Research Article

Evaluation of the Antioxidative Properties of *N*-Acylamino-Substituted Tricyclic Imides

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Received 25 April 2013; Revised 26 July 2013; Accepted 26 July 2013

Academic Editor: Esteban P. Urriolabeitia

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New *N*-acylamino-substituted tricyclic imides have been screened for scavenging ability against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH[•]), chelating activity on ferrous ions, and reductive potential. The results were compared with synthetic antioxidants BHT, BHA, and Trolox. The compounds exhibited different levels of antioxidant activity in all tests.

1. Introduction

N-substituted imides, such as maleimides [1], isohematinic acids [2], and especially bicyclic and tricyclic derivatives such as tandospirone derivatives [3, 4], were found to be remarkable due to variable pharmacological properties, and thus present antibiotic [5], fungicidal [6], analgesic [7], anxiolytic, and cytostatic effects [8]. The imide moiety is an integral structural part of various important bioactive molecules such as fumaramidmycin, granulatimide, isogranulatimide, and rebeccamycin. These molecules are reported to exhibit antidepressant, antitumor, anti-inflammatory, and antimicrobial activities [9, 10]. On the other hand, various bicyclic structures such as epiboxidine and epibatidine are biologically important molecules (Figure 1). A literature search reveals that certain compounds with antitumor activity, and in particular molecules able to interact with DNA, are characterized by the presence of both an extended π -system and an imide function [11–13]. Apart from biological activities, imide derivatives are useful in the reactions involving condensation, alkylation, acylation, and cyclocondensation.

Oxidation is essential to many living organisms for the production of energy to fuel biological processes. However, oxygen-centered free radicals or other reactive oxygen species (ROS), which are continuously produced *in vivo*, result in cell death and tissue damage. The role of oxygen radicals has been implicated in several diseases,

including cancer, diabetes, cardiovascular diseases, neural disorders, skin irritations, inflammations, and aging [14, 15]. Antioxidants deactivate and scavenge free radicals and inhibit the effect of oxidants by donating hydrogen atom or chelating metals. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are used as additives in foods to prevent oxidation of lipids. Besides, BHA and BHT are restricted by legislative rules because of doubts over their toxic and carcinogenic effects [16]. Therefore, there is a growing request and interest for safer antioxidants in food and pharmaceutical applications.

The Heck reaction, in particular, is widely used as an important method to build biologically active compounds in synthetic chemistry and the pharmaceutical industry [17, 18]. As an extension of the Heck reaction, Pd-catalyzed hydroarylation of alkynes and alkenes continues to attract researchers' interest in simple coupling processes and cyclization reactions [19, 20]. In the presence of triphenylarsine as a ligand [21, 22] the palladium-catalyzed hydroarylation of the easily accessible, unsaturated tricyclic *N*-substituted imides such as aryl- and methyl-substituted, epibatidine, epiboxidine, and tandospirone analogs have been proven to be a stereoselective, versatile, and high-yield approach for the synthesis of the corresponding aryl and heteroaryl derivatives [23–31].

Recently, we synthesized *N*-acylamino-substituted tricyclic imides by the palladium-catalyzed hydroarylation

FIGURE 1: (a) Rebeccamycin, (b) tandospirone, and (c) epibatidine.

reactions and characterized by spectral methods [32]. Here, we focused on the effect of these Heck compounds on the antioxidant activity. Using different chemical reaction-based assays, new synthesized *N*-acylamino-substituted tricyclic imide molecules have been screened for scavenging ability against the free radical 2,2-diphenyl-1-picryl-hydrazyl (DPPH[•]), chelating activity on ferrous ions, and reductive potential. The results were compared with the synthetic antioxidants BHT, BHA, and Trolox.

2. Results and Discussion

2.1. Chemistry. The synthesis of tricyclic imides were undertaken by Diels-Alder and acylation reactions by known procedures (Figure 2). Aryl- and heteroaryl-substituted *N*-acylamino-substituted tricyclic imide derivatives were synthesized by the reductive Heck conditions (Figure 3).

We also selected 5-iodo-3-methylisoxazole and 2-chloro-5-iodopyridine as the arylation reagents because of the structural similarity with epibatidine and epiboxidine [33, 34] which are known to behave as a potent $\alpha 4\beta 2$ nicotinic receptors. In this study, we analyzed the antioxidant effect of these reagents.

2.2. Antioxidant Activity Studies

2.2.1. Reducing Power. Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of antioxidant action, and can be strongly correlated with other antioxidant properties. In the reducing power assay, the presence of antioxidants results in the reduction of the Fe³⁺/ferricyanide complex to its ferrous form. Figure 4 shows the extension of the reduction, in terms of absorbance values at 700 nm, for the samples ranging in concentration from 25 to 100 $\mu\text{g/mL}$. From a comparison of the absorbance at 700 nm, the reducing power of the synthesized compounds was not concentration dependent. Compounds **4a-b**, **4d**, **5b-c**, and **6a-f** showed the best reducing activity when compared with other tested compounds. These activities were found significantly similar for all samples. This may be due to the fact that these molecules meet hydrophilicity, stereochemical, and electronic requirements

of the target in a better way as compared to other molecules. On the other hand, compounds **6a-f**, which possess an Ar¹ group introduced in position C5 of the heterocyclic ring, were more active than those in which such an Ar group is not attached in position C5, suggesting that conjugated double bonds and electronegative atoms in the ring system are involved in the reducing power activity. Compounds **6c** and **6f** that contain epibatidine and epiboxidine structure units, respectively, are known as biologically important groups and are expected to exhibit higher activity. This was confirmed by our results. The presence of alkene -O-, -S-, -N-, and -Cl- on the heterocyclic ring system seems to increase the activity of compounds. Compounds **1**, **3**, and **4c** showed the moderate activity. Compounds **2**, **5a**, and **5d** and all concentrations showed weaker activity than the other compounds and the standards. Standard antioxidants BHA, BHT and Trolox were approximately 2-fold more active than the samples.

2.2.2. Metal Chelating Activity. The ferrous ion (Fe²⁺) chelating effect of the newly synthesized compounds is presented in Figure 5. Compounds **4a**, **5a**, and **6a-f** showed moderate chelating activity on ferrous ions at an incubation time of 30 min. Other compounds tested gave an excellent chelating ability at the same conditions. The results were compared with EDTA at the same concentrations. None of the extracts appeared to be better chelators of iron(II) ions than the positive control EDTA in this assay system. At 100 $\mu\text{g/mL}$ concentration, EDTA was given 96% chelating effect on ferrous ions at an incubation time of 30 min.

2.2.3. Free Radical Scavenging Activity. The free radical scavenging action is known as an important mechanism of antioxidation. 1,1-diphenyl-2-picryl-hydrazyl (DPPH[•]) is used as a free radical to evaluate the antioxidative activity of some synthetic sources. The disappearance of DPPH[•] is directly proportional to the amount of antioxidants present in the reaction mixture. Antioxidants react with stable free radical 1,1-diphenyl-2-picryl-hydrazyl and convert it to a 1,1-diphenyl-2-picryl-hydrazine. The transfers of hydrogen or electron from antioxidant to DPPH[•] occur at different redox potentials and also depend on the structure of the antioxidant. Among all the samples, compounds **1**, **2**, **3**, **4b**,

FIGURE 2: Synthesis of tricyclic imides, 1, 2, and 3.

FIGURE 3: Synthesis of *N*-acylamino-substituted tricyclic imides.

4c, **5a**, **6a**, and **6d** showed the highest free radical scavenging activity at the concentration 25–100 $\mu\text{g/mL}$. The results for all the compounds are shown in Figure 6. The free radical scavenging effect was not concentration dependent. However, it was generally observed that the effect increased as the concentration of the compounds **6c–f** increased to a certain extent. Scavenging activity of BHA, BHT, and Trolox as known antioxidants was higher than that of samples. From these results, it can be stated that the samples tested have the moderate ability to scavenge free radicals and could serve as free radical inhibitors or scavengers according to the synthetic antioxidants.

The radical scavenging activities of the new compounds, expressed as an IC_{50} value, ranged from 6.46 to 81.63 $\mu\text{g/mL}$. IC_{50} values (the inhibitory concentration at which the DPPH radicals were scavenged by 50%) of compounds **6b**, **6c**, **6e**, and **6f** were higher than that of other compounds, which were comparable. A higher DPPH radical scavenging activity is associated with a lower IC_{50} value. It was evident that the compounds **6b**, **6c**, **6e**, and **6f** did show the radical scavenging ability to act as antioxidants (Table 1).

3. Conclusion

In summary, free radical scavenging, metal chelating, and reducing power activities of tested synthesized compounds were screened. Our results showed that the compounds have a mild antioxidant activity at various antioxidant systems *in vitro*. The newly compounds were potent radical scavengers, and their antioxidant capacities seem to be related to their

FIGURE 4: Reducing power activity of new compounds.

chemical compositions. Compounds **6c** and **6f** exhibited the highest radical scavenging activity than other compounds tested at 100 $\mu\text{g/mL}$ concentration. These compounds contain epibatidine and epiboxidine structure part, respectively. The further studies suggested the pharmacological and biological importance of the epibatidine and epiboxidine groups. Our results confirm their effect. Ar^1 substitutions at the C5 position are an attractive site for reductive potential. In addition, the benzyl-substituted compound **4a** also gave the highest chelating activity.

4. Experimental Section

4.1. Chemicals. The antioxidant activities of the *N*-acylamino-substituted tricyclic imides were evaluated

FIGURE 5: Metal chelating activity of new compounds.

based on the ability of the compounds to scavenge DPPH radicals, to reduce Fe(III) to Fe(II), and to bind to Fe(II) ions. The standard chemicals, 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), the stable free radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH^{*}), and trichloroacetic acid (TCA) were obtained from Sigma (Sigma-Aldrich GmbH, Germany). Butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were provided from Fluka (Buchs, Switzerland). Unless specified otherwise, all other reagents and solvents used were of analytical grade obtained from commercial suppliers.

4.2. Antioxidant Activity Studies

4.2.1. Reducing Power. In the reducing power assay, the presence of reductants (antioxidants) in the samples results in the reduction of the Fe³⁺/ferricyanide complex to its ferrous form. The reducing powers of the samples BHA, BHT, and Trolox were determined according to the method described by Oyaizu [35]. Various concentrations of the samples (25–100 µg) in 1 mL of distilled water were mixed with 2.5 mL of phosphate buffer (0.2 M, pH 6.6) and 2.5 mL potassium ferricyanide [K₃Fe(CN)₆] (1%, w/v), and the mixture was incubated at 50 °C for 30 min. Afterwards, 2.5 mL of trichloroacetic acid (10%, w/v) was added to the mixture and centrifuged at 3000 rpm for 10 min. Finally, 2.5 mL of upper-layer solution was mixed with 2.5 mL distilled water and 0.5 mL FeCl₃ (0.1%, w/v), and the absorbance was measured at 700 nm. Trolox, BHA, and BHT were used as standard antioxidants.

4.2.2. Metal Chelating Activity. The chelating activity of the new *N*-acylamino-substituted tricyclic imide compounds on ferrous ions (Fe²⁺) was measured according to the method of Decker and Welch [36]. Aliquots of 1 mL of different concentrations (25, 50, 75 and 100 µg/mL) of the samples were mixed with 3.7 mL of deionized water. The mixture was incubated with FeCl₂ (2 mM, 0.1 mL) for 30 min. After incubation, the reaction was initiated by addition of ferrozine (5 mM and 0.2 mL) for 10 min at room temperature, and then the absorbance was measured at 562 nm in a spectrophotometer. A lower absorbance indicates a higher chelating power. The chelating activity of the extract on Fe²⁺ was compared with

FIGURE 6: Radical scavenging activity (%) of different concentration of new compounds.

TABLE 1: Radical scavenging activity (IC₅₀: µg/mL) of new compounds.

Compounds	Free radical scavenging activity IC ₅₀ (µg/mL)
1	35.53
2	81.63
3	28.13
4a	25.78
4b	27.16
4c	16.95
4d	21.80
5a	36.77
5b	39.14
5c	35.58
5d	21.48
6a	19.08
6b	14.24
6c	6.79
6d	22.47
6e	14.10
6f	6.46
BHT	0.90
Trolox	1.85
BHA	1.72

that of EDTA at the same concentrations. Chelating activity was calculated using the following formula: metal chelating activity (%) = [1 – (absorbance of sample/absorbance of control)] × 100. Control test was performed without addition of the sample.

4.2.3. Free Radical Scavenging Activity. The free radical scavenging activity of the new *N*-acylamino-substituted tricyclic imide compounds were measured with 1,1-diphenyl-2-picryl-hydrazyl (DPPH^{*}) using the slightly modified methods of Brand-Williams et al. Briefly [37], 20 mg/L DPPH^{*} solution in methanol was prepared, and 1.5 mL of this solution was added to 0.75 mL of the sample, butylated hydroxyanisole

(BHA), butylated hydroxytoluene (BHT), and Trolox (25–100 $\mu\text{g/mL}$). The mixture was shaken vigorously, and the decrease in absorbance at 517 nm was measured at 30 min. Water (0.75 mL) in place of the sample was used as control. The percent inhibition activity was calculated using the following equation: free radical scavenging effect (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample solution. The sample concentration providing 50% inhibition (IC_{50}) was calculated from the graph of inhibition percentage plotted against sample concentration.

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