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Discovery of N-(benzyloxy)-1, 3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives as potential antiproliferative agents by inhibiting MEK

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ABSTRACT

Mitogen activated protein kinase (MAPK) signal transduction pathway has been proved to play an important role in tumorigenesis and cancer development. MEK inhibitor has been demonstrated significant clinical benefit for blocking MAPK pathway activation and possibly could block reactivation of the MAPK pathway at the time of BRAF inhibitor resistance. Twenty N-(benzyloxy)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives have been designed and synthesized as MEK inhibitors, and their biological activities were evaluated. Among these compounds, compound **7b** showed the most potent inhibitory activity with IC_{50} of 91 nM for MEK1 and GI_{50} value of 0.26 μM for A549 cells. The SAR analysis and docking simulation were performed to provide crucial pharmacophore clues that could be used in further structure optimization.

1. Introduction

In recent years, a number of key signaling pathways, membrane receptors, kinases and other biological macromolecules, which play an important role in tumorigenesis and cancer development, have been identified along with the further understanding of the pathogenesis of human cancer. Development of small molecular drugs specially targeting these specific macromolecules provides opportunities to overcome human cancers. MAPK signal transduction pathway is one of the most important signaling pathways inside the cell.[1-3]

BRAF, which is one of the most important pro-oncogenes in MAPK pathway, is mutated in approximately 8% of human tumors. Especially, BRAF mutations are frequently found in 40% to 60% of cutaneous melanoma patients.[4, 5] Most of these mutations cause substitutions of glutamic acid ($BRAF^{V600E}$, 70-90%) or lysine ($BRAF^{V600K}$, 10-30%) for valine, which constitutively activates MEK/ERK pathway in cancer cells, leading to tumor development, invasion, and metastasis.[6, 7] There is an accumulating body of experimental evidences validating treatment of selective class I BRAF inhibitors (Vemurafenib and Dabrafenib) as an effective therapeutic strategy for improved progression-free and overall survival in patients with $BRAF^{V600}$-mutated metastatic melanoma.[8-11] Although the overwhelming majority of patients benefit from BRAF inhibitors administration, unfortunately the therapeutic effects are often temporary (median time, 5 to 7 months) and limited by rapidly acquired resistance.[12] Multiple BRAF inhibitor resistance mechanisms in melanoma have been identified, including emergence of mutant BRAF-concurrent upstream RAS[13] or downstream MEK (MAP2K) mutations[14], amplification or alternative splicing of mutant BRAF[15], upregulation of RTKs (receptor tyrosine kinases)[16] and COT kinase (MAP3K8)[17].

As noted above, most reported resistance mechanisms were MAPK-dependent, which induced the reactivation of the MAPK pathways despite continued treatment with the drug. Furthermore, MEK is the downstream effector of BRAF, so that MEK inhibition is useful for blocking MAPK pathway activation and possibly could block reactivation of the MAPK pathway at the time of BRAF inhibitor resistance.[18, 19] Given these observations, several trials of MEK inhibitors administered alone or in combination with BRAF inhibitors were pursued in order to overcome resistance of $BRAF^{V600E}$ melanomas treated with RAF inhibitors. Indeed, several therapy approaches increased the

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Abbreviations: MEK, mitogen-activated protein kinase kinase; BRAF, V-RAF murine sarcoma viral oncogene homologue B1; ERK, extracellular regulated kinase; $BRAF^{V600E}$, V600E mutant BRAF; $BRAF^{V600K}$, V600K mutant BRAF; IC_{50}, half maximal inhibitory concentration; GI_{50}, the concentration that causes 50% growth inhibition; SAR, Structure-Activity Relationship.

magnitude and/or durability of response, resulted in more complete and durable inhibition of MAPK as well as greater tumor growth inhibition.[6, 20] There are several small molecule MEK inhibitors in clinical trials, such as CI-1040, PD0325901, U0126, PD98059, GSK1120212 and AZD2644. Trametinib (GSK1120212), a selective class I inhibitor of MEK1/2, is well tolerated and clinically active in a phase 3 open-label trial that patients could have been treated with $BRAF^{V600}$ mutant metastatic melanoma, with the exclusion of BRAF and MEK inhibitors and Ipilimumab.[11, 19] In a phase 3 trial, compared with Dabrafenib administrated alone, combination of Dabrafenib and Trametinib demonstrated a significant improvement in progression-free survival in previously untreated patients who had metastatic melanoma with $BRAF^{V600E/K}$ mutations.[21] This combined trial also could delayed the emergence of resistance and decreased the incidence of cutaneous hyperproliferative lesions in preclinical models. Due to MEK inhibitor has been demonstrated significant clinical benefit to reduce the emerged resistance， several studies are focus on the development of selectively MEK inhibitor. In continuation of our studies on the development of anti-cancer agents targeting MAPK pathway[22-25], we focus on the identification of novel highly specific and selected small molecule agents to inhibit the protein-kinase activity of MEK.

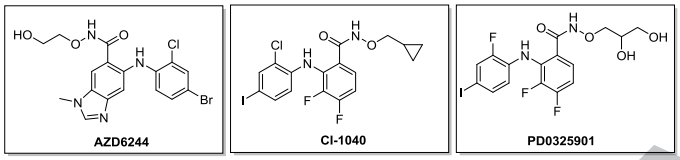
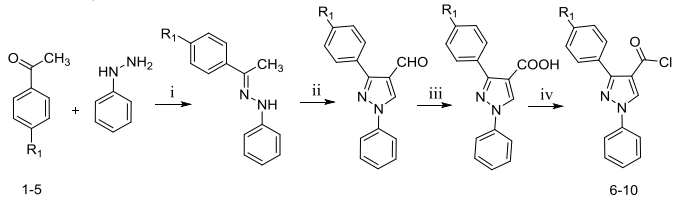


Figure 1. Reported MEK inhibitors owing N-methoxy methanamide moiety.

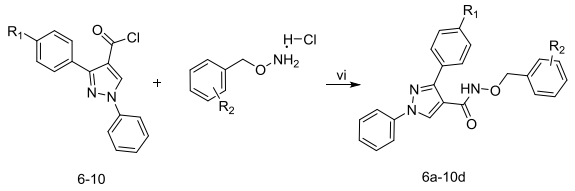
Many pyrazole derivatives have been reported to possess potent anticancer activities *in vitro* and *in vivo*, and much attention was paid to the discovery of Ser/Thr kinases inhibitors based on pyrazole core. As seen in Figure.1, several studies have shown that compounds owing N-methoxy methanamide moiety are known to exhibit attractive MEK inhibitory potency[26, 27]. Encouraged by above observations, in this thesis we designed and synthesized a serials of novel 1,3-diphenyl-1*H*-pyrazole derivatives containing derivatives N-methoxy methanamide moiety, in the hope of obtaining novel anticancer agents through inhibiting MEK kinase activity. Their biological activities *in vitro* are evaluated and the structure-activity relationships (SAR) are also discussed. Docking simulations were performed using the X-ray crystallographic structure of the MEK-1 to explore the binding modes of these compounds at the active site.

2. Results and discussion

2.1 Chemistry



Scheme 1. General synthesis of compounds **6-10**. Reagents and conditions: (i) ethanol, 50–60 ºC, 3 h; (ii) DMF, $POCl_3$, 50-60 ºC, 5 h; (iii) $KMnO_4$, 70-80 ºC, 3 h; (iv) $SOCl_2$, 70-80 ºC, 3 h.

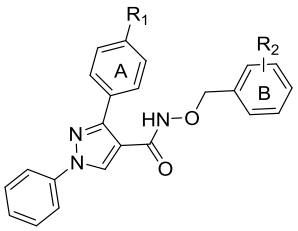


Scheme 2. Synthesis of compounds **6a-10d**. Reagents and conditions: (vi) THF, pyridine, rt, 2 h.

The 1,3-diphenyl-1H-pyrazole-4-carbonyl chloride derivatives (6-10) were prepared according to a previously reported synthetic route outlined in Scheme 1[28, 29]. The synthetic route of 1,3-diphenyl-N-benzyloxy-1*H*-pyrazole-4-carboxamide derivatives (**6a-10d**) was shown in Scheme 2. According to previously reported synthetic scheme [30, 31], the synthesis of compounds (**6a-10d**) began with the interaction of substituted 1,3-diphenyl-1*H*-pyrazole-4-carbonyl chloride（2.0 mmol）and various substituted *O*-benzyl hydroxylamine hydrochloride（2.0 mmol）with the help of pyridine (4.4 mmol) in anhydrous tetrahydrofuran. Then compounds **6a-10d** (Table 1) were obtained by subsequent purification with recrystallization from an ethanol-DMF mixture (2:1).

These compounds were reported for the first time except **6a** and **8a**. All of the synthesized compounds **6a-10d** (Table 1) gave satisfactory elementary analysis and spectroscopic data, which were full accordance with their depicted structures.

Table 1. Structures of compounds **6a-10d**.



Compound	R_1	R_2	Compound	R_1	R_2
6a	H	H	**8c**	OCH_3	2-Cl
6b	H	2-F	**8d**	OCH_3	2,4-Cl
6c	H	2-Cl	**9a**	Cl	H
6d	H	2,4-Cl	**9b**	Cl	2-F
7a	CH_3	H	**9c**	Cl	2-Cl
7b	CH_3	2-F	**9d**	Cl	2,4-Cl
7c	CH_3	2-Cl	**10a**	F	H
7d	CH_3	2,4-Cl	**10b**	F	2-F
8a	OCH_3	H	**10c**	F	2-Cl
8b	OCH_3	2-F	**10d**	F	2,4-Cl

2.2 Bioassays and SAR

All synthesized 1,3-diphenyl-N-benzyloxy-1*H*-pyrazole-4-carboxamide derivatives **6a-10d** were evaluated for their *in vitro* antiproliferative activities against three tumor cell lines, which were HeLa, MCF-7 and A549 cell lines, comparing with the positive control. The *in vitro* growth inhibitory activities of target compound were expressed as the concentration of the compound that inhibited each human cancer cells proliferation to 50 %

(GI_{50}) of the control value, and the results were summarized in Table 2.

Table 2. *In vitro* antiproliferation activities of compounds **6a-10d**.

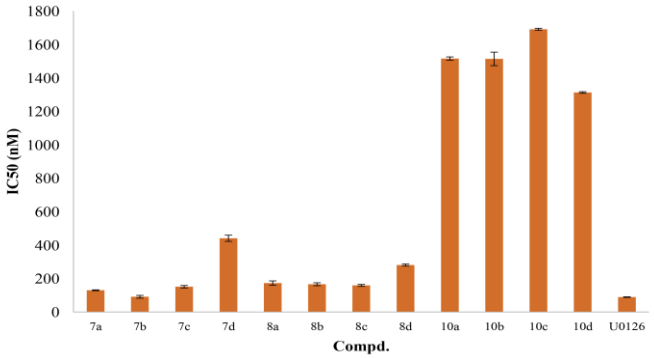
Compd.	GI_{50} (μM) [a, b]			CC_{50} (μM)
	HeLa	MCF-7	A549	293T
6a	8.83±0.64	13.38±0.72	5.00±0.26	121.61±10.16
6b	11.75±0.72	16.14±0.84	5.94±0.34	75.72±5.82
6c	17.84±0.84	7.41±0.38	10.29±0.62	82.05±6.60
6d	5.10±0.26	9.66±0.54	7.35±0.44	40.22±2.55
7a	6.69±0.45	1.87±0.12	1.14±0.06	40.81±2.67
7b	1.18±0.06	2.11±0.12	0.26±0.02	20.57±1.48
7c	4.68±0.25	2.64±0.14	0.38±0.03	38.69±2.65
7d	2.04±0.11	2.31±0.13	1.08±0.06	39.62±2.94
8a	2.64±0.14	2.77±0.14	1.94±0.09	200.7±12.25
8b	2.90±0.15	6.00±0.35	2.16±0.14	24.56±1.68
8c	3.48±0.18	3.73±0.21	2.45±0.16	586.45±36.37
8d	5.75±0.30	2.57±0.18	3.83±0.20	544.75±32.84
9a	8.35±0.48	10.78±0.61	12.98±0.69	51.93±3.62
9b	4.17±0.23	15.10±0.81	12.06±0.63	125.64±7.82
9c	8.85±0.45	16.10±0.92	15.45±0.86	128.64±8.04
9d	5.16±0.26	16.18±0.92	13.12±0.72	46.27±3.24
10a	6.11±0.32	15.30±0.87	10.38±0.65	217.04±13.53
10b	10.27±0.64	12.01±0.64	17.49±0.94	146.78±8.98
10c	36.42±2.42	11.37±0.62	8.33±0.67	44.69±3.06
10d	7.35±0.38	25.32±1.67	13.95±0.84	45.00±3.24
Gefitinib	1.52±0.08	6.71±0.34	2.86±0.18	

[a] Antiproliferation activity was measured using the MTT assay. Values are the average of three independent experiments run in triplicate.
[b] Errors were in the range of 5–10% of the reported values.

From the data obtained from cellular assay, it can be concluded that target 1,3-diphenyl-N-benzyloxy-1*H*-pyrazole-4-carboxamide derivatives showed moderate inhibitory activities against HeLa and MCF-7 cell lines, ranging from 1.18 to 36.42 μM. Compound **7b**, in which A ring was substituted by methyl group and B ring was substituted by *ortha*-fluorine, owed the most potent inhibitory activities (GI_{50} = 1.18 μM) against HeLa cells. In MCF-7 cells, compound **7a**, *N-(benzyloxy)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide*, in which A ring was substituted by methyl group as well, exhibited the lower GI_{50} value (1.87μM).

In comparison with HeLa and MCF-7 cells, A549 cell, which was bearing a constitutively active MAPK pathways, was more sensitive to most of target compounds, and showed low micromolar GI_{50} values. Compounds **7b** and **7c** exhibited GI_{50} value in submicromolar range (GI_{50} = 0.26 μM and 0.38 μM, respectively). Inspection of the chemical structure of all the compounds suggested that it could be divided into two subunits: A rings and B rings. A comparison of the *para* substituents on the A-ring demonstrated that an electron-withdrawing group has improved antiproliferation activity, in which introduction of methyl group generated the most potent activities. For the substituents on the ring B, compounds with *ortho* electron-withdrawing substitution showed stronger activities in the following order: F > Cl > H. Introduction of substituent to *para*-position of ring B results in less active analog.

Furthermore, the toxicity of compounds **6a-10d** was preliminary evaluated by a against human kidney epithelial cell 293T. The results were expressed as median cytotoxic concentration (CC_{50}) data obtained from the MTT assay (Table 2). As shown, these compounds were tested at multiple doses and demonstrated low cytotoxic activities *in vitro* against human kidney epithelial cell 293T.



[a] Values are the average of three independent experiments run in triplicate. Variation was generally 5–10%.

Figure 2. MEK1 inhibitory activities of selected compounds.

Table 3. pERK activity and kinase inhibitory activities against selected kinases of compound **7b**.

pERK	0.61±0.03
EGFR	>25
HER-2	>25
FAK	15.6±0.9
Aurora-A	8.30±0.5
VEGFR-2	>25

The inhibitory activities are displayed as IC50 (μM).

The inhibitory activities of selected compounds against MEK were evaluated by the Raf-MEK-ERK cascade kinase assay using recombinant proteins. Compounds owing the most potent activities in antiproliferation assay (serials 7 and 8), and owing less potent activities (serials 10), were selected to perform kinase assay. The results were summarized in Figure 2, it can be found that compounds in serials 10 showed poor MEK inhibitory activities while most of the compounds in serials 7 and 8 displayed potent inhibitory activities. That is to say, for tested 1,3-diphenyl-N-benzyloxy-1*H*-pyrazole-4-carboxamide derivatives, the SAR of MEK inhibitory activities were in accordance with their inhibitory activities of cancer cells proliferation. Among the tested compounds, **7b** showed the most potent inhibitory activity with IC_{50} of 91 nM, which was comparable to the positive control U0126 with IC_{50} of 89 nM.

The results suggested that the antiproliferative effects were mediated by direct interaction of target compounds with MEK.

Moreover, the phosphorylation level of extracellular signal-regulated kinase (ERK) was measured in a cell-based assay (IC_{50} pERK). As shown in Table 3, compound **7b** exhibited obviously inhibitory activity of ERK phosphorylation in BRAF mutant cell line. As shown in Table 3, the results of the kinase selectivity assay revealed that compound **7b** has an excellent selectivity profile.

To gain more understanding of the interaction between target compounds and MEK, we explored their binding modes generated by molecular docking based on the reported MEK1/inhibitor complex structure (PDB code: 3EQF) and preprocessed by the DS 3.1 (Discovery Studio 3.1, Accelrys, Inc., San Diego, CA)[32, 33]. Each ligand was docked as described previously [34], and the pose with the highest --ECD (cdocker interaction energy) was considered as the optimum pose for it. The binding models of **7b** with the MEK1 structure are shown in Figure 3. Visual inspection of the pose of **7b** into the MEK-binding site revealed that it has suitable shape complementarity with the ATP binding pocket. Especially the 1,3-diphenyl-1*H*-pyrazole skeleton was deeply embedded into the pocket (Figure 3a), and extensive hydrophobic interactions are formed between 1,3-diphenyl-1*H*-pyrazole skeleton and residues Val 82, Ala 95, Val 127, Met 143, Met 143 and Leu 197 of the ATP-binding pocket. On the other side, different interactions formed by N-(benzyloxy)amide side chain of **7b** with amino acid residues in binding site were stabilified the binding mode (Figure 3b). A cation-π interaction was established between Lys 156 and an electron-rich π system of aromatic ring B of **7b**. And an H-bond was also detected in the binding model (O···H-N/Ser 150, angle O···H-O = 108.4°, distance = 2.5 Å), which was simultaneously contributed to the combination. According to the above, the molecular docking result along with the biological assay data suggested that compound **7b** might be a potential inhibitor of the MEK1.

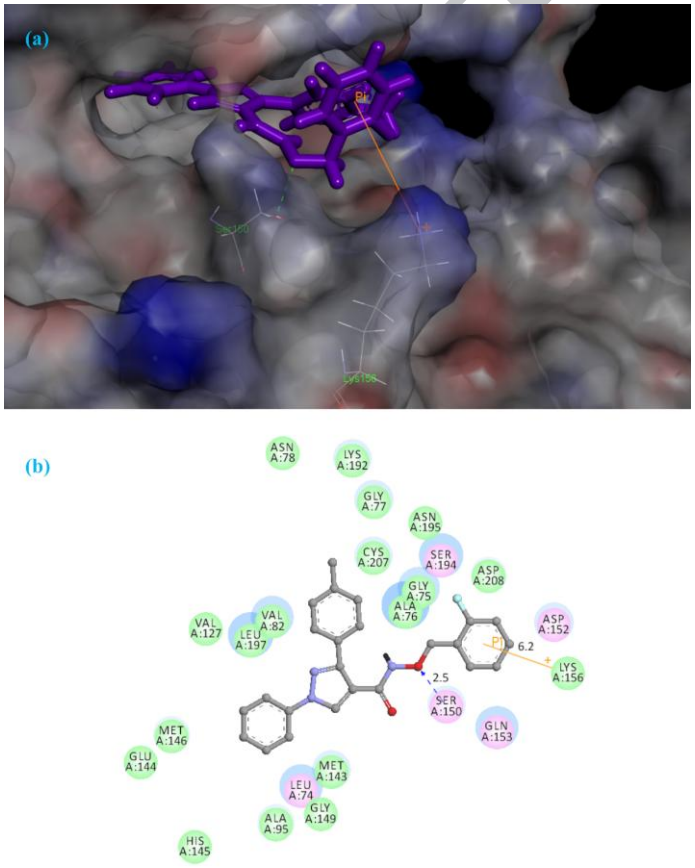


Figure 3. (a). Binding model of **7b** (purple) in the active site of the MEK1 protein-kinase. The H-bond is displayed as dashed line. (b) 2D projection drawing of **7b** docked into MEK1 active site.

3. Conclusion

In this study, a series of novel N-(benzyloxy)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives have been synthesized and evaluated for their antitumor activities as MEK inhibitors. These compounds exhibited potent inhibitory activities against various human cancer cell lines, especially in A549 cells which bears a constitutively active MAPK pathways. Compound **7b** and **7c** exhibited the most potent anti-proliferative activities against A549 cells (GI_{50} = 0.26 μM and 0.38 μM, respectively). In MEK inhibitory assay, the SAR of tested compounds was in accordance with the trend in cellular assay, among which **7b** showed the most potent inhibitory activity with IC_{50} of 91 nM, which was comparable to the positive control U0126 with IC_{50} of 89 nM. Docking simulation was performed to position compound **7b** into the active site of the MEK1 kinase to probe the binding mode. Analysis of the compound **7b** binding conformation in active site showed that it has suitable shape complementarity with the ATP binding pocket, and the conformation was stabilized by cation-π interaction with Lys 156 and an H-bond with Ser 150. Above all, the results obtained from this study suggest that N-(benzyloxy)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives skeleton may serve as a novel scaffold for the further development of more potent and selective MEK inhibitors which use as new therapeutic agent to fight against cancer.

4. Experimental section

4.1. General

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), proton nuclear magnetic resonance (^{1}H NMR) and elemental microanalyses (CHN). ^{1}H NMR and ^{13}C NMR spectra were measured on a Bruker AV-300 or a Agilent DD2 600Hz spectrometer AV-600 spectrometer at 25 ºC and referenced to Me4Si. Chemical shifts were reported in ppm (δ) using the residual solvent line as internal standard. Splitting patterns were designed as s, singlet; d, doublet; t, triplet; m, multiplet. ESI-MS spectra were recorded on a Mariner System 5304 Mass spectrometer. Elemental analyses were performed on a CHN-O-Rapid instrument and were within ±0.4% of the theoretical values. Melting points were determined on a XT4 MP apparatus (Taike Corp., Beijing, China) and were as read. Analytic thin-layer chromatography (TLC) was performed on the glass backed silica gel sheets (silica gel 60Å GF254). All compounds were detected by using UV light (254 nm or 365 nm).

4.2. General method for the preparation of target compounds 6-10

The starting material substituted 1,3-diphenyl-1*H*-pyrazole-4-carbonyl chloride derivatives (**6-10**) was synthesized as following: para-substituted acetophenone (**1-5**) (20mmol) interact with phenylhydrazine hydrochloride (25mmol) couple with sodium acetate (40mmol) in anhydrous ethanol to form 1-phenyl-2-(1-phenylethylidene) hydrazine, which was then dissolved in a cold mixed solution of DMF (20mL) and $POCl_3$ (16 mL), stirred at 50-60 ºC for 5 h. The resulting mixture was poured into ice-cold water, a saturated solution of sodium hydroxide was added to neutralize the mixture, then the obtained solid precipitate was oxidized to the corresponding carboxylic acids by treatment with potassium permanganate (10mmol), stirred at 70-80 ºC for 3 h while the transformation of acids into the appropriate acid

chlorides was accomplished with thionyl chloride in refluxing for 3h , and then thionyl chlorid was removed under reduced pressure to give the desired compounds **6**-**10**.

4.3. General procedure for 1,3-diphenyl-N-benzyloxy-1H-pyrazole-4-carboxamide derivatives 6a-10d

Compounds **6a-10d** were synthesized from a stirring mixture of the starting material substituted 1,3-diphenyl-1*H*-pyrazole-4-carbonyl chloride derivatives (**6-10**) (2 mmol) and substituted *O*-benzyl hydroxylamine hydrochloride (2.0mmol) with the help of pyridine (4.4 mmol) in anhydrous tetrahydrofuran (15 mL) at the room temperate for 2 h. The reaction mixture was poured into three times its volume of cold water when the product as a solid. The solid was filtered under vacuum, and then recrystallized from ethanol-DMF to afford the pure product **6a-10d**.

*4.3.1 N-(benzyloxy)-1,3-diphenyl-1H-pyrazole-4-carboxamide (**6a**)*

White solid. Yield: 80%. Mp: 125-127 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): 4.96 (s, 2H); 7.36-7.76 (m, 15H); 8.51 (s, 1H); 11.21 (s, 1H). MS (ESI): 370.1 ($C_{23}H_{19}N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{19}N_3O_2$: C, 74.78; H, 5.18; N, 11.37; Found: C, 74.81; H, 5.34; N, 11.29%.

*4.3.2 N-((2-fluorobenzyl)oxy)-1,3-diphenyl-1H-pyrazole-4-carboxamide (**6b**)*

White solid. Yield: 73%. Mp: 137-138 ºC. ^{1}H NMR(300 MHz, DMSO-d_6): 4.99 (s, 2H); 7.20-7.27 (m, 2H); 7.36-7.45 (m, 5H);7.79 (d, *J*=5.85Hz, 2H); 7.87 (d, *J*=8.43Hz, 2H); 8.79 (s, 1H); 11.48(s, 1H). MS (ESI): 388.1 ($C_{23}H_{18}FN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}FN_3O_2$: C, 71.31; H, 4.38; N, 10.85; Found: C, 71.38; H, 4.54; N, 11.01%.

*4.3.3 N-((2-chlorobenzyl)oxy)-1,3-diphenyl-1H-pyrazole-4-carboxamide (**6c**)*

White solid. Yield: 80%. Mp: 140-141 ºC. ^{1}H NMR(300 MHz, DMSO-d_6): 5.05 (s, 2H); 7.39-7.62 (m, 10H); 7.79 (d, *J*=6.03Hz, 2H); 7.88 (d, *J*=8.04Hz, 2H); 8.81 (s, 1H); 11.51 (s, 1H). MS (ESI): 404.1 ($C_{23}H_{18}ClN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}ClN_3O_2$: C, 68.40; H, 4.49; N, 10.40; Found: C, 68.53; H, 4.47; N, 10.296%.

*4.3.4 N-((2,4-dichlorobenzyl)oxy)-1,3-diphenyl-1H-pyrazole-4-carboxamide(**6d**)*

White solid. Yield: 87%. Mp: 167-169 ºC. ^{1}H NMR(300 MHz, $CDCl_3$): 4.96 (s, 2H); 7.27-7.56 (m, 13H); 7.75 (d, *J*=7.86Hz, 2H); 8.51 (s, 1H); 11.20 (s, 1H). MS (ESI): 438.1 ($C_{23}H_{17}Cl_2N_3O_2$, $[M+H]^+$). Anal. Calcd for ($C_{23}H_{17}Cl_2N_3O_2$: C, 63.03; H, 3.91; N, 9.59; Found: C, 63.25; H, 4.01; N, 9.48%.

*4.3.5 N-(benzyloxy)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (**7a**)*

White solid. Yield: 79%. Mp: 173-175 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): 2.41 (s, 3H); 4.96 (s, 2H); 7.15 (d, *J*=7.89Hz, 2H); 7.27-7.51 (m, 10H); 7.74 (d, *J*=8.22Hz, 2H); 8.51 (s, 1H); 10.20 (s, 1H). MS (ESI): 384.2 ($C_{24}H_{21}N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{21}N_3O_2$: C, 75.18; H, 5.52; N, 10.96; Found: C, 75.47; H, 5.56; N, 11.17%.

*4.3.6 N-((2-fluorobenzyl)oxy)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide(**7b**)*

White solid. Yield: 69%. Mp: 142-144 ºC. ^{1}H NMR(300 MHz, $CDCl_3$): 2.40 (s, 3H); 5.04 (s, 2H); 7.05-7.51 (m, 11H); 7.75 (d, *J*=8.22Hz, 2H); 8.52 (s, 1H); 10.21 (s, 1H). ^{13}C NMR (151 MHz, cdcl$_3$) δ 162.16, 160.51, 139.23, 139.18, 131.62, 131.16, 130.68, 130.63, 129.60, 129.55, 128.98, 128.66, 127.41, 124.21,124.18, 119.40, 115.58, 115.44, 71.81, 21.34. MS (ESI): 402.2 ($C_{24}H_{20}FN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}FN_3O_2$: C, 71.81; H, 5.02; N, 10.47; Found: C, 72.00; H, 4.98; N, 10.51%.

*4.3.7 N-((2-chlorobenzyl)oxy)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide(**7c**)*

White solid. Yield: 79%. Mp: 151-153 ºC. ^{1}H NMR(300 MHz, DMSO-d_6): 2.40 (s, 3H); 5.10 (s, 2H); 7.18-7.51 (m, 11H); 7.75 (d, *J*=8.04Hz, 2H); 8.52 (s, 1H); 10.21 (s, 1H). ^{13}C NMR (151 MHz, cdcl$_3$) δ 150.96, 139.25, 139.17, 134.34, 133.12, 131.20, 131.00, 129.89, 129.61, 129.56, 129.24, 128.98, 128.70, 127.42, 126.90, 199.42, 114.97, 75.43, 21.36. MS (ESI): 418.1 ($C_{24}H_{20}ClN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}ClN_3O_2$: C,68.98; H, 4.82; N, 10.06; Found: C, 68.74; H, 4.89; N, 10.27%.

*4.3.8 N-((2,4-dichlorobenzyl)oxy)-3-phenyl-1-(p-tolyl)-1H-pyrazole-4-carboxamide (**7d**)*

White solid. Yield: 85%. Mp: 169-171 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 2.37 (s, 3H); 5.01 (s, 2H); 7.22 (d, *J*=7.95Hz, 2H); 7.38 (t, *J*=7.30Hz, 1H); 7.47 (d, *J*=8.20Hz, 1H); 7.55 (t, *J*=7.92Hz, 3H); 7.61 (d, *J*=7.95Hz, 1H); 7.67 (d, *J*=7.65Hz, 3H); 7.87 (d, *J*=7.90Hz, 2H); 8.78 (s, 1H); 11.46 (s, 1H). MS (ESI): 452.2 ($C_{24}H_{19}Cl_2N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{19}Cl_2N_3O_2$: C, 63.73; H, 4.23; N, 9.29; Found: C, 63.69; H, 4.19; N, 9.57%.

*4.3.9 N-(benzyloxy)-1-(4-methoxyphenyl)-3-phenyl-1H-pyrazole-4-carboxamide(**8a**)*

White solid. Yield: 82%. Mp: 143-145 ºC. ^{1}H NMR (300 MHz, $CDCl_3$): 3.87 (s, 3H); 4.97 (s, 2H); 6.87 (d, *J*=8.58Hz, 2H); 7.36-7.41 (m, 6H); 7.48 (t, *J*=8.13Hz, 4H); 7.73 (d, *J*=8.25Hz, 2H); 8.50 (s, 1H); 10.21 (s, 1H). ^{13}C NMR (151 MHz, cdcl$_3$) δ 155.59, 134.42, 126.95, 126.50, 125.99, 125.94, 125.41, 124.83, 122.66, 119.50, 119.47, 119.39, 114.69, 110.86, 110.72, 109.58, 67.11, 50.58. MS (ESI): 400.2 ($C_{24}H_{21}N_3O_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{21}N_3O_3$: C, 72.16; H, 5.30; N, 10.52; Found: C, 72.38; H, 5.26; N, 10.78%.

*4.3.10 N-((2-fluorobenzyl)oxy)-1-(4-methoxyphenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**8b**)*

White solid. Yield: 70%. Mp: 144-146 ºC. ^{1}H NMR(300 MHz, DMSO-d_6): 3.86 (s, 3H); 5.05 (s, 2H); 6.91 (d, *J*=8.40Hz, 2H); 7.05-7.15 (m, 2H);7.36-7.54 (m, 7H); 7.75 (d, *J*=7.50Hz, 2H); 8.50 (s, 1H); 10.20 (s, 1H). MS (ESI): 418.1 ($C_{24}H_{20}FN_3O_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}FN_3O_3$: C, 69.05; H, 4.83; N, 10.07; Found: C, 69.29; H, 5.00; N, 10.04%.

*4.3.11 N-((2-chlorobenzyl)oxy)-1-(4-methoxyphenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**8c**)*

White solid. Yield: 78%. Mp: 121-123 ºC. ^{1}H NMR(300 MHz, DMSO-d_6): 3.87 (s, 3H); 5.03 (s, 2H); 7.17-7.52 (m, 11H); 7.75 (d, *J*=8.04Hz, 2H); 8.52 (s, 1H); 10.21 (s, 1H). MS (ESI): 434.1 ($C_{24}H_{20}ClN_3O_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{20}ClN_3O_3$: C, 66.44; H, 4.65; N, 9.68; Found: C, 66.67; H, 4.78; N, 9.56%.

*4.3.12 N-((2,4-dichlorobenzyl)oxy)-1-(4-methoxyphenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**8d**)*

White solid. Yield: 87%. Mp: 155-157 ºC. ^{1}H NMR(300 MHz, $CDCl_3$): 3.87 (s, 3H); 5.06 (s, 2H); 6.94 (t, *J*=8.72Hz, 2H); 7.24 (m, 1H);7.33-7.54 (m, 7H); 7.74 (d, *J*=7.47Hz, 2H); 8.50 (s, 1H); 10.19 (s, 1H). MS (ESI): 468.1 ($C_{24}H_{19}Cl_2N_3O_3$, $[M+H]^+$). Anal. Calcd for $C_{24}H_{19}Cl_2N_3O_3$: C, 61.55; H, 4.09; N, 8.97; Found: C, 61.78; H, 3.98; N, 9.23%.

*4.3.13 N-(benzyloxy)-1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**9a**)* White solid. Yield: 81%. Mp: 169-171 ºC.

^{1}H NMR (500 MHz, DMSO-d_6): 4.93 (s, 2H); 7.37-7.41 (m, 4H); 7.45 (s, 2H); 7.51 (d, *J*=8.50Hz, 2H); 7.56 (t, *J*=7.92Hz, 2H); 7.84 (d, *J*=8.55Hz, 2H); 7.88 (d, *J*=8.55Hz, 2H); 8.84 (s, 1H); 11.51 (s, 1H). MS (ESI): 404.1 ($C_{23}H_{18}ClN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}ClN_3O_2$: C, 68.40; H, 4.49; N, 10.40; Found: C, 68.65; H, 4.25; N, 10.56%.

*4.3.14 1-(4-chlorophenyl)-N-((2-fluorobenzyl)oxy)-3-phenyl-1H-pyrazole-4-carboxamide (**9b**)*

White solid. Yield: 65%. Mp: 152-154 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.03 (s, 2H); 7.24 (t, *J*=7.17Hz, 2H); 7.38-7.45 (m, 2H); 7.50 (d, *J*=8.50Hz, 2H); 7.53-7.58 (m, 3H); 7.84 (d, *J*=8.20Hz, 2H); 7.88 (d, *J*=7.90Hz, 2H); 8.83 (s, 1H); 11.53 (s, 1H). MS (ESI): 422.1 ($C_{23}H_{17}ClFN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{17}ClFN_3O_2$: C, 65.49; H, 4.06; N, 9.96; Found: C, 65.54; H, 4.03; N, 10.08%.

*4.3.15 N-((2-chlorobenzyl)oxy)-1-(4-chlorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**9c**)*

White solid. Yield: 78%. Mp: 177-178 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.05 (s, 2H); 7.40 (t, *J*=7.32Hz, 3H); 7.50 (d, *J*=8.50Hz, 3H); 7.55-7.60 (m, 3H); 7.85 (d, *J*=8.25Hz, 2H); 7.88 (d, *J*=7.65Hz, 2H); 8.84 (s, 1H); 11.55 (s, 1H). MS (ESI): 438.1 ($C_{23}H_{17}Cl_2N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{17}Cl_2N_3O_2$: C, 63.03; H, 3.91; N, 9.59; Found: C, 63.14; H, 4.03; N, 9.46%.

*4.3.16 1-(4-chlorophenyl)-N-((2,4-dichlorobenzyl)oxy)-3-phenyl-1H-pyrazole-4-carboxamide (**9d**)*

White solid. Yield: 81%. Mp: 172-174 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.02 (s, 2H); 7.40 (t, *J*=7.32Hz, 1H); 7.49 (t, *J*=9.00Hz, 3H); 7.55-7.67 (m, 4H); 7.82 (d, *J*=7.90Hz, 2H); 7.88 (d, *J*=7.60Hz, 2H); 8.82 (s, 1H); 11.52 (s, 1H). MS (ESI): 472.0 ($C_{23}H_{16}Cl_3N_3O_2$, $[M+H]^+$). Anal. Calcd for ($C_{23}H_{16}Cl_3N_3O_2$: C, 58.44; H, 3.41; N, 8.89; Found: C, 58.57; H, 3.26; N, 9.01%.

4.3.17 *N-(benzyloxy)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide*(**10a**)

White solid. Yield: 83%. Mp: 158-159 ºC. ^{1}H NMR (500 MHz, DMSO-d_6): 4.93 (s, 2H); 7.27 (t, *J*=9.00Hz, 2H); 7.36-7.46 (m, 6H); 7.56 (t, *J*=7.95Hz, 2H); 7.85 (d, *J*=8.20Hz, 2H); 7.88 (d, *J*=8.25Hz, 2H); 8.82 (s, 1H);11.48 (s, 1H). MS (ESI): 388.1 ($C_{23}H_{18}FN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{18}FN_3O_2$: C, 71.31; H, 4.68; N, 10.85; Found: C, 71.39; H, 4.55; N, 11.03%.

*4.3.18 N-((2-fluorobenzyl)oxy)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**10b**)*

White solid. Yield: 65%. Mp: 151-153 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.00 (s, 2H); 7.22-7.28 (m, 4H); 7.38-7.47 (m, 2H); 7.56 (t, *J*=7.95Hz, 3H); 7.84-7.98 (m, 4H); 8.81 (s, 1H); 11.50 (s, 1H). MS (ESI): 406.1 ($C_{23}H_{17}F_2N_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{17}F_2N_3O_2$: C, 68.14; H, 4.23; N, 10.37; Found: C, 68.27; H, 4.01; N, 10.53%.

*4.3.19 N-((2-chlorobenzyl)oxy)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**10c**)*

White solid. Yield: 80%. Mp: 135-136 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.05 (s, 2H); 7.27 (t, *J*=9.00Hz, 2H); 7.40 (m, 3H); 7.49-7.60 (m, 4H); 7.85 (d, *J*=8.20Hz, 2H); 7.88 (d, *J*=8.25Hz, 2H); 8.83 (s, 1H); 11.53 (s, 1H). MS (ESI): 422.1 ($C_{23}H_{17}ClFN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{17}ClFN_3O_2$: C, 65.49; H, 4.06; N, 9.96; Found: C, 65.53; H, 3.97; N, 10.12%.

*4.3.20 N-((2,4-dichlorobenzyl)oxy)-1-(4-fluorophenyl)-3-phenyl-1H-pyrazole-4-carboxamide (**10d**)*

White solid. Yield: 85%. Mp: 175-177 ºC. ^{1}H NMR(500 MHz, DMSO-d_6): 5.02 (s, 2H); 7.27 (t, *J*=8.82Hz, 2H); 7.40 (t, *J*=7.32Hz, 1H); 7.48 (d, *J*=7.00Hz, 1H); 7.54-7.67 (m, 4H); 7.84 (d, *J*=7.90Hz, 2H); 7.88 (d, *J*=7.60Hz, 2H); 8.81 (s, 1H); 11.51 (s, 1H). MS (ESI): 456.1 ($C_{23}H_{16}Cl_2FN_3O_2$, $[M+H]^+$). Anal. Calcd for $C_{23}H_{16}Cl_2FN_3O_2$: C, 60.54; H, 3.53; N, 9.21; Found: C, 60.75; H, 3.26; N, 9.37%.

4.4 Antiproliferative activity

The antiproliferative activities of the prepared compounds were evaluated by using a standard (MTT)-based colorimetric assay with some modification. Cell lines grew to log phase in DMEM supplemented with 10% fetal bovine serum, under a humidified atmosphere of 5% CO_2 at 37 ˚C. Cell suspensions were prepared and 100 μL/well dispensed into 96-well plates giving 10^5 cells/well. The plates were returned to the incubator for 24 h to allow the cells to reattach. Subsequently, cells were treated with the target compounds at increasing concentrations in the presence of 10% FBS for 48 h. Then, cell viability was assessed by the conventional 3-(4,5-dimethylthiazol- 2-yl)-2,5-diphenyltetrazolium bromide (MTT) reduction assay and carried out strictly according to the manufacturer instructions (Sigma). The absorbance (OD_{490}) was read on an ELISA reader (Tecan, Austria).

4.5 kinase assay

The MEK1 kinase assay was conducted by using a modified procedure as described by Toshiyuki *et al*. [35]. Tested compounds were dissolved in DMSO and were present at increasing concentrations. Briefly, non-phosphorylated myelin basic protein was coated onto an ELISA plate, and the active form of BRAF (Invitrogen) was mixed with unphosphorylated MEK1 (Millipore) and ERK2 (Invitrogen) in 10 μM ATP and 12.5 mM $MgCl_2$ containing MOPS buffer in the presence of various concentrations of tested compounds. The phosphorylation of MBP was detected by the anti-phospho-MBP antibody.

Detection of the effect of compounds on cell-based pERK1/2 activity in A549 cells was performed in ELISA kits (Invitrogen) and strictly according to the manufacturer instructions.

The kinase profiling of compound **7b** against EGFR, HER-2, FAK, Aurora-A, and VEGFR-2 were evaluated by the same protocol with our previously reported. [36-38]

4.6 Molecular docking

The X-ray crystal structure of the BRAF kinase domain in an active configuration (MEK1/ K252A, PDB code: 3EQF, Protein Data Bank) was used as the target structure in this approach. The molecular docking procedure was performed by using CDOCKER protocol for receptor-ligand interactions section of DS 3.1 (Discovery Studio 3.1, Accelrys, Inc., San Diego, CA).[33]

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References and notes

1. Cohen, P. *Nature Reviews Drug Discovery* **2002,** *1*, 309.
2. Sebolt-Leopold, J. S.; Herrera, R. *Nature Reviews Cancer* **2004,** *4*, 937.
3. Blume-Jensen, P.; Hunter, T. *Nature* **2001,** *411*, 355.
4. Davies, H.; Bignell, G. R.; Cox, C.; Stephens, P.; Edkins, S.; Clegg, S.; Teague, J.; Woffendin, H.; Garnett, M. J.; Bottomley, W.; Davis, N.; Dicks, E.; Ewing, R.; Floyd, Y.; Gray,

K.; Hall, S.; Hawes, R.; Hughes, J.; Kosmidou, V.; Menzies, A.; Mould, C.; Parker, A.; Stevens, C.; Watt, S.; Hooper, S.; Wilson, R.; Jayatilake, H.; Gusterson, B. A.; Cooper, C.; Shipley, J.; Hargrave, D.; Pritchard-Jones, K.; Maitland, N.; Chenevix-Trench, G.; Riggins, G. J.; Bigner, D. D.; Palmieri, G.; Cossu, A.; Flanagan, A.; Nicholson, A.; Ho, J. W.; Leung, S. Y.; Yuen, S. T.; Weber, B. L.; Seigler, H. F.; Darrow, T. L.; Paterson, H.; Marais, R.; Marshall, C. J.; Wooster, R.; Stratton, M. R.; Futreal, P. A. In *Nature*, 2002; Vol. 417, 949.

5. Flaherty, K. T.; Hodi, F. S.; Fisher, D. E. *Nature Reviews Cancer* **2012,** *12*, 349.

6. Lito, P.; Rosen, N.; Solit, D. B. *Nature Medicine* **2013,** *19*, 1401.

7. Wan, P. T. C.; Garnett, M. J.; Roe, S. M.; Lee, S.; Niculescu-Duvaz, D.; Good, V. M.; Jones, C. M.; Marshall, C. J.; Springer, C. J.; Barford, D.; Marais, R.; Cancer Genome, P. *Cell* **2004,** *116*, 855.

8. Sosman, J. A.; Kim, K. B.; Schuchter, L.; Gonzalez, R.; Pavlick, A. C.; Weber, J. S.; McArthur, G. A.; Hutson, T. E.; Moschos, S. J.; Flaherty, K. T.; Hersey, P.; Kefford, R.; Lawrence, D.; Puzanov, I.; Lewis, K. D.; Amaravadi, R. K.; Chmielowski, B.; Lawrence, H. J.; Shyr, Y.; Ye, F.; Li, J.; Nolop, K. B.; Lee, R. J.; Joe, A. K.; Ribas, A. *New England Journal of Medicine* **2012,** *366*, 707.

9. Falchook, G. S.; Long, G. V.; Kurzrock, R.; Kim, K. B.; Arkenau, T. H.; Brown, M. P.; Hamid, O.; Infante, J. R.; Millward, M.; Pavlick, A. C.; O'Day, S. J.; Blackman, S. C.; Curtis, C. M.; Lebowitz, P.; Ma, B.; Ouellet, D.; Kefford, R. F. *Lancet* **2012,** *379*, 1893.

10. Kefford, R.; Arkenau, H.; Brown, M. P.; Millward, M.; Infante, J. R.; Long, G. V.; Ouellet, D.; Curtis, M.; Lebowitz, P. F.; Falchook, G. S. *Journal of Clinical Oncology* **2010,** *28*.

11. Jang, S.; Atkins, M. B. *Lancet Oncology* **2013,** *14*, E60.

12. Bollag, G.; Hirth, P.; Tsai, J.; Zhang, J.; Ibrahim, P. N.; Cho, H.; Spevak, W.; Zhang, C.; Zhang, Y.; Habets, G.; Burton, E.; Wong, B.; Tsang, G.; West, B. L.; Powell, B.; Shellooe, R.; Marimuthu, A.; Nguyen, H.; Zhang, K. Y. J.; Artis, D. R.; Schlessinger, J.; Su, F.; Higgins, B.; Iyer, R.; D'Andrea, K.; Koehler, A.; Stumm, M.; Lin, P. S.; Lee, R. J.; Grippo, J.; Puzanov, I.; Kim, K. B.; Ribas, A.; McArthur, G. A.; Sosman, J. A.; Chapman, P. B.; Flaherty, K. T.; Xu, X.; Nathanson, K. L.; Nolop, K. *Nature* **2010,** *467*, 596.

13. Poulikakos, P. I.; Persaud, Y.; Janakiraman, M.; Kong, X.; Ng, C.; Moriceau, G.; Shi, H.; Atefi, M.; Titz, B.; Gabay, M. T.; Salton, M.; Dahlman, K. B.; Tadi, M.; Wargo, J. A.; Flaherty, K. T.; Kelley, M. C.; Misteli, T.; Chapman, P. B.; Sosman, J. A.; Graeber, T. G.; Ribas, A.; Lo, R. S.; Rosen, N.; Solit, D. B. *Nature* **2011,** *480*, 387.

14. Wagle, N.; Emery, C.; Berger, M. F.; Davis, M. J.; Sawyer, A.; Pochanard, P.; Kehoe, S. M.; Johannessen, C. M.; MacConaill, L. E.; Hahn, W. C.; Meyerson, M.; Garraway, L. A. *Journal of Clinical Oncology* **2011,** *29*, 3085.

15. Shi, H.; Moriceau, G.; Kong, X.; Lee, M.-K.; Lee, H.; Koya, R. C.; Ng, C.; Chodon, T.; Scolyer, R. A.; Dahlman, K. B.; Sosman, J. A.; Kefford, R. F.; Long, G. V.; Nelson, S. F.; Ribas, A.; Lo, R. S. *Nature Communications* **2012,** *3*.

16. Sun, C.; Bernards, R. *Trends in Biochemical Sciences* **2014,** *39*, 465.

17. Johannessen, C. M.; Boehm, J. S.; Kim, S. Y.; Thomas, S. R.; Wardwell, L.; Johnson, L. A.; Emery, C. M.; Stransky, N.; Cogdill, A. P.; Barretina, J.; Caponigro, G.; Hieronymus, H.; Murray, R. R.; Salehi-Ashtiani, K.; Hill, D. E.; Vidal, M.; Zhao, J. J.; Yang, X.; Alkan, O.; Kim, S.; Harris, J. L.; Wilson, C. J.; Myer, V. E.; Finan, P. M.; Root, D. E.; Roberts, T. M.; Golub, T.; Flaherty, K. T.; Dummer, R.; Weber, B. L.; Sellers, W. R.; Schlegel, R.; Wargo, J. A.; Hahn, W. C.; Garraway, L. A. *Nature* **2010,** *468*, 968.

18. Nazarian, R.; Shi, H.; Wang, Q.; Kong, X.; Koya, R. C.; Lee, H.; Chen, Z.; Lee, M.-K.; Attar, N.; Sazegar, H.; Chodon, T.; Nelson, S. F.; McArthur, G.; Sosman, J. A.; Ribas, A.; Lo, R. S. *Nature* **2010,** *468*, 973.

19. Kim, K. B.; Kefford, R.; Pavlick, A. C.; Infante, J. R.; Ribas, A.; Sosman, J. A.; Fecher, L. A.; Millward, M.; McArthur, G. A.; Hwu, P.; Gonzalez, R.; Ott, P. A.; Long, G. V.; Gardner, O. S.; Ouellet, D.; Xu, Y.; DeMarini, D. J.; Le, N. T.; Patel, K.; Lewis, K. D. *Journal of Clinical Oncology* **2013,** *31*, 482.

20. Flaherty, K. T.; Robert, C.; Hersey, P.; Nathan, P.; Garbe, C.; Milhem, M.; Demidov, L. V.; Hassel, J. C.; Rutkowski, P.; Mohr, P.; Dummer, R.; Trefzer, U.; Larkin, J. M. G.; Utikal, J.; Dreno, B.; Nyakas, M.; Middleton, M. R.; Becker, J. C.; Casey, M.; Sherman, L. J.; Wu, F. S.; Ouellet, D.; Martin, A.-M.; Patel, K.; Schadendorf, D.; Grp, M. S. *New England Journal of Medicine* **2012,** *367*, 107.

21. Long, G. V.; Stroyakovskiy, D.; Gogas, H.; Levchenko, E.; de Braud, F.; Larkin, J.; Garbe, C.; Jouary, T.; Hauschild, A.; Grob, J. J.; Sileni, V. C.; Lebbe, C.; Mandala, M.; Millward, M.; Arance, A.; Bondarenko, I.; Haanen, J. B. A. G.; Hansson, J.; Utikal, J.; Ferraresi, V.; Kovalenko, N.; Mohr, P.; Probachai, V.; Schadendorf, D.; Nathan, P.; Robert, C.; Ribas, A.; DeMarini, D. J.; Irani, J. G.; Casey, M.; Ouellet, D.; Martin, A. M.; Le, N.; Patel, K.; Flaherty, K. *New England Journal of Medicine* **2014,** *371*, 1877.

22. Shi, J. B.; Tang, W. J.; Li, R.; Liu, X. H. *European journal of medicinal chemistry* **2015,** *90*, 889.

23. Li, Q.-S.; Li, C.-Y.; Lu, X.; Zhang, H.; Zhu, H.-L. *European journal of medicinal chemistry* **2012,** *50*, 288.

24. Li, Q.-S.; Lv, X.-H.; Zhang, Y.-B.; Dong, J.-J.; Zhou, W.-P.; Yang, Y.; Zhu, H.-L. *Bioorganic & medicinal chemistry letters* **2012,** *22*, 6596.

25. Wang, Y.; Cheng, F. X.; Yuan, X. L.; Tang, W. J.; Shi, J. B.; Liao, C. Z.; Liu, X. H. *European journal of medicinal chemistry* **2016,** *112*, 231.

26. Sebolt-Leopold, J. S.; Dudley, D. T.; Herrera, R.; Van Becelaere, K.; Wiland, A.; Gowan, R. C.; Tecle, H.; Barrett, S. D.; Bridges, A.; Przybranowski, S.; Leopold, W. R.; Saltiel, A. R. *Nature medicine* **1999,** *5*, 810.

27. Dai, Y.; Yu, C.; Singh, V.; Tang, L.; Wang, Z.; McInistry, R.; Dent, P.; Grant, S. *Cancer research* **2001,** *61*, 5106.

28. Huang, X. F.; Lu, X.; Zhang, Y.; Song, G. Q.; He, Q. L.; Li, Q. S.; Yang, X. H.; Wei, Y.; Zhu, H. L. *Bioorganic & Medicinal Chemistry* **2012,** *20*, 4895.

29. Saeed, S.; Rashid, N.; Jones, P. G.; Ali, M.; Hussain, R. *Eur. J. Med. Chem.* **2010,** *45*, 1323.

30. Miyata, O.; Koizumi, T.; Asai, H.; Iba, R.; Naito, T. *Tetrahedron* **2004,** *60*, 3893.

31. Sun, J.; Lv, X. H.; Qiu, H. Y.; Wang, Y. T.; Du, Q. R.; Li, D. D.; Yang, Y. H.; Zhu, H. L. *Eur J Med Chem* **2013,** *68*, 1.

32. Discovery Studio 3.1, Accelrys Software Inc., San Diego, 2011.

33. Wu, G.; Robertson, D. H.; Brooks, C. L.; Vieth, M. *Journal of Computational Chemistry* **2003,** *24*, 1549.

34. Li, Q. S.; Ni, H. J.; Yang, Y.; Lv, X. H.; Ruan, B. F. *Medicinal Chemistry* **2015,** *11*, 305.

35. Yamaguchi, T.; Kakefuda, R.; Tajima, N.; Sowa, Y.; Sakai, T. *International Journal of Oncology* **2011,** *39*, 23.

36. Li, Q.-S.; Lv, P.-C.; Li, H.-Q.; Lu, X.; Li, Z.-L.; Ruan, B.-F.; Zhu, H.-L. *Journal of enzyme inhibition and medicinal chemistry* **2012,** *27*, 708.

37. Yang, X.-H.; Xiang, L.; Li, X.; Zhao, T.-T.; Zhang, H.; Zhou, W.-P.; Wang, X.-M.; Gong, H.-B.; Zhu, H.-L. *Bioorganic & medicinal chemistry* **2012,** *20*, 2789.

38. Yang, Y.; Shi, L.; Zhou, Y.; Li, H.-Q.; Zhu, Z.-W.; Zhu, H.-L. *Bioorganic & medicinal chemistry letters* **2010,** *20*, 6653.

Graphical Abstract

Discovery of N-(benzyloxy)-1, 3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives as potential antiproliferative agents by inhibiting MEK

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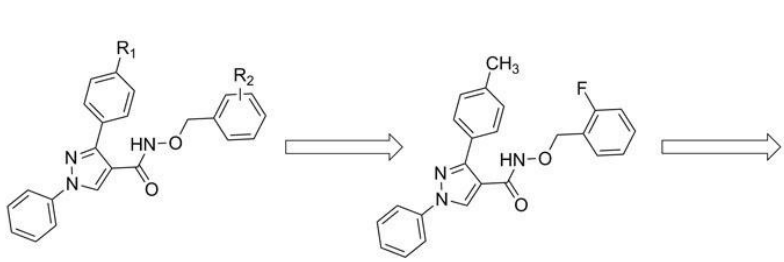
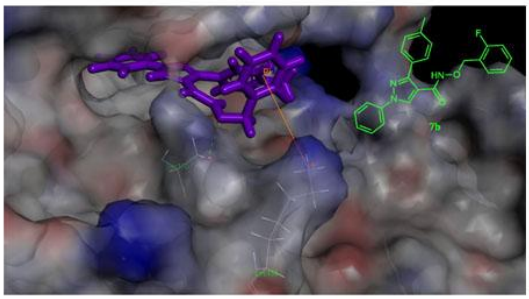
Twenty N-(benzyloxy)-1,3-diphenyl-1*H*-pyrazole-4-carboxamide derivatives have been designed and synthesized as MEK inhibitors, compound **7b** showed the most potent inhibitory activity with IC_{50} of 91 nM for MEK1 and GI_{50} value of 0.26 μM for A549 cells.





compound 7b showed the most potent inhibitory activity with IC_{50} of 91 nM for MEK1 and GI_{50} value of 0.26 μ M for A549 cells.