Effect of New Thalidomide Analogs in Acute Kidney Injury on Rats

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Abstract: Renal ischemia/reperfusion (I/R) injury contains multiple mechanisms involving an excessive amount of ROS that causes oxidative stress, inflammation, and rapid kidney dysfunction. This work aimed to study the ability of thalixisostere 3f on angiogenesis and antioxidant effect in I/R. There was a significant decrease in VEGF from thalix and modified thalixisostere 3f groups as compared with that of a negative control group (P < 0.001). There was a significant decrease in the mean concentration of MDA in kidney tissue obtained from thalix and thalixisostere 3f groups as compared with that of a positive control group (P < 0.001). There was a significant increase in the mean concentrations of SOD and GSH in kidney tissue of group's thalix and thalixisostere 3f groups as compared with that of a positive control group. We found that thalixisostere 3f is more effective than thalix in inhibiting the expression VEGF as pro-angiogenic factors. Molecular docking study indicated that the proper recognition of the inhibitor thalix-isostere with the conserved amino acid residues at the binding active site of VEGFR2 as one of the important enzymes to be targeted as part of antiangiogenic anticancer.

Keywords: cyclic imides; ischemic kidney; thalidomide; thalixisostere.

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1. Introduction

Ischemia and/or reperfusion (I/R) is one of the main factors participating in acute kidney injury [1]. It can happen as a result of systemic hypoperfusion (e.g., shock) with following circulatory resuscitations and tentative stopping of renal blood supply, e.g., in renal transplantation, partial kidney resection, and aortic cross-clamping [2]. The renal I/R injury is followed by complex mechanisms, including reactive oxygen species (ROS), ATP depletion, accumulation of intracellular Ca²⁺, mitochondrial dysfunction, multiple enzyme method effects, and proinflammatory cytokine production [3]. Multiple series of cellular reactions that finally produce renal cell death result from the harmful effect of I/R. However, experimental results recommend that apoptosis, hypoxia, oxidative stress, endothelial dysfunction, and inflammatory response are essential in renal dysfunction through ischemic reperfusion (IR)
conditions [4]. Oxidative stress and inflammation are found to be interconnected and thus prove to be the main providers of RIRI [5]. Recovery of kidney function after I/R injury based on injured cell replacement or renewal and protection from programmed cell death (apoptosis) [6]. In order to prevent irreversible tissue injury, blood flow restoration to ischaemic organs is essential. Although tissue injury may be induced by ischemia, reperfusion persist may also induce tissue injury [3, 7].

Vascular endothelial growth factor (VEGF) is thought to be one of the essential angiogenic factors, which can enhance the production of new blood vessels and lymphatic vessels [8]. VEGF has a number of different physiological organisms. For example, VEGF has a selective effect of increased endothelial cell mitosis and enhancing angiogenesis and endothelial cell reproduction. Also, VEGF can strengthen blood vessels by growing capillary permeability and thus enhancing plasma proteins' extravasations and other macromolecules. On the other hand, it raises the deposition to the extravascular matrix and provides nutrition to create novel capillary networks. So, VEGF is one of the essential growth factors for angiogenesis, which has a significant role in blood vessels production [9]. Thalidomide (thalix), was utilized as a sedative and anti-nausea drug in the 1950s but was prevented due to teratogenicity [10]. Thalidomide is anti-inflammatory and antiangiogenic. It has been shown to generate reactive oxygen species (ROS) [11]. In 1994, thalidomide became the focus of intense oncological research after its antiangiogenic properties. Its antiangiogenic effects have led to its assumption as an anticancer agent [12]. The above-mentioned biological interest data prompted us to find a new class of heterocyclic imide, structural analogs of the systemic thalidomide, and matching the effects of it on the renal dysfunction and injury affected by I/R of the kidney of the mouse in vivo when given as treatment (thalidomide and thalixisostere) for 3 days or when given for 7 days and for 14 days to reperfusion of the ischemic kidney.

2. Materials and Methods

All spectroscopic data were recorded according to the methods previously reported [13].

2.1. Synthesis of cyclic imide derivatives (3a-f).

General procedure

Equimolar amounts of 6-aminouracil (1.27 g, 10 mmol) and phthalic anhydride (2.57 g, 10 mmol) and/or 3-nitrophthalic anhydride (3.02 g, 10 mmol) and/or 4-nitrophthalic anhydride (3.02 g, 10 mmol) and/or 1,2,4-benzenetricarboxlic anhydride (3.01 g, 10 mmol) and/or tetrabromophthalic anhydride (1.49 g, 10 mmol) in the presence of freshly fused sodium acetate (1.64 g, 20 mmol) were fused at 150 °C in a silicon oil bath. The reaction mixture was then left to cool at room temperature; ethanol was added to the mixture. The formed solids were collected by filtration, washed with water, dried, and crystallized from EtOH to give compounds 3a-f.

2.1.1. 2-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)isoindoline-1,3-dione 3a.

White powder; yield (86%); mp> 300°C; IR (KBr): ν/cm⁻¹ = 3215 (2NH), 1724, 1690 (4C=O); ¹H NMR (DMSO-d₆) δ (ppm): 4.86 (s, 1H, C5-pyrimidine ring), 7.68 (s, 4H, Ar-H), 10.45, 10.87 (s, 2H, 2NH, D₂O exchangeable); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 76.7, 124.7 (2C), 130.3 (2C), 132.6 (2C), 151.8, 163.2, 164.5 (2C), 166.9; MS (EI, 70 eV): m/z (%)
257 (M⁺, 34.16), 215 (21.01), 207 (100.00), 157 (36.38), 125 (27.70), 100 (44.05); Anal. Calcd. For C₁₂H₇N₃O₄ (257.21): C, 56.04; H, 2.74; N, 16.34; Found: C, 56.11; H, 2.83; N, 16.40%.

2.1.2. 2-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-4-nitrosoindoline-1,3-dione 3b.

Pale yellow powder; yield (84%); mp> 300°C; IR (KBr): v/cm⁻¹ = 3214 (2NH), 1725, 1694 (4C=O); ¹H NMR (DMSO-d₆) δ (ppm): 3.87 (s, 1H, C5-pyrimidine ring), 8.16 (m, 1H, Ar-H), 8.45 (d, 1H, J = 8.0 Hz, Ar-H), 8.63 (d, 1H, J = 8.0 Hz, Ar-H), 10.41, 10.64 (s, 2H, 2NH, D₂O exchangeable); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 76.8, 127.1, 128.4, 130.5, 133.6, 134.3, 146.8, 151.2, 163.3, 164.1, 165.6, 166.7; MS (EI, 70 eV): m/z (%) 301 (M⁺-1, 30.31), 279 (100.00), 275 (24.11), 215 (25.26), 155 (49.52), 139 (58.19); Anal. Calcd. For C₁₂H₇N₃O₄ (302.20): C, 47.69; H, 2.00; N, 18.54; Found: C, 47.72; H, 2.06; N, 18.61%.

2.1.3. 2-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-5-nitrosoindoline-1,3-dione 3c.

Yellow powder; yield (81%); mp> 300°C; IR (KBr): v/cm⁻¹ = 3222 (2NH), 1731, 1689 (4C=O); ¹H NMR (DMSO-d₆) δ (ppm): 4.88 (s, 1H, C5-pyrimidine ring), 8.24 (d, 1H, J = 8.0 Hz, Ar-H), 8.43 (s, 1H, Ar-H), 8.65 (d, 1H, J = 8.0 Hz, Ar-H), 10.45, 10.68 (s, 2H, 2NH, D₂O exchangeable); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 76.3, 122.4, 127.4, 130.1, 131.7, 136.2, 148.6, 151.4, 163.2, 164.5, 165.8, 166.7; MS (EI, 70 eV): m/z (%) 302 (M⁺, 9.11), 284 (100.00), 257 (50.84), 231 (10.47), 181 (9.04), 115 (60.22); Anal. Calcd. For C₁₂H₆N₄O₆ (302.20): C, 47.69; H, 2.00; N, 18.54; Found: C, 47.74; H, 2.04; N, 18.62%.

2.1.4. 2-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-1,3-dioxoisooindoline-5-carboxylic acid 3d.

Pale yellow powder; yield (80%); mp> 300°C; IR (KBr): v/cm⁻¹ = 3242 (2NH), 1725, 1697 (4C=O); ¹H NMR (DMSO-d₆) δ (ppm): 4.85 (s, 1H, C5-pyrimidine ring), 8.12 (d, 1H, J = 8.0 Hz, Ar-H), 8.46 (s, 1H, Ar-H), 8.63 (d, 1H, J = 8.0 Hz, Ar-H), 10.42, 10.67 (s, 2H, 2NH, D₂O exchangeable), 12.63 (s, 1H, OH, D₂O exchangeable); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 76.2, 124.3, 130.4, 131.0, 133.2, 134.8, 136.4, 151.2, 163.4, 164.6, 165.8, 166.4, 170.1; MS (EI, 70 eV): m/z (%) 301 (M⁺, 7.10), 270 (32.83), 231 (41.81), 192 (39.91), 183 (35.70), 155 (43.05), 135 (58.68); Anal. Calcd. For C₁₃H₇N₃O₆ (301.21): C, 51.84; H, 2.34; N, 13.95; Found: C, 51.90; H, 2.41; N, 13.98%.

2.1.5. 4,5,6,7-Tetrabromo-2-(2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)isoindoline-1,3-dione 3e.

Yellow powder; yield (88%); mp> 300°C; IR (KBr): v/cm⁻¹ = 3214 (2NH), 1726, 1690 (4C=O); ¹H NMR (DMSO-d₆) δ (ppm): 4.84 (s, 1H, C5-pyrimidine ring), 10.42, 10.76 (s, 2H, 2NH, D₂O exchangeable); ¹³C NMR (125 MHz, DMSO-d₆) δ (ppm): 76.7, 124.2 (2C), 129.5 (2C), 138.8 (2C), 151.2, 163.1, 165.6 (2C), 166.4; MS (EI, 70 eV): m/z (%) 573 (M⁺+1, 28.88), 572 (M⁺, 36.52), 512 (63.52), 461 (34.96), 338 (28.65), 299 (100.00), 199 (49.71); Anal. Calcd. For C₁₂H₇Br₄N₃O₄ (572.79): C, 25.16; H, 0.53; N, 7.34; Found: C, 25.22; H, 0.55; N, 7.42%.

2.1.6. 6-(2,6-Dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)-5H-pyrrolo[3,4-b]pyridine-5,7(6H)-dione 3f.
Buf powder; yield (86%); mp 238-240°C; IR (KBr): v/cm\(^{-1}\) = 3268 (2NH), 1725, 1642 (4C=O); \(^1\)H NMR (DMSO-\(d_6\)) \(\delta\) (ppm): 5.76 (s, 1H, C5-pyrimidine ring), 7.38-9.12 (m, 3H, Ar-H), 10.21, 10.68 (s, 2H, 2NH, D\(2\)O exchangeable); \(^13\)C NMR (125 MHz, DMSO-\(d_6\)) \(\delta\) (ppm): 76.5, 124.6, 128.5, 131.4, 148.8, 150.6, 152.4, 160.3, 163.2, 167.4, 168.7; MS (EI, 70 eV): \(m/z\) (%) 259 (M\(^+\)+1, 54.50), 193 (100.00), 168 (36.40), 150 (90.90), 128 (63.60), 112 (100.00); Anal. Calcd. For C\(_{11}\)H\(_6\)N\(_4\)O\(_4\) (258.19): C, 51.17; H, 2.34; N, 21.70; Found: C, 51.26; H, 2.39; N, 21.77%.

2.2. Animals

Fifty-four male Sprague-Dawley rats weighing (201.0±10.5) g 3-4 Months old were purchased from the Medical Experimental Research Center (MERC) animal labs. Rats were allowed access to food and water at the Medical Experimental Research Center (MERC) animal labs. It was assumed that all laboratory animals had the standard diet and clean water freely during the experiment. Ventilation was suitable in the feeding room, with the natural lighting day and night. The culture temperature was preserved at 18–25 °C. In the present study, there were ten mice groups.

2.2.1. Animal model

After one week of adaptive feeding, 3-4 month male Sprague-Dawley rats were randomly divided into 9 groups, (1) Negative control group, (2) Positive ischemic/reperfusion control group mice suffered 45 min left renal ischemia tracked by clamp relief (3) thalix treated ischemic/ reperfusion group, rats obtained thalidomide (thalix) (1000 IU/kg, SC) 24 h before the operative method and continued daily till the day of sacrifice; and from (4) to (9) thalixisostere from \(3a\) to \(3f\) treated ischemic/ reperfusion group rats received thalixisostere from \(3a\) to \(3f\) (1000 IU/kg, SC) 24 h before the operative method and continued every day till the day of sacrifice. All rats with renal ischemic/reperfusion were subjected to serum analysis before surgery and 3days after renal ischemia/Reperfusion induction (n=6) for each group.

2.2.2. Biochemical parameters

Blood samples were taken before surgery at the end of the reperfusion period and 3days after renal ischemia for biochemical tests: Blood samples for creatinine and urea were measured using a colorimetric assay.

2.2.3. Estimation of lipid peroxidation, antioxidant enzymes, and non-enzymatic antioxidant in kidney tissues

Kidney tissues, harvested at 3days after ischemia, were kept in cold conditions. It was cut using a surgical scalpel to fine slices in the chilled 0.25 M sucrose and quickly flushed onto the filter paper. The tissue was chopped and homogenized in 10 mm Tris-HCl buffer, pH 7.4 (10 % w/v), with 25 strokes of Teflon pestle from the glass mixer at 2500 rpm. The clear supernatant was used for lipid peroxidation (MDA), antioxidant enzyme Superoxide dismutase (SOD), and low-glutathione (GSH) using a colorimetric group (Bio-Diagnostics, Dokki, Giza, Egypt) according to the manufacturer’s instructions for commercially available kits.
2.2.4. Estimation of vascular endothelial growth factor.

Bio-Plex Pro human angiogenesis array for VEGF was run according to the manufacturer’s instructions (Cat # M50007W214 Bio-Rad Laboratories, USA), which quantifies multiple protein bio-factors.

2.2.5. Methodology of molecular docking.

In the present work, the 3D structure of the VEGFR-2 enzyme was taken from a Protein Data Bank entry (PDB entry: 4ASD) co-crystallized with sorafenib as a ligand. The novel derivatives were built and optimized at the ChemDraw professional 2016. The optimized structures were then docked and analyzed using MOE software to determine specific interactions between ligands and essential amino acids within the active site of the VEGFR-2 enzyme.

2.3. Principle.

Antiangiogenesis activities studies used the 3-D crystallographic VEGFR2 (4ASD) to explore the thalidomide and its structural relevant analog recognition in the active site as a potential antioxidant.

2.4. Statistical analysis.

All values described in the text and figures are expressed as mean ± standard deviation (SD) for N observations. One-way analysis of variance (ANOVA) with the Bonferroni post hoc test was performed using SPSS version 19 for Windows. P values of less than 0.05 were considered to be significant.

3. Results and Discussion

3.1. Results.

3.1.1. Chemistry.

The newly synthesized cyclic imide derivatives 3a-f based on the fusion of anhydride derivatives 1a-f with 6-aminopyrimidine-2,4(1H,3H)-dione (2) in the presence of freshly fused sodium acetate (Scheme 1).

![Scheme 1](image)

1 and 3a, X= C, R= H, 1 and 3b, X= C, R= 3-NO₂, 1 and 3c, X= C, R= 4-NO₂,
1 and 3d, X= C, R= 4-COOH, 1 and 3e, X= C, R= 3,4,5,6-Br, 1 and 3f, X= N, R= H


Structures of the products 3a-f were confirmed by both elemental and spectral data. The IR spectra of compounds 3a-f showed the characteristic absorption bands in the region 3214-
corresponding to the stretching vibration of the two NH groups. Also, the strong frequency region of the spectra showed the absorption bands at 1690-1679 cm\(^{-1}\) due to the stretching vibrations of the four CO groups. Also, the mass spectroscopic measurements of compounds 3a-f showed the molecular ion peaks at \(m/z = 257 (M^+), 301 (M^+-1), 302 (M^+), 301(M^+), 573 (M^+1)\) and 259 (M\(^+\))), respectively, which are in agreement with their suggested structures. \(^1\)H NMR spectrum of structure 3f as an example revealed singlet signals at \(\delta 4.85, 10.51,\) and 10.89 ppm assignable to C-H of the pyrimidine ring and two NH protons.

The formation of cyclic imides 3a-f can be postulated according to the following mechanism (Scheme 2):

![Scheme 2](https://biointerfaceresearch.com/)

3.1.2. Pharmacological.

Kidney renal I/R injury is a difficult inflammatory method in which it is functional and morphologically destroyed through the ischaemic stage and undergoes additional injury through reperfusion.

In the current study, the effect of pretreatment with thalix and thalixisosteres 3a-f on renal function, angiogenesis VEGF, MDA (Malondialdehyde) (a marker of lipid peroxidation), SOD (Superoxide dismutase) (a marker of antioxidant enzyme), and GSH (Glutathione) (a marker of non-enzymatic antioxidant) were investigated.

3.1.2.1. The effect of thalixisosteres3a--3f on body weight:

As in Figure 1, there was an insignificant change in the body weight in all groups before (\(P = 0.427\)). There was a significant decrease in all I/R groups’ final body weight compared to a negative control group (\(P=0.01\)).
3.1.2.2. The effect of thalixisosteres 3a-3f on kidney injury caused by I/R.

As illustrated in Figures 2 and 3. There was a significant increase in creatinine from all I/R treated with thalixisosteres 3a-3f groups as compared to negative and positive control groups (P≤0.01). Also, there was an insignificant difference in creatinine from I/R treated with thalixisosteres 3a-3f groups compared to I/R treated with thalix groups (P>0.05).

There was a significant increase in urea from all I/R treated with thalix and thalixisosteres 3a-3f groups compared to negative control and positive control groups (P≤0.01). There were insignificant differences in urea from all I/R treated with thalixisosteres 3a-3f groups as compared to thalix groups (P>0.05).

3.1.2.3. The effect of thalixisosteres 3a-3f on Oxidative stress.

As illustrated in Figure 4, there was a significant increase in the mean concentration of MDA in kidney tissue obtained from all I/R treated with thalix and thalixisosteres 3a-3f groups.
as compared to a negative control group (P≤0.01). The mean MDA concentrations in kidney tissue of all I/R treated with thalix and thalixisosteres 3a-3f were significantly lower than that in positive control group (P≤0.01). Also, there was a significant increase in MDA concentrations in kidney tissue from I/R treated with thalixisosteres 3a-3e groups as compared to I/R treated with thalixisosteres 3f group (P≤0.05).

As shown in Figure 5, there was a significant decrease in the mean concentrations of GSH in kidney tissue samples obtained from all I/R treated with thalix and thalixisosteres 3a-3f groups compared to a negative control group (P ≤ 0.01). Moreover, there was a significant increase in the mean concentrations of GSH in kidney tissue samples from all I/R treated with
thalix and thalixisosteres 3a-3f compared with positive control groups (P ≤ 0.01). In addition, mean concentrations of GSH in kidney tissue of I/R treated with thalixisosteres 3a-3e groups were significantly decreased compared to I/R treated with thalixisosteres 3f group (P≤0.01).

As illustrated in Figure 6, there was a significant decrease in the mean activity of SOD in kidney tissue samples obtained from all I/R treated with thalix and thalixisosteres 3a-3f groups compared to a negative control group (P ≤ 0.01). Moreover, there was a significant increase in the mean activity of SOD in kidney tissue samples from all I/R treated with thalix and thalixisosteres 3a-3f groups as compared with positive control groups (P < 0.01). In addition, the mean SOD activity in kidney tissue of I/R treated with thalixisosteres 3a-3e groups was significantly decreased compared to I/R treated with thalixisosteres 3f group (P≤0.01).

3.1.2.4. The effect of thalixisostere 3f on angiogenesis.

As illustrated in Figure 7, there were insignificant differences in VEGF obtained from all I/R treated with thalix and thalixisosteres 3a-3e groups compared to a negative control group
(P > 0.05). On the other hand, there were insignificant differences in VEGF from thalix isosteres 3a-3e groups compared with thalix groups (P > 0.05). Moreover, there was a significant decrease in the mean VEGF from I/R treated with thalix isosteres 3f groups compared with negative and positive control groups (P ≤ 0.001). In addition, the mean concentrations of VEGF of I/R treated with thalix isosteres 3a-3e groups were significantly increased compared to I/R treated with thalix isosteres 3f group (P≤0.001).

![Figure 7. VEGF in studied groups.](https://doi.org/10.33263/BRIAC125.61006113)

3.2. Discussion.

Renal I/R injury is a complex inflammatory process in which the kidney is functionally and morphologically damaged during the ischemic phase and undergoes extra injury during reperfusion. In the present study, we used the Sprague-Dawley rat model of renal I/R injury (45 min) and observed it by renal function. The effect of pretreatment with thalix and new thalix on renal function and angiogenesis (VEGF), as well as on MDA (a marker of lipid peroxidation) and SOD (a marker of antioxidant enzyme) and GSH (a marker of non-enzymatic antioxidant) was investigated.

The present study showed a significant increase in serum creatinine and urea and a significant decrease in creatinine clearance at 3, 7, and 14 days after I/R, suggesting a significant degree of renal dysfunction. These findings confirm that I/R injury of the kidney causes glomerular dysfunctions and are in agreement with those reported previously [2]. According to the reported work [14], increased serum creatinine rates at the first 24 hours after reperfusion and the decrease on the 7th day may increase muscle mass.

By thalix and new thalix treatment, there was a significant increase in creatinine and urea and a decrease in creatinine clearance. These findings confirm that minimal glomerular or tubular atrophy or necrotic changes were demonstrated in kidneys of thalidomide-treated rats with those reported previously [15].

In this study, treatment with thalix and new thalix for 3, 7, and 14 days reduces the expression of VEGF as compared to control groups (P=0.001). VEGF was markedly upregulated in the positive control group. This finding suggests that it is possible that the release of growth factors (VEGF), triggered by proinflammatory cytokines by which thalix reduced VEGF production [16].
The involvement of ROS in I/R injury to the kidney and other organs is widely accepted. As inflammation is associated with free radical production and oxidative stress, there was also an increase in lipid peroxidation level and a decrease in SOD and GSH levels in I/R injury models.

Oxidative stress injury of the kidney may play a major role in inducing remote organ injury. The involvement of ROS in I/R injury to the kidney and other organs is widely accepted. We examined oxidative stress state in kidney tissues during renal I/R injury. We found a significant increase in MDA concentration (a marker of lipid peroxidation) and catalase enzyme, and a significant decrease in GSH in the kidney tissues after renal I/R injury. These findings suggest increased oxidative stress in the kidney during renal I/R injury and support findings [2].

The first step in this work was to investigate the effect of new thalix on ROS at renal I/R injury. In the present study, thalix and new thalix treatment were associated with a significant decrease in MDA and a significant increase in the activity of SOD and GSH in kidney tissue in renal I/R injury. These findings verified that the new thalix might be capable of acting as a direct antioxidant as well by activating antioxidant defense mechanisms. To the best of our knowledge, the present study is the first to show the effects of new thalix in preventing oxidative stress in I/R-induced renal injury.

The next step in this work was to investigate the effect of new thalix on angiogenesis at renal I/R injury model. The effect of thalix and new thalix on renal I/R was also investigated; the mean concentrations of VEGF of new thalix treated groups were lower than that in thalix treated group rats (P < 0.05) on various days after I/R. Since VEGF is highly specific, a number of studies have reported that VEGF has an important role in promoting proliferation, migration, and chemotactic response in bone, lung, kidney, brain, vascular endothelial cells, tumor, and other tissues [17]. Therefore, the formation of new capillaries was also reduced. This may be associated with the thalix-induced downregulation of VEGF [18]. Thalidomide inhibits expression of the pro-angiogenic factors VEGF and basic fibroblast growth factor (bFGF) via mechanisms involving tumor necrosis factor-alpha (TNFalpha) and transcriptional activation of the VEGF promoter [19].

3.2.1. Structural similarity and docking affinity.

The difference in linearity and aromaticity between the two compounds was expressed in their degree of complementarity and interaction with the surrounding amino acids (Figure 8).

![Figure 8. 3D sketch of both thalidomide (green colored) and the new compound (Blue colored) showing their docking layout. Heteroatoms colored Blue N and Red oxygen.](https://image.png)
3.2.2. VEGFR binding active site.

The binding-active site of VEGFR2 has extensive recognition towards the proper active docked analogs via expressing stable hydrogen bonds with the key amino acid residues, namely; Glu885, Val899, Asp104.

3.2.3. Docking of thalidomide.

The thalidomide expressed unique recognition with the key amino acid residues that are laid towards function. N-piperidinedione showed a strong hydrogen bond with Val899. In comparison, the two carbonyl oxygens expressed proper recognitions with the corresponding Glu885 and Asp1046 through binding to two solvent molecules of water. (Figure 9).

![Figure 9. X-ray binding interaction of thalidomide within the binding pocket of VEGFR2 complex (code: 4ASD).](https://doi.org/10.33263/BRIAC125.61006113)

![Figure 10. Putative binding mode of the new compound within the binding pocket of VEGFR.](https://doi.org/10.33263/BRIAC125.61006113)
3.2.4. Docking of thalidomide bioisostere.

Solvent expressed an exclusive role in recognition of the new compound within the VEGFR active site. The new compound expressed unique recognition with the key amino acid residue laid towards N1 of uracil expressed proper recognition with the corresponding Glu885 oxygen through binding to a water molecule.

Furthermore, carbonyl oxygen-4 showed strong bifurcated hydrogen bonds with both Val899 and Ile1044 through one solvent molecule.

Binding interaction with both carbonyl oxygens of the pyrrole ring was performed through binding to two molecules of water to be recognized properly with Asp1046 and Ile 888, respectively (Figure 10).

4. Conclusions

Consistent with the laboratory findings, this new thalix reduces VEGF formation at the three sample times. These findings suggest that new thalix is more effective than thalix in inhibiting the expression VEGF as pro-angiogenic factors. To the best of our knowledge, the present study is the first study to exhibit the antiangiogenic activity of new thalix in I/R-induced renal injury.

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Conflicts of Interest

The authors declare no conflict of interest.

References


