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

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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,

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http://dx.doi.org/10.1016/j.ejmech.2014.04.040
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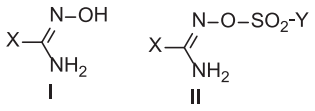


Fig. 1. General structures of amidoximes and Sulfonyl-Amidoximes.

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

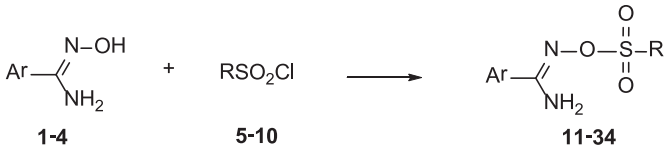
In this investigation, we synthesized a number of unknown, stable aromatic and hetero-aromatic sulfonyl-amidoxime derivatives which we envision to exhibit protection against radical attack. In general, the implication of free radicals in the pathways of the inflammatory process is particularly important. 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.



1-4 **5-10** **11-34**

No	Amidoximes Ar-	No	Sulfonyl chlorides R-	Sulfonyl-amidoximes	Yields %
1	4-nitro-phenyl-	**5**	phenyl-	**11**	82
1	"	**6**	4-tolyl-	**12**	78
1	"	**7**	4-nitro-phenyl-	**13**	96
1	"	**8**	propyl-	**14**	58
1	"	**9**	decyl-	**15**	37 (65)
1	"	**10**	hexadecyl-	**16**	27 (56)
2	4-pyridyl-	**5**	phenyl-	**17**	55
2	"	**6**	4-tolyl-	**18**	81
2	"	**7**	4-nitro-phenyl-	**19**	63
2	"	**8**	propyl-	**20**	20
2	"	**9**	decyl-	**21**	15
2	"	**10**	hexadecyl-	**22**	27
3	3-pyridyl-	**5**	phenyl-	**23**	96
3	"	**6**	4-tolyl-	**24**	98
3	"	**7**	4-nitro-phenyl-	**25**	77
3	"	**8**	propyl-	**26**	93
3	"	**9**	decyl-	**27**	25
3	"	**10**	hexadecyl-	**28**	39
4	2-pyridyl-	**5**	phenyl-	**29**	99
4	"	**6**	4-tolyl-	**30**	95
4	"	**7**	4-nitro-phenyl-	**31**	81
4	"	**8**	propyl-	**32**	80
4	"	**9**	decyl-	**33**	86
4	"	**10**	hexadecyl-	**34**	80

Yields refer to purified products.

Yields in parenthesis are based on recovered starting material.

Scheme 1. Reaction of Amidoximes with the corresponding Sulfonyl Chlorides.

Slight differences were observed over time. The best activity was observed by compounds **13**, **24** and **31** (~30–40% over time), whereas compounds **16**, **23**, **26** and **27** showed reducing activity of ~20% at 20 min. The same activity was observed for compounds **19**, **21** and **22**, however after relatively prolonged periods (60 min). The latter compounds, which seem to respond in longer interaction time, all possess the isonicotine-carbamidoxime (*p*-pyridine) structure, followed by *p*-nitro-phenyl (**13**, **14** and **16**). Interestingly, almost all nicotine-carbamidoxime sulfonyl derivatives (*m*-pyridine) exhibited reducing activity in the range of 20–40%, the best among all derivatives.

Regarding the substitution pattern of sulfonyl group, the best activity was observed for compounds bearing aromatic groups (**13**, *p*-nitro-phenyl and **24**, *p*-tolyl). Additionally, a number of aliphatic sulfonyl derivatives of the *p*-nitro, *p*-pyridyl and *m*-pyridyl amidoxime series showed reduced activities >20% (**26**, C_3, **21**, C_{10}, **27**, C_{10}, **16**, C_{16}, **22**, C_{16}). According to these results for the aliphatic series, increased lipophilicity does not seem to play a role, since chain length of 3, 10 or 16 carbon atoms does not influence dramatically the outcome of the experiments. This finding is in accordance with the results obtained for several amphiphilic benzamidoxime derivatives where expansion of the aliphatic chain from 5 to 13 carbon atoms did not dramatically influence the interaction with DPPH [43].

Lipophilicity, as clog *P* theoretically calculated values does not seem to influence the interaction of the studied compounds [44]. The absence of hydrogen atoms (phenolic hydroxyl groups) which could be donated to stabilize the DPPH radical has a direct impact on our results. The low interaction values of amidoximes and of sulfonyl-amidoximes compared to NDGA, should be mainly attributed to the absence of easily oxidized functionalities like the ones present in NDGA (two catechol subunits).

3.2. Free hydroxyl radical (HO•) scavenging ability

During the inflammatory process, phagocytes generate the superoxide anion radical at the inflamed site. At sites of inflammation superoxide anion reacts with superoxide dismutase and forms H_2O_2, which is dangerous in the cell because it can easily be transformed into the highly reactive hydroxyl radical, one of the most destructive free radicals [45].

Hydroxyl radicals formed in the body, can lead to the generation of carbon-centered and peroxyl radicals, which are considered to be responsible for several pathological conditions. It has been claimed that hydroxyl radical scavengers could offer against pathological disorders. The competition of sulfonyl-amidoximes with DMSO for HO• generated by the Fe^{3+}/ascorbic acid system, expressed as percent inhibition of formaldehyde production, was used for the evaluation of their hydroxyl radical scavenging activity [46].

All parent amidoximes **1**, **2**, **4** exhibited very good scavenging activities (>80%) almost equal to the activity of the standard compound Trolox (88%), with the exception of **3** which showed

Table 1
Interaction % with DPPH (RA%); Competition with DMSO for HO•; Inhibition of lipid peroxidation (LP); clog *P*.

Sulfonyl-amidoximes	RA (%) 20 min 100 μM	RA (%) 60 min 100 μM	HO• (%) 100 μM	% Inhibition of LP induced by AAPH 100 μM (IC_{50})	clog *P* [43]
1	3	4	**85**	41	0.77
2	3	7	**87**	no	1.68
3	12	15	**60**	no	−0.47
4	11	14	**81**	no	−0.47
11	4	8.4	34	5	1.92
12	10	11	**78**	25	2.42
13	34	38	no	**66.5 (IC_{50})**	1.66
14	10	19	**75**	22	1.48
15	3	12	48	no	5.18
16	21	24	**100**	3	8.35
17	2	11	44	22	0.68
18	no	no	32	no	1.18
19	13	20	0	49	0.42
20	no	4	**66**	no	0.24
21	14	21	**70**	28	3.94
22	14	21	no	12	7.11
23	24	25	28	21	0.68
24	34	41	**95**	43	1.18
25	8	11	18	**85 (IC_{50})**	0.42
26	23	27	**63**	16	0.24
27	20	24	**72**	33	3.94
28	7	16	**69**	**70 (IC_{50})**	7.11
29	4	no	32	30	0.68
30	5	4	**72**	**80 (IC_{50})**	1.18
31	32	32	**98**	**72 (IC_{50})**	0.42
32	6	1	**83**	no	0.24
33	11	10	**79**	46	3.94
34	4	1	no	21	7.11
NDGA	77.	81			
Trolox			88	55 **(IC_{50})**	

In the *in vitro* assays each experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.
no = no activity under the experimental conditions.
The bold numbers in the results presented, indicate a significant antioxidant activity.

moderate activity (60%). Their low lipophilicity as clog *P* values seems to be correlated with their high scavenging activity. Three out of the six *p*-nitro-phenyl-amidoxime sulfonyl derivatives had activity equal or over 75%, with the compound **16** to exhibit a complete scavenging effect (100%). Two derivatives of the isonicotinyl-series gave good results (**20**: 66% and **21**: 70%), whereas two others had moderate activity (**17** and **18**, ~30–45%). Four out of the six nicotinyl-derivatives exhibited scavenging activity in the range of 63–95% (compound **24** was the most potent, 95%). The same high activity was evidenced for *o*-pyridyl derivatives which exhibited activity in the range of 72–98% (**31**: 98%). The results given in several publications indicate the importance of pyridyl and/or sulphoxide groups for antioxidant activity [47]. It is referred that the aggressive hydroxyl radicals are successfully trapped by pyridyl substituted compounds, which exhibit potent antioxidative profile and protective effect against oxidative stress [48–50].

Considering the sulfonyl residue, we may assume that among the aromatic substituents, the presence of the electron-donating *p*-tolyl group led to a relatively positive effect in almost all cases (**12**: 78%, **18**: 32%, **24**: 95%, **30**: 72%), whereas most of the aliphatic ones showed considerably good scavenging activity [(**14**: C_3, 75%), (**16**: C_{16}, 100%), (**20**, C_3, 65%), (**21**: C_{10}, 70%), (**26**, C_3, 63%), (**27**, C_{10}, 72%), (**28**, C_{16}, 69%), (**32**, C_3, 83%), (**33**, C_{10}, 79%)]. Comparing the activity of parent compounds *m*- and *o*-pyridine-amidoxime with their substituted ones, the sulfonyl substitution seems to enhance scavenging activity. No role was found for the chain length size (3, 10 or 16 carbon atoms) as well as for the lipophilicity.

3.3. Inhibition of lipid peroxidation

AAPH induced linoleic acid oxidation has been developed as a quick and reliable method for measuring the antioxidant activity and provides a measure of how efficiently antioxidants protect against lipid oxidation *in vitro*. The use of AAPH is recommended as more appropriate for measuring radical-scavenging activity *in vitro*, because the activity of the peroxyl radicals produced by the action of AAPH shows a greater similarity to cellular activities such as lipid peroxidation. Oxidation of exogenous linoleic acid by a thermal free radical producer (AAPH) is followed by UV spectrophotometry in a highly diluted sample [51].

With the exception of compounds **13**, **25**, **28**, **30** and **31** for which IC_{50} values were able to be determined, all the others did not show significant anti-lipid peroxidation effect. Among these five amidoximes three presented a 4-nitro-phenyl substitution at R position underlying the positive influence of the electron withdrawing effect of NO_2 group. In compound **13** especially the combination of two 4-nitro-phenyl groups is correlated with the highest anti-lipid peroxidation response. The simultaneous presence of a pyridyl group enhances activity, independently of the attachment position (2- or 3-). Four out of the rest 23 derivatives inhibited lipid peroxidation in the range of ~40–50% (**1**, **19**, **24** and **33**).

Considering the influence of the electronic phenomena of the aromatic sulfonyl substituents we may conclude that the electron withdrawing effect of NO_2 group positively contributed to the outcome of the antioxidant activity (**19**: 48.7, **13**: 66.5-IC_{50}, **25**: 85-IC_{50}, **31**: 72-IC_{50}) comparing to reference compound Trolox. Lipophilicity does not play an important role, since the most potent derivative **13** presents low clog *P* value (1.66). The electron-donating effect influenced only the *o*-pyridine amidoxime derivative (**30**: 80-IC_{50}), whereas the long aliphatic chain seemed to interfer in the *m*-pyridine derivative (**28**: 70-IC_{50}). Finally, in general sulfonyl substitution of parent amidoximes and specifically the *p*-nitro-phenyl moiety enhanced lipid peroxidation activity.

4. Conclusion

Novel stable aromatic and heteroaromatic sulfonyl-amidoximes were synthesized in low to excellent yields. A preliminary biological evaluation concerning their antioxidant activity was attempted for the first time and was combined with a SAR study. The interaction of these compounds with the stable free radical DPPH was low, however, more than half of the 28 compounds, parent amidoximes included, highly compete DMSO (60–100%) for HO•. Compounds **1**, **2**, **4** and **32** exhibited activity close to the reference drug Trolox (>80%, compared to 88.2%) whereas compounds **16** and **24** showed 100% and 95% scavenging activity, respectively. Inhibition of lipid peroxidation was interesting for five compounds (**13**, **25**, **28**, **30** and **31**).

In our opinion, the herein presented results designate sulfonyl amidoximes as a new class of antioxidant compounds. Nicotine and picoline amidoximes were generally overall the most potent ones. Finally, combining reducing and anti-lipid peroxidation activity, compounds **13** and **31** could be used as lead molecules.

5. Experimental

5.1. Chemistry

Mps were measured on a Kofler hot-stage apparatus and are uncorrected. FT-IR spectra were obtained in a Perkin–Elmer 1310 spectrometer using potassium bromide pellets. NMR spectra were recorded on a Bruker AM 300 (300 MHz and 75 MHz for ^1H and ^{13}C

respectively) or Agilent 500/54 (500 MHz and 125 MHz for ^{1}H and ^{13}C respectively) spectrometer using $CDCl_3$, and/or DMSO-d_6 as solvent. *J* values are reported in Hz. Quint and sext are the abbreviations for quintet and sextet multiple NMR peaks. High resolution mass spectra (HRMS) were recorded on micrOTOF GC–MS QP 5050 Shimadzu single-quadrupole mass spectrometer. Mass spectra were determined on a Shimadzu LCMS-2010 EV system under Electrospray Ionization (ESI) conditions. Micro-analyses were performed on a Perkin–Elmer 2400-II Element analyzer. Analyses indicated by the symbols of the elements or functions were within 0.4% of the theoretical values. All reactions were monitored on commercial available pre-coated TLC plates (layer thickness 0.25 mm) of Kieselgel 60 F_{254}. Silica gel Merck 60 (40–60 μM) has been used for column chromatography. Yields were calculated after recrystallization or column chromatography.

5.1.1. General procedure

Parent amidoxime (2 mmol) was dissolved in the indicated solvent (0.15 M) and cooled to 0 °C under an argon atmosphere. Triethylamine (0.28 mL, 2 mmol) was added, followed by the required sulfonyl chloride (2 mmol) and the mixture was stirred for the indicated period allowing the temperature to slowly rise to 25 °C. Then, water (70 mL) was added and the mixture was extracted with dichloromethane (2 × 70 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under vacuum (rotary evaporator) to give a residue which was either recrystallized or purified using column chromatography.

5.1.1.1. 4-Nitro-N'-(phenylsulfonyloxy)benzimidamide (**11**).

Solvent: Chloroform; Reaction time: 6 h; Purification method: recrystallization; Pale yellow crystals, yield 82%, mp 133–135 °C (hexanes/ethyl acetate); IR (KBr): 3472, 3379, 1657, 1615, 1356 & 1188 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) δ 6.62 (br s, 2H), 7.50 (t, J = 7.8 Hz, 2H), 7.59 (t, J = 7.5 Hz, 1H), 7.72 (d, J = 8.8 Hz, 2H), 7.93 (d, J = 8.3 Hz, 2H), 8.11 (d, J = 8.5 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 122.6, 127.4, 127.8, 128.1, 133.0, 134.9, 135.8, 148.3, 155.4; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 322 $[M+H]^+$, 277 $[M+2H-NO_2]^+$, 166 (100%) $[M+2H-C_6H_5SO_3]^+$; negative = 157 (100%) $[C_6H_5SO_3]^-$; Elem. Anal.: calcd for $C_{13}H_{11}N_3O_5S$: C, 48.59; H, 3.45; N, 13.08; Found: C, 48.68; H, 3.46; N, 13.09.

5.1.1.2. 4-Nitro-N'-(tosyloxy)benzimidamide (**12**).

Solvent: Chloroform; Reaction time: 6 h; Purification method: recrystallization; Pale yellow crystals, yield 78%, mp 148–150 °C (hexanes/ethyl acetate); IR (KBr): 3498, 3391, 1637, 1598, 1359 & 1191 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$ + DMSO-d_6) δ 2.36 (s, 3H), 6.60 (br s, 2H), 7.28 (d, J = 8.2 Hz, 2H), 7.74 (d, J = 8.9 Hz, 2H), 7.80 (d, J = 8.3 Hz, 2H), 8.11 (d, J = 8.9 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$ + DMSO-d_6) δ 20.8, 122.5, 127.3, 127.7, 128.6, 131.9, 135.8, 143.9, 148.2, 155.2; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 390 $[M+Na+MeOH]^+$, 336 $[M+H]^+$, 166 $[M+2H-C_7H_7SO_3]^+$; negative = 171 (100%) $[C_7H_7SO_3]^-$; Elem. Anal.: calcd for $C_{14}H_{13}N_3O_5S$: C, 50.14; H, 3.91; N, 12.53; Found: C, 50.33; H, 3.92; N, 12.58.

5.1.1.3. 4-Nitro-N'-(4-nitrophenylsulfonyloxy)benzimidamide (**13**).

Solvent: Chloroform/DMF (8/1); Reaction time: 5 h; Purification method: recrystallization; Baize crystals, yield 96%, mp 142–144 °C (hexanes/ethyl acetate); IR (KBr): 3484, 3359, 1651, ~1350 & 1188 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 6.73 (br s, 2H), 7.73 (d, J = 8.9 Hz, 2H), 8.12 (d, J = 8.9 Hz, 2H), 8.16 (d, J = 8.9 Hz, 2H), 8.32 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 122.7, 123.3, 127.6, 129.4, 135.5, 140.9, 148.6, 150.0, 156.1; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 322 $[M+2H-NO_2]^+$, 166 (100%) $[M+2H-(O_2N-C_6H_4-SO_3)]^+$; negative = 202 (100%) $[(O_2N-C_6H_4-SO_3)]^-$; Elem. Anal.: calcd for $C_{13}H_{10}N_4O_7S$: C, 42.63; H, 2.75; N, 15.30; Found: C, 42.75; H, 2.74; N, 15.34.

5.1.1.4. 4-Nitro-N'-(propylsulfonyloxy)benzimidamide (**14**).

Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: recrystallization; Pale yellow crystals, yield 58%, mp 110–112 °C (hexanes/ethyl acetate); IR (KBr): 3474, 3368, 2972, 2933, 2874, 1640, 1347 & 1172 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, J = 7.6 Hz, 3H), 1.93 (sext, J = 7.6 Hz, 2H), 3.38 (t, J = 7.6 Hz, 2H), 5.40 (br s, 2H), 7.86 (d, J = 8.9 Hz, 2H), 8.27 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8, 17.1, 50.6, 124.0, 127.9, 136.0, 149.7, 155.7; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 288 $[M+H]^+$, 243 $[M+2H-NO_2]^+$, 166 (100%) $[M+2H-C_3H_7SO_3]^+$; negative = 123 (100%) $[C_3H_7SO_3]^-$; Elem. Anal.: calcd for $C_{10}H_{13}N_3O_5S$: C, 41.81; H, 4.56; N, 14.63; Found: C, 41.92; H, 4.57; N, 14.67.

5.1.1.5. N'-(decylsulfonyloxy)-4-nitrobenzimidamide (**15**).

Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: column chromatography; Baize crystals, yield 37% or 65% (based on recovered SM), mp 90–92 °C (hexanes/ethyl acetate); IR (KBr): 3482, 3353, 2954, 2921, 2851, 1639, 1360 & 1166 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.8 Hz, 3H), 1.25 (br s, 12H), 1.46 (quint, J = 7.5 Hz, 2H), 1.89 (quint, J = 7.3 Hz, 2H), 3.39 (t, J = 7.7 Hz, 2H), 5.39 (br s, 2H), 7.86 (d, J = 8.9 Hz, 2H), 8.28 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 23.3, 28.1, 28.9, 29.2, 29.2, 29.4, 31.8, 48.9, 124.0, 127.9, 136.0, 149.7, 155.7; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 386 $[M+H]^+$, 341 $[M+2H-NO_2]^+$, 166 (100%) $[M+2H-C_{10}H_{21}SO_3]^+$; negative = 221 (100%) $[C_{10}H_{21}SO_3]^-$; Elem. Anal.: calcd for $C_{17}H_{27}N_3O_5S$: C, 41.81; H, 4.56; N, 14.63; Found: C, 41.92; H, 4.55; N, 14.67.

5.1.1.6. N'-(hexadecylsulfonyloxy)-4-nitrobenzimidamide (**16**).

Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: column chromatography; Pale yellow crystals, yield 27% or 56% (based on recovered SM), mp 95–96 °C (ethyl acetate); IR (KBr): 3483, 3354, 2952, 2919, 2849, 1638, 1360 & 1167 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 0.80 (t, J = 6.2 Hz, 3H), 1.17 (br s, 24H), 1.38 (quint, J = 6.8 Hz, 2H), 1.80 (quint, J = 7.6 Hz, 2H), 3.31 (t, J = 7.7 Hz, 2H), 6.34 (br s, 2H), 7.88 (d, J = 8.9 Hz, 2H), 8.18 (d, J = 8.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 13.7, 22.2, 22.9, 27.7, 28.5, 28.8, 28.9, 29.0, 29.1, 29.16, 29.19, 31.4, 48.3, 123.2, 127.9, 136.2, 149.0, 155.8; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 524 $[M+Na+MeOH]^+$, 425 $[M+2H-NO_2]^+$, 166 (100%) $[M+2H-C_{16}H_{33}SO_3]^+$; negative = 305 (100%) $[C_{16}H_{33}SO_3]^-$; Elem. Anal.: calcd for $C_{23}H_{39}N_3O_5S$: C, 58.82; H, 8.37; N, 8.95; Found: C, 58.99; H, 8.39; N, 8.98.

5.1.1.7. N'-((phenylsulfonyl)oxy)isonicotinimidamide (**17**).

Solvent: Chloroform/DMF (8/1); Reaction time: 4 h; Purification method: recrystallization; White crystals, yield 55%, mp 130–132 °C (ethyl acetate); IR (KBr): 3409, 3361, 3103, 1654, 1602, 1364 & 1189 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 6.60 (br s, 2H), 7.46–7.53 (m, 4H), 7.59 (t, J = 7.7 Hz, 1H), 7.93 (d, J = 7.8 Hz, 2H) 8.55 (d, J = 5.6 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 120.3, 127.7, 128.0, 132.9, 135.0, 137.8, 148.8, 155.0; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 278 (100%) $[M+H]^+$, 122 $[M+2H-C_6H_5SO_3]^+$; negative = 157 (100%) $[C_6H_5SO_3]^-$; Elem. Anal.: calcd for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15; Found: C, 52.13; H, 4.01; N, 15.19.

5.1.1.8. N'-(tosyloxy)isonicotinimidamide (**18**).

Solvent: Chloroform/DMF (8/1); Reaction time: 4 h; Purification method: recrystallization; White crystals, yield 81%, mp 174–176 °C (ethyl acetate); IR (KBr): 3436, 3338, 3129, 2963, 1657, 1346 & 1188 (S=O) cm^{-1}; ^{1}H

NMR (500 MHz, DMSO-d_6) δ 2.39 (s, 3H), 7.36 (br s, 2H), 7.44 (d, J = 8.3 Hz, 2H), 7.50 (d, J = 7.3 Hz, 2H), 7.86 (d, J = 8.3 Hz, 2H), 8.64 (d, J = 7.3 Hz, 2H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.1, 121.0, 128.3, 129.7, 132.6, 138.0, 144.7, 150.2, 155.9; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 346 $[M+Na+MeOH]^+$, 314 $[M+Na]^+$, 292 (100%) $[M+H]^+$; negative = 290 $[M-H]^-$, 171 (100%) $[C_7H_7SO_3]^-$; Elem. Anal.: calcd for $C_{13}H_{13}N_3O_3S$: C, 53.60; H, 4.50; N, 14.42; Found: C, 53.75; H, 4.51; N, 14.46.

5.1.1.9. N′-(4-nitrophenylsulfonyloxy)isonicotinimidamide (***19***). Solvent: Chloroform/DMF (8/1); Reaction time: 5 h; Purification method: recrystallization; Extraction with an additional amount of ethyl acetate; Pale yellow crystals, yield 63%, mp 169–171 °C (ethanol); IR (KBr): 3457, 3308, 3104, 1657, 1376 & 1189 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 6.98 (br s, 2H), 7.44 (d, J = 5.9 Hz, 2H), 8.18 (d, J = 8.9 Hz, 2H), 8.34 (d, J = 8.7 Hz, 2H), 8.54 (d, J = 5.7 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ 119.9, 122.8, 128.9, 136.7, 140.3, 148.8, 149.4, 155.5; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 323 $[M+H]^+$, 122 (100%) $[M+2H-(O_2N-C_6H_4-SO_3)]^+$; negative = 202 (100%) $[(O_2N-C_6H_4-SO_3)]^-$; Elem. Anal.: calcd for $C_{12}H_{10}N_4O_5S$: C, 44.72; H, 3.13; N, 17.38; Found: C, 44.76; H, 3.14; N, 17.43.

5.1.1.10. N′-(propylsulfonyloxy)isonicotinimidamide (***20***). Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: column chromatography; White crystals, yield 20%, mp 134–136 °C (hexanes/ethyl acetate); IR (KBr): 3437, 3330, 3173, 2967, 1665, 1352 & 1172 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.02 (t, J = 7.4 Hz, 3H), 1.85 (sext, J = 7.6 Hz, 2H), 3.30 (t, J = 7.6 Hz, 2H), 6.33 (br s, 2H), 7.56 (d, J = 4.5 Hz, 2H), 8.61 (d, J = 4.5 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 12.4, 16.7, 49.9, 120.6, 137.8, 149.8, 155.5; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 244 (100) $[M+H]^+$, 122 $[M+2H-C_3H_7SO_3]^+$; negative = 123 (100%) $[C_3H_7SO_3]^-$; Elem. Anal.: calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; Found: C, 44.56; H, 5.40; N, 17.32.

5.1.1.11. N′-(decylsulfonyloxy)isonicotinimidamide (***21***). Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: column chromatography; White crystals, yield 15%, mp 86–88 °C (hexanes/ethyl acetate); IR (KBr): 3472, 3368, 2954, 2921, 2853, 1637, 1360 & 1174 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 0.79 (t, J = 6.1 Hz, 3H), 1.17 (br s, 12H), 1.38 (quint, J = 6.9 Hz, 2H), 1.76 (quint, J = 7.8 Hz, 2H), 3.30 (t, J = 7.8 Hz, 2H), 6.56 (br s, 2H), 7.58 (d, J = 6.1 Hz, 2H), 8.59 (d, J = 6.1 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ 13.3, 21.8, 22.5, 27.2, 28.1, 28.3, 28.4, 28.6, 31.0, 47.8, 120.4, 137.5, 149.4, 155.3; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 342 (100%) $[M+H]^+$, 122 $[M+2H-C_{10}H_{21}SO_3]^+$; negative = 221 (100%) $[C_{10}H_{21}SO_3]^-$; Elem. Anal.: calcd for $C_{16}H_{27}N_3O_3S$: C, 56.28; H, 7.97; N, 12.31; Found: C, 56.39; H, 7.99; N, 12.32.

5.1.1.12. N′-(hexadecylsulfonyloxy)isonicotinimidamide (***22***). Solvent: Chloroform/DMF (8/1); Reaction time: 20 h; Purification method: recrystallization; White crystals, yield 27%, mp 78–80 °C (hexanes/ethyl acetate); IR (KBr): 3469, 3365, 2953, 2921, 2847, 1637, 1359 & 1174 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$+DMSO-d_6) δ 0.80 (t, J = 6.9 Hz, 3H), 1.17 (br s, 24H), 1.38 (quint, J = 7.1 Hz, 2H), 1.80 (quint, J = 7.8 Hz, 2H), 3.30 (t, J = 7.8 Hz, 2H), 6.35 (br s, 2H), 7.56 (d, J = 5.8 Hz, 2H), 8.60 (d, J = 5.9 Hz, 2H); ^{13}C NMR (75 MHz, $CDCl_3$+DMSO-d_6) δ 13.3, 21.8, 22.5, 27.3, 28.1, 28.39, 28.44, 28.6, 28.7, 28.75, 28.78, 31.0, 47.9, 120.4, 137.5, 149.4, 155.3; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 448.5 $[M+Na]^+$, 426 (100%) $[M+H]^+$; negative = 305 (100%) $[C_{16}H_{33}SO_3]^-$; Elem. Anal.: calcd for $C_{22}H_{39}N_3O_3S$ C, 62.08; H, 9.24; N, 9.87; Found: C, 62.14; H, 9.26; N, 9.88.

5.1.1.13. N′-((phenylsulfonyl)oxy)nicotinimidamide (***23***). Solvent: Chloroform/DMF (8/1); Reaction time: 3 h; Purification method: recrystallization; Pale yellow crystals, yield 96%, mp 125–127 °C (ethyl acetate/ethanol); IR (KBr): 3418, 3325, 3150, 1657, 1350 & 1182 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 6.63 (br s, 2H), 7.26 (dd, J = 7.9, 4.8 Hz, 1H), 7.50 (t, J = 7.5 Hz, 2H), 7.60 (t, J = 7.9 Hz, 1H), 7.83 (d, J = 7.9 Hz, 1H), 7.94 (d, J = 7.6 Hz, 2H), 8.59 (d, J = 3.6 Hz, 1H), 8.69 (d, J = 1.4 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 122.2, 125.7, 127.5, 127.8, 132.7, 133.7, 135.0, 146.9, 150.5, 155.2; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 278 $[M+H]^+$, 122 $[M+2H-C_6H_5SO_3]^+$; negative = 157 (100%) $[C_6H_5SO_3]^-$; Elem. Anal.: calcd for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15; Found: C, 52.08; H, 4.01; N, 15.19.

5.1.1.14. N′-(tosyloxy)nicotinimidamide (***24***). Solvent: Chloroform/DMF (8/1); Reaction time: 4 h; Purification method: recrystallization; Pale yellow crystals, yield 98%, mp 140–142 °C (ethyl acetate); IR (KBr): 3408, 3310, 3114, 1654, 1343 & 1189 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 2.37 (s, 3H), 6.47 (br s, 2H), 7.25 (dd, J = 8.1, 4.9 Hz, 1H), 7.27 (d, J = 7.8 Hz, 2H), 7.82 (d, J = 8.1 Hz, 2H), 7.83 (dt, J = 7.2, 1.8 Hz, 1H), 8.56 (dd, J = 4.8, 1.5 Hz, 1H), 8.70 (d, J = 1.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 21.4, 123.0, 126.6, 128.5, 129.3, 132.8, 134.5, 144.5, 147.9, 151.4, 155.9; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 292 (100%) $[M+H]^+$, 122 $[M+2H-C_7H_7SO_3]^+$; negative = 171 (100%) $[C_7H_7SO_3]^-$; Elem. Anal.: calcd for $C_{13}H_{13}N_3O_3S$: C, 53.60; H, 4.50; N, 14.42. Found: C, 53.69; H, 4.49; N, 14.45.

5.1.1.15. N′-((4-nitrophenyl)sulfonyloxy)nicotinimidamide (***25***). Solvent: Tetrahydrofuran; Reaction time: 1 h; Purification method: recrystallization; Extraction with ethyl acetate instead of dichloromethane; Baize crystals, yield 77%, mp 110–111 °C (ethyl acetate), IR (KBr): 3425, 3323, 3106, 1659, 1367 & 1185 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 6.82 (br s, 2H), 7.27 (dd, J = 7.8, 4.8 Hz, 1H), 7.83 (dt, J = 8.1, 1.7 Hz, 1H), 8.17 (d, J = 8.9 Hz, 2H), 8.33 (d, J = 8.9 Hz, 2H), 8.57 (dd, J = 4.8, 1.5 Hz, 1H), 8.70 (d, J = 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 122.3, 123.0, 125.5, 129.1, 133.8, 140.7, 146.9, 149.6, 150.7, 155.8; HRMS (ESI) Calc $C_{12}H_{11}N_4O_5S$ $[M+H]^+$, 323.0445; found 323.0443.

5.1.1.16. N′-(propylsulfonyloxy)nicotinimidamide (***26***). Solvent: Chloroform; Reaction time: 20 h; Purification method: recrystallization; Pale yellow crystals, yield 93%, mp 105–106 °C (hexanes/ethyl acetate); IR (KBr): 3415, 3332, 3087, 2966, 1655, 1347 & 1197 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 1.03 (t, J = 7.6 Hz, 3H), 1.85 (sext, J = 7.6 Hz, 2H), 3.30 (t, J = 7.6 Hz, 2H), 6.46 (br s, 2H), 7.31 (dd, J = 7.9, 4.8 Hz, 1H), 7.98 (dt, J = 7.9, 1.8 Hz, 1H), 8.62 (dd, J = 4.8, 1.5 Hz, 1H), 8.88 (d, J = 1.7 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 12.2, 16.5, 49.7, 122.7, 126.1, 134.1, 147.5, 151.2, 155.6; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 244 $[M+H]^+$, 122 $[M+2H-C_3H_7SO_3]^+$; negative = 123 (100%) $[C_3H_7SO_3]^-$; Elem. Anal.: calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; Found: C, 44.51; H, 5.38; N, 17.22.

5.1.1.17. N′-(decylsulfonyloxy)nicotinimidamide (***27***). Solvent: Chloroform; Reaction time: 20 h; Purification method: recrystallization; Pale yellow crystals, yield 25%, mp 85–87 °C (hexanes/ethyl acetate); IR (KBr): 3411, 3325, 3125, 2925, 2848, 1653, 1347 & 1192 (S=O) cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (t, J = 6.4 Hz, 3H), 1.20 (br s, 12H), 1.39 (quint, J = 7.2 Hz, 2H), 1.71 (quint, J = 7.6 Hz, 2H), 3.43 (t, J = 7.9 Hz, 2H), 7.33 (br s, 2H), 7.50 (dd, J = 7.9, 4.8 Hz, 1H), 8.06 (dt, J = 7.9, 1.8 Hz, 1H), 8.70 (dd, J = 4.8, 1.5 Hz, 1H), 8.86 (d,

J = 1.8 Hz, 1H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.1, 23.0, 27.2, 28.4, 28.68, 28.71, 28.9, 31.3, 47.6, 123.6, 126.7, 134.7, 147.7, 151.7, 155.8; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 396 $[M+Na+MeOH]^+$, 364 $[M+Na]^+$, 342 (100%) $[M+H]^+$, 122 $[M+2H-C_{10}H_{21}SO_3]^+$; negative = 221 $[C_{10}H_{21}SO_3]^-$; Elem. Anal.: calcd for $C_{16}H_{27}N_3O_3S$: C, 56.28; H, 7.97; N, 12.31; Found C, 55.95; H, 8.00; N, 12.33.

*5.1.1.18. N′-((hexadecylsulfonyl)oxy)nicotinimidamide (**28**).* Solvent: Chloroform; Reaction time: 20 h; Purification method: recrystallization; Pale yellow crystals, yield 39%, mp 109–111 °C (hexanes/ethyl acetate); IR (KBr): 3416, 3332, 3121, 2918, 2849, 1655, 1351 & 1166 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 0.80 (t, J = 6.7 Hz, 3H), 1.18 (br s, 24H), 1.39 (quint, J = 7.0 Hz, 2H), 1.81 (quint, J = 7.5 Hz, 2H), 3.31 (t, J = 7.7 Hz, 2H), 6.28 (br s, 2H), 7.30 (dd, J = 8.0, 4.8 Hz, 1H), 7.97 (dt, J = 8.0, 2.0 Hz, 1H), 8.63 (dd, J = 4.8, 1.5 Hz, 1H), 8.87 (d, J = 1.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 13.6, 22.1, 22.9, 27.7, 28.5, 28.7, 28.8, 29.0, 29.06, 29.10, 29.12, 29.13, 31.4, 48.3, 122.8, 126.3, 134.2, 147.7, 151.5, 155.7; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 448 $[M+Na]^+$, 426 $[M+H]^+$; negative = 305 (100%) $[C_{16}H_{33}SO_3]^-$; Elem. Anal.: calcd for $C_{22}H_{39}N_3O_3S$ C, 62.08; H, 9.24; N, 9.87; Found: C, 61.90; H, 9.21; N, 9.88.

*5.1.1.19. N′-((phenylsulfonyl)oxy)picolininidamide (**29**).* Solvent: Chloroform; Reaction time: 5 h; Purification method: recrystallization; White crystals, yield 99%, mp 118–120 °C (hexanes/ethyl acetate); IR (KBr): 3457, 3301, 3138, 1645, 1364 & 1188 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.02 (br s, 2H), 7.33 (dd, J = 8.1, 5.4 Hz, 1H), 7.54 (t, J = 7.5 Hz, 2H), 7.63 (t, J = 6.9 Hz, 1H), 7.68 (dt, J = 7.9, 1.3 Hz, 1H), 7.84 (d, J = 8.1 Hz, 1H), 8.06 (d, J = 7.6 Hz, 2H), 8.51 (d, J = 4.5 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 121.1, 125.7, 128.82, 128.79, 133.7, 136.0, 136.7, 146.8, 148.5, 154.5; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 300 $[M+Na]^+$, 278 $[M+H]^+$, 122 (100%) $[M+2H-C_6H_5SO_3]^+$; negative = 157 (100%) $[C_6H_5SO_3]^-$; Elem. Anal.: calcd for $C_{12}H_{11}N_3O_3S$: C, 51.98; H, 4.00; N, 15.15; Found: C, 51.88; H, 3.99; N, 15.12.

*5.1.1.20. N′-(tosyloxy)picolinimidamide (**30**).* Solvent: Chloroform; Reaction time: 6 h; Purification method: recrystallization; White crystals, yield 95%, mp 144–146 °C (hexanes/ethyl acetate); IR (KBr): 3480, 3365, 1642, 1586, 1364 & 1181 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.41 (s, 3H), 5.97 (br s, 2H), 7.31–7.35 (m, 1H), 7.32 (d, J = 8.1 Hz, 2H), 7.67 (dt, J = 7.9, 1.6 Hz, 1H), 7.85 (d, J = 7.9 Hz, 1H), 7.93 (d, J = 8.2 Hz, 2H), 8.50 (d, J = 4.9 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.6, 121.1, 125.6, 128.8, 129.4, 132.9, 136.7, 144.7, 146.8, 148.4, 154.3; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 314 (100%) $[M+Na]^+$, 292 $[M+H]^+$, 122 $[M+2H-C_7H_7SO_3]^+$; negative = 171 (100%) $[C_7H_7SO_3]^-$; Elem. Anal.: calcd for $C_{13}H_{13}N_3O_3S$: C, 53.60; H, 4.50; N, 14.42; Found: C, 53.76; H, 4.49; N, 14.46.

*5.1.1.21. N′-(4-nitrophenylsulfonyloxy)picolinimidamide (**31**).* Solvent: Chloroform; Reaction time: 5 h; Purification method: recrystallization; Yellow crystals, yield 81%, mp 125–127 °C (hexanes/ethyl acetate); IR (KBr): 3494, 3384, 3108, 1652, 1377 & 1188 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6) δ 6.75 (br s, 2H), 7.32–7.37 (m, 1H), 7.64–7.71 (m, 2H), 8.20 (d, J = 9.1 Hz, 2H), 8.33 (d, J = 8.9 Hz, 2H), 8.48 (d, J = 5.1 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6) δ 119.9, 122.9, 124.8, 129.1, 135.8, 140.5, 145.5, 147.6, 149.5, 154.3; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 393 (100%) $[M+K+MeOH]^+$; negative = 321 (100%) $[M-H]^-$; Elem. Anal.: calcd for $C_{12}H_{10}N_4O_5S$: C, 44.72; H, 3.13; N, 17.38; Found C, 44.77; H, 3.12; N, 17.88.

*5.1.1.22. N′-(propylsulfonyloxy)picolinimidamide (**32**).* Solvent: Chloroform; Reaction time: 20 h; Purification method: recrystallization; White crystals, yield 80%, mp 65–66 °C (hexanes/ethyl acetate); IR (KBr): 3456, 3345, 2974, 2944, 2927, 1647, 1359 & 1169 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.10 (t, J = 7.4 Hz, 3H), 1.96 (sext, J = 7.6 Hz, 2H), 3.40 (t, J = 7.1 Hz, 2H), 6.11 (br s, 2H), 7.41 (dd, J = 7.1, 4.9 Hz, 1H), 7.76 (t, J = 7.7 Hz, 1H), 8.02 (d, J = 7.9 Hz, 1H), 8.59 (d, J = 4.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.9, 17.2, 50.5, 121.2, 125.8, 136.9, 146.8, 148.6, 154.7; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 266 $[M+Na]^+$, 244 $[M+H]^+$, 122 (100) $[M+2H-C_3H_7SO_3]^+$; negative = 123 (100%) $[C_3H_7SO_3]^-$; Elem. Anal.: calcd for $C_9H_{13}N_3O_3S$: C, 44.43; H, 5.39; N, 17.27; Found: C, 44.36; H, 5.40; N, 17.30.

*5.1.1.23. N′-((decylsulfonyl)oxy)picolinimidamide (**33**).* Solvent: Chloroform; Reaction time: 20 h; Purification method: recrystallization; White crystals, yield 86%, mp 66–67 °C (hexanes/ethyl acetate); IR (KBr): 3469, 3353, 2916, 2851, 1648, 1364 & 1178 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.87 (t, J = 6.9 Hz, 3H), 1.25 (br s, 12H), 1.42–1.51 (m, 2H), 1.91 (quint, J = 7.3 Hz, 2H), 3.41 (t, J = 7.7 Hz, 2H), 6.02 (br s, 2H), 7.40 (ddd, J = 7.3, 4.9, 1.0 Hz, 1H), 7.75 (dt, J = 7.7, 1.6 Hz, 1H), 8.02 (dd, J = 7.9, 0.8 Hz, 1H), 8.59 (d, J = 4.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 22.6, 23.3, 28.1, 28.9, 29.1, 29.2, 29.4, 31.8, 48.8, 121.1, 125.8, 136.8, 146.8, 148.6, 154.6; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 364 $[M+Na]^+$, 342 $[M+H]^+$, 122 (100%) $[M+2H-C_{10}H_{21}SO_3]^+$; negative = 221 $[C_{10}H_{21}SO_3]^-$; Elem. Anal.: calcd for $C_{16}H_{27}N_3O_3S$: C, 56.28; H, 7.97; N, 12.31; Found: C, 56.33; H, 7.96; N, 12.28.

*5.1.1.24. N′-((hexadecylsulfonyl)oxy)picolinimidamide (**34**).* Solvent: Chloroform; Reaction time: 20 h; Purification method: column chromatography; White crystals, yield 80%, mp 89–90 °C (hexanes/ethyl acetate); IR (KBr): 3470, 3353, 2916, 2850, 1650, 1364 & 1178 (S=O) cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.88 (t, J = 6.9 Hz, 3H), 1.26 (br s, 24H), 1.47 (quint, J = 7.4 Hz, 2H), 1.91 (quint, J = 7.3 Hz, 2H), 3.41 (t, J = 7.8 Hz, 2H), 6.07 (br s, 2H), 7.40 (dd, J = 7.4, 4.9 Hz, 1H), 7.76 (dt, J = 7.7, 1.5 Hz, 1H), 8.02 (d, J = 7.9 Hz 1H), 8.59 (d, J = 4.8 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 22.7, 23.4, 28.2, 29.0, 29.25, 29.33, 29.5, 29.58, 29.64, 31.9, 48.8, 121.2, 125.8, 136.9, 146.8, 148.6, 154.7; MS (LC–MS) (ESI, 1.65 eV): m/z positive = 448.5 $[M+Na]^+$, 426.5 $[M+H]^+$, 122 (100) $[M+2H-C_{16}H_{33}SO_3]^+$; negative = 305 $[C_{16}H_{33}SO_3]^-$; Elem. Anal.: calcd for $C_{22}H_{39}N_3O_3S$ C, 62.08; H, 9.24; N, 9.87; Found: C, 61.95; H, 9.25; N, 9.89.

5.2. Biological evaluation

All the reagents used were commercially available by Merck, 1,1-diphenyl-2-picrylhydrazyl (DPPH), nordihydroguaiaretic acid (NDGA) were purchased from the Aldrich Chemical Co. Milwaukee, WI, (USA). linoleic acid sodium salt, were obtained from Sigma Chemical, Co. (St. Louis, MO, USA).

5.2.1. Experiments in vitro

In the *in vitro* assays each experiment was performed at least in triplicate, and the standard deviation of absorbance was less than 10% of the mean.

5.2.1.1. Determination of the reducing activity of the stable radical 1,1-diphenyl-picrylhydrazyl (DPPH) [42]. To a solution of DPPH (final concentration 50 μM) in absolute ethanol was added an equal volume of the compounds dissolved in dimethylsulfoxide. As control solution ethanol was used. The final concentration of the tested compounds was 100 μM. After 20 and 60 min at room temperature, the absorbance was recorded at 517 nm (Table 1). All tests were

undertaken on three replicates and the results presented in Table 1 were averaged.

5.2.1.2. Competition of the tested compounds with DMSO for hydroxyl radicals [46]. The hydroxyl radicals generated by the Fe^{3+}/ascorbic acid system, were detected according to Nash, by the determination of formaldehyde produced from the oxidation of DMSO. The reaction mixture contained EDTA (0.1 mM), Fe^{3+} (167 μM), DMSO (33 mM) in phosphate buffer (50 mM, pH 7.4), the tested compounds (concentration 0.1 mM) and ascorbic acid (10 mM). After 30 min of incubation (37 °C) the reaction was stopped with CCl_3COOH (17%w/v). Trolox was used as an appropriate standard.

5.2.1.3. Inhibition of linoleic acid lipid peroxidation [51]. Production of conjugated diene hydroperoxide by oxidation of linoleic acid in an aqueous dispersion is monitored at 234 nm. 2,20-Azobis(2-amidinopropane) dihydrochloride (AAPH) is used as a free radical initiator. Ten microliters of the 16 mM linoleic acid dispersion was added to the UV cuvette containing 0.93 mL of 0.05 M phosphate buffer, pH 7.4 prethermostated at 37 °C. The oxidation reaction was initiated at 37 °C under air by the addition of 50 μL of 40 mM AAPH solution. Oxidation was carried out in the presence of aliquots (10 μL) of KukA and its analogues. In the assay without antioxidant, lipid oxidation was measured in the presence of the same level of DMSO. The rate of oxidation at 37 °C was monitored by recording the increase in absorption at 234 nm caused by conjugated diene hydroperoxides.

Acknowledgments

We would like to thank Dr A. Leo and Biobyte Corp. 201 West 4th Str., Suite 204, Claremont CA California, 91711, USA for free access to the C-QSAR program.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2014.04.040.

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