Communications to the Editor

Discovery of a Potent Substance P Antagonist: Recognition of the Key Molecular Determinant

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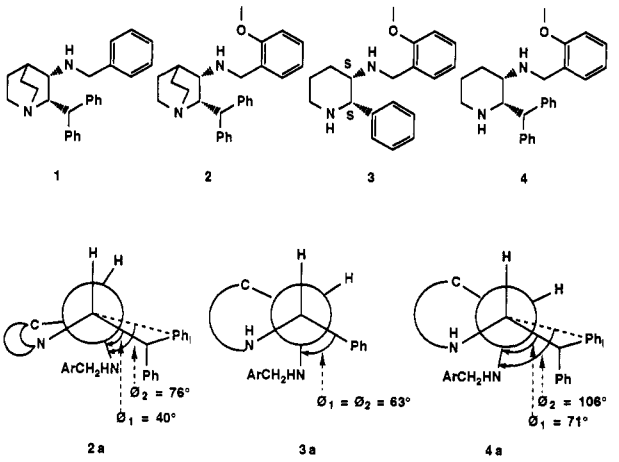
Substance P,[1] an 11 amino acid peptide,[2] is implicated in the pathogenesis of diverse diseases such as arthritis, asthma, and inflammatory bowel disease.[3] We used a chemical file screening approach that led to the discovery of (±)-1 as an initial lead structure; suitable structural modifications culminated in discovery of **2** (CP-96,345) as a potent inhibitor of binding of [^{3}H] SP at the NK-1 receptor.[4,5] We now reveal *specific geometrical parameters* that make the design of further substance P antagonists more facile. We disclose **3** (CP-99,994) in which the C-2 phenyl substituent is indicated by molecular modeling to occupy the same space as the inner phenyl ring of the benzhydryl group in **2**. *We report that* **3** *is the most potent SP antagonist yet discovered* and that the biological activity resides exclusively in (+)-(2*S*,3*S*)-**3** (Table I).

Following the identification of lead structure (±)-1, our approach to determining the three-dimensional pharmacophore structure initially involved assessment of the relative spatial orientation of the C-2 and C-3 substituents; we postulated that the dihedral angle (ϕ_1) between these groups was critical for activity. As a close approximation,



we argued that ϕ_1 determines the accesibility of the biorelevant conformation between free rotating C-2 and C-3 aromatic groups. Subtle trends in the receptor binding properties of several related analogs and insights gained from molecular modeling, however, suggested that this was an oversimplification. The benzhydryl group in **2** dictates that one of the phenyl rings will always be situated in front of the C-3 benzylamino group. We therefore surmised that the position of the inner phenyl ring (Ph_i) of the benzhydryl group relative to the C-3 benzylamino group must be important for activity; we further recognized that this relationship can be represented by the pseudo dihedral angle ϕ_2 rather then ϕ_1. ϕ_2 represents the dihedral angle between the center of the inner phenyl group, C-2, C-3 and the benzylamino nitrogen; ϕ_1 and ϕ_2 for **2** are 40° and 76°, respectively (Newman projection formulas **2a**).[6]

To explore the consequences of changing ϕ_1 and ϕ_2, we first synthesized **4** in which the piperidine ring (locked in a boat form in **2**) is now in a chair conformation. The position of Ph_i relative to the C-3 benzylamino group changes dramatically; the ϕ_2 for **4** is 106° as opposed to 76° for **2**. As a consequence, **4** has a greatly decreased affinity for the substance P receptor relative to **2** (Table I). To compensate for this increase in ϕ_2 in going from **2** to **4**, we conceived of **3**, in which ϕ_1 and ϕ_2 would be identical. Most importantly, the ϕ_2 (63°) for **3** is comparable to that of **2** (Newman projection formulas **2a**, **3a**, and **4a**). Significantly, the much smaller (±)-**3** had 2 times more affinity for the SP receptor than (±)-**2** (Table I).[7] The intersection map between **2** and **3** shows that the volume occupied by **3** is within that of **2** and that the C-2 phenyl group of **3** mimic the Ph_i of **2** (Figure 1). It is instructive to note that the locked-in boat piperidine ring of **2** accounts for this similarity. The NK-1 receptor, clearly, recognizes both aromatic groups of **3**. The freely rotating nature of the C-3 substituent makes it difficult to deduce the biorelevant orientation of aromatic rings when bound to the receptor. The critical aromatic rings in the X-ray crystals structures of **2** and **3** are parallel, and incidentally this is also the lowest-energy conformer

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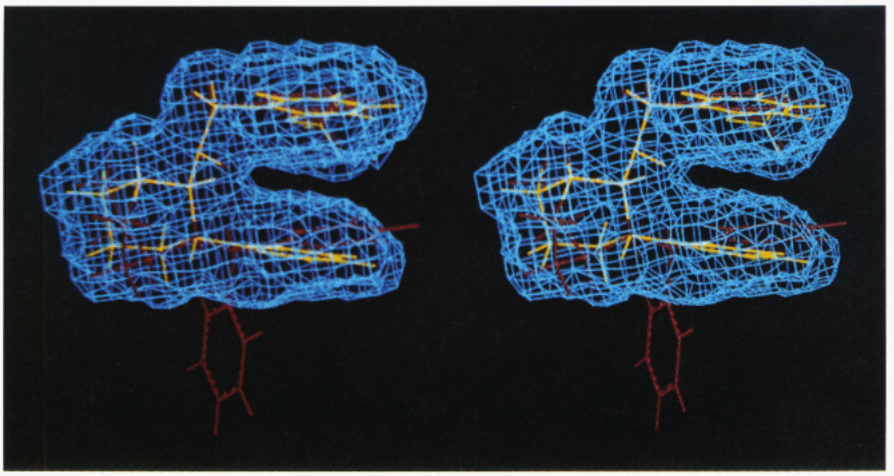


Figure 1. Stereoscopic view of van der Waals intersection map between **2** (red) and **3** (yellow).

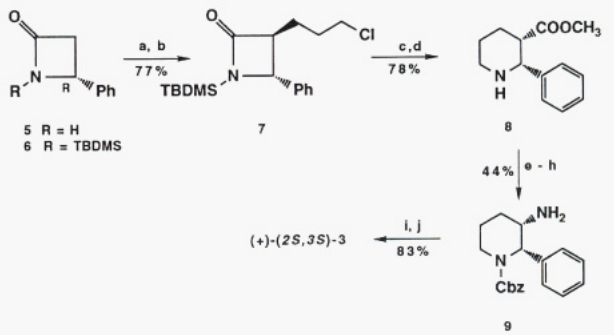
Table I. In Vitro Binding Affinity for the NK-1 Receptor in Human IM-9 Cell Using [^{125}I]-BH-SP of Substance P Antagonist[4,12]

compd	K_i (nM)	compd	K_i (nM)
(±)-**2**	1.1 ± 0.25	(±)-**3**	0.48 ± 0.2
(−)-**2**	0.66 ± 0.26	(+)-**3**	0.17 ± 0.04
(±)-**4**	49	(−)-**3**	>10 000

obtained for these compounds.[6] It is possible that steric factors at the receptor may induce parallel orientation between the two aromatic rings. Perhaps the two aromatic groups are stacked as shown in Figure 1, and the receptor surrounds the local lipophilic environment.

The enantiospecific synthesis of (+)-(2*S*,3*S*)-**3** commenced with the N-protection of (−)-(4*R*)-4-phenyl-2-azetidinone[8] (**5**, TBDMS-Cl, Et_3N, 25 °C, 99%) to give **6**, followed by a highly stereoselective alkylation with 1-bromo-3-chloropropane ($LiEt_2N$, −50 °C, THF) to furnish the trans product **7** ($J_{2,3}$ = 3 Hz) in 78% yield; none of the cis isomer could be detected in the ^{1}H NMR of the crude product. Compound **7** was transformed into **8** via a two step sequence: (1) simultaneous removal of the TBDMS group and hydrolysis of the β-lactam **7** (5% H_2SO_4 in methanol, reflux, 1 h) yielded the aminomethyl ester ($J_{2,3}$ = 11 Hz) in quantitative yield, and (2) cyclization of the crude product in DMF (100 °C, 15 min) in the presence of sodium iodide and sodium bicarbonate afforded **8** (78% from **7**).[9] The optical purity of **8** was assessed by

Scheme I [a]



[a] Reagents: (a) TBDMS-Cl, Et_3N; (b) $LiEt_2N$, −50 °C, $Br(CH_2)_3Cl$; (c) MeOH, H_2SO_4; (d) NaI, $NaHCO_3$, DMF, 100 °C; (e) Cbz-Cl, Et_3N, CH_2Cl_2; (f) Me_3Al, NH_4Cl, 50 °C, (g) LTA, *t*-BuOH; (h) EtOAc–HCl; (i) 2-(MeO)PhCHO, $NaBH_3CN$; (j) Pd/C, NH_4OCOH–Et_2O–HCl.

comparison of the ^{1}H-NMR spectra of MTPA amides of **8** and racemic **8** synthesized from racemic **5**; none of the other enantiomer could be detected. Next the stereospecific conversion of the carbomethoxy group into an amino group which was attained via a four-step sequence: (1) N-protection (benzyl chloroformate, TEA, 25 °C, 18 h), (2) conversion of the carbomethoxy group to a carboxamide (trimethylaluminum, ammonium chloride, 50 °C),[10] (3) oxidation of the carboxamide to an *N*-Boc group via Hoffmann degradation (lead tetraacetate, *t*-BuOH, reflux 3–5 h),[11] and (4) cleavage of the Boc group (EtOAc–HCl, 25 °C, 2–4 h) to yield **9** (44% from **8**). Reductive amination of **9** with *o*-methoxybenzaldehyde followed by cleavage of the Cbz group afforded (+)-(2*S*,3*S*)-**3** isolated as dihydrochloride salt (83%, mp 255 °C, ethanol $[\alpha]_D$ = +77° (*c* = 1, MeOH)). Similarly, following the chemistry described in Scheme I but starting with (−)-(3*S*)-4-phenyl-2-azetidinone, (−)-(2*S*,3*R*)-**3**, was obtained, mp 250 °C, ethanol $[\alpha]_D$ = −78° (*c* = 1, MeOH)). Significantly, the (+)-(2*S*,3*S*)-**3** isomer has greater than 1000-fold affinity for the SP receptor than (−)-(2*R*,3*R*)-**3**.[12] Additional work to further refine the geometrical properties influencing recognition of (+)-(2*S*,3*S*)-**3** will be reported in due course.

(6) In these studies structures were drawn and used as an initial input to the empirical energy program within SYBYL (version 5.5; Tripos Associates Inc.) and total geometry optimization was performed. Next the rotational energy profile of the C-2 and C-3 substituents was investigated. The Powell minimization method in vacuo and Tripos force field parameters were used. In these studies optimized geometries were obtained using SYBYL's systematic search algorithm which calculates energies as a function of torsional angle. For all compounds freely rotatable bonds were searched. The lowest-energy conformers thus obtained for each compound were used to calculate ϕ_1 and ϕ_2. These studies were performed using Silicon Graphic's IRIS-4D220 workstation.

(7) Syn orientation of the C-2 and C-3 substituents, as measured by ϕ_2, permits both the C-3 benzylamino side chain and C-2 phenyl to acquire biorelevant conformation. When this distinct spatial disposition is attained, an introduction of an ortho methoxy group consistently increases the potency. Indeed before the recognition of the beneficial effect of a ortho methoxy group,[4c] compounds with C-3 benzylamino side chain were synthesized; introduction of an ortho methoxy group invariably increased affinity of compounds for the substance P receptor.

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Supplementary Material Available: Crystallographic data for (±)-3 (9 pages). Ordering information is given on any current masthead page.