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Studies on Synthesis of Coumarin Derivatives. XX.[1] Synthesis and Antibacterial Activity of Derivatives of N-Substituted 3-Coumarincarboxamide

Masataka Ichikawa and Hisashi Ichibagase

Faculty of Pharmaceutical Sciences, Kumamoto University[2]

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N-Substituted 6-nitro- and 7-nitro-3-coumarincarboxamides were prepared from these corresponding acids by the Schotten–Baumann reaction. N-(2-Pyridyl)-7-nitro-8-hydroxy- and N-(2-pyridyl)-7-nitro-8-methoxy-3-coumarincarboxamide were also prepared by the fusing of these ethyl esters and 2-aminopyridine. All of N-substituted nitro-3-coumarincarboxamides were derived to the corresponding N-substituted amino-3-coumarincarboxamides by a catalytic reduction and then the acetamido and the nitrofurfurylidene derivatives were finally prepared. Antibacterial tests of these derivatives on tubercle bacilli were carried out and some N-(2-pyridyl) amide and nitrofurfurylidene derivatives showed a strong activity.

Possibly the most important coumarin–type antibacterial agent is the antibiotic, novobiocin, isolated as a fungal metabolite of *Streptomyces niveus*. By its excellent antibacterial spectrum, chiefly against gram–positive organisms, novobiocin has been led to its wide acceptance in medicine.

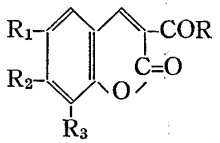
Recent patents[3,4] have claimed good antibacterial acitvity for 3-amino-4,7-dihydroxy-8-methylcoumarin and the amide derivatives derived from novobiocin.

Okumura, *et al.*[5–7] have reported the synthesis of a number of 3-acylamino-4-hydroxycoumarin derivatives related to novobiocin, several of which showed good antibacterial activity.

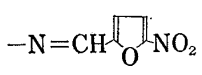
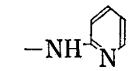
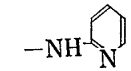
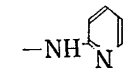
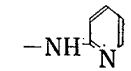
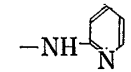
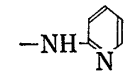
Rodighiero, *et al.*[8] have also examined derivatives of 3-aminocoumarin for antibacterial activity and have found the best compound to be 3-amino-7,8-dihydroxycoumarin which was particularly effective against *Streptococcus pyogenes* and moderatively effective against a variety of other bacteria.

1) Part XIX: H. Saikachi and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 1350 (1966).
2) Location: *Oehon-machi, Kumamoto.*
3) H. Charles, U.S. Patent 2966509 (1960).
4) W. Edward and A. Karl, Ger. Patent 1076141 (1960) [*C.A.*, **55**, 26378 (1961)].
5) K. Okumura, *Yakugaku Zasshi*, **80**, 525 (1960).
6) K. Okumura and I. Inoue, *Yakugaku Zasshi*, **81**, 453 (1961).
7) K. Okumura, K. Ashino, and T. Okuda, *Yakugaku Zasshi*, **81**, 1482 (1961).
8) G. Rodighiero and C. Antonello, *Boll. Chim. Farm.*, **97**, 592 (1958).

TABLE I. 6, 7 and 8–Substituted 3–Coumarincarboxamide Derivatives



Compd. No.	R_1	R_2	R_3	R	mp (°C)	Appearance	Formula	Analysis (%) Calcd. C	H	N	Found C	H	N
I	$-NO_2$	H	H	—N(H)〉	213	colourless needles (EtOH)	$C_{15}H_{14}O_5N_2$	59.60	4.63	9.27	59.75	4.72	9.26
II	$-NH_2$	H	H	—N(H)〉	161—162	yellow plates (benzene)	$C_{15}H_{16}O_3N_2$	66.17	5,88	10.29	66,37	6.02	10.27
III	$-NO_2$	H	H	—N(H)	199—200	light yellow needles (EtOH)	$C_{14}H_{12}O_5N_2$	58.75	4.31	9.46	58.33	4.17	9.38
IV	$-NH_2$	H	H	—N(H)	191	yellow plates [benzene–MeOH(9:1)]	$C_{14}H_{14}O_3N_2$	65.11	5.43	10.85	65.04	5.65	10.62
V	$-N=CH$(furan)$-NO_2$	H	H	—N(H)	260	brown yellow prisms (EtOH)	$C_{19}H_{15}O_6N_3$	59.84	3.93	11.02	59.92	3.98	10.91
VI	$-NO_2$	H	H	—NH(pyridyl)	268—269	light yellow needles (EtOH)	$C_{15}H_9O_5N_3$	57.88	2.90	13.49	57.81	2.67	13.29
VII	$-NH_2$	H	H	—NH(pyridyl)	263	brown yellow needles (EtOH)	$C_{15}H_{11}O_3N_3$	64.05	3.94	14.97	64.07	3.95	14.66
VIII	$-NHCOCH_3$	H	H	—NH(pyridyl)	>300	light yellow needles (AcOH)	$C_{17}H_{13}O_4N_3$	63.15	4.62	12.99	62.77	4.20	12.56
IX	$-N=CH$(furan)$-NO_2$	H	H	—NH(pyridyl)	256	yellow prisms (EtOH)	$C_{20}H_{12}O_6N_4$	59.40	2.97	13.86	59.27	2.85	13.34
X	H	$-NO_2$	H	—N(H)〉	185—186	yellow needles (EtOH)	$C_{15}H_{14}O_5N_2$	59.60	4.64	9.27	59.80	4.67	9.08
XI	H	$-NH_2$	H	—N(H)〉	202	brown yellow prisms (MeOH)	$C_{15}H_{16}O_3N_2$	66.17	5.88	10.03	66.44	5.83	9.73
XII	H	$-NO_2$	H	$-N(C_2H_5)_2$	173	light yellow needles (EtOH)	$C_{14}H_{14}O_5N_2$	57.93	4.86	9.65	57.78	4.57	9.70
XIII	H	$-NH_2$	H	$-N(C_2H_5)_2$	160	yellow prisms (AcOEt)	$C_{14}H_{16}O_3N_2$	64.68	6.19	10.76	64.68	6.02	10 75

XIV	H	$-NHCOCH_3$	H	$-N(C_2H_5)_2$	195	colourless prisms (EtOH)	$C_{16}H_{18}O_4N_2$	63.55	6.00	9.26	63.47	5.75	8.99
XV	H		H	$-N(C_2H_5)_2$	203	yellow prisms (EtOH)	$C_{19}H_{17}O_6N_3$	59.50	4.43	10.96	59.43	4.35	10.65
XVI	H	$-NO_2$	H	$-NHC_2H_5$	258—259	light yelllow needles (AcOEt)	$C_{12}H_{10}O_5N_2$	54.96	3.84	10.69	55.06	3.74	10.58
XVII	H	$-NH_2$	H	$-NHC_2H_5$	223	yellow needles (EtOH)	$C_{12}H_{12}O_3N_2$	62.06	5.21	12.06	61.84	5.21	11.81
XVIII	H	$-NHCOCH_3$	H	$-NHC_2H_5$	268–269.5	colourless plates (EtOH)	$C_{14}H_{14}O_4N_2$	61.31	5.14	10.22	61.51	4.89	10.24
XIX	H	$-NO_2$	H	$-NH-C_6H_5$	297—298	yellow needles ($CHCl_3$)	$C_{16}H_{10}O_5N_2$	61.93	3.22	9.03	61.91	3.22	8.89
XX	H	$-NH_2$	H	$-NH-C_6H_5$	292—293	yellow plates (EtOH)	$C_{16}H_{12}O_3N_2$	68.56	4.31	9.99	68.95	4.29	9.94
XXI	H	$-NO_2$	H		291—292	light yellow needles (AcOEt)	$C_{15}H_9O_5N_3$	57.88	2.90	13.49	57.87	2.89	13.26
XXII	H	$-NH_2$	H		276—277	brown yellow needles (AcOEt)	$C_{15}H_{11}O_3N_3 \cdot \frac{1}{2}H_2O$	62.07	4.14	14.48	62.37	4.38	14.25
XXIII	H	$-NO_2$	H	$-NH_2$	263—264	light yellow needles (acetone)	$C_{10}H_6O_5N_2$	51.29	2.58	11.96	51.44	2.43	12.15
XXIV	H	$-NH_2$	H	$-NH_2$	>300	brown yellow needles (EtOH)	$C_{10}H_8O_3N_2$	58.82	3.95	13.72	58.77	3.90	13.87
XXV	H	$-NO_2$	$-OH$		>300	yellow plates (AcOH)	$C_{15}H_9O_6N_3$	55.04	2.75	12.84	55.02	2.94	12.64
XXVI	H	$-NH_2$	$-OH$		>300	brown yellow prisms (AcOEt)	$C_{15}H_{11}O_4N_3$	60.60	3.70	14.14	60.41	3.93	13.82
XXVII	H	$-NO_2$	$-OCH_3$		246—247	light yellow plates (AcOH)	$C_{16}H_{11}O_6N_3$	56.30	3.22	12.31	56.78	3.29	12.17
XXVIII	H	$-NH_2$	$-OCH_3$		>300	brown yellow prisms (AcOEt)	$C_{16}H_{13}O_4N_3$	61.73	4.18	13.50	61.37	4.31	13.36

In previous reports, we have studied on the synthesis of oxazolocoumarin-type[9] derived from *o*-amino-hydroxycoumarin series, of the amide derivatives[10] and of 4-hydroxy-3-sulfanilamidocoumarin derivatives[11] related to novobiocin. In order to determine the structural features that contribute to antibacterial activity, we have also examined derivatives of 4-hydroxy-3-sulfanilamidocoumarin for antibacterial activity and have found that 4-hydroxy-7-amino-3-sulfanilamidocoumarin was able to inhibit growth of the tubercle bacillus significantly.[11]

In this paper, synthetic study of N-substituted 3-coumarincarboxamide derivatives was carried out to obtain some new chemotherapeutical agents in this field and, additionally, tuberculostatic activity for the compounds and for the prepared oxazolocoumarin series was observed.

I Chemical Items

1) All of nitro-3-coumarincarboxylic acids[12,13] were readily led to the acid chloride by refluxing with thionyl chloride and resulting acid chlorides were used for condensation with the amine series, respectively.

2) These N-substituted nitro-3-coumarincarboxamides obtained were hydrogenated using palladium on charcoal to give the corresponding amino-3-coumarincarboxamide in good yield.

3) These amino compounds were also derived to the acetamido and the nitrofurfurylidene compound, separately.

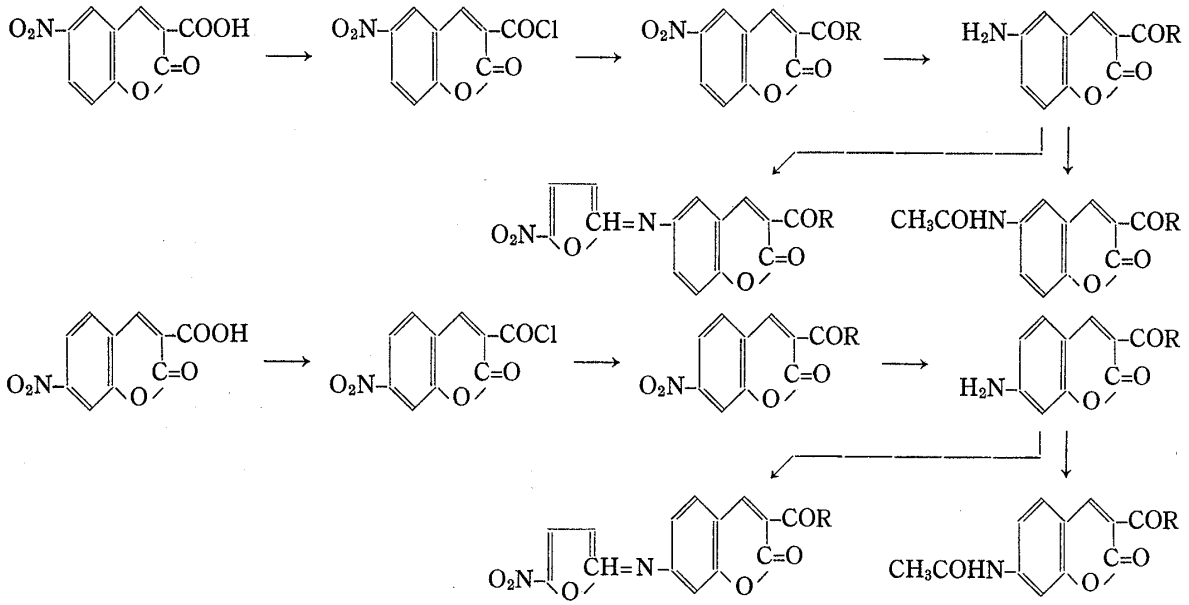


Chart 1

4) Both N-(2-pyridyl)-7-nitro-8-hydroxy- (XXV) and N-(2-pyridyl)-7-nitro-8-methoxy-3-coumarincarboxamide (XXVII) were directly derived from the corresponding ethyl ester[14] by fusing with 2-aminopyridine, separately.

5) Then, XXV and XXVII were reduced to the corresponding amino compounds (XXVI and XXVIII) by the same catalytic reduction as above mentioned, respectively.

9) H. Saikachi and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 1162 (1966).
10) H. Saikachi and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 1167 (1966).
11) M. Ichikawa and H. Ichibagase, *Yakugaku Zasshi*, **86**, 1064 (1966).
12) H. Ichibagase and S. Terada, *Yakugaku Zasshi*, **72**, 877 (1952).
13) H. Ichibagase, *Yakugaku Zasshi*, **75**, 1482 (1955).
14) M. Ichikawa, Y. Takaki, and H. Ichibagase, *Yakugaku Zasshi*, **81**, 769 (1961).

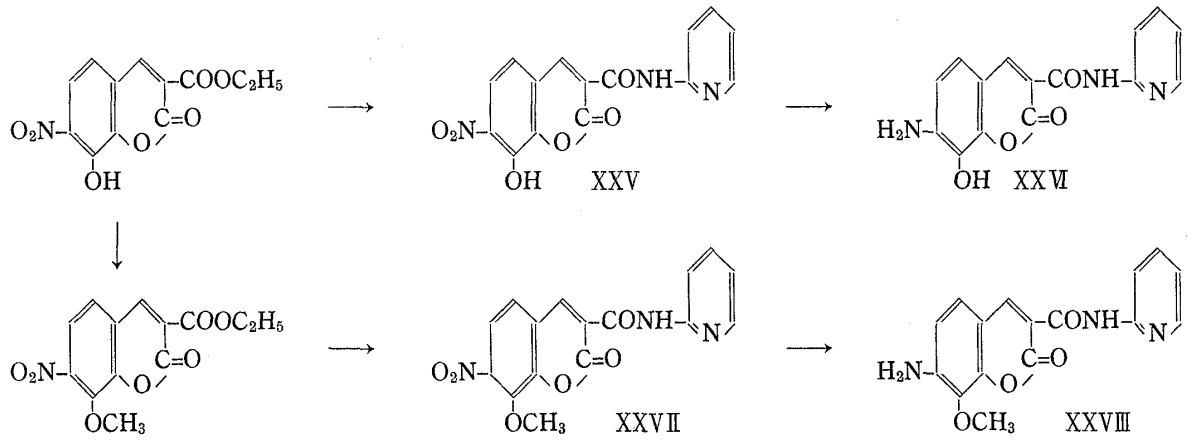


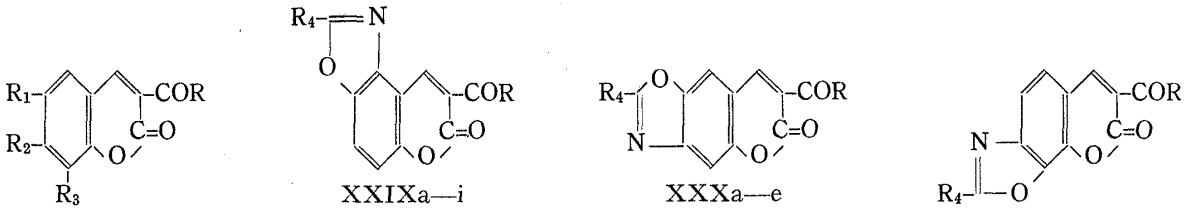
Chart 2

Their chemical properties together with their elemental analytical data are listed in Table I.

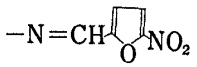
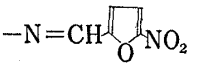
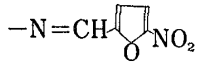
II Antibacterial Activity (*in Vitro*)

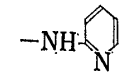
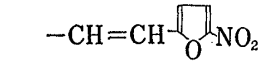
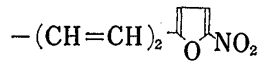
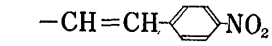
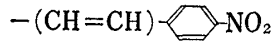
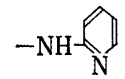
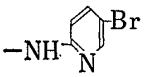
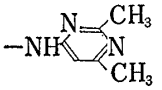
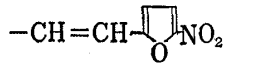
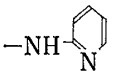
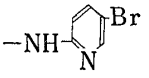
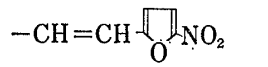
The results of the *in vitro* antibacterial activity against *Myco. tuberculosis* $H_{37}Rv$ are shown in Table II.

TABLE II. Minimum Inhibitory Concentration against *Myco. tuberculosis* $H_{37}Rv$



I—XXVIII XXIXa—i XXXa—e XXXIa—d

Compd. No.	Substituted groups					MIC[a] (μg/ml)	
	R_1	R_2	R_3	R_4	R	Solvent	$H_{37}Rv$[b]
IV	$-NH_2$	H	H	—		PG[c]	>100
V		H	H	—		PG	>100
VII	$-NH_2$	H	H	—		PG	50
IX		H	H	—		PG	6.3
XV	H		H	—		PG	25
XXVI	H	$-NH_2$	$-OH$	—		PG	>100
XXVIII	H	$-NH_2$	$-OCH_3$	—		PG	>100
XXIXa[g]	—	—	—	$-CH_3$	$-OC_2H_5$	PG	>100
XXIXb[d]	—	—	—	$-CH_3$		PG	>100
XXIXc[d]	—	—	—	$-CH_3$		PG	>100

XXIXd[d]	—	—	—	$-CH_3$	$-N(C_2H_5)_2$	PG	>100
XXIXe[10]	—	—	—	$-CH_3$		PG	50
XXIXf[e]	—	—	—		$-OC_2H_5$	PG	1.6
XXIXg[e]	—	—	—		$-OC_2H_5$	PG	50
XXIXh[e]	—	—	—		$-OC_2H_5$	PG	>100
XXIXi[e]	—	—	—		$-OC_2H_5$	PG	>100
XXXa[9]	—	—	—	$-CH_3$	$-OC_2H_5$	PG	>100
XXXb[10]	—	—	—	$-CH_3$		PG	>100
XXXc[10]	—	—	—	$-CH_3$		PG	>100
XXXd[10]	—	—	—	$-CH_3$		PG	>100
XXXe[e]	—	—	—		$-OC_2H_5$	PG	>100
XXXIa[9]	—	—	—	$-CH_3$	$-OC_2H_5$	PG	>100
XXXIb[10]	—	—	—	$-CH_3$		PG	3.2
XXXIc[10]	—	—	—	$-CH_3$		PG	>100
XXXId[e]	—	—	—		$-OC_2H_5$	PG	3.2

a) MIC=minimum inhibitory concentration
b) $H_{37}Rv$=*Mycobacterium tuberculosis var. hominis* $H_{37}Rv$
c) PG=propylene glycol
d) H. Saikachi and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 1350 (1966)
e) H. Saikachi and M. Ichikawa, *Chem. Pharm. Bull.* (Tokyo), **14**, 1347 (1966)

XXIXf showed the strongest action. Then, XXXIb and XXXId were next in order. However, compared with both XXIX-type and XXXI-type, any derivatives of XXX-type had no tuberculostatic activity.

From the above results, although carboethoxy group and the amide series such as diethylamino, pyrrolidino and morpholino at three position of coumarin ring have no tuberculostatic activity, the presence of the amide such as –NH–(2-pyridyl) and of nitrofurfurylidene group was observed to have tuberculostatic activity in this field.

It is also interested in structural features for antibacterial activity that their three isomeric oxazolocoumarins (XXIX–, XXX– and XXXI-type) have tuberculostatic activity in varying degrees, but it is seemed that cleavage of oxazole ring extremely decreases the activity.

Experimental

Synthesis

Most of all products are listed in Table I.

N,N-Pentamethylene- and N,N-Tetramethylene-nitro-3-coumarincarboxamide (I, X and III)——After a mixture of 1 g (4.3 mmoles) of the acid and 20 ml of $SOCl_2$ had been heated under reflux for 3 hr, the excess of $SOCl_2$ was removed *in vacuo*. The residue was suspended in 20 ml of dried benzene and then 1 g of the amine was gradually added to the suspension at room temperature. After standing at room temperature for 24 hr, benzene was evaporated *in vacuo* to leave a tarry residue, which was treated with 5% HCl in an ice bath. The resulting solid was collected by suction, washed with H_2O, dried and recrystallized, giving products (I, III and X) in 70—80% yield, respectively.

N,N-Diethyl-7-nitro-3-coumarincarboxamide (XII)——The acid chloride was prepared from 1 g (4.3 mmoles) of 7-nitro-3-coumarincarboxylic acid in the same manner as the above mentioned and then 1 g of diethylamine was gradually added to a suspension of the acid chloride in 20 ml of dried $CHCl_3$ at room temperature. After standing at room temperature for 48 hr, $CHCl_3$ was evaporated *in vacuo* to leave a tarry residue, which was treated with 2% aq. AcOH solution. The resulting solid was collected by suction, washed with H_2O, dried and recrystallized from EtOH, giving XII in 70% yield.

N-Ethyl-7-nitro-3-coumarincarboxamide (XVI)——The acid chloride (4.3 mmoles) prepared was suspended in 20 ml of dried ether and then a calculated amount of ethylamine etheral solution was gradually added to the suspension under stirring at below 5°. After stirring at below 10° for 6 hr, the resulting solid was collected by suction and recrystallized from AcOEt, giving XVI in 60% yield.

N-(2-Pyridyl)-nitro-3-coumarincarboxamides (VI and XXI) and N-Phenyl-7-nitro-3-coumarincarboxamide (XIX)——The acid chloride (4.3 mmoles) prepared was suspended in 50 ml of dried benzene and then 1 g of the amine was gradually added to the suspension under stirring at room temperature. After standing at room temperature for 48 hr, benzene was evaporated *in vacuo* to leave a tarry residue, which was treated with 2% aq. AcOH solution. The resulting solid was collected by suction, washed with H_2O, dried and recrystallized, giving products (VI, XIX and XXI) in 80% yield, respectively.

7-Nitro-3-coumarincarboxamide (XXIII)——The acid chloride (4.3 mmoles) prepared was gradually added to 20 ml of 28% aq. ammonia solution under stirring at 0°. After stirring at 0° for 2 hr, the resulting solid was collected by suction, washed with H_2O, dried and recrystallized from acetone, giving XXIII in 50% yield.

N-(2-Pyridyl)-7-nitro-8-hydroxy-3-coumarincarboxamide (XXV)——1 g of ethyl 7-nitro-8-hydroxy-3-coumarincarboxylate was fused with 1 g of 2-aminopyridine at 190°—200° for 30 min. After cooling, the resulting solid was extracted with 2% NaOH at room temperature. The alkaline solution was acidified by 10% HCl. Separated crystalls were collected by suction, washed with H_2O, dried and recrystallized, giving XXV in 60% yield.

N-(2-Pyridyl)-7-nitro-8-methoxy-3-coumarincarboxamide (XXVII)——1 g of ethyl 7-nitro-8-methoxy-3-coumarincarboxylate was fused with 1 g of 2-aminopyridine at 200° for 30 min. After cooling, the resulting solid was treated with a small amount of EtOH at room temperature. The insoluble material was collected by suction and recrystallized, giving XXVII in 70% yield.

Catalytic Reduction of N-Substituted Nitro-3-coumarincarboxamides——A solution of 2 g of the nitro compound in 300 ml of AcOH was hydrogenated using 1 g of 2% palladium on charcoal at room temperature under atmospheric pressure. After heating it for 30 min on water bath, the catalyst was removed and then the filtrate was evaporated *in vacuo*. The resulting residue was recrystallized, giving the amino-3-coumarincarboxamide in about 70% yield. XXVI and XXVIII were prepared from the corresponding nitro compounds (XXV and XXVII) by the same way as above mentioned, respectively.

Acetylation of N-Substituted Amino-3-coumarincarboxamides (VII, XIII and XVII)——A suspension of 1 g of the amino compound in 5 ml of Ac_2O was heated at 120° for 3 hr and then poured into ice water. The resulting solid was collected by suction, washed with H_2O, dried and recrystallized, giving products (VIII, XIV and XVIII) in 80% yield, respectively.

Condensation of N-Substituted Amino-3-coumarincarboxamides (IV, VII and XIII) with 5-Nitro-2-furaldehyde——To a suspension of 1 g of the amino compound in 10 ml of AcOH added 0.5 g of 5-nitro-2-furaldehyde at room temperature. The mixture was warmed on water bath for 20 min. After standing at room temperature for 24 hr, separated crystalls were collected by suction, dried and recrystallized, giving products (V, IX and XV) in 60% yield, respectively.

Antibacterial Activity Against *Myco. tuberculosis* $H_{37}Rv$ *in Vitro*——*Myco. tuberculosis* $H_{37}Rv$ was cultured for 4 weeks at 37° in Ogawa medium[15] and a suspension of the bacteria was prepared at concentration of 0.5 mg/ml. Test compounds were diluted by the two fold dilution method in the tubes containing

15) 1 g of KH_2PO_4 and 1 g of sodium glutamate were dissolved in 100 ml of H_2O. To the solution, 6 ml of glycerin, 6 ml of 2 %malachite green solution and 5 eggs (about 200 ml) were added.

5 ml of 5% serum Kirchner medium.[16] To these tubes, 0.1 ml of the suspension were added and then they were incubated for 3 weeks at 37° and the minimum inhibitory concentrations were determined.

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16) KH_2PO_4 4.0 g, $Na_2HPO_4 \cdot 12H_2O$ 3.0 g, sodium citrate 2.5 g, asparagine 5.0 g, glycerin 20 ml, $MgSO_4$ 0.6 g, total (H_2O) 100 g.