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Deciphering mechanism of anti-microbial action of non-antibiotic drugs Bupranolol and Propranolol against *E. coli*

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ABSTRACT

Rise of Antibiotic resistance has prompted scientific community to refocus on search of novel antibiotics. Although, knowledge about antibacterial action of non-antibiotic drugs over pathogens is decades old. Almost, no attention was paid in developing novel class of antibiotics using them. β-adrenergic receptors Bupranolol and Propranolol are known to be active against E. coli although precise mechanism is unknown. It was speculated their antimicrobial action is via membrane proteins involved in transport of molecules, drug efflux pumps and energy generation. Therefore, we screened membrane proteome of E. coli in order to decipher precise mechanism and targets of antimicrobial action of these non-antimicrobial drugs. Our findings are in agreement with the notion regarding antimicrobial action mentioned above and following are identified as potential targets for repurposing of the two molecules which includes Drug efflux pump AcrB, lipid importer Blc, Beta barrel assembly machinery complex and ATP Synthase.

INTRODUCTION

Paul Ehrlich "father of chemotherapy" had observed that non therapeutic chemicals are known to possess selective antimicrobial properties. However, his observations gained initial success with discovery of salvarsan and couple of other drugs to treat syphilis and urinary tract infections. But the encounter of antibiotic penicillin in 1928 by the Nobel laureate and Scottish scientist Alexander Fleming had changed the course of curing of contagious disease [1] till date more than 100 kinds of antimicrobial agents have been identified. These antibiotics can cure variety of transmittable disease caused by microorganisms, but the rise of drug resistant 'superbugs' has opened a Pandora box of new threats for researchers [2]. The spreading of drug resistance is accelerated due to human movement around the world and evolutionary selection against antibiotics [3]. Few important examples of antibiotic resistance are, erythromycin resistant *S. pneumoniae* and *Streptococcus pyogenes* [4], methicillin resistant *Staphylococcus aureus*, fluoroquinolone-resistant *S. aureus* [5] and vancomycin resistant *enterococci* [6]. The antibiotic resistance is considered as serious threat at global level by number of scientists [7]. The problem of resistance is so severe that the World Health Organization has warned that the infectious diseases are likely to become untreatable (World Health Organization; Press Release WHO/41. http://www.who.int, 2000).

Drugs are largely classified into antibiotics and non-antibiotics [8]. The medicines known to treat non-communicable diseases but are possessing antimicrobial properties are defined

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as non-antibiotics [9]. The display of microbe killing action of non-antibiotic drugs is observed over variable spectrum of Gram negative, Gram positive bacteria and some fungal species as shown in many investigations [10] [11,12] [13]. The spectrum of non-antibiotic drugs that display antimicrobial properties include for mucolytic agents, diuretic drugs, barbiturates, antihistamines, beta-adrenergic receptor antagonists, proton pump inhibitors, non-steroid anti-inflammatory drugs and psychotherapeutic drugs [14]. The precise mechanism of this phenomenon of non-antibiotics is unknown but it is hypothesized that they act on plasma membrane like the case of eukaryotic cells perhaps via changing cell permeability. The dose at which these drugs show anti-microbial activity is far higher than that is used to treat non-infectious disease. There had been some reports where these drugs are shown to enhance activity of antibiotics to a level that they were able to eliminate even the resistant microbes. To exemplify, combination of β-lactam antibiotics along with phenothiazines made methicillin resistant *S. aureus* susceptible. Few other examples in literature include diclofenac and streptomycin, promazine and tetracycline, trimeprazine and sulfatiazole, propranolol and tobramycin [15]. Few researchers have proposed the treatment of multi-drug resistant tuberculosis by combining phenothiazines like chlorpromazine, thioridazine and methdilazine as these combinations inhibited the microbe both in vitro and in vivo.

Although numerous studies had been made on non-antibiotic drugs still arbitrary understanding of their mechanism is available. In present investigation we have tried to dig deeper and our result not only explain earlier results but also suggest new targets for repurposing of Bupranolol and Propranolol (Figure 1) as an antimicrobial agent against *E. coli*.

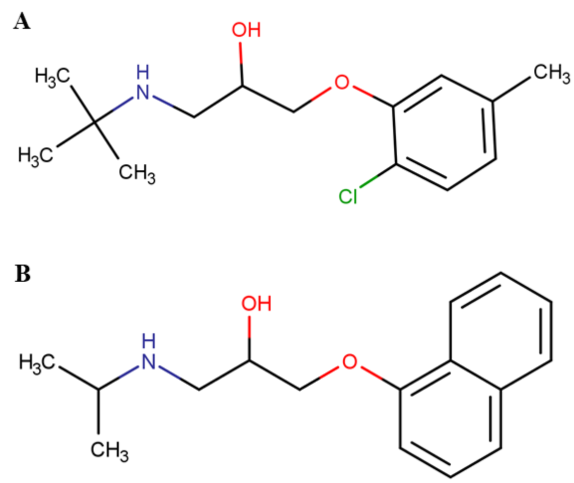


Fig. 1 β-adrenergic receptor antagonists A: Bupranolol and B: Propranolol.

Drug resistance in *E. coli*

E. coli is an essential component of gut microbiome and its pathological variants are known to cause health nuisance. The major infections include life threating blood stream infections and urinary tract infections. The leading cause of resistance in *E.coli* is due a side effect of antibiotic usage both human and veterinary medicine [16]. Numerous cases are reported about resistant *E. coli* outbreak in hospitals [17] [18] [19] and are also isolated from food sources [20] [21] [22] Thus, screening of novel antibiotics for resistant *E. coli* should be taken seriously.

Antimicrobial activity of β_2-adrenergic receptor antagonists bupranolol and propranolol

Both β_2-adrenergic receptor antagonists bupranolol and propranolol, are well known for their antinociceptive effects [23] and are also known to display antibacterial effects against *E. coli* [24]. The major hiccup in projecting these non-antimicrobial drugs as antimicrobial therapeutic agents is that they display antimicrobial activity nearly a hundred time's higher concentration than those found in plasma during standard treatment [14]. In other experiments these non-antimicrobial drugs have shown aggravate the effects of antibiotics [25]. This knowledge can be further explored for repurposing bupranolol and propranolol as an antibiotic compound.

METHODOLOGY

Dataset Preparation

Since most of the previous studies indicate that non antimicrobial studies indicate that action of non-antimicrobial drugs via efflux pumps, cross-membrane ions transport, cell energy transport, alteration of activity of membrane-bound enzymes [15]. Thus we screened membrane proteome of *E. coli* available in RCSB data base (rcsb.org) [26]. We had screened 133 RCSB entries related to *E. coli* membrane proteins with special emphasize on drug efflux pump as it was suggested to enhance microbial susceptibility towards antibiotics.

Ligand preparation

The ligands were prepared using MarvinSketch by referring DrugBank. They were converted into pdbqt format using Autodock tools (1.5.6). All rotatable bonds were made rotatable so that number of torsions was 6.

Protein preparation

All the proteins were segregated into individual chains and were made pure by removing water molecules and other ligands and were formatted into pdbqt format. They polar hydrogens and Kollman charges were added to ensure precise estimation of intermolecular interaction.

Protein-Ligand Docking

The single chain receptors and ligands were docked using Autodock Vina in a windows OS.

Active site cross check

The proteins were subjected to active site predictor [27] to verify predictions made by autodock vina.

RESULT AND DISCUSSION

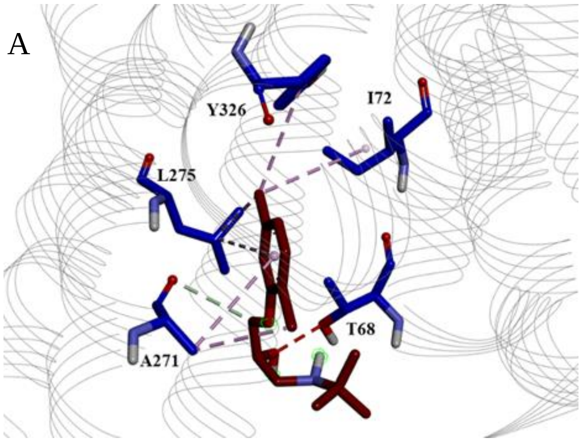
Interaction between Bupranolol and human beta-2 adrenergic receptor

Human beta-2 adrenergic receptor functions as a signal transductor along with G- proteins for the relaxation of smooth muscles and vasodilatory effects [28]. This protein mediates the activation of adenylate cyclase when epinephrine binds to it [29] [30]. [23] have shown that bupranolol more efficiently binds to the adrenergic receptor compared to propranolol.

Bupranolol binds to human beta-2 adrenergic receptor [3ny8] with the free energy of -6 kcal/mol (Table 1). The binding residues **I72, Y326** interacts with alkyl group of bupranolol, **L275** binds with both alkyl and aromatic group, **A271** binds to alkyl group and chloride group while **T68** binds to oxygen (Figure 2 A).

Similarly, 3ny8 binds with propranolol with the free energy of -6.7 kcal/mol (Table 2). **L275** and **A271** interacts with the aromatic rings of propranolol, **N69** binds to the oxygen group whereas **T68** binds to nitrogen group (Figure 2 B).

A



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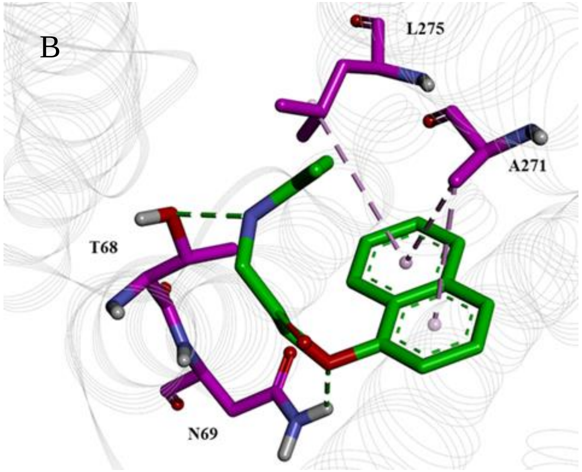

Fig. 2 Drug-Human target interactions: A. Blue coloured (sticks) amino acid residues binding interaction of bupranolol (red stick) with human beta-2 adrenergic receptor [3ny8 chain a] (grey line ribbon). L275, A271, I72, Y326, T68 are the binding residues with the free energy of -6 kcal/mol, B. Magenta coloured (sticks) amino acid residues binding interaction of propranolol (green stick) with human beta-2 adrenergic receptor [3ny8 chain a] (grey line ribbon). L275, A271, N69, T68 are the binding residues with the free energy of -6.7 kcal/mol.

Since, bupranolol and propranolol are known to display antibiotic activity against *E. coli* and speculated to act via inflecting the membrane function. Therefore, we have screened 130 PDB entries of *E. coli* membrane proteins which have lower free energy compared to 3ny8 were shortlisted. Following which we compared the binding residues of bupranolol and propranolol of 3ny8 with those of *E. coli* membrane proteins.

Interaction between Bupranolol with ATP synthase

ATP synthase is a ubiquitous molecular machine which is largely responsible for energy generation and is key to survival of the organism. This enzyme is situated in the inner cell membrane/periplasmic space of the microbe synthesizing ATP the energy currency by combining ADP and inorganic phosphate. This is done by the electrochemical proton gradient formed due to the pumping action of F_1F_0 complex. F_0 part is located in the

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membrane and F_1 subunit lies in periplasmic space [31].

Bupranolol interacts with A_1C_{12} sub complex of F_1F_0 ATP synthase [1c17 chain M] with free energy of -6.8 kcal/mol (Table 1). The binding residues **L237, V141** interacts with alkyl group and aromatic ring of bupranolol, while **A138, I223** binds to alkyl group only and **L126** binds with chloride group (Figure 3A).

Interaction between Bupranolol with lipid transporter Blc Dimer

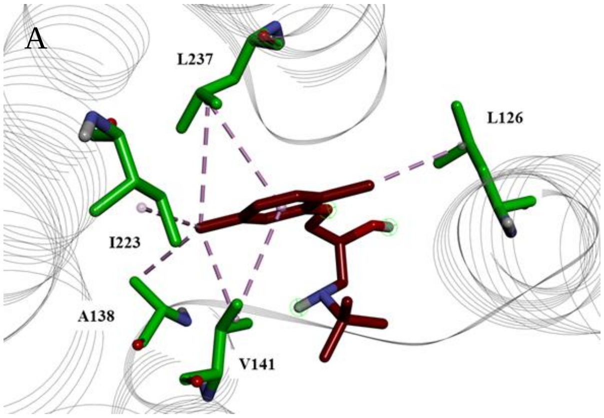
It comes from the class of bacterial lipocalins [32]. Blc is seen to be expressed in starvation conditions and stress like high osmolarity in the cell envelope and is active in its dimer form. Structural and physiological data shows that their role is in transport and storage of lipids specially lysophospholipids for membrane maintenance [33]. It is also speculated that they may contribute towards lipid trafficking in the membranal space. Hence, based on the proposals given by researchers it may act favorably towards the bacteria under stress conditions [34] [35]. Hence targeting this can kill otherwise surviving bacteria.

Bupranolol interacts with Blc dimer [2aco chain B] with free energy of -7.2 kcal/mol (Table 1). Protein residues **L141, V130, Y116** interacts with chloride group of bupranolol, **A62, N76** binds with the aromatic ring while **W139** interacts with both chloride and aromatic group of ligand (Figure 3 B).

Interaction of Bupranolol and Propranolol with Drug efflux pump

Acriflavine resistance protein B (AcrB) is a member of hydrophobic/amphiphile efflux - resistance nodulation cell division (HAE/RND) superfamily [36]. This type of protein generally works along with membrane fusion protein (MFP) and outer membrane fraction (OMF) protein [37]. Hence the functional heteromer is seen in conjugation with AcrA and TolC to extrude cytotoxic agents like antibiotics, dyes, detergents and salts etc. They have a wide substrate recognition hence called multi-drug efflux pump (MDEP). This polypeptide has the key site for ligand recognition and energy transduction. It gets its required energy via proton/drug antiport system [38].

AcrB is important to the survival of *E. coli* because it removes antibiotics and other harmful chemicals from the organism that would otherwise be toxic [39]. The multidrug resistance conferred by AcrB channel protein and its affiliate proteins is a serious medical concern, regarding the antibiotic treatment of different bacterial infections [40]. Hence targeting the MDEP itself provide a chance to overcome the resistance of the multiple drug resistant bacteria.



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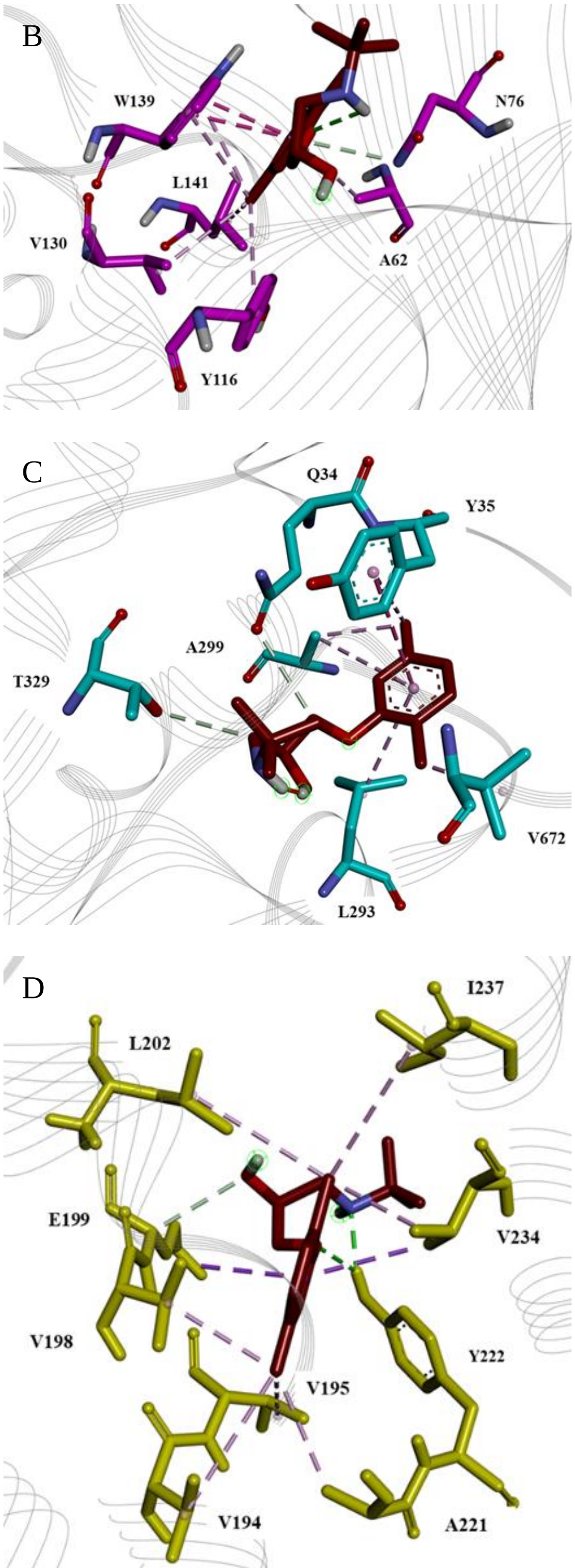

Fig. 3 Bupranolol (red stick) - bacterial target protein interactions: A. Green coloured amino acid residues A_1C_{12} sub complex of F_1F_0 ATP Synthase [1c17 chain M] where A138, L126, L237, V141, I223 are the binding residues with free energy of -6.8 kcal/mol, B. Magenta coloured amino acid residues Blc dimer in complex with vaccenic acid [2aco chain B] where A62, L141, V130, W139, N76, Y116 are interactions residues with bupranolol are with free energy -7.2 kcal/mol, C. Light blue coloured amino acid residues AcrB multidrug efflux pump [2hqd chain A] where L293, A299, V672, T329, Y35, Q34 binding residues with -6.9 kcal/mol free energy, D. Yellow coloured amino acid residues Bam ACDE complex [5d0q chain D] where V194, V195, V198, V234, A221, I237, L202, E199, Y222 are the binding residues with -6.4 kcal/mol free energy.

Bupranolol interacts with AcrB Multidrug Efflux Pump [2hqd] [41] [40] [39] with free energy of -6.9 kcal/mol (Table 1). The protein residues **V672** interacts with the chloride group, **A299, Y35** binds with aromatic and alkyl group of ligand, **L293** binds to aromatic group whereas **Q34** and **T329** binds to oxygen and nitrogen group respectively (Figure 3 C).

Propranolol also interact with Multidrug exporter AcrB [3aod chain B] with free energy of -7 kcal/mol (Table 2). The protein residues **L674** interacts with aromatic group, **S79** and **N81** binds to the oxygen groups whereas **E826** binds to alkyl group of ligand (Figure 4).

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Interaction of Bupranolol with beta-barrel assembly (BAM) machinery

β-barrel assembly proteins or BAM play critical role in inserting newly synthesized outer membrane proteins (OMPs) in the membrane. Due to steric difficulties they are not folded until they reach the periplasm, hence the protein BAM complex works to fold the OMPs into proper shape [42] which play crucial role in fundamental cellular functions varying from physiological, pathogenic and drug resistance.

BAM complex is a multimer composed of proteins Bam-A, B, C, D & E. It is still unclear how the machinery works however few models have been proposed according to which the process is catalyzed [43]. Since, this heteromer is a very essential for assembling the synthesized proteins in the bacterial outer membrane, it can be a very suitable for a drug target. By disabling this complex, the membrane proteins won't be inserted in the membrane appropriately and hence cells will lose their many of their vital functions such as drug efflux pumps, energy generation and transporters of small molecules/ions porins.

It is important to note that Bupranolol interacts with four same residues in BAM as that were found in 3NY8 cavity. It is quite possible that antimicrobial property displayed by bupranolol might be due to loss of the activity of BAM which might be resulting in concomitant disruption of other important cellular function.

Bupranolol interacts with Bam ACDE complex, outer membrane beta-barrel assembly machinery (BAM) complex [5d0q chain D] with the free energy of -6.4 kcal/mol (Table 1). The protein residues **V194, V195, A221** interacts with alkyl group **V198** is interacting with aromatic ring**, V234** interacts with both aromatic and chloride group**, I237, L202** interacts with chloride group, **E199** binds to oxygen at the 2nd carbon**,** whereas **Y222** interacts with both nitrogen and oxygen at 3rd carbon (Figure 3 D).

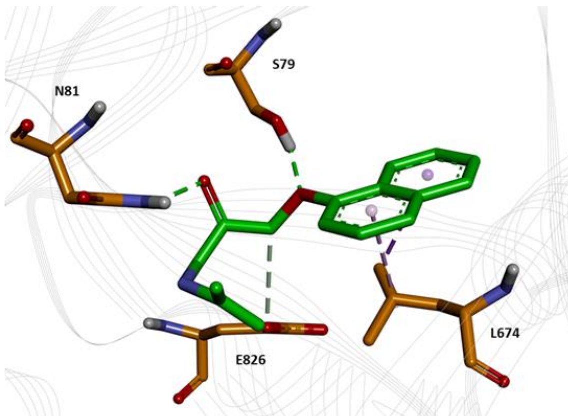

Fig. 4. Propranolol (green stick) - bacterial target protein interactions:

Brown coloured amino acid residues binding interaction of propranolol with multidrug exporter AcrB [3aod chain B]. The binding residues are L674, S79, N81, E826 with free of energy -7 kcal/mol.

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Table 1: Interaction energies and active residues of top targets with bupranolol.

Energy (kcal/mol)	PDB ID	Interacting Residues	Protein Name	Function
-6	3ny8 Chain A	L275,A271,I72,Y 326,T68	Human beta 2 adrenergic receptor	G protein-coupled receptor
-7.2	2aco Chain B	A62,L141,V130, W139,N76,Y116	Blc dimer	Helps in binding of fatty acids or phospholipids for transport, storage and membrane maintenance
-6.9	2hqd Chain A	L293,A299,V672, T329,Y35,Q34	AcrB Multidrug Efflux Pump	Proton motive force-dependent multidrug efflux
-6.8	1C17 Chain M	A138, L126, L237, V141, I223	A_1C_{12} sub complex of F_1F_O ATP synthase	Synthesis of cellular ATP
-6.4	5d0q Chain D	V194,V195,V198 ,V234,A221,I237, L202,E199,Y222	BamACDE complex	Mediates folding and insertion of outer membrane proteins, cobalamin uptake

Table 2: Interaction energies and active residues of top targets with propranolol.

Energy(kcal/mol)	PDB ID	Interacting Residues	Protein Name	Function
-6.7	3ny8 Chain A	L275,A271,N69 ,T68	Human beta-2 adrenergic receptor	Beta adrenergic receptor
-7	3aod Chain B	L674,S79,N81,E 826	Multidrug exporter AcrB	Proton motive force-dependent multidrug efflux

CONCLUSION

Our investigation has far reaching ramifications rather than just being means of investigation of mechanism of antibiotic action of non-antibiotic drug. Initially, Bupranolol and Propranolol were thought to act by mediating primarily with drug efflux pump but our finding indicates that these molecules can inhibit energy generation, proteins assembly and lipid metabolism also. The knowledge gained here can be channelized for development of novel antibiotics. This is need of hour because growing antibiotic resistance is cause of concern at global level. Many experts believe that newly evolved superbugs may go out of control to cause epidemic type of situation.

Further, in a recent study it was shown that non-antibiotic drugs can change composition of gut microbiome [44] via their antibiotic action. The investigators of this study feel that changes in microbiota composition can lead to the side effects which have been overlooked till now. We hope that knowledge gained through this investigation would be taken forward for understanding phenomenon mentioned above.

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Prediction of Skin Sensitization by *in-silico* tools : Today and future

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ABSTRACT

In this article we lay emphasis on using in-silico methods to identify substances for their skin sensitization potential. In vivo animal tests require huge time, constrained by ethical considerations and financial burden. To avoid such problems involved in animal models like LLNA, GPMT and h-CLAT assay computational methods are developed. In view of discussing the advantage of in-silico methods over in vivo animal testing methods in this article we have chosen model like QSAR and three expert systems viz VEGA, Derek Nexus and TIMES-SS and assessed their performance by the use of NICEATM LLNA database.

INTRODUCTION

SKIN SENSITIZATION : Substances that can cause an allergic response when come in contact with the skin are skin sensitisers and the phenomenon is known as skin sensitization. Skin sensitisers are chemicals of low molecular weight that covalently bind to the skin protein through Michael addition, bimolecular nucleophilic substitution (S_N2), Schiff base formation, nucleophilic aromatic substitution (S_NAr), or acyl transfer. Skin sensitization is a result of cumulative effect of many molecular mechanisms and cellular events. The following key events take place during skin sensitization process :

Key event 1: Attachment of allergen to skin protein and the process is known as haptenisation.

Key event 2: Release of pro-inflammatory signals by epidermal keratinocytes.

Key event 3: Activation and maturation of dendritic cells (DC)

Key event 4 : Movement of DC bearing hapten-protein complex from skin to draining local lymph node.

Key event 5 : Clonal expansion of hapten-peptide specific T-cells.

This sequence of pathway leads to allergic response on the skin. Here we draw our attention to the key event 3 of skin sensitization AOP, which is known as h-CLAT. When a compound comes in contact with the dendritic cells it leads to expression of two receptor proteins namely CD54 and CD86. Compounds which lead to the expression of CD54 and CD86 are skin sensitisers while the compounds that do not lead to the expression of CD54 and CD86 are non sensitisers. The pathway for skin sensitization is shown in fig 1. [1-6]

OECD and OECD guidelines for skin sensitization : The Organisation for Economic Co-operation and Development is an economic organisation. It was founded in 1961 with members from 37 countries to revive economic advancement and world trade. The organisation is responsible for providing the guidelines for testing chemicals. These guidelines are a set of specification accepted internationally for testing of chemicals decided on by OECD. Five sections are involved in the guidelines:

Section 1 : Physical Chemical Properties

Section 2 : Effects of Biotic System

Section 3 : Environmental Fate and Behaviour

Section 4 : Health Effects

Section 5 : Other Test Guidelines

OECD guidelines for skin sensitization fall under Section 5. The testing of skin sensitization is performed by GPMT (OECD TG 406) LLNA (OECD TG 429), h-CLAT (OECD 442 E) etc. The mechanism involved with the process of skin sensitization has been summed up as an Adverse Outcome Pathway (AOP). OECD has accepted six testing methods addressing the first three key events of AOP. DPRE marks the molecular initiation event (MIE) of the AOP (OECD TG 442C) by assessing a compound's reactivity towards cysteine and lysine containing peptides. LuSens and KeratinoSens marks the second key event of AOP (OECD) by examining the activation of Nrf2, the transcriptor factor in keratinocytes. h-CLAT marks the third key event by examining the expression of CD54 and CD86 proteins. (OECD 442E). [7-11]

OECD TOOL BOX : It is a software sketched to make predictions based on practical quantitative and qualitative relationship between structure and activity. It is developed and implemented in phases. The charge of its development is under the Laboratory of Mathematic Chemistry and OECD takes the charge of entire governance.[12] The toolbox is a stand-alone computational workflow with a number of advantages. The first and foremost merit of the toolbox being that it is freely available. The toolbox is also upgrated in regular interval. The basis of the prediction done by toolbox is the category approach. Experimental results that are available for the source substances are used to fill gaps for target substances. The parts involved for a logical workflow on the basis of category approach are : chemical input, profiling, endpoints, category definition, filling data and report.

Chemical Input : The module of 'Chemical Input' delivers the user means to enter target chemical. The aim of this part is to assure correct assignment of chemical structure to the target chemical.

Profiling : The module of 'Profiling' retrieves appropriate information electronically in automated alert forms on target compounds. Information about potency to destroy macromolecules, reactivity, reaction mechanism with receptors is provided.

End point : This part helps the user by providing an electronic process to fetch data. This module convert data into information.

Category definition : This module helps the users to group chemicals into significant category on the basis of similarity with target substance. Similarity does not mean only structural similarity but also chemical and physical properties, reaction profile and mechanism of interaction.

Data gap filling : For making prediction this module gives the user three options - trend analysis, read options and QSAR models. The concept for determining toxicity comprises of two factors – perturbation of toxic chemicals into biophase and their interaction with target sites.

Report : This module helps user to download audit trail of sequence and to arrive at the result.

In the new version of toolbox various modifications has been made to improve the core features. For instances two more descriptors were added to identify chemicals ID. Presently Daylight SMILES represents the connectivity of chemicals. Modifications in user interface has also been made.[12,13]

QSAR Model : To overcome many problems associated with animal testing methods certain models are developed. These models can short list compounds whose predicted activities are good from a large pool of wet lab studies. They can also be used to predict activity of unknown or new compounds. QSAR is such a model. It is a mathematical relationship which correlates measurable or calculable molecular properties to some specific biological activity in terms of an equation. QSAR models can either be local or global. This classification depends on the scale of the chemical space covered. Global models have a large domain of application in comparison to local model. On the contrary local models have high prediction accuracy than global models. Using QSAR we can estimate physical or chemical properties of unknown compounds based on known compounds. These properties are also called descriptors. There are many descriptors available and differentiated according to the type of information. 0D descriptor encodes properties like number of heavy atoms, molecular weight, 1D descriptor encodes whether a substructures is present or absent in a molecule. 2D descriptor encodes the connectivity of atoms present in molecules. 3D descriptor encodes properties such as HOMO-LUMO energies.[1], An approach of QSAR model is shown in fig 2.

DATABASES : A database is an arranged assembly of structured information or data electronically stored in a computer. Information about gene, genome, peptide sequence etc are stored in an organized manner. A perfect database should be comprehensive, annotated and should have a simple, easy way to understand structures. It consists of basic units called records or entries. Each record consists of fields, which hold predefined data related to the record. The data stored in the database should have minimum redundancy, should be executable, analysable, exchangeable. Data should be retrieved easily. NICEATM LLNA is one such database.

NICEATM LLNA Database : It comprises of 515 organic compounds of which 329 are sensitisers and 180 non-sensitisers. All the substances are provided with SMILES codes. Using CACTUS translator online the SMILES code are converted to a V2000 sdf file to process in different expert system.[14-16]

EXPERT SYSTEM : These are the computer applications developed to solve complex problems in a particular domain, at the level of extraordinary human intelligence and expertise. Dearden (1997) defined expert system as – "An expert system for predicting toxicity is considered to be any formalised system, not necessarily computer-based, which enables a user to obtain rational predictions about the toxicity of chemicals. All expert systems for the prediction of chemical toxicity are built upon experimental data representing one or more toxic manifestations of chemicals in biological; systems (the database), and/or rules derived from such data (the rulebase)". Expert systems maybe classified on the basis of the type of rules in the rulebase. The classifications are as follows :

i) Knowledge based expert system
ii) Statistical expert system
iii) Hybrid expert system

Knowledge based expert system : A knowledge based expert system has been developed with a knowledge of relationship between structure and toxicity. It lays emphasis on the need to understand the mechanism of action and metabolism. Derek Nexus is such a knowledge based expert system. It is marketed by LHASA Ltd. and was developed using dataset of GPMT in collaboration of Unilever in 1993. It contains 361 alerts. An alert is a substrate responsible for toxicity in company with associated literature, reference, comments and example. The common reaction mechanisms that are taken into consideration involves acylating agents, Michael electrophiles, alkylating agents, aldehyde etc. Files from NICEATM datasets are loaded in sdf files into Derek Nexus. However Derek Nexus is not a stand-alone tool as it is able to identify the potential for sensitization but not the absence of sensitization. [14-16]

Statistical expert system : One example of statistical expert system is VEGA. This model was developed on the basis of LLNA data from Gerberick et. al. under the project of CAESAR funded by EU. The system uses a misty algorithm based on eight descriptors. The algorithm designates substances as sensitisers and non-sensitisers. In VEGA substances are ranked as good, moderate and low on the basis of structural similarity of substances with substance in the training set. After the predictions are made using VEGA they are exported out as a text file (.txt) The dataset from NICEATM are loaded using the available SMILES code.[14-16]

Hybrid expert system : TIMES-SS is an example of hybrid expert system for predicting skin sensitization. It was developed by the Laboratory of Mathematical Chemistry at

University As. Zlatarov Bulgaria. The datasets used for its development was three main data sources- LLNA,GPMT and BgVV[1]. TIMES-SS enciphered structure skin metabolism and structure toxicity relationships. This expert system evaluates chemicals and distinguish them into 3 categories :

Category A : constitutes remarkable contact allergen.

Category B : constitutes substances showing potential for contact allergen.

Category C : represents controversial or unimportant contact allergenic potential.

In this expert system datasets are incorporated using the SMILES code derived from NICEATM dataset. The results are saved as text file (.txt).

The performance of these three expert systems was evaluated by calculating the sensitivity, accuracy, specificity and balanced accuracy. It was seen that TIMES-SS and Derek Nexus had higher balanced accuracy and accuracy than VEGA. Whereas sensitivity of VEGA was found to be higher than both TIMES-SS and Derek Nexus but associated with lower specificity.[14-16]

NEED AND ADVANTAGE OF USING IN-SILICO METHOD FOR SKIN-SENSITIZATION :

In recent years a large number of in-silico models are developed. These models can successfully replace animal models and in-vitro testing methods. In general animal experiments evokes ethical issues, they are also associated with human risk. The 7th amendment of Cosmetics Directive of European Union prohibited the sale of cosmetics tested on animals. This leads to the development of in-silico methods. The main advantage of using in-silico methods lies in the fact that the predictions are obtained in short time and it is also very cost effective. In addition they do not require materials for testing and also do not exhibit various practical problems like limited solubility, evaporation, aggregate formation. It is also seen that these tests provide accuracies upto approximately 65% to 80% when measured against LLNA and other human and animal data. [1]

CONCLUSION AND FUTURE OF USING IN-SILICO METHODS FOR SKIN SENSITIZATION :

At present no single algorithm or model exist that can consistently surpass all others. Strategies are followed to expand the accuracy of prediction of the models. For successful acceptance of the computational models and their use on regular basis they should obey the guidelines for linear QSAR models. The data used for testing and model building should be revealed for reproducibility. The domain of application should also be understandable. In-silico models and methods for skin sensitization are still not fully reliable. They cannot predict skin sensitization caused by metals and mixtures. However the available datasets offer opportunities for evolving new methods and models. Advance modelling techniques, growing accessibility of experimental data are expected to meet the demand of accuracy and applicability for using

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these methods for hazard assessment of skin in near future.[1]

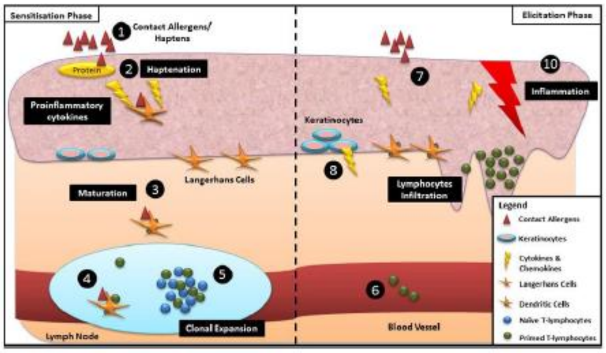

Fig 1: Key events associated with skin sensitization.[17]

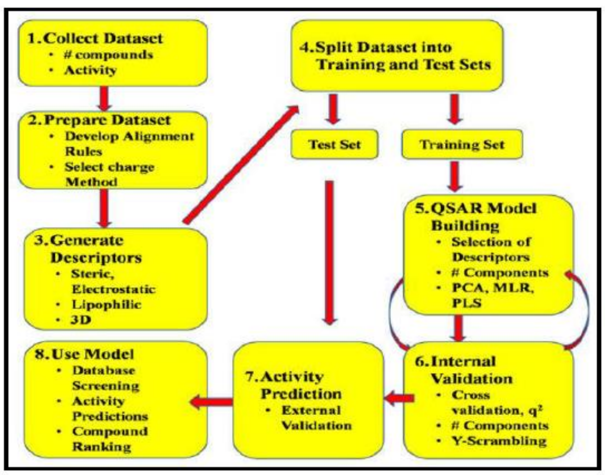

Fig 2: A schematic representation of QSAR model [18]

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A revisit to the tycoon race (MIC Switch) & It's status quo

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ABSTRACT

The largest portion of pharmaceutical sales comes from chiral drugs, either de novo or by developing them from formerly sold drugs. The economic relevance of these drugs are indicated by the fact that, worldwide sales of chiral drugs reached the figure of more than 300$ billion in 2013. When a drug comprising of two enantiomers (racemates) is substituted by a single purified version of itself (Enantiopure drug), it is called as "Chiral switch". Most individuals be it patients or physicians are unaware of this term. The switch has its own unique properties making it interesting from a legal standpoint, often found in context of patent expiration and was first exploited by Astra Zeneca over Nexium more than a decade ago. The issue struck headlines of many major newspaper and found its space in scientific journals too. Although, this issue is no more covered by either of them but the practice of patenting pure enantiomers continues to exist and end users (patients) bearing cost of it. Therefore, we have attempted to alarm both researchers and health professionals about the same, explaining enantiomers, their patentability and how the big companies are exploiting it.

INTRODUCTION

It has happened again, another pharmaceutical company has launched a drug that has been available as a generic elsewhere in the world for years at a surprisingly escalated price [1]. Have you ever thought about why we pay heftily for these life-saving prescription drugs? Do you really think it requires millions of dollars to make a new drug? [2] We as individuals pay the price again and again: firstly by promoting researches which are aided by the country authorities and secondly by purchasing those prescription drugs at higher rates [3]. The accurate figure might be about 1/5th of the total sum, it depends on what theory we decide to believe in [1]. Although, these facts appeared in the past decade but the trend still continues in the pharmaceutical industry.

The most sensitive topic for the pharmaceutical industry is Drug Pricing. When it comes to revenues, pharmaceutical giants undoubtedly lives up to their status. The gains from one generation of drugs are re-invested for the next, which provides a strong argument for high prices. The industry smartly tricks us through advertising campaigns into buying prescription drugs by changing (forcing) our perception into what they want us to see, thereby making the people feel content about the cost they pay [2, 4].

A chiral drug is a single molecule that has the ability to co-exist as two non-superimposable mirror images (owing to difference in spatial arrangements) known as enantiomers (**Fig. 1(a)**). These enantiomers have different biological properties but similar chemical structures and are distinguished as R- (*from Latin rectus meaning right*) and S- (*from Latin sinister for left*). When both these enantiomers are present as an equimolar mixture (50:50), it forms a racemate (**Fig. 1(b)**) and is designated by the prefix (±) or RS and has no overall optical activity [4-7].

Chirality is a rudimentary feature of environment and penetrates throughout the entire living world. We are under continuous impact of it since the beginning of time. The evidence surrounds us all, from amino acids having L-configuration to ordinary sugars of D-isomers. Our biological system displays 'handedness' influencing various drug-receptor interactions [5, 8]. It is important to note that, more than 50% of prescription drugs presently in use are chiral compounds and nearly 90% of them are racemates [9].

In a physiological environment, two enantiomers work differently. Although, each enantiomer has identical physical and chemical properties (boiling point, density & chemical reactivity) but has different biological activities (due to its different 3-dimensional arrangement) like pharmacology, mechanisms, toxicology, metabolism, pharmacokinetics etc. making each of them distinct from its counterpart [9-11]. Just like a right-handed person cannot wear the hand gloves of a left-handed person, one enantiomer cannot fit into the active site of another enzyme [12]. Chirality is an essential component for drug discovery [13] and is indicated by the examples given in **Table 1**. This characteristic feature of chiral drugs is exploited by the pharmaceutical industry to acquire patents and gaining profit by subsiding the generic competitions [14].

It becomes necessary to analyze a racemate in clinical laboratories as well as in pharma industries to eradicate the undesirable isomer. This increases potential benefits such as improved safety margin, different rates of metabolism for each enantiomer, decreased drug interactions, and obtains a favorable therapeutic drug with the right pharmacokinetic characteristics for the patient. At times, some chiral switches have led to unpredicted toxicity which has ensued in either removal of enantiomer from market or a pause in its manufacturing [18-21]. Drug companies should take up clinical trials before using chiral switching mechanisms and should have well conducted proofs that the chiral switched drug would be cost effective and would improve the condition of the patients rather than their patents [14, 22, 23].

Apart from enantiomers, we have diastereomers which exhibit different physical and chemical properties along with different biological activities. Together enantiomers, diastereomers and geometric isomers (cis-trans isomerism) are called Stereoisomers, molecules having same chemical formula but different spatial arrangement (**Fig. 2(a) & 2(b)**). Diastereomers are non-superimposable chiral centers but are not mirror images, unlike enantiomers (**Fig. 2(c)**) [24]. The configuration of double bond is specified with a prefix, E (*from Entgegen meaning in opposition to*) or Z (*from German Zusammen meaning together*) based on whether the substituents of higher priority are on opposite or same side respectively of the double bond. For example, **Triprolidine** is a H_1- antihistamine with anticholinergic and sedative properties, its E-configuration being 1000 times more active than the other is used for therapeutic purposes [25, 26].

Like other biological systems the human body too has an amazingly highly selective chiral environment leading to interaction between enantiomers and the target proteins, which produces a series of cascading events that eventually leads to synergistic or antagonistic effects. Thus, making Stereochemistry an important component of drug discovery [28, 29]. The enantiomer having higher pharmacological activity i.e. highest affinity is called "**Eutomer**", and one with least affinity is called "**Distomer**" [5, 30]. The more the chiral centers present in a drug molecule, the more complex the situation becomes for treating diseases [29, 16]. Enantiopure drugs are essential as the distomer metabolizes using a dissimilar pathway and generates unnecessary burden on the human system. Distomers leading to toxicity or adverse effects are known as teratogenic isomers, they create side effects, genetic diseases and at higher dosages may even lead to death [9, 22, 30]. Due to which the market authorization for enantiopure drugs has escalated rapidly with simultaneous downfall of belligerent racemates.

The shift in pharmacology from ''limited dimensionality'' to a specific spatial arrangement leads to a stress in the system due to the exposure of a 3-D world to the 2-D one. However, if dimensional considerations result in enhanced drug safety and therapeutic effect, then the double trouble involved is worthwhile [31].

Separation of racemates into respective enantiomers needs extra time, money and energy due to the additional steps involved. This is a major drawback for production of enantiopure drugs from the industrial standpoint [17]. When a drug has a patent protection, only the owner pharma company can manufacture, market and eventually earn profits over it. In most of the countries worldwide lifetime of a patent is 20 years [14, 32]. When the patent expires, the drug can be developed and vended by any other company- at this point the drug is known as generic drug. The availability of generic drugs removes the monopoly of the patent holder, leading to drop in prices (more competition) and ensuring the reach of life-saving drugs to the common people [32-34]. This way chiral switch has firmly established its role in the drug industry, they have merely become a tool for drug life-cycle management [35].

A synopsis of merits and demerits of Enantiopure drugs over Racemates are given below:

MERITS [4, 7, 14, 20]

- Absence of Distomers.
- More selective pharmacodynamic profile
- No enantiomer–enantiomer drug interactions
- Absence of bioinversion of enantiomers leading to toxicity
- Enhanced solubility in aqueous solution leading to better intravenous dosage
- Chemical feasibility due to lesser drug interactions
- Economically beneficial (clinically and in market)

DEMERITS [4, 7, 20, 36]

- No significant therapeutic advantage
- Financial risks of R&D
- Difficult to store and maintain its stability due to change in shelf life
- Spontaneous racemization might take place
- Higher purification cost
- Reduces affordability

THE MOST FAMOUS PRICEY PURPLE PILL

Over last 15 to 20 years chiral switch has been an area of substantial concern [16]. Chiral Switches has led to commercial accessibility of numerous drugs as both enantiopure and racemates at the same time [37]. AstraZeneca (AZ) earned $48 billion in the past decade without even actually investing in the drug discovery. The major contributor to the growth of chiral switch drug is **Nexium (Esomeprazole)**, [38] a proton pump inhibitor and a heartburn medication by AstraZeneca (AZ), a Eutomer. Nexium was the brainchild of many scientists, lawyers, strategists and marketers [39, 40]. AZ started working 6 years prior expiration of Prilosec, in 1995, on preparing a plan to avoid the threat of generic erosion [41].

Our stomach produces acid for proper digestion but surplus of it can create a disorder, inflammation, irritation and heartburns leading to formation of ulcers [42, 43]. Proton Pump Inhibitors (PPIs) subdue the gastric acid secretion by hindering the enzyme H^+/K^+ ATPase, mostly termed as proton pump and hence inhibiting both basal and stimulated acid secretion on the gastric parietal cells (**Fig. 3(a)**) [44]. The presence of Histamine, Acetylcholine and Gastrin activates the parietal cells [45, 46]. The PPIs bind non-competitively and has a dose dependent effect. They are also time dependent as their inhibition continues for 72 hours and it takes about 3 to 4 days for the acid secretory system to return to normal [47, 48]. They are the 2nd most commonly prescribed drug class worldwide, after cholesterol regulators [41, 42] and Nexium tops the chart. The formulations having PPIs as a constituent makes them gastro-resistant but too much of it can lead to an alkaline environment in the stomach leading to digestion related disorders, and therefore should be taken under the guidance of a doctor (**Fig. 3(b)**) [49]. It should be taken for a short duration only to cure the reflux symptoms (acidic regulation).

Getting back to Nexium story, AZ started a project and named it "**Shark Fin**" due to the graphical representation of income from the drug, [39, 50] Omeprazole, primarily used for treating **GERD (Gastro Esophageal Reflux Disease)**, Zollinger Ellison Syndrome, Ulcer burns caused by Helicobacter pylori, Crohn's disease ulcers etc. It is given as a Non-Steroidal Anti-Inflammatory Drug, NSAID to patients [33, 45]. There was a sharp increase then a sharp decline in the sales of Omeprazole drug on patent expiration, till AZ devised a strategy to combat it. Omeprazole is a racemate comprising both R- and S-Enantiomers & the company made a smart move by launching Nexium (purified S- Enantiomer) just about when the patent for **Prilosec (Omeprazole)** was on the verge of getting over [6, 51].

Nexium was only a "*doppelganger*" of Prilosec with systematic stereo-selective metabolism and tweaked chemical structure [52]. PPIs are metabolized by the enzyme CYP2C19 to a varying degree [53]. On basis of pharmacokinetic studies it was proposed that the efficiency of S-omeprazole undoubtedly depends on metabolic pathway (has more potential to interact with CYP2C19) and excretion from the human system [47, 54, 55]. AZ pointed out the fact that the CYP2C19 only metabolizes R-omeprazole to hydroxyl-omeprazole (hydrophilic) [56] and being hydrophilic it is easily excreted, whereas CYP3A4 metabolizes S-omeprazole to sulfone which is lipophilic [57, 58] and thus retained in the body for longer duration [59]. Since, the excretion of this metabolite is relatively slower it provides less discrepancy between individuals, [20, 47, 54] better control of intragastric pH [60] and higher bioavailability [61, 62]. This is why the approved dosage to treat GERD for esomeprazole is 40 mg whereas for omeprazole is 20mg. Yet another smart move, they compared higher dosage of Nexium with lesser doses of Prilosec, with the cards been marked in that way, Nexium looked like an upgrade [6, 51, 63-65].

They launched the new version of the drug in 2000 and had gained sales of $4.6 billion by 2005 [66]. The patent extension gave AZ 20 years of additional security on the same molecule [8]. Further the company claimed that Nexium could heal the esophageal erosion caused by GERD [49]. This led aside the generic competition and resulted in decline of sales of omeprazole [40]. Owing to the boost Nexium gave to AZ, it gained the title of “wonder drug”, [44] “the famous pricey purple pill” [39] and “the me-too pill” [67, 68]. It ranked 2nd with prescription sales clocking to $14.1 billion and 2.8% growth (**Fig. 4(a)**) [69]. AZ’s Nexium sales against Prilosec were on peak in 2013 and overall stock prices of the company were highest that year, according to various surveys conducted by Drugs.com (**Fig. 4(b)**).

Nexium symbolizes everything that is wrong with the pharma industry [50]. This scandalous practice claiming that the enantiopure drug had better clinical benefits can be cited as an instance of corporate waste of healthcare verticals. A nasty intelligent move extended their product life and their market monopoly, allowing them the evergreen profits. They not only earned by overcharging consumers but also tricked the insurance companies into believing the decorated twisted facts [70]. Thus, Nexium can be referred to as “Dark Phoenix” of the pharma industry.

But do you still believe that the rise of Nexium is completely the fault of the pharma industry alone? Is it? If the physicians consistently prescribe drugs, insurers regularly remunerate for them and individuals keep buying them at higher rates, then there is certainly more than one perpetrator in the prescription-drug scam [50].

OTHER ME-TOO PILLS

Chiral switch has helped in developing numerous drugs, it is amazing how two molecules that just differ in spatial arrangement of their atom can have extremely different biological activities and thus extraordinarily different effects on the human body! [17] Racemates exist as equimolar mixture of opposite enantiomers but in some cases, like ibuprofen [71] and thalidomide, [12] the enantiomers interconvert in vivo, which makes the preparation of a pure enantiomer for therapeutic purpose utterly ineffective. The progress in synthetic and analytical chemistry has made possible production of pure stereoisomeric drugs but it is very challenging to rationally assume the pharmacological or biological activity difference between two enantiomers [72, 73]. The cases (**Table 2**) are the individual enantiopure me-too drugs which have different or opposite effects [74] and therefore should be carefully manufactured by pharmaceutical industries for curing ailments unless its racemization conversion rate is slowed down [75].

The drugs mentioned in **Table 2** helped in escalating the stock market prices of the above mentioned pharma companies, leading to a boost in money plundering [84] and thereby welcoming more racemic switch marketing strategies. The belief that enantiopure drugs are safer and more effective against racemates, has led to constant pressure on the evaluation, development & manufacture of Enantiopure drugs as products [85]. More than 50% of the drugs, including many of the best sellers in the world are chiral drugs [86]. The timing of chiral switches by these pharma companies of these tycoon drugs was vital. The new single enantiomers were launched ideally before the expiration of the patents that covered the racemate, with extended distinctiveness and before the infiltrations of the respective generic drugs [87]. All these gave rise to a new era of **"Tycoon Race-mic Switch"**, which was no less than a race for money plundering schemes (**Table 3**).

It can be noted from both the Tables 4 & 5 that majority of chiral centre are found as NSAIDs. Mostly, the S-Enantiomer has the capacity to inhibit prostaglandin activity whereas the R-Enantiomer has poor inhibitory properties. At times, R-enantiomers are not inert and may cause different or opposite effects. The conversion of R- to S- depends upon the property of NSAID as well as the biological factors of our systems. The S- to R- conversion is next to very rare in NSAIDs, the enantiopure drugs like Dexketoprofen and Dexibuprofen are 100- fold more potent than their respective R-forms. They are as good as half doses of racemate with more efficiency and hence provides better treatment for inflammations [111].

For any given chiral drug, it is better to consider the enantiomers as distinct drugs with different properties unless proven otherwise. Hence, no generalizations can be made concerning enantiomers as they exhibit a wide distinction in their performing actions [112]. These actions can be categorized as:

1) *Equipotent enantiomers* (e.g. – **Carvedilol**, the (S)-(–)-isomer has 100 times greater potency as β-adrenoceptor blocker than (R)-(+)-isomer. However, both the isomers are approximately equipotent as α-adrenoceptor blockers and **Propranolol** as local anesthetic).
2) *One enantiomer with most activity* (e.g. - **NSAIDs, β-blockers**).
3) *Both active enantiomers with differing range in similar therapeutic and toxic effects* (e.g. - **Ketamine**).
4) *Both enantiomers show different pharmacological activities* (e.g. – **Thalidomide, Dopa, Ethambutol**) [9, 12, 15, 17, 81, 113].

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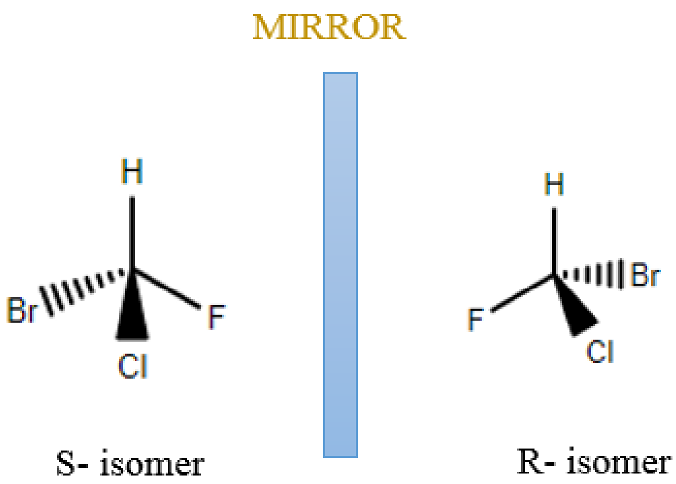

Fig. 1(a) Non- superimposable mirror images of Bromochlorofluoromethane

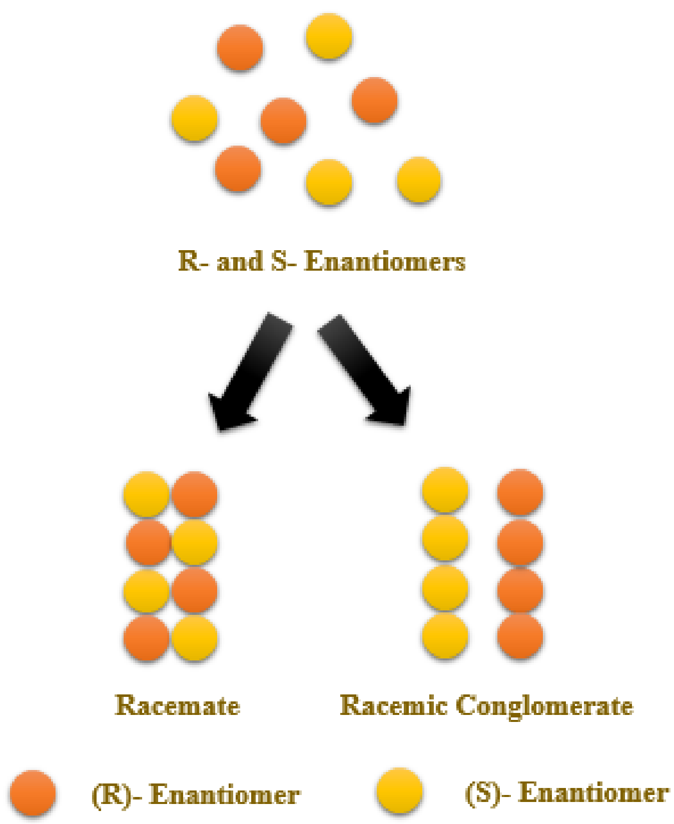

Fig. 1(b) Racemate vs Racemic Conglomerate.

The term 'racemic mixture' is to be avoided as it is most commonly used as a synonym for both 'racemate' and 'racemic conglomerate'. **Racemic Conglomerate** is formed when one form of enantiomer molecule possess higher affinity for the same form compared to the opposite enantiomer, the molecules form an ordered 1:1 complex. Whereas in **Racemate** mixtures, enantiomers of a chiral molecule has same affinity for opposite forms. The former can be separated with ease [4].

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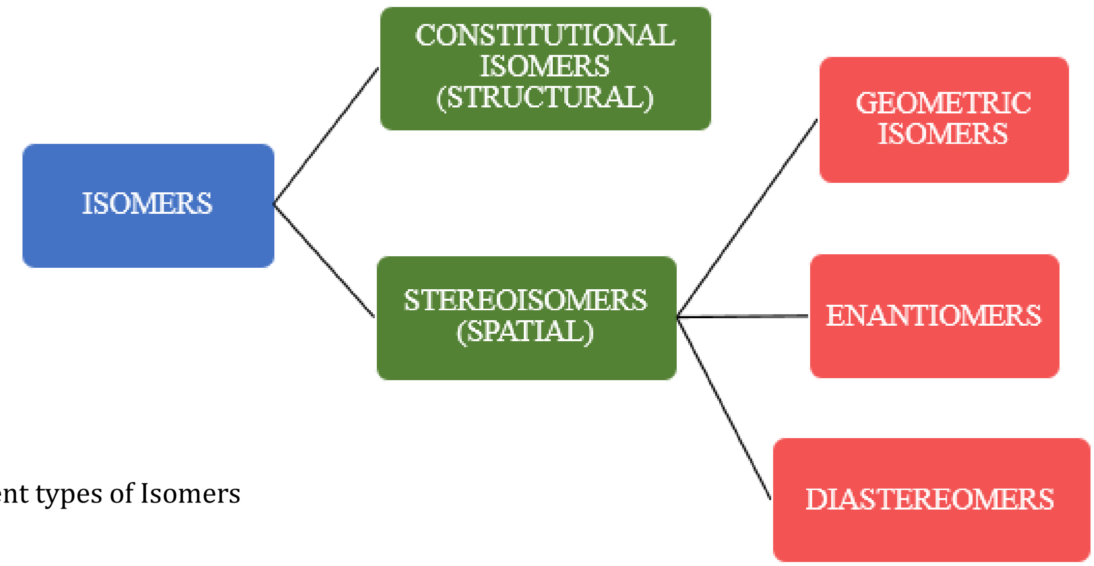
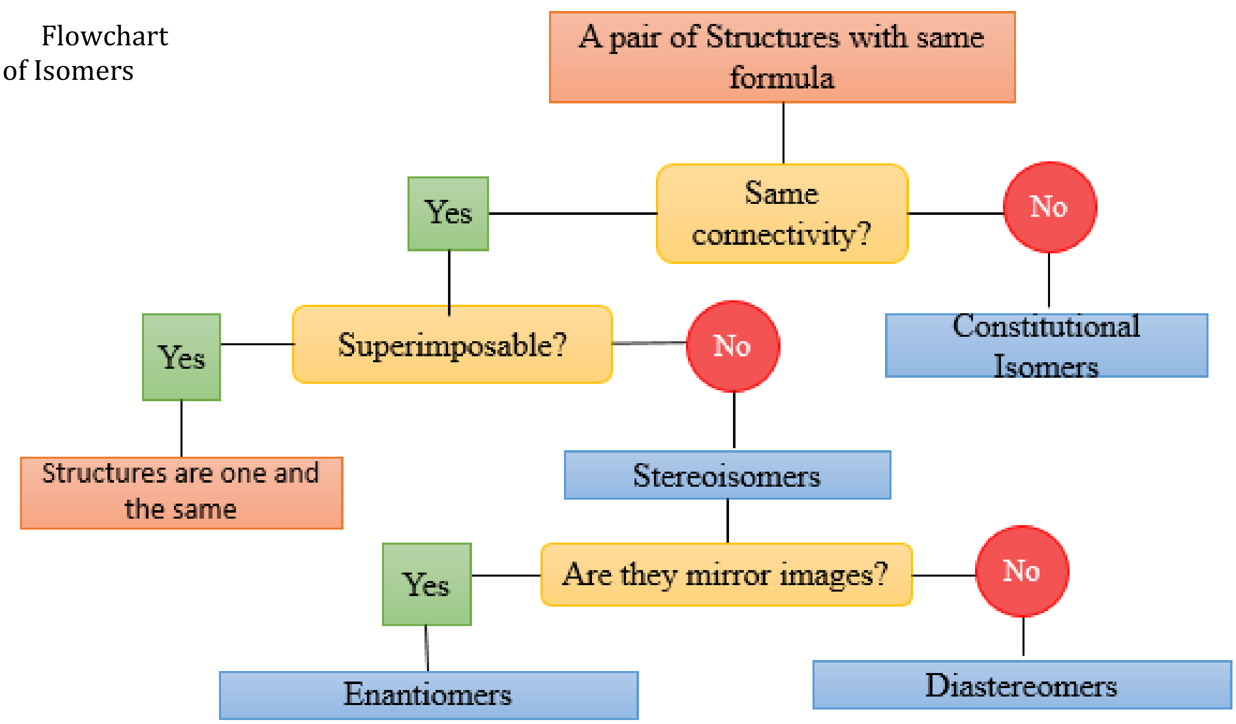


Fig. 2(a) Different types of Isomers

Fig. 2(b) Flowchart systemization of Isomers



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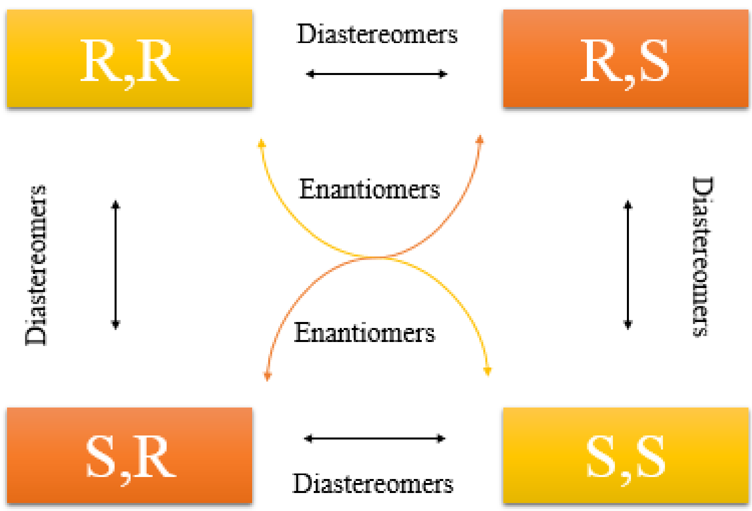
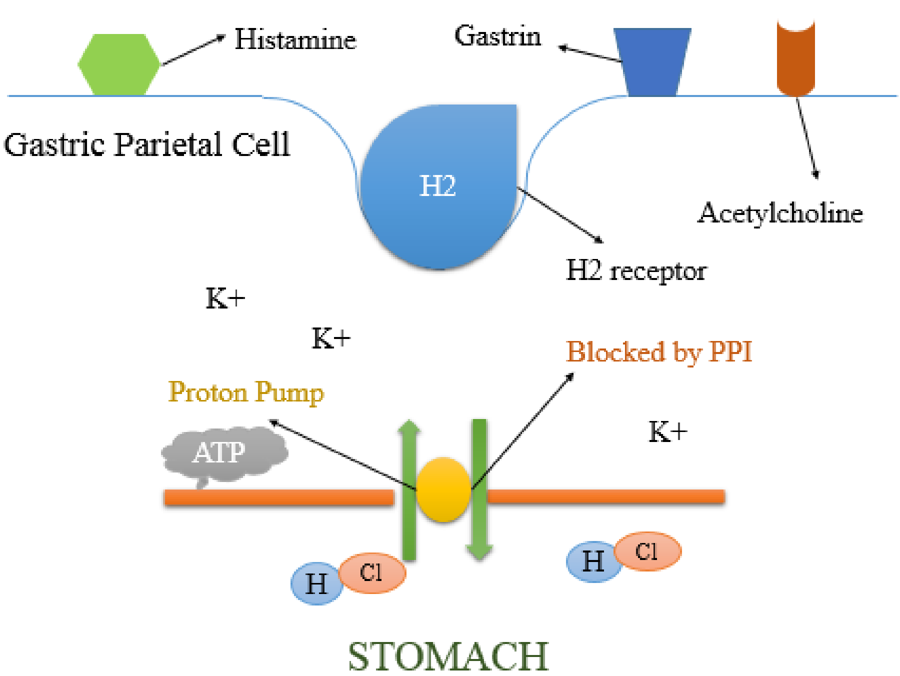


Fig. 2(c) Enantiomers vs Diastereomers Diagonal arrows are **Enantiomers** having non-superimposable mirror images. Parallel arrows are **Diastereomers** having one non-superimposable stereo center and are not mirror images [9, 27].

Fig. 3(a) Mechanism of Action of PPI



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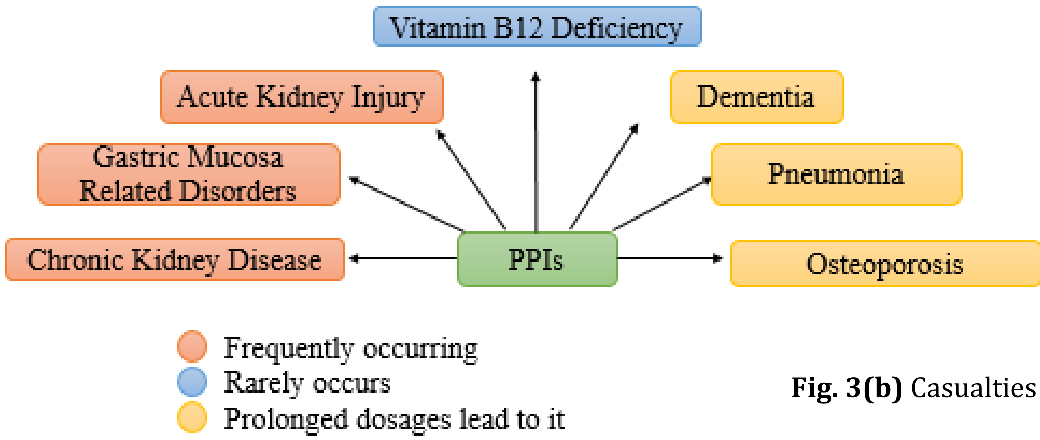

Fig. 3(b) Casualties due to PPI [45]

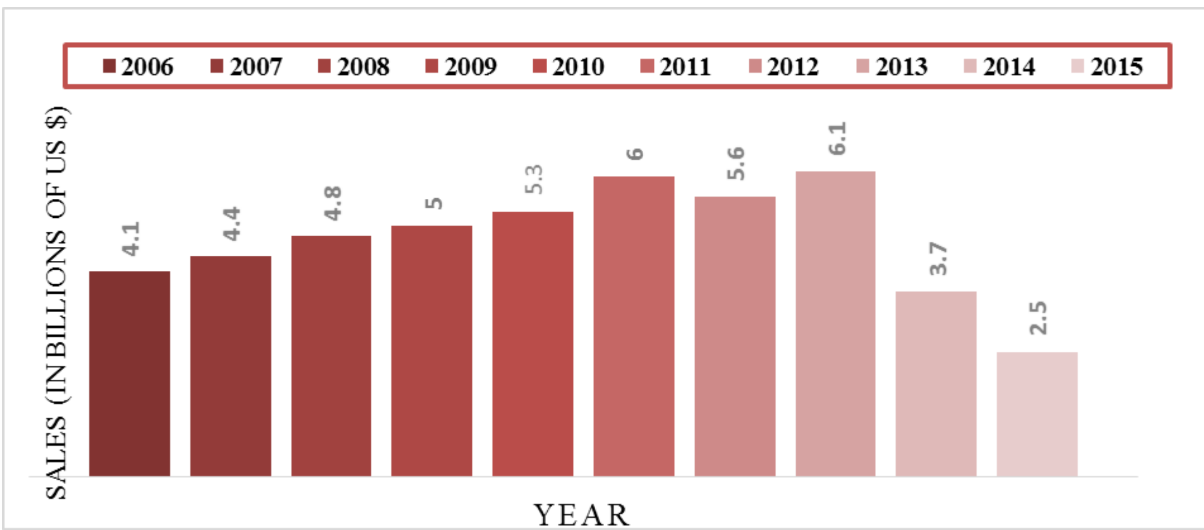

Fig. 4(a) Nexium Sales for the period of 2006-15

Fig. 4(b) Visual representation of surge in sales of Enantiopure Nexium against Prilosec in 2013.

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Table 1 – Various Therapeutic Classes Has Various Chiral Drugs [15-17]

Therapeutic Drug Classes	Examples
Antiarrhythmic	Propranolol, Propafenone, Sotalol
Antibiotics	Ofloxacin, Moxalactam
Anesthetic	Albuterol, Ketamine, Prilocaine
Antihistamine	Chlorphenamine, Terfenadine, Cetrizine
Antimalarial	Chloroquine, Halofantrine
NSAIDs	Ibuprofen, Ketoprofen, Naproxen
β- Blockers	Propranolol, Sotalol, Metoprolol
β-adrenergic	Salbutamol, Terbutaline
Opiate Analgesics	Methadone, Methorphan
Proton Pump Inhibitors	Omeprazole, Rabeprazole, Lansoprazole
Calcium Channel Blockers	Verapamil, Nimodipine

Table 2 – Different Physiological Properties Of The Enantiomers Of Some Drugs

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RACEMATE	PURPOSE	R- ENANTIOMER	S- ENANTIOMER	REMARKS	REFERENCE
Dopa	Effective in treatment of Parkinson's disease.	Used in curing Parkinson's disease	Causes deficiency of leukocytes and makes the person susceptible to infections.	Only L-Dopa is used in therapeutics as D-isomer leads to grave toxicity & agranulocytosis (lowered WBCs count)	[76]
Ethambutol	Anti-Mycobacterial Drug	Causes blindness	Used to treat Tuberculosis	D-Enantiomer helps in treatment of tuberculosis and the L-ethambutol causes blindness irrespective of R- and S- Enantiomeric form.	[77]
Ibuprofen	Analgesic and Non-Steroidal Anti-Inflammatory Drug	They are totally inactive	Dexibuprofen acts as the pain killer and is over 100-fold more potent	In human biological systems only inactive R-Ibuprofen undergoes chiral inversion into its active S-isomer. The vice versa is not possible	[76]
Ketamine	Anesthetic	Causes agitation, hallucination & restlessness (Stronger)	Causes less agitated behavior and better analgesia (Weaker)	Both causes dissociative hallucinations, agitated behavior and analgesia	[96]
Methorphan	Synthetic opioid	Levomethorphan is a potent opioid analgesic	Dextromethorphan is a dissociative cough suppressant as well as a hallucinogen.	The narcotic analgesic levomethorphan is not commercially available as it leads to various side effects (Hence got banned).	[78, 79]
Naproxen	NSAID	Used in treating arthralgia pain	Leads to liver poisoning with no analgesic effect	Compared to other NSAIDs, it possess low cardiovascular risk	[77, 80]
Propranolol	Antiarrhythmic (β-blocker)	Used as strong adrenoceptor antagonist	Not a strong adrenoceptor antagonist	Both provide local anesthetic effect.	[77, 114]
Salbutamol	Bronchodilator (β-adrenergic)	Levalbuterol is the active Broncho-dilating enantiomer	They have no adverse effect being the inactive enantiomer.	It is a short-acting β2 adrenergic receptor agonist. It helps in relaxing the smooth airway muscles.	[76]
Sotalol	Antiarrhythmic	α-blocker	β-blocker	Both the enantiomers have antiarrhythmic properties, but (−)-Sotalol can also act as β-blocker whereas (+)-Sotalol cannot.	[81, 82]
Thalidomide	Immunomodulatory and Selective Cytokine Inhibitor Drug that treats leprosy and multiple myeloma.	Used as a sedative to treat morning sickness	It is teratogenic (toxic) resulting in birth defects and range of malformations	Since it has the ability to racemize, it is to be avoided by pregnant women.	[83]

NOTE:
The enantiomer that rotates plane polarized light clockwise (+) is labeled as D and the other enantiomer that rotates light anticlockwise is L. Most frequently D-isomers are equivalent to R-Enantiomers and L-isomers are equivalent to S-Enantiomers. But this is not the case every time, at times the stereo centers might rotate the light in opposite direction leading to (+) and (-) R- Enantiomers and (+) and (-) S-Enantiomers

Table 3 - Enantiopure Drugs & Their Racemic Precursors Approved By 2018 Along With Their Purposes

ENANTIOPURE DRUG	BRAND NAME	MANUFAC-TURER	PURPOSE	RACEMIC PRECURSOR	BRAND NAME	MANUFAC-TURER	REFERENCE
Arformoteral (R)	Brovana	Sepracor	COPD*	Formoterol	Foradil	Novartis	[88]
Armodafinil (R)	Nuvigil	Cephalon	Narcolepsy	Modafinil	Provigil	Cephalon	[89]
Cisatracurium (R)	Nimbex	GlaxoSmithKline (GSK)	Neuromuscular blocker & Skeletal Muscle Relaxant	Atracurium besylate	Tracrium	Many	[90]
Dexchlorpheniramine (S)	Polaramine	Many	Anticholinergic & Antihistamine	Chlorphenamine	Chlor-Trimeton	Many	[91]
Dexfenfluramine (S)	Redux	Servier	Serotonergic anorectic drug	Fenfluramine	Pondimin	Servier	[92]
Dexibuprofen (S)	Seractil	Many	NSAID*	Ibuprofen	Advil/Motrin	Pfizer	[93]
Dexketoprofen (S)	Keral	Menarini	NSAID*	Ketoprofen	Actron	Many	[94, 95]
Dexlansoprazole (R)	Dexilant	Takeda	GERD* & Erosive Esophagitis (PPI*)	Lansoprazole	Prevacid	Takeda	[96]
Dexmethylphenidate (R)	Focalin	Novartis	ADHD*	Methylphenidate	Ritalin	Novartis	[97]
Dextroamphetamine (S)	Dexedrine	Barr and Mallinckrodt	ADHD* and Narcolepsy	Amphetamine	Benzedrine	GlaxoSmithKline (GSK)	[98]
Escitalopram (S)	Lexapro/ Cipralex	Lundbeck	Psychotropic Antidepressant	Citalopram	Celexa/ Cipramil	Forest	[99]
Esketamine (S)	Ketanest/ Spravato	Johnson & Johnson	General Anesthetic & Antidepressant	Ketamine	Ketalar/ Ketanest	J & J and many more	[100]
Eszopiclone (S)	Lunesta	Sunovion	Insomnia	Zopiclone	Imovane/ Zimovane	Rhone-Poulenc	[101, 102, 103]
Levalbuterol (R)	Xopenex	Many	Anesthetic and Bronchodilator	Albuterol	Ventolin	GlaxoSmithKline (GSK)	[104, 105]
Levamlodipine (S)	EsCordi Cor	Actavis Pharma	Antihypertensive & Antianginal agent	Amlodipine	Norvasc	Pfizer	[106]
Levobupivacaine (S)	Chirocaine	Abbvie Lt.	Local Anesthetic	Bupivacaine	Marcaine	Many	[107]
Levocetirizine (R)	Xyzal	Sanofi-Aventis	Rhinitis Allergy (H1-Antihistamine)	Cetrizine	Zyrtec/ Reactine	Pfizer	[105]
Levofloxacin (S)	Levaquin	Johnson & Johnson	Antimicrobial	Ofloxacin	Floxin	Johnson & Johnson	[108]
Levoleucovorin (S)	Fusilev	Spectrum	Rescue Therapy (Osteo-sarcoma)	Leucovorin	Wellcovorin	GlaxoSmithKline (GSK)	[109]

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Levomilnacipran (1S, 2R)	Fetzima	Allergan	Antidepressant	Milnacipran	Ixel/Savella	Laboratoires Pierre Fabre.	[110]

***Abbreviations:** *COPD - Chronic Obstructive Pulmonary Disease,*
NSAID – Non Steroidal Anti Inflammatory Drug,
GERD – Gastro Esophageal Reflux Disease,
PPI – Proton Pump Inhibitor and
ADHD – Attention Deficit Hyperactivity Disorder.

CONCLUSION

The field of stereoisomerism is all intriguing as well as perplexing. It opens up new avenues to enhance existing drugs as well as explore new ones. The pharma sector has its all eyes focused to compete and exploit the “RACE-mic switch”. The choice of developing an enantiopure drug or a racemate should be solely based upon the therapeutic effects supported by well conducted clinical proofs. The stereoisomerism, pharmacodynamics and pharmacokinetics of the drug should be studied accurately eliminating all adverse effects. The increasing trend of enhancing shelf life of patents worldwide should be restricted so that low cost generic medicines can be made available for the masses. Such patents are of dubious validity and thus shouldn’t be granted patent to suppress generic competition. The generic versions should be carefully manufactured for the treatment and not just low cost version of their branded counterparts.

There is crucial need for health practitioners to make themselves aware of issues relating to chiral switch. The flow of the biased data on racemic drugs in scientific literature should be reduced and should be more focused on specific chiral switch enantiomers as it is not only significant to science but also to patients. If both the enantiopure and the racemate formulation of a drug are available, FDA should investigate both of their clinical significance so that no window is left open for manipulating extended shelf life of patents.

So far, majority of the enantiopure drugs have shown no specific superiority over older racemates under FDA approved trials, although they were not directly compared. It is essential for the physician as well as the consumer to know more about the chiral drugs in order to help themselves to find an optimal treatment. Individuals should know about the stereochemistry concerning routes of dosage, formulation, administration, drug interactions, age gender disease etc. Moreover, the physicians should reassess the rationality of the existing racemates, after all it is somebody’s life that is at stake.

We emphasize that a special department in FDA should be setup which would look after all existing enantiomers and their efficacy. The chemist in academia should take initiative to work upon improved methods of analyzing and preparing enantiopure drugs so that pure enantiomers maybe available for evaluation. Our mechanistic understanding of the chiral structures and sites of drug action is continuously evolving. Continuous re-evaluation of older racemates should also be done to enable re-introduction of enantiopure drugs with cleaner pharmacological profile, improved safety and optimal therapeutic outcomes along with commercial advantages. This would result in pocket friendly as well as effective prescription drugs. A persistent concern in the marketing of "RACE-mic Switch" will undoubtedly have a drastic negative impact on the clinical practices and; to avoid that the above mentioned precautionary measures should be taken. We hope that only optimal therapeutic enantiopure drugs will thrive the market in future.

Thus it's either the **PATIENT** or the **PATENT**, the choice is in our hands.

ETHICAL STATEMENT

Authors declare that they have no conflict of interest among them. Wherever required, Ethical approval and Informed consent were taken for conducting above studies.

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