Bioorganic & Medicinal Chemistry 20 (2012) 4323–4329

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and antitumor activities of novel bis-aryl ureas derivatives as Raf kinase inhibitors

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ARTICLE INFO

Article history:
Received 18 November 2011
Revised 22 May 2012
Accepted 22 May 2012
Available online 30 May 2012

Keywords:
Bis-aryl ureas
Raf kinase
Synthesis
Antitumor activity

ABSTRACT

A series of novel bis-aryl ureas containing trifluoromethyl imidazolyl group targeting Raf kinase were designed and synthesized based on the lead compound of Sorafenib. All the prepared compounds were evaluated for their in vitro antiproliferative activities against three human cancer cell lines including MDA-MB-231 (breast), BGC-823 (gastric), and SMMC-7721 (liver). Several compounds from the series exhibited excellent antitumor activities against all three tested cancer lines. Further their inhibitory activities against Raf kinase were investigated, and three compounds (**11c**, **11d,** and **11p**) demonstrated better activities than contrast drug Sorafenib. Especially compound **11c** was found to be a potent and selective Raf kinase inhibitor and could be considered as a candidate compound for further development.

1. Introduction

Although cancer remains a devastating diagnosis, successfully developed target-based therapies have significantly changed cancer treatment.[1] The Ras/Raf/MEK/ERK mitogen-activated protein kinase (MAPK) signaling cascade transmits mitogenic stimuli to the nucleus through a series of phosphorylation events, which is critical to the survival, growth, proliferation and apoptosis of cells and has been implicated in up to 30% of human cancers.[2–5] Raf serine/threonine protein kinase, existing in three isoforms (A-Raf, B-Raf, C-Raf or Raf-1), is a crucial component of this signal transduction pathway. All three Raf isoforms are able to interact with Ras and activate the MAPK signaling pathway.[6] Mutations of Raf lead to an aberrant activation of the signaling pathway, which ultimately results in increased proliferation and survival of cancer cells. Activating mutations in Raf have been observed in malignant melanomas (66%), papillary thyroid cancers (40–70%), and ovarian cancers (35%).[7] Therefore Raf kinase has been recognized as an attractive target for drug discovery in the treatment of cancer.[8]

A number of small molecule Raf kinase inhibitors containing diverse scaffolds have emerged in the recent past, which can be divided into several structural classes such as 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 Sorafenb.[11] Sorafenib (BAY 43-9006) is the first and only Raf kinase inhibitor that has received clinical approval by the Food and Drug Administration (FDA) and European Medicine Agency (EMEA) for the treatment of advanced renal cell carcinoma (RCC) and unresectable hepatocellular carcinoma (HCC) thus far.[12,13] However, it has been pointed out that its activity against certain tumor types may be through inhibition of other kinases, for example, VEGFR, rather than Raf for its lack of efficacy in patients expressing the V600E (formerly termed V599E) B-Raf mutation.[14,15] This prompted our efforts for developing more potent and selective Raf kinase inhibitors.

According to the crystal structure of V600E B-Raf kinase domains in complex with Sorafenib, the distal pyridyl ring of Sorafenib occupies the ATP adenine binding pocket and interacts with three amino acids residues (Trp530, Phe582, and Phe594), the ring nitrogen atom accepts a hydrogen bond from main chain nitrogen of Cys531 and the methyl amide side group contracts the main carbonyl of Cys531. The lipophilic trifluoromethyl phenyl ring at end side of the molecule inserts into a hydrophobic pocket. Importantly, the urea moiety of Sorafenib forms two hydrogen bonds with B-Raf, one with the backbone aspartate, and the other with the glutamate side chain.[16] We explored structural modification of Sorafenib on the basis of retaining the pyridyl ring as well as the urea functional moiety with the goal to optimize the activity on Raf kinase even further. Principal changes focused on hinge binding moiety, the linear methyl amide side group of pyridyl moiety was replaced with rigid trifluoromethyl imidazolyl group for

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http://dx.doi.org/10.1016/j.bmc.2012.05.051

enhancing interaction with Raf. Subsequently, a variety of hydrophobic aromatic rings were introduced to the opposite end of the inhibitors for the study of structure–activity relationships (SAR). Moreover, we investigated chloro group on central phenyl ring with a view to adding potency of inhibitors (Fig. 1). Accordingly, a series of novel bis-aryl ureas derivatives were designed and synthesized. All the prepared compounds (Table 1) were evaluated for their antiproliferative activities against three human cancer cell lines including MDA-MB-231 (breast), BGC-823 (gastric) and SMMC-7721 (liver) in vitro. Several compounds from the series exhibited excellent antitumor activities against all three tested cancer lines. Further their inhibitory activities against Raf kinase were investigated, and the selective profile of the best compound **11c** was assessed against a panel of 20 protein kinases.

2. Chemistry

The synthetic route of the target compounds **11a–11r** is illustrated in Scheme 1. The commercially available starting material 4-chloropicolinic acid **1** was treated with $SOCl_2$ in the presence of a catalytic amount of DMF and then ammonolyzed in CH_2Cl_2 to give intermediate **2**. Next, compound **2** was treated with trifluoroacetic anhydride in dry EtOAc to afford compound **3** as a white solid. Compound **3** was reacted with corresponding acetylamino phenols of general formula by S_NAr reaction to produce compounds **4** and **5**. The trifluoromethyl imidazolyl rings on compound **6** and **7** were prepared according to the literature.[17] which were one-pot reaction of the corresponding nitrile **3** with NH_4Ac and 3-bromo-1,1,1-trifluoroacetone in the presence of CH_3ONa as catalyst. Further, the obtained compound **6** and **7** were hydrolyzed in the dilute sulfuric acid to give the key intermediates **8** and **9**. A series of substituted aromatic isocyanates **10** were synthesized by treating corresponding commercial aromatic amines with tricarbonyl chloride in toluene. Finally, the target compounds **11a–11r** were successfully obtained via the reaction of key intermediates **8** and **9** with corresponding substituted aromatic isocyanates **10** in the presence of Et_3N in DMF. The products obtained were purified by column chromatography on silica gel. The chemical structures of these novel compounds were all confirmed by ^1H NMR, MS spectra and elementary analysis and the results are presented in Section 5.

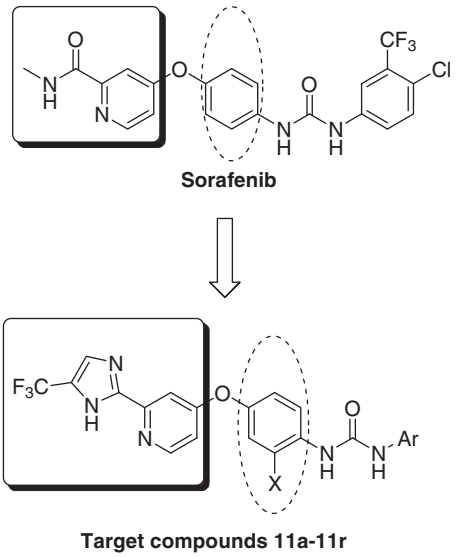
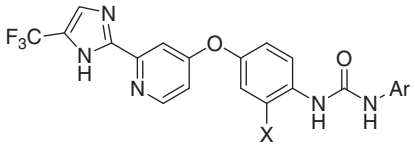
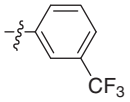
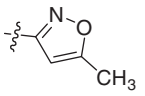
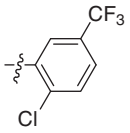
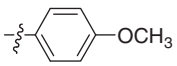
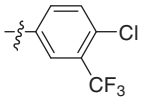
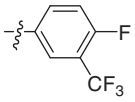
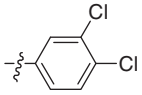
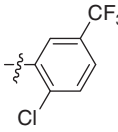
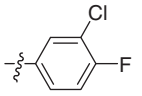
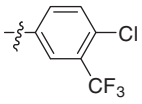
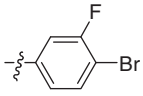
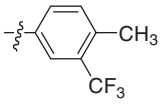
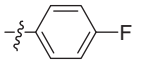
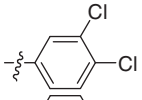
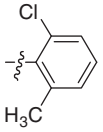
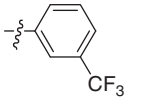
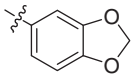
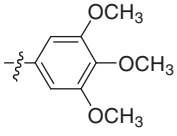


Figure 1. The structure of Sorafenib and the target compounds **11a–11r**.

Table 1
The preparation of target bis-aryl ureas compounds **11a–11r**

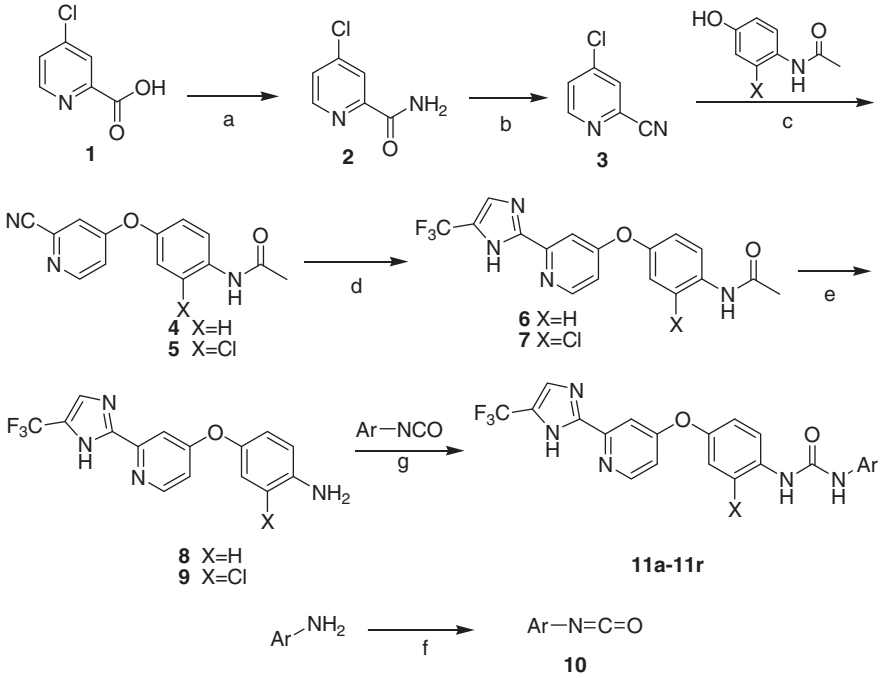


Compound	X	Ar	Compound	X	Ar
11a	H		**11j**	H	
11b	H		**11k**	H	
11c	H		**11l**	Cl	
11d	H		**11m**	Cl	
11e	H		**11n**	Cl	
11f	H		**11o**	Cl	
11g	H		**11p**	Cl	
11h	H		**11q**	Cl	
11i	H		**11r**	Cl	

3. Results and discussion

The in vitro antitumor activities of all the prepared compounds **11a–11r** were evaluated against three cancer cell lines consisting of MDA-MB-231 (breast), BGC-823 (gastric) and SMMC-7721 (liver) by the MTT assay and Sorafenib was used as the positive control drug. The IC_{50} (μM) values (concentration required to achieve 50% inhibition of the tumor growth) of the tested compounds on each cell line were presented in Table 2.

As shown in Table 2, most of the prepared compounds demonstrated moderate to excellent antiproliferative activities against three different cancer cell lines, and seven compounds (**11b–11d**, **11l–11n** and **11p**) exhibited more or similar potent activities against certain cancer lines in comparison with Sorafenib. Apparently, almost all of the tested compounds displayed better activities against MDA-MB-231 and SMMC-7721 than against BGC-823 cell line, which reflects selective behavior of the target compounds



Scheme 1. Synthetic route for the preparation of the target compounds. Reagents and conditions: (a) (1) $SOCl_2$, DMF, 80 °C, 3 h; (2) $NH_3{\cdot}H_2O$, CH_2Cl_2, 1 h; (b) Et_3N, trifluoroacetic anhydride, EtOAc, 0 °C; (c) K_2CO_3, DMF, 85 °C, 6 h; (d) NH_4Ac, CH_3ONa, 3-bromo-1,1,1-trifluoroacetone, *n*-PrOH, 85 °C, 21 h; (e) 20% H_2SO_4, 85 °C, 5 h; (f) tricarbonyl chloride, toluene, 80 °C, 8 h; (g) Et_3N, rt, 24 h.

Table 2
Antiproliferative activities of the target compounds **11a**–**11r** against MDA-MB-231, BGC-823 and SMMC-7721 cells in vitro

Compound	IC_{50}[a] (μM)		
	MDA-MB-231	BGC-823	SMMC-7721
11a	10.65 ± 0.27	26.19 ± 1.55	11.73 ± 0.69
11b	7.83 ± 0.31	18.37 ± 1.81	6.84 ± 0.54
11c	1.21 ± 0.14	13.15 ± 1.34	1.38 ± 0.11
11d	2.03 ± 0.40	10.70 ± 1.67	3.06 ± 0.33
11e	11.26 ± 0.48	15.66 ± 1.08	9.69 ± 1.20
11f	25.43 ± 1.13	66.80 ± 2.08	44.62 ± 2.03
11g	38.67 ± 1.87	23.74 ± 1.89	36.24 ± 1.83
11h	76.57 ± 2.39	83.98 ± 2.60	57.87 ± 1.92
11i	63.24 ± 2.13	>100[b]	92.36 ± 2.27
11j	44.80 ± 1.63	97.71 ± 2.03	87.66 ± 2.73
11k	95.36 ± 2.61	>100[b]	>100[b]
11l	8.21 ± 0.46	17.67 ± 1.57	5.72 ± 0.36
11m	7.03 ± 0.59	14.21 ± 1.32	5.29 ± 0.53
11n	3.77 ± 0.27	11.10 ± 1.03	6.52 ± 0.18
11o	10.11 ± 0.40	33.35 ± 1.20	8.38 ± 0.67
11p	1.77 ± 0.19	12.69 ± 1.96	2.49 ± 0.15
11q	14.24 ± 1.07	23.18 ± 2.57	9.72 ± 0.88
11r	>100[b]	>100[b]	>100[b]
Sorafenib	7.18 ± 0.17	10.91 ± 1.25	6.23 ± 0.21

[a] Results are expressed as means ± SD (standard deviation) of three independent experiments.
[b] Compounds with IC_{50} values >100 μM are considered to be inactive.

to some extent. To our delight, compound **11c** (with IC_{50} values = 1.21, 1.38 μM) displayed superior activities to Sorafenib (with IC_{50} values = 7.18, 6.23 μM) in both MDA-MB-231 and SMMC-7721 cell lines, which indicated that replacement of linear methylamide side group of pyridyl moiety with rigid trifluoromethyl imidazolyl ring was beneficial for improvement of antitumor activities. We hypothesized that the enhanced potency could arise from a series of contributing factors, including differences in size, electronic property, hydrogen-bond forming capability, and aqueous solubility.

A diverse electron withdrawing groups like fluoro, chloro, bromo, trifluoromethyl and electron donating groups like methyl, methoxy were introduced to the phenyl ring that connects with the urea group in the end of the inhibitors for investigation of structure–activity relationships (SAR). Compound **11a** bearing a trifluoromethyl group at the *meta* position of phenyl ring showed reasonable activities against both MDA-MB-231 and SMMC-7721 cell lines (with IC_{50} values = 10.65, 11.73 μM). Introduction of additional substituent like fluoro, chloro, methyl in other positions along with the trifluoromethyl group in the *meta* position were well tolerated (**11b**, **11c**, **11l**, **11o**). Introduction of a chloro group at *para* position (**11c**) was more effective than a fluoro group (**11l**) or a methyl group (**11o**), suggesting the importance of the substituent size at this position. Replacement of the trifluoromethyl group with chloro group at the *meta* position (**11d**) retained high potency. However, introduction of electron donating groups to phenyl ring or replacing phenyl ring with isoxazolyl ring led to remarkable decrease in antitumor activities (**11i**, **11k**, **11r**, **11j**). Surprisingly, introduction of a chloro group on the central phenyl ring did show moderate improvements in antitumor activities of the most molecules (compare **11a** and **11q**, **11b** and **11m**, and **11d** and **11p**), but led to a slight reduced potency of compound **11n** (compare **11c**).

In order to investigate whether these prepared compounds **11a**–**11r** inhibit Raf kinase or not, their inhibitory activities against C-Raf kinase further evaluated by the FRET assay.[18] Sorafenib was also used as the positive control drug. The IC_{50} (nM) values were summarized in Table 3. As clearly shown in Table 3, most of the tested compounds exhibited evident inhibitory potency against C-Raf. Moreover, compounds **11c**, **11d**, **11p** (with IC_{50} values = 10.6, 13.1, 16.5 nM) demonstrated better inhibitory activities than contrast drug Sorafenib (with IC_{50} values = 23.3 nM).

The kinase selectivity profile of these prepared compounds was assessed over 20 different protein kinases at a single dose concentration of 10 μM, using the best compound **11c** as a representative. The results listed in Table 4 revealed that compound **11c** has a very

Table 3
C-Raf kinase inhibitory activities of the target compounds **11a**–**11r** with Sorafenib as positive control

Compound	IC_{50}[a] (nM)	Compound	IC_{50}[b] (nM)
11a	87.8	**11k**	NT[b]
11b	75.9	**11l**	77.7
11c	10.6	**11m**	35.9
11d	13.1	**11n**	22.1
11e	69.2	**11o**	46.7
11f	81.5	**11p**	16.5
11g	94.9	**11q**	64.8
11h	120.3	**11r**	NT[b]
11i	NT[b]	Sorafenib	24.3
11j	147.9		

[a] Values are means of three experiments.
[b] NT: not tested.

good selectivity profile. While the compound displayed an inhibition percentage of 95.3% on C-Raf, the inhibitions exhibited on most other kinases were below 40%.

4. Conclusions

In conclusion, we have designed and synthesized a series of novel bis-aryl ureas derivatives, evaluated their antiproliferative activities against three human cancer cell lines (MDA-MB-231, BGC-823 and SMMC-7721) and inhibitory activities against C-Raf kinase in vitro. The test results showed that most of the prepared compounds demonstrated moderate to excellent antiproliferative activities and C-Raf inhibitory activities. Compounds **11c**, **11d**, **11p** exhibited more potent activities in comparison with contrast drug Sorafenib, especially compound **11c** showed a very good selectivity profile and could be considered as a candidate compound for further development. Moreover, from the structure–activity relationships (SAR) we may conclude that replacement of linear methylamide side group of Sorafenib with rigid trifluoromethyl imidazolyl ring was beneficial for improvement of antitumor activities, and introduction of a chloro group on the central phenyl ring further optimized inhibitory potency to some extent. The in vivo anticancer activities and detailed SAR of the compounds are currently under investigation in our laboratory.

5. Experimental section

5.1. Chemistry: General procedures

All reagents and solvents were purchased from commercial sources and used without further purification. Melting points were measured in open capillaries and are uncorrected. ^{1}H-NMR was recorded in DMSO-d_6 on a Bruker Avance 400 spectrometer; chemical shifts (δ) are reported in parts per million (ppm) relative to tetramethylsilane (TMS), used as an internal standard. Mass spectra (MS) were obtained from Agilent 1100 LC/MS Spectrometry Services. Elemental analyses were carried out on a CHN-O-rapid elemental analyzer (GmbH-Germany) for C, H and N, and the results are within ±0.4% of the theoretical values.

Table 4
Inhibition percentages of compound **11c** at a single dose concentration of 10 μM over 20 protein kinases

Kinase	Inhibition (%)	Kinase	Inhibition (%)
ABL1	44.3	IKKβ	5.6
AKT1	11.7	JAK2	0
c-Kit	23.9	PDGFRα	0
c-MET	2.2	PTK2	4.9
C-Raf	95.3	PKA	1.3
CDK2	7.8	PLK1	8.7
FLT3	17.7	KDR	3.5
FMS	50.8	SYK	20.4
FYN	0.4	TIE2	27.5
FGFR1	20.2	VEGFR2	36.8

5.1.1. 4-Chloropicolinamide (2)

A mixture of 4-chloropicolinic acid (10.0 g, 63.4 mmol), thionyl chloride (40 ml) and a catalytic amount of DMF were heated at 80 °C for 3 h. Then the reaction mixture was cooled to room temperature and evaporated under reduced pressure. The residue was dissolved in 30 ml of CH_2Cl_2 in an ice-bath, then added to a 25% aqueous solution of ammonia (60 ml) at a rate which kept the internal temperature below 7 °C with constant stirring. After 1 h, the precipitate was filtrated, washed with water (3 × 50 ml) and dried in vacuo to afford compound **2** (9.2 g, 93% yield) as a yellow solid, mp 161–162 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.74–7.76 (dd, J = 1.8 and 5.3 Hz, 1H), 7.80 (br s, 1H), 8.03 (d, J = 1.8 Hz, 1H), 8.20 (br s, 1H), 8.62 (d, J = 5.3 Hz, 1H); ESI-MS m/z: 157[M+H]$^+$; Anal. Calcd for $C_6H_5ClN_2O$ (%): C 46.03, H 3.22, N 17.89; Found: C 45.98, H 3.27, N 17.94.

5.1.2. 4-Chloropicolinonitrile (3)

The compound **2** (7.0 g, 44.7 mmol) and triethylamine (13 ml, 93.7mmol) were dissolved in dry EtOAc (50 ml) and the stirred solution was cooled to −5 °C in an ice-salt bath. Trifluoroacetic anhydride (13 ml, 92.2 mmol) was added dropwise to the chilled mixture over 45 min. The ice-salt bath was then removed and the reaction was warmed to room temperature and then stirred for another 30 min. The completion of reaction was detected by TLC, 10% aqueous K_2CO_3 (100 ml) was added, and the reaction mixture was allowed to stir for further 20 min. The mixture was extracted with EtOAc (3 × 50 ml), the combined organic layers were washed with brine (2 × 50 ml), dried over anhydrous Na_2SO_4, and concentrated under reduced pressure to give compound **3** (5.6 g, 90% yield) as a white solid, mp 84–85 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.54–7.56 (dd, J = 5.0 and 2.0 Hz, 1H), 7.74 (m, 1H), 8.65 (d, J = 5.0 Hz, 1H); ESI-MS m/z: 139[M+H]$^+$; Anal. Calcd for $C_6H_3ClN_2$ (%): C 52.01, H 2.18, N 20.22; Found: C 51.94, H 2.14, N 20.28.

5.1.3. Procedure for preparation of compound (4) and (5)

The compound **3** (2.8 g, 20.2 mmol) was added to a suspension of N-(4-hydroxyphenyl) acetamide (4.6 g, 30.3 mmol) and K_2CO_3 (5.6 g, 40.4 mmol) in dry DMF (30 ml), and the reaction was stirred at 85 °C for 5 h. The resulting mixture was cooled to room temperature, and then poured into ice water. The precipitate was collected by filtration and washed with water (3 × 50 ml) to give compound **4** (4.3 g, 85% yield) as a white solid, mp 173–175 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.08 (s, 3H), 7.14(d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 7.55–7.57 (dd, J = 2.1 and 5.3 Hz, 1H), 7.77 (d, J = 2.1 Hz, 1H), 8.59 (d, J = 5.3 Hz, 1H), 9.23 (s, 1H); ESI-MS m/z: 254[M+H]$^+$; Anal. Calcd for $C_{14}H_{11}N_3O_2$ (%): C 66.40, H 4.38, N 16.59; Found: C 66.33, H 4.35, N 16.65.

Compound **5**. Yield: 83%, off-white solid, mp 161–163 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.11 (s, 3H), 7.15 (m, 1H), 7.37–7.44 (m, 2H), 7.54–7.56 (dd, J = 1.8 and 4.9 Hz, 1H), 7.75 (d, J = 1.8 Hz, 1H), 8.57 (d, J = 4.9 Hz, 1H), 9.25 (s, 1H); ESI-MS m/z: 289[M+H]$^+$; Anal. Calcd for $C_{14}H_{10}ClN_3O_2$ (%): C 58.45, H 3.50, N 14.61; Found: C 58.39, H 3.54, N 14.68.

5.1.4. Procedure for preparation of compound (6) and (7)

A mixture of compound **4** (4.0 g, 15.8 mmol) and NH_4Ac (3.6 g, 47.4 mmol) in n-PrOH was stirred under nitrogen atmosphere at room temperature during addition of CH_3ONa (1.0 g, 19.0 mmol).

The reaction mixture was stirred at 70 °C for 1 h, then treated with dropwise addition of 3-bromo-1,1,1-trifluoroacetone (4 ml , 28.4 mmol). The mixture was heated to 85 °C and the completion of reaction was proved by TLC after stirring for another 20 h. The mixture was poured into water (50 ml), extracted with EtOAc (3 × 50 ml). The combined organic layers were dried over anhydrous Na_2SO_4, and evaporated under reduced pressure, the crude residue was purified by chromatography on a silica gel column using EtOAc/Hexane (1/2) as eluent to give 6 (3.3 g, 58% yield) as a light yellow solid, mp 182–184 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.09 (s, 3H), 7.05–7.07 (dd, J = 2.0 and 5.3 Hz, 1H), 7.22 (d, J = 8.7 Hz, 2H), 7.46 (d, J = 2.0 Hz, 1H), 7.60 (d, J = 8.7 Hz, 2H), 7.87 (s, 1H), 8.55 (d, J = 5.3Hz, 1H), 9.32 (s, 1H), 13.45 (s, 1H); ESI-MS m/z: 363[M+H]$^+$; Anal. Calcd for $C_{17}H_{13}F_3N_4O_2$ (%): C 56.36, H 3.62, N 15.46; Found: C 56.29, H 3.67, N 15.49.

Compound **7**. Yield: 63%, yellow solid, mp 197–199 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.06 (s, 3H), 7.04–7.06 (dd, J = 2.0 and 5.1 Hz, 1H), 7.19–7.22 (m, 1H), 7.34–7.46 (m, 3H), 7.84 (s, 1H), 8.53 (d, J = 5.3 Hz, 1H), 9.37 (s, 1H), 13.45 (s, 1H); ESI-MS m/z: 398[M+H]$^+$; Anal. Calcd for $C_{17}H_{12}ClF_3N_4O_2$ (%): C 51.46, H 3.05, N 14.12; Found: C 51.42, H 3.01, N 14.18.

5.1.5. Procedure for preparation of compound (8)and (9)

A mixture of compound **6** (3.3 g, 9.1 mmol) in 20% of dilute sulfuric acid (20 ml) was stirred at 85 °C under nitrogen atmosphere for 5 h. The resulting mixture was cooled to room temperature and alkalized with a 10% NaOH aqueous solution to pH 8–9. The precipitate was filtrated, washed with water (3 × 20 ml), and dried in vacuo to give 8 (2.6 g, 90% yield)as a red brown solid, mp 165–167 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.19 (br s, 2H), 6.82 (d, J = 9.5 Hz, 2H), 7.05–7.07 (dd, J = 1.8 and 5.2 Hz, 1H), 7.20 (d, J = 9.5 Hz, 2H), 7.41 (d, J = 1.8 Hz, 1H), 7.85 (s, 1H), 8.56 (d, J = 5.2 Hz, 1H), 13.43 (s, 1H); ESI-MS m/z: 321[M+H]$^+$; Anal. Calcd for $C_{15}H_{11}F_3N_4O$ (%): C 56.25, H 3.46, N 17.49; Found: C 56.22, H 3.50, N 17.46.

Compound **9**. Yield: 92%, red solid, mp 173–175 °C, ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.43 (br s, 2H), 6.82 (m, 2H) 7.04–7.06 (dd, J = 2.0 and 5.0 Hz, 1H), 7.20 (m, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.89 (s, 1H), 8.57 (d, J = 5.0 Hz, 1H), 13.51 (s, 1H); ESI-MS m/z: 356[M+H]$^+$; Anal. Calcd for $C_{15}H_{10}ClF_3N_4O$ (%): C 50.79, H 2.84, N 15.79; Found: C 50.84, H 2.80, N 15.77.

5.1.6. General procedure for preparation of substituted aromatic isocyanate (10)

To a stirred and cooled to 0 °C solution of tricarbonyl chloride (1 equiv) in toluene (20 ml), a solution of different aromatic amines (3 equiv) in 10 ml of CH_2Cl_2 was added dropwise under nitrogen atmosphere in an ice-bath. Upon completion of addition, the reaction mixture was stirred for 0.5 h at room temperature, and for another 6–8 h at 80–85 °C. The completion of reaction was detected by TLC, the solvent was removed under vacuum to obtain (**10**) as an oil which solidified and was stable at 0–7 °C.

5.1.7. General procedure for the synthesis of compounds (11a–11r)

To a solution of compound **8** or **9** (1 equiv) in DMF (20 ml), triethylamine (2 equiv) and corresponding substituted aromatic isocyanate **10** (1.1 equiv) were added under nitrogen atmosphere, and the mixture was stirred overnight at room temperature. The reaction mixture was quenched by addition of water and diluted with EtOAc. The organic layer was washed with brine and dried over anhydrous Na_2SO_4. After filtration and concentration, the crude product was purified by column chromatography (EtOAc/hexane) to obtain the desired bis-aryl ureas compounds **11a**–**11r** in 50–80% yield.

5.1.7.1. 1-(4-(2-(5-(Trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (11a).

White solid, yield: 55%, mp 115–118 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.09 (dd, J = 1.8 and 5.0 Hz, 1H), 7.22 (d, J = 7.7 Hz, 2H), 7.32 (d, J = 8.5 Hz, 1H), 7.40 (d, J = 1.7 Hz, 1H), 7.51–7.53 (m, 1H), 7.59–7.63 (m, 3H), 7.88 (s, 1H), 8.05 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.98 (s, 1H), 9.13 (s, 1H), 13.58 (s, 1H); ESI-MS m/z: 508[M+H]$^+$; Anal. Calcd for $C_{23}H_{15}F_6N_5O_2$ (%): C 54.44, H 2.98, N 13.80; Found: C 54.49, H 2.94, N 13.78.

5.1.7.2. 1-(2-Chloro-5-(trifluoromethyl)phenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11b).

White solid, yield: 57%, mp 219–220 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.09 (dd, J = 1.7 and 5.0 Hz, 1H), 7.24 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 1.7 Hz, 2H), 7.62–7.75 (m, 3H), 7.88 (s, 1H), 8.54–8.56 (m, 2H), 8.68 (s, 1H), 9.75 (s, 1H), 13.58 (s, 1H); ESI-MS m/z: 543[M+H]$^+$; Anal. Calcd for $C_{23}H_{14}ClF_6N_5O_2$ (%): C 50.98, H 2.60, N 12.93; Found: C 51.04, H 2.57, N 12.88.

5.1.7.3. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11c).

White solid, yield: 61%, mp 122–124 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.09 (dd, J = 1.7 and 5.1 Hz, 1H), 7.23 (d, J = 8.0 Hz, 2H), 7.40 (d, J = 1.7 Hz, 1H), 7.60–7.66 (m, 4H), 7.87 (s, 1H), 8.14 (d, J = 7.5 Hz, 1H), 8.55 (d, J = 5.1 Hz ,1H), 9.03 (s, 1H), 9.25 (s, 1H), 13.57 (s, 1H); ESI-MS m/z: 543[M+H]$^+$; Anal. Calcd for $C_{23}H_{14}ClF_6N_5O_2$ (%): C 50.98, H 2.60, N 12.93; Found: C 51.02, H 2.65, N 12.67.

5.1.7.4. 1-(3,4-Dichlorophenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11d).

White solid, yield: 70%, mp 116–118 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.40 (m, 5H), 7.53–7.62 (m, 3H), 7.88–7.91 (m, 2H), 8.55 (d, J = 5.2 Hz, 1H), 8.99 (s, 1H), 9.09 (s, 1H), 13.58 (s, 1H); ESI-MS m/z: 509[M+H]$^+$; Anal. Calcd for $C_{22}H_{14}Cl_2F_3N_5O_2$ (%): C 51.99, H 2.78, N 13.78; Found: C 52.02, H 2.74, N 13.73.

5.1.7.5. 1-(3-Chloro-4-fluorophenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11e).

White solid, yield: 75%, mp 210–211 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.06–7.07 (dd, J = 1.9 and 5.3 Hz, 1H), 7.20 (d, J = 7.7 Hz, 2H), 7.33–7.41 (m, 3H), 7.60 (d, J = 7.7 Hz, 2H), 7.81–7.85 (m, 2H), 8.55 (d, J = 5.3 Hz, 1H), 8.92 (s, 1H), 8.94 (s, 1H), 13.53 (s, 1H); ESI-MS m/z: 493[M+H]$^+$; Anal. Calcd for $C_{22}H_{14}ClF_4N_5O_2$ (%): C 53.73, H 2.87, N 14.27; Found: C 53.77, H 2.85, N 14.23.

5.1.7.6. 1-(4-Bromo-2-fluorophenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11f).

White solid, yield: 69%, mp 232–234 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.05–7.07 (dd, J = 1.8 and 5.2 Hz, 1H), 7.21 (d, J = 8.0 Hz, 2H), 7.36–7.40 (m, 2H), 7.57–7.60 (m, 3H), 7.85 (s, 1H), 8.12–8.17 (m, 1H), 8.54 (d, J = 5.2 Hz, 1H), 8.70 (s, 1H), 9.23 (s, 1H), 13.53 (s, 1H); ESI-MS m/z: 537[M+H]$^+$; Anal. Calcd for $C_{22}H_{14}BrF_4N_5O_2$ (%): C 49.27, H 2.63, N 13.06; Found: C 49.23, H 2.59, N 13.09.

5.1.7.7. 1-(4-Fluorophenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11g).

White solid, yield: 56%, mp 236–238 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.21 (m, 5H), 7.39 (d, J = 1.8 Hz, 2H), 7.60 (d, J = 7.7 Hz, 2H), 7.67–7.73 (m, 1H), 7.88 (s, 1H), 8.55 (d, J = 5.0 Hz, 1H), 8.78 (s, 1H), 9.25 (s, 1H), 13.57 (s, 1H); ESI-MS m/z: 458[M+H]$^+$; Anal. Calcd for $C_{22}H_{15}F_4N_5O_2$ (%): C 57.77, H 3.31, N 15.31; Found: C 57.74, H 3.36, N 15.33.

5.1.7.8. 1-(2-Chloro-6-methylphenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11h). White solid, yield: 77%, mp 243–245 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.28 (s, 3H), 7.03–7.41 (m, 7H), 7.60 (d, J = 7.9 Hz, 2H), 7.85 (s, 1H), 8.01 (s, 1H), 8.53 (d, J = 4.9 Hz, 1H), 9.06 (s, 1H), 13.52 (s, 1H); ESI-MS m/z: 489[M+H]$^+$; Anal. Calcd for $C_{23}H_{17}ClF_3N_5O_2$ (%): C 56.62, H 3.51, N 14.36; Found: C 56.65, H 3.48, N 14.39.

5.1.7.9. 1-(Benzo[*d*][1,3]dioxol-5-yl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11i). White solid, yield: 59%, mp 212–213 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 5.97 (s, 2H), 7.06–7.07 (dd, J = 1.9 and 5.3 Hz, 1H), 7.17–7.23 (m, 3H), 7.32–7.38 (m, 2H), 7.74 (d, J = 7.2 Hz, 2H), 7.87 (s, 1H), 8.31 (s, 1H), 8.50 (s, 1H), 8.53 (d, J = 5.3 Hz, 1H), 10.38 (s, 1H), 13.49 (s, 1H); ESI-MS m/z: 484[M+H]$^+$; Anal. Calcd for $C_{23}H_{16}F_3N_5O_4$ (%): C 57.15, H 3.34, N 14.49; Found: C 57.13, H 3.38, N 14.37.

5.1.7.10. 1-(5-Methylisoxazol-3-yl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11j). White solid, yield: 64%, mp 238–240 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.33–2.37 (s, 3H), 6.55 (s, 1H), 7.05–7.07 (dd, J = 1.9 and 5.3 Hz, 1H), 7.21 (d, J = 7.8 Hz, 2H), 7.40 (d, J = 1.9 Hz, 1H), 7.60 (d, J = 7.8 Hz, 2H), 7.85 (s, 1H), 8.54 (d, J = 5.3 Hz, 1H), 8.97 (s, 1H), 9.50 (s, 1H), 13.53 (s, 1H); ESI-MS m/z: 445[M+H]$^+$; Anal. Calcd for $C_{20}H_{15}F_3N_6O_3$ (%): C 54.06, H 3.40, N 18.91; Found: C 54.03, H 3.38, N 18.96.

5.1.7.11. 1-(4-Methoxyphenyl)-3-(4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11k). White solid, yield: 72%, mp 240–241 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.91 (s, 3H), 7.03–7.43 (m, 8H), 7.60 (d, J = 8.0 Hz, 2H), 7.86 (s, 1H), 8.03 (s, 1H), 8.53 (d, J = 5.2 Hz, 1H), 9.06 (s, 1H), 13.54 (s, 1H); ESI-MS m/z: 470[M+H]$^+$; Anal. Calcd for $C_{23}H_{18}F_3N_5O_3$ (%): C 58.85, H 3.86, N 14.92; Found: C 58.90, H 3.88, N 14.88.

5.1.7.12. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(4-fluoro-3-(trifluoromethyl)phenyl)urea (11l). White solid, yield: 67%, mp 132–135 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.08–7.10 (dd, J = 1.9 and 5.3 Hz, 1H), 7.25–7.28 (dd, J = 8.0 and 7.3 Hz, 1H), 7.44–7.65 (m, 4H), 7.87 (s, 1H), 8.02–8.04 (dd, J = 8.0 and 7.5 Hz, 1H), 8.24 (d, J = 7.5 Hz, 1H), 8.48 (s, 1H), 8.56 (d, J = 5.3 Hz, 1H), 9.79 (s, 1H), 13.56 (s, 1H); ESI-MS m/z: 561[M+H]$^+$; Anal. Calcd for $C_{23}H_{13}ClF_7N_5O_2$ (%): C 49.35, H 2.34, N 12.51; Found: C 49.38, H 2.29, N 12.56.

5.1.7.13. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(2-chloro-5-(trifluoromethyl)phenyl)urea (11m). White solid, yield: 68%, mp 249–250 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.11–7.12 (dd, J = 1.9 and 5.3 Hz, 1H), 7.26–7.55 (m, 4H), 7.75 (d, J = 7.8 Hz, 1H), 7.90 (s, 1H), 8.19 (m, 1H), 8.57–8.58 (m, 2H), 9.35 (s, 1H), 9.37 (s, 1H), 13.61 (s, 1H); ESI-MS m/z: 577[M+H]$^+$; Anal. Calcd for $C_{23}H_{13}Cl_2F_6N_5O_2$ (%): C 47.94, H 2.27, N 12.15; Found: C 47.91, H 2.24, N 12.19.

5.1.7.14. 1-(4-Chloro-3-(trifluoromethyl)phenyl)-3-(2-chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)urea (11n). White solid, yield: 75%, mp 200–203 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.10–7.12 (dd, J = 1.8 and 5.3 Hz, 1H), 7.26–7.28 (dd, J = 6.5 and 7.9 Hz, 1H), 7.53 (d, J = 8.0 Hz, 2H), 7.63–7.66 (m, 2H), 7.92 (s, 1H), 8.15 (s, 1H), 8.22 (d, J = 6.9 Hz, 1H), 8.57 (d, J = 5.2 Hz, 1H), 8.70 (s, 1H), 10.34 (s, 1H), 13.64 (s, 1H); ESI-MS m/z: 577[M+H]$^+$; Anal. Calcd for $C_{23}H_{13}Cl_2F_6N_5O_2$ (%): C 47.94, H 2.27, N 12.15; Found: C 47.97, H 2.24, N 12.19.

5.1.7.15. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(4-methyl-3-(trifluoromethyl)phenyl)urea (11o). White solid, yield: 64%, mp 263–264 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 2.50 (s, 3H), 7.09–7.11 (dd, J = 2.1 and 5.2 Hz, 1H), 7.25–7.28 (m, 1H), 7.39 (d, J = 2.1 Hz, 1H), 7.43–7.44 (m, 1H), 7.49–7.54 (m, 2H), 7.88 (s, 1H), 7.97 (d, J = 8.9 Hz, 1H), 8.25–8.27 (m, 1H), 8.44 (s, 1H), 8.57 (d, J = 5.2 Hz, 1H), 9.67 (s, 1H), 13.59 (s, 1H); ESI-MS m/z: 557[M+H]$^+$; Anal. Calcd for $C_{24}H_{16}ClF_6N_5O_2$ (%): C 51.86, H 2.90, N 12.60; Found: C 51.81, H 2.88, N 12.63.

5.1.7.16. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(3,4-dichlorophenyl)urea (11p). White solid, yield: 58%, mp 269–270 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.08–7.10 (dd, J = 2.0 and 5.0 Hz, 1H), 7.22–7.56 (m, 5H), 7.88 (s, 1H), 7.92 (d, J = 9.5 Hz, 1H), 8.22 (d, J = 9.5 Hz, 1H), 8.50 (s, 1H), 8.57 (d, J = 5.0 Hz, 1H), 9.75 (s, 1H), 13.56 (s, 1H); ESI-MS m/z: 544[M+H]$^+$; Anal. Calcd for $C_{22}H_{13}Cl_3F_3N_5O_2$ (%): C 48.69, H 2.41, N 12.90; Found: C 48.63, H 2.48, N 12.83.

5.1.7.17. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(3-(trifluoromethyl)phenyl)urea (11q). White solid, yield: 67%, mp 213–215 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 7.07–7.09 (dd, J = 2.0 and 5.1 Hz, 1H), 7.23 (d, J = 9.1 Hz, 2H), 7.32–7.34 (m, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.51–7.55 (m, 1H), 7.59–7.63 (m, 3H) , 7.88 (s, 1H), 8.04 (s, 1H), 8.55 (d, J = 5.1 Hz, 1H), 9.13 (s, 1H), 13.58 (s, 1H); ESI-MS m/z: 543[M+H]$^+$; Anal. Calcd for $C_{23}H_{14}ClF_6N_5O_2$ (%): C 50.98, H 2.60, N 12.93; Found: C 51.02, H 2.64, N 12.88.

5.1.7.18. 1-(2-Chloro-4-(2-(5-(trifluoromethyl)-1*H*-imidazol-2-yl)pyridin-4-yloxy)phenyl)-3-(3,4,5-trimethoxyphenyl)urea (11r). White solid, yield: 59%, mp 255–258 °C; ^{1}H NMR (DMSO-d_6, 400 MHz): δ 3.77 (s, 9H), 6.66 (s, 2H), 7.06–7.08 (dd, J = 1.9 and 5.2 Hz, 1H), 7.19–7.22 (m, 1H), 7.44–7.45 (m, 3H), 7.87 (s, 1H), 8.22 (s, 1H), 8.27 (s, 1H), 8.55 (d, J = 5.2 Hz, 1H), 13.60 (s, 1H); ESI-MS m/z: 565[M+H]$^+$; Anal. Calcd for $C_{25}H_{21}ClF_3N_5O_5$ (%): C 53.25, H 3.75, N 12.42; Found: C 53.22, H 3.74, N 12.49.

5.2. Biological assay

5.2.1. The assay of antiproliferative activities

The antiproliferative activities of the target compounds **11a**–**11r** were evaluated with MDA-MB-231, BGC-823 and SMMC-7721 cell lines by the MTT assay in vitro, with Sorafenib as the positive control drug. The cancer cell lines 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 5.0×10^3 cells/well and incubated for 24 h. The cells were then incubated for another 72 h with various concentrations of compounds **11a**–**11r**. Subsequently, 20 μL of fresh MTT solution (5 mg/ ml) was added to each well and incubated with cells at 37 °C for 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. All of the compounds were tested three times in each of the cell lines. The IC_{50} values were calculated by linear regression analysis, expressed in mean ± SD.

5.2.2. The assay of inhibitory activities against C-Raf kinase

The inhibitory activities against C-Raf kinase of the target compounds 11a–11r were investigated by the FRET assay, Sorafenib was also used as positive control drug. 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 myelin basic protein, 1 mM DTT, 0.1 mg/ml BSA) to 4 and 20 μg/ml, 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-[^{33}P] ATP (specific activity approx. 500 cpm/pmol) for incubation at 30 °C for 20 min. The reaction was spotted onto a phosphocellulose mat, washed with 1% phosphoric acid solution, and counted the radioactivity in the presence of scintillation fluid in a scintillation counter.

Acknowledgment

The generous financial support of this work by the National Natural Science Foundation of China (30973016) is gratefully acknowledged.

References and notes

1. Ma, W. W.; Adjei, A. A. *CA Cancer J. Clin.* **2009**, *59*, 111.
2. Weber, C. K.; Slupsky, J. R.; Herrmann, C.; Schuler, M.; Rapp, U. R.; Block, C. *Oncogene* **2000**, *19*, 169.
3. McCubrey, J. A.; Steelman, L. S.; Abrams, S. L.; Chappell, W. H.; Russo, S.; Ove, R.; Michele, M.; Tafuri, A.; Lunghi, P.; Bonati, A.; Stivala, F.; Nicoletti, F.; Libra, M.; Martelli, A. M.; Montalto, G.; Cervello, M. *Expert Opin. Emerg. Drugs* **2009**, *14*, 633.
4. McCubrey, J. A.; Steelman, L. S.; Chappell, W. H.; Abrams, S. L.; Wong, E. W. T.; Chang, F.; Lehmann, B.; Terrian, D. M.; Milella, M.; Tafuri, A.; Stivala, F.; Libra, M.; Basecke, J.; Evangelisti, C.; Martelli, A. M.; Franklin, R. A. *Biochim. Biophys. Acta* **2007**, *1773*, 1263.
5. Roberts, P. J.; Der, C. J. *Oncogene* **2007**, *26*, 3291.
6. Chong, H.; Vikis, H. G.; Guan, K. L. *Cell. Signalling* **2003**, *15*, 463.
7. Smith, A. L.; Demorin, F. F.; Paras, N. A.; Huang, Q.; Petkus, J. K.; Doherty, E. M.; Nixey, T.; Kim, J. L.; Whittington, D. A.; Epstein, L. F.; Lee, M. R.; Rose, M. J.; Babij, C.; Fernando, M.; Hess, K.; Le, Q.; Beltran, P.; Carnahan, J. *J. Med. Chem.* **2009**, *52*, 6189.
8. Sridhar, S. S.; Hedley, D.; Siu, L. L. *Mol. Cancer Ther.* **2005**, *4*, 677.
9. Li, H. F.; Lu, T.; Zhu, T.; Jiang, Y. J.; Rao, S. S.; Hu, L. Y.; Xin, B. T.; Chen, Y. D. *Eur. J. Med. Chem.* **2009**, *44*, 1240.
10. Wong, K. K. *Recent Patents Anticancer Drug Discov.* **2009**, *4*, 28.
11. Smith, R. A.; Dumas, J.; Adnane, L.; Wilhelm, S. M. *Curr. Top. Med. Chem.* **2006**, *1071*, 6.
12. Montagut, C.; Settleman, J. *Cancer Lett.* **2009**, *283*, 125.
13. Ramnath, N.; Adjei, A. *Update Cancer Ther.* **2007**, *2*, 111.
14. Eisen, T.; Ahmad, T.; Flaherty, K. T.; Gore, M.; Kaye, S.; Marais, R.; Gibbens, I.; Hackett, S.; James, M.; Schuchter, L. M.; Nathanson, K. L.; Xia, C.; Simantov, R.; Schwartz, B.; Poulin-Costello, M.; O'Dwyer, P. J.; Ratain, M. J. *Br. J. Cancer* **2006**, *95*, 581.
15. Ramurthy, S.; Subramanian, S.; Aikawa, M.; Amiri, P.; Costales, A.; Dove, J.; Fong, S.; Jansen, J. M.; Levine, B.; Ma, S.; Mcbride, C. M.; Michaelian, J.; Pick, T.; Poon, D. J.; Girish, S.; Shafer, C. M.; Stuart, D.; Sung, L.; Renhowe, P. A. *J. Med. Chem.* **2008**, *51*, 7049.
16. Wan, P. T.; Garnett, M. J.; Ros, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R. *Cell* **2004**, *116*, 856.
17. Dimitroff, M.; Miller, B. R.; Stillwell, B. S.; Siesel, D. A.; Swiftney, T.; Diaz, B.; Gu, D.; Ryckman, D.; Poon, D. J.; Pick, T. E. US20070049622A1.
18. Khire, U. R.; Bankston, D.; Barbosa, J.; Brittelli, D. R.; Caringal, Y.; Carlson, R.; Dumas, J.; Gane, T.; Heald, S. L.; Hibner, B.; Johnson, J. S.; Katz, M. E.; Kennure, N.; Kingery-Wood, J.; Lee, W.; Liu, X. G.; Lowinger, T. B.; Mcalexander, I.; Monahan, M. K.; Natero, R.; Renick, J.; Riedl, B.; Rong, H.; Sibley, R. N.; Smith, R. A.; Wolanin, D. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 783.