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Early stage repurposing of benzimidazole scaffolds towards breast cancer through *in-silico* tools

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Abstract

Back ground: The increased success and applications of drug repurposing can be considered as one of the consequences of poly pharmacology and it represents a manifestation of the shift from a single to multi target paradigm in drug discovery. Mebendazole, a well-known anti-helminthetic drug in wide clinical use, has anti-cancer properties that have been elucidated in a broad range of pre-clinical studies across a number of different cancer types. Significantly, there are also two case reports of anti-cancer activity in humans. Since, mebendazole has benzimidazole scaffold in its structural frame, it has been decided to choose a library of benzimdazole scaffold containing drugs for our study. Human epidermal growth factor receptor 2 (HER2) is over expressed in around 20–30% of breast cancer tumors, we have selected this protein for our *in silico* docking studies.

Objective: To select a specific benzimidazole scaffold containing drug as a specific ligand targeting HER-2 receptors through *in-silico* docking studies.

Materials and Methods: A library of existing drugs having benzimidazole in their structure would be generated on the computer and evaluated for their rigid/flexible docking with key enzyme HER-2 receptor. Suitable poses and binding interactions for specific protein/ receptor sites are studied by the use of drug design software *Accelrys discovery studio*.

Results: It has been concluded that the benzimidazole containing drugs pantoprazole, azeloprazole and etonitazene have shown significant inhibitory activity against HER-2 receptor.

Keywords: Repurposing, benzimidazole, Scaffold, breast cancer, HER-2, docking

INTRODUCTION

Drug repositioning or drug repurposing is an approach to accelerate the drug discovery process through the identification of a novel clinical use for an existing drug approved for a different indication [1]. A major problem of conventional cancer chemotherapy drugs (mainly DNA damaging agents) is notorious side effects that significantly reduce the quality of life of patients [2]. As most of non-cancer drugs have little or tolerable side effects in human, repositioning of non-cancer drugs for anticancer therapy will be an excellent strategy for future anticancer drug development [3].In addition, a few other non-cancer drugs are under clinical investigations (e.g. itraconazole, nelfinavir, digoxin, riluzole, mycophenolic acid and disulfiram) and numerous old drugs have demonstrated potent anticancer activities against a variety of human cancers [4].

Despite drug repositioning significantly reduces the investigational time and cost, this promising drug discovery approach still suffers many challenges and issues [5]. The limited intellectual property (IP) issue owing to the lack of a new composition of matter; Identification of exact anticancer acting mechanisms and target(s) for these drugs;The limited efficacy of repurposed drugs.

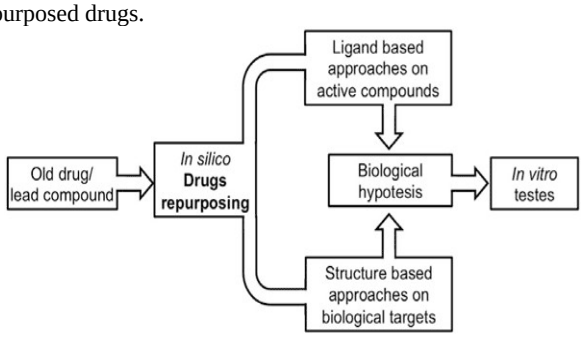


Figure 1:Overview of *in-silico* drug repurposing

In this line the scaffolds of the old drugs emerge as a great treasure-trove towards new cancer drug discovery [6]. This scaffold repurposing approach provides a new meaning on the old saying of **"to start with an old drug".**

The availability of several established clinical drug libraries and rapid advances in disease biology, genomics and bioinformatics has accelerated the pace of both activity and *in-silico* drug repositioning [7-8]. In activity based drug repositioning, the actual drugs may be required for screening. In contrast, *in-silico* drug repositioning (**Figure 1**) utilizes public data bases and bioinformatics tools to systematically identify interaction networks between drugs and protein targets[9]. Having handful amount of information on the structure of proteins and pharmacophores, this approach has been widely used for drug repositioning [10]. Various researchers confirmed the growth inhibitory effects of mebendazole against breast, ovary, colon carcinomas, and osteosarcoma, producing IC50s that varied from 0.1 to Mm[11]. Human epithelial receptor-2 (HER2) is overexpressed in a number of human cancers, including 20–40% of solid tumours including breast, ovarian, lung, gastric, and oral cancers where it correlates with poor prognosis. Given that HER2 is only expressed at low levels in normal human tissues, its overexpression in tumours makes it an attractive target for tumour-specific therapies[12].

Based on these views, it has been decided to propose scaffold repurposing by optimizing existing drugs as lead compounds with enhanced efficacy, target selectivity and clinical safety. A library of existing drugs having benzimidazole in their structure would be generated on the computer and evaluated for their rigid/flexible docking with key enzyme HER-2 receptor Suitable poses and binding interactions for specific protein/ receptor sites will be studied by the use of drug design software Accelrys discovery studio.At the end of this phase the drugs having significant inhibitory property against HER-2 would be selected for further *in-vitro* screening.

MATERIALS AND METHODS

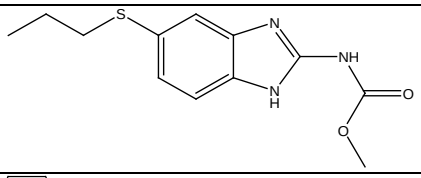
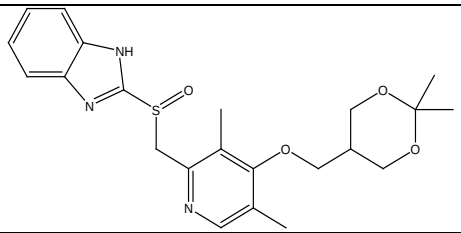
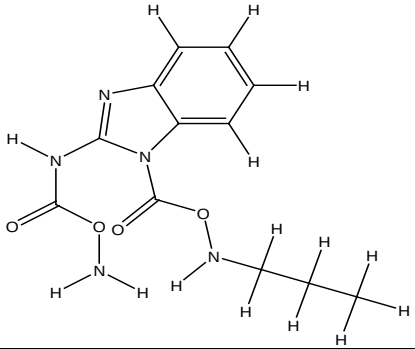
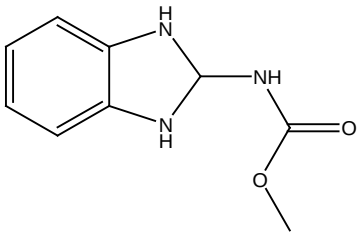
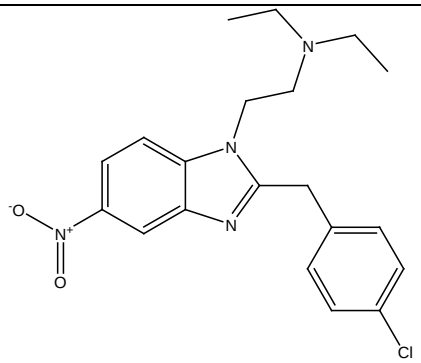
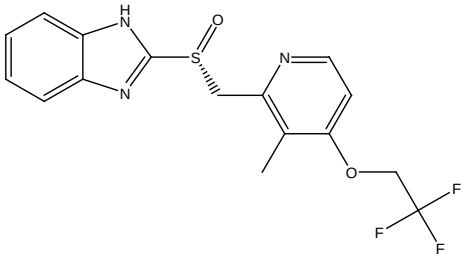
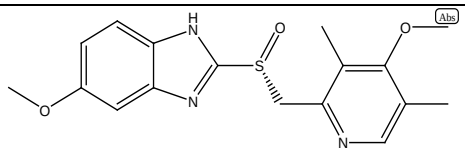
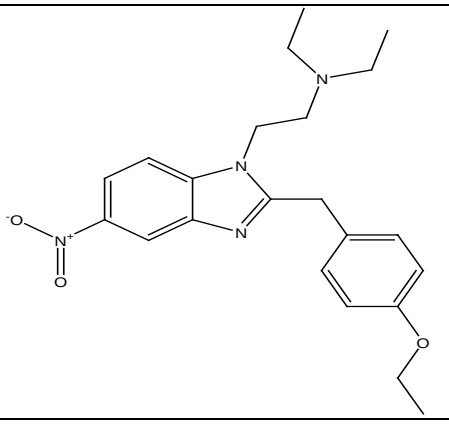
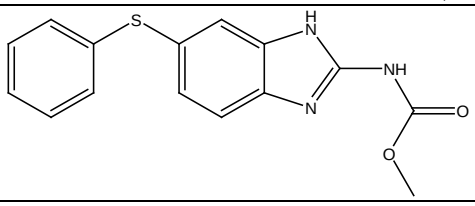
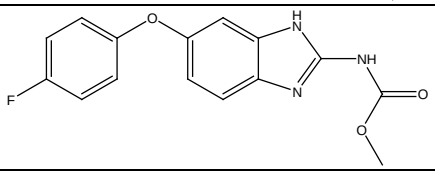
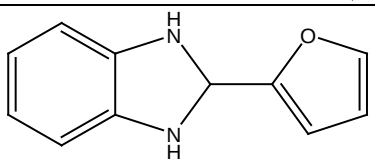
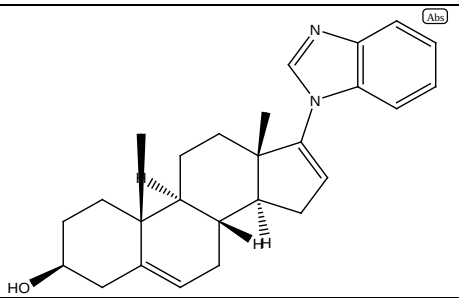
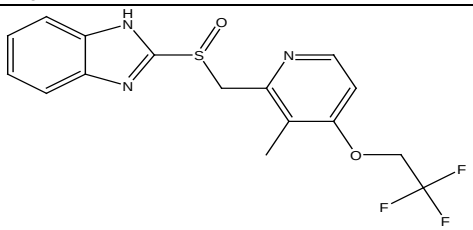
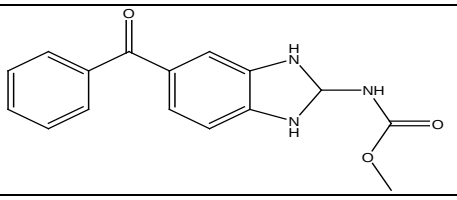
The docking studies had been carried out by using the docking software, Accelrys Discovery Studio 4.1 Client. The HER-2 receptor (PDB ID-4LQM) has been identified from protein data bank website.

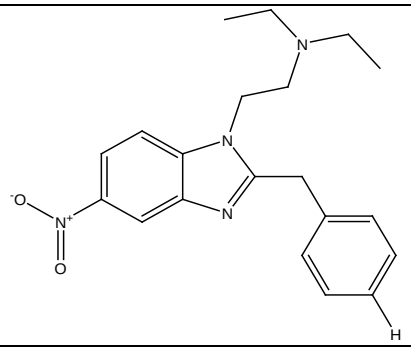
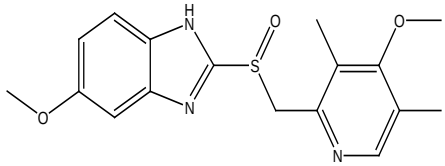
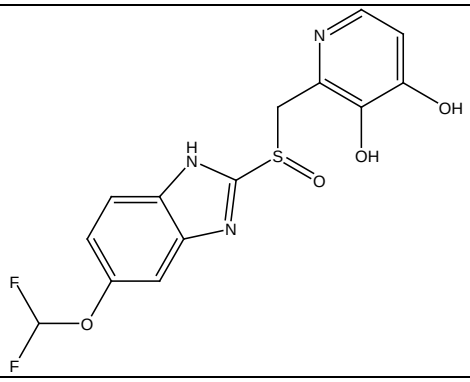
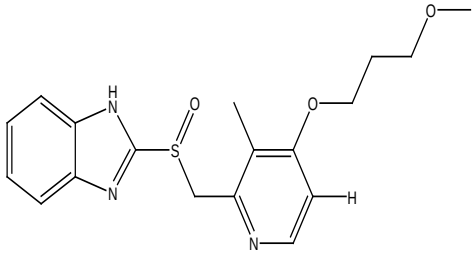
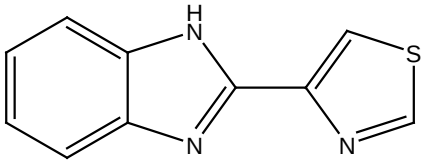
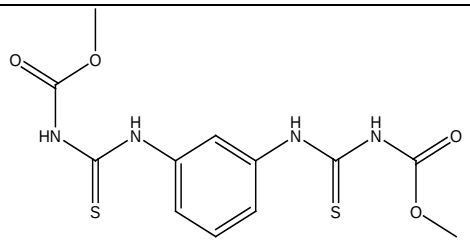
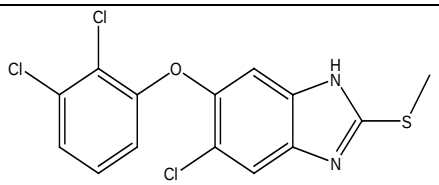
Ligand Preparation

Twenty one drugs having benzimidazole scaffold in their structures were selected for our study (**Table 1**). The ligand preparation included 2D-3D conversions, correcting structures, generating variation of these structures, verifying and optimizing the structure. All these tasks had been performed by using

ACD/Chemsketch software for drawing, displaying and characterizing chemical structures and substructures. The ligand structures were prepared using Discovery studio 4.1 through prepare ligand protocol.

Table 1: Selected drug candidates for *in-silico* drug design

Name of the drug	Chemical Structure
Albendazole	
Azeloprazole	
Benomyl	
Carbendazim	
Clonitazene	
Dexlanzoprazole	
Esomeprazole	
Etonitazene	
Fenbendazole	
Flubendazole	
Fuberidazole	
Galeterone	
Lanzaprazole	
Mebendazole	

Name of the drug	Chemical Structure
Nitazene	
Omeprazole	
Pantaprazole	
Rabeprazole	
Thiabendazole	
Thiophanate methyl	
Triclabendazole	

Protein preparation

The crystal structure of the investigational scaffold protein Human epidermal growth factor receptor -2 (HER-2) complexed with N-[4-(3-BROMO-PHENYLAMINO)-QUINAZOLIN-6-YL]-ACRYLAMIDE had been downloaded from RCSB protein data bank bearing the PDB code 4LQM (**Figure 2**). The protein was prepared by inserting missing atoms in incomplete residues, modeling missing loop regions based on information, deleting alternate conformations, removing waters, standardizing atom names, protonating titratable residues using predicted pKas. The potential energy, Van der Waals energy, Electrostatic energy and RMS gradient had been checked for the protein before and after minimization. The protein has 323 residues in which the complexes bound to the receptor molecule, All the hetero atoms and the non-essential water molecules were removed. Finally hydrogen atoms were merged to the target receptor molecule using Discovery Studio 4.1 Client. The missing residues at start of A chain GLY, SER, PRO, SER, GLY and GLN, GLY, GLY at end of the chain A were added. The prepared protein structure was validated with Ramachandran plot in which all the residues were in acceptable region (**Figure 3**).

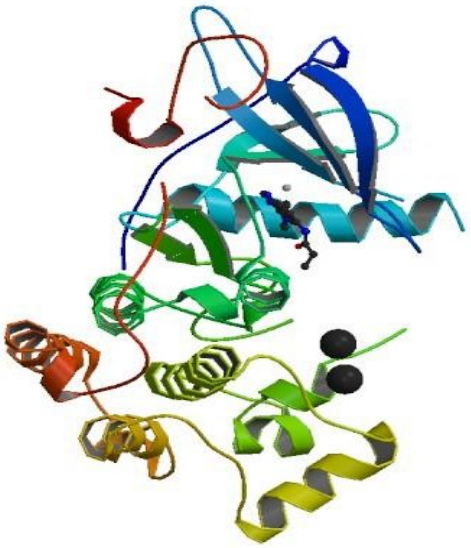


Figure 2: Three Dimensional Structure

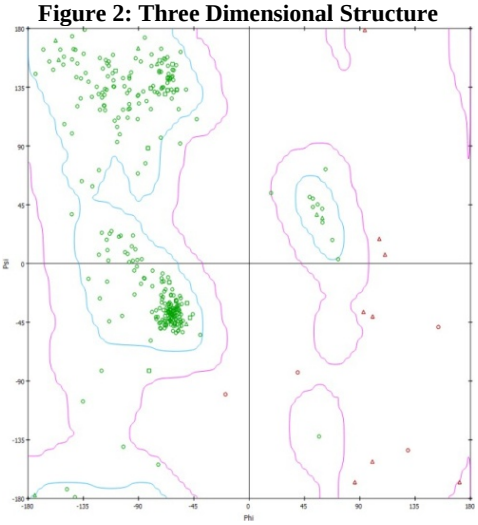


Figure 3: Ramachandran plot of 4LQM of HER-2 complex (4LQM

Active site identification of HER2 complex

The catalytic site prediction of Human HER2 complexed with the co-crysatl (4LQM) were analysed using Discovery Studio 4.1 Client. The following 11 residues were chosen as active site

residues (**Figure 4**). ALA743, LYS745, GLU762, LEU788, THR790, GLN791, MET793, GLY796, CYS797, ASP800, LEU844.

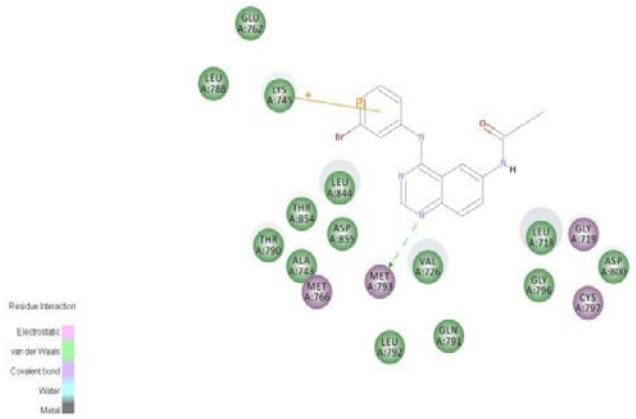


Figure 4: Active Binding Pocket using 35 residues of HER-2.

Molecular Docking Simulation

Molecular docking has been performed by the Libdock program and CDOCKER docking method implemented in Discovery Studio 4.1.CDOCKER is a simulated annealing based molecular docking method. In this docking method ligands are treated as fully flexible while protein is kept rigid. The minimized structures of the designed compounds were used as input ligand in the protocol explorer of CDOCKER. Each of them was given as input in another parameter meant for 'input ligands' and the protocol were run as many times as the number of inhibitors are selected for the experiment. The various conformations for ligand in this procedure had been generated by using molecular dynamics. The generated initial structures for the ligand were further refined using simulated annealing. The CDOCKER energy (protein-ligand interaction energies) of best configuration docked into the receptor of all the selected natural inhibitors, which had been calculated and compared with that of interacting residues at active site region with the crystallized inhibitors. Binding energy of protein and ligands had been calculated by following Calculation:
E binding = Ecomplex – (Ereceptor + Eligand)
The docking analysis of HER-2 complexed with the compounds was carried out by Discovery Studio 4.1 Client docking software. All the parameters used in Discovery Studio 4.1 Client docking were selected by default. Calculation type was set to dock mode and flexible mode was selected for the ligand. Grid resolution was set to 0.40 Å. The observed least energy indicated the easy binding character of ligand and receptor.

RESULTS AND DISCUSSION

Structure of the target protein

Human epidermal growth factor receptor 2 (HER2) (PDB id: 4LQM) in complex with the ligand N-[4-(3-BROMO-PHENYLAMINO)-QUINAZOLIN-6-YL]-ACRYLAMIDE has been exploited as a main therapeutic target for breast cancer. The three dimensional structure of HER-2 retrieved from the Protein Data Bank with PDB ID: 4LQM determined by X-Ray crystallography at a resolution of 2.0 (Å) was visualized in Discovery Studio 4.1 Client. 4LQM contains 323 acids and has been shown in the **Figure 2**.

Docking analysis

The predicted 11 active residues were used as the catalytic sites for the twenty one compounds used for docking studies. The results of libdock program and CDocker program were shown in **Table 2**. The results of the interaction between the active site residues of target HER-2 complex were given in **Figure 5-7** respectively. In the docking interactions, Pantoprazole, Etonitazene and Azeloprazole were found to have the highest libdock score of 120.079, 114.471 and 114.334 when compared with standard co crystal ligand of 4LQM having libdock score of 78.4739. On the other hand thiobendazole and thiophenate methyl had shown a weak libdock score of 68.863 and 60.0909 with respect to 4QLM (Table 3). These compounds have (-21.693, -35.505), (-20.481, -39.7272), (-17.0504,-44.5715) -Cdocker energy and -Cdocker interaction energy respectively (**Table 2**). The non bond interactions were shown clearly in the **Figure 5-7**. Pentaprazole, Etonitazene, Azeloprazole has interacted with the HER2 protein residues MET793, ASP855, LEU788, GLY796, ASP800, GLU762, ALA743, LEU844, LYS745, MET766, THR790, VAL726, LEU718, LEU792, MET766, GLU762, ASP855, CYS755, THR854. Finally, Pantoprazole,Etonitazene, Azeloprazole exhibits the best binding interaction with the HER2 complexed and further it could be useful for identification and development of new preventive and therapeutic drug against breast cancer.

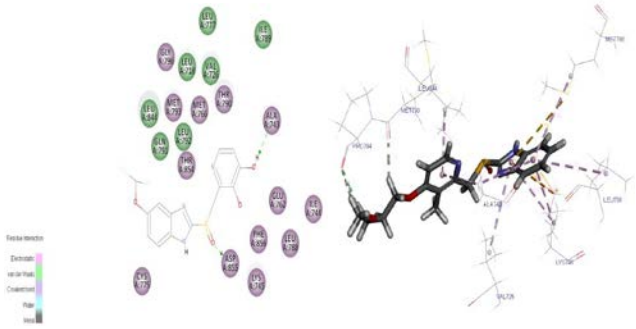


Figure 5: 2D & 3D docking interactions of Pantoprazole with HER2

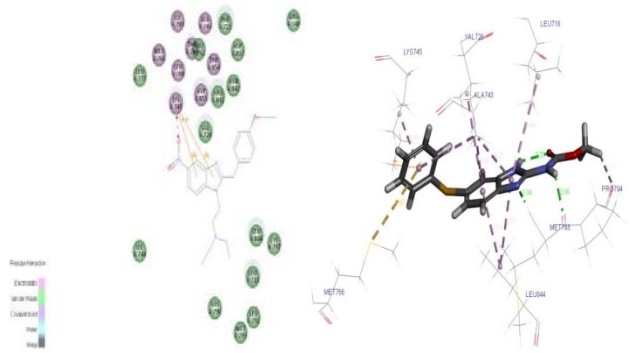


Figure 6: 2D & 3D docking interactions of Etonitazene with HER-2

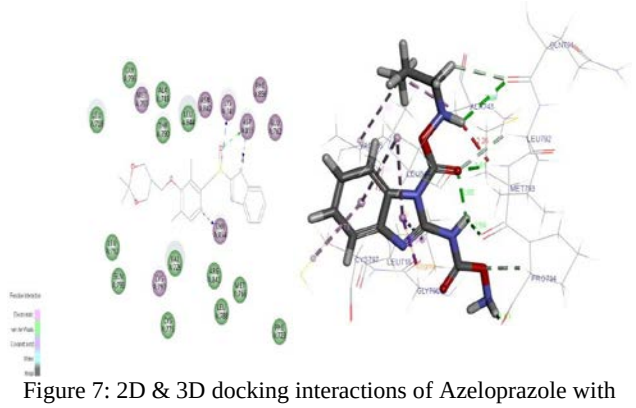


Figure 7: 2D & 3D docking interactions of Azeloprazole with HER-2

Table 2: Molecular libdock and Cdocker Scores

Molecules	Absolute Energy	Relative Energy	Libdock Score	-Cdocker Energy	-Cdocker Interaction Energy
Pantoprazole	57.8635	4.22677	120.079	21.693	35.505
Etonitazene	68.0693	9.93628	114.471	20.481	39.7272
Azeloprazole	77.6464	16.3954	114.334	17.0504	44.5715
Rabeprazole	59.4973	5.11636	112.322	21.3332	38.1977
Galeterone	91.6788	0	107.751	-59.8438	36.9532
Clonitazene	64.335	13.7107	106.907	24.7919	39.6644
cNitazene	60.0565	8.9348	106.28	27.5521	42.7311
Dexlansoprazole	60.6039	7.50975	103.906	15.2817	34.7431
Lansoprazole	60.6039	7.50975	103.906	15.684	34.027
Fenbendazole	73.0404	3.41836	91.7086	25.7361	33.8772
Esomeprazole	72.9989	4.53425	89.0222	15.399	34.2953
Omeprazole	72.9989	4.53425	89.0222	17.6135	37.8896
Mebendazole	82.4324	2.82536	88.1821	12.8252	34.4383
Flubendazole	70.2606	2.35663	87.9466	25.7667	37.2992
Ticlabendazole	75.9433	0.583514	84.7914	9.25192	35.6412
Benomyl	66.904	5.30057	83.1629	20.9357	36.5964
Albendazole	53.9781	0.128912	77.2177	27.7326	31.5392
Fuberidazole	43.8862	0	71.0114	7.19711	24.5018
Carbendazim	56.0938	7.46143	69.2818	9.44861	26.3454
Thiabendazole	48.2462	0	68.863	16.4281	23.1117
Thiophanate_methyl	72.3422	16.7899	60.0909	25.8713	33.7063
Anastrazole	58.1525	58.1525	91.4405	0.83143	36.0614
4LQM	65.6656	0	78.4739	26.4522	42.9923

CONCLUSION

It is well known that almost all drugs used in human therapy possess more than one target and thus can provide off target side effects in addition to their principal activity. If these drugs interact with an off-target pathway with sufficient potency, there is a high likelihood that they could be rapidly tested in patients. We hope that drug repurposing will play a high impact role in developing new cancer drug therapies and bringing these therapies rapidly to patients who are in great need of medicine to cure this deadly disease. It has been concluded that three benzimidazole containing drugs namely pantoprazole, azeloprazole and etonitazene may be taken up for further studies.

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