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EVALUATION OF BOUVARDIN, DEOXYBOUVARDIN, AND RA-I - RA-VII PARTIAL STRUCTURES: REASSIGNMENT OF THE PHARMACOPHORE

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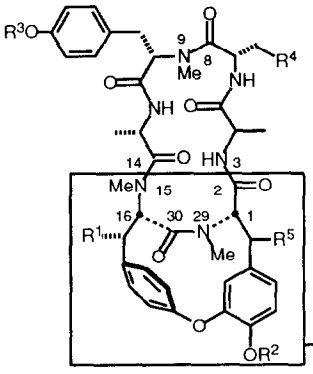
Abstract: The in vitro cytotoxic evaluation of a set of key partial structures and analogs of deoxybouvardin and RA-I - RA-VII is detailed and has permitted the reassignment of the agent pharmacophore.

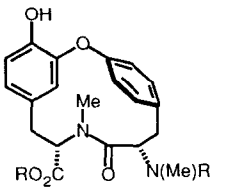
Bouvardin (**8**) and deoxybouvardin (**2**) constitute the initial members of a growing class of potent antitumor antibiotics now including RA-I - RA-VII.[1-7] Bouvardin has been shown to inhibit protein synthesis through eukaryotic 80S ribosomal binding resulting in inhibition of aminoacyl-*t*RNA binding and peptidyl-*t*RNA translocation and this is currently suggested to be the site of action for the agent's antitumor activity.[8] Initial[9-11] and more recent[12] studies on the agents have supported the original proposal[9] that the unusual 14-membered *N*-methyl cycloisodityrosine subunit of the agents may serve to induce a rigid, normally inaccessible conformation within the 18-membered cyclic hexapeptide that in turn constrains the biologically relevant D-Ala-Ala-*N*Me-Tyr(OMe)-Ala tetrapeptide to a biologically active conformation.[9-12]

However, until recently the role and importance of the 14-membered *N*-methyl cycloisodityrosine subunit was not easily addressed due to the synthetic inaccessibility of such agents.[13-15] Consequently, studies to date have relied on derivatization of the naturally occurring agents and the subsequent evaluation of the resulting agents. With access to **10-28** through the results of recent studies, key partial structures of the natural products incorporating the *N*-methyl cycloisodityrosine subunit and conformational analogs of the tetrapeptide subunit have become available for evaluation. As detailed herein, the evaluation of the full complement of agent partial structures proved more revealing than anticipated and suggests that a reversal of the assigned functional roles of the subunits is required.



Pharmacophore

R^1	R^2	R^3	R^4	R^5		
H	Me	Me	H	H	1	RA-VII
H	H	Me	H	H	2	RA-V, deoxybouvardin
H	H	Me	OH	H	3	RA-I
H	Me	H	H	H	4	RA-II
H	Me	Me	OH	H	5	RA-III
H	Me	Me	H	OH	6	RA-IV
OH	Me	Me	H	H	7	RA-VI
OH	H	Me	H	H	8	bouvardin



N-Me cycloisodityrosine

Key Partial Structures and Analogs Incorporating the Tetrapeptide. With indirect evidence[11] to support the inherent importance of the tetrapeptide to the biological properties of the agents **1-8**, structural and conformational analogs of **1-8** lacking the 14-membered *N*-methyl cycloisodityrosine subunit have been recognized as potentially useful and accessible analogs of **1-8**. To date, such efforts have resulted in the preparation of *O-seco*-deoxybouvardin (**10**)[10,16] and **11-13**[16] constituting the parent 18-membered, 14-membered, and 26-membered cyclic peptide incorporating the key D-Ala-Ala-*N*Me-Tyr(OMe)-Ala tetrapeptide as well as the more advanced agents **14-17**. The agents **14-15**[18] lack only the deoxybouvardin key N^{29}-C^{30} amide bond and the two additional agents, **16**[17] and **17**,[19] constitute conformationally flexible and rigid analogs of the agents which lack the 14-membered *N*-methyl cycloisodityrosine subunit. In **16**, the carbon-carbon double bond of the benzene ring serves to mimic the key cis N^{29}-C^{30} amide bond of the agents.[17] In **17**, the *N*-methyl β-lactam serves to mimic the structure and potential reactivity of the deoxybouvardin N^{29}-C^{30} *N*-methyl amide bond and the β-lactam linkage of the C^1-C^{16} carbons serves to restrict the number of accessible cyclic peptide conformations to those including those found for deoxybouvardin.[19]

The in vitro cytotoxic evaluation of **10-17** revealed no activity for the agents below the commonly accepted value of 4 μg/mL although detectable activity was observed for **12-17**, Table I. As such, the results underscore the importance of the intact 14-membered *N*-methyl cycloisodityrosine subunit for observation of potent cytotoxic activity (i.e. **10** and **15** versus **1-2**) Although the results may be interpreted in a manner that highlights the importance of the precise conformational features of the tetrapeptide subunit of the natural products, the lack of activity observed with **17** which possesses a restricted set of cyclic peptide conformations comparable to those of the natural products[19] and the results of additional studies detailed herein suggests this may not prove accurate

Figure I

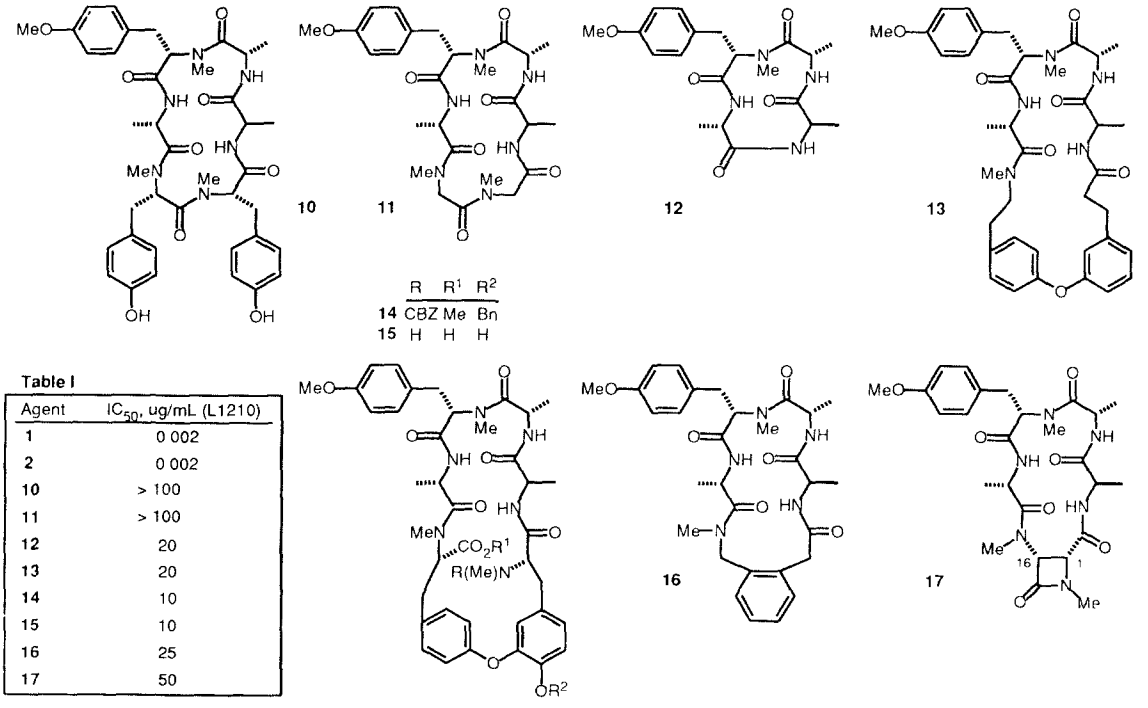


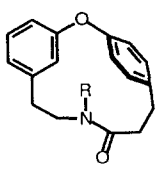
Table I

Agent	IC_{50}, ug/mL (L1210)
1	0 002
2	0 002
10	> 100
11	> 100
12	20
13	20
14	10
15	10
16	25
17	50

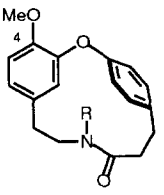
Key Partial Structures and Analogs Incorporating the Cycloisodityrosine 14-Membered Ring. A number of key partial structures of the 14-membered *N*-methyl cycloisodityrosine subunit of **1-8** became accessible as a consequence of the recent synthetic studies on the natural products and related analogs.[14,15,18,20] The evaluation of the subunits **18-27** including simple derivatives of *N*-methyl cycloisodityrosine (**24-25**) and the substructure possessing a C^{11}-N^{10} secondary amide (**26-27**)[20] proved revealing, Table II. While the simple derivatives **18-23** proved inactive in the in vitro cytotoxic assays and include derivatives possessing the cycloisodityrosine C4-hydroxy or alkoxy substituent, the C9-carboxy substituent, and/or the tertiary C^{11}-N^{10} amide, each member of a limited series of agents that include the functionalized C12 amine of cycloisodityrosine (**24-27**) proved to be potent cytotoxic agents. Moreover, the simple 14-membered cycloisodityrosine derivatives **24-27** were found to be only 10-30x less potent than the natural products **1-8**. As such, the experimental results suggest that it is the 14-membered ring of **1-8** that constitutes the pharmacophore of the natural products and that the tetrapeptide housed within the 18-membered ring potentiates the inherent biological properties of cycloisodityrosine. Consistent with this interpretation, the incorporation of **26-27** into N^{29}-desmethyl RA-VII (**28**)[20] in which the key N^{29}-C^{30} *N*-methyl amide of **1-8** was replaced with a secondary amide provided an agent that is twice as potent as the natural product.[21]

Thus, the in vitro cytotoxic evaluation of a full complement of partial structures and key analogs of **1-8** suggest that the assumed functional roles of the agent subunits should be reversed. The results of the studies suggest that the 14-membered *N*-methyl cycloisodityrosine subunit of the agents constitutes the pharmacophore and that the tetrapeptide housed within the 18-membered ring potentiates the inherent biological properties of cycloisodityrosine. These findings and their relationship to the proposed site of action of the natural products is under further investigation and will be disclosed in due course.

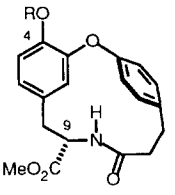
Figure II



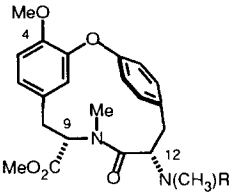
18 R = H
19 R = Me



20 R = H
21 R = Me



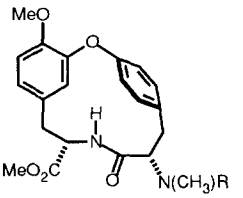
22 R = H
23 R = Me



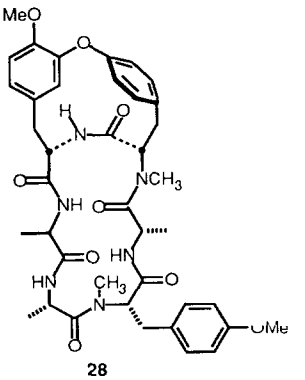
24 R = BOC
25 R = CBZ

Table II.

Agent	IC_{50}, ug/mL (L1210)
1	0 002
2	0 002
18	> 100
19	> 100
20	> 100
21	> 100
22	> 100
23	> 100
24	0.03
25	0 04
26	0.06
27	0 05
28	0.001



26 R = BOC
27 R = CBZ



28

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21. The in vitro cytotoxicity testing (L1210) was conducted as detailed, cf Boger, D. L.; Yasuda, M ; Mitscher, L. A.; Drake, S. D.; Kitos, P. A.; Thompson, S. C. *J. Med. Chem.* **1987**, *30*, 1918. The testing was conducted in duplicate with two separate sets of samples and agents **1-2**, **24-28** were tested simultaneously. Absolute values varied ± 50% of the average value reported and relative potency varied ± 10%.