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SYNTHESIS, CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZOTHIAZOLE ANALOGS AS POTENTIAL ANTITUBERCULAR AGENTS

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Keywords:

Benzthiazoles, Chalcones, Anti-tubercular, MABA

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ABSTRACT: Benzthiazole, Chalcones, and their analogs have a wide variety of biological activities like antihelminthic, antifungal, antibacterial, anti-diabetic, anti-tubercular, anti-inflammatory, anticonvulsant, diuretic *etc.*, In the present research work, an effort has been made to synthesize some new series of novel benzothiazole linked chalcones from 3-amino-acetophenone and potassium thiocyanate which were dissolved in glacial acetic acid at room temperature. Liquid bromine in glacial acetic acid was then added drop-wise, and finally, the reaction mass is quenched and basified with ammonia to obtain the desired Benzthiazole linked chalcone. The progress of the reaction is monitored through TLC. The purity of the compounds was identified by TLC and purified by recrystallization and column chromatography. The structures were determined by IR, 1H NMR, and Mass spectral data. The synthesized benthiazole linked chalcone analogues were screened for anti-tubercular activity by the use of MABA (Microplate Alamar Blue assay) analytical method on H37Rv strain of Mycobacterium tuberculosis. Based on the results of anti-tubercular activity, it can be seen that the compounds containing electron-withdrawing groups like chlorine, fluorine, nitrogen showed better activity than that of the other compounds in the series. The mechanism of action of the compounds can assassinate for their cell wall disruption by inhibiting the peptidoglycan synthesis as a potential antimicrobial agent or inhibiting the synthesis of mycolic acid as a potential anti-tubercular agent.

INTRODUCTION: Synthesis of hybrid drug technology paved the way for the development of innovative medicines, which are a sign of relief from several complex abnormalities / diseases, which include tuberculosis, cancer, and many other microbial infections.

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Single drug targets may not help in the treatment of complicated diseases that are difficult to diagnose or cure.

The first step toward this change is the hybrid drug or, even better and more affordable, the dual-target strategy, where two targets at different key points within the same or concurrent pathogenic pathways are carefully chosen for their potential additive effects or synergistic potentiating. In recent years, the chemistry of benzothiazoles captivated importance as these compounds have been found to exhibit several biological activities, such as Anti-inflammatory [1-2], Analgesic [3], Antitumor [4],

Anticonvulsant [5], Anti-diabetic [6], Antimicrobia [l7], Antifungal [8-9], CNS depressant [10], Antimalarial [11], Anthelmintic [12], Anticancer [13] Antiurease [14], Hypoglycemic agent [15], Anti-oxidant [16], Anti-helmenthic [17], Anti-histaminic [18], Insecticidal [19,] Anti-HIV [20], Antiprotozoal [21], Antitubercular [22].

Similarly, chalcones have also been found to exhibit several biological activities, such as anti-hyperglycemic, euglycemic, anti-inflammatory, anti-malarial, anti-oxidant, anti-tumor, cytotoxic, anti-microbial, anti-proliferative, PPARγ agonist, dual PPARα/γ activator, free radical scavenger, LDL oxidation inhibitor, glycogen synthase kinase (GSK) 3 inhibitor, aldose reductase inhibitor, cholesterol esterase inhibitor, 15-hydroxy-prosta-glandin dehydrogenase (15-PGDH) inhibitor, human β3 agonist, chymase inhibitor, bacterial arylamine N-Acetyltransferases (NATs) inhibitor, P2X7 receptor antagonist, thyroid hormone receptor antagonist, PTP1B inhibitor, human PTP1B and LMW-PTP inhibitor, Raf / MEK / Extracellular signal-regulated kinase (ERK1/2) inhibitor, dual inhibitor of the Raf/MEK/ERK and the PI3K/Akt signaling pathways, serine/threonine protein kinases Pim-1 and Pim-2 inhibitor, G-protein coupled receptor 40 (GPR40) agonist, MurD ligase inhibitor, monoamine oxidase B (MAO-B) inhibitor and neuroprotective.

In-addition, besides to benzothiazoles, chalcones and substituted pyrimidines have also been reported to exhibit diverse biological activities, such as such as Antiretroviral, Anti-tubercular, Antitumor, Anti-neoplastic, Anti-inflammatory, Diuretic, Anti-malarial, Cardiovascular, Cystic fibrosis trans-membrane conductance regulator inhibitors, β-site APP-cleaving enzyme 1 inhibitors, A3 adenosine receptor antagonists, Inhibitors of heat shock protein 90, Adenosine kinase inhibitory activity, EGFr and C-erbB-2 inhibitory activity, Anti-bacterial, Phosphodiesterase 5 inhibitory activity, Antifungal, Antiviral, Antihypertensive and Hepatoprotective respectively.

Having such a diverse range of pharmacological activities, these classes of the compound have attracted medicinal chemists, and consequently, a number of strategies based on hybrid drug discovery and development have been originated to synthesize them.

In the present study, the MH approach is followed as a novel and emerging drug discovery paradigm based on the selection of the above two pharmacophore fragments with superior therapeutic value and safety. This can be achieved by designing individual new chemical entities (hybrid drugs) by applying molecular hybridization techniques to the chosen bioactive fragment pharmacophores. This approach emerged based on the need for new methods to identify preliminary hit compounds and to validate against selected multifactorial diseases such as cancer, diabetes, multidrug-resistant microbial infections, *etc.* The relevant work will discuss the synthetic methodology used to prepare the designed hybrid molecules and the ease by which it may be cleaved to form the independent components *in-vivo*.

As a first step, two biologically relevant fragment pharmacophores benzothiazole and chalcone moieties were chosen, which are responsible for its pharmacological potential. Subsequently, the key pharmacophoric features are amalgamated in an individual molecule to obtain benzothiazole-linked chalcone hybrids. The decision to generate hybrid compounds is purely driven by the availability of reference compounds that earlier approved for clinical use, the chemical nature of the pharmacophores, and their synthetic feasibility. The rationale behind the selection of benzothiazole, chalcone pharmacophores for the construction of benzothiazole-linked chalcones to evaluate their and anti-tubercular activity using Mycobacterium tuberculosis H37Rv strain in the present study, the selection of *in-vitro* bioassay protocols are based on their pharmacological significance and reported literature in the relevant field of drug discovery and development. The structures of rationally designed benzothiazole analogs are exhibited as **Fig. 1**.

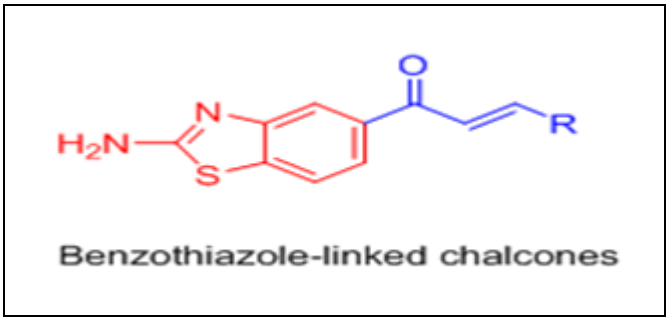


FIG. 1: GENERAL STRUCTURES OF RATIONALLY DESIGNED BENZOTHIAZOLE ANALOGS

It is proved from the literature that the compounds containing either benzothiazole/chalcone. Based on these observations, it was considered worthwhile to synthesize and characterize a series of benzothiazole-linked chalcones in the present investigation. As a part of a research program aimed at the search for new hybrid pharmacophores as anticancer and antitubercular agents, we are interested in having chalcone conjugation to the benzothiazole basic nucleus to give a series of benzothiazole-linked chalcones. Therefore, in the present study, an attempt has been made to synthesize and characterize a series of benzo-thiazole analogs of the key intermediate 1-(2-aminobenzo[d]thiazol-5-yl) ethan-1-one.

All the structures of the benzothiazole-linked chalcones (AC1-AC14) were appropriately established by melting point, IR, NMR, mass spectroscopic, and analytical data. To evaluate the synthesized benzothiazole-linked chalcones (AC1-AC14) for their *in-vitro* antitubercular activity using Mycobacterium tuberculosis H37Rv strain.

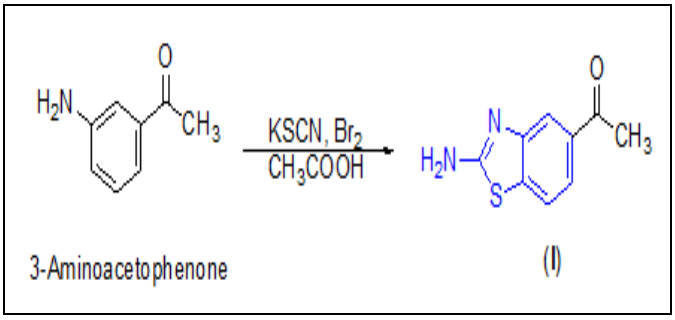
MATERIALS AND METHODS:

The Reaction Sequence Employed in the Synthesis of benzo-thiazole-linked chalcones (AC1-AC14). Synthesis of 1-(2-aminobenzo [d]thiazol -5-yl) ethan-1-one(I): The key intermediate in the present investigation is 1- (2-aminobenzo [d]thiazol-5-yl) ethan-1-one (I) which was prepared as per the method reported earlier from 3-aminoacetophenone (2.0 g, 14.8 mmol), potassium thiocyanate (3.9 g, 52.0 mmol) were dissolved in glacial acetic acid at room temperature. Liquid bromine (2.6 g, 16.3 mmol) in glacial acetic acid was then added drop-wise, maintaining the reaction temperature below 10 °C for a period of 90-180 min.

After the addition was complete, the reaction mixture was stirred, and the progress of the reaction was monitored by thin-layer chromato-graphy (TLC). After completion of the reaction as indicated by the disappearance of starting material on TLC analysis, the solids were filtered off and then washed first with glacial acetic acid and then with water. The filtrate was diluted with 300 ml of warm water, neutralized to pH 7 to 7.5 by using liquor ammonia and then cooled overnight in the refrigerator to allow the product to precipitate.

The product was filtered, washed with cold water, and dried under vacuum. The product was recrystallized using Methanol. Compound I, analyzed for $C_9H_8N_2OS$, which possess m.p. 242-245 ºC, which was consistent with the literature reported m.p. 246 ºC. The IR spectrum of compound I exhibited the characteristic absorption bands at 3356 cm^{-1} and 1647 $cm^{-1,}$ suggesting the presence of a primary amine group and carbonyl groups, respectively.

The characteristic band attributed to the presence of C=N stretch in the benzothiazole ring was observed at 1594 cm^{-1}. The 400 MHz 1H-NMR spectrum of the compound I in DMSO-d6with TMS as an internal standard exhibited characteristic peaks of primary amino (-NH) and acetyl protons (-COCH3) as two singlets, one at δ 7.85 ppm (1H, s) and the other one at δ 2.50 ppm (1H,s). The aromatic protons of benzothiazole nucleus accounted for in the range of δ 7.3 to 8.3. In the 13C-NMR spectrum, a carbonyl carbon appeared at δ 210 ppm. The ESI mass spectrum (negative ion mode) of compound I revealed a (M-H)- ion at m/z191. Eventually, all the spectra of the compound are in keeping consistent with the literature reported characterization data. Based on the above spectral data and elemental analysis, the structure of the compound was confirmed as 1-(2- aminobenzo [d] thiazol-5-yl) ethan-1-one (I). The reaction procedure for the synthesis of the intermediate is well illustrated in **Scheme 1**.



SCHEME 1: REACTION SCHEME FOR THE SYNTHESIS OF INTERMEDIATE (I)

General Procedure:

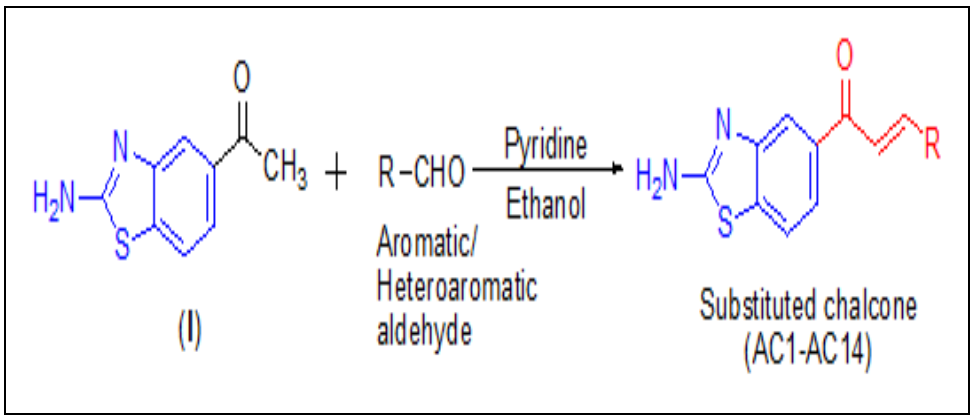
Synthesis of (E)-1-(2-aminobenzo[d]thiazol-5-yl) -3-(substituted)prop-2-en-1-ones (AC1-AC14): To a solution of 1-(2-aminobenzo [d] thiazol-5-yl) ethan-1-one (I) (0.005 M) and suitably substituted aldehydes (0.005 M) in ethanol (10 ml),catalytic amount of pyridine were added dropwise with

continuous stirring at room temperature over a period of 15 min. The reaction mixture was then kept at room temperature for about 48 h with occasional shaking.

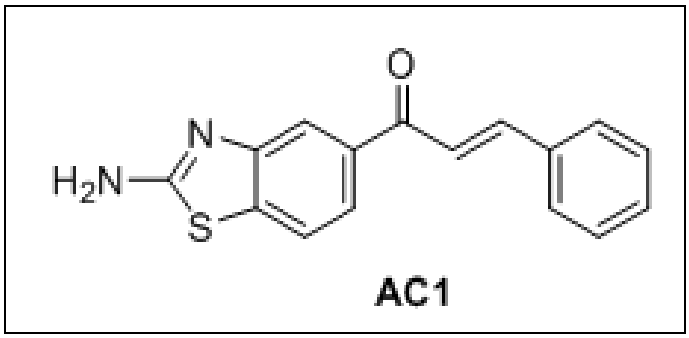
After 48 h it was poured into ice-cold water and then neutralized to pH 2 using 5 N hydrochloric acid. The yellow precipitate obtained was filtered, washed, dried, and recrystallized from dry ethanol.

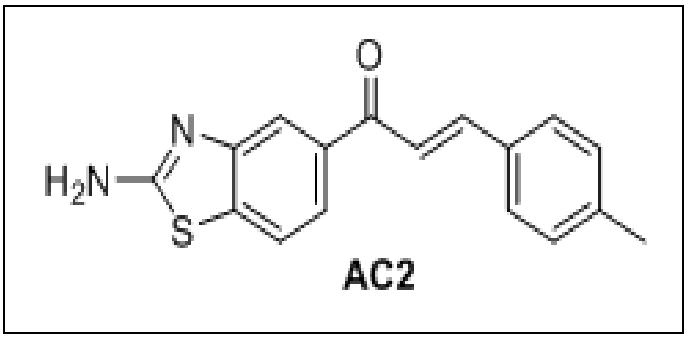
The (E)- 1- (2- aminobenzo [d] thiazol- 5- yl)- 3- (substituted) prop-2-en-1-ones AC1 to AC14 were obtained in good yield.

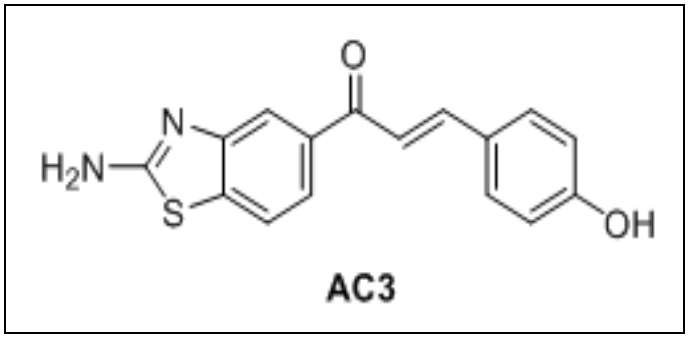
All the synthesized compounds, as mentioned in **Fig. 2** were characterized by spectroscopic methods such as FTIR, NMR, and mass spectral analysis. The reaction procedure for the synthesis of the intermediate is well illustrated in **Scheme 2**.

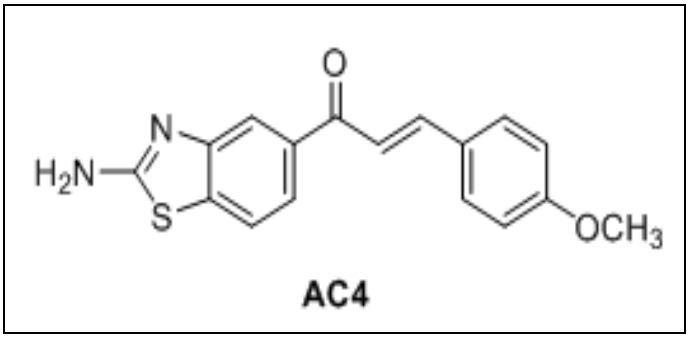


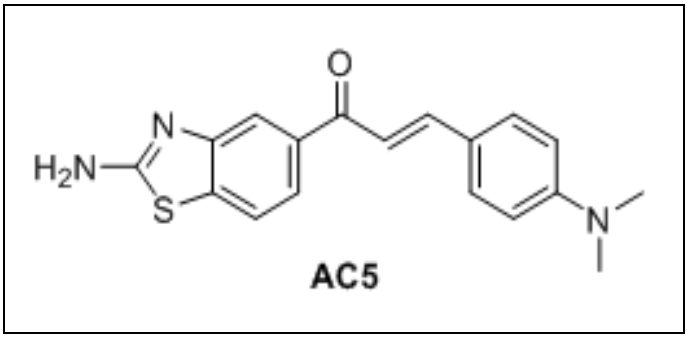
SCHEME 2: REACTION SCHEME FOR THE SYNTHESIS OF SUBSTITUTED CHALCONES

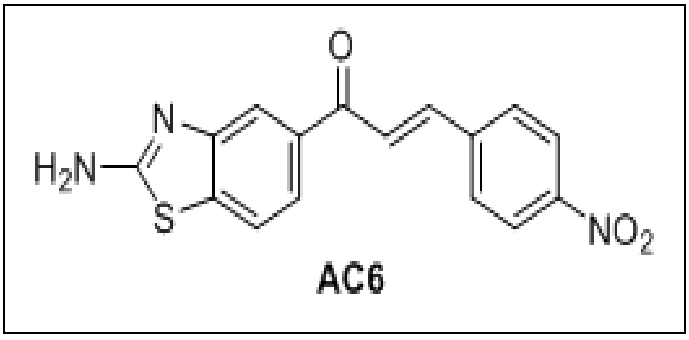


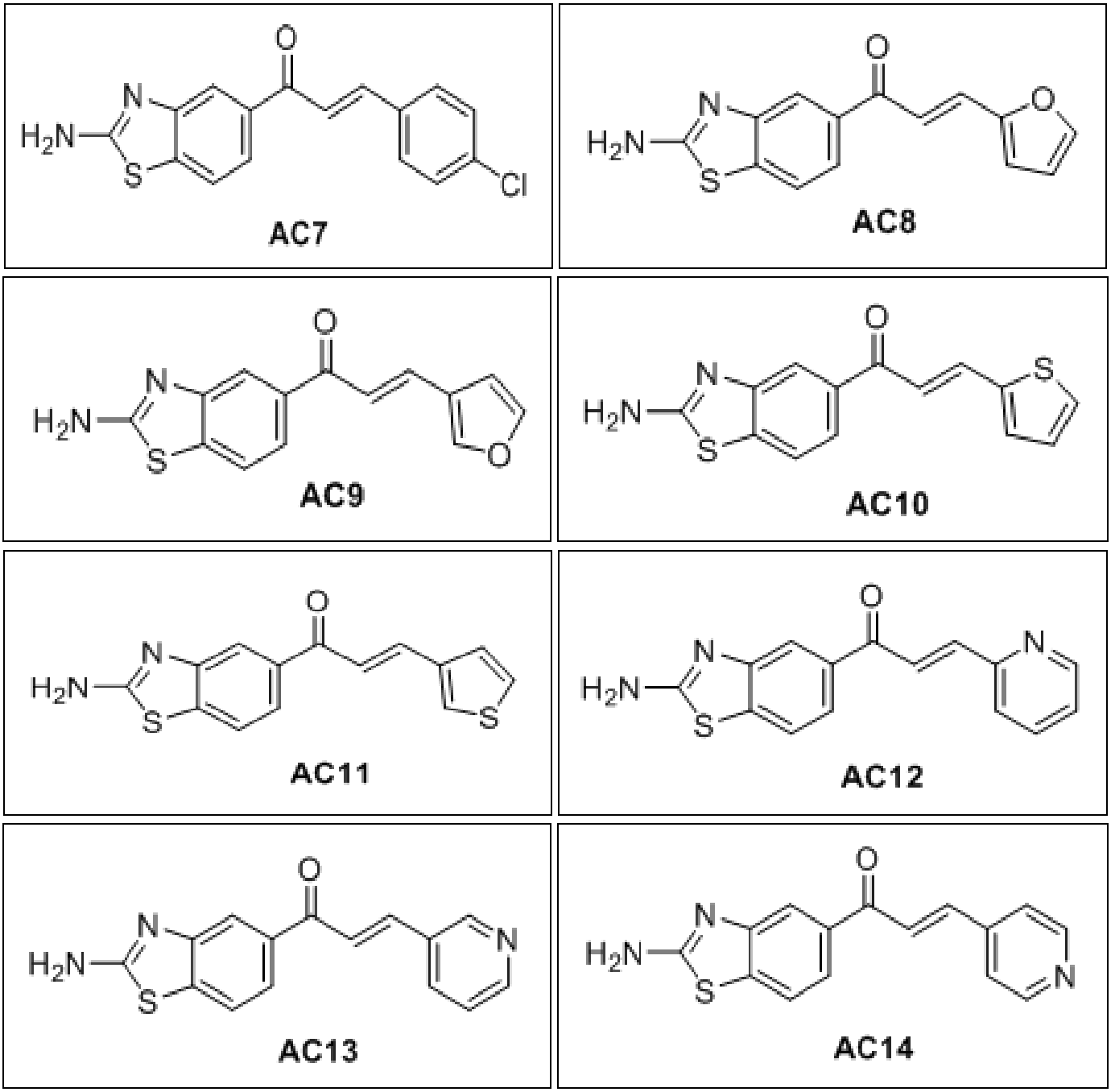












LIST OF BENZOTHIAZOLE-LINKED CHALCONES AC1-AC14 PRODUCED *VIA* SCHEME 2

Synthesis of (E)-1-(2-aminobenzo[d]thiazol-5-yl) -3-phenylprop-2-en-1-one (AC1): A mixture of 1-(2-aminobenzo [d] thiazol-5-yl) ethan-1-one (I) (0.005 mol.) and benzaldehyde (0.005 mol.) was stirred in ethanol (7.5 mL) and then catalytic amount of pyridine (3-5 drops)was added drop wise with continuous stirring at room temperature over a period of 15 min. The reaction mixture was then kept at room temperature for about 48 h with occasional shaking. After 48 h it was poured into ice-cold water, and then neutralized to pH 2 using 5 N hydrochloric acid.

The yellow precipitate obtained was filtered, washed, dried, and recrystallized from dry ethanol. Compound AC1, analyzed for $C_{16}H_{12}N_2OS$, m.p. 260-262 ºC. The IR spectrum of compound AC1 exhibited the characteristic absorption bands at 3442 cm^{-1} and 1641 $cm^{-1,}$ suggesting the presence of a primary amine group and carbonyl groups, respectively. The characteristic band attributed to the presence of C=C stretch in the α,β-unsaturated ketone, was observed at 1617 cm^{-1}. The 400 MHz 1H-NMR spectrum of the compound AC1 in DMSO-d6 with TMS as an internal standard

exhibited characteristic peaks of H-α and H-β protons of α,β-unsaturated carbonyl group as two doublets, one at δ 7.53 ppm (H-β, J=15.7 Hz) and the other one at δ 6.75 ppm (H-α, J=15.7 Hz). The large J value clearly reveals the trans geometry at the double bond. In the 13C-NMR spectrum, a carbonyl carbon (AC1) appeared at δ 190.4 ppm. The α and β carbon atoms with respect to the carbonyl group showed characteristic signals at δ 122.4 ppm (Cα) and δ 143.2 ppm (Cβ) respectively. The ESI mass spectrum (negative ion mode) of compound AC1 revealed an (M-H)- ion at m/z279.3. Eventually, all the spectra of the compound are in keeping consistent with the expected structure. The results of the elemental analysis were also in close agreement with those of the calculated values. Based on the above spectral data and elemental analysis, the structure of the compound was confirmed as (E)-1-(2-aminobenzo [d]thiazol-5-yl)-3-phenylprop-2-en-1-one (AC1).

By adopting the above the synthetic procedure, benzothiazole-linked chalcones AC2-AC14 were also synthesized. The physical and spectral characterization of all the compounds was presented individually as follows.

(E)-1 2-aminobenzo [d] thiazol-5-yl) -3-phenyl-prop-2-en-1-one (AC1): Yield: 55%, Cream colored powder, Melting point (m.p.): 260-262 ºC, Chemical Formula: $C_{16}H_{12}N_2OS$, Relative Molecular Mass: 280.35, Anal. Found. for $C_{16}H_{12}N_2OS$, %: C, 68.55; H, 4.31; N, 9.99; O, 5.71; S, 11.44,IR (KBr, v_{max} cm^{-1}): 3442 (N−H), 1641 (C=O), 1617 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.75 (1H, d, J = 15.7 Hz), 7.37-7.57 (7H, 7.48 (tt, J = 7.5, 1.5 Hz), 7.51 (dd, J = 8.6, 1.8 Hz), 7.53 (d, J = 15.7 Hz), 7.44 (dddd, J = 8.1, 2.3, 1.5, 0.5 Hz), 7.42 (dddd, J = 8.1, 7.5, 2.0, 0.5 Hz)), 7.61 (1H, dd, J = 8.6, 0.5 Hz), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 279 [M–H]–.

(E)-1- (2-aminobenzo[d]thiazol-5-yl) -3-(p-tolyl) prop-2-en-1-one (AC2): Yield: 61%, Yellow powder, Melting point (m.p.): 268-270 ºC, Chemical Formula: $C_{17}H_{14}N_2OS$, Relative Molecular Mass: 294.37, Anal. Found. for $C_{17}H_{14}N_2OS$, %: C, 69.36; H, 4.79; N, 9.52; O, 5.43; S, 10.89, IR (KBr, v_{max} cm^{-1}): 3355 (N−H), 1595 (C=O), 1516.05 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 2.33 (3H, s), 6.75 (1H, d, J = 15.7 Hz), 7.22 (2H, ddd, J = 8.0, 1.4, 0.5 Hz), 7.48-7.64 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.55 (ddd, J = 8.0, 1.8, 0.5 Hz), 7.53 (d, J = 15.7 Hz), 7.51 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 293 [M–H]–

(E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(4-hydroxyphenyl) prop-2-en-1-one (AC3): Yield: 62%, Cream colored powder, Melting point (m.p.): 245-247 ºC, Chemical Formula: $C_{16}H_{12}N_2O_2S$, Relative Molecular Mass: 296.34, Anal. Found. for $C_{16}H_{12}N_2O_2S$, %: C, 64.85; H, 4.08; N, 9.45; O, 10.80; S, 10.82, IR (KBr, v_{max} cm^{-1}): 3443 (N−H), 1630 (C=O), 1573 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.71 (1H, d, J = 15.6 Hz), 6.90 (2H, ddd, J = 8.3, 1.6, 0.4 Hz), 7.46-7.63 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.56 (ddd, J = 8.3, 1.9, 0.4 Hz), 7.57 (d, J = 15.6 Hz), 7.49 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 295 [M–H]–.

(E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(4-methoxyphenyl) prop-2-en-1-one (AC4): Yield: 39%, Lemon yellow powder, Melting point (m.p.): 257-259 ºC, Chemical Formula: $C_{17}H_{14}N_2O_2S$, Relative Molecular Mass: 310.37, Anal. Found. for $C_{17}H_{14}N_2O_2S$, %: C, 65.79; H, 4.55; N, 9.03; O, 10.31; S, 10.33, IR (KBr, v_{max} cm^{-1}): 3428 (N−H), 1645 (C=O), 1616 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 3.83 (3H, s), 6.71 (1H, d, J = 15.6 Hz), 7.19 (2H, ddd, J = 8.8, 1.2, 0.5 Hz), 7.46-7.63 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.56 (d, J = 15.6 Hz), 7.49 (ddd, J = 8.8, 1.9, 0.5 Hz), 7.49 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 309 [M–H]–.

(E)-1-(2-aminobenzo [d] thiazol-5-yl) -3-(p-tolyl) prop-2-en-1-one (AC2): Yield: 61%, Yellow powder, Melting point (m.p.): 268-270 ºC, Chemical Formula: $C_{17}H_{14}N_2OS$, Relative Molecular Mass: 294.37, Anal. Found. for $C_{17}H_{14}N_2OS$, %: C, 69.36; H, 4.79; N, 9.52; O, 5.43; S, 10.89, IR (KBr, v_{max} cm^{-1}): 3355 (N−H), 1595 (C=O), 1516.05 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 2.33 (3H, s), 6.75 (1H, d, J = 15.7 Hz), 7.22 (2H, ddd, J = 8.0, 1.4, 0.5 Hz), 7.48-7.64 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.55 (ddd, J = 8.0, 1.8, 0.5 Hz), 7.53 (d, J = 15.7 Hz), 7.51 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 293 [M–H]–.

(E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(4-hydroxyphenyl) prop-2-en-1-one (AC3): Yield: 62%, Cream colored powder, Melting point (m.p.): 245-247 ºC, Chemical Formula: $C_{16}H_{12}N_2O_2S$, Relative Molecular Mass: 296.34, Anal. Found. for $C_{16}H_{12}N_2O_2S$, %: C, 64.85; H, 4.08; N, 9.45; O, 10.80; S, 10.82, IR (KBr, v_{max} cm^{-1}): 3443 (N−H), 1630 (C=O), 1573 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.71 (1H, d, J = 15.6 Hz), 6.90 (2H, ddd, J = 8.3, 1.6, 0.4 Hz), 7.46-7.63 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.56 (ddd, J = 8.3, 1.9, 0.4 Hz), 7.57 (d, J = 15.6 Hz), 7.49 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 295 [M–H]–.

(E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(4-methoxyphenyl) prop-2-en-1-one (AC4): Yield: 39%, Lemon yellow powder, Melting point (m.p.): 257-259 ºC, Chemical Formula: $C_{17}H_{14}N_2O_2S$, Relative Molecular Mass: 310.37, Anal. Found. for $C_{17}H_{14}N_2O_2S$, %: C, 65.79; H, 4.55; N, 9.03; O, 10.31; S, 10.33, IR (KBr, v_{max} cm^{-1}): 3428 (N−H), 1645 (C=O), 1616 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 3.83 (3H, s), 6.71 (1H, d, J = 15.6 Hz), 7.19 (2H, ddd, J = 8.8, 1.2, 0.5 Hz), 7.46-7.63 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.56 (d, J = 15.6 Hz), 7.49 (ddd, J = 8.8, 1.9, 0.5 Hz), 7.49 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 309 [M–H]–.

(E)-1-(2-aminobenzo [d] thiazol-5-yl)-3-(4-(dimethylamino) phenyl)prop-2-en-1-one (AC5): Yield: 45%, Reddish yellow powder, Melting point (m.p.): 241-243 ºC, Chemical Formula: $C_{18}H_{17}N_3OS$, Relative Molecular Mass: 323.41, Anal. Found. for $C_{18}H_{17}N_3OS$, %: C, 66.85; H, 5.30; N, 12.99; O, 4.95; S, 9.91, IR (KBr, v_{max} cm^{-1}): 3450 (N−H), 1650 (C=O), 1626 (C=C)1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 3.83 (3H, s), 6.71 (1H, d, J = 15.6 Hz), 7.19 (2H, ddd, J = 8.8, 1.2, 0.5 Hz), 7.46-7.63 (5H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.56 (d, J = 15.6 Hz), 7.49 (ddd, J = 8.8, 1.9, 0.5 Hz), 7.49 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 322 [M–H]–.

E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(4-nitrophenyl) prop-2-en-1-one (AC6): Yield: 51%, Dark yellow powder, Melting point (m.p.): 263-265 ºC, Chemical Formula: $C_{16}H_{11}N_3O_3S$, Relative Molecular Mass: 325.34, Anal. Found. for $C_{16}H_{11}N_3O_3S$, %: C, 59.07; H, 3.41; N, 12.92; O, 14.75; S, 9.85, IR (KBr, v_{max} cm^{-1}): 3454 (N−H), 1633 (C=O), 1617 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 7.00 (1H, d, J = 16.6 Hz), 7.53 (1H, dd, J = 8.6, 1.8 Hz), 7.61 (1H, dd, J = 8.6, 0.5 Hz), 7.80 (1H, dd, J = 1.8, 0.5 Hz), 7.85 (2H, ddd, J = 8.7, 2.2, 0.5 Hz), 7.95 (1H, d, J = 16.6 Hz), 8.07 (2H, ddd, J = 8.7, 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 324 [M–H]–.

(E)-1- (2-aminobenzo [d] thiazol-5-yl)-3-(4-chlorophenyl) prop-2-en-1-one (AC7): Yield: 40%, Yellow powder, Melting point (m.p.):274-276 ºC, Chemical Formula: $C_{16}H_{11}ClN_2OS$, Relative Molecular Mass: 314.79, Anal. Found. for $C_{16}H_{11}ClN_2OS$, %: C, 61.05; H, 3.52; Cl, 11.26; N, 8.90; O, 5.08; S, 10.18, IR (KBr, v_{max} cm^{-1}): 3492 (N−H), 1634 (C=O), 1619 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.71 (1H, d, J = 15.7 Hz), 7.47-7.63 (7H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.55 (ddd, J = 8.1, 1.4, 0.5 Hz), 7.53 (ddd, J = 8.1, 1.5, 0.5 Hz), 7.51 (d, J = 15.7 Hz), 7.50 (dd, J = 8.6, 1.8 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 313 [M–H]–.

(E)-1-(2-aminobenzo [d] thiazol-5-yl)-3-(furan-2-yl)prop-2-en-1-one (AC8): Yield: 60%, Brown powder, Melting point (m.p.): 281-283 ºC, Chemical Formula: $C_{14}H_{10}N_2O_2S$ Relative Molecular Mass: 270.31, Anal. Found. for $C_{14}H_{10}N_2O_2S$, %: C, 62.21; H, 3.73; N, 10.36; O, 11.84; S, 11.86, IR (KBr, v_{max} cm^{-1}): 3241 (N−H), 1647 (C=O), 1591 (C=C), 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.32 (1H, dd, J = 3.4, 1.8 Hz), 6.82 (1H, d, J = 15.7 Hz), 7.03 (1H, dd, J = 3.4, 0.9 Hz), 7.25 (1H, d, J = 15.7 Hz), 7.51 (1H, dd, J = 8.6, 1.8 Hz), 7.56-7.64 (2H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.58 (dd, J = 1.8, 0.9 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 269 [M–H]–.

(E)-1-(2-aminobenzo [d] thiazol-5-yl)-3-(furan-3-yl)prop-2-en-1-one (AC9): Yield: 42%, Yellowish brown powder, Melting point (m.p.):275-277 ºC, Chemical Formula: $C_{14}H_{10}N_2O_2S$, Relative Molecular Mass: 270.31, Anal. Found. for $C_{14}H_{10}N_2O_2S$, %: C, 62.21; H, 3.73; N, 10.36; O, 11.84; S, 11.86, IR (KBr, v_{max} cm^{-1}): 3388 (N−H), 1649 (C=O), 1620 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.70-6.79 (2H, 6.74 (d, J = 15.6 Hz), 6.71 (dd, J = 1.8, 0.9 Hz)), 7.45 (1H, dd,

J = 1.8, 0.8 Hz), 7.48-7.58 (2H, 7.51 (dd, J = 8.6, 1.8 Hz), 7.53 (d, J = 15.6 Hz)), 7.61 (1H, dd, J = 8.6, 0.5 Hz), 7.80 (1H, dd, J = 1.8, 0.5 Hz), 7.86 (1H, dd, J = 0.9, 0.8 Hz). ESI-MS (m/z, negative ion mode): 269 [M–H]–.

(E)-1- (2-aminobenzo [d] thiazol-5-yl) -3-(thiophen-2-yl) prop-2-en-1-one (AC10): Yield: 47%, Yellow powder, Melting point (m.p.): 266-268 ºC, Chemical Formula: $C_{14}H_{10}N_2OS_2$, Relative Molecular Mass: 286.37 Anal. Found. for $C_{14}H_{10}N_2OS_2$, %: C, 58.72; H, 3.52; N, 9.78; O, 5.59; S, 22.39, IR (KBr, v_{max} cm^{-1}): 3443 (N−H), 1630 (C=O), 1573 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.80 (1H, d, J = 15.6 Hz), 7.28 (1H, dd, J = 7.5, 4.4 Hz), 7.40 (1H, dd, J = 7.5, 1.2 Hz), 7.48-7.64 (3H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.57 (d, J = 15.6 Hz), 7.51 (dd, J = 8.6, 1.8 Hz)), 7.55 (1H, dd, J = 4.4, 1.2 Hz), 7.79 (1H, dd, J = 1.8, 0.5 Hz). ESI-MS (m/z, negative ion mode): 285 [M–H]–.

(E)-1 -(2-aminobenzo [d] thiazol-5-yl) -3-(thiophen-3-yl) prop-2-en-1-one (AC11): Yield: 29%, Yellow powder, Melting point (m.p.): 259-261 ºC, Chemical Formula: $C_{14}H_{10}N_2OS_2$, Relative Molecular Mass: 286.37, Anal. Found. for $C_{14}H_{10}N_2OS_2$, %: C, 58.72; H, 3.52; N, 9.78; O, 5.59; S, 22.39, IR (KBr, v_{max} cm^{-1}): 3428.12 (N−H), 1645.58 (C=O), 1616.05 (C=C) , 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.79 (1H, d, J = 15.6 Hz), 7.22 (1H, dd, J = 5.2, 1.1 Hz), 7.45 (1H, dd, J = 5.2, 1.6 Hz), 7.51 (1H, dd, J = 8.6, 1.8 Hz), 7.61 (1H, dd, J = 8.6, 0.5 Hz), 7.71 (1H, d, J = 15.6 Hz), 7.80 (1H, dd, J = 1.8, 0.5 Hz), 8.12 (1H, dd, J = 1.6, 1.1 Hz).ESI-MS (m/z, negative ion mode): 285 [M–H]–.

(E)-1-(2-aminobenzo[d]thiazol-5-yl)-3-(pyridin-2-yl)prop-2-en-1-one (AC12): Yield: 37%, Light yellow powder, Melting point (m.p.): 284-286 ºC, Chemical Formula: $C_{15}H_{11}N_3OS$, Relative Molecular Mass: 281.33, Anal. Found. for $C_{15}H_{11}N_3OS$, %: C, 64.04; H, 3.94; N, 14.94; O, 5.69; S, 11.40, IR (KBr, v_{max} cm^{-1}): 3225 (N−H), 1655 (C=O), 1597 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.91 (1H, d, J = 14.8 Hz), 7.42 (1H, d, J = 14.8 Hz), 7.49-7.64 (3H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.57 (ddd, J = 7.4, 4.7, 1.4 Hz), 7.52 (dd, J = 8.6, 1.8 Hz)), 7.67-7.77 (2H, 7.74 (ddd, J = 7.7, 1.4, 0.5 Hz), 7.71 (ddd, J = 7.7, 7.4, 1.9 Hz)), 7.79 (1H, dd, J = 1.8, 0.5 Hz), 8.70 (1H, ddd, J = 4.7, 1.9, 0.5 Hz).ESI-MS (m/z, negative ion mode): 280 [M–H]–.

(E)-1-(2-aminobenzo[d]thiazol-5-yl)-3-(pyridin-3-yl)prop-2-en-1-one (AC13): Yield: 51%, Light yellow powder, Melting point (m.p.): 291-293 ºC, Chemical Formula: $C_{15}H_{11}N_3OS$, Relative Molecular Mass: 281.33, Anal. Found. for $C_{15}H_{11}N_3OS$, %: C, 64.04; H, 3.94; N, 14.94; O, 5.69; S, 11.40, IR (KBr, v_{max} cm^{-1}): 3298 (N−H), 1694 (C=O), 1600 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.73 (1H, d, J = 15.6 Hz), 7.41 (1H, ddd, J = 8.2, 4.7, 0.5 Hz), 7.48-7.64 (3H, 7.61 (dd, J = 8.6, 0.5 Hz), 7.58 (d, J = 15.6 Hz), 7.51 (dd, J = 8.6, 1.8 Hz)), 7.80 (1H, dd, J = 1.8, 0.5 Hz), 7.96 (1H, ddd, J = 8.2, 1.9, 1.6 Hz), 8.56 (1H, ddd, J = 4.7, 1.9, 1.9 Hz), 8.98 (1H, ddd, J = 1.9, 1.6, 0.5 Hz). ESI-MS (m/z, negative ion mode): 280 [M–H]–.

(E)-1-(2-aminobenzo [d] thiazol-5-yl)-3-(pyridin-4-yl) prop-2-en-1-one (AC14): Yield: 44%, Light yellow powder, Melting point (m.p.): 297-299 ºC, Chemical Formula: $C_{15}H_{11}N_3OS$, Relative Molecular Mass: 281.33, Anal. Found. for $C_{15}H_{11}N_3OS$, %: C, 64.04; H, 3.94; N, 14.94; O, 5.69; S, 11.40, IR (KBr, v_{max} cm^{-1}): 3368 (N−H), 1655 (C=O), 1593 (C=C) 1H NMR (400 MHz, DMSO-d6) δ (ppm): δ 6.80 (1H, d, J = 15.6 Hz), 7.52 (1H, dd, J = 8.6, 1.8 Hz), 7.61 (1H, dd, J = 8.6, 0.5 Hz), 7.75-7.84 (4H, 7.82 (ddd, J = 4.6, 1.6, 0.5 Hz), 7.80 (d, J = 15.6 Hz), 7.79 (dd, J = 1.8, 0.5 Hz)), 8.70 (2H, ddd, J = 4.6, 1.9, 0.5 Hz). ESI-MS (m/z, negative ion mode): 280 [M–H]–.

Mycobacterium Tuberculosis H37Rv (Mtb H37Rv) Inhibitory Activity (*In-vitro* anti-tubercular Activity): The Mycobacterium tuberculosis H37Rv (Mtb H37Rv) inhibitory activity for the synthesized benzothiazole-linked chalcones (AC1-AC14) are assessed by using microplate Alamar Blue assay (MABA) described by Maria *et al.* [23] This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method. Briefly, 200 µL of sterile deionized water was added to all outer perimeter wells of sterile 96 wells plate to minimized evaporation of medium in the test wells during incubation.

The 96 wells plate received 100 µL of the Middle brook 7H9 broth, and serial dilution of compounds was made directly on the plate. The final drug concentrations tested were 100 to 0.2 µg/mL. Plates were covered and sealed with parafilm and incubated at 37 °C for five days. After this time, 25 µL of freshly prepared 1:1 mixture of Alamar Blue reagent and 10% tween 80 was added to the plate and incubated for 24 h. Blue color in the well was interpreted as no bacterial growth, and pink color was scored as growth. The IC_{50} value, which prevented the color change from blue to pink. The results of Mtb H37Rv inhibitory activity studies are given in **Table 1**.

RESULTS:

Benzothiazole-Linked Chalcones:

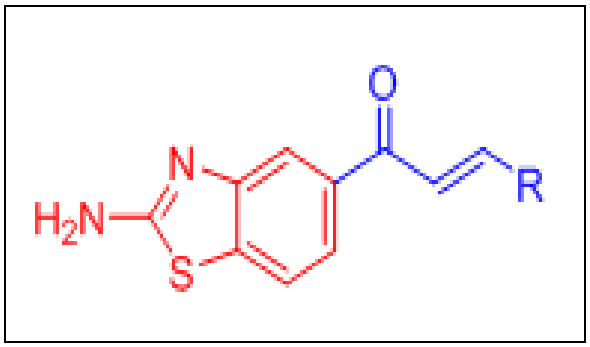


TABLE 1: MYCOBACTERIUM TUBERCULOSIS H37RV INHIBITORY ACTIVITY DATA OF BENZOTHIAZOLE-LINKED CHALCONES AC1-AC14

S. no.	Compound	R	MIC values (µg/mL) of M. tuberculosis H37Rv
1	AC1	C_6H_5	25
2	AC2	4-MeC_6H_4	25
3	AC3	4-OHC_6H_4	100
4	AC4	4-OMeC_6H_4	12.5
5	AC5	4-N$Me_2C_6H_4$	12.5
6	AC6	4-$NO_2C_6H_4$	50
7	AC7	4-ClC_6H_4	25
8	AC8	Furan-2-yl	25
9	AC9	Furan-3-yl	50
10	AC10	Thiophen-2-yl	6.25
11	AC11	Thiophen-3-yl	50
12	AC12	Pyridin-2-yl	6.25
13	AC13	Pyridin-3-yl	100
14	AC14	Pyridin-4-yl	50
15	Pyrazinamide	--	3.125

DISCUSSION: The investigation of *in-vitro* Mycobacterium tuberculosis H37Rv inhibitory activity screening data **Table 1** revealed that the compounds AC10 and AC12 demonstrated comparatively the most potent inhibitory activity, with values of 6.25 µg/mL respectively. It is interesting to note that the compounds AC4 and AC5 also showed appreciable inhibitory activity with values of 12.5 µg/mL, respectively. The other compounds such as AC1, AC2, AC7, and AC8 showed a moderate level of activity at concentrations value 25 µg/mL. The compounds AC6, AC14, AC11, AC9 Showed activity with concentration values 50 µg/mL. Whereas AC3 and AC13 exhibited comparatively less activity with IC_{50} value as 100 µg/mL in comparison with the standard drug (Pyrazinamide: 3.125 µg/mL). Structure-Activity Relationship (SAR) of these compounds exhibited the intrinsic phenomenon of Mycobacterium tuberculosis H37Rv inhibitory activity associated with the basic nucleus consisting of benzothiazole and chalcone moieties as seen in the case of the compounds AC1-AC14. In some cases, the activity was enhanced by the influence of some substituents and decreased by some other substituents, AC10 (Thiophen-2-yl) > AC12 (Pyridin-2-yl) > AC4 (4-OMeC_6H_4) > AC5 (4-N$Me_2C_6H_4$) > AC2 (4-MeC_6H_4) > AC7 (4-ClC_6H_4)>AC8 (Furan-2-yl) > AC1 (C_6H_5) > AC6 (4-$NO_2C_6H_4$) > AC14 (Pyridin-4-yl) > AC11 (Thiophen-3-yl) > AC9 (Furan-3-yl)>AC3 (4-OHC_6H_4) > AC13 (Pyridin-3-yl).

However, it was revealed that various aliphatic / aromatic / heteroaromatic functional group substitutions at position C-3 of α,β-unsaturated carbonyl system followed its activity order as AC10 (Thiophen-2-yl)>AC12 (Pyridin-2-yl)>AC4 (4-OMeC_6H_4) >AC5 (4-NMe2C_6H_4) >AC2 (4-MeC_6H_4) > AC7 (4-ClC_6H_4) > AC8 (Furan-2-yl) >

AC1 (C_6H_5) > AC6 (4-$NO_2C_6H_4$) > AC14 (Pyridin-4-yl) > AC11 (Thiophen-3-yl) > AC9 (Furan-3-yl) > AC3 (4-OHC_6H_4) > AC13 (Pyridin-3-yl) respectively.

CONCLUSION: Benzthiazole and Benzthiazole linked chalcones play an important role in the treatment of many disorders such as tuberculosis at present the envy to synthesize antitubercular moiety by the synthesis of Benzthiazole linked chalcone has paved the way to many of the researchers to synthesize the molecules of this kind.

The main activity of the Benzthiazole linked chalcone may be due to inhibition of cell wall synthesis, and some of the bioactive groups present in the molecule, such as hydroxyl group, may increase the penetration through some of the specialized channels (polar porin channels) present in gram-negative bacteria.

So, both electron-withdrawing and electron-donating groups are equally important in these synthesized Schiff bases. Compounds with electron-withdrawing groups on aryl aldehyde showed challenging activity as antifungal agents. Antiotubercular activity of the compound may be attributed to inhibition of cell wall component (Mycolic acid) synthesis.

FUTURE PERSPECTIVE: Benzthiazole is the order of interest to many of the researchers in recent days. Still, many of the pharmacological investigations to be carried out on the different hybrid molecules which are to synthesized in the near future.Benzothiazole is a high multifaceted molecule that provides a platform for the cure of many of the disorders present in this world. The researchers in the future have to concentrate on the modification of the free versatile amino group to synthesize Schiff bases or sulphonyl urease to get different pharmacological interest molecules and their derivatives.

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