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# New Cytotoxic *bis*-1,3-Thiazolidine-2,4-dione (TZD) Derivatives with *meta*-Xylyl and Propyl linkers: Synthesis and Characterization

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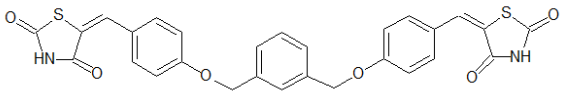
**Abstract:** Two new bi*s*-1,3-thiazolidine-2,4-dione (TZD) derivatives (**B** and **C**) with an aromatic and aliphatic linkers were synthesized from the TZD derivative precursor **A** in 61.36% and 21.62% yields respectively. The *meta*-xylyl and *propyl* linkers were incorporated in compounds **B** and **C** to increase the lipophilicity of the molecule. This property will make it better penetrate the cell membrane and reach the target receptor sites which will hopefully increase the potency of the compounds.

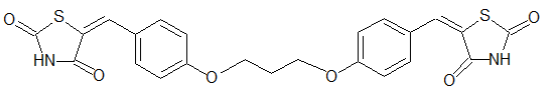
Precursor compound **A** was prepared via Knoevenagel condensation reaction of TZD and *para*-hydroxybenzaldehyde. Bimolecular Nucleophilic Substitution (Sn2) reaction of compound **A** with α,α'-dibromo-*m*-xylene or 1,3-dibromopropane produced compounds **B** and **C** respectively.

A second approach explored in the synthesis of compounds **B** and **C** was to prepare precursors **D** (43.70%) and **E** (64.95%), followed by the introduction of the TZD moieties by condensation using the heterocyclic base, Piperidine. The target compounds **B** and **C** however were not obtained using this method. The use of precursor D resulted in the formation of mono-TZD derivative **G** (98.00%) instead.

Cytotoxicity test was carried out on TZD derivatives **A**, **B** and **C** against HDFn (human dermal fibroblast), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell) using PrestoBlue™ Assay. All compounds (**A**, **B** and **C**) were shown to have no significant activity against normal cells unlike the Bleomycin standard with an $IC_{50}$ 9.6 μg/mL. All compounds (**A**, **B** and **C**) were found to inhibit the proliferation of both breast and colon cancer cells. Compound **B** exhibited greater inhibition of colon cancer cells ($IC_{50}$ 0.16 μg/mL), while compound **A** exhibited greater inhibition of breast cancer cells ($IC_{50}$ 3.97 μg/mL) compared to the standard. These data shows that compounds **B** and **A** are potential drugs for treatment of the two diseases.



(compound B)



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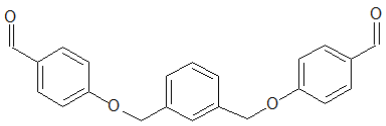

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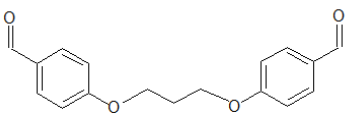

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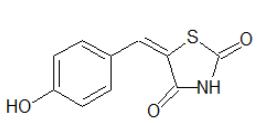


(compound A)

**Key Words:** Cytotoxic; Thiazolidinedione derivative; Synthesis and Characterization; Cancer, Diabetes

# 1. INTRODUCTION

World Health Organization (WHO) has accounted 36 million of the 57 million deaths worldwide to be due to non-communicable diseases (NCD) such as heart disease, stroke, cancer, chronic respiratory diseases and diabetes. Cardiovascular diseases was the leading cause of these deaths but cancer and diabetes also contributed a considerable number. In 2008, cancer claimed 7.6 million lives while diabetes caused 1.3 million deaths (NDEP, 2013).

Researchers all over the world are in constant search of a more effective cure to these diseases. One challenge is to prepare in the laboratory, a new breed of therapeutic medicines with no harmful side effects. If these medicines are made readily available and affordable then it would significantly decrease mortality due to these diseases.

One strategy is to produce a potential drug by manipulating the structure of an organic compound with a known bioactivity. Structure-activity studies on this compound involve the introduction of different moiety that may result in the generation of a new compound with increased activity and decreased side effects (Momose et al, 1990).

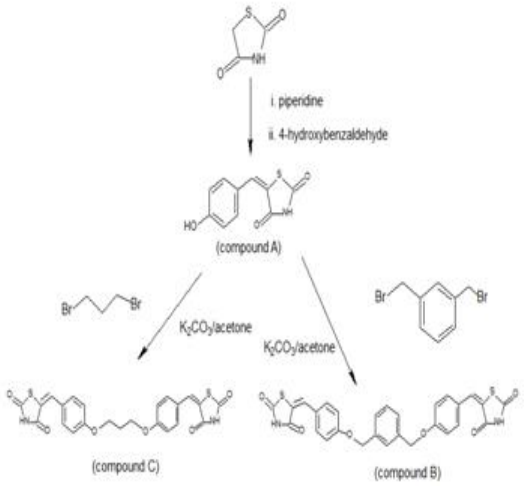
An organic molecule of interest that may be used towards this end is Thiazolidinedione (TZD) (Malik, 2011). Thiazolidinedione and some of its derivatives were found to be a preventive and potential cure to some diseases such as in diabetes mellitus (Type 2 Diabetes) (Niranjan, 2013; Rekha, 2011); various kinds of cancer including lung, breast and colon cancers (Blanquicett et al, 2008; Panigrahy et al, NA; Shimazaki et al, 2008); neurodegenerative diseases; hypertension (Abougalambou, 2013) and endothelial dysfunction (Matsumoto et al, 2007).

This study is concerned with the synthesis of *bis*-thiazolidinedione derivatives with an aliphatic or aromatic linker. The compounds contain two units of TZD with nonpolar linkers. The propyl and *meta*-xylyl linkers are incorporated to increase the lipophilicity of the molecule. This property will make it better penetrate the cell membrane and reach the target receptor sites. This will hopefully increase the potency of the drug.

# 2. METHODOLOGY

Two approaches were tried in the synthesis of compound **B** and **C**. Method 1 (Scheme 1) involved the substitution reaction of compound **A** with α,α'-dibromo-*m*-xylene and 1,3-dibromopropane respectively. Method 2 (Scheme 2) on the other hand involved a Knoevenagel condensation reaction of TZD to precursor compounds **D** and **E** to synthesize compound **B** and **C** respectively.

## 2.1. Method 1




Reaction Scheme 1. Synthesis of Compounds B and C (Method 1)

### 2.1.1. Synthesis of Compound **A:** (5-[(4-hydroxyphenyl)methylidene]-1,3- thiazolidine-2,4-dione)

*Para*-hydroxybenzaldehyde (1.2465 g, 10.21 mmol), 0.285 mL piperidine and 2,4-thiazolidinedione (1.2076 g, 10.29 mmol) were mixed with 8.89 mL ethanol in 100 mL round bottom flask and refluxed for six hours.

The mixture was cooled and poured into 10 mL iced water in 100 mL beaker, stirred and left to stand for 30 minutes. It was washed using ethanol and filtered using vacuum. Compound **A** was obtained as a yellow powdery compound (1.0122g, 44.38%): **M.P.**=278-282 (lit 280-284); **Rf**= 0.48 (15%EtOAc/DCM); **IR** (KBr) 3405 $cm^{-1}$ (N-H), , 3120 $cm^{-1}$ (-OH) , 1678 and 1720 $cm^{-1}$ (C=O str. TZD).); **MS**($ES^{-}$) [M-H] 220.

### 2.1.2. Synthesis of Compound **B:** ((E)-5-{[p-(m-{p-[(E)-(2,4-Dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}phenoxy) phenyl]methylidene}-1,3-thiazolidine-2,4-dione)

Mixture of α,α'-dibromo-*m*-xylene (0.678 g, 0.257 mmol) with compound **A** (0.101 g, 0.456 mmol) and potassium carbonate ($K_2CO_3$) (0.222 g, 1.60 mmol) was dissolved in 7 mL acetone in round bottom flask. The mixture was refluxed for six hours then poured into ice. Milky whitish-yellow compound was observed and was found to be slightly soluble in water/acetone mixture. The precipitate was washed with cold acetone, DCM & diethyl ether. Afterwards, it was washed with hot acetone, yielding amorphous yellow solid.

Compound **B** was obtained as a yellow powdery compound (76.2 mg, 61.36%): **M.P.=**200-203°C ; **Rf**= 0.7647 (15% ethyl acetate/hexane); **IR** (KBr) 3405 $cm^{-1}$ (N-H), 1681.25$cm^{-1}$, 1737.14$cm^{-1}$ (C=O str. TZD).

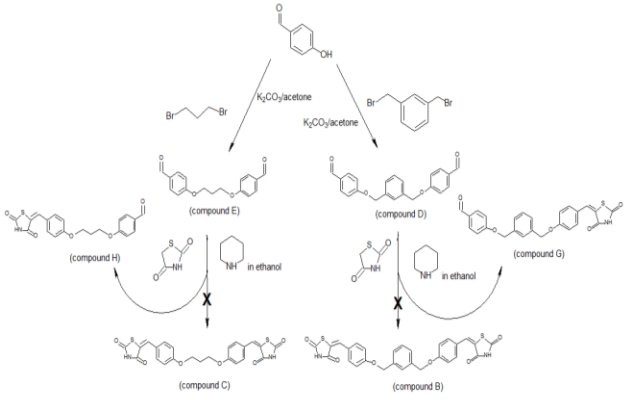
### 2.1.3. Synthesis of Compound **C:** ( (E)-5-{[p-(3-{p-[(E)-(2,4-Dioxo-1,3-thiazolidin-5-ylidene)methyl]phenoxy}propoxy)phenyl]methylidene}-1,3-thiazolidine-2,4-dione)

Measured amount of 1,3-dibromopropane (0.02 mL, 0.23 mmol) was added to compound **A** (0.1062 g, 0.48 mmol) with potassium carbonate ($K_2CO_3$) (0.2216 g, 1.60 mmol) was dissolved in 5mL acetone and was refluxed for four hours.

After four hours of reflux, mixture was poured into ice, allowed to precipitate and then filtered. Solid crystals were collected then washed with cold ethanol and water. It was filtered in vacuum yielding yellow powdery compound.

Compound **C** was obtained as a yellow powdery compound (24.0 mg, 21.62%): **M.P.=**167-171 °C; **Rf**= 0.8542 (15% ethyl acetate/DCM); **IR** (KBr) 3409.46 $cm^{-1}$ (N-H), 1680.53 $cm^{-1}$ and 1738.08$cm^{-1}$ (C=O str. TZD)

## 2.2. Method 2




Reaction Scheme 2. Synthesis of Compound B and C (Method 2)

### 2.2.1. Synthesis of Compound **D:** (*p*-[*m*-(*p*-Formylphenoxy)phenoxy]benzaldehyde)

Aromatic linker α,α'-dibromo-*m*-xylene (1.0855 g, 4.11 mmol) was added to 1,4-*p*-hydroxybenzaldehyde (1.0161 g, 8.32 mmol) with potassium carbonate,$K_2CO_3$ ( 3.5976 g, 26.03 mmol) mixed in 40mL acetone in a round bottom flask. The solution was refluxed for four hours and 30 minutes.

After cooling, the mixture was poured into iced water forming a milky white solution. It was then extracted with ethyl acetate (3x30mL) and water. The organic layer was collected and the solvent was evaporated *in vacuo*. The crude product was left to cool down and amorphous white solid crystals were obtained.

Compound **D** was obtained as a white amorphous crystals (622.1 mg, 43.70%): **M.P**.=88-89 °C; **Rf**= 0.54 (15%EtOAc/DCM); **IR** (KBr) 1693.5 $cm^{-1}$ (HC=O).

### 2.2.2. Synthesis of Compound **E:** (*p*-[3-(*p*-Formylphenoxy)propoxy]benzaldehyde) Formylphenoxy)propoxy]benzaldehyde)

Measured amount of dibromopropane (0.42 mL, 4.113 mmol) was added to *para*-hydroxybenzaldehyde (1.0091g, 8.26 mmol) with potassium carbonate ($K_2CO_3$) (4.5478 g, 32.90 mmol) dissolved in acetone, then refluxed for five hours.

After 5 hours of reflux, mixture was poured into ice, allowed to precipitate and then filtered. It was washed with cold ethanol and water, and white precipitates were obtained.

Compound **E** was obtained as a white needle-like crystals (759.50 mg, 64.95%): **M.P.=**123-124 °C; **Rf**= 0.511 (15%EtOAc/DCM); **IR** (KBr) 1691.72 $cm^{-1}$ (C=O).

### 2.2.3. Synthesis of Compound **B** (formation of Compound G)

Thiazolidinedione (0.0372 g, 0.3169 mmol) was added to compound **D** (0.0560 g, 0.1571 mmol), piperidine (14.2 µL , 0.91 mmol) and dissolved in 3 mL ethanol in a round bottom flask. The solution was refluxed for six hours.

After six hours of reflux, the mixture was poured into ice, stirred and left to stand for 30 minutes. It was then washed with cold ethanol and filtered *in vacuo*. A yellow powdery compound was collected.

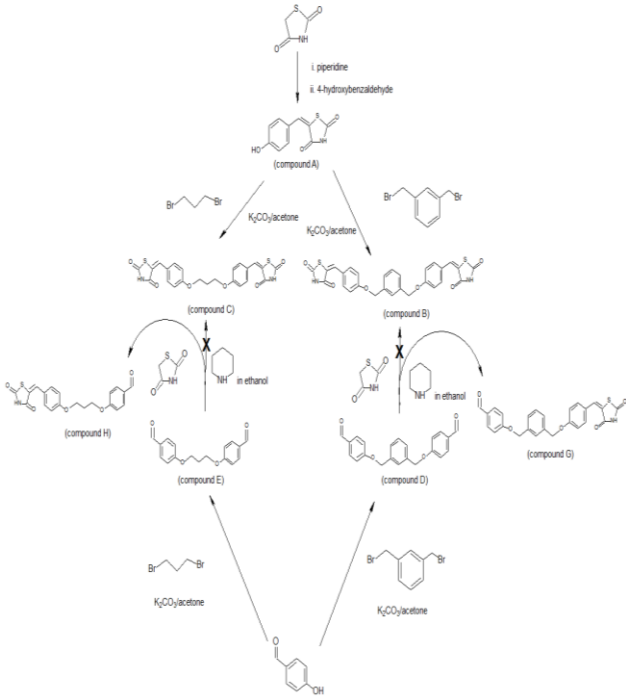
The product was obtained as a yellow powdery compound (67.40 mg): **M.P.=**159-161 °C; **IR** (KBr) 3400 $cm^{-1}$ (N-H), 1693 $cm^{-1}$ (CH=O), 1738.6 $cm^{-1}$ (C=O str. TZD).

# 3. RESULTS AND DISCUSSION

One of the most widely studied region in therapeutic drug discoveries are the different strategies on the synthesis of these drugs. It is done via manipulation of the structure of some organic compounds with a known bioactivity that boost the drug's potentials and is anticipated to have decreased side effects, more affordable, and readily available to everyone.[2]

Thiazolidinedione, a compound which is found to be a potential cure and prevention to some diseases (i.e. diabetes mellitus; various kinds of cancer including lung, breast and colon cancers; neurodegenerative diseases; and endothelial dysfunction), became one of the most studied compound since the early 90's.

In this research study, *bis*-thiazolidinedione derivatives with either aliphatic or aromatic linker were synthesized. The aliphatic linkers decrease the polarity of the compound which could make it better penetrate the cell membrane and allow it to reach its receptors.




Reaction Scheme 3: Synthesis of Compounds B and C

Two general strategies were done to synthesize the TZD derivatives. One of which was the addition of TZD to *para*-hydroxybenzaldehyde to produce Compound **A.** Further reaction of **A** with the linkers formed compounds **B** and **C**. The other strategy explored the attachment of *para*-hydroxybenzaldehyde to the linkers producing compounds **D** and **E**. Further reaction of **D** however produced the TZD derivative **G** rather than the target compound **B.** Reaction of **E** to form **C** was unsuccessful. The proposed overall synthesis is shown on the reaction scheme 3.

## 3.1. Method 1

Precursor compound **A** was first prepared via Knoevenagel condensation reaction of TZD and para-hydroxybenzaldehyde followed by Sn2 reaction with Xylyl and Propyl linkers for synthesis of compounds **B** and **C** respectively.

### 3.1.1. Synthesis of Compound **A**

Compound **A** has a TZD unit and phenol functionality. Its synthesis involved the Knoevenagel condensation reaction between TZD and *para*-hydroxybenzaldehyde.

Three trials were done in the synthesis of compound **A**. The percent yield ranges from 27% to 44%. The third trial gave the highest % yield. It also produced compound A with a melting point nearest the literature value. The different melting points may be attributed to the variation in packing during recrystallization. The IR spectrum of compound **A** showed a sharp peak at $3400^{-1}$ cm $^{-1}$ (N-H) which signifies the presence of TZD and showed an absorption at $1678^{-1}$ $cm^{-1}$ indicating the presence of the olefin(C=C) group. The mass spectrum showed a pseudomolecular ion of [M-H] = 220 which is consistent with the target molecule.

### 3.1.2. Synthesis of Compound **B**

Compound **B** was obtained as a yellow powdery solid (76.2 mg, 61%) with a melting point of 200-203°C that is considerably lower than compound **A** (278-282°C). This can be attributed to the less efficient packing of compound **B** due to size and free rotation on the $sp^3$ carbon and oxygen single bond ($C_{sp}3$-O).

Compound **B** was obtained TLC pure with an $R_f$ value of 0.76 (EtOAc/hexane). Compound **A** starting material did not travel the TLC plate using this same solvent system indicating that the product is much more nonpolar. This is expected due to the addition of the nonpolar xylyl group.

The IR spectrum does not show C-Br absorption confirming the complete reaction of the dibromide.

### 3.1.3. Synthesis of Compound C

Compound **C** was obtained as a yellow powdery solid with a melting point of 167-171°C

which is considerably lower than Compound A (278-282°C). This can be attributed to the less efficient packing of Compound C due to size and free rotation on the $sp^3$ carbon and oxygen single bond ($C_{sp}3$-O).

Compound **C** was obtained TLC pure with an $R_f$ value of 0.8542 (EtoAc/DCM). Compound A starting material did not travel the TLC plate using this same solvent system indicating that the product is much more nonpolar. This is expected due to the addition of the nonpolar propyl group.

The IR spectrum did not show C-Br absorption in confirming the complete reaction of the dibromide.

### 3.2. Method 2

Precursor compounds **D** and **E** were first prepared via Sn2 reaction of 2 equivalents para-hydroxybenzaldehyde with 1 equivalent of xylyl and propyl linkers respectively. Precursor compounds **D** and **E** were further reacted via Knoevenagel condensation reaction with TZD to synthesize compounds **B** and **C** respectively.

#### 3.2.1. Synthesis of Compound B using Method 2

The final product from method 2 was obtained as a yellow powdery solid (64.7 mg) with a melting point of 159-161°C. The observed melting point is lower than the product from method 1 indicating that the obtained product is *not* identical with compound **B**. In addition, the compound has a very low solubility in all solvents tried (hexane, DCM, EtOAc, Acetone, Ethanol).

The IR spectrum of the product from method 2 showed the characteristic absorptions of TZD. However, it also showed the absorption at 1693 $cm^{-1}$ which can be attributed to the carboxyl of an aldehyde. This is consistent with the aldehyde absorption of Compound **D** which is the starting material in the synthesis. These observations are in agreement with the proposed structure for compound G (Scheme 3).

Compound **G** was probably formed from the reaction of compound **D** with only one equivalent of TZD. Since the addition of a unit of TZD affects the solubility of the resulting compound, it may have precipitated and gone out of the solution before the attachment of the second TZD. Compound G was obtained in 98% yield.

#### 3.2.2. Synthesis of Compound C using Method 2

The final product from method 2 was obtained as a yellow powdery solid (10.9 mg) with a melting point of 206-207°C. The observed melting point is higher than compound **C** from method 1 and has low solubility in various solvents such as DCM, ethyl acetate, acetone, acetonitrile and ethanol. This indicates that the obtained product is not identical with compound **C**. The IR spectrum showed absorption at 1700 $cm^{-1}$ indicating the presence of the aldehyde functionality. A possible structure for the compound produced using this method is compound **H** (Scheme 3). However, the observed melting point seems inconsistent if this is the case because the addition of only one TZD should result to a lower melting point compared to **C.** Additional information should be gathered to determine the structure of this product.

### 3.3. Determination of cell viability and cytotoxicity on normal and cancer cell lines

TZD derivatives **A**, **B** and **C** were subjected to cytotoxicity test against HDFn (human dermal fibroblast), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell) using $IC_{50}$ PrestoBlue™ Assay. The activity is compared with Bleomycin which is a known drug for cancer. Data shows that the three compounds have no significant activity against normal cells unlike the standard drug (Bleomycin) with an $IC_{50}$ 9.78 μg/mL.

The three compounds (**A,B,C**) showed activity against MCF-7 (breast cancer) with an $IC_{50}$ (μg/mL) of 3.97, 39.2, 40 for A, B, and C respectively. It is worth noting that compound A exhibited the lowest concentration needed to inhibit cell proliferation of breast cancer cell, lower than the standard $IC_{50}$ of Bleomycin.

Compound **A**, **B**, & **C** showed activity against HT-29 (colon cancer cells) with an $IC_{50}$ (μg/mL) of 14.29, 0.15, 13.528 respectively. Compound **B** is the most active showing an inhibitory concentration lower than the standard with an $IC_{50}$ of 11.07 μg/mL.

From the information above, it can be said that compound **A** is selective in the inhibition of breast cancer cells while compound **B** is selective in the inhibition of colon cancer cells. Since both compounds are inactive against a normal cell then they are potential drugs for the treatment of the two diseases.

## 4. CONCLUSIONS

Two new bi*s*-1,3-thiazolidine-2,4-dione (TZD) derivatives (**B** and **C**) were synthesized. Compound **B** has a *meta* xylyl linker and obtained as yellow powder in 61% Yield. Compound **C** has a

propyl linker and obtained as yellow powder and obtained in 21% yield.

Two approaches were carried out in the synthesis. The more efficient method involved the preparation of Compound **A** (44%) via Knoevenagel Condensation reaction between *para*-hydroxybenzaldehyde and TZD. Reaction of compound **A** with *meta*-xylyldibromide and 1,3-dibromopropane yielded the target compounds **B** and **C** respectively. The second method required the preparation of compounds **D** (43%) and **E** (65%) from the Sn2 reaction of *para*-hydroxybenzaldehyde and *meta*-xylyldibromide or 1,3-dibromopropane. The target compounds **B** and **C** however were not obtained using this method. The synthesis of **B** from **D** however generated the TZD derivative **G** (98%).

Cytotoxicity test carried out on TZD derivatives **A**, **B** and **C** against HDFn (human dermal fibroblast), HT-29 (colon cancer cell) and MCF-7 (breast cancer cell) using PrestoBlue Assay showed that compound **A** is selective in the inhibition of breast cancer cells while compound **B** is selective in the inhibition of colon cancer cells. Since both compounds **A** and **B** were inactive against normal cells, then both are potential drugs for the treatment of the two diseases.

## 5. ACKNOWLEDGMENTS

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## 6. APPENDIX A.

Supplementary data for this article is available upon request.

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