Full Paper

Synthesis of 1*H*-Pyrazole-1-carboxamide Derivatives and Their Antiproliferative Activity against Melanoma Cell Line

Mohammed I. El-Gamal[1,2], Tae Bo Sim[1], Jun Hee Hong[3], Jung-Hyuck Cho[1], Kyung Ho Yoo[1], and Chang-Hyun Oh[1,2]

[1] Biomaterials Center, Korea Institute of Science and Technology, Cheongryang, Seoul, Republic of Korea
[2] Department of Biomolecular Science, University of Science and Technology, Daejeon, Republic of Korea
[3] College of Pharmacy, Chosun University, Gwangju, Republic of Korea

Synthesis of a new series of 1*H*-pyrazole-1-carboxamide derivatives is described. Their antiproliferative activity against A375 human melanoma cell line was tested and the effect of substituents on the diarylpyrazole scaffold was investigated. The pharmacological results indicated that most of the newly synthesized compounds showed moderate activity against A375, compared with sorafenib. Among all of these derivatives, compound **IIe** which has *N*-methylpiperazinyl and phenolic moieties showed the most potent antiproliferative activity against A375 human melanoma cell line.

Keywords: A375 / Antiproliferative activity / 3,4-Diarylpyrazole / Melanoma / 1*H*-Pyrazole-1-carboxamide

Received: February 23, 2010; Revised: April 26, 2010; Accepted: April 26, 2010

DOI 10.1002/ardp.201000057

Introduction

Melanoma is the most aggressive form of skin cancer and is the fastest growing cancer in the United States [1, 2]. Early stage melanoma can be cured surgically. However, melanoma metastasizing to major organs (stage IV) is virtually incurable [2]. Patients with advanced melanoma have a median survival time of less than one year, and the estimated 5-year survival time is less than 15% [2, 3]. With the incidence of melanoma rapidly rising in the United States and other developed countries, there is an urgent need to develop more effective drugs [4–6].

The current treatments involve surgical removal of the tumor, immunotherapy, radiotherapy, chemotherapy, various combinations, or the use of new treatments in clinical trials. As for immunotherapy, interferon alfa-2b (intron-A) [7] has been approved by both the FDA and EMEA for adjuvant treatment of melanoma patients, and aldesleukin (proleukin) [8, 9] is also approved for the treatment of metastatic melanoma in the USA.

Correspondence: Chang-Hyun Oh, Biomaterials Center, Korea Institute of Science and Technology, PO Box 131, Cheongryang, Seoul 130-650, Republic of Korea,
E-mail: choh@kist.re.kr
Fax: +82-2-958-5189.

After reviewing the literature, we found many promising, potent, and selective antiproliferative agents for treatment of melanoma. The most famous one of these agents is sorafenib [10–15]. Sorafenib is an oral multikinase inhibitor that targets 2 classes of kinases which are known to be involved in both tumor proliferation and angiogenesis [16]. It inhibits RAF kinases (RAF-1 and B-RAF), as well as proangiogenic receptor tyrosine kinases of the PDGFR and VEGFR family [10]. The antiproliferative activity of sorafenib against melanoma is assumed to be due to B-RAF inhibition and induction of apoptosis in a caspase-independent manner [17]. Sorafenib demonstrated high antiproliferative activity against different melanoma xenografts and cell lines [17], but not in case of advanced metastatic melanoma (stage IV) [16]. In addition, sorafenib has been implicated in the development of reversible posterior leukoencephalopathy syndrome and secondary erythrocytosis [18]. The poor efficiency of sorafenib in case advanced metastatic melanoma and its side effects encourage the search for new antiproliferative compounds for treatment of melanoma.

Moreover, antiproliferative agents with 3,4-diarylpyrazole scaffold targeting B-RAF kinase and can be efficient for treatment of melanoma have been identified [19, 20]. Among these derivatives, compound A [19] (Fig. 1) was considered as the lead compound in order to design our target compounds. In our efforts in order to develop new antiproliferative agents

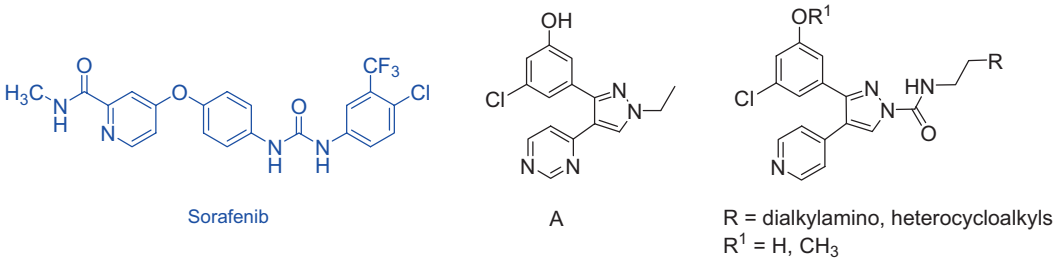


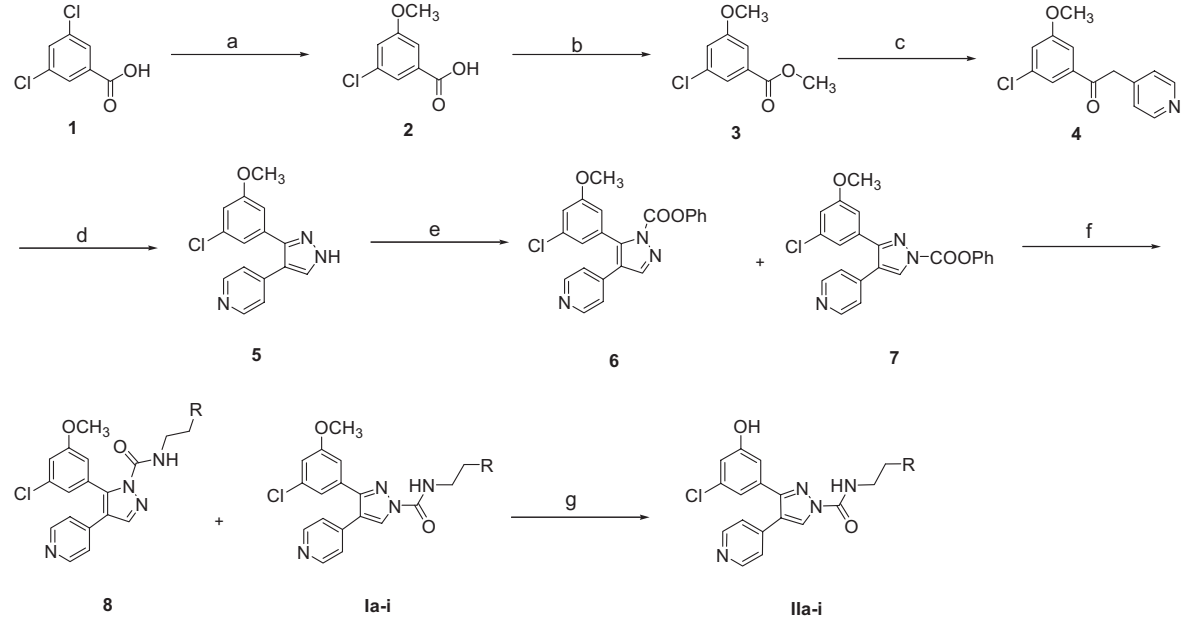
Figure 1. Structures of sorafenib, the lead compound A, and target compounds.

for treatment of melanoma, we designed and synthesized 18 new 3,4-diarylpyrazole derivatives possessing an amide moiety at position 1 of the pyrazole ring. The newly synthesized compounds **Ia–i** and **IIa–i** were designed by isosteric replacement of the pyrimidine ring of the lead compound A with a pyridine ring and introduction of different amide tails at position 1 of the pyrazole ring instead of the ethyl group. The phenolic hydroxy group was retained in compounds **IIa–i** and modified into methoxy group in compounds **Ia–i** in order to study its effect on the activity (Fig. 1). Herein, we report the synthesis and antiproliferative activity against A375 human melanoma cell line of these compounds.

Results and discussion

Chemistry

3,4-Diarylpyrazole derivatives **Ia–i** and **IIa–i** with amide moiety at position 1 of the pyrazole ring were prepared according to the sequence of reactions shown in Scheme 1. Heating 3,5-dichlorobenzoic acid (**1**) with three molecular equivalents of sodium methoxide in hexamethylphosphoramide (HMPA) followed by acidification with HCl gave 3-chloro-5-methoxybenzoic acid (**2**), which upon esterification with methanol in the presence of acetyl chloride afforded the corresponding methyl ester **3** [21]. The pyridyl derivative **4**



Reagents and conditions: (a) sodium methoxide, HMPA, 115–120°C, 15 h; (b) acetyl chloride, MeOH; (c) 4-picoline, LHMDS, THF; (d) (i) DMF-DMA, (ii) hydrazine monohydrate, EtOH; (e) phenyl chloroformate, TEA, THF; (f) substituted ethanamine, K_2CO_3, CH_2Cl_2; (g) BF_3 Me_2S, CH_2Cl_2.

Scheme 1. Synthesis of the target compounds **Ia–i** and **IIa–i**.

was obtained by treatment of **3** with 4-picoline in THF in the presence of lithium bis(trimethylsilyl)amide (LHMDS). Cyclization to the pyrazole compound **5** was carried out by treatment of **4** with dimethylformamide dimethyl acetal (DMF-DMA), and subsequent treatment with hydrazine monohydrate [19]. Interaction of **5** with phenyl chloroformate in the presence of TEA in dry THF gave a mixture of pyrazole-2-carboxylate derivative **6** and pyrazole-1-carboxylate **7** in an approximate ratio of 1:4. The mixture was then reacted with different ethanamines to furnish the target methoxy compounds **Ia–i** in combination with their regioisomers **8**. Compounds **Ia–i** with lower R_f values on TLC were obtained in the pure form after purification by flash column chromatography. Demethylation of the methoxy group of **Ia–i** using boron trifluoride–methyl sulfide complex afforded the corresponding hydroxy derivatives **IIa–i**.

Antiproliferative activity and discussion

The antiproliferative activity of the newly synthesized compounds against A375 human melanoma cell line was tested. The ability of the 1*H*-pyrazole-1-carboxamide derivatives to inhibit the growth of A375 cell line is summarized in Tables 1 and 2. The results are expressed as IC_{50} values. Sorafenib was selected as the reference standard, because it has been extensively used in clinical trials for melanoma [4, 22].

As listed in Tables 1 and 2, most of the compounds showed moderate activity, while compounds **Ie**, **IIa**, **IIb**, **IIe**, and **IIh** having IC_{50} values ranging from 4.2 to 6.9 μM exhibited similar activity to that of Sorafenib (IC_{50} = 5.6 μM). Compound **Ie** possesses a methoxy group on the benzene ring while compounds **IIa**, **IIb**, **IIe**, and **IIh** possess a hydroxy group. In addition, the R moiety of compounds **Ie** and **IIe** is *N*-methylpiperazinyl moiety while the R moieties of compounds **IIa**, **IIb**, and **IIh** are dimethylamino, diethylamino, and 2-methylpiperidinyl, respectively.

The compounds in Table 2 were more potent than those in Table 1, which suggests that the hydroxy group on position 3 of the benzene ring is optimal for the activity. This may be attributed to hydrogen bond formation at the receptor site.

The effect of the terminal substituents of the tail at position 1 of the pyrazole ring was also investigated. Compounds **Ii** and **IIi** having a 5-membered ring, pyrrolidine ring, showed diminished activity. We can conclude that this moiety is unfavorable for antiproliferative activity against melanoma compared with dialkylamino and 6-membered rings. By comparing compounds **Ia** and **Ib**, we find that smaller alkyl groups, two methyl groups, are more optimal for activity than the slightly longer groups, ethyl groups. Upon comparing the activity of the piperazinyl derivatives, it was found that the *N*-methyl derivatives (**Ie** and **IIe)** were more potent than that of *N*-acetyl derivatives (**If** and **IIf**). These results may be rationalized by the steric and/or electronic effects of the acetyl group, compared with the methyl group. Introduction of a methyl group on the piperidine ring (compound **IIh**) enhanced the activity compared with non-methylated piperidine ring (compound **IIg**). On the other hand, introduction of two methyl groups on the morpholine ring (compounds **Id** and **IId**) did not alter the activity, compared with derivatives with non-methylated morpholine ring (compounds **Ic** and **IIc**).

In conclusion, a new series of 3,4-diaryl-1*H*-pyrazole-1-carboxamide derivatives was synthesized based on our previous

Table 1. Antiproliferative activity of methoxy compounds **Ia–i** against A375P cell line

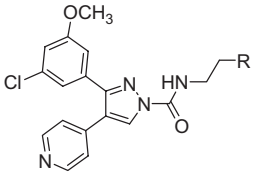
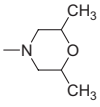
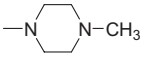
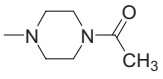
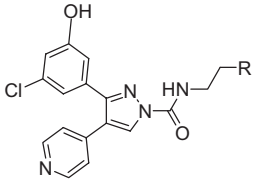
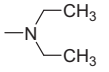
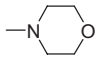
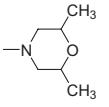
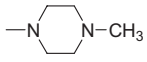
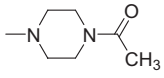
Structure	Comp. No.	R	IC_{50} (μM)
	**Ia**		9.6
	Ib		16.3
	Ic		>20
	Id		>20
	Ie		6.9
	If		>20
	Ig		>20
	Ih		>20
	Ii		>20
Sorafenib			5.6

Table 2. Antiproliferative activity of hydroxy compounds **IIa–i** against A375P cell line

Structure	Comp. No.	R	IC_{50} (μM)
	**IIa**		6.6
	IIb		6.8
	IIc		10.8
	IId		10.8
	IIe		4.2
	IIf		13.0
	IIg		17.2
	IIh		6.8
	IIi		>20
Sorafenib			5.6

literature studies. Among all of these derivatives, compound **IIe** having *m*-hydroxyphenyl substituted *N*-methylpiperazinyl moieties showed the most potent antiproliferative activity against A375 human melanoma cell line. One can conclude that these moieties are optimal for antiproliferative activity of this series of compounds. Further modification of these compounds in order to improve their potency is currently in progress. Our ultimate goal is to identify several compounds that are highly potent against melanoma cells.

Experimental

Chemistry

All melting points were obtained on a Walden Precision Apparatus Electrothermal 9300 apparatus and are uncorrected. Mass spectra (MS) were taken in ESI mode on a Waters 3100 Mass Detecter (Waters, Milford, MA, USA). Nuclear magnetic resonance (NMR) spectroscopy was performed using a Bruker ARX-300, 300 MHz spectrometers (Bruker Bioscience, Billerica, MA, USA) with TMS as an internal standard. IR spectra (KBr disks) were recorded with a Bruker FT-IR instrument (Bruker Bioscience, Billerica, MA, USA). Unless otherwise noted, all solvents and reagents were commercially available and used without further purification.

*3-Chloro-5-methoxybenzoic acid **2***

A mixture of 3,5-dichlorobenzoic acid (**1**, 2.0 g, 10.4 mmol) and NaOMe (6.74 mL, 25 wt-% solution in methanol, 31.2 mmol) in HMPA (40 mL) was heated at 120°C for 15 h. The mixture was poured into ice-water and acidified with conc. HCl to give 3-chloro-5-methoxybenzoic acid (**2**, 1.56 g, 80%) as a precipitate which was collected by filtration and used in the next step without purification. ^{1}H-NMR (DMSO-d_6) δ [ppm]: 3.82 (s, 3H), 7.30 (t, 1H, J = 2.0 Hz), 7.38 (q, 1H, J = 1.3 Hz), 7.47 (t, 1H, J = 1.6 Hz).

*Methyl 3-chloro-5-methoxybenzoate **3***

Acetyl chloride (1.9 mL, 28.1 mmol) was added dropwise to a solution of **2** (1.0 g, 5.4 mmol) in MeOH (40 mL) at 0°C and the reaction mixture was then stirred at room temperature for 15 h. After evaporation of the organic solvent, the residue was purified by flash column chromatography (silica gel, hexane/methylene chloride 5:1 v/v then switching to hexane/methylene chloride 1:1 v/v) to give **3** (0.7 g, 65.1%) as an oil. ^{1}H-NMR ($CDCl_3$) δ [ppm]: 3.85 (s, 3H), 3.92 (s, 3H), 7.09 (t, 1H, J = 2.2 Hz), 7.45 (q, 1H, J = 1.3 Hz), 7.61 (t, 1H, J = 1.6 Hz).

*1-(3-Chloro-5-methoxyphenyl)-2-(pyridin-4-yl)ethanone **4***

To a mixture of compound **3** (1.0 g, 5.0 mmol) and 4-picoline (0.5 mL, 5.6 mmol) in THF (5 mL) in a cooled bath at −25°C, LHMDS (3.7 mL, 1.0 M solution in THF, 19.9 mmol) was slowly added to maintain the temperature at −25°C. The resulting mixture was stirred overnight at room temperature. The mixture was quenched with saturated aqueous NH_4Cl. Ethyl acetate was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 10 mL). The combined organic layer extracts were washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was purified by flash column chromatography (silica gel, hexane/ethyl acetate 1:1 v/v then switching to hexane/ethyl acetate 1:5 v/v) to yield compound **4** (0.52 g, 40%). ^{1}H-NMR ($CDCl_3$) δ [ppm]: 3.85 (s, 3H), 4.25 (s, 2H), 7.12 (t, 1H, J = 2.0 Hz), 7.19 (d, 2H, J = 5.7 Hz), 7.39 (q, 1H, J = 1.4 Hz), 7.54 (t, 1H, J = 1.5 Hz), 8.58 (d, 2H, J = 5.7 Hz).

*4-[3-(3-Chloro-5-methoxyphenyl)-1H-pyrazol-4-yl] pyridine **5***

Compound **4** (1.0 g, 3.8 mmol) was added to DMF-DMA (5.14 mL, 38.2 mmol) and the mixture was stirred at room temperature for 18 h. The resulting solution was concentrated to dryness to

furnish an oil which was used in the next step without purification. To a portion of the oil from the previous step (0.137 g, 0.457 mmol) in EtOH (3 mL) was added hydrazine monohydrate (0.04 mL, 0.76 mmol) and the reaction mixture was stirred overnight at room temperature. Water (5 mL) was added to the reaction mixture and the organics were extracted with ethyl acetate (3 × 5 mL). The combined organic layer extracts were washed with brine and dried over anhydrous Na_2SO_4. After evaporation of the organic solvent, the residue was purified by column chromatography (silica gel, hexane/ethyl acetate 1:1 v/v then switching to hexane/ethyl acetate 1:5 v/v) to yield compound **5** (0.11 g, 81%). ^{1}H-NMR (DMSO-d_6) δ [ppm]: 3.77 (s, 3H), 6.85 (brs, 1H), 6.94 (brs, 1H), 7.06 (brs, 1H), 7.23 (d, 2H, J = 5.4 Hz), 7.83 (s, 1H), 8.55 (d, 2H, J = 5.3 Hz).

*Phenyl 3-(3-chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxylate **7***

To a solution of compound **5** (0.1 g, 0.35 mmol) in anhydrous THF (10 mL), triethylamine (0.112 g, 1.1 mmol) was slowly added at 0°C. Phenyl chloroformate (0.165 g, 1.05 mmol) was slowly added to the above solution at 0°C. The reaction mixture was stirred at the same temperature for 2 h. The mixture was diluted with H_2O (10 mL) and CH_2Cl_2 (15 mL). The organic layer was separated, washed with brine, and dried over anhydrous $MgSO_4$. The organic solvent was evaporated under reduced pressure and the residue (a mixture of compounds **6** and **7**) was used in the next step without further purification.

*General procedure for preparation of compounds **Ia–i***

To a solution of the crude product of the last step (0.1 g, 0.246 mmol) in dry CH_2Cl_2 (3 mL), a solution of the appropriate ethanamine derivative (0.738 mmol) in dry CH_2Cl_2 (2 mL), and anhydrous K_2CO_3 (68 mg, 0.492 mmol) were added. The reaction mixture was stirred at room temperature for 1 h. Water (5 mL) was added to the reaction mixture and the organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (2 × 3 mL) and the combined organic layer extracts were washed with brine and dried over anhydrous $MgSO_4$. The organic solvent was evaporated under reduced pressure and the residue was obtained.

*3-(3-Chloro-5-methoxyphenyl)-N-(2-(dimethylamino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **Ia***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 4:1 v/v). 58% Yield; oil; ^{1}H-NMR (CD_3OD) δ [ppm]: 2.37 (s, 6H), 2.66 (t, 2H, J = 6.7 Hz), 3.58 (t, 2H, J = 6.5 Hz), 3.75 (s, 3H), 6.96 (dd, 1H, J = 1.7 Hz, J = 2.0 Hz), 7.02 (dd, 1H, J = 2.0 Hz, J = 2.2 Hz), 7.14 (dd, 1H, J = 1.6 Hz, J = 2.0 Hz), 7.39 (d, 2H, J = 6.2 Hz), 8.51 (d, 2H, J = 6.1 Hz), 8.62 (s, 1H); ^{13}C-NMR (CD_3OD) δ [ppm]: 160.6, 150.2, 149.8, 141.9, 141.0, 134.8, 134.3, 129.3, 120.4, 120.3, 114.8, 112.7, 112.6, 57.9, 54.7, 44.1, 37.5; MS m/z: 400.9 [M + H$^+$].

*3-(3-Chloro-5-methoxyphenyl)-N-(2-(diethylamino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **Ib***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate-methanol 6:1 v/v). 70% Yield; m.p.: 104–107°C; IR (KBr) [cm^{-1}]: 3318.3, 2967.8, 2935.2, 1737.3, 1610.8, 1578.4, 1550.5, 1509.9, 1471.5, 1409.7, 1266.3, 1210.5, 1173.9, 1138.6, 1043.5; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 0.98 (t, 6H, J = 6.3 Hz), 2.47 (q, 4H, J = 6.3 Hz), 2.62 (t, 2H, J = 5.8 Hz), 3.15 (t, 2H, J = 5.2 Hz), 3.72 (s, 3H), 6.92 (dd, 1H, J = 2.0 Hz, J = 2.2 Hz), 7.13 (dd, 1H, J = 2.0 Hz, J = 2.2 Hz), 7.33 (dd, 1H, J = 1.9 Hz, J = 1.8 Hz), 7.36 (d, 2H, J = 4.3 Hz), 8.50 (brs, 1H), 8.54 (d, 2H, J = 4.2 Hz), 8.75 (s, 1H); ^{13}C-NMR (CD_3OD) δ [ppm]: 162.6, 152.4, 149.0, 143.0, 138.5, 135.8, 135.4, 134.8, 132.5, 120.4, 114.4, 112.6, 109.1, 54.7, 51.2, 48.4, 39.7, 10.2; MS m/z: 429.1 [M + H$^+$].

*3-(3-Chloro-5-methoxyphenyl)-N-(2-morpholinoethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **Ic***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 4:1 v/v). 45% Yield; m.p.: 103–105°C; IR (KBr) [cm^{-1}]: 3252.1, 2964.2, 2943.2, 1727.0, 1609.0, 1578.4, 1509.5, 1411.6, 1296.0, 1139.5, 1115.4, 1042.0; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.44-2.52 (m, 6H), 3.16 (t, 2H, J = 5.0 Hz), 3.58 (t, 4H, J = 4.1 Hz), 3.73 (s, 3H), 6.94 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.13 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 7.36 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.53 (d, 2H, J = 6.0 Hz), 8.56 (d, 2H, J = 6.0 Hz), 8.61 (brs, 1H), 8.80 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 160.6, 151.3, 149.7, 140.9, 134.8, 134.2, 129.3, 128.9, 121.4, 120.4, 114.4, 112.7, 109.2, 66.4, 57.6, 54.8, 53.3, 37.3; MS m/z: 443.0 [M + H$^+$].

*3-(3-Chloro-5-methoxyphenyl)-N-(2-(2,6-dimethylmorpholino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **Id***

It was purified by flash column chromatography (silica gel, ethyl acetate/hexane 2:1 v/v then switching to ethyl acetate). 54% Yield; m.p.: 80–82°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.18 (d, 6H, J = 6.3 Hz), 2.59–2.80 (m, 6H), 3.44 (t, 2H, J = 4.9 Hz), 3.71–3.77 (m, 5H), 6.94 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 7.13 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.26 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 7.63 (d, 2H, J = 5.1 Hz), 8.30 (brs, 1H), 8.42 (d, 2H, J = 5.0 Hz), 8.58 (s, 1H); MS m/z: 471.0 [M + H$^+$].

*3-(3-Chloro-5-methoxyphenyl)-N-(2-(4-methylpiperazin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **Ie***

It was purified by flash column chromatography (silica gel, ethyl acetate/hexane 1:1 v/v then switching to ethyl acetate). 66% Yield; m.p.: 112–115°C; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.28 (s, 3H), 2.37–2.58 (m, 8H), 2.62 (t, 2H, J = 6.0 Hz), 2.96 (t, 2H, J = 5.9 Hz), 3.69 (s, 3H), 6.98 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.04 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.24 (dd, 1H, J = 2.0 Hz, J = 1.9 Hz), 7.35 (d, 2H, J = 6.1 Hz), 7.90 (brs, 1H), 8.21 (s, 1H), 8.45 (d, 2H, J = 6.2 Hz); ^{13}C-NMR (CD_3OD) δ [ppm]: 160.6, 150.1, 149.7, 142.9, 140.9, 135.5, 134.8, 134.2, 121.4, 120.4, 114.4, 112.7, 109.4, 57.2, 54.8, 53.3, 53.2, 46.8, 37.3; MS m/z: 455.9 [M + H$^+$].

*N-(2-(4-Acetylpiperazin-1-yl)ethyl)-3-(3-chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide **If***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 4:1 v/v). 45% Yield; m.p.: 187–190°C; ^{1}H-NMR (CD_3OD) δ [ppm]: 2.11 (s, 3H), 2.49–2.62 (m, 6H), 3.33 (t, 2H, J = 4.9 Hz), 3.53 (t, 4H, J = 4.0 Hz), 3.75 (s, 3H), 7.09 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.14 (dd, 1H, J = 2.1 Hz, J = 1.9 Hz), 7.26 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.35 (d, 2H, J = 6.2 Hz), 8.50 (d, 2H, J = 6.1 Hz), 8.61 (s, 1H); MS m/z: 484.0 [M + H$^+$].

3-(3-Chloro-5-methoxyphenyl)-N-(2-(piperidin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***Ig***

It was purified by flash column chromatography (silica gel, ethyl acetate/hexane 1:1 v/v then switching to ethyl acetate). 41% Yield; m.p.: 39–41°C; IR (KBr) [cm^{-1}]: 3317.1, 2967.8, 1737.3, 1610.8, 1578.4, 1509.9, 1409.6, 1266.3, 1173.9, 1138.6, 1072.2, 1043.5; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.39–1.63 (m, 6H), 2.27 (t, 4H, J = 7.6 Hz), 2.72 (t, 2H, J = 5.9 Hz), 3.42 (t, 2H, J = 5.6 Hz), 3.80 (s, 3H), 6.88 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.07 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.22 (dd, 1H, J = 2.1 Hz, J = 2.0 Hz), 7.35 (d, 2H, J = 5.1 Hz), 7.79 (s, 1H), 8.51 (d, 2H, J = 4.9 Hz); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 160.3, 150.0, 149.8, 143.0, 138.6, 136.6, 135.2, 132.4, 122.7, 120.7, 114.5, 112.6, 98.3, 57.1, 55.6, 53.9, 36.8, 24.3; MS m/z: 440.95 [M + H^+].

3-(3-Chloro-5-methoxyphenyl)-N-(2-(2-methylpiperidin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***Ih***

It was purified by flash column chromatography (silica gel, ethyl acetate/hexane 1:1 v/v then switching to ethyl acetate). 40% Yield; m.p.: 73–75°C; ^{1}H-NMR (CD_3OD) δ [ppm]: 1.15 (d, 3H, J = 5.3 Hz), 1.38–1.66 (m, 6H), 2.17–2.63 (m, 5H), 3.31 (t, 2H, J = 5.0 Hz), 3.75 (s, 3H), 6.96 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.03 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 7.15 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.40 (d, 2H, J = 5.9 Hz), 8.52 (d, 2H, J = 6.1 Hz), 8.62 (s, 1H); MS m/z: 455.07 [M + H^+].

3-(3-Chloro-5-methoxyphenyl)-4-(pyridin-4-yl)-N-(2-(pyrrolidin-1-yl)ethyl)-1H-pyrazole-1-carboxamide ***Ii***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 3:1 v/v). 46% Yield; oil; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.83 (t, 4H, J = 5.3 Hz), 2.61 (t, 4H, J = 5.1 Hz), 2.77 (t, 2H, J = 6.3 Hz), 3.32 (t, 2H, J = 5.9 Hz), 3.73 (s, 3H), 6.83 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.11 (dd, 1H, J = 1.9 Hz, J = 2.1 Hz), 7.22 (dd, 1H, J = 1.6 Hz, J = 1.8 Hz), 7.27 (d, 2H, J = 5.8 Hz), 7.59 (brs, 1H), 8.43 (s, 1H), 8.58 (d, 2H, J = 6.0 Hz); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 160.3, 150.3, 149.3, 143.0, 139.7, 135.9, 135.3, 133.9, 121.7, 120.9, 115.4, 115.0, 112.8, 55.7, 55.6, 53.9, 39.4, 23.5; MS m/z: 426.93 [M + H^+].

General procedure for preparation of compounds ***IIa–i***

To a solution of compound **Ia–i** (0.1 mmol) in methylene chloride (3 mL), BF_3 Me_2S (0.13 g, 1 mmol) was added dropwise at room temperature under N_2 and the reaction mixture was stirred at the same temperature for 48 h. The mixture was quenched with saturated aqueous $NaHCO_3$. Ethyl acetate (3 mL) was added and the organic layer was separated. The aqueous layer was extracted with ethyl acetate (3 × 2 mL). The combined organic layer extracts were washed with brine and dried over anhydrous Na_2SO_4. The organic solvent was evaporated under reduced pressure and the residue was obtained.

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(dimethylamino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIa***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 4:1 v/v). 41% Yield; m.p.: 179–181°C; IR (KBr) [cm^{-1}]: 3391.6, 3126.9, 2970.7, 2924.2, 1730.0, 1610.5, 1593.1, 1512.0, 1417.5, 1345.1, 1293.0, 1205.5, 1153.7, 1003.4; ^{1}H-NMR (CD_3OD) δ [ppm]: 2.39 (s, 6H), 2.69 (t, 2H, J = 6.7 Hz), 3.59 (t, 2H, J = 6.5 Hz), 6.76 (dd, 1H, J = 2.1 Hz, J = 1.8 Hz), 6.85 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.05 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 7.41 (d, 2H, J = 5.9 Hz), 8.51 (d, 2H, J = 6.0 Hz), 8.63 (s, 1H); ^{13}C-NMR (CD_3OD) δ [ppm]: 158.5, 150.5, 149.9, 142.7, 141.0, 138.5, 134.6, 134.2, 123.3, 120.3, 119.1, 115.6, 114.0, 57.8, 46.7, 37.3; MS m/z: 386.95 [M + H^+].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(diethylamino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIb***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 6:1 v/v). 48% Yield; m.p.: 118–120°C; IR (KBr) [cm^{-1}]: 3392.3, 3127.0, 2970.7, 1729.7, 1610.4, 1593.5, 1512.0, 1417.6, 1345.1, 1293.0, 1205.6, 1153.7, 1003.4; ^{1}H-NMR (CD_3OD) δ [ppm]: 1.16 (t, 6H, J = 7.1 Hz), 2.78 (q, 4H, J = 7.1 Hz), 2.87 (t, 2H, J = 6.9 Hz), 3.57 (t, 2H, J = 6.9 Hz), 6.78 (dd, 1H, J = 1.4 Hz, J = 1.9 Hz), 6.85 (dd, 1H, J = 1.8 Hz, J = 2.0 Hz), 7.04 (dd, 1H, J = 1.3 Hz, J = 1.9 Hz), 7.40 (d, 2H, J = 4.8 Hz), 8.51 (d, 2H, J = 4.9 Hz), 8.63 (s, 1H); ^{13}C-NMR (CD_3OD) δ [ppm]: 158.6, 150.5, 149.9, 141.0, 135.9, 134.6, 134.2, 129.2, 123.3, 120.4, 119.1, 115.7, 114.0, 51.1, 48.4, 37.0, 9.8; MS m/z: 414.96 [M + H^+].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-morpholinoethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIc***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 6:1 v/v). 55% Yield; m.p.: 247–250°C; IR (KBr) [cm^{-1}]: 3400.0, 3124.7, 2973.7, 1732.8, 1610.6, 1593.5, 1510.3, 1404.7, 1324.0, 1293.7, 1154.0, 1094.1, 1002.1; ^{1}H-NMR (CD_3OD) δ [ppm]: 2.50 (t, 4H, J = 6.9 Hz), 2.61 (t, 2H, J = 6.6 Hz), 3.30–3.72 (m, 6H), 6.78 (dd, 1H, J = 1.5 Hz, J = 1.9 Hz), 6.87 (dd, 1H, J = 1.8 Hz, J = 2.0 Hz), 7.09 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.41 (d, 2H, J = 5.9 Hz), 8.45 (s, 1H), 8.62 (d, 2H, J = 6.0 Hz); MS m/z: 428.92 [M + H^+].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(2,6-dimethylmorpholino)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IId***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 10:1 v/v). 60% Yield; m.p.: 175–178°C; IR (KBr) [cm^{-1}]: 3399.4, 3124.7, 2936.2, 1732.8, 1610.8, 1593.6, 1510.2, 1404.8, 1324.2, 1293.7, 1153.9, 1094.1, 1002.1, ^{1}H-NMR (CD_3OD) δ [ppm]: 1.20 (d, 6H, J = 6.3 Hz), 2.48–2.70 (m, 6H), 3.36 (t, 2H, J = 4.8 Hz), 3.74 (m, 2H), 6.80 (dd, 1H, J = 2.0 Hz, J = 2.1 Hz), 6.93 (dd, 1H, J = 1.9 Hz, J = 1.8 Hz), 7.15 (dd, 1H, J = 1.9 Hz, J = 2.0 Hz), 7.54 (d, 2H, J = 5.0 Hz), 8.42 (d, 2H, J = 4.9 Hz), 8.55 (s, 1H); MS m/z: 456.97 [M + H^+].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(4-methylpiperazin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIe***

It was purified by flash column chromatography (silica gel, ethyl acetate). 63% Yield; m.p.: 120–122°C; IR (KBr) [cm^{-1}]: 3399.2, 3124.8, 2973.8, 1732.7, 1610.6, 1593.3, 1510.2, 1404.8, 1356.2, 1293.9, 1154.0, 1094.2, 1002.2; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 2.25 (s, 3H), 2.45–2.59 (m, 8H), 2.60 (t, 2H, J = 6.1 Hz), 3.02 (t, 2H, J = 5.9 Hz), 6.78 (dd, 1H, J = 1.8 Hz, J = 2.1 Hz), 6.84 (dd, 1H, J = 2.0 Hz, J = 2.0 Hz), 7.14 (dd, 1H, J = 2.0 Hz, J = 1.8 Hz), 7.53 (d, 2H, J = 6.2 Hz), 8.10 (brs, 1H), 8.43 (d, 2H, J = 6.2 Hz), 8.54 (s, 1H); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 159.8, 149.3, 142.6, 138.2, 135.2, 134.8, 134.1, 121.1, 119.9, 114.6, 113.0, 109.1, 57.2, 55.3, 54.7, 52.2, 42.8, 37.9; MS m/z: 441.95 [M + H^+].

N-(2-(4-Acetylpiperazin-1-yl)ethyl)-3-(3-chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIf***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 4:1 v/v). 50% Yield; m.p.: 197–199°C; IR (KBr) [cm^{-1}]: 3346.6, 3126.5, 2930.4, 1731.5, 1610.7, 1510.1, 1433.5, 1345.6, 1154.5, 1001.9; ^{1}H-NMR (CD_3OD) δ [ppm]: 2.04 (s, 3H), 2.53–2.72 (m, 6H), 2.93 (t, 2H, $J = 4.9$ Hz), 3.42 (t, 4H, $J = 5.0$ Hz), 6.87 (dd, 1H, $J = 1.7$ Hz, $J = 1.9$ Hz), 6.94 (dd, 1H, $J = 1.6$ Hz, $J = 2.0$ Hz), 7.10 (dd, 1H, $J = 1.7$ Hz, $J = 2.1$ Hz), 7.38 (d, 2H, $J = 4.6$ Hz), 8.10 (s, 1H), 8.59 (d, 2H, $J = 4.7$ Hz); MS *m/z*: 470.0 [$M + H^+$].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(piperidin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIg***

It was purified by flash column chromatography (silica gel, ethyl acetate). 50% Yield; m.p.: 205–208°C; IR (KBr) [cm^{-1}]: 3415.1, 2932.2, 1731.5, 1610.7, 1510.0, 1433.7, 1345.2, 1154.7, 1001.8; ^{1}H-NMR (CD_3OD) δ [ppm]: 1.37–1.59 (m, 6H), 2.23 (t, 4H, $J = 7.0$ Hz), 2.68 (t, 2H, $J = 6.2$ Hz), 3.28 (t, 2H, $J = 6.1$ Hz), 6.77 (dd, 1H, $J = 1.9$ Hz, $J = 2.1$ Hz), 6.91 (dd, 1H, $J = 1.9$ Hz, $J = 1.8$ Hz), 7.11 (dd, 1H, $J = 1.8$ Hz, $J = 2.0$ Hz), 7.58 (d, 2H, $J = 5.0$ Hz), 8.02 (s, 1H), 8.51 (d, 2H, $J = 5.1$ Hz); MS *m/z*: 426.95 [$M + H^+$].

3-(3-Chloro-5-hydroxyphenyl)-N-(2-(2-methylpiperidin-1-yl)ethyl)-4-(pyridin-4-yl)-1H-pyrazole-1-carboxamide ***IIh***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 10:1 v/v). 45% Yield; m.p.: 122–124°C; IR (KBr) [cm^{-1}]: 3406.0, 2932.2, 1731.5, 1610.7, 1510.0, 1433.7, 1345.1, 1154.7, 1001.8; ^{1}H-NMR (CD_3OD) δ [ppm]: 1.19 (d, 3H, $J = 6.2$ Hz), 1.35–1.68 (m, 6H), 2.44–2.73 (m, 5H), 3.06 (t, 2H, $J = 5.2$ Hz), 6.82 (dd, 1H, $J = 1.7$ Hz, $J = 1.9$ Hz), 6.86 (dd, 1H, $J = 1.9$ Hz, $J = 2.0$ Hz), 7.06 (dd, 1H, $J = 1.6$ Hz, $J = 1.8$ Hz), 7.41 (d, 2H, $J = 4.8$ Hz), 8.51 (d, 2H, $J = 4.8$ Hz), 8.63 (s, 1H); ^{13}C-NMR (CD_3OD) δ [ppm]: 158.6, 150.5, 149.8, 142.7, 135.9, 134.6, 134.2, 129.2, 120.3, 119.1, 115.7, 114.0, 110.6, 56.4, 52.1, 48.4, 39.8, 36.3, 33.6, 25.1, 23.2; MS *m/z*: 441.0 [$M + H^+$].

3-(3-Chloro-5-hydroxyphenyl)-4-(pyridin-4-yl)-N-(2-(pyrrolidin-1-yl)ethyl)-1H-pyrazole-1-carboxamide ***IIi***

It was purified by flash column chromatography (silica gel, ethyl acetate then switching to ethyl acetate/methanol 3:1 v/v). 49% Yield; m.p.: 182–185°C; IR (KBr) [cm^{-1}]: 3374.6, 2973.9, 2802.5, 1732.2, 1610.1, 1498.7, 1424.2, 1366.8, 1152.3, 1087.9; ^{1}H-NMR (CD_3OD) δ [ppm]: 1.77 (t, 4H, $J = 5.0$ Hz), 2.29 (t, 4H, $J = 5.1$ Hz), 2.63 (t, 2H, $J = 6.0$ Hz), 3.02 (t, 2H, $J = 6.1$ Hz), 6.81 (dd, 1H, $J = 1.8$ Hz, $J = 1.7$ Hz), 6.93 (dd, 1H, $J = 1.9$ Hz, $J = 1.8$ Hz), 7.12 (dd, 1H, $J = 2.0$ Hz, $J = 1.8$ Hz), 7.37 (d, 2H, $J = 5.6$ Hz), 8.54 (d, 2H, $J = 5.8$ Hz), 8.63 (s, 1H); MS *m/z*: 412.9 [$M + H^+$].

Evaluation of the biological activity

A375P cells were purchased from American Type Culture Collection (ATCC, Rockville, MD, USA) and maintained in Dulbecco's modified eagle medium (DMEM, Welgene, Daegu, Korea) supplemented with 10% fetal bovine serum (FBS, Welgene, Daegu, Korea) and 1% penicillin/streptomycin (Welgene, Daegu, Korea) in a humidified atmosphere with 5% CO_2 at 37°C. A375P cells were taken from culture substrate with 0.05% trypsin-0.02% EDTA and plated at a density of 5×10^3 cells/well in 96 well plates and then incubated at 37°C for 24 h in a humidified atmosphere with 5% CO_2 prior to treatment with various concentrations (3-fold serial dilution, 12 points) of test compounds. The cells were incubated for 48 h after treatment with the test compounds. The A357P cell viability was assessed by the conventional 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay. MTT assays were carried out with CellTiter 96® (Promega) according to the manufacturer's instructions. The absorbance at 590 nm was recorded using EnVision 2103 (Perkin Elmer; Boston, MA, USA). The IC_{50} was calculated using GraphPad Prism 4.0 software.

The authors have declared no conflict of interest.

References

[1] E. Atallah, L. Flaherty, *Curr. Treat. Options Oncol.* **2005**, *6*, 185–193.

[2] A. Barth, L. A. Wanek, D. L. Morton, *J. Am. Coll. Surg.* **1995**, *181*, 193–201.

[3] C. M. Anderson, A. C. Buzaid, S. S. Legha, *Oncol.* **1995**, *9*, 1149–1158.

[4] V. Gray-Schopfer, C. Wellbrock, R. Marais, *Nature* **2007**, *445*, 851–857.

[5] C. Garbe, T. K. Eigentler, *Melanoma Res.* **2007**, *17*, 117–127.

[6] H. B. Koon, M. B. Atkins, *Expert Rev. Anticancer Ther.* **2007**, *7*, 79–88.

[7] D. H. Lawson, *Cancer Control* **2005**, *12*, 236–241.

[8] S. A. Rosenburg, M. T. Lotze, J. C. Yang, P. M. Aebersold, W. M. Linehan, C. A. Seipp, D. E. White, *Ann. Surg.* **1989**, *210*, 474–484.

[9] M. B. Atkins, M. T. Lotze, J. P. Dutcher, R. I. Fisher, G. Weiss, K. Margolin, J. Abrams, M. Sznol, D. Parkinson, M. Hawkins, C. Paradise, L. Kunkel, S. A. Rosenberg, *J. Clin. Oncol.* **1999**, *17*, 2105–2116.

[10] S. M. Wilhelm, C. Carter, L. Tang, D. Wilkie, A. McNabola, H. Rong, *Cancer Res.* **2004**, *64*, 7099–7109.

[11] D. Strumberg, H. Richly, R. A. Hilger, N. Schleucher, S. Korfee, *J. Clin. Oncol.* **2005**, *23*, 965–972.

[12] J. W. Clark, J. P. Eder, D. Ryan, C. Lathia, H. J. Lenz, *Clin. Cancer Res.* **2005**, *11*, 5472–5480.

[13] M. Moore, H. W. Hirte, L. Siu, A. Oza, S. J. Hotte, O. Petrenciuc, F. Cihon, C. Lathia, B. Schwartz, *Ann. Oncol.* **2005**, *16*, 1688–1694.

[14] D. Strumberg, D. Voliotis, J. G. Moeller, R. A. Hilger, H. Richly, S. Kredtke, C. Beling, M. E. Scheulen, S. Seeber, *J. Clin. Pharmacol. Ther.* **2002**, *40*, 580–581.

[15] H. Richly, P. Kupsh, K. Passage, M. Grubert, R. A. Hilger, R. Voigtmann, B. Schwartz, E. Brendel, O. Christensen, C. G. Haase, D. Strumberg, *Int. J. Clin. Pharmacol. Ther.* **2004**, *42*, 650–651.

[16] F. Egberts, K. C. Kaehler, E. Livingstone, A. Hauschild, *Onkologie* **2008**, *31*, 398–403.

[17] S. M. Wilhelm, L. Adnane, P. Newell, A. Villanueva, J. M. Llovet, M. Lynch, *Mol. Cancer Ther.* **2008**, *7*, 3129–3140.

[18] D. T. Alexandrescu, R. McClure, H. Farzanmehr, C. A. Dasanu, *J. Clin. Oncol.* **2008**, *26*, 4047–4048.

[19] M. J. Bennett, S. Cho-Schultz, J. G. Deal, S. J. King, T. J. Marrone, C. L. Palmer, W. H. Romines, E. Y. Rui, S. C. Sutton, L. R. Zhender, WO 2007/105058 A2. **2007**, [Chem. Abstr. **2007**, *147*, 385972].

[20] M. Pulici, F. Zuccotto, A. Badari, S. Nuvoloni, G. Cervi, G. Traquandi, S. Biondaro, P. Trifiro', C. Marchionni, M. Modugno, WO 2010010154 A1. **2010**, [Chem. Abstr. **2010**, 115411].

[21] K. Takahashi, S. Shimizu, M. Ogata, *Heterocycles* **1985**, *23*, 1483–1491.

[22] T. Eisen, T. Ahmad, K. T. Flaherty, M. Gore, S. Kaye, R. Marais, I. Gibbens, S. Hackett, M. James, L. M. Schuchter, K. L. Nathanson, C. Xia, R. Simantov, B. Schwartz, M. Poulin-Costello, P. J. O'Dwyer, M. J. Ratain, *Br. J. Cancer* **2006**, *95*, 581–586.

