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PREFORMULATION STUDIES ON AG-392, A NOVEL ANTITUMOR COMPOUND.

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ABSTRACT

AG-392, a novel antitumor agent, was synthesized using a technology described as "Protein Structure-based Drug Design". A validated HPLC analytical method was developed to quantitate AG-392. Solubility studies conducted on the AG-392 free base, its glucuronate salt and mesylate salt showed solubilities less than 0.8 mg/mL in D_5W. However, the solubility in propylene glycol for the free base was 24 mg/ml and was 7 and 27 mg/mL for the glucuronate and mesylate salts of AG-392, respectively. Based on the results of preformulation studies, a formulation of glucuronate salt of AG-392 in propylene glycol at a concentration of 5 mg/mL was developed for the parenteral delivery. This formulation is compatible with D_5W and WFI, but not saline solution. The dilute solutions in WFI show pH values ranging from 4.7 to 5.5. The proposed formulation was tested for *in vivo* efficacy in a murine lymphoma model and was found to display antitumor activity.

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INTRODUCTION

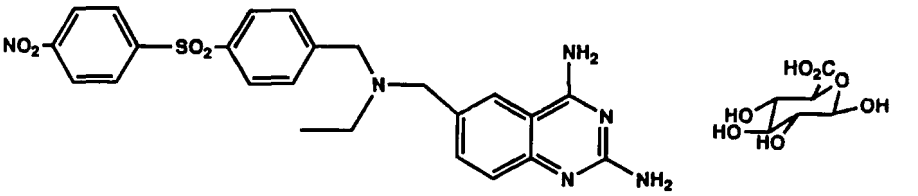
The enzymes, thymidylate synthase (TS) and dihydrofolate reductase (DHFR), are attractive targets for cancer chemotherapy because of the key role they play in DNA biosynthesis[1]. A series of 2,4-diamino quinazoline compounds was designed as bifocal antifolates based upon knowledge of the three dimensional structures of both enzymes. From this series, the compound 2,4-diamino-6-(N-(4-(4'(nitrophenyl)sulfonyl)-benzyl)-ethyl) aminoquinazoline glucuronate was chosen for further preclinical study. This compound inhibits both TS and DHFR, with inhibitory constants of 25 nM and 0.21 pM, respectively[2]. The glucuronate salt of AG-392 was found to be a potent inhibitor of cellular growth in a variety of human and murine cell lines and DHFR was determined as the primary intracellular locus of cytotoxicity. Antitumor activity was also observed *in vivo* when glucuronate salt was tested against P388 leukemia, colon 26 adenocarcinoma, and B16 melanoma tumors in mice[1,2]. In view of the promising antitumor potential of glucuronate salt of AG-392, preformulation studies were performed to develop an injectable formulation which would be suitable for further preclinical evaluation.

The purpose of the present work was to investigate the cosolvency effect of propylene glycol on the solubility of the free base of AG-392 and its glucuronate and mesylate salt. An injectable solution of the glucuronate salt was developed as a product. A filtration study was conducted to investigate if there was any loss during filtration through 0.2 μm filters to evaluate if aseptic processing would be possible. A binding of other drugs with some filters has been observed in our laboratories with other thymidylate synthase inhibitors[4]. Since the injectable product (glucuronate salt of AG-392 concentrate in propylene glycol) must be diluted prior to use, the stability in dilute solutions was also investigated.

EXPERIMENTAL

1. The free base (AG-392), glucuronate salt and mesylate salt of AG-392 were synthesized by Agouron Pharmaceuticals, Inc.

The chemical structure of the glucuronate salt is as follows:



Molecular weight: 672.68

2. HPLC Analysis:

HPLC:	Hewlett Packard 1090M HPLC equipped with a Diode-Array detector and an auto injector.
Column:	LiChrospher 100 RP-8 column (125x4 mm, 5 μm)
Wavelength:	240 nm
Mobile Phase:	Methanol:0.01M Phosphate Buffer (72:28)
Flow Rate:	0.8 mL/min

3. Filtration: The mesylate salt solution was filtered through three different 0.2 μm syringe filters, with 13 mm diameters. The filtration volume was 2 mL. The percentage of recovery was the ratio of its concentration in solution before and after filtration.

Filters used:	Gelman Polysulfone filters, 0.2 μm, Lot 2133
	Gelman PTFE filters, 0.2 μm, Lot 2745
	Gelman Nylon filters, 0.2 μm, Lot 1462

4. Solubility: An excess amount of drug was added to test tubes containing 5% dextrose in water (D_5W) with varying concentrations of propylene glycol (PG). The suspensions were sonicated for approximately one hour and then shaken overnight at room temperature before they were filtered through 0.45 μm polysulfone filters to remove any undissolved particles. After appropriate dilutions, the solutions were assayed by HPLC.

5. Dilution of Solution Formulations: The free base, glucuronate salt and

mesylate salt were dissolved in PG with sonication. The final drug concentrations were 10, 5 and 20 mg/mL, respectively. The solutions were then diluted with D_5W at a ratio of 1:1, 1:2, 1:3, 1:5, 1:10 and 1:20. The pH value of each solution was measured.

6. Antitumor Studies: The glucuronate salt was dissolved in PG and diluted to a ratio of 1:1 with D_5W to give a final concentration of 1.4 mg/ml. This formulation was administered to tumor-bearing male $B6D2F_1$ mice by daily intraperitoneal injection at a dose of 14 mg/kg for seven days. The thymidine kinase deficient murine lymphoma, L5178Y(TK^-), was inoculated intramuscularly into the gastrocnemius muscle at 5×10^6 cells per mouse on day 0. Treatment began on day 1. Tumor growth was monitored by changes in leg diameter at the site of inoculation.

RESULTS AND DISCUSSION

Filtration-Binding Study

The results of this study showed that there was no significant drug loss after filtration through any of the three membranes studied.

Solubility Study

The solubility of the AG-392 free base, the glucuronate and the mesylate salts is tabulated in Table 1. The solubility of these three compounds in PG and D_5W is apparently higher than the solubility in WFI. PG was added to the D_5W solution in order to enhance the solubility in D_5W. The cosolvency effect of PG on solubilities of the free base, glucuronate salt and mesylate salt of AG-392 in D_5W is shown in Figure 1. The results indicate that solubility of these compounds increases with increasing PG concentrations; the solubility of the free base being increased the most and that of the glucuronate salt the least. The solubilities of the AG-392 free base, glucuronate salt and mesylate salt in PG were 24, 7 and 27 mg/mL, respectively.

TABLE 1

Solubility of AG-392 free base, glucuronate and mesylate salts of AG-392

	Solubility (mg/mL)		
Chemical	WFI	D_5W	PG
AG-392 Free Base	nd	0.06	24
Glucuronate Salt of AG-392	0.07	0.72	7
Mesylate Salt of AG-392	0.12	0.54	27

nd = none detected

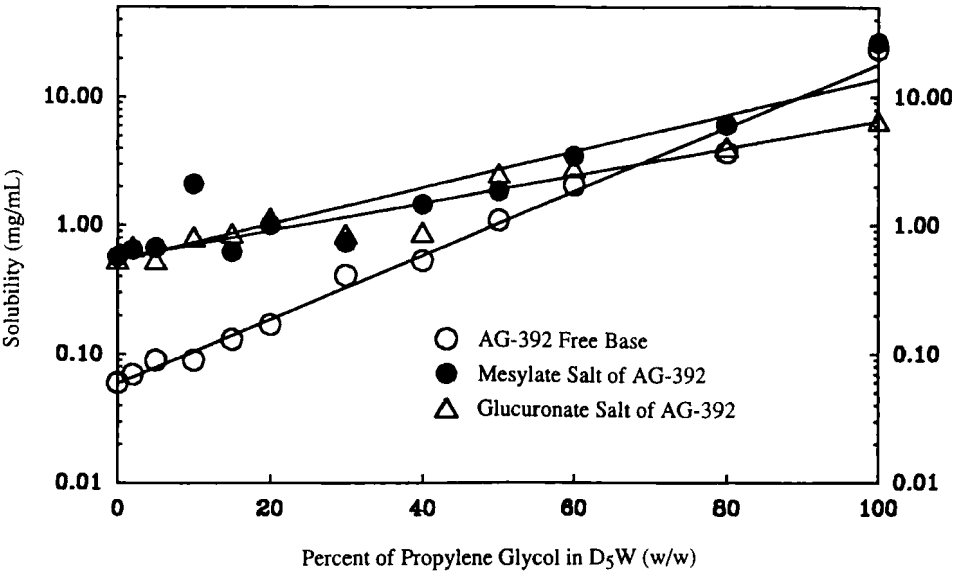


FIGURE 1

Effect of Propylene Glycol as cosolvent on Solubility of Glucuronate Salt and Mesylate Salt of AG-392.

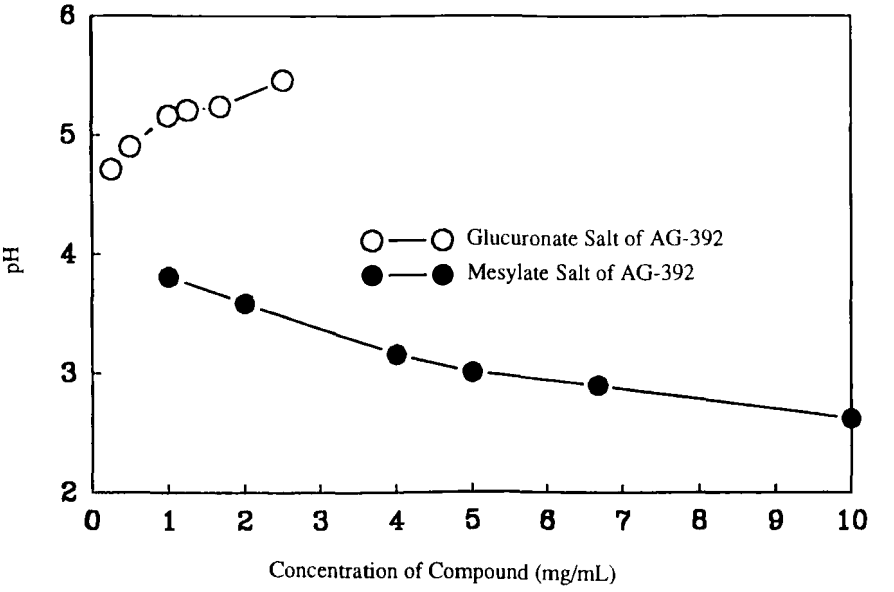


FIGURE 2

Dependence of pH on Concentration of Glucuronate Salt and Mesylate Salt of AG-392.

Dilution Study

The free base, glucuronate salt of AG-392 and mesylate salt of AG-392 were dissolved in PG at concentrations of 10, 5 and 20 mg/mL, respectively. These three solutions are compatible with both D_5W and WFI, but cause precipitation in saline solution.

Dilutions of these solutions were performed using D_5W at ratios of 1:1, 1:2, 1:3, 1:5, 1:10, and 1:20. The AG-392 free base, precipitated out immediately under every dilution condition. The mesylate salt of AG-392 precipitated slowly after dilution. Dilute glucuronate salt of AG-392 was stable for at least 10 days. The pH values of the freshly diluted solutions of glucuronate and mesylate salts were measured and the relationships between pH value and concentration are shown in Figure 2.

As is shown in Figure 2, pH values of mesylate solutions decrease for more dilute solutions. However, pH values of glucuronate solutions increase with dilution. The pH increased from 4.7 to 5.5 with an increase in the concentration of the glucuronate from 0.25 mg/mL to 2.5 mg/mL.

In vivo Antitumor Study

Treatment with the glucuronate salt of AG-392 PG/D_5W formulation at 14 mg/kg daily resulted in a tumor growth delay of more than 23.5 days as compared to vehicle-treated controls. In addition, at the end of the study (day 35) 100% of glucuronate salt of AG-392-treated mice were designated as "cured" meaning that there was no evidence of tumor at the site of inoculation.

CONCLUSION

An injectable formulation of glucuronate salt of AG-392 in PG at a concentration of 5 mg/mL was developed. The solution can be aseptically filtered using 0.2 μm polysulfone, PTFE or Nylon membrane. The product is compatible with D_5W and WFI and must be diluted with D_5W prior to use. After dilutions, physiologically acceptable pH values (between 4.7 and 5.5) result. This product was also shown to display biological activity in a murine tumor model.

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