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Synthesis and structure–activity relationships of novel cationic lipids with anti-inflammatory and antimicrobial activities

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ABSTRACT

Certain membrane-active cationic steroids are known to also possess both anti-inflammatory and antimicrobial properties. This combined functionality is particularly relevant for potential therapies of infections associated with elevated tissue damage, for example, cystic fibrosis airway disease, a condition characterized by chronic bacterial infections and ongoing inflammation. In this study, six novel cationic glucocorticoids were synthesized using beclomethasone, budesonide, and flumethasone. Products were either monosubstituted or disubstituted, containing one or two steroidal groups, respectively. In vitro evaluation of biological activities demonstrated dual anti-inflammatory and antimicrobial properties with limited cytotoxicity for all synthesized compounds. Budesonide-derived compounds showed the highest degree of both glucocorticoid and antimicrobial properties within their respective mono- and disubstituted categories. Structure–activity analyses revealed that activity was generally related to the potency of the parent glucocorticoid. Taken together, these data indicate that these types of dual acting cationic lipids can be synthesized with the appropriate starting steroid to tailor activities as desired.

Cystic fibrosis (CF) is a monogenic disorder characterized by persistent bacterial infections of the airway. Although gene therapy could be an ideal treatment for this single-gene disease, development of an effective gene delivery system is complicated by the chronically infected and inflamed airway environment typical of many CF patients. Infections inhibit proper gene transfer, regardless of whether they are on-going or recently resolved at the time of gene administration.[1] The mechanism behind this observed effect was linked to the immune response occurring at the time of gene delivery. Compounds with dual antimicrobial and anti-inflammatory properties may prove to be useful in preventing this effect from occurring, particularly if co-administered with the gene carrier during delivery. Additionally, such compounds may also be useful as locally applied antibiotics for prevention and symptomatic treatment of chronic bacterial lung infection, a dominant cause of death in CF subjects.

By the age of 17 years, nearly 70% of CF patients are infected with an opportunistic pathogen known as *Pseudomonas aeruginosa*. Once acquired, the organism is difficult to completely eradicate from the CF airway, leading to persisting infection and inflammation that eventually causes permanent pulmonary damage.[2] Pathophysiological symptoms of this condition are cyclic in intensity, with the more intense periods termed 'exacerbations'. Such exacerbations are associated with high bacterial (usually, *P. aeruginosa*) concentrations in the airway sputum, and aggressive antibiotic treatment is typically required to improve lung function.[3,4] However, while early *P. aeruginosa* infections are susceptible to common anti-pseudomonal antibiotics (e.g., β-lactam antibiotics, aminoglycosides, and fluoroquinolones), later infections are more difficult to treat as antibiotic resistance emerges with the patient's age.[2] Resistance develops under the pressure of continued heavy use of antibiotics, which facilitates the selection of resistant strains, and is additionally linked to increased occurrence of hypermutable *P. aeruginosa* isolates.[5] The emergence of these isolates is associated with the ongoing oxidative stress caused by the chronic polymorphonuclear leukocyte inflammation seen in many CF patients.[6] Thus, a novel antibiotic with a dual anti-inflammatory function may also prove useful for treating and preventing additional exacerbations of CF airway disease.

Previous cationic sterol-based cationic lipids synthesized by our laboratory, dexamethasone spermine (DS)[7] and disubstituted

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dexamethasone spermine (D_2S)[8], exhibited dual antimicrobial and anti-inflammatory functions. This study presents the synthesis and characterization of six new cationic steroids of similar structure to further understanding of this family of compounds. Instead of dexamethasone (dex), the six novel lipids presented (Fig. 1) here contain other glucocorticoids (GCs) as the steroidal side groups: flumethasone in compounds **1** and **2** (FS and F_2S), budesonide in compounds **3** and **4** (BuS and Bu_2S), and beclomethasone in compounds **5** and **6** (BeS and Be_2S). These cationic lipids were the result of linking a polyamine known as spermine to the 21-OH position of each steroid. Products from this linkage reaction were either monosubstituted or disubstituted, with linkages occurring at one or both terminal amino groups of spermine, respectively. Final products were evaluated for glucocorticoid, antimicrobial, and DNA lipofection activities, as well as for any potential cytotoxic effects on mammalian cells.

A one-pot reaction with the appropriate GC mesylate, Traut's reagent (TR), and spermine (shown in Fig. 2) yielded the monosubstituted steroid (compounds **1**, **3**, or **5**) as the major product and the disubstituted steroid (compounds **2**, **4**, or **6**) as the minor product. The primary amines on either end of spermine reacted with TR to cause a selective ring-opening, resulting in an exposed sulfhydryl (–SH) end group. This end group then interacted with the α-keto mesylate of the modified GCs to form an α-keto thioether linkage between the steroid and polycation tail (i.e., the spermine-TR conjugate), yielding the monosubstituted cationic steroid. To form the disubstituted product, the primary amine of the monosubstituted lipid reacted with another TR molecule to eventually result in another thioether linkage with a separate GC molecule.

Desired compounds were purified from excess starting reactants and unwanted reaction intermediates with semi-preparative HPLC, and then their molecular weights and chemical structures

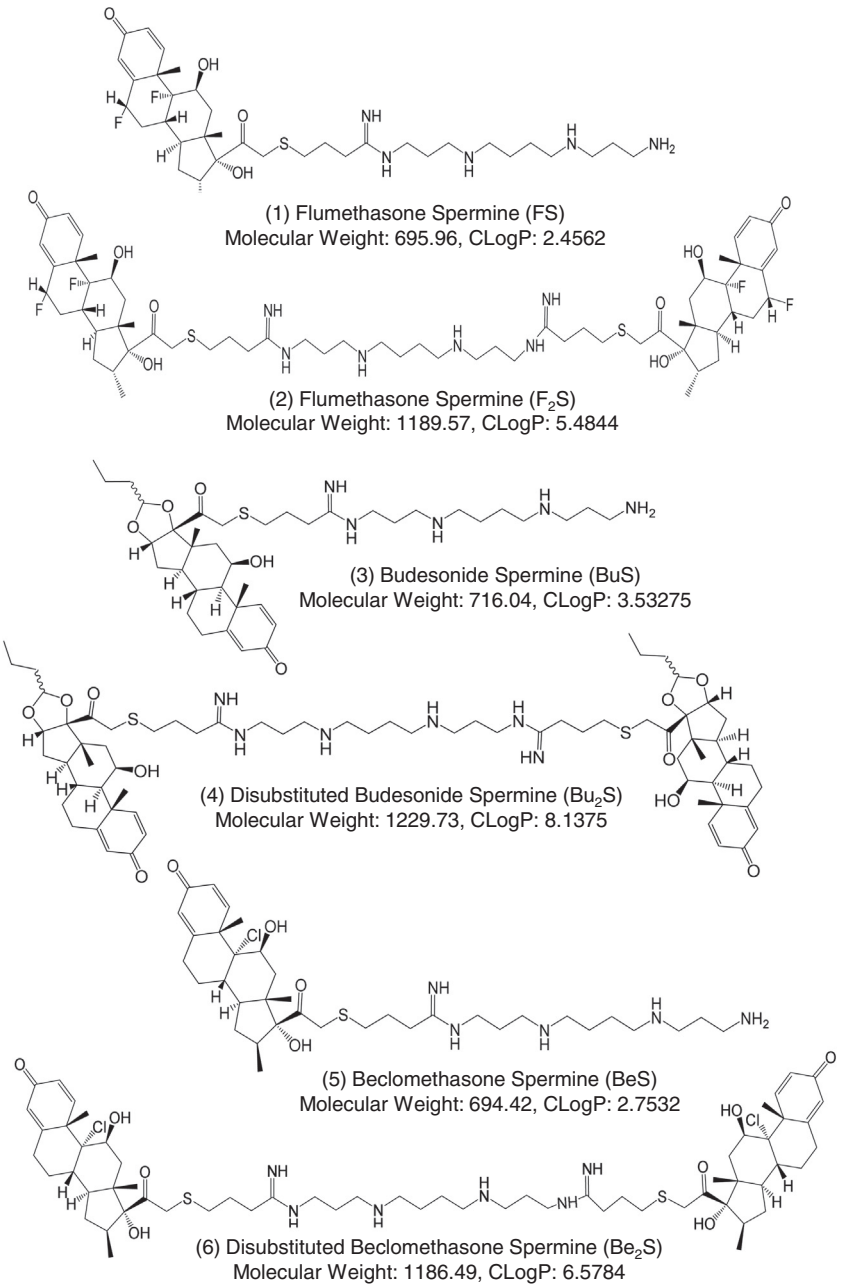

Figure 1. Structures, molecular weights, and *C*Log*P* values of FS (compound **1**), F_2S (compound **2**), BuS (compound **3**), Bu_2S (compound **4**), BeS (compound **5**), and Be_2S (compound **6**).