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A novel, broad-spectrum anticancer compound containing the imidazo[4,5-*e*][1,3]diazepine ring system

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ABSTRACT

Synthesis and broad-spectrum anticancer activity of a novel heterocyclic compound **1** containing the title imidazo[4,5-*e*][1,3]diazepine ring system have been reported. The compound shows potent in vitro antitumor activity with low micromolar IC_{50}'s against prostate, lung, breast, and ovarian cancer cell lines tested. The long alkyl chain attached to the six-position of the heterocyclic ring of **1** appears to be necessary for the observed biological activity.

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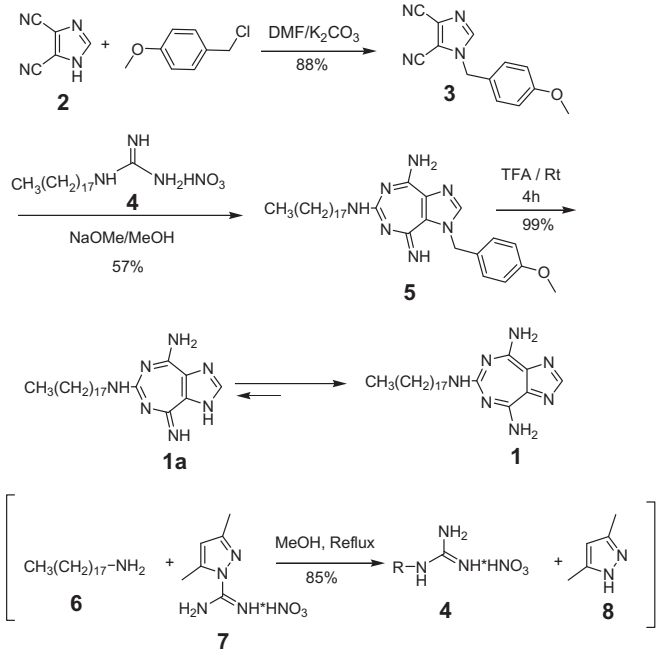
Cancer is the second leading cause of death worldwide after heart disease, claiming more than half a million deaths in US alone in 2009.[1] The major causes of cancer deaths are those related to lung (30%), prostate (9%) and colorectum (9%) in men, and lung (26%), breast (15%), colorectum (9%), and ovarian (5%) cancers in women.[1,2] Deaths from cancer worldwide are projected to continue rising with an estimated 12 million deaths in the year 2030.[2] Surgery, radiation, and chemotherapy are the principal modes of cancer treatment.[3] Newer agents, specifically targeted against detectable molecular abnormalities in certain tumors, and which minimize damage to normal cells, are emerging as valuable therapeutics.[4–9] Despite this progress, the majority of patients diagnosed with these major malignancies will die of their disease and therefore, there is a need for new agents with novel mechanisms of action. Though much effort has been focused on the development of novel tyrosine kinase inhibitors and antibodies directed at signal transduction,[10–15] exploration of new compounds directed against 'traditional' targets of DNA and tubulin continues to be important.[16,17]

In this Letter, we describe the synthesis and broad-spectrum anticancer activity of a novel heterocycle (**1**) containing the title



Scheme 1.

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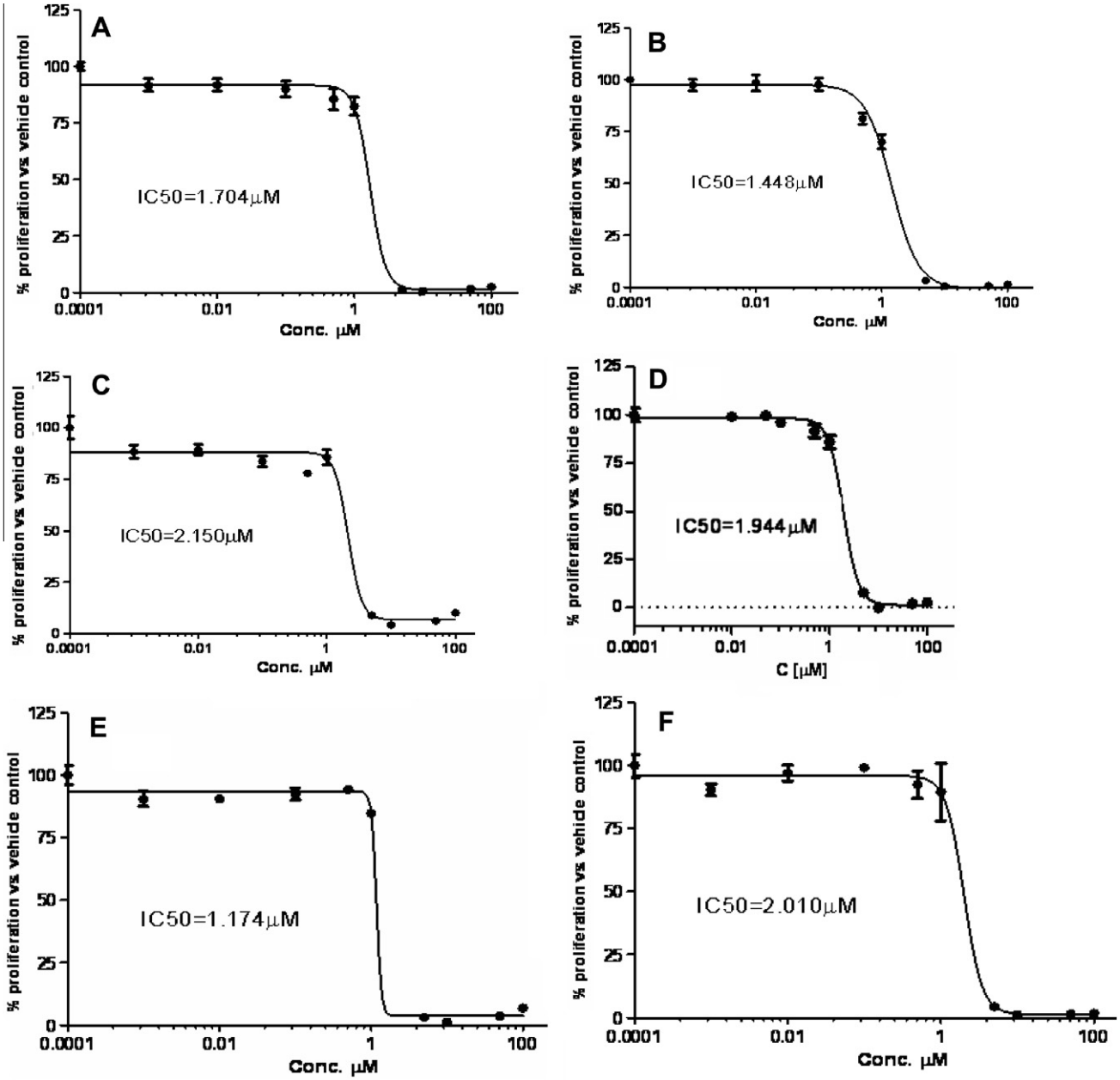
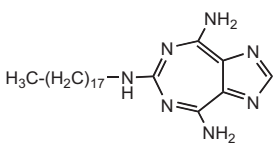


Figure 1. Antitumor activity of compound **1** in vitro against (A) A549 (lung), (B) H460 (lung), (C) MCF-7 (breast), (D) MDA-MB-231 (breast), (E) OVCAR-3 (ovarian), and (F) PC-3 (prostate) cell lines. Each compound was tested at nine different concentrations, and each drug dilution was repeated four times. Cells treated with DMSO (equiv volume) were used as a vehicle control.

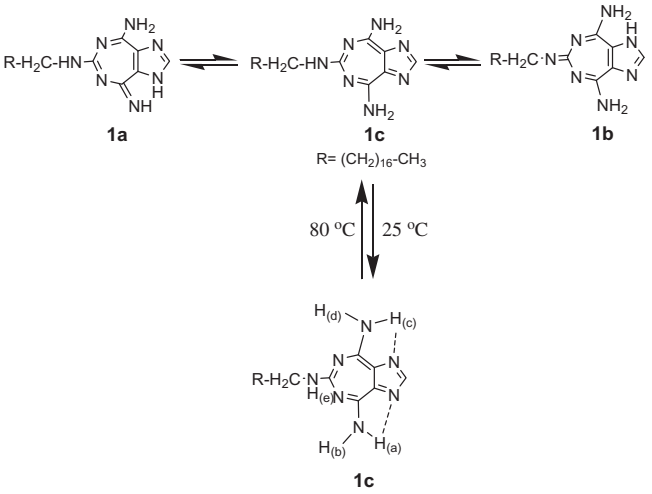
imidazo[4,5-*e*][1,3]diazepine ring system. Compound **1** is easy to synthesize from commercially available starting materials, can be conveniently scaled up to multigram quantities, is a stable, crystalline solid, soluble in aqueous acid, and was found to be highly active in vitro against all of the cancer cell lines tested, which include lung, breast, prostate, and ovarian cancers.



1

Compound **1** was synthesized[18] in three steps starting from 4,5-dicyanoimidazole (**2**) (Scheme 1). The NH group of the latter was protected by reaction with *p*-methoxybenzyl chloride in dimethylformamide, catalyzed by potassium carbonate, to afford **3** in 88% yield. Compound **3** was reacted with octadecyl-guanidinium nitrate (**4**), freshly prepared by the reaction of octadecylamine (**6**) with 3,5-dimethyl-1-pyrozolylamidinium nitrate (**7**), to produce the ring-closed product **5** in 57% yield. The deprotection of **5** was carried out by treatment with trifluoroacetic acid at room temperature for four hours, which gave the target product **1** in quantitative yield. Compound **1** was completely characterized[18] by ^{1}H and ^{13}C NMR, IR, and mass spectral data, as well as elemental microanalyses.

Compound **1** can exist in several different tautomeric forms, including but not limited to **1a**, **1b**, and **1c** as shown (Scheme 2). The ^{1}H NMR of the product in DMSO-d_6 showed five D_2O-exchangeable protons, three of which appeared as a broad singlet each at δ 7.79, 7.69 and 7.38 and the remaining two appeared as a triplet overlapped with a singlet centered at δ 7.45. As structure **1a** contains a free imidazole NH, which normally



Scheme 2.

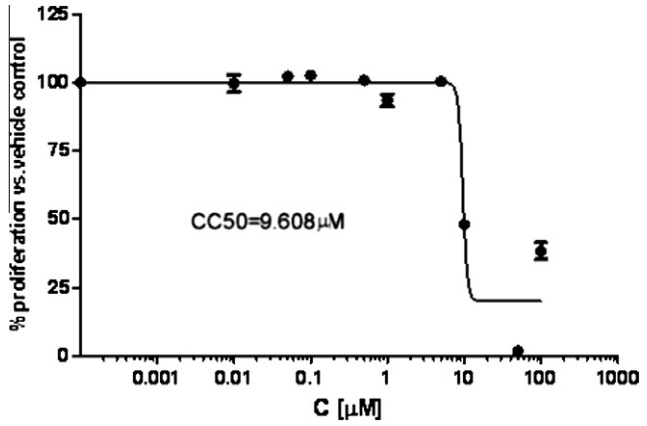


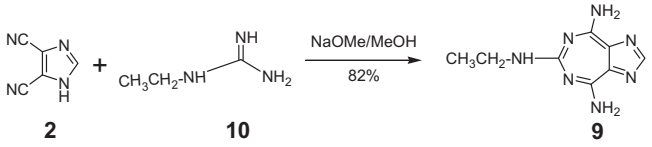
Figure 2. Effect of compound **1** on immortalized breast cancer cell line MCF10A. WST-1 cell proliferation assay, MCF-10A, 1250 cells treated with **1** for 72 h.

is expected to appear at >10 δ, and since structure **1c** lacks the $H_{(e)}$ proton to exhibit a triplet by coupling with an adjacent CH_2 group, either of the tautomeric forms **1a** or **1b** does not match the observed NMR data. On the other hand, structure **1c** is consistent with the observed data in that the two NH protons in each of the two NH_2 groups would be magnetically different because of the anticipated H-bonding with the imidazole N atoms, resulting in four different signals, and the remaining NH would be a triplet through coupling with the adjacent CH_2 group. Indeed, variable temperature NMR experiments conducted at 10° intervals between 25 and 80 °C confirmed this assignment by revealing gradual coalescence of signals at δ 7.79 and 7.69 (tentatively H_a and H_b protons) and at 7.45 and 7.38 (tentatively H_e, H_c, and H_d).

Compound **1** was screened in vitro against six cancer cell lines, including A549 and H460 (lung cancer), MCF-7 and MDA-MB-231 (breast cancer), OVCAR-3 (ovarian cancer), and PC-3 (prostate cancer). The results are graphically represented in Figure 1.

In order to study the effect of **1** on normal cell growth and proliferation, we also determined the CC_{50} value (cytotoxicity) of **1**, employing the immortalized normal breast cell line MCF10A. The results are shown in Figure 2.

As part of a preliminary structure–activity relationship study, we became interested in exploring the role of the long hydrophobic chain attached to the seven-membered heterocyclic ring of **1**. To this end, we synthesized an analogue of **1** containing a much



Scheme 3.

shorter alkyl chain, namely an ethyl group. Thus, compound **9** was synthesized in 82% yield by direct condensation of 4,5-dicyanoimidazole with ethylguanidine (Scheme 3). Compound **9** was fully characterized as before using spectral and microanalytical data.

Compound **9** was screened in vitro as before against six cancer cell lines. The compound was found to be inactive (IC_{50} >800 μM) against PC-3 (prostate) and MCF-7 (breast) cancer cell lines, and only weakly active against A549 (IC_{50} = 38 μM) (lung) and H460 (IC_{50} = 22 μM) (lung), and OVCAR-3 (ovarian) cancer cell lines. These results suggest that the long alkyl chain attached at the six-position of the heterocyclic ring plays a significant role in the observed biological activity.

In conclusion, we have discovered a novel, broad-spectrum antitumor compound that shows potent in vitro activity with low micromolar IC_{50}'s against all six cancer cell lines tested. The cytotoxicity (CC_{50}) of **1** to normal cells is at least at a four fold higher concentration than the therapeutic concentration levels. The long alkyl chain attached to the six-position of the heterocyclic ring of **1** appears to be necessary for the observed biological activity as compound **9** with an ethyl group failed to show good activity under the same experimental conditions. Further studies of structure–activity relationships (SAR) to enhance potency and decrease toxicity as well as mechanistic explorations of antitumor activity of **1** are currently in progress.

Acknowledgment

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18. ***Experimental***: *Organic synthesis procedure*: Preparation and physico-chemical properties of the compounds are as follows: *1-(4-Methoxybenzyl)-4,5-dicyanoimidazole* (**3**): A suspension of 4,5-dicyanoimidazole (1.49 g, 12 mmol) and potassium carbonate (1.99 g,

14.4 mmol, 1.2 equiv) in anhydrous DMF (15 mL) was stirred for 5 min. To the reaction mixture was added *p*-methoxybenzyl chloride (1.99 mL, 14.4 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was evaporated to leave 5 mL dimethyl-formamide. Ice was added to the residue to remove excess potassium carbonate, the solution was stirred for 1 h, leaving the precipitate which was filtered and washed successively with water and methanol. The reaction afforded a white solid (88%). ^{1}H NMR (DMSO-d_6): δ 8.457 (s, 1H, imidazole-CH), 7.26 (d, J = 8.7 Hz, 2H, Ph-H), 6.94 (d, J = 8.7 Hz, 2H, Ph-H), 5.36 (s, 2H, benzyl-CH_2), 3.72 (s, 3H, methoxy-CH_3). Anal. Calcd for $C_{13}H_{10}N_4O$: C, 65.54; H, 4.23; N, 23.52. Found: C, 65.45; H, 4.26; N, 23.46.

1-(p-Methoxybenzyl)-8-imino-4,6-diamino-N^6-octadecyl-1,8-dihydroimidazo[4,5-e][1,3]diazepine (**5**): In a flame-dried, two-necked round bottom flask, anhydrous methanol (30 mL), 3,5-dimethyl-1-pyrozolylamidinium nitrate (1.05 g, 5 mmol), and octadecylamine (3.0 g, 5 mmol) was added. The reaction mixture was refluxed for 5 h. Then the solvent was evaporated under reduced pressure to dryness. The octadecylguanidine nitrate was recrystallized from methanol in a quantitative yield. Anhydrous methanol (10 mL) was added to a flame-dried, two-necked round bottom flask equipped with a nitrogen gas inlet and a condenser, and fresh sodium metal (0.15 g, 5 mmol) was added to methanol and stirred for half an hour. The octadecylguanidine nitrate prepared previously was added to the freshly prepared sodium methoxide reaction mixture. After 0.5 h, the reaction mixture was transferred to a centrifuge tube to remove the sodium nitrate. The residue was transferred back to another flame-dried flask, 4,5-dicyano-1-*p*-methoxybenzyl-imidazole (0.83 g, 4 mmol) was added to the flask, and the reaction mixture was refluxed overnight. The reaction mixture was evaporated to dryness and the residue was recrystallized from anhydrous methanol to afford 1.26 g of a white solid. Yield 57%. ^{1}H NMR (DMSO-d_6): δ 8.0 (s, 1H, imidazole-CH), 7.41 (br, 1H, NH), 7.20 (d, J = 8.7 Hz, 2H, Ph-H), 7.10 (br, 2H, NH_2), 6.83 (d, J = 8.7 Hz, 2H, Ph-H), 6.30 (br, 1H, NH), 5.68 (s, 2H, benzyl-CH_2), 3.67 (s, 3H, –OCH_3), 1.37 (m, 2H, CH_2-NH), 1.19 (s, 32H, CH_2s), 0.81 (t, J = 6.4 Hz, 3H, CH_3). MS (ESI): 550 (MH^+).

8-Imino-4,6-diamino-N^6-octadecyl-1,8-dihydroimidazo[4,5-e][1,3]diazepine (**1**): Compound **5** (96.6 mg, 0.176 mmol) was added to a 25 mL round bottom flask with TFA (5 mL), and the reaction mixture was stirred for 3 h at room temperature. It was evaporated to dryness, the residue was treated with saturated sodium bicarbonate, filtered and washed with cold methanol to afford a white solid (75.5 mg, 99%). ^{1}H NMR (DMSO, 400 MHz): δ 7.80(s, 1H, NH), 7.70(s, 1H, NH), 7.52(s, 1H, imidazole-CH), 7.45(m, 2H, 2NH), 7.40(s,1H, NH), 3.29 (m, 2H, CH_2-NH), 1.49 (m, 2H, CH_2CH_2NH), 1.23(s, 30H, 15xCH_2), 0.86(t, J = 6.6 Hz, 3H, CH_3); ^{13}C NMR (DMSO, 400 MHz): δ 161.53, 159.72, 159.16, 136.50, 135.69, 41.87, 31.83, 29.57, 29.39, 29.24, 27.00, 22.64, and 14.50; UV (l_{max}) 249.5 nm ($e = 4.35 \times 10^4$, 280.5 nm (sh) ($e = 2.07 \times 10^4$); MS (ESI): 430 (MH^+); HRMS (FAB) Calcd for $C_{24}H_{43}N_7$: m/z 430.3658; found 430.3651; Anal. Calcd for $C_{24}H_{43}N_7 \cdot 2H_2O$: C, 61.90; H, 10.17; N, 21.05. Found: C, 61.87; H, 10.25; N, 20.83.

8-Imino-4,6-diamino-N^6-ethyl-1,8-dihydroimidazo[4,5-e][1,3]diazepine (**9**): Fine sodium metal was placed in a flame dried apparatus and anhydrous methanol (15 mL) was added and stirred at room temperature for 10 min in argon atmosphere. Then ethyl guanidine hemisulfate 1.092 g (0.008 mol) was added to the above sodium methoxide solution. This reaction mixture was stirred at room temperature for 1 h. Separated sodium sulfate was removed under centrifugation at 4 °C and then ethyl guanidine solution was added to the solution of dicyanoimmidazole 0.708 g (0.006 mol) in anhydrous methanol (15 mL). This reaction mixture was refluxed for 72 h. Reaction mixture was brought to room temperature and separated solid was filtered and washed with cold methanol. ^{1}H NMR (DMSO-d_6, 400 MHz) d 7.58 (br s, 4H, NH_2, D_2O exchangeable), 7.55 (s, 1H, imidazole CH), 3.28 (q, J = 7.32 Hz, 2H, CH_2), 1.02 (t, J = 7.32 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6, 400 MHz) d 161.3, 159.8, 159.2, 149.7, 136.4, 135.6, 36.5, 15.3; MS (ESI) m/z 206 (MH^+); Anal. Calcd for $C_8H_{11}N_7$: C, 46.82; H, 5.40; N, 47.78. Found: C, 46.70; H, 5.69; N, 48.01.

Biological screening procedure: New anticancer compounds were tested using following cell lines: lung cancer: A549 and H460; breast cancer: MCF-7 and MDA-MB-231; ovarian cancer: OVCAR-3 and prostate cancer: PC-3. Cells ($0.5–1.7 \times 10^3$ cells/50 μl/well) were seeded in RPMI + 10% FBS in 96-well plate the day before adding the drug dilutions. DMSO was used to dissolve all compounds (stock 3–200 mM). Each compound was tested at nine different concentrations: 100, 50, 10, 5, 1, 0.5, 0.1, 0.05 and 0.01 μM, final. Each drug dilution for each drug was tested in four-replicates within each experiment, and each experiment was repeated 1× or 2×. Cells treated with DMSO (equiv volume) were used as a 'vehicle control'. After addition of the drug, cells were cultured for 72 h at 37 °C, 5% CO_2. The experiment was terminated by adding WST-1 cell proliferation reagent (Roche, Mannheim, Germany) to each well and additional incubation for 4 h at 37 °C, 5% CO_2. The colorimetric readouts of cellular metabolic activity was performed by measuring absorbance at 450–690 nm using a Synergy HT Multi-Detection Microplate Reader and GEN5 software (Bio-Tek, Winoski, VT). Data analysis and IC_{50} calculation was done using GRAPHPAD PRISM software, v.5 (La Jolla, CA).