ference between the overall vehicle response and the mean response for the experiments containing the particular treatment. For example, for those treatments which appear only in experiments with lower than average responses, the adjusted harmonic mean is higher than the corresponding mean cell response; for those treatments which appear only in experiments with higher than average responses, the adjusted mean is lower than the corresponding cell means.

Each drug/dose combination was compared to the vehicle control response, the response for the appropriate dose of the standard reference compound, ciglitazone, and the lead compound 6. To adjust for multiple comparisons, the 20 and 75 mg/kg doses of each compound were grouped into one of the 12 different sets (see text). A two-sided significance level ($p < 0.05$) based on a Bonferroni adjustment[17] of multiple comparisons was calculated for each set of compounds by dividing 0.05 (nominal significance level) by the number of comparisons being made within the set. The Bonferroni adjustment was made separately for the comparisons to vehicle control (results in Tables I–IV) ciglitazone and 6 (see supplementary data). A comparison was declared significant (indicated by *) if the observed p value was less than the Bonferroni-adjusted p value for that group of compounds relative to vehicle control.

Acknowledgment. We thank Bruce Hofmann (NMR), Marie Politowski (IR, CHN, log p), Charles Kuhlman (HPLC) and their staffs for obtaining the analytical data and Cheryl Delfino, who prepared the manuscript.

Supplementary Material Available: A complete listing of the 12 groups of compounds which constitute Tables I–IV, harmonic and adjusted harmonic means for each compound at 75 and 20 mg/kg doses, and the relevant Bonferroni p values (14 pages). Ordering information is given on any current masthead page.

Synthesis and Antibacterial Activity of Some Novel 6-Methyl- and 6-Propenyl-Substituted Carbapenems

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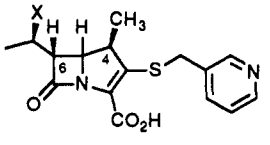
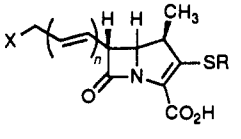
The synthesis and antibacterial activity of a number of 6-methyl- and 6-propenyl-substituted carbapenems is described. The 6-(hydroxymethyl)- and 6-(aminomethyl)carbapenems possessed more potent antibacterial activity in vitro than their respective 6-(1′(*R*)-hydroxyethyl) or 6-(1′(*R*)-aminoethyl) counterparts. However, because of reduced stability, the 6-(aminomethyl)carbapenem was found to be inactive in vivo. All 6-hydroxypropenyl or 6-aminopropenyl derivatives that were prepared were less active than their respective 6-heteroethyl-substituted analogues.

Introduction

Replacement of the hydroxyl function in the 6-(1′-hydroxyethyl) side chain of carbapenems of the thienamycin class (e.g. 1), with an amino group results in compounds (e.g. 2[1]) which possess improved activity against Gram-negative bacteria, notably *Pseudomonas aeruginosa*. A part of the work undertaken to elucidate the structure–activity relationship of this group of compounds involved efforts to prepare a 6-aminomethyl derivative (4, $n = 0$)[2] and its vinylogue, the 6-(3′-aminoprop-1′-enyl) derivative (4, $n = 1$). This work, along with the synthesis and antibacterial activity of some novel 6-(hydroxypropenyl) derivatives [e.g. 3 ($n = 1$)] is described.



1: X = OH
2: X = NH_2

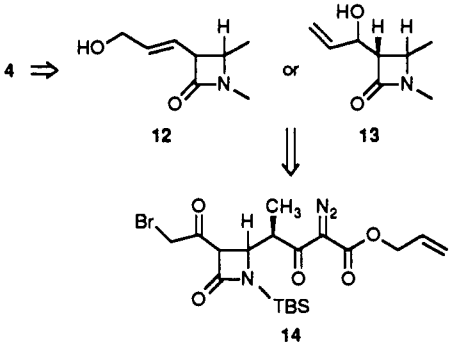


3: X = OH
4: X = NH_2

Chemistry

The preparation of the 6-(hydroxymethyl)carbapenem (5) has already been reported.[3] It was felt that this compound could be converted to the 6-aminomethyl analogue (7) using methodology first described by Bachi[4] and developed for use in the synthesis of the present series of compounds by Banville.[1] This involved the Mitsunobu[5,6] reaction of the alcohol 5 with hydrazoic acid to give the azide 9. Reaction of the azide with triphenylphosphine

Scheme I[a]



[a] TBS = *tert*-butyldimethylsilyl.

† Current address, Bristol-Myers Squibb Pharmaceutical Research Institute, Wallingford, CT.

‡ Wallingford, CT location.

(1) Banville, J.; Rémillard, R.; Fung-Tomc, J.; Desiderio, J.; Michel, A.; Mēnard, M.; Kessler, R.; Partyka, R. 6-(1-Aminoalkyl)-1β-methyl carbapenems: synthesis and in vitro and in vivo activities. 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, GA, Oct 21–24, 1990; Abstract 902. A manuscript describing this work is in preparation. 6-(Aminoethyl)carbapenems have been previously described. However, they lacked a 4β-methyl substituent and were described as being extremely labile: Corbett, D.; Coulton, S.; Southgate, R. Inversion of Configuration at C-8 in the Olivanic Acids: Conversion into the Thienamycins and Other Novel Derivatives. *J. Chem. Soc., Perkin Trans. 1* **1982**, 3011–3016.

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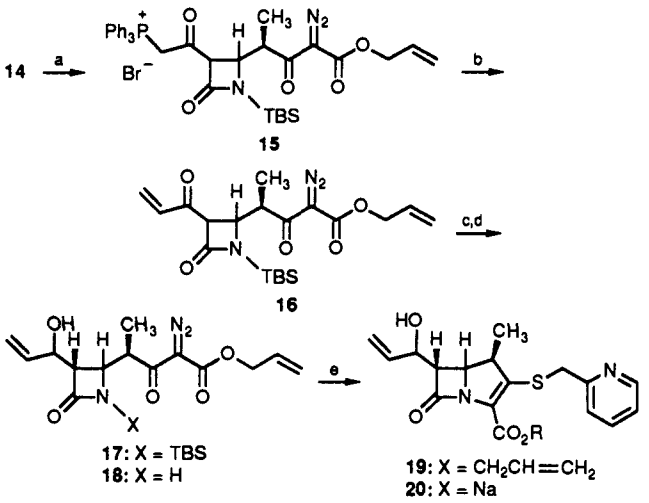
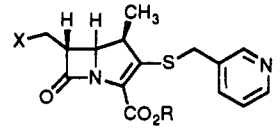
Scheme II[a]



[a] (a) Triphenylphosphine, $CHCl_3$ (83%). (b) Formalin, aqueous $NaHCO_3$, CH_2Cl_2 (63%). (c) $NaBH_4$, $CeCl_3 \cdot 7H_2O$, MeOH, −78 °C. (d) 10% HCl, MeOH (77%). (e) Rhodium(II) octanoate dimer, ethyl acetate–hexane (1:1), reflux; diphenyl chlorophosphate, Hunig's base, CH_3CN; trimethylsilyl chloride, Hunig's base; 2-(mercaptomethyl)pyridine Hunig's base, acetic acid, tetrahydrofuran–water (1:1) (48%).

gave the phosphinimine **10** which was treated directly with *p*-nitrobenzaldehyde to give the Schiff base **11**. Palladium-catalyzed deprotection[7] of the carboxyl group followed by hydrolysis of the Schiff base afforded the 6-(aminomethyl)carbapenem **8**.

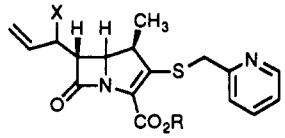


5: X = OH, R = $CH_2CH{=}CH_2$
6: X = OH, R = Na
7: X = NH_2, R = $CH_2CH{=}CH_2$
8: X = NH_2, R = H
9: X = N_3, R = $CH_2CH{=}CH_2$
10: X = $NPPh_3$, R = $CH_2CH{=}CH_2$
11: X = NCH-(*p*-NO_2)Ph, R = $CH_2C{=}CH_2$

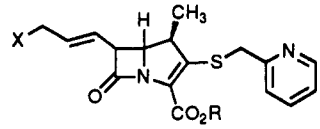
It was thought that the vinylogue of the 6-aminomethyl compound (**4**) could be obtained from the α-bromo ketone **14**, a useful precursor for the synthesis of novel 6-substituted carbapenems.[8] This was to be accomplished in analogous fashion to the transformation of **5** to **8** via the intermediates **12** or **13** (Scheme I).

Reaction of the α-bromoketone **14** with triphenylphosphine gave the phosphonium salt **15** (Scheme II). This was treated with sodium bicarbonate in the presence of aqueous formaldehyde to give α,β-unsaturated ketone **16**. Sodium borohydride reduction of the ketone was accomplished at −78 °C using the Luche[9] procedure. The reduction was not stereoselective but gave a 1:1 mixture of alcohol isomers, **17**, with remarkable regioselectivity. The *N*-silyl group was then removed and the resulting mixture of isomers, **18**, was converted to the mixture of isomeric carbapenems, **19**, using the Merck process.[10] At this point, it was found that one of the alcohol isomers of **19** (diastereomer A) could be cleanly obtained from the mixture by crystallization. Careful chromatography of the mother liquor allowed for the separation of the other isomer of **19** (diastereomer B) only slightly contaminated with its isomer. For the sake of comparison, both isomers were individually deprotected and the antibacterial activity of the resulting carboxylate salts **20** (diastereomers A and B) was determined.

The allylic transposition of the hydroxy group of **19** was effected using the procedure of Reich.[11] Thus the mixture of isomeric allylic alcohols **19** was converted[12] to a mixture of isomeric allyl selenides **21**. These were treated directly with peracid followed by piperidine to give the allyl alcohol **24**. Again, a portion of this material was deprotected and the antibacterial activity of the carboxylate salt **25** was determined. The ^1H NMR spectrum of this compound (**25**) showed a large vicinal coupling constant (15.6 Hz) for the olefinic protons. This value is consistent with the depicted *E*-olefin geometry.



21: X = SePh, R = $CH_2CH{=}CH_2$
22: X = OAc, R = $CH_2CH{=}CH_2$
23: X = OH, R = Na



24: X = OH, R = $CH_2CH{=}CH_2$
25: X = OH, R = Na
26: X = N_3, R = $CH_2CH{=}CH_2$
27: X = N_3, R = Na
28: X = NHPh, R = Na

Efforts were made to convert **19** into an allylic amine (**4**, *n* = 1). The Mitsunobu reaction of the mixture of isomeric allylic alcohols **19** with hydrazoic acid gave a mixture of isomeric allyl azides which was isolated as a group by rapid chromatography. Attempts to separate individual isomers were unsuccessful since equilibration[13] to the thermodynamically more stable 6-(3′-azidoprop-1′-enyl) isomer **26** was too rapid. The resulting mixture of azides, consisting predominantly of **26**,[14] was then carried through the sequence of reactions that had been used

(2) Mastalerz, H.; Banville, J.; Ruediger, E.; Fung-Tomc, J.; Desiderio, J.; Mênard, M.; Kessler, R.; Partyka, R. 6-(1-Aminoalkyl)-carbapenems: The effect of methyl substitution in the sidechain at position 6 on in vitro and in vivo antibacterial activity. 30th Interscience Conference on Antimicrobial Agents and Chemotherapy, Atlanta, GA, Oct 21–24, 1990; Abstract 903.
(3) Ruediger, E.; Solomon, C. Novel Conversion of a 3-(1-Hydroxyethyl)azetidinone to a 3-Hydroxymethylazetidinone: A Synthesis of 6-Hydroxymethyl-1β-methylcarbapenem. *J. Org. Chem.* **1991**, *56*, 3183–3187.
(4) Bachi, M.; Vaga, J. Phosphinimines as Useful Intermediates in the Synthesis of 3-(Acylamino)-β-lactams. *J. Org. Chem.* **1979**, *44*, 4393–4396.
(5) Mitsunobu, O. The Use of Diethyl Azodicarboxylate and Triphenylphosphine in Synthesis and Transformation of Natural Products. *Synthesis* **1981**, 1–28.
(6) Loibner, H.; Zbiral, E. *Helv. Chim. Acta* **1976**, *59*, 2100–2113.
(7) Jeffrey, P.; McCombie, S. Homogeneous, Palladium(0)-catalyzed Exchange Deprotection of Allylic Esters, Carbonates, and Carbamates. *J. Org. Chem.* **1982**, *47*, 587–590.
(8) Bouthillier, G.; Mastalerz, H.; Mênard, M. The Synthesis of an Asparenomycin Analog. *Tetrahedron Lett.* **1991**, *32*, 1023–1026.
(9) Luche, J. L. Lanthanides in Organic Chemistry. 1. Selective 1,2 Reductions of Conjugated Ketones. *J. Am. Chem. Soc.* **1978**, *100*, 2226–2227.
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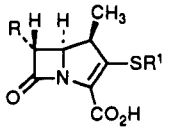
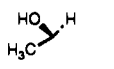
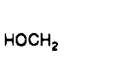
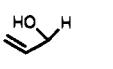
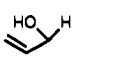
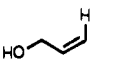
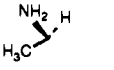
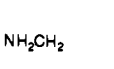
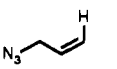
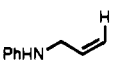
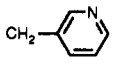
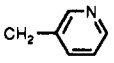
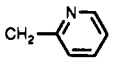
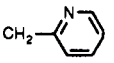
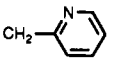
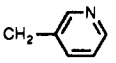
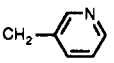
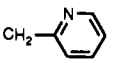
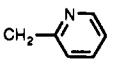
(11) Reich, H.; Schroeder, M.; Reich, I. Organoselenium Chemistry. Preparation of Allyl and Homoallyl Amines by Aminomethylation of Phenylseleno-substituted Allyltin Reagents. *Israel J. Chem.* **1984**, *24*, 157–161.
(12) Grieco, P.; Gilman, S.; Nishizawa, M. Organoselenium Chemistry. A Facile One-Step Synthesis of Alkyl Aryl Selenides from Alcohols. *J. Org. Chem.* **1976**, *41*, 1485–1486.
(13) Gagneux, A.; Winstein, S.; Young, W. Rearrangement of Allylic Azides. *J. Am. Chem. Soc.* **1960**, *82*, 5956–5957.
(14) It is assumed that the geometry of the double bond in the 6-side chain is the thermodynamically more stable *E* isomer.

Table I. In Vitro Antibacterial Activity, Oral Therapeutic Efficacy, and Chemical Stability



		1	6	20	20	25	2	8	27	28
	R									
	R^1									
				diast. A	diast. B					
MIC[a]										
S. pneumoniae	A9585	0.016	0.002	0.016	0.13	0.13	4	0.5	0.03	–
S. pyogenes	A9604	0.016	0.004	0.25	0.56	0.06	4	0.5	0.03	–
S. faecalis	A20688	2	4	16	125	125	63	125	32	>64
S. aureus	A9537	0.016	0.008	0.06	0.25	1	2	0.5	0.06	0.25
E. coli	A15119	0.016	0.016	0.25	1	8	0.03	0.008	8	32
K. pneumoniae	A9664	0.13	0.06	0.5	4	16	0.13	0.03	16	64
E. cloacae	A9659	0.13	0.06	1	4	32	0.06	0.016	32	64
P. vulgaris	A21559	0.03	0.13	0.03	0.25	8	0.016	0.016	8	64
P. retteri	A22424	16	0.5	2	2	16	2	0.25	32	64
P. aeruginosa	A9843	125	125	125	125	125	1	1	125	64
PD_{50}[b]		17	7	–	–	–	8	>25	–	–
stability[c]		65	45	74	72	21	3	1.5	21	35

[a] Minimum inhibitory concentrations, lowest antibiotic concentration inhibiting bacterial growth (μg/mL), were determined by the microdilution method using Nutrient-Broth and final bacterial concentrations of 5.0×10^5 CFu/mL. *Streptococci* were tested using Todd–Hewitt Broth. Microtiter trays were incubated at 35 °C for 18 h. [b] The protective dose (po) for 50% of the animals tested; mg/kg per dose; mice were treated twice, at 0 and 2 h after infection with E. coli. [c] Half-life determined by UV spectrophotometry for ca. 10^{-4}-M solutions in pH 7.4 phosphate buffer (0.07 M) at 37 °C; h.

to convert **9** into **8**. However, in this case the reaction mixtures were much more complex (TLC analysis) and no characterizable, β-lactam-containing products could be isolated. Similarly, the allyl azide carboxylate salt **27**,[14] obtained in one step by the palladium-catalyzed reaction[15] of the acetate derivatives **22** of the isomeric allylic alcohols **19** with aqueous sodium azide, simply afforded unidentifiable decomposition products on attempted reduction of the azide function. An allyl amine, albeit the *N*-phenyl derivative **28**, was prepared by taking advantage of the facile palladium-catalyzed reaction[7] of allyl esters with aromatic amines. Reaction of the allyl acetates **22** with an excess of aniline in the presence of a palladium catalyst gave the deprotected *N*-phenylamine **28** directly.

Biological Results and Discussion

Data on antibacterial activity and chemical stability of the compounds that were prepared together with data[1] for two reference compounds, **1** and **2**, is listed in Table I.

Of the compounds bearing a hydroxyl substituent in the 6-side chain, the 6-hydroxymethyl compound **6** possessed a slightly better spectrum of in vitro antibacterial activity than the 6-(1′(*R*)-hydroxyethyl) derivative **1**. As well, in spite of comparable MIC's, **6** was found to be more effective than **1** against *Escherichia coli* in systemically infected mice. The allylic alcohols **20** (diastereomers A and B) possessed overall reduced levels of antibacterial activity[16] relative to **6**. This decrease in activity was even more pronounced in the case of the allylic alcohol **25**.

It is interesting to note that of the two isomeric allylic alcohols **20**, diastereomer A possessed more potent antibacterial activity than diastereomer B. In the case of thienamycin, it has been observed[17] that the 6-(1′(*R*)-hydroxyethyl) compound is more active than its 6-(1′-(*S*)-hydroxyethyl) isomer. It therefore seems likely that the allylic alcohol **20** (diastereomer A) is the 6-(1′(*R*)-hydroxyprop-3′-enyl) isomer.

For the compounds bearing a nitrogen substituent in the 6-side chain, the 6-aminomethyl compound **8** was again found to possess the best spectrum of antibacterial activity.

(15) Murahashi, S.; Taniguchi, Y.; Imada, Y.; Tanigawa, Y. Palladium(0)-catalyzed Azidation of Allyl Esters. Selective Synthesis of Allyl Azides, Primary Allylamines, and Related Compounds. *J. Org. Chem.* **1989**, *54*, 3292–3303.

(16) It has been our experience that carbapenems bearing a 3-(pyridinylmethylthio) substituent which are identical in all respects except for the position of the pyridine nitrogen atom have very similar antibacterial activity. Hence, it is felt that comparisons which are made between compounds such as **6** and **20** are valid.

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However, it was essentially inactive in vivo against *E. coli*. This is presumably a result of its poor stability. The allyl azide **27** was comparable in activity with the allylic alcohol **25** while the *N*-phenyl derivative **28** was essentially devoid of activity.

In summary, the 6-(aminomethyl)- and 6-(hydroxymethyl)carbapenems, **6** and **8**, were found to be the more potent antibacterials in vitro. They are however chemically less stable than their hydroxy or aminoethyl counterparts. In the case of the 6-aminomethyl compound **8**, this is apparently responsible for its lack of activity in vivo.

Experimental Section

Melting points were taken on a Gallenkamp apparatus and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 141 polarimeter. The UV and IR spectra were recorded on Hewlett-Packard 8451 and Perkin-Elmer 781 spectrophotometers, respectively. The ^{1}H NMR spectra were obtained on a Bruker AC 200 or AMX 400 instrument using tetramethylsilane or sodium 3-(trimethylsilyl)propionate-2,2,3,3,-d_4 as the internal standard. High-resolution FAB-MS (nitrobenzyl alcohol matrix) were obtained with a Kratos MS50 mass spectrometer. Thin-layer chromatography was performed with Merck Art 5719 Kieselgel 60 F_{254} plates. Medium-pressure column chromatography employed Merck Art 9385 Kieselgel 60 (230–400 mesh) using ethyl acetate–hexane as eluent, or Waters C_{18} BondaPak using acetonitrile–water mixtures as eluent. The purity of the final carbapenems was determined by analytical HPLC using a Waters C_{18} Bondapak column (10-μm particle size, 3.8 mm × 30 cm) with a Waters 481 LC spectrophotometric detector. Where necessary, solvents were dried and reactions were conducted under an Ar atmosphere.

(4*R*,5*S*,6*S*)-6-(Aminomethyl)-4-methyl-7-oxo-3-[(pyridin-3-ylmethyl)thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid (8). A solution of allyl (4*R*,5*S*,6*S*)-6-(hydroxymethyl)-4-methyl-7-oxo-3-[(pyridin-3-ylmethyl)thio]-1-azabicyclo[3.2.0]-hept-2-ene-2-carboxylate[3] (**5**) (505 mg, 1.40 mmol), triphenylphosphine (367 mg, 1 equiv), and hydrazoic acid (1.0 mL, 1.4 M in toluene, 1 equiv) in dry tetrahydrofuran (15 mL) was cooled in an ice bath. Diisopropyl azodicarboxylate (280 μL, 1 equiv) was added, and the reaction was left stirring for 10 min. The solvent was removed, and the residual oil was chromatographed to afford allyl (4*R*,5*S*,6*S*)-6-(azidomethyl)-4-methyl-7-oxo-3-[(pyridin-3-yl)methyl)thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (**9**) as an oil (368 mg, 68%): ^{1}H NMR ($CDCl_3$) δ 1.26 (d, J = 7.3 Hz, 3 H), 3.33 (dq, J = 8.1, 7.3 Hz, 1 H), 3.43 (m, 1 H), 3.71 (dq, δ_A = 3.75, δ_B = 3.64, J_{AB} = 12.7 Hz, J_{AX} = 7.9 Hz, J_{BX} = 3.7 Hz, 2 H), 4.02 (dd, J = 8.1, 2.5 Hz, 1 H), 4.06 (q, δ_A = 4.08, δ_B = 4.04, J_{AB} = 13.0 Hz, 2 H), 4.62–6.02 (m, CH_2CH=CH_2), 7.24–8.57 (m, arom, 4 H). Triphenylphosphine (238 mg, 1.03 equiv) was added to a stirred solution of the azide **9** (339 mg, 0.88 mmol) in dry benzene (0.5 mL). After 2 h at room temperature, *p*-nitrobenzaldehyde (133 mg, 1 equiv) was added and the reaction was left stirring for 2 h. The solvent was removed, and the residual oil was taken up in CH_2Cl_2 (5 mL). A solution of potassium 2-ethylhexanoate (1.76 mL, 0.5 M in ethyl acetate, 1 equiv) and tetrakis(triphenylphosphine)palladium(0) (31 mg, 0.03 equiv) were added. After 10 min, a phosphate buffer (10 mL, 0.2 M, pH 6.0) was added and the resulting mixture was left stirring vigorously for 0.5 h in an ice bath. The organic phase was separated and further washed with buffer (2 × 5 mL). The combined aqueous phases were applied directly onto a reverse-phase silica gel column. This was eluted with water followed by CH_3CN–water mixtures (up to CH_3CN–H_2O = 1:4). Lyophilization of the appropriate fractions afforded the amino acid **8** (124 mg, 44%) as a fluffy, off-white solid: IR (KBr disk) ν 3420, 1760, 1600 cm^{-1}; UV (phosphate buffer, 0.05 M, pH 7.4) λ_{max} 304 nm (ϵ = 7500); ^{1}H NMR (400 MHz, D_2O) δ 1.17 (d, J = 7.2 Hz, 3 H), 3.36 (dq, J = 7.2, 8.9 Hz, 1 H), 3.46 (dd, J = 8.5, 13.4 Hz, 1 H), 3.50 (dd, J = 6.2, 13.4 Hz, 1 H), 3.67 (ddd, J = 2.4, 8.6, 6.2 Hz, 1 H), 4.05 (dd, J = 8.9, 2.4 Hz, 1 H), 4.06 (d, J = 14.0 Hz, 1 H), 4.18 (d, J = 14.0 Hz, 1 H), 7.47–8.54 (m, arom, 4 H); HRMS calcd for $C_{15}H_{18}N_3O_3S$ (M + 1)$^+$ 320.1069, found 320.1079; HPLC purity, 94%.

Allyl (4*R*,5*S*,6*S*)-6-[(1′*ξ*)-1′-Hydroxyprop-2′-enyl]-4-methyl-7-oxo-3-[(pyridin-2-ylmethyl)thio]-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate, Diastereomers A and B (19). A solution of (3*S*,4*R*)-3-(2′-bromo-1′-oxoethyl)-1-(*tert*-butyldimethylsilyl)-4-[1′(*R*)-methyl-3′-[(allyloxy)carbonyl]-2′-oxo-3′-diazopropyl]azetidin-2-one (**14**)[8] (6.19 g, 12.7 mmol) in $CHCl_3$ (15 mL) under Ar was cooled in an ice bath. Triphenylphosphine (3.34 g, 1 equiv) was added in portions over 10 min, and the reaction was left at 4 °C for 18 h. Most of the solvent was removed under vacuum, and the residual oil was triturated with diethyl ether followed by a mixture of diethyl ether–hexane (1:1). This left the phosphonium salt **15** as a pale yellow powder (7.93 g, 83%). A saturated aqueous solution of $NaHCO_3$ (20 mL) was added to a vigorously stirred solution of the salt **15** (11.6 g, 15.5 mmol) and formaldehyde (10 mL, 37 wt % aqueous solution) in CH_2Cl_2 (50 mL) at room temperature. After 15 min, the organic phase was removed, washed with water, and dried (Na_2SO_4), and the solvent was removed. Chromatography afforded (3*S*,4*R*)-1-(*tert*-butyldimethylsilyl)-3-(1′-oxoprop-2′-enyl)-4-[1′(*R*)-methyl-3′-[(allyloxy)carbonyl]-2′-oxo-3′-diazopropyl]azetidin-2-one (**16**) (4.07 g, 63%) as a yellow oil: IR (film) 2140, 1750, 1720, 1675, 1655 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.12 (s, 3 H), 0.22 (s, 3 H), 0.91 (s, 9 H), 1.16 (d, J = 7.0 Hz, 3 H), 4.14 (dq, J = 7.0, 4.6 Hz, 1 H), 4.24 (dd, J = 4.5, 2.8 Hz, 1 H), 4.73 (m, 2 H), 5.19 (d, J = 2.8 Hz, 1 H), 5.34 (m, 2 H), 5.93 (m, 1 H), 6.05–7.26 (m, 3 H). The ketone **16** (4.07 g, 9.7 mmol) and $CeCl_3 \cdot 7H_2O$ (3.63 g, 1.0 equiv) were dissolved in MeOH (100 mL) under Ar. This stirred solution was then cooled to −78 °C and powdered $NaBH_4$ (370 mg, 1.0 equiv) was added. After 5 min, acetone (8 mL) was added and the low-temperature bath was removed. Upon warming to room temperature the reaction was diluted with brine (15 mL) and ethyl acetate and a little water were added to give a two-phase solution. The organic phase was separated, washed with brine, and dried (Na_2SO_4). The solvents were removed, and the residual oil was taken up in methanol (15 mL). The resulting solution was cooled in an ice bath, and an aqueous solution of HCl (5 mL, 10%) was added. The bath was removed after 1 h and after an additional 1.5 h sufficient solid $NaHCO_3$ was added to neutralize the reaction. Most of the solvent was removed, and the residual material was partitioned between ethyl acetate and a little water. The organic phase was separated and dried (Na_2SO_4) and the solvent removed. The residual oil was chromatographed to afford a chromatographically homogeneous mixture (1:1) of alcohol diastereomers **18** as a white solid (2.29 g, 77%). A solution of this mixture of diastereomers (630 mg, 2.04 mmol) in ethyl acetate–hexane (48 mL, 1:1) was brought to a reflux. Rhodium(II) octanoate (30 mg) was carefully added, and the reaction was left at reflux for 5 min. After the reaction mixture was allowed to cool to room temperature, the solvent was removed. The residual oil was dissolved in dry CH_3CN (15 mL) and this was cooled in an ice bath. Diphenyl chlorophosphate (0.44 mL, 1.05 equiv), Hunig's base (0.37 mL, 1.05 equiv), and a pinch of (dimethylamino)pyridine was added. After 1 h, this was followed by trimethylsilyl chloride (0.34 mL, 1.3 equiv) and Hunig's base (0.44 mL, 1.3 equiv). After another 0.5 h, 2-(mercaptomethyl)pyridine (0.30 mL, 1.3 equiv) and Hunig's base (0.37 mL, 1.05 equiv) were added. The reaction was left for 3 h whereupon it was diluted with cold ethyl acetate (120 mL) and washed with water followed by brine. The organic phase was dried (Na_2SO_4), and the solvents were removed. The residual oil was taken up in tetrahydrofuran–water (6 mL, 1:1), and acetic acid (0.24 mL) was added. After 1.5 h, solid $NaHCO_3$ was added to neutralize the reaction mixture, most of the solvents were removed, and the residual material was extracted with ethyl acetate. The extracts were dried (Na_2SO_4), and the solvent was removed. Rapid chromatography afforded the mixture of allylic alcohol diastereomers **19**. These were dissolved in ethyl acetate and hexane was added to the point of incipient turbidity. The crystals which formed (90 mg) were the pure alcohol **19** (diastereomer A): mp 129–130 °C; $[\alpha]^{23}_D$ +82° (c 2.3, $CHCl_3$); IR (KBr disk) 3380, 1755, 1700 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.22 (d, J = 7.3 Hz, 3 H), 3.35 (dd, J = 5.8, 2.6 Hz, 1 H), 3.64 (dq, J = 7.3, 9.2 Hz, 1 H), 4.06 (d, J = 14.2 Hz, 1 H), 4.12 (dd, J = 9.2, 2.6 Hz, 1 H), 4.27 (d, J = 14.2 Hz, 1 H), 4.58–4.86 (m, 3 H), 5.20–5.48 (m, 4 H), 5.85–6.05 (m, 2 H), 7.18–8.52 (m, arom, 4 H). Anal. ($C_{20}H_{22}N_2O_4S$) C, H, N. The mother liquor was chromatographed (silica gel/hexane–benzene–2-propanol = 8:1:1) to give a further 62 mg of diastereomer A, 78 mg of a mixture of alcohol diastereomers A and B, and 150 mg of slightly impure alcohol diaste-

reomer B as an oil: IR (film) 3400, 1700, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (d, J = 7.3 Hz, 3 H), 3.41 (dd, J = 6.2, 2.6 Hz, 1 H), 3.65 (dq, J = 7.3, 9.1 Hz, 1 H), 4.01 (dd, J = 2.6, 9.1 Hz, 1 H), 4.05 (d, J = 14.2 Hz, 1 H), 4.26 (d, J = 14.2 Hz, 1 H), 4.50 (t, J = 6.2 Hz, 1 H), 4.59–4.87 (m, 2 H), 5.20–5.48 (m, 4 H), 5.86–6.06 (m, 2 H), 7.16–8.51 (m, arom, 4 H). The combined yield of alcohol diastereomers was 48%.

Sodium (4*R*,5*S*,6*S*)-6-(1′*ξ*-Hydroxyprop-2′-enyl)-4-methyl-7-oxo-3-[(pyridin-2-ylmethyl)thio]-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (20) (Diastereomers A and B). To a solution of the allyl alcohol 19 (diastereomer A) (88 mg, 0.23 mmol) and *N*-methylaniline (61 μL, 2.5 equiv) in dry tetrahydrofuran (1 mL) under Ar was added tetrakis(triphenylphosphine)palladium(0) (13 mg, 0.05 equiv) and triphenylphosphine (13 mg, 0.20 equiv). After 1 h, water (3 mL) and an aqueous solution of $NaHCO_3$ (0.41 mL, 0.5 M) were added. Most of the organic solvent was removed under high vacuum, and the resulting suspension was extracted with ethyl acetate. The aqueous phase was applied onto a reverse-phase column and gradient elution (water to CH_3CN–water = 1:4) followed by lyophilization of the desired fractions afforded the product 20 (diastereomer A) as a tan-colored powder (32 mg, 38%): IR (KBr disk) 3400, 1750, 1595 cm^{-1}; UV (phosphate buffer, 0.05 M, pH 7.4) 304 nm (ϵ = 9000); ^1H NMR (D_2O) δ 1.10 (d, J = 7.2 Hz, 3 H), 3.33 (dq, J = 7.2, 9.2 Hz, 1 H), 3.52 (dd, J = 2.5, 5.8 Hz, 1 H), 4.03 (dd, J = 2.5, 9.2 Hz, 1 H), 4.09 (d, J = 14.0 Hz, 1 H), 4.22 (d, J = 14.0 Hz, 1 H), 4.58 (t, J = 5.8 Hz, 1 H), 5.26–6.02 (m, 3 H), 7.33–8.47 (m, arom, 4 H); HRMS calcd for $C_{17}H_{18}N_2O_4SNa$ $(M + 1)^+$ 369.0885, found 369.0879; HPLC purity, 98%. Similar treatment of the allyl alcohol 19 (diastereomer B) afforded the salt 20 (diastereomer B) as a tan-colored powder (51%): IR (KBr disk) 3420, 1750, 1590 cm^{-1}; UV (phosphate buffer, 0.05 M, pH 7.4) 304 nm (ϵ = 7,700); ^1H NMR (D_2O) δ 1.12 (d, J = 7.2 Hz, 3 H), 3.33 (dq, J = 7.2, 9.0 Hz, 1 H), 3.55 (dd, J = 2.4, 5.6 Hz, 1 H), 3.99 (dd, J = 9.0, 2.4 Hz, 1 H), 4.09 (d, J = 14.1 Hz, 1 H), 4.23 (d, J = 14.1 Hz, 1 H), 5.26–6.06 (m, 3 H), 7.33–8.47 (m, arom, 4 H); HRMS calcd for $C_{17}H_{18}N_2O_4SNa$ $(M + 1)^+$ 369.0885, found 369.0893; HPLC purity, 93%.

Sodium (4*R*,5*S*,6*R*)-6-(3′-Hydroxyprop-1′(*E*)-enyl)-4-methyl-7-oxo-3-[(pyridin-3-ylmethyl)thio]-1-azabicyclo-[3.2.0]hept-2-ene-carboxylate (25). Tri-*n*-butylphosphine (143 μL, 1.2 equiv) was added to a solution of the mixture of diastereomeric allylic alcohols 19 (166 mg, 0.43 mmol) and *o*-nitrophenyl selenocyanate (110 mg, 1.1 equiv) in dry tetrahydrofuran under Ar. After 0.5 h, the solvent was removed and the residual oil was chromatographed to afford the selenide 21 as an impure mixture of diastereomers (112 mg, ca. 0.20 mmol). A solution of this mixture in CH_2Cl_2 (5 mL) was cooled to −35 °C and *m*-chloroperbenzoic acid (85 mg, 80%, 2 equiv) was added. After 1 min, piperidine (38 μL, 2 equiv) was added and after an additional 20 min, the reaction was allowed to warm to ice bath temperature. Phosphate buffer (5 mL, 0.1 M, pH 7.0) was then added with vigorous stirring and the aqueous phase was separated and extracted with additional CH_2Cl_2. The combined organic phases were dried (Na_2SO_4), and the solvent was removed. Chromatography [silica gel/cold (5 °C) ethyl acetate and then cold ethyl acetate–methanol = 9:1] afforded allyl (4*R*,5*S*,6*S*)-6-(3′-hydroxyprop-1′-enyl)-4-methyl-3-[(pyridin-2-ylmethyl)thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (24) as an oil (40 mg, 24%): IR (film) 3400, 1770, 1700 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.25 (d, J = 7.3 Hz, 3 H), 3.69 (dq, J = 7.3, 8.9 Hz, 1 H), 3.85 (dd, J = 2.4, 6.4 Hz, 1 H), 3.97 (dd, J = 2.4, 8.9 Hz, 1 H), 4.05 (d, J = 14.1 Hz, 1 H), 4.16 (m, 2 H), 4.25 (d, J = 14.1 Hz, 1 H), 4.60–4.86 (m, 2 H), 5.20–5.48 (m, 2 H), 5.76–6.02 (m, 3 H), 7.15–8.51 (m, arom, 4 H). A solution of the alcohol (40 mg, 0.10 mmol) and *N*-methylaniline (17 μL, 1.5 equiv) in dry tetrahydrofuran (2 mL) was transferred via cannula to a stirred, pale yellow solution of bis(dibenzylideneacetonato)palladium(0) (6 mg, 0.1 equiv) and triphenylphosphine (11 mg, 0.4 equiv) in dry tetrahydrofuran (2 mL) under Ar. After 5 min, the reaction was placed in an ice bath, and diethyl ether (4 mL) and water (4 mL) were added. The pH of the aqueous phase was adjusted to 7.0 by the addition of a few drops of saturated aqueous $NaHCO_3$. The organic phase was removed and extracted with additional water. The combined aqueous phases were placed under high vacuum to remove any organic solvent and the water was then removed by lyophilization. The residual brown glassy foam was then chromatographed (reverse-phase silica gel–water and then CH_3CN–water up to a 1:9 mixture) to give, after lyophilization, the sodium salt 25 as a tan-colored, fluffy solid (18 mg, 52%): IR (KBr disk) 3420, 1750, 1600 cm^{-1}; UV (phosphate buffer, 0.07 M, pH 7.4) 304 nm (ϵ = 9700); ^1H NMR (400 MHz, D_2O), δ 1.14 (d, J = 7.3 Hz, 3 H), 3.36 (dq, J = 8.7, 7.3 Hz, 2 H), 3.95 (dd, J = 2.3, 8.7 Hz, 1 H), 3.98 (dd, J = 2.3, 6.8 Hz, 1 H), 4.11 (d, J = 14.0 Hz, 1 H), 4.12 (dd, J = 5.2, 1.1 Hz, 2 H), 4.23 (d, J = 14.0 Hz, 1 H), 5.85 (ddt, J = 1.1, 6.8, 15.6 Hz, 1 H), 5.93 (dt, J = 5.2, 15.6 Hz, 1 H), 7.34–8.47 (m, arom, 4 H); HRMS calcd for $C_{17}H_{18}N_2O_4SNa$ $(M + 1)^+$ 369.0885, found 369.0893; HPLC purity, 95%.

Allyl (4*R*,5*S*,6*S*)-6-(3′-Azidopro-1′(*E*)-enyl)-4-methyl-3-[(pyridin-2-ylmethyl)thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (26), Impure. A solution of the mixture of isomeric allylic alcohols 19 (324 mg, 0.88 mmol), hydrazoic acid (0.44 mL, 2.0 M in toluene, 1.0 equiv), and triphenylphosphine (230 mg, 1.0 equiv) in dry tetrahydrofuran (9 mL) under Ar was cooled in an ice bath. Diisopropyl azodicarboxylate (177 μL, 97%, 1.0 equiv) was added, and the reaction was left for 15 min. The solvent was removed, and the residual oil was quickly chromatographed to isolate the two major products (R_f = 0.52 and 0.41, development with ethyl acetate–hexane = 3:1). Attempted preparative silica gel TLC separation (ethyl acetate–hexane = 1:1) resulted in the conversion of the two apparent components into a chromatographically homogeneous component (R_f = 0.41, as above). This component, obtained as an oil (188 mg), consisted predominantly of the allyl azide 26: IR (film) 2100, 1775, 1705 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.26 (d, 7.2 Hz, 3 H), 3.72 (dq, 7.2, 8.9 Hz, 1 H), 3.80 (d, J = 4.8 Hz, 2 H), 3.88 (dd, J = 5.9, 2.5 Hz, 1 H), 3.98 (dd, J = 8.9, 2.5 Hz, 1 H), 4.05 (d, J = 14.1 Hz, 1 H), 4.26 (d, J = 14.1 Hz, 1 H), 4.61–4.86 (m, 2 H), 5.21–5.49 (m, 2 H), 5.73–6.06 (m, 3 H), 7.16–8.52 (m, arom, 4 H).

Sodium (4*R*,5*S*,6*S*)-6-(3′-Azidoprop-1′-enyl)-4-methyl-3-[(pyridin-2-ylmethyl)thio]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (27). A solution of the mixture of isomeric allylic alcohols 19 (168 mg, 0.435 mmol) in a mixture of pyridine (1.0 mL) and acetic anhydride (0.5 mL) was left stirring at room temperature for 2 h. The mixture was then placed under high vacuum to remove the excess reagents. The residue was chromatographed to give the isomeric allylic acetates 22 as an oil (159 mg, 85%). A solution of NaN_3 (4.05 mL, 0.5 M in water, 2.2 equiv) and a solution of the isomeric allylic acetates (395 mg, 0.92 mmol) in tetrahydrofuran (5 mL) was added to a pale yellow solution of bis(dibenzylideneacetonato)palladium(0) (26 mg, 0.05 equiv) and triphenylphosphine (48 mg, 0.20 equiv) in tetrahydrofuran (5 mL) under Ar. After 1 h, diethyl ether (10 mL) and water (5 mL) were added. The organic phase was separated and washed with water (2 × 25 mL). The combined aqueous phases were put under high vacuum to remove any residual organic solvent and then applied onto a reverse-phase silica gel column. Gradient elution, beginning with water and ending with a mixture of water and CH_3CN (4:1) afforded, after lyophilization, the allyl azide 27 as a tan-colored, fluffy solid (216 mg, 62%): IR (KBr disk) 3400, 2100, 1750, 1600 cm^{-1}: UV (phosphate buffer, 0.07 M, pH 7.4) 340 nm (ϵ = 8700); ^1H NMR (D_2O) δ 1.16 (d, J = 7.2 Hz, 3 H), 3.39 (dq, J = 7.2, 8.5 Hz, 1 H), 3.88 (d, J = 5.2 Hz, 2 H), 3.95–4.07 (m, 2 H), 4.12 (d, J = 14.1 Hz, 1 H), 4.26 (d, J = 14.1 Hz, 1 H), 5.81–6.04 (m, 2 H), 7.34–8.48 (m, arom, 4 H); HRMS calcd for $C_{17}H_{17}N_5O_3SNa$ $(M + 1)^+$ 394.0950, found 394.0936; HPLC purity, 87%.

Sodium (4*R*,5*S*,6*S*)-4-Methyl-6-[3′-(phenylamino)prop-1′-enyl]-3-[(pyridin-2-ylmethyl)thio]-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (28). A mixture of isomeric allylic acetates 22 (355 mg, 0.83 mmol) and aniline (0.76 mL, 10 equiv) in dry tetrahydrofuran (5 mL) was transferred by cannula to a stirred, yellow solution of bis(dibenzylideneacetonato)palladium(0) (24 mg, 0.05 equiv) and triphenylphosphine (30 mg, 0.20 equiv) in dry tetrahydrofuran (1 mL) under Ar. After 1.5 h, diethyl ether (5 mL) and water (10 mL) were added and the pH of the aqueous phase was adjusted to 7.0 by the addition of a little saturated aqueous $NaHCO_3$ solution. The organic phase was separated and extracted with water (2 × 10 mL). The combined aqueous phases were placed under high vacuum to remove any residual organic solvent, and then the water was removed by lyophilization. This left a yellow powder which was chromato-

graphed on a reverse-phase column (gradient elution beginning with water and ending with CH_3CN–water = 1:4). Lyophilization of the desired fractions afforded the product **28** (111 mg, 32%) as a pale yellow, fluffy solid: IR (KBr disk) 3400, 1750, 1605 cm^{-1}; UV (phosphate buffer, 0.07 M, pH 7.4) 304 nm (ϵ = 8700); ^1H NMR (D_2O) δ 1.09 (d, J = 7.2 Hz, 3 H), 3.29 (quin, J = 7.2 Hz, 1 H), 3.76–3.91 (m, 4 H), 4.08 (d, J = 14.2 Hz, 1 H), 4.21 (d, J = 14.2 Hz, 1 H), 5.81–5.85 (m, 1 H), 6.83–8.45 (m, arom, 9 H); HRMS calcd for $C_{23}H_{23}N_3O_3SNa$ $(M + 1)^+$ 444.1358, found 444.1356; HPLC purity, 94%.

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Novel Benzothiophene-, Benzofuran-, and Naphthalenecarboxamidotetrazoles as Potential Antiallergy Agents[1]

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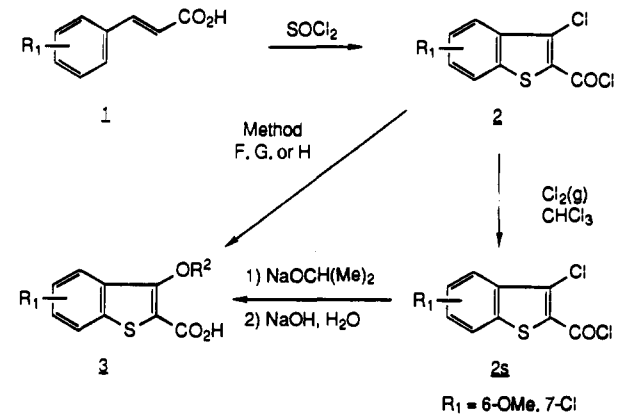
The synthesis and antiallergic activity of a series of novel benzothiophene-, benzofuran-, and naphthalenecarboxamidotetrazoles are described. A number of the compounds inhibit the release of histamine from anti-IgE stimulated basophils obtained from allergic donors. Optimal inhibition is exhibited in benzothiophenes with a 3-alkoxy substituent in combination with a 5-methoxy, 6-methoxy, or a 5,6-dimethoxy group. Compound **13c** (CI-959) also inhibited respiratory burst of human neutrophils and the release of mediators from anti-IgE-stimulated human chopped lung.

Current drug therapies for asthma have deficiencies, including, side effects, lack of compliance, lack of efficacy, lack of oral activity, and symptomatic relief without addressing the inflammatory component of the disease. For example, β-adrenergic stimulants only treat the symptoms; corticosteroids and theophylline have side effects that limit their use; and cromolyn sodium has to be taken by inhalation and is not universally effective. An orally active prophylactic agent with an antiinflammatory component would be a major advance in the therapy of asthma. The objective of our program was to discover compounds that would block the release of mediators from cells believed to play a fundamental role in the pathogenesis of allergic diseases. Mediators[2] that have been implicated in the pathogenesis of asthma or other allergic diseases include histamine, leukotrienes, platelet activating factor (PAF), and prostaglandins. Cells[3] postulated to play a key role in allergic diseases include mast cells and eosinophils, with basophils, neutrophils, and T-lymphocytes also being involved.

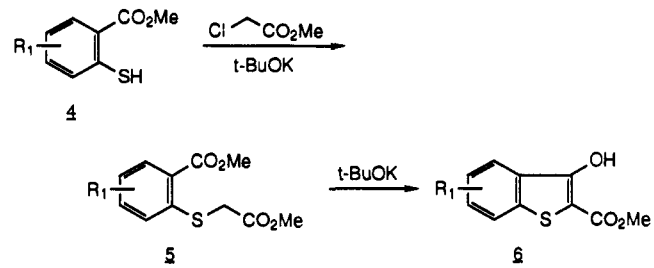
In searches for orally active cromolyn-type drugs, many potential antiasthmatic compounds[4] identified in programs using inhibition of histamine release from rat mast cells (passive cutaneous anaphylaxis test)[5] failed to show clinical efficacy.[6] The heterogeneity[7] of mast cells from different sources may be part of the reason for this failure.

To avoid these pitfalls our discovery effort focused on looking for compounds that would inhibit mediator release from a variety of human cells including mast cells and basophils. Because of their accessibility, we used human basophils for our initial screening to generate structure activity relationship (SAR) data. As a measure of mediator-release inhibition, compounds were tested for their ability to block the release of histamine from anti-IgE-stimulated human basophils. This test model, as developed by Lichtenstein and others,[8,9] has been employed in the evaluation of potential antiallergy compounds. Series of furoindoles,[10-12] indoles,[13] thiophenes, and related mo-

Scheme I



Scheme II. Method B



nocycles,[14] and triazolopyrimidines[15] have been identified previously as active in this screen.

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