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Aromatic And Quinone Derivatives Of Aspirin That Express Antibacterial Activity

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ABSTRACT

Two aspirin (acetylsalicylic acid) derivatives were synthesized which have an aromatic or quinone substituent that forms an ester group at the former site of the carboxyl group of aspirin. An aromatic substiuent was formed utilizing martius yellow and a quinone moiety by utilizing the dye alizarin. The former derivative contains one aspirin molecule while the latter incorporates two aspirin molecules. Both aspirin derivatives showed measurable growth inhibition and/or aggregation of bacteria when tested against gram negative bacteria Escherichia coli. Bacterial inhibition was expressed at all solubilizing concentrations. The aqueous solubility of aspirin-alizarin derivative is determined to be 11.88 μg/mL while aspirin-martius yellow solubilizes to 31.3 μg/mL. Molecular modeling determined properties such as parachor, Log P, polar surface area, molecular volume, rotatable bonds, numbers of -OH & -NH, and molar refractivity. The aromatic and quinone substituents significantly increase the lipophilic characteristic of these constructs. Calculated molecular descriptors and observed antibacterial activity demonstrates druglikeness attributes for both derivatives. The aspirin component may be released into its active form upon esterase hydrolysis of the ester group linking acetylsalicylic acid to the fused ring moiety.

KEYWORDS

Antibacterial;
Aspirin;
Alizarin;
Martius yellow.

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INTRODUCTION

Escherichia coli (*E.coli*) are a member of the genus Escherichia and the family. Enterobacteriaceae (enteric bacteria). *E.coli* exist as facultative gram negative rods that are a consistent inhabitant of the human intestinal tract. *E.coli* is responsible for three types of infections in humans: 1) Urinary tract infections (UTI); 2) Neonatal meningitis; and 3) Intestinal diseases (gastroenerities)[1,2]. *E.coli* is the most encountered bacterium in the clinical laboratory. In addition to being the primary cause of UTI, *E.coli* has been linked to diseases in most parts of the body[3]. Pneumonia and meningitis are among the many illnesses that pathogenic strains of *E.coli* can cause. *E.coli* accounts for over 28% of the cases of neonatal meningitis and over 90% of uncomplicated UTI. *E.coli* is generally sensitive to streptomycin and gentamycin[4].

Aspirin (acetylsalicylic acid) is a nonsteroidal anti-inflammatory drug (NSAID) which has been shown previously to inhibit the growth of some bacteria[5] and inhibits cyclooxygenase (COX) 1 and 2. The major metabolite of aspirin is salicylic acid, which has been shown to attenuate virulence in endovascular infections of *Staphylococcus aureus*[6]. COX is an enzyme that is a component of an enzyme complex which has been referred to as prostaglandin G/H synthetase, an important chemical for making prostaglandins[7]. Aspirin inhibits prostaglandin synthetase and consequently the manifestation of inflammation[7].

Quinones comprise one of the largest classes of antitumor agents[8] (ie. anthracycline antibiotics). In addition, quinone compounds have been shown to be effective against many disease causing organisms such as *Staphylococcus aureus*, and those causing anthrax, brucellosis, tuberculosis, and dysentery[9,10]. This work presents the synthesis, molecular properties, and in vitro appraisal of two aspirin derivatives that contain an aromatic or quinone substituent.

MATERIALS AND METHODS

Reagents and instrumentation

All reagents were obtained from Aldrich Chemical Co., P.O. Box 355, Milwaukee, WI 53201. Tissue culture evaluation was completed at the Dept. Of Veterinary & Biomedical Sciences, University of Nebraska at Lincoln.

Molecular modeling software

Partition coefficients, violations of Rule of 5, rotatable bonds, number of -NH, -OH, nitrogen atoms, oxygen atoms, PSA, index of refraction, molar volume, molar refractivity, parachor, and polarizability were calculated in combination or individually by software provided by Molinspiration (SK-841 04 Bratislava, Slovak Republic), Actelion (4123 Allschwil, Switzerland), Daylight Chemical Information Systems (27401 Los Altos, Mission Viejo, CA), EPISUITE (SRC, Suite 1975, Denver CO), Syracuse Research Corporation (Suite 1975, Denver CO), Interactive Analysis (6 Ruben Duren Way, Bedford, MA), and ChemSketch (90 Adalaide St West, M5H 3V9 Toronto Canada). Rate constants for hydrolysis and skin penetration calculated by EPISUITE. Statistical software utilized for Cluster Analysis, multiple regression, and correlations were by VISTA (Forrest Young, University of North Carolina, Chapel Hill NC), SSP Instat (Smiths Statistics, Pomona College, Claremont CA), GEPAS (Bioinformatics Unit, Centro Nacional de Investigaciones Oncologicas, 28039 Madrid, Spain), Finite Math & Applied Calculus (Stefan Warner, Steven Costenoble, Dept. Of Mathematics, Hofstra University). Multiple regression analysis utilized Numerical Mathematics Software (Orlando Mansur, Copyrighted 2001-2002).

Synthesis of Compounds

Aspirin-alizarin agent

Activate aspirin by placing 0.167 grams into 30 mL of CH_3CN (dried over molecular sieves), 0.5 mL to 1.0 mL of triethylamine, and 5.0 mL of $SOCl_2$, reflux 30 minutes. Distill off excess $SOCl_2$ and maintain volume by addition of CH_3CN. Add 0.5mL to 1.0 mL of triethylamine to act as proton sink, add 0.112 grams of alizarin. Reflux 40 minutes to one hour. Precipitate product over ice/water or -10° C overnight (overnight preferred). Store desiccated at -10° C.

Aspirin-martius yellow agent

Activate aspirin by adding 0.20 grams into 30 mL of CH_3CN (dried over molecular sieves) that has 0.50 mL to 1.0 mL of triethylamine. Add 5 mL of $SOCl_2$ and reflux 30 minutes. Distill out excess $SOCl_2$ adding additional CH_3CN to maintain volume. Add 0.26 grams martius yellow, 0.5 mL to 1.0 mL triethylamine, and reflux 30 minutes. Precipitate product over ice/water or overnight at -10°C. Store the product desiccated at -10°C.

Tissue culture evaluation

The two lead drug compounds were placed into Luria-Bertani (LB) media at various concentrations and inoculated to an optical density (OD_{600}) of 0.05 with penicillin susceptible *Escherichia coli* strain HB 101 that had been previously grown to late log phase (OD_{600} = 1.0-1.5) at 37° C. Duplicate cultures inoculated in this manner were then grown at 37° C for five hours, dilutions were plated on LB agar to determine CFU and the OD_{600} of each culture was determined.

RESULTS AND DISCUSSION

Aspirin is a multi-tasking pharmaceutical that is applied to treat a variety of illnesses. Aspirin is now accepted as an important weapon in the prevention of heart disease as well as reduces the risk of strokes. It reduces the risk of pre-eclampsia and will benefit conditions of diabetes and dementia. Increasing evidence shows that aspirin may reduce the risk of many common cancers.

Molecular structures of both aspirin derivatives are shown in figure 1 with carbon atoms numbered for C-13 NMR assignment (as parts per million, ppm). The presence of aromatic rings will increase the hydrophobicity of these compounds. The aspirin molecules are attached (linked) to the fused ring region by an ester group (R-C(O)-OR) that replaces the former carboxyl group (R-C(O)-OH) of aspirin. There is one aspirin molecule on the aspirin-martius yellow derivative and two on aspirin-alizarin. The aspirin-alizarin derivative has two aspirin molecules positioned on separte but adjacent carbons that are within the fused ring region. All oxygen atoms and nitrogen atoms found will provide sites that are hydrogen bond acceptors. Aqueous solubility was determined for aspirin-alizarin and aspirin-martius yellow as 11.88 microgram/milliliter and 31.3 microgram/milliliter, respectively.

The aromatic ring of aspirin does not lie within the same plane as the fused ring system. The actual length of the fused ring region of martius yellow within the aspirin-martius yellow derivative is calculated to be 8.401 angstroms by SPARTAN molecular modeling. Similarly the fused rings of aspirin-alizarin are 9.234 angstroms in length. The depth

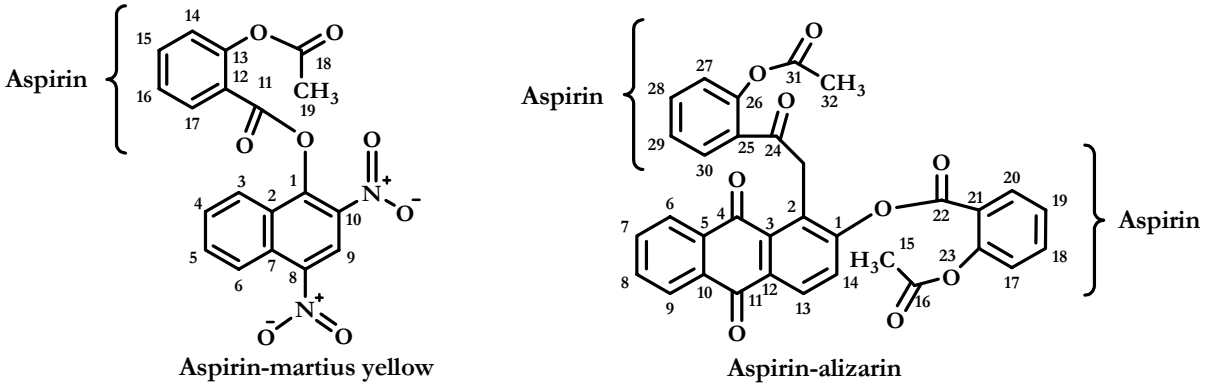

Figure 1: Structures of aspirin-martius yellow and aspirin-alizarin agent

Molecular structures of both constructs are presented here with carbon atoms numbered for C-13 assignments in ppm. Aspirin-Martius Yellow (2,4-DINI-TRO-1-NAPHTHYL-2-(ACETYLOXY) BENZOATE): 1) 149.7; 2) 126.2; 3) 123.7; 4) 129.8; 5) 133.7; 6) 123.0; 7) 128.2; 8) 146.1; 9) 116.5; 10) 136.0; 11) 164.0; 12) 123.4; 13) 154.3; 14) 121.3; 15) 133.2; 16) 125.2; 17) 130.1; 18) 168.0; 19) 16.9. Aspirin-Alizarin (9,10-DIOXO-9,10-DIHYDROANTHRACEN-1,2-YL DI[2-(ACETYL-OXY) BENZOATE]: 1) 150.0; 2) 147.1; 3) 127.5; 4) 187.0; 5) 134.2; 6) 129.6; 7) 132.5; 8) 132.5; 9) 129.6; 10) 134.2; 11) 187.0; 12) 131.4; 13) 126.8; 14) 125.8; 15) 16.9; 16) 168.0; 17) 130.1; 18) 125.2; 19) 133.2; 20) 121.3; 21) 123.4; 22) 164.0; 23) 154.3; 24) 164.0; 25) 123.4; 26) 154.3; 27) 121.3; 28) 133.2; 29) 125.2; 30) 131.1; 31) 168.0; 32) 16.9. Maximum length of flat fused ring region for aspirin-martius yellow agent is 8.401 Angstroms and for aspirin-alizarin agent is 9.234 Angstroms.

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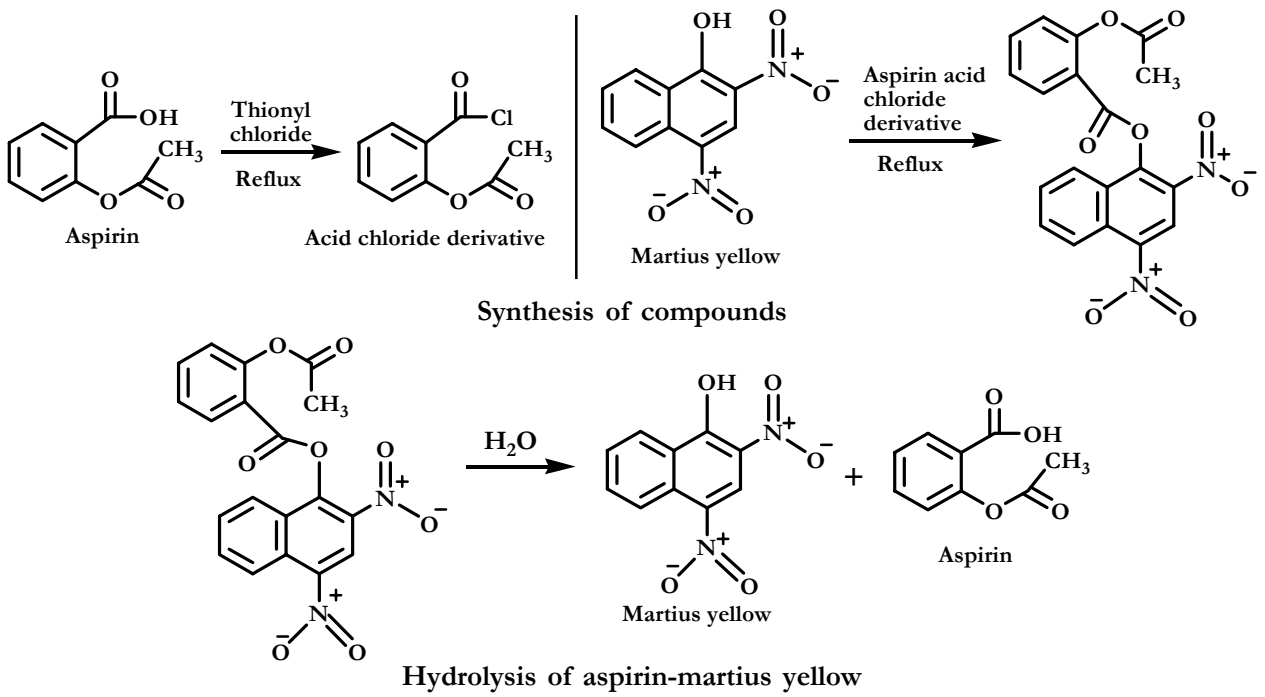

Figure 2

Major steps for synthesis of lead drugs are highlighted here where the activation of the carbonyl carbon of aspirin is accomplished with thionyl chloride. The activated aspirin molecule will attack hydroxyl groups (-OH) on either alizarin or martius yellow to form an ester group after mild reflux. The lower half shows the possible hydrolysis of the ester linkage by an esterase enzyme in the human blood system resulting in the generation of an active aspirin molecule.

for the martius yellow moiety within the aspirin-martius yellow derivative, which is 2.204 angstroms.

To accomplish the synthesis of the two aspirin derivatives the initial reaction is the activation of the aspirin carbonyl carbon by refluxing in organic solvent in the presence of excess thionyl chloride (see Figure 2). Unreacted thionyl chloride must be removed by simple distillation prior to the addition of the fused ring compounds or undesired reactions will occur. Then a final reflux step carried out in suitable organic solvent will produce the derivatives.

The two aspirin derivatives studied here have the hydrophobic fused ring system but also the aspirin substituent which has considerable water solubility (for aspirin at Log P=1.644, the solubility in water at 25° is 5.3 g/L). The hydrolysis of the ester groups may occur *in vivo* by esterase enzymes which are ubiquitous in the human blood system. This action would then release the parent structure of aspirin and restore the carboxylic acid group (see Figure2). Presumably the medicinal activity of aspirin is then restored and it functions as a nonsteroidal anti-inflammatory drug (NSAID) that irreversibly acetylates COX-1 and COX-2. COX-1 and COX-2 activities produce different types of prostaglan-dins. The inhibition of cyclooxygenase will inhibit the expression of inflammation. Aspirin is about 160x times for specific to COX-1 than for COX-2. The rate of skin penetration (Kp) for aspirin-alizarin is estimated by EPISUITE (see Materials and Methods) to be 0.00412 cm/hour. Rate of skin penetration by aspirin-martius yellow is estimated by EPISUITE to be 0.00206 cm/hour. These values fall among those of other medicinal compounds, for example the Kp values of styramate (muscle relaxant), methylsalicylate (NSAID), and 4-hydroxybenzoic acid (fungicide) are 0.000191 cm/hour, 0.0145 cm/hour, and 0.00362 cm/hour, respectively.

Important physico-chemical parameters of both derivatives are presented in TABLE 1, which includes descriptors of lipophilicity, polarizability (ie. parachor, molar volume, and molar refractivity), polar surface area, number of rotatable bonds, and num-

TABLE 1: Comparison of descriptors for aspirin-alizarin and aspirin-martius yellow agents.

[1]Descriptor	Aspirin-alizarin	Aspirin-martius yellow
miLOG P	6.329	4.602
Number of O and N	10	10
Number of –OH and –NH	0	0
Number of rotatable bonds	10	7
Violations of rule of 5	2	0
Index of refraction	1.640	1.700
Polar surface area	139.36 A^2	144.26 A^2
Molar refractivity	144.6 cm^3	100.17 cm^3
Molar volume	401.1 cm^3	268.2 cm^3
Parachor	1117.8 cm^3	760.9 cm^3

[1]Values for miLog P, Polar surface area1, number of oxygen and nitrogen, number of hydrogen donors, violations of rule of 5, and number of rotatable bonds calculated by methods of Molinspiration. Values of MR, MV, Parachor, and Index of Refraction calculated by method of ChemSketch.

ber of violations of Rule of 5[11]. The Rule of 5 describes a criteria for estimating effectiveness of bioavailability (the extent to which a drug is absorbed into the bloodstream) and has the following guidelines: 1) Molecular weight < 500; 2) Clog P< 5; 3) Fewer than 5 hydrogen bond donors; 4) Fewer than 10 hydrogen bond acceptors. Violation of two or more of the Rule of 5 guidelines suggest significant problems in bioavailability. The aspirin-alizarin derivative shows 2 violations and the aspirin-martius yellow has zero violations. Presented in TABLE 1 are values of partition coefficient, hydrogen bond donors, violations of Rule of 5, and polar surface area for aspirin-alizarin to be 6.329, 0, 2, 139.36 A^2, and 4.602, 0, 0, 144.26 A^2, respectively. High positive values for miLog P indicate a greater lipophilic nature (soluble in lipid by-layers) for the aspirin derivatives relative to aspirin alone (miLog P = 1.644) and consequently lower water solubility. Lower water solubility is also indicated when there are zero number of hydrogen bond donors. Polar surface area as a descriptor totals the surface area belonging to polar atoms and correlates well with predictions of transport efficiencies and properties of drugs[12-15]. The determined value of PSA for aspirin-alizarin (145.68 A^2) suggests absorption in the intestinal tract to be 10% to 15% of the total amount of this drug located in the intestinal tract[12, 13]. SPARTAN modeling software calculates the total surface area of aspirin-alizarin to be 571.03 A^2, indicating that 25.5% of the total area is polar and 74.5% is nonpolar. Likewise the PSA value for aspirin-martius yellow (144.26 A^2) indicates that a total of 10% of drug present will be absorbed while in the intestinal tract. Similarly SPARTAN calculates a total area of 404.47 A^2 for the aspirin martius yellow molecule, indicat-

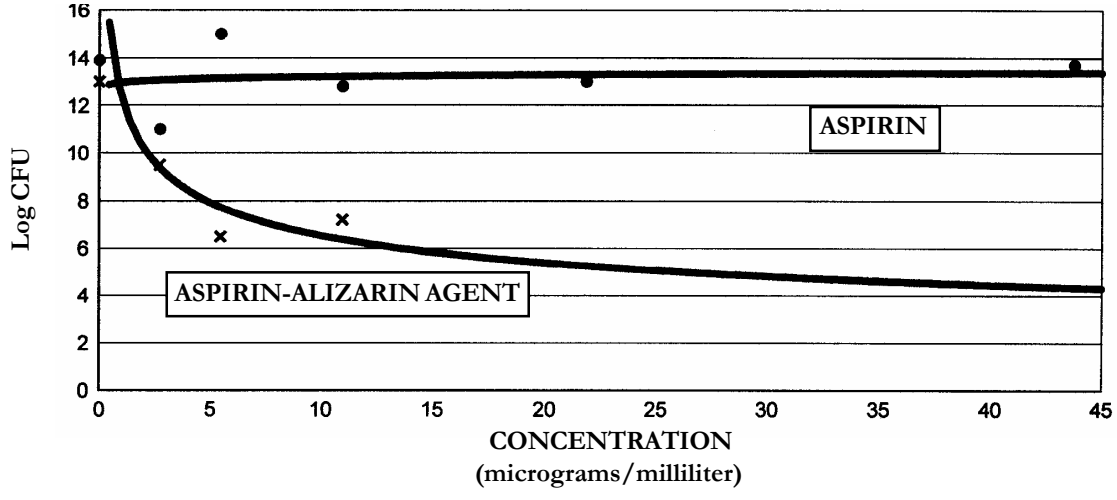

Figure 3: Inhibition of colony forming units (CFU) by aspirin and aspirin-alizarin agent

Shown as a plot of Log CFU versus concentration of aspirin-alizarin agent, a significant reduction of colony forming units is induced at levels of concentration within the solubility range of the agent (solublity 11.88 μg/mL). The CFU levels of the control aspirin remain relatively constant to more than threetimes the effective concentration of aspirin-alizarin.

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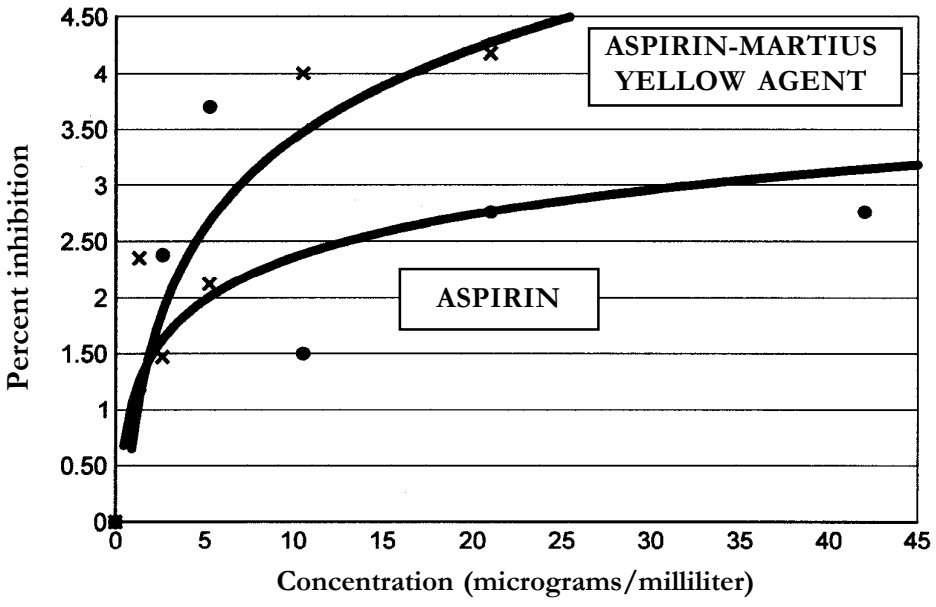
Comparison of growth inhibition of *escherichia coli* by aspirin and aspirin-martius yellow agent



Comparison of inhibition of colony forming units (CFU) Induced by aspirin and aspirin-martius yellow agent

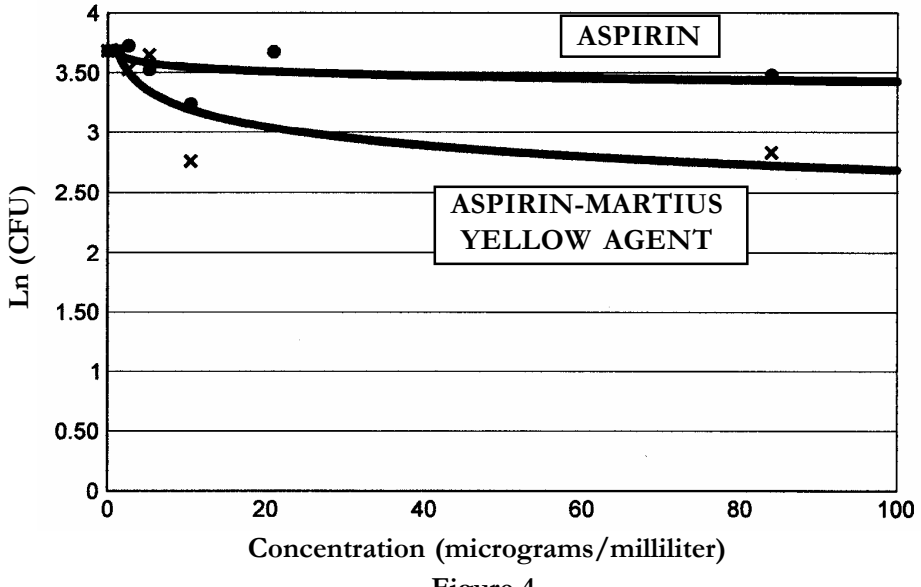


Figure 4

Plots of percent inhibition and Ln CFU show clearly the inhibition of *E. coli* induced by the aspirin-martius yellow agent.
Top plot: Percent inhibition of bacteria out paces the effects of aspirin alone.
Bottom plot: The reduction of colony forming units induced by aspirin-martius yellow is significant while the effects of aspirin alone are relative constant. The bacterial inhibition occurs within the solubility range of aspirin-martius yellow (solubility 31.3 μg/mL).

ing 35.7% of the surface area is polar and 64.3% is nonpolar. The higher PSA value for aspirin-martius yellow accompanies the result of zero violations of Rule of 5, indicating good bioavailability and good bioactivity. The agent aspirin-alizarin showed 2 violations of the Rule of 5, which when coupled with a high miLog P of 6.329 suggest this compound may have problems in bioavailability (ie. Poor water solubility and excessive lipid solubility).

Partition coefficients are important pharmacological descriptors and provide estimates of drug lipid solubility and water solubility. Drugs must utilize a

TABLE 2: Partition coefficients of aspirin agents.

Partition coefficient	Aspirin-alizarin	Aspirin-martius yellow
[1]miLog P	6.329	4.602
[2]iaLog P	4.32	1.24
[3]actCLog P	6.19	4.24
[4]epiLog P	5.32	3.45
[5]LogKowLog P	5.32	3.45

[1]By method of Molinspiraiton. [2]By method of Interactive. [3]By method of Actelion. [4]By method of EPISUITE. [5]By method of Syracuse Research Corporation.

water based blood circulation system to arrive at their site of medicinal activity and cross cell membranes. TABLE 2 presents partition coefficients which are calculated by various algorithms for comparison to others (see Materials and Methods). General trend is for higher positive values of calculated Log P (including Clog P), which for aspirin-alizarin has a mean of 5.48, median of 5.32, standard deviation of 0.8424, and with 90% confidence interval of 4.67 to 6.28. For aspirin-martius yellow the mean is 3.40, median of 3.45, standard deviation of 1.306, and 90% confidence interval of 2.15 to 4.64. The values of Log P can be utilized with the melting points of these derivatives to determine aqueous solubility from the equation:

$$\text{Log P} = 6.5 - 0.89\text{Log(S)} - 0.015\text{mpt}$$

Where Log(S) is solubility in micromoles/Liter and mpt is defined as the melting point. For aspirin-martius yellow a milog P of 4.602 coupled with a melting point of 191.89°C produces a solubility of 31.3 micrograms/milliliter. Likewise the solubility of asirin-alizarin is 11.9 micrograms/milliliter, utilizing miLog P of 6.329 and melting point of 288.9°C[16,17].

The agent aspirin-alizarin was placed into tissue culture and evaluated for its affect on gram negative *Escherichia coli*. This strain was penicillin susceptible and has DNA not encapsulated in a nuclear membrane. Although aspirin-alizarin has low solubility in aqueous solution (11.88 μg/mL) it showed significant antibacterial action below and at its maximum solubility, presented in figure 3. This antibacterial action is compared to that of aspirin alone which remains lower to over three times the effective concentration of aspirin-alizarin. Reduced colony forming units are effective in demonstrating growth inhibition of bacteria if the agent induces bacterial aggregation (see Figure 3). Hydrolysis of the ester groups of the aspirin-alizarin structure (linking aspirin to alizarin) is determined to be approximately 1.086 L/mole·sec (for aspirin molecule closest to the cyclic dione group) and 1.53 L/mole·sec (for aspirin furthest from the cyclic dione group).

A similar affect on *Escherichia coli* was observed with the aspirin martius yellow agent (Figure 4) when compared to the action of aspirin alone. Again significant antibacterial action was observed at the maximum solubility of aspirin-martius yellow (31.3 μg/mL) and below this value. A concentration of ~80 g/mL was attempted and showed similar bacterial inhibition as that found at ~20 μg/mL for CFU analysis. Significant bacterial aggregation measured as Colony forming units occurred with aspirin-martius yellow. Aspirin-martius yellow significantly inhibited *Escherichia coli* at solubilizing concentrations when measured in terms of percent inhibition and reduction of Colony forming units. The rate constant for hydrolysis at the linking ester group is 0.408 L/mole·sec. EPISUITE calculates the rate of skin permeation for aspirin-martius yellow to be 2.06E-03 cm/hour.

CONCLUSION

The two aspirin derivatives were determined to have several favorable pharmacological properties in addition to antibacterial activity at their aqueous solubilizing concentrations. The two derivatives were formed by utilizing an ester group as a linker to a single alizarin (a quinone) or martius yellow (aromatic) molecule. These two agents were analyzed and found to be highly lipophilic which indicates increased solubility in lipid by-layers. Aspirin-alizarin showed maximum aqueous solubility at 11.88 μg/mL and significant inhibition of gram negative penicillin susceptible *Escherichia coli* at that concentration and at less. Aspirin-martius yellow showed maximum aqueous solubility at 31.3 μg/mL with significant inhibition of *Escherichia coli* at that concentration and less. The Aspirin-martius yellow agent showed zero violations of the Rule of 5 which indicates effective bioavailability. Partition coefficient Log P was de-

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termined by several methods giving average values for aspirin-alizarin and aspirin-martius yellow to be 5.48 and 3.40, respectively.

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