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Supporting Information

Supporting Information

A Combinatorial Approach to 2,4,6-Trisubstituted Triazines with Potent Antimalarial Activity: Combining Conventional Synthesis and Microwave-Assistance

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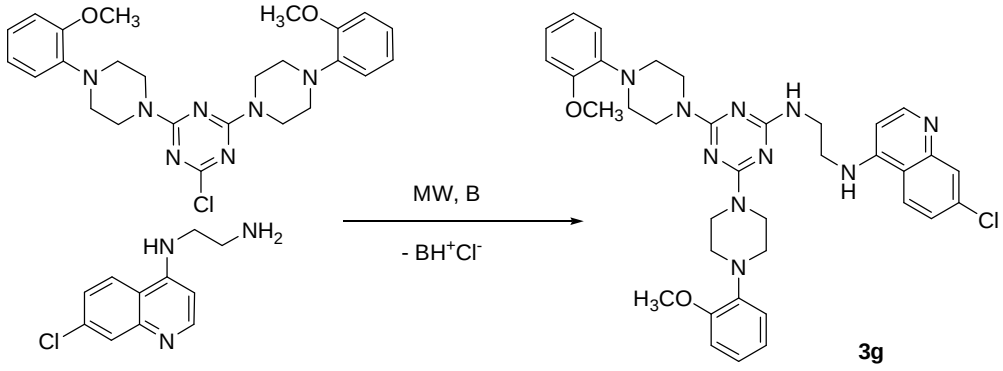
Materials and Methods

Solvents and reagents for chemical synthesis were purchased from Fluka (St. Gallen, Switzerland) and Riedel-de Haën (Seelze, Germany), and used as supplied. Analytical thin layer chromatography (TLC) was performed on Merck silica gel 60 F254 plates (0.25 mm). Compounds were visualized by UV irradiation or dipping the plate in a cerium sulfate-ammonium molybdate (CAM) solution or sulforic acid methanol solution. Flash column chromatography was carried out using forced flow of the indicated solvent on Kieselgel 60 (230-400 mesh). Microwave-assisted reactions were performed on a Biotage Initiator (Uppsala, Sweden) high-frequency microwave synthesizer working at 2.45 GHz, equipped with magnetic stirrer and sample processor (30 vials capacity, 5 mL each). Reaction vessels were Biotage microwave glass vials sealed with applicable cap. Temperature was controlled by the internal IR sensor of the microwave apparatus. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker AVANCE 400 instrument (Karlsruhe, Germany) in [D_6]DMSO with chemical shifts reported in parts per million (δ ppm) referenced to internal standards CD_3SOCD_3 (2.50 ppm ^{1}H, 39.5 ppm ^{13}C). Splitting patterns are indicated as s, singlet; d, doublet; t, triplet; q, quartet; brs, broad singlet for ^{1}H NMR data. Mass spectra were acquired on a Bruker Esquire 3000 instrument (ionic trap), equipped with an analytical HPLC Agilent 1100 using a Waters Xterra column, 4.6 × 50 mm, 3.5 µm granulometry as stationary phase and an acetonitrile/water gradient as elution solvent (10 to 100% acetonitrile in 6 min, 10 µL injection). HPLC purity of each final compound was > 99% at 254 nm. Elemental analyses were performed on a Perkin Elmer Series II CHNS/O 2400 analyser (Waltham, MA).

Optimization of the reaction conditions

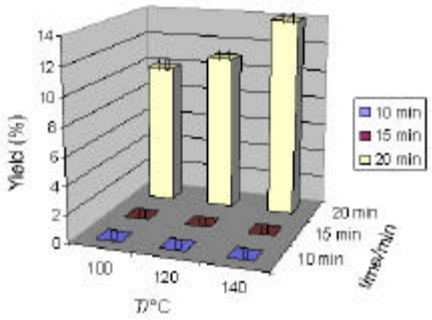
Experimental conditions for microwave irradiations were optimized using **3g** as the target compound, and the same conditions were applied to all compounds in Table 1 reported in the main text, except where noted.



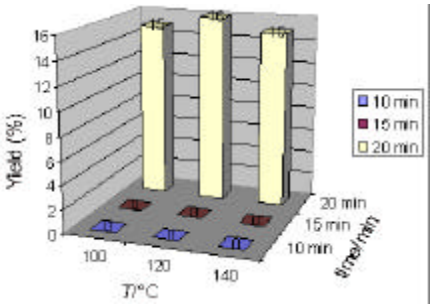
In the following section of this paragraph, 3D graphics illustrating variable quantities, such as temperature, reaction time and yield, are reported for each set of experiments. Related reaction conditions are also summarized.

First set of experiments:

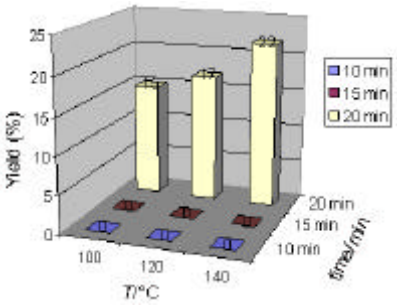
1. Pressure = 6 bar
   Triazine/aminoquinoline ratio = 1:1
   Solvent = dimethylformamide
   Base = NaOH (1 equiv)



2. Pressure = 6 bar
   Triazine/aminoquinoline = 1:2
   Solvent = dimethylformamide
   Base = NaOH (1 equiv)

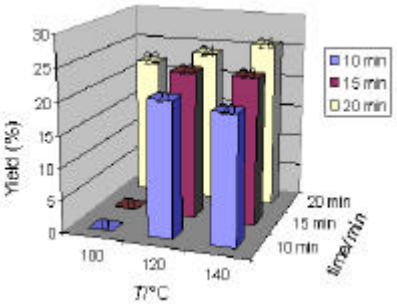


3. Pressure = 6 bar
   Triazine/aminoquinoline = 1:3
   Solvent = dimethylformamide
   Base = NaOH (1 equiv)

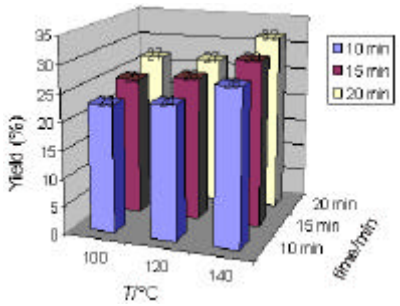


Second set of experiments:

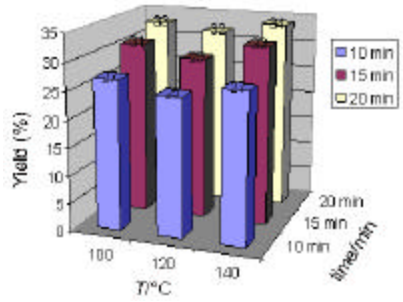
4. Pressure = 6 bar
   Triazine/aminoquinoline = 1:1
   Solvent = dimethylsulfoxide
   Base = diisopropylethylamine (1 equiv)



5. Pressure = 6 bar
   Triazine/aminoquinoline = 1:2
   Solvent = dimethylsulfoxide
   Base = diisopropylethylamine (1 equiv)

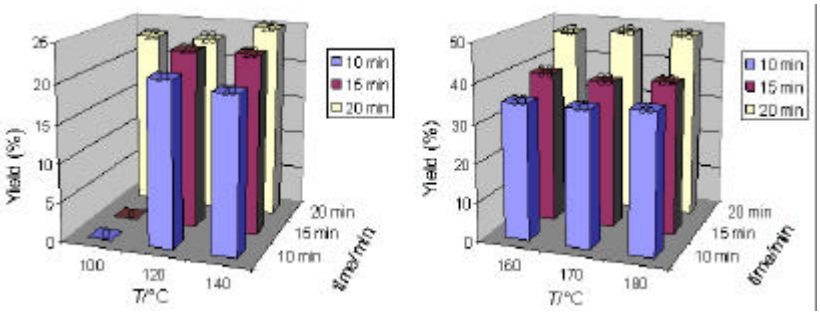


6. Pressure = 6 bar
   Triazine/aminoquinoline = 1:3
   Solvent = dimethylsulfoxide
   Base = diisopropylethylamine (1 equiv)

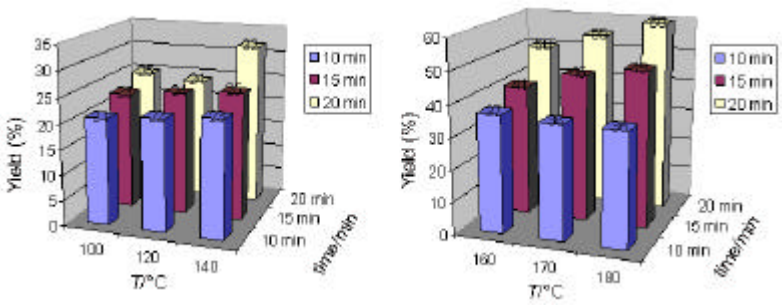


Third set of experiments

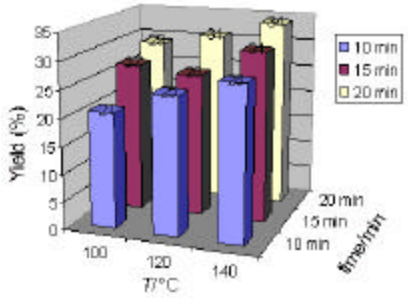
7. Pressure = 6 bar
   Triazine/aminoquinoline
   = 1:1
   Solvent = dimethylsulfoxide
   Base = *N*-methyl morpholine
   (1 equiv)



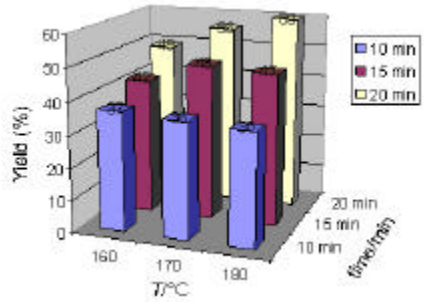
8. Pressure = 6 bar
   Triazine/aminoquinoline
   = 1:2
   Solvent = dimethylsulfoxide
   Base = *N*-methyl morpholine (1
   equiv)



9. Pressure = 6 bar
   Triazine/aminoquinoline = 1:3
   Solvent = dimethylsulfoxide
   Base = *N*-methyl morpholine (1 equiv)

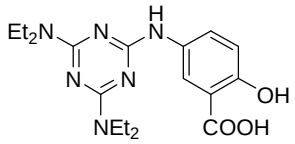


10. Pressure = 6 bar
    Triazine/aminoquinoline = 1:1.5
    Solvent = dimethylsulfoxide
    Base = *N*-methyl morpholine (1 equiv)



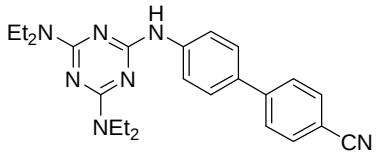
On the basis of the best conditions obtained (6 bar pressure, solvent DMSO, base NMM), we further adjusted time (18 min.), temperature (180 °C) and triazine/aminoquinoline ratio (1:1.06). Maximum yield 62%.

Compound Characterization.



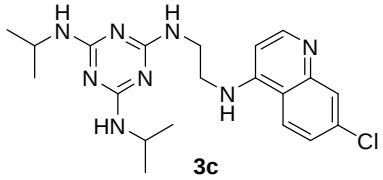
3a

5-(4,6-*bis*(Diethylamino)-1,3,5-triazin-2-ylamino)-2-hydroxybenzoic acid (3a). ^{1}H NMR ([D_6]DMSO): d 1.14 (t, *J* = 6.9 Hz, 12 H), 3.58 (m, 8 H), 6.96 (d, *J* = 8.8 Hz, 1 H), 7.51 (dd, *J* = 2.7, 8.8 Hz, 1 H), 8.42 (s, 1 H). ^{13}C NMR ([D_6]DMSO): d 13.20, 42.64, 113.15, 118.04, 121.72, 128.17, 129.31, 158.01, 171.99. HPLC-MS: *m/z* = 375.0 [M+1]. $C_{18}H_{26}N_6O_3$ (374.2): calcd. C 57.74, H 7.00, N 22.44; found C 57.91, H 6.94, N 22.36.



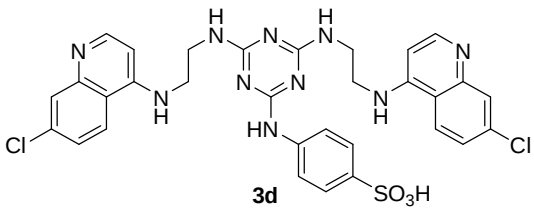
3b

4'-(4,6-*bis*(Diethylamino)-1,3,5-triazin-2-ylamino)biphenyl-4-carbonitrile (3b). ^{1}H NMR ([D_6]DMSO): d 1.14 (t, *J* = 6.6 Hz 12 H), 3.55 (m, 8 H), 7.68 (d, *J* = 8.0 Hz 2 H), 7.86 (m, 6 H). ^{13}C NMR ([D_6]DMSO): d 12.53, 41.31, 41.82, 110.05, 113.61, 116.48, 119.35, 120.79, 126.89, 127.71, 132.73, 143.55, 154.00, 157.85. HPLC-MS: *m/z* = 416.2 [M+1]. $C_{24}H_{29}N_7$ (415.2): calcd. C 69.37, H 7.03, N 23.60; found C 68.98, H 7.07, N 23.95.



3c

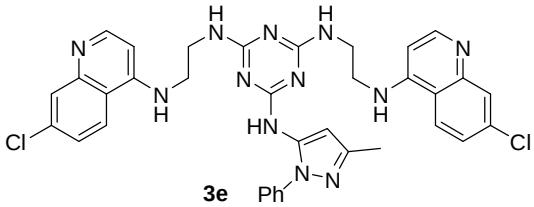
***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{4},*N*^{6}-diisopropyl-1,3,5-triazine-2,4,6-triamine (3c).** ^{1}H NMR ([D_6]DMSO): d 1.08 (d, 12 H), 3.38 (m, 2 H), 3.51 (m, 2 H), 4.03 (m, 2 H), 7.44 (m, 2 H), 7.78 (s, 1 H) 8.16 (d, *J* = 6.0 Hz, 1 H), 8.39 (d, *J* = 5.4 Hz, 1 H). ^{13}C NMR ([D_6]DMSO): d 22.83, 23.18, 38.62, 41.36, 99.09, 117.80, 117.99, 124.37, 124.47, 124.63, 127.97, 133.93, 149.48, 150.50, 150.57, 152.38, 165.37, 166.52. HPLC-MS: *m/z* = 414.9 [M+1]. $C_{20}H_{27}ClN_8$ (414.2): calcd. C 57.89, H 6.56, N 27.01; found C 58.35, H 7.02, N 26.85.



3d

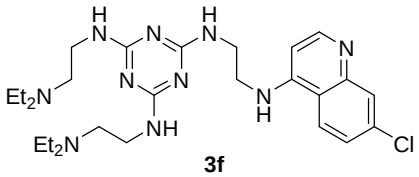
4-(4,6-*bis*(2-(7-Chloroquinolin-4-ylamino)ethylamino)-1,3,5-triazin-2-ylamino)benzenesulfonic acid (3d). Minor modifications to the general procedure were applied in this case. The first substitution in cyanuric

chloride took place in the presence of NaOH (2 equiv.) as base and a water/acetone 1:1 solution as solvent and the isolation was performed by adjusting the pH of the solution to pH 6 by dropwise precipitation of the product with 4 N HCl. Also the trisubstituted product was isolated by addition of 4 N HCl. Analogous conditions were used also for cpds. **3h**, **3i**, **3j** and **3n**. ^{1}H NMR ([D$_6$]DMSO): d 3.69 (m, 8 H), 6.80 (m, 1 H), 6.85 (m, 1 H), 6.95 (m, 1 H),7.24 (m, 1 H), 7.47 (m, 4 H), 7.64 (d, *J* = 7.4 Hz, 1 H), 7.70 (m, 1 H), 7.83 (m, 2 H), 7.94 (s,1 H), 8.54 (s, 1 H). ^{13}C NMR ([D$_6$]DMSO): d 43.20, 99.07, 115.90, 119.53, 120.12, 125.82, 126.31, 127.16, 138.37, 138.73, 138.87, 143.61, 156.10, 159.09, 159.42. HPLC-MS: *m/z* = 691.1 [M+1]. $C_{31}H_{28}Cl_2N_{10}O_3S$ (690.1): calcd. C 53.84, H 4.08, N 20.25; found C 53.53, H 4.02, N 20.54.



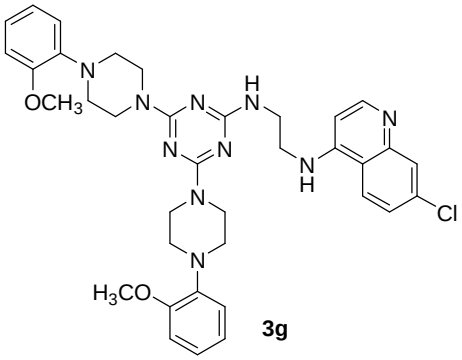
3e

***N*^{2},*N*^{4}-*bis*(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{6}-(3-methyl-1-phenyl-1*H*-pyrazol-5-yl)-1,3,5-triazine-2,4,6-triamine (3e).** ^{1}H NMR ([D$_6$]DMSO): d 2.10 (s, 3 H), 3.74 (m, 4 H), 3.82 (m, 4 H), 6.80 (m, 2 H), 6.92 (m, 2 H),7.26 (d, *J* = 7.8 Hz, 1 H), 7.38 (m, 4 H), 7.64 (m, 2 H), 7.88 (m, 2 H), 8.25 (m, 4 H). ^{13}C NMR ([D$_6$]DMSO): d 14.35, 38.74, 42.54, 99.12, 117.78, 121.22, 123.56, 124.42, 124.58, 126.98, 127.72, 129.05, 133.96, 138.22, 139.57, 148.01, 149.18, 150.64, 152.19, 165.78, 166.38. HPLC-MS: *m/z* = 691.0 [M+1]. $C_{35}H_{32}Cl_2N_{12}$ (690.2): calcd. C 60.78, H 4.66, N 24.30; found C 60.34, H 4.52, N 24.71.



3f

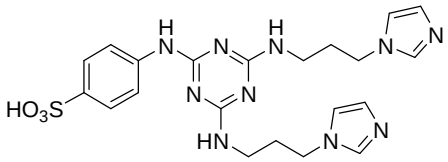
***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{4},*N*^{6}-bis(2-(diethylamino)ethyl)-1,3,5-triazine-2,4,6-triamine (3f).** ^{1}H NMR ([D$_6$]DMSO): d 0.93 (m, 12 H), 2.47 (m, 12 H), 3.23 (m, 8 H), 6.57 (d, J = 6.7 Hz, 1 H), 7.40 (m, 2 H),7.79 (s, 1 H), 8.15 (m, 1 H), 8.41 (m, 1 H). ^{13}C NMR ([D$_6$]DMSO): d 12.33, 38.63, 47.08, 52.38, 99.13, 117.82, 124.35, 124.48, 127.99, 133.80, 149.50, 150.54, 152.39, 166.09, 166.41. HPLC-MS: *m/z* = 529.3 [M+1]. $C_{26}H_{41}ClN_{10}$ (528.3): calcd. C 59.02, H 7.81, N 26.47; found C 59.51, H 7.86, N 26.21.



3g

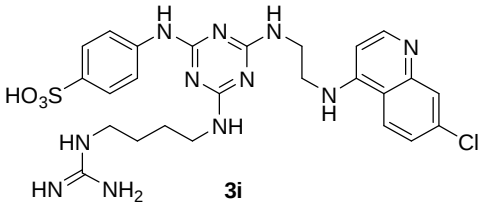
***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{4},*N*^{6}-bis(4-(2-methoxyphenyl)piperazin-1-yl)-1,3,5-triazine-2,4,6-triamine (3g).** ^{1}H NMR ([D$_6$]DMSO): d 1.18 (m, 2 H), 3.04 (m, 6 H), 3.70 (m, 2 H), 3.81 (s, 6 H),

3.90 (m, 4 H), 3.98 (m, 4 H), 6.47 (d, *J* = 5.2 Hz, 1 H), 6.83 (s, 4 H), 6.96 (m, 2 H), 7.35 (d, *J* = 7.2 Hz, 1 H), 8.00 (s, 1 H), 8.30 (d, *J* = 7.2 Hz, 1 H), 8.38 (d, *J* = 5.2 Hz, 1 H), 8.61 (s, 1 H). ^{13}C NMR ([D_6]DMSO): d 39.08, 43.43, 46.62, 50.55, 55.42, 98.13, 111.28, 116.96, 118.44, 121.08, 122.23, 123.16, 123.40, 125.64, 127.50, 135.36, 140.90, 147.74, 150.78, 152.32, 164.42, 165.13, 165.47, 166.60, 167.47, 169.69; HPLC-MS: *m/z* = 681.3 [M+1]. $C_{36}H_{41}ClN_{10}O_2$ (680.3): calcd. C 63.47, H 6.07, N 20.56; found C 63.62, H 5.97, N 20.44.



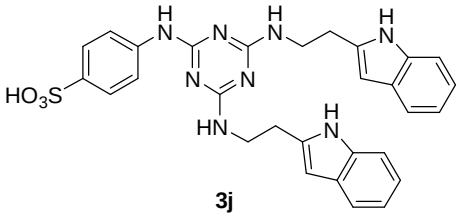
3h

4-(4-(4-(1*H*-imidazol-1-yl)butylamino)-6-(3-(1*H*-imidazol-1-yl)propylamino)-1,3,5-triazin-2-ylamino)benzenesulfonic acid (3h). ^{1}H NMR ([D_6]DMSO): d 2.09 (m, 4 H), 3.23 (m, 4 H), 4.26 (m, 4 H), 7.52 (m, 4 H), 7.63 (s, 2 H), 7.75 (m, 2 H), 9.12 (m, 2 H). HPLC-MS: *m/z* = 499.1 [M+1]. $C_{21}H_{26}N_{10}O_3S$ (498.2): calcd. C, 50.59; H, 5.26; N, 28.09; found C 50.92, H 5.17, N 27.74.



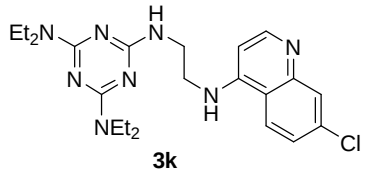
3i

4-(4-(2-(7-Chloroquinolin-4-ylamino)ethylamino)-6-(4-guanidinobutylamino)-1,3,5-triazin-2-ylamino)benzenesulfonic acid (3i). ^{1}H NMR ([D_6]DMSO): d 1.52 (m, 4 H), 3.11 (m, 2 H), 3.35 (m, 2 H), 3.69 (m, 5 H), 3.91 (m, 1 H), 6.78-8.58 (m, 10 H), 9.30 (m, 2 H). ^{13}C NMR ([D_6]DMSO): d 26.93, 27.10, 41.08, 43.67, 44.96, 99.17, 117.77, 118.83, 124.40, 124.45, 126.18, 127.84, 133.91, 141.13, 149.38, 150.52, 152.37, 157.11, 164.43, 166.30. HPLC-MS: *m/z* = 600.2 [M+1]. $C_{25}H_{30}ClN_{11}O_3S$ (599.2): calcd. C 50.04, H 5.04, N 25.67; found C 50.50, H 5.17, N 25.24.



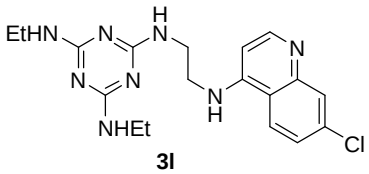
3j

4-(4,6-*bis*(2-(1*H*-indol-2-yl)ethylamino)-1,3,5-triazin-2-ylamino)benzenesulfonic acid (3j). ^{1}H NMR ([D_6]DMSO): d 3.00 (m, 4 H), 3.63 (m, 4 H), 6.87 (m, 1 H), 7.02 (m, 4 H), 7.19 (s, 1 H), 7.36 (d, *J* = 7.0 Hz, 2 H), 7.57 (m, 4 H), 7.80 (m, 2 H), 8.26 (m, 2 H). ^{13}C NMR ([D_6]DMSO): d 41.55, 42.31, 54.05, 111.88, 112.51, 118.31, 121.41, 123.09, 126.31, 127.75, 136.73, 141.08, 141.49, 164.21, 165.68. HPLC-MS: *m/z* = 569.2 [M+1]. $C_{29}H_{28}N_8O_3S$ (568.2): calcd. C 61.25, H 4.96, N 19.71; found C 60.80, H 5.09, N 20.14.



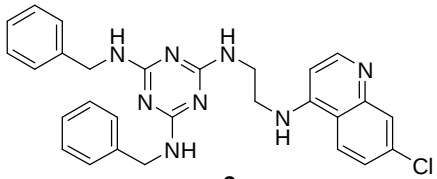
3k

***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{4},*N*^{4},*N*^{6},*N*^{6}-tetraethyl-1,3,5-triazine-2,4,6-triamine (3k).** ^{1}H NMR ([D_6]DMSO): d 1.03 (m, 12 H), 3.38 (m, 2 H), 3.51 (m, 2 H), 4.03 (m, 2 H), 6.94 (d, *J* = 5.5 Hz 1 H), 7.72 (dd, *J* = 7.7 and 2.1 Hz, 1 H), 7.97 (d, *J* = 2.1 Hz, 1 H), 8.43 (d, *J* = 7.7 Hz, 1 H), 8.56 (d, *J* = 5.5 Hz, 1 H). ^{13}C NMR ([D_6]DMSO): d 13.05, 41.85, 42.12, 42.77, 99.11, 111.45, 114.33, 116.00, 117.21, 119.50, 120.08, 125.80, 127.35, 138.67, 138.90, 143.42, 153.11, 156.28, 156.37, 160.75. HPLC-MS: *m/z* = 443.2 [M+1]. $C_{22}H_{31}ClN_8$ (442.2): calcd. C 59.65, H 7.05, N 25.29; found C 60.00, H 6.89, N 25.04.



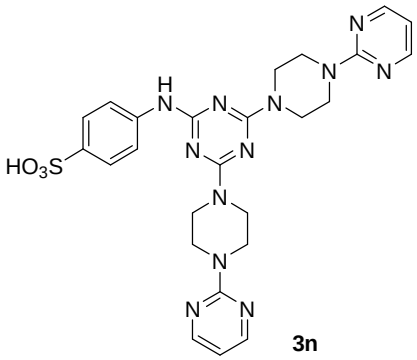
3l

***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*^{4},*N*^{6}-diethyl-1,3,5-triazine-2,4,6-triamine (3l).** ^{1}H NMR ([D_6]DMSO): d 1.07 (m, 6 H), 3.11 (m, 2 H), 3.26 (m, 2 H), 3.61 (m, 2 H), 3.74 (m, 2 H), 6.95 (m, 1 H), 7.73 (m, 1 H), 7.98 (s, 1 H), 8.43 (m,1 H), 8.55 (m, 1 H). ^{13}C NMR ([D_6]DMSO): d 14.51, 14.58, 35.50, 35.65, 42.54, 99.07, 115.10, 116.01, 118.00, 119.23, 125.91, 127.25, 127.35, 127.49, 138.51, 138.60, 138.95, 143.40, 143.62, 154.82, 155.50, 156.29. HPLC-MS: *m/z* = 387.1 [M+1]. $C_{18}H_{23}ClN_8$ (386.2): calcd. C 55.88, H 5.99, N 28.96; found C 56.10, H 6.08, N 28.73.



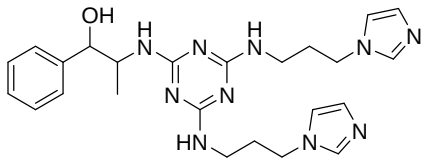
3m

***N*^{2},*N*^{4}-Dibenzyl-*N*^{6}-(2-(7-chloroquinolin-4-ylamino)ethyl)-1,3,5-triazine-2,4,6-triamine (3m).** ^{1}H NMR ([D_6]DMSO): d 3.37 (m, 2 H), 3.50 (m, 2 H), 4.42 (m, 4 H), 6.54 (m, 1 H), 6.89 (m, 1 H) 7.20 (m,8 H), 7.43 (m, 2 H), 7.77 (s, 1 H), 8.23 (m, 1 H), 8.34 (m, 1 H). ^{13}C NMR ([D_6]DMSO): d 38.71, 43.48, 59.26, 99.09, 117.73, 124.38, 126.81, 127.38, 127.73, 127.88, 128.39, 128.55, 129.06, 133.88, 141.33, 149.38, 150.58, 151.93, 162.70, 166.39, 166.67. H HPLC-MS: *m/z* = 510.9 [M+1]. $C_{28}H_{27}ClN_8$ (510.2): calcd. C 65.81 H 5.33 N 21.93; found C 66.13, H 5.18, N 21.70.



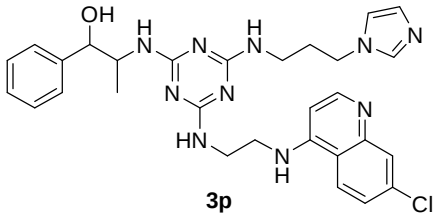
3n

4-(4,6-*bis*(4-(Pyrimidin-2-yl)piperazin-1-yl)-1,3,5-triazin-2-ylamino)benzenesulfonic acid (3n). ^{1}H NMR ([D_6]DMSO): d 3.10 (m, 4 H), 3.58 (m, 2 H), 3.84 (m, 8 H), 3.96 (m, 2 H), 6.67 (m, 2 H), 7.53 (d, *J* = 7.9 Hz, 2 H),7.62 (d, *J* = 7.9 Hz, 2 H), 8.39 (m, 4 H). HPLC-MS: *m/z* = 577.1 [M+1]. $C_{25}H_{28}N_{12}O_3S$ (576.2): calcd. C 52.07, H 4.89, N 29.15; found C 52.33, H 5.02, N 28.81.



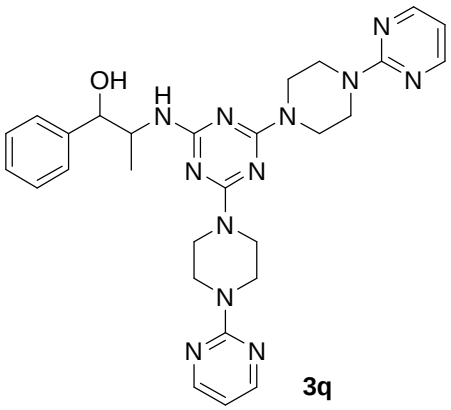
3o

2-(4-(4-(1*H*-Imidazol-1-yl)butylamino)-6-(3-(1*H*-imidazol-1-yl)propylamino)-1,3,5-triazin-2-ylamino)-1-phenylpropan-1-ol (3o). ^{1}H NMR ([D_6]DMSO): d 0.93 (t, *J* = 6.4 Hz, 3 H), 1.91 (m, 4 H), 3.15 (m, 4 H), 3.98 (m, 4 H), 4.13 (m, 1 H), 4.74 (m, 1 H), 5.47 (brs, 1 H), 6.88 (s, 2 H) 7.18 (m, 3 H), 7.28 (m, 4 H), 7.63 (s, 2 H). ^{13}C NMR ([D_6]DMSO): d 14.05, 30.92, 36.99, 43.76, 51.30, 55.99, 74.10, 119.31, 126.03, 126.12, 126.47, 127.38, 127.68, 128.29, 137.27, 143.72, 164.91, 165.62. HPLC-MS: *m/z* = 477.2 [M+1]. $C_{24}H_{32}N_{10}O$ (476.3): calcd. C 60.48, H 6.77, N 29.39; found C 60.33, H 6.81, N 29.51.



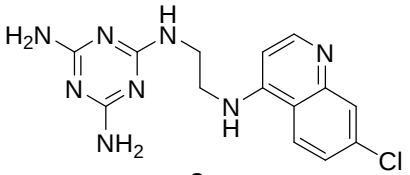
3p

2-(4-(3-(1*H*-Imidazol-1-yl)propylamino)-6-(2-(7-chloroquinolin-4-ylamino)ethylamino)-1,3,5-triazin-2-ylamino)-1-phenylpropan-1-ol (3p). ^{1}H NMR ([D_6]DMSO): d 0.91 (d, *J* = 6.4 Hz, 3 H), 1.91 (m, 2 H), 3.14 (m, 2 H), 3.50 (m, 4 H), 3.98 (m, 2 H), 4.11 (m, 1 H), 4.73 (m, 1 H), 5.48 (brs, 1 H), 6.56 (s, 1 H), 6.88 (s, 1 H) 7.17 (s, 2 H), 7.29 (m, 5 H), 7.63 (s, 1 H), 7.79 (s, 1 H), 8.19 (m, 1 H), 8.41 (s, 1 H). ^{13}C NMR ([D_6]DMSO): d 14.24, 30.92, 37,12, 38.20, 43.75, 51.36, 74.11, 98.64, 117.33, 119.32, 123.93, 126.09, 126.48, 127.48, 127.65, 128.30, 133.28, 137.28, 143.70, 148.99, 150.05, 151.90, 164.90, 165.62, 165.82. HPLC-MS: *m/z* = 573.2 [M+1]. $C_{29}H_{33}ClN_{10}O$ (572.3): calcd. C 60.78, H 5.80, N, 24.44; found C 60.41, H 5.89, N 24.61.



3q

2-(4,6-*bis*(4-(Pyrimidin-2-yl)piperazin-1-yl)-1,3,5-triazin-2-ylamino)-1-phenylpropan-1-ol (3q). ^{1}H NMR ([D$_6$]DMSO): d 0.96 (d, *J* = 6.5 Hz, 3 H), 3.35 (m, 4 H), 3.75 (m, 12 H), 4.17 (m, 1 H), 4.74 (m, 1 H), 5.33 (brs, 1 H), 6.52 (brd, *J* = 7.9 Hz, 2 H), 6.64 (s, 2 H) 7.20 (d, *J* = 7.7 Hz, 1 H), 7.30 (m, 4 H), 8.38 (s, 4 H). ^{13}C NMR ([D$_6$]DMSO): d 13.60, 13.86, 42.33, 42.43, 43.11, 51.48, 74.01, 110.23, 126.05, 126.50, 127.76, 143.73, 157.91, 161.19, 164.62, 164.71, 164.94. HPLC-MS: *m/z* = 555.1 [M+1]. $C_{28}H_{34}N_{12}O$ (554.3): calcd. C 60.63, H 6.18, N 30.30; found C 60.30, H 5.87, N 30.56.



3r

***N*^{2}-(2-(7-Chloroquinolin-4-ylamino)ethyl)-1,3,5-triazine-2,4,6-triamine (3r).** ^{1}H NMR ([D$_6$]DMSO): d 3.35 (t, *J* = 5.9 Hz, 2 H), 3.51 (t, *J* = 5.9 Hz, 2 H), 6.10 (s, 1 H), 6.32 (s, 1 H) 6.55 (d, *J* = 5.3 Hz, 1 H), 6.76 (s, 1 H) 7.39 (d, *J* = 8.9 Hz, 1 H), 7.46 (s, 1 H), 7.79 (s, 1 H), 8.15 (d, *J* = 8.9 Hz, 1 H), 8.43 (d, *J* = 5.3 Hz, 1 H). ^{13}C NMR ([D$_6$]DMSO): d 38.66, 99.15, 117.76, 124.44, 124.69, 127.92, 133.87, 149.41, 150.51, 152.40, 167.22, 167.50, 167.70. HPLC-MS: *m/z* = 331.0 [M+1]. $C_{14}H_{15}ClN_8$ (330.1): calcd. C 50.84, H 4.57, N 33.88; found C 50.65, H 4.66, N 33.99.

Cytotoxicity Assay.

Cytotoxicity was evaluated on human fibroblasts from skin biopsies or on human microvascular endothelial cells (HMEC-1, provided by the Centers for Disease Control, Atlanta, GA, USA). Fibroblasts were maintained in DMEM (EuroClone, Pero, Italy) supplemented with 10% fetal calf serum (HyClone, Logan, UT, USA), 2 mM glutamine, 100 U/mL of penicillin and 100 μg/mL of streptomycin. HMEC-1 were maintained in MCDB 131 medium (GIBCO-BRL, Paisley, Scotland) supplemented with 10% fetal calf serum (HyClone, Logan, UT, USA), 10 ng/mL of epidermal growth factor (PreproTech, Rocky Hill, NY, USA), 1 μg/mL of hydrocortisone, 2 mM glutamine, 100 U/mL of penicillin, 100 μg/mL of streptomycin and 20 mM Hepes buffer (EuroClone, Pero, Italy). For the cytotoxicity assay, cells were seeded in 96 well flat bottom tissue culture clusters (Costar, NY, USA) at 10^4 cells/well. Cell proliferation was measured after 72 h using the MTT (3-[4,5-

dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) test, in the absence or in the presence of different doses of tested compounds (up to 20 μg/mL). The results are expressed as IC_{50} which is the dose of compounds able to inhibit 50% cell growth. All test were performed three times in triplicate.