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ORIGINAL ARTICLE

Synthesis and evaluation of antioxidant activity of novel 3,5-disubstituted-2-pyrazolines

Akshay Kumar [a], Bhat G. Varadaraj [a], Rajeev K. Singla [b,*]

[a] *Department of Pharmaceutical Chemistry, Manipal College of Pharmaceutical Sciences, Manipal University, Manipal 576104, Karnataka, India*
[b] *Division of Biotechnology, Netaji Subhas Institute of Technology, Azad Hind Fauz Marg, Sector-3, Dwarka, 110078 New Delhi, India*

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KEYWORDS

3,5-Disubstituted-2-pyrazoline;
Antioxidant;
Reactive oxygen species

Abstract Driven by the increased demand of pyrazolines exhibiting biological activities like antioxidant, anti-inflammatory, antidepressant, antimicrobial, antitumor, and antitubercular drug activity as a stable fragment in biological moieties, lead us to synthesize 2-pyrazolines by the condensation of various substituted chalcones and hydrazine hydrate in the presence of ethanol. The structure of the synthesized molecules was confirmed on the basis of physical data and extensive spectral studies. All the 13 compounds have been screened for antioxidant activity using DPPH radical scavenging method, NO scavenging assay, superoxide radical scavenging assay and hydrogen peroxide radical scavenging assay. All the compounds showed good free radical scavenging activity which is comparable to that of the standard ascorbic acid, out of which ATP-1, ATP-2 and ATP-3 come out to be the best molecules with an IC50 less than 40 mcg/ml. The results indicated that 2-pyrazolines could be the potential candidates eliciting antioxidant activity, and further studies can be conducted using molecular modeling tools for designing 2-pyrazolines having better activity.

1. Introduction

Small ring heterocycles containing nitrogen, sulfur and oxygen have been under investigation for a long time because of their important medicinal properties.[1] Also, 2-pyrazolines have been reported to possess a variety of significant and diverse pharmacological activities such as antibacterial,[2–4] antifungal,[5,6] antiviral,[7] antitubercular,[8,9] antidepressant,[10,11] antiamoebic,[12,13] anti-inflammatory,[14] anticonvulsant,[15] analgesic,[16] anticancer[17] and antioxidant activities.

The reactive oxygen species, which include superoxide anions (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH), and Lipid peroxidation, which involves a series of free radical mediated chain reactions processes, are associated with several types of biological damage. Sodium nitroprusside, a vasodilator drug induces oxidative brain injury, which is actually mediated by the hydroxyl radicals generated by iron

* Corresponding author. Tel.: +91 98186 03719.
E-mail address: rajeevsingla26@gmail.com (R.K. Singla).
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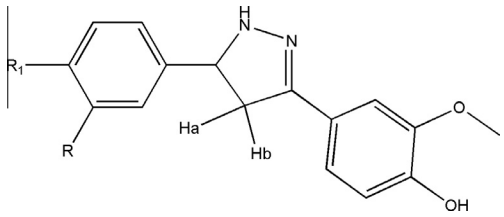


Figure 1 General structure of 3-[(4-hydroxy-3-methoxy)phenyl]-5-phenyl/substituted phenyl-2-pyrazoline derivatives.

element in sodium nitroprusside. Our defense system produces glutathione and other thiols which significantly reduce sodium nitroprusside and other reactive oxygen species. But for the neurodegenerative disorders, supplementation with external antioxidant agents is of need. But unfortunately, the clinically effective antioxidant drugs are scarce. Therefore much attention has been focused on the use of synthetic antioxidants to inhibit lipid peroxidation and to protect from damage due to free radicals.[18]

2-Pyrazolines seem to be the most frequently studied pyrazoline type compounds. After the pioneering work of Fischer and Knövenagel in the late nineteenth century, the reaction of α,β-unsaturated aldehydes and ketones and then with hydrazines became one of the most popular methods for the preparation of 2-pyrazolines. 2-pyrazolines have potential antioxidative effects, capable of preventing oxidative damage as well as clastogenic effects.[19,20] As a result, numerous substituted 2-pyrazolines have been synthesized. In light of these findings, it was felt worthwhile to synthesize some new 2-pyrazoline derivatives and evaluate them for their antioxidant potential.

So the current paper deals with the synthesis of novel 2-pyrazolines (Fig. 1) and statistical analysis of their anti oxidant potential.

2. Materials and Methods

2.1. Drugs and chemicals

Reactants and reagents for the synthesis of molecules (ATP 01–13) and for the evaluation of antioxidant activity were procured from Sigma–Aldrich Ltd. All the drugs and chemicals except the test compounds were dissolved or diluted in distilled water and used for the experimentation purpose. ATP 01–13 were dissolved in 100% DMSO and dilutions were made with distilled water so that the final concentration of DMSO did not exceed (0.1% v/v).

2.2. Instrumentation

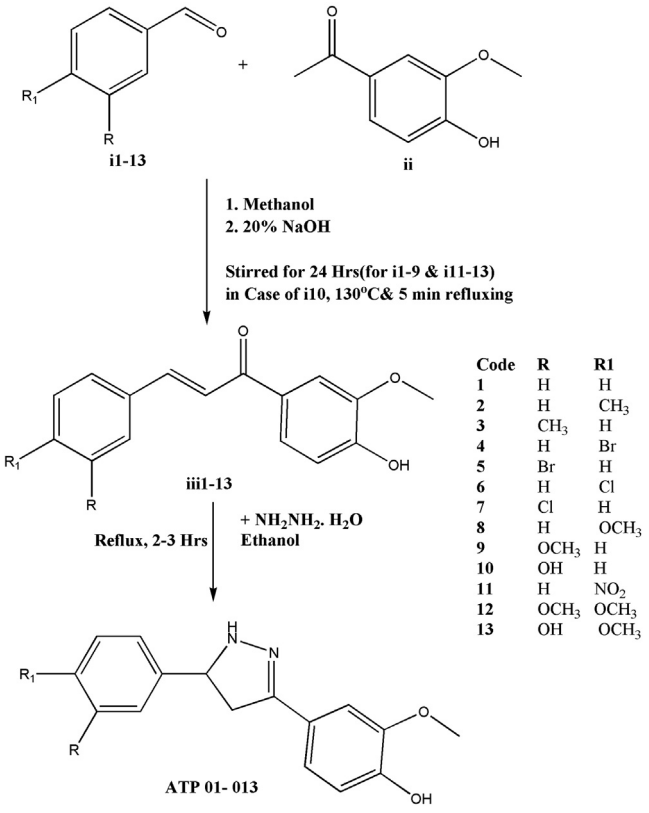
Melting points were determined using open capillary tube in Toshniwal melting point apparatus and are uncorrected. UV spectra were recorded on Shimadzu UV 1650. IR spectra were recorded on Shimadzu FTIR-8310 using KBr pellets. ^{1}H-NMR spectra were recorded on AMX 400 at 200 MHz using tetramethylsilane (TMS) as the internal standard and DMSO as solvent, collected from the Indian Institute of Science(IISc), India. LC-ESI-MS were recorded using Shimadzu LC-ESI-MS at Manipal Accunova, Manipal. Rota evaporator and UV chamber from Servewell Instruments Pvt. Ltd. The chemical shifts were expressed in part per million (ppm) downfield from the internal standard; the coupling constants are in Hz, and signals are quoted as *s* (singlet), *d* (doublet), *t* (triplet), *q* (quartet), or *m* (multiplet). The purity of the compounds was checked by TLC using Merck precoated silica gel GF aluminum plates and ethyl acetate: chloroform (15:85) as solvent system.

2.3. General procedure for synthesis of chalcone derivatives (Fig. 2)

A mixture of 1-(4-hydroxy-3-methoxyphenyl)ethanone (0.01 mol) and sodium hydroxide (20%, 5 ml) in methanol (5–10 ml) was added to the appropriate (un)substituted benzaldehyde (0.01 mol). After complete addition, the reaction mixture was stirred for 24 h at room temperature, with regular monitoring by TLC. This mixture was then poured into crushed ice (300 gm) and was neutralized with dil. HCl, resulting in the precipitation of chalcone. The precipitated solid was then filtered, washed and dried in a vacuum desiccator. Product was recrystallized using ethanol–water gradient. The purity of the each compound was checked by TLC using n-hexane: ethyl acetate (7:3).[21]

2.4. Procedure for synthesis of chalcone from 3-OH benzaldehyde, iii10 (Fig. 2)

A mixture of 1-(4-hydroxy-3-methoxyphenyl)ethanone (0.01 mol) and sodium hydroxide (20%, 5 ml) in methanol (5–10 ml) was added to the appropriate 3-hydroxybenzaldehyde (0.01 mol). After complete addition, the reaction mixture



Code	R	R1
1	H	H
2	H	CH_3
3	CH_3	H
4	H	Br
5	Br	H
6	H	Cl
7	Cl	H
8	H	OCH_3
9	OCH_3	H
10	OH	H
11	H	NO_2
12	OCH_3	OCH_3
13	OH	OCH_3

Figure 2 Schematic synthesis of 3,5-disubstituted-2-pyrazolines.

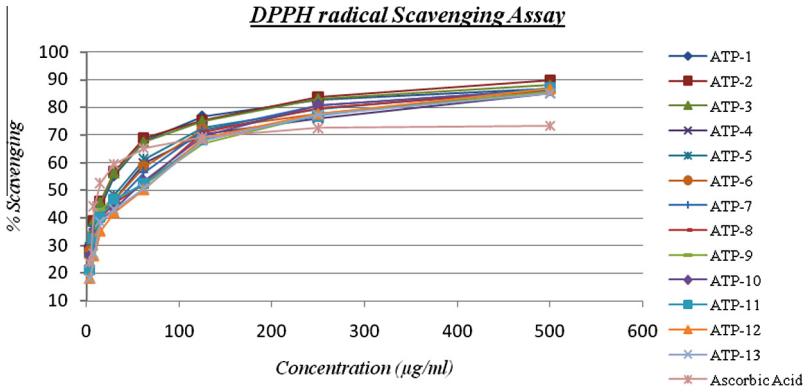


Figure 3 DPPH radical scavenging assay results of novel 3,5-disubstituted-2-pyrazolines.

was refluxed at 130 °C for 5 min. The mixture was then poured into crushed ice (300 ml) and was neutralized with dil. HCl, resulted in the precipitation of (E)-1-(4-hydroxy-3-methoxyphenyl)-3-(3-hydroxyphenyl)prop-2-en-1-one. The precipitated solid was filtered, washed and dried in a vacuum desiccator. Product was recrystallized using ethanol–water gradient. The purity of the each compound was checked by TLC using n-hexane: ethyl acetate (7:3).[22]

2.5. Procedure for synthesis of 3-[(4-hydroxy-3-methoxy) phenyl]-5-phenyl/substituted phenyl-2-pyrazoline derivatives (ATP 01–13) (Fig. 2)

A mixture of chalcone (0.001 mol) and hydrazine hydrate (99–100%, 0.003 mol) in methanol (5–10 ml) was refluxed for 2–3 h, followed by solvent evaporation using rota evaporator to yield solid product. The solid compound thus obtained was then filtered and washed with water followed by methanol. White crystalline powder was obtained. The purity of the compounds (Fig. 3) was checked by TLC using n-hexane:ethyl acetate (9:1).

2.5.1. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-phenyl-2-pyrazoline (ATP-1)

Molecular formula $C_{16}H_{16}N_2O_2$, Mol wt. 268, % yield 64.33, M. Pt. 170, UV (nm): 305.60, 208.60, FT-IR (KBr disc): 3331 (Ar O–H); 3080, 3032 (Ar–H); 2956, 2926, 2879 (C–H); 1645 (C═N); 1599, 1568, 1514, 1456 (Ar C═C); 1203 (Ar C–OH), ^{1}H-NMR: 2.80–2.74 (dd, 1H, CHH_a), 3.40–3.29 (dd, 1H, CH_bH), 3.78 (s, 3H, OCH_3), 4.73 (t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2 (s, 1H, ArOH).

2.5.2. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(4-methylphenyl)-2-pyrazoline (ATP-2)

Molecular formula $C_{17}H_{18}N_2O_2$, Mol wt. 282, % yield 55.50, M. Pt. 149, UV (nm): 306.60, 213.60, FT-IR (KBr disc): 3346.61 (Ar O–H); 3080, 3049 (Ar–H); 2962, 2924, 2879, 2850 (C–H); 1600, 1562, 1510, 1458 (Ar C═C); 1211 (Ar C–OH), ^{1}H-NMR: 2.28 (s, 3H, CH_3), 2.80–2.74 (dd, 1H, CHH_a), 3.40–3.29 (dd, 1H, CH_bH), 3.78 (s, 3H, OCH_3), 4.73 (t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2 (s, 1H, ArOH), 3.6 (d, 3H, Ar–CH_3).

2.5.3. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(3-methylphenyl)-2-pyrazoline (ATP-3)

Molecular formula $C_{17}H_{18}N_2O_2$, Mol wt. 282, % yield 35.33, M. Pt. 123, UV (nm): 306.60, 211.00, FT-IR (KBr disc): 3335 (Ar O–H); 3086, 3051 (Ar–H); 2955, 2922, 2856 (C–H); 1599, 1575, 1514, 1458 (Ar C═C); 1205 (Ar C–OH), ^{1}H-NMR: 2.80–2.74 (dd, 1H, *Jab* = 16.0 Hz, *Jax* = 8.0 Hz, CH_a); 3.40–3.29 (dd, 1H, *Jab* = 16.0 Hz, *Jbx* = 8.0 Hz, CH_b); 3.78 (s, 3H, OCH_3); 4.73 (t, 1H, *J* = 10 Hz CHAr); 6.75 (d, 1H, NH); 7.23–6.95 (m, 7H, Ar); 9.2 (s, 1H, ArOH), MS: $[M^+]$ 282; $[M^+ - CH_3] \rightarrow 267$; $[M^+ - OCH_3] \rightarrow 251$.

2.5.4. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(4-bromophenyl)-2-pyrazoline (ATP-4)

Molecular formula $C_{16}H_{15}BrN_2O_2$, Mol wt. 347, % yield 66.16, M. Pt. 146, UV (nm): 307.60, 208.40, FT-IR (KBr disc): 3340 (Ar O–H); 3097, 3055 (Ar–H); 2953, 2922, 2850 (C–H); 1600, 1573, 1512, 1460 (Ar C═C); 1205 (Ar C–OH); 545 (Ar–C–Br); ^{1}H-NMR: 2.80–2.74 (dd, 1H, CHH_a), 3.40–3.29 (dd, 1H, CH_bH), 3.78 (s, 3H, OCH_3), 4.73 (t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2 (s, 1H, ArOH), 3.9 (s, 1H, Ar–CH–Br).

2.5.5. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(3-bromophenyl)-2-pyrazoline (ATP-5)

Molecular formula $C_{16}H_{15}BrN_2O_2$, Mol wt. 347, % yield 37.66, M. Pt. 120, UV (nm): 306.60, 204.60, FT-IR (KBr disc): 3333 (Ar O–H); 3082, 3057 (Ar–H); 2960, 2924, 2852 (C–H); 1566, 1512, 1456 (Ar C═C); 1205 (Ar C–OH); 550 (Ar–C–Br), ^{1}H-NMR: 2.80–2.74 (dd, 1H, CHH_a), 3.40–3.29 (dd, 1H, CH_bH), 3.78 (s, 3H, OCH_3), 4.73 (t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2 (s, 1H, ArOH), 4.2 (s, 1H, Ar–CH–Br).

2.5.6. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(4-chlorophenyl)-2-pyrazoline (ATP-6)

Molecular formula $C_{16}H_{15}ClN_2O_2$, Mol wt. 302, % yield 68.66, M. Pt. 154, UV (nm): 306.40, 217.40, FT-IR (KBr disc): 3340 (Ar O–H); 2924 (C–H); 1597, 1575, 1512, 1487 (Ar C═CC), ^{1}H-NMR: 2.80–2.74 (dd, 1H, CHH_a), 3.40–3.29 (dd, 1H, CH_bH), 3.78 (s, 3H, OCH_3), 4.73 (t, 1H, CHAr), 6.75 (d, 1H, NH), 7.23–6.95 (m, 7H, Ar), 9.2 (s, 1H, ArOH).

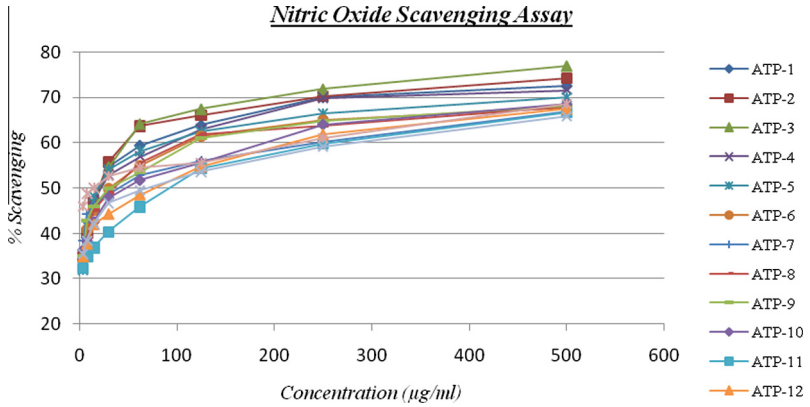


Figure 4 Nitric oxide scavenging assay results of novel 3,5-disubstituted-2-pyrazolines.

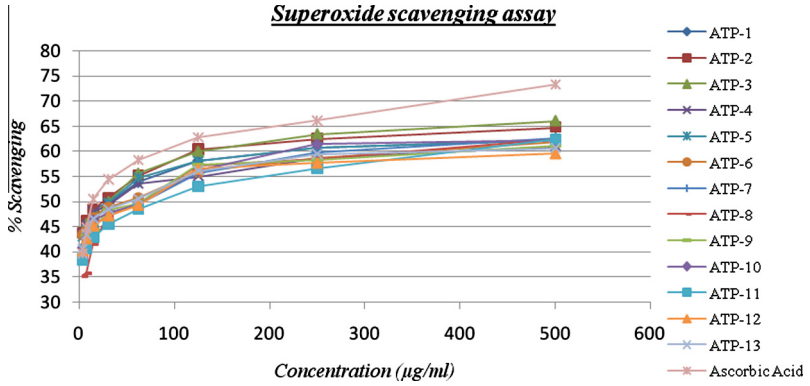


Figure 5 Superoxide scavenging assay results of novel 3,5-disubstituted-2-pyrazolines.

2.5.7. 3-[(4-Hydroxy-3-methoxy) phenyl]-5-(3-chlorophenyl)-2-pyrazoline (ATP-7)

Molecular formula $C_{16}H_{15}ClN_2O_2$, Mol wt. 302, % yield 40.00, M. Pt. 138, UV (nm): 306.00, 211.00, FT-IR (KBr disc): 3331 (Ar O–H); 3080 (Ar–H); 2960, 2926, 2862 (C–H); 1597, 1570, 1512, 1460 (Ar C═CC); 1203 (Ar C–OH); 645 (Ar–C–Cl), MS: $[M^+] \rightarrow 302$; $[M^+ - CH_3] \rightarrow 287$; $[M^+ - OCH_3] \rightarrow 271$.

2.5.8. 3-[(4-Hydroxy-3-methoxy)phenyl]- 5-(4-methoxyphenyl)-2-pyrazoline(ATP-8)

Molecular formula $C_{17}H_{18}N_2O_3$, Mol wt. 298, % yield 81.66, M. Pt. 136, UV (nm): 306.40, 208.40; FT-IR (KBr disc): 3320 (Ar–O–H), 3000, 2870 (C–H), 1600, 1525 (Ar C═CC), 1200 (Ar C–OH), ^{1}H-NMR: 2.7–3.4 (dd, 2H, pyrazoline CH_2), 3.71 (s, 3H, m-OCH_3), 3.78 (s, 3H, p-OCH_3), 4.79 (t, 1H, J = 10.4 Hz, CHAr), 6.5 (d, 1H, NH), 6.8–8.5 (m, 7H, Ar), 9.2 (s, 1H, ArOH).

2.5.9. 3-[(4-Hydroxy-3-methoxy)phenyl]-5-(3-methoxyphenyl)-2-pyrazoline (ATP-9)

Molecular formula $C_{17}H_{18}N_2O_3$, Mol wt. 298, % yield 87.00, M. Pt. 164, UV (nm): 306.80, 203.40; FT-IR (KBr disc): 3325 (Ar–O–H), 2928, 2847 (C–H), 1599, 1514 (Ar C═CC), 1195 (Ar C–OH), ^{1}H-NMR: 2.81–2.74 (dd, 1H, *Jax* = 5.6 Hz, *Jab* = 16 Hz, H_a), 3.41–3.29 (dd, 1H, *Jbx* = 5.6 Hz, *Jab* = 16 Hz, H_b), 3.73 (s, 3H, OCH_3[A]), 3.77 (s, 3H, OCH_3[B]), 4.74 (t, 1H, J = 10.4 Hz, CHAr), 6.75 (d, 1H, NH), 7.25–6.79 (m, 7H, Ar), 9.2 (s, 1H, ArOH); MS: $[M^+] \rightarrow 298$; $[M^+ - CH_3] \rightarrow 283$; $[M^+ - OCH_3] \rightarrow 267$.

2.5.10. 3-[(4-Hydroxy-3-methoxy)phenyl]-5-(3-hydroxyphenyl)-2-pyrazoline (ATP-10)

Molecular formula $C_{16}H_{16}N_2O_3$, Mol wt. 284, % yield 40.33, M. Pt. 222, UV (nm): 306.80, 206.60; FT-IR (KBr disc): 3300 (Ar O–H); 3100, 3050 (Ar–H); 2950, 2906 (C–H); 1670 (C═CN); 1599, 1514, 1456 (Ar C═CC); 1215 (Ar C–OH), 1245 (Asymmetric C–O–C stretch), 1043 (symmetric C–O–C stretch); ^{1}H-NMR: 2.3–3.2 (dd, 2H, Pyrazoline CH_2), 3.54 (s, 3H, m-OCH_3), 4.44 (t, 1H, CH–Ar), 6.2–7.5(m, 7H, Ar–H), 5.8 (d, 1H, NH), 9.1 (s, 1H, m-Ar-OH), 9.3 (s, 1H, p-Ar-OH).

2.5.11. 3-[(4-Hydroxy-3-methoxy)phenyl]-5-(4-nitrophenyl)-2-pyrazoline (ATP-11)

Molecular formula $C_{16}H_{15}N_3O_4$, Mol wt. 313, % yield 34.00, M. Pt. 177, UV (nm): 306.80, 206.20, FT-IR (KBr disc): 3331 (Ar O–H); 3080, 3032 (Ar–H); 2956, 2926, 2879 (C–H); 1645 (C═CN); 1599, 1514, 1456 (Ar C═CC); 1203 (Ar C–OH), 1250 (Asymmetric C–O–C stretch), 1048 (symmetric C–O–C stretch), 1568 [Asymmetric ($ArNO_2$) (N═CO)$_2$ stretch], 1345 [symmetric ($ArNO_2$) (N═CO)$_2$ stretch], 850 (C–N stretch for $ArNO_2$); ^{1}H-NMR: 2.5–3.5 (dd, 2H,

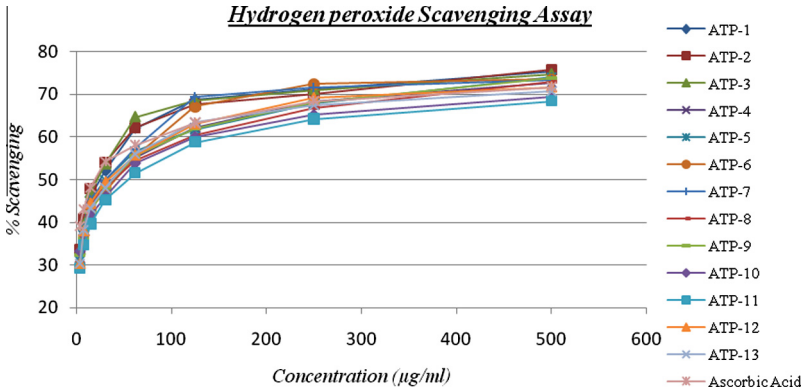


Figure 6 Hydrogen peroxide scavenging assay results of novel 3,5-disubstituted-2-pyrazolines.

Pyrazoline CH_2), 3.83 (s, 3H, m-OCH_3), 4.54 (t, 1H, CH–Ar), 6.5–7.7 (m, 7H, Ar–H), 6.3 (d, 1H, NH), 9.3 (s, 1H, p-Ar-OH).

2.5.12. 3-[(4-Hydroxy-3-methoxy)phenyl]-5-(3,4-dimethoxyphenyl)-2-pyrazoline (ATP-12)

Molecular formula $C_{17}H_{20}N_2O_4$, Mol wt. 329, % yield 17.33, M. Pt. 144, UV (nm): 286.80, 211.80; FT-IR (KBr disc): 3392, 3315 (Ar O–H); 2926 (C–H); 1577, 1512, 1456 (Ar C=CC); 1247 (Asymmetric C–O–C stretch); 1040 (symmetric C–O–C stretch); MS: $[M^+] \rightarrow 329$; $[M^+ - CH_3] \rightarrow 314$; $[M^+ - OCH_3] \rightarrow 298$.

2.5.13. 3-[(4-Hydroxy-3-methoxy)phenyl]-5-(3-hydroxy-4-methoxyphenyl)-2-pyrazoline (ATP-13)

Molecular formula $C_{17}H_{18}N_2O_4$, Mol wt. 314, % yield 58.66, M. Pt. 192, UV (nm): 286.40, 207.00; FT-IR (KBr disc): 3392, 3315 (Ar O–H); 2926 (C–H); 1577, 1512, 1456 (Ar C=CC); ^{1}H-NMR: 2.7–3.4 (dd, 2H, Pyrazoline CH_2), 3.65 (s, 3H, p-OCH_3), 3.83(s, 3H, m-OCH_3),4.63(t, 1H, CH–Ar), 6.8–7.5(m, 7H, Ar–H), 6.6 (d, 1H, NH), 9.1 (s, 1H, m-Ar-OH), 9.4 (s, 1H, p-Ar-OH).

2.6. Evaluation of antioxidant activity

2.6.1. Diphenylpicrylhydrazyl radical assay method (DPPH method)

The assay was carried out in a 96 well microtiter plate. To 100 µl of DPPH solution, 100 µl of each of the test sample or the standard drug was added separately in wells of the microtiter plate. The final concentrations in the wells for test and standard solutions were 500–4 µg. The plates were incubated at 37 °C for 20 min and the absorbance of each solution was measured at 540 nm, using ELISA microtiter plate reader. The experiment was performed in triplicate and% scavenging activity was calculated using the formula given below. IC50 (Inhibitory concentration) is the concentration of the sample required to scavenge 50% of DPPH free radicals and it was calculated from the graph, % scavenging vs. concentration.[23]

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2.6.2. Nitric oxide scavenging assay

The reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffered saline (PBS, 0.5 ml) and 0.5 ml of each test sample in DMSO was incubated separately at 25 °C for 150 min. After incubation, 50 µl of the reaction mixture containing nitrate was removed and 100 µl of sulfanilamide reagent was added and allowed to stand for 5 min for completion of diazotization, then 100 µl of NEDD was added and allowed to stand for 30 min in diffused light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA microtiter plate reader. The experiment was performed in triplicate and % scavenging activity was calculated using the formula given below. IC50 (Inhibitory concentration) is the concentration of the sample required to scavenge 50% of nitric acid and it was calculated from the graph, % scavenging vs. concentration.[24,25]

$$\% \text{ Scavenging} = \frac{\text{Control} - \text{Test}}{\text{Control}} \times 100$$

2.6.3. Superoxide scavenging assay

To 0.3 ml of various fractions of test and standard sol. in DMSO, 1 ml alkaline DMSO and 0.1 ml NBT were added. The absorbance was measured at 560 nm. The experiment was performed in triplicate.[26,27]

$$\% \text{ Scavenging} = \frac{\text{Test} - \text{Control}}{\text{Test}} \times 100$$

2.6.4. Hydrogen peroxide scavenging assay

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS) (pH 7.4). Various fractions of test and standard solution in DMSO were added to 2 ml of hydrogen peroxide solution in PBS. After 10 min, the absorbance was measured at 230 nm.[28,29]

$$\% \text{ Scavenging} = \frac{\text{Test} - \text{Control}}{\text{Test}} \times 100$$

3. Results and discussion

In the present study, chalcones iii were synthesized by condensing various (un)substituted benzaldehydes i with 3-methoxy-4-hydroxyacetophenone ii. Refluxing chalcones iii with hydrazine hydrate iv for 2–3 h in ethanol yielded the target compounds 2-pyrazoline derivatives v. All the 13 novel compounds were subjected to their structural

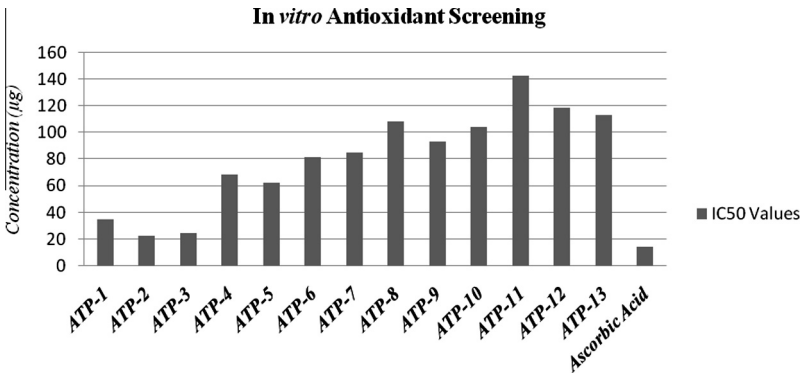


Figure 7 Mean antioxidant activity of novel 3,5-disubstituted-2-pyrazolines.

characterization using physical and spectral data(UV, FTIR, proton NMR and MS). 3,5-Disubstituted-2-pyrazolines have shown characteristic absorbance within the range of 305–307 nm when observed under UV spectroscopy. General vibrational frequencies of the parent molecule in the IR study were 3330 for the hydroxyl group in the aromatic ring, 3086, 3051 for the aromatic hydrogens, 2800–3000 for the C–H, 1458–1599 for the aromatic C═C stretching, 1200 for Ar–C–OH. Compounds had shown their characteristic peaks according to the substituents like 545 cm^{-1} for the bromine substitutions in ATP-4 and ATP-5, 645 cm^{-1} for aromatic chloro group in ATP-6 and ATP-7, 1568 and 1345 for the nitro substation in ATP-11 and so on. In the proton NMR spectra of the generalized parent molecule, a doublet of doublet peak at 2.28–2.74 for the methylene of pyrazoline, a singlet at 3.78 for the methoxy group, multiplet within the range of 6.5–8.0 for the aromatic hydrogens, a doublet at 6.75 for amino of pyrazoline and a singlet at 9.2 or 9.3 for the aromatic hydroxyl group were seen. Compounds had shown their characteristic peaks according to the substituents like a triplet at 4.73 for the methyl substitution at aromatic ring in ATP-2 and ATP-3 and so on.

All the 13 novel molecules were subject for their evaluation of antioxidant profile using four models DPPH radical scavenging assay method (Fig. 3), nitric oxide scavenging assay method (Fig. 4), superoxide scavenging assay method (Fig. 5) and hydrogen peroxide scavenging assay (Fig. 6), while the mean antioxidant activity of these molecules were represented by Fig. 7. All the compounds were shown to have excellent antioxidant activity when compared with standard ascorbic acid. After careful evaluation of DPPH assay, NO scavenging, superoxide scavenging and hydrogen peroxide scavenging assay results, it reveals that ATP-1, ATP-2 and ATP-3 have shown the potential antioxidant activity out of which ATP-2 is the best one when compared with ascorbic acid. It signifies the role of steric as well as electron releasing group CH_3 at the meta substituted group of 2-pyrazoline for the inhibition of free radicals. Electron withdrawing groups and high electron releasing groups like methoxy are not facilitators of free radical inhibition. Electron withdrawing groups reduce the efficiency of hydrogen release from the amino group of pyrazoline, so if halogens were the substituents on the 3-phenyl of pyrazoline, antioxidant activity greatly reduced revealing the inappropriateness of halogen in antioxidants. Moreover the nitro group containing molecule ATP-11 has shown the poor scavenging results for the nitric oxide scavenging assay revealing the inability of molecules(having nitro group) to scavenge nitric oxide radicals in the body. Hydroxyl and methoxy group substituents inducing the resonance thereby stabilize the molecules, so the molecules with above substituents have less antioxidant capacity. Least activity was observed with the hydroxyl and methoxy substitutions at the 3-phenyl of 2-pyrazoline, so these substitutions are not recommended further for the antioxidant molecules.

4. Conclusion

We reported the synthesis and evaluation of antioxidant activity of 3,5-disubstituted-2-pyrazoline analogs. The results were almost comparable to standard ascorbic acid. The findings are highly significant. It is proposed to prevent the use of electron withdrawing groups in the 3,5-disubstituted-2-pyrazoline analogs if aimed to be having a potential antioxidant. Based on the data of the present study, it is very difficult to suggest any possible mechanism for the antioxidant effects of the derivatives, as the present study is purely a preliminary investigation. But still the results of this study will provide useful information for the design of novel molecules as antioxidant agents.

5. Conflict of interest

None.

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