J. Biosci., Vol. 12, Number 3, September 1987, pp. 311-320. © Printed in India.

Interaction energy studies on pyrazolopyrimidine nucleoside antibiotics —A theoretical study: Oxoformycin B

RAJENDRA PRASAD OJHA, M. ROYCHOUDHURY and NITISH K. SANYAL
Department of Physics, University of Gorakhpur, Gorakhpur 273 009, India

MS received 26 May 1986; revised 10 June 1987

Abstract. The biological activity of oxoformycin B has been exafned on the basis of the model developed for the incorporation of nucleoside analogues during transcription. Claverie's simplified formula has been employed for intermolecular interaction energy calculation. The pairing energy of oxoformycin B base with complementary bases as well as the association energy with nucleic acid base pairs have been calculated. The results are compared with those of similar computation with normal bases. In addition to the in-plane interaction the vertical interaction energy between the analogue and the normal bases has been computed to specify the particular position of the analogue in the chain. On the basis of the model an attempt has been made to explain the mechanism of the biological action of oxoformycin B and to compare the biological activity of pyrazolopyrimidine nucleoside analogues.

Keywords. Interaction energy; nucleoside analogues; transcription; incorporation; inhibition.

Introduction

Certain modifications of the bases and corresponding nucleosides and nucleotides of RNA and DNA have been shown to confer very interesting biological and chemotherapeutic properties. The pyrazolopyrimidine antibiotics represent a class of modified nucleosides containing the unusual C-riboside linkage. The mechanism of biological activity of these analogues was not clear till the model of Sanyal *et al* (1977, 1981, 1984, 1985, 1986a–d, 1987) was proposed. In this model the quantum mechanical method (Claverie, 1978) has been used to obtain the binding energies and sites of association. The influence of environmental factors and thermodynamic parameters is very important in complex formation. Although enthalpy values for complex formation by the field ionization mass spectrometric technique are not available in the literature, this method has already been checked for the interaction of molecules (Langlet, 1981) for which experimental results were available. We have carried out a detailed study of the two modes of association (stacking and hydrogen bonding) *in vacuo* and investigated theoretically the biological activity of oxoformycin B (OFB) using the incorporation model (Sanyal *et al.,* 1981, 1986a-d, 1987). The theoretical results were in agreement with the experimental evidence and stimulated us to do further study. The activity of OFB has been compared with the activity of formycin and formycin B (FB) investigated by the same method (Sanyal *et al.,* 1986d, 1987).

Methodology

OFB, an analogue of xanthosine was isolated from the culture filtrate of *Nocardia interforma* and from the urine of mice and rabbits (Ishizuka *et al.,* 1968a, b; Sheen *et*

Abbreviations used: OHB, Oxoformycin B; HB, formycin B.

al., 1968, 1979). OFB is not inhibitory against yoshida sarcoma, *Xanthomonas oryzae* or influenza virus (Kunimoto *et al.,* 1968). Following the subcutaneous injection of FB, oxidation to OFB occurred rapidly in all organs except the peritoneum (Ishizuka 1968b). From the structural point of view, OFB differs from FB in having the hydrogen attached to C(2) replaced by an oxygen atom and a hydrogen atom attached to N(3). The glycosyl torsion angle of crystaline OFB is (χ =164·1) and places this nucleoside in the *syn* form (Koyama *et al.,* 1976). An intermolecular hydrogen bond between N-(3) and O(5') in OFB stabilizes the *syn* conformation. The conformation about the exocyclic C(4')-O(5') bond is *gg* and the sugar puckering is C(3′)-*endo* (Koyama *et al.,* 1976). The molecular orbital calculation (Saran *et al.,* 1977) by PCILO method has shown that the intrinsically preferred conformation is *syn* for χ_{CC} associated with *gg* for $\emptyset_{C(4')\text{-}C(5')}$.

The method of computation employed here is based on the expression due to Claverie (1978) and has already been discussed in detail (Sanyal *et al.,* 1986b, d). The crystal structure of the OFB nucleoside has been taken from literature (Koyama *et al.,* 1976). CNDO/2 atomic charges and atomic dipoles have been computed and used in further calculations. The stacking energy as well as the hydrogen bonding energy of OFB base with nucleic acid bases and base pairs have been calculated. The minimum energy values and the corresponding configurations are compared with those recommended for transcription.

Results and discussion

Table 1 shows the net charges and atomic dipoles at atomic centres of OFB base and figure 1 gives the structure and atomic indices. The structural changes in the analogue change the charge at the atomic centres to values different from those in adenine, formycin and FB. Using these charges, the interaction energy is computed by a minimization process (Sanyal *et al.,* 1986).

Stacking energy

The minimum energy values corresponding to the staked complexes are given in table 2 and the configurations of the staked complexes are presented in figure 2.

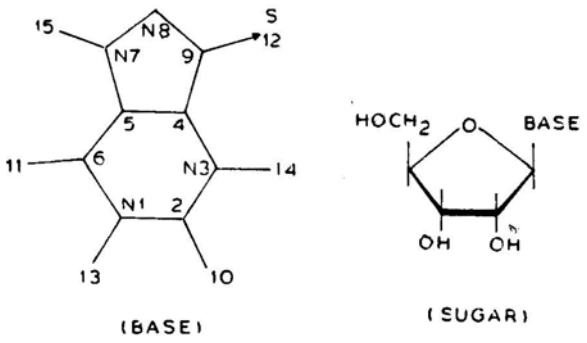

Figure 1. Chemical structure of OFB.

Table 1. Molecular charge distribution on OFB.

Atom No.	Atom	Atomic dipole components				Atomic charges nt analogues					
						Formycin			ge	Adenine	
		Charge	X	Y	Z	Atom	Charge	Atom		Atom	Charge
1	N	−0·248	−0·008	−0·064	−0·164	N1	−0·262	N1	84	N1	−0·285
2	C	0·443	−0·159	0·271	−0·003	C2	0·205	C2	02	C2	0·232
3	N	−0·197	−0·047	0·040	−0·064	N3	−0·218	N3	02	N3	−0·261
4	C	0·095	−0·042	−0·207	−0·002	C4	0·096	C4	48	C4	0·219
5	C	−0·036	0·032	0·335	0·016	C5	−0·014	C5	41	C5	−0·060
6	C	0·350	0·150	−0·179	0·029	C6	0·254	C6	73	C6	0·265
7	N	−0·011	0·083	−0·036	0·019	N7	−0·066	N7	23	N7	−0·204
8	N	−0·088	0·083	−0·931	−0·006	N8	−0·077	N8	10	C8	0·174
9	C	0·005	−0·309	−0·078	−0·010	C9	0·011	C9	51	N9	−0·145
10	O	−0·393	−0·675	1·251	0·006	N(6)	−0·236	O(6)	54	N(6)	−0·227
11	O	−0·333	1·387	0·127	0·098	H(9)	0·007	H(1)	24	H(9)	0·114
12	H	0·015	0·000	0·000	0·000	H(2)	−0·041	H(2)	18	H(8)	−0·017
13	H	0·139	0·000	0·000	0·000	H′(6)	0·126	H(7)	19	H′(6)	0·122
14	H	0·134	0·000	0·000	0·000	H″(N6)	0·116	H(9)	08	H″(6)	0·119
15	—	—	—	—	—	H(7)	0·100	—		H(2)	−0·045

Table 2. Stacking energy (in Kcal/mol) of OHB with nucleic acid bases.

E_{QQ}	E_{QM}	E_{MM}	Base	Separation Å	E_{el}	E_{pol}	E_{disp}	E_{rep}	E_{tot}
−2·56	−5·39	−0·77	G	3·1	−8·72	−3·19	−10·78	6·97	−15·65
−0·71	−3·34	−1·38	A	3·1	−5·43	−1·04	−5·82	2·68	−29·61
−2·32	−4·61	−0·86	C	3·2	−7·79	−2·08	−7·19	3·48	−13·58
−1·42	−2·05	−0·31	U	3·3	−3·78	−1·03	−4·37	1·15	−8·03
−1·53	−2·26	−0·34	T	3·2	−4·13	−1·19	−5·03	1·62	−8·72

E_{QQ} , Monopole-monopole; E_{QM} , monopole-dipole; E_{MM} , dipole-dipole energy terms; E_{el} , electrostatic energy; E_{pol}, polarization energy; E_{disp}, dispersion energy; E_{rep}, repulsion energy; E_{tot}, total energy terms. $E_{el} = E_{QQ} + E_{QM} + E_{MM}$; $E_{tot} = E_{el} + E_{pol} + E_{disp} + E_{rep}$.

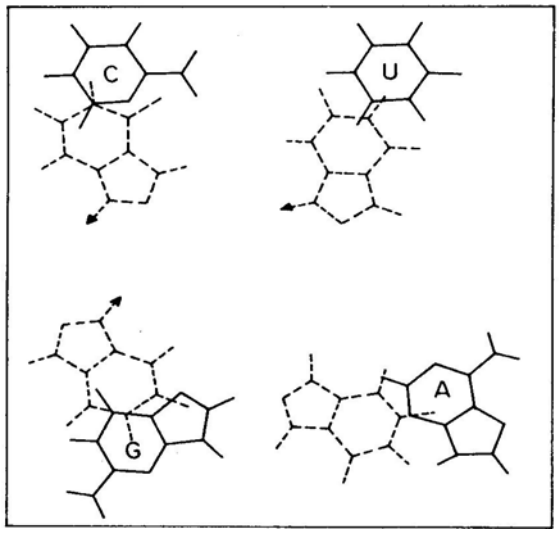

Figure 2. Minimum energy configuration of stacked complexes of OFB base with nucleic acid bases.

$$\binom{\text{OFB}}{\text{G}} > \binom{\text{OFB}}{\text{C}} > \binom{\text{OFB}}{\text{A}} > \binom{\text{OFB}}{\text{T}} > \binom{\text{OFB}}{\text{U}}.$$

It is clear from the table 2 that the stability of these complexes is in the order. This indicates the selectivity of the bases for binding with AFB base. The energy components in table 2 show that the dispersion terms are dominant for all the complexes, while the electrostatic interactions stabilize the minimum energy geometries. The polarization terms are very weak for all the complexes. It may be inferred that the binding for the polar bases guanine and cytosine is stronger than for adenine, thymine and uracil. The stacked complexes shown in figure 2 indicate that the polar atom of one molecule is oriented over that of the other.

Pairing energy

The pairing energy results from the in-plane interaction between the OFB and nucleic acid bases. Only the interactions with the complementary bases of the parent

Table 3. Hydrogen bonding energy (in Kcal/mol) of OFB with nucleic acid bases.

Interacting base	E_{QQ}	E_{QM}	E_{MM}	E_{el}	E_{pol}	E_{disp}	E_{rep}	E_{tot}	Complex
U	−2·15	−2·94	−0·92	−6·01	−1·53	−5·27	5·93	−6·87	I
T	−2·16	−2·98	−0·93	−6·07	−1·56	−5·29	5·93	−6·99	
U	−2·30	−3·46	−0·96	−6·71	−1·22	−4·96	5·67	−7·22	II
T	−2·29	−3·49	−0·97	−6·75	−1·24	−4·99	5·68	−7·30	
U	−2·32	−3·52	−0·87	−6·72	−1·34	−5·01	5·78	−7·28	III
T	−2·31	−3·47	−0·86	−6·63	−1·30	−5·13	5·79	−7·27	
U	−2·30	−3·03	−0·83	−6·16	−1·66	−5·32	6·04	−7·10	IV
T	−2·27	−2·97	−0·81	−6·05	−1·62	−5·44	6·05	−7·06	
C	−0·91	−1·35	−0·17	−2·43	−2·46	−5·76	4·95	−5·70	V
C	−0·20	−0·93	−0·08	−1·21	−2·15	−5·59	4·81	−4·14	VI

nucleoside (purine) have been considered. The minimum energy configurations of the complexes are illustrated in figure 3 for normal (*anti*) and inverted (*syn*) conformations of the nucleoside. The calculated energy values for these complexes (table 3) are compared with the standard energy values for the Watson-Crick pairs (table 4). The energy value for OFB : C is smaller than the recommended energy value and stable complex formation with cytosine is not possible. Of the 4 OFB: U/T complexes, two represent the *syn,* while the other two represent the *anti* conformation of the analogue. It is clear that the energy values for these complexes are comparable to those of A: U/T pairs although the complexes are less stable than the Watson-Crick pairs. From figure 3 it is clear that their geometries are different from those of standard base pairs because the glycosyl C(l')-N bond does not obey

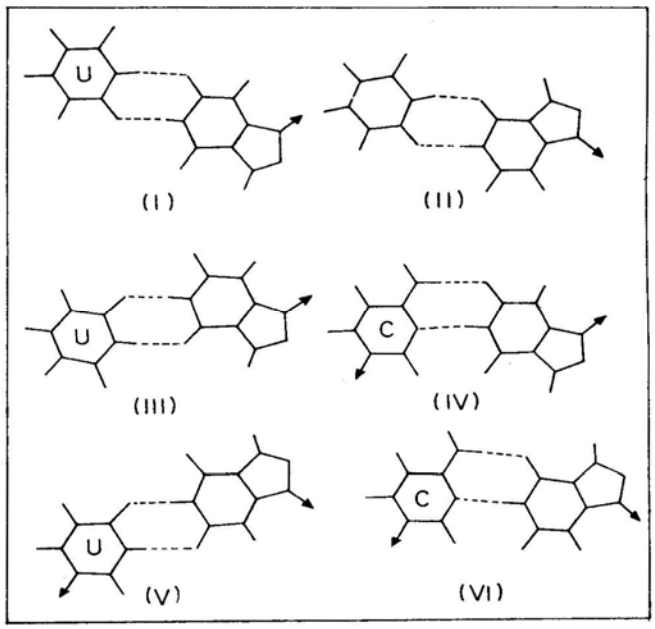

Figure 3. Dimer complexes of OFB with normal bases.

Table 4. Recommended energy values for nucleic acid bases.

Pairing energy		Trimer energy	
Complex	Energy (Kcal/mol)	Complex	Energy (Kcal/3 mol)
G-C	−20·55	(G-C)-G	−20·91
		(G-C)-C	−14·75
A-U	− 8·52	(A-U)-A	− 5·01
		(A-U)-U	− 8·55
A-T	− 8·49	(A-T)-A	− 4·42
		(A-T)-T	− 8·10

dyad symmetry and the isomorphism of A-U/T and G-C base pairs is lost (Crick, 1966). It is inferred that the AFB base may form 'wobble' base pairs in the nucleic acid chain.

Trimer energy

The in-plane interaction energies of the OFB base with nucleic acid base pairs are given in table 5 and the corresponding minimum energy configurations for these complexes are shown in figure 4. AFB base forms two complexes I and II with binding energies of – 7·00 Kcal/3 mol and – 8·69 Kcal/3 mol, respectively when it interacts with the guanine-cytosine base pair. These energies are much smaller than the recommended transcription energy values for (G-C)-G and (G-C)-C complexes (table 4). The association sites are identified in figure 5; the unmarked arrows represent the glycosyl bond of AFB and. the arrows marked with a star represent the spatial position of the nucleic acid bases interacting with DNA base pairs in the configurations recommended for the transcription process (Sanyal *et al.*, 1986a-d,

Table 5. Hydrogen bonding energy (in Kcal/3 mol) of OFB with nucleic acid base pairs.

Base pair	Complex	E_{QQ}	E_{QM}	E_{MM}	E_{el}	E_{pol}	E_{disp}	E_{rep}	E_{tot}
G-C	I	−1·98	−2·66	−0·37	−5·01	−2·08	−5·44	5·53	−7·00
	II	−2·57	−3·19	−0·25	−6·01	−2·49	−5·87	5·69	−8·69
A-U	III	−1·94	−3·65	−1·16	−6·75	−1·60	−6·88	6·79	−8·44
	IV	−1·60	−2·98	−0·98	−5·56	−1·15	−5·57	5·41	−6·87
	V	−1·62	−3·30	−1·08	−6·00	−1·27	−6·34	6·35	−7·27
	VI	−0·16	−0·16	−0·50	−0·82	−1·05	−6·27	5·65	−2·49
	VII	−1·82	−3·22	−0·52	−5·55	−1·22	−5·13	4·80	−7·11
	VIII	−2·01	−2·50	−0·65	−5·16	−1·83	−6·11	6·06	−7·04
	IX	−1·52	−3·31	−1·24	−6·07	−1·01	−5·52	5·13	−7·47
A-T	III	−1·87	−2·95	−0·64	−5·46	−2·29	−8·64	9·48	−6·91
	IV	−1·60	−3·01	−0·99	−5·60	−1·18	−5·57	5·41	−6·94
	V	−1·64	−3·34	−1·09	−6·07	−1·29	−6·36	6·35	−7·37
	VI	−0·20	−0·21	−0·51	−0·91	−1·07	−6·28	5·65	−2·61
	VII	−1·84	−3·24	−0·53	−5·61	−1·25	−5·14	4·80	−7·20
	VIII	−1·72	−4·18	−1·41	−7·30	−1·29	−5·55	5·81	−8·33
	IX	−1·52	−3·33	−1·24	−6·10	−1·02	−5·53	5·13	−7·52

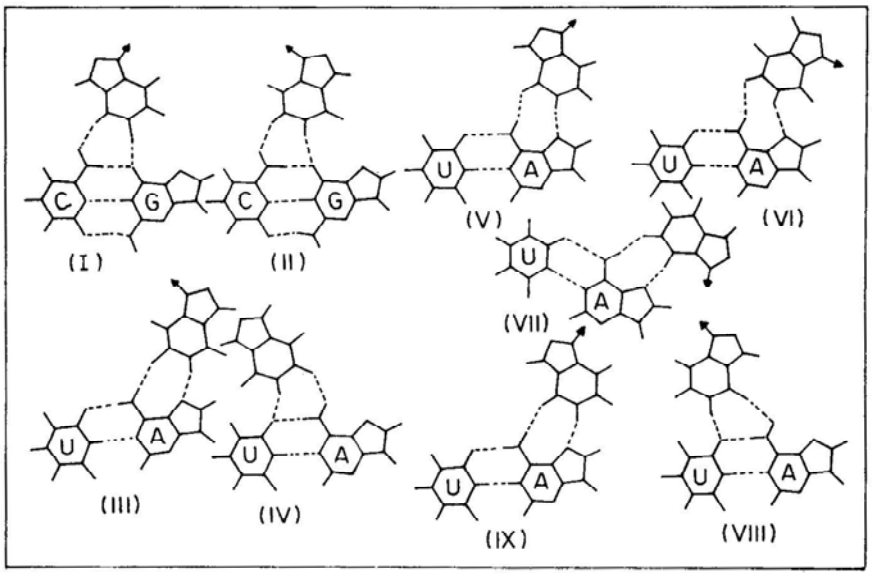

Figure 4. Minimum energy configuration of OFB with nucleic acid base pairs.

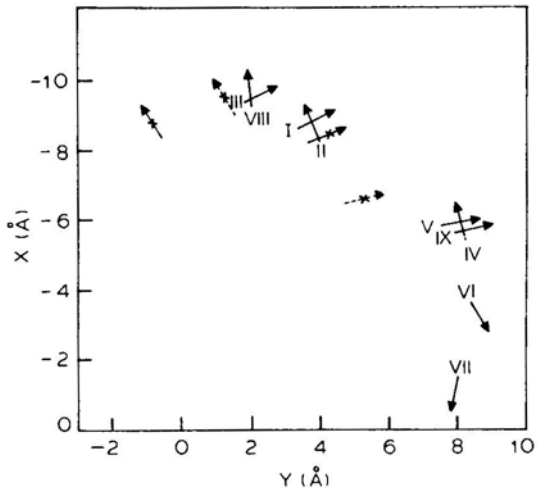

Figure 5. Arrow diagram corresponding to the sugar ends of the OFB base in the minimum energy configuration with base pairs. Arrows with a star represent the recommended site of association.

1987). The analysis of complexes I and II by means of figure 5 shows that the former differs appreciably from the recommended site, while the latter deviates from the Standard site of interaction. This shows that II (*anti*) presents no interesting condition while interaction of OFB in configuration I (*syn*) seems to be interesting. Although the interaction energy of this configuration is not sufficiency to replace G during transcription, binding of some of the analogue under high concentration of the drug is not improbable.

The interaction energies of (A-U)-OFB and (A-T)-OFB complexes are given in table 5 and their corresponding minimum energy complexes are illustrated in figure 4. The spatial positions for (A-U)-OFB and (A-T)-OFB complexes are similar and only the minimum energy configurations for (A-U)-OFB are shown. The spatial position of these complexes are compared in figure 5. Comparison of energy values of these complexes with the recommended energy value for (A-U)-A (table 4) shows that some of the complexes have sufficiency energy values. It is noteworthy that for A-U/A-T pair, the selection of entrant bases and base analogue depends more on the spatial position than the energy (Sanyal *et al.,* 1980a, b). It can be inferred from the arrow diagram of figure 5 that all the complexes except VIII are widely deviated/displaced from the recommended site of association and hence there is no possibility of association through these complexes, the *syn* conformation of the nucleoside decreasing their association power. The complex VIII fits with the spatial position to some extent. A slight displacement in its position, which reduces its energy values allows some flexibility for the association of the nucleoside. Again the configuration VIII shows the normal (*anti*) conformation of OFB, but the intrinsic conformation of OFB is *syn* (which is stabilized) and the *syn→anti* transition requires much energy (Saran *et al.,* 1977). This change in conformation decreases the energy such that its association capacity is reduced. This type of association cannot permit the growth of RNA chains during transcription (Sanyal *et al.,* 1980a, b, 1981). The inhibitory effect of OFB is due to the concentration of the molecule. Due to the lack of experimental results these speculations on the basis of theoretical interaction energy studies forbid us to compare. From these speculations it is clear that the conversion of FB→OFB stops or reduced drastically many inhibitory effects (Jain and Logothetopoulos, 1977).

Inhibitory power of pyrazolopyrimidine nucleoside

From the previous paper in this series (Sanyal *et al.,* 1986d) we have seen that formycin can incorporate in the RNA chain through the complex III with energy

Table 6. Association energy of formycin (Sanyal *et al.,* 1986d) and FB (Sanyal *et al.,* 1987) with nucleic acid base pairs.

	Formycin		FB	
Base pair	Complex	Total energy	Complex	Total energy
G-C	I	−12·63	I	−15·09
	II	−12·83	II	− 7·13
			III	− 8·62
			IV	− 6·57
A-U	III	− 5·47	V	− 8·22
	IV	− 5·90	VI	− 9·29
			VII	− 7·65
			VIII	− 6·63
A-T	III	− 5·70	V	− 8·32
	IV	− 6·01	VI	− 9·26
			VII	− 8·37
			VIII	− 6·73

value – 5·47/–5·70 Kcal/3 mol for AAU/A-T pair, where as the FB may also incorporate in the RNA chain through the complex VI with values –9·29/ –9·26 Kcal/3 mol (Sanyal *et al.,* 1987). The comparative energy values are as shown in table 6. This energy is slightly reduced to place the analogue at the recommended site, which implies that it requires some additional energy for its incorporation in the chain. The spatial positions of the complexes formed by formycin and FB are shown in figure 6. From the discussion in this paper we have that incorporation of OFB is not possible. It may bind in some cases when the concentration is very high. It is inferred that formycin is most active while OFB is least active among the drugs of the pyrazolopyrimidine class.

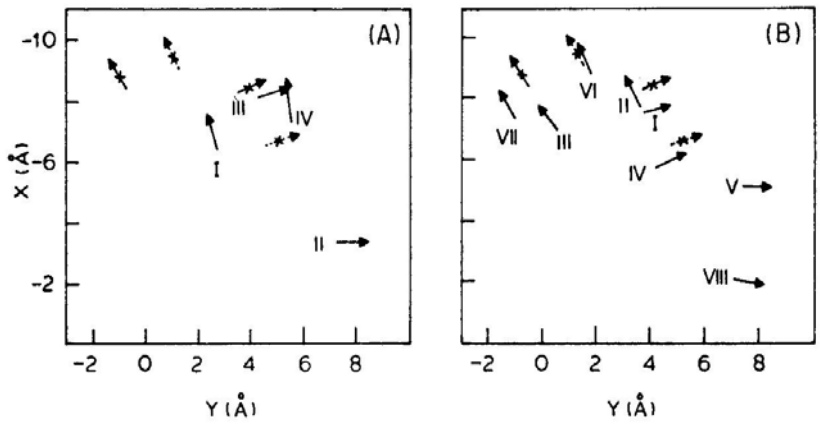

Figure 6. Arrow diagram corresponding to the sugar ends of the other analogues of pyrazolopyrimidine class of nucleoside in the minimum energy configurations of analogous base with base pairs (the energy values are given in table 6). The arrows with asterisks represent the recommended site of association. **A.** Hormycin (Sanyal *et al.,* 1986d). **B.** FB (Sanyal *et al.,* 1987).

Conclusion

From the above results we have concluded that the inhibitory power of the pyrazolopyrimidine nucleoside antibiotics is in the order

Formycin > FB > OFB,

which is in agreement with experimental results (Suhadolnik, 1970, 1979). These results also support the validity of the model of incorporation of analogue. This method may be helpful in drug design.

Acknowledgements

The authors thank Proof. G. Govil and Dr. A. Saran of the Tata Institute of Fundamental Research, Bombay, for their help, cooperation and discussions as well as for providing computational facilities. One of us (R.P.O.) is grateful to the Department of Science and Technology, and Council of Scientific and Industrial Research, New Delhi, for financial assistance.

References

Claverie, P. (1978) in *Intermolecular interactions: from diatomics to biopolymers* (ed. B. Pullman) (New York: Wiley) p. 69.
Crick, F. H. C. (1966) *J. Mol. Biol.* **19,** 548.
Ishizuka, M., Sawa, T., Koyama, G., Takuchi, T. and Umezawa, H. (1968a) *J. Antibiot.* (*Tokyo*), **A21,** 1.
Ishizuka, M., Sawa, T, Hori, S., Takayama, H., Takeuchi, T. and Umezawa, H. (1968b) *J. Antibiot* (*Tokyo*), **A21,** 5.
Jain, K. and Logothetopoulos, J. (1977) *Endocrinology,* **100,** 923.
Koyama, G., Nakamura, H., Umezawa, H. and Iitaka, Y. (1976) *Acta Crystalogr.,* **B32,** 813.
Langlet (1981).
Kunimoto, T., Wakashiro, T., Okamura, I., Asajima, T. and Hori, M. (1968) *J. Antibiot.* (*Tokyo*), **A21,** 468.
Sanyal, N. K., Kumar, U. and Sinha, R. P. (1977) *J. Theor. Biol.,* **66,** 39.
Sanyal, N. K., Kumar, U. and Roychoudhury, M. (1980a) *Nucleic Acid Res.,* **8,** 3975.
Sanyal, N. K., Kumar, U. and Roychoudhury, M. (1980b) *Nucleic Acid Res.,* **8,** 3983.
Sanyal, N. K., Roychoudhury, M. and Ojha, R. P. (1981) *Int. J. Quant. Chem.,* **20,** 69, 159, 179.
Sanyal, N. K., Roychoudhury, M. and Ojha, R. P. (1984) *J. Theor. Biol.,* **110,** 505.
Sanyal, N. K., Roychoudhury, M. and Ojha, R. P. (1985) *J. Theor. Biol.,* **116,** 195.
Sanyal, N. K., Ojha, R. P. and Roychoudhury, M. (1986a) *J. Comp. Chem.,* **8,** 113.
Sanyal, N. K., Ojha, R. P. and Roychoudhury, M. (1986b) *J. Comp. Chem.,* **8,** 120.
Sanyal, N. K., Ojha, R. P. and Roychoudhury, M. (1986c) *J. Comp. Chem.,* **8,** 130.
Sanyal, N. K., Ojha, R. P. and Roychoudhury, M. (1986d) *Int. J.Biol. Macromol.,* **8,** 183.
Sanyal, N. K., Ojha, R. P. and Roychoudhury, M. (1987) *Int. J. Biol. Macromol.,* **9,** 19.
Saran, A., Mitra, C. K. and Pullman, B. (1977) *Int. J. Quant. Chem. Quant. Biol. Symp.,* **4,** 43.
Sheen, M. R., Kim, B. K., Martin, H. and Parks, R. E. (1968) *Proc. Am. Assoc. Cancer Res.,* **9,** 63.
Sheen, M. R., Martin, H. F. and Parks, R. E. (1979) *Mol. Pharmacol.,* **6,** 99.
Suhadolnik, R. J. (1970) *Nucleoside Antibiotica* (New York: Wiley).
Suhadolnik, R. J. (1979) *Prog. Nucleic Acids Res. Mol. Biol.,* **22,** 193.