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A Novel Study on Bioactive 2-Substituted Amino-5-benzothiazol-2-oyl-4-phenylthiazoles

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Amino or substituted aminothiazoles exhibit bacterial, fungicidal, herbicidal and pesticidal properties. The (4+1) thiazole construction strategy adopted involves the synthesis of the [C-N-C-S] precursors, namely 1-aryl-3-(N-phenylbenzimidoyl)thiourea or 1-alkyl-3-(N-phenylbenzimidoyl)-thiourea and the preparation of the C5 synthone, the halo acetylhetaroyl derivative. The optimized reaction conditions developed have thus lead to the preparation of five 2-(N-arylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles and three 2-(N,N-dialkylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles. The structure of these compounds were assigned on the basis of elemental analysis, FTIR, ^{1}H NMR and ^{13}C NMR and screened for their antimicrobial activity.

Key Words: Heterocyclics, Thiazole, Bioactivity, Cytotoxicity, Antitumor activity.

INTRODUCTION

The significant and varied biological activities of thiazoles have stimulated extensive research on their synthesis. They found uses as analgesic, anaesthetic, anticonvulsant, antiallergic, antiinflammatory, immunoregulatory, blood cholesterol lowering, antitumor, antitubercular and several other activities. Certain 4-thiazoline derivatives show significant activity as anticonvulsant agents. Theonezolide A[1], a 2,4-disubstituted thiazole derivative, is a macrolide isolated from the marine sponge exhibits cytotoxicity against murine lymphoma and human epidermoid carcinoma. Amino or substituted amino thiazoles exhibit bactericidal, fungicidal, herbicidal and pesticidal properties. Molecules incorporating one or more thiazole unit in their system are found to possess a wide range of biological activity[2]. For example, nizatidine[3] in which 4-position carries an aliphatic side chain is an effective drug for peptic ulcer and tiazofurin[1] with an amide group as the 4-substituent is a thiazole derivative with antitumor activity against general uterine tumors. 4-Chloro-2-oxobenzothiazolin-3-yl acetic acid known as benzolin[4] is used as an ingredient of herbicides and methyl

ester of (2-benzothiazol-2-yl)alanyl-2-aminohexanoic acid was reported to have utility in treating Alzheimers disease[5]. Thiaflavin[6] is a benzothiazole salt used to visualize plaques composed of amyloid beta found in the brains of Alzheimere disease patients. Riluzole[7], containing a benzothiazole ring found uses as drugs against motor neurone desease. Benzothiazole derivatives, mercaptobenzothiazol known as captex[8] is widely used in industry as vulcanizers and accelerators. A large number of structural variations are possible on the thiazoles skeleton with a variety of substituents at the C2, C4 and C5 positions. Hence we decided to explore the possibility of a new series of compounds with a substituted amino group at C2, phenyl at C4 and a benzothiazole group at C5 to see the biological activity offered by them. The antibacterial screening was carried out following standard protocols using three bacterial strains namely *Bacillus substilis*, *Staphylococcus aureus* and *Escherachia coli*.

EXPERIMENTAL

All chemicals used were of commercial grade. Melting points were uncorrected and were determined by open capillary method using an immerson bath of silicon oil or sulphuric acid. Thin layer chromatography was performed using silica gel-G (E. Merck, India) coated on glass plates. The spots were visualized in iodine vapour or under UV-light. The spectra were recorded on, Bruker spectrospin-100 (100 MHz), Bruker WM-400 (400 MHz) for ^{1}H and 100 MHz for ^{13}C), Jeol GSX (400 MHz) or EM-390 NMR spectrometers and Jeol D-300, Jeol SX-102, Shimadzu QP-2000. All new compounds gave satisfactory results for C, H and N analysis (IIT, Chennai or CDRI, Lucknow).

Synthesis of 2-alkylamino-5-(benzothiazol-2-oyl)-4-phenylthiazoles

Preparation of N-benzoyl-N',N'-dialkylthiourea: Benzoyl chloride (7.5 mmol) in benzene was stirred well with aqueous solution of potassium thiocyanate (33 %, 5.5 mL) in presence of tetrabutylammonium bromide (TBAB, 2 g) during the course of 15 min. Stirring was continued for another 0.5 h. The aqueous layer was then removed using a Pasteur pipette. This was extracted with benzene and the benzene extract was added back to the main bulk. Dimethylamine (7.5 mmol) in benzene was added to the benzene layer in the flask and the mixture was stirred at room temperature for 2 h. It was then diluted slowly with pet. ether under slow stirring until the precipitation of thiourea was complete. The precipitate of N-benzoyl-N',N'-dimethylthiourea (**1a**) formed was then filtered, washed with pet. ether, dried and crystallized from EtOH/H_2O, yield 75 %, m.p. 138 ºC; (**1b**) N-benzoyl-N,N'-diethylthiourea, yield 71 %, m.p. 98 ºC; (**1c**) N-benzoyl- N,N'-methylphenylthiourea: yield 85 %, m.p. 134 ºC.

Synthesis of 2-(N,N-dialkylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: In this reaction, 2-bromoacetylbenzothiazole (1 mmol) was dissolved in dry acetone and reacted with N-benzoyl-N,N'-dialkylthiourea (1 mmol) at reflux for 1 h. The reaction mixture obtained was then added to ice cold distilled water. The pH was adjusted to *ca.* 6 and the precipitate obtained was collected and recrystallized from ethanol-water.

Synthesis of 2-(N-arylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles

Preparation of 2-bromoacetylbenzothiazole: The 2-bromoacetylbenzothiazole[9] was obtained by a reported method that involved three stages starting from 2-aminothiophenol. Lactic acid (56 mmol) and 2-aminothiophenol (20 mmol) were refluxed for 12 h and the mixture was then kept at 0 ºC for another 12 h. It was then treated with NaOH (10 %) solution, again cooled and the solid deposited was filtered, washed and dried to obtain 2-(2-hydroxyethyl)benzothiazole, which on oxidation with potassium dichromate in glacial acetic acid to give 2-acetylbenzothiazole. Bromination of 2-acetylbenzothiazole using bromine in glacial acetic acid in presence of AIBN to give the required 2-bromoacetylbenzothiazole.

Preparation of N-phenylbenzamidine[10]: Benzonitrile and aniline were treated in a 250 mL flask in presence of anhydrous $AlCl_3$. The reaction mixture was then heated at 180-200 ºC for 0.5 h. The hot reaction mixture was poured into water containing conc. HCl under constant stirring. The solution was decolourized by activated charcoal and poured in slow stream to a stirred solution of water containing NaOH. The flocculent precipitate was worked out as a white powder, yield 55 %; m.p. 105-110 ºC. The crude sample was crystallized from ethanol-water system m.p. 111-112 ºC.

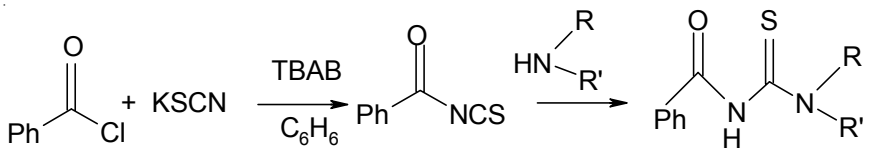
Preperation of 1-aryl-3-(N-phenylbenzimidoyl)thiourea[11]: 1-Phenyl-3-(N-phenylbenzimidoyl)thiourea (**3a**) was prepared as follows. N-phenylbenzamidine (5 mmol) was dissolved in N,N-dimethylformamide and phenyl isothiocyanate (5 mmol) was added and the workup gave 1-phenyl-3-(N-phenylbenzimidoyl)thiourea, crude yield 95 %; m.p. 132 ºC. It was then crystallized from ethanol-water system, yield 80 %; m.p. 140 ºC. The following imidoylthioureas were prepared using the above method: (**3b**) 1-(*o*-tolyl)-3-(N-phenylbenzimidoyl)thiourea: yield 56 %; m.p. 125 ºC; (**3c**) 1-(*m*-tolyl)-3-(N-phenylbenzimidoyl)thiourea: yield 81 %; m.p. 124 ºC; (**3d**) 1-(*p*-tolyl)-3-(N-phenylbenzimidoyl)thiourea: yield 85 %; m.p. 117 ºC and (**3e**)1-(*p*-chlorophenyl)-3-(N-phenylbenzimidoyl)thiourea: yield 96 %; m.p. 124 ºC.

Synthesis of 2-(N-arylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: As a typical example, 1-(N-phenylbenzimidoyl)-3-(*p*-tolyl)thiourea (0.5 mmol) was dissolved in N,N-dimethylformamide and warmed to 60-70 ºC. To this warm solution, 2-bromoacetylbenzothiazole (0.5 mmol) was added followed by triethylamine (1 mmol). The reaction mixture was poured

to ice-cold water with stirring, kept mildly acidic. The precipitate formed was filtered, washed with water and dried. The crude yield of the product was 89 %; m.p. 225 ºC. Crystallization from ethanol-acetone-water afforded yellow needles of 5-(benzothiazol-2-oyl)-4-phenyl-2-(*p*-tolylamino)-thiazole, yield 72 %, m.p. 230 ºC. Syntheses of other 2-arylamino-5-(benzothiazol-2-oyl)-4-phenylthiazoles (**4a-e**) were performed using a similar synthetic procedure.

RESULTS AND DISCUSSION

Synthesis of 2-alkylamino-5-(benzothiazol-2-oyl)-4-phenylthiazoles: The synthesis of 2-(N,N-dialkylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles requires the preparation of N,N-dialkyl-N'-benzoylthiourea. The required N,N-dialkyl-N'-benzoylthiourea was prepared by the reaction of benzoylisothiocyanate which in turn was prepared by using benzoyl chloride and potassium isothiocyanate in a phase transfer catalysis, with dialkylamine *in situ* (**Scheme-I**).

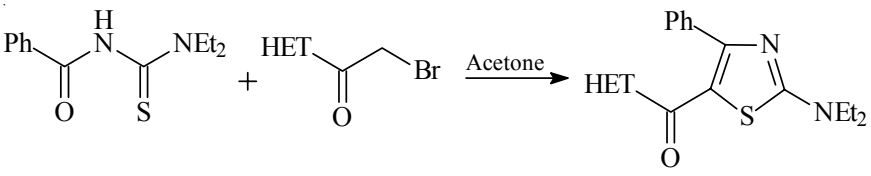


(a-c)

a : R = R' = Me; b : R = R'= Et; c : R = Me, R'= Ph

Scheme-I

The reaction of N-benzoyl-N',N'-dialkylthiourea with 3-(2-bromoacetyl)benzothiazole was carried out in dry acetone at reflux to obtain yellow crystals of a compound with molecular composition $C_{21}H_{19}N_2OS_2$. The reaction is as shown in **Scheme-II**.



1b **2b**

HET = benzothiazolyl

Scheme-II

The IR spectrum of the compound showed an absorption band due to ν(C=O) at 1612 cm^{-1} and due to phenyl δ(C-H) bands at 764 and 700 cm^{-1}.

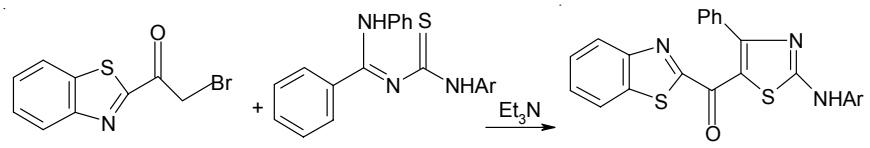
The ^{1}H NMR spectrum showed a triplet at δ 1.27 due to 6 H and a quartet at δ 3.65 due to four hydrogens indicating the presence of two ethyl groups. The multiplets at δ 7.32-7.45 corresponding to three hydrogens and at δ 7.55-7.70 with peak integral of four hydrogen and two doublet at δ 8.18 and 8.23 of one hydrogens each showed the presence of nine aromatic hydrogens in the system. The ^{13}C NMR spectrum of the compound also support the structure of the compound. Physical and spectral data of 2-dialkylamino-5-(benzothiazol-2-oyl)-4-phenylthiazoles (**2a-c**) are:

2a: 2-(N,N-Dimethylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: yield 63 %, m.p. 156 ºC, m.f.: $C_{19}H_{15}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3063, 2935, 2369, 1692, 1566, 1499, 1459, 1371, 1303, 1155, 1075, 947, 906, 818, 771, 722, 710.

2b: 2-(N,N-Diethylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: yield 69 %, m.p. 161 ºC, m.f.: $C_{21}H_{19}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3063, 2975, 1612, 1533, 1501, 1445, 1330, 1155, 1081, 1020, 912, 816, 764, 700. ^{1}H N M R (400 MHz, DMSO-d_6), δ: 1.15-1.35 (t, J = 6.7H = 2, 6H), 3.55-3.75 (t, J = 6.7 H_2, 4H), 7.28-7.46 (m, 4H), 7.28-7.46 (m, 3H), 7.51-7.70 (m, 4H), 8.10-8.28 (dd, 2H) ^{13}C NMR (125 MHz, DMSO-d_6), δ: 12.0 (2), 46.0 (2), 113.0, 124.6, 127.0, 127.5, 129, 129.5 (2), 136, 152.5, 165.3, 169, 172.7, 173.7.

2c: 2-(N,N-Methylphenylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: yield 70 %, m.p 181 ºC, m.f.: $C_{24}H_{17}N_3OS_2$; IR (KBr, ν_{max}, cm^{-1}): 3063, 2928, 1620, 1539, 1512, 1465, 1357, 1310, 1061, 919, 811, 703, 582.

Synthesis of 2-(N-arylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: In this synthetic procedure, the required 2-bromoacetylbenzothiazole was prepared and made to react with the 1-(*o*,*p*-dimethylphenyl)-3-(N-phenylbenzimidoyl)thiourea in DMF in the presence of triethylamine. The workup afforded a solid product in high yield, which on purification gave crystals having m.f. $C_{25}H_{19}N_3OS_2$. The IR spectrum of the compound showed vibration bands at 3137, 2928 and 1607 cm^{-1} which are due to ν(N-H), ν(C-H) and ν(C=O), respectively. The low stretching frequency of carbonyl band in the product when compared to that in the precursor 2-bromoacetylbenzothiazole at 1689 cm^{-1} was indicative of an increased conjugation as well as possibilities of hydrogen bonding. The ^{1}H NMR spectrum showed a singlet at δ 11.20 assignable to N-H and two singlets each with peak integral corresponding to three hydrogen at δ 2.30 and 3.34 were assigned to the two methyl groups. There were six multiplets in the aromatic region δ 7.04-8.30 with peak integral corresponding to 12 hydrogens. The ^{13}C NMR spectrum also showed the presence of two methyl carbons. The reaction path way is shown in **Scheme-III**.



3(a-e) **4(a-e)**

3 & **4** Ar = a : *o*-tolyl, b : *m*-tolyl, c : *p*-tolyl, d: *o*,*p*-dimethylphenyl e : *p*-phenetyl

Scheme-III

Physical and spectral data of 2-arylamino-5-(benzothiazol-2-oyl)-4-phenylthiazoles

4(a-e): 4a) 2-(*o*-Tolylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: Yield 89 %, m.p. 225 ºC, m.f.: $C_{24}H_{17}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3151, 3043, 2928, 1627, 1560, 1519, 1465, 1344, 1378, 1303, 1189, 1101, 912, 825, 771, 710, 602. ^{1}H NMR (300 MHZ, $CDCl_3$), δ: 2.39 (S, 3H), 7.22-7.25 (d, 2H), 7.35-7.45 (m, 5H), 7.45-7.55 (m, 2H),7.70-7.75 (m,2H), 7.90-8.00 (m, 1H), 8.12-8.20 (m, 1H), 8.20-8.30 (br, s, 1N-H).

4b) 2-(*m*-Tolylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: Yield 87 %, m.p. 202 ºC, m.f.: $C_{24}H_{17}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3171, 3043, 2928, 1593, 1560, 1512, 1169, 1108, 933, 916, 832, 771, 710, 602. ^{1}H NMR (300 MHZ, $CDCl_3$), δ: 2.41 (S, 3H), 6.97-7.09 (d, J = 7Hz, 1H), 7.12-7.15 (s, 1H), 7.35-7.45 (m, 5H, Ar-H),7.47-7.57 (m, 2H), 7.65-7.77 (m, 2H), 7.92-7.98 (m, 1H), 8.08-8.17 (m, 1H), 8.21 (br, s, 1N-H).

4c) 2-(*p*-Tolylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: Yield 89 %, m.p. 230 ºC, m.f.: $C_{24}H_{17}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3169, 3123, 3054, 2361, 2343, 1832, 1752, 1735, 1676, 1655, 1592, 1557, 1510, 1483, 1461, 1343, 1305, 1159, 1110, 920, 905, 867, 758, 747,727, 699,668, 616.

4d) 2-(*o*,*p*-Dimethylphenylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: Yield 95 %, m.p. 224 ºC, m.f.: $C_{25}H_{19}N_3OS_2$ IR (KBr, ν_{max}, cm^{-1}): 3137, 3036, 2928, 1607, 1560, 1512, 1465, 1351, 1310, 1216, 1108, 912, 818, 757, 703, 589; ^{1}H NMR (300 MHZ, $CDCl_3$), δ: 2.31 (S, 3H), 7.30-7.40 (m, 7H, Ar-H), 7.42-7.56 (m, 2H), 7.60-7.70 (m, 2H), 7.90-8.00 (m, 1H), 8.05-8.47 (m, 1H), 8.35 (br, s, 1N-H).

4e) 2-(p-Phenetylamino)-5-(benzothiazol-2-oyl)-4-phenylthiazoles: Yield 81 %, m.p. 239 ºC, m.f.: $C_{25}H_{19}N_3O_2S_2$; IR (KBr, ν_{max}, cm^{-1}): 3171, 3130, 2935, 1600, 1573, 1512, 1465, 1344, 1310, 1249, 1202, 1115, 1054, 938, 919, 831, 720, 589; ^{1}H NMR (400 MHz, DMSO-d_6), δ: 2.23-2.35 (d, 3H), 3.28-3.38 (d, 3H), 7.05-7.13 (m, 1H), 7.13-7.80 (S, 1H), 7.41-7.49 (m, 3H), 7.52-7.69 (m, 4H), 7.70-7.73 (m, 2H), 8.05-8.13 (m, 1H), 8.22-8.32 (m, 1H); ^{13}C NMR (125 MHz, DMSD-d_6), δ: 17.61, 20.50, 113.00,

118.00, 124.20, 124.55, 127.15, 127.36, 127.53, 128.93, 129.04, 129.16, 129.52, 129.61, 131.51, 152.49, 163.63, 164.48, 168.81, 169.05, 169.27, 172.96, 174.16, 174.47.

Antibacterial study: The antibacterial screening was carried out following standard protocols using three bacterial strains. The bacterial strains selected to screen the antibacterial activity of thiazoles were two Gram-positive bacteria, *Staphylococcus aureus*, *Bacillus subtilis* and one Gram-negative bacteria, *Escherachia coli*. Agar disc diffusion method was followed for antibacterial susceptibility test. Out of the eight thiazoles screened for microbial activity, two of them were found to be active against *Bacillus subtilis*. The cytotoxic activity studies are under way.

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