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Lipophilic derivatives of cyclam as new inhibitors of tumor cell growth

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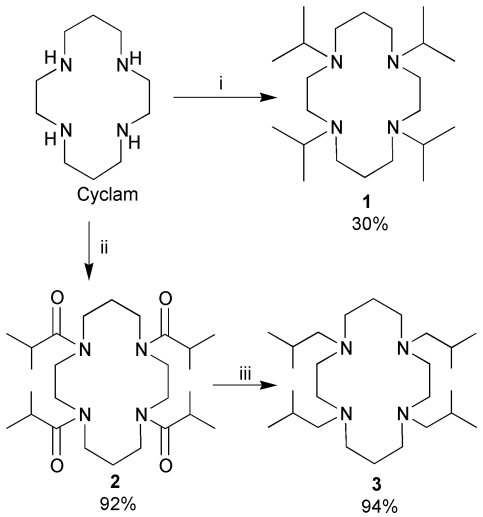
Two new lipophilic tetraazamacrocycles were prepared and, in contrast to non-lipophilic analogs, found to be potent inhibitors of tumor cell growth *in vitro* with IC_{50} values below 10 micromolar.

For the last three decades, macrocyclic compounds have found utility in a number of medical applications.[1] In particular, tetraazamacrocycles have been a primary focus owing to their proven ability to bind biomedically relevant metal ions and, in some cases, due to properties specific to the macrocycles themselves. For example, porphyrinic compounds and their metal complexes have been effective and continue to show promise in cancer therapy and/or diagnosis.[2] Additionally, saturated tetraazamacrocycles (*e.g.* 1,4,7,10-tetraazacyclododecane, cyclen, and 1,4,8,11-tetraazacyclotetradecane, cyclam) and their derivatives have been studied as carriers of metal ions in antitumor[3] and imaging applications[4] and, most recently, as anti-HIV agents.[5] In most cases, the cyclen and cyclam derivatives contain polar pendant functionalities to increase complex stabilities or allow for attachment of the macrocycle structure to other chemical species. Perhaps surprisingly, there is a paucity of studies on bulky-appended, lipophilic analogs of classic azacrown ethers despite the impact that lipophilicity has on biodistribution and, potentially, along with increased peripheral bulk, on the coordination chemistry of the macrocycle.[6] The lipophilicity should facilitate the transport of the macrocycles into the cell whereby normal cellular function can be engaged and, perhaps, disrupted. In fact, McKeage *et al.* have recently demonstrated a parabolic relationship between lipophilicity and antitumor activity for a homologous series of gold phosphine complexes toward the CH-1 cell line, suggesting optimal lipophilicity as a critical factor in drug design.[7] We are interested in lipophilic tetraazamacrocycles as new agents for tumor cell growth inhibition and as hosts for transition metal ions. As such, we have prepared two new derivatives of cyclam, compounds **1** and **3**† (Scheme 1) that contain bulky isopropyl and isobutyl substituents, respectively. We wish to report here on their synthesis and efficacy as inhibitors of L1210 tumor cell growth in culture. Further, a comparison of their inhibitory activity was made with respect to the parent cyclam macrocycle and a more polar amide-containing species, compound **2**, to emphasize the lipophilic structure–antitumor property relationship.[8]

Isopropyl and isobutyl substituents were chosen as lipophilic additions to the cyclam core because both are synthetically accessible in few steps[9] and were thought to impart a reasonable degree of lipophilicity without completely compromising water solubility. As shown in Scheme 1, the tetraisopropyl-appended cyclam, **1**, was prepared by treatment of commercially-available cyclam with an excess of 2-bromopropane in the presence of triethylamine–acetonitrile for 24 h at reflux. Despite the modest yield of 30%, the product was readily isolated as a colorless oil by column chromatography on alumina. Compound **3**, containing isobutyl appendages, was prepared by acylation of cyclam with isobutyryl chloride in the presence of triethylamine–dichloromethane for 30 min at rt to afford the tetraamide **2**. Global reduction using lithium aluminum hydride and purification by column chromatography on alumina



Scheme 1 *Reagents and conditions*: i, 2-bromopropane, TEA, CH_3CN, reflux; ii, isobutyryl chloride, TEA, CH_2Cl_2; iii, $LiAlH_4$, THF–CH_2Cl_2.

provided **3** in 86% yield (based on cyclam) as a white solid. The significantly higher yield of **3** *vs.* **1** is a result of the generally more efficient introduction of its pendant substituents. In the synthesis of **3**, the isobutyl groups are attached *via* acylation using a relatively potent electrophile with subsequent nearly quantitative reduction. This route is not available for the isopropyl-containing compound, so direct alkylation with a somewhat hindered alkyl halide, 2-bromopropane, was used. Therefore, given comparable activity, derivatization *via* isobutyl functionalities is preferred.

With compounds **1** and **3** in hand, we studied their effect on L1210 cell growth in culture in comparison to the less lipophilic cyclam and tetraamide **2**. Cyclam and compound **2** at 25 uM (highest concentration studied) had no effect on L1210 cell growth. Orioli and coworkers have noted a similar lack of toxicity for cyclam toward the human ovarian tumor cell line A2780 (IC_{50} > 100 uM).[10] However, compounds **1** and **3**, containing lipophilic side chains, were effective inhibitors of L1210 cell growth with IC_{50} values of 6.2 and 8.7 uM, respectively (Table 1). In contrast to the majority of antitumor

Table 1 Effect of tetraazamacrocycles on growth of mouse leukemia L1210 cells in culture

Compound	$IC_{50}/\mu M$[a]
Cyclam	> 25.0 (2)[b]
1	6.2 (4)
2	> 25.0 (2)[b]
3	8.7 (4)

[a] IC_{50}, concentration of drug required to inhibit L1210 cell growth by 50%. Cell counts were made 72 h after addition of drugs to the cells. Five different drug concentrations were used in each determination and triplicate wells, control (no drug) and each drug concentration were run. The number in parentheses is the number of independent determinations made. [b] There was no inhibition of L1210 cell growth at this concentration.

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studies involving azacrowns and their metal complexes,[3] it is notable that the activities of **1** and **3** were obtained using the free macrocycles. At this time we have no evidence as to whether the differences seen between inactive cyclam and compound **2** and the active compounds **1** and **3** relate to differences in the concentrations of each drug reached in the cells.

In conclusion, we have prepared isobutyl- and isopropyl-appended derivatives of cyclam. Both are effective growth inhibitors of L1210 cells. Importantly, in the absence of the lipophilic substituents the inhibitory properties of the macrocycle are greatly diminished. While the mechanism of inhibition of cell growth has not yet been defined, these data suggest that lipophilic tetraazamacrocycles offer a new series of compounds to be further studied in structure–function relationships and for mechanism(s) of action. Additionally, the opportunity for structural diversity in the title compounds is particularly noteworthy with compounds **1** and **3** representing a foundation for the development of lipophilic species with even greater activities/properties.

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Notes and references

† *Selected spectroscopic data*: for **1**: ^{1}H NMR ($CDCl_3$) δ 0.96 (24 H; d, CH_3), 1.61 (4 H, quintet, $CH_2CH_2CH_2$), 2.43 (8 H, t, NCH_2), 2.47 (8 H, s, NCH_2), 2.82 (4 H, septet, NCH); ^{13}C NMR ($CDCl_3$) δ 18.79, 27.13, 49.13, 50.35, 52.49; EI MS *m*/*e* 368 (M^+). For **2**: ^{1}H NMR ($CDCl_3$) δ 1.08 (24 H, overlapping d's, CH_3), 1.87 (4 H, quintet, $CH_2CH_2CH_2$), 2.72 (2 H, septet, CH), 2.86 (2 H, septet, CH), 3.33–3.63 (16 H, m, NCH_2; ^{13}C NMR ($CDCl_3$) δ 19.59, 19.86, 29.28, 30.10, 30.33, 45.92, 47.42, 47.75, 48.05, 177.79, 178.44; FAB MS *m*/*e* 481 (M^+). For **3**: ^{1}H NMR ($CDCl_3$) δ 0.86 (24 H; d, CH_3), 1.64 (8 H, m, $CH_2CH_2CH_2$, CH), 2.09 (8 H, d, NCH_2CH), 2.44–2.52 (16 H, m, NCH_2); ^{13}C NMR ($CDCl_3$) δ 21.00, 22.81, 26.58, 51.31, 52.06, 64.53; FAB MS *m*/*e* 425 ($M + H^+$).

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