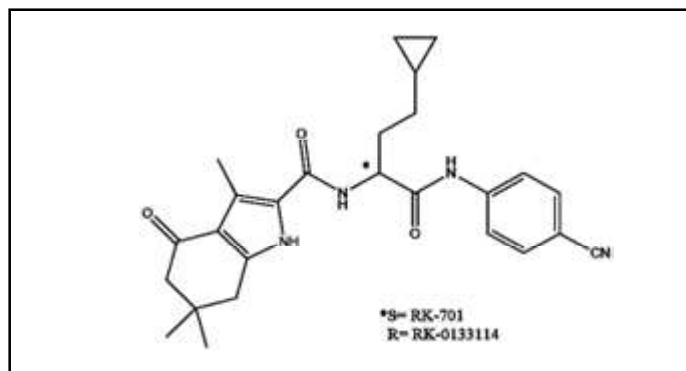


# CRIPS Digest

## A specific G9a inhibitor unveils BGLT3 lncRNA as a universal mediator of chemically induced fetal globin gene expression

Mutations in the  $\beta$ -globin gene results in the heritable illness known as sickle cell disease (SCD). Fetal  $\gamma$ -globin induction is a recognised therapeutic approach. Epigenetic modulators, such as G9a inhibitors, have recently been suggested as potential therapeutics. The molecular processes via which these little molecules revive  $\gamma$ -globin are still unknown. In this research, it is reported that RK701 is a highly selective and non-genotoxic G9a inhibitor.



In this study an enantiomer is also reported along with the molecule RK-701 which is RK-0133114 and it shows 100-fold weaker inhibition as compared to RK-701. The Caco-2 permeability assay and the parallel artificial membrane permeability assay (PAMPA) both showed that RK-701 had reasonable cell permeability. HUDEP-2 cells and primary human CD34+ hematopoietic cells were taken for induction of the fetal globin by treating with RK-701 and the activity was observed as enhanced percentage of cells producing HbF in a concentration-dependent manner and raised the RNA level of  $\gamma$ -globin but not  $\beta$ -globin (observed in case of HUDEP-2 cells).

Treatment with RK-701 causes the expression of foetal globin in mouse and human erythroid cells. It was discovered that BGLT3 long non-coding (lnc) RNA located inside  $\gamma$ -globin gene locus is upregulated selectively by RK-70 for the induction of  $\gamma$ -globin. By preventing the binding of two important  $\gamma$ -globin repressors in conjunction with G9a to the BGLT3 gene locus via CHD4, a member of the NuRD complex, RK-701 specifically upregulates BGLT3. Surprisingly, BGLT3 is required for the induction of  $\gamma$ -globin by inducers such as hydroxyurea, RK-701, and other inducers. The fact that BGLT3 is involved in  $\gamma$ -globin

induction everywhere suggests that treating Sickle Cell Disease (SCD) should be a priority (Nature Communications (2023), 14(1):1-8).

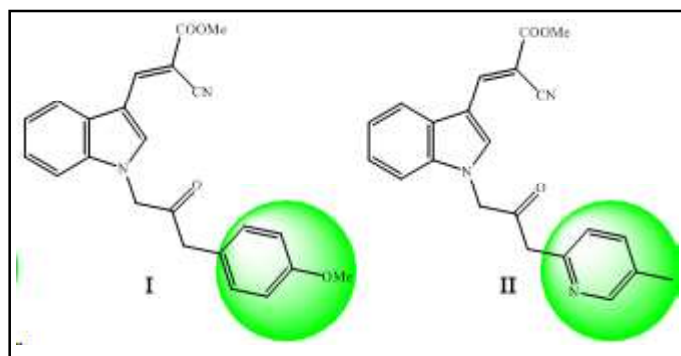
## Indole Derivatives as New Structural Class of Potent and Antiproliferative Inhibitors of Monocarboxylate Transporter 1 (MCT1; SLC16A1)

Cancer is one of the leading causes of death as multiple drug resistance (MDR) is developed by continuous adaptations in cancer cells for their survival; hence, cells keep renovating their metabolic pathway and mechanism of various transporter through mutations thereby most first-line and second-line treatment becomes ineffective. Thus, researchers are inclined more towards a novel target to win the battle against cancerous cells.

Monocarboxylate 1 (MCT1) is a novel drug target significant against cancer cells, inhibition of this target prevents the supply of lactate and pyruvate uptake by anaerobic and aerobic cells which ultimately results in the scarcity of glucose and causes apoptosis. There are limited MCT-1 inhibitors reported based on indole or indole-like scaffolds which include syrosingopine, lonidamine, and many more. Puri et al., synthesized a 16-indole-based active compound which has a significant  $IC_{50}$  value than the previously reported compounds.

All 16 indole-based compound shows inhibition of MCT-1 transporter in the sub-micromolar range, compound I with  $IC_{50}$  value of 81.0 nM was the most active amongst the series and compound II with  $IC_{50}$  value of 82.0 nM second most active as well as compared to the previously reported compound which has  $IC_{50}$  value 87.0 nM.

The antiproliferative activity of all 16 compounds was tested against MCT1 Expressing Cancer Cell



Lines and Cancer Cell viability was checked by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. Also, the Cell Cycle Distribution Analysis, Apoptotic assay Inhibitory Activity against Multidrug Transporters ABCB, ABCC1, and ABCG2 of compound I and it was found that all compounds exhibit cytotoxic activity.

Computational analysis showed the most potent lead compound I very low free binding energy of -9.5 kcal mol<sup>-1</sup> and made hydrogen bonds with Arg313, Ser154, and Lys38, along with one carbon-hydrogen bond with Ser371. Also, it exhibits two electrostatic  $\pi$ -cation interactions with Lys38 and hydrophobic interactions, with Leu66, Pro406, Pro37, and Val282.

The physicochemical properties of all compounds are determined by SwissADME software from which it is concluded that chiefly drug likeliness of all compounds according to Lipinski's rule of five. All compounds are superior to previously discovered compounds and possess the potential to serve as novel anticancer agents which can inhibit multiple cancer cell metabolism pathways. (J. Med. Chem. (2023), 66, 657-676)

## **The role of Pyrazolo[3,4 d]pyridazinone Derivatives as a selective DDR1 inhibitor for inflammatory bowel syndrome therapy**

Discovery of pyrazolo [3, 4-d] pyridazinone derivatives as selective DDR1 inhibitors via deep learning based design, synthesis, and biological evaluation.

Discoidin domain receptor 1 (DDR1) is a crucial member of the class of transmembrane receptor tyrosine kinases (RTKs) which exhibits a crucial role in several cellular processes includes cellular proliferation, migration, proliferation and invasion. The activation of DDR1 is associated with the generation of various kind of cytokines like IL and TNF and generates significant impact upon inflammatory disorders. A few inhibitors of Discoidin domain receptor 1 (DDR1) have been reported but most of those have specific disadvantages like poor target specificity. Therefore, a three-step scaffold-based molecular design process is used to create a highly selective antagonist of the Discoidin domain receptor 1 (DDR1). This three-step process is based upon the matched molecular pairs (MMP) algorithm to create a large library of fragments with different combinations of functional groups. Formation of scaffold-based virtual molecular library was carried out using chEMBL database, kinase activity virtual profiling was carried out by using KinomeX tool and molecular docking screening was carried out through the Glide program within the Schrodinger software.

A new series of new pyrazolo[3,4-d]pyridazinone derivatives were generated using a machine learning based activity score and through the process of virtual screening. Kinase activity virtual profiling was carried out using KinomeX tool. A total of 16 different types of kinases have been generated by this tool. In comparison to other kinases, the produced compounds displayed a greater than expected active probability towards DDR1. Tree maps (TMAPs) were plotted to generate a 2D layout of a minimum spanning tree that clusters molecules according to resemblance. This allowed us to see how structure similarity and docking scores relate to one another. Molecular docking result showed the pyridazine moiety of the lead compound (DC1) forms three hydrogen bonds with the residue Met704 and Asp702 in the hinge region of DDR1 receptor. In the c-Helix and the DFG motif, respectively, Glu672 and Asp784 of the linker amide created two hydrogen bonds. The N-Phenyl substituted portion of the lead molecule reached the solvent exposed area of DDR1. The modified benzofuran ring fills the hydrophobic pocket. The phenyl ring is modified to show prominent interactions with the allosteric site of the DDR1 receptor. On the basis of the docking score top 2 compounds were synthesized and bioactivity experiments were carried out.

With IC<sub>50</sub> values of 1.2 nM and 1.9 nM, respectively, the top 2 compounds showed strong inhibitory action against DDR1. A scaffold-based molecular design workflow was designed for finding potential promising drug candidates for DDR1 inhibition. Through this approach two compounds were discovered that exhibited potent inhibitory activity against DDR1 and inhibits the expression of pro-inflammatory cytokines and DDR1 autophosphorylation in cells. (J. Med. Chem. (2021), 65, 103-119)

## **Discovery, drug action and future aspects of MK-8189, a Potential and Selective inhibitor of PDE10A against Schizophrenia**

MK-8189 is a highly potent selective PDE10A inhibitor which is under phase 2b clinical studies (NCT04624243). It selectively inhibits PDE10A one of the phosphodiesterase enzymes which hydrolyses secondary messengers cAMP and cGMP involved in the neuronal signaling in striatum. Excessive expression of PDE10A results in abnormal striatal output which is strongly associated with schizophrenia pathophysiology. Thus, inhibition of striatal PDE10A can improve neuronal signaling, cure positive symptoms and improve cognition.

Fragment screening identified 4,6-dichloro-2-cyclopropyl-5-methylpyrimidine molecule as potential inhibitor of PDE10A having good efficiency of ligand

binding (LBE=0.57). Structure of PDE10A bound to inhibitor obtained by X-ray crystallography and rational design aided with parallel library synthesis resulted in molecules with PDE10A inhibitory activities at picomolar concentrations but suffered from weak pharmacokinetic profile including poor oral bioavailability, high unbound clearance, poor aqueous solubility, off target ion channel activity against hERG, reversible inhibition of CYP2C9 and CYP3A4 and activation of CYP3A4PXR. Rigorous derivatization and optimization at both the side chains as well as core portion resulted in MK-8189, which has pyrimidine core, 2(5-methylpyridin-2-yl)-cyclopropylmethoxy as side chain 1 and (5-methyl-1,3,4-thiadiazol-2-yl) methyl amino as side chain 2. It has 8 times better PDE10A inhibitory potency, moderately improved solubility at pH 7, reduced CP3A4 PXR activation, CYP2C9 and CYP3A4 inhibition and rat unbound clearance.

X-ray crystallography structure of catalytic domain (residues 439 to 779) of PDE10A in complex with MK-8189 having resolution of 2.1 Å (PDBID: 8DI4) was used to study molecular interactions. MK-8189 interacts via multiple interactions with the crucial residues at the active site of the target enzyme PDE10A. The 5-methylpyridine ring of chain A forms hydrogen bond interaction with the Tyr683 in selectivity domain. The 2-methylpyrimidine core forms  $\pi$ - $\pi$  stacking interactions with benzyl side chain of Phe719, whereas, N1 nitrogen forms hydrogen bond with Gln716 and N3 nitrogen along with 4-amino linker forms water mediated hydrogen bonds

with sidechain residues respectively Ser667 and Tyr514. The pyrimidine core's N3 nitrogen also forms auxiliary interactions with the binding site of PDE10A.

The small size (MW=382) and significant lipid solubility (LogD=2.1) of MK-8189 results in better efficiency of ligand binding (LBE = 0.54) and ligand lipophilicity efficiency (LLE=7.8). The pharmaceutical profile is sufficiently high throughput solubility at pH 7 (167 mM) crystalline free base solubility in simulated intestinal fluid (0.17 mg/mL) and in acidic simulated gastric fluid (5mg/mL) is excellent as well. MK-8189 has significant plasma clearance and smaller volume of distribution leading to a half-life of 4.8 h in rats and 4.2 h in rhesus monkeys. The oral bioavailability in rats was 46% and in monkeys 41%. It showed significant plasma protein binding in rat, monkey as well as human plasma with average unbound fraction in plasma of 8.2% in rat, 8.7% in monkey and 4.0% in human plasma. At the same time, MK-8189 does not potentially inhibit the CYP3A4 and CYP2C9 and in the PXR assay it was not active ( $EC_{50} > 30$  mM). MK-8189 has excellent off target profile. It exhibits excellent profile against ion channels (Iks, Cav1.2 and Nav 1.5 >30 mM and functional hERG Ikr  $IC_{50} = 33$  mM). MK-8189 has high passive permeability (35.4 to 42.6 X 10<sup>-6</sup> cm/s) and is not substrate for human and monkey P-gp (B-A/A-B ratio <2). The physicochemical and pharmacological profile, pharmacokinetics, high selectivity and interaction with PDE10A makes the MK8189 a potential therapeutic for schizophrenia acting as highly selective PDE10A inhibitor (Layton et al. J. Med. Chem. (2023)).

