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Research Article

Insilico study of anti-carcinogenic lysyl oxidase-like 2 inhibitors

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ABSTRACT

Lysyl oxidase homolog 2 (LOXL2), also known as lysyl oxidase-like protein 2 is recently been explored as regulator of carcinogenesis and has been shown to be involved in tumor progression and metastasis of several carcinomas. Therefore LOXL2 has been considered as potential therapeutic target. Doing so, its inhibitors as new chemotherapeutic lead molecules: 4-amino-5-(2-hydroxyphenyl)-1,2,4-triazol-3-thione (2a) and 4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol (2b) are synthesized by fusion method (refluxed at 160 °C). Spectral analysis of these triazole derivatives are characterized by FTIR and NMR. Active binding sites and quality of the LOXL2 model is assessed by Ramachandran plots and finally drug–target analysis is performed by computational virtual screening tools. Compounds 2a and 2b showed optimum target binding affinity with −6.2 kcal/mol and −8.9 kcal/mol binding energies. This *insilico* study will add to our understanding of the drug designing and development, and to target cancer-causing proteins more precisely and quickly than before.

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1. Introduction

Carcinogenesis is literally the development of cancer which can be characterized by a progression of changes at the cellular, genetic and epigenetic level and as a consequence the cells undergo abandoned cell division and form a malignant mass (Fearon and Vogelstein, 1990). During development of cancers, in common, tumor suppressor genes are silenced in cancer cells by biological enzymes which catalyze epigenetic histone modifications. Therefore these enzymes offer new therapeutic targets for anti-cancer drugs. The histone modification by lysyl oxidase homolog 2 (LOXL2) enzyme has been previously reported and its expression is shown to be up-regulated in several pathologic states and hence its contribution in development of fibrosis and cancer (Barry-Hamilton et al., 2010). In general, LOXL2 evidently over expressed in a majority of human cancers, these findings strengthened LOXL2 to be considered a putative target for cancer treatment (Jourdan-Le Saux et al., 1999).

LOXL2 enzyme is considered as novel drug target that promotes the cancer metastasis and it has been reported that LOXL2 is markedly overexpressed in carcinoma relative to normal condition and associated with tumor development. The inhibition of LOXL2 target in gene knockout studies significantly inhibited the tumor growth and metastasis (Peng et al., 2009; Chang et al., 2013). Therefore, LOXL2 is likely to be an excellent therapeutic target in many cancer types (Barker and Erler, 2011) and has been selected for this study due to its potential role.

Presently, it is unknown whether LOXL2 can be pharmacologically targeted for cancer treatment, nevertheless, small inhibitors molecule against histone deacetylases have been developed as an effective reagents to activate the expression of tumor suppressor gene in cancer chemotherapy, however, there is a need of chemical inhibitors that may counteract its activity (Bekircan and Gumrukcuoglu, 2005; Herranz et al., 2012).

The epigenetic studies underlying tumor genesis has been proved for the identification of new therapeutic targets for cancer drugs. Nevertheless, research to develop chemotherapeutic drugs for treatment of carcinogenesis is relatively slow due to expensive techniques, uncharacterized genetic basis of cancer and increased reports of mutagenesis. Thus, there is urgent need of establishing alternative drug development strategies to encounter the situation. In this regard, *in silico* methods have the advantages of speed, low cost and, even more importantly, enables researchers to raise questions that would otherwise be difficult to address experimentally.

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There is a progressive development in drug discovery procedures from conventional ligand based drug discovery to structural and targeted based drug designing approaches (Mdluli and Spigelman, 2006). These virtual techniques are widely used and have become vital component of drug discovery and development programs (Bajorath, 2002). The applicability of such methods in the new lead identification and optimization has served as an important tool in our quest to access novel drug like compounds (Reddy et al., 2007).

Here, we have proposed new triazole derivatives as LOXL2 inhibitors that showed optimum binding affinity with minimum binding energies. These triazole compounds can inhibit the cancer cell growth by modifying this enzyme as previous studies on mice models revealed that some clinically relevant chemotherapeutic agents have anti-cancer activity by blocking the action of LOXL2 enzyme (Erler et al., 2006; Baker et al., 2013). Triazoles have been used as a pharmacore for many years in order to develop novel ligands and have been proven useful, thus gained considerable attention in drug industries because of their effective biological activities. These derivatives have been shown to possess therapeutically interesting activities such as antimicrobial, and anti-cancer (Bekircan and Gumrukcuoglu, 2005). We have synthesized and analyzed the new efficient triazole derivatives that can inhibit the LOXL2 enzyme, thereby offering new avenues for cancer research and treatment. The current study was conducted with the objectives: synthesis of new triazoles derivatives, characterization and drug likeness evaluation, and finally these drugs were computationally designed against LOXL2 enzyme to arrest metastasis. Therefore, these new triazoles derivatives can get a pharmaceutical application on future directions in anti-cancer drug development.

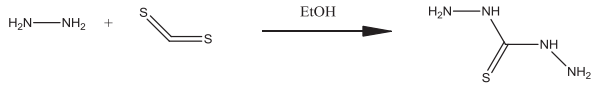
2. Materials and methods

2.1. Synthesis of lead compounds

Synthesis of 4-amino-5-(2-hydroxyphenyl)-1,2,4-triazol-3-thione (2a) (Fig. 5A) and 4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol (2b) (Fig. 5B) compounds (2a) and (2b) were synthesized according to three-steps experimental procedure.

2.1.1. Step 1: synthesis of thiocarbohydrazide (1a)

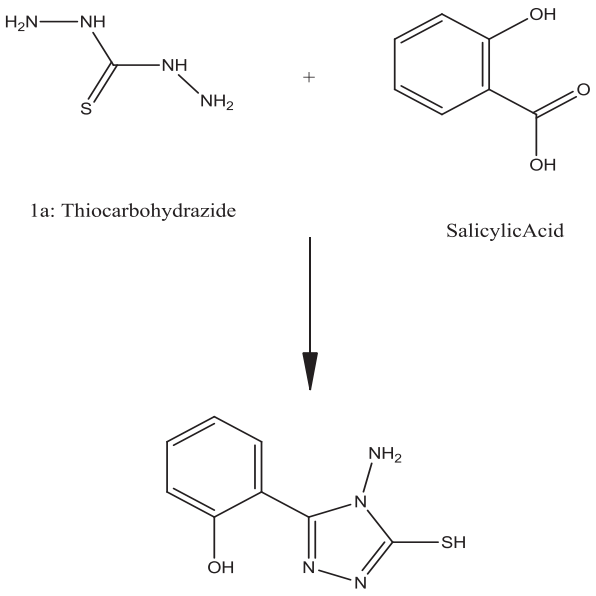
Hydrazine hydrate (30 ml) was heated at reflux condenser with carbon disulfide (15 ml) in the presence of ethanol (150 ml) for about 4 h. On cooling, thiocarbohydrazide was precipitated as solid. Excess of the solvent and thiocarbohydrazide was removed by heating on water bath at their boiling points unless free from both of the solvent and unreacted hydrazine hydrate.



Hydrazine Hydrate Carbon Disulfide 1a: Thiocarbohydrazide

2.1.2. Step 2: synthesis of 4 amino-5-(2-hydroxyphenyl)-1,2,4-triazol-3-thione (2a)

Equimolar quantities of thiocarbohydrazide (0.1 mol) and salicylic acid (0.1 mol) were fused at 160 °C for about 2 h in an oil bath by constant stirring. TLC was used to determine the progress of reaction. After completion of reaction the mixture was cooled and filtered and recrystallized from 70% ethanol.

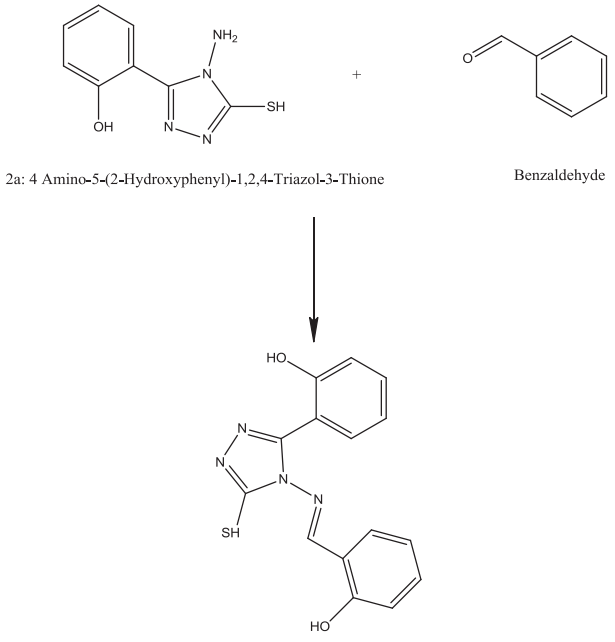


1a: Thiocarbohydrazide SalicylicAcid

2a: 4 Amino-5-(2-Hydroxyphenyl)-1,2,4-Triazol-3-Thione

2.1.3. Step 3: synthesis of 4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol (Schiff base) from (2b)

1 g of 2a was dissolved in ethanol while heating on water bath. Completely dissolved equimolar quantity of benzaldehyde added and reflux was started with continuous stirring for 4 h. The completion of reaction was monitored by TLC. The mixture then cooled to form solid product. It was then filtered and recrystallized by 70% ethanol.



2a: 4 Amino-5-(2-Hydroxyphenyl)-1,2,4-Triazol-3-Thione Benzaldehyde

2b: 4-(2-hydroxybenzalidine) amine-5-(2-hydroxy) phenyl-1,2,4-triazole-3-thiol

2.2. Structural characterization

Physical properties and structural characterization of these derivatives were carried out by FTIR (Bruker Germany Alpha