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SYNTHESIS OF NOVEL NITROGEN HETEROCYCLES BEARING BIOLOGICAL ACTIVE CARBOXAMIDE MOIETY AS POTENTIAL ANTITUMOR AGENTS

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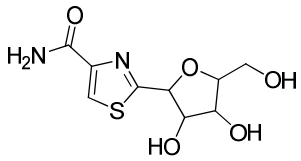
ABSTRACT. In the present study, synthesis of a simple series of nitrogen heterocycles containing *N*-(*p*-bromophenyl) carboxamide moiety, such as benzimidazole, benzoxazine, oxadiazole and triazole compounds, by using 4-bromo aniline and diethyl oxalate as a key starting material has been described. Five nitrogen heterocycles compounds moiety were evaluated for their anticancer activity against MCF-7 cell line. The results revealed that compound **8** (*N*-(4-bromophenyl)-5-thioxo-1,3,4-oxadiazole-2-carboxamide) was the most potent cytotoxic activity. Cell cycle analysis demonstrated that compound **8** induce cell cycle arrest at G1 phase with apoptosis inducing activity marked by increase in G0 phase.

KEY WORDS: Synthesis, Nitrogen heterocycles, Carboxamide, Antitumor agents

INTRODUCTION

The rapid spread of cancer has sparked an intense worldwide search for new nitrogen heterocycles containing carboxamide moiety, which may be used in designing novel antitumor drugs. Among these, tiazofurin (TR, 2-β-D-furanosylthiazole-4-carboxamide), a natural C-nucleoside, was reported to possess potential anticancer activity (Figure 1) [1]. Furthermore, the antitumor activities of the natural antineoplastic antibiotic bleomycin, netropsin, and thiazole netropsin have been reported [2].



Tiazofurin

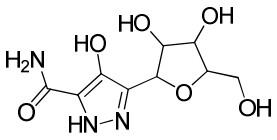


Figure 1. Structures of nitrogen heterocycles as antitumor agents.

Benzimidazole nucleus as nitrogen heterocycles is an important pharmacophore in modern drug discovery [3]. Substituted benzimidazole derivatives have found commercial application in veterinarian medicine as anthelmintic agents and used to act as topoisomerase inhibitors [4], selective neuropeptide II receptor antagonists [5], angiotensin II inhibitors [6], potential antitumor agents [7, 8], treatment for interstitial cystitis and ulcers [9, 10], antimicrobial agents [11], smooth muscle cell proliferation inhibitors [12], and diverse area of chemistry [13]. In addition, benzimidazole is very important intermediate in organic reaction [14]. Benzimidazole derivatives exhibit significant activity against several viruses such as HIV [15], herpes (HSV-1) [16], RNA [17], influenza [18] and human cytomegalovirus (HCMV) [19].

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Triazole nucleus as nitrogen heterocycles has an attracted wide attention of the medicinal chemists in search for new therapeutic molecules. Literature survey on triazoles has shown that these derivatives possess broad spectrum of biological activities including good P38 map kinase inhibition and anti-inflammatory activity [20-22].

Due to broad spectrum activities reported in the literature [23-28] so far, we have synthesized several nitrogen heterocycles containing carboxamide moiety, targeting for potent molecules possessing antitumor and kinase inhibitory activity [23].

We have described in this paper the synthesis of new nitrogen heterocycles bearing biologically active *N*-(4-bromophenyl) carboxamide moiety by using diethyl oxalate and *p*-bromoaniline as starting material. Some synthesized nitrogen heterocycles were evaluated against cancer cell lines.

EXPERIMENTAL

Chemistry

^{1}H-NMR spectra were recorded using Bruker 400 DRX-Avance NMR spectrometer. Chemical shifts of ^{1}H-NMR spectra were reported relative to tetramethyl silane (TMS). ^{13}C-NMR spectra were run using a Bruker DRX-Avance NMR spectrometer. The prepared compounds were dissolved in deuterated dimethyl sulfoxide (DMSO) as solvent. Splitting patterns were reported as s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. IR spectra were recorded using a broker FT-8000 spectrometer and frequencies were expressed in cm^{-1}. The molecular weight of the prepared compounds was determined by electron ionization (EI) mass spectrometer operating at 70 eV. Melting point of the synthesized compounds was measured with an electrothermal melting point apparatus and has not been corrected. All the prepared compounds give satisfactory elemental analyses within 0.04% of the theoretical values. The elemental analyses were carried out on a Perkin-Elmer 2400 series CHN. Chemicals and solvents were purchased from commercial sources in analytical grade purity.

Synthesis of ester oxalic acid amide

A mixture of equimolar quantity of diethyl oxalate (0.01 mol) and 4-bromoaniline (0.01 mol) in 50 mL acetic acid was heated under reflux for 2-3 h. The reaction mixture was cooled and poured into ice-water with stirring. The solid product formed was collected after filtration, washed with water, dried, and recrystallized from ethanol to give **1**.

As colorless crystals, yield 63%. m.p. 121-123 ^{o}C. IR (KBr) υ_{max}: 3265 (NH), 1753, 1705 (C=O), 1605, 1585 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.32 (t, 3H, CH_3), 4.32 (q, 2H, OCH_2), 7.58 (d, 2H, Ar-H), 7.86 (d, 2H, Ar-H), 11.03 (NHCO) ppm. Anal. calcd. For $C_{10}H_{10}BrNO_3$ (271): C, 44.28; H, 3.69; N, 5.17. Found: C, 44.09; H, 3.33; N, 5.01.

*Synthesis of 2-substituted aniline (**2**) and 2-substituted benzoic acid (**3**)*

A mixture of ester oxalic acid amide **1** (0.01 mol) and aromatic amine derivatives (namely, *o*-phenylene diamine and anthranilic acid (0.01 mol) in 50 mL glacial acetic acid was heated under reflux for 2-3 h, then cooled and poured into water. The resulting solid was filtered off, washed with water and dried. Finally, the product was crystallized from butanol to give **2** and **3**.

*2-[(4-Bromophenylamino)-carbonyl, (ethoxy)methylideneamino]aniline (**2**)*

As colorless crystals, yield 71%. m.p. 240-242 ^{o}C. IR (KBr) υ_{max}: 3231 (NH), 1757, 1707 (C=O), 1603, 1595 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (t, 3H, CH_3), 4.32 (q, 2H, OCH_2), 7.34-7.96 (m, 10H, Ar-H and NH_2), 10.88, 11.04 and 11.11 (NHCO, N=C-OH) ppm. ^{13}C-NMR (DMSO-d_6) δ: 162.44 (CO of amide), 160.90 (O-C=N), 159.01, 157.96 (N=C-OH), 145.79,

142.30, 138.20, 137.70, 137.51, 137.39, 132.11, 132.04, 132.00, 122.91, 122.86, 122.60, 122.57, 117.09, 116.73, 116.41, 115.70 (C-aromatic), 62.98 (OCH_2), 14.32 (CH_3) ppm. MS: m/z (%) = 361 (M^+). Anal. calcd. For $C_{16}H_{16}BrN_3O_2$ (361): C, 53.18; H, 4.43; N, 11.63. Found: C, 53.36; H, 4.56; N, 11.52.

*2-[(1-Ethoxy-2-oxo-2-(phenylamino)ethylideneamino]benzoic acid (**3**)*

As yellow crystals, yield 69%. m.p. 215-217 °C. IR (KBr) υ_{max}: 2930-3271 (br. OH), 3261 (NH), 1749, 1701 (C=O), 1603, 1587 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (t, 3H, CH_3), 4.29 (q, 2H, OCH_2), 7.21-8.02 (m, 7H, Ar-H), 8.61 (d, 1H, Ar-H), 10.86, 10.95 and 11.02 (NHCO and N=C-OH), 12.76 (s, 1H, COOH) ppm. Anal. calcd. For $C_{17}H_{15}BrN_2O_4$ (390): C, 52.31; H, 3.85; N, 7.18. Found: C, 52.18; H, 3.67; N, 7.07.

*Synthesis of N-(4-bromophenyl)-benzimidazole-2-carboxamide (**4**) and N-(4-bromophenyl)-3,1-benzoxazine-2-carboxamide (**5**)*

A solution of compounds **2** and **3** **(**0.01 mol) in acetic anhydride (20 mL) was heated under reflux for 2 h, then cooled and poured into ice-water. The reaction mixture was left for 24 h and the solid formed was filtered off, washed with water, and dried. Finally, the product was crystallized from ethanol to give **4** and **5**.

Compound **4** as colorless crystals, yield 63%. m.p. 260-262 °C. IR (KBr) υ_{max}: 3232 (NH), 1752, 1691 (C=O), 1604, 1587 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.23-7.87 (m, 8H, Ar-H), 10.95 and 11.02 (NHCO and N=C-OH), 13.41 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6) δ: 159.01 (NHCO), 157.96 (N=C-OH), 145.79 (C=N), 142.95 (C-N), 138.20, 137.51, 135.21, 133.03, 132.11, 132.00, 125.65, 125.04, 123.32, 122.91, 122.86, 120.98, 120.55, 117.09, 116.41, 113.18 (C-aromatic) ppm. MS: m/z (%) = 315 (M^+). Anal. calcd. For $C_{14}H_{10}BrN_3O$ (315): C, 53.33; H, 3.17; N, 13.33. Found: C, 53.11; H, 3.02; N, 13.17.

Compound **5** as colorless crystals, yield 61%. m.p. 239-241 °C. IR (KBr) υ_{max}: 3229 (NH), 1757, 1693 (C=O), 1600, 1583 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.49-8.16 (m, 8H, Ar-H), 10.92 and 10.95 (NHCO and N=C-OH) ppm. ^{13}C-NMR (DMSO-d_6) δ: 159.00 (NCO), 158.80 (O-C=O), 156.22 (N=C-OH), 150.08 (N=C-O), 144.99, 137.62, 137.57, 137.51, 137.39, 132.63, 132.11, 130.86, 130.38, 128.82, 128.01, 127.84, 123.17, 123.01, 122.91, 118.56, 117.09, 117.06 ppm. MS: m/z (%) = 345 (M^+). Anal. calcd. For $C_{15}H_9BrN_2O_3$ (345): C, 52.20; H, 2.63; N, 8.12. Found: C, 52.01; H, 2.52; N, 8.19.

*Synthesis of N-(4-bromophenyl)-3-(ethoxycarbonyl)methyl benzimidazole-2-carboxamide (**6**).*

A mixture of benzimidazole-2-carboxamide **4** (0.01 mol) and ethyl chloro acetate (0.01 mol) in dimethyl formamide (30 mL) was heated under reflux for 6 h, then cooled and poured into water. The reaction mixture was neutralized with dilute hydrochloric acid (1 M). The solid obtained was filtered off, washed with water, dried and purified by recrystallization from ethanol to give compound **6**.

As pale-yellow crystals, yield 61%. m.p. 190-192 °C. IR (KBr) υ_{max}: 3287 (NH), 1772, 1678 (C=O), 1632 (C=N), 1610, 1592 (C=C), 1425 (CH_2), 1171, 1098 (C-O) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 1.22 (t, 3H, CH_3), 4.18 (q, 2H, OCH_2), 5.55 (s, 2H, NCH_2CO), 7.41-7.95 (m, 8h, Ar-H), 11.04 and 11.15 (NHCO and N=C-OH) ppm.^{13}C-NMR (DMSO-d_6) δ: 168.79, 158.28 (C=O of ester and amide), 143.72 (N=C-O), 140.63 (N-C=N), 137.79, 137.31, 137.01, 132.11, 132.01, 125.52, 124.16, 123.01, 120.85, 117.19, 116.66, 111.78, (C-aromatic), 61.73, (OCH_2), 47.00 (NCH_2CO), 14.45 (CH_3) ppm. Anal. calcd. For $C_{18}H_{16}BrN_3O_2$ (385): C, 56.10; H, 4.16; N, 10.91. Found: C, 55.98; H, 4.02; N, 10.66.

Synthesis of N-(amino), N-(4-bromophenyl)oxalic acid dicarboxamides (7)

Reaction of ester oxalic acid carboxamide **1** (0.01 mol)with hydrazine hydrate (0.01 mol) in ethanol (30 mL) was heated under reflux for 2 h, the reaction mixture was cooled and poured into water, then neutralized with dilute hydrochloric acid (1%). Solid product formed was filtered off, washed with water, dried and recrystallized from ethanol to give **7**.

As pale-yellow crystals, yield 56%. m.p. 225-227 ^{o}C. IR (KBr) υ_{max}: 3356, 3285 (NH_2 and NH) 1689 (C=O), 1605, 1592 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 5.21 (s, 2H, NH_2), 7.57 (d, 2H, Ar-H), 7.87 (d, 2H, Ar-H), 10.51 (s, 1H, NH), 11.02, 11.17 (NHCO, N=C-OH) ppm. Anal. calcd. For $C_8H_8BrN_3O_2$ (257): C, 37.35; H, 3.11; N, 16.34. Found: C, 37.37; H, 3.02; N, 16.26.

*Synthesis of N-(4-bromophenyl)-5-thioxo-1,3,4-oxadiazole-2-carboxamide (**8**)*

A mixture of compound **7** (0.01 mol) and carbon disulphide (0.02 mol) was refluxed in pyridine (30 mL) for 8 h. The reaction mixture was cooled and poured into ice-water, then neutralized with dilute hydrochloric acid (1 M). The formed product was filtered off, washed with water, dried, and recrystallized from ethanol to give **8**.

As colorless crystals, yield 62%. m.p. 263-265 ^{o}C. IR (KBr) υ_{max}: 3281 (NH), 1687 (C=O), 1601, 1590 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 7.51-7.60 (d, 2H, Ar-H), 7.80-7.86 (d, 2H, Ar-H), 10.87 AND 11.03 (NHCO and N=C-OH), 11.14 (s, 1H, NH) ppm. ^{13}C-NMR (DMSO-d_6) δ: 178.62 (C=S), 159.84 (CONH), 154.84 (N=C-OH) 150.97 (N=C-O), 137.51, 137.34, 132.16, 132.11, 132.06, 123.17, 122.97, 122.91, 117.28, 117.09 (C-aromatic) ppm. MS: m/z (%) = 299 (M^+). Anal. calcd. For $C_9H_6BrN_3O_2$ (299): C, 36.12; H, 2.01; N, 14.05. Found: C, 36.08; H, 1.92; N, 13.88.

*Synthesis of N-(4-bromophenyl)-5-thioxo-4-amino-1,2,4-triazole-3-carboxamide (**9**)*

A mixture of *N*-(4-bromophenyl)-5-thioxo-1,3,4-oxadiazole-2-carboxamide (**8**) (0.01 mol) and hydrazine hydrate(2 mL)in absolute ethanol was heated under reflux for 4 h. the solvent and excess of hydrazine hydrate were removed under reduced pressure. The residue washed with ether and recrystallized from ethanol to give **9**.

As pale-yellow crystals, yield 63%. m.p. 263-265 ^{o}C. IR (KBr) υ_{max}: 3388, 3261, 3199 (NH_2 and NH) 1691 (C=O), 1589, 1583 (C=C) cm^{-1}. ^{1}H-NMR (DMSO-d_6) δ: 6.10 (s, 2H, NH_2), 7.52-7.80 (m, 4H, Ar-H), 10.34 (s, 1H, NH), 10.78 (s, 1H, SH), 11.09, 11.15 (s, 1H, NHCO and N=C-OH) ppm. ^{13}C-NMR (DMSO-d_6) δ: 166.50 (C=S), 159.90 (CONH), 158.18 (N=C-OH) 154.18 (N=C-N), 143.54 (N=C-SH), 137.59, 137.51, 137.42, 132.30, 132.11, 131.99, 122.91, 122.77, 122.60, 117.09, 116.98, 116.75 (C-aromatic) ppm. MS: m/z (%) = 313 (M^+). Anal. calcd. For $C_9H_8BrN_5OS$ (313): C, 34.50; H, 2.56; N, 22.36. Found: C, 34.33; H, 2.42; N, 22.16.

Anti-tumor activity against breast cancer cell line (MCF-7)

The cytotoxic activity was measured *in vitro* for the thiohydantoin derivatives using the MTT assay. Cells were plated in 96- multiwall plate (10^5 cells/well) for 24 h before treatment with the compounds. Test compounds were dissolved in dimethyl sulfoxide. Different concentrations of the compound under test (10, 25, 50, and 100 μM) were added to the cell's monolayer. Triplicate wells were prepared for each individual concentration. Monolayer cells were incubated with the compound(s) for 48 h at 37 ^{o}C and in atmosphere of 5% CO_2. After 48 h, cells were fixed, washed, and stained with 40 μL of MTT solution (5 mg/mL of MTT in 0.9% NaCl) in each well was added and incubated for an additional 4 h. MTT crystals were solubilized by adding 180 μL of acidified isopropanol/well and the plate was shaken at room temperature, followed by photometric determination of the absorbance at 570 nm using ELISA reader. The molar

concentration required to inhibit 50% of cell viability (IC_{50}) was calculated and compared with the reference drug doxorubicin. The surviving fractions were expressed as means ± S.E.M.

*Cell cycle analysis of compound **8***

MCF-7 cells, ($3.0x10^5$ cells/well) and incubated at 37 ^oC for 12 h. The target cells were then treated with the compound **8** at its IC_{50} concentration dose value for 48 h. After treatment, cells were collected and fixed with 75% ethanol at 20 ^oC overnight, then, cells were washed with PBS followed by centrifugation and incubated with (10 mg/mL) Rnase (Sigma, USA) and (5 mg/mL) propidium iodide (PI, Sigma) before flow cytometry analysis (*FACSCalibur* cytometer using Cell quest software, BD Bioscience, USA).

Apoptosis determination by Annexin-Vassay

The MCF-7 cells, (2×10^5 cells/well) were treated with compound **8** at its IC_{50} concentration value for 48 h. After treatment, cells were harvested and washed twice (180 g, 10 min, 4 °C) with PBS. Each cell well was resuspended in 100 µL of binding buffer, and 5 µL Annexin V-FITC were added. After an incubation time of 10 min at room temperature, additional 400 µL of binding buffer were added for a final volume of 500 µL. Cells were stained with PI immediately before measurement. Cells were the analyzed by using *FACSCalibur* Flow cytometer (Becton and Dickinson, Heidelberg, Germany). Data thus obtained were analyzed with Cell-Quest software (Becton and Dickinson, Heidelberg, Germany).

RESULTS AND DISCUSSION

Chemistry

In this investigation, a new simple series of nitrogen heterocycles containing *N*-(4-bromophenyl) carboxamide moiety were designed, synthesized **(**Scheme 1**)** and biologically evaluated for their invitro antitumor activity.

Thus, reaction of diethyl oxalate with *p*-bromoaniline in acetic acid under reflux gave the corresponding ester oxalic acid amide (**1**) as a key starting material. Condensation of ester oxalic acid amide (1) with *o*-phenylene diamine and anthranilic acid in glacial acetic acid led to the formation of 2-[(4-bromophenylamino)-carbonyl, (ethoxy)methylideneamino]aniline (**2**) and 2-[(1-ethoxy-2-oxo-2-(phenylamino)ethylideneamino]benzoic acid (**3**), respectively.

N-(4-bromophenyl)-benzimidazole-2-carboxamide (**4**) and *N*-(4-bromophenyl)-3,1-benz-oxazine-2-carboxamide (**5**) were obtained *via* cyclization of 2-substituted aniline (**2**) and 2-substituted benzoic acid (**3**) with acetic anhydride under reflux. Alkylation of benzimidazole-2-carboxamide derivative (**4**) with ethyl chloroacetate in dimethyl formamide to yield *N*-(4-bromophenyl)-3-(ethoxycarbonyl)methyl benzimidazole-2-carboxamide (**6**).

Condensation of ester derivative (**1**) with hydrazine hydrate in ethanol under reflux led to the formation ofoxalic acid hydrazide derivative (**7**). The compound **7** was reacted with carbon disulphide in pyridine under reflux gave the corresponding *N*-(4-bromophenyl)-5-thioxo-1,3,4-oxadiazole-2-carboxamide (**8**).

Treatment of 1,3,4-oxadiazole derivative (**8**) with hydrazine hydrate in ethanol under reflux afforded the corresponding *N*-(4-bromophenyl)-5-thioxo-4-amino-1,2,4-triazole-3-carboxamide (**9**).

*NMR spectra investigation of synthesized nitrogen heterocycles (**4-9**)*

To confirm the struct6ural features of the compounds, ^{1}H-NMR and ^{13}C-NMR spectra of the synthesized nitrogen heterocycles (**4-9**), recorded in DMSO-d_6 were examined.

*[1]H-NMR spectra investigation of nitrogen heterocycles (**4-9**)*

*Compounds **4**, **5** and **6***

From the data of [1]H-NMR spectra for the compounds **4** and **5** gave clear cut evidence singlet signals at δ 10.95, 11.02, 13.41 ppm and δ 10.92, 10.95 ppm due to the proton of NH function for the carboxamide group for the compounds **4** and **5**.

Also, compounds **4** and **5** containing the same protons of aromatic rings, which appeared in the [1]H-NMR spectra as multiplet signals in the expected region at δ 7.23-8.16, among total 8 protons for the aromatic rings.

In case of compound **6**, [1]H-NMR spectrum gave a new three signals at δ 5.55 as singlet, δ 4.20 as quartet signal and δ 1.22 ppm as triplet signal assigned to the protons of methylene and ethoxy function for the CH_2COOEt group, clearly confirmed the formation of compound **6**. Also, compound **6** containing the same protons of carboxamide and aromatic rings appeared in the [1]H-NMR spectrum at δ 11.15, 11.04 ppm (CONH) and δ 7.40-7.95 ppm as multiplet signals for the aromatic rings.

[1]H-NMR spectra of these compounds **4, 5** and **6** gives number of proton signals, which showed that the presented two isomer of these compounds as shown in Figure 2.

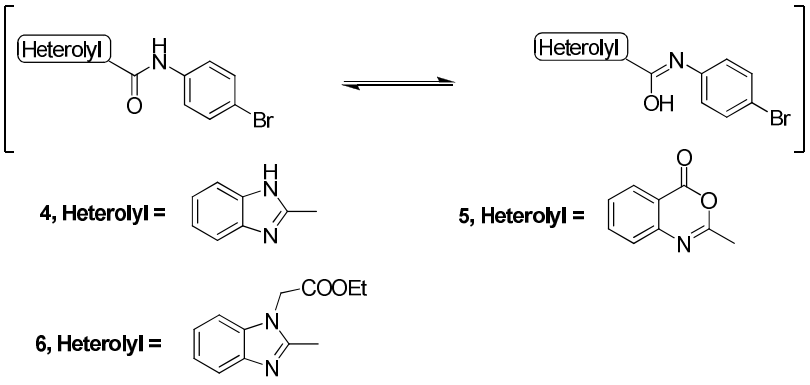


Figure 2. Keto-enol tautomers of compounds **4-6**.

*Compounds **8** and **9***

From study, the [1]H-NMR spectra of compounds **8** and **9** showed the structure of these compounds in thione-thiol and keto-enol tautomers. [1]H-NMR spectra of these compounds **8** and **9** showed the presented singlet signals in the region at δ 10.34-11.20 ppm refer to the protons of carboxamide (NHCO and N=C-OH) and thiosemicarbazide groups. In addition, the number of proton signals of the aromatic ring were observed within the expected chemical shift regions and showed that the presented four isomers of these compounds as shown in Figure 3.

*[13]C-NMR spectra investigation of nitrogen heterocycles (**4-9**)*

*Compounds **4**, **5** and **6***

From study, the [13]C-NMR spectra of compounds **4, 5** and **6** showed the structure of these compounds in two isomers as keto-enol tautomers (Figure 2).

^{13}C-NMR spectra of these compounds **4, 5** and **6** showed that the presented two characteristic signals at δ 159.01, 159.00 ppm refer to the protons of NHCO groups for the keto form, and at δ 157.96, 156.22 ppm attributed to the protons of OH (N=C-OH) groups for the enol form. Also, ^{13}C-NMR revealed a characteristic carbon signal in the region δ 145.00-112.00 ppm due to the carbons of aromatic ring, these signals further supported the formation of two isomers for these compounds **4**, **5** and **6**.

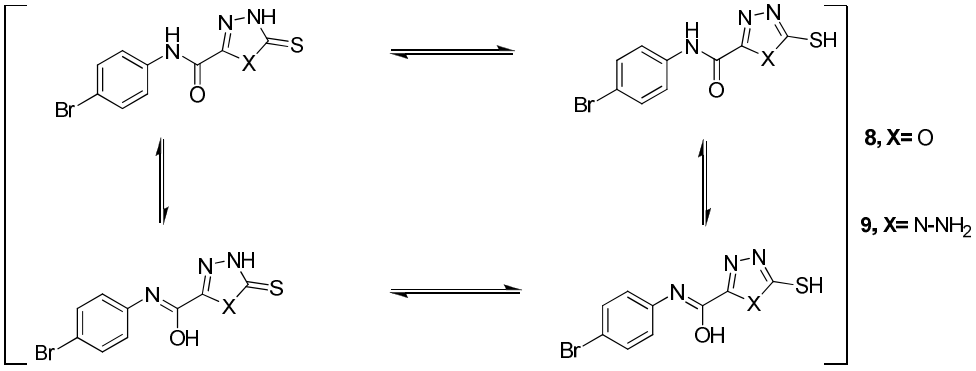


Figure 3. Different isomers of compounds **8** and **9**.

*Compounds **8** and **9***

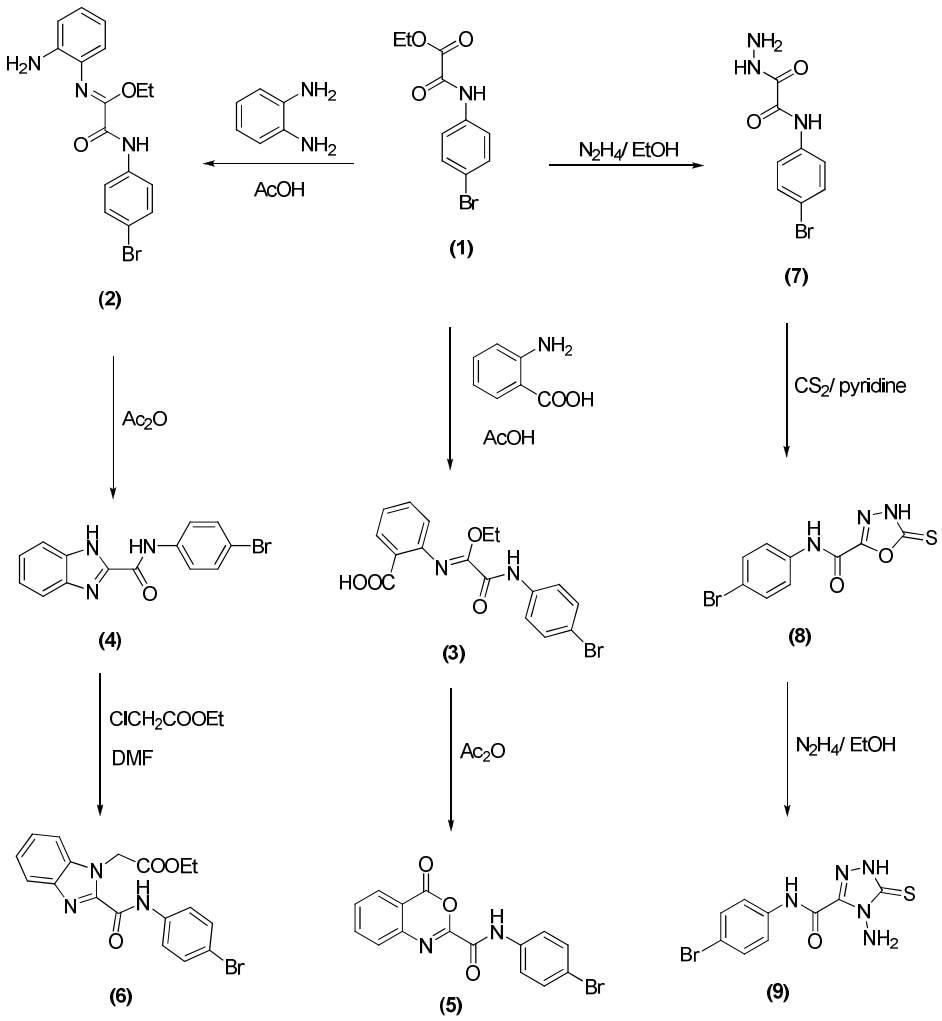
From the data of ^{13}C-NMR spectra for the compounds **8** and **9** gave and showed that the presented characteristic carbon signals in the region δ 179.00-142.00 ppm due to the carbons of two isomers for the carboxamide and thiocarboxamide groups as shown in Figure 3. The ^{13}C-NMR spectra of compounds **8** and **9** showed the presence of three values of each carbon signal of the aromatic ring carbon, these signals further confirmed the presented three isomers of these compounds.

Anti-tumor activity against breast carcinoma (MCF-7) cells

Compounds **4**, **5**, **6**, **8** and **9** were tested for their anti-proliferative activity against breast carcinoma (MCF-7) cell line using MTT assay method. Doxorubicin (Dox) was employed as positive reference. The IC_{50} values (μM) of the tested compounds and reference compound are listed in Table 1 and presented graphically in Figure 4. The most potent compound **8** was the most active with IC_{50} values less than 5 μM. In conclusion, compounds **8** and **9** were the most potent active compounds. The highest cytotoxic compound is **8** which had IC_{50} value of 3.81 μM compared with the value of 2.29 μM of Dox.

Table 1. Calculated IC_{50} (μM) for compounds **4, 5, 6,8** and **9** against breast carcinoma (MCF-7) cells.

Compound No.	IC_{50} (μM)/MCF-7	IC_{50} (μM)/Hs371T
4	10.01±0.08	-
5	11.22±0.61	-
6	29.27±0.19	-
8	3.81±0.11	75.21±0.21
9	5.32±0.14	-
Dox	2.29±0.09	112.06±0.12



Scheme 1. Synthesis of nitrogen heterocycles containing *N*-4-(bromophenyl)-carboxamide moiety (**4-9**).

*Cell cycle analysis of compound **8***

To study the mechanism of anticancer activity of compound **8**, cell cycle analysis was carried out using DNA flow cytometry analysis in MCF-7 cells. MCF-7 cells were incubated with IC_{50} concentration of compound **8** for 24 h and then subjected to DNA flow cytometry analysis. Compound **8** could enhance the G1 phase by 13.13% compared with the untreated control. This effect was accompanied by increase in cell percentage in G_0phase of the cell cycle. These results suggested that compound **8** induce cancer cell death via G1 phase arrest with apoptosis inducing activity marked by the presence G_0 peak in the cell cycle distribution profile of MCF-7 cells (Figure 5).

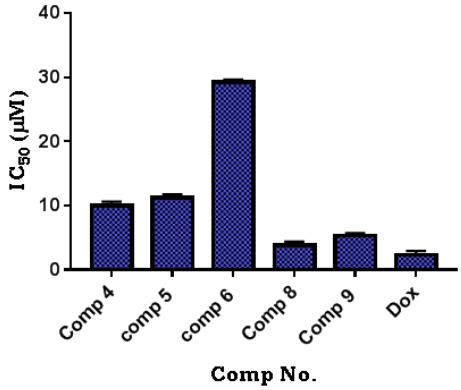


Figure 4. Calculated IC_{50} (μM) for compounds **4**, **5**, **6**, **8** and **9** against breast carcinoma (MCF-7) cells.

Figure 5. Effect of compound **8** on the cell cycle profile of MCF-7.

CONCLUSION

A new simple series of nitrogen heterocycles bearing biologically active carboxamide moiety were synthesized from *p*-bromo aniline and diethyl oxalate as a key starting material. The structure of these nitrogen heterocycles was confirmed by IR, ^{1}H-NMR, ^{13}C-NMR, MS and elemental analysis. In this work, the synthesized nitrogen heterocycles were screened for their anticancer activity against MCF-7 cells. The results of revealed that compound **8** was the most potent cytotoxic activity. Cell cycle analysis demonstrated that compound **8** induce cell cycle arrest at G1 phase with apoptosis inducing activity marked by increase in G0 phase.

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