6c (567 mg, 1.47 mmol) and lithium iodide trihydrate (8.62 g, 46 mmol) were added to dimethylformamide (15 mL). The mixture which resulted was brought to reflux and stirred for 72 h, after which the DMF was removed by Kugelrohr distillation. The residue which remained was dissolved in chloroform (250 mL), washed with 5% $Na_2S_2O_3$ (7 × 100 mL), and dried (Na_2SO_4). The solvent was then evaporated under reduced pressure, and ether (25 mL) was added. The precipitate which resulted was filtered, washed with ether (3 × 5 mL), and dried to yield the free base of **7c** (270 mg, 62%). Formation of the hydrochloride salt was accomplished with the addition of a cold saturated solution of methanol-hydrogen chloride to the free base in methanol: mp 250-252 °C; IR (KBr) 1651, 1644, 1633, 1488, 1424, 1383, 1318, 832, 756 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.62 (d, 2 H, *J* = 9.0 Hz), 7.88 (t, 1 H, *J* = 8.0 Hz), 7.98 (t, 1 H, *J* = 8.2 Hz), 8.06 (d, 2 H, *J* = 9.0 Hz), 8.28 (d, 1 H, *J* = 7.9 Hz), 8.36 (d, 1 H, *J* = 7.7 Hz), 9.03 (s, 1 H); MS (CI, CH_4) 296 (M + 1); high-resolution MS *m/e* 295.0495 ($C_{16}H_{10}N_3OCl$ requires 295.0512). Anal. ($C_{16}H_{10}N_3OCl$) C, H, N.

2-(*p*-Fluorophenyl)-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol Hydrochloride (7d). (*p*-Fluorophenyl)isoquinolinium hydroxide **6d** (100 mg, 0.271 mmol) and lithium iodide trihydrate (3.5 g, 18.7 mmol) were added to dimethylformamide (10 mL). The mixture which resulted was brought to reflux and stirred for 48 h, after which the DMF was removed by Kugelrohr distillation. The residue which remained was taken up in chloroform (50 mL), washed with 5% $Na_2S_2O_3$ (4 × 100 mL), and dried over sodium sulfate. The solvent was then removed under reduced pressure to yield an oil which was solidified upon the addition of ether. The solid was filtered, washed with ether (3 × 5 mL), and dried to provide the isoquinoline free base **7d** (49 mg, 64%). Upon the addition of a cold saturated solution of methanol-hydrogen chloride to the free base **7d** in methanol, the hydrochloride salt **7d** was isolated: mp 255-259 °C; IR (KBr) free base 1617, 1583, 1502, 1432, 1382, 1209, 1080, 836 cm^{-1}; ^1H NMR (Me_2SO-d_6) δ 7.40 (t, 2 H, *J* = 8.9 Hz), 7.87 (t, 1 H, *J* = 7.5 Hz), 7.98 (t, 1 H, *J* = 8.1 Hz), 8.03 (dd, 2 H, *J* = 9.1 Hz, 4.9 Hz), 8.28 (d, 1 H, *J* = 7.6 Hz), 8.35 (d, 1 H, *J* = 7.8 Hz), 9.04 (s, 1 H); MS (CI, CH_4) 280 (M + 1); high-resolution MS *m/e* 279.0818 ($C_{16}H_{10}N_3OF$ requires 279.0808). Anal. ($C_{16}H_{10}N_3OF \cdot HCl$) C, H, N.

2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinoline-3-thiol (8). To a stirred solution of anhydrous toluene (10 mL) and Lawesson's reagent (41 mg, 0.10 mmol) was added pyrazoloisoquinolin-3-ol hydrochloride **7a** (50 mg, 0.17 mmol). The mixture which resulted was brought to 110 °C under nitrogen with stirring. After 4 h the solution was cooled to room temperature, and the toluene was removed under reduced pressure. The residue was then purified by flash chromatography (SiO_2) with chloroform as the eluant to provide pure **8** (21 mg, 45%): mp 220 °C dec; IR (KBr) 2520, 1620, 1592, 1494, 1388, 1260, 1096, 1020, 801 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (s, 1 H), 7.14 (m, 3 H), 7.32 (d, 2 H, *J* = 8.1 Hz), 7.68 (t, 1 H, *J* = 8.6 Hz), 7.83 (t, 1 H, *J* = 8.0 Hz), 7.91 (d, 1 H, *J* = 7.9 Hz), 8.51 (d, 1 H, *J* = 8.1 Hz), 8.78 (s, 1 H); EIMS *m/z* 277 (M^+); high-resolution MS *m/e* 277.0674 ($C_{16}H_{11}N_3S$ requires 277.0674). The title compound **8** was shown to be homogeneous by TLC on silica gel (R_f = 0.11; ethyl acetate).

3-Chloro-2-phenyl-2*H*-pyrazolo[4,3-*c*]isoquinoline (9). 2-Phenyl-2*H*-pyrazolo[4,3-*c*]isoquinolin-3-ol hydrochloride **7a** (60 mg, 0.200 mmol) was dissolved in phenylphosphonic dichloride (7 mL). The resulting solution was stirred and warmed to 90 °C. After 30 min the temperature was increased to 125 °C for 2 h, after which the cooled reaction mixture was poured over ice water (50 mL). The resulting solution was basified to pH 8.5 with saturated aqueous Na_2CO_3, followed by extraction with chloroform (3 × 50 mL). The combined organic extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield the free base **9** (36 mg, 64%): mp 185-186 °C; IR (KBr) 1596, 1560, 1499, 1475, 1387, 754, 690, 574 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.55 (m, 3 H), 7.73 (m, 3 H), 7.83 (t, 1 H, *J* = 8.0 Hz), 8.03 (d, 1 H, *J* = 7.7 Hz), 8.55 (d, 1 H, *J* = 7.4 Hz), 8.99 (s, 1 H); EIMS *m/z* 279 (M^+), 244 (M^+ - Cl); high-resolution MS *m/e* 279.0563 ($C_{16}H_{10}N_3Cl$ requires 279.0563). The title compound **9** was shown to be homogeneous by TLC on silica gel [R_f 0.22; $CHCl_3$ (55%), hexane (45%)].

Acknowledgment. We wish to thank Jim Laloggia for excellent technical assistance and the NIMH (MH 36644) for generous financial support.

Registry No. 4, 137695-87-5; 5, 53726-69-5; 6a, 137695-77-3; 6b, 137695-78-4; 6c, 137695-79-5; 6d, 137695-80-8; 97a, 137695-81-9; 7b, 137695-82-0; 7c, 137695-83-1; 7d, 137695-84-2; 8, 137695-86-4; 9, 137695-85-3; 10, 77779-60-3; 11, 77779-36-3; 12, 77779-50-1; $PhNHNH_2$, 100-63-0; *p*-$MeOC_6H_4NHNH_2$, 3471-32-7; *p*-$ClC_6H_4NHNH_2$, 1073-69-4; *p*-$FC_6H_4NHNH_2$, 371-14-2; LiI, 10377-51-2; $PhPOCl_2$, 824-72-6; flunitrazepam-*t*, 80573-68-8; flunitrazepam, 1622-62-4.

Synthesis and Substance P Receptor Binding Activity of Androstano[3,2-*b*]pyrimido[1,2-*a*]benzimidazoles

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Several heterosteroids containing a dihydroethisterone skeleton were prepared and shown to displace substance P in a receptor binding assay. Further biochemical (kinetic and Scatchard analyses) and pharmacological evaluation (substance P-induced plasma extravasation and salivation in the rat) of a representative example in this series (**5a**) established that these compounds are competitive antagonists at the substance P receptor.

Introduction

Substance P (Figure 1) is an undecapeptide that belongs to a family of neurotransmitters known as neurokinins that includes the structurally related neurokinin A (NKA) and neurokinin B (NKB).[1] Based on the relative potencies of these agonists, three neurokinin receptors, generally referred as NK-1, NK-2, and NK-3, have been proposed. Recently three NK receptors have been cloned and sequenced,[2,3] validating this classification. Substance P (SP),

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† Sterling Research Group.

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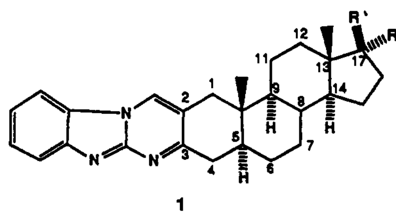
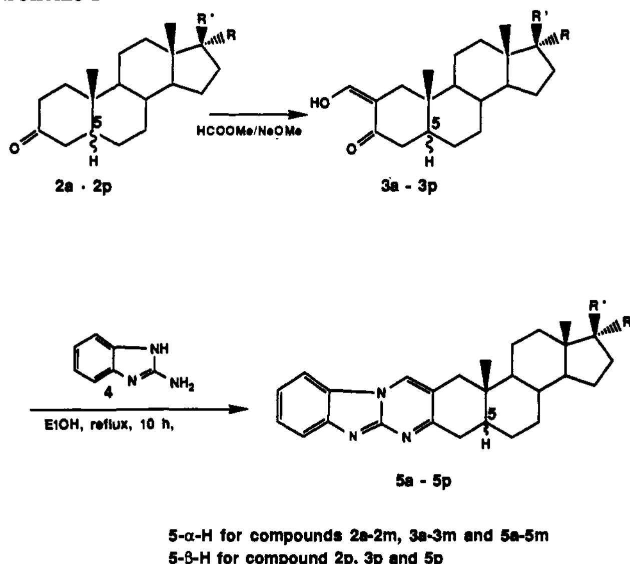


Arg·Pro·Lys·Pro-Gln-Gln-Phe-Phe-Gly-Leu·Met·NH_2
Substance-P (SP)

Figure 1. General structure of heterosteroids.

Scheme I



a moderately selective NK-1 receptor agonist, has a wide spectrum of pharmacological activity and is implicated in pain and inflammation.[4] However, the lack of selective and potent nonpeptidic compounds has been a limiting factor in fully exploring the therapeutic potential of SP receptor antagonists, except for the recently disclosed quinuclidine[5] and imidazoquinoxaline[6] derivatives. In an effort to develop more potent SP antagonists, we recently discovered that a unique steroid heterocycle, 17-β-hydroxy-17-α-ethynyl-5-α-androstano[3,2-*b*]pyrimido[1,2-*a*]benzimidazole (1, Figure 1) binds to the NK-1 receptor from rat brain. A series of analogues in this class of compounds was prepared to understand the structure–activity relationships and improve potency. The results of our synthetic efforts and biological data are presented here.

Chemistry

Most of the hetero-steroids described in this paper (Table I) were prepared by the condensation[7] of a 2-(hydroxymethylene)-3-keto steroid compound (3) with 2-aminobenzimidazole (4) (Scheme I). The hydroxymethylene compounds were prepared from the 3-keto compounds using standard formylation procedures.[7] In order to establish structure–activity relationships, structural changes were made in the steroid and heterocyclic portion of 1. To assess the significance of the hydroxyl group at C-17, it was converted to the methyl ether (**5b**). Alternatively, the C-17 α-ethynyl group was replaced with other substituents in order to understand the steric and electronic requirements at this position. Thus, 17-α-ethyl- and 17-α-vinylandrostan-3-one (**2g** and **2h**) were prepared by catalytic hydrogenation of **2a**. The 17-α-propynyl (**2i**), 17-α-propargyl (**2j**), and 17-α-phenyl (**2k**) 3-keto steroids were prepared from commercially available 3,17-androstanedione by nucleophilic addition to the 17-keto-

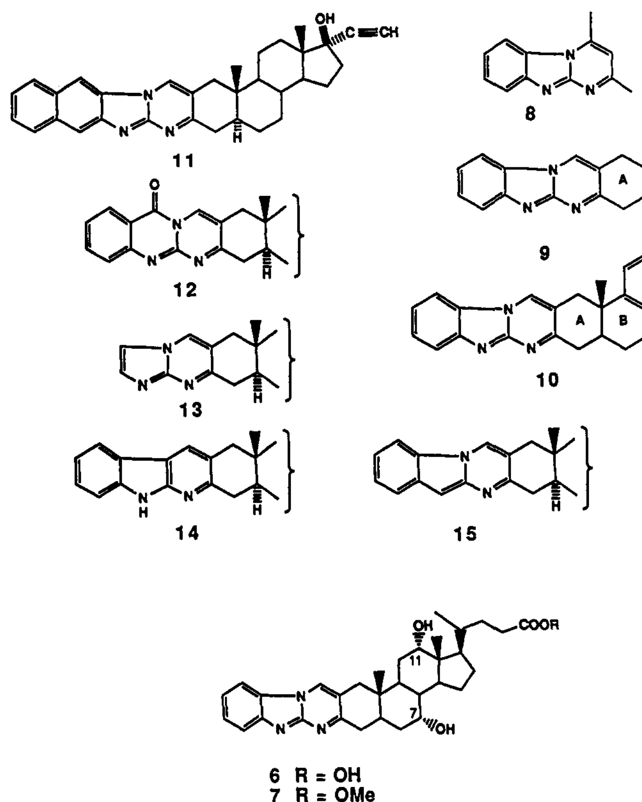


Figure 2. Other heterosteroids.

Scheme II

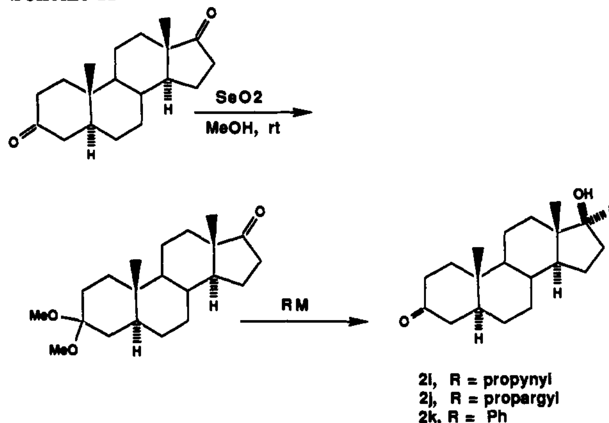


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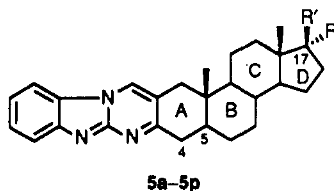
Table I. Physicochemical and Receptor Binding Data[a]



compd no.	R	R'	mp (°C)	% yield	formula	anal.	IC_{50}, nM, mean ± SEM for 3 or *n*
5a	ethynyl	OH	298–9	90	$C_{29}H_{33}N_3O{\cdot}0.2H_2O$	CHN	50 ± 11
5b	ethynyl	OMe	301 dec	64	$C_{30}H_{35}N_3O{\cdot}0.5H_2O$	CHN	>10000
5c	ethynyl	OH (oxalate salt)	177–9	95	$C_{31}H_{35}N_3O_5{\cdot}0.7H_2O$	CHN	24 ± 2
5d	H	OH	282–4[b]	73	$C_{27}H_{33}N_3O$	CHN	220 ± 40
5e	H	H	318–21 dec	85	$C_{27}H_{33}N_3$	CHN	>10000
5f	Me	OH	285–8 dec	21	$C_{28}H_{35}N_3O$	CHN	200 ± 20
5g	Et	OH	249–50	39	$C_{29}H_{37}N_3O{\cdot}1CH_3OH$	CHN	2900 ± 200
5h	vinyl	OH	285–8	48	$C_{29}H_{35}N_3O{\cdot}0.5CH_3OH$	CHN	86 ± 36
5i	propynyl	OH	295–7	22	$C_{30}H_{35}N_3O{\cdot}1H_2O$	CHN	180 ± 30 (4)
5j	propargyl	OH	241–3	51	$C_{30}H_{35}N_3O$	CHN	320 ± 80 (5)
5k	C_6H_5	OH	>310 dec	39	$C_{33}H_{37}N_3O{\cdot}0.3H_2O$	CHN	>10000
5l	$CH(Me)(CH_2)_3CH(Me)_2$	H	294–6[b]	94	$C_{35}H_{49}N_3$	CHN	>10000
5m	=O		>250 dec	43	$C_{27}H_{31}N_3O{\cdot}0.8H_2O$	CHN	>10000
5n(Δ^4)	ethynyl	OH	>300 dec	90	$C_{29}H_{31}N_3O$	CHN	48 ± 10
5o(Δ^4)	H	COOMe	315–6	66	$C_{29}H_{33}N_3O_2{\cdot}0.2H_2O$	CHN	>10000
5p(β-5H)	H	OH	>250 dec	50	$C_{27}H_{33}N_3O$	CHN	>10000
6	–	–	301–2	69	$C_{33}H_{43}N_3O_4{\cdot}0.4H_2O$	CHN	>10000
7	–	–	320–1	85	$C_{32}H_{41}N_3O_4{\cdot}1H_2O$	CHN	>2500
8	–	–	238	75	$C_{12}H_{11}N_3$	CHN	>10000
9	–	–	228–30	75	$C_{14}H_{13}N_3$	CHN	>10000
10	–	–	235–7 dec		$C_{21}H_{21}N_3$	CHN	>10000
11	–	–	302–5 dec	90	$C_{33}H_{35}N_3O$	CHN	320 ± 70
12	–	–	>300 dec	23	$C_{30}H_{33}N_3O_2{\cdot}0.5H_2O$	CHN	1200 ± 200
13	–	–	320–2 dec		$C_{25}H_{31}N_3O$	CHN	1600 ± 400
14	–	–	>300	18	$C_{30}H_{34}N_2O$	CHN	>10000
15	–	–	>300	18	$C_{30}H_{34}N_2O{\cdot}0.25CH_3OH$	CHN	>10000
spantide							500 ± 18
substance P							0.12 ± 0.06 (6)

[a] See Figure 2 for structures of 6–15. [b] Reference 7.

androstane 3,3-dimethyl ketal, followed by deprotection[8,9] (Scheme II).

Compounds 6 and 7 were prepared from 3-ketoallocholic acid (Figure 2).[10] These compounds have additional hydroxyl groups at C-7 and C-11, as well as no hydroxyl group and a 4-pentanoic acid side chain at C-17. In order to determine whether the complete steroid skeleton is necessary for activity, compounds 9 and 10 (corresponding to the steroid A and AB rings, respectively) were prepared from cyclohexanone and decalone.[11]

A few compounds were also prepared to assess the electronic and steric requirements of the heterocyclic portion of 1. Thus, compounds 11–15 (Figure 2) were synthesized by the condensation of the corresponding 2-amino heterocycles with 3a (Scheme I), following the standard procedure.[7]

Biological Results and Discussion

The biochemical evaluation of the compounds described above was carried out in a standard SP receptor binding assay, using ^{125}I-Bolton Hunter Substance P (^{125}I-BHSP) as the ligand in rat forebrain membranes.[12] The IC_{50}

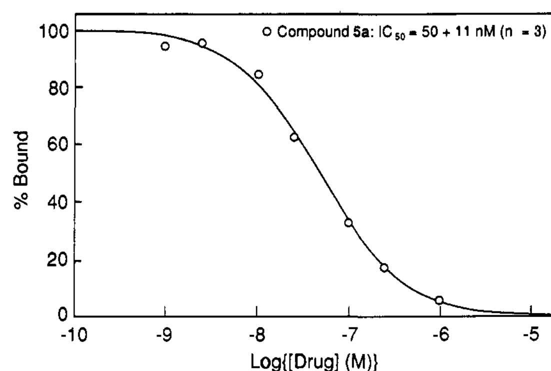


Figure 3. Displacement curve of ^{125}I-BHSP by **5a**.

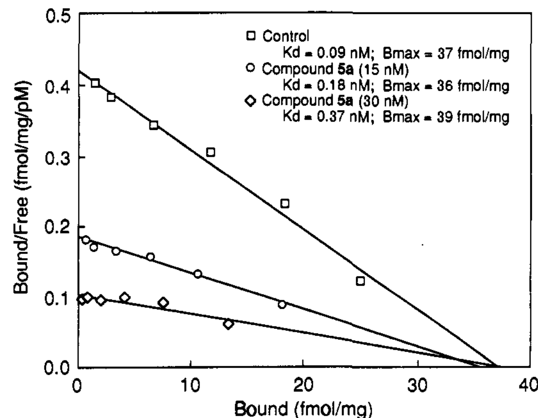


Figure 4. Scatchard analysis of **5a**.

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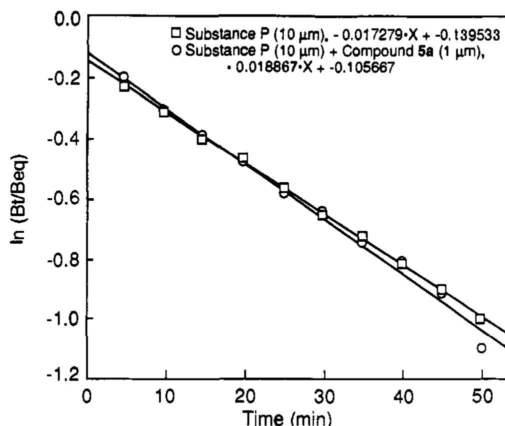

Figure 5. Dissociation of **5a** with ^{125}I-BHSP.

values were calculated from at least 6-point displacement curves and are reported in Table I. The most potent compound in this series was found to be **5a** (Figure 3, IC_{50} = 50 nM). The reversibility of receptor interaction with **5a** as a representative example in this series was assessed by Scatchard analysis experiment. A concentration dependent increase in apparent K_d for SP, with no change in the number of binding sites (B_{max}) was observed (Figure 4). To test the nature of the receptor interaction, kinetic analyses of the dissociation rates for SP were investigated in the presence and absence of test compound. In the presence of 1 µM **5a**, no apparent effect on the rate of dissociation of SP was observed (Figure 5). Thus, the fact that **5a** produced a concentration dependent increase in K_d with no change in B_{max} and no effect on the dissociation is consistent with a competitive interaction with the receptor.

The in vivo activity of these compounds was determined in two classical SP models in the rat. Compound **5a** was found to inhibit SP-induced plasma extravasation in the hindpaw (MED = 0.3 mg/kg, iv). This dose produced a 39% decrease in the extravasation of Evans blue dye following SP. The more soluble oxalate salt, **5c**, was also tested and found to inhibit SP-induced salivation (MED = 7.5 mg/kg, iv) in rats.[13] From the above biochemical and pharmacological experiments, **5a** appears to act as a competitive NK-1 antagonist. This led us to explore the SAR features in this series, in an attempt to prepare compounds more potent than **5a** in the receptor binding assays.

SAR at the C-17 Position. From the structure–activity studies, it is clear that substituents at C-17 are necessary for activity and in particular, the 17-β-hydroxy group is critical. Thus, 17-keto (**5m**), 17-β-methoxy (**5b**), and 17-deoxy (**5e**) compounds were all inactive in the binding assay. The nature of 17-α-substituents appear to be less critical, since **5d** that lacks the ethynyl group was only 5-fold less potent than **5a**. The steric nature of the 17-α-substituents may be playing a modulatory role in the biological activity, as evidenced by the decrease in binding potency with the introduction of bulky substituents at this position. Thus **5g**, with 17-α-ethyl substituent, showed marginal activity compared to either **5f** (17-α-methyl) or **5h** (17-α-vinyl).

(12) Park, C. H.; Massari, V. J.; Quirion, R.; Tizabi, Y.; Shults, C. W.; O'Donohue, T. L. Characteristics of ^{3}H-Substance P Binding Sites in Rat Brain Membranes. *Peptides* **1984**, *5*, 833–836.

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Steroid Skeleton. An important observation in this series was that active compounds had either an A/B trans ring junction or 4,5-unsaturation in the steroid A ring (**5n**). On the other hand, the compound having an A/B cis ring junction (**5p**) was completely inactive. This is due to the difference in shape between the A/B trans and unsaturated compounds, which are fairly planar and the A/B cis compound with an angular conformation. The allocholic acid analogues **6** and **7** were inactive. It is not known whether this is due to the additional hydroxyl groups at C-7 and C-11 or the absence of C-17 hydroxyl group or the altered C-17 side chain. The presence of the entire steroid appears to be necessary for activity, as compounds **8**,[14] **9**[15] (A ring only), and **10** (A,B ring) were inactive.

Heterocyclic Portion. Several compounds (**11–15**) were tested in which structural changes were made in the imidazopyridine ring system. While the introduction of an extra ring (**11**) was tolerated to some extent, removal of the existing aromatic ring (**13**) lowered the potency considerably. Replacement of the benzimidazole nucleus in **5a** with a quinazolinone (e.g. **12**) also resulted in reduced affinity. Similarly, **14** and **15**, which lacked the masked guanidine functionality, were completely inactive.

Conclusions

In summary, a series of heterosteroid compounds was evaluated for their ability to displace substance P in an NK-1 receptor binding assay using rat brain. The 17-α-ethynyl-β-hydroxy substituents were important for maximum binding potency, as was the pyrimidobenzimidazole. The stereochemistry of the A/B ring junction of the steroid was also critical for activity, the cis compounds being totally inactive. Compound **5a** was the most potent, with an IC_{50} of 50 nM. It is an NK-1 antagonist as evidenced by its ability to inhibit SP-induced plasma extravasation and salivation in rats.

Experimental Section

General Procedures. Melting points were recorded using a Thomas-Hoover melting point apparatus and are uncorrected. Proton NMR spectra were recorded on a GE QE-300 spectrometer. Chemical shifts are reported in ppm from tetramethylsilane on the δ scale with residual solvent as the internal standard (chloroform 7.26 ppm). Mass spectra were obtained in either electron impact or field desorption mode. When necessary, solvents and reagents were dried prior to use, following the known procedures. Moisture-sensitive reactions were carried out in an atmosphere of argon using oven-dried glassware.

General Synthesis of Heterosteroids (5). A solution of 2-(hydroxymethylene)-3-oxo steroid (**3**, 0.001 mol) and 2-aminobenzimidazole (**4**, 0.0012 mol) in absolute ethanol (50 mL) was heated at reflux for 7–20 h. Progress of the reaction was monitored by TLC, and once the reaction was completed, the reaction mixture was concentrated in vacuo to half its original volume. In many cases the product crystallized out at this point; the solid was then collected by filtration and dried. In a few cases, the reaction mixture was purified by column chromatography. In all the cases, compounds were recrystallized and characterized by spectral and analytical data (Table I). Spectral data of 17-β-hydroxy-17-α-ethynyl-5-α-androstano[3,2-*b*]pyrimido[1,2-*a*]-

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benzimidazole (**5a**) is given here as a representative example: ^{1}H NMR ($CDCl_3$) δ 0.82 (s, 3 H, CH_3), 0.89 (s, 3 H, CH_3), 1.21–3.02 (m, 21 H), 2.59 (s, 1 H, ethynyl-H), 7.31 (t, 1 H, J = 7.68 Hz), 7.46–7.50 (m, 1 H), 7.77 (d, 1 H, J = 8.1 Hz), 7.94 (d, 1 H, J = 8.1 Hz), and 8.39 (s, 1 H).

Receptor Binding Procedures.[12] ^{125}I-Bolton Hunter Substance P used in these experiments was purchased from New England Nuclear. The rat forebrain (whole brain minus cerebellum) of a male Sprague–Dawley rat was homogenized in 20 volumes of ice-cold 50 mM tris(hydroxymethyl)aminomethane hydrochloride (Tris·HCl), pH 7.4, at 4 °C, 5 mM KCl and 120 mM NaCl (wash buffer) with a Tekmar Tissumizer Mark II. The homogenate was centrifuged at 48000g for 10 min, and then the resulting pellet was resuspended in 50 mM Tris·HCl, pH 7.4, at 4 °C, 10 mM EDTA and 30 mM KCl, and incubated for 30 min at 4 °C. The homogenate was then centrifuged as above and washed twice by centrifugation in 50 mM Tris·HCl (pH 7.4, at 25 °C). The final pellet was resuspended in 60 volumes of Tris buffer.

The binding assay mixture (0.25 mL) contained 75–125 μg of membrane protein, 0.1 nM ^{125}I-BHSP, and test compound in 50 mM Tris·HCl, at pH 7.4, at 25 °C, 0.02% bovine serum albumin, 1 μg of chymostatin, 2 μg of leupeptin, 20 μg of bacitracin, 1.2 mM $MnCl_2$. Nonspecific binding was defined with 1 μM substance P. All assays were run in duplicate or triplicate, and the reaction mixtures were incubated for 20 min at 25 °C. The assay mixtures were then diluted with 2 mL wash buffer and filtered through GF/C glass fiber filters presoaked in 0.01% polyethylenimine. The filters were washed seven more times with 2 mL of wash buffer. The radioactivity trapped on these filters was counted in a Packard Cobra Gamma Counter. The competition curve data were analyzed by computer nonlinear least-squares best fit of the data to the Hill equation which determined the IC_{50} values from at least six concentrations of the test compound (1×10^{-10} M to 1×10^{-5} M).

Plasma Extravasation in Rats. Experiments were carried out on male Sprague–Dawley rats (150–175 g). Evans Blue dye (30 mg/kg) was administered iv through the tail vein. Compound **5a** was dissolved in a vehicle of 10% ethanol, 15% propylene glycol, and 75% water containing 0.15% TWEEN 80. The compound was administered (in one-half log unit doses) via the tail vein with the Evans Blue dye. Two minutes later the rats received an intradermal injection of SP (2.5 nM) into the left hind paw. An intradermal injection of saline (50 μL) was administered into the right paw as a vehicle and volume control. The animals were sacrificed 20 min after the intradermal injections and the paws removed at the hair line above the ankle. The paws were minced and placed into 4 mL of 99% formamide and incubated overnight at 60 °C. After incubation the samples were centrifuged at 1800 rpm for 5 min (Sorvall RT6000B). The concentration of extractable Evans blue dye was quantified spectrophotometrically at 620 nm (Thermomax Microplate quantified spectrophotometrically at 620 nm (Thermomax Microplate Reader, Molecular Devices, S/N UVT 05318). Plasma extravasation was assessed in the SP injected paw after subtraction of the Evans Blue value of the saline injected paw. Multiple comparisons were made with a one-way analysis of variance followed by the Dunnett test. Compound **5a** was compared to a control group which received the vehicle and Evans Blue iv.

Acknowledgment. We would like to acknowledge the assistance of Ms. Barbara Fragale, Ms. Colleen Duggan, and Ms. Cheryl Meravi for the biochemical evaluation and Mr. Larry Wagner for the pharmacological evaluation.

Chromophore-Modified Antineoplastic Imidazoacridinones. Synthesis and Activity against Murine Leukemias

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The synthesis of 8-hydroxy and 8-methoxy analogues of some substituted 5-aminoimidazoacridinones (**4**) is described. The synthesis was carried out by a three-step sequence from the corresponding 1-chloro-4-nitro-9(10H)-acridinone precursors (**1**). The annulation of the imidazolo ring onto the aminoacridinone chromophore was accomplished by heating the required aminoacridinone (**3**) with formic acid or, in the case of 1-methyl derivatives, with N,N-dimethylacetamide. Potent cytotoxic activity against L1210 leukemia, as well as antitumor activity against P388 leukemia in mice, was demonstrated for the 8-hydroxy analogues. The corresponding 8-methoxy derivatives were not cytotoxic. However, in some cases, they showed significant in vivo antileukemic activity.

Introduction

In our recent paper, the 5-[(aminoalkyl)amino]-6H-imidazo[4,5,1-de]acridin-6-ones were reported as a novel class of antineoplastic agents.[1] Further research on their structurally close analogues, the 5-[(aminoalkyl)amino]-6H-v-triazolo[4,5,1-de]acridin-6-ones, revealed the importance of the OH group at position 8 for their antineoplastic activity.[2] This finding, previously ascertained also for other structural groups of synthetic "DNA complexing agents", for example, the analogues of lucanthone,[3] ellipticine,[4,5] and benzothiopyranoindazoles,[6] prompted us to synthesize a number of substituted 5-amino-8-hydroxy-6H-imidazo[4,5,1-de]acridine-6-ones (**4i–p**).

It has been reported in the literature for several different groups of anticancer agents that, beside the hydroxy, the respective methoxy derivatives also show antitumor activity.[7-10] Additionally, in the case of 5-nitropyrazoloacridines, the methoxy derivatives were found to exhibit selectivity against solid tumors.[11,12] On the basis of these reports, we synthesized for comparison the corresponding

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