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(*E*)-1-((ARYLOXYQUINOLIN-3-YL)METHYLENE)THIOSEMICARBAZIDES: SYNTHESIS AND COMPARATIVE STUDY OF THEIR *IN VITRO* AND *IN SILICO* ACTIVITIES

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ABSTRACT

Ten novel (*E*)-1-((2-phenoxyquinolin-3-yl) methylene) thiosemicarbazides were prepared by reacting 2-chloroquinoline-3-carbaldehydes with phenols followed by coupling the products with thiosemicarbazide. The ten thiosemicarbazides 1-10 were subjected to both *in vitro* and *in silico* studies for their pharmacological properties. They were found to exhibit very good antioxidant, antibacterial, antifungal and anti-proliferative activities. A few of them show even better free radical scavenging activity as compared to ascorbic acid used as standard compound. The *in silico* studies include drug likeness and bioactivity on the basis of their compliance of Lipinski's Rule of Five, physical properties using Molinspiration programme and molecular docking with the enzyme EGFR (Epidermal growth factor receptor)tyrosine kinase. The results of molecular docking studies corroborate the results obtained from the *in vitro* anti-proliferative experiments. All these results indicate that aryloxyquinolinethiosemicarbazides have highly promising pharmacological properties and have the potential to be developed into therapeutically beneficial products. In particular, (*E*)-1-((2-(2-nitrophenoxy)-6-methylquinolin-3-yl) methylene) thiosemicarbazide (7) seems to have the potential for further investigation of its anticancer properties.

KEYWORDS

(*E*)-1-((2-phenoxyquinolin-3-yl) methylene) thiosemicarbazides, Antioxidant activity, Antimicrobial activity, Anti-proliferative activity and Molecular docking.

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INTRODUCTION

Chloroquinoline-3-carbaldehyde and its substituents are key intermediates for design of pharmacologically important compounds containing quinoline unit, which find broad pharmaceutical and therapeutic applications. In addition, the ease of their preparation from substituted acetanilides by Vilsmeier-Haack reaction may be attributed to the

popularity of studying their medicinal application[1]. Also, thiosemicarbazones constitute one of the most versatile classes of compounds that possess a wide spectrum of biological activities such as antimicrobial, antimalarial and antitumor activities. Because of their outstanding display of biological properties, the thiosemicarbazone derivatives incorporating the heterocyclic moiety have been the focus of investigation by medicinal chemists in the past decades[2]. In keeping with this trend, the present work is aimed at the design and synthesis of new compounds containing quinoline and thiosemicarbazide moieties and to study their biological activities.

Increasing resistance of pathogens to existing drugs is a serious problem with antimicrobial therapy and necessitates continuing research in new classes of antimicrobials and since quinoline compounds have the potential to act as inhibitor of antibiotic efflux pumps in cells[3], the possibility of these compounds being developed into drugs that can overcome antibiotic resistance is quite promising. Preliminary screening (calculation of zone of inhibition) of synthesized compounds against gram-positive and gram-negative bacteria and fungal species has been carried out in the present study.

Cancer remains a life threatening disease worldwide in spite of available conventional treatments such as surgery, radiotherapy or chemotherapy. The limitations of chemotherapy are mainly associated with low efficacy, high toxicity and high cost of the drugs used. Hence, a wide range of scientific approaches are attempted to find better chemotherapeutic agents[4]. Keeping this in view we synthesized compounds containing both quinoline and thiosemicarbazide moieties expecting them to turn out to be lead molecules in drug development efforts, because a number of widely used drugs contain one or the other of these in their molecules. Cervical cancer remains the most common cause of death from cancer in women. Since HeLa cell (derived from cervical cancer cells) is an immortal cell line used in medical research, it was thought worthwhile to perform the activity against these cell lines[5].

Protein kinases are viable target for anti-cancer drug development as they are involved in many pathophysiological problems. EGFR-TK (Epidermal Growth Factor - Tyrosine Kinase) plays an important role in signal transduction pathways and has been implicated in numerous tumors of epithelial origin. Several benzothiazole and quinozoline derivatives have been clinically validated as selective inhibitors of EGFR phosphorylation at the ATP binding site. With the above facts, in the present study we carried out virtual screening on EGFR-TK against the synthesized compounds using molecular docking to identify new anti-EGFR inhibitors[6].

Previous reports have revealed that various diseases are associated with free radicals and reactive oxygen species (ROS). The potential therapeutic or preventive effects of antioxidative agents may be included in the course of inhibition of carcinogenesis and cancer. It is thus to be expected that quinoline derivatives may contribute to good antioxidant activity[7], and as such quinoline structure is chosen in the present work as active pharmacophoric core with additional structural modifications are designed to explore their antioxidant activities.

With these facts as background, we synthesized several aryloxy quinoline thiosemicarbazides, namely (*E*)-1-((2-phenoxyquinolin-3-yl)methylene) thiosemicarbazide (1) and quinoline and phenol ring substituted derivatives employing simple experimental conditions and carried out comprehensive preliminary studies under *in vitro* as well as *in silico* settings of their pharmacological properties, such as antioxidant activity, antimicrobial activity, anti-proliferative, drug-likeness and molecular docking aspects. We found that all the aryloxy quinoline thiosemicarbazides (1-10) that we have studied exhibited excellent biological properties, with a few of them showing good potential to be developed into compounds of medicinal value. The results of these studies are the focus of the present report.

GENERAL NOMENCLATURE FOR COMPOUNDS 1-10

(*E*)- 1-((2-aryloxyquinolin-3-yl) methylene) thiosemicarbazide.

MATERIAL AND METHODS

Chemicals obtained from Sigma-Aldrich and SD Fine Chemicals companies were purified when needed or used without further purification. Melting points were determined using MR-Vis Visual melting range apparatus (LAB INDIA version 2.2.2). Proton and carbon-13 NMR spectra were recorded on 400 MHz Bruker FT-NMR using TMS as the internal standard and DMSO as solvent, and the chemical shifts (δ) are given in parts per million (ppm). The IR spectra (in KBr pellets) were recorded on a Shimadzu CVT-04 spectrophotometer. Merck silica gel 60 F254 TLC plates were used to monitor the progress of reactions and purity of products. The compounds were purified by chromatography on silica gel column using petroleum ether-ethyl acetate mixture as eluant. 2-Chloroquinoline-3-carbaldehydes were synthesized from corresponding acetanilides by Vilsmeier-Haack reaction following the literature procedure,[1e] which were then reacted with phenols to get intermediate aryloxy quinoline-3-carbaldehydes (1a–10a) as described in the literature[8]. The same reaction conditions were used for the synthesis of all thiosemicarbazide derivatives (1-10); the typical procedure is given below.

EXPERIMENTAL

Synthesis of (E)-1-((2-phenoxyquinolin-3-yl)methylene)thiosemicarbazide (1)

To a suspension of 500 mg (2.61 mmol) of 2-chloroquinoline-3-carbaldehyde in 5 mL of DMF, 368.58 mg (3.91 mmol) of phenol and 722 mg (5.22 mmol) of K_2CO_3 were added. The reaction mixture was refluxed for about 8 h, the progress of the reaction being monitored by periodically testing the mixture by TLC. At the end of the reaction, the reaction mixture was cooled to room temperature and then poured into chilled water (50 mL) with continuous stirring followed by neutralization with 1.5 N HCl until pH 7 resulted. The separated solid was collected by filtration and purified by flash column chromatography on silica gel using petroleum ether-ethyl acetate mixture (95:05 ratio) as eluting solvent, to yield 196 mg (30%) of intermediate 2-phenoxyquinoline-3-carbaldehyde (1a)[8]. Other aryloxy quinoline-3-carbaldehydes (2a–10a) were prepared and purified following the same procedure.

To a suspension of 100 mg (4.01 mmol) of 2-phenoxyquinoline-3-carbaldehyde (1a) in DMF (5 mL), 45.63 mg (5.01 mmol) of thiosemicarbazide was added. The reaction mixture was refluxed for 2 to 3 h, the progress of the reaction being monitored by periodically testing the mixture by TLC. At the end of the reaction, the mixture was added to crushed ice; the precipitated product was collected by filtration and washed with DCM to yield 120 mg (93%) of the title compound (1)[9].

Other aryloxy quinoline thiosemicarbazides (2–10) were prepared following the same procedure from corresponding aryloxy quinoline-3-carbaldehydes (2a–10a). The yields and melting points of the compounds 1-10 are provided in Table No.1.

SPECTRAL AND HRMS DATA

(*E*)-1-((2-phenoxyquinolin-3-yl)methylene)thiosemicarbazide (1)

^{1}H NMR(400 MHz, DMSO d_6):δ = 7.28 (m, 3H), 7.49 (m, 3H), 7.57 (d, *J* =8.4 Hz, 1H), 7.64 (t, 1H), 7.90 (d, *J* =8Hz, 1H), 8.23 (s, 1H), 8.41 (s, 1H), 8.54 (s, 1H), 9.23 (s,1H), 11.72 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 119.53, 122.25, 125.43, 126.00, 126.15, 127.35, 128.71, 130.10, 131.12, 136.18, 136.36, 146.07, 153.81, 159.35, 178.75;IR (KBr, cm^{-1}): ν_{max}3461, 3341, 3157, 3088, 1620, 1587, 1529, 1492, 1350, 1087; HRMS (ESI) *m/z*Calcd for$C_{17}H_{14}N_4OSNa^+$[M+Na]$^+$ 345.0786, Found: 345.0780.

(*E*)-1-((6-methyl-2-phenoxyquinolin-3-yl)methylene)thiosemicarbazide (2)

^{1}H NMR(400 MHz, DMSO d_6):δ = 2.49 (m, 3H), 7.27 (t, 3H), 7.47 (t, 3H), 7.67 (s, 1H), 8.22 (s, 1H), 8.41 (s, 1H), 8.52 (s,1H), 9.14 (s, 1H), 11.71 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.40, 119.43, 122.20, 125.34, 126.15, 127.16, 127.50,

130.09, 133.18, 135.26, 135.77, 136.31, 144.52, 153.92, 158.91, 178.72;IR (KBr, cm^{-1}): ν_{max}3425, 3306, 3157, 3016, 1587, 1535, 1492, 1344, 1089; HRMS (ESI) *m/z*Calcd for $C_{18}H_{16}N_4OSNa^+[M+Na]^+$ 359.0943, Found: 359.0953.

(*E*)-1-((6-methoxy-2-phenoxyquinolin-3-yl)methylene)thiosemicarbazide (3)

^{1}H NMR(400 MHz, DMSO d_6):δ = 2.49 (m, 3H), 7.27 (t, 3H), 7.47 (t, 4H), 7.66 (s, 1H), 8.21 (s, 1H), 8.40 (s, 1H), 8.52 (s,1H), 9.14 (s, 1H), 11.70 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.39, 119.41, 122.19, 125.33, 126.14, 127.15, 127.49, 130.08, 133.17, 135.25, 135.75, 136.29, 144.51, 153.91, 158.90, 178.70;IR (KBr, cm^{-1}): ν_{max}3493, 3343, 3154, 3014, 1618, 1573, 1538, 1492, 1348, 1092; HRMS (ESI) *m/z*Calcd for $C_{18}H_{16}N_4O_2SNa^+[M+Na]^+$ 375.0892, Found: 375.0889.

(*E*)-1-((2-(4-nitrophenoxy)quinolin-3-yl)methylene)thiosemicarbazide (4)

^{1}H NMR(400 MHz, DMSO d_6):δ = 7.59 (m, 3H), 7.69 (m, 2H), 7.95 (d, *J* = 8 Hz, 1H), 8.26 (s, 1H), 8.36 (d, *J* = 8 Hz , 2H), 8.45 (s, 1H), 8.51 (s,1H), 9.31 (s, 1H), 11.75 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 119.71, 122.85, 126.05, 126.61, 127.52, 128.80, 131.43, 135.72, 136.92, 144.61, 145.80, 158.45, 159.23, 178.76;IR (KBr, cm^{-1}): ν_{max}3446, 3327, 3158, 3012, 1590, 1511, 1412, 1341, 1089; HRMS (ESI) *m/z*Calcd Calcd for $C_{17}H_{13}N_5O_3SNa^+[M+Na]^+$ 390.0637, Found: 390.0637.

(*E*)-1-((2-(4-nitrophenoxy)-6-methylquinolin-3-yl)methylene)thiosemicarbazide (5)

^{1}H NMR(400 MHz, DMSO d_6):δ = 2.47 (d, *J* = 6.8 Hz, 3H), 7.53 (t, 4H), 7.69 (s, 1H), 8.21 (s,1H), 8.33 (d, *J* = 12 Hz, 2H), 8.41 (s, 1H), 8.46 (s, 1H), 9.19 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.44, 119.62, 122.62, 126.03, 126.63, 127.31, 127.52, 133.49, 135.85, 135.97, 136.29, 144.27, 144.49, 157.93, 159.39, IR (KBr, cm^{-1}): ν_{max} 3480, 3353, 3171, 3012, 1588, 1537, 1415, 1336, 1101;HRMS (ESI) *m/z*Calcd for $C_{18}H_{15}N_5O_3SNa^+[M+Na]^+$ 404.0793, Found: 404.0796.

(*E*)-1-((2-(2-nitrophenoxy)quinolin-3-yl)methylene)thiosemicarbazide (6)

^{1}H NMR(400 MHz, DMSO d_6):δ = 7.58 (m, 5H), 7.9 (m, 2H), 8.2 (d, *J* = 8Hz, 1H), 8.27 (s, 1H), 8.45 (s, 1H), 8.53 (s, 1H), 9.27 (s, 1H), 11.75 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 118.97, 126.13, 126.20, 126.40, 127.11, 127.24, 128.82, 131.46, 135.49, 136.05, 136.85, 142.71, 145.44, 145.93, 158.22, 178.82;IR (KBr, cm^{-1}): ν_{max}3499, 3371, 3170, 3034, 1590, 1544, 1414, 1351, 1093; HRMS (ESI) *m/z*Calcd for $C_{17}H_{13}N_5O_3SNa^+[M+Na]^+$ 390.0637, Found: 390.0644.

(*E*)-1-((2-(2-nitrophenoxy)-6-methylquinolin-3-yl)methylene)thiosemicarbazide (7)

^{1}H NMR(400 MHz, DMSO d_6):δ = 2.48 (d, *J* = 16 Hz, 3H), 7.41 (d, *J* = 8 Hz, 1H), 7.49 (m, 1H), 7.58 (t, 1H), 7.66 (t, 2H), 7.89 (m, 1H), 8.19 (dd, *J* = 1.2 Hz, 1.1 Hz, 1H) 8.27 (s, 1H), 8.46 (s, 1H), 8.53 (s, 1H), 9.19 (s, 1H), 11.76 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.38, 118.84, 126.14, 126.38, 127.02, 127.56, 133.46, 135.71, 135.96, 136.20, 142.74, 143.87, 146.01, 157.79, 178.81;IR (KBr, cm^{-1}): ν_{max} 3420, 3353, 3162, 3015, 3022, 1593, 1521, 1422, 1347, 1088; HRMS (ESI) *m/z*Calcd for $C_{18}H_{15}N_5O_3SNa^+[M+Na]^+$ 404.0793, Found: 404.0792.

(*E*)-1-((2-(4-chlorophenoxy)quinolin-3-yl)methylene)thiosemicarbazide (8)

^{1}H NMR(400 MHz, DMSO d_6):δ = 7.34 (d, *J* = 8 Hz, 2H), 7.58 (m, 5H), 7.9 (d, *J* = 11.12 Hz, 1H), 8.24 (s, 1H), 8.42 (s, 1H), 8.51 (s, 1H), 9.24 (s, 1H), 11.71 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 119.42, 124.30, 126.14, 126.23, 127.37, 128.72, 129.50, 130.01, 131.20, 136.00, 136.47, 145.91, 152.52, 159.14, 178.74;IR (KBr, cm^{-1}): ν_{max}3478, 3351, 3172, 3019, 1593, 1538, 1485, 1160, 1084, 821; HRMS (ESI) *m/z*Calcd for $C_{17}H_{13}ClN_4OSNa^+[M+Na]^+$ 379.0396, Found: 379.0392.

(*E*)-1-((2-(4-chlorophenoxy)-6-methylquinolin-3-yl)methylene)thiosemicarbazide (9)

^{1}H NMR(400 MHz, DMSO d_6):δ = 2.46 (d, *J* = 16 Hz, 3H), 7.30 (d, *J* = 12 Hz, 2H), 7.50 (d, *J* = 16 Hz, 4H), 7.65 (s, 1H), 8.19 (s,1H), 8.39 (s, 1H), 8.48 (s, 1H), 9.12 (s, 1H), 11.68 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.40, 119.31, 124.20,

126.22, 127.16, 127.49, 129.38, 129.97, 133.24, 135.40, 135.86, 136.15, 144.36, 152.64, 158.68, 178.73;IR (KBr, cm^{-1}): ν_{max} 3478, 3351, 3172, 3019, 1587, 1534, 1485, 1247, 817; HRMS (ESI) *m/z*Calcd for $C_{18}H_{15}ClN_4OSNa^+[M+Na]^+$ 393.0553, Found: 393.0553.

(*E*)-1-((2-(4-chlorophenoxy)-6-methoxyquinolin-3-yl)methylene)thiosemicarbazide (10)

^{1}H NMR(400 MHz, DMSO d_6):δ = 3.87 (s, 3H), 7.30 (m, 4H), 7.52 (t, 3H), 8.18 (s, 1H), 8.42 (s, 1H), 8.48 (s, 1H), 9.15 (s, 1H), 11.70 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 55.86, 106.82, 119.53, 123.12, 124.00, 127.11, 128.80, 129.22, 129.97, 135.40, 136.08, 141.55, 152.84, 157.04, 157.70, 178.77;IR (KBr, cm^{-1}): ν_{max}3487, 3320, 3161, 3022, 1612, 1578, 1541, 1492, 1353, 1089, 820.06; HRMS (ESI) *m/z*Calcd for $C_{18}H_{15}ClN_4O_2SNa^+[M+Na]^+$ 409.0502, Found: 409.0509.

ANTIOXIDANT ACTIVITY

Preparation of test solution

Each compound (10 mg) was dissolved in 10 mL of methanol to make a clear solution. In order to prepare test solutions varying in concentrations from 10-50 µg/mL , 10 µL, 20 µL, 30 µL, 40 µL and 50 µL equivalent to 10 µg, 20 µg, 30 µg, 40 µg and 50 µg respectively were pipetted out from each of the solutions and was made up to 100 µL with methanol .

Free radical scavenging activity on 1,1-diphenyl-2-picrylhydrazyl (DPPH)[10]

Methanolic solution of 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM, 5 mL)was added to each of the sample solutions. The mixtures were shaken vigorously and allowed to stand for 20 minutes at 27 ˚C, after which their light absorbance was measured at 517 nm. Blank absorbance was measured with pure methanol and the DPPH solution as control. Each experiment was performed in triplicate. The radical scavenging activity of the tested compound was expressed as the inhibition percentage of free radical by the sample and was calculated using the following formula.

DPPH radical scavenging activity (%) = [(control OD – Sample OD)/Control OD)] x100.

ABTS radical cation decolourization assay[11]

2,2-Azinobis(3-ethylbenzothiozoline-6-sulfonic acid) cation radical ($ABTS^{+\cdot}$) was produced by reacting ABTS (7.0 mM) with ammonium persulfate (2.45 mM) and was allowed to stand in dark at room temperature for 12-16 h before use. Solutions of test compounds at concentrations ranging from 10-50 µg/mL were made up to 500 µL with DMSO and to each one of these were added 300 µL of ABTS solution; the final volume was made up to 1.0 mL with ethanol. The solutions were incubated in dark for 30 min at room temperature. The absorbance was read at 745 nm with DMSO as blank. Each experiment was performed thrice. The radical cation decolourization activity was expressed as inhibition percentage of cations by the sample and was calculated using the following formula.

ABTS radical scavenging activity (%) = [(control OD – Sample OD)/Control OD)] x100.

The results of the observation are presented in Table No.2, and in graphical form in Figure No.1 and Figure No.2.

ANTIMICROBIAL ACTIVITY MEASUREMENT

Bacterial susceptibility test

The activity against Gram negative and Gram positive bacteria was tested using the agar well diffusion method[12]. Nutrient agar, purchased from HiMedia, India, was used as bacteriological medium. The test compounds were dissolved in 10% aqueous DMSO solvent to a final concentration of 100 µg/100 µL. Pure DMSO was taken as negative control and100 µg/100 µL solutions in 10% aqueous DMSO of ofloxacin and ampicillin were taken as the positive controls. Inoculum (100 µL) was aseptically introduced on to the surface of sterile agar plates and sterilized cotton swabs were used for the even distribution of the inoculum. Wells were prepared in the agar plates using a sterile cork borer of 6.0 mm diameter. Solutions (100 µL) of test and control compounds were introduced in the well. The same procedure was used for all the strains. The plates were incubated aerobically at 35 ˚C and examined after

24 h[13]. The diameter of the zone of inhibition produced by each test sample was measured and compared with those of commercial antibiotics ofloxacin and ampicillin.

Fungal susceptibility test

The antifungal activity of the title compounds was tested using agar well diffusion method. The potato dextrose agar plates were inoculated with 10 days old cultures of *Aspergillus niger* and *Aspergillus flavus* by point inoculation. A well of about 6.0 mm diameter with sterile cork borer was aseptically punched on each agar plate. The test compounds, each of 100 µg/100 µL concentration, were introduced into the well. A negative control well was prepared with 100 µL of pure DMSO and a positive control well of 100 µg/100 µL of fluconazole was also prepared. The plates were kept in laminar flow for 30 minutes for pre-diffusion of compounds to occur followed by incubation at 28 ˚C for 48 h. The resulting zones of inhibition were measured (in mm) using HiMedia zone scale[14].

The results of bacterial and fungal susceptibility tests are given in Table No.3.

ANTIPROLIFERATIVEACTIVITY MEASUREMENT

Cell culture

HeLa cell line was maintained in DMEM medium (GIBCO) supplemented with 10% (v/v) heat-inactivated fetal bovine serum (FBS) and 1% antibiotic solution (penicillin 100 Uml-1and streptomycin 100µgml-1) at 37 ˚C in a humidified atmosphere of 95% air/5% CO_2. The medium was changed at 2-day intervals until they reach 95% confluency. The confluent cells were subcultured with 0.25% trypsin containing 0.02% ethylene-diaminetetraacetic acid (EDTA) in PBS for 3 min at 37 ˚C.

MTT Assay

The MTT assay was carried out as described previously to measure cell viability[15]. Ten thousand cells in 100 µL of DMEM media were seeded in the wells of a 96-well plate. After 24 h, the existing media was removed and 100 µL of various concentrations of complexes were added and incubated for 48 h at 37 °C in a CO_2 incubator. Control cells were supplemented with 0.05% DMSO vehicle. At the 48th hour of incubation, MTT (3-(4,5-dimethylthaizol-2-yl)-2,5-diphenyl tetrazolium bromide, obtained from Sigma, 10 µL of 5 mg/mL) was added to the plate. The contents of the plate were pipetted out carefully, the formazan crystals formed were dissolved in 100 µL of DMSO, and the absorbance was measured at 550 nm in a microplate reader (Tecan, infinite F200 Pro). Experiments were performed in triplicate, and the results were expressed as mean of percentage inhibition. A graph of the concentration versus percentage growth inhibition was plotted, and the concentration at which 50% cell death occurred was considered as the IC_{50} value. Before adding MTT, bright field images (Olympus 1X81, cellSens Dimension software) were taken for visualizing the cell death.

The results of the observation are presented in Table No.4; Microscopy images representing the cell death caused by the compounds are shown in Figure No.3.

MOLECULAR DOCKING STUDIES

The three dimensional structure of target protein EGFR tyrosine kinase having keyword 2J5F was downloaded from PDB (www.rcsb.org/pdb) structural database (Figure No.6). This file was then opened in SPDB viewer edited by removing the hetero atoms and adding C terminal oxygen. The active pockets on target protein molecule were found out using CASTp server[16]. The ligands were drawn using ChemDraw Ultra 6.0 and assigned with proper 2D orientation (ChemOffice package). 3D coordinates were prepared using PRODRG server[17].Autodock V3.0 was used to perform Automated Molecular Docking in AMD Athlon (TM)2x2 215 at 2.70 GHz, with 1.75 GB of RAM. AutoDock 3.0 was compiled and run under Microsoft Windows XP service pack 3. For docking, grid map is required in AutoDock, the size of the grid box was set at 102, 126 and 118 Å (R, G, and B), and grid center -58.865, -8.115, -24.556 for x, y, and z-coordinates. All torsions were allowed to rotate during docking. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods

were applied for minimization, using default parameters[18]. The newly synthesized compounds were taken as ligands and docked against target molecule EGFR tyrosine kinase.

The results of the studies are presented in Table No.5 and in Figure No.4 and Figure No.5.

DRUG LIKENESS AND BIOACTIVITY ESTIMATION[19]

Lipinski's rule of five is commonly employed for evaluation of a chemical compound for its drug-like properties, which can be predicted by *in silico* methods. For the purpose of calculating drug-likeness and bioactivity of our test compounds 1-10, we have used the well-known Molinspiration software program. The structures of the compounds were entered through the use of text file containing SMILES (simplified molecular input line entry system) notations. The structures of the derivatives 1-10 in SMILES notations are given in Table No.6.

Drug likeness calculation on the basis of Lipinski's rule of five (RO5)

The drug likeness score was calculated by considering MilogP (partition coefficient calculated using molinspiration), number of heavy atoms, number of hydrogen donor, number of hydrogen acceptor and number of violation, number of rotatable bonds, molecular weight, and volume.

Lipinski's rule states:

- An octanol-water partition coefficient log P not greater than 5
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- A molecular mass less than 500 Daltons
- No more than one number of violations.

The estimated physical properties to check compliance of Lipinski's rule of five are presented in Table No.7.

Bioactivity estimation

Bioactivity of a drug can be checked by calculating the activity score of GPCR ligand, ion channel modulator, nuclear receptor legend, kinase inhibitor, protease inhibitor, enzyme inhibitor. Drug likeness score of each compound was calculated and compared with the specific activity of the compound, and the results were compared with the score for standard drug. For organic molecules the probability is as follows: if the bioactivity score is >0, then it is active, if it is -5.0-0.0 then it is moderately active, and if it is < -5.0 then it is inactive.

The estimated bioactivity values are provided in Table No.8.

RESULTS AND DISCUSSION

Preparation of aryloxy quinoline thiosemicarbazides (1-10)

For the present study we have synthesized(*E*)-1-((2-phenoxyquinolin-3-yl)methylene)thiosemicarbazide (1) and its substituted derivatives (2-10) by reacting 2-chloroquinoline-3-carbaldehydes and its 6-substituted derivatives with phenols and its 2- and 4- monosubstituted derivatives, and the products formed were then treated with thiosemicarbazide. The required 2-chloroquinoline-3-carbaldehydes were prepared from acetanilide and its 4-substituted derivatives employing Vilsmeier-Haack procedure. The synthetic route is presented in scheme No.1.

The starting material 2-chloro-3-formylquinolines were prepared according to literature procedure [1e]by Vilsmeier-Haack reaction and converted to aryloxyquinoline-3-carbaldehydes by nucleophilic displacement of chloro group at C2 in 2-chloro-3-formylquinolines with phenols in refluxing dimethylformamide using anhydrous potassium carbonate as base[8]. Subsequently, the obtained aryl ethers were condensed with thiosemicarbazide in DMF to afford the target compounds in good yields (Table No.1), which were identified by their IR, NMR and HRMS data.

BIOLOGICAL EVALUATION

To understand the potential pharmacological properties of the synthesized compounds, we carried out several *in vitro* as well as *in silico* studies. The *in vitro* investigations included free radical scavenging activity, antibacterial activity, fungal susceptibility and anti-proliferative activity. The *in silico* studies included calculation of

compliance of Lipinski's Rule of Five, bioactivity using Molinspiration program and docking on EGFR tyrosine kinase using Autodock V3.0 program. All the studies have clearly indicated that aryloxy quinoline thiosemicarbazides1-10have a good potential for being developed as therapeutically useful compounds.

Antioxidant activity

All the compounds1-10 were tested for their scavenging capacity of DPPH and ABTS free radicals, employing established procedures[10,11], with ascorbic acid serving as standard scavenging agent. The results are presented in Table No.2 and in graphical form in Figure No.1 and Figure No.2. The results show that all the compounds 1-10 possess good free radical scavenging property. However, the scavenging activity of DPPH free radicals markedly differs from that of ABTS free radicals, the former being generally more efficiently scavenged. An important outcome of these tests is that compounds1, 2, 8, 9 and 10 are more efficient (lowest IC_{50} value) than ascorbic acid in scavenging DPPH free radicals, while in the case of ABTS, the best scavenging efficiency is shown by the compound 9. Considering all the antioxidant investigation data, we can say that compound 9has the highest percentage of scavenging ability and the lowest IC_{50} value, including ascorbic acid used as standard.

Antimicrobial activity

Antimicrobial activity was studied using the Gram positive bacteria, *Staphylococcus aureus* and *Bacillus subtilis* and Gram negative bacterium *Escherichia coli*, and the fungi *Aspergillus niger* and *Aspergillus flavus*. Antibacterial activity was compared with the standard antibiotics ofloxacin and ampicillin; for antifungal activity fluconazole was used as standard. The activity values measured in terms of zones of inhibition are given in Table No.3. The results reveal that all the tested compounds possess reasonably good antibacterial and antifungal activity. In particular, the compounds 5, 6 and 10 (against *E. coli*) and 3, 6 and 9 (against *S. aureus)* and10 (against *B. subtilis*) exhibit good antibacterial activity which is close to that exhibited by the standard antibiotics. The compounds 4, 8 and 9also show the best antifungal activity against both *A. niger* and *A. flavus* which is very close to that observed for the standard compound fluconazole.

Antiproliferative activity

All the compounds have shown *in vitro* cytotoxic effect at different concentrations ranging from 100-500 µg/mL in a dose dependent manner, against human cervical carcinoma (HeLa) cell line. Out of the tested compounds 7 has shown to have good antiproliferative activity, which is followed by 3, 4 and 5when compared to standard drug doxorubicin. The test values obtained demonstrate that all the compounds possess cytotoxic effect in a dose dependent manner. All the tested compounds 1-10showed excellent inhibitory activity. Among these, the compound **7** showed IC_{50} at the lowest concentration of 25.9 ± 0.31. The results of these tests are given in Table No.4. Microscopy images representing the cell death caused by the compounds can be seen in Figure No.3. HeLa cells have been reported to contain human papilloma virus 18 (HPV-18) sequences, a low expression of p53 and normal expression of pRB (retinoblastoma suppressor)[20]. The cytotoxicity of the test compounds may be attributed to the possible effect of p53, which modulates several cellular processes including apoptosis, cell cycle arrest and DNA repair[21]. It is the target of point mutations and small deletions and insertions that lead to total or partial abolition of protein function. But the epidermal growth factor receptor is the first identified member of the type I receptor tyrosine kinase family and is a major regulator of several distinct, diverse cellular pathways. More experiments are required to understand the exact mechanism by which the cells are affected. It is important to correlate the structure of these compounds with their biological effect, which will be valuable to propose new lead compounds with better cytotoxic potential.

Molecular docking

Based on the promising *in vitro* anticancer results, it was thought worthwhile to perform molecular docking studies and screening by considering EGFR tyrosine kinase as the target receptor in order to uncover supportive correlation between the data from *in silico* studies and the *in vitro* results. Each

of the ten compounds was docked to get the best conformer. The results were analyzed for the binding energy, docking energy and the number of hydrogen bonds formed.

The docking of the ten compounds1-10and the standard (doxorubicin) with EGFR tyrosine kinase domain revealed that those compounds which have inhibitory capability are exhibiting interactions with one or the other amino acid in the active pockets as shown in Figure No.4andFigure No.5. The topology of the active site of EGFR tyrosine kinase was similar in all synthesized molecules, which is lined by interacting amino acids as predicted from the ligplot (Figure No.6).The docking results for inhibitor compounds are documented in Table No.5. Binding energies in the protein–ligand interactions explain how cogently the ligand binds with the protein. In this study ligands 8, 7 and 9 have shown better binding energies (-9.24, -8.42 and -8.29 $kJmol^{-1}$ respectively). The receptor-ligand complex is stabilized by several favorable intermolecular interactions such as hydrogen bonds and hydrophobic contacts. Among the ten compounds studied, ligands 2, 3, 4, 5 and 7 were found to form three hydrogen bonds with the receptor active site residues and to show effective bonding. Considering the data in Table No.5 it is clear that 7 is the most efficient ligand as it has the most favorable conditions to bind to the receptor protein with three hydrogen bonds, the lowest docking energy (-10.04$kJmol^{-1}$), the second lowest binding energy (-8.42 $kJmol^{-1}$), second the lowest intermolecular energy (-9.67$kJmol^{-1}$), and the highest inhibitory constant, which are much better than even those of the drug doxorubicin used here as standard for comparison. It is noteworthy that the cytotoxicity properties obtained from *in vitro* studies for 7match the bioactivity obtained from docking study. On these grounds we consider that 7is a good inhibitor of EGFR tyrosine kinase, and hence has good potential to study further as anticancer agent.

Drug-likeness and Bioactivity Estimation

All *in vitro* bioactivity results indicate that the compounds 1-10have good pharmacological potential which is supported by computation of various physical properties of these compounds using Molinspiration software programme (Table No.7), which very clearly demonstrate that nine of them comply with Lipinski's rule of five, while the other one exhibits only one violation which is acceptable even in the case of a drug in use. The estimated bioactivity values are provided in Table No.8. The data given in Table No.7 and Table No.8 are self-explanatory and they undoubtedly lead us to the fact that the ten tested compounds possess drug-like properties.

Contents

- Yields and melting points of the aryloxy quinoline-3-carbaldehyde intermediates (1a–10a).
- Representative spectral data of intermediates
- Scanned copies of ^{1}H and ^{13}C NMR spectras of final products.

Table No.1: Yields and melting points of the aryloxy quinoline-3-carbaldehyde intermediates (13a–21a)

S.No	Compound	R	R^1	R^2	mp (˚C)	Yield (%)
1	1a	H	H	H	123	30
2	2a	CH_3	H	H	154	35
3	3a	OCH_3	H	H	187	24
4	4a	H	NO_2	H	165-168	42
5	5a	CH_3	NO_2	H	182	44
6	6a	H	H	NO_2	166	42
7	7a	CH_3	H	NO_2	182	44
8	8a	H	Cl	H	166	45
9	9a	CH_3	Cl	H	157	52
10	10a	OCH_3	Cl	H	178-180	45

Table No.1: Yields and melting points of the aryloxy quinoline thiosemicarbazides 1-10

Compound	R	R^1	R^2	mp (˚C)	Yield (%)
1	H	H	H	252	93
2	CH_3	H	H	237 - 239	81
3	OCH_3	H	H	247	63
4	H	NO_2	H	230	64
5	CH_3	NO_2	H	251	73
6	H	H	NO_2	236 - 238	79
7	CH_3	H	NO_2	230 - 231	74
8	H	Cl	H	235 - 236	98
9	CH_3	Cl	H	240	44
10	OCH_3	Cl	H	241	96

Table No.2: Scavenging activity and IC_{50} values of aryloxy quinoline thiosemicarbazides (1-10)

S.No	Compounds	Scavenging (%)		IC_{50} µg/mL	
		DPPH	ABTS	DPPH	ABTS
1	1	48.95^{b}	12.23^{f}	41.29^{c}	107.1^{e}
2	2	47.66^{c}	13.6^{e}	48.73^{e}	120.33^{f}
3	3	47^{c}	5.85^{h}	100^{i}	225.34^{h}
4	4	45.78^{d}	5.73^{h}	110.18^{j}	399.05^{j}
5	5	47.36^{c}	11.18^{g}	55.23^{g}	294.06^{i}
6	6	47.61^{c}	$11.86^{f,g}$	53.30^{f}	154.67^{g}
7	7	46.25^{d}	15.03^{d}	81.36^{h}	75.32^{c}
8	8	48.75^{b}	16.72^{c}	39.53^{b}	98.04^{d}
9	9	52.42^{a}	44.62^{b}	21.14^{a}	33.5^{a}
10	10	46.12^{d}	14^{e}	45.6^{d}	98.73^{d}
11	Ascorbic acid (std)	32.09^{a}	52.082^{a}	49.73^{e}	55.66^{b}

Note: All values are mean of 15 replications for each sample. Mean values denoted by superscripts ($^{a–i}$) differ significantly at $P < 0.01$ by Tukey (HSD) test.

Table No.3: Antimicrobial activity of aryloxy quinoline thiosemicarbazides (1-10)

S.No	Compounds	Zone of Inhibition (mm) X*± SE				
		Bacterial strains			Fungal strains	
		S. aureus	*B. subtillis*	*E. coli*	*A. niger*	*A. flavus*
1	Ofloxacin	18±0.00	20±0.00	20±0.00		
2	Ampicillin	22±0.00	20±0.00	15±0.00		
3	Fluconazole				25±0.00	25±0.00
4	1	9.33±0.33	8±0.57	8.66±0.33	6±0.00	6±0.57
5	2	9±0.57	9±0.57	9±0.00	6.33±0.33	6±0.57
6	3	13±0.57	12±0.00	10±0.57	10±0.00	10±0.57
7	4	10±0.00	10±0.57	9.66±0.33	19±0.57	18±0.57
8	5	12±0.00	12.33±0.33	12.33±0.33	14±0.00	16.33±0.33
9	6	13±0.00	12.33±0.33	12±0.00	6.33±0.33	8±0.00
10	7	9±0.57	10±0.00	9±0.00	9±0.00	6.33±0.33
11	8	12±0.00	12.33±0.33	9±0.00	18±0.00	18±0.57
12	9	13±0.00	12.33±0.33	10±0.00	18±0.00	18.33±0.33
13	10	10±0.57	13±0.57	12.33±0.33	6±0.00	6±0.00

Note: * - Mean of 3 replications, SE- Standard Error

Table No.4: *In vitro* cytotoxicity of 1-10 on human carcinoma cell line

S.No	compounds	IC_{50} values (in µg/mL)
1	1	180.5 ± 0.70
2	2	132.3 ± 0.16
3	3	32.3 ± 0.17
4	4	42.5 ± 0.14
5	5	53.2 ± 0.78
6	6	96.6 ± 0.95
7	7	25.9 ± 0.31
8	8	88.2 ± 0.17
9	9	105.2 ± 0.59
10	10	96.6 ± 0.95
11	Doxorubicin	7.6 ± 0.12

IC_{50} values (in µg/mL) are indicated as mean ± SD of three independent experiments.

Table No.5: Molecular docking with EGFR tyrosine kinase

S.No	Compounds	B.E[a]	D.E[b]	I.C[c]	I.E[d]	H-B[e]	Bonding
1	1	-6.0	-7.05	4e-005	-6.93	1	**1**::DRG1:HAX:TK:A:GLU866:OE1
2	2	-6.92	-8.3	8.53e-006	-7.85	3	TK1:A:HIS773:HE2:**2**::DRG1:NAW TK1:A:GLN820:HE22:**2**::DRG1:NAG TK1:A:LYS823:HZ3:**2**::DRG1:NAW
3	3	-5.41	-7.02	0.000109	-6.65	3	**3**::DRG1:HA1:TK2:A:GLN849:O **3**::DRG1:HAW:TK2:A:GLN849:O TK2:A:HIS850:HD1:**3**::DRG1:OAK
4	4	-6.29	-8.0	2.46e-005	-7.53	3	**4**::DRG1:HAT:TK3:A:ALA859:O **4**::DRG1:HAO:TK3:A:GLY857:O TK3:A:ARG836:HH21:**4**::DRG1:OAX
5	5	-6.35	-7.95	2.2e-005	-7.6	3	**5**::DRG1:HAZ:TK4:A:GLU1015:OE1 TK4:A:ASN771:HN:**5**::DRG1:OBA TK4:A:ARG776:HN:**5**::DRG1:OAZ
6	6	-5.96	-8.02	4.27e-005	-7.21	2	TK5:A:GLN701:HN:**6**::DRG1:OAW TK5:A:ARG831:HH12:**6**::DRG1:NAG
7	7	-8.42	-10.04	6.73e-007	-9.67	3	TK6:A:GLY696HN2:**7**::DRG1:OAZ TK6:A:ALA698:HN:**7**::DRG1:OAZ TK6:A:ARG832:HH21:**7**::DRG1:OBA
8	8	-9.24	-9.22	1.02e-007	-9.8	1	**8**:: DRG:HAG:TK:B:ASP804:OD
9	9	-8.29	-8.46	3.46e-007	-9.03	1	**9**:: DRG:HAA:TK:B:ALA637:O
10	10	-6.04	-7.44	3.12e-006	-7.86	1	**10**:: DRG:HAC:TK:B:ASP742:OD2
11	Doxorubicin	-5.8	-7.55	5.61e-005	-7.36	3	DOX::DRG1:HAZ:TK10:A:VAL876:O TK10:A:ARG803::HH21:DOX::DRG1:OAK TK10:A:ILE878:HN:DOX::DRG1:OAZ

[a] Binding energy, [b] Docking energy, [c] Inhibitory constant, [d] Intermol energy, [e] Hydrogen bonds

Table No.6: Smiles representation of the derivatives 1-10 used for the calculations

compound	Smiles Notation
1	NC(=S)NN=Cc2cc1ccccc1nc2Oc3ccccc3
2	Cc3ccc2nc(Oc1ccccc1)c(C=NNC(N)=S)cc2c3
3	COc3ccc2nc(Oc1ccccc1)c(C=NNC(N)=S)cc2c3
4	NC(=S)NN=Cc2cc1ccccc1nc2Oc3ccc(N(=O)=O)cc3
5	Cc3ccc2nc(Oc1ccc(N(=O)=O)cc1)c(C=NNC(N)=S)cc2c3
6	NC(=S)NN=Cc2cc1ccccc1nc2Oc3ccccc3N(=O)=O
7	Cc3ccc2nc(Oc1ccccc1N(=O)=O)c(C=NNC(N)=S)cc2c3
8	NC(=S)NN=Cc2cc1ccccc1nc2Oc3ccc(Cl)cc3
9	Cc3ccc2nc(Oc1ccc(Cl)cc1)c(C=NNC(N)=S)cc2c3
10	COc3ccc2nc(Oc1ccc(Cl)cc1)c(C=NNC(N)=S)cc2c3

Table No.7: Estimated physical properties and Lipinski's RO5 for 1-10 by Molinspiration

S.No	Compounds	miLogP[a]	TPSA[b]	Natoms[c]	MW[d]	nON[e]	nOHNH[f]	nviol[g]	nrotb[h]	volume[i]
1	1	3.911	72.54	23.0	322.393	5	3	0	5	279.085
2	2	4.336	72.54	24.0	336.42	5	3	0	5	295.646
3	3	3.944	81.77	25.0	352.419	6	3	0	6	304.631
4	4	3.87	118.36	26.0	367.39	8	3	0	6	302.419
5	5	4.295	118.36	27.0	381.417	8	3	0	6	318.98
6	6	3.822	118.36	26.0	367.39	8	3	0	6	302.419
7	7	4.247	118.36	27.0	381.417	8	3	0	6	318.98
8	8	4.589	72.54	24.0	356.83	5	3	0	5	292.621
9	9	5.014	72.54	25.0	370.865	5	3	1	5	309.182
10	10	4.622	81.77	26.0	386.864	6	3	0	6	318.167
11	Ofloxacin	0.262	75.014	26	361.37	7	1	0	2	311.15
12	Ampicillin	-0.873	112.73	24	349.41	7	4	0	4	298.87
13	Flucanazole	-0.118	81.664	22	306.28	7	1	0	5	248.96
14	Doxorubicin	0.57	206.08	39	543.52	12	7	3	5	459.18

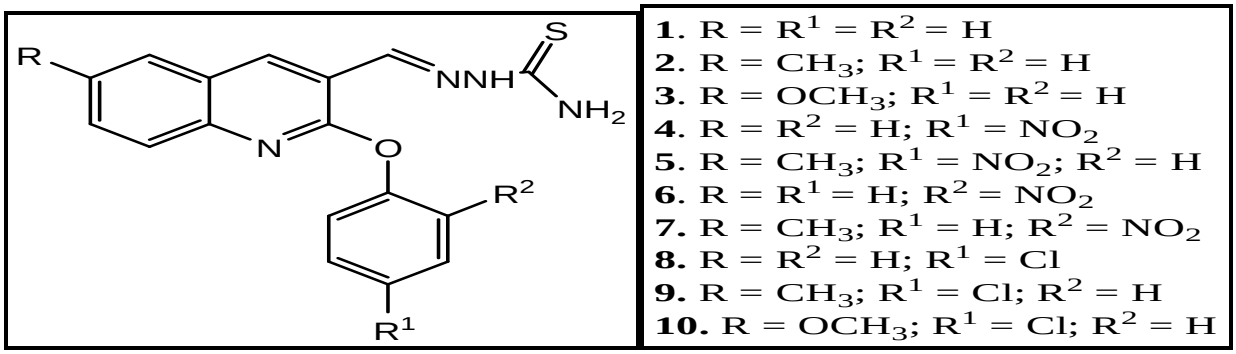
[a]Octanol-water partition coefficient.[b]Molecular polar surface area. [c]Number of non-hydrogen atoms. [d]Molecular weight. [e]Number of hydrogen bond acceptors (O and N atoms). [f]Number of hydrogen bond donors (OH and NH groups). [g]Number of Rule of 5 violations.

[h]Number of rotatable bonds. [i]Molecular volume

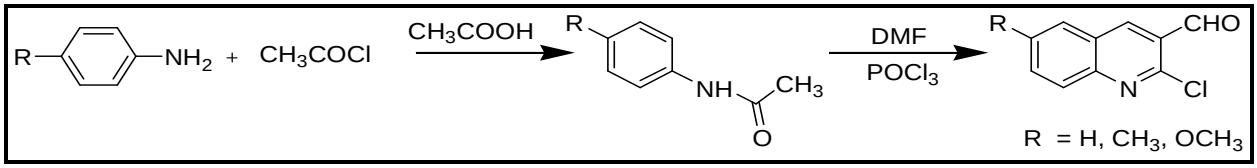
Table No.8: Bioactivity of compounds 1-10 calculated by Molinspiration

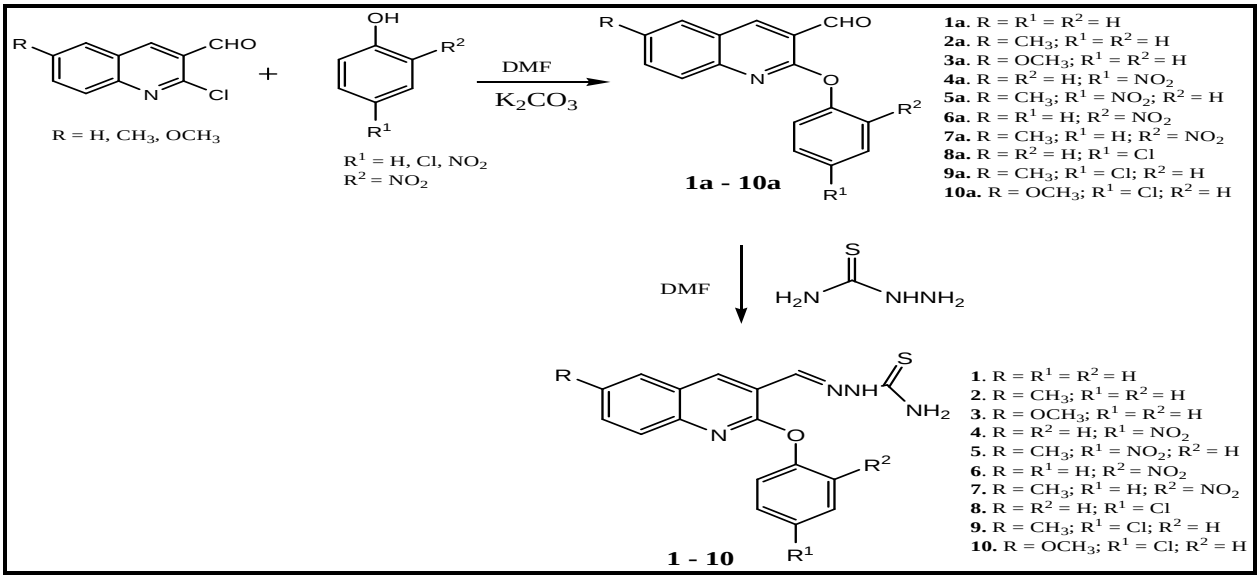
S.No	Compounds	GPCR ligand	Ion channel modulator	Kinase inhibitor	Nuclear receptor ligand	Protease inhibitor	Enzyme inhibitor
1	1	-0.53	-0.54	-0.27	-0.76	-0.39	-0.13
2	2	-0.56	-0.61	-0.32	-0.76	-0.42	-0.20
3	3	-0.52	-0.56	-0.26	-0.70	-0.39	-0.15
4	4	-0.62	-0.54	-0.38	-0.77	-0.47	-0.23
5	5	-0.65	-0.59	-0.42	-0.77	-0.51	-0.28
6	6	-0.67	-0.69	-0.41	-0.81	-0.52	-0.25
7	7	-0.69	-0.74	-0.45	-0.82	-0.55	-0.31
8	8	-0.51	-0.53	-0.28	-0.75	-0.40	-0.16
9	9	-0.54	-0.59	-0.33	-0.75	-0.44	-0.22
10	10	-0.50	-0.55	-0.27	-0.69	-0.42	-0.18
11	Ofloxacin	0.23	-0.14	-0.06	-0.13	-0.26	0.35
12	Ampicillin	0.04	-0.47	-0.71	-0.61	0.87	0.25
13	Fluconazole	0.04	0.01	-0.09	-0.23	-0.09	0.03
14	Doxorubicn	0.20	-0.20	-0.07	0.32	0.67	0.66

GENERAL STRUCTURE FOR COMPOUNDS 1-10



Scheme No.1





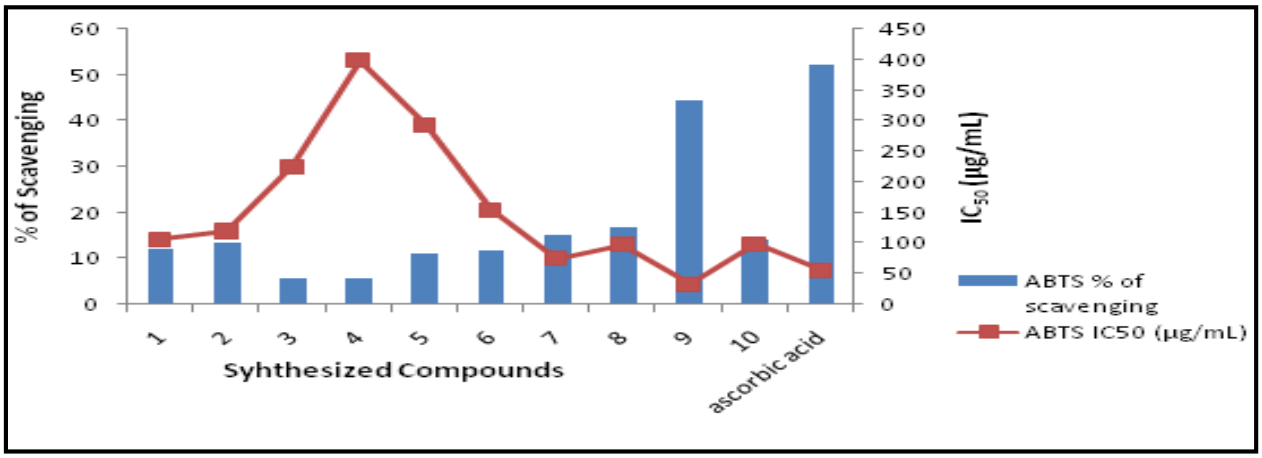


Figure No.1: ABTS scavenging activity of different compounds and its IC_{50} (µg/mL)

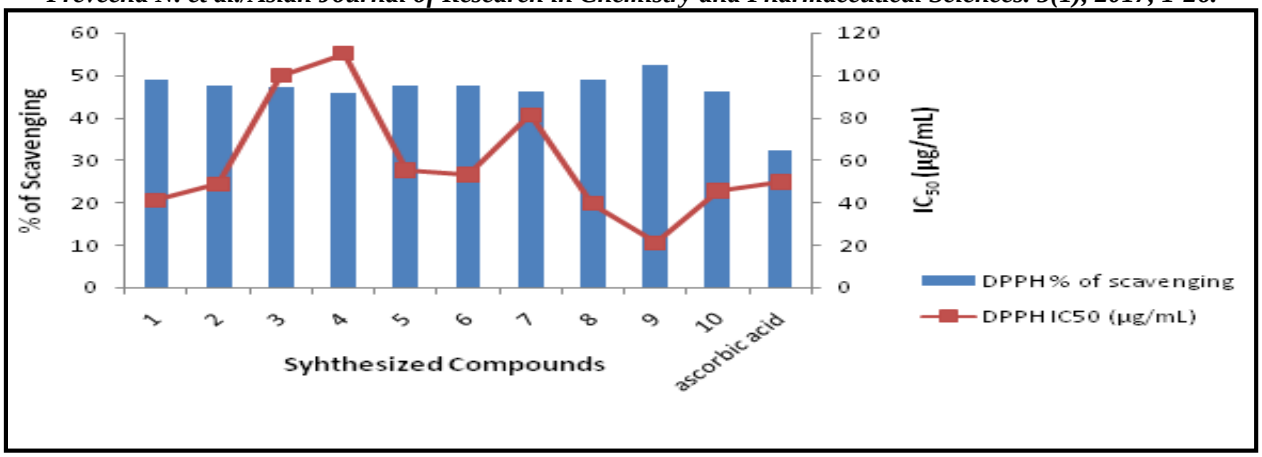


Figure No.2: DPPH scavenging activity of different compounds and its IC_{50} (µg/mL)

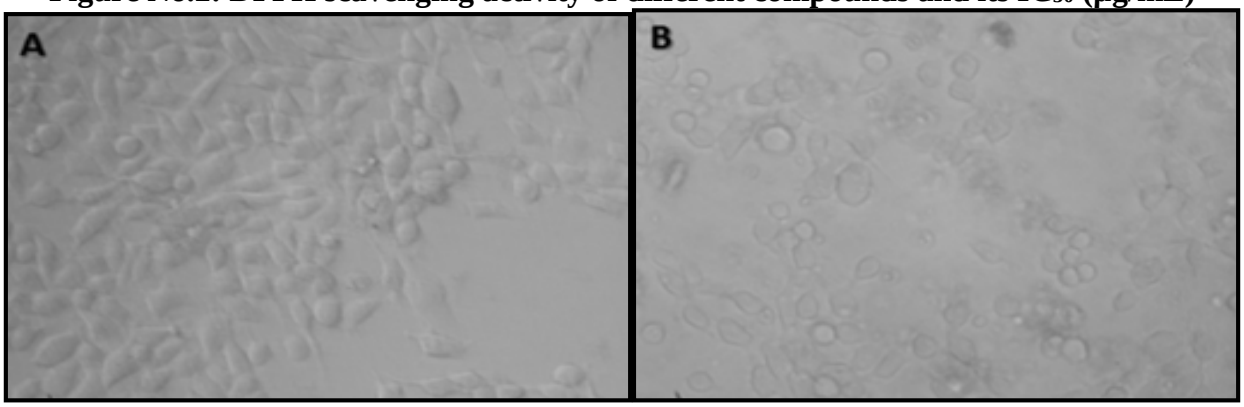


Figure No.3: Anticancer activity of compounds showing cell death, A-control; B-treated

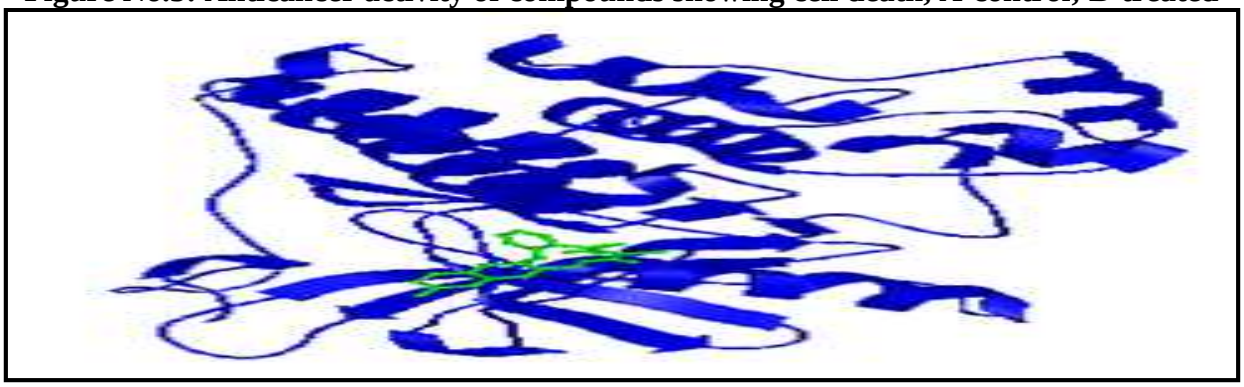


Figure No.4: Enfolding of compound 7 in the active pocket of EGFR tyrosine kinase

Figure No.5: Enfolding of Doxorubicin in the active pocket of EGFR tyrosine kinase

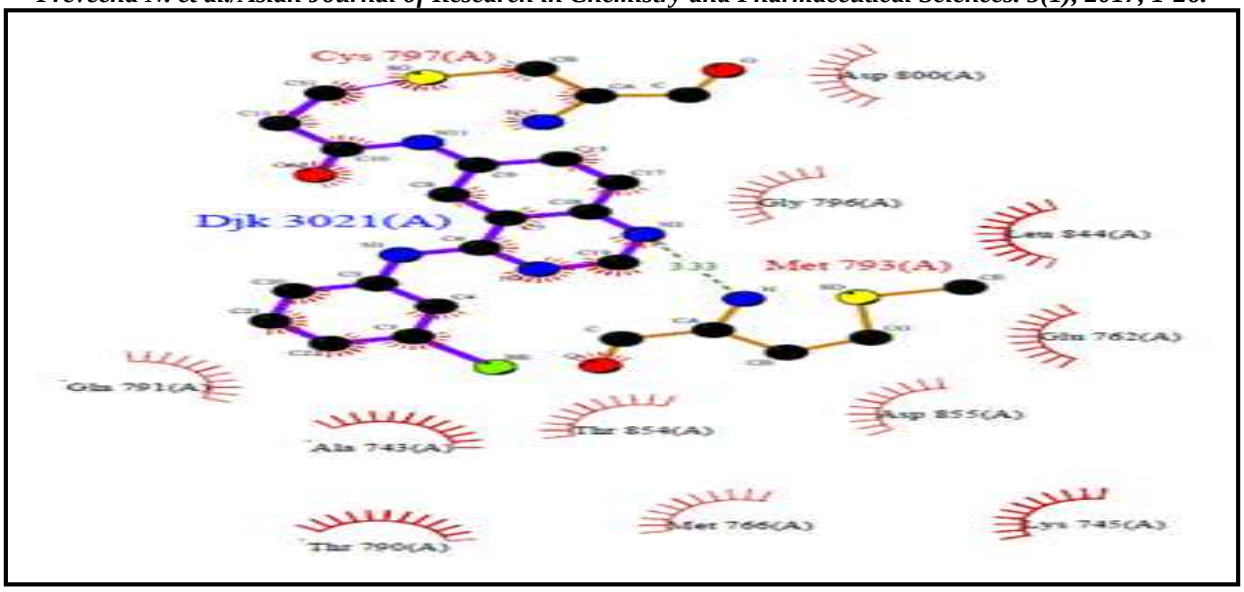
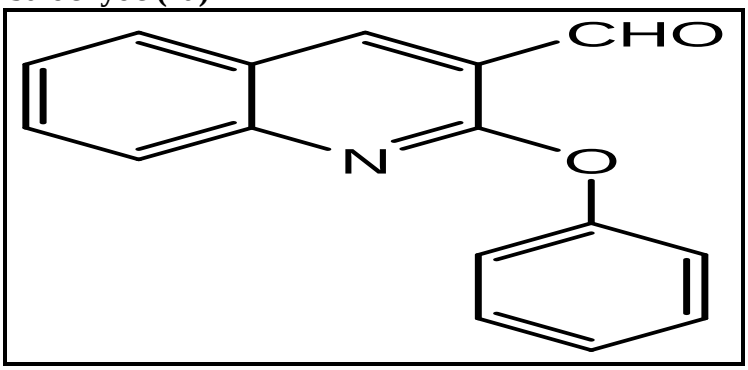


Figure No.6: Interacting amino acids as predicted from the ligplot

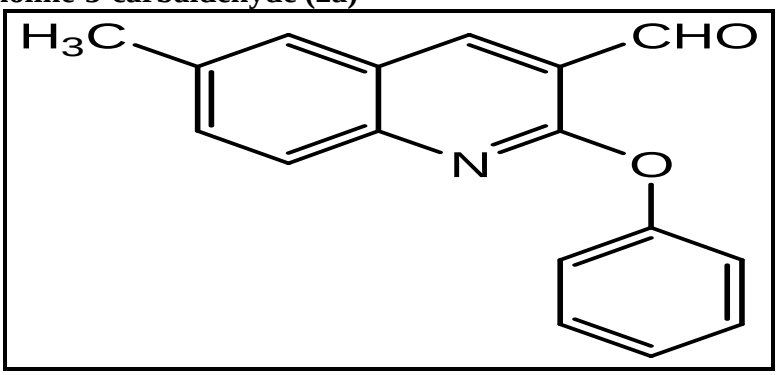
REPRESENTATIVE SPECTRAL DATA OF INTERMEDIATES

2-Phenoxyquinoline-3-carbaldehyde (1a)



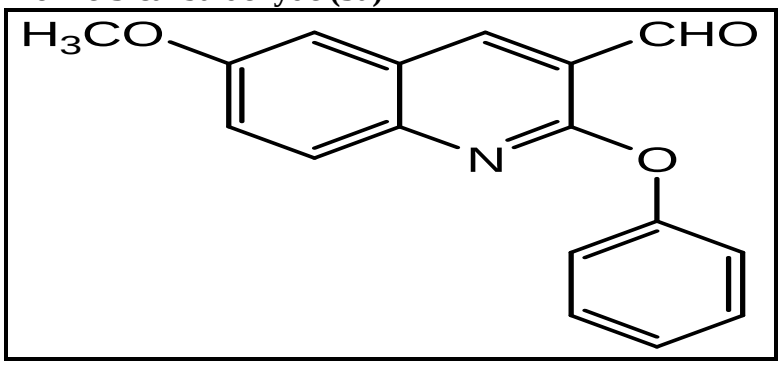
^{1}H NMR (400 MHz, DMSO d_6): δ = 7.45 (s, 1H), 7.54 (m, 3H), 7.77 (s, 2H), 7.95 (d, *J* = 8 Hz, 1H), 8.36 (d, *J* = 9.2 Hz, 2H), 8.81 (s, 1H), 10.62 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 115.70, 120.05, 122.40, 125.51, 125.61, 126.28, 126.60, 127.83, 129.83, 133.35, 141.50, 144.89, 148.21, 158.11, 159.33, 188.18.

6-Methyl-2-phenoxyquinoline-3-carbaldehyde (2a)



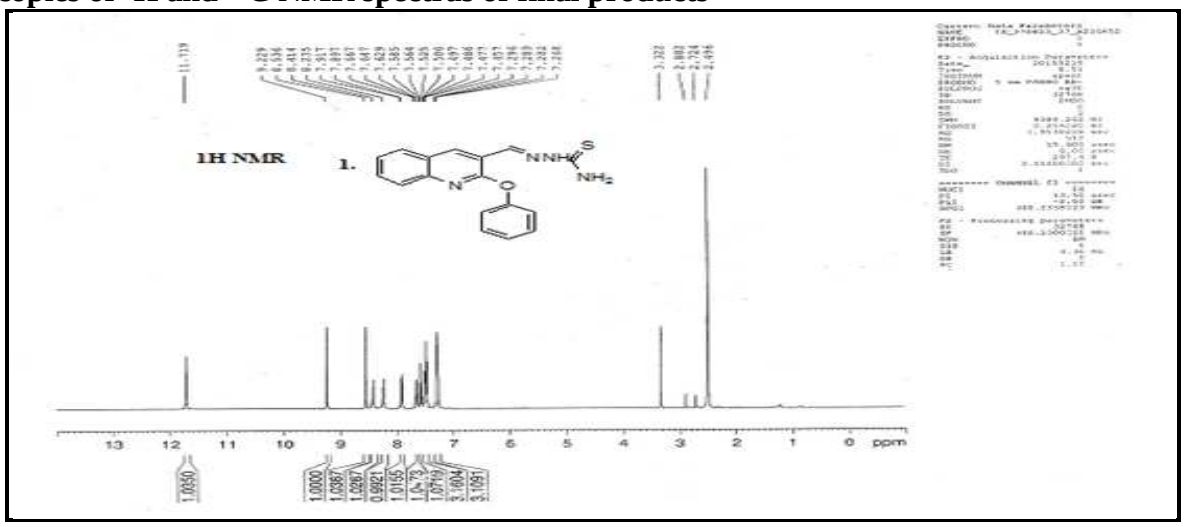
^{1}H NMR (400 MHz, DMSO d_6): δ = 2.49 (s, 3H), 7.28 (m, 3H), 7.46 (m, 2H), 7.53 (d, *J* = 8.44 Hz, 1H), 7.64 (d, *J* = 7.24 Hz, 2H), 8.65 (s, 1H), 10.64 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 21.37, 120.18, 121.80, 125.15, 125.22, 127.60, 128.52, 129.58, 135.05, 135.72, 139.92, 147.12, 153.25, 160.16, 189.23.

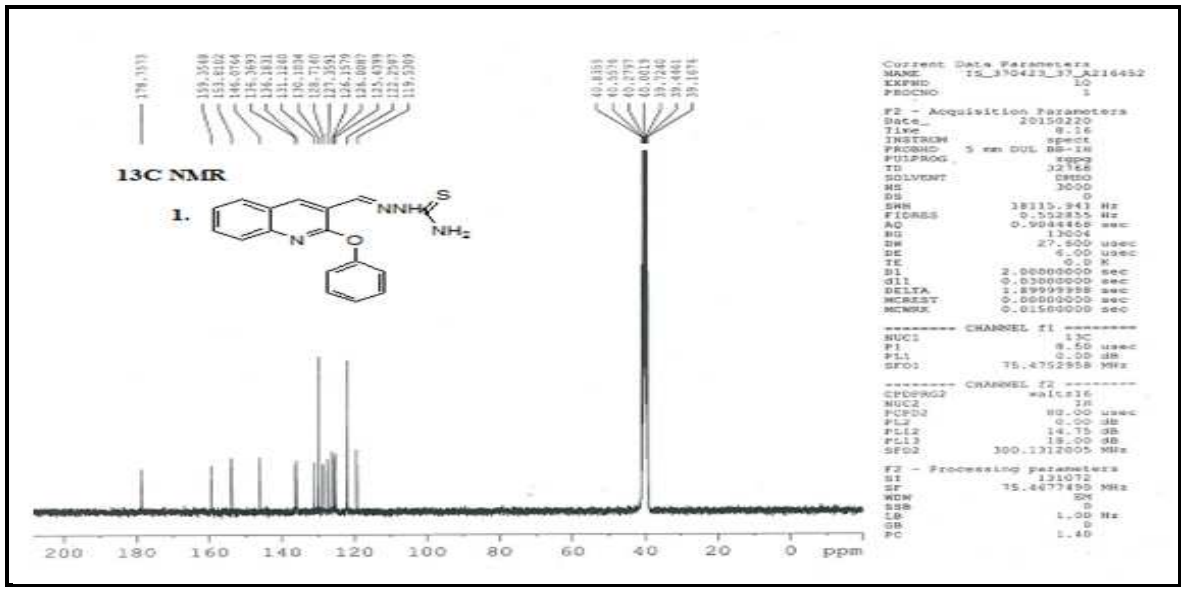
6-Methoxy-2-phenoxyquinoline-3-carbaldehyde (3a)

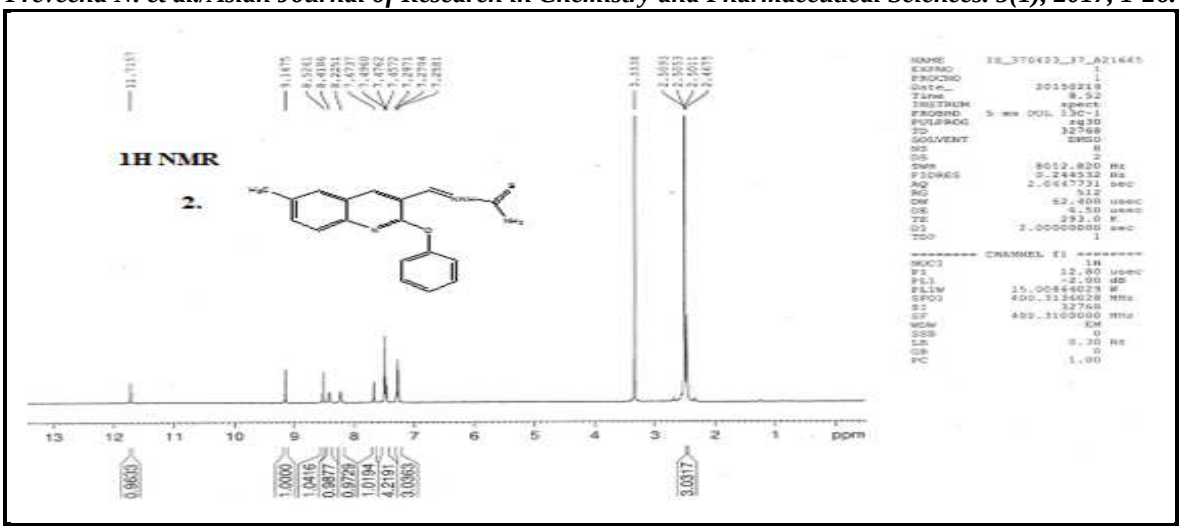


^{1}H NMR (400 MHz, DMSO d_6): δ =3.91 (s, 3H), 7.15 (s, 1H), 7.25 (m, 3H), 7.35 (m, 1H), 7.45 (m, 2H), 7.65 (d, *J* = 9.2 Hz, 1H), 8.64 (s, 1H), 10.64 (s, 1H); ^{13}C NMR (400 MHz, DMSO d_6): δ = 55.70, 106.98, 120.25, 121.67, 125.06, 125.32, 126.01, 129.24, 129.59, 139.09, 144.52, 153.41, 157.27, 159.33, 189.26.

Scanned copies of ^{1}H and ^{13}C NMR spectras of final products



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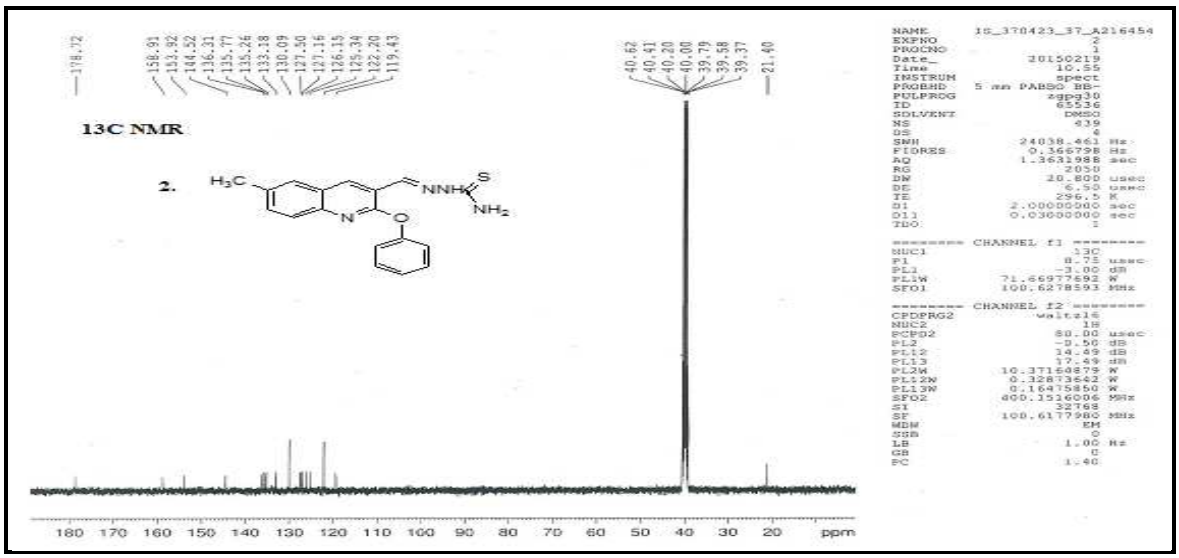
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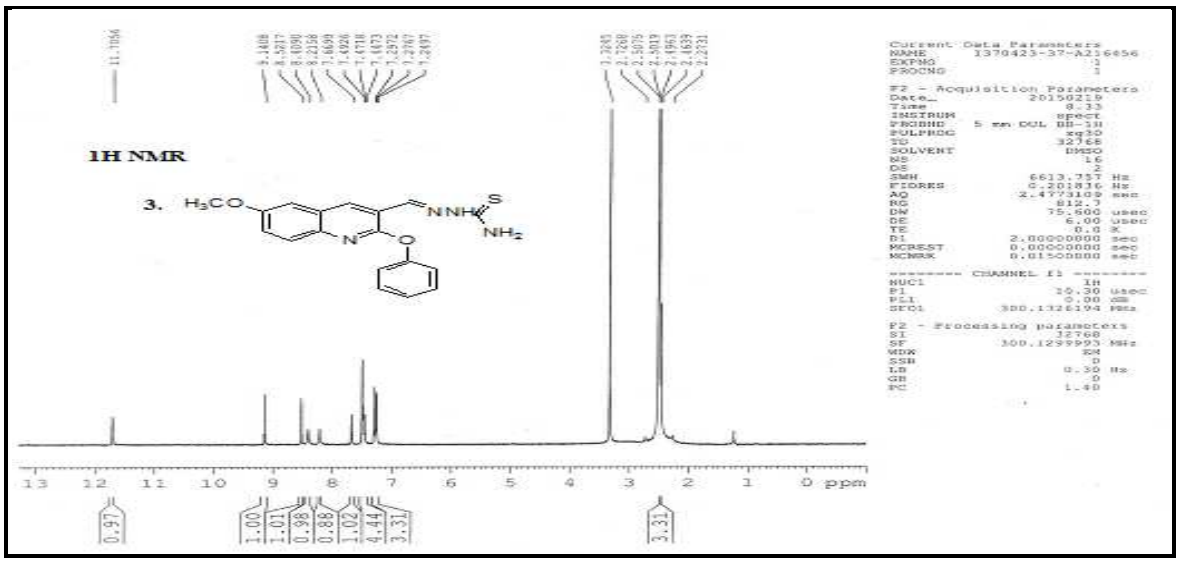
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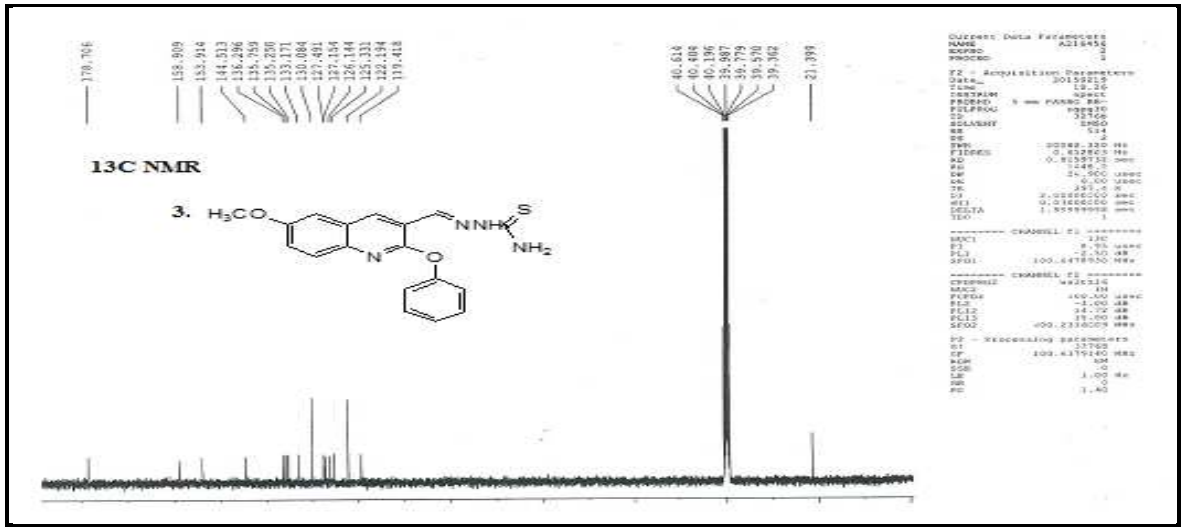
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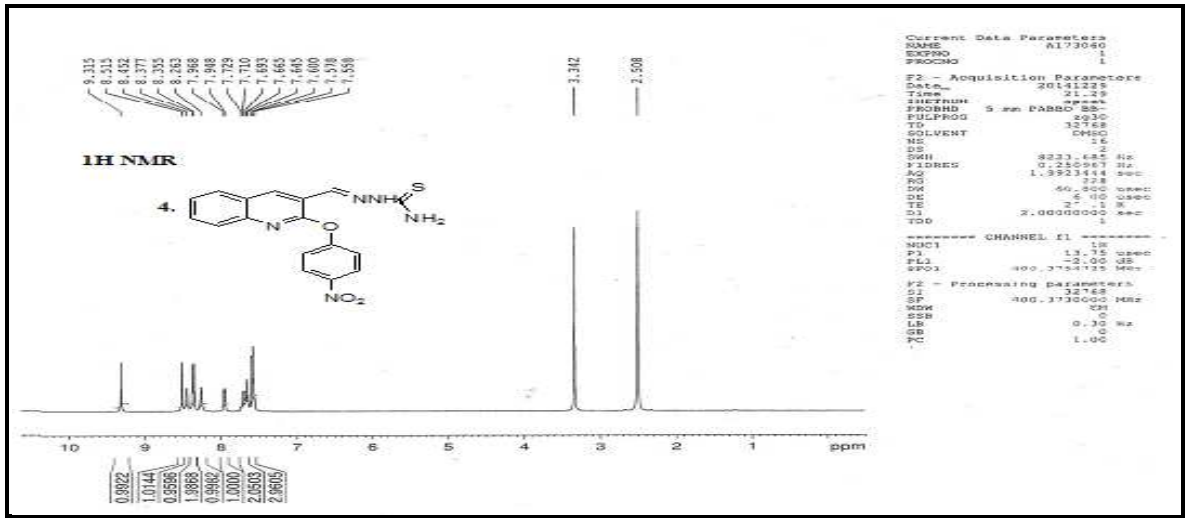
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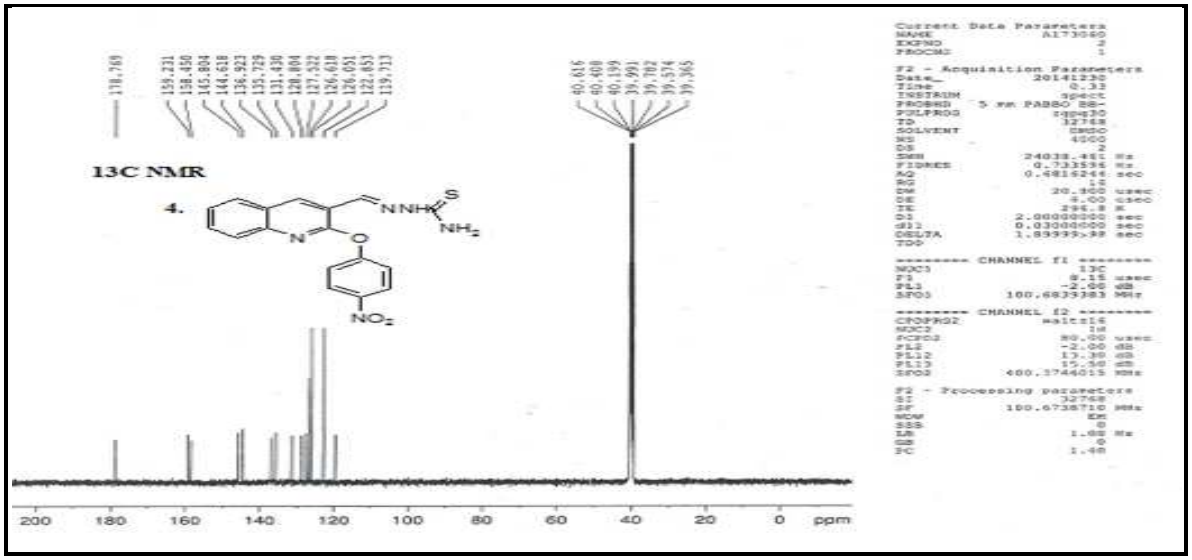
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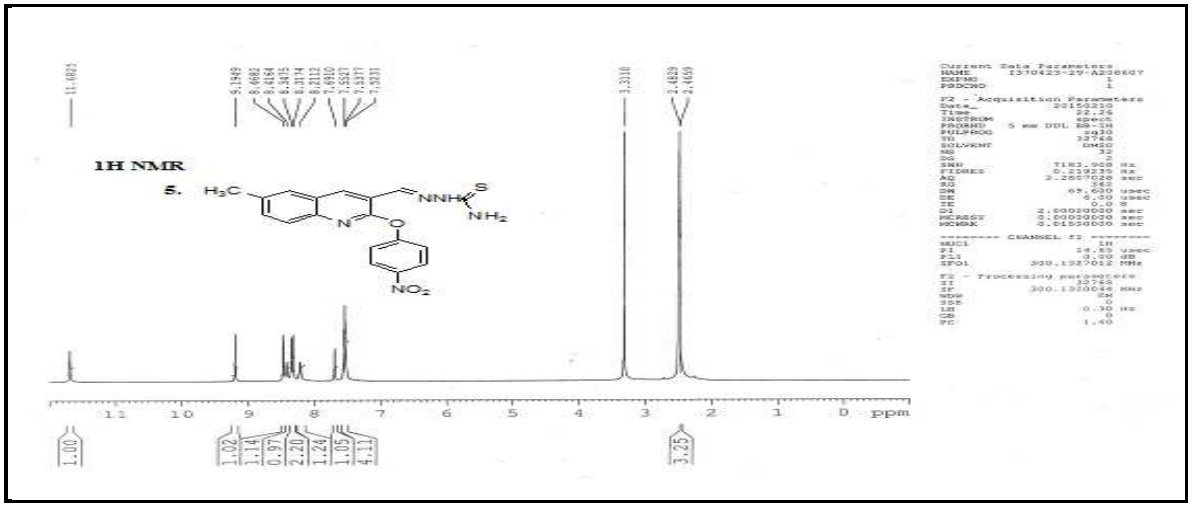
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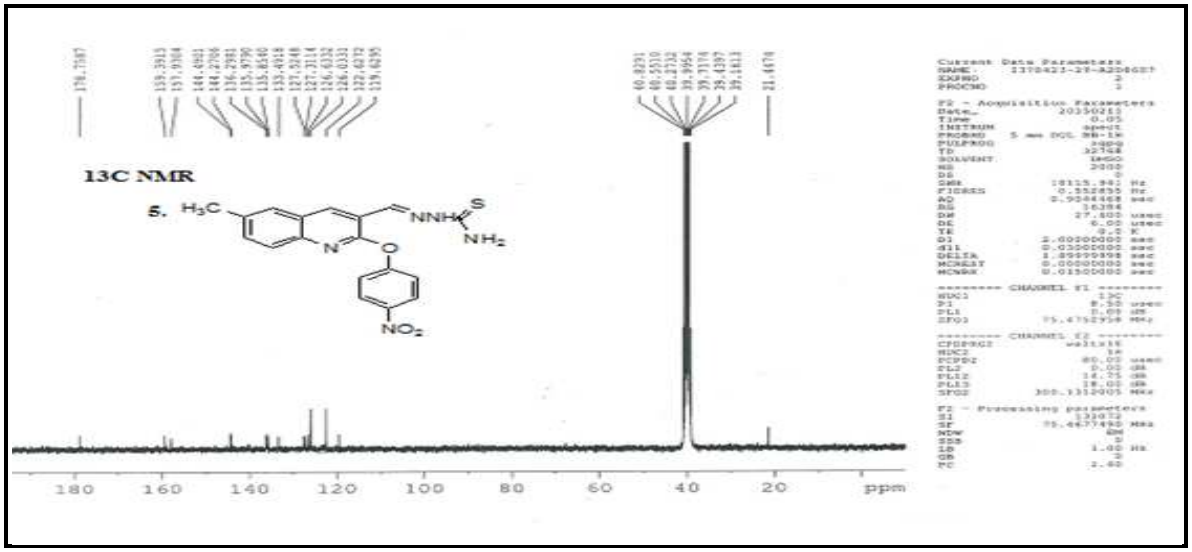
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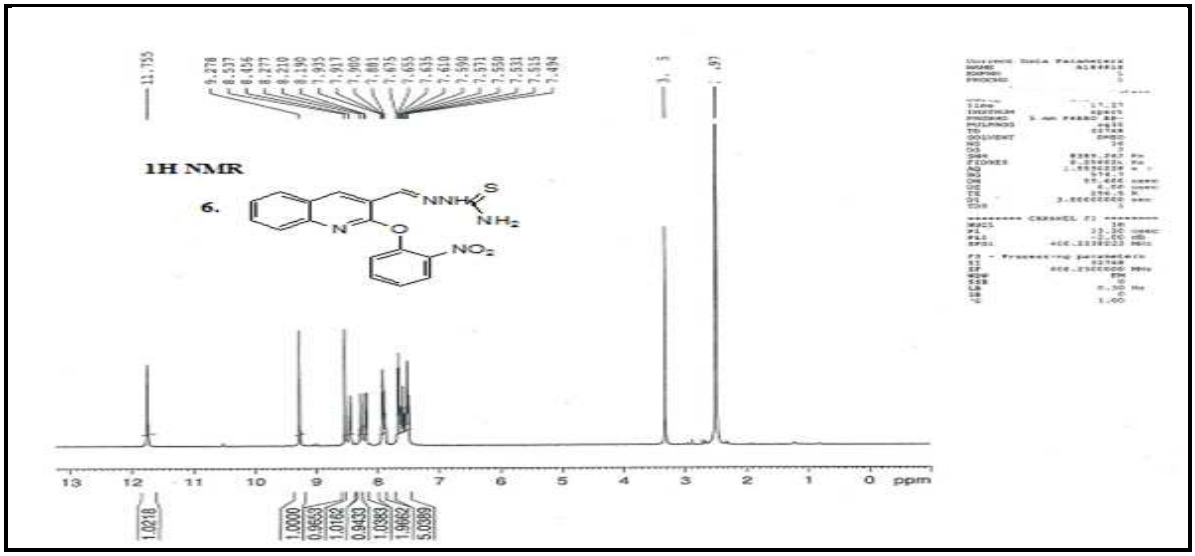
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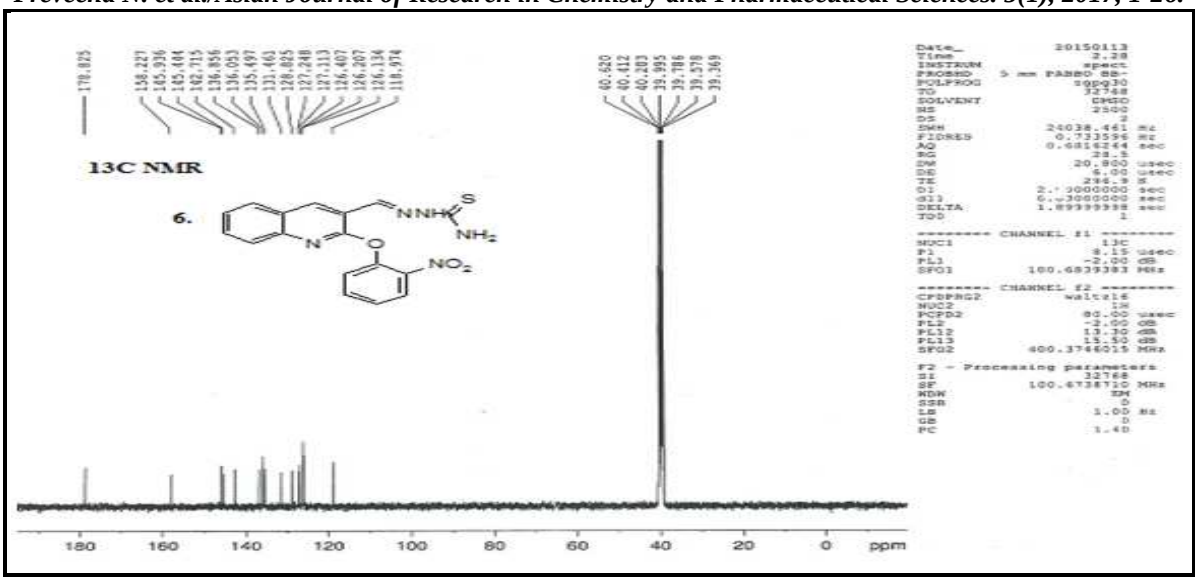
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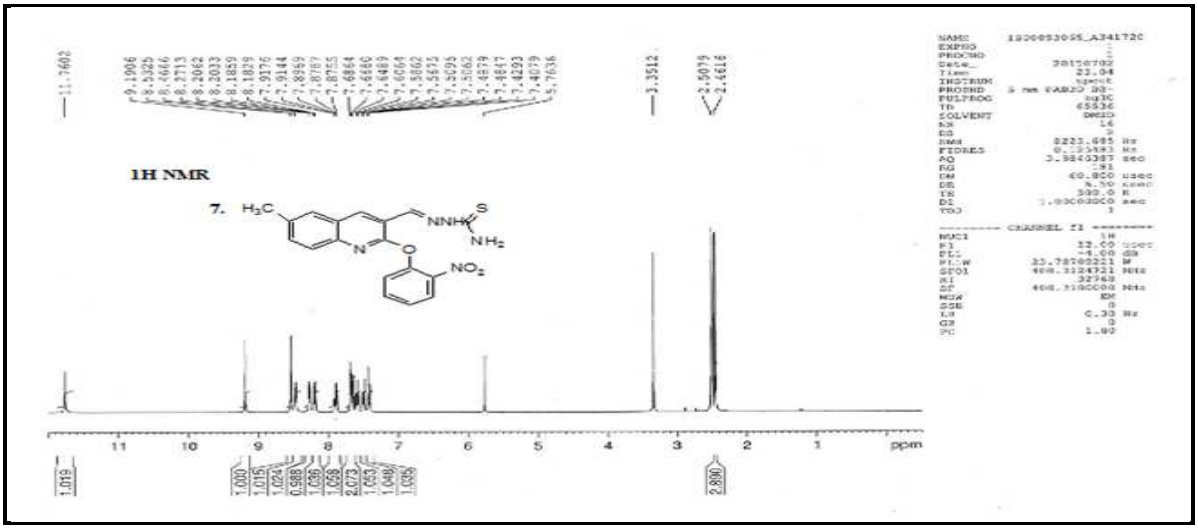
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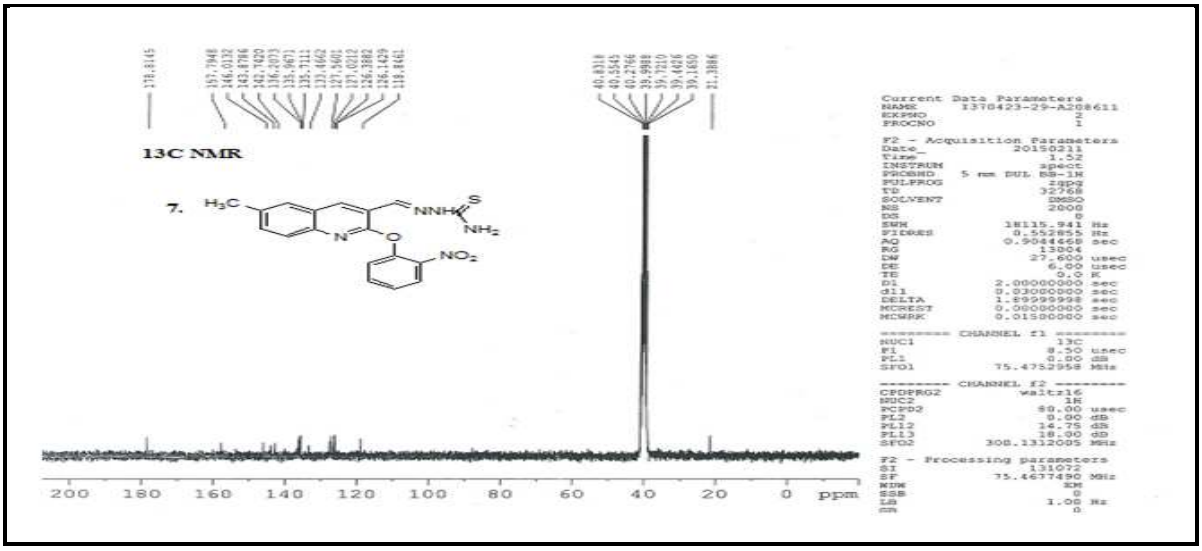
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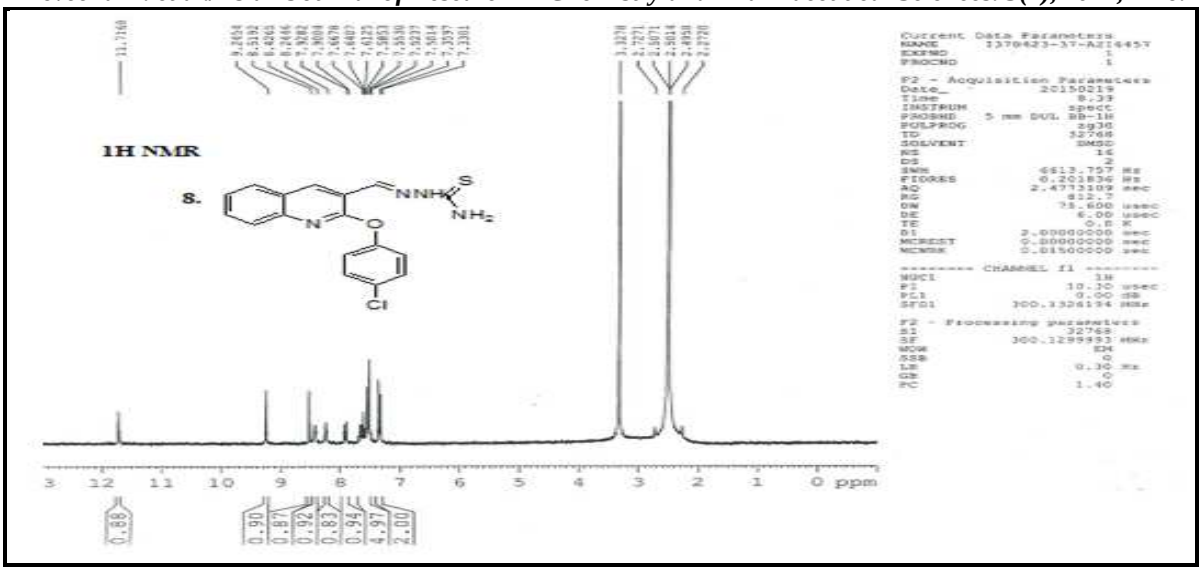
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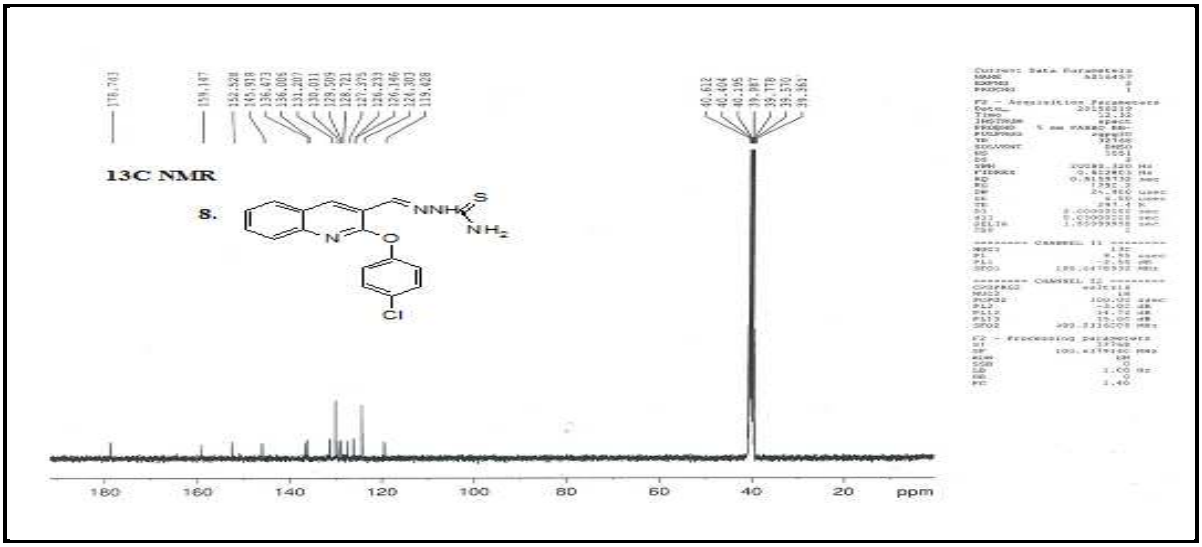
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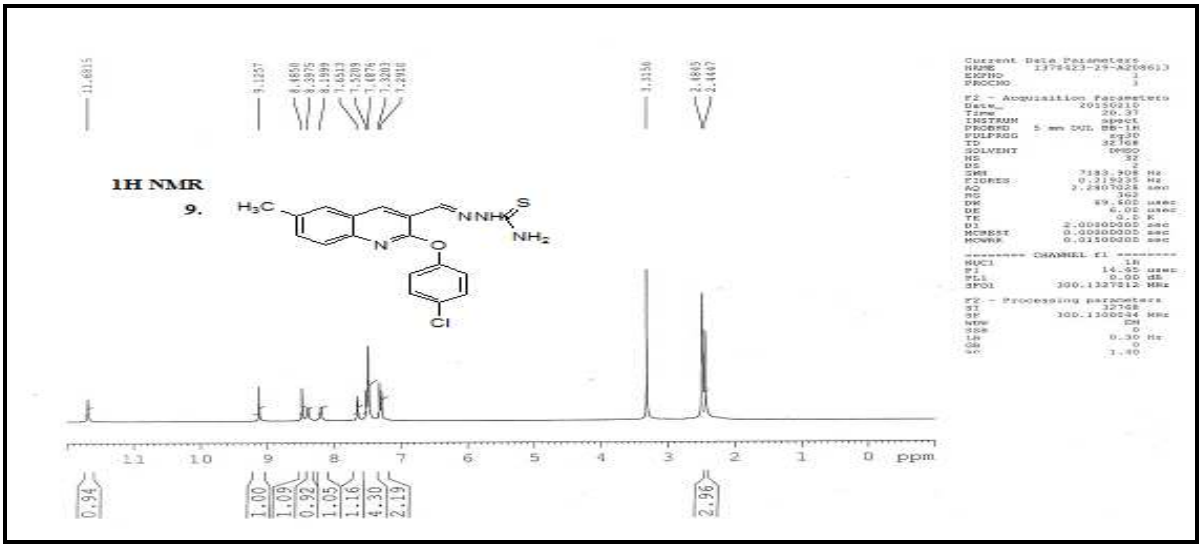
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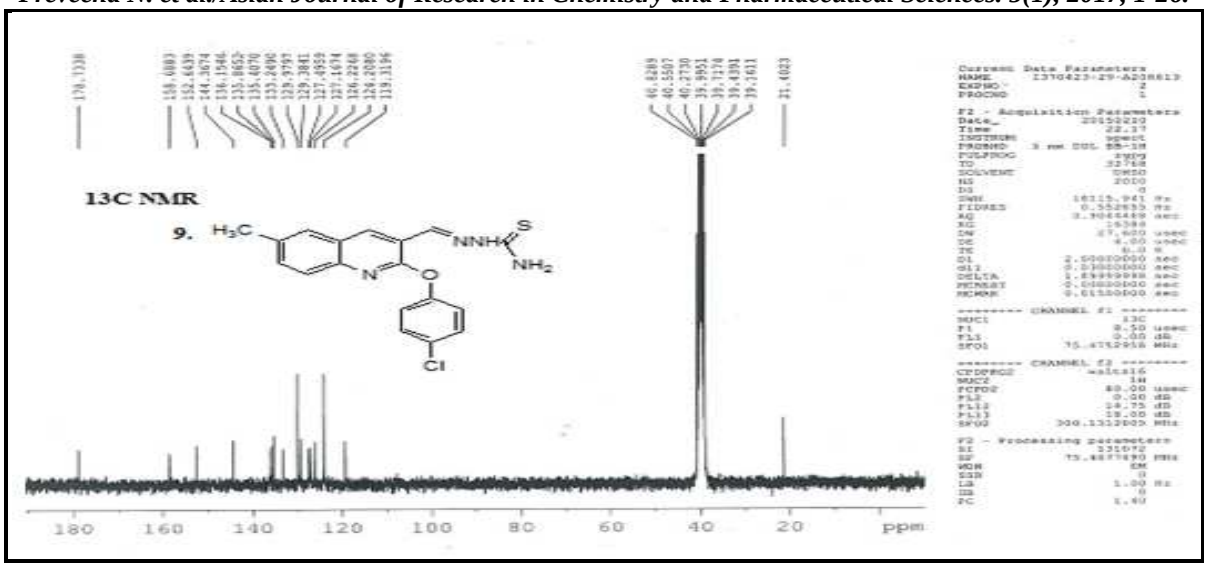
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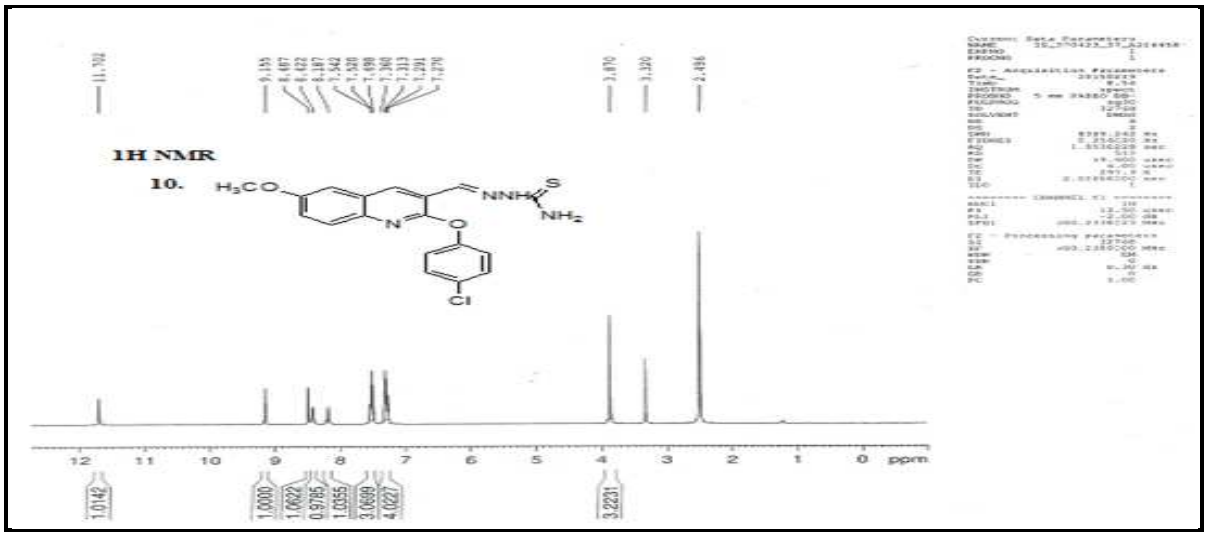
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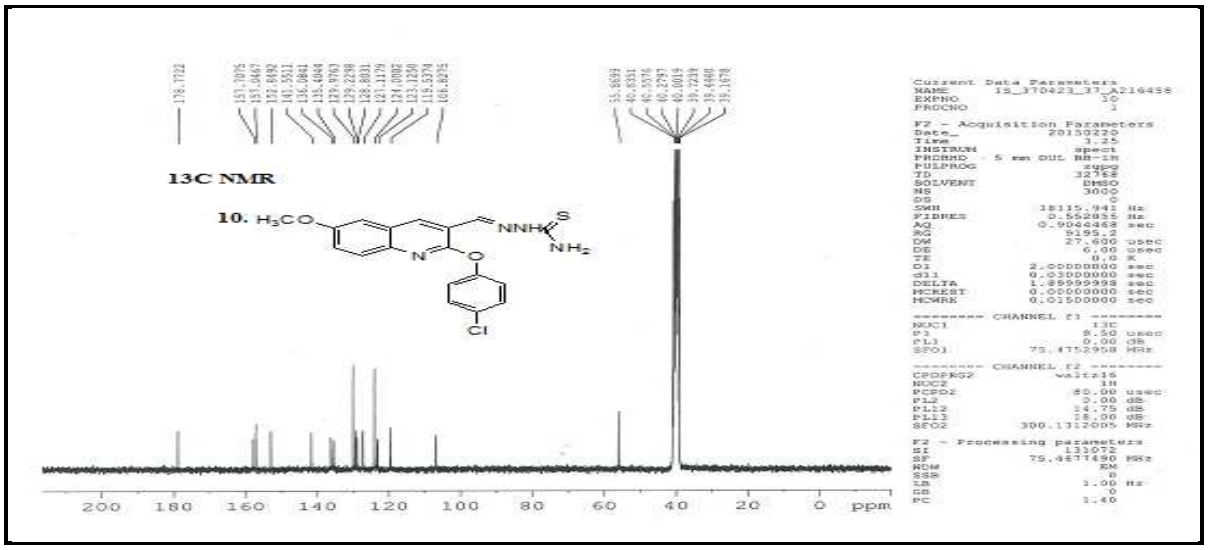
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CONCLUSION

Ten new aryloxy quinoline thiosemicarbazides (1-10),synthesized in three simple steps starting from readily available chemicals, show a number of useful pharmacological activities as evaluated by *in vitro* procedures, such as free radical scavenging activity, bacterial and fungal susceptibility measurements, and antiproliferative activity, which well match with the results obtained from *in silico* methods, such as compliance of Lipinski's rule of five, bioactivity calculated using Molinspiration programme, and data from docking them on to EGFR tyrosine kinase enzyme. From these results we are led to conclude that the compounds described here are good potential candidate molecules for further phases of bioactivity studies.

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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