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Synthesis and Characterization of Bis-thiazolidine-2,4-dione with Non-polar Linkers

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Abstract: Bis-thiazolidine-2,4-dione derivatives with propyl or *m*-xylyl non-polar linkers were synthesized. These derivatives were prepared with the intention to produce a more enhanced set of potential drugs in the treatment of cancer with reduced side effects. The synthesis of the two target TZD derivatives involved two preparatory steps. Each of the non-polar linkers, 1,3-dibromopropane and α, α'-dibromo-*m*-xylene, underwent nucleophilic substitution with two *p*-hydroxybenzaldehyde units and formed the precursor compounds **3a** (50.17%) and **3b** (97.35%). The precursors were then reacted with two TZD units via Knoevenagel condensation yielding 38.20% of the target product **4a** with **Y** and 58.27% of **4b** with **Z**. The techniques utilized in the characterization and structural confirmation of all the compounds mentioned involve Fourier-Transform Infrared Spectroscopy, Mass Spectrometry, and/or Nuclear Magnetic Resonance Spectrometry.

Key Words: Thiazolidine-2,4-dione; *p*-hydroxybenzaldehyde; Knoevenagel condensation

1. INTRODUCTION

World Health Organization (WHO) has deemed non-communicable diseases such as heart disease, stroke, cancer, chronic respiratory diseases, and diabetes as the cause of 40 million out of all the 56 million deaths. Out of those diseases mentioned, cardiovascular diseases were considered to be the leading cause of these deaths; however, a considerable number of deaths also come from cancer and diabetes (World Health Organization, 2017). Even with the current existence of multiple cancer drugs, the demand for the research of new drugs is still very high because of the known disadvantages of chemotherapeutic treatments and the persisting high mortality rate. In addition, not all existing drugs are compatible and effective with all kinds of cancer.

Thiazolidine-2,4-dione (TZD) or glitazone is a five-membered heterocyclic ring that has been one of the most studied compounds since the 90's due to its bioactivity (Mendgen, Steuer & Klein, 2012); they are a class of anti-diabetic agents that were initially discovered due to its ability to improve insulin action and sensitivity (Kahn, Chen & Cohen, n.d.). TZDs and some of its derivatives were found to be an inhibitory agent and a potential cure to some of the diseases such as diabetes mellitus (Type 2 Diabetes), several kinds of cancer (lung, breast, and colon cancers), neurodegenerative diseases, hypertension, and endothelial dysfunction (Dawood & Abu-Deif, 2017).

The biological activity of TZD is most probably caused by the presence of multiple functional groups, particularly two ketones and an amine. These functional groups target several proteins that are associated with certain conditions (Chadha, Bahia, Kaur & Silakari, 2015). Many researches were able to demonstrate the increased

biological activity of molecules with a TZD moiety (Jain, Vora & Ramaa, 2013). The compound *p*-hydroxybenzaldehyde (PHB) is commercially used by many industries and its production is constantly being optimized (Zhang, Liu, Li & Li, 2011). It is also commonly used to form the central aryl ring of various bioactive TZD derivatives (Laxmi et al., 2016). It will be used in the first preparation step of the target compound because of the reliability and effectiveness of the method involved.

The main aim of this research is to prepare two new derivatives of TZD in order to generate new anti-carcinogens, which have the potential to become drugs with higher biological activity and decreased side effects. The target derivatives have two TZD units with varying sizes of the non-polar linkers, specifically using propyl and *m*-xylyl components. The presence of a lipophilic linker may enable the molecule to have higher cell permeability (Bartolami, Bouillon, Dumy & Ulrich, 2016).

2. METHODOLOGY

2.1 Materials, Reagents, and Apparatus

All of the chemicals used in the synthesis of thiazolidine-2,4-dione derivatives were of analytical grade and were either purchased from Sigma-Aldrich Chemicals or from Univar. The melting points were determined using Fischer-Johns Mel-Temp apparatus. FTIR spectra were acquired using 550 Nicolet Magna IR spectrometer. MS spectra were obtained using Bruker Mass Spectrometer with Electron Spray Ionization. The H-NMR spectra were obtained using 300 MHz Bruker, 500MHz Bruker Avance III, and 400 MHz Varian Mercury AS-400 NMR Spectrometer models. Thin layer chromatography (TLC) experiments were carried out using silica gel plates (Fluka) and the spots were visualized using ultraviolet light or iodine (I_2).

*2.2 General Procedures for the Synthesis of Bis-O linked PHBs: Compound **3a-3b***

Initially, 1,3-dibromopropane/ α, α'-dibromo-*m*-xylene and distilled acetone were added to a two-necked heart-shaped flask (100 mL) containing *p*-hydroxybenzaldehyde, potassium carbonate, and a hexagonal magnetic stirring bar. The flask was sealed with a rubber septum and was purged with nitrogen gas. The mixture was stirred and heated using an oil bath at approximately 52-54ºC for 4-5 hours. The progress of reaction was monitored by taking the TLC profile of the reaction mixture every hour.

After 4-5 hours, the flask was removed from the oil bath and allowed to cool to room temperature. The crude product was quenched with distilled water (10 mL) and was transferred to a separatory funnel. The organic layer was extracted with DCM (10 mL, 3 times) and dried with anhydrous sodium sulfate. The solvent was removed in *vacuo* and the crude product was purified by column chromatography using 100% dichloromethane as mobile phase.

2.2.1 Synthesis of 4,4'-(propane-1,3-diylbis(oxy))dibenzaldehyde (3a)

The amounts used in the reaction were: K_2CO_3 (471.7 mg, 3.4 mmol), *p*-hydroxybenzaldyde (304.6 mg, 2.5 mmol), 1,3-dibromopropane (136.6 mg, 0.7 mmol), and acetone (5 mL). White crystalline solid (0.0965 g, 50.17%); **M.P.** 120 ºC; **Rf**= 0.30-0.34 (DCM); **IR** (KBr, cm^{-1}) 2954.86 – 2842.47 (C-H), 1247.97 – 1049.09 (C-O), 1697.31 (C=O), 1601.24 (C=C); **MS** [M+Na^+] 307 [M+K^+] 323; **^{1}H NMR** (300 MHz, DMSO-d*6*) showed a spectrum containing these peaks: δ 9.86 (s, 2H), 7.15 (d, *J* = 8.4 Hz, 4H), 7.86 (d, *J* = 8.7 Hz, 4H), 4.27 (t, *J* = 6.0, 6.6 Hz, 4H), 2.26 (p, *J* = 5.7, 6.9, 8.1 Hz, 2H).

2.2.2 Synthesis of 4,4'-((1,3-phenylenebis(methylene))bis(oxy))dibenzaldehyde (3b)

The amounts used in the reaction were: K_2CO_3 (1215 mg, 8.8 mmol), *p*-hydroxybenzaldyde (769.9 mg, 6.5 mmol), α, α'-dibromo-*m*-xylene (575.2 mg, 2.2 mmol), and acetone (20 mL). White solid (0.7348 g, 97.35%); **M.P.** 80-84 ºC; **Rf**= 0.43-46 (DCM); **IR** (KBr, $cm^{-1)}$ 2927.18 (C-H), 1251.53 – 1161.60 (C-O), 1693.37 (C=O), 1600.38 (C=C); **MS** [M+Na^+] 369; **^{1}H NMR** (400 MHz, Chloroform-d) δ 9.87 (s, 2H), 7.83 (d, J = 8.0 Hz, 4H), 7.07 (d, J = 8.0 Hz, 4H), 7.51 (s, 1H), 7.45 (m, 4H), 5.16 (s, 3H).

*2.3 General Procedures for the Synthesis of Bis-O linked TZD Derivatived: Compound **4a-4b***

Compound **3a/3b**, TZD, and urea were placed in a round bottom flask with a hexagonal magnetic stirrer. The solid mixture was stirred under nitrogen and heated at 110°C. After 30 minutes, it was cooled to room temperature. The crude product was filtered under vacuum and washed with water, cold ethyl acetate, and cold DCM. The product was recrystallized using DMF/ethanol. It was washed with cold ethanol and filtered.

2.3.1 Synthesis of (5Z,5'Z)-5,5'-(((propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(methanylylidene))bis(thiazolidine-2,4-dione) (4a)

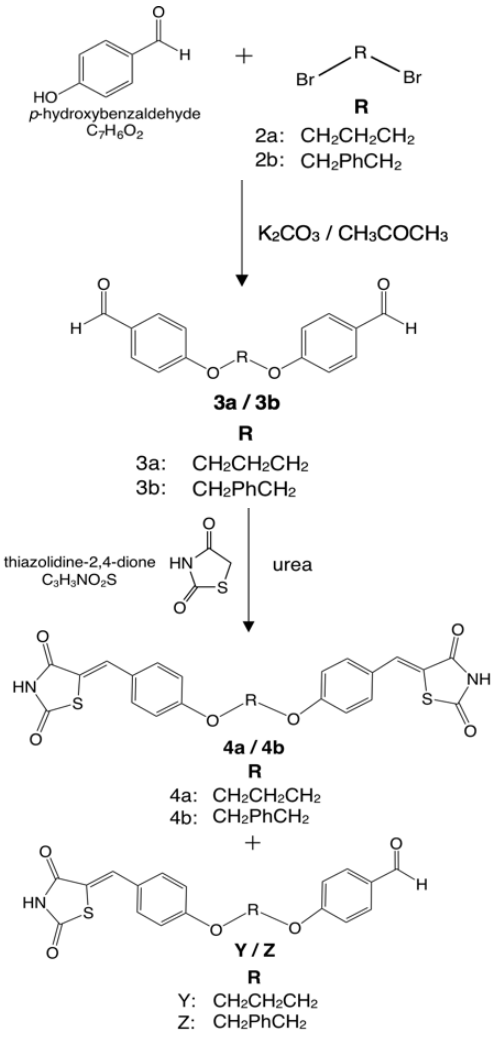
The amounts used in the reaction were: Compound **3a** (248.0 mg, 8.72 mmol), TZD (237.8 mg, 2.03 mmol), urea (34.9 mg, 0.581 mmol). Light yellow colored product (0.1843 g, 38.20%); **M.P.** 296->300 ºC; **Rf**= 0.61 and 0.50 (1:1 Ehtyl Acetate: Hexane); **IR** (KBr, cm^{-1}) 3389.90 (N-H), 3125.36(Alkene C-H), 2951.54 (Aliphatic C-H), 1700.22 (C=O), 1600.00 (C=C), 1252.81 – 1160.75 (C-O); **MS** [M+Na^+] 505 (**4a**) [M+Na^+] 406 (**Y**); **^{1}H NMR** (400 MHz, DMSO-d6) δ 12.51 (s, 1H), 9.84 (s, 1H), 7.84 (d, *J* = 8.0 Hz, 2H), 7.73 (s, 1H), 7.53 (d, J= 8.0 Hz, 2H), 7.12 (m, 4H), 4.22 (m, 4H), 2.21 (p, *J* = 8.0, 4.0 Hz, 2H).

2.3.2 Synthesis of (5Z,5'Z)-5,5'-((((1,3-phenylenebis(methylene))bis(oxy))bis(4,1phenylene))bis(methanylylidene))bis(thiazolidine-2,4-dione) (4b)

The amounts used in the reaction were: Compound **3b** (348.4 mg, 1.01 mmol), TZD (273.1 mg, 2.332 mmol), urea (41.0 mg, 0.682 mmol). Yellowish solid powder (0.3192 g, 58.27%); **M.P.** 280-284 ºC; **Rf**= 0.60 (1:1 Ethyl Acetate: Hexane); **IR** (KBr, cm^{-1}) 3181.81-3042.79 (Aromatic C-H), 1257.94 – 1179.26 (C-O); 1680.88 (C=O); 1509.42-1383.99 (C=C), 1595.77 (N-H bending), 3417.12 (N-H); **^{1}H NMR** (400 MHz, DMSO-d6) δ 12.51 (s, 3H), 9.84 (s, 1H), 7.85 (d, *J* = 12.0 Hz, 2H), 7.72 (d, 3H), 7.53 (m, 8H), 7.42 (d, *J* = 4.0 Hz, 6H), 7.19 (s, 2H), 7.15 (m, 2H), 5.21 (d, *J* = 16.0 Hz, 8H).

3. RESULTS AND DISCUSSION

The target of this research was to synthesize two new thiazolidinedione derivatives using two non-polar linkers. The linkage formed between the two non-polar linkers with two units of *p*-hydroxybenzaldehyde each will eventually serve as the lipophilic area of the target compounds, which may contribute significantly to the cell permeability of the possible drug.



Scheme 1. Overall Scheme for the synthesis of Bis-thiazolidine-2,4-dione derivatives with propyl (4a) or *m*-xylyl non-polar (4b) linkers

Two precursor products were formed under 1,3-dibromopropane, with the amount of

intermediate **X** being less than **3a**. In the second preparation step, an additional product (**Y** or **Z**) was formed under each precursor with the respective proposed structures.

*3.1 Synthesis of Bis-O linked PHBs: Compound **3a-3b***

The preparation step involves the bimolecular nucleophilic substitution (S_N2) of *p*-hydroxybenzaldehyde (PHB) with 1,3-dibromopropane and α, α'-dibromo-*m*-xylene to make precursors 3a and 3b, which l contain two PHB units bound by propyl and *m*-xylyl linkers respectively.

3.1.1 Synthesis of 4,4'-(propane-1,3-diylbis(oxy))dibenzaldehyde (3a)

Compound **3a** was obtained with a moderate yield of 50%. Possible reasons for not obtaining a higher yield may include the weak reactivity of the intermediate compound, the obstruction of other parameters that were not explored, and the losses from its purification using silica through column chromatography. The IR spectrum shows the presence of a strong peak at 2954.86 cm^{-1}, which firmly supports the production of the target compound containing the non-polar propyl linker. The strong peak at 1247.97 cm^{-1} signifies the resulting O-linkage from the reaction.

The relative molecular mass of the precursor compound given by the pseudomolecular ion peak [M+Na^+] at m/z at 307.11552 also correlates with its expected weight. The ^{1}H NMR spectrum supports the structure of **3a** since it was able to show that PHB was O-linked to the propyl linker at both sides.

3.1.2 Synthesis of 4,4'-((1,3-phenylenebis(methylene))bis(oxy))dibenzaldehyde (3b)

In the synthesis of compound **3b**, the yields from various trials ranged from 94% to 97% and with the highest yield obtained on the final trial. The remaining percentage representing the few that did not react may stand for the presence of an intermediate from the incomplete formation of the target precursor, which was not mentioned or characterized due to its negligible amount. The high reactivity of the *m*-xylyl linker may have contributed greatly to the success of this reaction.

Similar to **3a**, the IR spectrum of the compound lacks a peak below 667 cm^{-1}, which signifies the loss of the Br attached to the reactant. The mass spectrum shows the pseudomolecular ion peak at [M+Na^+] = 369 which is consistent with the molecular weight of the target precursor. The ^{1}H-NMR spectrum shows that PHB is O-linked to *m*-xylyl at both sides.

*3.2 Synthesis of Bis-O linked TZD Derivatived: Compound **4a-4b***

This step involves a Knoevenagel condensation reaction catalyzed by urea in order to attach two thiazolidine-2,4-dione (TZD) components to each of the starting materials **3a** and **3b** in the synthesis of the the final products, **4a** and **4b**, under solvent-free conditions (Shah & Singh, 2012).

3.2.1 Synthesis of (5Z,5'Z)-5,5'-(((propane-1,3-diylbis(oxy))bis(4,1-phenylene))bis(methanylylidene))bis(thiazolidine-2,4-dione) (4a)

The TLC pure product was obtained in 38.20% yield. Recrystallization in different solvents (ethanol, ethyl acetate, DMF) was utilized in the purification of the target product. The low % yield may be attributed to the losses in the multiple recrystallization process.

The IR spectrum shows a singular stretch at 3389.90 cm^{-1} – confirming the binding of the TZD unit. The peak at 1738.94 cm^{-1} may represent the aldehyde carbonyl indicating the presence of a product with only one TZD group attached. This is consistent with the observed base peak [M+Na^+] at 407 confirming the presence of product **Y.** The presence of the pseudomolecular ion peak of [M+Na^+] at 505 on the other hand was consistent with the target precursor.

The ^{1}H-NMR spectrum supports the formation of compound **Y** as shown in the presence of a singlet. The peaks at 9.84 ppm and at 12.51 ppm shown in the ^{1}H-NMR spectrum indicate the aldehyde proton and N-H proton of TZD peaks respectively.

2.3.2 Synthesis of (5Z,5'Z)-5,5'-((((1,3-phenylenebis(methylene))bis(oxy))bis(4,1phenyl

ene))bis(methanylylidene))bis(thiazolidine-2,4-dione) (4b)

The synthesis of compound **4b** resulted to a yield of 58%. The remaining percentage may have been due to an incomplete reaction or from losses incurred during product transfer. With that said, the product was expected to form purely due to the high reactivity of the aromatic nonpolar linker.

Based on the IR spectrum, the single extended peak at 3417.12 cm^{-1} indicates the presence of the secondary N-H group of TZD. Product **4b** was also obtained impure, so the peak at 1736.87 cm^{-1} may represent the aldehyde carbonyl of the compound with only one TZD group attached. Based on the ^{1}H-NMR spectrum, the product resulted to be a mixture containing the intermediate **Z** with only one TZD group linked and compound **4b** as shown in the presence of a singlet at 9.84 ppm corresponding to a single aldehyde proton.

4. CONCLUSIONS

This study was able to provide a working method that led to the synthesis of two new 1,3-thiazolidine-2,4-dione derivatives bound by non-polar linkers. The beginning precursors, **3a** and **3b**, were obtained in 50.17% and 97.35% yield respectively. The target products **4a** and **4b** were obtained with their respective yields of 38.20% and 58.27%. After the establishment of the results for each reaction, characterization was accomplished; all the acquired data have supported the structures provided. Both proposed compounds have straightforward syntheses and have simpler structures that made it good candidates for further testing as potential drugs.

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6. REFERENCES

Bartolami, E., Bouillon, C., Dumy, P., & Ulrich, S. (2016). Bioactive clusters promoting cell penetration and nucleic acid complexation for drug and gene delivery applications: From designed to self-assembled and responsive systems. *Chem. Commun.*, *52*(23), 4257–4273. doi:10.1039/c5cc09715k

Chadha, N., Bahia, M. S., Kaur, M., & Silakari, O. (2016). Thiazolidine-2, 4-dione derivatives: Programmed chemical weapons for key protein targets of various pathological conditions. *Bioorganic & Medicinal Chemistry*, *23*(13), 2953–2974. doi:10.1016/j.bmc.2015.03.071

Dawood, K., & Abu-Deif, H. (n.d.). Chemical and Pharmaceutical BulletinVol. 62 (2014) No. 5 p. 439- 445. Retrieved August 20, 2016, f rom http://doi.org/10.1248/cpb.c14-00031

Jain, V. S., Vora, D. K., & Ramaa, C. S. (2013). Thiazolidine-2, 4-diones: Progress towards multifarious applications. *Bioorganic & medicinal chemistry*,*21*(7), 1599- 1620. doi:10.1016/j.bmc.2013.01.029

Kahn, R., Chen, L., & Cohen, S. (n.d.). JCI - Unraveling the mechanism of action of thiazolidinediones. Retrieved August 20, 2016, http://dx.doi.org/10.1172/JCI11705

Laxmi, S., Anil, P., Rajitha, G., Rao, A., Crooks, P., & Rajitha, B. (2016). Synthesis of thiazolidine-2,4-dione derivatives: anticancer, antimicrobial and DNA cleavage studies. *Journal Of Chemical Biology*, *9*(4), 97-106. http://dx.doi.org/10.1007/s12154- 016-0154-8

Lipinski, C., Lombardo, F., Dominy, B., & Feeney, P. (2001). Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings1PII of original article: S0169- 409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3–25.1. Advanced Drug Delivery Reviews, 46(1-3), 3-26. http://dx.doi.org/10.1016/s0169-409x(00)00129-0

Mendgen, T., Steuer, C., & Klein, C. D. (2012). Privileged Scaffolds or promiscuous binders: A comparative study on Rhodanines and related Heterocycles in medicinal chemistry. *Journal of Medicinal Chemistry*, *55*(2), 743–753. doi:10.1021/jm201243p

Pavia, D., Lampman, G., Kriz, G., & Vyvyan, J. (2009). Introduction to spectroscopy (4th ed., p. 29). Stanford, CT: Cengage Learning.

Shah, S., & Singh, B. (2012). *Urea/thiourea catalyzed, solvent-free synthesis of 5-arylidenethiazolidine-2,4-diones and 5-arylidene-2-thioxothiazolidin-4-ones*. Retrieved December 2017,from http://dx.doi.org/10.1016/j.bmcl.2012.07.049

World Health Organization Home Page. Retrieved December 20, 2017 from http://www.who.int/gho/ncd/en/

Zhang, Q., Liu, Y., Li, G., & Li, J. (2011). Preparation of p-hydroxybenzaldehyde by hydrolysis of Diazonium salts using rotating packed bed. *Chinese Journal of Chemical Engineering*, *19*(1), 140–144. doi:10.1016/s1004-9541(09)60190-7