Synthesis and Biological Activities of Novel Indole-3-Carbinol and (Benzimidazolylmethyl) Triazole-3, 4-Diamine Derivatives

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ABSTRACT

Condensation of 2-(1-benzyl-1H-indole-2-carbonyl)-N-ethylhydrazinecarbothioamide 5 with hydrazine hydrate yielded 5-(1-benzyl-1H-indol-2-yl)-N3-ethyl-4H-1, 2, 4-triazole-3, 4-diamine 6. Cyclization of 5-fluoro-1H-indole-2-carbohydrazide 4a and 3-formyl-1H-indole-2-carboxamide 11 using triethoxymethane gave 8-fluoro-[1,2,4]triazino[4,5-a]indol-1(2H)-one 7 and 1-oxo-1H-imidazo[1,5-a]indole-9-carbaldehyde 12 respectively. Formylation of 7 using POCl3/DMF gave 8-fluoro-1-oxo-1, 2-dihydro-[1, 2, 4] triazino [4, 5-a] indole-10-carbaldehyde 8. Reduction of 8 and 12 with NaBH4 in methanol gave 8-fluoro-10-(hydroxymethyl)-[1,2,4]triazino[4,5-a]indol-1(2H)-one 9 and 9-(hydroxymethyl)-1H-imidazo[1,5-a]indol-1-one 13. On the other hand, refluxing of thiosemicarbazide and semicarbazide derivatives of benzimidazole hydrazides 17a-d with hydrazine hydrate afforded N3-substituted-5-((2-phenyl-1H-benzo[d]imidazol-1-yl)methyl)-4H-1,2,4-triazole-3,4-diamines 18a-d. The synthesized compounds were studied for their antioxidant and antimicrobial activities. The structures of new compounds were confirmed by both analytical and spectral data. The antitumor activity of certain selected new compounds was screened in vitro against human breast carcinoma (MCF-7) cell line. The antioxidant, anticancer and antimicrobial activities of the selected compounds showed promising results.

KEYWORDS: Triazoles, antioxidant, antiproliferative screening, antimicrobial activity
INTRODUCTION

The increasing diversity of small molecule libraries is an important source for the discovery of new drug candidates. In terms of this trend, the literature survey showed that indole derivatives possess anticancer (1-3), antioxidant (4-7), antibacterial (8-12), antifungal (13-14), antiviral (15-16) and antihypertensive activities (17). In addition, indole-3-carbinol, a naturally occurring autolysis product of glucobrassicin, that exhibits anti-proliferative activity in many types of human cancer cells (18-20) including estrogen responsive and estrogen-independent breast cancer cells (21-23) and human prostate cancer cells (24). Based on these findings, syntheses of some new indole heterocycles were carried out for the purpose of evaluation of their antioxidant, antimicrobial and antitumor activities.

Likewise, compounds with different substitution in the benzimidazole ring are associated with a wide range of biological activities including anticancer (2), antiviral (16), antibacterial (10-11), antifungal (14), antihelminthic (25-26), anti-inflammatory (27), antihistaminic (28), proton pump inhibition (29-30), antioxidant (4-7, 31), antihypertensive (17) and anticoagulation (32) properties. In addition, broad spectrum biological activities were associated with benzimidazole. Various semicarbazide, thiosemicarbazide (33) and their cyclized products such as oxazoles, oxadiazoles, triazoles and thiadiazoles have been reported to possess antibacterial (34), antifungal (35) and antioxidant activities (36). In the present work it was decided to synthesize compounds containing benzimidazole and triazole moieties in one frame and screen them for their antimicrobial, antioxidant and anticancer activities.

MATERIALS AND METHODS

Apparatus
Melting points were measured in open capillary tubes using Stuart melting point apparatus SMP10 (UK). Infrared (IR) spectra were recorded using KBr discs on a Shimadzu Spectrophotometer (υmax in cm⁻¹) (Kyoto, Japan). Proton Magnetic Resonance (1H-NMR) spectra were recorded on Mercury–300 BB (NMR300) spectrometer (300MHz). 13C NMR spectra were recorded on JNM-LA series FT NMR system (Lambda-100MHz). Chemical shifts are reported in δ values (parts per million, ppm) relative to tetramethylsilane (TMS) as internal standard. Abbreviations used in NMR analysis are as follows: d=doublet, m=multiplet, q=quartet, s=singlet, t=triplet. Electron impact mass spectra (EI-MS) were recorded on DI Analysis Shimadzu QP-2010 Plus mass spectrometer. Elemental analyses were recorded on Vario EL-CHNS Elemental Analyzer (GmbH, Germany). The results of elemental analyses (C, H, N) were found to be in good agreement (±0.4%) with the calculated values. IR, 1H -NMR, EI-MS and Elemental analyses were performed in the Microanalytical center, Cairo University, Egypt. Reactions were monitored by thin layer chromatography (TLC) on Merck silica gel 60 F254 and visualized with UV light.

Chemicals and Reagents
Ethyl isothiocyanate, triethoxymethane, dimethylsulphoxide, dimethyl formamide, phosphorous oxychloride, sodium borohydride, ethyl-2-chloroacetate, phenyl isocyanate, 4-bromophenyl isothiocyanate were obtained from Sigma, St. Louis, MO, USA and Merck, Darmstadt, Germany.
**Experimental Chemistry**

**Ethyl 5-substituted-1H-indole-2-carboxylate 1-2**

A mixture of p-Toluenesulfonic acid (3 g, 17.4 mmol) in dry benzene (50 ml) was heated under reflux using Dean-stark apparatus for 1.5 h. A suspension of ethyl pyruvate 4-substituted phenylhydrazone (10 mmol) in dry benzene (30 ml) was added and the whole mixture was refluxed for 5 h. The resulting solution was diluted with benzene, washed with aqueous NaHCO₃, dried over anhydrous Na₂SO₄, and evaporated to dryness. The resulting product was recrystallized from ethanol.

**Ethyl 1H-indole-2-carboxylate 1:** mp. 125-126°C (37, 47).

**Ethyl 5-fluoro-1H-indole-2-carboxylate 2:** mp. 150-152°C (37, 47).

**Ethyl 1-benzyl-1H-indole-2-carboxylate 3**

Dimethylsulfoxide (DMSO, 15 ml) was added to potassium hydroxide (1.51g, 27 mmol, crushed pellets) and the mixture was stirred for 15 min. Ethyl 1H-indole-2-carboxylate 1 (1.26g, 6.7 mmol) was added and then the mixture was stirred at room temperature for 2 h. Benzyl chloride (3.4 ml, 27 mmol) was added and the mixture was cooled briefly and stirred for further 2 h. Water was added and the mixture was extracted with ether. Ether layers were washed with water, dried, and solvent and excess of benzyl chloride was removed. The oily compound was obtained and used without further purification (38-39).

**1-Substituted-5-halo-1H-indole-2-carboxydrazides 4a-b**

A mixture of ethyl 1-substituted- 5-halo-indole-2-carboxylates 1, 3 (5.3 mmol) and hydrazine hydrate 99% (15 mmol, 0.75 ml) was refluxed in 20 ml ethanol for 6 h. The precipitate formed after cooling was collected by filtration and recrystallized from DMF/ethanol mixture.

**5-Fluoro-1H-indole-2-carbohydrazide 4a:** mp. 192-193°C (40-42).

**1-Benzyl-1H-indole-2-carbohydrazide 4b:** mp. 225-228°C (40-42).

**2-(1-Benzyl-1H-indole-2-carbonyl)-N-ethyl hydrazine carbothioamide 5**

A mixture of 1-benzyl-1H-indole-2-carboxydrazide 4b (0.8g, 3 mmol), absolute ethanol (20 ml), and ethyl isothiocyanate (0.4 ml, 4.5 mmol) was heated under reflux for 2 h. Precipitate formed was cooled, filtered and recrystallized from ethanol. mp. 200-203°C (31, 40, 43-47).

**5-(1-Benzyl-1H-indol-2-yl)-N³-ethyl-4H-1,2,4-triazole-3, 4-diamine 6**

A suspension of 2-(1-benzyl-1H-indole-2-carbonyl)-N-ethyl hydrazine carbothioamide 5 (0.35g, 1 mmol) in hydrazine hydrate 99% (0.1 ml, 2 mmol) was refluxed for 5 h. It was then diluted with water and acidified with HCl. The white solid separated out was filtered, washed with water and recrystallized from ethanol (48). mp. =165-167°C, (yield 80%). IR (cm⁻¹): 1596(C=N), 3408(NH), 300 MHz): δ(δppm) = 1.00(t, 3H, CH₂-ethyl), 3.97(q, 2H, NCH₃), 4.82(s, 3H, NH₂), 5.58(s, 2H, CH₂-CH₂), 6.91(s, 1H, H at C₃-indole), 6.93-7.73(m, 9H, Ar-H), 13-C-NMR (DMSO-d₆, 100 MHz): δ = 13.88, 38.12, 47.0, 100.22, 110.76, 120.52, 121.21, 121.96,
127.16, 127.33, 127.86, 128.35, 130.16, 135.95, 137.33, 139.49, 147.92 ppm.

8-Fluoro-[1, 2, 4] triazino [4, 5-a] indol-1(2H)-one 7

A mixture of 5-fluoro-1H-indole-2-carbohydrazide 4a (0.2g, 1mmol), N, N-dimethylformamide (DMF, 5 ml), and triethoxymethane (0.3 ml, 2 mmol) was boiled in an ice-bath for 30 min. Solvent was removed and the residue recrystallized from DMF / ethanol mixture (47, 3:1). mp. =188-190°C, (yield 75%). IR (cm⁻¹): 1550(C=N), 1664(C=O), 3176(NH). Mass spectrum: m/z(%): 231(M⁺, 100%), 203(M⁺, 100%), 193(8.55%), 178(2.37%), 162(94.34%), 147(38.86%), 133(45.30%), 121(16.52%), 99(15.97%), 73(4.26%), 57(10.87%).

8-Fluoro-1-oxo-1, 2-dihydro-[1, 2, 4] triazino [4, 5-a] indole-10-carbaldehyde 8

In a 250 ml two-necked round bottomed flask, dry N, N dimethylformamide (DMF, 2 ml, 27.3 mmol) was cooled in an ice-bath for 30 min. Phosphorus oxychloride (0.6 ml, 4 mmol) was dropped into the reaction flask in about 5 min. The cooling bath was removed and the reaction mixture was then stirred at room temperature for 30 min. The mixture was newly cooled in an ice-bath and a solution of ethyl 1H-carboxylate 10 (0.75g, 4 mmol) in DMF (2 ml) was dropped in about 5 min into the reaction flask. The mixture was then stirred in a water-bath for 2 h at 80-100°C. The colored solution obtained was poured over crushed ice. The mixture was extracted with ether. Ether layers were washed with water and dried over anhydrous Na₂SO₄ and evaporated to dryness. The product was filtered off and recrystallized from DMF / ethanol mixture (47, 51-55). mp. >300°C, (yield 60%). IR (cm⁻¹): 1664(C=O), 7.34-8.27(m, 3H, Ar-H), 9.08(s, 1H, H at C₄-triazine), 11.96(s, 1H, NH-triazine), 13.21, 134.96, 138.16, 151.33, 157.96 ppm.
bath for 2 hr at 80-100°C. The colored solution obtained was poured over crushed ice. The resulting orange precipitate was left overnight for complete precipitation. The collected product was filtered and washed first with warm H₂O and then with ethanol / water mixture. mp. 192 °C (47, 51-55).

3-Formyl-1H-indole-2-carboxamide 11

A mixture of ethyl 3-formyl-1H-indole-2-carboxylate 10 (0.4g, 1.8 mmol), ethanol (80 ml), and 32% NH₄OH (80 ml) was heated at 60°C for 3 h. after dilution with water; the amide was extracted with ethyl acetate. The organic solution was washed with brine then dried (56). mp. = 198-202°C, (yield 65%). Mass spectrum: m/z(%): 188(M⁺, 30.33%), 143(10.30%), 115(100%), 100(21.30%), 76(12.10%).

1H-NMR (DMSO-d₆, 300 MHz): δ(ppm) = 6.89(s, 2H, NH₂), 7.36-8.60(m, 4H, Ar-H), 10.22(s, 1H, CHO), 10.81(s, 1H, NH indole).

1-Oxo-1H-imidazo [1, 5-a] indole-9-carbaldehyde 12

A mixture of 3-formyl-1H-indole-2-carboxamide 11 (0.19g, 1mmol), N, N-dimethylformamide (5 ml), and triethoxymethane (0.3 ml, 2 mmol) was boiled for 6 h. Solvent was removed and the residue recrystallized from DMF / ethanol mixture (47, 49-51). mp. =190°C, (yield 70%). Mass spectrum: m/z(%): 198(M⁺, 12%), 169(0.23%), 143(100%), 127(10.10%). 114(5.25%), 100(2.36%). 76(62.30%). 

1H-NMR (DMSO-d₆, 300 MHz): δ(ppm) = 7.50-8.61(m, 4H, Ar-H), 9.60(s, 1H, H at C₃ imidazole), 10.30(s, 1H, CHO).

9-(Hydroxymethyl)-1H-imidazo[1,5-a]indol-1-one 13

Sodium borohydride (0.1g, 2.7 mmol) was added to 1-oxo-1H-imidazo [1, 5-a] indole-9-carbaldehyde 12 (0.45g, 2.25 mmol) in ethanol (25 ml) and stirred for 4–6 h at room temperature. Water (50 ml) was added and the solution was partitioned with ether (3× 50 ml). The combined organic phases were back extracted with water (2× 100 ml), dried over anhydrous Na₂SO₄, and concentrated to dryness (55). mp. =140°C, (yield 65%). IR (cm⁻¹): 1601(C=N), 1655(C=O), 3303(OH). Mass spectrum: m/z(%): 200(M⁺, 5.01%), 190(88.73%), 172(88.94%), 144(100%), 129(69.24%), 118(63.17%), 102(30.70%), 89(48.33%), 77(34.37%), 65(28.19%).

1H-NMR (DMSO-d₆, 100 MHz): δ(ppm) = 4.85(s, 2H, CH₂ carbinol), 5.71(s, 1H, OH), 7.03-7.71(m, 4H, Ar-H), 11.48(s, 1H, C₃ imidazole).

2-Substitutedphenyl benzimidazoles 14a-b

The mixture of o-phenylenediamine (1 mmol, 0.1g) and sodium metabisulfite adduct of substituted benzaldehydes (1.25 mmol) in N,N-dimethyl formamide (DMF, 5 ml) was heated at 110 °C for 5 h. Water was added to the reaction medium and the solid product was collected by filtration and washed with water. The crude product was recrystallized from ethanol.

2-(3-Nitrophenyl)-1H-benzo[d]imidazole 14a: mp. 204-206°C (57-61).

2-(4-Methoxyphenyl)-1H-benzo[d]imidazole 14b: mp. 226°C (57-61).
Ethyl 2-(2-(substituted phenyl)-1H-benzo[d]imidazol-1-yl)-acetates 15a-b

Dimethylsulfoxide (DMSO, 15 ml) was added to potassium hydroxide (1.5g, 27 mmol) (crushed pellets) and the mixture was stirred for 15 min, then related 2-substituted benzimidazoles 14a-b (6.7 mmol) was added and the mixture was stirred for 2 h. Ethyl-2-chloroacetate (3.3 ml, 27 mmol) was added and the mixture was cooled briefly and stirred for further 2 h. Water was added and the mixture was extracted with diethyl ether. Ether layers were washed with water, dried, and solvent and excess of ethyl-2-chloroacetate was removed. The residue was recrystallized from ethanol to give the desired esters.


Ethyl 2-(2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl) acetate 15b: mp. 151-153°C (61).

2-(2-(Substituted phenyl)-1H-benzo[d]imidazol-1-yl) acetohydrazides 16a-b

A mixture of hydrazine hydrate 99% (5 mmol, 0.25 ml) and related ethyl 2-(2-(substituted phenyl)-1H-benzo[d]imidazol-1-yl)-acetates 15a-b (1.5 mmol) in absolute ethanol (20 ml) were refluxed for 4 h. The reaction mixture was cooled and poured into water. The crude product was filtered off and recrystallized from ethanol to give the desired hydrazides.


2-(2-(4-Methoxyphenyl)-1H-benzo[d]imidazol-1-yl) acetohydrazide 16b: mp. 180-183°C (61).

N-substituted-2-(2-(2-(Substitutedphenyl)-1H-benzo[d]imidazol-1-yl) acetyl) hydrazinecarbo (ox/thio) amides 17a-d

A mixture of 2-(2-(substituted phenyl)-1H-benzo[d]imidazol-1-yl) acetohydrazides 16a-b (3 mmol), absolute ethanol (20 ml) and substituted isothiocyanate or phenyl isocyanate (4.5 mmol) was heated under reflux for 2 h. Precipitate formed was cooled, filtered and recrystallized from ethanol.

N-ethyl-2-(2-(4-methoxyphenyl)-1H-benzo[d]imidazol-1-yl)acetyl) hydrazinecarbothioamide 17a: mp. 215-218°C (31, 43).


2-(2-(2-(4-Methoxyphenyl)-1H-benzo[d]imidazol-1-yl)acetyl)-N-phenylhydrazine carboxamide 17d: mp. 110-113°C (43-45, 61).

5-(2-(Substituted phenyl)-1H-benzo[d]imidazol-1-yl) methyl)-N³-substituted-4H-1, 2, 4-triazole-3, 4-diamine 18a-d

A suspension of N-substituted-2-(2-(2-substitutedphenyl-1H-benzo[d]imidazol-1-yl)acetyl) hydrazinecarbo (ox/thio) amides 17a-d (1 mmol) in hydrazine hydrate 99% (0.1 ml, 2 mmol) was refluxed for 5 h. It was then diluted with water and acidified with HCl. The white solid separated out was filtered, washed with water and recrystallized from ethanol (48).
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\[ N^3\text{-ethyl-5-}(2-(4\text{-methoxyphenyl})-1H\text{-benzo[}\text{d} imidazol-1-yl)\text{-methyl}-4H-1, 2, 4\text{-triazole-3, 4- diamine 18a}} \]: mp. =250-252°C, (yield 75%). Mass spectrum: m/z(%): 363(M\(^+\), 6.26%), 334(12.17%), 319(21.12%), 303(8.19%), 277(27.92%), 263(12.30%), 237(100%), 223(19.37%), 192(12.63%), 116(20.36%), 90(50.30%), 76(66.62%). \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) (ppm) = 1.10(t, 3H, CH\(_3\)-ethyl), 3.10(q, 2H, CH\(_2\)-ethyl), 3.76(s, 3H, OCH\(_3\)), 4.55(s, 3H, NH\(_2\)/NH), 5.30(s, 2H, N-CH\(_2\)), 7.00-7.54(m, 8H, Ar-H).

\[^{13}\]C-NMR (DMSO-d\(_6\), 100 MHz): \(\delta\) = 13.01, 38.46, 55.35, 110.42, 110.76, 114.14, 114.35, 115.68, 119.09, 121.77, 122.33, 122.63, 130.49, 130.80, 135.86, 142.52, 147.87, 153.12, 160.57, 167ppm.

\[ N^3\text{-}(4\text{-bromophenyl})\text{-5-}(2-(3\text{-nitrophenyl})-1H\text{-benzo[}\text{d} imidazol-1-yl)methyl)-4H-1,2,4\text{-triazole-3,4- diamine 18b}} \]: mp. =200-202°C, (yield 73%). Mass spectrum: m/z(%): 505(M\(^+\), 0.93%), 492(1.46%), 479(0.52%), 463(2.55%), 449(2.98%), 435(1.43%), 421(1.07%), 407(3.18%), 393(2.18%), 379(1.44%), 364(0.43%), 351(1.29%), 337(1.25%), 309(0.87%), 295(1.44%), 281(5.92%), 266(1.45%), 256(100%), 222(11.82%), 209(7.14%), 184(4.37%), 176(8.58%), 155(10.58%), 149(4.98%), 134(5.99%), 118(13.81%), 90(23.91%), 71(52.72%), 57(36.09%). \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) (ppm) = 5.23(s, 2H, CH\(_2\)), 6.36(s, 3H, NH\(_2\)/NH), 7.15-8.80(m, 12H, Ar-H).

\[^{13}\]C-NMR (DMSO-d\(_6\), 100 MHz): \(\delta\) = 44.01, 118.85, 119.35, 120.22, 120.76, 122.02, 122.98, 125.56, 125.76, 130.66, 131.95, 133.33, 134.49, 134.92, 140.22, 141.52, 150.12, 151.32, 155.82, 160.57 ppm.

\[ 5\text{-}(2-(3\text{-Nitrophenyl})-1H\text{-benzo[}\text{d} imidazol-1-yl)methyl)-N^3\text{-phenyl-4H-1,2,4\text{-triazole-3,4- diamine 18c}} \]: mp. =284-286°C, (yield 78%). Mass spectrum: m/z(%): 426(M\(^+\), 0.89%), 410(0.44%), 384(0.43%), 358(1.29%), 318(1.25%), 291(0.87%), 265(100%), 238(5.92%), 224(1.45%), 178(5.36%), 102(1.82%), 76(6.09%). \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) (ppm) = 5.41(s, 2H, CH\(_2\)), 6.72(s, 3H, NH\(_2\)/NH), 6.89-8.60(m, 13H, Ar-H).

\[ 5\text{-}(2-(4\text{-Methoxyphenyl})-1H\text{-benzo[}\text{d} imidazol-1-yl)methyl)-N^3\text{-phenyl-4H-1,2,4\text{-triazole-3,4- diamine 18d}} \]: mp. =198-200°C, (yield 71%). Mass spectrum: m/z(%): 411(M\(^+\), 2.2%), 385(5.44%), 359(7.03%), 334(1.29%), 319(7.25%), 303(3.87%), 277(100%), 263(5.92%), 237(6.25%), 223(5.36%), 192(1.82%), 154(6.52%). 116(7.09%), 90(12.55%). \(^1\)H-NMR (DMSO-d\(_6\), 300 MHz): \(\delta\) (ppm) = 3.66(s, 3H, OCH\(_3\)), 5.13(s, 2H, CH\(_2\)), 6.52(s, 3H, NH\(_2\)/NH), 6.85-7.77(m, 13H, Ar-H).
Table 1: The found and calculated values of elemental analyses

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2. Biological studies

2.1. Antioxidant activity

DPPH is a stable free radical that can accept an electron or hydrogen radical to become a stable diamagnetic molecule. The radical scavenging potential of the compounds 6, 9, 13, 18a, 18b, 18c and 18d was determined by measuring the decrease in absorbance due to DPPH at 517 nm (63-70). Stock solution of tested compounds in methanol was freshly prepared (150 µg/ml), then diluting to make aliquots concentrations of 5, 10, 15, 20, 40, 60, 80, 100 and 120 µg/ml. To 2ml of each compound aliquot, 2ml of methanolic solution of DPPH (80 µg/ml) was added and left for 30 min until the reaction between the two substances was complete. A blank reference was prepared by adding 2ml of the DPPH solution to 2 ml of methanol. The absorbance of the samples was measured at 517 nm using spectrophotometer.

2.2 Antiproliferative screening

Compounds 6, 9, 13, 18a and 18b were tested against human breast carcinoma cell line (MCF-7) in vitro. The method applied is similar to that reported by Skehan P (71) using Sulfo-Rhodamine-B stain (SRB). Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compound to allow attachment of cell to the wall of the plate. Different concentrations of the compound under test (0, 5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer in triplicate wells. Individual dose, monolayer cells were incubated with the compounds for 48 h at 37°C in an atmosphere of 5 % CO₂. After 48 h, cells were fixed, washed and stained with SRB stain. Excess stain was washed with acetic acid and attached stain was recovered with Tris-EDTA buffer. Color intensity was measured in an ELISA reader. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line and the IC₅₀.

2.3 Antimicrobial screening

The antimicrobial activities of compounds 6, 9, 13, 18a, 18b, 18c and 18d were tested by agar plug technique (72) against the following indicator strains: Pr., Proteus vulgaris (clinical culture); Salm., Salmonella typhimurium (NCMB 74); Shig., Shigella bodydii (ATCC 9207); V., Vibrio sp. (clinical culture); Staph., Staphylococcus aureus (NCMB 6571); Cand., Candida albicans (clinical culture); G., Gardnerella vaginitis (clinical culture) and B., Bacillus subtilis (NCMB 3610). 150 µl of an overnight culture was grown as a lawn on Zobell media. Plugs of 11 mm in diameter were stanched out with a cork borer and placed with the bacterial side down onto agar plates, which had been seeded with a 1:100 dilution of a stationary phase culture of the above indicator strains. After overnight incubation at 37 °C, the plates were inspected for the formation of inhibition zones around the agar plugs. These measurements were carried out at Suez Canal University’s Center for Studies and Environmental Advisory.

RESULTS AND DISCUSSION

1-Chemistry

In the present investigation, ethyl 5-substituted-1H-indole-2-carboxylates 1-2 were obtained by applying improved Fischer indolization (37); 4-substituted anilines were converted into their diazonium salt with nitrous acid and this was followed by reduction with stannous chloride in acid medium to afford the corresponding aryldrazines. Condensation of aryldrazines with ethyl pyruvate gave the corresponding aryldrazones, and then refluxed with dehydrated p-TsOH in dry toluene using Dean-stark apparatus to give compounds 1-2. Ethyl 1-
benzyl-1H-indole-2-carboxylate 3 was synthesized by benzylation of ethyl 1H-indole-2-carboxylate 1 with benzyl chloride in the presence of dimethyl sulfoxide (DMSO) and KOH as basic catalyst (38-39). Reaction of 2-3 with hydrazine hydrate gave 1-substituted-5-halo-1H-indole-2-carboxyldrazines 4a-b (40-42). Condensation of 4b with ethylisothiocyanate in absolute ethanol gave 2-(1-benzyl-1H-indole-2-carbonyl)-N-ethylhydrazinecarbothioamide 5 (31, 40, 43-47). Condensation of 5 with hydrazine hydrate afforded 5-(1-benzyl-1H-indol-2-yl)-N3-ethyl-4H-1, 2, 4-triazole-3, 4-diamine 6 (48). Refluxing of 5-fluoro-1H-indole-2-carboxyldrazide 4a with a one carbon inserting reagent triethoxymethane in DMF gave 8-fluoro-[1,2,4]triazino[4,5-a]indol-1(2H)-one 7 (47, 49-51). The assignment of the structure 7 for that product, where the carbons was inserted between hydrazine moiety and the indole NH rather than the indole carbon was based on the absence, in its 1H-NMR Spectrum of the indole NH and the presence of the proton on indole C10 (δ=9.22 ppm). The Vilsmeier-Hack formylation of 7 using POCl3/DMF gave 8-fluoro-1-oxo-1, 2-dihydro-[1, 2, 4] triazino [4, 5-a] indole-10-carbaldehyde 8 (47, 51-55). Reduction of 8 with NaBH4 in methanol gave 8-fluoro-10-(hydroxymethyl)-[1,2,4]triazino[4,5-a]indol-1(2H)-one 9 (55). 3-Formyl-1H-indole-2-carboxamide 11 was prepared by reacting ethyl 3-formyl-1H-indole-2-carboxylate 10 with ammonium hydroxide in the presence of ethanol (56) and then cyclized to 1-oxo-1H-imidazo[1,5-a]indole-9-carbaldehyde 12 using triethoxymethane in DMF (47, 49-51). Reduction of 12 with NaBH4 in methanol afforded 9-(hydroxymethyl)-1H-imidazo [1, 5-a] indol-1-one 13 (55), (Scheme 1).
Scheme 1. The synthesis of indolyl heterocycles

1. The synthesis of indolyl heterocycles
The synthesis of 2-substitutedphenyl-1H-benzo[d]imidazoles 14a-b were prepared via the oxidative condensation of o-phenylenediamine with sodium metabisulphite (Na₂S₂O₅) adduct of appropriate substituted benzaldehydes in DMF (57-61). N- Alkylation of 14a-b with ethyl-2-chloroacetate in the presence of DMSO/KOH afforded ethyl 2[2-(substituted phenyl)-1H-benzo[d] imidazol-1-yl]-acetates 15a-b (31, 44, 61). Hydrazine hydrate and 15a-b in ethanol were refluxed for 4 h to give the desired hydrazides 16a-b (31, 44, 61).

Condensation of hydrazides 16a-b with ethylisothiocyanate,4-bromophenylisothiocyanate and phenylisocyanate in absolute ethanol afforded the corresponding thiosemicarbazide and semicarbazide derivatives 17a-d (31, 43-45, 61). Refluxing of 17a-d with hydrazine hydrate afforded 5-((2-(4-substitutedphenyl)-1H-benzo[d]imidazol-1-yl) methyl)-N³-substituted-4H-1, 2, 4-triazole-3, 4-diamine 18a-d (48), (Scheme 2).

**Scheme 2. Synthesis of 2[2-(substitutedphenyl)-1H-benzo-[d]imidazol-1-yl]1,2,4-triazole-3,4-diamine**
2- Biological results

2.1. Antioxidant activity

Compounds 6, 9, 13, 18a, 18b, 18c and 18d were evaluated for their antioxidant activity by measuring the change in the absorbance of DPPH (1,1-Diphenyl-2-picrylhydrazyl radical) at 517 nm by the spectrophotometric method (62). Results were plotted on the graph as the difference between the absorbance of the blank and the sample-reagent solution, (Figure 1)

It was found that vitamin C, the reference, showed the highest antioxidant activity while compounds 6, 18a, 18b, 18c, and 18d possess moderate antioxidant activity, on the other hand, compounds 9, 13 have minimal antioxidant activity. An essential structural requirement for antiradical activity is the availability of free electron species, compounds which possess removable hydrogen atoms can therefore easily react with DPPH radicals. During the present study, most of the antiradical compounds were found to possess NH group, which can easily donate hydrogen atom to the DPPH radical. The resulting unpaired electron, after the loss of a hydrogen atom, can be easily delocalized over a conjugated ring and provide necessary stability to the system. The concentration at which the compounds showed antioxidant activity that is half the value of the maximal activity is known as EC50, (Table 2).
Figure 1: The difference between the absorbance of the blank and the sample-reagent solution. Each data represents mean ± SD.
Table 2: **Efficacy, EC$_{50}$ and E$_{50}$ of compounds showing interesting antioxidant activity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Highest Efficacy Absorbance</th>
<th>E$_{50}$*</th>
<th>EC$_{50}$ (μg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C</td>
<td>1.1483 ± 0.0002</td>
<td>0.574</td>
<td>9.86</td>
</tr>
<tr>
<td>6</td>
<td>0.8067 ± 0.0011</td>
<td>0.4035</td>
<td>20.73</td>
</tr>
<tr>
<td>18a</td>
<td>0.937 ± 0</td>
<td>0.4685</td>
<td>24.51</td>
</tr>
<tr>
<td>18b</td>
<td>0.967 ± 0.001</td>
<td>0.4835</td>
<td>30.21</td>
</tr>
<tr>
<td>18c</td>
<td>0.9693 ± 0.0191</td>
<td>0.4836</td>
<td>22.52</td>
</tr>
<tr>
<td>18d</td>
<td>0.9106 ± 0.0045</td>
<td>0.4555</td>
<td>34.66</td>
</tr>
</tbody>
</table>

* The value of [Abs Blank-Abs Compound] at EC$_{50}$

2.2. Antiproliferative screening

Compounds 6, 9, 13, 18a and 18b were tested against human breast carcinoma cell line (MCF-7) *in vitro* using doxorubicin (DXR) as a reference drug. The measurements were carried out at the National Institute of Cancer, Cancer Biological Department, Cairo, Egypt. The relation between surviving fraction and drug concentration is plotted to get the survival curve of each tumor cell line and the IC$_{50}$, (Figure 2 and Table 3).
**Figure 2:** The relation between the surviving fractions of human breast carcinoma cell line (MCF-7) and drug concentration

![Graph showing the relation between surviving fractions and drug concentration](graph.png)

**Table 3:** In vitro cytotoxic activity (IC\(_{50}\)) of compounds 6, 9, 13, 18a and 18b and doxorubicin against a human breast carcinoma cell line (MCF-7)

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC(_{50}) (µg. mL(^{-1}))</th>
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<tbody>
<tr>
<td>Doxorubicin</td>
<td>4.4</td>
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<tr>
<td>6</td>
<td>10.7</td>
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<tr>
<td>9</td>
<td>11.8</td>
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<tr>
<td>13</td>
<td>10.8</td>
</tr>
<tr>
<td>18a</td>
<td>13.7</td>
</tr>
<tr>
<td>18b</td>
<td>16.2</td>
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</tbody>
</table>

IC\(_{50}\) is defined as the concentration, which results in a 50% decrease in cell number as compared with that of the control structures in the absence of an inhibitor.
It was found that compounds 6, 9, 13, 18a and 18b have significant anti-proliferative activities against human breast cell line MCF-7 compared to the reference standard drug doxorubicin. Compounds 6 showed high activity which can be attributed to the presence of the benzyl group on NH of indole [18-20] and triazole ring on 2-position of indole. Compounds 13 and 9 were found to have a promising activity due to the presence of a carbinol group on the 3-position of indole (18-20).

2.3 Antimicrobial screening
The antimicrobial activities of compounds 6, 9, 13, 18a, 18b, 18c and 18d were tested by agar plug technique (72). The results showed that compound 6 had a promising activity against Gram-positive bacteria, Gram-negative bacteria and Candida, and requires further investigations. Compounds 18b, 18c had activity against Gram-negative bacteria only. They may be promising as selective Gram-negative inhibitors and require further investigations. Finally compound 13 demonstrated better anticandidal activities if concentrated samples will be analyzed, (Table 4).
Table 4: Antimicrobial Activity of tested compounds using agar plug technique

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<td>3</td>
<td>3</td>
<td>2</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<td>3</td>
<td>-</td>
<td>-</td>
<td>-</td>
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<td>18d</td>
<td>-</td>
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<tr>
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<td>-</td>
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<td>-</td>
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<td>8</td>
<td>-</td>
<td>12</td>
<td>14</td>
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<td>6</td>
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<td>-</td>
<td>6</td>
<td>12</td>
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<tr>
<td>DMSO</td>
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</table>

The activities are based on the diameters of zones of inhibition in mm. Solvent: DMSO, Sample concentration [c] = 15 μL mL⁻¹ Gentamicin 10μg; Tobramycin 10 μg; Tetracycline 30 μg
CONCLUSION

In the present study, ten new compounds derived from indole, benzimidazole were synthesized and studied for their antioxidant, anticancer, and antimicrobial activities. The promising results demonstrate the necessity for further investigations.
REFERENCES


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