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# 11-Phenyl-[*b*,*e*]-dibenzazepine compounds: Novel antitumor agents

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ABSTRACT

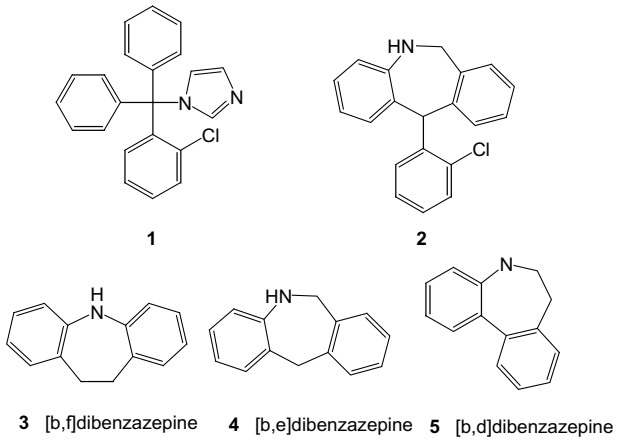
A series of 11-phenyl-[*b*,*e*]-dibenzazepine compounds were synthesized and shown to be inhibitors of tumor cell proliferation with $IC_{50}$ values ranging from submicromolar to micromolar concentrations. Flow cytometric analyses of several active compounds demonstrated inhibition of cell cycle progression at the $G_0$–$G_1$ phase transition resulting in $G_0$–$G_1$ arrest.



For small organic molecules, nitrogen-containing heterocycles have received considerable attention in the literature, mainly due to a broad range of biological properties and a detailed history as important medicinal pharmacophores.[1] Of these heterocycles, the synthesis, reaction, and biological activity of nitrogen containing seven-membered ring compounds is an expanding area of research in medicinal chemistry. There has been an extensive analysis of benzdiazepine compounds, which exhibit a wide range of biological activities including antidepressive, analgesic, antipsychotic, antihistaminic, and antimuscarinic activities.[2a–e] Although, various scaffolds of dibenzazepines 3, 4, and 5 (Fig. 1) show diverse biological activities,[2f–h] these compounds, especially scaffold **4**, have not been as widely investigated.

As an ongoing program aimed at discovery and development of effective antiproliferative agents, a study was initiated to assess clotrimazole **1** (Fig. 1) and its analogs for anticancer activity based on a novel mechanism of action.[3]

Clotrimazole (CLT) **1**, a drug marketed to fight fungal infections, inhibits cell growth and proliferation by blocking the cell cycle in $G_0$–$G_1$ through $Ca^{2+}$ store-mediated inhibition of translation initiation.[4] In particular, CLT reduces synthesis and expression of $G_1$ cyclins and thereby inhibits associated cyclin-dependent kinase activity, which is required for progression into S phase.[4] CLT induces the release of $Ca^{2+}$ from intracellular stores in the endoplasmic reticulum, blocks the influx of extracellular $Ca^{2+}$ through the $Ca^{2+}$ stores-regulated $Ca^{2+}$ channels, and inhibits $Ca^{2+}$ uptake into intracellular stores. The sustained depletion of intracellular $Ca^{2+}$ activates RNA-dependent protein kinase (PKR), resulting in phosphorylation of eIF2α at serine 51 and its concomitant inactivation. Inactivation of eIF2α inhibits formation of the ternary complex between Met-tRNA, eIF2α, and GTP, the rate-limiting step in translation initiation, and abrogates ribosomal synthesis of growth promoting proteins such as cyclin A, E, and D1 leading to cell growth arrest in early $G_1$.[5,3b]




**1** **2** **3** [b,f]dibenzazepine **4** [b,e]dibenzazepine **5** [b,d]dibenzazepine

**Figure 1.** Structure of Clotrimazole[1] and different scaffolds of dibenzazepine compounds.

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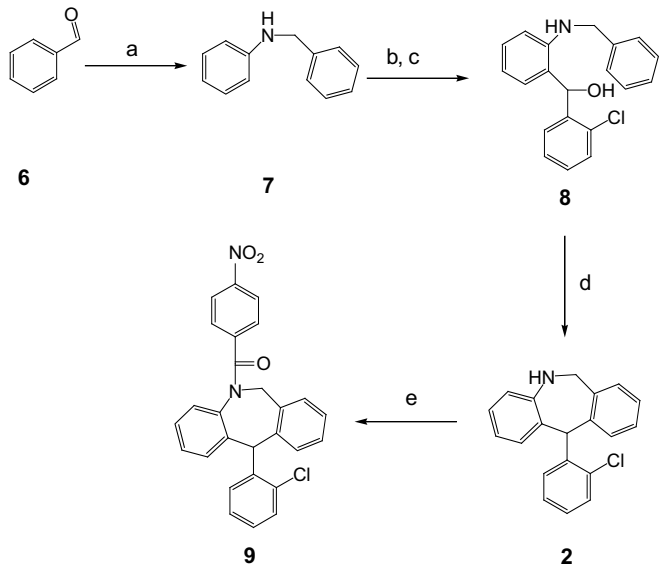
Previous studies on CLT concluded that the antiproliferative activity depends on the triaryl methane moiety and in particular the 'propeller' type configuration around the methane.[3a] In a continued effort aimed at generation of more active compounds with reduced toxicity, compounds were synthesized based on the 11-aryl-[*b*,*e*]-dibenzazepine **2** scaffold, resulting in a series of cytostatic antiproliferative agents. Herein, we describe the synthesis and in vitro antiproliferative activity of a series of the 11-aryl-substituted [*b*,*e*]-dibenzazepine compounds and their potential for use as anticancer agents. Given that the 11-aryl-[*b*,*e*]-dibenzazepine scaffold **2** and CLT **1** (Fig. 1) have a similar configuration at the central carbon *C-11*, while lacking the imidazole moiety associated with the known hepato-toxicity of CLT **1**, we hypothesized that the new compounds would have similar activity with lower toxicity.

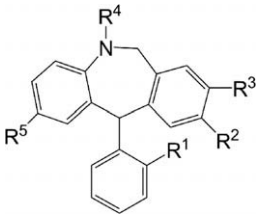
The dibenzazepine scaffold has been constructed as shown in Scheme 1.[6] The synthesis starts with benzaldehyde **6** and aniline, where reductive amination of aniline and the corresponding aldehyde gave the desired amino derivative **7** in a quantitative yield. The alkylation of secondary aniline was selectively achieved through the intermediate anilinodichloboranes derivatives.[7] The reaction of this intermediate, which formed in situ, with benzaldehyde derivative in the presence of tertiary amine yielded the secondary aniline derivative **8** in 70% yield.[8] Subsequently, the compounds were cyclized using concentrated sulfuric acid in dichloromethane at room temperature. The 11-substituted dibenzazepine system **2** was generated as a racemic mixture, which was used for further reactions. The final compounds were prepared through a standard method for alkylation and acylation of the azepine ring. Table 1 shows the compounds that were prepared for this study.

It has been demonstrated that the antiproliferative activity of CLT **1** depends, in large part, on the conformation of the triaryl methane moiety. This led to the hypothesis that the similar triaryl structure of the dibenzazepine at position 11 would have antiproliferative activity but decreased systemic toxicity compared to CLT due to the absence of the imidazole moiety of 1. A sulforhodamine B (SRB) assay,[9] using human colon adenocarcinoma cells (HT-29), was performed to test the antiproliferative activity of compound **2**, the core of the designed compounds. The $IC_{50}$ value of compound




**Scheme 1.** Reagents and conditions: (a) aniline, $Et_3N$, $NaBH_4$, MeOH; (b) $BCl_3$, toluene, −10 C; (c) 2-chlorobenzaldehyde, $Et_3N$; (d) $H_2SO_4$, DCM, rt, 2 h; (e) *p*-nitrobenzoic chloride, $Et_3N$, DCM.

**Table 1**
Various dibenzazepine compounds, synthesized in this study.




| Compound | $R^1$ | $R^2$ | $R^3$ | $R^{4a}$ | $R^5$ |
|---|---|---|---|---|---|
| **10** | Cl | H | H | $CH_3$ | H |
| **11** | H | H | H | $CO_2Me$ | H |
| **12** | Cl | H | H | $CO_2Ph$ | H |
| **13** | H | H | H | Bz | H |
| **14** | H | H | H | 3-$OMe_3$Bz | H |
| **15** | Cl | H | H | 3-$OMe_3$Bz | H |
| **16** | Cl | H | H | Bz | H |
| **17** | H | H | H | Bz | H |
| **18** | Cl | H | H | Bn | H |
| **19** | Cl | H | H | 4-$NH_2$Bz | H |
| **20** | H | H | H | 4-$NHMe_3$Bz | H |
| **21** | H | H | H | 4-$NMe_2$Bz | H |
| **22** | H | H | H | 4-$NO_2$Bz | H |
| **23** | Cl | H | H | 4-OMeBz | H |
| **24** | Cl | H | H | CONH(4-$NO_2$Ph) | H |
| **25** | Cl | H | OMe | 4-$NO_2$Bz | Cl |
| **26** | Cl | OMe | H | 4-$NO_2$Bz | Cl |

[a] Bz is COPh, Bn is $CH_2$Ph.

2 was 1.2 μM compared to 5.1 μM for CLT 1.[3] SRB assays performed with different cell lines demonstrated a broad range of activity for these compounds. Table 2 shows the $IC_{50}$ values in SRB assays of the synthesized compound tested against up to a total of 9 tumor cell lines. As shown in Table 2, compounds **9, 22, 23, 25**, and **26** have a submicromolar activity against different cell lines, while the rest of the synthesized compounds show an activity in the micromolar range.

Compounds **9, 25**, and **26** have been screened in the NCI 60 cell line assay for antitumor activity.[10] The results demonstrate promising activity with a unique spectrum of activity against the panel of cell lines. The NCI COMPARE algorithm[11] suggests that this class of compounds has distinguishing activity that may indicate a novel mechanism of action and subsequently novel anticancer activity.

To further investigate the effect of this class of compounds, the effect of compound **9** (chosen as a representative of the compounds with submicromolar activity against the panel of cell lines used) was tested on cell cycle progression. DNA content analysis of cultured human colon carcinoma cells (HT-29) was determined after treatment with or without compound **9**[12] at 25 and 50 μM (Fig. 2). When cultures were treated with vehicle alone (DMSO) in complete medium for 16 h, cell cycle progression was evident with 37.91% and 13.78% at S and $G_2$–M phases, respectively (A). Under identical conditions, cell cycle progression was dramatically blocked in $G_0$–$G_1$ when cells were treated with compound **9**. In the presence of 25 μM of compound **9**, fewer cells exited out of $G_0$–$G_1$ phase (B; 63.68%) and consequently fewer cells progressed to S phase (B; 25.79%). Higher concentrations of compound **9** (50 μM) resulted in a dose dependent increase in the number of cells remaining in $G_0$–$G_1$ phase (C; 69.22%). As expected, there was a concomitant decrease in the number of cells in S phase. There was no indication that treatment with compound **9** induced apoptosis, as there was no increase in the sub-$G_1$ population of cells.

In this study, the dibenzazepine compounds were prepared as a racemate. To see whether there is a differential effect for the pure enantiomers and whether it is worth separating the enantiomers for further development of this class of compound, both enantiomers

**Table 2**
$IC_{50}$ (μM) values of the synthesized compounds.[a]

| Compound | A549 | HT29 | MMRU | MCF7 | HepG2 | U118MG | Hela | HTB 174 | MDA-MB-231 |
|---|---|---|---|---|---|---|---|---|---|
| **2** | 5.4 | 1.2 | — | 2.7 | 0.9 | 1.4 | 1.1 | — | 1.3 |
| **9** | 0.8 | 0.3 | 0.2 | 0.6 | 0.3 | 0.5 | 0.3 | 0.1 | 0.5 |
| **10** | 11.4 | 9.7 | 4.6 | 1.7 | — | — | — | — | — |
| **11** | >10 | >10 | 10 | — | — | — | — | — | — |
| **12** | — | 5 | — | 4.2 | 3.6 | — | — | — | — |
| **13** | >10 | >10 | >10 | — | — | — | — | — | — |
| **14** | >5 | 1.7 | 2.5 | 4.5 | 2.6 | — | — | 3.6 | — |
| **15** | 3.7 | 1.7 | — | 3.4 | 1.2 | 2.5 | 1.4 | — | 1.5 |
| **16** | 2.5 | 0.6 | — | 1.5 | 0.8 | 1.3 | 0.9 | — | 1.2 |
| **17** | >5 | 2.6 | 2.7 | 4.2 | 3.4 | — | — | 2.6 | — |
| **18** | >5 | >5 | — | 4.3 | 4.5 | — | >5 | — | >5 |
| **19** | 3.7 | 1.7 | — | 1.1 | 1.5 | 1.6 | 0.5 | — | 1.2 |
| **20** | 2 | 1.1 | — | 1.7 | 1.0 | 1.8 | 0.4 | — | 1.3 |
| **21** | 2.1 | 1.0 | — | 1.7 | 1.0 | 2.6 | 0.9 | — | 1.7 |
| **22** | — | 0.7 | 0.5 | — | 0.9 | 0.9 | 0.6 | 0.7 | — |
| **23** | 1.1 | 0.4 | 0.2 | 0.5 | 0.4 | 0.4 | 0.2 | 0.3 | — |
| **24** | >5 | 4.8 | 3.1 | 2.4 | 4.5 | — | — | 4.0 | — |
| **25** | 1.5 | 0.6 | 0.5 | 1.3 | 0.9 | 0.8 | 0.5 | 0.5 | 0.9 |
| **26** | 0.6 | 0.2 | 0.2 | 0.7 | 0.4 | 0.4 | 0.2 | 0.2 | 0.4 |

[a] The assay was done using SRB assay method, (—), not tested.

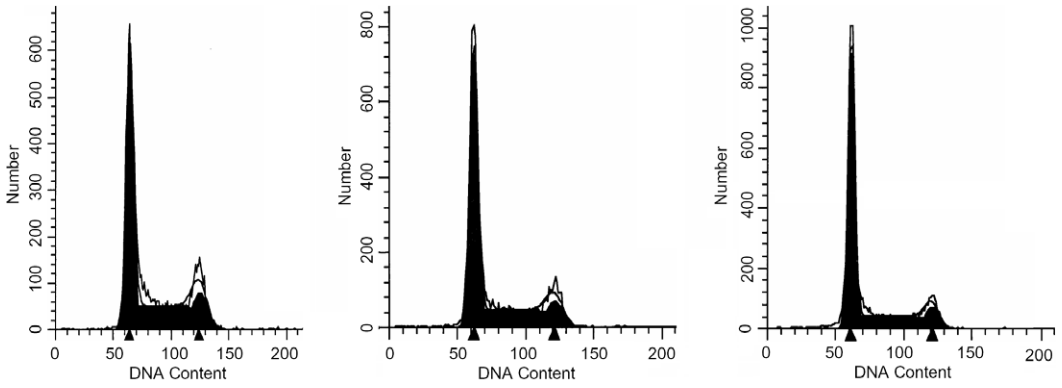



**Figure 2.** DNA content analysis of HT-29 colon cancer cells by flow cytometry. HT-29 cells were treated with various concentrations (25 and 50 μM) of compound **9** for 16 h. Cells were stained with propidium iodide and analyzed by flow cytometry. The percentage of cells at different stages of the cell cycle ($G_0$–$G_1$, S, $G_2$–M) is indicated below.

of compound **9** were separated (enantiomer **9a** and enantiomer **9b**; the enantiomeric identity was not characterized). Both enantiomers have been screened in the NCI screening assay, the results did not show any difference of either enantiomer to that of the racemate compound **9**. The average log$GI_{50}$ values of **9a** and **9b** were −5.36 and −5.18, respectively, while that of **9** was −5.71. To further confirm this finding, the cell cycle effect of **9a** and **9b** on the H-460 cell line was compared to that of 9 at a concentration of 50 μM following serum-starvation for 72 h. As shown in Table 3, no significant difference was observed between the two enantiomers and the racemate. The extent of the arrest was similar to that of the racemate (% of $G_0$–$G_1$ population in the range of ~70% for all compounds compared to ~40% for the vehicle control). These results confirmed that there is no significant difference between the two enantiomers and their ability to cause cell cycle arrest.

In conclusion, a series of 11-aryl-substituted [*b*,*e*]-dibenzazepine compounds were synthesized and the in vitro antiproliferative activity investigated. The synthetic dibenzazepine compounds were effective in growth inhibition at submicromolar to micromolar concentrations. Compound **9**, as a representative compound from those in the series with submicromolar activity, was shown to specifically arrest the cell cycle in $G_0$–$G_1$ phase. Pure enantiomers of **9** did not show any advantage over the racemate, in antiproliferative activity or in the ability to arrest the cell cycle at $G_0$–$G_1$ stage. These results together with those from an ongoing investigation into the

**Table 3**
Effect of **9a**, **9b**, and **9** on cell cycle progression.[a]

| Treatment | $G_0$–$G_1$ | $G_2$–M | S |
|---|---|---|---|
| DMSO | 40.92 | 11.55 | 47.53 |
| **9a** (50 μM) | 71.06 | 5.09 | 23.86 |
| **9b** (50 μM) | 74.96 | 5.76 | 19.29 |
| **9** | 73.95 | 5.24 | 20.81 |

[a] DNA content analysis of H-460 non-small cell lung cancer cells by flow cytometry. H-460 cells were serum-starved for 72 h, and then treated with 50 μM of **9a**, **9b**, and **9** for 16 h. Cells were stained with propidium iodide and analyzed by flow cytometry. Percentage of cells under different stages of cell cycles ($G_0$–$G_1$, S, $G_2$–M) is shown.

molecular mechanism of action will form the basis for further development of this class of compounds as anticancer therapeutics.

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8. All reactions were performed under dry conditions and the products gave a satisfactory physico-chemical analysis, herein the procedure for the synthesis of compound **2** and **8**. Boron trichloride (1 equiv, 1 M in Heptane) was added over 10 min via a canulla to stirred solution of **7** (1 mmol) at 0 °C in dry Toluene under nitrogen atmosphere. When the addition was completed, the ice water bath was replaced by an oil bath, and the reaction mixture was heated to reflux for 30 min. The heating bath was replaced by an ice bath to bring the internal temperature to 5 °C. A solution of 2-chlorobenzaldehyde (1.1 equiv) and triethylamine (2 equiv) in toluene (6 ml) was added over 30 min. The resulting slurry was stirred at room temperature for 1 h. Deionized water (20 ml) followed by 10% aqueous sodium hydroxide (100 ml) was added, the aqueous layer was adjusted to pH 8. The organic layer was isolated and the aqueous layer was extracted with toluene (2× 10 ml). The combined organic layers were washed with water, dried and concentrated to dryness by rotary evaporation. Column chromatography separation gave 70% yield. NMR (300 MHz, $CDCl_3$) 7.52 (1H, dd) 7.38 (1H, m), 7.1–7.4 (6H, m), 6.8 (2H, dm), 6.65 (2H, m), 6.1 (1H, bs), 5.1 (1H, bs), 4.6 (2H, bs). (2-chloro)-[2-(benzylamino)-phenyl]-methan-1-ol 8, (1 mmol) was suspended in 5 ml DCM. To the resulting slurry, sulfuric acid (95–98%, 5.5 equiv) was added over 5 min the reaction mixture turned from slurry to a solution. The viscous solution was stirred at room temperature for 2.5 h until TLC showed the consumption of the starting material. DCM (125 ml) was added and the biphasic reaction mixture was cooled to 10 °C. Ten percent aqueous sodium hydroxide was added to adjust the aqueous layer to pH 10. The organic layer was isolated and the aqueous layer was extracted with DCM (2× 60). The organic layer was combined and evaporated to dryness; the resultant material was subjected to chromatographic separation to give pure **2**, NMR (300 MHz, $CDCl_3$) 7.62 (bd, 1H), 7.48 (bd, 1H), 7.32–7.04 (m, 8H), 6.68 (bt, 1H), 6.58 (bd, 1H), 5.36 (bs,1H), 4.64 (d,1H), 4.25 (bs,1H), 3.65 (bd,1H).
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10. Based on the results of the 60-cell line assay performed by NCI, these three compounds have been referred to the biological committee in order to study their spectrum of activity as compounds with novel activity. This resulted in a recommendation by the NCI to further evaluate these compounds in preclinical stages.
11. http://dtp.nci.nih.gov/docs/compare/compare.html.
12. *Cell cycle analysis:* H460 and HT-29 cells were cultured in complete medium containing 0.1%DMSO, 10, 25, and 50 μM **9**. Cells were harvested 16 h following treatment, and washed with cold PBS twice. Cells were fixed in 70% ethanol at 4 °C at least 4 h. The fixed cells were centrifuged at 1500 rpm for 4 min at 4 °C, washed with cold PBS containing 2% FBS twice, treated with 3 mg/ml ribonuclease (Sigma Chemical Co) and 50 μg/ml propidium iodide (PI) (Sigma Chemical Co) for 30 min at 37 °C. Flow cytometry analysis was performed on the Becton Dickinson fluorescence-activated sorter FACS Scan by using the Becton Dickinson Cell Quest program. Data were evaluated using Modfit software (Verity Software House, Topsham, ME).