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SPIRO INDOLINONE BETA-LACTAMS, INHIBITORS OF POLIOVIRUS AND RHINOVIRUS 3C-PROTEINASES

Jerry W. Skiles* and Daniel McNeil

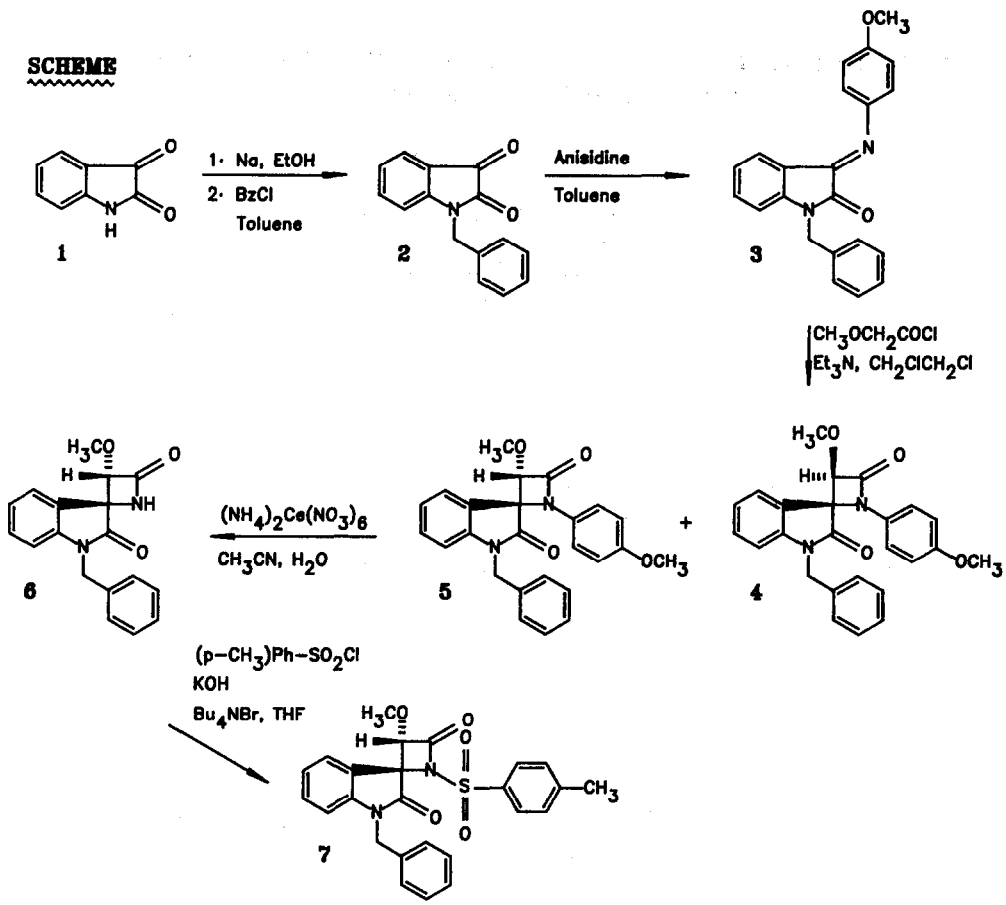
Department of Medicinal Chemistry
Boehringer Ingelheim Pharmaceuticals, Inc.
90 East Ridge, P.O. Box 368
Ridgefield, Connecticut 06877

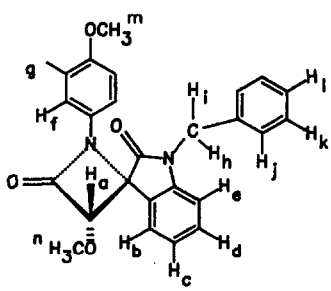
Summary: Preparation of the spiro beta-lactam **7** and its evaluation as a poliovirus and human rhinovirus 3C-proteinase inhibitor is described.

Controlled proteolysis of precursor polypeptides is crucial to the life cycle of picornaviruses[1] (e.g., human rhinovirus and poliovirus). The replication of many animal and plant viruses is entirely dependent on proteolytic processing, and proteolytic enzymes are involved in a variety of functions including separation of structural and non-structural proteins, generation of specific enzymes (RNA polypeptides), coordinated assembly of the virion and maturation. The evidence suggests the involvement of virus-encoded proteinases in the processing of viral precursor polypeptides. The rhinovirus, like other picornaviruses contains a single positive strand RNA genome that is translated into a large single precursor polyprotein (≈ 200,000 Daltons). This polyprotein can cleave itself into different domains without the help of cellular components. It does this so efficiently that under normal circumstances, the polyprotein cannot be observed at all. Most cleavages of the polyprotein occur between glutamine and glycine (Q-G) and two cleavages between tyrosine and glycine (Y-G). The Q-G cleavages are carried out by polypeptide "3C-proteinase" and the Y-G cleavage by polypeptide "2A-proteinase". Both of these proteinases can act *in cis* (intramolecular or auto catalytic) as well as *in trans* (intermolecular) fashion and both are thought to be sulphydryl[2] proteinases. All picornaviruses studied produce a 3C-proteinase which is required for the virus to undergo maturation.

As part of our continuing effort to synthesize antiviral agents of the picornavirus family, we were interested in preparing a series of spiro indolinone beta-lactams as potential mechanistic based inhibitors of viral 3C-proteinases. The effectiveness of N-methylisatin-β-thiosemicarbazone (methisazone)[3a] as an antiviral agent and of N-aminomethylisatins[3b] as retarding poliomyelitis Type II progression has previously been demonstrated. Cephalosporin sulfones[4a] and monocyclic azetidinones[4b] have previously been reported to be potent inhibitors of human neutrophil elastase, a serine proteinase. Since the hydrolytic mechanisms of serine and cysteine proteases are similar, we thought it might be of interest to see if beta-lactams such as **7** could inhibit viral 3C-proteinases.

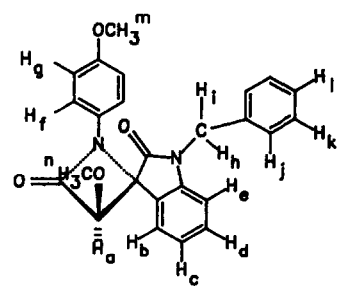
SCHEME





NOE

Atom Irrad·	Enhancement
b	none
n	a
a	n



NOE

Atom Irrad·	Enhancement
b	a,c
n	a
a	b,n

The synthesis of **7** begins with the benzylation of isatin (**1**) to give **2**. Equimolar amounts of 1-benzylisatin (**2**) and anisidine in ethanol were refluxed for 30 minutes to afford the Schiff base **3**.[5] The required 2,2'-spiro[indoline-3,3'-azetidine] intermediates **4** and **5** were obtained by the acid chloride-imidate cycloaddition route.[6] Thus the reaction of methoxyacetyl chloride with **3** under ketene generating conditions afforded a mixture of corresponding *E* and *Z*-2-azetidinones **4** and **5** respectively in 80 % overall yield. The isomers **4** and **5** were separated by chromatography over silica-gel employing methylene chloride/ ethyl acetate (97:3). Assignment of the relative stereochemistry of **4** and **5** was done by measuring NOE. The *Z*-isomer **5** exhibited an NOE effect between the hydrogen of the 2-azetidinone ring and that of the *ortho*-hydrogens of the indanone phenyl ring. The *E*-isomer **4** exhibited no NOE between these two protons.[7] The *(Z)*-N-aryl-2-azetidinone **5** was converted to the N-substituted-2-azetidinone **6** by treatment with ceric ammonium nitrate.[8] All attempts to acylate the 2-azetidinone **6** with *para*-methylphenylsulfonyl chloride under standard conditions failed (e.g., NaH, LDA, K_2CO_3, KOH, DBU). The desired sulfonamide **7**; however, was successfully obtained in 60 % purified yield by employing tetrabutylammonium bromide in THF in the presence of pulverized KOH at room temperature.

Compound **7** was found to be a good inhibitor[9] of both poliovirus and human rhinovirus 3C-proteinases (IC_{50} = 20 μg/mL). In addition **7** also inhibited human leukocyte elastase (HLE) (IC_{50} = 0.4 μg/mL) as well as Cathepsin G (IC_{50} = 4.0 μg/mL).

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2. Although this enzyme is thought to be a cysteine proteinase, thiol-reactive compounds such as leupeptin and E-64, with the exception of zinc salts, do not inhibit. In addition the protease is not sensitive to phosphonofluoridate, chloroisocoumarin, or trifluoromethyl ketones and is therefore unlike the classical serine-type enzymes. The catalytic site may be buried within the core of the enzyme and not easily accessible.

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7. a) Compound **5**: ^1H and ^{13}C NMR and MS data are listed below:
^1H NMR (250 MHz) ($CDCl_3$) δ 2.98 (s, OCH_3), 3.67 (s, OCH_3), 5.02 (s, H_a), 5.03 (d, J = - 15.8 Hz, H_i), 5.10 (d, J = -15.8 Hz, H_h), 6.81 (dt, J = 9.2, 2.5 Hz, H_g), 6.89 (dt, J = 9.2, 2.5 Hz, H_f) 7.11 (td, J = 7.7, 0.7 Hz, H_c), 7.21 (d, J = 7.7 Hz, H_e), 7.3 - 7.5 (m, H_b, H_d, H_{j-l}) ppm; ^{13}C NMR (25 MHz) (DMSO) δ 44.39 (benzylic CH_2), 55.45 (aromatic OCH_3), 58.97 (β-lactam OCH_3), 67.14 (spiro C), 90.07 (β-lactam CH), 109.77, 114.59, 119.31, 121.39 (quaternary C), 123.30, 126.58, 127.68, 128.08, 128.92, 129.78, 130.63, 135.46, 143.16, 157.10, 162.35 (C = O), 173.17 (C = O) ppm; MS (CI/NH_3) [$(M + H)^+$/(%)] 432 (14), 283 (10), 266 (100).

Compound **4**: ^1H and ^{13}C NMR and MS data are listed below:
^1H NMR (250 MHz) (DMSO) δ 3.21 (s, 3 H, OCH_3), 3.67 (s, 3 H, OCH_3), 5.17 (S, H_a), 5.04 (s, H_h, H_i), 6.80 (dt, J = 9.3, 2.7 Hz, H_g), 6.86 (dt, J = 9.3, 2.7 Hz, H_f), 7.09 (td, J = 7.6, 1.0 Hz, H_c), 7.21 (d, J = 7.6 Hz, H_e), 7.3 - 7.4 (m, H_d, H_{j-e}), 7.53 (d, J = 7.6 Hz, H_b) ppm; ^{13}C NMR (25 MHz) (DMSO) δ 44.49 (benzylic C), 55.43 (aromatic OCH_3), 59.54 (β-lactam OCH_3), 67.84 (spiro carbon), 93.00 (β-lactam CH), 110.02, 114.59, 119.37, 123.30, 127.81, 127.96, 128.84, 130.63, 124.28, 129.62, 135.53, 142.60, 157.05, 162.74 (C = O), 171.08 (C = O) ppm; MS (CI/NH_3) [$(M + H)^+$/(%)] 432 $(M + NH_4)^+$ (100), 226 (100).

Compound **7**: ^1H and ^{13}C NMR and MS data are listed below:
^1H NMR (250 MHz) ($CDCl_3$) δ (s, 3 H, CH_3), 3.12 (s, 3 H, OCH_3), 4.83 (s, β-lactam H), 5.03 (pair of doublets, 2 H, J_{gem} = - 15.0 Hz, $\Delta_{A,B}$ = 8.25 Hz, δ_B = 5.19, benzylic protons), 6.75 (d, 1 H), 7.00 (t, 1 H), 7.16 (d, 1 H), 7.25 - 7.40 (m, 8 H), 7.73 (d, 2 H) ppm; ^{13}C NMR (25 MHz) (DMSO) δ 21.61 (CH_3), 44.43 (CH_2), 59.20 (OCH_3), 67.91 (quaternary C), 89.04 (CH), 109.96 (CH), 120.48 (quaternary C), 122.93 (CH), 126.38 (CH), 127.22 (CH), 127.87 (CH), 128.18 (CH), 128.92 (CH), 129.75 (CH), 130.92 (CH), 135.11 (quaternary C), 135.74 (quaternary C), 143.86 (quaternary C), 145.67 (quaternary C), 162.12 (C = O), 172.0 (C = O) ppm; MS (CI/NH_3) [$(M + H)^+$/ (%)] 480 (M + $NH_4)^+$ (100), 266 (75).

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9. Curiously a typical cephalosporin reported in reference 3a above, while being a potent elastase inhibitor in our hands (IC_{50} = 0.2 μg/mL), does not inhibit poliovirus or human rhinovirus proteinases when assayed up to 100 μg/mL.

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