Free Radical Scavenging Effect of 2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid

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Abstract
2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid is a synthesized novel compound. Synthesis of compound was monitored using TLC and structure confirmed using FT-IR and UV/V analysis. The pH was observed to be 4.0. In vitro free radical scavenging activities was carried out by DPPH antioxidant assay which revealed significant activities against ascorbic acid, butylated hydroxylanisole and]-tocopherol employed as standards. This study shows that the synthesized compound has a free radical scavenging signature which could be a lead in the discovery of new drug in the face of multiple drug resistance of public concern.

Keywords: *Novel compound, Free radical scavenger*

Introduction

Organic chemistry has been a justified commercial tool in the synthesis of wide range of compounds. These sets of compounds have significantly led to prevention and cure of many terminal diseases due to oxidative stress. Free radicals are organic and inorganic species with at least an unpaired electron. Unpaired electrons when present in a biochemical system have been reported in literatures to be responsible for cell denaturing. Among reported ailments include ageing, cancer, cardiovascular and brain dysfunction, cataracts, immune system compromise etc. (Rao *et* al., 2006; Cantuti-Castelvetri *et al*., 2000; Neuzil *et al*., 1997; Stefanis *et al*., 1997; Esterbauer *et al*., 1991).

System biochemical failure is an endogenous process, attributed to free radical propagating nature of establishing chain reactions with an active site responsible for well-being, compromising it through covalent bonding or other forms biosynthetic mediated interactions. This leaves an unpaired electron at the active site which could be an enzyme responsible for a specific systemic function. This could only be terminated if a free radical scavenger is present to establish bonding. This process helps to ameliorate systemic failure.

2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid is a β-amino carbonyl compound often referred to as Mannich base. This reaction is based on the condensation of an active α-hydrogen containing compounds with formaldehyde and either a primary, secondary amine or ammonia. Some examples include: 4-{(dipropylamino){bis(methylene)}sulfanyl}-benzamide, 2-(3-Phenylaminopropionyloxy)-benzoic acid, 3-Phenylamino-1-(2,4,6-trimethoxy-phenyl)-propan-1-one etc. Mannich bases have a wide spectrum of physiological

submissions such as antimicrobial, antifungi, antiviral, anti-inflammatory, anticancer, antioxidant, anticonvulsant, muscle relaxant, antiplatelet, antimalarial, antihypertensive, antitumor, oxytocic, diuretic etc. (Oloyede *et al*., 2014; Valarmathi *et al*., 2011; Marikpuri *et al*., 2010; Saraswathi *et al*., 2010; Abdulrahman *et al*., 2008).

Compounds are usually elucidated by basic predictable methods which include: nuclear magnetic resonance (NMR), fourier transform infrared (FT-IR) and ultraviolet/visible (UV/V) absorption spectroscopies and mass spectrometry. Crystallography is another important technique among others. These basic techniques are based on interaction of matter with specific range of radiations from the electromagnetic zones (Pavia *et al*., 2001; Johnstone and Rose, 1996).

Drug resistance is a menace calling for new discoveries. The aim of this study is to ascertain the free radical scavenging effect of 2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid using standard protocol.

Materials and Methods

Chemicals and Reagents: Chemicals and reagents used for this study were analytical grade (BDH) which include: 2-acetoxybenzoic acid, formaldehyde, 3-nitroanline, methanol, ethanol, hexane, chloroform, potassium hydrogen phthalate, borax solution, ascorbic acid, α-tocopherol and butylated hydroxylanisole (BHA). 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was a product of Sigma-Aldrich, Germany.

Equipment/apparatus: HH-S water bath, pH meter (Hanna instrument), weighing balance (mettle H18), fourier transform infrared spectrophometer (Perkin-Elmer), ultraviolet/visible spectrophotometer (spectro UVD-2960), reflux condenser set-up, suction pump and glass wares, thin layer chromatography (TLC) F_{254} precoated plates and filter papers (grade no.1Whatmann).

General procedures:

Synthesis of 2-(3-[3-Nitrophenylamino]propanoyloxy) benzoic acid (SC)
Equivalent amount of 2-acetoxybenzoic acid, formaldehyde and 3-nitroaniline in absolute methanol were refluxed for two hrs, monitored with TLC and filtered above room temperature. The filtrate was kept below room temperature for crystallization. Recrystallization was done in ethanol (Mann and Saunders, 1978).

Spectroscopic analysis

The FT-IR spectrum of SC was recorded as KBr disc on a Perkin-Elmer FT-IR machine in the range of 4000-400 cm^{-1}. The compound was also scanned using spectro UVD-2960 for absorption in the uv/v range.

Purity test

TLC was employed using different solvent systems (ethyl acetate: hexane (3:1), ethyl acetate: chloroform (3:1) and methanol: ethyl acetate (1:3)).

pH determination

The pH of compound was determined using a pH meter standardized using standard buffers (0.05 M potassium hydrogen phthalate, pH 4.0 and 0.01 M borax solution, pH 9.0).

Free radical scavenging activity

The DPPH test with absorbance observed at 517 nm was used to evaluate the free radical scavenging effect of SC with ascorbic acid, α-tocopherol and BHA used as standards in a triplicate range dosed concentration (Oloyede *et al*., 2014; Mutee *et al*., 2010; Gulcin *et al*., 2002; Lugasi *et al*., 1999).

Results

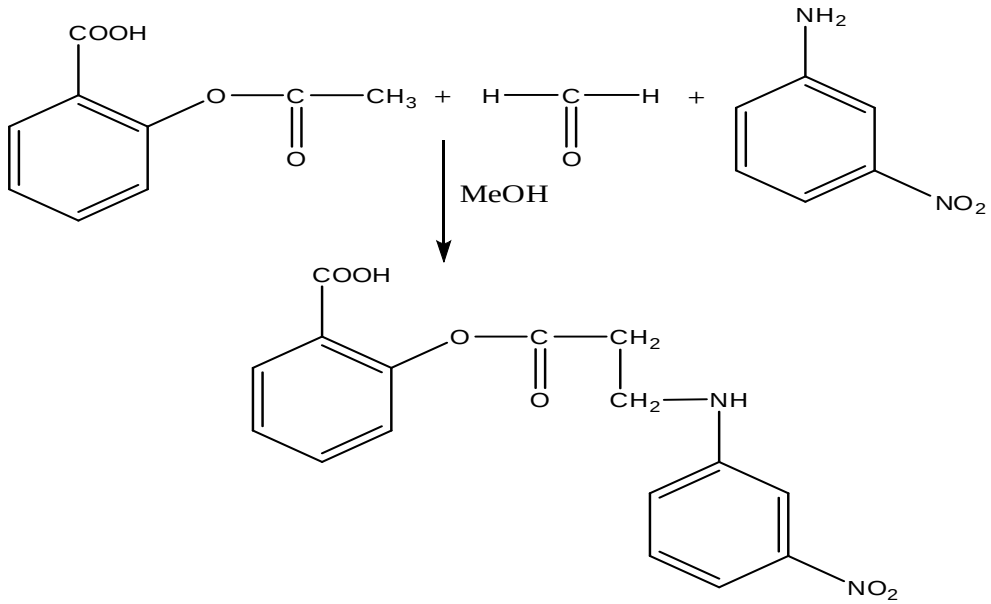
2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid

An orange solid with 75% yield which decolourizes on recrystallization and washing to form needle like crystals. The equation of reaction is shown in **scheme a**. The molecular formula of SC is $C_{16}H_{13}O_6N_2$ with an exact calculated molar mass of 305 $gmol^{-}$.

The FT-IR reveals the presence of the following stretches: 3493-3397 cm^{-1} (N-H str.), 3008cm^{-1} (C-H_{sp2} str.), 2870 cm^{-1} (C-H_{sp3} str.), 1758 and 1685 cm^{-1}(C=O str.) and 1606 cm^{-1} (C=C str.). Distinct absorptions were observed at 215 and 300 nm from the uv/v analysis.

The TLC profilings show a single spot using three different solvent systems (ethyl acetate: hexane (3:1), ethyl acetate: chloroform (3:1) and methanol: ethyl acetate (1:3). The pH was observed to be 4.0. Solubility in ethanol, methanol, ethyl acetate and chloroform was positive but negative in water and hexane.

Free radical scavenging effect of synthesized compound on DPPH with absorbance observed at 517 nm is reported in table 1.



Scheme a: equation of reaction for the synthesis of 2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid

Table 1: Free radical scavenging activity of SC on DPPH (absorbance at 517 nm)

Concentration mg/mL	%Inhibition			
	SC	α-tocopherol	Ascorbic acid	BHA
0.0625	91.95	23.73	96.78	95.17
0.1250	93.48	19.79	94.85	95.25
0.2500	94.42	31.62	96.86	95.82
0.5000	94.61	29.36	95.33	96.78
1.000	94.93	21.07	96.90	97.10

*SC- synthesized compound BHA- butylated hydroxylanisole. Absorbance measurement of DPPH standard is 1.243 at 517 nm

Discussion

2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid is an organic compound belonging to the class of β-amino carbonyl compounds. It is synthesized based on Mannich reaction involving the condensation of active methylene compound with formaldehyde and either a primary, secondary amine or ammonia (Oloyede *et al*., 2011). The structure of the compound was confirmed using peaks in the FT-IR spectrum obtained. The uv/v spectroscopic reveals the presence of electronic transitions supporting the unsaturated moieties in the synthesized compound. The degree of purity was ascertained using TLC profiling which indicate single spot in three solvent systems. The pH was observed to be 4.0 indicating of the presence of ionizable hydrogen atoms from a relatively weak acid. This could be attributed to the carboxylic, -COOH functional group present in the synthesized compound. It free radical scavenging potential (table 1.0) shows that, as the concentration increases it free radical scavenging activity increases. The set of electron pairs found around the electronegative oxygen and nitrogen species present in the basic functional groups, is responsible for its scavenging activities. When compared with standards it has considerably higher free radical scavenging property than α-tocopherol and significant comparable activities when compared with ascorbic acid and BHA.

Thus, 2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid is a prospective candidate for pharmacological and physiological signature.

Conclusion and recommendation

This study is a survey of organic synthesis as a comprehensive tool of synthesizing nature's hidden molecules from readily available factors. 2-(3-[3-Nitrophenylamino]propanoyloxy)-benzoic acid has been synthesized from 2-acetoxybenzoic acid, formaldehyde and 3-nitroaniline using Mannich reaction. Spectroscopic analysis revealed the following functional group moieties: C=O, C=C, N-H and C-H which were justified by the electronic transition obtained from uv/v analysis. The free radical scavenging activity is commendable and should be explored for mankind benefit. Thorough investigation into the characterization, toxicity and medicinal proclivity of this compound is opened for further work.

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