Bioorganic & Medicinal Chemistry Letters 21 (2011) 5975–5977

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

Synthesis and cytotoxicity of 8-cyano-3-substitutedalkyl-5-methyl-4-methylene-7-methoxy-3,4-dihydropyrido[4,3-*d*]pyrimidines

Wen-Yan Mo [a], Yong-Ju Liang [b], Yu-Cheng Gu [a,c], Li-Wu Fu [b,*], Hong-Wu He [a,*]

[a] Key Lab of Pesticide and Chemical Biology, Ministry of Education, School of Chemistry, Central China Normal University, Wuhan 430079, PR China
[b] State Key Laboratory of Oncology in South China, Cancer Center, Sun Yat-sen University, Guangzhou 510060, PR China
[c] Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire RG42 6EY, United Kingdom

ARTICLE INFO

Article history:
Received 1 June 2011
Revised 2 July 2011
Accepted 14 July 2011
Available online 28 July 2011

Keywords:
Pyrido[4,3-*d*]pyrimidine
Synthesis
Antitumor activity
MTT

ABSTRACT

Synthesis and cytotoxicity of 11 4-methylene pyrido[4,3-*d*]pyrimidines **5a–k** were described. Cytotoxicity assay results showed that some compounds had much stronger antitumor activity than Fluorouracil against KB cell lines. The most active compound **5i** exhibited high potency against KB, CNE2, MGC-803 cell lines with IC_{50} values of 0.48, 0.15, 0.59 μM, respectively. The preliminary structure–activity relationships indicated that the introduction of benzyl groups bearing electron-donating function groups is favorable for the activity.

Pyrido[4,3-*d*]pyrimidines display various remarkable biological activities, such as fungicidal, antiviral, antiinflammatory, and antimicrobial properties.[1–6] And some compounds have also been discovered as EGFr-TK inhibitors[7–9] or DHFR inhibitors,[10] which stimulated our interest intensely.

As known so far, the reported synthesis of pyrido[4,3-*d*]pyrimidine derivatives usually involved in the formation of a pyrimidine ring from a suitable substituted pyridine ring.[11–13] However, the preparation of the substituted pyridine or the formation of the pyrimidine ring required relatively strict reaction condition and long reaction time.

In our previous work, a protocol via the aza-Wittig reaction have been developed for the construction of pyrido[4,3-*d*]pyrimidin-4-ones in order to find compounds with some particular biological functions.[14] And recently, a highly efficient regioselective intramolecular ring closure reaction was also developed and utilized successfully for the synthesis of bioactive 4-methylene pyrido[4,3-*d*]pyrimidines.[15–17]

Based on our previous research work, pyrido[4,3-*d*]pyrimidine derivatives exhibited some interesting antitumor activity,[18] which attracted our attention. As literatures described,[9,11] this class of pyrido[4,3-*d*]pyrimidines are most likely to be the EGFr inhibitors. And reports ever pointed out that electron-donating groups in *ortho*-position of pyridine ring would contribute to the potency.[8] So here we designed and synthesized a series of 4-methylene pyrido[4,3-*d*]pyrimidines, in which the electron-donating methoxy group was introduced to the *ortho*-position of pyridine and a variety of the substituents were introduced to the pyrimidine ring.

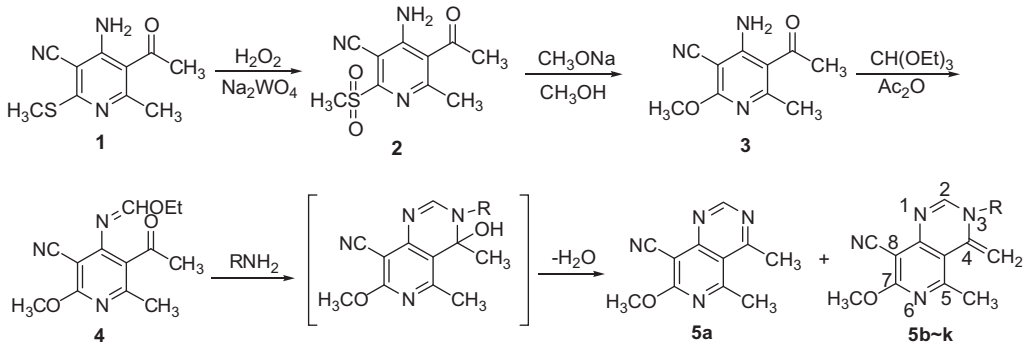
As shown in the Scheme 1, the intermediate **1** was prepared according to the literature.[16] The intermediate **1** was readily oxidated by hydrogen peroxide to give intermediate **2,** and then converted into 7-methoxy-4-amino-pyridine **3** by nucleophilic substitution reaction. Followed by the reaction of compound **3** with triethyl orthoformate, formamidate **4** was obtained in good yield. Then the formamidate **4** was cyclized smoothly with alkyl amines or ammonia at mild condition to afford title compounds **5** in moderate to good yields (Table 1).

All the structures of title compounds **5a–k** were elucidated by comprehensive ^{1}H NMR, IR and mass spectra, together with elemental analysis. In the ^{1}H NMR spectra, two protons of 4-methylene group in pyrimidine are nuclear isoequivalent, appear at δ 4.50–4.70 as two doublets. 2-H appears at δ 7.50 as singlet, 7-OCH_3 group at δ 4.03 and 5-CH_3 group at δ 2.60. For the ^{13}C NMR spectra, the carbon atoms in the pyridine and pyrimidine ring show representative bands. For example, C(5) appears at δ 30 ppm, C(7) at δ 55 ppm and C(4) at δ 82 ppm, and the carbon atom in cyano group appears at δ 85 ppm. The IR spectra shows typical absorption bands at 2220 (C≡N) and 1600, 1550, 1520 cm^{-1} (C=N, C=C).

The cytotoxicity of all the title compounds were evaluated against KB cells by MTT assay,[19] and two representative compounds **5i** and **5j** were further screened against CNE2, MGC-803 cell lines. All the IC_{50} values derived from the experimental data are summarized in Tables 1 and 2. As the results listed, some

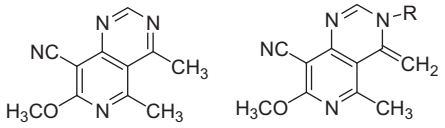
* Corresponding authors. Tel.: +86 20 87343163; fax: +86 20 87343170 (L.F.); tel./fax: +86 27 67867960 (H.H.).
E-mail address: he1208@mail.ccnu.edu.cn (H.-W. He).

0960-894X/$ - see front matter © 2011 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2011.07.067



Scheme 1. Synthesis of pyrido[4,3-*d*]pyrimidines.

Table 1
Yields and in vitro cytotoxicity of pyrido[4,3-*d*]pyrimidines **5**



5a **5b~k**

Compd	R	Yield[a] (%)	IC_{50} against KB[b,c] (μM)
5a	—	76	>50
5b	CH_3CH_2–	67	>50
5c	$CH_3CH_2CH_2$–	69	>50
5d	$CH_3(CH_2)_4CH_2$–	62	>50
5e	2-Furfuryl-CH_2–	85	>50
5f	$PhCH_2$–	83	>50
5g	4-$ClPhCH_2$–	71	20
5h	3-CH_3PhCH_2–	54	37
5i	4-CH_3PhCH_2–	49	0.48
5j	4-OCH_3PhCH_2–	65	0.67
5k	4-$ClPhCH_2CH_2$–	73	>50
Fluorouracil	—	—	12.5

[a] Isolated yields based on formamidate **3**.
[b] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.
[c] KB cells represent the drug sensitive human oral carcinoma cells.

Table 2
Antitumor activity of representative compounds **5i–j** in vitro

Compd	R	IC_{50} [a] (μM)	
		CNE2[b]	MGC-803[c]
5i	4-CH_3PhCH_2-	0.15	0.59
5j	4-OCH_3PhCH_2-	0.18	0.67

[a] IC_{50} values are presented as mean values of three independent experiments done in quadruplicates. Coefficients of variation were <10%.
[b] CNE2 cells represent the human nasopharyngeal carcinoma cells.
[c] MGC803 cells represent the human gastric carcinoma cells.

compounds showed excellent antiproliferative activity against human tumor cell lines. Among them, two representative compounds **5i** and **5j** displayed much higher antitumor activity against KB cell lines than positive control Fluorouracil. For example, compound **5i** showed pretty high potency against KB, CNE2 and MGC-803 cells with IC_{50} values of 0.48, 0.15, 0.59 μM, respectively. And compound **5j** also showed IC_{50} values against KB, CNE2 and MGC-803 cells low down as 0.67, 0.18 and 0.67 μM, respectively.

As indicated in Table 1, linear alkyl groups on the 3-position of pyrimidine ring have no good to the cytotoxicity. However, the introduction of aromatic alkyl groups, especially 4-substituted benzyl bearing electron-donating groups, contributes to the antitumor activity significantly. For example, the most active compounds **5i** and **5j** have 4-methyl or 4-methoxy benzyl groups on 3-position of pyrimidine ring, respectively. Moreover, for the aromatic alkyl groups, lengthening the methylene chain will decrease the activity. For instance, compound **5k** (4-$ClPhCH_2CH_2$–) shows much lower activity than compound **5g** (4-$ClPhCH_2$–).

In conclusion, we synthesized a series of 4-methylene pyrido[4,3-*d*]pyrimidines **5** and discovered two compounds **5i** and **5j** with promising high potency against KB, CNE2, MGC-803 cells. And cytotoxicity could be further improved by incorporating appropriate functional groups.

Acknowledgments

This work was financially supported by the National Basic Research Program of China (No. 2010CB126100), National Natural Science Foundation of China (No. 20772042, 21002037), the 863 Project (No. 2006AA09Z419) and a fellowship awarded to W.-Y. Mo by Syngenta Ltd, and the research was supported partly by the PCSIRT (No. IRT0953).

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.07.067.

References and notes

1. Mo, W. Y.; Yao, Y. Y.; Shen, Y. L.; He, H. W.; Gu, Y. C. *J. Heterocycl. Chem.* **2009**, *46*, 579.
2. Hacker, R. E.; Jourdan, G. P. EP 0414386, **1991**; Chem. Abstr. 1991, 115, 71630j.
3. Yamada, H. EP 0665224, **1994**; Chem. Abstr. 1994, 121, 230784e.
4. Elselarge, E. F.; Clarke, J.; Jacob, P. *J. Heterocycl. Chem.* **1972**, *9*, 1113.
5. Rosowsky, A.; Mota, C. E.; Queener, S. F. *J. Heterocycl. Chem.* **1995**, *32*, 335.
6. Hussein, E. S.; Suhair, M. A. Z.; Mona, A. M. *J. Med. Chem.* **2000**, *43*, 2915.
7. Thompson, A. M.; Murray, D. K.; Elliott, W. L.; Fry, D. W.; Nelson, J. A.; Showalter, H. D. H.; Roberts, B. J.; Vincent, P. W.; Denny, W. A. *J. Med. Chem.* **1997**, *40*, 3915.
8. Fry, D. W.; Nelson, J. A.; Slintak, V.; Keller, P. R.; Rewcastle, G. W.; Denny, W. A.; Zhou, H. R.; Bridges, A. *J. Biochem. Pharmacol.* **1997**, *54*, 877.
9. Smaill, J. B.; Palmer, D. B.; Rewcastle, G. W.; Denny, W. A.; McNamara, D. J.; Dobrusin, E. M.; Brodges, A. J.; Zhou, H. R.; Showalter, H. D. H.; Winters, R. T.; Fry, D. W.; Nelson, J. M.; Slintak, V.; Elliot, W. L.; Roberts, B. J.; Vincent, P. W.; Patmore, S. J. *J. Med. Chem.* **1999**, *42*, 1803.
10. Rosowsky, A.; Bader, H.; Moran, R. G. *J. Heterocycl. Chem.* **1989**, *26*, 509.
11. Rewcastle, W. G.; Palmer, D. B.; Thompson, A. M.; Bridges, A. J.; Cody, D. R.; Zhou, H. R.; Fry, D. W.; McMichael, A.; Denny, W. A. *J. Med. Chem.* **1996**, *39*, 1823.
12. Thompson, A. M.; Bridges, A. J.; Fry, D. W.; Kraker, A. J.; Denny, W. A. *J. Heterocycl. Chem.* **1995**, *38*, 3780.
13. Lanier, M. C.; Feher, M. N.; Ashweek, J.; Loweth, C. J.; Rueter, J. K.; Slee, D. H.; Williams, J. P.; Zhu, Y. F.; Sullivan, S. K.; Brown, M. S. *Bioorg. Med. Chem.* **2007**, *15*, 5590.
14. Ren, Q. Y.; Cui, Z. P.; He, H. W.; Gu, Y. C. *J. Fluorine Chem.* **2007**, *128*, 1369.

15. Mo, W. Y.; Liao, G. H.; Wang, T.; He, H. W. *J. Fluorine Chem.* **2008**, *129*, 519.
16. He, H. W.; Wang, T.; Liao, G. H.; Mo, W. Y.; Ren, Q. Y. CN 1746170, 2006; *Chem. Abstr.* **2006**, *145*, 124583.
17. He, H. W.; Mo, W. Y.; Ren, Q. Y. CN 101323617, 2008; *Chem. Abstr.* **2008**, *149*, 402379.
18. Ren, Q. Y.; Liang, Y. J.; He, H. W.; Fu, L. W.; Gu, Y. C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6713.
19. *Evaluation of cytotoxicity (MTT)*: The cancer cells harvested during the exponential growth phase were seeded into 96-well microtitre plates at 5×10^4–1×10^5 cells/mL in fresh medium. After overnight growth, the cells were treated with the compounds (dissolved in DMSO) at selected concentrations for a period of 3 days. At the end of the treatment period, MTT solution (10 μL, 10 mg/mL) in PBS (PBS without MTT as the blank) was fed to each well of the culture plate (containing 200 μL medium). After 3 h incubation, the formazan crystal formed in the well was solubilized with 100 μL DMSO for optical density reading at 540 nm. The inhibition rate was calculated according to Eq. 1. The assessments of cytotoxic activity were expressed as the concentration inhibiting 50% of cancer cell growth (IC_{50}).

$$IR\% = (1 - OD_{average,\ sample})/OD_{average,\ blank} \times 100\% \quad (1)$$