SYNTHESIS, CHARACTERIZATION AND IN-VITRO ANTIMICROBIAL AND ANTICANCER EVALUATION OF PYRAZOLE DERIVATIVE

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Abstract: *A unique synthetic pathway consisting of many steps was devised for the synthesis of a range of pyrazole derivatives, which were subsequently characterised based on their chemical structures. The experimental procedure involved a series of sequential reactions utilising Meldrum acid, HATU, and DIPEA. These reactions led to the synthesis of a range of compounds exhibiting distinct characteristics and demonstrating promising prospects for utilisation within the domain of organic chemistry. This study presents a comprehensive dataset comprising detailed spectral and biological examination of ten chemicals, denoted as B6 to B10. The compounds in question have been subjected to characterization through the utilisation of infrared spectroscopy (IR), nuclear magnetic resonance (NMR), and mass spectrometry techniques. Additionally, their antibacterial and anticancer properties have been assessed. The nuclear magnetic resonance (NMR) spectra of compounds B6 to B10 were analysed. The nuclear magnetic resonance (NMR) spectra of each substance exhibit the chemical shifts of different proton and carbon signals. Theoretical and actual mass spectra are shown for each molecule. The measured masses exhibit a high degree of proximity to the corresponding theoretical masses, hence suggesting the precise synthesis of the compounds. The antibacterial properties of compounds B6 to B10 were assessed against Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Aspergillus Niger. The findings are presented in the tabular format, displaying the zone of inhibition measurements in millimetres. The present study aimed to assess the impact of vitamin B6 to B10 on the MCF-7 cell line, which is commonly associated with breast cancer, utilising the MTT assay technique. The obtained outcomes encompass measurements of concentration, absorbance, percentage of inhibition, and IC50 values.*

Keyword: Synthesis of pyrazole, IR, NMR, Mass spectroscopy, Anti-inflammatory, Anticancer activity

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INTRODUCTION

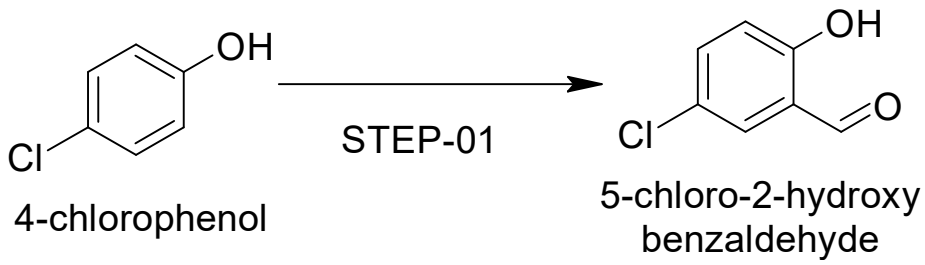
Exploring the world of pharmaceuticals with the help of basic chemistry concepts is the exciting and vital field of pharmaceutical chemistry. It explores their production, chemical makeup, compositions, architectures, and significant effects on living things. In-depth research on the chemical and physical characteristics of medications, quality control procedures, and storage requirements are all included in the field of pharmaceutical chemistry. Within the broad field of pharmaceutical sciences, it is a fundamental element that is deeply entwined with disciplines such as drug technology, toxicological chemistry, pharmacognosy, and pharmacy organisation. Pharmaceutical chemistry is fundamentally derived from the combination of medico-biological fields (pharmacology, physiology, and biological chemistry) with chemical sciences (inorganic, organic, analytical, physical, and colloidal chemistry).The science of pharmaceuticals made significant advancements in the 16th century, which is when pharmaceutical chemistry first emerged[1-3]. Medicinal chemistry was its baby, born out of the ever-growing hope that biochemical discoveries would unlock the door to drug discovery. Pharmacists have played a crucial part in the development and history of pharmaceutical chemistry. In the 19th century, synthetic molecules discovered a role in modifying the path of biological processes, marking a pivotal event in the history of this field. The development of therapeutic substances with a specific goal was made possible by advances

like the introduction of ether and chloroform for anaesthesia. Among these trailblazers, phenacetin is a monument, one of the first medications designed with the knowledge of biochemical changes in mind. Chemotherapeutic chemicals participate in chemical reactions with cell receptor sites—a basic idea clarified by Ehrlich. Furthermore, he made the hypothesis—supported by experimental data—that drug resistance developed when the parasite was unable to absorb the medication. Throughout the course of pharmaceutical chemistry, drug molecule modification has become a vital tactic for maximising the effects of pharmacological substances. Drug discovery still depends heavily on this procedure. Pharmaceutical chemistry gains from physicochemical insights that direct drug development by embracing the emergence of sophisticated analytical techniques like X-ray analysis, UV, IR, and NMR. Furthermore, at the cutting edge of biochemistry, the deep comprehension of drug-receptor interactions along with advances in enzymology and pharmacokinetics have enabled medicinal chemists to conjecture the complex processes of drug action. [3-6] In the fields of chemistry and pharmacology, heterocyclic molecules are essential. Based on their electrical arrangement, they can be further divided into aliphatic and aromatic types and further classified according to their structure. These heterogeneous molecules have a broad range of applications and are essential to the drug discovery process. This study examines the categorization and importance of heterocyclic chemicals, with a particular emphasis on flavonoids and benzopyrans, illuminating their prevalence in nature and possible therapeutic effects. [7-10].This study examines the synthesis and chemistry of pyrazolines and pyrazoles, heterocyclic chemical molecules with a variety of biological functions. This work explores the synthesis and different derivatives of pyrazoles, which are five-membered ring structures having two nitrogen and three carbon atoms. It also looks at the structural alterations and biological processes connected to pyrazolines—hydrogenated versions of pyrazoles. According to the study, these chemicals have anti-inflammatory, anti-cancer, and anti-microbial properties.[11–15]

MATERIALS

The required chemicals are purchased from local chemical suppliers of Merck or Cosmo Chem Pvt. Ltd.

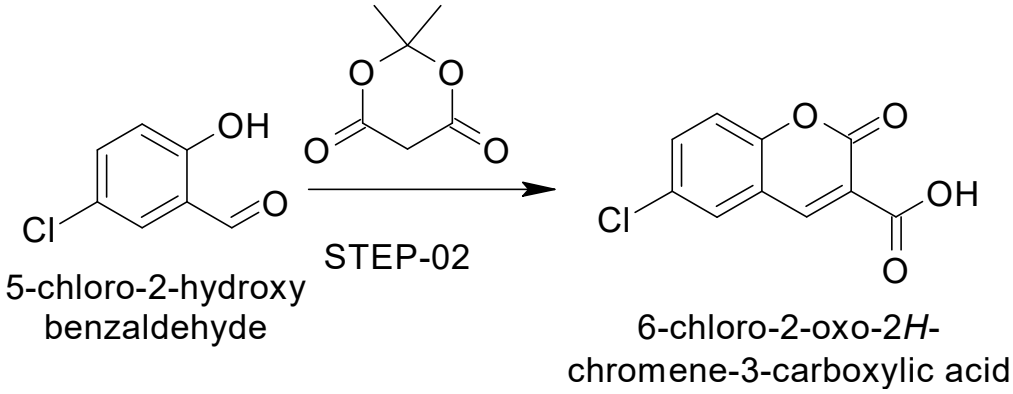
Synthesis & Characterization
Scheme
Step-01:



Compound A

1. To a stirred solution phenol 2.45g (1eq) in TFA (15mL) wad added HMTA (2.15g, 1.03eq) at 0°C.
2. Then reaction mixture was stirred at 80°c for 16h. Progress of the reaction was monitored by TLC (5% ethyl acetate in hexane).
3. After completion of starting material cool the reaction mixture, add crushed ice to it and then extracted with DCM 250 mL*3.
4. The organic layer was dried over sodium sulphate and concentrated under vacuum to afford crude material 2.5g which was purified by column chromatography silica 100-200 mesh using 5 to 10 % ethyl acetate in hexane as an eluent to afford 2 gm desired product as an light pink solid.

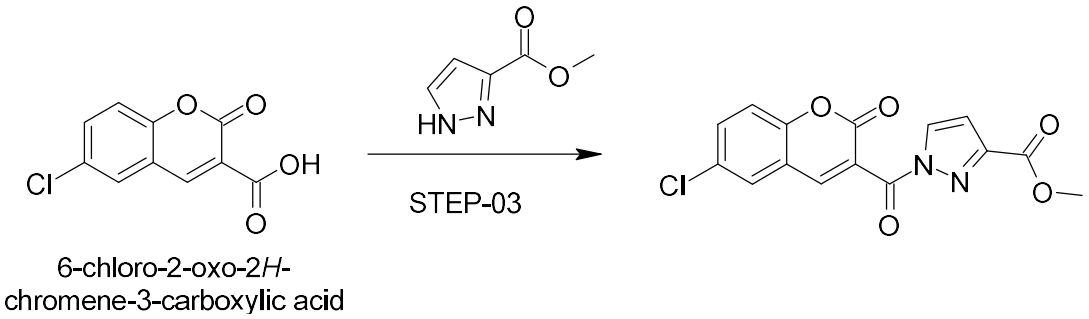
Step 02:



Compound B

1. To a stirred solution of step-01 product (1eq) in water was added Meldrum acid (1 eq) at 0°C followed by addition of 20% aq K2CO3 (0.1 eq).
2. The reaction mixture was stirred at rt for 20h vigorously.
3. Progress of the reaction was monitored by TLC.
4. After completion of starting material reaction mixture was acidified with 2N HCl there is off white solid precipitation, filter it and washed with cold water, dry it and recrystallized with ethyl acetate to afford white solid which is as such used for next reaction.

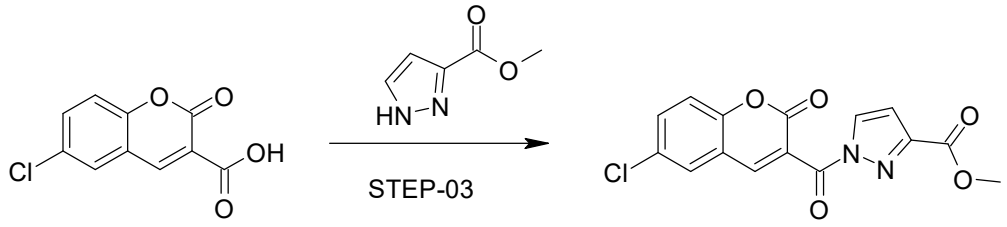
Step 03



Compound C

1. To a stirred solution of step-02 product (1eq) in DMF wad added HATU (1.5 eq) at 0°C followed by addition of DIPEA (3.0 eq).
2. After 10 min add pyrazole (1.2eq).
3. The reaction mixture was stirred at RT for 16h.
4. Progress of the reaction was monitored by TLC. After completion of starting material add crushed ice to it and then extracted with DCM 250 mL*3.
5. The organic layer was dried over sodium sulphate and concentrated under vacuum to afford crude material 2.5g which was purified by column chromatography silica 100-200 mesh using 5 to 10 % Methanol in DCM as an eluent to afford desired product as an off white solid.

Step 04

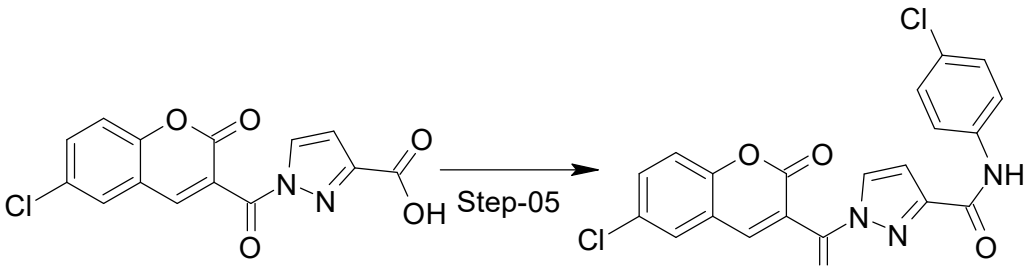


6-chloro-2-oxo-2*H*-chromene-3-carboxylic acid

methyl 1-(6-chloro-2-oxo-2*H*-1-benzopyran-3-carbonyl)-1*H*-pyrazole-3-carboxylate

1. In this step methyl 1-(6-chloro-2-oxo-2H-1-benzopyran-3-carbonyl)- 1H-pyrazole-3-carboxylate is reacted with lithium hydroxide to proceed for hydrolysis.

Compound D
Step 05



1-(6-chloro-2-oxo-2*H*-chromene-3-carbonyl) -1*H*-pyrazole-3-carboxylic acid

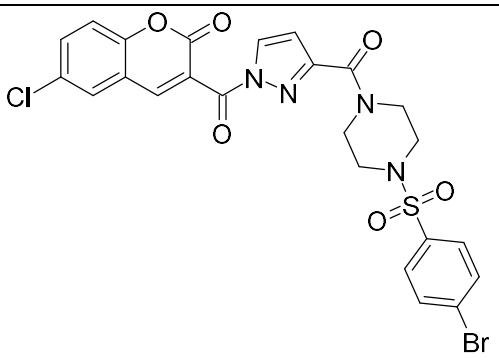
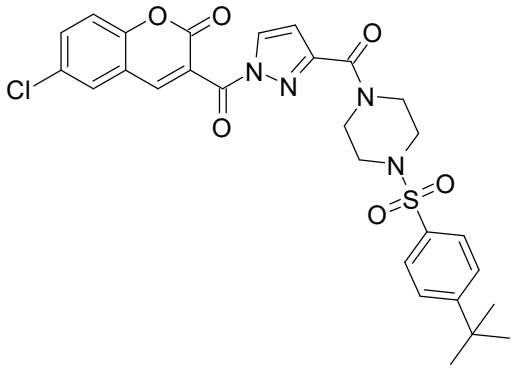
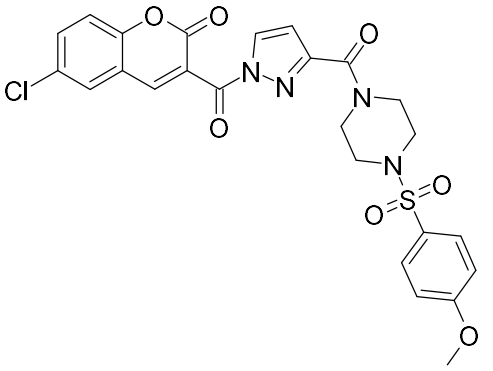
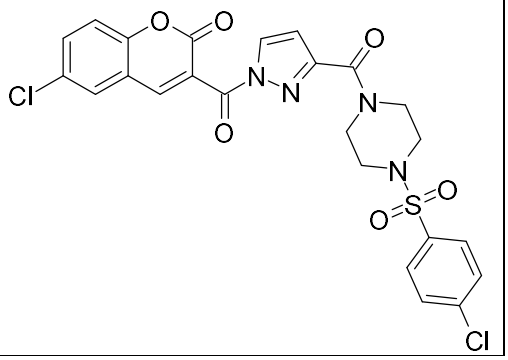
Target

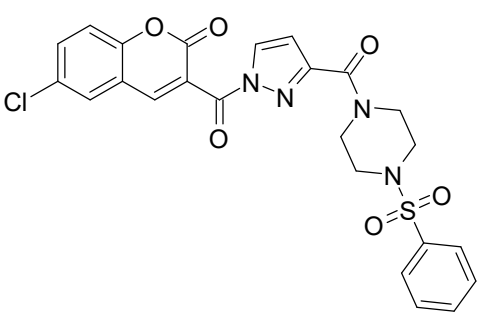
Compound E

1. To a stirred solution of acid (1.2 eq) in DMF wad added HATU (1.5 eq) at 0°C followed by addition of DIPEA (3.0 eq).
2. After 10 min add step-04 product (1eq).
3. The reaction mixture was stirred at RT for 16h.
4. Progress of the reaction was monitored by TLC.
5. After completion of starting material add crushed ice to it and then extracted with DCM 200 mL*3.
6. The organic layer was dried over sodium sulphate and concentrated under vacuum to afford crude material which was purified by column chromatography silica 100-200 mesh using 3 to 10 % Methanol in DCM as an eluent to afford desired product as an off white solid to light brown solid.

Table 1: Chemical Structures of Pyrazole Derivatives

Sr. no.	Compound Number	Molecular Formula	Molecular Weight	Chemical Structure

1.	**B6**	$C_{21}H_{11}Cl3N_3O_4$	605.84	
2.	**B7**	$C_{28}H_{27}ClN_4O_6S$	583.05	
3.	**B8**	$C_{25}H_{21}ClN_4O_7S$	556.97	
4.	**B9**	$C_{24}H_{18}Cl2N_4O_6S$	561.39	

5.	**B10**	$C_{24}H_{19}ClN_4O_6S$	526.94	

Characterization of synthesized compounds
Physical Characterization data of synthesized compounds
Spectral analysis of Pyrazole derivatives

a) Infrared Spectroscopy[16]

FT-IR Spectroscopy is a technique applied to determination of the interaction between an IR radiation and a sample that can be solid, liquid or gaseous. It measures the frequencies at which the sample absorbs, and also the intensities of these absorptions. The frequencies are helpful for the identification of the chemical functional groups that are responsible for the absorption of radiation at different frequencies. The concentration of component can be determined based on the intensity of the absorption. The 14 spectrum is a two-dimensional plot in which the axes are represented by intensity and frequency of sample absorption. Because all compounds show characteristic absorption/emission in the IR spectral region and based on this property they can be analyzed both quantitatively and qualitatively using FT-IR spectroscopy.

b) Nuclear Magnetic Resonance Spectroscopy (NMR)[17]

To characterize a molecule by NMR spectroscopy, record the difference in the magnetic properties of various magnetic nuclei present within the molecule. NMR is non-destructive method, with the help of new instruments may use less than one milligram of sample. Spectroscopy has made a wide spread tool in chemistry for the study of chemical structure.

c) Mass spectroscopic analysis[18]

Mass spectrometry is a powerful analytical technique used to quantify known materials, to identify unknown compounds within a sample, and to elucidate the structure and chemical properties of different molecules. The complete process involves the conversion of the sample into gaseous ions, with or without fragmentation, which are then characterized by their mass to charge ratios (m/z) and relative abundances.

d) Antimicrobial activity [19-21]

1. All the compounds synthesized (B1-B10) in the present investigation were screened for their anti-bacterial activity by subjecting the compounds to standard procedures.
2. Antibacterial activities were tested on nutrient medium against Aspergillus Niger, Staphylococcus aureus, Bacillus subtilis, Escherichia Coli that are representative types of gram positive and gram-negative organisms respectively.
3. The antibacterial activity of the compounds was assessed by well-diffusion method.
4. The inoculum of the microorganism was prepared from the bacterial cultures.
5. 15ml of nutrient agar (Hi media) medium was poured in clean sterilized Petri plates and allowed to cool and solidify.
6. Bacterial strain was spread over the medium evenly with a spreading rod.

7. Wells of 6mm in diameter were bored using a sterile cork borer. Solutions of all the compounds (5, 10µg/ml) in DMSO were prepared.
8. 5, 10µg/ml of solutions was added to the wells.
9. The petri plates incubated at 37^0C for 24 h. Amoxicillin (1mg/ml) was prepared as a positive control DMSO was taken as negative control.
10. Antibacterial activity was evaluated by measuring the diameters of the zone of inhibitions (ZI) all the determination was performed in triplicates

e) In-vitro Anticancer activity[22]

The MTT/MTS in vitro cell proliferation assay is one of the most widely used assays for evaluating preliminary anticancer activity of both synthetic derivatives and natural products and natural product extracts. The highly reliable, colorimetric based assay is readily performed on a wide range of cell lines. This assay gives an indication of whole cell cytotoxicity; however, to determine the exact molecular target further assays need to be performed. Of these, kinase inhibition assays are also one of the most widespread enzyme inhibition screening assays performed. Kinases are enzymes that play a key role in a number of physiological processes and their inhibitors have been found to exhibit anticancer activity against various human cancer cell lines. Herein, we describe the methods for performing both in vitro MTT/MTS cytotoxicity and kinase enzyme inhibition assays. These are two of the most useful anticancer screening techniques available that are relatively economical and can be easily and routinely performed in the laboratory to characterize anticancer activity. Both assays are highly versatile and can be modified to test against targeted disease processes by using specific kinase enzymes or cell lines.

MTT Assay Method

Principle of Assay

The assay is dependent on the reduction of the tetrazolium salt MTT (3-(4, 5-dimethylthazol-2-yl)-2,5-diphenyl tetrazolium bromide) by the mitochondrial dehydrogenase of viable cells to form a blue formazan product. The cells are then solubilized with an organic solvent (eg. Isopropanol, dimethylsulfoxide) and the released, solubilized formazan reagent is measured spectrophotometrically.

Chemical reagents

The chemicals used in the present work were AR grade and LR grade, purchased from Ranbaxy, Merck, S.D. Fine chemicals and Research Lab and used as received. The list of chemical used were Trypsin, Trypan blue, MTT salt, DMSO, methanol, ethanol.

Cell line used: MCF 7 (human cervical carcinoma)

All cell lines were grown and maintained in suitable (MEM-media and were grown and sub cultured in medium supplemented with 10% fetal bovine serum,1% L-Glutamine.1% penicillin streptomycin – streptomycin-amphotericine-B antibiotic solution. All cells were trypsinated using trypsin-EDTA solution and seeded in96 well plates.

Procedure

1. The MCF-7 cell line was maintained in MEM medium supplemented with 10 % fetal bovine serum.
2. The cells were plated at a density of 1×10^5 cells per well in a 96-well plate, and cultured for 24 h at 37 °C.
3. The cells were subsequently exposed to 10, 40 and 100µg/ml.
4. The plates were incubated for 24 h, and cell proliferation was measured by adding 10 µL of MTT (thiazolyl blue tetrazolium bromide) dye (5 mg ml^{-1} in phosphate-buffered saline) per well.
5. The plates were incubated for a further 4 h at 37°C in a humidified chamber containing 5% CO^2.
6. Formazan crystals formed due to reduction of dye by viable cells in each well were dissolved in 200µl DMSO, and absorbance was read at 490 nm.
7. The results were compared with the standard drug inhibitors Seliciclib. (10, 50 and 100µg/ml.)

Percent Cytotoxicity = Reading of control - Reading of treated cells / Reading of control X 100

f) Anti-inflammatory study [23]

Procedure

1. Wistar rats were divided into 12 groups, with 3 rats in each.
2. The first group was inflamed by carrageenan injection and did not undergo any treatment.
3. The inflammation of the second group, used as reference, and that of the third group was treated by a topically dermal application with Diclofam gel (2 mg/paw) and test item (2 mg/paw), respectively, 1/2 hour before the carrageenan injection.
4. The doses Test item and Diclofam chosen during treatments were proportional in the size of the edema and covered the whole swelling.
5. Test item and Diclofam were applied to the plantar surface of the hind paw by gentle rubbing 50 times with one's index finger.
6. In all treated groups, the size of the edema was measured before and after the inflammatory injection using a digital caliper.
7. Edema was expressed as the relative increase in paw volume induced by the inflammation injection (i.e., the edema was proportional to the volume difference between 0 hours and the other times, 30 min, 60, 90, 120, and 150 minutes after carrageenan injection).
8. Percentile edema inhibition was calculated according to following formula:

Percentile inhibition =[1-(VT/V0)] X 100

Where;

VT represents the edema volume in the drug treated group.

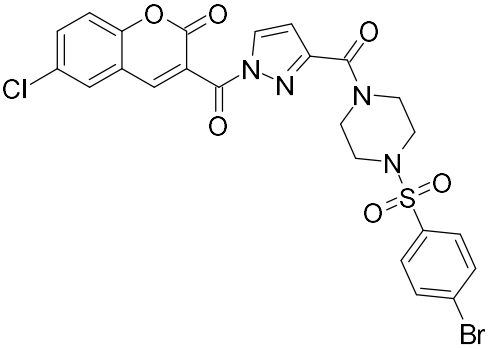
V0 represents the edema volume in the Carr group

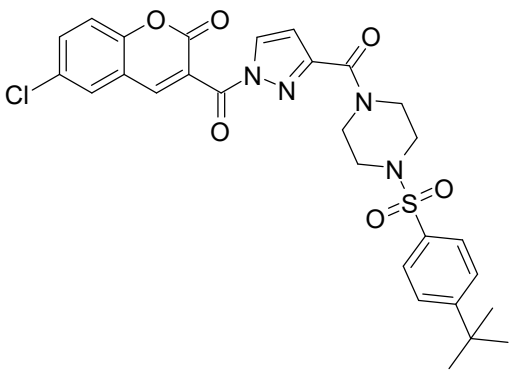
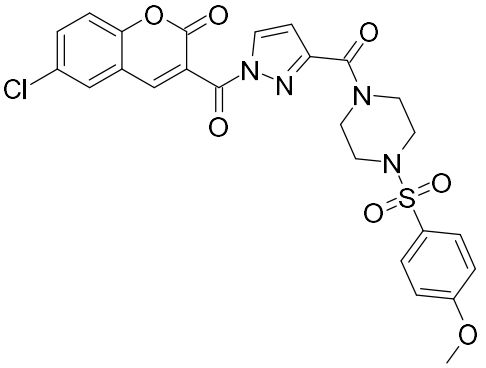
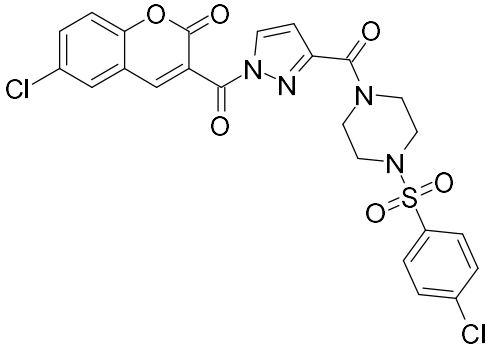
RESULTS AND DISCUSSION

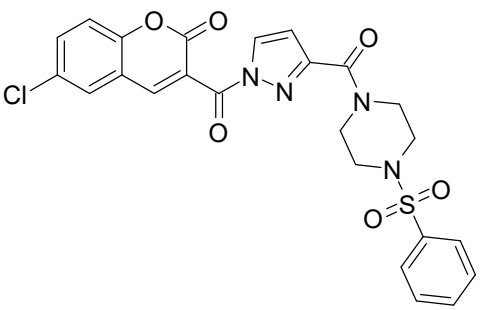
Physical Characterization of B6-B10 compounds

Melting points of the compounds B6-B10 were ranging from 100°C to >350°C. Yield of all the compounds were ranging from 55% to 90%. All the compounds were soluble in Methanol, Ethanol.

Table 2: Chemical structure and molecular weight of synthesized compound

Sr. no.	Compound Number	Molecular Weight	Percentage Yield	IUPAC Name and Chemical Structure
1.	**B6**	605.84	71.58	 3-[3-[4-(4-bromophenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one Molecular Weight: $C_{21}H_{11}Cl3N_3O_4$

2.	**B7**	583.05	70.14	 3-[3-[4-(4-tert-butylphenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one Molecular Weight: $C_{28}H_{27}ClN_4O_6S$
3.	**B8**	556.97	80.26	 6-chloro-3-[3-[4-(4-methoxyphenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]chromen-2-one Molecular Weight: $C_{25}H_{21}ClN_4O_7S$
4.	**B9**	561.39	85.48	6-chloro-3-[3-[4-(4-  chlorophenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]chromen-2-one Molecular Weight: $C_{24}H_{18}Cl2N_4O_6S$

5.	**B10**	526.94	82.36	 3-[3-[4-(benzenesulfonyl)piperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one Molecular Weight: $C_{24}H_{19}ClN_4O_6S$

Spectral analysis of B6- B10 Compounds

I.R. SPECTRA (B6- B10) Compounds

B6: 3-[3-[4-(4-bromophenyl) sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one

IR (cm^{-1}): 1648.52 (C=O), 3278.59 (N-H), 2847.55 (Aromatic C-H), 869.26(C-Cl), 664.15(Br), 2215.15 (SO_2)

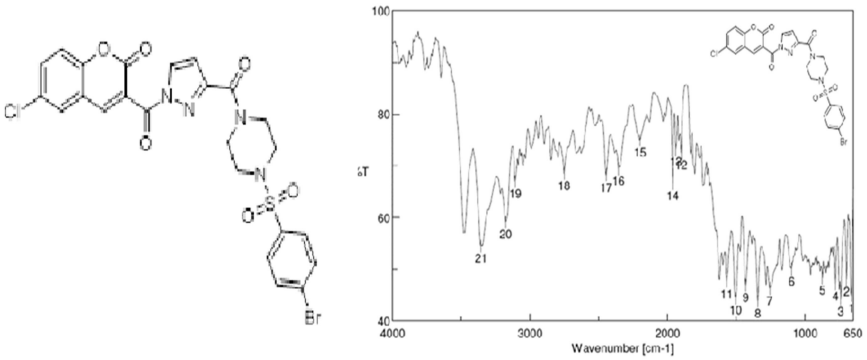


Figure 6: IR Spectrum of Compound B6

B7: 3-[3-[4-(4-tert-butylphenyl) sulfonylpiperazine-1-carbonyl] pyrazole-1-carbonyl]-6-chlorochromen-2-one

IR (cm^{-1}): 1745.85 (C=O), 3145.18 (N-H), 2945.11 (Aromatic C-H), 754.26(C-Cl), 2214.56 (SO_2)

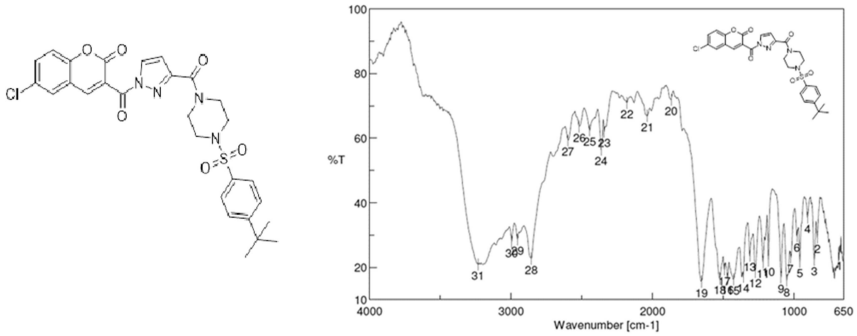


Figure 7: IR Spectrum of Compound B7

B8: 6-chloro-3-[3-[4-(4-methoxyphenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]chromen-2-one
IR (cm^{-1}): 1751.56 (C=O), 3214.55 (N-H), 2892.45 (Aromatic C-H), 751.59(C-Cl), 2210.56 (SO_2)

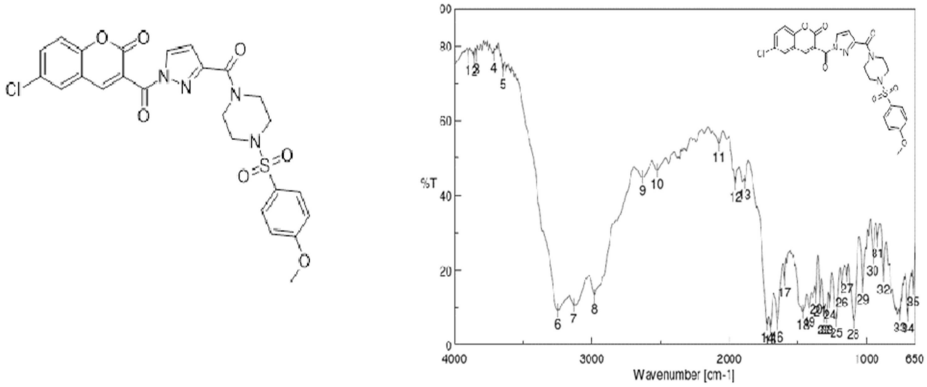


Figure 8: IR Spectrum of Compound B8

B9: 6-chloro-3-[3-[4-(4-chlorophenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]chromen-2-one
IR (cm^{-1}): 1745.69 (C=O), 3147.55(N-H), 2958.62 (Aromatic C-H), 658.95(C-Cl), 2135.26 (SO_2)

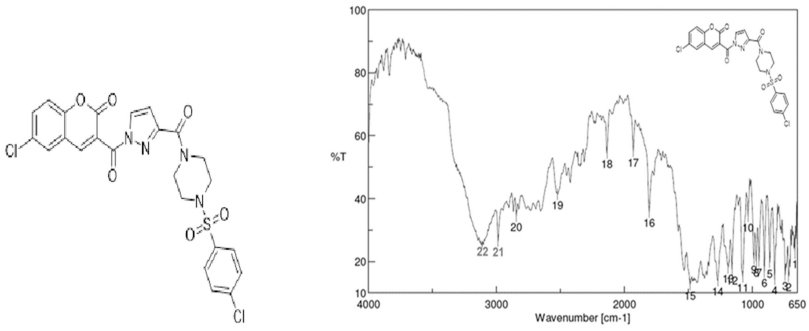


Figure 9: IR Spectrum of Compound B9

B10: 3-[3-[4-(benzenesulfonyl)piperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one
IR (cm^{-1}): 1658.74 (C=O), 3258.21(N-H), 2895.63 (Aromatic C-H), 712.58(C-Cl), 2256.23 (SO_2)

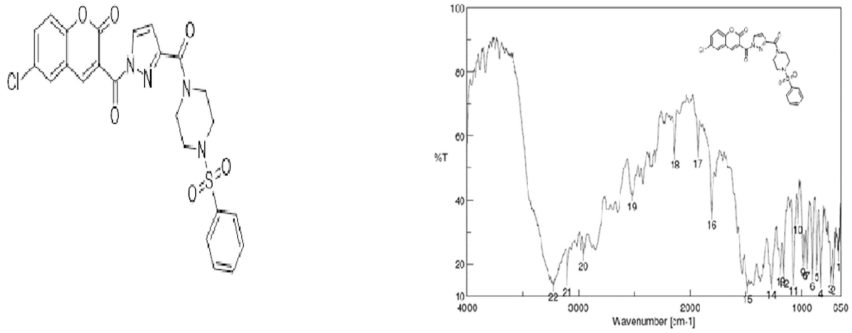


Figure 10: IR Spectrum of Compound B10

N.M.R. SPECTRA (B6-B10)
B6: 3-[3-[4-(4-bromophenyl) sulfonylpiperazine-1-carbonyl] pyrazole-1-carbonyl]-6-chlorochromen-2-one
1H NMR: δ 3.49-3.66 (8H, 3.57 (ddd, J = 15.6, 7.0, 2.9 Hz), 3.58 (ddd, J = 15.1, 7.0, 2.9 Hz)), 6.46 (1H, d, J = 4.3 Hz), 7.41-7.76 (7H, 7.47 (dd, J = 8.1, 0.5 Hz), 7.54 (dd, J = 1.7, 0.5 Hz), 7.57 (ddd, J = 7.9, 1.9, 0.4 Hz), 7.61 (dd, J = 8.1, 1.7 Hz), 7.69 (ddd, J = 7.9, 1.5, 0.4 Hz)), 7.84 (1H, d, J = 4.3 Hz), 8.77 (1H, s).
13C NMR: δ 45.4 (2C, s), 52.6 (2C, s), 102.5 (1C, s), 112.9 (1C, s), 116.8 (1C, s), 121.3 (1C, s), 122.3 (1C, s), 127.3 (2C, s), 129.3 (1C, s), 129.8 (1C, s), 130.4 (1C, s), 131.7 (1C, s), 132.7 (2C, s), 134.5 (1C, s), 136.9 (1C, s), 151.7 (1C, s), 154.1 (1C, s), 155.8 (1C, s), 156.3 (1C, s), 160.6 (1C, s).

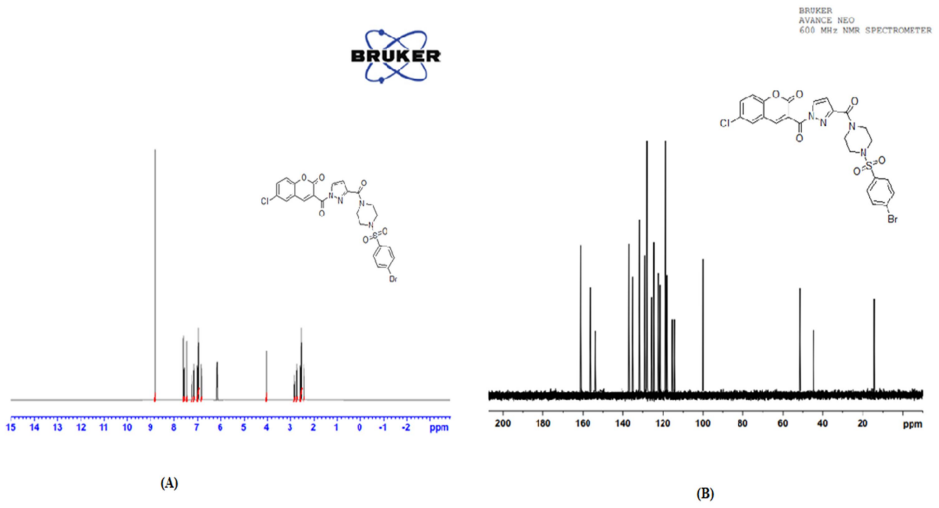


Figure 16: A-NMR Spectrum of Compound (B6),B-^{13}C NMR Spectrum of Compound B6

B7: **3**-[3-[4-(4-tert-butylphenyl)sulfonylpiperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one
1H NMR: δ 1.26 (9H, s), 3.50-3.67 (8H, 3.58 (ddd, J = 15.1, 7.0, 2.9 Hz), 3.59 (ddd, J = 15.6, 7.0, 2.9 Hz)), 6.46 (1H, d, J = 4.3 Hz), 7.34-7.67 (7H, 7.40 (ddd, J = 8.1, 1.7, 0.5 Hz), 7.47 (dd, J = 8.1, 0.5 Hz),

7.49 (ddd, J = 8.1, 1.5, 0.5 Hz), 7.54 (dd, J = 1.7, 0.5 Hz), 7.61 (dd, J = 8.1, 1.7 Hz)), 7.84 (1H, d, J = 4.3 Hz), 8.77 (1H, s).
13C NMR: δ 31.1 (3C, s), 34.7 (1C, s), 45.4 (2C, s), 52.6 (2C, s), 102.5 (1C, s), 112.9 (1C, s), 116.8 (1C, s), 121.3 (1C, s), 125.8 (2C, s), 127.2 (2C, s), 129.3 (1C, s), 129.8 (1C, s), 130.4 (1C, s), 131.7 (1C, s), 134.5 (1C, s), 136.9 (1C, s), 151.7 (1C, s), 152.6 (1C, s), 154.1 (1C, s), 155.8 (1C, s), 156.3 (1C, s), 160.6 (1C, s).

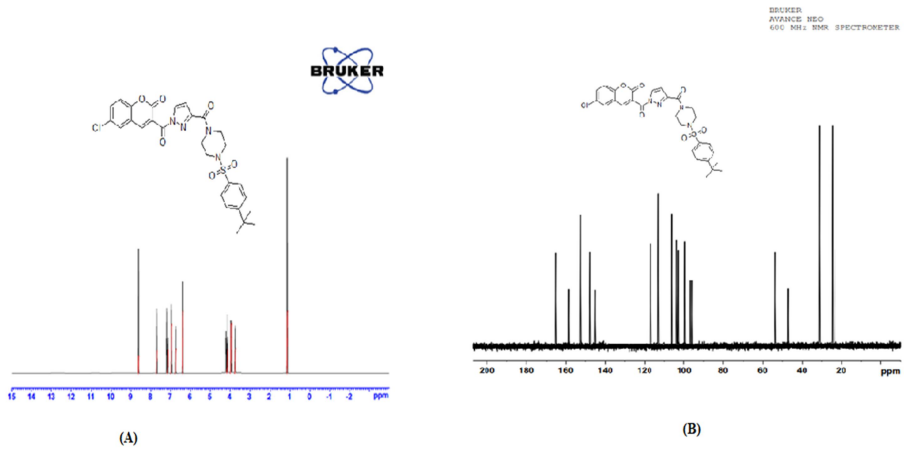


Figure 17: NMR Spectrum of Compound (B7), B-^{13}C NMR Spectrum of Compound B7

B8: 6-chloro-3-[3-[4-(4-methoxyphenyl) sulfonylpiperazine-1-carbonyl] pyrazole-1-carbonyl] chromen-2-one
1H NMR: δ 3.49-3.66 (8H, 3.57 (ddd, J = 15.6, 7.0, 2.9 Hz), 3.58 (ddd, J = 15.1, 7.0, 2.9 Hz)), 3.84 (3H, s), 6.46 (1H, d, J = 4.3 Hz), 6.97 (2H, ddd, J = 7.9, 1.3, 0.4 Hz), 7.41-7.67 (5H, 7.47 (dd, J = 8.1, 0.5 Hz), 7.54 (dd, J = 1.7, 0.5 Hz), 7.59 (ddd, J = 7.9, 1.5, 0.4 Hz), 7.61 (dd, J = 8.1, 1.7 Hz)), 7.84 (1H, d, J = 4.3 Hz), 8.77 (1H, s).
13C NMR: δ 45.4 (2C, s), 52.6 (2C, s), 56.0 (1C, s), 102.5 (1C, s), 112.9 (1C, s), 114.6 (2C, s), 116.8 (1C, s), 121.3 (1C, s), 128.3 (2C, s), 129.3 (1C, s), 129.8 (1C, s), 130.4 (1C, s), 131.7 (1C, s), 134.5 (1C, s), 136.9 (1C, s), 151.7 (1C, s), 154.1 (1C, s), 155.8 (1C, s), 156.3 (1C, s), 159.8 (1C, s), 160.6 (1C, s).

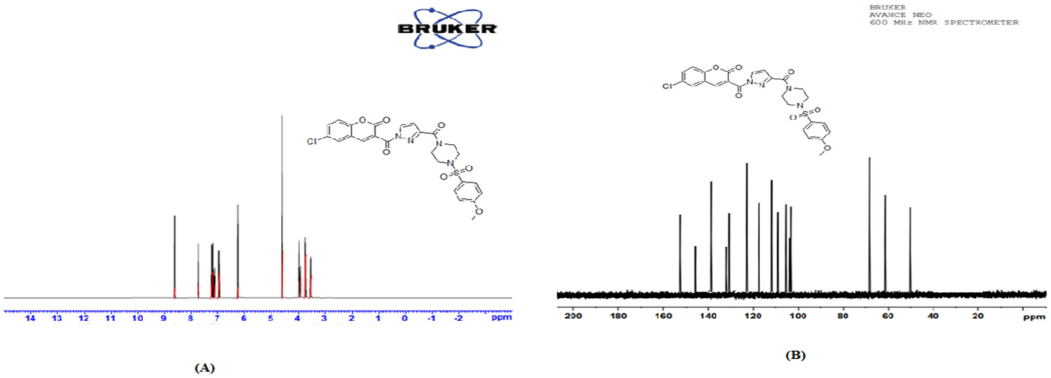


Figure 18: NMR Spectrum of Compound (B8),B-^{13}C NMR Spectrum of Compound B8

B9: 6-chloro-3-[3-[4-(4-chlorophenyl) sulfonylpiperazine-1-carbonyl] pyrazole-1-carbonyl] chromen-2-one
1H NMR: δ 3.49-3.66 (8H, 3.57 (ddd, J = 15.6, 7.0, 2.9 Hz), 3.58 (ddd, J = 15.1, 7.0, 2.9 Hz)), 6.46 (1H, d, J = 4.3 Hz), 7.41-7.70 (7H, 7.47 (dd, J = 8.1, 0.5 Hz), 7.54 (dd, J = 1.7, 0.5 Hz), 7.61 (ddd, J = 7.9, 1.9, 0.4 Hz), 7.61 (dd, J = 8.1, 1.7 Hz), 7.64 (ddd, J = 7.9, 1.6, 0.4 Hz)), 7.84 (1H, d, J = 4.3 Hz), 8.77 (1H, s).
13C NMR: δ 45.4 (2C, s), 52.6 (2C, s), 102.5 (1C, s), 112.9 (1C, s), 116.8 (1C, s), 121.3 (1C, s), 127.3 (2C, s), 129.1 (2C, s), 129.3 (1C, s), 129.8 (1C, s), 130.4 (1C, s), 131.7 (1C, s), 133.7 (1C, s), 134.5 (1C, s), 136.9 (1C, s), 151.7 (1C, s), 154.1 (1C, s), 155.8 (1C, s), 156.3 (1C, s), 160.6 (1C, s).

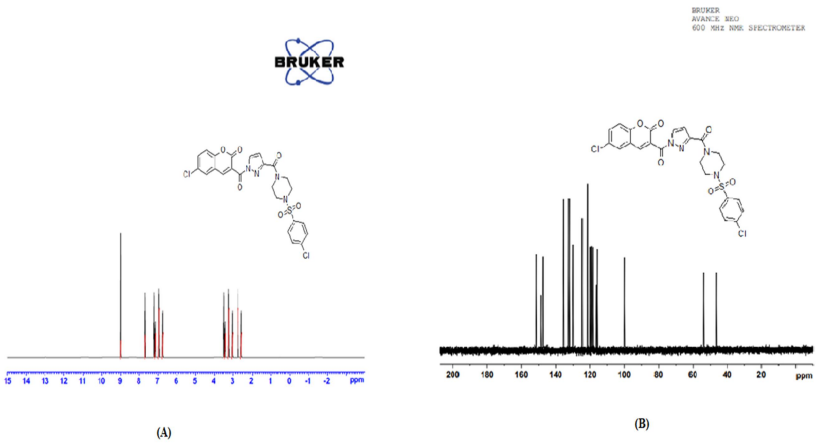


Figure 19: NMR Spectrum of Compound (B9),B-^{13}C NMR Spectrum of Compound B9

B10: 3-[3-[4-(benzenesulfonyl)piperazine-1-carbonyl]pyrazole-1-carbonyl]-6-chlorochromen-2-one
1H NMR: δ 3.50-3.67 (8H, 3.58 (ddd, J = 15.1, 7.0, 2.9 Hz), 3.59 (ddd, J = 15.6, 7.0, 2.9 Hz)), 6.46 (1H, d, J = 4.3 Hz), 7.41-7.90 (9H, 7.47 (dd, J = 8.1, 0.5 Hz), 7.52 (dddd, J = 8.0, 7.6, 1.6, 0.4 Hz), 7.54 (dd, J = 1.7, 0.5 Hz), 7.61 (dd, J = 8.1, 1.7 Hz), 7.62 (tt, J = 7.6, 1.5 Hz), 7.74 (dtd, J = 8.0, 1.5, 0.4 Hz), 7.84 (d, J = 4.3 Hz)), 8.77 (1H, s).
13C NMR: δ 45.4 (2C, s), 52.6 (2C, s), 102.5 (1C, s), 112.9 (1C, s), 116.8 (1C, s), 121.3 (1C, s), 126.8 (2C, s), 127.8 (1C, s), 128.5 (2C, s), 129.3 (1C, s), 129.8 (1C, s), 130.4 (1C, s), 131.7 (1C, s), 134.5 (1C, s), 136.9 (1C, s), 151.7 (1C, s), 154.1 (1C, s), 155.8 (1C, s), 156.3 (1C, s), 160.6 (1C, s).

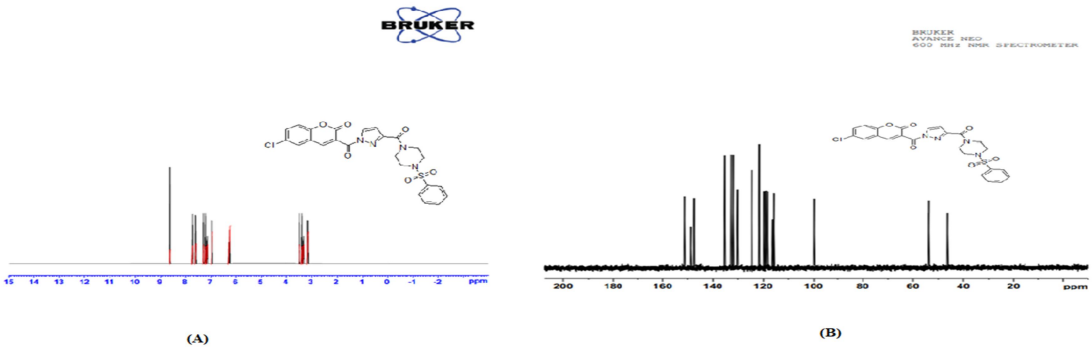


Figure 20: NMR Spectrum of Compound (B10), B-^{13}C NMR Spectrum of Compound B10

Mass Spectra (B1-B10)

Table 3: Mass spectra of Theoretical mass and observed mass

Formulation code	Theoretical Mass	Observed Mass
B6	599.4	604.78
B7	583.05	582.19
B8	556.97	557.02
B9	561.39	565.45
B10	526.94	526.17

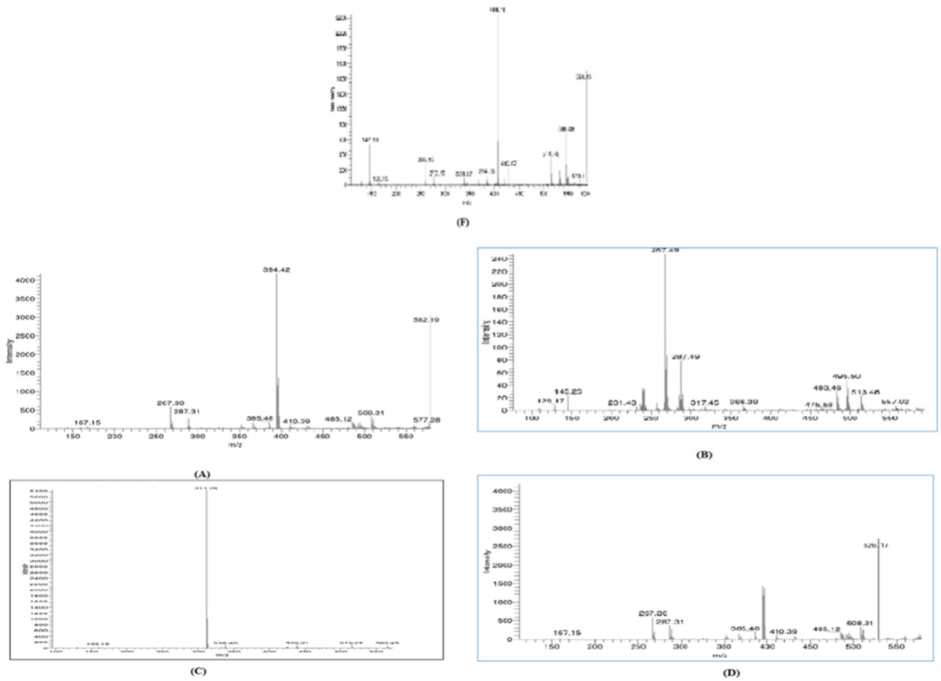


Figure 22: A- MASS Spectrum of Compound B7, B- Figure: MASS Spectrum of Compound B8, C- Figure: MASS Spectrum of Compound B9, D- Figure: MASS Spectrum of Compound B10

Antimicrobial activity

Table 4: Antimicrobial Activity of synthesized compounds (B1-B10) against Aspergillus Niger, Staphylococcus aureus, Bacillus subtilis, Escherichia Coli.

Sample ID	Conc. of Stocksolution	Zone of inhibition in mm			
		Bacillus subtilis	***Staphylococcus aureus***	***Escherichia coli***	***Aspergillus niger***
B6		14	18	17	23
B7		16	16	15	22
B8		12	20	12	21

B9		13	21	13	24
B10		14	18	15	23
Std.	**0.5 mg/ml**	19	24	19	30

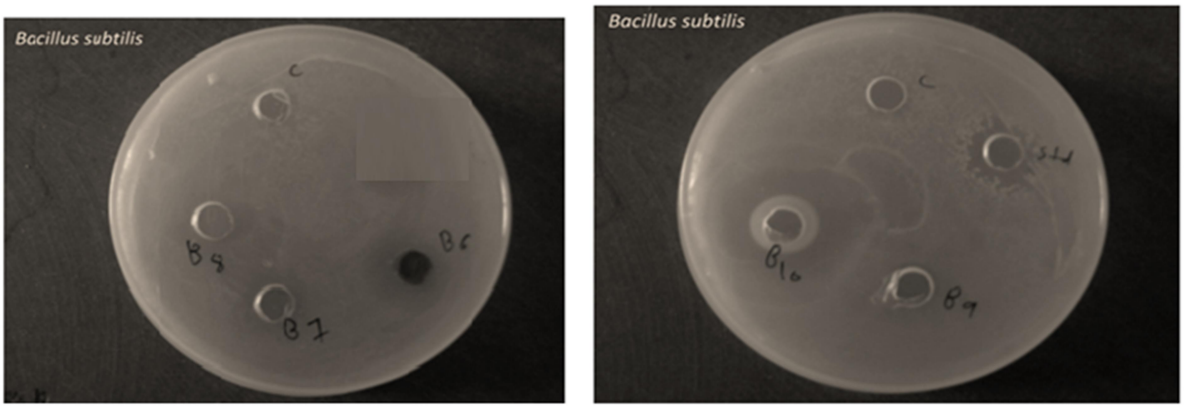


Figure 23: Microbial activities against *Bacillus subtilis* (B6 to B10)

Figure 24: Microbial activities against *Staphylococcus aureus* (B6 to B10)

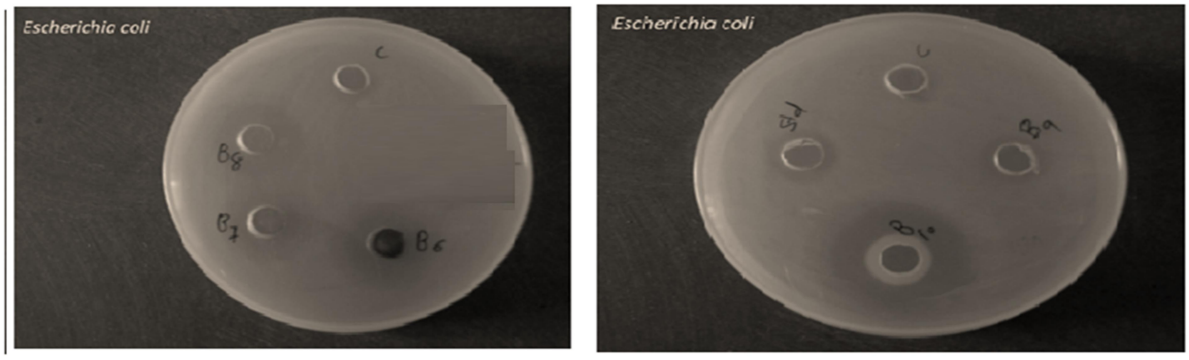


Figure 25: Microbial activities against *Escherichia coli* (B6 to B10)

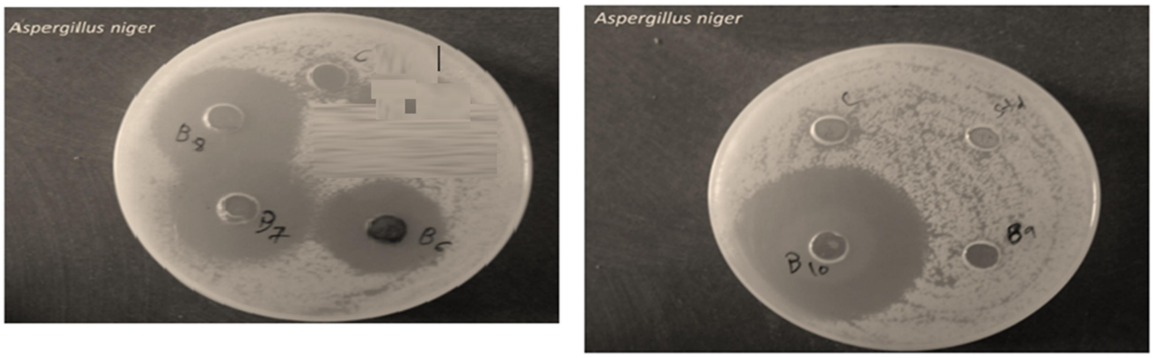


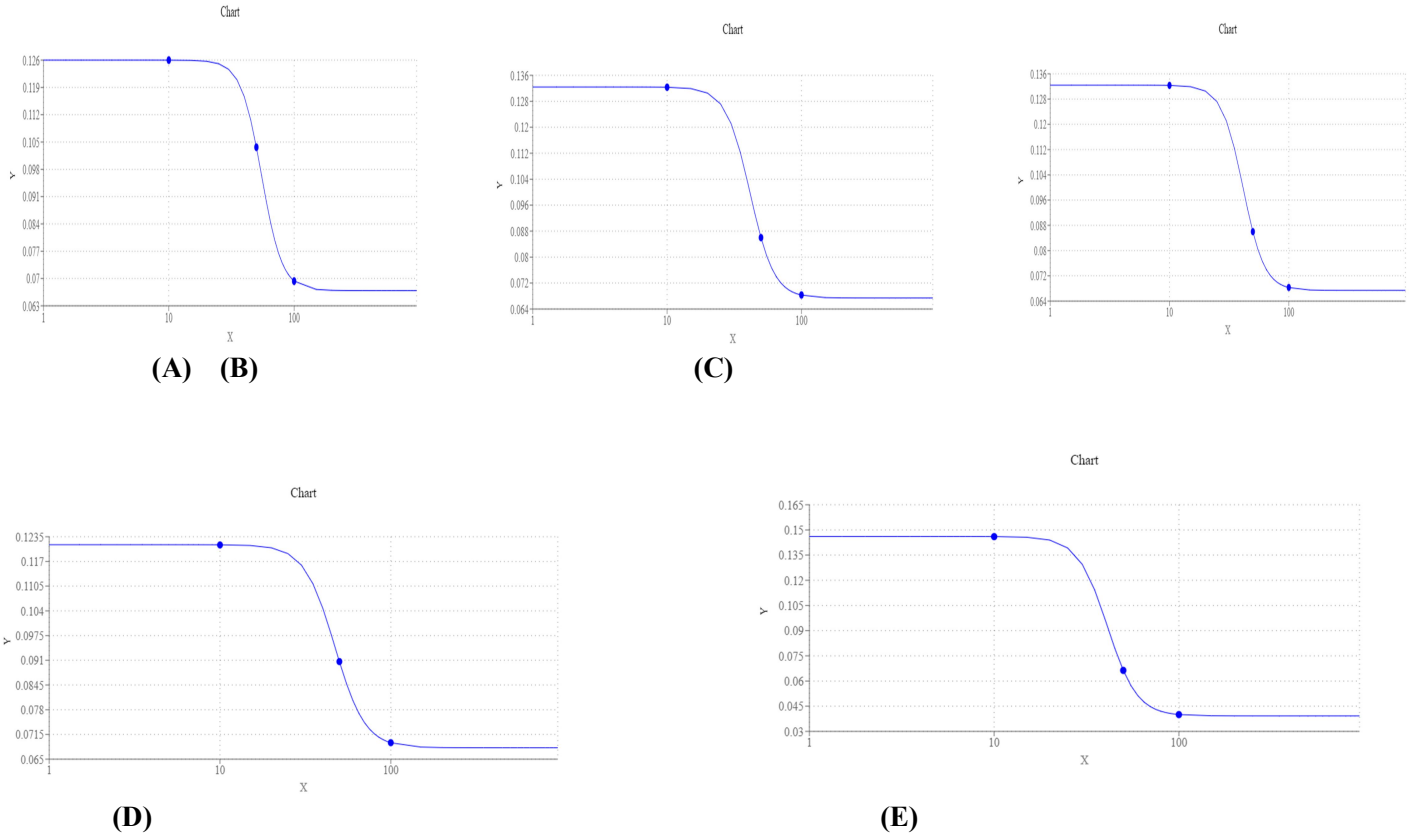
Figure 26: Microbial activities against *Aspergillus Niger* (B6 to B10)

Anticancer activity

Table 5: Effects of B6- B10 against MCF-7 Cell line (Breast cancer cell line) by MTT assay method.

Sr. no	Sample	Concentration(µg/ml)	Absorbance(nm)	Mean	%inhibition	IC50 (µg/ml)
1	**Control**	-	0.415 0.415 0.414	0.41466667		
2	**Standard**	10	0.068 0.072 0.078	0.07266667	82.47588424	65.15
		50	0.061 0.058 0.058	0.059	85.77170418	
		100	0.027 0.025 0.03	0.02733333	93.40836013	
8	**B6**	10	0.122 0.129 0.127	0.126	69.61414791	54.96
		50	0.101 0.106 0.104	0.10366667	75	
		100	0.068 0.069 0.071	0.06933333	83.27974277	
9	**B7**	10	0.132 0.129	0.13233333	68.08681672	41.38

			0.136			
		50	0.088 0.081 0.089	0.086	79.26045016	
		100	0.065 0.069 0.071	0.06833333	83.52090032	
10	**B8**	10	0.119 0.117 0.12	0.11866667	71.38263666	49.12
		50	0.087 0.081 0.08	0.08266667	80.06430868	
		100	0.051 0.049 0.055	0.05166667	87.54019293	
11	**B9**	10	0.122 0.119 0.123	0.12133333	70.73954984	46.97
		50	0.087 0.096 0.089	0.09066667	78.13504823	
		100	0.071 0.068 0.069	0.06933333	83.27974277	
12	**B10**	10	0.145 0.149 0.144	0.146	64.79099678	40.99
		50	0.059 0.069 0.071	0.06633333	84.00321543	
		100	0.036 0.041 0.043	0.04	90.35369775	



(A) (B) (C) (D) (E)

Figure 28: A- Graph (Concentration [µg/ml] Vs Mean Absorbance nm) IC50 of compound B6.B-Graph (Concentration [µg/ml] Vs Mean Absorbance nm) IC50 of compound B7.C- Graph (Concentration [µg/ml] Vs Mean Absorbance nm) IC50 of compound B8.D- Figure Graph (Concentration [µg/ml] Vs Mean Absorbance nm) IC50 of compound B9.E- Graph (Concentration [µg/ml] Vs Mean Absorbance nm) IC50 of compound B10.

Mtt Assay Results

Table7: Inhibitory effect of B6 to B10 Compounds

Compound/ Concentration	% inhibitory		
	10	50	100
B6	70.12	73.69	84.56
B7	69.56	73.01	83.26
B8	68.55	73.25	81.14
B9	71.23	74.15	82.49
B10	70.14	75.44	85.44
STD	81.59	88.45	91.26

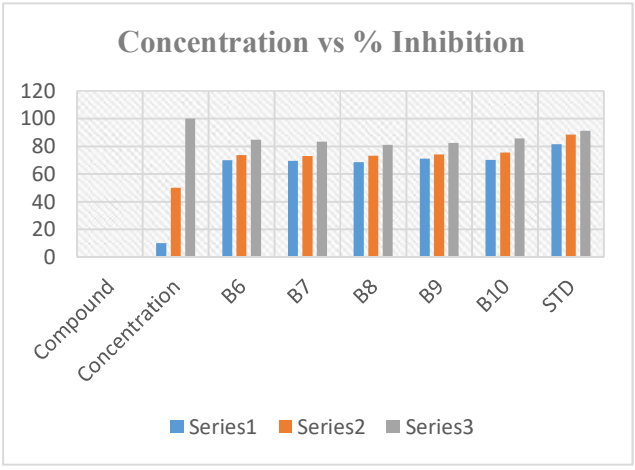


Figure 29:Inhibitory effect of B6 to B10 Compounds

Anticancer activity of B16to B10 Compounds on MCF 7 (human cervical carcinoma)

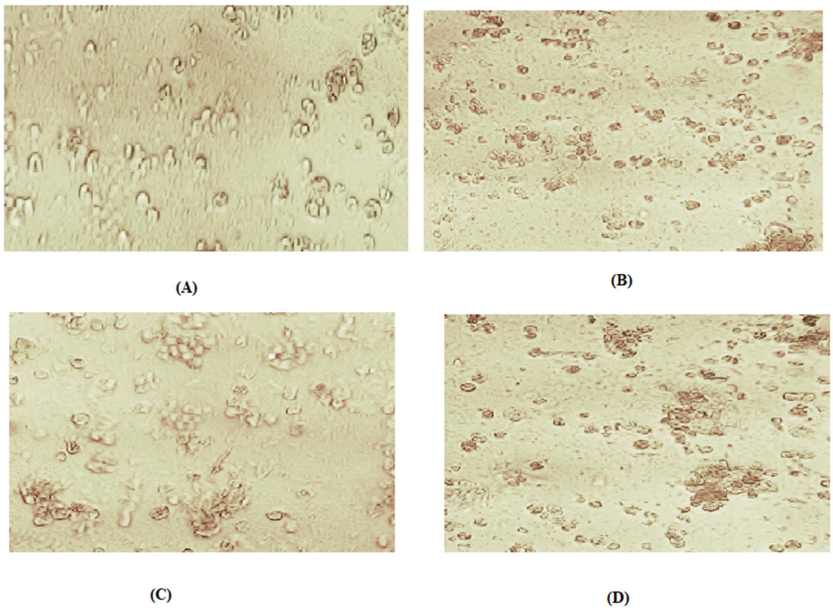


Figure 31: A-Compound B7 on MCF 7,B- Compound B8 on MCF 7, C- Compound B9 on MCF 7, D- Compound B8 on MCF 10

In-Vivo Study
Anti-inflammatory activity

Table 8:Anti-inflammatory activity of synthesized compound (B1-B10)

Time Interval	Control	Standard	B6	B7	B8	B9	B10
00 min	5.48	6.12	8.12	8.06	7.45	8.56	7.15
30 min	6.58	7.45	7.44	7.45	8.12	8.14	7.02
60 min	7.88	8.58	6.58	7.26	7.15	7.55	6.56
120 min	8.22	7.45	6.22	6.55	6.59	7.14	6.41

150 min	7.41	6.25	6.20	6.15	6.45	6.36	6.20

All Reading are in mm

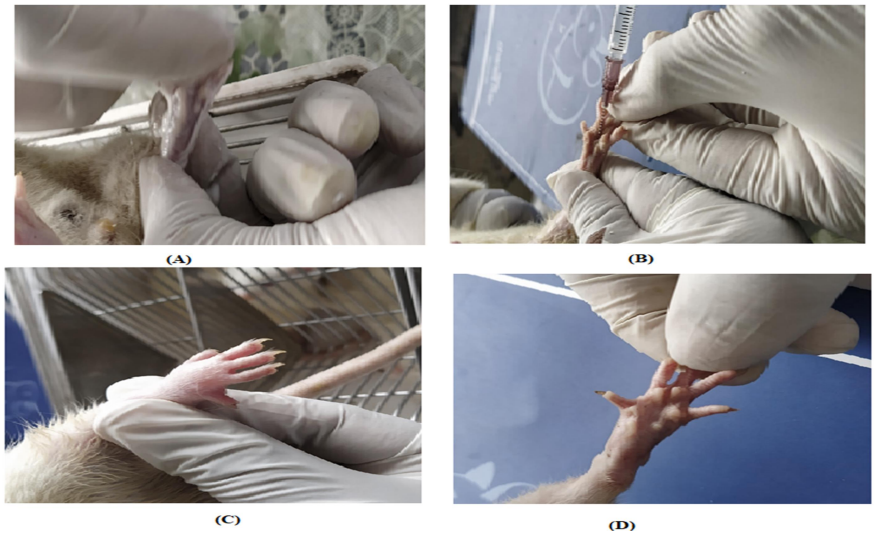


Figure 33: Application of synthesized compound- Induction of inflammation- At 30 min, D- at 150 min

CONCLUSION

To sum up, the recently created pyrazole derivative showed encouraging biological activity, indicating that more research into it for medicinal and drug development purposes may be necessary. Details regarding the antibacterial and anticancer properties of compounds B1 through B10, as well as their mass, nuclear magnetic resonance (NMR), and infrared (IR) spectra. Organic compound functional groups are frequently identified using infrared (IR) spectra. The C=O, N-H, O-H, aromatic C-H, and C-Cl bonds are associated with the main infrared peaks. Identification of the structure can be aided by these peaks. Information about a compound's hydrogen and carbon surroundings can be found in its NMR spectra. Understanding the atoms' connection within the molecule depends on these spectra. Mass Diagrams: Mass spectra reveal details about a compound's molecular weight. The compounds appear to have been properly synthesised, as evidenced by the observed masses of the compounds being near to their theoretical masses. Antimicrobial Activity: the ability of the substances to combat different microbes. The "Zone of inhibition" shows how much certain substances prevent these microbes from growing. Greater zones of inhibition may indicate more potent antibacterial activity in a compound. To ascertain a compound's cytotoxicity against cancer cells, the MTT assay is employed (in this example, MCF-7 breast cancer cells). The chemicals' potential as anticancer agents can be inferred from the percentage of inhibition and IC50 values.

Conflict of Interest

None. Declared by Authors.

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