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Synthesis, Characterization and Antioxidant Potency of Naturally Occurring Phenolic Monoterpenoids Based Hydrazide Motifs

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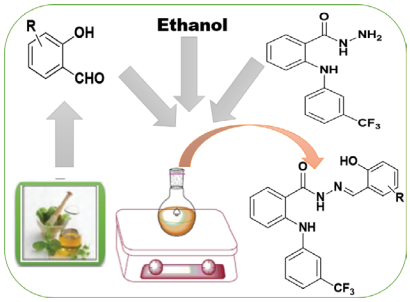
Abstract

Present article reports three novel compounds derived from flufenamic acid. Synthesis of hydrazide motifs was achieved by reaction between 2-((3-(trifluromethyl)phenyl)amino)benzoh drazide and naturally occurring phenolic monoterpenoids based aldehydes. The developed methodology offers mild and faster reaction conditions with excellent product yield. The synthesized compounds have been screened for their antioxidant activity using DPPH radical scavenger assay and ABTS assay. All the compounds exhibited good antioxidant activity as compared with standards that is, ascorbic acid and α-Tocopherol.



Keywords: Flufenamic acid; Antioxidant activity; DPPH; ABTS; Hydrazides

Introduction

Hydrazides are important key intermediates in the synthesis of biologically active scaffolds, and their synthesis has attracted noteworthy attention due to their utility as building blocks in medicinal chemistry [1-3]. Flufenamic acid, mefenamic acid, meclofenamic acid and numerous derivatives of anthranilic acid are Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) used as potent analgesic and anti-inflammatory agents in the treatment of various painful musculosketal illnesses [4-6].

In present era antioxidants motivate researcher's consensus towards both medicinal plants and synthetic compounds. Recently synthesis of a wide variety of biologically relevant scaffolds gained significant importance in medicinal chemistry as a tool for preparation of archives of compounds; considerable attention is focused on development of clean, high yielding, and environmentally friendly chemical processes and technologies [7]. The interference of free radicals has been responsible for causing the pathogenicity of numerous diseases [8-10], cardiovascular diseases [11-13], neural disorder and various chronic diseases [14-17]. The design of novel molecules to counteract harmful effect of free radicles and other oxidant is an important objective [18-20]. Antioxidants are recently invented as the drug candidates to counteract the diverse diseases, such as carcinogenesis, inflammation and aging in aerobic organism [21-23]. However, the widespread action of synthetic antioxidants is ruled out owing to their toxicity and unwanted side effects and there is a growing interest in the use of natural antioxidants and their derivatives for treatment of oxidative stress related diseases [24-26].

Natural phenolic compounds are known for their antimicrobial and antioxidant properties. They act as free radical scavengers and their antioxidant potential depends on the substituent present and the extent of structural conjugation [27-30]. Extract of Thymus vulgaris possesses wide variety of biological and pharmaceutical activities. Oils of thyme are used as food preservatives and for the treatment of several diseases [31,32]. The two isomer of thyme oil thymol and carvacrol exhibit antioxidant, free radical scavenger, antibacterial, antifungal, antiviral, antitumor and anti-inflammatory activities [33,34]. Eugenol is pale yellow, aromatic oily liquid extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf. It is a well-known natural product occurring in many angiospermic plants [35].

In present study we have prepared three phenolic monoterpenoids based novel derivatives as antioxidants; and assessed *in vitro* by using DPPH radical scavenging assay and ABTS assay. DPPH is a stable free radical which has an unpaired valence electron at one atom of nitrogen bridge; scavenging of DPPH radical is the basis of the prevalent DPPH antioxidant assay [36-39]. Stable radicals of ABTS are generated when ABTS is mixed with potassium persulfate and incubated in dark condition [40].

In this work, we have synthesized naturally occurring phenolic monoterpenoid based derivatives; characterized them by sophisticated analytical techniques and *in vitro* antioxidant

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potency of these compounds has been investigated through their interaction with 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2-azino-bis(3ethylbenzothiazoline-6-sulfonic acid) (ABTS).

Experimental

Melting points of all the synthesized compounds were determined by the open capillary method. The confirmation of synthesized compounds was carried out by thin layer chromatography on 200 µm thick aluminum sheets supported with silica gel 60 F254 as an adsorbent by developing the TLC plate using hexane:ethyl acetate (1:1) solvent system. Spots were visualized under UV-light. 1H and 13C NMR spectra were scanned at Bruker AC-400 MHz spectrometer FT NMR in DMSO-d_6 using TMS as an internal standard. The chemical shift values are on δ scale. The purity of synthesised compounds was measured by using GC Shimatzu 2010 plus having GC solution software. All the chemicals and solvents required for the experimental work were locally purchased from Sigma-Aldrich, S. D. Fine chemicals, Himedia and were used as usual without further purification.

Synthesis of 2-((3-(trifluromethyl)phenyl)amino)benzoyl chloride

Flufenamic acid (1 g, 0.0035 mole) was charged into round bottom flask and then thionyl chloride (1.5 mL, 0.0126 mole) was added to it. The reaction mixture was stirred for 10-15 min at room temperature, then maintained at reflux condition for 60-90 min; cooled and then used for next step.

Synthesis of 2-((3-(trifluromethyl)phenyl)amino)benzo hydrazide

In the product prepared in above step hydrazine hydrate (5 mL) was added drop wise and put on reflux condition for 90-120 min. The product obtained was filtered, washed 2-3 times with water and then dried at room temperature. The product was recrystallized by using suitable solvent.

Synthesis of (E)-N'-(5-allyl-2-hydroxy-3-methoxybenzy lidene)-2-((3 (trifluromethyl)phenyl)amino)benzo hydrazide

2-((3-(trifluromethyl)phenyl)amino)benzo hydrazide (0.5g, 0.0016 mole) in 10 ml ethanol was charged in round bottom flask, then ethanolic solution of 5-allyl-2-hydroxy-3-methoxybenzaldehyde (0.33 g, 0.0016 mole) was added to it and the reaction mixture was refluxed for 90-120 min. Recrystallization of the product was carried out with suitable solvent. M.P. 110-114°C, 1H NMR (400 MHz, DMSO-d_6) δ 3.65-3.71 (d, 2H), δ 3.88 (s, 3H), δ 5.04-5.10 (d, 2H), δ 5.99 (s, 1H), δ 6.74 (s, 1H Ar), δ 6.87 (s, 1H Ar), δ 6.96-6.99 (t, 1H Ar), δ 7.18-7.30 (m, 1H Ar), δ 7.45-7.48 (m, 5H Ar), δ 7.76-7.78 (d, 1H Ar), δ 8.57 (s, 1H OH), δ 9.48 (s, 1H imine CH), δ 11.00 (s, 1H NH), δ 12.06 (s, 1H NH).

Synthesis of (E)-N'-(2-hydroxy-6-isopropyl-3-methylbenzyli dene-2-((3-(trifluromethyl)phenyl)amino)benzohydrazide

To the solution of 2-((3-(trifluromethyl)phenyl)amino)benzo hydrazide (0.5 g, 0.0016 mole) dissolved in 20 mL ethanol; 20 mL ethanolic solution of 2-hydroxy-6-isopropyl-3-methylbenzaldehyde (0.30 g, 0.0016 mole) was added and reflux condition was maintained up to 90-120 min. After completion of the reaction product was filtered and washed with ethanol and dried at room temperature. M.P. 128-130°C, 1H NMR (400 MHz, DMSO-d_6) δ 1.22-1.27 (d, 6H), δ 2.18 (s, 3H), δ 3.24-3.29 (m, 1H), δ 6.70-6.72 (d, 1H Ar), δ 6.96-6.99 (t, 1H Ar), δ 7.10-7.12 (d, 1H Ar), δ 7.19-7.21 (d, 1H Ar), δ 7.39-7.48 (m, 6H Ar), δ 7.79-7.81 (d, 1H imine CH), δ 9.03 (s, 1H,OH), δ 9.64 (s, 1H NH), δ 12.67 (s, 1H NH).

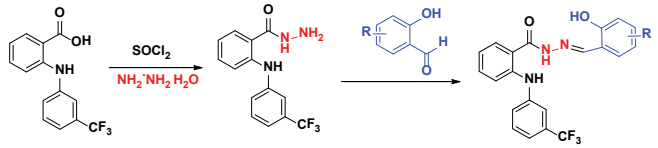
Synthesis of (E)-N'-(2-hydroxy-3-isopropyl-6-methylbenzyli dene-2-((3-(trifluromethyl)phenyl)amino)benzohydrazide

The ethanolic solution of 2-((3-(trifluromethyl)phenyl)amino) benzo hydrazide (0.5 g, 0.0016 mole) was prepared and drop wise added to the ethanolic solution of 2-hydroxy-3-isopropyl-6-methylbenzaldehyde (0.30 g, 0.0016 mole) and reflux condition was maintained for 90-120 min. The product was filtered and recrystallized with suitable solvent and dried at room temperature. M.P. 122-124°C, 1H NMR (400 MHz, DMSO-d_6) δ 1.19-1.24 (t, 6H), δ 2.38 (s, 3H), δ 3.54 (m, 1H), δ 6.64-6.66 (d, 1H Ar), δ 6.97-7.00 (t, 1H Ar), δ 7.07-7.09 (d, 1H Ar), δ 7.19-7.21 (d, 1H Ar), δ 7.38-7.47 (m, 6H Ar), δ 7.80-7.82 (s, 1H imine CH), δ 8.89 s, (1H OH), δ 9.64 (s, 1H NH), δ 12.56 (s, 1H NH) (Scheme 1).

Melting point and purity were recorded and the data is compiled in Table 1.

Antioxidant activity: DPPH free-radical scavenging is a method regularly used to evaluate the antioxidant property of natural or synthetic compounds. This method is based on reaction of DPPH with hydrogen donors to produce a stable product, causing a colour change from purple to faint yellow [14].

DPPH radical scavenging activity: The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging method described by Wu et al. [41,42] was used to assay the free radical scavenging ability of flufenamic hydrazide derivatives with certain modifications. 1 ml of 0.2 mM DPPH reagent prepared in methanol was added in test



Scheme 1: Synthesis of (E)-N'-(2-hydroxy-3-isopropyl-6-methylbenzylidene-2-((3-(trifluromethyl) phenyl) amino)benzohydrazide

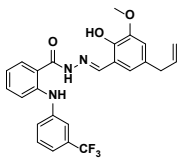
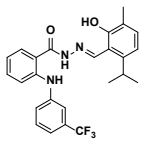
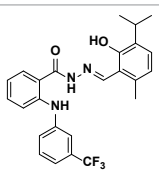
S. No.	Structure	M.P./B.P. (°C)	Purity (%)	% RSA (DPPH)	% RSA (ABTS)
1	 FLE	110-114	99.66	82.53	73.95
2	 FLC	128-130	99.69	78.64	86.30
3	 FLT	122-124	99.71	80.32	80.48

Table 1: Data of melting point and purity.

tubes containing 0.8 mL of each compound (1 mg mL^{-1} in DMSO), then mixture was kept in dark for 30 min at room temperature. Similar protocol was performed for α-Tocopherol and L-ascorbic acid as standard known antioxidant. The absorbance of the resulting mixture was measured at 517 nm by UV-Vis spectrophotometer. Control was prepared by composition of 0.8 ml of DMSO and 1 ml DPPH reagent, and analysed as described above. The % scavenging activity was determined by following equation:

$$\text{Radical scavenging activity (\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorption of control (blank, only DMSO) and A_1 is the absorption of respective compounds (Figure 1).

ABTS assay: The ABTS ability of the compounds was determined using the protocol described earlier [43]. Solution of 7 mM 2,2-azino-bis(3ethylbenzothiazoline-6-sulfonic acid) (ABTS) and potassium persulfate (2.45 mM) was prepared and incubated in the dark for 14-16 hrs, thereafter solution was diluted using ethanol until the absorbance reached to 0.7 at 734 nm. In a fresh test tube, one milliliter of diluted solution was mixed with 100 µL of (1 mg mL^{-1}) compound, and after 5 min the absorbance was measured at 734 nm. Ascorbic acid and α-Tocopherol were used as standards for comparison. The percentage reduction (I%) against ABTS was calculated using the following equation:

$$\text{Percentage reduction (I\%)} = [(A_0 - A_1)/A_0] \times 100$$

Where A_0 is the absorption of control (blank, only DMSO) and A_1 is the absorption for the compounds (Figure 2).

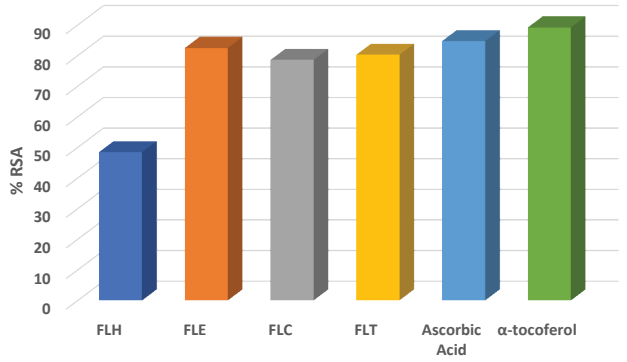


Figure 1: *In vitro* antioxidant activity of synthesized derivatives by using DPPH radical scavenging assay.

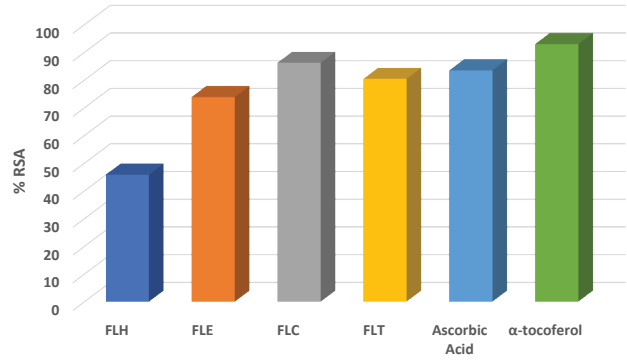


Figure 2: *In vitro* antioxidant activity of synthesized derivatives by using ABTS assay.

Conclusion

In summary, we have successfully developed three novel substituted hydrazides. The novelty of this work is the use of a nontoxic, low-cost, and environmentally benign reagents for synthesis of derivatives. The reaction conditions are clean and mild. Reaction time is short, and product yield is appreciable. Most important is that all compounds show remarkable and acceptable antioxidant activity; in some cases comparable with standards. Among all tested compounds FLE is more potent than others in DPPH assay; while in ABTS assay FLC shows more activity.

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