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NAD(P)H:Quinone Oxidoreductase 1 Inducer Activity of some Novel Anilinoquinazoline Derivatives

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Abstract

The Keap1/Nrf2/ARE pathway enables the cells to survive oxidative stress conditions through regulating the expression of cytoprotective enzymes such as NAD(P)H:quinone oxidoreductase 1 (NQO1). This work presents the design and synthesis of novel anilinoquinazoline derivatives (**2-16a**) and evaluation of their NQO1 inducer activity in murine cells. Molecular docking of the new compounds was performed to assess their ability to inhibit Keap1-Nrf2 protein-protein interaction through occupying the Keap1 Nrf2-binding domain which leads to Nrf2 accumulation and enhanced gene expression of NQO1. The docking results showed that all compounds can potentially interact with Keap1, however, 1,5-Dimethyl-2-phenyl-4-(2-phenylquinazolin-4-ylamino)-1,2-dihydropyrazol-3-one (**9**), the most potent inducer, showed the largest number of interactions with key amino acids in the binding pocket (Arg483, Tyr525 and Phe478) compared to the native ligand or any other compound in this series.

Keywords: anilinoquinazoline, molecular modeling, Keap1/Nrf2, cytoprotection, NQO1 induction.

Introduction

Antioxidants, from a chemical viewpoint, are considered electron donors to free radicals, molecular centers that tend to lose electrons initiating oxidations, and thus protecting the cells against oxidative stress[1-3]. Such oxidative stress situations, generated from imbalanced production of reactive oxygen species (ROS), e.g. superoxide anion radical (O^{2-}) and hydrogen peroxide (H_2O_2), is unfortunately associated with many pathological conditions including stroke, diabetes, Alzheimer's disease, cancer and chronic inflammation[4]. As a natural mechanism to counteract oxidative stress, aerobic cells express superoxide dismutase converting superoxide to hydrogen peroxide, which is subsequently disposed by catalase and peroxidases. In addition to these enzymatic defenses, there are the indirect antioxidants," namely, the cytoprotective enzymes, that catalyze a wide variety of chemical reactions, protecting cells and organisms and allowing their adaptation to many types of stress[5]. Among the most critical cytoprotective enzymes is the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor erythroid 2-related factor 2 (Nrf2)-antioxidant response elements (AREs) pathway[6,7]. The Keap1/Nrf2/ARE pathway enables the cells in adapting and surviving oxidative and inflammatory stress conditions through regulating the expression of a network of more than 100 cytoprotective genes. This pathway is inducible by various stress stimuli and small molecules (termed inducers), whereby the inducers react with specific cysteine residues of the protein sensor Keap1, which loses its ability to target Nrf2 for ubiquitination and proteasomal degradation, resulting in its stabilization, followed by binding to the ARE and transcriptional activation of cytoprotective genes, such as NAD(P)H: quinone oxidoreductase 1 (NQO1)[6, 8-11]. Recently, the Keap1-Nrf2 protein-protein interaction is viewed as a critical target for intervention and potential management of a variety of oxidative stress-related pathologies, including cancer, Parkinson's and Alzheimer's disease, and diabetes[12-15]. Design of non-covalent small molecule modulators of the Keap-Nrf2 interaction has been intensively explored[16,17]. Quinazoline derivatives are considered excellent bioactive substances where a number of biological activities have been associated with antioxidant activity[18-20].

In continuation of our work towards identification of NQO1 inducers[21, 22], we herein report the synthesis, NQO1 inducer activity and Keap1 binding in silico screening results for a novel class of anilinoquinazolines.

Materials and methods
Chemistry

Melting points (uncorrected) were determined in open capillaries on a Gallen Kamp melting point apparatus (Sanyo Gallen Kamp, UK). Pre-coated silica gel plates (Kieselgel 0.25 mm, 60 G F 254; Merck, Germany) were used for thin layer chromatography. A developing solvent system of chloroform/methanol (8:2 mL) mixture was used and the spots were detected by ultraviolet light. IR spectra (KBr disc) were recorded using an FT-IR spectrophotometer (Perkin Elmer, USA). NMR spectra were scanned on a NMR spectrophotometer (Bruker AXS Inc., Switzerland), operating at 500 MHz for ^{1}H spectra and 125.76 MHz for ^{13}C spectra. Chemical shifts are expressed in δ-values (ppm) relative to TMS as an internal standard, using DMSO-d_6 as a solvent. Elemental analyses were done on a model 2400 CHNSO analyzer (Perkin Elmer, USA). All the values were within ± 0.4 % of the theoretical values. All reagents used were of AR grads. The starting material 4-chloro-2-phenylquinazoline **1** was purchased from sigma (USA) and was directly used for preparation of the target compounds.

*General procedure for the Synthesis of 2-phenyl-quinazoline-4-amine derivatives (**2-16a**).*

A mixture of **1** (2.40 g, 0.01 mol) and different amines (0.012 mol) in dry dimethylformamide (10 mL) containing trimethylamine 3 drops was refluxed for 24 h. , then left to cool. The solid product formed was collected by filtration and recrystallized from acetic acid to give **2**-**16a**, respectively.

N-Heptyl-2-phenylquinazolin-4- amine (**2**)

Yield, 87%; m.p. 141.3 °C. IR (KBr, cm^{-1}): 3278 (NH), 3074 (CH arom.), 2924, 2950, 2851 (CH aliph.), 1639 (C=N). ^{1}H-NMR (DMSO-d_6): 0.9 (t, 3H, CH$_3$), 1.2 [m, 10H, 5CH$_2$], 3.8 [t, 2H, CH2-NH], 7.6-8.7 [m, 9H, Ar-H], 10.8 [s, 1H, NH exchangeable with D$_2$O]. ^{13}C-NMR (DMSO-d_6): 14.3, 22.5, 26.8, 28.9, 31.5, 31.6, 42.1, 112.6, 120.3, 124.8 (2), 128.2, 129.3, 129.5, 131.7, 133.8, 135.5, 139.5, 157.3, 160.2. MS m/z (%): 319 (M$^+$)

(3.23), 204 (100). Anal. Calcd. For $C_{21}H_{25}N_3$ (319): C, 78.96; H, 7.89; N, 13.15. Found: C, 78.59; H, 8.13; N, 12.81.

N-(Octan-2-yl)-2-phenylquinazolin-4-amine (**3**)

Yield, 80%; m.p. 192.8 °C. IR (KBr, cm^{-1}): 3191 (NH), 3100 (CH arom.), 2954, 2925, 2850 (CH aliph.), 1629 (C=N). ^{1}H-NMR (DMSO-d_6): 0.7 [t, 3H, CH$_3$], 0.8 [d, 3H, CH$_3$, *J* = 7.2 Hz], 1.2-1.8 [m, 10H, 5CH$_2$], 2.8 [m, 1H, CH], 7.6-8.8 [m, 9H, Ar-H], 10.0 [s, 1H, NH exchangeable with D$_2$O]. ^{13}C-NMR (DMSO-d_6): 14.3, 20.3, 21.2, 25.8, 28.8, 31.6, 36.4, 48.6, 112.5, 120.4, 124.9 (2), 128.2, 129.4, 129.6 (2), 131.9, 133.8, 136.0, 157.5, 159.8, 160.6. MS m/z (%): 333 (M$^+$) (12.7), 255 (100). Anal. Calcd. For $C_{22}H_{27}N_3$ (333.47): C, 79.24; H, 8.16; N, 12.60. Found: C, 79.50; H, 7.84; N, 12.25.

2-Phenyl-N-(2-(pyrrolidin-1-yl)ethyl)quinazolin-4-amine (**4**)

Yield, 90%; m.p. 93.9 °C. IR (KBr, cm^{-1}): 3325 (NH), 358 (CH arom.), 2935, 2846 (CH aliph.), 1617 (C=N). ^{1}H-NMR (DMSO-d_6): 1.4-1.5 [m, 4H, CH$_2$-CH$_2$ Cyclo], 2.4-2.5 [m, 4H, CH$_2$-N-CH$_2$ Cyclo], 2.6 [t, 2H, N-CH$_2$], 3.8 [t, 2H, CH$_2$-NH], 7.8-8.5 [m, 10H, Ar-H + NH]. ^{13}C-NMR (DMSO-d_6): 24.5 (2), 40.5, 54.7, 57.7 (2), 114.3, 123.0, 125.6 (2), 128.3, 128.6, 128.7 (2), 130.4, 133.0, 139.2, 150.3, 159.7, 160.1. MS m/z (%): 318 (M$^+$) (22.5), 247 (100). Anal. Calcd. For $C_{20}H_{22}N_4$ (318): C, 75.44; H, 6.96; N, 17.60. Found: C, 75.09; H, 6.63; N, 17.92.

N-(2-(1-Methylpyrrolidin-2-yl)ethyl)-2-phenylquinqzolin-4-amine (**5**)

Yield, 85%; m.p. >360 °C. IR (KBr, cm^{-1}): 3308 (NH), 3060 (CH arom.), 2950, 2819, (CH aliph.), 1618 (C=N). ^{1}H- NMR (DMSO-d_6): 1.7-2.3 [m, 6H, 3CH$_2$ Cyclo], 1.9 [m, 2H, CH$_2$-CH], 2.4 [m, 1H, CH Cyclo], 2.5 [s, 3H, N-CH$_3$], 3.0 [m, 2H, CH$_2$-NH], 7.4-8.6 [m, 10H, Ar-H + NH]. ^{13}C- NMR (DMSO-d_6): 21.4, 29.4, 29.5, 40.4, 40.5, 55.2, 66.0, 114.3, 123.4, 125.7 (2), 128.2, 128.3, 128.7 (2), 130.5, 133.2, 139.1, 150.3, 159.7, 160.2. MS m/z (%): 332 (M$^+$) (21.6), 316 (100). Anal. Calcd. For $C_{21}H_{24}N_4$ (332): C, 75.87; H, 7.28; N, 16.85. Found: C, 76.11; H, 7.57; N, 17.20.

N-(2-(1-Methyl-1H-pyrrol-2-yl)ethyl)-2-phenylquinqzolin-4-amine (**6**)

Yield, 82%; m.p. 172.7 °C. IR (KBr, cm^{-1}): 3334 (NH), 3059 (CH arom.), 2930, 2825 (CH aliph.), 1618 (C=N). ^{1}H-NMR (DMSO-d_6): 1.9 (s, 3H, CH$_3$), 2.8-3.9 [m, 4H, 2CH$_2$], 5.6-6.6 [m, 3H, 3CH Pyrrole], 7.5-8.5 [m, 10H, Ar-H + NH]. ^{13}C-NMR (DMSO-d_6): 25.9, 33.6, 41.0, 106.4, 106.7, 114.3, 121.8, 123.0, 125.5, 125.7 (2), 128.1, 128.3, 128.6 (2), 130.4, 133.1, 133.4, 150.4, 159.8, 162.7. MS m/z (%): 328 (M$^+$) (3.26), 248 (100). Anal. Calcd. For $C_{21}H_{20}N_4$ (328): C, 76.80; H, 6.14; N, 17.06. Found: C, 76.55; H, 6.47; N, 16.81.

1-(3-(2-Phenylquinazolin-4-ylamino)propylpyrrolidin-2-one (**7**)

Yield, 88%; m.p. >360 °C. IR (KBr, cm^{-1}): 3370(NH), 3100 (CH arom.), 2932, 2847, (CH aliph.), 1654 (C=O), 1572 (C=N). ^{1}H-NMR (DMSO-d_6): 1.5-3.2 [m, 6H, 3CH$_2$ Cyclo], 1.9 [m, 2H, CH$_2$-<u>CH$_2$</u>-CH$_2$], 3.3-3.5 [m, 4H, NH-CH$_2$ +N-CH$_2$], 7.3-8.8 [m, 10H, Ar-H + NH]. ^{13}C-NMR (DMSO-d_6): 18.5, 20.5, 29.1, 40.3, 40.4, 56.5, 114.3, 123.1, 125.8 (2), 128.3, 128.6, 130.5 (2), 133.1, 135.7, 139.1, 150.3, 159.6, 160.1, 183.2. MS m/z (%): 346 (M$^+$) (15.38), 317 (100). Anal. Calcd. For C$_{21}$H$_{22}$N$_4$O (346): C, 72.81; H, 6.40; N, 16.17. Found: C, 72.54; H, 6.08; N, 16.46.

N-(1-Ethyl-1H-pyrazol-5-yl)-2-phenylquinazolin-4-amine (**8**)

Yield, 92%; m.p. 243.5 °C. IR (KBr, cm^{-1}): 3414 (NH), 3062 (CH arom.), 2956, 2854 (CH aliph.), 1617 (C=N). ^{1}H-NMR (DMSO-d_6): 1.3 [t, 3H, CH$_3$], 4.3 [q, 2H, CH$_2$], 7.4-8.5 [m, 11H, Ar-H], 12.5 [s, 1H, NH exchangeable with D$_2$O]. ^{13}C-NMR (DMSO-d_6): 16.2, 40.4, 93.8, 114.6, 121.4, 126.3 (2), 127.0, 127.8, 128.2 (2), 129.0, 131.8, 133.2, 135.0, 149.1, 152.9, 162.8 (2). MS m/z (%): 315(M$^+$) (9.54), 286 (100). Anal. Calcd. For C$_{19}$H$_{17}$N$_5$ (315): C, 72.36; H, 5.43; N, 22.21. Found: C, 72.69; H, 5.16; N, 22.51.

1,5-Dimethyl-2-phenyl-4-(2-phenylquinazolin-4-ylamino)-1,2-dihydropyrazol-3-one (**9**).

Yield, 84%; m.p. 149.4 °C. IR (KBr, cm^{-1}): 3413 (NH), 3060 (CH arom.), 2923, 2839, (CH aliph.), 1654 (C=O), 1618 (C=N). ^{1}H-NMR (DMSO-d_6): 2.3 [s, 3H, CH$_3$], 3.1 [s, 3H, N-CH$_3$], 7.3-8.5 [m, 14H, Ar-H], 9.4 [s, 1H, NH exchangeable with D$_2$O]. ^{13}C-NMR (DMSO-d_6): 11.6, 31.2, 114.2 (2), 123.6, 124.0 (2), 126.2, 126.7 (2), 128.2, 128.4, 128.7 (2), 129.6 (2), 130.6 (2), 133.5, 135.9, 138.8, 150.8, 159.5, 160.1, 162.7. MS m/z (%): 407 (M$^+$) (5.98), 331 (100). Anal. Calcd. For C$_{25}$H$_{21}$N$_5$O (407): C, 73.69; H, 5.19; N, 17.19. Found: C, 73.44; H, 5.50; N, 17.56.

N-(3-(1H-Imidazol-1-yl)propyl)-2-phenylquinazolin-4-amine (**10**)

Yield, 79%; m.p. 174.5 °C. IR (KBr, cm-1): 3231 (NH), 3058 (CH arom.), 2927, 2866 (CH aliph.), 1617 (C=N). ^{1}H-NMR (DMSO-d_6): 2.1-4.1 [m, 6H, CH$_2$-CH$_2$-CH$_2$-N], 7.2-8.4 [m, 12H, Ar-H], 8.5 [s, 1H, NH exchangeable with D$_2$O]. ^{13}C-NMR (DMSO-d_6): 30.6, 40.5, 44.4, 114.3, 119.9, 123.1, 125.7 (2), 128.3, 128.6, 128.8, 130.5 (2), 133.1 (2), 137.8, 139.0, 150.3, 159.6, 160.1. MS m/z (%): 329 (M$^+$) (17.23), 288 (100). Anal. Calcd. For C$_{20}$H$_{19}$N$_5$ (329): C, 72.93; H, 5.81; N, 21.26. Found: C, 72.71; H, 5.49; N, 20.93.

N-(3-(2-Methylpiperidin-1-yl)propyl)-2- phenylquinazolin-4-amine (**11**)

Yield, 84%; m.p. 254.7 °C. IR (KBr, cm^{-1}): 3434(NH), 3089 (CH arom.), 2957, 2779, (CH aliph.), 1633 (C=N). ^{1}H-NMR (DMSO-d_6): 1.2 [s, 3H, CH$_3$], 1.3-2.6 [m, 9H, 4CH$_2$ + CH Cyclo], 1.6-3.8 [m, 6H, 3CH$_2$], 7.4-8.9 [m, 9H, Ar-H], 10.8 [s, 1H, NH

exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 12.3, 22.2, 23.5, 27.9, 34.4, 40.4, 50.1, 55.1, 59.0, 112.9, 120.9, 125.0 (2), 128.2 (2), 129.3 (2), 129.8, 133.7, 135.8, 157.6 (2), 160.6. MS m/z (%): 360 (M$^+$) (33.85), 344 (100). Anal. Calcd. For $C_{23}H_{28}N_4$ (360): C, 76.63; H, 7.83; N, 15.54. Found: C, 76.91; H, 7.49; N, 15.22.

2- Phenyl-N-(2-piperidin-1-yl)ethyl)quinazolin-4-amine (12)

Yield, 90%; m.p. 317.5 ^{o}C. IR (KBr, cm^{-1}): 3401 (NH), 3100 (CH arom.), 2936, 2713 (CH aliph.), 1630 (C=N). ^1H-NMR (DMSO-d_6): 2.2-2.7 [m, 10H, 5CH$_2$ Cyclo], 2.8 [t, 2H, N-CH$_2$], 4.3 [s, 2H, CH$_2$NH], 7.4-9.1 [m, 9H, Ar-H], 10.2 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 24.5, 28.0 (2), 47.4, 50.7, 59.4 (2), 112.6, 120.5, 125.0 (2), 128.1, 128.2, 129.4 (2), 130.4, 131.5, 133.9, 157.4, 160.2, 162.8. MS m/z (%): 332(M$^+$) (45.11), 219 (100). Anal. Calcd. For $C_{21}H_{24}N_4$ (332.20): C, 75.87; H, 7.28; N, 16.85. Found: C, 76.11; H, 7.55; N, 17.10.

N-(2-Morpholinoethyl)-2-phenylquinazolin-4-amine (13)

Yield, 76%; m.p. 273.4 ^{o}C. IR (KBr, cm^{-1}): 3413 (NH), 3076 (CH arom.), 2927, 2836 (CH aliph.), 1599 (C=N). ^1H-NMR (DMSO-d_6): 2.3-2.4 [m, 4H, CH$_2$-N-CH$_2$ morpholino], 2.5-3.2 [m, 4H, 2CH$_2$], 3.6-4.3 [m, 4H, CH$_2$-O-CH$_2$], 7.3-8.8 [m, 9H, Ar-H], 10.3 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 51.5, 54.9, 55.4 (2), 63.7 (2), 113.3, 126.9, 127.8 (2), 129.6 (2), 129.8 (2), 133.1 (2), 135.5, 159.1, 160.2, 163.4. MS m/z (%): 334 (M$^+$) (22.17), 256 (100). Anal. Calcd. For $C_{20}H_{22}N_4O$ (334): C, 71.83; H, 6.63; N, 16.75. Found: C, 71.50; H, 6.30; N, 16.45.

N-(3-Morpholinopropyl)-2-phenylquinazolin-4-amine (14)

Yield, 80%; m.p. 235.8 ^{o}C. IR (KBr, cm^{-1}): 3324 (NH), 3088 (CH arom.), 2954, 2864 (CH aliph.), 1610 (C=N). ^1H-NMR (DMSO-d_6): 1.8-1.9 [m, 2H, NH-CH$_2$-<u>CH$_2$</u>-CH$_2$], 2.2-2.3 [m, 4H, CH$_2$-N-CH$_2$ morpholino], 3.1 [t, 2H, N-CH$_2$], 3.2 [t, 2H, NH-CH$_2$], 3.8-3.9 [m, 4H, CH$_2$-O-CH$_2$], 7.5-8.5 [m, 9H, Ar-H], 11.2 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 23.7, 40.5, 51.4, 54.3 (2), 63.6 (2), 113.9, 123.8, 128.8 (2), 128.9 (2), 129.6 (2), 132.7, 133.1, 135.6, 152.2, 160.3, 161.7. MS m/z (%): 348 (M$^+$) (10.62), 221 (100). Anal. Calcd. For $C_{21}H_{24}N_4O$ (348): C, 72.39; H, 6.94; N, 16.08. Found: C, 72.08; H, 6.60; N, 16.35.

N-(1-Benzylpiperidin-2-yl)-2-phenylquinazolin-4-amine (15)

Yield, 83%; m.p. 250.9 ^{o}C. IR (KBr, cm^{-1}): 3380 (NH), 3077 (CH arom.), 2988, 2867 (CH aliph.), 1630 (C=N). ^1H-NMR (DMSO-d_6): 2.1-2.8 [m, 9H, 4CH$_2$ + CH Cyclo], 4.3 [s, 2H, CH$_2$-Ph], 7.4-8.9 [m, 14H, Ar-H], 10.2 [s, 1H, NH exchangeable with D_2O]. ^{13}C-NMR (DMSO-d_6): 22.6, 28.0, 34.5, 50.7, 59.4, 83.2, 112.6, 125.0, 128.2, 129.2 (2), 129.4 (3), 129.9 (3), 130.4 (2), 131.5, 132.0, 133.9, 136.1, 157.5, 160.2, 162.8. MS m/z

(%): 394 (M$^+$) (5.88), 314 (100). Anal. Calcd. For $C_{26}H_{26}N_4$ (394.22): C, 79.16; H, 6.64; N, 14.20. Found: C, 79.48; H, 6.36; N, 13.83.

6-(2-Penylquinazolin-4-ylamino)hexanoic acid (**16a**)

Yield, 76%; m.p. 164.6 °C. IR (KBr, cm-1): 3438 (OH), 3311 (NH), 3078 (CH arom.), 2939, 2854 (CH aliph.), 1687 (C=O), 1613 (C=N). 1H-NMR (DMSO-*d6*):1.4-1.8 [m, 6H, 3CH2], 2.2 [t, 2H, CH2CO], 3.8 [t, 2H, NH-CH2], 7.3-8.8 [m, 9H, Ar-H], 10.5 [s, 1H, NH exchangeable with D2O], 14.9 [s, 1H, OH exchangeable with D2O]. ^{13}C-NMR (DMSO-*d6*): 24.6, 26.3, 28.3, 34.0, 41.9, 112.6, 126.8, 128.2 (2), 129.4 (2), 129.6 (2), 133.7 (2), 135.8, 157.4 (2), 160.2, 174.8. MS m/z (%): 335 (M+) (39.45), 290 (100). Anal. Calcd. For $C_{20}H_{21}N_3O_2$ (335): C, 71.62; H, 6.31; N, 12.53. Found: C, 71.29; H, 6.60; N, 12.19.

Biological evaluation

Hepa1c1c7 murine hepatoma cells were grown in a humidified atmosphere at 37 °C, 5% CO_2. The cell culture medium was □-MEM supplemented with 10% (v/v) heat- and charcoal-inactivated fetal bovine serum. For evaluation of the potential NQO1 inducer activity, cells (10^4 per well) were grown in 96-well plates for 24 h, after which the cell culture medium was replaced with fresh medium containing each inducer (dissolved in DMSO and diluted in the medium 1:1000), and the cells were grown for a further 48 h. There were eight replicates of each treatment of serial dilutions of inducers. The final DMSO concentration in the cell culture medium was maintained 0.1% (v/v) in all wells. At the end of the treatment period, cell lysates were prepared in digitonin and the specific activity of NQO1 was determined using menadione as a substrate as described[23,24]. The Concentration which Doubles the specific activity of NQO1 (CD value) was used as a measure of inducer potency. Mean values for the eight replicate wells are shown for each data point. The standard deviation for each data point was within 5% of the mean value.

Molecular modeling study

The molecular model of all the new anilinequinazoline derivatives was built using MOE software suite version 10.2008 maintaining all the default parameters. The structures' geometry was optimized and a systematic conformational search was carried out to an RMS gradient of 0.01Å using the ConfSearch module implemented in MOE. Computations were set to be performed with the Merck Force Field (MMFF94s). The

new compounds' ability to access and block the Nrf2-binding site of Keap1 was evaluated by performing a molecular docking study using the crystallographic structure of Keap1 obtained from the Protein Data Bank (PDB ID: 4IQK). Following addition of the missing hydrogens and calculating the partial charges of the receptor, validation of the docking has been carried out by docking of the native ligand. Afterwards, the ligands were docked where they were left free to explore all conformations possible inside the enzyme. Multiple separate docking simulations using default parameters were performed followed by choosing the best conformations based on the combination of S score data, E conformation and appropriate fitting with the relevant amino acids in the binding pocket.

Results and Discussion
Chemistry

The new anilinoquinazolines derivatives **2**-**16a** that were designed for the aim of exploring their potential cytoprotective activity, were synthesized from the key starting material 4-chloro-2-phenylquinazoline **1** by allowing it to react with primary amines with their side chains bearing aliphatic groups (e.g. heptane, octane), aromatic groups (e.g. pyrrolidine, imidazole, piperidine) or substituted aromatic rings (e.g. methyl pyrrolidine, benzyl piperidine, methyl piperidine). Refluxing of **1** with the amines in dry dimethylformamide in the presence of triethylamine as a catalyst yielded the desired corresponding compounds **2-16a** with good yield values. (**Scheme 1 and 2**) The structures of the products were assigned on the basis of their analytical and spectral data. First, the IR spectra of the reaction products showed in all compounds an absorption band corresponding to NH function in the region 3434-3191 cm^{-1}, in addition to a C=N band in the region 1639-1572 cm^{-1}. Moreover, compounds **7**, **9** and **16a** have shown an extra carbonyl absorption band in the region 1687-1654 cm^{-1}. Finally, Compounds **4-16a** bearing aliphatic carbon chains have shown absorption peaks around 2988-2713 cm^{-1}. Moreover, ^{1}H-NMR spectra of compounds **2–16a** in (DMSO-d_6) revealed a singlet signal that was exchangeable with D_2O in the region 9.4-11.2 ppm corresponding to a NH group. Regarding compound **16**, compound **1** was reacted with 6-aminocaproic acid with the expected product to be azepan-2-one derivative **16b**, however, the hexanoic acid

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derivative **16a** was obtained instead. That was revealed on the basis of elemental analysis and IR spectrum which showed the presence of OH absorption band at 3438 cm^{-1}, NH band at 3311 cm^{-1} and a carbonyl band at 1687 cm^{-1}. That was further confirmed using spectral analysis where ^{1}H-NMR showed a triplet at 3.8 ppm for the NH-CH$_2$, signal at 10.5 ppm for the NH group which is exchangeable with D$_2$O and another signal at 14.9 ppm for the OH group which is exchangeable with D$_2$O. Moreover, ^{13}C- NMR of **16a** revealed signals at 24.6 ppm for $\underline{CH_2}$-CH$_2$-COOH, 26.3 ppm for $\underline{CH_2}$-CH$_2$-CH$_2$-COOH , 28.3 ppm for NH-CH$_2$-$\underline{CH_2}$, 34.0 ppm for $\underline{CH_2}$-COOH, 41.9 ppm for NH-CH$_2$, 174.8 ppm for C=O group. These assignments fully support and affirm the proposed structure.
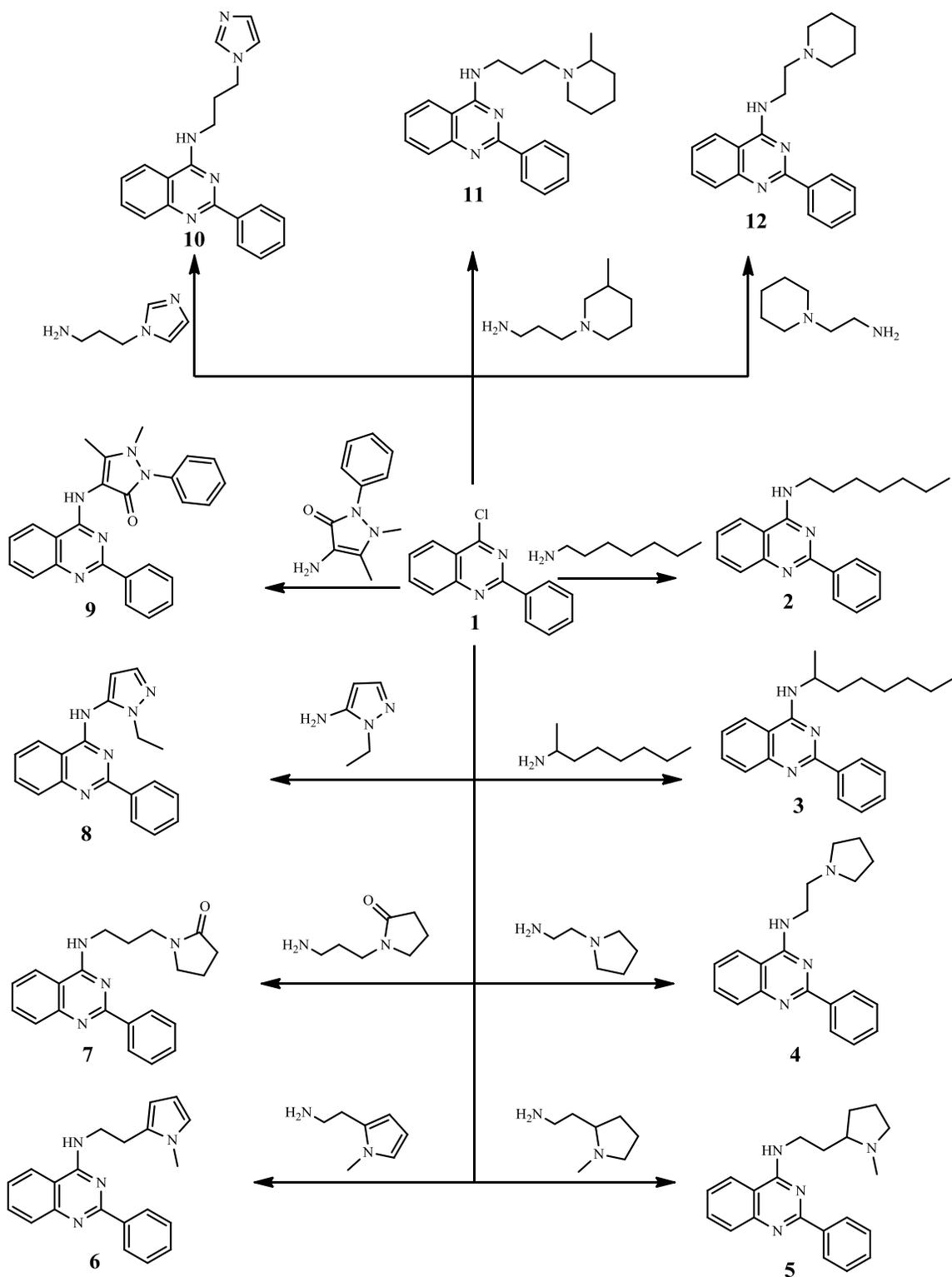
Scheme 1: reagents and conditions:A mixture of 1 (0.01mol) and different amines (0.012 mol) in dry DMF (10 mL) containing Et$_3$N 3 drops was refluxed for 24 h. , then left to cool. The solid product formed was collected by filtration and recrystallized from acetic acid.
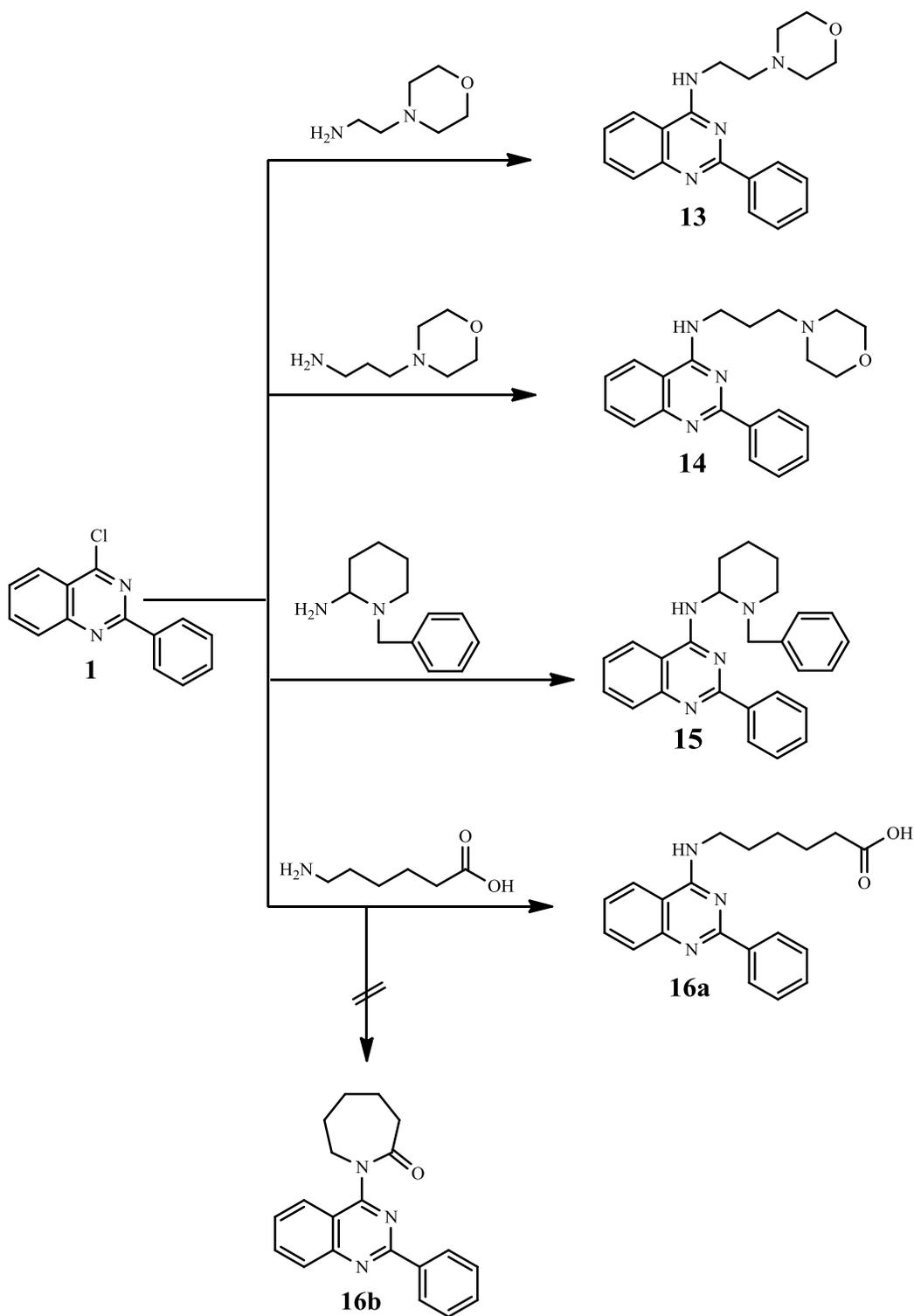
Scheme 2: reagents and conditions:A mixture of 1 (0.01mol) and different amines (0.012 mol) in dry DMF (10 mL) containing Et$_3$N 3 drops was refluxed for 24 h. , then left to cool. The solid product formed was collected by filtration and recrystallized from acetic acid.

Biological activity

The ability of the novel compounds to duplicate the activity of NAD(P)H:quinone oxidoreductase 1 (NQO1) was used as a measure of their cytoprotective activity (CD values). Evaluation of the NQO1 inducer activity showed that compounds **2**, **5** and **14** were inactive, whereas compounds **4**, **6**, **11**, **12**, **13**, and **16a** had weak activity, however, CD value was not reached (**Figure 1**). On the other hand, compounds **3** (CD = 14 µM), **7** (CD = 26 µM) and **15** (CD = 19 µM) had moderate inducer activity. Compounds **8** (CD = 5.2 µM) and **10** (CD = 5.5 µM) were of approximately equal potency. However, the cell responses to them were very different where the dose-response dependency was very clear for compound **8**, whereas it was completely absent for compound **10**. The most potent inducer in this series was compound **9** (CD = 3.9 µM), which also showed a very clear dose response with a magnitude of induction of ~6-fold at a concentration of 50 µM.

Conc. (uM)	Compound #														
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16a
0.1563	NR*	1.05	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
0.3125	NR	1.08	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
0.625	NR	1.07	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
0.781	1.25	NR	1.24	1.09	0.97	1.01	1.23	1.34	1.66	1.11	1.01	1.12	1.01	1.12	1.04
1.25	NR	1.19	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
1.563	1.25	NR	1.3	1.1	0.96	1.01	1.33	1.56	1.78	1.1	1.12	1.17	1.04	1.14	1.04
2.5	NR	1.28	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
3.125	1.21	NR	1.49	1.12	1.08	1.1	1.57	1.86	1.89	1.2	1.26	1.16	1.06	1.23	1.1
5	NR	1.56	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
6.25	1.2	NR	1.68	1.2	1.31	1.25	2.12	2.41	2.03	1.3	1.36	1.24	1.11	1.38	1.23
10	NR	1.93	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
12.5	NR	NR	1.76	1.28	1.54	1.48	2.63	3.23	2.1	1.5	NR	1.39	1.1	1.67	1.45
20	NR	2.13	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR	NR
25	NR	NR	NR	NR	NR	1.98	3.34	4.74	NR	NR	NR	1.41	1.06	2.22	1.53
50	NR	NR	NR	NR	NR	2.73	4.16	6.45	NR	NR	NR	NR	NR	3.19	NR
100	NR	NR	NR	NR	NR	3.63	4.25	NR	NR	NR	NR	NR	NR	4.02	NR
CD**	NR	14	NR	NR	NR	26	5.2	3.9	5.5	NR	NR	NR	NR	19	NR

Table 1: NQO1 inducer activity and CD values of the test compounds.

*NR = Not Recorded

** CD data presented are the averages of 3 independent experiments, each with 8 replicate wells of cells, and SD (standard deviation) for each data point was within 5% of the value.
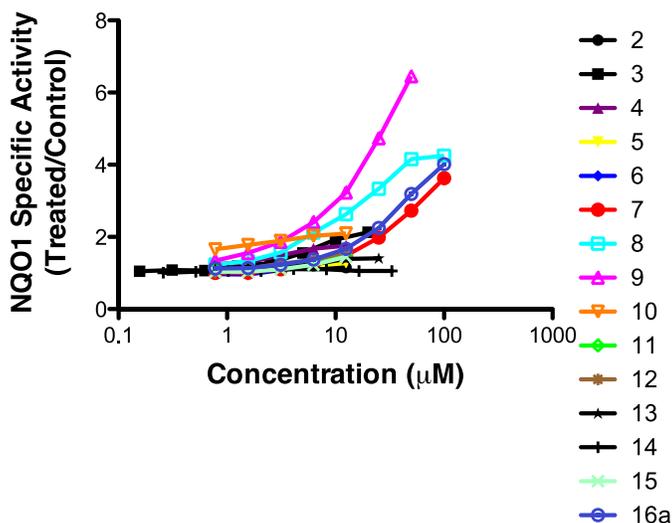
Figure 1: Concentration dependence of NQO1 inducer activity of quinazoline derivatives.

Molecular modeling

It has been established that binding of Keap1 to Nrf2 promotes its degradation, thus maintaining low levels of expression of cytoprotective gene products. Several small molecule compounds have been reported to have binding affinity to the Keap1 Kelch domain therefore antagonizing its activity[25, 26]. In order to assess the ability of the newly synthesized compounds to access and block the Kelch domain of Keap1, a molecular docking study was performed using the crystal structure obtained from the Protein Data Bank (PDB ID: 4IQK) using MOE software suite version 10.2008. The key interactions detected between the validated native ligand and the receptor are found to be arene-cation interaction with Arg415, arene-arene interaction with Tyr525 and 3 hydrogen bonds with Ser602, Ser508 and Ser555 with S = -13.306 Kcal/mol with a rmsd of 0.6635 Kcal/mol/Å (**Figure 2**). Docking of the synthesized compounds revealed that binding to Arg415 through an arene-cation interaction is an important common interaction among all compounds. Moreover, by observing the interactions of compounds **4-16a** it was found that compounds showing activity have a larger number of interactions with the binding site of the sensor protein *via* their side chain or via their main skeleton. That may emphasize the role of variation of the side chain in either making its own interactions or in pushing the phenylquinazoline moiety to more interactions with more amino acids.

By having a further insight of compound **9** that showed the best activity (CD = 3.9 μM), it was obvious that it also showed the best binding affinity with S = - 11.7347 Kcal/mol. Compound **9** is able to make two arene-cation interactions with Arg483. Although that Arg is different from Arg415 that the native ligand interact with, the overlap of compound **9** over the native ligand showed a change in the orientation of the compound to ensure better fitting in the pocket. (**Figure 3**). Moreover, an arene-arene interactions is noticed with Tyr525 similar to the native ligand. Finally an additional arene-arene interaction is noticed with Phe478; that additional interaction may be related to the high activity of that compound since it is only noticed in the interactions of compound **9** with the sensor. (**Figure 4**)

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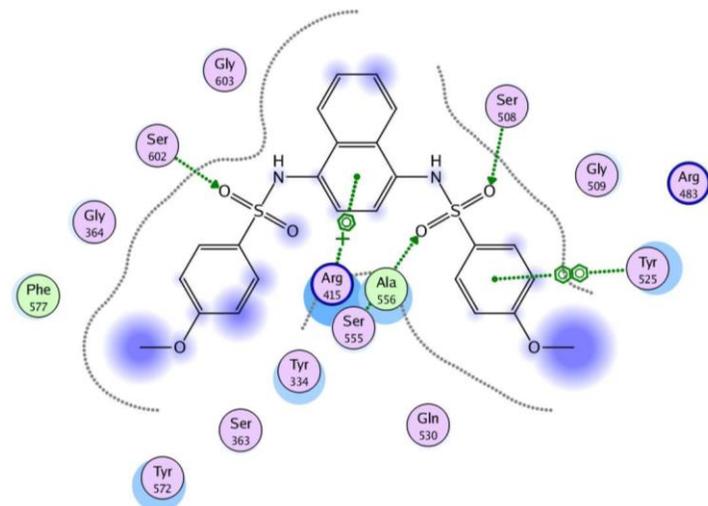
Figure 2: *Interactions of the native ligand with the Kelch domain of Keap1 (PDBID: 41QK)*
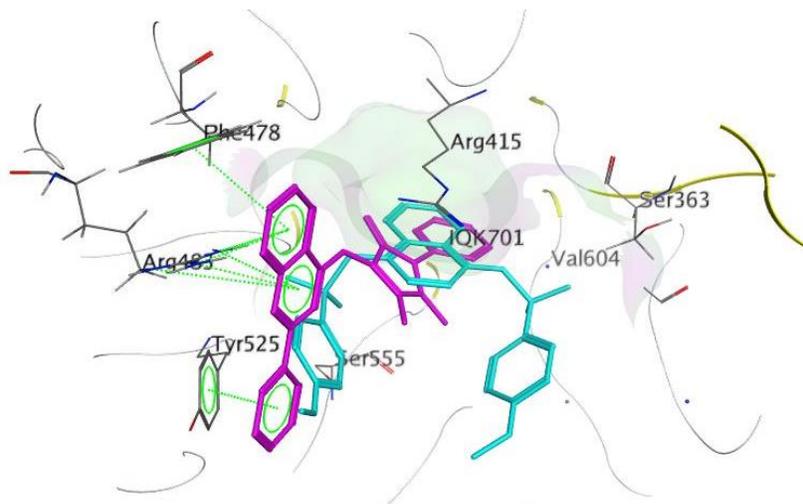
Figure 3: *Overlap of compound **9** (magenta) over native ligand (cyan) the Kelch domain of Keap1.*
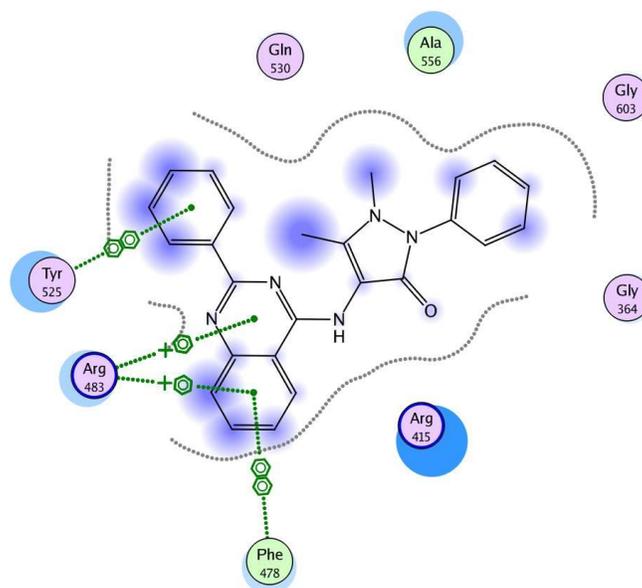
Figure 4: *Interactions of Compound **9** with the Kelch domain amino acids of Keap1.*

Conclusion

In conclusion, this study deals with the synthesis of novel anilinoquinazoline derivatives with potential cytoprotective NQO1 inducing activity. Among the derivatives **2-16a**, twelve compounds showed activity, with six of them showing CD values ranging between 3.9- 25 μM. Finally, the molecular docking study has shown that the most active derivative, compound **9** (CD = 3.9 μM), showed arene-arene interactions with key amino acids in the active pocket of Keap1. The obtained results introduce compound **9** as a lead for anilinoquinazoline scaffold-based cytoprotective agents thus serving as a starting point for lead optimization of new molecules based on the chemotype described herein.

Declaration of interest

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