Chinese Chemical Letters 25 (2014) 702–704

Contents lists available at ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Synthesis and *in vitro* cytotoxic activities of sorafenib derivatives

Ke Wang [b], Yan Li [a], Li-Jing Zhang [b], Xiao-Guang Chen [a], Zhi-Qiang Feng [b,*]

[a] State Key Laboratory of Bioactive Substances and Functions of Natural Medicines, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China
[b] Beijing Key Laboratory of Active Substance Discovery and Druggability Evaluation, Institute of Materia Medica, Chinese Academy of Medical Sciences & Peking Union Medical College, Beijing 100050, China

ARTICLE INFO

Article history:
Received 21 January 2014
Received in revised form 27 February 2014
Accepted 28 February 2014
Available online 15 March 2014

Keywords:
Sorafenib
Antiproliferative activity
A549
ACHN
MDA-MB-231

ABSTRACT

A series of novel sorafenib derivatives have been designed and synthesized. The cytotoxic activities of these compounds were tested in three tumor cell lines. Most of the compounds showed potent antiproliferative activity against the tested cell lines with IC_{50} = 0–20 μmol/L. Some compounds demonstrated competitive antiproliferative activities to sorafenib against all three cancer cell lines. Among them, compound **5g** demonstrated significant inhibitory activity against A549, ACHN and MDA-MB-231 cell lines with IC_{50} values of 1.29, 1.99, 3.11 μmol/L, respectively.

1. Introduction

Cancer is a major public health problem in the world. In 2008, 7.6 million people died of cancer (around 13% of all deaths), and this number is projected to increase with an estimated 13.1 million in 2030 [1]. Among the various types of malignant tumors, lung cancer is the most commonly diagnosed cancer, renal cell cancer is the most lethal of all urological cancers, while breast cancer causes the second leading deaths in women [2–4]. Although chemotherapy is one of the most frequently used forms of cancer therapy, the use of available chemotherapeutics is often limited mainly due to the adverse side effects and resistance of available anticancer agents. Therefore, searching for new anticancer agents with better activity remains important [5].

Diarylurea derivatives are of great interest due to their broad spectrum of biochemical effects and pharmaceutical functions. In particular, there have been intense research efforts in recent years in the design and development of diarylurea derivatives as a class of antitumor agents [6–8]. There are several diarylurea compounds that have been in clinical trials or clinical use, such as sorafenib, ABT-869, KRN-951 [9–11].

Sorafenib (Nexavar, Fig. 1), a diarylurea derivative, targets the RAS-RAF-MEK-ERK signaling pathway in numerous cancer cell lines *in vitro*. Sorafenib was approved by the U.S. Food and Drug Administration (FDA) for treatment of advanced renal cell carcinoma in 2005 and unresectable hepatocellular carcinoma in 2007 [12,13]. Currently, sorafenib is under investigation in several human malignancies, including lung and breast cancer [14,15]. However, sorafenib still has poor physicochemical properties and poor therapeutic activity in the treatment of malignant melanoma [10,11]. Then there are a considerable number and variety sorafenib derivatives identified as multi-targeted tyrosine kinase inhibitors [16–18]. In the present paper, a new series of sorafenib derivatives with 2-picolinylhydrazide moiety were designed and synthesized. The antiproliferative activities of 18 compounds against non-small cell lung carcinoma (A549), human kidney adenocarcinoma cell line (ACHN) and human breast cancer cell line (MDA-MB-231) were evaluated.

2. Experimental

Melting points were measured on a Yanaco micro melting point apparatus and uncorrected. ^{1}H NMR spectra were recorded at 400 MHz or 300 MHz spectrometer at 24 °C in the indicated solvent and are reported in ppm relative to tetramethylsilane and referenced internally to the residually protonated solvent. HRMS were carried out by Agilent LC/MSD TOF.

The target compounds were prepared as outlined in Scheme 1. Compound **2** was synthesized according to the protocol reported by Prof. Bankston *et al.* [19]. A solution of 4-aminophenol (4.80 g,

* Corresponding author.
E-mail address: fengzhq@imm.ac.cn (Z.-Q. Feng).

1001-8417/$ – see front matter © 2014 Zhi-Qiang Feng. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.
http://dx.doi.org/10.1016/j.cclet.2014.03.020

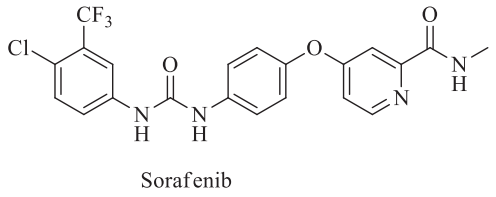


Fig. 1. The structure of sorafenib.

44.00 mmol) in dry *N*,*N*-dimethylformamide (DMF) (75 mL) was treated with potassium *tert*-butoxide (5.15 g, 45.85 mmol) and the reddish-brown mixture was stirred at room temperature for 2 h. The contents were treated with methyl 4-chloropicolinate (**1**, 7.50 g, 44.00 mmol) and potassium carbonate (3.25 g, 23.52 mmol) and then heated to 80 °C under argon. After 8 h, the mixture was cooled to room temperature and poured into ethyl acetate (250 mL) and brine (250 mL). The layers were separated, and the aqueous phase was back-extracted with ethyl acetate (150 mL). The combined organic phase were washed with brine (3× 500 mL), dried over sodium sulfate, filtered and concentrated to afford **2** (9.52 g, 89%) as a brown solid after vacuum-drying at 40 °C for 5 h.

The suspension of **2** (3.35 g, 13.77 mmol) in CH_2Cl_2 (10 mL) was treated with dropwise addition of 4-chloro-3-(trifluoromethyl)-phenyl isothiocyanate (3.10 g, 14.01 mmol) in CH_2Cl_2 (15 mL) 0 °C under argon. The mixture was stirred at room temperature, and a yellow solid precipitated after 15 min. The mixture was stirred for 24 h and then filtered. The solid was washed with CH_2Cl_2 and dried under vacuum for 12 h at 40 °C to afford **3** (6.07 g, 95%) as white solid [19].

The suspension of **3** (4.37 g, 9.40 mmol) and hydrazine monohydrate (5.00 g) in methanol (50 mL) was heated to reflux for 10 h. After cooling at room temperature, the precipitate formed was filtered and washed with water, to afford **4** (4.0 g, 91%) as white solid.

Added compound **4** (1 equiv.), appropriate acids (1.2 equiv.), 2-(7-aza-1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU) (1.2 equiv.), Et_3N (1.5 equiv.) to anhydrous DMF (5 mL) and stirred the solution at room temperature for 12 h. The reaction mixture was poured into H_2O (100 mL). The precipitates were collected by filtration and washed with water to give the target compound **5a–r** in a reasonable yield. Selected spectral data are listed below and the others were given in Supporting information.

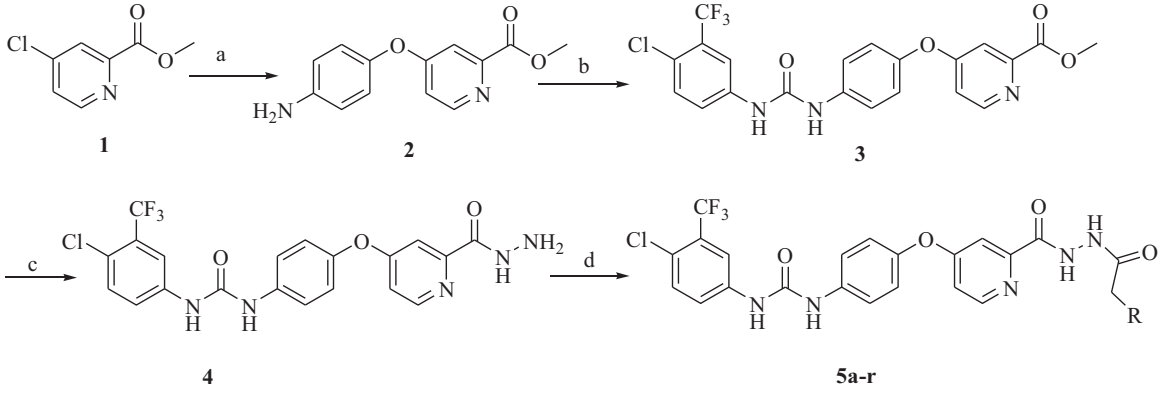
Compound **5a**: Yield: 93%; white solid. Mp 203–205 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 10.50 (s, 1H, –CONH–), 10.29 (s, 1H, –CONH–), 9.21 (s, 1H, –CONH–), 9.00 (s, 1H, –CONH–), 8.54 (d, 1H, J = 5.6 Hz, Ar-H), 8.12 (s, 1H, Ar-H), 7.61 (m, 4H, Ar-H), 7.32 (m, 5H, Ar-H), 7.20 (m, 4H, Ar-H), 3.52 (s, 2H, –CH_2–). HRMS (ESI) (*m/z*): Calcd. for $C_{28}H_{22}O_4N_5ClF_3$ 584.1307 $[M+H]^+$, found 584.1305.

Compound **5b**: Yield: 82%; white solid. Mp 182–184 °C ^{1}H NMR (300 MHz, DMSO-d_6): δ 10.52 (s, 1H, –CO–NH–), 10.29 (s, 1H, –CO–NH–), 9.25 (s, 1H, –CONH–), 9.03 (s, 1H, –CONH–), 8.54 (d, 1H, J = 5.7 Hz, Ar-H), 8.12 (d, 1H, J = 8.7 Hz, Ar-H), 7.64 (m, 4H, Ar-H), 7.35 (m, 4H, Ar-H), 7.17 (m, 3H, Ar-H), 3.52 (s, 2H, –CO–CH_2–). HRMS (ESI) (*m/z*): Calcd. for $C_{28}H_{21}O_4N_5Cl_2F_3$ 618.0917 $[M+H]^+$, found 618.0907.

3. Results and discussion

Cytotoxicity of these derivatives was evaluated against three human cancer cell lines by the MTT assay. These three cell lines are: non-small cell lung carcinoma (A549), human kidney adenocarcinoma cell line (ACHN) and human breast cancer cell line (MDA-MB-231). The results of cytotoxicity studies were summarized in Table 1.

As shown in Table 1, most of compounds exhibited potent antiproliferative activity against all three cell lines with IC_{50} = 1–20 μmol/L; 12 compounds demonstrated significant inhibitory activities against all three cell lines. Compound **5a** with unsubstituted aryl ring exhibited no cytotoxicity against all the tumor cell lines tested (IC_{50} > 20 μmol/L). Compound **5c** with 3-chlorophenyl moiety was more potent than **5b** and **5d** with 2-chlorophenyl and 4-chlorophenyl moiety. Compound **5g** and **5h** with 3-fluorophenyl moiety and 4-fluorophenyl moiety showed potent inhibitory activity against all three cell lines, with IC_{50} values of 1.29, 1.99, 3.11 μmol/L and 7.14, 4.47, 3.04 μmol/L, respectively. These results indicated that halogen substituent at the 3-position of phenyl ring is favorable for activity. Compound **5e** and **5f** with 3-trifluoromethylphenyl moiety and 4-trifluoromethylphenyl moiety showed the most potent inhibitory activity against MDA-MB-231 cell line with the inhibitory concentration (IC_{50}) values of 2.39 and 2.61 μmol/L, respectively. Compounds **5i**, **5j** and **5k**, with 3,5-dichlorophenyl moiety, 3-fluoro-4-bromophenyl moiety and 2,3-difluorophenyl moiety showed similar activity against tested all three cell lines to **5c**. Compound **5j** exhibited the most potent inhibitory activity against ACHN cell line with IC_{50} = 0.89 μmol/L. Compound **5l** with 3-chloro-4-hydroxylphenyl moiety was inactive to all tested cell lines (IC_{50} > 20 μmol/L). Bearing one or two methoxyl group on the aromatic derivatives, Compounds **5m** and **5n** with 2-methoxylphenyl moiety and 3,4-dimethoxylphenyl moiety only exhibited cytotoxicity against ACHN, with IC_{50} values of 9.88 and 10.48 μmol/L. Compound **5o** with 4-ethoxylphenyl moiety only exhibited cytotoxicity against



Scheme 1. Synthetic route for the preparation of title compounds **5a**–**5r**. Reagents and conditions: (a) DMF, *t*-BuOK, 4-aminophenol, K_2CO_3, 89%, (b) 4-chloro-3-(trifluoromethyl)phenyl isothiocyanate, DCM, 0 °C to r.t., 95%, (c) NH_2NH_2, MeOH, reflux, 12 h, 91%, (d) HATU, Et_3N, DMF, 73%-93%.

Table 1
The structures and IC_{50} values of the target compounds.

Compd.	Substituent (R)	IC_{50} (μmol/L)[a]		
		A549	ACHN	MDA-MB-231
5a	C_6H_5	>20.0	>20.0	>20.0
5b	4-ClC_6H_4	8.64	17.18	18.88
5c	3-ClC_6H_4	3.73	1.46	6.96
5d	2-ClC_6H_4	8.77	>20.0	7.46
5e	3-$CF_3C_6H_4$	8.18	2.58	2.39
5f	4-$CF_3C_6H_4$	7.09	2.02	2.61
5g	3-FC_6H_4	1.29	1.99	3.11
5h	4-FC_6H_4	7.14	4.47	3.04
5i	3-F-5-FC_6H_3	2.12	1.54	4.37
5j	3-F-4-BrC_6H_3	3.06	0.89	8.08
5k	2-F-3-FC_6H_3	3.46	3.48	8.17
5l	3-Cl-4-OHC_6H_3	>20.0	>20.0	>20.0
5m	2-$OMeC_6H_4$	>20.0	9.88	>20.0
5n	3-OMe-4-$OMeC_6H_3$	>20.0	10.48	>20.0
5o	4-$OEtC_6H_4$	7.39	>20.0	>20.0
5p	2-Naphthyl	4.97	5.14	7.89
5q	4-C_6H_5-C_6H_4	4.72	8.48	6.62
5r	4-MeC_6H_4	4.76	10.63	4.66
Sorafenib		7.22	12.80	9.24

[a] The IC_{50} values represent the compound concentration required to inhibit tumor cell proliferation by 50%.

A549, with IC_{50} value of 7.39 μmol/L. These results indicated that hydroxyl and methoxyl at the phenyl ring are unfavorable. Compounds **5p**, **5q** and **5r**, with naphthyl moiety, biphenyl moiety and 4-methylphenyl moiety showed similar activity against tested all three cell lines with IC_{50} = 4–11 μmol/L.

4. Conclusion

In summary, a new series of sorafenib derivatives were prepared. Most of compounds showed potent antiproliferative activity against all three cancer cell lines. Compounds **5c**, **5e**, **5f**, **5g**, **5i**, **5j**, **5k**, **5p**, **5q** and **5r** exhibited more potent activity against the tested three cancer lines as compared with sorafenib. Among them, compound **5g**, which was the most promising compound against the tested cell lines. The initial SARs showed that variations in substitutions of the phenyl ring had a significant impact on the cytotoxicity. Substitution of the phenyl ring at the 3-position was well tolerated and 3-Cl substitution produced the best potency. Moreover, the introduction of hydroxyl and methoxyl at the phenyl ring are unfavorable. All the above results demonstrated that these compounds could be promising lead compounds of novel antitumor drugs. Further studies about the cellular mechanism of the potent compounds are in progress.

Acknowledgment

We gratefully acknowledge the generous support provided by the National S&T Major Special Project on Major New Drug Innovation (No. 2012ZX09103-101-019).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.cclet.2014.03.020.

References

[1] Cancer – Fact sheet N°297, reviewed January 2013. WHO website: http://www.who.int/mediacentre/factsheets/fs297/en/.
[2] R.A. Smith, V. Cokkinides, O.W. Brawley, Cancer screening in the United States 2009: a review of current American Cancer Society guidelines and issues in cancer screening, CA Cancer J. Clin. 59 (2009) 27–41.
[3] D.M. Parkin, L.M. Fernandez, Use of statistics to assess the global burden of breast cancer, Breast J. 12 (2006) 570–580.
[4] R.C. Flanigan, A.J. Polcari, C.M. Hugen, Prognostic variables and nomograms for renal cell carcinoma, J. Urol. 18 (2011) 20–31.
[5] M.N. Noolvi, H.M. Patel, V. Bhardwaj, Synthesis and in vitro antitumor activity of substituted quinazoline and quinoxaline derivatives: searching for anticancer agent, Eur. J. Med. Chem. 45 (2010) 4188–4198.
[6] H.J. Kim, H.J. Cho, H. Kim, et al., New diarylureas and diarylamides possessing acet(benz)amidophenyl scaffold: design, synthesis, and antiproliferative activity against melanoma cell line, Bioorg. Med. Chem. Lett. 22 (2012) 3269–3273.
[7] M.X.Q. Sun, J.Q. Wu, et al., Design, synthesis, and in vitro antitumor evaluation of novel diaryl ureas derivatives, Eur. J. Med. Chem. 45 (2010) 2299–2306.
[8] C.R. Zhao, R.Q. Wang, G. Li, et al., Synthesis of indazole based diarylurea derivatives and their antiproliferative activity against tumor cell lines, Bioorg. Med. Chem. Lett. 23 (2013) 1989–1992.
[9] S. Wilhelm, C. Carter, M. Lynch, et al., Discovery and development of sorafenib: a multikinase inhibitor for treating cancer, Nat. Rev. Drug Discov. 5 (2006) 835–844.
[10] S. Ramurthy, S. Subramanian, M. Aikawa, et al., Design and synthesis of orally bioavailable benzimidazoles as Raf kinase inhibitors, J. Med. Chem. 51 (2008) 7049–7052.
[11] T. Eisen, T. Ahmad, K.T. Flaherty, et al., Sorafenib in advanced melanoma: a Phase II randomized discontinuation trial analysis, Br. J. Cancer 95 (2006) 581–586.
[12] R.C. Kan, A.T. Farrell, H. Saber, et al., Sorafenib for the treatment of advanced renal cell carcinoma, Clin. Cancer Res. 12 (2006) 7271–7278.
[13] G.M. Keating, A. Santoro, Sorafenib: a review of its use in advanced hepatocellular carcinoma, Drugs 69 (2009) 223–240.
[14] A. Rossi, P. Maione, M.L. Ferrara, et al., Angiogenesis inhibitors and vascular disrupting agents in non-small cell lung cancer, Curr. Med. Chem. 16 (2009) 3919–3930.
[15] V. Roy, E.A. Perez, Biologic therapy of breast cancer: focus on co-inhibition of endocrine and angiogenesis pathways, Breast Cancer Res. Treat. 116 (2009) 31–38.
[16] J.W. Yao, Z.P. He, J. Chen, et al., Design, synthesis and biological activities of sorafenib derivatives as antitumor agents, Bioorg. Med. Chem. Lett. 22 (2012) 6549–6553.
[17] Y.F. Zhao, Z.J. Liu, X. Zhai, et al., Synthesis and in vitro antitumor activity of novel diaryl urea derivatives, Chin. Chem. Lett. 24 (2013) 386–388.
[18] K.F. Chen, W.T. Tai, C.Y. Hsu, et al., Blockade of STAT3 activation by sorafenib derivatives through enhancing SHP-1 phosphatase activity, Eur. J. Med. Chem. 55 (2012) 220–227.
[19] D. Bankston, J. Dumas, R. Natero, et al., A Scaleable synthesis of BAY 43-9006: a potent raf kinase inhibitor for the treatment of cancer, Org. Process Res. Dev. 6 (2002) 777–781.