Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry Letters
ELSEVIER
Bioorganic & Medicinal Chemistry Letters 14 (2004) 147–150

Synthesis of antitumor 6-alkylidenepenicillanate sulfones and related 3-alkylidene-2-azetidinones

Grigory Veinberg,* Irina Shestakova, Maxim Vorona, Iveta Kanepe and Edmunds Lukevics

Latvian Institute of Organic Synthesis, 21 Aizkraukles Street, Riga, LV 1006, Latvia

Received 15 May 2003; revised 11 August 2003; accepted 30 September 2003

Abstract—6-Alkylidenepenicillanate sulfoxides and sulfones were synthesized on the base of 6-oxopenicillanate esters. The targeted splitting of their thiazolidine ring led to the formation of 3-alkylidene substituted 4-heteryldithio and 4-methylsulfonyl azetidin-2-ones. Some of mono and bicyclic β-lactams revealed potent cytotoxic properties towards monolayer tumor cells in <10-μM concentrations.

Recently discovered antitumor monocyclic and bicyclic β-lactam systems[1] in general are in a good agreement with the phenomenon of azetidin-2-one pharmacophore inexhaustible pharmacological potential due to specific ability of its numerous derivatives to inhibit not only bacterial transpeptidase but also mammalian serin and cystein proteases.[2]

Potent inhibiting properties exhibited by 7-alkylidene substituted cephalosporanate sulfones against tumor strains both in vitro and vivo[1c] encouraged us to subject penicillanate sulfones and 4-heteroaryldithio and 4-methylsulfonyl azetidin-2-ones containing alkylidene side chain at positions 6 and 3 to similar biological investigation.

The condensation of 6-oxopenicillanates obtained by two alternative methods[3,4] with phosporanes **2** according to published methodology[1c] resulted in the preparation of pure *Z* isomer **3a** and *Z*/*E* mixtures of 6-alkylidenepenicillanates **3b–e**. Oxidation of penicillanates **3b–e** with equivalent of *m*-CPBA due to the specific structure of the side chain similar to those described in Buynak work[5] produced sterically more preferable *R* sulfoxides **4a–e**, **6d**,**e**. However in the case of **4a** according to PMR and HPLC data there was obtained unseparable mixture of *R* and *S* sulfoxides in 86:14 ratio. The same reaction of **3a–e** with 3-fold excess of *m*-CPBA resulted in the formation of sulfones **5a–e**, **7d**,**e**. All *Z* and *E* isomeric sulfoxides and sulfones were successfully separated by column chromatography with the only exception in the case of **5d** and **7d** mixture (Scheme 1).

Reaction of *Z* and *E* isomeric sulfoxides **4a–e** and **6d**,**e** with 2-mercaptobenzothiazole (**8**) according to well known procedure[6] led to the splitting of thiazolidine ring in penicillin nucleus and to the formation of 3(*Z*) and 3(*E*)-alkylidene-4-heteroaryldithioazetidin-2-ones **9a–e** and **10d**,**e** (Scheme 2).

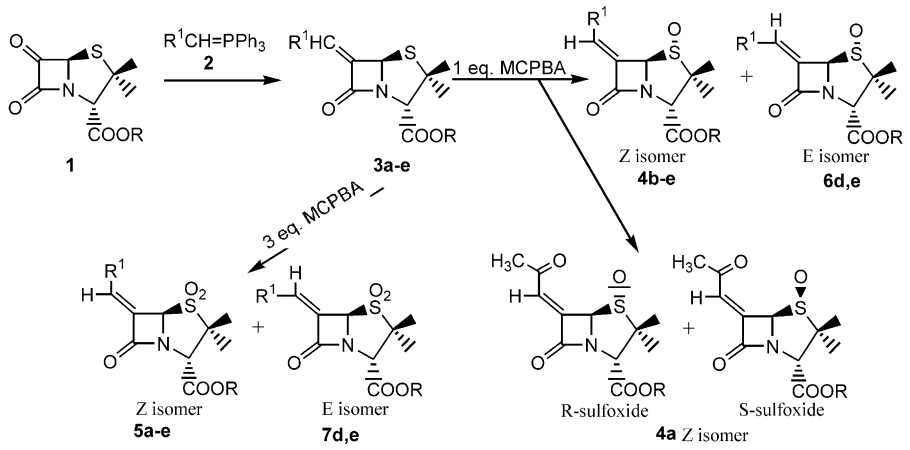
The treatment of 6-(*Z*)-alkylidenepenicillanate sulfones **5a**,**b**,**e** with DBU and alkylation of intermediate sulfinic anion **11** with methyliodide according to Lukič procedure[7] resulted in the formation of 3(*Z*)-alkylidene-4-methylsulfonylazetidin-2-ones **12a**,**b**. In the case of 6(*Z*)-4-nitrobenzylidenepenicillanate **5e** parallel to mentioned reaction there was observed side-chain transformation into the equilibrium mixture of 3(*Z*) and 3(*E*) isomers **12e** (Scheme 3).

For the purpose of SAR investigation, there were also synthesized mono and bicyclic β-lactams **13**, **14** and **16** by the deprotection of carboxyl and hydrogenation of double bond in **5e** and by the splitting of **4b** with 2-mercaptobenzimidazole (**15**) (Scheme 4).[8]

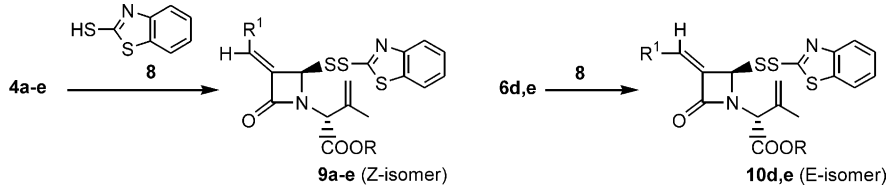
Cytotoxic properties of synthesized compounds were tested in vitro on standard monolayer tumor cell lines:

* Corresponding author. Tel.: +371-755-5946; fax: +371-755-0338; e-mail: veinberg@osi.lv

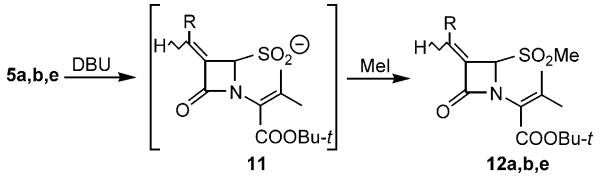
0960-894X/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2003.09.078



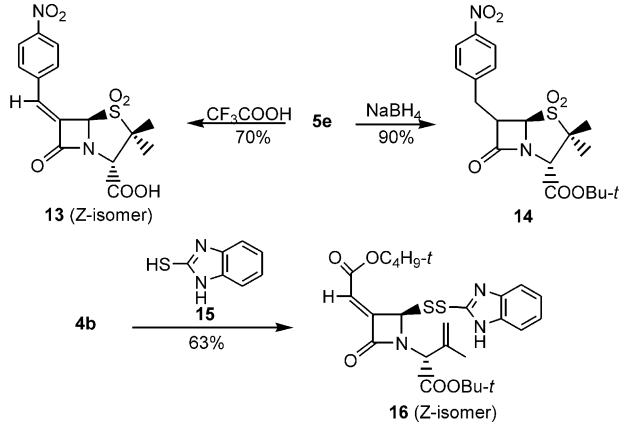
Scheme 1. **3a** R = *t*-Bu, R^1 = MeCO (*E*/*Z* = 0/100), 80%; **3b** R = *t*-Bu, R^1 = *t*-BuOCO (*E*/*Z* = 8/92), 15%; **3c** R = Bh, R^1 = *t*-BuOCO (*E*/*Z* = 4/96), 36%; **3d** R = *t*-Bu, R^1 = Ph (*E*/*Z* = 16/84), 49%; **3e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4 (*E*/*Z* = 40/60), 42%; **4a** R = *t*-Bu, R^1 = MeCO (*R*/*S* = 84/16), 85%; **4b** R = *t*-Bu, R^1 = *t*-BuOCO, 57%; **4c** R = Bh, R^1 = *t*-BuOCO, 48%; **4d** R = *t*-Bu, R^1 = Ph, 28%; **4e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 13%; **5a** R = *t*-Bu, R^1 = MeCO, 70%; **5b** R = *t*-Bu, R^1 = *t*-BuOCO, 27%; **5c** R = Bh, R^1 = *t*-BuOCO, 13%; **5e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 37%; **6d** R = *t*-Bu, R^1 = Ph, 21%; **6e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 19%; **7e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 37%; **5d**/**7d** R = *t*-Bu, R^1 = Ph (*Z*/*E* = 80/20), 32%.



Scheme 2. **9a** R = *t*-Bu, R^1 = MeCO, 75%; **9b** R = *t*-Bu, R^1 = *t*-BuOCO, 42%; **9c** R = Bh, R^1 = *t*-BuOCO, 36%; **9d** R = *t*-Bu, R^1 = Ph, 9%; **9e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 60%; **10d** R = *t*-Bu, R^1 = Ph, 14%; **10e** R = *t*-Bu, R^1 = 4-O_2N–C_6H_4, 45%.



Scheme 3. **12a** R = MeCO (*Z*/*E* = 100/0), 18%; **12b** R = *t*-BuOCO, (*Z*/*E* = 100/0), 23%; **12e** R = 4-O_2N–C_6H_4, (*Z*/*E* = 55/45), 23%.



Scheme 4.

MG-22A (mouse hepatoma), HT-1080 (human fibrosarcoma), B16 (mouse melanoma), Neuro 2A (mouse neuroblastoma) and on normal cell lines: 3T3 (mouse fibroblasts) and BHK (baby hamster kidney) using 96 well plates, CV, MTT and NR coloration.[9,10] Concentration of NO in supernatant generated by cells in the presence of tested compounds was determined by Greyss method.[9] Extrapolation of obtained values for 100% live cells allowed to calculate comparable Total Generation ability parameter (TG_{100}) for tested compounds.[1c]

IC_{50}-cytotoxic concentrations providing 50% cell inhibiting effect and TG_{100} data for synthesized compounds are presented in Table 1. Their analysis evidenced that the incorporation of *tert*-butoxycarbonylmethylene, benzylidene and 4-nitrobenzylidene structures at the C6 position of penicillanate sulfoxides and sulfones the same as at C3 positions of 4-heteroaryldithio and 4-methylsulfonyl azetidin-2-ones in many cases provided antitumor effect in $<10\,\mu M$ concentrations. However, only **5c** and **9c** demonstrated good selectivity towards tumor and normal cells. Isomeric mixture **5d**/**7d** inhibited tumor cells growth at six time lower concentrations than normal 3T3 cells only in the case of HT-1080. The same selectivity was observed for azedidinone **12e** towards MG-22A. The overwhelming majority of tested compounds with intensive antitumor properties caused similar NO generation in appropriate cells lines and vice versa.

Azetidinone **9e** with 3(*Z*)-4-nitrobenzylidene side chain turned out to be more active than its *E*-isomeric counterpart **10e** towards all tested tumor cell lines. Similar leadership of *Z*-isomer for the **5e** and **7e** pair was limited by three tumor species, but isomeric couple **9d** and

Table 1. In vitro cytotoxic and NO generating activity of 6-alkylidenepenicillanate sulfones and 3-alkylidene-2-azetidinones

Compd	Cell lines																
	HT-1080			MG-22A			B16			Neuro 2A			3T3			BHK 21	
	IC_{50} (CV)[a]	IC_{50} (MTT)[b]	TG_{100}[d]	IC_{50} (CV)	IC_{50} (MTT)	TG_{100}	IC_{50} (CV)	IC_{50} (MTT)	TG_{100}	IC_{50} (CV)	IC_{50} (MTT)	TG_{100}	IC_{50} (CV)	IC_{50} (MTT)	IC_{50} (NR)[c]	IC_{50} (CV)	IC_{50} (MTT)
4a	6.7	15.3	n.t.	61.2	79.5	n.t.	101	101	31	113	79.5	71	14.4	12.2		9.1	3.3
4c	24.1	20.2	750	12.1	16.1	750	12.1	20.2	566	24.2	>200	31	16.1	18.5		n.t.	n.t.
5a	93.3	189	33	>200	>200	5											
5b	1.2	<1.2	640	2.5	2.5	389	16.2	19.9	100	16.2	17.5	600			5.0	5.8	3.9
5c	10.5	10.5	800	7.8	7.4	533	3.9	3.9	800	>20	>200	18	>200	>200		n.t.	n.t.
5d/**7d**	2.6	2.6	700	6.4	12.2	800	7.9	15.9	800	12.2	15.9	800	18.6	21.2		n.t.	n.t.
5e	2.4	3.8	250	1.9	32.5	400	2.8	2.1	400	2.4	1.6	400	2.8	2.4		4.0	5.2
6d	90.0	106	73	116	154	58											
7e	0.9	0.9	300	2.3	32.2	300	4.7	6.2	350	3.8	3.8	400	3.3	2.4		3.3	2.4
9a	6.3	4.6	350	29.3	32.5	300	9.8	22.4	300	58.7	30.4	83	31.4	33.1		28.4	51.3
9b	64.6	8.90	1100	6.7	6.7	1050	6.7	6.7	800	71.4	62.5	850			17.9	6.7	4.5
9d	9.8	9.8	750	5.1	10.8	139	11.8	10.8	1000	11.8	13.7	750	11.8	19.6		n.t.	n.t.
9c	0.4	0.4	1500	0.8	0.7	440	0.5	0.5	375	>200	>200	27	12.4	15.5		n.t.	n.t.
9e	4.1	3.2	350	3.4	4.5	300	0.7	3.6	300	4.3	2.5	300	4.8	5.7		4.8	2.1
10d	9.8	8.8	700	10.6	9.8	900	13.7	10.6	1000	51.0	72.5	850	15.7	19.6		n.t.	n.t.
10e	9.9	9.3	400	9.9	15.2	450	8.3	9.3	600	12.2	10.4	350	n.t.	n.t.		n.t.	n.t.
12b	7.2	3.4	350	3.6	4.8	300	1.4	34.2	300	4.8	1.9	250	5.8	5.3		6.0	1.9
12e	3.7	2.7	450	1.6	2.3	450	4.6	8.0	63	13.8	8.5	250	11.0	16.7		13.1	32.1
14	>200	>200	9	>200	>200	13											
16	23.2	39.4	1400	23.2	39.4	1100	25.5	34.8	400	62.6	37.1	500			30.2	7.0	7.0
13	17.7	18.6	200	134	134	200											

n.t., not tested; IC_{50}, concentration (μM) providing 50% cell killing effect.
[a] CV, coloration.
[b] MTT, coloration.
[c] NR, coloration.
[d] Extrapolated total NO radicals generation ability at 100 μM concentration of tested compound.

10d was characterized practically by equal properties. Controversial tendencies in the activity of penicillanates and azetidinones with the same side chain did not allow to make definite judgement about the preference of closed or opened thiazolidine ring in β-lactam containing structures. The IC_{50} data obtained for **13** an **14** gave clear evidence about the inefficiency of the deprotection of carboxyl and of the side-chain hydrogenation.

Obtained results widen the structural diversity of antitumor β-lactams and confirm the perspectivity of further investigations in this area.

References and notes

1. (a) Otani, T.; Oie, S.; Matsumoto, H.; Tempest, M.; Micetich, R. G.; Singh, R.; Yamashita, T. WO 01,109, 1994; *Chem. Abstr*. **1994**, *121*, 57497. (b) Alpegiani, M.; Bissolino, P.; Perrone, E.; Pesenti E. WO 02,603 1995; *Chem. Abstr*. **1995**, *122*, 205179. (c) Veinberg, G.; Vorona, M.; Shestakova, I.; Kanepe, I.; Zharkova, O.; Mezapuke, R.; Turovskis, I.; Kalvinsh, I.; Lukevics, E. *Bioorg. Med. Chem*. **2000**, *8*, 1033. (d) Banik, I.; Becker, F. F.; Banik, B. B. *J. Med. Chem*. **2003**, *46*, 12.
2. (a) Doherty, J. B.; Ashe, B. M.; Barker, P. L.; Blacklock, T. J.; Butcher, J. W.; Chandler, G. O.; Dahlgren, M. E.; Davies, P.; Dorn, C. P., Jr.; Finke, P. E.; Firestone, R. A.; Hagmann, W. K.; Halgren, T.; Knight, W. B.; Maycock, A. L.; Navia, M. A.; O'Grady, L.; Pisano, J. M.; Shah, S. K.; Thompson, K. R.; Weston, H.; Zimmerman, M. *J. Med. Chem*. **1990**, *33*, 2513. (b) Finke, P. E.; Dahlegren, M. E.; Weston, H.; Maycock, A. L.; Doherty, J. B. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 2277. (c) Zhou, N. E.; Kaleta, J.; Prisima, E.; Menard, R.; Micetich, R. G.; Singh, R. *Bioorg. Med. Chem. Lett*. **2002**, *12*, 3417. (d) Zhou, N. E.; Guo, D.; Thomas, G.; Reddy, A. V. N.; Kaleta, J.; Prisima, E.; Menard, R.; Micetich, R. G.; Singh, R. *Bioorg. Med. Chem. Lett*. **2003**, *13*, 139.
3. Chen, Y. L., Eur. Patent 150,984, 1985; *Chem. Abstr*. **1986**, *104*, 50732.
4. Buynak, J. D.; Rao, A. S.; Nidamarthy, S. D. *Tetrahedron Lett*. **1998**, *39*, 4945.
5. Buynak, J. D.; Rao, A. S.; Doppalapudi, V. R.; Adam, G.; Petersen, P. J.; Nidamarthy, S. D. *Bioorg. Med. Chem. Lett*. **1999**, *9*, 1997.
6. Micetich, R. G.; Maiti, S. N.; Spevak, P.; Tanaka, M.; Yamazaki, T.; Ogawa, K. *Synthesis* **1986**, 292.
7. Lukič, I. *Org. Prep. Proc. Int. Briefs* **1999**, *31*, 352.
8. All products described in this manuscript have been characterized by elemental analysis and ^{1}H NMR spectra.
9. Fast, D. J.; Lynch, R. C.; Leu, R. W. J. *Leuckocyt. Biol*. **1992**, *52*, 255.
10. Freshney, P. J. *Culture of Animal Cells (A Manual of Basic Technique)*; Wiley-Liss: New York, 1994; p 296.