Reviewer #1:
The manuscript by Lee et al describes the synthesis and modifications of a battery of novel bis-benzamiudes. Functionally the compounds were tested as peptidomimetics to interfere with coactivator-androgen receptor interaction in the prostate cancer LNCaP cells.

Authors found bioactivity in the nanomolar range of derivatives with antiproliferative activity. Further, the interaction of the coactivator PELP is strongly reduced by one derivative (14d) that inhibits androgen receptor-mediated transactivation.

The compounds are interesting but they may act not only specific for the androgen receptor. The inhibition of cell proliferation might be due to general toxicity or to other pathways and not necessarily the inhibition of androgen receptor LXLL motif interactions. Since LNCaP cell growth is androgen-dependent it is unclear whether the compounds that reduce cell proliferation are interfering with the androgen –dependent growth.

Major points:

1. **To detect whether there is a general cell toxicity other prostate cancer cells lacking the expression of the androgen receptor must be tested, such as DU145 or PC3 cells using the MTT assay.**

Response: We thank the reviewers for raising questions on the general toxicity of the bis-benzamides 14d and 14s. As such, we have tested the effects of these compounds on the viability of AR-negative PC3 cells. As demonstrated in the Figure S1b, MTT based dose-response assay confirmed that these compounds do not have any effect on the growth of AR negative PC3 cells.

2. **To detect whether the androgen-signaling is indeed inhibited, cell growth of LNCaP cells must be tested with and without androgens**

Response: As requested by the reviewer, we have assessed the effects of the drugs on the viability of LNCaP cells in presence or absence of the bis-benzenamides. As shown in the Figure S1a, while DHT alone increased the viability of LNCaP cells, 14d or 14s decreased the % of DHT induced viable cells after 72 hours. However,
without DHT, these compounds did not affect the cell growth. Thus, our data indicate that bis-benzamides inhibit DHT dependent cell viability of LNCaP cells.

Figure S1. Dependence of compounds 14d and 14s on AR-signaling. (a) The effects of 14d and 14s for the growth inhibition of LNCaP cells in the presence or absence of DHT by MTT assay.

To describe this, the text was revised in page 7 line193-195.

“In addition, cell proliferation was unaffected by 14d and 14s in the absence of DHT (Supplementary Materials Figure S1a), suggesting compounds 14d and 14s are dependent on androgen for their inhibitory activity.”

3. To detect whether androgen-mediated transactivation is specifically inhibited, selected compounds shall be analyzed with and without androgen treatment for expression analysis of endogenous androgen receptor target genes, e.g. PSA, or other known target genes.

Response: We are thankful to the reviewers for asking questions pertaining to the effects of these compounds on the expression of AR targets. We demonstrate in the Figure 4c that 14d or 14s treatment profoundly inhibits the transactivation of PSA and TMPRSS2 by DHT in LNCaP cells. These data suggest that bis-benzamides 14s and 14d inhibit androgen-dependent AR transcriptional activity.

Figure 4. Inhibition of AR-coactivator interaction and AR-mediated transcriptional activity by compounds 14d and 14s. (c) The effects of 14d and 14s on the expression levels of AR target genes PSA and TMPRSS2 in LNCaP cells.

To describe this, the text was revised in page 6 line177-183.

“Expression of well-known AR target genes such as prostate specific antigen (PSA) and transmembrane protease serine 2 (TMPRSS2) was also measured by quantitative reverse transcription PCR (qRT-PCR). Compound 14s significantly reduced the expression of PSA and TMPRSS2 mRNA (5.2- and 5.0-fold, respectively) in the presence of DHT. Similarly, compound 14d decreased PSA and TMPRSS2 mRNA levels
by 2.7- and 2.3-fold, respectively (Figure 4c). These results indicate that blockage of the AR–coactivator interaction and AR transactivation accounts for the antiproliferative activities of compounds 14d and 14s.

4. **Authors should explain with compound 14s that the PELP-AR interaction is only slightly weakened while there is a strong inhibition of the luciferase units.**

Response: Data in Figure 4a and 4b show that compound 14s weakly inhibits the interaction between AR and PELP1 interaction but still has strong inhibitory activity on AR transactivation. In addition to PELP1, there are other coactivators including steroid receptor coactivators (SRCs) that possess the LXXLL motif for their interaction with AR and enhance the transcriptional activity of AR. Compound 14s may inhibit such coactivators in addition to PELP1 showing the inhibitory activity in luciferase assays. To clarify, we added the following statements in page 8, lines 265-269.

“On the other hand, compound 14s had similar effects on AR transactivation compared to 14d, but only weakly inhibited the recruitment of PELP1 to AR (Figure 4a). Since a number of coactivators including steroid receptor coactivators (SRCs) also possess the LXXLL motif for their interactions with AR and enhance the transcriptional activity of AR [8], compound 14s may block such coactivators in addition to PELP1 to display its inhibitory activity on AR transactivation.”

5. **Fig. 2: What is set as 100% if values are 160% for cell viability? Does it suggest growth promoting activity of compound 14d and 14s?**

Response: The growth of LNCaP cells is increased by DHT compared to untreated cells, and the DHT-induced growth can be suppressed by bis-benzamides. In Figure 3, cell viability observed in cells treated with DHT was set as 100% and data were normalized to the DHT treatment.

![Figure 3](image_url)

Figure 3. Dose-response experiments of bis-benzamides 14d and 14s for the growth inhibition of LNCaP cells by MTT assay.