Anthranilic Acid Analogues as Uncommonly Shifted HYdrogen bonded (U SHY) diamagnetic CEST (diaCEST) MRI Contrast Agents based on the N-H exchange

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Target Audience: Investigators and clinicians working in the areas of MR contrast agents, molecular imaging and CEST imaging.

Purpose: As an alternative to traditional MR agents based on water relaxivity, CEST agents have the advantages of 1) selective detection based on the frequencies of their labile protons, 2) amplification of signal from the low-concentration agents to water through multiple chemical-exchange events, and 3) possessing contrast sensitive to environmental parameters such as pH. Most natural diaCEST agents[1], such as glucose, glutamate, L-arginine, and a variety of proteins and peptides have labile protons with a small chemical shift difference from H_2O ($\Delta\omega < 4$ppm), raising several challenges for signal detection and quantification. To address this, recent efforts have been focused on developing agents with labile protons with larger chemical shifts[1], including thymidine analogues (5.5 ppm), iopamidol (4.2 and 5.5 ppm) and the salicylic acid analogues with the C2-OH resonating ~8–10.8 ppm from H_2O, which we have termed Uncommonly Shifted HYdrogen-bonded agents(U SHY).[2] To explore further the capabilities of the benzoic acid core for generating CEST contrast, we describe the anthranilic acid analogues: N-aryl, N-acyl and N-sulfonyl derivatives, as another class of U SHY agents, based on the exchange of N-H protons (**Scheme 1**).

Results and Discussion: In contrast to salicylic acid (**1**), its N-H analogue anthranilic acid (**2**), failed to produce CEST contrast (**Fig.1, Scheme 1**). Interestingly, significant contrast at 4.8 ppm was observed in N-phenylanthranilic acid (**4**), with the exchange rate k_{sw} = 2.0 kHz measuring using both QUESP and QUEST methods. Comparing **4** and **2**, the loss of CEST signal in **2** indicates that k_{sw} is too high. This is possibly due to the additional non-hydrogen-bonded C2 N-H proton undergoes a fast intramolecular exchange with the hydrogen-bonded proton. As N-phenylanthranilic acid analogues are commonly used as non-steroidal anti-inflammatory drugs (NSAIDs), we found two out of five NSAID drugs, flufenamic acid (**5**) and meclofenamic acid (**6**), also show CEST contrast at 4.8ppm , unfortunately both have low water solubility (10 mM or lower).

To further increase the chemical shift to fit the slow to intermediate detection window of CEST ($k_{sw} < \Delta\omega$) while still keeping k_{sw} slow enough for achieving efficient saturation using a B1 suitable for our 3T MR hardware[1], we investigated the C2 amide analogues of anthranilic acid. Amide N-H protons tend to be shifted further than amine protons, although they also tend to exchange with water slower. As expected, **10** did not show any contrast presumably because k_{sw} is too slow. However, after modification of the structure to **11**, an example of a more acidic N-H proton, we observed CEST contrast with the labile proton resonating at 9.3 ppm indicating a strong hydrogen bond interaction in water. The contrast produced by **11** is relatively low (6 % at 25 mM, B_1 = 3.6 μT), because k_{sw} is relatively slow (0.3 kHz). Further increasing the acidity through 2-(methyl-sulfonamido) benzoic acid (**12**) results in more substantial contrast at 7.3 ppm (~15 % at 25 mM, B_1 = 3.6 μT).with a k_{sw} = 0.6 kHz at pH = 7.1. Maximum contrast is achieved using B_1 = 6 μT or higher with ~90% of this contrast available at B_1 = 3.6 μT (Figure 3b), which is near the maximum power we can apply on our clinical scanners. More interestingly, the contrast and k_{sw} of **11** and **12** remained almost constant between the pH's 6 – 8 (**Fig.2**). Slightly modifying the structure of **12**, as in **13-16**, also shows similar contrast from 6.3 to 7.8ppm.

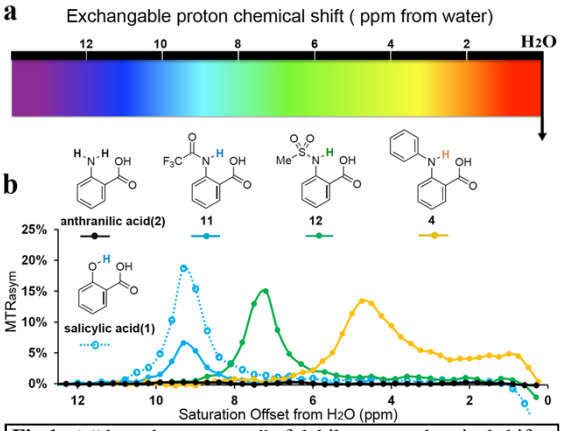
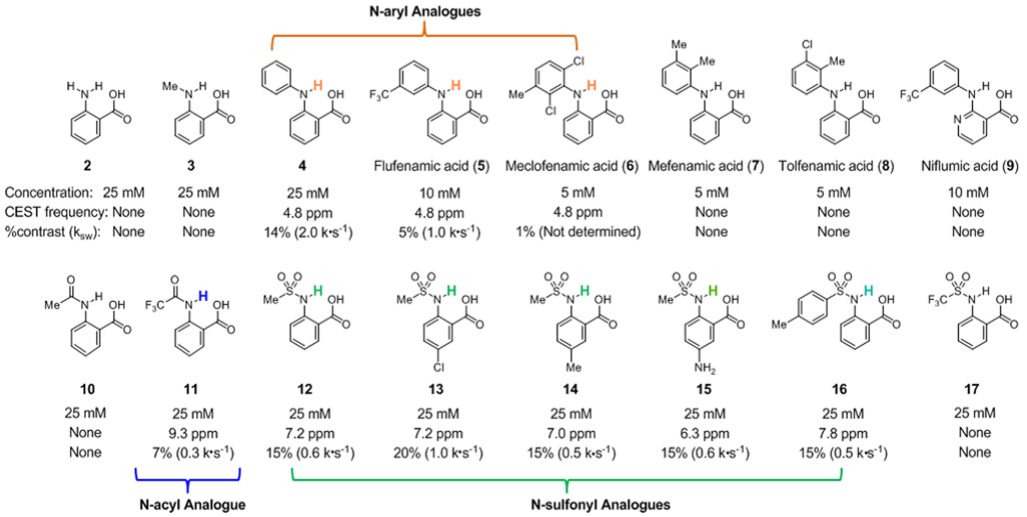
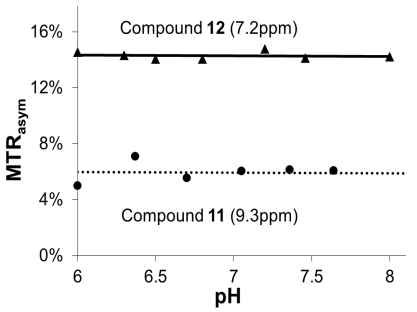


Fig.1. a) "the color spectrum" of labile proton chemical shifts for diaCEST agents; b) CEST contrast curves for representative salicylic acid (**1**) and anthranilic acid derivatives (**2, 4, 11** and **12**) using B_1 = 3.6 μT, t_{sat} = 3 s (25 mM , pH 7.1-7.4).

Conclusion: We have demonstrated that anthranilic acid provides a suitable scaffold for diaCEST agents. The N-H labile protons in N-aryl anthranilic acids (**4-6**) resonate at 4.8 ppm while for N-sulfonyl anthranilic acids (**12-16**) these resonate between 6 – 8 ppm and for **11** labile protons resonate at 9.3 ppm, which could be used for multi-color MR imaging and complementary to other existing diaCEST probes, with one NSAID, **5**, already administered to patients, having been identified among these analogues. The pH independence makes **11** and **12-16** ideal U SHY probes for *in vivo* quantification.

Reference [1]Van Zijl, P. et al,MRM.2011,65:927 [2]Yang, X., Song, X. et al, Angew. Chem. Int. Ed.,2013, 52: 8116. Supported by NIH grants R01EB015031 and R01EB015032.





Scheme1(Left): CEST frequency [ppm] and contrast [%] (k_{sw}, [k•s^{-1}]) of anthranilic acid and its analogues. (Conditions: pH 7.1 –7.5, using t_{sat} = 3 s, B_1 = 3.6 μT)

Fig.2(Above): CEST contrast changes as a function of pH value for compound **11** and **12.**