Data S1

Supplementary material is provided below on preliminary experiments concerning the effect of NSAIDs and prednisolone on the expression of *Runx2* and *Bsp* genes in MC3T3-E1 cells.

RNA extraction and reverse-transcription quantitative PCR (RT-q PCR). MC3T3-E1 cells were seeded at $5x10^5$ cells per well (6-well plate) in culture medium. On day 1, culture medium was changed into osteogenic medium (as described in the Materials and methods section) and cells were treated with drugs (10^{-6} M) for 1 or 7 days. Then, the cells were directly lysed by the addition of 1 mL Trizol (Invitrogen) and total RNA was extracted with the Miniprep RNA isolation kit (Macherey-Nagel EURL, France), followed by DNase treatment (Invitrogen). One μg RNA was subjected to Thermoscript RT-PCR (Invitrogen) using poly(dT) as primers. Real-time PCR was performed with KAPA SYBR Green Fast Universal qPCR Kit (KAPA Biosystems) in a Mx3000P apparatus (Stratagene) under the following conditions: 40 cycles of 95°C for 30 sec. Triplicate RNA samples of two different experiments were analysed with each set of primers for *Bsp* (F 5'-TCCATCGAAGAATCAAAG CA-3' and R 5'-ATGAGCGTGGCCGGTACTTA-3') and *Runx2* (F 5'-GACGTGCCCAGGCGTATTTC-3' and R 5'-AAGTCT GGGGTCCGTCAAGG-3') genes. The relative mRNA expression levels were calculated from the threshold cycle (Ct) value of each PCR product and normalized with that of the 18S gene (F 5'-TTGAACCCCATTCCATCCA-3' and R 5'-CCATCC AATCGGTAGTAGCG-3') using the comparative Ct method (51). The relative quantity of the expression of each gene from the control (untreated) cells was set to 1, and values from the drug-treated cells were expressed as a relative fold. Statistical analysis for the qPCR experiments was performed using t-test and comparison of nonparametric results between groups was performed with the Mann-Whitney U test (SPSS Software). A p value <0.05 was considered significant. Results are expressed as mean values ± standard error to the mean (SEM).

Gene expression of Runx2 and Bsp. We present preliminary results on the impact of the NSAID drugs and prednisolone treatment on the gene expression of differentiating MC3T3-E1 cells. The mRNA expressions of *Runx2* and *Bsp* bone related genes, were measured after treatment of cells with NSAIDs or prednisolone for 1 or 7 days, respectively. Data from quantitative real time PCR (Fig. S1) show that none of the tested drugs decreased the expression of either *Runx2* or *Bsp1*. On the contrary, gene expression of the early osteogenic transcription factor *Runx2* was induced by lornoxicam and parecoxib treatment. *Runx2* expression was even more significantly increased by paracetamol. The expression of bone sialoprotein (*Bsp1*) was increased only by the non-selective drugs diclofenac and lornoxicam among the NSAIDs investigated. Interestingly, prednisolone exerted the strongest effect, increasing *Bsp* mRNA levels by approximately 40-fold over the control.

Figure S1. mRNA expression levels of (A) *Runx2* and (B) *Bsp* in cells treated with NSAIDs or prednisolone for 1 or 7 days in osteogenic medium, respectively. Expression levels were normalized to the *18S* housekeeping gene. Cells in non-osteogenic medium (control) and cells in osteogenic medium (osteogen. M) are shown for comparison. Each bar represents the mean ± SE, of triplicate technical and duplicate biological samples in two independent experiments (n=12); statistical analysis was performed using a t-test. *P<0.05 vs. untreated control. NSAIDs, Nonsteroidal anti-inflammatory drugs.