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CYCLOPENTENONES AS POTENTIAL INHIBITORS OF PENICILLIN SENSITIVE ENZYMES

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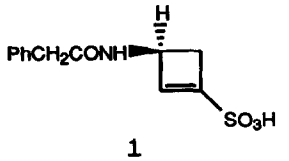
Abstract : 2-cyclopenten-1-ones bearing an acylamino side-chain at C-4 and an acetic acid residue at C-2 were designed as potential alkylating inhibitors of penicillin sensitive enzymes. Compounds 11 and 12 were efficiently prepared from the readily available 2-phenylsulfonyl-4-methoxycarbonyl-cyclopentan-1-one 3. They were inactive in all tests.

The discovery of new inhibitors of Penicillin Sensitive Enzymes (PSEs) represents an important therapeutic objective in view of the ever-increasing resistance of bacteria to existing beta-lactam antibiotics. Most of the research in that area has focused on β–lactam mimics which act as *acylating agents*[1]. Excellent antibacterial properties were exhibited by compounds containing pyrazolidinone or isoxazolidinone rings[1].

We recently became interested in the design and synthesis of *alkylating molecules* which could selectively block the active serine residue of PSEs[2]. In this process, the enzyme and the inhibitor would become linked by an ether function which is resistant to hydrolysis and, therefore, an *irreversible inhibition* of the enzyme should be observed. We first synthesized oxaziridines and epoxides related to penams or carbapenems[3,4]. However these compounds were very unstable and no biological activity was observed. We then decided to examine Michael acceptors as potential alkylating inhibitors of PSEs[5]. To us, these systems were particularly attractive because the geometry of attack of a nucleophilic reagent on a carbon-carbon double bond should be somewhat close to that on the carbonyl function of a beta-lactam.

We recently described the synthesis of a cyclobutene-1-sulfonate 1 (Scheme 1) mimicking a monobactam[5]. However the compound was too unstable in water or protic solvents to allow for biological evaluation. This led us to consider the expectedly more stable 2-cyclopenten-1-ones 2 as potential inhibitors of PSEs (Scheme 1). These molecules can be considered as alkylating analogs of beta-lactam antibiotics : they bear the acylamino- and the acetic acid side-chains requested for the recognition by the target enzymes, and a carbonyl group which will activate the carbon-carbon double bond towards nucleophilic reagents.



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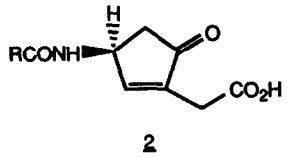

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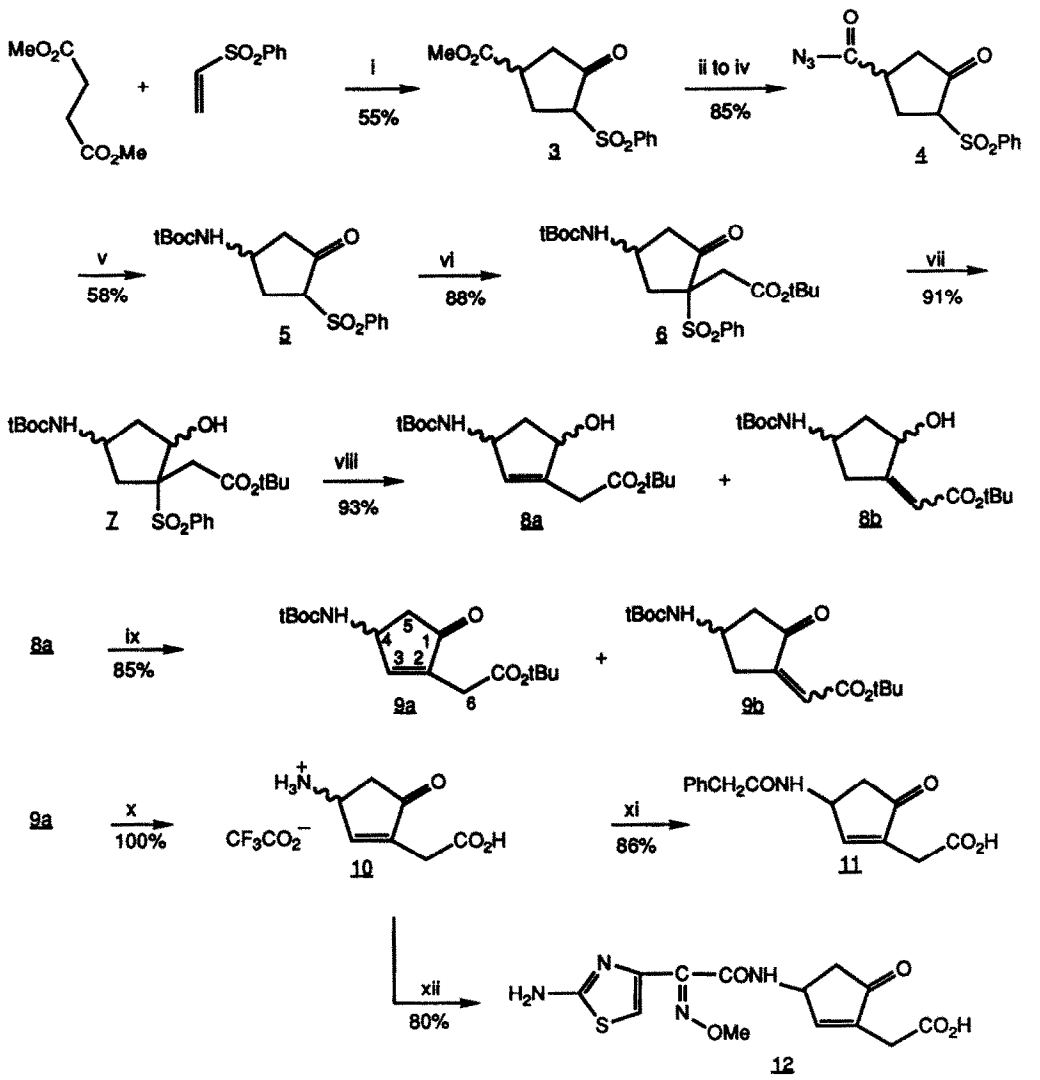
Scheme 1

The target molecules 2 (racemic mixtures) were readily prepared by the sequence of transformations outlined in Scheme 2.



(i) KHMDS (1.2 equiv.), THF, -78°C, 40 min., then addition of sulfone (1 equiv.), -78°C, 30 min.; (ii) HCl 6 N, acetone, 85°C, 5hrs; (iii) $Me_2C{=}C(Cl)NMe_2$ (2 equiv.), CH_2Cl_2, 20°C, 15hrs; (iv) NaN_3 (2 equiv.), 10% $nBu_4N^+Br^-$, CH_2Cl_2 - H_2O, 0°C to 20°C, 4hrs; (v) tBuOH (40 equiv.), $ClCH_2CH_2Cl$, 90°C, 24hrs; (vi) $BrCH_2CO_2tBu$ (2 equiv.), CH_2Cl_2 - H_2O,NaOH, $nBu_4N^+HSO_4^-$, 20°C, 3hrs; (vii) $NaBH_4$ (2 equiv.), MeOH, 0°C, 1hr; (viii) DBU (1.2 equiv.), CH_2Cl_2, 20°C, 7hrs; (ix) PDC (2 equiv.), CH_2Cl_2, 20°C, 7hrs; (x) CF_3CO_2H (20 equiv.), CH_2Cl_2, 20°C, 5hrs; (xi) R - COCl (2 equiv.), H_2O, K_2CO_3, 20°C, 1hr, then HCl 0.5 N; (xii) RCO_2 - benzotriazolyl (1.5 equiv.), K_2CO_3, H_2O - acetone, 20°C, 5hrs, then HCl 0.5 N.

Scheme 2

A cis : trans (1 : 2) mixture of cyclopentanones 3 was obtained by Michael addition of the potassium enolate derived from dimethyl succinate onto vinylphenylsulfone followed by spontaneous cyclization of the resulting adduct. Hydrolysis of the methyl ester of 3 could be successfully performed in acidic conditions. Basic solutions (e.g. LiOH) led to the cleavage of the ring by a retroaldol process. Reaction of the acid (cis : trans, 1 : 1) with tetramethyl-α-chloroenamine[6] quantitatively yielded the acid chloride which was directly treated with sodium azide under phase-transfer conditions[7]. The crude acyl azide 4 smoothly rearranged upon heating at 90° C in the presence of a large excess of t-butanol to yield the carbamate 5 (cis : trans, 1 : 1). Compound 5 was purified by flash chromatography on silica gel. Introduction of the acetic acid side-chain was effected according to Lamm's procedure[8]. All attempts to eliminate phenylsulphinic acid from 6 using various types of bases (e.g. DBU, tBuOK) only gave complex mixtures of products, probably as a result of the instability of the formed enone in these conditions[9]. Thus the carbonyl group was first reduced and the resulting alcohol 7 (mixture of diastereoisomers) was treated with DBU. A 85 : 15 mixture of endocyclic and exocyclic olefins 8a and 8b was obtained. Compound 8a was isolated by flash chromatography (79 %) and oxidized with pyridinium dichromate to the protected 4-aminocyclopentenone 9a which was contaminated by a small amount (≤ 6 %) of isomer 9b[10,11]. The aminoacid 10 was quantitatively obtained after cleavage of the t-Boc protecting group with trifluoroacetic acid. Acylation of 10 with phenylacetyl chloride under Schotten-Baumann conditions yielded 11[12]. The anchorage of the cefotaxime side-chain was effected using the N-hydroxybenzotriazole ester of 2-amino-α-(methoxyimino)-4-thiazole acetic acid[13]. The cephalosporin analog 12 was obtained in 80 % yield[14].

The antibacterial properties of compounds 10, 11 and 12 were tested against ten representative micro-organisms (E. coli ESS, Ps. aeruginosa 447B-ES48, Ent. faecalis I, Staph. aureus Oxford, B. subtilis ATCC 6633, H. influenzae Q1, M. catarrhalis 1502, Strep. agalactiae Hester, Strep. pneumoniae 1761, Strep. pyogenes CN10)[15]. Their enzymic inhibitory activity was examined against the D,D-carboxypeptidase of Streptomyces R39[16] and four β-lactamases (from S. albus G, E. coli (TEM-1), E. cloacae P99, B. cereus)[17]. The three compounds were inactive in all tests.

However our design of structures 2 neglected an important factor of the enzyme machinery, i.e. hydrogen bonding between the inhibitor and amide bonds of the protein. The lack of an oxygen atom on the electrophilic carbon atom C-3 in both 11 and 12 precludes any stabilizing interaction with the "oxyanion hole" of the protein[18]. This problem is presently being studied in our laboratory.

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11. Characterization of 9a (±) : mp 85-87° C; IR (KBr) ν 1730, 1710, 1645, 1530 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 7.37 (br s, 1, H-3); 4.93 (m, 1, NH); 4.72 (m, 1, H-4); 3.17 (s, 2, H-6); 2.92 (dd, 1, J = 18.8 and 6.6 Hz, H-5); 2.21 (dd, 1, J = 18.8 and 2.3 Hz, H-5'); 1.46 (s, 18, tBu 2x); ^{13}C NMR (50 MHz, $CDCl_3$) ppm 205.04 (C-1); 169.08 (CO ester), 157.54 (C-3), 155.06 (CO carbamate), 140.74 (C-2), 81.46, 80.09, 49.11 (C-4), 42.46, 31.10 (C-5), 28.31, 27.98; Analysis, calcd for $C_{16}H_{25}NO_5$: C, 61.72 %; H, 8.09 %; N, 4.50 %, found : C, 61.72 %; H, 8.24 %; N, 4.49 %.
12. Characterization of 11 (±) : IR (KBr) ν 3450, 3277, 1700, 1644, 1572, 1549 cm^{-1}; 1H NMR (200 MHz, CD_3OD) δ 7.32 (br s, 1, H-3); 7.18 (m, 5, Ph); 4.88 (m, 1, H-4); 3.41 (br s, 2, Ph $\underline{CH_2}$); 2.99 (br s, 2, H-6); 2.74 (dd, 1, J = 18.7 and 6.6 Hz, H-5); 2.13 (dd, 1, J = 18.7 and 2.2 Hz, H-5'); ^{13}C NMR (50 MHz, DMSO d^6) ppm 206.55 (C-1), 174.74 (CO acid), 170.29 (CO amide), 157.28 (C-3), 143.23 (C-2), 136.50, 129.28, 128.45, 126.59, 47.66 (C-4), 42.43, 41.75, 33.70 (C-5); Mass (FAB^-/glycol) $C_{15}H_{15}NO_4$: 272 (M-1).
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14. Characterization of 12 (±) : IR (KBr) ν 3070, 1745, 1710, 1650, 1630, 1170, 1035 cm^{-1}; ^1H NMR (200 MHz, D_2O) δ 7.56 (s, 1, H-3); 7.05 (s, 1, thiazolyl); 5.17 (m, 1, H-4); 3.97 (s, 3, OCH_3); 3.33 (s, 2, H-6); 3.00 (dd, 1, J = 18.9 and 6.5 Hz, H-5); 2.41 (dd, 1, J = 18.9 and 2.1 Hz, H-5'); ^{13}C-NMR (50 MHz, D_2O) ppm 209.80 (C-1), 174.98 (CO acid), 171.38 (CO amide), 161.79 (C = N), 160.48 (C-3), 143.07 (C-2), 141.48, 131.22, 111.60, 64.82, 49.55 (C-4), 42.00, 30.90 (C-5); Mass (FAB^+/glycol) $C_{13}H_{14}N_4O_5S$: 339 (M + 1).
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