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Synthesis Some New Benzoxazole Derivatives and Their Cytotoxicity to Human Cancer Cell Lines.

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ABSTRACT

A new series of benzoxazole derivatives were synthesized based on the reaction of 2-mercaptobenzoxazole as a core compound with some chloroacetamide derivatives and hydrazine. The structures of the newly synthesized compounds were secured by IR, ^{1}H NMR, MS and elemental analyses. The synthesized benzoxazole compounds have been evaluated for their cytotoxic effects toward four human cancer cell lines. They displayed moderate to strong cytotoxic effects against the tested cancer cell lines. Compounds **11** and **12** showed the stronger cytotoxic effects against hepatocellular carcinoma (IC_{50} 5.5±0.22 µg/ml) and breast cancer (IC_{50} 5.6±0.32 µg/ml) cell lines, respectively.

Keywords: 2-Mercaptobenzoxazole, N-chloroacetylamino-acetanilide, hydrazine hydrate, 2-hydrazinylbenzoxazole, ninhydrin, piperonal, anticancer activity.

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INTRODUCTION

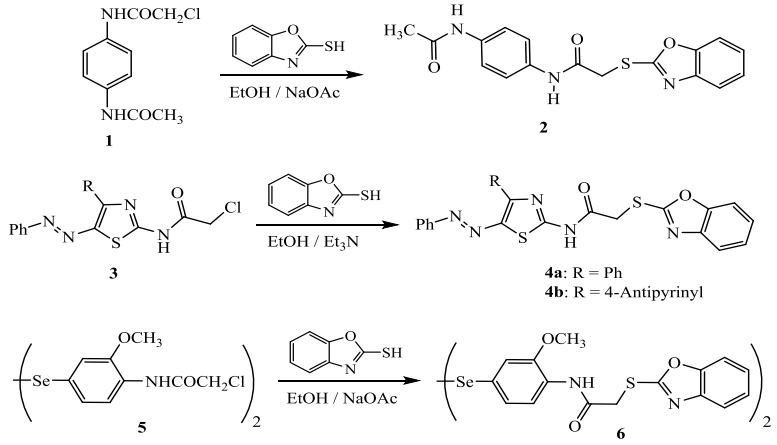
Oxazole derivatives are well-known five-membered nitrogen containing heterocyclic compounds. They are highly versatile intermediates used for the synthesis of several organic molecules, including amino acids, peptides, antimicrobial or antitumor compounds, immunomodulators, heterocyclic precursors for biosensors coupling, and photosensitive composition devices for proteins [1-3]. Benzoxazole and its derivatives are known to exhibit high therapeutic efficiency as antibacterial, antifungal, antitumor, anti-tubercular, anti-inflammatory and HIV-1 reverse transcriptase inhibitory activities [4-8]. 2-Mercaptobenzoxazoles have emerged as a potent medicinal scaffold and have remained the focus of drug discovery due to their important therapeutic values [9]. In the present study, we report on the synthesis of some new benzoxazole derivatives, their characterization by spectral data (IR, ^{1}H NMR and MS) and evaluation their cytotoxicity against several human cancer cell lines.

RESULTS AND DISCUSSION

N-(4-Acetamidophenyl)-2-(benzoxazol-2-ylthio)-acetamide **(2)** is formed by treatment of 2-mercaptobenzoxazole with p-(N-chloroacetylamino)-acetanilide **(1)** [10] in ethanol and sodium acetate under reflux. The chemical structure of **2** was established based on its elemental analysis and spectral data. The IR spectrum of **2** exhibited the characteristic absorption bands of NH groups at 3469 and 3297 cm^{-1} in addition to a broad absorption at 1663 cm^{-1} for the carbonyl groups (C=O). ^{1}H NMR spectrum displayed two singlet signals at 9.85 and 10.33 ppm for two (NH) protons, multiplet in the region 7.69-6.71 ppm for eight aromatic protons and two singlet signals at 2.01 and 4.35 ppm for the protons of (CH_3) and (CH_2) groups.

2-(Benzoxazol-2-ylthio)-N-(4-substituted-5-phenylazo-thiazol-2-yl)-acetamide derivatives **4** were synthesized by the reaction of 5-phenylazo-2-(chloroacetylamino)-thiazole derivatives **3** [11] with 2-mercaptobenzoxazole in hot ethanol containing catalytic amount of trimethylamine. Elemental and spectroscopic analyses support the suggested chemical structure of **4**. The IR spectrum of **4a** revealed the characteristic absorption band of NH group at 3157 cm^{-1} and carbonyl group (C=O) at 1691 cm^{-1}. ^{1}H NMR spectrum of **4b** exhibited the proton of (NH) function as singlet at 11.65 ppm, the aromatic protons as multiplet in the region 7.65-7.10 ppm and the protons of two methyl and one methylene groups as three singlet signals at 4.30, 3.20 and 2.25 ppm. . In the mass spectrum of **4a**, the molecular ion peak was recorded at m/z = 471 indicating the molecular weight of the formula $C_{24}H_{17}N_5O_2S_2$.

N,*N*'-(Diselanediyl-bis(2-methoxy-4,1-phenylene))-bis(2-(benzoxazol-2-ylthio)-acetamide) **(6)** has been obtained when 2-mercaptobenzoxazole was treated with N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis-(2-chloro-acetamide) **(5)** [12] in ethanol and sodium acetate under reflux. Elemental analysis and spectral data support the suggested chemical structure of **6**. The IR spectrum of **6** revealed the characteristic absorption bands of NH group at 3283 cm^{-1} and carbonyl group at 1696 cm^{-1}. ^{1}H NMR spectrum displayed singlet for two protons at 9.74 ppm of two NH groups, multiplet for fourteen aromatic protons in the region 7.97-7.18 ppm, singlet at 4.41 ppm for four protons of two methylene groups and singlet at 3.78 ppm for six protons of two methoxy groups.



Scheme 1: The reaction of 2-mercaptobenzoxazole with N-chloroacetamide derivatives

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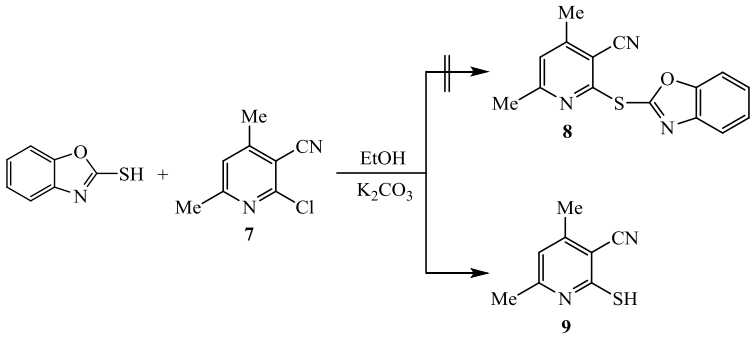
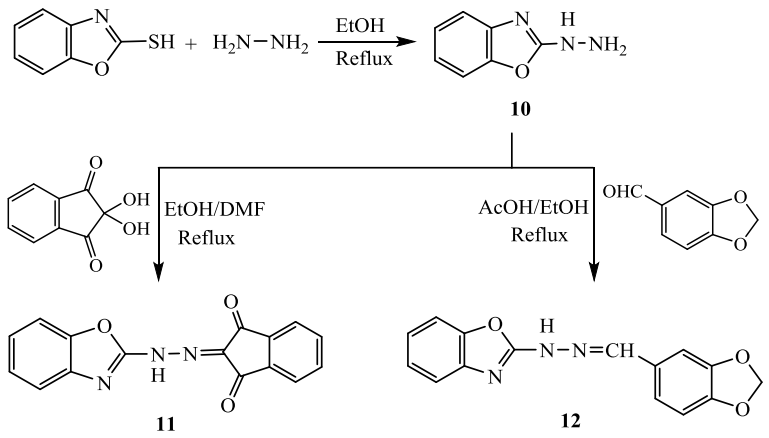
The reaction of 2-mercaptobenzoxazole with 2-chloro-4,6-dimethyl-nicotinonitrile in refluxing ethanol containing potassium carbonate failed to give the target sulfide derivative **8**. The isolated product was analyzed and identified as 2-mercapto-4,6-dimethyl nicotinonitrile **(9)**. The measured melting point was identical to the reported one in the literature [13] and the elemental analysis is in a good agreement with the calculated analysis. The IR spectrum of **9** revealed the characteristic absorption bands of (SH) at 3238 cm^{-1}, (C≡N) group at 2217 cm^{-1} and (C=N) group at 1658 cm^{-1}. Its ^1H NMR spectrum exhibited singlet at 12.35 ppm for one proton of (SH) function, singlet at 6.13 ppm for one proton of pyridine C-5 in addition to two singlet signals at 2.28 and 2.21 ppm for the protons of two methyl groups.



Scheme 2: The reaction of 2-mercaptobenzoxazole with 2-chloro-4,6-dimethyl-nicotinonitrile

The synthesis of 2-hydrazinylbenzoxazole **(10)** is reported in the literature by treatment 2-mercaptobenzoxazole with hydrazine hydrate in ethanol under reflux [14]. Heating of 2-hydrazinylbenzoxazole **(10)** with ninhydrin under reflux in ethanol and DMF mixture furnished the corresponding condensation product 2-(2-(benzoxazol-2-yl)hydrazono)-1H-indene-1,3(2H)–dione **(11)**. Elemental and spectroscopic analyses supported the chemical structure of **11.** The IR spectrum exhibited the characteristic absorption band of NH group at 3163 cm^{-1}, absorption frequencies of carbonyl groups at 1716 and 1680 cm^{-1} in addition to the absorption of (C=N) group at 1630 cm^{-1}. In the mass spectrum, the molecular ion peak was recorded at m/z = 291 indicating the molecular weight of the formula $C_{16}H_9N_3O_3$.

Finally, condensation of 2-hydrazinylbenzoxazole **(10)** with piperonal was achieved by heating in ethanol containing 0.5 ml acetic acid to afford the corresponding 2-(2-(benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl)benzoxazole **(12)** The chemical structure of **12** was secured by its correct elemental analysis and spectral data. The IR spectrum of **12** revealed the characteristic absorption band of NH group at 3209 cm^{-1} and absorption frequency of (C=N) group at 1593 cm^{-1}. Its ^1H NMR spectrum referred to singlet at 10.85 ppm (NH), singlet at 8.15 ppm (CH=N), multiplet in the region 7.10-7.70 ppm for seven aromatic protons and singlet at 5.80 ppm (O-CH_2-O).



Scheme 3: Condensation of 2-hydrazinylbenzoxazole with ninhydrin and piperonal

In vitro Antitumor activity: Benzoxazole compounds **2**, **4**, **6**, **11** and **12** were evaluated for their effects on the viability of various human cancer cell lines: HepG2 (hepatocellular carcinoma), HTC-116 (colorectal carcinoma),

PC-3 (human prostate cancer carcinoma) and MCF-7 (mammary gland breast cancer). Our results indicate that the synthesized benzoxazole derivatives exhibit moderate to strong cytotoxic effects toward the four tested human cancer cell lines (Table 1). However, the results also suggest no selective cytotoxic effects of benzoxazole derivatives toward cancer cells. Compound **4a**, **4b** and **6** showed moderate cytotoxic effects against the tested cancer cell lines. Similarly, compound 2 exhibited very strong cytotoxicity against HepG2 cells (IC_{50} 9.7±0.32) and strong effect among the other three cancer cell lines. Compounds **11** and **12**, derived from 2-hydrazinylbenzoxazole moiety, exhibited highest cytotoxic effects toward all tested cancer cell lines. Their IC_{50} values were very close to the standard anticancer drug 5-fluorouracil.

Table 1: Cytotoxic Activity of the synthesized benzoxazole compounds against human tumor cells

Compound	In vitro Cytotoxicity IC_{50} (µg/ml)			
	HepG2	HCT-116	PC-3	MCF-7
5-FU	7.9±0.28	5.2±0.14	8.3±0.25	5.5±0.21
2	9.7±0.32	10.6±0.60	11.0±0.96	11.9±0.97
4a	27.2±2.34	32.5±2.63	34.9±2.84	40.3±3.74
4b	21.4±2.09	29.7±2.16	32.7±2.43	32.3±2.47
6	34.8±2.11	40.2±3.21	38.4±2.88	45.9±3.86
11	5.5±0.22	5.4±0.22	7.9±0.30	7.1±0.41
12	7.7±0.19	8.0±0.43	7.2±0.24	5.6±0.32

IC_{50} (µg/ml): 1 – 10 (very strong), 11 – 20 (strong), 21 – 50 (moderate), 51 – 100 (weak) and above 100 (non-cytotoxic). **5-FU** = 5-Fluorouracil.
Data are reported as means ± Standard Deviation.

EXPERIMENTAL

All melting points were determined on Gallenkamp electric melting point apparatus and being incorrect. The IR spectra were recorded using FT-IR spectrometer (Thermo Scientific Nicolet iS 50) in KBr disks, microanalysis unit, Chemistry Department, Faculty of Science, Mansoura University. The ^{1}H NMR Spectra were measured on a Varian Spectrophotometer at 300 MHz, using TMS as an internal reference and DMSO-d_6 as solvent at the Chemistry Department, Faculty of Science, Cairo University. The mass spectra were performed using a Varian MAT 311 mass spectrometer at 70 eV. Elemental analyses were carried out at Microanalysis Center, Faculty of Science, Cairo University.

Synthesis of *N*-(4-acetamidophenyl)-2-(benzoxazol-2-ylthio)-acetamide (2):

A mixture of p-(N-chloroacetylamino)-acetanilide **(1)** (0.23 g, 0.001 mol) and 2-mercaptobenzoxazole (0.15 g, 0.001 mol) was heated under reflux for 4 hours in 15 mL absolute ethanol containing 0.2 g sodium acetate. The reaction mixture was allowed to cool at room temperature and then diluted with ice-cold water. The separated solid product was filtered and recrystallized from ethanol to give **2.**

Light brown crystals; Yield: 68%, m.p. = 216 -218 ºC. Retardation factor = 0.72 [petroleum ether / ethyl acetate (2:1)]. IR ($\overline{\nu}$ /cm^{-1}): 3469 (NH), 3297 (NH), 1663 (C=O), 1557 (C=C). ^{1}H NMR (DMSO-d_6): δ/ppm = 10.33 (s, 1H, NH), 9.85 (s, 1H, NH), 7.69-6.71 (m, 8H, Ar-H), 4.35 (s, 2H, CH_2), 2.01 (s, 3H, CH_3CO). Analysis for $C_{17}H_{15}N_3O_3S$ (341.38): Calcd. C, 59.81; H, 4.43; N, 12.31%. Found: C, 59.57; H, 4.48; N, 12.20%.

Synthesis of 2-(benzoxazol-2-ylthio)-N-(4-substituted-5-phenylazothiazol-2-yl)-acetamide derivatives 4a,b:

A mixture of 5-phenylazo-2-(chloroacetylamino)-thiazole derivatives **3** (0.001 mol) and 2-mercaptobenzoxazole (0.15 g, 0.001 mol) in 20 mL absolute ethanol containing triethylamine (0.5 mL) was heated under reflux for 6 hours. The reaction mixture was cooled at room temperature and the separated solid product was filtered off, dried and recrystallized from ethanol.

2-(Benzoxazol-2-ylthio)-N-(4-phenyl-5-phenylazo-thiazol-2-yl)-acetamide (4a):

Orange crystals; Yield: 56%, m.p. = 172-174 ºC. Retardation factor = 0.56 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{\nu}$ /cm^{-1}): 3157 (NH), 1691 (C=O), 1645 (C=N). MS (m/z, %): 471 (24), 366 (100), 192 (43), 174 (58),

150 (71), 96 (27), 76 (18). Analysis for $C_{24}H_{17}N_5O_2S_2$ (471.55): Calcd. C, 61.13; H, 3.63; N, 14.85%. Found: C, 61.31; H, 3.57; N, 14.73%.

2-(Benzoxazol-2-ylthio)-N-(4-antipyrinyl-5-phenylazo-thiazol-2-yl)-acetamide (4b):

Red crystals; Yield: 62%, m.p. = 158-160 ˚C. Retardation factor = 0.62[petroleum ether / ethyl acetate (4:2)]. IR ($\overline{V}$ /cm^{-1}): 3187 (NH), 1717 (C=O), 1677 (C=O, antipyrine). ^{1}H NMR (DMSO-d_6): δ/ppm = 11.65 (s, 1H, NH), 7.65-7.10 (m, 8H, Ar-H), 4.30 (s, 2H, CH_2), 3.20 (s, 3H, CH_3), 2.25 (s, 3H, CH_3). Analysis for $C_{29}H_{23}N_7O_3S_2$ (581.67): Calcd. C, 59.88; H, 3.99; N, 16.86%. Found: C, 59.73; H, 3.95; N, 16.71%.

Synthesis of *N*,*N*'-(diselanediyl-bis(2-methoxy-4,1-phenylene))-bis(2-(benzoxazol-2-ylthio)-acetamide) (6):

A mixture of N,N'-(diselanediylbis(2-methoxy-4,1-phenylene))bis-(2-chloro-acetamide) **(5)** (0.55 g, 0.001 mol) and 2-mercaptobenzoxazole (0.32 g, 0.002 mol) was refluxed for 4 hours in 15 mL ethanol containing sodium acetate (0.4 g). The reaction mixture was allowed to cool at room temperature and poured into cooled water. The solid product that formed was collected by filtration, dried and recrystallized from ethanol-DMF to give **6**.

White crystals; Yield: 54%, m.p. = 162-164 ºC. Retardation factor = 0.44 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{V}$ /cm^{-1}): 3283 (NH), 1696 (C=O), 1671 (C=N), 1590, 1524 (C=C), 820(C-Se). ^{1}H NMR (DMSO-d_6): δ/ppm = 9.74 (s, 2H, 2NH), 7.97-7.18 (m, 14H, Ar-H), 4.41 (s, 4H, 2CH_2), 3.78 (s, 6H, 2OCH_3). Analysis for $C_{32}H_{26}N_4O_6S_2Se_2$ (784.62): Calcd. C, 48.99; H, 3.34; N, 7.14%. Found: C, 48.84; H, 3.30; N, 7.19%.

Synthesis of 2-mercapto-4,6-dimethylnicotinonitrile *(9):*

A mixture of compound 2-chloro-4,6-dimethyl nicotinonitrile (0.33 g, 0.002 mol) and 2-mercaptobenzoxazole (0.32 g, 0.002 mol) was refluxed for 4 hours in 15 mL ethanol and 0.5 g potassium carbonate. The yellow crystals that separated was filtered off and recrystallized from ethanol to give **9**.

Light orange crystals; Yield: 85%, m.p. = 280 -282 ºC. Retardation factor = 0.62 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{V}$ /cm^{-1}): 3238 (SH), 2217 (C≡N), 1658 (C=N). ^{1}H NMR (DMSO-d_6): δ/ppm = 12.35 (s, 1H, SH), 6.13 (s, 1H, pyridine H-5), 2.28 (s, 3H, CH_3), 2.21 (s, 3H, CH_3). Analysis for $C_8H_8N_2S$ (164.23): Calcd. C, 58.51; H, 4.91; N, 17.06%. Found: C, 58.40; H, 4.86; N, 17.18%.

Synthesis of 2-hydrazinylbenzoxazole (10):

A mixture of 2-mercaptobenzoxazole (1.51 g, 0.01 mol) and hydrazine hydrate (5 mL) was refluxed for 3 hours, then 15 mL ethanol was added and refluxed for 4 hours. The separated precipitate was filtered and washed with cold water and recrystallized from ethanol to give **10**.

White crystal; Yield: 38%, m.p. = 214-215 ºC, Lit. m.p. = 210-212 ºC [14]. Retardation factor = 0.64 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{V}$ /cm^{-1}): 3266, 3212 (NH), 1645 (C=N), 1594 (C=C). Analysis for $C_7H_7N_3O$ (149.15): Calcd. C, 56.37; H, 4.73; N, 28.17%. Found: C, 56.13; H, 4.78; N, 28.01%.

Synthesis of 2-(2-(benzoxazol-2-yl)hydrazono)-1H-indene-1,3(2H)-dione (11):

A mixture of **10** (0.15 g, 0.00l mol) and ninhydrin (0.18 g, 0.00l mol) in ethanol absolute (15 ml) and (5.0 ml) DMF was heated and refluxed for 4 hours. The solid that formed after cooling was filtered to give **11**.

Orange crystals; Yield: 69%, m.p. = 278-280 ºC. Retardation factor =0.56 [petroleum ether / ethyl acetate (4:2)]. IR ($\overline{V}$ /cm^{-1}): 3163 (NH), 1716 (C=O), 1680 (C=O), 1630 (C=N), 1591 (C=C). MS (m/z, %): 291 (17), 158 (97), 130 (27), 102 (100.0), 76 (31). Analysis for $C_{16}H_9N_3O_3$ (291.27): Calcd. C, 65.98; H, 3.11; N, 14.43%. Found: C, 65.81; H, 3.15; N, 14.33%.

Synthesis of 2-(2-(benzo[d][1,3]dioxol-5-ylmethylene)hydrazinyl)-benzoxazole (12):

A mixture of **10** (0.15 g, 0.001 mol) and piperonal (0.15 g, 0.001 mol) in 20 mL absolute ethanol and 5 mL acetic acid was refluxed for 2 hours. The solid product that obtained on cooling was filtered off and dried to give **12**.

Yellow crystals; Yield: 48%, m.p. = 255-256 ºC. Retardation factor = 0.44 [petroleum ether / ethyl acetate (4:1)]. IR ($\overline{\nu}$ /cm^{-1}): 3209 (NH), 1593 (C=N). ^{1}H NMR (DMSO-d_6): δ/ppm = 10.85 (s, 1H, NH), 8.15 (s, 1H, CH=N), 7.10-7.70 (m, 7H, Ar-H), 5.80 (s, 2H, CH_2). Analysis for $C_{15}H_{11}N_3O$ (281.27): Calcd. C, 64.05; H, 3.94; N, 14.94%. Found: C, 64.18; H, 3.87; N, 14.82%.

Cytotoxicity assay

Cell Lines

Four human tumor cell lines namely; hepatocellular carcinoma HepG-2, colorectal carcinoma HCT-116, human prostate cancer PC-3 and mammary gland breast cancer MCF-7. The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical Reagents

The reagents RPMI-1640 medium, MTT, DMSO and 5-fluorouracil (Sigma Co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK). 5-Fluorouracil was used as a standard anticancer drug for comparison.
MTT assay [15]: The cell line mentioned above was utilized to determine the inhibitory effects of compounds on cell growth utilizing the MTT test. This colorimetric test is based on the transformation of the yellow 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in practical cells. Hep2 was refined in RPMI-1640 medium with 10% fetal bovine serum. Anti-toxins included were 100 units/ml penicillin and 100 µg/ml streptomycin at 37 °C in a 5% CO_2 incubator. The cell line was seeded in a 96-well plate at a density of 1 × 10^4 cells/well [16] at 37 °C for 48 hours under 5% CO_2. After incubation the cells were treated with distinctive concentration of compounds and incubated for 24 hours. After 24 hours of medication treatment, 20 µl of MTT solution at 5 mg/ml was added and incubated for 4 hours. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric test is measured and recorded at absorbance of 570 nm utilizing a plate reader (EXL 800) USA. The relative cell viability in percentage was ascertained as (A570 of treated samples/A570 of untreated sample) × 100.

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