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Original Article

Synthesis and characterisation of novel 2,4-diphenyloxazole derivatives and evaluation of their *in vitro* antioxidant and anticancer activity

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ABSTRACT

Background: In the present study, novel 2,4-disubstitued oxazoles were synthesised, characterised and screened for their biological activities.

Methods: Various substituted benzoic acids were refluxed with phenylacyl bromide in presence of triethylamine, to yield the respective phenylacyl esters, which on further refluxation with acetamide gave 2,4-disubstituted oxazole. The synthesised compounds were analysed by spectral studies to confirm their structure. Then, they were studied for their *in vitro* antioxidant activity by DPPH and nitric oxide radical scavenging methods. In addition, the *in vitro* anticancer activity was determined by MTT assay using HepG2 and HeLa cell lines.

Results: Totally, fifteen novel 2,4-disubstituted oxazole derivatives were synthesised, characterised and screened for their biological activities. Among the compounds tested, OXD-10, having 4-nitro-3-hydroxy phenyl substitution at the second position showed 50% free radical scavenging at 461.28 μg/ml by nitric oxide radical scavenging method. Other compounds were not found to have antioxidant activity by both methods. *In vitro* anticancer activity was performed on two different cancerous cell lines and compounds OXD-6 and OXD-15 showed promising activity on HepG2 cell line and their IC_{50} value was calculated as 16.89 and 16.65 μM respectively. Whereas, compound OXD-13 exhibited significant growth inhibition on HeLa cells and the IC_{50} value was 56.52 μM.

Conclusion: The above results concluded that, the compounds containing 2-nitro phenyl and 4-acetyloxyphenyl substituents at the second position in the oxazole scaffold played an important role in determining their anticancer potential and the 4-nitro-3-hydroxy phenyl group at second position in the scaffold could impart a major role in their radical scavenging property.

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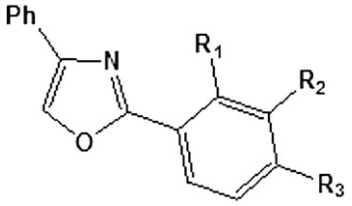
Oxazole is a five membered ring system containing N and O as heteroatoms at 1st and 3rd position. They have attracted great interest in recent years because of their various biological and analytical properties. Substituted oxazole derivatives were found to be associated with antibacterial, antifungal,[1] anti-tubercular,[2] anti-inflammatory,[3] analgesic, HIV inhibitor and muscle relaxant properties. Oxazoles functionalised at 2nd and 4th position with different oxidation state of appending carbon atom have found important application in the synthesis of more complex structures. Recently, much attention has been focused on the preparation of 2,4 and 2,4,5-substituted oxazoles because of their utilities as building blocks for complex natural products.[4]

Innovative therapeutic applications such as brain derived neurotrophic factor inducers,[5] as antibacterial in intraperitoneal sepsis,[6] prion disease therapeutics[7] and antiTB activities are also reported. Oxazole and their reduced derivatives are found in marine sources. Neopeltolide having potent *in vitro* action in lung adenocarcinoma, ovarian sarcoma.[8] In view of the above information we initiated a process of preparing novel 2,4-disubstitued oxazole analogues having the general structure of (A) and screening them for their antioxidant and anticancer activities.



1. Experimental

The melting point of the synthesised compounds was determined by using open capillary tubes in scientific melting point apparatus and was uncorrected. The progress of the reaction and the purity of the compounds was analysed by using precoated TLC plates; the solvent system used was petroleum ether and ethyl acetate (1:9). The spots were visualised under UV light. IR spectra of the synthesised compounds were recorded using Shimadzu FT-IR 8310 Japan and KBr press. Proton NMR spectra of the synthesised compounds were recorded on Bruker Biospin Avance-300 MHz at SAIF, IIT, Chennai. Mass spectra of the synthesised compounds were recorded on Shimadzu MS-MS QP5050 at SAIF, IIT, Chennai.

1.1. *Step 1: synthesis of phenylacyl esters (2)*

Various aromatic acids (**1**; 0.052 mol) in 30–40 ml of absolute alcohol, triethylamine (0.104 mol) were refluxed with phenacyl bromide (0.05 mol) for 1.5 h. The progress of the reaction was monitored by TLC analysis and after completion of the reaction, the reaction mixture was poured into ice cold water with constant stirring. The precipitate (**2**) was filtered, washed with water and recrystallised from 80% alcohol.

1.2. *Step 2: synthesis of 2,4-disubstituted oxazole (3)*

Phenylacyl ester (**2**; 0.01 mol) was added to a mixture of 20 ml xylene and 47% BF_3/Et_2O (0.7 ml). Then, the same was treated with acetamide (0.05 mol) and refluxed for over 20 h. The progress of the reaction was monitored by TLC analysis and after completion of the reaction, the reaction mixture was poured into ice cold water with constant stirring. Further, it was extracted with dichloromethane. The organic layer was collected and solvent was evaporated under reduced pressure. The crude product (**3**) was purified through silica gel column using petroleum ether: ethyl acetate as eluent.

1.3. *Spectral data of the reference compounds*

OXD-6: IR (cm^{-1}) (KBr): C=C (str) 1589.40, C=N (str) 1558.54, Ar C–H (str) 3047.63, C–Br (str) 688.61; ^{1}H-NMR (ppm) ($CDCl_3$): δ 8.02 (s, 1H), 8.02–7.99 (dd, J = 6 Hz, 3 Hz, 1H), 7.86–7.82 (m, 2H), 7.75–7.72 (dd, J = 7.29, 1.32 Hz, 1H), 7.74–7.40 (m, 3H), 7.37–7.29 (m, 2H); MS (m/z): $[M^+]$300.

OXD-7: IR (cm^{-1}) (KBr): C=C (str) 1580.01, C=N (str) 1548.89, Ar C–H (str) 3115.14, C–H (str) 2922.25; ^{1}H-NMR (ppm) (CDCl3): δ 7.96–7.90 (m, 3H), δ 7.85–7.81 (m, 2H), δ 7.46–7.27 (m, 5H), δ 7.44 (m, 3H); MS (m/z): M^+235.

OXD-9: IR (cm^{-1}) (KBr): C=C (str) 1620.26, C=N (str) 1566.25, Ar C–H (str) 3110.27, C–O (str) 1263.42, N=O 1518.03; ^{1}H-NMR (ppm) (CDCl3): δ 8.85 (d, J = 3 Hz, 1H), 8.31–8.27 (dd, J = 9Hz, 3 Hz, 1H), 7.97 (s, 1H), 7.83–7.79 (m, 2H), δ 7.47–7.49 (m, 2H), 7.47–7.42 (m, 2H), 7.38–7.32 (m, 1H), 4.04 (s, 3H); MS (m/z): M^+296.

OXD-11: IR (cm^{-1}) (KBr): C=C (str) 1604.83, C=N (str) 1581.68, Ar C–H (str) 3026.41; ^{1}H-NMR (ppm) (CDCl3): δ 8.05–8.02 (dd, J = 6 Hz, 3 Hz, 1H), 7.73–7.70 (m, 3H), 7.56–7.27 (m, 11H); MS (m/z): $[M^{+1}]^+$ 297, 165 (100%).

2. Biological activities

2.1. *Antioxidant activity-DPPH radical scavenging assay*

The assay was carried out in a 96 well microtitre plate. 100 μL of DPPH solution was added to 100 μL of each of the test sample of concentrations 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 μg/ml or the standard solution i.e., ascorbic acid, separately in each well of the microtitre plate. The plates were incubated at 37 °C for 20 min and the absorbance of each solution was measured at 540 nm, using Enzyme Linked Immuno Sorbent Assay (ELISA) microtitre plate reader. The absorbance of solvent control containing the same amount of methanol and DPPH solution was measured as well. The experiment was performed in triplicate and % scavenging activity was calculated using the formula given below. IC_{50} (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.[9]

2.2. *Antioxidant activity – nitric oxide scavenging assay*

The Nitric oxide scavenging activity of the compounds was tested at 500, 250, 125, 62.5, 31.25, 15.62 and 7.81 μg/ml