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Synthesis and characterization of novel fluorescent nitrogen-containing bisphosphonate imaging probes for bone active drugs

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Abstract

Progress in the synthesis of novel fluorescent conjugates of N-heterocyclic bisphosphonate drugs and related analogues, together with some recent applications of these compounds as imaging probes, are briefly discussed.

Keywords

Fluorescent; bisphosphonate; imaging; conjugates

Introduction

Bisphosphonates (BPs) are therapeutic agents for the treatment of bone disorders such as osteoporosis and Paget's disease and also find use in the cancer clinic.1 Several nitrogen-containing bisphosphonates (N-BPs) and phosphonocarboxylate (PC) analogues demonstrate antitumor effects *in vitro* and *in vivo*.1 However, details of the skeletal distribution, cellular uptake and mechanisms of these drugs remain to be elucidated, pointing to the need for fluorescent imaging probes which mimic some or all of their pharmacological properties. Of these, the affinity of the parent drugs for bone mineral is a key component of their mechanism of action, as well as their cellular effects.

Results and Discussion

Recently, we introduced a so-called "magic linker" synthesis of novel fluorescent conjugates formed from the N-BP drug risedronate or related phosphonocarboxyl (PC) analogues with a range of fluorescent dyes,2 including a near infrared (NIR) fluorescing dye, Alexa Fluor 647

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(AF647).3 The new fluorescent probes generally retain substantial affinity for bone mineral, reflecting the varying affinities of their parent drug components. Moreover, some of the conjugates retain the ability to inhibit protein prenylation, both *in vitro* and *in vivo* in osteoclasts, suggesting that they can mimic key pharmacological characteristics of the parent drug *in vivo*.2, 3

We have now extended the scope of this approach to seven new heterocyclic N-BP drug-dye combinations. In particular, with appropriate modifications, the "magic linker" synthesis provides a general methodology to label other known heterocyclic N-BP drugs in addition to risedronate. The conjugates can be prepared in good yield (50%–77% of isolated product) and high purity (typically > 98% by HPLC) and are fully characterized by HPLC, UV-VIS and fluorescence emission spectroscopy, ^{1}H and ^{31}P NMR and high-resolution MS.

Differential labeling of an N-BP drug and analogues having different bone affinities using dyes with distinguishable fluorescent emission spectra results in complementary fluorescent probes, allowing simultaneous detection of individual low and high affinity BPs and PCs in bone, bone tissues, and cells. Other new applications of the fluorescent N-BP imaging probes are also under development, e.g. their use to visualize distribution of a labeled N-BP drug in the otic capsule bone.

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