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REVIEW ARTICLE

REVIEW ANALYSIS ON SYNTHESIS, ENZYME INHIBITION AND HEMOLYTIC STUDY OF NOVEL ACETAMIDE DERIVATIVES

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ABSTRACT

The synthesis of 2- (2,4-dichloro-6-{[4-(2-furoyl)-1-piperazine]sulfonyl}phenoxy)-*N*-(aryl) acetamides was carried out under the controlled conditions as mentioned in the scheme. In the first step, the nucleophile is formed by reacting the calculated amount of *N*-(2-furoyl) piperazine **(1)** with 3,5-dichloro-2-hydroxybenzenesulfonylchloride **(2)** in the presence of an aqueous solution of sodium carbonate and stirred for three hours at room temperature to produce{4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furoyl)methanone **(3)**. In the parallel set of reactions, electrophile was prepared by reacting substituted aniline with acetyl bromide in the vicinity of an aqueous sodium carbonate solution. Vigorous shaking formed the precipitates of the electrophile. In the final step, nucleophile and electrophile reacted with each other in an acetonitrile/K_2CO_3 medium that produced various 2-(2,4-dichloro-6-{[4-(2-furoyl)-1-piperazine] sulfonyl}phenoxy)-*N*-(aryl) acetamides **(7a-c)** as outlined in scheme-1. Their structures are confirmed by spectral analysis techniques such as EI-MS, Infrared (IR), proton, and ^{13}C NMR. The synthesized compounds' enzyme inhibition potential was tested against *a*-glucosidase, AChE, and BChE enzymes. As illustrated from the results of $IC_{50,}$ these compounds are observed as the active inhibitory potential for these enzymes. The cytotoxic activity by hemolytic Assay revealed that these compounds have mild exposure to cytotoxicity on RBCs.

KEYWORDS

N-(2-furoyl)piperazine, 3,5-dichloro-2-hydroxybenzenesulfonylchloride, acetyl bromide, EI-MS, ^{13}C NMR,^{1}HNMR, Enzyme Inhibition, *a*-glucosidase, AChE, and BChE Enzymes, Cytotoxic Activity, Hemolytic Assay.

1. INTRODUCTION

Heterocyclic compounds have a characteristic feature of bioactivity perspective that has been significantly utilized in the pharmaceutical sector to reveal modern sedate candidates with curing advancements (Hardik et al., 2012; KnezHrnčič et al.,2019). Amides play a significant role as an essential component of natural substances, such as peptides and proteins, due to their stability and polarity (Joullié, and Lassen, 2010). Its significant contributions are involved in the production of pharmaceutical drugs and its role as a precursor for remedies manufacturing. Substituted polyamine moieties have a significant role in targeting the biochemical in all therapeutic treatments. Piperazine entitles heterocyclic moiety, the primary constituent of different dynamic particles related to organic substances (Patel and Won Park, 2013). The attraction of the piperazine molecule is governed by the ring that contains the polar nitrogen (Abdel-Hay et al., 2013). Compounds of the piperazine are considered one of the major classes of organic chemicals. Over time, too many structural modifications occurred that make them suitable for great pharmaceutical industry utilization for drug discovery and new substance development (Mosnaim et al., 2006).

Piperazine performs an integral part against the biological targets to overcome their drastic effects and recover a patient from certain diseases (Chow et al., 2020). Its chemical behavior is mainly related to piperidine. Piperazine is found as deliquescent crystals of alkaline nature that have a salty flavor (Jahanshahi et al., 2010). A certain degree of oxidization occurs when it is ingested as a remedy in the body and excretes fromthe body without minor change in structure and composition (Rudrapal, and De, 2013). During the era of its discovery, it was revealed that it could be helpful as a medication that has the potential to cause the removal of parasitic worms of the intestine in children (Villamizar et al., 1996). Later on, it was used adequately as a remedy for treating threadworm infections (Sharma et al., 2020). Piperazine's inherent healing ability for uric acid excretion has not been an effective drug. It was observed as a sanative drug in 1953 (Goodwin and Standen, 1954). The involvement as a catalyst produced many significant molecules on a commercial and industrial scale (Yousefi et al., 2018).

The effectiveness of the anticancer drugs enhanced by the presence of the piperazine ring and its sub-alternates is considered significant in curing diseases that resist protein inhibition and demolish impact (Walayat et al., 2019). It is a sedate used as an anticancer drug (Sahin and Durdagi, 2020). The parasitic worm on vertebrates' intestines is affected by γ-aminobutyric acid,which is agonistic by nature (Martin et al., 2002).

The piperazine compounds have an affinity for dealing with the membrane channels containing ions. Debilitated paralysis of the worm resulted from piperazine compound exposure that resists Ascaris's repulsive action through the acetylcholine (Tahseen, 2018). During the action, the worm gets crippled and escapes from the intestine's lumen wall and is removed through the body's feces due to peristalsis (Rips et al., 1973). At least 1/4th of the acetylcholine is consumed by the liver, transformed into *N*-mononitrosopiperazine, and fused into the liver's gastric juice. Within

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three to four hours, MNPz converted to*N*-nitroso-3-hydroxypyrrolidine. After approaching the kidneys, just 20% urinated with the pee within a day. Derivatives of the acetamide had a wide range of applications as an active biological agent for antifungal, antibacterial, pesticide properties effective for warm-blooded organisms, and involved in the development of specific enzymes that resist the replication of HIV (human immune deficiency syndrome) virus (Gold and Moellering Jr, 1996; Levy, 1994; Zhan et al., 2009).

A variety of acetamide compounds inhibit the farnesyltransferase process of enzymes, a vital strategy that involves producing potential anticancer remedies (Schlitzer et al., 2002). Certain derivatives of acetamide are employed therapeutically against cancer treatment and are observed to have some advantageous specialties as anticancer treatment (Ahmed et al., 2018). Antifungal compounds have the potential to destroy or kill fungus and its related fungal organisms by establishing the difference between cells of the host and incoming pathogenic organisms by imparting little exposure to the host (Woodhams et al., 2011).

2. Materials and Methods

2.1 Chemistry

Selected Chemicals for the synthesis of targeted compounds were purchased from vendor Sigma Aldrich, Alfa Aesar, located in Germany, and local suppliers were selected to take analytical grade solvents. Melting points of the prepared compounds are obtained by utilizing the technique of an open capillary tube. Griffin and George's apparatus used to determine the melting point was inaccurate. By employing TLC (thin layer chromatography) and utilizing different *n*-hexane and ethyl acetate ratios (used as mobile phase), the synthesized compounds' preliminary pureness was determined at 254 nm. Spectral peaks of IR by using the potassium bromide pellet method were taken on a Jasco-320-A spectrometer. Using Bruker spectrometers, different peaks of ^{1}H-NMR signals at a frequency of 500 MHz (^{13}C-NMR at 150 MHz) in $CDCl_3$ employing Bruker spectrometers. Fragmented peaks of the synthesized compounds were recorded by employing EIMS analysis on a JMS-HX-110 spectrometer.

Synthesis of {4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone (3)

The quantity of 12.8 mmol; 2.31 g,*N*-(2-Furoyl)piperazine **(1)**was taken in 100 mL RBC with distilled water, and the solution pH was adjusted in the range of 9.0 to 10.0 by the addition of an adequate amount of aqueous 10 % Na_2CO_3, with the continuous addition of dropwise 12.8 mmol; 3.33 g, of 3,5-dichloro-2-hydroxybenzenesulfonyl chloride **(2)** into the reaction mixture containing piperazine and sodium carbonate. The reaction mixture's stirring carried on until the reaction was accomplished for three hours at room temperature. TLC was employed at different time intervals to ensure the formation of the targeted compound. When the reaction was completed, the slow addition of hydrochloric acid was used to maintain the solution's strongly acidic (2.0) pH. The Reaction mixture containing the product was allowed to stand at room temperature (RT) for 15 minutes. The filtration is employed to obtain the precipitates, and washing takes place by distilled water to afford a white solid of {4-[(3,5-dichloro-2-hydroxyphenyl)sulfonyl]-1-piperazinyl}(2-furyl)methanone (**3**).

Synthesis Method for 2-Bromo-N-(aryl) acetamides(6a-c)

In an iodine flask containing 15 mL (6a-c; one in each reaction) of deionized water, 15 mmol of di-substituted aniline was added. The solution's pH was maintained in the basic range of 9-10 with the addition of 10% sodium carbonate solution (Na_2CO_3). After that, 15 mmol**(5)** of 2-bromoacetyl bromide were taken in the iodine flask dropwise for 2-5 minutes. The iodine flask's vigorous shaking is employed manually when the complete addition of calculated 2-bromoacetyl bromide is done, and then the reaction mixture stays at room temperature for 3-4 hours. Solid precipitates were formed in the iodine flask, and TLC (thin layer chromatography) was employed to ensure product formation until a single spot was detected. Precipitates of the product are separated by using the filtration method, and washing the precipitates is done with deionized water. These precipitates are dried at room temperature to get the maximum amount of the 2- Bromo-*N*-(aryl) acetamides **(6a-c)**.

Synthesis of 2-(2,4-dichloro-6-{[4-(2-furoyl)-1-piperazinyl] sulfonyl} phenoxy)-N-(aryl) acetamides (7a-c)

A round bottom flask of 100 mL was taken with 20-30 mL of acetonitrile and added 0.00024 mol of the nucleophile **(3)**. Solid potassium carbonate (K_2CO_3) of 0.0135 mol was added to the above mixture. The reaction mixture was refluxed for 30 minutes to activate the reactants, and then the addition of the desired electrophile of 0.0045 mol (**6a-c**) was added to the round bottom flask. The reaction is stained for a time interval of 4-5 hours and reflexed. The reaction monitoring for product formation was done by employing TLC and ensuring that the reaction was completed. Precipitates are formed by adding deionized or distilled water to the reaction mixture. When the precipitates are formed, filtration is separated and washed with an adequate amount of distilled water. The separated precipitates of the product are stand to dry off to get the desired derivatives of acetamides **(7a-c)**.

2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2methylphenyl) acetamide (7a)

Off-white amorphous solid; Yield: 78 %; m.p: 155-157 ^{o}C; Mol. F.: $C_{24}H_{23}Cl_2N_3O_6S$; Mol. Mass: 551;

IR(KBr, cm^{-1}) *v*: 3408 (N-H), 3086 (Ar C-H), 2887 (R C-H), 1658 (C=O), 1580 (Ar C=C), 1381 (S=O), 1195 (C-O-C), 1118 (C-N-C);

^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 8.37 (br.s, 1H, -NH), 7.95 (d, *J* = 8.0 Hz, 1H, H-6''''), 7.77 (d, *J* = 2.5 Hz, 1H, H-5''), 7.68 (d, *J* = 2.5 Hz, 1H, H-3''), 7.56 (d, *J* = 7.5 Hz, 1H, H-6''''), 7.40 (distorted d, *J*= 1.6 Hz, 1H, H-5), 7.25 (d, *J* = 6.3 Hz, 1H, H-5''''),7.12 (t, *J* = 6.2 Hz, 1H, H-4''''), 7.04 (d, *J* = 3.4 Hz, 1H, H-3), 6.47 (dd, *J* = 3.5, 1.8 Hz, 1H, H-4), 4.79 (s, 2H, CH_2-2'''), 3.89 (br.s, 4H, CH_2-2' CH_2-6'), 3.33 (br.t, *J* = 5.0 Hz, 4H, CH_2-3', CH_2-5'), 2.24 (s, 3H, CH_3-7'''');

^{13}C-NMR(150 MHz, $CDCl_3$, δ / ppm): 164.87 (C-1'''), 158.94 (C-1''), 149.78 (C-6), 147.37 (C-2), 144.01 (C-5), 135.32 (C-2''''), 134.80 (C-3''), 134.18 (C-5''), 131.38 (C-1''''), 130.84 (C-3''''), 130.63 (C-4''''), 129.42 (C-6''''),128.88 (C-5''''), 126.96 (C-6''), 125.61 (C-4''), 122.74 (C-2''), 117.63 (C-3), 111.61 (C-4),73.12 (C-2'''), 46.22 (C-2', C-3', C-5', C-6'), 17.69 (C-7'''');

EI-MS (*m/z*): 555 $[M + 4]^+$, 553 $[M + 2]^+$, 551 $[M]^+$, 403 $[C_{15}H_{14}Cl_2N_2O_5S]^+$, 372 $[C_{15}H_{12}Cl_2NO_4S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 148 $[C_9H_{10}NO]^+$, 134 $[C_8H_8NO]^+$, 106 $[C_7H_8N]^+$, 95 $[C_5H_3O_2]^+$.

2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2,6-dimethylphenyl) acetamide (7b)

Greyish white amorphous solid; Yield: 86%; m.p: 125-127 ^{o}C; Mol. F.: $C_{25}H_{25}Cl_2N_3O_6S$; Mol. Mass: 565;

IR (KBr, cm^{-1})*v*: 3408 (N-H), 3080 (Ar C-H), 2883 (R C-H), 1655 (C=O), 1587 (Ar C=C), 1381 (S=O), 1197 (C-O-C), 1116 (C-N-C);

^{1}H-NMR(600 MHz, $CDCl_3$, δ / ppm): 8.05 (br.s, 1H, -NH), 7.79 (d, *J* = 2.5 Hz, 1H, H-5''), 7.69 (d, *J* = 2.4 Hz, 1H, H-3''), 7.41 (d, *J* = 1.2 Hz, 1H, H-5), 7.17-7.11 (m, 3H, H-3'''' to H-5''''), 7.04 (d, *J* = 3.4 Hz, 1H, H-3), 6.47 (dd, *J* = 3.4, 1.7 Hz, 1H, H-4), 4.86 (s, 2H, CH_2-2'''), 3.91 (br.s, 4H, CH_2-2', CH_2-6'), 3.36 (br.t, *J* = 5.1 Hz, 4H, CH_2-3', CH_2-5'), 2.28 (m, 6H, CH_3-7'''', CH_3-8'''');

^{13}C-NMR(150 MHz, $CDCl_3$, δ / ppm): 165.32 (C-1'''), 158.95 (C-1''), 149.81 (C-6), 147.37 (C-2), 144.02 (C-5), 135.42 (C-1''''), 134.20 (C-3''), 132.65 (C-2'''', C-6''''), 131.21 (C-5''),130.59 (C-3'''', C-5''''), 129.54 (C-4''''),128.42 (C-6''), 128.38 (C-4''), 127.83 (C-2''), 117.73 (C-3), 111.68 (C-4), 72.99 (C-2'''), 46.27 (C-2', C-3', C-5', C-6'), 18.45 (C-7'''', C-8'''');

EIMS (*m/z*): 569 $[M + 4]^+$, 567 $[M + 2]^+$, 565 $[M]^+$, 403 $[C_{15}H_{14}Cl_2N_2O_5S]^+$, 386 $[C_{16}H_{14}Cl_2NO_4S]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 162 $[C_{10}H_{12}NO]^+$, 148 $[C_9H_{10}NO]^+$, 120 $[C_8H_{12}N]^+$, 95 $[C_5H_3O_2]^+$.

*2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2-*methoxycarbonylphenyl)acetamide (7c)

White brown amorphous solid; Yield: 86%; m.p: 173-175 ^{o}C; Mol. F.: $C_{25}H_{23}Cl_2N_3O_8S$; Mol. Mass: 595;

IR (KBr, cm^{-1})*v*: 3412 (N-H), 3089 (Ar C-H), 2881 (R C-H), 1655 (C=O), 1587 (Ar C=C), 1388 (S=O), 1196 (C-O-C), 1111 (C-N-C);

^{1}H-NMR (600 MHz, $CDCl_3$, δ / ppm): 9.06 (br.s, 1H, -NH), 8.75 (d, *J* = 8.5 Hz, 1H, H-6''''), 8.11 (d, *J* = 7.7 Hz, 1H, H-3''''), 7.78 (d, *J* = 2.8 Hz, 1H, H-5''), 7.63 (d, *J* = 2.7 Hz, 1H, H-3''), 7.49 (br.s, 1H, H-5), 7.30-7.14 (m, 2H, H-4'''', H-5''''), 7.03 (d, *J* = 3.5 Hz, 1H, H-3), 6.53 (dd, *J* = 3.5, 1.7 Hz, 1H, H-4), 4.94 (s, 2H, CH_2-2'''), 3.91 (br.s, 4H, CH_2-2', CH_2-6'), 3.95 (s, 3H, $COOCH_3$-7''''), 3.46 (br.t, *J* = 5.1 Hz, 4H, CH_2-3', CH_2-5');

^{13}C-NMR(150 MHz, $CDCl_3$, δ / ppm): 174.40 (C-7''''), 164.80 (C-1'''), 158.44 (C-1''),151.74 (C-6), 147.37 (C-2), 144.31 (C-5), 135.48 (C-1''''), 134.85 (C-3''), 134.68 (C-5''), 133.83 (C-5''''),127.95 (C-3''''), 126.56 (C-4''''), 126.06 (C-6''), 125.14 (C-4''), 123.51 (C-6''''), 122.74 (C-2''), 120.29 (C-2''''), 117.62 (C-3), 111.81 (C-4), 72.90 (C-2'''), 52.46 (C-8''''), 46.49 (C-2', C-3', C-5', C-6');

EI-MS (*m/z*): 599 $[M + 4]^+$, 597 $[M + 2]^+$, 595 $[M]^+$, 403 $[C_{15}H_{14}Cl_2N_2O_5S]^+$, 416 $[C_{16}H_{12}Cl_2NO_6S]^+$, 192 $[C_{10}H_{10}NO_3]^+$, 179 $[C_9H_{11}N_2O_2]^+$, 178 $[C_9H_8NO_3]^+$, 150 $[C_8H_8NO_2]^+$, 95 $[C_5H_3O_2]^+$.

4. Biochemistry

4.1 a-Glucosidase Assay

Different enzymes are used to determine the inhibitory action of the synthesized compounds. Compounds are employed regarding the cited method to examine the inhibitory potential toward *a*-glucosidase [25]. A total volume of100 µL of the solution was formed by mixing buffer of phosphate saline with the concentration of 50 mM at pH of 6.8 and a volume of 70 µL, 10 µL of 0.5 mM concentrated solution of the tested sample along with ten µLof enzymes of about 0.057 units. The above mixture is dissolved as well as possible. It was pre-incubated for ten minutes at a temperature equal to the homeostatic temperature of the human body. The absorbance of the mixture is determined at a wavelength of 400 nm. A microplate reader is used to record the mixture's absorbance at a wavelength of 400 nm. We added a 0.5 mL concentrated substrate with p-nitrophenylglucopyranosidase equal to the volume of about 10 µL by maintaining the temperature at 37 ºC for half an hour. Any sample's absorbance value fluctuation determines the concerned compound's percentage inhibition. Positive control of acarbose is used as a reference to evaluate the inhibitory potential of the enzyme. Overall, the experiment to determine inhibitory action was performed three times to minimize error and miscalculation. The below mathematical equation is being used to determine the percentage (%) inhibition:

$$\%\ age\ Inhibition\ = \frac{Control - Test}{Control} \times\ 100 \qquad (1)$$

The IC50 values of synthesized compounds were calculated using the EZ-Fit software to determine the enzyme kinetics (Perrella Scientific Inc. Amherst, USA). Adequate dilution of the studied compounds is done to evaluate the IC_{50} value, starting from the concentration of 0.5 mM to the concentration of a solution with a value of 0.01563 mM. The percentage inhibition value used to make the graph was the average value of the performed three experiments independently.

4.2 AChE and BChE Assay

The synthesized compounds' inhibition activity against the acetyl/butyrylcholinesterase (AChE/BChE) enzymes was determined by employing the reported technique [74]. The reference standard Eserine was utilized as a positive control to evaluate the inhibition potential toward cholinesterase enzymes. Percentage inhibition of the IC_{50} was determined by putting values in the below mathematical equation.

$$\text{Inhibition } (\%) = \frac{Control - Test}{Control} \times 100 \qquad (2)$$

4.3 Hemolytic Activity

Samples of Bovine blood were separated in EDTA, and the dilution was done by 0.9% NaCl (saline), and centrifugation took place for ten mint at 1000x. The dilution of separated erythrocytes took place in a buffer solution of phosphate saline with a pH of 7.4, resulting in a suspension. A volume of 20 µL that contains 10mg/mL of the synthesized chemical compound was mixed with the suspension of red blood cells of volume 180 µL and then incubated at room temperature for half an hour. The negative control used in the hemolysis assay is Phosphate Buffer Saline (PBS). Triton 100-X was used as the reference standard for positive control in the hemolytic activity. Percentage hemolysis of the sample is determined by putting the values in the below mathematical equation:

$$(\%)\ \text{of Hemolysis} = \frac{Absorbance\ of\ sample - Absorbance\ of\ negative\ control}{Absorbance\ of\ positive\ control} \times 100 \qquad (3)$$

4.4 Statistical Exploration

The resulted data from threeold performed experiments are mentioned as mean ± Scanning electron microscope (SEM), and Microsoft Excel 2010 was employed for arithmetical analysis. 50% inhibitory concentration (IC_{50}) of the synthesized compound was determined by EzFitPerrella Scientific Inc. Amherst USA software.

5. Result Analysis

5.1 Chemistry

The present research work involves the synthesis of several multifunctional acetamide derivatives **(7a-c)** with mild cytotoxicity and valuable enzyme inhibition activity of AChE, BChE, and a-glucosidase, prepared by the following scheme in Fig.1.The overall synthesis was completed in two steps. Firstly nucleophile is prepared by reacting a calculated amount of *N*-(2-furoyl) piperazine with 3,5-dichloro-2-hydroxybenzenesulfonyl chloride in the vicinity of aqueous sodium carbonate solution. Electrophile was prepared by reacting the calculated amount of substituted aniline with acetyl bromide in the presence of an aqueous sodium carbonate solution. The prepared electrophile and nucleophile are then coupled with each other in an acetonitrile/K_2CO_3 medium to produce different derivatives of 2-(2,4-dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(aryl) acetamides (**7a-c**). The targeted synthesized chemical compound structures were identified by ^{1}H-NMR, IR, and ^{13}C-NMR techniques of spectral analysis, and the spectral data obtained is mentioned in the experimental section.

IR spectroscopy reveals the presence of functional groups such as C=O, C-O, C=N, C-N, N-H, C=C, and aromatic by stretching peaks in the expected region of spectra. The aromatic and aliphatic protons were detected in the corresponding up field and downfield regions. The EI-MS did further clarify that the synthesized compounds show the relative [M+1] peak according to the relevant molecular peak.

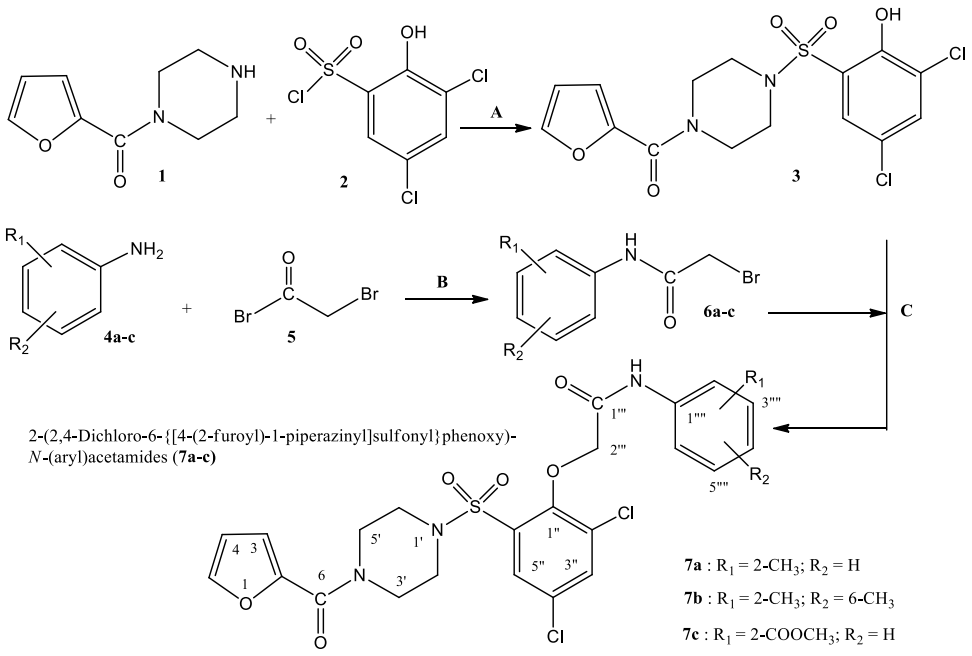

Figure 1: Schematic outline for the synthesis of 2-(2,4-dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(aryl)acetamides (7a-c). Reagents & Conditions: (A) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3 hrs. (B) Aq. Na_2CO_3 soln./pH 9-10/stirring at RT for 3-4 hrs. (C) Acetonitrile/K_2CO_3/refluxing for 4-5 h.

2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2-methylphenyl)acetamide (7a)

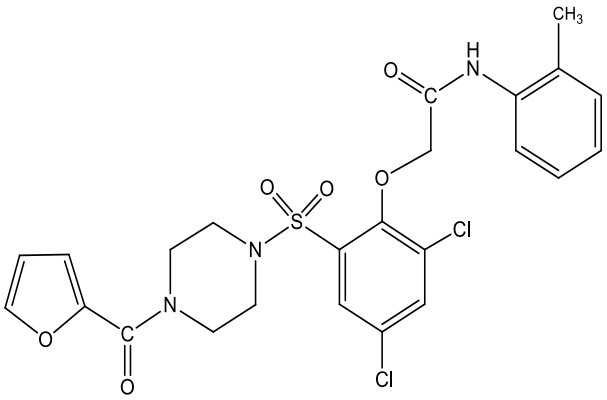

Figure 2: Structure of 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(2-methylphenyl) acetamide(7a).

5.2 IR Spectral Interpretation

Synthesized compound 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(2-methylphenyl)acetamide, further revealed to clarify the synthesis. Distinguishing peaks are found in the IR spectrum, in the functional group region with wavenumbers (cm^{-1}) such as 3408 (N-H) of amide, 3086 (Aromatic C-H), 1658 (C=O), 1580 (Aromatic C=C), 2887 (R C-H), 1381 (S=O), 1195 (C-O-C) and 1118 (C-N-C) that establish the conformation of compounds that contains above functional groups of acetamide, 2-furoyl-1-piperazine ring, and sulfonamide.

5.3 ^{1}H-NMR Analysis

Protons of CH_2-3' and CH_2-5' formed a broad triplet at chemical shift 3.33 in the up field, having a coupling constant of 5.0.H-3'' proton at δ 7.68 and H-5'' proton at δ 7.77 give duplet in the spectrum's downfield region with a coupling constant value of 2.5. These are less shielded by the induced magnetic field due to coupling with the electron-withdrawing group that causes the deshielding and high-frequency results. Protons on carbon 4 and 5 give distorted duplets at a chemical shift value of 6.47 and 7.40. H-4 proton has a coupling constant of 3.5 and 1.8, whereas H-5 is observed with 1.6. A triplet with a coupling constant of 6.2 in the downfield region shows H-4'''' proton, similarly protons of H-5'''', H-6'''' having *δ* 7.25 and 7.56 value with a chemical shift of 6.3 & 7.5. An upfield singlet has three protons of CH_3-7'''' observed at a chemical shift of 2.24. A broad singlet was demonstrated by four protons of CH_2-2' CH_2-6' at a chemical shift value of 3.89. NH gives a broad singlet of one proton at a chemical shift of 8.37 in the downfield due to the electron-withdrawing group coupling.

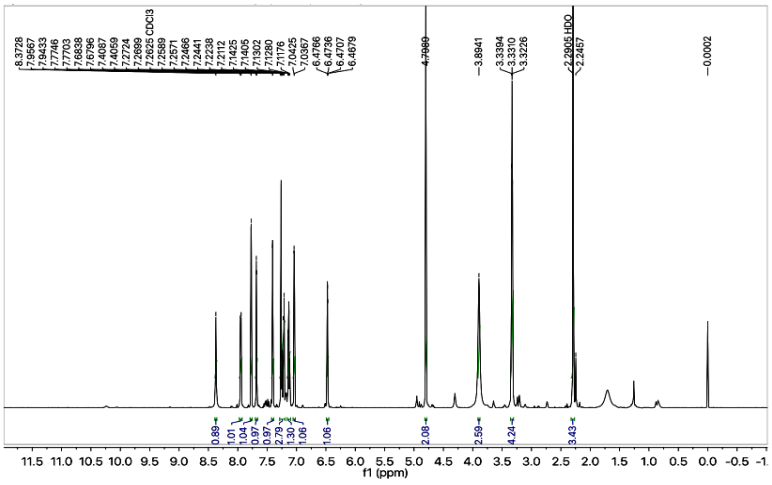

Figure 3a: ^{1}H-NMR spectrum of compound 7a.

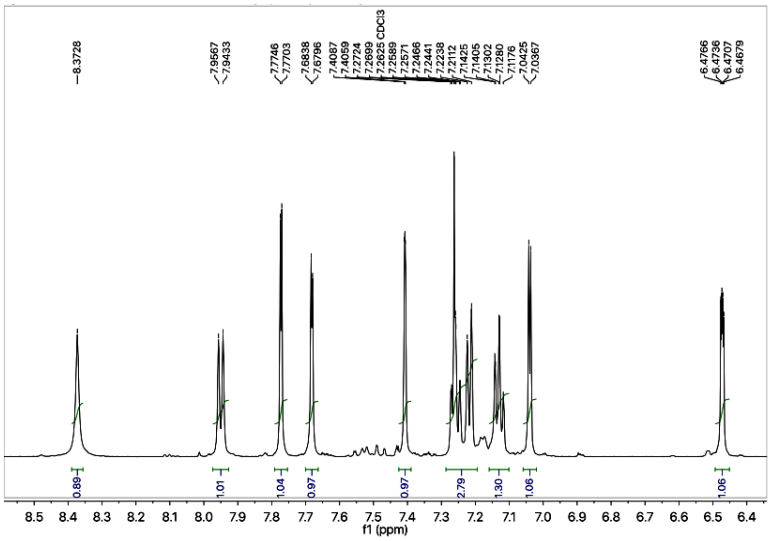

Figure 3b: Expanded whole aromatic region of the ^{1}H-NMR spectrum of compound 7a.

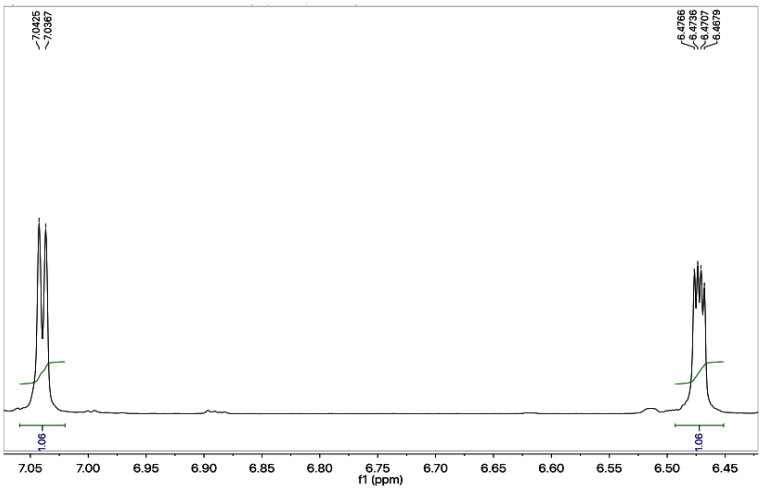

Figure 3c: Expanded aromatic part of a ^{1}H-NMR spectrum of compound 7a.

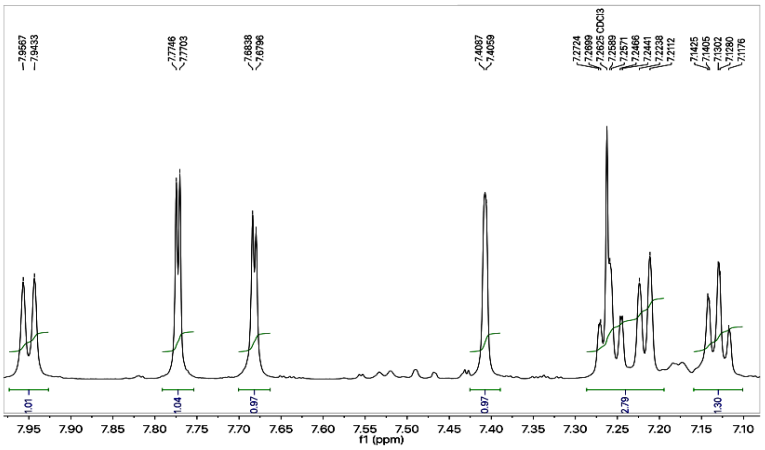

Figure 3d: Expanded downfield part of a ^{1}H-NMR spectrum of compound 7a.

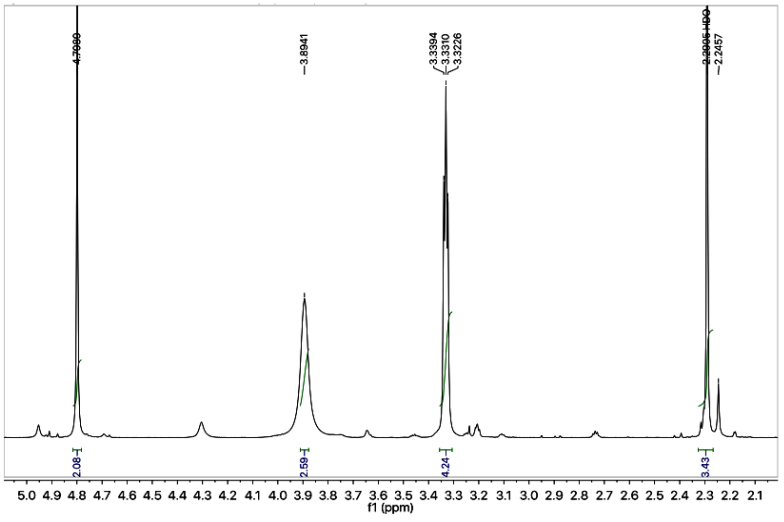

Figure 3e: Expanded downfield part of a ^{1}H-NMR spectrum of compound 7a.

5.4 C-NMR Spectral Analysis

There are twenty-one peaks of carbon demonstrated the resonance of several sets of symmetrical carbons that are present in the molecule, lower signal of carbon than original number of carbon atom in the molecular formula occur due to the same position in the molecule that resonate at the same value. Single signal at *δ value* 46.22 (C-2', C-3', C-5' and C-6'), confirmed the piperazine moiety, similarly the four signals of carbon 2, 3, 4, and 5 of the furoyl ring at chemical shift value 147.37, 117.63, 11.61 and 135.32 assured the presence of furoyl. Carbonyl carbon that linked the piperazine and furoyl found at 149.78. Six signals at 158.94 (C-1''), 122.74 (C-2''), 134.80 (C-3''), 125.61 (C-4''), 134.18 (C-5''), and 126.96 (C-6''), confirmed the presence of 2,4-Dichloro-2-oxyphenyl ring. Amide carbon signal found at 164.87 (C-1'''). Seven signals at 131.38 (C-1''''), 135.32 (C-2''''), 130.84 (C-3''''), 130.63 (C-4''''), 128.88 (C-5''''), 129.42 (C-6''''), and 17.69 (C-7'''') corroborated the presence of methylphenyl ring.

5.5 EI-MS Analysis

The peak at *m/z* of 179 justified the furoyl and piperazine ion component. The molecular ion peak at 403 and 148 represents the fragmentation between the C-O bond of amide and 3,5-dichloro-2-oxyphenyl ring that gives molecular ion formulas such as $[C_{15}H_{14}Cl_2N_2O_5S]^+$ and $[C_9H_{10}NO]^+$. The *m/z* peak at 372 and 179 represents the bond breaking between the S-N bond represented by molecular ions like $[C_{15}H_{12}Cl_2NO_4S]^+$ and $[C_9H_{11}N_2O_2]^+$. The molecular ion peak at 134 represents a fragment that contains methyl phenyl acetamide moiety $[C_8H_8NO]^+$. An m/z peak confirmed a fragment of 2-methyl aniline at 106 $[C_7H_8N]^+$. An m/z peak of 95 confirmed the furoyl fragment $[C_5H_3O_2]^+$.

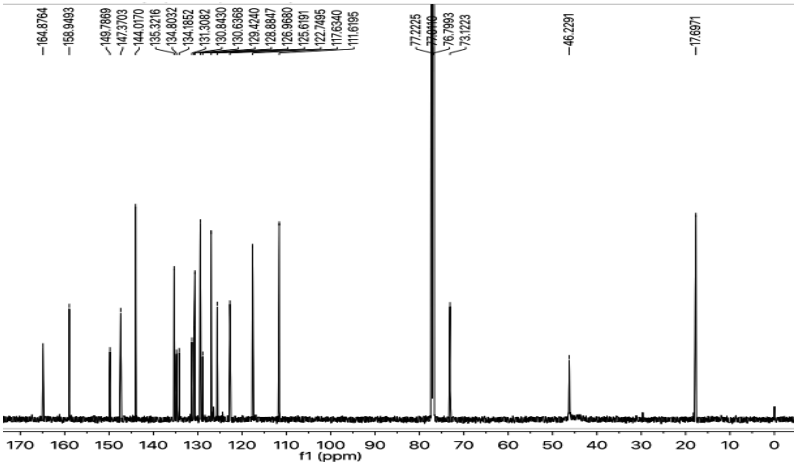

Figure 4: ^{13}C-NMR spectrum of compound 7a.

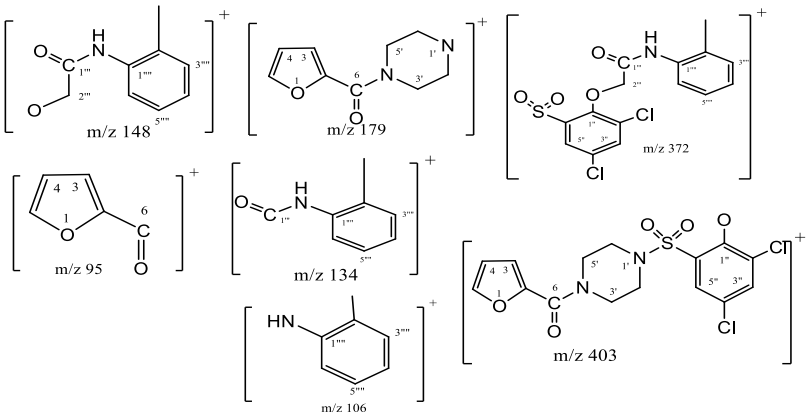

Figure 5a: Fragmentation of 2-(2,4-Dichloro-6-{[4-(2-furoyl)1 piperazinyl]sulfonyl}phen oxy)-*N*-(2-methylphenyl) acetamide(7a).

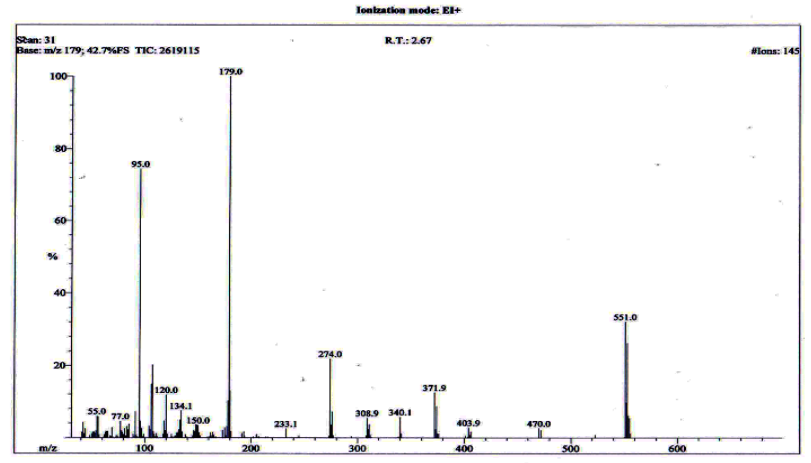

Figure 5b: EI-MS spectrum of compound 7a.

2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2,6-dimethylphenyl)acetamide (7b)

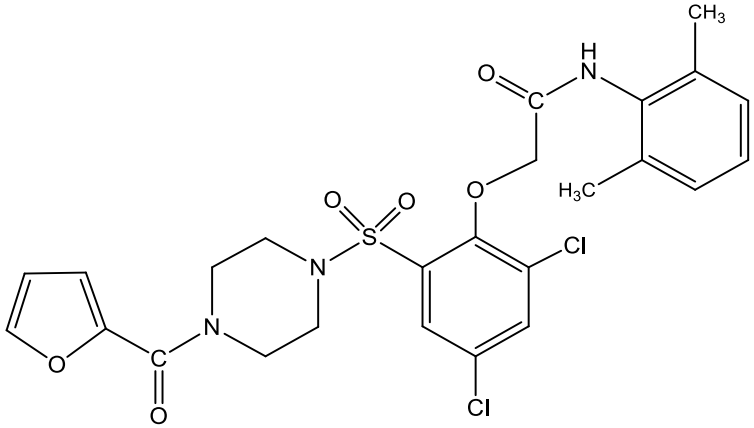

Figure 6: Structure of 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(2,6-dimethylphenyl)acetamide (7b).

5.6 H-NMR Spectral Analysis

Protons of CH_2-3' and CH_2-5' formed a broad triplet at chemical shift 3.36 in the up field, having a coupling constant of 5.1. The single proton ofH-3'' at δ 7.69 and H-5'' proton at δ 7.79 gives a duplet in the downfield region of the spectrum with a coupling constant value of 2.4 & 2.5. These are less shielded by the induced magnetic field due to coupling with the electron-withdrawing group that causes the deshielding and high-frequency results. Single protons on carbon 4 and 5 give distorted duplets at a chemical shift value of 6.47 and 7.41. H-4 proton has a coupling constant of 3.4 and 1.7, whereas H-5 is observed with 1.2. A triplet with a coupling constant of 6.2 in the downfield region shows H-4'''' proton, similarly protons of H-5'''', H-6'''' having δ 7.25 and 7.56 value with a chemical shift of 6.3 & 7.5. An up field singlet has three protons of CH_3-7'''' observed at a chemical shift of 2.24, and a broad singlet was demonstrated by four protons of CH_2-2' CH_2-6' at a chemical shift value of 3.91. NH proton gives one proton a broad singlet at a chemical shift of 8.05 in the downfield due to the electron-withdrawing group coupling. A multiplet of H-3'''' to H-5'''' was shown by the three protons at chemical shift values 7.17-7.11 in the spectrum's downfield region. An up field multiplet of CH_3-7'''' and CH_3-8'''' is given due to six protons in each neighboring at a chemical shift at 2.28.

5.7 IR Spectral Analysis

To ensure the functional groups of the targeted compound, a molecule of 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(2,6-dimethyl phenyl)acetamide, further subjected to IR analysis to clarify the synthesis. In the IR spectrum, characteristic peaks found at 3408 (N-H) amide, 1197 (C-O-C),3080 (Aromatic C-H) of benzene, 2883 (R C-H), 1655 (C=O) carbonyl groups, 1587 (Ar C=C), 1381 (S=O) and 1116 (C-N-C) that represents the functional groups of the compounds 2-furoyl-1-piperazine, acetamide, and sulfonamide.

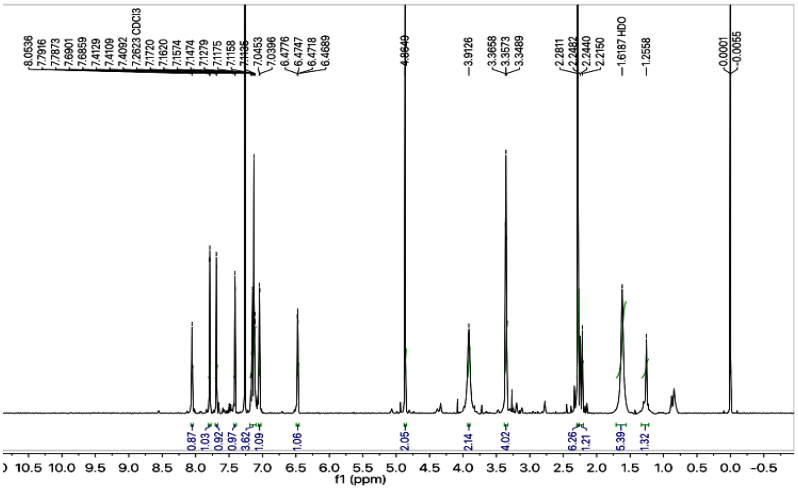

Figure 7a: ^{1}H-NMR spectrum of compound 7b.

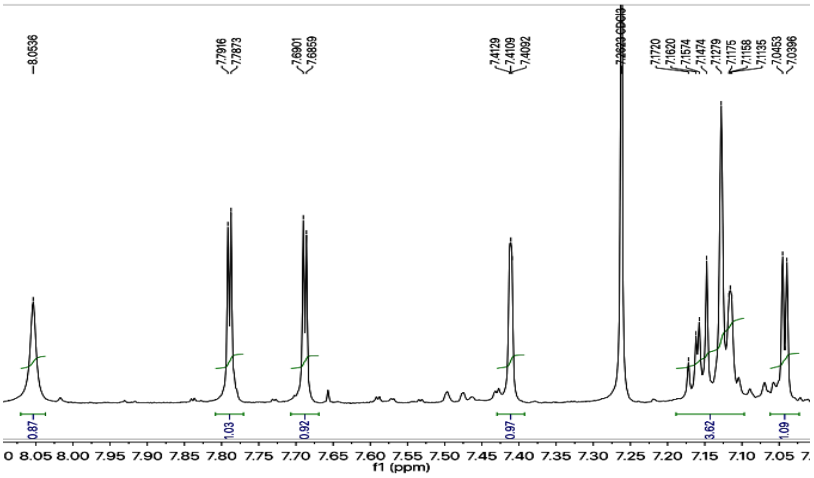

Figure 7b: Expanded aromatic region of the ^{1}H-NMR spectrum of compound 7b.

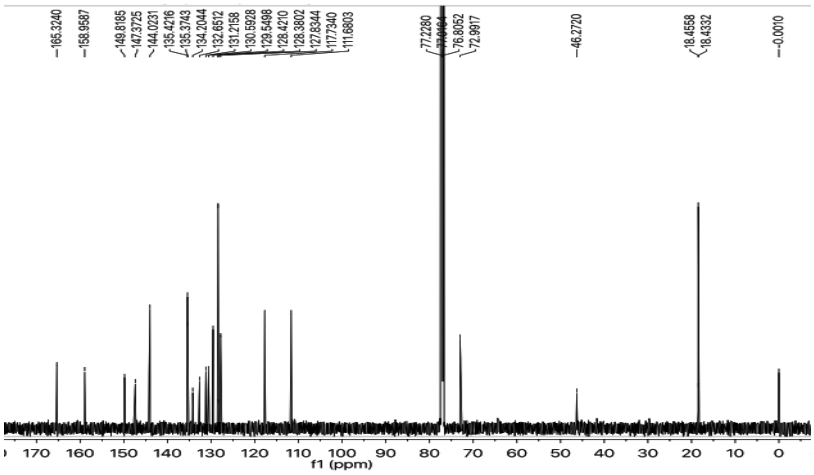

Figure 8: ^{13}C-NMR spectrum of compound 7b

5.8 EI-MS Analysis

The peak at *m/z* of 179 justified the furoyl and piperazine ion fragment. The molecular ion peak at 403 and 162 represents the fragmentation between the C-O bond of amide and 3,5-Dichloro-2-oxyphenyl ring that gives molecular ion formulas such as $[C_{15}H_{14}Cl_2N_2O_5S]^+$ and $[C_{10}H_{12}NO]^+$. The *m/z* peak at 386 and 179 represents the bond breaking between the S-N bond that forms the molecular ion like $[C_{16}H_{14}Cl_2NO_4S]^+$ and $[C_9H_{11}N_2O_2]^+$. The molecular ion peak at 148 represents a fragment that contains dimethyl phenyl acetamide moiety $[C_9H_{10}NO]^+$. An m/z peak confirmed a fragment of 2,6-dimethyl phenyl formamide at 120 $[C_8H_{12}N]^+$. An *m/z* peak of 95 confirmed the furoyl fragment $[C_5H_3O_2]^+$.

2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-N-(2-methoxycarbonylphenyl)acetamide (7c)

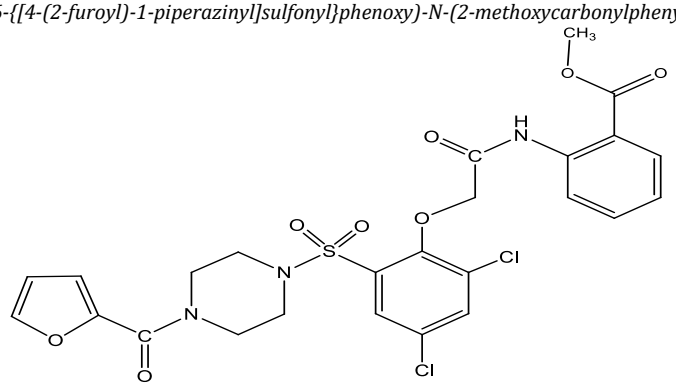

Figure 9: 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(2-methoxycarbonylphenyl) acetamide (7c).

5.9 IR Spectral Analysis

To ensure the functional groups of the targeted compound in the molecule of 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl] sulfonyl}phenoxy)-*N*-(2-methoxycarbonylphenyl) acetamide, it is further subjected to IR analysis to clarify the synthesis of the desired compound. During the analysis of the IR spectrum, distinctive peaks were found in the region of wavenumber (cm^{-1}) 3412 (N-H), 3089 (Aromatic C-H) of benzene, 2881 (R C-H), 1196 (C-O-C), 1655 (C=O) carbonyl groups, 1587 (Ar C=C), 1388 (S=O), and 1111 (C-N-C) which ensure the involvement of functional groups containing compounds like acetamide, 2-furoyl-1-piperazine and sulfonamide moieties.

5.10 ^{1}H-NMR Spectral Analysis

Four protons of CH_2-3' and CH_2-5' formed a broad triplet at a chemical shift value of 3.46 in the up filed region of the spectrum, having a coupling constant of 5.1 Hz. The single proton of H-3'' at δ 7.63 and H-5'' proton at δ 7.78 gives a duplet in the downfield region of the spectrum that has a coupling constant value of 2.7 & 2.8. These are less shielded by the induced magnetic field due to coupling with the electron-withdrawing group that causes the deshielding and high-frequency results. Single protons on H-5 show a broad singlet at a chemical shift value of 7.49. H-4 proton has a coupling constant of 3.5 and 1.7, forming a distorted duplet at a chemical shift of 6.53. A multiplet of two protons observed at δ 7.30-7.14 in the downfield region shows H-4'''' and H-5'''' proton similarly single protons of H-3'''', H-6'''' having δ 8.11 and 8.75 value have coupling constant 7.7 and 8.5 Hz have a duplet in the less shielded region of the spectrum. An upfield singlet has three protons of CH_3-7'''' observed at a chemical shift of 3.95, and a broad singlet was demonstrated by four protons of CH_2-2' CH_2-6' at a chemical shift value of 3.91. NH proton gives one proton a broad singlet at a chemical shift of 9.06 in the downfield due to the electron-withdrawing group coupling. Two protons of CH_2-2''' have a singlet at a chemical shift of 4.94.

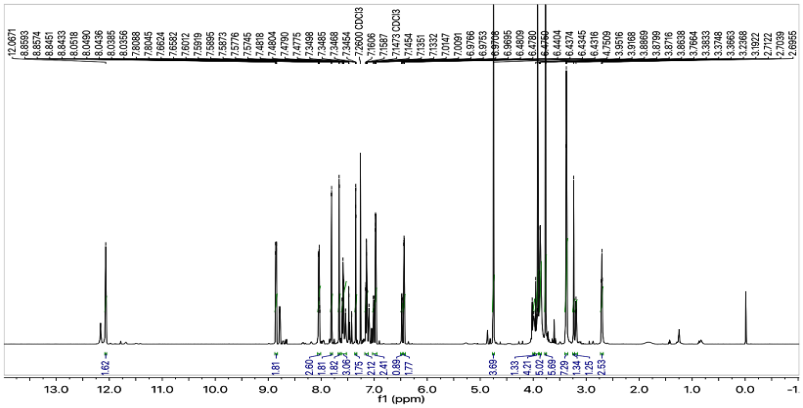

Figure 10 a: A ^{1}H-NMR spectrum of compound 7c.

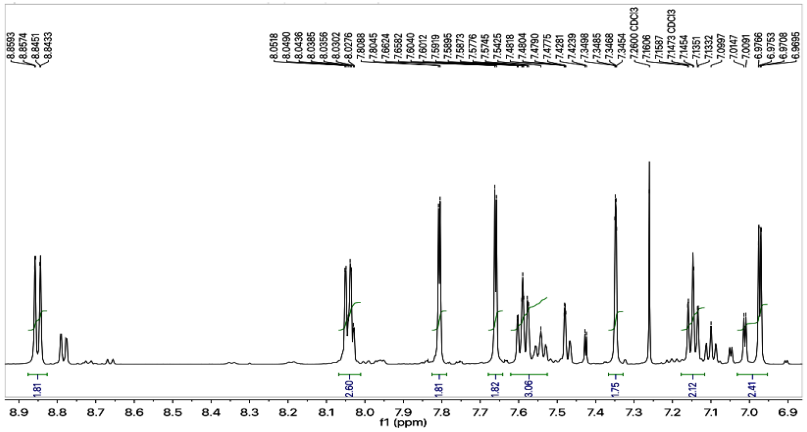

Figure 10b: Expanded aromatic region of the ^{1}H-NMR spectrum of compound 7c.

5.11 C-NMR Spectral Analysis

There are twenty-two peaks of carbon atoms in the ^{13}C-NMR spectrum that shows the resonance behavior of several sets of symmetrical carbons that are present in the molecule, lower signal of carbon than original number of carbon atom in the molecular formula occur due to the same position in the molecule that resonate with the same value. Single signal at δ *value* 46.49 (C-2', C-3', C-5' and C-6'), confirmed the piperazine moiety, similarly the five signals of carbon 2, 3, 4, 5 and 6 of the furoyl ring at chemical shift value 147.37, 117.62, 111.81, 144.31 and 151.74 assured the presence of furoyl moiety. Carbonyl carbon (C-6) that linked the piperazine and furoyl found at 151.74. Six signals at 158.44 (C-1''), 122.74 (C-2''), 134.85 (C-3''), 124.14 (C-4''), 134.68 (C-5''), and 126.06 (C-6''), confirmed the presence of 2,4-dichloro-2-oxyphenyl ring. Amide carbon signal found at 164.80 (C-1'''). Five signals at 135.48 (C-1''''), 120.29 (C-2''''), 127.95 (C-3''''), 126.56 (C-4''''), 133.83 (C-5''''), 123.51 (C-6''''), 174.40 (C-7''''), and 52.46 (C-8''''), corroborated the presence of di-substituted phenyl ring. A carbon signal at chemical shift 72.90 (C-2'''), justify the methyl carbon of the ester linkage

5.12 EI-MS Spectral Analysis

The peak at *m/z* of 179 justified the component of 2-Furoyl-1-piperazine $[C_9H_{11}N_2O_2]^+$. The molecular ion peak at 403 and 192 represents the fragmentation between the C-O bond of amide and 3,5-dichloro-2-oxyphenyl ring that gives molecular ion formulas such as $[C_{15}H_{14}Cl_2N_2O_5S]^+$ and $[C_{10}H_{10}NO_3]^+$. The *m/z* peak at 416 and 179 represents the bond breaking between the S-N bond that forms the molecular ion like $[C_{16}H_{12}Cl_2NO_6S]^+$ and $[C_9H_{11}N_2O_2]^+$. The molecular ion peak at 178 represents a fragment that contains methoxycarbonylphenyl acetamide moiety $[C_9H_8NO_3]^+$. An m/z peak confirmed a fragment of methoxycarbonyl phenyl formamide at 150 $[C_8H_8NO_2]^+$. An *m/z* peak of 95 ensures the furoyl fragment $[C_5H_3O_2]^+$.

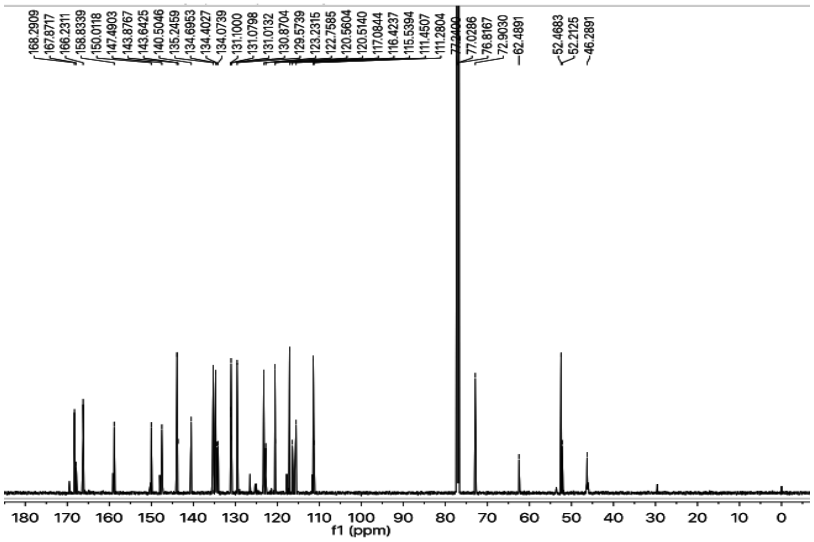

Figure 11: A^{13}C-NMR spectrum of compound 7c.

6. Biochemistry

6.1 Enzyme Inhibition Activity

The compound 7a showed effective inhibitory potential toward the *a*-glucosidase enzyme, and 7b & 7c observed less inhibitory potential toward the *a*-glucosidase. While compound 7b was observed to be more potent against both the AChE and BChE enzymes may be due to the dimethyl phenyl ring involvement. 7ais less potent against AChE and has the least inhibitory potential for BChE regarding the standard control. A similar case occurs in the case of 7c, and it is less active for BChE and least active for the AChE enzyme.

6.2 Hemolytic Activity

To determine the cytotoxicity of the synthesized **7a-c** compounds were subjected to a hemolysis assay. The outcome results of the % inhibition of the compounds are shown in the table below. These compounds revealed a moderate rate of toxicity to the membrane of the red blood cell. The highest toxicity rate demonstrated by the **7a** is 46.34%, but this value is lower than the positive control (Triton-X-100), while 7c has the most negligible toxicity effect, 7.58, but higher than the PBS-0.09 on the red blood cell membrane. Based on increased toxicity, these can be arranged in the order of; **7a > 7b > 7c.** Bioactivity studies of 2-(2,4-Dichloro-6-{[4-(2-furoyl)-1-piperazinyl]sulfonyl}phenoxy)-*N*-(aryl)acetamides(**7a-c**).

Table 1: All compounds were dissolved in methanol, and experiments were performed in triplicate (mean±SEM, n=3). a = Acarbos, b = Eserine, AChE= Acetylcholinesterase, BChE =Butyrylcholinesterase.

Compounds	Enzymatic analysis						Hemolytic activity
	α-Glucosidase		AChE		BChE		
	Inhibition (%)	IC_{50} (*μ*M)	Inhibition (%)	IC_{50} (*μ*M)	Inhibition (%)	IC_{50} (*μ*M)	(%)
7a	7.12±0.18	-	37.82±0.25	-	48.33±0.25	-	46.34
7b	96.52±0.24	332.12±0.12	29.83±0.28	-	21.44±0.28	-	40.55
7c	87.95±0.23	359.86±0.12	43.68±0.23	-	32.56±0.13	-	7.58
Control	**92.23±0.14**^{a}	**38.25±0.12**^{a}	**91.27±1.17**^{b}	**0.04±0.0001**^{b}	**82.82±1.09**^{b}	**0.85±0.0001**^{b}	
PBS							**0.09**
Triton							**100**

7. Conclusion

It was observed from the Hemolytic assay and enzyme inhibition activity results that the synthesized derivatives of acetamides compounds that were authenticated by the IR, ^{13}C-NMR, and ^{1}H-NMR spectral analysis techniques could behave as valuable inhibition against the *a*-glucosidase, AChE, and BChE enzymes. These have mild cytotoxicity and are effective against microbes. These compounds could be utilized as a therapeutic substance in the pharmaceutical industry for human beings welfare.

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