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Original article

Synthesis and antitumor activity of α-aminophosphonate derivatives containing thieno[2,3-d]pyrimidines

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ABSTRACT

Two series of thieno[2,3-d]pyrimidine derivatives were designed and synthesized, in which bioactive α-aminophosphonate subunits were introduced at the N3 position through an N–N bond connection. The *in vitro* cytotoxic activity of the novel compounds was tested against human esophageal carcinoma cells (EC109), human hepatocarcinoma cells (HepG2), human gastric carcinoma cells (MGC-803), respectively, by the MTT method. The evaluation results revealed that compounds **6mb**, **6mf**, **6mg**, **6nd** and **6nh** exerted the most potent inhibition against HepG2, MGC-803 and EC109 cells, respectively. In particular, compound **6mg** presented excellent inhibitory effect against HepG2 (91.2%) and MGC-803 (94.4%) cells.

1. Introduction

It is well known that fused pyridines and thienopyrimidines have many valuable pharmacological properties, such as antioxidative [1,2], antimalarial [3,4], antibacterial [5], and antiviral effects [6]. The pyrimidine-based derivatives, *e.g.* thieno[2,3-d]pyrimidines, were proved to have pharmacological and therapeutic properties in medicinal chemistry, such as anti-inflammatory [7], antibacterial [8,9], antifungal [10,11], antitumor [12], antidepressant [13], antiplatelet [14], antihypertensive [15], herbicidal [16] and plant growth regulatory [17] properties.

As phosphorus analogs of α-amino acids and their esters, α-aminophosphonates have received much attention owing to their potential biological activities such as antibacterial, anticancer, as well as insecticidal and herbicidal properties, [18–22] and were investigated as starting materials for the syntheses of phosphonopeptides. Moreover, if the aminophosphonate subunit was suitably embedded into potential antitumor agents, it could effectively improve their bioactivity [23], *e.g.* benzothiazole moiety or fluorine moiety containing α-aminophosphonates [24–27] were proved to have high antitumor activity.

Based on the concept of bioisosterism and inspired by the above observations, we were led to the proposal to incorporate thieno[2,3-*d*]pyrimidine ring into the α-aminophosphonate compounds, to construct novel classes of thieno[2,3-d]pyrimidine moiety containing α-aminophosphonate derivatives, and to evaluate their antitumor activity. Therefore, two series of such compounds, incorporating the α-aminophosphonate and the thieno[2,3-d]pyrimidines, were designed and synthesized and their antitumor properties were investigated.

Twenty-two new diethyl or diisopropyl [(3-substituted or 4-substituted or 2,4-disubstituted) (2-methyl-6-ethyl-4-oxo-4-*H*-thieno[2,3-d]pyrimidin-3-yl-amino) methyl] phosphonates were synthesized and their *in vitro* antitumor activity under cell membrane conditions were evaluated by the MTT method. Compounds **6ma-6mk** and **6na-6nk**, as well as the intermediates **4a-4k**, were tested for their anti-proliferation activity against three human cancer cell lines (EC109, HepG2 and MGC-803) at different concentrations. This represents the first report about the synthesis and *in vitro* antitumor activity evaluation of thieno[2,3-d]pyrimidines containing α-aminophosphonate derivatives.

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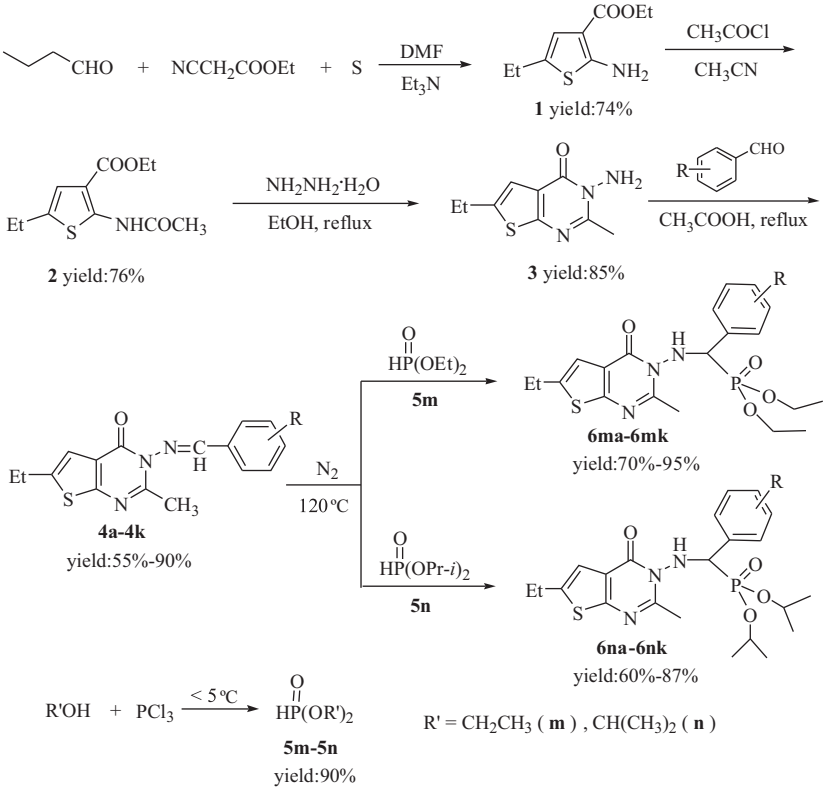
2. Experimental

All starting materials were commercially available and analytically pure. ^{1}H NMR spectra were recorded on a BRUKER DPX-400 spectrometer (Bruker Company, Germany), using TMS as an internal standard and $CDCl_3$ as a solvent. Chemical shifts (δ values) and coupling constants (J values) are given in ppm and Hz, respectively. TLC analysis was carried out on silica gel plates GF254 (Qindao Haiyang Chemical, China). High-resolution mass spectra were performed on an ESI Q-TOF MS spectrometer (Micromass, England). Melting points were determined on an XT4A apparatus (Beijing Keyi Electro-optic Instrument Plant, China) and were uncorrected. The intensity data were collected on an Agilent xcalibur Eos-II- CCD-diffractometer (Agilent Technologies).

Twenty-two α-aminophosphonate derivatives (**6ma-6mk** and **6na-6nk**) were prepared according to the reaction sequences shown in Scheme 1. The starting materials, ethyl 2-amino-5-ethylthiophene-3-carboxylate (**1**) [28] and dialkyl phosphite (**5**), were prepared according to the literature methods. The target analogs were easily obtained in high yields with the established reaction conditions. The acetylation of compound (**1**) with acetyl chloride in acetonitrile gave the ethyl ester of 2-acetylamino-5-ethylthiophene-3-carboxylic acid (**2**) [29]. The acetylated intermediate (**2**) then underwent condensation-cyclization reactions with hydrazine hydrate to afford compound (**3**), 3-amino-6-ethyl-2-methylthieno[2,3-d]pyrimidin-4-one, in good yield [30]. The Schiff base type key intermediates (**4**), obtained by the reaction of (**3**) with various substituted benzaldehydes, reacted with different dialkyl phosphite (**5**) under nitrogen atmosphere to generate the corresponding α-aminophosphonate derivatives (**6ma-6mk** and **6na-6nk**). All the new compounds were characterized using ^{1}H NMR and ^{13}C NMR, mass spectrometry and high resolution mass spectrometry, respectively (see Supporting information for structure characterization, yields and melting points).

Fortunately, the single crystals of compound **6mi** and **6mj** were obtained. The molecular structures of the two compounds are illustrated in Figs. 1 and 2. Colorless single crystals of the compound **6mi** (0.35 mm × 0.30 mm × 0.30 mm) and **6mj** (0.30 mm × 0.25 mm × 0.22 mm) were selected for X-ray diffraction analysis. The data were collected on an Agilent xcalibur Eos-II-CCD-diffractometer equipped with a graphite-monochromatic Cu Kα radiation (λ = 1.5418 Å) by using a ω scan mode in the range of $4.46 < \theta < 72.28^\circ$ for **6mi** and $4.42 < \theta < 67.07^\circ$ for **6mj** at 291(2) K. The entire structure was solved by the direct methods using the SHELXS-97 program [31] and refined by the full-matrix least-squares method on F2 with anisotropic thermal parameters for all non-hydrogen atoms using SHELXL-97. [32] For compound **6mi**, a total of 8957 reflections were recorded and 4476 were unique ($R_{int.}$ = 0.0225), among which 4245 ($-13 \le h \le 13$, $-9 \le k \le 14$, $-19 \le l \le 21$) were observed. For compound **6mj**, a total of 8872 reflections were recorded and 4183 were unique ($R_{int.}$ = 0.0232), among which 3930 ($-12 \le h \le 8$, $-14 \le k \le 13$, $-19 \le l \le 21$) were observed. X-ray diffraction analysis revealed that the thieno [2,3-d]pyrimidine ring in two compounds did not exhibit good coplanarity and the molecular structure is stabilized by intermolecular N(1)–H(1)...N(3) hydrogen bonds. Thereby, the structure of **6mi** and **6mj** were further confirmed.

A 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay was performed to investigate *in vitro* cytotoxic activity. Human esophageal carcinoma cells (EC109), human hepatocarcinoma cells (HepG2) and human gastric carcinoma cells (MGC-803) seeded into the 96-well plate (100 μL each well) were incubated at 37 °C under 5% CO_2 and 95% O_2 until cell adherence was observed. Cells were exposed to suspension of



Scheme 1. Synthetic routes for the targeted compounds.

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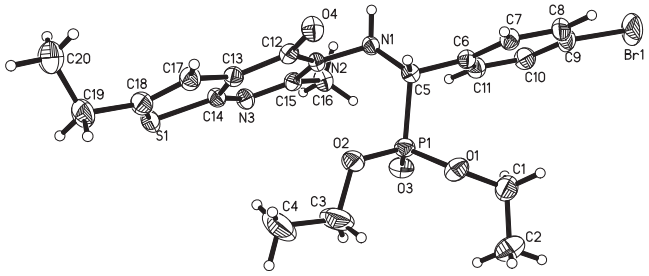

Fig. 1. X-ray structure of compound **6mi**.

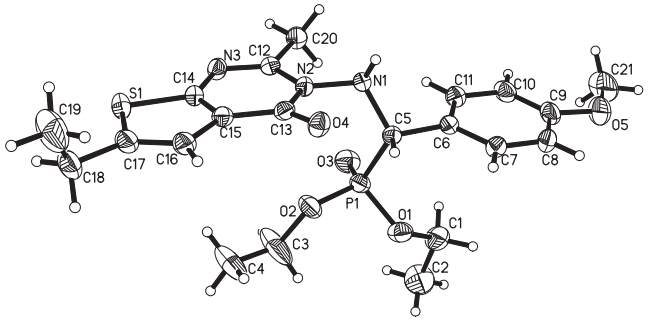

Fig. 2. X-ray structure of compound **6mj**.

compounds **6ma-6mk**, **6na-6nk**, as well as the intermediates **4a-4k** at different concentrations. After 48 h, 20 μL of MTT with a concentration of 5 μg/mL was added into the 96-well plate and incubated for 4 h at 37 °C. Then, the supernatant was aspirated, 150 μL of DMSO was added to each well, and the absorbance was measured at 490 nm. The formula for calculating inhibition of cell proliferation was as follows: inhibition of cell proliferation rate (%) = (OD value of the control group - OD value of drug group)/control OD value × 100%. This was used to plot an inhibition curve.

3. Results and discussion

The *in vitro* cytotoxic activity of compounds **6ma-6mk**, **6na-6nk**, as well as the intermediates **4a-4k** against human esophageal carcinoma cells (EC109), human hepatocarcinoma cells (HepG2), human gastric carcinoma cells (MGC-803) was evaluated by the MTT method and the potency was expressed as inhibition rate. The results are summarized in Table 1.

Results in Table 1 show that the changes of substituents affected the antitumor activity. Among analogs **6ma-6mk** and **6na-6nk**, even intermediates Schiff bases **4a-4k**, compounds **6ma-6mk** and **6na-6nk** have a relatively higher antitumor activity than that of Schiff bases **4a-4k**. These observations indicate that the introduction of phosphoryl group improves antitumor activity to EC109, HepG2 and MGC-803 cells.

The antitumor data also indicate that the substitutes of phosphonate has no apparent influence on antitumor activity to EC109, HepG2 and MGC-803 cells. For example, when R was 4-NO_2 and R′ was Et, compound **6me** exhibited moderate antitumor activity with an inhibition rate of 54.0% against EC109 cells at 50 μg/mL; the antitumor activity of **6ne**, where the phosphonate was substituted by an *i*-Pr, was little higher than the activity of **6me** in EC109 with the inhibition rate of 58.5%. Compound **6mk** with 2-Cl (R), 4-Cl(R), and Et (R') has antitumor activity in HepG2 cells, with an inhibition rate of 77.8% at the concentration of 50 μg/mL. Compound **6nk** with 2-Cl (R), 4-Cl(R), and *i*-Pr (R′) has a lower antitumor activity (60.8%) than that of **6mk**. This same trend was observed for compounds **6md/6nd** and **6mh/6nh** against EC109, HepG2 and MGC-803 cells.

Compounds **6mg** (R:4-Cl, R′:Et) and **6nh** (R:3-Br, R′:*i*-Pr) showed potent inhibition against EC109 cells with the inhibition rate of 86.8% and 85.4% at 50 μg/mL, respectively. Compounds **6mg** (R:4-Cl, R′:Et) and **6nd** (R:3-NO_2, R′:*i*-Pr) showed potent inhibition against HepG2 cells with the inhibition rate of 91.2% and 90.8% at 50 μg/mL, respectively. Compounds **6mb** (R:3-CH_3, R′:Et),**6mf** (R:3-Cl, R′:Et),**6mg** (R:4-Cl, R′:Et) and **6nh** (R:3-Br, R′:*i*-Pr) showed potent inhibition against MGC-803 cells with the

Table 1
Antitumor activity of target compounds (inhibitory ratio/%).

Comp.	EC109			HepG2			MGC-803		
	50 μg/mL	10 μg/mL	5 μg/mL	50 μg/mL	10 μg/mL	5 μg/mL	50 μg/mL	10 μg/mL	5 μg/mL
6ma	66.3	26.1	27.5	66.0	35.4	32.4	50.3	29.0	20.6
6mb	79.8	43.7	11.5	81.6	41.9	31.4	91.3	53.1	44.7
6mc	64.4	8.0	1.0	62.3	12.8	6.6	64.6	8.2	1.1
6md	43.3	16.4	17.2	72.9	43.3	32.9	49.6	29.2	27.8
6me	54.0	10.8	4.4	61.1	15.3	9.5	59.8	−3.0	−10.4
6mf	81.4	36.1	30.3	86.7	43.7	28.2	94.4	41.4	41.5
6mg	86.8	37.6	36.7	91.2	39.7	32.8	94.4	42.2	25.8
6mh	40.4	44.8	20.9	53.8	23.7	14.9	46.3	27.0	18.2
6mi	76.5	15.3	7.6	74.3	12.3	4.6	65.4	0.6	−7.5
6mj	58.2	23.0	26.6	70.3	42.4	35.9	65.4	19.2	8.7
6mk	73.6	57.7	25.6	77.8	64.4	34.3	75.3	62.6	16.6
6nd	45.3	11.2	−2.0	90.8	40.3	26.0	63.6	40.1	27.7
6ne	58.5	5.3	−1.8	58.1	24.3	6.9	84.3	39.2	31.8
6nh	85.4	13.8	11.2	48.7	39.7	21.3	93.7	39.9	41.6
6nk	51.0	41.7	36.7	60.8	48.1	36.2	64.5	63.3	36.9
4a	51.4	2.9	−8.9	57.0	19.5	16.7	74.6	20.3	22.3
4b	49.5	14.7	−6.9	38.7	25.9	26.1	35.5	28.2	22.8
4d	29.2	17.7	16.7	39.6	29.1	26.4	33.3	10.5	9.4
4e	18.8	14.9	17.8	37.8	20.9	9.0	55.9	21.8	2.8
4f	17.0	9.3	5.6	40.7	18.9	4.6	28.2	20.5	6.0
4g	32.0	24.4	5.4	74.8	55.2	43.1	80.7	40.5	−3.0
4h	19.3	19.7	7.9	33.1	9.0	22.8	30.6	27.1	11.2
4i	43.1	8.5	3.4	64.8	39.9	17.7	63.4	57.3	33.2
4j	3.54	0.2	64.7	77.0	66.2	37.3	74.8	62.5	16.8
4k	−7.0	−4.5	−27.5	33.1	23.8	6.1	41.1	9.0	−4.6

Results compared with the blank control group.

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inhibition rate of 91.3%, 94.4%, 94.4% and 93.7% at 50 μg/mL, respectively. It is worth mentioning that compound **6mg** presented the highest inhibition against EC109 (IC_{50} = 10.82 μ‴ mol/L), HepG2 (IC_{50} = 10.44 μmol/L) and MGC-803 (IC_{50} = 10.59 μmol/L) cells.

Analogs **6ma-6mk** and **6na-6nk**, with different substituents on the phenyl ring, showed various degrees of inhibitory activity against EC109, HepG2 and MGC-803 cells. Compounds **6mb/6nb** and **6mc/6nc** with hydrophobic alkyl chains ($-CH_3$) exerted moderate inhibitory activity except for **6mb**. Analogs **6md/6nd** and **6me/6ne** with strong electron-withdrawing groups $-NO_2$ as *meta*-substitutions or *para*-substitutions showed favorable antibacterial activity, but only **6nd** demonstrated potent inhibition against HepG2 cells. Analogs **6mf/6nf** (3-Cl), **6mg/6ng** (4-Cl), **6mh/6nh** (3-Br) and **6mi/6ni** (4-Br) showed different inhibitory activity against EC109, HepG2 and MGC-803 cells. The antitumor inhibition of **6mf/6nf** (3-Cl) and **6mg/6ng** (4-Cl) with the substituents at *meta*- or *para*-position were better than **6mh/6nh** (3-Br) and **6mi/6ni** (4-Br), respectively. No activity improvement was exhibited by **6mj/6nj** (4-OCH_3) and **6mk/6nk** (2,4-dichloride) compared to **6ma**(H). The results indicate that different substituents at different positions of the benzene ring significantly affect the antitumor activity of these compounds and the introduction of the electron-withdrawing groups to the benzene ring led to an increase of the antitumor activity. As to compound **6mg**, it is possible that the electronic and steric effects of chlorine atoms were more complementary to the receptors. Further studies will focus on structural optimization and structure–activity relationships of these compounds.

4. Conclusion

In summary, two series of novel α-aminophosphonate derivatives containing thieno[2,3-d]pyrimidines were synthesized and their antitumor activity *in vitro* against EC109, HepG2 and MGC-803 cells has been evaluated. Biological assays revealed that the substitutions on the phenyl ring influenced the antitumor activity remarkably, while the substitutes of phosphonate had no apparent influence on the antitumor activity. Analogs **6mf/6nf** (3-Cl) **and 6mg/6ng** (4-Cl) with the substituents at *meta*- or *para*-position showed better inhibition against all of the tested tumor cells. Amongst them, compound **6mg** demonstrated excellent inhibitory effect against HepG2 (91.2%) and MGC-803(94.4%) cells. These results are useful for the design of more potent novel antitumor compounds and further study are ongoing.

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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.2015.03.026.

References

[1] Y. Kotaiah, N. Harikrishna, K. Nagaraju, C. Venkata Rao, Synthesis and antioxidant activity of 1,3,4-oxadiazole tagged thieno[2,3-d] pyrimidine derivatives, Eur. J. Med. Chem. 58 (2012) 340–345.
[2] H.A. Stefani, C.B. Oliveira, R.B. Almeida, et al., Dihydropyrimidin-(*2H*)-ones obtained by ultrasound irradiation: a new class of potential antioxidant agents, Eur. J. Med. Chem. 41 (2006) 513–518.
[3] H. Kikuchi, K. Yamamoto, S. Horoiwa, et al., Exploration of a new type of antimalarial compounds based on febrifugine, J. Med. Chem. 49 (2006) 4698–4706.
[4] B. Singh, A. Maheshwari, G. Dak, K. Sharma, G.L. Talesara, Studies of antimicrobial activities of some 4-thiazolidinone fused pyrimidines, [1,5]-benzodiazepines and their oxygen substituted hydroxylamine derivatives, Indian J. Pharm. Sci. 72 (2010) 607–612.
[5] S. Rostamizadeh, M. Nojavan, R. Aryan, H. Sadeghian, M. Davoodnejad, A novel and efficient synthesis of pyrazolo[3,4-d]pyrimidine derivatives and the study of their anti-bacterial activity, Chin. Chem. Lett. 24 (2013) 629–632.
[6] H.N. Hafez, H.A. Hussein, A.R. El-Gazzar, Synthesis of substituted thieno[2,3-d]pyrimidine-2,4-dithiones and their S-glycoside analogues as potential antiviral and antibacterial agents, Eur. J. Med. Chem. 45 (2010) 4026–4034.
[7] V. Alagarsamy, S. Meena, K.V. Ramseshu, et al., Synthesis, analgesic, anti-inflammatory, ulcerogenic index and antibacterial activities of novel 2-methylthio-3-substituted-5,6,7,8-tetrahydrobenzo (*b*) thieno[2,3-*d*]pyrimidin-4(3H)-ones, Eur. J. Med. Chem. 41 (2006) 1293–1300.
[8] M.D. Bhuiyan, K.M. Rahman, M.D. Hossain, et al., Synthesis and antimicrobial evaluation of some new thienopyrimidine derivatives, Acta Pharm. 56 (2006) 441–450.
[9] M.R. Prasad, J. Prashanth, K. Shilpa, D.P. Kishore, Synthesis and antibacterial activity of some novel triazolothieno-pyrimidines, Chem. Pharm. Bull. (Tokyo) 55 (2007) 557–560.
[10] H.M. Aly, N.M. Saleh, H.A. Elhady, Design and synthesis of some new thiophene, thienopyrimidine and thienothiadiazine derivatives of antipyrine as potential antimicrobial agents, Eur. J. Med. Chem. 46 (2011) 4566–4572.
[11] B.V. Ashalatha, B. Narayana, K.K. Vijaya Raj, N.S. Kumari, Synthesis of some new bioactive 3-amino-2-mercapto-5,6,7,8-tetrahydro[1]benzothieno[2,3-d]pyrimidin-4(3H)-one derivatives, Eur. J. Med. Chem. 42 (2007) 719–728.
[12] X.J. Song, P. Yang, H. Gao, et al., Facile synthesis and antitumor activity of novel 2-trifluoromethyl-thieno[2,3-d]pyrimidine derivatives, Chin. Chem. Lett. 25 (2014) 1006–1010.
[13] T. George, C.L. Kaul, R.S. Grewal, R. Tahilramani, Antihypertensive and monoamine oxidase inhibitory activity of some derivatives of 3-formyl-4-oxo-4H-pyrido[1,2-a]pyrimidine, J. Med. Chem. 14 (1971) 913–915.
[14] O. Bruno, C. Brullo, A. Ranise, et al., Synthesis and pharmacological evaluation of 2,5-cycloamino-5H-[1]benzopyrano[4,3-d]pyrimidines endowed with in vitro antiplatelet activity, Bioorg. Med. Chem. Lett. 11 (2001) 1397–1400.
[15] R.K. Russell, J.B. Press, R.A. Rampulla, et al., Thiophene systems. 9. Thienopyrimidinedione derivatives as potential antihypertensive agents, J. Med. Chem. 31 (1988) 1786–1793.
[16] H. Liu, H.Q. Wang, Z.J. Liu, Synthesis and herbicidal activity of novel pyrazolo[3,4-d]pyrimidin-4-one derivatives containing aryloxyphenoxypropionate moieties, Bioorg. Med. Chem. Lett. 17 (2007) 2203–2209.
[17] J.M. Wang, T. Asami, S. Yoshida, N. Murofushi, Biological evaluation of 5-substituted pyrimidine derivatives as inhibitors of brassinosteroid biosynthesis, Biosci. Biotechnol. Biochem. 65 (2001) 817–822.
[18] W.M. Abdou, R.F. Barghash, M.S. Bekheit, Carbodiimides in the synthesis of enamino- and α-aminophosphonates as peptidomimetics of analgesic/antiinflammatory and anticancer agents, J. Arch. Pharm. Chem. Life Sci. 345 (2012) 884–895.
[19] U.R.S. Arigala, C. Matcha, K.R. Yoon, $Zn(OAc)_2{\cdot}2H_2O$-catalyzed synthesis of α-aminophosphonates under neat reaction, Heteroat. Chem. 23 (2012) 160–165.
[20] M.V.N. Reddy, A. Balakrishna, M.A. Kumar, et al., One-Step synthesis and bioassay of N-phosphoramidophosphonates, Chem. Pharm. Bull. 57 (2009) 1391–1395.
[21] X.P. Rao, Z.Q. Song, L. He, Synthesis and antitumor activity of novel α-aminophosphonates from diterpenic dehydroabietylamine, Heteroat. Chem. 19 (2008) 512–516.
[22] S.R. Devineni, S. Doddaga, R. Donka, N.R. Chamarthi, $CeCl_3{\cdot}7H_2O$-SiO_2: catalyst promoted microwave assisted neat synthesis, antifungal and antioxidant activities of α-diaminophosphonates, Chin. Chem. Lett. 24 (2013) 759–763.
[23] M. Gnant, P. Clézardin, Direct and indirect anticancer activity of bisphosphonates: a brief review of published literature, Cancer Treat. Rev. 38 (2012) 407–415.
[24] B.A. Song, G.P. Zhang, S. Yang, D.Y. Hu, L.H. Jin, Synthesis of *N*-(4-bromo-2-trifluoromethylphenyl)-1-(2-fluorophenyl)-*O*,*O*-dialkyl-α-aminophosphonates under ultrasonic irradiation, Ultrason. Sonochem. 13 (2006) 139–142.
[25] L.H. Jin, B.A. Song, G.P. Zhang, et al., Synthesis, X-ray crystallographic analysis, and antitumor activity of N-(benzothiazole-2-yl)-1-(fluorophenyl)-O,O-dialkyl-a-aminophosphonates, Bioorg. Med. Chem. Lett. 16 (2006) 1537–1543.
[26] B. Kaboudin, N.J. As-habei, Microwave-assisted synthesis of α-aminophosphinic acids from hypophosphorus acid salts under solvent free conditions, Tetrahedron Lett. 44 (2003) 4243–4245.
[27] B.C. Ranu, A. Hajra, A simple and green procedure for the synthesis of α-aminophosphonate by a one-pot three-componentcondensation of carbonyl compound, amine and diethyl phosphite without solvent and catalyst, Green Chem. 4 (2002) 551–554.
[28] S. Liu, Studies on Synthesis and Properties of Thienopyrimidine and Pyridopyrimidine Derivatives, (Master thesis), Jiangxi Normal University, Nanchang, 2009.
[29] K. Clausen, S.O. Lawesson, Studies on organophosphorus compounds synthesis of ethyl-2-(thioacylamino)-5-ethyl-3-thiophenecarboxylates and 2-substituted 6-ethylthieno [2,3-d] thiazine-4-thiones, Nouv. J. Chem. 4 (1980) 43–46.
[30] R.V. Chambhare, B.G. Khadse, A.S. Bobde, et al., Synthesis and preliminary evaluation of some N-[5-(2-furanyl)-2-methyl-4-oxo-4H-thieno[2,3-d] pyrimidin-3-yl]-carboxamide and 3-substituted-5-(2-furanyl)-2-methyl-3H-thieno[2,3-d] pyrimidin-4-ones as antimicrobial agents, Eur. J. Med. Chem. 38 (2003) 89–100.
[31] G.M. Sheldrick, SHELXS-97, Program for the Solution of Crystal Structure, University of Göttingen, Germany, 1997.
[32] G.M. Sheldrick, SHELXL-97, Program for the Crystal Structure Solution and Refinement, University of Göttingen, Germany, 1997.

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