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# Putative high-pIC50 SpDHBP synthase inhibitors against multi-drug resistant S. pneumoniae

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#### ABSTRACT

Streptococcus pneumoniae (MDRSp) has emerged to be multi-drug resistant to a wide range of antibiotics such as erythromycin and trimethoprim. The Center for Disease Control now lists MDRSp as one of the twelve most serious antibiotic resistance threats. Previous studies have sought to develop new therapies based on existing antibiotics, but these therapies are susceptible to the same resistance that MDRSp has built up. An emerging approach is to find inhibitors of MDRSp pathways, such as riboflavin synthesis that is present only in MDRSp and not in humans. Recently, researchers have elucidated 3,4-dihydroxy-2-butanone-4-phosphate (SpDHBP) synthase that is critical in riboflavin synthesis. This study exploits this new crystal structure and a number of recent advances, such as protein-protein interaction binding pockets and epoxidation site predictions, to screen for compounds. Two inhibitors, N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-pyrazole-4-carboxamide and N-[(1S)-2-[2-fluoro-5-(2-furyl)anilino]-1-methyl-2-oxo-ethyl]cyclobutanecarboxamide, are putative leads with pIC50 values that are greater than many common and currently available antibiotics.

Keywords: Streptococcus pneumoniae, virtual screening, riboflavin synthesis, pharmacophore, drug discovery

#### **INTRODUCTION**

Multi-drug resistant *Streptococcus pneumoniae* (MDRSp) is a facultative encapsulated Gram-positive alphahemolytic bacterial pathogen that invades the mucosal epithelium of the human nasopharyngeal cavity and respiratory airway, where it causes virulent forms of infectious diseases such as otitis media (ear infection), pneumonia, peritonitis, and sinusitis. Over the last two decades, the pathogen has acquired resistance to a wide range of antibiotics such as erythromycin, trimethoprim, sulfamethoxazole, vancomycin, tetracycline, chloramphenicol, and ofloxacin [1]. The Centers for Diseases Control and Prevention (CDC) now lists MDRSp as one of the twelve serious antibiotic resistance threats in the U.S.

This study builds upon earlier work seeking new therapies. One class of therapies seeks compounds based on existing antibiotics, such as 4-quinolones (targeting penicillin resistance)2, desmethyl macrolides3 and  $\alpha$ -amino- $\gamma$ -lactone ketolides4 (targeting erythromycin resistance), and C-4"-substituted azithromycins (based on azithromycin and quinolone SAR-compatible hybrids) [2]. This approach has the important advantage that the new compounds are likely to be as safe as the ones of which they are related, but they could also be susceptible to the same resistance that MDRSp has developed against existing drugs [3]. An alternative approach seeks inhibitors of new MDRSp pathways. A prominent example is the inhibition of Type II fatty acid synthesis. However, this pathway has

recently been found to be an unsuitable antibiotic target for Gram-positive pathogens because the pathogens could incorporate extracellular fatty acids to circumvent the lack of de novo synthesis [4].

Recently, researchers have elucidated through X-ray crystallography 3,4-dihydroxy-2-butanone-4-phosphate (SpDHBP) synthase (PDB 4FFJ), which catalyzes the biosynthesis of riboflavin, or 7,8- dimethyl-10-(10-D-ribityl) isoalloxazine [5]. Targeting SpDHBP synthase as an MDRSp anti-infective has several advantages. First, it does not suffer from extracellular incorporation as in the Type II fatty acid synthesis pathway. Second, SpDHBP synthase does not belong to any existing class of antibiotics, so it is less susceptible to the resistance that MDRSp has developed for existing drugs. Third, the gene coding SpDHBP synthase is highly conserved in a clinically relevant spectrum of species. Figure 1 shows that the sequence alignment of SpDHBP with the equivalent chain A from *Escherichia Coli* having 67% positive matches and small deviations. Specifically, the RMSD was 1.2 A<sup>o</sup> for the 191 alpha carbons and 1.24 A<sup>o</sup> for 764 backbone atoms. The homology is similar for *Vibrio cholerae* (70% positive matches), *Methanococcus jannaschii* (54%), *Candida albicans* (67%), and *Yersinia pestis* CO92 (67%). A fourth and important reason for targeting SpDHBP synthase is that riboflavin synthesis is endogenous in MDRSp but absent in humans, reducing the chance of cytotoxicity.

Score		Expect	Method		Identities	Positives	Gaps
202 bi	ts(51	5) 2e-69	Compositional r	natrix adjust.	95/202(47%)	137/202(67%	6) 1/202(0%)
Query	3	YRKIQEALE + +++ AL	EALQKGRLVLVIDDK AL++GR V+V+DD+	DRENEGDLICSAQ DRENEGD+I A+	AATTENVNFMATY	AKGLICMPMSE G++C+ ++E	62
Sbjct	13	FERVENAL	AALREGRGVMVLDDE	DRENEGDMIFPAE	TMTVEQMALTIRH	GSGIVCLCITE	72
Query	63	SLANQLMLS	SPMVENNTDNHKTAF MVENNT + T F	TVSIDYKE-TTTG	ISAEERGLTARMC +SA +R T R	VAEDITPSDFR +A+ PSD	121
Sbjct	73	DRRKQLDL	PMMVENNTSAYGTGF	TVTIEAAEGVTTG	VSAADRITTVRAA	IADGAKPSDLN	132
Query	122	RPGHMFPLI RPGH+FPI	IAKKGGVLERNGHTE A+ GGVL R GHTE	ATVDLLKLAGLKE AT+DL+ LAG K	CGLCCEIMNHDGK	MMRTDDLIQFS	181
Sbjct	133	RPGHVFPLF	RAQAGGVLTRGGHTE	ATIDLMTLAGFKP	AGVLCELTNDDGT	MARAPECIEFA	192
Query	182	KKHNIPLI KHN+ L+	TIKELQEYRKVYDQ TI++L YR+ +++	203			
Sbjct	193	NKHNMALV	TIEDLVAYRQAHER	214			

(a) Sequence alignment.



Figure 1. Conservation between DHBP synthase for S. pneumonia (4FFJ chain A, in red) and E. Coli (1G57 chain A, in magenta)

The riboflavin biosynthesis pathway (Figure 2) begins with MDRSp's *ribB* gene at chromosome IV:1428354-1428980, which codes for 214 amino acid residues. In a catalytic cycle, riboflavin produces flavin mononucleotide

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(FMN), which attenuates the mRNA translation of sroG sRNA (ending at 3182592) via a responsive riboswitch (RFN element) in the 5'-UTR (253 nt). sroG activates the ribBp operon and the central open reading frame of *ribB*, which codes for SpDHBP synthase through a  $\rho$ -dependent terminator. SpDHBP synthase catalyzes the substrate D-Ribulose 5-phosphate (Ru5P) in its conversion to SpDHBP and formate. As a metalloproteinase, SpDHBP synthase conversion is facilitated by divalent cations, usually Mg<sup>2+</sup>. Ru5P then undergoes an intramolecular rearrangement, with C-3 and C-5 breaking their bonds with C-4 and reconnecting between themselves. The C-4 and a hydroxyl are then released to form formate and the phosphorylated SpDHBP. The SpDHBP dimer comprises 8  $\alpha$ -helices surrounding a central 5-strand  $\beta$ -sheet, in a 3-layered  $\alpha$ - $\beta$ - $\alpha$  sandwich fold, whose layer interfaces are held by hydrophobic side chains. Hydrogen bonds form the dimer aggregation with 6 residues: Ala100, Asn46, Arg103, Glu101, Lys53, and Thr96. SpDHBP ultimately serves as a precursor for the xylene ring in riboflavin. With butanedionetransferase, it produces a phosphate group, 2 water molecules, and the 6,7-Dimethyl-8-(D-ribityl)lumazine precursor. Homotrimeric biboflavin synthase then catalyzes the dismutation of 6,7-Dimethyl-8-(D-ribityl)lumazine to afford riboflavin and butanedionetransferase.



Figure 2. The riboflavin biosynthesis pathway involving SpDHBP synthase

This paper reports the discovery of two new inhibitors of SpDHBP synthase. The discovery approach is distinctive on several fronts. First, while some virtual screening is ligand-based, seeking compounds which are similar to and hopefully better than an existing competent antibiotic [6], the screening here exploited the opportunity to inhibit SpDHBP synthase with a *de novo* structured-based approach. Second, contemporary target-based methods tend to rely on a single pocket for binding. For many years, it has been known that a superior method is to bind to a region of protein-protein interactions (PPI) [7]. The contact surfaces are larger (~1,500–3,000 Å<sup>2</sup>, compared with ~300– 1,000 Å<sup>2</sup>). Furthermore, PPI regional sites are usually contained within deep clefts that shield the binding from disruptive water molecules that aqueous bath of the ligand interaction. However, the PPI approach has been called "the Mount Everest" [8] of drug discovery because of its computational demands. A recent breakthrough comes from the realization that much of the energy of a PPI is contributed by just a few 'hot spot' residues. This PPI

cluster approach was used, with the computational demands significantly reduced through the chemical mimicry of just a small cluster of such residues [9]. A third departure from the literature is to screen from a very large dataset, involving 215,407,096 conformations of 22,723,923 compounds. This compares favorably with recent virtual screenings, such as the 260,000 compounds in the screening for penicillin-binding inhibitors [10] and the 200,000 in a screening for histidine kinase inhibitors. [11]

#### MATERIALS AND METHODS

#### Preparing target for docking.

The SpDHBP lyase crystallography structure from X-ray diffraction was used. It has a 1.95 Å resolution, a Cruickshank DPI of 0.153 Å, and only chain A, which was sufficient given that SpDHBP is a homodimer. Docking preprocessing was conducted using CHARMM 31b1 [12] and Chimera 1.10.1 [13]. This involved removing the ligand, deleting the solvent, and replacing incomplete side chains with those from Dunbrack's rotamer library [14]. Histidine protonation states were used and standard residues were from AMBER ff14SB [15]. Hydrogen were added to generate protonation states at physiological pH. Partial charges were assigned for standard residues such as water and standard nucleic acids.

#### Determining binding region of hotspot residues.

Clusters of PPI anchor residue hotspots were obtained from PocketQuery [16], which used a machine learning algorithm to train a support vector machine on a benchmark set of small-molecule inhibitor starting points (SMISPs). These SMISPs were obtained from known PPIs that had interface residues that overlap high-affinity ligands, thus providing validated starting points for the design of small-molecule inhibitors. For each residue, an aggregate was calculated based on six scores resulting from the residue going from the bound conformation in the complexed state to that as an independent chain. The first was the change,  $\Delta$ GFC, in Gibbs free energy. The second,  $\Delta\Delta$ GR, was the change in free energy of an alanine scanning mutagenesis. The third,  $\Delta$ SASA, was the solvent accessible surface area (SASA) change, and the fourth was related, in the percent change  $\Delta$ SASA%. The final two changes were evolutionary-based. One was the evolutionary rate and the other a conservation score based on multiple sequence alignment of related sequences. Residues with the highest aggregate were selected as targets.

### Employing pharmacophores for virtual screening.

A 3-D pharmacophore model was built with ZincPharmer [17], whose distinctive feature was its indexing approach. This approach allowed for searches at O(query) complexity, rather than the O(library) typically associated with fingerprint-based or alignment-based pharmacophores. The pharmacophore required hydrogen bond donors/acceptors of the ligand and receptor to be within 4Å of each other. Similarly, opposite charge features on the ligand and receptor must be within 5Å of each other, and aromatic features also within 5Å. Buriedness required the ligand hydrophobic feature to be within 6Å of at least three hydrophobic receptor features.

Virtual screening was done with a 2,256 core 63-DDR/21-QDR Infiniband supercomputer, screening 215,407,096 conformations of 22,723,923 compounds. The filter required a 1 maximum hit per conformation, 1 maximum hit per molecule, a maximum RMSD of 0.01, and a maximum of 4 rotatable bonds.

#### Screening for ADME-Tox properties.

Even though SpDHBP synthase is not present in human pathways, the lead compounds might still have inadvertent toxicities on other pathways. To check against ADME-Tox (absorption, distribution, metabolism, and excretion – toxicity) pharmacokinetics, a comprehensive set of prediction models from Chemicalize [18] was used. These included Lipinski's rule of five (molecular mass <= 500 Da, octanol-water partition coefficient log P <= 5, hydrogen bond acceptor count <= 10), a bioavailability criteria (satisfying at least 6 of the criteria of a set from Lipinski, rotatable bond count <= 10, polar surface area PSA <= 200 Å<sup>2</sup>, and the count of fused aromatic rings <= 5), the Ghose filter (molecular mass >= 160 Da and <= 480, atom count >= 20 and <= 70, log P >= -0.4 and <= 5.6, and refractivity >= 40 and <= 130), lead likeness criteria (molecular mass <= 450 Da, lipophilicity logD(pH 7.4) >= -4 and <= 4, ring count <= 4, rotatable bond count <= 10, hydrogen bond donor count <= 5, atom count <= 8), the Muegge filter (molecular mass >= 200 and <= 600, ring count <= 7, 6-atom count >= 5, atom count – 6-atom count – hydrogen count >= 2, rotatable bond count <= 15, hydrogen bond acceptor count <= 10, log P >= -2 and <= 5, and PSA <= 150), the Veber filter (rotatable bond count <= 10 and PSA <= 140).

#### Identifying CYP-mediated sites of metabolism.

While the ADME-Tox properties provided rules of thumb in identifying toxicity, Xenosite's CYP (cytochrome P450) quantified the metabolism of xenobiotic molecules, which are responsible for metabolizing 90% of commercial drugs [19]. Xenosite improves on previous sites of metabolism (SOMs) and sites of epoxidation (SOEs) predictions using machine learning fingerprint-based search built on deep neural convolution networks. Electrophilic reactivity, often caused by reactive metabolites that bind covalently to proteins, was indicated by the quantitative strength of a lead compound's conjugation at SOMs with glutathione (GSH) through cysteine, and with uridine diphosphate gluconosyltransferases (UGTs). Electrophilic reactivity can also be caused by the reaction of epoxides, a large class of 3-membered cyclic ethers formed by CYPs acting on aromatic or double bonds, due to ring tension and polarized C-O bonds at SOEs.

#### Docking ligand and analyzing receptor-ligand interactions.

Each lead compound from virtual screening that passed the ADME-Tox tests was validated to see if it could be docked with the receptor inhibitor. The initial candidate pocket was determined using a new measure of residue depth that correlates significantly better with conformation changes and protein stability in protein-protein interactions than accessible surface area [20]. This is because the procedure could uncover binding sites even if residues are buried in the protein core. Using the Voronoi procedure, this depth procedure first solvated the receptor in a pre-equilibrated box of solvent filled with atomic water model SPC216 from the GROMACS genbox [21]. It then removed clashing (within 2.6 Å) and non-bulk isolated (within a 4.2 Å solvent neighborhood) water molecules. Finally, the procedure mimicked the free diffusion dynamics of bulk water with a Monte-Carlo-like simulation that involved rotations and translations, with 25 solvation iterations.

Using the pocket thus found as a grid, molecular docking was conducted with Autodock Vina [22] Unlike molecular dynamics with explicit solvent, docking is governed not only by minimizing the energy profile but also by the morphology of the profile and temperature. The docked ligands were represented on a  $pK_a$  solvent surface of ionizable residues. These  $pK_a$  values revealed desolvation effects, hydrogen bonding, and Coulomb interactions. These values were derived by solving the linearized Poisson-Boltzmann equation (LPBE). The receptor-ligand interactions were mapped out using Discovery Studio from *Accelrys*. Receptor-ligand poses were generated with LigPlotPlus [23].

#### Quantifying drug potency with binding energy and pIC50.

One measure of the feasibility of the compound with respect to its docking was its binding affinity with the receptor. To calculate the change in free energy associated with binding, a conformation-dependent score was first calculated for each intermolecular pair of atoms:

$$c = \sum_{i < j} f_{t_i t_u}(r_{ij}),$$

where each atom *I* is of type  $t_i$ , with interatomic distance  $r_{ij}$ , and *f* is a symmetric set of interaction functions. The sum is over all pairs of intermolecular atoms with variable covalent lengths and dihedral angles except for 1-4 interactions—*i.e.*, those pairs separated by three consecutive covalent bonds. Autodock Vina calculated the free binding energy using a strictly increasing smooth function of *c* for the lowest-scoring conformation.

Drug potency was measured with the pIC50 from eADMET GmbH. For comparison, the pIC50 value was also obtained for a wide range of common antibacterial drugs: penicillin, erythromycin, trimethoprim, sulfamethoxazole, vancomycin, tetracycline, chloramphenicol, and ofloxacin.

#### **RESULTS AND DISCUSSION**

Figure 3 shows the PPI cluster of residues identified as the binding region. This highest-scoring cluster of residues identified in SpDHBP synthase has three residues: a methoionine, a proline, and a phenylalanine. It has a total  $\Delta G$  (change in free energy of an alanine scanning mutagenesis) of -6.61 kcal/mol and a minimum of -2.47 and a maximum of -1.91. These indicate strong interactions; the next cluster has a total  $\Delta G$  of -4.7 kcal/mol. As the figure shows, all residues also have with favorably low  $\Delta GFC$  and especially high  $\Delta SASA$ . They are rendered against a  $\Delta G$  surface on a rainbow spectrum, with red representing the lowest values and blue, the highest.



All energies in kcal/mol.

Figure 3. PPI Cluster Identified as Binding Region

Figure 4 shows the pharmacophore model that satisfied the conditions described in the "Methods" section. While there are other candidate pharmacophore classes, including hydrogen acceptors and donors, the 3 shown in the figure constitute the smallest set with the highest score. The pharmacophore was used with Bloom fingerprints and an index tree for rapid virtual screening.

Pharmacophore Class	х	у	Z	Radius
Aromatic	6.00	5.01	-2.30	1.10
Hydrophobic	6.08	14.14	-3.64	1.00
Hydrophobic	11.04	8.36	0.56	1.00

#### Figure 4. Pharmacophore Points

Figure 5 reports the hits from the Zinc library of compounds. All do not yet have common names in PubChem or ChemDB. Each hit notably has a small RMSD of at worst 0.010 Å. Further, the compounds exhibit heterogeneity, with the largest pair-wise Tanimoto coefficient at 0.3697, between ZINC05442755 and ZINC40157354. This provides some variation to the ways in which inhibition occurs, a favorable result of enlarging the target from just a single residue to a cluster of residues.

Compound		RM SD	Ma	Rotatable bonds	
ZINC05442755 N-[1-phenyl-2-(tetralin-1-ylcarbamoyl)ethyl]benzamide	O N H N H N H	0.01	39 8	4	
ZINC40157354 N-[3-[(1-phenylcyclopropyl)methylcarbamoyl]-5,6-dihydro-4H- cyclopenta[b]thiophen-2-yl]furan-2-carbox		0.01	40 6	4	
ZINC66796285 4-[4-(2-fluorophenyl)piperazin-1-yl]-2-(4-pyridyl)-6,7-dihydro-5H- cyclopenta[d]pyrimidine		0.00 8	37 5	3	
ZINC45972400 (2R)-N-(3,5-dimethylphenyl)-2-(phenylcarbamoylamino)propanamide	H <sub>3</sub> C	0.01 0	31 1	4	
ZINC77230615 N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-pyrazole-4- carboxamide	F NH	0.00 7	35 2	4	
ZINC80719114 N-[(1S)-2-[2-fluoro-5-(2-furyl)anilino]-1-methyl-2-oxo- ethyl]cyclobutanecarboxamide	H H H H H H H H H H H H H H H H H H H	0.00 9	33 0	4	

### Figure 5. Pharmacophore-based Hits

Figure 6 shows the ADME-Tox pharmacokinetic properties for the six hits. Although the first three hits do not look like lead compounds, the last three fulfilled all criteria. For stringent results, the 3 non-leads were discarded, although it should be noted that two (ZINC05442755 and ZINC66796285) of the them still passed all other criteria and could be candidates should more are needed.

Compound	Lipinski's rule of 5	Bio- availability	Ghose filter	Lead like-	Mue- gge	Veber filter
				ness	filter	
ZINC05442755	Yes	Yes	Yes	No	Yes	Yes
N-[1-phenyl-2-(tetralin-1-ylcarbamoyl)ethyl]benzamide						
ZINC40157354	No	Yes	Yes	No	No	Yes
N-[3-[(1-phenylcyclopropyl)methylcarbamoyl]-5,6-dihydro-4H-						
cyclopenta[b]thiophen-2-yl]furan-2-carbox						
ZINC66796285	Yes	Yes	Yes	No	Yes	Yes
4-[4-(2-fluorophenyl)piperazin-1-yl]-2-(4-pyridyl)-6,7-dihydro-						
5H-cyclopenta[d]pyrimidine						
ZINC45972400	Yes	Yes	Yes	Yes	Yes	Yes
(2R)-N-(3,5-dimethylphenyl)-2-						
(phenylcarbamoylamino)propanamide						
ZINC77230615	Yes	Yes	Yes	Yes	Yes	Yes
N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-						
pyrazole-4-carboxamide						
ZINC80719114	Yes	Yes	Yes	Yes	Yes	Yes
N-[(1S)-2-[2-fluoro-5-(2-furyl)anilino]-1-methyl-2-oxo-						
ethyl]cyclobutanecarboxamide						

#### Figure 6. ADME-Tox Properties

Figure 7 shows possible sites of epoxidation. Only ZINC80719114 has a ring with a bond epoxidation score that appears serious enough to be discarded as a candidate compound. This left two leads: (2R)-N-(3,5-dimethylphenyl)-2-(phenylcarbamoylamino)propanamide (ZINC45972400) and N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-pyrazole-4-carboxamide (ZINC77230615).



Figure 8 shows an example docking, of ZINC45972400. Specifically, it reports eight candidate conformations. The highest affinity conformation was selected for each lead compound. Their dockings are shown in Figure 9. They are rendered against rendered against pK<sub>a</sub> solvent surfaces with probe radius of 1.4 Å, with a rainbow spectrum in which red represents the lowest values and blue, the highest. The fit of the compounds against the surface is particularly striking.



Figure 8. Candidate Docking Confirmations of (2R)-N-(3,5-dimethylphenyl)-2-(phenylcarbamoylamino)propanamide (ZINC45972400)



ZINC45972400 (2R)-N-(3,5-dimethylphenyl)-2-(phenylcarbamoylamino)propanamide



ZINC77230615 N-(3-acetamido-4-methyl-phenyl)-3-(4fluorophenyl)-1H-pyrazole-4-carboxamide

Figure 9. Docking of the Two Lead Compounds, Against pKa Solvent Surfaces



(phenylcarbamoylamino)propanamide



ZINC77230615 N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-pyrazole-4-carboxamide

Figure 10. Receptor-ligand interactions



Figure 10 shows the receptor-ligand interactions. ZINC45972400 has two hydrophobic  $\pi$ -Alkyl bonds (from the ligand ring to CYS57, and to LEU55), an electrostatic  $\pi$ -anion bond (ring to GLU163:OE2), a hydrophobic  $\pi$ - $\pi$  T-shaped bond (ring to PHE85), and two conventional H bonds (from the H13 donor and H12, both to THR83:OG1).

Trimethoprim Sulfamethoxazole

Vancomycin

Tetracycline

Ofloxacin

Chloramphenicol

4.44 3.62

2.0

5.13

8.3 8.3

2.62

ZINC77230615 has a hydrophobic  $\pi$ -Alkyl bond (ligand ring to LEU129), a hydrophobic  $\pi$ - $\sigma$  bond (C1 to PHE85), a fluorine bond (ARG1:CZ to the F1 acceptor), an electrostatic  $\pi$ -cation bond (ARG1:SNH1 to the ligand ring), a carbon bond (C12 to the H acceptor GLU30:OE1), a conventional H-F bond (ARG139:HE to F1(, and 3 conventional H bonds (H8 to GLU163:OE2, H12 to HIS142:ND1, H1 to ASP23:OD2,). In addition to these interactions, the poses in Figure 11 show the residues making non-bonded contacts with the ligands. Taken together, these interactions and non-bonded contacts demonstrate the biology behind the high-affinities of the lead compounds.

Compound	Energies, kcal/mol						Ref	
		Kl	Inter-mole-	Inter-	Torsio-	Unbond	RMS	
		mM	cular	nal	nal	exten-ded		
ZINC4597240	-1.16	140.5	-2.35	0.57	1.19	0.57	14.4	
(2R)-N-(3,5-dimethylphenyl)-2-								
(phenylcarbamoylamino) propanamide								
ZINC77230615	1.23	-	0.03	0.23	1.19	0.23	13.7	
N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-								
1H-pyrazole-4-carboxamide								
Figure 12. Measures of Binding Affinity								

Compound	pIC50, -log M
ZINC45972400	3.86
(2R)-N-(3,5-dimethylphenyl)-2-(phenylcarbamoylamino)propanamide	
ZINC77230615	7.4
N-(3-acetamido-4-methyl-phenyl)-3-(4-fluorophenyl)-1H-pyrazole-4-carboxamide	
Penicillin V	5.04
Erythromycin	4.44

Figure 12	nIC50 Values	fow I and	Commounds and	Due evicting	Antibiotion
rigure 15.	DICEV values	for Leau	Compounds and	Pre-existing	Anuploucs

The affinity was quantified with energy levels, shown in Figure 12. In particular, the binding energies are low, and even negative for ZINC45972400. Figure 13 shows pIC50 values. Remarkably, the lead compounds have high values, with ZINC45972400's exceeding those of 3 known antibiotics now in use (trimethoprim, sulfamethoxazole, ofloxacin) and ZINC77230615 exceeding all but two. Notably, ZINC77230615's pIC50 exceeds even those of the commonly prescribed penicillin, erythromycin, and vancomycin.

#### CONCLUSION

The present study exploited the recent X-ray crystallography of SpDHBP synthase to identify two new compounds that hold promise against MDRSp. SpDHBP synthase is a vital enzyme in the riboflavin synthesis pathway, and has the advantages of being highly conserved in a clinically relevant set of Gram-negative bacterial pathogens and yet is absent in humans. The identified compounds were shown to have both high binding affinity and potency. Indeed, they exhibit higher pIC50 than the majority of current antibiotics.

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<sup>[8]</sup> Dömling, op. cit.