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Synthesis and biological activities of novel dialkyl (4-trifluoromethylphenylamino)-(4-trifluoromethyl or 3-fluorophenyl) methylphosphonates

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Abstract

A series of novel dialkyl (4-trifluoromethylphenylamino)-(4-trifluoromethyl or 3-fluorophenyl) methylphosphonates **3** were synthesized through the reaction of 3-fluorobenzaldehyde or 4-trifluoromethylbenzaldehyde and 4-trifluoromethylaniline with dialkyl phosphite by microwave irradiation using boron trifluoride-ether catalyst and their structures were clearly verified by spectroscopic data (IR, ^{1}H NMR and elemental analysis). The results of bioassay showed that these title compounds possess potential anticancer activities in vitro by MTT method. At the same time, we found these title compounds exhibit moderate antiviral activity against tobacco mosaic virus.

Keywords: Aminoalkylphosphonates; 4-Trifluoromethylphenyl; 3-Fluorophenyl; Synthesis; Anticancer activity; Anti-TMV activity; Microwave irradiation

1. Introduction

The importance of 1-aminoalkylphosphonic acids and their derivatives is commonly known [1]. Compounds of this type are widely used in agrochemistry as plant growth regulators [2], anti-fungal agents [3] and herbicides [4]. Some representatives find application in therapy and diagnostics medicine [5,6]. According to recent data, α-aminoalkylphosphonate derivatives are quite perspective for the design of therapeutic agents, including antitumor drugs [7–9]. In order to find new plant virucides, we had designed and synthesized a series of 1-(4-trifluoromethylphenyl)-1-aminoalkylphosphonate, some of which displayed good anti-TMV activity [10–13]. Fluorinated compounds in general and fluorinated heterocycles in particular are the focus of much interest in modern medicinal chemistry. A variety of pharmacological agents bearing a trifluoromethyl group are of special interest as they exhibit broad biological properties [14,15]. The trifluoromethyl-substituted compounds have been reported to possess biological activities as herbicides [16], fungicides [17], analgesic agents [18], antipyretic agents [19] and inhibitors for platelet aggregation [20]. Recently, much attention has been focused on their antiviral and antitumor agents after the discovery of the trifluoromethyl-substituted pyrazole C-glycoside pyrazofurin [21,22]. Considering the wide application of these compounds and potential to serve as antiviral and anticancer agents, we decided to reserve of bioactivity unit and replace of phenyl group by 4-trifluoromethylphenyl or 3-fluorophenyl in dialkyl (phenylamino)-(fluorophenyl) methylphosphonate. Thus, we designed a series of novel dialkyl (4-trifluoromethylphenylamino)-(4-trifluoromethyl or 3-fluorophenyl) methylphosphonates.

A typical method for the synthesis of substituted α-aminoalkylphosphonate is the three-component reaction of aldehydes, amine and dialkyl phosphite by one-pot Mannich type method [23]. However, the reported method involved high reaction temperature, poisonous solvent and expensive reagents, long reaction time, low yields and complex handling. To avoid these disadvantages, a couple of modifications including using montmorillonite clay [24] and alumina [25] in dry media under microwave irradiation have been reported recently. Using montmorillonite clay and alumina catalyst for the reported method [24,25], the reaction of fluorobenzaldehydes and

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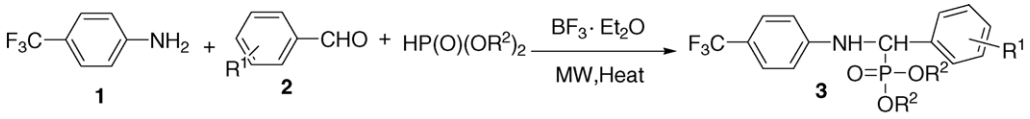

R^1=4-CF_3, 3-F; R^2=Me, Et, i-Pr, n-Pr, n-Bu

Scheme 1.

4-trifluoromethylaniline with dialkyl phosphite by microwave irradiation can be unpredictable. The title compound was thus obtained in low yield. As a part of our green technology program, we would also like to disclose here a more practical green alternative for a new method to synthesize α-aminoalkylphosphonate by a three-component condensation of aldehydes, amine and dialkyl phosphite at 75–80 °C under microwave irradiation using $BF_3{\cdot}Et_2O$ catalyst without any solvent. The synthetic route is shown in Scheme 1. The advantages of the procedure are short reaction period, high yield and simple working up.

The structures of **3** were firmly established by well-defined IR, ^{1}H NMR, ^{13}C NMR, ^{19}F NMR and elemental analysis. The anticancer activity is assayed by the MTT method for all products. Some of the compounds displayed good anticancer activity for two cells (PC3 and A431) in vitro.

2. Results and discussion

2.1. Chemistry

In order to optimize the reaction conditions, the synthesis of **3e** was carried out under several conditions. Firstly, the reaction technique should be used for microwave irradiation method. In the normal reaction conditions without microwave irradiation, this reaction was much slow and the yield of the product **3e** was decreased, for example, when the time was prolonged to 14 h compound **3e** could be obtained in the yield of 87.8%, as compared with the yield of 92.1% in 20 min under microwave irradiation (Table 3, entry 5). In addition, we also examined the effects of reaction temperature and reaction time on the Mannich-addition reactions (Table 1, entries 1–3 and 9–14). When the reaction time was prolonged from 5 to 30 min, the yield of **3e** was increased from 83.1 to 93.5% (Table 1, entries 1–4). When the reaction time was prolonged further to 30 min under microwave irradiation, tiny improvement of yield (93.5%, Table 1, entry 4) was obtained compared to that of 20 min (92.1%, Table 1, entry 3). As for the reaction temperature, it could be seen that the yield was relatively lower when the reaction was taken out at room temperature (Table 1, entry 9) than that at 80 °C (Table 1, entry 3). No substantial improvement was observed when the reaction system was heated to 90 °C (Table 1, entry 14). Hence, it is better for the reaction to be proceeded at 80 °C than to be placed in lower or higher temperature. As for the reaction power of microwave irradiation, it could be seen that when the amount of reaction power was increased from 500 to 600, 750, 800 and 900 W, the yield of **3e** was 56.1, 63.3, 92.1, 91.3 and 87.8%, respectively (Table 1, entries 3 and 5–8). Hence, it is better for the reaction to be proceeded in 750 W than to be placed in lower power. No substantial improvement was observed when the reaction power varied from 800 to 900 W under microwave irradiation (Table 1, entries 7 and 8).

In our experiment, we also screened $BF_3{\cdot}Et_2O$, $Sc(OT_f)_3$ and TsOH catalysts. The results demonstrated that the presence of $BF_3{\cdot}Et_2O$ can accelerate the addition reaction. Moreover, when TsOH and $Sc(OT_f)_3$ instead of $BF_3{\cdot}Et_2O$ were used as catalyst in the present reaction, no remarkable improvement of the yield of product was observed. For instance, when the catalyst was $Sc(OT_f)_3$, **3e** was obtained in the yield of 75.1% within 20 min (Table 2, entry 3), while for TsOH, the yield of **3e** was 79.7% (Table 2, entry 1). When $BF_3{\cdot}Et_2O$ was used as catalyst and the reaction was induced by microwave irradiation, the great improvement in the yield, approximately 20%, was realized (Table 2, entry 2 compared to entry 8). When the reaction was catalyzed by $BF_3{\cdot}Et_2O$ alone without microwave, the yield was only 38.0% (Table 2, entry 9), approximately 56% lower compared to that with microwave irradiation (Table 2, entry 2). Further, the effects of the amount of catalyst on the reaction were also examined (Table 2, entries 2, 4–7). It could be seen from Table 2 that when the amount of catalyst was decreased from 10 to 8, 6, 4 and 2%, the yield of **3e** was 94.1, 93.7, 93.2, 92.2 and 80.1%, respectively (entries 2 and 4–7). The product was obtained when 4% catalyst's amount was used (entry 6), indicated that there is a best catalyst quantity for this reaction.

Table 1
Different conditions used for the microwave-assisted synthesis of **3e**

Entry	Reaction time (min)	Power (W)	Reaction temperature (°C)	Yield[a] (%)
1	5	750	80	83.1
2	10	750	80	85.5
3	20	750	80	92.1
4	30	750	80	93.5
5	20	500	80	56.1
6	20	600	80	63.3
7	20	800	80	91.3
8	20	900	80	87.8
9	20	750	25	33.1
10	20	750	40	50.1
11	20	750	50	61.1
12	20	750	60	69.9
13	20	750	70	80.5
14	20	750	90	82.2

Reaction condition: Stirred under microwave irradiation by 4% $BF_3{\cdot}Et_2O$ in a Model XH-100C microwave synthesis instrument at 750–900 W.
[a] Yields of isolated products.

Table 2
Effect of the different catalyst and the amount of catalyst on the synthesis of **3e**[a]

Entry	Catalyst	Amount of the catalyst (mol%)	Yield[b] (%)
1	TsOH	10	79.7
2	$BF_3{\cdot}Et_2O$	10	94.1
3	$Sc(T_fO)_3$	10	75.1
4	$BF_3{\cdot}Et_2O$	8	93.7
5	$BF_3{\cdot}Et_2O$	6	93.2
6	$BF_3{\cdot}Et_2O$	4	92.2
7	$BF_3{\cdot}Et_2O$	2	80.1
8	$BF_3{\cdot}Et_2O$	0	74.4
9[c]	$BF_3{\cdot}Et_2O$	10	38.0

[a] All reactions were carried out at 78–80 °C for 20 min under microwave irradiation at 750 W.
[b] Isolated yields.
[c] The reactions were carried out at 78–80 °C for 20 min without microwave irradiation.

As shown in Table 3, while the substituted fluorine atom at 3-position of benzaldehyde, it was easily reaction with 4-trifluoromethylaniline and dialkyl phosphite in the presence of $BF_3{\cdot}Et_2O$ with microwave irradiation, afford the desired α-aminoalkylphosphonates product in excellent yields. In same class of compounds, the yield of products **3a–3e** is obvious dependent on the alkyl substituent on the phosphite. Selected the aldehyde with R^1 = 3-F was then subjected to addition at 78–80 °C to give **3e** and **3b** in high yield. The yields of the title compounds decrease with the order of **3e** > **3b** > **3a** > **3c** > **3d**.

The related analogues **3f–3i** for the same R^1 = 4-CF_3, it was directly reacted with dialkyl phosphite by one-pot method. Unfortunately, our trials on typical method, it was unsuccessful and low yields of product **3f–3i** could be isolated. Our research has shown that a convenient method for preparation of this class of title compounds **3f–3i**. In our experiment, we also found the reactivity of Mannich-addition for the dialkyl phosphite attached to 4-trifluoromethylaniline and 4-trifluoromethylbenzaldehyde were reduced by carbon number of dialkyl phosphite, such as dimethyl phosphite, diethyl phosphite, di-*n*-propyl, phosphite, di-isopropyl phosphite were subjected to the same reaction conditions. The yields of the title products decrease with the order **3g** > **3h** > **3i** > **3f**. It was probably explained by the steric effects that 4-trifluoromethylaniline and 4-trifluoromethylbenzaldehyde was treated with phosphite, bulky substituents of alkyl group of phosphite caused its reaction nucleophilicity to be reduced. Besides this, hot stable may also play an important role, for example, dimethyl phosphite is very easily active and unstable at high temperature and long reaction time. In conclusion, we have explored the addition reaction of dimethyl phosphite with 4-trifluoromethylaniline and 4-trifluoromethylbenzaldehyde to obtain dimethyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl) methylphosphonate in moderate yield. Under microwave irradiation using $BF_3{\cdot}Et_2O$ catalyst condition, the great improvement in the yield was achieved to afford **3** in 65.8–92.1% yield within a shorter reaction time (20 min) than those conducted with $BF_3{\cdot}Et_2O$ as catalyst without microwave irradiation (without $BF_3{\cdot}Et_2O$ as catalyst). The best result was obtained when 4-trifluoromethylaniline was reacted with 1 equiv. of dialkyl phosphite and 0.04 equiv. $BF_3{\cdot}Et_2O$ and 1 equiv. of 4-trifluorobenzaldehye or 3-fluorobenzaldehyde in solvent-free at 78–80 °C for 20 min. Under this reaction condition, Mannich-addition reaction proceeded smoothly.

Table 3
Yields[a] and reaction conditions used for the microwave-assisted synthesis of **3a–3i**

Entry	Product	Microwave method[b]		Classical method[c]	
		Reaction time (min)	Yield (%)	Reaction time (h)	Yield (%)
1	**3a**	20	89.0	14	78.3
2	**3b**	20	90.9	14	79.0
3	**3c**	20	86.2	14	68.9
4	**3d**	20	83.1	14	67.1
5	**3e**	20	92.1	14	87.8
6	**3f**	20	65.8	14	50.1
7	**3g**	20	80.0	14	66.1
8	**3h**	20	75.9	14	61.8
9	**3i**	20	71.0	14	56.8

[a] Yields of isolated products.
[b] *Reaction condition*: 4% $BF_3{\cdot}Et_2O$, 78–80 °C, under stirring under microwave irradiation in a Model XH-100C microwave synthesis instrument at 750 W.
[c] *Reaction condition*: First reaction step—a mixture of 4-trifluoromethylaniline (5 mmol) and 4-trifluoromethylbenzaldehyde (5 mmol) and TsOH (0.2 mmol) in toluene (15 mL) was refluxed for 7 h. Second reaction step—a mixture of *N*-(4-trifluoromethylphenyl)-2-(4-trifluoromethylphenyl)imine (5 mmol), toluene (20 mL) and dialkyl phosphite (5 mmol) and 4-dimethylaminopyridine (DMAP, 0.15 mmol) was heated at 110–115 °C for 7 h.

The structures of all of the products were confirmed by ^1H NMR, ^{13}C NMR, elemental analysis, IR and mass spectroscopy. The IR spectra of products **3** exhibited bands at about 3275.1–3358.0 s cm^{-1}, indicating the presence of NH. The signals at 1609.2–1618.2 cm^{-1} were assigned to C=O vibrations. While the absorption at 1240.2–1286.7 s cm^{-1} was assigned to be P=O stretching absorption bands, 1062.7–1086.7 s cm^{-1} to the C–O stretching absorption bands in the P–O–C group. In ^1H NMR spectra, all phenyl proton showed multiple at 6.577–7.642 ppm. The chemical shift, of PCH of ester was about 4.512–5.122 ppm, respectively, the H atom at the α-C exhibited a single or doublet or dd peak due to the coupling of the P atom. The products showed the NH proton at 5.206 and 5.356 ppm as a broad singlet or triplet due to the existence of hydrogen bond between P=O of phosphonate and NH of trifluoromethylphenylamino group in the molecule structure of **3** which leads to its chemical shift value of NH move to lower field. All of the carbon atoms in the compounds have been identified and the total number of protons calculated from the integration curve tallies with what has been expected from the molecular formula. The MS spectra revealed that the molecular ion peaks and fragmentation peaks were in accordance with the given structures of product **3**.

2.2. Biological activity

The antitumor activity is assayed by the MTT method [26,27]. It is listed in Table 4. The results showed that these compounds exhibit certain activities against the two cancer

Table 4
Inhibition ratio (%) (10 μM) of **3a–3i**

Compound	PC3 cells	A431 cells
3a	39.6	33.7
3b	46.4	39.9
3c	42.1	47.2
3d	41.2	32.0
3e	33.2	31.2
3f	49.7	48.2
3g	70.1	65.2
3h	59.2	58.1
3i	55.2	50.2

Inhibition ratio (%) = $(A_1 - A_2)/A_1 \times 100\%$. A_1: the mean optical densities of untreated cells; A_2: the mean optical densities of drug-treated cells.

cells in vitro. The compounds **3f–3i** have relatively higher antitumor activity than **3a–3e**. The antitumor data given in Table 4 indicate that the nature of fluorine and alkyl affects antitumor activity of the compounds. For example, the inhibition rate of compound **3g** with R^1 being 4-CF_3 group, R^2 being Et to PC3 attains 70.1% and the inhibition rate of **3g** to A431 attains 65.2% at 10 μM.

The preliminary biological tests showed that antiviral activities of the products are low or moderate. For example, the extent of inhibition of compound **3g** against TMV was 34.2% at a concentration of 500 mg/L.

3. Conclusion

A series of novel dialkyl (4-trifluoromethylphenylamino)-(4-trifluoromethyl or 3-fluorophenyl) methylphosphonate were synthesized by microwave irradiation in one step. This method is an easy, rapid, one-pot and good-yielding reaction for the synthesis of α-aminophosphonates. Their structures were verified by spectroscopic method. The results of bioassay showed that these title compounds exhibit certain activity against PC3 and A431 cancer cells in vitro and anti-TMV activities. Diethyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl) methylphosphonate **3g** has better biological activity than its structurally related analogues **3a–3f**, **3h** and **3i**.

4. Experimental

4.1. Instruments

The melting points of the products were determined on an XT-4 binocular microscope (Beijing Tech. Instrument Co., China) and are not corrected. The IR spectra were recorded on a Bruker VECTOR22 spectrometer in KBr disks. ^{1}H NMR (solvent $CDCl_3$) and ^{13}C NMR (solvent $CDCl_3$) spectra were performed on a Varian-Inova 400 MHz spectrometer at room temperature using TMS as internal standard. D_2O exchange was applied to confirm the assignment of the signals of NH protons. ^{19}F NMR spectra were obtained on a Varian EM-360A Spectrometer using CF_3COOH (TFA) as an external standard, positive for downfield shift. The mass spectra were taken on an HP5988A spectrometer. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. Microwave reaction was performed on a Beijing XH-100A microwave catalysis synthesis and extracts appearance (with a power of 750–900 W). Analytical TLC and column chromatography were performed on silica gel GF_{254}. Column chromatographic purification was carried out using silica gel. The reagents were all analytical reagent-grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use.

4.2. General procedure for the preparation of products *3a–3e*

A mixture of 4-trifluoromethylaniline (5 mmol), 3-fluorobenzaldehyde (5 mmol) and dialkyl phosphite [28] (5 mmol) and $BF_3{\cdot}Et_2O$ (0.2 mmol) was irradiated in the microwave catalysis synthesis and extracts appearance at 78–80 °C for 20 min. The reaction was followed and monitored by TLC (petroleum ether + ethyl acetate = 2 + 1 by volume). After the reaction was completed (1 h), the residue washed with water, filtered, dried, then the crude solid was purified by column chromatograph on silica gel using petroleum ether/ethyl acetate (v/v, 2:1) as eluent to give **3a–3e**.

4.2.1. Di-i-propyl (4-trifluoromethylphenylamino)-(3-fluorophenyl)-methylphosphonate *(3a)*

Colorless crystals; yield 89.0%, mp 113–114 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.602–7.351 (m, 8H, Ar–H), 5.338 (b, 1H, NH), 4.701–4.767 (m, 1H, OCH), 4.666 (s, 1H, CH–P), 4.471–4.550 (m, 1H, OCH), 0.967–1.342 (m, 12H, 4× CH_3); ^{13}C NMR: δ 164.43, 162.00, 149.31, 149.18, 138.77, 138.67, 130.42, 130.39, 130.33, 126.86, 126.33, 123.91, 123.88, 123.85, 123.64, 120.49, 120.16, 115.42, 115.39, 115.26, 115.21, 115.03, 114.98, 113.23, 72.86, 72.79, 72.65, 72.59, 56.80, 55.29, 24.44, 24.41, 24.06, 24.01, 23.52, 23.46, 14.46; ^{19}F NMR ($CDCl_3$, TFA): δ −62.12, −121.01; IR (KBr) (cm^{-1}): 3275.1 (NH), 1616.3 (C=O), 1282.6 (P=O), 1062.7 (P–O–C). Anal. calc. for $C_{20}H_{24}F_4NPO_3$: C 55.43; H 5.58, N 3.23. Found: C 55.40, H 5.45, N 3.20.

4.2.2. Di-n-propyl (4-trifluoromethylphenylamino)-(3-fluorophenyl)-methylphosphonate *(3b)*

Colorless crystals; yield 90.9%, mp 108–109 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.632–7.493 (m, 8H, Ar–H), 5.352 (b, 1H, NH), 4.800 (d, 1H, J = 23.1 Hz, CH–P), 4.421–4.765 (m, 4H, 2× OCH_2), 1.521–1.653 (m, 4H, 2× CH_2), 0.862–1.451 (m, 6H, 2× CH_3); ^{19}F NMR ($CDCl_3$, TFA): δ −62.09, −120.21; MS: 433 (M^+), 268, 248, 172, 145, 123, 109, 95, 83, 82, 81; IR (KBr) (cm^{-1}): 3306.2 (NH), 1616.3 (C=O), 1282.6 (P=O), 1062.7 (P–O–C). Anal. calc. for $C_{20}H_{24}F_4NPO_3$: C 55.43, H 5.58, N 3.23. Found: C 55.40, H 5.45, N 3.20.

4.2.3. Dimethyl (4-trifluoromethylphenylamino)-(3-fluorophenyl)-methylphosphonate *(3c)*

Colorless crystals; yield 86.2%, mp 105–107 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.637–7.520 (m, 8H, Ar–H); 5.341–5.361 (d, 1H, J = 8.0 Hz, NH), 5.082 (dd, 1H, J = 24.8, 7.6 Hz, CH–P), 3.426–3.819 (m, 6H, 2× OCH_3); ^{19}F NMR ($CDCl_3$, TFA): δ

−62.12, −119.11; IR (KBr) (cm^{-1}): 3286.7 (NH), 1618.2 (C=O), 1286.5 (P=O), 1062.7 (P–O–C). Anal. calc. for $C_{16}H_{16}F_4NPO_3$: C 50.94, H 4.27, N 3.71. Found: C 50.90, H 4.31, N 3.62.

4.2.4. Di-n-butyl (4-trifluoromethylphenylamino)-(3-fluorophenyl)-methylphosphonate (3d)

Colorless crystals; yield 83.1%, mp 100–101 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.531–7.340 (m, 8H, Ar–H), 5.311–5.321 (d, 1H, J = 8.0 Hz, NH), 5.121 (dd, 1H, J = 25.0, 7.9 Hz, CH–P), 4.336–4.621 (m, 4H, 2× OCH_2), 3.632–3.771 (m, 4H, 2× CH_2), 1.451–1.660 (m, 4H, 2× CH_2Me), 1.009–1.421 (m, 6H, 2× CH_3); ^{19}F NMR ($CDCl_3$, TFA): δ −61.90, −118.90; IR (KBr) (cm^{-1}): 3281.0 (NH), 1609.2 (C=O), 1281.5 (P=O), 1086.7 (P–O–C). Anal. calc. for $C_{22}H_{28}F_4NPO_3$: C 57.27, H 6.11, N 3.04. Found: C 57.27, H 6.12, N 3.04.

4.2.5. Diethyl (4-trifluoromethylphenylamino)-(3-fluorophenyl)-methylphosphonate (3e)

Colorless crystals; yield 92.1%, mp 79–81 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.607–7.341 (m, 8H, Ar–H), 5.339 (b, 1H, NH), 4.516 (s, 1H, CH–P), 4.471–4.550 (m, 4H, 2× OCH_2), 0.967–1.342 (m, 6H, 2× CH_3); MS: 405 (M^+), 318, 268, 172, 145, 109, 95, 81; ^{19}F NMR ($CDCl_3$, TFA): δ −62.20, −119.00; IR (KBr) (cm^{-1}): 3358.0 (NH), 1618.2 (C=O), 1270.5 (P=O), 1073.7 (P–O–C). Anal. calc. for $C_{18}H_{20}F_4NPO_3$: C 53.34, H 4.97, N 3.45. Found: C 53.27, H 4.99, N 3.34.

4.3. General method for the preparation of dialkyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl)-methylphosphonate (3f–3i)

A mixture of 4-trifluoromethylaniline (5 mmol), 4-trifluoromethylbenzaldehyde (5 mmol) and dialkyl phosphite [28] (5 mmol) and $BF_3{\cdot}Et_2O$ (0.2 mmol) was irradiated in the microwave catalysis synthesis and extracts appearance at 78–80 °C for 20 min. The reaction was followed and monitored by TLC (petroleum ether + ethyl acetate = 5 + 1 by volume). After the reaction was completed, the residue washed with water, filtered, dried, then the crude solid was purified by recrystallized from ethanol three times to give **3f–3i** as a colorless solid.

4.3.1. Dimethyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl)-methylphosphonate (3f)

Colorless crystals; yield 65.8%, mp 121–123 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.580–7.642 (m, 8H, Ar–H), 5.206 (t, 1H, J = 7.8 Hz, NH), 4.964 (dd, 1H, J = 24.8, 7.2 Hz, CH–P), 3.807 (d, 3H, J = 10.8 Hz, CH_3), 3.548 (d, 3H, J = 10.8 Hz, CH_3); ^{13}C NMR: δ 148.34, 148.20, 139.22, 128.01, 127.96, 126.69, 126.66, 125.84, 120.79, 113.03, 55.75, 54.26, 54.05, 53.98; ^{19}F NMR ($CDCl_3$, TMS): δ −118.90, −120.09; IR (KBr) (cm^{-1}): 3292.4 (NH), 1614.4 (C=O), 1240.2 (P=O), 1064.7 (P–O–C); MS: 427 (M^+), 318, 298, 248, 172, 145, 127, 109, 95, 81, 79, 63, 45, 31, 18. Anal. calc. for $C_{17}H_{16}F_6NPO_3$: C 47.78, H 3.77, N 3.27. Found: C 47.69, H 3.60, N 3.12.

4.3.2. Diethyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl)-methylphosphonate (3g)

Colorless crystals; yield 80.0%, mp 118–119 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.577–7.630 (m, 8H, Ar–H), 5.222 (b, 1H, NH), 4.864 (d, 1H, J = 24.8 Hz, CH–P), 3.755–4.176 (m, 4H, 2× OCH_2), 1.288–1.324 (m, 3H, CH_3), 1.041–1.176 (m, 3H, CH_3); ^{13}C NMR: δ 148.46, 128.04, 127.98, 126.66, 126.62, 125.69, 125.65, 112.39, 63.66, 63.57, 63.59, 56.15, 54.66, 16.42, 16.36, 16.19 and 16.13; ^{19}F NMR ($CDCl_3$, TFA): δ −118.30, −120.11; IR (KBr) (cm^{-1}): 3288.6 (NH), 1614.4 (C=O), 1280.7 (P=O), 1064.7 (P–O–C); MS: 455 (M^+), 318, 298, 248, 198, 172, 145, 127, 109, 95, 81, 65, 43, 29. Anal. calc. for $C_{19}H_{20}F_6NPO_3$: C 50.12, H 4.42, N 3.07. Found: C 50.31, H 4.30, N 2.99.

4.3.3. Di-n-propyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl)-methylphosphonate (3h)

Colorless crystals; yield 75.9%, mp 90–92 °C; ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.583–7.627 (m, 8H, Ar–H), 5.346 (t, 1H, J = 7.9 Hz, NH), 4.882 (d, 1H, J = 24.8 Hz, CH–P), 3.647–4.074 (m, 4H, 2× OCH_2), 1.474–1.713 (m, 4H, 2× CH_2), 0.790–0.937 (m, 6H, 2× CH_3); ^{13}C NMR δ 198.62, 148.47, 139.70, 128.06, 128.01, 126.65, 126.61, 125.94, 125.66, 125.25, 113.00, 69.07, 69.00, 68.95, 68.88, 56.16, 54.67, 23.86, 23.81, 23.68, 23.62, 9.91, 9.79; ^{19}F NMR ($CDCl_3$, TFA): δ −119.00, −121.22; IR (KBr) (cm^{-1}): 3277.0 (NH), 1616.3 (C=O), 1282.6 (P=O), 1066.6 (P–O–C); MS: 483 (M^+), 318, 298, 248, 172, 145, 133, 123, 107, 95, 83, 65, 43, 27. Anal. calc. for $C_{21}H_{24}F_6NPO_3$: C 52.18, H 5.00, N 2.89. Found: C 52.29, H 4.91, N 2.92.

4.3.4. Di-i-propyl (4-trifluoromethylphenylamino)-(4-trifluoromethylphenyl)-methylphosphonate (3i)

Colorless crystals; yield 71.0%, mp 126–128 °C. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 6.579–7.616 (m, 8H, Ar–H), 5.310 (b, 1H, NH), 4.794 (d, 1H, J = 23.5 Hz, CH–P), 4.495–4.794 (m, 2H, 2× OCH), 0.968–1.346 (m, 12H, 4× CH_3), ^{13}C NMR: δ 148.80, 139.96, 128.17, 128.12, 126.62, 126.59, 125.50, 120.42, 112.92, 72.61, 72.53, 72.46, 56.64, 55.13, 24.12, 24.08, 24.05, 23.74, 23.69, 23.23 and 23.1; ^{19}F NMR ($CDCl_3$, TMS): δ −118.99, −120.12; IR (KBr) (cm^{-1}): 3286.7 (NH), 1618.2 (C=O), 1286.5 (P=O), 1062.7 (P–O–C); MS: 483 (M^+), 318, 248, 172, 159, 145, 124, 109, 95, 82, 69, 57 and 43. Anal. calc. for $C_{21}H_{24}F_6NPO_3$: C 52.18, H 5.00, N 2.89. Found: C 52.29, H 4.91, N 2.92.

4.4. MTT assay against cell proliferation

All compounds tested were dissolved in DMSO (1–100 μM solutions) and subsequently diluted in the culture medium before treatment of the cultured cells. Tested cells were plated in 96-well plates at a density of 3×10^3 cells/well/100 μL of the proper culture medium and treated with the compounds at concentration of 1–100 μM for 48 h. In parallel, the cells were treated with 0.1% of DMSO as control. A MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay (Roche Molecular Biochemicals) was performed according to the instructions

provided by Roche. This assay is based on the cellular cleavage of the tetrazolium salt, MTT, into a formazan that is insoluble in the cell culture medium and is measured at 550 nm directly in 96-well assay plates. Absorbance is directly proportional to the number of living cells in culture. Two types of cells were used in these studies, PC3 (prostate cancer) and A431 (uterus cancer) cell lines (provided by Cell Bank of Committee on Type Culture Collection of Chinese Academy of Science) were cultivated in F-12 medium (for PC3) or RPMI 1640 medium (for A431) supplemented with 10% fetal bovine serum (provided by TBD & HY Bio. Co.) and 2 mM of L-glutamine. Tissue culture reagents were obtained from Gibco Co.

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Reference

[1] R.A. Cherkasov, V.I. Galkin, Usp. Khim. 67 (1998) 940–968.
[2] L. Maier, P. Diel, J. Phosphorus Sulfur Silicon 90 (1994) 259–279.
[3] L. Maier, P.J. Diel, Phosphorus Sulfur Silicon 57 (1991) 57–64.
[4] R.Y. Chen, Q. Dai, Sci. China Ser. B 25 (1995) 591–595.
[5] R. Engel, Chem. Rev. 77 (1977) 349–367.
[6] S.W.A. Bligh, N. Choi, D.S.C. Green, Polyhedron 12 (1993) 2887–2890.
[7] (a) A.A. Cordi, P. Desos, Eur. Patent Appl. EP 754, 693, 1997.
(b) A.A. Cordi, P. Desos, Chem. Abstr. 126 (1997) (157635d).
[8] R.Y. Chen, L. Mao, J. Phosphorus Sulfur Silicon 89 (1994) 97–104.
[9] L. Tusek-Bozic, M. Curic, J. Balzarini, E. De Clerq, Nucleosides Nucleotides 14 (1995) 777–781.
[10] B.A. Song, M.G. Jiang, Y.L. Wu, X.Q. He, S. Yang, L.H. Jin, G. Liu, D.Y. Hu, Chin. J. Org. Chem. 9 (2003) 967–972.
[11] B.A. Song, Y.L. Wu, S. Yang, D.Y. Hu, X.Q. He, L.H. Jin, P. Lu, Molecules 1 (2003) 186–189.
[12] S. Yang, B.A. Song, Y.L. Wu, L.H. Jin, G. Liu, D.Y. Hu, P. Lu, Chin. J. Org. Chem. 10 (2004) 1292–1295.
[13] S. Yang, Ph.D. Disseration, Guizhou University, Guiyang, 2004.
[14] L.F. Lee, F.M. Schleppnik, R.W. Schneider, D.H. Campbell, J. Heterocycl. Chem. 27 (1990) 243–245.
[15] P. Bravo, D. Dillido, G. Resnati, Tetrahedron 50 (1994) 8827–8836.
[16] J.C. Jung, E.B. Watkins, M.A. Avery, Tetrahedron 58 (2002) 3639–3646.
[17] K.L. Kees, J.J. Fitzgerald, K.E. Steimer, J.F. Mattes, B. Mihan, T. Tosi, D. Mondoro, M.L. McCaleb, J. Med. Chem. 39 (1996) 3920–3928.
[18] T.P. Gregory, C.D. Darlene, M.S. David, Pestic. Biochem. Phys. 60 (1998) 177–185.
[19] S.G. Kucukguzel, S. Rollas, H. Erdeniz, A.C. Kiraz, M. Ekinci, A. Vidin, Eur. J. Med. Chem. 35 (2000) 761–771.
[20] A.E. Mutalib, S.Y. Chen, R.J. Espina, J. Shockcor, S.R. Prakash, L.S. Gan, Chem. Res. Toxicol. 15 (2002) 63–75.
[21] G. Daidone, B. Maggio, S. Plescia, D. Raffa, C. Musiu, C. Milia, G. Perra, M.E. Marongiu, Eur. J. Med. Chem. 33 (1998) 375–382.
[22] S.A. Gamage, J.A. Spicer, G.W. Rewcastle, J. Milton, S. Sohal, W. Dangerfield, P. Mistry, N. Vicker, P.A. Charlton, W.A. Denny, J. Med. Chem. 45 (2002) 740–743.
[23] Q.M. Wang, Z.G. Li, R.Q. Hung, J.R. Cheng, Heteroatom. Chem. 12 (*2*) (2001) 68–72.
[24] I.S. Yadav, B.V.S. Reddy, C. Madan, Synletters (2001) 1131–1133.
[25] B. Kaboudin, R. Nazari, Tetrahedron Lett. 42 (2001) 8211–8213.
[26] F. Denizot, R. Lang, J. Immunol. Methods 89 (1986) 271–277.
[27] P. Skehan, R. Storeng, D. Scudiero, A. Monks, J. McMahon, D. Vistica, J.T. Warren, H. Bokesch, S. Kenney, Natl. J. Cancer Inst. 82 (1990) 1107–1112.
[28] H. Mccombie, B.C. Sauders, G.J. Stacey, J. Chem. Soc. (1945) 380–382.