BMS-192548, a Tetracyclic Binding Inhibitor of Neuropeptide Y Receptors, from *Aspergillus niger* WB2346

II. Physico-chemical Properties and Structural Characterization

YUE-ZHONG SHU, JINGFANG QIAN CUTRONE, STEVEN E. KLOHR and STELLA HUANG

Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492, U.S.A.

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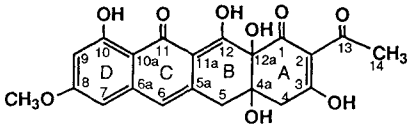
The structure of BMS-192548, a tetracyclic binding inhibitor of neuropeptide Y receptors, was established by spectroscopic methods. The compound has an unusul B-C-D ring β-diketone moiety.

During the screening of microbial fermentation extracts for their ability to inhibit the binding of ^{125}I-peptide YY (PYY) to the neuropeptide Y (NPY) receptor using the scintillation proximity assay (SPA), we have isolated BMS-192548 (**1**) from the extract of *Aspergillus niger* WB2346 by bioassay-guided fractionation. Compound **1** showed the inhibitory activity against ^{125}I-PYY binding to SK-N-MC and SMS-KAN cells, which expressed NPY_1 and NPY_2 receptors, respectively. The taxonomy, fermentation, isolation and biological activities are the subjects of the preceding paper.[1] While elucidating its structure we recognized the identical molecular formula and structural similarity between **1** and TAN-1612 (**2**), a substance P inhibitor (Fig. 1) recently isolated from *Penicillium claviforme*,[2] the clear difference in physico-chemical properties, however, did indicate that **1** is a regio- and possibly stereo- isomer of **2**. Herein we wish to report our studies of the structural characterization of **1**.

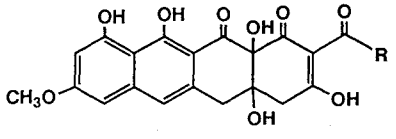
Results and Discussion

BMS-192548 (**1**) isolated as yellow prisms showed a molecular formula of $C_{21}H_{18}O_9$, identical to that of **2**, by high resolution MS analysis (Table 1). The UV spectrum (Fig. 2) also resembled that of **2** with the λ_{max} at 414 nm due to a partially reduced naphthacenone/naphthacenol chromophore.[3] However, some critical physico-chemical properties of **1** such as mp, $[\alpha]_D$ (Table 1), IR (Fig. 3) and NMR data (Figs. 4 and 5, Tables 2 and 3) are markedly different from those of **2**. Compound **1** was soluble in dimethyl sulfoxide and methanol, but was scarcely soluble in chloroform, whereas **2** was well dissolved in chloroform so that its NMR data were acquired in $CDCl_3$.[2] All of these evidence suggested that **1** may be a regio and/or stereo isomer of **2**. Efforts were made to obtain a desirable crystal of **1** or its methyl ether for X-ray crystallographic analysis, but were unsuccess-

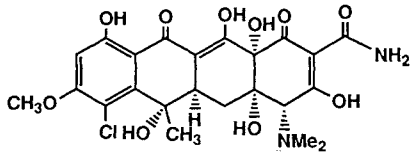
Fig. 1. Structures of related compounds.



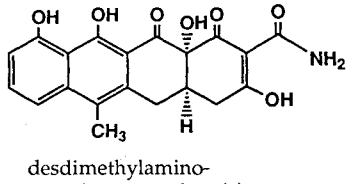
BMS-192548 (**1**)



R=CH_3 TAN-1612 (**2**)
R= NH_2 TAN-1652 (anthrotainin, **3**)



dactylocyclinone (**4**)



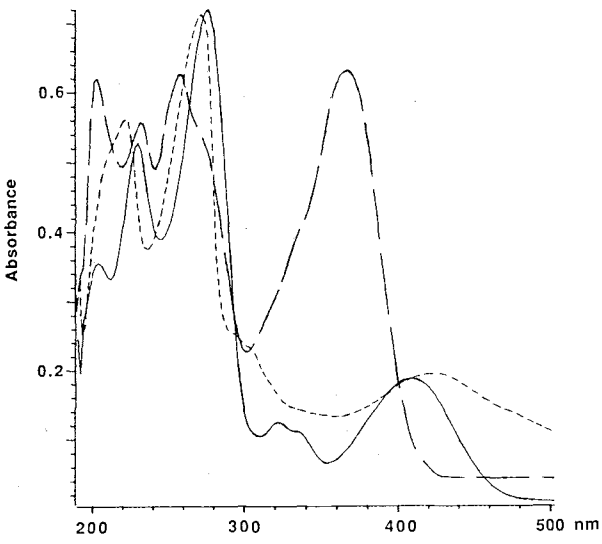
desdimethylamino-anhydrotetracycline (**5**)

Table 1. Physico-chemical properties of BMS-192548 (**1**).

Appearance	Yellow prisms from toluene-ethanol (5:1)
MP	254°C (dec)
Molecular formula	$C_{21}H_{18}O_9$
High resolution MS	Found 414.0965 Calcd. 414.0950
$[\alpha]_D$ (20°C)	+359° (*c* 0.04, CH_3OH)
UV λ_{max} (CH_3CN) nm (log ε)	280 (4.4440), 320 (sh 3.5548), 414 (3.8822)
IR (KBr) ν cm^{-1}	3420, 1640, 1590, 1530, 1395, 1355, 1230, 1155
CD λ_{max} (CH_3OH-0.05 N HCl, 1:1) nm	$[\theta]_{223}$ 2.702×10^4 $[\theta]_{241}$ -1.773×10^4 $[\theta]_{291}$ 2.327×10^4 $[\theta]_{322}$ 6.247×10^4

Fig. 2. UV spectra of BMS-192548 (**1**), dactylocyclinone (**4**) and des-*N*-dimethylaminoanhydrotetracycline (**5**).

—— (**1**), – – – (**4**), ------ (**5**).



ful. On the other hand, the ^1H NMR data and ^{13}C NMR signal assignments were not described for **2**,[2] making the rigorous spectral comparison of **1** with **2** difficult. We thus elected to carry out our own thorough NMR spectral analysis for the structural characterization of **1**.

The ^1H and ^{13}C NMR chemical shifts of **1** (Tables 2 and 3) showed some similarity to those reported for **2**[2] and **3**, the closely related compound anthrotainin (TAN-1652).[4] The signals due to the following characteristic functional groups were observed; an acetyl (C_{13}, δ 196.7; C_{14}, δ 31.2; 14-H, δ 2.31), a 1,2,3,5-tetrasubstituted phenolic ring containing a strongly hydrogen-bonded phenol (C_{10}, δ 163.4; 10-OH, δ 14.56), an aromatic methoxy (C_8, δ 162.3; O<u>C</u>H_3, δ 55.1; OC<u>*H*</u>$_3$, δ 3.75) and two *meta*-coupled aromatic CH (C_7, δ 97.6; 7-H, δ 6.33 and C_9, δ 97.3; 9-H, δ 6.06), a non-coupled vinyl CH (C_6, δ 111.7; 6-H, δ 6.32), two methylenes (C_4, δ 46.3 and C_5, δ 38.9), two quaternary carbinols (C_{4a}, δ 71.1; 4a-OH, δ 4.99 and C_{12a}, δ 80.7; 12a-OH δ 5.30), an enol carbon (C_{12}, δ 176.4) and three carbons having ketone nature (C_1, δ 194.1; C_3, δ 192.6; C_{11}, δ 194.4). The ^1H-^{13}C long range couplings (Fig. 6) observed from HMBC and COLOC spectra unambiguously confirmed the substitution pattern on the phenolic ring (D ring); the coupling between 7-H and C_6 indicated the D ring adjacent to the C_6 vinyl of C ring; the couplings of 6-H *versus* C_5 and C_{11a} in turn revealed the connectivity of the C_{5a}-C_6 double bond extended to the isolated C_5 methylene in B ring. The presence of β-keto enol groups at C_1 and C_3 was suggested by the ^{13}C chemical shifts of C_1, C_2 and C_3 (Table 3); the two isolated methylenes at C_5 and C_4 and two hydroxyl hydrogen at C_{4a} and C_{12a} showed extensive long range correlations (Fig. 6)

Fig. 3. IR Spectrum of BMS-192548 (**1**) (KBr pellet).

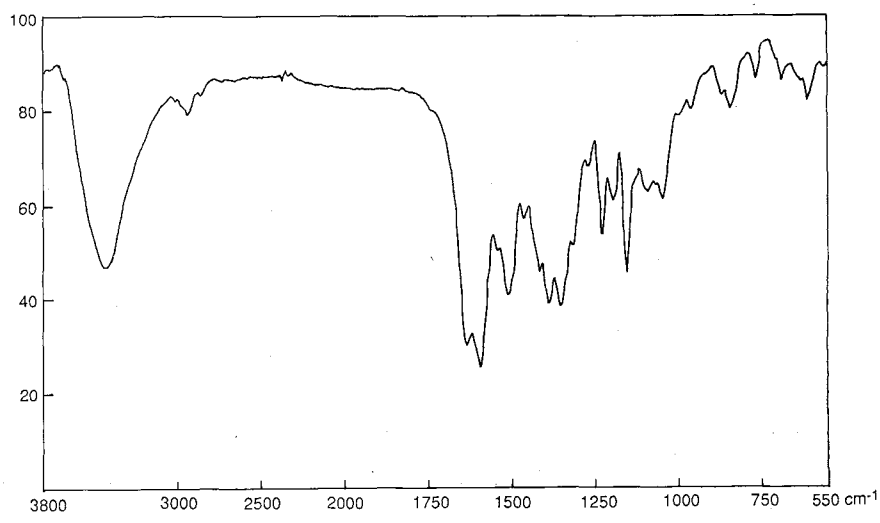


Fig. 4. ^{1}H NMR spectrum of BMS-192548 (**1**) (500 MHz, DMSO-d_6).

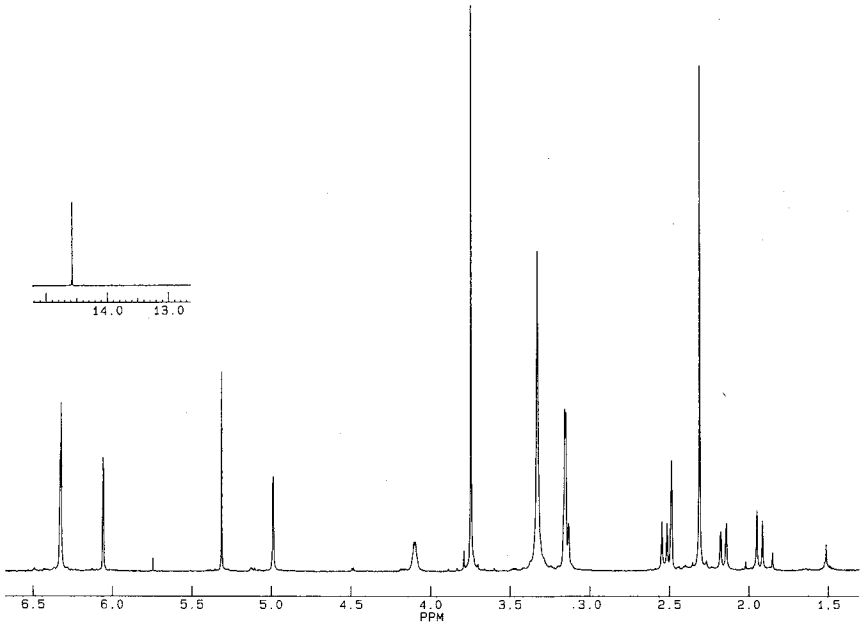
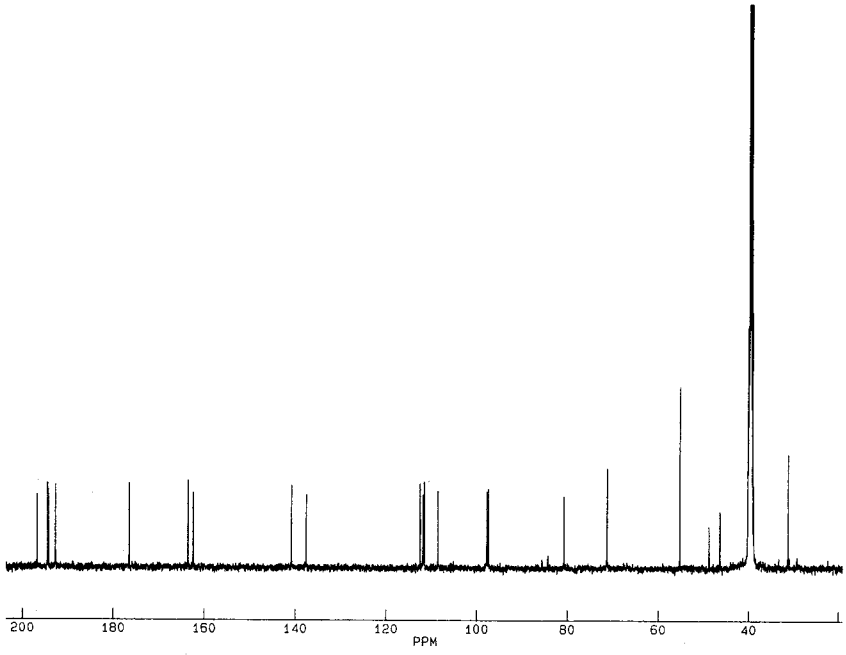


Fig. 5. ^{13}C NMR spectrum of BMS-192548 (**1**) (125 MHz, DMSO-d_6).



with neighboring carbons in HMBC and *vice versa* in COLOC including the critical correlation between C_2 and 4-H_A; these findings enabled the establishment of the A-B ring structural segment except the long range couplings across the double bond C_1-C_2 and of the quarternary carbon C_{12} were not observed. Thus, the remaining structural fragments to be placed into the backbone and side chain of **1** were the ketone carbonyl at δ 194.4, the enol carbon at δ 176.4, and the acetyl group (δ 196.7 and δ 31.2), which biosynthetically seems most reasonable to be attached at C_2.

The ketone and enol groups in **1** could have been placed at C_{12} and C_{11}, respectively, same as those in **2** and **3**, but such assignment was not consistent with the NMR data of **1** when compared with those of **3** (assigned NMR data were not available for **2**). First, the distinct differences in the chemical shifts of C, D ring protons (6-H, 7-H and 9-H) and C_6, C_{10} carbons between **1** and

3 were observed (Tables 2 and 3), these are most likely attributed to a different functionality at C_{11} in **1** from that in **3**. Secondly, the chemical shift of C_{12} (δ 176.4) in **1** was remarkably downfield shifted by *ca.* 10 ppm relative to the C_{11} enol carbon in **3** (δ 166.7), the resonance however appeared to be same to that of the C_{12} enol carbon (δ 176.4) in dactylocyclinone (**4**), a naturally occurring tetracycline analog,[5] implying that the enol functionality in **1** may be located at C_{12} rather than at C_{11}. Finally, the chemical shift of C_{10} phenolic hydroxyl proton (δ 14.56) in **1** strongly suggested that this hydroxyl existed in hydrogen-bonding with a *peri*-ketone group similar to the C_{10} hydroxyl (δ 12.5) in **4**, since the tautomeric form of the C_{11}-C_{11a}-C_{12} β-diketone segment in **2** and **3** should render the 10-OH participating a hydrogen-bonding with a *peri*-hydroxyl group to resonate at 9~10 ppm in ^{1}H NMR as those observed for des-*N*-dimethylaminoanhydrotetracycline (**5**) and chromomycinone derivatives,[6] unfortunately the chemical shifts of the corresponding 10-OH in both **2** and **3** were not documented.[2,4] Thus, the NMR data of **1** seemed to be most consistent with the C_{11}-keto C_{12}-enol tautomer of **2**. (Fig. 1)

It is particularly of interest that the B-C-D ring phenolic diketone tautomer like **1** can stably exist and be isolated. Though the tautomerism of 11,12-diketone is conceivable, one often infers that the tautomer form in **2** would prevail over that in **1**. In fact, the energy difference (5.6 kcal/mol) between **1** (heat of formation, $\Delta H = -296.9$ kcal/mol) and **2** ($\Delta H = -302.5$ kcal/mol), which were estimated by the AM1 molecular orbital calculation method, indicated that **2** is more thermodynamically favorable than **1**. The reason for the existence of **1** is at present unknown, but it may be partially due to a different stereochemistry of **1** at C_{4a} and C_{12a} from that of **2**, which gives rise the stabilization of the

Table 2. ^{1}H NMR data of BMS-192548 (**1**) and anthrotainin (TAN-1652, **3**).

Proton	**1** δ_H (mult, J=Hz)	**3*** δ_H (mult, J=Hz)
4-H_A	1.92 (d, 17.8)	2.84 (s)
4-H_B	2.15 (d, 17.8)	2.84 (s)
4a-OH	4.99 (s)	
5-H_A	2.55 (d, 16.5)	3.41 (br s)
5-H_B	3.13 (d, 16.5)	3.41 (br s)
6-H	6.32 (s)	6.90 (s)
7-H	6.33 (d, 2.3)	6.49 (d, 2.0)
8-OCH_3	3.75 (s)	3.87 (s)
9-H	6.06 (d, 2.3)	6.55 (d, 2.0)
10-OH	14.56 (s)	
12a-OH	5.31 (s)	
14-H_3	2.31 (s)	
13-NH_2		9.01 (s)

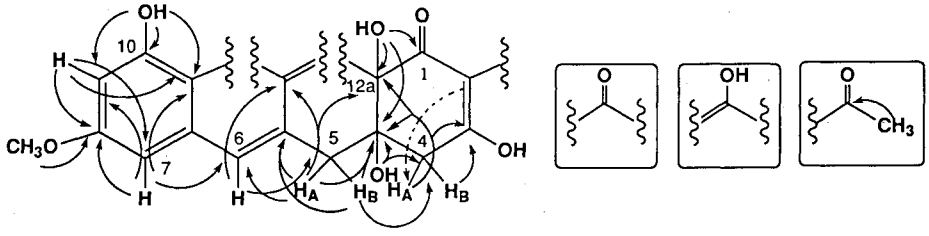
* Data taken from Ref. 4.

Table 3. ^{13}C NMR data of BMS-192548 (**1**) and related compounds.

Carbons	**1*** $^{13}\delta$ (m)	**3** $^{13}\delta$ (m)	**4*** $^{13}\delta$ (m)	Carbons	**1*** $^{13}\delta$ (m)	**3** $^{13}\delta$ (m)	**4*** $^{13}\delta$ (m)
1	194.1 (s)	192.1 (s)	193.1 (s)	8	162.3 (s)	163.2 (s)	163.6 (s)
2	112.4 (s)	98.0 (s)	101.0 (s)	8-OCH_3	55.1 (q)	55.6 (q)	56.9 (q)
3	192.6 (s)	194.3 (s)	191.7 (s)	9	97.3 (d)	101.1 (d)	100.5 (d)
4	46.3 (t)	42.1 (t)	69.7 (d)	10	163.4 (s)	159.3 (s)	162.0 (s)
4a	71.1 (s)	72.3 (s)	78.5 (s)	10a	111.4 (s)	108.2 (s)	112.1 (s)
5	38.9 (t)	37.9 (t)	26.4 (t)	11	194.4 (s)	166.7 (s)	190.1 (s)
5a	137.4 (s)	135.4 (s)	40.8 (d)	11a	108.4 (s)	106.8 (s)	104.4 (s)
6	111.7 (d)	117.5 (d)	74.5 (s)	12	176.4 (s)	196.6 (s)	176.4 (s)
6a	140.6 (s)	141.4 (s)	149.2 (s)	12a	80.7 (s)	82.1 (s)	74.3 (s)
7	97.6 (d)	99.5 (d)	109.5 (s)	13	196.7 (s)	173.5 (s)	172.2 (s)
				14	31.2 (q)		

* in DMSO-d_6; data of **3** taken from Ref. 4; data of **4** taken from Ref. 5.

Fig. 6. Selected long range ^{1}H-^{13}C correlations in structural moieties of BMS-192548 (**1**) observed in HMBC (H⟶C) and COLOC (C⇢H).

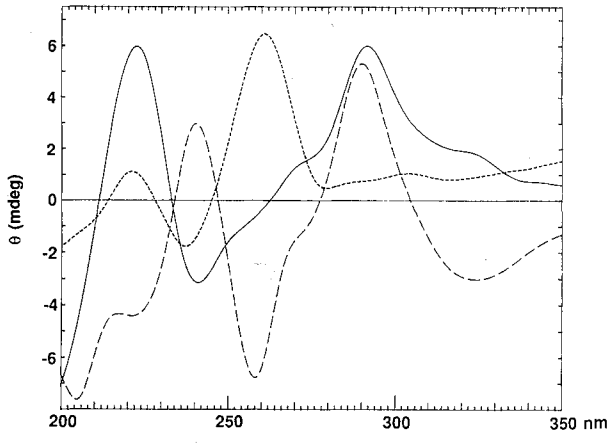


tautomeric form of **1**. While the relative stereochemistry of A-B ring junction in **3** was studied by X-ray crystallographic analysis and was shown to be *cis*, the stereochemistry of **2** was not reported, providing no directly comparable evidence to that of **1**. The circular dichroism (CD) spectra of tetracycline analogs including **4** were well studied,[7] the stereochemistry at ring junctures C_{4a}, C_{12a} and C_{5a} are the principal factors contributing to the chirality of the B-C-D ring chromophore of tetracycline. Therefore, BMS-192548 (**1**), by lacking the C_{5a} asymmetry, should demonstrate some differences in CD spectrum from those of typical tetracyclines, for the purpose of comparison two stereochemically known compounds with or without C_{5a} asymmetry (**4** and **5**, respectively) were also used in the CD study based on exciton coupling theory.[7] As previously described,[7,8] compounds **4** (dashed line in Fig. 7) and **5** (dotted line in Fig. 7), both having *cis* A-B ring juncture showed intense positive Cotton effect at 290 nm and 261 nm, respectively, due to the so-called exciton coupling of A ring chromophore to the B-C-D ring chromophore. Compound **1** demonstrated the CD spectrum (solid line) with the first positive Cotton effect at 291 nm and the second negative one at 241 nm, (Fig. 7, Table 1) which appeared to be similar to that of **4** (dashed line), and grossly similar to but bathochromically shifted from that of **5** (dotted line), suggesting that the absolute stereochemistry of **1** at A-B ring juncture may possibly resemble that of **4**, *i.e.* the 4a-*S*- and 12a-*S*-configuration. To clarify this possibility and the difference of stereochemistry at C_{4a} and C_{12a} between **1** and **2**, further direct comparison of the two compounds is needed.

In conclusion, we have established the structure of **1** by spectroscopic methods as shown in Fig. 1, the structure represents an unusual C_{11}-keto C_{12}-enol tautomer and possibly diastereomer of **2**.

Fig. 7. CD spectra of BMS-192548 (**1**), dactylocyclinone (**4**) and des-*N*-dimethylaminoanhydrotetracycline (**5**).

—— (**1**), – – – (**4**), ------ (**5**).



Experimental

Materials

BMS-192548 was isolated as described in the preceding paper.[1] Des-*N*-dimethylaminoanhydrotetracycline (**5**) was synthesized from tetracycline (Aldrich) according to the previously described procedures.[9] Dactylocyclinone was provided by Dr. A. TYMIAK and Ms. H. AX of the Research Institute.

Instrumental Analysis

Specific rotations ($[\alpha]_D$) were measured on a Perkin Elmer 241 polarimeter. UV spectra were recorded in MeOH - 0.05 N HCl (1 : 1, v/v) at a Shimadzu UV2100 spectrophotometer. IR spectra were recorded at a Perkin Elmer FT-IR 1800 spectrometer. NMR data, including COSY, DEPT, HETCOR, COLOC and HMBC (increment delay, 0.06 seconds) were taken at a Brucker AM-500 spectrometer (^1H, 500 MHz; ^{13}C, 125 MHz). High resolution mass spectra (HR-MS) were obtained at a Kratos MS50 mass spectrometer with FAB ionization mode at the acceleration voltage of 8.0 kV. Circular dichroism (CD) spectra were measured in MeOH - 0.05 N HCl (1 : 1, v/v) with a Jasco J500A spectropolarimeter, and the sample concentration was 0.01 mg/ml.

Acknowledgment

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