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1,2,3-TRIAZOLO[4,5-f]QUINOLINES
II. Preparation and antimicrobial evaluation of 6-ethyl-6,9-dihydro-1(2)(3)-R-1(2) (3)H-triazolo [4,5-f]quinolin-9-one-8-carboxylic acids as anti-infectives of the urinary tract (1).

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SUMMARY — *Some 6-ethyl-1(2)(3)-R-1(2)(3)H-triazolo[4,5-f]quinolin-9-one-8-carboxylic acids were prepared as novel analogues of oxolinic acid in order to evaluate the effect on antibacterial activity of the isosteric replacement of the dioxolic moiety with the triazole ring substituted in position 1 or 2.* In vitro *tests showed a good and selective activity against* Escherichia coli *(MIC 12.5 μg/ml) of compound (XVI).*

RIASSUNTO — *Sono stati preparati alcuni acidi triazolo[4,5-f]chinolin-9-one-8-carbossilici analoghi dell'acido ossolinico allo scopo di verificare l'influenza della sostituzione isosterica dell'anello diossolico con quello triazolico sull'attività antibatterica sopratutto anti Gram negativi. L'attività riscontrata* in vitro *sembra elettiva nei confronti dell'*Escherichia coli *per il composto (XVI) che presenta una MIC di 12.5 μg/ml. Vengono descritti i metodi di preparazione degli acidi e degli intermedi, nonché le condizioni dei saggi microbiologici.*

Introduction

Recent elucidation of the inhibitory effect of oxolinic and nalidixic acids on DNA gyrase (2) has stimulated extensive structure-activity studies on new fluoroquinolone derivatives as a source of new antibacterial agents (3).

Successful variations in either oxolinic or nalidixic acid have led to more

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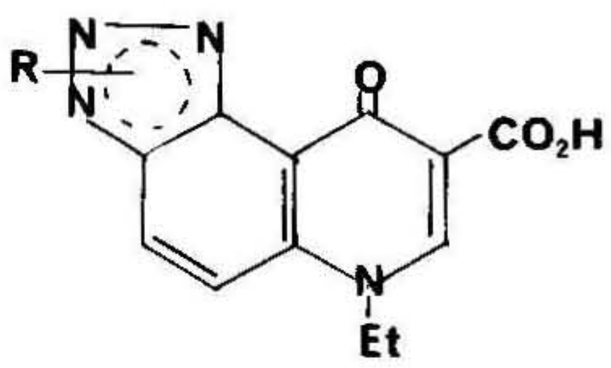
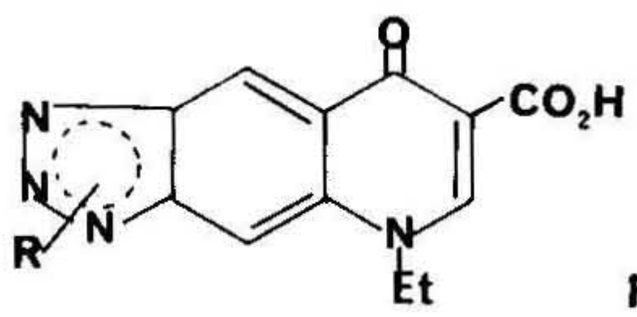
potent orally active compounds like norfloxacin and enoxacin now being marketed as broad spectrum anti-infective agents. In spite of this success different approaches to the development of new compounds have never ceased to appear in the literature (4) and this contribution is made in that context. In connection with the research program outlined in the preceding paper of this journal we have undertaken the preparation and the antimicrobial evalutaion of triazoloquinolinone carboxylic acids of structures A, B and C as bioisosters of oxolinic acid where the triazole and its annelation position might play an important role on their potential activity as anti-infective agents of the urinary tract.

In this paper we wish to report the synthesis of compounds A and preliminary results on the antimicrobial activity found *in vitro* against Gram(+) and (−) bacteria and *Candida albicans*. Further considerations will be presented as soon as the preparation and the screening of compounds B and C comes to completion.



A



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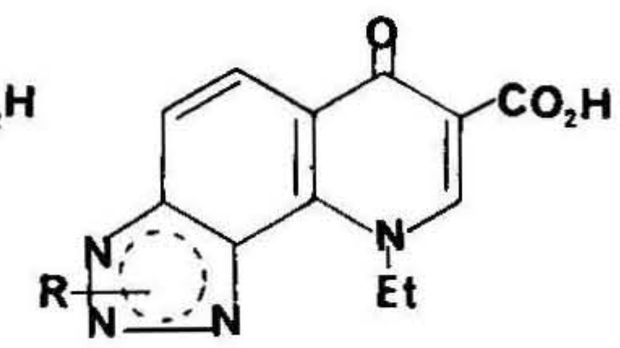


C

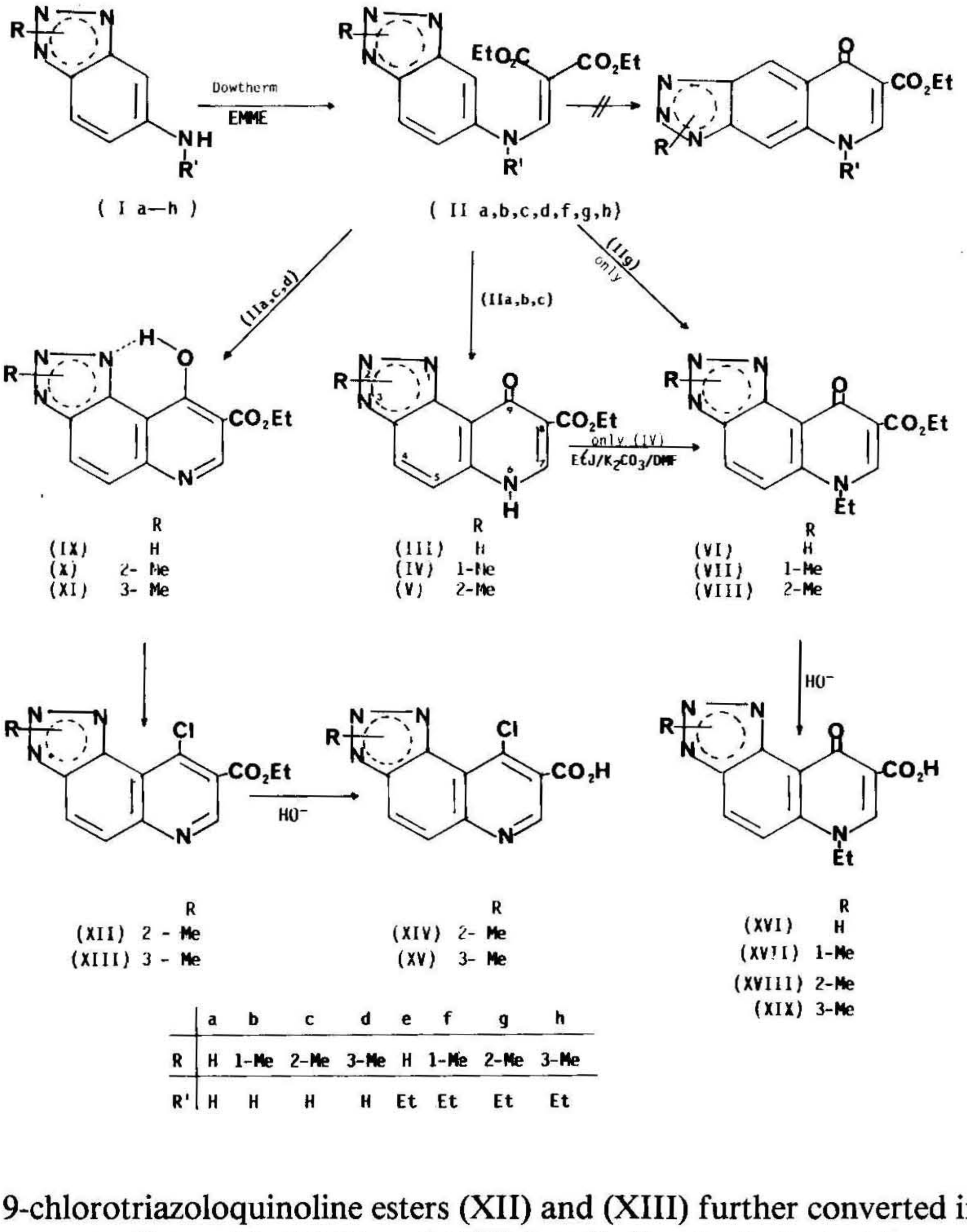
Chemistry

A synthetic approach to the preparation of compounds of structure A has already been outlined in (1) and is now reproduced in Scheme 1.

The reaction of amines (I a-h) with diethyl ethoxymethylenemalonate (EMME) was carried out in Dowtherm A and afforded aminomethylenemalonates (II a,b,c,d,f,g,h) in good yields (Table I). The cyclisation of these intermediates did not produce any linear triazoloquinolinones of B type in accordance with what we have already observed (1), but our experiments show that even angular ring closure is difficult when the ethyl group is present on the side chain nitrogen.

This step requested a variety of conditions that are indicated in Table I. According to these cyclisation of malonates took place yielding in some cases either triazoloquinolin-9-one or 9-hydroxytriazoloquinoline esters. Compounds (II a,b,c) gave respectively the derivatives (III) (IV) (V), while (IId) gave (XI). In addition from (IIa) and (IIc), under different conditions, we isolated respectively compounds (IX) and (X) which showed different physical properties (M.p., I.R. and N.M.R. spectra) and reactivity from their tautomers (III) and (V). In fact, treatment of (X) and (XI) with $POCl_3$

SCHEME I



	a	b	c	d	e	f	g	h
R	H	1-Me	2-Me	3-Me	H	1-Me	2-Me	3-Me
R'	H	H	H	H	Et	Et	Et	Et

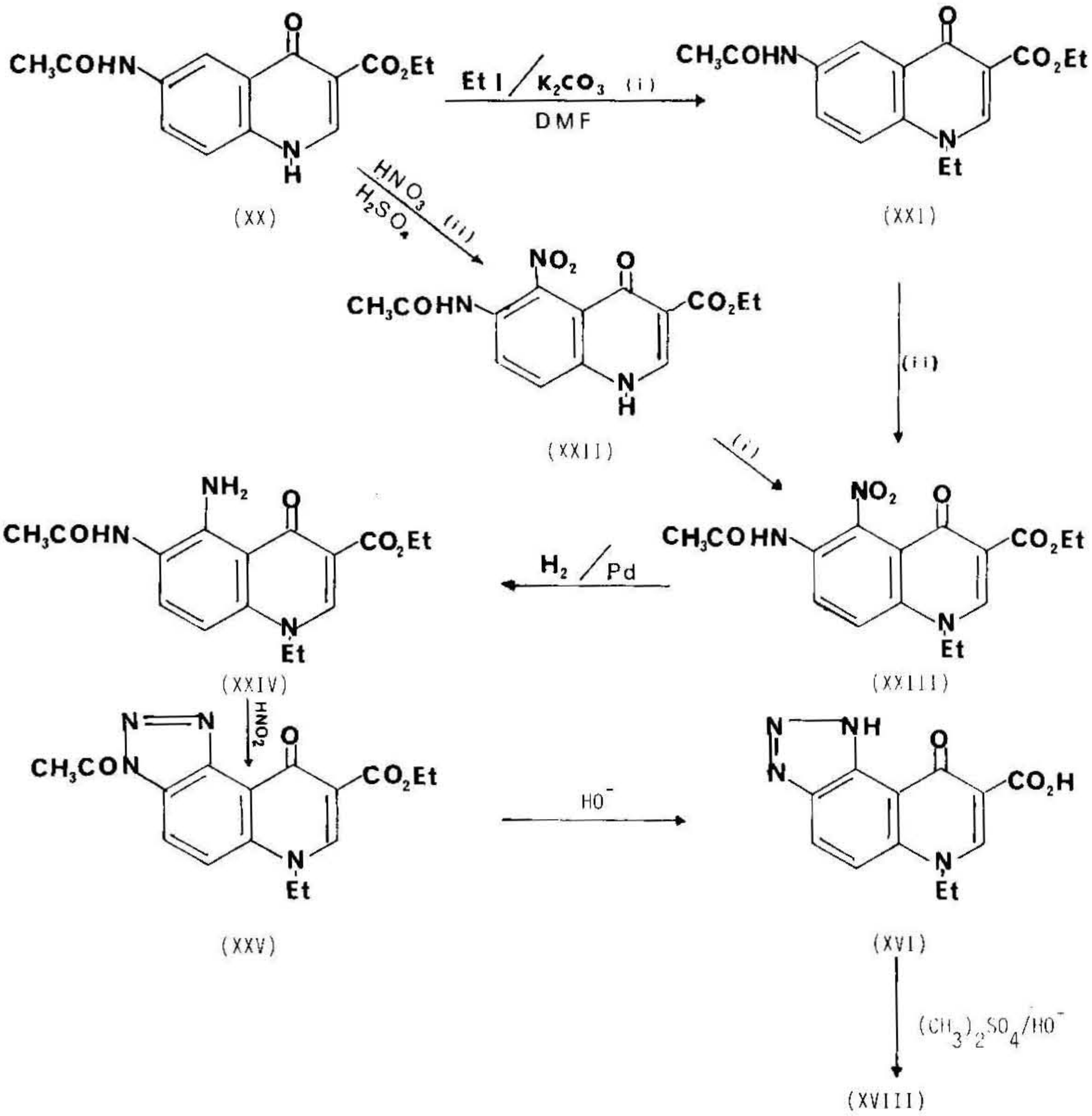
gave 9-chlorotriazoloquinoline esters (XII) and (XIII) further converted into the corresponding acids (XIV) and (XV) by alkaline hydrolysis. The esters (III) (IV) and (V) were instead resistant to chlorination.

A critical step was the attempt of ethylation of triazoloquinolinones (III-:-V) or their tautomers (IX-:-XI) using ethyl iodide and potassium carbonate in dry dimethylformamide. Only compound (VII) was obtained under these conditions. In all other cases when different procedures, claimed more successful by several authors (5), were employed, the reaction failed. This fact would suggest that whenever, owing to tautomeric equilibrium, hydrogen bonding occurs between triazole and hydroxy group in position 9 the reaction of ethylation is disfavoured. In order to overcome this inconvenience we attempted the cyclisation of compounds (II f,g,h) where the

ethyl group was already attached to the aminomethylenemalonate side chain, but in these cases the cyclisation proved to be very difficult and yielded compound (VIII) only, in accordance with our previous observation (1) that angular ring closure is more favoured when conjugation between side chain amine and 2-methylbenzotriazole exists.

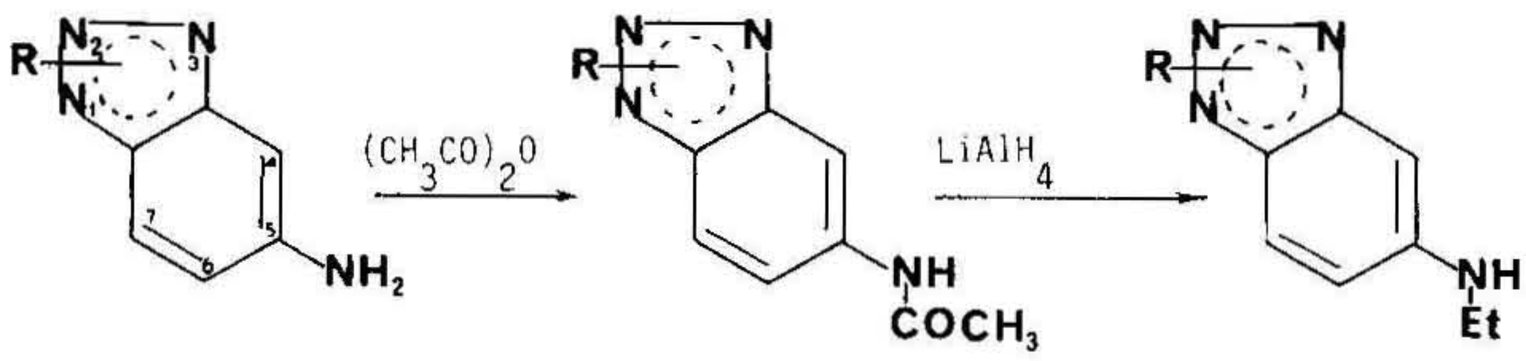
The unavailability of the intermediate (VI) for obtaining the desired acid (XVI) suggested an alternative route for its preparation (Scheme 2). The known compound (XX) (6) easily underwent ethylation to give (XXI) and after nitration (XXIII). An identical results was obtained when (XX) was first nitrated to (XXII) and then ethylated to (XXIII). Reduction of the latter followed by diazotisation of (XXIV) afforded compound (XXV) that on alkaline hydrolysis yielded the desired (XVI). Attempts at obtaining the acid (XIX) *via* a straightforward methylation of compound (XVI) on the basis of a facile alkylation of all atoms of nitrogen in the triazole ring (7), have led instead to 2-methyl derivative (XVIII) identical with that obtained by an alternative route.

SCHEME 2



Amines (I e-h) are new compounds and were prepared by $LiAlH_4$ reduction of acetyl derivatives (a,b,c,d) according to Scheme 3.

SCHEME 3



(I a—d) (I e—h)

R	R	R
a, H	(a) 2-$COCH_3$	e, H
b, 1- Me	(b) 1-Me	f, 1-Me
c, 2- Me	(c) 2-Me	g, 2-Me
d, 3- Me	(d) 3-Me	h, 3-Me

In the case of (Ia), acetylation gave a diacetylderivative (a) the m.p. of which was identical with that reported in the literature (8) but the exact position of the acetyl group in the triazole ring was not defined. In the light

TABLE I

Methods and time of reaction, melting points, yields, crystallization solvent, analytical data of compounds (II a-d), (II f-h), (III-V), (VIII-XI).

Comp.	Method	T °C	Reaction time	M.P. °C	Yield %	Solvent	Empirical formula	References
(IIa)	A	200	20'	178-80	86	a	$C_{14}H_{16}N_4O_4$	(1,11b)
(IIb)	A	110	12h	123-25	84	b	$C_{15}H_{18}N_4O_4$	
(IIc)	A	110	24h	101-03	60	a	$C_{15}H_{18}N_4O_4$	(1,11b)
(IId)	A	110	12h	122-24	76	b	$C_{15}H_{18}N_4O_4$	
(IIf)	A	150	24h	73-75	69	c	$C_{17}H_{22}N_4O_4$	
(IIg)	A	150	90'	78-79	65	c	$C_{17}H_{22}N_4O_4$	
(IIh)	A	150	24h	86-87	83	c	$C_{17}H_{22}N_4O_4$	
(III)	C	144	3h	199-200	16	d		(1)
(IV)	B	259	15'	273-75	86	e	$C_{13}H_{12}N_4O_3 \cdot 0.25\ H_2O$	
(V)	D	160	45'	126-28	54	a		(1,11b)
(VIII)	C	144	3h	208-11	71	g	$C_{15}H_{16}N_4O_3 \cdot 0.25\ H_2O$	
(IX)	E	150	3h	320	27	f	$C_{12}H_{10}N_4O_3$	
(X)	A	260	5'	292-96	63	e	$C_{13}H_{12}N_4O_3$	
(XI)	B	259	15'	290[d]	94	e	$C_{13}H_{12}N_4O_3$	

a, ethylether; b, $EtOH/Et_2O$; c, Et_2O/light petroleum 60-80°C; d, acetone/Et_2O; e, DMF/H_2O; f, dimethylsulfoxide; g, acetone.

of its chemical behavior [insolubility in mineral acid as reported for all 2-alkyl substituted benzotriazoles(7)] and for its U.V. and N.M.R. spectra we were able to establish that compound (a) corresponds to the structure assigned and that the acetyl group is easily removed during reduction with $LiAlH_4$ to give (I e).

The structure of the described compounds has been achieved from the analytical data and from their proton N.M.R. spectra. Angular triazolo [4,5-f]quinolinones showed an unambiguous AB system for the aromatic protons in C-4 and C-5 with a coupling constant of 9 Hz as observed in analogous cases (1,9,10). ^{1}H-N.M.R. spectra of (III) and (V) had been previously reported (1), while the spectrum of (IV) did not show the expected ortho coupling between N-6 and C-7 protons, an intense exchange being observed when recorded in DMSO-d_6 owing to its insolubility in $CDCl_3$. On the other hand its I.R. spectrum clearly shows both NH and OH absorption bands due to the aforementioned equilibrium. The I.R. spectra of (IX) and (X) clearly show a strong OH absorption band.

Experimental

A) CHEMISTRY

Melting points are uncorrected and were recorded on a Kofler apparatus. Elemental analyses (C,H,Cl,N) were performed at Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Padova University, and analytical results were within ±0.3% of theoretical values. ^{1}H-N.M.R. spectra were recorded at 60 MHz with a Perkin-Elmer Hitachi R-24A spectrometer and at 200 MHz with a Varian XL-200 instrument in both cases using TMS as internal standard of the solvent indicated in Tables II and III. U.V. spectra are given for solutions in ethanol and were recorded in nm (log ϵ) with a Perkin Elmer Lambda 5 spectrophotometer. I.R. spectra are for Nujol mulls and were recorded on a Perkin-Elmer 297 and 781 instruments.

Intermediates

Starting material was 5-nitrobenzotriazole (Aldrich) and its methylderivatives prepared according to known methods (1,11). 1-Methyl-5-nitro-1H-benzotriazole was also obtained by diazotisation of 2-amino-4-nitro-N-methylaniline as reported (12). Amines (I a-d) were prepared by catalytic hydrogenation over palladised charcoal as described: (Ia) (8), (Ib) (13), (Ic) (14), (Id) (12).

2-Acetyl-5-acetylamino-2H-benzotriazole (a)

A suspension of (I a) (1.03 g, 7.79 mmol) in acetic anhydride (15 ml) was kept under stirring at room temperature for 4 h. After the first 30 min. the clear solution got cloudy. Then a solid (a) (0.46 g, 27% yield) was collected: m.p. 182-85°C [lit. 184-85°C (8)]. Evaporation of the mother liquors *in vacuo* gave a residue that when washed with water, dried and recrystallized from ethyl ether, yielded further acetyl-derivative (a) (0.97 g, 57%) identical with a specimen as above.

I.R.: ν 3260, 1730, 1670 cm^{-1}; U.V.: λ_{max} 284,210; ^{1}H-N.M.R. ($CDCl_3$): δ 2.18 (3H,s, N-COCH_3), 2.85 (3H, s, NH-COCH_3), 7.60 (1H, d, J = 9 Hz, C-7 H), 7.90 (1H, dd, J = 9 and 2 Hz, C-6 H), 8.15 (1H, d, J = 2 Hz, C-4 H), 10.05 (1H, br s, NH).

5-Ethylamino-1H-benzotriazole (Ie)

Compound (a) (1.39 g, 6.37 mmol) in dry THF (75 ml) was added dropwise to a suspension of $LiAlH_4$ (0.97 g, 25.5 mmol) in the same solvent (25 ml) externally cooled with an iced bath and stirred. Once the addition was over the mixture was refluxed for 20 h. After cooling at 0°C the excess of hydride and the complex were destroyed using in the sequence water (2 ml), 20% NaOH solution (4.5 ml), water (6.2 ml). A solid was collected and thoroughly washed with THF. The filtrate, dried over anhydrous sodium sulfate, when evaporated *in vacuo* gave an oily residue (1.62 g) which was chromatographed over silica gel column (50:1) eluting with ethyl ether to yield (I e) (0.79 g, 77% yield), as white solid, m.p. 128-29°C from ether.

Analysis (C_8 $H_{10}N_4$): C,H,N.

I.R.: ν3370, 1635 cm^{-1}; U.V.: λ_{max} 321, 240, 206.

^{1}H-N.M.R. ($CDCl_3$): δ 1.20 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 3.05 (2H, q, J = 7 Hz, CH$_2$CH$_3$), 6.38 (1H, d, J = 2Hz, C-4 H), 6.55 (1H, dd, J = 9 and 2 Hz, C-6 H), 7.45 (1H, d, J = 9 Hz, C-7 H), 7.0-7.60 (1H, br s, NH).

1-Methyl-5-ethylamino-1H-benzotriazole (I f)

To a suspension of (b) (2 g, 10.5 mmol), prepared as described (14), in diglyme (50 ml) was added in small portions at room temperature an excess of $LiAlH_4$ (1.5 g, 39.5 mmol). During the addition all the solid passed into solution which became pale green. Then the mixture was heated at 100°C for 4 h. After cooling following the above procedure the excess of hydride and the complex were destroyed and the filtrate was extracted with chloroform. The extract, dried over anhydrous sodium sulfate, on evaporation in vacuo yielded (If) (1.72 g, 93% yield) m.p. 116-18°C from $CHCl_3$-ethyl ether.

Analysis ($C_9H_{12}N_4$):C,H,N.

I.R.:ν3330, 1630, 1590 cm^{-1}; U.V.:λ_{max} 344 (3.60), 229 (4.45), 201 (4.08).

^{1}H-N.M.R. ($CDCl_3$): δ 1.25 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 3.10 (2H, q, J = 7 Hz, CH$_2$CH$_3$), 3.72-3.20 (1H, br s, NH,exchanges with D_2O), 4.09 (3H, s, N-CH_3), 6.67 (1H, dd, J = 9 and 2 Hz, C-6 H), 6.80 (1H, d, J = 2 Hz, C-4 H), 7.08 (1H,d,J = 9 Hz, C-7 H).

2-Methyl-5-ethylamino-2H-benzotriazole (Ig)

This compound was obtained from (c), prepared as described (11b), in a manner similar to (Ie) in 81% yield: m.p. 84-86°C from ethyl ether-light petroleum 60-80°.

Analysis ($C_9H_{12}N_4$):C,H,N.

I.R.: ν3370 cm^{-1}; U.V.: λ_{max} 327, 216.

^{1}H-N.M.R. ($CDCl_3$): δ 1.28 (3H, t, J = 7 Hz, C*H*$_3$-CH$_2$), 3.15 (2H, q, J = 7 Hz, CH$_2$CH$_3$), 4.30 (3H, s, N-CH_3), 6.55 (1H, d, J = 2 Hz, C-4 H), 6.65 (1H, dd, J = 9 and 2 Hz, C-6 H), 7.50 (1H, d, J = 9 Hz, C-7 H).

3-Methyl-5-ethylamino-3H-benzotriazole (I h)

This compound was obtained from (d), prepared as described (12), in a similar manner as for the preparation of (If) in 91% yield: 103-05°C, from ethanol-ethyl ether.

Analysis ($C_9H_{12}N_4$): C,H.N.

I.R.: ν3300, 1670, 1590 cm^{-1}; U.V.: λ_{max} 323 (3.90), 267 (3.75), 234 (4.28), 203 (4.20).

^{1}H-N.M.R. ($CDCl_3$): δ 1.25 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 3.15 (2H, q, J = 7 Hz, CH$_2$CH$_3$), 3.50 (1H, br s, NH, collapses with D_2O), 4.10 (3H, s, N-CH_3), 6.25 (1H, d, J = 2 Hz, C-4 H), 6.52 (1H, dd, J = 9 and 2 Hz, C-6 H), 7.55 (1H, d, J = 9 Hz, C-7 H).

General methods for the synthesis of the compounds of Table I

Method A: Equimolar amounts of amine (I) (10 mmol) and EMME in Dow-

therm A (15-20 g) were heated, under stirring, at the temperature indicated in the Table I. After cooling the mixture was taken up with n-hexane and the solid filtered off and thoroughly washed with ethyl ether and eventually recrystallized.

Method B: In a two necked round bottom flask, equipped with a condenser, to 50 ml of refluxing diphenyl ether was added the ester (29 mmol). Heating was maintained for the time indicated, then the mixture was poured into a flask and diluted with light petroleum 60-80°C and stirred until a solid formed. Filtration, followed by recrystallisation from the indicated solvent, afforded the cyclic compound.

Method C: To a mixture of polyphosphoric acid (PPA) (10 g) in xylene (20 ml) was slowly added the ester (II g) (1 g, 29 mmol) dissolved in xylene (20 ml). The mixture was heated up to the temperature indicated in Table I and then poured into iced water and extracted with $CHCl_3$. The organic layer, dried over anhydrous sodium sulfate and evaporated, afforded a solid which was further recrystallized as indicated.

Method D: To ethyl polyphosphate (EPP) (10 g), previously heated as indicated, was added compound (IIc) (1g, 3.14 mmol) and the mixture was maintained at the temperature and for the time indicated in Table I. After cooling it was poured onto ice and brought to pH 5 with ammonia. The solid formed, after filtration and washing with water, was recrystallized.

Method E: This was identical with method C but xylene was replaced by DMF.

Ethyl 1-ethyl-6-acetylamino-1,4-dihydroquinolin-4-one-3-carboxylate (XXI)

Compound (XX) (0.46 g, 1.6 mmol), prepared as described (6), suspended in a mixture of DMF and potassium carbonate (5 ml + 0.69 g) was heated at 60°C for 30 min. Ethyl iodide (0.2 g, 2.47 mmol) was added and the heating was continued for 3 h. After cooling the inorganic phase was filtered off and the mother liquors poured into ice and extracted with $CHCl_3$. The extract, washed with water and dried over sodium sulfate, on evaporation afforded (XXI) (0.41 g, 90% yield), m.p. 265°C dec.

Analysis ($C_{16}H_{19}N_2O_4$): C,H,N.

I.R.: ν3450, 3290, 1690, 1630, 1615, 1590 cm^{-1}.

U.V.: λ_{max} 318 (4.17), 263 (4.35), 257 (4.42), 233 (4.45).

^{1}H-N.M.R. (DMSO-d_6): δ 1.5-1.0 (6H, m, CH_3CH$_2$), 2.0 (3H, s, CH_3CO), 4.5-3.8 (4H, m, CH_2CH$_3$), 7.56 (1H, d, H = 9 Hz, C-8 H), 7.90 (1H, dd, J = 9 and 2 Hz, C-7 H), 8.25 (1H, d, J = 2 Hz, C-5 H), 8.42 (1H, s, C-2 H), 10.20 (1H, s, NH).

Ethyl 1-ethyl-5-nitro-6-acetylamino-1,4-dihydroquinolin-4-one-3-carboxylate (XXIII)

a) To compound (XXI) (3.22 g, 10.6 mmol) in concd sulfuric acid (35 ml), externally cooled with an iced bath, was added under stirring concd nitric acid (d = 1.40) (40 ml) so that the temperature of the solution did not exceed 30°C. After 2 h at this temperature the mixture was poured onto ice and extracted with chloroform. The extract, dried and evaporated, gave a residue which after recrystallization from EtOH afforded (XXIII) (1.62 g, 44% yield), m.p. 265-67°C.

Analysis ($C_{16}N_{17}N_3O_6$): C,H,N.

I.R.: ν3450, 3180, 1735, 1660, 1635, 1610 cm^{-1}.

U.V.: λ_{max} 319 (4.15), 261 (4.23), 220 (4.57).

^{1}H-N.M.R. ($CDCl_3$ + DMSO-d_6): δ 1.6-1.1 (6H, m, CH_3CH$_2$), 2.0 (3H, s, CH_3CO), 4.5-4.0 (4H, m, CH_2CH$_3$), 7.84 (2H, at, J = 9 Hz, C-6 and C-7 H), 8.55 (1H, s, C-2 H), 9.59 (1H, s, NH-CO).

b) In a manner similar to the preparation of (XXI) compound (XXIII) was obtained from (XXII) (0.5 g, 1.56 mmol) in 50% yield.

Ethyl 6-acetylamino-5-nitro-1,4-dihydroquinolin-4-one-3-carboxylate (XXII)

To compound (XX) (1 g, 36 mmol), dissolved in sulfuric acid (d = 1.84) externally cooled at 0°C, was added HNO_3 (d = 1.40) (12 ml) keeping the temperature below 30°C. The mixture was stirred at room temperature for 3 h and eventually poured onto ice. The precipitate collected, washed and dried, gave (XXII) (0.8 g, 68% yield) m.p. 319-21°C from DMF.

I.R.: ν 3270, 3150, 3080, 1705, 1670 cm^{-1}; U.V.: λ_{max} 319, 263, 219.

^{1}H-N.M.R. (DMSO-d_6); δ 1.18 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 1.90 (3H, s, C*H*$_3$CO), 4.05 (2H, q, J = 7 Hz, C*H*$_2$CH$_3$), 7.62 (2H, at, J = 9 Hz, C-7 and C-8 H), 8.35 (1H, m, C-2 H), 9.55 (1H, s, NH-CO), 12.55 (1H, m, NH-CH =).

Ethyl 1-ethyl-5-amino-6-acetylamino-1,4-dihydroquinolin-4-one 3-carboxylate (XXIV)

Compound (XXIII) in ethanol (300 ml) was hydrogenated in the presence of 5% palladised charcoal (200 mg) under moderate pressure (3 atm.) at room temperature. The catalyst was removed by filtration and thoroughly washed with hot ethanol. The filtrate on evaporation *in vacuo* afforded (XXIV) (0.91 g, 94% yield), m.p. 248-50°C.

Analysis ($C_{16}H_{19}N_3O_4$): C,H,N.

I.R.: ν 3400, 1712, 1685, 1635 cm^{-1}; U.V.: λ_{max} 354, 275, 235.

^{1}H-N.M.R. ($CDCl_3$ + DMSO-d_6): δ 1.62-1.2 (6H, m, C*H*$_3$CH$_2$), 2.1 (3H, s, C*H*$_3$CO), 4.5-4.0 (4H, m, C*H*$_2$CH$_3$), 6.56 (1H, d, J = 9 Hz, C-8 H), 7.42 (1H, d, J = 9 Hz, C-6 H), 7.60 (2H, s, NH_2), 8.38 (1H, s, C-2 H), 8.92 (1H, br s, NH-CO).

Ethyl 3-acetyl-6-ethyl-6,9-dihydro-3H-triazolo[4,5-f] quinolin-9-one-8-carboxylate (XXV)

To a stirred suspension of (XXIV) (0.2 g, 0.63 mmol) in a mixture of acetic acid and water (1:1) was added at once at 0°C sodium nitrite (50 mg, 7.2 mmol) in a small volume of water. The solution was stirred for 1 h. On neutralisation a precipitate of (XXV) (0.17 g, 82% yield) was obtained. M.p. 255-57°C from EtOH.

Analysis ($C_{16}H_{16}N_4O_4$): C,H,N.

I.R.: ν 1720, 1620, cm^{-1}; U.V.: λ_{max} 335 (4.24), 321 (4.23), 308 sh (4.15), 274 (4.30), 257 (4.38), 232 (4.27), 217 (4.31).

^{1}H-N.M.R. (DMSO-d_6): δ 1.5-1.1 (6H, m, C*H*$_3$CH$_2$), 2.40 (3H, s, C*H*$_3$CO), 4.50-4.0 (4H, m, CH$_3$C*H*$_2$), 7.63 (1H, d, J = 9 Hz, C-5 H), 8.24 (1H, d, J = 9 Hz, C-4 H), 8.60 (1H, s, C-7 H).

Ethyl 1-methyl-6-ethyl-6,9-dihydro-1H-triazolo[4,5-f] quinolin-9-one-8-carboxylate (VII)

This compound was obtained in 75% yield by ethylation of (IV) (0.73 g, 2.7 mmol) at 70°C overnight using the same procedure as for the preparation of (XXI). M.p. 180-186°C. Characterisation was achieved through its hydrolysis into acid (XVII).

Ethyl 2-methyl- and 3-methyl-9-chloro-2(3)H-triazolo [4,5-f]quinoline-8-carboxylates (XII) and (XIII)

A mixture of compound (X) (1 g, 3.67 mmol) and $POCl_3$ (2.36 ml) was heated under reflux for 3 h. After evaporation of the excess of $POCl_3$ *in vacuo* the residue was poured onto ice and water (30 ml). The precipitate filtered off and washed with water gave (XII) (0.9 g, 84% yield), m.p. 121-23°C from Et_2O.

Analysis ($C_{13}H_{11}ClN_4O_2$): C,H,Cl,N.

I.R.: ν 3400-2500 (COOH), 1730, 1590 cm^{-1}; U.V.: λ_{max} 300, 260, 228.

^{1}H-N.M.R. ($CDCl_3$): δ 1.43 (3H, t, J = 7 Hz, C*H*$_3$CH$_2$), 4.42 (2H, q, J = 7 Hz, C*H*$_2$CH$_3$), 4.50 (3H, s, N-CH$_3$), 7.93 (1H,d,J = 9 Hz, C-5 H), 8.12 (1H, d, J = 9 Hz, C-4 H), 9.0 (1H, s, C-7 H).

A similar run starting from compound (V) gave only 50 mg of (XII).

In an identical manner from (XI) was obtained (XIII) (95% yield) m.p. 185-187°C from DMF.

Analysis ($C_{13}H_{11}ClN_4O_2$): C,H,Cl,N.

I.R.: ν1720, 1590 cm^{-1}; U.V.: λ_{max} 300, 253, 236.

^{1}H-N.M.R. ($CDCl_3$): δ 1.43 (3H, t, J=7 Hz, CH_3CH_2), 4.32 (3H, s, N-CH_3), 4.42 (2H, q, J=7 Hz, CH_2CH_3), 7.65 (1H,d,J=9 Hz, C-4 H), 7.85 (1H, d, J=9 Hz, C-5 H), 8.90 (1H, s, C-7 H).

General procedure for the preparation of acids (XIV) (XV) (XVI) (XVII) (XVIII)

The ester (1-3.33 mmol) in 2N NaOH solution (10-100 ml) was heated at 100°C under stirring for 2-3 h. After removal of the insoluble residue, the filtrate was acidified to give a precipitate which was collected and dried. Data of the obtained acids are reported in Table III.

Methylation of acid (XVI)

The acid (XVI) (0.6 g, 2.3 mmol) suspended in N NaOH solution (10 ml) was added of dimethyl sulfate (0.45 g, 4.6 mmol). The mixture was heated at 100°C overnight. After cooling it was diluted with water then acidified with 6N HCl solution and eventually extracted with $CHCl_3$. The evaporation of the dried extract yielded a residue of (XVIII) (0.35 g, 55%) identical with an authentic specimen as earlier described.

B) MICROBIOLOGY

Test organisms and culture media

Escherichia coli ATCC25922; *Escherichia coli* (Hospital isolate); *Pseudomonas aeruginosa* ATCC27853; *Pseudomonas aeruginosa* (Hospital isolate); *Streptococcus faecalis* ATCC33186; *Staphylococcus aureus* ATCC25923; *Candida albicans* (Hospital isolate).

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 Gavan and Town (16).

Each compound was dissolved in dimethylsulfoxide (DMSO) (10 mg/ml) and diluted with tripticase-soybroth (TSB,DIFCO) containing 1% glucose and 0.02% phenol red in order to obtain concentrations of drug ranging from 500 μg to 0.98 μg/ml. Inocula of bacteria and *Candida albicans* were prepared from overnight growth cultures diluted such that the final inoculum size was of 10^5 cells/ml.

Inoculated plastic trays added of the diluted antimicrobial agent were then incubated at 37°C for 18 h. The MIC was defined at the lowest amount of compound preventing growth or at the chance of colour of the indicator from red to yellow.

TABLE II

I.R., U.V. and ^{1}H-N.M.R. spectra of compounds of Table I.

Comp.	I.R. (nujol) (cm^{-1})	U.V. (Et. OH) λ_{max}	^{1}H-N.M.R. δ (J in Hz)
(IIb)	3400, 3300, 1680, 1630, 1600;	328, 269, 207;	1.35 and 1.40 (6H, dt, J = 7, *CH*$_3$-CH$_2$), 4.05-4.50 (7H, m + s, *CH*$_2$-CH$_3$ + N-CH$_3$) 7.26 (1H, dd, J = 9 and 2, C-5 H), 7.58 (1H, d, J = 9, C-4 H), 7.70 (1H, d, J = 2, C-7 H), 8.50 (1H, d, J = 13, N-*CH* = C =), 11.20 (1H, d, J = 13 *NH*-CH = C)[a];
(IId)	3250, 1695, 1650, 1615, 1585;	332 (4.50), 272 (4.00), 222 (4.50);	1.37 (6H, dt, J = 7, *CH*$_3$-CH$_2$), 4.20 (3H, s, N-CH$_3$), 4.25 (4H, dq, J = 7, *CH*$_2$-CH$_3$), 7.08 (1H, dd, J = 9 and 2, C-6 H), 7.13 (1H, s, C-4 H), 7.92 (1H, d, J = 9, C-7 H), 8.48 (1H, d, J = 13, N-*CH* = C =), 11.25 (1H, d, J = 13, *NH*-CH = C)[a];
(IIf)	1730, 1685, 1620, 1600, 1580;	314 infl., 287, 205;	1.05 (3H, t, J = 7, *CH*$_3$-CH$_2$), 1.22 (6H, t, J = 7, *CH*$_3$-CH$_2$), 3.50 (2H, q, J = 7, *CH*$_2$-CH$_3$), 3.75 (2H, q, J = 7, *CH*$_2$-CH$_3$), 4.15 (2H, q, J = 7, *CH*$_2$-CH$_3$), 4.22 (3H, s, N-CH$_3$), 7.25 (1H, dd, J = 9 and 2, C-6 H), 7.45 (1H, d, J = 9, C-7 H), 7.62 (1H, s, N-*CH* = C =), 7.72 (1H, d, J = 2, C-4 H)[a];
(IIg)	1740, 1660, 1630, 1580;	321, 281, 211;	1.02 (3H, t, J = 7, *CH*$_3$-CH$_2$), 1.20 (6H, t, J = 7, *CH*$_3$-CH$_2$), 3.55-3.75 (4H, m, *CH*$_2$-CH$_3$), 4.15 (2H, q, J = 7, *CH*$_2$-CH$_3$), 4.41 (3H, s, N-CH$_3$), 7.10 (1H, dd, J = 9 and 2, C-6 H), 7.50 (1H, d, J = 2, C-4 H), 7.60 (1H, s, N-*CH* = C =), 7.72 (1H, d, J = 9, C-7 H)[a];
(IIh)	1710, 1690, 1600, 1580;	321, 275, 203;	0.90-1.40 (9H, m, *CH*$_3$-CH$_2$), 3.20-4.20 (6H, m, *CH*$_2$-CH$_3$), 4.20 (3H, s, N-CH$_3$), 7.10 (1H, dd, J = 9 and 2, C-5 H), 7.26 (1H, d, J = 2, C-4 H), 7.60 (1H, s, N-*CH* = C =), 7.85 (1H, d, J = 9, C-7 H);
(IV)	3450, 3190, 3120, 3070, 1740, 1640;	334, 330, 312 infl., 296 infl.;	1.25 (3H, t, J = 7, *CH*$_3$-CH$_2$), 4.15 (4H, q, J = 7, *CH*$_2$-CH$_3$), 4.65 (3H, s, N-CH$_3$), 7.38 (1H, d, J = 9, C-5 H), 8.07 (1H, d, J = 9, C-4 H), 8.34 (1H, s, N-CH = C)[b*];
(VIII)	1730, 1690;	347, 332, 318, 295, 279, 267, 213;	1.40 (3H, t, J = 7, *CH*$_3$-CH$_2$), 1.52 (3H, t, J = 7, *CH*$_3$-CH$_2$), 4.30 (4H, dq, J = 7, *CH*$_2$-CH$_3$), 4.50 (3H, s, N-CH$_3$), 7.95 (1H, d, J = 9, C-5 H), 8.30 (1H, d, J = 9, C-4 H), 8.90 (1H, s, C-7 H)[a];
(IX)	3600-2500 (ass. OH and NH), 1700, 1680;	354, 331, 319, 302, 276 infl.;	1.37 (3H, t, J = 7, *CH*$_3$-CH$_2$), 3.30 (2H, br. s, NH + OH), 4.25 (2H, q, J = 7, *CH*$_2$-CH$_3$), 7.41 (1H, d, J = 9, C-4 H), 8.09 (1H, d, J = 9, C-5 H), 8.48 (1H, s, C-7 H)[c];
(X)	3450, 1740, 1710, 1630, 1610, 1590;	340, 326, 300 infl., 285 infl.;	1.32 (3H, t, J = 7, *CH*$_3$-CH$_2$), 4.35 (4H, q, J = 7, *CH*$_2$-CH$_3$), 4.45 (3H, s, N-CH$_3$), 7.90 (1H, d, J = 9, C-4 H), 8.30 (1H, d, J = 9, C-5 H), 9.20 (1H, s, C-7 H), 11.95 (1H, br s, OH);
(XI)	3450, 1710, 1630, 1590;	342, 328, 299, 289, 269;	1.43 (3H, t, J = 7, *CH*$_3$-CH$_2$), 4.43 (2H, q, J = 7, *CH*$_2$-CH$_3$), 4.58 (3H, s, N-CH$_3$), 8.12 (1H, d, J = 8.5, C-4 H), 8.45 (1H, d, J = 8.5, C-5 H), 9.02 (1H, s, C-7 H)[d];

[a]$CDCl_3$; [b]DMSO-d_6; [c]DMSO-d_6 + $CDCl_3$; [d]DMSO-d_6 + $CDCl_3$ + CF_3-COOD drops;
*NH not detected because of solvent exchange;
infl. = inflexion.

TABLE III

Analytical and spectroscopical data of acids (XIV-XVIII)

Comp.	Yield (%)	M.p. (°C)	Cryst. Solvent	Formula	I.R. (cm^{-1})	U.V. λ_{max} (log ϵ) (nm)	^{1}H-N.M.R. δ (J in Hz)
(XIV)	92	313-314	A	$C_{11}H_7ClN_4O_2$	3500-2600 (ass. COOH), 1700 (COOH), 1590	335, 321, 272 sh, 269, 214	4.60 (3H,s,N-CH_3), 7.72 (1H,d,J = 9,C-4H), 8.25 (1H,d,J = 9,C-5H), 8.73 (1H,s,C-7H)[a]
(XV)	74	>340	A	$C_{11}H_7ClN_4O_2$	3600-2500 (ass. COOH), 1730 (COOH), 1610	325, 308 infl., 298, 288, 249, 228	not recorded for its insolubility
(XVI)	71	325 (d)	A	$C_{12}H_{10}N_4O_3 \cdot 0.75\ H_2O$	3450, 3200, 1710, 1630	334(4.16), 320(4.10), 308(3.95), 273(4.24), 258(4.33), 233(4.24), 202(4.17)	1.517 (3H,t,J = 7.4,CH_3-CH_2), 4.666 (2H,q,J = 7.4,CH_2-CH_3), 7.829 (1H,d,J = 9.4,C-4H), 8.445 (1H,d,J = 9.4,C-5H), 9.101 (1H,s,C-7H)[b]
(XVII)	85	330-332	B	$C_{13}H_{12}N_4O_3$	1725, 1639, 1600	337(4.12), 323(4.11), 310 sh(3.94), 278(4.20), 262(4.28), 236(4.28)	1.669 (3H,t,J = 7.3,CH_3-CH_2), 4.540 (2H,q,J = 7.3,CH_2-CH_3), 4.931 (3H,s,N-CH_3), 7.630 (1H,d,J = 9.4,C-5H), 8.468 (1H,d,J = 9.4,C-4H); 8.873 (1H,s,C-7H)[c]
(XVIII)	73	278-281	A	$C_{13}H_{12}N_4O_3$	1710, 1690	341, 326, 277, 266, 207	1.50 (3H,t,J = 7,CH_3-CH_2), 3.25 (1H,br.s,COOH), 4.40-4.90 (5H,s + q,N-CH_3 + CH_2-CH_3), 7.82 (1H,d,J = 9,C-5H), 8.25 (1H,d,J = 9,C-4H), 8.90 (1H,s,C-7H)[d]

A, for acidification of sodium hydroxide solution of the crude acid;
[a]recorded at 60 MHz in DMSO-d_6; [b]recorded at 200 MHz in DMSO-d_6; [c]recorded at 200 MHz in $CDCl_3$; [d]recorded at 60 MHz in DMSO-d_6 + $CDCl_3$.

Results and discussion

Table IV reports the results of the *in vitro* microbiological activity of acids (XVI), (XVII), (XVIII) and their precursors (XXV), (XXIII), (VIII) against strains of Gram positive and negative bacteria and *Candida albicans* in comparison with nalidixic acid.

TABLE IV

Anti-microbial activity of acids (XVI), (XVII), (XVIII) and some precursors (XXV), (XXIII), (VIII). MIC (μg/ml) in comparison with nalidixic acid (A).

	(A)	(XVI)	(XVII)	(XVIII)	(XXV)	(XXIII)	(VIII)
Escherichia coli ATCC 25922	6,25	25	25	25	>100	>100	>100
Escherichia coli (Hospital is.)	3,17	12,5	25	100	>100	>100	>100
Pseudomonas aeruginosa ATCC 27853	>100	>100	>100	>100	>100	>100	>100
Pseudomonas aeruginosa -3-	>100	>100	25	>100	>100	>100	>100
Proteus Vulgaris	12,5	>100	—	>100	>100	>100	>100
Streptococcus faecalis	500	>500	—	500	>500	>500	500
Staphilococcus aureus ATCC 33186	125	>500	250	500	>500	>500	>500
Candida albicans (Hospital is.)	>500	>500	—	>500	>500	>500	>500

The data clearly indicate that growth inhibition occurs at very high MIC ranging from 100 μg to 150 μg against bacteria to 500 μg against *Candida* with the only exception of *Escherichia coli ATCC 25922*.

The acids (XVI), (XVII), (XVIII) showed against this microorganism an equivalent activity (MIC = 25 μg/ml) and compound (XVI) had twice the activity of its methyl derivatives against a strain of *Escherichia coli* from Hospital isolation. The other compounds were all inactive.

At this stage, we can conclude that compounds (XVI, XVII, XVIII) exhibited some selectivity against *E.coli* and in the case of (XVII) against *Pseudomonas aer.* Owing to the limited number of compounds tested, it is difficult to point out any structure-activity relationship. This aspect will be discussed in next future, when a comparison with compounds B and C will allow us to establish whether the annelation position of the triazole ring may influence activity as whole.

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