Synthesis of 2-Cyanoacrylates Containing Pyridinyl Moiety Under Ultrasound Irradiation

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Reaction of ethyl cyanoacetate with carbon disulfide and dimethyl sulfate in the presence of sodium methoxide in anhydrous methanol yields ethyl 2-cyano-3,3-dimethyl- thioacrylate, followed by the nucleophilic substitution with 2-amino-3-chloro-4- methylpyridine under ultrasonic irradiation affording the key intermediate, ethyl 3-(2-chloro-4-methylpyridin-3-ylamino)-2-cyano-3-methylthioacrylate. The title compounds were then obtained through the reaction of the key intermediate with the aliphatic amine under reflux condition. All 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 moderate antitumor activities against PC3 and A431 cells.

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Introduction.

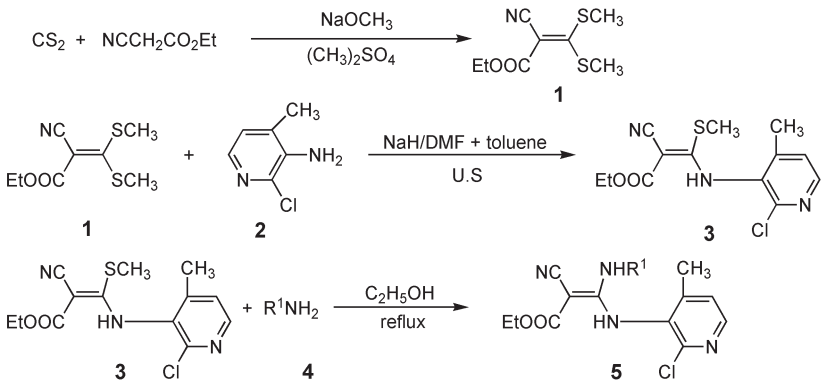
2-Cyanoacrylate derivatives are of considerable interests due to their potential biological properties. Various related compounds have herbicidal activity [1] and insecticidal activity [2]. It is reported that the ethoxyethyl *(Z)*-3-(4-chlorophenyl- methylamino)-2-cyano-3-isopropylacrylate (CPNPE) has the highest Hill inhibitory activity [3-4].

As far as we are aware, the introduction of pyridinyl group into organic molecules usually results in the enhancement of the biological activity of parent compounds [5-7]. Hence, in a search for new anticancer and antivirual agent, we thought that the replacement of the methylthio moiety by pyridinyl group in some 2-cyano-3,3-dimethylthioacrylate may result in the improvement of bioactivity. Therefore, several 2-cyanoacrylates containing pyridinyl moiety were synthesized and their anticancer bioactivities were evaluated. Use of the typical methods [8-9], a mixture of ethyl 2-cyano-3,3-dimethylthioacrylate, 3-amino-2-chloro-4-methylpyridine, 60% sodium hydride was stirred at 10-20 °C for 10-16 h in the mixture solvents of DMF and toluene. However, the typical method involved longer reaction time, low yield of products and complex handling. For this reaction, recently we have developed a new method to synthesize the intermediate **3** under ultrasonic irradiation in the mixture solvents of DMF-toluene. The method was very easy and mild and environmentally friendly. In this article we describe the preparation of 2-cyanoacrylate containing pyridinyl moiety and their antitumor bioactivities. The reaction route is shown in Scheme 1.

Results and Discussion.

In order to optimize the reaction conditions, the synthesis of **3** was carried out under several conditions. The effects of different solvents, reaction times, temperatures, or use of ultrasonic irradiation on the reactions are shown in Table 1. It could be seen that the presence of ultrasonic irradiation could accelerate the reaction (Table 1, entries 1-10, 18-20). When ultrasound is not used, the reaction was relatively slow and no product was detected within 1-4 h (Table 1, en tries 11, 12, 13). When the reaction time was prolonged to 16 h, **3** could be obtained in 57.2% (Table 1, entry 16) without ultrasonic irradiation. When the reaction was carried out from 1 h to 3 h under ultrasonic irradiation at room temper-

Scheme 1



ature, the yield of **3** was increased from 44.2% to 55.9% (Table 1, entries 1-3). Tiny improvement of the yield was obtained when the reaction time was prolonged to 4 h and 5 h (Table 1, entries 4 and 5). As for the reaction temperature, it could be seen that when the reaction was taken out at room temperature or even lower (Table 1, entries 1-5, and 6), the yield was lower than that at 40-45 °C (Table 1, entry 8). While interestingly the yield was decreased when the reaction temperature was increased to 50-60 °C (Table 1, entries 9-10). Hence, 40-45°C is the optimum temperature for the reaction. No substantial improvement was observed when the reaction time varied from 1 h to 5 h at 40-45 °C under ultrasound irradiation (Table 1, entries 17-20).

The effect of the solvent amount was also investigated. It could be seen that when the amount of solvent was decreased from 12 mL to 8, 6, 4, 2, and 0 mL, the yield of **3** was 69.7%, 73.1%, 79.2%, 68.8%, 65.0% and 31.2%, respectively (Table 2, entries 1-6). In conclusion, the best result was obtained when compound **3** was reacted with 1 equiv of amine and 2 equiv of NaH under ultrasonic irradiation with DMF-toluene as co-solvent at 40-45°C for 1 h. Under these reaction conditions, the amination reaction proceeded smoothly and moderate yields were obtained.

Table 1

Results at D ifferent Reaction Conditions for the Synthesis of **3**

Entry	Solvent	Time/h	Temperature / °C	Yield/% [c]
[a] 1	DMF/toluene	1	20-25	44.2
[a] 2	DMF/toluene	2	20-25	50.3
[a] 3	DMF/toluene	3	20-25	55.9
[a] 4	DMF/toluene	4	20-25	56.1
[a] 5	DMF/toluene	5	20-25	59.0
[a] 6	DMF/toluene	4	0-10	29.8
[a] 7	DMF/toluene	4	30-35	63.4
[a] 8	DMF/toluene	4	40-45	72.1
[a] 9	DMF/toluene	4	50-55	64.1
[a] 10	DMF/toluene	4	55-60	60.9
[b] 11	DMF/toluene	1	20-25	No product
[b] 12	DMF/toluene	4	20-25	No product
[b] 13	DMF/toluene	1	40-45	No product
[b] 14	DMF/toluene	4	40-45	34.8
[b] 15	DMF/toluene	10	20-25	48.1
[b] 16	DMF/toluene	16	20-25	57.2
[a] 17	DMF/toluene	1	40-45	78.8
[a] 18	DMF/toluene	2	40-45	76.7
[a] 19	DMF/toluene	3	40-45	77.0
[a] 20	DMF/toluene	5	40-45	73.5

[a] The reactions were carried out in 6mL DMF/toluene (1/1 by *V/V*) under ultrasound irradiation with NaH as base; [b] The reactions were carried out in 6mL DMF/toluene (1/1 by *V/V*) with stirring but without ultrasound irradiation with NaH as base; [c] Isolated yields.

Table 2

Effect of the Amount of Solvent on the Synthesis of **3** [a]

Entry	Amount of the solvent/mL	Yield/% [b]
1	12	69.7
2	8	73.1
3	6	79.2
4	4	68.8
5	2	65.0
6	0	31.2

[a] All reactions were carried out at 40-45°C for 1h under ultrasound irradiation with NaH as base; [b] Isolated yields.

As shown in Table 3 and Scheme 1, the synthesis of 3-aliphatic amino-3-(2-chloro-4-methylpyridin-3-ylamino)-2-cyanoacrylate **5** was carried out in moderate yields at 78-80 °C in ethanol for 4 h. Dramatic improvement of the yields were obtained when the reaction temperature was increased. When the reaction was carried out at 78-80 °C in ethanol for 4 h, **5a**, **5b**, **5c** and **5d** were obtained in the yields of 54.7%, 74.5%, 56.0% and 63.1%, respectively. While the corresponding numbers at 40-50 °C were 34.2%, 54.2%, 34.7% and 39.6%, respectively. Hence, the aminiation reaction can be finished under reflux condition with moderate yields.

As could be seen from the structure, there could be two configurations related to compound **3** and **5**. Actually, the configuration of compound **3** had been discussed which an *(E)* configuration was verified according to the single crystal structure determination [9]. While the attacking aliphatic amine **4** will occupy the position of the methylthio group which resulting in a *(Z)* configuration of compound **5**.

The antitumor activity was assayed by the MTT method [10-11]. The results presented in Table 4 indicate that these newly synthesized compounds exhibit promising anticancer activities to two cancer cells *in vitro*. The compound

Table 3

Synthesis of 3-Aliphatic Amino-3-(2-chloro-4-methylpyridin3-ylamino)-2-cyano- acrylate **5** Under Different Conditions

Entry	Compound	R	Time/h	Temperature/°C	Yield/%
1	**5a**	-Pr-*n*	4	78-80	54.7
2	**5b**	-Bu-*n*	4	78-80	74.5
3	**5c**	-CH_2Ph	4	78-80	56.0
4	**5d**	-Pr-*i*	4	78-80	63.1
5	**5a**	-Pr-*n*	4	40-50	34.2
6	**5b**	-Bu-n	4	40-50	54.2
7	**5c**	-CH_2Ph	4	40-50	34.7
8	**5d**	-Pr-*i*	4	40-50	39.6

Table 4

Bioassay of Cyanoacrylates with a Pyridinyl Moiety to PC3 and A431 Cell lines at 10μM

Compound	R	Inhibitor rate (%)	
		PC3	A431
5a	-Pr-*n*	34.6	52.1
5b	-Bu-*n*	23.1	12.4
5c	-CH_2Ph	80.1	87.5
5d	-Pr-*i*	63.2	71.3

5c has more potent antitumor activity than **5a**, **5b** and **5d**, which shows that the nature of R group affects antitumor activity to some extent. For example, when R is benzyl, compound **5c** has relatively higher activity to PC3 and A431 cells than its less active analogues **5a** and **5b**, in which the R groups were *n*-Pr- and *n*-Bu-, respectively.

Conclusion.

In summary, we described a practical and efficient procedure for the preparation of ethyl 2-cyano-3-(2-chloro-4-methylpyridin-3-ylamino)-3-methylthioacrylate through the two-component reaction of ethyl 2-cyano-3,3-dimethylthioacrylate and 2-amino-3-chloro-4-methylpyridine using NaH as the base under ultrasonic irradiation with DMF-toluene as co-solvent for 1 h at 40-45 °C. The reactions were, in general, fast, and efficient. Followed by amination with aliphatic amines, the target compounds were obtained in moderate yields.

In the MTT bioassay, these new compounds have moderate antitumor activities to PC3 and A431 cells. For example, the inhibitive activity of compound **5c** to PC3 and A431 cells at 10μM were 80.1% and 87.5%, respectively.

EXPERIMENTAL

The reagents and solvents were all analytical grade or chemically pure and were obtained from Shanghai Reagent Company. All solvents were dried, deoxygenated and redistilled before use. All melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and are not corrected. The infrared spectra were recorded on a Bruker VECTOR22 spectrometer in KBr pellets. ^{1}H NMR (solvent $CDCl_3$) and ^{13}C NMR spectra (solvent $CDCl_3$) 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. The mass spectra were taken on an HP5988A spectrometer. Elemental analysis was performed by an Elementar Vario-III CHN analyzer. Sonication was performed on a Shanghai Branson-CQX ultrasonic cleaner (with frequencies of 25 KHz and a nominal power of 500 W). Analytical TLC was conducted on GF_{254} plastic sheets at room temperature.

Ethyl 2-cyano-3,3-dimethylthioacrylate (**1**)

This compound was obtained as a white solid (36.7 g), yield 56.2%; m.p. 53.5-54.5°C (lit.ref. [12-13], m.p. 53-54°C).

3-Amino-2-chloro-4-methylpyridine (**2**).

A solution of 15% sodium hydroxide (530.0 g, 2.0 mol) was cooled to 0-5 °C. Bromine (80.0 g, 0.5 mol) was added dropwise with the temperature maintained at 0-5°C. To the resulting pale yellow solution was added 2-chloro-4-methylpyridine-3-carboxamide (85.0 g, 0.5 mol) in 2 h at 0-5 °C. The ice bath was then removed and the reaction mixture warmed to 65-70 °C for 2 h and maintained at 75 °C for an additional 2 h. The mixture was cooled and the crystalline product collected by filtration. The solid was dried and recrystallized from *n*-hexane affording white solid, yield 92.1%, m.p. 68-69 °C (lit. ref. [14], m.p. 62-64 °C).

(E)-Ethyl 3-(2-chloro-4-methylpyridin-3-yl-amino)-2-cyano-3-methylthioacrylate (**3**).

To an oven-dried three-necked 50 mL round-bottom flask was added ethyl 2-cyano-3,3-dimethylthioacrylate (**1**) (2.17 g, 0.01 mol), 3-amino-2-chloro-4-methylpyridine (**2**) (1.43 g, 0.01 mol), 60% sodium hydride (0.80 g, 0.02 mol), DMF (3 mL) and toluene (3 mL). The resulting mixture was placed in the ultrasonic cleaning bath at 40-45 °C for 1 h. The reaction progress 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 73.5%, m.p. 113-114 °C; IR: 3284.7, 2200.8, 1637.5, 1620.2, 1608.6, 1585.4, 1531.4, 1458.1, 1446.6, 1431.1, 1386.8, 1369.4, 1298.0, 1269.1, 1120.6, 1109.0, 1085.9, 779.2 cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 11.17(s, 1H, NH), 7.22-8.26(m, 2H, Py-H), 4.31(q, 2H, CH_2), 2.55(s, 3H, SCH_3), 2.34(s, 3H, Py-CH_3), 1.38(t, 3H, CH_3-C, *J*=7.9Hz); EIMS: m/z 311(M^+, 12.2).

Anal. Calcd. for $C_{13}H_{14}ClN_3O_2S$ (311): C, 50.08; H, 4.53; N, 13.48. Found: C, 50.00; H, 4.49; N, 13.48.

General Procedure for the Preparation of Products **5a-5d**.

A solution of ethyl 3-(2-chloro-4-methylpyridin-3-ylamino)-2-cyano-3-methyl- thioacrylate (**3**) (0.75 mmol) in EtOH (20 mL) was stirred, followed by the addition of amine (**4**) (0.82 mmol). The mixture was refluxed at 78-80 °C for 4 h. The solvent was then removed under reduced pressure. The crude solid was purified by column chromatography on a silica gel (eluent: ethyl acetate /petroleum ether, 2/8 by v/v) to give the title compounds.

(Z)-Ethyl 3-(2-Chloro-4-methylpyridin-3-ylamino)-3-propylamino-2-cyanoacrylate (**5a**).

This compound was obtained as white crystal; yield 54.7%; m.p. 143-145°C. IR: 2196.9, 1666.5, 1600.2, 1595.1, 1448.5, 1327.0, 1284.5, 1255.6, 1166.9, 1114.8, 1097.5, 1066.6cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ10.78(s, 1H, NH-Py), 9.37(s, 1H, NH-C), 7.17-8.23(m, 2H, Py-H), 4.21(q, 4H, NCH_2+OCH_2), 2.66 (d, 3H, OCH_2, J=8.1Hz), 2.34 (s, 3H, Py-CH_3), 1.31~1.68(m, 5H, CH_3+CH_2); 0.88 (d, 3H, CH_3, J=8.0Hz). ^{13}C NMR ($CDCl_3$): 170.63, 163.24, 148.36, 147.62, 125.11, 60.19, 18.39, 14.44; EIMS: m/z 325(M^+, 14.8).

Anal. Calcd. for $C_{15}H_{19}ClN_4O_2$ (323): C, 55.81; H, 5.93; N, 17.36. Found: C, 55.81; H, 5.89; N, 17.36.

(Z)-Ethyl 3-Butylamino-3-(2-chloro-4-methylpyridin-3-ylamino)-2-cyanoacrylate (**5b**).

This compound was obtained as white crystal; yield 74.5%; m.p. 143-145 °C; IR: 2208.4, 1653.0, 1543.0, 1396.4, 1377.1, 1311.5, 1269.1, 1232.5, 1211.3, 1172.7, 1124.5, 1024.2, 881.4cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 10.76(s, 1H, NH-Py), 8.35(s, 1H, NH-C), 7.28-8.22(m, 2H, Py-H), 4.20(q, 4H, -OCH_2+NCH_2), 2.72(d, 5H, CH_2+SCH_3, J=8.2Hz), 2.35(s, 3H, Py-CH_3), 0.8~1.81(m, 5H, CH_2-CH_3); ^{13}C NMR ($CDCl_3$): 125.07, 60.12, 18.37, 14.41, 13.36; EIMS: m/z 339(M^+, 17.1).

Anal. Calcd. for $C_{16}H_{21}ClN_4O_2$ (337): C, 57.06; H, 6.28; N, 16.63. Found: C, 57.06; H, 6.24; N, 16.64.

(Z)-Ethyl 3-Benzylamino-3-(2-chloro-4-methylpyridin-3-ylamino)-2-cyanoacrylate (**5c**).

This compound was obtained as a white solid; yield 55.7%; m.p. 140-142 °C; IR: 3197.9, 3107.3, 3030.1, 2985.8, 2191.1,

1662.6, 1608.6, 1570.0, 1527.6, 1506.4, 1481.3, 1456.2, 1436.9, 1386.7, 1371.3, 1325.1, 1294.2, 1276.8, 1207.4, 1168.8, 1132.2, 1114.8, 1105.2, 1000.14, 885.3, 775.3, 759.9cm^{-1}; ^{1}H NMR (400MHz, $CDCl_3$): δ 10.82(s, 1H, NH-Py), 9.87(s, 1H, NH-C), 6.65-8.14(m, 7H, Py-H+Ar-H), 4.09-4.20(m, 4H, NCH_2+OCH_2), 2.61(s, 3H, SCH_3), 2.18(s, 3H, Py-CH_3), 1.21(d, 3H, C-CH_3, *J*=7.4Hz); ^{13}C NMR ($CDCl_3$): 129.01, 128.26, 126.81, 125.05, 60.34, 18.29, 14.42; EIMS: m/z 370(M^+, 16.1).

Anal. Calcd. for $C_{19}H_{19}ClN_4O_2$ (370): C, 61.54; H, 5.16; N, 15.11. Found: C, 61.27; H, 5.34; N, 15.03.

(Z)-Ethyl 3-(2-Chloro-4-methylpyridin-3-ylamino)-2-cyano-3-isopropylaminoacrylate (**5d**).

This compound was obtained as a white solid; yield 63.8%; m.p. 107-109 °C. IR: 3234.6, 2981.9, 2200.7, 1658.7, 1633.7, 1591.2, 1523.7, 1506.4, 1415.7, 1375.2, 1323.1, 1280.7, 1247.9, 1207.4, 1161.1, 1112.9,1064.7, 1016.4, 837.1, 781.1cm^{-1}. ^{1}H NMR (400MHz, $CDCl_3$): δ 10.91(s, 1H, NH-Py), 7.41-8.26(m, 2H, Py-H), 4.20(q, 4H, NCH_2+OCH_2), 2.52(s, 3H, SCH_3), 2.26(s, 3H, Py-CH_3), 2.01(d, 1H, CH, *J*=7.0Hz), 1.21(t, 9H, $3CH_3$, *J*=12.0Hz). ^{13}C NMR ($CDCl_3$): 149.21, 148.17, 132.56, 125.43, 60.68, 17.99, 14.27; EIMS: m/z 325(M^+, 16.9).

Anal. Calcd. for $C_{15}H_{19}ClN_4O_2$ (323): C, 55.81; H, 5.93; N, 17.36. Found: C, 55.50; H, 5.87; N, 17.14.

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