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Original paper

Synthesis and antimicrobial properties of substituted 3-aminoxy-(*E*)-2-methoxyiminopropionyl penicillins and cephalosporins*

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Summary — The 3-aminoxy-*(E)*-2-methoxyiminopropionyl penicillins **13** and cephalosporins **14** and **15** were synthesized and assayed for their antimicrobial activity on Gram-positive and Gram-negative bacteria whether producers of ß-lactamases or otherwise. Compounds **13**, **14**, and **15** exhibited an activity which was generally lower than that of the corresponding phenylacetyl derivative: penicillin G (**4**), cephaloram (**5**), and phenylacetamidodesacetoxycephalosporanic acid (**6**). Furthermore, the comparison of the minimum inhibitory concentration values of some of the new 2-methoxyimino 3-aminoxypropionyl derivatives **13–15** with those of the corresponding ones **1–3** lacking the 2-methoxyimino substituent showed that the introduction of the 2-methoxyimino group of *E* configuration on the aminoxypropionamido side chain of **1–3** gives compounds (**13–15**) which do not generally possess better antimicrobial properties.

Résumé — Synthèses et propriétés antimicrobiennes de *(E)*-3-aminoxy-2-méthoxyimino propionyl pénicillines et céphalosporines. *Les* (E)*-3-iminoxy-2-méthoxyimino propionyl pénicillines **13** et céphalosporines **14** et **15** ont été synthétisées et testées quant à leur activité antimicrobienne sur les bactéries Gram-positive et Gram-négative produisant ou non des ß-lactamases. Les composés **13**, **14**, et **15** ont montré une activité généralement plus faible que celle du dérivé phénylacétylé correspondant: pénicilline G (**4**), céphalorame (**5**), et acide phénylacétamidodésacétoxycéphalosporanique (**6**). En outre, la comparaison des valeurs de concentration inhibitrice minimale de certains dérivés nouveaux: 2-méthoxyimino-3-aminoxy propionyl **13–15** avec celles des dérivés correspondants **1–3** sans substituant 2-méthoxyimino ont révélé que l'introduction du groupe 2-méthoxyimino de configuration* E *sur la chaîne latérale aminoxypropionyle de **1–3** donne des composés (**13–15**) qui ne possèdent généralement pas de meilleures propriétés antimicrobiennes.*

ß-lactams/penicillins/cephalosporins

Introduction

Numerous penicillins and cephalosporins that are commonly used in therapy are characterised by the presence in position 6 or 7, respectively, of acetamido functions, substituted by an aryl or by an aromatic heterocycle. Starting from this observation, some 3-aminoxypropionyl penicillins (**1**) and cephalosporins (**2** and **3**) [1], designed on the basis of the hypothesis that the (methylene aminoxy)methyl group (C=$NOCH_2$) could be a 'bioisoster' [2, 3] of either aryls or other aromatic groups [4], were prepared and tested for their antimicrobial properties. Compounds **1–3** exhibited an activity trend which was not substantially different from that of the corresponding phenylacetamido derivatives **4**, **5**, and **6**, respectively, taken as terms of comparison [1].

Several cephalosporins of significant therapeutic value see for example cefuroxime (**7**) [5], cefotaxime (**8**) [6], and ceftizoxime (**9**) [7, 8], present, as their

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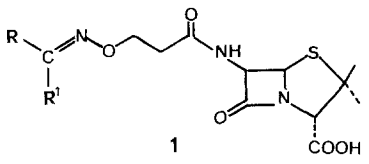

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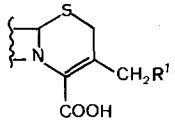
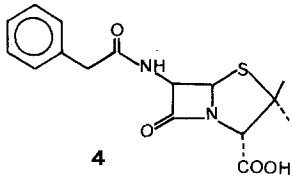


2, R^1 = OAc
3, R^1 = H



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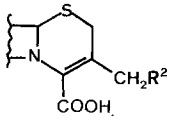
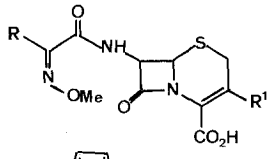


5, R^2 = OAc
6, R^2 = H

side chain on C(7), an acetamido-type moiety, substituted in the α position not only by an aromatic heterocycle, but also by a methoxyimino group. The latter substituent is commonly believed to play an important role in increasing certain antibiotic properties such as the activity towards Gram-negative bacteria and the resistance to enzyme inactivation [9–11].

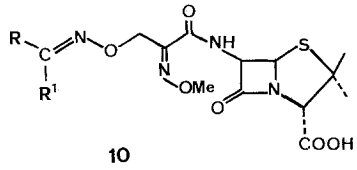


7, R =  ; R^1 = $CH_2OC(O)NH_2$

8, R =  ; R^1 = $CH_2OC(O)Me$

9, R = ; R^1 = H

It thus appeared to be of interest to verify whether the introduction of a methoxyimino function in the α position of the 3-aminoxypropionyl side chain of β-lactam derivatives of type **1–3** might make it possible to obtain new antibiotics (**10–15**) with a wider action spectrum and a greater resistance to enzyme inactivation.



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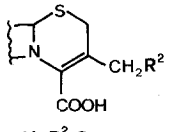
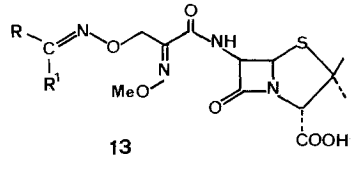
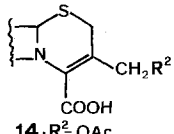


11, R^2 = OAc
12, R^2 = H

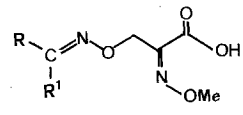


13



14, R^2 = OAc
15, R^2 = H

The synthesis of these new compounds (**10–15**), in which the methoxyimino group may exhibit the *Z* (as in **10–12**) or the *E* (as in **13–15**) configuration around the C=N double bond, required the availability of both 3-aminoxy-*(Z)*- (**16**) and 3-aminoxy-*(E)*-2-methoxyiminopropionic (**17**) acids, to be used as acylating agents for 6-aminopenicillanic acid (6-APA), 7-aminocephalosporanic acid (7-ACA), and 7-aminodesacetoxy-cephalosporanic acid (7-ADCA). Whereas the acids of type **17** were easily synthesized and characterized from the configurational point of view [12], the synthesis of the acids **16** could not be effected*.



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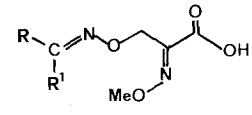


17

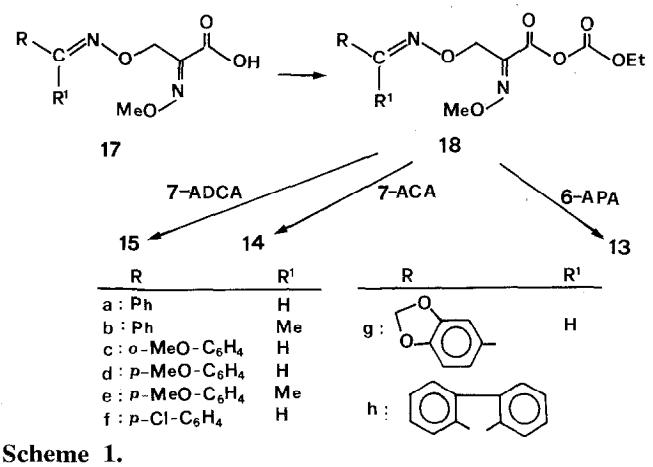
The present paper reports the synthesis and the evaluation of the antimicrobial properties of the 3-aminoxy-2-methoxyiminopropionyl-APAs (**13**), -ACAs (**14**), and -ADCAs (**15**), in which the configuration around the 2-methoxyimino double bond is *E*.

Chemistry

The penicillin (**13**) (table I) and cephalosporin (**14** and **15**) (table II) derivatives were obtained as described in scheme 1. The 3-aminoxy-*(E)*-2-methoxyimino-propionic acids (**17a–h**) [12] were transformed by reaction with ethyl chloroformate into the corresponding mixed anhydrides (**18a–h**). Reaction of the appropriate **18** with the sodium salts of 6-APA, 7-ACA, and 7-ADCA, directly afforded the penicillins (**13**) and the cephalosporins (**14** and **15**) which were isolated as free acids in the case of **13a–h, 14g** and **15g**, or as dicyclohexylammonium salts in the case of the remaining compounds.

The geometry of the 2-methoxyimino moiety of **13-15** and, when the *cis–trans* isomerism is possible, of their 3-aminoxy group, was assumed on the basis of

*The acids **17** were well stable and could be prepared by saponification of the corresponding ethyl esters [12]. Also the ethyl esters of the acids **16** were easily prepared. However, attempts to saponify these esters under several experimental conditions were unsuccessful due to the instability of the acids **16**. Only the 3-fluorenylidenaminoxy-*(Z)*-2-methoxyimino-propionic acid (**16h**) was obtained in very low yield. This compound, however, was found to decompose rapidly both on standing and when dissolved in an aprotic solvent (unpublished results from this laboratory).



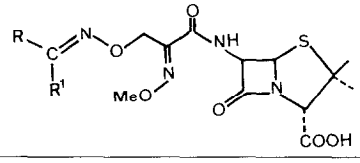
Scheme 1.

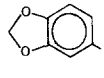
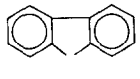
the knowledge of that of the starting acids **17** [12]. These compounds have been proved to be configurationally stable under the reaction conditions employed for the acylation of 6-APA, 7-ACA and 7-ADCA, leading to **13, 14,** and **15**, respectively.

Results and Discussion

Table III shows the minimum inhibitory concentrations (MICs) of the new penicillins (**13**) and cephalosporins (**14** and **15**) towards Gram-positive and Gram-negative bacteria, whether sensitive to enzyme inactivation or otherwise, together with those of 6-phenylacetamidopenicillanic acid (**4**, penicillin

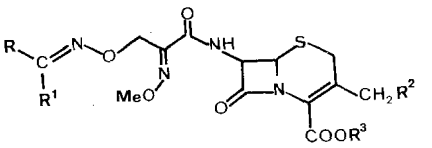
Table I. 3-Aminoxy-*(E)*-2-methoxyiminopropionyl penicillin derivatives.

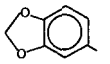
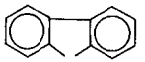
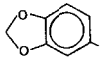
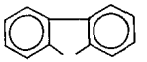


Compd	*R*	*R¹*	*mp(°C)*	*Recrystn solvent* [a]	*Yield, %* [b]	*Formula*[c]	*¹H NMR, δ*[d]
13a	Ph	H	65-66	A	35	$C_{19}H_{22}N_4O_6S$	1.62 and 1.70 2s, 6, $SC(CH_3)_2$, 4.15 (s, 3, $NOCH_3$), 5.13 (s, 2, OCH_2), 5.57 (d, 1, *J* = 4.4 Hz, CHS), 5.77 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*), 8.10 (s, 1, CH=N)
13b	Ph	Me	103-105	A	45	$C_{20}H_{24}N_4O_6S$	1.56 and 1.66 2s, 6, $SC(CH_3)_2$, 2.20 (s, 3, CH_3C=N), 4.12 (s, 3, $NOCH_3$), 5.13 (s, 2, OCH_2), 5.58 (d, 1, *J* = 4.4 Hz, CHS), 5.80 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*)
13c	*o*-MeO-C_6H_4	H	79-81	B	28	$C_{20}H_{24}N_4O_7S$	1.53 and 1.61 2s, 6, $SC(CH_3)_2$, 3.77 (s, 3, $NOCH_3$), 5.00(s, 2, OCH_2), 5.47 (d, 1, *J* = 4.6 Hz, CHS), 5.68 (dd, 1, *J* = 4.6 and 8.8 Hz, NHC*H*), 8.39 (s, 1, CH=N)
13d	*p*-MeO-C_6H_4	H	84-86	C	30	$C_{20}H_{24}N_4O_7S$	1.60 and 1.67 2s, 6, $SC(CH_3)_2$, 3.86 (s, 3, $NOCH_3$), 5.08 (s, 2, OCH_2), 5.57 (d, 1, *J* = 4.4 Hz, CHS), 5.75 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*), 8.08 (s, 1, CH=N)
13e	*p*-MeO-C_6H_4	Me	76-77	A	28	$C_{21}H_{26}N_4O_7S$	1.62 and 1.64 2s, 6, $SC(CH_3)_2$, 2.17 (s, 3, CH_3C=N), 3.81 (s, 3, $NOCH_3$), 5.04 (s, 2, OCH_2), 5.55 (d, 1, *J* = 4.4 Hz, CHS), 5.75 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*)
13f	*p*-Cl-C_6H_4	H	83-84	A	42	$C_{19}H_{21}ClN_4O_6S$	1.40 and 1.65 2s, 6, $SC(CH_3)_2$, 4.10 (s, 3, $NOCH_3$), 5.07 (s, 2, OCH_2), 5.57 (d, 1, *J* = 4.4 Hz, CHS), 5.75 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*), 7.73 (s, 1, CH=N)
13g		H	75-76	A	35	$C_{20}H_{22}N_4O_8S$	1.58 and 1.67 2s, 6, $SC(CH_3)_2$, 4.12 (s, 3, $NOCH_3$), 5.07 (s, 2, OCH_2), 5.60 (d, 1, *J* = 4.4 Hz, CHS), 5.87 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*), 8.04 (s, 1, CH=N)
13h			141-143	A	30	$C_{25}H_{24}N_4O_6S$	1.53 and 1.63 2s, 6, $SC(CH_3)_2$, 4.17 (s, 3, $NOCH_3$), 5.33 (s, 2, OCH_2), 5.52 (d, 1, *J* = 4.4 HZ, CHS), 5.73 (dd, 1, *J* = 4.4 and 8.4 Hz, NHC*H*)

[a] A = Et_2O-Pet Ether; B = CCl_4-Pet Ether; C = Et_2O-Heptane. [b] No efforts were made to optimize yields. [c] Anal C, H, N. [d] Spectra were obtained in $CDCl_3$ solutions.

Table II. 3-Aminoxy-*(E)*-2-methoxyiminopropionyl cephalosporin derivatives.



Compd	*R*	R^1	R^2	R^3	*mp, °C*	*Recrystn solvent* [a]	*Yield, %* [b]	*Formula* [c]	*^1H NMR, δ* [d]
14a	Ph	H	OAc	[e]	101–103	A	48	$C_{33}H_{45}N_5O_8S$	2.05 (s, 3, CH_3CO), 3.20 and 3.58 (2d, 2, J = 18.0 Hz, SCH_2), 4.10 (s, 3, $NOCH_3$), 5.03 (s, 2, CH_2OCO), 5.10 (br s, 3, OCH_2 and CHS), 5.90 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*), 8.13 (s, 1, CH=N)
14b	Ph	Me	OAc	[e]	156–157	B	57	$C_{34}H_{47}N_5O_8S$	2.05 (s, 3, CH_3CO), 2.21 (s, 3, CH_3C=N), 3.17 and 3.60 (2d, 2, J = 18.0 Hz, SCH_2), 4.08 (s, 3, $NOCH_3$), 5.02 (s, 2, CH_2OCO), 5.10 (br s, 3, OCH_2 and CHS), 5.83 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*)
14c	*o*-MeO-C_6H_4	H	OAc	[e]	124 (dec)	A	49	$C_{34}H_{47}N_5O_9S$	2.06 (s, 3, CH_3CO), 3.23 and 3.66 (2d, 2, J = 18.0 Hz, SCH_2), 3.81 (s, 3, $NOCH_3$), 5.01 (s, 2, CH_2OCO), 5.06 (br s, 3, OCH_2 and CHS), 5.93 (dd, 1, J = 4.8 and 9.0 Hz, NHC*H*), 8.50 (s, 1, CH=N)
14d	*p*-MeO-C_6H_4	H	OAc	[e]	169 (dec)	C	34	$C_{34}H_{47}N_5O_9S$	2.05 (s, 3, CH_3CO), 3.16 and 3.62 (2d, 2, J = 17.8 Hz, SCH_2), 3.83 (s, 3, $NOCH_3$), 4.98 (s, 2, CH_2OCO), 5.05 (br s, 3, OCH_2 and CHS), 5.88 (dd, 1, J = 4.8 and 8.8 Hz, CHC*H*), 8.08 (s, 1, CH=N)
14e	*p*-MeO-C_6H_4	Me	OAc	[e]	164 (dec)	C	29	$C_{35}H_{49}N_5O_9S$	2.10 (s, 3, CH_3CO), 2.23 (s, 3, CH_3C=N), 3.20 and 3.63 (2d, 2, J = 18.0 Hz, SCH_2), 3.86 (s, 3, $NOCH_3$), 5.03 (s, 2, CH_2OCO), 5.10 (br s, 3, OCH_2 and CHS), 5.83 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*)
14f	*p*-Cl-C_6H_4	H	OAc	[e]	162 (dec)	D	37	$C_{33}H_{44}ClN_5O_8S$	2.05 (s, 3, CH_3CO), 3.20 and 3.63 (2d, 2, J = 18.0 Hz, SCH_2), 4.08 (s, 3, $NOCH_3$), 5.02 (s, 2, CH_2CO), 5.08 (br s, 3, OCH_2 and CHS), 5.86 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*), 8.08 (s, 1, CH=N)
14g		H	OAc	H	116–118	E	55	$C_{22}H_{22}N_4O_{10}S$	2.03 (s, 3, CH_3CO), 3.23 and 3.61 (2d, 2, J = 18.0 Hz, SCH_2), 4.06 (s, 3, $NOCH_3$), 4.86 (s, 2, CH_2OCO), 4.95 (br s, 3, OCH_2 and CHS), 5.71 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*), 8.07 (s, 1, CH=N)
14h			OAc	[e]	170 (dec)	C	30	$C_{39}H_{47}N_5O_8S$	2.05 (s, 3, CH_3CO), 3.17 and 3.60 (2d, 2, J = 18.0 Hz, SCH_2), 4.10 (s, 3, $NOCH_3$), 5.06 (d, 1, J = 4.8 Hz, CHS), 5.12 (s, 2, CH_2OCO), 5.27 (s, 2, OCH_2), 5.83 (dd, 1, J = 4.8 and 8.8 Hz, NHC*H*)
15a	Ph	H	H	[e]	163 (dec)	C	36	$C_{31}H_{43}N_5O_6S$	2.08 (s, 3, CCH_3), 2.96 and 3.53 (2d, 2, J = 18.0 Hz, SCH_2), 4.10 (s, 3, $NOCH_3$), 5.10 (br s, 3, OCH_2 and CHS), 5.78 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*), 8.13 (s, 1, CH=N)
15b	Ph	Me	H	[e]	157 (dec)	D	47	$C_{32}H_{45}N_5O_6S$	2.06 (s, 3, CCH_3), 2.23 (s, 3, CH_3C=N), 3.00 and 3.53 (2d, 2, J = 18.0 Hz, SCH_2), 4.10 (s, 3, $NOCH_3$), 5.11 (br s, 3, OCH_2 and CHS), 5.73 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*)
15c	*o*-MeO-C_6H_4	H	H	[e]	161 (dec)	A	56	$C_{32}H_{45}N_5O_7S$	2.06 (s, 3, CCH_3), 3.02 and 3.53 (2d, 2, J = 18.0 Hz, SCH_2), 3.83 (s, 3, $NOCH_3$), 5.10 (br s, 3, OCH_2 and CHS), 5.77 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*), 8.53 (s, 1, CH=N)
15d	*p*-MeO-C_6H_4	H	H	[e]	163 (dec)	C	59	$C_{32}H_{45}N_5O_7S$	2.08 (s, 3, CCH_3), 3.00 and 3.53 (2d, 2, J = 18.0 Hz, SCH_2), 3.83 (s, 3, $NOCH_3$), 5.08 (br s, 3, OCH_2 and CHS), 5.77 (dd, 1, J = 4.6 and 8.8. Hz, NHC*H*), 8.07 (s, 1, CH=N)
15e	*p*-MeO-C_6H_4	Me	H	[e]	162 (dec)	A	50	$C_{33}H_{47}N_5O_7S$	2.06 (s, 3, CCH_3), 2.15 (s, 3, CH_3C=N), 3.00 and 3.50 (2d, 2, J = 18.0 Hz, SCH_2), 3.83 (s, 3, $NOCH_3$), 5.10 (br s, 3, OCH_2 and CHS), 5.80 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*)
15f	*p*-Cl-C_6H_4	H	H	[e]	180 (dec)	A	58	$C_{31}H_{42}ClN_5O_6S$	2.06 (s, 3, CCH_3), 3.01 and 3.53 (2d, 2, J = 18.0 Hz, SCH_2), 4.08 (s, 3, $NOCH_3$), 5.10 (br s, 3, OCH_2 and CHS), 5.73 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*), 8.07 (s, 1, CH=N)
15g		H	H	H	174 (dec)	F	48	$C_{20}H_{20}N_4O_8S$	1.87 (s, 3, CCH_3), 3.03 and 3.20 (2d, 2, J = 18.0 Hz, SCH_2), 3.83 (s, 3, $NOCH_3$), 4.73 (s, 2, OCH_2), 4.83 (d, 1, J = 4.6 Hz, CHS), 5.37 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*), 7.87 (s, 1, CH=N)
15h		H	[e]		179 (dec)	D	66	$C_{37}H_{45}N_5O_6S$	2.06 (s, 3, CCH_3), 3.00 and 3.50 (2d, 2, J = 18.0 Hz, SCH_2), 4.10 (s, 3, $NOCH_3$), 5.00 (d, 1, J = 4.6 Hz, CHS), 5.27 (s, 2, OCH_2), 5.73 (dd, 1, J = 4.6 and 8.8 Hz, NHC*H*)

[a] A = AcOEt–hexane; B = CCl_4–Pet Ether; C = *i*-PrOH; D = AcOEt; E = Et_2O–Pet Ether; F = EtOH–Et_2O. [b–d] Footnotes as in table I. [e] $(c\text{-}C_6H_{11})_2NH_2$.

Table III. Antimicrobial activity of penicillin (**13**) and cephalosporin (**14, 15**) derivatives towards Gram-positive and Gram-negative bacteria.

	MIC [a] *μg/ml*												
	Gram-positive							*Gram-positive resistant*				*Gram-negative* [b]	*Gram-negative resistant* [c]
Compd	S aureus[d]	S pyogenes[e]	S pyogenes *Grunenthal*	S pneum *Felton*	S faecalis *ATCC 6057*	S lutea *ATCC 9341*	*geom averages*	S aureus *F2*	S aureus *6014668*	S aureus *39/II FBF*	*geom averages*	*geom averages*	*geom averages*
13a	0.097	0.097	200	0.39	50	25	0.64	6.25	6.25	0.78	3.1	146	>200
13b	0.19	0.19	200	0.19	200	0.19	0.67	200	200	200	200	106	>200
13c	0.26	0.24	100	0.39	100	0.19	0.68	100	50	6.25	31.5	166	>200
13d	0.097	0.097	200	0.097	100	0.097	0.36	12.5	12.5	1.56	6.2	155	>200
13e	0.16	0.14	50	0.39	50	1.56	0.56	50	50	6.25	25	129	>200
13f	0.39	0.39	100	0.78	50	0.19	1.0	100	6.25	0.78	7.9	146	>200
13g	0.097	0.097	200	100	100	0.097	0.69	25	25	6.12	12.5	155	>200
13h	0.097	0.097	50	0.097	25	0.097	0.28	25	6.25	0.78	5.0	56.7	>200
4	0.012	0.012	6.25	0.012	0.78	0.012	0.03	25	50	3.12	15.7	8.6	>200
1a	0.060	0.068	0.012	0.012	6.25	0.012	0.060	25	25	1.56	9.90	28.35	>200
1h	0.015	0.024	0.048	0.048	3.12	0.097	0.040	3.12	1.56	0.78	1.60	28.35	>200
14a	1.56	0.39	200	1.56	200	0.39	2.0	50	0.39	6.25	5.0	121	>200
14b	1.04	0.39	200	3.12	200	0.39	1.9	25	25	3.12	12.5	94	>200
14c	2.60	0.97	3.12	6.25	200	0.39	2.3	100	100	12.5	50	113	>200
14d	1.04	0.39	200	3.12	200	0.39	1.9	50	25	6.25	19.8	106	>200
14e	9.11	0.39	200	25	200	0.39	3.1	0.78	25	3.12	3.9	113	>200
14f	0.65	1.17	6.12	1.56	25	0.39	1.4	25	25	12.5	19.8	155	>200
14g	0.39	0.42	200	50	200	0.39	1.9	25	25	6.25	15.7	106	>200
14h	0.19	0.19	200	0.19	200	0.19	0.67	6.25	3.12	3.12	3.9	94	>200
5	0.036	0.015	25	0.097	200	0.012	0.10	25	25	0.19	4.9	4.3	59.5
2a	0.002	0.020	0.012	0.024	12.5	0.012	0.020	3.12	3.12	0.39	1.60	11.67	>200
2h	0.001	0.001	0.012	0.001	1.56	0.001	0.002	0.78	0.39	0.01	0.10	6.14	>200
15a	16.7	2.53	200	50	200	6.25	12.5	200	200	50	126	>200	>200
15b	25	2.53	200	50	200	6.25	13.3	200	200	50	126	>200	>200
15c	67	2.53	200	100	200	12.5	20.7	200	200	50	126	>200	>200
15d	25	0.68	200	50	200	6.25	9.1	200	200	50	126	>200	>200
15e	33.3	1.27	200	50	200	12.5	12.5	200	200	50	126	>200	>200
15f	16.7	0.68	200	25	200	6.25	7.5	200	200	25	100	>200	>200
15g	25	0.68	200	50	200	6.25	9.1	200	200	50	126	>200	>200
15h	1.8	0.19	200	1.56	200	3.12	1.9	200	200	3.12	50	>200	>200
6	0.58	0.17	200	1.56	200	0.78	1.1	200	200	3.12	50	47	126
3a	0.98	0.93	1.56	0.78	100	0.78	1.46	50	50	3.12	19.8	146	>200

[a]The *in vitro* antibacterial activities were evaluated by 2-fold serial dilution method with a multiinocular device (see [13]). [b]Strains tested: *E coli* 120, *S typhi* 6/12, *S typhimurium* No, *S typhimurium* To, *S enteritidis* To, *S paratyphi* B To, *S disenteriae* NCTC 4837, *N meningitidis* To, *K pneumoniae* ATCC 10031, *P. providence* To, *P mirabilis* OSCB 2. [c]Strains tested: *E coli* R^+ TEM, *E cloacae* 214, *A cloacae* 653, *P aeruginosa* ISF 1. [d]Strains of *S aureus* tested: Smith, 9144, 6538/P. Results are reported as arithmetic averages of MIC's. [e]Strains of *S pyogenes* tested: ISM 68/231, ISM 68/241, *β*-haem A, UC 41. Results are reported as arithmetic averages of MIC's.

G), of 7-phenylacetamidocephalosporanic acid (**5**, cephaloram), and 7-phenylacetamidodesacetoxycephalosporanic acid (**6**), taken as terms of comparison. As for the Gram-positive microorganisms, an examination of both the MICs towards the single strains tested and of the geometrical averages of these MIC values (see table III) indicates that the new compounds **13–15** possess an antimicrobial activity towards this kind of bacteria, which is generally lower or comparable to that of the corresponding reference compounds **4–6**. The only exceptions are represented by the penicillins **13a, d, f,** and **h** which proved to be 2–5 times more active than penicillin G (**4**) towards Gram-positive β-lactamase-producing germs.

As regards Gram-negative bacteria, the new compounds **13–15** exhibited MIC values, towards the strains screened, which were generally ≥200 μg/ml, with the only exceptions of the penicillins **13** and cephalosporins **14** towards *N meningitidis* To, for which the MIC values were between 0.19 (for **13b** and **14g**) and 32 (for **14e**). This very poor activity of **13–15** on Gram-negative bacteria can be seen from a comparison of the geometrical averages of the MICs (see table III) which are generally much higher than those of the corresponding reference compounds **4–6**.

In table III are also reported the MICs of the previously prepared [1] 3-aminoxypropionyl penicillins (**1a, h**) and cephalosporins (**2a, h** and **3a**) which present the same R and R^1 substituents as the 2-methoxyimino-substituted 3-aminoxypropionyl penicillins **13a, h,** and cephalosporins **14a, h** and **15a**, but not their 2-methoxyimino group. In order to have homogeneous data for all compounds shown in table III, the antimicrobial activity of the previously examined penicillin (**1a, h**) and cephalosporin (**2a, h** and **3a**) derivatives has been newly determined. A comparison of these data shows that generally, when the MIC values are below 200 μg/ml, the methoxyimino-substituted β-lactam derivatives of type **13–15** present an activity which is lower than that of the unsubstituted compounds of type **1–3**. The only exception refers to the penam derivative **13a**, which proved to be about 3 times more active than **1a** towards Gram-positive resistant germs.

The present results indicate that the introduction of the methoxyimino group with the *E* configuration in the α position of the 3-aminoxypropionyl side chain of **1–3** gives compounds (**13–15**) which do not generally possess any better antimicrobial properties than those of the unsubstituted compounds (**1–3**), either as regards their action spectrum or their resistance to enzyme inactivation. A possible explanation for these findings may be offered by the configuration of the methoxyimino group of **13–15**, which is of the *E* type. It must be noted that in the most important methoxyimino-substituted cephalosporins of therapeutic interest, such as cefuroxime (**7**), cefotaxime (**8**), and ceftizoxime (**9**), the configuration of this group is of the *Z* type. Unfortunately, as pointed out above, the synthesis of the compounds **10–12** having the 2-methoxyimino group in the *Z* configuration was not possible due to the unavailability of the required acids of type **16***.

Experimental protocols

Melting points were determined on a Kofler hot–stage apparatus and are uncorrected. IR spectra for comparison of compounds were taken as paraffin oil mulls on a Perkin–Elmer model 1310 instrument. ^{1}H NMR spectra were obtained in *ca* 10% $CDCl_3$ solutions with a Varian EM 360 A spectrometer, using Me_4Si as the internal standard. The proton magnetic resonance assignments were established on the basis of the expected chemical shifts and the multiplicity of the signals. Evaporations were made *in vacuo* (rotating evaporator). $MgSO_4$ was always used as a drying agent. $CHCl_3$, triethylamine, and acetone were refluxed over P_2O_5, phenylisocyanate, and $KMnO_4$, respectively, and then rectified. Petroleum ether refers to the fraction boiling at 40–60°C. Elemental analyses were performed in our analytical laboratory and agreed with the theoretical values to within ±0.4%.

General procedure for the preparation of 3-aminoxy-(E)*-2-methoxyiminopropionyl penicillin (**13a–h**) and cephalosporin (**14a–h** and **15a–h**) derivatives*

A stirred solution of the appropriate acid **17a–h** (1.1 mmol) and triethylamine (1.1 mmol) in anhydrous $CHCl_3$ (10 ml) was treated dropwise at 0°C with a solution of ClCOOEt (1.1 mmol) in anhydrous $CHCl_3$ (2 ml). After 3 h at room temperature the mixture was evaporated. The residue was suspended in acetone (10 ml), cooled at 0°C and then treated dropwise under stirring with a solution of the sodium salt of 6-APA, 7-ACA, and 7-ADCA, prepared by dissolving these compounds (1.1 mmol) and solid $NaHCO_3$ (1.1 mmol) in a 2:1 acetone–H_2O mixture (15 ml). The resulting solution was stirred at room temperature for 4 h and the organic solvent was evaporated at reduced pressure. The resulting aqueous phase was cooled at 0°C, acidified with 20% aqueous H_3PO_4 to pH 2.8, and then extracted with $CHCl_3$. Evaporation of the washed (H_2O) organic extracts gave a residue which was crystallized from the proper solvent to yield the acids **13a–h, 14g** and **15g**, or was treated with a stoichiometric amount of dicyclohexylamine in AcOEt to afford the dicyclohexylammonium salts of **14a–f, h** and **15a–f, h** (see tables I and II).

The configurational stability of the acids **17a–h** was tested by dissolving the appropriate acid **17** (1.0 mmol) and triethylamine (1.0 mmol) in anhydrous $CHCl_3$ (10 ml) and treating the resulting solution, as described above, with ClCOOEt (1.0 mmol) in anhydrous $CHCl_3$ (2 ml). After 3 h at room temperature the mixture was evaporated at reduced pressure and the residue was dissolved in a 2:1 acetone–H_2O mixture (15 ml). Solid $NaHCO_3$ (1.0 mmol) was then added to this solution and the mixture was left standing for 4 h at room temperature and then concentrated at reduced pressure. The resulting aqueous phase was cooled at 0°C, acidified with 20% aqueous H_3PO_4 to pH 2.8, and extracted with $CHCl_3$. Evaporation of the filtered organic layer made it possible to recover the unaltered starting acids **17a–h**.

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