Design, synthesis and evaluation of novel 2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one based chalcones as cytotoxic agents

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Abstract

We designed and synthesised a series of novel chalcones, incorporating the heterocyclic framework of 2,2-dimethyl-2,3-dihydro-4(1H)-quinolinone, which was prepared via Sonogashira coupling of a substituted orthoaniline under aqueous conditions using Pd catalysis followed by acid-mediated cyclisation. The compounds were screened against the NCI-N87 and DLD-1 cancer cell lines, with most compounds showing low micromolar cytotoxic activity.

Keywords: Cancer research, Pharmaceutical chemistry

1. Introduction

Tetrahydroquinolines and their derivatives have long been of interest to medicinal chemists and pharmacologists, and several examples of this broad class possess useful activity in clinic [1]. Both natural products and synthetic compounds have been
developed commercially, for primarily antimicrobial, cardiovascular and neurological properties. A subset of the diverse quinolone collective is the 2,3-dihydro-4(1H)-quinolinones, of particular synthetic interest in the preparation of aza-analogues of flavanones, thus incorporating a 2-phenyl substitution. We were particularly interested in the 2,2-dimethyl-2,3-dihydro-4(1H)-quinolinones, which are less common and little studied. However, certain complex natural products (Fig. 1), such as the cyclopiamine (1) and Viola alkaloids (2) contain this structural fragment, and the motif has also been incorporated into compounds synthesised *de novo*, for example (3), designed as PAK (p21-activated kinase) inhibitors for the treatment of CNS disorders, or (4), chromenoquinolines intended as a steroid hormone receptor modulator [2, 3, 4, 5].

Hamada et al [6] described a series of quinoylacetic acids, illustrated by (5), which inhibited acetic acid-induced writhing in mice, and inhibited carrageenan-induced oedema in rats. These compounds all possessed an acetic acid ester side chain at C6. More recent publications focus on the cytotoxic potential of quinolinones, notably simple halo-substituted examples such as (6), which exhibited cytotoxicity, most notably against the RPMI human myeloma cell line with IC50s of 13.0–17.5 μM [7].

Further to such reports of cytotoxic activity, we became interested in the concept of incorporating the 2,2-dimethyl-2,3-dihydro-4(1H)-quinolinone motif within a more established cytotoxic pharmacophore, namely that of the chalcones. Chalcones, with their signature α,β-unsaturated ketone system, are a group of compounds studied for many diverse applications, and are considered privileged structures [8]. Many thousands of these compounds are known, and they may be further sub-divided into...
various structural classes and analogues. A subset of the chalcone derivatives are hybridised systems, though incorporation of another heterocycle. Some of these compounds are depicted in Fig. 2. Examples include the chromanochalcones, which have been utilised as intermediates both in the synthesis of natural products such as the parvisoflavones [9], and synthetic analogues thereof, such as aurone and flavanone derivatives, through oxidation under microwave irradiation [10]. Chromanochalcones have also been employed in the preparation of antimicrobial pyrimidines [11]. Related spirochromanone-chalcones have been shown to possess antitubercular activity [12]. Some nitrogenated derivatives have been evaluated for anti-cancer activity. Chinthala et al prepared a series of chromanochalcone-triazole derivatives and evaluated their anticancer activity on several cell lines [13]. The most promising example displayed anticancer activity across all cell lines tested, with IC$_{50}$s ranging from 35 to 66 µM. Noting these results, and the observation that potential anti-cancer activity has also been demonstrated among thiophenyl, indolyl, quinolyl and quinoxaline hybridised examples [14], we designed our target molecules, as to our knowledge, no 4-quinolinoyl analogues have been described to date. Our target compounds featured a 2,2-dimethyl substitution while remaining unsubstituted at C3. Of the diverse aromatic substitution patterns that may be envisaged on the parent skeleton, we focussed on those bearing a 6-substitution, inspired by their chromanochalcone analogues.

2. Results & discussion

2.1. Chemistry

Synthetic approaches towards the 4-quinolinone skeleton include the approach of Politanskaya [7], who used a p-toluene sulfonic acid-catalyzed cyclocondensation reaction of o-alkynylanilines, in turn prepared by the PdCl$_2$(PPh$_3$)$_2$—catalysed Sonogashira reaction of iodoanilines with 2-methylbut-3-yn-2-ol. Similarly, Pisansechi et al [15] used o-halo or o-trifl oxy-N-acetylanilines in the coupling step and

![Fig. 2. Diversity among hybridised chalcone structures in the literature.](https://doi.org/10.1016/j.heliyon.2018.e00767)
hydrochloric acid to induce cyclisation. Such reactions are believed to involve acid-catalyzed tandem Rupe rearrangement-Donnelly-Farrell ring closure. Alternatively, tertiary quinolinones may be accessed through a tandem Michael-SNAr reaction [16]. A more indirect route via initial cyclization of N-(1,1-dimethylpropargyl) anilines, using cuprous chloride in refluxing toluene, yields 6-substituted-2,2-dimethyl-1,2-dihydroquinolines, which are further oxidised [17]. A similar approach from 2,2-dimethyl-1,2,3,4-tetrahydroquinolines using sequential Boc protection, BH$_3$/H$_2$O$_2$ mediated 4-hydroxylation and oxidation was discussed by Clarke [18]. An alternate method which generates carbamate derivatives of quinolinones features a Bi(OTf)$_3$-catalyzed Meyer–Schuster rearrangement of propargyl alcohols followed by 1,4-addition of the resulting vinyl ketone [19]. Our synthetic approach is shown in Fig. 3, and was primarily based on the work of Polintaskaya and Pisaneschi, although using non-copper based Sonogashira coupling.

Commercial 4-acetylaniline (7) was regioselectively iodinated following a literature procedure [20] in good yield to afford (8). The literature reports that the coupling reaction proceeds more smoothly with protection of the aniline nitrogen, so this was accomplished quantitatively using acetic anhydride under acid catalysis. The desired acetamide (9), a white crystalline solid, was subjected to Sonogashira coupling with 2-methylbut-3-yn-2-ol. Classical Sonogashira couplings employ catalytic palladium alongside a metal co-catalyst and a base, but are air-sensitive, and competing side reactions can be problematic. With increasing emphasis on ‘green’ approaches, we were interested in the work of B. Liang et al [21], who published a mild protocol for copper-free Sonogashira coupling of aryl iodides with terminal acetylenes in water under aerobic conditions. The authors used 1 mol % PdCl$_2$ as catalyst, in the presence of pyrrolidine at room temperature or under gentle heating. A year later, Y.
Liang [22] et al documented that PdCl₂(PPh₃)₂ combined with TBAF as a promoter could allow cross coupling of terminal alkynes with diverse ArX species (X = I, Br or Cl), and that with iodides, the reaction was rapid and quantitative. Intrigued by these reports, we first attempted the coupling of iodoacetamide (9) with 2-methylbut-3-yn-2-ol. Interestingly, we found that use of the PdCl₂(PPh₃)₂/TBAF system, while resulting in 40% of desired product (10), also resulted in the formation of indole (11).

Indeed, cyclization of 2-alkynylanilines is an oft-used approach to the synthesis of indoles, as typified by the work of Politanskaya et al [23], who used KOH as optimal base to effect the cyclisation step, while PdCl₂ had no effect on substrates comparable to our butynol. In our hands, reaction of (9) with 2-methylbut-3-yn-2-ol using PdCl₂ and pyrrolidine in aqueous conditions obtained (10), without any concomitant formation of undesired indole. Cyclisation of (10) was effected in 55% yield using HCl to afford the novel quinolinone (12). Choice of acid for these cyclisations appears important; we also cyclised the benzylic alcohol and amine analogues of acetophenone (9); with the former, use of alcohol as co-solvent resulted in the isolation of methyl ethers, while with trifluoroacetic acid, reaction of the latter resulted in acid-catalysed rearrangement without ring formation. Monitoring of the cyclisation reaction was facilitated by the strongly fluorescent nature of the quinolinone (12) formed. Indeed, an analogous approach was undertaken by Majumdar et al [24], who used acid coupled with conventional or microwave heating in the cyclisation of tricyclic pyrano [3,2-f]quinolones intended as fluorophores in mammalian cell imaging. Finally, Claisen-Schmidt condensation of (12) under basic conditions afforded the desired quinlinoylchalcones (13a-l).

2.2. Pharmacological activity

We screened selected compounds from our panel for cytotoxic activity against two cancer cell lines one gastric (NCI-N87) and one colorectal (DLD-1), and the results are listed in Table 1. The unsubstituted compound (13a), although active against both cell lines, was more active against the N87 cell line. Introduction of electron

### Table 1. Cytotoxic activity of selected compounds in DLD-1 and N87 cells.

| Compound | R¹ | IC₅₀ (µM) DLD-1 | IC₅₀ (µM) N87 | clogP* |
|----------|----|----------------|---------------|--------|
| (13a)    | H  | 7.5            | 4.5           | 4.21   |
| (13b)    | 3-Cl| 4              | 3.5           | 4.81   |
| (13c)    | 4-OMe | 8.5          | 8             | 4.05   |
| (13e)    | 4-F | 4.5            | 4             | 4.35   |
| (13i)    | 2,4-Dimethoxy | 17      | ND            | 3.89   |
| (13j)    | 4-OH | 11            | 11            | 3.90   |
| (13l)    | 3-Pyridyl | 2.5      | 2.5           | 2.99   |

* clogP values calculated using MarvinSketch 18.11 from ChemAxon.
donating or withdrawing groups was then investigated to probe the effect on activity. With chlorine as an electron withdrawing group at position 3 ($13b$), activity in both cell lines was enhanced, particularly in the DLD-1 line. Similar activity was noted through fluorination at C4 ($13e$), perhaps reflecting enhanced lipophilicity of these derivatives, while introduction of either 4-hydroxy ($13j$) or methoxy ($13c$) substitution reduced activity in both cell lines. Indeed, lipophilicity of chalcones has been cited as an important parameter for observed Pgp inhibitory activity [25]. Multiple ring substitution with methoxy substituents was detrimental to activity, as noted by compound ($13i$). Interestingly, potent activity was noted with compound ($13l$), containing a 3-pyridyl system. Of note in this regard is the published results of Wen [26], who prepared a series of 3',5'-diprenylated chalcones and noted marked activity within an analogous 3-pyridyl derivative.

Chalcones are generally considered to act as cytotoxic agents through one of three main pathways, namely as antioxidants, via direct cytotoxic effects or via induction of apoptosis. However, heterocyclic and fused chalcones may show other interesting mechanisms of action, as their structures may possess more than one pharmacophore. More studies are required to evaluate the potential of 2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one based chalcones as cytotoxic agents and to establish their mechanism(s) of action.

3. Experimental

3.1. General

All required chemicals, solvents, and reagents were purchased from Sigma-Aldrich and were of reagent grade. Reaction progress was monitored on pre-coated thin layer chromatographic aluminum sheets (Silica Gel Merck 60 F254), and TLC visualization was done using a UV lamp. Fourier transform infrared spectra were carried out with neat film coated samples on diamond using a Nicolet™ iST™ 10 FT-IR spectrophotometer (Thermo Fisher). Significant absorption peak ($v_{max}$) values are given in cm$^{-1}$. $^1$H and $^{13}$C NMR spectra were recorded on Bruker Avance 400 spectrometer at 400 MHz and 100 MHz, respectively, in CDCl$_3$ and CD$_3$OD using tetramethylsilane (TMS) as the internal standard. Chemical shift values are given on the $\delta$ (ppm) scale, with signals are described as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), br. (broad signal), m (multiplet), with coupling constants ($J$) expressed in Hz. Mass spectral analyses were recorded using a Waters LCT Premiere XE (ESI-TOF MS) instrument. All calculated exact mono isotopic mass distributions were calibrated against internal reference standards.

1-(4-amino-3-iodophenyl)ethan-1-one (8) To a solution of 4-acetylaniline (9.43 g, 69.8 mmol) in methanol (50 mL), was slowly added a solution of calcium carbonate (12.8 g, 128.3 mmol) in water (30 mL), followed by a solution of iodine monochloride...
(15.04 g, 92.6 mmol) in methanol (50 mL). The mixture was stirred at room temperature for 15 hours, diluted with ether (100 mL) and quenched with water (100 mL). The aqueous layer was extracted with ether (4 × 100 mL) and the combined organic phases dried over anhydrous sodium sulfate. After filtration and removal of the solvent in vacuo, the iodinated product was obtained as a yellow crystalline solid (14.6 g, 80%) [26]. IR ν_max (neat) 1242, 1577, 1609, 1657, 3198, 3326, 3447 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ_H 2.52 (3H, s, CH₃), 6.74 (1H, d, J = 8.5 Hz, H₆), 7.79 (1H, dd, J = 8.5, 2 Hz, H₅), 8.29 (1H, d, J = 1.8 Hz, H₃).

**N-(4-acetyl-2-iodophenyl)acetamide (9)** To a solution of (8) (14.6 g, 55.9 mmol) in acetic anhydride (90 mL, 952 mmol) at room temperature was slowly added conc. sulfuric acid (8 mL). The reaction was stirred for twelve hours, and extracted with ethyl acetate/water (4 × 50 mL). The combined organic phases were dried over anhydrous sodium sulfate to yield the acetamide as a white solid (16.4 g, 97%) [27]. IR ν_max (neat) 581, 822, 902, 1179, 1244, 1356, 1382, 1507, 1579, 1611, 1660, 3339 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ_H 2.22 (3H, s NHCOC₆H₅), 2.50 (3H, s CH₃), 7.60 (1H, br. s, NH), 7.84 (1H, dd, J = 8.8, 2 Hz, H₅), 8.31 (1H, d, J = 1.8 Hz), 8.34 (1H, d, J = 8.8 Hz), H₆. ¹³CNMR δ_C 25.1, 26.5, 89.3, 120.4, 129.8, 134.1, 139.1, 142.2, 168.6, 195.7.

Acetamide (9) (0.15 g, 0.5 mmol), PdCl₂(PPh)₃ (3 mol %) and TBAF.₃H₂O (0.47 g, 1.5 mmol) were placed in a two necked flask under a nitrogen atmosphere. To this mixture was added 2-methylbut-3-yn-2-ol (0.06 mL, 0.6 mmol). The reaction was heated to 80 °C for 1 hour until TLC demonstrated completion of the reaction. The mixture was washed with water, extracted with ether (2 × 30 mL) and evaporated, and the residue was purified by flash column chromatography (hexane/ethyl acetate) to afford two products:

**N-(4-acetyl-2-(3-hydroxy-3-methylbut-1-yn-1-yl)phenyl)acetamide (10)** Brown oil: IR ν_max (neat) 1166, 1229, 1289, 1359, 1514, 1577, 1676, 2979, 3378 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ_H 1.62 (6H, s, (CH₃)₂), 2.18 (3H, s, NHCOC₆H₅), 2.50 (3H, s, CH₃), 7.84 (1H, dd, J = 8.7, 2 Hz, H₅), 7.92 (1H, d, J = 2 Hz, H₃), 8.01 (1H, br. s, NH), 8.43 (1H, d, J = 8.8 Hz, H₆). ¹³CNMR δ_C 25.0 (NHCOCH₃), 26.4 (ArCOCH₃), 31.5 ((CH₃)₂), 65.8 (COH), 76.5 (C≡CCOH), 102.3 (C≡CCOOH), 111.2 (ArC), 118.4 (ArC), 129.8 (ArC), 130.2 (ArC), 132.0 (ArC), 142.7 (ArC), 168.5 (NHC=O), 196.3 (ArC=O).

**1-(2-(prop-1-en-2-yl)-1H-indol-5-yl)ethan-1-one (11)** Brown solid: ¹H NMR (CDCl₃, 400 MHz) δ_H 2.12 (3H, s, CH₃), 2.58 (3H, s, CH₃), 5.06 (1H, s, C=CH₂), 5.30 (1H, s, C=CH₂), 6.56 (1H, d, J = 1.8 Hz, C=CH), 7.27 (1H, d, J = 8.56 Hz, H₇), 7.78 (1H, dd, J = 8.6, 1.7 Hz, H₆), 8.17 (1H, d, J = 0.8 Hz, H₄), 8.56 (1H, br. s, NH). ¹³CNMR δ_C 20.5, 26.6, 102.4, 110.5, 110.6 (C=CH₂), 122.9, 123.1, 128.4, 130.0, 134.8, 139.3, 140.2, 198.4 (C=O) [28].
Alternative procedure for (10): To 0.35 g (1.35 mmol) of (9) were added water (5 mL), PdCl₂ (1 mol %) and pyrrolidine (0.83 mL, 10 mmol) under aerobic conditions. The resulting mixture was stirred at 50 °C for 5 min. To this mixture was added 2-methylbut-3-yn-2-ol (0.18 mL, 1.8 mmol), and the reaction mixture was stirred at 50 °C for 24 h. The reaction was then extracted with EtOAc (3 × 10 mL), and the combined organic layers dried with anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue purified by flash chromatography to give (10) without formation of (11).

6-acetyl-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (12) To a solution of (10) (0.90 g, 3.47 mmol) in methanol (2 mL) was added conc. HCl/H₂O (1:1, to a final concentration of 0.1 M). The reaction was heated under reflux for 2 hours, after which it was extracted with EtOAc (3 × 20 mL), and the combined organic layers dried with anhydrous sodium sulfate. The solvent was removed in vacuo, and the residue purified by flash chromatography to give (12), a fluorescent brown oil (0.42 g, 55%). IR νmax (neat) 819, 1132, 1163, 1291, 1212, 1249, 1354, 1384, 1519, 1608, 1655, 2924, 2966, 3324 cm⁻¹; 1H NMR (CDCl₃, 400 MHz) δH 1.30 (6H, s, C₆H₃C,H₃), 2.47 (3H, s, CH₃CO), 2.57 (2H, s, CH₂), 4.60 (1H, br. s, NH), 6.56 (1H, d, J = 8.8 Hz, H₈), 7.90 (1H, dd, J = 8.8, 2 Hz, H₇), 8.33 (1H, d, J = 2 Hz, H₅). 13CNMR δC 26.1 (CH₃CO), 27.7 (CH₃C,H₃), 50.2 (C₃), 53.6 (C₂), 115.9 (C₈), 116.2 (C₄a), 127.0 (C₆), 129.6 (C₅), 134.6 (C₇), 152.7 (C₈a), 193.0 (C₄), 196.3 (CH₃CO). HRMS (ESI⁺): m/z calcld for C₁₃H₁₆NO₂ 218.1175; found: 218.1181 [M+H]⁺.

3.1.1. Synthesis of quinolinoylchalcones (13a-l)

6-cinnamoyl-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13a) To a solution of (12) (81 mg, 0.37 mmol) and benzaldehyde (40 mg, 0.37 mmol) in 5 mL ethanol, KOH (1 pellet) was added, and the reaction mixture was stirred at room temperature for 48 h. After neutralization of the excessive KOH with 1 M dilute hydrochloric acid, the resulting mixture was extracted with ethyl acetate (2 × 20 mL), and the organic phase was washed with brine and dried over MgSO₄. After solvent evaporation, the crude residue was purified by flash chromatography to give (13a) as a yellow oil (50 mg, 44%). IR νmax (neat) 765, 1195, 1225, 1337, 1520, 1592, 1605, 1652, 3308 cm⁻¹; 1H NMR (CDCl₃, 400 MHz) δH 1.38 (6H, s, C(C₆H₃)₂), 2.66 (2H, s, CH₃), 4.97 (1H, br. s, NH), 6.71 (1H, d, J = 8.7 Hz, H₈), 7.39−7.43 (3H, m, 3 × Ar-H), 7.63 (1H, d, J = 15.5 Hz, CH=CH), 7.64−7.67 (2H, m, 2 x Ar-H), 7.82 (1H, d, J = 15.5 Hz, CH=CH), 8.10 (1H, d, J = 8.7, 2.1 Hz, H₇), 8.55 (1H, d, J = 2.2 Hz, H₅). 13CNMR δC 27.7 (2C), 50.2, 53.7, 116.2, 116.3, 121.3, 127.5, 128.5 (2C), 128.9 (2C), 130.3, 135.1, 135.5, 143.8, 152.9, 187.7, 193.2. HRMS (M+H)⁺ 306.1481, C₂₀H₂₀NO₂ requires 306.1494.
(E)-6-(3-(3-chlorophenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13b) 3-chlorobenzaldehyde (40 mg, 0.28 mmol) was reacted with quinolinone (12) (40 mg, 0.184 mmol) as for (13a), to afford (13b) as a yellow oil (31 mg, 50%). IR $\nu_{\max}$ (neat) 616, 817, 1173, 1192, 1253, 1364, 1564, 1602, 3314 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$H 1.39 (6H, s, C(CH$_3$)$_2$), 2.67 (2H, s, CH$_2$), 4.67 (1H, br. s, NH), 6.70 (1H, d, $J$ = 8.8 Hz, H8), 7.35–7.37 (2H, m, 2 × Ar-H), 7.53 (1H, m, Ar-H), 7.61 (1H, d, $J$ = 15.5 Hz, CH=CH), 7.65 (1H, m, Ar-H), 7.74 (1H, d, $J$ = 15.5 Hz, CH=CH), 8.10 (1H, d, $J$ = 8.7, 2.1 Hz, H7), 8.53 (1H, d, $J$ = 2.2 Hz, H5). $^{13}$CNMR $\delta$C 27.8 (2C), 50.2, 53.7, 116.2, 116.3, 122.5, 126.7, 127.5, 128.1, 129.2, 130.1 (2C), 134.9, 135.5, 137.0, 142.1, 152.9, 187.2, 193.0. HRMS (M+H)$^+$ 340.1106, C$_{20}$H$_{19}$ClNO$_2$ requires 340.1104.

(E)-6-(3-(4-methoxyphenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13c) 4-methoxybenzaldehyde (25 mg, 0.184 mmol) was reacted with quinolinone (12) (40 mg, 0.184 mmol) as for (13a), to afford (13c) as a yellow solid (51 mg, 82%). IR $\nu_{\max}$ (neat) 819, 1021, 1109, 1166, 1225, 1510, 1601, 1652, 3324 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$H 1.38 (6H, s, C(CH$_3$)$_2$), 2.66 (2H, s, CH$_2$), 3.86 (3H, s, OCH$_3$), 4.70 (1H, br. s, NH), 6.69 (1H, d, $J$ = 8.8 Hz, H8), 6.92–6.96 (2H, m, 2 × Ar-H), 7.51 (1H, d, $J$ = 15.5 Hz, CH=CH), 7.61–7.65 (2H, m, 2 × Ar-H), 7.80 (1H, d, $J$ = 15.5 Hz, CH=CH), 8.11 (1H, d, $J$ = 8.7, 2.1 Hz, H7), 8.53 (1H, d, $J$ = 2.2 Hz, H5). $^{13}$CNMR $\delta$C 27.8 (2C), 50.2, 53.7, 55.4, 114.4 (2C), 116.2, 116.3, 118.9, 127.9, 128.0, 128.9, 130.3 (2C), 135.5, 143.6, 152.6, 161.5, 187.6, 193.2. HRMS (M+H)$^+$ 336.1576, C$_{21}$H$_{22}$NO$_3$ requires 336.1600.

(E)-6-(3-(2,4-dichlorophenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13d) 2,4-dichlorobenzaldehyde (40 mg, 0.23 mmol) was reacted with quinolinone (12) (50 mg, 0.23 mmol) as for (13a), to afford (13d) as a yellow solid (61 mg, 71%). IR $\nu_{\max}$ (neat) 819, 1021, 1109, 1166, 1225, 1510, 1601, 1652, 3324 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$H 1.39 (6H, s, C(CH$_3$)$_2$), 2.67 (2H, s, CH$_2$), 4.77 (1H, br. s, NH), 6.71 (1H, d, $J$ = 8.8 Hz, H8), 7.32 (1H, dd, $J$ = 8.4, 2.1 Hz, Ar-H), 7.46 (1H, d, $J$ = 2.1 Hz, Ar-H), 7.51 (1H, d, $J$ = 15.5 Hz, CH=CH), 7.74 (1H, d, $J$ = 8.4 Hz, Ar-H), 8.10 (1H, dd, $J$ = 8.7, 2.1 Hz, Ar-H), 8.12 (1H, d, $J$ = 15.5 Hz, CH=CH), 8.51 (1H, d, $J$ = 2.1 Hz, Ar-H). $^{13}$CNMR $\delta$C 27.8 (2C), 50.1, 53.7, 116.2, 116.4, 124.2, 127.3, 127.5, 128.6, 129.2, 130.0, 132.1, 135.6, 136.0, 136.2, 138.2, 152.9, 187.0, 193.0. HRMS (M+H)$^+$ 374.0664, C$_{20}$H$_{17}$Cl$_2$NO$_2$ requires 374.0715.

(E)-6-(3-(4-fluorophenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13e) 4-fluorobenzaldehyde (35 mg, 0.28 mmol) was reacted with quinolinone (12) (60 mg, 0.28 mmol) as for (13a), to afford (13e) as a yellow solid (54 mg, 61%). IR $\nu_{\max}$ (neat) 502, 816, 1198, 1224, 1413, 1507, 1587, 1651, 3240 cm$^{-1}$; $^1$H NMR (CDCl$_3$, 400 MHz) $\delta$H 1.39 (6H, s, C(CH$_3$)$_2$), 2.67 (2H, s, CH$_2$), 4.97 (1H, br. s,
(E)-6-(3-(2-chlorophenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13f) 2-chlorobenzaldehyde (0.096 g, 0.68 mmol, 1.31 eq.) was reacted with quinoline (12) (114 mg, 0.52 mmol) as for compound (13a), to afford (13f) (92 mg, 55%). IR 668, 710, 816, 1266, 1314, 1473, 1591, 1667, 1683, 2556, 2650, 2684, 2923 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δH 1.38 (6H, s, C(CH₃)₂), 2.66 (2H, s, C₂H), 4.92 (1H, br. s, NH), 6.71 (1H, d, J = 8.8 Hz, H₈), 7.33 (2H, m, 2 × ArH), 7.43 (1H, dd, J = 5.8, 3.5 Hz, ArH), 7.59 (1H, d, J = 15.6 Hz, CH=CH), 7.80 (1H, dd, J = 5.7, 3.7 Hz, ArH), 8.10 (1H, dd, J = 8.7, 2.1 Hz, ArH), 8.20 (1H, d, J = 15.6 Hz, CH=CH), 8.55 (1H, d, J = 2 Hz, H₅). ¹³CNMR δC 27.7 (C₂), 50.2, 53.7, 116.2, 116.4, 124.0, 127.1, 127.4, 127.9, 129.3, 130.2, 131.0, 133.5, 135.4, 135.5, 139.5, 152.9, 187.3, 193.1. HRMS (M+H)⁺ 340.107, C₂₀H₁₀FNO₂ requires 340.1400.

(E)-6-(3-(2,6-dichlorophenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13g) 2,6-dichlorobenzaldehyde (175 mg, 1.00 mmol) was reacted with quinoline (12) (186 mg, 0.86 mmol, 1.00 eq.) as for compound (13a), to afford (13g) (256 mg, 80%). IR 671, 727, 773, 830, 1164, 1196, 1226, 1259, 1519, 1597, 1658, 2924, 3315 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δH; 1.28 (6H, s, C(CH₃)₂), 2.55 (2H, s, C₂H), 5.34 (1H, br. s, NH), 6.66 (1H, d, J = 8.8 Hz, ArH), 7.10 (1H, dd, J = 8 Hz, ArH), 7.28 (2H, d, J = 8.1 Hz, 2 × ArH), 7.61 (1H, J = 16.0 Hz, CH=CH), 7.73 (1H, d, J = 16.0 Hz, CH=CH), 7.96 (1H, dd, J = 8.8, 2.0 Hz, H₇), 8.39 (1H, d, J = 1.8 Hz, ArH); ¹³CNMR δC; 27.7 (C₂), 50.2, 53.6, 116.1, 116.5, 126.8, 128.8 (C₂), 129.7, 129.8, 130.2, 133.0, 135.1 (C₂), 135.4, 136.8, 153.3, 187.5, 193.2. HRMS (M+H)⁺ 374.0725, C₂₀H₁₂Cl₂NO₂ requires 374.0715.

(E)-6-(3-(2-methoxyphenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one (13h) 2-methoxybenzaldehyde (223 mg, 1.64 mmol) was reacted with quinoline (12) (183 mg, 0.84 mmol) as for compound (13a), using methanol as alkanol, to yield (13i) (194 mg, 69%). IR 680, 830, 1023, 1162, 1192, 1244, 1377, 1517, 1601, 1651, 2927, 3307 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δH: 1.35 (6H, s, C(CH₃)₂), 2.63 (2H, s, C₂H), 3.87 (3H, s, OCH₃), 5.54 (1H, br. s, NH), 6.74 (1H, d, J = 8.7 Hz, H₈), 6.91 (1H, d, J = 8.3 Hz, ArH), 6.98 (1H, m, ArH), 7.35 (1H, m, ArH), 7.66 (1H, d, J = 7.5 Hz, ArH), 7.70 (1H, d, J = 15.5 Hz, CH=CH), 8.06 (1H, dd, J = 8.7, 1.9 Hz, H₇), 8.15 (1H, d, J = 15.8 Hz, CH=CH), 8.54 (1H, s, H₅); ¹³CNMR δC; 27.5 (C₂), 50.2, 53.5, 55.5, 111.2, 116.0, 116.4, 120.7, 121.9, 124.1, 127.4, 128.9, 129.2, 131.6, 135.4, 139.1,
153.2, 158.7, 188.3, 193.5. HRMS (M+H)+ 336.1591, C21H21NO3 requires 336.1600.

\((E)-6-(3-(2,4-dimethoxyphenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one\) (13i) 2,4-dimethoxybenzaldehyde (0.190 g, 1.14 mmol, 1.10 eq.) was reacted with quinolinone (12) (177 mg, 0.81 mmol) as for compound (13a), using methanol as alkanol, to yield (13j) (215 mg, 72%). IR \(\nu_{\text{max}}\) (neat) 782, 819, 1034, 1162, 1191, 1268, 1292, 1332, 1364, 1502, 1674, 2925, 3330 cm\(^{-1}\). \(^1\)H NMR (CDCl\(_3\), 400 MHz) \(\delta\) \(H\); 1.37 (6H, s, C(CH\(_3\))\(_2\)), 2.65 (2H, s, CH\(_2\)), 3.86 (3H, s, OCH\(_3\)), 3.90 (3H, s, OCH\(_3\)), 4.93 (1H, br. s, NH), 6.47 (1H, d, \(J = 2.2\) Hz, ArH), 6.54 (1H, dd, \(J = 8.6, 2.2\) Hz, ArH), 6.70 (1H, d, \(J = 8.8\) Hz, H8), 7.62 (2H, m, CH=CH and ArH), 8.05 (2H, m, CH=CH and ArH), 8.53 (1H, d, \(J = 2.0\) Hz, H5); \(^13\)CNMR \(\delta\) \(C\); 27.8 (2C), 50.4, 53.7, 55.6, 55.7, 98.5, 105.4, 116.2, 116.3, 117.5, 119.6, 128.0, 129.0, 130.7, 135.6, 139.3, 152.8, 160.4, 163.0, 188.3, 193.4. HRMS (M+H)+ 366.1716, C\(_{22}\)H\(_{23}\)NO\(_4\) requires 366.1705.

\((E)-6-(3-(4-hydroxyphenyl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one\) (13j) To a solution of 2-(diethylamino)ethanethiol (0.525 g, 3.09 mmol, 1.83 eq.) in anhydrous DMF (15 mL) was added potassium tert-butoxide (0.682 g, 6.08 mmol, 3.60 eq.) at 0°C. The resulting reaction mixture was allowed to heat to room temperature. To the reaction was added a solution of (13c) (0.567 g, 1.69 mmol, 1 eq.) in DMF (5 mL). The reaction was allowed to proceed at 160°C for 1.5 hours. The resulting mixture was partitioned between water (20 mL) and ethyl acetate (80 mL), the water layer was acidified with HCl (2 mL, 3 M). The organic layer was then washed with water (20 mL), dried over magnesium sulfate, filtered under gravity and concentrated under reduced pressure to yield a yellow crystalline solid. The crude sample was eluted from silica gel with an ethyl acetate/petroleum ether solution (0–50% ethyl acetate in petroleum ether). The desired fractions were concentrated to yield (13k) as a yellow crystalline solid (0.506 g, 93%). IR \(\nu_{\text{max}}\) (neat) 811, 1023, 1162, 1197, 1221, 1341, 1367, 1509, 1574, 1599, 1642, 1655, 2853, 2925, 2962, 3302 cm\(^{-1}\). \(^1\)H NMR (MeOD, 400 MHz) \(\delta\) \(H\); 1.31 (6H, s, C(CH\(_3\))\(_2\)), 2.59 (2H, s, CH\(_2\)), 6.77 (1H, d, \(J = 8.9\) Hz, H8), 6.82 (2H, m, 2 × ArH), 7.55 (3H, m, CH=CH and 2 × ArH), 7.67 (1H, d, \(J = 15.5\) Hz, CH=CH), 7.97 (1H, dd, \(J = 8.9, 2.2\) Hz, H7); \(^13\)CNMR \(\delta\) \(C\); 27.8 (2C), 50.8, 54.0, 116.7, 116.9 (2C), 117.4, 119.0, 127.5, 127.9, 130.3, 131.7 (2C), 136.2, 145.4, 155.6, 161.5, 189.8, 196.1. HRMS (M+H)+ 322.1451, C\(_{20}\)H\(_{20}\)NO\(_3\) requires 322.1443.

\((E)-6-(3-(benzo[d][1,3]dioxol-5-yl)acryloyl)-2,2-dimethyl-2,3-dihydroquinolin-4(1H)-one\) (13k) Benzo[d][1,3]dioxole-5-carbaldehyde (0.139 g, 0.93 mmol) was reacted with quinolinone (12) (0.172 g, 0.79 mmol) as for compound (13a), to yield (13l) (0.143 g, 52%). IR \(\nu_{\text{max}}\) (neat) 1035, 1163, 1187, 1241, 1368, 1501, 1599,
1663, 2854, 2920, 2963, 3315 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ_H: 1.38 (6H, s, C(CH₃)₂), 2.66 (2H, s, CH₂), 4.93 (1H, br., NH), 6.02 (2H, s, OCH₂O), 6.70 (1H, d, J = 8.7 Hz, ArH), 6.84 (1H, d, J = 7.9 Hz, ArH), 7.13 (1H, dd, J = 8.0, 1.2 Hz, ArH), 7.19 (1H, s, ArH), 7.46 (1H, d, J = 15.5 Hz, CH=CH), 7.74 (1H, d, J = 15.4 Hz, CH=CH), 8.08 (1H, dd, J = 8.7, 2.1 Hz, ArH), 8.52 (1H, d, J = 2.0 Hz, ArH); ¹³CNMR δ_C: 27.7 (2C), 50.2, 53.7, 101.6 (OCH₂O), 106.9, 108.6, 116.2, 116.3, 119.3, 125.0, 127.7, 129.0, 129.6, 135.5, 143.6, 148.4, 149.7, 152.8, 187.6, 193.2. HRMS (M+H)⁺ 350.1372, C₂₁H₁₉NO₄ requires 350.1392.

(E)-2,2-dimethyl-6-(3-(pyridin-3-yl)acryloyl)-2,3-dihydroquinolin-4(1H)-one (13l) Pyridine-3-carboxaldehyde (0.427 g, 3.99 mmol) was reacted with quinoline (12) (0.472 g, 2.17 mmol) as for compound (13a), with an extended reaction time of 216 h, to yield (13m) (129 mg, 21%). IR ν_max (neat) 802, 815, 830, 1160, 1197, 1229, 1338, 1411, 1520, 1595, 1609, 1652, 2883, 2924, 2962, 3311 cm⁻¹; ¹H NMR (CDCl₃, 400 MHz) δ_H: 1.39 (6H, s, C(CH₃)₂), 2.67 (2H, s, CH₂), 5.67 (1H, br. s, NH), 6.76 (1H, d, J = 8.8 Hz, H₈), 7.40 (1H, dd, J = 7.8, 4.9 Hz, ArH), 7.71 (1H, d, J = 15.7 Hz, CH=CH), 7.79 (1H, d, J = 15.7 Hz, CH=CH), 8.03 (1H, d, J = 8.0 Hz, ArH), 8.07 (1H, dd, J = 8.8, 2.1 Hz, H₇), 8.54 (1H, d, J = 2.0 Hz, H₅), 8.64 (1H, d, J = 3.8 Hz, ArH), 8.85 (1H, s, ArH); ¹³CNMR δ_C: 27.6 (2C), 50.1, 53.6, 116.0, 116.5, 123.5, 124.0, 126.7, 129.4, 131.1, 134.7, 135.4, 139.5, 149.8, 150.5, 153.4, 186.9, 193.3. HRMS (M+H)⁺ 307.1451, C₁₉H₁₉N₂O₂ requires 307.1447.

3.2. Proliferation assay

1 × 10³ cells/well were seeded plates into flat-bottomed, 96-well plates and allowed to attach overnight. Compounds were made up in DMSO. Wells were treated in triplicate with serial dilutions of drug in a final volume of 200 μL. Drug-free controls were included in each assay. Drugs were added to the plates at specific concentrations and incubated at 37 °C. Plates were incubated for a further 5 days at 37 °C in a humidified atmosphere with 5% CO₂ and cell viability was determined using an acid phosphatase assay as previously described [29]. The IC₅₀ was determined from the regression of a plot of the logarithm of the concentration versus percent inhibition at the time point of 120 hours using GraphPad Prism (GraphPad Software, California, United States).

Declarations

Author contribution statement

Julie Jean, David S. Farrell: Performed the experiments.

Angela M. Farrelly: Performed the experiments; Analyzed and interpreted the data.
Sinead Toomey: Conceived and designed the experiments; Performed the experiments; Contributed reagents, materials, analysis tools or data.

James Barlow: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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The authors declare no conflict of interest.

**Additional information**

No additional information is available for this paper.

**References**

[1] V. Sridharan, P.A. Suryavanshi, J.C. Menéndez, Advances in the chemistry of tetrahydroquinolines, Chem. Rev. 111 (11) (2011) 7157–7259.

[2] R.F. Bond, J.C.A. Boeyens, C.W. Holzapfel, P.S. Steyn, Cyclopiamines A and B, novel oxindole metabolites of Penicillium cyclopium westling, J. Chem. Soc., Perkin Trans. 1: Org. and Bio-Org. Chem. (0) (1979) 1751–1761.

[3] A. Aisa, Q. Chen, Alkaloid Compounds of Viola Tianshanica and Preparation Method Thereof, CN106946911A, July 14, 2017, https://worldwide.espacenet.com/publicationDetails/originalDocument?CC=CN&NR=106946911A&KC=A&FT=D&ND=&date=20170714&DB=&locale=. (Accessed 29 September 2018).

[4] D. Campbell, S.G. Duron, B. Vollrath, W. Wade, 8-(2’-Heterocycl)pyrido[2,3-D]pyrimidin-7(8H)-ones for the Treatment of CNS Disorders, U.S. Pat. Appl. 2013/0338153 A1, Dec. 19, 2013, http://appft1.uspto.gov/netacgi/nph-Parser?Sect1=PTO1&Sect2=HITOFF&d=PG01&p=1&u=/netahtml/PTO/srchnum. html&r=1&f=G&l=50&s1=20130338153.PGNR. (Accessed 29 September 2018).

[5] T.K. Jones, M.E. Goldman, C.L.F. Pooley, D.T. Winn, J.P. Edwards, S.J. West, C.M. Tegley, L. Zhi, L.G. Hamann, L.J. Farmer, R.L. Davis, Steroid Receptor
Modulator Compounds and Methods, US5696133A, Dec. 9, 1997, http://patft.uspto.gov/netacgi/nph-Parser?Sect2=PTO1&Sect2=HITOFF&p=1&u=/netahtml/PTO/search-bool.html&r=1&f=G&l=50&d=PALL&RefSRch=yes&Query=PN/5696133. (Accessed 29 September 2018).

[6] M. Hamada, K. Okamoto, T. Kurosaki, New Quinolylacetic Acid Compounds and Pharmaceutical Compositions Containing Them, Eur. Pat. Appl., 1983. EP0083222A1, https://worldwide.espacenet.com/searchResults?search=EP0083222A1&DB=EPODOC&submitted=true&locale=en_EP&ST=singleline&compact=false&DB=EPODOC&query=EP0083222A1. (Accessed 29 September 2018).

[7] L.V. Politanskaya, I.P. Chuikov, E.V. Tretyakov, V.D. Shteingarts, L.P. Ovchinnikova, O.D. Zakharova, G.A. Nevinsky, An effective two-step synthesis, fluorescent properties, antioxidant activity and cytotoxicity evaluation of benzene-fluorinated 2,2-dimethyl-2,3-dihydro-1H-quinolin-4-ones, J. Fluorine Chem. 178 (2015) 142–153.

[8] P. Singh, A. Anand, V. Kumar, Recent developments in biological activities of chalcones: a mini review, Eur. J. Med. Chem. 85 (2014) 758–777.

[9] M. Tsukayama, Y. Kawamura, H. Tamaki, T. Horie, Synthesis of parvisoflavones A and B, Chem. Pharm. Bull. 39 (7) (1991) 1704–1706.

[10] D. Ashok, R.S. Kumar, D. Mohan Gandhi, A. Jayashree, Solvent-free microwave-assisted synthesis and biological evaluation of aurones and flavanones based on 2, 2-dimethylchroman-4-one, Chem. Heterocycl. Comp. 52 (7) (2016) 453–459.

[11] D. Ashok, R.S. Kumar, D. Mohan Gandhi, A. Jayashree, Synthesis of novel 2,4,6-trisubstituted pyrimidine derivatives and their in vitro antimicrobial activity, Russ. J. Gen. Chem. 86 (6) (2016) 1396–1404.

[12] M. Mujahid, P. Yogeeswari, D. Sriram, U.M.V. Basavanag, Erik Diaz-Cervantes, Luis Cordoba-Bahena, Juvencio Robles, R.G. Gonnade, M. Karthikeyan, Renu Vyas, M. Mathukrishnan, Spirochromone-chalcone conjugates as antitubercular agents: synthesis, bio evaluation and molecular modeling studies, RSC Adv. 5 (2015) 106448–106460.

[13] Y. Chinthala, S. Thakur, S. Tirunagari, S. Chinde, A.K. Domatti, N.K. Arigari, K.V.N.S. Srinivas, S. Alam, K.K. Jonnala, F. Khan, A. Tiwari, P. Grover, Synthesis, docking and ADMET studies of novel chalcone triazoles for anticancer and anti-diabetic activity, Eur. J. Med. Chem. 93 (2015) 564–573.

[14] V. Sharma, V. Kumar, P. Kumar, Heterocyclic chalcone analogues as potential anticancer agents, Anticancer Agents Med. Chem. 3 (2013) 422–432.
[15] F. Pisaneschi, J.J.P. Sejberg, C. Blain, W.H. Ng, E.O. Aboagye, A.C. Spivey, 2-Substituted-2,3-dihydro-1H-quinolin-4-ones via acid-catalyzed tandem Rupe rearrangement-Donnelly-Farrell ring closure of 2-(3’-hydroxypropynyl) anilines, Synlett (2) (2011) 241–244.

[16] R.A. Bunce, T. Nago, S. Abuskhuna, Steric and electronic requirements in the synthesis of 2,3-dihydro-4(1H)-quinolinones by the tandem Michael-SNAr reaction, J. Heterocycl. Chem. 52 (2015) 1143.

[17] N.M. Williamson, A.D. Ward, The preparation and some chemistry of 2,2-dimethyl-1,2-dihydroquinolines, Tetrahedron 61 (1) (2005) 155–165.

[18] I.P. Clarke, O. Meth-Cohn, The synthesis of 1,2,3,4-tetrahydro-2,2-dimethylquinolin-4-ones as antioxidant precursors, ARKIVOC 2000 (3) (2000) 372–381.

[19] N. Okamoto, T. Sueda, R. Yanada, Bi(OTf)3-catalyzed tandem Meyer-Schuster rearrangement and 1,4-addition to the resulting vinyl ketone, J. Org. Chem. 79 (20) (2014) 9854–9859.

[20] K. Fujita, Y. Takahashi, M. Owaki, K. Yamamoto, R. Yamaguchi, Synthesis of five-, six-, and seven-membered ring lactams by Cp*Rh complex-catalyzed oxidative N-heterocyclization of amino alcohols, Org. Lett. 6 (2004) 2785.

[21] B. Liang, M. Dai, J. Chen, Z. Yang, Copper-free Sonogashira coupling reaction with PdCl2 in water under aerobic conditions, J. Org. Chem. 70 (2005) 391–393.

[22] Y. Liang, Y.-X. Xie, J.-H. Li, Modified palladium-catalyzed Sonogashira cross-coupling reactions under copper-, amine-, and solvent-free conditions, J. Org. Chem. 71 (2006) 379–381.

[23] L.V. Politanskaya, P. Chuikov, V.D. Shteingarts, Synthesis of indoles with a polyfluorinated benzene ring, Tetrahedron 69 (2013) 8477–8486.

[24] K.C. Majumdar, S. Ponra, T. Ghosh, R. Sadhukhan, U. Ghosh, Synthesis of novel pyrano[3,2-f]quinoline, phenanthroline derivatives and studies of their interactions with proteins: an application in mammalian cell imaging, Eur. J. Med. Chem. 71 (2014) 306–315.

[25] Z. Wen, Y. Zhang, X. Wang, X. Zeng, Z. Hu, Y. Liu, Y. Xie, G. Liang, J. Zhu, H. Luo, B. Xu, Novel 3’,5’-diprenylated chalcones inhibited the proliferation of cancer cells in vitro by inducing cell apoptosis and arresting cell cycle phase, Eur. J. Med. Chem. 133 (2017) 227–239.

[26] P.R. Kym, K.E. Carlson, J.A. Katzenellenbogen, Progestin 16.alpha.,17.alpha.-dioxolane ketals as molecular probes for the progesterone receptor:
synthesis, binding affinity, and photochemical evaluation, J. Med. Chem. 36 (9) (1993) 1111–1119.

[27] S. Kotha, V.R. Shah, Design and synthesis of 1-benzazepine derivatives by strategic utilization of Suzuki–Miyaura cross-coupling, aza-Claisen rearrangement and ring-closing metathesis, Eur. J. Org. Chem. (2008) 1054–1064.

[28] I. Ambrogio, S. Cacchi, G. Fabrizi, A. Prastaro, 3-(o-Trifluoroacetamidoaryl)-1-propargylic esters: common intermediates for the palladium-catalyzed synthesis of 2-aminomethyl-, 2-vinyl, and 2-alkylindoles, Tetrahedron 65 (44) (2009) 8916–8929.

[29] A. Martin, M. Clynes, Comparison of 5 microplate colorimetric assays for in vitro cytotoxicity testing and cell proliferation assays, Cytotechnology 11 (1993) 49–58.