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Free-radical scavenging and α-glucosidase inhibitory activities of pyrazoline and pyrazole heterocyclic compounds

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A total of fifty pyrazolines **3a-y** and pyrazoles **4a-y** have been evaluated for their free-radical scavenging and α-glucosidase inhibitory activities. The pyrazolines **3e**, **3h**, **3p**, **3s** and pyrazole **4e** have been identified as potent ABTS.$^+$ free-radical scavengers. The thiophene substituted pyrazoline **3s** has shown potent ABTS.$^+$ free radical scavenging activity compared to thiophene substituted pyrazole **4s**. Furanyl (**4r**), pyrazolyl (**4t**), 2-chloropyridinyl (**4u-w**), 2-chloroquinolinyl (**4x**) and 4-chlorocoumarin (**4y**) pyrazoles have displayed moderate ABTS.$^+$ free-radical scavenging activity. Pyrazoline **3i** and pyrazole **4b** have shown moderate DPPH free-radical scavenging activity.

Keywords: Pyrazolines, pyrazoles, free-radical scavenging, α-glucosidase inhibitory activity

Heterocycles are promising compounds known for displaying a variety of biological properties[1]. Pyrazolines are biologically important nitrogen heterocycles[2]. Ampyrone, phenazone and propyphenzone are the pyrazoline drug molecules. The pyrazolines were prepared by the cyclization of chalcones with hydrazine and its derivatives[2]. On the other hand; pyrazoles are an important heterocyclic compounds[3] plays a key role in the pharmaceutical and agrochemical industries. Celecoxib, Lonazolac, Viagra, Ruxolitinib, Ipazilide and Fezolamine are well known pyrazole drug molecules[3]. The synthetic methods for the preparation of pyrazoles were well documented in the literature[4-6]. The other way is to prepare the pyrazoles are by the oxidation of pyrazolines with oxidizing agents[7,8]. As part of our ongoing research, we have prepared chromeno-pyrazolone heterocyclic compounds and studied their biological properties[9]. Recently, we have reported Regio- and Stereoselective synthesis of *N*-alkenylpyrazoles and chromenopyrazoles[10]. The present manuscript describes the preparation of pyrazolines, pyrazoles including heterocycles such as furanyl, thiophene, pyrazolyl, 2-chloropyridinyl, 2-chloroquinolinyl and 4-chlorocoumarin pyrazolines and pyrazoles and studied their biological properties (free-radical scavenging and α-glucosidase inhibitory).

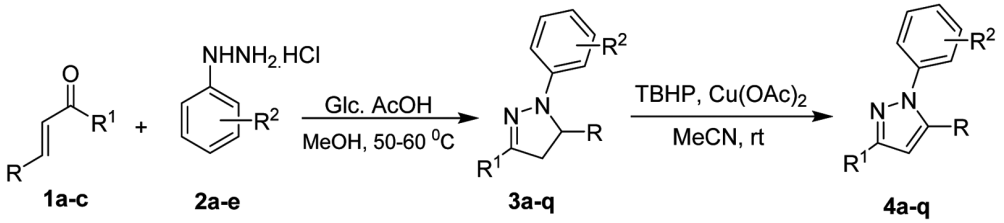
Results and Discussion

The pyrazolines and pyrazoles were prepared as depicted in Scheme I as per our earlier reported method[8]. The pyrazolines **3a-q** were prepared by reacting pent-3-en-2-one **1a**, 4-phenylbut-3-en-2-one **1b** and chalcone **1c** with phenylhydrazine hydrochlorides **2a-e** in dry methanol in the presence of glacial acetic acid at 50-60°C (Table I). Thus obtained pyrazolines **3a-q** (1.0 equiv.) were treated with $Cu(OAc)_2$ (30 mol%) in the presence of TBHP (*tert*-butyl hydroperoxide, 70 wt% in water, 5.0 equiv.) in acetonitrile at RT[8]. All these reactions were provided the corresponding pyrazoles **4a-q** (Table II).

Further, furanyl (**3r**), thiophene (**3s**), pyrazolyl (**3t**), 2-chloropyridinyl (**3u-w**), 2-chloroquinolinyl (**3x**) and 4-chlorocoumarin (**3y**) pyrazolines were prepared by reacting corresponding carboxaldehydes with phenylhydrazine hydrochlorides **2a-c** (Table II)[8]. Thus obtained pyrazoline compounds **3s-y** (1.0 equiv.) were subjected for oxidation under above conditions afforded the corresponding pyrazoles **4s-y** (Table II)[8].

Biology

All the prepared pyrazolines **3a-y** and pyrazoles **4a-y** were evaluated for their free- radical scavenging



Scheme I

Table I — The pyrazolines **3a-y**

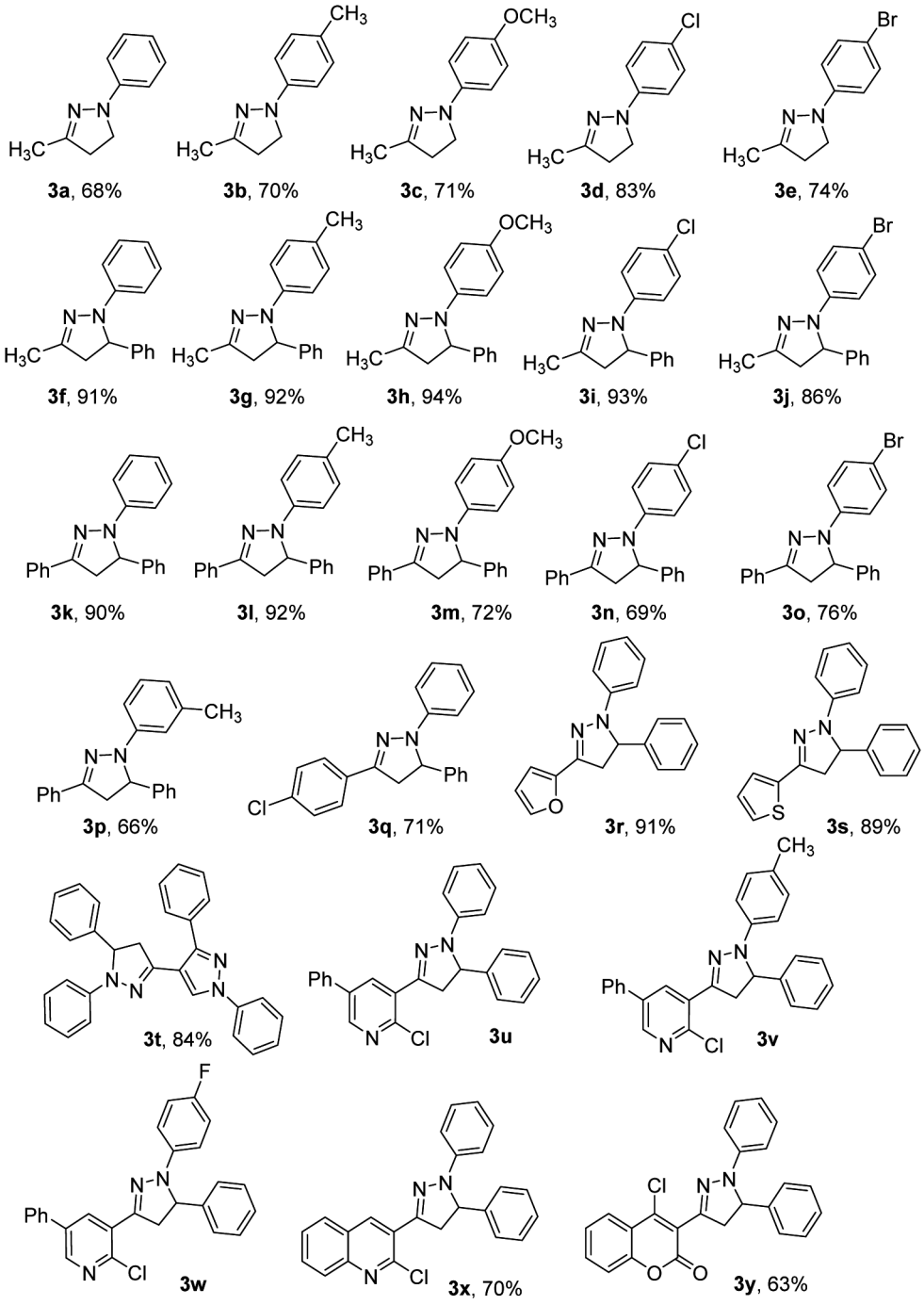
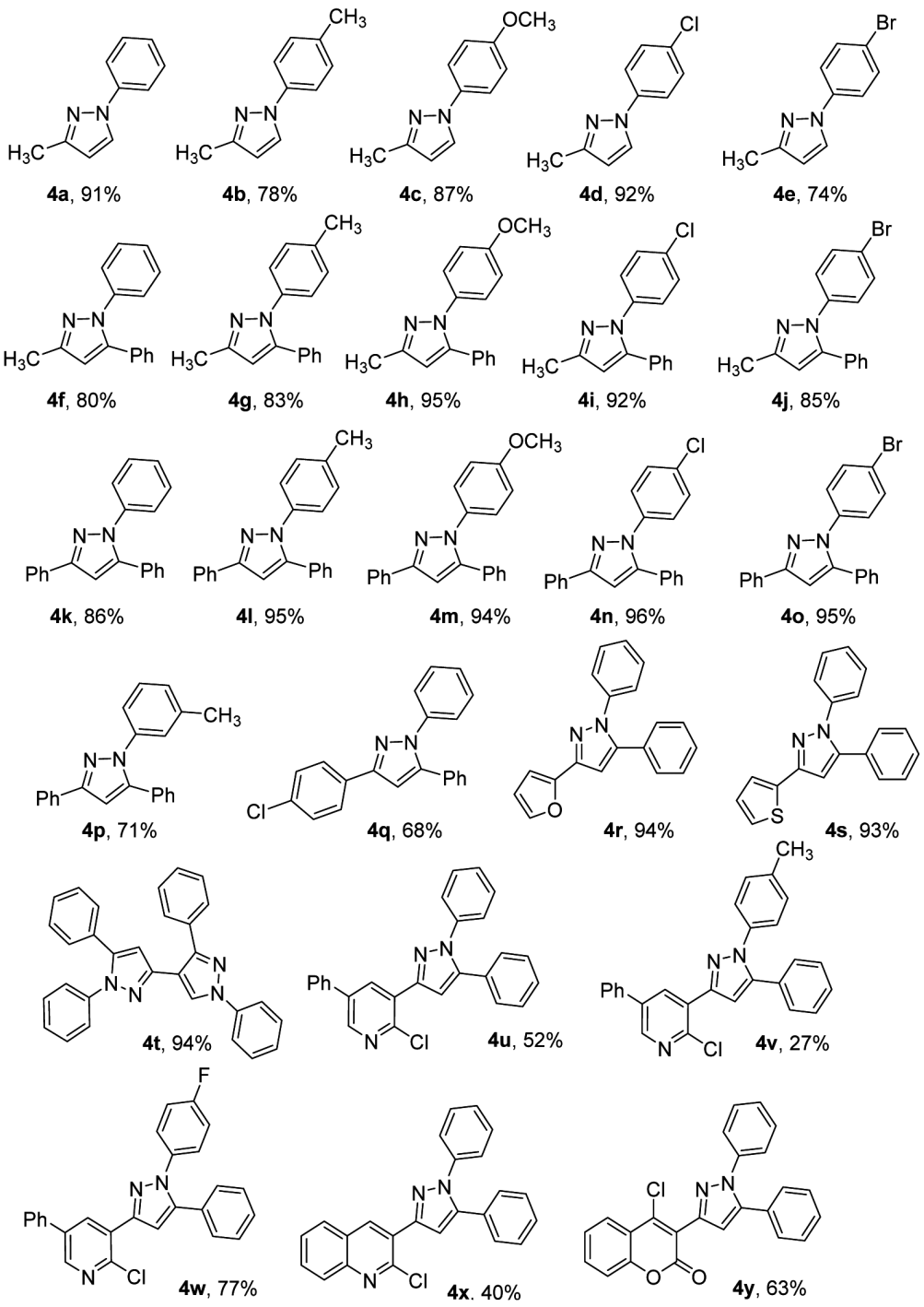


Table II — The pyrazoles **4a-y**



and α-glucosidase inhibitory activities and compared with the standard drugs (Table III and Table IV). The results are described below.

Free Radical Scavenging Activity

The $ABTS^{\cdot+}$ and DPPH free-radical scavenging activity of pyrazoline and pyrazole heterocyclic compounds were presented in Table III and Table IV along with the standard drugs Ascorbic acid and Trolox. The 3-methyl 4-bromophenyl pyrazoline **3e** (95.05) and 4-methoxyphenyl 5-methyl-3-phenyl pyrazoline **3h** (98.67), 3,5-diphenyl *m*-tolyl pyrazoline **3p** (92.39) and 1,5-diphenyl 3-thiophenyl

pyrazoline **3s** (92.39) were identified as potent $ABTS^{\cdot+}$ free-radical scavengers in comparison with standard drug Ascorbic acid (99.87). The pyrazolines **3b**, **3g**, **3i-j** and furyl **3r**, pyrazolyl **3t**, and 4-chlorocoumarin **3y** pyrazolines were displayed moderate $ABTS^{\cdot+}$ free-radical scavenging activity. Only one chloro substituted pyrazoline **3i** was shown moderate DPPH free-radical scavenging inhibitory activity (65.80) in present series of the compounds compared to Trolox (85.42).

The structure activity relationships of the pyrazolines revealed that the compound **3e** with bromo substitution displayed potent $ABTS^{\cdot+}$ free-radical scavenging activity and noticed that the corresponding chloro **3d** compound could not detect $ABTS^{\cdot+}$ activity. Similarly, methyl substituted pyrazoline **3b** shown mild activity compared to methoxy **3c** substitution. Interestingly, 4-methoxyphenyl pyrazoline **3h** displayed potent $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity when compared to compound **3c**. 4-Methylphenyl pyrazoline **3g**, 4-chloro/bromophenyl pyrazolines **3i-j** were shown mild $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. The compounds **3k-o** did not shown the activity when the phenyl group was present at 3rd and 5th position. However, when methyl group present on phenyl ring **3p** was shown potent $ABTS^{\cdot+}$ free-radical scavenging activity.

In case of heterocyclic moieties present at 3rd position of pyrazolines **3r-y**; the thiophene pyrazoline **3s** was shown potent $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. Furanyl **3r**, pyrazolyl **3t** and 4-chlorocoumarin **3y** shown moderate $ABTS^{\cdot+}$ free-radical scavenging activity. However, 2-chloropyridinyl **3u-w** and 2-chloroquinolinyl **3x** pyrazolines did not shown $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity.

The 3-methyl 4-bromophenylpyrazole **4e** was identified as potent $ABTS^{\cdot+}$ free-radical scavenger in comparison with standard drug Ascorbic acid. 3-Methyl/phenyl 4-methoxypyrazoles **4c, 4h** were

Table III — DPPH, $ABTS^{\cdot+}$ and α-glucosidase inhibitory activity of pyrazolines **3a-y**

Compd	$ABTS^{\cdot+}$ % Inhibition (20 µg /mL)	DPPH % Inhibition (25 µg/mL)	α-GI % Inhibition (20 µg/mL)
3a	44.22 ± 1.56	23.42 ± 0.42	23.88 ± 0.23
3b	58.69 ± 1.45	47.21 ± 0.11	ND
3c	ND	9.66 ± 0.01	ND
3d	ND	ND	ND
3e	95.05 ± 0.87	ND	ND
3f	ND	ND	ND
3g	56.60 ±1.88	29.57 ± 0.11	ND
3h	98.67 ± 1.54	17.33 ± 0.42	ND
3i	64.02 ± 0.84	65.80 ± 2.12	ND
3j	72.21 ± 0.94	13.41 ± 0.12	10.69 ± 0.11
3k	ND	ND	ND
3l	ND	ND	ND
3m	ND	27.64 ± 0.88	ND
3n	ND	43.55 ± 0.54	ND
3o	ND	36.08 ± 0.89	ND
3p	92.39 ± 1.59	7.06± 0.12	ND
3q	ND	10.57 ± 0.22	ND
3r	73.35 ± 2.98	19.46 ± 0.21	ND
3s	92.39 ± 1.44	ND	30.34 ± 0.54
3t	69.03 ± 0.68	ND	38.79 ± 0.80
3u	ND	26.12 ± 0.10	43.10 ± 0.05
3v	ND	ND	28.78 ± 0.01
3w	ND	ND	25.00 ± 0.50
3x	ND	ND	46.90 ± 0.61
3y	69.03 ± 0.35	ND	ND
Ascorbic acid	99.87 ± 0.45	–	–
Trolox	–	85.42 ± 0.82	–
Acarbose	–	–	64.22 ± 1.89

GI = α-Glucosidase; ND = Not detected

Table IV — DPPH, $ABTS^{\cdot+}$ and α-glucosidase inhibitory activity of pyrazoles **4a-y**

Compd	ABTS.+ % Inhibition (20 µg /mL)	DPPH % Inhibition (25 µg/mL)	α-GI % Inhibition (20 µg/mL)
4a	33.56 ± 0.54	ND	ND
4b	ND	71.04 ± 0.98	ND
4c	68.15 ± 0.94	3.66 ± 0.01	ND
4d	3.93 ± 0.88	ND	ND
4e	97.14 ± 3.54	9.71 ± 0.12	ND
4f	16.81 ± 0.12	26.05 ± 0.33	15.52 ± 0.22
4g	ND	10.37 ± 1.23	ND
4h	87.44 ± 2.54	13.01 ± 0.55	ND
4i	8.61 ± 0.89	ND	32.50 ± 0.21
4j	ND	ND	27.33 ± 0.15
4k	ND	8.23 ± 0.14	ND
4l	ND	ND	ND
4m	ND	ND	ND
4n	72.72 ± 2.88	ND	ND
4o	15.10 ± 0.12	ND	30.43 ± 0.44
4p	ND	ND	9.14 ± 0.11
4q	ND	ND	6.38 ± 0.12
4r	72.21 ± 2.12	ND	ND
4s	ND	ND	ND
4t	84.52 ± 3.12	ND	10.52 ± 0.01
4u	65.23 ± 1.04	ND	ND
4v	67.89 ± 0.48	ND	ND
4w	63.20 ± 0.56	ND	ND
4x	67.77 ± 0.94	ND	ND
4y	84.39 ± 0.88	ND	ND
Ascorbic acid	99.87 ± 0.45	–	–
Trolox	–	85.42 ± 0.82	–
Acarbose	–	–	64.22 ± 1.89

GI = α-Glucosidase; ND = Not detected

shown moderate $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. However, methyl and halo substituted pyrazoles **4b**, **4g**, **4d-e**, **4i-j** did not shown $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. Interestingly, 3,5-diphenyl substituted 4-chlorophenylpyrazole **4n** displayed moderate $ABTS^{\cdot+}$ free-radical scavenging activity. The heterocyclic furanyl **4r**, pyrazolyl **4t**, 2-chloropyridinyl **u-w**, 2-chloroquinolinyl **4x** and 4-chlorocoumarin **4y** pyrazolines were shown moderate $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. However, thiophene pyrazole **4s** could not detect the $ABTS^{\cdot+}$ free-radical scavenging activity. Only one pyrazole **4b** was shown moderate DPPH free-radical scavenging inhibitory activity (71.04) in present series of the compounds compared to Trolox (85.42).

Over all, four pyrazolines **3e**, **3h**, **3p**, **3s** and one pyrazole **4e** identified as potent $ABTS^{\cdot+}$ free-radical scavengers. Seven pyrazolines and ten pyrazoles were displayed mild to moderate $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity. One pyrazoline **3i** and pyrazole **4b** were displayed moderate DPPH free-radical scavenging inhibitory activity. Its noteworthy that bromo substituted pyrazoline **3e** and pyrazole **4e** shown potent $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity.

α-Glucosidase inhibitory activity

α-Glucosidase activity of pyrazolines **3a-y**, pyrazoles **4a-y** and their inhibitory values are presented in Table III and Table IV along with the standard drug Acarbose. The pyrazolines **3a-3r**, **3y** could not display α-glucosidase inhibitory activity (Table III), whereas compounds **3s-x** showed mild to moderate α-glucosidase inhibitory activity. The pyrazoles **4i-j** and **4o** showed mild α-glucosidase inhibitory activity.

Experimental Section

All the chemicals and reagents were purchased from Aldrich (Sigma-Aldrich, USA), AVRA Chemicals Pvt. Ltd (Hyderabad, India) and were used without further purification. Reactions were monitored by thin layer chromatography (TLC) on pre-coated silica gel 60 F254 (mesh); spots were visualized under UV light. Melting points were determined on a Stuart melting point apparatus and are uncorrected. IR spectrum was recorded with a Thermo Nicolet Nexus 670 FT spectrometer. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 300, 400 and 500 MHz spectrometers. Chemical shifts (δ) are quoted in parts per million and are referenced to tetramethylsilane (TMS) as internal standard. ESI-MS obtained on quarto micro spectrometer.

DPPH free-radical scavenging assay

Assay for the scavenging of stable free-radical based on DPPH [1,1-diphenyl-2-picrylhydrazyl] was done as reported earlier was performed[11]. Briefly, in a 96-well micro plate, 25 µL of test sample dissolved in DMSO (1 mg/mL), 125 µL of 0.1 M tris-HCl buffer (*p*H 7.4) and 125 µL of 0.5 mM DPPH solution dissolved in absolute ethyl alcohol were added. The reaction mixture was shaken well and incubated in dark condition for 30 min and read at 517 nm spectrophotometrically (Spectra Max plus384, Molecular Devices Corporation, Sunnyvale, CA, USA). Percentage of DPPH scavenging was calculated as (1-B/ A) × 100 where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples.

$ABTS^{\cdot+}$ free-radical scavenging assay

Scavenging of the $ABTS^{+}$ [2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)] was performed[11]. Briefly, 100 mL stock solution of $ABTS^{+}$ (0.5 mM) was prepared by addition of 1 mL potassium persulfate (6.89 mM PBS, *p*H 8.0). The mixture was stored in the dark for 16 h. Test compounds were dissolved in DMSO (5mg/mL). Primary screening was done by mixing 10 µL of test compound in 100 µL of methanol followed by 190 µL of $ABTS^{+}$ in a 96-well microplate. Absorbance of decolorized $ABTS^{+}$ was measured at 734 nm after 15 min incubation in the dark on a BioTek ^{synergy4} multi-mode microplate reader. For each test sample a separate blank sample (devoid of $ABTS^{+}$) was used for background subtraction. The percentage of $ABTS^{+}$ scavenging was calculated applying following formula;

$$\%\ ABTS^{+}\ \text{scavenging} = [(Absorbance_{control}\text{-}Absorbance_{test})/Absorbance_{control} \times 100].$$

Anti-hyperglycemic assay

α-Glucosidase inhibitory activity was determined as per earlier reported method[11]. Rat intestinal acetone powder in normal saline (100:1; w/v) was sonicated properly and the supernatant was used as a source of crude intestinal α-glucosidase after centrifugation. In brief, 20 µL of test samples (5 mg/mL DMSO solution) were reconstituted in 100 µL of 100 mM-phosphate buffer (*p*H 6.8) in 96-well microplate and incubated with 50 µL of crude intestinal

α-glucosidase for 5 min before 50 µL substrate (5 mM, *p*-nitrophenyl- α-D-glucopyranoside prepared in same buffer) was added. Release of *p*-nitrophenol was measured at 405 nm spectrophotometrically (Spectra Max plus 384), Molecular Devices Corporation, Sunnyvale, CA, USA) 5 min after incubation with substrate. Individual blanks for test samples were prepared to correct background absorbance where substrate was replaced with 50 µL of buffer. Control sample contained 10 µL DMSO in place of test samples. Percentage of enzyme inhibition was calculated as (1-B/A) × 100 where 'A' represents absorbance of control without test samples and 'B' represents absorbance in presence of test samples. For calculation of 50% enzyme inhibitory activity (IC50%) more than five dilutions of primary screening concentration (5 mg/mL DMSO solution) of test compounds were prepared.

Conclusions

In conclusion, total fifty pyrazolines, pyrazoles including heteroaryl pyrazolines and pyrazoles were evaluated for free-radical scavenging (DPPH, $ABTS^{\cdot+}$) and α-glucosidase inhibitory activities. Pyrazolines **3e**, **3h**, **3p**, **3s** and pyrazole **4e** were identified as potent $ABTS^{\cdot+}$ free-radical scavengers. Bromo substituted pyrazoline **3e** and pyrazole **4e** shown potent $ABTS^{\cdot+}$ free-radical scavenging inhibitory activity.

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