Chemistry Research Journal, 2020, 5(5):51-59

Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Synthesis of Novel Conjugated using Knoevenagel condensation for Quinoxaline 1,4-di-N-Oxide Derivatives as Anti-leishmania Agents

Khalil A.L. Alabdullah[1]*, Farouk Kandil[1], Sameh Hamo[1], Ch. Soukkarieh[2]

[1]Department of Chemistry, Faculty of Science, University of Damascus, Syria
[2]Department of Animal Biology, Faculty of Sciences, Damascus University, Syria

Abstract In this paper, we report and development of new and effective anti-leishmania agents, and with the aim of obtaining new and more potent anti-leishmania compounds which can improve the current chemotherapy anti-leishmania treatments, were synthesized connected of substituted side chains by Knoevenagel condensation to shape electron-donating groups conjugated with quinoxaline 1,4-di-*N*-oxide derivatives nucleus and evaluated of one new quinoxaline 1,4-di-*N*-oxide derivatives *in vitro* as an antileishmanial agent and the effect of different concentration of the synthesized compound (III) on the viability of *L. tropica* parasite was evaluated. The results obtained indicate that pyridine derivative (IC_{50}=25.05µg/ml) against *L. tropica* parasite.

Keywords Synthesis; Characterization; Quinoxaline-1,4-dioxides; Beirut reaction; Knoevenagel condensation; anti- leishmania

1. Introduction

Leishmaniasis is of the world's most important tropical parasitic disease and cause social and economical health problems, particularly in the tropical countries [1]. There are about 13 million people are infected in the worldwide [2]. The emergence of resistant parasites, the high cost and toxicity of current treatments call for the discovery of new drugs [3]. Quinoxaline 1,4-di-N-Oxidederivatives representing large class of hetero aromatic N-Oxides, That possess two N $\rightarrow$ O bonds at positions N_1 and N_4 cosecutive[4]. The oxidation of both nitrogen atoms of quinoxaline structure considerably broadened variety of biological properties [5-7].

Nowadays, there is a lot of active interest in medicinal chemistry because of posses pharmacological activity [8] As: antibacterial [9], antiparasitic [3], antiviral [10], anti- leishmania [1]antitumor [11].A heterocyclic di-N-oxide that is selectively toxic to oxygen-poor (hypoxic) tumor cells [12]. The massively spectrum of biological activities of quinoxaline -1,4- dioxides derivatives has been connected to its ability to generate reactive oxygen species (ROS). Through product radical pieces after biotransformation under hypoxic conditions leading to DNA damage [13].

Most of quinoxaline derivatives are synthetic and natural quinoxaline derivatives are rare [14]. The main synthetic approaches for the preparation quinoxaline -1,4-dioxides derivatives until 1965 were prepared by oxidation of quinoxaline derivatives with per acetic acid or with hydrogen peroxide in acetic acid [8]. Afterwards a superior synthetic route for the preparation of quinoxaline-1,4-dioxides has been devised by Haddadin and Issidorides, this method which has often been referred to as Beirut reaction involve the reaction the cycloaddition between benzofurazan N-oxide as a substrate with enamines or enolate anions generated from α-methylene ketones, 1,3-diketones or β-keto esters, 1,3-dinitriles in the presence of a base, leading to formation of quinoxaline-1,4-di-N-

oxide derivatives [8,10,13].The present work reports the synthesis of four new compound, by the Knoevenagel condensation of novel ring substituted the corresponding side chains [2-[2-(pyridin-2-yl) ethenyl] and 2-[4 phenylbuta-1,3-dien-1-yl] and 2-[2-(thiophen-2-yl) ethenyl] and 2-[2-(2 furyl) ethenyl] quinoxaline 1,4-di-oxide**]**quinoxaline 1,4-di-oxide.

2. Materials and Methods

2.1. General Remarks

All the used chemicals were purchased from Aldrich or Merck. The IR spectra of the ligands were recorded with a Midac 1700 instrument in KBr pellets, peak intensities are represented by: w (weak), m (medium), s (strong). ^{1}HNMR spectra of the all synthesized compounds in DMSO-*d6* or CD_3CN solution were recorded on a Bruker 400 MHz spectrometer and chemical shifts are indicated in ppm (δ), signal multiplicities are represented by: S (singlet), d (doublet), dd (doublet of doublets), t (triplet), and m (multiplet). Mass spectra were recorded using a KRATOS MS50TC spectrometer and mass spectra (MS), ESI (Positive) were recorded on an Esquire-LC-00075 spectrometer.
The purity of the synthesized compounds was evidenced by thin layer chromatography (TLC), and were analyzed by HPLC, was performed to establish the purity of the compounds. HPLC conditions: Shimadzu HPLC model CBM 20-A, equipped with UV-VIS detector (model SPD-20A) [KROMASIL 100 C_{18} (150mm×4.6mm, 5 micron)column] techniques, mobile phase: [acetonitrile/water (65/ 35)]. The compounds synthesized during the intermediate stage of this project were identified and were chemically characterized by infrared spectroscopy, nuclear magnetic resonance, Mass spectra. Also final products (III-VI), were determined by IR, ^{1}HNMR, MS, and determined the physical properties Table 1. By those measurements it was generally found to be in agreement with a quinoxalinedi-*N*-oxide structure and the corresponding side chains. The biological activity measurement was carried out at the Department of Faculty of pharmacy in Damascus University.

2.2. Chemical Synthesis

2.2.1. benzofurazan N-Oxide(I):

A mixture of (6.90) g (0.05) mol of 2-nitroaniline in absolute ethanol (35) ml and (2.80) g (0.05) mol of KOH was stirred until dissolved.
The mixture was cooled at (0-5) ^oC, (150) ml of sodium hypochlorite was added slowly while stirring at (0-5) ^oC for 90 min, until a yellow precipitate appeared the solid obtained was filtered off and washed with (300) ml distilled water and recrystallized from methanol.
Infrared spectra (KBr) cm^{-1}: 3079 (w, C-H-Ar)**;** 1615 (m, C=N-Ar); 1483 (s, N $\longrightarrow$ O); 1014 (s, N-O).
The ^{1}HNMR (CD_3CN) δ **PPM**: δ7.36-7.53 (4H, S, $CH_{4+5+6+7}$ BFO).
MS: Confirm the probable formula by showing a peak atm/z 136 $[M]^-$

2.2.2. 2-methylquinoxaline 1,4-Dioxide(II)

A solution of benzofurazan N-Oxide (3.4) g of (0.01) mol in (10) Acetonitrile and (15) ml Acetone and (1) ml pyrrolidine. The mixture was heated at reflux for 20 min at (68-70) ^oC temperature and the solution was allowed to stand at room temperature for 24 hour. The yellow precipitate was collected by filtration, washed methanol and dried.
Infrared spectra (KBr) cm^{-1}: 3023 (w, Ar-C-H), 2965(w, Methyl C-H), 1541 (m, C=N), 1333 (S, N $\longrightarrow$ O).
The ^{1}H NMR (DMSO) δ **PPM**: 2.49 (3H, s, N=C-CH_3); 8.81 (1H, s, H-3); 8.47 (2H, s, H-5+H-8); 7.97 (2H, s, H-6+H-7).
MS: Confirm the probable formula by showing a peak at m/z 176 $[M]^-$

2.2.3. General procedure for preparation of 2-methylquinoxaline 1,4-Dioxide Derivatives (scheme 1)

A mixture of 2-methylquinoxaline 1,4-Dioxide (II) (0.35) g, (0.002mol) in (20) ml methanol and appropriate aldehyde **(**0.006 mol) was stirred in 250 ml Erlenmeyer flask, which sodium hydroxide 5% in ethanol (10) ml was

added dropwise and the flask tightly stoppered at room temperature. Bright yellow crystals separated depending aldehyde within 1 hour. Filtered and washed with water and recrystallized from acetonitrile.

2-[2-(pyridin-2-yl)ethenyl]quinoxaline1,4-Di-N-oxides (III)

Infrared spectra (KBr) cm^{-1}: 3050 (w, C-H-Ar); 3000 (w, C-H in double bond); 1630 (s, C=C in double bond); 1533 (m, C=N-Ar); 1370 (s, N ⟶ O).

The ^{1}HNMR (DMSO) δ PPM: 9.21 (1H, S, H-3), 8.67 (1H, d, H-2'), 8.45-8.50 (2H, dd,H-5+H-8), 8.06-8.23 (2H, dd, H-6+H-7), 7.85-7.98 (3H, m, H-3'+H-5'+H-6'), 7.57 (1H, d, H-1'), 7.38 (1H, t, H-4') [Fig.1].

MS: Confirm the probable formula by showing a peak atm/z 265[M]$^{-}$[Fig.1].

2-[4-phenylbuta-1,3-dien-1-yl]quinoxaline 1,4-Di-N-oxides (IV)

Same as previous steps but in this experiment the mixture of compound (II) (0.002mol) and (0.002mol) for cinnamaldehyde.

Infrared spectra (KBr) cm^{-1}: 3070 (w, C-H-Ar); 2925 (w, C-H in double bond); 1632 (s, C=C in double bond); 1530 (m, C=N-Ar); 1376 (s, N ⟶ O).

The ^{1}HNMR (DMSO) δ PPM: 9.13 (1H, S, H-3), 8.45 (2H, s, H-5+H-8), 7.93 (3H, m,H-1'+H-2'+H-3'), 7.62 (2H, s, H-6+H-7), 7.22-7.39 (5H, m, H-4'+H-5'+H-6'+H-8'+H-9'), 6.99 (1H, S, H-7') [Fig.2].

MS: Confirm the probable formula by showing a peak atm/z =290[M]$^{-}$ [Fig.2].

2-[2-(thiophen-2-yl)ethenyl]quinoxaline1,4-Di-oxide (V)

Infrared spectra (KBr) cm^{-1}: 3067 (w, C-H-Ar); 3020 (w, C-H in double bond); 1614 (s, C=C in double bond); 1535 (m, C=N-Ar); 1376 (s, N ⟶ O).

The ^{1}HNMR (DMSO) δ PPM: 9.14 (1H, S , H-3), 7.96-7.87 (2H, m, H-6+H-7), 8.50-8.42 (3H, m, H-2'+H-5+H-8) 7.72-7.71 (1H, d, H-1'), 7.43-7.41 (1H, d, H-5'), 7.29-7.33 (1H, d, H-3'), 7.18-7.15 (1H, t, H-4') [Fig.3].

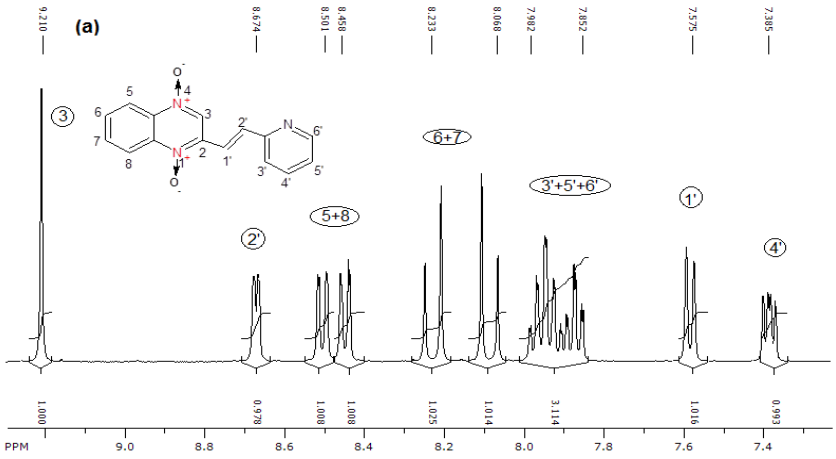
ESI-MS: Confirm the probable formula by showing a peak at m/z=271[M+H]$^{+}$[Fig.3].

2-[2-(furan-2-yl)ethenyl]quinoxaline1,4-Di-N-oxides (VI)

Infrared spectra (KBr) cm^{-1}: 3074 (w, C-H-Ar); 3016 (w, C-H in double bond); 1625 (s, C=C in double bond); 1532 (m, C=N-Ar); 1371 (s, N ⟶ O); 1239 (s, C-O-C).

The ^{1}HNMR (DMSO) δ PPM: 9.14 (1H, S, H-3); 8.48-8.43 (2H, m, H-5+H-8); 8.21-8.17 (1H, d, H-2'); 7.94-7.89 (3H, m, H-1'+H-6+H-7); 7.36 (1H, d, H-5'); 6.86 (1H, S, H-4'); 6.66 (1H, S, H-3') [Fig. 4].

ESI-MS: confirm the probable formula by showing a peak at m/z=255 [M+H]$^{+}$ [Fig. 4].



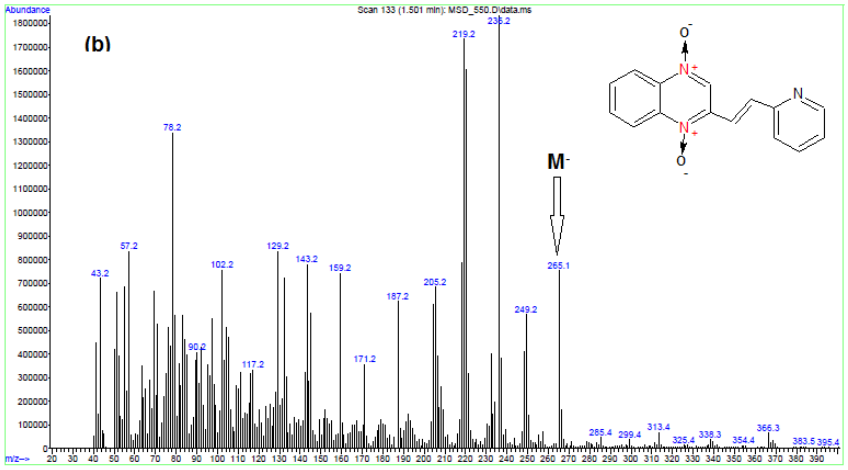
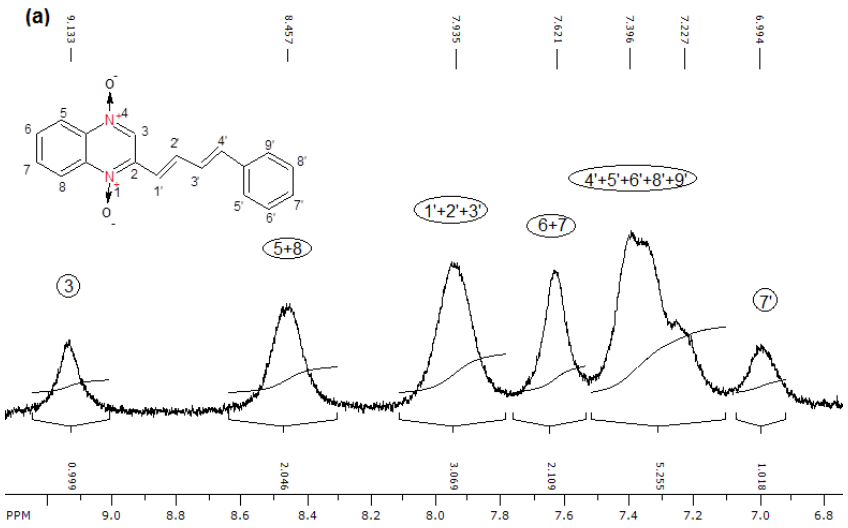


Figure 1: [1]H NMR (a) and (b) MS spectra of compound (III)



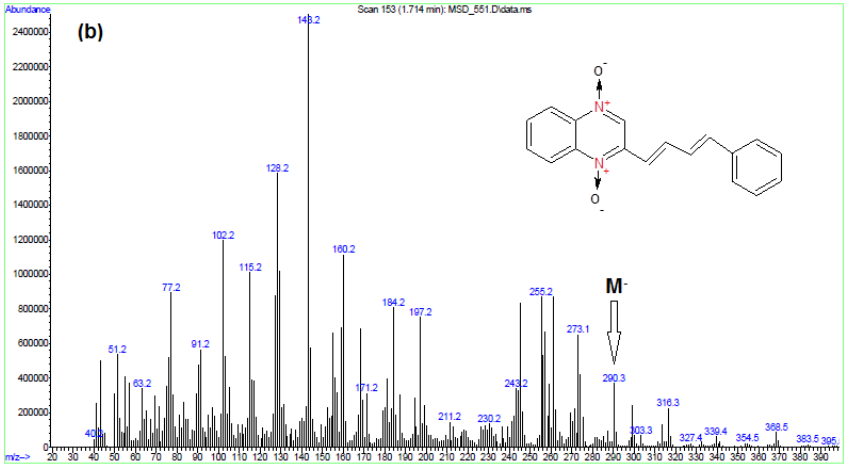


Figure 2: [1]H NMR (a) and (b) MS spectra of compound (IV)

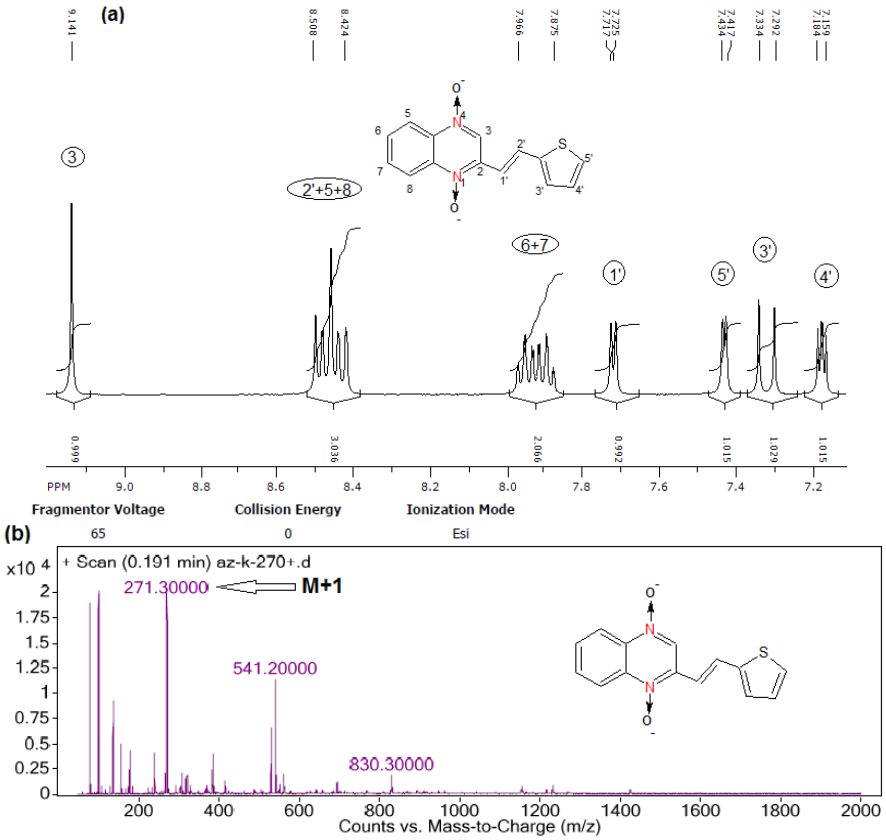


Figure 3: [1]H NMR (a) and (b) MS spectra of compound (V)

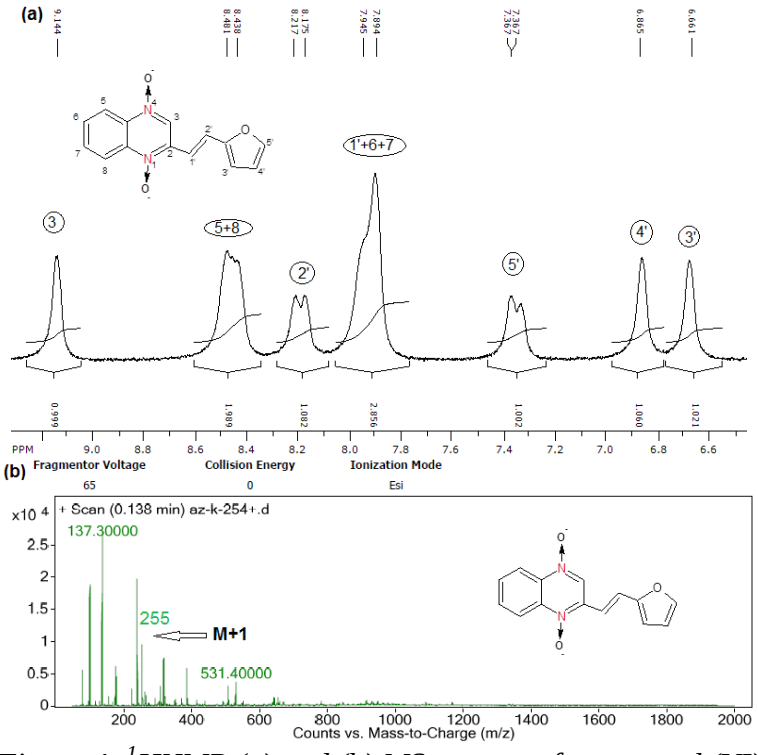


Figure 4: [1]HNMR (a) and (b) MS spectra of compound (VI)

Table 1: Physical properties and molecular formula of the synthesized compounds (I-VI)

Compound	Formula	R_f	M.P (C^o)	yield%
I	$C_6H_4N_2O_2$	0.65[a]	(69-70)	73
II	$C_9H_8N_2O_2$	0.42[a]	(184-185)	61
III	$C_{15}H_{11}O_2N_3$	0.52[b]	219	52
IV	$C_{18}H_{14}O_2N_2$	0.62[b]	225	22
V	$C_{14}H_{10}N_2O_2S$	0.78[a]	209	28
VI	$C_{14}H_{10}N_2O_3$	0.43[b]	204	55

R_f[a]: (Methanol : Dichloromethane) (2 : 8)
R_f[b]: (Toluene : Dioxane) (60 : 40)

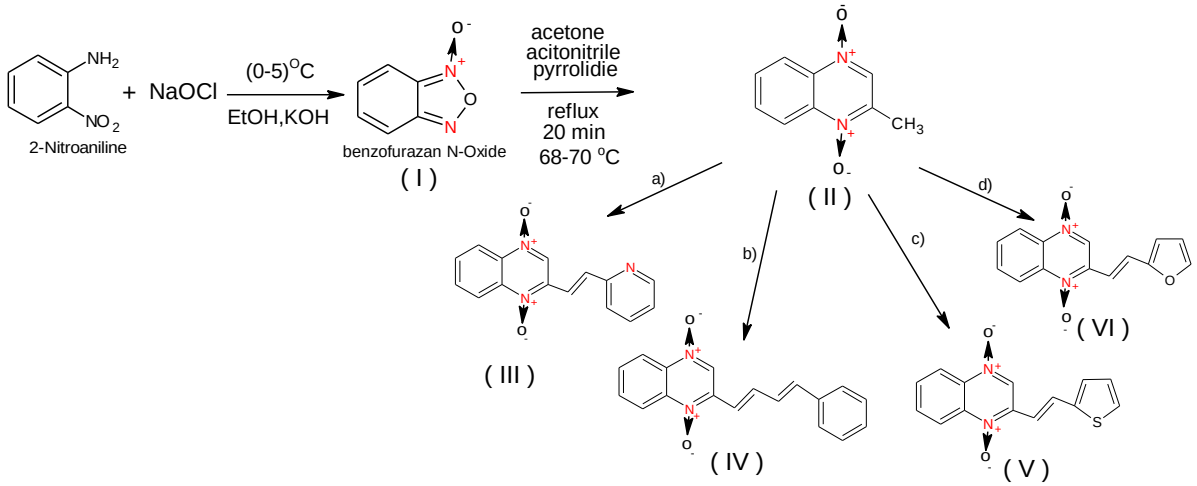
3. Results and Discussion

3.1. Chemistry

Scheme 1 shows the synthetic pathways to prepare benzofurazan N-Oxide (I) starting 2-Nitroaniline and synthesis the key substrate compounds (II).

The first substrate 2-Methylquinoxaline1,4-dioxide (II) were obtained by the well- known Beirut reaction between benzofurazan N-Oxide (I) and acetone, Compounds (II) was employed as key intermediate in the synthesis of the new quinoxaline 1,4-di oxides derivatives.

Reaction 2-methy quinoxaline 1,4 –dioxide (II) with appropriate aldehyde by the Knoevenagel condensation produced compound (III-VI), When amounts 1 mol of compound (II) and 3 mol (Furfural) or (2-thiophenecarboxaldehyde) or (2-pyridinecarboxaldehyde) or equimolar amount of (cinnamaldehyde) were reacted in the presence of 5% sodium hydroxide were obtained as yellow crystalline products (III-VI).



a) ethanol, 2-Pyridinecarboxaldehyde, 25C°, 1h **b)** ethanol,Cinnamaldehyde, 25C°,2h **C)** ethanol, 2-Thiophenecarboxaldehyde, 25 C°, 4h **d)** ethanol,furfural,25C°,3h

Scheme 1: Method of preparation of the target quinoxaline 1,4-di oxides derivatives (III-VI)

4. Biological Evaluation

4.1. Antileishmanial Activity

In vitro evaluation of anti-leishmania activity is significantly affected by substituents on the quinoxaline nucleus [15]. The compound (III) were evaluated for Antileishmanial activity against *L. tropica* parasite (promastiogote).

4.2. *In vitro* Antileishmanial Drug Assay

Study effect of compound (III) on reproducibility of promastigote *in vitro*

12 ml of 1640-RPMI medium each containing 2×10^6 motifs of *Leishmania tropica* were prepared. We distributed the previous medium over the wells of the planting plates, contain 12 wells, with an amount of 975µl in each well.

We left wells F_3 and F_4 for the witness and set aside two wells of the remaining wells to study the concentrations of compound (III). We added 25µl of DMSO to the control wells. we also added 25µl of compound (III) concentrate solution 1000 µg/ml, this gives a final concentration of compound (III) in the culture well of equal to 20µg/ml. We add 25µl of compound (III) concentrated solution 400 µg/ml to obtain a final concentration of compound (III) in the culture well equal to 8 µg/ml.

We added 25µl of compound (III) concentrated solution 300 µg/ml to obtain a final concentration of compound (III) in the culture well equal to 6 µg/ml, and 25µl of compound (III) concentrated solution 200 µg/ml to obtain a final concentration of compound (III) in the culture well equal to 4µg/ml, and 25µl of compound (III) concentrated solution 100 µg/ml to obtain a final concentration of compound (III) in the culture well equal to 2 µg/ml.

The previous culture plates, after being treated as mentioned above are incubated 26C^o for 24 hours and then we perform a cell viability assay by cell proliferation kitt II.

Cell viability assay by cell proliferation kit II

Titration is based on the cleavage of tetrazolium salts XTT yellow – colored result of being metabolized by living cells. This causes an orange pigment to form Formazon dye. The intensity of its color is proportional to the number of living cells.

This transformation takes place only with the presence of live cells and the intensity of the formed color is related to the number of live cells, or exactly how effective is the mitochondrial dehydrogenase enzyme. Read dye absorbance of Formazon, dissolved in aqueous medium by reader Elisa.

cell proliferation kit II consisted of 25 ml of reagent consisting of a solution containing XTT at a concentration of 1 mg/ml and 0.5 ml of the electron binding reagent that it consists of N-methyl debenzopyrazin methyl-sulphate, dissolved at a concentration of 0.383mg/ml in a sterile PBS phosphate binder 5 ml of XTT reagent are mixed with 0.1 ml of the double electronic detector to obtain a sufficient.

Solution for the assay in 96 wells, An Elisa plate is prepared and the required number of wells shall be allocated to calibrate cell viability in the previous culture try.

100 µl of culture media containing a specified number of parasites are added to each well. Then, different concentration of compound (III) were added in the corresponding wells, and incubated for 24 hours at a temperature of 26 C^o.

Then we add 50 µl of the previous two reagents mixture to each 100 µl of the culture medium and incubate the Elisa plate for 4 hour at temperature 37 C^o

We then read the absorbance of the color formed by an Elisa reader at a wavelength of 450 nm versus reference wavelength of 450 nm.

The percentage of a viability in each well of culture wells treated with a different concentration of compound (III) with the control is calculated (DMSO) according to the following equation:

$$\text{Viability \%} = \left(\frac{\text{sample absorbance}}{\text{control absorbance}}\right) \times 100$$

Calculate IC_{50} (50% inhibitory concentration) of the parasite cell using a program Excel [Fig. 5]. The viability curve is drawn in terms of the absorbance of the concentration: 2, 4, 6, 8, 20 µg/ml. from the equation of the curve we calculate the value IC_{50}.

He gave the value IC_{50}=25.05 to the compound (III).

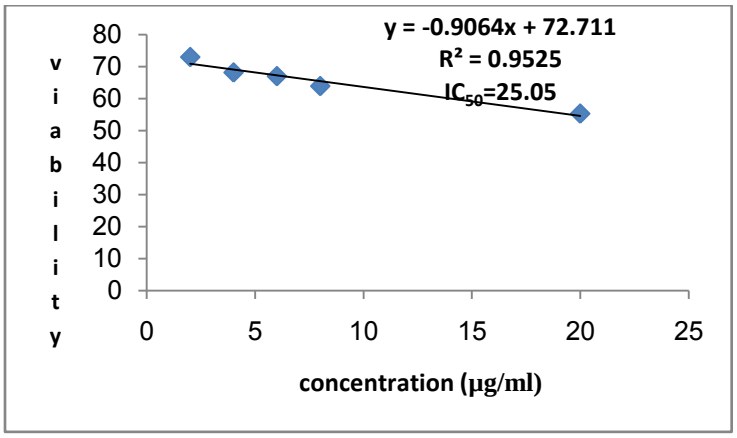


Figure 5: Effect of compound (III) on the motile forms of Leishmania

5. Conclusions

in summary, we have the synthesis of a series of quinoxaline 1,4-di oxides derivatives from 2-nitro aniline. The structure of the target products were characterized by ^{1}HNMR, MS, IR and compound 2-[2-(pyridin-2-yl)ethenyl]quinoxaline1,4-Di-N-oxides has been evaluated for effectiveness against leishmania style *L. tropica*. Drugs were then tested at increasing concentrations: 2, 4, 6, 8, 20 µg/ml. He gave the value IC_{50}=25.05.

Acknowledgments

Khalil A.L. Alabdullah is indebted to the (Department of Chemistry, Faculty of Science, University of Damascus and Department of Faculty of pharmacy, University of Damascus) This work was supported by Dr. Hassan alkhorry.

References

[1]. Barea, C., Pabón, A., Pérez-Silanes, S., Galiano, S., Gonzalez, G., Monge, A., Deharo, E. and Aldana, I., 2013. New amide derivatives of quinoxaline 1, 4-di-N-oxide with leishmanicidal and antiplasmodial activities. Molecules, 18(4), pp.4718-4727.

[2]. Chacón-Vargas, K.F., Andrade-Ochoa, S., Nogueda-Torres, B., Juárez-Ramírez, D.C., Lara-Ramírez, E.E., Mondragón-Flores, R., Monge, A., Rivera, G. and Sánchez-Torres, L.E., 2018. Isopropyl quinoxaline-7-carboxylate 1, 4-di-N-oxide derivatives induce regulated necrosis-like cell death on Leishmania (Leishmania) mexicana. Parasitology research, 117(1), pp.45-58.

[3]. Barea, C., Pabón, A., Galiano, S., Pérez-Silanes, S., Gonzalez, G., Deyssard, C., Monge, A., Deharo, E. and Aldana, I., 2012. Antiplasmodial and leishmanicidal activities of 2-cyano-3-(4-phenylpiperazine-1-carboxamido) quinoxaline 1, 4-dioxide derivatives. Molecules, 17(8), pp.9451-9461.

[4]. Mu, P., Zheng, M., Xu, M., Zheng, Y., Tang, X., Wang, Y., Wu, K., Chen, Q., Wang, L. and Deng, Y., 2014. N-oxide reduction of quinoxaline-1, 4-dioxides catalyzed by porcine aldehyde oxidase SsAOX1. Drug Metabolism and Disposition, 42(4), pp.511-519.

[5]. Carta, A., Paglietti, G., Nikookar, M.E.R., Sanna, P., Sechi, L. and Zanetti, S., 2002. Novel substituted quinoxaline 1, 4-dioxides with in vitro antimycobacterial and anticandida activity. European journal of medicinal chemistry, 37(5), pp.355-366.

[6]. Vicente, E., Villar, R., Perez-Silanes, S., Aldana, I., C Goldman, R. and Monge, A., 2011. Quinoxaline 1, 4-di-N-oxide and the potential for treating tuberculosis. Infectious Disorders-Drug Targets (Formerly Current Drug Targets-Infectious Disorders), 11(2), pp.196-204.

[7]. Gomes, J.R., Monteiro, A.R., Campos, B.B., Gomes, P. and da Silva, M.D.R., 2009. The enthalpies of dissociation of the N-O bonds in two quinoxaline derivatives. Journal of Physical Organic Chemistry, 22(1), pp.17-23.

[8]. González, M., Cerecetto, H. and Monge, A., 2007. Quinoxaline 1, 4-dioxide and phenazine 5, 10-dioxide. Chemistry and biology. In Bioactive Heterocycles V (pp. 179-211). Springer, Berlin, Heidelberg.

[9]. Carta, A., Loriga, M., Paglietti, G., Mattana, A., Fiori, P.L., Mollicotti, P., Sechi, L. and Zanetti, S., 2004. Synthesis, anti-mycobacterial, anti-trichomonas and anti-candida *in vitro* activities of 2-substituted-6, 7-difluoro-3-methylquinoxaline 1, 4-dioxides. European journal of medicinal chemistry, 39(2), pp.195-203.

[10]. Carta, A., Corona, P. and Loriga, M., 2005. Quinoxaline 1, 4-dioxide: a versatile scaffold endowed with manifold activities. Current medicinal chemistry, 12(19), pp. 2259-2272.

[11]. Amin, K.M., Ismail, M.M., Noaman, E., Soliman, D.H. and Ammar, Y.A., 2006. New quinoxaline 1, 4-di-N-oxides. Part 1: Hypoxia-selective cytotoxins and anticancer agents derived from quinoxaline 1, 4-di-N-oxides. Bioorganic & medicinal chemistry, 14(20), pp.6917-6923.

[12]. Ganley, B., Chowdhury, G., Bhansali, J., Daniels, J.S. and Gates, K.S., 2001. Redox-activated, hypoxia-selective DNA cleavage by quinoxaline 1, 4-di-N-oxide. Bioorganic & medicinal chemistry, 9(9), pp.2395-2401.

[13]. dos Santos Fernandes, G.F., Moreno-Viguri, E., Santivañez-Veliz, M., Paucar, R., Chin, C.M., Pérez-Silanes, S. and dos Santos, J.L., 2017. A Comparative Study of Conventional and Microwave-Assisted Synthesis of Quinoxaline 1, 4-di-N-oxide N-acylhydrazones Derivatives Designed as Antitubercular Drug Candidates. Journal of Heterocyclic Chemistry, 54(4), pp.2380-2388.

[14]. Moreno-Viguri, E. and Pérez-Silanes, S., 2013. Quinoxaline 1, 4-di-N-oxide derivatives: Interest in the treatment of chagas disease. Rev. Virtual Quím, 5, pp.1101-1119.

[15]. Wang X, Martínez MA, Cheng G, Liu Z, Huang L, Dai M, Chen D, Martínez-Larrañaga MR, Anadón A, Yuan Z. The critical role of oxidative stress in the toxicity and metabolism of quinoxaline 1, 4-di-N-oxides *in vitro* and *in vivo*. Drug Metabolism Reviews. 2016 Apr 2; 48(2):159-82.