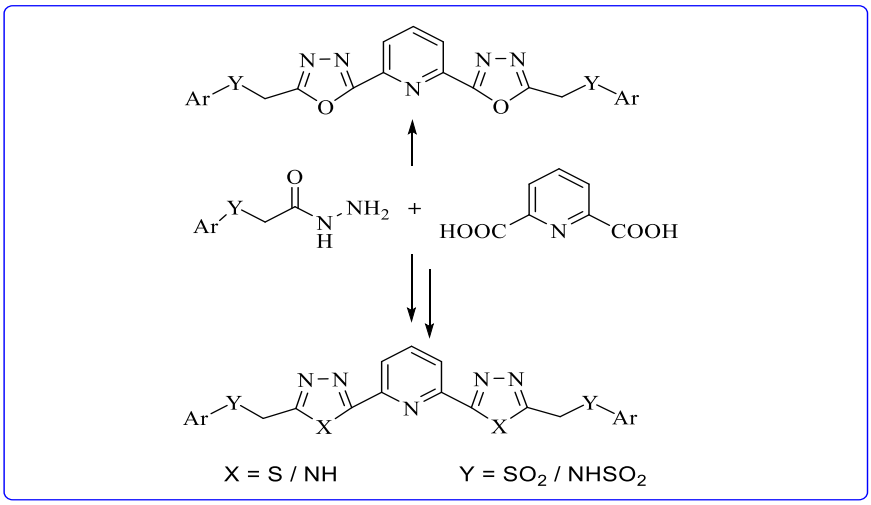
Synthesis, characterization and antioxidant activity of *bis* (arylsulfonylmethyl / arylaminosulfonylmethylazolyl) pyridines

Anil Kumar Gunthanakkala, Madhu Sekhar Mangali, Padmavathi Venkatapuram, Padmaja Adivireddy*

Department of Chemistry, Sri Venkateswara University, Tirupati 517 502, Andhra Pradesh, India, Tel: +91 877 2289303; Fax: +91 877 2249532, E-mail: adivireddyp@yahoo.co.in



A new class of *bis*(arylsulfonylmethylazolyl)pyridines and *bis*(arylaminosulfonylmethyl-azolyl)pyridines were synthesized from the synthetic intermediates methyl arylsulfonylacetic acid hydrazide and methyl arylaminosulfonylacetic acid hydrazide adopting a green methodology-ultrasonication. All the synthesized compounds were resulted in higher yield and in shorter reaction times. The spectral parameters such as IR, ^{1}H NMR, ^{13}C NMR, mass and microanalyses were used to determine the structures of all the synthesized compounds and were assayed for antioxidant activity. The *bis*(arylaminosulfonylmethylazolyl)pyridines showed higher radical scavenging activity than the *bis*(arylsulfonylmethylazolyl)pyridines. Besides, unsubstituted and methyl substituted compounds exhibited greater activity. Amongst all the tested compounds **8b** and **11b** were identified as potential antioxidants.

This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1002/jhet.4123

INTRODUCTION

Antioxidants are one of the prominent disciplines of scientific research as they can protect organism and cell from damage that are induced by oxidative stress. Scientists in different areas become interested in new compounds which prevent or reduce the impact of oxidative stress on cell.[1,2] Therefore, many chemists are searching for new antioxidants which can be structurally modified from the basic structures and effectively inhibit the radical scavenging activity. Heterocyclic compounds, particularly azoles, are of great scientific importance since they are used as key starting compounds for many pharmaceuticals.[3] One such type of compounds are oxadiazoles, thiadiazoles and triazoles. 1,3,4-Oxadiazoles and their derivatives show a wide spectrum of biological activities, including antioxidant, antibacterial, anti-inflammatory, antifungal, and antitubercular etc., Besides, oxadiazole is a privileged scaffold in many drugs *viz.*, raltegravir, zibotentan, tiodazosin and furamizole.[4] Moreover, 1,3,4-oxadiazoles are very good bioisosteres of amides and esters, which enhance pharmacological activities by participating in hydrogen bonding interactions with the receptors.[5] It is reported that 2,5-disubstituted 1,3,4-oxadiazoles are prepared either by thermal/acid catalyzed reaction of 1,2-diacylhydrazines[6] or by reaction of semicarbazone/hydrazone promoted by an oxidant[7] or by irradiation of a mixture of acid hydrazide and carboxylic acid by microwaves.[8] 1,3,4-Thiadiazole exhibit antioxidant, antimicrobial, anticancer, anti-inflammatory, herbicidal and insecticidal activities.[9, 10] The reaction of acylthiosemicarbazides with acid reagents such as trifluoroacetic acid[11] and methanesulfonic acid[12] is most frequently used procedure for the preparation of 1,3,4-thiadiazoles. Thiadiazole moiety makes the core structure of most familiar drugs-acetazolamide, megazol, sulfamethazole and cefazedone.[13] Besides, 1,2,4-triazoles possess antibacterial,[14] antifungal[15] and anti-inflammatory[16] activities. Some chemotherapeutics like vorozole, letrozole and anastrozole contain 1,2,4-triazole.[17] One of the synthetic methods for the synthesis of triazoles involves the utilization of N, N$^{'}$ -dimethylformamide dimethyl acetals.[18] Replacement of –O– by –S– or –NH– in some heterocycles is reported *viz.*, Bordners[19] synthesis of pyrroles from furan and the conversion of epoxide to episulfides by the treatment of thiocyanates or thiourea.[20-22] However, reports about the conversion of 1,3,4-oxadiazoles to 1,3,4-thiadiazoles and 1,2,4-triazoles are relatively less.[23, 24] Besides, pyridine is a ubiquitous moiety in coenzyme vitamin B_6 family and in numerous alkaloids.[25] Pyridine and its derivatives display antioxidant,[26] antimicrobial,[27] and anti-inflammatory activities.[28] The sulfone and sulfonamide are important functional groups found in a wide variety of natural products, drugs and materials.[29] The combination of different pharmacophores in one molecular frame could lead to a novel entity with increased biological properties. Hence, the development of green techniques for the preparation of azoles remains an

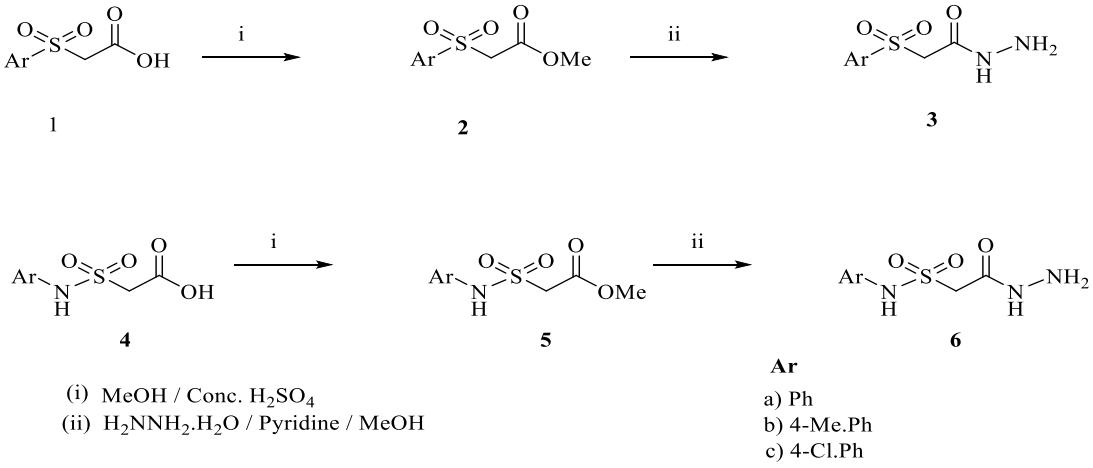
important task for synthetic community. In the recent years, ultrasound irradiation methodology offers high yield, shorter reaction time and milder reaction conditions[30] when compared with the traditional method.[31] Motivated by these observations and our ongoing efforts towards development of bioactive heterocycles,[32-34] it is planned to prepare hitherto unknown *bis* heterocycles with a variety of pharmacophoric units under ultrasonication and to study their antioxidant activity.

RESULTS AND DISCUSSION

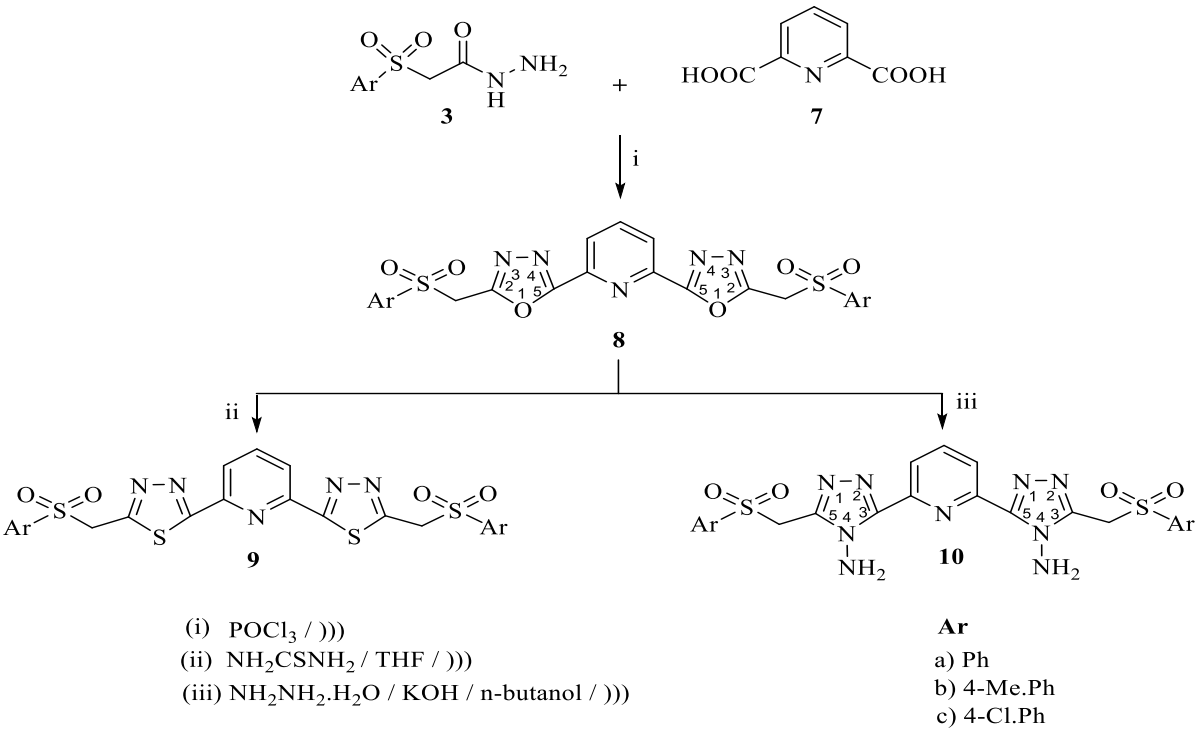
The methyl arylsulfonylacetic acid hydrazide (**3**) and methyl arylaminosulfonylacetic acid hydrazide (**6**) were used as synthons to prepare the target compounds *bis*(arylsulfonylmethyl oxadiazolyl / thiadiazolyl / triazolyl)pyridines and *bis*(arylaminosulfonylmethyl oxadiazolyl / thiadiazolyl / triazolyl)pyridines. Esterification of arylsulfonylacetic acid (**1**) and arylaminosulfonylacetic acid (**4**) with methanol in the presence of concentrated sulphuric acid gave methyl arylsulfonylacetic acid ester (**2**) and methyl arylaminosulfonylacetic acid ester (**5**). The treatment of compounds **2** and **5** with hydrazine hydrate in the presence of pyridine resulted in **3** and **6** (Scheme 1). The reaction of 2 mmol of compound **3** with 1 mmol of **7** in the presence of $POCl_3$ under ultrasonication produced 2,6-*bis*(2-arylsulfonylmethyl-1,3,4-oxadiazol-5-yl)pyridine (**8**). The oxadiazole ring present in compounds **8** and **11** were interconverted to thiadiazole and triazole rings by reaction with appropriate nucleophiles. In fact, the reaction of **8** with thiourea in THF under ultrasonication yielded 2,6-*bis*(2-arylsulfonylmethyl-1,3,4-thadiazol-5-yl)pyridine (**9**). On the other hand, 2,6-*bis*(3-arylsulfonylmethyl-4-amino-1,2,4-triazol-5-yl)pyridine **(10)** was obtained by the treatment of **8** with hydrazine hydrate in *n*-butanol (Scheme 2). The ^{1}H NMR spectra of **8a, 9a** and **10a** displayed a singlet at δ 5.25, 5.45 and 5.40 due to CH_2 protons attached to C-2 / C-3 respectively. Moreover, a broad singlet appeared at δ 5.62 ppm in **10a** was attributed to NH_2 and disappeared on deuteration. Furthermore, 2,6-*bis*(2-arylaminosulfonylmethyl-1,3,4-oxadiazol-5-yl)pyridine (**11)** was synthesized by the cyclocondensation of 2 mmol of compound **6** with 1 mmol of **7** in the presence of $POCl_3$ under ultrasonication. In a similar way, 2,6-*bis*(2-(arylaminosulfonylmethyl)-1,3,4-thadiazol-5-yl)pyridine (**12**) was prepared by the reaction of compound **11** with thiourea in THF. Likewise, 2,6-(*bis*(3-arylaminosulfonylmethyl-4-amino-1,2,4-triazol-5-yl)pyridine (**13**) was synthesized by the treatment of **11** with hydrazine hydrate in *n*-butanol (Scheme 3). The ^{1}H NMR spectra of **11a** and **12a** displayed a singlet at δ 5.45 and 5.30

(CH_2) and a broad singlet at 10.30 and 10.32 ppm (NH) in addition to aromatic protons. The ^{1}H NMR spectrum of **13a** exhibited a singlet at δ 5.04 due to CH_2 protons attached to C-3 and two broad singlets at 5.60 (NH_2) and 10.15 ppm (NH). The signals of NH and NH_2 disappeared upon addition of D_2O. IR, ^{13}C NMR, mass spectra and microanalyses were also utilized to establish the structures of all the synthesized compounds.

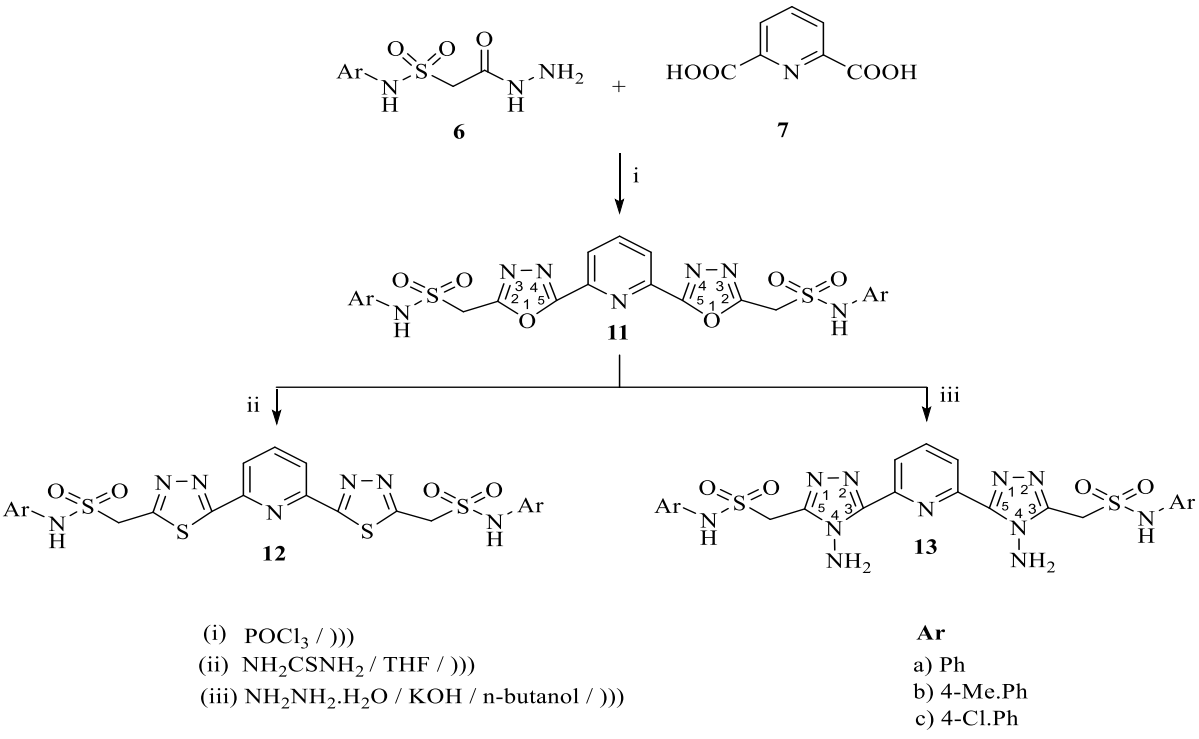
Scheme 1. Preparation of arylsufonyl/arylaminosufonylacetic acid hydrazide.



Scheme 2. Ultrasound assisted synthesis of *bis*(arylsufonylmethylazolyl) pyridines.



Scheme 3. Ultrasound assisted synthesis of *bis*(arylaminosufonylmethylazolyl) pyridines.



ANTIOXIDANT ACTIVITY

Compounds **8-13** were assayed for antioxidant activity by three methods (DPPH, NO, and H_2O_2) at 50, 75 and 100 µM concentrations (Tables 1, 2 & 3) using ascorbic acid as the standard drug. The results indicated that compounds **8a**, **8b**, **10b**, **11a**, **11b**, **13a** and **13b** displayed good activity. The compounds **9c**, **10c**, and **12c** showed low activity whereas the remaining compounds exhibited moderate activity. The structure-activity relationship of the tested compounds indicated that the compounds containing oxadiazole units (**8** and **11**) showed higher antioxidant activity than those containing thiadiazole (**9** and **12**) and triazole (**10** and **13**) moieties. It was observed that compounds having triazole displayed slightly higher radical scavenging activity than thiadiazole. The sulfonamide *bis* heterocycles (**11-13**) displayed comparatively higher antioxidant activity than the sulfone *bis* heterocycles (**8-10**). This may be due to the presence of increasing number of chromophoric groups. Moreover, electron donating methyl substituent on the phenyl ring enhanced the radical scavenging activity when compared with the electron withdrawing chloro substituted compounds. In fact, the radical scavenging activity increases with increase in concentration. In all three methods, the IC_{50} values of **8b** and **11b** are 32.95, 31.64, 32.42 and 32.01, 29.90, 31.34. Amongst all the tested compounds **8b** and **11b** exhibited excellent antioxidant activity.

CONCLUSION

In conclusion, some new *bis*(arylsulfonylmethylazolyl)pyridines and *bis*(arylaminosulfonylmethylazolyl)pyridines were synthesized from the synthetic intermediates, methyl arylsulfonylacetic acid hydrazide and methyl arylaminosulfonylacetic acid hydrazide adopting a green approach – ultrasonication and resulted in high yield in shorter reaction time. IR, NMR, mass and elemental analyses were used to elucidate the structures of all the synthesized compounds and were also tested for antioxidant activity. The compounds having oxadiazole ring displayed greater antioxidant activity than those having thiadiazole and triazole units. It was also noticed that compounds bearing sulfonamide moiety displayed higher radical scavenging activity than those with sulfone. This may be due to the presence of more chromophore units. Moreover, unsubstituted and methyl substituted compounds showed higher radical scavenging activity than the chloro substituted compounds. Amongst all the tested compounds, **8b** and **11b** were found to be potential antioxidant agents.

EXPERIMENTAL

General. Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. The homogeneity of the compounds was determined by TLC (silica gel H, BDH, hexane/ethyl acetate, 3:1). The IR spectra were run on a Thermo Nicolet IR 200 FT-IR spectrometer as KBr pellets and the wave numbers were reported in cm^{-1}. The ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 on a Jeol JNM spectrometer operating at λ-400 and λ-100 MHz. High-resolution mass spectra were run on Micro mass Q-TOF spectrometer using electrospray ionization. All chemical shifts were given in δ (ppm) using TMS as an internal standard. The elemental analyses were carried out on a Perkin-Elmer 240C elemental analyzer. The temperature was measured by flexible probe throughout the reaction. Ultrasound irradiation was performed in a Bandelin Sonorex RK 102H ultrasonic bath operating at frequency of 35 KHz. The synthetic intermediates methyl arylsulfonylacetic acid hydrazide (**3**) and methyl arylaminosulfonylacetic acid hydrazide (**6**) were synthesized as per the literature procedures. [32, 35]

General procedure for the synthesis of 2,6-*bis*(2-arylsulfonylmethyl-1,3,4-oxadiazol-5-yl)pyridine (**8)**. The compound **3** (2 mmol), **7** (1 mmol) and $POCl_3$ (5 mL) were subjected to ultrasound irradiation at a frequency of 35 KHz for 35-60 min at laboratory temperature. The progress of the reaction was checked by TLC. The excess $POCl_3$ was removed *in vacuo* and the resultant residue was poured onto crushed ice. The separated solid was filtered, washed with saturated sodium bicarbonate solution followed by water, dried and recrystallized from 2-propanol.

2,6-Bis(2-(phenylsulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (8a). Yield 92%; White solid, mp 171-173 ^{o}C; IR (KBr cm^{-1}): 1574 (C=N), 1312 & 1152 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.25 (s, 4H, CH_2-(C-2)), 7.25-7.81 (m, 13H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.6 (CH_2), 159.2 (C-2), 157.0 (C-5), 122.4, 125.7, 128.8, 132.4, 136.9, 140.7, 152.2 ppm (aromatic carbons); HRMS (*m/z*): 546.0518 $[M+ Na]^{+}$. *Anal*. Calcd. for $C_{23}H_{17}N_5O_6S_2$: C 52.77, H 3.27, N 13.38; Found: C 52.86, H 3.30, N 13.60%.

2,6-Bis(2-(4-methylphenylsulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (8b). Yield 80%; White solid, mp 177-179 ^{o}C; IR (KBr cm^{-1}): 1562 (C=N), 1308 & 1148 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.51 (s, 6H, Ar-CH_3), 5.42 (s, 4H, CH_2-(C-2)), 7.35-8.07 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.4 (Ar-CH_3), 54.0 (CH_2), 158.8 (C-2), 156.9 (C-5), 121.2, 125.0, 127.2, 132.0, 136.0, 140.6, 151.5 ppm (aromatic carbons); HRMS (*m/z*): 574.0831 $[M+ Na]^{+}$. *Anal*. Calcd. for $C_{25}H_{21}N_5O_6S_2$: C 54.44, H 3.84, N 12.70; Found: C 54.54, H 3.83, N 12.84%.

2,6-Bis(2-(4-chlorophenylsulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (8c). Yield 85%; White solid, mp 192-194 ^{o}C; IR (KBr cm^{-1}): 1575 (C=N), 1316 & 1157 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.52 (s, 4H, CH_2-(C-2)), 7.40-8.16 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.5 (CH_2), 159.6 (C-2), 157.4 (C-5), 122.7, 126.2, 128.9, 133.2, 137.8, 141.5, 153.6

ppm (aromatic carbons); HRMS (*m/z*): 613.9738 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{15}Cl_2N_5O_6S_2$: C 46.63, H 2.55, N 11.82; Found: C 46.75, H 2.57, N 11.98%.

General procedure for the synthesis of 2,6-*bis*(2-arylsulfonylmethyl-1,3,4-thiadiazol-5-yl)pyridine (9). In a sealed tube, a mixture of compound **8** (0.5 mmol), thiourea (2 mmol) and THF (6 mL) was taken and heated to reflux under ultrasonication at a frequency of 35 KHz for 80-110 min. The progress of the reaction was checked by TLC. The contents of the flask were extracted with dichloromethane, washed with water followed by brine solution. The organic layer was dried over anhydrous sodium sulfate and filtered. Removal of the solvent under reduced pressure gave a residue which was purified by column chromatography (silica gel, 60-120 mesh) using hexane-ethyl acetate (3:1) as eluent.

2,6-Bis(2-(phenylsulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (9a). Yield 88%; White solid, mp 169-171 ^{o}C; IR (KBr cm^{-1}): 1568 (C=N), 1321 & 1138 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.45 (s, 4H, CH_2-(C-2)), 7.34-7.96 (m, 13H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.1 (CH_2), 164.4 (C-2), 155.6 (C-5), 122.5, 125.4, 128.2, 134.2, 138.0, 141.7, 152.5 ppm (aromatic carbons); HRMS (*m/z*): 578.0061 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{17}N_5O_4S_4$: C 49.72, H 3.08, N 12.60; Found: C 49.81, H 3.09, N 12.78%.

2,6-Bis(2-(4-methylphenylsulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (9b). Yield 86%; White solid, mp 166-168 ^{o}C; IR (KBr cm^{-1}): 1561 (C=N), 1319 & 1131 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.48 (s, 6H, Ar-CH_3), 5.39 (s, 4H, CH_2-(C-2)), 7.25-7.83 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.8 (Ar-CH_3), 53.5 (CH_2), 163.9 (C-2), 154.4 (C-5), 122.0, 125.2, 128.0, 133.7, 137.3, 140.3, 151.3 ppm (aromatic carbons); HRMS (*m/z*): 606.0374 [M+ Na]$^+$. *Anal*. Calcd. for $C_{25}H_{21}N_5O_4S_4$: C 51.44, H 3.63, N 12.00; Found: C 51.55, H 3.66, N 12.20%.

2,6-Bis(2-(4-chlorophenylsulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (9c). Yield 89%; White solid, mp 178-180 ^{o}C; IR (KBr cm^{-1}): 1572 (C=N), 1327 & 1128 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.42 (s, 4H, CH_2-(C-2)), 7.50-7.98 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.3 (CH_2), 164.0 (C-2), 154.8 (C-5), 123.0, 126.6, 128.7, 135.6, 138.5, 142.0, 152.7 ppm (aromatic carbons); HRMS (*m/z*): 645.9282 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{15}Cl_2N_5O_4S_4$: C 44.23, H 2.42, N 11.21; Found: C 44.17, H 2.41, N 11.35%.

General procedure for the synthesis of 2,6-(*bis*(3-arylsulfonylmethyl-4-amino-1,2,4-triazol-5-yl)pyridine (10). The compound **8** (1 mmol), hydrazine hydrate (4 mmol) and *n*-butanol (5 mL) were kept under ultrasonication at a frequency of 35 KHz for 55-75 min. Then, potassium hydroxide (2 mmol) was added to the reaction media. The precipitate obtained was separated by filtration and treated with concentrated hydrochloric acid to pH ≈ 3. It was washed

with water, dried and purified by column chromatography (silica gel, 160-120 mesh) using hexane-ethyl acetate (3:1) as eluent.

2,6-(Bis(3-(phenylsulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (10a). Yield 89%; White solid, mp 174-176 ^{o}C; IR (KBr cm^{-1}): 3446 & 3338 (NH_2), 1559 (C=N), 1324 & 1132 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.40 (s, 4H, CH_2-(C-3)), 5.62 (bs, 4H, NH_2), 7.29-7.75 (m, 13H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 53.2 (CH_2), 154.7 (C-3), 150.2 (C-5), 121.5, 126.0, 128.5, 133.4, 137.1, 142.2, 152.3 ppm (aromatic carbons); HRMS (*m/z*): 574.1056 [M+ Na]$^{+}$. *Anal*. Calcd. for $C_{23}H_{21}N_9O_4S_2$: C 50.08, H 3.84, N 22.85; Found: C 50.18, H 3.88, N 23.01%.

2,6-(Bis(3-(4-methylphenylsulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (10b). Yield 85%; White solid, mp 188-190 ^{o}C; IR (KBr cm^{-1}): 3439 & 3333 (NH_2), 1561 (C=N), 1321 & 1126 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.44 (s, 6H, Ar-CH_3), 5.33 (s, 4H, CH_2-(C-3)), 5.63 (bs, 4H, NH_2), 7.20-7.69 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.5 (Ar-CH_3), 54.7 (CH_2), 154.6 (C-3), 150.1 (C-5), 121.6, 125.5, 128.3, 133.6, 136.8, 140.8, 152.1 ppm (aromatic carbons); HRMS (*m/z*): 602.1369 [M+ Na]$^{+}$. *Anal*. Calcd. for $C_{25}H_{25}N_9O_4S_2$: C 51.80, H 4.35, N 21.75; Found: C 51.92, H 4.37, N 21.97%.

2,6-(Bis(3-(4-chlorophenylsulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (10c). Yield 89%; White solid, mp 198-200 ^{o}C; IR (KBr cm^{-1}): 3453 & 3345 (NH_2), 1567 (C=N), 1330 & 1137 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.37 (s, 4H, CH_2-(C-3)), 5.68 (bs, 4H, NH_2), 7.31-7.95 (m, 11H, Ar-H) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 53.6 (CH_2), 155.5 (C-3), 150.7 (C-5), 121.8, 126.4, 129.0, 134.7, 138.6, 142.6, 153.2 ppm (aromatic carbons); HRMS (*m/z*): 642.0276 [M+ Na]$^{+}$. *Anal*. Calcd. for $C_{23}H_{19}Cl_2N_9O_4S_2$: C 44.52, H 3.09, N 20.32; Found: C 44.61, H 3.07, N 20.47%.

General procedure for the synthesis of 2,6-*bis*(2-arylaminosulfonylmethyl-1,3,4-oxadiazol-5-yl)pyridine (11). A mixture of compound **6** (2 mmol), **7** (1 mmol) and $POCl_3$ (5 mL) was subjected to ultrasound irradiation at a frequency of 35 KHz for 30-50 min at laboratory temperature. The progress of the reaction was monitored by TLC. Removal of the excess $POCl_3$ *in vacuo* yielded a residue which was poured onto crushed ice. The separated solid collected by filtration. It was washed with saturated sodium bicarbonate followed by water, dried and recrystallized from 2-propanol.

2,6-Bis(2-(phenylaminosulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (11a). Yield 87%; White solid, mp 167-169 ^{o}C; IR (KBr cm^{-1}): 3248 (NH), 1581 (C=N), 1335 & 1141 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.45 (s, 4H, CH_2-(C-2)), 7.26-7.89 (m, 13H, Ar-H), 10.30 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.4 (CH_2), 159.9 (C-2), 156.4 (C-5), 120.5, 124.8, 127.5,

132.7, 136.5, 141.4, 151.8 ppm (aromatic carbons); HRMS (*m/z*): 576.0736 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{19}N_7O_6S_2$: C 49.90, H 3.46, N 17.71; Found: C 50.03, H 3.47, N 17.90%.

2,6-Bis(2-(4-methylphenylaminosulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (11b). Yield 89%; White solid, mp 161-163 ^{o}C; IR (KBr cm^{-1}): 3242 (NH), 1577 (C=N), 1327 & 1136 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.26 (s, 6H, Ar-CH_3), 5.43 (s, 4H, CH_2-(C-2)), 7.21-7.91 (m, 11H, Ar-H), 10.25 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.3 (Ar-CH_3), 53.2 (CH_2), 158.3 (C-2), 156.3 (C-5), 120.0, 124.3, 127.3, 132.4, 137.4, 140.4, 151.0 ppm (aromatic carbons); HRMS (*m/z*): 604.1049 [M+ Na]$^+$. *Anal*. Calcd. for $C_{25}H_{23}N_7O_6S_2$: C 51.63, H 3.99, N 16.86; Found: C 51.71, H 4.01, N 17.03%.

2,6-Bis(2-(4-chlorophenylaminosulfonylmethyl)-1,3,4-oxadiazol-5-yl)pyridine (11c). Yield 91%; White solid, mp 182-184 ^{o}C; IR (KBr cm^{-1}): 3248 (NH), 1581 (C=N), 1335 & 1141 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.51 (s, 4H, CH_2-(C-2)), 7.15-7.79 (m, 11H, Ar-H), 10.36 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 53.7 (CH_2), 159.4 (C-2), 157.8 (C-5), 120.8, 125.8, 127.8, 133.8, 137.6, 141.8, 153.4 ppm (aromatic carbons); HRMS (*m/z*): 643.9956 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{17}Cl_2N_7O_6S_2$: C 44.38, H 2.75, N 15.75%; Found: C 44.48, H 2.74, N 15.94%.

General procedure for the synthesis of 2,6-*bis*(2-arylaminosulfonylmethyl-1,3,4-thiadiazol-5-yl)pyridine (12). The compound **11** (0.5 mmol), thiourea (2 mmol) and THF (6 mL) were taken in a sealed tube and heated to reflux under ultrasonication at a frequency of 35 KHz for 60-70 min. The progress of the reaction was monitored by TLC. The reaction mixture was extracted with dichloromethane, and washed with water followed by brine solution. The organic layer was dried over anhydrous sodium sulfate. The solvent was removed under vacuum and the resultant residue was purified by column chromatography (silica gel, 60-120 mesh) using hexane- ethyl acetate (3:1) as eluent.

2,6-Bis(2-(phenylaminosulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (12a). Yield 91%; White solid, mp 192-194 ^{o}C; IR (KBr cm^{-1}): 3228 (NH), 1591 (C=N), 1324 & 1141 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.30 (s, 4H, CH_2-(C-2)), 7.33-7.72 (m, 13H, Ar-H), 10.32 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.3 (CH_2), 164.1 (C-2), 155.1 (C-5), 120.5, 124.9, 127.0, 134.3, 136.6, 141.6, 152.0 ppm (aromatic carbons); HRMS (*m/z*): 608.0279 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{19}N_7O_4S_4$: C 47.17, H 3.27, N 16.74; Found: C 47.24, H 3.30, N 16.90%.

2,6-Bis(2-(4-methylphenylaminosulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (12b). Yield 88%; White solid, mp 199-201 ^{o}C; IR (KBr cm^{-1}): 3211 (NH), 1583 (C=N), 1322 & 1133 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.29 (s, 6H, Ar-CH_3), 5.37 (s, 4H, CH_2-(C-2)), 7.26-7.90 (m, 11H, Ar-H), 10.41 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 22.7 (Ar-CH_3), 53.6 (CH_2), 164.8 (C-

2), 154.5 (C-5), 119.5, 124.5, 127.7, 132.8, 136.2, 140.5, 151.4 ppm (aromatic carbons); HRMS (*m/z*): 636.0592 [M+ Na]$^+$. *Anal*. Calcd. for $C_{25}H_{23}N_7O_4S_4$: C 48.93, H 3.78, N 15.98; Found: C 49.06, H 3.82, N 16.19%.

2,6-Bis(2-(4-chlorophenylaminosulfonylmethyl)-1,3,4-thiadiazol-5-yl)pyridine (12c). Yield 92%; White solid, mp 211-213 ^{o}C; IR (KBr cm^{-1}): 3236 (NH), 1594 (C=N), 1331 & 1143 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.41 (s, 4H, CH_2-(C-2)), 7.33-8.01 (m, 11H, Ar-H), 10.62 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 54.5 (CH_2), 164.3 (C-2), 156.0 (C-5), 120.6, 125.6, 128.4, 134.6, 137.5, 142.5, 152.8 ppm (aromatic carbons); HRMS (*m/z*): 675.9500 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{17}Cl_2N_7O_4S_4$: C 42.20, H 2.62, N 14.98; Found: C 42.31, H 2.64, N 15.16%.

General procedure for the synthesis of 2,6-(*bis*(3-arylaminosulfonylmethyl-4-amino-1,2,4-triazol-5-yl)pyridine (13). A solution of compound **11** (1 mmol) and hydrazine hydrate (4 mmol) in *n*-butanol (5 mL) was subjected to ultrasound irradiation at a frequency of 35 KHz for 30-45 min. Then, potassium hydroxide (2 mmol) was added to reaction media and the precipitate obtained was separated by filtration. The resultant solid was treated with concentrated hydrochloric acid to pH ≈ 3. It was washed with water, dried and purified by column chromatography (silica gel, 160-120 mesh) using hexane- ethyl acetate (3:1) as eluent.

2,6-(Bis(3-(phenylaminosulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (13a). Yield 91%; White solid, mp 189-191 ^{o}C; IR (KBr cm^{-1}): 3478 & 3361 (NH_2), 3243 (NH), 1596 (C=N), 1334 & 1135 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.04 (s, 4H, CH_2-(C-3)), 5.60 (bs, 4H, NH_2), 7.36-7.64 (m, 13H, Ar-H), 10.15 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.2 (CH_2), 155.6 (C-3), 150.3 (C-5), 120.7, 125.9, 129.2, 134.0, 138.1, 142.3, 153.1 ppm (aromatic carbons); HRMS (*m/z*): 604.1274 [M+ Na]$^+$. *Anal*. Calcd. for $C_{23}H_{23}N_{11}O_4S_2$: C 47.50, H 3.99, N 26.49; Found: C 47.62, H 4.00, N 26.73%.

2,6-(Bis(3-(4-methylphenylaminosulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (13b). Yield 84%; White solid, mp 180-182 ^{o}C; IR (KBr cm^{-1}): 3471 & 3365 (NH_2), 3237 (NH), 1583 (C=N), 1326 & 1132 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.40 (s, 6H, Ar-CH_3), 5.30 (s, 4H, CH_2-(C-3)), 5.59 (bs, 4H, NH_2), 7.38-7.94 (m, 11H, Ar-H), 10.28 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 23.2 (Ar-CH_3), 54.7 (CH_2), 154.8 (C-3), 149.7 (C-5), 119.8, 124.2, 128.1, 133.5, 137.2, 141.2, 152.3 ppm (aromatic carbons); HRMS (*m/z*): 632.1587 [M+ Na]$^+$. *Anal*. Calcd. for $C_{25}H_{27}N_{11}O_4S_2$: C 49.25, H 4.46, N 25.27; Found: C 49.34, H 4.49, N 25.46%.

2,6-(Bis(3-(4-chlorophenylaminosulfonylmethyl)-4-amino-1,2,4-triazol-5-yl))pyridine (13c). Yield 89%; White solid, mp 199-201 ^{o}C; IR (KBr cm^{-1}): 3480 & 3382 (NH_2), 3251 (NH), 1598 (C=N), 1338 & 1139 (SO_2); ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.38 (s, 4H, CH_2-(C-3)), 5.70 (bs, 4H, NH_2), 7.57-8.10 (m, 11H, Ar-H), 10.30 (bs, 2H, NH) ppm; ^{13}C NMR (400 MHz, DMSO-d_6): δ 55.4

(CH_2), 155.2 (C-3), 151.6 (C-5), 121.2, 124.7, 130.0, 134.8, 138.4, 142.8, 153.8 ppm (aromatic carbons); HRMS (*m/z*): 672.0494 $[M+ Na]^+$. *Anal*. Calcd. for $C_{23}H_{21}Cl_2N_{11}O_4S_2$: C 42.47, H 3.25, N 23.69; Found: C 42.57, H 3.26, N 23.85%.

Antioxidant assays. The compounds **8-13** were evaluated for antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl,[36, 37] nitric oxide[38, 39] and hydrogen peroxide[40] methods at three 50,75 and 100 µg/mL concentrations.[33]

Acknowledgements. One of the authors M. Madhu Sekhar grateful to University Grants Commission (UGC), New Delhi, for the sanction of UGC-SRF fellowship. The authors are also thankful to Prof. Ch. Appa Rao, Department of Biochemistry, S. V. University, Tirupati for providing necessary facilities to carry out the antioxidant activity.

REFERENCES AND NOTES

[1] A. A. Al-Amiery, *Med Chem Res* **2012**, *21*, 3204.

[2] G. F. Zha. K. P. Rakesh, H. M. Manukumar, C. S. Shantharam, S. Long, *Eur J Med Chem* **2019**, *162*, 465.

[3] A. C. Sauer, J. G. Leal, S. T. Stefanello, M. T. B. Leite, M. B. Souza, F. A. A. Soares, O. E. D. Rodrigues, L. Dornelles, *Tetrahedron Lett* **2017**, *58*, 87.

[4] G. Yamini, M. Madhusekhar, G. Sravya, V. Padmavathi, A. Padmaja, *Monatsh Chem* **2018**, *149* (12), 2311.

[5] J. -J. Zhao, X. -F. Wang, B. -L. Li, R. -L. Zhang, B. Li, Y. -M. Liu, C. -W. Li, J. -B. Liu, B. -Q. Chen, *Bioorg Med Chem Lett* **2016**, *26*, 4414.

[6] S. Liras, M. P. Allen, B. E. Segelstein, *Synth Commun* **2000**, *30*, 437.

[7] S. L. Gaonkar, K. M. L. Rai, B. Prabhuswamy, *Eur J Med Chem* **2006**, *41*, 841.

[8] K. M. Khan, Z. Ullah, M. Rani, S. Perveen, S. M. Haider, M. I. Choudhary, A. Rahman, W. Voelter, *Lett Org Chem* **2004**, *1*, 50.

[9] T. A. Farghaly, M. A. Abdallah, G. S. Masaret, Z. A. Muhammad, *Eur J Med Chem* **2015**, *97*, 320.

[10] M. Zarei, *Tetrahedron* **2017**, *73*(14), 1867.

[11] E. Palaska, G. Sahin, P. Kelicen, N. T. Durlu, G. Altinok, *Il Farmaco* **2002**, *57*, 101.

[12] L. I. Lutwick, M. W. Rytel, J. P. Yanez, J. N. Galgiani, D. A. Stevens, *J Am Med Assoc* **1979**, *241*, 272.

[13] (a) S. Haider, M. S. Alam, H. Hamid, *Eur J Med Chem* **2015**, *92*, 156. (b) F. A. A. El-Hag, S. M. Gomha, T. A. Farghaley, *J Heterocyclic Chem* **2017**, *54*(2), 1172.

[14] T. Plech, B. Kapron, A. Paneth, U. Kosikowska, A. Malm, A. Strzelczyk, P. Staczek, L. Swiatek, B. Rajtar, M. Polz-Dacewicz, *Eur J Med Chem* **2015**, *97*, 94.

[15] Q. Y. Sun, J. M. Xu, Y. B. Cao, W. N. Zhang, Q. Y. Wu, D. Z. Zhang, J. Zhang, H. Q. Zhao, Y. Y. Jiang, *Eur J Med Chem* **2007**, *42*, 1226.

[16] A. M. Abdel-Megeed, H. M. Abdel-Rahman, G. S. Alkaramany, M. A. El-Gendy, *Eur J Med Chem* **2009**, *44*(1), 117.

[17] M. Clemons, R. E. Coleman, S. Verma, *Cancer Treat Rev* **2004**, *30*, 325.

[18] M. J. Stocks, D. R. Cheshire, R. Reynolds, *Org Lett* **2004**, *6*, 2969.

[19] C. A. Bordner, *US Patent June 10*, **1952**, *2*, 600, 689, *Chem Abstr* **1953**, *47*, 4373.

[20] E. E. Van Tamelen, *J Am Chem Soc* **1951**, *73*, 3444.

[21] C. C. Culvenor, W. Davies, W. E. Savige, *J Chem Soc* **1952**, 4480.

[22] C. C. Price, P. F. Kirk, *J Am Chem Soc* **1953**, *75*, 2396.

[23] F. Kiyoshi, T. Senji, *Chem Pharm Bull* **1960**, *8*, 908.

[24] N. Linganna, K. M. LokanathaRai, *Synth Commun* **1998**, *28*, 4611.

[25] G. D. Henry, *Tetrahedron* **2004**, *60*(29), 6043.

[26] C. Ajit, S. N. Pandey, *Asian J Pharm Clin Res* **2011**, *4*, 5.

[27] D. H. Vyas, S. D. Tala, J. D. Akbari, M. F. Dhaduk, K. A. Joshi, H. S. Joshi, *Indian J Chem* **2009**, *48*, 833.

[28] T. Murata, M. Shimada, S. Sakakibara, T. Yoshino, H. Kadono, T. Masuda, M. Shimazaki, T. Shintani, K. Fuchikami, K. Sakai, H. Inbe, K. Takeshita, T. Niki, M. Umeda, K. B. Bacon, K. B. Ziegelbauer, T. B. Lowingera, *Bioorg Med Chem Lett* **2003**, *13*, 913.

[29] A. Rodriguez, W. J. Moran, *J Org Chem* **2016**, *81*, 2543.

[30] M. Rouhani, A. Ramazani, S. W. Joo, *Ultrason Sonochem* **2015**, *22*, 391.

[31] S. Vahid, M. Ali, *Ultrason Sonochem* **2012**, *19*, 300.

[32] G. M. Reddy, A. Muralikrishna, V. Padmavathi, A. Padmaja, T. K. Tilak, C. A. Rao, *Chem Pharm Bull* **2013**, *61*, 1291.

[33] U. Nagarjuna, G. Sravya, V. Padmavathi, A. Padmaja, Med Chem Res 2017, 26, 1574.

[34] M. Madhu Sekhar, U. Nagarjuna, V. Padmavathi, A. Padmaja, N. Vasudeva Reddy, T. Vijaya, *Eur J Med Chem* **2018**, 145, 1-10.

[35] V. Padmavathi, G. Sudhakar Reddy, A. Padmaja, P. Kondaiah, AliShazia, *Eur J Med Chem* 2009, 44, 2106–2112.

[36] M. Burits, F. Bucar, *Phytother Res* **2000**, *14*, 323.

[37] M. Cuendet, K. Hostettmann, O. Potterat, W. Dyatmiko, *Helv Chim Acta* **1997**, *80*, 1144.

[38] L. C. Green, D. A. Wagner, J. Glogowski, P. L. Skipper, J. S. Wishnok, S. R. Tannenbaum, *Anal Biochem* **1982**, *126*, 131.

[39] L. Marcocci, J. J. Maguire, M. T. Droy-Lefaix, L. Packer, *Biochem Biophys Res Commun* **1994**, *201*, 748.

[40] R. J. Ruch, S. J. Cheng, J. E. Klaunig, *Carcinogenesis* **1989**, *10*, 1003.

Fig. 1 *In vitro* antioxidant activity of **8-13** by DPPH method

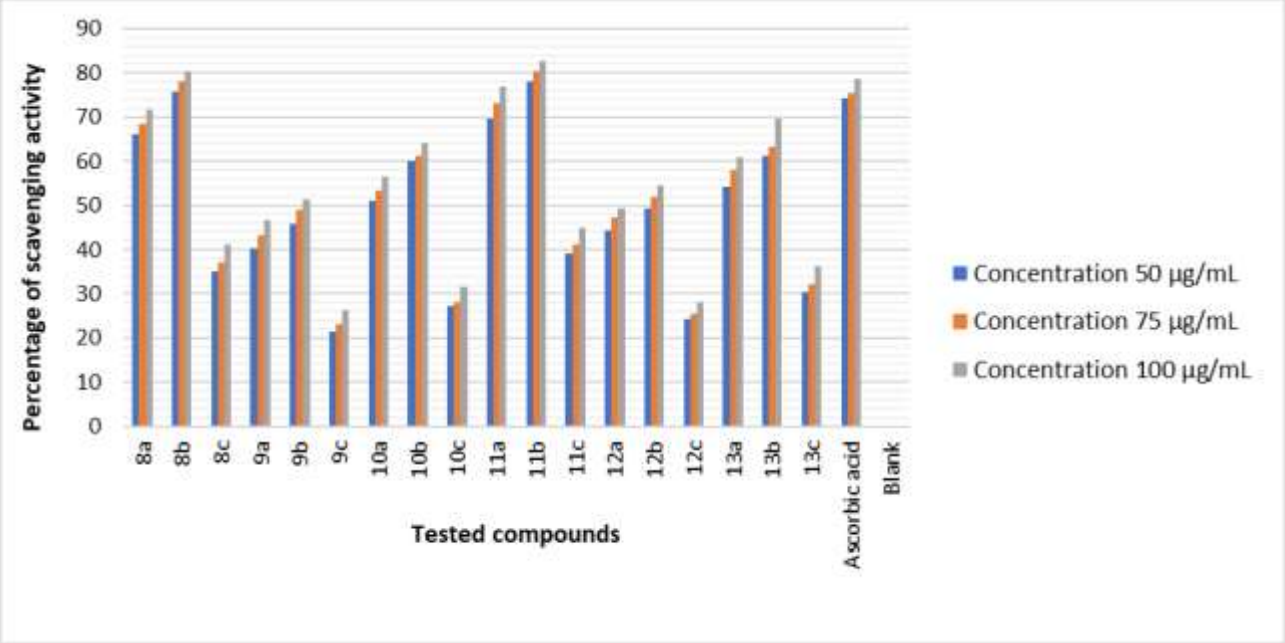


Fig. 2 *In vitro* antioxidant activity of **8-13** by NO method

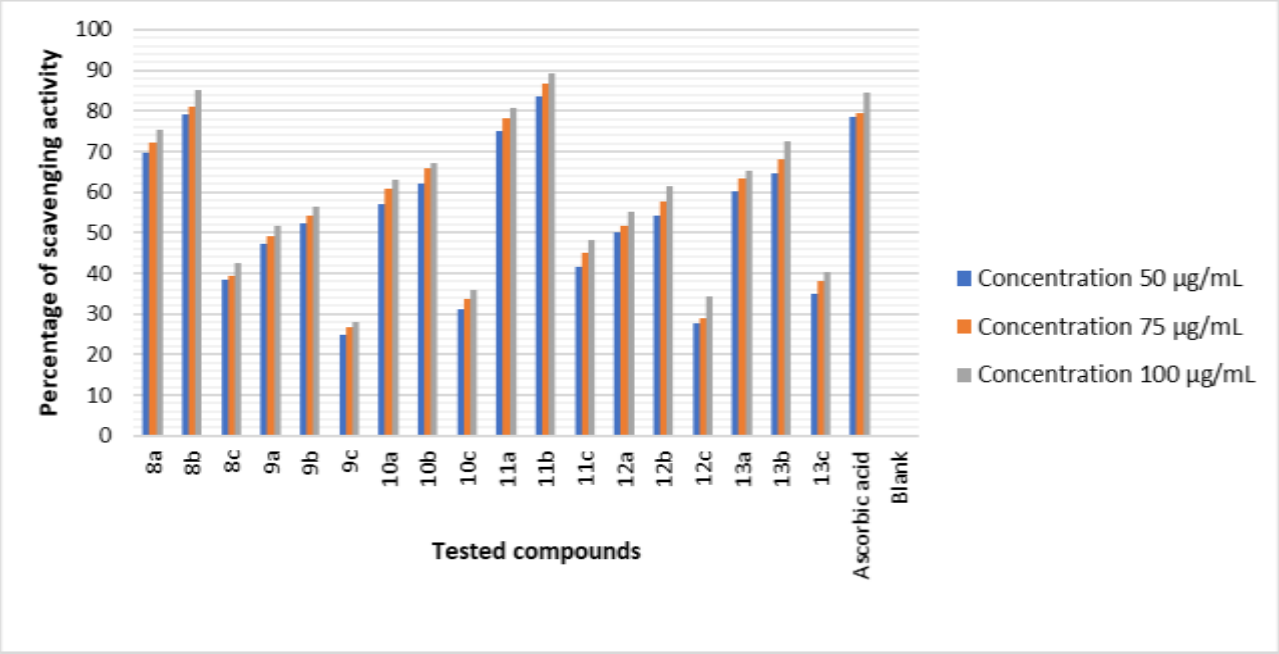


Fig. 3 *In vitro* antioxidant activity of **8-13** by H_2O_2 method

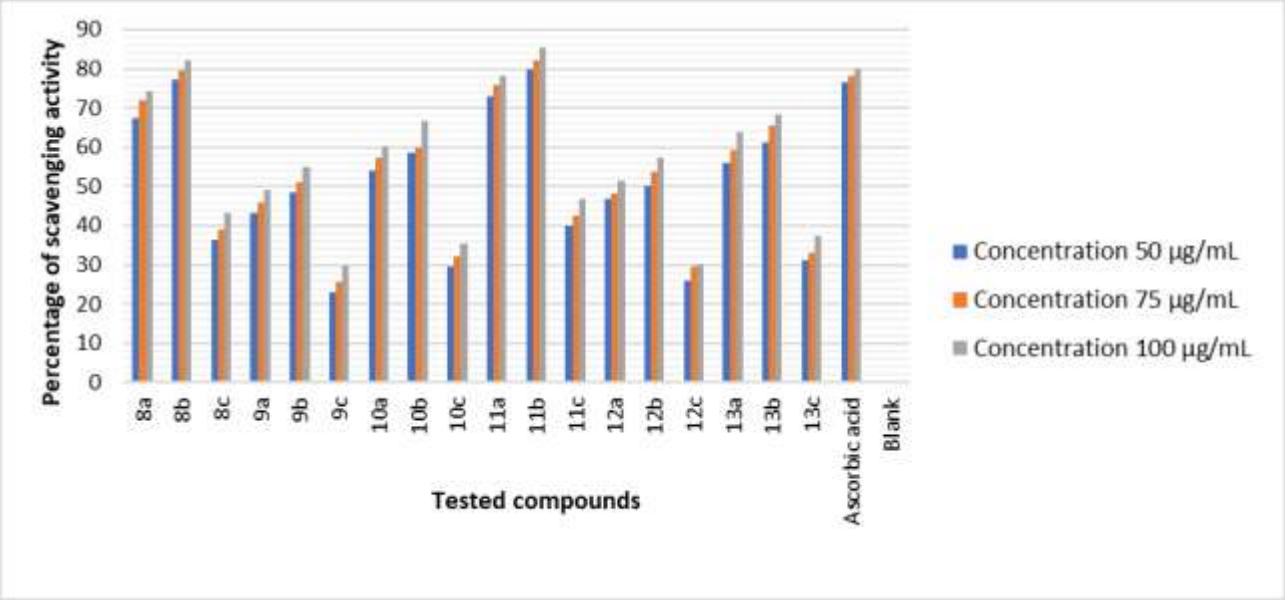


Table 1 *In vitro* antioxidant activity of **8-13** by DPPH method.

Compound	Concentration (μg/mL)			IC_{50} (μg/mL)
	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD	Mean ± SD
8a	66.26 ± 0.17	68.35 ± 0.15	71.56 ± 0.21	37.73 ± 0.06
8b	75.87 ± 0.11	78.09 ± 0.27	80.46 ± 0.28	32.95 ± 0.08
8c	35.19 ± 0.30	37.22 ± 0.18	41.28 ± 0.42	121.12 ± 0.28
9a	40.23 ± 0.21	43.21 ± 0.12	46.77 ± 0.17	106.90 ± 0.14
9b	45.74 ± 0.16	48.98 ± 0.15	51.27 ± 0.13	97.52 ± 0.16
9c	21.30 ± 0.46	23.19 ± 0.38	26.35 ± 0.33	189.75 ± 0.28
10a	50.96 ± 0.42	53.28 ± 0.36	56.43 ± 0.10	49.05 ± 0.04
10b	59.98 ± 0.20	61.16 ± 0.23	64.04 ± 0.12	41.68 ± 0.06
10c	27.15 ± 0.25	28.11 ± 0.25	31.54 ± 0.40	158.52 ± 0.25
11a	69.50 ± 0.34	73.06 ± 0.28	76.93 ± 0.29	35.97 ± 0.22
11b	78.08 ± 0.21	80.45 ± 0.17	82.65 ± 0.14	32.01 ± 0.10
11c	39.11 ± 0.23	41.06 ± 0.16	44.89 ± 0.13	111.38 ± 0.21
12a	44.43 ± 0.38	47.19 ± 0.35	49.27 ± 0.28	101.48 ± 0.27
12b	49.37 ± 0.26	52.04 ± 0.29	54.50 ± 0.07	50.63 ± 0.05
12c	24.38 ± 0.18	25.36 ± 0.14	28.10 ± 0.05	177.93 ± 0.02
13a	54.13 ± 0.12	58.10 ± 0.23	60.86 ± 0.08	46.18 ± 0.81
13b	61.18 ± 0.25	63.29 ± 0.09	69.74 ± 0.24	40.86 ± 0.11
13c	30.33 ± 0.44	32.10 ± 0.21	36.29 ± 0.16	137.89 ± 0.37
Ascorbic acid	74.19 ± 0.19	75.36 ± 0.16	78.62 ± 0.15	33.69 ± 0.07
Blank	-	-	-	-

(-) No activity, (±) SD

Table 2 *In vitro* antioxidant activity of **8-13** by NO method.

Compound	Concentration (μg/mL)			IC_{50} (μg/mL)
	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD	Mean ± SD
8a	69.66 ± 0.14	72.25 ± 0.12	75.20 ± 0.06	35.88 ±0.23
8b	79.01 ± 0.09	81.18 ± 0.04	85.29 ± 0.07	31.64 ±0.31
8c	38.29 ± 0.21	39.25 ± 0.19	42.47 ± 0.22	117.73 ±0.51
9a	47.42 ± 0.18	49.15 ± 0.24	51.55 ± 0.15	76.29 ± 0.17
9b	52.19 ± 0.14	54.26 ± 0.10	56.49 ± 0.04	47.90 ± 0.63
9c	24.89 ± 0.29	26.87 ± 0.30	28.11 ± 0.29	177.87 ± 0.61
10a	57.12 ± 0.31	60.80 ± 0.28	63.07 ± 0.18	43.76 ± 0.65
10b	62.11 ± 0.22	65.74 ± 0.17	67.13 ± 0.07	40.25 ± 0.45
10c	31.24 ± 0.15	33.68 ± 0.12	36.05 ± 0.11	138.69 ± 0.24
11a	75.16 ± 0.26	78.07 ± 0.22	80.59 ± 0.21	33.26 ± 0.46
11b	83.60 ± 0.27	86.63 ± 0.31	89.36 ± 0.19	29.90 ± 0.97
11c	41.72 ± 0.18	45.15 ± 0.15	48.23 ± 0.08	103.66 ± 0.80
12a	50.11 ± 0.22	51.79 ± 0.27	55.25 ± 0.27	49.89 ± 0.69
12b	54.33 ± 0.29	57.77 ± 0.34	61.41 ± 0.29	46.01 ± 0.57
12c	27.71 ± 0.13	28.83 ± 0.08	34.21 ± 0.05	146.15 ± 0.40
13a	60.11 ± 0.09	63.33 ± 0.11	65.21 ± 0.07	41.59 ± 0.87
13b	64.77 ± 0.10	68.20 ± 0.09	72.46 ± 0.12	38.59 ± 0.34
13c	35.10 ± 0.26	38.23 ± 0.21	40.45 ± 0.15	123.60 ± 0.43
Ascorbic acid	78.43 ± 0.16	79.57 ± 0.12	84.39 ± 0.13	31.87 ± 0.84
Blank	-	-	-	-

(-) No activity, (±) SD

Table 3 *In vitro* antioxidant activity of **8-13** by H_2O_2 method.

Compound	Concentration (μg/mL)			IC_{50} (μg/mL)
	50 Mean ± SD	75 Mean ± SD	100 Mean ± SD	Mean ± SD
8a	67.59 ± 0.21	71.92 ± 0.25	74.13 ± 0.31	36.98 ± 0.30
8b	77.11 ± 0.28	79.37 ± 0.18	82.12 ± 0.22	32.42 ± 0.41
8c	36.25 ± 0.30	39.00 ± 0.05	43.30 ± 0.31	115.47±0.54
9a	43.17 ± 0.23	45.85 ± 0.06	49.22 ± 0.38	101.58 ± 0.45
9b	48.32 ± 0.10	51.25 ± 0.21	55.09 ± 0.07	73.17 ± 0.28
9c	23.13 ± 0.21	25.58 ± 0.10	29.97 ± 0.32	166.83 ± 0.51
10a	54.13 ± 0.34	57.32 ± 0.09	60.15 ± 0.11	46.18 ± 0.69
10b	58.60 ± 0.21	60.03 ± 0.16	66.93 ± 0.07	42.66 ± 0.14
10c	29.63 ± 0.15	32.15 ± 0.13	35.44 ± 0.49	141.08 ± 0.90
11a	73.13 ± 0.08	76.08 ± 0.04	78.26 ± 0.30	34.18 ± 0.84
11b	79.76 ± 0.21	82.15 ± 0.14	85.56 ± 0.23	31.34 ± 0.78
11c	40.03 ± 0.27	42.50 ± 0.15	46.98 ± 0.10	106.42 ± 0.60
12a	46.76 ± 0.22	48.20 ± 0.22	51.32 ± 0.26	97.42 ± 0.96
12b	50.08 ± 0.07	53.75 ± 0.23	57.22 ± 0.08	49.92 ± 0.72
12c	25.84 ± 0.23	29.58 ± 0.08	30.34 ± 0.09	164.79 ± 0.66
13a	56.08 ± 0.31	59.28 ± 0.11	63.77 ± 0.37	44.57 ± 0.62
13b	61.31 ± 0.29	65.36 ± 0.04	68.47 ± 0.40	40.77 ± 0.35
13c	31.27 ± 0.16	33.15 ± 0.21	37.38 ± 0.31	133.76 ± 0.55
Ascorbic acid	76.42 ± 0.08	78.10 ± 0.30	80.29 ± 0.19	32.71 ± 0.21
Blank	-	-	-	-

(-) No activity, (±) SD