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IN VITRO ANTIOXIDANT ACTIVITY OF NOVEL 4-HYDROXY-1-METHYL-3-(3-SUBSTITUTED)-4,5-DIHYDROISOXAZOL-5-YL)QUINOLIN-2(1H)-ONE DERIVATIVES

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ABSTRACT

A series of novel 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives were synthesized from chalcones (α, β-unsaturated compounds) well known compounds to exhibit antioxidant activities. All the synthesized compounds were characterized by spectral and analytical data and were evaluated for antioxidant activity by DPPH method using standard Ascorbic acid. All the compounds are known to exhibit antioxidant activity. Among them, compound (5f) exhibited the IC_{50} = 7.2 µM, standard (IC_{50} = 5.85 µM)

Keywords: Chalcones, Quinolinone, Isoxazolines, Hydroxylamine hydrochloride, Antioxidant.

INTRODUCTION

Reactive oxygen species generated by cell metabolism or by exogenous factors include hydrogen peroxide (H2O2), the hydroxyl radical (HO), the superoxide anion radical (O2•⁻). These free radicals have essential roles in cell signaling, apoptosis and gene expression. On the other hand, excessive free radical attack can damage DNA, proteins and lipids resulting in diseases like cancer, neurological degeneration, arthritis as well as the process of aging. Therefore, considerable speculation has been directed towards the identification of antioxidants for use in preventive medicine. The radical mediated oxidation of DNA is related to many diseases. In addition, the radical scavenging capacities of antioxidants can be estimated by reacting with 2,2–diphenyl-1-picrylhydrazyl radical (DPPH). During the inflammatory process many reactive species are produced, among them reactive oxygen species (ROS) such as superoxide radical (O2•⁻), hydrogen peroxide (H2O2), hypochlorous acid (HOCl), singlet oxygen (1O2) and peroxyl radical (ROO•) as well as reactive nitrogen species (RNS), like nitric oxide (•NO) and peroxynitrite anion ($ONOO^-$). Indeed, ROS and RNS are also produced by the endothelial cells, Kupffer cells, neutrophils and macrophages as mechanism of defense against foreign infectious pathogens. To date, the prevention of oxidative stress related diseases has been tentatively achieved by the development of antioxidant compounds that are able to scavenge ROS and RNS and thus avoid radical-induced oxidation damage. The natural antioxidant Mechanisms may be insufficient in variety of conditions and hence dietary intake of antioxidant compounds are important.[1] When a validated scaffold has been identified, the study of structure reactivity relationship (SAR) may provide useful information for understanding of its mode of action and to the improvement of the antioxidant activity of future generated compounds. From the literature survey, it was found that heterocyclic compounds play important role in the drug discovery process and analysis of drugs in late development or on the market shows that 68% of them are heterocycles. Therefore, it is

not surprising that research on the synthesis of polyfunctionalized heterocyclic compounds has received special attention .Of these heterocycles, isoxazoline derivatives which are a rarity in nature have been reported to possess a wide range of biological activities which include possess antioxidant, antimicrobial[2], antituberculosis[3] anti-inflammatory[4], anti-viral[5] ,analgesic[6] ,antitumor[7], chemotherapy, antinociceptive[8], macrophage migration inhibitory factor activity, protein tyrosine phosphatases 1B inhibitors, antipsychotic, anticonvulsant, thrombosis, antianxiety [9], antidepressant[10], 5 HT_2A, $5HT_2C$ receptor ligands.[11] On the other hand quinolinone structure is characteristic of numerous natural products and synthetic analogues that exhibit a wide variety of biological activities. Compounds possessing the quinolinone moiety as a key structural feature TA 270 (4-hydroxy-1-methyl-3-octyloxy-7-sinapinoylamino-2(1H)-quinolinone was designed as an antioxidant. A naturally occurring quinolinone alkaloid has been recently isolated from the aleurone layer of *Oryza sati*V*a* cv *Hengjinmi*. This compound exhibited moderate antioxidant activity in a DPPH free radical scavenging assay.

.In view of the these observations, the present work aimed to synthesize novel 4-Hydroxy-1-methyl-3-(substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives from chalcones on treatment with the hydroxylamine hydrochloride in the presence of the base, which could allow further chemical modifications by cyclization. Moreover, the combination of two pharmacophores with biological importance on the same scaffold is a well established approach for the synthesis of more potent drugs with antioxidant activity.

MATERIALS AND METHODS

All the chemicals are of analytical grade. All the reactions were monitored by analytical thin layer chromatography (TLC) using E-Merck silica plates (60G-254), visualization was accomplished with UV light (256mm) and Iodine. Melting points were recorded and are uncorrected. Nuclear Magnetic Resonance spectra were recorded on GEMINI-200 MHz (Varian), AVANCE-300MHz (Bruker) spectrometer using tetramethylsilane (TMS) as the internal standard. Chemical shifts have been expressed in (δ) ppm units downfield from TMS. Mass spectra were recorded on CEC-21-11013 or Finningan Mat 1210 double focusing mass spectrometer operating at 70 eV using direct inlet system.

Experimental

Procedure for the synthesis of 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives.

A mixture of chalcone (0.01 mole) and hydroxylamine hydrochloride (0.01mole) in 2% ethanolic sodium hydroxide solution (5ml) was heated to reflux for 6-7 hours. The progress of the reaction was monitored by TLC. After completion of the reaction the solution was poured into ice water (15ml).The resulting solution was neutralized with dilute HCl. The resulting solid so obtained was filtered, washed several times with distilled water, dried and crystallized from ethanol.

Evaluation of Antioxidant Activity

α,α-Diphenyl-β-picryl hydrazyl (DPPH; 0.2 mM in methanol), a stable free radical was used for the evaluation of antioxidant activity of these test compounds (Blios, 1958). Briefly, to 0.1 mL of test compound (at different concentrations), 1.5 mL of methanol and 0.5mL of DPPH solution were added, mixed thoroughly and absorbance (OD) read at 517 nm against blank. The percentage reduction of free radical concentration (OD) with different concentration of test compounds was calculated and compared with standard, ascorbic acid. Results were expressed as IC_{50} values (concentration of test required to scavenge 50% free radicals).

RESULTS AND DISCUSSION

Novel isoxazoline derivative were synthesized by cyclization of substituted chalcone derivatives in the presence of hydroxylamine hydrochloride (Figure 1). Physiochemical properties of synthesized compounds were determined in terms of melting point & % yield (Table 1). Synthesized compounds were also characterized using FT-IR and 1HNMR. The results of *in-vitro* antioxidant

activity of the test compounds were depicted in Figure 2. DPPH radical scavenging is considered a good *in-vitro* model and is widely used to conveniently assess antioxidant efficacy. From the results it could be seen that most of the compounds showed significant antioxidant activity. The results of this study revealed that all synthesized compounds significantly scavenged DPPH free radicals in a concentration-dependant manner. Among all compounds 5e displayed more potent antioxidant activity. This may be due to increased lipophilicity of molecules because of substitution with electronegative atom such as chloro/bromo at the C5 position of the aromatic ring. The IC_{50} values of all the test compounds were found between 7.2 and 67.56 mM ,this was expected due to the presence of electronegative and electron with drawing substituents which facilitates the release of hydrogen atom along with an electron bonded to C5 atom of isoxazoline ring. The compounds 5g showed lesser radical scavenging ability; this might be due to un-substitution. Presence of electron donating substituents in the benzene ring 5b, 5h which retards the release of hydrogen atoms. Substitution with heterocyclic ring system did not alter the activity of isoxazolines to any greater extent, Results indicate that the compounds 5f and 5e containing electron withdrawing groups on the aromatic ring shows potential electron donating ability(Table 2, Figure 2).

Spectral Data

4-hydroxy-1-methyl-3(5-phenyl-4,5dihydro isoxazol-3-yl) quinolin-2(1H) one (5a)

IR(KBr, cm^{-1}): 3276, 2925.46, 1635.80, 1497.96, 1040; ^1H NMR ($CDCl_3$, 300 MHz, δ) : 8.19-5.80 (m, 9H, Ar), 4.83-4.90 (t, 1H ,Ar), 4.08-3.98 (dd, 1H, Ar) 3.67 (s, 3H, N-CH_3), 3.64-3.54 (dd, 1H, Ar) Mass(ESI-MS) 320:$[M]^+$

3-(5(2,5-dimethoxyphenyl)-4,5dihydroisoxazol-3-yl)4-hydroxy-1-methyl-quinolin-2(1H)one (5b)

IR (KBr, cm^{-1}): 3279, 2927.40, 1636.49, 1497.29; ^1H NMR ($CDCl_3$, 300MHz, δ): 8.27-7.19 (m, 7H,Ar), 5.44-4.44 (t, 1H, Ar, *J*=6.79, 12.08 Hz), 4.41-4.32 (dd, 1H, Ar, *J*=12.08, 6.79 Hz), 3.95 (s, 3H, OCH_3), 3.79 (s, 3H, O-CH_3), 3.61(s, 3H, N-CH_3), 3.62-3.49(dd, 1H, Ar *J*=6.79, 12.08 Hz); Mass (EI-MS): 380 $[M]^+$

4-hydroxy-3-(5-(3-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)-1-methylquinolin-2(1H)-one (5c)

IR (KBr, cm^{-1}): 3180, 2925.46, 1632.77, 1044.81; ^1H NMR ($CDCl_3$+DMSO-d6, 200MHz, δ): 8.13-6.87 (m, 8H, Ar), 5.16-5.05 (t, 1H, *J*=6.79, 12.08 Hz) 4.0-4.6 (dd, 1H, *J*=12.08 , 6.79 Hz), 3.64 (s, 3H, N-CH_3), 3.55-3.39 (dd, 1H, *J*=6.79, 12.08); Mass (EI-MS) : 336 $[M^+]$

4-hydroxy-3-(5-(4-hydroxyphenyl)-4,5-dihydroisoxazol-3-yl)-1-methylquinolin-2(1H)-one(5d)

IR (KBr,cm^{-1}): 3293, 2921, 1636.35, 1010; ^{1}HNMR ($CDCl_3$+DMSO-d6, 300MHz, δ): 8.10-7.37 (m, 8H, Ar), 5.65-5.77 (t, 1H, Ar, *J*=11.02 , 2.20 Hz), 4.11-3.95 (dd, 1H, Ar, *J*=12.48, 13.22 Hz), 3.79-3.70 (dd, 1H , Ar, *J*=2.93, 7.34, 11.02 Hz), 3.64 (s, 3H, N-CH_3); Mass (ESI, m/z): $[M+1]^+$ 388

3-(5-4-chlorophenyl)-4,5-dihydroisoxazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one(5e)

IR (KBr, cm^{-1}): 3420, 3293, 2921, 636.35 ,1499.98, 821.30, 1047 ;^1H NMR ($CDCl_3$+ DMSO-d6, 300 MHz, δ): 8.04-7.30 (d, 8H, Ar), 4.78-4.71 (t, 1H, Ar, *J*=8.14 Hz), 3.98-3.83 (dd, 1H, Ar, J=9.49 , 8.76 Hz), 3.58 (s, 3H, N-CH_3), 3.51-3.41(dd, 1H, Ar, J=9.497, 8.766 Hz); Mass (ESI, m/z): $[M+1]^+$ 354

3-(5-(4-bromophenyl)-4,5-dihydroisoxazol-3-yl)-4-hydroxy-1-methylquinolin-2(1H)-one (5f)

IR (KBr, cm^{-1}): 3420, 3293, 2921, 1636.35, 1499.98,1044, 821.30; ^1H NMR ($CDCl_3$+DMSO-d6, 200MHz,δ): 8.04-7.02 (m , Ar, 8H) , 4.78-4.67 (t, 1H, Ar, *J*=9.49 Hz), 3.98-3.83 (dd, Ar, 1H,*J*=9.49, 8.76), 3.58 (s, 3H, N-CH_3) 3.51-3.41 (dd, 1H, Ar, *J*=9.49, 8.76 Hz); Mass (ESI, m/z): $[M+1]^+$ 398

4-hydroxy-1-methyl-3-(5-(napthalen-2yl)-4,5-dihydroisoxazol-3-yl)quinolin-2(1H)-one (5g)

IR (KBr, cm^{-1}): 3179.09, 2926.98, 1632.77, 1507, 1008 ; ^1H NMR ($CDCl_3$+DMSO-d6, 200MHz, δ): 8.16-7.79 (m, 11H, Ar), 4.86-4.74 (t, 1H, Ar, *J*=11.02, 10.28Hz), 4.07-3.93 (dd, 1H,

Ar, *J*= 10.28 Hz), 3.40-3.26 (dd, 1H , Ar, *J*=11.02, 10.28 Hz), 3.62 (s, 3H, N-CH_3) ; Mass (ESI, m/z): $[M+1]^+$ 370

4-hydroxy-3-(5-(4-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)-1-methylquinolin-2(1H)-one (5h)

IR (KBr,cm^{-1}): 3179, 2925.56, 1634.93, 1507.74, 1035.50,1174.97; ^{1}H NMR ($CDCl_3$+DMSO-d6,200MHz, δ) : 8.13-6.87 (m, 8H,Ar), 5.16-5.05 (t, Ar, 1H, *J*=6.79,12.08 Hz), 4.0-4.6 (dd, 1H,Ar *J*=12.08, 6.79 Hz), 3.91 (s, 3H, OCH_3), 3.64 (s, 3H, N-CH_3), 3.55-3.39 (dd, 1H, *J*=6.79, 12.08); Mass (ESI, m/z): $[M+1]^+$ 350

4-hydroxy-1-methyl-3-(5-thiophen-2yl)-4,5-dihydroisoxazol-3-yl)quinolin-2(1H)-one (5i)

IR (KBr, cm^{-1}): 3400, 3180, 2925.46, 1632.77, 1579.15, 1008; ^{1}H NMR ($CDCl_3$+DMSO-d6, 300MHz, δ) : 8.11-7.19 (m, 7H, Ar), 5.14-5.00 (t, 1H, Ar, *J*=8.59 Hz), 4.04-3.90 (dd, 1H, Ar, *J*=7.81,10.94 Hz), 3.54-3.35 (dd, 1H, Ar, *J*=8.59, 10.94 Hz), 3.62 (s, 3H, N-CH_3); Mass (ESI, m/z) $[M+1]^+$: 325

4-hydroxy-3-(5-(2-methoxyphenyl)-4,5-dihydroisoxazol-3-yl)-1-methylquinolin-2(1H)-one (5j)

IR (KBr, cm^{-1}) : 3339.15, 2930.88, 1636.64, 1494.91, 1242.20,110.71; ^{1}H NMR ($CDCl_3$+DMSO-d6, 200MHz ,δ): 8.13-6.87 (m, 8H, Ar), 5.16-5.05 (t, 1H, Ar, *J*=6.79, 12.08 Hz), 4.0-4.6 (dd, 1H, Ar, *J*=12.08, 6.79 Hz), 3.93 (s, 3H, OCH_3), 3.64 (s, 3H, N-CH_3), 3.55-3.39 (dd, 1H, *J*=6.79,12.08 Hz); Mass (ESI, m/z): $[M+1]^+$ 350

CONCLUSION

Present research work involves synthesis of novel 4-Hydroxy-1-methyl-3-(substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives to explore their antioxidant activity. Compound exhibited potent antioxidant activity hence, it is concluded that there is ample scope for further study in developing these lead compounds for the treatment of inflammation and Analgesia.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

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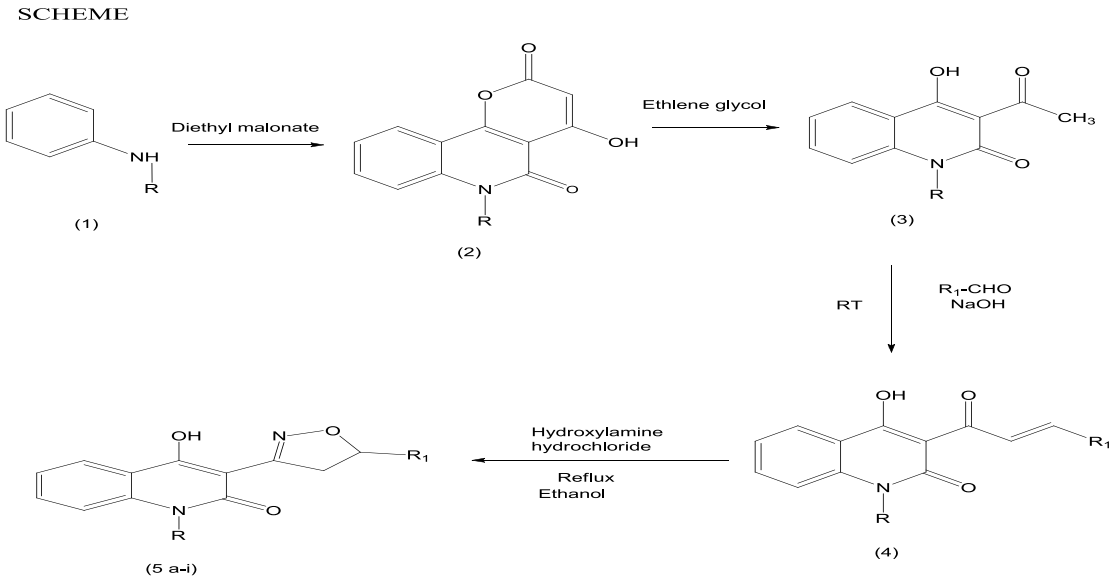

Figure 1: Synthesis of Novel 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives

Table 1: Physicochemical properties of 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl) quinolin-2(1H)-one derivatives

Sample code	Nature	Molecular formula	Molecular weight	m.p. (^{0}C)	% yield
5a	solid	$C_{19}H_{16}N_2O_3$	320	134-136	85
5b	solid	$C_{21}H_{20}N_2O_5$	380	158-160	62
5c	solid	$C_{19}H_{16}N_2O_4$	336	143-145	60
5d	solid	$C_{19}H_{16}N_2O_4$	336	180-182	74
5e	solid	$C_{19}H_{15}ClN_2O_3$	354	152-154	67
5f	solid	$C_{19}H_{15}BrN_2O_3$	399	120-122	53
5g	solid	$C_{23}H_{18}N_2O_3$	370	165-168	65
5h	solid	$C_{20}H_{18}N_2O_4$	350	161-163	65
5i	solid	$C_{17}H_{14}N_2O_3S$	326	171-174	48
5j	solid	$C_{20}H_{18}N_2O_4$	350	160-162	74

Table 2: Antioxidant activity of 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl) quinolin-2(1H)-one derivatives

Sample code	IC $_{50}$ (µmol)
5a	49.74
5b	57.23
5c	52.89
5d	60.23
5e	8.5
5f	7.2
5g	67.56
5h	34.2
5i	23.76
5j	28.89
Ascorbic acid	5.84

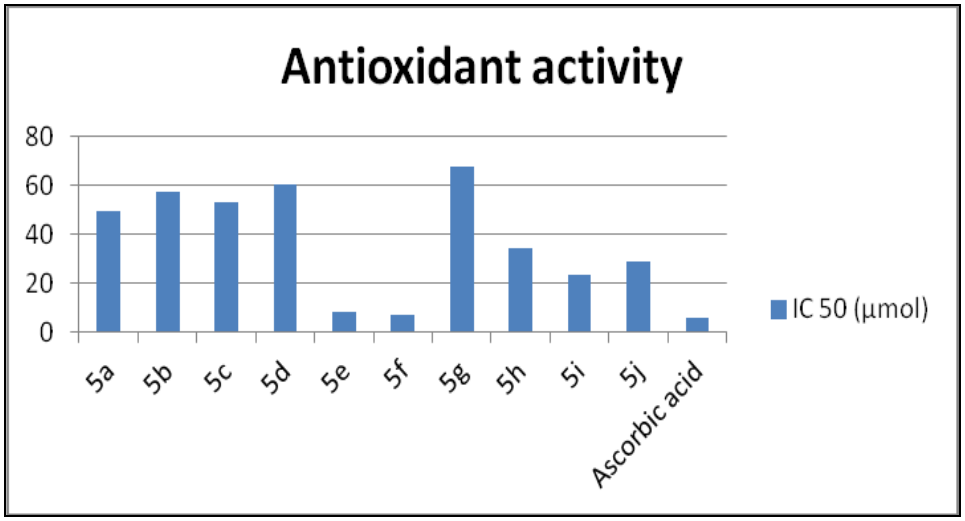

Figure 2: Graphical representation of antioxidant activity of 4-Hydroxy-1-methyl-3-(3-substituted)-4,5-dihydroisoxazol-5-yl)quinolin-2(1H)-one derivatives

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