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Imidazo[1,2] Coumarin analogues: Synthesis and antioxidant properties

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Research Article

Abstract

Two coumarin derivatives, namely 3-benzo[d]imidazo[2,1-b]thiazol-2-yl)-chromen-2-one (IBT) and 3-(8-hydroxy-imidazo[1,2-a]pyridin-2-yl)-chromen-2-one (IMPY), were synthesized and their antioxidant properties were investigated using spectrophotometric methods. The percentage of radical scavenging activity (%RSA) for each compound was determined using the α, α-diphenyl-β-picrylhydrazyl (DPPH) assay method. A comparative analysis of the %RSA values obtained with those of ascorbic acid, a well-known antioxidant, revealed that both IBT and IMPY exhibited considerable antioxidant activity, indicating their potential as antioxidants. IBT demonstrated moderate antioxidant properties, with an %RSA value of 70.65% at a concentration of 10^{-3} M, which was slightly lower than that of ascorbic acid, but still significant.

Keywords: Coumarin, Antioxidant, Radical scavenging activity, DPPH, Imidazobenzothiazole, Spectrophotometric Study

INTRODUCTION

Coumarins are classified as a member of the benzopyrone family which were isolated in 1820 from Tonka beans whose vernacular name is 'Coumarou'. Coumarins are of four main subtypes, namely the simple coumarins, furanocoumarins, pyranocoumarins and the pyrone-substituted coumarins. Simple coumarins comprise of hydroxylated, alkoxylated and alkylated derivatives of the parent compound, coumarin, along with their glycosides. While, furanocoumarin comprises of a five-membered furan ring attached to the coumarin nucleus, divided into linear or angular types with substitution at one or both of the remaining benzoid positions. Members which are analogous to the furanocoumarins, but contain a six-membered ring are called pyranocoumarins. Coumarins substituted in the pyrone ring include 4-hydroxycoumarin. The synthetic compound, Warfarin, belongs to this coumarin subtype.

A very large class of compounds found throughout the plant kingdom contains Coumarin as a component. The coumarins are found at high levels in some essential oils, particularly cinnamon bark oil (7,000 ppm), cassia leaf oil (up to 87,300 ppm) and lavender oil. Coumarin is also found in green tea, fruits (e.g. bilberry, cloudberry) and other foods such as chicory. Higher plants usually contain a large variety of Coumarin with the richest sources being the Rutaceae and Umbelliferon.

Coumarins are present at the highest levels in the fruits, followed by the roots, stems and leaves i.e. it is distributed throughout all parts of the plant. But environmental conditions and seasonal changes greatly influence its occurrence in the diverse parts of the plants. Recently, six new minor coumarins have been isolated from the fruits and the stem bark of *Calophyllum dispar* (Clusiaceae). The genus *Calophyllum*, which comprises 200 species, is widely distributed in the tropical rain forest where several species are used in folk medicine. Coumarins are known for their diverse pharmacological properties.[1]

Molecular oxygen enables the formation of reactive oxygen species (ROS), This is essential for many physiological processes in small quantities hence is an indispensable element for the life of aerobic organisms. But these are very toxic at high doses. The major ROS species in humans are the superoxide anion ($O_2^{\bullet-}$), hydrogen peroxide ($H_2O_2^{\bullet}$), radical hydroxide ($OH^{\bullet-}$) and the reactive nitrogen species (RNS), and nitric monoxide (NO). These are constantly generated within the cells during transfer of electrons upon exposure to various pollutants and harmful radiations like the ultraviolet rays. These free radicals presumably have deleterious effects in the human body such as deterioration of fats and other constituents of foodstuffs. There is an increasing interest in antioxidants, particularly in those intended to prevent these hazardous effects. Moreover, there is a preference for antioxidants from natural rather than from synthetic sources. In view of this, coumarin and its derivatives prove a good choice provided they show significant antioxidant activity.[2-4]

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It has been reported that oxidative stress, which is responsible for inflammation, atherosclerosis, and Alzheimer's disease, can be caused by the formation of hydroxyl radical ($^{\bullet}OH$) from hydrogen peroxide (H_2O_2) in the presence of a reducing agent such as superoxide ion ($O^{\bullet-2}$). This ultimately leads to damage to cells and tissue. It has been known that aromatic compounds such as phenols, phenolic acids, and amino acids like coumarin, benzothiazole, tyrosine, phenylalanine react rapidly with $^{\bullet}OH$ radical to form a hydroxylated product.[5-8] Hence an effective site specific $^{\bullet}OH$ radical scavenger has been synthesized by conjugation of coumarin with aromatic heterocyclic derivatives which can be expected as an antioxidant.[9-12]

Imidazo[1,2]coumarin analogues are a class of compounds that have received attention due to their potential therapeutic applications, such as antitumor, antiviral, antibacterial, and antioxidant properties. The synthesis of imidazo[1,2]coumarin analogues involves the reaction of imidazole and coumarin derivatives, which are readily available and inexpensive. Several synthetic strategies have been developed to prepare imidazo[1,2]coumarin analogues. One such approach involves the reaction of coumarin derivatives with o-phenylenediamine in the presence of a suitable acid catalyst, followed by cyclization with formaldehyde. Another method involves the reaction of coumarin derivatives with 1,2-diamines in the presence of a suitable acid catalyst, followed by cyclization with formaldehyde. In addition, imidazo[1,2]coumarin analogues can also be synthesized through a one-pot, three-component reaction using coumarin derivatives, aldehydes, and ammonium acetate.[13]

Imidazo[1,2]coumarin analogues have been reported to possess significant antioxidant activity. The antioxidant activity of these compounds is attributed to their ability to scavenge free radicals and inhibit lipid peroxidation. Studies have also suggested that imidazo[1,2]coumarin analogues can protect against oxidative stress-induced cellular damage, such as DNA damage and protein oxidation. In conclusion, the synthesis of imidazo[1,2]coumarin analogues is a promising area of research due to the potential therapeutic applications of these compounds. Their significant antioxidant activity highlights their potential as candidates for the development of new drugs for the treatment of diseases associated with oxidative stress.

In the present study, coumarin-conjugated benzothiazole heterocyclics have been synthesized and their radical scavenging activity has been analyzed by DPPH assay spectrophotometrically, which suggested significant antioxidant behavior towards free radicals.

EXPERIMENTAL

SYNTHETIC METHODOLOGY

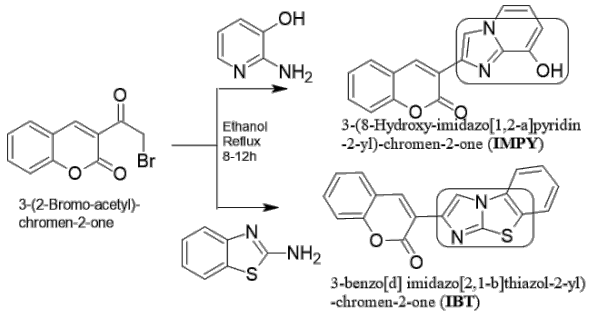
Synthesis of 3-benzo[d] imidazo[2,1-b]thiazol-2-yl)-chromen-2-one (IBT), and 3-(8-Hydroxy-imidazo[1,2-a]pyridin-2-yl)-chromen-2-one (IMPY)

A solution of 3-bromoacetyl coumarin (1.872 mmoles, 500 mg) in ethanol was stirred on a magnetic stirrer. In the reaction mixture of 2-amino-benzothiazole (1.872 mmoles, 311.14 mg) was added.[5] Reaction mixture (RM) was refluxed at 80 °C for 8 h. Brown color precipitates were obtained in the RM which was filtered and repeatedly washed with diethyl ether. These precipitates were dried under a vacuum.

3-benzo[d] imidazo[2,1-b]thiazol-2-yl)-chromen-2-one (IBT): ^{1}H NMR (400 MHz, d_6-DMSO): δ=8.60 (s, 1H, ArH), 8.49 (s, 1H, ArH), 7.86-7.84 (d, 1H, ArH), 7.74-7.72 (d, 1H, ArH), 7.51-7.47 (t, 1H, ArH), 7.45-7.15 (m, 4H, ArH), 6.87-6.84 (d, 1H, ArH); ^{13}C NMR (100 MHz, d_6-DMSO): δ=158.95, 155.67, 152.37, 147.03, 139.05, 136.09, 134.79, 131.41, 131.03, 130.56, 128.56, 124.81, 120.30, 119.45, 116.02, 114.71, 113.42, 110.73;

For the synthesis of IMPY, a solution of 2-amino-3-hydroxy-pyridine (1.387 mmoles, 250 mg) in ethanol was stirred on a magnetic stirrer[13] and 3-bromoacetyl coumarin (1.387 mmoles, 370.38 mg) was added. RM was kept on refluxing at 80 °C for 24 h. Yellow color precipitates were obtained in the reaction mixture which was filtered and repeatedly washed with diethyl ether. Precipitates obtained were dried under a vacuum.

3-(8-Hydroxy-imidazo[1,2-a]pyridin-2-yl)-chromen-2-one (IMPY): ^{1}H NMR (400 MHz, d6-DMSO): δ=8.97 (s, 1H, ArH), 8.89 (s, 1H, ArH), 8.45 (d, 1H, ArH), 7.79 (t, 1H, ArH), 7.72 (t, 1H, ArH), 7.51 (m, 1H, ArH), 7.25 (t, 1H, ArH), 7.21 (t, 1H, ArH); ^{13}C NMR (100 MHz, d6-DMSO): δ=158.25, 153.21, 143.77, 141.07, 135.70, 133.62, 129.59, 125.69, 120.32, 118.82, 117.66, 116.73, 116.20, 115.69.



Scheme 1. Synthetic Methodology for IBT and IMPY

SPECTROPHOTOMETRIC STUDIES

The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. This spectrophotometric assay uses the stable radical, 1,1-diphenyl-2-picrylhydrazyl (DPPH$^{\bullet}$), as a reagent. The DPPH radical absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, its absorption decreases. When a hydrogen atom or electron was transferred to the odd electron in DPPH$^{\bullet}$, the absorbance at 517 nm decreased proportionally to the increases of non-radical forms of DPPH. The DPPH$^{\bullet}$ solution was freshly prepared daily, stored in a flask covered with aluminum foil, and kept in the dark at 4 °C.[13] The assay was carried out in quartz cuvette and the final reaction mixture was 3 mL in a cuvette was taken. A methanol DPPH solution (1000 µM, final concentration) (2 mL) was mixed with different concentration of the heterocyclic molecules (IBT, and IMPY) (1000, 500, 100, 50, 10, 5, 1, 0 µM, final concentration) (2 mL) freshly prepared in methanol. The resulting mixture was incubated at RT in the dark

and after 0, 30, 60 and 90 min, 3 mL of reaction mixture was placed in a cuvette and the absorbance of DPPH radical was measured at 517 nm in a double beam spectrophotometer. The antioxidant activity was determined as the %RSA (% radical scavenging activity), calculated as follows:

$$\%RSA = [(A_0-A_i)/A_0] \times 100$$

where A_0 and A_i are the DPPH absorbance in the absence and in presence of added heterocyclic molecules (IBT and IMPY) at different concentrations respectively.[14-17]

Result and Discussion

Synthesis

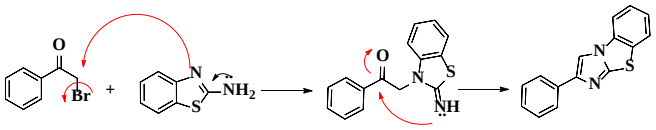
IBT was synthesized by the cyclization reaction between 3-bromoacetyl coumarin and 2-amino benzothiazole as shown in Scheme 1. The product was obtained with yield of 40.16% and its structure has been confirmed by ^{1}HNMR, and ^{13}CNMR spectroscopy.

Scheme 1 also revealed the synthesis of IMPY via a cyclization reaction between 3-bromoacetyl coumarin and 2-amino-3-hydroxy pyridine. Synthesized product was obtained with a yield of 45.62% and characterized by ^{1}H NMR, and ^{13}C NMR spectroscopy techniques.

Synthesis of these heterocyclic compounds can be explained by the cyclization mechanism shown in Scheme 2, where 3-bromoacetyl coumarin react with 6-amino benzothiazole (or 2-amino pyridine-2-ol) and 2-(2-iminobenzo[d]thiazol-3(2H)-yl)-1-phenylethan-1-one is formed as an intermediate through nucleophilic substitution reaction. The intermediate 2-(2-iminobenzo[d]thiazol-3(2H)-yl)-1-phenylethan-1-one can undergo cyclization in the presence of a suitable solvent to form the desired product, 2-phenylbenzo[d]imidazo[2,1-b]thiazole.

These synthesized molecules were further studied for their antioxidant property and compared with that of Ascorbic acid, a well-known antioxidant using the DPPH assay Scavenging Method. This method has been widely used to evaluate the free radical scavenging effect of various antioxidant substances. The basic principle behind this assay is the color change of DPPH solution from purple to yellow as the radical is quenched by the antioxidant and this color change was measured at 517 nm from the spectrophotometer. This color change involves the reduction of DPPH in alcoholic solution in the presence of a hydrogen-donating antioxidant due to the formation of the non-radical form DPPH-H in the reaction.[13c] Usually DPPH is used as a reagent to evaluate free radical scavenging activity of antioxidants. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule.

Absorbance at 517 nm was noted at different concentrations for the heterocyclic molecules (IBT, and IMPY) and ascorbic acid, taken as reference antioxidant for this experiment. The observed Radical scavenging activity (%RSA) at different concentrations for both heterocyclic molecules (IBT and IMPY) and ascorbic acid are shown in Figure 1 and Table 1.



Scheme 2. Plausible synthetic mechanism for IBT

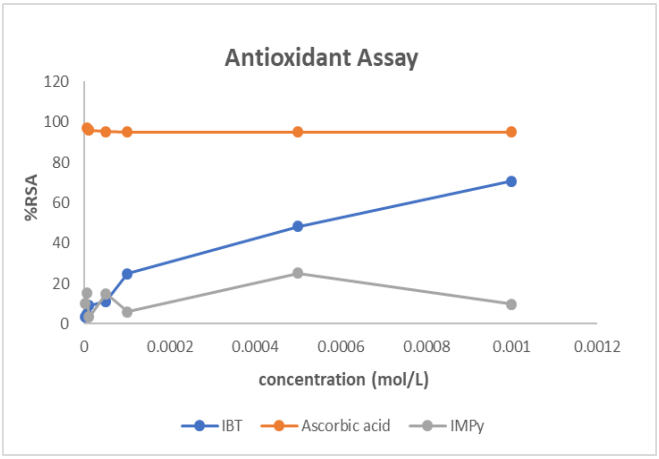


Figure 1. Percentage RSA *vs* Concentration plot for the synthesized heterocyclic molecules and Ascorbic acid

Table 1. Percentage RSA at different Concentration for the synthesized heterocyclic molecules and Ascorbic acid

Concentration	IBT	IMPY	Ascorbic acid
0.001	70.65	9.8	94.99
0.0005	48.32	25.17	94.89
0.0001	24.89	5.98	94.99
0.00005	11.26	15	95.19
0.00001	9.04	3.48	95.89
0.000005	4.97	15.14	96.79
0.000001	3.6	10.34	

Conclusion

Two molecules (IBT and IMPY) were synthesized successfully in the wet chemistry lab and their antioxidant properties were analyzed spectrophotometrically. IBT is observed to have comparatively higher radical scavenging activity than the other synthesized molecule, IMPY. The reason for this may be attributed to the coumarin moiety attached to the fused heterocyclic system. IBT is found to have a Percentage RSA value of 70.65%, a value, not better than that of ascorbic acid, but a significantly good value to behave as a potential antioxidant.

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SUPPLEMENTARY INFORMATION

The spectra of synthesized compounds is available as Supporting Information file and can be downloaded from article page.

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