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SYNTHESIS OF 2-QUINOLINE-CARBOXAMIDE DERIVATIVES AS POTENTIAL HDAC INHIBITORS

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Inhibition of histone deacetylase activity appears as an original and effective approach for the treatment of cancer. A series of novel quinoline-containing derivatives has been synthesized and found that some of these compounds possess nanomolar histone deacetylase inhibitory activity.

Keywords: inhibitors of histone deacetylase, quinoline, Zn^{2+} chelating groups.

Histone deacetylases (HDAC) are a group of enzymes that catalyze and regulate the process of deacetylation of histones. The process of acetylation-deacetylation of histones is involved in the regulation of chromatin structure and gene expression. Numerous studies have shown that HDAC inhibition leads to the tumor cell differentiation, proliferation, and apoptosis [1, 2]. These findings immensely stimulated a search for substances, both natural and synthetic, which could serve as HDAC inhibitors. As a result of these studies a number of HDAC inhibitors are currently under clinical trials as anticancer agents [3].

A widely accepted pharmacophoric description of simple HDAC inhibitors can be conditionally represented by three linearly bonded groups **A–L–X**, where **A** represents a surface recognition zone (CAP), usually an aryl group, which provides potency and selectivity, **L** is predominantly a hydrophobic linking group, and **X** represents a moiety that interacts with the catalytic Zn^{2+} ion at the HDAC active site, usually a hydroxamic acid group [3].

During our recent search for new HDACs inhibitors that would contain a quinoline cycle in the **A** part of the molecule [4], a new active 2-quinoline derivative **1** (Figure 1) with IC_{50} = 4 nM for HeLa extract was found (HeLa extract is a cell type in an immortal cell line that is used in cancer research).

A beneficial role of the 2-quinolinyl cycle presence in the **A** part of the structure **1** on the increase of HDAC inhibitory activity can be clearly demonstrated by comparing the activities of the compounds **1**–**3**. Thus, the hydroxamate **2** [5] with the phenyl moiety is a more than 200 times weaker HDAC inhibitor in comparison to the quinoline derivative **1**. Although a significant increase of the activity can be gained by changing the phenyl group of the structure **2** to the 2-naphthyl group, the naphthyl derivative **3** [6] is still 5 times less active than the compound **1**. One can quite easily recognize the above-mentioned pharmacophoric regions **A**–**L**–**X** in the structures **1**–**3**.

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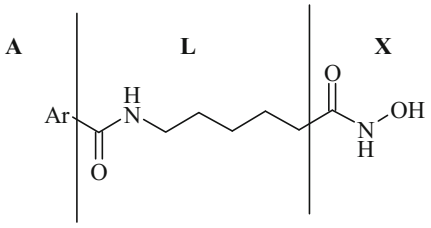


1–3

1 Ar = quinolin-2-yl, IC_{50} = 4 nM,
2 Ar = phenyl, IC_{50} = 900 nM,
3 Ar = naphth-2-yl, IC_{50} = 21 nM

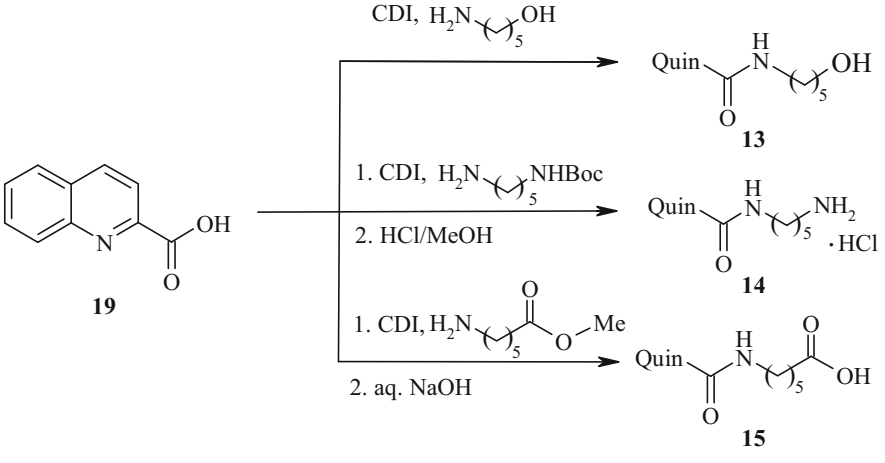
Fig. 1 General structure of a potential HDAC inhibitors

A great majority of known HDAC inhibitors contain hydroxamic acid as a Zn^{2+} binder. Although many hydroxamic acid-based HDAC inhibitors possess strong *in vitro* and *in vivo* activity, they in general exhibit unfavorable pharmacokinetic properties [7], including the potential for chronic toxicities.

The main purpose of the present work was to synthesize and assess the HDAC inhibitory potential of a series of such analogs of the compound **1**, in which the hydroxamic acid part of the molecule would be replaced by diverse functional groups *a priori* capable for more or less effective binding with the Zn^{2+} ion.

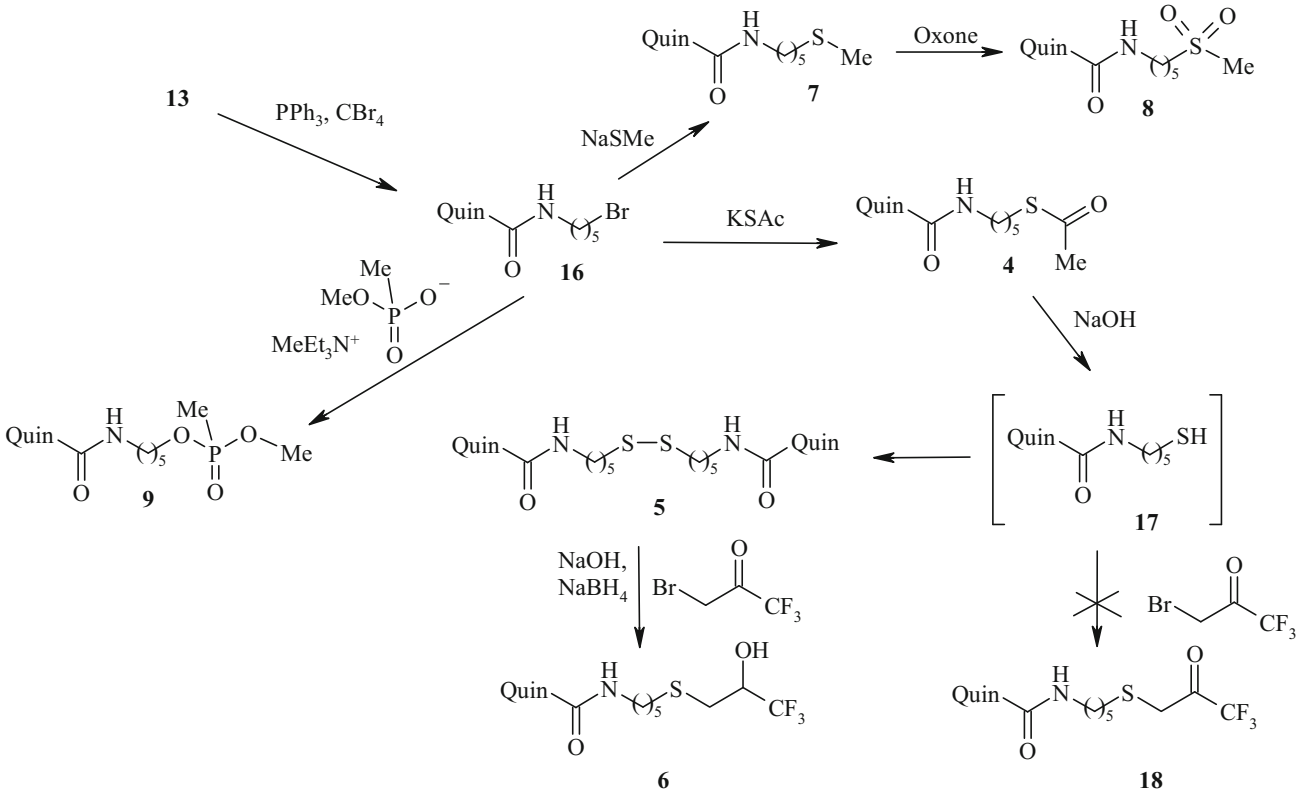
The compounds **4**–**12** prepared for this study are shown in Table 1. The routes used for the synthesis of the compounds **4**–**18** are depicted in Schemes 1–4. The intermediates **13**–**15** were prepared from carboxylic acid **19** as shown in Scheme 1. Thus, condensation of the carboxylic acid **19** with 5-aminopentan-1-ol in the presence of N,N'-carbonyldiimidazole (CDI) afforded the hydroxy derivative **13**. Similar condensation of the acid **19** with *tert*-butyl 5-aminopentylcarbamate followed by acidic elimination of the N-*tert*-butoxycarbonyl (Boc) protective group gave the amine hydrochloride **14**, but the use of methyl 6-aminohexanoate in the reaction with the acid **19** after ester hydrolysis gave the expected carboxylate **15**.

Scheme 1



Bromination of the hydroxy derivative **13** yielded bromide **16**, which in turn served as a common intermediate for further synthesis of compounds **4**–**9** (Scheme 2).

Scheme 2



Thus, the bromide **16** was treated with potassium ethanethioate to give S-ethanethioate derivative **4**, which was converted by a solution of sodium hydroxide in methanol into, presumably, thiol **17**. However, all attempts to obtain the thiol **17** in a pure state were unsuccessful, and only disulfane **5** was isolated from the reaction mixture. It is noteworthy that we were not able to obtain the expected S_N-substitution product **18** of this envisaged thiol **17** with such a reactive electrophile as 3-bromo-1,1,1-trifluoroacetone even upon one-pot conditions by adding the latter to the reaction mixture directly after the removal of the acetyl group from the ethanethioate **4**. As the only product of the reaction the disulfane **5** was isolated. Treatment of the dimeric compound **5** with sodium borohydride and sodium hydroxide in the presence of 3-bromo-1,1,1-trifluoroacetone yielded 2-hydroxypropylsulfanyl derivative **6**. Although ^{1}H NMR spectra of the compound **5** did not provide unequivocal evidence in favor of a dimeric structure –S–S– to a monomer –SH, the dimer structure of the substance **5** was assigned on the basis of its electrospray ionization mass spectrum and chemical behavior.

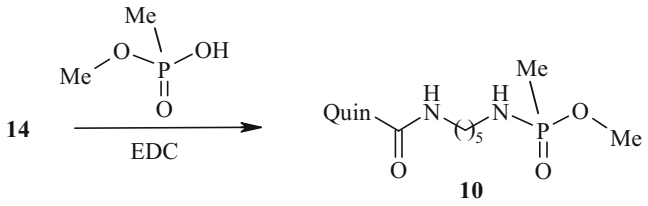
The methylsulfanyl derivative **7** was obtained by alkylation of sodium methanethiolate with the bromide **16**. Further oxidation of the compound **7** with 2 equivalents of oxone gave the sulfonyl derivative **8** (Scheme 2).

The ester **9** was prepared by the reaction of the bromide **16** with N,N-diethyl-N-methylethanaminium methyl methylphosphonate (Scheme 2).

The phosphonamidoate **10** was obtained by condensation of the amine **14** and methyl hydrogen methylphosphonate in the presence of 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide (Scheme 3).

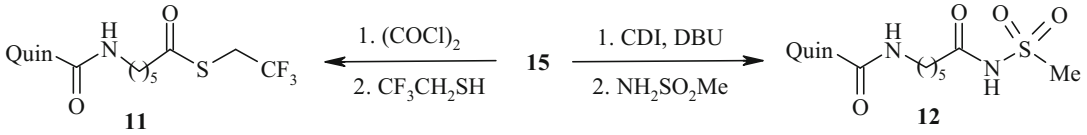
The compounds **11** and **12** were synthesized from the carboxylic acid **15** as depicted in Scheme 4. The acid **15** was converted by oxalyl chloride into the corresponding acid chloride, which, without additional purification, upon treatment with 2,2,2-trifluoroethanethiol yielded *S*-trifluoroethyl thioate **11**. The obtained compound **11** was found to be unstable under chromatographic purification conditions, and a significant amount of the product **11** was converted back to the starting acid **15,** reducing the yield of the reaction. The N-acylmethanesulfonamide **12** was obtained by condensing the acid **15** and methanesulfonamide in the presence of CDI and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU).

Scheme 3



EDC – 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide

Scheme 4

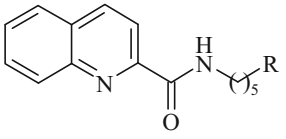


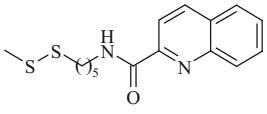
The compounds **4**–**12** synthesized in this study were tested *in vitro* using HeLa nuclear extract assay. In all cases, the enzyme activity was determined with a fluorometric assay. The results are summarized in Table 1. Trichostatin A (TSA) was used as a positive control that inhibited 100% of the HDAC activity at 1 μM.

Among the synthesized compounds **4**–**12** the most active turned out to be the sulfanyl derivatives **4**–**7**. In the case of the compounds **4** and **5,** as a possible mechanism for their action, an initial thioate hydrolysis or reduction of the disulfanyl group could not be ruled out. In the next step the thiol group creates a monodentate complex with the Zn^{2+} ion in the active center of the enzyme [8].

Within this project the sulfone **8** and sulfonamide **12** were found to be inactive, although sulfonamides are well-known inhibitors of carbonic anhydrase and other Zn^{2+} ion-dependent enzymes [9, 10].

TABLE 1. HDAC Enzyme Inhibition Data for Compounds **4–12**



Compound	R	Inhibition (HeLa), %*		
		1 μM	5 μM	20 μM
4	–SAc	33	48	105
5		20	37	71
6	$–SCH_2CH(OH)CF_3$	0	18	55
7	–SMe	0	0	23
8	$–SO_2Me$	NT	NT	0
9	–OP(O)Me(OMe)	NT	NT	0
10	–NHP(O)Me(OMe)	NT	NT	0
11	$–C(O)SCH_2CF_3$	NT	NT	0
12	$–C(O)NHSO_2Me$	NT	NT	0

* NT = not tested.

Phosphonic acid derivatives are known as metalloenzyme (thermolysin and carboxypeptidase A) inhibitors [11, 12]. Unfortunately, the phosphorus-based compounds **9** and **10** were found to be completely inactive in the experiments with the HeLa extract.

Compounds **8**–**12** were found to be inactive at the 20 μM concentration. Because of their weak inhibition potential, further studies of this group of compounds were stopped.

In conclusion, we have obtained and tested new HDAC inhibitors with low micromolar activity against HeLa extract. The active compounds are worthy of further investigation and optimization.

EXPERIMENTAL

Final compounds were assessed for their ability to inhibit HDAC activity. Enzyme activity was determined with a fluorometric assay.

The activity of the compounds as HDAC inhibitors was determined with a commercially available fluorescent assay kit Fluor de Lys^{TM}, BioMol Research Labs, Inc. (Plymouth Meeting, USA). HeLa extract was incubated for 30–60 min at 37ºC in assay buffer (25 mM HEPES, 137 mM NaCl, 2.7 mM KCl, 1 mM $MgCl_2$, pH 8.0) with 15 μM acetylated substrate in the presence of the test compound (HDAC inhibitor). The extent of deacetylation was determined by the addition of 50 μM of 1-in-500 dilution of developer and measurement of the fluorescence (excitation 355 nm, emission 460 nm), according to the instructions provided with the kit.

Percent activity (% activity) was calculated for each test compound ($[(S^C–B)/(S^0–B)]\cdot100$, wherein S^C denotes the signal measured in the presence of the compound being tested, S^0 the signal measured in the absence of the compound being tested, and B the background signal measured in blank wells containing the medium only).

^{1}H NMR spectra were recorded on Varian 200 and Varian 400 Oxford NMR spectrometers (200 and 400 MHz respectively) in solvent as indicated. Chemical shifts are referenced to HMDSO as internal standard (δ = 0.055 ppm) or using the signal of the corresponding residual protonated solvent (for DMSO-d_6 δ = 2.50, for $CDCl_3$ δ = 7.26 ppm). Melting points were determined using a Gallenkamp melting point apparatus. High-resolution mass spectra (HRMS) analyses were performed using a Micromass Q-TOF Micro quadrupole-time-of-flight high-resolution mass spectrometer. Leucine enkephalin was used as internal lock mass for accurate mass calculation (*m/z* 566.2771). LC-MS analyses were performed on an Acquity UPLC system (Waters) connected to the Micromass Q-TOF micro hybrid quadrupole-time-of-flight mass spectrometer operating in the electrospray ionization (ESI) positive ion mode and using a reverse-phase Acquity UPLC BEH C18 column (1.7 μm, 2.1×50 mm) on a gradient of 5–98% acetonitrile–water–0.1% formic acid. HPLC analysis was performed on the Waters HPLC equipped with a LiChrospher RP Select B column (eluent acetonitrile – 0.1% H_3PO_4). Elemental analyses were performed on Carlo Erba CHNS-O EA-1108 apparatus. Organic solvents were dried prior to use by standard procedures. Reagents and solvents were purchased from *Aldrich*, *Acros*, and *Alfa Aesar Chemical Industries*.

N-(5-Hydroxypentyl)-2-quinolinecarboxamide (13). 2-Quinolinecarboxylic acid (**19**) (0.50 g, 2.89 mmol) was dissolved in 20 ml THF, then CDI (0.56 g, 3.47 mmol) was added, and the solution was stirred at room temperature for 1 h. After addition of 5-amino-1-pentanol (0.36 g, 3.47 mmol), the mixture was stirred at room temperature for 3 h. The reaction mixture was evaporated and after addition of EtOAc, the organic phase was washed with H_2O, 3% HCl, dried over Na_2SO_4, filtered, and evaporated. Yield 0.68 g (91%); mp 63–64ºC. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.35 (2H, m, CH_2); 1.46 (2H, quint, *J* = 6.9, CH_2); 1.59 (2H, quint, *J* = 7.3, CH_2); 3.28–3.44 (4H, m overlapped with H_2O, $2CH_2$); 4.35 (1H, t, *J* = 5.2, OH); 7.72 (1H, ddd, *J* = 1.1, *J* = 7.0, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.3, *J* = 7.0, *J* = 8.3, H Quin); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.3, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.55 (1H, d, *J* = 8.5, H Quin); 8.90 (1H, t, *J* = 6.0, NH). Mass spectrum, *m/z*: 259 $[M+H]^+$. Found, %: C 69.45; H 7.02; N 10.79. $C_{15}H_{18}N_2O_2$. Calculated, %: C 69.75; H 7.02; N 10.84.

N-(5-Aminopentyl)-2-quinolinecarboxamide Hydrochloride (14). Starting *tert*-butyl N-{5-[(2-quinolinylcarbonyl)amino]pentyl}carbamate was obtained as described for the compound **13**. Yield 86% of a wax-like

product. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.25–1.46 (4H, m, $2CH_2$); 1.35 (9H, s, $3CH_3$); 1.58 (2H, quint, *J* = 7.3, CH_2); 2.91 (2H, q, *J* = 6.5, CH_2); 3.29–3.40 (2H, m overlapped with H_2O); 6.76 (1H, t, *J* = 5.7, NH); 7.72 (1H, ddd, *J* = 1.1, *J* = 6.9, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.4, *J* = 6.9, *J* = 8.4, H Quin); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.55 (1H, d, *J* = 8.5, H Quin); 8.89 (1H, t, *J* = 6.0, NH).

tert-Butyl N-{5-[(2-quinolinylcarbonyl)amino]pentyl}carbamate (2.40 g, 6.71 mmol) was dissolved in 50 ml of 1 N HCl solution in MeOH and stirred at room temperature for 1 h. The reaction mixture was evaporated and the residue was kept for 12 h at 0ºC. The obtained wax-like solid was recrystallized at 0–5ºC from MeCN–EtOH (10:1). Yield 1.07 g (54%). ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.44 (2H, m, CH_2); 1.67 (4H, m, $2CH_2$); 2.82 (2H, sextet, *J* = 6.4, CH_2); 3.42 (2H, q, *J* = 6.7, CH_2); 4.00–4.50 (3H, br. s overlapped with H_2O, NH_3); 7.77 (1H, t, *J* = 7.7, H Quin); 7.93 (1H, t, *J* = 7.7, H Quin); 8.14 (1H, d, *J* = 8.0, H Quin); 8.21 (1H, d, *J* = 8.4, H Quin); 8.22 (1H, d, *J* = 8.5, H Quin); 8.64 (1H, d, *J* = 8.5, H Quin); 9.04 (1H, t, *J* = 6.0, NH). HRMS (ESI), *m/z*: Found: 258.1646 $[M+H]^+$. $C_{15}H_{19}N_3O$. Calculated: M = 258.1606.

6-[(2-Quinolinylcarbonyl)amino]hexanoic acid (15). Starting methyl 6-[(2-quinolinylcarbonyl)amino]hexanoate was obtained as described for the compound **13**. Yield 60% of a wax-like product. ^{1}H NMR spectrum (200 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.27–1.43 (2H, m, CH_2); 1.48–1.68 (4H, m, $2CH_2$); 2.32 (2H, t, *J* = 7.1, CH_2); 3.36 (2H, q overlapped with H_2O, *J* = 6.7, CH_2); 3.57 (3H, s, CH_3); 7.72 (1H, ddd, *J* = 1.2, *J* = 6.9, *J* = 8.2, H Quin); 7.87 (1H, ddd, *J* = 1.5, *J* = 6.9, *J* = 8.4, H Quin); 8.08 (1H, dd, *J* = 1.5, *J* = 8.2, H Quin); 8.14 (1H, dd, *J* = 1.2, *J* = 8.4, H Quin); 8.16 (1H, d, *J* = 8.5, H Quin); 8.56 (1H, *J* = 8.5, H Quin); 8.93 (1H, t, *J* = 6.1, NH).

Methyl 6-[(2-quinolinylcarbonyl)amino]hexanoate (0.52 g, 1.73 mmol) was added to a solution of NaOH (0.25 g, 6.25 mmol) in 10 ml of EtOH and stirred at room temperature for 15 min. The solvent was evaporated. The residue was dissolved in 10 ml of H_2O, acidified to pH 3 with 18% HCl, and extracted with EtOAc. The organic phase was washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. Yield 0.31 g (62%); mp 70–71ºC. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.26–1.42 (2H, m, CH_2); 1.55 (2H, quint, *J* = 7.6, CH_2); 1.59 (2H, quint, *J* = 7.6, CH_2); 2.22 (2H, t, *J* = 7.3, CH_2); 3.35 (2H, q overlapped with H_2O, *J* = 6.7, CH_2); 7.72 (1H, ddd, *J* = 1.2, *J* = 6.9, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.6, *J* = 6.9, *J* = 8.5, H Quin); 8.08 (1H, dd, *J* = 1.6, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.5, H Quin); 8.15 (1H, d, *J* = 8.6, H Quin); 8.56 (1H, d, *J* = 8.6, H Quin); 8.94 (1H, t, *J* = 6.2, NH); 12.00 (1H, br. s, OH). HRMS (ESI), *m/z*: Found: 287.1365 $[M+H]^+$. $C_{16}H_{18}N_2O_3$. Calculated: M = 287.1396.

N-(5-Bromopentyl)-2-quinolinecarboxamide (16). Compound **13** (2.50 g, 9.68 mmol) was dissolved in 20 ml of MeCN, then Ph_3P (3.05 g, 11.62 mmol) and CBr_4 (3.90 g, 11.76 mmol) were added, and the solution was stirred at room temperature for 24 h. The reaction mixture was evaporated and after addition of EtOAc, the organic phase was washed with H_2O, brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography with EtOAc–light petroleum (1:2) as eluent. Yield 1.71 g (54%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.44 (2H, m, CH_2); 1.61 (2H, quint, *J* = 7.3, CH_2); 1.85 (2H, quint, *J* = 7.1, CH_2); 3.37 (2H, q overlapped with H_2O, *J* = 6.8, CH_2); 3.55 (1H, t, *J* = 6.7, CH_2); 7.72 (1H, ddd, *J* = 1.2, *J* = 7.0, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.4, *J* = 7.0, *J* = 8.4, H Quin); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.55 (1H, d, *J* = 8.5, H Quin); 8.94 (1H, t, *J* = 5.8, NH). HRMS (ESI), *m/z*: Found: 321.0568 $[M+H]^+$. $C_{15}H_{17}BrN_2O$. Calculated: M = 321.0602.

S-{5-[(2-Quinolinylcarbonyl)amino]pentyl} Ethanethioate (4). Compound **16** (0.10 g, 0.31 mmol) was dissolved in 7 ml of MeCN under Ar atmosphere, and potassium ethanethioate (0.07 g, 0.62 mmol) was added. The obtained solution was stirred at room temperature for 24 h. The reaction mixture was filtered, the filtrate was evaporated, dissolved in ethyl acetate, and washed with H_2O, 10% solution of citric acid, H_2O, dried over Na_2SO_4, filtered, and evaporated. Yield 0.08 g (84%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.33–1.42 (2H, m, CH_2); 1.56 (2H, quint, *J* = 7.4, CH_2); 1.59 (2H, quint, *J* = 7.4, CH_2); 2.30 (3H, s, CH_3); 2.84 (2H, t, *J* = 7.2, CH_2); 3.35 (2H, q overlapped with H_2O, *J* = 6.9, CH_2); 7.72 (1H, ddd, *J* = 1.2, *J* = 7.0, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.5, *J* = 7.0, *J* = 8.4, H Quin); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.56 (1H, d, *J* = 8.5, H Quin); 8.92 (1H, t,

J = 5.9, NH). Mass spectrum, *m/z*: 317 $[M+H]^+$. Found, %: C 63.40; H 6.01; N 8.55. $C_{17}H_{20}N_2O_2S \cdot 0.3H_2O$. Calculated, %: C 63.45; H 6.45; N 8.70.

N,N'-[Disulfanediyldi(pentane-5,1-diyl)]diquinoline-2-carboxamide (5). Compound **4** (0.27 g, 0.85 mmol) was dissolved in 10 ml of MeOH under Ar atmosphere, and a solution of NaOH (0.04 g, 1.00 mmol) in 4 ml of MeOH was added and stirred at room temperature for 1 h. The reaction mixture was evaporated, and the obtained residue was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography with EtOAc–light petroleum (1:2) as eluent. Yield 0.14 g (60%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.45–1.62 (4H, m, $2CH_2$); 1.62–1.84 (8H, m, $4CH_2$); 2.70 (4H, t, *J* = 7.2, $2CH_2$); 3.53 (4H, q, *J* = 6.6, $2CH_2$); 7.60 (2H, ddd, *J* = 1.3, *J* = 7.0, *J* = 8.1, H Quin); 7.75 (2H, ddd, *J* = 1.5, *J* = 7.0, *J* = 8.4, H Quin); 7.86 (2H, dd, *J* = 1.5, *J* = 8.1, H Quin); 8.09 (2H, d, *J* = 8.4, H Quin); 8.28 (2H, d, *J* = 8.6, H Quin); 8.29 (2H, d, *J* = 8.6, H Quin); 8.33 (2H, unresolved t, 2NH). HRMS (ESI), *m/z*: Found: 547.2144 $[M+H]^+$. $C_{32}H_{34}N_4O_2S_2$. Calculated: M = 547.2201.

N-{5-[(3,3,3-Trifluoro-2-hydroxypropyl)sulfanyl]pentyl}-2-quinolinecarboxamide (6). Compound **5** (0.13 g, 0.24 mmol) was dissolved in 5 ml of EtOH under Ar atmosphere at 0ºC, and to this solution solid NaOH (0.02 g, 0.47 mmol) and $NaBH_4$ (0.02 g, 0.52 mmol) were added, and the reaction mixture was stirred for 1 h at 0ºC. Then 3-bromo-1,1,1-trifluoropropan-2-one (0.06 ml, 0.56 mmol) was added, and the reaction mixture was stirred for 3 h at room temperature. The solvent was evaporated and the obtained residue was dissolved in EtOAc, washed with H_2O, dried over Na_2SO_4, filtered, and evaporated. Yield 0.11 g (58%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.46–1.60 (2H, m, CH_2); 1.65–1.80 (4H, m, $2CH_2$); 2.69 (2H, t, *J* = 7.3, CH_2); 2.93 (1H, br. s, OH); 3.48 (1H, dd, *J* = 8.4, *J* = 11.1, CH); 3.52 (2H, q, *J* = 6.8, CH_2); 3.62 (1H, dd, *J* = 3.0, *J* = 11.1, CH); 4.23 (1H, m, CH); 7.60 (1H, ddd, *J* = 1.2, *J* = 7.0, *J* = 8.2, H Quin); 7.75 (1H, ddd, *J* = 1.4, *J* = 7.0, *J* = 8.4, H Quin); 7.86 (1H, ddd, *J* = 0.6, *J* = 1.4, *J* = 8.2, H Quin); 8.09 (1H, d, *J* = 8.4, H Quin); 8.26–8.34 (3H, m, 2H Quin and NH). HRMS (ESI), *m/z*: Found: 387.1354 $[M+H]^+$. $C_{18}H_{21}F_3N_2O_2S$. Calculated: M = 387.1354.

N-[5-(Methylsulfanyl)pentyl]-2-quinolinecarboxamide (7). Compound **16** (0.50 g, 1.55 mmol) was dissolved in 15 ml of EtOH, a solution of sodium methanethiolate (0.23 g, 3.28 mmol) in 15 ml of EtOH–H_2O (1:1) was added, and the reaction mixture was stirred for 12 h at 50ºC. The solvent was evaporated, and the obtained residue was dissolved in EtOAc, washed with H_2O, brine, dried over Na_2SO_4, filtered, and evaporated. Yield 0.32 g (71%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.36–1.47 (2H, m, CH_2); 1.54–1.65 (4H, m, $2CH_2$); 2.02 (3H, s, CH_3); 2.47 (2H, t overlapped with DMSO, *J* = 7.3, CH_2); 3.37 (2H, q overlapped with H_2O, *J* = 6.8, CH_2); 7.72 (1H, ddd, *J* = 1.1, *J* = 7.0, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.4, *J* = 7.0, *J* = 8.4, H Quin); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.56 (1H, d, *J* = 8.5, H Quin); 8.92 (1H, t, *J* = 6.0, NH). HRMS (ESI), *m/z*: Found: 289.1361 $[M+H]^+$. $C_{16}H_{20}N_2OS$. Calculated: M = 289.1375.

N-[5-(Methylsulfonyl)pentyl]-2-quinolinecarboxamide (8). To a solution of compound **7** (0.15 g, 0.52 mmol) in 5 ml of DMF, a solution of oxone ($2KHSO_5 \cdot KHSO_4 \cdot K_2SO_4$) (0.64 g, 1.04 mmol) in 5 ml of H_2O was added, and the reaction mixture was stirred for 12 h at room temperature. To the reaction mixture 15 ml of H_2O was added; the product was extracted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. Yield 0.07 g (44%); mp 84–86ºC. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.61 (2H, m, CH_2); 1.77 (2H, quint, *J* = 7.4, CH_2); 1.95 (2H, m, CH_2); 2.90 (3H, s, CH_3); 3.04 (2H, m, CH_2); 3.57 (2H, q, *J* = 6.7, CH_2); 7.62 (1H, t, *J* = 7.4, H Quin); 7.77 (1H, t, *J* = 7.6, H Quin); 7.87 (1H, d, *J* = 8.0, H Quin); 8.11 (1H, d, *J* = 8.5, H Quin); 8.30 (1H, d, *J* = 8.6, H Quin); 8.31 (1H, d, *J* = 8.6, H Quin); 8.34 (1H, unresolved t, NH). Mass spectrum, *m/z*: 321 $[M+H]^+$. Found, %: C 59.91; H 6.01; N 8.62. $C_{16}H_{20}N_2O_3S$. Calculated, %: C 59.98; H 6.29; N 8.74.

Methyl 5-[(2-quinolinylcarbonyl)amino]pentyl Methylphosphonate (9). To a solution of compound **16** (0.34 g, 1.06 mmol) in 5 ml of $CHCl_3$, N,N-diethyl-N-methylethanaminium methyl methylphosphonate (0.28 g, 2.12 mmol) was added, and the reaction mixture was stirred in a closed vessel for 12 h at 100ºC. The reaction mixture was evaporated, the obtained residue was dissolved in $CHCl_3$, washed with H_2O, brine, dried

over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography with $CHCl_3$–MeOH (10:1) as eluent. Yield 0.24 g (65%) of a wax-like product. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.46 (3H, d, *J* = 17.4, CH_3); 1.50–1.58 (2H, m, CH_2); 1.70–1.80 (4H, m, $2CH_2$); 3.55 (2H, q, *J* = 6.8, CH_2); 3.70 (3H, d, *J* = 11.1, OCH_3); 4.00–4.10 (2H, m, CH_2); 7.61 (1H, ddd, *J* = 1.2, *J* = 6.9, *J* = 8.1, H Quin); 7.76 (1H, ddd, *J* = 1.5, *J* = 6.9, *J* = 8.4, H Quin); 7.88 (1H, d, *J* = 8.1, H Quin); 8.10 (1H, d, *J* = 8.4, H Quin); 8.28–8.33 (3H, m, 2H Quin and NH). HRMS (ESI), *m/z*: Found: 351.1448 $[M+H]^+$. $C_{17}H_{23}N_2O_4P$. Calculated: M = 351.1474.

Methyl P-Methyl-N-{5-[(2-quinolinylcarbonyl)amino]pentyl}phosphonamidoate (10). To a solution of N-(5-aminopentyl)-2-quinolinecarboxamide hydrochloride (**14**) (0.20 g, 0.68 mmol) in 10 ml of DMF were added successively EDC (0.18 g, 0.94 mmol), triethylamine (0.13 ml, 0.94 mmol), and methyl hydrogen methylphosphonate (0.11 g, 0.86 mmol), and the reaction mixture was stirred for 15 h at 100ºC. To the reaction mixture 20 ml of H_2O was added, the product was extracted with EtOAc, washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was purified by flash chromatography with $CHCl_3$–MeOH (10:1) as eluent. Yield 0.21 g (88%); mp 75–77ºC. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.27 (3H, d, *J* = 16.4, CH_3); 1.31–1.39 (2H, m, CH_2); 1.44 (2H, quint, *J* = 7.2, CH_2); 1.59 (2H, quint, *J* = 7.4, CH_2); 2.50–2.56 (2H, m overlapped with DMSO, CH_2); 3.32–3.39 (2H, m overlapped with H_2O, CH_2); 3.44 (3H, d, *J* = 11.0, OCH_3); 7.72 (1H, ddd, *J* = 1.1, *J* = 7.0, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.4, *J* = 7.0, *J* = 8.4, H Quin); 7.99 (1H, br. s, NH); 8.08 (1H, d, *J* = 8.1, H Quin); 8.14 (1H, d, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5, H Quin); 8.56 (1H, d, *J* = 8.5, H Quin); 8.91 (1H, t, *J* = 5.9, NH). Found, %: C 57.19; H 6.72; N 11.60. $C_{17}H_{24}N_3O_3P \cdot 0.4H_2O$. Calculated, %: C 57.26; H 7.01; N 11.78.

S-(2,2,2-Trifluoroethyl) 6-[(2-Quinolinylcarbonyl)amino]hexanethioate (11). To a solution of compound **15** (0.40 g, 1.40 mmol) in 10 ml of THF were added dropwise oxalyl dichloride (0.34 ml, 3.96 mmol) and two drops of DMF. The reaction mixture was stirred for 10 min at room temperature, and the precipitated crystals were collected by filtration to give 0.35 g (81%) of 6-[(2-quinolinylcarbonyl)amino]hexanoyl chloride which was used in the next reaction immediately. To a solution of 6-[(2-quinolinylcarbonyl)amino]hexanoyl chloride (0.35 g, 1.40 mmol) in 10 ml of CH_2Cl_2 under Ar atmosphere triethylamine (0.20 ml, 1.42 mmol) was added dropwise, the reaction mixture was stirred for 20 min at room temperature, then 2,2,2-trifluoroethanethiol (0.23 ml, 2.58 mmol) was added, and the reaction mixture was stirred for 6 h at room temperature. The reaction mixture was evaporated and the obtained residue was dissolved in CH_2Cl_2, washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The residue was purified by flash chromatography with EtOAc–light petroleum (1:2) as eluent, yielding 0.11 g of the starting acid **15** (30%) and 0.07 g of the product **11** (13%) with mp 39–41ºC. ^{1}H NMR spectrum (400 MHz, $CDCl_3$), δ, ppm (*J*, Hz): 1.44–1.54 (2H, m, CH_2); 1.72 (2H, quint, *J* = 7.5, CH_2); 1.78 (2H, quint, *J* = 7.6, CH_2); 2.67 (2H, t, *J* = 7.4, CH_2); 3.54 (2H, q, *J* = 6.8, CH_2); 3.57 (2H, q, *J* = 9.9, CH_2); 7.61 (1H, ddd, *J* = 1.2, *J* = 7.0, *J* = 8.1, H Quin); 7.76 (1H, ddd, *J* = 1.4, *J* = 7.0, *J* = 8.4, H Quin); 7.88 (1H, d, *J* = 8.1, H Quin); 8.10 (1H, d, *J* = 8.4, H Quin); 8.26–8.33 (3H, m, 2H Quin and NH). Mass spectrum, *m/z*: 385 $[M+H]^+$. Found, %: C 56.32; H 5.10; N 7.17. $C_{18}H_{19}F_3N_2O_2S$. Calculated, %: C 56.24; H 4.98; N 7.29.

N-{6-[(Methylsulfonyl)amino]-6-oxohexyl}-2-quinolinecarboxamide (12). To a solution of compound **15** (0.40 g, 1.40 mmol) in 15 ml of DMF was added CDI (0.27 g, 1.68 mmol), and the reaction mixture was stirred for 1 h at room temperature. Then DBU (0.21 ml, 1.40 mmol) and methanesulfonamide (0.16 g, 1.68 mmol) were added and the reaction mixture was stirred for 2 h at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc; the extract was washed with H_2O and brine, dried over Na_2SO_4, filtered, and evaporated. The crude product was recrystallized from Et_2O. Yield 0.44 g (86%); mp 165–167ºC. ^{1}H NMR spectrum (400 MHz, DMSO-d_6), δ, ppm (*J*, Hz): 1.27–1.37 (2H, m, CH_2); 1.57 (2H, quint, *J* = 7.6, CH_2); 1.59 (2H, quint, *J* = 7.5, CH_2); 2.28 (2H, t, *J* = 7.2, CH_2); 3.21 (3H, s, CH_3); 3.35 (2H, q overlapped with H_2O, *J* = 6.7, CH_2); 7.72 (1H, ddd, *J* = 1.2, *J* = 6.9, *J* = 8.1, H Quin); 7.87 (1H, ddd, *J* = 1.4, *J* = 6.9, *J* = 8.4, H Quin); 8.08 (1H, dd, *J* = 1.4, *J* = 8.1, H Quin); 8.14 (1H, dd, *J* = 1.2, *J* = 8.4, H Quin); 8.15 (1H, d, *J* = 8.5,

H Quin); 8.55 (1H, J = 8.5, H Quin); 8.92 (1H, t, J = 5.9, NH); 11.65 (1H, br. s, NH). Mass spectrum, *m/z*: 364 $[M+H]^+$. Found, %: C 55.93; H 5.47; N 11.33. $C_{17}H_{21}N_3O_4S$. Calculated, %: C 56.18; H 5.82; N 11.56.

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