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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 previous study. Some compounds were confirmed to be moderately potent RSK2 inhibitors with IC_{50} values of about 0.5 μM. Structure–activity relationship analysis and binding mode studies by molecular docking were performed.

Introduction

RSK1–4 (p90 ribosomal S6 protein kinases) belong to a family of 4 serine/threonine kinases, in which RSK2 plays a crucial role in a 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 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 (Fig. 1).[10–12] SL0101, isolated from the tropical plant *Forsteronia refracta*, 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 non-selectively 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} values.[12] However, none of these RSK2 inhibitors have been used in clinical trials until now. Therefore, there is an urgent need to develop other scaffold RSK2 inhibitors as anticancer drugs.

We recently developed the 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 the SHAFTS program in our previous study.[18–21] Among them, a hit compound (**1**) with an isoindole-1,3-dione scaffold (one drug-like chemical fragment[22,23]) showed some RSK2

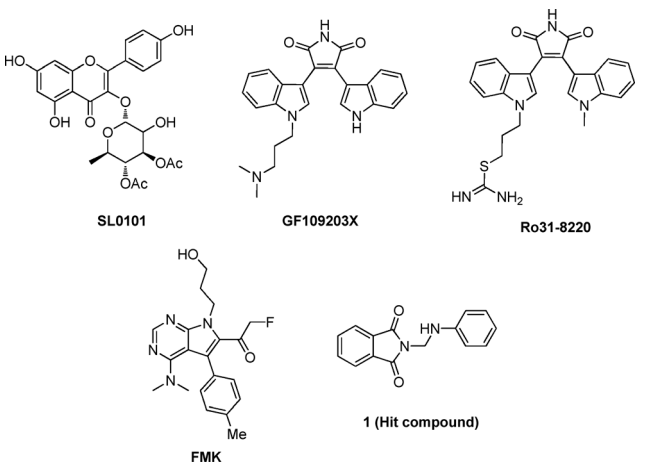

Fig. 1 Examples of reported RSK2 inhibitors and the hit compound **1**.

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affinity with an inhibitory ratio of 44% at 10 μM. In this study, structural optimization and synthesis from the hit compound 1 of 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 the RSK2 activity with an IC_{50} value of up to 0.47 μM (inhibitory ratio of 97% at 10 μM). Structure–activity relationship (SAR) analyses and molecular docking simulations were also performed to search for more potent RSK2 inhibitors.

Results and discussion

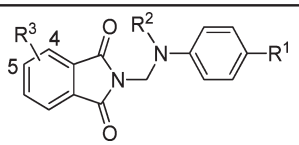
Structural optimization and SAR interpretation

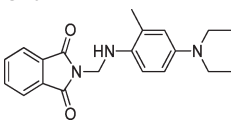
To improve the inhibitory potency of the hit compound and explore the structure–activity relationships, we designed a series of isoindole-1,3-dione derivatives as novel RSK2 inhibitors. The enzyme inhibitory activities toward RSK2 of the designed compounds were evaluated and the results are summarized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

We first investigated the effects of substituent R^1 on the arylamine part. Replacing R^1 in the hit compound 1 with electron-withdrawing halogen atoms (2 and 3) did not induce an 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 when a strong electron-donating methoxy group (7, IC_{50} = 0.47 μM) was introduced to the R^1 position. The same results were obtained for other typical electron-donating groups such as hydroxyl (9), ethoxy (10), diethylamino (11) and morpholinyl (12); they exhibited inhibitory activities against RSK2, with IC_{50} values of 0.52 μM, 0.59 μM, 0.86 μM and 0.79 μM, respectively. This tendency was tested by replacing the methoxy group (7) with an electron-withdrawing 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 activity against RSK2. When R^2 was methyl (13), the inhibitory activity decreased more than 7 times compared with compound 7. When R^2 was an acetyl group (14),

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] The inhibitory rate was measured at a concentration of 10 μM inhibitor. IC_{50} values were measured when the inhibitory rate at 10 μM exceeded 50%. [b] ND, not determined.

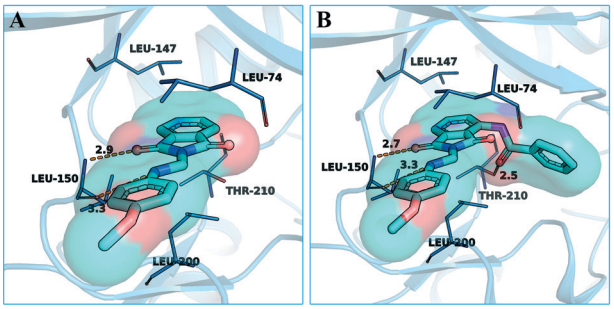
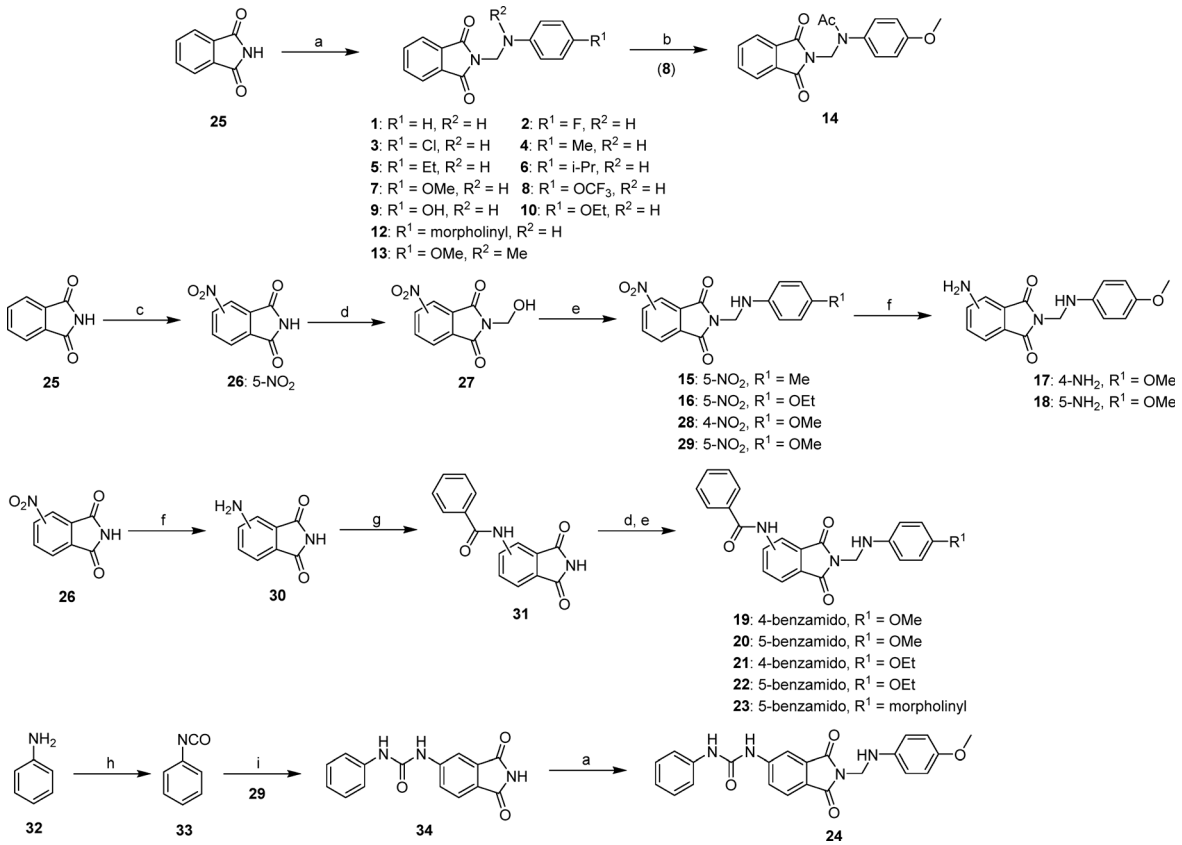


Fig. 2 The proposed binding modes for representative compounds **7** (A) and **19** (B). The X-ray crystal structure of the RSK2 NTKD (PDB ID: 4NW6) is shown as a light blue cartoon, and the docked inhibitors are represented by cyan sticks. Key residues (thin sticks) in the ATP binding site are colored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

the inhibitory potency against RSK2 completely disappeared. These results suggested that the size of substituents at R^2 might affect the spatial molecular structure. More probably, the impact on hydrogen bond formation might be the key factor; thus the hydrogen bonding tendencies were compared N–H > N–CH_3 > N–$COCH_3$. The investigations indicated that R^2 = H was necessary to keep the inhibitory activity against RSK2.

Since compound **7** mainly stretches along the hinge region of the RSK2 NTKD (Fig. 2A) and enough space in the binding pocket remains to be explored, we then focused on the substituents R^3 at the C4 position or the C5 position of the isoindole-1,3-dione scaffold. When an electron-withdrawing nitro group was introduced onto 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 the C5 position of compounds **17** and **18** were better tolerated with IC_{50} values of 3.53 μM and 0.59 μM against RSK2, 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** and just gave similar inhibitory activities. The results suggested that electron-withdrawing groups such as nitro on the C4 position or the 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 big groups such as benzamide were all tolerated, at least, no obvious adverse effects appeared compared with compound **7**. Furthermore, when a longer group such as phenylcarbamido was introduced onto the C5 position (**24**), its inhibitory activity against RSK2 diminished completely, illustrating that the volume



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%.

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

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 Fig. 2A and B with docking energies of −9.36 kcal mol^{-1} and −9.21 kcal mol^{-1}, 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 atoms in isoindole-1,3-dione was capable of hydrogen bonding to the 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 the backbone carbonyl group of Leu150 would be destroyed, leading to a decreased inhibitory activity. In addition, the oxygen atom in the 4-benzamido moiety of **19** acted as a hydrogen bond acceptor to the side chain hydroxyl group of Thr210 with a hydrogen bond distance of 2.5 Å, and the phenyl group in the 4-benzamido moiety was in contact with the residues scattered around through van der Waals interactions. However, compared with **7**, those extra hydrogen bonds and VDW contacts in **19** did not contribute to the binding affinity of the compounds of this series. Nevertheless, electron-donating groups at R^1 play an important role in the improvement of the inhibitory activity of the isoindole-1,3-dione derivatives, which could make VDW contacts with residues Leu74, Phe149 and Gly153 combine with the connected benzene ring. Therefore, compared with compounds **2**, **3** and **8**, compounds **9**, **10**, **11** and **12** all exhibited relatively high activity against the RSK2 enzyme with IC_{50} values around 0.5 μM.

Chemistry

The synthesis of the isoindole-1,3-dione derivatives used in this study is shown in Scheme 1. Several final products were prepared by coupling the starting material phthalimide with a variety of arylamines.[24] Acetylation of compound **7** produced the 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 the hydroxymethyl intermediate **27**, followed by coupling with *p*-anisidine to yield compound **28**. The nitro group of **28** was reduced by Pd/C hydrogenation in methanol to produce compounds **17** and **18** in good yields. The nitro compound **26** was reduced by Pd/C hydrogenation and further acylated by benzoyl chloride to yield **30**. Final compounds **19–23** were prepared from **30** by the method similar to that for compound **28**. For the synthesis of the desired compound **24**, isocyanatobenzene **32** was prepared from phenylamine by using triphosgene and subsequently coupled with the amino compound **29** to produce the phenylurea compound **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**. SAR analyses were conducted and the proposed binding modes were explored to further elucidate the SAR. Electron-donating substituents in R^1 were favorable for inhibitory activity, and un-substituted R^2 was beneficial to increase affinity with RSK2 by forming a H-bond. Compound **7** showed a much improved inhibitory activity compared with the initial hit, with an IC_{50} value of 0.47 μM. It is necessary to further optimize this scaffold to achieve more potent RSK2 inhibitors.

Acknowledgements

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