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Syntheses and Antimicrobial Activities of α-Isocyanoacrylic Acid Derivatives[1]

Mamoru Suzuki, Ken-ichi Nunami, Kazuo Matsumoto, Naoto Yoneda,[2a] Osamu Kasuga, Hirotsugu Yoshida, and Totaro Yamaguchi[2b]

Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd.[2a] *and Microbiological Research Laboratory, Tanabe Seiyaku Co., Ltd.*[2b]

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A series of α-isocyanoacrylic acid derivatives were prepared *via* the α-formylaminoacrylic acid derivatives, which were obtained by the reaction of isocyanoacetic acids and carbonyl compounds, in order to examine their antimicrobial activities. The structure-activity relationships are presented; it was found that (*Z*)-α-isocyanoacrylic acid esters possessing halogeno phenyl or thienyl groups exhibited fairly strong antifungal activities. Of these, (*Z*)-methyl α-isocyano-β-(2-thienyl)acrylates (**5s** and **5u**) showed the highest activity (1.56 μg/ml MIC) against *Tricophyton mentagrophytes*.

Keywords——formylaminoacrylic acids; isocyanoacrylic acids; antimicrobial activity; *Tricophytons*; structure–activity relationship

Isocyano compounds possess unusual structures and interesting chemical properties[3]: in particular, the reactivity of isocyanoacetic acids possessing polyfunctional moieties is of current interest in amino acid and heterocyclic chemistry.[4] On the other hand, isocyano compounds have been frequently isolated from natural products such as marine sponges[5] and bacterias,[6] and some of them exhibit biological activities. Of these, α,β-unsaturated compounds such as xanthocillins from *Penicillium notatum*[7] or *Aspergillus candidus*,[8] A32390A from an unidentified species of *Pyrenochaeta*,[9] and B371 from *Pseudomonas* species,[10] possess interesting antibacterial and/or antifungal activities. Thus, α,β-unsaturated isocyano compounds are of considerable interest from a pharmacological viewpoint.

On the other hand, in a series of studies on syntheses of amino acids and related compounds using isocyanoacetate, α-isocyanoacrylic acids, which are analogs of unsaturated amino acids, were found to be reactive intermediates for the syntheses of pyrrole,[11] pyrrolo[1,2-*c*]-

1) This paper constitutes Part XXII of the series entitled "Synthesis of Amino Acids and Related Compounds," Part XXI: K. Matsumoto, Y. Ozaki, T. Iwasaki, H. Horikawa, and M. Miyoshi, *Experientia*, **35**, 850 (1979).
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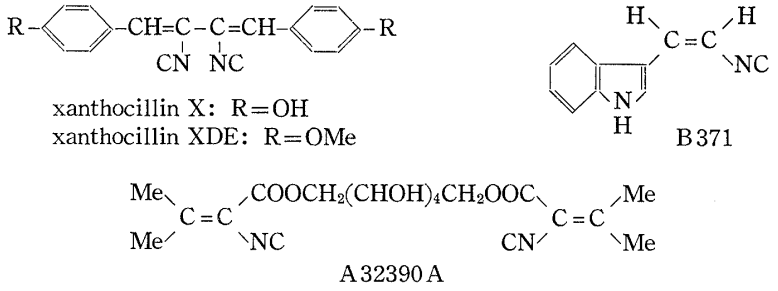

Fig. 1

pyrimidine,[12] and amidine derivatives.[13] We considered that these isocyano compounds themselves may possess biological activities; in this work, various α-isocyanoacrylic acid derivatives were systematically synthesized in order to investigate the structure-activity relationship for antimicrobial activities.

Synthesis

Methyl α-isocyanoacrylates (**5a**—**y**) were synthesized *via* methyl α-formylaminoacrylates (**3a**—**y**) by a method similar to that described by Schöllkopf *et al.*[14] and the authors,[13] as shown in Chart 1. Namely, the reaction of methyl isocyanoacetate (**2a**) with carbonyl compounds (**1**) was carried out in the presence of sodium hydride (NaH) in THF. Purification by recrystallization or column chromatography gave (*E*)- or (*Z*)-formylamino compounds (**3a**—**y**). The geometric structures of these compounds were determined from the anisotropic effect of the adjacent benzene ring in the nuclear magnetic resonance (NMR) spectra.[13-15] Subsequently, **3a**—**y** were treated with phosphoryl chloride and triethylamine to afford the corresponding methyl α-isocyanoacrylates (**5a**—**y**) (see Tables III and IV).

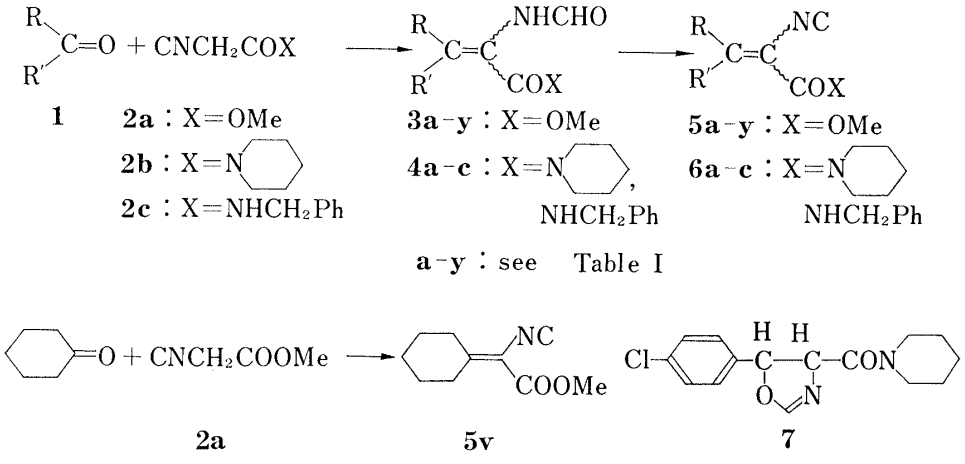

Chart 1

In the case of acrylic acid amide compounds, for example, (*Z*)-α-formylamino-4-chlorocinnamoylpiperidine (**4a**) was synthesized by the reaction of 4-chlorobenzaldehyde with isocyanoacetylpiperidine (**2b**) in the presence of NaH in DMF followed by recrystallization from AcOEt. On the other hand, the use of THF instead of DMF as a solvent interestingly

12) M. Suzuki and N. Yoneda, *J. Org. Chem.*, **41**, 1482 (1976).
13) M. Suzuki, K. Nunami, T. Moriya, K. Matsumoto, and N. Yoneda, *J. Org. Chem.*, **43**, 4933 (1978).
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15) R. Glaser, S. Geresh, U. Schöllkopf, and R. Meyer, *J. Chem. Soc., Perkin I*, **1979**, 1746.

gave only 5-(4-chlorophenyl)-2-oxazoline-4-carboxamoylpiperidine (**7**) in 80% yield.[16] These results were suggestive of lower reactivity of the α-carbon in the isocyanoacetamide in comparison with the isocyanoacetate.[17] Next, compound (**4a**) was converted to the corresponding α-isocyanoacrylic acid amide (**6a**) using phosphoryl chloride and triethylamine. Other isocyano compounds (**6b** and **6c**) were similarly prepared, as shown in Chart 1 (see Table IV).

On the other hand, a more convenient method for the synthesis of isocyanoacrylic acid compounds is required in practice. Although attempts to prepare isocyanoacrylates by a direct method using the reaction of **2a** with aldehydes have been made under various conditions, the desired products have not been obtained.[11,13]

In the present study, a direct synthesis of the unsaturated isocyano compounds was attempted using cyclic ketones such as cyclohexanone. It was found that the reaction proceeded smoothly in the presence of pyrrolidine in DMF to afford methyl α-isocyanocyclohexylideneacetate (**5v**) directly in 41% yield. This method was extended to other carbonyl compounds (**1**), but only amidine compounds were isolated,[13] due to insertion of the amino group into the isocyano group.

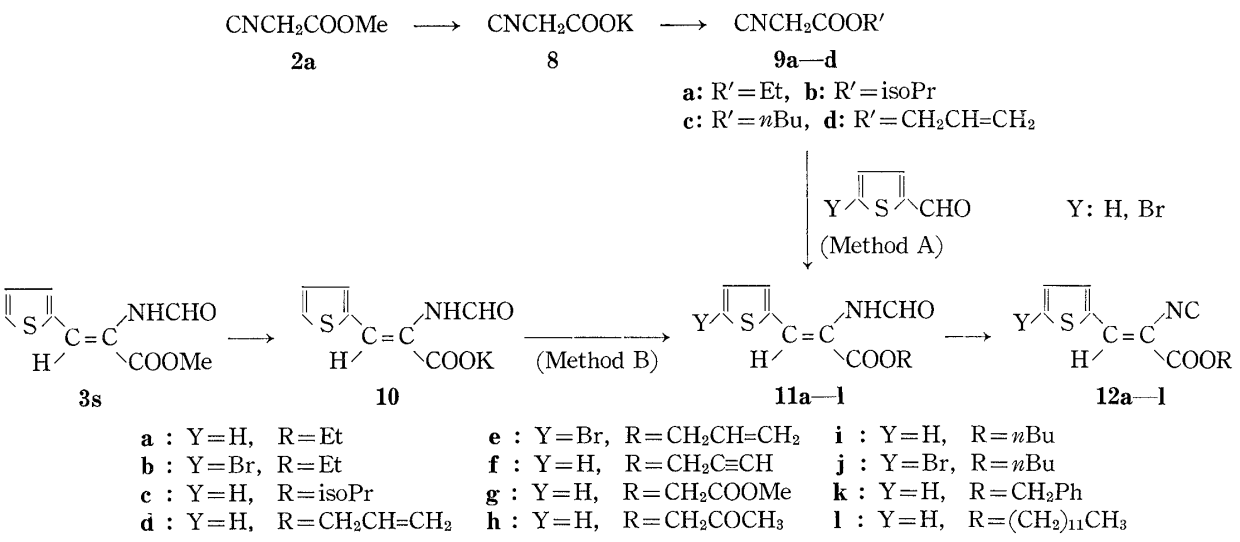

Chart 2

Moreover, thienylacrylic acid derivatives possessing various kinds of ester moieties (**12a—k**) were synthesized to examine the effects of various ester groups on the antimicrobial activity, using method A and method B shown in Chart 2. In the case of method A, at the first stage, the corresponding isocyanoacetic acid ester (**9**) was prepared from potassium isocyanoacetate (**8**) and alkylhalides in DMF, and then, as described above, α-isocyanoacrylate derivatives (**12a—k**) were formed *via* the α-formylamino compounds (**11a—k**) using these isocyanoacetates (**9**). On the other hand, in method B, (*Z*)-methyl α-formylamino-β-(2-thienyl)acrylate (**3s**) was first converted to the potassium salt (**10a**) by treatment with KOH–MeOH. Subsequently, esterification with alkyl halides followed by treatment with phosphoryl chloride gave the corresponding (*Z*)-α-isocyanoacrylates (**12d—k**). These results are summarized in Tables V and VI.

Biological Activity

All of the α-isocyanoacrylates obtained in this study were screened for antibacterial and antifungal activities against *Candida albicans*, *Trichophyton mentagrophytes*, *Aspergillus*

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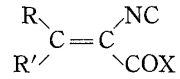
17) Y. Ozaki, K. Matsumoto, and M. Miyoshi, *Agr. Biol. Chem.* (Tokyo), **42**, 1565 (1978).

Table I. Antimicrobial Activities of α-Isocyanoacrylic Acid Derivatives (**5**, **6**, **12**)



Compd.	R	R′	X	Minimum inhibitory concentration (μg/ml)					
				C. alb.[a]	*T. men.*[b]	*A. bla.*[c]	*p. cit.*[d]	*S. aur.*[e]	*E. coli*[f]
5a	Ph	H	OMe	12.5	12.5	50	50	10	10
5b	α-Naphthyl	H	OMe	100	6.25	>100	>100	10	50
5c	2-F-Ph	H	OMe	>50	>50	>50	>50	>50	>50
5d	4-F-Ph	H	OMe	6.25	6.25	25	25	10	10
5e	2-Cl-Ph	H	OMe	3.12	6.25	50	50	6.25	25
5f	3-Cl-Ph	H	OMe	12.5	6.25	25	>100	3.12	100
5g	4-Cl-Ph	H	OMe	3.12	3.12	12.5	12.5	10	50
5h	2-Br-Ph	H	OMe	6.25	25	50	100	10	50
5i	2,4-diCl-Ph	H	OMe	6.25	6.25	50	>100	10	>50
5j	3,4-diCl-Ph	H	OMe	>100	6.25	>100	>100	10	10
5k	2,6-diCl-Ph	H	OMe	6.25	25	>100	>100	10	50
5l	4-Me-Ph	H	OMe	6.25	12.5	50	50	10	>50
5m	4-iso-Pr-Ph	H	OMe	50	50	50	>50	50	>50
5n	4-NO_2-Ph	H	OMe	100	12.5	100	100	50	50
5o	3,4-OCH_2O-Ph	H	OMe	3.12	3.12	12.5	>100	10	10
5p	H	3,4-OCH_2O-Ph	OMe	>50	>50	>50	>50	>50	>50
5q	4-Me_2N-Ph	H	OMe	>100	12.5	>100	12.5	>50	>50
5r	2-Furyl	H	OMe	12.5	25	>100	25	50	>50
5s	2-Thienyl	H	OMe	6.25	1.56	12.5	6.25	10	10
5t	H	2-Thienyl	OMe	>100	>100	>100	>100	>50	>50
5u	2-(5-Br)thienyl	H	OMe	12.5	1.56	12.5	12.5	1.56	50
5v	-$(CH_2)_5$-		OMe	>50	>50	>50	>50	>50	>50
5w	Ph	Ph	OMe	>100	>100	>100	>100	50	>50
5x	4-Cl-Ph	Me	OMe	50	>50	>50	>50	>50	>50
5y	2-Thienyl	Me	OMe	50	>50	>50	>50	>50	>50
6a	4-Cl-Ph	H		>50	>50	>50	>50	>50	>50
6b	2-Thienyl	H	$NHCH_2Ph$	>50	>50	>50	>50	>50	>50
6c	3,4-di-Cl-Ph	H		>50	50	>50	>50	10	>50
12a	2-Thienyl	H	OEt	6.25	3.12	25	12.5	3.12	50
12b	2-(5-Br)thienyl	H	OEt	12.5	3.12	50	>100	3.12	200
12c	2-Thienyl	H	OisoPr	12.5	6.25	50	50	3.12	50
12d	2-Thienyl	H	$OCH_2CH=CH_2$	12.5	6.25	25	50	6.25	25
12e	2-(5-Br)thienyl	H	$OCH_2CH=CH_2$	100	12.5	>100	>100	6.25	>200
12f	2-Thienyl	H	$OCH_2C\equiv CH$	12.5	1.56	12.5	25	3.13	50
12g	2-Thienyl	H	OCH_2COOMe	50	6.25	>100	>100	12.5	100
12h	2-Thienyl	H	OCH_2COCH_3	>50	>50	>50	>50	>50	>50
12i	2-Thienyl	H	O*n*Bu	50	25	50	>50	25	100
12j	2-(5-Br)thienyl	H	O*n*Bu	>50	>50	>50	>50	>50	>50
12k	2-Thienyl	H	OCH_2Ph	>50	>50	>50	>50	>50	>50
12l	2-Thienyl	H	$O(CH_2)_{11}CH_3$	>50	>50	>50	>50	>50	>50

a) *Candida albicans.*
b) *Trichophyton mentagrophytes.*
c) *Aspergillus blavus.*
d) *Penicillium citrinum.*
e) *Staphylococcus aureus.*
f) *Escherichia coli.*

flavus, *Penicillium citrinum*, *Staphylococcus aureus*, and *Escherichia coli* by the method described in the experimental section. These results are summarized in Table I.

As expected, a series of (*Z*)-methyl α-isocyanocinnamates (**5a**—**o** and **5q**) exhibited antimicrobial activities against various microbes: in particular, the antifungal activity against *Tricophyton mentagrophytes* or *Candida albicans* was fairly strong. Of these compounds, the chlorinated compounds (**5e**—**g**) showed higher activities, whereas other halogeno compounds such as fluoro (**5c** and **5d**) and bromo (**5h**) derivatives did not exhibit marked activity. Among the chloro compounds, *para* substitution increased the antifungal activity in comparison with the corresponding *ortho* and *metha* analogs. Unfortunately, the dichloro compounds (**5i**—**k**) showed rather weaker activities. The 3,4-methylenedioxy group (**5o**) significantly increased the antifungal activity.

Interestingly, the α-isocyanoacrylate compounds (**5r**, **5s**, and **5u**) substituted with furan and thiophene moieties with aromaticity similar to that of the phenyl group also showed marked antifungal activity; (*Z*)-methyl α-isocyano-β-(2-thienyl)acrylates (**5s** and **5u**) were the most active of the compounds tested in this study. On the other hand, the corresponding (*E*)-isomer (**5t**) was inactive; a similar result was also obtained with the phenyl compound **5p**. Thus, in order to investigate the effect of ester groups on the activity, the methyl ester moiety of **5s** and **5u**, in which the strongest antifungal activity was observed, was replaced with various other ester groups. As shown in Table I (**12a**—**l**), similar high activity was exhibited by low alkyl esters such as ethyl or propargyl esters, while an increase of the carbon chain length reduced the activity. Moreover, compound (**6b**) with a benzyl amide group instead of the methyl group and compound (**5y**) bearing a methyl group at the β-position (R′) of the acrylate (**5s**) showed greatly reduced antimicrobial activities. A similar tendency was also observed in the phenyl derivatives (**5w**, **5x**, **6a**, and **6c**). A cyclohexylidene derivative (**5v**), which is structurally unique, was inactive.

Judging from these results, (*Z*)-α-isocyanoacrylic acid ester structures which possess a halogeno phenyl or thienyl group and have no substituent at the β-position seem to be essential for antimicrobial activity. Among these compounds, (*Z*)-methyl α-isocyano-β-(2-thienyl)-acrylates (**5s** and **5u**) exhibited the strongest activities against *Tricophyton mentagrophytes* (MIC 1.56 μg/ml).

We also investigated the susceptibility distribution to the methyl α-isocyanoacrylate derivatives (**5d**, **5g**, and **5s**), which showed strong antimicrobial activities, using clinically isolated strains (19 samples) of *Tricophyton asteroides*. The MICs of these compounds against most of the strains were about 3.12 μg/ml, as shown in Table II. These compounds exhibited weak toxicity; the maximum doses tolerated in rats were above 300 mg/kg (*i.p.*).

Table II. Susceptibility Distribution of Clinically Isolated Strains (19 Samples) of *Trichophyton Asteroides* to α-Isocyanoacrylic Acid Derivatives (**5d**, **g**, **s**)

Compd.	Minimum inhibitory concentration (μg/ml)						Acute toxicity mg/kg, *i.p.* (rat)
	25	12.5	6.25	3.12	1.56	0.78	
5d	0	0	2	15	0	2	>300
5g	0	0	0	12	4	1	>300
5s	0	0	0	13	3	3	>300

Experimental

Melting points (which were measured with a Yamato melting point apparatus) and boiling points are uncorrected. The infrared (IR) spectra were recorded on a Shimadzu IR-27G infrared spectrophotometer. The NMR spectra were obtained using a Hitachi Perkin-Elmer R-20A high resolution NMR spectrometer

with tetramethylsilane as an internal standard. Column chromatography was carried out on silica gel (Kieselgel 60, 0.063—0.200 mm, E. Merck).

General Procedure for the Preparation of Methyl α-Formylaminoacrylates (3a—y)——These compounds were prepared by a method similar to that described by Schöllkopf *et al.* and the present authors.[13,14] A mixture of methyl isocyanoacetate (**2a**, 2 g, 0.02 mol) and the appropriate aldehyde or ketone (**1**, 0.02 mol) in THF (20 ml) was added dropwise to a suspension of NaH (65% in oil) (0.89 g, 0.024 mol) in THF (20 ml) at 30—35°. After stirring for 2 hr at room temperature, 10% AcOH (20 ml) was added to the mixture under cooling and the solvent was removed under reduced pressure. The residue was extracted with $CHCl_3$ and the extract was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. Et_2O was added to the resulting residue. The insoluble precipitates were filtered off by suction, and recrystallization from an appropriate solvent gave the (*Z*)-methyl α-formylaminoacrylate.[13-15] On the other hand, the (*E*)-isomer was isolated from the Et_2O filtrate by silica gel chromatography, eluting with $CHCl_3$–AcOEt (10: 1). These results are summarized in Table III.

General Procedure for the Preparation of Methyl α-Isocyanoacrylates (5a—y)——These compounds were

Table III. Formation of Methyl α-Formylaminoacrylates (**3**) and α-Formylaminoacrylic Acid Amides (**4**)

Compd.	Yield (%)	mp (°C)	Recryst. solvent	IR ν_{max}^{Nujol} cm^{-1}	Formula	Analysis (%) Calcd (Found) C	H	N
3a	37	90— 92	Benzene	3230, 1710, 1650	$C_{11}H_{11}NO_3$	64.38 (64.50	5.40 5.47	6.83 6.77)
3b	60	139—141	AcOEt	3250, 1720, 1655	$C_{15}H_{13}NO_3$	70.58 (70.18	5.13 5.11	5.49 5.42)
3c	44	93— 94	Et_2O	3230, 1720, 1660	$C_{11}H_{10}FNO_3$	59.19 (59.19	4.51 4.50	6.27 6.24)
3d	39	133—134	AcOEt–Et_2O	3220, 1725, 1660	$C_{11}H_{10}FNO_3$	59.19 (59.07	4.51 4.51	6.27 6.22)
3e	42	115—116	Benzene	3260, 1725, 1658	$C_{11}H_{10}ClNO_3$	55.13 (55.42	4.21 4.30	5.84 5.96)
3f	46	140—141	Benzene	3210, 1725, 1660	$C_{11}H_{10}ClNO_3$	55.13 (55.23	4.21 4.31	5.84 5.80)
3h	60	140—141	Benzene	3250, 1720, 1660	$C_{11}H_{10}BrNO_3$	46.50 (46.38	3.55 3.52	4.93 4.91)
3i	62	140—142	Benzene	3210, 1725, 1656	$C_{11}H_9Cl_2NO_3$	48.20 (48.32	3.31 3.46	5.11 5.07)
3j	52	157—158	AcOEt	3240, 1710, 1655	$C_{11}H_9Cl_2NO_3$	48.20 (48.07	3.31 3.42	5.11 5.04)
3k	48	99—100	EtOH–H_2O	3150, 1725, 1670, 1640	$C_{11}H_9Cl_2NO_3$	48.20 (48.17	3.31 3.33	5.11 5.03)
3m	37	90— 92	Benzene–hexane	3140, 1711, 1640	$C_{14}H_{17}NO_3$	68.00 (68.11	6.93 7.01	5.66 5.62)
3n	32	157—159	AcOEt	3250, 1720, 1670	$C_{11}H_{10}N_2O_5$	52.80 (52.86	4.03 4.14	11.19 11.54)
3r	37	85— 87	iso-Pr_2O	3240, 1728, 1653	$C_9H_9NO_4$	55.38 (55.21	4.64 4.70	7.17 7.17)
3u	35	166—167	AcOEt	3300, 1710, 1660, 1630	$C_9H_8BrNO_3S$	37.26 (37.12	2.78 2.69	4.83 4.81)
3w	76	188—189	MeOH	3200, 1725, 1650	$C_{17}H_{15}NO_3$	72.58 (72.27	5.37 5.43	4.98 4.87)
3x	32	129—130	Benzene	3200, 1728, 1655	$C_{12}H_{12}ClNO_3$	56.81 (56.91	4.77 4.75	5.52 5.42)
3y	38	125—127	AcOEt	3300, 1710, 1670	$C_{10}H_{11}NO_3S$	53.32 (53.19	4.92 4.90	6.22 6.15)
4a	39	168—169	AcOEt	3160, 1700, 1642, 1615	$C_{15}H_{17}ClN_2O_2$	61.54 (61.50	5.85 5.96	9.57 9.62)
4b	58	182—184	MeOH	3430, 1685, 1655, 1610	$C_{15}H_{14}N_2O_2S$	62.91 (63.09	4.92 4.89	9.78 9.76)
4c	53	171—172	Benzene	3270, 1675, 1650, 1603	$C_{15}H_{16}Cl_2N_2O_2$	55.06 (55.15	4.93 5.13	8.56 8.64)

prepared by a method similar to that described in the previous reports.[13,18] A solution of phosphoryl chloride (4.6 g, 0.03 mol) in CH_2Cl_2 (4 ml) was added dropwise to a mixture of **3** (0.02 mol) and triethylamine (9.8 ml, 0.07 mol) in CH_2Cl_2 (20 ml) at 25—30° over a period of 20 min with stirring. After stirring for 1 hr at room temperature, 15% K_2CO_3 (30 ml) was added to the mixture under cooling. The separated organic layer was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The resulting residue was chromatographed on silica gel (100 g), eluting with $CHCl_3$, to afford **5**. These results are summarized in Table IV.

Direct Synthesis of Methyl α-Isocyanocyclohexylideneacetate (5v)——A solution of cyclohexanone (1.96 g, 0.02 mol) and **2a** (2 g, 0.02 mol) in DMF (10 ml) was added dropwise to a solution of piperidine (1.7 g, 0.02 mol) in DMF (10 ml) at room temperature. After stirring for 3 hr at the same temperature, the solution was neutralized with 10% AcOH under cooling and the solvent was removed *in vacuo*. The residue was extracted

Table IV. Formation of Methyl α-Isocyanoacrylates (**5**) and α-Isocyanoacrylic Acid Amides (**6**)

Compd.	Yield (%)	mp (°C)	Recryst. solvent	IR ν_{max}^{Nujol} cm^{-1}	Formula	Analysis (%) Calcd (Found) C	H	N
5a	84	58— 60	Benzene–hexane	2120, 1735, 1622	$C_{11}H_9NO_2$	70.58 (70.39	4.85 4.80	7.48 7.44)
5b	89	82— 84	Benzene–hexane	2110, 1730, 1625	$C_{15}H_{11}NO_2$	75.94 (75.86	4.67 4.87	5.90 5.76)
5c	88	60.5— 61	Et_2O–hexane	2110, 1727	$C_{11}H_8FNO_2$	64.38 (64.23	3.92 3.98	6.82 6.72)
5d	67	98— 99	Hexane	2110, 1725, 1625	$C_{11}H_8FNO_2$	64.38 (64.35	3.92 3.96	6.82 6.72)
5e	87	86— 87	Et_2O	2110, 1727	$C_{11}H_8ClNO_2$	59.61 (59.70	3.64 3.77	6.32 6.26)
5f	74	94— 96	Et_2O	2110, 1727	$C_{11}H_8ClNO_2$	59.61 (59.56	3.64 3.79	6.32 6.21)
5h	62	77— 78	Benzene–hexane	2120, 1730	$C_{11}H_8BrNO_2$	49.65 (49.60	3.03 3.12	5.26 5.18)
5i	88	87— 89	iso-Pr_2O	2100, 1730, 1620	$C_{11}H_7Cl_2NO_2$	51.59 (51.62	2.76 3.03	5.47 5.45)
5j	81	116—118	Benzene	2100, 1730, 1620	$C_{11}H_7Cl_2NO_2$	51.59 (51.46	2.76 3.02	5.47 5.36)
5k	91	76— 77	Benzene–hexane	2120, 1745	$C_{11}H_7Cl_2NO_2$	51.59 (51.50	2.76 2.81	5.47 5.39)
5m	94	Syrup[a]		2100, 1730, 1620, 1605[b]	$C_{14}H_{15}NO_2$	73.34 (73.22	6.59 6.65	6.11 6.02)
5n	80	132—135	Benzene–hexane	2110, 1730, 1623	$C_{11}H_8N_2O_4$	56.89 (56.73	3.47 3.53	12.06 12.00)
5r	76	59— 60	iso-Pr_2O	2240, 1723, 1618	$C_9H_7NO_3$	61.03 (60.83	3.98 4.12	7.90 7.78)
5u	72	113—114	Benzene–hexane	2100, 1730, 1610	$C_9H_6BrNO_2S$	39.72 (39.59	2.22 2.11	5.15 5.03)
5w	93	96— 98	Benzene–hexane	2100, 1730, 1585	$C_{17}H_{13}NO_2$	77.55 (77.28	4.98 5.16	5.32 5.29)
5x	98	Syrup[a]		2100, 1730,[b] 1610	$C_{12}H_{10}ClNO_2$	61.16 (61.01	4.28 4.28	5.94 5.94)
5y	97	55— 57	Benzene–hexane	2100, 1720, 1570	$C_{10}H_9NO_2S$	57.95 (57.79	4.38 4.39	6.76 6.70)
6a	66	51— 52	Hexane	2100, 1650, 1590	$C_{15}H_{15}ClN_2O$	65.57 (65.38	5.50 5.56	10.20 10.08)
6b	56	135—137	Benzene–hexane	2100, 1660, 1605	$C_{15}H_{12}N_2OS$	63.36 (63.18	4.25 4.31	9.85 9.75)
6c	90	76— 78	Benzene–hexane	2100, 1648, 1610	$C_{15}H_{14}Cl_2N_2O$	58.27 (58.21	4.56 4.60	9.06 9.03)

a) Purified by column chromatography.
b) Taken as a film.

18) U. Schöllkopf, R. Harms, and D. Hoppe, *Ann. Chem.*, **1973**, 611.

with AcOEt and the extract was washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*. The oily residue was distilled to give **5v** as a colorless oil (1.47 g, 41%), bp 83° (2 mmHg) [lit.[19] bp 83° (2 mmHg)].

Synthesis of 5-(4-Chlorophenyl)-2-oxazoline-4-carboxamoylpiperidine (7)——A mixture of 4-chlorobenzaldehyde (1.4 g, 0.01 mol) and isocyanoacetylpiperidine[17,20] (**2b**, 1.52 g, 0.01 mol) in THF (10 ml) was added dropwise to a suspension of NaH (65% in oil) (0.44 g, 0.012 mol) in THF (10 ml) at 30—35°. After stirring for 2 hr at room temperature, 10% AcOH (10 ml) was added to the mixture under cooling and the solvent was removed under reduced pressure. The residue was extracted with AcOEt and the extract was washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*. Recrystallization of the resulting crystals from Et_2O–hexane gave **7** as colorless prisms (2.34 g, 80%), mp 118—119°. IR ν_{max}^{Nujol} cm^{-1}: 3080, 1640, and 1620. NMR (in $CDCl_3$) δ: 7.28 (4H, s, arom-H), 6.95 (1H, d, $J=3$ Hz, C_2–H), 6.16 (1H, d, $J=8$ Hz), 4.60 (1H, dd, $J=8$ Hz, C_4–H), 4.00—3.10 (4H, broad, $2\times CH_2$), and 1.61 (6H, broad s, $3\times CH_2$).[21] *Anal.* Calcd for $C_{15}H_{17}ClN_2O_2$: C, 61.54; H, 5.85; Cl, 12.11; N, 9.57. Found: C, 61.39; H, 5.91; Cl, 11.92; N, 9.45.

Typical Procedure for the Preparation of (*Z*)-α-Formylaminoacrylic Acid Amides (4a—c)——A mixture of **2b** (1.1 g, 0.007 mol) and 4-chlorobenzaldehyde (1.0 g, 0.007 mol) in DMF (10 ml) was added dropwise to a suspension of NaH (65% in oil) (0.33 g, 0.009 mol) in DMF (10 ml) at 30—35°. After stirring for 2 hr at room temperature, the solution was neutralized with 10% AcOH under cooling and the solvent was removed under reduced pressure. The residue was extracted with $CHCl_3$ and the extract was washed with H_2O, dried over Na_2SO_4, and concentrated *in vacuo*. The resulting residue was triturated with iso-Pr_2O to give a mixture of (*E*)- and (*Z*)-α-formylamino-4-chlorocinnamoylpiperidine as crystals (1.55 g, 71%). Recrystallization of these crystals from AcOEt gave only (*Z*)-**4a** as colorless needles (0.8 g, 39%). NMR (in DMSO-d_6) δ: 8.10 (1H, s, CHO), 7.91 (1H, broad s, NH), 7.48 (4H, s, arom-H), 6.08 (1H, s, –CH=), 3.70—3.30 (4H, m, $2\times CH_2$), and 1.80—1.20 (6H, m, $3\times CH_2$). **4b** and **4c** were obtained by a similar method. The results are summarized in Table III.

General Procedure for the Preparation of (*Z*)-α-Isocyanoacrylic Acid Amides (6a—c)——**4a—c** were treated according to the general procedure for the preparation of **5a—y** to afford **6a—c**, which were recrystallized from an appropriate solvent to obtain the (*Z*)-isomer; the results are summarized in Table IV.

Typical Procedure for the Preparation of Alkyl Isocyanoacetates (9a—d)——A solution of potassium isocyanoacetate[22] (**8**, 2.46 g, 0.02 mol) and allyl bromide (2.42 g, 0.02 mol) in DMF (15 ml) was stirred for 3 hr at 55—60°. The reaction mixture was concentrated *in vacuo* and the resulting residue was extracted with AcOEt. The extract was washed with H_2O, dried over $MgSO_4$, and concentrated *in vacuo*. The oily residue was distilled to give allyl isocyanoacetate (**9d**) as a colorless oil (2.3 g, 92%), bp 80° (4 mmHg). IR ν_{max}^{film} cm^{-1}: 2160, 1760, and 1670. NMR (in $CDCl_3$) δ: 6.25—5.60 (1H, m, –CH=C), 5.50—5.12 (2H, m, CH_2=), 4.70 (2H, d, $J=6$ Hz, OCH_2), and 4.37 (2H, s, NCH_2). Other esters (**9a—c**) were synthesized by the reaction of **8** with appropriate alkyl halides in a similar way. **9a**: 90%, bp 76—78° (4 mmHg) [lit.,[23] bp 76—78° (4 mmHg)]. **9b**: 92%, bp 70—72° (4 mmHg).[24] **9c**: 95%, bp 78—80° (4 mmHg). IR ν_{max}^{film} cm^{-1}: 2160 and 1755. NMR (in $CDCl_3$) δ: 4.25 (2H, s, NCH_2), 4.21 (2H, t, $J=7$ Hz, OCH_2), 1.90—1.20 (4H, m, $2\times CH_2$), and 0.95 (3H, t, $J=7$ Hz, CH_3).

Synthesis of (*Z*)-Potassium α-Formylamino-β-(2-thienyl)acrylate (10)——Compound **3s** (2.11 g, 0.01 mol) was added to a solution of KOH (1.68 g, 0.03 mol) in EtOH (30 ml), and the mixture was stirred for 5 hr at 50°. The solution was cooled to 0° and the resulting crystals were filtered off by suction. Recrystallization from EtOH gave **10** as colorless needles (2 g, 85%), mp 193° (dec.). IR ν_{max}^{KBr} cm^{-1}: 3250, 1758, and 1575. NMR (in D_2O) δ: 8.08 (1H, s, CHO) and 7.60—7.00 (4H, m, –CH= and arom-H). *Anal.* Calcd for $C_8H_6KNO_3S$: C, 40.83; H, 2.57; N, 5.95; S, 13.63. Found: C, 40.59; H, 2.67; N, 5.79; S, 13.48.

General Procedure for the Preparation of (*Z*)-Alkyl α-Formylamino-β-(2-thienyl)acrylates (11a—l). Typical Examples: Method A. (*Z*)-Ethyl α-Formylamino-β-[2-(5-bromo)thienyl]acrylate (11b)——A solution of **9a** (2.26 g, 0.02 mol) and 5-bromothien-2-aldehyde (3.82 g, 0.02 mol) in THF (30 ml) was added dropwise to a suspension of NaH (65% in oil) (0.89 g, 0.024 mol) in THF (20 ml) at 30—35°. The reaction mixture was treated according to the general procedure for the preparation of **3**, and recrystallization from AcOEt–iso-Pr_2O afforded **11b** as colorless needles (1.82 g, 30%), mp 137—138°. NMR (in $CDCl_3$) δ: 8.31 (1H, s, CHO), 8.40—8.00 (1H, broad, NH), 7.61 (1H, s, –CH=), 7.20—6.90 (2H, m, arom-H), 4.26 (2H, q, $J=7$ Hz, CH_2), and 1.32 (3H, t, $J=7$ Hz, CH_3).

Method B. (*Z*)-Propargyl α-Formylamino-β-(2-thienyl)acrylate (11f)——A solution of **10** (0.94 g, 0.004 mol) and propargyl bromide (0.71 g, 0.006 mol) in DMF (8 ml) was stirred for 2 hr at 55—60°. The reaction mixture was concentrated *in vacuo* and the resulting residue was extracted with AcOEt. The extract was

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20) K. Matsumoto, M. Suzuki, N. Yoneda, and M. Miyoshi, *Synthesis*, **1977**, 247.
21) The NMR spectrum suggested that this oxazoline compound was the *trans* form.[16]
22) D. Hoppe and U. Schöllkopf, *Chem. Ber.*, **109**, 482 (1976).
23) I. Ugi, U. Fetzer, U. Eholzer, H. Knupfer, and K. Offermann, *Angew. Chem.*, **77**, 492 (1965).
24) M. Suzuki, M. Miyoshi, and K. Matsumoto, *J. Org. Chem.*, **39**, 1980 (1974).

Table V. Formation of Alkyl α-Formylamino-β-(2-thienyl)acrylates and Alkyl α-Formylamino-β-[2-(5-bromo)thienyl]acrylates (**11**)

11	Method	Yield (%)	mp (°C)	Recryst. solvent	IR ν_{max}^{Nujol} cm^{-1}	Formula	Analysis (%) Calcd (Found) C	H	N	S
11a	A	28	111—113	AcOEt–iso-Pr_2O	3200, 1700, 1645, 1630	$C_{10}H_{11}NO_3S$	53.32 (53.19	4.92 5.01	6.22 6.20	14.23 14.00)
11b	A	30	137—138	AcOEt–iso-Pr_2O	3203, 1710, 1660, 1632	$C_{10}H_{10}BrNO_3S$	39.49 (39.41	3.31 3.33	4.61 4.60	10.54 10.34)
11c	A	30	97— 98	iso-Pr_2O	3200, 1708, 1665, 1630	$C_{11}H_{13}NO_3S$	55.21 (55.31	5.48 5.47	5.85 5.71	13.40 13.19)
11d	B	73	91— 93	AcOEt–iso-Pr_2O	3200, 1710, 1668, 1625	$C_{11}H_{11}NO_3S$	55.68 (55.51	4.67 4.78	5.90 5.73	13.51 13.28)
11e	A	28	118—120	iso-Pr_2O	3200, 1712, 1660, 1625	$C_{11}H_{10}BrNO_3S$	41.79 (41.78	3.19 3.08	4.43 4.29	10.14 10.03)
11f	B	69	118—120	AcOEt–iso-Pr_2O	3250, 2120, 1710, 1650	$C_{11}H_9NO_3S$	56.16 (56.10	3.86 3.71	5.95 5.90	13.63 13.49)
11g	B	79	92— 94	AcOEt–iso-Pr_2O	3210, 1765, 1710, 1660	$C_{11}H_{11}NO_5S$	49.06 (49.00	4.12 4.19	5.20 5.20	11.91 11.79)
11h	B	84	155—157	AcOEt	3230, 1730, 1715, 1660	$C_{11}H_{11}NO_4S$	52.16 (52.00	4.38 4.45	5.53 5.52	12.66 12.41)
11i	B	78	52— 54	Hexane	3205, 1702, 1662, 1630	$C_{11}H_{15}NO_3S$	56.90 (56.79	5.97 6.05	5.53 5.43	12.66 12.59)
11j	A	40	130—131	EtOH–H_2O	3200, 1710, 1660, 1632	$C_{12}H_{14}BrNO_3S$	43.38 (43.19	4.25 4.20	4.22 4.14	9.65 9.53)
11k	B	76	121—122	AcOEt–iso-Pr_2O	3230, 1713, 1666, 1628	$C_{15}H_{13}NO_3S$	62.70 (62.59	4.56 4.56	4.87 4.80	11.15 11.15)
11l	B	86	76— 78	AcOEt–iso-Pr_2O	3220, 1700, 1660, 1630	$C_{20}H_{31}NO_3S$	65.72 (65.59	8.55 8.62	3.83 3.80	8.77 8.59)

Table VI. Formation of Alkyl α-Isocyano-β-(2-thienyl)acrylates and Alkyl α-Isocyano-β-[2-(5-bromo)thienyl]acrylates (**12**)

12	Yield (%)	mp (°C)[a]	IR ν_{max}^{Nujol} cm^{-1}	Formula	Analysis (%) Calcd (Found) C	H	N	S
12a	84	64— 66	2120, 1720, 1615	$C_{10}H_9NO_2S$	57.95 (57.90	4.38 4.37	6.76 6.71	15.47 15.29)
12b	74	83— 84	2100, 1710, 1610	$C_{10}H_8BrNO_2S$	41.97 (41.82	2.82 2.93	4.90 4.87	11.21 11.29)
12c	85	87— 88	2110, 1710, 1615	$C_{11}H_{11}NO_2S$	59.71 (59.56	5.01 5.09	6.33 6.22	14.49 14.31)
12d	85	45— 46	2100, 1725, 1640, 1613	$C_{11}H_9NO_2S$	60.26 (60.21	4.14 4.15	6.39 6.27	14.62 14.40)
12e	80	59— 62	2100, 1720, 1610	$C_{11}H_8BrNO_2S$	44.31 (44.28	2.70 2.76	4.70 4.59	10.75 10.71)
12f	81	85— 86	2110, 1730, 1610	$C_{11}H_7NO_2S$	60.81 (60.59	3.25 3.29	6.45 6.29	14.76 14.58)
12g	89	106—108	2120, 1765, 1725, 1610	$C_{11}H_9NO_4S$	52.58 (52.63	3.61 3.70	5.57 5.39	12.76 12.51)
12h	86	106—108	2100, 1740, 1720, 1610	$C_{11}H_9NO_3S$	56.16 (56.11	3.86 3.93	5.95 5.90	13.63 13.55)
12i	89	51— 53	2100, 1720, 1610	$C_{12}H_{13}NO_2S$	61.25 (61.19	5.57 5.41	5.96 5.80	13.63 13.39)
12j	70	75— 76	2100, 1720, 1605	$C_{12}H_{12}BrNO_2S$	45.87 (45.59	3.85 3.92	4.46 4.40	10.21 9.99)
12k	77	87— 89	2100, 1715, 1610	$C_{15}H_{11}NO_2S$	66.89 (66.73	4.12 4.20	5.20 5.11	11.91 11.79)
12l	92	71— 73	2100, 1720, 1610	$C_{20}H_{29}NO_2S$	69.12 (69.20	8.41 8.45	4.03 4.00	9.23 9.15)

a) Recrystallyzed from benzene-hexane.

washed with H_2O and dried over $MgSO_4$, then concentrated *in vacuo*. The residue was recrystallized from AcOEt–iso-Pr_2O to afford **11f** as colorless needles (0.65 g, 69%), mp 118—120°. NMR (in DMSO-d_6) δ: 9.65 (1H, broad s, NH), 8.31 (1H, s, CHO), 7.85 (1H, s, –CH=), 8.10—7.70 (1H, m, thiophene C_5–H), 7.70—7.50 (1H, m, thiophene C_3–H), 7.30—7.10 (1H, m, thiophene C_4–H), 4.82 (2H, d, $J=3$ Hz, OCH_2), and 3.70—3.50 (1H, m, CH≡).

Other compounds were prepared in similar ways; the results are summarized in Table V.

General Procedure for the Preparation of (*Z*)-Alkyl α-Isocyano-β-(2-thienyl)acrylates (12a—l)——The compounds (**11**) were treated according to the general procedure for the preparation of **5a—y** to afford **12**; the results are summarized in Table VI.

Antifungal and Antibacterial Activity Testing——Antimicrobial activities of the test compounds are shown as the minimum inhibitory concentration (MIC) which was determined by an agar dilution method using the serial twofold dilution technique. Fungal spore suspension or broth culture of bacteria was streaked on Sabouraud's agar plates for fungi and on Hear infusion agar plates for bacteria. The concentrations of the compounds in the plates used were 100, 50, 25, ...1.56, 0.78 μg/ml (serial twofold dilutions). MIC was defined as the lowest concentration of a compound that prevented visible growth after incubating fungi at 27° for one week or bacteria at 37° for 18 hr.

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