Synthesis, Characterization and Biological Activity of 4-Imino-3-substituted-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide

Rajab Abu-El-Halawa[a*], Mohammad M. Ibrahim[a], Fatheya B. Rabi[a], Mohammad A. Alkenji[a], Qosay Al-Balas[b], Iman A. Mansi[c], Mahmoud Al-Refai[a]

[a] *Department of Chemistry, Faculty of Science, Al al-Bayt University, Jordan.*
[b] *Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.*
[c] *Faculty of Pharmaceutical sciences, The Hashemite University, Zarqa, Jordan.*

Received on Dec. 3, 2017 **Accepted on** Jan. 17, 2018

Abstract

A new series of 4-imino-3-substituted-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide derivatives **(3a-s)** were prepared by the cycloaddition of alkyl/aryl isothiocyanate to 3-aminoquinoxaline-2-carbonitrile-1,4-dioxide derivatives **(2a-c)**. All new compounds gave satisfactory analytical and spectral data in accordance with their assigned structures. Of the tested compounds for glyoxalase-I inhibitory activity, compound **3r** has showed the highest activity with an IC_{50} of 31.5 µM.

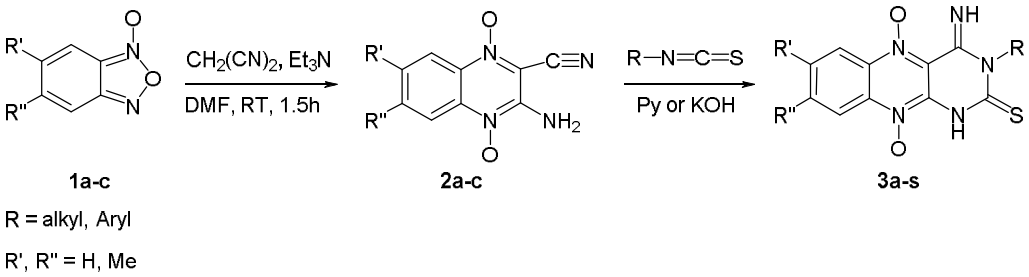
Keywords: *Pyrimidine; Quinoxaline; Pteridine; Isothiocyanate; Glyoxalase-I.*

Introduction

Quinoxaline heterocycles, including their fused-ring derivatives, display diverse pharmacological activities. Their 1,4-di-*N*-oxides have demonstrated excellent activities as antiviral, anticancer, antibacterial and antiparasitic agents, as well as anti-mycobacterium tuberculosis and against *T. cruzi*[1]. On the other hand, aromatic and heteroaromatic compounds bearing an *o*-aminonitrile group are useful substrates for the preparation of various condensed fused pyrimidine heterocyclic systems[2]. The facile conversion of aminonitrile derivatives to heterocyclic systems which are analogues of biomolecules prompted to the synthesis of potential antitumor and antiviral agents by this route[3-5].

Because of their biological importance and in continuation to our previous work on quinoxaline derivatization[6,7], the present work reports the synthesis of 3-substituted 4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[*g*]pteridine 5,10-dioxide **(3a-s)** by the reaction of alkyl/aryl isothiocyanate with 2-amino-3-cyanoquinoxaline 1,4-dioxide derivatives **(2a-c)** in the presence of pyridine or ethanolic potassium hydroxide as shown in scheme 1.

* **Corresponding author:** Rajab Abu-El-Halawa **E-mail:** halawarajab@yahoo.com



Scheme 1: Synthesis of pteridine derivatives **3a-s**.

Materials and methods

All chemicals used in this work were purchased from Aldrich or Acros and were of analytical grade or higher.

Instrumentation

Melting points were determined on an Electrothermal-9100 apparatus. IR spectra were obtained as KBr discs on a Nicolet Impact-400 FT-IR spectrometer. ^{1}H- and ^{13}C NMR spectra were recorded on a Bruker 300 MHz ultrashield instrument. Elemental analysis were recorded on Euro-vector EA 3000A analyser. All analyses were performed at the Central Laboratories of Al al-Bayt University, Mafraq, Jordan. Benzofurazan oxides **(1a-c)** and 2-amino-3-cyanoquinoxaline 1,4-dioxide **(2a-c)** were prepared according to literature procedures[8].

The biological activity measurements were carried out at the Department of Medicinal Chemistry and Pharmacognosy, Faculty of Pharmacy, Jordan University of Science and Technology, Irbid, Jordan.

Synthesis

*General procedures for the preparation of 3-substituted-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide derivatives **3a-s***

Method A

Substituted 2-amino-3-cyanoquinoxaline 1,4-dioxide **2a-c** (0.005 mol) and the appropriate alkyl/aryl isothiocyanate (0.006 mol) were mixed in THF (20 mL) followed by the addition of KOH (0.56 g, 0.005 mol) in hot ethanol (10 mL). The reaction mixture was stirred overnight at room temperature. The reaction mixture was then diluted with aqueous ethanol and cooled. The resulting precipitate was collected by filtration, washed with ethanol, ether and petroleum ether and recrystallized from ethanol–DMF (10:1).

Method B

A mixture of appropriate 2-amino-3-cyanoquinoxaline 1,4-dioxide derivative **2a-c** (0.005 mol) and the appropriate alkyl/aryl isothiocyanate (0.006 mol) in anhydrous pyridine (10 mL) was refluxed for 4 h and allowed to stand overnight at room

temperature. The reaction mixture was diluted with aqueous ethanol and cooled. The resulting precipitate was collected by filtration, washed with ethanol, ether, petroleum and ether and recrystallized from ethanol–DMF (10:1).

*4-Imino-3-methyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide (**3a**)*

According to method A. Red precipitate, 92% yield, m.p >300 ºC. IR (KBr, cm^{-1}): ν = 3330 (N-H), 3190 (C-H), 1610 (C=N), 1505 (NH-C=S) and 1130 (C=S). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.42 (s, 3H, CH_3), 4.03 (sb, 1H, NH), 7.33-7.68 (m, 4H, Ar-H), 10.22 (s, 1H, C=NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 179.2 (C=S), 154.7 (C=NH), 143.3, 134.3, 132.9, 127.6, 127.9, 125.6, 120.1, 119.2, 32.2 (CH_3). Anal. Calc. for $C_{11}H_9N_5O_2S$ (275.29): C, 47.99%; H, 3.29%; N, 25.44%; S, 11.62%. Found: C, 48.12%; H, 3.40%; N, 25.26%; S, 11.55%

*3-Ethyl-4-Imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide **(3b)***

According to method A. Red precipitate, 65% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 1.25 (t, J = 6.7 Hz, 3H, CH_3), 3.33 (s, 1H, NH), 4.63 (q, J = 6.7 Hz, 2H, CH_2), 7.63-8.41 (m, 4H, Ar-H), 11.79 (s, 1H, =NH). 13^{C} NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.1 (C=S), 151.0 (C=NH), 142.9, 137.7, 133.5, 133.2, 132.7, 128.0, 120.0, 119.2, 42.6 (CH_2), 14.6 (CH_3). Anal. Calc. for $C_{12}H_{11}N_5O_2S$ (289.32): C, 49.82%; H, 3.83%; N, 24.21%; S, 11.08 %. Found: C, 50.11%; H, 3.66%; N, 24.61%; S, 11.22%

*3-Butyl-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide **(3c)***

According to method A. Red precipitate, 72% yield, m.p >300 ºC. IR (KBr, cm^{-1}): ν = 3350 (N-H), 3190 (C-H), 1615 (C=N), 1500 (NH-C=S) and 1195 (C=S). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 1.05 (t, 3H, CH_3), 1.34 (m, 2H, CH_2), 1.69 (m, 2H, CH_2), 3.42 (s, 1H, NH), 4.53 (t, 2H, CH_2), 7.21-8.40 (m, 4H, Ar-H), 11.89 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.6 (C=S), 151.0 (C=NH), 149.7, 142.7, 137.4, 137.0, 133.6, 129.8, 128.1, 117.0, 56.5, 47.0, 27.9, 14.4. Elemental Anal. Calc. for $C_{14}H_{15}N_5O_2S$ (317.37): C, 52.98%; H, 4.76%; N, 22.07%; S, 10.10%. Found: C, 53.10%; H, 4.85%; N, 21.93; S, 9.85%.

*3-Hexyl-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide **(3d)***

According to method B. Red precipitate, 70 % yield, m.p >300 ºC. IR (KBr, cm^{-1}): ν = 3390 (N-H), 3200 (C-H), 1617 (C=N), 1575 (NH-C=S) and 1200 (C=S), 1300 (N-O). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 0.85-3.65 (m, 13H, Hexyl), 4.30 (s, 1H, NH), 7.35-8.66 (m, 4H, Ar-H), 10.86 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 182.0 (C=S), 156.1 (C=NH), 150.0, 146.3, 137.2, 134.6, 132.1, 127.9, 120.1, 118.4, 45.17, 30.8, 29.6, 26.1, 22.4, 14.4. Elemental Anal. Calc. for $C_{16}H_{19}N_5O_2S$ (345.42): C, 55.63%, H, 5.54, N, 20.28%, S, 9.28%. Found: C, 55.70%, H, 5.80%, N, 20.50%, S, 9.04%.

3-Cyclohexyl-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3e)***

According to method B. Red precipitate, 67% yield, m.p >300 ºC. IR (KBr, cm^{-1}): ν = 3385 (N-H), 2995 (C-H), 1630 (C=N), 1580 (NH-C=S) and 1190 (C=S), 1510 (C=C), 1355 (N-O). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 1.10-1.85 (m, 11H, *cyclohexyl*), 3.90 (s, 1H, NH), 7.07-8.30 (m, 4H, Ar-H), 8.62 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.0 (C=S), 157.1 (C=NH), 154.1, 149.6 137.1, 132.3, 132.2, 128.9, 120.3, 118.1, 55.4, 33.1, 33.0, 29.0, 28.8, 23.1. Elemental Anal. Calc. for $C_{16}H_{17}N_5O_2S$ (343.41): C, 55.96%; H, 4.99%; N, 20.40%; S, 9.33%. Found: C, 56.11%; H, 5.29%; N, 20.13%; S, 9.07%.

3-Benzyl-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3f)***

According to method B. Red precipitate, 41% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.38 (s, 2H, CH_2-Ph), 5.87 (s, 1H, NH), 7.18-8.40 (m, 9H, Ar-H), 11.78 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 181.2 (C=S), 152.7 (C=NH), 151.0, 146.4, 137.1, 134.2, 132.7, 132.4, 131.6, 130.1, 128.7, 128.1, 118.7, 109.2, 50.1 (CH_2Ph). Elemental Anal. Calc. for $C_{17}H_{13}N_5O_2S$ (351.38): C, 58.11%; H, 3.73%; N, 19.93%; S, 9.12%. Found: C, 58.33%, H, 4.01%, N, 20.21%, S, 8.98%.

4-Imino-3-phenyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3g)***

According to method A. Red precipitate, 67% yield, m.p >300 ºC. IR (KBr, cm^{-1}): ν = 3390 (N-H), 2990 (C-H), 1620 (NH-C=S), 1565 (C=N) and 1196 (C=S). ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 4.50 (s, ^{1}H, NH), 7.14-8.4 1 (m, 9H, Ar-H), 11.55 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 181.0 (C=S), 153.0 (C=NH), 146.3, 143.2, 142.9, 139.7, 137.6, 134.6, 132.4, 129.1, 128.2, 119.2, 118.0, 110.2. Elemental Anal. Calc. for $C_{16}H_{11}N_5O_2S$ (337.36): C, 56.96%; H, 3.29%; N, 20.76%; S, 9.51%. Found: C, 57.04%; H, 3.15%; N, 20.97%; S, 9.76%.

4-Imino-2-thioxo-3-(p-tolyl)-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3h)***

According to method A. Violet precipitate, 71% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.28 (s, 3H, CH_3), 6.93-8.27 (m, 8H, Ar-H), 4.50-4.47 (br, 1H, NH), 11.54 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 181.6 (C=S), 151.8 (C=NH), 145.5, 139.5, 138.1, 134.3, 131.6, 130.3, 129.1, 128.3, 126.5, 118.6, 117.2, 110.6, 21.4 (CH_3). Elemental Anal. Calc. for. $C_{17}H_{13}N_5O_2S$ (351.34): C, 58.10%; H, 3.73%; N, 19.93%; S, 9.12%. Found: C, 58.27%; H, 3.91%; N, 19.72%; S, 8.92%.

3-(4-Chlorophenyl)-4-imino-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3i)***

According to method B. Red precipitate, 81% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.17 (s, 1H, NH), 7.31-8.35 (m, 8H, Ar-H), 10.14 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.0 (C=S), 146.5 (C=NH), 146.8, 139.0, 137.5, 135.1, 129.6, 128.3, 126.5, 121.4, 118.4, 112.5, 112.2, 109.0. Elemental

Anal. Calc. for $C_{16}H_{10}ClN_5O_2S$ (371.8): C, 51.68%; H, 2.71%; N, 18.84%; S, 8.62.% Found: C, 51.41%; H, 2.53%; N, 19.13%; S, 8.81%.

4-Imino-3-(2-methoxyphenyl)-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3j)***

According to method B. Red precipitate, 54% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.83 (s, 3H, OCH_3), 6.93-8.27 (m, 6H, Ar-H), 4.18 (br, 1H, NH) 9.37 (br, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 181.0 (C=S), 156.0 (C=NH), 152.3, 146.3, 137.2, 135.1, 134.6, 132.1, 130.3, 129.6, 128.5, 124.3, 120.4, 116.2, 111.0, 105.2, 56.6 (OCH_3). Elemental Anal. Calc. for $C_{17}H_{13}N_5O_3S$ (367.38): C, 55.57%; H, 3.57%; N, 19.07%; S, 8.73%. Found: C, 55.29%; H, 3.82%; N, 19.31%; S, 9.03%.

4-imino-3,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3k)***

According to method A. A dark red precipitate, 73% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.48 (s, 3H, CH_3), 3.75 (s, 1H, NH), 7.32-8.41 (m, 3H, H-6,7,9), 11.79 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 185.5 (C=S), 167.3 (C=NH), 150.4, 143.2, 138.1, 135.2, 134.2, 119.3, 118.3, 117.2, 35.6, 20.9. Elemental Anal. Calc. for $C_{12}H_{11}N_5O_2S$ (289.32): C, 49.81%; H, 3.83%; N, 24.21%; S, 11.08%. Found: C, 49.72%; H, 3.97%; N, 24.41%; S, 11.27%.

4-Imino-3,7,8-trimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3l)***

According to method A. Amethystine precipitate, 74% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.52 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.87 (s, 3H, CH_3), 3.65 (br, 1H, NH), 8.34 (s, 1H, H-6), 8.41 (s, 1-H, H-9), 11.66 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.2 (C=S), 151.3 (C=NH), 145.8, 137.5, 135.2, 131.4, 129.8, 120.7, 118.5, 117.7, 35.4, 20.1, 19.6. Elemental Anal. Calc. for $C_{13}H_{13}N_5O_2S$ (303.34): C, 51.47%; H, 4.32%; N, 23.09%; S, 10.57%. Found: C, 51.69%; H, 4.48%; N, 22.19%; S, 10.34%.

3-Hexyl-4-imino-7,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3m)***

According to method A. A dark blue precipitate, 74% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 0.85-3.3 (m, 13H, Hexyl), 4.50 (s, 1H, NH), 2.51 (s, 3H, CH_3), 2.49 (s, 3H, CH_3), 7.35-8.66 (m, 4H, Ar-H), 11.60 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 179.7 (C=S), 150.2 (C=NH), 143.3, 138.2, 137.8, 135.6, 120.9, 118.5, 117.4, 46.7, 35.4, 31.1, 29.6, 26.2, 25.1, 20.1, 19.6, 13.9. Elemental Anal. Calc. for $C_{18}H_{23}N_5O_2S$ (373.47): C, 57.88%; H, 6.21%; N, 18.76%; S, 8.58%. Found: C, 57.99%; H, 6.38%; N, 18.55%; S, 8.76%.

4-Imino-3-(2-methoxyphenyl)-7,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3n)***

According to method A. Dark blue precipitate, 65% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.53 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 3.63 (s, 3H, OCH_3), 6.93-8.27 (m, 6H, Ar-H), 4.41-4.50 (br, 1H, NH), 11.48-11.51 (bs, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.0 (C=S), 153.6 (C=NH), 152.3, 146.3, 143.2, 137.2, 134.6, 133.1, 132.6, 130.3, 129.6, 128.5, 124.3, 120.4, 116.2, 106.2, 55.4 (OCH_3), 19.6, 19.2. Elemental Anal. Calc. for $C_{19}H_{17}N_5O_3S$ (395.44): C, 57.71%; H, 4.33%; N, 17.71%; S, 8.11%. Found: C, 57.92%; H, 4.49%; N, 17.54%; S, 8.39%.

4-Imino-7,8-dimethyl-2-thioxo-3-(p-tolyl)-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3o)***

According to method A. Dark blue precipitate, 75% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.35 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.93-8.27 (m, 6H, Ar-H), 4.64-4.47 (br, 1H, NH), 11.52 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.6 (C=S), 152.5 (C=NH), 145.5, 139.8, 138.2, 131.5, 130.0, 129.5, 129.2,128.6, 120.4, 118.5, 117,1, 110.6, 21.5, 19.5, 19.2. Elemental Anal. Calc. for $C_{19}H_{17}N_5O_2S$ (379.45): C, 60.14%; H, 4.52%; N, 18.46%; S, 8.45%. Found: C, 60.32%; H, 4.31%; N, 18.26%; S, 8.36%.

3-(4-Fluorophenyl)-4-imino-7,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3p)***

According to method A. Purple precipitate, 86% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.59 (s,1H, NH), 2.52 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 6.55-8.14 (m, 8H, Ar-H), 11.51 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 180.2 (C=S), 164.2, 152.3 (C=NH), 143.4, 138.3, 135.2, 133.0, 132.1, 128.7, 128.0, 127.5, 126.8, 125.2, 124.9, 118.0, 115.0, 19.7, 19.3. Elemental Anal. Calc. for $C_{18}H_{14}FN_5O_2S$ (383.40): C, 56.38%; H, 3.68%; N, 18.27%; S, 8.36%. Found: C, 56.45%; H, 3.49%; N, 18.48%; S, 8.17%.

3-(4-Chlorophenyl)-4-imino-7,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3q)***

According to method A. Orange precipitate, 72% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.15 (s, 1H, NH), 2.52 (s, 3H, CH_3), 2.48 (s, 3H, CH_3),7.36-8.33 (m, 6H, Ar-H), 11.04 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 181.1 (C=S), 147.2 (C=NH), 145.9, 139.2, 137.4, 135.1, 129.5, 128.3, 126.5, 121.4, 118.4, 112.5, 112.3, 109.0, 19.4, 19.0. Elemental Anal. Calc. for $C_{18}H_{14}ClN_5O_2S$ (399.85): C, 54.06%; H, 3.53%; N, 17.52%; S, 8.02%. Found: C, 54.62%; H, 3.35%; N, 17.73%; S, 7.86%.

3-(4-Bromophenyl)-4-imino-7,8-dimethyl-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3r)***

According to method A. Dark blue precipitate, 70% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 3.19 (s, 1H, NH), 2.53 (s, 3H, CH_3), 2.52 (s, 3H, CH_3), 7.64 (s, 1H, H-6), 7.63 (s, 1H, H-9), 11.48 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 179.6 (C=S), 152,6 (C=NH), 145.5, 138.5, 137.6, 134.5, 130.3, 129.9, 128.5, 122.5, 120.0, 118.3, 114.4, 109.9, 19.6, 19.1. Elemental Anal. Calc. for $C_{18}H_{14}BrN_5O_2S$ (444.30): C, 48.66%; H, 3.18%; N, 15.77%; S, 7.21%. Found: C, 48.41%; H: 3.35%; N, 15.49%; S, 7.02%.

4-Imino-7,8-dimethyl-3-(4-nitrophenyl)-2-thioxo-1,2,3,4-tetrahydrobenzo[g]pteridine 5,10-dioxide ***(3s)***

According to method A. Violet precipitate, 77% yield, m.p >300 ºC. ^{1}H NMR (300 MHz, DMSO-*d6*): δ (ppm) = 2.52 (s, 3H, CH_3), 2.51 (s, 3H, CH_3), 4.27 (s, 1H, NH), 8.09 (s, 1H, H-6), 8.27 (s, 1H, H-9), 11.43 (s, 1H, =NH). ^{13}C NMR (75 MHz, DMSO-*d6*): δ (ppm) = 178.8 (C=S), 152.4 (C=NH), 150.2, 145.9, 138.5, 135.5, 131.7, 131.4, 130.1, 126.6, 124.9, 122.0, 120.2, 110.4, 19.6, 19.1. Elemental Anal. Calc. for $C_{18}H_{14}N_6O_4S$ (409.40): C, 52.80%; H, 3.20%; N, 20.53%; S, 7.83%. Found: C, 52.52%; H, 3.46%; N, 20.27%; S, 7.89%.

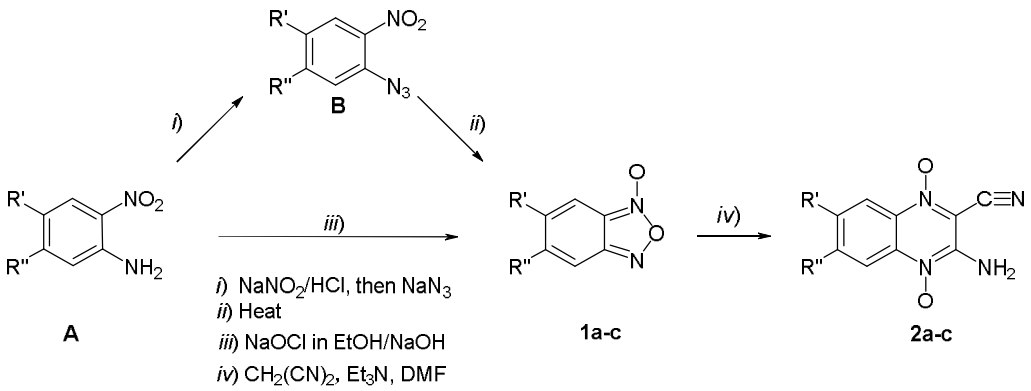
In vitro glyoxalase-i (Glo-I) enzyme assay

The enzymatic assay was carried out according to the manufacturer protocol. The inhibition activities of the eight selected compounds against Glo-I was tested as mentioned in Al-Balas et al[9]. Briefly, the enzyme was diluted using sterile deionized water to a concentration of 0.5 mg/mL, portioned, stored at -70 ^{o}C and thawed just before use. The selected compounds were prepared as 10 mM stock solutions in DMSO and diluted in assay buffer to have an end concentration of 50 µM in the enzyme assay. MG and glutathione were used as substrates and myricetin was used as standard inhibitor. The absorbance was measured at λ_{max} of 240 nm for 120 seconds at 25^{o}C. The assay was performed in triplicate and the mean was recorded.

Results and Discussion

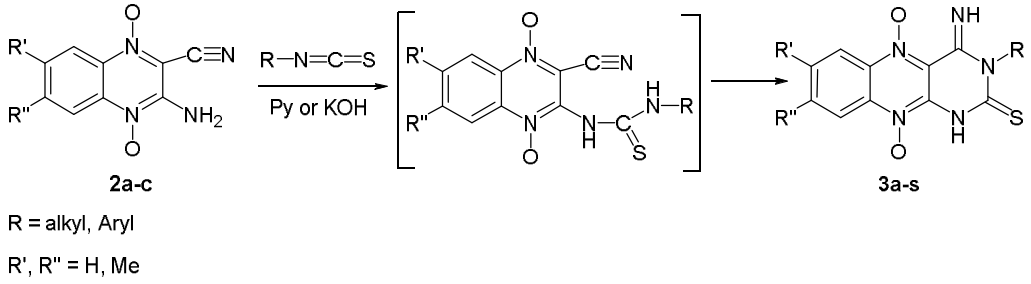
Synthesis and characterization

The 2-amino-3-cyanoquinoxaline 1,4-dioxide derivatives **(2a-c)** were obtained according to the literature by the reaction of corresponding benzofurazan oxides **(1a-c)** with malononitrile using *N*,*N*-dimethylformamide (DMF) as a solvent and triethylamine as a catalyst. The substituted benzofurazan oxides **(1a-c)** have been prepared using previously described methods using two different routes as shown in scheme 2[8].



Scheme 2: Preparation of 2-amino-3-cyanoquinoxaline 1,4-dioxide derivatives **2a-c**.

The new heterocyclic pteridines derivatives **(3a-s)** have been prepared by the reaction of alkyl/aryl isothiocyanate with appropriate 2-amino-3-cyanoquinoxaline 1,4-dioxide **(2a-c)** derivatives in the presence of pyridine or ethanolic potassium hydroxide without isolation of the corresponding 2-cyano-3-thioureidoquinoxaline 1,4-dioxide intermediate derivatives to yield the pteridines derivatives **(3a-s)** in moderate to very good yields (Table 1; Scheme 3).



Scheme 3: Synthesis of pteridine derivatives **3a-s**.

The spectral and analytical data of all new compounds agree with assigned structures. The infrared spectra of **3** showed the absence of the absorption band corresponding to the vibration of the CN group (2210 cm^{-1}) along with the presence of characteristic absorptions at 3350-3370 cm^{-1} (NH, H-bond), 1665–1680 cm^{-1} (C=N), 1500-1580 cm^{-1} (NH-C=S) and 1190-1200 cm^{-1} (C=S). The presence of a very small broad absorption band at 2450-2600 cm^{-1} arising from the S-H stretching vibration raises the possibility of the formation of traces of the thioenol tautomer, 4-imino-2-mercapto-3,4-dihydrobenzo[*g*]pteridine 5,10-dioxide derivatives (**3''**).

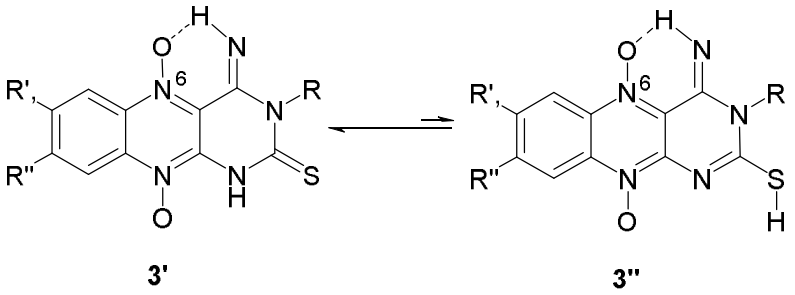
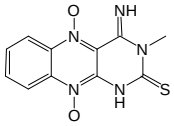
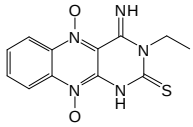
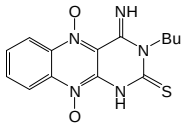
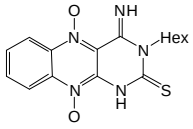
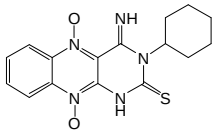
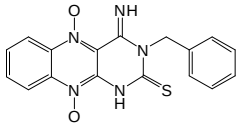
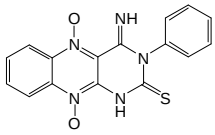
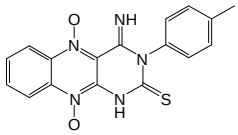
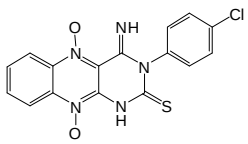
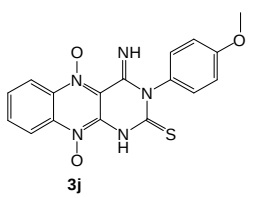
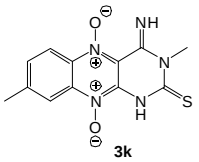
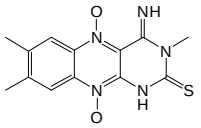
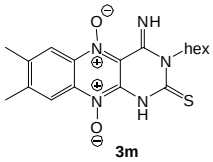
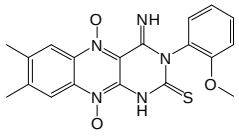
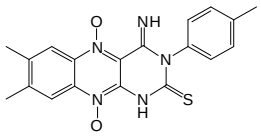
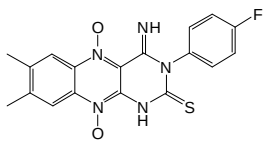
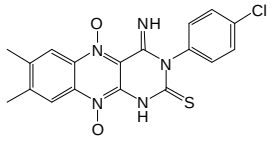
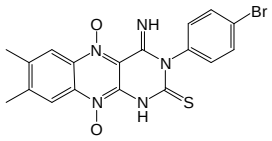
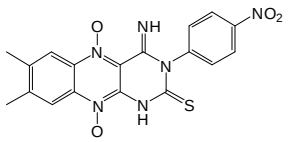


Table 1: Substrates scope and yields.

Entry	R	R`	R``	Method	Product	Yield (%)[a]
a	Me	H	H	A	 **3a**	92
b	Et	H	H	A	 **3b**	65
c	Bu	H	H	A	 **3c**	72
d	Hex	H	H	B	 **3d**	70
e	Cyclohex	H	H	B	 **3e**	67
f	Bn	H	H	B	 **f**	41
g	Ph	H	H	A	 **3g**	67
h	*p*-CH_3-C_6H_4	H	H	A	 **3h**	71
i	*p*-Cl-C_6H_4	H	H	B	 **3i**	81
j	*p*-MeO-C_6H_4	H	H	B	 **3j**	54

Entry	R	R`	R``	Method	Product	Yield (%)[a]
k	Me	H	Me	A	 **3k**	73
l	Me	Me	Me	A	 **3l**	74
m	Hex	Me	Me	A	 **3m**	74
n	*o*-MeO-C_6H_4	Me	Me	A	 **3n**	65
o	*p*-CH_3-C_6H_4	Me	Me	A	 **3o**	75
p	*p*-F-C_6H_4	Me	Me	A	 **3p**	86
q	*p*-Cl-C_6H_4	Me	Me	A	 **q**	72
r	*p*-Br-C_6H_4	Me	Me	A	 **3r**	70
s	*p*-NO_2-C_6H_4	Me	Me	A	 **3s**	77

[a] Yield after recrystallization.

The ^{1}H-NMR (DMSO-d_6) spectra of **3** show the presence of one downfield broadened signal at δ_H ~11 ppm with the integral intensity of 1H and exchangeable with D_2O which is attributed to the presence of C=NH‧‧‧O-N^{6} H-bonding (**3'**), and another broadened signal at δ_H ~3.3 ppm with the integral intensity ~1H corresponding to NH (D_2O exchange experiment).

The ^{13}C-NMR (DMSO-d_6) of compounds **3** show a signal at δ_C ~180 ppm corresponding to C^{2}=S and at another one at δ_C ~ 150 ppm belonging to the carbon atom of the imine group C^{4}=NH. The complete data for all proton and carbon atoms are given in the experimental part.

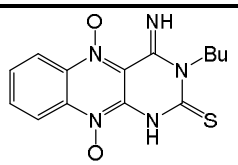
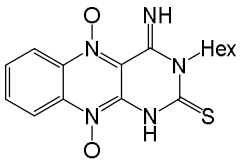
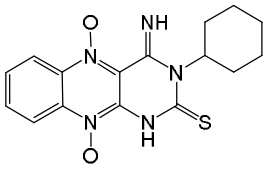
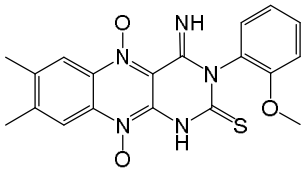
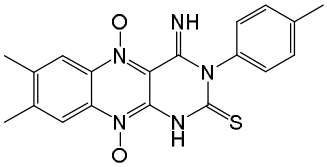
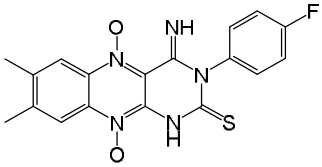
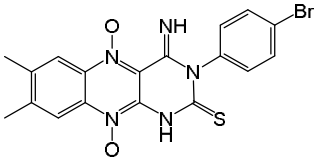
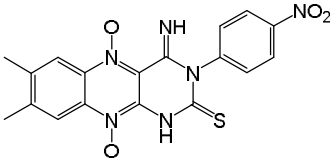
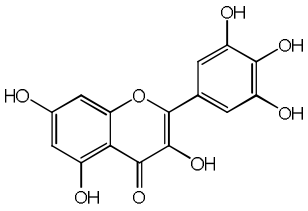
Glyoxalase inhibitory activity:

The glyoxalase system, consisting of glyoxalase-I (Glo-I) and glyoxalase-II (Glo-II), is responsible for the detoxification of methylglyoxal (MG) by converting it to lactate in presence of glutathione. MG is a highly reactive dicarbonyl compounds which can be toxic to the cell[8]. Glyoxalase-I and II enzymes were found to be abnormally over expressed in breast cancer cells. In addition, low levels of MG were observed compared to normal cells leading to increased proliferation and drug resistance in those tumor cells[11]. Inhibition of Glo-I enzyme in cancer cells will lead to the accumulation of MG, increasing its cytotoxicity which suggests a promising mechanism against cancer cells and specifically breast cancer.[11] Out of the 19 compounds **(3a-s)** prepared in this study, eight were chosen to be evaluated for their glyoxalase inhibition activity, among which compound **3r** was found to be the most active with %inhibition of 80% at a concentration of 50 µM and IC_{50} of 31.5 µM (Table 2).

Due to the limited number of compounds which showed prominent activity against Glo-I enzyme in this research, a straight forward structure-activity relationship could not be generalized. However, compound **3r** could be a considered as a leading compound with unique scaffold that has not been mentioned before in the literature against this enzyme. Obviously, the presence of para-substituted aromatic ring with Br atom has an influential role in the activity against Glo-I. This is supported by the fact that the compounds **3p** and **3q** containing F or Cl, respectively, showed low or negligible activity. The presence of non-aromatic (linear or cyclic) alkyl groups have showed no activity against this enzyme (**3c**, **3d**, **3e**) which supports the conclusion that aromatic amino acids could have a binding role inside the active site. Moreover, a para substitution is required as ortho substitution has showed weak activity (compound **3n**)**.**

Only the compound which contains Br has showed prominent activity but not the other halogenated compounds, this could be related to the size of the hydrophobic pocket inside the active site which is tolerable to large substituents such as Br. Cl and F are much smaller in size and will not fill the hydrophobic pocket optimally. In addition, the lipophilicity of Br is more than that of F or Cl; it is also less electronegative. The pocked size and active site mapping are given in reference[12].

Table 2: Glyoxalase inhibitory activity at 50µM concentration for the tested compounds.

Compound	Structure	Glyoxalase inhibitory activity (%)	IC_{50} (µM)
3c		**-3.3**	**-**
3d		**-16.3**	**-**
3e		**6.7**	**-**
3n		**3.3**	**-**
3o		**30**	**-**
3p		**15**	**-**
3r		**80**	**31.5**
3s		**35**	**-**
Myricetin		**95**	**3.5**

The phenomenon of the negative inhibition values for the compounds **3c** and **3d** suggests that these compounds might increase the activity of the *Glyoxala* enzyme by speeding up its metabolic rate.

Conclusions

A new series of pteridine derivatives were successfully synthesized in good to very good yields. All new compounds were characterized by standard spectroscopic techniques. Glyoxalase inhibitory evaluations showed that the compound **3r** was the most active with % inhibition of 80% and IC_{50} of 31.5 µM.

References

[1] a) Gil, A.; Pabón A.; Galiano, A.; Burguete, A.; Deharo, S.; Monge, A.; Aldana, I., *Molecules*, 2014, 19, 2166-2180 (and references therein); b) You, L.; Cho, E. J.; Leavitt, J.; Mad, L.-C; Montelione, G. T.; Anslyn, E. V.; Krug, R. M.; Ellington, A.; Robertus, J. D., *Bioorg. Med. Chem Lett.*, 2011, 21, 3007–3011; c) Torres, E.; Moreno-Viguri, E.; Galiano, S.; Devarapally, G.; Crawford, P. W.; Azqueta, A.; Arbillaga, L.; Varela, J.; Birriel, E.; Di Maio, R.; Cerecetto, H.; González, M.; Aldana, I.; Monge, A.; Pérez-Silanes, S., *Eur. J. Med. Chem.*, 2013, 66, 324-334; d) Barea, C; Pabon, A; Castillo, D; Zimic, M; Quiliano, M; Galiano, S; Perez-Silanes, S; Monge, A; Deharo, E; Aldana, *Bioorg. Med. Chem. Lett.*, 2011, 21, 4498-4502; e) González, M.; Cerecetto, H.; Monge, A.; Quinoxaline 1,4-dioxide and Phenazine 5,10-dioxide. Chemistry and Biology. In Topics in Heterocyclic Chemistry, 2007; 11, p. 179-211; f) Seng, F Bioactive Heterocycles V.; Khan, M.T.H.; 1st Ed.; Springer: Berlin, Germany, 2007; f) Seng, F.; Scheinpflug, H.; Kraus, P.; EP0001423, 1979, g) Seng, F.; Scheinpflug, H.; Kraus, P.; BR 7806618, 1979.

[2] a) Taylor, E. C.; Ravindranathan, R. V., *J. Org. Chem.*, 1962, 27, 2622-2627; b) Pazdera, P.; Novacek, E.; Ondracek, D., *Chem. Papers*, 43, 465-470 (1989); c) Pazdera, P.; Ondracek, D.; Novacek, E., *Chem. Papers*, 43(6), 771-781, d) Papadopoulos, E. P., *J. Heterocycl. Chem.*, 1989, 18, 515-518; e) Armarego, W. L. F., *Adv. Heterocycl. Chem.*, 1953, 1, 253; f) Pfleiderer, W., *Angew. Chem. Int. Ed.*, 1964, 3,114.

[3] a) DeGraw, J. I.; Tagawa, H.; Christie, P. H.; Kisliuk, R. L.; Gaumont, Y., *J. Heterocycl. Chem.*, 1986, 23, 1; b) Dave, C. G.; Shah, P. R.; Shah, G. K.; Pandya, P. S.; Dave, K. C.; Patel, V. J., *Indian J. Pharm. Sei.*, 1986, 48, 75.

[4] EL Ashry, E.; El-Rafi, M.; Elnagdi, M.; Abou-Elnaga, H.; Abdel Azim, W.; Boghdadi, Y., *Jord. J. Chem.,* 2009. 4, 223-231

[5] El-Saghier, A.; Matough, F.; Mahmoud, F.; Farhat M.; Nabil A.; Saleh N.; Kreddan, K.; El-Tier, S.; Hind Bin Hussien, H., *Jord. J. Chem.,* 2008, 3, 223-232.

[6] Abu-El-Halawah, R.; Ali, B. F.; Ibrahim, M. M.; Zahra, J. A.; Frey, W., *Acta Crystallogr Sect E*, 2008, 64, o571–o572.

[7] Maha, A.; Abu-El-Halawa, R.; Zabin, S. A.; Ibrahim, M. M.; Al-Refai, M.; Kaimari, T., *World Journal of Organic Chemistry*, 2015, 3(1), 1-8.

[8] a) Ley, K.; Seng. H., *Synthesis*, 1975, 415-422; b) Ley, K.; Seng. F.; Eholzer H.; Nast, R.; Schubart R., *Angew. Chemie*, 1969, 81, 569; c) Jarrar, A. A., *J. Chem. Edu*, 1974, 51, 755; d) Mallory, F. B., *Org. Synth. Coll.*, 1963, 4, 74; d) Ortega, M. A.; Sainz, Y.; Montoya, M. E.; Jaso, A.; Zarranz, B.; Aldana, I.; Monge, A., *Arzneim.-Forsch.*, 2002, 52, 113-119; e) Benítez D.; Lavaggi. M. L.; Gambino D.; Torre M. H.; Cerecetto, H.; González, M., *Med. Chem. Res.*, 2012, 21, 1439-1444.

[9] Al-Balas, Q. A.; Hassan, M. A.; Al-Shar'I, N. A.; Mhaidat, N. M.; Almaaytah, A. M.; Al-Mahasneh, F. M.; Isa, I. H., *Drug Des. Devel. Ther.*, 2016, 10, 2623–2629

[10] a) Distler, M. G., *J. Clin. Invest.*, 2012, 122(6), 2306–2315. doi:10.1172/JCI61319; b) Thornalley, P. J., *Biochem. Soc. Trans.*, 2003, 31(6), 1343-1348.

[11] Guo, Y.; Zhang, Y.; Yang, X.; Lua, P.; Yana, X.; Xiaoa, F.; Zhoua, H.; Wena, C.; Shia, M.; Lua, J.; Meng QH., *Cancer Biol. Ther.*, 2016, 17(2), 169–180.

[12] Al-Balas Q.; Hassan M., Al-Shar'i N.; El-Elimat T.; Almaaytah A., *Drug Dev. Res.*, DOI: 10.1002/ddr.21421.