INTRODUCTION:
Osteoarthritis (OA) is the most common joint disorder especially in the aging population. The major characteristic of OA is the loss of articular cartilage primarily due to the degradation of aggrecan and collagen, the major extracellular matrix components of cartilage. The catabolic enzymes which are believed to be responsible for this cartilage degradation include aggrecanases and matrix metalloproteinases (MMPs). Therefore, inhibition of these proteolytic enzymes would be important for therapeutic intervention of OA. The purpose of this present study was to analyze cartilage degradation in late stage OA. We analyzed aggrecanase activity as well as characterized the proteoglycan (PG) released by OA cartilage. We also analyzed the effect of a small molecule compound on the inhibition of both PG and collagen loss by OA cartilage.

METHODS:
In vitro inhibitory activity was determined for small molecule compounds against MMP-1, MMP-2, MMP-13, MMP-14 and ADAM-TS4. Human osteoarthritic cartilage from 3 donors was removed from knee joints from patients undergoing total knee-replacement surgery. The cartilage was sliced and cut into appropriate pieces and allowed to equilibrate for 48-72 h in serum-free media. The cartilage pieces were further cultured in the absence and presence of the compound at 0.2, 1.0 and 5.0 µg/ml. Media was collected at days 3 and 6. The cartilage was solubilized by digestion with proteinase K. PG and collagen content of media and cartilage samples were determined by the dimethylmethylene blue (DMMB) assay and hydroxyproline assay respectively. Media was also analyzed by western blots for the presence of aggrecanase-cleaved aggrecan fragments and high molecular weight aggrecan (HMW-aggrecan) molecules by monoclonal antibodies BC3 (kindly provided by Prof. B Caterson and Dr. C Hughes, Cardiff University, UK) and 3B3 respectively.

ESSENTIAL RESULTS:
Compound BB-94 was selected as a representative of the compounds with IC_{50} in the nanomolar range for all enzymes tested and analyzed for its inhibitory activity in human cartilage explant cultures. The results show that BB-94 was able to inhibit the loss of both PG and collagen. The inhibition of PG and collagen loss by BB-94 was about 40% and 44% respectively (Figure A and B). By western analysis, we were able to detect both BC3-positive fragments of aggrecan as well as 3B3-positive HMW-aggrecan in the explant culture media.

DISCUSSION: The results show that human articular cartilage explant assay provided an useful method to analyze PG released by late stage OA as well as an useful system to screen for small molecule inhibitors. It had been postulated that PG loss is an early event in the disease (1), but our results show that even late in the disease there is continuous PG loss and accompanying aggrecanase activity. We further confirm that HMW-aggrecan molecules are released by OA cartilage. This may be due to the fact that the continuous degradation of the matrix results in the release of aggrecan and/or that newly synthesized aggrecan is unable to get incorporated into the matrix. BB-94 was able to inhibit about 40% of PG loss. It is possible that BB-94 was unable to completely inhibit aggrecanase activity, or the presence of HMW-aggrecan accounts for a substantial percentage of PG loss, or there is a role of other non-aggrecanases in the release of PG by OA cartilage. Similar to PG inhibition, we observed that BB-94 was able to inhibit collagen loss by 4%. The possibilities include that BB-94 was unable to completely inhibit the enzymes responsible for collagen loss and also the role of non-metalloproteinases. These results add to the contention that further knowledge about the pathophysiology of OA is essential for significant therapeutic intervention of this joint disease.

REFERENCES: