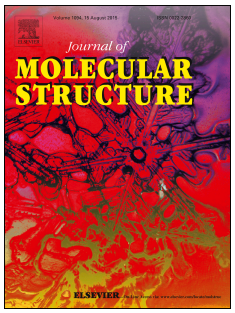

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PII: S0022-2860(19)31632-1

DOI: https://doi.org/10.1016/j.molstruc.2019.127523

Reference: MOLSTR 127523

To appear in: *Journal of Molecular Structure*

Received Date: 22 August 2019

Revised Date: 18 November 2019

Accepted Date: 2 December 2019

Please cite this article as: Ö. Gunduğdu, S.A.A. Noma, T. Taskin-Tok, B. Ateş, N. Kishali, Evaluation of xanthine oxidase inhibitor properties on isoindoline-1,3-dion derivatives and calculation of interaction mechanism, *Journal of Molecular Structure* (2020), doi: https://doi.org/10.1016/j.molstruc.2019.127523.

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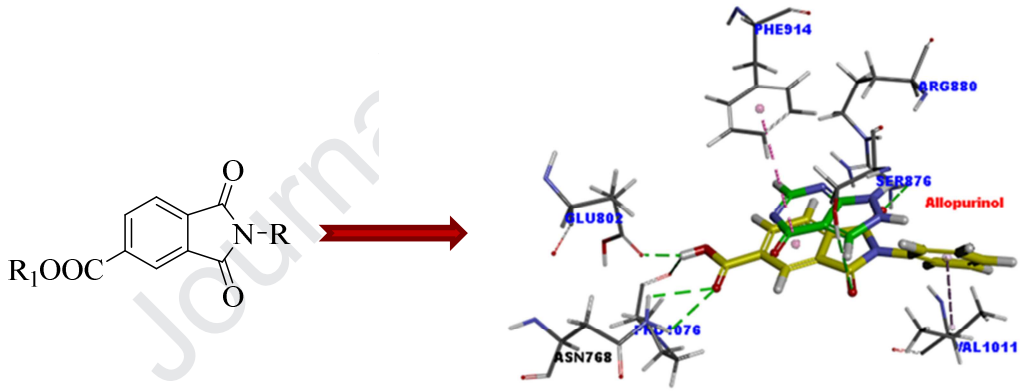

Evaluation of Xanthine Oxidase Inhibitor Properties on isoindoline-1,3-dion derivatives and Calculation of Interaction Mechanism

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Abstract

The aim of the present study was to synthesis of isoindole-1,3(2*H*)-dione (Phthalimides) derivatives and to investigation the inhibition of xanthine oxidase (XO). In study, xanthine oxidase inhibitory activities of complexes were observed in the range from 7.15 to 22.56 μM for isoindole-1,3-dione (**2a-c** and **3a-c**). N-phenyl isoindole-1,3-dione derivatives (**2c, 3c**) showed better activity (almost two times) than the other two derivatives (N-methyl (**2a, 3a**), N-ethyl (**2b, 3b**). It means that phenyl ring (R) remarkably enhances the xanthine oxidase inhibitory effect of complexes. In the meantime, molecular docking studies of these compounds against XO were also investigated by providing the inhibitory efficiency and estimating the interaction mechanisms of isoindol-1,3-dion derivatives with XO.

Keywords: Isoindoline-1,3-dione, Phthalimide, Xanthine Oxidase, Molecular Docking, Biological Activity

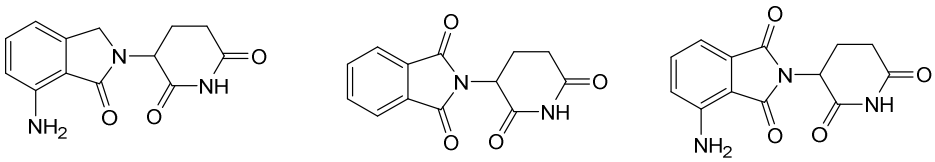
1. Introduction

Phthalimides are systematically known as isoindole derivatives. Phthalimides are molecules of interest in organic and medicinal chemistry due to their biological activity. They are building blocks of some biologically active compounds. Interest in the synthesis of isoindoline-1,3-dione derivatives is intensely increased due to its recently discovered action against inflammatory diseases and cancer [1-4].

Researchers have generally investigated the activity of isoindole in two different classes. The first group: Derivatives via N-atom, The second group: Derivatives via isoindole-aromatic ring. Activity studies on N-methyl and ethyl isoindole derivatives have been performed and efficient results have been obtained [5-11]. In this study, we tried to investigate the XO enzyme inhibition effect by synthesizing an aromatic N-derivative (phenyl) to compare with aliphatic –N derivatives. In addition, several groups have also studied various activity studies of many derivatives in the aromatic ring in N-substituted isoindole compounds [12-13]. In this study, derivatives with high H-binding capacity (acid group) and lower hydrogen bonding capacity (ester group) with enzyme were synthesized and XO enzyme activity was investigated. For XO enzyme inhibition, the presence of groups capable of H-bonds (weak intermolecular bond generally formed between oxygen or nitrogen heteroatoms and protons) is preferred. As is known, non-covalent interactions between inhibitors and enzymes include hydrogen bonds, hydrophobic interactions, and ionic bonds. Many of these weak bonds bind to produce strong and specific binding between enzyme and the inhibitor. Enzymes prefer hydrogen bonds to make the transition state more stable. Thus, isoindole derivatives (2a-c, 3a-c) were preferred since they contain both the N atom and the carboxyl group [14].

The biological activities of a great deal of isoindole-1,3-dione derivatives have been studied wide range of such as anti-inflammatory, anti-convulsant, anticancer activity, and anti-microbial activity, antioxidant, and analgesic activities. Furthermore, different modifications of the phthalimide structure have been found to affect biological activity [15-19]. These effect is seen in drugs such as Lenalidomide (Revlimid ™), Thalidomide and Pomalidomide (Actimid ™) [20-21].

Also, 2-(3-diethyl aminoalkyl)-isoindoline-1,3-dione derivatives could be described as selective acetylcholinesterase inhibitors by Malawska et. all. They suggested that these compounds could be used as a potential drug to treat Alzheimer's disease [22].



Scheme 1. Some drugs of the isoindole-1,3-dione derivative.

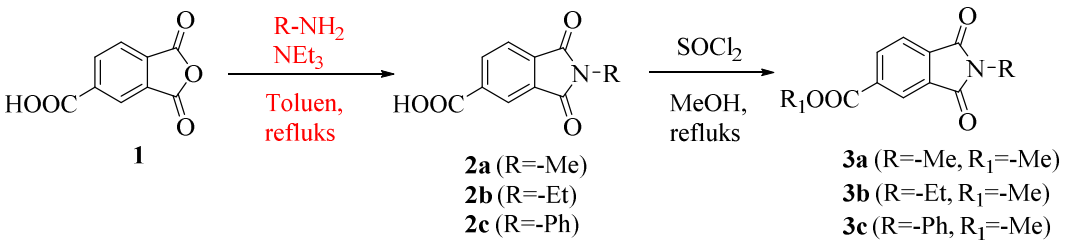
Recently, we synthesized isoindole-1,3-dione amine derivatives via 1,2-addition reaction of amines to α, β-unsaturated double bond in cyclohexane system and investigated their fluorescence properties. [23].

In this paper, we describe the synthesis a series of N-substituted isoindoline-1,3-dione derivatives and their inhibitory activity on xanthine oxidase. Furthermore, the docking calculations were exerted to define docking interactions of isoindole-1,3-dione derivatives-xanthine oxidase (XO) complexes.

2. Results and discussion

2.1. Chemistry

The chemical structure of isoindole-1,3-dione have hydrophobic character. This structure increases their potential to cross different biological membranes in *in vivo* [24]. Similarly, enzyme inhibition is indicative of biological activity. For this reason, derivatives of isoindole-1,3-dione were synthesized to examine XO inhibition activity. Isoindoline-1,3-dione derivatives were synthesized by starting from 1,3-dioxo-1,3-dihydro-isobenzofuran-5-carboxylic acid (**1**). For this purpose, corresponding amide derivatives were obtained by reacting 1,3-dioxo-1,3-dihydro-isobenzofuran-5-carboxylic acid (**1**) with methyl, ethyl, phenyl amine in the presence of NEt_3 in catalytic amounts (Scheme 1). Thus, 5-hydroxy-N-(methyl / ethyl / phenyl) isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**) were synthesized and then converted to the methyl ester derivative methyl 2-(R)-1,3-dioxoisoindoline-5-carboxylate (**3a**, **3b**, **3c**)[25].



Scheme 2. Isoindoline-1,3-dione derivatives synthesis.

2.2. Biological activity

Xanthine oxidase is known to play an important role in the synthesis of uric acid. XO is widely distributed throughout various organs including the liver, gut, lung, kidney, heart, brain and plasma. High levels of xanthine oxidase in some tissues have been associated with tissue damage and serious illnesses due to the relationship with production cytotoxic oxygen metabolites (H_2O_2, O_2^- and OH^-) cause oxidative damage on the cells [26]. Allopurinol, a purine derivative with the aim of inhibiting XO, has also been used in therapy for many years. However, it is not desirable that allopurinol may be converted to 4,6-dihydroxy pyrazole (3,4-δ) pyrimidine, which is high in toxicity level and is not as effective as itself in vivo. Moreover, allopurinol has to compete with xanthine in order to be able to inhibit the enzyme xanthine oxidase, depending on its chemical structure, and thus the yield decreases [27]. In addition, several studies have shown that hypersensitivity reactions of allopurinol and inducing side effects of Stevens-Johnson syndrome are present [28]. Therefore, there is a need for the synthesis of new non-purine compounds which are more efficient and have low side effects which will inhibit XO. For this reason, it has been decided to the investigated of XO activity of isoindole-1,3-dione derivatives.

2.2.1. Xanthine Oxidase Inhibition Properties of Complexes

The IC_{50} values for isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c**) and allopurinol as a positive control were given in Table 1. The isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c** derivatives showed a good inhibition activity toward against XO, the lowest IC_{50} ratio was for 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) compound as 7.15 μM and the highest one was 22.56 μM for 2-methyl-1,3-dioxoisoindoline-5-carboxylic acid (**2a**), and for allopurinol positive control was 6.34 μM. All the isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c**) derivatives showed inhibition of XO enzyme by decreasing the uric acid formation. Zafar H., et al prepared isonicotinohydrazides compounds and investigated their inhibitory effect on XO enzyme [29]. They found the range of IC_{50} value from 0.9 ± 0.01 μM to 330.4 ± 2.1 μM. The inhibition activities of benzimidazole derivatives against XO enzyme were determined in the range from 4.3 μM to 9.7 μM [30].

Ting-jian Z. *et al.* synthesized N-(4-alkoxy-3-cyanophenyl)isonicotinamide/nicotinamide derivatives as novel xanthine oxidase inhibitors, and the IC50 value range was from 0.3 ± 0.03 μM to 19.2 ± 1.2 μM [31]. Aktas et al. synthesized 2-hydroxyethyl substituted N-heterocyclic carbine to investigate the enzymes inhibition effect and the IC_{50} value range for

XO was found in between 1.253 ± 0.084 μM and 4.992 ± 0.113 μM [32]. In other study by Burmaoglu et al. [33], they tested novel bis-chalcone derivatives to determine their biological activity toward enzyme and cancer cells. They found IC_{50} for XO inhibition in the range from 0.728 ± 0.009 to 6.058 ± 0.095 μM. Kıbrız et al. [34] investigated the effect of pyrrole carboxamide rings on XO activity and it was reported that the IC_{50} values were of 4.608 - 7.084 μM.

Table 1. IC50 values of complexes on XO activities.

Compounds	IC_{50} μM	r^2
2a	22.56	0.9902
3a	21.19	0.9447
2b	20.788	0.9912
3b	15.794	0.976
2c	7.15	0.982
3c	10.94	0.9755
Allopurinol	6.34	0.986

2.2.2. Structure-activity relationship

In order to determine the relationship between the structures of the synthesized compounds and their XO enzyme inhibition, it would be rational to classify these compounds into two main groups: *i*) The substituted groups bonded to the nitrogen atom of the imide. There are three different groups bonded to the nitrogen atom. These are methyl, ethyl and phenyl groups. *ii*) The substituted groups bonded to aromatic ring. For this purpose, two derivatives were prepared for each group bonded to the nitrogen atom. These are 5-carboxylic acid (**2a-c**) and 5- methyl-carboxylate (**3a**, **3b**, **3c**) derivatives. In literature [35] observation revealed that substitution of phenyl ring R remarkably enhances the xanthine oxidase inhibitory effect, whereas the placement of phenyl ring with aliphatic groups diminishes this effect. Similar results were observed in this study; It was found that N-phenyl isoindole-1,3-dione derivatives (**2c**, **3c**) showed better activity than the other two derivatives (N-methyl (**2a**, **3a**), N-ethyl (**2b**, **3b**). In addition, it was observed that compounds (**2a**, **2b**, **2c**) with acid groups in the aromatic ring exhibited better activity than esters derivatives (**3a**, **3b**, **3c**). This circumstance indicates that the interaction between the enzyme and the isoindole derivative is more hydrophobic. As

the hydrophobic interactions in the ester derivatives are reduced according to the acid, it can be thought that there is a decrease in activity.

2.3. In silico details

In this study molecular docking applications were performed with DS 2018 SP1 to predict the interaction mechanisms of isoindol-1,3-dion derivatives with XO. Additionally, allopurinol compound for XO was used positive control in this research. Not only the docking results were evaluated based on docking score and binding energy values, but also compared with the selected positive controls.

2.3.1. Docking results between isoindol-1,3-dion derivatives and xantine oxidase (XO)

In this part, control compound, allopurinol was docked with XO. The results demonstrate that interaction mechanism and which type of non-bonding interactions involve in the binding site of the target protein, XO. In docking studies, the defined compounds, isoindol-1,3-dion derivatives were applied same process based on the positive control. Their binding affinity were likewise calculated and compared with allopurinol as XO inhibitor.

The positive control, allopurinol forms three hydrogen bonds with Ser876 (2.949 Å), Glu879 (2.224 Å) and Arg880 (2.706 Å) residues of the enzyme and five hydrophobic interactions with Phe914, Phe1009, Ala1078 and Ala1079 residues. The three dimensional (3D) orientation of the allopurinol and detail interaction types were given in Figure S7 and Table S7 of the supporting information part.

As known that selected compounds, isoindol-1,3-dion derivatives involve six structures were docked with XO. 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) which is one of the isoindol-1,3-dion derivatives, has six hydrogen bonds with Asn768, Ser876, Arg880, Glu802 and Pro1076 residues of the XO, (Figure 1**)**. Beside hydrogen bonds, there are two hydrophobic interactions with Phe914 and Val1011. Furthermore, the residue compounds (five structures) which display different non-bonding interactions with residues in the active site of the same enzyme. The docking calculations and views showed that a compounds form was more affinity than others that were given in Figure S8 and Table S8 of the supporting information part.

Especially, binding energy values of isoindol-1,3-dion derivatives are also reinforced the compound 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) (-16.1342 kcal/mol) is better interaction and affinity than others. Even this binding energy value of the 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) is exhibited better tendency than the positive control, allopurinol (-14.0382 kcal/mol) in Table 2. Moreover, orientations of each structure are very

important to do good interaction and show the biological functionality. Figure 2 shows the orientation of the ligands in XO as compared with the positive control. The results displayed that 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) has almost more similar orientation than other ones with positive control, allopurinol. Thus, the data obtained in the molecular docking calculations help to reveal the results of the XO inhibition analysis.

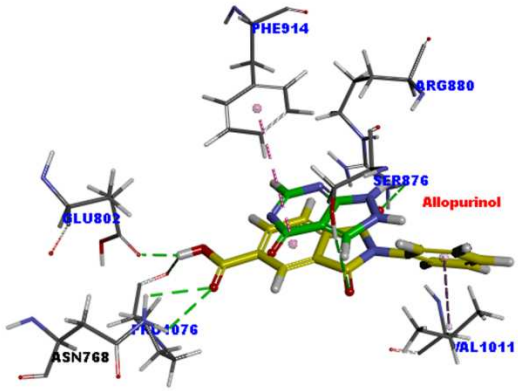


Figure 1. The best docking orientations of 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) (yellow and stick) and allopurinol (green and stick) in XO.

Table 2. The binding energy analysis of ligands and allopurinol* as positive control with that of XO.

Ligand Name-CDOCKER	Binding Energy (kcal/mol)	CDOCKER	CDOCKER intr	Ligand Energy (kcal/mol)	Protein Energy (kcal/mol)	Complex Energy (kcal/mol)	Entropic Energy (kcal/mol)
2a	-5.1495	-9.99882	-28.7439	-10.8867	-32,554.60	-32,570.70	18.6678
3a	-5.3659	-5.84289	-27.9546	6.9048	-32,554.60	-32,553.10	19.0259
2b	-6.5306	-9.05045	-27.0582	-2.3801	-32,554.60	-32,563.50	18.4715
3b	-10.8327	-6.82023	-25.0198	-8.8453	-32,554.60	-32,574.30	18.2815
2c	-16.1342	-10.8283	-30.1796	-17.5508	-32,554.60	-32,588.30	18.4968
3c	-11.0454	-3.98577	-27.0448	-1.5854	-32,554.60	-32,567.30	19.2069
Allopurinol*	-14.0382	-17.3939	-19.1738	41.7355	-57,006.30	-56,978.60	17.0079

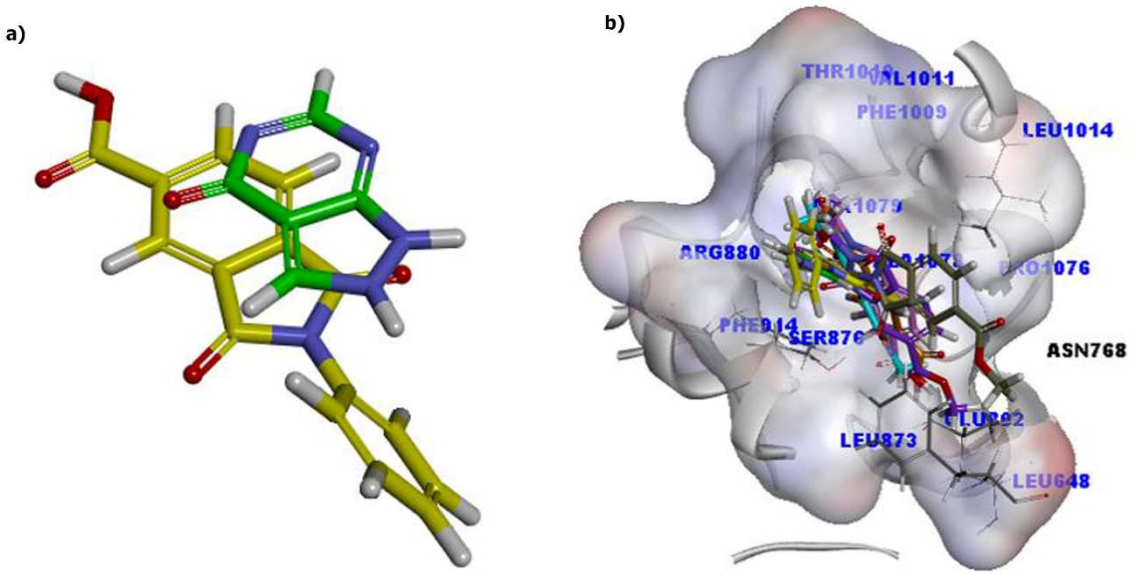


Figure 2. (a) The relative orientation of ligands, 1,3-dioxo-2-phenylisoindoline-5-carboxylic acid (**2c**) (yellow, stick) and allopurinol (green, stick) in XO. (b) A different view, superimposed form of the all ligands (**2a**, orange; **3a**, light blue; **2b**, light pink; **3b**, purple; **3c**, dark straw color, stick) and allopurinol (green, stick) in XO.

3. Experimental

3.1. General Procedure for 2

Onto the solutions of starting compound (1 equiv.) in toluene Et_3N (3 equiv.) was added at rt. Then R-NH_2 (1.2 equiv.) was added and refluxed for 24 hours. After that the reaction was cooled to room temperature. EtOAc (50mL) was added to the mixture and it was extracted with saturated NH_4Cl solution (3 x 50 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed in evaporator. The crude product was filtered from a small column with EtOAc / petroleum ether (60:40). The product was crystallized from EtOAc / petroleum ether (Yield: 90-95%) [25].

2a; M.p: 232-233 ^oC (lit: 240^oC) [36], IR (KBr): 3775, 3444, 3185, 1773, 1717, 1695, 1607, 1570, 1478, 1449, 1378, 1253, 1214, 1164. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.57 (s, 1H); 8.48 (dd, 1 H, *J*=7.7, 1.5 Hz); 7.97 (d, 1 H, *J*=7.7 Hz); 3.23 (s, 3 H). ^{13}C-NMR (100 MHz, CDC_{l3}) δ: 169.4 (2C); 167.5; 136.5; 136.2; 134.7; 132.8; 125.1; 123.6; 24.5. Anal. calc. for $C_{10}H_7NO_4$ (205.17): C, 58.54; H, 3.44; N, 6.83; found: C 58.20; H 3.66; N 6.41.

2b; M.p: 158-159 ^{o}C, IR (KBr): 3772, 3443, 2982, 2947, 1772, 1702, 1441, 1400, 1385, 1308, 1257, 1199, 1077. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.57 (s, 1H); 8.48 (dd, 1 H, *J*=7.7, 1.1 Hz), 7.97 (d, 1 H, *J*=7.7 Hz); 3.79 (q, 2 H, *J*=7.3 Hz); 1.30 (t, 3H, *J*= 7.3 Hz). ^{13}C-NMR (100 MHz, CDC_{l3}) δ: 169.8 (2C); 167.3; 136.6; 136.2; 134.7; 132.8; 125.1; 123.6; 33.6; 14.1. Anal. calc. for $C_{11}H_9NO_4$ (219.20): C, 60.28; H, 4.14; N, 6.39; found: C 60.62; H 4.70, N 6.48.

2c; M.p: 259-260 ^{o}C (lit: 257-259 ^{o}C [37]), IR (KBr): 3445, 3053, 1787, 1720, 1504, 1392, 1296, 1221, 1124, 1104, 1072. ^{1}H-NMR (400 MHz, CD_3OD) δ: 8.48 (m, 2H); 8.44 (m, 1 H); 7.64-7.33 (m, 5H).

^{13}C-NMR (100 MHz, CD_3OD) δ 166.7; 166.6; 166.3; 135.5; 135.0; 132.1; 131.9; 128.6; 128.4; 127.9; 126.68; 123.90; 123.24. Anal. calc. for $C_{15}H_9NO_4$ (267.24): C, 67.42; H, 3.39; N, 5.24; found: C 67.62; H 3.21; N 5.70.

3.2. General Procedure for 3

The starting compound (1 eq.) was dissolved in methanol and cooled to 0 °C. Then $SOCl_2$ (1.2 eq.) was added and refluxed for 18 hours. After that, the reaction mixture was cooled to room temperature and the solvent removed in evaporator. The solid was extracted by addition of EtOAc (2 x 20 mL) and saturated NH_4Cl solution (20 mL). The organic layer was dried over Na_2SO_4 and the solvent was removed in an evaporator. The crude product was purification with column chromatography with EtOAc / petroleum ether (30:70). The product was crystallized from EtOAc / petroleum ether (Yield: 90-95%) [25].

3a; M.p: 122-123 ^{o}C, IR (KBr): 3455, 3078, 2956, 1773, 1738, 1717, 1480, 1438, 1383, 1288, 1261, 1188, 1161. ^{1}H-NMR (400 MHz, $CDCl_3$)δ: 8.47, (dd, 1H, *J* = 1.5, 0.7 Hz); 8.39 (dd, 1H, *J* = 7.7, 1.5 Hz); 3.98 (s, 3H); 3.21 (s, 3H). ^{13}C-NMR (100 MHz, CDC_{l3}) δ: 167.7; 167.7; 165.5; 135.7; 135.6; 132.7; 129.1; 124.5; 123.5; 53,1; 24.5. Anal. calc. For $C_{11}H_9NO_4$ (219,20): C, 60.28; H, 4.14; N, 6.39; found: C 60.01; H 4.15; N 5.99.

3b; M.p: 96-97 ^{o}C, IR (KBr) δ: 3457, 3115, 2980, 2960, 1773, 1725, 1459, 1431, 1399, 1380, 1330, 1295, 1256, 1197, 1183. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.47 (s, 1H); 8.39 (d, 1 H, *J* =7.8 Hz); 7.90 (d, 1H, *J* = 7.7 Hz); 3.98 (s, 3H); 3.76 (q, 1 H, *J* =7.2 Hz); 1.28 (t, 3H, *J* = 7.2 Hz). ^{13}C-NMR (100 MHz, CDC_{l3}) δ: 167.3; 167.2; 165.3; 135.6; 135.5; 135.3; 132.5; 124.2; 123.2; 52.8; 33,2; 13.9 Anal. calc. For $C_{12}H_{11}NO_4$ (233,22): C, 61.80; H, 4.75; N, 6.01; found: C 62.22; H 4.21; N 6.31.

3c; M.p: 210-211 ^{o}C, IR (KBr): 3444, 2964, 1776, 1730, 1622, 1597, 1505, 1493, 1458, 1424, 1386, 1353, 1293, 1278, 1217, 1112, 1097. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 8.60 (d, 1H, *J* = 0.7 Hz); 8.50 (dd, 1H, *J* =7.7, 1.5 Hz); 8.04 (d, 1H, *J* = 7.7 Hz); 7.55-7.43 (m, 5 H); 4.01 (s, 3H); 1.55 (s, 3H). ^{13}C-NMR (100 MHz, CDC_{l3}) δ:166.7; 166.6; 166.2; 136.2; 136.0; 135.3; 132.5; 129.5; 128.6; 126.7; 125.1; 124.1; 123.2; 53.2. Anal. calc. For $C_{16}H_{11}NO_4$ (281,27); C, 68.33; H, 3.94; N, 4.98; found: C 68.62, H 3.81, N 4.75.

3.3. Xantine Oxidase (XO) Inhibition Assay

Bovine milk XO activity was determined spectrophotometrically by measuring uric acid formation at 295 nm at 37 °C, xanthine was used as substrate. For enzyme assay protocol 50 mM (pH=7.5) phosphate buffer, 1mM xanthine, and 0.2 U of XO enzyme were used. The inhibition of XO by isoindol-1,3-dion compounds derivatives were measured by the reduction of the uric acid concentration. Different concentrations of isoindol-1,3-dion derivatives were added for XO inhibition activity. The enzyme was pre-incubated for 10 min, with tested compounds, then the reaction was started by addition xanthine to reaction mixture. XO activity was determined by measuring the changes in absorbance of uric acid formation at 295 nm at 37 °C. The tested compounds were dissolved in DMSO, then diluted with phosphate buffer 50 mM (pH=7.5), the final concentration of DMSO in the reaction mixture was less than (0.01% v/v) and this ration does not interface with enzyme assay. A blank sample was measured without tested compounds. All the experiments were performed in triplicates, and values were expressed as means of three experiments. Allopurinol was used as a positive control. The IC_{50} values were determined for all based compounds were measured as percent inhibition of XO was studied in terms of decrease in uric acid formation as compared to the product formation in absence of inhibitor. The percent inhibition of XO activity was calculated as the following:

% Inhibition = (A-B)/A×100

where A = the absorbance at 295 nm without the test compound,

B = the absorbance at 295 nm with the test compound.

3.4. In silico details

Molecular docking calculations

Discovery Studio (DS) 2018 SP1 [38] were used to arrange and exert the docking calculations to define docking interactions of isoindol-1,3-dion derivatives-xanthine oxidase (XO)

complexes. The crystal structures of target, XO (pdb: 3NVY) was downloaded from the protein data bank (http://www.rcsb.org) was used for docking processes. The ligands, isoindol-1,3-dion derivatives were drawn and optimized at DFT/B3LYP/6-31G (d) basis set using Gaussian 09 (G09) [39] software. The optimized structures and data were also shown at Figure S1-S6 and Table S1-S6 in supporting information part of this study. Their conformational analyses were also investigated using DS 2018 SP1. On the other hand, the enzyme was prepared using DS tools and minimized until the root mean square deviation (RMSD) reaches the lower value of 0.05 kcal/mol $Å^2$. Define and edit binding site tool was used to detect binding site of the XO against isoindol-1,3-dion derivatives structures. Dock Ligands (CDOCKER) was performed to predict the conformations and binding interactions for the ligands. The best pose for each complex was chosen according to the docking score and energy.

4. Conclusion

We report an efficient synthesis of 5-carboxylic acid and 5-carboxymethyl ester derivatives of isoindole-1,3-dion. This method has potential to be widely used to synthesis 5- carboxylic acid and 5-carboxymethyl ester substituted isoindole-1,3-dione. Inhibitor properties of these derivatives of isoindole-1,3-dion were then evaluated against XO activity. The isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c)** were showed a good inhibition activity toward against XO. All the isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c**) showed inhibition of XO enzyme by decreasing the uric acid formation. Then, isoindole-1,3-dione derivatives were applied docking studies. In docking study, their binding affinity were calculated and compared with allopurinol as XO inhibitor. The results revealed that 1,3-dioxo-2-phenyl isoindoline-5-carboxylic acid (**2c**) had more similar orientation with allopurinol than other synthesized isoindole-1,3-dione derivatives (**2a, 2b, 3a, 3b, 3c**). These results in molecular docking are compatible the results of XO inhibition assay. This interaction studies with biological activity and molecular docking offer the opportunity to know about the effects of the isoindoline-1,3-dione derivatives in XO target and mechanism of interaction and it may be useful to design new compounds.

5. Acknowledgments

We would like to thank Ataturk University (Project number: 2014/178), (Project number: FBG-2018-6476) for its financial support of this work. The numerical calculations reported in

this study were performed at TUBITAK ULAKBIM, High Performance and Grid Computing Center (TRUBA resources).

4. References

[1] P. M. Lorz, F. K. Towae, W. Enke, R. Jackh, N. Bhargava Ullmann's Encyclopedia of Industrial Chemistry, 27, (2000) 131- 169. https://doi.org/10.1002/14356007.a20_181.pub2.

[2] M. K. Hargreaves, J. G. Pritchard, H. R. Dave, Cyclic Carboxylic Monoimides, Chem. Rev. 70, (1970) 439-469. https://doi.org/10.1021/cr60266a001.

[3] Y. Kato, H. Ebiike, K. Achiwa, N. Ashizawa, T. Kurihara, F. Kobayashi, Synthesis And Inhibitory Activity Of Isoindolinone Derivatives On Thromboxane A_2 Analog (U-46619)-Induced Vasocontraction, Chem. Pharm. Bull. 38, (1990) 2060-2062, https://doi.org/10.1248/cpb.38.2060.

[4] E. Valencia, I. Weiss, S. Firdous, A. J. Freyer, M. Shamma, A. Urzu´s, V. Fajardo, The isoindolobenzazepine alkaloids, Tetrahedron 40, (1984) 3957-3962, https://doi.org/10.1016/0040-4020(84)85073-5.

[5] Y. Li, T. T. Cao, S. Guo, Q. Zhong, C. H. Li, Y. Li, L. Dong, S. Zheng, G. Wang, S.F. Yin, Discovery of Novel Allopurinol Derivatives with Anticancer Activity and Attenuated Xanthine Oxidase Inhibition, Molecules 21, (2016) 1-16, https://doi.org/10.3390/molecules21060771.

[6] C. Hoarau, A. Couture, E. Deniau, P. Grandclaudon, A Versatile Synthesis of Poly- and Diversely Substituted Isoindolin-1-ones, Synthesis, 5, (2000) 655-660, https://doi.org/10.1055/s-2000-6385.

[7] A. Couture, E. Deniau, P. Grandclaudon, C. Hoarau, Total Syntheses of Taliscanine, Velutinam, and Enterocarpam II, J. Org. Chem. 63, (1998) 3128-3132, https://doi.org/10.1021/jo972247t.

[8] N. Guzior, M. Bajda, J. Rakoczy, B. Brus, S. Gobec, B. Malawska, Isoindoline-1,3-dione derivatives targeting cholinesterases: design, synthesis and biological evaluation

of potential anti-Alzheimer's agents, Bioorg. Med. Chem. 23, (2015) 1629-1637, https://doi.org/10.1016/j.bmc.2015.01.045.

[9] N.S. El-Gohary, M.I. Shaaban, Synthesis, Antimicrobial, Antiquorum□Sensing, and Cytotoxic Activities of New Series of Isoindoline□1,3□dione, Pyrazolo[5,1□*a*]isoindole, and Pyridine Derivatives, Arch. Pharm. 348, (2015) 666-680, https://doi.org/10.1002/ardp.201500037.

[10] A. Gupta, P. Singh, B. Kamble, A. Kulkarni, M.J.N. Chandrasekar, Synthesis, Docking and Biological Evaluation of Some Novel 5-bromo-2- (5-aryl-1,3,4-thiadiazol-2-yl)isoindoline-1,3-dione Derivatives Targeting ATP-binding Site of Topoisomerase II, Lett. Drug Des. Discovery, 9, (2012) 668-675, https://doi.org/10.2174/157018012801319463.

[11] A. Luniwal, L. Wanga, A. Pavlovsky, P. W. Erhardt, R. E. Viola, Molecular docking and enzymatic evaluation to identify selective inhibitors of aspartate semialdehyde dehydrogenase, Bioorg. Med. Chem., 20, (2012) 2950-2956, DOI: 10.1016/j.bmc.2012.03.013.

[12] F. Hassanzadeh, M. Rabbani, G. A. Khodarahmi, M. Moosavi, Synthesis and Evaluation of the Anxiolytic Activity of Some Phthalimide Derivatives in Mice Model of Anxiety, IJPR 11, (2012) 109-115.

[13] W. Zhou, S. Li, W. Lu, J.Yuan, Y. Xu, H. Li, J. Huang, Z. Zhao, Isoindole-1,3-dione derivatives as RSK2 inhibitors: synthesis, molecular docking simulation and SAR Analysis, Med. Chem. Commun., 7, (2016) 292-296, https://doi.org/10.1039/C5MD00469A.

[14] W. W. Cleland, Low-Barrier Hydrogen Bonds and Enzymatic Catalysis[1], Arch. Biochem. Biophys., 382, (2000), 1-5, https://doi.org./10.1006/abbi.2000.2011.

[15] A. Kamal, N.R. Bolla, P.S. Srikanth, Naphthalimide derivatives with therapeutic characteristics: a patent review, Expert Opin. Ther. Pat., 23, (2013) 299-317, https://doi.org/10.1517/13543776.2013.746313.

[16] K.M. Amin, A.H. El-masry, N.A. Mohamed, G.A. Awad, B.S. Habib, Synthesis, characterization and antimicrobial activity of some novel isoindole-1,3-dione derivatives**,** Der Pharma Chemica, 5 (5), (2013) 97-108.

[17] M.S. el-Gaby, M.A. Zahran, M.M. Ismail, Y.A. Ammar, A novel synthesis of dibenzo[c,f]chromenes, dibenzo[c,h]chromenes and benzo[7,8]chromeno[3,4-f]isoindoles as antimicrobial agents, Farmaco 55, (2000) 227–232, https://doi.org/10.1016/S0014-827X(00)00008-2.

[18] S. Rajasekaran, R. Krishna, P. Sanjay, A. Ranjan, Design, Synthesis, Antibacterial and invitro Antioxidant activity of substituted 2HBenzopyran-2-one derivatives, Int. J. Chem. Tech. Res., 3, (2011) 555–559.

[19] R.A. Pophale, M.N. Deodhar, Synthesis and evaluation of novel phthalimide derivatives as analgesic and antiinflammatory agents, Der Pharma Chemica., 2, (2010) 185–193.

[20] G. W. Muller, R. Chen, S.-Y. Huang, L. G. Corral, L. M. Wong, R. T. Patterson, Y. Chen, G. Kaplan, D. I. Stirling, Amino-substituted thalidomide analogs: Potent inhibitors of TNF-α production, Bioorg. Med. Chem. Lett. 9, (1999) 1625-1630. https://doi.org/10.1016/S0960-894X(99)00250-4.

[21] A. Palumbo, S. V. Rajkumar, Treatment of newly diagnosed myeloma, Leukemia 23, (2009) 449-456 https://doi.org/10.1038/leu.2008.325,

[22] M. Ignasik, M. Bajda, N. Guzior, M. Prinz, U. Holzgrabe, B. Malawska, Design, Synthesis and Evaluation of Novel 2□(Aminoalkyl)□isoindoline□1,3□dione Derivatives as Dual□Binding Site Acetylcholinesterase Inhibitors, Arch. Pharm. 345, (2012) 509-516, https://doi.org/10.1002/ardp.201100423.

[23] A. Tan, E. Bozkurt, N. Kishali, Y. Kara, A New and Convenient Synthesis of Amino□ phthalimide (1*H*□Isoindole□1,3(2*H*)□dione) Derivatives and Their Photoluminescent Properties, Helv. Chim. Acta., 97, (2014) 1107-1114, https://doi.org/10.1002/hlca.201300394.

[24] T.N. Bansode, J.V. Shelke, V.G. Dongre, Synthesis and antimicrobial activity of some new N-acyl substituted phenothiazines, Eur. J. Med. Chem., 44, (2009) 5094-098, https://doi.org/10.1016/j.ejmech.2009.07.006.

[25] H. Nadaroglu, A. A. Gungor, O. Gundogdu, N. H. Kishali, B. Sever, M. D. Altintop, Investigation of the inhibitory effects of isoindoline-1,3-dion derivatives on hCA-I and

hCA-II enzyme activities, J. Mol. Struct., 1197, (2019) 386-392, https://doi.org/10.1016/j.molstruc.2019.07.070.

[26] J. George, A. D. Struthers, Role of urate, xanthine oxidase and the effects of allopurinol in vascular oxidative stress, Vasc. Health. Risk. Manag. 5, (2009) 265-272, https://doi.org/10.2147/VHRM.S4265 .

[27] V. D. Ratnam, D. D. Ankola, D. K. Bhardwaj, M. N. V. Sahana, K. Ravi, Role of antioxidants in prophylaxis and therapy: A pharmaceutical perspective, J. Control. Release, 113, (2006) 189-207, https://doi.org/10.1016/j.jconrel.2006.04.015.

[28] A. Couture, E. Deniau, P. Grandclaudon, C. Hoarau, New Approach to Isoindolobenzazepines. A Simple Synthesis of Lennoxamine, Tetrahedron, 56, (2000) 1491-1499, https://doi.org/10.1016/S0040-4020(00)00067-3.

[29] H. Zafar, M. Hayat, S. Saied, M. Khan, U. Salar, R. Malik, I. M. Choudhary, M. K. Khan, Xanthine oxidase inhibitory activity of nicotino/isonicotinohydrazides: A systematic approach from in vitro, in silico to in vivo studies, Bioorg. Med.Chem., 25, (2017) 2351-2371, https://doi.org/10.1016/j.bmc.2017.02.044.

[30] H. S. Nile, B. Kumar, W.S. Park, *In Vitro* Evaluation of Selected Benzimidazole Derivatives as an Antioxidant and Xanthine Oxidase Inhibitors, Chem. Biol. Drug. Des., 82, (2013) 290–295, https://doi.org/10.1111/cbdd.12141.

[31] T.J. Zhang, S.Y. Li, L. Wang, Q. Sun, Q.X. Wu, Y. Zhang, F.H. Meng, Design, synthesis and biological evaluation of N-(4-alkoxy-3-cyanophenyl)isonicotinamide/nicotinamide derivatives as novel xanthine oxidase inhibitors, Eur. J. Med. Chem., 141, (2017) 362-372, https://doi.org/10.1016/j.ejmech.2017.09.051.

[32] A. Aktas, S.A.A. Noma, B.D. Celepci, F. Erdemir, Y. Gok, B. Ates, New 2-hydroxyethyl substituted N-Heterocyclic carbene precursors: Synthesis, characterization, crystal structure and inhibitory properties against carbonic anhydrase and xanthine oxidase, J. Mol. Structure. 1184, (2019) 487-494. https://doi.org/10.1016/j.molstruc.2019.02.063.

[33] S. Burmaoglu, S. Ozcan, S. Balcioglu, M. Gencel, S, S.A.A. Noma, S. Essiz, B Ates., Algul O. Synthesis, biological evaluation and molecular docking studies of bis-

chalcone derivatives as xanthine oxidase inhibitors and anticancer agents, Bioorg. Chem. 91, (2019) 103149. https://doi.org/10.1016/j.bioorg.2019.103149.

[34] E.İ. Kıbrız, M. Saçmacı, İ. Yıldırım, S.A.A. Noma, T.T. Tok, Ateş B., Xanthine oxidase inhibitory activity of new pyrrole carboxamide derivatives: *In vitro* and *in silico* studies, Arch. Pharm. Chem. Life Sci. (2018) 1800165. https://doi.org/10.1002/ardp.201800165

[35] M. Kaur, A. Kaur, S. Mankotia, H. Singh, A. Singh, J. V. Singh, M. K. Gupta, S. Sharma, K. Nepali, P. M. S., Bedi, Synthesis, screening and docking of fused pyrano[3,2-d]pyrimidine derivatives as xanthine oxidase inhibitor, Euro. J. Med. Chem. 131, (2017) 14-28, https://doi.org/10.1016/j.ejmech.2017.03.002.

[36] H. Schindlbauer, Zur Reaktion von Carbonsäureanhydriden mit Formamid und N-Methylformamid, Monatshefte für Chem., 104, (1973) 848-853.

[37] G-H. Kuo, C. Prouty, W. V. Murray, V. Pulito, L. Jolliffe, P. Cheung, S. Varga, M. Evangelisto, J. Wang, Design, Synthesis, and Structure−Activity Relationships of Phthalimide-Phenylpiperazines: A Novel Series of Potent and Selective α_{1a}-Adrenergic Receptor Antagonists, J. Med. Chem. 43, (2000) 2183-2195, https://doi.org/10.1021/jm9905918.

[38] Dassault Systemes Biovia, Discovery Studio Modeling Environment Release, Dassault Systemes, San Diego (2016).

[39] Gaussian 09, Revision E.01, Frisch, M. J. Trucks, G. W. Schlegel, H. B. Scuseria, G. E. Robb, M. A. Cheeseman, J. R. Scalmani, G. Barone, V. Mennucci, B. Petersson, G. A. et al. Gaussian, Inc., Wallingford, CT, USA, 2009.

HIGHLIGHTS:

1. Isoindoline-1,3-dione derivatives (**2a**, **2b**, **2c**, **3a**, **3b**, **3c**) were synthesized by starting from 1,3-dioxo-1,3-dihydro-isobenzofuran-5-carboxylic acid.
2. All of the synthesized isoindoline-1,3-dione derivatives showed a good inhibition of XO by reducing uric acid formation.
3. The highest IC_{50} ratio was observed in 2-methyl-1,3-dioxoisoindoline-5-carboxylic acid (**2a**) at 22.56 μM. The IC_{50} ratio for the positive control allopurinol was 6.34 μM.
4. The molecular docking results displayed that 1,3-dioxo-2-phenyl isoindoline-5-carboxylic acid (**2c**) has almost more similar orientation than other ones with positive control, allopurinol.

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: