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ARTICLE

Isoindole-1,3-dione Derivatives as RSK2 Inhibitors: Synthesis, Molecular Docking Simulation and SAR Analysis

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RSK2 (p90 ribosomal S6 kinase 2) is a serine/threonine kinase expressed in a variety of cancers. Molecular-targeted inhibition of RSK2 as a potential therapeutic strategy for human cancers has been documented. In this work, a series of isoindole-1,3-dione derivatives as novel RSK2 inhibitors were designed and synthesized from a hit discovered in our previously study. Some compounds were confirmed to be moderate potent RSK2 inhibitors with the IC_{50} values at about 0.5 μM range. The structure-activity relationship analysis and binding mode studies by molecular docking were performed.

Introduction

RSK1~4 (p90 ribosomal S6 protein kinase) belong to a family of 4 serine/threonine kinases, in which RSK2 plays a crucial role in the mitogen-activated protein kinase (MAPK) signaling pathway.[1, 2] It has been verified that the overexpression and aberrant activation of RSK2 are related to many human cancers and other diseases, such as breast cancer, prostate cancer,[3] myeloid leukemia,[4] HIV infection[5] and Coffin-Lowry syndrome (CLS).[6] Therefore, RSK2 is a promising anti-cancer target and the discovery of potent inhibitors against RSK2 is of great significance.

RSK2 has a unique structure that it possesses an N-terminal kinase domain (NTKD) and a C-terminal kinase domain (CTKD) connected by a linker region.[7] Thus there are two binding sites for ATP-competitive inhibitors in the NTKD and the CTKD, respectively.[8, 9] To date, some RSK2 inhibitors with diverse scaffolds have been developed (Figure 1).[10-12] SL0101 isolated from the tropical plant *Forsteroniarefracta* is the first reported RSK2 specific inhibitor. SL0101 was reported to interact with RSK2 at the NTKD ATP-binding pocket and showed good inhibitory activity with an IC_{50} value of 89 nM.[13, 14] Several other RSK2 inhibitors were also found to potently but nonselectively inhibit RSK2, such as PKC (protein kinase C) inhibitors GF109203X (IC_{50} = 50 nM against RSK2) and Ro31-8220 (IC_{50} = 3 nM against RSK2).[15] In addition, FMK is a selective and irreversible RSK2 CTKD inhibitor with an IC_{50} value of 15 nM.[16] Most recently, Shafer et al. reported a series of 2-Amino-7-substituted benzoxazole compounds as potent and selective RSK2 inhibitors with nanomolar IC_{50} value.[12] However, none of these RSK2 inhibitors have been used in the clinical trials until now. Therefore, there is urgent need to develop other scaffold RSK2 inhibitors as anticancer drugs.

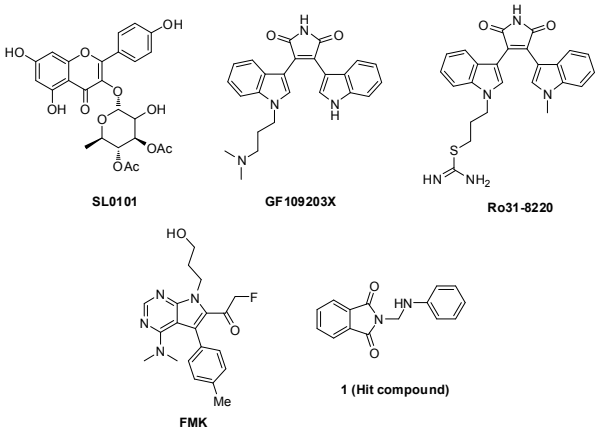

Fig. 1 Examples of reported RSK2 inhibitors and the hit compound **1**.

We recently developed SHAFTS program, a hybrid 3D similarity calculation method combining chemical feature matching and molecular shape superposition.[17] Several inhibitors for the NTKD of RSK2 were identified successfully using SHAFTS program in our previous study.[18-21] Among them, a hit compound **1** with isoindole-1,3-dione scaffold (one drug-like chemical fragment[22, 23]) showed some RSK2 affinity with an inhibitory ratio of 44% at 10 μM. In this study, the structural optimization and synthesis from the hit compound **1** as novel RSK2 inhibitors were carried out. Some compounds exhibited much improved inhibitory activity against RSK2 compared with the hit, the most active compound **7** inhibited RSK2 activity with an IC_{50} value up to 0.47 μM (inhibitory ratio of 97% at 10

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μM). The structure-activity relationship (SAR) analyses and molecular docking simulation explanations were also performed for searching more potent RSK2 inhibitors.

Results and discussion

Structural Optimization and SAR Interpretation

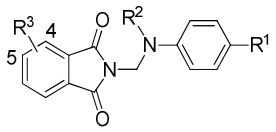
To improve the inhibitory potency of hit compound and explore the structure-activity relationships, we designed a series of isoindole-1,3-dione derivatives as novel RSK2 inhibitors. The enzyme inhibitory activities toward RSK2 of the designed compounds were evaluated and the results were summarized in Table 1.

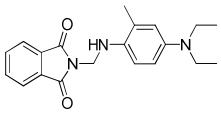
We first investigated the effects of substituent R^1 on arylamine part. Replacing the R^1 in hit compound **1** with electron-withdrawing halogen atoms (**2** and **3**) did not induce the improvement of inhibitory activities. Compounds substituted with weak electron-donating alkyl moieties, such as methyl (**4**), ethyl (**5**) and isopropyl (**6**), presented slightly improved potency against RSK2. We were pleased to find that the enzymatic inhibitory activities increased significantly while a strong electron-donating methoxyl group (**7**, IC_{50} = 0.47 μM) was introduced to the R^1 position. Same results reappeared for other typical electron-donating groups such as hydroxyl (**9**), ethoxyl (**10**), diethylamino (**11**) and morpholinyl (**12**), their inhibitory activities against RSK2 IC_{50} values were 0.52 μM, 0.59 μM, 0.86 μM and 0.79 μM, respectively. This tendency was tested by replacing the methoxyl group (**7)** with an electron-withdrawing group trifluoromethoxy moiety (**8**), its inhibitory potency against RSK2 nearly disappeared. The results indicated that electron-donating substituents in the R^1 position might be more favoured for improving the inhibitory potency against RSK2.

Next, we examined the impact of different substituents R^2 on the inhibitory against RSK2. When R^2 was methyl (**13**), the inhibitory activity lowered more than 7 times by comparing with compound **7**. When R^2 was acetyl group (**14**), the inhibitory potency against RSK2 completely disappeared. These results suggested that the volume of substituents at R^2 might affect the spatial molecular structure. More probably, the impact on hydrogen bond formation might be the key factor by comparing hydrogen bond tendency N-H>N-CH_3>N-$COCH_3$. The investigations indicated that R^2=H was necessary for keeping the inhibitory activity against RSK2.

Since compound **7** mainly stretches along the hinge region of RSK2 NTKD (Figure 2A) and enough space of the binding pocket remain to be explored, we then focused on the substituents R^3 at C4 position or C5 position of the isoindole-1,3-dione scaffold. While introducing an electron-withdrawing group nitro on the C5 position, the enzymatic inhibitory activities of compounds **15** and **16** decreased obviously compared with the corresponding compounds **4** and **10**. In contrast, amino substituents at the C4 position and C5 position

Table 1 In Vitro Enzymatic Inhibitory Activities of Isoindole-1,3-dione Compounds against RSK2[a]



Compd	R^1	R^2	R^3	Inhibition (%)	IC_{50} (μM)
1	H	H	H	43.57	ND[b]
2	F	H	H	56.66	8.74
3	Cl	H	H	8.52	ND
4	Me	H	H	54.00	4.3
5	Et	H	H	62.99	4.31
6	i-Pr	H	H	46.54	ND
7	OMe	H	H	97.19	0.47
8	OCF_3	H	H	28.05	ND
9	OH	H	H	100.92	0.52
10	OEt	H	H	100.12	0.59
11				81.04	0.86
12	morpholinyl	H	H	101.43	0.79
13	OMe	Me	H	86.99	3.50
14	OMe	Ac	H	17.17	ND
15	Me	H	5-NO_2	35.69	ND
16	OEt	H	5-NO_2	86.96	2.83
17	OMe	H	4-NH_2	95.64	3.53
18	OMe	H	5-NH_2	109.10	0.59
19	OMe	H	4-benzamido	99.51	0.99
20	OMe	H	5-benzamido	82.00	2.16
21	OEt	H	4-benzamido	105.41	1.34
22	OEt	H	5-benzamido	69.47	4.58
23	morpholinyl	H	5-benzamido	102.51	1.01
24	OMe	H	5-phenyl-carbamido	34.79	ND
Ro31-8220				98.21	0.01

[a]Inhibitory rate was measured at the concentration of 10 μM inhibitor. IC_{50} value was measured if the inhibitory rate at 10 μM exceeded 50%. [b]ND, Not determined.

of compounds **17** and **18** were better tolerated with IC_{50} values against RSK2 of 3.53 μM and 0.59 μM, respectively. We expected that introducing hydrophobic groups could enhance the binding affinity by increasing the hydrophobic interactions, but benzolation of the R^3 amino groups (**17** and **18**) led to corresponding compounds **19-23**, just brought similar inhibitory activities. The results suggested that electron-withdrawing group such as nitro on C4 position or C5 position of the isoindole-1,3-dione scaffold had negative effects on the inhibitory activity, while electron-donating groups such as NH_2, or a big group benzamide were all tolerated, at least, no obvious adverse effects appeared comparing with compound **7**. Furthermore, when introducing a longer group phenylcarbamido onto C5 position (**24**), its inhibitory activity against RSK2 diminished completely, this illustrated that the

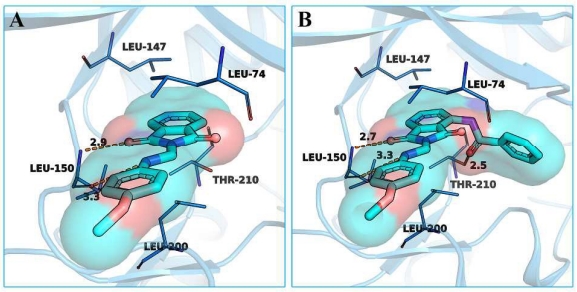
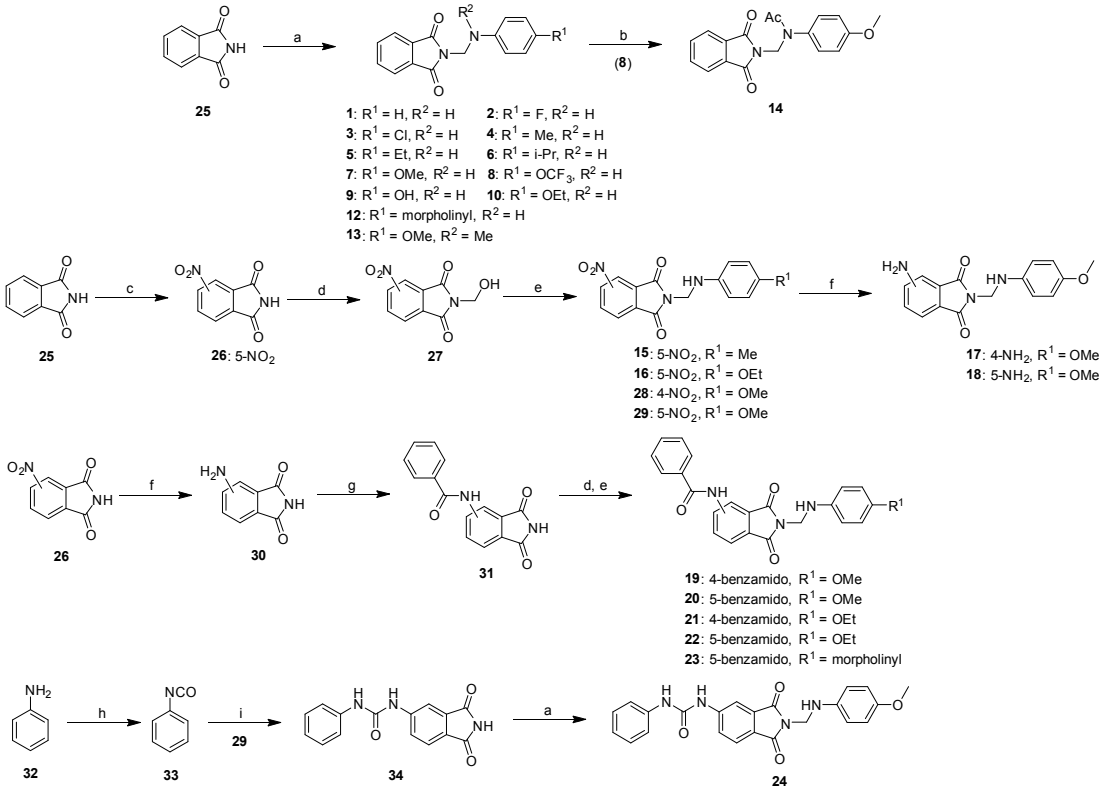


Fig. 2 The proposed binding modes for representative compounds **7** (A), **19** (B). The X-ray crystal structure of RSK2 NTKD (PDB ID: 4NW6) is shown as light blue cartoon, and the docked inhibitors are represented as cyan sticks. Key residues (thin sticks) in the ATP binding site are colored in blue. Hydrogen atoms are hidden for clarity. Potential intermolecular hydrogen bonds are showed in orange dashed lines.

volume and spatial orientation of phenylcarbamido didn't fit to the enzyme active pocket.

Binding Mode Analysis

To further study the action mechanism of these series of derivatives, the predicted binding modes of compounds **7** and **19** are shown in Figure 2A and 2B with the docking energy of -9.36 kcal/mol and -9.21 kcal/mol respectively. Just like the other RSK2 inhibitors we reported previously,[18-21] both of the compounds occupied the ATP binding site and generated a bi-dentate hydrogen bond with Leu150 at the hinge region. Specifically, one of the oxygen atom in isoindole-1,3-dione was capable to be hydrogen bonded to backbone nitrogen atom of Leu150, and the NR^2 group in the linker of **7** could also form a hydrogen bond with the backbone carbonyl group of Leu150 spontaneously. If the NR^2 group of **7** is substituted by other groups (R^2=CH_3 **13** and R^2=$COCH_3$ **14**), the hydrogen bond with backbone carbonyl group of Leu150 would be destroyed, leading to a decreased inhibitory activity. In addition, the oxygen atom in 4-benzamido moiety of **19** acted as a hydrogen bond acceptor to the side chain hydroxyl group of Thr210 with the hydrogen bond distance of 2.5Å, and the phenyl group in the 4-benzamido moiety contacted the residues scattering around with van der Waals interactions. However, compared with **7**, those extra hydrogen bond and VDW contacts of **19** did not contribute to the binding affinity for the compounds of this series. Nevertheless, electron-donating groups at R^1 plays an important role for the improvement of inhibitory activity of the isoindole-1,3-dione derivatives, which could make VDW contacts with residues Leu74, Phe149 and Gly153, combines with the connected benzene ring. Therefore, compared with



Scheme 1 Reagents and conditions: (a) formaldehyde, arylamine, EtOH, H_2O, reflux, 50-85%; (b) CH_3COOCl, Et_3N, CH_2Cl_2, rt, 69%; (c) HNO_3, H_2SO_4, 40 °C, 65%; (d) formaldehyde, 1,4-dioxane, H_2O, reflux; (e) $ArNH_2$, EtOH, 50 °C, 51-80%; (f) 10%Pd/C, H_2, MeOH, 97-99%; (g) acyl chloride, 1-methyl-2-pyrrolidinone, CH_3CN, 0 °C to rt, 90–93%; (h) triphosgene (BTC), PhMe, reflux; (i) DMF, PhMe, 110 °C, 48%.

compounds **2**, **3** and **8**, compounds **9**, **10**, **11** and **12** all exhibited relatively high activity against RSK2 enzyme with IC_{50} value around 0.5 μM.

Chemistry

The synthesis of the isoindole-1,3-dione derivatives used in this study was shown in Scheme 1. Several final products were prepared by coupling of the starting material phthalimide with a variety of arylamines.[24] Acetylation of compound **7** produced amide compound **14** as a white solid. The nitro compound **26** was prepared by nitration of phthalimide **25**.[25] Treating compound **26** with aqueous formaldehyde obtained hydroxymethyl intermediate **27** which was followed by coupling with p-anisidine to yield compound **28**. The nitro group of **28** was reduced with Pd/C hydrogenation in methanol to produce compound **17** and **18** in good yields. Nitro compound **26** was reduced with Pd/C hydrogenation and further acylated by benzoyl chloride to yield **30**. Final compounds **19**-**23** were prepared from **30** by a method similar to that for compound **28**. For the synthesis of the desired compound **24**, isocyanatobenzene **32** was prepared from phenylamine by using triphosgene, subsequently coupled with amino compound **29** to produce phenylurea compounds **33** which further reacted with formaldehyde and p-anisidine to give compound **24**.[26]

Conclusions

In summary, a series of isoindole-1,3-dione derivatives were designed and synthesized as RSK2 inhibitors through structural optimization of the hit compound **1**. The SAR analyses and the proposed binding modes were explored to further elucidate the SAR. Electron-donating substituents for R^1 were favorable for inhibitory activity, and un-substituted R^2 was beneficial to increase affinity with RSK2 by forming H-bond. Compound **7** showed much improved inhibitory activity compared with the initial hit, with an IC_{50} value of 0.47 μM. It is necessary to further optimization of this scaffold to achieve more potent RSK2 inhibitors.

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Isoindole-1,3-dione Derivatives as RSK2 Inhibitors: Synthesis, Molecular Docking Simulation and SAR Analysis

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The present study reports a series of novel potent RSK2 inhibitors from structure modifications of the virtual screening hit.