(S)-4-Chloro-6-[[1,4-bis(benzyloxy)but-2-oxy]amino]-2,5-diformamidopyrimidine (38c). A solution of (S)-1,4-bis(benzyloxy)but-2-oxyamine (**37c**) (2.17 g, 7.2 mmol), 4,6-dichloro-2,5-diformamidopyrimidine (1.7 g, 7.2 mmol), and triethylamine (3 mL, 2.4 mmol) in dioxane (50 mL) was stirred at 100 °C for 1.5 h. The reaction was cooled to 20 °C and filtered, and the solvent was removed in vacuo. The residue was chromatographed on silica gel eluting with chloroform–methanol (30:1) to give, as an oil, **38c** (2.59 g, 71%): IR (film) ν_{max} 3241, 3062, 3031, 2924, 2863, 1695, 1635, 1587, 1567, 1496, 1477, 1454, and 1417 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.98 (2 H, m, CH_2CH_2O), 3.65 (4 H, m, 2 × CH_2OCH_2Ar), 4.28 (1 H, m, CH), 4.50 (2 H, s, OCH_2Ar), 4.55 (2 H, s, OCH_2Ar), 7.30 (10 H, m, ArH), 7.84 (1 H, s, HCO), and 8.5–9.5 (3 H, m, D_2O exchange leaves s at 9.30, HCO and 2 × NH). Anal. ($C_{24}H_{25}ClN_5O_5$) C, H; N: calcd 14.04; found 13.40.

(R)-4-Chloro-6-[[1,4-bis(benzyloxy)but-2-oxy]amino]-2,5-diformamidopyrimidine (38b) was obtained in an identical fashion from **37b**, in 83% yield; IR and ^1H NMR are identical with those of the S enantiomer.

(S)-6-Chloro-9-[1,4-bis(benzyloxy)but-2-oxy]-2-formamidopurine (39c). A solution of (S)-4-chloro-6-[[1,4-bis(benzyloxy)but-2-oxy]amino]-2,5-diformamidopyrimidine (**38c**; 0.7 g, 1.4 mmol) in diethoxymethyl acetate (15 mL) was stirred at 100 °C for 1.5 h. The solvent was removed in vacuo, the residue was dissolved in methanol (20 mL) and concentrated aqueous ammonia (0.5 mL) and stirred for 0.5 h at 20 °C. The solvent was removed in vacuo and the residue was coevaporated with methanol (3 × 20 mL). The residue was chromatographed on silica gel eluting with chloroform–methanol (60:1) to give, as an oil, **39c** (0.57 g, 84%): IR (film) ν_{max} 3226, 3168, 3120, 3089, 3063, 3030, 3007, 2920, 2864, 1704, 1612, 1576, 1504, 1476, 1454, and 1439 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 2.04 (2 H, m, CH_2CH_2O), 3.55–3.80 (4 H, m, 2 × CH_2OCH_2Ar), 4.30–4.55 (4 H, m, OCH_2Ar), 4.76 (1 H, m, CH), 7.05–7.40 (10 H, m, ArH), 8.64 (1 H, s, 8-H), 9.33 (1 H, s, HCO), and 11.21 (1 H, s, D_2O exchangeable, NH); HRMS calcd for $C_{24}H_{24}ClN_5O_4$ 481.1517, found 481.1515.

(R)-6-Chloro-9-[1,4-bis(benzyloxy)but-2-oxy]-2-formamidopurine (39b) was obtained in an identical fashion from **38b**, in 91% yield; IR and ^1H NMR are identical with those of the S enantiomer.

(S)-9-(1,4-Dihydroxybut-2-oxy)guanine (41c). A solution of (S)-6-chloro-9-[1,4-bis(benzyloxy)but-2-oxy]-2-formamidopurine (**39c**; 0.4 g, 0.8 mmol) in 80% formic acid (20 mL) was stirred at 100 °C for 1 h and cooled to 20 °C. To the solution was added 10% palladium-on-charcoal (200 mg), the mixture was hydrogenated under atmospheric pressure for 1 h and then filtered, and the solvent was removed in vacuo. The residue was dissolved in methanol (2 mL) and concentrated aqueous ammonia (2 mL) and stirred at room temperature for 0.5 h. The solvent was removed in vacuo and the residue was chromatographed on reverse-phase silica gel eluting with water, 5% methanol–water, and 10% methanol–water. The product eluted in the 5% and 10% methanol fractions and crystallized from these fractions, giving **41c** (0.12 g, 57%): mp 248 °C dec; $[\alpha]^{25}_D$ = −32.3° (c 0.16, H_2O); UV, IR, and ^1H NMR data are identical with those of **41a**; HRMS calcd for $C_9H_{13}N_5O_4$ 255.0968, found 255.0965. Anal. ($C_9H_{14}N_5O_4 \cdot 0.5H_2O$) C, H, N.

(R)-9-(1,4-Dihydroxybut-2-oxy)guanine (41b) was obtained in an identical fashion from **39b**, in 45% yield: $[\alpha]^{25}_D$ = +33.8° (c 0.24, H_2O); IR and ^1H NMR are identical with those of the S enantiomer. Anal. ($C_9H_{13}N_5O_4 \cdot 0.5H_2O$) C, H, N.

Acknowledgment. We thank T. H. Bacon and D. Sutton for the antiviral testing results and K. Cox, S. Humphries, and C. Patience for expert technical assistance in the biological studies.

Synthesis and Structure–Activity Relationships of Benzo[*b*]thienylallylamine Antimycotics

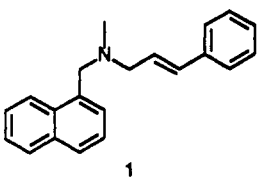
Peter Nussbaumer, Gabor Petranyi, and Anton Stütz*

Department of Dermatology, SANDOZ Forschungsinstitut, A-1235 Wien, Austria. *Received January 24, 1990*

Benzo[*b*]thiophene analogues of the allylamine antimycotic terbinafine (**2**) bearing the side chain at various positions and optionally substituted by halogen have been prepared and their antifungal activity studied. Derivatives bearing the side chain at positions 3, 4, or 7 are bioequivalents of **2**. Compounds containing the allylamine side chain at position 7, with a further substituent at position 3, showed significantly enhanced activity against *Candida albicans*, an effect which appears to be specifically linked only to this particular substitution pattern. 3-Chloro-7-benzo[*b*]thienyl derivative **7m** was found to be the most potent allylamine antimycotic identified so far. In general, substituted benzo[*b*]thiophenes can be used not only as potential equivalents of naphthalene in bioactive compounds but also as a tool to selectively modify biological activities.

Introduction

The allylamine derivatives are a new class of synthetic antifungal agents selectively inhibiting fungal squalene epoxidase.[1] The first representative, naftifine [(*E*)-*N*-methyl-*N*-(3-phenyl-2-propenyl)-1-naphthalenemethanamine, **1**], has recently become commercially available as



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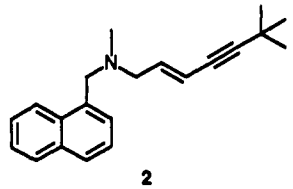

2

a topical antimycotic. Exploration of structure–activity relationships (SAR) on the basis of naftifine, together with new synthetic strategies, has led to the discovery of terbinafine (SF 86-327, Lamisil, **2**), the first pharmaceutical agent to contain an (*E*)-1,3-enyne structural element.[2] Terbinafine exhibits considerably higher activity than the original lead structure naftifine both in vitro and in vivo; it is also up to 1 order of magnitude more effective than clinical standards in various chemotherapeutic animal tests after oral or topical administration.[3,4] According to ad-

* To whom correspondence should be addressed.

(1) For recent reviews, see: (a) Stütz, A. *Antifungal Drugs. Ann. N.Y. Acad. Sci.* **1988**, *544*, 46–62. (b) Ryder, N. S. *Ibid.* 208–220. (c) Stütz, A.: Allylamine Derivatives. In *Molecular Aspects of Chemotherapy*; Borowski, E., Shupar, D., Ed.; Pergamon Press: New York, 1990; pp 205–214.
(2) Stütz, A. *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 320–328.
(3) Petranyi, G.; Meingassner, J. G.; Mieth, H. *Antimicrob. Agents Chemother.* **1987**, *31*, 1365–1368.
(4) Petranyi, G.; Meingassner, J. G.; Mieth, H. *Antimicrob. Agents Chemother.* **1987**, *31*, 1558–1561.

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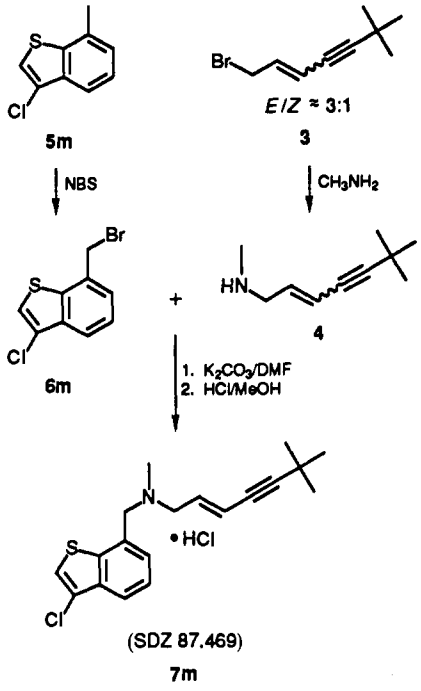
Scheme I. General Procedure for the Synthesis of Benzo[*b*]thiopheneallylamines



vanced clinical experience gained thus far, terbinafine is well-tolerated and shows good to excellent activity against various types of mycoses.[5-8]

Within allylamine antimycotics, SAR studies have concentrated thus far on the side chain and have revealed that the (*E*)-*tert*-butyl-enyne structural element is of crucial importance for the oral activity of these molecules.[2,9]

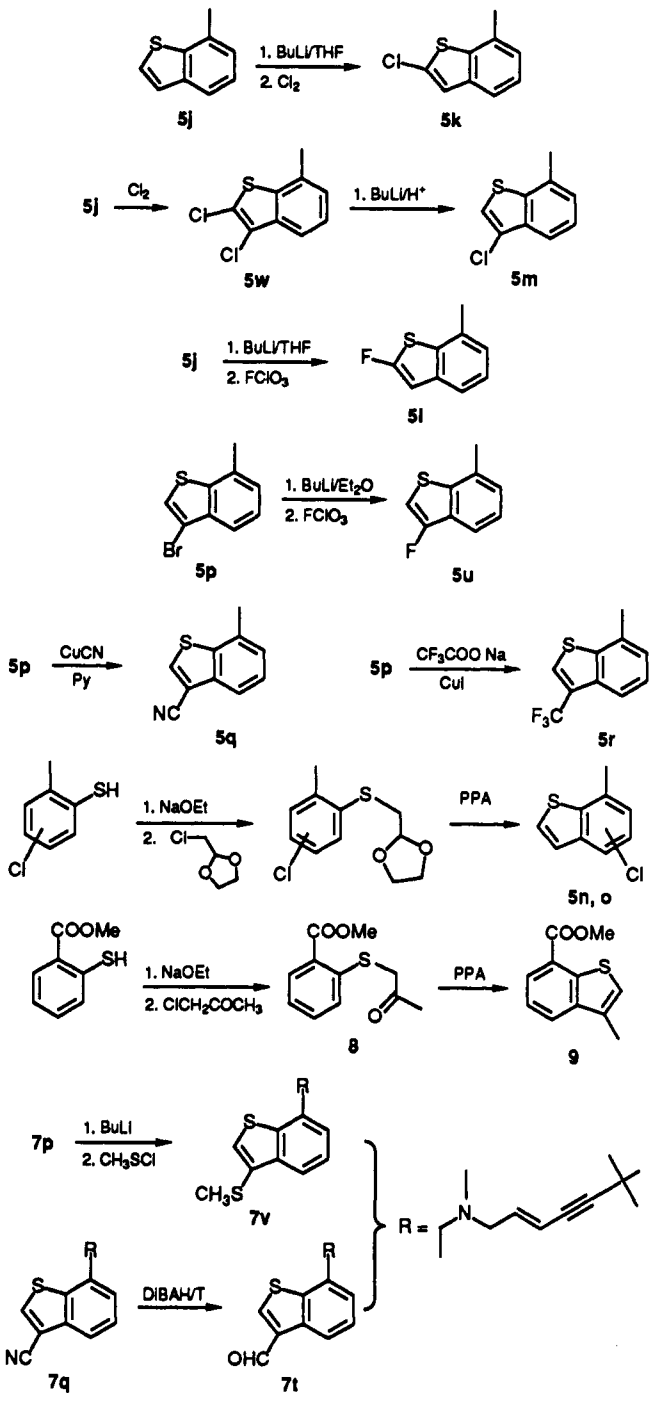
The aim of the present study was to examine whether replacement of the naphthalene part in terbinafine (**2**) by optionally substituted benzo[*b*]thiophenes modifies the antifungal activity, in particular against *Candida albicans*.[10]

Chemistry

Two versatile and general routes for the synthesis of antifungal allylamine derivatives bearing the (*E*)-1,3-enyne structural element have been developed.[9,11] The following general strategy was also shown to be of use for the synthesis of benzo[*b*]thienyl-related derivatives (Scheme I).

Compound **4**, obtained by reacting an *E*/*Z* mixture (~ 3/1) of **3**[9] and excess methylamine, was isolated either as an *E*/*Z* mixture or as the pure *E* isomer after selective crystallization of its corresponding hydrochloride salt. N-Alkylation of **4** by the appropriate (bromomethyl)-benzo[*b*]thiophenes (**6**), which in turn had been synthesized from the corresponding methylbenzo[*b*]thiophenes (**5**) by radical bromination, afforded benzo[*b*]thiopheneallyl-

Scheme II. Key Steps in the Synthesis of Novel Substituted 7-Benzo[*b*]thiophene Derivatives



amines (**7**). The pure *E* isomers were isolated by chromatography or selective crystallization of the hydrochloride salts.

Most of the substituted benzo[*b*]thiophene intermediates (**5**) are novel. Scheme II shows the key steps in the synthesis of additionally substituted 7-benzo[*b*]-thiopheneallylamines.

Consecutive treatment of 7-methylbenzo[*b*]thiophene[12] (**5j**) with *n*-butyllithium and chlorine afforded 2-chloro derivative **5k**.

3-Chloro-7-methylbenzo[*b*]thiophene (**5m**) was synthesized from 7-methylbenzo[*b*]thiophene (**5j**) by chlori-

(5) Savin, R. C. *Clin. Exp. Dermatol.* **1989**, *14* (2), 116–119.
(6) Zaias, N.; Serrano, L. *Ibid.* **1989**, *14* (2), 120–123.
(7) Villars, V.; Jones, T. C. *Ibid.* **1989**, *14* (2), 124–127.
(8) Jones, T. C.; Villars, V.: Terbinafine. In *Handbook of Experimental Pharmacology, Chemotherapy of Fungal Diseases*; Ryley, J. F., Ed.; Springer Verlag: London, New York, Heidelberg, in press.
(9) Stütz, A. *J. Med. Chem.* **1984**, *27*, 1539–1543.
(10) Part of this work was presented at the Xth Congress of the International Society for Human and Animal Mycology in Barcelona, Spain, 1988; (a) Stütz, A.; Nussbaumer, P. *Rev. Ibér. Micol.* **1988**, *5*, Suppl. 1, 89. (b) Petranyi, G.; Meingassner, J. G.; Schaude, M. *Ibid.* **1988**, *5*, Suppl. 1, 88.
(11) Stütz, A.; Granitzer, W.; Roth, S. *Tetrahedron* **1985**, *41*, p 5685–5696.
(12) Loozen, H. J. J.; Godefroi, E. F. *J. Org. Chem.* **1973**, *38* (5), 1056–1057.

Table I. In Vitro Activity (MIC, mg/L) of Benzo[b]thiopheneallylamines

	substit pattern[a]	organisms[b]					
		T. ment.	M. canis	A. fum.	Sp. sch.	C. a. 124	C. par. 39
2	terbinafine	0.003	0.006	0.8	0.4	25	0.8
7a	2, H	>100	>100	>100	>100	>200	>200
7b	3, H	0.01	0.02	3.13	0.8	100	3.13
7c	3, 2-Cl	0.1	0.1	>200	>200	>200	6.25
7d	3, 5-Cl	0.006	0.006	3.13	0.4	12.5	0.4
7e	3, 7-Cl	0.02	0.05	>100	0.8	25	1.56
7f	4, H	0.006	0.01	0.8	0.4	50	1.56
7g	4, 2-Cl	0.006	0.006	6.25	0.2	25	0.2
7h	4, 3-Cl	0.05	0.1	25	0.8	50	6.25
7i	5, H	0.4	1.56	>200	1.56	200	100
7j	7, H	0.006	0.01	0.1	0.2	25	0.8
7k	7, 2-Cl	0.01	0.01	>100	>100	>100	0.8
7l	7, 2-F	0.006	0.01	3.13	0.05	25	6.25
7m	7, 3-Cl	0.0015	0.003	0.1	0.2	0.8	0.1
7n	7, 4-Cl	0.1	0.4	>200	>200	100	0.8
7o	7, 5-Cl	0.05	0.1	>200	3.13	>200	0.2

[a] Position of allylamine side chain and substituents at the benzo[b]thiophene. [b] Abbreviations: *T. mentagrophytes*, T. ment.; *M. canis*, M. canis; *S. schenckii*, Sp. sch.; *A. fumigatus*, A. fum.; *C. albicans* Δ124, C. a. 124; and *C. parapsilosis* Δ39, C. par. 39.

nation, leading to 2,3-dichloro-7-methylbenzo[b]thiophene (**5w**), which was then selectively dechlorinated at position 2 by treatment with *n*-butyllithium and subsequent hydrolysis.

The 2-fluoro-7-methylbenzo[b]thiophene (**5l**), and its regioisomer 3-fluoro-7-methylbenzo[b]thiophene (**5u**) were synthesized from the appropriately lithiated 7-methylbenzo[b]thiophene (**5j**) by treatment with perchloryl fluoride (**Caution**![13]). The lithium salts were in turn obtained from 7-methylbenzo[b]thiophene (**5j**) (for the 2-isomer) and 3-bromo-7-methylbenzo[b]thiophene (**5p**) (for the 3-isomer) with *n*-butyllithium.

Compounds **5n** and **5o** were synthesized by ring closure of the *S*-methyldioxolanes of the appropriate chloro-2-methylthiophenols using polyphosphoric acid.

3-Bromo-7-methylbenzo[b]thiophene (**5p**) was converted to 3-cyano-7-methylbenzo[b]thiophene (**5q**) by treatment with copper(I) cyanide in anhydrous pyridine and to 7-methyl-3-(trifluoromethyl)benzo[b]thiophene (**5r**) by reaction with sodium trifluoroacetate and copper(I) iodide in 1-methyl-2-pyrrolidone.

An alternative strategy was developed for the synthesis of (*E*)-*N*,3-dimethyl-*N*-(6,6-dimethyl-2-hepten-4-ynyl)-benzo[b]thiophene-7-methanamine (**7s**). Methyl 2-(2-oxopropylthio)benzoate (**8**),[14] obtained by reaction of the sodium salt of methyl thiosalicylate and chloro-2-propanone, was cyclized to methyl 3-methyl-7-benzo[b]-thienylcarboxylate (**9**) using polyphosphoric acid. Subsequent ester reduction of **9** (diisobutylaluminum hydride/toluene) yielded 3-methylbenzo[b]thiophene-7-methanol (**10**), which upon treatment with bromine/1,2-bis(diphenylphosphino)ethane gave bromo derivative **6s**.

Methylthio derivative **7v** was obtained by lithiation of **7p** (*n*-butyllithium/−78 °C) followed by treatment with methanesulfenyl chloride. Reduction of the cyano function in **7q** with diisobutylaluminum hydride, followed by acidic hydrolysis, yielded formyl derivative **7t**.

Mycology

The in vitro antifungal activity of the allylamine derivatives was investigated against isolates of *Trichophyton mentagrophytes*, *Microsporum canis*, *Sporothrix schenckii*, *Aspergillus fumigatus*, *Candida albicans* Δ124, and *Candida parapsilosis* Δ39. Minimum inhibitory concentrations (MIC) were determined with Sabouraud's dextrose broth (pH 6.5) for dermatophytes, aspergilli, and *S. schenckii* and malt extract broth (pH 4.8) for yeasts in glass tubes. The test compounds were dissolved in DMSO and serially diluted with the growth media. The growth control was read after 48 h (yeasts), 72 h (molds), and 7 days (*S. schenckii* and dermatophytes) incubation at 30 °C. The MIC was defined as the lowest substance concentration at which no signs of fungal growth were detectable macroscopically.

The fungal strains were obtained either from the American Type Culture Collection, Rockville, MD, from the Centraalbureau voor Schimmelcultures, Baarn, The Netherlands, from the Hygiene-Institut, Würzburg, FRG, or the II. Universitäts-Hautklinik, Vienna, Austria. Filamentous fungi were harvested with a spatula from 21-day-old cultures grown on Kimming agar (E. Merck AG, Darmstadt, FRG) at 30 °C, whereas yeast blastospores were taken from approximately 30-h-old shaken cultures incubated at 37 °C in yeast nitrogen base (Difco Laboratories, Detroit, MI).

The clinical isolates were obtained from the II. Universitäts-Hautklinik of Vienna, Austria.

Results

Unsubstituted benzo[b]thiophene derivatives with the allylamine side chain at position 3, 4, or 7 (compounds **7b,f,j**) were found to be highly active against a broad range of human pathogenic fungi in vitro and comparable to the activity of terbinafine (**2**) (Table I). In contrast, derivative **7i**, with the side chain at position 5, showed a much reduced activity and analogue **7a** (side chain at position 2) turned out to be completely inactive in the test systems used.

Additional substitution of the active compounds **7b**, **7f**, and **7j** at the heteroaromatic ring system by halogen in various positions led to derivatives with comparable (**7d,e,g,l**) or somewhat decreased (**7c,h,k,n,o**) antifungal activity with one exception: 3-chlorobenzo[b]thiophene derivative **7m** (side chain at position 7) showed improved in vitro efficacy against all strains tested.

Considering particularly the changes of activity against *C. albicans*, a tendency toward increased sensitivity caused by additional halogen substituents at different positions was observed, e.g. **7d** and **7e** > **7b**, **7g** > **7f** (Figure 1). The substance **7m** turned out to be an outstanding compound,

(13) For example: (a) Peet, J. H. J.; Rocket, B. W. *J. Organomet. Chem.* **1974**, *82*, C54. (b) Adcock, W.; Khor, T. C. *Ibid.* **1975**, *91*, C20. (c) Wang, C.-L. J.; Grieco, P. A.; Okuniewicz, F. J. *J. Chem. Soc. Chem. Commun.* **1976**, 468. (d) Schroepfer, G. J.; Parish, E. J.; Tsuda, M.; Kandutsch, A. A. *Biochem. Biophys. Res. Commun.* **1979**, *91*, 606.

(14) Görlitzer, K. *Arch. Pharm.* **1974**, *307*, 523–539.

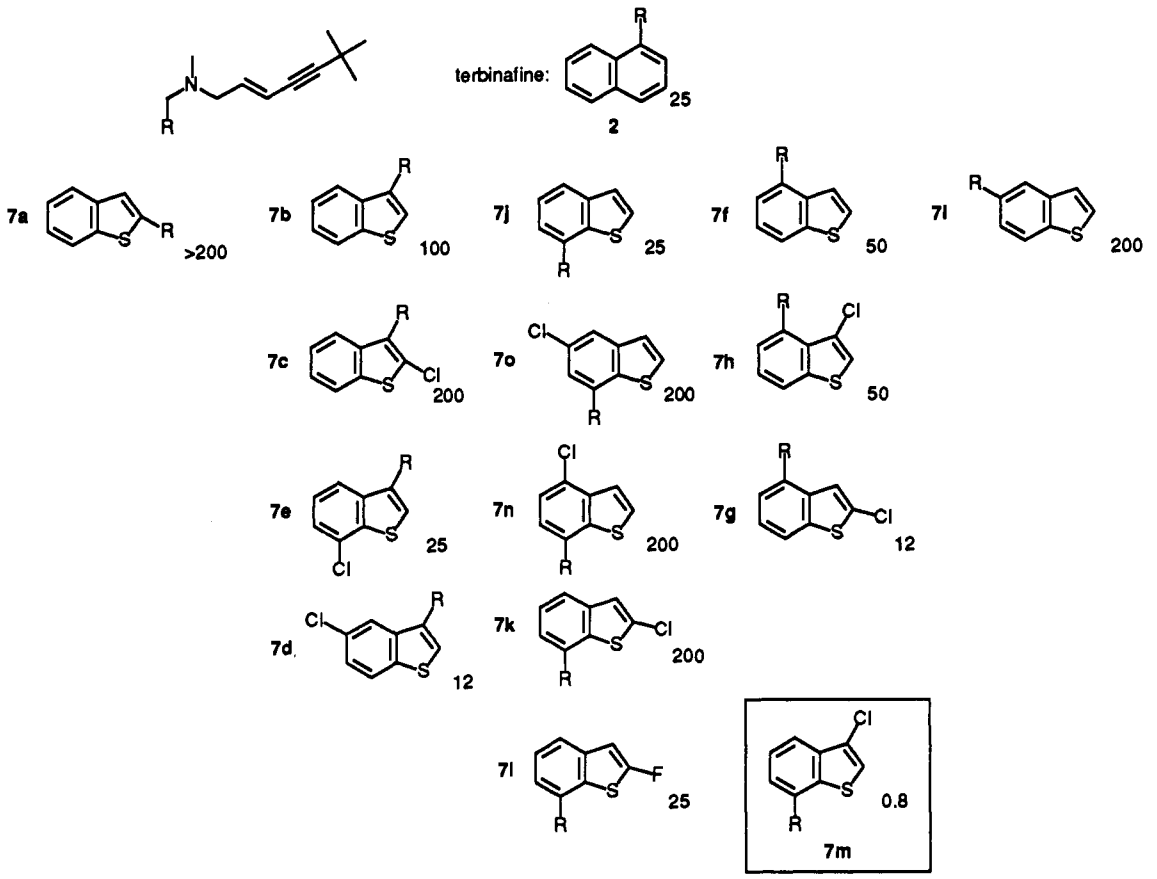


Figure 1. Structure–activity relationships of unsubstituted and halogen-substituted benzo[*b*]thienylallylamine derivatives [values represent MIC (mg/L) against *C. albicans*].

Table II. In Vitro Activity (MIC, mg/L) of Benzo[*b*]thiopheneallylamines[a]

	substit pattern	T. ment.	M. canis	A. fum.	Sp. sch.	C. a. 124	C. par. 39
7m	7, 3-Cl	0.0015	0.003	0.1	0.2	0.8	0.1
7p	7, 3-Br	0.006	0.006	0.4	0.8	0.8	0.2
7q	7, 3-CN	0.02	0.01	0.4	3.13	3.13	0.2
7r	7, 3-CF3	0.05	0.1	50	12.5	3.13	1.56
7s	7, 3-CH3	0.006	0.006	0.1	0.4	3.13	0.4
7t	7, 3-CHO	0.05	0.02	1.56	0.4	6.25	0.4
7u	7, 3-F	0.0008	0.0015	0.05	0.1	0.8	0.1
7v	7, 3-SCH3	0.02	0.1	>200	6.25	6.25	0.8

[a] With the side chain at position 7 and a substituent at position 3. See Table I, footnote *b*, for definition of abbreviations.

being about 15 times more active than the other compounds studied and about 30 times more effective than terbinafine (**2**).

Consequently, a second series of compounds (**7p–v**) with the same substitution pattern as **7m** but bearing substituents at position 3, varying in size and electronic effects, were prepared and tested. All of these compounds demonstrate significantly enhanced in vitro activity against *C. albicans* compared to the unsubstituted derivative **7j** (Figure 2). The most active compounds proved to be the 3-halo derivatives **7m**, **7p**, and **7u**, showing MICs of 0.8 mg/L.

Considering the spectrum and intensity of in vitro efficacy in general, compounds **7m**, **7p**, **7s**, and **7u** are the most effective allylamine derivatives reported to date (Table II).

3-Chloro derivative **7m** (SDZ 87-469) was selected for further investigations.[15] Excellent to outstanding in vitro activity was confirmed by testing a series of laboratory-

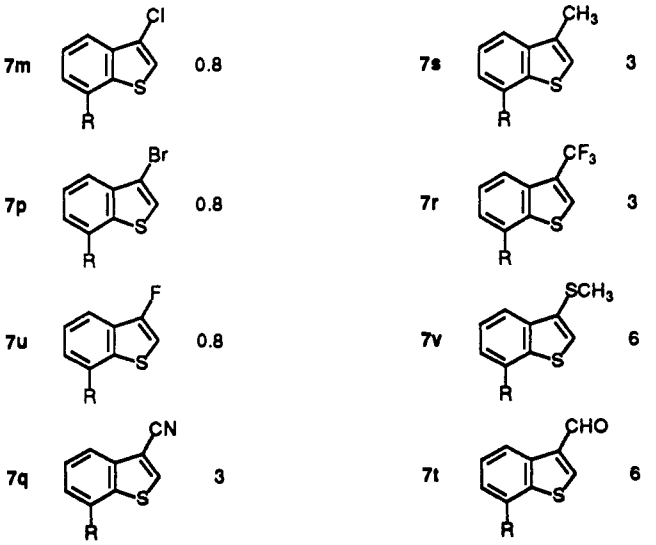


Figure 2. MICs (mg/L) of 3-substituted 7-benzo[*b*]thiopheneallylamines against *C. albicans*.

(15) Stütz, A.; Nussbaumer, P. *Drugs Future* **1989**, *14* (7), 639–642.

Table III. Minimum Inhibitory Concentrations (mg/L) of SDZ 87-469 (**7m**)

strains	no. tested	SDZ 87-469	terbinafine
Laboratory-Adapted Strains			
dermatophytes	12	0.0004–0.003	0.003–0.006
Aspergillus fumigatus	6	0.01–0.05	0.05–1.56
Aspergillus niger	1	0.02	0.02
Scopulariopsis brevicaulis	2	0.05	0.8
Sporothrix schenckii	6	0.02–0.1	0.1–0.4
Candida albicans	3	0.2–0.4	6.25–50
Candida parapsilosis	1	0.02	0.8
Torulopsis glabrata	2	>100	>100
Clinical Isolates			
dermatophytes	112	0.0008–0.02	0.0015–0.01
Candida albicans	29	0.1–3.13	6.25–100

adapted strains and clinical isolates (Table III). For example, dermatophytes were completely inhibited between 0.0004 and 0.003 mg/L, *Aspergillus* species between 0.01 and 0.05 mg/L, and *S. schenckii* between 0.02 and 0.1 mg/L, and clinical isolates of *C. albicans* showed an MIC range of 0.1–3.1 mg/L. Compound **7m** is the most potent inhibitor of fungal squalene epoxidase in enzyme preparations of *C. albicans* known at this time (IC_{50} = 0.011 μM).[16]

Compound **7m** has also been found to be highly effective in vivo.[9b,15] In the guinea pig trichophytosis model (treatment once daily for 9 days starting on the day of inoculation), **7m** proved to be significantly superior to the standards griseofulvin and ketoconazole after oral application: ED_{50} (mg/kg per day) = 3.1 (**7m**), 40.7 (griseofulvin), 50.8 (ketoconazole). With **7m**, 100% mycological cure was already achieved at a dose of 6 mg/kg per day.

Topical treatment of guinea pig microsporosis with 1% solution of **7m** once daily for 7 days starting 72 h after inoculation resulted in complete mycological cure of all animals. Under the same experimental conditions, neither clotrimazole nor econazole effected cure of animals.

In the guinea pig skin candidosis model (topical treatment twice daily for 5 days starting 3 days after inoculation) 90% mycological cure for **7m** vs 57% for terbinafine was determined with a 1% solution of **7m** or terbinafine.

Systemic efficacy of **7m** against *S. schenckii* was shown in subcutaneously implanted diffusion chambers in mice: treatment with 10 and 30 mg/kg six times (2 h before, 2, 4, 24, 48 and 72 h after implantation) achieved 57% and 98.5% CFU reductions (determined 24 h after last treatment), respectively. Corresponding CFU reductions of 49% and 84% were observed in parallel experiments with ketoconazole.

Details of these in vivo investigations will be reported elsewhere.

Discussion

In analogy to the well-known bioisosteric relationship between thiophene and benzene,[17,18] benzo[*b*]thiophene may be considered as bioisostere of naphthalene. Due to differences in physicochemical properties (electronic, steric, and lipophilic factors) benzo[*b*]thiophene analogues of biologically active naphthalene derivatives may have modified biological activity per se and/or different pharmacokinetic properties. Further changes of electronic density distribution and/or steric requirement caused by appropriate substituents may increase these differences or result in selective modification of the biological activity profile.

The results within the first series of analogues **7a**, **7b**, **7f**, **7i**, and **7j** clearly verify that benzo[*b*]thiophene can act as biosiostere of naphthalene, depending on the position of the side chain. Biological activity is restricted to compounds **7b**, **7f**, and **7j** (side chain at position 3, 4, or 7) in accordance with the finding that only 1-substituted naphthaleneallylamines are significantly active.[1a,19] Additional halogen substituents in various positions of the heteroaromatic ring system of highly active benzo[*b*]thiopheneallylamines cause major changes of the antifungal activity (Table I). The parameter responsible for loss of activity within compounds **7c** and **7h** seems to be the steric requirement of the neighboring halogen. In contrast, enhancement of the antifungal activity by an additional halogen substituent is shown for **7d** (in comparison with **7b**) and in particular for the 3-chloro-7-benzo[*b*]thiophene derivative **7m**.

Figure 1 summarizes the structure–activity relationships of unsubstituted and halogen substituted benzo[*b*]thienyl allylamine derivatives with respect to *C. albicans* and demonstrates a unique situation for derivative **7m**.

In vitro investigation of an additional series of compounds with the side chain at position 7 and further substituents varying in size and electronic effects at position 3 of the benzo[*b*]thiophene indicated that derivatives with this specific substitution pattern possess enhanced efficacy against *C. albicans* in general (Figure 2). Within the halogen derivatives **7m**, **7p**, and **7u**, showing the lowest MICs (0.8 mg/L) against *C. albicans*, no influence by the size of the halogen was observed. Even considerable changes in polarity (**7r**,**q**,t,v), electronic effects (**7q**,**r**,t,v), or size of the 3-substituent resulted only in minor decreases of sensitivity. On the other hand, these structural changes led to significantly decreased efficacy of **7r**,t,v against dermatophytes, of **7r**,v against *A. fumigatus*, and of **7q**,**r**,v against *S. schenckii*.

In conclusion, within the class of allylamine antimycotics, naphthalene could be effectively replaced by benzo[*b*]thiophene when bearing the side chain at position 3, 4, or 7. Additional substitution of the latter compound at position 3 led to derivatives with significantly enhanced sensitivity against *C. albicans*. In particular 3-chloro-7-benzo[*b*]thiophene derivative **7m** turned out to be the most potent allylamine antimycotic identified so far. Therefore substituted benzo[*b*]thiophenes may not only be used as potential equivalents of naphthalene in bioactive compounds but also as a tool to selectively modify or even enhance biological activities.

Experimental Section

3-Methylbenzo[*b*]thiophene, 5-methylbenzo[*b*]thiophene, 2-chloro-3-methylbenzo[*b*]thiophene, and 5-chloro-3-methylbenzo[*b*]thiophene were purchased from Maybridge. 2-Methylbenzo[*b*]thiophene,[20] 7-chloro-3-methylbenzo[*b*]-thiophene,[21] 4-methylbenzo[*b*]thiophene,[22] 7-methylbenzo[*b*]-thiophene,[12] and 3-bromo-7-methylbenzo[*b*]thiophene[23] were prepared according to published procedures.

(16) Ryder, N. S. *Biochem. Soc. Trans.* **1990**, *18*, 45–46.
(17) Erlenmeyer, H.; Berger, E.; Leo, M. *Helv. Chim. Acta* **1933**, *16*, 733.
(18) Martin-Smith, M.; Reid, S. T. *J. Med. Pharm. Chem.* **1959**, *1* (*6*), 507–564.
(19) Stütz, A.; Georgopoulos, A.; Granitzer, W.; Petranyi, G.; Berney, D. *J. Med. Chem.* **1986**, *29*, 112–125.
(20) Vio, L. *C. R. Seances Acad. Sci.* **1963**, *257*, 459–460.
(21) Chapman, N. B.; Clarke, K.; Sawhney, S. N. *J. Chem. Soc. C* **1968**, 518–532.
(22) Noyce, D. S.; Forsyth, D. A. *J. Org. Chem.* **1974**, *39* (*19*), 2828–2831.
(23) Faller, P. *Bull. Soc. Chim. Fr.* **1966**, *11*, 3618–3625.

Table IV. Physicochemical Properties of Benzo[b]thiopheneallylamines (7)

no.	morphology, mp, °C	NMR ($CDCl_3$) δ	% yield[a]	MS,[d] m/e	anal.
7a	colorless crystals, 97–99	7.58–7.88 (m, 2 H), 7.20–7.34 (m, 2 H), 7.06–7.12 (m, 1 H), 6.10 (dt, J = 16 + 6.5 Hz, 1 H), 5.65 (dt, J = 16 + 1.2 Hz, 1 H), 3.76 (d, J = 1 Hz, 2 H), 3.10 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.27 (s, 3 H), 1.24 (s, 9 H)	59[b]		$C_{19}H_{23}NS$ (297.46): C, H, N, S
7b	colorless crystals, 59–60, (HCl) 160–165	7.8–8.05 (m, 2 H), 7.25–7.5 (m, 3 H), 6.16 (dt, J = 16 + 6.5 Hz, 1 H), 5.66 (dt, J = 16 + 1.2 Hz, 1 H), 3.7 (s, 2 H), 3.1 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.24 (s, 3 H), 1.24 (s, 9 H)	61[b]		$C_{19}H_{23}NS{\cdot}HCl$ (333.92): C, H, Cl, N, S
7c	colorless crystals, 47–48	7.85–8.06 (m, 1 H), 7.6–7.8 (m, 1 H), 7.2–7.5 (m, 2 H), 6.15 (dt, J = 16 + 6.5 Hz, 1 H), 5.68 (dt, J = 16 + 1.5 Hz), 1 H); 3.71 (s, 2 H), 3.12 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.21 (s, 3 H), 1.24 (s, 9 H)	49[b]		$C_{19}H_{22}ClNS$ (331.91): C, H, Cl, N, S
7d	colorless crystals, 77	7.95 (d, J = 1.8 Hz, 1 H), 7.75 (d, J = 9 Hz, 1 H), 7.32 (s, 1 H), 7.3 (dd, J = 9 + 1.8 Hz, 1 H), 6.13 (dt, J = 16 + 6.5 Hz, 1 H), 5.65 (dt, J = 16 + 1.5 Hz, 1 H), 3.68 (s, 2 H), 3.08 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.23 (s, 3 H), 1.25 (s, 9 H)	51[b]	331 (M^+), 330, 316, 274, 181	$C_{19}H_{22}ClNS$ (331.91): C, H, Cl, N, S
7e	colorless crystals, 59–62, (HCl) 188–191	7.8–8.02 (m, 1 H), 7.2–7.45 (m, 3 H), 6.14 (dt, J = 16 + 6.5 Hz, 1 H), 5.67 (dt, J = 16 + 1.5 Hz, 1 H), 3.71 (s, 2 H), 3.1 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.23 (s, 3 H), 1.25 (s, 9 H)	64[b]		$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, Cl, N, S
7f	oil, (HCl) 182–187	7.5–7.85 (m, 2 H), 7.2–7.45 (m, 3 H), 6.12 (dt, J = 16 + 6.5 Hz, 1 H), 5.64 (dt, J = 16 + 1 Hz, 1 H), 3.76 (s, 2 H), 3.10 (dd, J = 6.5 + 1 Hz, 2 H), 2.18 (s, 3 H), 1.2 (s, 9 H)	62[b]		$C_{19}H_{23}NS{\cdot}HCl$ (333.92): C, H, Cl, N, S
7g	oil, (HCl) 195–206	7.63 (m, 1 H), 7.49 (d, J = 1 Hz, 1 H), 7.25 (m, 2 H), 6.14 (dt, J = 16 + 6.5 Hz, 1 H), 5.66 (dt, J = 16 + 1.5 Hz, 1 H), 3.68 (s, 2 H), 3.08 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.2 (s, 3 H), 1.24 (s, 9 H)	57[b]		$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C,[e] H, Cl, N, S
7h	oil, (HCl) 181–183	7.73 (dd, J = 7.5 + 2 Hz, 1 H), 7.42 (dd, J = 7.5 + 2 Hz, 1 H), 7.32 (t, J = 7.5 Hz, 1 H), 7.31 (s, 1 H), 6.12 (dt, J = 16 + 6.5 Hz, 1 H), 5.65 (dt, J = 16 + 1.5 Hz, 1 H), 4.15 (s, 2 H), 3.16 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.23 (s, 3 H), 1.24 (s, 9 H)	55[b]		$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, Cl, N, S
7i	colorless crystals, 70–72	7.67–7.85 (m, 2 H), 7.22–7.46 (m, 3 H), 6.13 (dt, J = 16 + 6.5 Hz, 1 H), 5.67 (dt, J = 16 + 1.2 Hz, 1 H), 3.60 (s, 2 H), 3.07 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.20 (s, 3 H), 1.23 (s, 9 H)	64[b]	297 (M^+), 147	$C_{19}H_{23}NS$ (297.46): C, H, N, S
7j	oil, (HCl) 148–158	7.7 (dd, J = 7 + 2 Hz, 1 H), 7.2–7.5 (m, 4 H), 6.16 (dt, J = 16 + 6.5 Hz, 1 H), 5.66 (dt, J = 16 + 1 Hz, 1 H), 3.74 (s, 2 H), 3.10 (dd, J = 6.5 + 1 Hz, 2 H), 2.2 (s, 3 H), 1.22 (s, 9 H)	58[b]		$C_{19}H_{23}NS{\cdot}HCl$ (333.92): C, H, Cl, N, S
7k	colorless crystals, 67–70, (HCl) 140–146	7.58 (dd, J = 7 + 2 Hz, 1 H), 7.1–7.4 (m, 3 H), 6.20 (dt, J = 16 + 7 Hz, 1 H), 5.65 (dt, J = 16 + 1 Hz, 1 H), 3.68 (s, 2 H), 3.08 (dd, J = 7 + 1 Hz, 2 H), 2.2 (s, 3 H), 1.22 (s, 9 H)	59[b]	331 (M^+), 326, 295, 288, 274, 236, 181	$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, N
7l	oil, (HCl) 141–151	7.52 (dd, J = 7.5 + 2 Hz, 1 H), 7.28 (t, J = 7.5 Hz, 1 H), 7.12 (dd, J = 7.5 + 2 Hz, 1 H); 6.69 (d, J = 3 Hz, 1 H), 6.18 (dt, J = 16 + 6.5 Hz, 1 H), 5.68 (dt, J = 16 + 1.5 Hz, 1 H), 3.68 (s, 2 H), 3.1 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.21 (s, 3 H), 1.23 (s, 9 H)	47[b]	315 (M^+), 300, 258, 165	$C_{19}H_{22}FNS{\cdot}HCl$ (351.91): C,[f] H, N
7m	colorless crystals, 54–56, (HCl)[c] 180–184	7.78 (dd, J = 7 + 2 Hz, 1 H), 7.25–7.5 (m, 3 H), 6.16 (dt, J = 16 + 6 Hz, 1 H), 5.65 (dt, J = 16 + 1 Hz, 1 H), 3.76 (s, 2 H), 3.1 (dd, J = 6 + 1 Hz, 2 H), 2.2 (s, 3 H), 1.22 (s, 9 H)	59[b]		$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, Cl, N, S
7n	colorless crystals, 55, (HCl) 170–173	7.53 (s, 2 H), 7.33 (d, J = 7.5 Hz, 1 H), 7.17 (d, J = 7.5 Hz, 1 H), 6.18 (dt, J = 16 + 6 Hz, 1 H), 5.67 (dt, J = 16 + 1.5 Hz, 1 H), 3.73 (s, 2 H), 3.11 (dd, J = 6 + 1.5 Hz, 2 H), 2.22 (s, 3 H), 1.24 (s, 9 H)	48[b]		$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, N
7o	oil, (HCl) 185–190	7.71 (d, J = 2 Hz, 1 H), 7.49 (d, J = 5.5 Hz, 1 H); 7.27 (d, J = 5.5 Hz, 1 H), 7.25 (d, J = 2 Hz, 1 H), 6.16 (dt, J = 16 + 6 Hz, 1 H), 5.66 (dt, J = 16 + 1.5 Hz, 1 H), 3.71 (s, 2 H), 3.1 (dd, J = 6 + 1.5 Hz, 2 H), 2.21 (s, 3 H), 1.23 (s, 9 H)	49[b]	331 (M^+), 316, 286, 274, 181	$C_{19}H_{22}ClNS{\cdot}HCl$ (368.37): C, H, Cl, N, S
7p	colorless crystals, 51–57, (HCl) 174–178	7.77 (dd, J = 7 + 2 Hz, 1 H), 7.2–7.55 (m, 3 H), 6.18 (dt, J = 16 + 6.5 Hz, 1 H), 5.66 (dt, J = 16 + 1 Hz, 1 H), 3.8 (s, 2 H), 3.1 (dd, J = 6.5 + 1 Hz, 2 H), 2.22 (s, 3 H), 1.24 (s, 9 H)	56[b]		$C_{19}H_{22}BrNS{\cdot}HCl$ (412.82): C, H, Br, Cl, N, S
7q	oil, (HCl) 188–192	8.15 (s, 1 H), 7.84–8.05 (m, 1 H), 7.40–7.62 (m, 2 H), 6.18 (dt, J = 16 + 6.5 Hz, 1 H), 5.69 (dt, J = 16 + 1.2 Hz, 1 H), 3.89 (s, 2 H), 3.21 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.28 (s, 3 H), 1.23 (s, 9 H)	46[b]		$C_{20}H_{22}N_2S{\cdot}HCl$ (358.93): C, H, Cl, N, S
7r	oil, (HCl) 175–188	7.78–7.96 (m, 2 H), 7.18–7.52 (m, 2 H), 6.17 (dt, J = 16 + 6.5 Hz, 1 H), 5.67 (dt, J = 16 + 1.2 Hz, 1 H), 3.78 (s, 2 H), 3.11 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.21 (s, 3 H), 1.23 (s, 9 H)	39[b]	365 (M^+), 350, 308, 270, 215	$C_{20}H_{22}F_3NS{\cdot}HCl$ (401.93): C, H, Cl, F, N, S
7s	oil, (HCl) 185–187	7.66 (dd, J = 7 + 2.5 Hz, 1 H), 7.24–7.50 (m, 2 H), 7.11 (q, J = 1.2 Hz, 1 H), 6.20 (dt, J = 16 + 6.5 Hz, 1 H), 5.69 (dt, J = 16 + 1.2 Hz, 1 H), 3.77 (s, 2 H), 3.11 (dd, J = 6.5 + 1.2 Hz, 2 H), 2.47 (d, J = 1.2 Hz, 3 H), 2.24 (s, 3 H); 1.25 (s, 9 H)	55[b]	311 (M^+), 296, 254, 216, 161, 150	$C_{20}H_{25}NS{\cdot}HCl$ (347.95): C, H, Cl, N, S

Table IV (Continued)

no.	morphology, mp, °C	NMR ($CDCl_3$) δ	% yield[a]	MS,[d] m/e	anal.
7t	oil	10.18 (s, 1 H), 8.62 (dd, J = 7.5 + 2 Hz, 1 H), 8.35 (s, 1 H), 7.47 (t, J = 7.5 Hz, 1 H), 7.31 (dd, J = 7.5 + 2 Hz, 1 H), 6.18 (dt, J = 16 + 6.5 Hz, 1 H), 5.67 (dt, J = 16 + 1.5 Hz, 1 H), 3.80 (s, 2 H), 3.12 (dd, J = 6.5 + 1.5 Hz, 2 H), 2.23 (s, 3 H), 1.24 (s, 9 H)	72	325 (M^+), 310, 273, 268, 230, 175, 150, 147	$C_{20}H_{23}NOS$ (325.47)[g]
7u	oil, (HCl) 167–171	7.7 (dd, J = 7.5 + 2 Hz, 1 H), 7.38 (t, J = 7.5 Hz, 1 H); 7.28 (dd, J = 7.5 + 2 Hz, 1 H), 6.84 (d, J = 2.5 Hz, 1 H); 6.19 (dt, J = 16 + 6.5 Hz, 1 H), 5.68 (dt, J = 16 + 1.5 Hz, 1 H), 3.76 (s, 2 H), 3.1 (dd, J = 6.5 + 1.5 Hz, 2 H); 2.21 (s, 3 H), 1.22 (s, 9 H)	36[b]	315 (M^+), 300, 258, 165	$C_{19}H_{22}FNS$·HCl (351.91): C,[h] H, Cl,[h] N, S
7v	oil, (HCl) 163–164	7.82 (dd, J = 7.2 + 2 Hz, 1 H), 7.18–7.52 (m, 3 H), 6.19 (dt, J = 16.2 + 6 Hz, 1 H), 5.69 (dt, J = 16.2 + 1 Hz, 1 H), 3.78 (s, 2 H), 3.12 (dd, J = 6 + 1 Hz, 2 H), 2.52 (s, 3 H), 2.23 (s, 3 H), 1.25 (s, 9 H)	54		$C_{20}H_{25}NS_2$·HCl (380.01): C, H, Cl, N, S

[a] Yields (not optimized) of isolated, analytically pure products. [b] The reactions were carried out with an E/Z mixture (≈3/1) of 4; the yield given refers to pure E isomer only. [c] Crystal modification starting at about 130 °C. [d] Free bases were used, intensive ions are given. [e] Calcd, 61.95; found, 61.36. 7g could only be obtained with 96% purity, as shown by HPLC. [f] Calcd, 64.85; found, 63.18. 7l could only be obtained with 90% purity, as shown by HPLC. [g] Not determined, due to the sensitivity for oxidation of 7t. [h] Calcd, 64.85 (C), 10.08 (Cl); found, 63.68 (C), 11.46 (Cl). 7u could only be obtained with 93% purity, as shown by HPLC.

Melting points were determined on a Reichert Thermovar microscope and are not corrected. The temperature is given in Celsius units. The purity of the compounds was determined by GLC (Siemens Sichromat 1) using quartz columns (stationary phase OV-101) or RP-HPLC (pump, Waters M 6000; columns, 18 or 10 μm Partisil ODS-10) using a water/acetonitrile gradient and a Schoeffel SF 770 UV detector (270 nm).

Thin-layer chromatography was performed with silica gel F_{254} plates (Merck) with visualization by UV or iodine vapor. Column chromatography was performed with silica gel 60 (0.040–0.063 mm, Merck) at a pressure of 3–5 bars.[24]

^1H NMR spectra were recorded at 90 MHz (Bruker WH 90) or 250 MHz (Bruker WM 250) in $CDCl_3$ with $(CH_3)_4Si$ as internal standard. Chemical shifts are given as δ units. Mass spectra were recorded on a MAT 311A instrument with EI ion source (70 eV and 250 °C) and direct inlet system by Dr. A. Nikiforov at the Institute of Organic Chemistry, University of Vienna. Elemental analyses were performed by Dr. O. Zak, microanalytical laboratory at the University of Vienna, Institute of Organic Chemistry.

Synthesis of Benzo[*b*]thienylallylamines (7). 1. N-Alkylation of *N*,6,6-Trimethyl-2-hepten-4-yn-1-amine (4) with (Bromomethyl)benzo[*b*]thiophenes (6). General Procedure. In a typical procedure, 7-(bromomethyl)-3-chlorobenzo[*b*]-thiophene (1.3 g, 5 mmol, **6m**) was dissolved in dry dimethylformamide (10 mL) and added slowly to a mixture of *N*,6,6-trimethyl-2-hepten-4-yn-1-amine (4) (750 mg, 5 mmol, *E*/*Z* = 3/1) and potassium carbonate (900 mg, 6.5 mmol) in dry dimethylformamide at 0 °C. After stirring overnight at room temperature, the solvent was evaporated in vacuo and the residue was partitioned between ether and water. The separated aqueous phase was extracted once with ether, and the combined organic extracts were dried over anhydrous sodium sulfate and concentrated in vacuo. The residue was chromatographed (toluene/ethyl acetate, 95/5) to give **7m** (980 mg, 59%) as colorless crystals (mp 56–58 °C from ethanol; for NMR, see Table IV), followed by the oily *Z* isomer (300 mg, 18%): NMR δ 7.78 (dd, J = 7 + 2 Hz, 1 H), 7.2–7.5 (m, 3 H), 6.05 (dt, J = 11 + 6.5 Hz, 1 H), 5.63 (dt, J = 11 + 1 Hz, 1 H), 3.8 (s, 2 H), 3.33 (dd, J = 6.5 + 1 Hz, 2 H), 2.24 (s, 3 H), 1.24 (s, 9 H).

2. Organometallic Reactions. (*E*)-*N*-(6,6-Dimethyl-2-hepten-4-ynyl)-*N*-methyl-3-(methylthio)benzo[*b*]-thiophene-7-methanamine (7v). Compound **7p** (380 mg, 1 mmol) was dissolved in dry ether (10 mL) and treated with *n*-butyllithium (0.68 mL, 1.09 mmol, 1.6 M solution in hexane) at −78 °C under argon. After 20 min at this temperature, methanesulfenyl chloride (0.15 mL, 1.3 mmol) was added and the mixture was stirred for 30 min at −78 °C. The reaction mixture was allowed to warm slowly to room temperature and stirred overnight. The mixture was then poured onto ice/water and extracted with ether. The combined organic layers were washed with saturated aqueous sodium bicarbonate, dried over magnesium sulfate, and concentrated in vacuo. The residue was chromatographed (toluene) to give the title compound **7v** (187 mg, 54%) as a colorless oil: mp (hydrochloride) 163–164 °C (2-propanol/ether).

(*E*)-*N*-(6,6-Dimethyl-2-hepten-4-ynyl)-*N*-methyl-3-formylbenzo[*b*]thiophene-7-methanamine (7t). Diisobutylaluminum hydride (2 mL, 2.4 mmol, 1.2 M solution in toluene) was added to **7q** (700 mg, 2.2 mmol) in dry toluene (20 mL) under argon at −50 °C. After stirring for 1 h at −50 °C and then 30 min at room temperature, 2 N acetic acid (10 mL) was added and stirring continued for 40 min. The reaction mixture was made alkaline by careful treatment with solid sodium bicarbonate and then extracted with ether. The combined organic layers were washed with brine, dried over magnesium sulfate, and concentrated. The crude product was chromatographed (toluene/ethyl acetate, 95/5) to give the title compound **7t** (509 mg, 72%) as a colorless oil.

Synthesis of Starting Materials. *N*,6,6-Trimethyl-2-hepten-4-yn-1-amine (4). Crude 1-bromo-6,6-dimethyl-2-hepten-4-yne[11] (3; 20 g, 99 mmol, *E*/*Z* ≈ 3/1), dissolved in 50 mL of dimethylformamide, was added to methylamine (250 mL, 2 mol, 8.03 M solution in ethanol) over 1 h at 0 °C and the mixture was stirred at room temperature overnight. The solvent was evaporated in vacuo and the residue was partitioned between ether and water. The aqueous layer was decanted, brought to pH 9 with 1 N aqueous sodium hydroxide, and then extracted with ether. The combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The crude product was purified by column chromatography (methylene chloride/methanol, 98/2) or by vacuum distillation (bp 76–78 °C at 18 mbar). Chromatographic purification gave amine 4 (11.5 g, 77%) as a slightly yellow oil possessing the same *E*/*Z* ratio as the starting material.

The isomeric mixture was used for the alkylation with (bromomethyl)benzo[*b*]thiophenes (6) to yield both isomers of benzo[*b*]thienylallylamines (7), which were separated by chromatography or selective crystallization of the trans isomer as its hydrochloride salt.

Pure (*E*)-*N*,6,6-trimethyl-2-hepten-4-yn-1-amine (4a) was obtained by treatment of the *E*/*Z* mixture with methanolic hydrogen chloride solution and selective crystallization: mp (hydrochloride) 163–166 °C (ethanol/ether); NMR [(*E*)-base] δ 6.07 (dt, J = 16 + 6.25 Hz, 1 H), 5.62 (dt, J = 16 + 1 Hz, 1 H), 3.22 (dd, J = 6.25 + 1 Hz, 2 H), 2.41 (s, 3 H), 1.23 (s, 9 H), 1.15 (s, 1 H); NMR [(*Z*)-base] δ 5.92 (dt, J = 10 + 6.25 Hz, 1 H), 5.58 (dt, J = 10 + 1 Hz, 1 H), 3.44 (dd, J = 6.25 + 1 Hz, 2 H), 2.44 (s, 3 H), 1.25 (s, 9 H), 1.1 (s, 1 H).

(24) Loibner, H.; Seidl, G. *Chromatographia* **1979**, *12*, 600.

Reaction of 4a with (bromomethyl)benzo[b]thiophenes (6) resulted in pure (E)-allylamine derivatives 7.

Synthesis of (Bromomethyl)benzo[b]thiophenes (6). 1. Radical Bromination of Methylbenzo[b]thiophenes (5). General Procedure. Methylbenzo[b]thiophene (5) and N-bromosuccinimide (1.0 equiv) were refluxed in tetrachloromethane after addition of a catalytic amount of dibenzoyl peroxide for 1–6 h (conversion monitored by thin-layer chromatography). The mixture was cooled in an ice bath, filtered, and concentrated in vacuo. Crude products so obtained were generally used in the following alkylation procedure without purification.

2. 7-(Bromomethyl)-3-methylbenzo[b]thiophene (6s). (a) Methyl 3-Methyl-7-benzo[b]thiophenecarboxylate (9). Methyl 2-[(2-oxopropyl)thio]benzoate[14] (8; 0.5 g, 2.2 mmol) was introduced with vigorous stirring into preheated polyphosphoric acid at 90 °C. After 20 min at this temperature, the mixture was poured onto ice and extracted with ethyl acetate. The combined organic layers were washed with saturated aqueous sodium bicarbonate and dried over magnesium sulfate, and the solvent was evaporated. The residue was chromatographed (hexane/ethyl acetate, 6/1) to give 9 (205 mg, 45%): NMR δ 8.11–8.17 (m, 1 H), 7.93 (dd, J = 7.5 + 1 Hz, 1 H), 7.48 (dd, J = 2 + 7.5 Hz, 1 H), 7.20–7.23 (m, 1 H), 4.02 (s, 3 H), 2.47 (d, J = 1.2 Hz, 3 H).

(b) 3-Methylbenzo[b]thiophene-7-methanol (10). Diisobutylaluminum hydride (2 mL, 2.4 mmol, 1.2 M solution in toluene) was added to a solution of 9 (200 mg, 1 mmol) in dry toluene (15 mL) at −40 °C. The mixture was stirred for 30 min at this temperature, diluted with ether, and added to ice-cold 1 N hydrochloric acid. The separated organic layer was washed successively with water and brine, dried, and concentrated in vacuo to give compound 10 (168 mg, 97%): NMR δ 7.6–7.8 (m, 1 H), 7.33–7.5 (m, 2 H), 7.08–7.18 (m, 1 H), 5.0 (d, J = 6 Hz, 2 H), 2.48 (s, 3 H), 1.84 (t, J = 6 Hz, 1 H).

(c) 7-(Bromomethyl)-3-methylbenzo[b]thiophene (6s). Bromine (0.047 mL, 0.9 mmol) was added to a solution of 1,2-bis(diphenylphosphino)ethane (0.2 g, 0.5 mmol) in dry methylene chloride (15 mL) at −10 °C. After stirring for 10 min, 10 (160 mg, 0.9 mmol) was added and the mixture was stirred for 40 min at −10 °C. The solvent was evaporated and the residue was extracted with 20 mL of ether. The mixture was filtered, the solid was washed with ether, and the combined ether filtrates were evaporated in vacuo to give the crude bromomethyl derivatives 6s (200 mg, 91%), which was used without further purification.

Synthesis of Methylbenzo[b]thiophenes (5). 2-Chloro-4-methylbenzo[b]thiophene (5g). A solution of 4-methylbenzo[b]thiophene (5f; 2 g, 13.5 mmol) in dry ether (20 mL) was treated with n-butyllithium (8.4 mL, 13.5 mmol, 1.6 M solution in hexane) at −10 °C under argon. After stirring for 1 h at 0 °C, the mixture was charged with chlorine (1 g, 14 mmol) at −30 °C and stirred at room temperature for another hour. The organic layer was washed several times with water and dried over anhydrous sodium sulfate, and the solvent was evaporated in vacuo. The crude, oily product (2.5 g) was used without further purification.

3-Chloro-4-methylbenzo[b]thiophene (5h). Chlorine gas was passed into a 30-mL carbon tetrachloride solution of 4-methylbenzo[b]thiophene (5f; 3.3 g, 22 mmol) at 0 °C until 4 g (56 mmol) had been taken up. After stirring for 3 h at room temperature, the solvent was distilled off and the residue was taken up in methylene chloride. The organic solution was washed with saturated aqueous sodium bicarbonate, dried over anhydrous sodium sulfate, and concentrated in vacuo to obtain crude 2,3-dichloro-4-methylbenzo[b]thiophene (5.1 g): NMR δ 6.85–7.45 (m, 3 H), 2.7 (s, 3 H).

The crude dichloro compound (5.1 g) was dissolved in dry ether (70 mL) and n-butyllithium (14.4 mL, 23 mmol, 1.6 M solution in hexane) was added under argon at 0 °C. After 1 h, the mixture was poured onto 2 N HCl and the separated organic layer was washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness. The crude, oily product (4.2 g) was used without further purification: NMR δ 7.55–7.75 (m, 1 H), 7.02–7.4 (m, 3 H), 2.87 (s, 3 H).

2-Chloro-7-methylbenzo[b]thiophene (5k). Crude material, which was used without further purification, was obtained in almost quantitative yield starting from 7-methylbenzo[b]thiophene (5j) following the procedure described for 2-chloro-4-methylbenzo[b]thiophene (5g): NMR δ 7.67–7.82 (m, 1 H), 7.18–7.60 (m, 3 H), 2.58 (s, 3 H).

2-Fluoro-7-methylbenzo[b]thiophene (5l). n-Butyllithium (30 mL, 48 mmol, 1.6 M solution in hexane) was added to a solution of 7-methylbenzo[b]thiophene (5j, 7 g, 47 mmol) in dry THF (80 mL) under argon at −70 °C. After stirring for 20 min at this temperature, perchloryl fluoride (**Caution! This reagent is extremely hazardous.**[13]) (5.5 g, 54 mmol) was condensed into the reaction mixture and the temperature was maintained below −60 °C. The mixture was stirred for 30 minutes at −70 °C and then slowly allowed to warm. At 0 °C, the mixture was treated with water (50 mL), washed with brine, dried over magnesium sulfate, and concentrated in vacuo. The residue was column chromatographed (hexane) to give 5l (60%) as a colorless oil. This was used without further purification.

3-Chloro-7-methylbenzo[b]thiophene (5m). This compound was prepared according to the procedure described for 3-chloro-4-methylbenzo[b]thiophene (5h) starting from 7-methylbenzo[b]thiophene (5j), but the intermediate 2,3-dichloro-7-methylbenzo[b]thiophene (5w) was purified by crystallization from methanol or by distillation in vacuo (mp 48–50 °C; bp 110–115 °C/0.03 mbar): NMR δ 7.55–7.7 (m, 1 H), 7.41 (t, J = 7.2 Hz, 1 H), 7.15–7.3 (m, 1 H).

3-Chloro-7-methylbenzo[b]thiophene (5m) was purified by vacuum distillation (60–62 °C/0.15 mbar) and obtained as a colorless oil in 56% yield for two steps: NMR δ 7.64–7.82 (m, 1 H), 7.41 (t, J = 7.5 Hz, 1 H), 7.30 (s, 1 H), 7.14–7.32 (m, 1 H), 2.59 (s, 3 H).

4-Chloro-7-methylbenzo[b]thiophene (5n). Following the procedure for the synthesis of 5o, 5n was isolated as a colorless oil in 52% overall yield after chromatographic purification (hexane) starting from 5-chloro-2-methylthiophenol:[25] NMR δ 7.37 (s, 2 H), 7.15 (d, J = 7.5 Hz, 1 H), 6.93 (d, J = 7.5 Hz, 1 H), 2.49 (s, 3 H).

5-Chloro-7-methylbenzo[b]thiophene (5o). Sodium (1.97 g, 85.5 mmol) dissolved in dry ethanol (150 mL) was treated with 4-chloro-2-methylthiophenol (12.4 g, 78 mmol). After stirring for 30 min at room temperature, bromoacetaldehyde dimethyl acetal (9.9 mL, 85.5 mmol) was added and the mixture was refluxed for 4 h. The solvent was then evaporated; the residue was treated with water and then extracted with ether. The combined organic layers were washed with saturated aqueous sodium carbonate and dried over anhydrous sodium sulfate, and the solvent was removed in vacuo. The crude product [19 g; NMR δ 6.93–7.40 (m, 3 H), 4.47 (t, J = 6.5 Hz, 1 H), 3.33 (s, 6 H), 3.03 (d, J = 6.5 Hz, 2 H), 2.36 (s, 3 H)] was added slowly to a mixture of orthophosphoric acid (150 mL) and phosphorus pentoxide (125 g) (this mixture had been previously stirred at 130 °C for 3 h) at 170–180 °C under diminished pressure (20 mbar), thus allowing the crude product to distill off (110–135 °C) during the reaction. The distillate was dissolved in ether, washed with water, dried over magnesium sulfate, and fractionally distilled to give the oily title compound 5o (7.4 g, 50%): bp 68–72 °C (0.1 mbar); NMR δ 7.6–7.7 (m, 1 H), 7.49 (dd, J = 5.5 + 0.6 Hz, 1 H), 7.3 (d, J = 5.5 Hz, 1 H), 7.1–7.2 (m, 1 H), 2.54 (s, 3 H).

3-Cyano-7-methylbenzo[b]thiophene (5q). 3-Bromo-7-methylbenzo[b]thiophene (5p; 3 g, 12 mmol) and copper(I) cyanide (1.1 g, 12 mmol) were dissolved in dry pyridine (20 mL) and heated in an autoclave for 12 h to 220 °C. The solvent was evaporated and the residue was taken up in methylene chloride, which was washed successively with diluted aqueous hydrochloric acid and water, dried over anhydrous sodium sulfate, and concentrated in vacuo. The crude product was purified by chromatography (toluene) to give yellowish crystals (720 mg, 34%): mp 82–84 °C; NMR δ 8.14 (s, 1 H), 7.79–7.96 (m, 1 H), 7.50 (t, J = 7.5 Hz, 1 H), 7.22–7.39 (m, 1 H), 2.60 (s, 3 H).

7-Methyl-3-(trifluoromethyl)benzo[b]thiophene (5r). 3-Bromo-7-methylbenzo[b]thiophene (5p; 2 g, 8.8 mmol), sodium trifluoroacetate (4.8 g, 35 mmol), copper(I) iodide (3.3 g, 17 mmol), and 1-methyl-2-pyrrolidone (20 mL) were heated under argon to 160 °C with vigorous stirring. Gas evolution started and the mixture was stirred for 1 h at 160 °C and for 1 additional hour at 180 °C. The cold reaction mixture was poured onto water and

(25) Archer, S.; Suter, C. M. J. Am. Chem. Soc. 1952, 74, 4307.

extracted with ether/hexane (v/v = 1/1). The combined organic layers were washed several times with water, dried over anhydrous sodium sulfate, and concentrated in vacuo. The residue was chromatographed (hexane) to obtain **5r** (1.03 g, 54%) as a colorless oil: NMR δ 7.92 (d, J = 1 Hz, 1 H), 7.72-7.94 (m, 1 H), 7.43 (t, J = 7.5 Hz, 1 H), 7.14-7.32 (m, 1 H), 2.59 (s, 3 H).

3-Fluoro-7-methylbenzo[*b*]thiophene (5u). 3-Bromo-7-methylbenzo[*b*]thiophene (**5p**; 9.05 g, 40 mmol) was dissolved in dry ether (35 mL), and *n*-BuLi (25 mL, 40 mmol, 1.6 M solution in hexane) was added under argon at -78 °C. After stirring for 20 min at this temperature the mixture was charged slowly with perchloryl fluoride (**Caution! This reagent is extremely hazardous.**[13]) (4.5 g, 44 mmol) and the temperature was maintained below -60 °C. After an additional 30 min at -78 °C, the temperature was allowed to rise to 0 °C during 1 h. The reaction mixture was quenched with water, the separated organic layer was dried over magnesium sulfate, and the solvent was removed in vacuo. The crude product was chromatographed (hexane) to give 5u (4.6 g, 35%) as a colorless oil.

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Design of Potent Protein Kinase Inhibitors Using the Bisubstrate Approach

A. Ricouart, J. C. Gesquiere, A. Tartar, and C. Sergheraert*

Faculté de Pharmacie de Lille, URA CNRS 1309, Institut Pasteur de Lille, rue Calmette, 59019 Lille, France.
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A new class of serine/threonine protein kinase inhibitors was designed by associating, in the same structure, mimics of both the ATP binding site and a protein substrate. Among the several potent antagonists which were obtained, the most active consists of isoquinoline-5-sulfonamide, as ATP mimic, and Ser-Arg_6, as peptidic moiety, bound by a $-NH(CH_2)_2NH(CH_2)_2CO-$ linker. This compound, with a K_i of 0.1 μM toward protein kinase C (PKC) and 0.004 μM toward cyclic AMP dependent protein kinase (PKA), is respectively 60- and 750-fold more active than the commercial inhibitor H-7.

The physiological importance of protein kinase C (PKC) activation is widely appreciated and well-documented; a number of recent reviews has covered various aspects of the enzymology of this kinase and its role as a signal-transducing protein in a plethora of physiological responses.[1-5] Several discrete subspecies have been defined: all these proteins, derived from multiple genes or from alternative splicing, have in common the presence of a regulatory and of a catalytic domain.

Activators of PKC such as phospholipids or diacylglycerol interact with the regulatory domain, while both ATP and the protein substrate interact with the catalytic domain.[6,7] The different inhibitors or activators of PKC reported to date are known to interact with a single of these four binding sites. We wish to report in this paper our efforts to design new PKC inhibitors able to interact simultaneously with two of these sites according to the bisubstrate concept. In this approach, the combination of two substrates required by the enzyme to form a single molecule allows the advantage of not only at least partially additive binding energies but also a significant entropic contribution.

We have focused our efforts on the two entities which are known to interact with the catalytic domain: the peptidic substrate and ATP.

In order to prepare such inhibitors, it is necessary not only to design structures which are suitable to interact with both binding sites but also to covalently link these two moieties with a spacer which could reproduce the distance between the binding sites in the enzyme structure.

(i) Substrate Mimics. The substrate specificity of PKC has been studied by many groups using, in general, a series of synthetic peptides.[8-12] These studies have shown the importance of basic residues close to the phosphorylated serine (or threonine) either on its C or N terminus side or both. We have therefore decided to use a cluster of arginine residues (Arg_4 or Arg_6) to interact with the peptidic recognition site. A second question was whether a phosphorylable residue should or should not be included. In favor of the former is the fact that when serine is substituted for alanine in a high-affinity substrate, the corresponding peptide obtained, which is devoid of phosphorylable residue, proves to be a poor inhibitor.[13] On the basis that a high-affinity substrate can become an inhibitor, provided the simultaneous binding of an ATP mimic prevents its phosphorylation, a serine residue was added in some analogues to the cluster of arginines.

(ii) ATP Mimics. No X-ray structural data are available for protein kinases; however, the crystal structure of phosphoglycerate kinase with bound ATP has been determined by Bryant et al.[14] This structure shows the presence of many hydrophobic side chains surrounding the adenine ring of ATP. The presence of such an hydro-

* To whom correspondance should be addressed.

(1) Nishizuka, Y. *Science* **1986**, *233*, 305.
(2) Nishizuka, Y. *Nature* **1988**, *334*, 661.
(3) Ashendel, C. L. *Biochem. Biophys. Acta* **1985**, 822, 219.
(4) Kikikawa, U.; Kishimoto, A.; Nishizuka, Y. *Annu. Rev. Biochem.* **1989**, *58*, 31.
(5) Parker, P. J.; Kour, G.; Marais, R. M.; Mitchell, F.; Pears, C.; Schaap, D.; Stabel, S.; Webster, C. *Mol. Cell. Endocrinol.* **1989**, *65*, 1.
(6) Nakadate, T.; Jeng, A. Y.; Blumberg, P. M. *Biochem. Pharmacol.* **1988**, 8, 1541.
(7) Hidaka, H.; Inagaki, M.; Kawamoto, S.; Sasaki, Y. *Biochemistry* **1984**, *23*, 5036.
(8) O'Brien, C. A.; Laurence, D. S.; Kaiser, E. T.; Weinstein, I. B. *Biochem. Biophys. Res. Commun.* **1984**, *124*, 296.
(9) Turner, R. S.; Kemp, B. E.; Su, H.; Kuo, J. F. *J. Biol. Chem.* **1985**, *260*, 11503.
(10) House, C.; Wettenhall, E. H.; Kemp, B. E. *J. Biol. Chem.* **1987**, *262*, 772.
(11) Woodgett, J. R.; Gould, K. L.; Hunter, T. *Eur. J. Biochem.* **1986**, *161*, 177.
(12) Ferrari, S.; Marchiori, F.; Borin, G.; Pinna, L. A. *FEBS Lett.* **1985**, *184*, 72.
(13) Unpublished results.
(14) Bryant, T. N.; Watson, H. C.; Wendell, P. C. *Nature* **1974**, *257*, 17.
(15) House, C.; Kemp, B. E. *Science* **1987**, *238*, 1726.

0022-2623/91/1834-0073$02.50/0