Bioorganic & Medicinal Chemistry Letters, Vol.2, No 12, pp. 1603-1606, 1992
Printed in Great Britain

0960-894X/92 $5.00 + .00

SYNTHESIS OF THIAZOLE AND SELENAZOLE DERIVATIVES WITH AFFINITY FOR THE ODORANT-BINDING PROTEIN.

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(*Received* 10 *September* 1992)

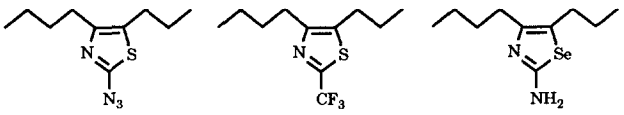
Abstract. Three new ligands for odorant-binding proteins (OBPs) have been prepared, to be used in protein structure studies: the photoaffinity label 2-azido-4-butyl-5-propylthiazole, the NMR probe 2-trifluoromethyl-4-butyl-5-propylthiazole and the X-ray probe 2-amino-4-butyl-5-propylselenazole. All three compounds bind the bovine OBP with dissociation constants in the micromolar range.

Odorant-binding proteins (OBPs) are soluble proteins, abundant in the nasal mucosa of several vertebrates and involved in odour recognition and transduction.[1] The first member of this family was purified from cow nasal tissue;[2-4] other odorant-binding proteins were later identified and purified from different species of vertebrates.[1,5-7]

All the OBPs studied so far bind several classes of odorants with affinities in the micromolar range and broad specificity.[8,9] Thiazoles are among the best ligands, provided they carry hydrophobic substituents. Several derivatives have been synthesized and used in biochemical measurements, in order to define the structural requirements for a tight binding.[10,11] The results have indicated that the best ligands bear an hydrophobic region, either as open hydrocarbon chains attached to positions 4 and 5 of the ring, or as cycloalkanes condensed with the thiazole in the same positions. On the other hand, binding does not seem to be affected by the presence of polar groups in position 2.

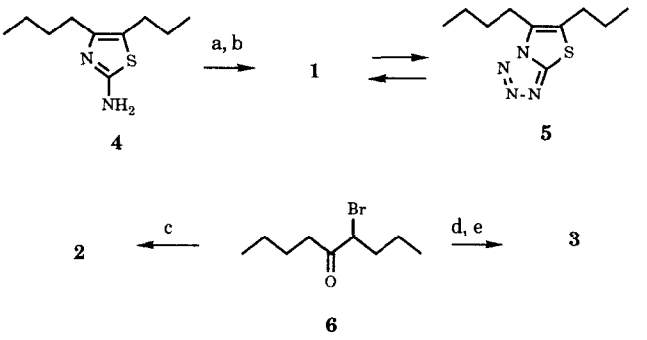
The molecular structure of the bovine OBP has been thoroughly investigated: both the aminoacid sequence[12] and the tridimensional structure in the solid state[13] have been determined. The protein crystallizes as a dimer with a cavity between the two subunits of the right size to accommodate small molecules like odorants. The identification of the actual binding site is the main structural aspect, that remains to be clarified. Its elucidation could represent an important contribution towards the definition of the physiological function of OBPs in the process of odour recognition.

We wish to report here the synthesis and the binding properties to the bovine OBP of azole derivatives **1-3**, which have been designed as probes for the investigation of the OBP binding site.



1 **2** **3**

In particular, azido derivative **1** is a candidate for photoaffinity labelling studies;[14] trifluoromethyl derivative **2** was conceived as a tool to study the receptor site by mean of the NMR spectroscopy;[15] finally, selenium containing derivative 3 should be able to make the identification of the ligand much easier when interpretating X-ray diffraction data of protein crystals grown in the presence of this ligand.[16]



Scheme. Reaction conditions: a) H_2SO_4, $NaNO_2$; b) NaN_3; c) Trifluoroacetamide, P_2S_5; d) selenurea; e) NaOH.

The azido compound **1**[17] was obtained from the 2-aminoderivative **4**[10], by conversion to the diazonium salt and reaction with sodium azide (Scheme), following classical procedures.[10,18,19] As expected,[20,21] this sequence actually yielded a slowly equilibrating mixture of **1** and its tetrazole cyclization product **5**. This was evidenced by two-dimensional TLC (silica gel, hexane : diethyl ether = 1 : 1 as eluant): the purified main reaction product (Rf = 0.6) still exhibited an impurity of Rf = 0.85; a second eluition after a few minutes showed that each single spot, separated in the first eluition, gave rise to the same mixture of two components of Rf 0.6 and 0.85. No attempt was made to determine the equilibrium constant; however, based on the relative intensity of the spots in the TLC, the component of Rf 0.6 seemed to be largely predominant; the ^{1}H-NMR indicated the main product being at least 95% pure. Based on the weak intensity of the band at 2131 cm^{-1} in the IR spectrum of the mixture, which is attributable to the azido group, we believe that the main component in the equilibrium mixture is **5** rather than **1**. Anyway, when this equilibrium mixture was irradiated with UV light (254 nm) in the presence of 1 M lysine, it gave rise to chemical species non migrating on TLC, with the same eluent. Furthermore, neither the azido nor the tetrazole compounds could be detected after UV irradiation, thus demonstrating that both forms can generate nitrene species (as reported in the literature for similar compounds)[22] and are suitable for photoaffinity labelling studies.

The trifluoromethyl substituted thiazole **2**[23] was prepared using one of the well known synthetic routes for the thiazole ring.[24] Accordingly, 4-bromo-5-nonanone **6** was reacted with

trifluoroacetamide and phosphorous pentasulphide, to give the desidered **2** in 27% (Scheme). Using an adaptation of a general synthetic route for 2-aminothiazoles,[18] that also proved effective for 2-aminoselenazoles,[25] the selenazole derivative **3** was synthesized,[26] by reaction of **6** with selenourea and obtained in 73% yield as the hydrobromide (Scheme). The free base was generated from the salt by alcalinization with NaOH, extraction with diethyl ether and recrystallization from isooctane.The same compound was also prepared by reaction of 5-nonanone with selenourea in the presence of iodine, according to a method reported for other selenazole derivatives,[27] but the yield was much lower (around 15%).

All three ligands present a strong and characteristic odour of bell peppers, typical of 2-isobutyl-3-methoxypyrazine, the natural aroma component of bell peppers, and of 4-butyl-5--propylthiazole, the compound taken as a reference in designing the structures of these odorants.

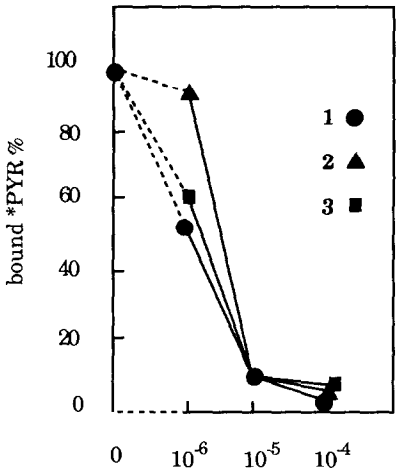


Figure . Competitive binding of ligands **1-3** to a purified sample of bovine odorant-binding proteine (OBP), in the presence of tritiated 2-isobutyl-3-methoxypyrazine (*PYR).

The affinity of these ligands towards the bovine OBP was measured in competitive binding experiments,[28] using tritiated 2-isobutyl-3-methoxypyrazine as the radioactive ligand; the relative inhibition curves are shown in the Figure. The three ligands show significant competition with the radioactive pyrazine, with dissociation constants in the micromolar range. Such values indicate binding affinities, strong enough for intended biochemical studies.

Acknowledgements. We thank Gianfranco Denti (Laboratorio di Chimica Inorganica, Istituto di Chimica Agraria, University of Pisa) for the NMR spectra and Roberto Lorenzi (Dipartimento di Biologia delle Piante Agrarie, University of Pisa) for the mass spectra. This work was supported by a CNR Special Project "Chimica Fine".

References and Footnotes

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17 **2-azido-4-butyl-5-propylthiazole 1** and **tetrazole 5** Viscous brown liquid. MS (20 eV)· m/z 224(M^+,17), 196(100), 181(14), 167(98), 154(75), 139(38), 87(90). IR (film). ν_{max} 2131 (weak), 1464, 1442, 1398, 1192, 1062, 972 cm^{-1}.^{13}C NMR ($CDCl_3$, 20 MHz). δ 13.57, 13 66, 22 35, 24.01, 25.39, 29.25, 29 71, 127 36(C5), 133.35(C4), 156.36(C2) ppm ^1H NMR ($CDCl_3$, 80 MHz) δ1 04(6 H, t, J=6 8 Hz, CH_3), 1.15-1 95(m, 6H), 2 77 (2 H, t, J=7 8 HzCH$_2$ bound to C5), 2.97(2 H, t, J=8.4 Hz, CH_2 bound to C4).
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23 **2-trifluoromethyl-4-butyl-5-propylthiazole 2** Colorless liquid b p 62 °C/1 mmHg The compound was further purified by column chromatography on silica gel, using a linear gradient from pure light petrol to pure benzene MS (20 eV). m/z 251(M^+,11), 236(16), 222(17), 209(36), 194(32), 181(100), 167(20)), 149(23), 140(43), 11(61), 85(41), 83(51), 71(59), 55(92), 43 (64) ^1H NMR ($CDCl_3$, 80 MHz) δ 1 00 (6 H, t, J=6 8 Hz, CH_3), 1 10-1 76 (6H, m), 2 61-2.86 (4 H, m) ppm.
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26. **2-amino-4-butyl-5-propylselenazole hydrobromide 3**. ^{13}C NMR ($CDCl_3$, 20 MHz) δ 13 55, 13.73, 22.21, 25.11, 26.57, 29.47, 30.76, 123.17 (C5), 133 39 (C4), 172.41 (C2) ppm ^1H NMR ($CDCl_3$, 80 MHz) δ 0 86-1.04 (6H, m), 1.10-1.75 (6H, m), 2.47 (2 H, t, J=6.2 Hz, CH_2 bound to C5), 2 56 (2 H, t, J=7.3 Hz, CH_2 bound to C4) ppm. Free base· MS (20 eV): m/z 248(M^+, 8), 246(M^+, 48), 244(M^+, 24), 243(M^+, 8), 242(M^+, 11), 231(6), 219(16), 217(72), 215(39), 206(11), 204(57), 202(34), 177(18), 176(62), 175(100), 173(65), 164(11), 162(53), 160(29), 123(13), 82(15), 81(14).
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28.Tritiated 2-isobutyl-3-methoxypyrazine was prepared by isotopic exchange at the Radiochemical Centre, Amersham, and had, at the time of the experiments, a specific activity of 1.2 Ci/mmol. The odorant-binding protein (OBP) was purified from cow nasal tissue, according to the published procedure.[3] Binding experiments were performed using the filtration method[29] and along with the following procedure· the purified bovine OBP (3 μM) was incubated at 4 °C with the radioactive pyrazine (3 μM) and the competitive ligand (0-0.1 mM) for 10 minutes in 20 mM Tris/HCl buffer, pH 7.4. 0.2 ml aliquots were then rapidly filtered through glass fiber filters treated with 2% aqueous solution of polyethylenimine; the filters were immediately washed with 5 ml of cold buffer and counted for bound radioactivity Non specific binding was measured in the presence of 1 mM cold 2-isobutyl-3-methoxypyrazine and accounted for less than 5% of total binding The data shown are means of duplicates, differing by less than 5% Although the figure reports the results of a single experiment, such measurements were repeated, using different preparations of the bovine OBP, and produced very similar results
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