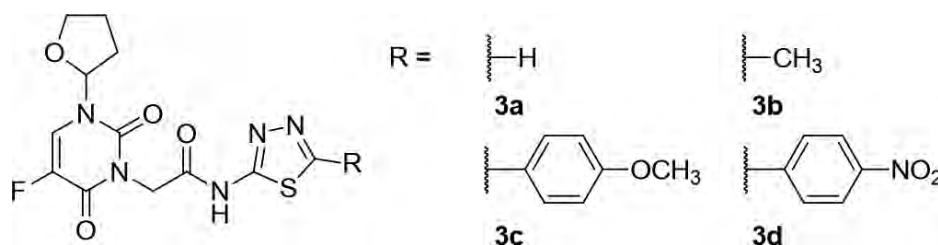


Synthesis and Evaluation of New Tegafur Derivatives Containing 1,3,4-Thiadiazole Moiety

Bo Liu, Sanhai Qin, Shenghai Yu, Zhongyu Wu, Aiqin Liu*

Institute of Materia Medica, Shangdong Academy of Medical Sciences, Jinan 250062, Shandong Province, P. R. China

ABSTRACT Tegafur is an oral chemotherapy prodrug converted to 5-fluorouracil which is activated and kills tumor cells mainly through inhibition of thymidylate synthase. In the present study, four new tegafur derivatives bearing 1,3,4-thiadiazole heterocycles were synthesized and investigated for their growth inhibitory effects on tumor cell lines and therapeutically effects on S180 xenografts.



KEYWORDS Antitumor activity, Synthesis, Tegafur, 1,3,4-Thiadiazole, 5-Fluorouracil

INTRODUCTION

5-Fluorouracil (5-FU) is a member of the fluoropyrimidine class of antineoplastic agents. Although it is a core anticancer agent for the past five decades in the treatment of solid tumors, it is difficult to sustain the effective plasma concentration of 5-FU with orally administered 5-FU itself. To enhance the efficacy, tumor selectivity and oral bioavailability of 5-FU, some new orally administrable 5-FU prodrugs and drug combinations have been developed and in clinical application. Among these drugs, tegafur is an oral chemotherapy prodrug converted to 5-FU *in vivo* that has been used for cancer chemotherapy, and it is also an important component of adjuvant therapies (S-1) for the treatment of various cancers such as colorectal cancer, stomach, colon, breast, lung, and bladder.^[1] S-1 has been used limitedly only in Japan and some other Asian countries so far,

and growing evidence has been reported recently on the disadvantage of 5-FU in neurotoxic effects, such as cerebellar syndrome and encephalopathy.^[2] Currently, a large number of fluoropyrimidine prodrugs have been developed to enhance the anticancer efficacy of 5-FU and to reduce its adverse reactions.^[3]

1,3,4-Thiadiazoles are a class of heterocycles which exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory, antiviral, and anti-HIV. Thus, the 1,3,4-thiadiazoles scaffold has attracted significant interest in medicinal and bioorganic chemistry.^[4,5] Considering the fact that tegafur has anticancer activities, and 1,3,4-thiadiazoles can introduce conformational constraints to tegafur that enhance the activity and alter the structure-activity relationships, a new series of tegafur derivatives bearing 1,3,4-thiadiazole heterocycles was designed and synthesized as potential anticancer agents.

*Corresponding author: Email: liuaiqin6@126.com

RESULTS AND DISCUSSION

Chemistry

The target compounds **3a-3d** were synthesized according to the synthetic route outlined in **Scheme 1**. The tegafur (**1**) was reacted with 1.0 equivalent of methyl chloroacetate in the presence of methanolic NaOH at 70°C to afford corresponding tegafur ester. Next, hydrolysis was accomplished by reacting tegafur ester first with NaOH solution and then acidified with 10% HCl to produce the key intermediate tegafur carboxylic acid (**I**) in 81% yield. On the other hand, treatment of carboxylic acid (**2a-2d**) with thiosemicarbazide and phosphorus oxychloride resulted in the formation of the corresponding intermediate 1,3,4-thiadiazoles (**IIa-IId**).^[6,7] Then, the condensation between intermediates **I** and **IIa-IId** resulted in the formation of the target tegafur derivatives (**3a-3d**).

Antitumor activity

In vitro antiproliferative activity assay on tumor cell lines

The antiproliferative activities of all synthesized tegafur derivatives (**3a-3d**) were evaluated against human gastric cancer cell line (HGC27), human liver cancer cell line (SMMC7721), and human colon cancer cell line (HCT15) using an MTT assay with cisplatin as reference. The obtained IC₅₀ values are shown in **Table 1**. Although these four tegafur derivatives showed dose-dependent (0.16~100 µg/mL) anticancer activities against the three cancer cells, they have lower toxicity on the three human cancer cell lines. In addition, to test the toxicity of these tegafur derivatives, the non-tumor Beas-2B cell line was used, and the result showed that these compounds displayed lower toxicities (IC₅₀ >100 µg/mL) on human non-tumor cell line. As prodrugs, these tegafur derivatives are stable and cannot convert to 5-FU and 1,3,4-thiadiazoles *in vitro* which are activated and kills tumor cell, maybe that is the main reason for the decreased antiproliferative activity *in vitro*. Further

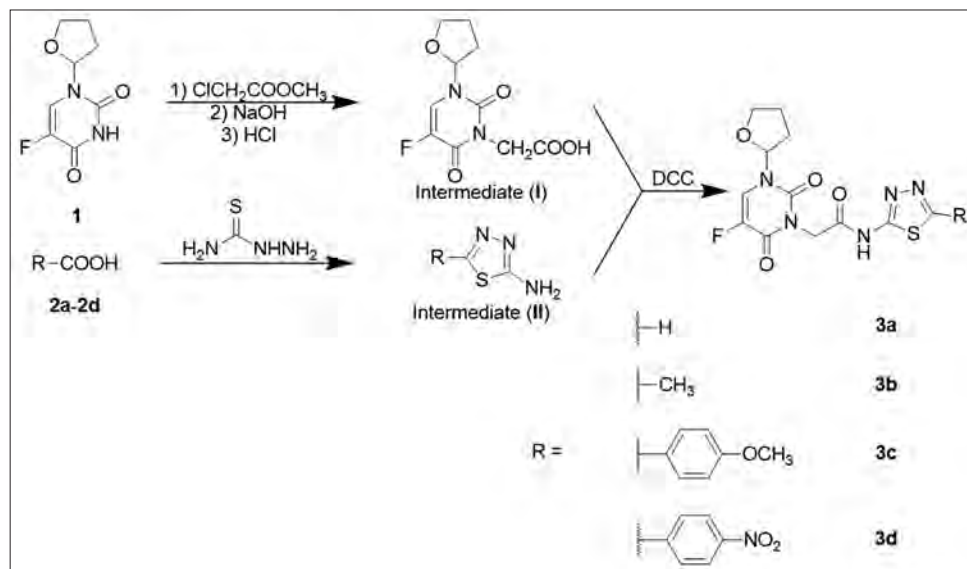
exploration of the mechanisms of their antiproliferative activities is currently underway.

In vivo xenograft experiments

To further confirm the antitumor efficacy of the tegafur derivatives (**3a-3d**) *in vivo*, we administered these compounds as monotherapy against S180 xenografts at 50 mg/kg/day, 25 mg/kg/day, 12.5 mg/kg/day, respectively, for 10 days. The tumor shrinkages were observed after intraperitoneal injection of these synthetic compounds after 10 days using floxuridine as reference [**Table 2**]. To our delight, the *in vivo* antitumor efficacy is not consistent with the *in vitro* cellular activity, which may be because these compounds act as fluoropyrimidine prodrugs *in vivo*. What's more, the mice in compound **3a/3c/3d** treated group have a better appetite than floxuridine, which implies that the synthesized compound **3a/3c/3d** may have lower side effects in mice *in vivo*. Compound **3a** displayed similar activity with floxuridine at 50 mg/kg/day, and *p*-methoxyphenyl substituted **3c** displayed a dramatic decrease in its activity. The results of *in vivo* activities showed that compound **3d** was more active than compound **3a** and floxuridine at 50 mg/kg/day. Replacement of the *p*-methoxyphenyl scaffold with the *p*-nitrophenyl tended to greatly enhance the activity (**3d** vs. **3c**), suggesting that the electron-withdrawing nitro group is favored. On the other hand, compound **3b** displayed lower activity than floxuridine.

EXPERIMENTAL SECTION

All commercial materials were used without further purification. All solvents were analytical grade. All melting points were determined in a Büchi capillary melting point apparatus and are uncorrected. The infrared (IR) spectra were recorded on a Nicolet Nexus 670FT-IR spectrometer using KBr disks and were expressed in cm⁻¹. The proton nuclear magnetic resonance (¹H NMR) spectra were recorded with a Bruker Avance DRX600 instrument with



Scheme 1: Synthesis of target tegafur derivatives

Table 1: The *in vitro* cytotoxic activity of 3a-3d against HGC27, SMMC7721, HCT15, and Beas-2B cell lines

Compound	HGC27	SMMC7721 IC ₅₀ ±SD (μg/mL)	HCT15	Beas-2B
3a	>100	>100	>100	>100
3b	>100	>100	>100	>100
3c	>100	>100	>100	>100
3d	>100	>100	>100	>100
Cisplatin	0.58	0.66	7.29	n.t. ^a

^an.t.: Indicate not tested**Table 2: The therapeutically effects of 3a-3d on S₁₈₀ xenograft**

Group	Dose (mg/kg)	Tumor weight (g) x±SD	Inhibition rate (%)
DMSO	–	1.81±0.77	–
Floxuridine	50	0.79±0.26*	56.35
3a	50	0.82±0.34*	54.70
	25	0.97±0.39**	46.41
	12.5	1.36±0.44	24.86
3b	50	1.31±0.45	27.62
	25	1.36±0.27	24.86
	12.5	1.47±0.22	18.78
3c	50	0.98±0.46	45.86
	25	1.02±0.55	43.65
	12.5	1.43±0.26	20.99
3d	50	0.68±0.33*	62.43
	25	0.88±0.52**	51.38
	12.5	1.33±0.38	26.52

P*<0.05, *P*<0.01 versus the negative group. SD: Standard deviation, DMSO: Dimethyl sulfoxide

tetramethylsilane as the internal standard. The chemical shifts (δ) were reported in parts per million and were relative to the central peak of the solvent, which was dimethyl sulfoxide-*d*₆ (DMSO-*d*₆) or CDCl₃. Coupling constants (*J*) were given in Hz. Mass spectra (MS) were measured with an API 4000. Thin-layer chromatography (TLC) was performed on silica gel GF254 plates (layer thickness, 0.2 mm). Column chromatography was carried out with silica gel in the solvents indicated.

Preparation of intermediate I

2-[5-fluoro-2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl]acetic acid (**I**)

Methanolic NaOH (0.3 M, 30 mL, and 10 mmol) was added to a solution of tegafur (2.0 g, 10 mmol) in methanol (20 mL) at 70°C, and then, methyl chloroacetate (0.5 M solution in methanol, 20 mL, and 10 mmol) was added. The resulting solution was stirred for 6 h. The mixture was concentrated under reduced pressure, diluted with EtOAc, filtered, and concentrated under reduced pressure. Then, 1.0 M NaOH (12 mL, 12 mmol) was added to the solution of this crude mixture in methanol (5 mL). After stirring at 45°C for 3 h, the resulting mixture was concentrated under reduced pressure. The residue was diluted with EtOAc, and

then extracted with water. The aqueous phase was acidified with 10% HCl, extracted with EtOAc. The organic layer was dried over Na₂SO₄, filtered, and concentrated under reduced pressure to give intermediate **I** (2.09 g, 81%) as a white solid, mp 289–290°C.

Preparation of intermediate II

To a solution of thiosemicarbazide (1.25 eq) and appropriate carboxylic acid (1.0 eq) in 1,4-dioxane (12.5 M^{−1}) was added phosphorus oxychloride (1.35 eq) dropwise at 0°C. The resulting solution was stirred at 85°C. The reaction mixture was cooled to room temperature once the starting material was consumed completely detected by TLC. The reaction mixture was poured into ice water, basified with ammonium hydroxide till pH = 8, filtered, and washed with water. The obtained crude product intermediate **II** (**IIa**, **IIb**, **IIc**, and **IId**) was used without future purification. **IIa**, colorless solid, mp 190–191°C [lit.^[6] mp 190–192°C.]; **IIb**, colorless solid, mp 235–236°C [lit.^[6] mp 231–235°C.]; **IIc**, colorless solid, mp 189–190°C [lit.^[7] mp 188–190°C.]; and **IId**, colorless solid, mp 233–234°C [lit.^[6] mp 232–235°C.].

Synthesis of target compounds

General procedure

A solution of dicyclohexylcarbodiimide (2.06 g, 10 mmol) in dimethylformamide (DMF) (100 mL) was added dropwise into a solution of intermediate **I** (2.58 g, 10 mmol) and intermediate **II** (12.5 mmol) in DMF (200 mL) at 0°C. The reaction mixture was stirred at 0°C for 3 h, and then kept at room temperature for another 8 h. The reaction mixture was filtered and concentrated. The crude product was purified by recrystallization from 95% ethanol to give pure product.

2-[5-fluoro-2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl]-N-(1,3,4-thiadiazol-2-yl)acetamide (**3a**)

White powder (2.77 g, 81%). IR (KBr, cm^{−1}): 3480 (ν_{N-H}), 2895 (ν_{CH2}), 1680 (ν_{C=O}), and 1574 (ν_{C=N}). ESI-MS (*m/z*): 341.9 [M+H]⁺, 363.9 [M+Na]⁺. ¹H-NMR (600 MHz, DMSO-*d*₆) δ : 1.92–1.97 (2H, m), 2.00–2.05 (1H, m), 2.24–2.30 (1H, m), 3.83 (1H, dt, *J* = 7.2 Hz, 15.0 Hz), 4.28 (1H, dt, *J* = 6.0 Hz, 13.8 Hz), 4.77 (2H, d, *J* = 4.2 Hz), 5.95–5.97 (1H, m), 8.09 (1H, d, *J* = 6.6 Hz), 9.20 (1H, s). Analysis calculated for C₁₂H₁₂FN₅O₄S: C, 42.23%; H, 3.54%; F, 5.57%; N, 20.52%; S, 9.39%. Found: C, 42.03%; H, 3.57%; F, 5.33%; N, 20.65%; S, 9.51%.



2-[5-fluoro-2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl]-N-(5-methyl-1,3,4-thiadiazol-2-yl)acetamide (**3b**)

White powder (2.73 g, 77%). IR (KBr, cm^{-1}): 3449 ($\nu_{\text{N-H}}$), 2997 (ν_{CH_3}), 2880 (ν_{CH_2}), 1682 ($\nu_{\text{C=O}}$), 1572 ($\nu_{\text{C=C}}$). ESI-MS (m/z): 356.0 [$\text{M}+\text{H}$] $^+$, 378.0 [$\text{M}+\text{Na}$] $^+$. $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 1.90–1.96 (1H, m), 2.04–2.12 (2H, m), 2.32–2.41 (1H, m), 2.67 (3H, s), 3.97–4.01 (1H, m), 4.23 (1H, td, $J = 4.8$ Hz, 8.4 Hz), 5.17 (2H, s), 5.98 (1H, dd, $J = 2.0$ Hz, 6.0 Hz), 7.47 (1H, d, $J = 6.0$ Hz). Analysis calculated for $\text{C}_{13}\text{H}_{14}\text{FN}_5\text{O}_4\text{S}$: C, 43.94; H, 3.97; F, 5.35; N, 19.71; S, 9.02. Found: C, 43.82; H, 3.80; F, 5.49; N, 19.90; S, 8.79.

2-[5-fluoro-2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl]-N-(5-(4-methoxyphenyl)-1,3,4-thiadiazol-2-yl)acetamide (**3c**)

White powder (3.79 g, 85%). IR (KBr, cm^{-1}): 3194 ($\nu_{\text{N-H}}$), 2888 (ν_{CH_2}), 1655 ($\nu_{\text{C=O}}$), 1600 ($\nu_{\text{C=C}}$). ESI-MS (m/z): 448.0 [$\text{M}+\text{H}$] $^+$. $^1\text{H-NMR}$ (600 MHz, CDCl_3) δ : 1.95–2.16 (4H, m), 3.87 (3H, s), 3.98–4.03 (1H, m), 4.25–4.27 (1H, m), 5.23 (2H, s), 6.01 (1H, s), 6.98 (2H, d, $J = 7.8$ Hz), 7.50 (1H, br s), 7.84 (2H, d, $J = 7.8$ Hz). Analysis calculated for $\text{C}_{19}\text{H}_{18}\text{FN}_5\text{O}_5\text{S}$: C, 51.00; H, 4.05; F, 4.25; N, 15.65; S, 7.17. Found: C, 51.28; H, 3.83; F, 4.11; N, 15.78; S, 7.24.

2-[5-fluoro-2,6-dioxo-3-(tetrahydrofuran-2-yl)-2,3-dihydropyrimidin-1(6H)-yl]-N-(5-(4-nitrophenyl)-1,3,4-thiadiazol-2-yl)acetamide (**3d**)

White powder (4.02 g, 87%). IR (KBr, cm^{-1}): 3432 ($\nu_{\text{N-H}}$), 2892 (ν_{CH_2}), 1657 ($\nu_{\text{C=O}}$), 1577 ($\nu_{\text{C=C}}$), 1349 ($\nu_{\text{N-N}}$), 718 ($\nu_{\text{C-S-C}}$). ESI-MS (m/z): 463.0 [$\text{M}+\text{H}$] $^+$, 484.9 [$\text{M}+\text{Na}$] $^+$. $^1\text{H-NMR}$ (600 MHz, $\text{DMSO}-d_6$) δ : 1.93–1.97 (2H, m), 2.02–2.07 (1H, m), 2.25–2.28 (1H, m), 3.84–3.85 (1H, m), 4.27–4.30 (1H, m), 4.81 (2H, d, $J = 15.6$ Hz), 5.98 (1H, s), 8.10 (1H, s), 8.22 (2H, d, $J = 6.0$ Hz), 8.37 (2H, d, $J = 6.6$ Hz). Analysis calculated for $\text{C}_{18}\text{H}_{15}\text{FN}_6\text{O}_6\text{S}$: C, 46.75; H, 3.27; F, 4.11; N, 18.17; S, 6.93. Found: C, 46.67; H, 3.32; F, 4.35; N, 18.09; S, 6.75.

Bioassay

Cell culture

HGC27, SMMC7721, and HCT15 (purchased from the Cell Bank of the Chinese Academy of Sciences, Shanghai, China) were cultured in RPMI-1640 medium supplemented with 100 $\mu\text{g}/\text{mL}$ streptomycin, 100 U/mL penicillin, and 10% (v/v) fetal bovine serum. All the cells were cultured in a humidified atmosphere containing 5% CO_2 at 37°C .

MTT cytotoxicity assay

The antitumor activities against HGC27, SMMC7721, and HCT15 human cancer cell lines were estimated using the 3-[4,5-dimethyl-2-thiazolyl]-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Cells were plated in each well of a 96-well plate and incubated at 37°C with different concentrations (100, 20, 5, 4, 0.8, and 0.16 $\mu\text{g}/\text{mL}$) of each compound or cisplatin (positive control) for 48 h.

0.5% MTT solution (10 μL) was added per well, and the cultures were continued for an additional 4 h. The medium was aspirated, the cells were dissolved in 200 μL of DMSO, and the optical density (OD) at 535 nm was determined in each well with a 96-well plate reader. The percent cell growth inhibition rate was calculated as $[(\text{ODc}-\text{ODt})/\text{ODc}]/100$. ODc represents the OD of the control group and ODt represents the OD of the treated group. The concentration inhibiting 50% of the cell proliferation (IC_{50}) was calculated.

In vivo xenograft experiments

Groups of 14 nude mice (6 weeks, Kunming strain, Animal Center of Shandong Lukang Pharmaceutical Co., Ltd) were used. The mice were inoculated subcutaneously into the right axilla with S180 (*Institute of Materia Medica, Chinese Academy of Medical Sciences*) cells (5×10^6 cells, 0.2 mL), respectively. After 7 days, the mice were treated intragastrically with compounds **3a**, **3b**, **3c**, and **3d** (50 mg/kg/day, 25 mg/kg/day, and 12.5 mg/kg/day of each compound), **floxuridine** (50 mg/kg/day), or the same volume of vehicle (1% DMSO) for consecutive 10 days. Inhibition rate of tumor by the following equation: Inhibition rate = $1-(\text{T}/\text{C}) \times 100\%$ (T: Tumor weight of the treatment group; C: Tumor weight of the control group).

ACKNOWLEDGMENTS

The authors would like to thank the Innovation Project of Shandong Academy of Medical Sciences for financial support. This research was supported by grants from Shandong Natural Science Foundation (Grant No. ZR2017PH003 and ZR2014YL035) and Shandong Academy of Medical Sciences Foundation (Grant No. 2016-5).

REFERENCES

- [1] Miura, K., Shirasaka, T., Yamaue, H., Sasaki, I. S-1 as a core anticancer fluoropyrimidine agent, *Expert Opin. Drug Deliv.*, **2012**, 9, 273–286.
- [2] Lucato, L.T., McKinney, A.M., Short, J., Teksam, M., Truwit, C.L. Reversible findings of restricted diffusion in 5-fluorouracil neurotoxicity, *Australas. Radiol.*, **2006**, 50, 364–368.
- [3] Liu, C., Dumbre, S.G., Pannecouque, C., Korba, B., Jonghe, S.D., Herdewijn, P. Synthesis and antiviral evaluation of base-modified deoxythreosyl nucleoside phosphonates, *Org. Biomol. Chem.*, **2017**, 15, 5513–5528.
- [4] Nagendra, G., Lamani, R.S., Narendra, N., Sureshbabu, V.V. A convenient synthesis of 1,3,4-thiadiazole and 1,3,4-oxadiazole based peptidomimetics employing diacylhydrazines derived from amino acids, *Tetrahedron Lett.*, **2010**, 51, 6338–6341.
- [5] Haider, S., Alam, M.S., Hamid, H. 1,3,4-thiadiazoles: A potent medicinal scaffold, *Eur. J. Med. Chem.*, **2015**, 92, 156–177.

- [6] Carvalho, A.S., Gibaldi, D., Pinto, A.C., Bozza, M., Boechat, N. Synthesis and Trypanocidal evaluation of new 5-[N-(3-(5-substituted)-1,3,4-thiadiazolyl)]amino-1-methyl-4-nitroimidazoles, *Lett. Drug. Des. Discov.*, **2006**, 3, 98–101.
- [7] Zheng, K.B., He, J., Zhang, J. Synthesis and antitumor activity of *N*¹-acetylamino-(5-alkyl/aryl-1,3,4-thiadiazole-2-yl)-5-fluorouracil derivatives, *Chin. Chem. Lett.*, **2008**, 19, 1281–1284.

Received: 17 Sep 2017; Accepted: 24 Oct 2017

