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CONCISE ARTICLE

Design, synthesis and biological evaluation of thiourea and nicotinamide-containing sorafenib analogs as antitumor agents

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A series of thiourea and nicotinamide-containing sorafenib analogs (**7a-n**) were designed and synthesized and their antiproliferative activities were tested against HCT116, MDA-MB-231, PC-3 and HepG2 cell lines. Most compounds showed potent activities against four cell lines, compound **7h** showed better activities than sorafenib against all four cell lines, and compound **7a**, **7e** showed better activities against HCT116 and MDA-MB-231 cell lines. The anti-angiogenic activities of **7e** and **7h** are also better than that of sorafenib in both *in vitro* HUVEC tuber formation assay and *ex vivo* rat thoracic aorta rings assay.

Introduction

It is well known that inhibition of two or more tumor-related enzymes is effective in the treatment of cancers. More and more attentions are being paid to multi-target drugs, because they can impact multiple targets simultaneously, and are better at controlling complex disease systems and are less prone to drug resistance[1]. One of the most famous multiple-targeted drugs, sorafenib, shows many advantages in the treatment of primary kidney cancer (advanced renal cell carcinoma) [2,3] and advanced primary liver cancer[4,5] because it can inhibit several kinases involved in tumor proliferation and angiogenesis including Raf, VEGFR, PDGFR and KIT[3,6,7]. It has been considered as a lead compound for further optimization in the past several years[8].

Hitherto, many efforts have been undertaken in modification of sorafenib to find better multiple-targeted drugs[9-11]. And most of the reported sorafenib derivatives are based on diaryl urea skeleton[12-13]. Inspired by the classic bioisosteric paradigm of thiopental replacing urea in pentobarbital with thiourea, a series of diaryl thiourea-containing sorafenib derivatives (as shown in **Fig. 1B** and **1C**) were synthesized in our group [14-16], and showed stronger antiproliferative activities against HCT-116 or MDA-MB-231 cells than sorafenib. And some of them also showed inhibitory activities against the phosphorylation of VEGFR and the anti-angiogenic activities to rat aortic ring.

In our previous work, a primary structure and activity relationships (SARs) of diaryl thiourea-containing sorafenib derivatives can be summarized: 1) Substitution of urea with thiourea can enhanced the antiproliferative activities of target compounds. 2) Compounds with a methyl group on the terminal amide have the best antiproliferative activities. 3) Compounds with 1,3-substitution on the B ring showed stronger anti-angiogenic activities than those with 1,4-substitution.

Based the above mentioned structure and activity relationships of diaryl thiourea-containing sorafenib derivatives, in this report, we describe a novel series of thiourea-containing derivatives of sorafenib (as shown in **Fig. 1D**). To enhance their biological activities, the thiourea group and formamide terminal are reserved, and 1,3-substitution mode is chosen on the B ring. Especially the 2,4-substitution mode on C ring is changed with 2,5-substitution mode for the following reasons: a) Modification on this part may change the binding mode and get better antitumor agents. b) 2,5-substitution mode could increase the water solubility of target compounds because there is no intramolecular hydrogen bond between the amide and the nitrogen atoms on the pyridine. c) The 2,5-substitution mode (nicotinamide) can increase the drug-likeness of target compounds because nicotinamide is already used clinically.

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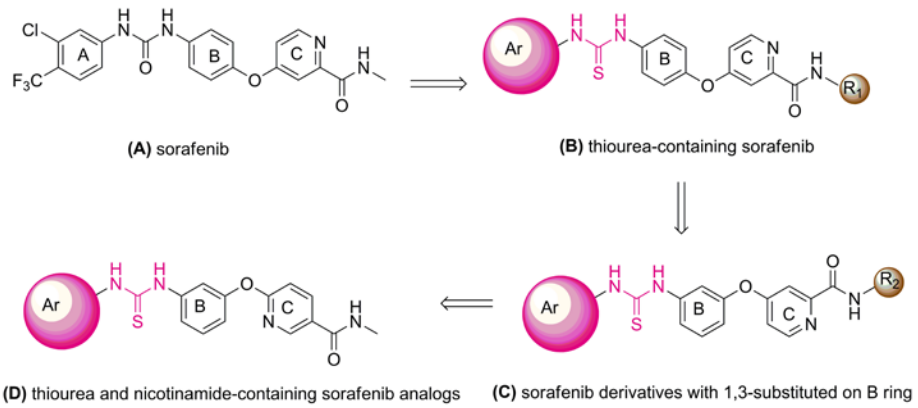
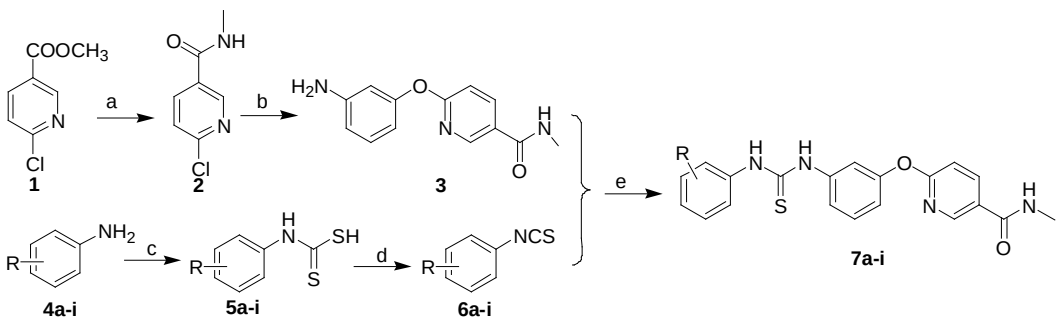


Fig.1 Modifications of sorafenib



Scheme 1 Reagents and conditions: (a) CH_3NH_2 (b) 3-aminophenol, DMF, *t*-BuOK (c) CS_2, toluene (d) BTC, DCM (e) DMF

Results and discussions:

Chemistry:

The synthetic routes to the target compounds were illustrated as outlined in **Scheme 1**.

6-chloronicotinic acid methyl ester (**1**) reacts with methylamine to generate 6-chloronicotinic acid methyl amide (**2**), which was treated with 3-aminophenol to get diaromatic ether (**3**). Furthermore, substituted anilines **4** were treated with CS_2 to generate **5**, and then treated with triphosgene (BTC) to produce various isothiocyanates (**6**). Finally, these isothiocyanates were reacted with **3** in DMF to afford **7** in the total yield of 19–48%. The final products were purified by column chromatography and their structures were characterized by ^{1}H NMR, ^{13}C NMR, MS and elemental analysis.

Biological evaluation

In vitro antiproliferative activity

In vitro cell cytotoxicity of the new nicotinamide-containing derivatives was initially evaluated against HCT116, MDA-MB-231, PC-3 and HepG2 cells lines by MTT assay using sorafenib and BMCL9o[16] as positive controls. As show in table 1, most compounds showed potent activities against all four cell lines. Compound **7a**, **7e**, **7h** showed better antiproliferative activities than sorafenib and compound **7d**, **7i**, **7l**, **7m**, **7n** showed relative weaker activities than others. This suggested that compounds which had two strong electron-withdrawing groups (such as F, Cl, Br, CF_3) on their terminal phenyl ring have better activities than those with only one substitution. Compound **7h** showed better antiproliferative activities than BMCL9o, this indicated that the nicotinamide moiety may enhance the cell cytotoxicity of this series of compounds than pyridine moiety. Compared to the thiourea and

pyridine-2-carboxamide containing compounds in our previous work[15], the inhibitory activities against MDA-MB-231 cells of the target compounds in this paper were much better, which indicated that the different aromatic C ring may change the binding mode of compounds to their specific targets .

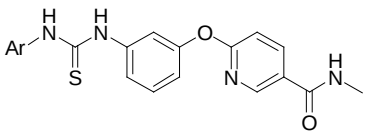
***In vitro* HUVEC tuber formation assay**

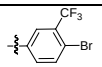
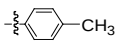
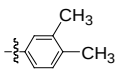
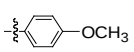
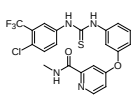
In view of their significant antiproliferative activities to tumor cells, compound **7e** and **7h** were chosen to test their anti-angiogenic activities by *in vitro* HUVEC tuber formation assay using sorafenib as positive controls. As shown in **Fig. 2**, elongated and robust tube-like structures were well established in the negative control (DMSO); The HUVEC tuber formation was partially inhibited by sorafenib at 0.05 μM and completely inhibited at 0.1 μM. Surprisingly, both **7e** and **7h** can inhibit tuber formation completely at 0.05 μM.

***Ex vivo* rat thoracic aorta rings (TARs) assay**

Just as compound **7e** and **7h** can well inhibit the formation of HUVECs tubular structure, *ex vivo* TARs assay was also employed to further study the anti-angiogenic activity of compound **7e** and **7h** because of TARs model involving multiple steps in angiogenesis: sprouting, proliferation, migration and differentiation and being more close to conditions *in vivo*. As shown in **Fig. 3**, Compound **7e** and **7h** have better inhibitory activities than sorafenib at 0.1 μM, and have comparable inhibitory activities at 0.05 μM.

Table 1 Structures and IC_{50} values of target compounds [a]



Compounds NO.	Substituent Ar-	IC_{50} (μM)[a] HCT116	MDA-MB-231	PC-3	HepG2
7a		3.12 ± 0.55	3.02 ± 0.42	2.67 ± 0.02	8.53 ± 0.56
7b		19.01 ± 1.03	13.29 ± 0.09	7.95 ± 0.05	20.20 ± 0.21
7c		25.41 ± 1.19	28.59 ± 1.87	18.35 ± 0.73	> 100
7d		36.65 ± 3.87	38.08 ± 2.21	15.36 ± 2.37	23.32 ± 2.82
7e		2.50 ± 0.35	2.67 ± 0.15	5.86 ± 0.25	4.55 ± 0.34
7f		42.51 ± 0.38	66.48 ± 3.27	28.36 ± 0.46	29.37 ± 1.08
7g		38.19 ± 0.37	69.34 ± 3.22	23.14 ± 0.63	26.72 ± 1.53
7h		2.21 ± 0.09	2.33 ± 0.22	1.98 ± 0.23	5.35 ± 0.21
7i		54.68 ± 0.53	55.95 ± 0.57	> 100	> 100
7j		20.89 ± 0.58	20.67 ± 0.78	19.34 ± 0.96	21.56 ± 1.62
7k		53.72 ± 0.93	29.46± 0.84	> 100	65.38 ±0.64
7l		48.23 ± 0.36	38.56 ± 0.89	67.38 ± 1.32	> 100
7m		39.37 ± 0.45	21.65 ± 0.57	32.67 ± 0.48	42.54 ± 0.69
7n		49.84 ± 0.24	31.54 ± 0.63	> 100	85.34 ± 1.43
BMCL9o		14.58 ± 0.34	42.43 ± 0.68	16.38 ± 0.38	24.89 ± 1.45

sorafenib	___	8.41 ± 0.19	17.84 ± 1.93	10.98 ± 0.67	10.48 ± 1.52

[a] Assays were performed in triplicate (n≥3); values were shown as mean ± SD.

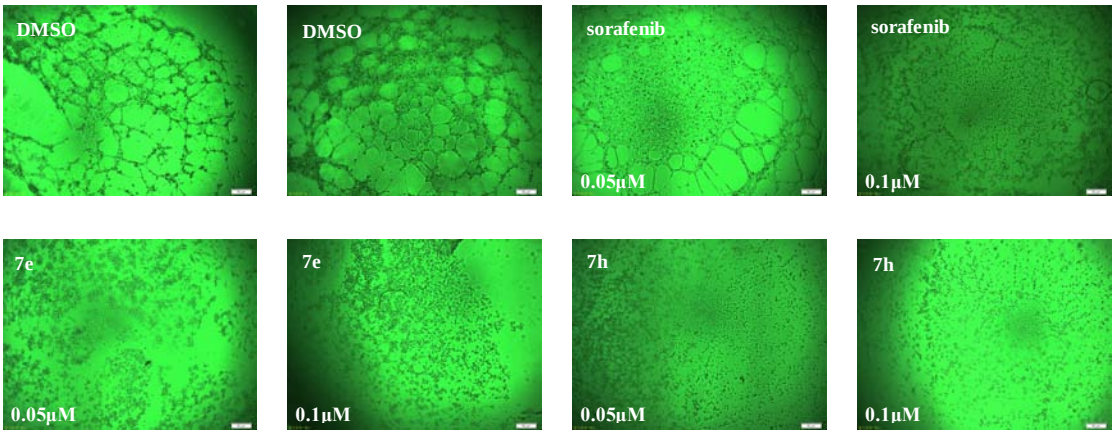


Fig.2 Representative images of tubes formed on Matrigel after seeding with HUVEC and treating with DMSO, sorafenib, **7e** and **7h** at different concentrations.

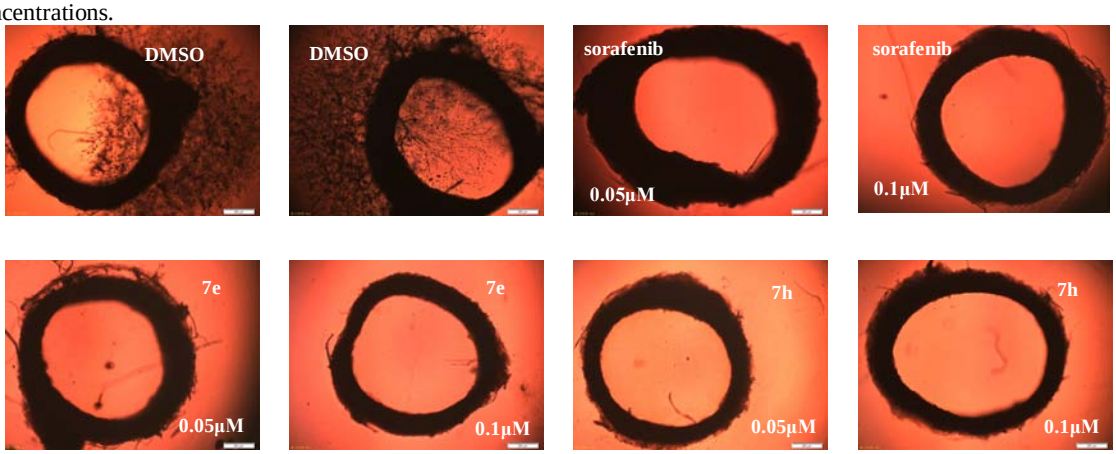


Fig.3 Microvessle growth *ex vivo.* Aortic segments isolated from Sprague-Dawley rats were placed in growth factor-reduced Matrigel-covered wells in the absence (DMSO) or presence of the compounds (sorafenib, **7e**, **7h**) for 6 days at different concentrations.

Conclusion

In summary, a novel series of sorafenib analogs were designed and synthesized as antitumor agents. Compared to sorafenib, most of the compounds showed potent inhibitory activities against four cell lines and compound **7a**, **7e**, **7h** with two strong electron-withdrawing groups on their terminal phenyl ring exhibited better inhibitory activities. Compared to the reported compound BMCL9o, compound **7h** showed better antiproliferative activities against all four cell lines. It indicated that the nicotinamide moiety may enhance the cell cytotoxicity of this series of compounds. It can also be inferred that the nicotinamide-containing derivates may lead a new binding mode to specific target in MDA-MB-231 cells because of the dramatic changes of the antiproliferative activities against MDA-MB-231 cell lines compared to the diaryl thiourea-containing sorafenib derivatives previously reported[16]. Furthermore, As shown *in vitro* HUVEC tuber formation assay and *ex vivo* rat thoracic aorta rings assay, **7e** and **7h** have excellent anti-angiogenic activities, and may be developed as potent anticancer agents in the future. And their mechanisms is now under investigated.

Acknowledgements

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