**In-silico Druggability Studies of 4-hydroxy-α-tetralone and its Derivatives with RND Efflux pump of E. coli**

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**Abstract**

The compound 4-hydroxy-α-tetralone (1) has been reported to possess potent anti-tubercular, anti-diabetic and anti-leishmanial activities. In our earlier studies the compound 1 and its various semi-synthetic derivatives showed potent bioenhancing activity in combination with nalidixic acid (NAL) and tetracycline (TET) reducing the minimum inhibitory concentration (MIC) of antibiotics up to 8 folds by inhibition of ABC efflux pump. However, in gram negative bacteria, resistance nodulation division (RND) family are considered as major efflux pump responsible for multidrug resistance (MDR). Hence, the current study was carried out to access the in-silico docking potential of compound 1 and its cinnamoyl (1a), 3, 4, 5-trimethoxybenzoyl (1b) derivatives against RND efflux pump target proteins AcrA, AcrB, TolC of E. coli. The docking study showed that the test compounds have good binding affinity with target proteins. The derivative 1a showed highest interaction with AcrA followed by AcrB showing binding energies -8.7 and -8.2 kcal/mol respectively. The low molecular weight ≤500, high hydrogen bonding, high log p value (>1) with hydroxy, methoxy and aromatic group of ligands make these compounds as effective efflux pump inhibitor. In drug likeliness studies, these compounds pass the safety criteria with enhanced bioavailability and absorption, less acute oral toxicity, less hepatotoxicity. This study promises that the compound 1 and its derivatives (1a & 1b) might be RND efflux pump inhibitors providing the initial platform for development of safer and cost-effective antibacterial drug to manage MDR infections.

**Keywords:** 4‐hydroxy‐α‐tetralone, Multi drug resistance, Efflux pumps, Molecular Docking, ADMET

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**1 Introduction**

The drug discovery and development programme is complicated process involving multidisciplinary, multi-sector cooperation and regulations of the United States Food and Drug Administration (USFDA) which ultimately makes it time consuming and expensive. In general, the process of drug discovery involves target identification, validation, target and cell-based screening, lead compound identification its optimization, preclinical and clinical trials, FDA approval and marketing.1,2

In recent years the computer aided drug design (CADD) has become a powerful tool which certainly has accelerated the drug discovery process by minimizing the time as well as reducing the cost. The druggability of the lead molecule can be assessed in silico by homology modeling, docking, multi-target searching & design, pharmacophore development, conformation generation and quantitative structure activity relationship (QSAR) for the optimization of novel molecules with affinity to a target by the pharmacokinetics, absorption, distribution, metabolism, excretion and toxicity properties as well as physicochemical characterization of phytomolecules.3,4

Despite their use as folklore remedy since beginning of civilization, the medicinal plants have historically proven value as a source of molecules with therapeutic potential.5 Even today the
plants being used in folklore remedy continue to be of great interest in drug discovery because of better biologically relevant chemistry, hence lesser side effects. The plant based bioactive leads find either direct use as drug or as novel chemophore which may be converted into druggable compounds by chemical transformation. However, the recent reports on emerging and sustained resistance to antibiotics and depleted pipeline of new antibacterial are creating a major health issue worldwide. Over the years, continued selective pressure by different antibacterial drugs has resulted in emergence of new strains of organisms that are multidrug resistance (MDR). In view of drug resistance and lack of novel antibiotics, WHO listed 12 human pathogenic bacteria (dirty dozen) including Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter species which urgently require novel antibiotics. In spite of recent advances in therapy, the diseases caused by them cause over 50% of deaths in hospital settings. Moreover, the economic impact of ‘superbug’ outbreaks could top $100 trillion; low-income countries would suffer disproportionately. WHO estimates the global typhoid fever disease burden at 11-20 million cases annually, resulting in about 128,000–161,000 deaths per year. The resistance among common pathogens causing community and hospital-associated infections has increased significantly worldwide, though regional patterns of resistance vary.

Several drug resistance mechanisms such as target alteration, drug inactivation, decreased permeability and increased efflux has been reported, among these multidrug efflux pumps are supposed as key players for MDR. These active efflux pumps of pathogenic bacteria extrude structurally unrelated group of drugs. In prokaryotes, five different types of efflux pumps are reported namely major facilitator (MF), multidrug and toxic efflux (MATE), resistance-nodulation-division (RND), small multidrug resistance (SMR) and ATP binding cassette (ABC). Amongst these, RND efflux pumps extrude wide range of compounds. In the post genomic era, these efflux pumps are promising drug target. However, there is major gap in developing strategies to control the spread of drug resistance caused by efflux pumps.

The compound 4-hydroxy-α-tetralone is a marker compound of genus Ammmania and has been reported to possess potent anti-tubercular, anti-diabetic and anti-leishmanial activities. In our earlier studies 4-hydroxy-α-tetralone and its various semi-synthetic derivatives showed potent bioenhancing activity in combination with nalidixic acid (NAL) and tetracycline (TET) reducing the minimum inhibitory concentration (MIC) of antibiotics up to 8 folds by inhibition of ABC efflux pump. However, in gram negative bacteria, RND family is considered as major efflux pump responsible for MDR. This prompted us to carry out the in-silico docking studies of 4-hydroxy-α-tetralone and its semi-synthetic derivatives with efflux pump proteins to assess statistical parameters for screening test set of RND efflux pump of E. coli.

2 Methodology

2.1 Protein characterization

The amino acid sequence of AcrA, AcrB, TolC proteins in FASTA format were retrieved from UniProt KB database (https://www.uniprot.org). Physicochemical characterization was done by ProtParam tool (https://web.expasy.org) and secondary structure analysis by GorIV tool (https://npsa-prabi.ibcp.fr).

2.2 Selection of ligands

The isolation of test compound 4-hydroxy-α-tetralone (1) from many species of genus Ammmania and its semi-synthetic myristoyl, palmitoyl, cinnamoyl, anisoyl and 3, 4, 5-trimethoxybenzoyl derivatives were reported in our earlier studies. Compound 1 and its cinnamoyl (1a), 3, 4, 5-trimethoxybenzoyl (1b) derivatives were selected for current study (Fig 1).

2.3 Drug likeliness profiling

The drug likeliness profiling of the test molecules was studied by ADMETSAR2 tool (http://lmmnd.ecust.edu.cn). Molecular weight, A log P, hydrogen bond acceptor, hydrogen bond donor, human intestinal absorption, Caco-2, human oral bioavailability, subcellular localization, carcinogenicity, eye corrosion, Ames mutagenesis, hepatotoxicity, acute oral toxicity was taken into consideration.

2.4 Molecular docking

Molecular docking of the ligands to the efflux pump proteins was carried out by Vina tool of PyRx virtual screening software v 0.8 (https://pyrx.sourceforge.io). 3D structure of AcrA, AcrB, TolC proteins were retrieved from Protein Data Bank (http://www.rcsb.org) with respective PDB IDs: 5V5S, 1OY6, 1TQQ. Both the retrieved proteins and synthesized ligands were converted into pdbqt format and docking was carried out. The configurations for each protein selected for docking are given in table 1. Interaction of protein and ligand was visualized using BIOVIA Discovery Studio Visualizer v 16.1.0.15350 (https://www.3dsbiovia.com).

3 Result & Discussions

3.1 Analysis of proteins

Fig 1: Structure of chemical descriptors (Ligands)
The physicochemical properties of all the retrieved proteins were studied for various parameters. As evident from table 2, the AcrB is the largest amongst the all three with 1049 amino acid residues and has a molecular weight of 113573.65 Dalton. The isoelectric point (pl) of AcrB and TolC suggests the acidic nature of proteins whereas AcrA being basic (pl = 7.69). Though it remains a connecting link between AcrB and TolC, the reason for its basic nature is not clear enough.

Table 1: Configurations of target proteins for docking

| Protein | X   | Y   | Z   | X   | Y   | Z   |
|---------|-----|-----|-----|-----|-----|-----|
| AcrA    | 229.508 | 220.476 | 160.3606 | 15.4054 | 19.1771 | 14.9788 |
| AcrB    | 42.4938  | 42.7927  | 71.4860  | 34.3867  | 63.6792  | 70.4601  |
| TolC    | 192.878  | 49.5180  | -11.5398 | 21.2743  | 17.1074  | 15.5843  |

Table 2: Physicochemical properties of target proteins

| Proteins | AA | MW (Da) | pl | -R | +R | EC | II | Al | GRAVY |
|----------|----|---------|----|----|----|----|----|----|-------|
| AcrA     | 397 | 42196.71 | 7.69 | 39 | 40 | 18910 | 29.31 | 91.18 | -0.256 |
| AcrB     | 1049 | 113573.65 | 5.39 | 97 | 81 | 89855 | 28.6 | 101.52 | 0.266 |
| TolC     | 493  | 53740.72  | 5.46 | 45 | 40 | 36790 | 34.53 | 86.57 | -0.427 |

AA: Amino acid residues; MW: Molecular weight; pl: Isoelectric point; -R: Negatively charged residues; +R: Positively charged residues; EC: Extinction coefficient; II: Instability index; GRAVY: Grand Average of Hydropathy

Table 3: Secondary structural characteristics of target proteins

| Protein | Alpha helix (%) | Extended strand (%) | Random coil (%) |
|---------|----------------|--------------------|-----------------|
| AcrA    | 35.01          | 17.63              | 47.36           |
| AcrB    | 40.51          | 17.25              | 42.23           |
| TolC    | 53.55          | 10.14              | 36.31           |

3.2 Drug likeliness properties

It has been seen that most drug candidates fail clinical trials because of unsatisfactory pharmacokinetics. The in silico screening of drug likeliness properties of the lead molecules is largely helpful to overcome such problem. In our study, the parameters used for in silico screening were absorption, distribution, metabolism, excretion and toxicity (ADMET). The results indicated that the drug likeliness properties of these compounds are within the domain of applicability to be considered as potent drug candidates (Table 4).

Table 4: Drug likeliness profile of ligands

| Lig. | MW  | ALP | HBA | HBD | HIA | Caco2 | HOB | SbL | CAR | EC | AM | HpT | AOT |
|------|-----|-----|-----|-----|-----|-------|-----|-----|-----|----|----|-----|-----|
| 1    | 162.19 | 1.7 | 2   | 1   | 0.99 | 0.87  | 0.8 | Mito| 0.91| 0.92| 0.96| 0.80| 0.78|
| 1a   | 292.33 | 3.96| 3   | 0   | 0.98 | 0.49  | 0.55| Mito| 0.822| 0.95| 0.54| 0.70| 0.52|
| 1b   | 356.37 | 3.59| 6   | 0   | 0.99 | 0.69  | 0.72| Mito| 0.90| 0.98| 0.58| 0.77| 0.51|

Lig.: Ligand; Mito: Mitochondria; MW: Molecular weight; ALP: A log P; HBA: H-Bond Acceptor; HBD: H-Bond Donor; HIA: Human Intestinal Absorption; HOB: Human Oral Bioavailability; SbL: Subcellular Localization; CAR: Carcinogenicity; EC: Eye Corrosion; AM: Ames Mutagenesis; HpT: Hepatotoxicity; AOT: Acute Oral Toxicity
3.3 Molecular docking studies

In gram negative bacteria, RND families are considered as major efflux pump responsible for MDR. The current study was carried out to access the in-silico docking potential of compound 1 and its semi-synthetic derivatives (1a & 1b) against RND efflux pump target proteins AcrA, AcrB, TolC of E. coli. The docking study showed that the test compounds have good binding affinity with target proteins (Table 5).

The derivative 1a showed highest interaction with AcrA followed by AcrB with binding energies -8.7 and -8.2 kcal/mol respectively. However, the binding affinity of 1a was less in case of TolC protein of E. coli. The interaction of amino acids with the ligands is given in table 6. The parent compound 1 and the derivative 1b showed same binding affinity with AcrA protein (binding energies -7.3 kcal/mol) while the binding affinity of 1b was more than parent compound 1 for the target protein AcrB.

From the results obtained it is noteworthy to observe that all the compounds are interacting well with amino acids of receptor proteins (Fig 2); however, due to presence of aromatic amino acids in receptor AcrB the ligands bind more efficiently with AcrB. The protein AcrB has different group of amino acids which are helpful in interaction through H-bonding as well as hydrophobic interaction. However, both in-vitro and in-vivo validation of results are required to establish these molecules as novel drug resistance reversal agent against critical bacterial superbugs.

Table 5: Molecular docking studies of protein and ligands

| Protein | Binding energy of ligands (kcal/mol) |
|---------|-------------------------------------|
|         | 1       | 1a       | 1b       |
| AcrA    | -7.3    | -8.7     | -7.3     |
| AcrB    | -6.5    | -8.2     | -7.5     |
| TolC    | -7.3    | -4.7     | -7.0     |

Table 6: Interaction of Ligands with amino acids

| Ligands | AcrA | AcrB | TolC |
|---------|------|------|------|
| 1       | Leu (287); Val (293) | Lys (267); Trp (187); Ala (777); Arg (780); | Ala (414); Gln (397); |
| 1a      | Val (214); Asp (214); Gly (290); Met (291); Leu (246); Ile (212); Leu (287) | Ile (306); Tyr (325); Leu (310); Arg (307); Phe (610); Pro (326); Gly (288); Leu (289) | Leu (415); Ala (414); Lys (401); |
| 1b      | Thr (248); Met (291); Leu (287); Val (293); Leu (246); Ile (212); Val (214) | Thr (329); Ile (291); Pro (326); Phe (610) | Asn (200); Lys (202); Gln (397); Leu (415); Ala (418); |

Fig 2: Interaction of ligand 1b with target proteins AcrA (a), AcrB (b) & TolC (c)

4 Conclusion

The docking studies of 4-hydroxy-a-tetralone (1) and its semi-synthetic derivatives (1a & 1b) showed high binding affinity against RND efflux pump target proteins AcrA, AcrB, TolC of E. coli. The calculated parameters of test compounds for drug likeness are within the acceptable limit. If we relate our earlier published wet lab results on account of these compounds, the
story becomes much interesting. These test compounds were able to reduce the mutation prevention concentration of TET, showed significant down expression of ATP dependent efflux pump gene (yojI) encoding multidrug ATP binding cassettes (ABC) transporter protein in real time polymerase chain reaction (RT-PCR) study.30

Finally, our continued efforts on 4-hydroxy-α-tetralone and its semi-synthetic derivatives indicate that these compounds are able to inhibit a variety of efflux pumps responsible for drug resistance in critical superbug, creating a new platform for development of cost-effective antibacterial combinations for the management of MDR infections.

5 Conflict of interest

The authors declare that there are no conflicts of interest among us.

6 Author’s contributions

SS & HCU carried out literature review, interpretation of results and draft the manuscript. ASS & GRD carried out the experiments. The manuscript has been checked and approved by all authors before submission to the journal.

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