Synthesis of Imidooxy Derivatives as Potential Anticancer Agents

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Objective

The objective was to synthesize imidooxy compounds and evaluate their growth suppression and cell killing properties in different cancer cell lines. Preliminary results from our laboratory indicate that certain imidooxy compounds possess promising anticancer activities against breast, lung, colon, prostate and brain cancers. The model compound, E49 (N-phthalimidooxy-2-methylacrylate), demonstrates potential anticancer activity in different cell lines at concentrations ranging from 10 -100 nanomolar.

Introduction

E49

- *N*-hydroxyphthalimide with a methyl acrylate side chain
- The first imidooxy compound to show pharmacotherapeutic potential
- Initially studied as a potential anti-convulsant due to optimum lipophilicity
  - High penetration into the CNS
- Later found to have anticancer properties

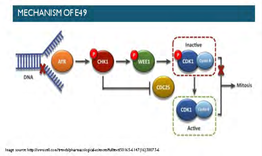
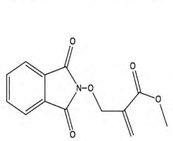
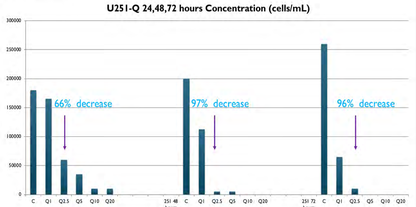


Image source:http://www.cell.com/trends/pharmacological-sciences/fulltext/S0165-6147(12)00072-6

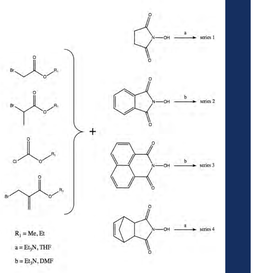




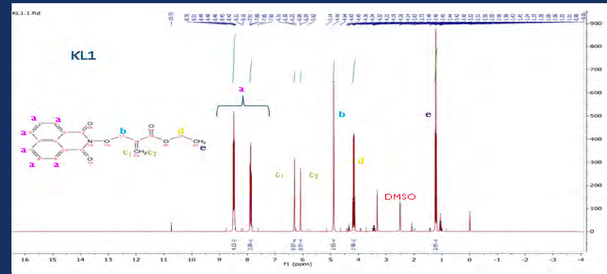
Methods

The reaction of N-hydroxyphthalimide with 2-bromomethylacrylate in triethylamine yielded the typical imidooxy analog E49. Using similar synthetic schemes, imidooxy formates, acetates, propionates, and methylacrylates were prepared from N-hydroxynaphthalimide, N-hydroxyph-thalimide, N-hydroxysuccinimide, and endo-N-hydroxy-5-norbornene. Eleven compounds AA1, E1, E3, E16, E29, E33, E38, E49, E62, GF1, and KL1 were evaluated for anticancer activity in different cell lines up to 48 hours of exposure. We determined the structural activity relationship with different ring sizes, side chain extensions, and bioisosteric substitutions on the anticancer activity of the imidooxy compounds.

The preliminary cell tissue studies of the imidooxy derivatives involved crystal violet (CV) assays that were used to assess growth suppression in glioblastoma cell lines at E49 doses between 2.5 and 20 μM and evaluated after 48 hours of exposure. The control group is the amount of growth in each cell line after 48 hours in the absence of drug. Growth after 48 hours is expressed as a comparative percentage of growth compared to the control. A value of 0% represents no growth after addition of drug and a negative value denotes complete growth suppression and killing the cells that are present



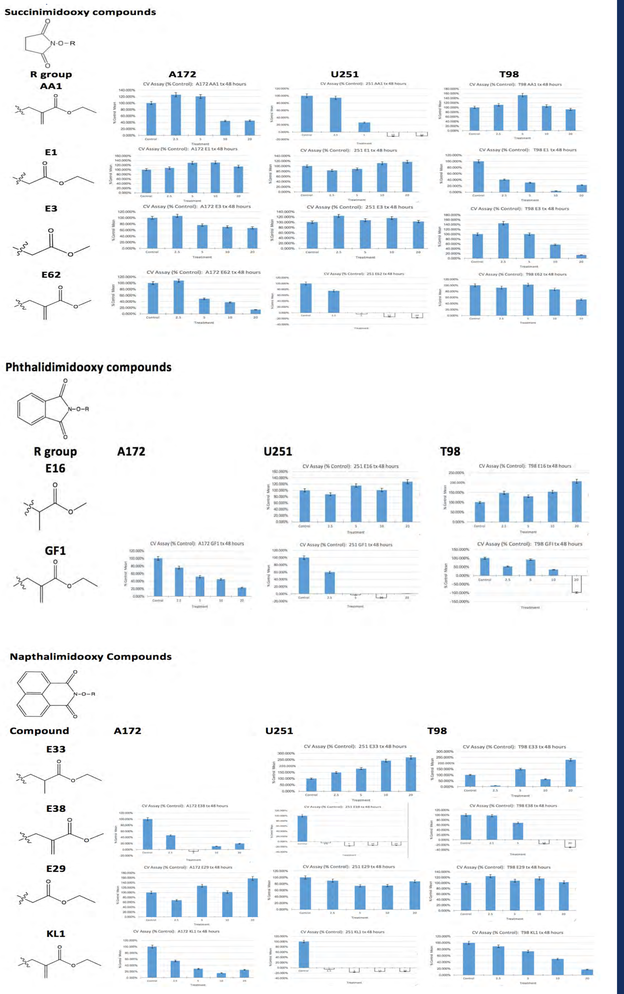
Results



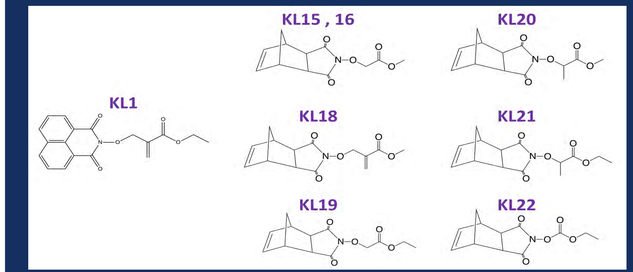
Succinimidooxy derivatives: each compound (AA1, E1, E3, and E62) showed significant growth reduction in at least one cell line (A172, U251, and/or T98). Compound AA1 showed significant growth suppression of 55.476% and 54.798% at a dose of 10 and 20 μM respectively. E62 significantly suppressed growth in line A172 at a dose of 5 μM. E62 also suppressed cell growth by 100% in line U251 and caused cell death at doses of 5 μM or greater.

Phtalidimidooxy derivatives: E16 did not suppress growth in either cell line in which it was tested (U251 and T98). GF1 showed growth reduction across all doses, reaching a level of significance at a dose of 10 μM in A172, 5 μM in U251 and 10 μM in T98. In cell lines U251 and T98, GF1 suppressed 100% of cell growth at 5 and 20 μM respectively and killed cells by 2.9 and 98% at these respective doses.

Napthalidimidooxy derivatives: Growth in T98 cell was suppressed up to 90% by E33 at 2.5 μM but was not suppressed at higher doses. E38 significantly suppressed growth in all cell lines. In A172, E38 was effective at a minimum dose of 2.5 μM and killed cells by a dose of 5 μM. At 10 μM, cell killing ceased but the growth suppression was still significant. In U251, all doses suppressed 100% of cell growth and killed by a minimum dose of 2.5 μM. T98 was significantly suppressed by 5 μM and started killing cells by 10 μM. E29 significantly suppressed growth in cell line A172 at a dose of 2.5 μM. KL1 significantly repressed growth in all cell lines without appreciable cell growth at any doses. A172 was suppressed by 72% at 5 μM, U251 was suppressed by 100% at all doses and resulted in cell killing at a minimum dose of 2.5 μM, and T98 was suppressed at a minimum dose of 10 μM.



Lead compounds for the future



Conclusions

This series of imidiooxy compounds shows varying activity across the cell lines tested. With this data, it is possible to evaluate how different side chains and ring structures influence the anti-cancer activity of each compound. Overall, the phthalimidooxy and napthalimidooxy compounds show the greatest effect on growth suppression across cell lines. This trend suggests that the larger ring structures increase the potency of the compounds and ability to inhibit cell growth. Although the succinimidooxy compounds did not demonstrate a comparable effect, there is a difference between the different side chains; a difference which is evident across the different ring structures as well. Based on the CV assay, there is a trend which suggests that an ethyl side chain provides more activity than the corresponding methyl side chains. Furthermore, adding a double bond to the structure appears to offer additional activity. Compounds the possess the ethyl acrylate side chain, a combination of the extended side chain and a double bond, show remarkable activity, exhibited in compounds AA1, GF1, and KL1. With this information, we can begin to establish a structure activity relationship which strengthens the potential of these compounds as anti-cancer compounds.

The *endo*-norbornene compounds are an exciting series of imidooxy derivatives. These novel compounds include KL15, KL18, KL19, KL20, KL21 and KL22. Compound KL18 is expected have potency similar to E49 and KL1 because it possesses the same imidooxy acrylate side chain in the chemical structure. However, KL18 also includes the norbornene moiety in the imidooxy group which could provide a more favorable safety profile as a potential anticancer agent.

Implications

The imidooxy compounds demonstrated anticancer activity in T98, U251, and A172 glioblastoma cancer cell lines at 2.5 to 5 micromolar concentrations. Overall, the phthalimidooxy and naphthalimidooxy compounds offered more potent anticancer activity across cell lines. Our initial *in vitro* evaluations indicate that E49 does not function as a DNA alkylator nor as DNA intercalator but it acts to disrupt cell cycle progression at the G2M boundary. The compound E49, and presumably, the imidooxy compounds presented here, utilize a unique mechanism of action that is unlike other chemotherapeutic compounds available on the market. Along with a novel mechanism, the imidoxy compounds could provide synergy with other anti-cancer agents, reduced resistance, and improved tolerability to chemotherapy regimens.