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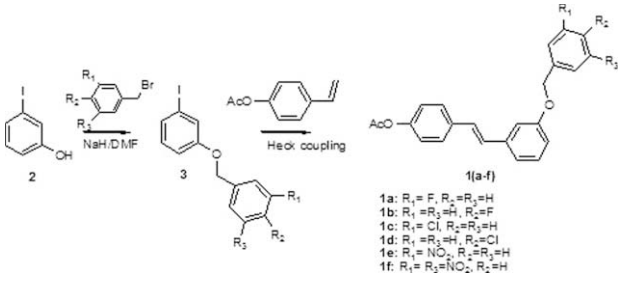
Design and synthesis of modified resveratrol analogues with potential apoptosis activity

T.T. Htar[1], J.F.F. Weber[2]. [1]*Monash University (Sunway campus), Jeffrey Cheah School of Medicine and Health Sciences, Kuala Lumpur, Malaysia;* [2]*University Technologi MARA, Institute Kajian Ubat Semulajadi (iKUS), Puncak Alam, Malaysia*

Background: Resveratrol (*trans* 3,5,4′-trihydroxystilbene) as a promising anticarcinogenetic and chemopreventive agent has been correlated with its inhibitory effect on cyclooxygenase, ornithine decarboxylase through the inhibition of protein kinase activity, angiogenosis and reactive oxygen species resulting from oxidation. A number of studies have shown the inhibitory effect of resveratrol on cell growth and its apoptosis inducing ability in various cancer cell lines. Secondly, the efficacy of resveratrol treatment certainly depends on the dosage, pharmacokinetics factors, routes of delivery and bioavailability. *In-vivo* studies on resveratrol pharmacokinetics profile reported that resveratrol is metabolized rapidly and extensively into its sulphate and glucuronide conjugates within 30 mins of an intravenous dose. Therefore, this project is aiming at synthesising novel modified resveratrol analogues with protecting groups on metabolically accessible hydroxyl groups at C-3 position of resveratrol to improve bioavailability and selectivity for apoptosis activity.
Material and Methods: Resveratrol analogues 1(a−f) were synthesised by Heck coupling of acetoxystyrene with 3-monosubstituted-iodophenol 3, yielded from protection of 3-iodophenol 2 with respective substituted-benzyl protecting group in the presence of sodium hydride (Scheme 1).
Results: The advantage of this scheme was that it minimised the product loss due to the involvement of only two steps of reactions and the absence of Wittig reaction. Protection with benzyl-protecting group on 3-iodophenol in the presence of sodium hydride gave a good yield range of product (67−99%). Derivatives of 3-monosubstituted-12-acetoxystilbene 1(a−f) were obtained with the yield of (40−77%) from Heck coupling reaction. The coupling constant of the vinylic protons at d 7.1 with a value of J = 11−13 Hz assured for the '*trans*' configuration in these modified-stilbenes.
Conclusions: Modified resveratrol analogues, 3-monosubstituted-4′-acetoxystilbenes were synthesised successfully.



Scheme 1. Synthesis of 3-monosubstituted-4′-acetoxystilbenes.

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Identification of synergistic drugs using combination high-throughput screening (cHTS) and breadth of activity in diverse cancer cell networks

R.J. Rickles[1], W.F. Tam[1], G.F. Short III[1], A. Myaskovsky[1], K.E. DeRoche[1], T.P. Giordono 3rd[1], B.G. Hoffstrom[1], P. Grossman[1], M.S. Lee[1].
[1]*CombinatoRx Inc., Oncology, Cambridge, USA*

The adaptive nature of tumor cells makes them difficult to kill. The cancer cell genome has remarkable plasticity and can make changes at the genome level (amplification, mutation or epigenetic) that results in adaptability and protection from insult by cancer drugs. An obvious and proven path to better cancer treatment is the use of drugs in combination. Focus on the coordinated action at multiple molecular targets provides unique therapeutic benefit not achievable with the "one-drug, one-target" paradigm. We have developed a proprietary combination high throughput screening platform (cHTS) to rapidly identify novel drug synergies of potential clinical utility. In this approach, we evaluate the activity of each chemical combination at multiple ratios, capturing the combined activity of two or more compounds over a broad range of single agent concentrations for comparison to models of drug interaction. This enables the systematic and empirical identification of potency and efficacy increases that result in enhanced, selective killing of tumor cells. Thousands of combinations can be evaluated in a cell line panel of varying genetic backgrounds within weeks. In depth analysis of interesting combinations in dozens of cells lines can be performed within a few months and may provide mechanistic insight into target-relevant network biology with the potential to influence clinical hypotheses. Such information can inform preclinical development paths as well as potential patient sub-populations that may respond favorably to particular drug combinations. One topical example is the PI3K and RAS signaling pathways which contain a number of important therapeutic targets and where inhibition of one pathway can induce compensatory changes in the other that blunt single agent anti-tumor activity. We have examined PI3K and MEK inhibitor single agent potency and breadth of combination synergy in a panel of cancer cell lines with varying genetic backgrounds to identify situations where this pairing might be most efficacious. Our results indicate that simple pathway mutational analysis is insufficient to predict the outcome of concurrent inhibition of these pathways and that systematic combination studies reveal unexpected patterns of sensitivity and resistance. In another example, we have utilized cHTS to make the surprising discovery that adenosine A2A and beta-2 adrenergic receptor agonists are highly synergistic, selective and novel targets that enhance glucocorticoid activity in B-cell malignancies. Our results underscore the value of systematic combination screening in the discovery and evaluation of new targets and therapies that may alter cancer treatment paradigms and improve patient outcomes.

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A new and fast in vitro screen to predict in vivo drug efficacy using cell primary culture from patient derived tumor established xenografts

O. Deas[1], G. Stephant[1], E. LeVen[1], M.F. Poupon[1], J.G. Judde[1].
[1]*XenTech, Cell Biology, Evry, France*

Background: We have developed a collection of 75 patients' derived tumorgrafts for in vivo preclinical evaluation of drug efficacy. These *in vivo* models reproduce accurately the behavior of the original tumors, including responses to chemotherapies (Marangoni et al. Clin Cancer Res 2007; 13: 3989–3998). In vivo drug efficacy assays are time, money and animal consuming. Therefore, we thought to develop a convenient *in vitro* screen to accelerate the identification of efficient compounds.
Methods: Primary cell cultures were obtained from tumors freshly explanted from mice in sterile condition and isolated by mechanical and enzymatic dissociation. Tissue was minced with scalpels and dissociated with a tissue shredder (gentleMACS™, Miltenyi Biotech) in standard culture medium supplemented with dispase, collagenase IV, hyaluronidase and DNAse. The cell suspension was filtered through a 100-mm nylon mesh. Cells were adapted for 2−5 days in standard culture conditions in Ham's F12/DMEM medium supplemented with 10% fetal bovine serum. Adherent cells were harvested by trypsinization. For the assay, cells were seeded in 96-well plates at a density of 2.5×10^3 to 10×10^3 cells/well, and incubated overnight at 37ºC prior to addition of test compounds. Each drug was tested at 10 concentrations covering five log. After 3 days, cell viability and doubling time were assessed by measuring ATP cell content (CellTiter-Glo®, Promega) IC50s were calculated as the concentration of drugs that inhibits cell growth by 50%. Growth inhibition is calculated as a percentage of ATP value compared to vehicle-treated controls.
Results: Today, we have tested more than 60 different tumorgrafts including breast, lung, colon, melanoma and glioblastoma and succeeded in a series of 20 models. For each model doubling time was assessed. A large panel of standard chemotherapies was tested in vitro to generate a drug associated IC50. Tested drugs were: adriamycin, 5-fluorouracil, irinotecan, topotecan, oxaliplatin, cisplatin and etoposide. As compared to established human cell lines, the doubling time of primary cultures was constantly longer, median: 60 hours (range: 33 to 70) vs 30 hours. The doubling time specific for each model was used as an internal quality parameter. A profile of drug sensitivity according to IC50 was also defined for each model. The correlation between in vitro and in vivo drug sensitivity data will be presented. Satisfying concordance was observed in several cases.
Conclusion: This assay allows classifying the different tumor models according to ex-vivo drug-specific IC50. Important parameters are discussed such as drug metabolism and the relationship between IC50 and in vivo tolerability issues. This ex vivo model system will be useful for rapid screening and early detection of drug efficacy in patient-derived tumorgrafts.