Tetrahedron Letters, Vol.22, No.20, pp 1871 - 1874, 1981
Printed in Great Britain

0040-4039/81/201871-04$02.00/0

CARBACYCLIC ISOSTERES OF PENICILLANIC AND CARBAPENEMIC ACIDS. SYNTHESIS OF BICYCLO[3.2.0]HEPTAN-6-ONES AS POTENTIAL ENZYME INHIBITORS.

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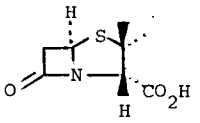
Summary: Carbacyclic ketone analogs of penicillanic and carbapenemic acids were synthesized and tested for inhibition of β-lactamase and DD-carboxypeptidase/transpeptidase enzymes.

Recent investigations in these laboratories have centered on the design of novel β-lactamase and bacterial cell-wall DD-carboxypeptidase/transpeptidase enzyme inhibitors. In the course of these studies we have attempted to define optimal steric and electronic criteria necessary for binding of substrates to these enzyme systems. The substrate specificity of the β-lactamases appears to require a bicyclic β-lactam nucleus for binding[1], a feature also possessed by the most notable inhibitors of DD-carboxypeptidase/transpeptidase. One hitherto unaddressed question pertains to the role of β-lactam nitrogen in substrate/inhibitor binding. To probe this point we chose to synthesize non-nitrogen containing analogs of penicillanic (1) and carbapenemic acid[2] and determine whether such bicyclic isosteres would manifest enzyme binding. This communication describes our results concerning penicillanic and carbapenemic acid models 2 and 4. One salient feature of these target molecules is the reactive cyclobutanone carbonyl which could interact with the aforementioned serine proteases to form a relatively stable hemi-ketal linkage, not dissimilar to the presumed mode of action of other protease inhibitors[3].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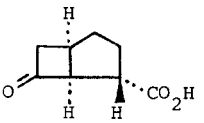
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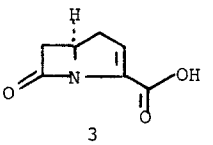
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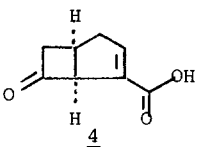
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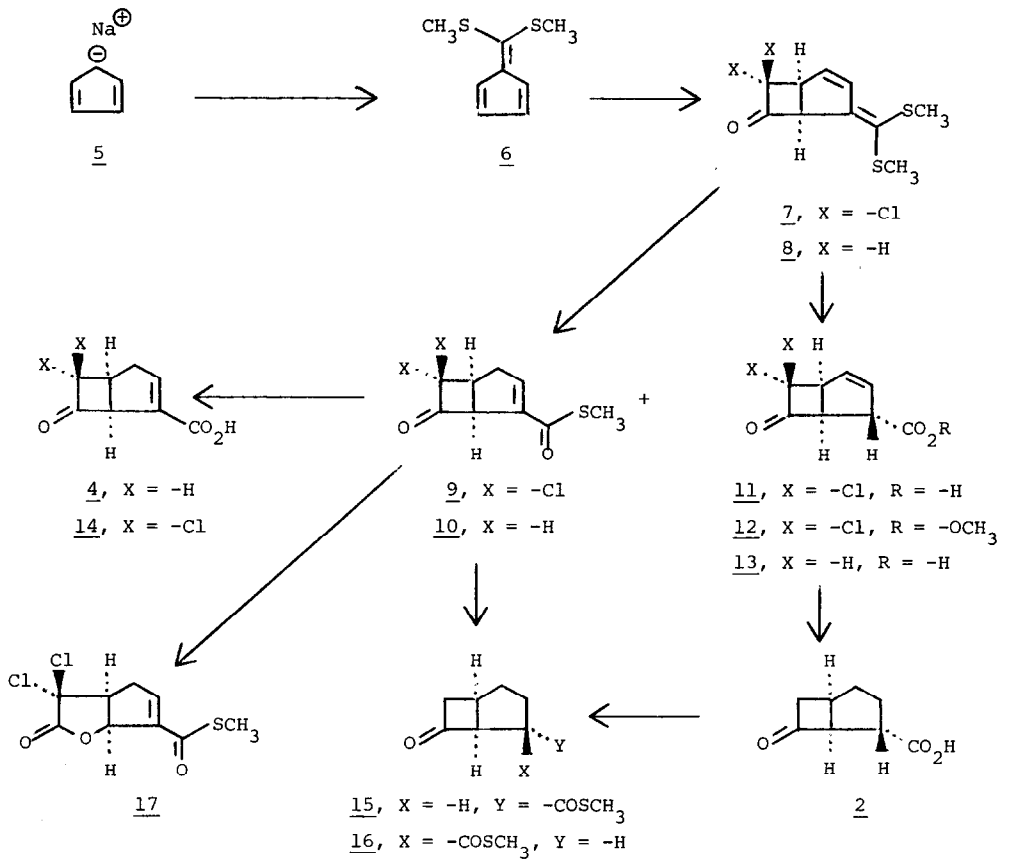
The selection of 4α-carboxybicyclo[3.2.0]heptan-6-one (2) as a suitable isosteric model for penicillanic acid (1) was based on a compromise between synthetic practicality and biological suitability. Our approach to this and the 4-carboxybicyclo[3.2.0]hepten-6-one (4) system was predicated on the well documented 2+2 cycloaddition of dichloroketene to reactive olefins.[4,5] 6,6-Bis-methylmercaptofulvene 6 was prepared by treatment of sodium cyclopentadienide with carbon disulfide (-15°C) followed by alkylation with methyl iodide[6]. Dichloroketene reacted ($Cl_2CHCOCl/Et_2N/Et_2O$/-10°) readily with freshly prepared 6 to produce 7 as a yellow oil of limited stability[7] [IR ($CHCl_3$) 1810 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.30 (s,3H), 2.35 (s,3H), 4.18 (octet,1H), 5.15 (d of d,1H), 6.6 (octet,1H), 6.91 (d of d,1H); m/e 281]. Thus,



5 6

7, X = -Cl
8, X = -H

4, X = -H
14, X = -Cl

9, X = -Cl
10, X = -H

11, X = -Cl, R = -H
12, X = -Cl, R = -OCH3
13, X = -H, R = -H

17

15, X = -H, Y = -COSCH3
16, X = -COSCH3, Y = -H

2

18 19

in this step all requisite carbon-carbon bonds are assembled with appropriate regiochemistry. The regiospecificity of this process is expected to be as shown by analogy to previous cycloadditions of dichloroketenes and fulvenes.[8,9]

Compound 7 showed considerable resistance to known dithioketeneacetal hydrolytic methods;[10] however, heating under reflux in 88% formic acid containing 2 equivalents of mercuric chloride (15 min) produced crystalline 11 [m.p. 136-7.5°C; IR (KBr) 1808, 1710 cm^{-1}; m/e 222 (M^+); ^{13}C (acetone-d_6) 52.7, 59.1, 61.2, 87.6, 130.7, 135.3, 170.9, 195.6] and thioester 9 as a viscous yellow oil [distilled 140-50°C/0.15 mm; IR ($CHCl_3$) 1805, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) 2.40 (s, 3H), 3.16 (m,2H), 3.70 (octet,1H), 5.00 (m,1H), 6.90 (m,1H); ^{13}C NMR ($CDCl_3$) 11.4, 36.6, 49.6, 67.9, 90.0, 137.9, 142.7, 186.6, 189.7; m/e 251 (M^+); UV (MeOH) 230 nm (ε=6400), 262 nm (ε=4100)] in approximately 1/4 ratio, in 16% isolated yield from cyclopentadiene. The gross structure, regiochemistry and stereochemistry of 11 was assumed by comparison (^1H NMR) to 18;[11] and was

unequivocally established by an X-ray crystallographic analysis.

Alternatively, prior reduction of 7 to 8 (Zn/HOAc/70°C/45 min), followed by the hydrolysis procedure, gave only 10 in low yield. Treatment of 11 with CH_3I/Et_3N afforded ester 12 as a distillable oil. On the other hand, esterification with diazomethane gave a mixture of expected 12 and ring expanded bicyclooctanone 19 [IR ($CHCl_3$) 1770 cm^{-1}, m/e 250 (M^+)]. Interestingly, 12 failed to isomerize to its α,β-unsaturated analog, under either basic or acidic conditions. Similar difficulties were encountered in converting a β,γ-unsaturated thienamycin derivative to its α,β-unsaturated form.[12] Reductive dechlorination of 11 (Zn/HOAc/70°/45 min) produced 13 which was crystallized as its dicyclohexylamine salt (66%, m.p. 167-9°C). Catalytic hydrogenation (10% Pd/C/EtOAc) of 13 afforded the desired target 2 (82%) also crystallized as its DCHA salt [m.p. 150-1°C; IR (KBr) 1770, 1625 cm^{-1}; ^{13}C (acetone-d_6, free acid) 28.5, 29.1, 31.0, 46.2, 51.2, 67.4, 174.3, 210.2].

Turning to the preparation of carbapenemic acid model 4, we found that 9 was unstable to basic hydrolysis, and extremely resistant to acid catalyzed hydrolysis of the unsaturated thioester function. Attempts at thioester hydrolysis using mercuric acetate in aqueous THF led to the interesting, but unwanted lactone 17[13] [35%; IR ($CHCl_3$) 1795, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) 2.43 (s,3H), 3.00 (m,2H), 3.78 (m,1H), 5.80 (d,1H,J=6 Hz), 7.13 (t,1H,J=2 Hz); ^{13}C NMR (acetone-d_6) 10.7, 35.9, 54.3, 81.2, 84.3, 139.8, 148.6, 167.5, 186.6; m/e 266 (M^+)]. Refluxing 9 in 1:1 concentrated hydrochloric acid/glacial acetic acid, however, produced acid 14 in 27% yield [m.p. 165-8°C; IR (KBr) 1805, 1680 cm^{-1}; ^{13}C (acetone-d_6) 36.8, 50.4, 68.9, 90.9, 132.2, 146.4, 163.5, 191.3].

Reductive dechlorination of 14 (Zn/HOAc/70°) yielded carbapenem analog 4, which crystallized as its dicyclohexylamine salt [m.p. 149-51°C; IR (KBr) 1770, 1625 cm^{-1}; UV (MeOH, 212 nm (ε=6000); ^{13}C NMR (acetone-d_6, free acid) 26.5, 40.4, 53.6, 72.2, 132.2, 145.1, 164.4, 204.0]. In order to relate the gross chemical structures of carbapenem analog 4 and penicillanic acid analog 2, substance 10, accessible by reductive dechlorination of 9 (Zn/HOAc/70°), was hydrogenated (10% Pd/C, HOAc, 50 psi) to produce two major products of differing R_f. These were assigned as 15 and 16 by ^{13}C NMR. Upon contact with DBU/$CHCl_3$ the isomer of lower R_f was converted to the higher R_f isomer. Since the thioester substituent is expected to be considerably more stable in the exo configuration, the stereochemistry of 15 (higher R_f) and 16 (lower R_f) is assigned as shown. Conversion of 2 to its acid chloride [$(COCl)_2/Et_2O$] followed by reaction with methanethiol permitted the low yield isolation of a thioester. This substance was identical to 15 (^{13}C NMR, IR, TLC), further confirming the stereochemistry assigned to this substance material and relating the overall structures of 2 and 4.

In conclusion, we note that carbapenem nucleus 3 has been prepared[14] and exhibits considerable antibacterial activity against only non-β-lactamase producing microorganisms (*i.e.*, 3 is a good substrate for β-lactamases). Substance 4, however, when tested for β-lactamase inhibition *vs.* the R-TEM enzyme,[15] and for DD-carboxypeptidase/transpeptidase inhibition *vs.* the R61 enzyme[16] was not a significant inhibitor. Compounds 2, 11 and 13 were also tested against these isolated enzymes and did not exhibit significant reversible or irreversible enzyme inhibition. Assuming that 2 and 4 are reasonable 3-dimensional models for penicillanic and carbapenemic acids, we conclude that β-lactam nitrogen plays a crucial role in the

recognition and binding of β-lactam antibiotics to these enzymes.

Acknowledgements. We are grateful to Drs. K. Bush and N. H. Georgopapadakou for biological testing data and the Squibb Institute Analytical Department for assistance in this project. The X-ray crystallographic study was performed by Ms. Mary Malley.

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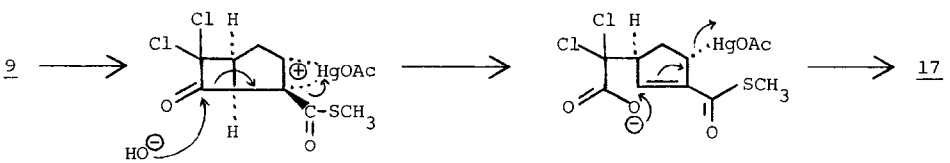

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(Received in USA 8 September 1980)