouraud broth, centrifuged to collect the pellet, and buffered with saline. The fungal pellet was homogenized in a

sterile hand-held homogenizer. This suspension was then plated onto SDA using a fungal spreader to obtain an even growth field. Sterile 6 mm Whatmann filter paper was impregnated with 250 µg/mL concentration of the various test compounds and standard drug griseofulvin. These discs were then placed in the center of each quadrant of an SDA plate. Each plate had one control disc impregnated with DMSO. The plates were incubated at 30°C. After 48 h, the plates were removed.

RESULTS AND DISCUSSION

All the newly synthesized compounds (1-16) were screened for their *in vitro* antifungal activity against different strains of fungi by measuring the zone of inhibition in mm. The results for the antifungal activity of the compounds of this series are depicted in Table 3. Compounds 15 and 16 were found to be the most potent compounds of the present series. Compound 16 was found to be posses more potent antifungal activity in comparison to the standard drug against *C. albicans* (29 mm), *C. krusei* (22 mm), *C. glabrata* (17 mm) and *C. parapsiolsis* (25 mm). Compounds 12, 13 and 14 also revealed satisfactorily good antifungal activity but not better than standard drug. All the other compound of this series were found to be active against one or the other fungal strains used and displayed mild to moderate as well as optimal activity.

CONCLUSION

It is interesting to point out those compounds 15 and 16 have more potent than all clinically useful antifungal standard drug. p-Hydroxy and m-methoxy substitution is beneficial from the biological activity point of view. The chloro substituents (due to presence of electronegative atom) also showed good activity.

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