# Substituted 2-Pyrones and 5,6-Dihydropyrones as Inhibitors of the Serine Proteases



William C. Groutas*, Tien L. Huang, Michael A. Stanga,
Michael J. Brubaker and Min K. Moi

Department of Chemistry, Wichita State University,
Wichita, Kansas 67208

Received September 10, 1984

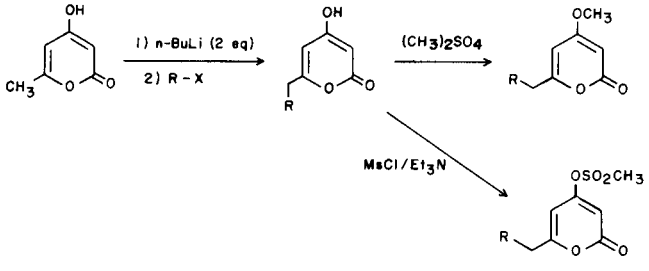
A series of substituted 2-pyrones and 5,6-dihydropyrones have been synthesized and investigated for their inhibitory activity toward human leukocyte elastase, procine pancreatic elastase and chymotrypsin.

*J. Heterocyclic Chem.*, **22**, 433 (1985).

It is currently accepted that the pathogenesis of pulmonary emphysema is due to a protease-protease inhibitor imbalance in the lungs [1]. An excess of human leukocyte elastase (HLE), a serine protease that is released by neutrophils in the lungs, initiates the degradation of lung connective tissue. The development of synthetic reversible and irreversible inhibitors of HLE might lead to some valuable thereapeutic agents.

We have recently shown that properly-designed heterocycles can function as highly selective inhibitors of HLE [3-4]. In continuing our studies in this area [5-6], we wish to report on the synthesis of a series of substituted 2-pyrones and dihydropyrones which a) incorporate in their structure a specificity moiety which takes into account the known substrate specificities of the various serine proteases [7] and makes possible the selective inhibition of HLE and none of the other serine proteases and b) have a latent functionality capable of undergoing an enzyme-induced transformation to yield a reactive moiety. Further reaction of this moiety with the active site serine *via* an irreversible Michael addition reaction leads to irreversible inactivation of the enzyme.

Scheme I




Synthesis.

Compounds **1** through **9** (Table 1) were synthesized according to Scheme I. 6-Methyl-4-hydroxy-2-pyrone was treated with 2 equivalents of *n*-butyl lithium in THF/-HMPT and the resulting dianion was alkylated by using the appropriate alkyl or benzyl halide [8]. The mesylate of the resulting product was readily obtained by reacting with methanesulfonyl chloride in triethylamine. The C-4 hydroxyl group was methylated with dimethyl sulfate by refluxing in 2-butanone.

The synthesis of compounds **10** through **13** was accomplished as shown in Scheme II. The dianion of methyl acetoacetate was generated through successive deprotonations using sodium hydride, followed by *n*-butyl lithium. Reaction with the appropriate aldehyde yielded the 5,6-dihydropyrones. Methylation and/or tosylate formation yielded the desired compounds [9].

The enzyme assays and inhibition studies were carried out as described previously [3-4]. None of the synthesized compounds showed any inhibition toward human leukocyte elastase, porcine pancreatic elastase and chymotrypsin. The lack of inhibitory activity toward chymotrypsin is surprising in light of the reported inhibition of chymotrypsin by 5-butyl-3*H*-1,3-oxazine-2,6-dione [10].

Scheme II

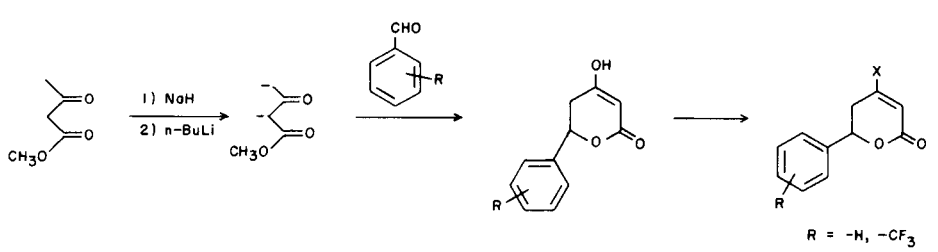
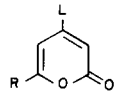


Table I

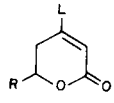
Substituted 2-Pyrones



| Compound No. | R | L | Mp °C | Yield % | Molecular Formula | Analyses %, Calcd./Found C | H | S | F |
|---|---|---|---|---|---|---|---|---|---|
| **1** | $CH_3(CH_2)_2$ | OH | 50-65 | 66 | $C_8H_{10}O_3$ | 62.33 | 6.54 | | |
| | | | | | | 62.58 | 6.69 | | |
| **2** | $CH_3(CH_2)_3$ | OH | partly liquid | 62 | $C_9H_{12}O_3$ | 64.27 | 7.19 | | |
| | | | | | | 64.06 | 7.28 | | |
| **3** | $CH_3(CH_2)_4$ | OH | 37-39 | 33 | $C_{10}H_{14}O_3$ | 65.95 | 7.74 | | |
| | | | | | | 65.80 | 8.07 | | |
| **4** | $CH_3(CH_2)_2$ | $OSO_2CH_3$ | liquid | 66 | $C_9H_{12}O_5S$ | 46.55 | 5.17 | 13.79 | |
| | | | | | | 46.37 | 5.25 | 13.60 | |
| **5** | $CH_3(CH_2)_3$ | $OSO_2CH_3$ | liquid | 60 | $C_{10}H_{14}O_5S$ | 48.78 | 5.69 | 13.01 | |
| | | | | | | 48.69 | 5.78 | 13.11 | |
| **6** | $CH_3(CH_2)_4$ | $OSO_2CH_3$ | liquid | 84 | $C_{11}H_{16}O_5S$ | 50.77 | 6.15 | 12.31 | |
| | | | | | | 50.64 | 6.32 | 12.22 | |
| **7** | $m$-$CF_3C_6H_4(CH_2)_2$ | OH | 174-176 | 37 | $C_{14}H_{11}O_3F_3$ | 59.16 | 3.89 | | 6.68 |
| | | | | | | 59.37 | 3.93 | | 6.71 |
| **8** | $m$-$CF_3C_6H_4(CH_2)_2$ | $OSO_2CH_3$ | 54-55 | 20 | $C_{15}H_{13}O_5F_3S$ | 49.72 | 3.61 | 8.85 | 15.73 |
| | | | | | | 49.86 | 3.79 | 8.71 | 15.66 |
| **9** | $m$-$CF_3C_6H_4(CH_2)_2$ | $OCH_3$ | 54-56 | 45 | $C_{15}H_{13}O_3F_3$ | 60.40 | 4.39 | | 19.11 |
| | | | | | | 60.36 | 4.36 | | 19.16 |

Table II

Substituted Dihydropyrones



| Compound No. | R | L | Mp °C | Yield % | Molecular Formula | Analyses %, Calcd./Found C | H | S | F |
|---|---|---|---|---|---|---|---|---|---|
| **10** | $m$-$CF_3C_6H_4$ | OH | 78-80 | 8.1 | $C_{12}H_9O_3F_3$ | 55.81 | 3.49 | | 22.09 |
| | | | | | | 55.88 | 3.58 | | 21.96 |
| **11** | $C_6H_5$ | OH | 137-139 | 44 | $C_{11}H_{10}O_3$ | 69.47 | 5.55 | | |
| | | | | | | 69.47 | 5.34 | | |
| **12** | $C_6H_5$ | $OCH_3$ | 140-142 | 39 | $C_{12}H_{12}O_3$ | 70.57 | 5.92 | | |
| | | | | | | 70.73 | 6.15 | | |
| **13** | $C_6H_5$ | $OSO_2$- -$CH_3$ | 79-81 | 23 | $C_{18}H_{16}O_5S$ | 62.85 | 4.69 | 9.32 | |
| | | | | | | 62.88 | 4.77 | 9.38 | |

## EXPERIMENTAL

Melting points were recorded on a Mel-Temp apparatus and are uncorrected. The infrared spectra were recorded on a Perkin-Elmer 1330 infrared spectrophotometer and the $^1H$ nmr spectra were recorded on a Hitachi Perkin-Elmer spectrometer using tetramethylsilane as internal standard. A Beckman Acta III uv/visible spectrophotometer was used in the enzyme assays. Elementary analyses were performed by Galbraith Laboratories, Knoxville, TN and M-H-W Laboratories, Phoenix, AZ.

4-Hydroxy-6-*n*-propyl, *n*-butyl and *n*-pentyl-2*H*-pyran-2-ones **1-3** and 4-hydroxy-6-2-(*m*-trifluoromethylphenylethyl)-2*H*-pyran-2-one **7** were prepared according to the procedure of Poulton and Cyr [8].

4-Mesyloxy-6-*n*-propyl-2*H*-pyran-2-one (**4**).

Triethylamine (5.56 ml, 0.04 mole) was added to compound **1** (3.08 g, 0.02 mole) in 25 ml of dry tetrahydrofuran under nitrogen. Methanesulfonyl chloride (1.56 g, 0.02 mole) was added dropwise to the stirred reaction mixture. After stirring overnight, the precipitate was filtered off, the filtrate was evaporated off and the oily residue was taken up in 100 ml of methylene chloride. After washing with water and drying over anhydrous sodium sulfate, the solvent was removed on the rotovac. The product was purified by flash chromatography using silica gel and hexane/ethyl acetate as eluents (3.05 g, 66% yield); ir (neat): 1725 $cm^{-1}$ (C=O), 1370 and 1180 (S=O); nmr (deuteriochloroform): $\delta$ 6.0 (1H, s, =CH), 5.3 (1H, s, =CH), 3.3 (3H, s, $CH_3SO_3$-), 2.5 (2H, t, =CH-$CH_2$-), 1.7 (2H, septet, -$CH_2$-), 1.0 (3H, t, $CH_3$-).

4-Mesyloxy-6-(*m*-trifluoromethylphenethyl)-2*H*-pyran-2-one (**8**).

Compound **7** (5.44 g, 0.019 mole) was dissolved in 50 ml of THF and mixed with triethylamine (5.56 ml, 0.04 mole) under nitrogen. Methane sulfonyl chloride (1.56 ml, 0.02 mole) was added dropwise and, after stir-

ring overnight, the reaction mixture was treated with water (80 ml). Extraction with ethyl acetate (3 × 75 ml) gave a crude product which was purified by flash chromatography using methylene chloride and ethyl acetate as eluents. The isolated product was purified further by recrystallization from hexane-ethyl acetate affording pure **8**, 1.36 g; ir (potassium bromide): 2950 $cm^{-1}$ (br, OH), 1650 (C=O); nmr (deuteriochloroform): δ 7.50 (4H, m, arom), 6.20 (1H, d, =CH), 6.05 (1H, d, =CH), 3.35 (3H, s, $CH_3SO_3$-), 3.1 (4H, sextet, -$CH_2CH_2$-).

4-Methoxy-6-(*n*-trifluoromethylphenethyl)-2*H*-pyran-2-one (**9**).

Compound **7** (3.4 g, 0.012 mole), anhydrous potassium carbonate (15.2 g, 0.108 mole) and dimethyl sulfate (2.72 g, 0.02 mole) in 200 ml of 2-butanone were refluxed under nitrogen for 3 hours. An additional amount of dimethyl sulfate (2.72 g) was added, and the heating continued for 20 hours. After filtration and evaporation of the solvent, the residue was purified by column chromatography on silica gel using hexane and methylene chloride as the eluents, yielding 1.61 g of **9**, mp 54-56°; ir (potassium bromide): 1730 $cm^{-1}$ (C=O); nmr (deuteriochloroform): δ 7.43 (4H, m), 5.75 (1H, d =CH), 5.45 (1H, d, =CH), 3.80 (3H, s, -$OCH_3$), 3.0 (4H, sextet, -$CH_2CH_2$-).

4-Hydroxy-6-phenyl-5,6-dihydro-2*H*-pyran-2-one (**11**).

Freshly distilled methyl acetoacetate (20.8 g, 0.18 mole) was added dropwise to a slurry of sodium hydride (4.20 g, 0.18 mole) in 400 ml of anhydrous tetrahydrofuran at 0°. *n*-Butyl lithium (114.5 ml of 1.6 *M* solution in hexane, 0.18 mole) was then added at 0°. Benzaldehyde (19.1 g, 0.18 mole) in 100 ml of tetrahydrofuran was added dropwise to the dianion and the reaction allowed to stir for 0.5 hours at 0° and then 0.5 hour at room temperature. After pouring the reaction mixture into ice-water and extracting with ethyl ether (4 × 125 ml), the aqueous layer was acidified with 6*N* hydrochloric acid to *p*H 2 and extracted with ethyl ether (4 × 150 ml). Evaporation of the solvent gave 15 g (44% yield) of a pale yellow solid, mp 137-139°; ir (potassium bromide): 1580 $cm^{-1}$ (C=O); nmr (DMSO-$d_6$): δ 7.50 (5H, s, arom), 5.6 (1H, q, ArCHO-), 5.22 (1H, s, =CH), 2.8 (2H, d, =CH), 2.8 (2H, d, =CH-$CH_2$-).

4-Methoxy-6-phenyl-5,6-dihydro-2*H*-pyran-2-one (**12**).

Using the same procedure as for compound **9**, compound **12** was obtained in pure form (1.34 g, 39% yield), mp 140-142°, after column chromatography on silica gel using methylene chloride and ethyl acetate as eluents; ir (potassium bromide): 1705 $cm^{-1}$ (C=O); nmr (deuteriochloroform): δ 7.4 (5H, s, arom), 5.5 (1H, t, =CH), 5.3 (1H, s, =CH), 3.8 (3H, s, -$OCH_3$), 2.7 (2H, d, =CH-$CH_2$-).

4-*p*-Toluenesulfonyloxy-6-phenyl-5,6-dihydro-2*H*-pyran-2-one (**13**).

*p*-Toluenesulfonyl chloride (6.35 g, 0.033 mole) was added to compound **11** (3.8 g, 0.02 mole) in 130 ml of dry tetrahydrofuran at −20°. Pyridine (1.6 ml, 0.02 mole) was added dropwise to the stirred reaction mixture. After stirring for 6 hours at 20°, the solvent was removed under vacuum and the residue was dissolved in 100 ml of ethyl ether. The ethereal solution was washed with water (100 ml), 5% hydrochloric acid (50 ml) and water (50 ml). After drying, the solvent was evaporated and the residue was chromatographed on silica gel using hexane and methylene chloride as eluents. The compound was further purified by recrystallization from diethyl ether/ethyl acetate yielding 1.57 g of **13**, mp 79-81°; ir (potassium bromide): 1720 $cm^{-1}$ (C=O), 1360 and 1180 (S=O); nmr (deuteriochloroform): δ 7.85 (4H, d, arom), 7.45 (4H, d, arom), 5.9 (1H, s, =CH), 5.5 (1H, t, =CH), 3.8 (2H, d, =CH-$CH_2$-), 2.5 (3H, s, -$CH_3$).

Acknowledgements.

We thank the National Institutes of Health for generous financial support (NHLBI grant HL30798-02).

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