# A Novel Selective $EP_4$ Prostaglandin Receptor Agonist, KMN-159, Induces Osteogenesis in Bone Marrow Stem Cells

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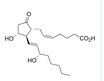
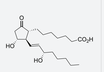
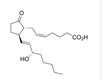
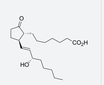
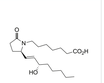
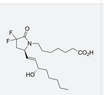
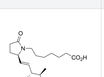
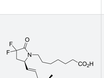
## KEY FINDING

KMN-159 induces osteogenesis through $EP_4$ receptor activation.

## Introduction

Prostaglandin $E_2$ ($PGE_2$) is a crucial regulator of bone remodeling, helping to maintain the balance between bone formation and resorption. $PGE_2$ exerts its biological effects by binding to four GPCRs designated $EP_1$ - $EP_4$, with its bone anabolic effects mediated through $EP_4$. This makes $EP_4$ a potential target for the development of agonists for bone growth and repair. Cayman Chemical's medicinal chemistry program developed a series of over 300 small molecule difluorolactam full agonists of the $EP_4$ receptor and identified KMN-159 as an early lead compound. KMN-159 is very stable and has increased binding selectivity for $EP_4$ over other EP receptors, excellent potency for activation of $EP_4$, and desirable pharmacological properties. KMN-159 also induces the differentiation of human and rat bone marrow stem cells, independent of the age or sex of the donor, as measured by the dose-dependent induction of cellular, biochemical, and molecular markers of osteoblastogenesis.

### Binding, Stimulation of cAMP Production, and Activation of a CRE-SEAP Reporter by Selected Compounds

| Compound | Structure | Rat $EP_4$ receptor binding $K_i$ (nM) | Rat $EP_2$ receptor cAMP formation $EC_{50}$ (nM) | Rat $EP_2$ receptor CRE activation $EC_{50}$ (nM) | Rat $EP_4$ receptor cAMP formation $EC_{50}$ (nM) | Rat $EP_4$ receptor CRE activation $EC_{50}$ (nM) |
|---|---|---|---|---|---|---|
| $PGE_2$ |  | 0.19 | 88.4 ± 4.2 (n = 5) | 2.8 ± 0.7 (n = 9) | 0.48 ± 0.09 (n = 5) | 0.03 ± 0.002 (n = 56) |
| $PGE_1$ |  | 0.20 | | 4.5 ± 0.46 (n = 3) | 0.51 ± 0.07 (n = 9) | 0.02 ± 0.004 (n = 3) |
| 11-deoxy $PGE_2$ |  | 0.42 | | 38.7 ± 4.5 (n = 3) | 1.1 ± 0.16 (n = 3) | 0.02 ± 0.007 (n = 3) |
| 11-deoxy $PGE_1$ |  | 0.21 | | 37.2 ± 6.3 (n = 3) | 1.3 ± 0.24 (n = 3) | 0.06 ± 0.012 (n = 3) |
| 11-deoxy-8-aza $PGE_1$ |  | 1.61** | >10,000 | 2,410 ± 50 (n = 3) | 2.6 ± 0.62 (n = 3) | 0.16 ± 0.049 (n = 3) |
| KMN-165 |  | 0.20** | >10,000 | 851 ± 63 (n = 3) | 0.30 ± 0.025 (n = 3) | 0.03 ± 0.007 (n = 3) |
| KMN-80 |  | 1.11 | >10,000 | >10,000 (n = 3) | 2.6 ± 0.31 (n = 6) | 0.17 ± 0.014 (n = 6) |
| KMN-159 |  | 0.24 | >10,000 (n = 3) | >10,000 (n = 3) | 0.50 ± 0.08 (n = 4) | 0.03 ± 0.003 (n = 7) |

** human $EP_4$ binding

### Acknowledgments

We would like to acknowledge the contributions of the following team members at Cayman Chemical who contributed to this work: Fred Ciske, Joe Colombo, Brad Germain, Melissa Holt, Jeff Johnson, Paul Kennedy, Andrei Kornilov, Jim Kramer, Adam Uzieblo, and Ting Zhao.

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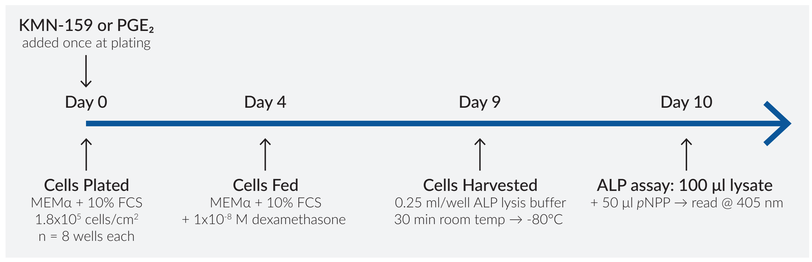
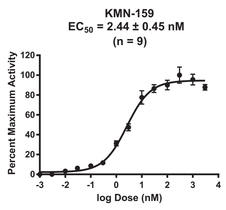
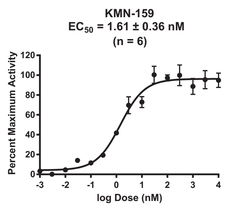
### KMN-159 Stimulates Osteoblastic Differentiation in Young and Old Female and Young Male Rat BMCs

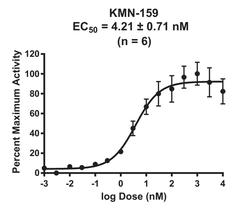



Young Female (<6 months)



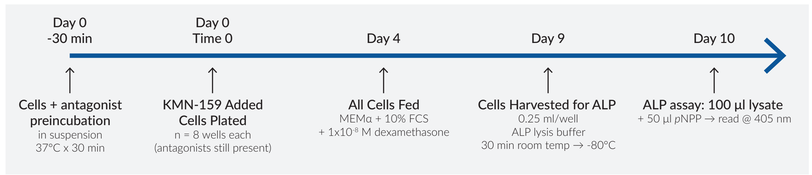

Old Female (>12 months)

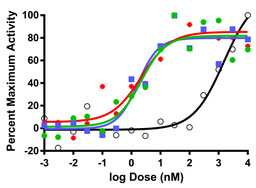



Young Male (<6 months)

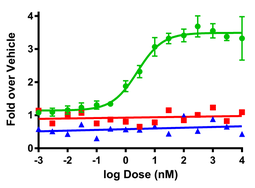
### Stimulation of Osteoblastic Differentiation in Rat Bone Marrow Cells Occurs *via* $EP_4$ Activation

These data demonstrate that KMN-159 stimulates osteogenesis *via* activation of the $EP_4$ receptor; specific agonists for the related $EP_2$ and $EP_3$ receptors do not stimulate osteogenesis and only an $EP_4$-selective antagonist blocks the osteogenesis induced by KMN-159.




Cells were stimulated with the indicated doses of KMN-159 following a 30-minute pretreatment with either vehicle (●); ONO-8711, an $EP_1$ selective antagonist (✱); PF-04418948, an $EP_2$ selective antagonist (■); or ONO-AE3-208, an $EP_4$ selective antagonist (○). Cells were cultured as above and alkaline phosphatase activity was measured on day 9 after plating.

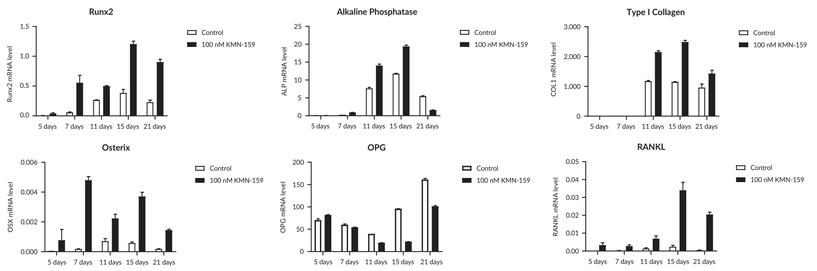



In order to further demonstrate that the induction of osteoblastic differentiation in rat bone marrow cells occurs *via* the $EP_4$ receptor, we first stimulated cells isolated from young female rats with the indicated doses of either Butaprost, an $EP_2$ selective agonist (■); Sulprostone, an $EP_3$ selective agonist (▲); or our $EP_4$ selective agonist, KMN-159 (●).

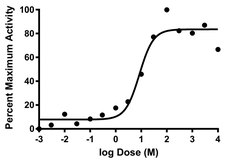
### KMN-159 Upregulates the mRNA Levels of Osteogenic Marker Genes in hBM-MSCs

hBM-MSCs were plated and treated with 100 nM KMN-159 as above and, beginning on day five, fed every three days with 100 nM KMN-159 in osteogenic medium. Cells were harvested on days 5, 7, 11, 15, and 21, and mRNA levels of osteogenic marker genes were determined by RT-qPCR using β-actin as a reference gene.

Early-stage osteoblastic markers including Runx2, alkaline phosphatase, type I collagen, and osterix were upregulated by KMN-159 compared to vehicle control. Runx2 and osterix mRNA were detectable by day 5, and alkaline phosphatase and type I collagen mRNA were detectable by day 7 or 11, respectively. OPG, an osteoclastogenesis inhibitory factor, was downregulated by KMN-159, whereas RANKL was significantly upregulated, suggesting that KMN-159 stimulates hBM-MSCs to upregulate genes necessary for crosstalk in promoting osteoclast differentiation.




### KMN-159 Stimulates ALP Activity Even After Dry Plate Storage at Room Temperature




KMN-159 dilutions were made in ethanol and 0.5 ml of each dilution was added to a well of a 24-well plate (n = 8 wells/dilution). Plates were dried overnight in a cell culture hood and then stored dry, in room light at room temperature for 24 days. Freshly isolated rat BMCs were plated into the wells containing the dried dilutions of KMN-159 and cultured for 9 days as above. ALP activity was assayed, and the $EC_{50}$ for KMN-159 under these conditions was determined to be 8.4 nM.

## Conclusions

- KMN-159, a novel difluorolactam $EP_4$ receptor agonist, specifically binds to and activates the rat $EP_4$ receptor with potency similar to the natural ligand $PGE_2$.
- KMN-159 induces osteoblastic differentiation of BMCs regardless of the age or sex of the donor rats, and this occurs solely through stimulation of the $EP_4$ receptor.
- Osteoblast phenotype marker genes are expressed at higher levels in hBM-MSCs treated with KMN-159 as compared to the vehicle-treated controls.
- KMN-159 retains its potency and efficacy even when dried on the surface of cell culture wells and stored at room temperature for at least 24 days.
- The specificity of KMN-159 for the $EP_4$ receptor will avoid the undesirable side effects of $PGE_2$ administration such as flushing, lethargy, and diarrhea.
- These studies strongly suggest that KMN-159 may increase bone formation *in vivo* and therefore may be a potential candidate for augmentation of bone mass.