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# 3,3-Dimethyl-1*H*-pyrrolo[3,2-*g*]quinolin-2(3*H*)-one derivatives as novel Raf kinase inhibitors

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A series of quinoline derivatives were designed and synthesized as tyrosine kinase inhibitors. Exploration of the structure–activity relationships resulted in compounds that are potent *in vitro*. In addition, compound **10f** was found to be a potent and selective Raf kinase inhibitor.



The relative mortality rate caused by cancer is still very high, but successfully developed target-based therapies have significantly changed cancer treatment. The Ras/Raf/MEK/ERK mitogen-activated protein (MAP) kinase signal transduction pathway transmits signals from cell-surface receptor tyrosine kinases to the nucleus, which is critical to the survival, growth, and proliferation of cells and has been implicated in several human cancers.[1–4] Raf serine/theronine protein kinases, existing in three isoforms, A-Raf, B-Raf, and C-Raf (Raf-1), is a crucial component of this signal transduction pathway. The three Raf isoforms are all able to interact with Ras and activate the MAP kinase pathway. Aberrant activation of the Ras/Raf/MEK/ERK pathway is commonly observed in various cancers.[5–7] Thus, therapeutic targeting of individual components of the Ras/Raf/MEK/ERK pathway has attracted much attention in the development of anti-cancer drugs.[8]

To date, a number of small molecule Raf kinases inhibitors containing diverse scaffolds have received U.S. Food and Drug Administration approval as cancer treatments in the recent past, which can be divided into ureas, urea bioisosteres, imidazoles, benzamides, oxindoles and aza-stilbenes.[9,10] Among them, ureas, especially bis-aryl ureas, have been most extensively investigated following the success of Sorafenib (tosylate salt of Bay 43-9006).[11] With the considerable number and variety of ureas identified as inhibitors of C-Raf kinase, Sorafenib, initially and mainly targeting C-Raf, has finally emerged as a successful representative of ureas, approved by the FDA for the treatment of advanced renal cell carcinoma (RCC) in 2005 and unresectable hepatocellular carcinoma (HCC) in 2007.[12,13] However, despite some motivating factors, Sorafenib faces significant challenges including drug resistance, lack of inhibitor selectivity, lack of inhibitor efficacy and difficulty in drug target validation for particular disease settings.[14] Sorafenib was developed initially as an inhibitor of Raf kinase, but its efficacy in renal and hepatocellular cancer was later attributed to inhibition of VEGFR2 and PDGFR and potentially other targets.[15] Therefore, we explored structural modifications of Sorafenib with the goal of optimizing the activity on C-Raf even further.

According to the crystal structure of B-Raf in complex with Bay 43-9006, the distal 4-pyridyl ring occupies the ATP adenine binding pocket of the kinase domain and the lipophilic trifluoromethyl phenyl ring at the opposite end of the molecule inserts into a hydrophobic pocket. Importantly, the urea moiety forms two hydrogen bonds with B-Raf, one with the backbone aspartate, and the other with the glutamate side chain.[12,16] As the residues of B-Raf that contact with Bay 43-9006 are conserved in C-Raf, consistent with much of the structure–activity relationship of C-Raf inhibition by Bay 43-9006 and its derivatives, the interaction mode can provide significant information for the conduction and validation of ligand-based and structure-based studies. Systematic analysis of the crystal structures of protein kinases led us to explore a series of quinoline derivatives as novel Raf kinase inhibitors with more potent and selective antitumor activities.

According to the research on nonclassical bioisosteric theory and structure–activity relationships (SAR) of Sorafenib, we explored structural modification of Sorafenib on the basis of retaining the urea functional moiety with the goals of improving the antitumor activities and optimizing the activity on Raf kinase even further. Principle changes focused on the hinge binding moiety – the linear methyl amide side group of the pyridyl moiety was changed to quinoline derivatives to enhance the interaction with Raf. In addition, a variety of hydrophobic aromatic rings were introduced into the end of the inhibitors for the study of structure–activity relationships (SAR). Subsequently, we investigated hydrogen, fluoro and chloro groups on the central phenyl ring with a view to increasing the potency of inhibitors (Fig. 1).

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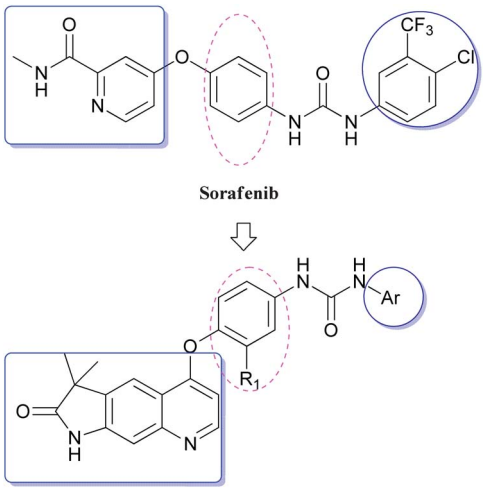
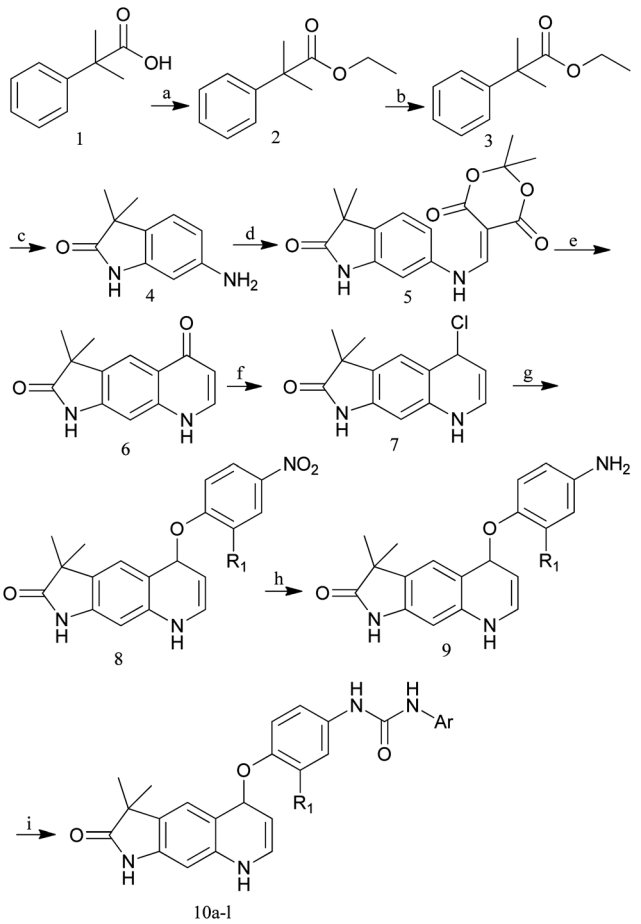



**Fig. 1** The structure and the target compounds **10a–l**.

In this paper, based on Sorafenib as the lead compound, we designed and synthesized a series of quinoline derivatives and tested their antitumor activities on the *in vitro* growth of human cancer cell lines HepG2, A549 and KCC-853 using the MTT assay.[17] Most of the compounds exhibited excellent antiproliferative activities against all three tested cell lines. Further, their inhibitory activities against Raf kinase were investigated, and the selective profile of the best compound **10f** was assessed against a panel of 20 protein kinases.

The general synthesis method for the target compounds **10a–l** was devised and is shown in Scheme 1. The ethyl ester **2** can be prepared by treating **1** with thionyl chloride in ethanol. Synthesis of compound **3** can be achieved by treating **2** with nitric acid and sulphuric acid. Cyclization to intermediate **4** can be performed by refluxing intermediate **3** in a mixed solvent of ethanol and $H_2O$ with iron powder (reduced) and ammonium chloride. Compound **5** can be synthesized by refluxing intermediate **4** with 5-(methoxymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione in isopropanol. Preparation of compound **6** was achieved by heating intermediate **5** in biphenyl and diphenyl ether. Compound **7** was obtained by chlorination of **6**. Compound **8** can be synthesized by reacting **7** with the 4-nitrophenol derivatives in diphenyl ether. Preparation of compound **9** was accomplished by refluxing **8** with powdered iron and ammonium chloride in water and alcohol. Synthesis of compounds **10a–l** was carried out using the arylamine derivatives and phenyl chloroformate in *N*,*N*-dimethylaniline and pyridine.

To confirm that the cellular antiproliferative activity is due to cellular inhibition of C-Raf kinase activity, all compounds were tested in both a cellular C-Raf assay and cell proliferation assays. The antiproliferative activities of compounds **10a–l** against HepG2, A549 and KCC-853 together with that of Sorafenib as reference standard and the $IC_{50}$ (μM) values (concentration required to achieve 50% inhibition of the tumour growth) of the tested compounds on each cell line were presented in Table 1. As shown in Table 1, most of compounds **10a–l** showed better or similar antiproliferative activities against cancer cell lines Hep G2, A549 and KCC-853 in

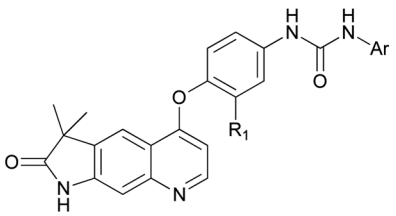


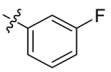
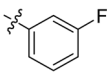
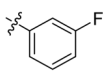
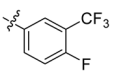
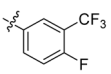
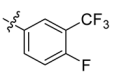
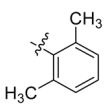
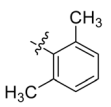
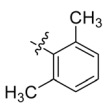
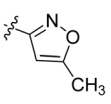
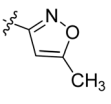
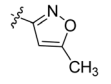

**Scheme 1** Reagent and conditions: (a) $SOCl_2$, ethanol, reflux, 88.0%; (b) nitric acid, sulphuric acid, rt, 54.5%; (c) Fe, $NH_4Cl$, EtOH, $H_2O$, reflux, 64.1%; (d) isopropanol, 5-(methoxymethylene)-2,2-dimethyl-1,3-dioxane-4,6-dione, reflux, 78.2%; (e) biphenyl, diphenyl ether, 240 °C, 48.2%; (f) $POCl_3$, $PCl_5$, reflux, 83.2%; (g) diphenyl ether, 160 °C, 47.1%; (h) Fe, $NH_4Cl$, EtOH, $H_2O$, reflux, 80.2%; (i) *N*,*N*-dimethylaniline, pyridine, phenyl chloroformate, 77.2%; (j) 5-nitropyridin-2-ol, diphenyl ether, 160 °C, 42.3%; (k) Fe, $NH_4Cl$, EtOH, $H_2O$, reflux, 78.5%; (l) *N*,*N*-dimethylaniline, pyridine, phenyl chloroformate, 80.3%.

comparison with Sorafenib. The best compound **10f** (with $IC_{50}$ values = 6.356, 0.916 and 1.102 μM) exhibited superior antiproliferative activities to Sorafenib (with $IC_{50}$ values = 12.669, 5.231 and 6.779 μM) in Hep G2, A549 and KCC-853. Moreover, most of the prepared compounds exhibited superior activities against A549 and KCC-853 than Hep G2. This provides valuable information, demonstrating the selective behaviour of the target compounds to some extent. We hypothesized that the enhanced potency could arise from a series of contributing factors, including size, electronic properties, and hydrogen-bond forming capabilities.

Diverse electron withdrawing groups (*e.g.* fluoro, trifluoromethyl) and electron donating groups (*e.g.* methyl) were introduced into the phenyl ring that connects with the urea group at the end of the inhibitors for investigation of structure–activity relationships (SARs). Compounds bearing a fluoro group at the *para* position of phenyl ring exhibited reasonable antitumor activities against the three cell lines (**10a**, **10b**, **10c**).

**Table 1** Antiproliferative activity of quinoline derivatives **10a–l**



| Compound | $R_1$ | Ar | $IC_{50}$ (μM) HepG2 | A549 | KCC-853 |
|---|---|---|---|---|---|
| **10a** | H |  | 12.042 | 5.463 | 10.587 |
| **10b** | F |  | 15.464 | 7.712 | 5.642 |
| **10c** | Cl |  | 7.353 | 4.385 | 6.493 |
| **10d** | H |  | 9.561 | 3.196 | 4.227 |
| **10e** | F |  | 8.090 | 2.155 | 2.849 |
| **10f** | Cl |  | 6.356 | 0.916 | 1.102 |
| **10g** | H |  | 20.340 | 10.537 | 10.265 |
| **10h** | F |  | 10.272 | 4.069 | 3.746 |
| **10i** | Cl |  | 11.197 | 3.852 | 4.391 |
| **10j** | H |  | 31.687 | 6.473 | 10.073 |
| **10k** | F |  | 12.005 | 5.026 | 4.179 |
| **10l** | Cl |  | 8.464 | 5.759 | 5.864 |
| **Sorafenib** | | | 12.669 | 5.231 | 6.779 |

Systematic evaluation of additional substituent groups at the *meta* position indicted that trifluoromethyl led to higher antitumor activities than other substituents, suggesting the importance of the substituent size at the aromatic ring (**10d**, **10e**, **10f**). However, introduction of electron donating groups or replacing with an isoxazolyl ring led to a decrease in antiproliferative activities. In particular, addition of a fluoro group to the central phenyl ring improved antitumor activities compared to a hydrogen substituent, whilst a chloro group further improved potency. Unfortunately, the chloro group led to a slight reduction in the potency of compound **10k**. In this case, changing the substituent on the end of the phenyl ring and the central phenyl ring gave improved potency.

**Table 2** C-Raf kinase inhibitory activities of the target compounds **10a-l**

| Compound | Inhibition (nM) | Compound | Inhibition (nM) |
|---|---|---|---|
| **10a** | 37.3 | **10h** | 30.3 |
| **10b** | 25.2 | **10i** | 28.1 |
| **10c** | 22.1 | **10j** | 58.5 |
| **10d** | 14.7 | **10k** | 27.2 |
| **10e** | 15.9 | **10l** | 18.7 |
| **10f** | 8.7 | **Sorafenib** | 28.5 |
| **10g** | 52.1 | | |

**Table 3** Inhibition percentages of compound **10f** at a single dose concentration of 10 μM over 20 protein kinases

| Kinase | Inhibition (%) | Kinase | Inhibition (%) |
|---|---|---|---|
| ABL1 | 26.2 | IKKβ | 4.7 |
| AKT1 | 16.5 | JAK2 | 0.2 |
| c-Kit | 18.7 | PDGFRα | 0.3 |
| c-MET | 1.5 | PTK2 | 3.6 |
| C-Raf | 94.6 | PKA | 1.3 |
| CDK2 | 7.8 | PLK1 | 4.9 |
| FLT3 | 15.3 | KDR | 1.4 |
| FMS | 46.9 | SYK | 15.0 |
| FYN | 0.6 | TIE2 | 22.3 |
| FGFR1 | 20.2 | VEGFR2 | 34.1 |

To test the potent antiproliferative activities, the inhibitory activities of these compounds against C-Raf kinase were further evaluated *in vitro* at a fixed concentration of 1 μM by the FRET assay,[18] Sorafenib was also used as the positive control drug. The results summarized in Table 2 show that most of the tested compounds exhibited a reasonable inhibitory potency against C-Raf kinase. Moreover, compound **10f** (with $IC_{50}$ = 8.7 nM) demonstrated more potent inhibitory activities against C-Raf than the positive control drug Sorafenib (with $IC_{50}$ values = 28.5 nM). Introduction of the 3,3-dimethyl-1*H*-pyrrolo[3,2-*g*]quinolin-2(3*H*)-one moiety led to a significant improvement in C-Raf potency and measurable cellular inhibition.

The kinase selective profile of these prepared compounds was assessed over 20 protein kinases at a single dose concentration of 10 μM, using compound **10f** as a representative. The dose response data are shown in Table 3. Within this table, which includes a range of protein kinases targets of therapeutic relevance, compound **10f** was found to demonstrate excellent selectivity over the majority of the targets. Based on the known pathway biology these particular off-target activities are not expected to contribute to inhibition of C-Raf mediated signaling.

In conclusion, the quinoline derivatives presented here are potent inhibitors of C-Raf protein, which results in blockade of signaling through the Ras/Raf/MEK/ERK pathway in Hep G2, A549 and KCC-853 cell lines *in vitro*. Moreover, from the structure–activity relationships (SARs) we may conclude that certain members of this series have selective properties that render them suitable for further evaluation and the results of such studies will be reported in due course.

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17 **Cell assay:** Hep G2, A549 and KCC-853 were cultured in RPMI-1640 medium, supplemented with 10% fetal calf serum (FCS), and maintained in a 5% $CO_2$, 95% humidity atmosphere at 37 °C. In 96-well plates were seeded $5.0 \times 10^3$ cells per well and incubated for 24 h. The cells were then incubated for another 72 h with various concentrations of compounds **10a–l**. Subsequently, 20 μL of fresh MTT solution (5 mg $ml^{-1}$) was added to each well and incubated with cells at 37 °Cfor an additional 4 h. The supernatant was carefully removed from each well and 100 μL of DMSO was added to each well to dissolve the formazan crystals which were formed by the cellular reduction of MTT. After mixing with a mechanical plate mixer, the absorbance of each well was measured by a plate reader at a test wavelength of 570 nm. $IC_{50}$ data were calculated by linear regression analysis as described above.
18 **FRET assay:** Active C-Raf and inactive Mek were diluted together with kinase dilution buffer (25 mM Tris, pH 7.5, 0.02 mM EGTA, 0.66 mg $mL^{-1}$ myelin basic protein, 1 mM DTT, 0.1 mg $mL^{-1}$ BSA) to 4 and 20 μg $mL^{-1}$, respectively, and 20 μL of this enzyme-substrate mixture was added to each well of a 96-well plate. Test compound (5 μL) of desired concentration was added to the mixture and the kinase reaction was initiated by adding 25 μL of 10 μM γ-[33P] ATP (specific activity approx. 500 cpm $pmol^{-1}$) for incubation at 30 °C for 20 min. The reaction was spotted onto a phosphocellulose mat, washed with 1% phosphoric acid solution, and the radioactivity counted in the presence of scintillation fluid in a scintillation counter.

 