International Journal of Pharmaceutics, 19 (1984) 17–25
Elsevier

IJP 00638

Solubility enhancement of some developmental anti-cancer nucleoside analogs by complexation with nicotinamide

J. Truelove, R. Bawarshi-Nassar, N.R. Chen and A. Hussain

College of Pharmacy, University of Kentucky, Lexington, KY 40536-0053 (U.S.A.)

(Received June 9th, 1983)
(Modified version received September 28th, 1983)
(Accepted October 3rd, 1983)

Summary

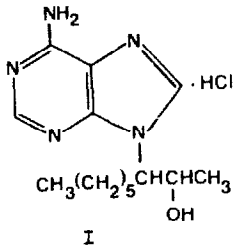
The use of the non-toxic vitamin, nicotinamide, as a solubilizing agent for the poorly water-soluble anti-cancer agents, erythro-9-(2-hydroxy-3-nonyl)adenine (NSC 263164), 3-deazauridine (NSC 126849) and thioquanine (NSC 752), is presented. NSC 263164 is shown to form 1:1 and 1:2 drug–nicotinamide complexes while NSC 126849 and NSC 752 probably form 1:1 complexes only. Nicotinamide complexation appears to be a useful approach to overcoming the most commonly encountered formulation problem in cancer chemotherapy, i.e. the lack of adequate solubility in physiologically acceptable systems.

Introduction

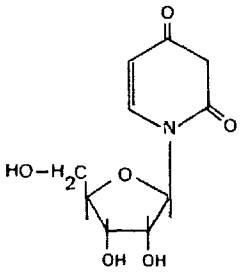
Most antitumor agents are administered intravenously in relatively large doses. The solubility of these compounds, however, is often so low that unreasonably large volumes would be required for their administration. The agent erythro-9-(2-hydroxy-3-nonyl)adenine (I) hydrochloride (NSC 263164) exhibits such poor water-solubility, even in its salt form, that practical parenteral dosage forms cannot be prepared.

Common approaches to enhancing the water-solubility of such compounds are to formulate them in highly acidic or basic solutions or in aqueous–non-aqueous

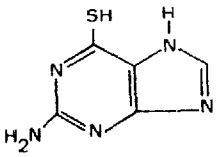
Correspondence: J. Truelove, College of Pharmacy, University of Kentucky, Lexington, KY 40536-0053, U.S.A.



I



II



III

solvent systems. Since such formulations are often found to be irritating upon administration, other approaches to enhancing compound solubility are often needed.

For example, in the case of I, the concentration desired in the dosage form was 5 mg/ml. This concentration could only be obtained if the solution pH was $\simeq 2$. Therefore, in order to achieve the desired concentration while maintaining a solution pH ≥ 4, the utility of non-toxic complexing agents was investigated.

Nicotinamide, a B vitamin, has been shown to be such an agent (Higuchi, 1959; Khalafallah, 1973). The formation of complex species between nicotinamide and certain heteroaromatic drug molecules has been shown (Fawzi et al., 1980), by molecular orbital calculation, to occur via a π-donor π-acceptor mechanism. Fawzi et al. (1980) also presented 1 : 1 and 1 : 2 drug–ligand equilibrium constants obtained by phase solubility methods for the interaction of π-donor heteroaromatic drug molecules and π-acceptor ligand molecules (nicotinamide). Since the structure of I includes the heteroaromatic adenine moiety, complexation via a similar π-donor π-acceptor mechanism might be expected to occur.

The primary objective, then, of this study was to examine the nature of the interaction of nicotinamide and I. A secondary objective was to determine the utility of the vitamin as a solubilizing agent for the other heteroaromatic anti-cancer compounds 3-deazauridine (NSC 126849, II) and thioquanine (NSC 732, III).

Experimental

Materials

Erythro-9-(2-hydroxyl-3-nonyl)adenine (I) hydrochloride, 3-deazauridine (II) and thioquanine (III) were supplied by the Pharmaceutical Resources Branch of the National Cancer Institute. The free base, I, of erythro-9-(2-hydroxyl-3-nonyl) adenine was prepared by NH_4OH neutralization of an aqueous solution of the hydrochloride salt. I (mp = 77°C) precipitated spontaneously and was analyzed spectrophotometrically and by HPLC. The chromatogram consisted of a single peak and the spectrophotometric absorbance observed was 100% of that expected based on the absorptivity of the hydrochloride. II and III were used as received. All other materials were of reagent or chromatographic grade and used as received.

Methods

The equilibrium aqueous solubility of I was determined by shaking an excess of the compound with water for 72 h at 25°C and filtering the resulting mixture. Portions of the filtrate were then subjected to pH measurement and diluted 40-fold with 0.1 N HCl prior to spectrophotometric analysis. The solubility determination was completed by comparing the observed absorbance with those obtained for similarly prepared standard solutions of I.

The pK_a of I was determined by a solubility method (Krebs and Speakman, 1945) since the absorption spectrum of the protonated and unprotonated species are virtually identical. Excess amounts (50–150 mg) of I were equilibrated at 25°C for 48 h with 5 ml portions of 0.1 N HCl (pH 2) or 0.25 M phosphate buffers at pH values 2.3–7 and, after filtration, I in solution was determined spectrophotometrically.

The effect of nicotinamide concentration on the solubilities of I, II and III was examined by the phase solubility method. Excess amounts of the drugs were equilibrated at 25°C for 48 h with 5 ml portions of 0.1 M phosphate buffer solutions at pH values of 3 or 5 and containing up to 400 mg/ml nicotinamide. At the end of the equilibration period, the mixtures were filtered and the filtrates analyzed for drug and nicotinamide by HPLC.

The effect of the co-solvent ethanol on the total solubility of I in the presence of nicotinamide at pH 5 was examined as follows. Flasks containing 10 or 20% (v/v) ethanol were diluted to volume with 0.1 M phosphate buffer and adjusted to an apparent pH value of 5. Solutions containing 300 mg/ml nicotinamide in the alcoholic buffers were then prepared and serially diluted with additional portions of the appropriate alcoholic buffer to provide final solutions containing 0, 100, 200 and 300 mg/ml nicotinamide. Excess quantities of I were then equilibrated at 25°C for 48 h with 5 ml portions of the final nicotinamide solutions. At the end of the equilibration period, the mixtures were filtered and the filtrates subjected to pH measurements and HPLC analysis.

HPLC method of analysis of I–III and nicotinamide

Analyses of I, II and III and nicotinamide were carried out at ambient temperature by reverse-phase HPLC. The column was a 30 cm × 3.9 mm μ-Bondapak-C_{18}

TABLE 1

THE HPLC MOBILE PHASES AND RETENTION TIMES

Drug	Mobile phase (v/v)	Flow rate (ml/min)	Retention time (min)	
			Drug	Nicotinamide
I	MeOH/H_2O 60:40	0.9	10.3	3.3
II	Phosphate 0.1 M pH 7	1.5	2.4	10.0
III	MeOH/H_2O 5:95	2.0	4.5	5.5

(Waters, Milford, MA). Detection was at 254 nm. The injection volumes were 20 μl and quantitation was done by comparing sample peak heights with those obtained from standard solutions. Mobile phase compositions and retention times for each system are shown in Table 1.

Results and Discussion

In order to determine the nature of the interaction between nicotinamide and I, II and III and to examine the possible utility of that interaction as a means of solubilizing heteroaromatic anti-cancer compounds in general, the interaction of nicotinamide and I was examined in detail.

The Beer's law plot for I was found to be linear and is described by $A_{260} = 1.374 \times 10^4 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ([I]) + 8.5×10^{-3} ($r = 1.000$). The equilibrium aqueous solubility of I was found to be 7.6×10^{-4} M at the pH of its saturated solution (7.8).

The solubility of I as a function of pH is shown in Table II. The solubility at pH 7 was taken to represent the intrinsic solubility and the pK_a values were calculated from Eqn. 1 (Krebs and Speakman, 1945)

$$pK_a = pH + \log\left[\frac{S_T - S_0}{S_0}\right] \quad (1)$$

TABLE 2

THE SOLUBILITY OF I AS A FUNCTION OF pH AND THE pK_a VALUES CALCULATED BY THE METHOD OF KREBS AND SPEAKMAN (1945)

Sample	Amount of I (mg)	pH	Total solubility, ($M \times 10^4$)	pK_a calculated
1	150	2.0	948	4.29
2	100	2.3	405	4.22
3	100	3.1	56.6	4.13
4	100	4.1	12.3	4.29
5	50	7.0	4.82	–

where S_T is the total solubility and S_0 is the intrinsic solubility. Using the pK_a value found (4.2), the theoretical aqueous solubility was calculated to be 0.55 mg/ml. It was apparent from these data that pH adjustment could not produce the desired concentration at pH values ≥ 4.

The solubility of I was found, however, to increase at pH values 3 and 5 in the presence of nicotinamide as shown in Figs. 1 and 2, respectively. As shown in these Figs., however, the increase in total solubility was not a linear function of nicotinamide concentration suggesting the formation of higher-order complexes.

The formation of these complexes may be expressed as:

$$[I] + [Ni] \overset{K_{1:1}}{\rightleftharpoons} [I \cdot Ni] \tag{2}$$

$$[I] + 2[Ni] \overset{K_{1:2}}{\rightleftharpoons} [I \cdot Ni_2] \tag{3}$$

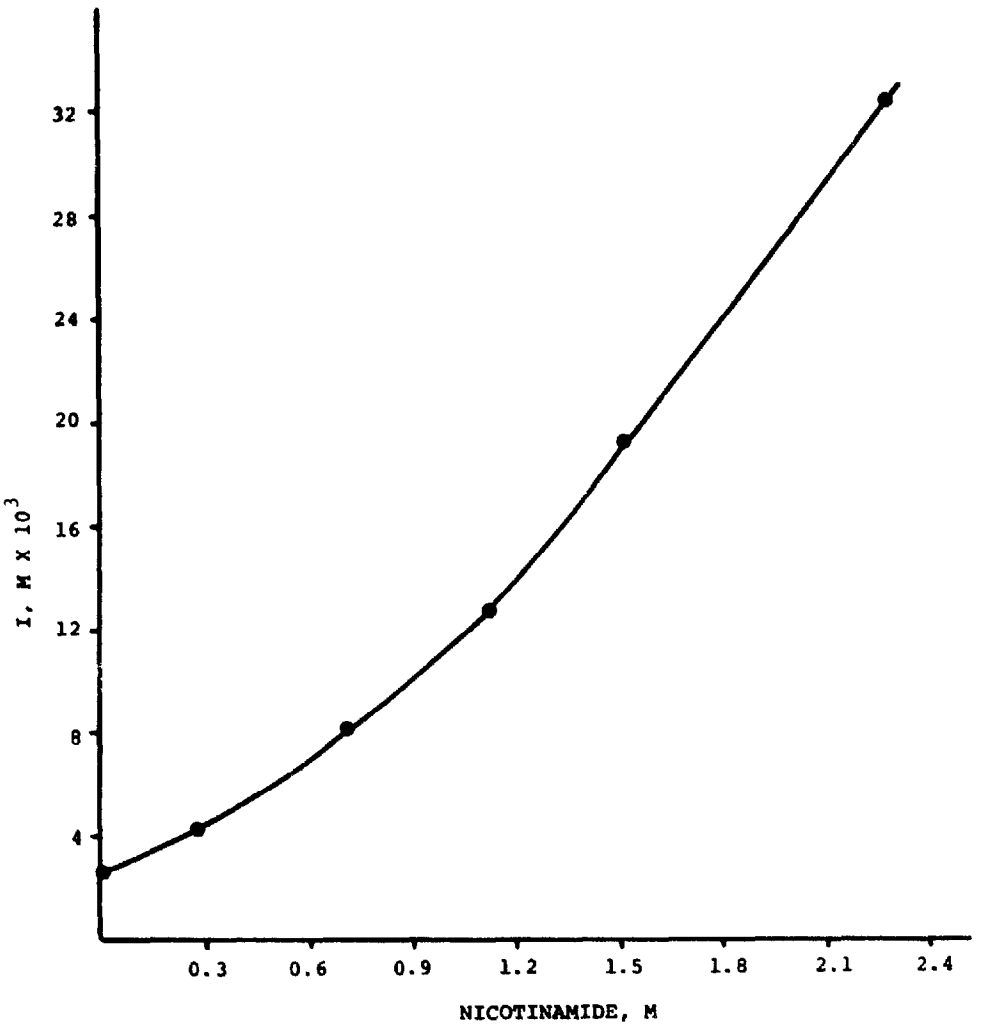


Fig. 1. The influence of nicotinamide concentration on the solubility of I in 0.1 M phosphate buffer at pH 3 and 25°C.

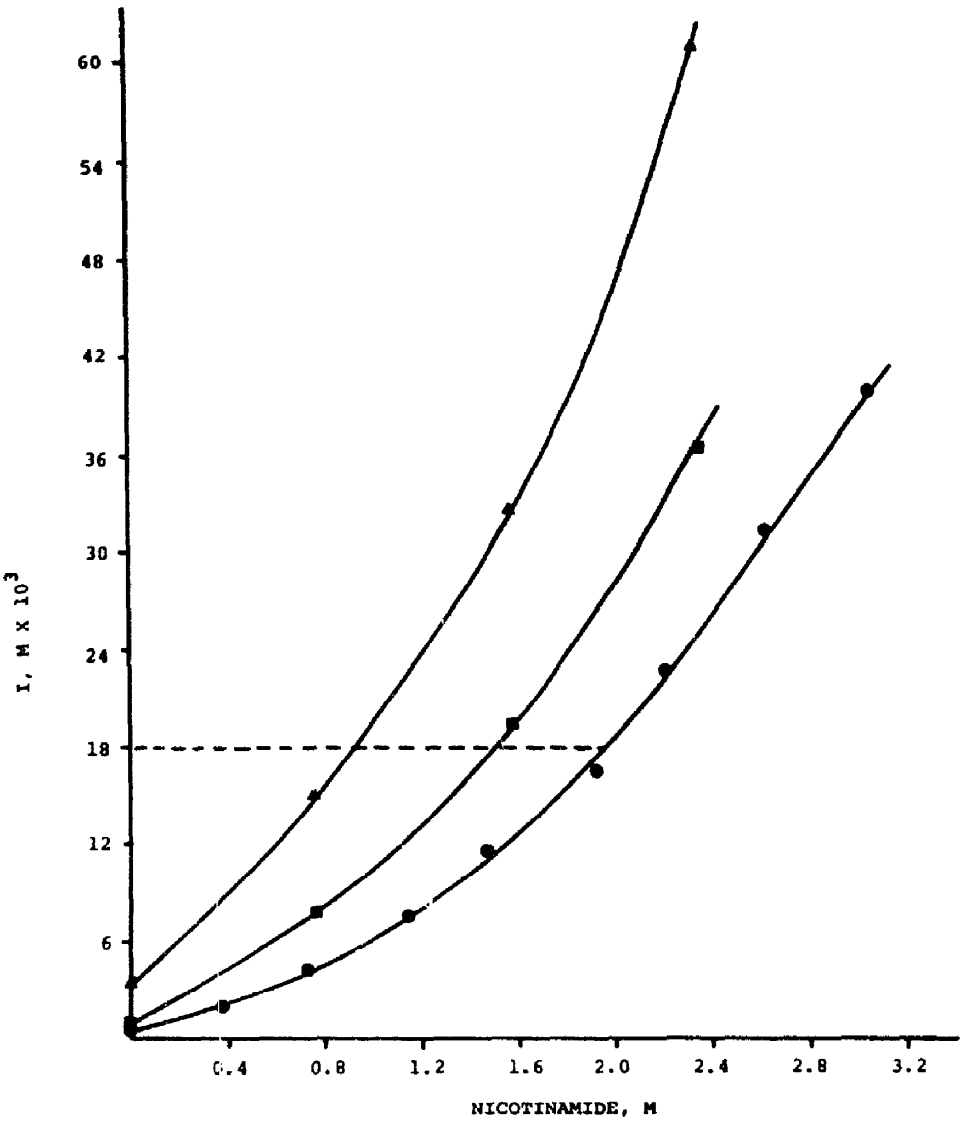


Fig. 2. The influence of nicotinamide concentration on the solubility of I in alcoholic 0.1 M phosphate buffer at pH 5 and 25°C: 0% ethanol, ●; 10% ethanol, ■; and 20% ethanol, ▲. (The dashed line represents the solubility desired in the dosage form.)

where [I] is the concentration of free I; [Ni] is the concentration of free nicotinamide; $[I \cdot Ni]$, $[I \cdot Ni_2]$, $K_{1:1}$ and $K_{1:2}$ are the concentrations of and formation constants for the 1 : 1 and 1 : 2 complexes, respectively. It follows that the total solubility of I is:

$$[I]_T = [I] + [I \cdot Ni] + [I \cdot Ni_2] \tag{4}$$

or

$$[I]_T - [I] = K_{1:1}[I][Ni] + K_{1:2}[I][Ni]^2 \tag{5}$$

It can be shown (Iga et al., 1981) that:

$$[Ni] = \frac{[Ni]_T - 2([I]_T - [I])}{1 - K_{1:1}[I]} \tag{6}$$

where $[Ni]_T$ is the total nicotinamide concentration. Substituting Eqn. 6 into Eqn. 5 and rearranging gives:

$$\frac{[I]_T - [I]}{[Ni]_T - 2([I]_T - [I])} = \frac{K_{1:1}[I]}{1 - K_{1:1}[I]} + \frac{K_{1:2}[I]}{(1 - K_{1:1}[I])^2}\{[Ni]_T - 2([I]_T - [I])\} \tag{7}$$

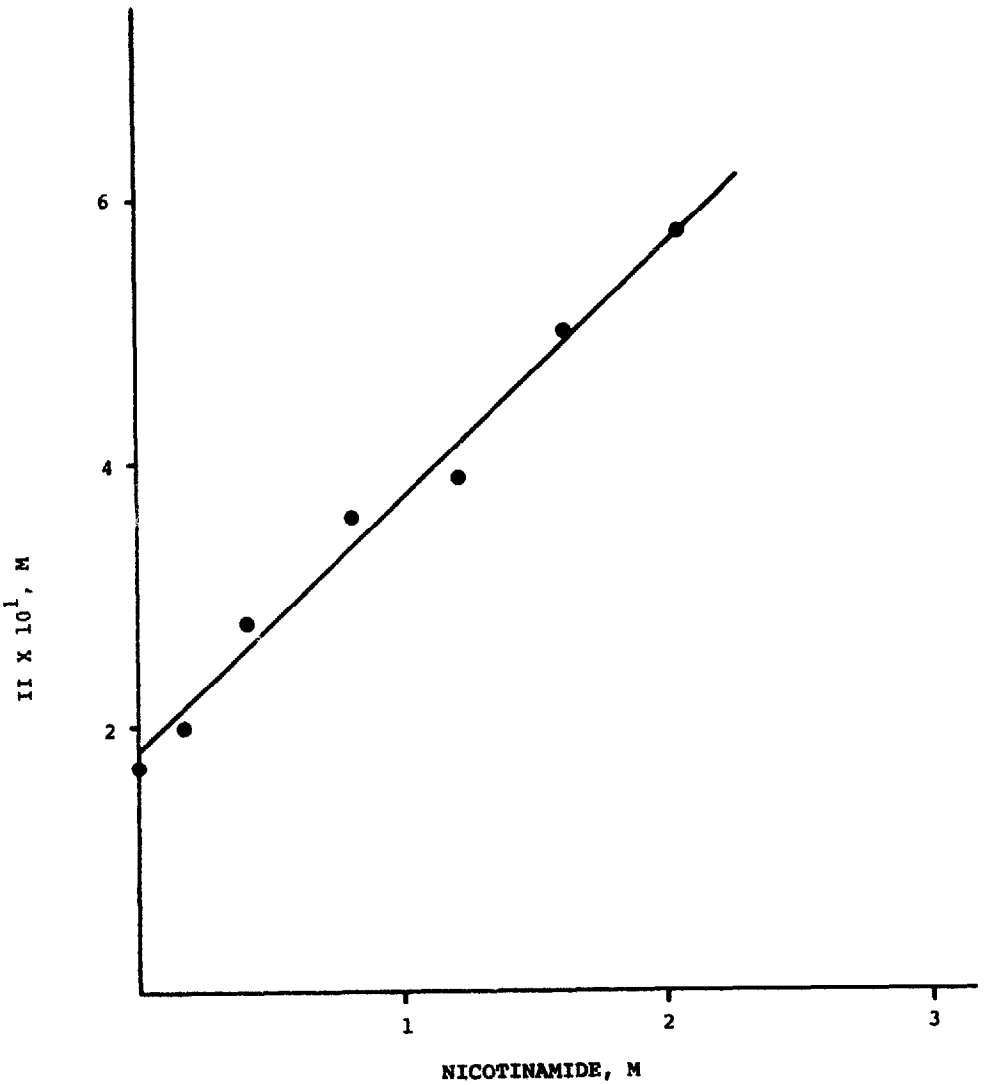


Fig. 3. The influence of nicotinamide concentration on the solubility of II in 0.1 M phosphate buffer at pH 5 and 25°C.

Eqn. 7 suggests that a plot of $[I]_T - [I]/\{[N_i]_T - 2([I]_T - [I])\}$ versus $[Ni]_T - 2([I]_T - [I])$ should be linear with a slope of $K_{1:2}[I]/(1 - K_{1:1}[I])^2$ and an intercept of $K_{1:1}[I]/(1 - K_{1:1}[I])$. Such a plot of the data at pH 5 and 0% ethanol was found to be linear ($r = 0.9943$) with a slope of 3.76×10^{-3} M^{-1} and an intercept of 1.99×10^{-3}. The values of $K_{1:1}$ and $K_{1:2}$ obtained from the intercept and slope were 3.6 M^{-1} and 6.8 M^{-1}, respectively.

The use of an ethanol co-solvent further enhances the solubility of I in solutions of nicotinamide. As shown in Fig. 2, the use of 20% ethanol, for example, reduces the amount of nicotinamide required to achieve the desired solubility by a factor of 2.

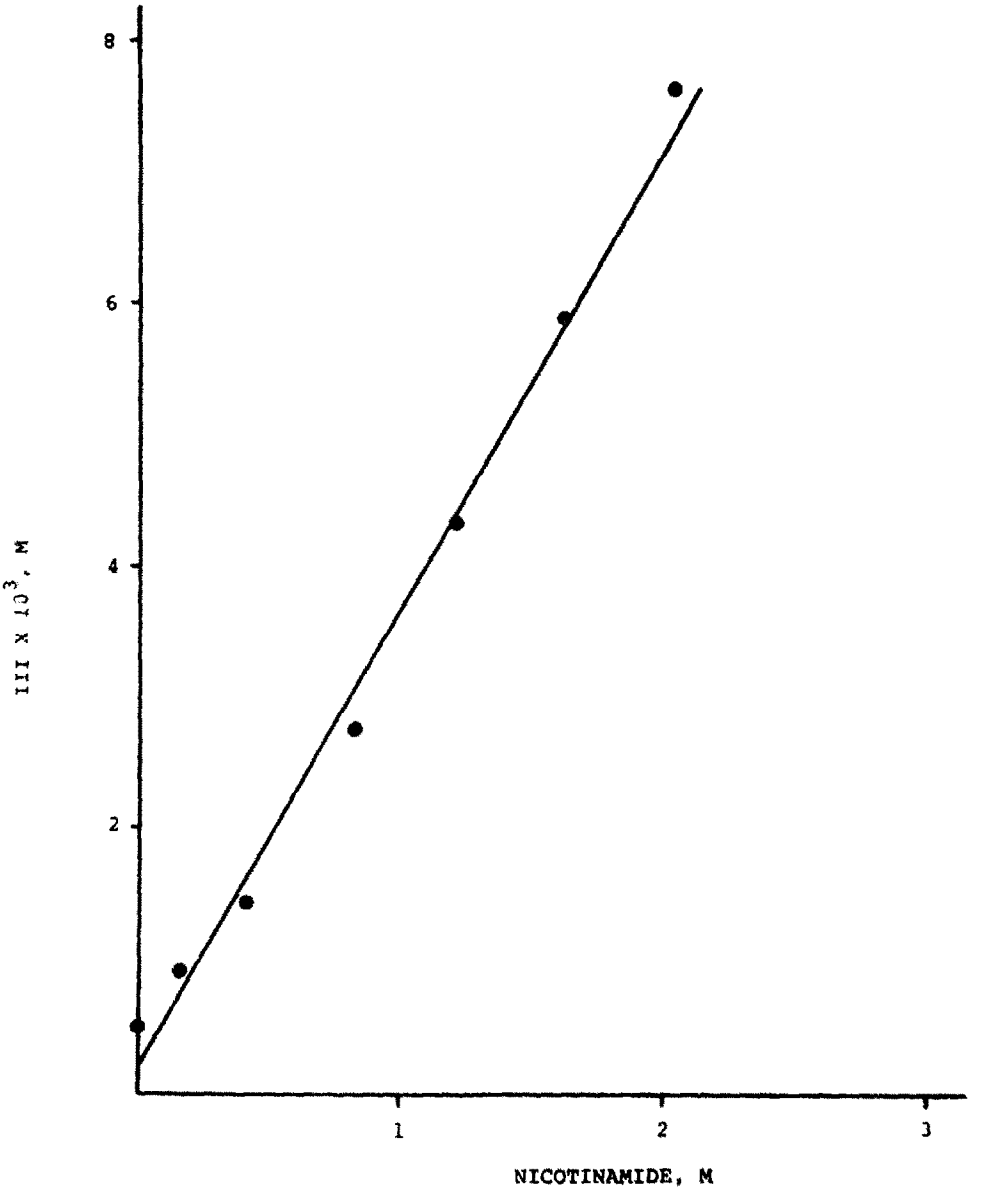


Fig. 4. The influence of nicotinamide concentration on the solubility of III in 0.1 M phosphate buffer at pH 5 and 25°C. The line is the least-squares linear regression of $[III]_T$ versus $[Ni]_T$.

The influence of nicotinamide on the total solubility of II and III is shown in Figs. 3 and 4, respectively. It is apparent that nicotinamide complexation also enhances the solubility of these anti-cancer agents. The solubility of II appears to increase in a linear fashion, consistent with the formation of a 1 : 1 drug–nicotinamide complex. The 1 : 1 stability constant calculated for the complex was 1.33 M^{-1}. As shown in Fig. 4, the solubility of III increases with nicotinamide concentration. The data, while clearly linear at higher ligand concentrations, may exhibit slight curvature at the lower nicotinamide concentrations. The line shown in Fig. 4 is the result of least-squares linear regression of $[III]_T$ versus $[Ni]_T$ ($r = 0.9961$, $K_{1:1} = 17.3$ M^{-1}). The data were also fitted to a plot of $([III]_T - [III])/\{[Ni]_T - 2([III]_T - [III])\}$ versus $[Ni]_T - 2([III]_T - [III])$ in accordance with Eqn. 7. The result was, however, somewhat less satisfactory ($r = 0.976$). Although the exact nature of the interaction occurring between III and nicotinamide may not be that resulting in exclusive formation of a 1 : 1 complex, it is clear that significant solubility enhancement has occurred.

Conclusions

The results described here indicate that nicotinamide forms complexes with 3 heteroaromatic anti-cancer agents. Erythro-9-(2-hydroxy-3-nonyl)adenine (NSC 263164) was found to form 1 : 1 and 1 : 2 complexes whereas 3-deazauridine (NSC 126849) was found to form a 1 : 1 complex only. The solubility of thioquanine (NSC 732) was found to be enhanced by nicotinamide, although the nature of the complex(es) formed is less clear. It appears that nicotinamide complexation may be a useful approach to the enhancement of the solubility of heteroaromatic anti-cancer compounds.

Acknowledgement

This work was supported in part by NCI Contract NOI-CM-07381.

References

Fawzi, M., Davison, E. and Tute, M., Rationalization of drug complexation in aqueous solution by use of Hückel frontier molecular orbitals. J. Pharm. Sci., 69 (1980) 104–106.

Higuchi, T. and Bolton, S., The solubility and complexing properties of oxytetracycline and tetracycline III. J. Am. Pharm. Assoc., Sci. Edn., 48 (1959) 557–564.

Iga, K., Hussain, A. and Kashihara, T., New direct calculation of $K_{1:1}$ and $K_{1:2}$ complexation constants using solubility method. J Pharm. Sci., 70 (1981) 108–109.

Khalafallah, N., El-Shibini, F., El-Khawas, F., Daabis, N. and Saleh, A., The influence of nicotinamide on the solubility of certain drugs. J. Drug Res. Egypt, 5 (1973) 175–188.

Krebs, H. and Speakman, J., Dissolution constant, solubility, and the pH value of the solvent. J. Chem. Soc., (1945) 593–595.