

Supporting Information

Identification of Novel 2-((1-(benzyl (2-hydroxy-2-phenylethyl) amino)-1-oxo-3-phenylpropan-2-yl)carbamoyl)benzoic Acid Analogues as BMP-2 Stimulators

Vishal M. Balaramnavar,[†]Imran A. Khan,[†]Jawed Akhtar Siddiqui,[§]Mohd Parvez Khan,[§]Bandana Chakravarti,[§]Kunal Sharan,[§]Gaurav Swarnkar,[§]Namrata Rastogi,[§]H.H. Siddiqui,^ψDurga Prasad Mishra,[§]Naibedya Chattopadhyay,[§]Anil K. Saxena^{†}*

[†]Division of Medicinal and Process Chemistry, [§]Division of Endocrinology, CSIR-Central Drug Research Institute, Lucknow - 226001, India. ^ψDepartment of Pharmacy, Integral University Lucknow.

Running title: Identification of BMP-2 promoters

Title pageS1
MTT Assay for Cell Viability (Figure 1)S3
Relative BMP-2 transcript level in RCOs (Table 1)S4
Absolute BMP-2 levels (pg/ml) in the conditioned medium (Table 2)S5

MTT Assay for Cell Viability

Calvaria derived primary osteoblasts cells were plated in a 96-well plate at 2×10^3 cells/well. When they attained ~60% confluence, they were pre-starved for about 3 h in 0.5% FCS medium and incubated with different concentrations (1nM, 10nM and 100nM) of compounds or vehicle in 2% FCS phenol red free DMEM for 24 hr. On termination of incubation, 5 mg/mL of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazoliumbromide (MTT) solution was added and incubated for another 4 hr. Formazan crystals were dissolved in DMSO and optical density (OD) was measured at 550 nm on an ELISA reader. The results of the MTT assay were represented in the Figure 1 (**S3**).

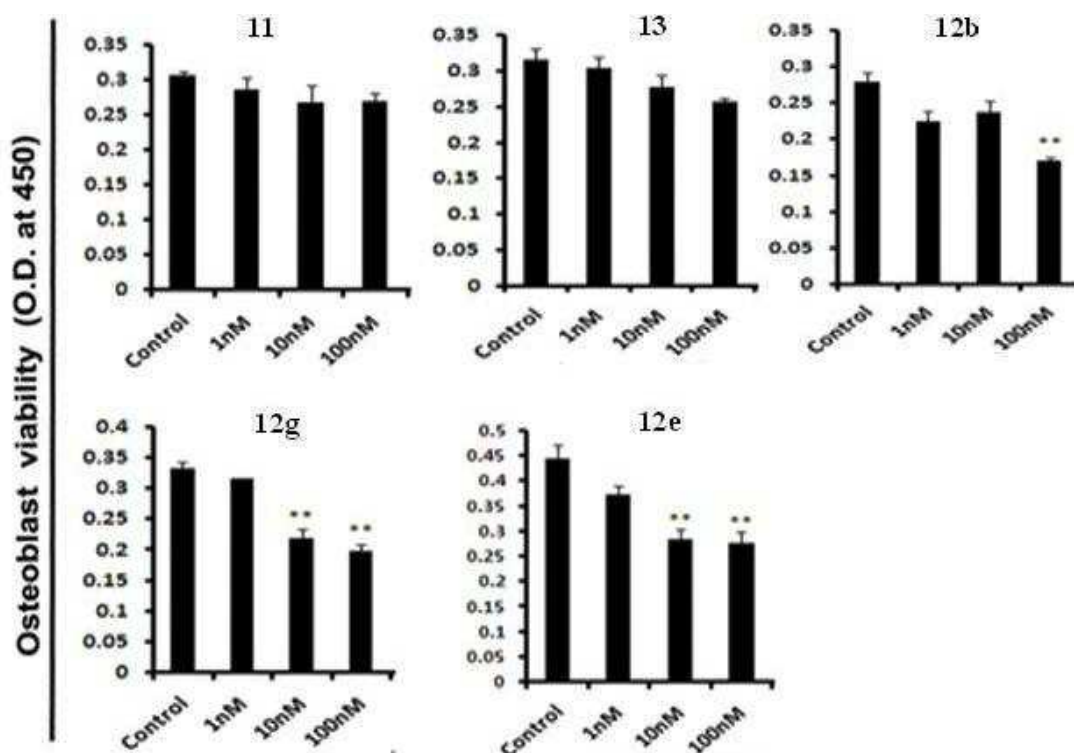


Figure 1. Cytotoxicity evaluation of compounds. Osteoblasts purified from neonatal calvariae of rats (RCO) were plated directly in 96-well plates after collagenase digestion at 2×10^3 /well and cultured for 3–5 d. At ~70% confluence, they were pre-starved for about 3 h in 0.5% FCS medium and incubated with different concentrations (1-, 10- and 100nM) of compounds (**11**, **13**, **12b**, **12g** and **12e**) or vehicle in 2% FCS containing phenol red free DMEM for 24 h. Cell viability was measured with MTT assay as described in Materials and Methods. Each assay was performed in triplicate and results are represented as mean \pm SEM. *P < 0.05; **P < 0.01; ***P < 0.001.

Table 1. Relative BMP-2 transcript level in RCOs.

Comp.	0.1nM	1nM	10nM	100nM	1000nM
11	2.50± 0.91	5.07±3.01*	10.81±1.19***	14.63±1.01***	18.27±1.45***
13	0.96±0.61	1.16±0.31	1.44±0.15	1.58±0.54	2.27±0.28*

Each assay was performed in triplicate at 48 h and results are expressed as mean ± S.E.M. * $P < 0.05$; *** $P < 0.001$ vs. control.

Table 2. Absolute BMP-2 levels (pg/ml) in the conditioned medium

Comp.	Control	0.1nM	1nM	10nM	100nM	1000nM
11	119.14±4.7	166.79±4.6**	189.43±3.2***	185.38±5.3**	163.46±4.1**	158.45±3.9*
12a	119.14±4.7	106.98±3.9	119.14±3.8	117.11±3.4	122.95±3.1	110.32±3.1
12b	119.14±4.7	117.23±6.3	127.47±2.9	122.71±4.7	122.49±6.4	133.43±5.4
12c	119.14±4.7	99.60±3.8	115.92±4.9	106.36±5.6	115.19±6.4	110.32±5.8
12d	119.14±4.7	105.67±4.6	106.55±6.9	99.42±4.6	117.13±5.3	119.85±4.7
12e	112.76±3.9	137.56±3.5	158.99±4.6**	122.90±5.7	155.60±2.2**	125.16±5.2
12f	112.76±3.9	119.52±6.3	111.26±5.4	110.87±6.9	108.28±4.7	116.14±4.6
12g	112.76±3.9	134.18±3.9	148.56±4.9**	126.04±5.3	129.53±5.1	117.27±6.2
12h	112.76±3.9	99.00±4.5	120.65±3.5	113.88±6.5	131.92±5.8	105.54±6.3
13	112.76±3.9	125.16±4.8	137.56±5.2	128.54±5.7	187.18±7.2**	148.84±5.5*

Each assay was performed in triplicate and results are expressed as mean ± S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. control.

