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Short communication

Synthesis of potential Rho-kinase inhibitors based on the chemistry of an original heterocycle: 4,4-Dimethyl-3,4-dihydro-1*H*-quinolin-2-one

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Abstract

A new series of substituted 4,4-dimethyl-3,4-dihydro-1*H*-quinolin-2-one have been prepared via condensation of 3,3-dimethylacryloyl chloride with aniline. Details of synthetic procedures are shown. Our aim was to investigate the potency of our original heterocycle in the inhibition of the Rho-kinase enzyme, known to be of major importance in the cascade reactions leading to arterial hypertension. Biological activity for the seven compounds has been investigated and is presented.

Keywords: Rho-kinase inhibitors; Hypertension; Heterocyclic chemistry

1. Introduction

Hypertension, commonly referred to as ''high blood pressure'', is a medical condition where the blood pressure is chronically elevated. It is one of the most important cardiovascular risk factors (as diabetes mellitus, dyslipidemia and tobacco) all over the world. Twenty percentage of the industrial population is concerned according to the World Health Organization.

This disease is defined by a rise in the blood pressure in the arteries due to the abnormal contraction of the smooth muscle. The contraction being regulated by the cytosolic calcium concentration to which the Rho/Rho-kinase intracellular pathway is associated, designing compounds that inhibit the enzyme seem to be of great interest [1–5]. Different kinds of molecules have already been studied. Two of most effective inhibitors of Rho-kinase are now considered as the research leaders: Y-27632, a pyridine derivative which selectively inhibits smooth-muscle contraction by inhibiting Ca^{2+} sensitization [6–8], and HA-1077, an isoquinolinesulfonamide derivative which has an antispatic effect on the cerebral artery [9–11] (Fig. 1).

Considering these two molecules Takami et al. [12] studied Rho-kinase ligand-binding site. They described it as a three region (A, F and D) pocket. The A region, composing the bottom of the cavity, is flat and possesses an amine function (NH of Met 167) allowing the formation of a hydrogen bond with any heteroatom correctly oriented. The F region is spherical and relatively spacious. Docking simulation indicated that several chemical fragments could occupy this space especially planar linkers such as amide or urea moieties. The third region (D region) being also hydrophobic and cleft-like in shape, substituents should preferably be aromatic (Fig. 2).

Inspired by Takami et al.'s work [13,14], we elaborated new potential Rho-kinase inhibitors investigating the synthesis of 4,4-dimethyl-3,4-dihydro-1*H*-quinolin-2-one [15]. This 6–6 ring system should enable the substrate to settle Rho-kinase's active site twice by the presence of its two hydrogen acceptors. Hopefully thinking of imitating the HA-1077 sequence we

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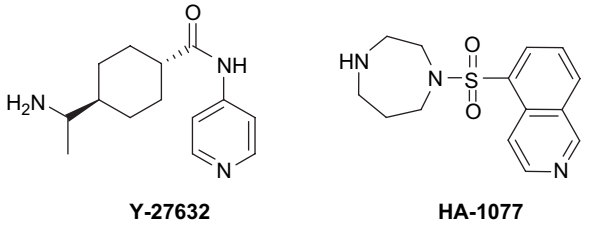


Fig. 1. Two most effective inhibitors of Rho-kinase.

also wished to evaluate the relevance of the same sulfonamide linker.

2. Chemistry

The sulfonyl chloride key intermediate was synthesized in three steps starting from the commercially available aniline. Condensation of 3,3-dimethylacryloyl chloride in pyridine yielded the desired amide **1** [16] which was further cyclized by a Friedel and Crafts reaction [17]. Chlorosulfonylation of the quinolinone **2** was then carried out under rather drastic conditions: direct introduction into pure chlorosulfonic acid (Scheme 1).

The last step to afford the desired sulfonamide derivatives was carried out either in dichloromethane with triethylamine or by using pyridine as the solvent as well as the required base.

It is noteworthy we investigated among others the insertion of 2-methylpiperazine described by Hidaka [18,19] as a more rigid configuration of the homopiperazine moiety of HA-1077 (Scheme 2).

In order to investigate the relevance of the linker's sequence, the synthesis of a molecule containing a switched linker was

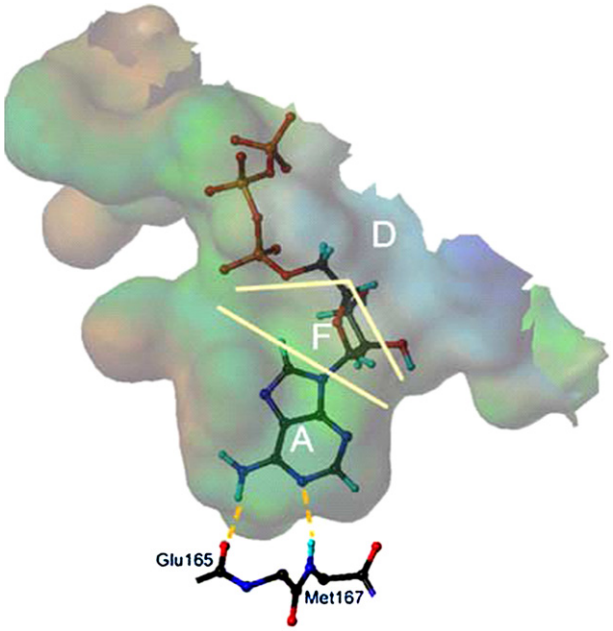
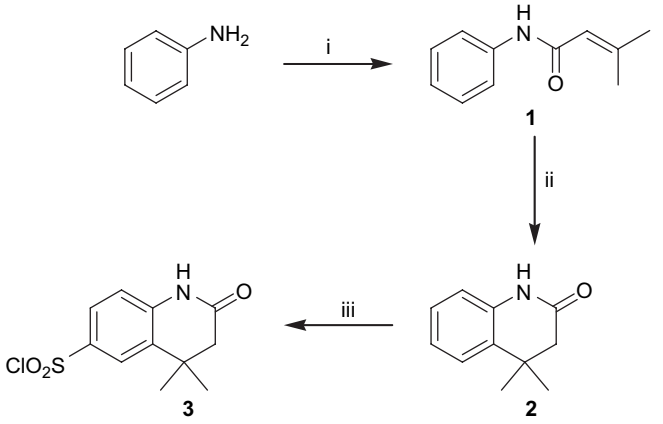


Fig. 2. Ligand-binding pocket of Rho-kinase homology model. A docking model of ATP is shown. Hydrogen bonds between N1 of ATP and HN of Met167, and the amino group at the 6-position and C=O of Glu165 are indicated as dashed lines [12].



Scheme 1. Synthesis of 4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonyl chloride. *Reagents*: (i) 3,3-dimethylacryloyl chloride, pyridine; (ii) $AlCl_3$, DCM; (iii) $ClSO_3H$.

also elaborated as shown in Scheme 3. Classical methylation reaction provided the expected product starting from the amide **2**. Several attempts were necessary to find the right conditions in order to afford the correctly substituted nitro-intermediate **11**. First cyclization of 3-methyl-*N*-(4-nitrophenyl)but-2-enamide was investigated but never we were able to achieve the reaction within the conditions previously elaborated. Nitration of the intermediate **10** was therefore carried out in an inert solvent where the nitronium ion NO_2^+ is directly generated by concentrated nitric acid (Scheme 3).

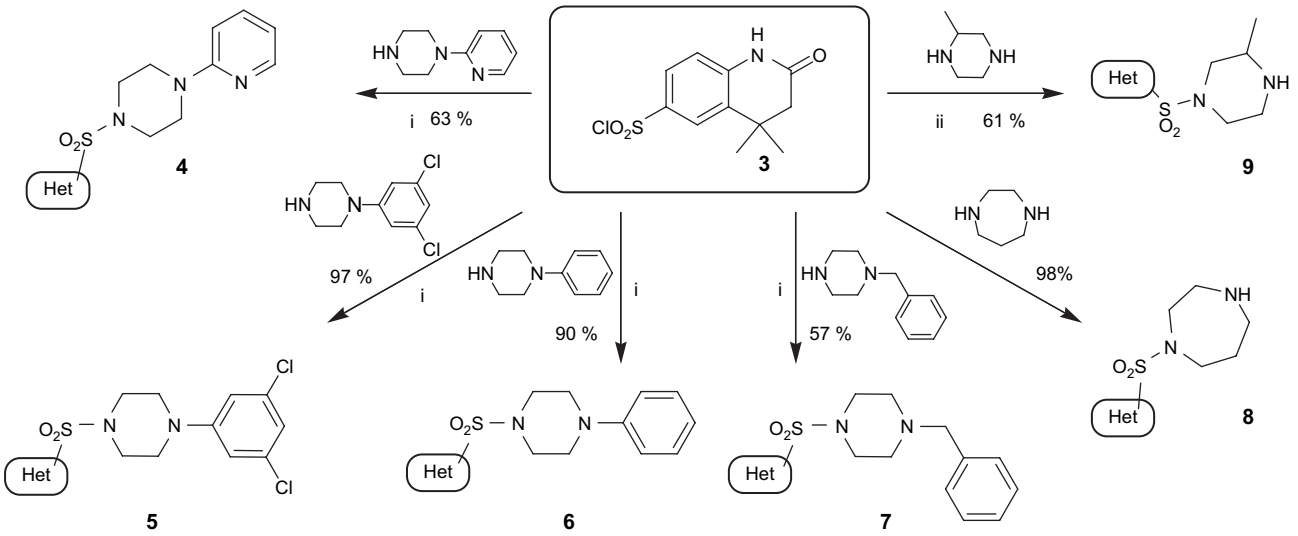
The best conditions were identified as being the addition of nitric acid at 0 °C in dichloromethane. The reduction was next carried out under rather mild conditions with tin(II) chloride dihydrate to afford the amine derivative **12** in a good yield. Treatment with a pretty bulky and flat sulfonyl chloride led to the desired sulfonamide **13** (Scheme 4).

3. Biological activity

The seven synthesized compounds have been studied for possible future biological functions.

3.1. Purification of the recombinant Rho-kinase

The plasmid encoding for human Rho-kinase as a fusion protein with glutathione-*S*-transferase was a generous gift from Prof. Pierre Pacaud (Nantes). The protein was expressed by baculovirus infection of the Tn5 insect cell line. Fifty milliliters of Tn5 cells (1 L of culture) was solubilized in 150 mL of extraction buffer (20 mM Tris–HCl, pH 7.2, 10 mM glycerophosphate, 10 mM $MgCl_2$, 100 μM EGTA, 1 mM DTT, including complete Roche® protease inhibitor cocktail). The suspension was sonicated three times for 15 s in cold. The extract was then centrifuged (10,000*g*, 15 min, 4 °C) and the pellet thereof was re-suspended in the same buffer, sonicated and centrifuged as above. Both supernatants were saved and used as material for the purification of the enzyme. In brief, affinity chromatography was achieved on a glutathione–sepharose column equilibrated in the extraction buffer. Four hundred

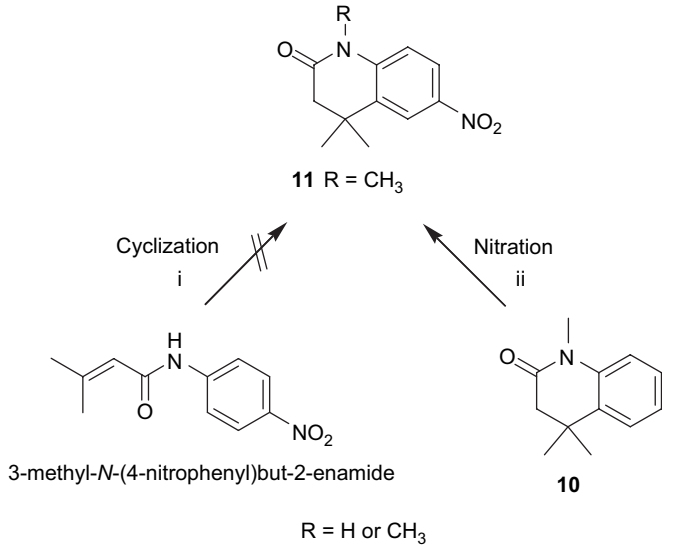


Scheme 2. Synthesis of 4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonamide derivatives. *Reagents*: (i) pyridine; (ii) Et_3N, DCM; (iii) DCM.

milliliters of extraction fluid, obtained above, was chromatographed in a 100 mL column at 4 mL/min flow speed. The column was then developed with a rinsing buffer (extraction buffer supplemented with 150 mM NaCl) and then with an elution buffer (20 mM Tris–HCl, pH 7.2, 10 mM glycerophosphate, 10 mM $MgCl_2$, 100 μM EGTA, 1 mM DTT, and 10 mM GSH). The elution fractions, containing the Rho-kinase activity, were then concentrated on PEG 20000 bead bed, pooled and snap frozen at −80 °C until further use.

3.2. Measurement of Rho-kinase catalytic activity

The phosphorylation of the biotinylated substrate S6 (biotinyl Ala-Lys-Arg-Arg-Arg-Leu-Ser-Ser-Leu-Arg-Ala) was performed in the wells of a 96-well plate coated with streptavidin (Flasplate), in which the enzyme was added with 1 μM ATP containing 0.2 μCi of [γ-^{33}P]ATP in a total volume of 100 μL, with or without the tested compound solubilized in pure DMSO at a final concentration leading to a DMSO concentration not exceeding 2%. The reaction was allowed to continue for 15 min and stopped by eliminating the fluids from the wells. The wells were then washed four times with PBS buffer with 0.01% Tween 20. The radioactivity associated with the substrate in the plates was evaluated by counting in a TopCount-NXT (Packard) counter.



Scheme 3. Study for the achievement of the intermediate **11**. *Reagents and conditions*: (i) $AlCl_3$, DCM; (ii) HNO_3, DCM, 0 °C.

4. Results and discussion

In order to validate the relevance of our tests we also evaluated the IC_{50} of the most effective compound among Takami's molecules. Despite the introduction of various substituents on our original heterocycle and the inversion into the linker's chain none of our compounds showed significant activity for the inhibition of Rho-kinase.

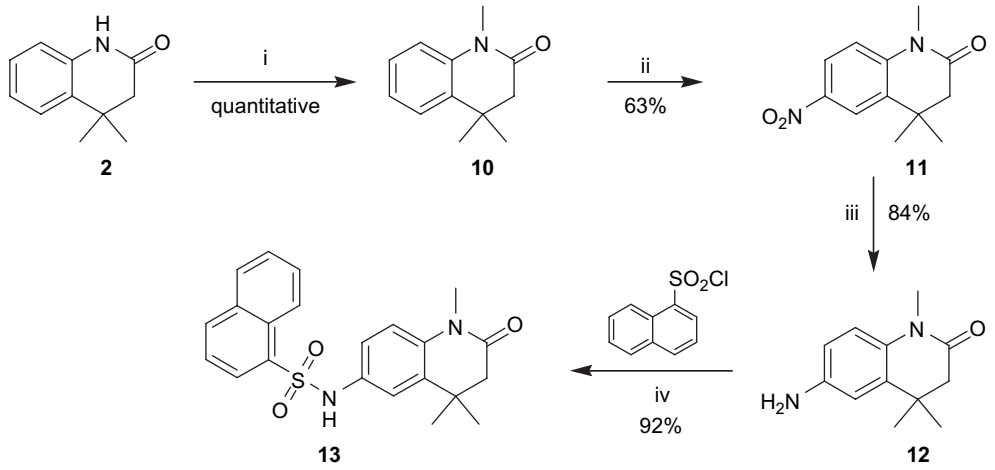
5. Conclusion

To conclude, we described the synthesis of seven analogs of Rho-kinase inhibitors which possess an original heterocyclic skeleton. Unfortunately, among all the molecules synthesized and tested none showed significant activity. Neither our novel 4,4-dimethyl-3,4-dihydro-1*H*-quinolin-2-one heterocycle nor the introduction of the sulfonamide linker improved affinity for the Rho-kinase binding site. Another novel heterocycle is currently under investigation whose results will be reported elsewhere.

6. Experimental

6.1. Chemicals

Starting materials, aniline, 3,3-dimethylacryloyl chloride, chlorosulfonic acid, 1-(2-pyridyl) piperazine, *N*-(3,5-dichlorophenyl)piperazine, *N*-phenylpiperazine, 1-benzylpiperazine, homopiperazine, 2-methylpiperazine, naphthalene-1-sulfonyl



Scheme 4. Synthesis of the switched linker compound. *Reagents and conditions*: (i) MeI, NaH, DMF; (ii) HNO_3, DCM, 0 °C; (iii) $SnCl_2 \cdot 2H_2O$, EtOH; (iv) pyridine.

chloride, tin chloride dihydrate and solvents (chloroform, petroleum ether, diethyl ether, ethyl acetate, dichloromethane, pyridine, heptane, triethylamine, methanol, 2-propanol and ethanol) were all purchased from either Aldrich Ltd., Acros or VWR and were used as received.

6.2. Equipment

Melting points are uncorrected. MS results were recorded on a Perkin–Elmer SCIEX API 3000 spectrometer. Reaction products were purified, unless otherwise notified, by flash chromatography using silica gel (Merck 230–400 mesh). Analytical TLC was carried out on silica gel F_{254} plates. All anhydrous reactions were performed in oven-dried glassware under an atmosphere of argon.

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker 200 MHz using $CDCl_3$ as a solvent. The solvent characteristic signals were observed, respectively, as a singlet at 7.26 ppm and as a triplet at 77.1 ppm.

6.3. Compound synthesis and analyses

6.3.1. General procedure for the preparation of 4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonyl chloride: synthesis of compounds *1–3*

Under an atmosphere of argon, aniline (5 g, 53.7 mmol) was dissolved in dry pyridine (15 mL), cooled down to 0 °C and treated with 3,3-dimethylacryloyl chloride (7.17 mL, 64.4 mmol). After 1 h at room temperature, the reaction was completed. A solution of HCl 1 N was added and the resulting orange precipitate was filtered on Büchner filter and washed with water and heptane. Recrystallisation was then carried out in 2-propanol and 3-methyl-but-2-enoic acid phenylamide **1** was isolated as a white solid.

Yield 95%. M.p. 129–130 °C (lit. 129–130 °C [7]). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.90 (d, 3H, $J_{11-9} = 0.9$ Hz, H_{11}); 2.21 (d, 3H, $J_{12-9} = 0.9$ Hz, H_{12}); 5.70 (t, 1H, $J_{9-11} = J_{9-12} = 0.9$ Hz, H_9); 7.07 (t, 1H, $J_{4-3} = J_{4-5} = 7.5$ Hz, H_4); 7.15 (s, 1H, N*H*); 7.30 (t, 2H, $J_{3-4} = J_{3-2} = J_{5-4} = J_{5-6} = 7.5$ Hz, $H_3 + H_5$); 7.52 (d, 2H, $J_{2-3} = J_{6-5} = 7.5$ Hz, $H_2 + H_6$). ^{13}C NMR ($CDCl_3$) δ (ppm): 20.4 (C_{11} or C_{12}); 27.8 (C_{12} or C_{11}); 119.0 (C_9); 120.2 ($C_2 + C_6$); 124.4 (C_4); 129.4 ($C_3 + C_5$); 138.6 (C_1); 153.9 (C_{10}); 165.5 (C_8). MS (IC): $m/z = 175$ [M].

Under an inert atmosphere, a solution of 3-methyl-but-2-enoic acid phenylamide **1** (5 g, 28.5 mmol) in anhydrous dichloromethane (50 mL) was cooled down to 0 °C. Aluminum chloride (15.2 g, 114 mmol) was added portionwise for at least 1 h period of time and the mixture was allowed to warm to room temperature. The reaction was completed within the hour. The mixture was then poured onto ice and the product was extracted with diethyl ether. The organic layers were combined and successively washed with water, a saturated solution of sodium hydrogenocarbonate and a saturated solution of sodium chloride. They were then dried over $MgSO_4$ and the solvents were removed under reduced pressure. Recrystallisation in absolute ethanol provided 4,4-dimethyl-3,4-dihydro-1*H*-quinolin-2-one **2** as a white solid.

Yield 90%. M.p. 111–112 °C (lit. 116 °C [8]). ^{1}H NMR ($CDCl_3$) δ (ppm): 1.33 (s, 6H, $2 \times CH_3$); 2.50 (s, 2H, H_3); 6.83 (dd, 1H, $J_{5-6} = 7.6$ Hz, $J_{5-7} = 1.3$ Hz, H_5); 7.05 (ddd, 1H, $J_{7-6} = 7.5$ Hz, $J_{7-8} = 7.5$ Hz, $J_{7-5} = 1.3$ Hz, H_7); 7.18 (dd, 1H, $J_{6-5} = 7.6$ Hz, $J_{6-7} = 7.5$ Hz, H_6); 7.29 (d, 1H, $J_{8-7} = 7.5$ Hz, H_8); 8.84 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 28.1 ($2 \times CH_3$); 34.4 (C_4); 45.7 (C_3); 116.2 (C_8); 124.0 (C_6); 124.9 (C_5); 127.9 (C_7); 132.9 (C_a); 136.2 (C_b); 171.5 (C_2). MS (IC): $m/z = 175$ [M].

Chlorosulfonic acid (0.38 mL, 5.71 mmol) was cooled down to 0 °C under an inert atmosphere. 4,4-Dimethyl-3,4-dihydro-1*H*-quinolin-2-one **2** was added portionwise over a 15 min period. The reaction was allowed to warm to room temperature and stirred for 1 h. The mixture was then poured onto ice and extracted with ethyl acetate. The organic layer was successively washed with a saturated solution of sodium hydrogenocarbonate and a saturated solution of sodium chloride. After drying the organic extract over anhydrous $MgSO_4$, evaporation of the solvent afforded the desired

4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonyl chloride **3** as a beige solid used without further purification.

Yield 70%. M.p. 177–178 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.45 (s, 6H, $2 \times CH_3$); 2.63 (s, 2H, H_3); 7.07 (d, 1H, $J_{8-7} = 8.4$ Hz, H_8); 7.90–7.98 (m, 2H, $H_5 + H_7$); 9.55 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.9 ($2 \times CH_3$); 34.8 (C_4); 44.9 (C_3); 116.9 (C_8); 124.6 (C_5); 127.9 (C_7); 134.2 (C_6); 139.2 (C_b); 142.5 (C_a); 171.6 (C_2). MS (IC): $m/z = 274$ [M + 1].

6.3.2. General procedure for the preparation of 4,4-dimethyl-2-oxo-1,2,3,4-tetrahydroquinoline-6-sulfonamide derivatives **4–9**

*6.3.2.1. General procedure for compounds **4–7** [20].* Under an argon atmosphere, the appropriate amine (0.36 mmol) was dissolved in dry pyridine (3 mL). The sulfonyl chloride derivative **3** (0.1 g, 0.36 mmol) was added portionwise and the reaction mixture was allowed to stir at room temperature until the reaction was completed (0.5–4 h). Then water was added and the product was extracted with ethyl acetate. The organic layers were washed several times with a solution of HCl 1 N and finally with a saturated solution of sodium chloride. The extract was dried over anhydrous $MgSO_4$ and solvents were removed under reduced pressure. Recrystallisation in 2-propanol afforded the desired sulfonamides **4–7**.

*6.3.2.1.1. 4,4-Dimethyl-6-(4-(pyridin-2-yl)piperazin-1-ylsulfonyl)-3,4-dihydroquinolin-2(1H)-one **4**.* White solid. Yield 63%. M.p. 232–233 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.40 (s, 6H, $2 \times CH_3$); 2.57 (s, 2H, H_3); 3.12–3.17 (m, 4H, H_{14}); 3.66–3.71 (m, 4H, H_{13}); 6.56–6.67 (m, 2H, $H_{17} + H_{19}$); 6.93 (d, 1H, $J_{8-7} = 8.2$ Hz, H_8); 7.43–7.51 (m, 1H, H_{18}); 7.60 (dd, 1H, $J_{7-8} = 8.2$ Hz, $J_{7-5} = 1.9$ Hz, H_7); 7.66 (d, 1H, $J_{5-7} = 1.9$ Hz, H_5); 8.13–8.15 (m, 1H, H_{20}); 8.92 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 28.0 ($2 \times CH_3$); 34.6 (C_4); 45.1 (C_{13} or C_{14}); 45.2 (C_{14} or C_{13}); 46.2 (C_3); 107.6 (C_{17}); 114.5 (C_{19}); 116.4 (C_8); 125.1 (C_5); 128.2 (C_7); 130.5 (C_6); 133.7 (C_b); 138.1 (C_{18}); 140.5 (C_a); 148.4 (C_{20}); 159.1 (C_{16}); 171.1 (C_2). MS (IC): $m/z = 400$ [M].

*6.3.2.1.2. 6-(4-(3,5-Dichlorophenyl)piperazin-1-ylsulfonyl)-4,4-dimethyl-3,4-dihydroquinolin-2(1H)-one **5**.* Beige solid. Yield 97%. M.p. 218–219 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.42 (s, 6H, $2 \times CH_3$); 2.59 (s, 2H, H_3); 3.18–3.29 (m, 8H, $4 \times CH_2$); 6.73 (d, 1H, $J_{19-17} = J_{19-21} = 8.9$ Hz, H_{19}); 6.97 (d, 1H, $J_{8-7} = 8.2$ Hz, H_8); 7.31 (d, 2H, $J_{17-19} = J_{21-19} = 8.9$ Hz, $H_{17} + H_{21}$); 7.65 (dd, 1H, $J_{7-8} = 8.2$ Hz, $J_{7-5} = 2.0$ Hz, H_7); 7.72 (d, 1H, $J_{5-7} = 2.0$ Hz, H_5); 8.70 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 28.0 ($2 \times CH_3$); 34.6 (C_4); 45.1 (C_3); 46.1 (C_{13}); 49.1 (C_{14}); 116.5–116.7 ($C_{17} + C_{21}$); 118.6 (C_{19}); 124.0 (C_a); 125.0 (C_5); 128.2 (C_7); 130.3 (C_6); 131.0 (C_8); 133.4–133.7 ($C_{18} + C_{20}$); 140.7 (C_b); 150.3 (C_{16}); 171.8 (C_2). MS (IC): $m/z = 469$ [M + 1].

*6.3.2.1.3. 4,4-Dimethyl-6-(4-phenylpiperazin-1-ylsulfonyl)-3,4-dihydroquinolin-2(1H)-one **6**.* Beige solid. Yield 90%. M.p. 224–225 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.42 (s, 6H, $2 \times CH_3$); 2.60 (s, 2H, H_3); 3.25 (dd, 8H, $J_{13a-14a} = 15.3$ Hz, $J_{13a-14b} = 5.6$ Hz, $H_{13} + H_{14}$); 6.90–6.98 (m, 3H, $J_{17-18} = J_{21-20} = 8.5$ Hz, $H_{17} + H_{19} + H_{21}$); 7.02 (d, 1H, $J_{8-7} = 8.2$ Hz, H_8); 7.30 (dd, 2H, $J_{18-17} = J_{20-21} = 8.5$ Hz, $J_{18-19} = J_{20-19} = 7.4$ Hz, $H_{18} + H_{20}$); 7.67 (dd, 1H, $J_{7-8} = 8.2$ Hz, $J_{7-5} = 1.9$ Hz, H_7); 7.72 (d, 1H, $J_{5-7} = 1.9$ Hz, H_5). ^{13}C NMR ($CDCl_3$) δ (ppm): 28.0 ($2 \times CH_3$); 34.6 (C_4); 45.1 (C_3); 46.4 (C_{13}); 49.6 (C_{14}); 116.6 (C_{19}); 117.3 ($C_{17} + C_{21}$); 121.3 (C_8); 125.0 (C_5); 128.3 (C_7); 129.7 ($C_{18} + C_{20}$); 130.5 (C_6); 133.7 (C_a); 140.6 (C_b); 151.0 (C_{16}); 171.7 (C_2). MS (IC): $m/z = 399$ [M].

*6.3.2.1.4. 6-(4-Benzylpiperazin-1-ylsulfonyl)-4,4-dimethyl-3,4-dihydroquinolin-2(1H)-one **7**.* Beige solid. Yield 57%. M.p. 95–96 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.40 (s, 6H, $2 \times CH_3$); 2.55–2.60 (m, 6H, $H_3 + H_{14}$); 3.04–3.09 (m, 4H, H_{13}); 3.53 (s, 2H, H_{16}); 6.97 (d, 1H, $J_{8-7} = 8.2$ Hz, H_8); 7.29–7.33 (m, 5H, H_{18}–H_{22}); 7.61 (dd, 1H, $J_{7-8} = 8.2$ Hz, $J_{7-5} = 1.9$ Hz, H_7); 7.68 (d, 1H, $J_{5-7} = 1.9$ Hz, H_5); 9.03 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 28.0 ($2 \times CH_3$); 34.6 (C_4); 45.1 (C_3); 46.4 (C_{13}); 52.5 (C_{14}); 63.1 (C_{16}); 116.4 (C_8); 125.1 (C_5); 127.8 (C_{20}); 128.2 (C_7); 128.8 ($C_{19} + C_{21}$); 129.6 ($C_{18} + C_{22}$); 130.6 (C_6); 133.6 (C_a); 137.7 (C_{17}); 140.4 (C_b); 171.4 (C_2). MS (IC): $m/z = 413$ [M].

*6.3.2.2. 6-(1,4-Diazepan-1-ylsulfonyl)-4,4-dimethyl-3,4-dihydroquinolin-2(1H)-one **8**.* Under an inert atmosphere, homopiperazine (73 mg, 0.73 mmol) was dissolved in dry dichloromethane (5 mL) and the reaction mixture was cooled down to 0 °C. The sulfonyl chloride **3** (0.1 g, 0.36 mmol) was also dissolved in dry dichloromethane (5 mL), cooled down to 0 °C and canulated over the homopiperazine solution. The reaction mixture was allowed to warm to room temperature and stirred for 0.5 h. After removal of the solvent under reduced pressure, the crude product was purified by column chromatography on silica gel (DCM/MeOH).

Beige solid. Yield 98%. M.p. 177–178 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.41 (s, 6H, $2 \times CH_3$); 1.76 (s, 1H, N*H*); 1.84–1.90 (m, 2H, H_{14}); 2.57 (s, 2H, H_3); 2.94–3.04 (m, 4H, $H_{15} + H_{17}$); 3.34–3.45 (m, 4H, $H_{13} + H_{18}$); 6.95 (d, 1H, $J_{8-7} = 8.3$ Hz, H_8); 7.66 (dd, 1H, $J_{7-8} = 8.3$ Hz, $J_{7-5} = 2.0$ Hz, H_7); 7.75 (d, 1H, $J_{5-7} = 2.0$ Hz, H_5); 8.87 (s, 1H, N*H*). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.8 ($2 \times CH_3$); 34.6 (C_4); 41.6 (C_{14}); 45.0 (C_3); 46.2 (C_{13}); 48.3 (C_{15}); 50.1 (C_{17}); 51.5 (C_{18}); 116.8 (C_8); 124.0 (C_5); 127.5 (C_7); 132.5 (C_6); 133.7 (C_a); 141.9 (C_b); 170.4 (C_2). MS (IC): $m/z = 338$ [M + 1].

*6.3.2.3. 4,4-Dimethyl-6-(2-methylpiperazin-1-ylsulfonyl)-3,4-dihydroquinolin-2(1H)-one **9** [21,22].* Under an inert atmosphere, 2-methylpiperazine (73 mg, 0.73 mmol) was dissolved in dry dichloromethane (10 mL) and triethylamine (0.1 mL, 0.73 mmol) was added. The mixture was cooled down to 0 °C. The sulfonyl chloride **3** (0.1 g, 0.36 mmol) was also dissolved in dichloromethane (10 mL) and the solution was cooled down using an ice/water bath. The sulfonyl chloride solution was canulated over piperazine and base mixture. After 3 h at 0 °C, the reaction was completed and water was added. The product was extracted with dichloromethane and the combined organic layers were successively washed

with water and a saturated solution of sodium chloride, dried over anhydrous $MgSO_4$ and solvents were evaporated. The crude product was purified by column chromatography on silica gel (DCM/MeOH).

Beige solid. Yield 61%. M.p. 198–199 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.08 (d, 3H, $J_{13'-13} = 6.3$ Hz, $H_{13'}$); 1.41 (s, 6H, $2 \times CH_3$); 1.94 (t, 1H, $J_{17a-16a} = J_{17a-16b} = 10.6$ Hz, H_{17a}); 2.33 (dt, 1H, $J_{16a-17a} = J_{16a-17b} = 10.6$ Hz, $J_{16a-16b} = 4.2$ Hz, H_{16a}); 2.58 (s, 2H, H_3); 2.92–3.04 (m, 3H, $H_{13} + H_{14}$); 3.62–3.67 (m, 2H, $H_{16b} + H_{17b}$); 6.98 (d, 1H, $J_{8-7} = 8.2$ Hz, H_8); 7.62 (dd, 1H, $J_{7-8} = 8.2$ Hz, $J_{7-5} = 1.9$ Hz, H_7); 7.68 (d, 1H, $J_{5-7} = 1.9$ Hz, H_5); 9.07 (s, 1H, NH). ^{13}C NMR ($CDCl_3$) δ (ppm): 19.6 ($C_{13'}$); 27.7 ($2 \times CH_3$); 34.3 (C_4); 44.8 (C_3); 45.3 (C_{14}); 46.2 (C_{16}); 50.3 (C_{13}); 52.8 (C_{17}); 116.2 (C_8); 124.7 (C_5); 127.9 (C_7); 130.3 (C_6); 133.3 (C_a); 140.1 (C_b); 171.1 (C_2). MS (IC): $m/z = 338$ [M + 1].

6.3.3. General procedure for the preparation of N-(1,4,4-trimethyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl) naphthalene-1-sulfonamide: synthesis of compounds ***10–13***

Under an argon atmosphere, a solution of 4,4-dimethyl-3,4-dihydro-1H-quinolin-2-one **2** (0.525 g, 3 mmol) in dry DMF (10 mL) was cooled down to 0 °C and canulated dropwise over a suspension of sodium hydride, a 60% dispersion in mineral oil (0.120 g, 3 mmol), in dry DMF (5 mL) at 0 °C. After 5 min, methyl iodide (0.28 mL, 4.5 mmol) was added dropwise to the mixture still at 0 °C. The reaction mixture was allowed to warm up to room temperature and stirring was maintained for 2 h. After hydrolysis, the product was extracted with ethyl acetate and the combined organic layers were washed several times with a saturated solution of ammonium chloride, dried over anhydrous $MgSO_4$ and solvents were removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EP/DCM) to provide the desired 1,4,4-trimethyl-3,4-dihydroquinolin-2(1H)-one **10** as a yellow oil [15].

Yield 99%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.33 (s, 6H, $2 \times CH_3$); 2.55 (s, 2H, H_3); 3.43 (s, 3H, $H_{1'}$); 7.06–7.15 (m, 2H, $H_7 + H_8$); 7.27–7.36 (m, 2H, $H_5 + H_6$). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.5 ($2 \times CH_3$); 29.5 ($C_{1'}$); 33.1 (C_4); 46.0 (C_3); 115.1 (C_8); 123.4 (C_6); 124.3 (C_5); 127.4 (C_7); 134.8 (C_a); 139.4 (C_b); 169.7 (C_2). MS (IC): $m/z = 189$ [M].

A solution of 1,4,4-trimethyl-3,4-dihydroquinolin-2(1H)-one **10** in dry dichloromethane was cooled down to 0 °C under an argon atmosphere. Extra pure nitric acid was added carefully and the mixture was allowed to stir at 0 °C for 3 h before hydrolysis over ice. The product was extracted with dichloromethane and the combined organic layers were successively washed with a solution of sodium hydrogenocarbonate and sodium chloride, and dried over $MgSO_4$. The solvents were then evaporated. The crude product was purified by column chromatography on silica gel (EP/AcOEt) to afford 1,4,4-trimethyl-6-nitro-3,4-dihydroquinolin-2(1H)-one **11** as a yellow solid.

Yield 68%. M.p. 130–131 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.36 (s, 6H, $2 \times CH_3$); 2.58 (s, 2H, H_3); 3.44 (s, 3H, $H_{1'}$); 7.09 (d, 1H, $J_{8-7} = 9.5$ Hz, H_8); 8.14–8.19 (m, 2H, $H_5 + H_7$). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.6 ($2 \times CH_3$); 30.2 ($C_{1'}$); 33.7 (C_4); 45.6 (C_3); 115.4 (C_7); 120.7 (C_5); 124.0 (C_8); 136.0 (C_b); 143.6 (C_a); 145.1 (C_6); 169.6 (C_2). MS (IC): $m/z = 234$ [M].

Tin chloride dihydrate (0.482 g, 2.14 mmol) was added to a solution of **11** (0.1 g, 0.43 mmol) in absolute ethanol (10 mL). A few milliliters of ethyl acetate was added to activate tin chloride and the reaction mixture was refluxed for 3 h. The solvents were then removed under reduced pressure and the crude residue was taken up in a solution of sodium hydroxide 10% and extracted with ethyl acetate. The combined organic layers were washed with a saturated solution of sodium chloride and the solvent was evaporated. The product was purified by column chromatography on silica gel (DCM) to provide 6-amino-1,4,4-trimethyl-3,4-dihydroquinolin-2(1H)-one **12** as a yellow oil.

Yield 88%. ^{1}H NMR ($CDCl_3$) δ (ppm): 1.25 (s, 6H, $2 \times CH_3$); 2.45 (s, 2H, H_3); 3.34 (s, 3H, $H_{1'}$); 3.58 (s, 2H, NH_2); 6.57 (dd, 1H, $J_{7-8} = 8.4$ Hz, $J_{7-5} = 2.6$ Hz, H_7); 6.66 (d, 1H, $J_{5-7} = 2.6$ Hz, H_5); 6.81 (d, 1H, $J_{8-7} = 8.4$ Hz, H_8). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.7 ($2 \times CH_3$); 29.8 ($C_{1'}$); 33.4 (C_4); 46.4 (C_3); 111.9 (C_5); 113.8 (C_7); 116.5 (C_8); 131.9 (C_b); 136.5 (C_a); 142.6 (C_6); 169.4 (C_2). MS (IC): $m/z = 204$ [M].

Under an inert atmosphere, to a solution of **12** (0.06 g, 0.29 mmol) in dry pyridine (3 mL) was added naphthalene-1-sulfonyl chloride (0.067 g, 0.29 mmol). After 0.5 h at room temperature, water was added to the reaction mixture and the product was extracted with ethyl acetate. The organic layer was then washed successively with a solution of HCl 1 N and a saturated solution of sodium chloride. Recrystallisation in 2-propanol afforded the desired N-(1,4,4-trimethyl-2-oxo-1,2,3,4-tetrahydroquinolin-6-yl)naphthalene-1-sulfonamide **13** as an orange solid.

Yield 92%. M.p. 218–219 °C. ^{1}H NMR ($CDCl_3$) δ (ppm): 0.99 (s, 6H, $2 \times CH_3$); 2.34 (s, 2H, H_3); 3.27 (s, 3H, $H_{1'}$); 6.62 (d, 1H, $J_{5-7} = 2.4$ Hz, H_5); 6.75 (d, 1H, $J_{8-7} = 8.6$ Hz, H_8); 6.85 (dd, 1H, $J_{7-8} = 8.6$ Hz, $J_{7-5} = 2.4$ Hz, H_7); 7.45 (dd, 1H, $J_{15-16} = 8.2$ Hz, $J_{15-14} = 7.5$ Hz, H_{15}); 7.65 (dddd, 2H, $J_{19-18} = 7.6$ Hz, $J_{20-21} = 7.2$ Hz, $J_{20-18} = 1.8$ Hz, $J_{19-21} = 1.3$ Hz, $H_{19} + H_{20}$); 7.95 (dd, 1H, $J_{18-19} = 7.6$ Hz, $J_{18-20} = 1.8$ Hz, H_{18}); 8.04 (d, 1H, $J_{16-14} = 8.0$ Hz, H_{16}); 8.14 (dd, 1H, $J_{21-20} = 7.2$ Hz, $J_{21-19} = 1.3$ Hz, H_{21}); 8.65 (d, 1H, $J_{14-16} = 8.0$ Hz, H_{14}). ^{13}C NMR ($CDCl_3$) δ (ppm): 27.4 ($2 \times CH_3$); 29.8 ($C_{1'}$); 33.2 (C_4); 45.8 (C_3); 115.9 (C_5); 120.2 (C_7); 123.0 (C_8); 124.5 (C_{20}); 124.6 (C_{19}); 127.4 (C_{14}); 128.7 (C_b); 128.9 (C_{15}); 129.6 (C_{21}); 131.0 (C_{18}); 131.6 (C_{22}); 134.3 (C_{17}); 134.5 (C_6); 135.0 (C_{16}); 136.0 (C_a); 137.8 (C_{13}); 169.5 (C_2). MS (IC): $m/z = 394$ [M].

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