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Structural studies of 2,5-disubstituted 1,3,4-thiadiazole derivatives from dithioesters under the mild condition: Studies on antioxidant, antimicrobial activities, and molecular docking

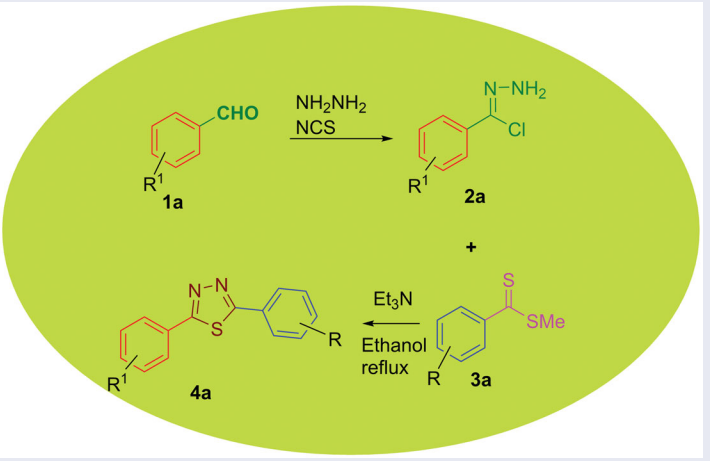
Kemparaje Gowda[a], Hassan A. Swarup[a], Sandhya C. Nagarakere[b], Shobith Rangappa[c], Rangappa S. Kanchugarkoppal[b], and Mantelingu Kempegowda[a]

[a]Department of Studies in Chemistry, University of Mysore, Mysuru, India; [b]Department of Chemistry, St. Philomena's College, Mysuru, India; [c]Adichunchanagiri Institute for Molecular Medicine, Mandya, India

ABSTRACT

A series of 2,5-disubstituted 1,3,4-thiadiazole was synthesized and evaluated for their antioxidant and molecular docking studies. These molecules were efficiently synthesized under mild and inexpensive starting material. Construction of these molecules developed using substituted aldehydes and substituted dithioesters in presence of NCS (N-Chorosuccinimide). The compounds **4a**, **4b**, **4c**, **4f**, and **4k** showed good antibacterial and antioxidant activity among which, 4k possess excellent antibacterial and antioxidant activity. The results of antioxidant activity studies revealed that compound **4k** manifested profound antioxidant potential. The molecular docking study was performed with respective newly synthesized compounds to ascertain the binding mode of **4k** with respect to the critical proteins involved in biosynthesis and metabolic pathways.

GRAPHICAL ABSTRACT



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KEYWORDS
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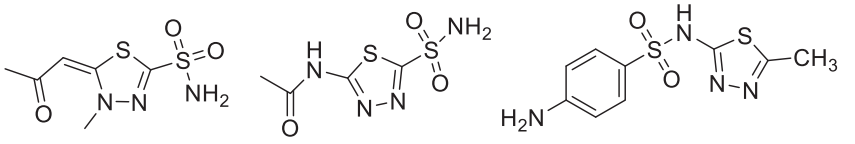
CONTACT Mantelingu Kempegowda kmlingu@gmail.com; Rangappa Kanchugarakoppal S rangappaks@gmail.com
Synthetic Laboratory, DOS in Chemistry, University of Mysore, Mysuru, Karnataka, 570006, India

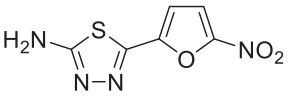
Supplemental data for this article can be accessed on the publisher's website.

Introduction

Among the heterocycles 1,3,4-thiadiazole having wide applications in the field of biological systems.[1,2] These 1, 3, 4-thiadiazole ring has been found to be vital in pharmacological activities because of their pharmaceutical interest.[3,4] It shows a broad spectrum of biological activities like anticancer,[5–7] antioxidant,[8] antimicrobial,[9] antihypertensive activity,[10] anti-inflammatory,[11] anticonvulsants[12] and antifungal activity.[13,14] Some of the important drugs containing 1,3,4-thiadiazole ring are methazolamide, acetazolamide, sulfamethizole, and furidiazine.



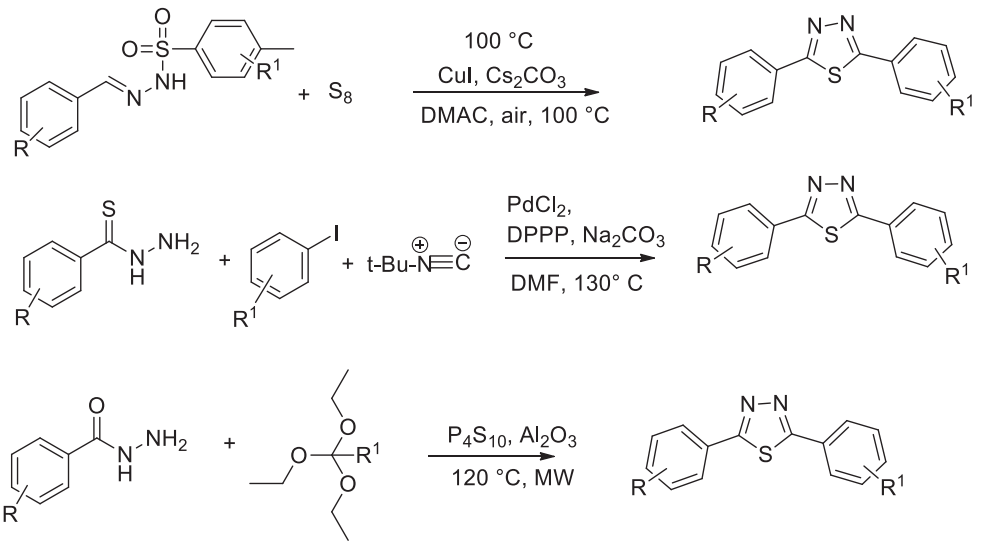
Methazolamide Acetazolamide Sulfamethizole

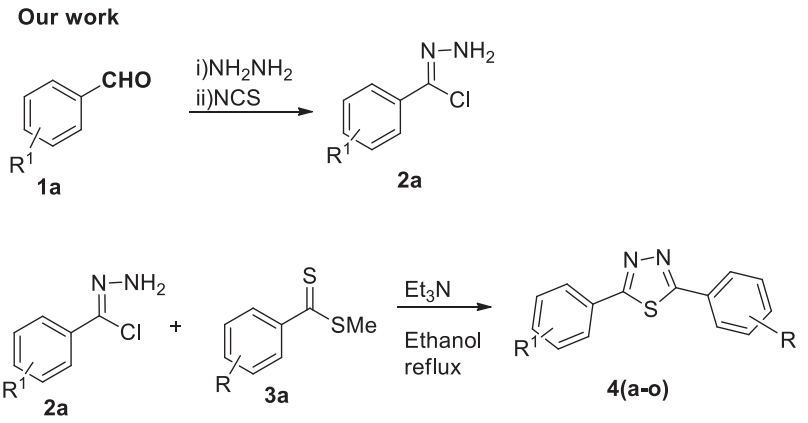


Furidiazine

Apart from pharmaceutical interests, 1,3,4-thiadiazole plays an important role in the field of material science like it is used in photoconductivity,[15] corrosion inhibitor[16] and also in liquid crystals.[17] Distinct methods have been reported for the synthesis of 2,5-disubstituted 1,3,4-thiadiazole in conventional methods like the use of metal catalysts under harsh reaction conditions. Eycken and group[18] reported the synthesis of 2,5-disubstituted 1,3,4-thiadiazole using N-tosylhydrazones and elemental sulfur in the presence of CuI and Cs_2CO_3. In addition to this reaction between an aryl halide and tert-butyl isocyanide via isocyanide cyclization using $PdCl_2$ and DPPP were also reported by Zhu and coworkers.[19]

Previous work





The use of the microwave irradiation method was also developed by Varma and group[20] for the synthesis of 2,5-disubstituted 1,3,4-thiadiazole through a condensation reaction of triethyl orthoalkanates and acid hydrazide in presence of Nafion NR50 and phosphorus pentasulfide with alumina. The drawbacks with these protocols are, it requires the use of transition-metal catalysts which are toxic in nature under harsh reaction conditions like microwave radiation followed by tedious work-up procedure and poor functional-group tolerance, have a narrow scope. Dithioesters occupy a foremost position in organic synthesis and these are adaptable starting materials in the preparation of many heterocyclic since their impartially stable and methods for the preparation of dithioesters are easy. In continuation of our interest in the synthesis of a heterocyclic ring, cycloaddition reaction compounds[21–26] and also based on our experiences on thioesters,[22,27–32] we explored a method to construct 2,5-disubstituted 1,3,4-thiadiazole which were synthesized from simple and inexpensive starting materials and reagents like substituted aldehydes and substituted dithioesters using Chlorosuccinimide.

Result and discussion

Before optimizing the method for the synthesis of 2,5-disubstituted 1,3,4-thiadiazole, we synthesized benzohydrazonoyl chloride **2a** by using hydrazine hydrate and NCS. We took up **2a** and **3a** as the pilot substrate for optimizing the method. During optimization, primarily we choose DMF as solvent and TEA as a base, the suspension was stirred at 90 °C for 2 days (Table 1, entry 1), fortunately, we observed a new spot between **2a** and **3a** while monitoring the reaction. After the spectral analysis, we came to know that the generated spot was our desired compound **4a**. With our interest to determine suitable solvent for cyclization, we have screened the solvents like DMF, Ethanol, DMSO, THF, methanol, CH_3CN, Toluene, and water (Table 1, entries 1, 5 and 13–18) and we found that there was no reaction taking place when the solvent system became acetonitrile/toluene/water, in DMF, DMSO, THF, MeOH yields were low and in ethanol, the reaction went smoothly to give a good yield. Further, We want to optimize the base by using different bases like Na_2CO_3, K_2CO_3, NaOH, TEA, N,N-Diisopropylethylamine (DIPEA), KOH and LiOH (Table 1, entries 2–5, 10–12). We observed that TEA was a suitable base for the cyclization and formation of the product

was isolated in 80% yield (Table 1, entry 5). In order to know the stoichiometric effect of TEA on the yield of product which we performed the reaction by changing the equivalence of TEA (Table 1, entries 5–7), and maximum yield of the product **4a** was observed at 1 Eq. of TEA. Finally, the effect of temperature was also tested by varying the temperature, at lower temperature yields less comparing to reflux conditions (Table 1, entries, 5, 6 and 8). Eventually, we found that reaction of **2a** (1 Eq.) and dithioesters (1 Eq.) with base TEA (1 Eq.) by using Ethanol (10 vol) as a solvent at reflux condition for 48 h was ideal (Table 1, entry 6). With the aforementioned optimized condition we want to check the substrate scope of the method by varying the substitution at the para position of the aryl group of dithioesters **3**, in that OMe, Me, F, Cl, Et give corresponding products **4(b–f)** with the yield of 89–95%.

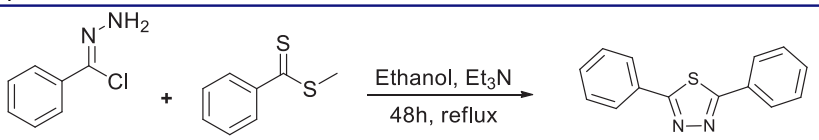
Similarly, we checked the substrate scope by varying different substitution at aryl group of benzohydrazonoyl chloride **2**, such as OMe, Me, F, Cl and 3,4 dimethoxy groups which give corresponding products **4(f–o)** with the yield of 88–95% (Table 2).

Biology

Antioxidant activity

Antibacterial and antioxidant activity are closely related.[33] Hence, we further aimed to access the antioxidant activity for **4(a–o).** Radical scavenging potency of all the compounds **4(a–o)** was assessed *in vitro* by various antioxidant activities like superoxide radical (Fig. 1a), hydroxyl radical (Fig. 1b), lipid peroxidation (Fig. 1c), and DPPH

Table 1. Optimization of reaction conditions.

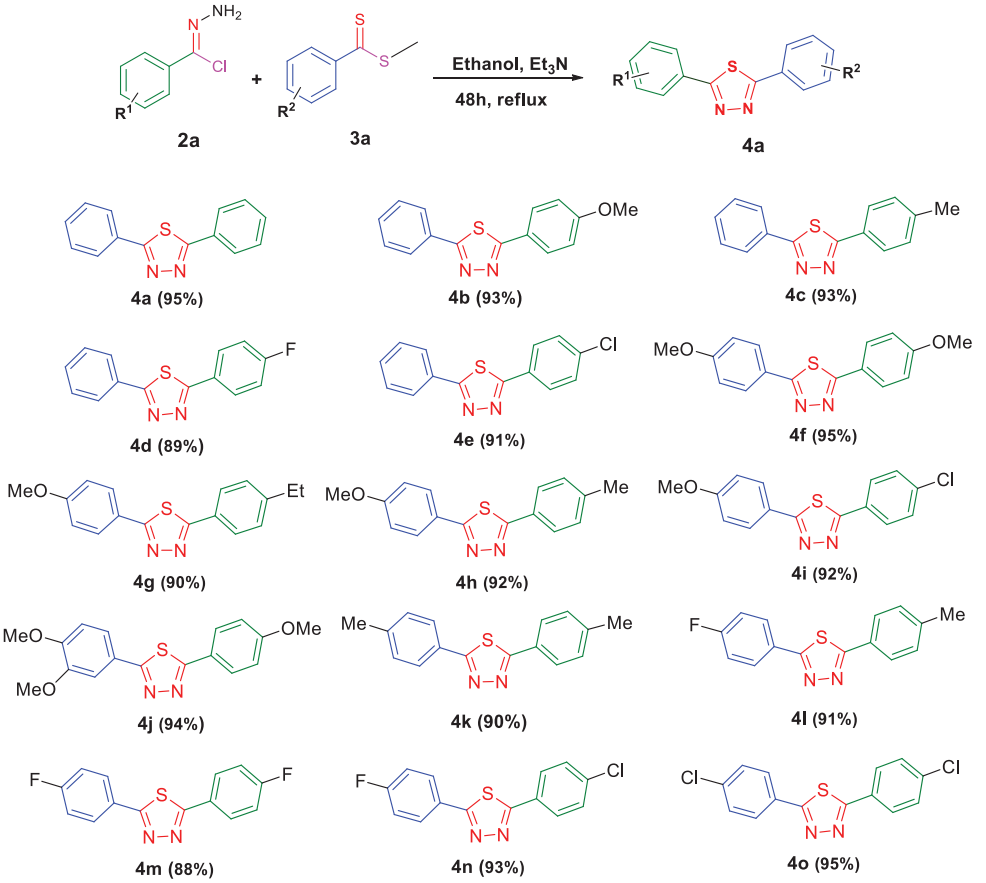


2a **3a** **4a**

Entry	Base	Equivalence (Eq.)	Solvent	Temp (°C)	Time (hr)	Yield (%)
1	Et_3N	2.5	DMF	90	48	10
2	Na_2CO_3	2.5	Ethanol	90	48	20
3	K_2CO_3	2.5	Ethanol	90	48	23
4	NaOH	2.5	Ethanol	90	48	22
5	Et_3N	2.5	Ethanol	90	48	80
6	**Et_3N**	**1.0**	**Ethanol**	**Reflux**	**48**	**95**
7	Et_3N	0.5	Ethanol	50	48	85
8	Et_3N	1.0	Ethanol	40	30	70
9	Et_3N	0.5	Ethanol	Reflux	20	60
10	DIPEA	2.5	Ethanol	Reflux	20	21
11	KOH	2.5	Ethanol	Reflux	48	46
12	LiOH	2.5	Ethanol	Reflux	48	48
13	Et_3N	2.5	DMSO	Reflux	10	10
14	Et_3N	2.5	THF	Reflux	48	20
15	Et_3N	2.5	MeOH	Reflux	48	20
16	Et_3N	2.5	MeCN	Reflux	48	0
17	Et_3N	2.5	Toluene	Reflux	48	0
18	Et_3N	2.5	H_2O	Reflux	48	0

Bold values represent the best optimized condition obtained for the synthesis of compounds.

Table 2. The substrate scope of reactions.



(Fig. 1d).[34] The experiments were performed in triplicate at five different concentrations. The results were taken as a mean ± standard deviation (SD). In complex metabolic system drives the most common superoxide anions to generate in a variety of biological systems which result in oxidative stress. Hence, an NBT assay gives a whether the synthesized compounds are capable to scavenge superoxide anions. Results deliberate that the **4k** shows potent superoxide anion scavenge activity compared to **4(a–c)**, and **4f** (Fig. 1a). Another devastating free radical is hydroxyl radicals which are known to initiate cell damage, break DNA and cause strand breakage which, in turn, result in carcinogenesis, mutagenesis, and cytotoxicity *in vivo*. Thus we attempt to explore the effect of **4(a–o)** on hydroxyl radicals generated by Fe^{3+} ions were measured by the extent of deoxyribose degradation, as an indicator of TBA–MDA adducts formation, among the series **4(a–o)**. Among the studied, compounds **4b** and **4f** exhibited a remarkable capacity for scavenging hydroxyl radical, significantly higher than that of the standard BHA, whereas compound **4a** showed moderate scavenging activity. **4k** exerts more inhibition of hydroxyl radicals among the series (Fig. 1b). To determine the production of malondialdehyde and related lipid peroxides in the RBC membrane, the lipid peroxidation assay was carried out. The reactive system of TBA generates lipid peroxides as a

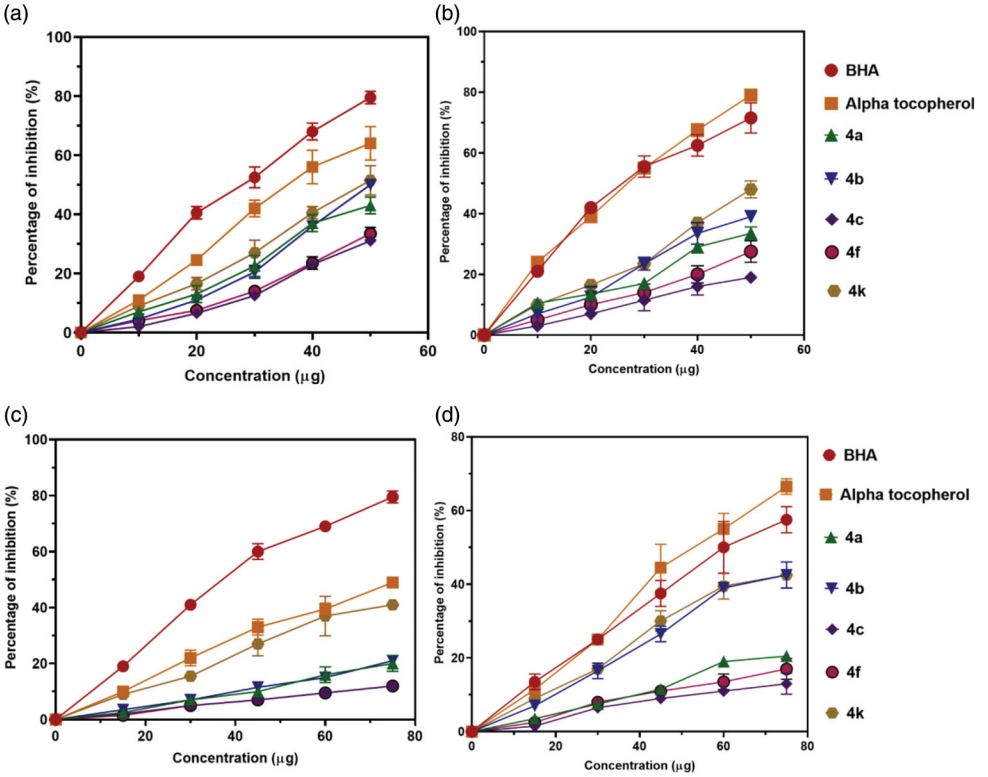


Figure 1. Comparison of radical scavenging activities like superoxide radical (a), hydroxy radical (b), lipid peroxidation (c), and DPPH (d) of compounds **4a**, **4b**, **4c**, **4f**, and **4k**.

byproduct induced by Ferrous sulfate: Ascorbate system. **4k** showed the highest inhibition compared with the **4a, 4b**, **4c**, and **4f** of ghost lipid peroxidation, about 36 ± 3% at a lower concentration (75 µg) (Fig. 1c). The freshly prepared DPPH solution shows a deep purple color with an absorption maximum at 517 nm. Based on the experimental results, compound **4f** and **4k** having Methyl and methoxy substituent showed stronger DPPH scavenging activity than others (Fig. 1d). Compounds **4b** and **4c** having a methoxy substitution showed poor antioxidant properties, whereas the rest of compounds showed less activity compared to the standard BHA and tocopherol

Antimicrobial activities

The results of the antimicrobial activities of the synthesized compounds **4(a–o)** are summarized in Table 3. Results confirm that the newly synthesized compounds **4(a–o)** exert a good antibacterial activity ranging from 12.5–100 µg/mL against bacterial species. Among the series **4(a–o),** compounds **4(a–c)**, **4f**, and **4k**, poses the highest antibacterial activity against *Escherichia coli, S. pyogenes*, and *S. aureus* (Table 3). Substitution at R^1 and R^2 with Methyl (**4k**) and Methoxy (**4f**) at para position (Table 3) possess good antimicrobial activity against the test bacterial species, Whereas the increase of hydrophobic at R^1, and R^2 results in a decrease in the activity which is seen in compound **4k** at para and meta position, Hence the introducing methyl or methoxy at meta

Table 3. Minimum inhibitory concentrations (MIC) (in μg/mL*) of the synthesized compounds **4(a–o)** against bacterial species.

Compound	*S. aureus*	*S. pyogenes*	*E. coli*
4a	75.0 ± 0.16	12.5 ± 1.05	25.0 ± 0.26
4b	50.0 ± 0.21		25.0 ± 0.17
4c		25.0 ± 0.44	50.0 ± 0.82
4d	50.0 ± 0.41	50.0 ± 0.87	
4e			
4f	50.0 ± 0.15	25.0 ± 0.75	
4g	50.0 ± 0.44	25.0 ± 0.27	25.0 ± 0.23
4h	12.5 ± 1.05	50.0 ± 0.22	50.0 ± 0.15
4i	12.5 ± 0.71	50.0 ± 0.13	12.5 ± 0.16
4j	25.0 ± 0.16	50.0 ± 1.42	12.5 ± 0.32
4k	50.0 ± 0.03	50.0 ± 0.96	75.0 ± 0.12
4l			
4m			50.0 ± 0.42
4n	75.0 ± 1.05		
4o	75.0 ± 0.44	100.0 ± 0.27	75.0 ± 0.23
Ciprofloxacin	12.5 ± 1.04	50.0 ± 1.23	25.0 ± 1.16

*Values are mean ± SD of three replicates. Ciprofloxacin was used as a positive control against bacteria species.

position has resulted in low activity. When the same organism was tested on the agar-well diffusion test, resulting in the same activity as seen in the serial dilution method of determining antimicrobial activity is (Fig. 2a–c). Methyl or methoxy group at R^2 at para position alone will bear little activity (**4b** and **4c**). These validate that the methyl/Methoxy group very much essential for antibacterial activity. The poorer inhibitory effect of these compounds might be attributed to the presence of fluoro, chloro, and combination of methyl or methoxy group also in poor activity.

Molecular docking

In order to understand possible mechanisms of **4(a–o)** synthesized compounds which exerted antioxidant and antibacterial activity, we have performed molecular docking studies to ascertain the probable bind of a potent molecule at the active site. The molecular docking studies showed an interacting map of AmpC β-lactamase and catalase. β-Lactamases are the most culprit that cause resistance to β-lactam containing antibiotics. Several β-Lactamases inhibitors along with antibiotics are an attractive target for drug discovery and have been determined, in making this enzyme an attractive target for a computational biologist. Hence we attempted to check the mode of binding with β-lactamase (Fig. 3), with a hydrogen bonding with Ala307, Ser257, and Ala79. Whereas ascertain the binding mode with catalase in exerting antioxidant activity, we had, dock **4k** into the active site of catalase. Ligand **4k** interacted catalase with Leu134 and Ser196 with hydrogen bonding and with Phe140 it interacted via π–π stacking with benzene ring of Phe140 with 1,3,4-thiadiazole ring (Fig. 3). Based on docking score, ligand **4j** and **4k** possess higher bioactivity with promising scoring function, when compared to other structurally related compounds as tabulated in Table 4.

Conclusion

In the present study highlights the new synthesis route of 2,5-disubstituted 1,3,4-thiadiazole from dithioesters in mild condition. The method is a simple and reliable approach toward the synthesis of 2,5-disubstituted 1,3,4-thiadiazole, and paves the way for further

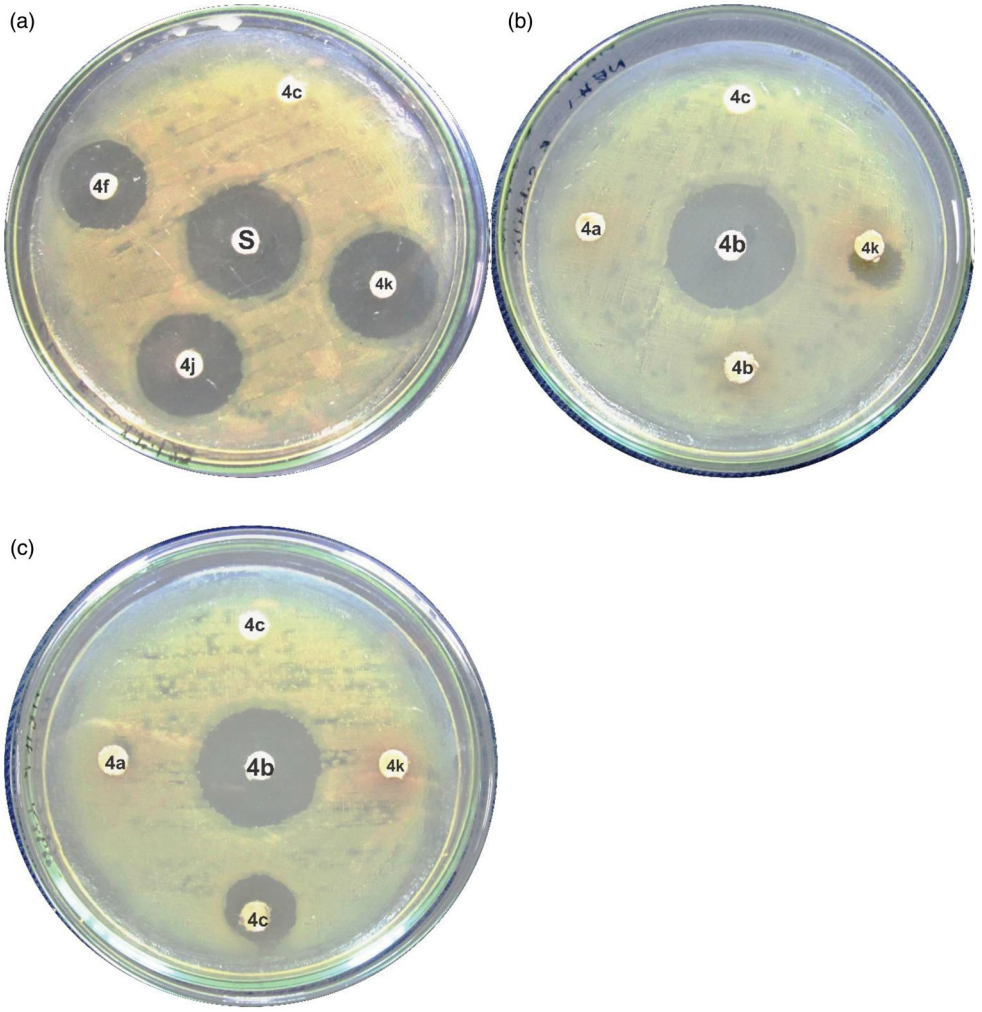


Figure 2. Inhibition zone of newly synthesized molecules against *E. coli* (a), *S. pyogenes* (b), and *S. aureus* (c) bacterial strains.

development of thiadiazole and dithioesterschemistry. The presence of 1,3,4-thiadiazole and methyl/methoxy substitutions in an aromatic ring is posited to be the key feature for the biological potency of the synthesized compounds. Preliminary studies show that the compounds **4(a–d)**, and **4k** possess excellent antimicrobial and antioxidant activities.

General experimental conditions

Materials

All work relating to analytical thin-layer chromatography was performed with Merck silica gel 60 F_{254} aluminum plates and were visualized with UV light. The following mobile phases were employed for TLC: chloroform, methanol, hexane, and ethyl acetate in different ratios. The instrumental techniques employed for the characterization of the newly synthesized compounds include ^{1}H and ^{13}C NMR and mass spectroscopy. ^{1}H

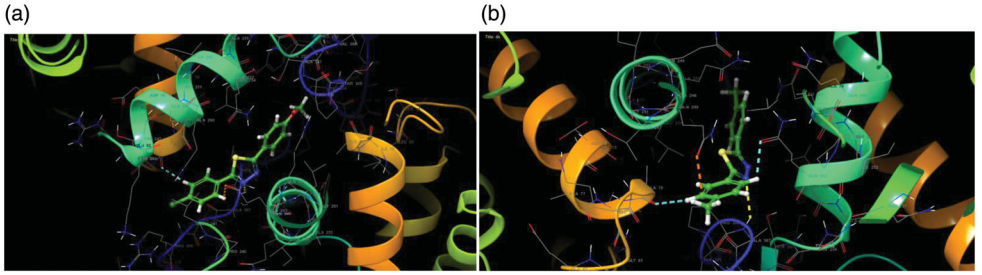


Figure 3. Molecular docking interactive map of compounds **4j** (a) and **4k** (b) into the AmpCβ-lactamase (PDB id 1KE4), binding deep inside the active site, depicting the best docking pose.

and ^{13}C NMR spectra were recorded on a Bruker-AV (500, 400 and 126, 101 MHz, respectively) and Agilent WM (400 and 100 MHz) Fourier transforms spectrophotometer in $CDCl_3$ or DMSO-d_6 solution using tetramethylsilane (TMS) as an internal standard. Chemical shifts were recorded in ppm relative to TMS. Mass and purity were recorded on an LC–MSD-Trap-XCT (Agilent Technologies, Inc., Santa Clara, CA). All the reagents and chemicals used were from Sigma Aldrich chemicals.

General procedure for the preparation of product

Step 1: General procedure for the synthesis of benzylidene hydrazine using aldehyde and hydrazine hydrate

To the solution of aryl aldehyde **1** (5.0 mmol, 1 Eq.) in Ethanol (10 vol), Hydrazine hydrate (5.0 mmol, 1 Eq.) was added and stirred at room temperature for 6 h. Then the reaction was monitored by TLC, after the completion of the reaction, the reaction mixture was allowed to cool at room temperature, which was diluted with water and the aqueous layer was extracted with ethyl acetate (25 mL × 3), dried over anhydrous sodium sulfate, the solvent was removed under reduced pressure to get benzylidene hydrazine which was then taken to the next step without any purification.

Step 2: General procedures for the synthesis of benzohydrozonychloride using chlorosuccinamide andbenzylidene hydrazine

To the solution of benzylidenehydrazine(4.8 mmol, 1 Eq.) in DCM (10 vol), N-Chlorosuccinamide (NCS) was added (4.8 mmol, 1 Eq.) and stirred the reaction mixture at room temperature for 6 h. Then the reaction was monitored by TLC, after the completion of the reaction, the reaction mixture was allowed to cool at room temperature, which was then diluted with water, the aqueous layer was extracted with ethyl acetate (25 mL × 3), dried over anhydrous sodium sulfate, the solvent was removed under reduced pressure to get benzohydrozonychloride, which was then taken to the next step without any purification.

Step 3: General procedure for the synthesis of 2,5-disubstituted 1,3,4-thiadiazoles using benzohydrozonychloride and dithioesters

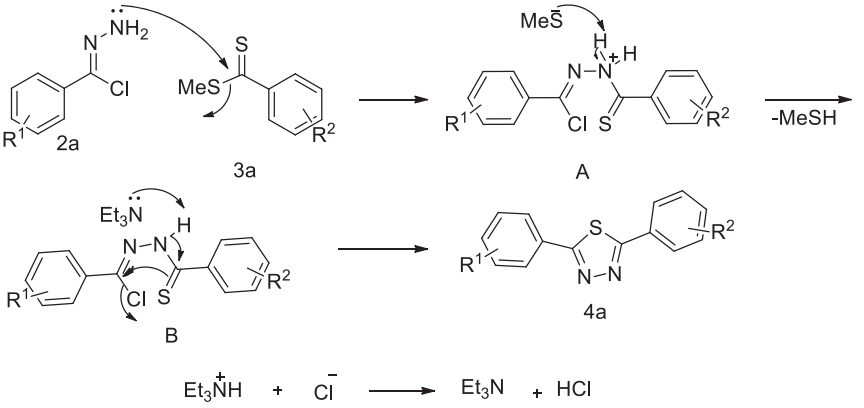
To the solution of benzohydrozonylchloride (1.2 mmol, 1 Eq.) in Ethanol, dithioester (1.2 mmol, 1 Eq.) and triethylamine (1.2 mmol, 1 Eq.) were added and refluxed for 48 h. Then the reaction was monitored by TLC. After the completion of the reaction, the

Table 4. Molecular docking scores of all the synthesized compounds against Catalase, DNA Gyrase, YmaH and MurB as obtained through docking.

	Antioxidant			Antibacterial								
	PDB-2CAG (Catalase)			PDB-2XCT (DNA Gyrase)			PDB-3HSB (YmaH)			PDB-1MBT (Oxidoreductase)		
Ligand	Docking score	E-model	Energy	Docking score	E-model	Energy	Docking score	E-model	Energy	Docking score	E-model	Energy
4a	−4.89	−40.33	−30.59	−6.13	−49.40	−36.20	−5.65	−36.67	−27.49	−5.36	−48.50	−36.22
4b	−5.18	−54.52	−40.83	−5.22	−45.07	−35.74	−5.18	−39.09	−29.61	−3.46	−34.97	−30.52
4c	−5.16	−51.98	−39.02	−5.54	−47.60	−37.01	−5.50	−38.60	−29.02	−4.28	−39.46	−33.17
4d	−4.76	−48.83	−37.45	−5.23	−43.62	−37.00	−5.47	−39.75	−30.06	−3.96	−37.14	−31.75
4e	−4.98	−51.92	−39.69	−5.76	−43.14	−34.60	−5.62	−41.60	−31.10	−4.23	−38.71	−33.86
4f	−4.82	−46.73	−35.59	−5.87	−51.66	−38.45	−5.90	−39.99	−29.64	−3.91	−35.43	−29.62
4g	−5.22	−50.27	−37.33	−5.53	−41.23	−30.85	−5.70	−39.07	−29.13	−5.07	−45.47	−36.65
4h	−5.54	−58.05	−45.02	−5.68	−53.34	−43.12	−5.23	−41.55	−32.05	−4.60	−45.40	−37.96
4i	−4.85	−45.58	−34.56	−5.45	−44.65	−34.90	−5.96	−38.30	−28.28	−5.33	−46.63	−35.11
4j	−4.83	−48.12	−36.76	−5.04	−46.33	−37.00	−5.89	−39.66	−29.43	−5.12	−45.45	−36.81
4k	−5.62	−49.19	−36.62	−6.19	−49.65	−36.78	−5.97	−38.91	−28.84	−6.02	−50.39	−39.13
4l	−4.80	−45.81	−34.93	−5.52	−44.60	−34.95	−5.85	−37.76	−28.01	−4.10	−35.05	−29.32
4m	−4.99	−48.60	−36.72	−5.54	−44.08	−35.12	−5.90	−38.90	−28.82	−3.76	−35.67	−31.03
4n	−3.02	−31.03	−22.59	−4.78	−41.06	−28.54	−3.52	−20.79	−22.61	−2.01	−21.83	−28.02
4o	−3.82	−36.73	−28.59	−4.27	−38.26	−21.45	−4.90	−29.99	−20.74	−2.35	−25.43	−31.62
Ciprofloxacin				−5.36	−51.33	−53.12	−5. 98	−49.66	−49. 34	−5.12	−45.45	−36.81
BHA	−5. 61	−55.82	−49.86									

reaction mixture was allowed to cool at room temperature, which was then diluted with water, the aqueous layer was extracted with ethyl acetate (25 mL × 3), dried over anhydrous sodium sulfate, the solvent was removed with reduced pressure and obtained residue was purified by silica gel chromatography to get 2,5-disubstituted 1,3,4-Thiadiazole.

Mechanism



Lone pair of electrons from Amine group in the benzohydrazonoyl chloride attacks electrophilic carbon of dithioester to give intermediate A which eliminates upon MeSH to give intermediate B. Then TEA abstract proton form B and in situ cyclization taking place to generate final product 2,5-disubstituted 1,3,4-thiadiazole with the elimination of Hydrochloric acid.

Antimicrobialactivity

Minimum inhibitory concentrations (MICs) were determined by serial dilution technique.[35] The nutrient broth, which contains the logarithmic serially two-fold diluted amount of test compound **4(a–o)** and controls were inoculated with approximately 1 to 2×10^5cells/mL of actively dividing the bacteria cells. The bacterial culture was incubated for 24 h at 37 °C, the growth was monitored visually. The lowest concentration required to arrest the growth of microorganisms which regarded as minimumin hibitory concentration. The synthesized compounds were screened for their antibacterial activity against *E. coli*, *S. pyogenes*, and *S. aureus.* The antibiotics Ciprofloxacin was used as a positive control against bacterial species. Dimethyl sulfoxide was used as solvent control. The experiments were carried out in triplicate; the results were taken as a mean ± standard deviation (SD).

Agar-well diffusion testing

The agar well diffusion method was followed according to Marasini, et al.[36] Antibacterial activities of the **4(a–o)** were investigated by the agar well diffusion method. *Staphylococcus aureus* and *E. coli* test cultures were first spread into the surface of sterilized Mueller Hinton Agar plates using sterile cotton swabs. Five wells were made on each plate with a sterile cork borer instrument. The potent among the serially

diluted test compounds were chosen for agar–well diffusion. 15 µg in DMSO (<5%) of each of **4(a–c)**, **4f**, and **4k** were added in one well of each plate. The antibiotics Ciprofloxacin was used as a positive control against bacterial species. Diffusion of compound, antibiotics, and DMSO were allowed at room temperature for 1 h. All of the plates were then covered with lids and incubated at 37 °C for 24 h. After incubation, plates were observed for the zone of bacterial growth inhibition. The size of inhibition zones was measured and antimicrobial activity of the **4(a–c)**, **4f**, and **4k** was expressed in terms of an average diameter of inhibition zone in millimeters.

Superoxide scavenging activity

The Superoxide radical ($O2^{\bullet-}$) scavenging activity of **4(a–o)** was measured according to the method of Lee et al.[37] The reaction mixture containing 100 µL of 30 mM EDTA (pH 7.4), 10 µL of 30 mM hypoxanthine in 50 mM NaOH, and 200 µL of 1.42 mM nitro blue tetrazolium with or without **4(a–o)** and SOD serving as a positive control in various concentrations ranging from 0–50µg. After a pre-incubated solution at ambient temperature for 3 min, 100 µL of xanthine oxidase solution (0.5 U/mL) was added to the mixture and incubated for 1 h at 37 °C and the volume were made up to 3 mL with 20 mM phosphate buffer (pH 7.4). The solution was incubated at room temperature for 20 min, absorbance was measured at 560 nm. Appropriate controls were included ruling out the artifacts induced reaction. The control was without any inhibitor. Inhibitory effect of **4(a–o)** on superoxide radicals was calculated as follows:

$$\text{Superoxide radical scavenging activity}(\%) = \frac{\text{Absorbance of control} - \text{Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Hydroxyl radical scavenging activity of 2,5-disubstituted 1,3,4-thiadiazole

The hydroxyl radical scavenging activity of **4(a–o)** was according to the method of Halliwell and Gutteridge.[38] The reaction mixture containing $FeCl_3$ (100 µM), EDTA (104 µM), H_2O_2 (1 mM) and 2-deoxy- D-ribose (2.8 mM) were mixed **4(a–o)** (0–50 µM) in 1 mL final reaction volume made of potassium phosphate buffer (20 mM pH 7.4) and incubated for one hour at 37 °C. α-tocopherol and BHA were used as a positive control. The mixture was heated at 95 °C in a water bath for 15 min followed by the addition of 1 mL each of TCA (2.8%) and TBA (0.5% TBA in 0.025 M NaOH containing 0.02% BHA). eventually, the reaction mixture was cooled on ice and centrifuged at 5000 rpm till 15 min. The absorbance of the supernatant was measured at 532 nm using the negative control without any antioxidant considering 100% oxidation. The percentage hydroxyl radical scavenging activity of **4(a–o)** was determined.

Lipid peroxidation inhibition assay

The evaluation of the antioxidant activity of **4(a–o)** based on the inhibition of peroxidation in RBC ghost was done according to Shimasaki et al.[39] The evaluation of oxidation was done measure by the TBA reactive substances.[40] The human erythrocyte ghost was isolated according to the method of Dodge et al. 100 µL of ghost suspension (300 µg membrane protein equivalent) was subjected to peroxidation by ferrous sulfate

and ascorbic acid (10:100 μmol) in a final volume of 1 mL of Tris-buffered saline (20 mM, pH 7.4,150 mM NaCl). The reaction mixture was treated with or without **4(a–o)** (0–75 μM), α-tocopherol and BHA (400 μM) were used as the positive control. The contents were incubated for 1 h at 37 °C. The reaction was terminated by the addition of 10 μL of 5% phenol and 1 mL of 1% TCA. For each system 1 mL of 1% TBA was added, the contents were kept in a boiling water bath for 15 min, cooled and centrifuged at 6000 rpm for 10 min. The absorbance of supernatants was measured colorimetrically at 535 nm. Appropriate blanks were included for each measurement. The negative control sample was considered as 100% peroxidation. Without any test. The % inhibition of lipid peroxidation was determined accordingly by comparison of the absorbance of the test samples with a negative control.

DPPH radical scavenging activity

DPPH radical scavenging activity was assessed according to the method of Shimada et al.[41] The **4(a–o)** at concentrations ranging from 0 to 75 μg were mixed in 1 mL of freshly prepared 0.5 mM DPPH ethanolic solution and 2 mL of 0.1 M acetate buffer pH 5.5. The resulting solution was then incubated at 37 °C for 30 min and measured colorimetrically at 517 nm. α-tocopherol and BHA (0–75 μg) was used as positive control under the same assay conditions. Negative control was without any inhibitor or **4(a–o)**. Lower absorbance at 517 nm represented higher DPPH scavenging activity. The % DPPH radical scavenging activity of **4(a–o)** was calculated from the decrease in absorbance at 517 nm in comparison with the negative control.

Molecular docking studies

Molecular docking protocol was followed according to the reported method by Vivek et al.[42] Briefly, The structural drawing and geometry cleaning of novel potentials of **4(a–o)** subjected to other parameters, viz., energy minimization by using OPLS 2005 force field, the addition of hydrogen atoms, neutralization of charged groups, generation of ionization states and set pH 7.5 using Epik. Generation of tautomers and stereoisomers of 32 per ligand and low-energy ring conformations and optimize the geometries followed by generating low energy ring conformation per ligand were computed, optimized by LigPrep and used for molecular docking.

The crystal structure of AmpC beta-lactamase from *E. coli,* and Catalasewere obtained from the Brookhaven Protein Data Bank, whose PDB id, 1KE4 (AmpC β-lactamase),[43] and 2CAG (Catalase).[44] The crystal structure was imported and refined by a multistep process through the protein preparation wizard of workspace, which includes energy minimization using OPLS-2005 force field, correct bond orders were assigned, hydrogen atoms were added and the water molecules were removed from 5 Å hetero atom, formal charges, amide groups of Asn and Gln were optimized. All amino acid flips were assigned to correct geometry and hydrogen bonds were optimized. Using PROPKA, pH was fixed and optimized to 7.5. Non-hydrogen atoms were minimized by restrained minimization to default RMSD to 0.3 Å. Using Extra-precision (XP) docking and scoring each compound docking into the receptor grid of radii 20 Å × 20 Å × 20 Å and the docking calculation was judge based on the score.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

ORCID

Rangappa S. Kanchugarkoppal http://orcid.org/0000-0003-0572-6305

References

[1] Sharma, B.; Verma, A.; Prajapati, S.; Sharma, U. K. Synthetic Methods, Chemistry, and the Anticonvulsant Activity of Thiadiazoles. *Int. J. Med. Chem.* **2013**, *2013*, 1–16. DOI: 10.1155/2013/348948.

[2] Gomha, S.; Kheder, N.; Abdelhamid, A.; Mabkhot, Y. One Pot Single Step Synthesis and Biological Evaluation of Some Novel Bis(1,3,4-Thiadiazole) Derivatives as Potential Cytotoxic Agents. *Molecules* **2016**, *21*, 1532. DOI: 10.3390/molecules21111532.

[3] Jain, A. K.; Sharma, S.; Vaidya, A.; Ravichandran, V.; Agrawal, R. K. 1,3,4-Thiadiazole and Its Derivatives: A Review on Recent Progress in Biological Activities. *Chem. Biol. Drug Des.* **2013**, *81*, 557–576. DOI: 10.1111/cbdd.12125.

[4] Ferrer, S.; Jiménez, A.; Borrás, J. Synthesis and Characterization of the Acetazolamide Complexes of Co(II) and Zn(II). *Inorg. Chim. Acta.* **1987**, *129*, 103–106. DOI: 10.1016/S0020-1693(00)85910-1.

[5] Rzeski, W.; Matysiak, J.; Kandefer-Szerszeń, M. Anticancer, Neuroprotective Activities and Computational Studies of 2-Amino-1,3,4-Thiadiazole Based Compound. *Bioorg. Med. Chem.* **2007**, *15*, 3201–3207. DOI: 10.1016/j.bmc.2007.02.041.

[6] Noolvi, M. N.; Patel, H. M.; Singh, N.; Gadad, A. K.; Cameotra, S. S.; Badiger, A. Synthesis and Anticancer Evaluation of Novel 2-Cyclopropylimidazo[2,1-b][1,3,4]-Thiadiazole Derivatives. *Eur. J. Med. Chem.* **2011**, *46*, 4411–4418. DOI: 10.1016/j.ejmech.2011.07.012.

[7] Terzioglu, N.; Gürsoy, A. Synthesis and Anticancer Evaluation of Some New Hydrazone Derivatives of 2,6-Dimethylimidazo[2,1-b][1,3,4]Thiadiazole-5-Carbohydrazide. *Eur. J. Med. Chem.* **2003**, *38*, 781–786. DOI: 10.1016/S0223-5234(03)00138-7.

[8] Khan, I.; Ali, S.; Hameed, S.; Rama, N. H.; Hussain, M. T.; Wadood, A.; Uddin, R.; Ul-Haq, Z.; Khan, A.; Ali, S.; et al. Synthesis, Antioxidant Activities and Urease Inhibition of Some New 1,2,4-Triazole and 1,3,4-Thiadiazole Derivatives. *Eur. J. Med. Chem.* **2010**, *45*, 5200–5207. DOI: 10.1016/j.ejmech.2010.08.034.

[9] Yusuf, M.; Jain, P. Synthetic and Biological Studies of Pyrazolines and Related Heterocyclic Compounds. *Arabian J. Chem.* **2014**, *7*, 553–596. DOI: 10.1016/j.arabjc.2011.09.013.

[10] Clerici, F.; Pocar, D.; Guido, M.; Loche, A.; Perlini, V.; Brufani, M. Synthesis of 2-Amino-5-Sulfanyl-1,3,4-Thiadiazole Derivatives and Evaluation of Their Antidepressant and Anxiolytic Activity. *J. Med. Chem.* **2001**, *44*, 931–936. DOI: 10.1021/jm001027w.

[11] Kadi, A. A.; Al-Abdullah, E. S.; Shehata, I. A.; Habib, E. E.; Ibrahim, T. M.; El-Emam, A. A. Synthesis, Antimicrobial and anti-Inflammatory Activities of Novel 5-(1-Adamantyl)-1,3,4-Thiadiazole Derivatives. *Eur. J. Med. Chem.* **2010**, *45*, 5006–5011. DOI: 10.1016/j.ejmech.2010.08.007.

[12] Jatav, V.; Mishra, P.; Kashaw, S.; Stables, J. P. CNS Depressant and Anticonvulsant Activities of Some Novel 3-[5-Substituted 1,3,4-Thiadiazole-2-yl]-2-Styryl Quinazoline-4(3H)-Ones. *Eur. J. Med. Chem.* **2008**, *43*, 1945–1954. DOI: 10.1016/j.ejmech.2007.12.003.

[13] Chen, C.-J.; Song, B.-A.; Yang, S.; Xu, G.-F.; Bhadury, P. S.; Jin, L.-H.; Hu, D.-Y.; Li, Q.-Z.; Liu, F.; Xue, W.; et al. Synthesis and Antifungal Activities of 5-(3,4,5-Trimethoxyphenyl)-2-Sulfonyl-1,3,4-Thiadiazole and 5-(3,4,5-Trimethoxyphenyl)-2-Sulfonyl-1,3,4-Oxadiazole Derivatives. *Bioorg. Med. Chem.* **2007**, *15*, 3981–3989. DOI: 10.1016/j.bmc.2007.04.014.

[14] Liu, X.-H.; Shi, Y.-X.; Ma, Y.; Zhang, C.-Y.; Dong, W.-L.; Pan, L.; Wang, B.-L.; Li, B.-J.; Li, Z.-M. Synthesis, Antifungal Activities and 3D-QSAR Study of N-(5-Substituted-1,3,4-Thiadiazol-2-yl)Cyclopropanecarboxamides. *Eur. J. Med. Chem.* **2009**, *44*, 2782–2786. DOI: 10.1016/j.ejmech.2009.01.012.

[15] Hu, Y.; Li, C.-Y.; Wang, X.-M.; Yang, Y.-H.; Zhu, H.-L. 1,3,4-Thiadiazole: Synthesis, Reactions, and Applications in Medicinal, Agricultural, and Materials Chemistry. *Chem. Rev.* **2014**, *114*, 5572–5610. DOI: 10.1021/cr400131u.

[16] Sherif, E. M.; Park, S.-M. 2-Amino-5-Ethyl-1,3,4-Thiadiazole as a Corrosion Inhibitor for Copper in 3.0% NaCl Solutions. *Corros. Sci.* **2006**, *48*, 4065–4079. DOI: 10.1016/j.corsci.2006.03.011.

[17] Peng, X.; Gao, H.; Xiao, Y.; Cheng, H.; Huang, F.; Cheng, X. Synthesis and Self-Assembly of Photoresponsive and Luminescent Polycatenar Liquid Crystals Incorporating an Azobenzene Unit Interconnecting Two 1,3,4-Thiadiazoles. *New J. Chem.* **2017**, *41*, 2004–2012. DOI: 10.1039/C6NJ02604D.

[18] Vachhani, D. D.; Sharma, A.; Van der Eycken, E. Pd/Cu-Catalyzed C-H Arylation of 1,3,4-Thiadiazoles with (Hetero)Aryl Iodides, Bromides, and Triflates. *J. Org. Chem.* **2012**, *77*, 8768–8774. DOI: 10.1021/jo301401q.

[19] Fan, X.-Y.; Jiang, X.; Zhang, Y.; Chen, Z.-B.; Zhu, Y.-M. Palladium-Catalyzed One-Pot Synthesis of Diazoles via Tert-Butyl Isocyanide Insertion. *Org. Biomol. Chem.* **2015**, *13*, 10402–10408. DOI: 10.1039/C5OB01328C.

[20] Polshettiwar, V.; Varma, R. S. Greener and Rapid Access to Bio-Active Heterocycles: One-Pot Solvent-Free Synthesis of 1,3,4-Oxadiazoles and 1,3,4-Thiadiazoles. *Tetrahedron Lett.* **2008**, *49*, 879–883. DOI: 10.1016/j.tetlet.2007.11.165.

[21] Mantelingu, K.; Lin, Y.; Seidel, D. Intramolecular [3 + 2]-Cycloadditions of Azomethine Ylides Derived from Secondary Amines via Redox-Neutral C-H Functionalization. *Org. Lett.* **2014**, *16*, 5910–5913. DOI: 10.1021/ol502918g.

[22] Swarup, H. A.; Gowda, K.; Mantelingu, K. G.; Rangappa, K. S. Effective and Transition-Metal-Free Construction of Disubstituted, Trisubstituted 1,2,3-NH-Triazoles and Triazolo Pyridazine via Intermolecular 1,3-Dipolar Cycloaddition Reaction. *ChemistrySelect* **2018**, *3*, 703–708. DOI: 10.1002/slct.201702547.

[23] Raghavendra, G. M.; Ramesha, A. B.; Revanna, C. N.; Nandeesh, K. N.; Mantelingu, K.; Rangappa, K. S. One-Pot Tandem Approach for the Synthesis of Benzimidazoles and Benzothiazoles from Alcohols. *Tetrahedron Lett.* **2011**, *52*, 5571–5574. DOI: 10.1016/j.tetlet.2011.08.037.

[24] Ramesha, A. B.; Raghavendra, G. M.; Nandeesh, K. N.; Rangappa, K. S.; Mantelingu, K. Tandem Approach for the Synthesis of Imidazo[1,2-a]Pyridines from Alcohols. *Tetrahedron Lett.* **2013**, *54*, 95–100. DOI: 10.1016/j.tetlet.2012.10.112.

[25] Nandeesh, K. N.; Swarup, H. A.; Sandhya, N. C.; Mohan, C. D.; Pavan Kumar, C. S.; Kumara, M. N.; Mantelingu, K.; Ananda, S.; Rangappa, K. S. Synthesis and Antiproliferative Efficiency of Novel Bis(Imidazol-1-yl)Vinyl-1,2,4-Oxadiazoles. *New J. Chem.* **2016**, *40*, 2823–2828. DOI: 10.1039/C5NJ02925B.

[26] Hegde, M.; Mantelingu, K.; Swarup, H. A.; Pavankumar, C. S.; Qamar, I.; Raghavan, S. C.; Rangappa, K. S. Novel PARP Inhibitors Sensitize Human Leukemic Cells in an Endogenous PARP Activity Dependent Manner. *RSC Adv.* **2016**, *6*, 6308–6319. DOI: 10.1039/C5RA19150E.

[27] Kumar, S. V.; Yadav, S. K.; Raghava, B.; Saraiah, B.; Ila, H.; Rangappa, K. S.; Hazra, A. Cyclocondensation of Arylhydrazines with 1,3-Bis(Het)Arylmonothio-1,3-Diketones and 1,3-Bis(Het)Aryl-3-(Methylthio)-2-Propenones: Synthesis of 1-Aryl-3,5-Bis(Het) Arylpyrazoles with Complementary Regioselectivity. *J. Org. Chem.* **2013**, *78*, 4960–4973. DOI: 10.1021/jo400599e.

[28] Raghava, B.; Parameshwarappa, G.; Acharya, A.; Swaroop, T. R.; Rangappa, K. S.; Ila, H. Cyclocondensation of Hydroxylamine with 1,3-Bis(Het)Arylmonothio 1,3-Diketones and 1,3-Bis(Het)Aryl-3-(Methylthio)-2-Prop-Enones: Synthesis of 3,5-Bis(Het)Arylisoxazoles with Complementary Regioselectivity. *Eur. J. Org. Chem.* **2014**, *2014*, 1882–1892. DOI: 10.1002/ejoc.201301667.

[29] Sandhya, N. C.; Nandeesh, K. N.; Rangappa, K. S.; Ananda, S. One-Pot Synthesis of 2,3-Substituted Benzo[b]Thiophenes via Cu(i) Catalysed Intramolecular Cyclisation from Dithioesters. *RSC Adv.* **2015**, *5*, 29939–29946. DOI: 10.1039/C5RA02114F.

[30] Ramesha, A. B.; Sandhya, N. C.; Pavan Kumar, C. S.; Hiremath, M.; Mantelingu, K.; Rangappa, K. S. A Novel Approach for the Synthesis of Imidazo and Triazolopyridines from Dithioesters. *New J. Chem.* **2016**, *40*, 7637–7642. DOI: 10.1039/C6NJ01038E.

[31] Ramesha, A. B.; Pavan Kumar, C. S.; Sandhya, N. C.; Kumara, M. N.; Mantelingu, K.; Rangappa, K. S. Tandem Approach for the Synthesis of 3-Sulfenylimidazo[1,5-a]Pyridines from Dithioesters. *RSC Adv.* **2016**, *6*, 48375–48378. DOI: 10.1039/C6RA03771B.

[32] Jenifer Vijay, T. A.; Nandeesh, K. N.; Raghavendra, G. M.; Rangappa, K. S.; Mantelingu, K. Transition Metal Free Intramolecular S-Arylation: One-Pot Synthesis of Thiochromen-4-Ones. *Tetrahedron Lett.* **2013**, *54*, 6533–6537. DOI: 10.1016/j.tetlet.2013.09.094.

[33] Randhawa, G. K.; Sharma, R. Chemotherapeutic Potential of Cow Urine: A Review. *J. Intercult. Ethnopharmacol.* **2015**, *4*, 180–186. DOI: 10.5455/jice.20150222100320.

[34] Nagamallu, R.; Srinivasan, B.; Ningappa, M. B.; Kariyappa, A. K. Synthesis of Novel Coumarin Appended Bis(Formylpyrazole) Derivatives: Studies on Their Antimicrobial and Antioxidant Activities. *Bioorg. Med. Chem. Lett.* **2016**, *26*, 690–694. DOI: 10.1016/j.bmcl.2015.11.038.

[35] Prabhudeva, M. G.; Vivek, H. K.; Kumar, K. A. Synthesis of Novel Pyrazole Carboxamides Using Reusable Catalyst as Antimicrobial Agents and Molecular Docking Studies. *Chem. Data Collect.* **2019**, *20*, 100193. DOI: 10.1016/j.cdc.2019.100193.

[36] Marasini, B. P.; Baral, P.; Aryal, P.; Ghimire, K. R.; Neupane, S.; Dahal, N.; Singh, A.; Ghimire, L.; Shrestha, K. Evaluation of Antibacterial Activity of Some Traditionally Used Medicinal Plants against Human Pathogenic Bacteria. *BioMed. Res. Int.* **2015**, *2015*, 1–6. DOI: 10.1155/2015/265425.

[37] Lee, J. C.; Kim, H. R.; Kim, J.; Jang, Y. S. Antioxidant Property of an Ethanol Extract of the Stem of *Opuntia ficus-indica* Var. saboten. *J. Agric. Food Chem.* **2002**, *50*, 6490–6496. DOI: 10.1021/jf020388c.

[38] Halliwell, B.; Gutteridge, J. M. The Importance of Free Radicals and Catalytic Metal Ions in Human Diseases. *Mol. Aspects Med.* **1985**, *8*, 89–193. DOI: 10.1016/0098-2997(85)90001-9.

[39] Shimasaki, H.; Ueta, N.; Mowri, H. O.; Inoue, K. Formation of Age Pigment-like Fluorescent Substances during Peroxidation of Lipids in Model Membranes. *Biochim. Biophys. Acta.* **1984**, *792*, 123–129.

[40] Dahle, L. K.; Hill, E. G.; Holman, R. T. The Thiobarbituric Acid Reaction and the Autoxidations of Polyunsaturated Fatty Acid Methyl Esters. *Arch. Biochem. Biophys.* **1962**, *98*, 253–261. DOI: 10.1016/0003-9861(62)90181-9.

[41] Shimada, K.; Fujikawa, K.; Yahara, K.; Nakamura, T. Antioxidative Properties of Xanthan on the Autoxidation of Soybean Oil in Cyclodextrin Emulsion. *J. Agric. Food Chem.* **1992**, *40*, 945–948. DOI: 10.1021/jf00018a005.

[42] Kameshwar, V. H.; Kumar, J. R.; Priya, B. S.; Swamy, S. N. Synthesis, Characterization and Bioactivity Studies of Novel 1,3,4-Oxadiazole Small Molecule That Targets Basic Phospholipase A2 from Vipera Russelli. *Mol. Cell. Biochem.* **2017**, *426*, 161–175. DOI: 10.1007/s11010-016-2888-6.

[43] Powers, R. A.; Shoichet, B. K. Structure-Based Approach for Binding Site Identification on AmpC Beta-Lactamase. *J. Med. Chem.* **2002**, *45*, 3222–3234. DOI: 10.1021/jm020002p.

[44] Gurunanjappa, P.; Hamse, V.; Kumar, K. *Bioactive Formylpyrazole Analogues: Synthesis, Antimicrobial, Antioxidant and Molecular Docking Studies*. Editor, 2017, Vol. 29, pp. 1549–1554. DOI: 10.14233/ajchem.2017.20562.