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Research Article

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Synthesis and anti-oxidant activity of certain chalcone based acetyl and N-phenyl substituted pyrazolines

Sudhakara Rao G.[1], Kalaichelvan V. K.[1] and Ganguri Sudhakara Rao[2]

[1]*Department of Pharmacy, Annamalai University, Chidambaram, Tamilnadu, India*
[2]*Department of Pharmacy, Vishwa Bharathi College of Pharmaceutical Sciences, Perecherla, Guntur, A. P., India*

ABSTRACT

A free radical is a molecular species having an unpaired electron and it is a highly reactive entity and unstable. Free radicals are formed constantly in human system during metabolism or during the process of phagocytosis. Free radicals can also be generated from ionizing radiations, ozone, heavy metal poisoning, cigarette smoking and chronic alcohol intake. These free radicals are highly reactive and oxidise biomolecules leading to tissue injury and cell death. If these free radicals are removed by enzymes or nonenzymes involved in defense mechanism. To overcome this problem many antioxidants has been prepared in world. Here in order to determine the antioxidant properties, these compounds are evaluated. The compounds which are chalcones and chalcone based acetyl substituted and n-phenyl substituted pyrazolines such as (Ph1-Ph4 & Py1-Py4) were screened by using the process of DiPicryl Hydrazyl Radical (DPPH) model for antioxidant activity. Among the 16 compounds, Chalcone based acetyl and n-phenyl substituted pyrazolines are evaluated for antioxidant activity.

Keywords: Chalcones, acetyl and n-phenyl substituted pyrazolines, Antioxidant Activity, (DPPH) Scavenging Activity.

INTRODUCTION

Free radicals are an atom or molecule that bears an unpaired electron and is extremely reactive, capable of engaging in rapid change reaction that destabilize other molecules and generate many more free radicals. In plants and animals these free radicals are deactivated by antioxidants. These antioxidants act as an inhibitor of the process of oxidation, Even at relatively small concentration and thus have diverse physiological role in the body. The body is constantly exposed to the negative and sometimes lethal effects of oxidants during normal physiological processes. The harmful free radicals such as hydroxyl, peroxyl and the superoxide anion are constantly being produced as a result of metabolic reactions in living systems. On a daily basis, up to 5% of inhaled oxygen may be converted to reactive oxygen species(ROS). These ROS have the ability to bind to cellular structures, and have been implicated in number of pathological processes such as aging, inflammation, reoxygenation of ischemic tissues, atherosclerosis, cancer and even Parkinson's disease in men. [1]Two processes, which produce free radicals *in vivo*, have been identified and named the Fenton reaction and the Haber-weiss reaction. [2]Antioxidants play an important role in animal health. Conventional antioxidants have been shown to improve animal performance during conditions characterized by increased tissue oxidant levels such as stress, injury and infections. [3]The3 (4-(1-phenyl- 5-(p-chlorophenyl) 4,5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one is an electron withdrawing group and exhibited antioxidant activity. Favorable substitution may increase their free radical scavenging effect[4].

α,β–unsaturated ketone group containing moiety (Chalcones)has shows significant position in medicinal chemistry. The electron rich olefinic bond has shown involvement in Michel interaction and divers class of biological activities. To evaluate the role of reactive α,β–unsaturated ketone group in antioxidant activity, it is necessary to screen and compare tetrahydrochalcone with respective chalcones [5-9]. Several catalytic reducing agents have been employed for selective reduction including palladium/ vinyl acetate, rhodium (I)complexes, pincer-aryl ruthenium (II) complexes, magnesia, iridium/ formic acid, sodium hypophosphite, palladium/ethylene atmosphere, CeO2-ZnO complex etc [10].

EXPERIMENTAL SECTION

Synthesis of a new series of pyrazoline derivatives (Q1-4,P1-4,Ph1-4& Py1-4) have been obtained from the starting materials anthranilic acid (A) and benzoyl chloride (B) to 2[phenyl]-benzo(1,3)oxazine-4-one(C) in pyridine further reaction with *p*-amino acetophenone gives 3-(4-acetyl phenyl)-2-(phenyl)-3Hquinazoline-4-one (D) derivatives. Then on condensation with different substituted aromatic aldehydes afforded four Q1-4 compounds. Then further incorporated into pyrazoline, N-phenyl pyrazoline and N-acetylpyrazoline ring systems at position 3 of the quinazoline ring. The newly synthesized compounds have been supported by spectral data IR, H1NMR and Mass spectra.

Melting points of the synthesized compounds were determined in open capillary tubes and were uncorrected. IR spectra were recorded on BRUKER FT-IR spectrometer using ATR. ^{1}H-NMR spectra of the compounds in deutiriated dimethyl sulfoxide (DMSO) and $CDCl_3$ was recorded on BRUKER Av 400 spectrometer. Mass spectra were recorded on LCMS QP 5000 Shimadzu. Thin layer chromatography was performed using pre-coated aluminium plates, coated with silica gel GF_{254} [E. Merck]. Ethylacetate : Methanol in the ratio of 3 : 2 was used as the eluent. The spots were visualized in the UV/Iodine chamber.

Synthesis of chalcones : Synthesis of 3(4(3(4-substituted phenyl) acrolyl) phenyl) 2-phenyl quinazoline (4*H*)-one Equimolar mixture of compound D (0.01mole) and the appropriate aromatic aldehydes (0.01mole) p-chloro benzaldehyde, p-nitro benzaldehyde, p-methylbenzaldehyde and p-methoxy benzaldehyde were dissolved in ethanol and cold solution of 40% NaOH (15mL) was added in portion keeping the temperature below 10C with continuous stirring. The reaction mixture was kept overnight. Then it was acidified with dilute HCl and poured ice cold water with stirring. The product obtained was filtered, washed with cold water dried and recrystallised from ethanol.

Synthesis of pyrazolines

To a mixture of compound Q1-4 (0.01mole) and hydrazine hydrate (0.01m) in ethanol and added a few drops of glacial acetic acid and refluxed for 8hrs. The reaction mixture was poured in to crushed ice. The separated solids were filtered and recrystallised form ethanol.

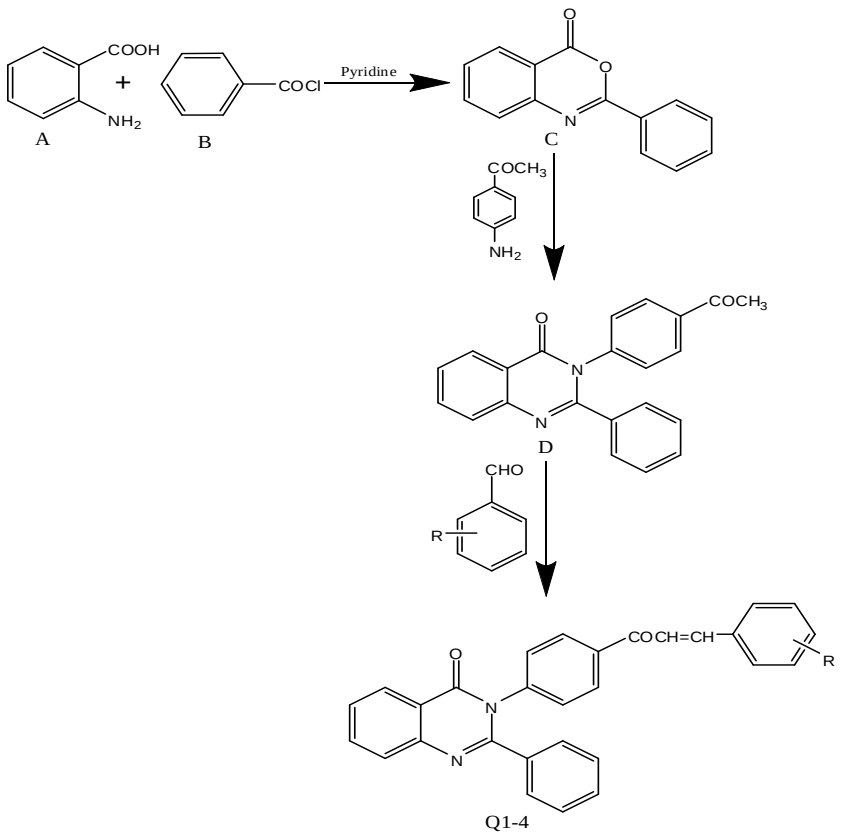
Synthesis of N-phenylpyrazolines

Mixture of compound Q1-4 (0.01mole) and phenyl hydrazine dissolved in 20 ml of 1, 4 dioxane. To this reaction added 2-3 drops of sulphuric acid and the contents were refluxed for 4hrs. Then add 5ml of glacial acetic acid and continue the reflux another 2 hrs. After cooling the reaction mixture pour the contents in ice cold water. The obtained solid allow drying and recrystallized from ethanol.

Synthesis of N-acetylpyrazolines

The mixture of compound Q1-4 (0.01mole), hydrazine hydrate (0.01m) and glacial acetic acid (10ml) refluxed for 8hrs. The reaction mixture was poured in to crushed ice and the obtained mass was dried and recrystallised form ethanol.

Scheme-I



Scheme-II

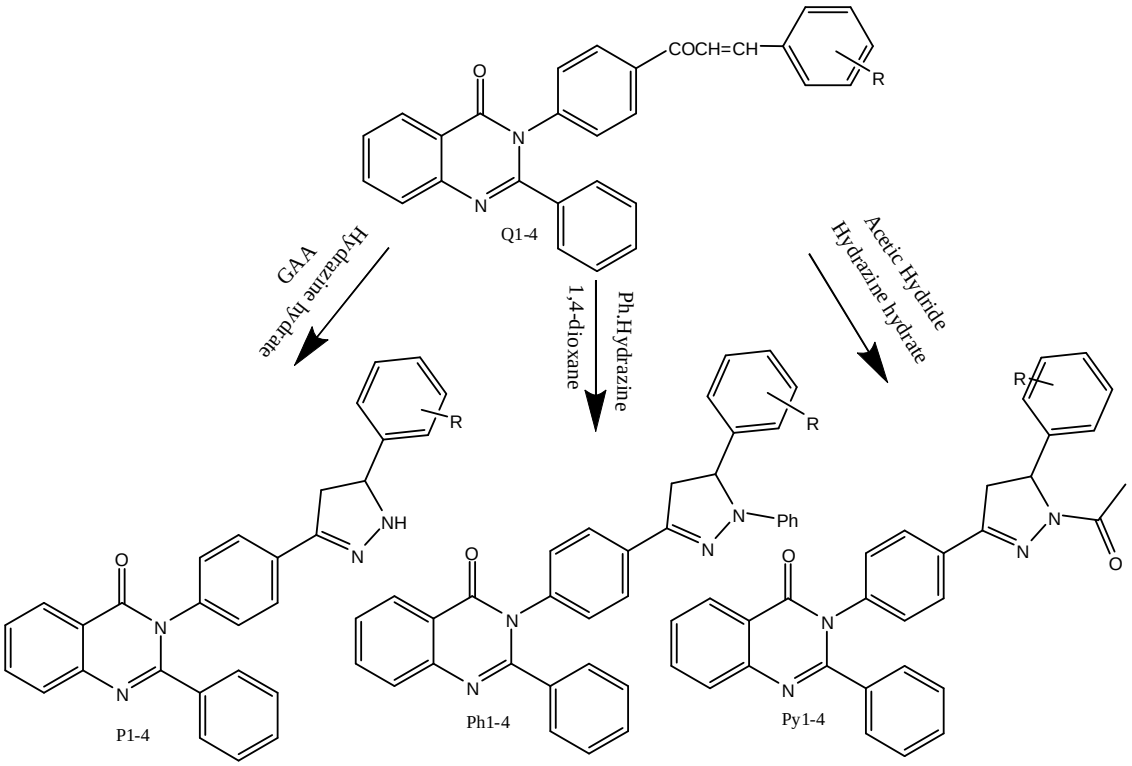


Table 1. Physical data

S.No	Code	Mol.wt	Mol.form	M.P	% yield
1	Q_1	450.5	$C_{28}H_{19}N_2O_2Cl$	140-142ºC	63
2	Q_2	461	$C_{28}H_{19}N_3O_4$	168-170 ºC	73
3	Q_3	430	$C_{29}H_{22}N_2O_2$	210-212 ºC	79
4	Q_4	446	$C_{29}H_{22}N_2O_3$	178-180 ºC	83
5	P1	478.5	$C_{29}H_{21}N_4OCl$	190-192 ºC	43
6	P2	473	$C_{29}H_{21}N_4O_3$	110-112 ºC	85
7	P3	470	$C_{30}H_{24}N_5O$	234-236 ºC	56
8	P4	472	$C_{30}H_{24}N_4O_2$	161-163 ºC	78
9	Ph1	554.5	$C_{35}H_{25}N_4OCl$	176-178 ºC	59
10	Ph2	549	$C_{35}H_{25}N_4O_3$	267-269 ºC	81
11	Ph3	532	$C_{36}H_{28}N_4O$	155-157 ºC	63
12	Ph4	548	$C_{36}H_{28}N_4O_2$	122-124 ºC	72
13	Py1	520.5	$C_{31}H_{23}N_4O_2Cl$	190-192 ºC	68
14	Py2	529	$C_{31}H_{23}N_5O_4$	217-219 ºC	63
15	Py3	498	$C_{32}H_{26}N_4O_2$	132-134 ºC	78
16	Py4	514	$C_{32}H_{26}N_4O_3$	97-99 ºC	80

SPECTRAL STUDY OF NEWLY SYNTHESIZED COMPOUNDS

P_{h1}:3 (4-(1-phenyl- 5-(p-chlorophenyl) 4,5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 176-178^{o}C; yield (%): 59; R_f: 0.93; IR (ATR,Cm-^{1}): 1706 (C=O of quinazolinone, str), 1556 (C=C, str), 1598 (C=N, str), 2908 (C-H Ali, str), 3110 (C-H Aro, str), 820 (C-Cl, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 7.32-9.01 (23H, m, Ar-H), 3.10 (2H, d, CH_2 of pyrazole), 4.36 (1H, s, CH-pyrazole), 1.56 (3H, s, Ar-methyl); Mass: m/z 178.

P_{h2}:3 (4-(1-phenyl- 5-(p-nitrophenyl) 4,5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 267-269^{o}C; yield (%): 81; R_f: 0.82; IR (ATR,Cm-^{1}): 1710 (C=O of quinazolinone, str), 1522 (C=C, str), 1610 (C=N, str), 2918 (C-H Ali, str), 3120 (C-H Ar, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 7.32-9.01 (23H, m, Ar-H), 2.96 (2H, d, CH_2 of pyrazole), 4.05 (1H, s, CH-pyrazole), 1.23 (3H, s, Ar-methyl); Mass: m/z 269.

P_{h3}:3 (4-(1-phenyl- 5-(p-methylphenyl) 4,5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 155-157^{o}C; yield (%): 63; R_f: 0.43; IR (ATR,Cm-^{1}): 1712 (C=O of quinazolinone, str), 1530 (C=C, str), 1588 (C=N, str), 2899 (C-H Ali, str), 3100 (C-H Ar, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 7.32-9.01 (23H, m, Ar-H), 3.12 (2H, d, CH_2 of pyrazole), 4.16 (1H, s, CH-pyrazole), 1.81 (3H, s, Ar-methyl); Mass: m/z 157.

P_{h4}: 3 (4-(1-phenyl- 5-(p-methoxyphenyl) 4, 5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 122-124^{o}C; yield (%): 72; R_f: 0.91; IR (ATR,Cm-^{1}): 1706 (C=O of quinazolinone, str), 1556 (C=C, str), 1598 (C=N, str), 2908 (C-H Ali, str), 3110 (C-H Aro, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 7.32-9.01 (23H, m, Ar-H), 2.96 (2H, d, CH_2 of pyrazole), 4.21 (1H, s, CH-pyrazole), 1.28 (3H, s, Ar-methyl); Mass: m/z 124.

P_{y1}:3 (4-(N-acetyl-5-(chlorophenyl)-4, 5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 190-192^{o}C; yield (%): 68; R_f: 0.49; IR (ATR,Cm-^{1}): 1698 (C=O, str), 1706 (C=O of quinazolinone, str), 1592 (C=N, str), 1459 (C=C, str), 2930 (C-H Ali, str), 3097 (C-H Aro, str), 828 (C-Cl, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 2. 91 (2H, d, CH_2 of pyrazoline), 4.66 (1H, s, CH of pyrazoline), 7.2-7.4 (17H, m, Ar-H), 2.06 (3H, s, CH_3 of acetyl); Mass: m/z 192.

P_{y2}:3(4-(N-acetyl-5-(nitrophenyl)-4,5dihydro-1H-pyrazol-3-yl)phenyl quinazoline-4(3H) one m. p. 217-219^{o}C; yield (%): 63; R_f: 0.43; IR (ATR,Cm-^{1}): 1698 (C=O, str), 1706 (C=O of quinazolinone, str), 1592 (C=N, str), 1459 (C=C, str), 2930 (C-H Ali, str), 3097 (C-H Aro, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 3.08 (2H, d, CH_2 of pyrazoline), 4.45 (1H, s, CH of pyrazoline), 7.2-7.4 (17H, m, Ar-H), 1.90 (3H, s, CH_3 of acetyl); Mass: m/z 219.

P_{y3}:3-(4-(N-acetyl-5-(methylphenyl)-4,5 dihydro-1*H*-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 132-134^{o}C; yield (%): 78; R_f: 0.84; IR (ATR,Cm-^{1}): 1698 (C=O, str), 1706 (C=O of quinazolinone, str), 1592 (C=N, str), 1459 (C=C, str), 2930 (C-H Ali, str), 3097 (C-H Aro, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 2.81 (2H, d, CH_2 of pyrazoline), 4.72 (1H, s, CH of pyrazoline), 7.2-7.4 (17H, m, Ar-H),1.89 (3H, s, CH_3 of acetyl); Mass: m/z 134.

P_{y4}: 3 (4-(N-acetyl-5-(methoxyphenyl)-4, 5 dihydro-1H-pyrazol-3-yl) phenyl quinazoline-4(3H) one m. p. 97-99^{o}C; yield (%): 80; R_f: 0.59; IR (ATR,Cm-^{1}): 1698 (C=O, str), 1706 (C=O of quinazolinone, str), 1592 (C=N, str), 1459 (C=C, str), 2930 (C-H Ali, str), 3097 (C-H Aro, str); ^{1}H NMR (δppm; $CDCl_3$/DMSO-d_6) : 2. 80 (2H, d, CH_2 of pyrazoline), 4.69 (1H, s, CH of pyrazoline), 7.2-7.4 (17H, m, Ar-H),1.96 (3H, s, CH_3 of acetyl); Mass: m/z 99.

ANTIOXIDANT ACTIVITY

***In-vitro* antioxidant activity of newly synthesized chalcones and chalcone based pyrazolines:**

In the present study, *in-vitro* antioxidant activity of newly synthesized compounds was performed by DPPH model[11-12]The method employed was by determining the free radical inhibitory ability of different antioxidant by using very stable free radical such as 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol. Stock solution of DPPH (1.3 mg/mL) in methanol was prepared. Stock solution of DPPH 100 µL was added to 3.0 mLof methanol and absorbance was recorded at 516 nm. The various concentrations of chalcone based acetyl substituted and n-phenyl substituted pyrazoline compounds (25, 50 and 100µg/mL) were prepared. All sample solutions 1.0 mL each is diluted with 3.0 mL with methanol and 100 µL of stock solution of DPPH was added. Test tubes were kept for 30 min in light to complete the reaction. After 30min, absorbance of each test tube was recorded at 516 nm on UV-VIS spectrophotometer against methanol as a blank. The effective concentration of sample required to scavenge DPPH radical by 50% (IC_{50} value) was obtained by linear regression analysis of dose-response curve plotting between % inhibition and concentrations. Regression equations to derived IC_{50} values showed inverse relationship between IC_{50} values and percentage scavenging potential of compound.

The DPPH free radical scavenging activity was calculated using the following formula:

$$\% \text{ scavenging} = \frac{\text{Absorbance of control - Absorbance of test sample}}{\text{Absorbance of control}} \times 100$$

Where; Control is absorbance of a DPPH solution without compound;
Test is the absorbance of the test compounds with DPPH.

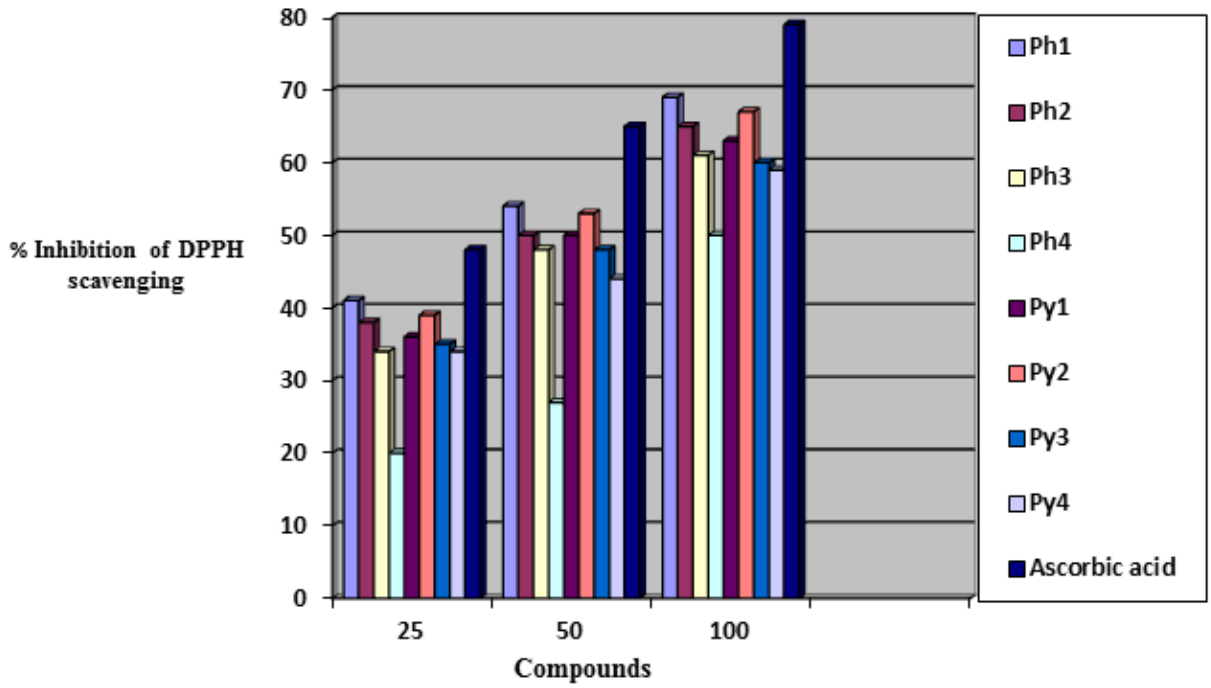
The degree of discoloration indicates the free radical scavenging efficiency of the compound. Ascorbic acid was used as the free radical scavenger reference compound.

Table 2. *In-Vitro* antioxidant activity of newly synthesized chalcone based n- acetyl substituted and n-phenyl substituted pyrazoline compounds

S.no	Compound code no	% Inhibition of DPPH-Scavenging			IC_{50} (µg/mL)
		25 µg/mL	50 µg/mL	100 µg/mL	
1.	Ph1	41.05±0.02	54.12±0.04	69.08±0.5	40.52
2.	Ph2	38.04±0.01	50.05±0.23	65.05±0.1	47.45
3	Ph3	34.22±0.37	48.44±0.15	61.09±0.16	57.98
4	Ph4	20.01±0.12	27.00±0.2 32	50.0±0.09	98
5	Py1	36.80±0.14	50.60±0.03	63.54±0.12	49.12
6	Py2	39.16±0.14	53.06±0.3	67.07±0.1	42.90
7	Py3	35.01±0.06	48.30±0.01	60.13±0.02	58.64
8	Py4	34.89±0.21	44.51±0.12	59.29±0.11	70.50
9	Std. drug Ascorbic acid	48.12±0.16	65.14±0.1	79.30±0.0	31.48

Data represents mean ±S.D. of triplicate analysis

Figure 1. Compounds Vs % Inhibition of DPPH scavenging activity



RESULTS AND DISCUSSION

In this study, the synthesis of chalcone compounds has been carried out successfully from the starting material 2,4-dihydroxy acetophenone. The purity of the newly synthesized compounds was established by TLC and melting point. The structures of chalcone based acetyl substituted and n-phenyl substituted pyrazolines were confirmed on the basis of spectral data (IR,1H NMR). Chalcones showed the IR absorptions characteristics of phenolic -OH (3200-3350 cm-1), carbonyl>C=O (1685-1600 cm-1) and aromatic C=C (1580-1400 cm-1) functionalities. The 1H NMR spectra of chalcones displayed multiplet due to aromatic protons at 6.92-8.00 δ (m, Ar-H) and singlet due to phenolic –OH at 11.94-12.75 δ (s, 2H, 2 x OH). The characteristics signals for a chalcone moiety appeared as two doublets at 7.55-7.79δ (d, 1H, *α*-H) and 7.82-7.95 δ (d, 1H, *β*-H). The characteristics signals for -CO-C*H*=C< appeared as singlets at 6.73-6.86 δ (s, 1H, *α*-H) and also absence of peak for -CO-CH=C*H*- proton gave evidence for the use of *β*-H in the cyclization process of chalcone. All the compounds (Ph1-Ph4 & Py1-Py4) given satisfactory IR, 1H NMR, Mass spectra of compounds and elemental analysis data correlation with the assign structure. The antioxidant activity results in table(2) show that most of the compounds exhibited moderate to good antioxidant activity compared to that of standard (ascorbic acid). As a typical compound, Ph3,Ph4, Py3 and Py4 was produced the good activity when compared to standard drug. By contrast, compounds Ph1 and Py2, with a meta electron-withdrawing nitro group, displayed weak activity. These results suggested that the substitution pattern of acetyl groups and n-phenyl groups may be crucial for their antioxidant activity enhancement. However, in the evaluation of chalcones, chalcone based acetyl substituted and n-phenyl substituted pyrazolines considered to good antioxidant properties.

CONCLUSION

In conclusion on the basis of above findings of chalcone based acetyl substituted and n-phenyl substituted pyrazolines produced the good antioxidant activity. Among them Ph3, Ph4, Py3 and Py4 was produced the good activity when compared to standard drug. By contrast, compounds Ph1 and Py2, with a meta electron-withdrawing nitro group, displayed weak activity These results suggested that the substitution pattern of acetyl groups and n-phenyl groups may be crucial for their antioxidant activity enhancement In spite of this, chalcones could also be selected in our synthetic lead optimization study because they were also shown good antioxidant activity. However chalcone based pyrazoline derivatives having the antioxidant properties.

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REFERENCES

[1]. Setiadi DH;Chass GA, Torday LL, Varro A, Papp JG. *Journal of molecular structure*, **2003**; 620:93-106.
[2]. Halliwell B, Gutteridge JMC. Free radicals in biology and medicines. Oxford University Press, Oxford **1985**, 4th edition,pp 120-123.
[3]. Nickels W. *European journal of biochemistry*" **2003**; 270: 2109-2119.
[4]. Dutta S, Padhye S, Priyadarsini KI, Newton C. *Bioorganic &Medicinal Chemistry Letters* **2005**, 15: 2738–2744.
[5] H.P. Avila HP, E. de Fátima, A Smania, *et al*. *Bio.Med.Chem.*, **2008**, 16:9790-9794.
[6]. Y. Kong, K. Wang, M.C. Edler, *et al. Bio.Med. Chem.*, **2010**, 18: 971-977.
[7]. L. Jayasinghe , B. Balasooriya , W.C. Padmini, *et al. Phytochem.*, **2004**,65: 1287-1290.
[8]. A. Bhattacharya, L.C. Mishra, M. Sharma, *et al*. *Euro.J.Med.Chem*., **2009**, 44: 3388-3393.
[9]. A. Valla, B. Valla, D. Cartier, *et al*. *Euro. J. Med.Chem.*, **2006**, 41: 142-146.
[10]. L.J. Yamin, E.I. Gasull, S.E. Blanco *et al*. *J. Mol. Str.*, **1998**, 428: 167-174.
[11]. Murti Y., Yogi B. and Pathak D., *J. Pharm. Res*., **2011**; 4(10), 3452-3454.
[12]. Matsubara N., Fuchimoto S., Iwagaki H., Nonaka Y., Kimura T., Kashino H., Edamatsu R., HiramatsuM. and Orita K., *Res. Commun. Chem. Pathol. Pharmacol*. **1991**, 71,PP. 239-242.