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RESEARCH ARTICLE

Synthesis of new 1,3,4-benzotriazepin-5-one derivatives and their biological evaluation as antitumor agents

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Abstract New derivatives of 1,3,4-benzotriazepin-5-one were designed and synthesized as structural analogues to the antitumor agents devazepide and asperlicin. An efficient and novel approach to the synthesis of 2-amino-1,3,4-benzotriazepin-5-one **2** was developed and its structure was confirmed. The newly synthesized derivatives were evaluated for their in vitro antitumor activity on 60 different cell lines. Compounds **8** and **9** displayed the most potent antitumor activity against several cell lines specifically ovarian cancer, renal cancer and prostate cancer, while compounds **5, 10** and **12** showed significant activities against UO-31 renal cancer cell line.

Keywords 1,3,4-benzotriazepin-5-one derivatives · Synthesis · Antitumor activity

Introduction

Benzotriazepinones and benzodiazepinones are commonly known therapeutic agents and have long been used in medicine for their anxiolytic, sedative, anticonvulsant, antidepressant and hypnotic and anticancer activity (Osman et al. 2002; Araujo et al. 2008; Ophardt 2003). Although there are many anticancer drugs in the market, a considerable amount of research focuses on benzotriazepinones and benzodiazepinones due to their specificity. They have distinct mechanism of action which may vary in their effects on different types of normal and cancer cells. A single "cure" for cancer has been proven elusive since there are more than 100 different types of cancer present. Unfortunately, the majority of drugs currently in the market are not specific, which leads to the many common side effects associated with cancer chemotherapy (Escherich et al. 2001).

There is an increasing interest for oncological applications of drugs which target specific receptors like cholecystokinin (CCK) receptors, due to their over expression in cancer cells (De-Luca et al. 2007). CCK antagonists were studied as growth inhibitors in certain forms of cancer (Lattmann et al. 2006). Amongst the many non-peptide ligands that have been devised for CCK receptors, those based on benzodiazepine ring systems are by far the most prominent (Sinha et al. 1999). 3-Amino-1,4-benzodiazepines as well as chemically related diverse amines were screened for the cholecystokinin receptor inhibition in a radiolabel binding assay (Offel et al. 2006). Devazepide **I** (formerly MK-329) (Fig. 1) is considered the most potent and highly specific CCK_A receptor antagonist, with almost 160 times greater affinity for CCK_A receptors than for CCK_B receptors (Tashiro et al. 1999). In addition, asperlicin **II** (Fig. 1) proved to be a potent nonpeptidal CCK antagonist where the 1,4-benzodiazepine ring system has served as a useful tool for delineating the pharmacological actions of CCK (Saemian et al. 2006). Focusing on the 1,4-benzodiazepine ring system that comprises part of the structure of asperlicin and devazepide and combining the elements of diazepam and tryptophan which mimic asperlicin (McDonald et al. 2006; Herranz 2003). The synthesis of novel derivatives of 1,3,4-benzotriazepinone analogous to asperlicin and devazepide were described in order to achieve new lead compounds with enhanced the potency of antitumor agents. In Scheme 1 was introduced new derivatives an analogues to asperlicin comprising the cyclization of five and/or six membered rings with 1,3,4-benzotriazepin-5-one

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to study the effect of ring size and different substituent's on antitumor activity. Schemes 2 and 3 outline the synthesis of new derivatives analogues to devazepide as different spacer were engaged 1,3,4-benzotriazepin-5-one as a bioisoster of 1,4-benzodiazepine and different indole derivatives (Andreani et al. 2010; João et al. 2008) to study their biological effect on several tumor cell lines.

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

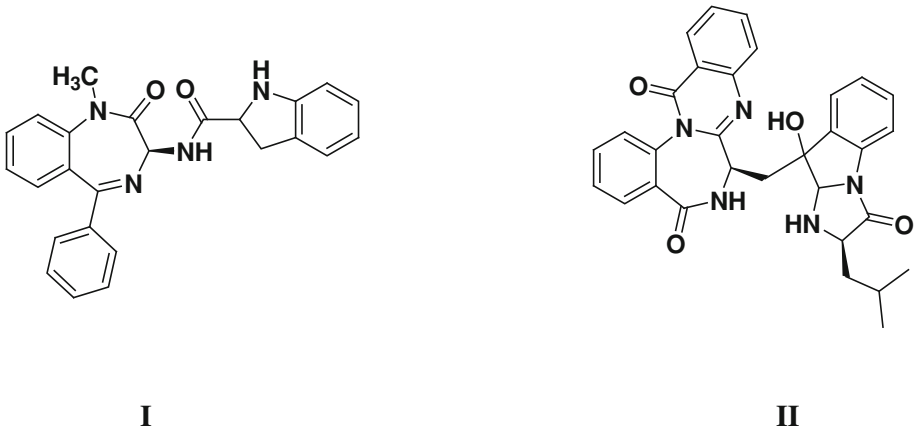
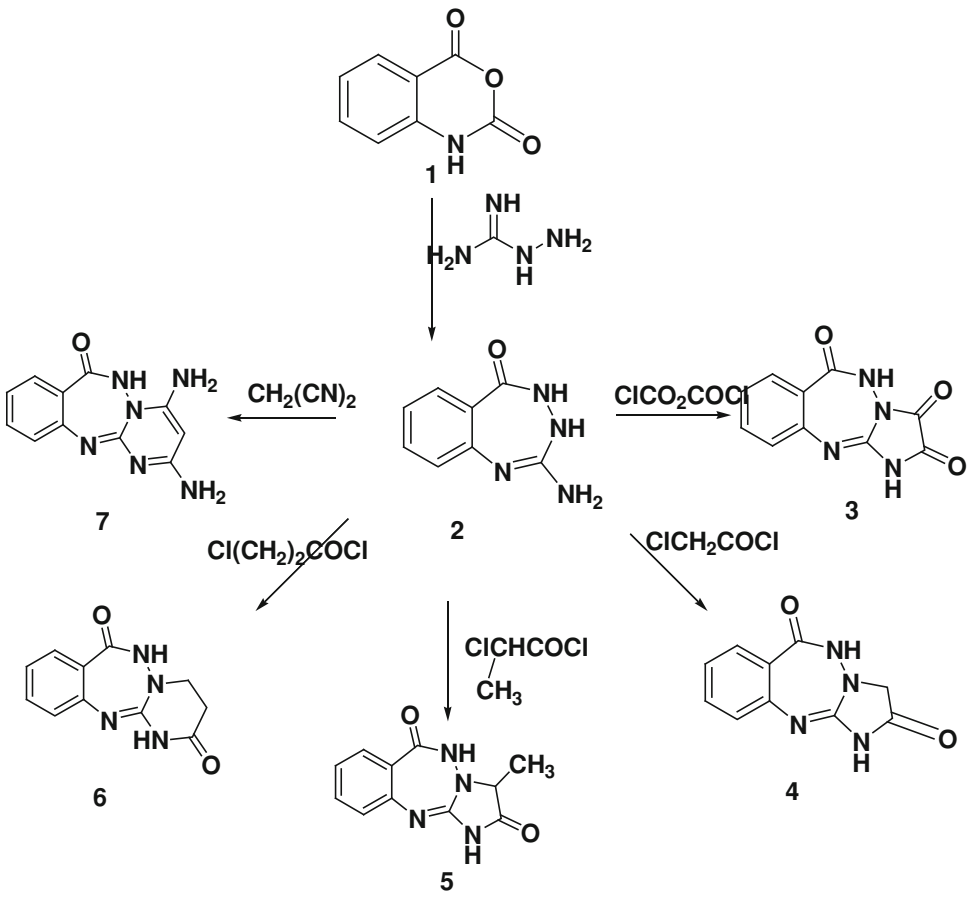
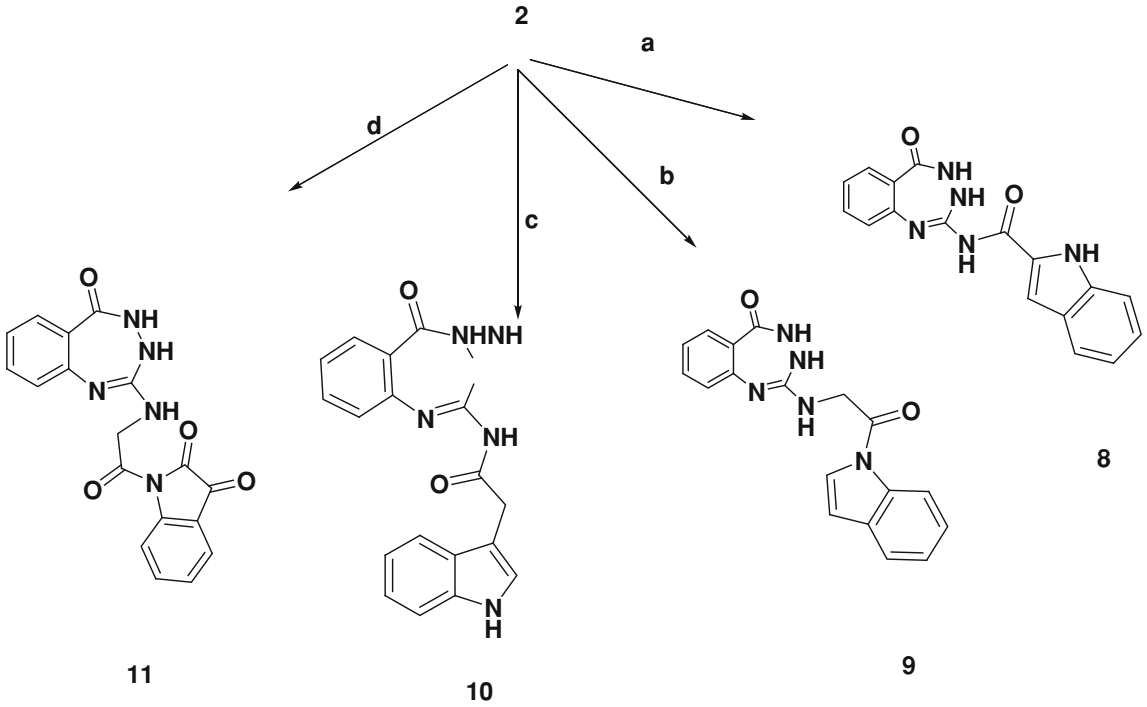


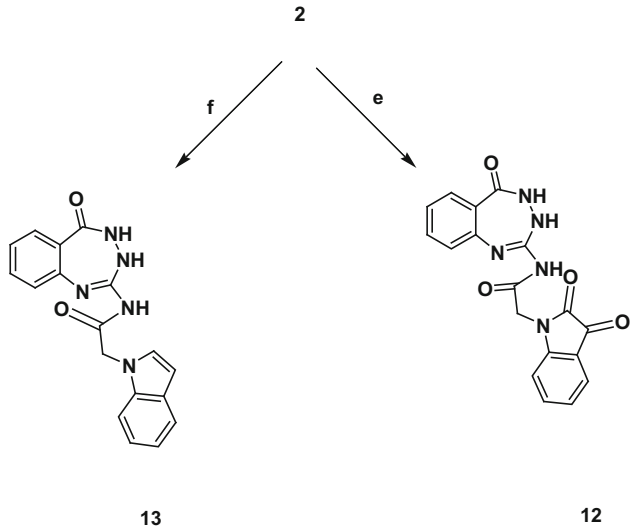
Fig. 1 Structures of devazepide I and asperlicin II



Scheme 1 Synthesis of target compounds **3–7**



Scheme 2 Synthesis of target compounds **8**–**11**. *a* 1*H*-Indole-2-carbonyl chloride. *b* 2-Chloro-1-(1*H*-indol-1-yl)ethanone. *c* 2-(1*H*-Indol-3-yl)acetyl chloride. *d* 1-(2-Chloroacetyl)indoline-2,3-dione



Scheme 3 Synthesis of target compounds **12** and **13**. *e* Ethyl 2-(2,3-dioxoindolin-1-yl)acetate and *f* ethyl 2-(1*H*-indol-1-yl)acetate

spectrophotometer using potassium bromide discs. The H-NMR spectra were recorded on a Varian Gemini 300 MHz using DMSO-d_6 as solvent. The chemical shifts were reported as parts permillion 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 ready-made Silica–gel plates fluorescent (Merck) using $CHCl_3$/CH_3OH (9.5:0.5) as solvent using, UV lamp.

Indole-2-carbonyl chloride (**a**) (Shakila et al. 2010), indole-1-acetyl chloride (**b**) (Mutschler and Winkler 1978), indole-3-acetyl chloride (**c**) (Rogers and Stern 1992), ethyl 2-(1H-indol-1-yl)acetate (**d**) (Huntress and Bornstein 1949), ethyl 2-(2,3-dioxoindolin-1-yl)acetate (**e**) (Rekhter 2005) and1-(2-chloroacetyl)indoline-2,3-dione (**f**) (Abd-Elrahman et al. 2008) were prepared according to reported procedures.

*2-Amino-3,4-dihydro-1,3,4-benzotriazepin-5-one (**2**)*

To a suspension of isatoic anhydride **1** (0.01 mol, 1.63 g) in acetic acid (25 mL), aminoguanidine bicarbonate (0.01 mol, 0.77 g) was added and the mixture was heated under reflux for 4 h.The reaction mixture was cooled and poured into ice-cold water (50 mL), the solution was then neutralized with sodium bicarbonate. Aqueous ammonia (10 mL) was added and the solution was left overnight. The precipitated product was then filtered, washed with ice-cold water (20 mL) and crystallized from dimethyl-formamide/water (1:1).

m.p. 240–241 °C, yield 82 %, IR(KBr, cm^{-1}): 3425, 3248, 3221(NH and NH_2), 3150 (CH aromatic), 1647 (C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 2.02 (s, 2H, NH_2 exchanged by D_2O), 6.48–6.76 (m., 1H, C_7 ArH),

7.02–7.29 (m., 1H, C_8 ArH), 7.89 (s., 1H, C_6 ArH), 8.57 (s., 1H, C_9 ArH), 15.95, 16.21 (2 s., 2H, NH exchanged by D_2O); EIMS: M+ (*m/z*) 176 (9.44 %). Anal Calcd. for $C_8H_8N_4O$ (176.18): C, 54.54; H, 4.58; N, 31.80; Found C, 54.44; H, 4.31; N, 31.50.

General synthetic procedure for **3, 4, 5** and **6**

A mixture of **2** (1.76 g, 0.01 mol) and each of oxalyl chloride, chloroacetyl chloride, 2-chloropropionyl chloride and 3-chloropropionyl chloride (0.015 mol) in dry benzene (25 mL) and anhydrous potassium carbonate (0.2 mol) was heated under reflux for 4, 5, 5, 7 h respectively. The solvent was evaporated under reduced pressure to dryness. The residue was dissolved in water (10 mL), filtered, dried and crystallized from methanol.

*1H-Imidazo[2,1-b]1,3,4-benzotriazepine-2,3,6-(5H)-trione (**3**)*

m.p.>300 °C, yield 77 %, IR(KBr, cm^{-1}): 3406, 3221(NH), 3087 (CH aromatic), 1710, 1690, 1651(3C=O); ^{1}H-NMR 300 MHz (DMSO-d_6): δ 6.47–6.78 (m., 1H, C_8 ArH), 7.03–7.23 (m., 1H, C_9 ArH), 7.55 (s., 1H, C_7 ArH), 8.57 (s., 1H, C_{10} ArH), 11.43, 12.29 (2s., 2H, NH exchanged by D_2O); EIMS: M-1 *m/z* 229 (4.72 %), M+ (*m/z*) 230 (1.50 %). Anal Calcd. for $C_{10}H_6N_4O_3$ (230.18): C, 52.18; H, 2.63; N, 24.34; Found C, 52.39; H, 2.74; N, 24.82.

*1H-Imidazo[2,1-b]1,3,4-benzotriazepine-2,6-(3H,5H)-dione (**4**)*

m.p. 232–233 °C, yield 75 %, IR(KBr, cm^{-1}): 3441, 3221 (NH), 3147 (CH aromatic), 2978, 2939 (CH aliphatic),1708, 1651 (2C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 3.06 (s., 2H, N–CH_2–CO), δ 7.07–7.25 (m., 1H, C_8 ArH), 7.43–7.62 (m., 1H, C_9 ArH), 7.80 (s., 1H, C_9 ArH), 8.35 (s., 1H, C_{10} ArH), 10.59, 11.78 (2 s., 2H, NH exchanged by D_2O); EIMS: M+ (*m/z*) 216 (22.75 %), M+ 1 *m/z* 217 (12.60 %). Anal Calcd. for $C_{10}H_8N_4O_2$ (216.20): C, 55.55; H, 3.73; N, 25.91; Found C, 55.18; H, 3.53; N, 26.11.

*3-Methyl-1H-imidazo[2,1-b]1,3,4-benzotriazepine-2,6-(5H)-dione (**5**)*

m.p. 238–240 °C, yield 80 %, IR(KBr, cm^{-1}): 3422, 3221 (NH), 3128 (CH aromatic), 2982 (CH aliphatic),1711, 1651(2C=O); ^{1}H-NMR 300 MHz (DMSO-d_6): δ 1.19 (d., 3H, CH_3), 3.07 (q., 1H, CH), 6.71–6.82 (m., 1H, C_8 ArH), 7.36–7.61 (m., 1H, C_9 ArH), 7.67 (s., 1H, C_6 ArH), 8.39 (s., 1H, C_{10} ArH), 10.22, 11.61 (2 s., 2H, NH exchanged by D_2O); EIMS: M+ 1 (*m/z*) 231 (23.26 %), M+ *m/z* 230 (22.09 %). Anal Calcd. for $C_{11}H_{10}N_4O_2$ (230.22): C, 57.39; H, 4.38; N, 24.34; Found C, 57.57; H, 4.58; N, 24.74.

*3,4-Dihydropyrimido[2,1-b]1,3,4-benzotriazepine-2,7-(1H,6H)-dione (**6**)*

m.p. >300 °C, yield 75 %, IR(KBr, cm^{-1}): 3414, 3224(NH), 3107 (CH aromatic),1695, 1647 (2C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 2.26 (t., 2H, $NHCH_2$), 3.59 (t., 2H, CO–CH_2), 6.98–7.16 (s., 1H, C_9 ArH), 7.45 (m., 1H, C_{10} ArH), 7.68 (s., 1H, C_8 ArH), 8.58 (s., 1H, C_{11} ArH), 10.44, 11.64 (2 s., 2H, NH exchanged by D_2O); EIMS: M-1 (*m/z*) 229 (2.10 %), M+ *m/z* 230 (1.50 %). Anal Calcd. for $C_{11}H_{10}N_4O_2$ (230.22): C, 57.39; H, 4.38; N, 24.34; Found C, 57.66; H, 4.18; N, 24.11.

*2,4-Diaminopyrimido[2,1-b]1,3,4-benzotriazepine-7-(1H)-one (**7**)*

A mixture of **2** (1.76 g, 0.01 mol), malononitrile (6.60 g, 0.01 mol) and triethylamine (3 mL) in absolute ethyl alcohol (30 mL) was heated under reflux for 12 h. The solvent was evaporated under reduced pressure to dryness and cooled. The precipitate formed was filtered, washed with water (2 × 10 mL), dried and crystallized from methanol.

m.p. 270–271 °C, yield 54 %, IR(KBr, cm^{-1}): 3480, 3252, 3224 (NH and NH_2), 3077 (CH aromatic), 1652 (C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 1.99 (br.s, 4H, $2NH_2$ exchanged by D_2O), 6.49-6.66 (m., 1H, C_9 ArH), 7.03-7.48 (m., 1H, C_{10} ArH), 7.81 (s., 1H, C_8 ArH), 8.40 (s., 1H, C_{11} ArH), 8.55 (s., 1H, C_3 ArH), 15.22 (s., 1H, NH exchanged by D_2O); EIMS: M + (m/z) 242 (33.43 %); Anal Calcd. for $C_{11}H_{10}N_6O$ (242.24): C, 54.54; H, 4.16; N, 34.69; Found C, 54.83; H, 4.32; N, 34.95.

General synthetic procedure for **8, 9, 10** and **11**

A mixture of **2** (1.76 g, 0.01 mol) and freshly prepared **a, b, c** and **d** (0.01 mol) of each in dry benzene (25 mL)/triethylamine (0.5 mL) was heated under reflux for 2,3,2,4 h respectively. The solvent was evaporated under reduced pressure to dryness. Water (5 mL) was added and the formed precipitate was filtered, dried and crystallized from ethanol.

*N-(5-Oxo-4,5-dihydro-3H-1,3,4-benzotriazepin-2yl)-1H-indole-2-carboxamide (**8**)*

m.p. 250–252 °C, yield 50 %, IR (KBr, cm^{-1}): 3380, 3221(NH), 3103 (CH aromatic), 1690, 1651 (2C=O); ^{1}H-NMR 300 MHz (DMSO-d_6): δ 6.42–6.79 (m., 4H, $C_{4'}$,$_{5'}$, $_{6'}$, C_7 ArH), 7.11 (s, 1H, $C_{3'}$ ArH), 7.25–7.60 (m., 1H, C_8 ArH), 7.79 (s., 1H, C_6 ArH), 8.23 (br s., 1H, $C_{7'}$ ArH), 8.48 (s., 1H, C_9 ArH), 10.40, 11.41, 11.62, 12.24 (4 s., 4H, NH

exchanged by D_2O); EIMS: M+ (*m/z*) 242 (33.43 %); Anal Calcd. for $C_{17}H_{13}N_5O_2$ (319.32): C, 63.94; H, 4.10; N, 21.93; Found C, 64.17; H, 4.08; N, 21.53.

*2-(2-(1H-indol-1-yl)-2-oxoethylamino)-3,4-dihydro-1,3,4-triazepin-5-one (**9**)*

m.p. 155–156 °C, yield 49 %, IR(KBr, cm^{-1}): 3400–3221 (NH), 3101 (CH aromatic), 2986 (CH aliphatic), 1692, 1651 (2C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 3.07 (s, 2H, CO–$\underline{CH_2}$NH), 6.48–7.43 (m., 4H, $C_{4',5'}$, $_{6'}$, C_7 ArH), 7.46 (s, 1H, C_8 ArH), 7.59–7.68 (m., 1H, $C_{3'}$ ArH), 7.85 (s., 1H, C_6 ArH), 8.15 (br s., 1H, $C_{7'}$ ArH), 8.24 (s., 1H, C_9 ArH), 8.44 (s., 1H, $C_{2'}$ ArH),10.23, 11.33, 12.00 (3s., 3H, NH exchanged by D_2O); EIMS: M − 1 (*m/z*) 332 (1.59 %), M+ (*m/z*) 333 (1.81 %), M + 1 (*m/z*) 334 (8.29). Anal Calcd. for $C_{18}H_{15}N_5O_2$ (333.34): C, 64.86; H, 4.54; N, 21.01; Found C, 64.88; H, 4.32; N, 20.97.

*N-(5-Oxo-4,5-dihydro-3H-1,3,4-benzotriazepin-2yl)-1H-indole-3-acetamide (**10**)*

m.p. 195–197 °C, yield 52 %, IR(KBr, cm^{-1}): 3406, 3224, 3213 (NH), 3127 (CH aromatic), 2974, 2939 (CH aliphatic), 1691,1643 (2C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 3.09 (s, 2H, CO–$\underline{CH_2}$), 6.57–7.43 (m., 4H, $C_{4',5'}$, $_{6'}$, C_7 ArH), 7.46–7.53 (m., 1H, C_8 ArH), 7.68 (s., 1H, C_6 ArH), 8.15 (br s., 1H, $C_{7'}$ ArH), 8.24 (s., 1H, C_9 ArH), 8.49 (s., 1H, $C_{2'}$ ArH), 10.41, 11.60, 11.95, 12.42 (4s., 4H, NH exchanged by D_2O). Anal Calcd. for $C_{18}H_{15}N_5O_2$ (333.34): C, 64.86; H, 4.54; N, 21.01; Found C, 65.09; H, 4.32; N, 21.32.

*1-[2-(4,5-Dihydro-5-oxo-3H-1,3,4-benzotriazepin-2yl-amino) acetyl] indoline-2,3-dione (**11**)*

m.p. 220–222 °C, yield 47 %, IR(KBr, cm-1): 3500–3350 (NH), 3123 (CH aromatic), 2978, 2931, 2893 (CH aliphatic), br. 1700–1635 (4C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 3.91 (s, 2H, CO–$\underline{CH_2}$NH), 6.91–6.96 (m., 1H, $C_{5'}$ ArH), 7.23–7.28 (m., 1H, $C_{6'}$ ArH), 7.33–7.48 (m., 1H, C_7 ArH), 7.62–7.66 (m., 1H, C_8 ArH), 7.75 (s., 1H, C_6 ArH), 7.82 (d., 1H, $C_{4'}$ ArH), 8.54 (br s., 1H, C_9 ArH), 8.70 (d., 1H, $C_{7'}$ ArH), 11.56, 11.94, 12.19 (3s., 3H, NH exchanged by D_2O); EIMS: M + 4 (*m/z*) 367 (0.94 %). Anal Calcd. for $C_{18}H_{13}N_5O_4$ (363.33): C, 59.50; H, 3.61; N, 19.28; Found C, 59.18; H, 4.02; N, 19.42.

General synthetic procedure for **12** and **13**

A mixture of **2** (1.76 g, 0.01 mol) and freshly prepared **e** and **f** (0.01 mol) of each in absolute ethanol (25 mL) was heated under reflux for 7 h. The reaction mixture was cooled and the formed precipitate was filtered, dried and crystallized from ethanol.

*N-(4,5-dihydro-5-oxo-3H-1,3,4-benzotriazepin-2-yl)-2-(2,3-dioxoindolin-1-yl)acetamide (**12**)*

m.p. 120–122 °C, yield 42 %, IR (KBr, cm^{-1}): 3418, 3395 (NH), 3039 (CH aromatic), 2985, 2947 (CH aliphatic), 1751, 1740, 1675, 1647 (4C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 1.23 (s, 1H, NH exchanged by D_2O); 4.13, 4.60 (2s, 2H, CO–$\underline{CH_2}$), 7.15–7.19 (m., 4H, ArH), 7.59–8.01 (m., 4H, ArH); EIMS: M − 2 *m/z* 361 (75.48 %), M − 1 362 (22.50 %), M + 363 (6.10 %). Anal Calcd. for $C_{18}H_{13}N_5O_4$ (363.33): C, 59.50; H, 3.61; N, 19.28; Found C, 59.72; H, 3.92; N, 19.02.

*N-(4,5-dihydro-5-oxo-3H-1,3,4-benzotriazepin-2-yl)-2-(1H-indol-1yl)acetamide (**13**)*

m.p. 203–204 °C, yield 46 %, IR(KBr, cm^{-1}): 3421, 3385 (NH), 3066 (CH aromatic), 1671, 1647 (2C=O); ^{1}H NMR 300 MHz (DMSO-d_6): δ 4.23, 4.77 (2s, 2H, CO–$\underline{CH_2}$), 6.59–7.19 (m., 4H, $C_{4',5'}$, $_{6'}$, C_7 ArH), 7.34–7.39 (m., 1H, C_8 ArH), 7.55 (br s., 1H, C_6 ArH), 7.88 (s., 1H, $C_{7'}$ ArH), 8.21 (br s., 1H, C_9 ArH), 8.46 (d., 1H, $C_{3'}$ ArH), 8.59 (d., 1H, $C_{2'}$ ArH), 10.63, 11.83, 12.41 (3s., 3H, NH exchanged by D_2O); EIMS: *m/z* M + 333 (19.48 %). Anal Calcd. for $C_{18}H_{15}N_5O_2$ (333.34): C, 64.86; H, 4.54; N, 21.01; Found C, 64.72; H, 4.36; N, 21.02.

Antitumor screening

Under a sterile condition, cell lines were grown in RPMI 1640 media (Gibco, NY, USA) supplemented with 10 % fetal bovine serum (Biocell, CA, USA), 5×10^5 cell/ml was used to test the growth inhibition activity of the synthesized compounds. The concentrations of the compounds ranging from 0.01 to 100 μM were prepared in phosphate buffer saline. Each compound was initially solubilised in dimethyl sulfoxide (DMSO), however, each final dilution contained less than 1 % DMSO. Solutions of different concentrations (0.2 mL) were pipetted into separate well of a microtiter tray in duplicate. Cell culture (1.8 mL) containing a cell population of 6×10^4 cells/ml was pipetted into each well. Controls, containing only phosphate buffer saline and DMSO at identical dilutions, were also prepared in the same manner. These cultures were incubated in a humidified incubator at 37 °C. The incubator was supplied with 5 % CO_2 atmosphere. After 48 h, cells in each well were diluted 10 times with saline and counted by using a coulter counter. The counts were corrected for the dilution. The data reported as mean graph of the percent growth inhibition of the treated cells, and presented as percentage

growth inhibition (GI %). The obtained results of the tested triazepinone analogues **2–13**; showed distinctive potential pattern of selectivity, as well as broad-spectrum antitumor activity. (Grever et al. 1992; Monks et al. 1991; Boyd and Paull 1995; Skehan et al. 1990) (Table 1).

Results and discussion

Synthetic routes for novel compounds are depicted in Schemes 1, 2 and 3. The structure and synthesis of 2-amino-1,3,4-benzotriazepin-5-one (**2**) was unavailable through previously described methods. Literature survey revealed that, the reaction of isatoic anhydride with glycine afforded 3*H*-1,4-benzodiazepin-2,5-(1*H*,4*H*)-dione (**III**) (Fig. 2) (Mohiuddin et al. 1985). Moreover, derivatives of 4-methyl benzotriazepinone (**IV**) (Fig. 2) were reported by two steps reaction (Leiby and Heindel 1977).

From the previous findings the unsubstituted 2-amino-1,3,4-benzotriazepin-5-one was synthesized using one pot reaction of isatoic anhydride with 1-aminoguanidine bicarbonate affording 82 % yield of the target compound. The possible mechanism of the regioselective formation of **2** is shown in (Fig. 3).

In order to prove the structure of 2-amino-1,3,4-benzotriazepin-5-one IR, mass, ^{1}H NMR spectra and microanalysis were performed. In addition, the reaction of isatoic anhydride with 1-aminoguanidine bicarbonate in refluxing ethanol (a previously reported procedure for the synthesis of **V** (Fig. 4) has been used as an evidence for the cyclization to eliminate the possible formation of the open structure (Bozena and Stanislaw 1962).

According to the fact that several diamino compounds upon reaction with chloroacid chlorides leads to the formation of cyclised derivatives,(Taher and Raafat 2005) the reaction of compound **2** with oxalyl chloride, chloroacetyl chloride, 2-chloropropionyl chloride and 3-chloropropionyl chloride in dry benzene in presence of potassium carbonate resulted in the formation of the cyclised target compounds **3, 4, 5** and **6** in 75–85 % yield.The IR spectra of the products revealed the disappearance of absorption bands of NH_2 group and the appearance of one absorption band at 3,450–3,220 cm^{-1} due to NH group. Beside the appearance of an absorption band at 1690–1670 cm^{-1} due to C=O groups. ^{1}H NMR was also used as an evidence for the formed derivatives structure, ^{1}H NMR of **4** showed a singlet peak at δ 2.99 ppm corresponding to CH_2 group, while compound **5** showed doublet peak at 1.19, quartet signal at 3.07 ppm due to CH_3 and CH groups. Compound **6** showed two signals at 2.26–3.59 ppm due to 2 CH_2 groups. Moreover, the cyclization of several compounds by the use of malononitrile in the presence of triethylamine was previously reported (El-Enany et al. 2011; Ried and Aboul-Fetouh 1988). Consequently, the reaction of **2** with malononitrile in absolute ethanol and calculated amount of triethylamine afforded **7** with IR spectrum showing forked bands at 3330–3250 cm^{-1} corresponding to 2 NH_2 groups and mass spectrum showing molecular ion peak in 33.43 % abundance. In addition to ^{1}H NMR spectrum which showed distinct two singlet signals exchangeable with D_2O at 1.9 ppm equivalent to the 2 NH_2 groups. In Schemes 2 and 3, target compounds **8–13** were prepared by reaction of **2** with freshly prepared reported indole acid chlorides and/or esters. Firstly, the reagents **a** and **c** were prepared by reaction of either 1*H*-indole 2-carboxylic acid or indole 3-acetic acid reacted with thionyl chloride. Secondly, indole and isatin (indole 2,3-dione) reacted with chloroacetyl chloride producing intermediates **b** and **d**. Finally, **e** and **f** intermediates were obtained upon the reaction of indole and isatin with ethyl chloroacetate in boiling ethanol [c.f experimental part]. The new final compounds **8–13** were synthesized via the reaction of compound **2** with each of the previously obtained intermediates **a–f**. The structure of the new derivatives was consistent with the proposed structures and was proved via IR spectra by the disappearance of the forked peak at 3248, 3221 equivalent to NH_2 group and via ^{1}H-NMR spectra showed a peak of CH_2 of acetyl linkage around 3.06–4.77 ppm, in addition to the molecular ion peak obtained in each case.

Preliminary in vitro antitumor screening

The antitumor screening of all novel synthesized compounds **2–13** was carried out in the National Cancer Institute (NCI), Bethesda, MD, USA. They were subjected to the NCI's disease-oriented human cell lines screening assay to be evaluated for their in vitro antitumor activity. A single dose (10 μM) of the test compounds were used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely; leukaemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate, and breast cancer cells (Grever et al. 1992; Monks et al. 1991; Boyd and Paull 1995; Skehan et al. 1990).

The results revealed that compound **2** showed moderate activity against non cell small lung cancer HOP-92 and renal cancer UO-31 with percentage growth inhibition (GI %) 16 %. Compound **5** showed moderate activity against ovarian cancer and breast cancer MCF7, GI % 21–26 %, respectively and displayed promising activity against renal cancer UO-31, GI % 36 %. Compound **8** possessed moderate activity against lung cancer HOP-92, H-322M and H-522, GI % 15, 19 and 17 % respectively. In addition, it recorded significant activity against leukaemia CCRF-CEM, CNS cancer U-251, colon cancer HCT-116 and ovarian LGROVI, GI % 26, 28, 23 and 22 %, respectively. Moreover, compound **8** showed an excellent

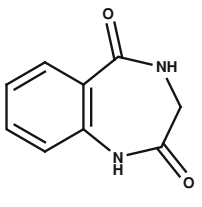
Table 1 Inhibition percent of the tested compounds **2–13** (10 μMolar) on different 60 cell lines

Subpanel tumor cell lines	% Growth inhibition (GI %)[a]											
	2	3	4	5	6	7	8	9	10	11	12	13
Leukemia												
CCRF-CEM	–	–	–	–	–	–	26	–	–	–	–	–
HL-60(TB)	–	–	–	–	–	–	–	–	–	–	–	–
K-562	–	–	–	–	–	–	–	–	–	15	–	–
MOLT-4	–	–	–	–	–	–	–	–	–	17	–	–
RPMI-8226	–	–	–	10	–	–	–	–	15	–	–	–
SR EKVX	–	–	–	9	–	–	–	–	–	–	–	31
Non-small cell lung cancer												
Hop-62	–	–	–	13	–	–	–	–	–	–	–	–
Hop-92												
ATTC	–	14	–	12	–	–	14	10	–	–	–	–
Hop-92	–	16	–	–	10	–	15	**41**	–	–	–	–
NCI-H460	–	–	–	–	–	–	–	10	–	–	–	–
NCI-H226	–	–	–	–	–	–	19	10	–	–	–	–
NCI-H23	10	–	–	–	–	–	–	–	–	–	–	–
NCI-322M	–	–	–	–	–	–	–	–	–	–	–	–
NCI-H522	–	–	–	–	–	–	17	–	–	–	10	–
COLO-205												
Colon cancer												
HCT-116	–	–	10	–	–	23	10	–	–	–	–	19
HCT-15	–	–	–	–	–	–	–	–	–	–	–	–
HT29	–		–	–	–	–	–	–	–	–	–	–
KM12	–	–	–	–	–	–	–	–	–	–	–	–
HCC-2988												
SW-620												
CNS cancer												
SF-268	–	–	–	–	–	10	–	–	–	–	–	–
SF-295	–	–	–	–	–	–	–	–	–	–	–	–
SF-539	–	–	–	–	–	11	–	–	–	–	–	18
SNB-19	–	–	–	–	–	–	12	–	–	–	–	–
SNB-75	–	–	–	14	11	–	–	–	–	22	–	–
U251	–	–	–	–	–	–	28	13	–	–	–	–
Melanoma												
LOX IMVI	–	–	–	–	–	–	–	10	–	–	–	–
MALME-3 M	–	–		–	–	–	–	–	–	18	–	–
M14	–	–	–	–	–	–	–	–	–	–	–	–
MDA-MB-435	–	–	–	–	–	–	–	–	–	–	–	–
SK-MEL-2	–	–	–	–	–	–	–	10	–	–	–	–
SK-MEL-28	–	–	–	–	–	–	–	–	–	–	–	–
SK-MEL-5	–	–		–	–	–	–	–	–	–	–	–
UACC-257	–	–	–	–	–	–	–	–	–	–	–	–
UACC-62	–	–	–	–	–	–	–	10	–	–	–	–
Ovarian cancer												
IGORV-1	–	–	–	21	–	–	**40**	31	13	11	–	–
OVCAR-3												
OVCAR-4												
OVCAR-5												

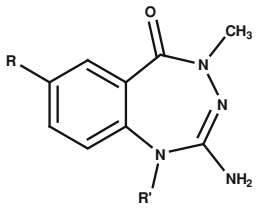
Table 1 continued

Subpanel tumor cell lines	% Growth inhibition (GI %)[a]											
	2	3	4	5	6	7	8	9	10	11	12	13
OVCAR-3	–	–	–	–	–	–	22	16	–	–	–	–
OVCAR-4	–	–	–	–	–	–	15	16	–	–	–	–
OVCAR-5	–	–	–	–	–	–	–	–	–	–	–	–
OVCAR-8	–	–	–	–	–	–	–	–	–	–	–	–
NCI/ADR-RES	–	–	–	–	–	–	–	–	–	–	–	–
SK-OV-3												
Renal cancer												
786-0	–	–	–	–	–	–	–	–	–	–	–	–
A498	–	12	–	–	13	–	–	–	–	–	–	–
ACHN	–	–	–	–	–	–	–	–	–	13	–	–
CAKI-1	–	–	–	–	10	–	16	15	11	10	19	–
SN12C	–	–	–	–	–	–	–	–	–	–	–	–
TK-10	–	–	–	–	–	–	–	–	–	–	–	–
UO-31	–	16	–	36	13	–	31	30	35	18	**41**	14
Prostate cancer												
PC-3	–	–	–	–	–	–	32	29	21	–	–	–
DU-145												
Breast cancer												
MCF7	–	–	–	26	–	–	33	18	–	–	–	–
MDA-MB-231/ATCC	–	–	–	10	–	–	19	13	–	–	–	–
MDA-MB-231/ATCC												
HS-578T	–	–	–	–	–	–	–	–	–	–	–	–
BT-549	–	–	–	–	–	–	–	–	–	–	–	–
T-47D	–	10	–	–	–	–	–	11	13	–	–	–
MDA-MB-468	–	–	–	19	–	–	–	–	–	–	–	–

– dashes means; growth inhibition GI % < 10



III



IV

R= H, Cl, Br, NO_2
R'= H,CH_3

Fig. 2 Structures of previously prepared some benzoazepine derivatives

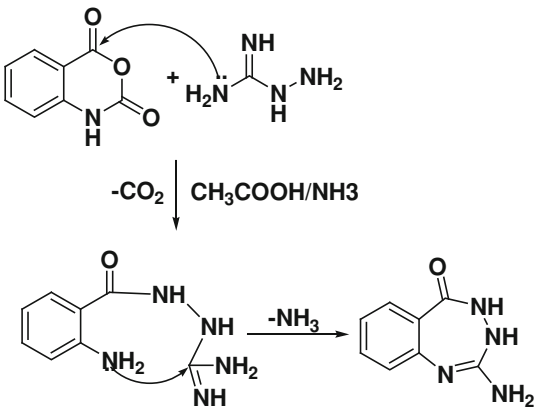
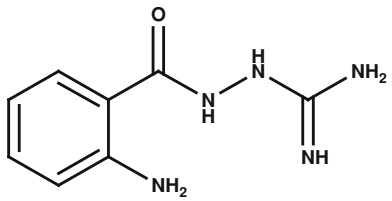


Fig. 3 Proposed mechanism of synthesis of 2-amino-1,3,4-benzotriazepin-5-one

activity against ovarian cancer, renal cancer UO-31, prostate cancer PC-3 and breast cancer MCF-7, GI % 40, 31, 31, 33 %, respectively. Compound **9** showed highly activity against non small cell lung HOP-92, ovarian cancer, renal cancer UO-31 and prostate cancer PC-3 of GI % 41, 31, 30 and 29 %, respectively. Compound **10** displayed high activity against renal cancer UO-31 in GI % 35 % while it registered moderate activity against prostate cancer PC-3 in ratio 21 %. Compound **11** showed moderate activity against leukaemia K-562 and MOLT-4 in ratios 15 and 17 %, respectively. In addition, it possessed good



V

Fig. 4 Structure of *N*-(o-aminobenzoyl)-hydrazinecarboximidamide

activity toward CNS CNB-75, melanoma MAL-3M an d renal cancer UO-31, GI % 22, 18 and 18 %, respectively. Compound **12** showed high activity against renal cancer UO-31 in GI 41 % inhibition, while displayed moderate activity toward renal cancer CAKI-1 in 18 % inhibition. Compound **13** showed promising activity against leukaemia RPMI-88226 in GI % 31 % while showed moderate inhibition activity toward CNS-SF539 and colon cancer HCT-116 in GI % 18 and 19 %, respectively. From all the data presented by NCI, it appears that amongst the tested compounds, compounds **8** and **9** displayed the higher inhibition against most of the tested cell lines as leukaemia, ovarian, renal UO-31, prostate and breast cancer. While higher activity against leukaemia CCRF-EM and SR is displayed by compounds **8** and **13.** Best inhibition activity towards ovarian and prostate PC-3 is presented by compounds **8** and **9**. While the most promising inhibition activity against renal cancer UO-31 is demonstrated by compounds **5, 8–12**. Compounds **5** and **8** showed the most inhibition against breast cancer MCF-7.

These results revealed that compound **8** is the most active derivative. This may be attributed to the linkage between triazepine and indole moieties at position 2 of indole ring giving maximum activity. The activity decreases when the linkage at position 1 of indole rings as in compound **9**. The activity destroyed when the linkage between indole moiety and triazepine at position 3 of indole ring as in compound **10**. The activity also decreases when the spacer elongated up to three atoms in the same compound. Fusion of triazepine and five membered ring and six membered ring in compounds **2** and **5** showed less activity than other compounds with spacer linkage. Isatin moiety improved the anticancer activity against leukaemia, CNS and renal UO-31 at position 1of isatin linkage with triazepine moiety as in compounds **11** and **12**. The new derivatives have advantage of achirality specifically at position 3 in which NH group was incorporated in the new structures rather than the chiral carbon in the previous antitumor agents **I** and **II**.

Research Highlights

Synthesis of novel 1,3,4-benzotriazepines derivatives.
In vitro antitumor evaluation of all the synthesized compounds was on 60 different cell lines.
Compounds **8** and **9** displayed the most potent antitumor activity.
Elemental analysis and spectroscopic characterization of newly synthesized compounds.

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