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Synthesis and evaluation of 2-(1*H*-indol-3-yl)-4-phenylquinolines as inhibitors of cholesterol esterase

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ABSTRACT

A series of 2-(substituted) phenyl and 2-indolyl quinoline derivatives (**10a–l**) was synthesized by an efficient microwave-assisted, trifluoroacetic acid-catalyzed, solvent-free method. Evaluation of the inhibitory activity led to the identification of two quinoline inhibitors of cholesterol esterase. 2-(1*H*-Indol-3-yl)-6-nitro-4-phenylquinoline (**10l**; IC_{50} = 1.98 μM) was characterized as a mixed-type inhibitor with a pronounced competitive binding mode.

The quinoline scaffold is prevalent in a variety of biologically active compounds. This heterocyclic class became well known for the antimalarial activities of several of its representatives, such as chloroquine (**1**, Fig. 1).[1] Quinolines also possess outstanding activity against leishmaniasis, and the 6-methoxy-8-alkylaminoquinoline derivative sitamaquine has been evaluated in clinical trials with a positive outcome.[2] The introduction of 2-alkylaminomethyl, 2-aryl, 6-halo, or 8-nitro groups has recently been examined with respect to the quinolines' trypanocidal or antileishmanial activities.[3,4] Bedaquiline (**2**), an inhibitor of the mycobacterial ATP synthase, was approved to treat multi-drug-resistant tuberculosis.[5] Quinolines, mainly 5-hydroxy derivatives, have been known for years as iron chelating therapeutics and antifungals.[6,7] Several quinoline-based compounds also show effective anticancer activity,[8,9] and cabozantinib (**3**), a multitargeted receptor tyrosine kinase inhibitor, has reached the market for the treatment of medullary thyroid cancer.[10] The antibiotic and cytotoxic drug nitroxoline (8-hydroxy-5-nitroquinoline) and some if its derivatives, for example **4**, were shown to inhibit the human cysteine protease cathepsin B.[11] Quinolines have been explored as anti-inflammatory agents interacting with different targets,[12] as well as inhibitors of monoamine oxidase A and B,[13] or phosphodiesterase type 4B, as shown, for example, for the 1*H*-indol-3-yl derivative **5**.[14]

Moreover, quinolines have been described as inhibitors of the cholesteryl ester transfer protein (CETP). It mediates the transfer of neutral lipids such as triglycerides, free cholesterol, and cholesteryl esters in the blood among various lipoprotein particles. CETP facilitates the transfer of cholesteryl esters packaged in high-density lipoproteins (HDL) to low-density lipoproteins (LDL) and very-low-density lipoproteins (VLDL). CETP inhibitors, such as **6**, are expected to provide a potential therapeutic benefit for patients with coronary artery disease.[15]

This broad spectrum of biological and biochemical activities of quinolines has attracted much attention in drug research. The facile synthetic accessibility and the versatile possibilities to modify the quinoline core have further contributed to the wide utilization of this heterocycle. It became obvious that the quinoline scaffold can be regarded as a 'privileged structure' in medicinal chemistry.[9,16]

In the present study, a series of 2,4-diarylquinolines was evaluated with respect to their inhibitory activity against a panel of proteases and esterases, that is the serine esterases cholesterol esterase (CEase) and acetylcholinesterase, the serine proteases thrombin, trypsin, chymotrypsin, and human leukocyte elastase (HLE), as well as the cysteine protease cathepsin L. The selected enzymes share an acyl transfer mechanism by which the reaction is catalyzed. This mechanism involves the nucleophilic attack of the active site serine (or cysteine) at the substrate's carbonyl carbon, the release of the first cleavage product and the hydrolysis of the (thio)ester bond to form the second product. Although an impressive number of reports underlie the manifold bioactivities of quinoline derivatives, an investigation to survey the putative

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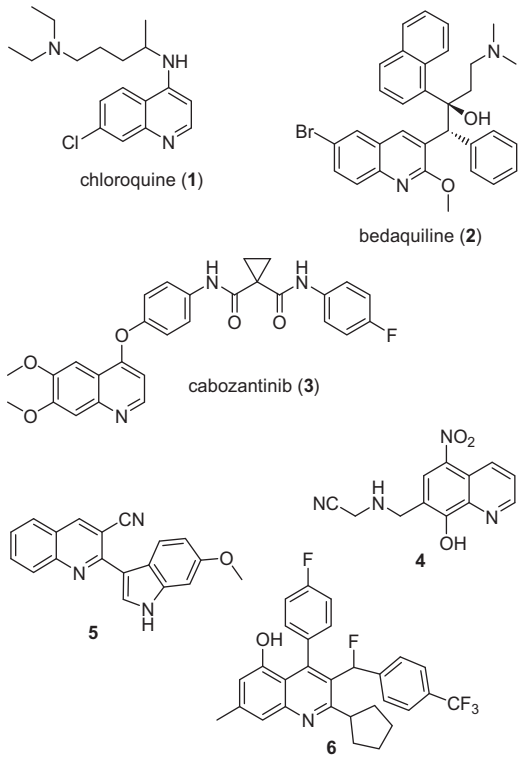
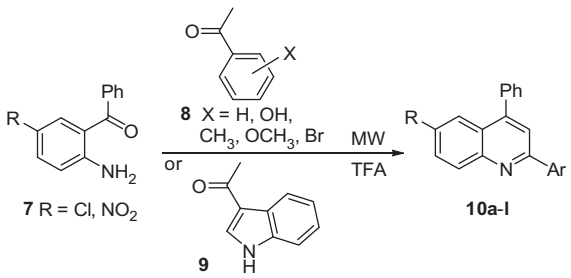


Figure 1. Examples of bioactive quinoline derivatives.



Scheme 1. Synthesis of quinolines **10**.

inhibition of serine and cysteine hydrolases has not been undertaken so far.

We envisaged the preparation of the target compounds by Friedländer reaction under microwave conditions, in continuation of previous studies.[3,17,18] In position 2 of the target 4-phenylquinolines, several aromatic groups should be introduced, that is (subst.) phenyl and indol-3-yl residues. The latter group was chosen on the basis of the structure of corresponding bioactive compounds, such as **5** (Fig. 1), and the occurrence of indole substructures in alkaloids found in the leaves and stem bark extracts of the Bolivian plant *Peschiera van heurkii* (Muell. Arg.) which have been screened for antileishmanial and antibacterial properties.[19] Position 6 of the target quinolines should be occupied by a halo (i.e. chloro) or nitro group in due consideration of corresponding bioactive representatives.[3–5]

The Friedländer annulation of *o*-aminobenzophenones with ketones is a particularly straightforward approach for the synthesis of polysubstituted quinolines. Friedländer reactions afford high temperatures in the absence of catalyst, but catalytic systems have been continuously explored in search of improved efficiencies showing that acid catalysts are mostly more effective than base catalysts. Several transition-metal-catalyzed or metal-triflate-catalyzed Friedländer reaction processes have been developed. In addition, microwave (MW)-assisted or ionic liquid-promoted Friedländer annulations have been employed.[20]

Thus, 2-amino-5-chloro-benzophenone or 2-amino-5-nitrobenzophenone (**7**) was reacted under microwave irradiation with a set of substituted acetophenones **8** in order to obtain 2-phenylquinolines **10a–e** and **10g–k**, and the acetylindole **9** was employed to prepare the 2-(3-indolyl)quinolines **10f** and **10l** (Scheme 1 and Table 1). In these syntheses, trifluoroacetic acid was used as catalyst, exhibiting a more suitable and efficient performance compared with hydrochloric acid. The final products **10a–l** were afforded in high yields and the reaction times were between 2 and 14 min.[21–33]

The two indolyl-substituted quinolines **10f** and **10l** were identified as inhibitors of porcine CEase with IC_{50} values of 1.33 and 1.98 μM, respectively (Fig. 2, Table 1).[36] Most of the inhibitors of CEases bear an electrophilic warhead structure, prone to covalently interact with the enzyme upon being attacked by the active site serine.[37] Noteworthy, the two identified quinoline derivatives lack such an electrophilic site. Whereas CEase inhibition was attained with both, the 6-chloro and the 6-nitro derivative, the 2-indolyl moiety was essential for inhibitory activity; the presence of (substituted) monocyclic aryl groups at 2-position led to inactive compounds.

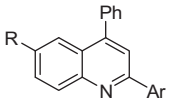
Further characterization of the CEase inhibition was exemplarily carried out with **10l** by performing the assay in the presence of different substrate concentrations (Figs. 3 and 4). A K_i value of 660 nM and a K_i' value of around 7 μM were obtained, where K_i and K_i' represent the equilibrium constants for competitive and uncompetitive inhibition, respectively. Thus, the analysis revealed mixed-type inhibition with a pronounced competitive component. Future investigations are planned to extend the variability of the quinolines' substitution pattern. Such envisaged new derivatives might be designed with respect to a closer similarity to the structure of cholesterol (esters) and to confirm their putative accommodation within the active site of CEase in a substrate like manner.

CEase catalyses the hydrolysis of dietary cholesteryl esters, besides a variety of other substrates, and plays an important role in cholesterol absorption. The inhibition of CEase may therefore render a way to limit the bioavailability of dietary cholesterol. Circulating CEase accumulates in atherosclerotic lesions and is supposed to have deleterious effects in atherosclerosis. Accordingly, CEase was considered to be a potential target for the development of inhibitors for the treatment for diseases such as hypercholesterolemia and coronary heart disease.[37]

Evaluation of the enzyme-inhibiting properties of **10a–l** showed no inhibitory activity against acetylcholinesterase from *Electrophorus electricus,* human thrombin, bovine trypsin, bovine chymotrypsin and human cathepsin L for any of the quinolines (Table 1).[38–42] Three derivatives, that is **10f**, **10i** and **10l**, exhibited inhibitory activity against HLE. The kinetic analysis revealed some deviations from noncooperative binding. The underlying mode of the elastase-inhibitor interactions was not further investigated in the course of this study.

In summary, we have employed a MW-assisted, solvent-free method to afford 2,4-diarylquinoline derivatives in high yields and short reaction times under eco-friendly conditions. As an outcome of a screening against selected hydrolases, we identified representatives of the 'privileged' quinoline structure as inhibitors of CEase. It would be attractive to combine their structural feature with those of quinoline-type CETP inhibitors. Such an approach might lead to the development of bifunctional compounds addressing two different, therapeutically relevant targets of the cholesterol homeostasis.

Table 1
2,4-Diarylquinolines prepared and enzyme-inhibitory properties



Compd	R	Ar	IC_{50} values[a,b,c,d]					
			CEase	Acetylcholin-esterase	Thrombin	Trypsin	Chymo-trypsin	HLE
10a	Cl	Ph	⩾20 μM	n.i.	n.i.	n.i	n.i.	⩾28 μM
10b	Cl	C_6H_4(4-CH_3)	⩾20 μM	n.i.	n.i.	⩾51 μM	n.i.	⩾28 μM
10c	Cl	C_6H_4(4-OH)	⩾20 μM	n.i.	⩾30 μM	⩾51 μM	n.i.	n.i.
10d	Cl	C_6H_4(4-OCH_3)	⩾20 μM	n.i.	⩾30 μM	n.i.	n.i.	⩾28 μM
10e	Cl	C_6H_4(3-Br)	⩾20 μM	n.i.	n.i.	⩾51 μM	n.i.	⩾28 μM
10f	Cl	Indol-3-yl	1.33 ± 0.12 μM	⩾37 μM	⩾30 μM	n.i.	n.i.	4.22 ± 0.02 μM[e]
10g	NO_2	Ph	⩾20 μM	⩾37 μM	n.i.	⩾51 μM	n.i.	n.i.
10h	NO_2	C_6H_4(4-CH_3)	⩾20 μM	n.i.	⩾30 μM	n.i.	n.i.	⩾28 μM
10i	NO_2	C_6H_4(4-OH)	⩾20 μM	n.i.	⩾30 μM	n.i.	n.i.	2.91 ± 0.14 μM[e]
10j	NO_2	C_6H_4(4-OCH_3)	n.i.	⩾37 μM	⩾30 μM	n.i.	n.i.	n.i.
10k	NO_2	C_6H_4(3-Br)	⩾20 μM	n.i.	n.i.	n.i.	n.i.	⩾28 μM
10l	NO_2	Indol-3-yl	1.98 ± 0.11 μM	22.9 μM	⩾30 μM	n.i.	⩾30 μM	0.63 ± 0.02 μM[e]

[a] n.i. no inhibition. More than 90% residual activity determined in duplicate experiments in the presence of the inhibitor at a single concentration (25 μM in the case of CEase, 5 μM in the case of chymotrypsin, 50 μM in the case of cathepsin L, 10 μM in the case of the other enzymes).
[b] Limits without standard error were determined from duplicate experiments at a single inhibitor concentration (as noted above).
[c] IC_{50} values were determined in duplicate experiments in the presence of five different inhibitor concentrations.
[d] None of the compounds inhibited human cathepsin L.
[e] IC_{50} values were obtained by linear regression according to the equation $v = v_0/(1+([I]/IC_{50})^x)$.

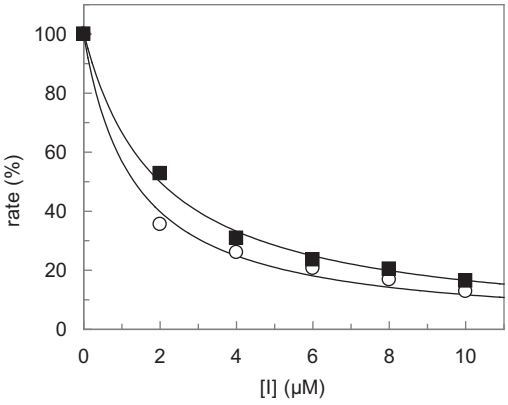


Figure 2. Inhibition of porcine CEase by compounds **10f** (open circles) and **10l** (closed squares). Data are means of duplicate measurements. Non-linear regression according to the equation $v = v_0/(1+[I]/IC_{50})$ gave values IC_{50} = 1.33 ± 0.12 μM and IC_{50} = 1.98 ± 0.11 μM, respectively.

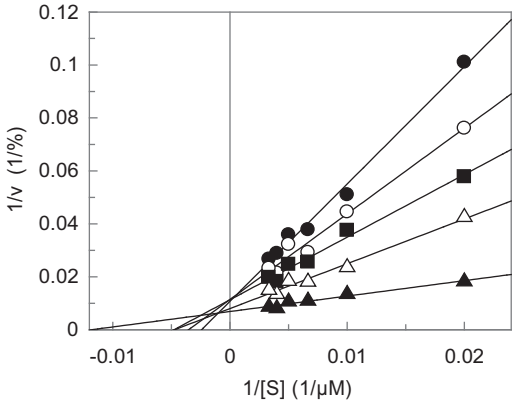


Figure 3. Inhibition of porcine CEase by compounds **10l**. Lineweaver-Burk plots from measurements in the presence of six different concentrations of the substrate *para*-nitrophenyl butyrate (50, 100, 150, 200, 250, and 300 μM). Concentrations of **10l** were 0 μM (closed triangles), 1 μM (open triangles), 2 μM (closed squares), 3 μM (open circles), and 4 μM (closed circles). Data are means of sextuplicate measurements. Linear regression gave values for slopes and intercepts, respectively.

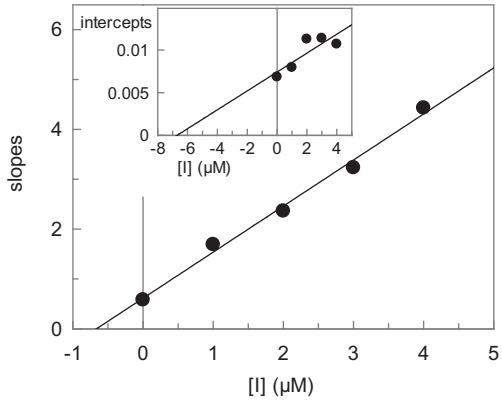


Figure 4. Replots of the data obtained for the inhibition of CEase by **10l** as shown in Figure 3. Slopes and intercepts (inset) were plotted versus concentrations of **10l**. Linear regression gave values K_i = 0.66 μM and $K_i' \approx 7$ μM.

Acknowledgments

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21. Melting points were determined in a capillary Electrothermal 9100 SERIES-Digital apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker 300 MHz spectrometer at 300.13 and 75.46 MHz for ^{1}H and ^{13}C NMR, respectively. IR spectra (KBr) were recorded on a FT Perkin Elmer Spectrum One. Elemental analyses were performed on an Exeter CE 440 apparatus. Microwave-assisted reactions were carried out in a CEM Discover oven.
22. *General procedure for the preparation of compounds* ***10***: A mixture of **7** (1.0 mmol), **8** or **9** (1.50 mmol) and TFA (0.1 mL) was subjected to MW irradiation, at 300 W and 250 °C. After reaction completion (TLC), the mixture was diluted with CH_2Cl_2, washed with water, HCl (5%, 10 mL) and brine, dried (Na_2SO_4) and concentrated under reduced pressure to give a solid which was triturated with hot EtOH. The product was isolated by suction filtration. 6-Chloro-2,4-diphenylquinoline (**10a**) was prepared as described.[17]
23. *6-Chloro-2-(4-methylphenyl)-4-phenylquinoline* (***10b***): Reaction time 5 min; yield 67%; mp 129–131 °C. ^{1}H NMR ($CDCl_3$) δ 2.44 (s, 3H), 7.33 (d, J = 7.9 Hz, 2H), 7.54–7.58 (m, 5H), 7.66 (dd, J = 2.3 Hz, 9.0 z, 1H), 7.82 (s, 1H), 7.85 (d, J = 2.3 Hz, 1H), 8.09 (d, J = 8.2 Hz, 2H), 8.17 (d, J = 9.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 164.42, 156.66, 148.38, 139.78, 137.76, 136.26, 131.98, 131.52, 130.38, 129.62, 129.42, 128.78, 128.66, 127.40, 126.38, 124.45, 119.88, 21.5; FT-IR (KBr) cm^{-1} 699, 771, 835, 1590, 3120. Anal. Calcd for $C_{22}H_{16}ClN$: C 80.11, H 4.89, N 4.25. Found: C 80.07, H 4.92, N 4.29.
24. *6-Chloro-2-(4-hydroxyphenyl)-4-phenylquinoline* (***10c***): Reaction time 5 min; yield 67%; mp 221–224 °C. ^{1}H NMR (DMSO-d_6): δ 6.90 (d, J = 8.5 Hz, 2H), 7.56–7.59 (m, 5H), 7.71 (s, 1H), 7.77 (d, J = 9.0 Hz, 1H), 7.98 (s, 1H), 8.10 (d, J = 8.9 Hz, 1H), 8.20 (d, J = 8.5 Hz, 2H), 9.94 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 159.43, 156.24, 146.58, 137.01, 131.56, 130.50, 130.20, 129.48, 129.03, 128.88, 125.50, 123.80, 119.09, 115.67; FT-IR (KBr) cm^{-1} 699, 751, 834, 1172, 1283, 1594, 3320; GC/MS m/z 331(M$^+$). Anal. Calcd for $C_{21}H_{14}ClNO$: C 76.02, H 4.25, N 4.22. Found: C 76.05, H 4.22, N 4.25.
25. *6-Chloro-2-(4-methoxyphenyl)-4-phenylquinoline* (***10d***): Reaction time 7 min; yield 94%; mp 138–141 °C. ^{1}H NMR ($CDCl_3$) δ 3.91 (s, 3H), 7.09 (d, J = 8.9 Hz, 2H), 7.55–7.65 (m, 5H), 7.84–7.88 (m, 2H), 7.94 (d, J = 2.0 Hz, 1H), 8.14 (d, J = 8.9 Hz, 2H), 8.55 (d, J = 9.2 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 163.21, 154.99, 154.28, 140.73, 135.76, 134.45, 133.66, 130.66, 130.17, 129.29, 129.21, 126.29, 125.94, 125.24, 125.15, 121.15, 115.07, 55.61; FT-IR (KBr) cm^{-1} 702, 755, 836, 1187, 1597, 1671, 3250; GC/MS m/z 345 (M$^+$). Anal. Calcd for $C_{22}H_{16}ClNO$: C 76.41, H 4.66, N 4.05. Found: C 76.45, H 4.70, N 4.01.
26. *2-(3-Bromophenyl)-6-chloro-4-phenylquinoline* (***10e***): Reaction time 7 min; yield 79%; mp 129–131 °C. ^{1}H NMR ($CDCl_3$) δ 7.38 (t, J = 7.8 Hz, 1H), 7.50–7.60 (m, 6H), 7.66 (dd, J = 2.1 Hz, 9.2 Hz, 1H), 7.78 (s, 1H), 7.85 (d, J = 2.3 Hz, 1H), 8.0 (d, J = 7.7 Hz, 1H), 8.15 (d, J = 9.0 Hz, 1H), 8.35 (s, 1H); ^{1}H NMR (DMSO-d_6) δ 7.51 (t, J = 7.9 Hz, 1H), 7.59–7.65 (m, 5H), 7.71 (d, J = 7.7 Hz, 1H), 7.78 (d, J = 2.0 Hz, 1H), 7.84 (dd, J = 2.2 Hz, 8.9 Hz, 1H), 8.19 (t, J = 8.9 Hz, 2H), 8.35 (d, J = 7.8 Hz, 1H), 8.55 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 154.63, 148.29, 146.48, 140.41, 136.70, 132.63, 132.04, 131.67, 131.05, 130.60, 129.93, 129.63, 128.99, 128.90, 126.47, 126.14, 123.91, 122.49, 119.79; FT-IR (KBr) cm^{-1} 697, 709, 760, 1357, 1542, 1588, 3170; GC//MS m/z 395 (M$^+$). Anal. Calcd for $C_{21}H_{13}BrClN$: C 63.90, H 3.32, N 3.55. Found: C 63.93, H 3.28, N 3.57.
27. *6-Chloro-2-(1H-indol-3-yl)-4-phenylquinoline* (***10f***): Reaction time 3.5 min; yield 84%; mp 155–158 °C. ^{1}H NMR ($CDCl_3$) δ 7.16–7.25 (m, 2H), 7.46–7.47 (m, 1H), 7.48–7.56 (m, 2H), 7.64–7.66 (m, 3H), 7.81–7.87 (m, 3H), 8.02 (s, 1H), 8.55 (d, J = 9.1 Hz, 1H), 8.90 (d, J = 3.2 Hz, 1H), 11.30 (s, 1H, NH); ^{1}H NMR (DMSO-d_6) δ 7.22–7.24 (m, 2H), 7.48–7.50 (m, 1H), 7.64–7.68 (m, 6H), 7.79 (d, J = 8.9 Hz, 1H), 8.07 (s, 1H), 8.17 (d, J = 8.9 Hz, 1H), 8.51 (s, 1H), 8.78 (s, 1H) 11.88 (s, 1H, NH); ^{13}C NMR (DMSO-d_6) δ 155.11, 137.28, 136.86, 130.33, 129.86, 129.48, 128.91, 125.41, 124.99, 123.95, 123.04, 122.50, 122.32, 120.82, 120.32, 114.16, 112.03; FT-IR (KBr) cm^{-1} 699, 745, 1204, 1602,1634,1675, 2873; GC/MS m/z 354 (M$^+$); Anal. Calcd for $C_{23}H_{15}ClN_2$: C 77.85, H 4.26, N 7.89. Found: C 77.89, H 4.22, N 7.86.
28. *6-Nitro-2,4-diphenylquinoline* (***10g***): Reaction time 10 min; yield 71%; mp 206–208 °C. ^{1}H NMR (DMSO-d_6) δ 7.54–7.75 (m, 8H), 8.26 (s, 1H), 8.33 (d, J = 9.3 Hz, 1H), 8.41 (m, 2H), 8.51 (dd, J = 2.6 Hz, 9.1 Hz, 1H), 8.68 (d, J = 2.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 159.66, 151.19, 150.79, 145.45, 138.11, 136.77, 132.05, 131.17, 130.22, 129.84, 129.52, 128.34, 124.61, 123.71, 122.85,120.91; FT-IR (KBr) cm^{-1} 685, 752, 1338, 1594,2953. Anal. Calcd for $C_{21}H_{14}N_2O_2$: C 77.29, H 4.32, N 8.58. Found: C 77.26, H 4.36, N 8.54. Analytical data are in accordance with the literature.[34,35]
29. *2-(4-Methylphenyl)-6-nitro-4-phenylquinoline* (***10h***): Reaction time 12 min; yield 72%; mp 207–208 °C. ^{1}H NMR (DMSO-d_6) δ 2.37 (s, 3H), 7.38 (d, J = 8.1 Hz, 2H), 7.59–7.76 (m, 5H), 8.24 (s, 1H), 8.28 (d, J = 2.5 Hz, 1H), 8.35–8.37 (m, 2H), 8.48 (dd, J = 2.6 Hz, 9.1 Hz, 1H), 8.68 (d, J = 2.5 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 159.56, 151.04, 150.84, 145.29, 141.13, 136.83, 135.34, 131.91, 130.20, 130.09, 129.79, 129.50, 128.26, 124.50, 123.64, 122.83, 120.67, 21.44; FT-IR (KBr) cm^{-1} 699, 820, 1337, 1593, 2931. Anal. Calcd for $C_{22}H_{16}N_2O_2$: C 77.63, H 4.74, N 8.23. Found: C 77.67, H 4.72, N 8.26.
30. *2-(4-Hydroxyphenyl)-6-nitro-4-phenylquinoline* (***10i***): reaction time 2.5 min; yield 60%; mp 292–295 °C. ^{1}H NMR (DMSO-d_6) δ 6.92 (d, J = 8.8 Hz, 2H), 7.41–7.85 (m, 5H), 8.13 (s, 1H), 8.21 (d, J = 9.3 Hz, 1H), 8.26 (d, J = 8.8 Hz, 2H), 8.43 (dd, J = 2.6 Hz, 9.3 Hz, 1H), 8.64 (d, J = 2.4 Hz, 1H), 10.07 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 160.65, 159.50, 150.94, 150.68, 144.86, 136.92, 131.56, 130.13, 129.71, 129.47, 128.90, 124.09, 123.52, 122.77, 120.19, 116.26,115.60; FT-IR (KBr) cm^{-1}; 701, 833, 1172, 1336, 1590, 3415. Anal. Calcd. for $C_{21}H_{14}N_2O_3$: C 73.68, H 4.12, N 8.18. Found: C 73.72, H 4.09, N 8.21.
31. *2-(4-Methoxyphenyl)-6-nitro-4-phenylquinoline* (***10j***): Reaction time 14 min; yield 56%; mp 217–218 °C. ^{1}H NMR (DMSO-d_6) δ 3.02 (s, 3H), 7.12 (d, J = 8.8 Hz, 2H), 7.58–7.75 (m, 5H), 8.23 (s, 1H), 8.28 (d, J = 9.2 Hz, 2H), 8.41 (d, J = 9.1 Hz, 1H), 8.48 (dd, J = 2.6 Hz, 9.3 Hz, 1H), 8.66 (d, J = 2.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 163.72, 159.27, 159.91, 150.91, 145.09, 136.90, 134.42, 131.73, 130.48, 130.19, 130.01, 129.76, 129.49, 124.29, 123.64, 122.84, 120.40, 114.8, 55.89; FT-IR (KBr) cm^{-1} 702, 836, 1028, 1172, 1336, 1589,2920; GC/MS m/z 356(M$^+$). Anal. Calcd for $C_{22}H_{16}N_2O_3$: C 74.15, H 4.53, N 7.86. Found: C 74.18, H 4.56, N 7.83.
32. *2-(3-Bromophenyl)-6-nitro-4-phenylquinoline* (***10k***): Reaction time 12 min; yield 40%; mp 232–233 °C. ^{1}H NMR (DMSO-d_6) δ 7.54 (t, J = 8.9 Hz, 1H), 7.65–7.77 (m, 6H), 8.35 (d, J = 2.6 Hz, 1H), 8.39 (s, 1H), 8.42 (d, J = 9.1, 1H), 8.52 (dd, J = 2.6 Hz, 9.3 Hz, 1H), 8.62 (m, 1H), 8.69 (d, J = 2.4, 1H); ^{13}C NMR (DMSO-d_6) δ 157.9, 151.5, 150.6, 145.7, 140.4, 136.6, 133.8, 132.2, 131.6, 130.8, 130.3, 129.9, 129.5, 127.4, 124.8, 123.8,123.0, 122.8, 121.0; FT-IR (KBr) cm^{-1}; 697, 701, 1352, 1536, 2988. Anal. Calcd for $C_{21}H_{13}BrN_2O_2$: C 62.24, H 3.23, N 6.91. Found: C 62.27, H 3.19, N 6.94.
33. *2-(1H-Indol-3-yl)-6-nitro-4-phenylquinoline* (**10l***):* Reaction time 6 min; yield 60%; mp 276–279 °C. ^{1}H NMR (DMSO-d_6) δ 7.17–7.29 (m, 2H), 7.40–7.47 (m, 1H), 7.63–7.66 (m, 5H), 8.16 (s, 1H), 8.23 (d, J = 9.4 Hz, 1H), 8.42 (dd, J = 2.5 Hz, 9.1 Hz, 1H), 8.54–8.61 (m, 2H), 8.90–8.92 (m, 1H), 11.91 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 159.15, 151.62, 149.14, 144.05, 137.84, 137.07, 130.93, 130.85, 130.06, 129.50, 129.42, 126.11, 123.48, 123.36, 123.28, 123.05, 122.55, 121.46, 121.10, 115.55, 112.46; FT-IR (KBr) cm^{-1} 699, 745, 1371, 1531, 1634, 2895, 3103; Anal. Calcd. for $C_{23}H_{15}N_3O_2$: C 75.60, H 4.14, N 11.50. Found: C 75.57, H 4.17, N 11.46.
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36. Porcine cholesterol esterase inhibition was assayed spectrophotometrically at 405 nm at 25 °C. Assay buffer was 100 mM sodium phosphate, 100 mM NaCl, pH 7.0. A stock solution of CEase (122 μg/mL) was prepared in 100 mM sodium phosphate buffer, pH 7.0 and kept at 0 °C. A 1:122 dilution was done with the same buffer immediately before starting the measurement. Sodium taurocholate (12 mM) was dissolved in assay buffer and kept at 25 °C. A stock solution of *para*-nitrophenyl butyrate (20 mM) was prepared in acetonitrile. The final concentration of acetonitrile was 3%, of DMSO 3%, of the substrate *para*-nitrophenyl butyrate 200 μM, and of sodium taurocholate 6 mM. Assays were performed with a final concentration of 10 ng/mL of CEase. Into a cuvette containing 430 μL assay buffer, 500 μL of the sodium taurocholate solution, 20 μL acetonitrile, 10 μL of the *para*-nitrophenyl butyrate solution, and 30 μL of an inhibitor solution in DMSO were added and thoroughly mixed. After incubation for 5 min at 25 °C, the reaction was initiated by adding 10 μL of the enzyme solution (1 μg/mL).
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38. Enzyme assays were performed as described.[39–42]
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