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EVALUATION OF SOME GENERIC DRUGS FOR REVERSAL OF MULTIDRUG-RESISTANCE IN *PSEUDOMONAS AERUGINOSA* USING COMPUTER-AIDED DRUG DESIGN

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Abstract – The alarming rate of the increase in multidrug-resistant cases of Pseudomonas aeruginosa is a growing clinical issue necessitating the urgent need for new antibiotics and alternative strategies to combat the bacterial pathogen. Repurposing approved drugs in clinical use with known pharmacology and toxicology is one such cost-effective alternative approach. In this study, four essential protein targents for P. aeruginosa involved in the development of multidrug resistance were selected from Protein Data Bank using a validated method. Structure-based virtual screening method with PyRx was used to screen a database of 175 approved drugs. Celecoxib and meloxicam (marketed inhibitors of cyclooxygenase-2), fluconazole (an antifungal), desloratadine (antihistamine) and nitrofurantoin were found to bind with all the protein targets with binding energy greater than that obtained with the respective cocrystallized ligands.

Keywords: *P. aeruginosa,PyRx,multidrug-resistant, Molecular docking*

I. INTRODUCTION

With the discovery of antibiotics in the 20th century, they were believed to be an excellent innovation and a turning point in the history of medicine [1, 2]. They have helped in defeating most infectious diseases that had plagued humankind and had significantly assisted in reducing mortality and morbidity related to the illnesses[1, 3]. Shortly afterwards, bacteria began to show resistance to antimicrobials [1]. That was not a problem during the earlier half of the 20th century because there were other antimicrobials to change to [4]. However, in recent times, antimicrobial resistance has become a major problem [2]. In most parts of the world, infection with *Pseudomonas aeruginosa* (*P. aeruginosa*) a gram-negative opportunistic pathogen has now been noted to be on the increase, causing high mortality and morbidity particularly in patients with immune-compromise, burns, wound infections and cystic fibrosis [5-8]. Many studies of clinical isolates from hospitalized patients have also found *P. aeruginosa* among the leading causes of hospital-acquired infections that are resistant to antibiotics[7, 9, 10], resulting in hard-to-treat infections due to the limited number of effective antipseudomonal drugs as well as the inherent potentials of the organisms in acquiring resistance to those drugs [5, 6]. This scenerio left us with the limited options of using either new investigational drugs or old and toxic drugs (e.g. polymyxins) or in some cases, inefficient drugs [11-13].

The Development of new drugs is a time-consuming and a very costly interprise, which takes up to 10-15 years and requires about US1.8b [14].

Virtual screening is a computer-aided drug design that is used to screen a large database of small molecular chemicals/drugs to predict how those chemicals interact (binding affinity or orientation) with a particular protein target. It is a time-saving and cost-effective modern method of drug discovery and drug repositioning (finding new therapeutic indications). This method could be an important weapon against the multidrug-resistant *P. aeruginosa.*

Most of the published studies on virtual screening of drugs against Pseudomonas aeruginosa were aimed at repositioning drug candidates or lincenced drugs against single protein targets, such as quorum sensing signal protein [15, 16], biofilm formation [17] and AmpC/β-lactamase [18]. However, no work has been published on multiple protein targets involved in multidrug resistance in *P. aeruginosa* using structure-based virtual screening for drug repositioning. This research therefore aims at evaluating some generic drugs for their potential for repositioning against multidrug-resistant *P. aeruginosa.*

II. MATERIALS AND METHODS

A. Virtual screening

1) Selection of protein structure (target) and development of local database

The crystal structures of *P. aeruginosa* proteins were identified and downloaded from the Protein Data Bank

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(PDB) (www.rcsb.org) using the search terms MexB, MexY, efflux pump, *Pseudomonas aeruginosa*, β-lactamase and hydrolase. Entries for *Pseudomonas aeruginosa* were selected. Four suitable protein structures with PDB ID 3W9I[19], 1X92[20], 3WXC [20]and 4XMH were chosen based on their critical role in causing multidrug resistance in *Pseudomonas aeruginosa*, according to the following criteria highlighted by Warren, and his colleague [21] for the selection of crystal structure for molecular docking: (a) availability of experimental data for the protein, (b) R-free value of < 0.45, (c) a difference between R and R-free value ≤ 0.05, (d) density precision index of < 0.5 and (e) structure with higher resolution of < 3.5Å.

2) Criteria for drugs selection and development of local database

The following two different types of selection criteria were used:

a. Drugs with excellent safety profile of LD_{50} > 2000, expired patency and no known pharmacokinetic interaction with anti-Pseudomonas antibiotics. Antibacterial agents were also exclude [22]. One hundred drugs in clinical usage that are not used for the treatment of *Pseudomonas aeruginosa* were selected, downloaded from the DrugBank[23] and saved in Structure Data Format (sdf).
b. Marvin Chemaxon was employed to perform a structure similarity search between the drugs in the DrugBank and co-crystallized ligands/inhibitors of the selected protein targets using Tanimoto coefficient using and setting the similarity threshold at 0.5 [24].

3) *In silico* docking and selection of best performing drugs for *in-vitro* studies.

Docking simulation of the drugs (selected using the criteria highlighted above), were run against each of the four selected protein targets (with PBD ID: 4MXH, 3W9I, 1X92, and 3WXC), using an automated docking software, PyRx version 0.9.2 [25-27].

4) Importation of drugs and protein molecules

The protein targets in PDB format and the ligands in sdf format were imported from the local database created into PyRx workspace using load molecule and openbabel icon.

5) Preparation of the protein targets

Water molecules, cofactors and co-crystallized ligands were removed from each of the protein molecules (targets) according to the software manual, by deleting the "HETATM" records from the protein structure document files. The structures were then converted into AutoDock macromolecules.

6) Preparation of the ligands

Preparation of the ligands was done according to the software manual by minimizing the energy of each ligand so that the net interatomic force is close to zero, and then converted to AutoDock ligands.

7) Molecular docking

Docking simulation was done using Vina wizard of PyRx 0.9.2. Each of the selected targetswas docked against the selected ligands and a respective co-crystallized ligand which served as a control. In all situations, the entire receptor conformational space was searched, using grid boxes with measurements 60×60×60 and 30×30×30Å. The exhaustiveness was set at 100. Any drug with low binding energy equal to or less than that of its corresponding co-crystallized ligand were chosen and then filtered using Microsoft Excel. Subsequently, drugs that show low binding energy in all the four protein targets were selected for *in vitro* studies.

B. Antimicrobial Susceptibility Testing

1. Disc Diffusion Method

Antibiotic stock solutions were made to the maximum concentration of 10mg/ml and then diluted to an appropriate concentration in the broth. A sterile distilled water was used for the dilutions except for drugs that cannot be dissolved in water. Accordingly, suitable solvents were used for the initial dissolution dropwise. Once the powder was dissolved, the final volume was made up with distilled water.

Stock solutions were prepared using the following formula:

$$\frac{1000}{p} \times c \times v = w$$

Where P = potency (mg/g), C = required concentration of the solution in multiples of 1000mg/L, V = volume (ml) and W is the weight of the drug (mg).

a. Preparation of Discs for Antibiotics and Selected Drugs

Whatman filter paper no. 1 was used to prepare discs using an office file puncture approximately 6 mm in diameter. Discs were placed in a Petri dish and sterilized in a hot air oven. The respective stock solutions of antibiotics and of drugs selected from the docking studies at C-Max concentration were delivered to the discs using 20-gauge wire loop with a diameter of 2 mm and then oven dried.

b. Preparation of Bacterial Suspensions

The cultures were grown on non-selective agar for 18 hours at 37°C and thegrowthwere re-suspended in sterile 0.85 % saline, according to EUCAST guidelines for colony suspension method. The concentrated bacterial suspensions obtained were diluted with sterile 0.85 % saline to match the turbidity of a 0.5 McFarland standard (bacterial suspension containing approximately 1.5 x 10^8 CFU/ml).

c. Inoculation of Test Plates

Within 15 minutes after adjusting the turbidity (0.5 McFarland) of the inoculum suspension, a sterile cotton swab was immersed into the suspension. The swab was rotated about three times and pressed firmly on the inside wall of the tube above the fluid level so as to remove the excess inoculum. The dried surface of a Mueller-Hinton agar plate prepared above was inoculated by streaking the swab over the entire sterile agar surface. The procedure was repeated by streaking the swab two more times, rotating the plate approximately 60° each time to ensure an even distribution of the inoculum.

d. Application of Discs to Inoculated Agar Plates

Each disc for the selected antibiotics (ciprofloxacin, tetracycline, ceftriaxone, amoxicillin, and co-trimoxazole) was put onto the surface of separately inoculated agar plate

about 2 mm adjacent to the respective discs of the selected drugs. Each disc was pushed down to ensure complete contact with the agar surface. The plates were covered, inverted and incubated at 37°C for 24 hours.

e. Reading Plates

After 24 hours of incubation, each plate was inspected. The diameters of the zones of inhibition (judged by the unaided eye) including that of the disc were measured to the nearest whole millimeter, using a ruler, which was held on the back of the inverted plate. The Petri-dishes were held few inches above a black background.

C. Synergy Testing

Macro-broth dilution and determination of the effect on *P. aeruginosa* of different concentrations of ciprofloxacin alone and in combination with the selected drugs

Mueller-Hinton broth was prepared and sterilized according to the manufacturer's instructions, and in line with the procedure described by the EUCAST. Nine tubes of different ciprofloxacin concentrations made directly in Mueller-Hinton broth were prepared in triplicates, starting with 4 ml of 5.25 µg/ml of ciprofloxacin, put in tube 1. Sterile Mueller-Hinton broth (2 ml) was put into tubes 2 to 11. Two ml of the solution in tube 1 was transferred into tube 2, making the concentration of ciprofloxacin in the tube 2.625 µg/ml, i.e. double dilution. Further serial double dilutions were performed in the same way up to tube 9, covering a range of dilutions from 1:1 to 1:256. Tube 10 contained inoculated broth and served as a positive control while tube 11 contained uninoculated broth, serving as a negative control. All tubes contained 2 ml of Mueller-Hinton broth, with 10 and 11 containing no ciprofloxacin. The bacterial suspensions obtained with the direct colony suspension method contained approximately 1.5 x 10^8 CFU/ml. volume of 2 ml broth (except the negative control tube) resulting in an approximate count of 3 x 10^5 CFU/ml per tube. The tubes were incubated for 24 hours at 37°C. Thereafter, the optical density (OD) of each tube was obtained using spectrophotometry, and extrapolated to find bacterial colony forming units from the standard curve.

D. Creation of standard curve for extrapolation of *P. aeruginosa* CFU from optical density

A serial dilution of *Pseudomonas aeruginosa* inoculum corresponding to the turbidity of McFarland scale ten which is equivalent to 3.00 X 10^9 bacterial CFU/ml, was done in Muller Hinton broth in a dilution ranges of 1, 1/2, 1/4, 1/8, 1/16, 1/32, 1/64, and 1/128. The ODs of the respective dilutions were obtained using a spectrophotometer calibrated by setting the wavelength at 600nm. A plain sterile Muller Hinton broth was used to set the transmittance to 100%.

E. Statistical Analysis

Statistical analysis was done using Graph-pad Prism version 6 (demo) and Microsoft excel 2016. A p-value ≤ 0.05 was considered statistically significant. All data were expressed as mean ± standard error of mean. Data was presented in tables and charts as appropriate.

III. RESULTS

A. Selected drugs and proteins used for docking studies

Tables 1 and 2 below show the list of one hundred and seventy-five drugs and four protein targets (PDB ID) respectively selected using the criteria highlighted in the methodology. Table 2 also shows respective co- crystallized ligands for each target that were used as a control in the docking studies.

TABLE 1 SELECTED DRUGS USED IN THE DOCKING STUDIES

1	Ropivacaine	36	Dicloxacillin	71	Betaxolol	106	Chlorpheniramine	141	Cimetidine
2	Bupivacaine	37	Cloxacillin	72	Tamoxifen	107	Sulindac	142	Albendazole
3	Levobupivacaine	38	Cephaloglycin	73	Darifenacin	108	Hydroxychloroquine	143	Piroxicam
4	Mepivacaine	39	Flucloxacillin	74	Pralatrexate	109	Chloroquine	144	Voriconazole
5	Degarelix	40	Cefaclor	75	Pemetrexed	110	Furazolidone	145	Enalapril
6	Benazepril	41	Dalfopristin	76	folic acid	111	Sumatriptan	146	Diclofenac
7	Polymyxin B	42	Cephalexin	77	Promethazine	112	Ramipril	147	Cisapride
8	Cetrorelix	43	Ticarcillin	78	Thiamine	113	Nitrofuratoin	148	Metoclopramide
9	Fentanyl	44	Azidocillin	79	Pyridoxine	114	Rabeprazole	149	Posaconazole
10	Ropivacaine	45	Cyaclacillin	80	Baclofen	115	Nedocromil	150	Magnesium sulphate
11	Desmopresin	46	Mg hydroxide	81	Tramadol	116	Lisinopril	151	Probenecid
12	Prilocaine	47	Terbutaline	82	Fluconazole	117	Thiabendazole	152	Lopinavir
13	Ivaceftor	48	Granisetron	83	Acetaminophen	118	Esomeprazole	153	Arthemeter
14	Trimetrexate	49	Ondansetron	84	Ascorbic acid	119	Meclizine	154	Lumefantrine
15	Tadalafil	50	Magnesium Salicylate	85	Pantoprazole	120	Scopolamine	155	Lamivudine
16	Alosetron	51	Amantadine	86	Furosemide	121	Naprozen	156	Penicillin V
17	Cefpiramide	52	Naloxone	87	Clotrimazole	122	Rivabarin	157	Hetacillin
18	Reserpine	53	Buprenorphine	88	Acebutalol	123	Meloxicam	158	Amoxicillin
19	Indomethacin	54	Misoprostol	89	Aciclovir	124	Spironolactone	159	Azlocillin
20	Lactulose	55	Meclofenamic acid	90	Primaquine	125	Cetirizine	160	Pivampicillin
21	Inulin	56	Azocillin	91	Indomethcin	126	Ranitidine	161	Atomoxetine
22	Glucosamine	57	Piperacillin	92	Omeprazole	127	Nifedipine	162	Propofol
23	Kanamycin	58	Becampicillin	93	Pyrazinamide	128	Carbamazepine	163	Pentamidine
24	Neomycin	59	Methicillin	94	Doxylamine	129	Ipratropium	164	Gemfibrozil
25	Paromomycin	60	Nafcillin	95	Amlodipine	130	Itraconazole	165	Metoprolol
26	Framycetin	61	Oxacillin	96	Proguanil	131	Chloroprocaine	166	Ketoconazole
27	Artemether	62	Mezlocillin	97	Diphenhydramine	132	Amitriptyline	167	Ibuprofen
28	Topiramate	63	Pivmecillinam	98	Atorvastin	133	Cyproheptadine	168	Carbidopa
29	Boceprevir	64	Fesoterotidine	99	Fluvastin	134	Allopurinol	169	Praziquantel
30	Azeteonam	65	Mexiletine	100	Amodiaquine	135	Desloratidine	170	Methyldopa
31	Tazobactam	66	Tolterodine	101	Aspirin	136	Quinidine	171	Quinine
32	Benzylpenicillin	67	Tyloxapol	102	Hydrocodone	137	Lansoprazole	172	Tenoxicam
33	Carbenicillin	68	Alprenolol	103	Salbutamol	138	Loratidine	173	Celocoxib
34	Cyalacillin	69	Penbutolol	104	Ketoprofen	139	Nabumetone	174	Mefloquine
35	Ampicillin	70	Oxybenzone	105	Miconazole	140	Ketorolac	175	Procaine

Protein name	PDB ID	Co-crystallized ligand
Metallo-β-lactamase	4XMH	Moxalactam
Multidrug efflux transporter AcrB	3W9I	Dodecyl-beta-d-maltoside
Phosphoheptose isomerase	1X92	d-glycero-d-mannopyranose-7-phosphate
β-lactamase	3WXC	Aminophtalic acid

TABLE 2 BINDING ENERGIES OF BEST PERFORMING DRUGS

		Binding energy, Kcal			
	Drugs	Targets			
		1x92	3xwc	3w9I	4mxh
	Co-crystallize ligand	-6.4	-5.0	-6.5	-6.9
1	Tadalafil	-6.9	-6.4	-10.9	-9.3
2	Posaconazole	-7.9	-6.2	-10.7	-8.3
3	Spironolactone	-7.4	-6.2	-9.0	-7.6
4	folic acid	-7.4	-5.8	-8.6	-8.2
5	Cyproheptidine	-6.4	-6.7	-8.9	-7.6
6	Prazequantel	-6.5	-6.4	-9.2	-7.4
7	Loratidine	-7.2	-6.1	-8.7	-7.3
8	Desloratidine	-7.2	-6.1	-8.7	-7.2
9	Darefenacin	-6.5	-6.1	-8.2	-8.4
10	Piroxicam	-6.5	-6.9	-8.2	-7.5
11	Lopinavir	-7.5	-6.3	-8.2	-7.0
12	Celocoxib	-6.6	-5.5	-9.1	-7.2
13	Mefloquine	-6.7	-6.3	-8.1	-7.1
14	Alosetron	-6.7	-5.9	-8.0	-7.4
16	Lansoprazole	-6.6	-5.5	-7.9	-7.6
17	Hydrocodone	-6.5	-6.0	-8.2	-6.9
18	Meloxicam	-6.5	-6.0	-7.8	-7.3
19	Cetirizine	-6.6	-5.1	-8.0	-7.8
20	Pantoprazole	-6.6	-5.3	-7.7	-7.8
21	Nitrofurantoin	-7.0	-5.4	-6.9	-7.1
22	Omeprazole	-6.4	-5.1	-7.3	-7.6
23	Fluconazole	-6.7	-5.4	-7.1	-6.9

Qualitative test for antipseudomonal effect and synergy testing (disc diffusion test)

Out of the 19 drugs tested qualitatively for antipseudomonal effect, none shows a visible inhibition of P. aeruginosa growth when tested alone. However, five drugs (Table 4) have demonstrated synergistic activity with ciprofloxacin by elongating the zone of inhibition of ciprofloxacin towards the respective drug disc. Furthermore, among the antibiotics tested, only ciprofloxacin, shows clearzone ofP. aeruginosa inhibition (table 4)

TABLE 3 DISC DIFFUSION TEST OF CIPROFLOXACIN AND BEST PERFORMING DRUGS IN PSEUDOMONAS AERUGINOSA CULTURE MEDIUM

	Ampicillin	Tetracycline	Ciprofloxacin	Cotrimoxazole	Ceftriaxone
Tadalafil	+++	+++	---	+++	+++
Posaconazole	NT	NT	NT	NT	NT
Spironolactone	+++	+++	---	+++	+++
folic acid	+++	+++	---	+++	+++
Cyproheptadine	+++	+++	---	+++	+++
Praziquantel	+++	+++	---	+++	+++
Loratidine	+++	+++	---	+++	+++
Desloratadine	+++	+++	---*	+++	+++
Darifenacin	NT	NT	NT	NT	NT
Piroxicam	+++	+++	---	+++	+++
Lopinavir	NT	NT	NT	NT	NT
Celecoxib	+++	+++	---*	+++	+++
Mefloquine	+++	+++	---	+++	+++
Alosetron	+++	+++	---	+++	+++
Lansoprazole	+++	+++	---	+++	+++
Hydrocodone	+++	+++	---	+++	+++
Meloxicam	+++	+++	---	+++	+++
Cetirizine	+++	+++	---	+++	+++
Pantoprazole	NT	NT	NT	NT	NT
Nitrofurantoin	+++	+++	---*	+++	+++
Omeprazole	+++	+++	---	+++	+++
Fluconazole	+++	+++	---*	+++	+++

--- = zone of inhibition, +++ = no zone inhibition,
---* = synergy, and NT = not tested

QUANTITATIVE TESTING OF THE INHIBITORY EFFECT ON* P. AERUGINOSA *OF COMBINING CIPROFLOXACIN WITH EACH OF THE SELECTED DRUGS (BROTH MACRO-DILUTION)

Table 5 shows the respective number of bacterial colony forming units obtained following 24 hours incubation of *Pseudomonas aeruginosa* in different concentrations of ciprofloxacin alone, and in various combinations of ciprofloxacin with drugs highlighted in table 4 above that show probable synergistic action.

TABLE 4 NUMBER OF **P. AERUGINOSA** *CFU AFTER 24 HOURS OF INCUBATION IN DIFFERENT CONCENTRATION CIPROFLOXACIN AND IN COMBINATIONS WITH FIXED CONCENTRATION OF MELOXICAM, FLUCONAZOLE, NITROFURANTOIN, DESLORATADINE AND CELECOXIB*

CPR	CPR	CPR+ cel(0.50mcg/ml)	CPR+ deslo(0.004 mcg/ml)	CPR+ ntf(0.4mcg/ml)	CPR+ flu(6.2mcg/ml)	CPR+ mel(1.3mcg/ml)
µmol/ml	Bacteria CFU/ml X10^6					
0.00	122.15	130.59	127.92	128.56	118.04	118.21
0.01	85.06	33.38	34.60	67.02	17.30	33.71
0.02	37.65	25.75	38.67	29.61	28.51	35.25
0.04	20.59	11.30	32.90	1.05	6.09	16.89
0.08	23.63	4.31	10.16	3.18	2.51	2.92
0.16	13.15	4.64	0.09	8.69	1.05	1.13
0.33	11.81	4.56	0.65	1.51	1.95	0.57
0.66	14.62	3.74	2.53	1.71	2.60	0.33
1.31	16.20	4.15	2.04	1.67	0.58	1.06
2.63	14.01	1.23	2.93	1.83	3.33	2.92
5.25	11.45	0.82	3.99	0.44	8.36	6.74

Note: - CPR = ciprofloxacin; cel = celecoxib; deslo = desloratadine; ntf = nitrofurantoin; flu = fluconazole; mel = meloxicam

Table 6 IC50, AREA UNDER CURVE AND MIC OF CIPROFLOXACIN ALONE AND IN COMBINATION WITH THE SELECTED DRUGS

	CPR	CPR + celecoxib	CPR+ desloratidine	CPR+ Nitrofurantoin	CPR + fluconazole	CPR+ meloxicam
LogIC_{50}	-0.475	-0.758	-0.495	-0.590	-0.980	-0.699
IC_{50}	0.335	0.174	0.320	0.257	0.105	0.200
Span	115.8	130.2	132.2	128.5	124.2	125.6
Bottom	5.9	3.673	3.617	5.766	2.794	3.166
Top	10.78	6.86	6.62	10.64	5.274	5.889
R^2	0.750	0.9064	0.912	0.793	0.939	0.924
AUC	1121	800	811	964	728	807
MIC (mcg/ml)	0.164	0.041	0.082	0.041	0.041	0.041

IV. DISCUSSION

In this work, we searched forcommercially available drugs approved for other pharmacological indications, which may have the potential to interfere with four essential proteins involved in the mechanism of resistance of *P. aeruginosa* using Structure-based virtual screening (SBVS) and validated the potential of the anti-pseudomonal effect of the selected drugs (with best binding energies)*invitro* using *P. aeruginosa* ATTC 27853.

This study reaffirmed that structure-based virtual screening is a valid and efficient means for the discovery of drugs with secondary pharmacological effect against *P* aeruginosa. From the library of 175 approved drugs we created, 23 drugs (Table 3) were found to bind to all the four *P. aeruginosa* targets (PDB ID 3WI9, 1X92, 3WXC, and 4XMH), with binding energy less than the set cut-off energy (binding energy of the respective co-crystallized ligands), indicating a better binding affinity than the ligands. Of the 23 drugs, 19 were tested further *in vitro*(because they are the only ones accessible in our locality)for inherent antibacterial activity using disc diffusion test individually and in combination with ciprofloxacin, amoxicillin, ceftriaxone, cotrimoxazole and tetracycline.

Five (fluconazole, celecoxib, nitrofurantoin, desloratadine and meloxicam) out of the nineteen drugswere found to influencethe antimicrobial activity of ciprofloxacin qualitatively (a hit rate of 26.3%), even though they did not seem to have intrinsic anti-pseudomonal activity when tested alone at the dose used in this study, i.e the maximum plasma concentration achievable with a standard dosage of the various drugs.This hit rate was high, which was encouraging, indicating the utility of this approach compared with the random in vitro high throughput method.

Quantitative assay of the drugs revealed that fluconazole, celecoxib, meloxicam, nitrofurantoin and desloratadine increased the sensitivity of *P. aeruginosa* to ciprofloxacin.

Ciprofloxacin-celecoxib,ciprofloxacin-meloxicam, ciprofloxacin- fluconazole, ciprofloxacin- desloratadine and ciprofloxacin-nitrofurantoin produced significantly lowerIC_{50} when compared with ciprofloxacin alone. This finding indicated that the drugs potentiated the activity of ciprofloxacin (since the agents possessed no antimicrobial activity when given singly). Additionally, the effect of ciprofloxacin on the population of *P. aeruginosa*after 24 hours of incubationrevealedlarger AUC compared with ciprofloxacin in combinations with any of the 5 drugs, signifying the presence of more bacterial population. Of the 5 drugs, ciprofloxacin-desloratadine combination exhibited the lowest percentage antibacterial inhibition compared to other combinations. This can be explained by the fact that desloratadine had the least number of polar contacts with each of the targets.

Thangamani*et al.* [28] reported anti-Pseudomonal activity of celecoxib when used in combination with colistin. It has also been documented that celecoxib, a cyclooxigenase-2 (COX2) inhibitor, increased the sensitivity of *S. aureus*, MRSA and *M. smegmatis* to ciprofloxacin through the inhibition of the efflux pump leading to accumulation of the drugs inside the bacteria cell [29]. However, the current study is the first to document the ability of the drug to increase the susceptibility of P. aerugenosa to ciprofloxacin. The increase in sensitivity of *P. aeruginosa* to ciprofloxacin, that we observed in this study, may not be attributable to celecoxib binding to efflux pump protein (3w9I) only as documented above by Thangamani*et al.*, since celecoxib also shows a good binding affinity to other proteins used in this study (1x92,3xwc, 4mxh).

On the other hand, meloxicam, another COX2 inhibitor, has been shown to act as a potential inhibitor of regulatory proteins of the *Pseudomonas* quorum signal mechanism: LasR and PqsE genes[30]. Several studies have shown fluconazole and nitrofurantoin to have antibacterial activity [31, 32]. However, no study has investigated their effectiveness against multidrug-resistant *P. aeruginosa* either singly or in combination with ciprofloxacin.

V. CONCLUSION

In conclusion, this study has demonstrated the utility of using computer-aided approach in repositioning approved drugs to reverse antimicrobial resistance by clinically important pathogens such as P. aerugenosa. *In vivo* studies areneeded to validate the findings.

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