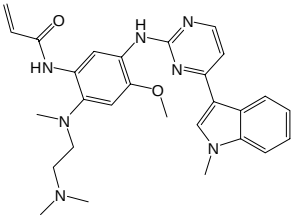
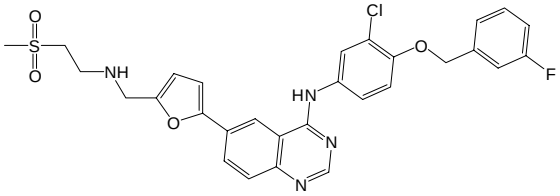
Development of Dual Inhibitors Targeting Epidermal Growth Factor Receptor in Cancer Therapy

Dual activator design is one of the important strategies of drug discovery. Multi target drug discovery is an extension of the same. In this process, a single chemical species which positively influences more than one drug target needs to be identified. For example, dual activators of PPAR and PPAR produce the anti-obesity as well as anti-diabtic effects. In fact the dual activators often provide synergic effect. Pan PPAR agonistic activity also has been reported. Tan et al. reported the development of dual inhibitors. The macromolecular target is the EGFR (Epidermal Growth Factor Receptor) and the therapeutic area is cancer. This target is important in mediating the signaling transduction (cell) and influences the behaviour of the tumors.

Osimertinib is a lead molecule for the treatment of EGFR-mutant non-small-cell lung cancer (NSCLC). Lapatinib is also an approved drug for breast cancer. Designing dual inhibitors for cancer is being done



Osimertinib

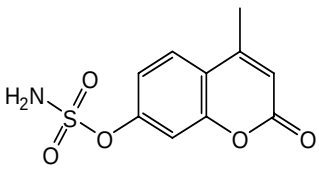


Lapatinib

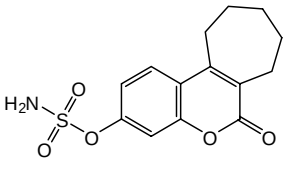
with this pair of targets (i) EGFR/HER2, (ii) EGFR/VEGFR-2, (iii) EGFR/FGFR (iv) EGFR/IGF-1R (v) l EGFR/c-MET (vi) EGFR/ALK (vi) EGFR/NF-?B (vii) EGFR/BRA (viii) EGFR/HDAC (ix) EGFR/Microtubule (x) EGFR/PARP. Osimertinib and lapatinib are among many other drugs which are being tried for dual activity involving EGFR as one of the targets. (J. Med. Chem. 2022, :https://doi.org/10.1021/acs.jmedchem.1c01714)

Triazole based steroid sulfatase (STS) inhibitors for the treatment of breast cancer

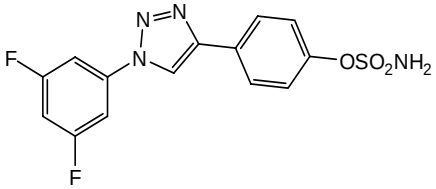
Breast cancer is the most common type of cancer found in females worldwide and occurs due to overexpression of estrogens. Steroid sulfatase (STS) is an enzyme which regulates the metabolism of estrogens. Steroid sulfatase (STS) inhibition can be considered as one of the important strategy for the treatment of hormone dependent breast cancer as it will block the production of estrogen. Coumate is a lead molecule having IC_{50} = 380 nM in placental microsomes. The modification of coumate led to the development of irosustat which also proved to be a potent STS inhibitor having IC_{50} value of 8 nM and this drug reached phase - II clinical trials. The introduction of fluorine in molecules during drug design is an approach for drug discovery people as it makes the molecule a suitable drug candidate. A series of triazole based compounds have been synthesized and the most potent STS inhibitor was found to be 4-(1-(3,5-difluorophenyl)-1H-1,2,3-triazol-4-yl)phenyl sulfamate (Compound D1) with an IC_{50} value of 36.78 nM. The inhibition rate of all synthesized compounds was carried out using radioisotope assay in MCF-7 cells. The structure-activity relationship (SAR) studies suggest that the introduction of fluorine atoms lead to the increase in hydrophobicity which can provide stability to the enzyme-inhibitor complex due to hydrophobic interactions. Molecular modelling studies revealed that the triazole and phenyl rings in the synthesized compounds show van der Waals interactions with Leu103, Leu167, Phe178, Phe182, Phe237, Val486, Phe488, and Phe553. In this work, new triazole based steroid sulfatase (STS) inhibitors were designed and synthesized. (J. Med. Chem. 2022, 65, 6, 5044-5056).



Coumate



Irosustat



Compound D1

Fasting attenuated inflammasome activation: a novel target for the treatment of inflammatory disorders

Inflammasome can be defined as the protein complexes that mediate the activation of cytokines by caspase-1 dependent pathway. It plays a malicious role in initiating pyroptosis and pathogenesis of type-2 diabetes, Muckle-Wills Syndrome, familial cold auto inflammatory syndrome, atherosclerosis, multiple sclerosis, Alzheimer disease, age related functional decline, bone loss, gout and liver fibrosis. An inflammasome consists of a sensor

molecule connected to caspase-1 by an adaptor protein known as ASC (apoptosis-associated speck-like protein containing CARD). ASC has two domains: a pyrin domain and caspase activation and recruitment domain (CARD). Interaction of ASC with inflammasome sensor molecule results into assembly of ASC into dimers. By utilising CARD, ASC brings monomers of procapase-1 to proximity and activates by cleavage, which further activates various proteins such as IL-1 β and IL-18. These proteins are responsible for genesis of inflammation.

Inflammasomes are formed by the proteins of two families: NLR (NOD like receptors) and PYHIN (pyrin and HIN domain-containing protein). NLR proteins are made up of three domains:

a) C- terminal leucine rich repeat (LRR) domain that interacts with putative ligands and essential for autoregulation,

b) Intermediate nucleotide binding and oligomerization domain (NOD, also called NACHT domain) that binds to ribonucleotides and regulates self-olgiomerisation and inflammasome assembly,

c) N-terminal pyrin (PYD), caspase activation and recruitment domain (CARD) responsible for mediating protein-protein interactions and downstream signalling.

Three NLR proteins are known to form inflammasomes. These are: NLRP 1, and 3 (NOD-, LRR- and pyrin domain containing 1, and 3, respectively) as well as NLRPC4 (NOD-, LRR- and CARD domain containing 4). NLRP1 is activated by muramyl dipeptide (MDP), a bacterial peptidoglycan and can also sense toxins of Bacillus anthracis. NLRP4 inflammasome senses flagellin, bacterial flagellar protein. While the NLRP3 inflammasome can be triggered by various agents like microbial stimuli (virus, bacteria, protozoans and fungi), crystalline or aggregated substances (amyloids, cholesterol crystals, asbestos, silica, uric acid), pore forming toxins, excess glucose, extracellular ATP, and necrotic cell components. PYHIN (pyrin and HIN domain-containing) protein also assembles an inflammasome called as AIM2 (absent in melanoma 2), which is responsible for sensing of foreign double stranded DNA. NLRP3 is most studied and its role in various diseases is well characterised. Prolonged fasting is known for reducing inflammation and produces various ketone bodies such as betahydroxybutyrate and acetoacetate in liver to meet energy requirements by serving as alternative source of ATP. Betahydroxybutyrate (BHB) suppresses NLRP3 activation and inhibits IL-1β processing due to activation of Toll like receptor 4. BHB maintains intracellular K^+ levels. As K^+ efflux activates NLRP3, its inhibition has a beneficial effect in inflammation. BHB also prevents ATP induced ASC oligomerisation and speck formation. Above mechanisms plays crucial role in NLRP3 activation. BHB is highly specific for NLRP3 as it does not inhibit any other inflammasomes. Its mechanism of action is independent of chirality of molecule, reactive oxygen species, AMPK, autophagy and glycolytic inhibition.

As BHB has inhibitory effect on NLRP3, it can be utilised as a tool for preventing activation of NLRP3 and can aid in the treatment of type-2 diabetes, Alzheimer disease, liver fibrosis and other inflammatory diseases. So NLRP3 can be a attractive drug target, opening new avenues for application of dietary or pharmacological approaches, which elevates the BHB without starvation for treatment of inflammatory diseases (Curr. Opin. Immunol. 2010, 22, 28-33; Hepatology 2014, 59, 898-910; Nat. Med. 2015, 21, 263-269; Nat. Rev. Immunol. 2013, 13, 397-411).

Peptidomics: Field branching from proteomics

The methods used for measuring cellular molecules such as RNA, proteins, metabolites are termed as 'Omics'. Proteomics attempts to identify all proteins in organisms by two step approach including separation and then sequencing using mass spectrometric methods. The major limitation of this method is that high molecular weight protein are not trapped in gel. Also, the proteins lower than 10 kDa are generally not retained and overlook in proteomics study. Nevertheless, this mass region contains very important group of peptides.

Peptides are known to exert potent biological effects on vital system of an organism (respiratory, cardiovascular, endocrine, inflammatory, and nervous system). Examples includes insulin C-peptide, calcitonin, substance-P, collagen fragments, amyloid and angiotensin II. Predicting peptide function is challenging because the peptides with similar functions might have very different sequence and structure. "Peptidomics" is the field that deals with the comprehensive, qualitative and quantitative analysis of peptides in biological samples. These peptides are either intact small molecules, such as hormones, cytokines, and growth factors, peptides that are released form larger protein precursors during protein processing or may represent degradation products of proteolytic activity.

The human peptidome ($\leq$ 10000) and metabolome are relatively small compared to the transcriptome ($\geq$ 100000) or the proteome ($\geq$ 1000000) therefore

can be used with great advantage for high-throughput screening of proteins in a microarray format. Two approach for peptidomics are (1) Affinity peptidomics: composition of a protein mixture is determined by directly assaying the peptides from crude tryptic digestion using antibody. (2) Combinatorial peptidomics: includes assaying of peptides directly from crude tryptic digestion without using antibody or affinity selection, depletion of peptide pool done through chemical crosslinking of subset of peptides to solid support.

Similarly, as peptides are much more stable and robust than proteins they can be detected by mass spectrometric techniques. In biological fluids, peptides represent protein synthesis, processing, and degradation. Peptidomics methodology enabled us to detect and identify more than 550 endogenous neuropeptides in 1 mg of hypothalamic extracts from rats and mice.

In addition, several novel post-translational modifications of the neuropeptides are being identified. Post-translational modifications (PTM's) are covalent processing events that change the properties of a protein, or peptide, by cleavage or by addition of a modifying group to one or more amino acids. PTMs can also act as protector of the peptide against proteolytic degradation for example, at N-terminus neuropeptides carry a pyroglutamic acid or C-terminal amide-formation. These modifications can be determined using MS by comparison of experimental data to a known amino acid sequence. In this case, the peptide can be sequenced and identified and the mass increment between the obtained sequence and the measured parent mass will be that of the PTM.

Since the amount of peptides in the circulation changes dynamically according to the physiological or pathological state of an individual, it is possible that comprehensive peptide analysis (exploitation of the "peptidome") may lead to discovery of novel biomarkers or to new diagnostic approach. For example,Peptidomics can be used as diagnostic tool for cancer. The novel aspect of these is that the biomarkers are not present in serum at the time of blood collection but they generate after coagulation and complement system gets activated. It is postulated that tumour specific exoproteases generate series of peptides with diagnostic potential. The diagnostic sensitivity of this method for prostate cancer was claimed to be 100%. Similarly, increased in carboxypeptidase N activity in myocardial infarction have also been published.

Conventional food processing releases peptides from food proteins, and these peptides can be examined with peptidomics. For example, in cheese production, casein is often curdled through the use of exogenous enzyme. Peptidomics can be applied to identify which peptides have been released during production. Once the released fragments are identified, they can be also be examined for potential functional activity.

There are variety of issues in peptidomic analysis which are yet to be addressed. It is the need of the hour to search for faster means for facilitating faster isolation, fragmentation, and detection of more peptides in less time, as well as to have more sensitive detectors that will allow the detection of less abundant peptides. Improved software for identification of peptides with complex modifications is necessary. Even if a peptide occurs for only a short time before further degradation, it may still transmit a signal. With peptidomics we can now monitor peptide release across time and physiological or sub-cellular location to reveal their roles in complex biological interaction networks. This information can open new avenues for detection of new classes of biomarkers and aid in faster detection of diseases. (J. Proteome Res. 2006, 5, 2079; J. Chromatogr. B. Analyt. Technol. Biomed. Life Sci. 2005, 815, 11-24; Proteomics 2006, 6, 744-747; J. Proteome Res. 2003, 2, 213-219).

Stem Cells Therapy for the Treatment of Type-1 Diabetes Mellitus

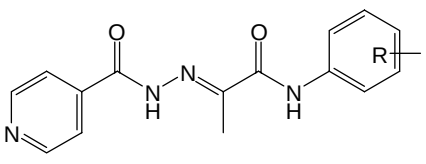
Type-1 diabetes mellitus (T1DM) is chronic autoimmune disorder characterized by chronic hyperglycemia due to permanent destruction of the β-cells and lack of insulin. Hyperglycemia causes life threatening diabetic complications like neuropathy, retinopathy and nephropathy. Exogenous insulin administration is the primary and standard therapy to control the hyperglycemia in T1DM patients. Currently available genetically synthesized exogenous insulin regimens like short-acting and long-acting failed to match the endogenous insulin and not able to completely overcome the diabetic complications. Another gold standard therapy for T1DM is whole pancreas transplantation. Drawbacks of this therapy are limited donor availability, requires lifelong immunosuppressant, cardiac morbidity. Hence, the attractive alternative for definitive cure of T1DM is differentiation of stem-cells into functional β-cells

using stem cells.

Stem-cells are unspecialized cells that are capable of renewing themselves through cell division but can differentiate into tissue- and organ-specific specialized cells under certain physiologic or experimental conditions. Stem cells include embryonic stem cells (ESC), induced pluripotent stem cells (iPS), tissue derived stem cells such as mesenchymal stem cells, hepatic stem cells etc. ESCs are isolated from blastocysts and differentiated into definitive endoderm, which can be further differentiated into insulin producing beta-cells. Current research and clinical studies focusing on permanent cure for T1DM by stem cell based therapy reduced the autoimmune destruction of β cells in T1DM, while tissue-derived stem cells like MSCs reduce the T-cell-mediated immune response against newly formed β-cells. Together, stem cell research and therapy are viable and novel approach for the treatment of the T1DM (Diabet Med 2012: 29, 14-23; Frontiers in Cell and Developm Biol 2014: 2; 9, Nat Rev Endocrinol 2010: 6; 139-148 and QJM 2014: 107; 253-259).

Design and synthesis of 2-(2-isonicotinoylhydrazineylidene) propanamides as InhA inhibitors with high antitubercular activity

Tuberculosis is one of the top infectious killers in the world. Every year 10 million people fall ill with TB and 1.5 million people die from it. About one-quarter of the world's population is estimated to be infected by TB bacteria, according to WHO. In the discovery of novel antituberculosis medications, one of the design strategies is to change old pharmaceuticals or current compounds with proven bioactivity to obtain optimally improved antiTB potency, effectiveness against drug-resistant strains, and short treatment duration. Isoniazid (INH) is a major anti-TB medicine with a multitarget mode of action, although it has been linked to substantial hepatotoxicity and increased drug resistance. Its primary mode of action against Mycobacterium tuberculosis (Mtb.) is the suppression of enoyl-acyl-carrier protein reductase (called InhA), which disrupts the formation of mycolic acids and hence the bacterial cell wall. Anti-TB drugs have been shown to work on InhA. INH is a prodrug that needs the mycobacterial enzyme catalase-peroxidase to make it work (KatG). Most cases of INH resistance are caused by changes in a gene called katG.To improve the pharmaco-toxicological profile of INH, the Author designed new INH analogues linked via a pyruvic acid-based bridge with halogenated anilines to obtain structures with higher antitubercular activity against sensitive strain Mtb and some other drug-resistant strains, such as multidrug-resistant and extensively drug-resistant TB strains (MDR-TB and XDR-TB, respectively), as well as nontuberculous (atypical) mycobacteria (NTM).



(R = F, Cl, Br, I, OCF3, CF3)

2-(2-isonicotinoylhydrazineylidene) propanamides as InhA inhibitors

The compounds demonstrated outstanding antimycobacterial action against MtbH37Rv (2 and 3;MIC≤ 0.03 μM), and their activity against NTM (MIC =2 μM) was considerable and in some cases superior to INH. Eight of the most efficient derivatives were evaluated further against six MDR-TB strains and one XDR-TB strain of that 4-iodo aniline substituted molecule 4 exhibited potent anti-TB activity. (MIC=8μM). As previously stated, the findings suggest a possible cross-resistance to parent INH. The mechanism of action analysis employed two of the most efficient derivatives (three against sensitive strains and four against resistant strains), which demonstrated lower production of all forms of mycolic acids while not affecting the synthesis of shorter fatty acids. The activity of InhA-overproducing strains was likewise decreased, but KatG overproduction increased their antimycobacterial characteristics. The novel series, when combined, suppresses the formation of mycolic acids in Mtb H37Ra cells via InhA inhibition. Antitubercular drugs are among the most common forms of hepatotoxic medications. Therefore, the cytotoxic properties of the HepG2 human hepatocellular carcinoma cell line were examined in vitro (IC_{50}~50 μM). The MTT SI results indicate little toxicity to liver cells, suggesting low cytotoxic action. Therefore, (E)-2-(2-isonicotinoylhydrazineylidene) propanamides may serve as a basis for the development of innovative antitubercular medicines with a much superior pharmacotoxicological profile than the original isoniazid. (Eur. J. Med. Chem., 2021, 223, 113668).