DOI: 10.1002/cbdv.202100066

FULL PAPER

CHEMISTRY & BIODIVERSITY

Design, Synthesis, Antimicrobial Evaluation and *in Silico* Studies of Eugenol-Sulfonamide Hybrids

Helloana Azevedo-Barbosa,[a] Bianca Pereira do Vale,[a] Grazielle Guidolin Rossi,[b] Fallon dos Santos Siqueira,[b] Kevim Bordignon Guterres,[b] Marli Matiko Anraku de Campos,[b] Thiago dos Santos,[c] Jamie Anthony Hawkes,[a] Danielle Ferreira Dias,[d] Stefânia Neiva Lavorato,[e] Thiago Belarmino de Souza,[f] and Diogo Teixeira Carvalho*[a]

[a] Faculdade de Ciências Farmacêuticas, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva 700, Alfenas 37130-001, MG, Brazil, e-mail: diogo.carvalho@unifal-mg.edu.br

[b] Departamento de Análises Clínicas e Toxicológicas, Universidade Federal de Santa Maria, Av. Roraima No. 1000, Cidade Universitária, Camobi, Santa Maria 97105-900, RS, Brazil

[c] Faculdade de Ciências Farmacêuticas de Ribeirão Preto, Universidade de São Paulo, Avenida do Café, Ribeirão Preto 14040-903, SP, Brazil

[d] Instituto de Química, Universidade Federal de Alfenas, Rua Gabriel Monteiro da Silva, 700, Alfenas 37130-001, MG, Brazil

[e] Centro das Ciências Biológicas e da Saúde, Universidade Federal do Oeste da Bahia, Rua Professor José Seabra de Lemos, 316, Recanto dos Pássaros, Barreiras 47808-021, BA, Brazil

[f] Escola de Farmácia, Universidade Federal de Ouro Preto, Morro do cruzeiro, Bauxita, Ouro Preto 35400-000 MG, Brazil

Using molecular hybridization, specific sulfonamide derivatives of eugenol were synthesized with subtle modifications in the allylic chain of the eugenol subunit (and also in the nature of the substituent group in the sulfonamide aromatic ring) which allowed us to study the influence of structural changes on the antimicrobial potential of the hybrids. Antimicrobial test results showed that most of the synthesized hybrid compounds showed good activity with better results than the parent compounds. Molecular docking studies of the hybrids with the essential bacterial enzyme DHPS showed complexes with low binding energies, suggesting that DHPS could be a possible target for the antibacterial sulfonamide-eugenol hybrids. Furthermore, most of the final compounds presented similar docking poses to that of the crystallographic ligand sulfamethoxazole. The results obtained allow us to conclude that these are promising compounds for use as new leads in the search for new antibacterial sulfonamides.

Keywords: sulfonamides, eugenol, antimicrobial, molecular hybridization, dihydropteroate synthase, molecular docking.

Introduction

Research aimed at discovering new antimicrobial agents, especially new antibacterial agents, represents one of the most urgent aspects in the field of new drug research. The evolution of microorganisms has surpassed the discovery of new active substances for drugs, whilst at the same time the rise in the misuse of antibacterial compounds has led to a worrying increase in microbial resistance.[1–3] Several strategies are employed in the discovery of new antibacterial drug candidates; i) the systematic screening of synthetic compound libraries; ii) the investigation of natural products guided by ethnopharmacology; iii) the structural modification of known drugs; and iv) the *in silico* design of new molecules.[4–6]

Supporting information for this article is available on the WWW under https://doi.org/10.1002/cbdv.202100066

In this context, the process known as molecular hybridization has great appeal and importance, enabling the discovery of excellent candidates for anti-infectious agents. This approach is characterized by the connection (or fusion) of distinct pharmacophoric subunits, derived from synthetic or natural bioactive substances, in order to create new chemical entities which are referred to as 'hybrids', These hybrids can present improved properties compared to the pre-existing pharmacophores individually.[7–9]

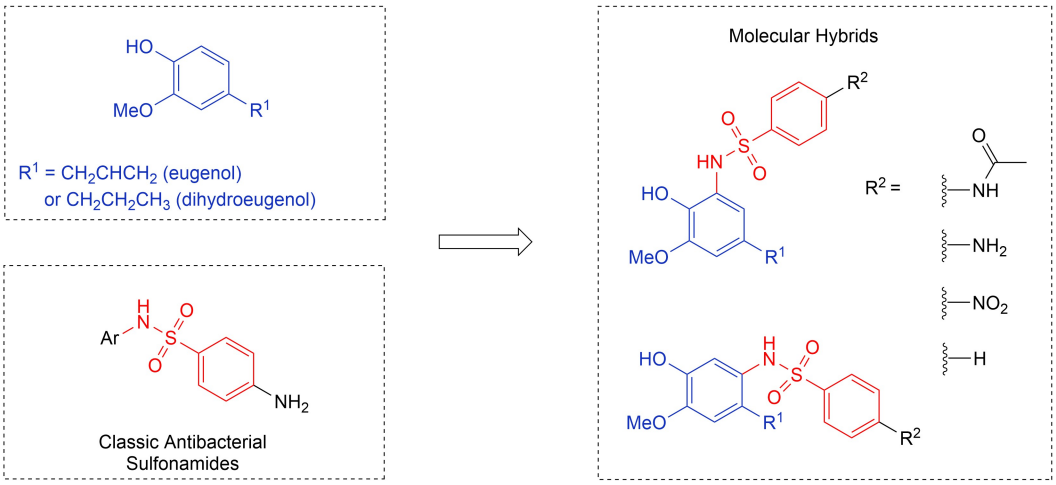
The introduction of sulfonamide-based antibacterial compounds into the therapeutic arsenal represented one of the most important milestones in advancing the treatment of infectious diseases, and contributed to the reduction in mortality rates.[10] Numerous sulfonamide-based compounds have been used to develop potent lead substances with better efficacy and less toxicity.[11] Indeed, bioactive molecules possessing the sulfonamide group have been reported to demonstrate potent properties such as antibacterial,[12] antifungal,[13] antiviral,[14] antiparasitic,[15] and anti-inflammatory properties[16] amongst others. Pharmacological properties of eugenol have been extensively highlighted, especially involving its antibacterial and antifungal properties.[17–22] Our research group has previously demonstrated that synthetic compounds obtained from natural phenylpropanoids, such as eugenol or its analogs (dihydroeugenol and isoeugenol), have important antimicrobial activity and that hybrids obtained from phenylpropanoids and sulfonamide moieties have important antitumor activity.[23–30] Taking advantage of the molecular hybridization strategy (and the well-established antibacterial potential of eugenol and sulfonamide-based drugs separately) herein we present for the first time the antibacterial activity of eugenol and dihydroeugenol-based hybrids bearing the sulfonamide functionality (*Scheme 1*).

In order to better understand the influence of structural changes on the antibacterial potential of the hybrid molecules, certain derivatives were also synthesized with subtle modifications in the allylic chain of the eugenol subunit, and in the nature of the substituent group in the aromatic ring of sulfonamide. Furthermore, we present molecular docking results that evaluate the ability of these compounds to interact with dihydropteroate synthase (an enzyme essential for bacterial multiplication), which gives a direct indication of the antibacterial properties of the tested compound.

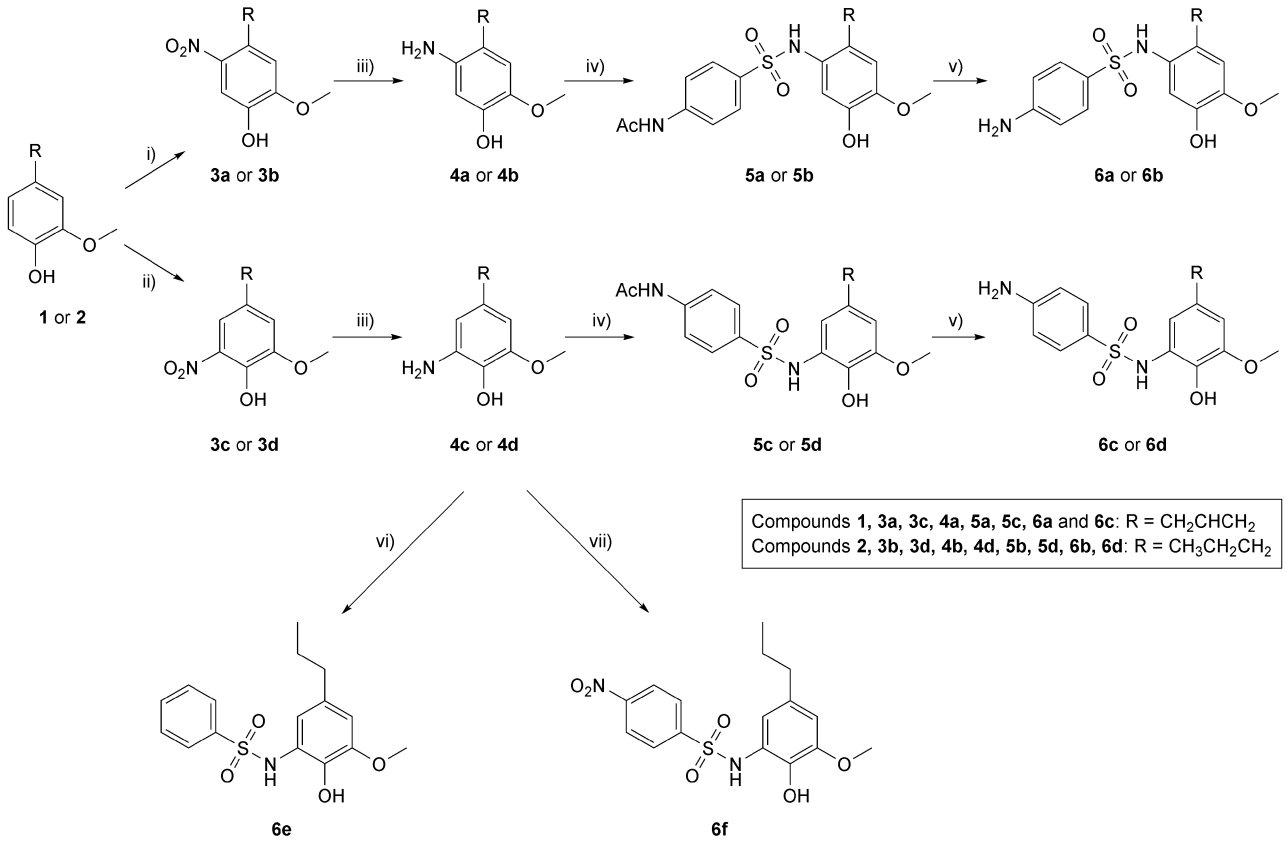
Results and Discussion

Chemistry

The substances evaluated in this study were synthesized according to the synthetic route shown in *Scheme 2*, and this route has previously been reported by Azevedo-Barbosa *et al.*[30] In summary, the starting molecules **1** and **2** were nitrated to produce intermediate compounds **3a**–**3d** that were further reduced to aryl amines **4a**–**4d** with tin chloride. These amines, which are key intermediates for obtaining the desired products, were reacted with the respective sulfonyl chlorides to form the known compounds **5a**–**5d** and **6a**–**6d**, as well as the newly presented compounds **6e** and **6f**.



Scheme 1. Rationale for the design of new sulfonamide-based molecular hybrids as antibacterial compounds.



Scheme 2. Synthetic route to the phenylpropanoid-based sulfonamide derivatives: i) $Bi(NO_3)_3 \cdot 5H_2O$, SiO_2, $CHCl_3$, 70 °C; ii) $NaNO_3$, $KHSO_4$, SiO_2/H_2O (1:1), CH_2Cl_2, 25 °C; iii) $SnCl_2 \cdot 2H_2O$, EtOH, 80 °C; iv) 4-acetamidobenzenesulfonyl chloride, pyridine, 100 °C; v) $SOCl_2$, MeOH, 25 °C; vi) benzenesulfonyl chloride, triethylamine, 25 °C, vii) 4-nitrobenzenesulfonyl chloride, triethylamine, 25 °C.

The characterization data for the previously known compounds are in agreement with the literature. The structural characterization of the newly reported compounds **6e** and **6f** was confirmed by Fourier transform infrared spectroscopy (FT-IR), ^1H and ^{13}C nuclear magnetic resonance spectroscopy (NMR) and high-resolution mass spectrometry (HR-MS) as described in the experimental section. In summary, the successful formation of sulfonamide linkages was confirmed by its characteristic bands in FT-IR spectra (3441 or 3485 cm^{-1} for NH and 1168 or 1225 cm^{-1} for SO_2). The presence of the propyl side chain in these compounds was confirmed (using NMR) by the presence of the group of signals between 2.46–0.87 ppm for **6e** and 2.49–0.90 for **6f**. The existence of seven aromatic hydrogens in each compound may be observed as signals between 7.80–6.42 for **6e** and 8.23–6.48 for **6f**. Small values of coupling constants (<2) were found for the doublets related to the aromatic hydrogens in the dihydroeugenol subunit. This confirms that the *ortho* relationship exists between the sulfonamide and the hydroxy group. Finally, the HR-MS data is in strict agreement with the calculated molecular masses.

Therefore, from the analytical data collected (a supplementary file is available containing all of the relevant spectra) it can be concluded that the structures of the synthesized compounds are those shown in *Scheme 2*. These compounds were then evaluated in antibacterial studies as well as molecular docking studies as shown in the following sections.

Antibacterial In Vitro Studies

The susceptibility profiles against Gram-negative (*Klebsiella pneumoniae*, *Pseudomonas aeruginosa*), Gram-positive (*Staphylococcus aureus*, *Staphylococcus saprophyticus*) and non-tuberculosis *Mycobacterium* microorganisms (*Mycobacterium abscessus*, *Mycobacterium massiliense*) were determined for sulfamethoxazole, eugenol and the synthesized hybrid compounds following known protocols.[31,32] The minimum inhib-

itory concentration (MIC) values are shown in *Table 1* with the best results shown in the shaded boxes.

As a typical antimicrobial agent of the sulfonamide class, sulfamethoxazole was one of the first systemic bacteriostatic drug used in the treatment of different human infections.[33] Sulfamethoxazole is one of the broadest spectrum sulfonamide drugs, and has been widely used to control infections caused by fast growing non-tuberculosis mycobacteria, mainly due to its easy oral administration.[34] It is worth noting that on first appearance, the MIC figures for sulfamethoxazole above are relatively high against *K. pneumoniae*, *P. aeruginosa*, *S. aureus* and *S. saprophyticus* which may be mistaken for potentially resistant species. However, sulfamethoxazole (whilst being a broad-spectrum antibacterial agent) is not used to treat these species due to its low level of activity. In the case of *M. abscessus* and *M. massiliense*, resistance mechanisms are known to have been developed by microorganisms of the genus *Mycobacterium* against sulfamethoxazole. This is confirmed by our results, which show a resistance profile with *M. massiliense* and a sensitivity profile with *M. abscessus*.

Overall, the results obtained in our study demonstrate high MIC values for sulfamethoxazole against Gram-negative and Gram-positive microorganisms (3125 $\mu g\,mL^{-1}$). Moreover, considering the dosage breakpoints listed in the standard protocols,[31] we observed a resistance profile to sulfamethoxazole presented by *M. massiliense*, as already reported in the studies by Siqueira *et al.*[35] and Flores *et al.*[36] These values corroborate the decrease in the clinical use of sulfamethoxazole, alone or in combination with trimethoprim, due to the development of resistance to this agent.[37] As seen with the sulfamethoxazole, eugenol also did not show noteworthy activity against any of the tested microorganisms (both resulting in high MIC values).

In contrast, most of the tested hybrid compounds showed low MIC values – indicating good antimicrobial activity. It is possible to see that compounds **5a**, **5c** and **6a**–**6e** had low MIC values against at least one studied pathogen. As the best results, we cite compounds **5c**, **6b** and **6d** against *S. aureus*, which show MIC values lower than 40 $\mu g\,mL^{-1}$. Therefore, we conclude that the inhibition of the microorganisms in their planktonic form occurred due to its new structural pattern, since the MIC values were much lower than the MIC values of sulfamethoxazole and eugenol alone (3125 and 2500 $\mu g\,mL^{-1}$, respectively).

The same was detected for compounds **6a**, **6b**, **6c** and **6e** with MIC values below 80 $\mu g\,mL^{-1}$ against *S. saprophyticus*, whilst eugenol and sulfamethoxazole had values higher than 2500 $\mu g\,mL^{-1}$. For the Gram-negative microorganisms, it is important to note that none of the hybrids showed good (i.e., low) MIC values, although all of them inhibited *P. aeruginosa* with lower MIC values than the parent compounds. For microorganisms of the genus *Mycobacterium*, we observed a satisfactory inhibitory effect of the new compounds against *M. massiliense*, highlighting the values obtained for **6a**, **6c** and **6e**, all of them lower than those obtained for sulfamethoxazole and eugenol alone.

The general aspects that could be observed regarding the influence of the structure on antimicrobial activity were that the substitution of aromatic amino or acetamide groups by the electron-withdrawing nitro group was detrimental, as can be seen between derivatives **6d** and **6f**, which showed the overall highest MIC results. On the other hand, the removal of both the amino and acetamide groups still allowed for good activity, as seen by the antibacterial

Table 1. Minimum inhibitory concentration (MIC) values for the synthesized compounds and parent compounds.

Compounds	Minimum Inhibitory Concentration ($\mu g\,mL^{-1}$)					
	Gram-negative		Gram-positive		Mycobacteria	
	K. pneumoniae	*P. aeruginosa*	*S. aureus*	*S. saprophyticus*	*M. abscessus*	*M. massiliense*
Eugenol	625	2500	2500	2500	156	156
Sulfamethoxazole	2500	2500	3125	3125	8	128
5a	2500	1250	**78**	312	2500	1250
5c	1250	625	**39**	312	625	312
6a	1250	625	156	**78**	156	**78**
6b	2500	1250	**19**	**78**	**78**	312
6c	1250	625	312	**19**	156	**78**
6d	625	312	**39**	625	**39**	312
6e	625	625	**78**	**78**	**78**	**78**
6f	1250	625	156	156	312	312

action of **6e** against *S. aureus*, *S. saprophyticus*, *M. abscessus* and *M. massiliense*. The interchange from an allyl to a propyl side chain in **6a** to **6b** and **6c** to **6d**, positively affected the antimicrobial action against *S. aureus* and *M. abscessus*. Considering the relative position of the sulfonamide group, its change from *meta* to *ortho* position in the phenolic ring increased antibacterial potency, as seen for **6c** against *S. saprophyticus* and for **6d** against *K. pneumoniae*, *P. aeruginosa* and *M. abscessus*.

Molecular Docking and Prediction Studies

Since classic sulfonamides are known as inhibitors of the essential bacterial enzyme dihydropteroate synthase (DHPS), it was hypothesized that the antibacterial activity exhibited by the sulfonamide-eugenol hybrids described in this work may be correlated to their ability to inhibit DHPS. Thus, the interaction of the designed compounds with this enzyme was predicted by docking studies and the main results are shown in *Table 2*. The crystal structure coordinates of *Y. pestis* dihydropteroate synthase complexed with sulfamethoxazole and dihydropteroate diphosphate (DHP) were obtained from the Protein Data Bank (PDB) online database using the identification number '3TZF',[38]

It is worth noting that although we are comparing data from cell-based experimental biological activities with theoretical enzyme-based studies (which do not take into account physico-chemical properties, membrane transposition capacity or the differences between Gram-positive and Gram-negative bacteria), previously published studies show a good correlation between MIC-based experimental trends and theoretical ones.[39–41] From the results shown in *Table 2*, the comparison between the binding energies of the enzyme-ligand complexes and the MIC data in the *in vitro* assays shows a partial correlation between the experimental antibacterial activity and the *in silico* enzyme inhibition potential. It is important to reaffirm that whilst 'low' MIC values indicate a 'high' inhibitory activity, 'low' binding energy values (i.e., more negative) refer to a 'high' affinity between the enzyme and ligand.

Compound **5a** was one of the least active compounds against the evaluated bacterial strains (other than against *K. pneumoniae* where it showed some activity), which correlates with the docking studies. In general, the *in vitro* studies indicate that the compounds with free amines tend to have greater antibacterial activity than the corresponding amides, which was also detected via the *in silico* studies and corroborates what is known from structure–activity relationships in sulfa drugs.[42] Although compounds **6e** and **6d** present low *in vitro* MIC values and low *in silico* binding energies complexing with DHPS, compounds **5a** and **6a** do not follow this correlation.

Considering the conformations assumed by the ligands into the enzyme active site, we found three different interaction profiles (*Figure 1A–C*). Compounds **6a**–**6e** present similar docking poses (*Figure 1A*). In addition, their poses are similar to the pose of crystallographic ligand sulfamethoxazole (*Figure 1D*). Since complexes with the lowest binding energies were found for DHPS and these ligands, and considering their pose similarity with sulfamethoxazole (a well-known DHPS inhibitor), suggesting that DHPS could be a possible target of the sulfonamide-eugenol hybrids.

For compounds **5a**, **5c** and **6f**, another two interaction profiles were found (shown in *Figure 1B* and *1 C*), resulting in the highest binding energy complexes among the evaluated ones. The energy differences could be explained based on the extra interaction between the sulfonamide group and the enzyme or the π-π stacking interaction with PHE190

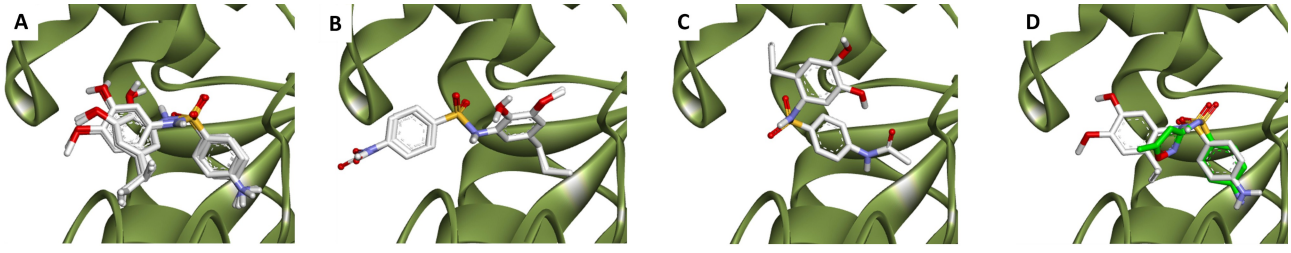


Figure 1. Docking results for the most stable complexes formed from sulfonamides and the dihydropteroate synthase (DHPS) active site (PDB ID: 3TZF),[38] where (A) are sulfonamides **6a**–**6e**, (B) is sulfonamide **5c**, (C) is sulfonamide **5a** and (D) is sulfonamide **6c** and crystallographic sulfamethoxazole (green carbons). The DHPS active site is represented by green ribbons, and atoms are white = carbon, yellow = sulfur, blue = nitrogen, and red = oxygen.

Table 2. Docking results in *Y. pestis* DHPS active site

Compound	Binding Energy (kcal/mol)	Ligand-Enzyme Interaction				
			Hydrogen bond	π-π T-shaped	π-π Stacking	Cation-π
5a	−6.9		H_3CO:GLN226 SO:ARG235	–	CONHPh:PHE28	–
5c	−6.9		SO:ARG235	HOPh:PHE28	–	–
6a	−7.7		NH_2:THR62 SO_2:SER222	NH_2Ph:PHE28 NH_2Ph:PHE190	–	–
6b	−7.6		NH_2:THR62 SO_2:SER222	NH_2Ph:PHE28 NH_2Ph:PHE190	–	–
6c	−7.7		HO:ARG235 SO_2:SER222	NH_2Ph:PHE28 NH_2Ph:PHE190	HOPh:PHE28	–
6d	−7.6		SO_2:SER222	NH_2Ph:PHE28 NH_2Ph:PHE190	HOPh:PHE28	–
6e	−7.8		HO:ARG235 SO_2:SER222	Ph:PHE28 Ph:PHE190	HOPh:PHE28	–
6f	−7.0		SO:ARG235	HOPh:PHE28	–	–
6a-DHP conjugate	−10.3	Heteroaromatic	NH_2:ASP185, NH:ASP185, NH_2:ASN115, NH:ASP96, C=N:LYS221, C=O:LYS221	–	–	Ar:ARG255
		Sulfonamide moiety	HO:ARG235 SO:SER222	NHPh:PHE28, NHPh:PHE190	–	NHPh:LYS221 –
6b-DHP conjugate	−10.2	Heteroaromatic	NH:ASP185 NH:ASP96 C=N:LYS221 C=O:LYS221	–	–	Ar:ARG255
		Sulfonamide moiety	SO:SER222 –	NHPh:PHE28, NHPh:PHE190	–	NHPh:LYS221 –
6c-DHP conjugate	−10.1	Heteroaromatic	NH_2:SER27 NH_2:GLU60 NH: SER27 NH:ASP96	–	–	Ar:ARG255
		Sulfonamide moiety	HO:LYS192, HO:GLY189 SO:SER222	NHPh:PHE28 NHPh:PHE190	–	–
6d-DHP conjugate	−10.0	Heteroaromatic	NH_2:SER27 NH_2:GLU60 NH: SER27 NH:ASP96	–	–	Ar:ARG255
		Sulfonamide moiety	HN:GLY189 *HO:GLY189* SO:SER222	NHPh:PHE28 *NHPh:PHE190* –	–	–

predicted to the five other compounds (*Figure 2* and *Table 2*).

For compounds **6a**–**6d** we also proposed the interaction between DHPS and the reaction products between dihydropteroate diphosphate, one of its substrates, and the 4-aminobenzenesulfonamides, which we refer to as dihydropterin-sulfonamide conjugates (*Figure 3*).

Roland *et al.*[42] demonstrated that sulfonamides could act as substrates for DHPS, being recognized by the *p*-aminobenzoic acid (PABA) binding site and conjugated to dihydropteroate diphosphate. Recently, Zhao *et al.*[43] demonstrated the potential of structure-related pterin-sulfa conjugates to inhibit such enzyme, which inspired us to evaluate the activity of these conjugates possibly generated *in situ*.

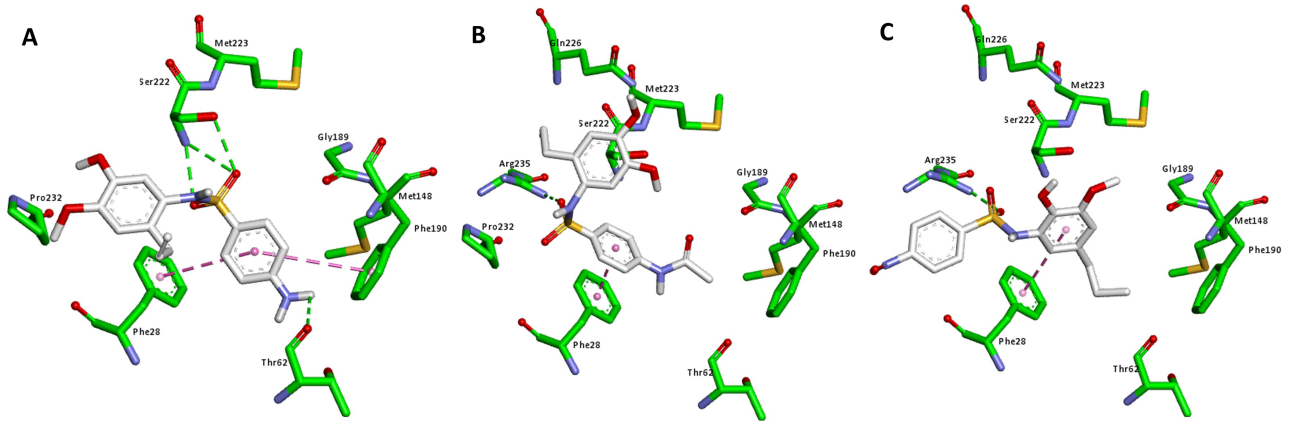


Figure 2. Interaction of compounds **6a**, **5a** and **6f** (A–C, respectively) with the dihydropteroate synthase (DHPS) active site. The carbon chain of the DHPS active site is represented as green ribbons, and the atoms are white = carbon, yellow = sulfur, blue = nitrogen, and red = oxygen.

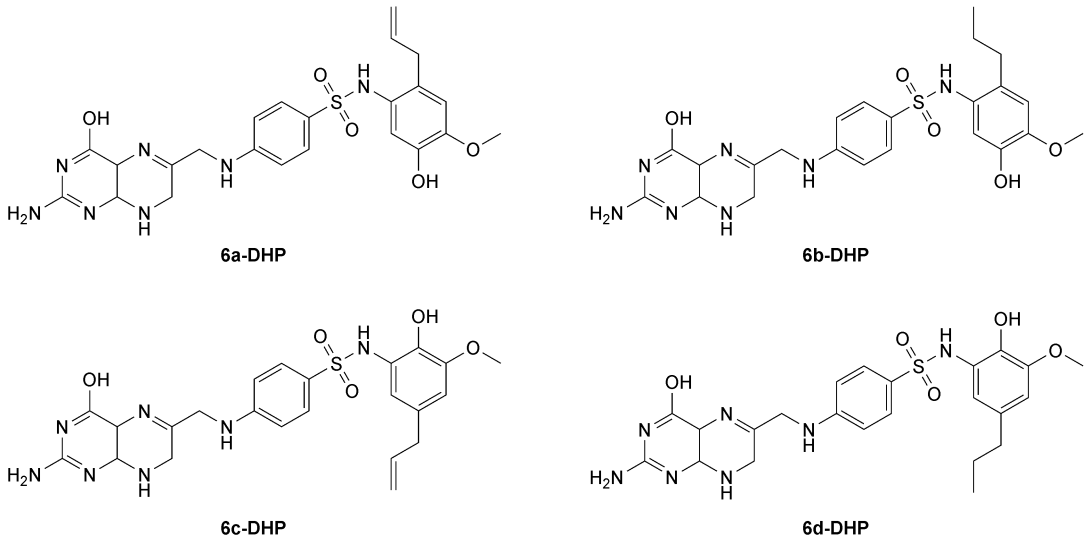


Figure 3. Structures of dihydropteroate diphosphate (DHP)-aniline adducts.

For the proposed conjugates, two interaction profiles were found: one for compounds **6a** and **6b** (*Figure 4A*) and another for compounds **6c** and **6d** (*Figure 4B*). This can be expected due to the structural similarity between these pairs. The complexes formed by compound **6a-DHP** or **6b-DHP** with DHPS were more energetically stable than with **6c-DHP** or **6d-DHP**. The heteroaromatic systems of compounds **6a-DHP** and **6b-DHP** were docked in a similar way to the same group in crystallographic DHP, as well as its aromatic rings found in the same position of crystallographic sulfamethoxazoles aromatic system (*Figure 4C*). This pose is also similar to the one found for pterin-sulfa conjugate compound 16 in DHPS active site as described by Zhao *et al.*[43] (*Figure 6D*). The DHP unit, as well as the central aromatic ring and the sulfonamide group, assume similar positions differing slightly only in the position of the *N*-phenyl group of the sulfonamides.

Subsequently, the physicochemical properties and toxicity risks of the tested compounds were predicted (*Table 3*) using the OSIRIS Property Explorer.[44] The toxicity risks predicted for eugenol seem to be suppressed in the hybrids, since none of eugenol-sulfonamide hybrids presents mutagenic, tumorigenic, or irritant potential like eugenol. In comparison to sulfamethoxazole, all of the synthesized sulfonamides present higher lipophilicity ($C_{log}P$) than this drug. $C_{log}P$

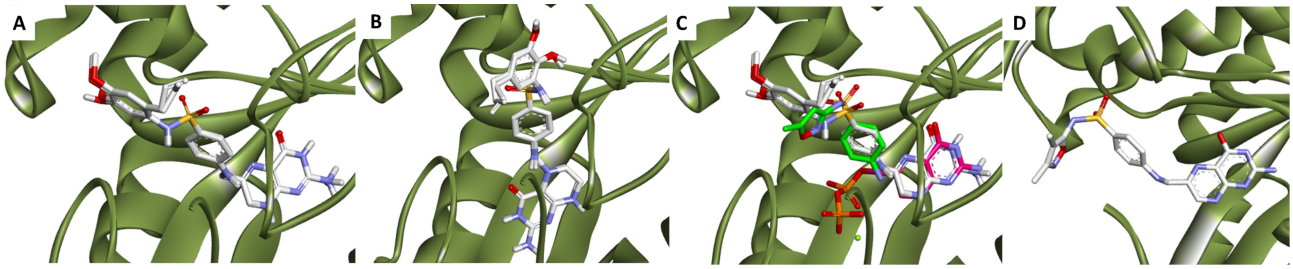


Figure 4. Interaction of DHP-sulfonamide conjugates (white = carbon, yellow = sulfur, blue = nitrogen, red = oxygen) with *Y. pestis* DHPS active site (green ribbon, PDB ID: 3TZF)[38] where (A) is the conformation of **6a-DHP** and **6c-DHP** found in their most stable complex with DHPS active site. (B) is the conformation of **6c-DHP** and **6d-DHP** found in their most stable complex with DHPS active site. (C) is the conformation comparison among ligands **6a-DHP** and **6b-DHP** (white = carbon) and crystallographic sulfamethoxazole (green = carbon) and DHP (pink = carbon) interacting with DHPS active site. (D) is the pose of pterin-sulfa conjugate compound 16 in *Y. pestis* DHPS active site (green ribbon, PDB ID: 5JQ9).[40]

Table 3. Predicted physicochemical properties, toxicity risks and drug score of eugenol-sulfonamide hybrids, eugenol, and reference drug sulfamethoxazole [*values closer to 1 indicate the greater the potential of a compound to become a drug].

Compound	Toxicity Risks				Physicochemical properties			Drug Likeness	Drug Score*
	Mutagenic	Tumorigenic	Irritant	Reproductive effect	ClogP	Solubility	MW		
Eugenol	+	+	+	–	2.27	−2.05	164	−2.78	0.11
Sulfamethoxazole	–	–	–	–	0.44	−3.02	253	2.77	0.88
5a	–	–	–	–	2.28	−3.72	376	1.30	0.70
5c	–	–	–	–	2.28	−3.72	376	3.09	0.77
6a	–	–	–	–	1.89	−3.46	334	−0.17	0.61
6b	–	–	–	–	2.08	−3.52	336	0.38	0.66
6c	–	–	–	–	1.89	−3.46	334	1.67	0.77
6d	–	–	–	–	2.08	−3.52	336	2.22	0.79
6e	–	–	–	–	2.76	−3.44	321	−7.29	0.41
6f	–	–	–	–	1.84	−3.90	366	−8.55	0.39

values close to (or above) 2 indicate that the designed compounds may have better gastrointestinal absorption profiles, as well as having greater potential to access the central nervous system, which are important features for the treatment of systemic and central bacterial infections, respectively. The combination of values obtained from drug likeness, $C_{log}P$, solubility, molecular weight (MW) and the toxicity risks calculation, results in the Drug Score parameter, which is important to assess the potential of a new substance to become a drug. It is important to remember that the closer the value is to 1, the greater the potential of a compound has to become a drug. The eugenol-sulfonamide hybrids present Drug Score values which are higher than eugenol itself, indicating an improvement in their general properties compared to the parent compound. It is possible to observe that the aromatic amino and amide groups contribute positively to this parameter, since lower values were predicted for compounds **6e** and **6f**, which present none of these groups. In general, the best Drug Score values are associated to the sulfonamides that also demonstrated the best (i.e., lowest) MIC values: **5c**, **6b**, **6c** and **6d**.

Conclusions

A set of sulfonamides, designed as hybrid compounds from either eugenol or dihydroeugenol were screened for the first time as antibacterial agents. Most of the tested hybrid compounds showed good antimicrobial activity, with the best results being demonstrated by the compounds with a free amino group-as is usual with the classic antibacterial sulfonamide drugs. However, this seems not to be a requirement since the unsubstituted compound **6e** had good (i.e., low) MIC values against *S. aureus*, *S. saprophyticus*, *M. abscessus* and *M. massiliense*.

In general, the most active compounds showed excellent (i.e., low) MIC values when compared to eugenol alone, which had an MIC greater than 2000 $\mu g\,mL^{-1}$ against *S. aureus* and *S. saprophyticus*. For the Gram-negative microorganisms, it is important to note that none of the hybrids showed good (i.e., low) MIC values, although all of them inhibited *P. aeruginosa* with lower MIC values than the parent compounds.

Docking studies of these sulfonamides with the essential bacterial enzyme DHPS showed complexes with favorable binding energies and these results suggest that DHPS could be a possible target of the antibacterial sulfonamide-eugenol hybrids. Furthermore, compounds **6a**–**6e** presented similar docking poses which are also very close to that with the crystallographic ligand sulfamethoxazole. Docking studies also indicate that dihydropterin-sulfonamide conjugates interact with DHPS active site in a similar way to pterin-sulfa conjugates known to act as competitive DHPS inhibitors.[40] These results indicate promise, which leads us to conclude that compounds **6a**–**6e** are the most promising compounds for use as new leads in the search for new antibacterial sulfonamides.

Experimental Section

General

Eugenol, dihydroeugenol and other reagents were purchased from Sigma (São Paulo, Brazil) and were used as supplied. Thin-layer chromatography (TLC) on silica gel plates (Macherey–Nagel, DC-Fertigfolien ALUGRAM® Xtra Sil G/UV254) was used to monitor the progress of reactions and to prove purity. Column chromatography was performed using column grade silica gel (Sorbiline; 0.040–0.063 mm mesh size). Melting points of the compounds were obtained on a Büchi 535 melting-point apparatus and are uncorrected. Fourier transform infrared spectroscopy (FT-IR) spectra were recorded on a Shimadzu FT-IR Affinity-1 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AC-300 spectrometer (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR experiments) using either deuterated chloroform or deuterated dimethyl sulfoxide. Chemical shifts (δ) were reported in parts per million (ppm) with reference to tetramethylsilane (TMS) as the internal standard and coupling constants (J) were reported in Hertz (Hz). High resolution mass (HR-MS) spectra were obtained on a quadrupole time-of-flight instruments Waters Xevo G2-S QTOF and Bruker Daltonics micro TOF QII/ESI-TOF, both equipped with ESI positive and negative ion sources.

Synthesis and Characterization

The synthesis and characterization of the amino key intermediates **4a**–**4d** and the sulfonamide compounds **5a**–**5d** and **6a**–**6d** has previously been published by this research group.[30] The synthesis of the new compounds **6e** and **6f** were done as follows:

- The amino derivative **4b** (1 equiv.) and the respective benzenesulfonyl chloride (3 equiv.) were solubilized in triethylamine (5 ml) and vigorously stirred overnight at room temperature.
- The progress of the reaction was monitored by TLC using a hexane/ethyl acetate solvent mixture (8:2 ratio v/v).
- The reaction mixture was poured into ice/water and the pH was adjusted to pH 1–2 using hydrochloric acid before being extracted with ethyl acetate (3× 50 ml aliquots).
- The organic phase was dried over anhydrous sodium sulfate and concentrated under reduced pressure to produce the crude product, which in turn was purified by column chromatography (silica gel, hexane/ethyl acetate (6:4 v/v) solvent mixture).

The analytical results generated for the newly presented synthesized hybrid compounds **6e** and **6f** are as follows:

***N*-(2-Hydroxy-3-methoxy-5-propylphenyl) benzenesulfonamide** (**6e**). Beige solid. Yield: 30%. Melting Point: 120–125 °C. FT-IR (cm^{-1}): 3441 (N–H), 3275 (O–H), 2953–2847 (C–H sp^3), 1616, 1512 (C=C), 1330, 1168 (S=O), 1311 (C–O–C). ^{1}H-NMR (δ ppm; $CDCl_3$, 300 MHz): 7.80–7.76 (m; 2H; H2′), 7.51–7.46 (m; 1H; H4′), 7.41–7.35 (m; 2H; H3′), 6.95 (d; 1H; $J=1.8$; H4), 6.94 (s; 1H; OH), 6.42 (d; 1H; $J=1.8$; H6), 5.49 (s; 1H; $NHSO_2$), 3.78 (s; 3H; OCH_3), 2.46 (t; 2H; $J=7.6$; CH_3–CH_2–CH_2), 1.62–1.49 (sex; 2H; CH_3–CH_2–CH_2), 0.87 (t; 3H; $J=7.3$; CH_3–CH_2–CH_2). ^{13}C-NMR (δ ppm; $CDCl_3$, 75 MHz): 146.1 (C3), 139.0 (C1′), 134.5 (C1), 134.1 (C5), 132.8 (C4′), 128.7 (C2′), 127.2 (C3′), 123.4 (C2), 113.8 (C4), 107.8 (C6), 56.0 (OCH_3), 37.8 (CH_3–CH_2–CH_2), 24.6 (CH_3–CH_2–CH_2), 13.5 (CH_3–CH_2–CH_2). HR-MS ESI/Q-TOF (*m/z*): $[M+Na]^+$ calculated for $C_{16}H_{19}NNaO_4S=344.0927$; Found 344.0925.

***N*-(2-Hydroxy-3-methoxy-5-propylphenyl)-4-nitrobenzenesulfonamide** (**6f**). Yellow solid. Yield: 33%. Melting Point: 176–180 °C. FT-IR (cm^{-1}): 3485 (N–H), 3240 (O–H), 2958–2859 (C–H sp3), 1613, 1528 (C=C), 1514, 1462 (NO_2), 1340, 1225 (S=O), 1314 (C–O–C). ^{1}H-NMR (δ ppm; $CDCl_3$, 300 MHz): 8.23–8.19 (m; 2H; H3′), 7.95–7.90 (m; 2H; H2′), 6.98 (d; 1H; $J=1.7$; H4), 6.95 (s; 1H; OH), 6.48 (d; 1H; $J=1.7$; H6), 5.40 (s; 1H; $NHSO_2$), 3.79 (s; 3H; OCH_3), 2.49 (t; 2H; $J=7.6$; CH_3–CH_2–CH_2), 1.64–1.52 (sex.; 2H; CH_3–CH_2–CH_2), 0.90 (t; 3H; $J=7.3$; CH_3–CH_2–CH_2). ^{13}C-NMR (δ ppm; $CDCl_3$, 75 MHz): 150.1 (C4′), 146.2 (C3), 144.8 (C1′), 134.9 (C1), 134.7 (C5), 128.5 (C3′), 123.9 (C2′), 122.1 (C2), 114.7 (C4), 107.8 (C6), 56.0 (OCH_3), 37.7 (CH_3–CH_2–CH_2), 24.6 (CH_3–CH_2–CH_2), 13.6 (CH_3–CH_2–CH_2). HR-MS ESI/Q-TOF (*m/z*): $[M+K]^+$ calculated for $C_{16}H_{18}KN_2O_6S=$ 405.0517; Found 405.0518.

From the analytical data generated (a supplementary file is available containing all of the relevant spectra), it can be concluded that the structures of the synthesized compounds are those shown in *Figure 2*. These compounds were then evaluated in antibacterial studies as well as molecular docking studies as shown in the following sections.

Antimicrobial Evaluation

Microorganisms

For this study three groups of standard strains were used from the Bacteria Bank of the Mycobacteriology Laboratory (LABMYCO), Department of Clinical and Toxicological Analysis, Federal University of Santa Maria. The first group comprised of the standard strains of rapidly growing mycobacteria (*M. fortuitum* ATCC 6841 and *M. abscessus* ATCC 19977). The second group included Gram-positive bacteria (*Staphylococcus aureus* ATCC 29213 and *Staphylococcus saprophyticus* ATCC 15305). The third group comprised the Gram-negative bacteria, *Pseudomonas aeruginosa* (PAO1) and *Klebsiella pneumonia* (KPC-type carbapenemase). The mycobacteria strains were kept in a Löwenstein-Jensen agar (Hi Media Laboratories Pvt. Ltd., India) and the Gram-positive and Gram-negative strains remained stored in BHI broth (brain heart infusion broth) supplemented with glycerol at −20 °C until use.

Determination of the Minimum Inhibitory Concentration (MIC)

Susceptibility tests were performed using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M24-A2 standard[31] protocol for the mycobacteria group and according to CLSI M100-S23 standard[32] for the other bacteria. The compounds were used at different concentrations, according to the mass obtained in the synthesis stage and the quantity available for the test, from serial dilutions in Mueller–Hinton Broth (Hi Media Laboratories Pvt. Ltd., India). The density of the inoculum was standardized according to the 0.5 MacFarland scale and transferred to the microplates. The plates were incubated at 30 °C for 72 h for the mycobacteria group and at 35 °C during 24 h for the Gram-positive and Gram-negative bacteria. MIC was considered as the lowest concentration, given as $\mu g\,mL^{-1}$ values, of the compound that inhibited visible bacterial growth. The compound 2,3,5-triphenyltetrazolium chloride (TTC) (Vetec®) was used as an indicator of microbial growth.

Molecular Docking and Prediction Studies

The structures of the sulfonamide derivatives and the dihydropteroate diphosphate (DHP)-sulfonamide conjugates were drawn and optimized with a Dreiding-like force field in the Biovia Discovery Studio software program.[45] The crystal structure coordinates of *Y. pestis* dihydropteroate synthase complexed with sulfamethoxazole and dihydropteroate diphosphate (DHP) were obtained from the Protein Data Bank (PDB) online database using the identification number '3TZF',[38]

AutoDock PDBQT files of macromolecules and ligands were prepared using AutoDock Tools Software v1.5.2[46] using the method described by Morris *et al.*[47] For the docking studies with the sulfonamide derivatives, the macromolecule structure was prepared by keeping only chain B and their crystalized DHP and Mg^{2+} ligands. The dimensions of the docking grid were 1.6 nm × 1.6 nm × 1.6 nm (16 Å × 16 Å × 16 Å) and the coordinates of the center point were (29.951) × (−1.452) × (8.959), covering the area occupied by the crystallographic ligand sulfamethoxazole. For the docking studies with the DHP-sulfonamide adducts, crystal DHPS was prepared by keeping only the chain B and removing all ligands, water molecules and chain A. The dimensions of the docking grid were 2.4 nm × 2

nm × 20 nm (24 Å × 20 Å × 20 Å) and the coordinates of the center point were (24.878) × (−1.871) × (8.959), covering the area occupied by crystallographic ligands sulfamethoxazole and DHP.

Docking studies were performed with AutoDock Vina Software v1.1.2[48] using an exhaustiveness of 8 (default value) and generating a maximum of 10 binding modes as previously reported in a method by Trott *et al.*[49] Results were analyzed and figures generated using BIOVIA Discovery Studio software.[45] The toxicity risks, physicochemical properties ($C_{log}P$, Solubility and molecular weight) and the Drug Score of eugenol-sulfonamide hybrids were predicted using the online OSIRIS Property Explorer software program.[44]

Acknowledgements

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES)-Finance Code 001 and Scholarship 88882.429942/2019-01.

Author Contribution Statement

The conception and design of the work was carried out by D. T. Carvalho, H. Azevedo-Barbosa, T. B. de Souza, and J. A. Hawkes. The synthetic experimental work and structural characterization was performed by H. Azevedo-Barbosa, B. P. do Vale, and T. dos Santos. Data collection was carried out by F. S. Siqueira and S. N. Lavorato. Analysis and interpretation of the data was undertaken by D.T. Carvalho, H. Azevedo-Barbosa, J. A. Hawkes, and D. F. Dias. Computational studies were conducted by S. N. Lavorato. The antimicrobial evaluation and subsequent data analysis was done by F. S. Siqueira, M. M. A. Campos, G. G. Rossi, and K. B. Guterres. Drafting of the manuscript was conducted by D. T. Carvalho, H. Azevedo-Barbosa, J. A. Hawkes, and S. N. Lavorato. The critical revisions of the manuscript were conducted by D. T. Carvalho, J. A. Hawkes, and F. S. Siqueira. All authors approved the submitted manuscript.

References

[1] S. B. Levy, B. Marshall, 'Antibacterial Resistance Worldwide: Causes, Challenges and Responses', *Nat. Med.* **2004**, *10*, 122–129. DOI: 10.1038/nm1145.

[2] B. Catry, J. Dewulf, D. Maes, B. Pardon, B. Callens, M. Vanrobaeys, G. Opsomer, A. de Kruif, F. Haesebrouck, 'Effect of Antimicrobial Consumption and Production Type on Antibacterial Resistance in the Bovine Respiratory and Digestive Tract', *PLoS One* **2016**, *11*, e0146488. DOI: 10.1371/journal.pone.0146488.

[3] B. Aslam, W. Wang, M. I. Arshad, M. Khurshid, S. Muzammil, M. H. Rasool, M. A. Nisar, R. F. Alvi, M. A. Aslam, M. U. Qamar, M. K. F. Salamat, Z. Baloch, 'Antibiotic resistance: a rundown of a global crisis', *Infect. Drug Resist.* **2018**, *11*, 1645–1658. DOI: 10.2147/IDR.S173867.

[4] G. M. Keserü, G. M. Makara, 'The Influence of Lead Discovery Strategies on the Properties of Drug Candidates', *Nat. Rev. Drug Discovery* **2009**, *8*, 203–212. DOI: 10.1038/nrd2796.

[5] S. Ou-Yang, J. Lu, X. Kong, Z. Liang, C. Luo, H. Jiang, 'Computational Drug Discovery', *Acta Pharmacol. Sin.* **2012**, *33*, 1131–1140. DOI: 10.1038/aps.2012.109.

[6] D. J. Newman, G. M. Cragg, 'Natural Products as Sources of New Drugs from 1981 to 2014', *J. Nat. Prod.* **2016**, *79*, 629–661. DOI: 10.1021/acs.jnatprod.5b01055.

[7] M. d. O. Pedrosa, R. M. D. da Cruz, J. d. O. Viana, R. O. d. Moura, H. M. Ishiki, J. M. B. Filho, M. F. F. M. Diniz, M. T. Scotti, L. Scotti, F. J. B. Mendonca, 'Hybrid Compounds as Direct Multitarget Ligands: A Review', *Curr. Top. Med. Chem.* **2017**, *17*, 1044–1079. DOI: 10.2174/1568026616666160927160620.

[8] U. R. Anumala, J. Waaler, Y. Nkizinkiko, A. Ignatev, K. Lazarow, P. Lindemann, P. A. Olsen, S. Murthy, E. Obaji, A. G. Majouga, S. Leonov, J. P. von Kries, L. Lehtiö, S. Krauss, M. Nazaré, 'Discovery of a Novel Series of Tankyrase Inhibitors by a Hybridization Approach', *J. Med. Chem.* **2017**, *60*, 10013–10025. DOI: 10.1021/acs.jmedchem.7b00883.

[9] V. Ivasiv, C. Albertini, A. E. Gonçalves, M. Rossi, M. L. Bolognesi, 'Molecular Hybridization as a Tool for Designing Multitarget Drug Candidates for Complex Diseases', *Curr. Top. Med. Chem.* **2019**, *19*, 1694–1711. DOI: 10.2174/1568026619666190619115735.

[10] D. Davenport, 'The war against bacteria: how were sulphonamide drugs used by Britain during World War II?', *Medical Humanities* **2012**, *38*, 55–58. DOI: 10.1136/medhum-2011-010024.

[11] F. A. Khan, S. Mushtaq, S. Naz, U. Farooq, A. Zaidi, S. M. Bukhari, A. Rauf, M. S. Mubarak, 'Sulfonamides as Potential Bioactive Scaffolds', *Curr. Org. Chem.* **2018**, *22*, 818–830. DOI: 10.2174/1385272822666180122153839.

[12] F. Naaz, R. Srivastava, A. Singh, N. Singh, R. Verma, V. K. Singh, R. K. Singh, 'Bioorganic and Medicinal Chemistry Molecular modeling, synthesis, antibacterial and cytotoxicity evaluation of sulfonamide derivatives of benzimidazole, indazole, benzothiazole and thiazole', *Bioorg. Med. Chem.* **2018**, *26*, 3414–3428. DOI: 10.1016/j.bmc.2018.05.015.

[13] S. K. Badampudi, R. G. P. Aluru, R. R. Papammagari, R. L. R. K. Rao, 'Synthesis, characterization and pharmaco-

logical evaluation of certain sulfonamide containing heterocyclic motifs', *Pol. Annals Med.* **2014**, *21*, 75–81. DOI: 10.1016/j.poamed.2014.07.006.

[14] G. Chen, H. Ren, A. Turpoff, A. Arefolov, R. Wilde, J. Takasugi, A. Khan, N. Almstead, Z. Gu, T. Komatsu, C. Freund, J. Breslin, J. Colacino, J. Hedrick, M. Weetall, G. M. Karp, 'Discovery of *N*-(4′-(indol-2-yl)phenyl)sulfonamides as novel inhibitors of HCV replication', *Bioorg. Med. Chem. Lett.* **2013**, *23*, 3942–3946. DOI: 10.1016/j.bmcl.2013.04.050.

[15] C. R. Andrighetti-Fröhner, K. N. de Oliveira, D. Gaspar-Silva, L. K. Pacheco, A. C. Joussef, M. Steindel, C. M. O. Simões, A. M. T. de Souza, U. O. Magalhaes, I. F. Afonso, C. R. Rodrigues, R. J. Nunes, H. C. Castro, 'Synthesis, biological evaluation and SAR of sulfonamide 4-methoxychalcone derivatives with potential antileishmanial activity', *Eur. J. Med. Chem.* **2009**, *44*, 755–763. DOI: 10.1016/j.ejmech.2008.04.016.

[16] J. Lal, S. K. Gupta, D. Thavaselvam, D. D. Agarwal, 'Biological activity, design, synthesis and structure-activity relationship of some novel derivatives of curcumin containing sulfonamides', *Eur. J. Med. Chem.* **2013**, *64*, 579–588. DOI: 10.1016/j.ejmech.2013.03.012.

[17] S. Hemaiswarya, M. Doble, 'Synergistic interaction of eugenol with antibiotics against Gram negative bacteria', *Phytomedicine* **2009**, *16*, 997–1005. DOI: 10.1016/j.phymed.2009.04.006.

[18] D. Campaniello, M. R. Corbo, M. Sinigaglia, 'Antifungal Activity of Eugenol against *Penicillium*, *Aspergillus*, and *Fusarium* Species', *J. Food Prot.* **2010**, *73*, 1124–1128. DOI: 10.4315/0362-028X-73.6.1124.

[19] J. Qiu, H. Feng, J. Lu, H. Xiang, D. Wang, J. Dong, J. Wang, X. Wang, J. Liu, X. Deng, 'Eugenol reduces the expression of virulence-related exoproteins in *Staphylococcus aureus*', *Appl. Environ. Microbiol.* **2010**, *76*, 5846–5851. DOI: 10.1128/AEM.00704-10.

[20] H. Carrasco, M. Raimondi, L. Svetaz, M. di Liberto, M. V. Rodriguez, L. Espinoza, A. Madrid, S. Zacchino, 'Antifungal activity of eugenol analogs. Influence of different substituents and studies on mechanism of action', *Molecules* **2012**, *17*, 1002–1024. DOI: 10.3390/molecules17011002.

[21] B. Das, D. Mandal, S. K. Dash, S. Chattopadhyay, S. Tripathy, D. P. Dolai, S. K. Dey, S. Roy, 'Eugenol Provokes ROS-Mediated Membrane Damage-Associated Antibacterial Activity against Clinically Isolated Multidrug-Resistant *Staphylococcus aureus* Strains', *Infect. Dis.: Res. Treat.* **2016**, *9*, 11–19. DOI: 10.4137/idrt.s31741.

[22] A. Marchese, R. Barbieri, E. Coppo, I. E. Orhan, S. F. Nabavi, M. Izadi, M. Abdollahi, M. Nabavi, M. Ajami, 'Critical Reviews in Microbiology Antimicrobial activity of eugenol and essential oils containing eugenol: A mechanistic viewpoint', *Crit. Rev. Microbiol.* **2017**, *43*, 668–689. DOI: 10.1080/1040841X.2017.1295225.

[23] T. B. De Souza, M. Orlandi, L. F. L. Coelho, L. C. C. Malaquias, A. L. T. Dias, R. R. Carvalho, N. C. Silva, D. T. Carvalho, 'Synthesis and in vitro evaluation of antifungal and cytotoxic activities of eugenol glycosides', *Med. Chem. Res.* **2014**, *23*, 496–502. DOI: 10.1007/s00044-013-0669-2.

[24] T. B. De Souza, P. O. B. Raimundo, S. F. Andrade, T. M. M. Hipólito, N. C. Silva, A. L. T. Dias, M. Ikegaki, R. P. Rocha, L. F. L. Coelho, M. P. Veloso, D. T. Carvalho, D. F. Dias, 'Synthesis and antimicrobial activity of 6-triazolo-6-deoxy eugenol glucosides', *Carbohydr. Res.* **2015**, *410*, 1–8. DOI: 10.1016/j.carres.2015.04.002.

[25] T. B. De Souza, K. M. O. Brito, N. C. Silva, R. P. Rocha, G. F. Sousa, L. P. Duarte, L. F. L. Coelho, A. L. T. Dias, M. P. Veloso, D. T. Carvalho, D. F. Dias, 'New eugenol glucoside-based derivative shows fungistatic and fungicidal activity against opportunistic Candida glabrata', *Chem. Biol. Drug Des.* **2016**, *87*, 83–90. DOI: 10.1111/cbdd.12625.

[26] P. H. O. Abrão, R. B. Pizi, T. B. De Souza, N. C. Silva, A. M. Fregnan, F. N. Silva, L. F. L. Coelho, L. C. C. Malaquias, A. L. T. Dias, D. F. Dias, M. P. Veloso, D. T. Carvalho, 'Synthesis and biological evaluation of new eugenol Mannich bases as promising antifungal agents', *Chem. Biol. Drug Des.* **2015**, *86*, 459–465. DOI: 10.1111/cbdd.12504.

[27] T. M. M. Hipólito, G. T. L. Bastos, T. W. L. Barbosa, T. B. De Souza, L. F. L. Coelho, A. L. T. Dias, I. C. Rodríguez, M. H. Dos Santos, D. F. Dias, L. L. Franco, D. T. Carvalho, 'Synthesis, Activity, and Docking Studies of Eugenol-Based Glucosides as New Agents against *Candida* sp.', *Chem. Biol. Drug Des.* **2018**, *92*, 1514–1524. DOI: 10.1111/cbdd.13318.

[28] T. Dos Santos, C. M. Coelho, T. C. Elias, F. S. Siqueira, E. S. S. D. Nora, M. M. A. De Campos, G. A. P. de Souza, L. F. L. Coelho, D. T. Carvalho, 'Synthesis and biological evaluation of new eugenol-derived 1,2,3-triazoles as antimycobacterial agents', *J. Braz. Chem. Soc.* **2019**, *30*, 1425–1436. DOI: 10.21577/0103-5053.20190038.

[29] D. J. Alvarenga, L. M. F. Matias, L. M. Oliveira, L. P. M. O. Leão, J. A. Hawkes, B. V. B. Raimundo, L. F. D. Castro, M. M. A. De Campos, F. S. Siqueira, T. Dos Santos, D. T. Carvalho, 'Exploring how structural changes to new Licarin A derivatives effects their bioactive properties against rapid growing mycobacteria and biofilm formation', *Microb. Pathog.* **2020**, *144*, 104203. DOI: 10.1016/j.micpath.2020.104203.

[30] H. Azevedo-Barbosa, G. Á. Ferreira-Silva, C. F. Silva, T. B. de Souza, D. F. Dias, A. C. C. de Paula, M. Ionta, D. T. Carvalho, 'Phenylpropanoid-based sulfonamide promotes cyclin D1 and cyclin E down-regulation and induces cell cycle arrest at G1/S transition in estrogen positive MCF-7 cell lines', *Toxicol. in Vitro* **2019**, *59*, 150–160. DOI: 10.1016/j.tiv.2019.04.023.

[31] Clinical and Laboratory Standards Institute (CLSI) Database, 'Susceptibility Testing of Mycobacteria, *Nocardia* spp. and Other Aerobic Actinomycetes', 3rd Edition: M24-A2. 2011. Available online: https://clsi.org/standards/products/microbiology/documents/m24/. Accessed 02/10/2020.

[32] Clinical and Laboratory Standards Institute (CLSI) Database, 'Performance Standards for antimicrobial susceptibility testing: M100-S23. 2013'. Available online: https://clsi.org/standards/products/microbiology/documents/m100/. Accessed 02/10/2020.

[33] M. J. García-Galán, M. S. Díaz-Cruz, D. Barceló, 'Identification and determination of metabolites and degradation products of sulfonamide antibiotics', *TrAC Trends Anal. Chem.* **2008**, *27*, 1008–1022. DOI: 10.1016/j.trac.2008.10.001.

[34] 'Manual Nacional de Vigilância Laboratorial da Tuberculose e Outras Micobactérias', 2008. Ministério da Saúde, Brasília, Brazil. (Translated from Portuguese: 'National Manual for Laboratory Surveillance of Tuberculosis and Other Myco-

bacteria', 2008. Ministry of Health, Brasília, Brazil). ISBN 978–85-334-1447-1.

[35] F. D. S. Siqueira, G. G. Rossi, A. K. Machado, C. F. S. Alves, V. C. Flores, V. D. Somavilla, V. A. Agertt, J. D. Siqueira, R. de S. Dias, P. M. Copetti, M. R. Sagrillo, D. F. Back, M. M. A. de Campos, 'Sulfamethoxazole derivatives complexed with metals: a new alternative against biofilms of rapidly growing mycobacteria', *Biofouling*. **2018**, *34*, 893–911. DOI: 10.1080/08927014.2018.1514497.

[36] V. C. Flores, F. d. S. Siqueira, C. R. Mizdal, P. C. Bonez, V. A. Agertt, S. T. Stefanello, G. G. Rossi, M. M. A. d. Campos, 'Antibiofilm effect of antimicrobials used in the therapy of mycobacteriosis', *Microb. Pathog.* **2016**, *99*, 229–235. DOI: 10.1016/j.micpath.2016.08.017.

[37] P. Houvinen, 'Resistance to Trimethoprim-Sulfamethoxazole', *Clin. Infect. Dis.* **2001**, *32*, 1608–14. DOI: 10.1086/320532.

[38] Protein Data Bank (PDB) online database. https://www.rcsb.org. Accessed 02/10/2020.

[39] S. Mondal, S. M. Mandal, T. K. Mondal, C. Sinha, 'Structural characterization of new Schiff bases of sulfamethoxazole and sulfathiazole, their antibacterial activity and docking computation with DHPS protein structure', *Spectrochim. Acta A Mol. Biomol. Spectrosc.* **2015**, *150*, 268–279. https://doi.org/10.1016/j.saa.2015.05.049.

[40] M. B. Pisano, A. Kumar, R. Medda, G. Gatto, R. Pal, A. Fais, B. Era, S. Cosentino, E. Uriarte, L. Santana, F. Pintus, M. J. Matos, 'Antibacterial Activity and Molecular Docking Studies of a Selected Series of Hydroxy-3-arylcoumarins', *Molecules*. **2019**, *24*, 2815. https://doi.org/10.3390/molecules24152815.

[41] B. Jia, Y. M. Ma, B. Liu, P. Chen, Y. Hu, R. Zhang, 'Synthesis, Antimicrobial Activity, Structure-Activity Relationship, and Molecular Docking Studies of Indole Diketopiperazine Alkaloids', *Front. Chem.* **2019**, *7*, 837. https://doi.org/10.3389/fchem.2019.00837.

[42] S. Roland, R. Ferone, R. J. Harvey, V. L. Styles, R. W. Morrison, 'The characteristics and significance of sulfonamides as substrates for *Escherichia coli* dihydropteroate synthase', *J. Biol. Chem.* **1979**, *254*, 10337–10345. PMID: 385600.

[43] Y. Zhao, W. R. Shadrick, M. J. Wallace, Y. Wu, E. C. Griffith, J. Qi, M. K. Yun, S. W. White, R. E. Lee, 'Pterin-sulfa conjugates as dihydropteroate synthase inhibitors and antibacterial agents', *Bioorg. Med. Chem. Lett.* **2016**, *26*, 3950–3954. DOI: 10.1016/j.bmcl.2016.07.006.

[44] OSIRIS Property Explorer. Available Online: www.organic-chemistry.org/prog/peo/. Accessed 01/10/2020.

[45] BIOVIA, Dassault Systèmes, 'Biovia Discovery Studio software v19.1.0.18287', San Diego: Dassault Systèmes. 2019. Available online: https://www.3ds.com/products-services/biovia/. Accessed 01/10/2020.

[46] 'AutoDock Tools Software v1.5.2', Available online: http://autodock.scripps.edu/resources/adt. Accessed 01/10/2020.

[47] G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell, A. J. Olson, 'AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility', *J. Comput. Chem.* **2009**, *16*, 2785–2791. DOI: 10.1002/jcc.21256.

[48] 'AutoDock Vina Software v1.1.2', Available online: http://autodock.scripps.edu/resources/adt. Accessed 01/10/2020.

[49] O. Trott, A. J. Olson, 'AutoDock Vina: Improving the Speed and Accuracy of Docking with a New Scoring Function, Efficient Optimization, and Multithreading', *J. Comput. Chem.* **2010**, *31*, 455–461. DOI: 10.1002/jcc.21334.

Received January 25, 2021
Accepted March 4, 2021

