Supporting Material

SYNTHESIS, BIOLOGICAL EVALUATION, AND IN SILICO STUDIES OF NEW HETEROCYCLES INCORPORATING 4,5,6,7-TETRABROMOPHTHALIMIDE MOITEY AS POTENTIAL ANTIBACTERIAL AND ANTICANCER AGENTS

General protocol of the chemical experiments

All reactions were carried out with the exclusion of moisture. All solvents were dried. All melting points are uncorrected. The IR spectra were recorded as potassium bromide pallets on Aldrich FT-IR spectrometer (Central lab at Faculty of Science, Benha, Ain Shams, and Cairo universities). Mass spectra were recorded on GCMS (Gas Chromatography-Mass Spectrometer) Shimadzu QP-2010 Plus (Microanalytical center, Ain shams University). Elemental Analysis was determined on an elementary analysis system at Ain Shams University. using UV light. Bruker Spectro spins DPX-400MHz were used to record the ^{1}HNMR (500 MHz, DMSO-d_6) and ^{13}CNMR (125 MHz, Chloroform-*d)* spectra. Chemical shift (d) values were stated in parts per million (ppm) using internal standard tetramethylsilane. The D_2O exchange confirmed the exchangeable protons (OH and NH). LC-MS/MS (PerkinElmer) was used to record the mass spectra, presented as m/z. Elemental analyses were achieved by using PerkinElmer 240 analyzer. The purity of synthesized compounds as well as the progress of the reaction was assessed by ascending thin layer chromatography (TLC) (silica gel Fluka, 706, 43-50 EA) by using methanol/chloroform (9:1 v/v) and methylene chloride/chloroform (4:1 v/v) combination as solvent system.

General Protocols of biological studies

Antibacterial assay

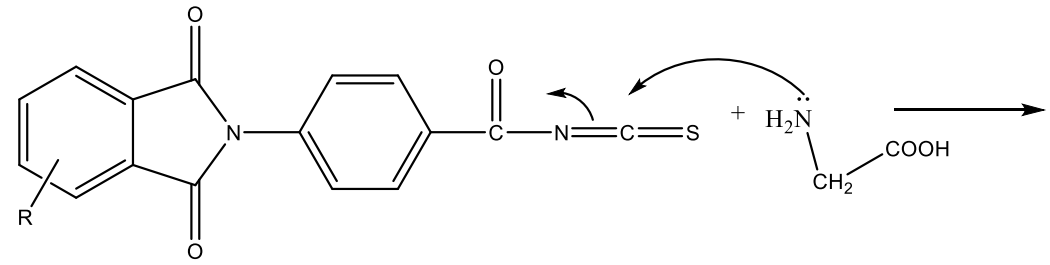
All investigations were executed at the biology department, Faculty of Science, Benha University, Egypt. The antimicrobial activities of all the synthesized molecules were determined in vitro, using the hole-plate and filter disc methodologies. The investigated compounds were dissolved in 10% acetone (V/V). The width of the inhibition zone indicated the potency of the antimicrobial activity: (-) no antimicrobial activity, (+) mild activity with the diameter of the zones equal to (0.5 – 0.7

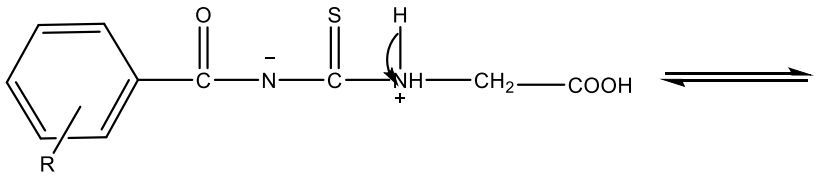
cm), (++) moderate activity with the diameter of the zones equal to (1.1 – 1.2 cm), and (+++) marked activity with the diameter of the zones equal to (1.6 – 1.8 cm). The results of the control samples are not included in Table (1), as they revealed a negative response

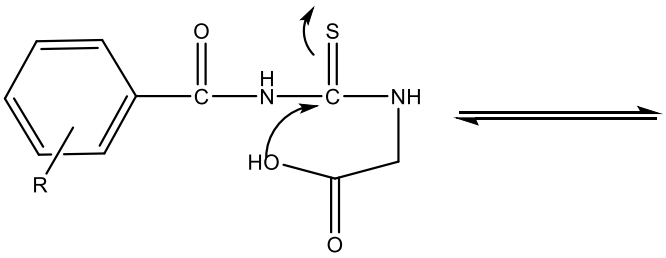
Anticancer assay

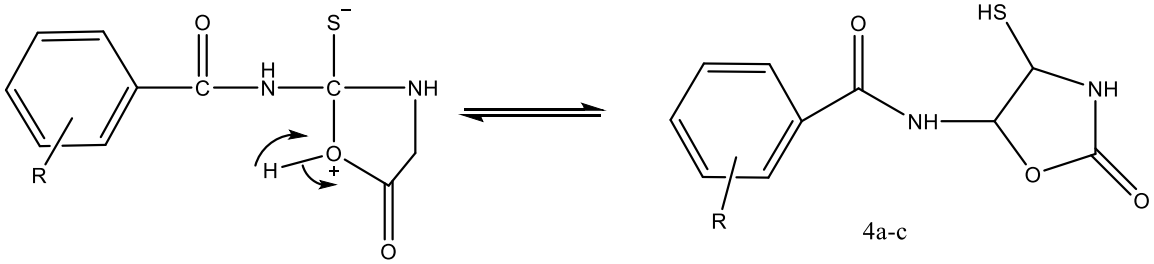
The cells were supplied by the Egyptian Holding Company for Biological Products and Vaccines (VACSERA) and then kept in the tissue culture unit. The growth of the cells was affected in RPMI-1640 medium, supplemented with 10% heat-inactivated FBS, 50 units/mL of penicillin, and 50 mg/mL of streptomycin, and maintained in a humidified atmosphere with 5% carbon dioxide. The cells were maintained as monolayer cultures by serial sub-culturing, with cell culture reagents obtained from Lonza (Basel, Switzerland). The antitumor activities of the complexes were assessed against OVCAR-3 (ovarian cancer) and HOPE-62 (small cell lung cancer) cell lines. The sulforhodamine B (SRB) assay method was applied to determine the cytotoxicity, as described in the literature. Exponentially growing cells were collected using 0.25% Trypsin-EDTA and seeded in 96-well plates at 1000–2000 cells/well in RBMI-1640 supplemented medium. The cells were kept in the medium for 24 h and then they were incubated for 3 days with various concentrations of the copper complexes. Following 3 days of treatment, the cells were fixed with 10% trichloroethanoic acid for 1 h at 4 °C. Wells were stained for 10 min at room temperature with 0.4% SRBC dissolved in 1% acetic acid. The plates were air-dried for 24 h and the dye was dissolved in Tris-HCl for 5 min with shaking at 1600 rpm. The optical density (OD) of each well was assessed spectrophotometrically at 564 nm with an ELISA microplate reader (ChroMate-4300, FL, USA). The IC_{50} values were calculated from a Boltzmann sigmoidal concentration-response curve using the nonlinear regression fitting models (Graph Pad, Prism Version 9).

Mechanism of reactions

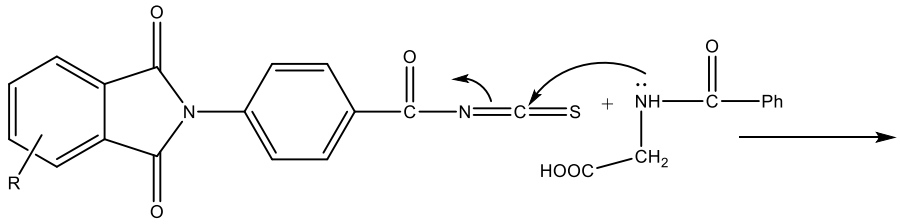








4a-c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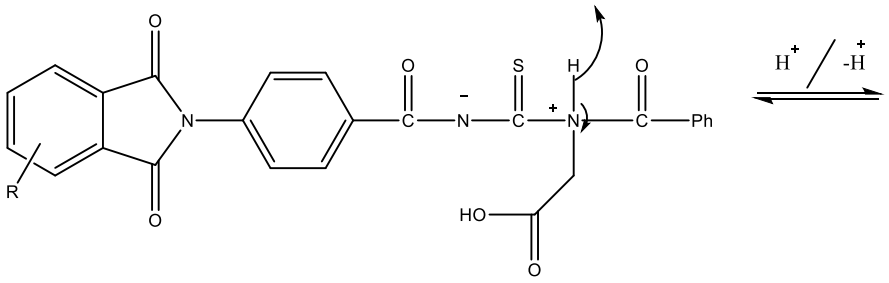
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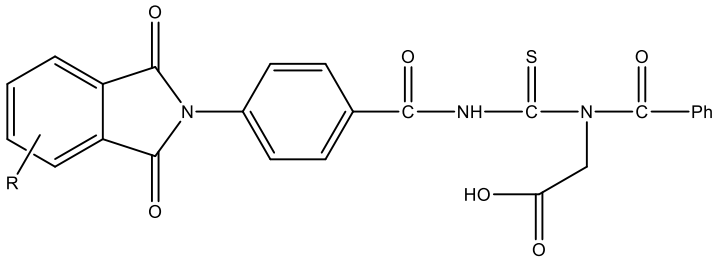
-H+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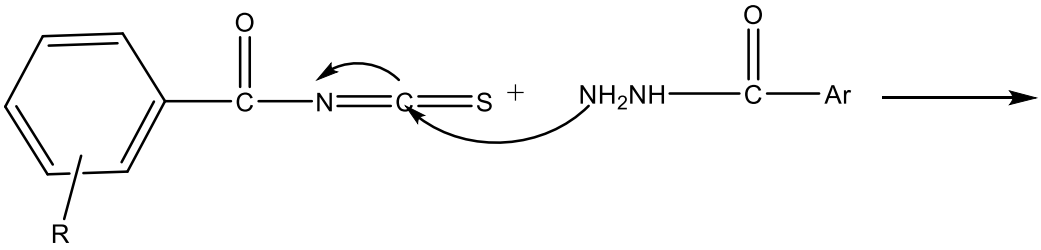
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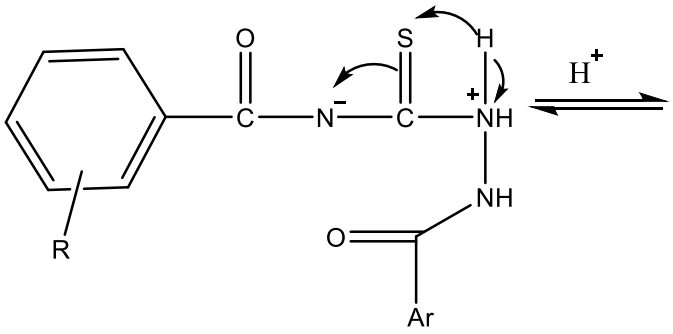
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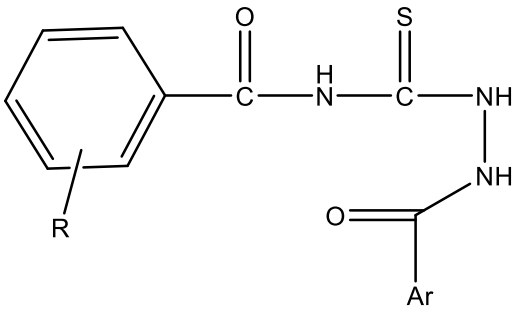
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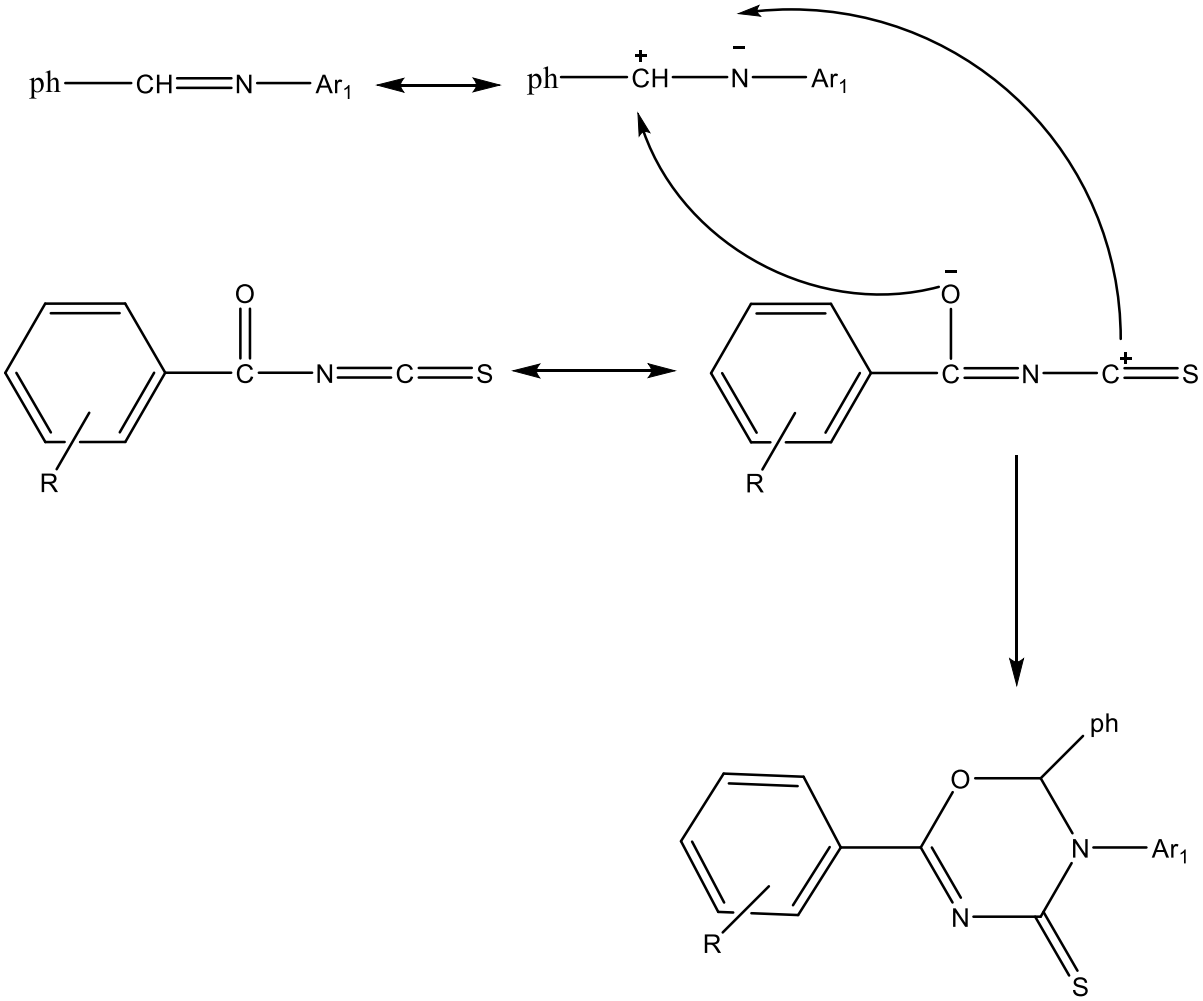
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