AN OVERVIEW OF NOVEL KETOHEXOKINASE INHIBITORS WITH STRUCTURE ACTIVITY RELATIONSHIP & ITS' MOLECULAR FRAMEWORK

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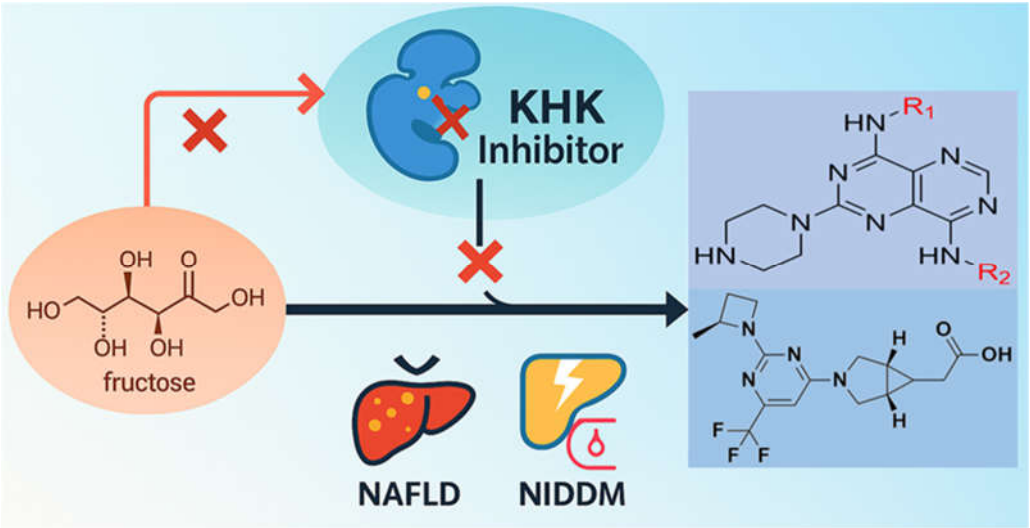
Abstract: An enzyme called ketohexokinase (KHK) is very indispensable for the metabolism of glucose and fructose. Overpowering consumption of fructose can eventually give rise to several poor health outcomes, which includes obesity, low insulin levels, and an increased risk of these two various diseases such as non-alcoholic fatty liver disease (NAFLD) & noninsulin-dependent diabetes mellitus (NIDDM). In recent years, researchers have crafted highly effective and selective inhibitors for KHK using various innovative techniques, leading to the emergence of small-molecule derivatives as promising lead compounds. These studies highlight the importance of heterocyclic cores with substituents, hydrophobic moieties, and hydrogen bond acceptors in enhancing the efficacy and selectivity of KHK inhibitors. Structural modifications through the introduction of amide linkers, alteration of aromatic rings, and fluorine substitution at strategic positions have been found to enhance enzyme binding. Lots of chemical moiety such as indazole ring, pyrimidino-pyrimidine, triazolopyrimidine, pyrrolopyridine etc. were attached with various substituents and response several IC_{50} value. In addition to explaining the scaffolds' design, this study used the structure-activity connection to define a chemical entity that is both effective and extremely hazardous. In order to help professionals build and optimize those innovative KHK inhibitors, we investigate the insights regarding the SARs of ketohexokinase inhibitors in this study.



Graphical Abstract [KHK Inhibitor]

Keywords: Ketohexokinase inhibitor, Fructose metabolism, Structure Activity & Relationship, IC_{50}

INTRODUCTION: The enzyme ketohexokinase (KHK, sometimes called fructokinase) catalyses the transformation of D-fructose from its furanose form to fructose-1-phosphate using adenosine triphosphate (ATP) with a potassium ion (K+).[1] It is a key modulator of hepatic glucose metabolism and starts the intracellular breakdown of many ingested carbohydrates.[2] There are two different 'isoform' such as ketohexokinase-A (KHK-A) & ketohexokinase-C (KHK-C), are encoded by two alternative mRNA transcript variants that express human KHK (hKHK). A significantly lower Km (0.73 mM for KHK-C vs. 28.6 mM for KHK-A), a higher kcat ($3.14 \times 103\ s^{-1}$ for KHK-C vs. $3.04 \times 102\ s^{-1}$ for KHK-A), and a 405-fold higher overall.

Catalytic efficiency ($4.30 \times 106\ M^{-1}\ s^{-1}$ for KHK-C vs $1.06 \times 104\ M^{-1}\ s^{-1}$ for KHK-A) demonstrates the much higher affinity and capacity for fructose phosphorylation.[3] Although KHK-A is widely expressed, KHK-C expression is restricted to areas of the liver, kidney, as well as the intestine the body's three main sites for fructose metabolism.[4]

Because of its perceived sweetness, humectant qualities, and affordability, fructose has emerged as a key component in processed meals. These characteristics have caused the consumption of fructose to rise in tandem with the consumption of foods that have been extensively processed.[5] In the United States, about 10% of calories are now sugar, with 13% of people ingesting more than 25% of their daily calories from added sugar.[5,6] Usually, fructose is added to food in the form of table sugar, Corn syrup containing high level of fructose (generally made-up by 45% of glucose as well as 55% of fructose), or a fructose/glucose disaccharide.[6] Sugar intake has been linked in numerous studies to cardiovascular

disease risk factors,[7-9] overweight and obesity, & NIDDM,[9,6] NAFLD),[10-13] & Non-alcoholic steatohepatitis which is also known as NASH.[14] Although the American Heart Association (AHA) & World Health Organization (WHO) recommend consuming no more than 5% of daily calories from sugar, it is still difficult to put these recommendations into practice globally.[15,16] It is still up for debate whether sugar causes metabolic dysfunction because it contains excess calories that are concealed in processed food items or whether fructose metabolism in sugar is specifically harmful. However, other research indicates that consuming too much fructose can be harmful since it contains too many calories.[17] Recently published calorie-matched studies show that fructose overconsumption is more likely than glucose overconsumption to cause adverse adaptative thermogenesis in individuals, insulin intransigence, including steatosis, hyperlipidaemia, elevated de novo lipogenesis, risk aspects for cardiovascular disease, and adiposity.[18-20] Brief study, it is found that human participant fat content in their livers and DNL rise after nine days of fructose consumption.[21] In a different study, consuming carbohydrates raised the level of plasma triglycerides (TGs), uric acids, along with apolipoprotein C3 (ApoC3) in a dose-responsive manner at various fructose dosage levels.[22] Children who were given short-term fructose restriction showed improvements in insulin sensitivity, hepatic steatosis, ApoC3, and other cardiovascular risk variables, which further supports the idea that fructose consumption causes metabolic disease in people.[21-24] Fructose metabolism begins with the phosphorylation of fructose by ketohexokinase (KHK), which is expressed as two different enzyme isoforms, KHK-A and KHK-C. Fructose metabolism begins with the phosphorylation of fructose by ketohexokinase (KHK), which has been identified as two different enzyme isoforms, KHK-A and KHK-C.[25] Compared to KHK-A, KHK-C has a higher affinity and ability for fructose phosphorylation. KHK-A is widely expressed at low levels, but KHK-C is only expressed in the liver, kidney, and along the intestine—the main sites of fructose metabolism.[26] Since humans with loss-of-function KHK variants and KHK-null mice are unable to metabolize fructose, fructose builds up in plasma and is excreted in urine; KHK is necessary for fructose metabolism.[27-29] Therapeutic targeting of KHK in the aim of minimizing metabolic diseases like NAFLD/NASH, T2D, as well cardiovascular disease is also supported by the fact that mice protected by genetic elimination of KHK or knocking with small interfering RNA are not affected by the detrimental metabolic consequences caused by fructose, such as overweight, insulin-resistant steatosis, high cholesterol levels, and hepatic inflammation.[29-32] *In-vitro* selectivity tests have previously shown that PF-06835919 is a highly selective inhibitor of KHK-A/C, and toxicological investigations in rats and dogs did not reveal any development-limiting effects.[33] In this study, we show that the tiny-molecule KHK-A/C inhibitor PF-PF-06835919 restores the metabolic dysfunction, such as insulin resistance, hepatic steatosis, and hyperlipidaemia, that is brought on by fructose consumption in rats. Crucially, we observed that these metabolic syndrome characteristics can be restored by KHK inhibition and that they additionally occur in rats fed a diet that is comparable in macronutrient composition to the usual American diet (AD), even though they are typically investigated in rodents administered fructose at supraphysiologic amounts.[31-33]

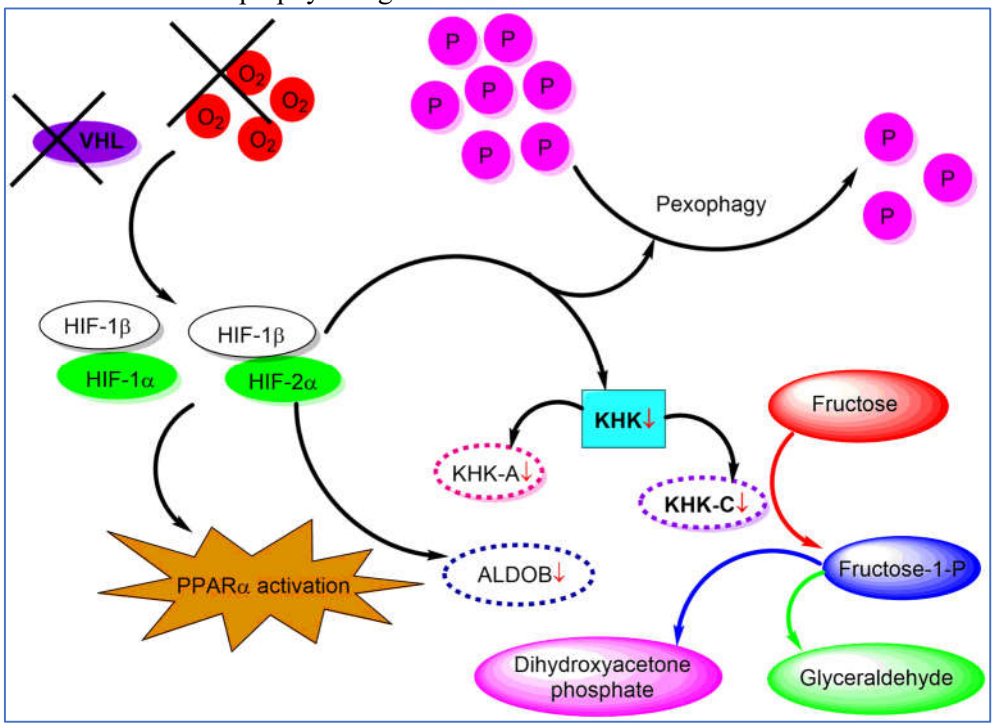


Fig-1: Fructokinase inhibitor workflow [where KHK denotes ketohexokinase enzyme, Fructose-1-P is Fructose-1-phosphate, ALDOB is aldolase-B enzyme, KHK-A/C- Ketohexokinase-A/C]

The prevalence statistics of non-alcoholic fatty liver disorder (NAFLD), PPARα (Peroxisome Proliferator-Activated Receptor Alpha) is a nuclear receptor that controls lipid oxidation and energy levels. When fructose metabolism is impeded, liver metabolism shifts to fatty acid oxidation. This shift activates PPARα, restoring lipid homeostasis and reducing hepatic steatosis. PPARα activation promotes mitochondrial biogenesis and fatty acid β-oxidation.

Another key part of this NAFLD, HIF-1α and HIF-2β (Hypoxia-Inducible Factors) are transcription factors that stabilize under low oxygen or changing metabolic circumstances. Excess fructose metabolism can cause pseudo-hypoxia by depleting ATP and increasing uric acid synthesis, which stabilizes HIF-1α and HIF-2β. These variables subsequently activate glycolysis and inflammatory pathways, which contribute to metabolic stress and liver damage by preventing fructose metabolism. Inhibiting fructose metabolism with ketohexose inhibitors can improve PPARα activity and lower maladaptive HIF-1α and HIF-2β responses, potentially leading to therapeutic benefits for metabolic disorders.[34]

The high incidence of nonalcoholic steatohepatitis (NASH) and non-alcoholic fatty liver disease (NAFLD) in the general population is estimated to be between 3% and 5% and 10% to 30%, respectively.[34] Obese and overweight people have far higher prevalence rates, which range from 10% to 50% and 50% to 90%, respectively. About 60% to 70% of those people with type 2 diabetes mellitus (T2DM) additionally have an overabundance of non-alcoholic fatty liver disease (NAFLD).[35,36] While treatment for NAFLD must focus on those who are most at risk of long-term problems, we must take into account the spectrum of NAFLD in light of its extremely high prevalence.[36]

NAFLD is an ongoing medical condition marked by steatosis and an excessive buildup of triglycerides in the liver. A new term, metabolic-associated fatty liver disease, has been created as a result of the identification of NAFLD as a metabolic condition that commonly co-occurs with obesity, metabolic syndrome, and type 2 diabetes.[37] Patients may develop NASH, which can lead to fibrosis of the liver, cirrhosis, and hepatocellular cancer if there is additional inflammation and liver damage. Steatosis can lead directly to hepatic fibrosis or NASH.[38] The disease's development process varies; in an investigation of 108 NAFLD patients, 42% showed fibrosis progression and 18% showed fibrosis regression within 6.6 years.[39] The degree of fibrosis represents an indicator of both mortality and the eventual need for liver transplantation.[40] The most recent meta-analysis found that among individuals with NAFLD, biopsy report confirmed fibrosis was linked to both overall mortality and liver-related morbidity during the course of long-term follow-up.[41] There are four subtypes of fibrosis: cirrhosis (F4), advanced fibrosis (F3), substantial fibrosis (F2), and early fibrosis (F0/1).[42] Thus, pharmacologic treatment is primarily limited to patients with fibrosis or NASH. No particular therapies have yet been endorsed by regulatory bodies, despite improvements in our knowledge of pathogenic pathways and the discovery that liver fibrosis is the best indicator of the course of the disease. Treatment options other than randomized controlled trials are mostly restricted to lifestyle changes meant to encourage physical activity and weight loss.

The aggressive tumor known as pancreatic ductal adenocarcinoma (PDAC) has one of the worst prognoses because it is frequently discovered at an advanced stage, and because it doesn't show any symptoms. By 2030, it is expected to have the shortest survival rate after five years of all cancers and rank as the second most common cause of cancer-related deaths.[43,44] About 80% of patients exhibit oncogenic KRAS mutations during the earliest stages of tumor growth, and between 90% and 50% of patients suffer from loss-of-function mutations in the tumor inhibitors TP53, SMAD4, and CDKN2A, which results in PDAC.[45] During carcinogenesis, PDAC tumors exhibit a distinct metabolic phenotype and develop metabolic plasticity to increase cellular fitness and give cancer cells a selective advantage.[46,47] To enable the synthesis of tumor biomass, intrinsic triggering of KRAS is essential for metabolic reprogramming, specifically the glycolytic switch.[48] Therefore, identifying how metabolism is altered in pancreatic cancer could help develop novel interventional treatment approaches. Through increased glycolysis, carbohydrates provide energy for the synthesis of building blocks that support the unchecked growth of cancer cells.[49,50] Over the past forty years, dietary sugar intake has skyrocketed in the Western world, coinciding with a rise in the prevalence of obesity, cardiovascular disease, diabetes, and cancer, suggesting a potential causative link.[51-53]

Nevertheless, it is uncertain how particular carbs contribute to various illness conditions. Significant amounts of both as well as glucose are found in foods like sucrose, which has a glucose content of 50% and 50% fructose, as well as high fructose syrup made from corn (HFCS), which contains 55% fructose as well as 42% glucose. Different tissues can use glucose directly as an energy source. When glucose levels are too high, it can also be stored within the liver as glycogen or transformed into fructose via the polyol pathway.[54-56] High doses of fructose (R1g/kg) can feel overwhelmed gastrointestinal fructose absorption as well as clearance, causing fructose to reach both the liver and the colon, where it can impact gut microbiota and impair intestinal barrier function. In contrast, low doses of fructose are mainly eliminated by the liver (90%) as well.[57,58] Consequently, this may result in endotoxemia, hepatic inflammation, and the stimulation of *De-novo* lipogenesis.[59] The primary distinction between fructose and glucose metabolism is that fructose breakdown to triosephosphates is unconstrained, whereas glycolysis is strictly controlled by the cell's energy condition at the level of phosphofructokinase (PFK).[60] The effective conversion of fructose into fat is facilitated by the absence of inhibitory feedback systems. Additionally, unrestricted KHK-driven fructose phosphorylation can promote high glycolytic flow by limiting ATP levels and ATP-mediated negative feedback regulation of PFK.[61] The rate-limiting initial enzyme of fructose metabolism, ketohexokinase (KHK), transforms fructose into fructose-1-phosphate (F1P), and it is subsequently broken down by aldolase B to initiate glycolysis.[62] KHK comes in two forms: low-affinity KHK-C and high-affinity KHK-A. KHK-A isoforms are produced via the mutually exclusive transcription of the KHK gene's exons, which are 3C and 3A, respectively.[63] The APOBEC1 complementation factor (A1CF) regulates alternative gene splicing of KHK-C in the liver.[60,61] whereas in dedifferentiated hepatic carcinoma (HCC)

cells, heterogeneous nuclear ribonucleoprotein H1/2 (hnRNPH1/2) drives ac-myc-driven transition of KHKC to the KHKA isoform.[55-62]

According to recent research, consuming fructose has been connected to a variety of malignancies. In human colorectal, breast, lung, and glioma cancers, as well as pancreatic cancer, increased fructose absorption via GLUT5 overexpression reinforces glycolysis, promoting cell survival and proliferation.[54,64] Irrespective of being overweight or metabolic syndrome, a daily modest dosage of high-fructose corn syrup accelerated the formation of intestinal cancerous tumors in mice with *adenomatous polyposis coli* (APC) mutations.[65] Similarly, fructose absorption boosted tumor growth *in-vivo* and cancer proliferation and migration of cells *in-vitro* in both prostate cancer and acute myeloid leukemia.[66-68] Loss of KHK function is linked to neoplastic disease since most malignancies have been found to have decreased KHK enzymatic activity or transcript levels. Even so, there is a shortage of research examining the cell-autonomous effects of various KHK-isoforms upon cancer cells regarding fructose metabolism.

Structural activity relationship of ketohexokinase inhibitors: -

In recent years, researchers have crafted highly effective and selective inhibitors for ketohexokinase (KHK) using various innovative techniques, leading to the emergence of small-molecule derivatives as promising lead compounds. Thanks to the synthesis and screening of fructokinase inhibitors, we've been able to dive deep into understanding how structure relates to activity, all made possible through innovative, structure-based optimization strategies.

To improve binding affinity and selectivity, various research teams have been exploring KHK inhibitors by tweaking the core structure, adding polar and hydrophobic groups, and fine-tuning hydrogen-bonding interactions. With the help of in vitro enzyme assays, a 1-member panel of ketohexokinase inhibitors (1a–1r) was synthesized, and their inhibitory profile was determined. Systematic alteration of electronic and steric properties of critical functions gave insights into molecular parameters necessary to bring about potent KHK inhibition (Table 1), as reported by **Zhang et al., 2011**.

These studies highlight the importance of heterocyclic cores with substituents, hydrophobic moieties, and hydrogen bond acceptors in enhancing the efficacy and selectivity of KHK inhibitors. Structural modifications through the introduction of amide linkers, alteration of aromatic rings, and fluorine substitution at strategic positions have been found to enhance enzyme binding and inhibition. Furthermore, polar functional groups of hydroxyl and carboxyl also ensure increased interaction with active site residues and stabilization of the inhibitor-enzyme complex. Potentially of use in the treatment of such diseases as diabetes and metabolic syndrome, the developments open up a pathway towards creating new treatments with the target for fructose metabolic abnormalities.

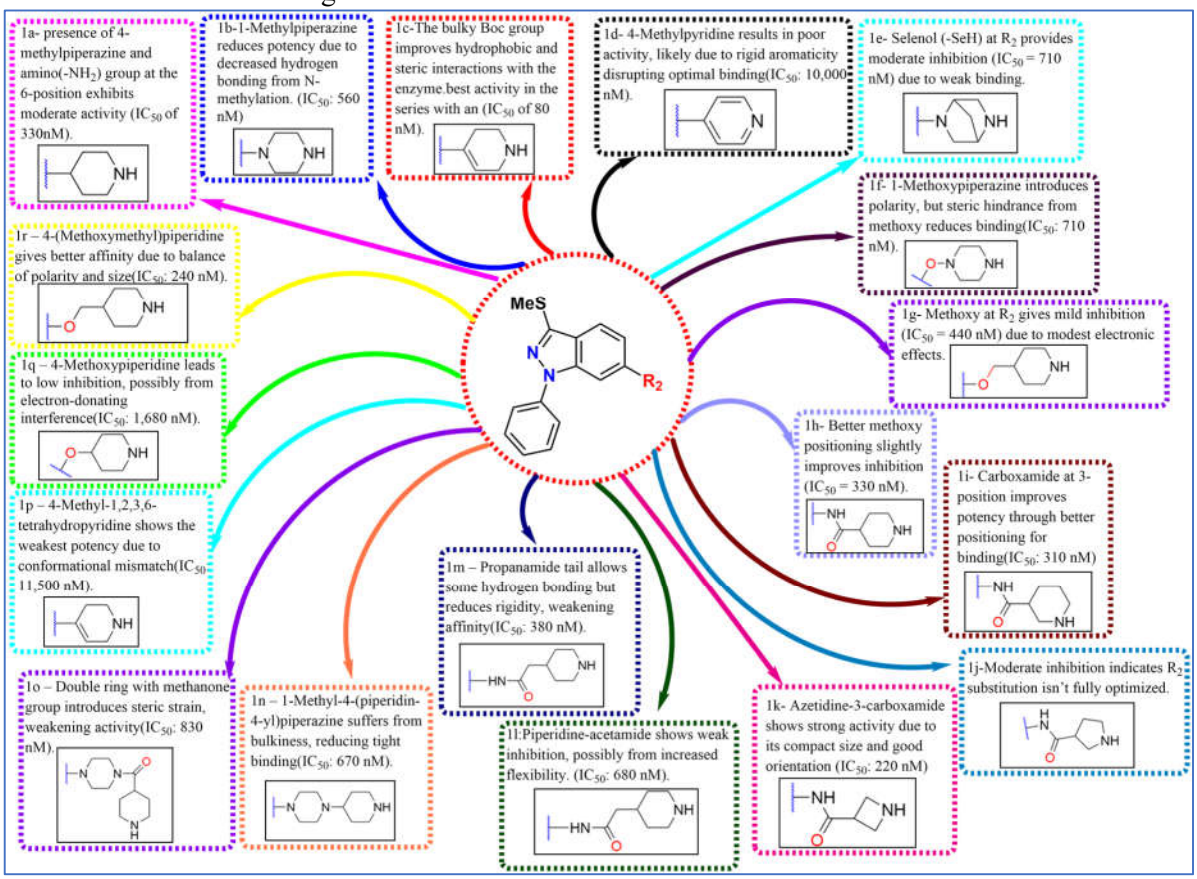


Fig-2: Structure Activity Relationship of several substituent based indazole derivative

Due to the presence of 4-methylpiperazine (**Fig-2**), the **1a** compound has its IC_{50} value of 330 nM. At this position, it suggests that the specific electronic and steric environment also allows a moderate level of interaction with the active site of the enzyme. This substitution is essential for determining activity since [hydrophobic/hydrophilic] interactions likely influence the binding affinity. 1a, this compound has an amino (-NH_2) functional group at the 6-position (R_2), which is significant in inhibiting the enzyme. The amine functionality can form stable hydrogen bonds

with the active site residues of the KHK enzyme, which stabilizes the inhibitor-enzyme pair. The low IC_{50} value of 330nM shows that hydrogen bonding plays a vital role in molecule recognition and binding efficacy. **1b**, when amines are methylated to form N-methyl (-NHMe), inhibition is reduced to some extent (IC_{50} rises from 330 to 560 nM). This rise suggests that, although the NH group is still engaged in hydrogen bonding, the methyl group adds steric hindrance, reducing optimal enzyme binding. Additionally, the increased hydrophobicity of -NHMe might alter water solubility, affecting bioavailability. **1c**, this derivative contains an amine (-NHBoc) protected with a bulky tert-butoxycarbonyl (Boc) group, which maximizes inhibition and possesses the lowest IC_{50} value of any compound tested (80 nM). The large tert-butoxycarbonyl (Boc) group may enhance binding interactions by filling hydrophobic cavities in the active site of the enzyme and, by its bulk, stabilize the entire enzyme-inhibitor complex through hydrophobic and steric interactions. The significant enhancement over **1a** and **1b** suggests that larger, hydrophobic protecting groups enhance activity. Hydrogenation and Boc removal greatly decrease inhibitory activity, with an IC_{50} value >10 µM. Elimination of steric bulk and hydrophobic interactions is expected to reduce binding to the enzyme, highlighting the significance of structural rigidity in effective inhibition. The compound 1e, which contains a selenol (-SeH) moiety at the R_2 position, produces moderate inhibition (IC_{50} = 710 nM). Selenium compounds often possess unique binding characteristics based on selenium's polarizability and capacity to form chalcogen bonds. Nonetheless, in comparison with amine derivatives, selenium seems less effective in the establishment of stable hydrogen bonds, decreasing binding affinity. This compound, **1f**, is also like **1e**, and this compound has moderate inhibition, and the SeH group does not significantly enhance binding. The fact that there is no improvement over the 1e compound suggests that further modifications are necessary to enhance selenium's role in molecular recognition. In a 1g compound, Methoxy ($-OCH_3$) replacement at the R_2 position gives mild inhibition. The electron-donating characteristics of methoxy may influence enzyme interactions through enhanced electron density on the molecule. The IC_{50} value of 440 nM indicates that methoxy inhibits, though not as efficiently as NH or NHBoc groups. This molecule (1h) maintains the methoxy ($-OCH_3$) group, but is more inhibited than 1g, with an IC_{50} of 330 nM. This suggests that the orientation or position of the methoxy group affects enzyme binding, perhaps optimizing fit within the active region of the enzyme. The best of the methoxy-substituted derivatives, **1i**, has an IC_{50} of 310 nM and is among the most potent inhibitors in this series. The greater relative potency compared to **1g** and **1h** suggests that positional factors and electronic distribution affect enzyme interactions. The IC_{50} of compound **1j** is consistent with moderate inhibition. The R_2 group is not high-potency, as with 1c, but it could possess steric and electrical effects that affect enzyme binding.

It could be beneficial to enhance hydrogen bond acceptor/donor activity at this position. Its IC_{50} of **1k** reveals the minimum level of inhibition of the enzyme of **1k's** R_2 mutation. The efficiency of this site may be enhanced through a modification of its electrical context to encourage constructive interactions within the active site of the enzyme. The quite modest IC_{50} of compound **1l** indicates that it positively contributes to enzyme binding, even though its R_2 substitution might not be in its optimal conformation for maximum efficacy. One may incorporate groups capable of forming hydrogen bonds or electron withdrawal to further enhance their inhibitory activity. The R_2 group is communicating with the enzyme to some extent, by the **1m** IC_{50} value, but it does not contain the structural features necessary for strong binding. Activity could be enhanced by adapting electronic distribution and the steric barrier in this position. Compound **1n** highlights how R_2 modification specifies biological activity, with an IC_{50} value indicating strong inhibition. To enhance enzyme affinity, the steric and electronic properties of this substituent may be optimized. The level of inhibition of compound **1o** reveals that while its R_2 substitution is responsible for enzyme interaction, it is not the optimal candidate for binding. Potential adjustments to SAR may consider hydrogen bonding interactions, electrical effects, or hydrophobicity. Its R_2 subfamily is a **1p** IC_{50} ketohexokinase inhibitor, but structural modification could increase binding contacts for improved efficacy. Eighth, with a moderate inhibitory IC_{50}, highlights the significance of R_2 substitution on the enzyme interaction. Potency at this site may be increased by optimizing the steric and electronic properties. Though **1r** has a moderate inhibition, the R_2 group of **1r** influences binding to the enzyme, but not to a significant extent. The optimization of substituent properties at this position would lead to enhanced biological activity.

To compare the effect of R_3 substitutions, the unsubstituted molecule **1c**, with an IC_{50} of 80 nM, can be employed as a control. The fact that there is no second functional group at this position indicates that the core structure by itself accounts for a large amount of inhibition. Compound **2a**. It is more active to replace a fluorine atom at position three (IC_{50} = 42 nM). This enhanced activity may be due to fluorine's high electron-withdrawing ability, which may increase the dipole moment or stabilize hydrogen bonding interactions with the enzyme. In compound **2b**, shifting fluorine to the 4-position (IC_{50} = 23 nM) also enhances the inhibitory effect. Enhanced activity may result from better binding to the active site of the enzyme, maximizing steric accommodation and electrostatic interaction. In Compound **2c**, an IC_{50} value of 34 nM is achieved by replacing a hydrogen atom at the 3-position with a methyl group. The steric interactions and electronic environment of the compound are slightly affected by the electron-donating methyl group. The moderate activity indicates that fluorine is better than methyl substitution at this position, although methyl substitution could enhance affinity. In Compound **2d**, Activity is reduced to some extent from the 3-position when the methyl group occupies the 4-position (IC_{50} = 40 nM).

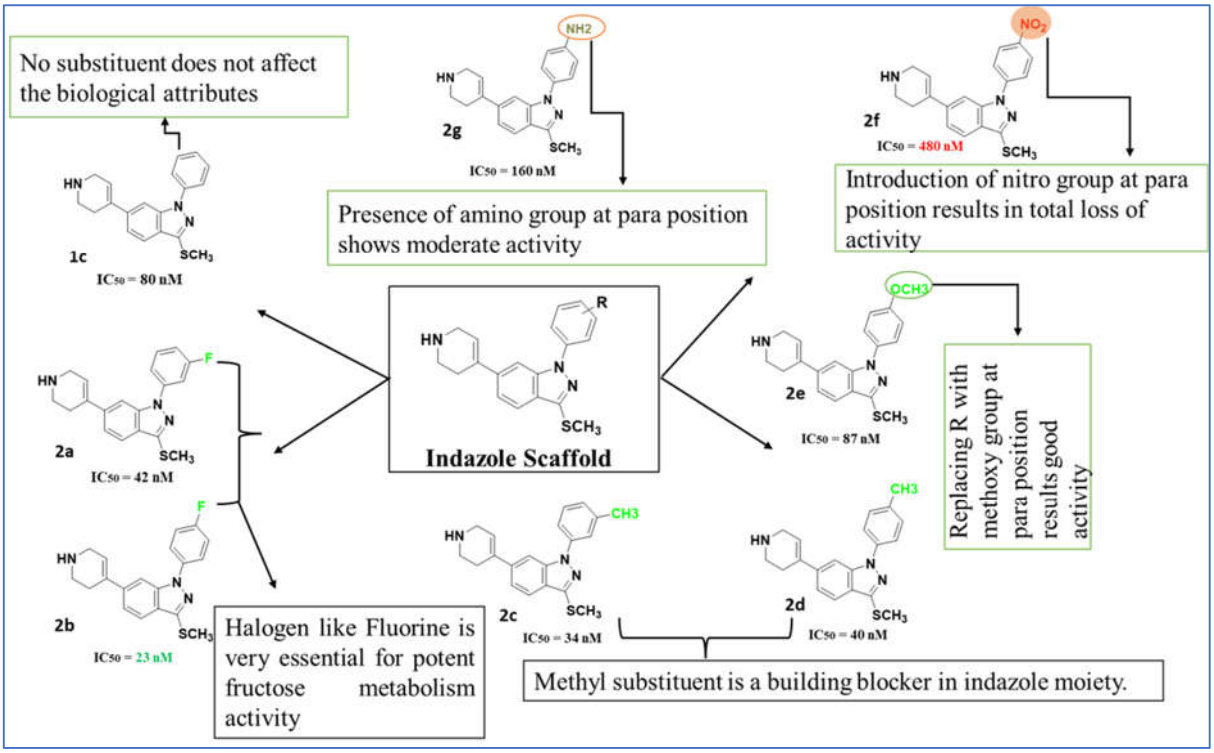
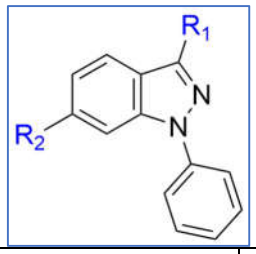
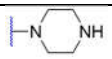
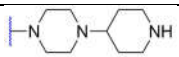
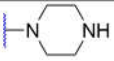
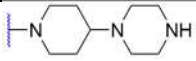
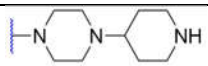


Fig-3: SAR studies focusing on substitutions at the R position of the indazole core.

Changes in electron distribution within the molecule or steric interference with the best enzyme binding may be the cause of this. Substitution of the 4-position of the R_3 group largely changes the inhibitory activity of the compounds. Due to the steric hindrance or disruption of beneficial hydrophobic contacts induced by the electronegative oxygen within the enzyme pocket, compound **2e** with methoxy (-OMe) at position 4 shows lower activity (IC_{50} = 87 nM) than the parent molecule. Having a nitro (-NO_2) functionality in the same position, compound **2f** is very much weaker in activity (IC_{50} = 480 nM). This is presumably because of the high electron-withdrawing ability of the nitro group, which could interfere with important enzyme interactions or cause steric clashes to interfere with binding. In comparison, compound **2g** with the amine (-NH_2) modification has a moderate loss in activity (IC_{50} = 160 nM); while the amine group does have the potential to engage hydrogen bonds, their influence on the electronic nature of the compound as well as patterning in bulk may not serve the optimal interaction with the enzyme.



ID	R_1	R_2	R_2 substituent name	KHZ enzyme IC_{50}(nM)
1b	thiomethyl		1-methylpiperazine	560
1n	thiomethyl		1-[4-(4-aminopiperidin-1-yl) piperidin-1-yl]	670
3a	Methyl		1-methylpiperazine	1280
3b	Methyl		1-(1-methylpiperidin-4-yl) piperazine	3200
4a	Methoxy		1-[4-(4-aminopiperidin-1-yl) piperidin-1-yl]	1380

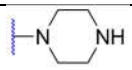
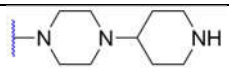
5a	Ethyl		1-methylpiperazine	570
5b	Ethyl		1-[4-(4-aminopiperidin-1-yl) piperidin-1-yl]	560

Table 1: The structure-activity relationship (SAR) of the R1 and R2 positions in indazole derivatives.

In this series of analogues prepared from the parent indazole template, a detailed investigation on the effects of the R^1 position at the pyrazole ring and R^2 position at the aryl ring on inhibition of the KHK enzyme was explored. Compound 1b with the thiomethyl (SMe) group possessed potent inhibitory activity with an IC_{50} value of 560 nM. This property emphasizes the beneficial role of the SMe (thiomethyl) substituent, which can enhance lipophilicity and possibly form beneficial hydrophobic contacts in the active site of the target protein. The pyrazole ring is also able to form favorable hydrogen bonding or π-π stacking interactions, stabilizing binding. With an IC_{50} of 670 nM after the heterocyclic core modification, compound 1n, which maintains the SMe group but contains a triazole ring as the heterocyclic core instead of a pyrazole ring, showed a comparatively decreased activity. The triazole ring will not bind as strongly as pyrazole because of ring electronics or structural reasons that change hydrogen bonding or binding orientation within the enzyme active site, even though the SMe group will remain catalytically active, based on this minor loss of activity. Compound **3a**, in which the R-substituent is a methyl group and the heterocycle is a pyrazole ring, had an IC_{50} of 1280 nM. The methyl group does not seem to contribute as much to binding because of the absence of polar or electron-donating properties for effective binding. Compound **3b** also demonstrates the importance of the heterocycle at the centre for its activity by replacing the pyrazole ring with the triazole ring. This conformational adjustment led to IC_{50} increasing dramatically to 3200 nM, which resulted in a significant decrease in binding capacity. Thus, the heterocyclic ring and R-substituent features each have a regulatory role in providing an inhibitory effect. The IC_{50} value of compound **4a**, which contained a triazole ring and a methoxy group, was 1380 nM. Even though the methoxy group is an electron donor, it could not offset the reduced activity of the triazole core, which implies that the triazole ring is not an alternative for the pyrazole ring's binding property in the binding pocket. Conversely, compound **5a**, with an IC_{50} of 570 nM and an ethyl group and a pyrazole ring, was potent. Compound 5b with the same ethyl group but a triazole ring showed a little more potency (IC_{50} of 500 nM), which suggests that the triazole core can be applied in certain situations. This highlights the importance of structural and electronic factors in modulating biological activity.

On R_2 substituents of the indazole core emphasize bulkiness, hydrogen bond ability, and electronic character as KHK enzyme inhibitors. Those indazole moieties IC_{50} value is given below and discuss about their SARs (**Fig-4**).

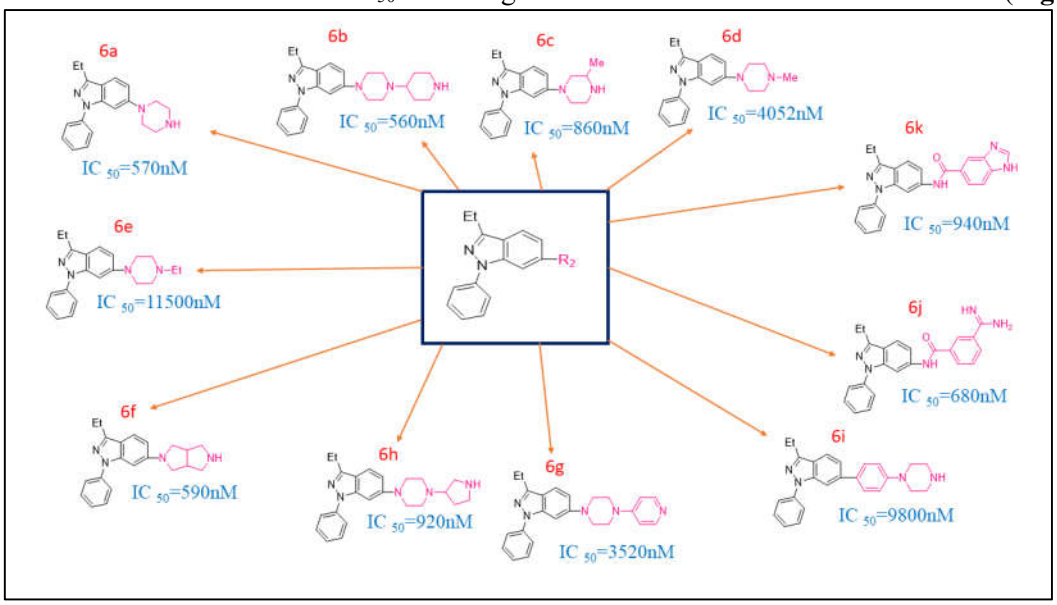


Fig: 4 – Several substituents have varying IC_{50} values of the same indazole scaffold.

At IC_{50} 570 nM, compound **6a** with the 1H-pyrazole ring at R_2 was very active and well-interacting owing to the theoretically ideal planarity and hydrogen bond ability of the pyrazole heterocycle. Surprisingly, **6b** exhibits similar activity (IC_{50} = 560 nM) to the pyrazole, which was replaced with a 1H-1,2,3-triazole. Due to its similar nitrogen positioning and ability to form hydrogen bonds with active site residues, a triazole ring could reproduce a pyrazole's binding interactions under certain conditions. Conversely, methylation of the triazole nitrogen atom of **6c** resulted in reduced activity (IC_{50} = 860 nM), probably due to steric blocking or changed electronic orientation. Substantial contact of the enzyme-binding site is averted with **6d** because the N-methyltriazole group occupies the position of the NH hydrogen-bond-donating group, so further activity reduction (IC_{50} = 4520 nM) occurs. In **6e**, where an N-ethyltriazole had a much lower IC_{50} of 11,500 nM, the effect was more significant. This indicates that bulkier alkyl groups at this position are harmful to activity, possibly because they introduce steric clashes or conformational penalties. An alternate

connectivity of a pyrazole ring in compound **6f**, the regioisomer of compound **6a**, still revealed a decent inhibitory activity (IC_{50} = 590 nM) and tolerance toward ring modifications. Compound **6g**, the isomer of **6b** involving a triazole, due to its reduced activity (IC_{50} = 920 nM), confirmed that the geometry of nitrogens within the triazole ring dramatically influences the binding interaction. A significant loss of activity in **6h**, which bears a tetrazole ring (IC_{50} = 3520 nM), is observed. It may be a result of either too much polarity or poor spatial positioning for contact with the target. A further indication that there are specific structures of triazole that are highly tolerated is the compound **6i**, which exerted a weak inhibitory effect (IC_{50} = 9800 nM) owing to its unique triazole isomer. One was amazed to discover that amino-substituted heterocycles **6j** and **6k** possess substantial potency (IC_{50} = 680 nM and 900 nM, respectively). Despite the absence of optimum aromatic or planar heterocycles, these findings imply that polar hydrogen-bonding functionalities can, in part, reestablish binding affinity.

We also analysed the SAR of some of the KHK inhibitors from **Futatsugi et al.** (2020) and discovered that PF-06835919 (**Compound 14**) is an extremely potent clinical candidate with better pharmacokinetics and optimized binding activity.

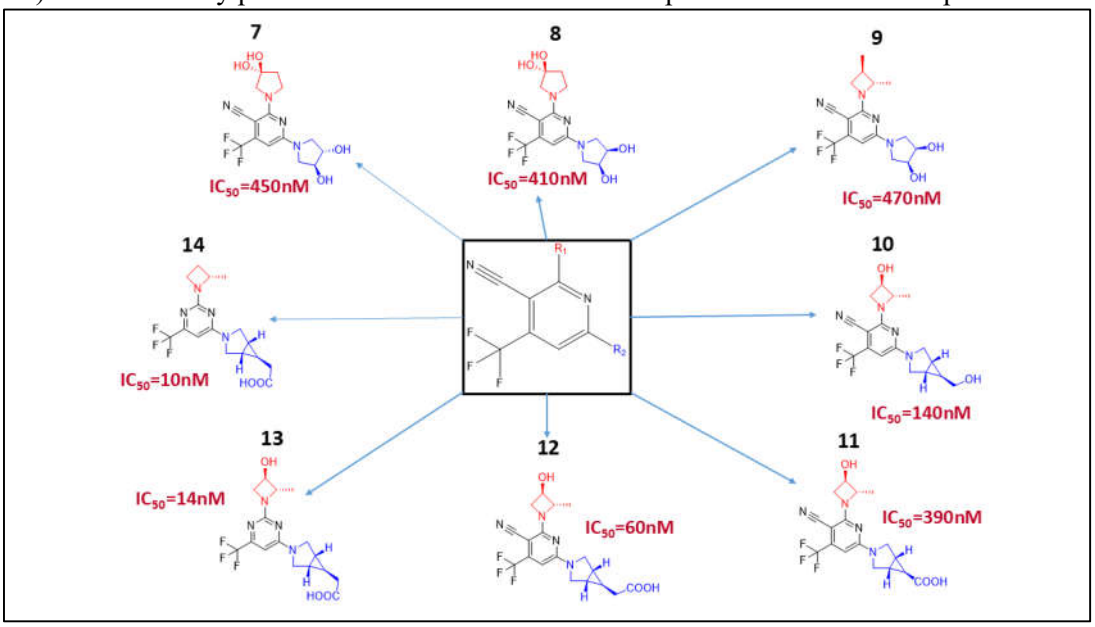


Fig-5: 2,6-dimethyl-4-(trifluoromethyl) nicotinonitrile derivatives show good binding activity. Moreover, **Compound 14** trifluoromethyl pyrimidine analogue is a most optimised compound.

To enhance pharmacokinetic properties and potency, the SAR of KHK inhibitors started from a fragment-derived lead and introduced some rational modifications. The goal was to maximize interactions within the KHK ATP-binding pocket, that is, through beneficial hydrophobic contacts and the adjustment of polar residues. The first lead **Compound, 7**, was quite an active inhibitor derived from pyrrolidine (IC_{50} = 450 nM). The trifluoromethyl and pyrrolidine diol activity of KHK characterized important contacts in its ATP-binding region. It was metabolically stable and permeable, but its activity had to be optimized. **Compound 8** substituted the trans-diol pyrrolidine with an achiral *cis*-analogue with comparable potency (IC_{50} = 410 nM) and binding geometry, simplifying production without loss of activity. **Compound 9,** with a 2-methylazetidin-3-ol replacing the pyrrolidine ring, came from additional structural development. With this new vector, the 3-azabicyclo[3.1.0]hexane unit of **Compound 10** enhanced its spatial contact with Arg-108 by water-mediated hydrogen bonding, resulting in a significant enhancement of potency (IC_{50} = 140 nM). A carboxylic acid was appended for additional optimization to engage ionically with Arg-108. This modification was in **Compound 11**, but the activity only slightly improved (IC_{50} = 390 nM), indicating that the spacer between the acid moiety and core was not optimal. This led to a staggering rise in potency (IC_{50} = 6 nM), which became the series' defining enhancement. Although of very high potency, molecule 6's hydroxyl group left it susceptible to glucuronidation. **Compound 13** was designed to decrease this metabolic liability by substituting the cyanopyridine ring with a less lipophilic pyrimidine core. This resulted in improved stability without sacrificing good activity (IC_{50} = 14 nM), although slightly lower than that of **Compound 12**. **Compound 14** (PF-06835919), the result of final optimization, had the hydroxyl group eliminated to get rid of the glucuronidation hotspot. **Compound 8** had a good potency (IC_{50} = 10 nM), enhanced permeability (Papp = 25 × 10^6 cm/s), and showed outstanding pharmacokinetic and safety profiles. Following that, fructose was finally selected as the clinical candidate for further investigations, **Phase 2** clinical trials are ongoing nowadays to treat metabolic disorders associated with excessive fructose intake.

KHK's ATP binding domain was studied by **B.E. Maryanoff** and others about ketohexokinase inhibitors and the interactions with the 27B aspartate residue. They focused on the possible interactions and binding with all the pyrimidine-pyrimidine inhibitors of ketohexokinase relating to the R_3 substituent 2 position. These are inhibitors of ketohexokinase (KHK), an enzyme that plays a major role in fructose metabolism. KHK inhibition may offer treatment for fructose-induced conditions such as obesity and associated metabolic disorders. Each compound has a leading pyrimidino-pyrimidine core, and scientists varied one region of the molecule, referred to as R_3, in Portz and McKenzie

to determine how these changes affect inhibition of the enzyme. R_3 is a group attached to a nitrogen atom in the piperazine ring. This group reaches into the enzyme's binding pocket where it can interact with a particular amino acid, Aspartate-27B (Asp-27B). Meanwhile, the pyrimidino-pyrimidine was identified as a lead compound that possesses inhibitory activity.

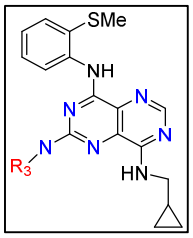


Fig: 6 - Structures and KHK inhibition results for pyrimidino-pyrimidine

In **Compound 15**, the original compound (just piperazine) has an IC_{50} of 12 nM, which is already pretty good. It slots snugly into the enzyme's pocket and forms good hydrogen bonds with Asp-27 B. By using spirocyclic piperazine-mimetic, in **Compound 16**, provided great KHK inhibition (IC_{50} = 8 nM). In the 4-7 compound, which was meant to test the C-methylation of piperazine ring effect, the distal nitrogen binds to ASP-27 B. In **Compound 17**, Potency was reduced sixfold when a single methyl group was placed next to the interacting piperazine nitrogen (IC_{50}=70nM). A single remote methyl group in **Compound 18** loss potency due to fivefold (IC_{50}= 58nM). The two methyl groups located on opposite sides of the interacting piperazine nitrogen brought about a nine-fold reduction in efficacy (**Compound 19**) [IC_{50}=110nM]. Only a twofold decrease in potency was caused by gem dimethyl groups (**Compound 20**) [IC_{50}=40nM]. The compound's effectiveness began to decline with an increasing distance between the pyrimidinopyrimide scaffold and the nitrogen of the Asp-27B interaction. The same was true with compound 21, [IC_{50}= 66nM].

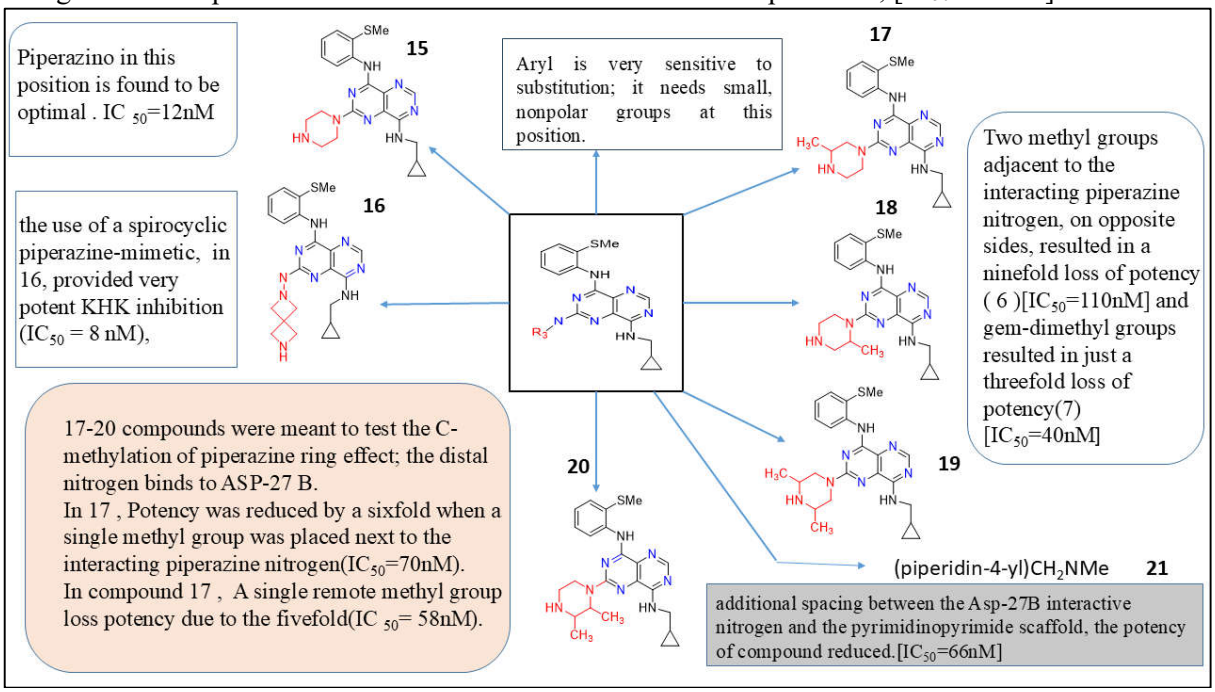


Fig: 7 – Structure activity & relationship of pyrimidino-pyrimidine derivatives

In the **Compound 22 & Compound 23**, the potency of the compound was reduced due to conformational constraint. The compound's effectiveness began to decline with an increasing distance between the pyrimidinopyrimide scaffold and the nitrogen of the Asp-27B interaction. The same was true with compound 21 [IC_{50}=66nM]. In 25 compounds, Bulky bicyclic amine was much less potent (IC_{50}=800nM). In compound number **Compound 26**, the efficacy of the compound (IC_{50} = 6000nM) was further reduced due to the presence of a phenyl ring in the R_3 position, which has a fused interaction, decreasing the basicity of the nitrogen's interaction. In **Compound 27**, which has given good KHK inhibition (IC_{50}=43nM). **Compound 28** has an IC_{50} value of 2 nM and is the strongest known inhibitor of KHK. The high potency for (IC_{50} = 15 nM) was surprising because the interacting nitrogen atom is now situated far outside of the Asp-27 B-dominated region of the active site. Excellent KHK inhibition is demonstrated by additional research employing **Compound 30-33**, with IC_{50} values ranging from 30 to 50 nM.

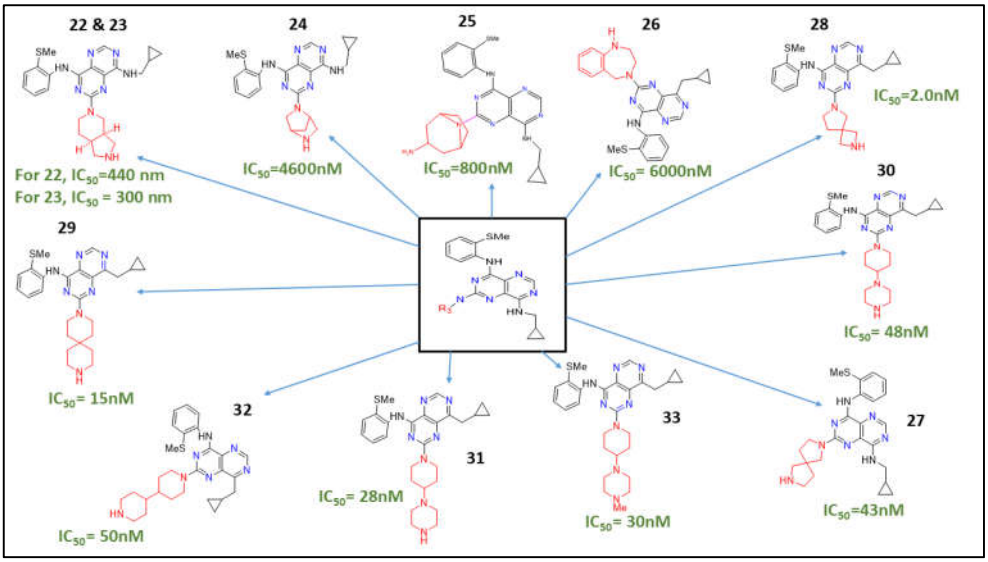
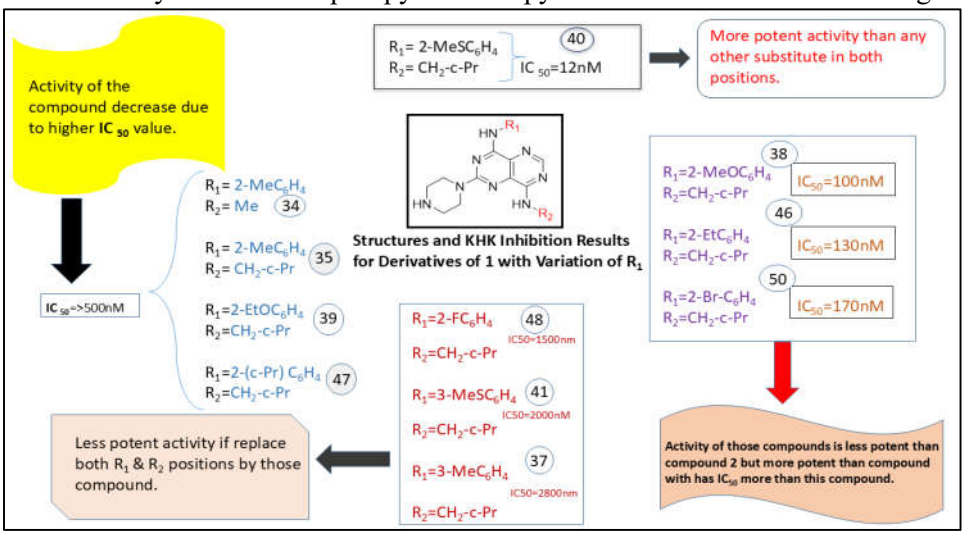


Fig: 8- Structure activity & relationship of pyrimidino-pyrimidine derivatives with biological attributes



Some research groups have been exploring KHK inhibitors through the modification of hydrogen-bonding interactions, incorporation of polar and hydrophobic substituents, and alteration of the core structure for enhanced binding affinity and selectivity. Pyrimidino-pyrimidine scaffolds were discovered to substantially inhibit the enzyme ketohexokinase (KHK) by **Bruce E. Maryanoff et al**. They demonstrated that in an effort to counteract modern diets, body weight, fatty acids, and triglycerides can be reduced by inhibiting KHK-mediated suppression of fructose metabolism. This would provide a new method for the treatment of obesity and diabetes. They identified pyrimidine derivatives that are potent and specific human liver KHK inhibitors, validating the scaffold as an antidiabetic therapeutic lead.

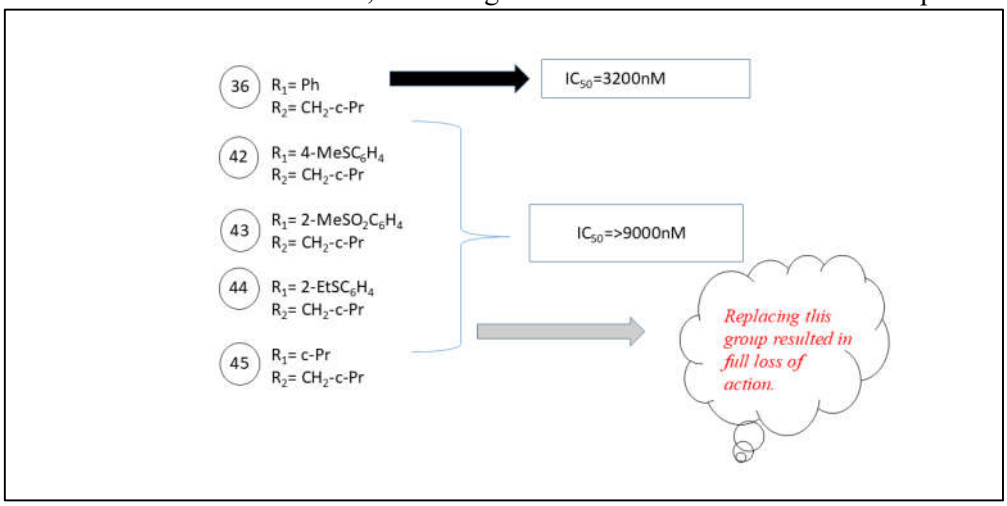


Fig: 9 – SARs of pyrimidino-pyrimidine derivatives with half-inhibitory concentration value.

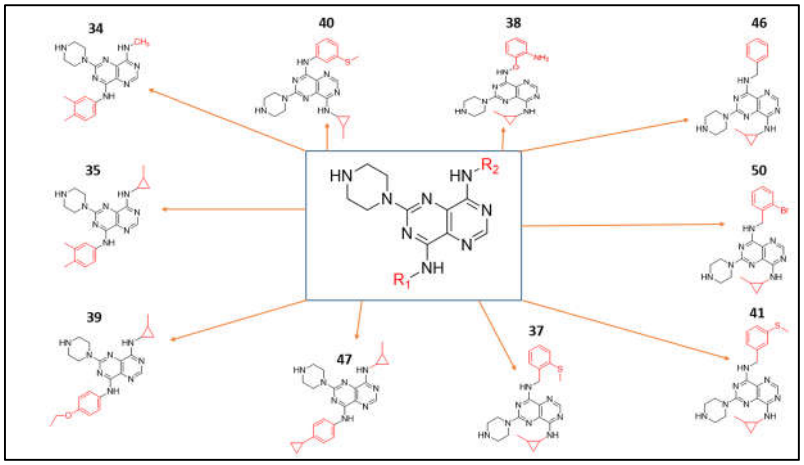


Fig: 10 - Structure activity & relationships of novel KHK inhibitors with pyrimidino-pyrimidine derivatives.

Potency is greatly increased by methoxy (**Compounds 38 and 39**) and methyl-thio (**Compound 40**) substituents in the ortho position: 2-MeOC$_6$H$_4$ (**Compound 38**): IC_{50} = 100nM, 2-EtOC$_6$H$_4$ (**Compound 39**): IC_{50} = 200nM, 2-MeSC$_6$H$_4$ (compound 40): IC_{50} = 12 nM yields the best results. The activity dramatically drops when these groups move to meta or para locations (**Compounds 41 and 42**): 3-MeSC$_6$H$_4$ (**Compound 41**): IC_{50} = 2000 nM, 4-MeSC$_6$H$_4$ (**Compound 42**): IC_{50} > 9000 Nm. The ortho position is crucial, probably because of the closeness of intramolecular interactions or ideal binding alignment. Sulfonyl groups located at the ortho (**Compound 43**) and meta (**Compound 44**) positions result in total loss of activity: IC_{50} > 9000 nM. These groups are excessively polar or sterically obstructive for the binding site. CF_3 (**Compound 48**): Activity moderate (IC_{50} = 1500 nM) Cl or Br (**Compound 49, 50**): activity major (IC_{50} = 540 & 170 nM, respectively). EWGs diminish potency; halogens exhibit moderate effects, potentially because of their size and polarizability. Linear/cyclic alkyls (**Compound 51 & Compound 52**) yield minimal activity. C-Pr and c-hexyl (**Compound 51, 52**): IC_{50} exceeds 9000 nM. Aromaticity and particular electronic effects are essential for binding. Alkyl groups do not possess these characteristics. 2-i-PrC$_6$H$_4$ (**Compound 46**): IC_{50} = 5000 nM 2-(c-Pr)C$_6$H$_4$ (**Compound 47**): Improved to 380 nM, indicating that cyclization may enhance binding, potentially by restricting conformational flexibility. Strongest group: 2-MeSC$_6$H$_4$ (**Compound 40**, IC_{50} = 12 nM). Ortho substitution is essential for effectiveness. Electron-donating groups (EDGs), particularly those based on sulfur or oxygen, improve potency at the ortho position. EWGs and alkyl groups greatly diminish activity. The aromatic nature and substitution arrangement at R_1 are critical factors in determining potency.

They are also reported that the following scaffold Analogues have potent KHK inhibition activity. Small alkyl group substitutes NH-Methyl (**Compound 34**), NH-Propyl (**Compound 53**) have relatively small efficacy and are also more lipophilic and less ideal for the binding pocket (IC_{50} = 400 nM). Potency significantly decreases with increasing alkyl chain length and substituting bulky alicyclic groups like NH-hexyl **Compound 54** (IC_{50}=1600nM), NH-(c-hexyl) **Compound 55** (IC_{50}=2300nM), Net_2 compound 56 (IC_{50} = 1700 nM). NH-$CH_2C{\equiv}CH$ **Compound 57**, contains a triple bond which adds rigidity, also fits better in the hydrophobic pocket (IC_{50}= 300nM), and Benzalyamine **Compound 58**, contains an aromatic ring which may add pi-stacking (IC_{50} = 400), retains good potency. 2-Thiophenylmethylamine **Compound 59** is the most potent modification in this group (IC_{50} = 60 nM), suggesting a strong electronic or hydrogen-bonding interaction.

Changing piperazine to homopiperazine (**Compound 61**) retains potency (IC_{50} = 300 nM). Replacing piperazine with N-Methyl-piperazine (**Compound 62**) decreases potency (IC_{50} = 1500 nM). Morpholino substitution (**Compound 63**) significantly reduces potency (IC_{50}=>7000 nM), indicating that piperazine is crucial for activity. Cyclopropylmethylamine (**Compound 64**) enhances potency (IC_{50} = 70 nM), suggesting a beneficial interaction. 4-(NH_2)-piperidino (**Compound 65**) and 4-piperidinyl-NH (**Compound 66**) show moderate potency (IC_{50} = 200–710 nM). The N-R_2 group strongly affects activity, with small alkyl groups maintaining potency, whereas bulky/hydrophobic groups decrease it. The piperazine ring at N-R_3 is crucial for activity; modifications like morpholino drastically reduce potency. The presence of specific heterocycles (e.g., 2-thiazolyl, piperidino derivatives) enhances.

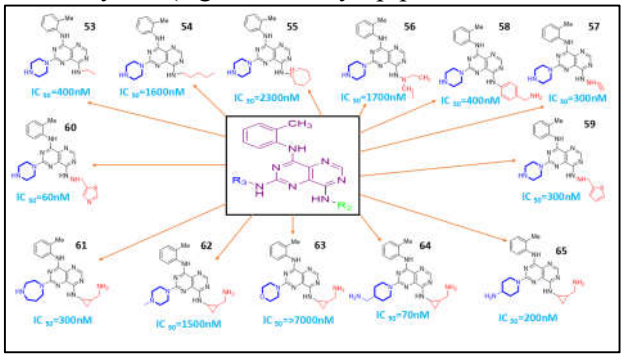
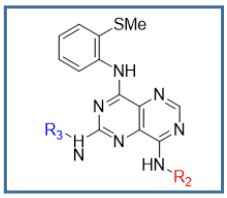


Fig: 11- Diverse molecular framework of pyrimidino-pyrimidine derivatives for KHK inhibitors.



Structures and KHK Inhibition Results for analogues of 7 with Variation of R_2 and R_3

COMPOUND	R_2	R_3	IC_{50}(nM)
67	Cyclo-butylmethyl		18
68	Cyclo-propylethyl		50
69	Ethoxy-methyl		7.0
70	2-thienylmethyl		30
71	2-thiazolylmethyl		16
72	2-pyridylmethyl		9.8
73	Hydrogen		7.1

Table-2: Several substitutes present in compound **67-73** and their potent biological attributes

They also found that the triazolopyrimidine scaffold, which is a fused compound of triazole and pyrimidine ring which has optimal KHK inhibition activity. In contrast to other compounds in the same series, piperazino derivatives containing CH_2CH_2OMe (**Compound 69**) have improved potency (IC_{50} = 7.0 nM). Activity is significantly impacted if cyclopropylmethyl is replaced with different groups at R_2, like 2-thienylmethyl (Compound 70, IC_{50}=30nM), 2-thiazolylmethyl (Compound 71, IC_{50}=16nM) 2-pyridylmethyl.

COMPOUND	R_2	R_3	IC_{50}(nM)
74	Cyclo-propylmethyl	(R)-3-Aminopiperidine	18
75	Cyclo-propylmethyl	(s)-3-Aminopiperidine	23
76	Cyclo-propylmethyl	4-(Aminomethyl)-piperidine	10
77	Cyclo-propylmethyl	3-(Aminomethyl)-azetidine	30
78	Cyclo-propylmethyl	2,6-diazaspiro[3.3]heptane	8.0
79	Cyclo-propylmethyl	N-methyl-ethylenediamine	130
80	Cyclo-propylmethyl	4-(Di-methylaminomethyl)-piperidine	140
81	Cyclo-propylmethyl	N-Methylpiperazine	110

Table-3: Different substituent represents their IC_{50} numerical regarding novel KHK inhibitors.

Substitution gives better activity due to electronic or hydrogen bonding effects (**Compound 72**, IC_{50}=9.8nM). The most potent compound (IC_{50} = 7.0 nM) is **Compound 69** with a CH_2CH_2OMe group. Strong potency is similarly revealed via the 2,6-diazaspiro[3.3]hept-2-yl substitution (**Compound 78**) (IC_{50} = 8.0 nM). **Compounds 67-72**, which are analogs of piperazine, tend to have moderate activity (7.1-18 nM). 4-(Aminomethyl)-piperidine (**Compound 76**, IC_{50}=10nM) and azetidino (**Compound 77**) show improved potency due to favorable orientation and size. Activity is typically increased by the presence of restricted bicyclic amines (like the spirocyclic system in **Compound 78**) or substituents that contain oxygen (like methoxy group in **Compound 69**). Potency is affected by variations in piperazine substitution; certain changes marginally increase IC_{50}. They also further investigate that this Triazolopyrimidine scaffold has inhibitory activity if the mentioned group is attached in that position, which is mentioned in the table below, which they investigate by cell lysates by using LC-MS to quantify F1P.

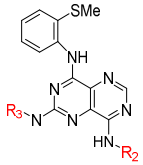


Fig: 11a- Parent pyrimidino-pyrimidine scaffold with thiomethyl group

COMPOUNDS	R_2	R_3	IC_{50}(nM)
69	2-Methoxyethyl		140
71	2-Thiazolymethyl		270
72	2-Pyridylmethyl		270
73	Hydrogen		78
77	Cycloproplymethyl	3-(Aminomethyl)-azetidino	590
78	Cycloproplymethyl	2,6-Diazaspiro[3.3]hept-2-yl	360

Table: 4- Represents the substituents and its biological activity

When compounds 40, 77, and 78 include a cyclopropylmethyl (-CH_2-c-Pr) group, the activity is moderate (IC_{50} = 400, 590, 360 nM). Potency (IC_{50} = 140 nM) is enhanced by changing R_2 to CH_2CH_2OMe (**Compound 69**), showing that stronger polarity at this position may improve activity. The moderate activity (IC_{50} = 270 nM) is preserved by adding electron-donating groups such as CH_2(2-thiazolyl) (**Compound 71**) or CH_2(2-pyridyl) (**Compound 72**). Potency is greatly increased (IC_{50} = 78 nM) when the R_2 group (**Compound 73**, H) has been eliminated, demonstrating that steric hindrance at this site may decrease activity. **Compounds 40, 69, 71, 72, and 73** are members of the piperazine group, which offers a wide variety of IC_{50} values and is well tolerated. Potency (IC_{50} = 590 nM) is decreased when piperazine is substituted with a 3-(NH_2CH_2)-azetidine group (**Compound 77**), indicating that binding is influenced by the ring's size or flexibility. **Compound 78**'s modest activity (IC_{50} = 360 nM) upon the introduction of a 2,6-diazabicyclo[3.3]heptane moiety may suggest a new binding mechanism or steric effects. **Compound 73**'s potency is greatly increased by removing R_2. R_2 groups that release electrons or are slightly polar raise activity. Generally speaking, the piperazine moiety is advantageous, although stiff or large modifications (such as azetidine or spiro systems) tend to reduce efficacy.

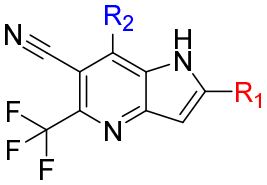


Fig: 12- General structure of pyrimidino-pyrrole derivatives with trifluoromethyl group

COMPOUNDS	R_1	R_2	IC_{50}(nM)
82	Ethyl	 3-methylpyrrolidin-3-ol	1480
84	H	 (S)-3-methylpyrrolidin-3-ol	670
85	H	 (*R*)-3-methylpyrrolidin-3-ol	24,210

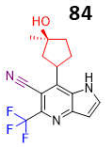
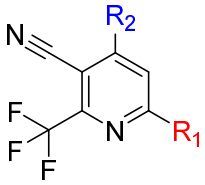
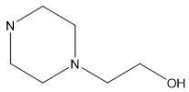
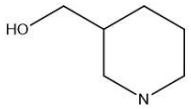
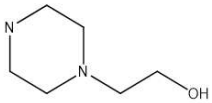
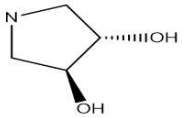
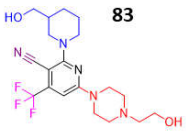


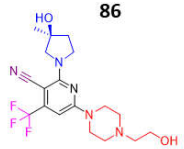
Fig: 13- SARs of pyrimidino-pyrrole derivatives

According to Kim Huard et al., the enzyme ketohexokinase (KHK) is the rate-limiting enzyme in fructose metabolism and helps phosphorylate fructose to fructose 1-phosphate (F1P). The increase in fructose consumption over the past century may be a contributing factor to obesity, insulin resistance, dyslipidemia, and nonalcoholic fatty liver disease (NAFLD). Feedback inhibition does not govern fructose metabolism like it does glucose. They found that the pyrimidinopyrimidine structure can inhibit KHK. Pyrrolopyridine, a KHK inhibitor found in **Compound 82**, exhibits moderate potency and low metabolic stability in tests using human liver microsomes (HLMs). The ethyl group at the C_2 position was removed without lowering potency because it was discovered during the crystal structure analysis that it does not contribute any significant interactions. It was found that the methylpyrrolidinol group improved the ribose pocket; compound 84's S enantiomer was more effective than **Compound 85**'s R enantiomer. Pyrrolopyridine (compound 84) was found to be a reasonably strong and effective inhibitor of human and rat KHK with sufficient microsomal stability, permeability, and thermodynamic solubility.



COMPOUNDS	R_1	R_2	IC_{50}(nM)
83			16,790
86		 (S)-3-methylpyrrolidin-3-ol	1030
87		 (S)-3-methylpyrrolidin-3-ol	450





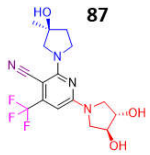


Fig-14: Represents the substituents and its biological activity

Pyridine (In **Compound 83)** offers an additional, synthetically facilitated pathway meant for further optimization, despite acting as a less effective KHK inhibitor, most likely due to the functionalized piperidine ring's less optimal interaction within the ribose pocket. With an hKHK IC_{50} of 1.03 µM, **Compound 86** demonstrated a moderate capacity to inhibit KHK. *Trans*-dihydroxypyrrolidine analog (**Compound 87**) was found to have the best potency, physical properties, and clearance, with an hKHK IC_{50} value of 0.45 µM. The two hydroxyl groups generate hydrogen bonds with the solvent-exposed polar side chains Glu227 and Asn107, along with a bound water molecule. **Compound 87** showed outstanding selectivity for KHK with no appreciable activity against a range of transporters, receptors, ion channels, and enzymes, including the hERG channel and significant human CYP450s. When the crystal structures of pyrrolopyridine (**Compound 82** and pyridine **Compound 83** were compared, both scaffolds successfully aligned, displaying all three essential interactions with the ribose pocket, the conserved water molecule, and the hydrophobic pocket of KHK.

Conclusion: Ketohexokinase inhibitors are essential for the metabolism of both fructose and glucose. A number of scaffolds, including the pyrimidino-pyrimidine, pyrrolopyridine moiety, and indazole ring, have demonstrated the effects of KHK inhibitors. According to our review, the indazole moiety is more powerful than the other two. The molecule 2b's fluorine substituent in the indazole moiety has an IC_{50} value of 23 nM, however the pyrimidine derivative 1c, which has an IC_{50} value of 80 nM, is more significant than the other indazole derivatives. The pyrimidino-pyrimidine derivative **PF-06835919**, which has an IC_{50} value of 10 nM and is now undergoing clinical trials, is beneficial against two main diseases: non-alcoholic fatty liver disease (NAFLD) and noninsulin-dependent diabetic mellitus (NIDDM). Accordingly, we believe that the **1c** and **2b** compounds should exhibit the same action against NAFLD and NIDDM in terms of IC_{50} values. In future aspects, these two new indazole scaffold (**1c & 2b**) may be utilised in other diseases by molecular modelling approach.

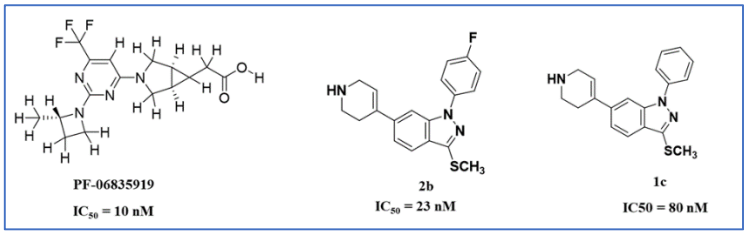


Fig-15: Chemical structure of PF-06835919, 2b & 1c

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