Pure & Appl. Chem., Vol. 58, No. 5, pp. 737–744, 1986.
Printed in Great Britain.

Contribution of an organic chemist to the resolution of some biological problems: consequences

Pierre Potier

Institut de Chimie des Substances Naturelles du C.N.R.S.
91190 - Gif-sur-Yvette, France

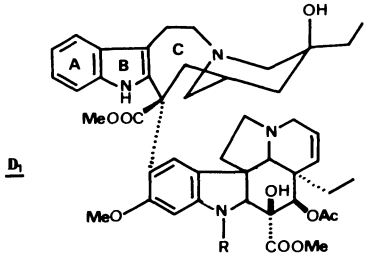
Abstract - In our efforts aimed at the discovery of new natural products of biological and, eventually, therapeutic interest, we have become particularly interested in the following three areas: (i) Anticancer chemotherapy: the derivatives of vinblastine 1 and those of ellipticine 13 have been especially studied; (ii) The central nervous system, wherein we have looked for new substances which can interact with the benzodiazepine receptor. These studies in turn led us to investigate; (iii) Interactions between the nervous system and the immune system with the resulting discovery of some interesting properties of several compounds. In all three cases, the contribution of the chemist has been essential.

ANTICANCER CHEMOTHERAPY

Recent progress in understanding the subtle mechanisms underlying the development of cancerous cells (oncogenes) allows us to hope for new, specific cancer therapies. To date, chemotherapy still remains one of the principal treatments for this type of disease, together with surgery, radiotherapy, and, to a smaller extent, at least for the moment, immunotherapy.

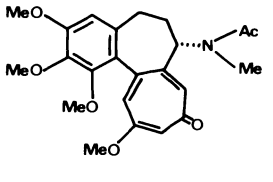
Among the substances used in the chemotherapy of cancer, the "spindle poisons" hold a prominent position : vinblastine 1 and its derivatives ; podophyllotoxin 4 and its derivatives ; colchicine 3, the oldest of these compounds, has not yet provided sufficiently useful derivatives which would permit as wide a range of use as the preceding compounds. In addition, maytansine 5, raised hopes which, presently, have been disappointing in view of this compound's poor therapeutic index and long-term toxic effects. Two series of products have been particularly studied at Gif-sur-Yvette :

. that of new derivatives of the vinblastine group 1.
. that of derivatives of ellipticine 13 and its isomer, olivacine 16.

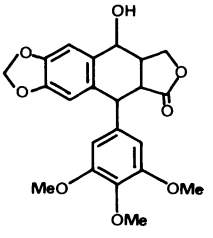


1 R=CH_3 : Vinblastine
= Velbé®

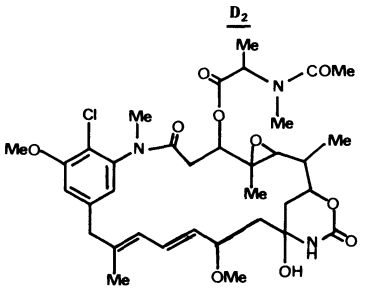
2 R=CHO : Vincristine
= Oncovin®



3 Colchicine



4 Podophyllotoxine



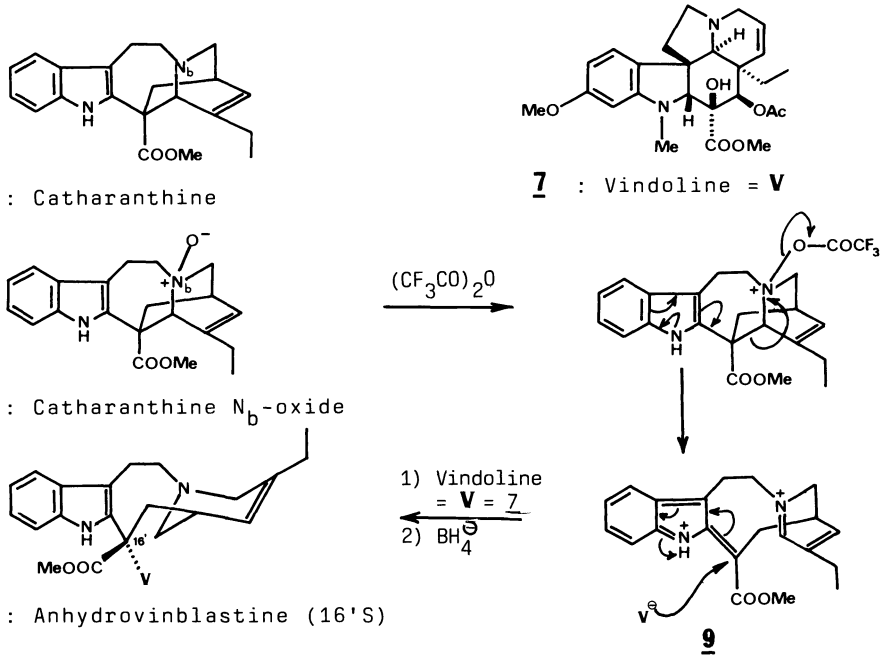
5 : Maytansine

Derivatives of Vinblastine

The discovery, in our laboratory, of a new, "biomimetic" synthesis of the dimeric indole alkaloids of the vinblastine group (ref. 1,2) has permitted access to original derivatives heretofore unobtainable by simple chemical modification of the natural extraction products. Several of these new derivatives have shown potent antitumor activity and one of these, navelbine 12, which is currently undergoing phase II clinical trials, raises serious hopes of eventual therapeutic applications.

Catharanthine N-oxide 8, easily obtained from catharanthine 6, treated with trifluoroacetic anhydride, undergoes a fragmentation reaction, leading to an iminium intermediate 9 ; attack of this intermediate 9 by the vindoline 7, acting as a large nucleophile, leads to the 16'S derivative ("natural" series) whose reduction in situ with sodium borohydride provides anhydrovinblastine 10 (Scheme 1). This latter derivative can be transformed into various dimeric alkaloids discovered in some species of Catharanthus, in particular C. roseus, and thus constitutes a key intermediate in the biosynthesis of these complex natural products (ref. 3).

Scheme 1



6 : Catharanthine

7 : Vindoline = **V**

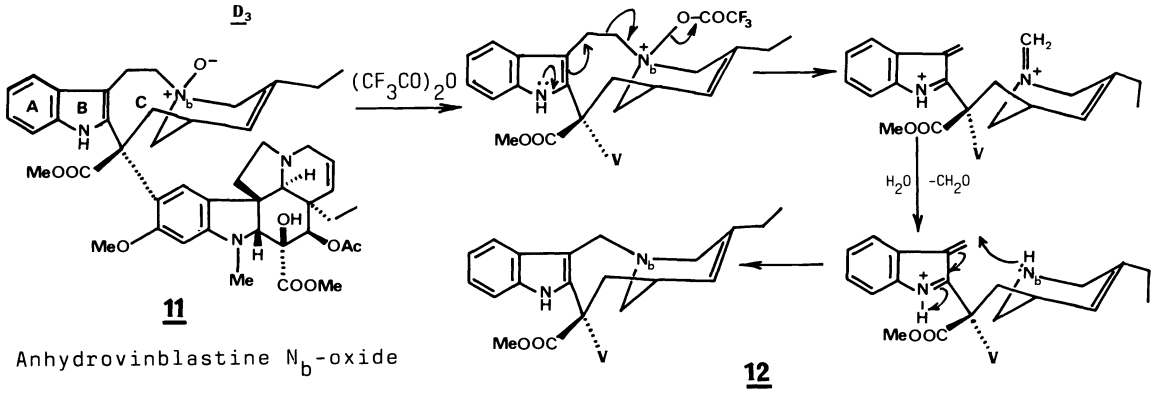
8 : Catharanthine N_b-oxide

10 : Anhydrovinblastine (16'S)

9

Anhydrovinblastine 10 can, in turn, be converted to its N-oxide 11. The latter, treated with trifluoroacetic anhydride, leads to nor-anhydrovinblastine or navelbine 12 (Scheme 2).

Scheme 2



11

Anhydrovinblastine N_b-oxide

12

Nor-anhydrovinblastine

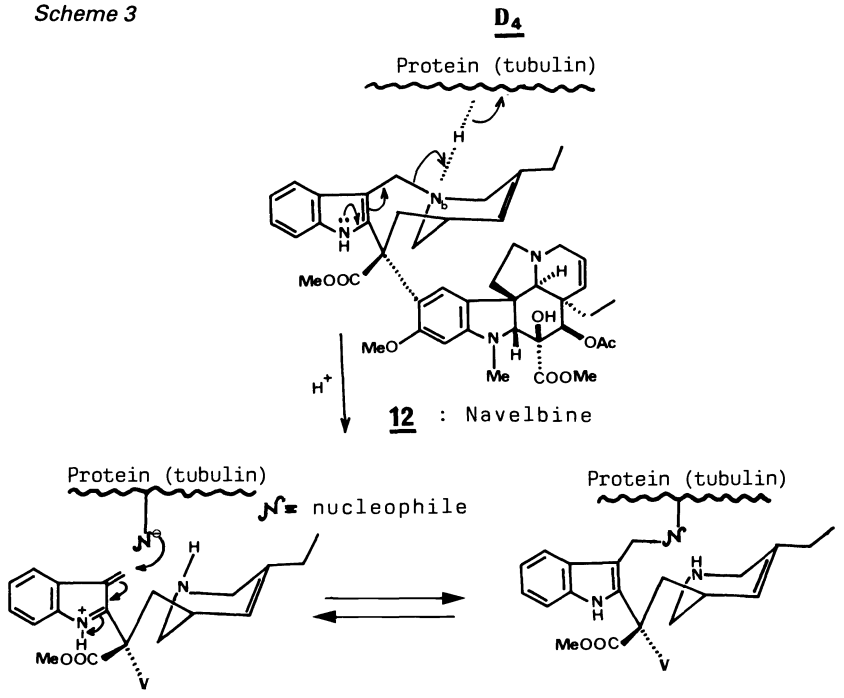
= Navelbine®

Navelbine has demonstrated an ability to inhibit in vitro the polymerisation of tubulin, superior or equal to that of vinblastine or vincristine, respectively (ref. 4). This compound is actually undergoing phase II clinical trials and already appears to be an antitumor agent likely to reinforce our therapeutic arsenal.

The essential structural difference between navelbine 12 and the vinblastine derivatives 1 or 2 resides in the contraction of ring C of this molecule (9-membered ring → 8-membered ring) and, especially, of the presence on this ring of a gramine-type partial structure. Now, the little we know of the relations between structure and biological activity of the compounds of the vinblastine series indicates that the pK of the N_b nitrogen atom is very important : this nitrogen atom must be capable of being protonated.

Unfortunately, we know very little about the precise nature of the interactions of vinblastine-type molecules with tubulin. This, of course, is partly due to the fact that the exact structure of tubulin is not presently known and it is thus impossible to fully appreciate the nature of the interactions between these "spindle poisons" and their fixation sites on this protein. It is, however, possible that the essential interaction of tubulin and the spindle poisons results from hydrophobic type binding between the "diphenylmethane" (or biphenyl) type of partial structure, which is found in many of these compounds, and some part of the protein. This hydrophobic interaction could be complemented by interactions of another type, for example, those resulting from protonation of the N_b nitrogen atom by an acidic site on tubulin. This protonation could provoke, in the case of navelbine 12, the opening of the C-ring and the formation of an iminium species particularly susceptible to attack, for example, by a nucleophilic entity situated close-by on the protein (Scheme 3). The intervention of such a mechanism,

Scheme 3



giving rise to a new reversible covalent bond between the protein (tubulin) and the inhibitors of its polymerisation (e.g. navelbine), could account (partially or totally) for the biological activity of these latter compounds.

This notion of reversible covalent binding should be applicable to other biological systems ; the compounds which would result could constitute an effective transitory blocking of the active sites of the protein or enzyme and thus provide an interesting alternative to classic "suicide-inhibitors", by definition irreversible.

The preparation of radioactive derivatives of navelbine should allow us to verify the validity of these hypotheses.

It should be added that the C-nor-vinblastine-type structure present in navelbine has not yet been found in nature while anhydrovinblastine 10, from which it is derived, was first

prepared by synthesis (ref. 2) and is considered a key intermediate in the biosynthesis of indole alkaloids of the vinblastine group (ref. 3). Anhydrovinblastine 10 has subsequently been shown to be a constituent of Catharanthus roseus (ref. 5).

Derivatives of Pyrido[4,3-b]carbazole: ellipticine and olivacine

Ellipticine 13 was first isolated from Ochrosia elliptica Labill. (Apocynaceae) by Goodwin and coll. (ref. 6). As early as 1875, Barquisseau described the same Ochrosia in his treatise, naming it Ochrosia borbonica and collected in the Reunion Islands (Mascareinhas) (ref. 7,8). The genus Ochrosia stretches from the Indian Ocean to the Hawaiian confines of the Pacific Ocean : this constitutes, in fact, an immense dispersion. This dispersion is facilitated by the nature of the fruit which is a fibrous drupe, capable of floating, thus enabling its step-wise spread to other islands (or continents).

The structure of ellipticine was confirmed by the total synthesis of Woodward and coll. In 1967, Dalton and coll. (ref. 9) showed that ellipticine 13 and 9-methoxyellipticine 15 possess interesting antitumor properties. As a result, much work was undertaken by many groups to develop a therapeutically useful antitumor derivative belonging to this class of pyrido [4,3-b] carbazoles (ref. 7). This research culminated in 1973 with the development of "Celiptium" ® or 2-methyl-9-methoxyellipticine acetate 17.

The derivatives of an isomer of ellipticine, olivacine 16, are also active antitumor agents but have not to this day reached the level of development of certain of the ellipticine derivatives.

The mechanism of action in vivo of these pyridocarbazole derivatives is not well known even though many in vitro experiments have permitted these compounds to be classed as DNA intercalating agents.

The discovery of the alkylating properties of sile oxidized derivatives of ellipticine by Paoletti, Meunier and coll. constitutes an important step in understanding the mechanism of action of these compounds (ref. 10). In effect, these authors showed that compounds as structurally varied as amino acids, glutathione and nucleosides could add to position 10 of the iminoquinone species, the latter obtained by oxidation of hydroxyellipticine-type derivatives (Scheme 4). However, the structures proposed for these adducts formed between hydroxyellipticine and nucleophiles have been modified as a result of our own work (ref. 11,12).

The high affinity of ellipticine and its derivatives for DNA-type structures (10^{-5} to 10^{-6} M) and the intercalation of these types of molecules within the DNA structure were long considered the two most important factors in explaining their biological and therapeutic activities.

In fact, derivatives of ellipticine are known (9-amino or 9-fluoro) which bind to DNA with as high an affinity as 9-hydroxyellipticine but which demonstrate little or no antitumor activity. This observation led to the proposal that DNA may not be the only biological target of ellipticine-type derivatives.

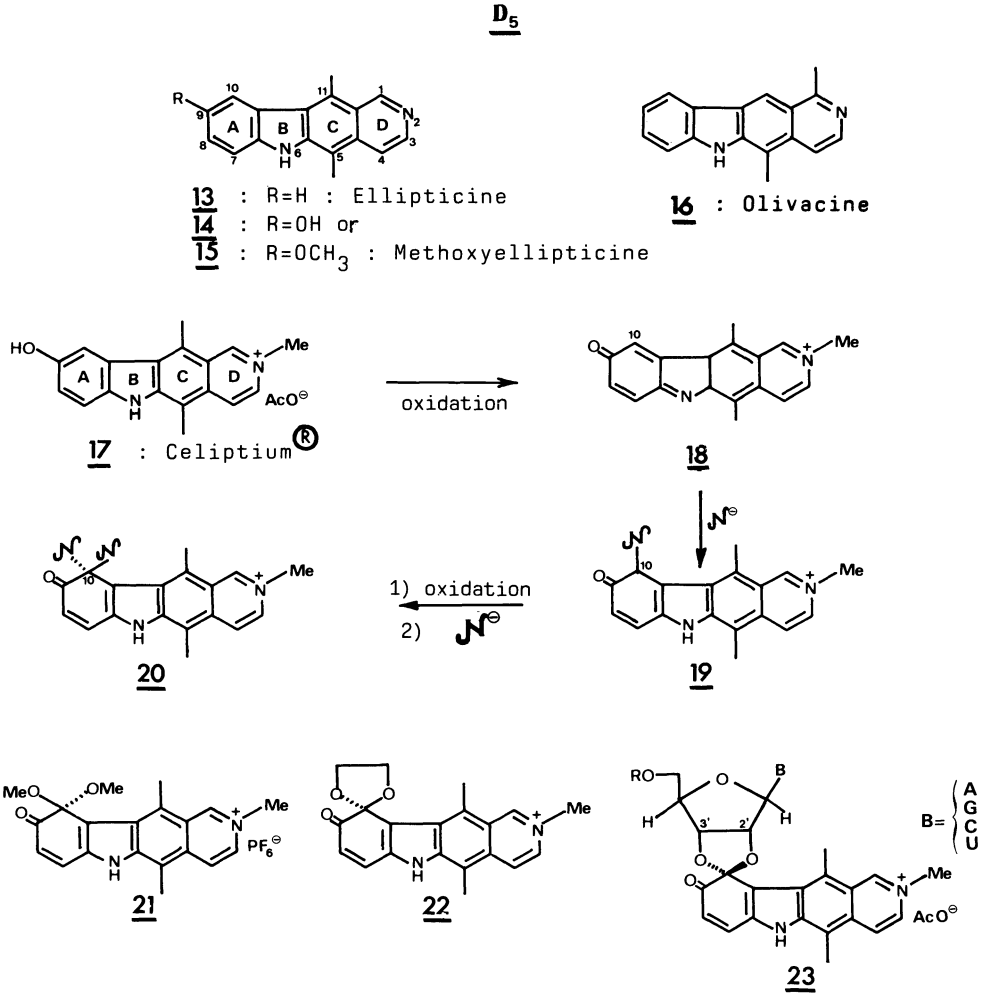
In effect, 9-hydroxyellipticine or its quaternary ammonium derivative 17 can be easily oxidized to give iminoquinone derivatives such as 18 (ref. 10). These iminoquinone species are excellent electrophiles to which extremely varied nucleophiles (amino acids, thiol derivatives, alcohols, amines, nucleosides, etc.) can add to position 10 (Scheme 4) (ref. 7,8,10,11,12).

Among the excellent biological nucleophiles which can add to position 10 of the iminoquinone species formed by oxidation are, in fact, the ribonucleosides. Thus, 9-hydroxyellipticine is easily oxidized (for example, by hydrogen peroxide and by Horse-radish peroxidase) to the iminoquinone 18 which typically can undergo a first nucleophilic addition (N^-), leading to a primary adduct 19 (not always isolable in view of its instability). This adduct gives rise to a phenolic derivative by proton shift which, in turn, is again oxidized, leading to a new iminoquinone structure capable of undergoing a second nucleophilic addition (e.g. 21 or 22). The case of glycol addition 22 is particularly interesting since such a cis 1,2-glycol group is found in the ribose unit of ribonucleosides and nucleotides which compose RNA.

Obviously, it can easily be seen why the many studies concerning the interaction between small molecules and nucleotide substrates have dealt mainly with DNA, whose preparation is relatively simple, beginning, for example, with calf thymus. The preparation of RNA of purity comparable to that of DNA is much more difficult.

If a compound such as 17 is oxidized (for example, with hydrogen peroxide and Horse-radish peroxidase) and the product reacted with adenine (A), guanine (G), cytosine (C) or uracil (U), we note that the reactivities decrease in the order A > G >> C > U. There exists a large difference in reactivity between the purine bases A and G,

Scheme 4



which react very rapidly, and the pyrimidine bases, C and T, which react much more slowly. Furthermore, A reacts better than G. Finally, a regioselectivity is observed in this reaction between 17 and each of the four bases, such that only position 10 of 17 is affected. This regioselectivity can be explained by an appropriate positioning of the two reactive species, the heterocycles of the purine (and pyrimidine) bases being stacked above (and below) rings C and D of the pyridocarbazole derivatives (e.g. 17). Thus, the two hydroxyl groups at positions 2' and 3' of the ribose moiety of purine (or pyrimidine) nucleosides also react solely at position 10 of the iminoquinone (e.g. 18) to give structures of type 23. If the same reaction is attempted with 2-deoxy-adenosine (DNA-type structure), no addition is observed (ref. 8). The simultaneous presence of two cis-glycol hydroxyls at positions 2' and 3' of the ribose portion is thus necessary.

As the result of these observations, we were led to propose that the antitumor activity of the pyridocarbazole class of compounds (and perhaps of other related structures such as the anthracyclinones) could result from this type of reaction on the A or poly A' terminals which are found in cellular ribonucleic structures. Thus, it is conceivable that all or part of the biological activity of these antitumor products is a result of a blocking of the nucleotide terminals which are found in t-RNAs or m-RNAs. The cellular machinery would thus be completely disorganized (ref. 13,14).

The inhibition of polyribonucleotide synthesis and of the elongation of polypeptide chains by olivacine 16 (ref. 15) can also be explained by this type of reaction and reinforces our hypothesis.

In conclusion, we can affirm that mere intercalation of ellipticine-type molecules with the DNA structure does not sufficiently account for the biologial activity of these compounds. The formation of covalent compounds between these pyridocarbazole (or related) structures and ribonucleotide structures must not only also be taken into account but may, in fact, represent the principal mode of action in explaining the antitumour properties of these compounds.

SOME β-CARBOLINE DERIVATIVES ACTIVE ON THE NERVOUS SYSTEM

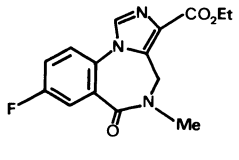
The 1,4-benzodiazepines (e.g. flunitrazepam, diazepam, Scheme 5) are a class of compounds widely used therapeutically for their anxiolytic, sedative, anticonvulsant and muscle relaxant properties. These various activities of the benzodiazepines are apparently mediated by specific central-type receptors, discovered independently by Möhler and Okada (ref. 16) and by Braestrup and Squires (ref. 17) in 1977. These benzodiazepine receptors, for which at least two subclasses have been described (the BZ_1 and the BZ_2 receptor) (ref. 18) are coupled both to the GABA receptor and the chloride ionophore in the form of some supramolecular complex. It is not known whether all the above-mentioned properties of benzodiazepines can be explained by means of their ability to facilitate the actions of GABA, a neuroinhibitor, but at least their anticonvulsant and anxiolytic activities seem to be largely accounted for by this coupling. It is also not known to this day whether each of the BZ_1 and BZ_2 subtypes of benzodiazepine receptors is associated with different, distinct biological activities.

The discovery of these receptors for synthetic benzodiazepines naturally led to the search for the endogenous ligand or ligands of the receptors. One such ligand, isolated by Braestrup and coworkers, but since shown to be an artefact of the isolation procedure, is ethyl β-carboline-3-carboxylate 24 (ref. 19). This compound, as well as the homologous methyl and propyl esters, display very high affinity for the central benzodiazepine receptors. Interestingly enough, however, we and others have been able to show, using a photoactivated model of epilepsy in baboons, that the methyl and ethyl esters of β-carboline-3-carboxylate (25, 24) are respectively highly convulsant and proconvulsant agents (ref. 20,21) while the propyl ester 26 antagonises the anticonvulsant activity of benzodiazepines (ref. 20). Since then, it has been shown that these β-carboline derivatives also either antagonise the anxiolytic, sedative and muscle relaxant properties of benzodiazepines or else demonstrate completely opposite effects (e.g., anxiogenic, stimulating effects).

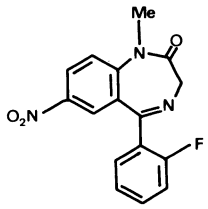
In searching for more selective antagonists of the benzodiazepines, we were led to synthesize analogues of these β-carbolines, in particular, a series of molecules belonging to the class of 3-amino-β-carbolines 27. Although these derivatives generally had a lower affinity for the benzodiazepine receptor than the parent 3-carboxy-β-carbolines, one of these compounds, 3-methoxycarbonylamino-β-carboline (β-CMC, 28) had a sufficiently important affinity (IC_{50}=4 x 10^{-8} M) and merited a closer study of its in vivo activity. We have thus been able to show that, at least in the mouse, β-CMC, while displaying neither convulsant/anticonvulsant activity nor anxiolytic/anxiogenic activity, is able to effectively inhibit the sedative properties of diazepam (ref. 22). Consequently, β-CMC represents the first selective antagonist of these sedative actions of benzodiazepines and may prove to be a valuable tool in studying the mechanisms of sleep and the relationship with the

Scheme 5

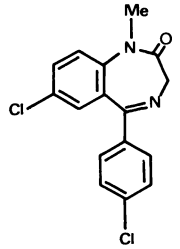
MOLECULES "Central" "Mixed" "Peripheral"



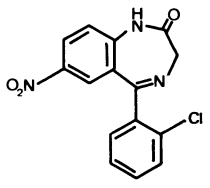
Ro 15-1788



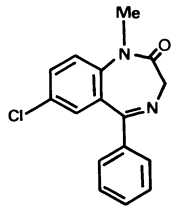
Flunitrazepam



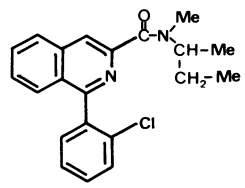
Ro 5-4864



Clonazepam

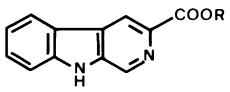


Diazepam

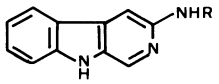


PK 11-195

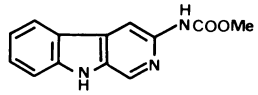
benzodiazepine receptor. For instance, our in vitro and in vivo studies (including positron emission tomography) have revealed that β-CMC is highly selective for the type of benzodiazepine receptors found in the cerebellum. Now, it is known that these cerebellar receptors belong almost exclusively to the BZ_1 subclass of receptor, which heretofore had been associated, on the basis of disputable evidence, with the anticonvulsant/anxiolytic actions of benzodiazepines. In light of the very limited and selective action of β-CMC on sedation, it would seem more likely that the BZ_1 receptors are, in fact, implicated in sleep mechanisms. More research will be needed to clarify this problem.



24 R=Et
25 R=Me
26 R=Prop.



27 R=H, Et, $COCH_3$, $COCF_3$, SO_2CH_3, CHO, aso...



28 β-CMC

RELATIONS BETWEEN NERVOUS AND IMMUNE SYSTEMS: INTERACTION OF BENZODIAZEPINES AND RELATED SUBSTANCES WITH MACROPHAGES

Numerous clinical observations have led to the hypothesis that nervous and immune systems might respond to identical stimuli (ref. 23). This conclusion has been substantiated by a number of experimentalists. For example, neuropeptides such as endorphin, vasopressin, substance P., etc., are known to modify the phagocyting properties of macrophages. This suggests the presence of common receptors on the respective cell types, or, at least, that these receptors could have common sub-structural features. In the light of the fact that a number of receptors (or binding sites) are known to be formed by several structural sub-units, one can well envisage that a given "complex" receptor (or binding site) present in the nervous system can be structurally related, in part, with another receptor present, for instance, on one of the cellular components of the immune system (for example, macrophages).

It is also a common clinical observation that the evolution of some diseases is obviously under the influence, if not the control, of the nervous system. Anxiety undoubtedly favours the development of diseases known to be highly influenced by the status of the patient's immunosystem. An "anxiety peptide" has recently been claimed to be discovered in the brain (ref. 24).

It was therefore interesting to try to see whether some psychoactive drugs such as benzodiazepines, β-carbolines or related compounds could also interfere in some way with the immune system.

Indeed, we have been able to show that, among the various substances that we have examined, some belonging to the category of "peripheral-type benzodiazepines" (i.e. ligands known to interact with the "peripheral" benzodiazepine receptors) could stimulate, both in vitro and in vivo, murine macrophages (ref. 25). This stimulation is effective using a single low dose (1 mg/kg) of "peripheral" drugs, such as Ro 5-4864, PK 11195 and to a smaller extent, diazepam (Scheme 5).

This observation is important when one takes into account the state of development of our knowledge of the "benzodiazepine receptor". A recent paper by H. Möhler and coll. has shown a colocalization and structural homogeneity of $GABA_A$ receptors and benzodiazepine receptors in the brain (ref. 26,27,28). It is therefore quite possible that these central receptors (in the brain) could share some structural features in common with "peripheral receptors". This should be easily reconciled if one admits that all benzodiazepine receptors are made of several structural sub-units which have come together ; a part of this complex receptor (or binding site) should represent a "recognition unit" able to bind endogenous (or external) ligands. This first event would not be enough to trigger a physiological response. This response could now follow after "triggering" another sub-unit of the complex receptor [for example, the $GABA_A$ receptor linked to the benzodiazepine receptor (ref. 26)].

With this hypothesis, the apparent diversity of receptors could be simply the result of a sort of "mecano-type" building-up of receptors based on the aggregation of various sub-units.

With this idea in mind, we can now try to explain our results. We have shown that there are "peripheral" benzodiazepine binding sites on murine macrophages. The occupation of these binding sites by compounds like Ro 5-4864, PK 11195 or diazepam (Scheme 5) stimulates the macrophages. The exact nature of this stimulation is under current study.

We have also prepared a number of β-carboline derivatives. It has been shown by Braestrup and coll. (ref. 19) that the ethyl ester of β-carboline-3-carboxylic acid (ref. 24) binds very tightly to the central-type benzodiazepine receptor. Some of the compounds that we have prepared (described above) also exhibit a high affinity for the same receptors.

However, the β-carboline derivatives that we have synthesized do not have a great affinity for peripheral benzodiazepine binding sites and consequently do not affect murine macrophages.

Acknowledgements

The results described above have been obtained during the last few years and are due to the Skilfull expertise and patience of many coworkers, most of them being listed in the accompanying references. I wish to thank all of them very heartfully. Thanks are also due to Dr. R.H. Dodd for reviewing the manuscript in English and to F. Hoël and Mrs C. Mongé for typing the manuscript. C.N.R.S., La Ligue Nationale Française contre le Cancer, l'Association pour la Recherche sur le Cancer (Villejuif), le PIRMED have supported this work.

Last, but not least, this lecture is dedicated to Professor Sir Derek Barton, Director of the Institut de Chimie des Substances Naturelles, for his continuous interest and for the numerous stimulating discussions that I have had with him, during these last eight years.

REFERENCES

1. P. Potier, Ann. Pharm. Fr., 38, 407 (1980).
2. N. Langlois, F. Guéritte, Y. Langlois and P. Potier, J. Am. Chem. Soc., 98, 7017 (1976).
3. F. Guéritte, N.V. Bac, Y. Langlois and P. Potier, J.C.S. Chem. Comm., 452 (1980).
4. P. Potier, D. Guénard and F. Zavala, C. R. Soc. Biol. Fr., 173, 414 (1979).
5. K. Jovanovics, G. Fekete, L. Dancji, E. Dezseri, C. Lorincz, B. Szarvädy, G. Dobo and C. Szantay, Chem. Abstracts, 90, 61230e (1979).
6. S. Goodwin, A.F. Smith and E.C. Horning, J. Am. Chem. Soc., 81, 1903 (1959).
7. V.K. Kansal and P. Potier, in preparation for J. Nat. Prod. and references therein.
8. B. Chanel-Gillet, Thèse de Doctorat ès-Sciences, Paris-Orsay (1985).
9. L.K. Dalton, R.S. Demerac, B.C. Elmes, J.W. Loder, J.W. Swan and T. Teitei, Aust. J. Chem., 20, 2715 (1967).
10. G. Meunier, B. Meunier, C. Auclair, J. Bernadou and C. Paoletti, Tetrahedron Letters, 3655 (1983).
11. V.K. Kansal, S. Funakoshi, P. Mangeney, P. Potier, B. Gillet, E. Guittet and J.-Y. Lallemand, Tetrahedron Letters, 2351 (1984).
12. V.K. Kansal, R. Sundramoorthi, B.C. Das and P. Potier, Tetrahedron Letters, in press.
13. V.K. Kansal, P. Potier, B. Gillet, E. Guittet, J.-Y. Lallemand, T. Huyn-Dinh and J. Igolen, Tetrahedron Letters, 2891 (1985).
14. V.K. Kansal, S. Funakoshi, P. Mangeney, B. Gillet, E. Guittet, J.-Y. Lallemand and P. Potier, Tetrahedron, in press.
15. P. Tovaty and M. Simon, Biochem. Biophys. Acta, 697, 313 (1982).
16. H. Möhler and T. Okada, Science, 198, 849 (1977).
17. R. Squires and C. Braestrup, Nature, 266, 732 (1977).
18. W. Sieghart and M. Karobath, Nature, 286, 285 (1980).
19. C. Braestrup, M. Nielsen and C.E. Olsen, Proc. Natl. Acad. Sci. USA, 77, 2288 (1980).
20. A. Valin, R.H. Dodd, D.R. Liston, P. Potier and J. Rossier, Eur. J. Pharmacol., 85, 93 (1982).
21. C. Cépeda, T. Tanaka, R. Besselièvre, P. Potier, R. Naquet and J. Rossier, Neurosci. Lett., 24, 53 (1981).
22. R.H. Dodd, C. Ouannès, L. Prado de Carvalho, A. Valin, P. Venault, G. Chapouthier, J. Rossier and P. Potier, J. Med. Chem., 28, 824 (1985).
23. F. Zavala, J. Haumont and M. Lenfant, European J. Pharmacol., 106, 561 (1985) and references therein.
24. P. Ferrero, A. Guidotti, B. Conti-Tronconi and E. Costa, Neuropharmacology, 23, 1359 (1984) and subsequent references.
25. M. Lenfant, F. Zavala, J. Haumont and P. Potier, C. R. Acad. Sc. Paris, 300, série III, 309 (1985).
26. P. Schoch, J.G. Richards, P. Häring, B. Takacs, C. Stähli, T. Staehelin, W. Haefely and H. Möhler, Nature, 314, 168 (1985).
27. P. Häring, C. Stähli, P. Schoch, B. Takacs, T. Staehelin and H. Möhler, Proc. Natl. Acad. Sc., USA, 82, 4837 (1985).
28. S. Martin Shreeve, Claire M. Fraser and J. Craig Venter, Proc. Natl. Acad. Sc., USA, 82, 4842 (1985).