Chinese Chemical Letters 24 (2013) 299–302

Contents lists available at SciVerse ScienceDirect

Chinese Chemical Letters

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

Original article

Synthesis and cytotoxic evaluation of new colchicine derivatives bearing 1,3,4-thiadiazole moieties

Li-Hong Shen [a,*], Hong-Yu Li [a], Hui-Xia Shang [a], Shu-Ting Tian [a], Yi-Sheng Lai [b], Li-Jie Liu [c]

[a] Handan Key Laboratory of Organic Small Molecule Materials, Handan College, Handan 056005, China
[b] Center of Drug Discovery, China Pharmaceutical University, Nanjing 210009, China
[c] Hebei Yiling Medicine Corporation Ltd., Shijiazhuang 050035, China

ARTICLE INFO

Article history:
Received 12 December 2012
Received in revised form 9 January 2013
Accepted 21 January 2013
Available online 15 March 2013

Keywords:
Colchicine
N-Methyl colchiceinamide
1,3,4-Thiadiazole
Cytotoxicity

ABSTRACT

In search of novel anticancer agents, a series of *N*-methyl colchiceinamide derivatives (**7a**–**7i**) containing 1,3,4-thiadiazole moieties were synthesized, and their structures were confirmed by spectral analysis. Cytotoxicity of these compounds was evaluated by MTT assay *in vitro* against four human tumor cell lines, *i.e.* A2780, A549, BEL7402, and MCF-7. The results indicated that most of the derivatives showed significant anticancer activities, particularly, compounds **7h** and **7i** showed more potent cytotoxic activities of all screened cancer cells than colchicine.

1. Introduction

Colchicine (**1** in Fig. 1) and many of its derivatives are powerful mitotic poisons, anti-inflammatories, and inhibitors of tumor growth [1,2]. Most biological effects of colchicine are probably related to the formation of a colchicine–tubulin complex which prevents microtubule polymerization [3,4]. Although colchicine is a potent antimitotic agent, its medical uses in cancer chemotherapy are limited due to its high toxicity. Therefore, many attempts have been made to discover more effective and less toxic analogues of colchicine by modifying the substituents of its basic structure [5]. *N*-Methyl colchiceinamide (**2** in Fig. 1), a semisynthetic derivative of colchicine, showed considerably higher stability toward acid hydrolysis and is a slightly less active antitumor and toxicity agent than colchicine [6].

In recent years, different classes of thiadiazole compounds have been investigated, many of which have been found to be important scaffolds with broad spectrum of pharmacological activities [7–9]. Particularly, 1,3,4-thiadiazoles are much explored for their broad spectrum of biological activities including anti-inflammatory, antiviral, antimicrobial, antidepressants, antileishmanial and anticancer [10–13]. The action connected with the apoptotic mechanisms and angiogenesis, which is a crucial step in the tumorigenesis, seems to be very promising in anticancer therapy [14,15].

With these facts, and in order to obtain compounds with better anticancer activities, in this study we synthesized a variety of novel colchicine derivatives by combining *N*-methyl colchiceinamide and differently substituted 1,3,4-thiadiazole moieties and tested their anticancer activities against a selected panel of human cancer cell lines.

2. Experimental

2.1. Chemistry

Solvents and reagents were analytical reagents and when necessary, were purified and dried by standard methods. IR spectra (KBr) were recorded on a Nicolet Impact 410 spectrometer. The ^{1}H NMR spectra were recorded on a Brucker spectrometer (300 MHz) with $CDCl_3$ as the solvent and TMS as the internal standard. Chemical shifts reported in δ (parts per million) values. Mass spectra were performed on Agilent 1100 series LC/MSD Trap (SL) spectrometer. Elemental analyses were performed with an Elementar Vario EL III instrument and analyses for C, H, and N were within ±0.4% of the theoretical values. Flash column chromatography was performed on a column packed with silica gel 60 (200–300 mesh).

* Corresponding author.
E-mail address: lhshen1974@gmail.com (L.-H. Shen).

1001-8417/$ – see front matter © 2013 Li-Hong Shen. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.
http://dx.doi.org/10.1016/j.cclet.2013.01.052

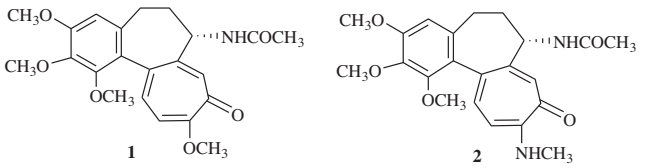


Fig. 1. Structures of colchicine (**1**) and *N*-methyl colchiceinamide (**2**).

2.2. General procedure for the synthesis of compounds (*7a–7i*)

The reaction mixture of succinate **4** (18 mg, 0.04 mmol), compounds **6a–6i** (0.06 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) (25 mg, 0.13 mmol), DMAP (2 mg, 0.019 mmol) and dry dichloromethane (5 mL) was stirred at room temperature for 24 h. Then, additional dichloromethane (20 mL) was added. The organic layer was washed with water and brine, and then dried over $MgSO_4$. After the solvent was removed in vacuo, the residue was separated by column chromatography (eluent: ethyl acetate/petroleum ether = 1/1) to yield the target compounds **7a–7i** (shown in Scheme 1).

2.3. Selected data of the target compounds *7a–7i*

*N-Deacetyl-N-{[5-(benzylsulfanyl)-1,3,4-thiadiazol-2-yl]carbamoyl pionyl} N-methyl colchiceinamide (***7a***):* Yellow syrup, yield 48%. IR (KBr, cm^{-1}): ν 3329, 1704, 1692, 1615, 1600, 1580, 1492. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.35 (m, 5H), 3.07 (d, 3H, *N*-CH_3), 3.67 (s, 3 H, MeO-1), 3.91 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.25–4.29 (t, 2H, OCH_2), 4.44–4.48 (m, 4H, SCH_2, OCH_2), 4.60–4.72 (m, 1H, H-7), 6.54 (s, 1H, H-4), 7.14 (m, 2H, H-11 and H-12), 7.49 (s, 1H, H-8), 7.45–7.78 (m, 5H), 12.80 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 661.2 $[M]^+$; Anal. calcd. for $C_{33}H_{35}N_5O_6S_2$: C 59.89, H 5.33, N 10.58; found: C 60.07, H 5.49, N 10.64.

*N-Deacetyl-N-({5-[(3-chlorobenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7b***):* Yellow syrup, yield 50%. IR (KBr, cm^{-1}): ν 3325, 2932, 1700, 1692, 1613, 1507, 1394. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.40 (m, 5H), 3.07 (d, 3H, *N*-CH_3), 3.69 (s, 3H, MeO-1), 3.95 (s×2, 6H, MeO-2 and MeO-3), 4.25–4.29 (t, 2H, OCH_2), 4.45–4.49 (m, 4H, SCH_2, OCH_2), 4.67 (m, 1H, H-7), 6.52 (s, 1H, H-4), 7.12 (m, 2H, H-11 and H-12), 7.49 (s, 1H, H-8), 7.70–7.75 (m, 2H), 8.04–8.06 (m,2H), 12.56 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 695.1 $[M]^+$; Anal. calcd. for $C_{33}H_{34}ClN_5O_6S_2$: C 56.93, H 4.92, N 10.06; found: C 57.06, H 5.09, N 10.18.

*N-Deacetyl-N-({5-[(4-chlorobenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7c***):* Yellow syrup, yield 46%. IR (KBr, cm^{-1}): ν 3330, 2919, 1703, 1692, 1606, 1546, 1467. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.38 (m, 5H), 3.08 (d, 3H, *N*-CH_3), 3.65 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.96 (s, 3H, MeO-3), 4.35 (t, 2H, OCH_2), 4.48 (m, 4H, SCH_2, OCH_2), 4.65 (m, 1H, H-7), 6.55 (s, 1H, H-4), 7.10 (m, 2H, H-11 and H-12), 7.52 (s, 1H, H-8), 7.74–7.79 (m, 2H), 8.04–8.07 (m, 2H), 13.01 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 695.2 $[M]^+$; Anal. calcd. for $C_{33}H_{34}ClN_5O_6S_2$: C 56.93, H 4.92, N 10.06; found: C 57.09, H 5.07, N 9.90.

*N-Deacetyl-N-({5-[(3-methylbenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7d***):* Yellow syrup, yield 54%. IR (KBr, cm^{-1}): ν 3326, 2927, 1708, 1693, 1602, 1567, 1496. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.36 (m, 5H), 3.08 (d, 3H, *N*-CH_3), 3.68 (s, 3H, MeO-1), 3.91 (s, 3H, MeO-2), 3.97 (s, 3H, MeO-3), 4.35 (m, 4H, SCH_2, OCH_2), 4.48 (m, 2H, OCH_2), 4.65 (m, 1H, H-7), 6.58 (s, 1H, H-4), 7.15 (m, 2H, H-11 and H-12), 7.43–7.47 (m, 2 H), 7.60–7.65 (m, 2H), 7.74–7.79 (m, 1 H), 13.02 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 675.2 $[M]^+$; Anal. calcd. for $C_{34}H_{37}N_5O_6S_2$: C 60.43, H 5.52, N 10.36; found: C 60.66, H 5.47, N 10.18.

*N-Deacetyl-N-({5-[(4-methylbenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7e***):* Yellow syrup, yield 60%. IR (KBr, cm^{-1}): ν 3328, 2925, 1710, 1695, 1612, 1511, 1396. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.35 (m, 5H), 3.09 (d, 3H, *N*-CH_3), 3.63 (s, 3H, MeO-1), 3.89 (s, 3H, MeO-2), 3.94 (s, 3H, MeO-3), 4.30 (m, 4H, SCH_2, OCH_2), 4.46 (m, 2H, OCH_2), 4.63 (m, 1H, H-7), 6.59 (s, 1H, H-4), 7.16 (m, 2H, H-11 and H-12), 7.50 (s, 1H, H-8), 7.62–7.66 (m, 2H), 7.84–7.90 (m, 2 H), 13.12 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 675.2 $[M]^+$; Anal. calcd. for $C_{34}H_{37}N_5O_6S_2$: C 60.43, H 5.52, N 10.36; found: C 60.24, H 5.49, N 10.48.

*N-Deacetyl-N-({5-[(3-methoxybenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7f***):* Yellow syrup, yield 58%. IR (KBr, cm^{-1}): ν 3326, 2923, 1705, 1692, 1632, 1561, 1462. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.34 (m, 5H), 3.07 (d, 3H, N-CH_3), 3.68 (s, 3H, MeO-1), 3.95 (s×2, 6H, MeO-2 and MeO-3), 4.38 (m, 4H, SCH_2, OCH_2), 4.45 (m, 2H, OCH_2), 4.63 (m, 1H, H-7), 6.59 (s, 1H, H-4), 7.16 (m, 2H, H-11 and H-12), 7.50 (s, 1H, H-8), 7.51–7.54 (m, 1H), 7.60–7.65 (m, 1H), 7.73–7.79 (m, 2H), 13.14 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 691.2 $[M]^+$; Anal. calcd. for $C_{34}H_{37}N_5O_7S_2$: C 59.03, H 5.39, N 10.12; found: C 60.14, H 5.59, N 10.08.

*N-Deacetyl-N-({5-[(4-methoxybenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7g***):* Yellow syrup, yield 50%. IR (KBr, cm^{-1}): ν 3327, 2923, 1707, 1696, 1639, 1562, 1461. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.34 (m, 5H), 3.07 (d, 3H, *N*-CH_3), 3.58 (s, 3H, MeO-1), 3.90 (s, 3H, MeO-2), 3.96 (s, 3H, MeO-3), 4.32 (m, 4H, SCH_2, OCH_2), 4.44 (m, 2H, OCH_2), 4.63 (m, 1H, H-7), 6.54 (s, 1H, H-4), 7.12 (m, 2H, H-11 and H-12), 7.53 (s, 1H, H-8), 7.60–7.65 (m, 2H), 7.74–7.79 (m, 2H), 13.10 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 691.1 $[M]^+$; Anal. calcd. for $C_{34}H_{37}N_5O_7S_2$: C 59.03, H 5.39, N 10.12; found: C 60.16, H 5.28, N 10.06.

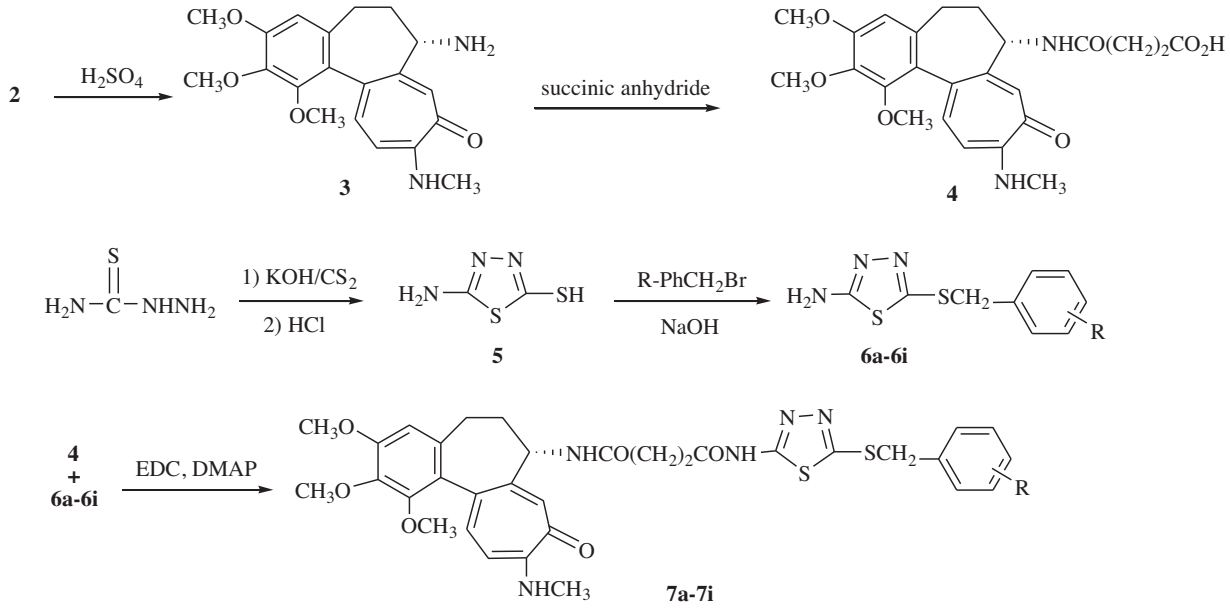
*N-Deacetyl-N-({5-[(3-nitrobenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7h***):* Yellow syrup, yield 55%. IR (KBr, cm^{-1}): ν 3327, 2923, 1707, 1691, 1635, 1552, 1491. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.35 (m, 5H), 3.08 (d, 3H, *N*-CH_3), 3.62 (s, 3H, MeO-1), 3.89 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.32 (m, 2H, OCH_2), 4.46 (m, 4H, SCH_2, OCH_2), 4.63 (m, 1H, H-7), 6.53 (s, 1H, H-4), 7.09 (m, 2H, H-11 and H-12), 7.53 (s, 1H, H-8), 7.49–7.54 (m, 1H), 7.60–7.66 (m, 1H), 7.75–7.80 (m, 1H), 8.04–8.08 (m, 1H), 12.60 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 706.1 $[M]^+$; Anal. calcd. for $C_{33}H_{34}N_6O_8S_2$: C 56.08, H 4.85, N 11.89; found: C 56.21, H 4.98, N 11.67.

*N-Deacetyl-N-({5-[(4-nitrobenzyl)sulfanyl]-1,3,4-thiadiazol-2-yl}carbamoyl pionyl) N-methyl colchiceinamide (***7i***):* Yellow syrup, yield 56%. IR (KBr, cm^{-1}): ν 3325, 2925, 1709, 1698, 1637, 1565, 1492. ^1H NMR (300 MHz, $CDCl_3$): δ 1.50–2.35 (m, 5H), 3.08 (d, 3H, *N*-CH_3), 3.62 (s, 3H, MeO-1), 3.89 (s, 3H, MeO-2), 3.95 (s, 3H, MeO-3), 4.30 (m, 2H, OCH_2), 4.45 (m, 4H, SCH_2, OCH_2), 4.63 (m, 1H, H-7), 6.53 (s, 1H, H-4), 7.09 (m, 2H, H-11 and H-12), 7.53 (s, 1H, H-8), 7.74–7.78 (m, 2H), 8.02–8.06 (m, 2H), 13.02 (brs, 2H, NHCO); MS (ESI, *m*/*z*): 706.2 $[M]^+$; Anal. calcd. for $C_{33}H_{34}N_6O_8S_2$: C 56.08, H 4.85, N 11.89; found: C 56.02, H 4.78, N 11.96.

2.4. Biological activity

Human cancer cell lines were purchased from the Key Gen Serving Science Company (Nanjing, China). Cancer cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum (FBS) and cells were routinely cultured in a humidified incubator at 37 °C with 5% carbon dioxide.

Cancer cells were seeded in 96-well microtiter plates and attached to the bottom of the well overnight. After 24 h, the compounds (10^{-4}–10^{-8} mol/L) dissolved in DMSO (5%) were added to each well and incubated for 3 days. At the end of 3 days incubation, the medium in each well was replaced by fresh



Scheme 1. Synthesis of the target compounds **7a**–**7i**.

medium (200 μL) containing 5 mg/mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium hydrobromide (MTT). The formazan product of MTT reduction was dissolved in DMSO 3 h later, and absorbance was measured using an ELISA micro-plate reader. Growth of tumoral cells was quantitated by the ability of living cells to reduce the yellow dye MTT to a purple formazan product. Drug effect was quantified as the percentage of control absorbance of reduced dye at 570 nm.

3. Results and discussion

3.1. Chemistry

The synthetic route of these target compounds is outlined in Scheme 1. *N*-Methyl colchiceinamide (**2**) was refluxed with concentrated H_2SO_4 in water to give *N*-methyl deacetyl colchiceinamide (**3**). Compound **3** was acylated by succinic anhydride in dry pyridine at 60 °C to give succinate **4** in 95% yield. The physical and spectral properties of compounds **3** and **4** were in accordance with the literature [16,17]. The compound 5-amino-1,3,4-thiadiazole-2-thiol (**5**) was prepared by cyclization of thiosemicarbazide with CS_2. Treatment of **5** with corresponding benzyl bromides ($RPhCH_2Br$) in presence of NaOH in 80% EtOH at room temperature afforded *S*-benzyl intermediates **6a**–**6i** according to the literature [18,19] in 68%–90% yields.

By the acylation of compounds **6a**–**6i** with succinate **4** in the presence of a coupling agent EDC and a catalyst, 4-(dimethylamino)pyridine (DMAP), in CH_2Cl_2 at room temperature, nine new final compounds **7a**–**7i** were synthesized. All of the newly synthesized compounds **7a**–**7i** were identified by IR, ^{1}HNMR and MS spectra and confirmed by elemental analysis.

The elemental analysis data for each compound were in good agreement with the empirical formula proposed. In the IR spectra, newly synthesized compounds **7a**–**7i** exhibited characteristic υ (C=O) bands at 1690–1695 cm^{-1} and 1700–1710 cm^{-1} for amide side chains and tropolone rings, respectively. The υ (N–H) stretching bands were centered at 3324–3330 cm^{-1}.

The ^{1}H NMR spectral data of compounds **7a**–**7i** are presented in Section 2. The ^{1}H NMR spectra of all complexes were consistent with their corresponding protons as chemical shift values and numbers of hydrogen.

3.2. Biological studies

In vitro evaluation of the target compounds **7a**–**7i** for their cytotoxic properties was performed by means of MTT assays in triplicate using four different human cancer cell lines: A2780 (human ovary cancer), A549 (human lung cancer), BEL7402 (human hepatoma), and MCF7 (human breast carcinoma). Colchicine was the positive control for the cytotoxic effect. The biological activity data are presented in Table 1.

As shown in Table 1, these novel derivatives **7a**–**7i** showed superior or comparable cytotoxic activity to colchicine and **2** against four tumor cells. Among these compounds **7d**, **7e**, **7f** and **7g** exhibited potent anticancer activities similar to that of colchicine, while the activities of **7h** and **7i** are stronger than that of colchicine.

The preliminary structure–activity relationships (SARs) revealed that the substituent on benzene ring of benzene alkyl groups plays an important role in activity. The compounds with nitro or methoxy groups displayed more potent anticancer activities than those with chlorine or methyl groups. In particular, electron-withdrawing substituents such as a nitro group (**7h** and **7i**) showed better anti-tumor activity than **7f** and **7g** with electron-donating groups. Furthermore, the anti-tumor activities were governed by the position of substituents. *para*-Substituted compounds as **7c**, **7e**, **7g** and **7i** showed higher antitumor activities than **7b**, **7d**, **7f**, and **7h** with *meta*-substituents.

Table 1
The cytotoxicity data of the target compounds.

Compounds	R	IC_{50} (μmol/L)/cell line			
		A2780	A549	BEL7402	MCF7
7a	H	0.134	0. 156	0.212	0.163
7b	3-Cl	0.112	0.108	0.125	0.235
7c	4-Cl	0.109	0.106	0.113	0.208
7d	3-Me	0.097	0.085	0.086	0.108
7e	4-Me	0.096	0.082	0.084	0.085
7f	3-MeO	0.095	0.080	0.080	0.084
7g	4-MeO	0.092	0.078	0.079	0.082
7h	3-NO_2	0.024	0.015	0.016	0.018
7i	4-NO_2	0.023	0.011	0.009	0.014
2		0.102	0.082	0.085	0.090
Colchicine		0.094	0.078	0.080	0.084

4. Conclusion

In summary, a series of colchicine analogues (**7a**–**7i**) by hybridization of *N*-methyl colchiceinamide with 1,3,4-thiadiazole were investigated. Preliminary bioassay data indicated that all of the target compounds exhibited cytotoxic activity against the tumor cells to varied extent. Compared with colchicine, compounds **7h** and **7i** were more potent. Our results may provide some guidance for the development of novel colchicine anticancer agents. Further SARs and mechanistic studies on this new class of anticancer compounds are currently in progress and will be reported in due course.

References

[1] B. Bhattacharyya, D. Panda, S. Gupta, et al., Anti-mitotic activity of colchicine and the structural basis for its interaction with tubulin, Med. Res. Rev. 28 (2008) 155–183.
[2] B. Yang, Z.C. Zhu, H.V. Goodson, et al., Syntheses and biological evaluation of ring-C modified colchicine analogs, Bioorg. Med. Chem. Lett. 20 (2010) 3831–3833.
[3] Y. Lu, J. Chen, M. Xiao, et al., An overview of tubulininhibitors that interact with the colchicine binding site, Pharm. Res. 29 (2012) 2943–2971.
[4] J.T. Huzil, J. Mane, J.A. Tuszynski, Computer assisted design of second-generation colchicine derivatives, Interdiscip. Sci. 2 (2010) 169–174.
[5] K.H. Lee, Current developments in the discovery and design of new drug candidates from plant natural product leads, J. Nat. Prod. 67 (2004) 273–283.
[6] M. Cifuentes, B. Schilling, R. Ravindra, et al., Synthesis and biological evaluation of B-ring modified colchicine and isocolchicine analogs, Bioorg. Med. Chem. Lett. 16 (2006) 2761–2764.
[7] M. Wu, Q. Sun, C. Yang, et al., Synthesis and activity of combretastatin A-4 analogues: 1 2,3-thiadiazoles as potent antitumor agents, Bioorg. Med. Chem. Lett. 17 (2007) 869–873.
[8] M.N. Noolvi, H.M. Patel, N. Singh, et al., Synthesis and anticancer evaluation of novel 2-cyclopropylimidazo[2, 1-b][1,3,4]-thiadiazole derivatives, Eur. J. Med. Chem. 46 (2011) 4411–4418.
[9] L. Shen, Y. Zhang, A. Wang, et al., Synthesis and identification of [1,2,4]thiadiazole derivatives as a new series of potent and orally active dual agonists of peroxisome proliferator-activated receptors alpha and delta, J. Med. Chem. 50 (2007) 3954–3963.
[10] R. Sharma, J. Sainy, S.C. Chaturvedi, 2-Amino-5-sulfanyl-1,3,4-thiadiazoles: a new series of selective cyclooxygenase-2 inhibitors, Acta Pharm. 58 (2008) 317–326.
[11] D. Kumar, B.R. Vaddula, K.H. Chang, et al., One-pot synthesis and anticancer studies of 2-arylamino-5-aryl-1, 3,4-thiadiazoles, Bioorg. Med. Chem. Lett. 21 (2011) 2320–2323.
[12] M.S. Alam, L. Liu, D.U. Lee, Cytotoxicity of new 5-phenyl-4, 5-dihydro-1,3,4-thiadiazole analogues, Chem. Pharm. Bull. (Tokyo) 59 (2011) 1413–1416.
[13] D. Kumar, N.M. Kumar, B. Noel, et al., A series of 2-arylamino-5-(indolyl)-1 3,4-thiadiazoles as potent cytotoxic agents, Eur. J. Med. Chem. 55 (2012) 432–438.
[14] T. Kawaguchi, S. Yamamoto, S. Kudoh, et al., Tumor angiogenesis as a major prognostic factor in stage I lung adenocarcinoma, Anticancer Res. 17 (1997) 3743–3746.
[15] S.P. Lin, Y.T. Lee, S.H. Yang, et al., Colon cancer stem cells resist antiangiogenesis therapy-induced apoptosis, Cancer Lett. 328 (2013) 226–234.
[16] L.H. Shen, Y. Li, D.H. Zhang, et al., Synthesis and evaluation of nitrate derivatives of colchicine as anticancer agents, Chin. Chem. Lett. 22 (2011) 768–770.
[17] K. Akiyama, N-methyldeacetylcolchiceinamide derivatives, European Patent EP 0607647 A1 (1994).
[18] T. Wang, Y.H. Zhang, S. Yu, et al., Synthesis and biological evaluation of novel thalidomide analogues as potential anticancer drugs, Chin. Chem. Lett. 19 (2008) 928–930.
[19] X.W. Kong, Y.H. Zhang, T. Wang, et al., Synthesis and cytotoxic evaluation of novel dimethyl [11′-biphenyl]-2,2′-dicarboxylates bearing 1,3,4-thiadiazole moieties, Chem. Biodivers. 5 (2008) 1743–1752.