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Research article

# Structure-based design and evaluation of novel *N*-phenyl-1H-indol-2-amine derivatives for fat mass and obesity-associated (FTO) protein inhibition

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ABSTRACT

Fat mass and obesity-associated (FTO) protein contributes to non-syndromic human obesity which refers to excessive fat accumulation in human body and results in health risk. FTO protein has become a promising target for anti-obesity medicines as there is an immense need for the rational design of potent inhibitors to treat obesity. In our study, a new scaffold *N*-phenyl-1H-indol-2-amine was selected as a base for FTO protein inhibitors by applying scaffold hopping approach. Using this novel scaffold, different derivatives were designed by extending scaffold structure with potential functional groups. Molecular docking simulations were carried out by using two different docking algorithm implemented in CDOCKER (flexible docking) and AutoDock programs (rigid docking). Analyzing results of rigid and flexible docking, compound MU06 was selected based on different properties and predicted binding affinities for further analysis. Molecular dynamics simulation of FTO/MU06 complex was performed to characterize structure rationale and binding stability. Certainly, Arg96 and His231 residue of FTO protein showed stable interaction with inhibitor MU06 throughout the production dynamics phase. Three residues of FTO protein (Arg96, Asp233, and His231) were found common in making H-bond interactions with MU06 during molecular dynamics simulation and CDOCKER docking.



## 1. Introduction

Obesity and weight gain are the main risk factors associated with diseases like diabetes mellitus, cardiovascular disease, and nonalcoholic fatty liver disease, with an increased risk of disability (Ogden et al., 2007). In 2007, several studies described that a cluster of single nucleotide polymorphism (SNPs) in the first intron of fat mass and obesity-associated (FTO) protein was highly correlated with obesity-related traits (Müller et al., 2013). FTO protein has been shown to influence obesity and energy utilization in human up to half the world's population (Fischer et al., 2009; Church et al., 2010). Additionally, FTO protein has been identified to be involved in various disease processes which include cardiovascular diseases (Pausova et al., 2009), Alzheimer disease (Keller et al., 2011), type II diabetes (Wehr et al., 2010), and breast cancer (Kaklamani et al., 2011). This makes FTO protein an interesting target to study with respect to its involvement in human diseases.

FTO protein contains a double-stranded β-helix fold which is typical for the member in the Fe(II) and 2-oxoglutarate (2OG)-dependent AlkB dioxygenase family which also includes human homologues ALKBH1-8 (Fu et al., 2010). FTO protein can oxidatively demethylate single-stranded nucleic acids *in vitro* (Gerken et al., 2007), but it has relatively lower repair activities compared to other member proteins of AlkB family (Lee et al., 2005). However, the physiological function and *in vivo* substrates of FTO protein have remained largely unclear. The function of obesity-risk factor FTO protein is to demethylate $N^6$-methyladenosine ($m^6A$) in mRNA. And this apparently indicates a novel and reversible regulatory mechanism in mammalian cells. Additionally, FTO protein has been defined to demethylate diverse mRNAs which shows that the regulation of $N^6$-methyladenosine ($m^6A$) by FTO protein likely influences various biological pathways related to diseases and cellular signaling (Meyer et al., 2012). This knowledge suggests that FTO protein plays an important role in controlling other gene expression and protein translation processes involved

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in the regulation of diseases like obesity and cancer (Tung and Yeo, 2011). These studies have advanced the possibilities of novel therapeutics which involves, targeting FTO protein with small molecules.

Recently revealed crystal structure of FTO protein provides basis for its substrate specificity and binding sites (Han et al., 2010). Moreover, these studies enable the rational design of new inhibitors targeting FTO protein. Inhibiting activity of FTO protein by small molecules has been proposed as a potential treatment for extreme obesity (Thalhammer et al., 2012). However, validation of this approach requires the development of small molecule inhibitors for this protein. The natural product rhein has been identified as the first inhibitor of FTO protein through structure-based virtual screening (Chen et al., 2012). Rhein competitively disrupts binding of FTO protein to the $m^6A$ substrate, and enhances thermal stability of FTO protein by direct binding. Rhein actively increases cellular levels of $m^6A$ in mRNA and the structural complex illustrate that rhein does indeed bind to the nucleic acid binding site (Aik et al., 2013). However, rhein characterized little selectivity for the AlkB subfamily which eclipses its applicability as a specific functional probe of FTO protein inside the cell. The 2OG-tethering approach was also applied to develop the cell active FTO protein inhibitors and these inhibitors proved highly selective *in vitro* for the AlkB subfamily. However, selectivity of these inhibitors remains unclear *in vivo* (Toh et al., 2015). Moreover, the molecular modeling of FTO/rhein complex suggests that rhein has a smaller and more compact chemical structure to achieve potency and selectivity through favorable interactions at the active site (Chen et al., 2012).

In order to avoid competition with internal 2OG, an alternative approach has been applied to identify selective inhibitors of FTO protein in studies done by Huang et al. (2015). In that approach, a high-throughput fluorescence polarization assay has been performed to compare differences in the displacement of $m^6A$-containing ssDNA binding to FTO protein and ALKBH5, respectively. And the screening done by Huang et al. (2015) was resulted in the discovery of meclofenamic acid (MA) which specifically inhibits FTO protein over ALKBH5 (Huang et al., 2015). Results obtained from their study (Huang et al., 2015) establish MA as a highly selective inhibitor of FTO protein demethylation *in vitro*. The structural complex of FTO/MA provides molecular insight into the mechanism of competitive inhibition of FTO protein and also reveals a novel binding site to further design specific inhibitors of FTO protein (Huang et al., 2015).

### 1.1. A new chemical scaffold for FTO protein

Heterocyclic chemistry is one of the most substantial source of novel compounds with diverse biological activity, mainly because of their unique ability to bind reversibly to proteins (Kaushik et al., 2013). The true applicability of heterocyclic structures is their ability to design one library based on one core scaffold structure and to screen it against receptor structure. The fusion of several rings lead to geometrically well-defined structures and holds the ability to orient substituents in three dimensional space. Thus, biologically active heterocyclic templates are always of interest to both organic and medicinal chemists (Kaushik et al., 2013; Kalathiya et al., 2014). Newly designed scaffold structure for FTO protein involves indole in its structure. Indole is the parent substance of a large number of essential compounds that occur naturally. This structure enabled many different structures to be rationalized on the basis of widely distributed heterocyclic compounds in nature having medical importance (Kaushik et al., 2013). Tryptophan contains indole in its structure as major constituent and in the past, this constituent has been used widely in medicines because of its potent physiological properties (Kaushik et al., 2013; Kalathiya et al., 2014; Saxton, 2008; Radwanski and Last, 1995). The indole-based pharmaceutical comprises very important class of therapeutic molecules and are likely to replace many existing pharmaceuticals in the future. The biological properties of these indoles represent much progress with regard to older compounds (Kaushik et al., 2013; Biswal et al., 2012; Yutkin and Chin, 2012).

Scaffold hopping is one of the central tasks in rational drug design. This method typically starts with known active compounds and resulted in a novel chemotype by modifying the core structure of molecule (Böhm et al., 2004). Although the concept of scaffold hopping is comparatively new, this strategy has been applied from beginning of drug discovery (Cramer et al., 1999; Schneider et al., 1999). The concept of scaffold hopping was introduced by Schneider et al. (1999), as a method to identify isofunctional molecular structures with significant different molecular backbones. There are many cases confirming that very small changes in lead structure can have dramatic effects on molecular properties. Thus, a pharmacologist might judge from the function of a compound and will regard an agonist as being different from an antagonist, even though the compounds differ only by one minor substituent (Sun et al., 2012).

An example for successful scaffold hopping is the discovery of GABA-receptor ligands starting from the benzodiazepine core. Examples of compounds with new scaffold structure are Zopiclone, Zolpidem, and Zaleplon (Teuber et al., 1999). Further interesting set of examples are dopamine agonists. These molecules nicely describe that starting from the natural ligand, both ligands having high structural similarity to dopamine (i.e. Fenoldopam) but also completely novel structures can be discovered (i.e. Quinpirole) (Andersen and Jansen, 1990; Kebabian et al., 1997). A distinct set of scaffolds was obtained in the anti-inflammatory field of the cyclooxygenase (COX) ligands and interestingly, the recently approved all COX-2 inhibitors have rather similar structures (Lednicer, 2002; Trummlitz and van Ryn, 2002). In our work, new scaffold structure (*N*-phenyl-1H-indol-2-amine) has been designed considering structure and activity of two already available inhibitors rhein (Chen et al., 2012) and meclofenamic acid (Huang et al., 2015) for FTO protein. Certain type of different compounds were designed based on conformation of an active part of distinct group of inhibitors and all of these eleven compounds contains designed scaffold (*N*-phenyl-1H-indol-2-amine) (Fig. 1) in their structure, and were tested towards FTO protein using molecular docking and simulation approaches.

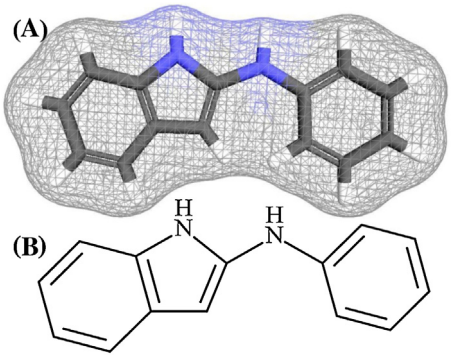


**Fig. 1.** Chemical structure of designed scaffold (*N*-phenyl-1H-indol-2-amine). (A) Stick and solid surface representation (*color:* nitrogen atom in blue and carbon in grey) and (B) 2D representation of designed scaffold. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)