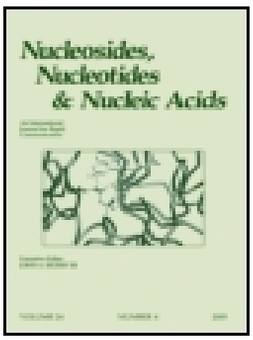

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# A First Microwave-Assisted Synthesis of a New Class of Purine and Guanine Thioglycoside Analogs

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**ABSTRACT**

A first microwave-assisted synthesis of a new class of novel purine thioglycoside analogs from readily available starting materials has been described. The key step of this protocol is the formation of sodium pyrazolo[1,5-a]pyrimidine-7-thiolate and 7-mercaptopyrazolo[1,5-a]pyrimidine derivatives via condensation of 5-amino-1H-pyrazoles with sodium 2,2-dicyanoethene-1,1-bis(thiolate) salts or 2-(dimercaptomethylene)malononitrile, respectively, under microwave irradiation, followed by coupling with halo sugars to give the corresponding purine thioglycoside analogs. The obtained purines and purines thioglycosides derivatives were evaluated in vitro against lung (A549), colon (HCT116), liver (HEPG2), and prostate (PC3) cancer cell lines. Some of these compounds (**5b**, **5d, 5f**, and **9a–d**) exhibited little potency toward the four cell lines. On the other hand, compound **5a** elicited higher cytotoxicity on both prostate (PC3) and colon (HCT116), respectively, while it was found moderate on lung (A549), and inactive on liver (HEPG2). Moreover, compound **5c** was found moderate with $LC_{50}$ values 52.0–88.9 $\mu$M for almost all the cell lines.

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Microwave synthesis; purine thioglycoside analogs; thioglycosides; ketene dithiolate salts; antimetabolites; nucleoside analogs

## 1. Introduction

Purines are important in biological systems since two of the four DNA bases are purine derivatives, and many modern pharmaceutical agents contain purine fragments. Several examples of biologically important purine derivatives have been identified, such as antagonists of nucleic acid synthesis (6-mercaptopurine) which is highlighted as one of the most significant new purine derivatives to have been identified in the last years,[1] Also 6-mercaptopurine is used an antileukemic agent.[2–4] in addition, the pharmacological activity of thiopurines includes antifungal, antimicrobial, antiviral, and antitumor activities.[5,6] Not only do some purine derivatives exhibited cytotoxic effects against cancer cell lines, but also the

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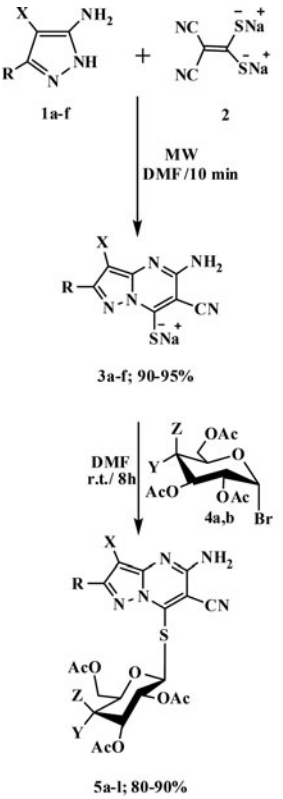
antiproliferative effect of many derivatives was evaluated on a panel of tumor cell lines.[7] Recently, it has been reported that mercaptopurine (6MP) and thioguanine (6TG) derivatives, bearing a purine moiety possess many types of biological activities,[8,9] including anticancer activity.[10] Consequently, many mercaptopurine derivatives were designed and synthesized as antimetabolic agents. We have recently reported different successful approaches for the synthesis of purine and pyrimidine nucleoside analogs.[11–14] Derivatives of these ring systems are interesting as antimetabolites in biochemical reactions.[15–21] Continuing our efforts for the development of simple, eco-friendly and cost-effective methodologies, we report herein a novel microwave-assisted synthesis of thioguanine and mercaptopurine analogs and their thioglycosides as antimetabolic agents. All examples reported in the literature are for *N*-glycosides and very few examples are reported for guanine or purine thioglycosides.[22–24] As far as we know, this is the first method to be reported for the preparation of this ring system. In this study, a number of derivatives have been found to possess antitumor activity of sufficient interest to warrant further investigation.

## 2. Results and discussion

### 2.1. Chemistry

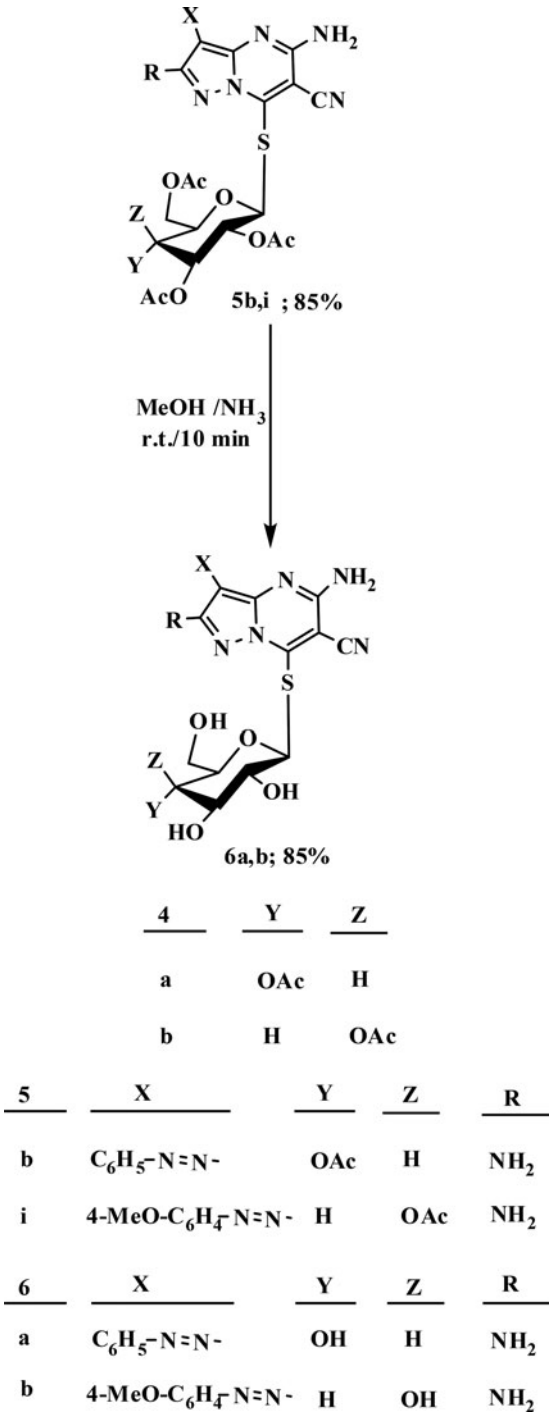
It has been found that reaction of malononitrile with carbon disulfide in the presence of sodium ethoxide gives sodium 2,2-dicyanoethene-1,1-bis(thiolate) **2**. Compound **2** is readily reacted with one equivalent of 5-aminopyrazole derivatives **1a–f** in the presence of dimethylformamide under microwave activation 600 W for 10 minutes at 154°C, to give the corresponding sodium pyrazolo[1,5-*a*]pyrimidine-7-thiolate derivatives **3a–f** in good yields. Compounds **3a–f** reacted with halosugars **4** in dimethylformamide at room temperature to give the corresponding *S*-glycosides **5a–l** with high yields (Scheme 1). It has been suggested that the *cis*-($\alpha$) sugars react by a simple $SN^2$ reaction to give the $\beta$-glycoside products.[25] The structures of **5a-l** were established on the basis of their elemental analysis and spectral data (IR, $^1$H NMR, $^{13}$C NMR). For example, the analytical data for **5a** revealed a molecular formula $C_{22}H_{22}N_6O_9S$, its $^1$H NMR spectrum showing the anomeric proton as a doublet at $\delta$ 5.55–5.56 ppm with a spin-spin coupling constant of 8.70 Hz indicating the $\beta$-configuration. The other six glucose protons resonated at $\delta$ 4.21–5.23 ppm. When glycosides **5b** and **5i** were treated with methanolic ammonia at room temperature for 10 minutes, the deprotected derivatives **6a** and **6b** were obtained in almost quantitative yields (Scheme 2), the structures of which were established on the basis of elemental analysis and spectral data. Thus, the analytical data for **6a** reveal the molecular formula $C_{19}H_{20}N_8O_5S$. The $^1$H NMR spectrum shows the anomeric proton as a doublet at $\delta$ 5.56–5.58($J_{1',2'}$ = 9.95 Hz), indicating the presence of only the $\beta$ -*D*-configuration. Encouraged by these results, we decided to synthesize the *S*-glycosides **5** using the reaction of **1** with [bis(mercapto)methylene]malononitrile **7** and comparing the resulting products for




| 1,3 | X | R | 5 | X | Y | Z | R | 5 | X | Y | Z | R |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| a | CN | H | a | CN | OAc | H | H | g | CN | H | OAc | H |
| b | $C_6H_5$-N=N- | $NH_2$ | b | $C_6H_5$-N=N- | OAc | H | $NH_2$ | h | $C_6H_5$-N=N- | H | OAc | $NH_2$ |
| c | 4-MeO-$C_6H_4$-N=N- | $NH_2$ | c | 4-MeO-$C_6H_4$-N=N- | OAc | H | $NH_2$ | i | 4-MeO-$C_6H_4$-N=N | H | OAc | $NH_2$ |
| d | 4-Me-$C_6H_4$-N=N- | $NH_2$ | d | 4-Me-$C_6H_4$-N=N- | OAc | H | $NH_2$ | j | 4-Me-$C_6H_4$-N=N- | H | OAc | $NH_2$ |
| e | 4-Cl-$C_6H_4$-N=N- | $NH_2$ | e | 4-Cl-$C_6H_4$-N=N- | OAc | H | $NH_2$ | k | 4-Cl-$C_6H_4$-N=N- | H | OAc | $NH_2$ |
| f | 4-Br-$C_6H_4$-N=N- | $NH_2$ | f | 4-Br-$C_6H_4$-N=N- | OAc | H | $NH_2$ | l | 4-Br-$C_6H_4$-N=N- | H | OAc | $NH_2$ |

**Scheme 1.** Synthetic pathway for purine thioglycoside analogs.

stereochemical considerations. Thus, in a simple experimental procedure, treatment of compounds **1b–f** with **7** in DMF solvent under microwave activation 600 W for 10 minutes at 154°C, afforded the corresponding 7-mercaptopyrazolo[1,5-*a*]pyrimidine derivatives **9a–e**. The latter compounds were treated with sodium ethoxide followed by addition of the peracetylated sugars **4a,b** in DMF at room temperature to afford the *S*-glycosyl compounds **5b–f,h–l**. The latter were shown to be the same as those obtained from the reaction of **3** with **4** by comparing the obtained melting points and spectral data. Methylation of compounds **9a–e** with methyl iodide in sodium ethoxide gave the 7-methylthiopyrazolo[1,5-*a*]pyrimidine products **10a–e**. Compounds **10a–e** can also be prepared by reaction of **1b–f** with 2-[bis(methylthio)methylene]malononitrile **8** in dimethylformamide under microwave activation 600 W for 10 minutes at 154°C. When compounds **5b–f,h–l**



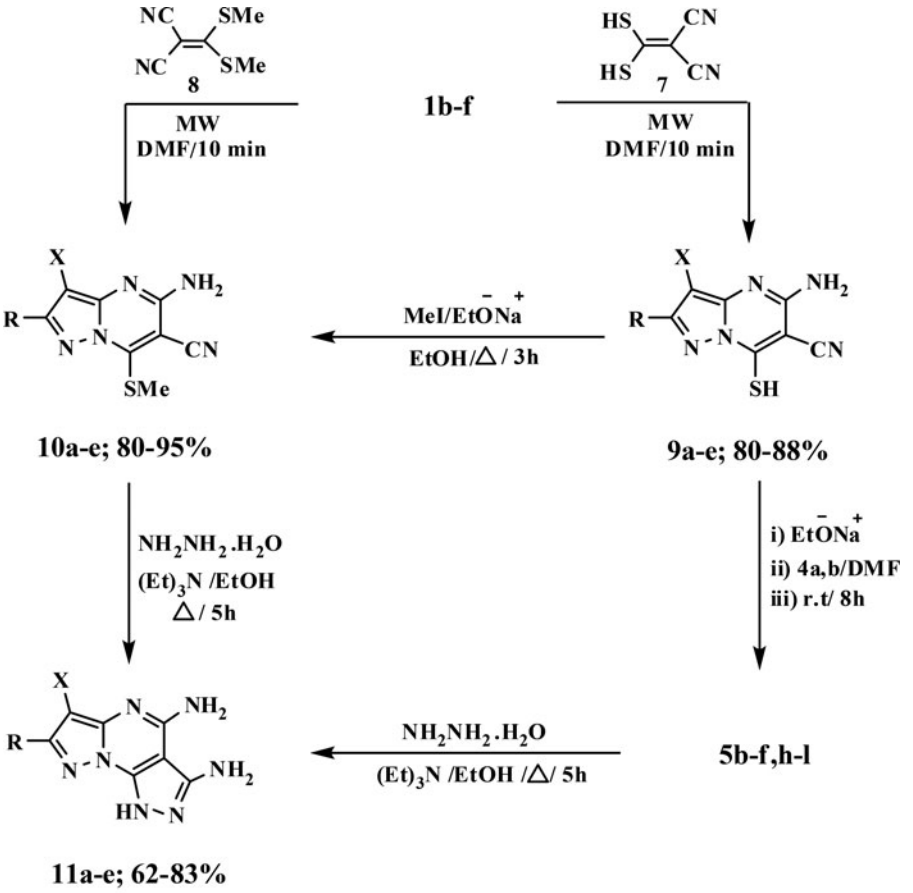

| 4 | Y | Z |
|---|---|---|
| a | OAc | H |
| b | H | OAc |

| 5 | X | Y | Z | R |
|---|---|---|---|---|
| b | $C_6H_5$-N=N- | OAc | H | $NH_2$ |
| i | 4-MeO-$C_6H_4$-N=N- | H | OAc | $NH_2$ |

| 6 | X | Y | Z | R |
|---|---|---|---|---|
| a | $C_6H_5$-N=N- | OH | H | $NH_2$ |
| b | 4-MeO-$C_6H_4$-N=N- | H | OH | $NH_2$ |

**Scheme 2.** Synthetic pathway for purine thioglycoside free sugar.

and **10a–e** were subjected to the reaction with hydrazine, the dipyrazolo[1,5-a:4′,3′-*e*]pyrimidine derivatives **11a–e** were obtained (Scheme 3).[26] The structure of compounds **11** were established on the basis of elemental analysis and spectral data (MS, $^1$H NMR and IR). Thus, the IR spectrum of **11a** revealed the absence of a CN band,




| 9,10,11 | X | R |
|---|---|---|
| a | $C_6H_5$-N=N- | $NH_2$ |
| b | 4-MeO-$C_6H_4$-N=N- | $NH_2$ |
| c | 4-Me-$C_6H_4$-N=N- | $NH_2$ |
| d | 4-Cl-$C_6H_4$-N=N- | $NH_2$ |
| e | 4-Br-$C_6H_4$-N=N- | $NH_2$ |

**Scheme 3.** Synthetic pathway for mercaptopyrazolo[1,5-a]pyrimidines and dibyrazolopyrimidines.

and $^1$H NMR spectrum contained a broad band at $\delta = 8.74$ ppm assignable to NH group, a multiplet at $\delta = 7.32–7.59$ ppm assigned to the aromatic protons and three broad singlets at $\delta$ 6.61 and 7.71 ppm assignable for three $NH_2$ groups.

### 2.2. Antitumor evaluation

Potential cytotoxicity effect of the ten of the newly synthesized compounds and doxorubicin drug were evaluated in the National Research Centre (Bio-assay–Cell Culture Laboratory) using MTT assay. The synthesized compounds **5a–f, 9a–d**

**Table 1.** Cytotoxicity of the synthesized candidates on lung (A549), colon (HCT116), liver (HEPG2), and prostate (PC3) cancer cell lines.

| Compound No. | Cytotoxic activity for A549 cell line | | Cytotoxic activity for HCT116 cell line | | Cytotoxic activity for HEPG2 cell line | | Cytotoxic activity for PC3 cell line | |
|---|---|---|---|---|---|---|---|---|
| | 100 $\mu$M (%) | $LC_{50}$ ( $\mu$M) | 100 $\mu$M (%) | $LC_{50}$ ( $\mu$M) | 100 $\mu$M (%) | $LC_{50}$ ( $\mu$M) | 100 $\mu$M (%) | $LC_{50}$ ( $\mu$M) |
| 5a | 75.0 | **68.5** | 80.4 | **59.4** | 42.4 | — | 100 | **32.7** |
| 5b | 54.6 | — | 48.8 | — | 62.6 | — | 55.7 | —- |
| 5c | 88.2 | **55.4** | 56.6 | **88.9** | 89.4 | **52.0** | 78.5 | **70.2** |
| 5d | 51.2 | — | 56.3 | — | 49.3 | — | 51.2 | — |
| 5e | 42.4 | — | 54.2 | — | 57.6 | — | 48.6 | — |
| 5f | 52.3 | — | 45.6 | — | 41.4 | — | 28.7 | — |
| 9a | 47.8 | — | 57.1 | — | 56.6 | — | 59.0 | — |
| 9b | 44.2 | — | 49.5 | — | 33.7 | — | 48.2 | — |
| 9c | 52.4 | — | 44.7 | — | 58.6 | — | 12.0 | — |
| 9d | 49.2 | — | 57.2 | — | 37.5 | — | 39.8 | — |
| Doxorubicin | | 48.8 | | 65.1 | | 37.8 | | 41.1 |

$LC_{50}$: Lethal concentration of the sample which causes the death of 50% of cells in 48 hours.

were subjected to in vitro antitumor screening against human cancer cell lines using cell- based disease.[27–31] The compounds were evaluated for their cytotoxicity against four human tumor cell lines namely hepatocellular carcinoma HEPG2, colon carcinoma HCT116, prostate carcinoma PC3, and lung carcinoma A549. Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan.[25,27] A probit analysis was carried for $LC_{50}$ determination using SPSS 11 program. The antitumor drug doxorubicin was used as positive control. In regard to the antitumor selectivity among the used tumor cell lines, the chemical compounds showed weak, moderate as well as strong inhibition ability against most of the tested cancer cells as revealed by the $LC_{50}$.The tested compounds **5b, 5d, 5e, 5f**, and **9a–d** exhibited low antitumor activity toward both the four cell lines (at 100 $\mu$M the values of mortality of cancer cell lines range between 12% and 59%) (Table 1). However compound **5c** proved to be slightly active toward all cancer cell lines with $LC_{50}$ values of 55,4 $\mu$M for lung cell line (A549) (Figure 1), 88.9 $\mu$M for colon cell line (HCT116) (Figure 2), 52.0 $\mu$M for hepatocellular cell line (HEPG2) (Figure 4) and 70.2 $\mu$M for prostate cell line (PC3) (Figure 3) respectively. On the other hand, compound **5a** exhibited slight activity against the lung cell line with an $LC_{50}$ value of 68.5 $\mu$M and higher activity against the colon cell line HCT116, and the prostate cell line PC3 with $LC_{50}$ values of 59.4 and 32.7 $\mu$M, respectively, compared to the standard drug doxorubicin. Looking at the structure-activity relationships of the tested compounds which contain a purine nucleus, it was found that the substance **5c**, possessing a *p*-methoxyphenyl ring appended to the C-3 position of the purine nucleus via a diazenyl linker and a thioglycosidic linkage at C-7 exhibited a minor amount of antitumor activity. In contrast, linking a phenyl, *p*-methyl, *p*-chloro, *p*-bromophenyl ring to the purine nucleus (**5b, 5d, 5e, 5f**, and **9a–d**) leads to essentially no antitumor activity compared with the standard drug. The loss of potency may be due to a decrease of cellular penetration or an inability to making effective contacts with

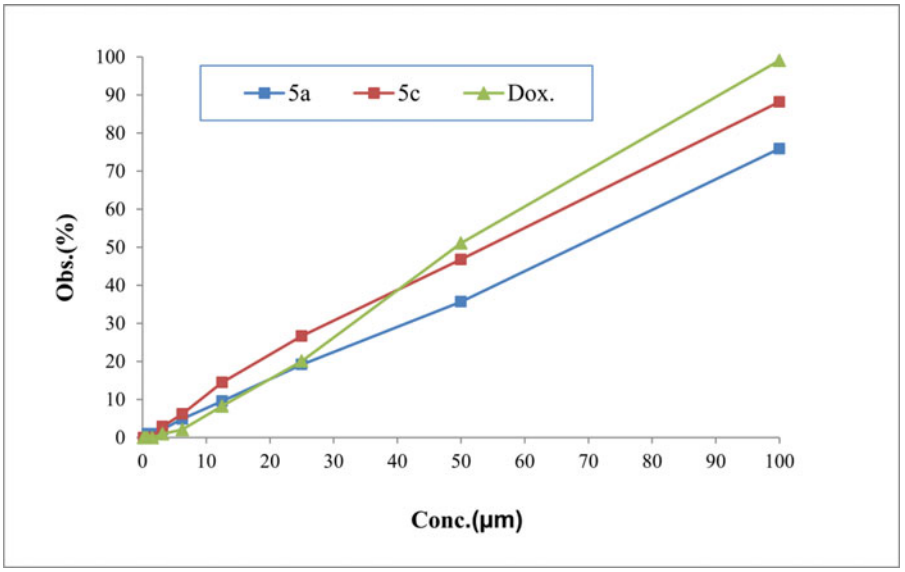



**Figure 1.** Representative graph showing observed responses of **A549** cell in the presence of increasing concentrations of compounds **5a** and **5c**.

the active site. On the other hand, the corresponding purine nucleus carrying two substituents especially with a thioglycosidic linkage at C-7 and cyano group at position 3 (**5a**) do have some activity. Such thioglycosides may act in the same way as antimetabolites by inhibiting key enzymes in the biosynthesis of purine and pyrimidine that are incorporated in the DNA molecule, resulting in cell death.

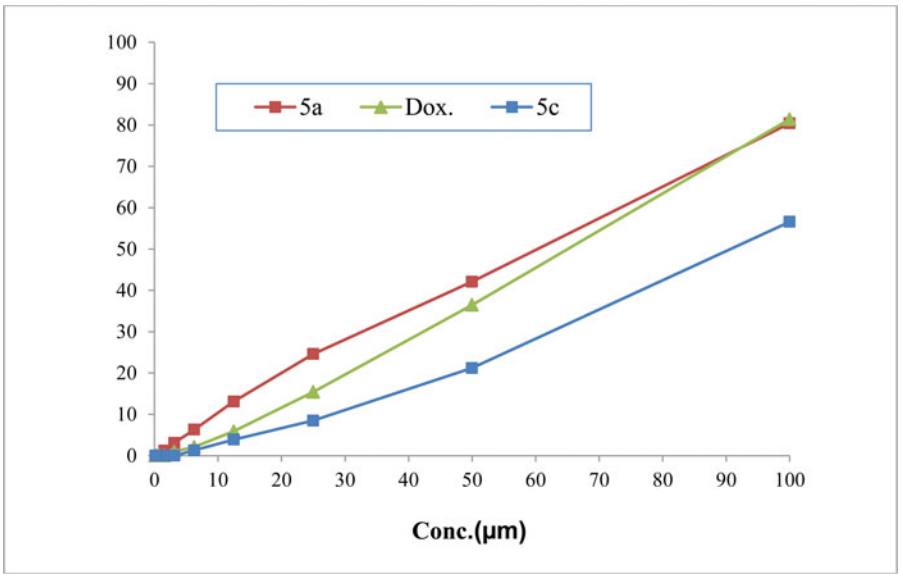



**Figure 2.** Representative graph showing observed responses of **HCT116** cell in the presence of increasing concentrations of compounds **5a** and **5c**.

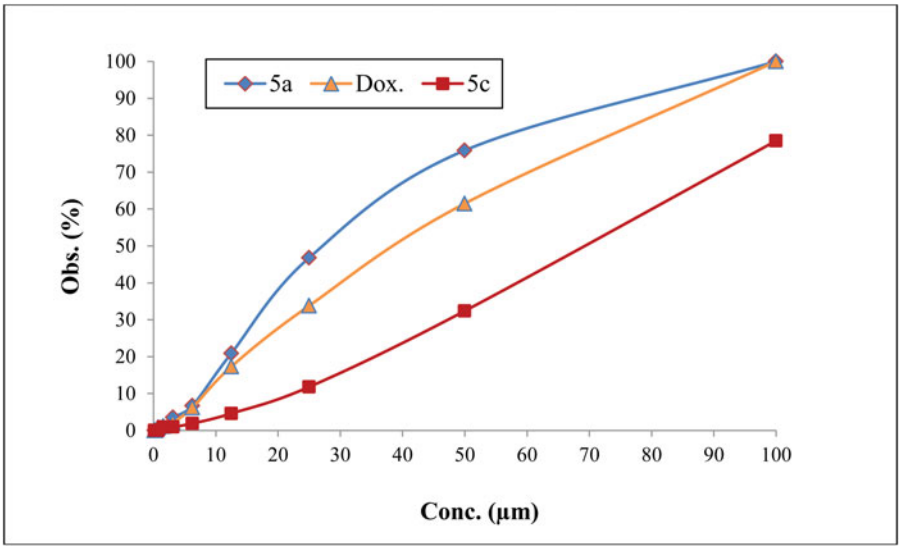



**Figure 3.** Representative graph showing observed responses of **PC3** cell in the presence of increasing concentrations of compounds **5a** and **5c**.

## 3. Experimental

### 3.1. Chemistry

All melting points were measured on a Gallenkamp melting point apparatus. The $^{1}H$ NMR spectra were measured on a Jeol-500 MHz spectrometer for solutions DMSO-$d_6$ and $CDCl_3$ using $Si(CH_3)_4$ as an internal standard at National Research Center, Cairo, Egypt. Progress of the reactions was monitored by TLC using aluminum sheets coated with silica gel F254 (Merck). Viewing under a short-wavelength UV lamp affected detection. Microwave reactions were conducted using Microwave.

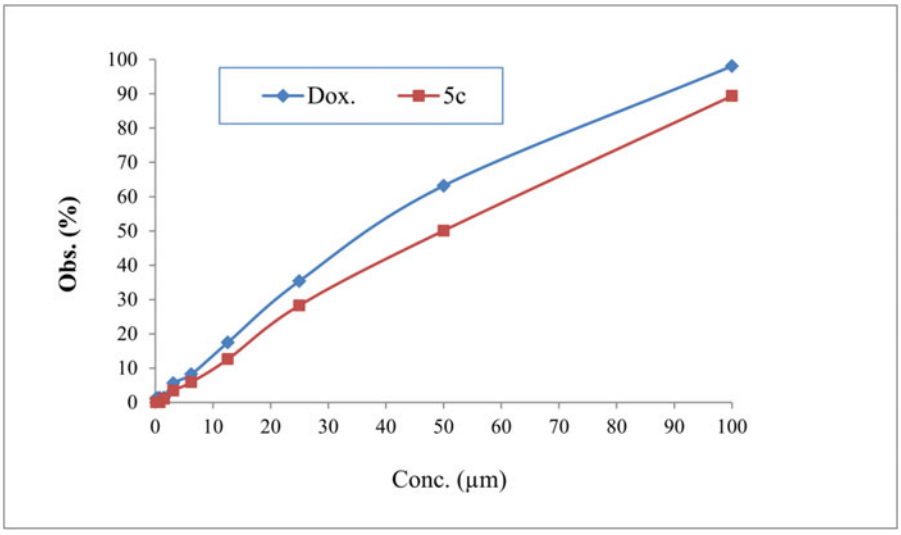



**Figure 4.** Representative graph showing observed responses of **HEPG2** cell in the presence of increasing concentrations of compound **5c**.

### 3.1.1. General procedure for the synthesis of 3a–f

A mixture of 4-(aryldiazenyl)-1*H*-pyrazole-3,5-diamine or 5-amino-1*H*-pyrazole-4-carbonitrile **1a–f** (10 mmol) and sodium 2,2-dicyanoethene-1,1-bis(thiolate) **2** (10 mmol) in DMF (20 mL) irradiated in a microwave oven 600 W for 10 minutes at 154°C. The reaction mixtures were cooled to room temperature and triturated with MeOH to afford compounds **3a–f** which was collected by filtration and recrystallized from the appropriate solvent. Yield (92%).

**3.1.1.1.** ***Sodium 5-amino-3,6-dicyanopyrazolo[1,5-a]pyrimidine-7-thiolat(3a).*** Brown solid; (MeOH); yield (92%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3465, 3284, 2213, 2216, 1607, 1598. $C_8H_3N_6NaS$.

**3.1.1.2.** ***Sodium 2,5-diamino-6-cyano-3-(phenyldiazenyl)pyrazolo[1,5-a] pyrimid-ine-7-thiolate(3b).*** Brown solid; (MeOH); yield (90%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3466, 3439, 3025, 2206, 1609, 1595. $C_{13}H_9N_8NaS$.

**3.1.1.3.** ***Sodium 2,5-diamino-6-cyano-3-((4-methoxyphenyl)diazenyl)pyrazolo[1, 5-a]pyrimidine-7-thiolate (3c).*** Brown solid; (MeOH); yield (95%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3465, 3433, 3286, 2927, 2879, 2205, 1606, 1596. $C_{14}H_{11}N_8NaOS$.

**3.1.1.4.** ***Sodium 2,5-diamino-6-cyano-3-(p-tolyldiazenyl)pyrazolo[1,5-a] pyrimid-ine-7-thiolate(3d).*** Brown solid; (MeOH); yield (95%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3455, 3427, 3065, 2956, 2206, 1719, 1601. $C_{14}H_{11}N_8NaS$.

**3.1.1.5.** ***Sodium 2,5-diamino-6-cyano-3-((4-chlorophenyl)diazenyl)-6-cyanopyrazolo[1,5-a]pyrimidine-7-thiolate(3e).*** Brown solid; (MeOH); yield (95%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3468, 3452, 3036, 2221, 1638, 1596. $C_{13}H_8ClN_8NaS$.

**3.1.1.6.** ***Sodium 2,5-diamino-6-cyano-3-((4-bromophenyl)diazenyl)-6-cyanopyrazolo[1,5-a]pyrimidine-7-thiolate(3f.).*** Brown solid; (MeOH); yield (93%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3435, 3389, 3028, 2209, 1645, 1613. $C_{13}H_8BrN_8NaS$.

### 3.1.2. General procedures for the synthesizing of (5a–l)

***Method A.***

To a solution of **3a–f** (10 mmol) in dry DMF (20 mL) a solution of halosugars **4** in dry DMF (10 mL) was dropped within 30 minutes and the reaction mixture was stirred at room temperature until completion (TLC. 6–8 hours). After completion, the reaction mixture was poured on ice water. A solid product precipitate was filtered off and washed with water, dried and purified by column chromatography using an appropriate solvent system giving compounds **5a–l**.

***Method B.***

To a solution of **9** (10 mmol) in equivalent sodium ethoxide was stirred for 30 minutes at room temperature. Then the reaction mixture was evaporated under reduced pressure and it dissolved in dry DMF (20 mL) followed by addition of the activated cyclic sugars of (2,3,4,6-tetra-*O*-acetyl-$\alpha$-D-gluco or galacto)pyronosyl bromide **4** in dry DMF (10 mL) within 30 minutes and the reaction mixture was stirred at room temperature until completion (TLC, 3–8 hours). After completion, the reaction mixture was poured on water. The aqueous phase was extracted with ethyl acetate (3 × 20 mL), the combined organic phase was washed with water, dried over anhydrous sodium sulphate. Removal of solvent gave a residue which was purified by column chromatography using an appropriate solvent system to give compounds **5**.

**3.1.2.1.** ***5-Amino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-pyrazolo [1,5-a]pyrimidine-3,6-dicarbonitrile(5a).*** Compound **5**a was purified by column chromatography (petroleum. ether /ethyl acetate 6:1, $R_f = 0.35–0.37$ region); yellow solid; (EtOH); yield (89%); mp154°C; $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 2.05–2.09 (4s, 12H, 4xOAc), 4.21, 4.23 (m, 2H, 2H-6′), 4.24 (m, 2H, H-4′, H-5′), 4.62 (*t*, 1H, H-3′), 5.23 (*t*, 1H, H-2′), 5.55–5.56 (d, 1H, $J_{1'-2'} =$ 8.70 Hz, H-1′), 6.63 (s, 2H, $NH_2$), 8.21 (s, 1H, pyrazole H-2); $^{13}$C NMR: $\delta$ 20.18 ($4CH_3CO$), 62.72 (C-6′), 68.82 (C-4′), 70.24 (C-2′), 72.62 (C-3′), 76.51 (C-5′), 81.46 (C-1′), 69.15 (C-3), 90.35 (C-6), 115.25 (CN), 116.42 (CN), 131.63 (C-3$^a$), 154.34 (C-2), 161.82 (C-5), 170.76 (4CO), 172.44 (C-7). Anal. Calcd. For $C_{22}H_{22}N_6O_9S$ (546.51): C, 48.35; H, 4.06; N, 15.38; S, 5.87%. Found: 48.28; H, 4.02; N, 15.25; S, 5.72%.

**3.1.2.2.** ***2,5-Diamino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-3-(phenyldiazenyl) pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5b).*** Compound **5**b was purified by column chromatography (petroleum. ether/ethyl acetate 8:2, $R_f =$ 0.74–0.76 region); yellow solid; (EtOH; yield (85%); mp165°C; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 1.94–2.09 (4s, 12H, 4xOAc), 4.01–4.09 (m, 3H, 2H-6′ and H-5′), 4.99 (*t*, 1H, H-4′), 5.13 (*t*, 1H, H-3′), 5.40 (*t*,1H, H-2′), 5.66–5.68 (*d*, 1H, $J_{1'-2'} =$ 9.95 Hz, H-1′), 6.66 (*s*, 2H, $NH_2$), 6.86 (*s*, 2H, $NH_2$), 7.34–7.89 (m, 5H, $C_6H_5$); $^{13}$C NMR; $\delta$ 21.22 ($4CH_3CO$), 62.62 (C-6′), 69.64 (C-4′), 70.43 (C-2′), 74.21 (C-3′), 77.11 (C-5′), 84.21 (C-1′), 91.21 (C-6), 93.51 (C-3), 117.23 (CN), 128.51 (Ar-C), 133.51 (C-3$^a$), 156.12 (C-2), 163.53 (C-5), 171.51 (4CO), 173.31 (C-7). Anal. Calcd. For $C_{27}H_{28}N_8O_9S$ (640.62): C, 50.62; H, 4.41; N, 17.49; S, 5.01%. Found: C, 50.51; H, 4.36; N, 17.40; S, 5.11%.

**3.1.2.3.** ***2,5-Diamino-3-((4-methoxyphenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio) pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5c).*** Compound **5**c was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.45–0.47region); yellow solid; (EtOH); yield (90%); mp177°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3455, 3397, 3029, 2223, 1734, 1649; $^1$H-NMR (500 MHz,$CDCl_3$)

$\delta$ 1.95–2.08 (4s, 12H, 4xOAc), 3.68 (s, 3H, $OCH_3$), 4.15 (m, 2H, 2H-6′), 4.23 (m, 1H, H-5′), 4.42 (t, 1H, H-4′), 4.56 (*t*, 1H, H-3′), 5.18–5.20 (t, 1H, H-2′), 5.23–5.24 (d, 1H, $J_{1'-2'} =$ 5.00 Hz, H-1′), 6.32 (s, 2H, $NH_2$), 6.52 (s, 2H, $NH_2$), 6.99–7.68 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{28}H_{30}N_8O_{10}S$ (670.65): C, 50.15; H, 4.51; N, 16.71; S, 4.78%. Found: C, 50.25; H, 4.55; N, 16.62; S, 4.62%.

**3.1.2.4.** ***2,5-Diamino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)-3-(p-tolyldiazenyl) pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5d).*** Compound **5**d was purified by column chromatography (petroleum ether/ethyl acetate 8:2,$R_f$ = 0.70–0.72 region); yellow solid; (EtOH); yield (82%); mp180°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3452, 3410, 3380, 2947, 2221, 1751, 1620. $^1$H-NMR (500 MHz, $CDCl_3$) $\delta$ 2.01–2.04 (4s, 12H, 4xOAc), 2.80 (s, 3H, $CH_3$), 4.14–418 (m, 2H,2 H-6′,), 4.25 (m, 1H, H-5′), 5.22 (*t*, 1H, H-4′), 5.35 (*t*,1H, H-3′), 5.58 (*t*, 1H, H-2′), 5.82–5.84 (d, 1H, $J_{1'-2'} =$ 14.15 Hz, H-1′), 6.61 (brs, 2H, $NH_2$), 6.81 (brs, 2H, $NH_2$), 7.22–7.36 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{28}H_{30}N_8O_9S$ (654.65): C, 51.37; H, 4.62; N, 17.12; S, 4.90%. Found: C, 51.40; H, 4.53; N, 17.15; S, 4.85%.

**3.1.2.5.** ***2,5-Diamino-3-((4-chlorophenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5e).*** Compound **5**e was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.45–47 region); yellow solid; (EtOH); yield (81%); mp185°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3475, 3416, 3242, 2210, 1745, 1604; $^1$H-NMR (500 MHz, $CDCl_3$)$\delta$ 2.02–2.07 (4s, 12H, 4xOAc), 3.98–4.26 (m, 1H, H-5′), 4.27–4.28 (m, 2H, 2H-6′), 4.80–5.00 (*t*, 1H, H-4′), 5.22 (*t*, 1H, H-3′), 5.38 (*t*,1H, H-2′), 5.79–5.80 (d, 1H, $J_{1'-2'} =$ 5.12 Hz, H-1′), 6.83 (brs, 2H, $NH_2$), 6.99 (brs, 2H, $NH_2$), 7.45–7.70 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{27}H_{27}ClN_8O_9S$ (675.07): C, 48.04; H, 4.03; N, 16.60; S, 4.75%. Found: C, 48.01; H, 4.01; N, 16.52; S, 4.71%.

**3.1.2.6.** ***2,5-Diamino-3-((4-bromophenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-glucopyranosylthio)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5f).*** Compound **5**f was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.65–67 region); yellow solid; (EtOH); yield (90%); mp19°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3455, 3401, 2218, 1751, 1620; $^1$H-NMR (500 MHz, $CDCl_3$)$\delta$ 1.95–2.08 (4s, 12H, 4xOAc), 3.92–394 (m, 2H, H-6′), 4.15 (m, 1H, H-5′), 4.73 (*t*,1H, H-4′), 4.95 (*t*, 1H, H-3′), 5.35 (*t*, 1H, H-2′), 5.55–5.57 (d, 1H, $J_{1'-2'} =$ 9.2 Hz, H-1′), 6.62 (brs, 2H, $NH_2$), 6.81 (brs, 2H, $NH_2$), 7.41–7.68 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{27}H_{27}BrN_8O_9S$ (719.52): C, 45.07; H, 3.78; N, 15.57; S, 4.46 5. Found: C, 45.20; H, 3.68; N, 15.60; S, 4.40%.

**3.1.2.7.** ***5-Amino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-pyrazolo[1,5-a]pyrimidine-3,6-dicarbonitrile(5g).*** Compound **5**g was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.70–72

region); yellow solid; (EtOH); yield (89%); mp154°C; $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ 2.03–2.11 (4s, 12H, 4xOAc), 4.11 (m, 2H, 2H-6′), 4.29–4.30 (m, 1H, H-5′), 5.15 (*t*, 1H, H-4′), 5.23 (*t*, 1H, H-3′), 5.31 (*t*, 1H, H-2′), 5.74–5.76 (d, 1H, $J_{1'-2'}$ = 10.70 Hz, H-1′), 6.73 (brs, 2H, $NH_2$), 8.34 (s, 1H, pyrazole H-2); $^{13}$C NMR: $\delta$ 21.25 ($4CH_3CO$), 62.54 (C-6′), 68.22 (C-4′), 71.44 (C-2′), 73.32 (C-3′), 77.42 (C-5′), 81.22 (C-1′), 92.25 (C-6), 93.12 (C-3), 116.33 (CN), 117.24 (CN), 132.52 (C-3$^a$), 155.44 (C-2), 160.46 (C-5), 170.66 (4CO), 172.22 (C-6). Anal. Calcd. For $C_{22}H_{22}N_6O_9S$ (546.51): C, 48.35; H, 4.06; N, 15.38; S, 5.87%. Found: C, 48.45; H, 4.16; N, 15.40; S, 5.85%.

**3.1.2.8.** ***2,5-Diamino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-3-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5h).*** Compound **5**h was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.45–47 region); yellow solid; (EtOH); yield (80%); mp172°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3470, 3436, 3029, 2207, 1743, 1596; $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ 1.94–2.19 (4s, 12H, 4xOAc), 4.06–4.15 (m, 3H,2H-6′ and H-5′), 4.94–4.97 (*t*, 1H, H-4′), 5.28–5.38 (m, 2H, H-3′ and H-2′), 5.52–5.54 (d, 1H, $J_{1'-2'}$ = 10.3 Hz, H-1′), 6.10 (s, 2H, $NH_2$), 6.43 (s, 2H, $NH_2$), 7.37–7.89 (m, 5H, $C_6H_5$). Anal. Calcd. For $C_{27}H_{28}N_8O_9S$ (640.62): C, 50.62; H, 4.41; N, 17.49; S, 5.01%. Found: C, 50.50; H, 4.35; N, 17.37; S, 5.11%.

**3.1.2.9.** ***2,5-Diamino-3-((4-methoxyphenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5i).*** Compound **5**i was purified by column chromatography (petroleum. ether/ethyl acetate 8:2,$R_f$ = 0.62–64 region); yellow solid; (EtOH); yield (85%); mp201°C; $^1$H-NMR (500 MHz, DMSO-$d_6$): $\delta$ 1.92–2.10 (4s, 12H, 4xOAc), 3.41 (s, 3H, $OCH_3$), 3.98–4.10 (m, 2H, 2H-6′), 4.11–4.12 (m, 1H, H-5′), 4.92–4.94 (*t*, 1H, H-4′), 5.11–5.13 (*t*, 1H, H-3′), 5.38 (*t*, 1H, H-2′), 5.79–5.81 (d, 1H, $J_{1'-2'}$ = 10.7 Hz, H-1′), 6.35 (s, 2H, $NH_2$), 6.64 (s, 2H, $NH_2$), 7.31–7.54 (m, 4H, $C_6H_4$); $^{13}$C NMR: $\delta$ 20.17 ($4CH_3CO$), 62.12 (C-6′), 68.62 (C-4′), 70.31 (C-2′), 73.32 (C-3′), 76.32 (C-5′), 82.21 (C-1′), 89.41 (C-6), 90.21 (C-3), 115.45 (2C, Ar-C), 117.11 (CN), 123.51 (Ar-C), 127.68 (2C, Ar-C), 134.52 (C-3$^a$), 158.10 (C-2), 167.68 (C-5), 169.82 (4CO), 171.17 (C-6). Anal. Calcd. For $C_{28}H_{30}N_8O_{10}S$ (670.65): C, 50.15; H, 4.51; N, 16.17; S, 4.78%. Found: C, 50.35; H, 4.45; N, 16.78; S, 4.62%.

**3.1.2.10.** ***2,5-Diamino-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio)-3-(p-tolyldiazenyl)pyrazolo [1,5-a]pyrimidine-6-carbonitrile(5j).*** Compound **5**j was purified by column chromatography (petroleum ether/ethyl acetate 8:2,$R_f$ = 0.61–63 region); yellow solid; (EtOH); yield (82%); mp186°C; $^1$H-NMR (500 MHz, DMSO-$d_6$)$\delta$ 2.05–2.09 (4s, 12H, 4xOAc), 2.69–2.70 (s, 3H, $CH_3$), 4.12 (m, 1H, H-5′), 4.23 (*t*, 1H, H-4′), 4.33–4.40 (m, 2H, 2H-6′), 4.67 (*t*,1H, H-3′), 5.23 (*t*, 1H, H-2′), 5.55–5.56 (d, 1H, $J_{1'-2'}$ = 3.8 Hz, H-1′), 6.53 (s, 2H, $NH_2$), 6.65 (s, 2H, $NH_2$),

7.25–7.44 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{28}H_{30}N_8O_9S$ (654.65): C, 51.37; H, 4.62; N, 17.12; S, 4.90%. Found: C, 51.20; H, 4.70; N, 17.22; S, 4.76%.

**3.1.2.11.** ***2,5-Diamino-3-((4-chlorophenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio) pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5k).*** Compound **5**k was purified by column chromatography (petroleum ether/ethyl acetate 8:2,$R_f$ = 0.55–57 region); yellow solid; (EtOH); yield (86%); mp188°C; $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ 2.02–2.36 (4s, 12H, 4xOAc), 3.89 (m, 1H, H-5′), 4.12 (*t*, 1H, H-4′), 4.24–4.26 (*t*, 1H, H-3′), 5.11–5.31 (*t*, 1H, H-2′), 5.33–5.34 (m, 2H, 2H-6′), 5.49–5.51 (d, 1H, $J_{1'-2'}$ = 10.7 Hz, H-1′), 6.20 (s, 2H, $NH_2$), 6.34 (s, 2H, $NH_2$), 7.25–7.71 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{27}H_{27}ClN_8O_9S$ (675.07): C, 48.04; H, 4.03; N, 16.60; S, 4.75%. Found: C, 48.24; H, 4.27; N, 16.25; S, 4.80%.

**3.1.2.12.** ***2,5-Diamino-3-((4-bromophenyl)diazenyl)-7-(2′,3′,4′,6′-tetra-O-acetyl-β-D-galactopyranosylthio) pyrazolo[1,5-a]pyrimidine-6-carbonitrile(5l).*** Compound **5**l was purified by column chromatography (petroleum. ether/ethyl acetate 8:2, $R_f$ = 0.65–0.67 region); yellow solid; (EtOH); yield (80%); mp191°C; $^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ 1.99–2.05 (4s, 12H, 4xOAc), 3.71 (m, H, 2H-5′), 4.12–4.23 (m, 2H, H-6′), 5.13 (*t*, 1H, H-2′), 5.32–5.34 (m, 2H, H-3′, H-4′), 5.49–5.51 (d, 1H, $J_{1'-2'}$ = 9.9 Hz, H-1′), 6.29 (s, 2H, $NH_2$), 6.64 (s, 2H, $NH_2$), 7.25–7.48 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{27}H_{27}BrN_8O_9S$ (719.52): C, 45.07; H, 3.78; N, 15.57; S, 4.46%. Found: C, 45.25; H, 3.58; N, 15.45; S, 4.32%.

### *3.1.3. Ammonolysis of 5b and 5i*

Dry gaseous ammonia was passed through a solution of protected nucleoside **5b** or **5i** (10 mmol)in dry methanol (20 mL) at room temperature for 10 minutes the reaction mixture was stirred until the reaction was judged complete by TLC (10–12 hours) using ($CHCl_3$/ MeOH 9:1) (Rf, 0.76–0.78). The resulting mixture was then concentrated under reduced pressure to afford (**6a** or **6b**) as solid residue that was crystallized from appropriate solvent.

**3.1.3.1.** ***2,5-Diamino-7-(β-D-glucopyranosylthio)-3-(phenyldiazenyl)pyrazolo [1,5-a]pyrimidine-6-carbonitrile(6a).*** Yellow solid; (EtOH); yield (85%); mp178°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3631, 3407, 3331, 3102, 2220, 1620, 1573. $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.85–3.92 (m, 2H, 2H-6′), 4.11 (m, 1H, H-5′), 4.50–4.60 (m, 2H, H-4′, H-3′), 4.70 (s, 1H, 2′-OH), 5.12 (m, 3H, 3′-OH, 4′-OH, and 6′-OH), 5.32 (*t*, 1H, H-2′), 5.56–5.58 (d, 1H, $J_{1'-2'}$ = 9.95 Hz, H-1′), 6.45 (s, 2H, $NH_2$), 6.75 (s, 2H, $NH_2$), 7.42–7.68 (m, 5H, $C_6H_5$). Anal. Calcd. For $C_{19}H_{20}N_8O_5S$ (472.48): C, 48.30; H, 4.27; N, 23.72; S, 6.79%. Found: C, 48.40; H, 4.16; N, 23.65; S, 6.72%.

**3.1.3.2.** ***2,5-Diamino-3-((4-methoxyphenyl)diazenyl)-7-(β-D-galactopyranosyl-hio)pyrazolo[1,5-a] pyrimidine-6-carbonitrile(6b).*** Yellow solid; (EtOH); yield

(85%); mp185°C; $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 3.70–374 (m, 3H, 2H-6′, H-5′), 3.82 (s, 3H, $OCH_3$), 4.34 (m, 2H, H-4′, H-3′), 4.61 (d, 2H, 2′-OH, 3′-OH), 4.84 (d, 1H, 4′-OH), 5.47 (d, 1H, 6′-OH), 5.52 (*t*, H, H-2′), 5.61–5.62 (d, 1H, $J_{1'-2'}$ = 10.95 Hz, H-1′), 6.62 (s, 2H, $NH_2$), 6.84 (s, 2H, $NH_2$), 7.43–7.67 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{20}H_{22}N_8O_6S$ (502.14): C, 47.80; H, 4.41; N, 22.30; 6.38%. Found: C, 47.72; H, 4.35; N, 22.28; S, 6.35%.

### 3.1.4. General procedure for the synthesizing of 9a–e

A mixture of each of 4-(aryldiazenyl)-1H-pyrazole-3,5-diamine **1** (10 mmol) and 2-(dimercaptomethylene)malononitrile) **7** (10 mmol) was dissolved in DMF (20 mL) in small beaker. The reaction mixture was put in a domestic microwave oven 600 W and irradiate for 10 minutes at 154°C. The progress of the reaction was monitored by TLC until the reactants disappeared, then the solution was concentrated and the remaining residue was triturated with MeOH to afford **9** which was purified by column chromatography (3:1 petroleum ether –EtOAc) and recrystallized from the appropriate solvent.

**3.1.4.1.** ***2,5-Diamino-7-mercapto-3-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(9a).*** Brown solid; (MeOH); yield (82%); mp 284°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3417, 3309, 3207, 3147, 3109, 2206, 1620, 1563; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 6.47 (s, 2H, $NH_2$), 6.75 (s, 2H, $NH_2$), 7.24–7.67 (m, 5H, $C_6H_5$), 12.21 (s, 1H, SH); $^{13}$C NMR: $\delta$ 89.39 (C-6), 94.21 (C-3), 117.24 (CN), 128.61(4C, Ar-C), 128.82 (2C, Ar-C), 135.14 (C-3$^a$), 156.45 (C-2), 166.36 (C-5), 191.51 (C-7); MS. m/z (%) 307 (20) [$M^{+3}$]. Anal. Calcd. For $C_{13}H_{10}N_8S$ (310.34): C, 50.31; H, 3.25; N, 36.11; S, 10.33%. Found: C, 50.30; H, 3.12; N, 36.21; S, 10.20%.

**3.1.4.2.** ***2,5-Diamino-7-mercapto-3-((4-methoxyphenyl)diazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitril (9b).*** Brown solid; (MeOH); yield (85%); mp 272°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3433, 3406, 3296, 2909, 2210, 1673, 1649. $^1$H NMR (500 MHz, $CDCl_3$)$\delta$ 3.80 (s, 3H, $OCH_3$), 6.51 (s, 2H, $NH_2$), 6.68 (s, 2H, $NH_2$), 7.25–7.33 (m, 4H, $C_6H_4$), 7.61 (s, 1H, SH); $^{13}$C NMR: $\delta$ 56.71 ($CH_3$), 88.75 (C-6), 93.23 (C-3), 114.53 (2C, Ar-C), 117.62 (CN), 122.12 (4C, Ar-C), 129.61 (2C, Ar-C), 133.14 (C-3$^a$), 155.13 (C-2), 166.26 (C-5), 194.53 (C-7); MS. *m/z* (%) 341 (18) [$M^{+2}$]. Anal. Calcd. For $C_{14}H_{12}N_8OS$ (340.36): C, 49.40; H, 3.55; N, 32.92; S, 9.42%. Found: C, 49.35; H, 3.40; N, 32.85; S, 9.25%.

**3.1.4.3.** ***2,5-Diamino-7-mercapto-3-(p-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(9c).*** Yellow solid; (MeOH); yield (88%); mp 298–300°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3407, 3306, 3117, 3062, 2206, 1596, 1696. $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 2.69 (s, 3H, $CH_3$), 6.23 (s, 2H, $NH_2$), 6.62 (s, 2H, $NH_2$), 7.15–7.55 (m, 4H, $C_6H_4$), 7.91 (s, 1H, SH); $^{13}$C NMR: $\delta$ 40.22 ($CH_3$), 89.35 (C-6), 93.21 (C-3), 117.12 (2C, Ar-C), 126.27 (CN), 128.36 (4C, Ar-C), 131.61 (2C, Ar-C), 132.31 (C-3$^a$), 156.33

(C-2), 165.21 (C-5), 190.58 (C-7). Anal. Calcd. For $C_{14}H_{12}N_8S$ (324.36): C, 51.84; H, 3.73; N, 34.55; S, 9.89%. Found: C, 51.80; H, 3.61; N, 34.46; S, 9.77%.

**3.1.4.4.** ***2,5-Diamino-3-((4-chlorophenyl)diazenyl)-7-mercaptopyrazolo[1,5-a]pyrimidine-6-carbonitrile (9d).*** Brown solid; (MeOH); yield (75%); mp 286°C; $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 6.61 (s, 2H, $NH_2$), 6.75 (s, 2H, $NH_2$), 7.45–7.58 (m, 4H, $C_6H_4$), 11.99 (SH); $^{13}$C NMR: $\delta$ 89.35 (C-6), 93.22 (C-3), 117.12 (CN), 126.27 (Ar-C), 128.36 (2C, Ar-C), 131.61 (2C, Ar-C), 132.31 (C-3$^a$), 156.33 (C-2), 165.21 (C-5), 190.58 (C-7); MS. m/z (%) 347 (25) [$M^{+3}$]. Anal. Calcd. For $C_{13}H_9ClN_8S$ (344.78): C, 45.29; H, 2.63; N, 32.50; S, 9.30%. Found: C, 45.20; H, 2.55; N, 32.43; S, 9.25%.

**3.1.4.5.** ***2,5-Diamino-3-((4-bromophenyl)diazenyl)-7-mercaptopyrazolo[1,5-a]pyrimidine-6-carbonitrile (9e).*** Brown solid; (MeOH); yield (86%); mp 282°C; $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ 6.61 (s, 2H, $NH_2$), 6.81 (s, 2H, $NH_2$), 7.25–7.40 (m, 4H, $C_6H_4$), 11.12 (s, 1H, SH); $^{13}$C NMR: $\delta$ 89.35 (C-6), 93.22 (C-3), 117.12 (CN), 123.24 (Ar-C), 130.13 (4C, Ar-C), 130.78 (C-3$^a$), 128.11 (C, Ar-C), 157.13 (C-2), 163.22 (C-5), 191.33 (C-7). Anal. Calcd. For $C_{13}H_9BrN_8S$ (389.23): C, 40.11; H, 2.33; N, 28.79; S, 8.24%. Found: C, 40.06; H, 2.30; N, 28.65; S, 8.18%.

### *3.1.5. General procedure for the synthesizing of (10a-e)*

***Method A***:

A solution of compounds **1** (10 mmol) and 2-(bis(methylthio)methylene) malononitrile **8** (10 mmol) dissolved in DMF (20 mL) and boiled at 154°C under microwave 600 W for 10 minutes. The reaction was monitored by TLC (petroleum ether–EtOAc 3;1) until the reactants disappeared. The solvent was then evaporated and the remaining residue was triturated with MeOH to afford compounds **10** which was separated by column chromatography and recrystallized from the appropriate solvent. Yield (86%).

***Method B***:

A solution of compounds **9** (10 mmol) in sodium ethoxide(10 mmol) was refluxed for 30 minutes allowed to cool to room temperature and methyl iodide (10 mmol) then was added slowly to this mixture, then the reaction mixture was refluxed with stirred for 3 hours. The formed solid product was collected by filtration and recrystallized from appropriate solvent.

**3.1.5.1.** ***2, 5-Diamino-7-(methylthio)-3-(phenyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(10a).*** Brown solid; (MeOH); yield (95%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3468, 3397, 2978, 2220, 1620, 1611. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 2.81 (S, 3H, $SCH_3$), 6.62 (s, 2H, $NH_2$), 6.85 (s, 2H, $NH_2$) 7.33–7.49 (m, 5H, $C_6H_5$); $^{13}$C NMR: $\delta$ 14.12 ($CH_3$), 91.52 (C-6), 93.21 (C-3), 117.52 (CN), 128.91 (4C, Ar-C), 130.23 (2C, Ar-C), 133.51 (C-3$^a$), 156.57 (C-2), 165.25 (C-5), 173.52 (C-6). Anal. Calcd.

For $C_{14}H_{12}N_8S$ (324.36): C, 51.84; H, 3.73; N, 34.55; S, 9.89%. Found: C, 51.75; H, 3.70; N, 34.52; S, 9.80%.

**3.1.5.2.** ***2,5-Diamino-3-((4-methoxyphenyl)diazenyl)-7-(methylthio)pyrazolo [1,5-a]pyrimidine-6-carbonitrile(10b).*** Brown solid; (MeOH); yield (95%); mp >300°C. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 2.33 (S, 3H, $SCH_3$), 3.85 (s, 3H, $OCH_3$), 6.30 (s, 2H, $NH_2$), 6.51 (s, 2H, $NH_2$), 7.33–7.61 (m, 4H, $C_6H_4$); $^{13}$C NMR: $\delta$ 14.53 ($SCH_3$), 57.21 ($OCH_3$), 91.22 (C-6), 95.11 (C-3), 115.12 (2C, Ar-C), 117.63 (CN), 122.4 (Ar-C), 128.79 (2C, Ar-C), 134.22 (C-3$^a$), 157.11 (C-2), 161.53 (Ar-C), 165.65 (C-5), 174.24 (C-6). Anal. Calcd. For $C_{15}H_{14}N_8OS$ (354.39): C, 50.84; H, 3.98; N, 31.62; S, 9.05%. Found C, 50.77; H, 3.78; S, 9.01%.

**3.1.5.3.** ***2,5-Diamino-7-(-methylthio)-3-(p-tolyldiazenyl)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(10c).*** Brown solid; (MeOH); yield (90%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3452, 3412, 3382, 2933, 2893, 2220, 1604, 1596. $^1$H NMR (500 MHz, $CDCl_3$): $\delta$ 2.51 (s, 3H, $CH_3$), 2.90 (S, 3H, $SCH_3$), 6.64 (s, 2H, $NH_2$), 6.86 (s, 2H, $NH_2$), 7.32–7.79 (m, 4H, $C_6H_4$); $^{13}$C NMR: $\delta$ 13.13 ($SCH_3$), 21.23 ($CH_3$), 90.82 (C-6), 96.12 (C-3), 117.33 (CN), 125.71 (Ar-C), 128.72 (2C, Ar-C), 129.22 (2C, Ar-C), 134.11 (C-3$^a$), 138.23 (Ar-C), 154.11 (C-2), 164.45 (C-5), 172.34 (C-7). Anal. Calcd. For $C_{15}H_{14}N_8S$ (324.36): C, 53.24; H, 4.17; N, 33.11; S, 9.48%. Found: C,53.35; H, 4.20; N, 33.25; S, 9.35%.

**3.1.5.4.** ***2,5-Diamino-3-((4-chlorophenyl)diazenyl)-7-(methylthio)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(10d).*** Brown solid; (MeOH); yield (90%); mp 300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3472, 3417, 3018, 2943, 2218, 1614, 1598. $^1$H NMR (500 MHz, DMSO-$d_6$): $\delta$ 2.68 (s, 3H, $SCH_3$), 6.66 (s, 2H, $NH_2$), 6.87 (s, 2H, $NH_2$), 7.32–7.76 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{14}H_{11}ClN_8S$ (358.81): C, 46.86; H, 3.09; N, 31.23; S, 8.94%. Found: C,46.70; H, 3.05; N, 31.20; S, 8.85%.

**3.1.5.5.** ***2,5-Diamino-3-((4-bromophenyl)diazenyl)-7-(methylthio)pyrazolo[1,5-a]pyrimidine-6-carbonitrile(10e).*** Brown solid; (MeOH); yield (90%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3458, 3402, 2943, 2883, 2219, 1620, 1596. $^1$H NMR (500 MHz, DMSO-$d_6$)$\delta$ 2.83 (s, 3H, $SCH_3$), 6.65 (s, 2H, $NH_2$), 6.88 (s, 2H, $NH_2$) 7.34–7.78 (m, 4H, $C_6H_4$). Anal. Calcd. For $C_{14}H_{11}BrN_8S$ (403.26): C, 41.70; H, 2.75; N, 27.79; S, 7.95%. Found: C,41.76; H, 2.60; N, 27.68; S, 7.82%.

### *3.1.6. General procedure for the synthesizing of (11a–e)*

A solution of **5** or **10** (10 mmol) and hydrazine hydrate (10 mmol) in ethanol (20 mL) containing a few drops of triethylamine was refluxed for 5 hours, cooled, the precipitate was filtered off and crystallized from the appropriate solvent.

**3.1.6.1.** ***6-(Phenyldiazenyl)-1H-dipyrazolo[1,5-a:4′,3′-e]pyrimidine-3,4,7-triamine(11a).*** Yellow solid; (EtOH); yield (80%); mp >300°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3480, 3390 ($NH_2$, NH); $^1$H NMR (DMSO)$\delta$ 6.61 (brs, 4H, $2NH_2$), 7.32–7.59 (m, 5H, $C_6H_5$), 7.71 (brs, 2H, $NH_2$), 8.74 (s, 1H, NH); MS. m/z (%) 308 (22) [$M^+$]. Anal. Calcd. for $C_{13}H_{12}N_{10}$ (308.30): C, 50.64; H, 3.92, N, 45.43%. Found: C, 50.49; H, 3.87; N, 45.36%.

**3.1.6.2.** ***6-((4-Methoxyphenyl)diazenyl)-1H-dipyrazolo[1,5-a:4′,3′-e]pyrimidine-3,4,7-triamine(11b).*** yellow solid; (EtOH); yield (83%); mp >300°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3577, 3408, 3314; $^1$H NMR (DMSO) $\delta$ 3.81 (s, 3H, $OCH_3$), 6.36 (brs, 2H, $NH_2$), 6.48 (brs, 2H, $NH_2$), 6.92 (m, 2H, phenyl protons), 7.38 (brs, 2H, $NH_2$), 7.61 (m, 2H, phenyl protons), 8.44 (brs, 1H, NH); MS. m/z (%) 338 (12) [$M^+$]. Anal. Calcd. for $C_{14}H_{14}N_{10}O$ (338.33): C,49.70; H, 4.71; N, 41.40%. Found: C, 49.62; H, 4.57; N, 41.36%.

**3.1.6.3.** ***6-(p-Tolyldiazenyl)-1H-dipyrazolo[1,5-a:4′,3′-e]pyrimidine-3,4,7-triamine(11c).*** yellow solid; (EtOH); yield (76%); mp >300°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3327. $^1$H NMR (DMSO) $\delta$ 3.91 (s, 3H, $OCH_3$), 6.56 (s, 4H, $2NH_2$), 7.23–7.26 (m, 2H, $C_6H_2$), 7.36 (s, 2H, $NH_2$), 7.54–7.57(m, 2H, $C_6H_2$), 8.44 (s, 1H, NH). Anal. Calcd. for $C_{14}H_{14}N_{10}$ (322.33): C, 52.17; H, 4.38; N, 43.45%. Found: C, 52.12; H, 4.25; N, 43.35%.

**3.1.6.4.** ***6-((4-Chlorophenyl)diazenyl)-1H-dipyrazolo[1,5-a:4′,3′-e]pyrimidine-3,4,7-triamine(11d).*** yellow solid; (EtOH); yield (82%); mp >300°C; IR (KBr, $cm^{-1}$)$\upsilon$ 3530, 3440 ($NH_2$, NH). Anal. Calcd. for $C_{13}H_{11}ClN_{10}$ (342.75): C, 45.56; H, 3.23, N, 40.87%. Found: C, 45.42; H, 3.15; N, 40.77%.

**3.1.6.5.** ***6-((4-Bromophenyl)diazenyl)-1H-dipyrazolo[1,5-a:4′,3′-e]pyrimidine-3,4,7-triamine(11e).*** yellow solid; (EtOH); yield (75%); mp >300°C; IR (KBr, $cm^{-1}$) $\upsilon$ 3458, 3400, 2220. $^1$H NMR (DMSO)$\delta$ 7.09 (brs, 4H, $2NH_2$), 7.28–7.49 (m, 4H, $C_6H_4$), 7.67 (brs, 2H, $NH_2$), 10.01 (s, 1H, NH). Anal. Calcd. for $C_{13}H_{11}BrN_{10}$ (387.20): C, 40.33; H, 2.86, N, 36.17%. Found: C, 40.25; H, 2.78; N, 36.10%.

## 3.2. Biological assays

### 3.2.1. Antitumor screening

Cell viability was assessed by the mitochondrial dependent reduction of yellow MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) to purple formazan.[23]

***Procedure***: All the following procedures were done in a sterile area using a Laminar flow cabinet biosafety class II level (Baker, SG403INT, Sanford, ME, USA).

Cells were suspended in RPMI 1640 medium for HePG2- PC3 and HCT116–DMEM for A549. The media are supplemented with 1% antibiotic-anti mycotic mixture(10,000U/ml Potassium Penicillin, 10,000 $\mu$g/ml Streptomycin Sulfate and 25 $\mu$g/ml Amphotericin B), 1% L-glutamine and 10% fetal bovine serum and kept at 37°C under 5% $CO_2$. Cells were batch cultured for 10 days, then seeded at concentration of $10 \times 10^3$ cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 hours under 5% $CO_2$ using a water jacketed Carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA). Media was aspirated, fresh medium (without serum) was added and cells were incubated either alone (negative control) or with different concentrations of sample to give a final concentration of (100–50-25–12.5–6.25–3.125–0.78 and 1.56 ug/ml). After 48 hours of incubation, medium was aspirated, 40ul MTT salt (2.5 $\mu$g/ml) were added to each well and incubated for further four hours at 37°C under 5% $CO_2$. To stop the reaction and dissolve the formed crystals, 200 $\mu$L of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37°C. A positive control which composed of 100 $\mu$g/ml was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions.[19,24] The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent *t*-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula: ((Reading of extract / Reading of negative control) -1) x 100.A probit analysis was carried for $LC_{50}$ and $LC_{90}$ determination using SPSS 11 program.

## 4. Conclusion

We have developed a new and simple method for the synthesis of the purine and guanine thioglycoside analogs under microwave activation. The mild reaction conditions, clean reaction profiles, zero side product and cost efficiency render this approach as a useful and innovative to the existing methods for glycoside formation. Further studies on the application of this method for the synthesis of other highly functionalized biologically active glycosides are underway. The antitumor activities of the synthesized compounds were tested against human tumor cell lines: (lung (A549), colon (HCT116), liver (HEPG2), and prostate (PC3)).

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