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Synthesis and Antifungal Activity of 1,4-Benzothiazine Derivatives

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ABSTRACT

A series of [Alkyl or un/substituted phenyl]-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide were designed and synthesized. In vitro antifungal activity assay indicates that the 1,4-Benzothiazine has good antifungal activity against most of the tested pathogenic fungi i. e. *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur.* Some few compounds such as BTA-43 and BTA-56 have significant antifungal activity and broader spectrum, which are promising leads for the development of novel antifungal agents. The structures of the synthesized compounds were established on the basis of IR, NMR, ^{13}CNMR and Mass spectra data.

Keyword: Antifungal activity, 1,4-Benzothiazine, Dermatophytes.

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INTRODUCTION

4*H*-1,4-Benzothiazines possess a wide spectrum of biological and pharmacological activities due to presence of a fold along the nitrogen and sulfur axis, which is considered to be responsible as one of the structural features to impart their activities. [1-3] During the past two decades, the frequency of invasive and systemic fungal infections has increased dramatically in the population with altered immunity.[4-5] Current available therapy in treating fungal infections can suffer from drug related toxicity, hazardous drug–drug interactions, non-optimal pharmacokinetics and development of drug resistance.[6] Fungal infections remain a significant cause of morbidity and mortality, specially in immune compromised host where the incidence of life threatening fungal infections has risen dramatically.[7]

With the aim of developing a new class of antifungal drugs with more potent and broad spectrum, some new compounds 4*H*-1,4-benzothiazines were designed and synthesized for screening of antifungal activities against four microorganism such as, *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur*.

MATERIALS AND METHODS

All chemicals used are LR grade and were purchased from Research laboratory Pvt ltd. All solvents used for chromatography are AR grade were purchased from Rankem Ltd. Melting points of all the synthesized compounds are uncorrected. The progress of reaction was monitored by TLC silica gel adsorbent on coated aluminium plates (Merck) and UV light as a visualizing agent. The purity of synthesized compounds was checked by thin-layer chromatography. The IR spectra were recorded on Shimadzu FTIR spectrophotometer in the range of 4000-400 cm^{-1}. ^{1}HNMR spectra were scanned at 300 MHz on Varian-NMR-Mercury 300 FT NMR spectrophotometer using DMSO d-6 as solvent and TMS as an internal standard. ^{13}CNMR spectra on Bruker Avance-II spectrometer were scanned at 500 MHz using DMSO d-6 as solvent and mass spectra recorded on Applied Biosystems 4800 plus MALDI TOF analyzer.

Experiment:

(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetic acid (3)

To a solution of maleic anhydride (1) (5.0g, 0.05 mole) in THF (30mL) a solution of *o*-aminothiophenol (2) (6.25g, 0.05mole) in THF (15mL) was added with stirring and the solution was stirring continue for 10 minutes at room temperature, the crystalline product that precipitated out, was filtered off and washed with THF.

Yield; 10g(96%), Melting point: 172-174 ^{O}C, IR(cm^{-1}): 3000-2550 (COOH) Broad, 3274 (N-H)*s*,

3010 (C-H) *s*, 1710 (C=O) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.85 (*d*, 2H, CH_2), 3.800 (*t*, H, CH), 6.98-7.327 (*m*, 4H. ArH), 10.69 (*s*, 1H, NH), 12.43 (*s*, 1H, COOH). Mass (m/e): M^+ = 223.25, 205.028(100%), 124.014, 116.027.

Methyl 2-(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetate (4)

(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetic acid **(3)** (0.01 mol), dry methanol (25 mL) and few drops of Conc. H_2SO_4 (98%) were taken in a 100 mL round bottomed flask and was heated at reflux for 4 h on a water bath. Progress of reaction was monitored by TLC. The reaction mixture was concentrated, cooled, the crystalline product was separated by filtration, dried.

Yield: 85 %, Melting point: 130-132 ^{O}C, IR (cm^{-1}): 1740 (C=O) *m*, 3010 (CH) s, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.750 (*d*, 2H, CH_2), 3.170 (t, 1H, CH), 4.120 (s, 1H, CH_3), 7.000-7.421 (*m*, 4H. ArH), 10.220 (*s*, 1H, NH). Mass (m/e): M^+ = 237.05, 206.028(100%),

General procedure for synthesis of *N*-[Alkyl or un/substituted phenyl]-2-(3-oxo-3, 4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA) (5)

In a round bottomed flask (100 mL) fitted with a reflux condenser, a mixture of Methyl 2-(3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetate (2.37 g, 0.01mole) (**4**) and alkyl/ Arylamine (0.01mole) in dry methanol (50 mL) was heated on a water bath for 6 hr. After completion of the reaction (monitored by TLC), the reaction mixture was poured into cold water and extract with dichloromethane and wash with dil HCl. Extract was then dry, concentrated, cooled and recrystallized to get the product. Analytical sample was prepared by column chromatograph eluting with methanol: chloroform (3:7).

***N*-Methyl-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-4)** Yield: 80%, Melting Point: 185-188 ^{O}C, IR (cm^{-1}): 1700(–CONH-) *s*, 1770 (-CO-) *m*, 3250 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.723 (*d*, 2H, CH_2), 3.831 (*t*, 1H, CH), 2.500 (*s*, 3H, N-CH_3), 6.845-7.400 (*m*, 4H, ArH), 9.279 (*s*, 1H, NH amide), 10.700 (*s*, 1H, NH lactam), ^{13}C NMR (DMSO-d_6, 500 MHz,): δ 33.370 (CH_2), 37.117 (CH ring), 51.635 (amine methyl gr), 117.043, 117.955, 123.031, 126.285, 127.184, 130.717 (6 peak of aromatic) 165.441 (C=O, lactam), 170.066 (C=O, amide), Mass (m/e): M^+ = 236.56, 206.00(100%), 164.27, 124.87.

2.1.3.2 *N*-tert-Butyl-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-8)

Yield: 55%, Melting Point: 190-192 ^{O}C, IR (cm^{-1}): 1640 (–CONH-) *m*, 1760 (-CO-) *s*, 3066 (C-H) s, 3290 (N-H) *s*,^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 1.523 (*s*, 9H, $3CH_3$), 2.710 (*d*, 2H, CH_2), 3.701 (*t*, 1H, CH), 7.020-7.525 (*m*, 4H, ArH), 8.310 (*s*, 1H, NH amide), 10.723 (*s*, 1H, NH lactam), ^{13}C NMR (DMSO-d_6, 500 MHz,): δ 26.136 (methyl gr of t-butyl), 33.355(CH_2), 37.182

(CH ring), 51.635 (amine t-butyl carbon), 117.073, 117.996, 123.077, 127.241, 127.522, 136.271 (6 peak of aromatic) 165.448 (C=O, lactam), 170.136 (C=O, amide), Mass (m/e) : M^+ = 278.4660, 206.085(100%), 221.26, 164.82, 124.67.

***N*-Ethyl-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-5)**

Yield: 56%, Melting Point: 180-183 ^{O}C, IR (cm^{-1}): 1770(-CO-) *m*, 1704 (–CONH-) *m*, 2931(C-H) *s*, 3270 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 1.123 (*t*, 3H, CH3), 1.814 (*q*, 2H, CH_2), 2.835 (*d*, 2H, CH_2), 3.721 (*t*, 1H, CH), 7.010-7.421 (*m*, 4H, ArH), 8.351 (*s*, 1H, NH amide), 10.721 (*s*, 1H, NH lactam).

***N*-Butyl-2-(3-oxo-3, 4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-47)**

Yield: 70%, Melting Point: 190-192 ^{O}C, IR (cm^{-1}): 1760(-CO-) *s*, 1700(–CONH-) *s*, 2960 (C-H) *m*, 3310 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 0.912 (*t*, 3H, CH_3), 1.414-1.122 (*m*, 6H, CH_2), 2.735 (*d*, 2H, CH_2), 3.721 (*t*, 1H, CH), 6.910-7.521 (*m*, 4H, ArH), 8.211 (*s*, 1H, NH amide), 10.721 (*s*, 1H, NH lactam).

***N*-(2-Hydroxyethyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-20)**

Yield: 65%, Melting Point: 185-188 ^{O}C, IR (cm^{-1}): 1700(–CONH-) *s* , 2950 (C-H) *s*, 3600-3300 (-OH) Broad *s*, 3250 (N-H) *m*, ^{1}HNMR (DMSO-d_6, 300 MHz): δ 2.621 (*d*, 2H, CH_2), 2.9 (*t*, 2H, CH_2), 3.5 (*t*, 2H, CH2), 3.850 (*t*, 1H, CH), 4.145 (*s*, 1H, OH), 6.910-7.520 (*m*, 4H, ArH), 8.423 (*s*, 1H, NH amide), 10.978 (*s*, 1H, NH lactam).

***N*-(2-Hydroxyphenyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-64)**

Yield: 45%, Melting Point: 204-207 ^{O}C, IR (cm^{-1}): 1670(–CONH-) *s*, 1770(-CO-) *s*, 3250 (N-H) *m*, 3550-3280(O-H broad peak) *m*, ^{1}HNMR (DMSO-d_6, 300 MHz): δ 2.625 (*d*, 2H, CH_2), 3.901 (*t*, 1H, CH), 4.524 (*s*, 1H, OH), 6.845-7.461 (*m*, 8H, ArH), 9.56 (*s*, 1H, NH amide), 10.590 (*s*, 1H, NH lactam).

***N*-(3-Chloro-4-hydroxyphenyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-35)**

Yield: 50%, Melting Point: 208-210 ^{O}C, IR (cm^{-1}): 1670(–CONH-) *m*, 1760(-CO-) *s*, 3250 (N-H) *m*, 3560-3320 (OH broad peak) *s*, ^{1}HNMR (DMSO-d_6, 300 MHz): δ 2.700 (*d*, 2H, CH_2), 3.890 (*t*, 1H, CH), 4.152 (*s*, 1H, OH), 6.645-7.321 (*m*, 7H, ArH), 9.209 (*s*, 1H, NH amide) 10.779 (*s*, 1H, NH lactam).

***N*-(1-Hydroxypropan-2-yl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-25)**

Yield: 60%, Melting Point: 204-206 ^{O}C, IR (cm^{-1}): 1700(–CONH-) *s*, 3060 (–CH_2) *m*, 2931(-C-H) *m*, 3210 (N-H) *m*,3600-3300 (OH broad peak) *s*, ^{1}HNMR (DMSO-d_6, 300 MHz); δ 2.9 (*d*, 2H, CH_2), 1.545 (*m*, 1H, CH), 1.245 (*d*, 3H, CH3), 2.780 (*d*, 2H, CH_2), 3.857 (*t*, 1H, CH), 4.120 (*s*, OH, 1H), 6.910-7.240 (*m*, 4H, ArH), 8.723 (*s*, 1H, NH amide), 11.208 (*s*, 1H, NH lactam).

***N*-(2-Methyl-3-nitrophenyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-66)**

Yield: 42%, Melting Point: 155-159 ^{O}C, IR (cm^{-1}): 1760(-CO-) *s*, 1712(-CONH-) *s*, 1340, 1410 (-NO_2) *m*, 3300 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.552 (*s*, 3H, CH3), 2.910 (*d*, 2H, CH_2), 3.740(*t*, 1H, CH), 6.911-7.390 (*m*, 7H, ArH), 9.309 (*s*, 1H, NH amide), 10.590 (*s*, 1H, NH lactam).

***N*-(3-Nitrophenyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-69)**

Yield: 67%, Melting Point: 185-190 ^{O}C, IR (cm^{-1}): 1750 (-CO-) *m*, 1670(-CONH-) *s*, 1340, 1410 (-NO_2) *w*, 3260 (N-H) *s*,^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.800 (*d*, 2H, CH_2), 3.765 (*t*, 1H, CH), 6.911-7.421 (*m*, 8H, ArH), 9.209 (*s*, 1H, NH amide), 10.590 (s, 1H, NH lactam).

***N*-(4-Nitrophenyl)-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-63)**

Yield: 52%, Melting Point: 240-242 ^{O}C, IR (cm^{-1}): 1760(-CO-) *s*, 1670(-CONH-) *s*, 1340, 1420 (-NO_2) *w*, 3250 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 *MHz*) δ 2.885 (*d*, 2H, CH_2), 3.787(*t*, 1H, CH), 6.870-7.250 (*m*, 8H, ArH), 9.200 (*s*, 1H, NH amide), 10.600 (*s*, 1H, NH lactam).

***N*-(2-Methylphenyl)-2-(3-Oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-57)**

Yield: 80%, Melting Point: 240-245 ^{O}C, IR (cm^{-1}): 1708(-CO-) *s* , 1610 (-CONH-) *m*, 2911 (-C-H) *s*, 3250 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 MHz,): δ 2.1 (s, 3H, CH_3), 2.880 (*d*, 2H, CH_2), 3.765(*t*, 1H, CH), 6.970-7.456 (*m*, 8H, ArH), 9.390 (*s*, 1H, NH amide), 10.780 (*s*, 1H, NH lactam), ^{13}CNMR (DMSO-d_6, 500 MHz,): δ 18.733 (CH_3) 33.406 (CH_2), 39.400 (CH ring), 100.616, 104.370, 116.654, 123.040, 125.373, 125.690, 126.916, 130.670, 131.208, 134.829, 136.423, 138.241 (12 peak of aromatic) 158.355 (C=O, lactam), 168.6227 (C=O, amide), Mass (m/e): M^+ = 312.0200, M^+ + 2 =314.0486, 206.234 (100%), 207.0392, 164.0235, 165.1051, 106.1555.

***N*-(3-Methylphenyl)-2-(3-Oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-58)**

Yield: 52%, Melting Point: 210-215 ^{O}C, IR (cm^{-1}): 1710(-CO-) *s*, 1610 (-CONH-) *m*, 2911, (C-H) *s*, 3225 (N-H) *s*, ^{1}HNMR (DMSO-d_6, 300 MHz): δ 1.9 (*s*, 3H, CH_3), 2.750 (*d*, 2H, CH_2),

3.750 (*t*, 1H, CH), 6.811-7.321 (*m*, 8H, ArH), 9.350 (*s*, 1H, NH amide), 10.600 (s, 1H, NH lactam).

_N_-(4-Methylphenyl)-2-(3-Oxo-3,4-dihydro-2_H_-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-56)

Yield: 70%, Melting Point: 212-215 ^{O}C, IR (cm^{-1}): 1710 (-CO-) *s*, 1610 (-CONH-) *m*, 2920 (C-H) *w*, 3290 (N-H) *m*, ^{1}HNMR (DMSO-d$_6$, 300 MHz): δ 2.1 (*s*, 3H, CH$_3$), 2.880 (*d*, 2H, CH$_2$), 3.765(*t*, 1H, CH), 7.150-7.550 (*m*, 8H, ArH), 9.134 (*s*, 1H, NH amide), 10.750 (s, 1H, NH lactam). ^{13}CNMR (DMSO-d$_6$, 500 MHz,): δ 21.631 (CH$_3$) 33.370 (CH$_2$), 37.170 (CH ring), 103.950, 112.077, 117.143, 117.955, 123.070, 126.285, 127.184, 127.559, 130.710, 131.376, 134.870, 137.635, (12 peak of aromatic) 165.550 (C=O, lactam), 171.285 (C=O, amide), Mass (m/e): M^{+} = 312.5678, 206.0086 (100%), 164.3156.

_N_-(2-Ethylphenyl)-2-(3-oxo-3,4-dihydro-_2H_-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-65)

Yield: 35%, Melting Point: 195-197 ^{O}C, IR (cm^{-1}): 1710(-CO-) *s* , 1620 (-CONH-) *m*, 2930 (C-H) *s*, 3260 (N-H) *m*, ^{1}HNMR (DMSO-d$_6$, 300 MHz) δ 1.200 (*t*, 3H, CH$_3$), 1.800 (*q*, 2H, CH$_2$), 2.880 (*d*, 2H, CH$_2$), 3.765 (*t*, 1H, CH), 7.050-7.560 (*m*, 8H, ArH), 9.490 (*s*, 1H, NH amide), 10.800 (*s*, 1H, NH lactam)..

_N_-(2-Chlorophenyl)-2-(3-oxo-3, 4-dihydro-_2H_-benzo[b][1, 4]thiazin-2-yl) acetamide (BTA-59)

Yield: 41%, Melting Point; 235-239 ^{O}C, IR (cm^{-1}): 1710(-CO-) *s* , 1620 (-CONH-) *m*, 2930 (C-H) *s*, 3200 (N-H) *s*, ^{1}HNMR (DMSO-d$_6$, 300 MHz): δ 2.900 (*d*, 2H, CH$_2$), 3.856 (*t*, 1H, CH), 7.070-7.445 (*m*, 8H, ArH), 9.400 (*s*, 1H, NH amide), 10.890(*s*, 1H, NH lactam).

_N_-(3-Chlorophenyl)-2-(3-oxo-3,4-dihydro-2_H_-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-60)

Yield: 52%, Melting Point: 215-218 ^{O}C, IR (cm^{-1}): 1710 (-CO-) *s*, 1610 (-CONH-) s, 2980 (C-H) *s*, 3250 (N-H) *s*, ^{1}HNMR (DMSO-d$_6$, 300 MHz) δ 2.780 (*d*, 2H, CH$_2$), 3.790 (*t*, 1H, CH), 6.970-7.450 (m, 8H, ArH), 9.200 (*s*, 1H, NH amide), 10.600 (*s*, 1H, NH lactam).

_N_-(4-Chlorophenyl)-2-(3-oxo-3,4-dihydro-_2H_-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-61)

Yield: 60%, Melting Point: 230-232^{O}C, IR (cm^{-1}): 1725 (-CO-) *s*, 1620 (-CONH-) *s*, 2970 (-C-H) *w*, 3200 (N-H) *m*, ^{1}H NMR (DMSO-d$_6$, 300 MHz): δ 2.550 (*d*, 2H, CH$_2$), 3.765 (*t*, 1H, CH), 6.980-7.321 (m, 8H, ArH), 9.057 (s, 1H, NH amide), 10.950 (*s*, 1H, NH lactam).

_N_-(2-Methoxyphenyl)-2-(3-oxo-3,4-dihydro-_2H_-benzo[b][1, 4]thiazin-2-yl) acetamide (BTA-67)

Yield: 52%, Melting Point: 180-185 ^{O}C, IR (cm^{-1}): 1620 (C=O) *s*, 1730(-CONH-) *s*, 2970 (C-H) *s*, 3220 (N-H) *m*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.880 (*d*, 2H, CH_2), 3.770 (*t*, 1H, CH), 4.500 (*s*, 3H, CH_3), 6.975-7.450 (*m*, 8H, ArH), 9.400 (*s*, 1H, NH amide), 10.800 (*s*, 1H, NH lactam).

***N*-(4-Methoxyphenyl)-2-(3-oxo-3,4-dihydro-*2H*-benzo[b][1,4]thiazin-2-yl) acetamide (BTA-68)**

Yield: 64%, Melting Point: 188-192 ^{O}C, IR (cm^{-1}): 1610(-CO-) *s* , 1700(-CONH-) *s*, 2970 (C-H) *s*, 3250 (N-H) *m*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 2.900 (*d*, 2H, CH_2), 3.665 (*t*, 1H, CH), 4.150 (*s*, 3H, CH_3), 6.975-7.450 (*m*, 8H, ArH), 9.400 (*s*, 1H, NH amide), 10.800 (*s*, 1H, NH lactam).

N-Cyclohexyl-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-24)

Yield: 52%, Melting Point:185-190 ^{O}C IR (cm^{-1}): 1640(-CO-) *s*, 1730(-CONH-) *s*, 2850 (C-H) *s*, 3200 (N-H) *m*, ^{1}HNMR (DMSO-d_6, 300 *MHz*): δ 1.2 (*s*, 11H, cyclohexane), 2.719 (*d*, 2H, CH_2), 3.521 (*t*, 1H, CH), 6.910-7.490 (*m*, 4H, ArH), 8.540 (*s*, 1H, NH amide), 10.653 (*s*, 1H, NH lactam).

***N*-Phenyl-2-(3-Oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA-70)**

Yield: 62%, Melting Point: 239-245 ^{O}C IR (cm^{-1}): 1630(-CO-) *m*, 1730(-CONH-) *s*, 2930 (C-H) *m*, 3220 (N-H) *m*, ^{1} H NMR (DMSO-d_6, 300 *MHz*): δ 2.600 (*d*, 2H, CH_2), 3.890 (*t*, 1H, CH), 7.040-7.750 (*m*, 9H, ArH), 9.350 (s, 1H, NH amide), 10.850 (*s*, 1H, NH lactam).

Pharmacological activity

Preparation of nutrient broth media for the growth of dermatophytes:

Composition of Double Strength Sabouraud's broth

i. Peptone – 20gm
ii. Dextrose – 80gm
iii. Distilled water –Qs to 1000 mL

Peptone and dextrose were dissolved in distilled water with heating. Then it was cooled and the P_H was adjusted to 5.4 with lactic acid and filtered.

The prepared medium and the suspension tubes were sterilized at 120^{o}C for 15 minutes in autoclave. The stock solution 1µmole/ml compound (equimolar) was prepared in DMSO. All the compounds were screened individually by making serial dilutions containing 0.5, 0.25, 0.125, 0.0625, 0.0312 and 0.0156 µmole/ml of the compound. To each tube containing 2ml of Sabouraud's liquid medium, 2ml of the drug solution (BTA compounds) of 1µmole/ml was added. The tubes were inoculated using microbial suspension in saline solution. The standard drugs used are Ketoconazole (1 µmole/ml) for comparison. The positive control (Organism +

broth + DMSO) and the blank negative control (broth + DMSO) were also prepared. The dermatophytes were incubated at 28 ^{0}C. Growth, MIC was determined at 24 h for *C. albicans*, at 72 h for *T. rubrum, E. floccosum* and *M. furfur*. The growth in the tubes was observed visually for the turbidity.

RESULTS AND DISCUSSION:

Chemistry:

Target compound were prepared by synthetic scheme described in fig no. 1. (3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetic acid (3) was synthesized by mixing equimolar quantities of maleic anhydride and *o*-aminothiophenol in THF at room temperature. Obtained 3 reflux with methanol in the presence of sulphuric acid for 6 hr to form ester (4). The resultant ester (4) were condense with aliphatic or aromatic amine into *N*-[Alkyl or un/substituted phenyl]-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide (BTA) (5) by simple refluxing ester (4) with different alkyl or aryl amines in methanol for 6 hrs. All compounds were obtaining in good yield. All reactions were monitored by analytical thin-layer chromatography. The structures of the compounds were established with IR and ^{1}HNMR, ^{13}CNMR and Mass spectra.

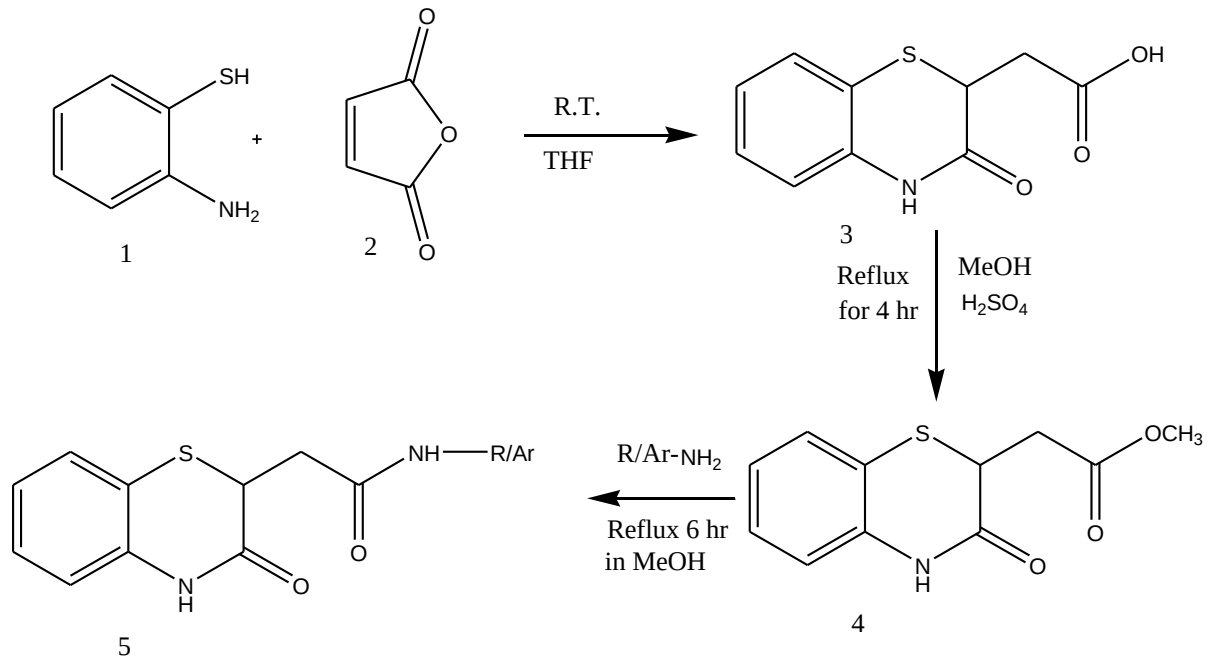

Figure 1: Scheme of Synthesis of 1,4-Benzothiazine derivatives.

The formation of 1,4-Benzothiazine ring is confirm by IR spectra of (3-Oxo-3,4-dihydro-2*H*-1,4-benzothiazin-2-yl)acetic acid (**3**) with COOH (-OH) peak at 3000-2550 broad & strong and C=O peak at 1710. The NMR spectra of ester (4) were show a consistence result with the structure; peak for CH-CH$_2$ group found at δ 2.75 *d* & 3.170 *t*, aromatic proton in the range of δ 7.000-

7.421, lactam proton on nitrogen at δ 10.220 *s*, and methoxy proton at 4.120. The ester of 3 with methanol also confirm with IR by the absence of OH peak at 3000-2550 and in NMR show the absence of carboxylic proton at δ 12.430 ppm and presence of singlet at 4.120 for the methoxy group. The formation of amide linkage in 1,4-Benzothiazine derivatives is confirm by NH-CO peak at 1700-1650 and N-H peak at 3200-3400 and in NMR two singlet peak around at δ 8.5-9.5 and 10.0-11.0 ppm for the NH of amide linkage and lactam respectively. The confirmation of structure of title compounds with IR, ^{1}HNMR, Mass and ^{13}CNMR spectra of some representative derivatives.

In-vitro antifungal activity:

A group of synthesized compounds were screened for antifungal activity against *Candida albicans, Epidermophyton floccosum, Trychophyton rubrum, and Malassazia furfur.* The evaluation of antifungal activity was carried out by the tube dilution method (turbidometric method). The turbidometric method depends upon the inhibition of growth of a microbial culture in the uniform solution of drug in a fluid medium (broth) that is favorable for its growth. The nutrient medium used for fungi was *Sabouraud's broth*.[8-9] The activities of each compound are expressed in terms of the minimum inhibitory concentration (MIC) means minimum concentration of compounds that inhibit the growth of fungus. The activity of each synthesized compound is given in Table 1.

Table 1: Antifungal activity data of 1,4-Benzothiazine derivatives against four fungus species

Sr.No.	Compound Code	MIC			
		C. albicans	*E. floccosum*	*T. rubrum*	*M. furfur*
1	BTA-4	0.250	0.125	0.250	0.125
2	BTA-8	0.125	0.125	0.125	0.250
3	BTA-5	0.125	0.125	0.125	0.125
4	BTA-47	0.0625	0.0625	0.0625	0.125
5	BTA-20	0.0625	0.0625	0.125	0.125
6	BTA-43	0.0312	0.0625	0.125	0.125
7	BTA-64	0.250	0.250	0.250	0.250
8	BTA-35	0.125	0.125	0.125	0.0625
9	BTA-25	0.125	0.250	0.0625	0.125
10	BTA-66	0.250	0.250	0.125	0.250
11	BTA-69	0.125	0.125	0.125	0.250
12	BTA-63	0.250	0.125	0.250	0.250
13	BTA-57	0.250	0.250	0.125	0.250
14	BTA-58	0.250	0.250	0.125	0.125
15	BTA-56	0.0625	0.0625	0.0312	0.0312
16	BTA-65	0.250	0.250	0.250	0.250
17	BTA-59	0.125	0.125	0.250	0.250

18	BTA-60	0.250	0.250	0.250	0.125
19	BTA-61	0.250	0.0625	0.125	0.250
20	BTA-67	0.250	0.250	0.125	0.125
21	BTA-68	0.125	0.250	0.125	0.125
22	BTA-24	0.0625	0.0625	0.0625	0.0625
23	BTA-70	0.125	0.125	0.250	0.250
24	Ketaconazole (std)	0.0312	0.0312	0.0312	0.0312

All MIC value in µmol/ml

*Each result represents the average of triplicate reading.

E. Floccusom= Epidermophyton. floccusom; M. ruburum = Microsporum. rubrum: M. furfur = Malassazia furfur

Antifungal activity of all synthesized compounds were evaluated against four microbial species, viz. *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur,* it was found that few compound of 1,4-Benzothiazine derivatives has good in-vitro antifungal activity. BTA-47, BTA-20, BTA-56, BTA-24 with MIC-0.0625 µgm/ml, BTA-43 with MIC-0.0312 µgm/ml against *Candida albicans*; BTA-47, BTA-20, BTA-43, BTA-56, BTA-61, BTA-24 with MIC-0.0625 µgm/ml against *Trichophyton rubrum;* BTA-47, BTA-25, BTA-24 with MIC-0.0625 µgm/ml, BTA-56 with MIC-0.0312 µgm/ml against *Epidermophyton floccosum* and BTA-35, BTA-24 with MIC-0.0625 µgm/ml, BTA-56 with MIC-0.0312 µgm/ml against Malassazia *furfur*. The 1,4-Benzothiazine derivatives had significant activity against all fungal species.

CONCLUSION

In the present investigation, the synthesized [Alkyl or un/substituted phenyl]-2-(3-oxo-3,4-dihydro-2*H*-benzo[b][1,4]thiazin-2-yl)acetamide derivatives showed promising antifungal activity against four fungal species, *Candida albicans, Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur*. The 1,4-Benzothiazine derivatives had significant activity against all fungal species hence these derivatives are broad spectrum for antifungal activity.

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