2020

From Nature to Medicinal Chemistry: Anti-Cancer Activity of Novel Indane Scaffolds

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The indane scaffold is of key importance in the natural world. It occurs in a range of biologically active natural products. The natural structures range from simple indanes and indanones with antibacterial activity e.g. indane isolated from the cyanobacterium *Nostoc* to the pterin family of fern and fungal metabolites, which show cytotoxicity toward the Human leukemic HL-60 cell line, to oligomers of resveratrol (3,4'-trihydroxystilbene) e.g. the indane Puclatoxofuran isolated from the Dipterocarpaceae. The therapeutic potential of indanes becomes more evident in the area of medicinal chemistry where this scaffold is incorporated into structures that demonstrate therapeutic properties e.g. the indanone Sulfadin (Clinfor™, Merck), a monoteran anti-inflammatory that has recently demonstrated anti-proliferative and apoptotic effects. More recently the tubulin polymerisation inhibitor Indanocine (NIH) has been receiving attention. During the course of our research on indane monomers and dimers, we have noted that the potential of novel indane scaffolds for the treatment of inflammatory disease and cancer has not been fully exploited. It is also well established that inflammation plays a significant role in the development of cancer. The aim of this research project is to combine, experience led and evidence based, theories with our knowledge of small molecule drug development to generate novel hybrid scaffolds with therapeutic potential for the treatment of inflammatory disease. The second fragment of interest, in this study comes from the naturally occurring benzophenones. This class of molecules features widely in plants used as anti-tumour agents in Traditional Herbal Medicinal Systems. A range of bioactivities has been established for members of this chemical class, including tubulin binding and P-glycoprotein (P-gp) modulating activity, the prototypical efflux transporter, implicated in multidrug resistance (MDR).

Results and Discussion

A novel scaffold designed around an indane nucleus was identified as the chemical target in this study (Fig.1). The scaffold was synthesised by combining a series of small chemical fragments using a range of spacer molecules. The second major fragment incorporated into the molecule is centred on the small naturally occurring bioactive benzophenone monoy. Depending on substitution pattern demonstrates a variety of biological properties. Initial studies have led to the development of a synthetic approach that has thus far yielded eight final products with the desired final chemical scaffold. All products and intermediates have been characterized spectroscopically. The cytotoxicity of the starting fragments and products were evaluated using the Acid Phosphatase assay, in a series of in vitro cancer cell lines. Coupling of the main molecular fragments and spacers gave rise to compounds with a novel chemical scaffold. These molecules displayed significant increases in IC₅₀ values in the cell lines under investigation, relative to their component fragments (Figs.2 and 3). Compounds with the new scaffold induce apoptosis to varying degrees and also demonstrate the inhibition of ROS production os a free radical scavenger cell cycle arrest. A lead compound has been identified within the series which has displayed the highest IC50 value (1.0 μM) against the HER2+ cell line SKBR3. Current studies are now focussed on this cell line.

![Figure 1: Target Hybrid Scaffold](image1)

![Figure 2: Cytotoxicity of parent hybrid and natural precursors in MCF7 and SKBR3 (Breast), DU145 (Prostate) and A549 (Lung) cell lines](image2)

![Figure 3: IC50 comparison of lead compounds in HER2+ SKBR3 breast cancer cell line](image3)

![Figure 4: Flow cytometry analysis of Apoptosis induction of lead compound over time in SKBR3 cells](image4)

![Figure 5: Flow cytometry analysis of ROS accumulation of leading compounds in SKBR3 cells (24 h, μM)](image5)

![Figure 6: Flow cytometry analysis of Apoptosis induction and Cell cycle arrest in HEK-293 cells (72 h, μM)](image6)

Conclusion and Perspectives: We have identified a novel chemical scaffold, that demonstrates cytotoxic activity in vitro in a range of human cell lines, including MCF7 and SKBR3 (Breast), DU145 (Prostate) and A549 (Lung). Excitingly, 012L has not shown cytotoxicity to normal HEK cells even at a concentration 30 times higher than its IC50 on SKBR3. Tamoxifen, on the other hand, at its IC50 concentration, has induced apoptosis on 85% of the HEK cells. The decrease in ROS accumulation in a dose-dependent manner for the compounds of this novel indane scaffold suggests that it might be involved in the cellular apoptosis induction. Our working hypothesis has been that hybridization of several bioactive fragments results in synergized bioactivity (Figure 2 and 3). The results demonstrate the validity of this hypothesis, showing that the nature and position of substitution together with stereo-isomeric specificity results in significant increases in bioactivity: e.g. coupling of indan-1-one (IC50 100 μM in SKBR3, A549, MCF7 and IC50 40.88 μM in DU145) with fragment A (IC50 100 μM in SKBR3 and MCF7, and IC50 40.84 μM in A549, IC50 35.54 μM in DU145) yields a novel chemical scaffold with IC50 1.04 μM in SKBR3, 14.9 in A549, 26.9 in DU145 (Prostate) and 35.7 in MCF7. One hybrid 14bz1 compares well with Tamoxifen across the cell lines. Several hybrids are more potent in the SKBR3 cell line. Future chemical synthesis: We are preparing to couple indane monomers with new fragments we have prepared, that display nanomolar activity across the four cell lines, to generate what we believe will be derivatives with significant nanomolar dual activities. Future bioactivity evaluation: Initial studies also support the effects of scaffolds against other HER2+ breast cancer cells, including resistant to conventional treatments. A series of in dendr in vitro anti-cancer assessments and in vitro and in vitro anti-inflammatory studies will be carried out over the next months, to establish MOE and selectivity of these scaffolds.

Methods:

Synthesis and specific compounds: Will not be discussed at this point. Cell Culture: All the cell lines were maintained in a humid chamber at 37 °C and 5% CO₂. The SKBR3 (ATCC CCL-144) and A549 (ATCC CCL-185) cells were cultured in RPMI-1640 medium (Becton Dickinson, Milan) supplemented with 5% fetal bovine serum (FBS) and 1% L-Glutamine. A549 (ATCC CCL-185) lung carcinoma cells were grown in Dubecso's Modified Eagle Medium supplemented with 5% FBS. HER2+ (ATCC CRL-1577™ embryonic kidney cells) was grown in Minimum Essential Medium Eagle supplemented with 10 % FBS. Acid Phosphatase assay: After 5 days of incubation of cells with the drugs, the medium was removed and each well was washed with 100 μL of PBS. Then, 100 μL of freshly prepared phosphate substrate (10 mM p-nitrophenyl phosphate, Sigma) in 0.1 M sodium acetate (Sigma). 0.1% triton X-100 pH 5 (BDH) was added to each well. The plates were wrapped in tin foil and incubated in the dark at 37°C for 1 hour. The reaction was stopped by the addition of 50 μL of NaOH 1 M to each well and measuring was obtained in a dual beam plate reader at 405 nm with a reference wavelength of 620 nm.

Bibliography