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Synthesis and antimicrobial evaluation of some 1-(4-arylthiazol--2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols

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Abstract: A series of sixteen 1-(4-arylthiazol-2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a**–**p**) was synthesized starting from dehydroacetic acid (DHA, **1**) *via* the stepwise formation of thiosemicarbazone (**2**), 3-(1-(2-(4-arylthiazol-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran--2-ones (**4a**–**d**) and 1-(1-(4-arylthiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol--4-yl)butane-1,3-diones (**5a**–**d**) in high yields. The *in vitro* antibacterial and antifungal activities of the synthesized bipyrazoles **7a**–**p** were investigated against two Gram-positive bacterial strains, *viz*. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 7443), one Gram-negative bacterial strain, *viz*. *Escherichia coli* (MTCC 42), and two fungal strains, *viz*. *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 282). The compounds **7a** and **7e** were found to exhibit better inhibitory activity against *A. niger* than the reference fluconazole. Moreover, the antifungal activities of the title compounds were more prolific than their antibacterial activities. Furthermore, in order to study binding interactions, docking simulations of compounds **7a**, **7m** and **7o** were performed into the active site of *S. aureus* 1,4-dihydroxy-2-naphthoyl--CoA synthase.

Keywords: bipyrazoles; antibacterial; antifungal; docking simulations.

INTRODUCTION

Infectious diseases caused by various microbes, such as bacteria, fungi, viruses and parasites, are still a leading cause and major threat to public health, despite much important progress having been made in recent years.[1–3] The discovery, development and synthesizing a new efficient, active and less toxic molecules for systemic activities remains the aim and subject of many molecular architectures and drug researchers. The growing clinical importance of drug resistant bacterial and fungal pathogens has lent an additional urgency in the field

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of microbiological research and development of new antimicrobial agents. Bipyrazoles, in particular, have been proven to be useful as potential antitumor, anti-inflammatory, antibacterial, antifungal, cytotoxic and CNS active agents.[4–9] Their usefulness is reported in the synthesis of heat resistant polymers.[10] Bipyrazolyl derivatives have also been reported to act as active components in the capture of active oxygen and free radicals *in vivo* and act as useful agents for preventing and treating various diseases induced by active oxygen, while some of them have found application as agents for detecting singlet oxygen.[11] On the other hand, thiazole derivatives also constitute an attractive class of organic heterocycles as they have been reported to exhibit a wide range of pharmacological properties, including anticonvulsant,[12] analgesic,[13] antimicrobial,[14] anti-inflammatory,[15] antitumor,[16] antipyretic,[17] antitubercular,[18] anti-HIV,[19] antioxidant,[20] diuretic[21] *etc*. Likewise, benzothiazole derivatives have attracted continuing interest because of their widespread biological activities, *viz*. anticancer, antimicrobial, anticonvulsant, antiviral, antitubercular, antimalarial, anthelmintic, analgesic and anti-inflammtory, antidiabetic, antidepressant, anti-HIV, anti-Alzheimer's disease (AD), antihistaminic, antioxidant, anti-leishmanial, enzyme inhibitors and receptor agonists/antagonists, fungicidal activities and many more.[22–27]

The pervasiveness of the bipyrazole nucleus in bioactive molecules has stimulated the need for elegant and different ways for their synthesis. Recently, synthetic methodologies to bipyrazoles along with their applications have been reviewed as a new class of supramolecular complexes, organometallics, cage-like structures and self-assembling, metallomacrocycles with bipyrazole ligands as promising catalysts, molecular mimics, molecular magnetic devices and sensors.[28] The synthesis of bipyrazoles derived from dehydroacetic acid (DHA) was studied by Gelin *et al*[29] and other researchers.[30–32]

Protein and small molecule docking is a potent strategy that has grown into an essential component of computer-aided drug design.[33] It gives an appropriate picture of protein−ligand interactions and facilitates the design of potential active leads. Docking tools normally explore ligand conformations to generate a list of docked poses along with scores and detect a single highest scoring pose as the best solution.[34]

Owing to the importance of bipyrazoles, thiazoles and bezothiazoles that qualify them as excellent scaffolds in therapeutic and medicinal research and in continuation of an ongoing research program on the synthesis of pyrazoles,[35–37] herein, the synthesis, antimicrobial evaluation and molecular docking studies of several 1-(4-arylthiazol-2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a**–**p**) are reported.

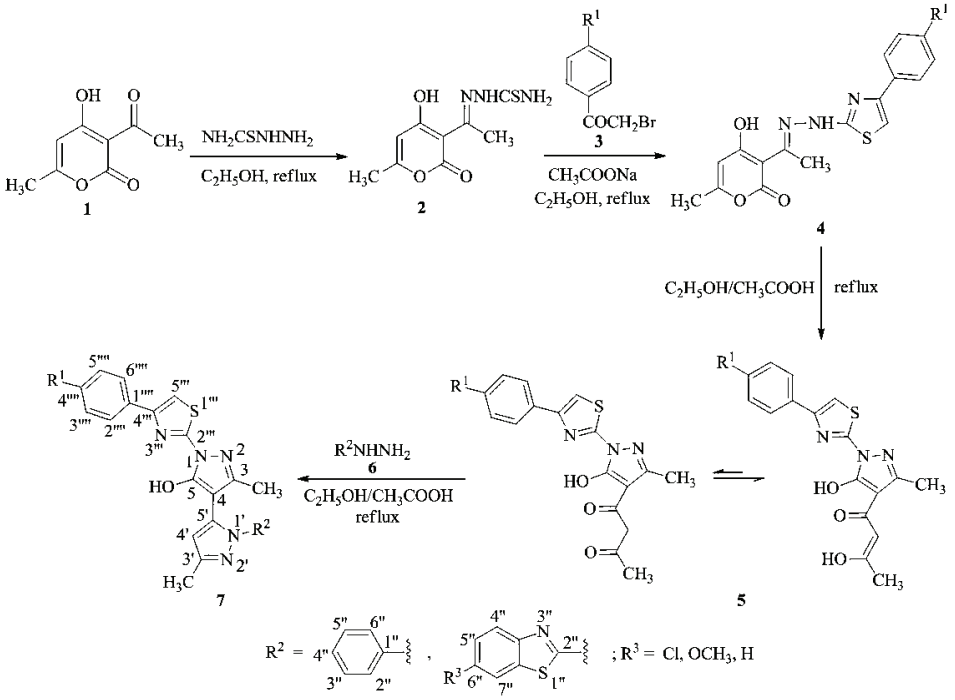
RESULTS AND DISCUSSION

Chemistry

Dehydroacetic acid (**1**) has been reported to generate a number of heterocyclic compounds through ring opening and recyclization upon treatment with a variety of binucleophiles.[38–43] The approach towards the synthesis of 1-(4-arylthiazol-2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a**–**p**) commenced with the readily available dehydroacetic acid (**1**). Dehydroacetic acid upon condensation with equimolar quantities of thiosemicarbazide in refluxing absolute ethanol afforded thiosemicarbazone **2** that on gentle refluxing with the appropriate phenacyl bromide/4-substituted phenacyl bromides (**3a**–**d**) in dry ethanol in presence of sodium acetate yielded the corresponding 3-(1-(2-(4-arylthiazol-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-ones (**4a**–**d**), which in turn underwent rearrangement in ethanol–acetic acid mixture to afford the key intermediate 1-(1-(4-arylthiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)butane-1,3-diones (**5a**–**d**) in good yields, as described in the literature.[37,44] Thereafter, a solution of equimolar quantities of (**5a**–**5d**) and an appropriate phenylhydrazine/2-hydrazinylbenzothiazole/6-substituted-2-hydrazinylbenzothiazoles (**6a**–**d**) in ethanol and acetic acid was heated at reflux for 2–3 h to furnish the desired 1-(4-arylthiazol-2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a**–**7p**) in good yields (69–91 %), Scheme 1.

The structures of all the bipyrazoles **7a**–**p** were well characterized by satisfactory spectroscopic (IR, ^{1}H-NMR, ^{13}C-NMR and mass) and analytical data. The IR spectra of the bipyrazoles **7a**–**d** exhibited characteristic broad absorption bands of medium intensity in the region 3099–3107 cm^{-1} due to the C5–OH of the pyrazole moiety. The ^{1}H-NMR spectra of the bipyrazoles **7a**–**d**, in each case, displayed two sharp singlets in the δ regions 1.87–1.88 and 2.39–2.43 ppm, each integrating for three protons due to methyl groups situated at the 3- and 3′-positions, respectively. In the aromatic region, at the highest field, a singlet integrating for one proton in the δ region 6.29–6.30 ppm was displayed, which was assigned to C4′–H of the pyrazole moiety. All these assignment were in obeisance with the chemical shifts of similar protons, as reported in literature.[45–47] The broad signal exhibited in the δ region 13.05–13.16 ppm (exchangeable with D_2O) was safely assigned to C5–OH. The other aliphatic and aromatic protons were observed in the expected regions. The structures of the bipyrazoles **7e**–**p**, tethered with a benzothiazole/6-substituted benzothiazole moiety were corroborated by employing spectral as well as analytical results such as those for **7a**–**d**. The compounds **7e**–**p** exhibited the characteristic broad absorption bands of medium intensity in the region 3055–3107 cm^{-1} due to C5–OH stretching in their IR spectra. The ^{1}H-NMR spectra of **7e**–**p** displayed two sharp singlets in the δ regions 2.24–2.26 and 2.33–2.39 ppm, integrating for three protons each due to methyl groups situated at the 3- and 3′-positions, respectively. In the aromatic

region, the key proton of the pyrazole ring C4′–H appeared as a singlet in the δ region 6.52–6.57 ppm. In the most downfield region, the broad signal exhibited in the δ region 13.02–13.17 ppm (exchangeable with D_2O) was easily assigned to C5–OH. The other aliphatic and aromatic protons were observed in the expected regions. Furthermore, the ^{13}C-NMR and mass spectral results of the bipyrazoles **7a**–**p** were also found satisfactory (*vide* experimental).



3a, **4a**, **5a**, $R^1 = OCH_3$; **3b**, **4b**, **5b**, $R^1 = CH_3$; **3c**, **4c**, **5c**, $R^1 = Cl$; **3d**, **4d**, **5d**, $R^1 = H$; **6a**, $R^2 = C_6H_5$;
6b, R^2 = 6-Chlorobenzo[*d*]thiazol-2-yl; **6c**, R^2 = 6-Methoxybenzo[*d*]thiazol-2-yl; **6d**, R^2 = Benzo[*d*]thiazol-2-yl;
7a, $R^1 = OCH_3$, $R^2 = C_6H_5$; **7b**, $R^1 = CH_3$, $R^2 = C_6H_5$; **7c**, $R^1 = Cl$, $R^2 = C_6H_5$; **7d**, $R^1 = H$, $R^2 = C_6H_5$;
7e, $R^1 = OCH_3$, R^2 = 6-Chlorobenzo[*d*]thiazol-2-yl; **7f**, $R^1 = CH_3$, R^2 = 6-Chlorobenzo[*d*]thiazol-2-yl;
7g, $R^1 = Cl$, R^2 = 6-Chlorobenzo[*d*]thiazol-2-yl; **7h**, $R^1 = H$, R^2 = 6-Chlorobenzo[*d*]thiazol-2-yl;
7i, $R^1 = OCH_3$, R^2 = 6-Methoxybenzo[*d*]thiazol-2-yl; **7j**, $R^1 = CH_3$, R^2 = 6-Methoxybenzo[*d*]thiazol-2-yl;
7k, $R^1 = Cl$, R^2 = 6-Methoxybenzo[*d*]thiazol-2-yl; **7l**, $R^1 = H$, R^2 = 6-Methoxybenzo[*d*]thiazol-2-yl;
7m, $R^1 = OCH_3$, R^2 = Benzo[*d*]thiazol-2-yl; **7n**, $R^1 = CH_3$, R^2 = Benzo[*d*]thiazol-2-yl;
7o, $R^1 = Cl$, R^2 = Benzo[*d*]thiazol-2-yl; **7p**, $R^1 = H$, R^2 = Benzo[*d*]thiazol-2-yl

Scheme 1. Synthesis of bipyrazoles **7a**–**p**.

In vitro *antimicrobial activities*

The *in vitro* antibacterial and antifungal activities of all the sixteen synthesized 4,5′-bipyrazol-5-ol derivatives (**7a**–**p**) were screened against two Gram-positive bacterial strains, *viz*. *Bacillus subtilis* (MTCC 441) and *Staphylococcus aureus* (MTCC 7443), one Gram-negative bacterial strain, *viz*. *Escherichia coli*

(MTCC 42), and two fungal strains, *viz*. *Candida albicans* (MTCC 183) and *Aspergillus niger* (MTCC 282). Ciprofloxacin and fluconazole were used as standards against the bacteria and fungi, respectively. The serial tube dilution technique[48] was used to determine the minimum inhibitory concentrations (*MIC*) of the test compounds. The *MIC* values of **7a**–**p** against the tested microorganisms are depicted in Table I.

TABLE I. *In vitro* antimicrobial activity of **7a**–**p**; minimum inhibitory concentration (*MIC*), μmol mL^{-1}

Entry	Microorganism				
	Gram-positive bacteria		Gram-negative bacterium	Fungi	
	B. subtilis	*S. aureus*	*E. coli*	*C. albicans*	*A. niger*
7a	0.0282	0.0141	0.0282	0.0141	0.0035
7b	0.0292	0.0292	0.0146	0.0073	0.0073
7c	0.0111	0.0279	0.0279	0.0069	0.0069
7d	0.0075	0.0302	0.0302	0.0302	0.0075
7e	0.0234	0.0936	0.0234	0.0468	0.0029
7f	0.0241	0.0241	0.0060	0.0060	0.0060
7g	0.0464	0.0232	0.0232	0.0057	0.0057
7h	0.0247	0.0247	0.0247	0.0061	0.0061
7i	0.0117	0.0235	0.0235	0.0058	0.0058
7j	0.0121	0.0243	0.0243	0.0486	0.0972
7k	0.0234	0.0234	0.0117	0.0058	0.0058
7l	0.0249	0.0249	0.0249	0.0062	0.0062
7m	0.0249	0.0124	0.0124	0.0124	0.0062
7n	0.0258	0.0258	0.0258	0.0064	0.0516
7o	0.0247	0.0123	0.0247	0.0061	0.0061
7p	0.0531	0.0265	0.0265	0.0066	0.0066
Ciprofloxacin	0.0047	0.0047	0.0047	–	–
Fluconazole	–	–	–	0.0050	0.0050

The data presented in Table I revealed that bipyrazoles **7g** and **7p** exhibited the least inhibitory activity, **7a**, **7b**, **7e**, **7f**, **7h** and **7k**–**o** exhibited low activity, **7c**, **7i** and **7j** were found to be moderately active whereas **7d** (*MIC*, 0.0075 μmol mL^{-1}) was found to exhibit noticeable inhibitory activity against *B. subtilis*; against *S. aureus*, **7e** was found the least active, **7b**–**d**, **7f**–**l**, **7n** and **7p** exhibited low activity whereas **7a**, **7m** and **7o** were found moderately active; **7a**, **7c**–**e**, **7g**–**j**, **7l** and **7n**–**p** were found less effective, and **7b**, **7k** and **7m** were found moderately active. Compound **7f** (*MIC*, 0.0060 μmol mL^{-1}) exhibited considerable inhibitory activity against *E. coli* as compared to the reference, *i.e.*, ciprofloxacin (*MIC*, 0.0047 μmol mL^{-1}). The compounds **7e** and **7j** were found least effective, **7d** was found less effective, **7a** and **7m** were found moderately active, and **7b**, **7c**, **7f**–**i**, **7k**, **7l** and **7n**–**p** exhibit appreciable inhibitory activity against *C. albicans*, while **7j** and **7n** were found least effective, and **7b**–**d**, **7f**–**i**, **7k**–**m**, **7o** and **7p**

were found to exhibit considerable inhibitory activity against *A. niger* as compared to the reference, *i.e*., fluconazole. It is interesting to mention here that **7a** (*MIC*, 0.0035 μmol mL^{-1}) and **7e** (*MIC*, 0.0029 μmol mL^{-1}) were found to exhibit greater inhibitory activity than fluconazole against *A. niger*.

From the antimicrobial activity data, the following structure–activity relationships were established:

a) Substitution of hydrogen by larger groups, such as methyl, methoxy and chloro, resulted in increased activity against *E. coli.*

b) Against *B. subtilis*, compounds containing a methoxy group were generally more active.

c) Derivatives containing chloro groups were found to exhibit greater inhibitory activity against *C. albicans.*

d) In case of *S. aureus* and *A. niger*, compounds with chloro or methoxy groups were more active.

Docking simulations

In order to determine the probable mechanism of action responsible for the antimicrobial activity of the synthesized compounds, the common structural skeleton was screened using the BAITOC web server (www.scfbio-iitd.res.in/ /software/drugdesign/baitocnew.jsp (March, 2017)), which provides probable biological targets for organic compounds. The structural framework under study was found to be active against *S. aureus* 1,4-dihydroxy-2-naphthoyl-CoA synthase. Therefore, the most active compounds against *S. aureus*, namely **7o**, **7m** and **7a**, were docked into the active site of 1,4-dihydroxy-2-naphthoyl-CoA synthase (Fig. 1). The X-ray crystal structure of the enzyme was obtained from the RCSB Protein Data Bank (PDB ID: 2UZF) and the Autodock Vina docking program[49] was used for performing the docking simulations.

The most preferred solutions of most active compound **7o** displayed various types of interactions with residues of the active site (Fig. 2). The hydroxyl group of this compound displayed a hydrogen bond with Ser73 and the nitrogen of the thiazole ring created a hydrogen bond with Thr143. The hydroxypyrazole ring and the phenyl ring exhibited π-donor type hydrogen bonding with Thr143 and Ser149, respectively.

The benzothiazole and thiazole rings were involved in T-shaped π–π interactions with Phe258 while the hydroxyl on the pyrazole ring showed π–π stacking interactions with the same residue. The pyrazole ring and thiazole ring made π–σ bonding with Val119 and Val147, respectively. The same conformation of compound **7m** exhibited most of the interactions (Fig. 3) as displayed by compound **7o**. However, one hydrogen bond with Ser73 and the T-shaped π–π interactions with Phe258 were missing in case of **7m**. However, the benzothiazole ring presented three π–alkyl type interactions with Val33, Arg34 and Ala36. Similarly, com-

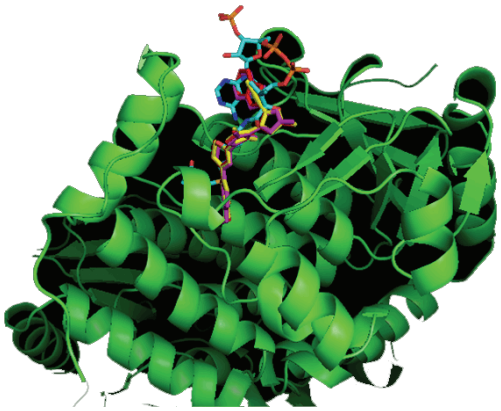


Fig. 1. Compounds **7o** (magenta), **7m** (pink) and **7a** (yellow) docked in *S. aureus* 1,4-dihydroxy-2-naphthoyl-CoA synthase (cartoon) along with co-crystallized ligand CAA (cyano).

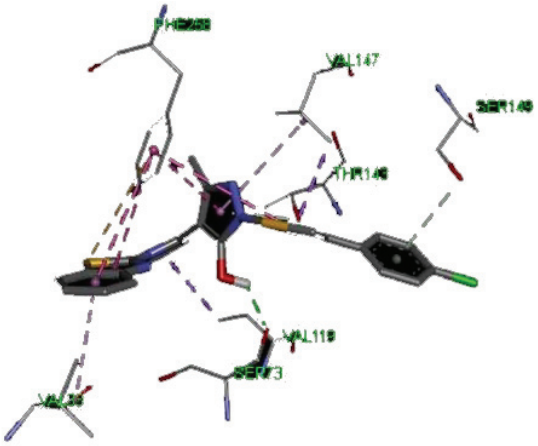


Fig. 2. Binding interactions (dotted lines) of compound **7o** in binding site of enzyme *S. aureus* 1,4-dihydroxy-2-naphthoyl-CoA synthase (hydrogen bond: green, π–π: magenta, π–σ: violet, sulphur–π: yellow and π–alkyl: light pink).

pound **7a** exhibited interactions with the same residues as that of **7o** except one hydrogen bond with Ser73, one T-shaped π–π interaction with Phe258 (Fig. 4). In addition, one π–sulphur interaction existed between the sulphur atom of the benzothiazole ring and the π electrons of Phe258. Thus, the latter features may be the cause of the higher activity of compound **7o**. The greater activity of compound **7m** than **7a** may be due to the shorter length of the hydrogen bond with Thr143 (H···N = 2.92 for **7m**; H···N = 2.97 for **7a**), which causes tighter binding of the compound with the enzyme, resulting in greater inhibition.

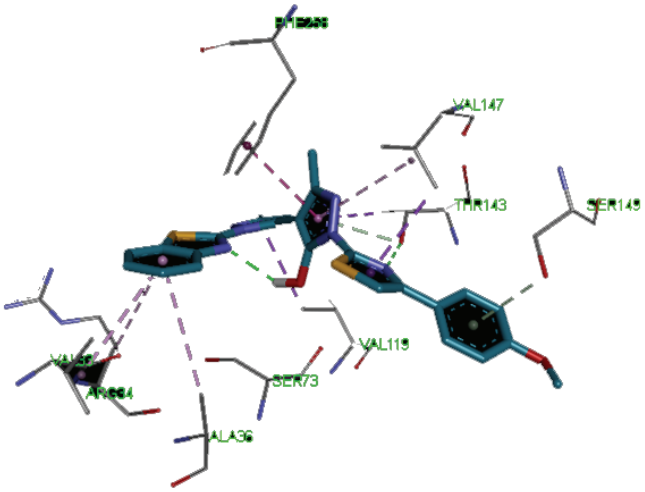


Fig. 3. Binding interactions (dotted lines) of compound **7m** in binding site of enzyme *S. aureus* 1,4-dihydroxy-2-naphthoyl-CoA synthase (Hydrogen bond: green, π–π: magenta, π–σ: violet and π–alkyl: light pink).

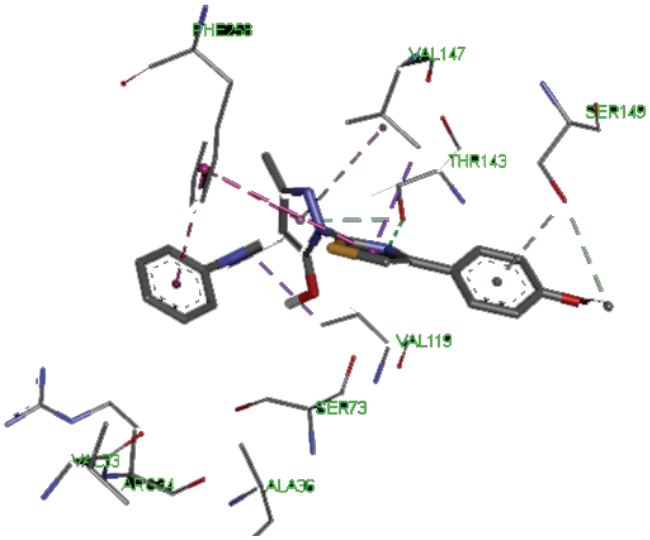


Fig. 4. Binding interactions (dotted lines) of compound **7a** in binding site of enzyme *S. aureus* 1,4-dihydroxy-2-naphthoyl-CoA synthase (hydrogen bonds: green, π–π: magenta, π–σ: violet and π–alkyl: light pink).

EXPERIMENTAL

Chemistry

Melting points (°C) were determined in open capillaries and are uncorrected. The IR absorption spectra were scanned on a Perkin Elmer Spectrum, BX II FTIR spectrometer using potassium bromide (KBr) pellets and the wavenumbers ($\bar{\nu}$) are given in cm^{-1}. The ^{1}H-NMR

spectra were recorded on a Bruker Avance II 400 spectrometer at 400 MHz, using dimethyl sulfoxide-d_6 (DMSO-d_6) as solvent. The chemical shifts (δ) are reported in ppm using tetramethylsilane (TMS) as an internal standard. Coupling constants (J) are valued in Hz. The mass spectra were recorded on a Waters Micromass Q-Tof Micro (ESI) spectrometer. The figure given in parentheses represents relative intensities corresponding to the base peak taken as 100. Elemental analysis was carried out using Vario Micro Cube Elementar CHNS analyser. Analytical results for C, H and N were within ±0.4 % of the theoretical values. The purity of the compounds was checked by thin layer chromatography (TLC) using readymade silica gel (SIL G/UV254, ALUGRAM) plates. The spots were visualized under a ultraviolet (UV) lamp. Solvents were dried using standard literature procedures.

Physical and spectral data for the synthesised bipyrazoles are given in the Supplementary material to this paper.

Synthetic procedures

The thiosemicarbazone **2** was prepared by the condensation of equimolar quantities of 3-acetyl-4-hydroxy-6-methyl-2*H*-pyran-2-one (DHA) and thiosemicarbazide in absolute alcohol as per a literature procedure.[37,50]

*General procedure for the synthesis of 3-(1-(2-(4-arylthiazol-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-ones (**4a–d**)*

To a solution of **2** (2.41 g, 0.01 mol) and anhydrous sodium acetate (0.82 g, 0.01 mol) in absolute ethanol (50 mL) was added the appropriate phenacyl bromide/4-substituted phenacyl bromide (**3**, 0.01 mol) slowly under stirring. Thereafter, the reaction mixture was heated gently at reflux for 25–35 min. The thus obtained yellowish crystalline solid was filtered, washed with cold ethanol, and recrystallized from dimethylformamide (DMF) and water to furnish the corresponding 3-(1-(2-(4-arylthiazol-2-yl)hydrazono)ethyl)-4-hydroxy-6-methyl-2*H*-pyran-2-ones (**4a**–**d**) in high yields.[37,50]

*General procedure for the synthesis of 1-(1-(4-arylthiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)butane-1,3-diones (**5a–d**)*

A solution of an appropriate hydrazone (**4**, 0.01 mol) in glacial acetic acid (50 mL) was refluxed for 2 h. The reaction mixture was kept overnight at room temperature, the thus obtained solid was filtered, washed with a little cold ethanol and crystallized from acetonitrile to afford the corresponding 1-(1-(4-arylthiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)-butane-1,3-diones (**5a**–**d**) in good yields.[37,50]

*General procedure for the synthesis of 1-(4-arylthiazol-2-yl)-1′-(aryl/heteroaryl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a–p**)*

To a solution of 1-(1-(4-arylthiazol-2-yl)-5-hydroxy-3-methyl-1*H*-pyrazol-4-yl)butane-1,3-diones (**5**, 0.005 mol) and an appropriate phenylhydrazine/2-hydrazinylbenzothiazole/6-substituted-2-hydrazinylbenzothiazoles (**6**, 0.005 mol) in dry ethanol (50 mL) was added 1–2 mL of glacial acetic acid. Thereafter, the contents were heated at reflux for 2–3 h. The crude solid obtained after concentrating and cooling the reaction mixture was filtered, which upon crystallization from acetonitrile afforded the corresponding 1-(4-(phenyl/4-substituted phenyl)thiazol-2-yl)-2-(phenyl/benzothiazol-2-yl/6-substitutedbenzo[*d*]thiazol-2-yl)-3,3′-dimethyl-[4,5′-bi-1*H*-pyrazol]-5-ols (**7a**–**p**) in high yields.

Antimicrobial activity

The *in vitro* antibacterial and antifungal activities of all sixteen synthesized bipyrazoles **7a**–**p** were tested against five microorganisms, *i.e.*, two Gram-positive bacteria, *viz. B. subtilis*

(MTCC 441) and *S. aureus* (MTCC 7443), one Gram-negative bacterium, *viz*. *E. coli* (MTCC 42), and two fungi, *viz*. *C. albicans* (MTCC 183) and *A. niger* (MTCC 282), by the serial tube dilution technique[48] using two solid media, double strength nutrient broth and Sabouraud dextrose broth for bacterial and fungal growth, respectively. The stock solutions (100 μg mL^{-1}) of all the test compounds were prepared by dissolving 1 mg of the test compound in 10 mL of dimethyl sulphoxide (DMSO). Ciprofloxacin and fluconazole were used as references against the bacterial and fungal strains, respectively. Fresh cultures were obtained by inoculation of the respective microorganism in a suitable media (double strength nutrient broth in case of the bacteria and Sabouraud dextrose broth in the case of the fungi) followed by incubation at 37±1 °C. The stock solutions of the test compounds were serially diluted in test tubes containing 1 mL of sterile medium to obtain concentrations of 50–3.12 μg mL^{-1} and then inoculated with 100 μL of a suspension of the respective microorganism in sterile saline. The inoculated test tubes were incubated at 37±1 °C for 24 h in case of *B. subtilis*, *S. aureus* and *E. coli*, at 37±1 °C for 48 h in case of *C. albicans* and at 37±1 °C for 120 h in case of *A. niger* and their minimum inhibitory concentration (*MIC*) values were determined. In microbiology, *MIC* is the lowest concentration of an antimicrobial agent that will inhibit the visible growth of a microorganism after incubation. The reference compounds ciprofloxacin and fluconazole were also tested under similar conditions to compare with the results of tested compounds.

Docking studies

The course of action for the docking studies was taken in accordance with the *modus operandi* given by Kumar *et al*.[51] The structures of the molecules were drawn with MavinSketch 5.10.[52] The 3D X-ray crystallographic arrangement of *Staphylococcus aureus* enzyme, 1,4-dihydroxy-2-naphthoyl-CoA synthase, together with co-crystallized ligand acetoacetyl--CoA (CAA, PDB ID: 2UZF) was taken from the Brookhaven Protein Databank (http:////www.rcsb.org/pdb). The necessary changes in enzyme were realised with UCSF Chimera 1.10.[53] The Dunbrack rotamer library[54] and Antechamber,[55] and were applied to correct imperfect side chains and to compute Gasteiger charges. AutoDock tools[56] were exercised to make pdbqt files.

AutoDock Vina was chosen for performing molecular docking. The parameters of Vina search box selected were centre_x = 6.00, centre_y = –18.22, centre_z = –29, and size_x = = 37.11, size_y = 37.53, size_z = 48.04. The exhaustiveness for the docking was set to be 8.

Co-crystallized ligand CAA was redocked into the active site of the enzyme for validation of the docking protocols. The accurate docked conformation confirmed that the opted protocols were correct for the docking simulations against this enzyme. Visualization of results was realised with the PyMOL[57] and Discovery Studio 4.0.[58]

CONCLUSIONS

In conclusion, sixteen bipyrazoles (**7a**–**p**) were prepared using dehydroacetic acid (**1**), thiosemicarbazide and phenacyl bromide/4-substituted phenacyl bromide (**3**) in good yields. The bipyrazoles were screened for their *in vitro* antimicrobial activities. Compounds **7d** against *B. subtilis* and **7f** against *E. coli* exhibited significant antibacterial activity. The compounds **7b**, **7c**, **7f**–**i**, **7k**, **7l**, **7n**–**p** against *C. albicans* and **7b**–**d**, **7f**–**i**, **7k**–**m**, **7o** and **7p** against *A. niger* showed appreciable antifungal activity while **7a** and **7e** against *A. niger* showed greater inhibitory activity than the reference, *i.e*., fluconazole. Generally, the compounds

containing chloro or methoxy groups exhibited better antimicrobial activity. Docking studies of compounds **7a, 7m** and **7o** against *S. aureus* 1,4-dihydroxy--2-naphthoyl-CoA synthase showed that hydrogen bonding and T-shaped π–π interactions were responsible for the antibacterial activity. Thus, some of these studied compounds may emerge as potential molecules for further development as antimicrobial drug candidates.

SUPPLEMENTARY MATERIAL

The physical and spectral data of the synthesized bipyrazoles are available electronically at the pages of the journal website: http://www.shd.org.rs/JSCS/, or from the corresponding author on request.

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ИЗВОД

СИНТЕЗА И ИСПИТИВАЊЕ АНТИМИКРОБНЕ АКТИВНОСТИ НЕКИХ 1-(4-АРИЛТИАЗОЛ-2-ИЛ)-1′-(АРИЛ/ХЕТЕРОАРИЛ)-3,3′-ДИМЕТИЛ--[4,5′-БИ-1*H*-ПИРАЗОЛ]-5-ОЛА

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Синтетисана је серија од шеснаест 1-(4-арилтиазол-2-ил)-1′-(арил/хетероарил)-3,3′--диметил-[4,5′-би-1*H*-пиразол]-5-ола, полазећи од дехидросирћетне киселине (DHA, **1**), преко тиосемикарбазона **2**, 3-(1-(2-(4-арилтиазол-2-ил)хидразоно)етил)-4-хидрокси--6-метил-2*H*-пиран-2-она (**4a**–**d**) и 1-(1-(4-арилтиазол-2-ил)-5-хидрокси-3-метил-1*H*--пиразол-4-ил)бутан-1,3-диона (**5a**–**d**), у високом приносу. Испитана је *in vitro* антимикробна и антифунгална активност синтетисаних бипиразола **7a**–**p** према два соја грам--позитивних бактерија *Bacillus subtilis* (MTCC 441) и *Staphylococcus aureus* (MTCC 7443), једном соју грам-негативних бактерија *Escherichia coli* (MTCC 42), и два соја гљивица *Candida albicans* (MTCC 183) и *Aspergillus niger* (MTCC 282). Два једињења, **7a** и **7e**, показала су бољу активност према *A. niger* у поређењу са стандардом флуконазолом. Осим тога, једињења показују бољу антифунгалну него антибактеријску активност. Такође, извршено је моделовање везивања једињења **7a**, **7 m** и **7o** у активно место 1,4-дихидрокси-2-нафтоил-CoA синтазе *Staphylococcus aureus*.

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