Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 5, No. 4, pp. 377-380, 1995

0960-894X/95 $9.50+0.00

0960-894X(95)00039-9

STRUCTURE-ACTIVITY STUDIES OF THE NATURAL PRODUCT SUBSTANCE P ANTAGONIST WIN 64821

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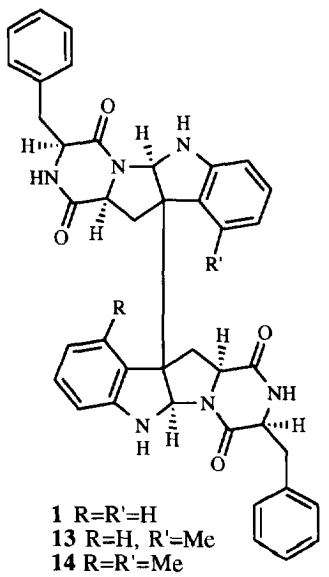
Abstract: Tryptophyl alkylation at C3a of the diketopiperazine **2** gave compounds related to the substance P antagonist WIN 64821 (**1**). The biological activities of these compounds, and those of methyl analogs **13** and **14** produced by directed biosynthesis, gives structure-activity information for **1**.

Substance P (SP) is the endogenous ligand for the neurokinin-1 (NK-1) receptor and plays an important role in pain and inflammation.[1] A competitive antagonist to SP at the NK-1 receptor is potentially useful as a non-narcotic analgesic or as an anti-inflammatory agent. Recently we reported the discovery of WIN 64821 (**1**), a potent and competitive antagonist to SP at the NK-1 receptor.[2] WIN 64821 was isolated from an *Aspergillus* sp. and is a dimeric diketopiperazine as shown. Retrosynthetically **1** can be envisioned to arise from dimerization of two molecules of **2**, which is the biosynthetic mechanism.[2b] Formation of a C-C bond at C3a of the indole in **2** is non-trivial. Owing to the structural complexity of WIN 64821 and the anticipated difficulty in preparing analogs, we decided to produce analogs of **1** via directed biosynthesis.[2b]



1 R=R'=H
13 R=H, R'=Me
14 R=R'=Me

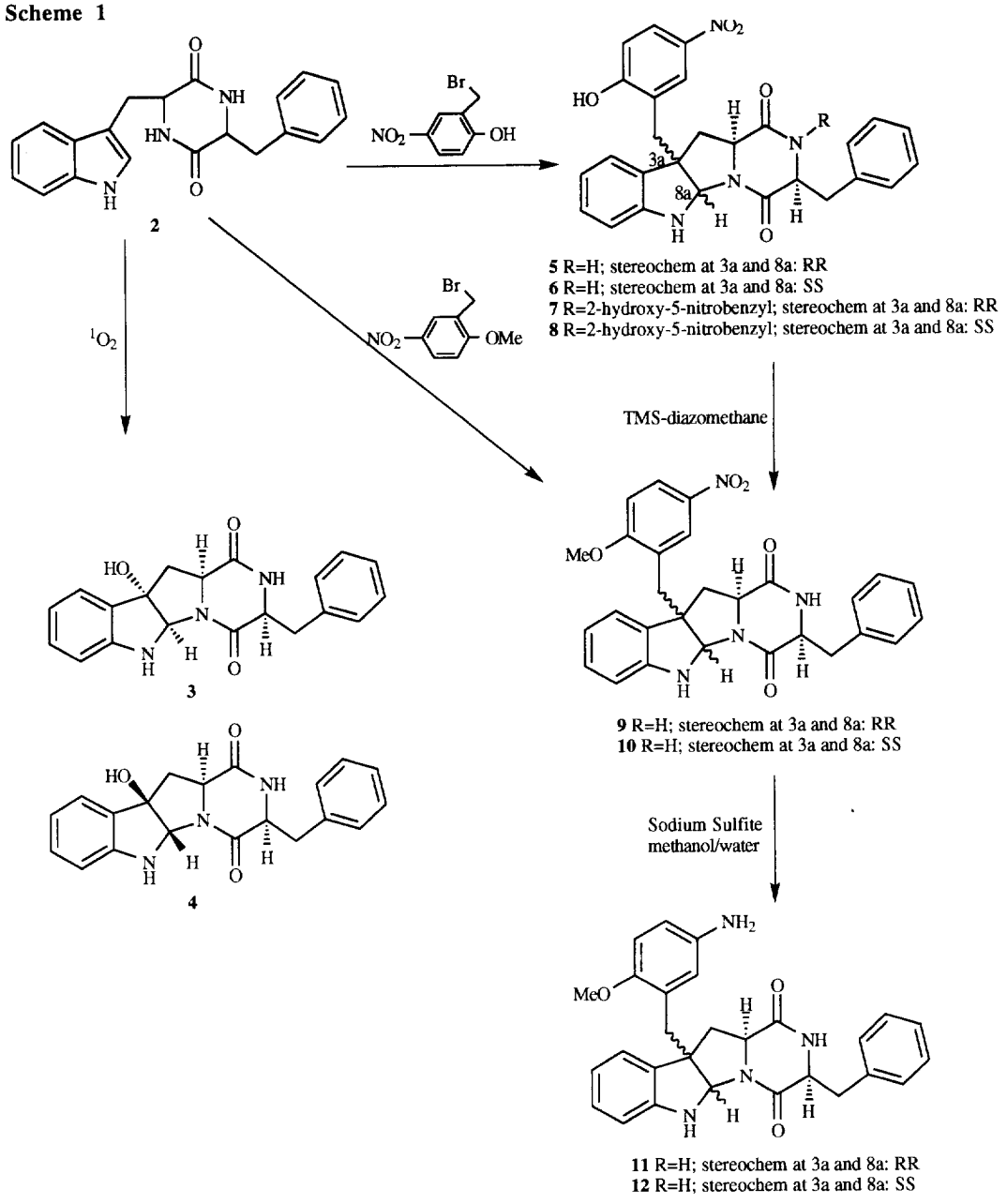
Feeding 4-methyltryptophan into the fermentation broth gave a mixture of **1**, **13** and **14**. These compounds were separated using HPLC with a C-18 column. Compounds **13** and **14** are equivalent to **1**, but have a bulky methyl substituent on one or both of the indoline moieties. NMR analysis (as performed for **1**)[2a] confirmed that the solution conformations of **13** and **14** are not significantly different to the solution conformation of **1**, indicating that the 4-methyl substituents do not influence global molecular conformation. Compounds **1** and **13** are potent substance P antagonists (IC_{50}'s of 0.24 μM and 0.44 μM respectively), while **14** (IC_{50}'s of 5.7 μM) is considerably less potent than **1**. The fact that the single methyl substituent of **13** does not diminished binding activity and the second methyl group of **14** does, indicates that either only one indoline moiety is involved in receptor binding, or that the binding pocket for one of the indoline groups is more sensitive to steric changes than is the other. In either case **1** must bind non-symmetrically to the NK-1 receptor. Therefore, the symmetry of **1** is not essential for high binding affinity.

The fact that **1** binds in a non-symmetrical manner encouraged us to produce simplified synthetic compounds for structure activity studies, by extending the monomeric subunit of **1**. Firstly, we produced compounds **3** and **4**, which are equivalent to one half of **1**, by reaction of **2** with oxygen in the presence of Rose Bengal. Diastereoisomers **3** and **4** were separated using HPLC and identified by comparison with literature data.[2a,3] Both **3** and **4** were found to be inactive (IC_{50} > 100 μM) in the substance P binding assay,[2c] indicating that at least part of the second diketopiperazine unit is necessary for the biological activity of **1**.

To mimic the second indoline of **1** we attempted to introduce a third aromatic group into **2** via benzylation at the 3a position. Only benzylation with 2-hydroxy-5-nitrobenzyl bromide was successful, yielding the monoalkylation products **5** and **6** (each in 30% yield)[5] and the dialkylation products **7** and **8** (each in 15% yield) (Scheme 1). Successful benzylation required the presence of both the 2-hydroxy and 5-nitro substituents, suggesting that benzylation goes through an intermediate quinone methide. The relative stereochemistries of these compounds were assigned by comparisons of their CD spectra with those of **1**, **3**, **4** and ditryptophenaline.[2a] Compound **5**, which has the same ring junction stereochemistry as **1**, was found to be the most active of the four compounds in our substance P bind assay (IC_{50} 48 μM). The IC_{50} for **6** was 98 μM and compounds **7** and **8** were inactive (IC_{50} > 100 μM). Low energy conformations of **5**, generated using the MULTIC method with MACROMODEL,[2a] indicate good overlap of the aromatic functionality's with the corresponding groups in **1**. The 2-hydroxy group was anticipated to be a good mimic for the amino group on the second indoline of **1**, and these functionality's overlapped in one of the low energy structures for **5** overlaid with **1**. Conversely, the *N*-benzyl group of **7** does not overlap with any part of structure **1**. This lack of overlap, in conjunction with the low receptor affinity of **7**, (relative to **5**) indicates that N-alkylation leads to a decrease in receptor affinity for this series of compounds.

The low binding affinities of **5** and **6**, as compared with **1**, indicates that the phenolic group is probably not a good mimetic for the second indoline nitrogen of **1**, even though these groups can occupy similar spatial positions. In light of the relatively low binding affinity of **5** and **6**, and indications that a methoxy substituents on an aromatic ring enhances the receptor affinity of other SP inhibitors,[6] we sought to methylate the phenolic group in compounds **5** and **6**. Alkylation of **2** with 2-methoxy-5-nitrobenzyl bromide at elevated temperatures gave low yields of **9** and **10** (<10% in each case). However, treatment of **5** and **6** with TMS-diazomethane gave **9** (90% yield) and **10** (95% yield), respectively. Disappointingly, the biological activities of **9** (54 μM) and **10** (88 μM) were not significantly different from those of **5** and **6**.

Scheme 1



In an attempt to mimic the indoline of **1** with an analine, compounds **9** and **10** were converted to **11** and **12**, respectively. Reduction of the nitro group in **9** and **10** to the corresponding amino group appears to increase receptor affinity. However, the receptor affinity of **11** (IC_{50} 26 μM) and **12** (IC_{50} 38 μM) is still approximately 100-fold less than that of **1** (IC_{50} 0.24 μM). The lower receptor affinity of **11**, relative to **1**, could be attributed

to: (1) the amino group of **11** not being able to occupy the same spatial position as the second indoline amino group of **1**; (2) the methoxy group of **11** sterically inhibiting binding; or (3) the second phenylalanyl group of **1** being crucial for biological potency. It is also possible that these monomeric compounds are binding to a completely different site on the NK-1 receptor than is **1**.

In summary, the assay results for **13** and **14** indicate that the symmetry of **1** is not necessary for high binding affinity. The lack of activity of compounds **3** and **4** indicates that more than half of the symmetrical dimer **1** is necessary for bioactivity. The relatively low receptor affinity of compounds **5-12** as compared to **1** indicates that the substituted benzyl moieties in **5-12** are not good mimetics, in terms of their binding properties, for the second monomeric subunit of **1**. Further work is needed to map out the binding site of the second monomeric subunit of **1**, and to produce a potent non-symmetrical synthetic analog of **1**.

Acknowledgment: We thank Jan Popp and David Houck for the production of compounds **13** and **14**. We also thank David Sedlock, Howard Sun, Amanda Gillum and Raymond Cooper for helpful discussions.

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5. Characterization data for selected compounds: Compound **5**. MP 186-188°C; UV δ_{max} (MeOH) 218 nm (ε 28 000), 235 (14000), 302 (7800); CD [θ] (nm) 28500 (239), 3500 (302); IR (KBr) 3420, 1695, 1440, 1350, 1285; HRFABMS (M^+) 484.1727 (Δ 3.7 ppm): ^{1}H NMR (CD_3CN) δ(J) 2.22 dd (8.4, 13.3), 2.61 dd (8.2, 13.2), 2.94 dd (7.1, 14.8), 3.03 d (13.5), 3.21 dd (4.8, 14.8), 3.23 d (13.5), 4.16 dd (8.1, 8.4), 4.23 dd (4.8, 7.1), 5.51 s, 5.59 s, 5.87 s, 6.52 d (8.1), 6.71 dd (7.7, 8.2), 6.92 d (9.0), 7.01 dd (7.6, 8.2), 7.14 d (8.2), 7.20 s, 7.72 d (2.9), 7.92 dd (2.9, 9.0); ^{13}C NMR δ 36.2, 36.4, 39.5, 57.1, 57.6, 58.5, 81.9, 110.7, 116.9, 120.0,125.1, 125.8, 128.2, 129.3, 129.9, 130.0, 130.0, 130.9,130.9, 131.2, 133.7, 138.2, 141.0, 149.8, 164.7, 168.8, 170.9.
   Compound **6**. MP 193-194°C; UV δ_{max} (MeOH) 214 nm (ε 26500), 238 (12800), 302 (6400); CD [θ] (nm) -21000 (241), -7400 (315); FTIR (KBr) 3425, 1680, 1445, 1350, 1298; HRFABMS (M^+) 484.1722 (Δ 4.7 ppm); ^{1}H NMR (CD_3CN) δ(J) 1.78 dd (11.4, 12.3), 2.45 dd (5.6 12.3), 2.91 d (13.4), 3.02 dd (6.3, 14.3), 3.08 d (13.5), 3.15 dd (4.8, 14.3), 3.77 dd (5.6, 11.4), 4.28 dd (4.8, 6.3), 5.22 s, 5.44 s, 6.07 s, 6.48 d (7.6), 6.70 dd (8.2, 8.4), 6.92 d (9.0), 7.00 dd (7.6, 8.2), 7.05 d (8.4), 7.26 m, 7.55 d (2.9), 7.93 dd (2.8, 9.0); ^{13}C NMR δ 36.9, 37.8, 40.6, 57.2, 59.8, 61.7, 80.0, 110.3, 116.5, 120.0, 125.3, 125.8, 128.2, 129.3, 129.9, 130.1, 130.1, 131.2, 131.2, 131.4, 133.6, 138.0, 141.2, 151.9, 164.1, 169.8, 171.7.
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(*Received in USA* 24 *October* 1994; *accepted* 3 *January* 1995)