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The synthesis and biological evaluation of some caffeic acid amide derivatives: *E*-2-Cyano-(3-substituted phenyl) acrylamides

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ABSTRACT

A series of caffeic acid amide derivatives 2-cyano-(3-substituted phenyl)acrylamides were synthesized via Knoevenogal condensation of substituted benzaldehydes with cyanoacetamides. The structure of compound **1f** was determined as *E*-isomer by X-ray diffractive analysis. The biological screening tests in vitro showed that compound **1b** has obvious inhibitory activities against human gastric carcinoma cell line BGC-823, human nasopharyngeal carcinoma cell line KB and human hepatoma cell line BEL-7402 with IC_{50} values of 5.6 μg/mL, 13.1 μg/mL and 12.5 μg/mL, respectively. Some preliminary structure–activity relationships (SAR) were also proposed which may provide a direction for further study.

To find the synthetic or naturally occurring small molecules which have significant biological activity or therapeutic use still deserves current interests. In the last decade, much attention has been focused on the caffeic acid and its derivatives. Lots of researches have shown that some caffeic acid derivatives possess a wide range of biological activities such as anti-cancer activity,[1–5] anti-bacterial activity,[6] anti-oxidation,[7,8] and anti-viral activity.[9] Typically, the caffeic acid phenylethanolester (CAPE), firstly isolated from beeswax in 1988, was proved to have remarkable anti-cancer activities.[5] A derivative of caffeic acid amide, Entacapone, chemically named *E*-2-cyano-*N*,*N*-diethyl-3-(3,4-dihydroxy-5-notrophenyl)acrylamide, was a selective catechol *O*-methyltransferase (COMT) inhibitory agent and has been clinically used as an anti-parkinsonism drug for near ten years.[10–12] Besides, there are researches revealed that some derivatives of Entacapone possess obvious anti-cancer activity.[13,14] Although there have been lots of reports involving the biological activities of CAPE and its analogues, study on the relationship between the structure and its biological activity for this kind of compounds is still in demand. Due to our interest in this, we have synthesized ten caffeic acid amides derivatives 2-cyano-(3-substituted phenyl)acrylamides and further investigated their biological activities. We report here the synthesis, X-ray structure and inhibitory activities against BGC-823, KB, BEL-7402 and acetylcholinesterase of these compounds.

Initially, we try to prepare the title compounds by two steps: firstly by Knoevenagel condensation of substituted benzaldehydes with cyanoacetic esters to give 2-cyano-(3-substituted phenyl) acrylic esters, then followed by condensation with various aryl or alicyclic amines to form amides. In the second step, however, the condensation proceeded in a poor yield for the lack of enough activity of the ester group caused by its delocalization with vicinal pi-bond. Then we try to synthesize the 2-cyano-(3-substituted phenyl)acrylamides by Knoevenagel condensations of substituted benzaldehydes with cyanoacetamides.

Most of N-substituted cyanoacetamides are not available and need to be prepared. Although there have been several literature methods available for its preparation, there are some disadvantages in these methods such as needing high temperature and high pressure,[15] needing microwave,[16] using poisonous reagent[17] or using lithium amide at very low temperature,[18] which are inconvenient in the laboratory preparation. Here we developed a convenient method for the preparation of cyanoacetamides, that is, by condensing cyanoacetic acid with amines under the presence of DCC as coupling agent. By means of this method, the intermediate cyanoacetamides (**3k**–**3q**) were synthesized in a moderate yield except the cyanoacetamide (**3r**), which prepared by reacting ethyl cyanoacetate with aqueous ammonia according to the standard procedure.[19] The starting materials nitro-substituted benzaldehydes were prepared from vanillin according to the literature methods.[20]

As a result, the synthesis of 2-cyano-(3-substituted phenyl)-acrylamides were achieved in moderate to high yield via

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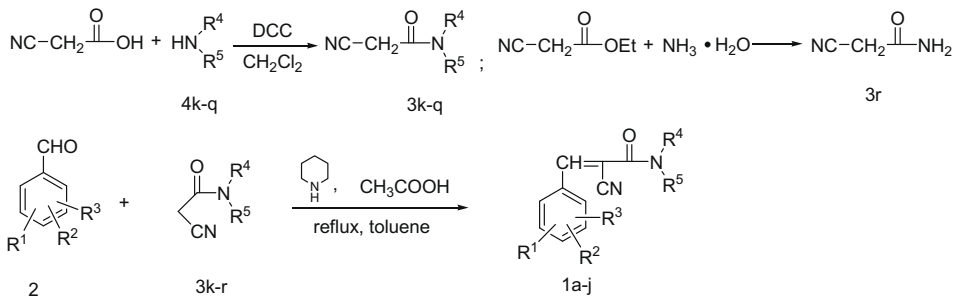


Scheme 1. Synthetic route of 2-cyano-(3-substituted phenyl)acrylamides.

Knoevenagel condensations of substituted benzaldehydes with cyanoacetamides in the presence of piperidine as catalyst (Scheme 1).[25]

The whole synthetic route was shown in Scheme 1. The synthesis of all title compounds were summarized in Table 1. The synthesis of cyanoacetamides and their characterizations were summarized in Table 2.

The structures of all title compounds were characterized by IR, ^{1}H NMR and EI-MS, besides, four new compounds were additionally confirmed by elemental analysis. For these compounds, it seemed that they can exist in either configuration of cis(*Z*) or trans(*E*) for a C–C double bond involving in the molecular structure. Unfortunately, it is difficult to determine their geometries based on the direct spectroscopic evidences since the α-cyanocaffeamido products contain single vinylic hydrogen that does not allow facile NMR assignment based on vicinal proton–proton coupling. According to the observation of a single vinylic signal

Table 1
Synthesis and ^{1}H NMR data of 2-yano-(3-substituted phenyl)acrylamides

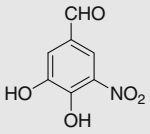
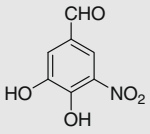
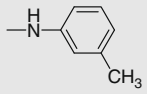
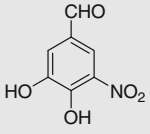
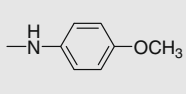
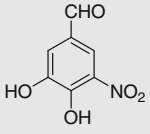
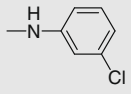
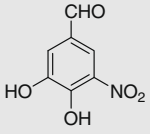
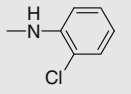
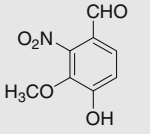
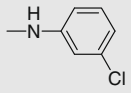
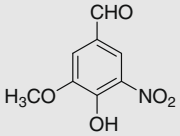
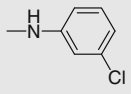
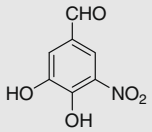
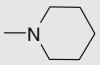
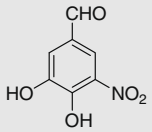
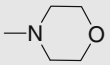
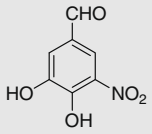
Entry	2	$-NR^4R^5$	Yield (%)	Mp, °C [lit]	^{1}H NMR, σ (DMSO-d_6, 400 MHz)
1a			88	265–267	7.29–7.33 (m, 5H), 7.41–7.45 (m, 5H), 7.61 (d, *J* = 2.0 Hz,1H), 7.83 (d, *J* = 2.0 Hz, 1H), 7.95 (s, 1H, C=CH)
1b*			89	217–219	2.31 (s, 3H), 6.96 (d, 1H), 7.25 (t, 1H), 7.46 (d, 1H), 7.49 (s, 1H), 7.84 (d, *J* = 2.0 Hz, 1H), 7.99 (d, *J* = 2.0 Hz, 1H), 8.16 (s, 1H, C=CH), 10.25 (s, 1H, –NH)
1c			95	211–214 [210–214][21]	3.75 (s, 3H), 6.94 (d, 2H), 7.56 (d, 2H), 7.83 (d, *J* = 1.6 Hz, 1H), 7.99 (d, *J* = 1.6 Hz, 1H), 8.14 (s, 1H, C=CH), 10.20 (s, 1H, –NH)
1d			98	244–247 [242–248][13]	7.20 (d, 1H), 7.40 (t, 1H), 7.60 (d, 1H), 7.82 (s, 1H), 7.84 (d, *J* = 2.0 Hz, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 8.18 (s, 1H, C=CH), 10.48 (s, 1H, –NH)
1e*			40	225–226	7.29 (t, 1H), 7.38 (t, 1H), 7.55 (d, 1H), 7.63 (d, 1H), 7.83 (d, *J* = 2.0 Hz, 1H), 8.00 (d, *J* = 2.0 Hz, 1H), 8.21 (s, 1H, C=CH), 9.95 (s, 1H, –NH)
1f*			42	217–218	3.90 (s, 3H), 7.21 (d, 1H), 7.29 (d, 1H), 7.40 (t, 1H), 7.57 (d, 1H), 7.79 (s, 1H), 7.88 (s, 1H, C=CH), 7.90 (d, 1H), 10.52 (s, 1H, –NH)
1g			82	231–234 [229–231][13]	3.95 (s, 3H), 7.20 (d, 1H), 7.41 (t, 1H), 7.60 (d, 1H), 7.83 (s, 1H), 7.94 (d, *J* = 1.6 Hz, 1H), 8.18 (d, *J* = 1.6 Hz, 1H), 8.27 (s, 1H, C=CH), 10.52 (s, 1H, –NH)

Table 1 (*continued*)

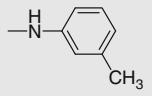
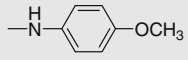
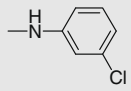
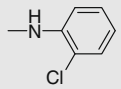
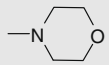
Entry	2	$-NR^4R^5$	Yield (%)	Mp, °C [lit]	^{1}H NMR, σ (DMSO-d_6, 400 MHz)
1h			67	207–208 [206–207][21]	1.55–1.63 (m, 6H), 3.51 (t, 4H), 7.63 (s, 1H, C=CH), 7.75 (d, J = 2.4 Hz, 1H), 7.90 (d, J = 2.4 Hz, 1H)
1i			78	186–190 [190–192][21]	3.59 (m, 4H), 3.62 (m, 4H), 7.68 (s, 1H, C=CH), 7.77 (d, J = 2.0 Hz, 1H), 7.93 (d, J = 2.0 Hz, 1H)
1j		$-NH_2$	53	300–303 [296–298][22]	7.73 (s, 1H, –NH), 7.77 (d, J = 2.0 Hz, 1H), 7.85 (s, 1H, –NH), 7.93 (d, J = 2.0 Hz, 1H), 8.05 (s, 1H, C=CH)

* New compound.

in the ^{1}H NMR spectrum (see Table 1), we believe that only one geometric isomer exists in each compound. Furthermore, by means of the X-ray diffractive analysis of single-crystal, the structure of compound **1f** was undoubtedly confirmed as trans-configuration (Fig. 1).[23] Based on the above facts, accordingly, these title compounds prepared in this paper were assumed to be in trans-geometry. The selectivity in configuration may derive from the repulsion between the substituted phenyl group and amide group, which are two bulky moieties bonding on C–C double bond.

The screening tests of antitumor activities in vitro for all title compounds and *cis*-diamminedichloroplatinum (DDP) as a positive control were carried out on human gastric carcinoma cell line BGC-823, human nasopharyngeal carcinoma cell line KB and human hepatoma cell line BEL-7402 with MTT assay. The IC_{50} values of

Table 2
Synthesis and characterizations of the intermediate cyanoacetamides

Entry	$-NR^4R^5$	Reaction time (h)	Yield (%)	Mp (°C) [lit]	Solvent for recryst.	IR σ (cm^{-1})	^{1}H NMR, δ $CDCl_3$
3k		6	52	154–156 [151–152][18]	CH_2Cl_2	2275, 1676	3.43 (s, 2H), 7.25–7.45 (m, 10H)
3l		4	61	134–137	CH_2Cl_2	3273, 2256, 1668	2.35 (s, 3H), 3.54 (s, 2H), 7.02–7.33 (m, 4H)
3m		1.5	73	128–131 [131][24a]	Petro. ether	3309, 2280, 1657	DMSO-d_6, 3.72 (s, 3H), 3.84 (s, 2H), 6.8–7.4 (m, 4H)
3n		4	73	135–137	THF	3273, 2256, 1668	DMSO-d_6, 3.92 (s, 2H), 7.15–7.74 (m, 4H)
3o		4	69	116–119 [118][24b]	CH_2Cl_2	3254, 2261, 1671	3.61 (s, 2H), 7.11–7.42 (m, 4H)
3p		7	60	87–89 [85–86][24c]	CH_2Cl_2	2256, 1643	1.57–1.65 (m, 6H), 3.38 (t, 2H), 3.47 (s, 2H), 3.55 (t, 2H)
3q		5	73	86–88 [85–86][18]	EtOH	2280, 1660	3.90–3.45 (m, 4H), 3.63 (t, 2H), 3.46 (t, 2H), 3.48(s, 2H)
3r	$-NH_2$	2	70	120–121 [119–120][19]	EtOH (95%)	3408, 3203, 2271, 1686	3.58 (s, 2H), 7.32 (br d, 1H), 7.64 (br d, 1H)

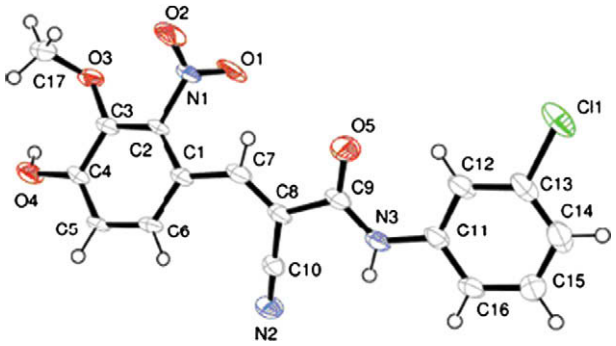


Figure 1. Molecular structure diagram of compound **1f** with 30% probability displacement ellipsoids.

Table 3
Values IC_{50} of all title compounds against BGC-823, KB and BEL-7402

Compound	BGC-823 (μg/mL)	KB (μg/mL)	BEL-7402 (μg/mL)
1a	>100	56.2	104
1b	5.6	13.1	12.5
1c	45.4	>100	29.2
1d	>100	40.1	72.7
1e	10.5	24	37.4
1f	>100	65.3	>100
1g	26.1	21.1	56.6
1h	>100	>100	>100
1i	>100	>100	>100
1j	>100	>100	>100
DDP	0.7	0.6	1.77

inhibitory cell growing for these compounds were summarized in Table 3.

In Table 3, it is clear that those compounds with phenyl or substituted phenyl in the amide moiety of the molecular structure(compound **1a**, **1b**, **1c**, **1d**, **1e**, 1f and **1g**) possess more strong inhibitory activities than that with alicyclic substituents (compound 1h and **1i**) or hydrogen (compound **1j**). This suggests that an aryl substituent in the molecular amide moiety maybe more preferred than an aliphatic or alicyclic substituent. Comparing their structures and IC_{50} values of compound **1b**–**1e**, we can conclude that the nature and position of substituent in the phenyl of amide moiety exert an effect on the molecule's inhibitory activities, and the m-methyl substituent in phenyl is more preferred than p-methoxy and m or o-chloro substituent. Besides, compound **1g** shows more strong inhibitory activities than compound **1d** and **1f**, which indicates that the substituents on the other phenyl bonding to the C–C double bond also affect its activities.

In these compounds, compound **1b** shows a better results than others—its IC_{50} values against BGC-823, KB and BEL-7402 reach to 5.6 μg/mL, 13.1 μg/mL and 12.5 μg/mL, respectively. So, it would be a valuable lead compound for further study, although its IC_{50} is larger approximately by an order of magnitude than that of the general antitumor agent DDP.

Inspired by the fact that Entacapone has selective COMT inhibitory activity and has been clinically used as anti-parkinsonism drug, we have carried out the screening test of inhibitory activities against acetylcholinesterase for these compounds. Unfortunately, all compounds showed a very limited activity—the inhibitory rate being under 7.38% in the concentration of 2.5×10^{-5} mol/L (Table 4). Maybe, this indicates that the aryl substituent in the amide moiety of these compounds should be replaced by the aliphatic substituent, such as the diethyl substituent in the Entacapone. The further investigation on the SAR is ongoing.

Table 4
Inhibitory rates of all title compounds against acetylcholinesterase

Compound	Concentration (mol/L)	Inhibitory rate (%)
1a	2.5×10^{-5}	7.38
1b	2.5×10^{-5}	2.73
1c	2.5×10^{-5}	4.64
1d	2.5×10^{-5}	4.37
1e	2.5×10^{-5}	0.95
1f	2.5×10^{-5}	2.19
1g	2.5×10^{-5}	1.64
1h	2.5×10^{-5}	0.27
1i	2.5×10^{-5}	3.00
1j	2.5×10^{-5}	1.09

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.081.

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23. *Crystals of compound* **1f** *for structural analysis were obtained by slow evaporation of acetonitrile. Crystal data*: $C_{17}H_{12}N_3ClO_5$, $M = 373.75$, triclinic, $a = 8.166(3)$, $b = 10.320(4)$, $c = 10.696(4)$ Å, $\alpha = 90.040(5)$, $\beta = 108.302(5)$, $\gamma = 102.979(5)°$, $U = 808(5)$ Å^3, $T = 293(2)$ K, space group $P\bar{1}$, $Z = 2$, $D_c = 1.536$ g/cm^3, μ(Mo-Kα) = 0.273 mm^{-1}, 3460 reflections measured, 2860 unique ($R_{int} = 0.065$) which were used in all calculations. Fine $R_1 = 0.091$, $wR(F^2) = 0.256$ (all data). Full crystallographic details of **1f** have been deposited at the Cambridge Crystallographic Data Center and allocated the deposition number CCDC 634551.

24. Sadtler Research Laboratories, SADTLER Standard Infrared Grating Spectra, Philadelphia: Researchers, Editors and Publishers, 1976, (a) 50056K, (b) 50057K, and (c) 45799P.

25. *Representative synthetic procedure for compound* **1b**: (a) To a mixture of 5.4 g(50 mmol) 3-methylaniline in 15 mL dichloromethane was added drop wise a solution of 4.2 g (50 mmol) cyanoacetic acid in 30 mL dichloromethane at room temperature. After the addition, a solution of 10.5 g (50 mmol) DCC in 20 mL dichloromethane was added to the mixture. Then the mixture was heated to reflux until the TLC test shows the reaction come to the end. Cool the mixture to room temperature, then the resulted precipitate was filtered and washed with dichloromethane. The filtrate was concentrated under vacuum to give crude product, which was recrystallized with dichloromethane to afford 5.3 g crystal product *N*-(3-methylphenyl)-2-cyanoacetamide, yield 61%, mp 134–139 °C. (b) To a mixture of 1.8 g (10 mmol) *N*-(3-methylphenyl)-2-cyanoacetamide, 2.0 g (11 mmol) 3,4-dihydroxy-5-nitrobenzaldehyde and 50 mL toluene were added 0.35 mL piperidine and 1.3 mL acetic acid. Then the mixture was heated to reflux until the TLC test shows the reaction is complete. Cooled the mixture to room temperature, then the precipitate was filtered, washed and then recrystallized with absolute alcohol to afford 3.0 g crystal compound **1b**, yield 89%, mp 217–219 °C. IR(KBr, cm^{-1}): 3368, 3315, 2215, 1666, 1614, 1582, 1546, 1490, 1249, 738, 625. ^{1}H NMR, δ (DMSO-d_6, 400 MHz): 2.31 (s, 3H), 6.96 (d, J = 7.6 Hz, 1H), 7.25 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.49 (s, 1H), 7.84 (d, J = 2.0 Hz, 1H), 7.99 (d, J = 2.0 Hz, 1H), 8.16 (s, 1H, C=CH), 10.25 (s, 1H, –NH). EI-MS: 339 (M^+), 321, 263, 233, 185, 159, 131, 107, 91, 77, 65, 53. Elemental analysis (calcd data in parentheses): C: 60.25 (60.18), H: 3.78 (3.86), N: 12.29 (12.38). For other experimental details, see Supplementary data.