Drug-Receptor Interactions of New Isoxazole Scaffold against Breast and Skin Cancer Cell Lines

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Abstract

The electron rich oxygen and nitrogen exists at 1, 2 positions in a five membered ring system is known as isoxazole, an active pharmacophore. They are reported to be interesting synthons to synthesize various useful scaffolds of interesting biological activities. Herein, we report the synthesis of isoxazoles from ethoxy substituted chalcone derivatives on reaction with hydroxylamine hydrochloride in basic medium. All the compounds are characterized by spectroscopic methods. The present study is an effort to extend the utility of virtual drug-receptor interaction of isoxazoles on the active site of target inhibitor bound cytochrome P450 family oxidoreductase [PDB: 3PM0] for breast cancer cell line and pirin inhibiting target [PDB: 3ACL] for skin cancer cell lines respectively. Both the chloro and bromo substituted derivatives showed maximum interactions with minimum docking scores. *In silico* ADME–toxicity evaluation to predict the preliminary pharmacokinetic and toxicity profile of the synthesized compounds is also conducted.

Keywords: Isoxazole, molecular docking, ADME-toxicity.

Introduction

The research and progress or advancement of organic chemistry contributes permanently for the development in multiple fields of science. The presence of at least one heterocyclic ring is an interesting feature found in most of the synthesized novel compounds. Hence, heterocyclic chemistry plays a salient role in searching active synthons having number of potential applications. Isoxazole is one such pharmacologically important molecule in medicinal chemistry field. Due to the presence of electron rich oxygen and nitrogen at 1, 2 position makes the bond less stable resulting in activity towards biological targets which made them to be used as pharmacological agents.

Naturally ocuuring ibotenic acid is an amino acid with hydroxy isoxazole ring system used as psychoactive drug. The marketed drugs which contain isoxazole core are valdecoxib, flucloxacillin, cloxacillin, dicloxacillin, and danazol, sulfamethoxazole, sulfisoxazole, oxacillin, and acivicin (An antitumour, and antileishmanial drug) [**1, 2**]. Isocarboxazide a monoamine oxidase inhibitor and isoxazoline-5-one present in Legume seeds are found to be active isoxazole derivatives [**3**]. The popular antibiotic drug cycloserine was used as antibiotic, antitubercular and antibacterial agent especially used in the treatment of leprosy [**4**].

In the view of the reported biological activities of these compounds, synthesis of such derivatives has attracted chemists to carry out quite number of experiments in recent years. This prompted to carry out further work on these novel isoxazoles derivatives. In order to know their potency towards the binding to the selected targets 3PM0 (breast cancer) and 3ACL (skin) cancer pathway enzymes are evaluated by docking studies.

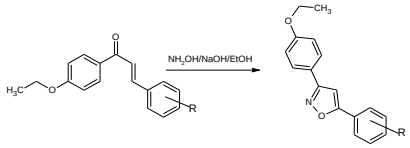
Experimental section

The melting point was determined using capillary tube in Digital melting point apparatus and reported without correction. Reaction progress was tracked by TLC.A ^{1}HNMR spectrum was documented on Bruker instrument at 400 MHz with 5mm PABBO BB tubes in $CDCl_3$. The CHNSO analyzer of FLASH EA 1112 model was used to enumerate the composition of elements.

Synthesis of target isoxazoles

An equimolar mixture of ethoxy substituted chalcone and hydroxylamine hydrochloride in absolute ethanol with 5ml of sodium hydroxide (10%) was subjected for reflux (12 hour). The solid precipitate obtained on pouring reaction mixture onto 50 ml ice-cold water was collected by filteration. Recrystallisation of the crude products from ethanol yielded pure samples. Analytical and spectral data are given in **Table 1** and **Table 2** respectively.

Reaction scheme



R=2-Cl, 4-Br

Table 1. Analytical data.

Code	Molecular Formula	Composition (%)		Melting Point (ºC)	Yield (%)
		Calculated	Obtained		
I	$C_{17}H_{14}ClNO_2$	C:68.12 H:4.71 Cl:11.83 N:4.67 O:10.68	C:67.95 H:4.58 Cl:11.77 N:4.62 O:10.56	168-170	62
II	$C_{17}H_{14}BrNO_2$	C:59.32 H:4.10 Br:23.21 N:4.07 O:9.30	C:59.11 H:3.97 Br:23.16 N:3.96 O:9.26	172-175	58

Table 2. Spectral data.

Compound	Spectral Data ^{1}H NMR in $CDCl_3$ performed at 400 MHz (δ ppm)
5-(2-Chlorophenyl)-3-(4-ethoxyphenyl)-1,2-oxazole **(I)**	1.44 (3H, t, J=7Hz, CH_3); 4.09 (2H, q, J=7Hz, OCH_2); 7.48(2H, d, J=8.8Hz, Aromatic hydrogen of ortho protons of 4-ethoxyphenyl); 6.95 (2H, d, J=8.8Hz Aromatic hydrogen of meta protons of 4-ethoxyphenyl); 7.59 (1H, s, oxazole Aromatic hydrogen); 7.75 (1H, d, J=9Hz, Aromatic hydrogen of 2-chlorophenyl); 7.24(1H, d, J=7.68 Hz, Aromatic hydrogen of 2-chlorophenyl); 7.37-7.35 (2H, m, Aromatic hydrogen of 2-chlorophenyl); 7.30-7.28 (2H, m, Aromatic hydrogen of 2-chlorophenyl).
5-(4-Bromophenyl)-3-(4-ethoxyphenyl)-1,2-oxazole **(II)**	1.45 (3H, t, J=7Hz, CH_3); 4.10 (2H, q, J= 7Hz, OCH_2); 7.36-7.25 (2H, m, Aromatic hydrogen of p-ethoxyphenyl); 6.99-6.77(2H, m, Aromatic hydrogen of 4-ethoxyphenyl; 7.55-7.41(4H, m, Aromatic hydrogen of 4-bromophenyl); 8.02(1H, s, oxazole Aromatic hydrogen).

Molecular docking studies

In silico drug designing and computational pharmacokinetic properties were performed using Schrödinger, LLC, New York, NY, 2017 at Mangalore University.

Drug-receptor interaction studies

The isoxazole analogues I and II were taken for docking studies in order to know the binding efficiency to understand drug receptor interactions. In the present work it was decided to select 3PM0 breast cancer target to test the binding efficiency of the isoxazole scaffold based on the mechanism of inhibiting CYP1 enzymes by flavanoids in cancer chemotherapy. Docking was done for a inhibitor (*N*-{[3-(Benzyloxy)phenyl](methyl) -Λ^4-sulfanylidene} -4 – methylbenzene -1 -sulfonamide) bound nuclear proteinPirin (PIR)(PDB: 3ACL), which inhibits melanoma cell progression and hence chosen as target. The interactions with 3PM0 and 3ACL are shown in **Fig. 1** and **Fig. 2** respectively. The binding modes with PDB:3PM0 and 3ACL and their docking score of isoxazoles along with their respective interacting residues are given in **Table 3**.

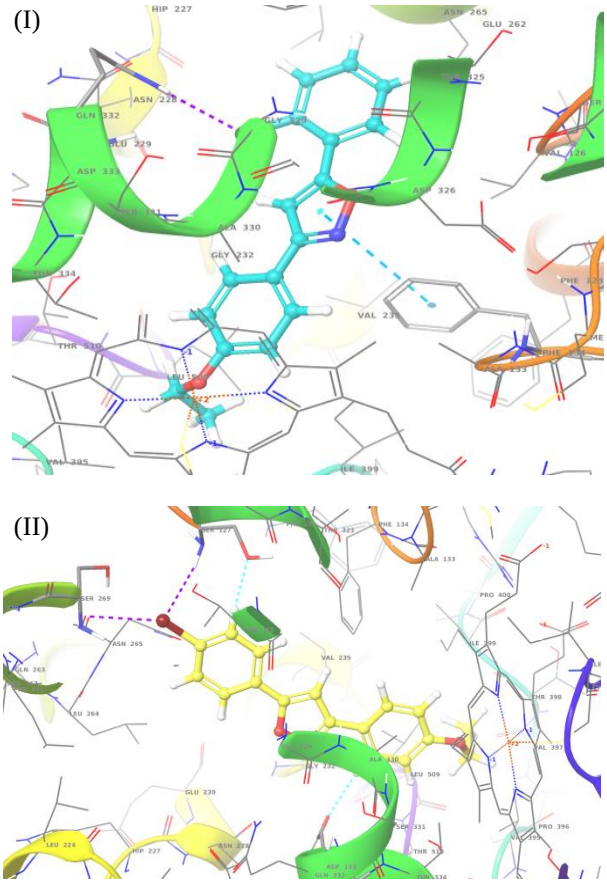
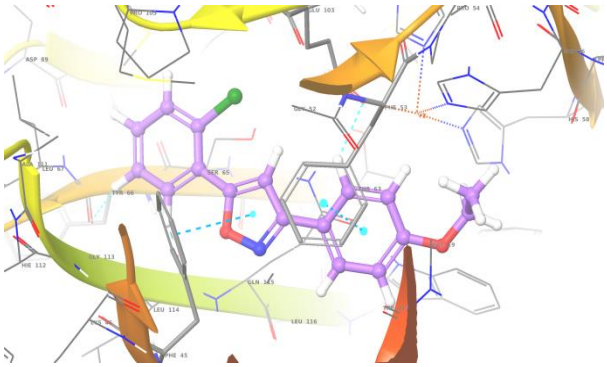


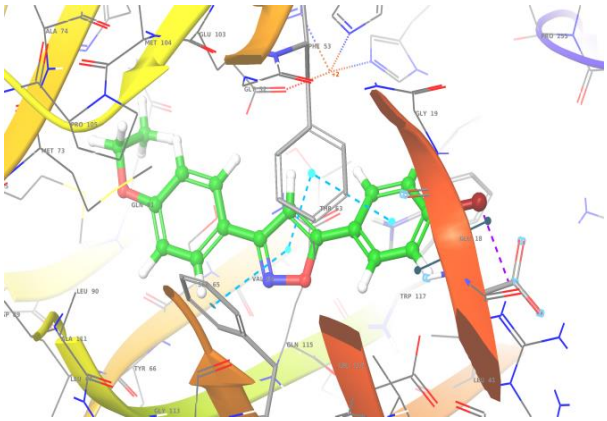
Fig. 1. Compound **I** (blue stick model) and compound **II** (yellow stick model) interactions with residues of 3PM0.

Table 3. Parameters of drug receptor interactions.

Docking score of isoxazoles against human breast cancer cells (PDB: 3PM0)				
Code	**R**	**Gide Score**	**Interacting Residue**	**Interaction**
I	2-Cl	-9.386	Phe134, Gln332	π –π, Salt bridge
II	4-Br	-9.029	Phe 134, Ser 127, Ser269, Thr 325	π –π, Salt bridge, H bond
Docking score of isoxazoles against human metastatic melanoma cells (PDB: 3ACL)				
I	2-Cl	-7.884	Phe 53, Glu 103, Tyr66	π -π , Salt bridge,H bond
II	4-Br	-7.523	Phe 45, Glu 103	π –π, Salt bridge



(I)



(II)

Fig. 2. Compound **I** (violet stick model) and compound **II** (green stick model) interactions with residues of 3ACL.

Pharmacokinetics

The pharmacokinetic profiling of isoxazole scaffold against two targets was calculated. Enumerated molecular weight and obtained molecular weights are in good agreement. Lipinski's rule of five is considered to evaluate the drug likeness and the computed value is within the acceptance limit. Pharmacokinetic parameters found to be within acceptable limits and are shown in **Table 4.**

Table 4. Calculated Pharmacokinetic properties.

Calculated Pharmacokinetic properties of isoxazole against 3PM0									
Code	**R**	**[a] Molecular weight**	**[b] Van der Waals surface area**	**[c] LogS**	**[d] Donor HB**	**[e] Acceptror HB**	**[f] Log P**	**[g] % of HOA**	**[h] Rule of Five (number of Voilation)**
I	2-Cl	299.75	38.8	-4.8	0	2.7	4.64	100	0
II	4-Br	344.20	38.4	-5.1	0	2.7	4.72	100	0
Calculated Pharmacokinetic properties of isoxazole against 3ACL									
1	2-Cl	299.75	39.3	-5.3	0	3.5	4.20	100	0
2	4-Br	344.20	40.2	-5.1	0	3.7	4.25	100	0

Summary

In summary, the isoxazole scaffold derived from 4-ethoxysubstituted chalcone was synthesized.These molecules were characterized by spectral data and molecular docking was carried out on the active site of target inhibitor bound cytochrome P450 family oxidoreductase [PDB:3PM0] for breast cancer cell line and pirin inhibiting target [PDB: 3ACL] for skin cancer cell lines respectively. The interacting residue at the binding site of 3PM0 was found to be Phe134 with docking score -9.386 for 2-chloro and -9.029 for 4-bromo derivative. Similarly the interacting residue at binding site of 3ACL is Phe 53 and Phe 45 for compounds I and II respectively. The ligand molecules bound to the receptor site through π-π interactions, H bond and salt bridge with near parallel orientation.

Acknowledgements

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