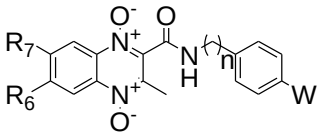
Graphical Abstract:

36 new amide quinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated as potential anti-tubercular agents by the TAACF, obtaining biological values in the same order as the reference compound rifampin.



New 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives as anti-*Mycobacterium tuberculosis* agents.

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Abstract:

Mycobacterium tuberculosis (M.Tb) is a bacillus capable of causing a chronic and fatal condition in humans known as tuberculosis (TB). It is estimated that there are 8 million new cases of TB per year and 3.1 million infected people die annually. Thirty-six new amide quinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated as potential anti-tubercular agents, obtaining biological values similar to the reference compound, Rifampin (RIF).

Key words: anti-tuberculosis agents, quinoxaline 1,4-di-*N*-oxide derivatives, *Mycobacterium tuberculosis (M.Tb.)*, Rifampin.

1. Introduction

Tuberculosis (TB) is a respiratory disease mainly caused by the bacillus *Mycobacterium tuberculosis (M.Tb.)*. It is responsible for 8 million new cases and 3.1 million deaths per year, mostly occurring in developing countries, although there are over 400,000 new cases annually in industrialized countries. As infection with human immunodeficiency virus (HIV) becomes more prevalent, tuberculosis is becoming a serious problem in developed countries as well[1], as can be observed in Figure 1. Of particular concern is the development of drug-resistant forms of the disease, Multidrug-Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB). These forms of the disease are more often fatal and are difficult and expensive to treat. It has been estimated that up to 50 million people are infected with drug-resistant forms of TB.[2,3]

Due to the problems related to MDR-TB and XDR-TB, it is necessary to develop new, potent, fast-acting anti-tuberculosis drugs with low-toxicity profiles that can be given in conjunction with drugs used to treat HIV infections.[4,5] New drugs and improved delivery methods will be integral parts of a strategy to fully control future outbreaks of TB, particularly MDR-TB, which has severely challenged the limited number of effective treatment options.[3]

Figure 1:

Quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives display excellent biological activities (antiviral, anticancer, antibacterial, anti-parasitic… etc.) with application in many different therapeutic areas.[6-8] As a result of the anti-tuberculosis research project, our group has published several papers in which synthesis and biological activity assessments of a large number of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives have been described[9-17], identifying some structural requirements for optimal activity.[13-19]

A few years ago, a group of thirty-one 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives were prepared and tested against *M.Tb.*, obtaining some interesting results.[13] Three of these compounds presented enough selectivity and good results in macrophage assay so as to merit continuation of their study. With the aim of improving the previous results, new amide quinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated as potential anti-tubercular agents, obtaining excellent results as can be observed in this report.

2. Results and Discussion

Thirty-six new 1,4-di-*N*-oxide-3-methylquinoxaline-2-carboxylic acid aryl amide derivatives were prepared through the synthetic route as follows:

First, the starting benzofuroxanes (BFX) were obtained by previously described methods.[20,21] Next, the initial *β*-acetoacetamide derivatives (**1-4**) were synthesized through acetoacetylation of corresponding aryl amines by diketene, using methanol as the solvent as shown in Scheme 1.[22]

Scheme 1.

Finally, the new 1,4-di-*N*-oxide-quinoxaline derivatives were prepared using a variation of the Beirut reaction, where the different BFX react with the corresponding *β*-acetoacetamide in the presence of calcium chloride and ethanolamine as catalysts (Scheme 2).[23]

Scheme 2.

Quinoxaline derivatives were un-substituted or substituted in positions 6 and 7 by chloro, fluoro or trifluoromethyl moiety as electron-withdrawing groups and by methyl or methoxy moiety as electron-releasing groups. When the new quinoxalines were prepared from monosubstituted-BFX, mixtures of positional isomers were obtained. Generally, it could be observed that the 7-substituted isomer prevailed over 6-substituted isomer.[24]

Table 1 summarizes the biological values of the synthesized compounds. The primary screening level determines the activity of the compounds against *M.Tb.* in H37Rv strain. Samples showing a percentage of TB growth inhibition greater than or equal to 90% are considered active and therefore, move on to the secondary screening. Active compounds are tested in order to determine the actual minimum inhibitory concentration (MIC), and simultaneously cytotoxicity (IC_{50}) in VERO cells is evaluated. Next, the MIC and IC_{50} values are formed into a ratio termed Selectivity Index (SI). If the SI level is 10 or greater, a compound is considered active at the second level.

Table 1:

As can be observed in table 1, compounds **10** and **27** were identified as the most interesting derivatives, with great antitubercular activity and low values of cytotoxicity, as can be observed by their SI values. Derivatives **9**, **13**, **28**, **29**, **36** and **38** have also shown very good results. Some structure-activity relationships could be established.

Looking at the values for compounds **9-13**, **20-22**, **27-31** and **36-40,** it can be said that, in general, the introduction of an electron-withdrawing substituent in the quinoxaline ring results in an increment in the antitubercular activity of the derivatives. On the contrary, the insertion of an electro-releasing moiety results in a reduction of this activity, as can be observed by the results

obtained for the compounds **6-8**, **15-17**, **24-26** and **33-35**. In short, it can be concluded that the insertion of an electron-withdrawing moiety in the quinoxaline ring is an essential requirement in order to improve the antitubercular activity, as established in previous works reported by the group.[13]

To further explore the SAR of these compounds, a methoxy group was substituted in the para position of the benzene ring. This derivatization led to an increase of the cytotoxicity of the compounds, as can be observed by comparing the following derivatives **5** vs **14**, **6** vs **15**, **11** vs **20**, **13** vs **22**, **24** vs **33**, **23** vs **32**, **27** vs **36** and **29** vs **38**. With the aim of improving these results, current studies are ongoing to modify not only the substituent on the benzene ring but also the aromatic ring itself.

Finally, looking at the values of the compounds **10** vs **28** and **13** vs **31**, it can be observed that lengthening the aliphatic chain can also result in a greater value of cytotoxicity. Taking into account these results and previous ones obtained by the group[13], the most suitable linker is established as a single methylene group between the carboxamide and the benzene ring.

3. Conclusions

Thirty-six new 1,4-di-*N*-oxide-3-methylquinoxaline-2-carboxylic acid aryl amide derivatives were synthesized using a variation of the Beirut reaction. Thirty-four of these compounds were evaluated against *M.Tb.* in H37Rv strain; all of them showed a TB growth inhibition percentage greater than or equal to 90%. These compounds were considered active at first level and moved on to the secondary screening, where the actual minimum inhibitory concentration (MIC) in H37Rv strain and cytotoxicity (IC_{50}) in VERO cells were determined. In this case, twenty three of the twenty-six evaluated derivatives showed an SI greater than 10, being considered to be active at the second level.

Taking into account the SI values shown in Table 1, it can be said that these values are similar to the reference compound, Rifampin (RIF). In conclusion, the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.

4. Experimental Section

4.1. General Remarks

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and elemental microanalyses (CHN).

Alugram SIL G/UV254 (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG., Düren, Germany) was used for TLC, and Silica gel 60 (0.040-0.063 mm, Merck) was used for Flash Column Chromatography. The ^{1}H MNR spectra were recorded on a Bruker 400 Ultrashield instrument (400 MHz), using TMS as internal standard and with DMSO-d_6 or $CDCl_3$ as solvents; the chemical shifts are reported in ppm (δ) and coupling constant (*J*) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), ddd (double double doblet), t (triplet), q (quadruplet) and m (multiplet). The IR spectra were recorded on a Nicolet Nexus FTIR (Thermo, Madison, USA) in KBr pellets. Elemental microanalyses were obtained on a CHN-900 Elemental Analyzer (Leco, Tres Cantos, Spain) from vacuum-dried samples. The analytical results for C, H and N were within ± 0.4 of the theoretical values. Chemicals were purchased from Panreac Química S.A. (Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Spain), Acros Organics (Janssen Pharmaceuticalaan, Geel, Belgium) and Lancaster (Bischheim-Strasbourg, France).

4.2. General procedure of synthesis

*4.2.1. Synthesis of β-acetoacetamide derivatives **(1-4)***

The corresponding aryl amines (20.0 mmol) were diluted in methanol (20.0 mL) in N_2 atmosphere and cooled in ice bath until 0ºC. Next, diketene (22.0 mmol) was added dropwise and the reaction was stirred for 2-3h. The obtained residue was precipitated with cold diethyl ether and filtered in order to obtain a red-brown solid. The compound was used without further purification.

4.2.1.1. *N-benzyl-3-oxobutanamide* **(1)**. Yield 25%. IR (KBr): 3318 (m, NH); 1617 (s, CO); 1540 (s, CO). ^{1}H MNR (400 MHz, $CDCl_3$) δ ppm: 9.52 (bs, 1H, NH); 7.33-7.21 (m, 5H, Ph); 4.56-4.35 (m, 4H, CH_2-NH-CO-CH_2-CO); 1.89 (s, 3H, CH_3).

4.2.1.2. *N-(4-methoxybenzyl)-3-oxobutanamide* **(2).** Yield 77%. IR (KBr): 3317 (s, NH); 1619 (s, CO); 1539 (s, CO). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.46 (t, 1H, $J_{NH\text{-}CH2}$=5.0 Hz, NH); 7.20 (d, 2H, $J_{H2\text{-}H3}$=8.6 Hz, H_2+H_6); 6.89 (d, 2H, H_3+H_5); 4.22 (d, 2H, CH_2-NH); 3.73 (s, 2H, CO-CH_2-CO); 3.35 (s, 3H, OCH_3); 2.15 (s, 3H, CH_3).

4.2.1.3. *3-Oxo-N-phenethylbutanamide* **(3)**. Yield 46%. IR (KBr): 3257 (s, NH); 1714 (s, CO); 1644 (s, CO). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.13 (t, 1H, $J_{NH\text{-}CH2}$=5.2 Hz, NH); 7.34-7.14 (m, 5H, Ph); 3.33-3.30 (m, 2H, CH_2-NH); 3.27 (s, 2H, CO-CH_2-CO); 2.72 (t, 2H, $J_{CH2\text{-}Ph}$=7.2 Hz, CH_2-Ph); 2.10 (s, 3H, CH_3).

4.2.1.4. *N-(4-methoxyphenethyl)-3-oxobutanamide* **(4).** Yield 56%. IR (KBr): 3263 (m, NH); 1719 (m, CO); 1641 (s, CO). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.10 (t, 1H, $J_{NH\text{-}CH2}$=5.3 Hz, NH); 7.13 (d, 2H, $J_{H2\text{-}H3}$=8.4 Hz, H_2+H_6); 6.85 (d, 2H, H_3+H_5); 3.72 (s, 3H, OCH_3); 3.32-3.18 (m, 4H, CH_2-NH-CO-CH_2-CO); 2.65 (t, 2H, $J_{CH2\text{-}Ph}$=7.3 Hz, CH_2-Ph); 2.10 (s, 3H, CH_3).

4.2.2. Synthesis of 1,4-di-N-oxide-3-methyl-quinoxaline-2-carboxylic acid aryl amide derivatives **(5-40)**

The corresponding BFX (1.0 mmol) and the corresponding *β*-acetoacetamide (1.2 mmol) were dissolved in the minimum amount of methanol. Next, calcium chloride (0.1 mmol) and ethanolamine (5 drops) were added as catalyst, as described by Stumm and Niclas.[23] The reaction was stirred at room temperature from 1 to 5 hours, filtered, and washed with cold ethanol. The solid was dissolved in dichloromethane and quenched with water. The organic phase was dried with sodium sulphate and filtered. The solvent was removed in vacuo and precipitated with cold diethyl ether in order to obtain a yellow solid. The solid was purified by column chromatography, if necessary.

4.2.2.1. *1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide* **(5).** Yield: 47%. IR (KBr): 3218 (m, NH); 1677 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.36 (t, 1H, $J_{NH\text{-}CH2}$=5.8 Hz, NH); 8.52-8.49 (m, 2H, H_5+H_8); 8.02-7.95 (m, 2H, H_6+H_7); 7.46-7.28 (m, 5H, Ph); 4.57 (d, 2H, CH_2); 2.43 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{15}N_3O_3$: C: 66.02%; H: 4.85%; N: 13.59%; Found: C: 66.02%; H: 4.96%; N: 13.69%.

4.2.2.2. *3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-benzylamide* **(6).** Yield: 1.3%. IR (KBr): 3222 (m, NH); 1661 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.37 (t, 1H, $J_{NH\text{-}CH2}$=5.9 Hz, NH); 8.39 (d, 1H, $J_{H5\text{-}H6}$=8.8 Hz, H_5); 8.31 (d, 1H, $J_{H8\text{-}H6}$=1.5 Hz, H_8); 7.83 (dd, 1H, H_6); 7.46-7.28 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 2.59 (s, 3H, $CH_{3\text{-}C3}$); 2.41 (s, 3H, $CH_{3\text{-}C7}$). Calculated analysis for $C_{18}H_{17}N_3O_3$: C: 66.87%; H: 5.26%; N: 13.00%; Found: C: 66.79%; H: 5.26%; N: 12.91%.

4.2.2.3. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-benzylamide **(7).** Yield: 21%. IR (KBr): 3199 (m, NH); 1679 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, $CDCl_3$) δ ppm: 8.94 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.05 (s, 1H, H_5); 7.98 (s, 1H, H_8); 7.49-7.34 (m, 5H, Ph); 4.67 (d, 2H, CH_2); 2.65 (s, 3H, $CH_{3\text{-}C3}$); 2.51 (s, 3H, $CH_{3\text{-}C7}$); 2.48 (s, 3H, $CH_{3\text{-}C6}$). Calculated analysis for $C_{19}H_{19}N_3O_3$: C: 67.66%; H: 5.64%; N: 12.46%; Found: C: 67.89%; H: 5.50%; N: 12.45%.

4.2.2.4. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(8).** Yield: 8%. IR (KBr): 3276 (m, NH); 1655 (s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.37 (t, 1H, $J_{NH\text{-}CH2}$=5.9 Hz, NH); 8.42 (d, 1H, $J_{H5\text{-}H6}$=9.5 Hz, H_5); 7.81 (d, 1H, $J_{H8\text{-}H6}$=2.5 Hz, H_8); 7.60 (dd, 1H, H_6); 7.47-7.28 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 4.00 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_4$: C: 63.53%; H: 5.00%; N: 12.35%. Found: C: 63.92%; H: 5.05%; N: 12.48%.

4.2.2.5. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(9).** Yield: 35%. IR (KBr): 3235 (m, NH); 1669 (s, CO); 1321 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.55-8.52 (m, 2H, H_5+H_8); 8.49 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 7.81 (dd, 1H, $J_{H6\text{-}H5}$=9.2 Hz, $J_{H6\text{-}H8}$=2.2 Hz, H_6); 7.45-7.32 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.79 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{14}ClN_3O_3$: C: 59.38%; H: 4.07%; N: 12.23%; Found: C: 59.06%; H: 4.19%; N: 12.13%.

4.2.2.6. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(10).** Yield: 61%. IR (KBr): 3274 (m, NH); 1649 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.72 (s, 1H, H_5); 8.68 (s, 1H, H_8); 8.41(t, 1H, $J_{NH\text{-}CH2}$=5.8 Hz, NH); 7.44-7.32 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.79 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_4$: C: 54.11%; H: 3.44%; N: 11.14%; Found: C: 54.08%; H: 3.38%; N: 10.87%.

4.2.2.7. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(11).** Yield: 45%. IR (KBr): 3300 (m, NH); 1671 (s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.61 (dd, 1H, $J_{H5\text{-}H6}$=9.5 Hz, $J_{H5\text{-}F}$=5.0 Hz, H_5); 8.52 (d, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.19 (dd, 1H, $J_{H8\text{-}F}$=8.5 Hz, $J_{H8\text{-}H6}$=2.7 Hz, H_8); 7.62 (ddd, 1H, $J_{H6\text{-}F}$=7.3 Hz, H_6); 7.46-7.28 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.77 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{14}FN_3O_3$: C: 62.37%; H: 4.28%; N: 12.84%; Found: C: 62.28%; H: 4.32%; N: 12.96%.

4.2.2.8. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(12).** Yield: 38%. IR (KBr): 3292 (m, NH); 1670 (s, CO); 1529 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6): 9.38 (t, 1H, $J_{NH\text{-}CH2}$=6.0 Hz, NH); 8.59-8.48 (m, 2H, H_5+H_8); 7.46-7.26 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 2.42 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{13}F_2N_3O_3$: C: 59.13%; H: 3.77%; N: 12.17%; Found: C: 59.36%; H: 3.62%; N: 11.90%

4.2.2.9. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-benzylamide **(13).** Yield: 28%. IR (KBr): 3294(m, NH); 1670(s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.92 (d, 1H, $J_{H8\text{-}H6}$=1.7 Hz, H_8); 8.70 (d, 1H, $J_{H5\text{-}H6}$=9.0 Hz, H_5); 8.38 (bs, 1H, NH); 8.03 (dd, 1H, H_6); 7.45-7.32 (m, 5H, Ph); 4.75 (d, 2H, CH_2); 2.82 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{14}F_3N_3O_3$: C: 57.29%; H: 3.71%; N: 11.14%; Found: C: 57.25%; H: 3.62%; N: 11.15%.

4.2.2.10. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(14).** Yield: 82%. IR (KBr): 3174 (m, NH); 1674 (f, CO); 1329 (f, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.26 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.52-8.50 (m, 2H, H_8+H_5); 8.02-7.95 (m, 2H, H_6+H_7); 7.37 (d, 2H, $J_{H2'\text{-}H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.95 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.42 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_4$: C: 63.71%; H: 5.05%; N: 12.38%; Found: C: 63.43%; H: 5.11%; N: 12.03%

4.2.2.11. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(15**). Yield: 71%. IR (KBr): 3206 (m, NH); 1674 (f, CO); 1326 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.27 (t, 1H, $J_{NH\text{-}CH2}$=5.9 Hz, NH); 8.39 (d, 1H, $J_{H5\text{-}H6}$=8.8 Hz, H_5); 8.30 (s, 1H, H_8); 7.82 (dd, 1H, $J_{H6\text{-}H8}$=1.7 Hz, H_6); 7.36 (d, 2H, $J_{H2'\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$), 6.94 (d, 2H, $H_{3'}$+$H_{5'}$), 4.48 (d, 2H, CH_2), 3.76 (s, 3H, OCH_3), 2.59 (s, 3H, $CH_{3\text{-}C7}$), 2.39 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.58%; H: 5.42%; N: 11.89 %; Found: C: 64.83%; H: 5.61%; N: 11.50%.

4.2.2.12. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(16).** Yield: 64%. IR (KBr): 3210 (m, NH); 1677 (f, CO); 1332 (f, N^+O). ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 9.28 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.27 (s, 1H, H_5); 8.25 (s, 1H, H_8); 7.37 (d, 2H, $J_{H2'\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.50 (s, 6H, $CH_{3\text{-}C6}$+$CH_{3\text{-}C7}$); 2.39 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_4$: C: 65.38%; H: 5.76%; N: 11.44%; Found: C: 65.09%; H: 5.62%; N: 11.63%.

4.2.2.13. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(17).** Yield: 79%. IR (KBr): 3283 (m, NH); 1652 (f, CO); 1329 (f, N^+O). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 9.28 (t, 1H, J_{NH-CH2}=5.7 Hz, NH); 8.40 (d, 1H, J_{H5-H6}=9.5 Hz, H_5); 7.79 (d, 1H, J_{H8-H6}=2.6 Hz, H_8); 7.59 (dd, 1H, H_6); 7.37 (d, 2H, $J_{H2'-H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2), 3.99 (s, 3H, $OCH_{3\text{-}C7}$), 3.75 (s, 3H, $OCH_{3\text{-}C4'}$), 2.38 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{19}N_3O_5$: C: 61.78%; H: 5.18%; N: 11.38%; Found: 61.58%; H: 5.21%; N: 11.32%.

4.2.2.14. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(18).** Yield: 30%. IR (KBr): 3270 (m, NH); 1654 (f, CO); 1330 (f, N^+O). ^1H MNR (400 MHz, DMSO-d_6): 9.27 (t, 1H, J_{NH-CH2}=5.6 Hz, NH); 8.53-8.47 (m, 2H, H_5+H_8); 8.03 (dd, 1H, J_{H6-H5}=9.2 Hz, J_{H6-H8}=2.3 Hz, H_6); 7.36 (d, 2H, $J_{H2'-H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}ClN_3O_4$: C: 57.84%; H: 4.31%; N: 11.24%; Found: C: 57.92%; H: 4.32%; N: 11.22%.

4.2.2.15. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(19**). Yield: 18%. IR (KBr): 3199 (m, NH); 1643 (f, CO); 1323 (f, N^+O). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 9.30 (t, 1H, J_{NH-CH2}=5.8 Hz, NH); 8.67 (s, 2H, H_5+H_8); 7.35 (d, 2H, $J_{H2'-H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 1H, $H_{3'}$+$H_{5'}$), 4.48 (d, 2H, CH_2), 3.75 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_4$: C: 57.84%; H: 4.31%; N: 11.24%; Found: C: 57.92%; H: 4.32%; N: 11.22%.

4.2.2.16. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(20).** Yield: 31%. IR (KBr): 3213 (m, NH); 1677 (f, CO); 1323 (f, N^+O). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 9.28 (t, 1H, J_{NH-CH2}=5.9 Hz, NH); 8.57 (dd, 1H, J_{H5-H6}=9.5 Hz, J_{H5-F}=5.2 Hz, H_5); 8.24 (dd, 1H, J_{H8-F}=8.8, J_{H8-H6}=2.8 Hz, H_8); 7.92 (ddd, 1H, J_{H8-F}=8.0 Hz, H_6); 7.36 (d, 2H, $J_{H2'-H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}FN_3O_4$: C: 60.50%; H: 4.51%; N: 11.76%; Found: C: 60.74%; H: 4.51%; N: 11.73%.

4.2.2.17. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(21).** Yield: 71%. IR (KBr): 3199 (m, NH); 1645 (f, CO); 1333 (f, N^+O). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 9.29 (t, 1H, J_{NH-CH2}=5.8 Hz, NH); 8.56-8.50 (m, 2H, H_5+H_8); 7.36 (d, 2H, $J_{H2'-H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H,

OCH_3); 2.41 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}F_2N_3O_4$: C: 57.60%; H: 4.03%; N: 11.20%; Found: C: 57.33%; H: 3.91%; N: 11.14%.

4.2.2.18. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(22).** Yield: 78%. IR (KBr): 9.88%. IR (KBr): 3276 (m, NH); 1648 (f, CO); 1133 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.29 (t, 1H, $J_{NH\text{-}CH2}$=6.1 Hz, NH); 8.75 (s, 1H, H_5); 8.70-8.69 (m, 1H, H_8); 8.32-8.26 (m, 1H, H_6); 7.36 (d, 2H, $J_{H2'\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.95 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.44 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{16}F_3N_3O_4$: C: 56.02%; H: 3.93%; N: 10.32%; Found: C: 56.39%; H: 3.85%; N: 10.44%.

4.2.2.19. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(23).** Yield: 55%. IR (KBr): 3213 (m, NH); 1668 (s, CO); 1334 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.50-8.45 (m, 2H, H_5+H_8); 8.02-7.95 (m, 2H, H_6+H_7); 7.35-7.22 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-*CH_2*); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH_2*-Ph), 2.25 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_3$: C: 66.87%; H: 5.26%; N: 13.00%; Found: C: 66.50%; H: 5.30%; N: 12.79%.

4.2.2.20. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-phenylethylamide **(24).** Yield: 67%. IR (KBr): 3203 (m, NH); 1666 (s, CO); 1326 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.38 (d, 1H, $J_{H5\text{-}H6}$=8.8 Hz, H_5); 8.23 (s, 1H, H_8); 7.82 (d, 1H, H_6); 7.35-7.21 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-*CH_2*); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH_2*-Ph); 2.59 (s, 3H, $CH_{3\text{-}C7}$); 2.23 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{19}H_{19}N_3O_3$: C: 67.65%; H: 5.64%; N: 12.46%; Found: C: 67.28%; H: 5.87%; N: 12.16%.

4.2.2.21. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-phenylethylamide **(25).** Yield: 61%. IR (KBr): 3203 (m, NH); 1663 (s, CO); 1328 (s, N^+O^-). ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.26 (s, 1H, H_5), 8.23 (s, 1H, H_8); 7.36-7.22 (m, 5H, Ph); 3.61-3.56 (m, 2H, NH-*CH_2*); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH_2*-Ph); 2.50 (s, 6H, $CH_{3\text{-}C6}$+$CH_{3\text{-}C7}$); 2.22 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_3$: C: 68.38%; H: 5.98%; N: 11.97%; Found: C: 68.47%; H: 6.08%; N: 11.75%

4.2.2.22. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(26).** Yield: 36%. IR (KBr): 3180 (m, NH); 1665 (s, CO); 1329 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.40 (d, 1H, $J_{H5\text{-}H6}$=9.5 Hz, H_5); 7.77 (d, 1H, $J_{H8\text{-}H6}$=2.5 Hz, H_8); 7.60 (dd, 1H, H_6); 7.36-7.23 (m, 5H, Ph); 3.99 (s, 3H, OCH_3);

3.61-3.56 (m, 2H, NH-CH_2); 2.87 (t, 2H, J_{CH2-Ph}=7.02 Hz, CH_2-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.59%; H: 5.38%; N: 11.90%. Found: C: 64.46%; H: 5.44%; N: 11.66%

4.2.2.23. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(27).** Yield: 44%. IR (KBr): 3224 (m, NH); 1667 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, J_{NH-CH2}=5.2 Hz, NH); 8.49 (d, 1H, J_{H5-H6}=9.2 Hz, H_5); 8.45 (d, 1H, J_{H8-H6}=2.3 Hz, H_8); 8.03 (dd, 1H, H_6); 7.36-7.29 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, J_{CH2-Ph}=7.1 Hz, 2H, CH_2-Ph); 2.23 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}ClN_3O_3$: C: 60.42%; H: 4.47%; N: 11.75%; Found: C: 60.14%; H: 4.50%; N: 11. 58%

4.2.2.24. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(28).** Yield: 38%. IR (KBr): 3231 (m, NH); 1666 (s, CO); 1322 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 88.93 (t, 1H, J_{NH-CH2}=5.6 Hz, NH); 8.66 (s, 1H, H_5); 8.64 (s, 1H, H_8); 7.34-7.22 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, 2H, J_{CH2-Ph}=7.0 Hz, CH_2-Ph); 2.24 (s, 3H, CH_{3-C3}). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_3$: C: 55.10%; H: 3.82%; N: 10.71%; Found: C: 54.70%; H: 3.92%; N: 10.44%

4.2.2.25. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(29).** Yield: 77%. IR (KBr): 3231 (m, NH); 1669 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.92 (t, 1H, J_{NH-CH2}=5.7 Hz, NH); 8.56 (dd, 1H, J_{H5-H6}=9.5 Hz, J_{H5-F}=5.2 Hz, H_5); 8.20 (dd, 1H, J_{H8-F}=8.8 Hz, J_{H8-H6}=2.5 Hz, H_8); 7.91 (ddd, 1H, J_{H6-F}=8.0 Hz, H_6); 7.36-7.23 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, 2H, J_{CH2-Ph}=7.0 Hz, CH_2-Ph); 2.23 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}FN_3O_3$: C: 63.34%; H: 4.69%; N: 12.32%; Found: C: 63.02%; H: 4.80%; N: 12.32%.

4.2.2.26. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(30).** Yield: 39%. IR (KBr): 3240 (m, NH); 1668 (s, CO); 1332 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, J_{NH-CH2}=5.6 Hz, NH); 8.53-8.47 (m, 2H, H_6+H_8); 7.36-7.20 (m, 5H, Ph); 3.61-3.56 (m, 2H, NH-CH_2); 2.87 (t, 2H, J_{CH2-Ph}=7.1 Hz, CH_2-Ph); 2.24 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}F_2N_30_3$: C: 60.17%; H: 4.18%; N: 11.70%; Found: C: 60.32%; H: 4.34%; N: 11.87%.

4.2.2.27. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-phenylethylamide **(31).** Yield: 9%. IR (KBr): 3243 (m, NH); 1669 (s, CO); 1323 (s, N^+O^-). ^{1}H MNR

(400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{NH\text{-}CH2}$=5.4 Hz, NH); 8.74 (s, 1H, H_8); 8.66 (d, 1H, $J_{H5\text{-}H6}$=9.2 Hz, H_5); 8.27 (d, 1H, H_6); 7.33-7.31 (m, 5H, Ph); 3.58-3.63 (m, *2*H, NH-*CH_2*); 2.88 (t, $J_{CH2\text{-}Ph}$=7.0 Hz, 2H, *CH_2*-Ph); 2.27 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{16}F_3N_3O_3$: C: 58.31%; H: 4.09%; N: 10.74%; Found: C: 58.10%; H: 4.025; N: 10.76%.

4.2.2.28. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(32).** Yield: 10,90%. IR (KBr): 3212 (m, NH); 1670 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.48-8.46 (m, 2H, H_5+H_8); 8.00-7.98 (m, 2H, H_6+H_7); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$), 3.72 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-*CH_2*); 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH_2*-Ph); 2.28 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.59%; H: 5.38%; N: 11.90%; Found: C: 64.25%; H: 5.24%; N: 11.81%

4.2.2.29. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(33).** Yield: 19.11%. IR (KBr): 3209 (m, NH); 1666 (s, CO); 1329 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.38 (d, 1H, $J_{H5\text{-}H6}$=8.9 Hz, H_5); 8.26 (s, 1H, H_8); 7.81 (d, 1H, H_6); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.2 Hz, $H_{2'}$+$H_{6'}$); 6.89 (d, 2H, $H_{3'}$+$H_{5'}$), 3.72 (s, 3H, OCH_3); 3.56-3.52 (m, 2H, NH-*CH_2*); 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.0 Hz, *CH_2*-Ph); 2.58 (s, 3H, $CH_{3\text{-}C7}$); 2.25 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_4$: C: 65.40%; H: 5.72%; N: 11.44%; Found: C: 65.62%; H: 5.66%; N: 11.58%.

4.2.2.30. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(34).** Yield: 52.94%. IR (KBr): 3203 (m, NH); 1665 (s, CO); 1326 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.26 (s, 1H, H_5); 8.22 (s, 1H, H_8); 7.22 (d, 2H, $J_{H2'\text{-}H3}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.73 (s, 1H, OCH_3); 3.56-3.51 (m, 2H, NH-*CH_2*); 2.79 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH_2*-Ph); 2.46 (s, 6H, $CH_{3\text{-}C7}$+$CH_{3\text{-}C6}$); 2.25 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{21}H_{23}N_3O_4$: C: 66.14%; H: 6.04%; N: 11.02%; Found: C: 66.02%; H: 6.09%; N: 10.99%.

4.2.2.31. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(35).** Yield: 20.97%. IR (KBr): 3221 (m, NH); 1666 (s, CO); 1325 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.89 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.40 (d, 1H, $J_{H5\text{-}H6}$=9.5 Hz, H_5); 7.76 (d, 1H, $J_{H8\text{-}H6}$=2.7 Hz, H_8); 7.59 (dd, 1H, H_6); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.98 (s, 3H, $OCH_{3\text{-}C7}$); 3.72 (s, 3H, $OCH_{3\text{-}C4'}$); 3.57-3.52 (m, 2H,

NH-*CH*$_2$); 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH*$_2$-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{20}H_{21}N_3O_5$: C: 62.66%; H: 5.48%; N: 10.97%; Found: C: 62.37%; H: 5.66%; N: 10.66%

4.2.2.32. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(36).** Yield: 48.00%. IR (KBr): 3287 (m, NH); 1655 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.87 (s, 1H, NH); 8.49 (d,1H, $J_{H5\text{-}H6}$=9.3 Hz, H_5); 8.45 (d, 1H, $J_{H8\text{-}H6}$=2.1 Hz, H_8); 8.02 (dd, 1H, H_6); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.73 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-*CH*$_2$); 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.0 Hz, *CH*$_2$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{18}ClN_3O_4$: C: 58.84%; H: 4.65%; N: 10.84%; Found: C: 58.62%; H: 4.72%; N: 10.40%.

4.2.2.33. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(37).** Yield: 28.40%. IR (KBr): 3230 (m, NH); 1667 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.66 (s, 1H, H_5); 8.64 (s, 1H, H_8); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.72 (s, 1H, OCH_3); 3.57-3.52 (m, 2H, NH-*CH*$_2$); 2.79 (t, 2H, $J_{CH2\text{-}Ph}$=6.9 Hz, *CH*$_2$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{17}Cl_2N_3O_4$: C: 54.03%; H: 4.03%; N: 9.95%; Found: C: 53.66%; H: 3.92%; N: 9.65%.

4.2.2.34. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(38).** Yield: 18.76%. IR (KBr): 3223 (m, NH); 1668 (s, CO); 1330 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.56 (dd, 1H $J_{H5\text{-}H6}$=9.4 Hz, $J_{H5\text{-}F}$=5.2 Hz, H_5); 8.20 (dd, 1H, $J_{H8\text{-}F}$=8.8 Hz, $J_{H8\text{-}H6}$=2.8 Hz, H_8); 7.94-7.81 (m, 1H, H_6); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.72 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-*CH*$_2$); 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, *CH*$_2$-Ph); 2.28 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{18}FN_3O_4$: C: 61.46%; H: 4.85%; N: 11.32%; Found: C: 61.10%; H: 4.78%; N: 11.54%.

4.2.2.35. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(39).** Yield: 12.75%. IR (KBr): 3242 (m, NH); 1669 (s, CO); 1334 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.48-8.46 (m, 2H, H_5+H_8); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.4 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.76 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-*CH*$_2$); 2.79 (t, 2H, $J_{CH2\text{-}CH2}$=7.0 Hz, CH_2-*CH*$_2$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{17}F_2N_3O_4$: C: 58.61%; H: 4.37%; N: 10.80%Found: C: 58.86%; H: 4.435%; N: 10.55%.

4.2.2.36. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(40).** Yield: 6.79%. IR (KBr): 3243 (m, NH); 1669 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.74 (s, 1H, H_5); 8.66 (d, 1H, $J_{H8\text{-}H6}$=1.8 Hz, H_8); 8.27 (dd, 1H, $J_{H6\text{-}H5}$=9.1 Hz, H_6); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.71 (s, 3H, OCH_3); 3.58-3.53 (m, 2H, NH-CH_2), 2.80 (t, 2H, $J_{CH2\text{-}Ph}$=7.0 Hz, CH_2-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{20}H_{18}F_3N_3O_4$: C: 57.01%; H: 4.28%; N: 9.98%; Found: C: 57.33%; H: 4.22%; N: 9.88%.

4.3. General procedure of anti-tuberculosis activity

In vitro evaluation of the anti-tuberculosis activity was carried out at the GWL Hansen's Disease Center within the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the treatment of tuberculosis. Under the direction of the U.S. National Institute of Allergy and Infectious Disease (NIAID), the Southern Research Institute coordinates the overall program. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capability to inhibit the growth of virulent *M.Tb.*[25]

4.3.1. Determination of growth inhibition percentage via MABA

The initial screen is conducted against *M.Tb.* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[26] Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system. Compounds effecting <90% inhibition in the primary screen (MIC >6.25 μg/mL) were not further evaluated.

4.3.2. Determination of minimum inhibitory concentration (MIC) via MABA

Compounds demonstrating at least 90% inhibition in the primary screen were re-tested against *M.Tb.* H37Rv at lower concentrations in order to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampicin was used as the reference compound (RIF 0.015-0.125 μg/mL).

4.3.3. Determination of cytotoxicity in VERO cells

Compounds are screened by serial dilution to assess toxicity to a VERO cell line at concentrations less than or equal to 6.25 µg/ml or 10 times the MIC for *M.Tb.* H37Rv if sample solubility in culture medium permits. After 72 hours of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. RIF was used as the reference compound (RIF IC_{50}>100 µg/mL).

4.3.4. Determination of selectivity index (SI)

The selectivity index (SI) is defined as the ratio of the measured IC_{50} in VERO cells to the MIC for *M.Tb.* H37Rv. In general, requirements for moving a compound into *in vivo* testing include: MIC ≤ 6.25 µg/mL and SI ≥ 10 (occasionally lower). RIF was used as the reference compound (RIF SI>800).

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6. References

[1]Milstien, J. *Immunological Basis for Immunization / Module 5: Tuberculosis.* WHO/EPI/GEN/93.15 (World Health Organization) Global Programme for Vaccines and Immunization/Expanded Programme on Immunization: Switzerland 1993.

[2]TAACF: http://www.taacf.org/about-TB-background.htm

[3] NIAID: http://www3.niaid.nih.gov/topics/tuberculosis/

[4]Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Hevener, K.; Jones, V. C.; Lenaerts, A. J.; McNeil, M. R.; Wang, Y.; Franzblau, S.; Lee, R. E. *J. Med. Chem.* **2004,** *47*, 5276.

[5]Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *J. Med. Chem.* **2005,** *48*, 2019.

[6]Jan, B.; Erik De, C.; An, C.; Vikki, B.; Jörg-Peter, K. *AIDS Res Hum Retroviruses* **2000,** 16, 517.

[7]Vicente, E.; Villar, R.; Solano, B.; Burguete, A.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *An. R. Acad. Nac. Farm.* **2007,** 73, 927.

[8]Waring, M.; Ben-Hadda, T.; Kotchevar, A.; Ramdani, A.; Touzani, R.; Elkadiri, S.; Hakkou, A.; Bouakka, M.; Ellis, T. *Molecules* **2002,** 7, 641.

[9]Ortega, M. A.; Sainz, Y.; Montoya, M. E.; López de Ceráin, A.; Monge, A. *Pharmazie* **1999,** *54*, 24.

[10]Sainz, Y.; Montoya, M. E.; Martínez-Crespo, F. J.; Ortega, M. A.; López de Ceráin, A.; Monge, A. *Arzneimittel-Forsch.* **1999,** *49*, 55.

[11]Ortega, M. A.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Tirapu, I.; Aldana, I.; Monge, A. *Pharmazie* **2001,** *56*, 205.

[12]Ortega, M.A.; Sainz, Y.; Montoya, M.E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneimittel-Forsch.* **2002,** *52*, 113.

[13]Zarranz, B.; Jaso, A.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* **2003,** *11*, 2149.

[14]Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *E. J. Med. Chem.* **2003,** *38*, 791.

[15]Villar, R.; Vicente, E.; Solano, B.; Pérez-Silanes, S.; Aldana, I.; Maddry, J. A.; Lenaerts, A. J.; Franzblau, S. G.; Cho, S. H.; Monge, A.; Goldman, R. C. *J. Antimicrob. Chemother.* **2008,** *62*, 547.

[16]Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Pérez-Silanes, S.; Aldana, I.; Maddry, J. A.; Lenaerts, A. J.; Franzblau, S. G.; Cho, S. H.; Monge, A.; Goldman, R. C. *Antimicrob. Agents Chemother.* **2008,** *52*, 3321.

[17]Vicente, E.; Pérez-Silanes, S.; Lima, L.M.; Ancizu, S.; Burguete, A.; Solano, B.; Villar, R.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* **2009,** *17*, 385.

[18]Vicente, E.; Duchowicz, P. R.; Castro, E. A.; Monge, A. *J. Mol. Graphics Modell.* **2009,** *28*, 28-36.

[19]Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *Molecules* **2008,** *13*, 86.

[20]González, M.; Cerecetto, H. In *Topics in Heterocyclic Chemistry;* Khan M. T. H., Ed.; Bioactive Heterocycles IV. Benzofuroxan and Furoxan. Chemistry and Biology; Springer Berlin: Heidelberg, 2007; Vol. 10, pp 265.

[21]Ortega, M. A.; Sainz, Y.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneimittel-Forsch.* **2002,** *52*, 113.

[22]Clemens, R. J. *Chem. Rev.* **1986,** *86*, 241.

[23]Stumm, G.; Niclas, H. J. *J. Prakt. Chem.* **1989,** *331*, 736.

[24]Cheeseman, G. W. H.; Cookson R. F. In *Condensed Pyrazines*; Willey J. and sons, Ed.; New York: NY, USA, 1979; Vol. 35, pp 35.

[25]TAACF: http://www.taacf.org/Process-text.htm#assays

[26]Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemotherapy* **1997,** *41*, 1004.

New 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives as anti-*Mycobacterium tuberculosis* agents.

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Abstract:

Mycobacterium tuberculosis (M.Tb) is a bacillus capable of causing a chronic and fatal condition in humans known as tuberculosis (TB). It is estimated that there are 8 million new cases of TB per year and 3.1 million infected people die annually. Thirty-six new amide quinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated as potential anti-tubercular agents, obtaining biological values similar to the reference compound, Rifampin.

Key words: anti-tuberculosis agents, quinoxaline 1,4-di-*N*-oxide derivatives, *Mycobacterium tuberculosis (M.Tb.)*, Rifampin.

1. Introduction

Tuberculosis (TB) is a respiratory disease mainly caused by the bacillus *Mycobacterium tuberculosis (M.Tb.)*. It is responsible for 8 million new cases and 3.1 million deaths per year, mostly occurring in developing countries, although there are over 400.000 new cases annually in industrialized countries. As infection with human immunodeficiency virus (HIV) becomes more prevalent, tuberculosis is becoming a serious problem in developed countries as well[1], as can be observed in Figure 1. Of particular concern is the development of drug-resistant forms of the disease, Multidrug-Resistant Tuberculosis (MDR-TB) and Extensively Drug-Resistant Tuberculosis (XDR-TB). These forms of the disease are more often fatal and are difficult and expensive to treat. It has been estimated that up to 50 million people are infected with drug-resistant forms of TB.[2,3]

Because of the problems related to MDR-TB and XDR-TB, it is necessary to develop new, potent, fast-acting anti-tuberculosis drugs with low-toxicity profiles that can be given in conjunction with drugs used to treat HIV infections.[4,5] New drugs and improved delivery methods will be integral parts of a strategy to fully control future outbreaks of TB, particularly MDR-TB, which has severely challenged the limited number of effective treatment options.[3]

Figure 1:

Quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives display excellent biological activities (antiviral, anticancer, antibacterial, anti-parasitic… etc.) with application in many different therapeutic areas.[6-8] As a result of the anti-tuberculosis research project, our group has published several papers in which synthesis and biological activity assessments of a large number of quinoxaline and quinoxaline 1,4-di-*N*-oxide derivatives have been described[9-17], identifying some structural requirements for optimal activity.[13-19]

A few years ago, a group of thirty-one 3-methylquinoxaline-2-carboxamide 1,4-di-*N*-oxide derivatives were prepared and tested against *M.Tb.*, obtaining some interesting results.[13] Three of these compounds presented enough selectivity and good results in macrophage assay so as to merit continuation of their study. With the aim of improving the previous results, new amide quinoxaline 1,4-di-*N*-oxide derivatives have been synthesized and evaluated as potential anti-tubercular agents, obtaining excellent results as can be observed in this report.

2. Results and Discussion

Thirty-six new 1,4-di-*N*-oxide-3-methylquinoxaline-2-carboxylic acid aryl amide derivatives were prepared through the synthetic route as follows:

First, the starting benzofuroxanes (BFX) were obtained by previously described methods.[20,21] Next, the initial *β*-acetoacetamide derivatives (**1-4**) were synthesized through acetoacetylation of corresponding aryl amines by diketene, using methanol as the solvent as shown in Scheme 1.[22]

Scheme 1.

Finally, the new 1,4-di-*N*-oxide-quinoxaline derivatives were prepared using a variation of the Beirut reaction, where the different BFX react with the corresponding *β*-acetoacetamide in the presence of calcium chloride and ethanolamine as catalysts (Scheme 2).[23]

Scheme 2.

Quinoxaline derivatives were un-substituted or substituted in positions 6 and 7 by chloro, fluoro or trifluoromethyl moiety as electron-withdrawing groups and by methyl or methoxy moiety as electron-releasing groups. When the new quinoxalines were prepared from monosubstituted-BFX, mixtures of positional isomers were obtained. Generally, it could be observed that the 7-substituted isomer prevailed over 6-substituted isomer.[24]

Table 1 summarizes the biological values of the synthesized compounds. The primary screening level determinates the activity of the compounds against *M.Tb.* in H37Rv strain. Samples showing a percentage of TB growth inhibition greater than or equal to 90% are considered active and therefore, move on to the secondary screening. Active compounds are tested in order to determine the actual minimum inhibitory concentration (MIC), and simultaneously cytotoxicity (IC_{50}) in VERO cells is evaluated. Next, the MIC and IC_{50} values are formed into a ratio termed Selectivity Index (SI). If the SI level is 10 or greater, a compound is considered active at the second level.

Table 1:

3. Conclusions

Thirty-six new 1,4-di-*N*-oxide-3-methylquinoxaline-2-carboxylic acid aryl amide derivatives were synthesized using a variation of the Beirut reaction. Thirty-four of these compounds were evaluated against *M.Tb.* in H37Rv strain; all of them showed a TB growth inhibition percentage greater than or equal to 90%. These compounds were considered active at first level and moved on to the secondary screening, where the actual minimum inhibitory concentration (MIC) in H37Rv

strain and cytotoxicity (IC_{50}) in VERO cells were determinated. In this case, twenty three of the twenty-six evaluated derivatives, showed a SI greater to 10, being considered active at the second level.

The compounds **10** and **27** were identified as the most interesting derivatives, with great antitubercular activity and low values of cytotoxicity as observed by their SI values (Table 1). The derivatives **9**, **13**, **28**, **29**, **36** and **38** have also shown very good results. Some structure-activity relationships could be established. Looking at the values of compounds **9-13**, **20-22**, **27-31** and **36-40,** it can be said that, in general, the introduction of an electron-withdrawing substituent in the quinoxaline ring results in an increment in the antitubercular activity of the derivatives. On the contrary, the insertion of an electro-releasing moiety results in a reduction of this activity as can be observed by the results obtained for the compounds **6-8**, **15-17**, **24-26** and **33-35**. Comparing the cytotoxicity data of the derivatives **5** vs **14**, **6** vs **15**, **11** vs **20**, **13** vs **22**, **24** vs **33**, **23** vs **32**, **27** vs **36** and **29** vs **38** it can be concluded that the substitution of the para position of the benzene ring with a methoxy group increases the cytotoxicity of the compounds. Finally, looking at the values of the compounds **10** vs **28** and **13** vs **31**, it can be observed that lengthening the aliphatic chain can also result in a greater value of cytotoxicity.

Taking into account the SI values shown in Table 1, it can be said that these values are similar to the reference compound, Rifampin (RIF). In conclusion, the potency, selectivity, and low cytotoxicity of these compounds make them valid leads for synthesizing new compounds that possess better activity.

4. Experimental Section

4.1. General Remarks

All of the synthesized compounds were chemically characterized by thin layer chromatography (TLC), infrared (IR), proton nuclear magnetic resonance (^{1}H-NMR) and elemental microanalyses (CHN).

Alugram SIL G/UV254 (Layer: 0.2 mm) (Macherey-Nagel GmbH & Co. KG., Düren, Germany) was used for TLC, and Silica gel 60 (0.040-0.063 mm, Merck) was used for Flash Column Chromatography. The ^{1}H MNR spectra were recorded on a Bruker 400 Ultrashield instrument (400 MHz), using TMS as internal standard and with DMSO-d_6 or $CDCl_3$ as solvents; the chemical shifts are reported in ppm (δ) and coupling constant (*J*) values are given in Hertz (Hz). Signal multiplicities are represented by: s (singlet), bs (broad singlet), d (doublet), dd (double doublet), ddd (double double doblet), t (triplet), q (quadruplet) and m (multiplet). The IR spectra

were recorded on a Nicolet Nexus FTIR (Thermo, Madison, USA) in KBr pellets. Elemental microanalyses were obtained on a CHN-900 Elemental Analyzer (Leco, Tres Cantos, Spain) from vacuum-dried samples. The analytical results for C, H and N, were within ± 0.4 of the theoretical values. Chemicals were purchased from Panreac Química S.A. (Barcelona, Spain), Sigma-Aldrich Química, S.A. (Alcobendas, Spain), Acros Organics (Janssen Pharmaceuticalaan, Geel, Belgium) and Lancaster (Bischheim-Strasbourg, France).

4.2. General procedure of synthesis

*4.2.1. Synthesis of β-acetoacetamide derivatives **(1-4)***

The corresponding aryl amines (20.0 mmol) were diluted in methanol (20.0 mL) in N_2 atmosphere and cooled in ice bath until 0ºC. Next, diketene (22.0 mmol) was added dropwise and the reaction was stirred for 2-3h. The obtained residue was precipitated with cold diethyl ether and filtered in order to obtain a red-brown solid. The compound was used without further purification.

4.2.1.1. *N-benzyl-3-oxobutanamide* **(1)**. Yield 25%. IR (KBr): 3318 (m, NH); 1617 (s, CO); 1540 (s, CO). ^{1}H MNR (400 MHz, $CDCl_3$) δ ppm: 9.52 (bs, 1H, NH); 7.33-7.21 (m, 5H, Ph); 4.56-4.35 (m, 4H, C$\underline{H_2}$-NH-CO-C$\underline{H_2}$-CO); 1.89 (s, 3H, CH_3).

4.2.1.2. *N-(4-methoxybenzyl)-3-oxobutanamide* **(2).** Yield 77%. IR (KBr): 3317 (s, NH); 1619 (s, CO); 1539 (s, CO). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 8.46 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.0 Hz, NH); 7.20 (d, 2H, $J_{\underline{H2}\text{-}H3}$=8.6 Hz, H_2+H_6); 6.89 (d, 2H, H_3+H_5); 4.22 (d, 2H, C$\underline{H_2}$-NH); 3.73 (s, 2H, CO-C$\underline{H_2}$-CO); 3.35 (s, 3H, OCH_3); 2.15 (s, 3H, CH_3).

4.2.1.3. *3-Oxo-N-phenethylbutanamide* **(3)**. Yield 46%. IR (KBr): 3257 (s, NH); 1714 (s, CO); 1644 (s, CO). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 8.13 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.2 Hz, NH); 7.34-7.14 (m, 5H, Ph); 3.33-3.30 (m, 2H, C$\underline{H_2}$-NH); 3.27 (s, 2H, CO-C$\underline{H_2}$-CO); 2.72 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.2 Hz, C$\underline{H_2}$-Ph); 2.10 (s, 3H, CH_3).

4.2.1.4. *N-(4-methoxyphenethyl)-3-oxobutanamide* **(4).** Yield 56%. IR (KBr): 3263 (m, NH); 1719 (m, CO); 1641 (s, CO). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 8.10 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.3 Hz, NH); 7.13 (d, 2H, $J_{\underline{H2}\text{-}H3}$=8.4 Hz, H_2+H_6); 6.85 (d, 2H, H_3+H_5); 3.72 (s, 3H, OCH_3); 3.32-3.18 (m, 4H, C$\underline{H_2}$-NH-CO-C$\underline{H_2}$-CO); 2.65 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.3 Hz, C$\underline{H_2}$-Ph); 2.10 (s, 3H, CH_3).

4.2.2. Synthesis of 1,4-di-N-oxide-3-methyl-quinoxaline-2-carboxylic acid aryl amide derivatives **(5-40)**

The corresponding BFX (1.0 mmol) and the corresponding *β*-acetoacetamide (1.2 mmol) were dissolved in the minimum amount of methanol. Next, calcium chloride (0.1 mmol) and ethanolamine (5 drops) were added as catalyst, as described by Stumm and Niclas.[23] The reaction was stirred at room temperature from 1 to 5 hours, filtered and washed with cold ethanol. The solid was dissolved in dichloromethane and quenched with water. The organic phase was dried with sodium sulphate and filtered. The solvent was removed in vacuo and precipitated with cold diethyl ether in order to obtain a yellow solid. The solid was purified by column chromatography, if necessary.

4.2.2.1. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(5).** Yield: 47%. IR (KBr): 3218 (m, NH); 1677 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.36 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.8 Hz, NH); 8.52-8.49 (m, 2H, H_5+H_8); 8.02-7.95 (m, 2H, H_6+H_7); 7.46-7.28 (m, 5H, Ph); 4.57 (d, 2H, CH_2); 2.43 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{15}N_3O_3$: C: 66.02%; H: 4.85%; N: 13.59%; Found: C: 66.02%; H: 4.96%; N: 13.69%.

4.2.2.2. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-benzylamide **(6).** Yield: 1.3%. IR (KBr): 3222 (m, NH); 1661 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.37 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.9 Hz, NH); 8.39 (d, 1H, $J_{\underline{H5}\text{-}H6}$=8.8 Hz, H_5); 8.31 (d, 1H, $J_{\underline{H8}\text{-}H6}$=1.5 Hz, H_8); 7.83 (dd, 1H, H_6); 7.46-7.28 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 2.59 (s, 3H, $CH_{3\text{-}C3}$); 2.41 (s, 3H, $CH_{3\text{-}C7}$). Calculated analysis for $C_{18}H_{17}N_3O_3$: C: 66.87%; H: 5.26%; N: 13.00%; Found: C: 66.79%; H: 5.26%; N: 12.91%.

4.2.2.3. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-benzylamide **(7).** Yield: 21%. IR (KBr): 3199 (m, NH); 1679 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, $CDCl_3$) δ ppm: 8.94 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.05 (s, 1H, H_5); 7.98 (s, 1H, H_8); 7.49-7.34 (m, 5H, Ph); 4.67 (d, 2H, CH_2); 2.65 (s, 3H, $CH_{3\text{-}C3}$); 2.51 (s, 3H, $CH_{3\text{-}C7}$); 2.48 (s, 3H, $CH_{3\text{-}C6}$). Calculated analysis for $C_{19}H_{19}N_3O_3$: C: 67.66%; H: 5.64%; N: 12.46%; Found: C: 67.89%; H: 5.50%; N: 12.45%.

4.2.2.4. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(8).** Yield: 8%. IR (KBr): 3276 (m, NH); 1655 (s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.37 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.9 Hz, NH); 8.42 (d, 1H, $J_{\underline{H5}\text{-}H6}$=9.5 Hz, H_5); 7.81 (d, 1H, $J_{\underline{H8}\text{-}H6}$=2.5

Hz, H_8); 7.60 (dd, 1H, H_6); 7.47-7.28 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 4.00 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_4$: C: 63.53%; H: 5.00%; N: 12.35%. Found: C: 63.92%; H: 5.05%; N: 12.48%.

4.2.2.5. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(9).** Yield: 35%. IR (KBr): 3235 (m, NH); 1669 (s, CO); 1321 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.55-8.52 (m, 2H, H_5+H_8); 8.49 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 7.81 (dd, 1H, $J_{\underline{H6}\text{-}H5}$=9.2 Hz, $J_{\underline{H6}\text{-}H8}$=2.2 Hz, H_6); 7.45-7.32 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.79 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{14}ClN_3O_3$: C: 59.38%; H: 4.07%; N: 12.23%; Found: C: 59.06%; H: 4.19%; N: 12.13%.

4.2.2.6. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(10).** Yield: 61%. IR (KBr): 3274 (m, NH); 1649 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.72 (s, 1H, H_5); 8.68 (s, 1H, H_8); 8.41(t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.8 Hz, NH); 7.44-7.32 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.79 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_4$: C: 54.11%; H: 3.44%; N: 11.14%; Found: C: 54.08%; H: 3.38%; N: 10.87%.

4.2.2.7. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(11).** Yield: 45%. IR (KBr): 3300 (m, NH); 1671 (s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.61 (dd, 1H, $J_{\underline{H5}\text{-}H6}$=9.5 Hz, $J_{\underline{H5}\text{-}F}$=5.0 Hz, H_5); 8.52 (d, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.19 (dd, 1H, $J_{\underline{H8}\text{-}F}$=8.5 Hz, $J_{\underline{H8}\text{-}H6}$=2.7 Hz, H_8); 7.62 (ddd, 1H, $J_{\underline{H6}\text{-}F}$=7.3 Hz, H_6); 7.46-7.28 (m, 5H, Ph); 4.74 (d, 2H, CH_2); 2.77 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{14}FN_3O_3$: C: 62.37%; H: 4.28%; N: 12.84%; Found: C: 62.28%; H: 4.32%; N: 12.96%.

4.2.2.8. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-benzylamide **(12).** Yield: 38%. IR (KBr): 3292 (m, NH); 1670 (s, CO); 1529 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6): 9.38 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=6.0 Hz, NH); 8.59-8.48 (m, 2H, H_5+H_8); 7.46-7.26 (m, 5H, Ph); 4.56 (d, 2H, CH_2); 2.42 (s, 3H, CH_3). Calculated analysis for $C_{17}H_{13}F_2N_3O_3$: C: 59.13%; H: 3.77%; N: 12.17%; Found: C: 59.36%; H: 3.62%; N: 11.90%

4.2.2.9. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-benzylamide **(13).** Yield: 28%. IR (KBr): 3294(m, NH); 1670(s, CO); 1323 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.92 (d, 1H, $J_{\underline{H8}\text{-}H6}$=1.7 Hz, H_8); 8.70 (d, 1H, $J_{\underline{H5}\text{-}H6}$=9.0 Hz, H_5); 8.38 (bs, 1H, NH); 8.03 (dd, 1H, H_6); 7.45-7.32 (m, 5H, Ph); 4.75 (d, 2H, CH_2); 2.82 (s, 3H, CH_3).

Calculated analysis for $C_{18}H_{14}F_3N_3O_3$: C: 57.29%; H: 3.71%; N: 11.14%; Found: C: 57.25%; H: 3.62%; N: 11.15%.

4.2.2.10. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(14).** Yield: 82%. IR (KBr): 3174 (m, NH); 1674 (f, CO); 1329 (f, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.26 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.52-8.50 (m, 2H, H_8+H_5); 8.02-7.95 (m, 2H, H_6+H_7); 7.37 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.95 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.42 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_4$: C: 63.71%; H: 5.05%; N: 12.38%; Found: C: 63.43%; H: 5.11%; N: 12.03%

4.2.2.11. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(15).** Yield: 71%. IR (KBr): 3206 (m, NH); 1674 (f, CO); 1326 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.27 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.9 Hz, NH); 8.39 (d, 1H, $J_{\underline{H5}\text{-}H6}$=8.8 Hz, H_5); 8.30 (s, 1H, H_8); 7.82 (dd, 1H, $J_{\underline{H6}\text{-}H8}$=1.7 Hz, H_6); 7.36 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$), 6.94 (d, 2H, $H_{3'}$+$H_{5'}$), 4.48 (d, 2H, CH_2), 3.76 (s, 3H, OCH_3), 2.59 (s, 3H, $CH_{3\text{-}C7}$), 2.39 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.58%; H: 5.42%; N: 11.89 %; Found: C: 64.83%; H: 5.61%; N: 11.50%.

4.2.2.12. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(16).** Yield: 64%. IR (KBr): 3210 (m, NH); 1677 (f, CO); 1332 (f, N^+O). ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 9.28 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.27 (s, 1H, H_5); 8.25 (s, 1H, H_8); 7.37 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.50 (s, 6H, $CH_{3\text{-}C6}$+$CH_{3\text{-}C7}$); 2.39 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_4$: C: 65.38%; H: 5.76%; N: 11.44%; Found: C: 65.09%; H: 5.62%; N: 11.63%.

4.2.2.13. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(17).** Yield: 79%. IR (KBr): 3283 (m, NH); 1652 (f, CO); 1329 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 9.28 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.40 (d, 1H, $J_{\underline{H5}\text{-}H6}$=9.5 Hz, H_5); 7.79 (d, 1H, $J_{\underline{H8}\text{-}H6}$=2.6 Hz, H_8); 7.59 (dd, 1H, H_6); 7.37 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2), 3.99 (s, 3H, $OCH_{3\text{-}C7}$), 3.75 (s, 3H, $OCH_{3\text{-}C4'}$), 2.38 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{19}N_3O_5$: C: 61.78%; H: 5.18%; N: 11.38%; Found: 61.58%; H: 5.21%; N: 11.32%.

4.2.2.14. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(18).** Yield: 30%. IR (KBr): 3270 (m, NH); 1654 (f, CO); 1330 (f, N^+O). ^{1}H

MNR (400 MHz, DMSO-d_6): 9.27 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.53-8.47 (m, 2H, H_5+H_8); 8.03 (dd, 1H, $J_{\underline{H6}\text{-}H5}$=9.2 Hz, $J_{\underline{H6}\text{-}H8}$=2.3 Hz, H_6); 7.36 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}ClN_3O_4$: C: 57.84%; H: 4.31%; N: 11.24%; Found: C: 57.92%; H: 4.32%; N: 11.22%.

4.2.2.15. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(19).** Yield: 18%. IR (KBr): 3199 (m, NH); 1643 (f, CO); 1323 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 9.30 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.8 Hz, NH); 8.67 (s, 2H, H_5+H_8); 7.35 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 1H, $H_{3'}$+$H_{5'}$), 4.48 (d, 2H, CH_2), 3.75 (s, 3H, OCH_3), 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_4$: C: 57.84%; H: 4.31%; N: 11.24%; Found: C: 57.92%; H: 4.32%; N: 11.22%.

4.2.2.16. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(20).** Yield: 31%. IR (KBr): 3213 (m, NH); 1677 (f, CO); 1323 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d6) δ ppm: 9.28 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.9 Hz, NH); 8.57 (dd, 1H, $J_{\underline{H5}\text{-}H6}$=9.5 Hz, $J_{\underline{H5}\text{-}F}$=5.2 Hz, H_5); 8.24 (dd, 1H, $J_{\underline{H8}\text{-}F}$=8.8, $J_{\underline{H8}\text{-}H6}$=2.8 Hz, H_8); 7.92 (ddd, 1H, $J_{\underline{H8}\text{-}F}$=8.0 Hz, H_6); 7.36 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.7 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.40 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}FN_3O_4$: C: 60.50%; H: 4.51%; N: 11.76%; Found: C: 60.74%; H: 4.51%; N: 11.73%.

4.2.2.17. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(21).** Yield: 71%. IR (KBr): 3199 (m, NH); 1645 (f, CO); 1333 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.29 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.8 Hz, NH); 8.56-8.50 (m, 2H, H_5+H_8); 7.36 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.94 (d, 2H, $H_{3'}$+$H_{5'}$); 4.48 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.41 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{15}F_2N_3O_4$: C: 57.60%; H: 4.03%; N: 11.20%; Found: C: 57.33%; H: 3.91%; N: 11.14%.

4.2.2.18. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-(4-methoxybenzyl)-amide **(22).** Yield: 78%. IR (KBr): 9.88%. IR (KBr): 3276 (m, NH); 1648 (f, CO); 1133 (f, N^+O). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 9.29 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=6.1 Hz, NH); 8.75 (s, 1H, H_5); 8.70-8.69 (m, 1H, H_8); 8.32-8.26 (m, 1H, H_6); 7.36 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.95 (d, 2H, $H_{3'}$+$H_{5'}$); 4.49 (d, 2H, CH_2); 3.76 (s, 3H, OCH_3); 2.44 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{16}F_3N_3O_4$: C: 56.02%; H: 3.93%; N: 10.32%; Found: C: 56.39%; H: 3.85%; N: 10.44%.

4.2.2.19. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(23).** Yield: 55%. IR (KBr): 3213 (m, NH); 1668 (s, CO); 1334 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.50-8.45 (m, 2H, H_5+H_8); 8.02-7.95 (m, 2H, H_6+H_7); 7.35-7.22 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, CH_2-Ph), 2.25 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{17}N_3O_3$: C: 66.87%; H: 5.26%; N: 13.00%; Found: C: 66.50%; H: 5.30%; N: 12.79%.

4.2.2.20. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-phenylethylamide **(24).** Yield: 67%. IR (KBr): 3203 (m, NH); 1666 (s, CO); 1326 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, $J_{NH\text{-}CH2}$=5.7 Hz, NH); 8.38 (d, 1H, $J_{H5\text{-}H6}$=8.8 Hz, H_5); 8.23 (s, 1H, H_8); 7.82 (d, 1H, H_6); 7.35-7.21 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, CH_2-Ph); 2.59 (s, 3H, $CH_{3\text{-}C7}$); 2.23 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{19}H_{19}N_3O_3$: C: 67.65%; H: 5.64%; N: 12.46%; Found: C: 67.28%; H: 5.87%; N: 12.16%.

4.2.2.21. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-phenylethylamide **(25).** Yield: 61%. IR (KBr): 3203 (m, NH); 1663 (s, CO); 1328 (s, N^+O^-). ^{1}H RMN (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{NH\text{-}CH2}$=5.6 Hz, NH); 8.26 (s, 1H, H_5), 8.23 (s, 1H, H_8); 7.36-7.22 (m, 5H, Ph); 3.61-3.56 (m, 2H, NH-CH_2); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.1 Hz, CH_2-Ph); 2.50 (s, 6H, $CH_{3\text{-}C6}$+$CH_{3\text{-}C7}$); 2.22 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_3$: C: 68.38%; H: 5.98%; N: 11.97%; Found: C: 68.47%; H: 6.08%; N: 11.75%

4.2.2.22. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(26).** Yield: 36%. IR (KBr): 3180 (m, NH); 1665 (s, CO); 1329 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{NH\text{-}CH2}$=5.5 Hz, NH); 8.40 (d, 1H, $J_{H5\text{-}H6}$=9.5 Hz, H_5); 7.77 (d, 1H, $J_{H8\text{-}H6}$=2.5 Hz, H_8); 7.60 (dd, 1H, H_6); 7.36-7.23 (m, 5H, Ph); 3.99 (s, 3H, OCH_3); 3.61-3.56 (m, 2H, NH-CH_2); 2.87 (t, 2H, $J_{CH2\text{-}Ph}$=7.02 Hz, CH_2-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.59%; H: 5.38%; N: 11.90%. Found: C: 64.46%; H: 5.44%; N: 11.66%

4.2.2.23. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(27).** Yield: 44%. IR (KBr): 3224 (m, NH); 1667 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.91 (t, 1H, $J_{NH\text{-}CH2}$=5.2 Hz, NH); 8.49 (d, 1H, $J_{H5\text{-}H6}$=9.2 Hz, H_5); 8.45 (d, 1H, $J_{H8\text{-}H6}$=2.3 Hz, H_8); 8.03 (dd, 1H, H_6); 7.36-7.29 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-CH_2); 2.87 (t, $J_{CH2\text{-}Ph}$=7.1 Hz, 2H, CH_2-Ph); 2.23 (s, 3H, CH_3). Calculated analysis for $C_{18}H_{16}ClN_3O_3$: C: 60.42%; H: 4.47%; N: 11.75%; Found: C: 60.14%; H: 4.50%; N: 11. 58%

4.2.2.24. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(28).** Yield: 38%. IR (KBr): 3231 (m, NH); 1666 (s, CO); 1322 (s, N^+O^-). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 88.93 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.66 (s, 1H, H_5); 8.64 (s, 1H, H_8); 7.34-7.22 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-C$\underline{H_2}$); 2.87 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, C$\underline{H_2}$-Ph); 2.24 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{18}H_{15}Cl_2N_3O_3$: C: 55.10%; H: 3.82%; N: 10.71%; Found: C: 54.70%; H: 3.92%; N: 10.44%

4.2.2.25. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(29).** Yield: 77%. IR (KBr): 3231 (m, NH); 1669 (s, CO); 1328 (s, N^+O^-). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 8.92 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.56 (dd, 1H, $J_{H5\text{-}H6}$=9.5 Hz, $J_{\underline{H5}\text{-}F}$=5.2 Hz, H_5); 8.20 (dd, 1H, $J_{\underline{H8}\text{-}F}$=8.8 Hz, $J_{\underline{H8}\text{-}H6}$=2.5 Hz, H_8); 7.91 (ddd, 1H, $J_{\underline{H6}\text{-}F}$=8.0 Hz, H_6); 7.36-7.23 (m, 5H, Ph); 3.62-3.57 (m, 2H, NH-C$\underline{H_2}$); 2.87 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, C$\underline{H_2}$-Ph); 2.23 (s, 3H, C$\underline{H_3}$). Calculated analysis for $C_{18}H_{16}FN_3O_3$: C: 63.34%; H: 4.69%; N: 12.32%; Found: C: 63.02%; H: 4.80%; N: 12.32%.

4.2.2.26. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-phenylethylamide **(30).** Yield: 39%. IR (KBr): 3240 (m, NH); 1668 (s, CO); 1332 (s, N^+O^-). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.53-8.47 (m, 2H, H_6+H_8); 7.36-7.20 (m, 5H, Ph); 3.61-3.56 (m, 2H, NH-C$\underline{H_2}$); 2.87 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.1 Hz, C$\underline{H_2}$-Ph); 2.24 (s, 3H, C$\underline{H_3}$). Calculated analysis for $C_{18}H_{15}F_2N_30_3$: C: 60.17%; H: 4.18%; N: 11.70%; Found: C: 60.32%; H: 4.34%; N: 11.87%.

4.2.2.27. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-phenylethylamide **(31).** Yield: 9%. IR (KBr): 3243 (m, NH); 1669 (s, CO); 1323 (s, N^+O^-). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 8.93 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.4 Hz, NH); 8.74 (s, 1H, H_8); 8.66 (d, 1H, $J_{\underline{H5}\text{-}H6}$=9.2 Hz, H_5); 8.27 (d, 1H, H_6); 7.33-7.31 (m, 5H, Ph); 3.58-3.63 (m *2*H, NH-C$\underline{H_2}$); 2.88 (t, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, 2H, C$\underline{H_2}$-Ph); 2.27 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{16}F_3N_3O_3$: C: 58.31%; H: 4.09%; N: 10.74%; Found: C: 58.10%; H: 4.025; N: 10.76%.

4.2.2.28. 1,4-Di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(32).** Yield: 10,90%. IR (KBr): 3212 (m, NH); 1670 (s, CO); 1328 (s, N^+O^-). ^1H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.5 Hz, NH); 8.48-8.46 (m, 2H, H_5+H_8); 8.00-7.98 (m, 2H, H_6+H_7); 7.22 (d, 2H, $J_{H2'\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$), 3.72 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.1 Hz, C$\underline{H_2}$-Ph); 2.28 (s, 3H, CH_3).

Calculated analysis for $C_{19}H_{19}N_3O_4$: C: 64.59%; H: 5.38%; N: 11.90%; Found: C: 64.25%; H: 5.24%; N: 11.81%

4.2.2.29. 3,7-Dimethyl-1,4-di-N-oxidequinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(33).** Yield: 19.11%. IR (KBr): 3209 (m, NH); 1666 (s, CO); 1329 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.5 Hz, NH); 8.38 (d, 1H, $J_{\underline{H5}\text{-}H6}$=8.9 Hz, H_5); 8.26 (s, 1H, H_8); 7.81 (d, 1H, H_6); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.2 Hz, $H_{2'}$+$H_{6'}$); 6.89 (d, 2H, $H_{3'}$+$H_{5'}$), 3.72 (s, 3H, OCH_3); 3.56-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, C$\underline{H_2}$-Ph); 2.58 (s, 3H, $CH_{3\text{-}C7}$); 2.25 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{20}H_{21}N_3O_4$: C: 65.40%; H: 5.72%; N: 11.44%; Found: C: 65.62%; H: 5.66%; N: 11.58%.

4.2.2.30. 1,4-Di-N-oxide-3,6,7-trimethylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(34).** Yield: 52.94%. IR (KBr): 3203 (m, NH); 1665 (s, CO); 1326 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.26 (s, 1H, H_5); 8.22 (s, 1H, H_8); 7.22 (d, 2H, $J_{H2'\text{-}H3}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.73 (s, 1H, OCH_3); 3.56-3.51 (m, 2H, NH-C$\underline{H_2}$); 2.79 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.1 Hz, C$\underline{H_2}$-Ph); 2.46 (s, 6H, $CH_{3\text{-}C7}$+$CH_{3\text{-}C6}$); 2.25 (s, 3H, $CH_{3\text{-}C3}$). Calculated analysis for $C_{21}H_{23}N_3O_4$: C: 66.14%; H: 6.04%; N: 11.02%; Found: C: 66.02%; H: 6.09%; N: 10.99%.

4.2.2.31. 1,4-Di-N-oxide-7-methoxy-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(35).** Yield: 20.97%. IR (KBr): 3221 (m, NH); 1666 (s, CO); 1325 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.89 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.5 Hz, NH); 8.40 (d, 1H, $J_{\underline{H5}\text{-}H6}$=9.5 Hz, H_5); 7.76 (d, 1H, $J_{\underline{H8}\text{-}H6}$=2.7 Hz, H_8); 7.59 (dd, 1H, H_6); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.98 (s, 3H, $OCH_{3\text{-}C7}$); 3.72 (s, 3H, $OCH_{3\text{-}C4'}$); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.1 Hz, C$\underline{H_2}$-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{20}H_{21}N_3O_5$: C: 62.66%; H: 5.48%; N: 10.97%; Found: C: 62.37%; H: 5.66%; N: 10.66%

4.2.2.32. 7-Chloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(36).** Yield: 48.00%. IR (KBr): 3287 (m, NH); 1655 (s, CO); 1328 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.87 (s, 1H, NH); 8.49 (d,1H, $J_{\underline{H5}\text{-}H6}$=9.3 Hz, H_5); 8.45 (d, 1H, $J_{\underline{H8}\text{-}H6}$=2.1 Hz, H_8); 8.02 (dd, 1H, H_6); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.73 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, C$\underline{H_2}$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{18}ClN_3O_4$: C: 58.84%; H: 4.65%; N: 10.84%; Found: C: 58.62%; H: 4.72%; N: 10.40%.

4.2.2.33. 6,7-Dichloro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(37).** Yield: 28.40%. IR (KBr): 3230 (m, NH); 1667 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.66 (s, 1H, H_5); 8.64 (s, 1H, H_8); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.5 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.72 (s, 1H, OCH_3); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.79 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=6.9 Hz, C$\underline{H_2}$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{17}Cl_2N_3O_4$: C: 54.03%; H: 4.03%; N: 9.95%; Found: C: 53.66%; H: 3.92%; N: 9.65%.

4.2.2.34. 1,4-Di-N-oxide-7-fluoro-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(38).** Yield: 18.76%. IR (KBr): 3223 (m, NH); 1668 (s, CO); 1330 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.88 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.6 Hz, NH); 8.56 (dd, 1H $J_{\underline{H5}\text{-}H6}$=9.4 Hz, $J_{\underline{H5}\text{-}F}$=5.2 Hz, H_5); 8.20 (dd, 1H, $J_{\underline{H8}\text{-}F}$=8.8 Hz, $J_{\underline{H8}\text{-}H6}$=2.8 Hz, H_8); 7.94-7.81 (m, 1H, H_6); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.72 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.1 Hz, C$\underline{H_2}$-Ph); 2.28 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{18}FN_3O_4$: C: 61.46%; H: 4.85%; N: 11.32%; Found: C: 61.10%; H: 4.78%; N: 11.54%.

4.2.2.35. 6,7-Difluoro-1,4-di-N-oxide-3-methylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(39).** Yield: 12.75%. IR (KBr): 3242 (m, NH); 1669 (s, CO); 1334 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.5 Hz, NH); 8.48-8.46 (m, 2H, H_5+H_8); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.4 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.76 (s, 3H, OCH_3); 3.57-3.52 (m, 2H, NH-C$\underline{H_2}$); 2.79 (t, 2H, $J_{\underline{CH2}\text{-}\underline{CH2}}$=7.0 Hz, CH_2-C$\underline{H_2}$-Ph); 2.26 (s, 3H, CH_3). Calculated analysis for $C_{19}H_{17}F_2N_3O_4$: C: 58.61%; H: 4.37%; N: 10.80%Found: C: 58.86%; H: 4.435%; N: 10.55%.

4.2.2.36. 1,4-Di-N-oxide-3-methyl-7-trifluoromethylquinoxaline-2-carboxylic acid 2-(4-methoxyphenyl)-ethylamide **(40).** Yield: 6.79%. IR (KBr): 3243 (m, NH); 1669 (s, CO); 1324 (s, N^+O^-). ^{1}H MNR (400 MHz, DMSO-d_6) δ ppm: 8.90 (t, 1H, $J_{\underline{NH}\text{-}CH2}$=5.7 Hz, NH); 8.74 (s, 1H, H_5); 8.66 (d, 1H, $J_{\underline{H8}\text{-}H6}$=1.8 Hz, H_8); 8.27 (dd, 1H, $J_{\underline{H6}\text{-}H5}$=9.1 Hz, H_6); 7.22 (d, 2H, $J_{\underline{H2'}\text{-}H3'}$=8.6 Hz, $H_{2'}$+$H_{6'}$); 6.88 (d, 2H, $H_{3'}$+$H_{5'}$); 3.71 (s, 3H, OCH_3); 3.58-3.53 (m, 2H, NH-C$\underline{H_2}$), 2.80 (t, 2H, $J_{\underline{CH2}\text{-}Ph}$=7.0 Hz, C$\underline{H_2}$-Ph); 2.22 (s, 3H, CH_3). Calculated analysis for $C_{20}H_{18}F_3N_3O_4$: C: 57.01%; H: 4.28%; N: 9.98%; Found: C: 57.33%; H: 4.22%; N: 9.88%.

4.3. General procedure of anti-tuberculosis activity

In vitro evaluation of the anti-tuberculosis activity was carried out at the GWL Hansen's Disease Center within the Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF) screening program for the discovery of novel drugs for the treatment of tuberculosis. Under the direction of the U.S. National Institute of Allergy and Infectious Disease (NIAID), the Southern Research Institute coordinates the overall program. The purpose of the screening program is to provide a resource whereby new experimental compounds can be tested for their capability to inhibit the growth of virulent *M.Tb.*[25]

4.3.1. Determination of growth inhibition percentage via MABA

The initial screen is conducted against *M.Tb.* H37Rv (ATCC 27294) in BACTEC 12B medium using the Microplate Alamar Blue Assay (MABA).[26] Compounds exhibiting fluorescence were tested in the BACTEC 460-radiometric system. Compounds effecting <90% inhibition in the primary screen (MIC >6.25 μg/mL) were not further evaluated.

4.3.2. Determination of minimum inhibitory concentration (MIC) via MABA

Compounds demonstrating at least 90% inhibition in the primary screen were re-tested against *M.Tb.* H37Rv at lower concentrations in order to determine the actual minimum inhibitory concentration (MIC) in the MABA. The MIC was defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls. Rifampicin was used as the reference compound (RIF 0.015-0.125 μg/mL).

4.3.3. Determination of cytotoxicity in VERO cells

Compounds are screened by serial dilution to assess toxicity to a VERO cell line at concentrations less than or equal to 6.25 μg/ml or 10 times the MIC for *M.Tb.* H37Rv if sample solubility in culture medium permits. After 72 hours of exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay. RIF was used as the reference compound (RIF IC_{50}>100 μg/mL).

4.3.4. Determination of selectivity index (SI)

The selectivity index (SI) is defined as the ratio of the measured IC_{50} in VERO cells to the MIC for *M.Tb*. H37Rv. In general, requirements for moving a compound into *in vivo* testing include: MIC ≤ 6.25 μg/mL and SI ≥ 10 (occasionally lower). RIF was used as the reference compound (RIF SI>800).

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6. References

[1]Milstien, J. *Immunological Basis for Immunization / Module 5: Tuberculosis.* WHO/EPI/GEN/93.15 (World Health Organization) Global Programme for Vaccines and Immunization/Expanded Programme on Immunization: Switzerland 1993.

[2]TAACF: http://www.taacf.org/about-TB-background.htm

[3] NIAID: http://www3.niaid.nih.gov/topics/tuberculosis/

[4]Tangallapally, R. P.; Yendapally, R.; Lee, R. E.; Hevener, K.; Jones, V. C.; Lenaerts, A. J.; McNeil, M. R.; Wang, Y.; Franzblau, S.; Lee, R. E. *J. Med. Chem.* **2004,** *47*, 5276.

[5]Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *J. Med. Chem*. **2005,** *48*, 2019.

[6]Jan, B.; Erik De, C.; An, C.; Vikki, B.; Jörg-Peter, K. *AIDS Res Hum Retroviruses* **2000,** 16, 517.

[7]Vicente, E.; Villar, R.; Solano, B.; Burguete, A.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *An. R. Acad. Nac. Farm.* **2007,** 73, 927.

[8]Waring, M.; Ben-Hadda, T.; Kotchevar, A.; Ramdani, A.; Touzani, R.; Elkadiri, S.; Hakkou, A.; Bouakka, M.; Ellis, T. *Molecules* **2002,** 7, 641.

[9]Ortega, M. A.; Sainz, Y.; Montoya, M. E.; López de Ceráin, A.; Monge, A. *Pharmazie* **1999,** *54*, 24.

[10]Sainz, Y.; Montoya, M. E.; Martínez-Crespo, F. J.; Ortega, M. A.; López de Ceráin, A.; Monge, A. *Arzneimittel-Forsch.* **1999,** *49*, 55.

[11]Ortega, M. A.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Tirapu, I.; Aldana, I.; Monge, A. *Pharmazie* **2001,** *56*, 205.

[12]Ortega, M.A.; Sainz, Y.; Montoya, M.E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneimittel-Forsch.* **2002,** *52,* 113.

[13]Zarranz, B.; Jaso, A.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* **2003,** *11,* 2149.

[14]Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *E. J. Med. Chem.* **2003,** *38,* 791.

[15]Villar, R.; Vicente, E.; Solano, B.; Pérez-Silanes, S.; Aldana, I.; Maddry, J. A.; Lenaerts, A. J.; Franzblau, S. G.; Cho, S. H.; Monge, A.; Goldman, R. C. *J. Antimicrob. Chemother.* **2008,** *62,* 547.

[16]Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Pérez-Silanes, S.; Aldana, I.; Maddry, J. A.; Lenaerts, A. J.; Franzblau, S. G.; Cho, S. H.; Monge, A.; Goldman, R. C. *Antimicrob. Agents Chemother.* **2008,** *52,* 3321.

[17]Vicente, E.; Pérez-Silanes, S.; Lima, L.M.; Ancizu, S.; Burguete, A.; Solano, B.; Villar, R.; Aldana, I.; Monge, A. *Bioorg. Med. Chem.* **2009,** *17,* 385.

[18]Vicente, E.; Duchowicz, P. R.; Castro, E. A.; Monge, A. *J. Mol. Graphics Modell.* **2009,** in press.

[19]Vicente, E.; Villar, R.; Burguete, A.; Solano, B.; Ancizu, S.; Pérez-Silanes, S.; Aldana, I.; Monge, A. *Molecules* **2008,** *13,* 86.

[20]González, M.; Cerecetto, H. In *Topics in Heterocyclic Chemistry;* Khan M. T. H., Ed.; Bioactive Heterocycles IV. Benzofuroxan and Furoxan. Chemistry and Biology; Springer Berlin: Heidelberg, 2007; Vol. 10, pp 265.

[21]Ortega, M. A.; Sainz, Y.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A. *Arzneimittel-Forsch.* **2002,** *52,* 113.

[22]Clemens, R. J. *Chem. Rev.* **1986,** *86,* 241.

[23]Stumm, G.; Niclas, H. J. *J. Prakt. Chem.* **1989,** *331,* 736.

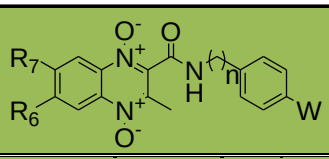
[24]Cheeseman, G. W. H.; Cookson R. F. In *Condensed Pyrazines*; Willey J. and sons, Ed.; New York: NY, USA, 1979; Vol. 35, pp 35.

[25]TAACF: http://www.taacf.org/Process-text.htm#assays

[26]Collins, L. A.; Franzblau, S. G. *Antimicrob. Agents Chemotherapy* **1997,** *41*, 1004.

Table(s)

Table 1.

					Secondary Screening		
Comp.	**R_7**	**R_6**	**n**	**W**	**MIC(µg/mL) H37Rv[a]**	**IC_{50}(µg/mL) VERO[b]**	**SI (IC_{50}/MIC)[c]**
5	H	H	1	H	2.606	>100	>38.372
6	CH_3	H	1	H	4.207	>100	>23.77
7	CH_3	CH_3	1	H	>100	NT	NT
8	OCH_3	H	1	H	12.346	NT	NT
9	Cl	H	1	H	0.433	>100	>230.94
10	Cl	Cl	1	H	<0.2	>100	>500
11	F	H	1	H	1.522	>100	>65.70
12	F	F	1	H	1.727	18.378	10.64
13	CF_3	H	1	H	0.406	>100	>246.30
14	H	H	1	OCH_3	2.847	>30	>10.537
15	CH_3	H	1	OCH_3	8.903	>30	>3.3696
16	CH_3	CH_3	1	OCH_3	>100.00	NT	NT
17	OCH_3	H	1	OCH_3	12.007	NT	NT
18	Cl	H	1	OCH_3	NT	NT	NT
19	Cl	Cl	1	OCH_3	NT	NT	NT
20	F	H	1	OCH_3	1.508	>30	>19.893
21	F	F	1	OCH_3	0.764	16.605	21.734
22	CF_3	H	1	OCH_3	0.999	>30	>30.03
23	H	H	2	H	3.048	>100	>32.81
24	CH_3	H	2	H	8.621	>100	>11.60
25	CH_3	CH_3	2	H	16.359	NT	NT
26	OCH_3	H	2	H	13.657	NT	NT
27	Cl	H	2	H	<0.2	>100	>500
28	Cl	Cl	2	H	<0.195	>30	>153.84
29	F	H	2	H	0.504	>100	>198.41
30	F	F	2	H	0.517	>30	>58.027
31	CF_3	H	2	H	1.153	>30	>26.019
32	H	H	2	OCH_3	3.464	>30	>8.6605
33	CH_3	H	2	OCH_3	8.852	>30	>3.389
34	CH_3	CH_3	2	OCH_3	>100.00	NT	NT
35	OCH_3	H	2	OCH_3	16.866	NT	NT
36	Cl	H	2	OCH_3	<0.195	>30	>153.84
37	Cl	Cl	2	OCH_3	0.454	>30	>66.079
38	F	H	2	OCH_3	<0.195	>30	>153.84
39	F	F	2	OCH_3	2.058	>30	>14.577
40	CF_3	H	2	OCH_3	1.427	>30	>21.023
RIF	-	-	-	-	0.015-0.125	>100	>800

Figure(s)

Legends:

Scheme 1. Synthetic scheme of initial *β*-acetoacetamide derivatives (**1-4**).

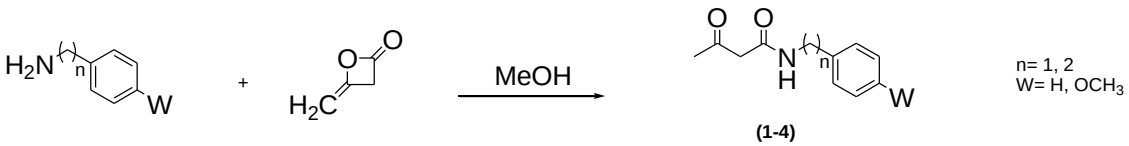
Scheme 2. Synthetic route of 1,4-di-*N*-oxide-3-methylquinoxaline-2-carboxylic acid aryl amide derivatives (**5-40**).

Table 1: Biological values of compounds (**5-40**) at secondary screening.

[a]Actual Minimum Inhibitory Concentration in *M.Tb.* H37Rv strain. [b]Cytotoxicity in VERO cells. [c]Selectivity Index. NT: Non Tested.

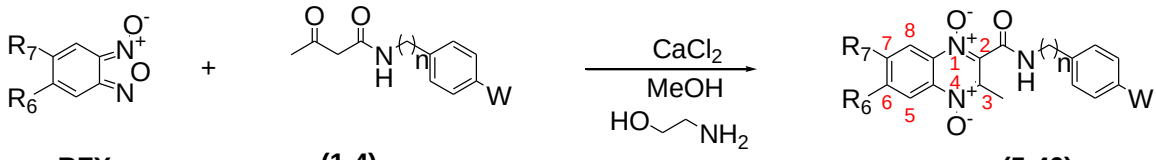
In the first screening, all of the compounds were evaluated against *M.Tb.* in H37Rv strain. All of them were active at this level. In the secondary screening, the actual minimum inhibitory concentration (MIC) in H37Rv strain and cytotoxicity (IC_{50}) in VERO cells were determined. Finally, the SI was calculated and the compounds containing SI level ≥ 10 were considered active at the second level. All of these active compounds are highlighted in green in the table, with the most active ones being marked in dark green.

Scheme 1.



(1-4)

Scheme 2.



BFX **(1-4)** **(5-40)**

n= 1, 2
W= H, OCH_3
R_6, R_7= H, Cl, F, OCH_3, CH_3, CF_3

Figure(s)

Legends:

Figure 1: Estimated number of new TB cases, by country, 2007

[Source: Global Tuberculosis Control WHO REPORT 2009]

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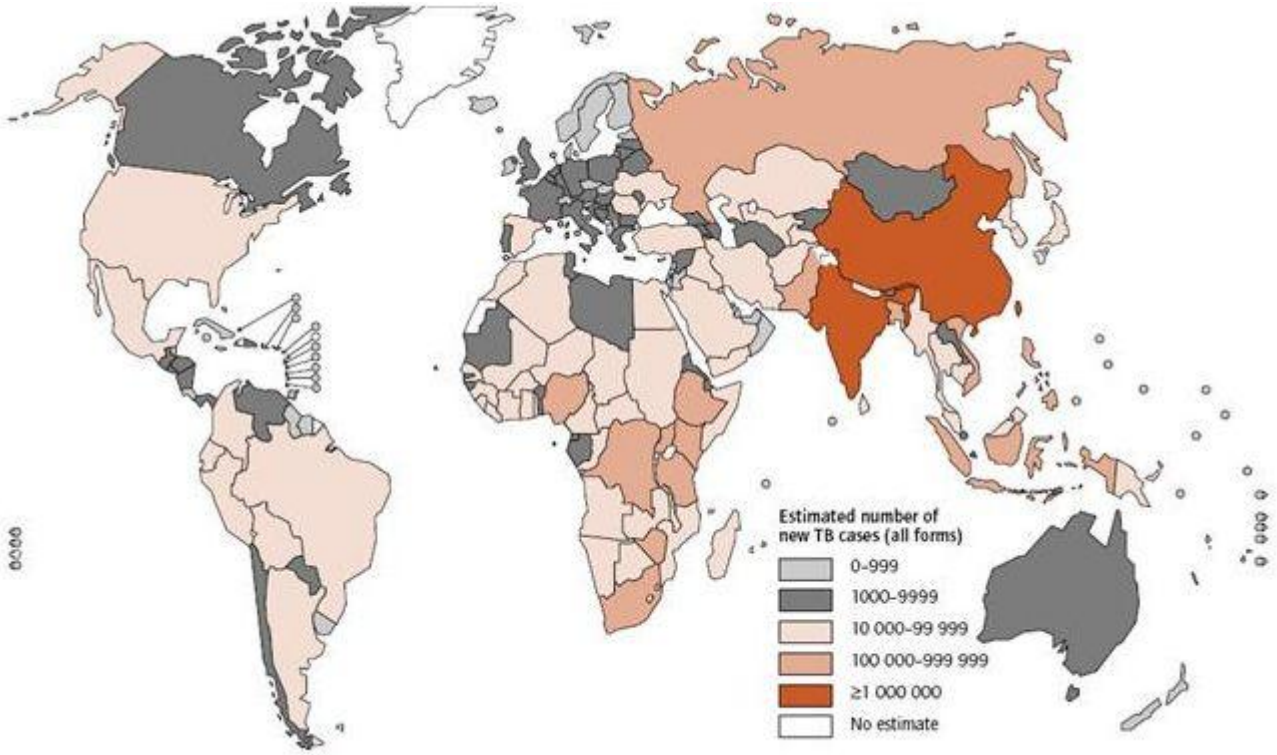
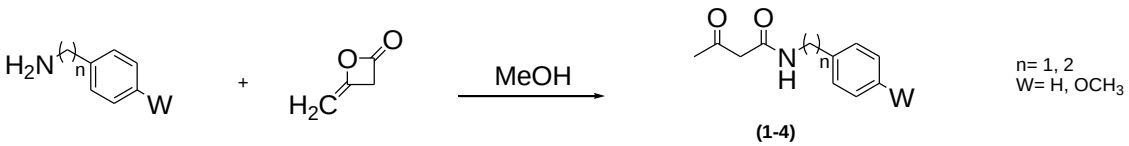
Figure 1.

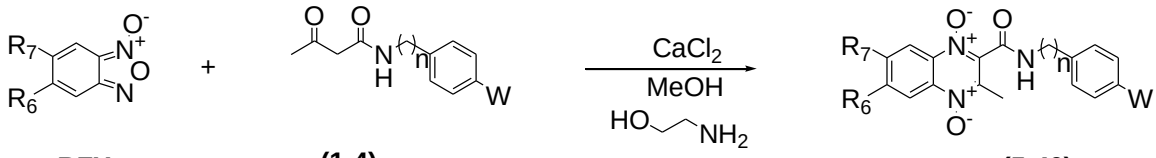

Estimated number of new TB cases (all forms)
0–999
1000–9999
10 000–99 999
100 000–999 999
≥1 000 000
No estimate

Scheme 1.



(1-4)

Scheme 2.



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R_6, R_7= H, Cl, F, OCH_3, CH_3, CF_3