

Synthesis and Antifungal Activity of 3-[(3-Phenyl-5-Thioxo-1,5-Dihydro-4h-1,2,4-Triazol-4-Yl)Imino]-1,3-Dihydro-2h-Indole-2-One

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Abstract: A series of 3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indole-2-one derivatives were synthesised through the nucleophilic substitution at carbonyl carbon of Isatin. Structure of synthesized compounds was elucidated by using IR, ¹H NMR & ¹³C NMR spectrometry. Synthesised compounds showed significant antibacterial activity against E.coli (ATCC 35218), S.aureus (ATCC 25323), E.faecalis (Clinical isolate), K. Pneumonia, P. aeruginosa (ATCC 27893) using agar well diffusion method.

Keywords: Indole, Triazole, nucleophilic substitution, Antibacterial activity.

INTRODUCTION

In recent year heterocyclic compounds analogues and derivatives have attracted strong interest due to their useful biological and pharmacological properties [1, 2]. The small and simple triazole nucleus is present in compounds involved in research aimed at evaluating new products that possess biological activities, such as, antimicrobial, anti-tumor, anthelmintic, anti-leishmanial, anti-convulsant and anti-inflammatory [3]. Extensive use of antimicrobial drugs also favour the emergence of resistant strains [4,5]. The overuse and misuse of antimicrobials have led to the death of sensitive strains leaving resistant strains to survive, multiply and infect new hosts [6]. This has opened a new field for the researchers to prepare the mimic of already existing compounds. Biological activity of these compounds was enhanced by using complexes of already available drugs [7]. Present study was focused to prepare, characterised and biological assay of novel substituted compounds of 3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indole-2-one to overcome the problems of resistance produced in microorganisms.

MATERIALS AND METHODS

SYNTHESIS OF TRIAZOLE DERIVATIVES

The derivatives were prepared according to the reaction sequences depicted in Scheme 1. All chemicals and reagents for synthesis of triazole derivatives were obtained from commercial suppliers and were used without further purification. Reactions were monitored by thin layer chromatography (TLC). All melting points were recorded are uncorrected. The structure of the compounds were confirmed by IR and ¹HNMR and ¹³CNMR spectra.

DERIVATIVES OF 3-[(3-PHENYL-5-THIOXO-1,5-DIHYDRO-4H-1,2,4-TRIAZOL-4-YL)IMINO]-1,3-DIHYDRO-2H-INDOL-2-ONE

The synthesis of 3-[(3-Phenyl-5-Thioxo-1,5-Dihydro-4h-1,2,4-Triazol-4-Yl)Imino]-1,3-dihydro-2H-Indol-2-one derivatives were initiated with the synthesis of 4-amino-5-phenyl-2,4-dihydro-3H-1,2,4-triazole-3-thione (2a) according to the Fig1

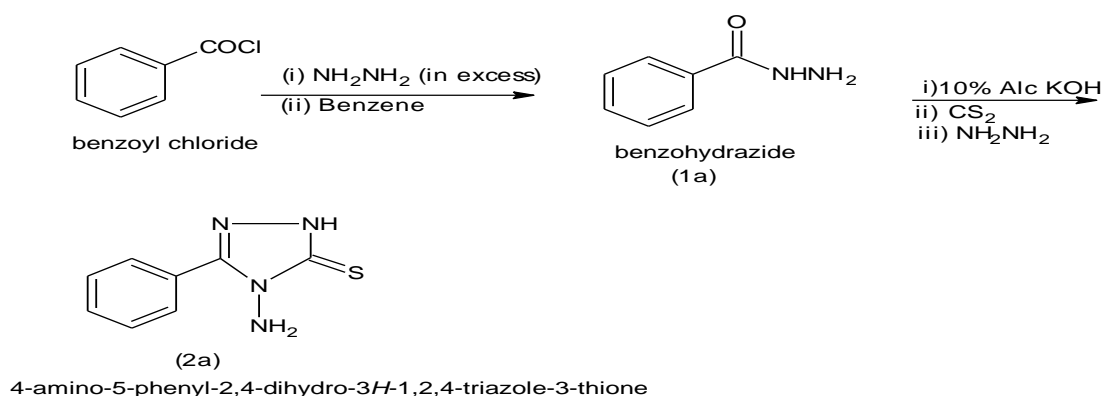
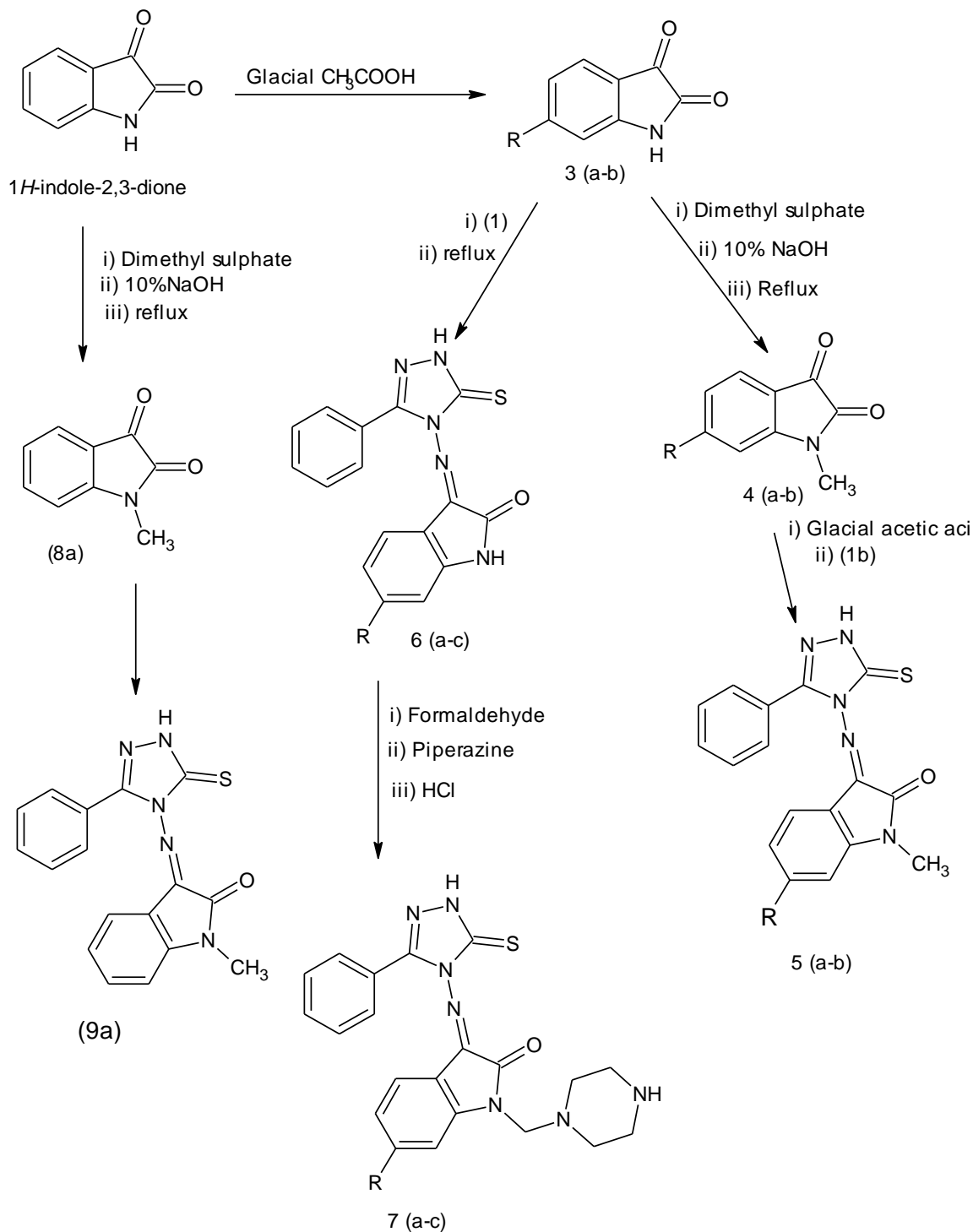


Fig 1: schematic diagram to prepare 2(a)

The starting compound 2a was prepared by the dropwise addition of Benzene to the ice cool benzoyl chloride followed by excess of Hydrazine hydrate with continuous stirring. The content was filtered to obtain benzohydrazide (1a). 10% ethanolic solution of KOH was added to 2gm of product (1a) followed by carbon disulphide and then hydrazine hydrate in equimolar quantity. Mixture was heated at 50 °C to obtain product (2a).



Where R

- a) Br
- b) NO₂
- c) H

Fig 2:- Schematic diagram of synthesis of 3-[(3-phenyl-5-thioxo-1,5-dihydro-4*H*-1,2,4-

Further preparation were done according the figure 2 triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one derivatives.

3a and 3b were produced by the bromination and nitration of 1 H-indole-2,3-dione (3c). Methylation of 3(a-c) was done to obtain 4(a-c).

General procedure for preparation of 5(a-c)

Equimolar amount of 4(a-c) was mixed with (2a) in glacial acetic acid. Solution mixture was refluxed, allowed to cool and then filtered to get 5(a-b).

General procedure for preparation of 6(a-c)

Equimolar amount of 3(a-b) was mixed with (2a) in 10 ml of glacial acetic acid. Solution mixture was refluxed, allowed to cool and then filtered to get 6(a-b).

General procedure for preparation of 7(a-c)

6 (a-c) and piperazine were dissolved in equimolar quantity in formaldehyde. Reaction mixture was refluxed and the resulting solid was filtered and recrystallised from alcohol to afford 7(a-c).

General procedure for preparation of 8(a-c)

6(a-c) and piperidine was mixed in equimolar quantity in formaldehyde. The reaction mixture was refluxed in acidic medium for 5hr at 50-60°C. The resulting mixture was crystallized from benzene to obtain 8(a-c).

SCREENING OF ANTIFUNGAL ACTIVITY

The newly synthesized compounds were screened for their in vitro antifungal activity against total of 3 fungal strains *C.albicans* ATCC 90028, *C. tropicalis* ATCC 750, *C.krusie*. The antifungal activity was assayed by using agar well diffusion method by measuring the zone of inhibition in mm [8,9]. Standard drug Fluconazole was used for the comparison purpose. The synthesised compounds was weighed and dissolved in DMSO as diluent to yield the required concentration of 1µg/ml, using sterile glassware.

Formation of plaque was observed after 48h for antifungal activities [10].

RESULTS

Physical properties and characterisation of synthesised triazole derivatives have been given below.

5a). 6-bromo-1-methyl-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one : Molecular Weight = 414.27908, Chemical composition = C(49.29%), H (2.92%), Br (19.29%), N (16.90%), O(3.86%), S (7.74%), m.p- 142°C, yield-78%, ¹H-NMR(DMSO δ ppm): 11.098 (1H,s,NH), 10.893 (1H,s,NH), 7.981 (1H, d, ArH), 7.957(1H,d,ArH), 7.543 (1H,d,ArH), 7.487 (2H,m,ArH), 7.436 (1H,s,ArH), 6.884 (1H,d,ArH), 6.857 (1H,m,ArH). ¹³C-NMR(DMSO-d) δppm: 165.911, 131.914, 131.271, 129.038, 128.337, 127.801, 127.126, 38.487. IR $\bar{\nu}$ (cm⁻¹) : 3205.91 (NH), ~3050 (CH Aromatic), ~2950(CH,Methyl), 1697.6(C=O), 1631.17 (C=N), 1465.13 (C=C Ar), 1120.22 (C=S), 1060.4 (C-N), 688.39 (C-Br).

5b). 1-methyl-6-nitro-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one :

Molecular Weight = 380.38058 g, Chemical Composition = C(53.68%) H(3.18%) N(22.09%) O(12.62%) S(8.43%), m.p – 155 °C, yield- 87.4%, ¹H-NMR(DMSO δ ppm): 10.493 (1H,s,NH), 7.920 (1H,s, ArH), 7.895(2H,m,ArH), 7.562 (1H,d,ArH), 7.506 (2H,m,ArH), 7.482 (1H,d,ArH), 7.292 (1H,m,ArH), 4.024 (3H,s,Alkyl).

¹³C-NMR(DMSO-d) δ ppm : 165.951, 145.20, 142.545, 129.268, 128.592, 127.504, 111.392, 132.606, 131.946. IR $\bar{\nu}$ (cm⁻¹) : ~3150 (NH), 3054.64 (CH Aromatic), 2878.83 (CH,Methyl), 1773.88 (C=O), 1632.09 (C=N), 1531.45 (NO Nitro) , 1466.13 (C=C Ar), 1285.54 (C=S), 1234.65 (C-N).

5c). 1-methyl-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one:

Molecu Weight = 335.38302 g , m.p - 185-187 °C , yield-84.6%, Chemical Composition = C(53.68%) H(3.18%) N(22.09%) O(12.62%) S(8.43%), C(60.88%) H(3.91%) N(20.88%) O(4.77%) S(9.56%), ¹H-NMR (DMSO d) δ ppm:

10.483 (1H,s,NH), 7.926 (2H, d, ArH), 7.588(1H,m,ArH), 7.509 (2H,m,ArH), 7.287 (1H,d,ArH), 7.265 (1H,d,ArH), 7.117 (1H,m,ArH), 7.094 (1H,m,ArH), 3.543 (3H,s,Alkyl). ¹³C-NMR(DMSO-d) δppm : 165.828, 138.391, 132.556, 131.839, 128.493, 127.430, 38.668. IR $\bar{\nu}$ (cm⁻¹) : 3197.04 (NH), 3053.49 (CH Aromatic), 3004.99 (CH,Methyl), 1709.33(C=O), 1632.45 (C=N), 1488.28 (C=C Ar), 1285.80 (C=S), 1232.80 (C-N).

6a) 6-bromo-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one :

Molecular Formula = C₁₆H₁₀BrN₅OS, Formula Weight = 400.2525, Chemical Composition = C (48.01%), H (2.52%), Br (19.96%), N (17.50%), O (4.00%), S(8.01%), m.p - 162°C, yield – 76.6%, ¹H-NMR(DMSO d) δ ppm: 11.198 (1H,s,NH), 10.527 (1H,s,NH), 7.956 (1H,d,ArH), 7.556 (2H,m,ArH), 7.509 (2H,m,ArH), 7.746 (1H,d,ArH), 7.488 (1H,d,ArH), 7.647 (1H,s,ArH). ¹³C-NMR(DMSO-d) δ ppm: 183.201, 165.836, 159.342, 158.946, 150.729, 149.583, 140.064, 138.383, 132.573, 131.856, 128.501, 127.496, 126.911, 126.820, 124.669, 122.749, 119.526, 117.787, 112.216. IR $\bar{\nu}$ (cm⁻¹) : 3199.80 (NH), 3060.8 (CH Aromatic), 1747.73(CO), 1615.29 (C=N), 1467.63 (C=C Ar), 1288.47 (C=S), 1208.92 (C-N), 686.85 (C-Br).

6b) 6-nitro-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Formula Weight = 746.73458, Chemical Composition = C(53.08%) H(2.97%) N(22.51%) O(12.86%) S(8.59%), m.p – 186 °C, yield – 81%, ¹H-NMR(DMSO d) δ ppm: 11.037 (1H,s,NH), 10.530 (1H,s,NH), 7.956 (1H,d,ArH), 7.588 (2H, m, ArH), 7.511(1H,d,ArH), 7.456 (2H,m,ArH), 7.313 (1H,d,ArH), 7.290 (1H,m,ArH), 7.175 (1H,m,ArH). ¹³C-NMR(DMSO-d) δ ppm: 184.446, 165.885, 150.762, 145.586, 144.762, 132.581, 131.872, 128.534, 127.529, 124.702, 122.790, 117.795, 111.177. IR $\bar{\nu}$ (cm⁻¹) : 3156.72 (NH), 3056.54 (CH Aromatic), 1733.03(CO), 1617.33 (C=N), 1464.55 (C=C Ar), 1287.66 (C=S), 1195.16 (C-N).

6c). 3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Mole Weight = 321.35644, Chemical Composition = C(59.80%)

H(3.45%) N(21.79%) O(4.98%) S(9.98%), m.p-176-180, yield – 79.6%, ¹H-NMR (DMSO δ ppm): 11.037 (1H,s,NH), 10.530 (1H,s,NH), 7.956 (1H,d,ArH), 7.588 (2H, m, ArH), 7.511(1H,d,ArH), 7.456 (2H,m,ArH), 7.313 (1H,d,ArH), 7.290 (1H,m,ArH), 7.175 (1H,m,ArH). ¹³C-NMR(DMSO-d) δ ppm: 184.446, 165.885, 150.762, 145.586, 144.762, 132.581, 131.872, 128.534, 127.529, 124.702, 122.790, 117.795, 111.177. IR $\bar{\nu}$ (cm⁻¹) : 3156.72 (NH), 3056.54 (CH Aromatic), 1733.03(CO), 1617.33 (C=N), 1464.55 (C=C Ar), 1287.66 (C=S), 1195.16 (C-N).

7a) 6-bromo-1-(piperazinemethyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl) imino]-1,3-dihydro-2H-indol-2-one : Molecular Weight = 498.3988, Chemical Composition = C(50.61%) H(4.04%) Br(16.03%) N(19.67%) O(3.21%) S(6.43%), 110 °C, yield – 76.2%, ¹H-NMR(DMSO d) δ ppm: 10.275 (1H,s,NH), 7.966 (2H,d, ArH), 7.651(1H,d,ArH), 7.522 (2H,m,ArH), 7.490 (1H,s,ArH), 7.465 (1H,m,ArH), 7.441 (1H,d,ArH), 3.476 (2H,s,CH₂), 3.082(2H,t,CH₂), 2.791(2H,t, CH₂), 2.617(2H,m, CH₂), 2.160 (2H,m, CH₂). ¹³C NMR (DMSO -d) δ ppm - 165.688, 145.586, 131.749, 130.908, 127.463, 126.854, 51.138, 43.077, 39.888. IR $\bar{\nu}$ (cm⁻¹) : 3306.37-3226.79 (NH), 3011.89 (CH Aromatic), 2980.17 (CH,Methyl), 1723.58 (C=O), 1632.70 (C=N), 1506.52-1463.28 (C=C Ar), 1285.81 (C=S), 1022.24 (C-N), 690.41 (C-Br).

7b) 1-(piperazinemethyl)-6-nitro-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl) imino]-1,3-dihydro-2H-indol-2-one : Molecular Weight = 464.5003, Chemical Composition = C(54.30%) H(4.34%) N(24.12%) O(10.33%) S(6.90%), m.p - 82°C, yield – 58.6%, ¹H NMR (DMSO d) δ ppm - 10.530 (1H, s, NH), 8.059 (1H, s, Ar) , 7.948 (1H, d, Ar), 7.924 (1H, d, Ar), 7.585 (1H, d, Ar), 7.553 (1H, d, Ar), 7.529 (1H, m, Ar) , 3.521 (2H, m, CH₂), 2.754, 2.713, 2.642, 2.549 and 2.510 (2H, t). ¹³C NMR (DMSO d) δ ppm - 165.869, 161.245, 145.199, 140.475, 132.573, 131.889, 128.543 127.488 and 123.293, 52.497, 50.346, 42.558. IR (KBr) $\bar{\nu}$ cm⁻¹ : 3479.34-3350.05 (NH), 3176.10 (Ar CH), 3107.16 (CH₃), 1962.35-1801.17 (C=O), 1632.49 (C=N), 1512.72-1385.92 (C=C Ar), 1283.95 (C=S), 1016.95 (CN) and 1569.17 (NO) .

7c). 1-(piperazin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Formula Weight = 419.50274, Chemical Composition = C(60.12%) H(5.05%) N(23.37%) O(3.81%) S(7.64%), m.p - 80-85°C, yield – 58.6%, ¹H NMR (DMSO d) δ ppm- 10.499 (1H,s,NH), 7.903 (2H,d,ArH), 7.506(2H,m,ArH), 3.455(2H,s,CH₂), 3.124 (4H,t, CH₂), 2.728, 2.594, 2.528 and 2.490(4H,m,CH₂) . ¹³C NMR (DMSO d) δ ppm -145.546, 132.573, 131.864, 129.251, 128.518, 128.370, 127.471, 65.869, 50.948,

42.673. IR (KBr) $\bar{\nu}$ cm⁻¹ : 3306.37-3226.79 (NH), 3011.89 (Ar CH), 2980.17 (CH₂), 1723.58 (C=O), 1632.70 (C=N), 1506-1463.28 (Ar C=C), 1285.81 (C=S), 1022.24 (C-N), 690.41 (C-Br).

8a). 6-bromo-1-(piperidin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Molecular Weight = 497.41074, Chemical Composition = C(53.12%) H(4.26%) Br(16.06%) N(16.90%) O(3.22%) S(6.45%), m.p - 87-90°C, yield – 56.4%, ¹H NMR (DMSO d) δ ppm - 10.504 (1H,s,NH), 7.642 (1H,s,ArH), 7.478(2H,d,ArH), 7.185 (2H,d,ArH), 7.907(2H,d,ArH), 3.363 (2H,s,CH₂), 2.711, 2.686, 2.653, 2.528 (4H,m,CH₂). ¹³C NMR (DMSO d) δ ppm: 145.504, 129.095, 127.669, 68.76, 22.448, 38.668. IR (KBr) $\bar{\nu}$ cm⁻¹ : 3382.10 (NH), 3012.80 (Ar CH), 2979.01-2563.27 cm⁻¹ (CH₃), 1716.99-1667.65 cm⁻¹ (C=O), 1632.23 (C=N), 1464.72 (C=C Ar), 1287.65 (C=S), 1023.19 (C-N), 688.37 (C-Br) .

8b). 6-nitro-1-(piperidin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Molecular Weight = 463.51224, Chemical Composition = C(57.01%) H(4.57%) N(21.15%) O(10.36%) S(6.92%), m.p - 82-85°C, yield – 59.6%, ¹H NMR (DMSO d) δ ppm -10.493 (1H,s,NH), 7.292(1H,s,ArH), 7.920(2H,d,ArH), 7.895, 7.585(2H,d,ArH), 7.562(2H,m,ArH), 7.531(2H,m,ArH), 4.029 (2H,s,CH₂). ¹³C NMR (DMSO d) δ ppm: 150.721, 138.392, 124.669, 123.202, 117.787, 112.232, 110.568, 52.497, 38.668, 26.017. IR (KBr) $\bar{\nu}$ cm⁻¹ : 3346.44 (NH), 3059.69 (Ar CH), 3029.55 (C₂H₂), 1966.98 (C=O), 1621.40 (C=N), 1448.48 (Ar C=C), 1286.76 (C=S), 1034.08 (CN), 1578.86 (NO).

8c). 1-(piperidin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one: Molecular Weight = 418.51468, Chemical Composition = C(63.14%) H(5.30%) N(20.08%) O(3.82%), S(7.66%), m.p - 72-76°C, yield – 52.6%, ¹H NMR (DMSO d) δ ppm 10.438 (1H,s,NH), 7.902(2H,d,ArH), 7.902(2H,m,ArH), 7.265(1H,m,ArH), 7.287(2H,d,ArH), .534(2H,m,ArH), 7.509(1H,m,ArH), 3.832 (2H,s,CH₂). ¹³C NMR (DMSO d) δ ppm :145.20, 138, 124.669, 123.202, 122.773, 117.787 and 112.23, 66.420, 38.940, 26.017. IR (KBr) $\bar{\nu}$ cm⁻¹ : 3198.99 (NH), 3075.35 (Ar CH), 2995.15 (C₂H₂), 1714.62 (C=O), 1614.61 (C=N), 1469.65 (Ar C=C), 1290.04 (C=S), 1069.37 s(C-N).

These compounds target the biosynthesis of ergosterol by inhibiting the cytochrome P450-dependent lanosterol 14 α -demethylase resulting in accumulation of toxic methyl sterols in membranes that may culminate in fungi static effect or fungal death [11].

Table 4.7 antifungal activity of [(3-Phenyl-5-Thioxo-1,5-Dihydro-4H-1,2,4-Triazol-4-yl) Imino] -1,3-Dihydro-2H-Indole-2-one Derivatives

Compounds	Inhibition zone (mm)		
	C.albicans	C.krusie,	C.tropicalis
5a	16.27	11.30	11.20

5b	13.37	12.86	11.38
5c	12.67	9.47	9.72
6a	10.34	8.56	10.26
6b	8.72	7.26	9.53
6c	7.03	7.18	8.65
7a	19.0	12.50	9.77
7b	14.80	12.53	12.83
7c	12.80	16.53	09.56
8a	11.0	11.50	19.77
8b	8.80	9.53	12.50
8c	9.80	10.53	10.56
Fluconazole	25.33	24.65	24.06
DMSO	-	-	-

Inhibition zone values refer that the activity of compounds are: (i) not active (0-6 mm), (ii) slight active (7-8 mm), (iii) moderate active (9-11 mm), (iv) active (12-15 mm) and (v) high active (≥ 16 mm).

Compound (7a) 6-bromo-1-(piperazin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one was the most active against *C.albicans*, (7c) 1-(piperazin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl) imino]-1,3-dihydro-2H-indol-2-one showed the significant antifungal activity against *C.krusicie* and (8b) 6-nitro-1-(piperidin-1-yl-methyl)-3-[(3-phenyl-5-thioxo-1,5-dihydro-4H-1,2,4-triazol-4-yl)imino]-1,3-dihydro-2H-indol-2-one against *C.tropicalis*.

Electron withdrawing NO_2 - group of (7c) was most active from each derivatives. NO_2 - group increases the hydrophobicity of compound enhancing the penetration of the fungal cell membrane. Antifungal activity of (7c) without any substituent on the phenyl ring of indole has higher activity than that of with Br- substituent [12]. Derivatives 7a, 7b and 7c with two active centres of piperazine ring to form H-bond with the target sites were found to be more potent antifungal agent than 8a, 8b and 8c with one active centre of piperidine ring [13]. Compound 5a, 5b, 5c having Methyl substituent at N- results in increased fungicidal activity slightly in compare to 6a ,6b, 6c because alkylation increases the lipophilicity of compound and hence penetration power.

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