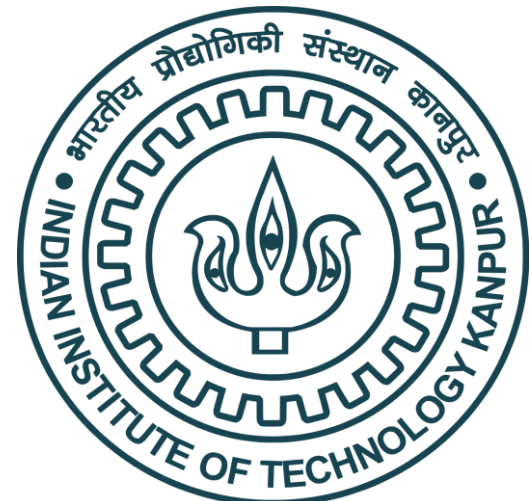




2,6,9-Trisubstituted Purine Derivatives inducing osteogenic potential of C2C12 Cells and MC3T3 cells

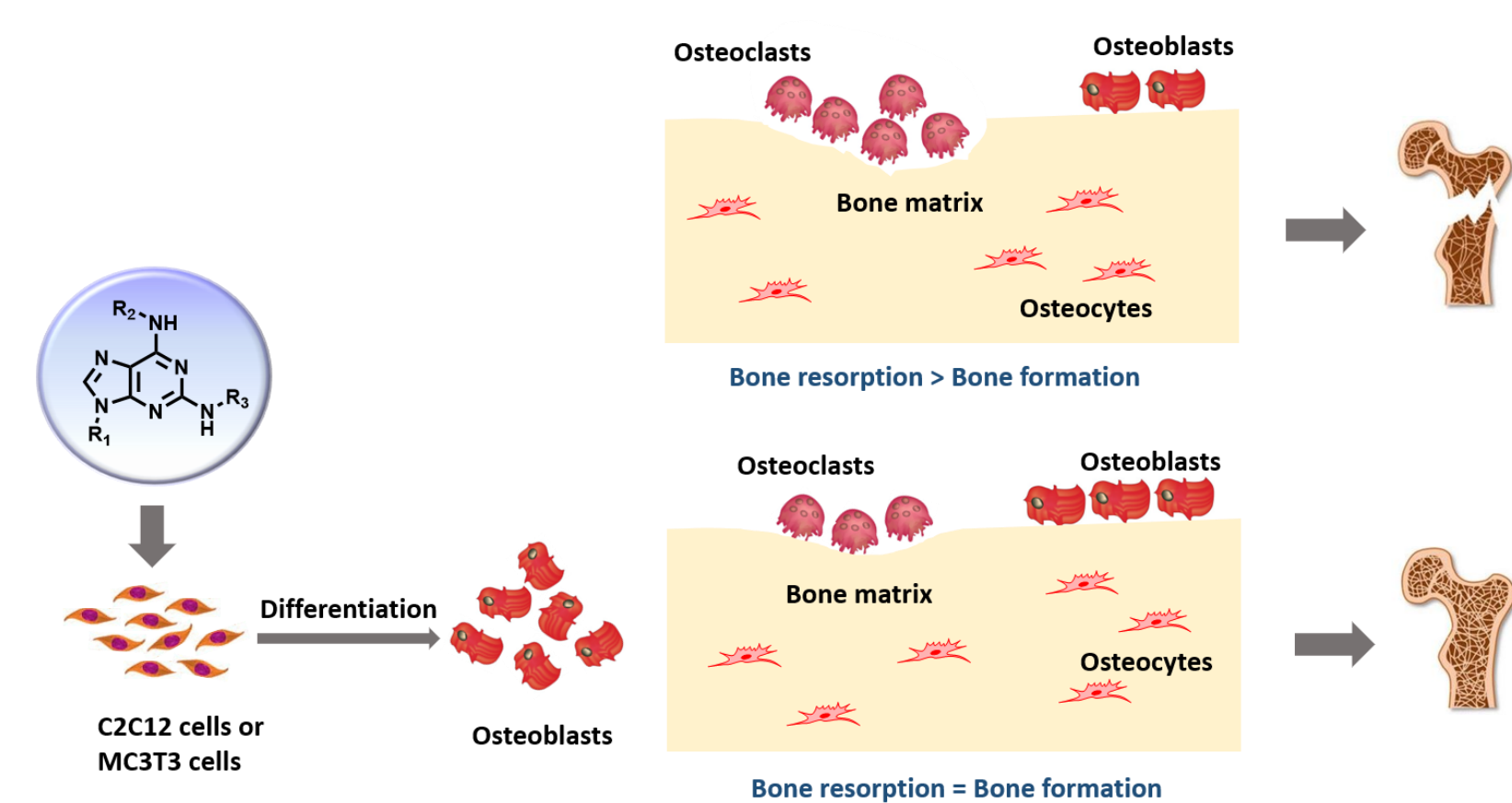
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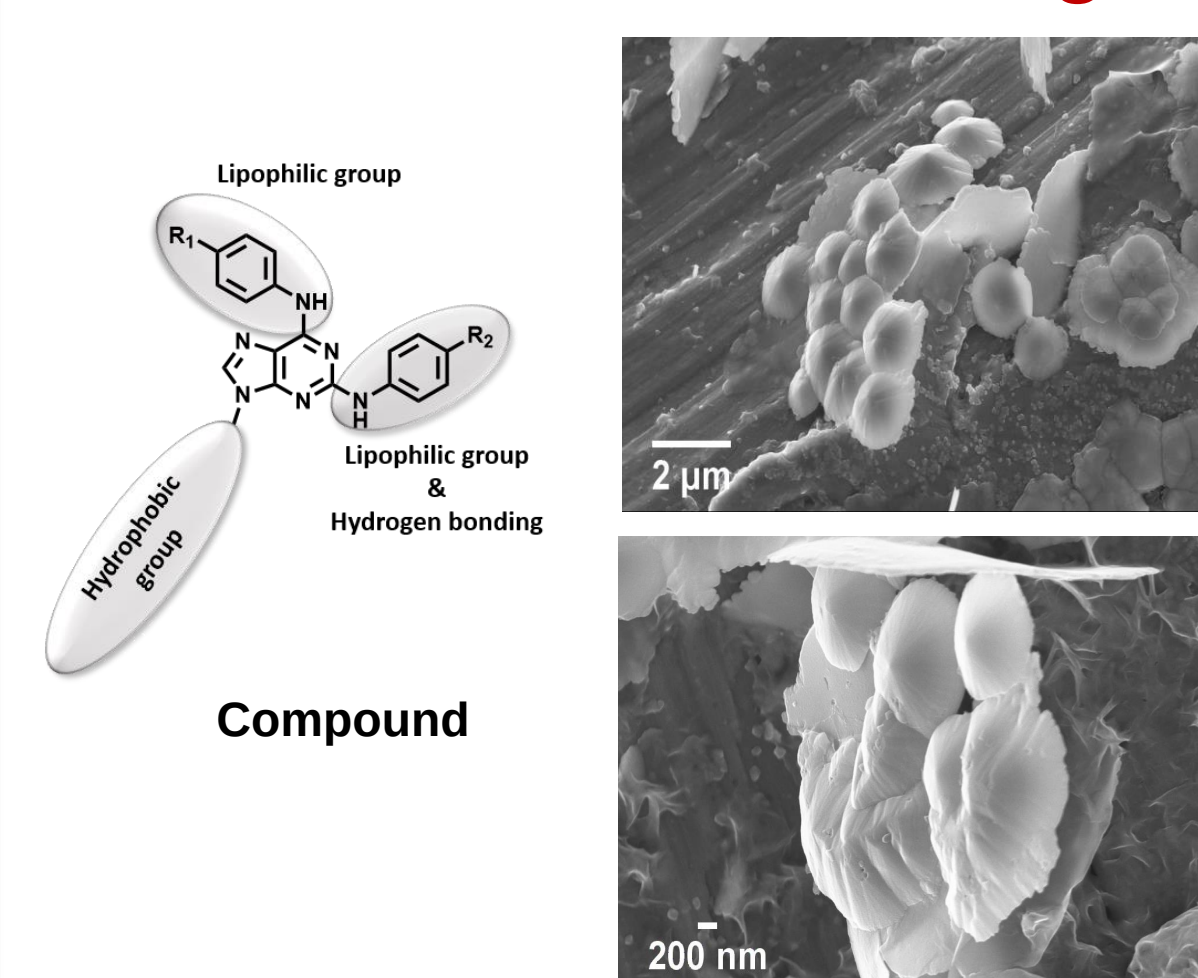
4th Commonwealth
Chemistry poster
4-5 October 2023

Introduction

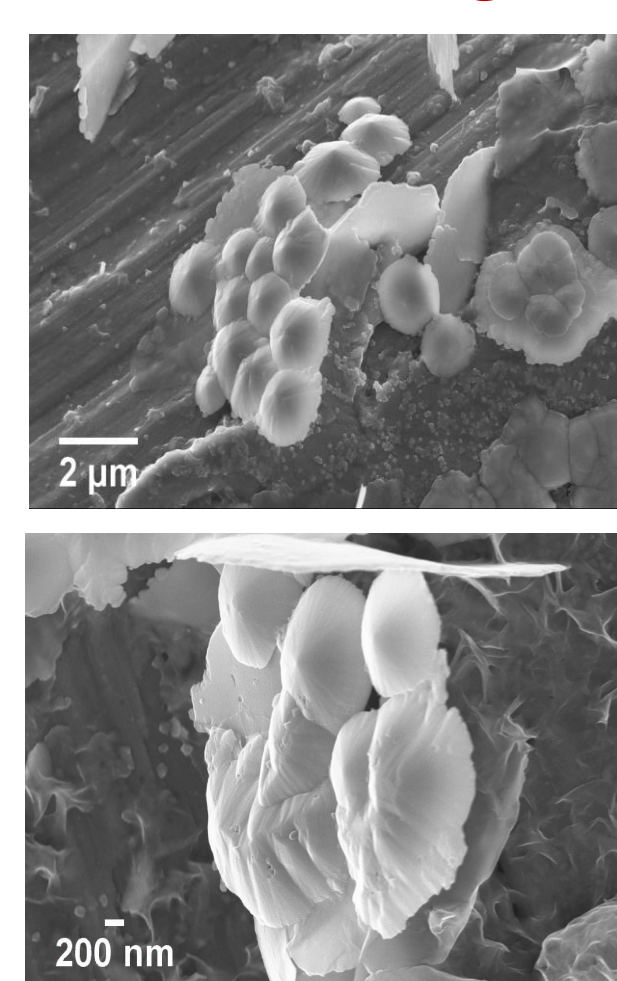
- Imbalance of bone formation and bone resorption causes bone-related disorders.
- Most of the therapies use antiresorptive agents such as bisphosphonate drugs (alendronate, zoledronic acid, ibandronate, etc).
- Purine-based compounds such as purmorphamine and reversine are reported for the osteogenesis of stem cells.
- We have designed and synthesized two 2,6,9-trisubstituted purine derivatives and checked their osteogenic potential in the C2C12 cell line (myoblast cell line) and MC3T3 cell line (osteoblast precursor cell line).



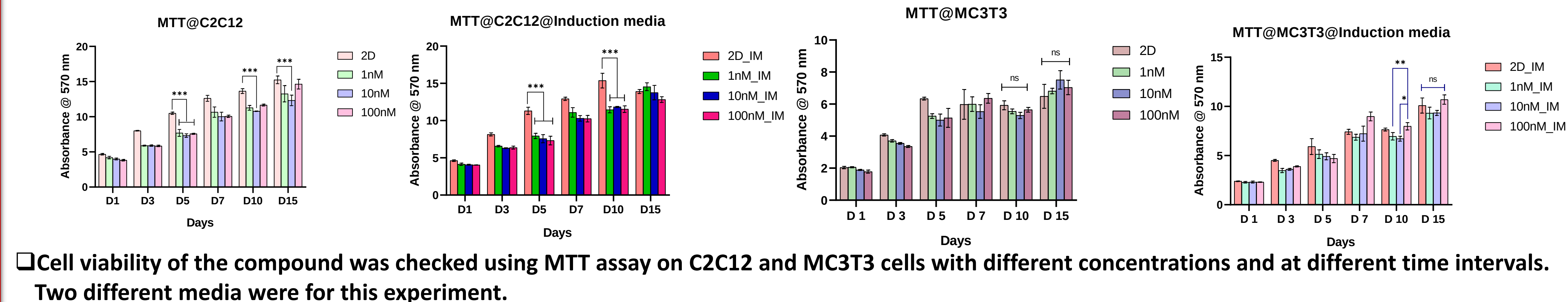
SEM images



- The morphological structures were captured using scanning electron microscopy (SEM) at a concentration of 10 μ M.



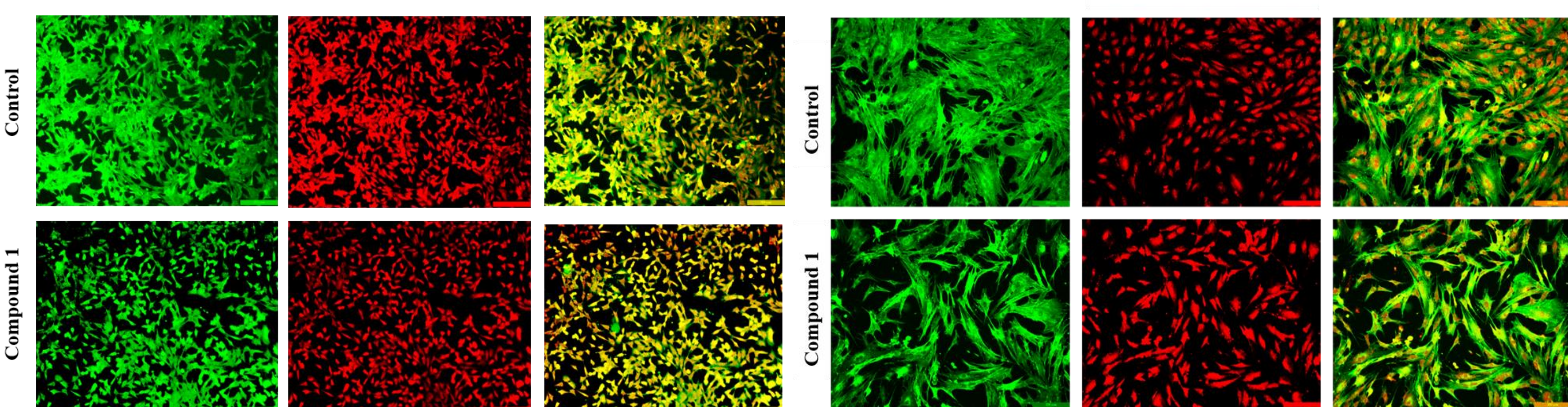
Cell viability



Immunofluorescence Staining

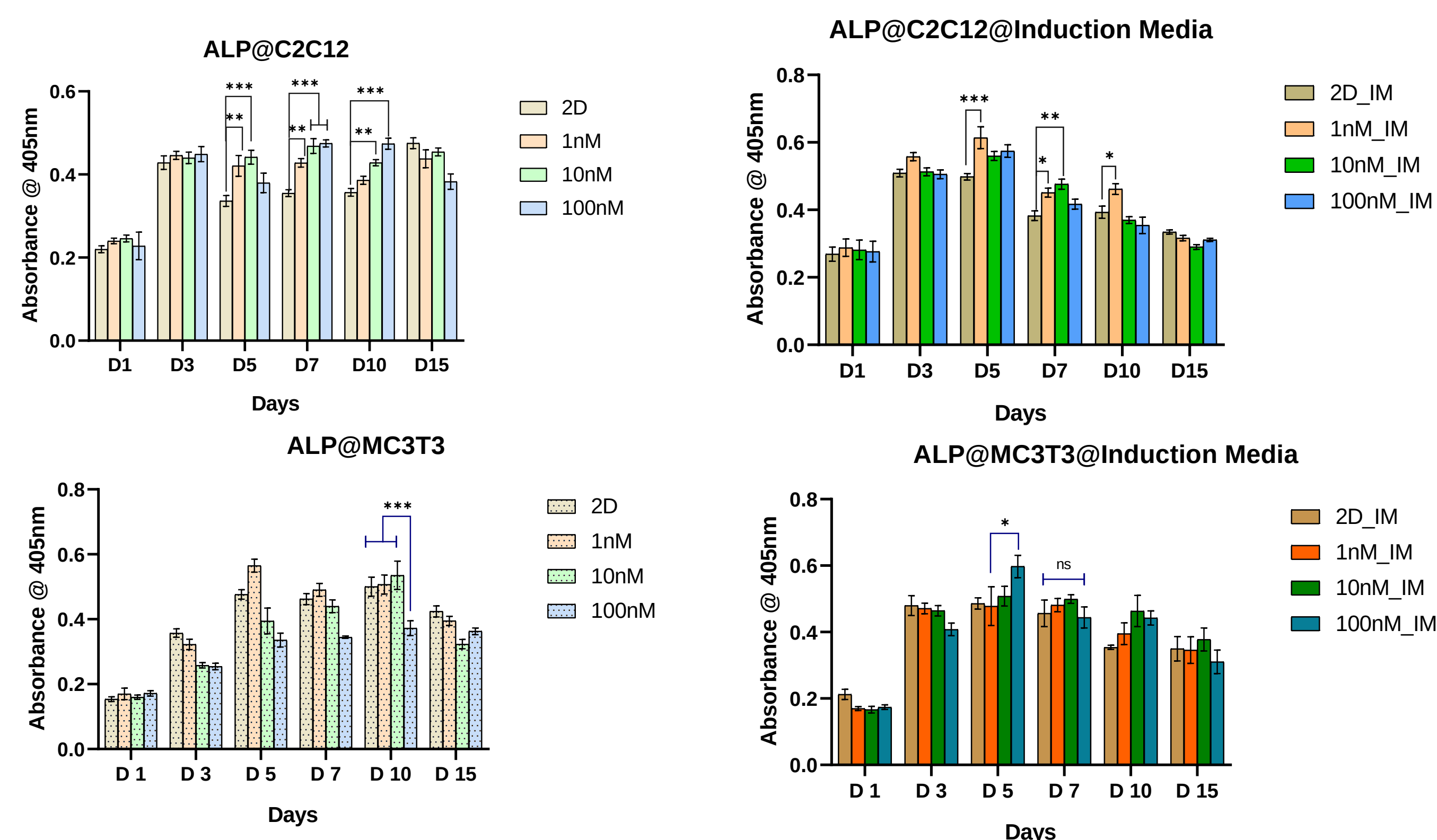
C2C12 cells @day 7

MC3T3 cells @day 7



- Cell morphology was checked using FITC/PI staining for both cells i.e., C2C12 and MC3T3 cells after 7 days of treatment with 10 nM of the compound.
- The images deciphered intact morphologies of healthy cells after osteogenic differentiation.

ALP assay

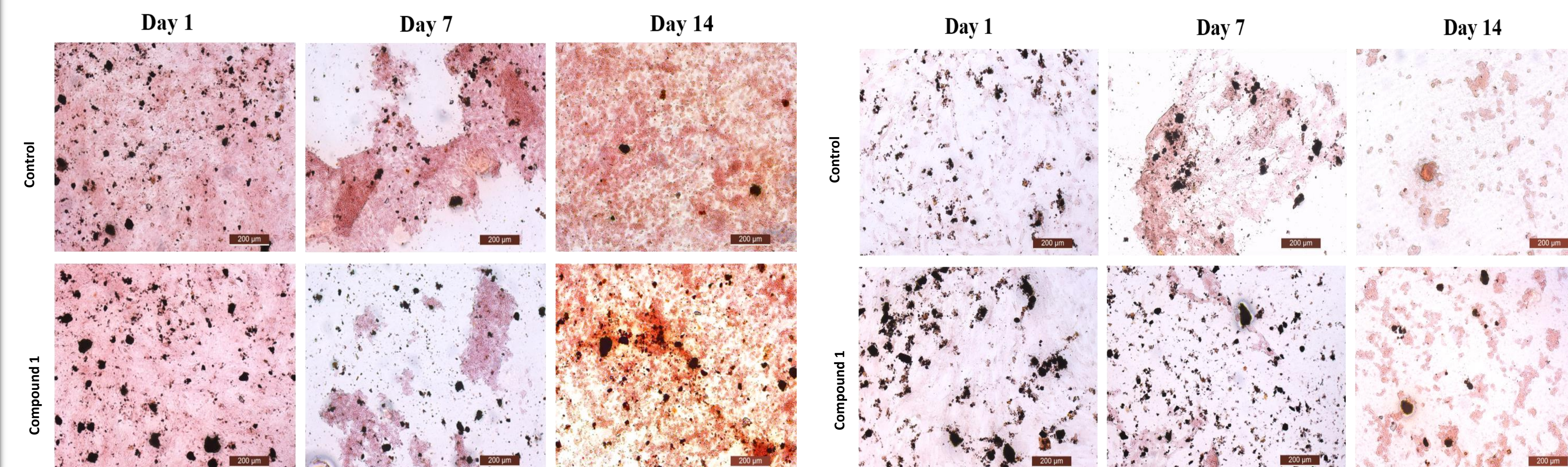


- ALP activity of the compound was checked using C2C12 and MC3T3 cells with different concentrations and at different time intervals.

Alizarin Red S Staining

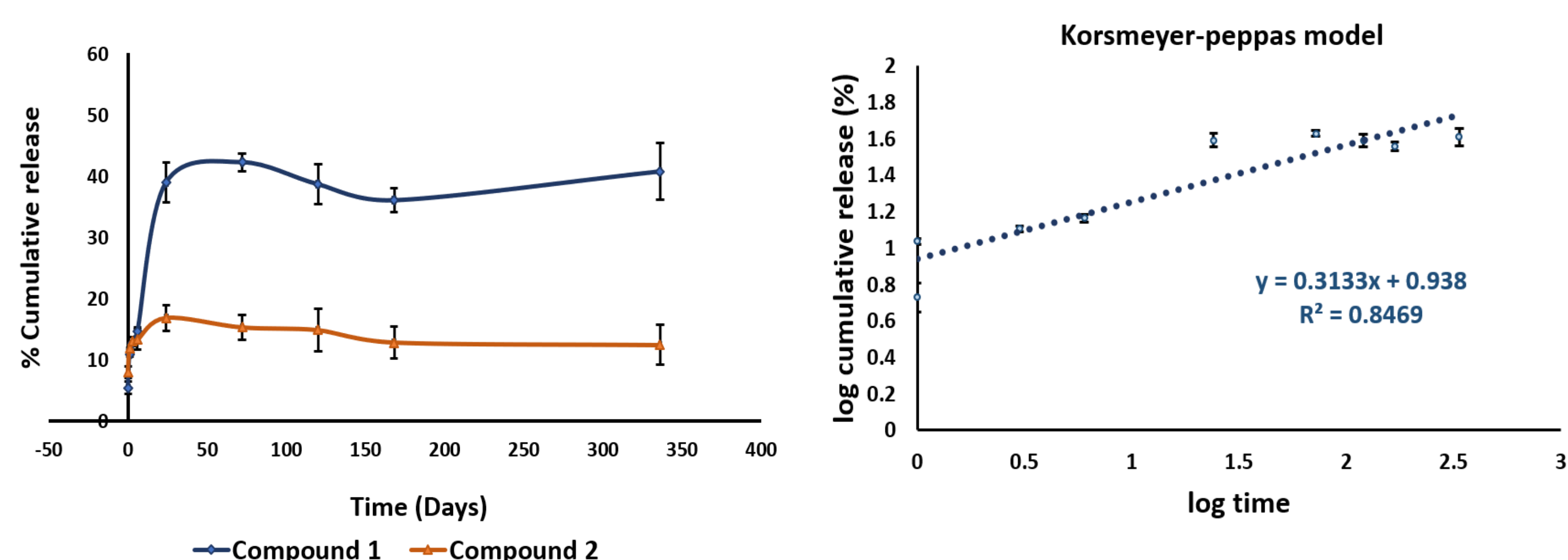
C2C12 cells

MC3T3 cells



- Calcium deposition was checked with 10 nM of the compound in both C2C12 and MC3T3 cells using alizarin red S staining.
- Deposition of calcium nodules indicates that the cells have achieved the potential to enhance bone regeneration.

In vitro drug release experiment



- Drug release experiment of compounds 1 & 2 from Cryogel scaffold using UV-Vis spectroscopy
- Compound 1 shows absorbance peak at 290 nm and Compound 2 at 300 nm
- Compound 1 showed sustained release from Cryogel at pH 7.4 at 37°C
- The release kinetics follows Korsmeyer-peppas model with a rate constant, $k=0.144 \text{ min}^{-1}$ and a release exponent, $n=0.3133$
- With $n<0.43$, the release is described as Fickian diffusion.

Conclusion

- The compound showed potent osteogenic induction *in vitro* when tested on C2C12 cells and MC3T3-E1 cells with no cytotoxicity.
- The compound showed increased ALP activity and calcium deposition at 10 nM concentration in both cells.
- A sustained release of compound 1 was observed from Cryogel scaffold describing Fickian diffusion.
- This kind of purine derivative can have a high potential for bone tissue engineering.

References

- Jiang, M.; Liu, R.; Liu, L.; Kot, A.; Liu, X.; Xiao, W.; Jia, J.; Li, Y.; Lam, K. S.; and Yao, W. *Nature Communications* 2020, 11, 4278.
- Arjmand, B.; Sarvari, M.; Alavi-Moghadam, S.; Payab, M.; Goodarzi, P.; Gilany, K.; Mehrdad, N.; and Larijani B. *Frontiers in Endocrinology* 2020, 11, 430.

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