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Synthesis and evaluation of 1-(benzo[*b*]thiophen-2-yl)ethanone analogues as novel anti-osteoporosis agents acting on BMP-2 promotor

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ABSTRACT

A novel series of 1-(benzo[*b*]thiophen-2-yl)ethanone analogues were prepared and evaluated for enhancing BMP-2 expression. Compounds **1**–**5**, **7**, **8**, **12**, **13** and **16**, with upregulation rate values of 35.6%, 27.9%, 39.8%, 32.0%, 37.1%, 30.2%, 28.0%, 33.5%, 22.8% and 27.3% in vitro, respectively, at a concentration of 4 μM, exhibited potent effect for enhancing BMP-2 expression. We also found that compounds **1** and **12** produced a dose-dependent increase on bone histology and histomorphometry, and effectively reduced bone defects induced by ovariectomy in an ovariectomized rat model (OVX).

Osteoporosis is a reduction in skeletal mass due to an imbalance between bone formation modulated by osteoblasts and bone resorption modulated by osteoclasts.[1] There are no safe and effective methods to help porous bone to recover. The mainstay of therapy for osteoporosis is bone resorption. The effects of currently available drugs in increasing or recovering bone mass are relatively small, generally less than 10% over 3 years. Therefore, there is a need for developing new bone-building (anabolic) agents. Anabolic agents directly stimulate bone formation and correct the imbalance of bone formation and resorption in established osteoporosis.[2] Not only are anabolic agents able to increase bone mass, but they also have the capacity to improve bone quality and increase bone strength. The only anabolic agent currently available is recombinant human parathyroid hormone PTH.

Bone morphogenetic protein (BMP) plays important roles in osteoblastic differentiation and bone formation.[2a,3] Among the BMP family members, BMP-2 has been extensively studied. BMP-2 is expressed by normal osteoblasts and has been shown to stimulate osteoblast differentiation and bone formation during embryonic skeletal development and postnatal bone remodeling in vitro, as well as bone formation in vivo.[2a,4] Application of rhBMP-2 (recombinant human bone morphogenetic protein-2) to an open tibial fracture has been reported, however, this product is very expensive.[5]

Significant progress has been made in osteoporosis therapy, but drugs used in the clinic lack specificity, and generally have severe side effects. Owing to the inherent difficulties associated with these current anabolic treatments (dose route, narrow therapeutic index, bone quality), there remains considerable interest in small molecules which produce new bone growth as potential treatments for osteoporosis. Mundy et al. reported that statins enhanced new bone formation in vitro and in rodents. Such effects were associated with increased expression of the BMP-2 gene in bone cells.[6] Within a program of identifying agents with strong tissue specificity in improving metabolic balance of osteoporosis, and with the help of an anti-osteoporosis screening model targeting the promoter of the BMP-2 gene, we have evaluated thousands of compounds and observed that 1-(benzo[*b*]thiophen-2-yl)ethanone (**1**) has potent activity in enhancing BMP-2 expression and has the potential to be developed in the future as a clinical drug. To develop a more thorough understanding of the SAR, we wish to explore substituent effects on the phenyl ring, and both the C-2 and C-3 positions of compound **1**. We now wish to report our findings for these compounds which enhance BMP-2 expression in vitro. Compounds **1** and **12** have also shown bone anabolic effects in vivo in the ovariectomized rat model.

The synthesis of substituted 1-(benzo[*b*]thiophen-2-yl)ethanone analogues **1–11** is shown in Scheme 1. The key intermediates 2-(ethylthio)benzaldehyde analogues **1b**–**11b** were obtained in good yield from commercially available substituted 2-chlorobenzaldehyde and ethanethiol sodium salt, using tetra-*n*-butylammonium bromide as a phase transfer catalyst. The ethanethiol sodium salt was made from ethanethiol and a sodium hydroxide solution. Reaction of the 2-(ethylthio)benzaldehyde analogues

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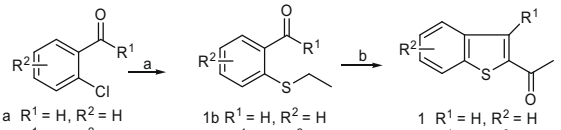


1a R^1 = H, R^2 = H	1b R^1 = H, R^2 = H	1 R^1 = H, R^2 = H
2a R^1 = H, R^2 = 6-Cl	2b R^1 = H, R^2 = 6-Cl	2 R^1 = H, R^2 = 4-Cl
3a R^1 = H, R^2 = 5-Me-6-Cl	3b R^1 = H, R^2 = 5-Me-6-Cl	3 R^1 = H, R^2 = 4-Cl-5-Me
4a R^1 = H, R^2 = 3-Me-6-Cl	4b R^1 = H, R^2 = 3-Me-6-Cl	4 R^1 = H, R^2 = 4-Cl-7-Me
5a R^1 = H, R^2 = 4,5-$(OMe)_2$	5b R^1 = H, R^2 = 4,5-$(OMe)_2$	5 R^1 = H, R^2 = 5,6-$(OMe)_2$
6a R^1 = H, R^2 = 5-CF_3	6b R^1 = H, R^2 = 5-CF_3	6 R^1 = H, R^2 = 5-CF_3
7a R^1 = H, R^2 = 3-CF_3	7b R^1 = H, R^2 = 3-CF_3	7 R^1 = H, R^2 = 7-CF_3
8a R^1 = Me, R^2 = H	8b R^1 = Me, R^2 = H	8 R^1 = Me, R^2 = H
9a R^1 = Me, R^2 = 6-Cl	9b R^1 = Me, R^2 = 6-Cl	9 R^1 = Me, R^2 = 4-Cl
10a R^1 = Me, R^2 = 5-Me-6-Cl	10b R^1 = Me, R^2 = 5-Me-6-Cl	10 R^1 = Me, R^2 = 4-Cl-5-Me
11a R^1 = Me, R^2 = 5-CF_3	11b R^1 = Me, R^2 = 5-CF_3	11 R^1 = Me, R^2 = 5-CF_3

Scheme 1. Reagents and conditions: (a) CH_3CH_2SH, NaOH, H_2O, 1 h, then **1a**, $(n\text{-}Bu)_4NBr$, reflux 4 h; (b) $ClCH_2COCH_3$, CaO, reflux 3 h.

with chloroacetone in the presence of calcium oxide as a solid base furnished the corresponding target compounds 1-(benzo[*b*]thiophen-2-yl)ethanone analogues **1–11** using well established literature methodology.[7]

The activities of 18 compounds (compounds **12–18** were bought from Acros Organics) in enhancing BMP-2 expression were evaluated using lovastatin as a positive control employing recombinant plasmid PMB and rat skull cells MC3T3-E1. The results are summarized in Table 1.[6,8] Many compounds exhibited strong activities in enhancing BMP-2 expression with upregulation rate >21% (**1–5**, **7**, **8**, **12**, **13**, **16**) or >10% to <21% (**6**, **9**, **11**, **14**, **15**, **17**). The lead compound 1-(benzo[*b*]thiophen-2-yl)ethanone (**1**), at a concentration 10-fold higher than that of the positive control, lovastatin, significantly enhanced BMP-2 expression as reflected by the upregulation rate value of 35.6%, almost twofold higher than that of lovastatin.

We first evaluated the effects of substituents and their location on the phenyl ring of compound **1** on enhancing BMP-2 expression. 4-Cl, 4-Cl-5-Me, 4-Cl-7-Me, 5,6-$(OMe)_2$, 7-CF_3 substituted compounds (**2–5**, **7**) showed potent activities, with upregulation rate values of 27.9%, 39.8%, 32.0%, 37.1%, 30.2%. The effect of the 5,6-$(OMe)_2$-substituted compound **5** is similar to that found for compound **1**. The 4-Cl-5-Me-substituted compound **3** showed a little bit higher activity than compound **1**, and its isomer 4-Cl-7-Me-substituted compound **4** showed a little bit lower activity compared with compound **1**. The 7-CF_3-substituted compound **7** is more potent than its 5-CF_3 isomer compound **6**. These results show that electron donating groups cause higher upregulation rate values than electron-withdrawing groups in this system.

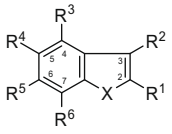
By comparing the results for **3** and **4**, one can see that C-5 position is more favorable for electron donating group attachment than the C-7 position. However, C-7 position is more favorable for electron-withdrawing groups than the C-5 position based on the results for **6** and **7**.

Next we evaluated the effects of different heterocycles on enhancing BMP-2 expression. When the sulfur atom in compound **1** was replaced by oxygen to yield **12**, the upregulation rate value is similar with that found for compound **1**. When the sulfur atom in compound **1** was replaced by nitrogen, and the position of the acetyl group was changed from C-2 to C-3 to yield **13**, the upregulation rate value decreased to 22.8%. Furthermore, when the position of the acetyl group of compound **1** was changed from C-2 to C-3 to yield **14**, the upregulation rate value decreased to 11.9%. The results demonstrated that the 2-acetyl benzoheterocycle is much better than the 3-acetyl benzoheterocycle.

We also evaluated analogues of compound **1** with different substituents at C-2 position on enhancing BMP-2 expression. Replacement of the 2-acetyl group in compound **1** with a carboxyl group, to yield **15**, resulted in decreased activity. Replacing the 2-acetyl group of compound **1** with methyl formate, formamide and formhydrazide (yielding **16–18**) resulted in decreased activities compared with compound **1**. These results demonstrate that the 2-acetyl group is very important for the potent effect of compound **1**.

In an effort to further understand the substituent effects, compounds **8–11** were synthesised. All those compounds have a methyl

Table 1
BMP-2 expression enhancing activities of 1-(benzo[*b*]thiophen-2-yl)ethanone analogues [a] in vitro



Compound	X	R^1	R^2	R^3	R^4	R^5	R^6	Upregulation rate (%)
1	S	COMe	H	H	H	H	H	35.6
2	S	COMe	H	Cl	H	H	H	27.9
3	S	COMe	H	Cl	Me	H	H	39.8
4	S	COMe	H	Cl	H	H	Me	32.0
5	S	COMe	H	H	OMe	OMe	H	37.1
6	S	COMe	H	H	CF_3	H	H	18.5
7	S	COMe	H	H	H	H	CF_3	30.2
8	S	COMe	Me	H	H	H	H	28.0
9	S	COMe	Me	Cl	H	H	H	16.5
10	S	COMe	Me	Cl	Me	H	H	6.5
11	S	COMe	Me	H	CF_3	H	H	16.7
12[c]	O	COMe	H	H	H	H	H	33.5
13[c]	NH	H	COMe	H	H	H	H	22.8
14[c]	S	H	COMe	H	H	H	H	11.9
15[c]	S	CO_2H	H	H	H	H	H	10.8
16[c]	S	CO_2Me	H	H	H	H	H	27.3
17[c]	S	$CONH_2$	H	H	H	H	H	11.2
18[c]	S	$CONHNH_2$	H	H	H	H	H	7.8
Lovastatin[b]								20.9

[a] Compound concentration for **1–18** is 4 μM.
[b] Compound concentration for lovastatin is 0.4 μM.
[c] Commercially available.

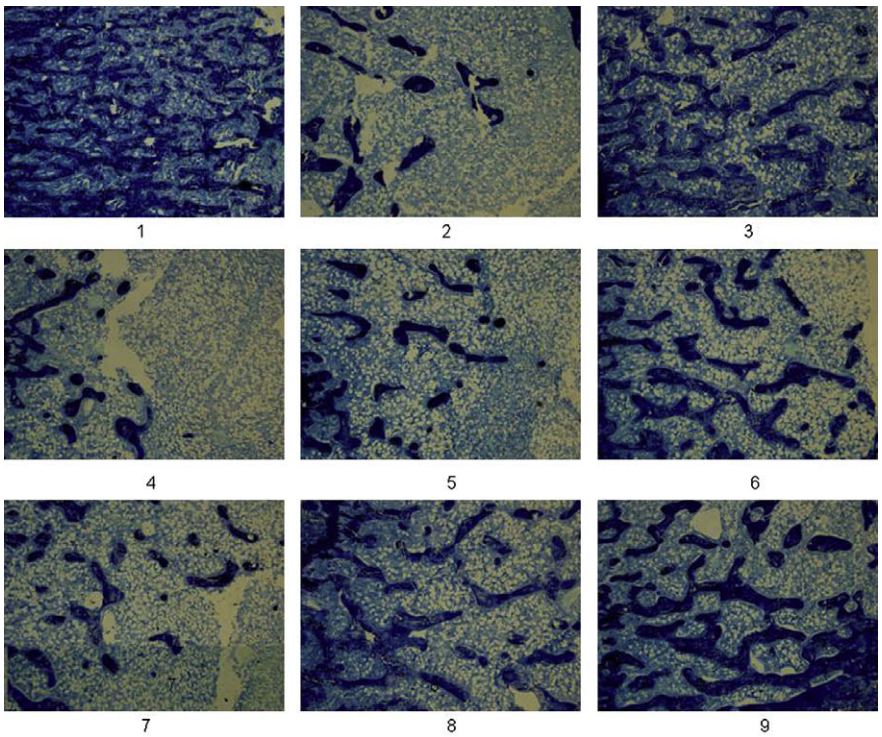


Figure 1. Histology of left proximal tibia, illustrating trabecula (dark blue). (1) Sham operated group. (2) OVX group. (3) Raloxifene (5 mg/kg/day, positive control) treated group. (4) **12L** (compound **12** 10 mg/kg/day) treated group. (5) **12M** (compound **12** 20 mg/kg/day) treated group. (6) **12H** (compound **12** 40 mg/kg/day) treated group. (7) **1L** (compound **1** 7.5 mg/kg/day) treated group. (8) **1M** (compound **1** 15 mg/kg/day) treated group. (9) **1H** (compound **1** 30 mg/kg/day) treated group.

group at the C-3 position. However, the activities of **8–11** decreased when compared their respective analogs, **8** versus **1**, **9** versus **2**, **10** versus **3**, and **11** versus **6**. This finding revealed that the C-3 methyl group is not favored for enhancing BMP-2 expression.

Compounds **1** and **12** were chosen for in vivo efficacy evaluation using the ovariectomized Sprague–Dawley rat model (OVX).[9] Test rats were assigned groups: sham-operated (Sham), OVX with vehicle (OVX, control); OVX with raloxifene (5 mg/kg/day, orally, for 90 days, positive control); OVX with compound **1** (orally for 90 days); and OVX with compound **12** (orally for 90 days).

Bone histology under light microscopy is shown in Figure 1. The trabecula is dense and compact in the sham-operated group, but for the OVX group with vehicle, it is turbulent and results in trabecula sparse rarefaction. In the OVX groups receiving raloxifene and compound **1** (30 mg/kg/day), the number, the thickness, the area and connectivity of the trabecula have significantly increased compared with the control. In the OVX groups receiving **1** (7.5 mg/kg/day, 15 mg/kg/day, 30 mg/kg/day) and **12** (10 mg/kg/day, 20 mg/kg/day and 40 mg/kg/day), the number, area, thickness and connectivity of trabecula increased compared with the OVX group in a dose-dependent manner.

Table 2 summarizes the effects of the OVX group, the groups after treatment with raloxifene, compound **1** and compound **12** on bone histomorphometry of rats evaluated in terms of trabecular bone volume percentage (TBV%), trabecula resorption surface percentage (TRS%), trabecula formation surface percentage (TFS%), mineral apposition rate (MAR, μM/d), osteo cortex mineralization rate (mAR, μM/d) and average osteoid width (OSW, μM).

Clearly, all parameters in the femora of OVX are statistically significant compared with the sham group, $p < 0.05$ on TFS% and mAR,

Table 2
Effects of OVX, oral treatment with raloxifene, compound **1** and compound **12** on bone histomorphometry

Group	TBV (%)	TRS (%)	TFS (%)	MAR(μM/d)	mAR (μM/d)	OSW (μM)
SHAM	27.38 ± 1.67	3.49 ± 1.19	3.97 ± 1.33	1.61 ± 0.24	2.42 ± 0.36	8.65 ± 0.99
OVX	10.34 ± 1.62[b]	8.18 ± 1.75[b]	6.16 ± 1.76[a]	2.28 ± 0.36[b]	3.22 ± 0.68[a]	11.33 ± 1.91[b]
Ralo	22.31 ± 1.93[d]	3.34 ± 1.03[d,e]	6.52 ± 1.76	2.14 ± 0.49	3.15 ± 0.84	11.08 ± 1.59
12L	12.39 ± 1.50[c]	7.84 ± 1.85	7.26 ± 1.80	2.31 ± 0.49	3.28 ± 0.70	11.25 ± 1.38
12M	13.13 ± 1.39[d]	7.15 ± 1.93	6.75 ± 1.90	2.34 ± 0.33	3.51 ± 0.68	11.62 ± 1.60
12H	17.50 ± 1.62[d]	5.30 ± 0.79[d]	6.74 ± 0.93	2.20 ± 0.18	3.30 ± 0.21	10.99 ± 0.66
1L	14.41 ± 1.50[d]	7.81 ± 1.67	7.40 ± 1.60	2.32 ± 0.32	3.42 ± 0.50	11.44 ± 1.52
1M	19.39 ± 1.95[d]	5.16 ± 1.34[d]	6.64 ± 1.95	2.19 ± 0.29	3.14 ± 0.61	10.86 ± 1.09
1H	21.02 ± 1.85[d]	4.74 ± 1.62[d,e]	6.39 ± 1.85	2.18 ± 0.51	3.22 ± 0.33	10.76 ± 1.49

12L, 12M and **12H:** separately treated with compound **12** 10 mg/kg/day, 20 mg/kg/day and 40 mg/kg/day for 90 days; **1L, 1M** and **1H:** separately treated with compound **1** 7.5 mg/kg/day, 15 mg/kg/day and 30 mg/kg/day for 90 days.

[a] $p < 0.05$.
[b] $p < 0.01$ compared with sham group.
[c] $p < 0.05$.
[d] $p < 0.01$ compared with OVX group.
[e] $p > 0.05$ compared with sham group.

$p < 0.01$ on other parameters. Compounds **1** and **12** showed a dose-dependent increase in all the parameters as compared to OVX.

Ovariectomy is associated with a lower trabecular bone volume and a higher trabecula resorption surface percentage compared with sham ($p < 0.01$). All doses of compounds **1** and **12** showed statistically significant ($p < 0.05$) effects on TBV% compared with OVX. **12**H and **1**M (see Table 2) groups showed higher TRS% ($p < 0.01$) than OVX. The raloxifene and **1**L (see Table 2) groups showed strong anti-osteoclast activities compared with OVX ($p < 0.01$) and they returned the trabecula resorption surface percentage back to a level similar to sham ($p > 0.05$).

Ovariectomy resulted in a statistically significant ($p < 0.05$) increase of TFS%, mAR, and showed a very significantly ($p < 0.01$) increased MAR and OSW compared with sham. The treatment with different doses of compound **1**, compound **12** and raloxifene had no significant effect on these parameters.

The LD_{50} of compound **1** for Kuming mice (female, 18–20 g in weight) was over 5 g/kg (orally), and was 1.58 g/kg (intraperitoneally). In order to further understand the toxicity of compound **1**, we treated the sham rats with a dosage of 150 mg/kg/day (orally) for 90 days. Compound **1** at the treatment dosage showed no significant effect on general behavior, blood parameters including glutamine–oxaloacetic transaminase (GOT), glutamic–pyruvic transaminase (GPT), total bilirubin (TBIL), phosphatases (ALP), total protein (TP), albumin (Alb), triglycerides (TG), total cholesterol (CHO), direct high density lipoprotein (D-HDL), direct low density lipoprotein (D-LDL), glucose (Glu), blood urea nitrogen (B.U.N.), creatinine (Cre), Ca^{2+} and bone histomorphometry (TBV, TRS, TFS, MAR, mAR, OSW) of the tested rats.

It is interesting to note that compounds **1** and **12** had similar activities in vitro, but different activities in vivo. One can envision situations where delivery properties can contribute to this observed in vivo potency differences. The partition coefficients (log *P*) of compounds **1** and **12** between *n*-octanol/water were 4.37 and 3.41, respectively, determined by HPLC on a C_{18} column with a mobile phase of CH_3OH–H_2O (85:15, v/v; flow rate 1.0 ml/min; UV detection wavelength 296 nm). The different log *P*s for **1** and **12** suggest that they have different pharmacokinetic properties, which could contribute to the observed difference in activities. However, detailed animal studies are needed to confirm this hypothesis.

In conclusion, the BMP pathway is a promising new area to target therapeutic agents for the treatment of low bone mass. A new class of small molecules was found to be potential anabolic agents targeting BMP-2 in this work. We have described the synthesis and SAR studies of 1-(benzo[*b*]thiophen-2-yl)ethanone analogues as potent anti-osteoporosis agents. At a concentration 10-fold higher than that of lovastatin, compound **1**–**5**, **7**, **8**, **12**, **13** and **16** exhibit more potent activities on enhancing BMP-2 expression in vitro than lovastatin. We also have found that 1-(benzo[*b*]thiophen-2-yl) ethanone (**1**) and its analogue **12** produced a dose-dependent increase on bone histology and histomorphometry and effectively reduced bone defects induced by ovariectomy in a OVX rat model. Compounds **1** and **12** showed similar activities in vitro, however, compound **12** showed lower activities in vivo than compound **1**. The LD_{50} of compound **1** on mice was over 5 g/kg (orally), and 1.58 g/kg (intraperitoneally). These results suggest that compounds of this class may be useful in the treatment of bone degenerative disease, including osteoporosis. Further investigation of the bioavailabilities and activities of compounds **1** and **12** in senescence accelerated mouse and the bioavailabilities and activities of compounds **3** in OVX are underway.

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