Available online at www.sciencedirect.com
SCIENCE DIRECT®
Journal of Fluorine Chemistry 126 (2005) 87–92
JOURNAL OF FLUORINE CHEMISTRY
www.elsevier.com/locate/fluor

Synthesis and bioactivity of 2-cyanoacrylates containing a trifluoromethylphenyl moiety

Baoan Song*, Song Yang, Huimin Zhong, Linhong Jin, Deyu Hu, Gang Liu

Key Laboratory of Green Pesticide and Agricultural Bioengineering, Ministry of Education, Center for Research and Development of Fine Chemicals, Guizhou University, Huaxi District, Guiyang 550025, PR China

Received 1 September 2004; received in revised form 19 October 2004; accepted 23 October 2004
Available online 15 December 2004

Abstract

A series of 2-cyanoacrylates containing a trifluoromethylphenyl moiety were synthesized in three steps: ethyl 2-cyano-3,3-dimethylthioacrylate was prepared from ethyl cyanoacetate with carbon disulfide and dimethyl sulfate, then its reaction with 4-trifluoromethylaniline yielded ethyl 2-cyano-3-(4-trifluoromethylphenylamino)-3-methylthioacrylate, and then reacted with an alkylamine. The new structures were verified by elemental analysis, IR, ^{1}H NMR and mass spectra. In the MTT test, these new compounds were found to possess high antitumor activities against PC3 and A431 cells.

Keywords: Trifluoromethylphenylcyanoacrylate; Synthesis; Antitumor bioactivity

1. Introduction

2-Cyanoacrylates are of considerable importance because of their versatile biological activity and the possibility for application in agrochemistry, e.g. herbicides that disrupt photosynthetic electron transportation at a common binding domain on the 32 kDa polypeptide of the photosystem II (PSII) reaction center [1–2]. Among these cyanoacrylates, ethoxyethyl (*Z*)-3-(4-chlorophenylmethylamino)-2-cyano-3-isopropyl-acrylate (CPNPE) exhibits the highest Hill inhibitory activity yet reported [3–7].

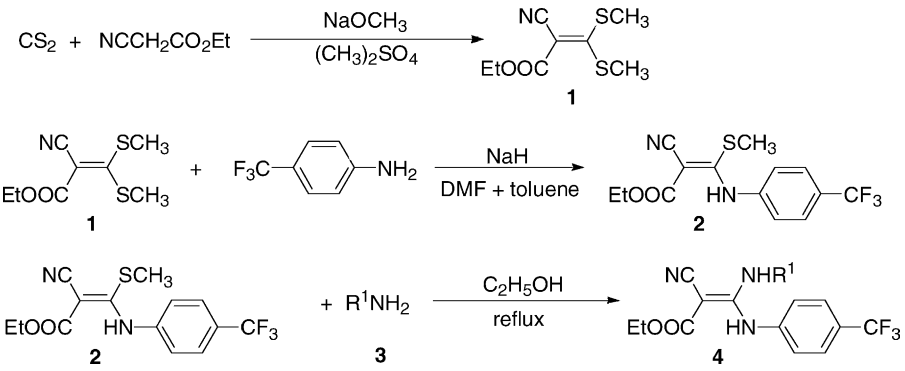
On the other hand, fluorinated compounds, in general, are the focus of much interest in modern medicinal chemistry and are ideal for use in drug design because of the good biological activity and low toxicity of molecules containing the trifluoromethyl moiety plus the ease of substitution of a conventional phenyl or heteroaromatic group with trifluoromethyl. Moreover, trifluoromethyl-containing compounds often possess unexpected biological activity [8–9]. Indeed, the substitution of a main group by a trifluoromethyl group in a bioactive compound might be expected to induce great changes in molecular properties, such as the hydrophobicity, solubility, special biomimetic effect and electronegativity [10]. Hence, in a search for new anticancer drug, we thought that the replacement of the methylthio moiety by 4-trifluoromethylphenyl-amino group in some 2-cyano-3,3-dimethylthioacrylate may change the bioactivity. Therefore, several trifluoromethyl-substituted cyanoacrylates were synthesized and their anticancer bioactivities were evaluated. The reaction route is shown in Scheme 1.

2. Results and discussion

Ethyl 2-cyano-3,3-dimethylthioacrylate (**1**) was prepared in 56.2% yield from ethyl cyanoacetate, carbon disulfide, dimethyl sulfate with sodium methoxide as the base. It was found that the dropwise adding of sodium methoxide was better than pouring in at one time since, many side products were observed if the sodium methoxide was added at once.

* Corresponding author. Tel.: +86 851 3620521; fax: +86 851 3622211.
E-mail addresses: songbaoan22@yahoo.com, gzgdjh@tmail.gzu.edu.cn (B. Song).

0022-1139/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.jfluchem.2004.10.041



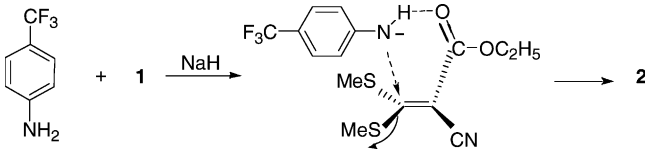
Scheme 1.

Compound **1** was then reacted with 4-trifluoromethylaniline to yield ethyl (*E*)-2-cyano-3-(4-trifluoromethylphenylamino)-3-methylthioacrylate (**2**). In this reaction step, sodium hydride was used as deprotonator for the aryl amine so that the nucleophilicity of the amine would be enhanced (no reaction was observed even after refluxing the reaction mixture for 10 h, if sodium hydride was not used). Through using the solvent mixture DMF and toluene, the mineral oil coated on the sodium hydride powder is dissolved in toluene, so the reaction system could be more homogenized. When the reaction was completed and the mixture was treated with water, the organic impurity is dissolved by toluene.

When the amine attacks the double bond to form a transition state, the orientation of 4-trifluoromethylaniline and ester carbonyl should be of a *cis* configuration due to the presence of hydrogen bond, as shown in Scheme 2. Also, the configuration of the target compound was kept due to the loss of a methylthio group, which resulted in an (*E*) configuration of the compound and this was confirmed by the X-ray single crystal analysis of **2**.

Compound **2** was treated with the aliphatic amine (**3**) to obtain 3-aliphatic amino-2-cyano-3-(4-trifluoromethylphenylamino) acrylate (**4**). In our experiment, we found the nucleophilicity of the aliphatic amine attached to the α,β-unsaturated double bond of *S,N*-kentene acetals was reduced. When amine (**3**) was treated with **2**, the reaction was relatively slow, probably due to the steric hindrance of the alkyl group, inhibiting the reactivity of nucleophilic substitution. Therefore, the order of reactivity of aliphatic amine is probably as follows:

$$NH_3 > NH_2{-}CH_2Ph > NH(CH_3)_2 > NH_2{-}Pr - n > NH_2{-}Pr - i > NH_2{-}Bu - n$$



Scheme 2.

From the yields given below, it could be seen that the yields of the title compounds decreased with the order: **4a** > **4e** > **4f** > **4b** > **4d** > **4c**.

The structures of all of the products were confirmed by ^{1}H NMR, ^{13}C NMR, elemental analysis, IR and mass spectroscopy. From the spectra data of **4**, it can be seen that the IR spectra of **4** exhibited bands at about 3204.7–3287.9 cm^{-1}, indicating the presence of NH. The signals at 1627.9–1660.4 cm^{-1} were assigned to C=O vibrations. The strong absorptions C=C appeared at 1531–1548 cm^{-1}. A set of peaks at 2193.0–2204.6 cm^{-1} was due to C=N vibrations. The signals at 1112.9–1165.0(s) were assigned to C–F vibrations. In ^{1}H NMR data, all phenyl protons showed multiplet at δ, 7.12–7.74. While chemical shifts of CH_2 of ester were at about 4.06 and 4.15 ppm, respectively, exhibiting doublet. The NH proton of **4** was at 9.52–11.07 ppm in a singlet probably due to the existence of hydrogen bonding between ester carbonyl and NH of 4-trifluoromethylphenylamino group, which leads to its chemical shift moving to lower field. As for the ^{13}C NMR data of **4**, all of the carbon atoms were identified and the numbers of protons calculated from the integration curve were tallied with what was expected from the molecular formula. The MS spectra revealed that the molecular ion and fragmentation peaks were in accordance with the proposed structure of compound **4**.

It could be seen from the X-ray single crystal analysis that compound **2** maintains a planar structure (see Fig. 1). It can be seen that the bond length of C(1)–C(2) (1.386 Å) is longer than normal C=C (1.34 Å), C(3)–O(2) (1.331 Å) is shorter than normal single C–O (1.44 Å), C(2)–C(3) (1.453 Å) and C(2)–C(6) are shorter than normal C–C (1.54 Å), C(3)–O(1) (1.213Å) is longer than normal C=O (1.34 Å), C(1)–S(1) (1.754 Å) is shorter than the normal covalent single S–C bond (1.801–1.825 Å), C(1)–N(1) (1.333 Å) bond is shorter than the normal C–N single bond (1.49 Å) and C=N is longer than the normal C=N, which suggest that the electron density is localized among C(8)–N(1)–C(1)–C(2)–C(3)–O(1). From the view of Fig. 2 and Table 1, it is known that the two neighboring molecules are linked by hydrogen bonds, in the form of N(1)–H(1)···O(1). The donor and

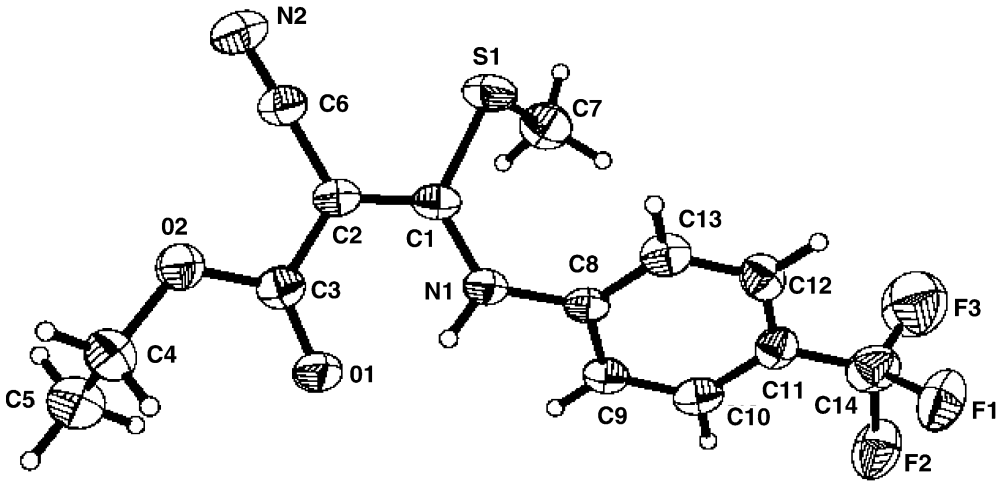


Fig. 1. Molecular structure of compound **2**.

acceptor distance is N(1)–O(1) 2.644(5) Å and the bond angle is 135.3(7)°. The crystal packing is stabilized by these extensive hydrogen bonds.

2.1. Anticancer bioactivity assay

The inhibitive effects of cyanoacrylates, **4a–f,** against cell proliferation of PC3 and A431 cell lines were evaluated. The IC_{50} values for compounds, **4a–f,** indicated that PC3 cells were more sensitive to compound **4** than A431 cells were.

The results presented in Table 2 indicate that these newly synthesized compounds exhibited promising anticancer activities against two cancer cells in vitro. The compounds **4e** and **b** have most potent antitumor activity than **4a**, **c**, **d** and **f** do. The bioassay indicated that the nature of the R group affected anticancer activity. For example, when R is

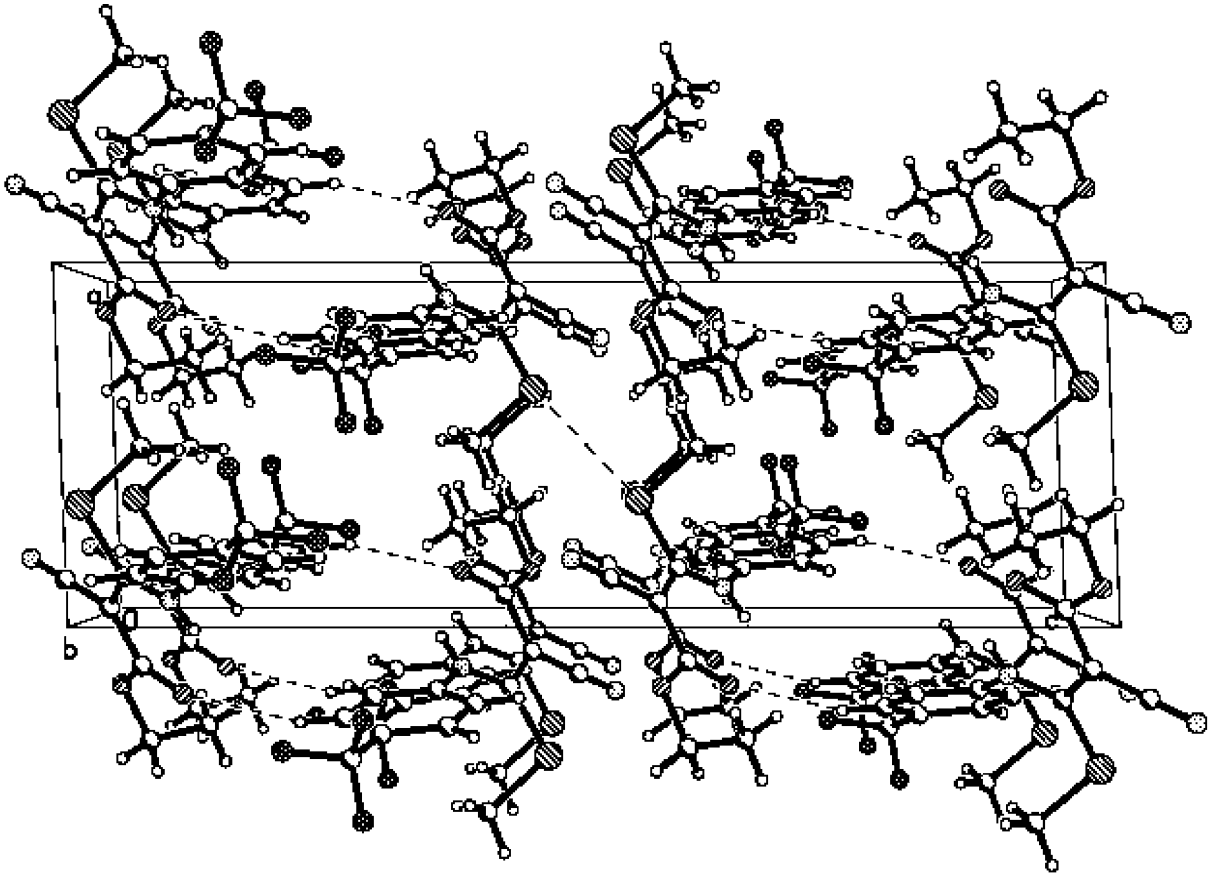


Fig. 2. Packing diagram of the unit cell of compound **2**.

Table 1
Hydrogen bonds for compound **2** (Å and degree (°))

D–H···A	N(1)–H(1)···O(1)
d(D–H)	0.86
d(H···A)	1.96
d(D···A)	2.644(5)
<(DHA)	135.3

Table 2
Bioassay of cyanoacrylates with a trifluoromethylphenyl moiety against PC3 and A431 cell lines

Compounds	R	IC_{50} (μM)	
		PC3	A431
4a	–H	32.1 ± 2.1	36.1 ± 1.1
4b	–Pr-*n*	18.2 ± 0.3	22.1 ± 2.5
4c	–Bu-*n*	23.1 ± 1.1	32.1 ± 1.7
4d	–Pr-*i*	21.1 ± 2.2	26.1 ± 2.6
4e	$–CH_2Ph$	12.1 ± 1.6	12.1 ± 2.4
4f	$(CH_3)_2$	42.1 ± 2.1	39.2 ± 4.3

benzyl, compound **4e** has relatively higher activity to PC3 and A431 cells.

3. Conclusion

A series of 3-aliphatic amino-2-cyano-3-(4-trifluoromethylphenylamino) acrylate (**4**) containing a trifluoromethylphenyl moiety were synthesized in three steps. Their structures were verified by spectroscopic method. In the MTT bioassay, these new compounds were found to possess high antitumor activities against PC3 and A431 cells. As far as we know, it is the first report that the substituted 2-cyanoacrylates have anti-cancer bioactivity.

4. Experimental

4.1. Instruments

The melting points of the products were determined on a 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. ^1H NMR (solvent $CDCl_3$) and ^{13}C (solvent $CDCl_3$) NMR spectra were performed on a Varian–Inova 500 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 as an external standard, positive for downfield shift. The mass spectra were taken on an HP5988A spectrometer. Elemental analysis was performed by an Elementar Vario-III CHN analyzer. The reagents were all analytical reagent-grade or chemically pure. The thin layer chromatography was conducted on GF_{254} plastic sheets at room temperature. The samples were applied in methanol and the chromatograms were developed in the following eluting systems: (a) benzene–methanol (10:1) and (b) ethyl acetate–tetrahydrofuran–methanol (12:3:1). The spots were visualized with UV light and with iodine vapor. All solvents were dried, deoxygenated and redistilled before use.

4.2. Synthesis

4.2.1. Ethyl 2-cyano-3,3-dimethylthioacrylate (*I*)

To a mixture of anhydrous ethanol (400 ml), ethyl cyanoacetate (34.0 g, 0.301 mol) and carbon disulfide (23.0 g, 0.303 mol) under 20 °C was added dropwise a solution of sodium methoxide (38.48 g, 0.610 mol) in anhydrous methanol (360 ml) for 1 h. After adding the solution, the mixture was stirred for 8 h at room temperature. Then, dimethyl sulfate was added dropwise during 0.5 h and the reaction mixture was heated under reflux for 2.5 h. The solvent was removed under reduced pressure and then water (40 ml) was added and filtered. The residue was dried and recrystallized twice from a mixture of *n*-hexane/diethyl ether, 1/1 (v/v) to give a white solid (36.7 g): yield, 56.2%; m.p., 53.5–54.5 °C (lit. ref. [11], m.p., 53–54 °C).

4.2.2. Ethyl 2-cyano-3-(4-trifluoromethylphenylamino)-3-(methylthio)acrylate (*2*)

To an oven-dried three-necked 100-ml round-bottom flask fitted with a magnetic stirring bar and charged with dry N_2 was added intermediates **1** (2.17 g, 0.01 mol), 4-trifluoromethylaniline (1.61 g, 0.01 mol), 60% sodium hydride (0.80 g, 0.02 mol), DMF (20 ml) and toluene (20 ml). The resulting mixture was then stirred at 10–20 °C for 40 h. The extent of the reaction was monitored by TLC. The mixture was poured into ice water (100 ml) and separated. The aqueous phase was acidified with 10% HCl to pH 6–7, and filtered. The residue was dried and recrystallized from anhydrous ethanol to give a white solid, yield 63.5%, m.p., 78–79 °C. IR (KBr): 3005.1, 2208.4, 1653.0, 1593.2, 1537.2, 1396.4, 1377.1, 1313.5, 1307.7, 1269.1, 1172.7, 1124.5, 1118.7, 1024.2, 881.4, 810.1, and 675.0 cm^{-1}. ^1H NMR ($CDCl_3$): δ 11.40(s, 1H, NH), 7.82–7.69(m, 4H, Ar–H), 4.24(q, 2H, CH_2), 2.05(s, 3H, SCH_3), and 1.27(t, 3H, CH_3–C). MS *m/z*: 330, 311, 283, 270, 255, 237, 212, 198, 184, 160, 145, 125, 111, 95, and 75. Anal. Calc. for $C_{14}H_{13}F_3N_2O_2S$: C, 50.90; H, 3.97; N, 8.48. Found: C, 50.73; H, 3.81; N, 8.30.

4.2.3. General procedure for the preparation of products *4a–f*

A solution of ethyl 2-cyano-3-(4-trifluoromethylphenylamino)-3-methylthio-acrylate (**2**) (0.75 mmol) in EtOH (40 ml) was stirred, followed by the addition of amine (**3**) (0.82 mmol). The mixture was then heated under reflux for 8–12 h. The extent of the reaction was monitored by TLC. The solvent was removed under reduced pressure. The

product was purified by column chromatography on a silica gel (eluent: ethyl acetate/petroleum ether, 2/8, v/v) to give the title compounds.

*4.2.3.1. Ethyl 3-amino-2-cyano-3-(4-trifluoromethylphenylamino)acrylate (**4a**).* White crystal; yield: 72.6%; m.p., 193.5–194.5 °C. IR (KBr): 3204.7, 2976.1, 2200.7, 1635.6, 1620.2, 1608.6, 1585.4, 1531.4, 1458.1, 1446.6, 1492.2, 1386.8, 1369.4, 1298.0, 1269.1, 1120.6, 1109.0, 1085.9, and 779.2 cm^{-1}; ^1H NMR ($CDCl_3$/TMS): δ 11.07(s, 1H, NH–Ar), 9.21(s, 2H, NH_2), 7.26–7.74(m, 4H, Ar–H), 4.18–4.25(q, 2H, –OCH_2), and 1.29–1.65(m, 3H, CH_3). ^{13}C NMR ($CDCl_3$/TMS): 169.36, 161.83, 161.26, 138.40, 127.48, 127.44, 127.40, 125.18, 118.82, 60.32, and 14.47. ^{19}F NMR ($CDCl_3$/TFA): δ −11.9. Anal. Calc. for $C_{13}H_{12}F_3N_3O_2$: C, 52.18; H, 4.04; N, 14.04. Found: C, 52.13; H, 4.00; N, 13.98.

*4.2.3.2. Ethyl 2-cyano-3-(4-trifluoromethylphenylamino)-3-(propylamino)acrylate (**4b**).* White crystal; yield: 58.6%; m.p., 95.5–96.5 °C. IR (KBr): 3213.4, 2995.4, 2970.3, 2200.7, 1651.0, 1587.4, 1537.2, 1444.6, 1427.3, 1384.8, 1369.4, 1294.2, 1274.9, 1244.0, 1226.7, 1188.1, 1165.0, 1107.1, 1089.7, 1002.9, 873.7, 775.3, 734.8, and 698.2 cm^{-1}. ^1H NMR ($CDCl_3$/TMS): δ 10.70(s, 1H, NH–Ar), 9.25(s, 1H, NH–C), 7.13–7.62(m, 4H, Ar–H), 4.06–4.26(q, 2H, O–CH_2), 2.86(d, J = 7.3 Hz, 2H, NCH_2), 1.54–1.68(m, 2H, CH_2), 1.31–1.35(m, 3H, CH_3), and 0.87–0.91(m, 3H, CH_3). ^{13}C NMR ($CDCl_3$/TMS): 169.33, 162.15, 126.71, 122.89, 120.42, 60.37, 47.37, 22.83, 14.44, and 11.11. ^{19}F NMR ($CDCl_3$/TFA): δ −11.3. MS *m/z*: 341, 326, 313, 298, 287, 273, 254, 237, 226, 210, 199, 187, 174, 161, 145, 135 125, 111, 95, 85, and 69. Anal. Calc. for $C_{16}H_{18}F_3N_3O_2$: C, 56.30; H, 5.32; N, 12.31. Found: C, 56.17; H, 5.20; N, 12.30.

*4.2.3.3. Ethyl 3-butylamino-2-cyano-3-(4-trifluoromethylphenylamino)acrylate (**4c**).* White solid; yield: 45.0%; m.p., 105–106 °C. IR (KBr): 3304.1, 3053.3, 2975.9, 2200.8, 1627.9, 1589.3, 1548.8, 1473.6, 1438.9, 1417.6, 1375.2, 1294.2, 1112.9, 1085.9, 858.3, 819.7, 655.8, and 526.5 cm^{-1}; ^1H NMR ($CDCl_3$/TMS): δ 10.70(s, 1H, NH–Ar), 9.24(s, 1H, NH–C), 7.12–7.62(m, 4H, Ar–H), 4.22(t, J = 8.1 Hz, 2H, O–CH_2), 2.89–3.94(m, 2H, NCH_2), and 0.83–1.62(m, 10H, CH_2–CH_2–CH_3+CH_3). ^{13}C NMR ($CDCl_3$/TMS): 169.33, 162.16, 142.37, 126.78, 122.86, 120.51, 60.40, 45.32, 31.45, 19.65, 14.45, and 13.46. ^{19}F NMR ($CDCl_3$/TFA): δ −11.2. MS *m/z*: 355, 332, 322, 312, 296, 282, 273, 264, 254, 237, 226, 210, 199, 187, 172, 161, 145, 134, 125, 111, 102, 93, 83, and 68. Anal. Calc. for $C_{17}H_{20}F_3N_3O_2$: C, 57.46; H, 5.67; N, 11.82. Found: C, 57.59; H, 5.71; N, 11.74.

*4.2.3.4. Ethyl 2-cyano-3-isopropylamino-3-(4-trifluoromethylphenylamino)acrylate (**4d**).* White solid; yield: 56.1%; m.p., 100.5–102 °C. IR (KBr): 3219.9, 2204.6, 1668.4, 1583.5, 1531.4, 1479.4, 1440.8, 1408.0, 1369.4, 1348.2, 1303.8, 1273.0, 1253.7, 1170.7, 1157.2, 1026.1, and 701.1 cm^{-1}. ^1H NMR ($CDCl_3$/TMS): δ 9.56(s, 1H, NH–Ar), 8.87–8.89(d, J = 7.4 Hz, 1H, NH–C), 7.20–7.67(m, 4H, Ar–H), 4.04–4.10(q, 2H, OCH_2), 3.82–3.87(m, 1H, NCH), and 1.04–1.19(m, 9H, 3 × CH_3). ^{19}F NMR ($CDCl_3$/TFA): δ −11.4. MS *m/z*: 341, 332, 322, 312, 296, 282, 273, 264, 254, 237, 226, 210, 199, 187, 172, 161, 145, 134, 125, 111, 102, 93, 83, and 68. Anal. Calc. for $C_{16}H_{18}F_3N_3O_2$: C, 56.30; H, 5.32; N, 12.31. Found: C, 56.36; H, 5.11; N, 12.39.

*4.2.3.5. Ethyl 3-benzylamino-2-cyano-3-(4-trifluoromethylphenylamino)acrylate (**4e**).* White solid; yield: 71.5%; m.p., 139–141 °C. IR (KBr): 3287.9, 3012.8, 2997.3, 2980.0, 2937.5, 2204.6, 1668.4, 1558.5, 1531.4, 1479.4, 1436.9, 1408.0, 1369.4, 1348.2, 1303.8, 1273.0, 1253.7, 1186.2, 1165.0, 1157.2, 1026.1, and 781.1 cm^{-1}. ^1H NMR ($CDCl_3$/TMS): δ 9.52(s, 1H, NH–C–Ar), 9.32(s, 1H, NH–C–Ph), 7.13–7.63(m, 9H, Ar–H), 4.45(d, J = 8.2 Hz, 2H, O–CH_2), 4.07(dd, J = 10.2, 7.1 Hz, 2H, NCH_2), and 1.16(t, J = 7.2 Hz, 3H, CH_3). ^{13}C NMR ($CDCl_3$/TMS): 169.14, 161.17, 137.35, 136.90, 134.38, 129.29, 128.96, 128.66, 127.63, 127.45, 125.84, 125.80, 123.96, 117.18, 59.40, 57.34, 46.22, and 14.36. ^{19}F NMR ($CDCl_3$/TFA): δ −11.0. MS *m/z*: 389, 370, 358, 344, 328, 316, 299, 299, 288, 276, 264, 251, 235, 223, 210, 187, 172, 155, 142, 128, 106, 91, 77, and 65. Anal. Calc. for $C_{20}H_{18}F_3N_3O_2$: C, 61.69; H, 4.66; N, 10.79. Found: C, 61.52; H, 4.52; N, 10.62.

*4.2.3.6. Ethyl 2-cyano-3-(dimethylamino)-3-(4-trifluoromethylphenylamino)acrylate (**4f**).* White solid; yield: 63.1%; m.p., 126–128 °C. IR (KBr): 3263.5, 2987.7, 2927.9, 2193.0, 1662.6, 1614.4, 1537.2, 1431.1, 1388.7, 1332.1, 1265.1, 1161.7, 1118.7, 1066.6, 1060.8,1014.5, and 839.0 cm^{-1}. ^1H NMR ($CDCl_3$/TMS): δ 9.52(s, 1H, NH–Ar), 7.01–7.62(m, 4H, Ar–H), 4.23–4.18(q, 2H, O–CH_2), 2.96(s, 6H, 2CH_3), and 1.31(t, J = 7.4 Hz, 3H, CH_3–C). ^{13}C NMR ($CDCl_3$/TMS): 169.17, 164.34, 142.71, 126.88, 126.85, 126.82, 126.58, 120.93, 119.48, 77.32, 77.00, 76.68, 63.43, 60.59, 41.40, and 14.36. ^{19}F NMR ($CDCl_3$/TFA): δ −11.6. Anal. Calc. for $C_{15}H_{16}F_3N_3O_2$: C, 55.04; H, 4.93; N, 12.84. Found: C, 55.06; H, 4.89; N, 12.84.

4.3. Crystal structure determination

For the determination of structure, using a single crystal, X-ray intensity data were recorded on a Rigaku Raxis-IV diffraction meter using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). In the range $1.76^\circ \leq \theta \leq 25.01^\circ$, 2739 independent reflections were obtained. Intensities were corrected for Lorentz and polarization effects and empirical absorption, and all data were corrected using SADABS [12] program. The structure was solved by direct methods SHELXS-97 program [13]. All the non-hydrogen atoms were refined on $F2$ anisotropically by the full-matrix least squares method. The hydrogen atoms were located from the difference Fourier map, but their positions were not

refined. The contributions of these hydrogen atoms were included in structure–factor calculations. The final least-squares cycle gave $wR = 0.1793$, $R = 0.1107$ for 7835 reflection with $I > 2\sigma(I)$; the weighting scheme, $w = 1/[\sigma^2(F_o^2) + (0.0940P)^2 + 1.0028P]$, where $P = (F_o^2 + 2F_c^2)/3$. The maximum and minimum difference peaks and holes are 0.346 and -0.227 e A^{-3}, respectively, $s = 1.034$ and $(\Delta/\sigma)_{max} = 0.0100(16)$. Atomic scattering factors and anomalous dispersion corrections were taken from *International Table for X-ray Crystallography* [14]. Crystallographic data (excluding structure factors) for the structure have been deposited to the Cambridge Crystallographic Data Center as supplementary publication no. CCDC-245102.

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-2yl)-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.

Acknowledgements

The authors wish to thank the National Key Project for Basic Research (Grant no. 2003CB114404), High-Tech Research and Development Program of China (Contract no. 2002AA217131, 2002AA649190) and the National Natural Science Foundation of China (Grant no. 20362004) for financial support.

Reference

[1] W.K. Banham, J.L. Huppatz, J.N. Phillips, Z. Naturforsch. 48c (1993) 136–139.
[2] H.G. McFadden, D.C. Craig, J.L. Huppatz, J.N. Phillips, Z. Naturforsch. 46c (1991) 93–98.
[3] J.L. Huppatz, Weed Sci. 44 (1996) 743–748.
[4] J.N. Phillips, W.K. Banham, J.N. Phillips, Z. Naturforsch. 48c (1993) 132–135.
[5] S.-Y. Yu, Z.-M. Li, Chin. J. Pestic. Sci. 3 (2001) 18–22.
[6] H.-Y. Liu, Y.-L. Sha, G.-G. Dai, H.-F. Tan, H.-Z. Yang, L.-H. Lai, Phosphorus, Sulfur Silicon 148 (1999) 235–241.
[7] H.-K. Sun, Q.-M. Wang, R.-Q. Huang, H. Li, Y.-H. Li, J. Organomet. Chem. 655 (2002) 182–185.
[8] G. Magueur, B. Crousse, S. Charneau, P. Grellier, J.P. Begue, D. Bonnet-Delpon, J. Med. Chem. 47 (2004) 2694–2699.
[9] 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.
[10] I.M. Abdou, A.M. Saleh, H.F. Zohdi, Molecules 9 (2004) 109–116.
[11] X.-J. Wang, Z.-T. Huang, Acta Chim. Sinica 47 (1989) 890–895.
[12] G.M. Sheldrick, Program for Empirical Absorption Correction of Area Detector Data, University of Gottingen, Germany, 1996.
[13] G.M. Sheldrick, SHELXTL V5.1, Software Reference Manual, Bruker AXS Inc., Madison, Wisconsin, USA, 1997.
[14] A.J. Wilson, International Table for X-ray Crystallography, vol. C, Kluwer Academic Publishers, Dordrecht, Tables 6.1.1.4 (pp. 500–502) and 4.2.6.8 (pp. 219–222), respectively, 1992.