Synthesis and biological studies of new quinazolines with ether functions in position 2

Dabbugoddu Brahmaiah, a,b Anagani Kanaka Durga Bhavani, *c Pasula Aparna, a Nangunoori Sampath Kumar, a Hélène Solhi, d Rémy Le Guevel, d Blandine Baratte, e Sandrine Ruchaud, e Stéphane Bach, e Paul Mosset, f and René Grée* f

a Chemveda Life Sciences India Pvt. Ltd., #B-11/1, IDA Uppal, Hyderabad-500039, Telangana, India
b Jawaharlal Nehru Technological University Hyderabad, Kukatpally, Hyderabad-500 085, Telangana, India
c Osmania University, Department of Chemistry, Hyderabad 500007, Telangana, India
d Univ Rennes, Plateform ImPACcell, BIOSIT, F-35000 Rennes, France
e Sorbonne Université, CNRS, Plateforme de Criblage KISSf (Kinase Inhibitor Specialized Screening facility), Protein Phosphorylation and Human Diseases Unit, Station Biologique de Roscoff, place G. Tessier, 29688 Roscoff Cedex, France
f Univ Rennes, CNRS, ISCR (Institut des Sciences Chimiques de Rennes), UMR 6226, F-35000 Rennes, France
Email: rene.gree@univ-rennes1.fr

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Abstract

A series of new quinazolines linked to triazoles through an ether chain in position 2 has been designed and synthetized through a flexible route. Cytotoxicity assays on selected cancer cell lines and inhibition studies toward a panel of representative mammalian kinases have been performed on these molecules.

Keywords: Quinazolines, triazoles, cytotoxicity, kinases
Introduction

Many quinazoline alkaloids and derivatives have been isolated already, and the antimalarial (+)-Febrifugine 1 and the cytotoxic Luotonin A 2 are representative examples of this family of natural products (Figure 1).\(^1\) From a structural point of view, almost all of them possess a quinazoline-4(3H)-one ring system. On the other hand, the quinazoline nucleus is also a privilege scaffold found in the basic skeleton of various types of drugs and/or bioactive molecules.\(^2\) Representative examples are indicated in Figure 1 and it is worth noting that all compounds 3-8, which are potent kinase inhibitors used as anticancer drugs, have an aniline-type substituent in position 4 of this structure. Some other quinazolines, like Doxazosine 9 and Prazosin 10 (these two derivatives are used against hypertension) have both a primary amine in position 4 and a substituted piperazine in position 2.

![Figure 1. Representative examples of bioactive natural products and drugs with quinazoline cores.](image)

Further, extensive literature search shows that much less is known about type-A molecules with X-R\(^1\) chains in position 2 (X being heteroatom like O, S and N and R\(^1\) = alkyl or substituted alkyl chains, aromatics, heteroaromatics.....) (Figure 2). Therefore, we embarked on a program involving the synthesis and biological evaluation of such derivatives. The goal of this paper is to report the first part of this research, dealing with the

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synthesis of a focused library of oxygen-linked molecules 11 with a short linker and triazoles in terminal position. The choice of the triazole nucleus is based upon the fact that this core structure is found also in many biologically active molecules.\(^5\)

![Figure 2. Our target molecules.](image)

**Chemical synthesis**

Various strategies and disconnections have been reported for the synthesis of quinazolines.\(^6\)-\(^7\) In our case we favored the approach through two C-N bond disconnections and starting from an ortho-aminobenzaldehyde derivative 13 (Scheme 1). Reaction with urea gave in good yield quinazoline 14 which was further treated by POCl\(_3\) to give chloroquinazoline 14a. This intermediate was reacted with propargyl alcohol to isolate in 65% yield propargyl ether 15. Final click-type reactions,\(^8\)-\(^11\) with various azido derivatives 16a-j,\(^12\) afforded the target molecules 11a-j in good yields (Table 1).

**Results and Discussion**

**Scheme 1. Synthesis of quinazoline-based triazoles.**
Table 1. Synthesis of our target molecules

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All intermediates and final products have spectral and analytical data in agreement with their structures.

Biological studies

Based on the known biological properties of molecules with quinazoline scaffolds, we performed a few primary biological screenings of the ten molecules 11a-11j.

First, their cytotoxicity has been checked on seven representative cancer cell lines (HuH7, CaCo-2, MDA-MB231, HCT116, PC3, NCI-H727, MCF7). All compounds were found to be devoid of any significant cytotoxicity at 10 µM concentration (See Supplementary Material).

Their activity was also screened against a panel of eight representative kinases (HsCDK5/p25, HsCDK9/CyclinT, HsPIM1, MmCLK1, RnDYRK1A, HsHASPIN, HsGSK3β, HsCK1ε) and the results are reported in Table 2. Interestingly only one of these kinases (MmCLK1) was found to be responding to these derivatives. A few molecules (11a, 11c, 11d, 11e, 11f, 11j) exhibited a moderate inhibition of this kinase at 10µM. On the other hand, these molecules were found to be devoid of any significant activity at 10µM concentration on the other kinases.
Table 2. Study of the inhibition of quinazolines 11 against a representative panel of mammalian kinases

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Percentages of residual kinase activity were determined at 1 and 10 µM concentration for each compound. Kinase activities were assayed in duplicate.

Conclusions

In summary, we designed a short and convergent strategy toward the desired quinazolines linked to triazoles through an ether chain in position 2 of the quinazoline. Some of these derivatives exhibit a moderate, but significant and selective, activity against the MmCLK1 kinase. Extension of this approach to other quinazoline-derived derivatives is under active study and will be reported in due course.

Experimental Section

Chemical Synthesis

**General.** All anhydrous reactions were performed in heat gun-dried round-bottomed flasks under a dry argon or nitrogen atmosphere. Air and moisture-sensitive compounds were introduced via syringes or cannula, using
standard inert atmosphere techniques. In addition, the gas stream was passed through glass cylinder filled with P₂O₅ to remove any traces of residual moisture. Reactions were monitored by thin layer chromatography (TLC) using E. Merck silica gel plates and components were visualized by illumination with short wavelength UV light and/or staining (Ninhydrin or basic KMnO₄). THF and Et₂O were dried over sodium-benzophenone and distilled prior to use. Anhydrous CH₂Cl₂ was prepared by refluxing in the presence of CaH₂ and distilled right before use unless otherwise noted. Infrared spectra have been recorded on a Bruker alpha II FTIR spectrometer. ¹H NMR spectra were recorded at 300 and 400 MHz, and ¹³C NMR spectra at 75 and 100 MHz, in CDCl₃ or DMSO-d₆ using tetramethylsilane (TMS) as an internal standard on Bruker spectrometers (Avance 300III and Avance 300I and AvanceII 400). Assignments were made using standard 2D NMR techniques (COSY, HMQC/HSQC, HMBC). High resolution mass spectra were performed using a time of flight Maxis 4G (Bruker Daltonik Gmbh, Bremen, Germany) in Electrospray positive ionization mode. LC-MS analyses were carried out on a Shimadzu [LCMS-2020], SHIMPAK, XR ODS-II column (50 x 2 mm) utilizing the following method. Solvent A = Acetonitrile, B = 0.1% TFA in water; Initial 95% of solvent B, then run gradient, which should reach 90% solvent A within 10 min and hold 90% solvent A for another 10 min. Flow Rate: 0.2 ml/min.

**Synthesis of 2-amino-3-methoxybenzaldehyde (13).** To a stirred solution of 3-methoxy-2-nitrobenzaldehyde (12) (10 g, 54 mmol) in a mixture of methanol (100 mL) and glacial acetic acid (100 mL) and water (50 mL) was added reduced iron powder (16 g, 144 mmol) in lot wise manner. The resulting suspension was stirred at 30-35°C for 2h. The reaction mixture was filtered on Celite bed and washed with methanol (50 mL). The filtrate was concentrated to remove volatiles and it was partitioned between NaHCO₃ (150 mL) and ethyl acetate (200 mL). The basic layer was further extracted with ethyl acetate (100 mL). The combined organic extracts were washed with brine (50 mL), dried over Na₂SO₄ and concentrated to dryness and purified by chromatography on silica gel eluting with 20% EtOAc in hexane giving a compound 13 (6.5 g, 78% Yield) as a off white solid. ¹H NMR (400 MHz, CDCl₃) δ ppm: 9.93 (s, 1H), 8.45 (d, 1H), 7.82 (d, 1H), 6.80 (t, 1H); Mass (m/z): 165.2 (M+H).

**Synthesis of 8-methoxyquinazolin-2-ol (14).** 2-Amino-3-methoxybenzaldehyde (13) (5 g, 33.1 mmol), urea (20 g, 336.3 mmol) and cat. NH₄OAc (10 mg) were thoroughly mixed together in a round bottom flask. The solid mixture was heated to 160 °C and the solids quickly melted and stirring was continued for 15 min. A solid started to precipitate from the hot solution, NMP (25 mL) was added to dissolve the solids, the reaction was heated with stirring for an additional 2 h at 155-160 °C. The hot reaction mixture was poured into vigorously stirred ice water (150 mL). The reaction mixture was filtered, washed with water (50 mL) and ethyl acetate (15 mL) giving a brown color solid which was dried to give compound 14 (4.9 g, 82% yield) as a off white solid. ¹H NMR (500 MHz, DMSO-d₆) δ ppm: 11.3 (br, 1H), 9.23 (s, 1H), 7.43 (t, 1H), 7.34 (d, 2H), 3.90 (s, 3H); Mass (m/z): 177.2 (M+H).

**Synthesis of 2-chloro-8-methoxyquinazoline (14a).** A mixture of compound 14 (4 g, 22 mmol) and 2,3,5-collidine (5 mL, 38.8 mmol) in acetonitrile (80 mL) was stirred at room temperature for 10 min. POCl₃ (22 mL, 222 mmol) was added to reaction mixture at 0 °C and the reaction mixture was heated for 10 h at reflux. The mixture was concentrated to dryness. The obtained residue was partitioned between EtOAc (200 mL) and sat. NaHCO₃ (200 mL). The mixture was stirred cautiously watching gas evolution until the pH reached to 8. The layers were separated and the organic layer was washed with sat. NaHCO₃ (75 ml), brine (50ml), dried (Na₂SO₄), and concentrated to dryness, giving compound 14a (2.8 g, 63.5% yield) as a pale yellow solid. ¹H NMR (400 MHz, CDCl₃) δ ppm: 9.25 (s, 1H), 7.61-7.57 (t, J 16.0 Hz, 1H), 7.51-7.48 (t, J 8.4, 1H), 7.28 (dd, J 8.0, 0.8 Hz, 1H), 4.07 (s, 3H); ¹³C NMR (400 MHz, CDCl₃): δ 162.72, 156.93, 154.08, 143.80, 128.59, 124.33, 118.41, 113.08, 56.26. Mass (m/z): 195.1 (M+H).
Synthesis of 8-methoxy-2-(prop-2-yn-1-yloxy)quinazoline (15). Propargyl alcohol (518 mg, 9.24 mmol) was added to a stirred suspension of Cs2CO3 (3g, 9.24 mmol) and DMF (10 mL), after stirring 15 minutes at 30 °C compound 14a (600 mg, 3.08 mmol) was added. After stirring at 100 °C for 16h, the reaction mixture was quenched with water (30 mL) and extracted with DCM (2x25 mL). The combined organic extracts were washed with brine (5%, 15 mL), dried over Na2SO4 and concentrated to dryness. Purification by chromatography on silica gel (elution with EtOAc in hexane) gave compound 15 (450 mg, 65% yield) as a off white solid. 1H NMR (400 MHz, CDCl3) δ ppm: 9.23 (s, 1H), 7.46-7.38 (m, 2H), 7.21 (d, J 7.6, 1.6 Hz, 1H), 5.21 (s, 2H), 4.06 (s, 3H), 2.49 (t, J 2.4 Hz, 1H); 13C NMR (400 MHz, CDCl3): δ 163.62, 161.11, 153.84, 143.67, 125.43, 122.96, 118.90, 113.06, 78.55, 74.63, 56.33, 55.18. Mass (m/z): 215.1 (M+H).

General procedure for preparation of compounds, 11a-j. To the stirred solution of compound 15 (0.1 mmol, 1.0 eq.) and compound 16a-j [prepared using general protocol,12 (0.1 mmol, 1 eq.) in a 1:1 mixture of t-butanol and water (0.56 mL) was added copper sulfate pentahydrate (0.01 mmol, 0.1 eq.) and sodium ascorbate (0.03 mmol, 0.3 eq.) at room temperature. The resulting mixture was stirred for overnight. After completion of the reaction, it was diluted with a mixture of ethyl acetate and water (7.5 ml in a 1:1 ratio), the organic layer was separated and washed with 5% ammonium hydroxide solution (0.5 mL) and followed by brine (0.5 mL). The combined organic layers were dried over Na2SO4. The solvents were evaporated under reduced pressure to get the crude product, which was purified by chromatography on silica gel affording target compounds 11a-j (65-75% yield) as light yellow to brown solids.

8-Methoxy-2-((1-(4-methoxyphenyl)-1H-1,2,3-triazol-4-yl)methoxy)quinazoline (11a). Reaction of 15 (25 mg, 0.11 mmol) with 16a (17.4 mg, 0.11 mmol) gave 11a: 31.6 mg, 75% yield. mp 179-181 °C; FT-IR (KBr, cm−1): 3137, 2958, 2841, 1584, 1516, 1470, 1419, 1292, 1261, 1175, 1117, 1034, 836, 763. 1H NMR (300 MHz, CDCl3): δ 9.23 (s, 1H, H-quinazoline), 8.37 (br t, J 0.5 Hz, 1H, H-triazole), 7.62 (half part of an A2X2 system, 2H meta to OMe), 7.46 (dd, J 8.1, 1.6 Hz, 1H, H-C7 of quinazoline), 7.40 (dd, J 8.1, 7.4 Hz, 1H, H-C6 of quinazoline), 7.22 (dddd, J 7.4, 1.6, 0.2 Hz, 1H, H-C5 of quinazoline), 7.01 (half part of an A2X2 system, 2H ortho to OMe), 5.80 (d, J 0.5 Hz, 2H, CH2), 4.09 (s, 3H, CH3, OMe on quinazoline), 3.86 (s, 3H, CH3, OMe of 4-methoxyphenyl). 13C NMR (75 MHz, CDCl3): δ 163.72 (CH, α to N of quinazoline), 161.65 (C, C2 of quinazoline), 159.81 (C, α to OMe), 153.83 (C, C8 of quinazoline), 144.19 (C of triazole), 143.59 (C, C8a of quinazoline), 130.67 (C, δ to OMe), 125.28 (CH, C6 of quinazoline), 122.92 (CH of triazole), 122.89 (C, C4a of quinazoline), 122.15 (2CH, meta to OMe), 118.99 (CH, C7 of quinazoline), 114.78 (2CH, ortho to OMe), 112.87 (CH, C5 of quinazoline), 61.33 (CH2), 56.23 (CH3, OMe on quinazoline), 55.64 (CH3, OMe of 4-methoxyphenyl). LC-MS: 363.13 [M]. HRMS-ESI (m/z [M+Na]+) calcd for C19H17N3NaO3: 386.12236, found 386.1222, [M+K]+ calcd for C19H17KN5O3: 402.09630, found 402.0957.

2-((1-(4-Chlorophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-8-methoxyquinazoline (11b). Reaction of 15 (25 mg, 0.11 mmol) with 16b (17.92 mg, 0.11 mmol) gave 11b: 28 mg, 65% yield. mp 130 °C (dec); FT-IR (KBr, cm−1): 3339, 3332, 2999, 1581, 1493, 1421, 1382, 1293, 1171, 1108, 1029, 830, 756. 1H NMR (400 MHz, CDCl3): δ 9.24 (s, 1H, H-quinazoline), 8.47 (s, 1H, H-triazole), 7.68 (half part of an A2X2 system, 2H meta to Cl), 7.49 (half part of an A2X2 system, 2H ortho to Cl), 7.46 (dd, J 8.2, 1.4 Hz, 1H, H-C7 of quinazoline), 7.42 (dd, J 8.2, 7.5 Hz, 1H, H-C6 of quinazoline), 7.23 (dd, J 7.6, 1.4 Hz, 1H, H-C5 of quinazoline), 5.80 (d, J 0.6 Hz, 2H, CH2), 4.10 (s, 3H, CH3, OMe), 13C NMR (100 MHz, CDCl3): δ 163.77 (CH, α to N of quinazoline), 161.58 (C, C2 of quinazoline), 153.80 (C, C8 of quinazoline), 144.68 (C of triazole), 143.53 (C, C8a of quinazoline), 135.66 (C, δ to Cl), 134.50 (C, α to Cl), 129.94 (2CH, ortho to Cl), 125.36 (CH, C6 of quinazoline), 122.91 (C, C4a of quinazoline), 122.77 (CH of triazole), 121.65 (2CH, meta to Cl), 119.04 (CH, C7 of quinazoline), 112.93 (CH, C5 of quinazoline), 61.17
2-((1-(2-Chlorophenyl)-1H,1,2,3-triazol-4-yl)methoxy)-8-methoxyquinazoline (11c). Reaction of 15 (20 mg, 0.093 mmol) with 16c (14.28 mg, 0.11 mmol) gave 11c: 22.1 mg, 64% yield. mp 134-136 °C; FT-IR (KBr, cm⁻¹): 3146, 3004, 1580, 1486, 1417, 1382, 1288, 1168, 1119, 1022, 757, 665. ¹H NMR (300 MHz, CDCl₃): δ 9.23 (s, 1H, H-quinazoline), 8.48 (br t, J 0.5 Hz, 1H, H-triazole), 7.66-7.59 (m, 1H, H-4 of 2-chlorophenyl), 7.59-7.53 (m, 1H, H-5 of 2-chlorophenyl), 7.48-7.40 (m, 3H, H-3 and H-6 of 2-chlorophenyl and H-C7 of quinazoline), 7.40 (dd, J 8.1, 7.4 Hz, 1H, H-C6 of quinazoline), 7.20 (dd, J 7.4, 1.6 Hz, 1H, H-C5 of quinazoline), 5.84 (d, J 0.5 Hz, 2H, CH₂), 4.06 (s, 3H, CH₃, OMe). ¹³C NMR (75 MHz, CDCl₃): δ 163.74 (CH, α to N of quinazoline), 151.54 (C, C of quinazoline), 153.73 (C, C8 of quinazoline), 143.42 (C, C8a of quinazoline), 143.32 (C of triazole), 134.93 (C, α to Cl), 130.75 (CH, C5 of 2-chlorophenyl), 130.62 (CH, C6 of 2-chlorophenyl), 128.29 (C, C1 of 2-chlorophenyl), 127.94 (CH, C3 of 2-chlorophenyl), 127.76 (CH, C4 of 2-chlorophenyl), 126.89 (CH of triazole), 125.29 (CH, C6 of quinazoline), 122.80 (C, C4a of quinazoline), 118.90 (CH, C7 of quinazoline), 112.70 (CH, C5 of quinazoline), 61.03 (CH₂), 56.13 (CH₃, OMe). LC-MS: 367.79 [M]. HRMS–ESI (m/z) [M+Na]⁺ calcd for C₁₈H₁₄⁻ClN₅O₂Na: 390.07282, found 390.0729, [M+K]⁺ calcd for C₁₈H₁₄⁻ClKN₂O₂: 406.04676, found 406.0460.

8-Methoxy-2-(((1-(2-methoxyphenyl)-1H,1,2,3-triazol-4-yl)methoxy)quinazoline (11d). Reaction of 15 (25 mg, 0.11 mmol) with 16d (17.4 mg, 0.11 mmol) gave 11d: 29.4 mg, 68% yield. mp 147-149 °C; FT-IR (KBr, cm⁻¹): 3157, 2963, 2838, 1580, 1470, 1417, 1286, 1170, 1017, 753, 668. ¹H NMR (300 MHz, CDCl₃): δ 9.23 (s, 1H, H-quinazoline), 8.44 (br t, J 0.6 Hz, 1H, H-triazole), 7.77 (ddd, J 7.8, 1.7, 0.3 Hz, 1H, H₆ of 2-methoxyphenyl), 7.45 (dd, J 8.2, 1.5 Hz, 1H, H-C7 of quinazoline), 7.41 (ddd, J 8.2, 7.6, 1.7 Hz, 1H, H-C6 of quinazoline), 7.39 (dd, J 8.1, 7.4 Hz, 1H, H₄ of 2-methoxyphenyl), 7.20 (br dd, J 7.5, 1.5 Hz, 1H, H-C5 of quinazoline), 7.09 (td, J 7.7, 1.3 Hz, 1H, H₅ of 2-methoxyphenyl), 7.07 (br dd, J 8.3, 1.1 Hz, 1H, H₃ of 2-methoxyphenyl), 5.84 (d, J 0.6 Hz, 2H, CH₂), 4.07 (s, 3H, CH₃, OMe on quinazoline), 3.84 (s, 3H, CH₃, OMe of 2-methoxyphenyl). ¹³C NMR (75 MHz, CDCl₃): δ 163.63 (CH, α to N of quinazoline), 161.70 (C, C2 of quinazoline), 153.85 (C, C8 of quinazoline), 151.23 (C α to OMe of 2-methoxyphenyl), 143.69 (C, C8a of quinazoline), 143.03 (C of triazole), 130.04 (CH, C4 of 2-methoxyphenyl), 126.47 (CH of triazole), 126.41 (C, C1 of 2-methoxyphenyl), 125.62 (CH, C6 of 2-methoxyphenyl), 125.20 (CH, C6 of quinazoline), 122.85 (C, C4a of quinazoline), 121.22 (CH, C5 of 2-methoxyphenyl), 118.94 (CH, C7 of quinazoline), 112.83 (CH, C5 of quinazoline), 112.27 (CH, C3 of 2-methoxyphenyl), 61.38 (CH₂), 56.23 (CH₃, OMe on quinazoline), 55.94 (CH₃, OMe of 2-methoxyphenyl). LC-MS: 363.13 [M]. HRMS–ESI (m/z) [M+Na]⁺ calcd for C₁₉H₁₇N₅SNaO₃: 386.12236, found 386.1228, [M+K]⁺ calcd for C₁₉H₁₇N₅K₂O₃: 402.09630, found 402.0963, [M+H]⁺ calcd for C₁₉H₁₈N₅O₂: 364.14041, found 364.1404.

8-Methoxy-2-((1-(3-methoxyphenyl)-1H,1,2,3-triazol-4-yl)methoxy)quinazoline (11e). Reaction of 15 (25 mg, 0.11 mmol) with 16e (17.4 mg, 0.11 mmol) gave 11e: 30 mg, 71% yield. mp 137-139 °C; FT-IR (KBr, cm⁻¹): 3144, 3002, 1594, 1483, 1420, 1289, 1165, 1015, 1031, 855, 764. ¹H NMR (300 MHz, CDCl₃): δ 9.23 (s, 1H, H-quinazoline), 8.48 (br t, J 0.6 Hz, 1H, H-triazole), 7.46 (dd, J 8.2, 1.6 Hz, 1H, H-C7 of quinazoline), 7.44-7.36 (m, 2H, H-C6 of quinazoline at 7.43 ppm and H₅ of 3-methoxyphenyl at 7.39 ppm), 7.34 (dd, J 2.4, 2.1 Hz, 1H, H₂ of 3-methoxyphenyl), 7.26 (ddd, J 8.0, 2.1, 1.0 Hz, 1H, H₆ of 3-methoxyphenyl), 7.23 (br dd, J 7.5, 1.6 Hz, 1H, H-C5 of quinazoline), 6.96 (ddd, J 8.3, 2.5, 1.0 Hz, 1H, H₄ of 3-methoxyphenyl), 5.81 (d, J 0.5 Hz, 2H, CH₂), 4.10 (s, 3H, CH₃, OMe on quinazoline), 3.87 (s, 3H, CH₃, OMe of 3-methoxyphenyl). ¹³C NMR (75 MHz, CDCl₃): δ 163.74 (CH, α to N of quinazoline), 161.63 (C, C2 of quinazoline), 160.64 (C, α to OMe of 3-methoxyphenyl), 153.83 (C, C8 of quinazoline), 144.34 (C of triazole), 143.56 (C, C8a of quinazoline), 138.20 (C, C1 of 3-methoxyphenyl), 130.49 (CH, C5 of 3-methoxyphenyl), 125.30 (CH, C6 of quinazoline), 122.96 (CH of triazole), 122.90 (C, C4a of quinazoline), 119.00 (CH, C7 of quinazoline), 114.60 (CH, C4 of 3-methoxyphenyl), 112.88 (CH, C5 of

2-((1-(4-Bromophenyl)-1H-1,2,3-triazol-4-yl)methoxy)-8-methoxyquinazoline (11f). Reaction of 15 (25 mg, 0.11 mmol) with 16f (23.1 mg, 0.01 mmol) gave 11f: 31 mg, 64% yield. mp 144-146 °C; FT-IR (KBr, cm−1): 3061, 3007, 1581, 1491, 1418, 1291, 1172, 1024, 829, 762. 1H NMR (400 MHz, CDCl3): δ 9.24 (s, 1H, H-quinazoline), 8.47 (br t, J 0.6 Hz, 1H, H-triazole), 7.67-7.60 (symmetrical m, 4H of 4-bromophenyl), 7.47 (dd, J 8.1, 1.4 Hz, 1H, H-C7 of quinazoline), 7.42 (dd, J 8.1, 7.5 Hz, 1H, H-C6 of quinazoline), 7.23 (dd, J 7.6, 1.3 Hz, 1H, H-C5 of quinazoline), 5.80 (br d, J 0.6 Hz, 2H, CH2), 4.10 (s, 3H, CH3, OMe). 13C NMR (100 MHz, CDCl3): δ 163.78 (CH, α to N of quinazoline), 161.57 (C, C2 of quinazoline), 153.79 (C, C8 of quinazoline), 144.70 (C of triazole), 143.51 (C, C8a of quinazoline), 136.13 (C, δ to Br), 132.91 (2CH, ortho to Br of 4-bromophenyl), 125.36 (CH, C6 of quinazoline), 122.91 (C, C4a of quinazoline), 122.73 (CH of triazole), 122.34 (C, α to Br), 121.88 (2CH, meta to Br of 4-bromophenyl), 119.07 (CH, C7 of quinazoline), 112.92 (CH, C5 of quinazoline), 61.17 (CH2), 56.23 (CH3, OMe). LC-MS: 412.24 [M]. HRMS–ESI (m/z) [M+Na]+ calcld for C18H1479BrN5NaO2: 343.02231, found 343.0225, [M+K]+ calcld for C18H1479BrKN5O2: 449.99624, found 449.9959.

8-Methoxy-2-((1-phenyl-1H-1,2,3-triazol-4-yl)methoxy)quinazoline (11g). Reaction of 15 (25 mg, 0.11 mmol) with 16g (13.90 mg, 0.01 mmol) gave 11g: 29.1 mg, 74% yield. mp 168-170 °C; FT-IR (KBr, cm−1): 3143, 3070, 3003, 1585, 1484, 1418, 1292, 1171, 1117, 1030, 764, 690. 1H NMR (400 MHz, CDCl3): δ 9.24 (s, 1H, H-quinazoline), 8.48 (s, 1H, H-triazole), 7.76-7.71 (m, 2H, H ortho of Ph), 7.55-7.49 (m, 2H, H meta of Ph), 7.48-7.38 (m, 3H, H-C6 of quinazoline, H-C7 of quinazoline and H para of Ph), 7.23 (dd, J 7.6, 1.3 Hz, 1H, H-C5 of quinazoline), 5.82 (s, 2H, CH2), 4.10 (s, 3H, CH3, OMe). 13C NMR (75 MHz, CDCl3): δ 163.74 (CH, α to N of quinazoline), 161.64 (C, C2 of quinazoline), 153.83 (C, C8 of quinazoline), 144.40 (C of triazole), 143.57 (C, C8a of quinazoline), 137.20 (C ipso of Ph), 129.74 (2CH, meta of Ph), 128.69 (CH, para of Ph), 125.30 (CH, C6 of quinazoline), 122.90 (CH of triazole), 122.85 (C, C4a of quinazoline), 120.52 (2CH, ortho of Ph), 119.01 (CH, C7 of quinazoline), 112.88 (CH, C5 of quinazoline), 61.28 (CH2), 56.22 (CH3, OMe). LC-MS: 333.3 [M]. HRMS–ESI (m/z) [M+Na]+ calcld for C18H15N3NaO2: 356.11179, found 356.1121, [M+K]+ calcld for C18H15KN3O2: 372.08573, found 372.0856.

2-((1-Benzyl-1H-1,2,3-triazol-4-yl)methoxy)-8-methoxyquinazoline (11h). Reaction of 15 (20 mg, 0.093 mmol) with 16h (12.38 mg, 0.093 mmol) gave 11h: 24.2 mg, 74% yield. mp 109-111 °C; FT-IR (KBr, cm−1): 3066, 1579, 1468, 1414, 1280, 1166, 1117, 1002, 816, 742. 1H NMR (300 MHz, CDCl3): δ 9.19 (s, 1H, H-quinazoline), 7.84 (s, 1H, H-triazole), 7.43 (dd, J 8.1, 1.4 Hz, 1H, H-C7 of quinazoline), 7.39 (dd, J 8.1, 7.5 Hz, 1H, H-C6 of quinazoline), 7.37-7.32 (m, 3H, 2H meta and 1H para of benzyl), 7.29-7.23 (m, 2H, 2H ortho of benzyl), 7.18 (dd, J 7.5, 1.3 Hz, 1H, H-C5 of quinazoline), 5.71 (s, 2H, CH2 between O and triazole), 5.52 (s, 2H, CH2 of benzyl), 3.99 (s, 3H, CH3, OMe). 13C NMR (75 MHz, CDCl3): δ 163.63 (CH, α to N of quinazoline), 161.52 (C, C2 of quinazoline), 153.71 (C, C8 of quinazoline), 144.07 (C of triazole), 143.49 (C, C8a of quinazoline), 134.54 (C ipso of Ph), 129.09 (2CH, meta of benzyl), 128.73 (CH, para of benzyl), 128.15 (2CH, ortho of benzyl), 125.24 (CH, C6 of quinazoline), 124.19 (CH of triazole), 122.77 (C, C4a of quinazoline), 118.89 (CH, C7 of quinazoline), 112.72 (CH, C5 of quinazoline), 61.32 (CH2 between O and triazole), 56.12 (CH3, OMe), 54.19 (CH2 of benzyl). LC-MS: 347.37 [M]. HRMS–ESI (m/z) [M+Na]+ calcld for C19H17N5NaO2: 370.12744, found 370.1277, [M+K]+ calcld for C19H17KN5O2: 386.10138, found 386.1013.
9.43 (s, 1H, H-quinazoline), 8.41 (s, 1H, H-triazole), 8.21 (half part of an A2X2 system, 2H ortho to NO2 of 4-nitrobenzyl), 7.62 (dd, J 8.1, 1.3 Hz, 1H, H-C7 of quinazoline), 7.53 (half part of an A2X2 system with small coupling with CH2, 2H meta to NO2 of 4-nitrobenzyl), 7.48 (dd, J 8.0, 7.9 Hz, 1H, H-C6 of quinazoline), 7.40 (dd, J 7.9, 1.2 Hz, 1H, H-C5 of quinazoline), 5.81 (s, 2H, CH2 of 4-nitrobenzyl), 5.55 (s, 2H, CH2 between O and triazole), 3.96 (s, 3H, CH3, OMe). 13C NMR (100 MHz, DMSO-d6): δ 164.22 (CH, α to N of quinazoline), 160.86 (C, C2 of quinazoline), 153.11 (C, C8 of quinazoline), 147.14 (C, α to NO2), 143.28 (C para to NO2 of 4-nitrobenzyl), 142.70 (C of triazole), 142.32 (C, C8a of quinazoline), 128.94 (2CH, meta to NO2 of 4-nitrobenzyl), 125.73 (C of triazole), 125.39 (CH, C6 of quinazoline), 123.79 (2CH, ortho to NO2 of 4-nitrobenzyl), 122.35 (C, C4a of quinazoline), 118.96 (CH, C7 of quinazoline), 113.54 (CH, C5 of quinazoline), 60.09 (CH2 between O and triazole), 55.84 (CH3, OMe), 51.84 (CH2 of 4-nitrobenzyl). LC-MS: 392.37 [M]. HRMS–ESI (m/z) [M+Na]+ calcd for C19H16N2NaO4: 415.11252, found 415.1126, [M+K]+ calcd for C19H16KN2O4: 431.08646, found 431.0861.

2-((1-(2,6-Dichlorobenzyl)-1H-1,2,3-triazol-4-yl) methoxy)-8-methoxyquinoxaline (11j). Reaction of 15 (25 mg, 0.11 mmol) with 16j (23.57 mg, 0.11 mmol) gave 11j: 29.3 mg, 60% yield. mp 186-188 °C; FT-IR (KBr, cm−1): 3002, 1578, 1426, 1268, 1166, 1114, 1016, 758. 1H NMR (300 MHz, CDCl3): δ 9.19 (s, 1H, H-quinazoline), 7.85 (br s, 1H, H-triazole), 7.44 (dd, J 8.2, 1.7 Hz, 1H, H-C7 of quinazoline), 7.42-7.36 (m, 3H, 2H ortho to Cl at 7.38 ppm and H-C6 of quinazoline at 7.40 ppm), 7.29 (dd, J 9.1, 6.8 Hz, 1H, H meta to Cl), 7.19 (dd, J 7.3, 1.7 Hz, 1H, H-C5 of quinazoline), 5.85 (s, 2H, CH2 of 2,6-dichlorobenzyl), 5.70 (d, J 0.6 Hz, 2H, CH2 between O and triazole), 4.03 (s, 3H, CH3, OMe). 13C NMR (75 MHz, CDCl3): δ 163.61 (CH, α to N of quinazoline), 161.50 (C, C2 of quinazoline), 153.73 (C, C8 of quinazoline), 143.62 (C of triazole), 143.53 (C, C8a of quinazoline), 136.85 (2C, α to Cl), 131.05 (CH, meta to Cl), 130.08 (C, β to Cl), 128.87 (2CH, ortho to Cl), 125.23 (CH, C6 of quinazoline), 123.76 (CH of triazole), 122.78 (C, C4a of quinazoline), 118.89 (CH, C7 of quinazoline), 112.71 (CH, C5 of quinazoline), 61.39 (CH2 between O and triazole), 56.19 (CH3, OMe), 49.03 (CH2 of 2,6-dichlorobenzyl). LC-MS: 416.26 [M]. HRMS–ESI (m/z) [M+Na]+ calcd for C19H1535Cl2N3NaO2: 438.04950, found 438.0495, [M+K]+ calcd for C19H1535Cl2KN2O2: 454.02344, found 454.0229.

Cytotoxicity studies

Cell culture. Skin normal fibroblastic cells are purchased from Lonza (Basel, Switzerland), HuH7, Caco-2, MDA-MB-231, HCT116, PC3, MCF7 and NCI-H727 cancer cell lines were obtained from the ECACC collection (Porton Down, UK). Cells are grown at 37°C, 5% CO2 in ECACC recommended media: DMEM for HuH7, MDA-MB-231 and fibroblast, EMEM for MCF7 and CaCo-2, McCoy’s for HCT116 and RPMI for PC3 and NCI-H727. All culture media are supplemented by 10% of FBS, 1% of penicillin-streptomycin and 2 mM glutamine.

Cytotoxic assay. Chemicals are solubilized in DMSO at a concentration of 10 mM (stock solution) and diluted in culture medium to the desired final concentrations. The dose effect cytotoxic assay of chemical is performed at 25 μM. Cells are plated in 96 wells plates (4000 cells/well). Twenty-four hours after seeding, cells are exposed to chemicals. After 48h of treatment, cells are washed in PBS and fixed in cooled 90% ethanol/5% acetic acid for 20 minutes and the nuclei are stained with Hoechst 33342 (B2261 Sigma). Image acquisition and analysis are performed using a Cellomics ArrayScan VTI/HCS Reader (ThermoScientific). The survival percentages are calculated as the percentage of cell number after compound treatment over cell number after DMSO treatment.

Protein kinase assays

Kinase enzymatic activities were assayed in 384-well plates using the ADP-Glo assay kit (Promega, Madison, WI) according to the recommendations of the manufacturer. This assay is a luminescent ADP detection assay that provides a homogeneous and high-throughput screening method to measure kinase activity by quantifying the amount of ADP produced during a kinase reaction. Briefly, the reactions were carried out in a
final volume of 6 µl for 30 min at 30°C in the following buffer: 10 mM MgCl2, 1 mM EGTA, 1 mM DTT, 25 mM Tris-HCl pH 7.5, 50 µg/ml heparin; with either protein or peptide as substrate in the presence of 10 µM ATP. After stopping the kinase reaction, Kinase Detection Reagent was added for one hour at RT. The transmitted signal was then measured using an Envision microplate luminometer (PerkinElmer, Waltham, MA) and expressed in Relative Light Unit (RLU). HsCDK5/p25 (human, recombinant, expressed in bacteria) was assayed on 0.8 µg/µl of histone H1 as substrate. HsCDK9/CyclinT (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed on 0.27 µg/µl of the following peptide: YSPTSPSYSPSYSPSYPSKKK, as substrate. HsGSK3β (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed on 0.010 µg/µl of GS-1 peptide, a GSK-3-selective substrate (YRRAVPPSPSRHSPHQSPEDEEE). HsCK1ε (human, recombinant, expressed by baculovirus in Sf9 insect cells) was assayed on 0.022 µg/µl of the following peptide: RRKHAAIGSPAYSITA (“Sp” stands for phosphorylated serine) as CK1-specific substrate. RnDYRK1A-kd (Rattus norvegicus, kinase domain aa 1 to 499, expressed in bacteria, DNA vector kindly provided by Dr. W. Becker, Aachen, Germany) was assayed on 0.033 μg/µl of the following peptide: KKISGRLSPIMTEQ as substrate. MmCLK1 (from Mus musculus, recombinant, expressed in bacteria) was assayed on 0.027 µg/µl of the following peptide: GRSRSRSRSRSR as substrate. HsPim-1 (human proto-oncogene, recombinant, expressed in bacteria) was assayed on 0.8 µg/µl of histone H1 (Sigma #H5505) as substrate. HsHaspin-kd (human, kinase domain, amino acids 470 to 798, recombinant, expressed in bacteria) was assayed on 0.007 µg/µl of Histone H3 (1-21) peptide (ARTKQTARKSTGGKAPRKQLA) as substrate. Peptide substrates were obtained from Proteogenix (Schiltigheim, France).

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Supplementary Material

Electronic Supplementary Information (ESI) available: cytotoxic studies of the quinazolines 11 plus copies of the ¹H and ¹³C NMR spectra for all new compounds

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