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Synthesis and Biological Evaluation of Novel 2-(5-(butylamino)-1,3,4-thiadiazol-2- ylthio)-N-(4-(4-bromophenyl) thiazol-2-yl) Acetamide as Potential Anti-Cancer Drug”

Shweta Rajaram Shinde, Brahmavalley Arts, Commerce and Science College, Nashik

Makrand Balasaheb Karwal, Research Scholar

Nikita Rushikesh Handore, Brahmavalley Arts, Commerce and Science College, Nashik

1. Introduction:

During the last two decades, tetrabutylammonium bromide (TBAB) has gained significant attention as an efficient metal-free homogeneous phase-transfer catalyst [1]. TBAB is an environmentally benign, non-volatile, non-corrosive, non-flammable, low-cost, commercially available ammonium salt with high chemical stability [2]. It has been successfully used in the liquid–liquid or solid–liquid phase-transfer alkylation of the NH groups of aromatic amine[3], amides[4], lactams[5], sulfonamides[6] and other heterocyclic nitrogen containing compounds[7].However, from literature survey, TBAB play limited. role either phase-transfer catalyst or catalyst but not having any other significance.

Thiazoles are nitrogen and sulphur containing heterocyclic ring and its derivatives have wide spectrum of biological activities, including anticancer [8], anti-tuberculosis agents [9], antibacterial [10], anti-inflammatory [11], antiviral [12], antimicrobial [13], ecto-50-nucleotidase inhibitors [14], and many other important medicinal activities.

Direct alkylation/benzylation of thiol and amine is most commonly used for the preparation of S and N substituted amine derivative respectively. In 2021, Ekta Verma and co-workers reported that the N-alkylation of heterocyclic compounds such as isatoic anhydride and isatin derivatives and purification of product done by a greater number of steps [15]. In 2022, Ricardo Acosta Ortiz and co-workers reported the synthesis of a curing agent derived from limonene and used for alkylation of primary amino groups with allyl bromide, TBAB and sodium hydroxide [16]. Consequently, there is only role of TBAB act as phase transfer catalyst. Here, we reported the TBAB in THF presented in intimate ion pair form at room temperature and it is electrophilic source of butylation for the synthesis of novel 2-(5-(butylamino)-1,3,4-thiadiazol-2-ylthio)-N- (thiazol-2-yl) acetamide derivatives having high yield of the product with no other products.

However, reports on alkylation of 5-amino-1,3,4-thiadiazole-2-thiol derivatives are still limited. Therefore, new ambient nucleophile like 5-amino-1,3,4-thiadiazole-2-thiol for organic synthetic are still in great demand. However, some procedures suffer from drawbacks including low yields, drastic reaction conditions, poor functional groups, tolerance, less selectivity, and the use of highly expensive reaction components. In these reports on 5-amino-1,3,4-thiadiazole-2-thiol derivatives are used as nucleophile precursor for alkylation.

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2. Application of Thiazole

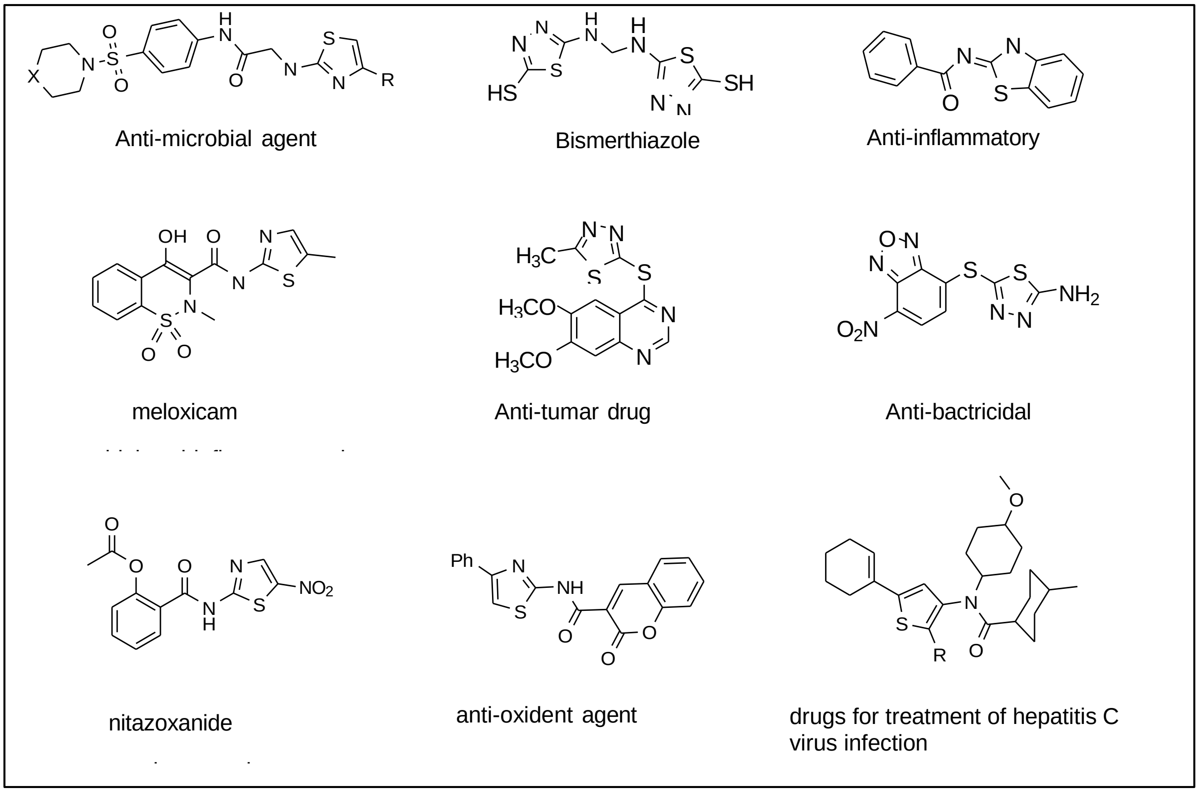
Anti-microbial agent
Bismerthiazole
Anti-inflammatory
meloxicam
Anti-tumar drug
Anti-bactricidal
nitazoxanide
anti-oxident agent
drugs for treatment of hepatitis C virus infection

Figure 1: Thiazole Drugs

Having the above aspects in mind we report a series of novel 2-(5-(butylamino)-1,3,4-thiadiazol- 2-ylthio)-N-(thiazol-2-yl)acetamide derivatives (9a-l) by TBAB in THF as source of butylation clubbing 5-amino-1,3,4-thiadiazole-2-thiol with 2-chloro-N-(thiazol-2-yl)acetamide(8a-l) (Scheme 1). The most well-known synthetic methods for 2-aminothiazoles have utilized the direct condensation of halocarbonyl compounds with thiourea (Hantzsch reaction, Products 3a– 3l) and then followed acylation by using chloroacetyl chloride (Products 5a–l).The potential utility of these methods in organic synthesis for alkylation of nitrogen template having alkyl as well as coumaril substituted thiazole. (Scheme 2).

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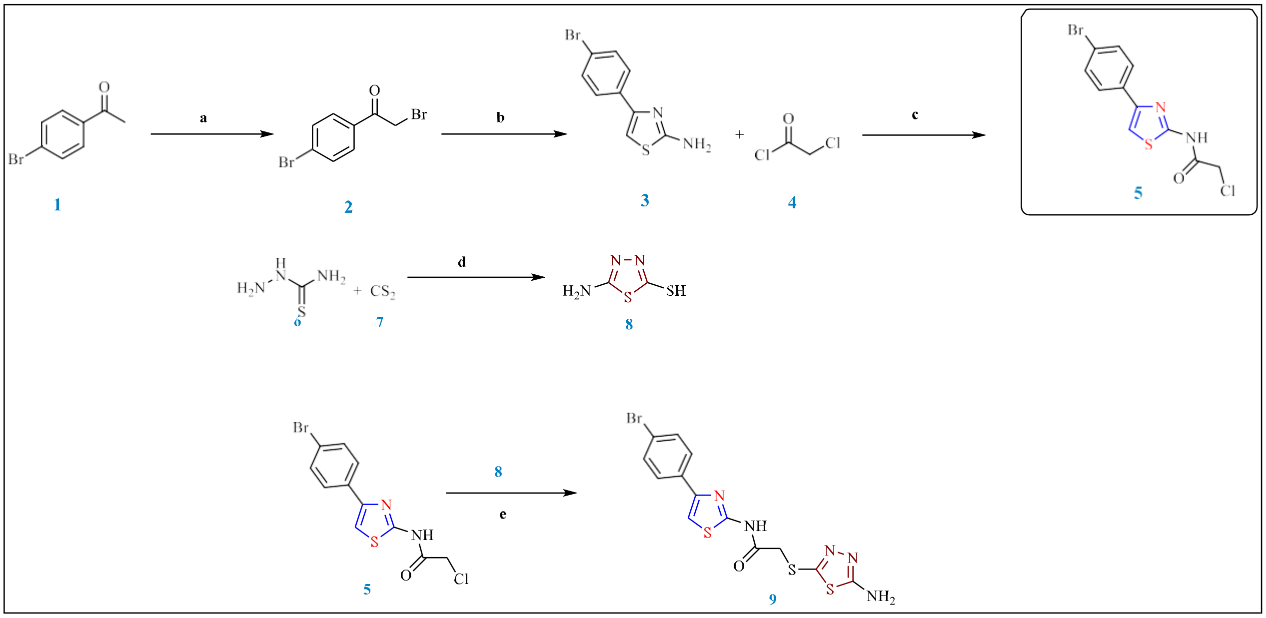
3. PRESENT WORK:



Scheme 1: "Synthesis of Novel 2-(5-(butylamino)-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-bromophenyl)thiazol-2-yl)acetamide"

4. RESULTS AND DISCUSSION

The product (9) is readily prepared from N-thiazol-2-yl-2-chloroacetamide derivatives (5) with butylated catatonic source from mixture of TBAB and non protic solvent THF at room temperature.

Table 1: "Synthesis of Novel 2-(5-(butylamino)-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-bromophenyl)thiazol-2-yl)acetamide"

Compound Number	**Compound Name**	**% Yield**	**Color**	**M.P/ B. P (In ^{0}C)**	**Reaction Time**
2	2-bromo-1-(4-bromophenyl)ethanone	80.20	Brown	108 – 110	120 min
3	4-(4-bromophenyl)thiazol-2-amine	85.50	Light Brown	183-187	60 min
5	2-chloro-N-(4-(4-bromophenyl)thiazol-2-yl)acetamide	86.25	Light Brown	241-242	30 min
8	5-amino-1,3,4-thiadiazole-2-thiol	86.88	Beige	232-234	420 min
9	2-(5-(butylamino)-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-bromophenyl)thiazol-2-yl)acetamide	87.90	Dark Brown	278-280	180 min

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4.1 Molecular Docking Study:

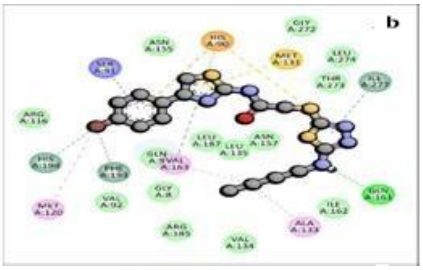

Figure 2: 2D representation of the docking compounds **9**

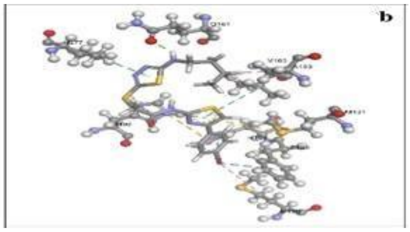

Figure 3: 3D representation of the docking compounds **9**

4.4.1 Docking Results and Interaction Analysis:

Since drug **9** is inhibited in studies, docking results are consistent. MCAT is a high-confidence pharmacological target according to the Uni Prot K.B. reviewed (Swiss-Prot) entry (Uni Prot ID: P9WNG5). It also states that Ser91 and His194 are active site amino acids using manual homology-based inference. The docking data (**Table 6 and Figures 5-6**) show that these amino acids interact with ligands often. His90, located next to Ser91 in the active site cavity, interacts with all '**9**' compounds. This suggests that the chemicals modulate the active site and prevent malonyl-CoA binding. The compound **9** has the best MIC value of compound **9** and docking results support that. This shows that these compounds may target MCAT most likely. In compound **9**, alkyl and Pi-alkyl interactions stabilize the protein-ligand complex. The hydrophobic pocket of Met120, Phe193, and His194 allow compound 9's bromine atom to bond to these amino acids via alkyl and pi-alkyl interactions. The docking results suggest that a halogen or carbon atom at this location in the Ligand affects binding.

Table 2: B.E of Compounds of 4a-4k by Molecular Docking Screened

Compound	**B. E**
9	-7.54
Doxorubicin (Standard Compound)	3.26

B.E.: Binding efficiency (kcal/mol)

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5. EXPERIMENTAL SECTION

5.1 General Information

All reagents and solvents were commercially available in analytical grade and used as received. The used solvents were purified and dried according to common procedures. were obtained with a FTICR-MS (Ionspec 7.0T) spectrometer. Mass spectra (MS) and Fourier-transform infrared spectroscopy (FTIR) (KBr disc) (, cm1) were recorded at the Laboratory, Punjab University Chandigarh. The ^{1}H and ^{13}C NMR spectra were recorded in CDCl$_3$ or DMSO-d6 solutions on a Brukera Vance Neo 500 MHz NMR Spectrometer (Saif, P.U.) spectrometer. Chemical shifts are reported in parts per million (ppm) relative to CDCl$_3$ (7.27 ppm) for ^{1}H NMR data and CDCl$_3$ (77.0 ppm) for ^{13}C NMR data or the peak of DMSO-d6, defined at = 2.50 (^{1}H NMR) or = 39.5 (^{13}C NMR). The following abbreviations were used to explain multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, and br = broad.

5.2 Synthetic procedure for 2-bromo-1-(4-bromophenyl)ethan-1-one derivative (2)

2-bromo-1-(4-bromophenyl)ethan-1-one derivative (2) were prepared by the methods reported in the literature [20]. A four-necked, round-bottomed flask was charged with acetophenone or the acetophenone derivative **1**(10 mmol) and glacial acetic acid (20 ml). The flask was immersed in an ice salt mixture (0–5 °C), and bromine (0.01 mol) was added from the pressure equalizing funnel at such a rate that the temperature of the reaction mixture did not exceed 5^{0}C. After completion of the addition, it was stirred at RT for 2-3 hours. The suspension was poured onto crushed ice. The precipitate was filtered, repeatedly washed with water, and dried at room temperature.

Mobile phase: chloroform

5.3 Synthetic procedure for thiazol-2-amino derivatives (3)

Thiazol-2-amino derivatives (3) were prepared by the methods reported in the literature [21]. The mixture of 2-bromo-1-(4-bromophenyl)ethan-1-one (2), thiourea (11 milimole), and ethanol (10 ml) was refluxed in four necked round-bottomed flasks at 78 ^{0}C. The reaction was completed within 1–2 hours. The reaction mixture was poured into the crushed ice. The products are filtered and washed with water and ethanol. The products were recrystallized by using ethanol as a solvent.

Mobile phase: n-hexane: ethyl acetate = 6:4.

5.4 Synthetic procedure for acylation of thiazol-2-amino derivatives (5)

The mixture of thiazol-2-amino derivatives (**3**) (1 millimole), and triethylamine (1.3 millimole) was taken in a round bottom flask. Stirred the reaction mass at room temperature for 30 minutes. The chloroacetyl chloride (**4**) (2.2 millimoles) was added at 0–5 ^{0}C. The reaction was completed within 1–4 hours. The reaction mixture is washed with a brine solution and then water. The solution was neutralized by a 1:1 HCl solution. The products were filtered and washed with water.

Mobile phase: n-hexane: ethyl acetate = 6:4.

5.5 Synthesis of 5-amino-1,3,4-thiadiazole-2-thiol (8)

2-amino-1,3,4-thiadiazole-2-thiol was prepared by the methods reported in the literature [22]. Thiosemicarbazide (10 millimoles) was suspended in absolute ethanol and anhydrous Na$_2$CO$_3$ (10 millimoles) with carbon disulfide (10 milimoles). The mixture was warmed under stirring and refluxed for 1 h, and then heated in a steam bath for 4 h. Completion of the reaction was indicated by TLC with toluene, ethyl acetate, and aq. formaldehyde (TEF) 5:4:1 as mobile phases. The solvent was evaporated under vacuum and the residue dissolved in water (20 ml), acidified with conc. HCl to give the product (12) (54 g), 86. 88 %, m. p. 232 ^{0}C.

5.6 Synthetic of Novel 2-(5-(butylamino)-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-bromophenyl)thiazol-2-yl)acetamide (9)

Compounds 5 were prepared by the methods reported in the literature [23,24]. A solution of 5- amino-

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1,3,4-thiadiazole-2-thiol (**8**) (1 millimole), potassium carbonate (1.1 millimoles) in THF (10 ml) was stirred at ambient temperature for 30 minutes. Subsequently, prepared the solution of acylated 2-aminothiazole derivative (**5**) (1 millimole) and TBAB (0.5milimole) in THF (10 ml) and was added drop-wise by dropping a funnel into the reaction mass. Stirred the reaction mass at room temperature and confirmed the completion of the reaction by TLC. After the reaction was completed, the reaction mixture was poured into crushed ice. The product was filtered, washed with brine solution, and recrystallized from ethyl acetate to give a pure product.

5.7 In-Silico studies

5.7.1 Target prediction

Target was identified with help of Pharmacophore mapping and the process was carried out by Pharm Mapper web server.[5] The core compound was supplied as a 'sdf' file, and the energy minimization was enabled, and the algorithm was asked to perform GA match. other parameters were set to default.[15] From the obtained result a plausible drug target in Mycobacterium tuberculosis was searched from PDB (Full form of PDB).

5.7.1 Molecular docking

Molecular docking studies were performed following the literature reports. [6,7,8] The results are summarized below.

5.7.2 Compound library preparation

The series of compounds were drawn in 2D in online version of Marvin Sketch (marvinjs-demo.chemaxon.com/latest/demo.html) and 1D string of 'SMILES' were generated. Then the file containing SMILES was submitted to FROG2 web server [9] to generate 3D conformers. The resulting 'sdf' file containing energy optimized 3D conformers were obtained.

5.7.3 Ligand preparation

From Protein Data Bank (PDB) **2AF6** entry was retrieved. The identifier corresponds to the crystal structure of *Mycobacterium tuberculosis.* Flavin dependent thymidylate synthase (Mtb ThyX) in the presence of co-factor FAD and substrate analogy 5-Bromo-2'-Deoxyuridine-5'- Monophosphate (BrdUMP). The complexed structure and its cofactor were separated in two different files, containing the protein in its apo-form and the separated ligand BrdUMP. Eight chains in the protein structure were observed with the protein identified as a dimer of two homomeric domain containing 4 chains each. Therefore, chain A, B, C and D were retained, and remaining portion of the protein coordinates were ignored from the PDB file.

5.7.4 System validation and virtual screening

For virtual screening and molecular docking PyRx, a virtual screening software for computer aided drug discovery was used. First, the apo form of the receptor was loaded and polar hydrogens and Kollman charges were added to convert it into 'pdbqt' format. Subsequently, using the openable tab of the GUI, compound library was loaded, minimized, and converted in the 'pdbqt' format for docking. The grid box was generated using Auto grid program. For reproducibility of the result the dimension of the grid box was set to default 50x50x50, and the centroid of the grid box was fixed at the centre of the ligand so the grid box can cover the whole active site of the receptor. Lamarckian Genetic algorithm was utilized for the docking keeping the other parameters unchanged. Prior to the screening of the newly synthesized ligand, the system was validated for assuring the ability of the software to reproduce the experimental pose of the ligand. The predicted pose by the embedded Auto dock 4.2 shows 1.38 Å root mean square deviation (RMSD) which is less than 2 Å thereby confirming the successful validation of the system. [10]

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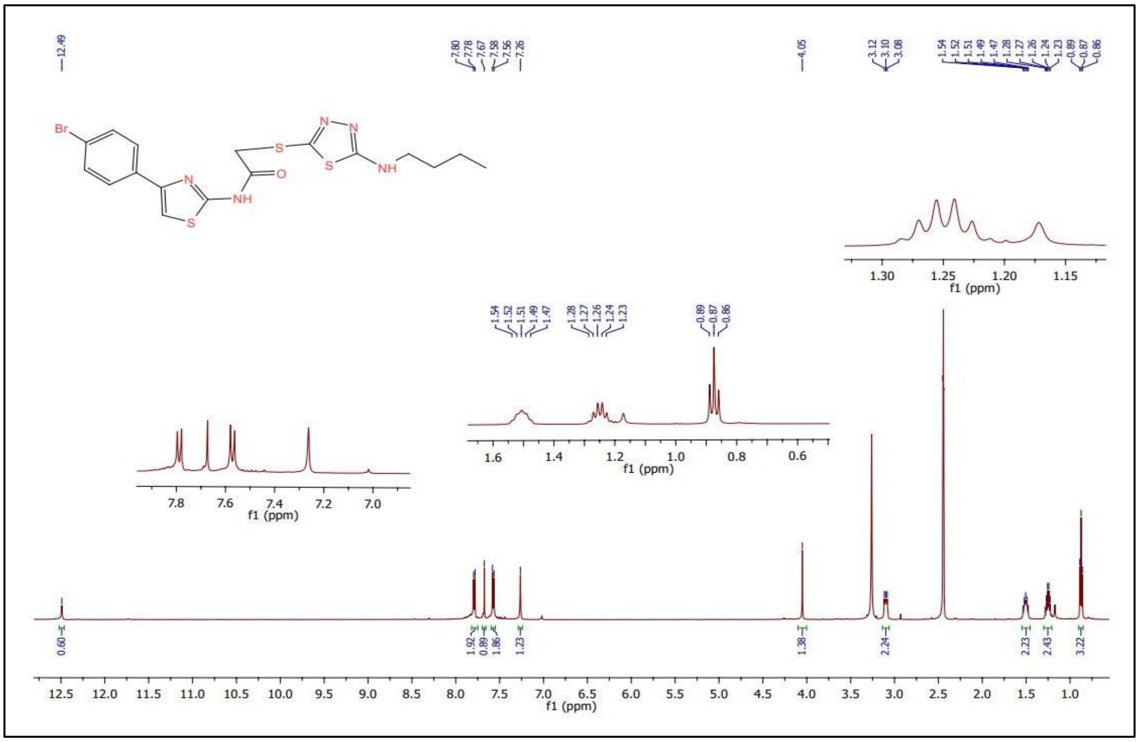
5.7.5 Post processing and analysis

Auto dock generates Docking log files (.dlg) to predict the poses of the ligand and it assigns docking score according to the binding affinity. The best pose corresponding to the structure with lowest binding free energy converted to PDB file with help of Open Babel software.

Subsequently, in the BIOVIA discovery Studio, complexed structure with the receptor and the active conformation of the drug molecules. Two-dimensional interaction images were processed from the same software using receptor ligand interaction utility. According to the shown interaction in the 2D images, the 3D high quality interaction images were generated with help of the PyMol software.

6. Spectral Analysis

6.1 ^{1}H-NMR



Spectra 1: PMR spectra of compound 9.

M.P. 278-280 °C, Yield: 86 %, brown powder; FT.IR: 620.73, 1177.97, 1383.05, 1516.65,1617.57, 3470.61 cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d$_{6}$): δ 0.87 (t, J = 7.3 Hz, 3H), 1.26 (sextate,J = 7.2 Hz, 2H), 1.51 (quintet, J = 15.5, 8.0 Hz, 2H), 3.14 (t, 2H), 4.05 (s, 2H), 7.26 (s, 1H), 7.57(d, J = 8.5 Hz, 2H), 7.67 (s, 1H), 7.79 (d, J = 8.5 Hz, 2H), 12.49 (s, 1H); MS: Exact Mass: 484.98; Observed Mass: 487

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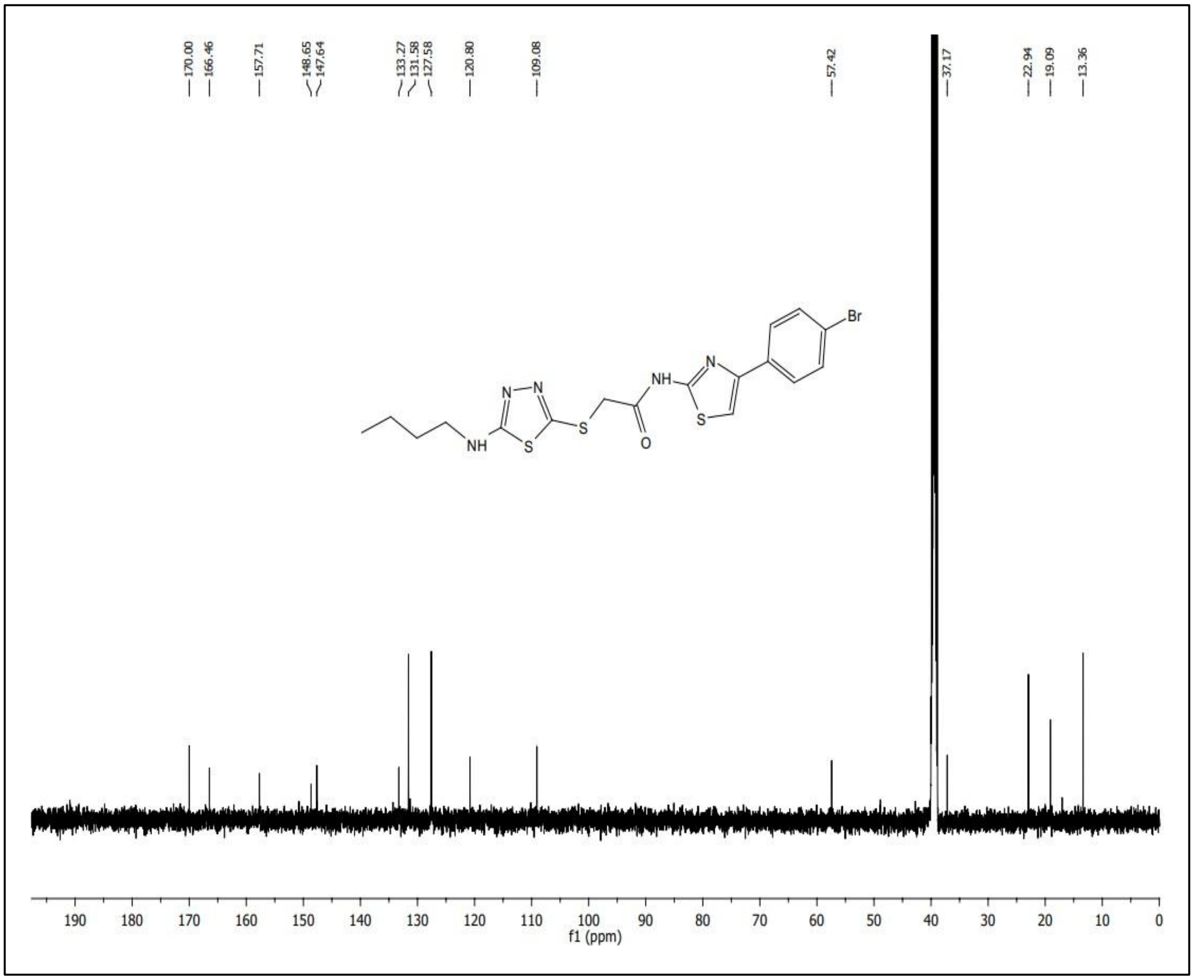

Spectra 2: CMR spectra of compound 9.

^{13}C NMR (500 MHz, DMSO-d_6): δ 13.36,19.09, 22.94, 37.17, 57.42, 109.08, 120.80, 127.58(Strong), 131.58(Strong), 133.27, 147.64, 148.65, 157.71, 166.46, 170.00;

7. CONCLUSIONS

The novel method for the synthesis of thiadiazol-thiazole-acetamide derivatives 9 by using TBAB as catalyst and butylating of amine provides a high yield, simple product isolation and low cost. The structure of the newly synthesized compounds was confirmed by different spectroscopic techniques such as- ^{1}H-NMR and ^{13}C-NMR. Thus, the overall study concluded that thiazole- thiadiazole compounds could act as a hit lead for further developments of potential anticancer and antimycobacterial agent.

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8. Photos:

 Product 2-(5-amino-1,3,4-thiadiazol-2-ylthio)-N-(4-(4-fluorophenyl)thiazol-2-yl)acetamide	 TLC **Mobile Phase:** n-hexane :Ethyl acaetate : Acetic Acid - 5:4:1

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