Material and Methods: 40 rats were injected (i.v.) with BNML cells and divided into 4 groups treated with: 1) EC (40 mg/kg b.w. by gavage) for 19 consecutive days, 2) Eto (14 mg/kg b.w., i.p.) for 3 consecutive days, from day 17 to 19 of the experiment, 3) EC + Eto, or 4) water as a solvent of EC (control group). The rats were killed 2 hours after the last dose of EC and/or 1 hour after Eto administration. The spleen and the bone marrow were isolated. The BNML cells were identified using an RM-124 antibody and analysed by flow cytometry. The mean concentrations of EC and *O*-methylated EC (Methyl-EC) in plasma were analyzed by HPLC-ECD.
Results: The mean concentration of EC in the plasma was 4.7 mM, and that of Methyl-EC was 5.5 mM. After simultaneous treatment (EC+Eto), the concentration of EC in the plasma decreased significantly to a level of 2.3 mM and that of Methyl-EC to 2.5 mM. The weight of the spleens isolated from the rats of each experimental group, was significantly diminished in comparison to the control group. EC significantly diminished the extent of BNML cells in the spleen but not in the bone marrow of rats in comparison to the control. The simultaneous treatment of rats (EC+Eto) led to a significant decrease in the number of BNML cells in the spleen of rats compared to Eto or EC applied alone. EC did not affect significantly, the number of leukemic cells diminished by Eto in the bone marrow of BNML rats.
Conclusions: Complementary treatment enhanced the anti-cancer effect of Eto in the spleen, although the bioavailability of EC decreased by about of 50% when it was administered simultaneous with Eto. It can be surmised that the increase in bioavailability of EC during Eto administration can enhance the anti-cancer effect of this polyphenol.

1031 POSTER

Fast-forwarding kinase drug discovery: aurora and EGFR kinase inhibitor lead development

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Background: Drug discovery is a complex, risky, costly and time consuming process, nevertheless essential. Means to improve the efficiency and shorten the time frame required for drug discovery are being sought after continuously by researchers. Here we present our synthetic, screening and design strategies to speed-up drug discovery lead identification process for kinase inhibitors, using Aurora A and EGFR kinase inhibitor development as a prototype.
Material and Methods: Compounds were synthesized using batch reactor and screened on 96-well plates. X-ray co-crystal studies were performed as reported by us previously (J Med Chem, 2009, 52 (4), 1050–1062).
Results: Furano-pyrimidine compound BPR1K224 was identified as an Aurora kinase A inhibitor with an IC_{50} of ~300 nM, by screening in house compound library. Over 350 analogs of the hit were rapidly synthesized in batch reactor and screened for Aurora inhibition without purification to identify 2nd generation lead compounds. Based on the X-ray co-crystal structure of the lead compounds in complex with Aurora A, a potent Aurora A inhibitor BPR1K432 (IC_{50} ~50 nM), which possessed anti-proliferative activity in HCT-116 cell line (IC_{50} ~400 nM) was synthesized. Counter-screening of 350 library compounds for EGFR kinase inhibition, led to the identification of an EGFR kinase inhibitor BPR1K0317 with an IC_{50} ~200 nM. Based on the molecular docking studies of BPR1K0317 in the active site of EGFR kinase protein, acrylamide michael acceptor group was introduced, which resulted in the identification of a potent EGFR kinase inhibitor BPR3K007, which inhibited both wild type (IC_{50} 7 nM) and DM (IC_{50} 22 nM) EGFR kinase and possessed anti-proliferative activity in HCC827 cell line (IC_{50} 8 nM).

Compound	Inhibition, IC_{50} (nM)				
	Aurora A based		EGFR based		
	Kinase	HCT-116	Kinase		HCC827
			WT	DM	
BPR1K224	309	>10000	>10000	–	–
BPR1K432	43	400	>10000	–	–
BPR1K317	>10000	–	223	>10000	518
BPR3K007	~10000	–	7	22	8

WT – Wild type EGFR kinase; DM – Double mutant (L858R/T790M) EGFR kinase.

Conclusion: Rapid synthesis of 350 compounds and screening them without purification, led to the identification of potent Aurora A and EGFR kinase inhibitors with furano-pyrimidine scaffold. Similar synthesis and screening protocols could be applied to diverse scaffolds and could help in identifying inhibitors for other kinase in shorter periods, relative to traditional methods resulting in newer targeted anticancer therapy.

1032 POSTER

Apoptosis-inducing effects of morinda citrifolia and doxorubicin on the Ehrlich ascites tumour in balb-c mice

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Background: Morinda Citrifolia (noni) is a herbal remedy with promising anti-cancer properties. However, its effects on various cancers are to be investigated to make a firm conclusion before implementing it into the clinical practice. Therefore, we investigated the cytotoxic potential of noni on Ehrlich ascites tumor grown in female Balb/c mice and also combined it with a potent anti-cancer agent, doxorubicin.
Materials and Methods: One group received noni only (n = 8), another one doxorubicine (n = 8), and the other one noni+doxorubicine (n = 8) for 14 days after the inoculation of cells. The control group (n = 7) received 0.9% NaCl only.
Results: We found that short and long diameters of the tumor tissues were about 40−50% smaller, compared to those in control group. This anti-growth effect was resulted from the induction of apoptosis, which was proved by the positivity of TUNEL and active caspase-3 cells in tissues and confirmed by caspase-cleaved cytokeratin 18 elevation in serum of the treated groups. In addition, the proliferation was decreased, which is immunohistochemically shown by the PCNA staining.
Conclusion: We conclude that noni may be useful in the treatment of breast cancer either on its own or in combination with doxorubicine, which seems to warrant further clinical studies.

1033 POSTER

Study of adapter protein Ruk/CIN85 subcellular redistribution after stimulation of HeLa cells with activators of PI3K

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Background: Ruk/CIN85 belongs to a family of ubiquitously expressed adaptor molecules. Adaptor protein Ruk/CIN85 is important component of different cellular pathways and directly involved in regulation of multiple cellular functions, including proliferation, adhesion, invasion, and survival.
Material and Methods: Serum-starved human cervical cancer (HeLa) cells were treated with 5α-dihydrotestosterone (DHT), interferon-gamma (IFNγ) and isoproterenol (ISO). Expression of adapter protein Ruk/CIN85 full-length form was studied using Western-blot analysis as well as confocal microscopy.
Results: There are data that stimulation of HeLa cells with DHT lead to the activation of PI3K-dependent signaling. It was established that the content of full-length CIN85/Ruk form in Triton-X-100-soluble fraction of untreated human cervical adenocarcinoma HeLa cells in logarithmic growth phase was very low. Interestingly, stimulation of cells with DHT resulted in up-regulation of the full-length form content. The maximal effect of DHT on p85 content (10 min of stimulation) was observed at 0.01 nM of ligand concentration followed by the decrease of this effect at 30 min. It should be stressed, that 10 min stimulation of HeLa cells with high (100 nM) or intermediate (1 nM) concentration of DHT led to the least up-regulation of p85 content, while the maximal p85 level after 30 min stimulation with DHT was revealed at 0.1 nM ligand concentration. In transfected HeLa cells, a punctate Ruk/CIN85 localization pattern, common for proteins involved in membrane trafficking was revealed using confocal microscopy. In addition, the obtained data demonstrate partial colocalization between Ruk/CIN85 and actin in untreated HeLa cells. After DHT treatment partial redistribution of Ruk/CIN85 signal was observed. The main changes were connected with decrease of Ruk/CIN85-actin colocalization in near membrane area and corresponding accumulation of protein in perinuclear region. The same time- and concentration-dependent changes in Ruk/CIN85 content were characteristic to IFNγ and ISO action.
Conclusions: The obtained results suggest that changes in the expression level as well as subcellular redistribution of Ruk/CIN85 in human cervical adenocarcinoma can lead to loss of consistent control of both apoptosis