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1,2,3-TRIAZOLO[4,5-h]QUINOLINES. III. PREPARATION AND ANTIMICROBIAL EVALUATION OF 4-ETHYL-4,7-DIHYDRO-1(2)-R-1(2)H TRIAZOLO[4,5-h]QUINOLIN-7-ONE-6-CARBOXYLIC ACIDS AS ANTI-INFECTIVES OF THE URINARY TRACT (*) (**)

PAOLO SANNA (***) - ANTONIO CARTA - GIUSEPPE PAGLIETTI

ISTITUTO DI CHIMICA FARMACEUTICA E TOSSICOLOGICA - UNIVERSITÀ DI SASSARI
VIA MURONI 23, 07100 SASSARI

STEFANIA ZANETTI - GIOVANNI FADDA

ISTITUTO DI MICROBIOLOGIA E VIROLOGIA DELLA FACOLTÀ DI MEDICINA
UNIVERSITÀ DI SASSARI - VIALE S. PIETRO, 07100 SASSARI

SUMMARY — *Some 4-ethyl-1(2)-R-1(2)H-4,7-dihydro-triazolo[4,5-h]-quinolin-7-one-6-carboxylic acids were prepared as novel analogues of oxolinic acid, in order to discover the influence of the annelation position of the triazole ring on the antimicrobial activity that, in some isomers triazolo[4,5-f]quinolinone carboxylic acids, is selective against* Escherichia coli. *Some interesting side reactions in the cyclization of 1(2)-R-1(2)H-benzotriazol-4-yl-aminomethylenemalonate are also described. The biological results indicate that this type of annelation is not profitable for antimicrobial activity.*

RIASSUNTO — *Viene descritta la sintesi di alcuni acidi 4-etil-1(2)-R-1(2)H-4,7-diidro-triazolo[4,5-h]chinolin-7-one-6-carbossilici come nuovi analoghi dell'acido ossolinico allo scopo di verificare l'influenza della posizione dell'anello triazolico, isostero del diossolo, sull'attività antimicrobica che in composti isomeri, in cui l'anello triazolico si trova in [4,5-f], era sembrata selettiva nei confronti dell'*Escherichia coli. *Vengono inoltre descritte alcune interessanti reazioni collaterali verificatesi nel corso della ciclizzazione dei 1(2)-R-1(2)H-benzotriazol-4-il-amminometilenemalonati. I saggi* in vitro *dimostrano che tale posizione angolare del triazolo in [4,5-h] non è proficua per questa attività.*

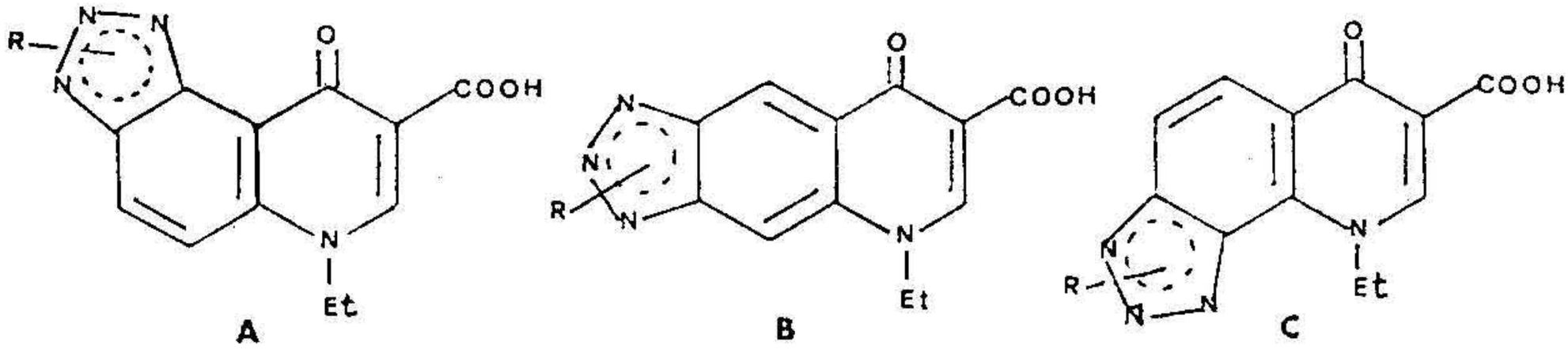
(*) Part of this work was presented as a poster communication at the 1st. Congreso Conjunto Hispano-Italiano de Quimica Terapeutica - Granada, 19-22 September 1989, pag. 312.

(**)-II see Reference (1).

(***) To whom correspondence should be addressed.

Introduction

In a previous paper (1) we reported that some triazolo[4,5-f]-quinolinone carboxylic acids of structure **A**, closely related to oxolinic acid, showed encouraging *in vitro* antimicrobial activity against *E. coli* at 12.5-25 μg/ml MIC.



In that context, we also planned to synthetise compounds of structures **B** and **C** in order to evaluate the influence of linear [4,5-g] or angular [4,5-h] annelation of the triazole ring on the activity of these isomers.

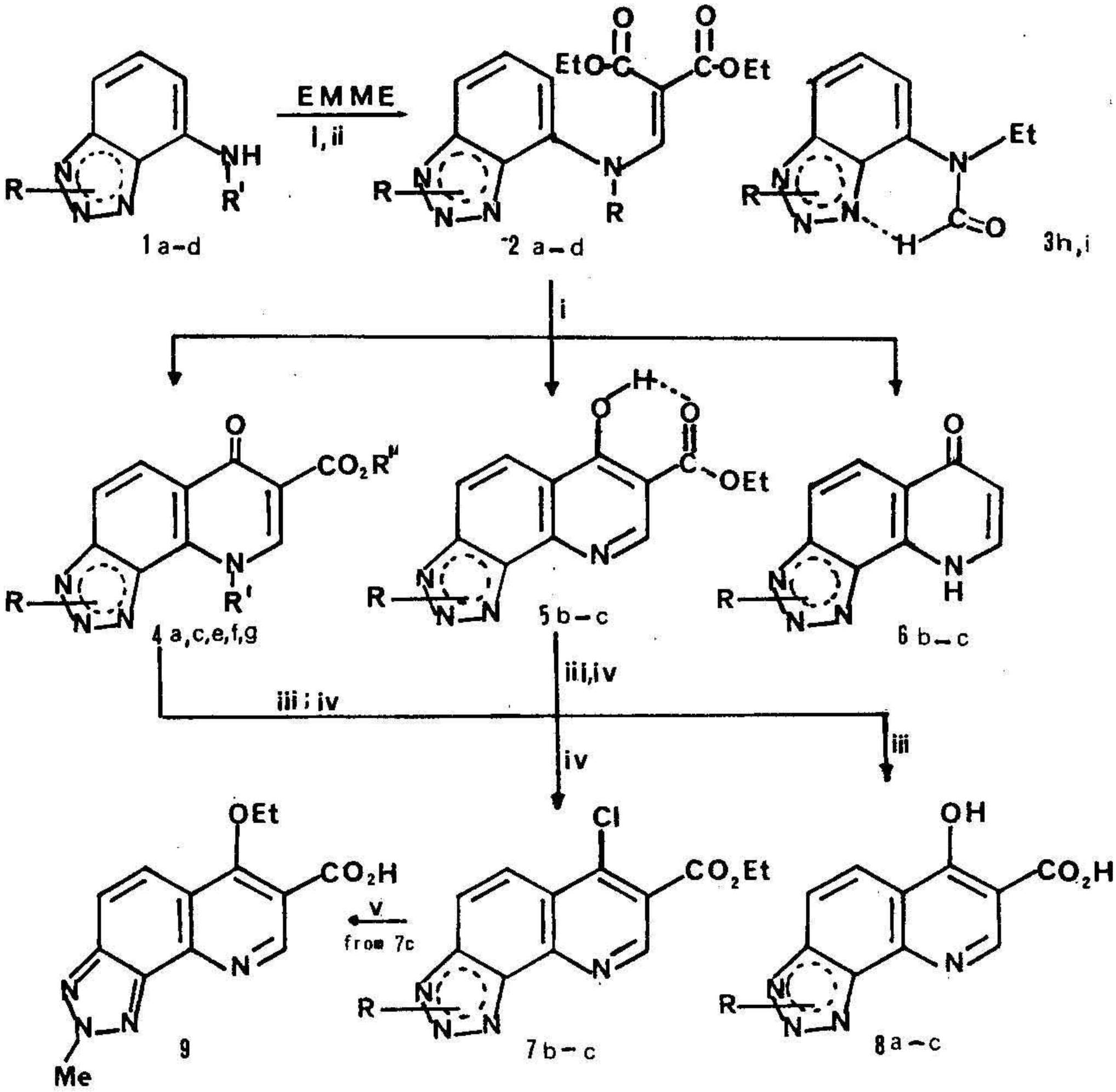
In this paper we report the synthesis of the acids of structure **C** and results on antimicrobial activity found *in vitro* against Gram (+) and Gram (-) bacteria.

Chemistry

The synthetic approach to compounds of structure **C** is depicted in Schemes 1 and 3. We must first point out that condensation of amines **1a-d** with diethyl ethoxymethylenemalonate (EMME), in Dowtherm, gave high yields of aminomethylenemalonates **2a-c** (Table I).

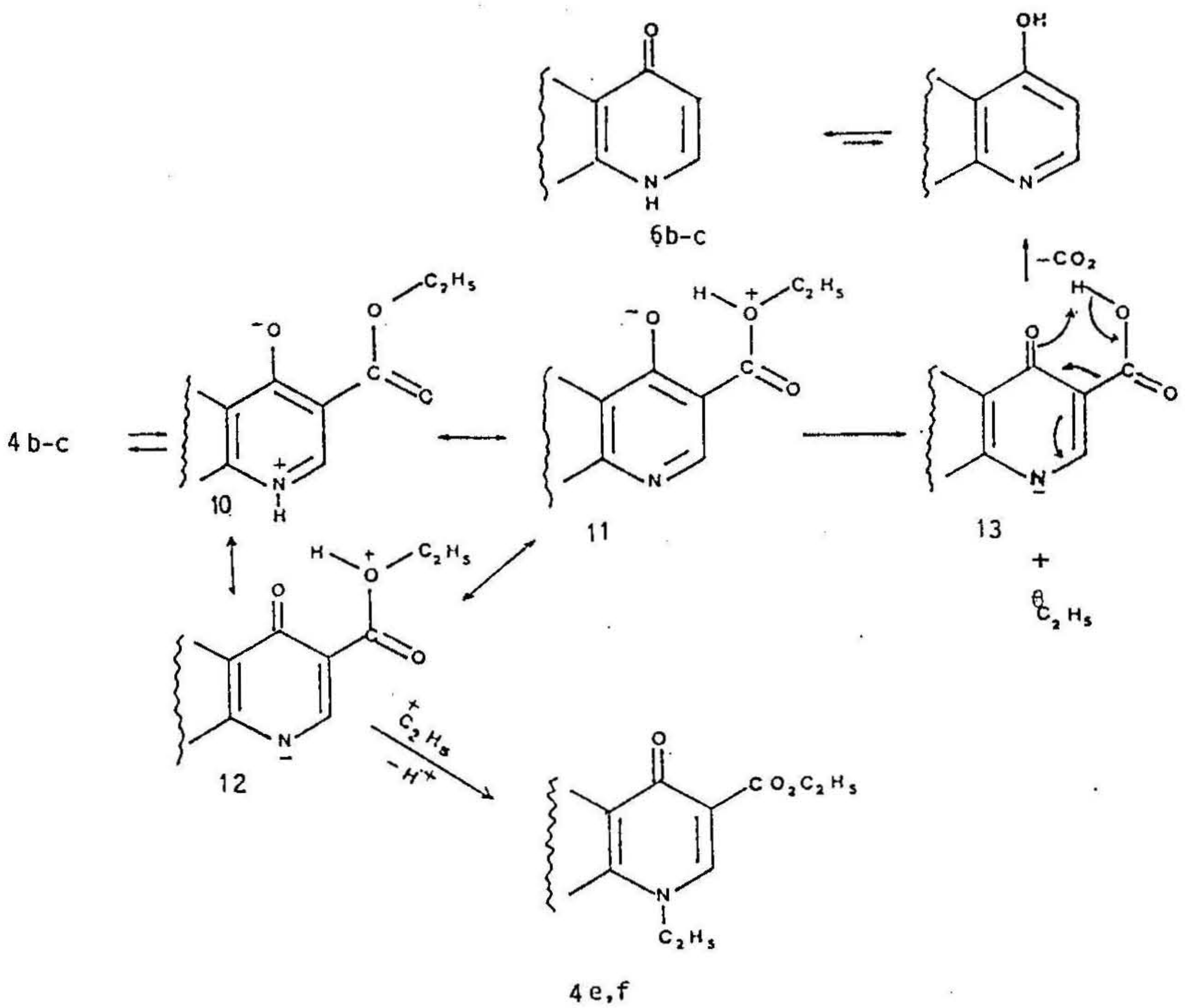
This reaction failed with amine **1d**. However, when this compound was heated under reflux with an excess of EMME in the absence of solvent, we were able to isolate the desired **2d** in very moderate yield (10%), accompanied by its acylation products **3i** (23% yield) and **3h** (16.5% yield), the structure of which was unambiguously established by its UV and ^{1}H and ^{13}C spectra (Table 1) and its different behavior towards an ethereal solution of hydrogen chloride, in which compound **3h** formed an insoluble salt in accordance with the general properties of 1-alkyl-benzotriazoles (2). Since compound **2d** in Dowtherm at 250°C gave the expected ring closure into quinolinone, the formation of compounds **3h,i** seems rather peculiar and ascribable both to acylation of the secondary amine and ethylation of the triazole ring in both positions 1 and 2. This may be due to partial decomposition of EMME at the reflux temperature, thus originating either the formic acid or the ethylcarbocation intermediates for the above reactions.

SCHEME 1



	a	b	c	d	e	f	g	h	i
R	H	1-Me	2-Me	H	1-Me	2-Me	H	1-Et	2-Et
R'	H	H	H	Et	Et	Et	Et	-	-
R"	Et	Et	Et	Et	Et	Et	H		

i, Dowtherm at 250°C; ii, in excess of EMME at 100°C; iii, NaOH; iv, $POCl_3$; v, EtONa.



Scheme 2

Compounds **2a** and **2c** have been previously described by other Authors respectively in references (3) and (4). The physical properties of **2a** were coincident with those found by us, while for compound **2c** the above Authors indicate either a lower melting point or a lower yield, spectroscopic data being in accordance with ours.

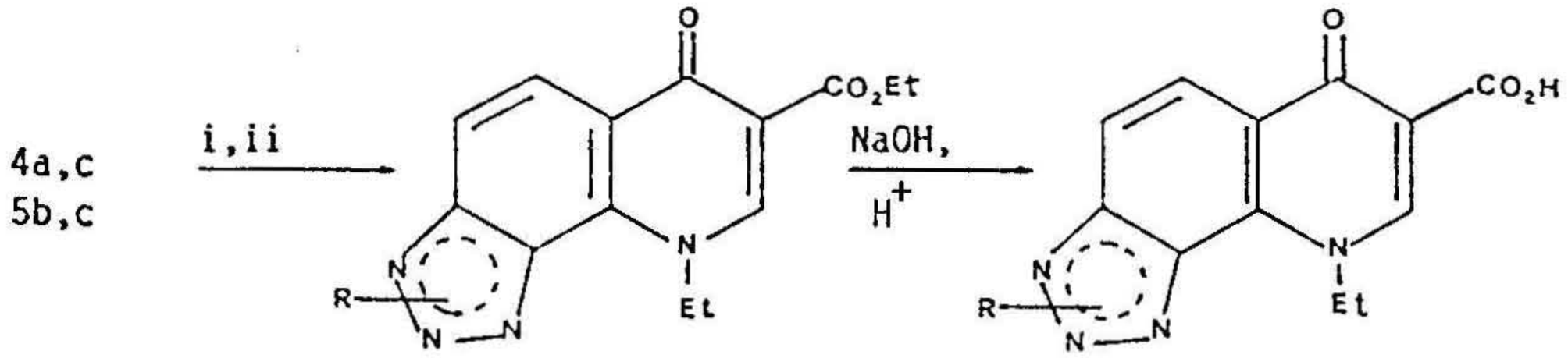
The ring closure of **2a-d** carried out in Dowtherm under reflux mostly proceeded according to a Conrad-Limpach mechanism and also gave rise to different results for each compound examined, as summarised in Table II.

In particular, the cyclization reaction of **2a** led to triazolo[4,5-h]-quinoline **4a** (98% yield) identical to that described (5), whereas compound **2b** mainly gave **5b** (34.5%) accompanied by its decarboxylation product **6b** (15%) and N_4-ethyl derivative **4e** (12.6%). Compound **2c** in turn gave the expected **4c** (50%) as major compound, together with compounds **5c** and **6c** and a small amount of N_4-ethyl derivative **4f** (9.4%). Our results differ from those reported by Milata *et al.* (4), who obtained only **4c** in 64% yield but with a lower mp.

Thermal cyclization of **2d** gave acid **4g** in satisfactory yield possibly because hydrolysis took place easily, thanks to treatment with an alkaline solution during the course of its purification. Comparison of the results, in the case of the cyclization of aminomethylenemalonates **2b** and **2c**, suggests that quinolone by itself is not formed but is often accompanied by side-reactions which partly involve bond fission between the C-OEt moiety in the ester group, with the formation of intermediates **11** and **12**. These respectively undergo decarboxylation to **6b-c** and electrophilic attack from the generated ethyl cation, to give compounds **4e-f** (Scheme 2). Similar behavior of the above-mentioned reaction has been observed by other Authors (6) during cyclisation of *para*-chloroanilinomethylenemalonate as reported by Jones (7). It is noteworthy that the isolation of both tautomers **4c** and **5c** showed different mps and infrared spectra but an identical UV spectrum. Their ^{1}H-NMR spectra, run at 200 MHz in $CDCl_3$ (see Table III), were substantially consistent with the assigned structures. Because of the presence of NH stretching absorption at 3500 cm^{-1}, we attribued the structure **4c** to the highest melting point tautomer, whereas **5c** was assigned to the other isomer for the presence of a low-field proton located at 12.31δ indicating O-H hydrogen bonding. However, when reacted with $POCl_3$, compounds **4c** and **5c** yielded the sole derivative **7c**. This fact indicates that they may exist as tautomers in the solid state, as observed in other cases (8), because of the stabilisation due to the different type of hydrogen bonding occuring while in solution the equilibrium is markedly influenced by the reagent and lies well in favour of the phenol form. In a parallel way, compound **5b** was converted into **7b**. On the other hand it is to be noted that compounds **6b-c** do exist in quinolone form and their structure is confirmed by ^{13}C-NMR spectra which showed C = O resonances at very low field ($\sim$ 175δ). Alkaline hydrolysis of esters **4a** and **5b** gave respectively acids **8a** and **8b**, while compounds **4c** and **5c** gave the sole compound **8c**, thus confirming that the strong alkaline medium favours the formation of the *ortho*-hydroxy acid.

N_4-Ethylation of compounds **4a-c** and **5b-c** was carried out with ethyl iodide in DMF in the presence of either potassium carbonate (**4a,c**) or sodium hydride (**5b,c**). In particular, compound **4a** underwent multiple alkylation, giving rise to the desired **4d** and the further alkylated derivatives **14** and **15** which were separated by flash chromatography. The determination of the structure of the single isomers was deduced by comparison of their UV spectra with those of N_1- or N_2-methyl derivatives **4e** and **4f** which were unambiguously obtained, according to Scheme 3, by ethylation of compounds **5b** and **5c** and proved to be identical (mixed mp, UV, NMR) to the cyclisation products coming respectively from **2b** and **2c**. This observation allowed us to confirm the assigned structures and the proposed mechanism. No formation of O-ethyl derivatives, as claimed in other similar cases (6), was observed. However, in order to exclude that this type of ethylation was taking place, we submitted compound **7c** to reaction with sodium ethox-

SCHEME 3



4d	R=H;	4g
4e	R=1-Me;	16
4f	R=2-Me;	17
14	R=1-Et;	18
15	R=2-Et;	19

i, K_2CO_3/EtI/DMF/100°C; ii, NaH/EtI/DMF/90°C.

ide and obtained acid **9**, which was different from acid **17** obtained from the alkaline hydrolysis of **4f**. An identical hydrolysis as above was applied to esters **4d, 4e, 4f, 14, 15**, which gave respectively acids **4g,16,17,18,19** in good yields.

Amine **1d** is a new compound and was prepared by $LiAlH_4$ reduction of the known 1(2)-acetyl-4-acetylamino-1(2H)benzotriazole (9).

The structures of the described products were unambiguously deduced from the analytical data and the whole of their spectroscopic properties (Tables I, III and IV).

Experimental

A) CHEMISTRY

Apparatus, instruments and procedures were described in a previous paper (1). Elemental analyses (C,H,N.Cl) were performed at the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, University of Padova, and analytical results were within $\pm 0.4\%$ of theoretical values.

Intermediates

Starting material was *4-nitrobenzotriazole*, prepared according to the literature (9). Methylation of this compound in alkaline medium with dimethyl sulfate gave three isomers, which were separated and which were identical to those described by Milata *et al.* (5). Amines (**1a-c**) were obtained by catalytic hydrogenation of the corresponding nitro compounds and were identical with those described: **1a**, mp 142-145°C [lit. 149°C (9)]; **1b**, mp 118-119°C [lit. 121°C (10)]; **1c**, mp 45-48°C [lit. 48°C (11)]. Amine **1d** was obtained by reproducing a reaction scheme used for the synthesis of its previously described isomer 5-ethylamino-1H-benzotriazole (1). Its preparation is reported below.

4-Ethylamino-1H-benzotriazole (1d)

A solution of 1-acetyl-4-acetylamino-1H-benzotriazole, obtained according to Fries *et al.* (9), [(3 g, 14 mmole) dissolved in dry THF (120 ml)] was added slowly under stirring to a suspension of $LiAlH_4$ (2.66 g, 70 mmole) in dry THF (40 ml), externally cooled with an ice bath. The mixture was then refluxed for 20 h and eventually the complex was destroyed according to a previously described procedure (1). The precipitate was filtered off and thoroughly washed with THF. The mother liquors, dried over anhydrous Na_2SO_4, on evaporation gave a crude oil which, after chromatography through silica-gel, eluting in order with diethyl ether and diethyl ether: acetone (7:3), gave crystals of **1d** (1.71 g, 77% yield): mp 162-165°C.

Anal. $C_8H_{10}N_4$ (C,H,N).

I.R: cm^{-1} 3420, 3380 (NH), 1640 (C=C).

U.V. [EtOH]: 323, 270, 226 nm.

^{1}H-NMR [200 MHz-$CDCl_3$]: δ7.28 (t, 1H, *J* 8.12, H-5), 6.89 (d, 1H, *J* 8.12, H-7), 6.33 (d, 1H, *J* 8.12, H-6), 5.30 (flat s, 1H, NH, exchange with D_2O), 3.36 (q, 2H, *J* 7, N-C*H*$_2$Me), 1.36 (t, 3H, *J* 7, N-CH$_2$*Me*).

*General methods for synthesis of aminomethylene malonates (**2a-d**) of Scheme 1*

Method A: To amines **1a,b,c** (26-44 mmole) suspended or dissolved in Dowtherm (tenfold of used weight) at 50°C, EMME in slight excess (20%) was added. Then the mixture was heated at 150°C under stirring for 4 h. On cooling, it was taken up with ten times its volume of hexane and stirred for another 30 min. The precipitate formed (**2a-c**) was filtered off and recrystallised or chromatographed as indicated in Table I. Yields, mps and spectroscopic and analytical data are reported in Table I.

Method B: Amine **1d** (14.8 mmole) was refluxed with a large excess of EMME (28.4 mmole) for 68 h. After cooling, the mixture was chromatographed over silica-gel, eluting as follows: with light petroleum (40-60°C) to give compound **3i** as an oil (0.71 g), which crystallised after long standing in the freezer from a mixture of light petroleum and diethyl ether (2:1 ratio); with diethyl ether and an increasing amount of acetone (up to 10%), to yield in succession compound **2d** (0.51 g), which crystallised after trituration with hexane, and compound **3h** (0.51 g) which instead crystallised from a mixture of diethyl ether-light petroleum (40-60°C). Yields, mps, analytical and spectroscopic data are reported in Table I.

TABLE I

Products obtained by reaction of amines **1a-d** *with* ***EMME*** *(Physical and spectroscopic data)*

Compd	Method	Yield %	Mp °C (from)	Analyses for	ν_{max} cm^{-1}	λ_{max} [EtOH] nm	^1H NMR [200MHz] J in Hz; chemical shift in ppm(δ); solvent= $CDCl_3$ ^{13}C [50 MHz]
2a	A	81	215-216 (c)	a			
2b	A	96	118-119 (a)	$C_{15}H_{18}N_4O_4$	1695,1650,1620, 1580	345,292,282sh, 264sh,258,218	11.63(d,1H,J 13, NH), 9.48(d,1H,J 13,N-CH=C), 7.45(t, 1H,J 8, H-7), 7.24(t, 1H, J 8, H-5), 7.01(d,1H, J 8, H-6), 4.35(m, 4H, OCH_2Me), 4.31 (s,3H,N-Me), 1.41(dt,6H, OCH_2Me)
2c	A	90	80-81 (a)	b			
2d	B	11	72-73 (b)	$C_{16}H_{20}N_4O_4$	1710,1700,1630, 1590	345,291,282sh, 264sh,258,217	11.65(d,1H,J 13.5, NH), 9.52(d,1H, J 13.5,NCH=C 7.44(t,1H,J 8,H-7), 7.23(d, 1H,J 8,H-5), 7.01 (d, 1H, J 8,H-6),4.70(q, 2H, J 7, N-CH_2Me),4.38 (q,2H, J 7, OCH_2Me), 4.23(q,t, J 7, OCH_2Me), 1.64(t,3H,J 7,N-CH_2Me), 1.43(t,3H,J 7,O-CH_2Me) 1.41(t,3H,J 7, O-CH_2Me).

TABLE I *(continuation)*

3h	B	16.5	101-102 (d)	$C_{11}H_{14}N_4O$	3070,1670,1610, 1590	289,273,266infl, 216	8.99(s,1H,CHO),7.49(d,1H,J 9, H-7), 7.46(t,1H, J 9, H-5), 7.08(d,1H,J 9, H-6),4.73(q, 2H,J 7.3,N_1-CH_2Me), 4.28(q, 2H, J 7.3,N_4-CH_2Me), 1.67(t,3H, J 7.3,Me), 1.23(t, 3H, J 7.3, Me). ^{13}C:162.97(d,C=O),140.75(s,C-7a), 134.25(s, C-3a), 132.53(s,C-4), 127.63(d,C-6), 116.48(d, C-5), 107.02(d, C-7), 43.36 (t,N_2-CH_2), 39.71 (t,CH_2), 14.80(q,Me), 12.98(q,Me).
3i	B	23	47-49 (a/d)	$C_{11}H_{14}N_4O$	3060,1670,1620, 1580,1520	292infl,284,278sh, 214	8.80(s,1H,CHO), 7.78(d,1H,J 8.6,H-7), 7.38 (t, 1H,J 8.6,H-5), 7.07(d, 1H,J 8.6, H-6), 4.79(q, 2H, J 7.3, N_2-CH_2Me), 4.15(q, 2H, J J 7.3, N_4-CH_2Me), 1.73(t, 3H, J 7.3, Me), 1.20(t, J 7.3, Me). ^{13}C :162.69(d,C=O), 145.64(s, C-3a), 139.85 (s, C-7a), 130.78(s, C-4), 126.11(d, C-6), 118.98(d, C-7), 116.07(d, C-5), 51.78(t,N_2-CH_2), 39.44(t, CH_2), 14.91(q,Me), 12.95(q,Me).

a, yield 84%;mp 218-222°C ref (3); b, yield 53%; mp 72-74°C. ref (4). (a)=diethyl ether; (b)=hexane; (c)=acetone; (d)=light petroleum (bp 40-60°C).

TABLE II

Products obtained by cyclization of aminomethylenemalonates **2a-d** *according to Scheme 1*

Compound	Method	Compounds isolated (yields %)
2a	C	**4a**(98)
2b	C	**4e**(12.6), **5b**(34.5), **6b**(15)
2c	C	**4c**(50), **4f**(9.4), **5c**(14.5), **6c**(20)
2d	C	**4g**(22)

TABLE III

Reaction conditions, yields and physical and spectroscopic data of compounds obtained according to Scheme 1

Compd	Method	Yield %	M.p. °C from	Analyses for	ν_{max} cm^{-1}	λ_{max} [EtOH] nm	^{1}H-NMR [200 MHz]with internal lock J in Hz	Solv.
4a	C	98	295-298 a	$C_{12}H_{10}N_4O_3^+$ 0.25 H_2O	3420,1720,1700, 1645,1625,1585	320sh, 316,262sh, 248sh,240,202	16.30(brs,1H, NH), 13.30(brs,1H,NH),8.46 (s, 1H,H-5), 8.21(d,1H, J 9,H-8),7.43(d, 1H, J 9,H-9), 4.25((q, 2H,J 7, CH_2Me), 1.31(t,3H,J 7,CH_2Me)	DMSO-d_6
4c	C	11.8	>330 a	$C_{13}H_{12}N_4O_3^+$ 1.75 H_2O	3500,1660,1620, 1585	356sh,339,326sh, 304sh,290sh,241, 203	8.89(s, 1H,H-5), 8.24(d,1H, J 9,H-8),7.62 (d,1H, J 9,H-9), 4.53(s,3H, N-Me), 4.27 (q, 2H,J 7, CH_2Me), 1.28(t, 3H,J 7,CH_2Me)	DMSO-d_6
4e	C F	12.4 81.3	210-212 b	$C_{15}H_{16}N_4O_3$	1720,1680,1620, 1590, 1570	336,324,315sh,266, 252,233,203	8.67(d,1H,J 9,H-8), 8.54(s,1H, H-5), 7.49 (d,1H,J 9,H-9), 5.14(q,2H,J 7,N-CH_2Me), 4.44(q,2H,J 7,O-CH_2Me), 4.38(s,3H,N-Me), 1.62(t,3H, J 7, N-CH_2Me), 1.44(t,3H,J 7, O-CH_2Me)	$CDCl_3$
4f	C F	9.4 19.1	180-182 b	$C_{15}H_{16}N_4O_3$	1680,1625,1615, 1580,1550	344,331,312sh,300sh 260sh, 248,242,206	8.49(d,1H,J 9,H-8), 8.48(s,1H,H-5),7.75 (d,1H,J 9,H-9), 4.91(q,2H,J 7,CH_2Me),4.58 (s,3H,N-Me), 4.43(q,2H,J 7,CO_2CH_2Me),1.56 (t,3H,J 7, N-CH_2Me), 1.44(t,3H,CO_2CH_2Me)	$CDCl_3$
5b	C	34.5	275-278 c	$C_{13}H_{12}N_4O_3$	3450br,1730,1645 1630,1590,1555	328,318,264sh,248sh 235	8.44(s,1H,H-5), 8.22(d,1H,J 9,H-8),7.77 (d,1H,J 9,H-9), 4.39(s,3H,N-Me),4.24(q,2H J 7.5,CH_2Me), 1.30(t,3H,J 7.5,CH_2Me)	DMSO-d_6

TABLE III *(continuation)*

5c	C	14.5	232-235 b	$C_{13}H_{12}N_4O_3$	3400-:-2600, 1720, 1650, 1610, 1575, 1535	356sh,339,326sh, 304sh,290sh,240, 205	12.31(s,1H,OH), 9.25(s,1H,H-5),8.18(d,1H, J~9,H-8), 7.85(d,1H,J~9,H-9), 4.60(s, 3H, N-Me), 4.54(q,2H,J 7,CH_2Me), 1.50 (t,3H,J 7, CH_2Me)	$CDCl_3$
6b	C	14.6	>320 b	$C_{10}H_8N_4O$ + 0.75 H_2O	3400 br,1650, 1630,1590	321sh,316,303,286sh, 264,256,246,229, 200	12.75(brs,1H,NH), 8.18(d,1H,J 9,H-8), 7.92 (d,1H,J 7.5,H-5), 7.74(d,1H,J 9,H-9), 6.27 (d,1H,J 7.5,H-6), 4.39(s,3H,N-Me) ^{13}C: 175.87 (C=O)	DMSO-d_6
6c	C	20	261-264 b	$C_{10}H_8N_4O$ + 0.33 H_2O	3440 br,1650 w, 1625,1550	321,308,300sh,264, 242,231,200	12.65(brs,1H,NH), 8.10(d,1H,J 9,H-8),7.9 (d,1H,J 7.5,H-5), 7.72(d,J 9,H-9), 6.31(d, 1H,J 7.1,H-6), 4.63(s,3H,N-Me). ^{13}C: 175.87(C=O).	DMSO-d_6
7b	D	35.3	> 300 c	$C_{13}H_{11}ClN_4O_2$	3440 (overtone), 1720,1700,1640, 1620, 1565	312,300,237,217	9.41(s,1H,H-5), 8.46(d,1H,J 9,H-9), 7.79 (d,1H,J 9,H-8), 4.53(q,2H,J 7, CH_2Me), 4.47(s,3H,N-Me), 1.49(t,3H,J 7,CH_2-Me)	$CDCl_3$
7c	D	73.8	147-148 c	$C_{13}H_{11}ClN_4O_2$	3440(overtone) 1720,1600	280sh,260,227	9.28(s,1H,H-5), 8.27(d,1H,J 10,H-8),7.98 (d,1H,J 10, H-9), 4.63(s,3H,N-Me), 4.56 (q,2H,J 7, CH_2Me), 1.49(t,3H,J 7,CH_2Me)	$CDCl_3$
8a	H	98.5	> 300 a	$C_{10}H_6N_4O_3$	3410,1690,1640, 1620,1590,1565	323,310,300sh,260sh, 248,242,200	8.89(s,1H,H-5), 7.98(d,1H,J 9, H-8),7.42 (d,1H,J 9, H-9), 4.04(s,3H, N-Me)	DMSO-d_6 + NaOD

TABLE III *(continuation)*

8b	H	97	300-305 a	$C_{11}H_8N_4O_3$	3500-2600,1710, 1620,1585,1560	321,308,298sh, 262sh,250,245,234	15.46(s,1H,OH), 14.40(brs,1H,COOH), 8.68 (s,1H,H-5), 8.31(d,1H, J 9, H-8), 7.98 (d,1H,J 9, H-9), 4.43(s,3H,N-Me)	DMSO-d_6
8c	H	94.4* 89**	291-293 a	$C_{11}H_8N_4O_3$	3500-2600,1720, 1640,1610,1565, 1560,1500	342sh,332,320, 308sh,280sh,249,	9.27(s,1H,H-5), 8.34(d,1H,J 9., H-8), 8.17(d,1H,J 9 , H-9), 4.67(s,3H,N-Me)	DMSO-d_6 TFA
9	G	16.7	> 320 b	$C_{13}H_{12}N_4O_3$	3500-2700,1700, 1620,1590,1560, 1520	324,309,276sh,256, 218	9.10(s,1H,H-5), 8.12(d,1H,J 9, H-8),7.99 (d,1H,J 9, H-9), 4.58(s,3H,N-Me), 4.36 (q, 2H, J 7, O-CH_2Me), 4.50-3.50(brs,1H, COOH, exchanges with D_2O), 1.44(t, 3H, J 7, OCH_2Me) ^{13}C : 168.29(C=O); 163.71(C-OCH_2Me) OH	DMSO-d_6

Crystallization solvent: a=dimethylsulfoxide; b=ethanol; c=acetone.
* yield obtained from **5c**; ** yield obtained from **4c**.

General procedures for cyclization of **2a-d**

Method C: Compounds **2a-d** (4-22 mmole) were added dropwise to a large excess (10 fold) of preheated Dowtherm at 250°C and the mixture refluxed for 4 h. The work-up of the mixture was carried out as under method A to give the compounds of Table III. It is to be noted that, in the case of cyclization of **2d**, the work-up of the oily residue, obtained after addition of hexane onto the reaction mixture, was carried out treating it with 2M NaOH aqueous solution. After removal of the insoluble residue, the alkaline mother liquors were made acid with concentrated HCl aqueous solution to give compound **4g**. Mps, yields, analytical and spectroscopic data of compounds **4a,4c,4e,4f,5b,5c,6b,6c** are reported in Table III, those of **4g** in Table IV.

Chlorination of compounds **5b** *and* **5c**

Method D: A mixture of compound **5b** or **5c** (21 mmole) and $POCl_3$ (4 ml) was heated at 110-115°C, under stirring, for 2.5 h. Then the excess of $POCl_3$ was removed *in vacuo* and the oily residue was poured into crushed ice and stirred overnight. The precipitate was filtered off, thoroughly washed with water and eventually recrystallised to give chloroderivatives **7b** and **7c**. Mps, yields, analytical and spectroscopic data are reported in Table III.

General procedures for ethylation of compounds **4a,4c,5b,5c,7c**

Method E: To compounds **4a,c** (2.5-7.8 mmole), dissolved in a mixture of DMF (12 fold its weight) and potassium carbonate in excess (20%) heated to 100°C, a large excess of ethyl iodide (100%) was added under stirring and the mixture kept at this temperature for 4 h. After cooling, the inorganic phase was filtered off and the mother liquors, taken up with diethyl ether, were chromatographed on a silica-gel column, eluting with diethyl ether-ethanol to give compounds **4d,4f,14** and **15**. Yields, mps, analytical and spectroscopic data are reported in Table III and IV.

Method F: To compound **5b,c** (2.5-3.7 mmole), suspended in DMF (25-40 ml), 50% sodium hydride dust (5-7 mmole) was added and the mixture heated to 90°C, under stirring, for 2.5 h. After cooling, ethyl iodide (5-7.4 mmole) in DMF was added and heating was continued for 20 h. The inorganic phase was filtered off and the mother liquors chromatographed as above to give esters **4e** and **4f**, which were identical to those previously described.

Method G: To a solution of sodium ethylate (prepared from 36 ml of absolute ethanol and 0.36 g of sodium) compound **7c** (0.45 g, 15.5 mmole) dissolved in absolute ethanol (35 ml) was added. The mixture was refluxed under stirring for 2.5 h. After cooling, the inorganic phase was filtered off and the mother liquors evaporated under vacuum. The solid residue was redissolved with water and made acidic with concentrated HCl aqueous solution. The solid obtained was chromatographed over silica gel, eluting with diethyl ether-ethanol to give compound **9**. Yield, physical data and NMR are reported in Table III.

General procedures for preparation of acids **8a-c** *of Scheme 1 and* **4g, 16-19** *of Scheme 3.*

Method H: Esters **(4a,c) (5b-c) (4d-f) (14-15)** were suspended in 2M NaOH aqueous solution (1 ml for each 10 mg of ester) and the mixture heated to 100°C and kept under stirring for 4 h. After cooling, the solution was made acidic with 1M

TABLE IV

Methods, yields, physical, analytical and spectroscopic data of compounds obtained in Scheme 3

Compd	Method	Yield %	Mp °C from	Analyses for	ν_{max} cm^{-1}	λ_{max}[EtOH] nm	^{1}H-NMR [200MHz] with internal lock; J in Hz; Chemical shifts in ppm ( ). Solv. $CDCl_3$=a; DMSO-d_6=b; TFA=c; Acetone-d_6=d	
4d	C	3.2	178-180 a	$C_{14}H_{14}N_4O_3$	1720,1640,1615, 1590,1560,1550	354sh,338,325,312sh, 296sh,248sh,241, 203	12.30(s,1H,NH),9.24(s,1H,H-5),8.17(d,1H, J 9,H-8), 7.85(d,1H,J 9,H-9), 4.86(q,2H,J 7,N-CH_2Me), 4.51(q,2H,J 7,OCH_2Me), 1.77 (t, 3H,J 7, N-CH_2Me), 1.49(t,3H,J 7,OCH_2-Me). ^{13}C [50 MHz]($CDCl_3$): 170(C=O), 167(COOEt)	a
4g	C	22	287-291 b	$C_{12}H_{10}N_4O_3 + H_2O$	1690,1640,1620, 1550	332,320,280sh,253, 250	15.51(s,1H,COOH), 14.23(s,1H,NH),8.68(s,1H, H-5), 8.18(d,1H, J 9,H-8), 7.92(d,1H, J 9, H-9), 4.92(q,2H,J 7, N -CH_2Me), 1.72(t, 3H, J 7, N-CH_2Me).	b
14	E	16.3	259-262 c	$C_{16}H_{18}N_4O_3$	1700,1620,1605, 1575	333,323,312infl, 265,250,231,201	8.75(s,1H, H-5), 8.36(d,1H, J 9,H-8), 7.92 (d,1H, J 9, H-9), 5.12(q, 2H, J 7, N_1-CH_2Me), 4.83(q, 2H, J 7,N_4-CH_2Me), 4.27(q, 2H,J 7,O-CH_2Me), 1.56(t,3H, J 7, N_1-CH_2Me), 1.47(t,3H, J 7,N_4-CH_2Me), 1.31(t, 3H, J 7, OCH_2Me).	b
15	E	19.6	140-142 d	$C_{16}H_{18}N_4O_3$	1695,1630,1620, 1590,1570,1495	344,331,310,272infl 260sh,249,242,206	8.57(s,1H, H-5), 8.29(d, 1H, J 9,H-8), 7.73 (d, 1H, J 9, H-9), 4.99(q, 2H, J 7,N_2-CH_2Me) 4.90(q, 2H, J 7,N_4-CH_2Me), 4.29(q, 2H, J 7, OCH_2Me), 1.73(t,3H, J 7, N_2-CH_2Me), 1.53(t, 3H, J 7,N_4-CH_2Me), 1.34(t,3H,J 7, OCH_2Me).	d

TABLE IV *(continuation)*

16	H	88	286-290 b	$C_{13}H_{12}N_4O_3$	3450,1720,1625, 1570,1515	332, 321,310infl, 266sh,257,252,237, 202	15.47(s, 1H, COOH), 9.15(s, 1H, H-5),8.42 (d, 1H, J 9, H-8), 8.06(d, 1H, J 9, H-9), 5.29(q, 2H, J 7,NCH_2Me), 4.46(s, 3H, N-Me), 1.53(t, 3H, J 7,NCH_2Me),	b
17	H	88	310 b	$C_{13}H_{12}N_4O_3$	1735,1625,1605, 1515	341,327,312,260infl 247,203	13.78(s, 1H, COOH), 9.43(s, 1H, H-5), 8.49 (d, 1H, J 9.5, H-8), 8.30(d, 1H, J 9.5, H-9), 5.52(q, 2H, J 7,CH_2Me), 4.73(s, 3H, N-Me), 1.75(t, 3H, J 7, CH_2Me).	b
18	H	88	245-246 a	$C_{14}H_{14}N_4O_3$	1710,1690,1625, 1570,1520	332,321,308infl, 266sh,258,252,237, 201	15.52(s,1H, COOH), 9.16(s, 1H,H-5), 8.44 (d, 1H, J 9, H-8), 8.12(d, 1H, J 9, H-9), 5.29(q, 2H, J 7, N_1-CH_2Me), 4.89(q, 2H, J 7.3, N_4-CH_2Me),1.58(t, 3H, J 7, N_1-CH_2Me), 1.52(t, 3H, J 7.3, N_4-CH_2Me).	b
19	H	96	294-295 b	$C_{14}H_{14}N_4O_3$ + 0.66 H_2O	1720, 1625,1600, 1560	340,326,310,266infl, 258sh,247,202	9.44(s, 1H, H-5),8.57(d, 1H, J 9.3, H-8), 8.38(d, 1H, J 9.3, H-9), 5.53(q, 2H, J 7, N_2-CH_2Me), 5.08(q, 2H, J 7.3, N_4-CH_2Me), 1.88(t, 3H, J 7.3, N_2-CH_2Me), 1.80(t, 3H, J 7, N_4-CH_2Me).	a + c

a=ethanol; b=dimethylsulfoxide; c=acetone; d=diethyl ether.

HCl aqueous solution and the acids precipitated were collected and recrystallised. Tables III and IV report yields, mps and analytical and spectroscopic data.

B) MICROBIOLOGY

Test organisms and culture media

Escherichia coli ATCC 25922; *Escherichia coli* (Hospital isolate); *Pseudomonas aeruginosa* ATCC 27853; *Proteus mirabilis; Streptococcus faecalis* ATCC 33186; *Staphylococcus aureus* ATCC 25923.

Antimicrobial assay

Antimicrobial activity was evaluated *in vitro*. Nalidixic acid was used as growth inhibitor standard. MIC (μg/ml) values were determined according to a microdilution method in broth described by Gavant and Town (12). Each compound was dissolved in dimethylsulfoxide (DMSO) (10 mg/ml) and diluted with trypticase soy broth (TSB, DIFCO) containing 1% glucose and 0.02% phenol red in order to obtain drug concentrations ranging from 500 μg to 0.98 μg/ml. Inocula of bacteria were prepared from overnight growth cultures diluted so that the final inocolum size was 10^5 cells/ml. Inoculated plastic trays with the diluted antimicrobial agent added were then incubated at 37°C for 18 h. The MIC was defined as the lowest amount of compound preventing growth or as the change of colour of the indicator from red to yellow.

Results are reported in Table V.

Results and discussion

Table V reports the results of the *in vitro* antimicrobial activity of acids **4g,8a,8b,8c,16,17,18** and **19** against strains of Gram(+) and Gram(-) bacteria in comparison with nalidixic acid.

The data clearly indicate that all these acids were only poorly or completely inactive. In particular, acid **4g**, an isomer of a previously described compound, in which the annelation of the triazole occurred at position [4,5-f] and which showed a certain selective activity (MIC = 25 μg/ml) against *Escherichia coli* (1), seemed to be devoid of any activity against this strain, but was only just equiactive against both *Streptococcus faecalis* and *Proteus mirabilis* (MIC = 125 μg/ml).

The results obtained seem to indicate that the annelation position of the triazole ring in [4,5-h] in quinolinone carboxylic acids of structure **C**, in comparison with the previously described isomers of structure **A** (1), negatively influences activity as a whole. At this stage, the inactivity demonstrated by our compounds only seems attributable to steric effects due to this type of annelation.

TABLE V .

Anti-microbial evaluation of acids ***4g,8a,8b,8c,16,17,18,19.***
MIC (μg/ml) in comparison with nalidixic acid (A)

Compd	Escherichia coli (clin.isol)	Escherichia coli ATCC25922	Pseudomonas aeruginosa ATCC27853	Proteus mirabilis ATCC13315	Streptococcus faecalis ATCC29212	Staphilococcus aureus ATCC25923
A	3.17	6.25	100	12.5	500	125
4g	250	500	250	125	125	500
8a	-	500	500	250	500	500
8b	-	500	500	500	500	500
8c	-	500	500	500	500	500
16	-	250	250	250	500	500
17	-	500	500	250	500	500
18	-	500	500	500	500	500
19	-	500	500	500	500	500

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REFERENCES

1) NUVOLE A., SANNA P., PAGLIETTI G., JULIANO C., ZANETTI S., CAPPUCCINELLI P., *Il Farmaco,* **44**, (6)619; 1989.
2) PAGLIETTI G., SPARATORE F., *Ann. Chim.*, (Rome), **62**, 128; 1972.
3) MILATA V., ILAVSKY D., GOLJER I., *Chem. Papers*, **42**, 801; 1988.
4) MILATA V., ILAVSKY D., GOLJER I., LESKO J., CHAHINIAN M., HENRY-BASCH E., *Coll. Czech. Chem. Comm.*, **55**, 1038; 1990.
5) MILATA V., ILAVSKY D., *Coll. Czech. Chem. Comm.*, **52**, 2918; 1987.
6) RAYCHANDHURI A., BASU U.P., *J. Indian Chem. Soc.*, **47**, 25; 1970.
7) JONES G.: Quinoline Part I, in "The Chemistry of Heterocyclic Compounds", E.C. TAYLOR & A. WEISSBERGER Eds, J. Wiley-Interscience-New York.
8) SINSKY M.S., BASS R.G.*J. Heterocycl. Chem.*, **21**, 759; 1984 and references cited therein.
9) FRIES K., GÜTERBOCK H., KUHN H., *J. Liebig's Ann. Chem.*, **511**, 213; 1934.
10) KAMEL M., SHERIF S., KAMEL M.M., *Tetrahedron*, **20**, 211; 1964.
11) KAMEL M., ALI M.I., KAMEL M.M., *Tetrahedron*, **22**, 3351; 1966.
12) JONES R.N., BARRY A.L., GAVANT T.L., WASHINGTON J.A., Susceptibility tests: Microdilution and macrodilution broth procedures, p. 972-977. In LENNETTE E.H., BALOWS A., HAUSLER W.J., Jr., SHADOMY H.J., (Editors), Manual of Clinical Microbiology, 4 th Edition, American Society for Microbiology, Washington, D.C.; 1985.

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