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Design, Synthesis And Biological Evaluation of New Benzotriazepine Derivatives As Cytotoxic Agents

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Abstract : *New benzotriazepine derivatives were synthesized and evaluated for their cytotoxic activity against human breast cancer cell line MCF-7, The new derivatives were found to inhibit cancer growth at three different concentrations with derivative* ***2****-((4-(dimethylamino) benzylidene) amino)-3H- benzo[e] [1,2,4] triazepin-5(4H)-one* ***(IIIo)*** *as the most potent with IC_{50} value 49,9 µg/ml compared to doxurubucin 3.83 µg/ml.*

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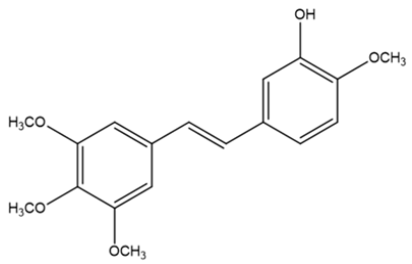
I. Introduction

Despite intensive research, cancer is one of the main causes of death globally with considerable economic and social burden. [1] Cancer has been and continues to be one of the major areas offocus for drug discovery.[2]Breast cancer is a complex group of diseases and is one of the most common cancers diagnosed worldwide. [3]Breast cancer is the second leading cause of cancer death in women, affecting 1.7 million patients every year worldwide. [4] Exploration of new strategies for the prevention of breast cancer metastasis is justifiably at the center of clinical attention. [5] Therefore, it is necessary to identify novel tumor associated molecules to target for biomarker development and immunotherapy.[6]

Basic activity of benzodiazepines (BzDs) is associated with central nervous system (CNS) and the most abundant group of BZDs are 1,4-benzodiazepine derivatives. [7]A variety of biologicalactivities and synthetic routes have also been established for the related benzotriazepines.[8]

Combretastatin A4 (Fig1) is a *cis*-stilbene and its related compound, colchicine (Fig1), belong to a class of compounds referred to as colchicinoids. [9]Combretastatin is an anti-mitotic agent that gained rapid recognition among cancer biologists and clinicians as one of the newer vascular disrupting agentsfor cancer therapy. [10]

From the above and by combining structures of benzotriazepine and structures of combretastatin to design the structure of the new derivatives using the common structural analogs of the two phenyl systems separated by an alkene bridge bearing methoxy and hydroxyl substituents. These compounds were screened for their *in vitro* antitumor activity on breast cancer cell line MCF-7.



Combretastatin Colchicine

Fig 1: Structure of Combretastatin A4 and colchicine as colchicinoids

II. Materials and Methods

Chemistry

All melting points were determined in open glass capillaries on a Gallenkamp melting point apparatus and are uncorrected. The IR spectra were recorded on a Perkin-Elmer spectrophotometer using potassium bromide discs. The ^{1}H-NMR and ^{13}CNMR spectra were recorded on a Varian Gemini 300 MHz and Bruker 400MHz using DMSO- d_6 as solvent. The chemical shifts were reported as parts per-million δ ppm, tetramethylsilane (TMS) as an internal standard. Mass spectra were obtained on a Jeol-SX-102 instrument. Elemental analysis was performed on a Perkin-Elmer 2400 C, H, N analyzer and values were within ±0.4% of

theoretical percentages. The progress of the reaction was monitored on readymade Silica –gel plates fluorescent (Merck) using $CHCl_3/CH_3OH$ (9.5:0.5) as solvent using, UV lamp.
2-Amino-3,4-dihydro-1,3,4-benzotriazepin-5-one**I** was synthesizedaccording to reported procedure [11]

General procedure for the synthesis of (IIIa-o)
A suspension of **II (0.005mol, 0.88g)** in ethanol was heated under reflux then the appropriate aldehyde (0.005mol) was added. The reflux was continued for 7 hs then the reaction mixture was cooled, filtered. The separated solid was dried and crystallized from ethanol.

2-((4-hydroxybenzylidene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIa
Mp 242°C, yield 85%, IR(KBr, cm^{-1}): 3361(OH), 3213(NH), 3066 (CH aromatic),2923(CH aliphatic), 1647 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 6.58(d,*j=6 Hz*, 2H, Ar-H),6.95 (d, *j=6Hz*, 2H, Ar-H), 7.20 (t,2H,Ar-H), 8.44(s, 1H, Ar-H), 8.59(s, 1H, Ar-H), 9.70 (s, 1H, =CH), 11.48 (s,1H,OH, D_2O exchangeable),11.63 (s,1H,NH, D_2O exchangeable), 12.27 (s,1H,NH, D_2O exchangeable).Anal.Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.26; H, 4.33; N, 19.98.

2-((3-hydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIb
Mp 248°C, yield 65%, IR(KBr, cm^{-1}): 3444(OH), 3213(NH), 3064 (CH aromatic),2933(CH aliphatic), 1683 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 7.03(d, *j=7.2 Hz*, 1H, Ar-H),7.11 (s, 1H, Ar-H), 7.35 (t,2H, Ar-H), 7.86(m, 2H, Ar-H), 8.21(s, 1H, Ar-H), 8.59(d, *j=7.2 Hz*, 1H, Ar-H),9.90 (s, 1H, =CH), 11.64 (s,1H,OH, D_2O exchangeable),11.63 (s,1H,NH, D_2O exchangeable), 12.17 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.25; H, 4.30; N, 19.96.

2-((2-hydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIc
Mp 260°C, yield 57%, IR(KBr, cm^{-1}): 3425(OH), 3211(NH), 3064 (CH aromatic),2966(CH aliphatic), 1651 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 6.88(d, *j=6.9 Hz*, 1H, Ar-H),7.05-7.14 (m, 4H, Ar-H), 7.47-7.60 (m,2H, Ar-H), 8.86(d, *j=7.8 Hz*, 1H, Ar-H),10.20(s, 1H, =CH), 11.72 (s,1H,OH, D_2O exchangeable),11.82 (s,1H,NH, D_2O exchangeable), 12.12 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.26; H, 4.31; N, 19.98.

2-((2,3-dihydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIId
Mp 248°C, yield 72%, IR(KBr, cm^{-1}): 3385(OH), 3213(NH), 3061 (CH aromatic),2926(CH aliphatic), 1651 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 6.78(d, *j=7.8 Hz*, 1H, Ar-H),7.19-7.33 (m, 3H, Ar-H), 7.86-7.93 (m,2H, Ar-H), 8.57(d, *j=8.1 Hz*, 1H, Ar-H),10.20 (s, 1H, =CH), 11.72 (s,2H,OH, D_2O exchangeable),11.82 (s,1H,NH, D_2O exchangeable), 12.22 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_3$: C, 60.81; H, 4.08; N, 18.91. Found: C, 60.82; H, 4.09, N, 18.92.
2-((2,4-dihydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIe
Mp 258°C, yield 73%, IR(KBr, cm^{-1}): 3420(OH), 3209(NH), 3066 (CH aromatic),2926(CH aliphatic), 1650 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 6.38(d, *j=7.5 Hz*, 1H, Ar-H),7.21-7.25 (m, 2H, Ar-H), 7.51(d, *j=8.4 Hz*, 1H, Ar-H), 7.86 (d, *j=7.2 Hz*, 1H, Ar-H), 8.05(d, *j=6.6 Hz*, 1H, Ar-H), 8.21 (s,1H, Ar-H), 9.91 (s, 1H, =CH), 11.00 (s,1H,OH, D_2O exchangeable), 11.73 (s,1H,OH, D_2O exchangeable),11.82 (s,1H,NH, D_2O exchangeable), 12.12 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_3$: C, 60.81; H, 4.08; N, 18.91. Found: C, 60.80; H, 4.08, N, 18.91.

2-((3,4-dihydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIf
Mp 275°C, yield72%, IR(KBr, cm^{-1}): 3420(OH), 3369(NH), 3064 (CH aromatic),2924(CH aliphatic), 1647 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6): 6.48(d, *j=6.7 Hz*, 1H, Ar-H),6.57-6.66 (m, 2H, Ar-H), 6.86 (d, *j=6.84 Hz*, 1H, Ar-H), 7.20 (d, *j=5 Hz*, 1H, Ar-H), 8.44 (s,1H, Ar-H), 8.57(d, *j=7.7 Hz*, 1H, Ar-H), 9.66 (s, 1H, =CH), 10.44 (s,1H,OH, D_2O exchangeable), 11.49 (s,1H,OH, D_2O exchangeable),11.67 (s,1H,NH, D_2O exchangeable), 12.31 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_3$: C, 60.81; H, 4.08; N, 18.91. Found: C, 60.79; H, 4.07, N, 18.90.

2-((4-hydroxy-3-methoxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIg
Mp 232°C, yield 65%, IR(KBr, cm^{-1}): 3420(OH), 3367(NH), 3066 (CH aromatic),2926(CH aliphatic), 1647 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 3.56(s, 3H, OCH_3),6.95(d, *j=6.7 Hz*, 1H, Ar-H),7.17-7.25 (m, 2H, Ar-H), 7.31 (d, *j=7.8 Hz*, 1H, Ar-H), 7.39 (d, *j=5 Hz*, 1H, Ar-H), 7.55 (d, , *j=7.8 Hz*, 1H, Ar-H), 8.22(s,1H, Ar-H), 9.77 (s, 1H, =CH), 10.74 (s,1H,OH, D_2O exchangeable),11.83(s,1H,NH, D_2O exchangeable), 12.18 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 455; N, 18.06. Found: C, 61.99; H, 4.57, N, 18.05.

2-((4-methoxybenzylidene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIh
Mp 254°C, yield 63%, IR(KBr, cm^{-1}): 3217(NH), 3070 (CH aromatic),2976(CH aliphatic), 1647 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 3.87 (s, 3H, OCH_3), 6.49(s, 1H, Ar-H), 6.73(s, 1H, Ar-H), 7.15(s, 1H, Ar-H), 7.57(s, 1H, Ar-H),7.88 (s, 1H, Ar-H), 8.05(s,1H, Ar-H), 8.21(s, 1H, Ar-H), 8.59(s, 1H, Ar-H), 9.88 (s, 1H, =CH), 11.64 (s,1H,NH, D_2O exchangeable), 12.35 (s,1H,NH, D_2O exchangeable). m/z293 (M^+-1 , 5.7%). Anal.Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.19; H, 4.65; N, 18.94.

2-((2-methoxybenzylidene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIi
Mp 270°C, yield 55%, IR(KBr, cm^{-1}): 3215(NH), 3064 (CH aromatic),2976(CH aliphatic), 1647 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 3.90 (s, 3H, OCH_3), 6.71(d, *j=7.7 Hz*, 1H, Ar-H),7.01-7.08 (m, 2H, Ar-H), 7.32(d, *j=5.9 Hz*, 1H, Ar-H), 7.68(d, *j=6.7 Hz*, 1H, Ar-H),7.85 (t, 1H, Ar-H), 8.42(d, *j=6.7 Hz*, 1H, Ar-H), 8.57(d, *j=6.8 Hz*, 1H, Ar-H),10.35(s, 1H, =CH), 11.64 (s,1H,NH, D_2O exchangeable), 12.32 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 64.99; H, 4.51; N, 18.98.

2-((3,4-dimethoxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIj
Mp 275°C, yield 76%, IR(KBr, cm^{-1}): 3448(NH), 3067 (CH aromatic),2924(CH aliphatic), 1647 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6): 3.81 (s, 6H, $2OCH_3$), 6.48 (s, 1H, Ar-H), 6.71(d, *j=6.9 Hz*, 1H, Ar-H), 7.02(d, *j=6.3 Hz*, 1H, Ar-H), 7.15(d, *j=7.9 Hz*, 1H, Ar-H),8.03 (d, *j=7.5 Hz*, 1H, Ar-H), 8.42 (d, *j=7.5 Hz*, 1H, Ar-H), 8.57(d, *j=7.5 Hz*, 1H, Ar-H), 9.82 (s, 1H, =CH),11.53 (s,1H,NH, D_2O exchangeable), 12.33 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{17}H_{16}N_4O_3$: C, 62.95; H, 4.79; N, 17.27. Found: C, 62.78; H, 4.67, N, 17.00.

2-((3,4,5-trihydroxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIk
Mp 242°C, yield 70%, IR(KBr, cm^{-1}): 3566(OH), 3444(NH), 3064 (CH aromatic),2926(CH aliphatic), 1647 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6):3.96 (s,2H,2OH, D_2O exchangeable), 6.30(s,1H, Ar-H), 6.47(s,1H, Ar-H),7.85 (d, *j=7.76 Hz*, 1H, Ar-H), 8.01 (t,1H, Ar-H), 8.18 (t,1H, Ar-H), 8.57(d, *j=8.2 Hz*, 1H, Ar-H), 9.52 (s, 1H, =CH), 10.43 (s,1H,OH, D_2O exchangeable),11.65 (s,1H,NH, D_2O exchangeable), 12.30 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{15}H_{12}N_4O_4$: C, 57.69; H, 3.87; N, 17.94. Found: C, 57.49; H, 3.57, N, 17.80.

2-((3,4,5-trimethoxybenzylidene)amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIL
Mp 272°C, yield 85%, IR(KBr, cm^{-1}): 3421(NH), 3064 (CH aromatic),2937(CH aliphatic), 1649 (CO). ^{1}H-NMR 300 MHz (DMSO-d_6): 3.76 (s, 3H, OCH_3), 3.85 (s, 6H, $2OCH_3$), 6.48 (s, 1H, Ar-H), 6.71(s, 1H, Ar-H),8.20(d, *j=7.2 Hz*, 1H, Ar-H),8.35 (d, *j=9.0 Hz*, 1H, Ar-H), 8.43 (d, *j=7.8 Hz*, 1H, Ar-H), 8.58 (d, *j=7.8 Hz*, 1H, Ar-H), 9.88 (s, 1H, =CH),11.49 (s,1H,NH, D_2O exchangeable), 11.63 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{18}H_{18}N_4O_4$: C, 61.01; H, 5.12; N, 15.81. Found: C, 60.89; H, 5.03, N, 15.60

2-((pyridin-4-ylmethylene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIm
Mp 252°C, yield 62%, IR(KBr, cm^{-1}): 3213(NH), 3066 (CH aromatic),2922(CH aliphatic), 1651 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6): 6.68(s, 1H, Ar-H),7.03 (s, 1H, Ar-H), 7.35(s, 1H, Ar-H), 7.57(s, 1H, Ar-H),8.03 (s, 1H, Ar-H), 8.19 (s, 1H, Ar-H), 8.41(s, 1H, Ar-H), 8.87(s, 1H, Ar-H),10.08 (s, 1H, =CH), 11.64 (s,1H,NH, D_2O exchangeable), 12.24 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{14}H_{11}N_5O$: C, 63.39; H, 4.18; N, 26.40. Found: C, 63.59; H, 4.08; N, 26.01.

2-((pyridin-3-ylmethylene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIn
Mp 260°C, yield 52%, IR(KBr, cm^{-1}): 3213(NH), 3064 (CH aromatic),2927(CH aliphatic), 1647 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6): 6.70(s, 1H, Ar-H),7.03 (s, 1H, Ar-H), 7.14(s, 1H, Ar-H), 7.35(s, 1H, Ar-H),7.52 (s, 1H, Ar-H), 8.03 (s, 1H, Ar-H), 8.24(s, 1H, Ar-H), 8.58(s, 1H, Ar-H),10.10 (s, 1H, =CH), 11.63 (s,1H,NH, D_2O exchangeable), 12.24 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{14}H_{11}N_5O$: C, 63.39; H, 4.18; N, 26.40. Found: C, 63.61; H, 4.02; N, 26.05.

2-((4-(dimethylamino) benzylidene) amino)-3H-benzo[e][1,2,4]triazepin-5(4H)-one IIIo
Mp 252°C, yield 75%, IR(KBr, cm^{-1}): 3209(NH), 3064 (CH aromatic),2956(CH aliphatic), 1647 (CO). ^{1}H-NMR 400 MHz (DMSO-d_6): 3.02 (s, 6H, $2CH_3$), 6.47(s, 1H, Ar-H),6.70 (t, 1H, Ar-H), 7.34(t, 1H, Ar-H), 7.55(t, 1H, Ar-H),7.85 (t, 1H, Ar-H), 8.06 (s, 1H, Ar-H), 8.32(s, 1H, Ar-H), 8.43(s, 1H, Ar-H),10.43 (s, 1H, =CH), 11.62 (s,1H,NH, D_2O exchangeable), 12.26 (s,1H,NH, D_2O exchangeable). Anal.Calcd for $C_{17}H_{17}N_5O$: C, 66.43; H, 5.58; N, 22.79. Found: C, 66.51; H, 5.72; N, 22.85.

Pharmacology
***In vitro* anticancer testing**

The breast tumor cell line (MCF-7), was obtained frozen in liquid nitrogen (-180 ˚C) from the American Type Culture Collection (ATCC) and was maintained in the National Cancer Institute, Cairo, Egypt, by serial sub-culturing.
All chemicals used in this study are of high analytical grade. They were obtained from either Sigma-Aldrich or Bio-Rad.

Measurement of potential cytotoxicity

The cytotoxic activity of the newly synthesized compounds was measured *in vitro* on breast tumor cell line (MCF-7) using Sulforhodamine-B stain (SRB) assay applying the method of Skehan*et al*. [12]
The cells were plated in a 96-multiwell plate (104 cells/well) for 24 h before treatment with the test compounds to allow attachment of the cells to the wall of the plate. The test compounds were dissolved in DMSO and diluted with saline to the appropriate volume. Different concentrations of the test compounds (0, 5, 12.5, 25 and 50 µg/ml) were added to the cell monolayer. Triplicate wells were prepared for each individual dose. Monolayer cells were incubated with the test compounds for 48 h at 37˚ C in atmosphere of 5 % CO_2. After 48 h, the cells were fixed withtrichloroacetic acid, washed with water, and stained for 30 min with 0.4 % (wt/vol) Sulforhodamine-B stain dissolved with 1 % acetic acid. Excess stain was removed by four washes with 1 % acetic acid and the attached stain was recovered with Tris EDTA buffer. The colour intensity was measured in ELISA reader. For each compound, the relation between the surviving fraction and the drug concentration was plotted to get the survival curve of each tumor cell line. The IC50 values (the concentration required for 50 % inhibition of cell viability) were calculated using sigmodial dose response curve-fitting models (GraphPad, Prizm software incorporated), each concentration was repeated three times. The results are given in **Table 1.**

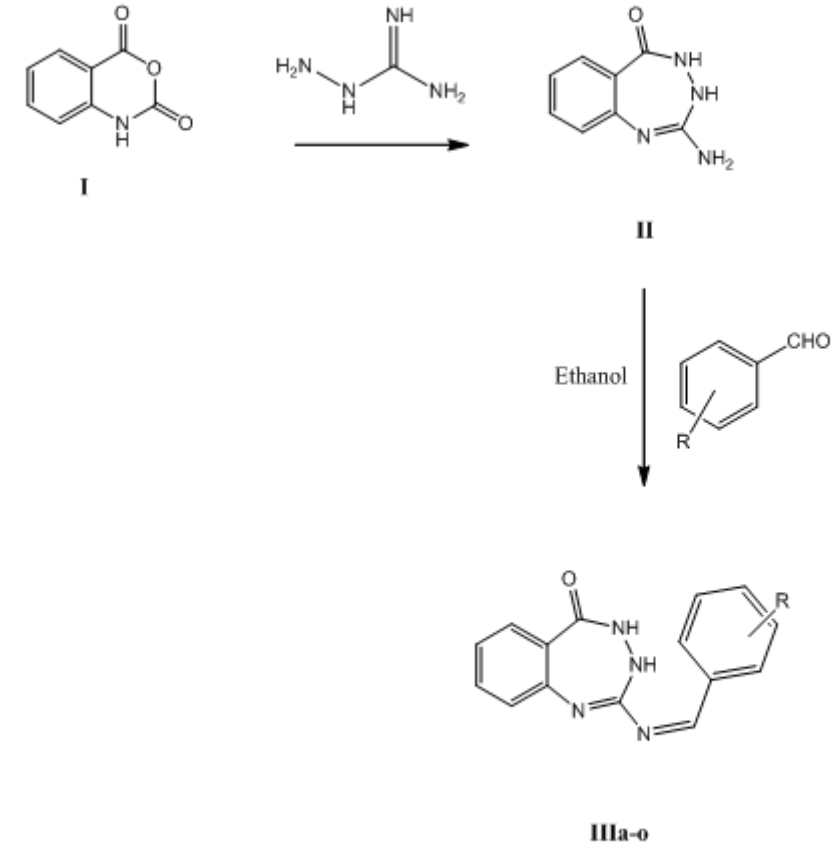
Conc. / Compound	0 µg/ml	5 µg/ml	12.5 µg/ml	25 µg/ml	50 µg/ml
IIIa	1.000	0.865	0.697	0.706	0.736
IIIb	1.000	0.931	0.835	0.634	0.562
IIIc	1.000	0.817	0.713	0.645	0.852
IIId	1.000	0.961	0.875	0.564	0.508
IIIe	1.000	0.875	0.724	0.514	0.507
IIIf	1.000	0.943	1.014	0.830	0.731
IIIg	1.000	0.913	0.724	0.687	0.673
IIIh	1.000	0.941	0.754	0.528	0.509
IIIi	1.000	0.817	0.713	0.645	0.852
IIIj	1.000	0.968	0.951	0.825	0.708
IIIk	1.000	0.888	0.769	0.718	0.793
IIIL	1.000	0.970	0.888	0.692	0.709
IIIm	1.000	1.000	1.000	0.727	0.635
IIIn	1.000	1.000	0.920	0.630	0.546
IIIo	1.000	1.000	1.000	0.850	0.493 / IC50 49.9
Doxorubucin	1.000	0361	0.385	0.332	0.299 / IC50 3.83

III. Results and discussion

Chemistry

The target derivatives were synthesized according to scheme 1. First, isatoic anhydride reacted with 1-aminoguanidine bicarbonate in a one pot reaction producing 2-Amino-3,4-dihydro-1,3,4-benzotriazepin-5-one **I** [11]. Further reaction of **I** with number of selected aromatic aldehydes was performed in ethanol to afford derivatives **IIIa-o**, the structure of the newly synthesized derivatives was confirmed by IR, ^{1}HNMR, mass apectrum and microanalyses. The IR spectral bands showed distinct bands equivalent to OH group at 3566-3361cm^{-1} in addition to the sharp peak of NH at 3448-3209cm^{-1}. Moreover ^{1}H NMR showed the appearance of a singlet signal equivalent to (=CH) proton proving the stereospecific purity of the product which appeared at 9.52-10.43 ppm, the D_2O exchangeable singlet signals of NH protons appeared at 11.49-12.35ppm. Finally the OH singlet signals appeared at 10.43-11.73ppm in addition to a singlet signal at 3.96ppm for **IIIk.**

Scheme 1:



III= *p*-OH, *m*-OH *o*-OH, 2,3-OH, 2,4-OH, 3,4-OH

4-OH,3-OCH_3

Pharmacology

All the newly synthesized derivatives were evaluated for their cytotoxic activity against human breast cancer cell line (MCF-7) compared to doxorubicin with four different concentrations (0, 5, 12.5, 25 and 50 µg/ml)to elaborate their activity. The best activity was found at 25 µg/ml and50 µg/ml. Most of the derivatives were found to have good to moderate activity with compound **IIIo** with the highest activity among all the derivatives with IC_{50} value of 49.9 µg/ml bearing the *p*- dimethyl amino group. Followed by **IIIe and IIId** with their disubstituted hydroxyl group having percentage inhibition0.507 and 0.508 respectively, then *p*-methoxy derivative**IIIh** with closer activity showing 0.509 inhibition. Moderate inhibition was shown by **IIIn, IIIb, IIIm** and **IIIg** having 0.546, 0.562, 0.635 and 0.673 percentage inhibition. A lower activity was presented by **IIIj, IIIL, IIIf, IIIa and IIIk**having percentage inhibition 0.708, 0.709, 0.731, 0.736 and 0.793. Finally the lowest activity was found by **IIIc and IIIi**having equal percentage inhibition of 0.852. From the above the highest activity was presented by the disubstituted derivatives while the lowest was shown by the ortho substitution.

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Conflict of Interest

The author have declared no conflict of interest.

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