European Journal of Medicinal Chemistry 80 (2014) 145–153

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis of stable aromatic and heteroaromatic sulfonyl-amidoximes and evaluation of their antioxidant and lipid peroxidation activity

Ismini Doulou [a], Christos Kontogiorgis [b], Alexandros E. Koumbis [c], Eleni Evgenidou [c], Dimitra Hadjipavlou-Litina [b], Konstantina C. Fylaktakidou [a,*]

[a] Laboratory of Organic, Bioorganic and Natural Products Chemistry, Molecular Biology and Genetics Department, Democritus University of Thrace, 68100 Alexandroupolis, Greece
[b] Department of Pharmaceutical Chemistry, School of Pharmacy, Faculty of Health Sciences, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece
[c] Laboratory of Organic Chemistry, Department of Chemistry, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece

ARTICLE INFO

Article history:
Received 16 December 2013
Received in revised form
26 March 2014
Accepted 11 April 2014
Available online 13 April 2014

Keywords:
Antioxidant
Hydroxyl radicals
Lipid peroxidation
Amidoximes
Sulfonyl-amidoximes

ABSTRACT

We describe herein the synthesis of stable aromatic and heteroaromatic sulfonyl-amidoximes, from the reaction of amidoximes with the corresponding sulfonyl chlorides, in low to excellent yields. Evaluation of their antioxidant activity has shown that 17 out of 28 compounds highly compete DMSO for hydroxyl radicals, while five of them inhibit lipid peroxidation. Combining the reducing and anti-lipid peroxidation ability it seems that compounds **13** and **31** could be used as lead molecules.

1. Introduction

Cell metabolism of aerobic organisms has as an unavoidable consequence the formation of Reactive Oxygen Species (ROS). Normally, the organism defense against these highly reactive species involve enzymes, like superoxide dismutase and glutathione peroxidase, and naturally occurring antioxidants such as ascorbic acid (vitamin C), α-tocopherol (vitamin E), β-carotene and polyphenolic flavonoids. Nevertheless, in many pathophysiological conditions the excessive production of ROS overwhelms the natural antioxidant defense mechanisms and essentially biological molecules, such as lipids, proteins and DNA can be modified by those persistently high levels of ROS. This imbalance is termed oxidative stress (OS) and has been associated with several human diseases such as cancer, neurodegenerative syndromes and inflammation. It is consistent that rates of ROS production are increased in most diseases [1]. As a result, natural and synthetic small molecules possessing antioxidant activity are becoming increasingly important in this kind of disease prevention and therapy.

Amidoximes (**I**, Fig. 1) are bi-functional molecules exhibiting a rich and fertile chemistry, which provides one of the shortest ways to reach various heterocycles [2–5]. They possess varied and diverse biological activities, which makes them an important and attractive pharmacophore in medicinal chemistry. A plethora of publications refers to individual amidoximes bearing anticoagulant–antiaggregatory, antimicrobial, antituberculotic, anthelminthic, herbiside, insecticide, antihistaminic, antineoplastic, antiarrythmic, anxiolytic–antidepressant, antihypertensive, hypoglycemic, antiamnesia and antiinflammatory biological properties [6]. Others were found to inhibit glycosidase, squalene hopene cyclise, gastric juice secretion and thrombin, to regulate plant growth and to complex with metals and, thus, act as radiopharmaceuticals and anti-pollutants [6]. Some of the above activities are related to their recently discovered ability to release NO [6–8] or to act as prodrugs of amidines.

Surprisingly, although known for many decades, sulfonyl-amidoximes (**II**, Fig. 1) have received limited biological attention [9,10] comparing to other *O*-substituted derivatives of amidoximes. In one of the two cases studied, an *O*-methanosulfonyl-amidoxime derivative exhibited the best antimalarial activity, in a nanomolar range, comparing with other amidoxime derivatives [9]. Sulfonyl-amidoximes are known synthones for various organic scaffolds,

* Corresponding author.
E-mail address: kfylakta@mbg.duth.gr (K.C. Fylaktakidou).

http://dx.doi.org/10.1016/j.ejmech.2014.04.040
0223-5234/

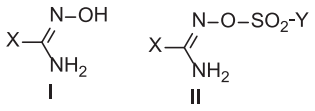

Fig. 1. General structures of amidoximes and Sulfonyl-Amidoximes.

like *N*-substituted asymmetric ureas [11] and cyanamides [11–13], azirines [14,15], amidines [16] and 5-amino-1,2,4-thiadiazoles [17]. Nevertheless, besides aromatic amidoximes which are referred as unstable [11,18], benzylic and aliphatic ones seem to be isolable [9,11,13,17], however, in most of the cases they were prepared and used *in situ*.

While exploring in our laboratories possible transformations of sulfonyl-amidoximes, we have discovered that *p*-nitro-phenyl and *o*-, *m*-, *p*-pyridine amidoxime sulfonyl derivatives were tolerant to treatment with base, chromatographic separation and long lasting storage. Interestingly, to the best of our knowledge, none of the parent amidoximes have ever been sulfonylated before. Our interest in the chemistry and biology of amidoximes and their derivatives [6,19–24] and in antioxidant–antiinflammatory phenomena [19,24–34] has prompted us to investigate the antioxidant activity of these new and stable sulfonyl-amidoximes.

In order to have a diverse pool of compounds to examine, we have synthesized derivatives with electron donating and electron withdrawing substituent aromatic sulfonyl groups, as well as aliphatic ones bearing short to long linear chains. This allowed us to examine the influence of both electronic effects and lipophilicity in an effort to establish a Structure Activity Relationship (SAR). The biological experiments described herein examine the capability of the title compounds, as well as their parent amidoximes, to interact with the stable radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH), to compete with DMSO for HO• and to inhibit lipid peroxidation. The results are discussed in terms of SAR and an attempt is made to define the necessary structural features for active compounds.

2. Chemistry

Each one of amidoximes **1** [11], **2** [35], **3** [35] and **4** [36] reacted with sulfonyl chlorides **5**–**10** in chloroform, or a mixture of chloroform and dimethylformamide (DMF) or tetrahydrofuran (THF), in the presence of triethylamine, to give the corresponding sulfonyl-amidoximes in low to excellent yields, Scheme 1.

After aqueous work-up the crude material was recrystallized, or in certain few cases purified with column chromatography. Yields of aliphatic sulfonyl-amidoximes were generally low, due to the sensitivity of the reagents and their low solubility in the reaction solvents. An exception was observed in the case of aliphatic-sulfonyl *o*-pyridine amidoximes (**32**–**34**), probably because, in contrary to the other three, their parent amidoxime (**4**) was highly soluble in chloroform. These products needed chromatographic purification in order to remove the remained residues of the reagents used and their by-products. On the other hand, the aromatic sulfonyl-chlorides (**5**–**7**) gave the corresponding sulfonyl-amidoximes in good to excellent yields and purity, and in none of these cases a column chromatography purification was necessary. Additionally, the reaction of *m*-pyridine amidoxime with *p*-nitro-phenyl-sulfonyl chloride occurred in a much better yield when THF was used as a solvent. To our delight a simple aqueous work-up was sufficient to remove the unreacted starting material (if any) when *p*- and *m*-pyridine amidoximes were used. All compounds were found to be quite stable after long storage, except of compound **25** which was relatively unstable, compared to the rest of the compounds.

Spectroscopic data unambiguously verified the proposed structures. In IR spectra all compounds gave absorptions in around 1360 and 1180 cm^{-1}, characteristic of the SO_2 bond [9,13,17,18]. In the negative electrospray LC–MS spectra a single fragment corresponding to $[R-SO_3]^-$ was observed in all but one cases (in compound **31** the main peak corresponded to the $[M-H]^-$·fragment). Additionally, the majority of the positive electrospray LC–MS spectra exhibited as the main fragment (100%) the one corresponding to *m*/*z* 166 for *p*-nitrophenyl amidoxime derivatives and 122 for the pyridine amidoxime derivatives ($[M+2H-RSO_3]^+$). The hydroxyl-imino structure of these amidoxime derivatives was verified in their ^1H NMR spectra from the existence of a broad singlet peak, at 6–7 ppm integrated for two protons (NH_2). In accordance to the literature, these chemical shifts are found relatively downfield compared to their corresponding parent amidoximes [18]. Additionally, we observed the corresponding two absorptions at the IR spectra for the NH_2 group, as well as absorptions around 1630–1670 cm^{-1} due to the C=N bond [9,13,17,18].

3. Biological assays

In this investigation, we synthesized a number of unknown, stable aromatic and hetero-aromatic sulfonyl-amidoxime derivatives which we envision to exhibit protection against radical attack. In general, the implication of free radicals in the pathways of the inflammatory process is particularly important. Antioxidants are defined as substances that, even at low concentrations, significantly delay or prevent oxidation of easily oxidizable substrates. Many non-steroidal anti-inflammatory drugs have been reported to act either as inhibitors of free radical production or as radical scavengers [37]. Consequently, compounds with antioxidant properties could be expected to offer protection in rheumatoid arthritis and inflammation and to lead to potentially effective drugs [38]. For the estimation of the antioxidative potential of chemical components, different experimental approaches were used [39]. Most of them require a spectrophotometric measurement and a certain reaction time in order to obtain reproducible results [40].

The radical scavenging ability of compounds **11**–**34** as well as of the parent amidoximes **1**–**4** was tested against the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical as well as against the hydroxyl radical (HO•) generated by the Fe^{3+}/ascorbic acid system. Finally, the ability of the synthesized sulfonyl-amidoximes to inhibit lipid peroxidation induced by the thermal free radical producer 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH) was evaluated.

3.1. Interaction with the DPPH stable free radical

We have performed a radical scavenging measuring method using the stable radical DPPH [41] according to the methods of Hadjipavlou et al. [42]. The compounds were tested at a final concentration of 100 μM after 20 and 60 min (Table 1). This interaction indicates the reducing ability (RA) of the tested compounds in an iron-free system. The DPPH test is very useful in the micromolar range, demanding a very short period of time for the outcome of the results, for both hydrophilic and lipophilic samples. Due to its odd number of electrons DPPH gives a strong absorption band at 517 nm.

Perusal of the % RA values, showed that 9 out of 28 compounds exhibited limited reducing ability less than 10% at 60 min, whereas 3 out of 28 more than 30% in both 20 and 60 min. Nordihydroguaiaretic Acid (NDGA) has been used as a reference compound.