Egypt. J. Chem. **Vol. 65,** No. 3 pp. 161 - 169 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Synthesis and Cytotoxic Activity of New Substituted Pyrazolo[3,4-*b*]pyridine Derivatives and Their Acyclic Nucleoside Analogs

Mohamed A. Hawata[a*], Farag A. El-Essawy[a,b], Wael A. El-Sayed[c,d] and Mohamed N. El-Bayaa[c]

[a] *Chemistry Department, Faculty of Science, Menoufia University, Shebin El-Kom 32511, Egypt.*

[b] *Basic Science Department, Preparatory Year Deanship, Prince Sattam Bin Abdulaziz University, Al Kharj 16278, Saudi Arabia.*

[c] *Photochemistry Department, National Research Centre, Dokki, Giza 12622 Egypt,*

[d] *Department of Chemistry, College of Science, Qassim University, Buraidah, 51452 Saudi Arabia*

Abstract

As an important strategy, including incorporation of more than active core in one molecule, for finding potent candidates against cancer cells, a number of functionalized pyrazolopyridin derivatives linked to oxadiazole, dioxolane, acyclic sugar and fluorene ring systems, were synthesized through heterocyclization reactions. The derived sugar hydrazone and the corresponding acyclic *C*-nucleoside analog in addition to acyclic *N*-nucleoside were also prepared. The behavior of the afforded compounds as possible cytotoxic agents against human HTC116 and MCF7 cancer cells was investigated and the results showed that compounds **11-13** showed the highest activities against the two cancer cells. Other compounds revealed a type of selectivity toward one cancer cell while the activity was relatively lost against the other cancer cell line.

Keywords: pyrazolopyridine; sugar; hydrazone; oxadiazole; acyclic nucleoside; anticancer.

1. INTRODUCTION

Cancer is one of the most serious diseases and we can treat with chemotherapy, but this method is still rare as there are few chemotherapy agents with potent activities and also because of severs toxicities to normal cells in addition to undesirable side effects of these agents. We need to improve novel chemotherapeutic agents which are more active as antitumor and taking into consideration decrease side effects which represent a great attention and actual challenge to medicinal chemists to develop a safe and no-side effected anticancer drug. In such direction, an interesting strategy involving the synthesis of novel hybrid molecules incorporating varied well-known active cores, was found efficient in designing and discovering potent anticancer leads [1,2].

Recently pyrazole derivatives are catching attention because of its biological activities [3-8]. They are also acknowledged for the reported anticancer activities of pyrazole motif incorporating compounds [9-11]. Fused heterocycles possessing the pyrazole ring, such as those incorporating the pyrazolopyridine system were reported by their broad spectrum of important bioactivities [12-14]. The pyrazolo[3,4-b]pyridine system constitute an interesting group of heterocyclic compounds and a number of their derived compounds are more eminent as kinase, such as Pazopanib [15] inhibitors for "CDK2" such as Roscovitine (**Figure 1**) [16-18] in addition to the reported blood platelet aggregation inhibitory activity [19], the effect of improving "bonemetabolism" [20] and adenosine antagonist activity.

*Corresponding author e-mail: drmohamedhwata@gmail.com; (Mohamed A. Hawata)

EJCHEM use only: Receive Date: 09 August 2021, Revise Date: 21 August 2021, Accept Date: 06 September 2021

DOI: 10.21608/ejchem.2021.90009.4302

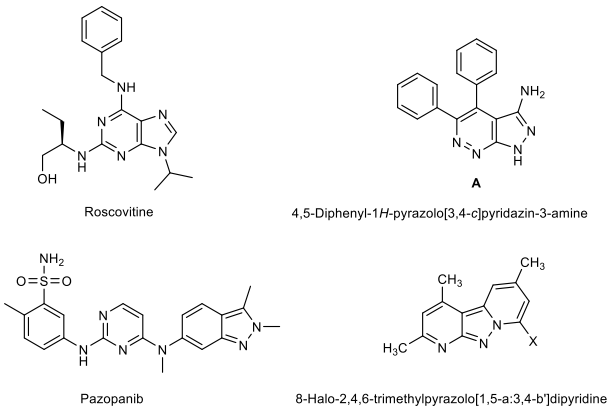


Figure 1: Anticancer pyrazolopyridine derivatives and related compounds

Another promising five membered core is 1,3,4-oxadiazole because of its application in new agrochemicals and bioactive compounds [21,22] including anticancer activities [23].

On the other hand, acyclic *C*-nucleosides, being iso-steric and structural analogs of modified nucleosides were reported with their antiviral, anticancer and antimicrobial activities [24]. The promoted structural modification involving the heterocyclic nucleobase (as aglycon part) and/or the sugar moiety (as the glycon part) was found efficient in designing and synthesizing potent anticancer and antiviral compounds and we have been interested in designing new heterocyclic sugar derivatives [25]. In an ongoing research, we document in the current work the synthesis and cytotoxic activity of new hybrid compounds incorporating the pyrazolopyridine and 1,3,4-oxadiazole ring systems attached to acyclic sugar moieties.

2. EXPERIMENTAL

Chemistry

Kofler block apparatus was utilized to determine Melting points and are un-corrected. The IR spectra were documented on a perkin-Elmer 1720 FTIR spectrometer (cm^{-1}), using KBr disks. ^{1}H NMR and ^{13}C NMR spectra were determined on a varian Gemini spectrometer (300 MHz) using DMSO-d_6 or $CDCl_3$ as a solvent and TMS (δ) as the internal standard. Mass spectra were obtained using Mass spectra were obtained using a CC 2010 Shimadzu Gas chromatography instrument mass spectrometer (70 eV). The progress of the reactions was monitored by TLC using aluminum silica gel plates 60 F245.

General procedures for the preparation of 2a-2c. A solution of **1** (3.6 mmol, 0.58g) and the substituted malonate reagents (3.8 mmol) in CH_3COOH (10 mL) was refluxed for 5-8 h (TLC). The r.m. allowed to reach r.t. and poured on to crushed ice. The ppt. was filtered and cleaned by column chromatography using 5% MeOH in CH_2Cl_2 as eluent.

2,4-Dimethyl-8-oxo-5,8-dihydro-1,5,8*a*,9-tetraazafluorene-7-carbonitrile (2a). Yield: 51%, mp > 300ºC. IR (KBr) cm^{-1}, *v*: 3159 (NH), 2226 (C≡N), 1638 (C=O). ^{1}H NMR spectrum, δ, ppm: 2.55 s (3H, CH_3), 2.60 s (3H, CH_3), 4.40 brs (1H, NH), 6.85 s (1H, Ar-H) 8.51 s (1H, CH). ^{13}C NMR spectrum, δc, ppm: 18.56, 24.73 (2xCH_3), 61.08, 92.94, 102.72, 147.70, 148.80, 149.57, 153.49, 161.92, 165.75, 174.72 (Ar-Carbons). Mass spectrum: *m/z* = 239 [M^+]. Found, %: C C 60.35; H 3.89; N 29.17. $C_{12}H_9N_5O$. Found, %: C 60.35; H 3.89; N 29.17. $C_{12}H_9N_5O$. Calculated, %: C 60.25; H 3.79; N 29.27.

8-Imino-2,4-dimethyl-5,8-dihydro-1,5,8*a*,9-tetraazafluorene-7-carbonitrile (2b). Yield: 40%, mp > 300ºC. IR (KBr) cm^{-1}, *v*: 3338 (NH), 2228 (C≡N). ^{1}H NMR spectrum, δ, ppm: 2.55 s (3H, CH_3), 2.60 s (3H, CH_3), 4.40 brs (1H, NH), 6.81 s (1H, Ar-H) 8.50 s (1H, CH) 9.11 s (1H, NH). Mass spectrum: *m/z* = 238 [M^+]. Found, %: C 60.57; H 4.28; N 35.17. $C_{12}H_{10}N_6$. Calculated, %: C 60.50; H 4.23; N 35.27. *M* 238.25.

2,4-Dimethyl-8-oxo-5,8-dihydro-1,5,8*a*,9-tetraazafluorene-7-carboxylic acid ethyl ester (2c). According to the previously published procedure [27], the preparation was done.

2,4-Dimethyl-8-oxo-5,8-dihydro-1,5,8*a*,9-tetraazafluorene-7-carboxylic acid hydrazide (3). A mix of **2c** (0.28 g, 1 mmol) and $N_2H_4.H_2O$ (0.1 mL, 3 mmol) in C_2H_5OH (20 mL) was refluxed for 6 h. Then ethanol was evaporated by rotary evaporator and the ppt. was washed with C_2H_5OH, filtered, dried and recrystallized from methanol to afford **3** as white crystals. Yield: 75%, mp 288-290ºC. IR (KBr) cm^{-1}, *v*: 3428-3298 (NH_2), 3159 (NH), 1638(C=O). ^{1}H NMR spectrum, δ, ppm: 2.49 brs (2H, NHN*H*$_2$), 2.51 s (3H, CH_3), 2.74 s (3H, CH_3), 3.31 brs (1H, NH), 6.67 s (1H, Ar-H), 8.71 s (1H, NC*H*) 10.29 brs (1H, CON*H*). Found, %: C 52.86; H 4.40; N 30.75. $C_{12}H_{12}N_6O_2$. Calculated, %: C 52.94; H 4.44; N 30.87.

2,4-Dimethyl-8-oxo-5,8-dihydro-1,5,8*a*,9-tetraazafluorene-7-carboxylic acid (4-nitrobenzylidene)hydrazide (4). To a stirred solution of compound **3** (0.27 g, 1 mmol) in C_2H_5OH (30 mL), *p*-nitrobenzaldehyde (0.3 g, 2 mmol) and a catalytic amount of acetic acid were inserted at r.m. The reaction mixture was refluxed for six hours then ethanol was evaporated and the remaining ppt. was collected and dried. Recrystallization from ethanol afforded the tetraazafluoren derivative **4** as a yellow powder. Yield: 86%, mp 160-162ºC. IR (KBr) cm^{-1}, *v*: 3159 (NH), 1638 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.49 s (3H, CH_3), 2.50 s (3H, CH_3), 3.29 brs (1H, NH), 6.92 s (1H, Ar-H), 8.15-8.31 m (4H, Ar-H), 8.59 s (1H, N*CH*), 8.91 s (1H ,CH), 9.28 s (1H, NH). Found, %: C 56.39; H 3.62; N 24.26. $C_{19}H_{15}N_7O_4$. Calculated, %: C 56.30; H 3.73; N 24.19.

General procedures for the preparation of triazenes 5a-5c. A mix of the pyrazolpyridine **1** (1.5 g, 9.35 mmol), conc. HCl (3 mL) and H_2O (3 m) was cooled to 0 ºC, and a solution of $NaNO_2$ 0.8 g (15.2 mmol) in H_2O (10 mL) was added. After swirling for 30 min, a mix of the appropriate amine (12 mmol) and K_2CO_3 (2.5 g, 18.1 mmol) in H_2O (25 mL) was included. The r.m. was swirled at r.t. until diazonium salt had disappeared by (TLC). The solution was extracted with $CHCl_3$ (3×75 mL). The organic phases were cleaned with H_2O (3×30 mL), dried over Na_2SO_4, and steam *in vacuo* to give the product which was washed with Et_2O/petrolum ether (65-70^{O}C) (1:1, v/v) to give products **5a-5c** in 50-70% yield.

3-(3-ethyl-3-methyltriaz-1-en-1-yl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine (5a). Yield: 70%, mp 123-125ºC. IR (KBr) cm^{-1}, *v*: 3435 (NH). ^{1}H NMR spectrum, *δ*, ppm: 1.24 s (6H, 2×CH_3), 2.50 s (3H, CH_3), 2.57 s (3H, CH_3), 3.78 q (2H, CH_2), 6.81 s (1H, Ar-H), 12.79 brs (1H, NH). ^{13}C NMR spectrum, *δ*c, ppm: 19.19 (2xCH_3), 23.91 (2xCH_3), 106.56 (CH_2), 117.55, 117.76, 142.08, 151.38, 152.35, 157.47 (Ar-Carbons). Mass spectrum: *m/z* = 232 [M^+]. Found, %: C 56.79; H 6.82; N 36.08. $C_{11}H_{16}N_6$. Calculated, %: C 56.88; H 6.94; N 36.18. *M* 232.29.

4,6-dimethyl-3-(3-methyl-3-phenyltriaz-1-en-1-yl)-1H-pyrazolo[3,4-b]pyridine (5b). Yield: 60%, mp 130-132ºC. IR (KBr) cm^{-1}, *v*: 3404 (NH). ^{1}H NMR spectrum, *δ*, ppm: 2.67 s (3H, CH_3), 2.76 s (3H, CH_3), 2.88 s (3H, CH_3), 3.75 brs (1H, NH), 6.82 s (1H, Ar-H), 7.25-7.68 m (5H, Ar-H). ^{13}C NMR spectrum, *δ*c, ppm: 19.66 (CH_3), 21.88 (CH_3), 33.70 (N-CH_3) 109.15, 118.24, 118.52, 121.93, 125.18, 128.15, 129.46, 129.51, 129.96, 143.97, 149.18, 157.44 (Ar-Carbons). Found, %: C 64.36; H 5.85; N 29.90. $C_{15}H_{16}N_6$. Calculated, %: C 64.27; H 5.75; N 29.98.

3-(3,3-diphenyltriaz-1-en-1-yl)-4,6-dimethyl-1H-pyrazolo[3,4-b]pyridine (5c). Yield: 65%, mp 138-140ºC. IR (KBr) cm^{-1}, *v*: 3395 (NH). ^{1}H NMR spectrum, *δ*, ppm: 2.58 s (3H, CH_3), 2.74 s (3H, CH_3), 3.66 brs (1H, NH), 6.79 s (1H, Ar-H), 6.81-7.87 m (10H, Ar-H). ^{13}C NMR spectrum, *δ*c, ppm: 21.32 (CH_3), 23.74 (CH_3), 114.96, 116.68, 119.30, 119.57, 120.10, 121.86, 124.64, 129.06, 129.30, 141.31, 143.40, 145.29, 158.38 (Ar-Carbons). Found, %: C 70.25; H 5.34; N 24.59. $C_{20}H_{18}N_6$. Calculated, %: C 70.16; H 5.30; N 24.54.

(*E*)-*N*-benzylidene-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (6). A mix of **1** 1.6 g (10 mmol), benzaldehyde 1 mL (10 mmol), and CH_3COOH 0.06 mL (1 mmol) in toluene (50 mL) was refluxed with azeotropic removal of water for 10 h. The mixture was reached to 5 ºC, gave crude **6** by filtration. The compound was washed with cold toluene and ether [15].

[3-(Benzylidene-amino)-4,6-dimethyl-3*a*,7*a*-dihydropyrazolo[3,4-*b*]pyridin-1-yl]acetic acid ethyl ester (7). To a stirred suspension of **6** (1.20, 5 mmol) in 10 mL dimethylformamide, K_2CO_3 (7.5 mmol, 1.04g,) ethyl chloroacetate was added dropwise (0.9 mL, 7.5 mmol). The r.m. was stirred at r.t. for 24 h., and then poured into crushed ice with stirring. The ppt. was cleaned with H_2O and recrystalized from C_2H_5OH to give the white crystals of **7**. Yield: 91%, mp 96-98ºC. IR (KBr) cm^{-1}, *v*: 2965, 2929 (CH aliphatic), 1742 (COOEt), 1589 (C=N). ^{1}H NMR spectrum, *δ*, ppm: 1.27 t (3H, *J* = 3.5 Hz, CH_3), 2.52 s (3H, CH_3), 2.63 s (3H, CH_3), 4.44 q (2H, *J* = 3.5 Hz, CH_2), 5.28 s (2H, NCH_2), 6.60 s (1H, H-5, ArH), 7.24-8.02 m (5H, Ar-H), 9.09 s (1H, N=CH). Found, %: C 67.92; H 5.91; N 16.57. $C_{19}H_{20}N_4O_2$. Calculated, %: C 67.84; H 5.99; N 16.66.

(*E*)-2-[3-(Benzylideneamino)-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl)acetohydrazide (8). The pyrazolopyridine ester **7** (2.18 g, 6.5 mmol) and $N_2H_4.H_2O$ (0.7 mL, 25 mmol) in C_2H_5OH (20 mL) was refluxed for 6 h. ethanol was evaporated and the ppt. cleaned with filtered off and

recrystalized from methanol to afford a white powder of **8.** Yield: 92 %, mp 258-260ºC. IR (KBr) cm^{-1}, *v*: 3437-3305 (NH*NH*$_2$), 3194-3056 (CH aromatic), 2979, 2931 (CH aliphatic), 1652 (CONH), 1633 (C=N). ^{1}H NMR spectrum, *δ*, ppm: 2.47 brs (2H, NHN*H*$_2$), 2.55 s (3H, CH_3), 2.61 s (3H, CH_3), 5.27 s (2H, NCH_2), 6.62 s (1H, H-5, ArH), 7.22-8.01 m (5H, Ar-H), 9.07 s (1H, N=CH), 10.09 brs (1H, CON*H*). Found, %: C 63.40; H 5.54; N 26.17. $C_{17}H_{18}N_6O$. Calculated, %: C 63.34; H 5.63; N 26.07.

D-(+)-Mannose {2-[3-(benzylideneamino)-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl]}acetohydrazone (9). A solution of the D-mannose (1.8 g, 10 mmol) in water (3 mL) was treated with a solution of **8** (3.2 g, 10 mmol) in C_2H_5OH (75 mL) and 1-3 drops of CH_3COOH. The solution was refluxed for 6 h. then the solvent was evaporated and the ppt. was washed with little amount of distilled water and recrystalized from dimethylformamide to give a white powder of compound **9**. Yield: 65 %, mp 164-166ºC. IR (KBr) cm^{-1}, *v*: 3440-3410 (OH, NH), 3113-3056 (CH aromatic), 2959, 2931 (CH-aliph.), 1678 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 2.42 s (3H, CH_3), 2.55 s (3H, CH_3), 3.30-4.28 m (5H, alditoyl protons), 4.32-4.75 brs (5H, 5xOH), 5.11 s (2H, NCH_2), 5.17 m (2H, CH_2), 6.62 s (1H, H-5, ArH), 7.26-7.39 m (5H, Ar-H), 7.49 s (1H, N=CH), 11.28 brs (1H, NH). Found, %: C 57.12; H 5.90; N 17.27. $C_{23}H_{28}N_6O_6$. Calculated, %: C 57.02; H 5.82; N 17.35.

2,3,4,5,6-Penta-*O*-acetyl-D-mannose {2-[3-(benzylideneamino)-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl]}acetohydrazone (10). A mixture of **9** (0.48 g, 1 mmol), acetic anhydride (10 mL), and pyridine was swirled at room temperature for overnight. The r.m. was poured onto crushed ice with stirring, the ppt. collected and cleaned by a solution of Na_2CO_3 then H_2O and recrystalized from methanol to afford product **10** as a pale yellow gum. Yield: 51 %. IR (KBr) cm^{-1}, *v*: 3110-3058 (CH aromatic), 2933, 2911 (CH aliphatic),1755 (C=O), 1665 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 1.86, 1.90, 1.95, 1.98, 2.02 s (15H, 5 $OCOCH_3$), 2.36 s (3H, CH_3), 2.49 s (3H, CH_3), 4.15 m (2H, CH_2), 4.32 s (2H, NCH_2), 5.01-5.44 m (5H, alditoyl protons), 6.88 s (1H, H-5, ArH), 7.01-7.44 m (5H, Ar-H), 8.12 s (1H, N=CH hydrazide). 8.22 s (1H, N=CH), 10.06 brs (1H, NH). Found, %: C 57.14; H 5.61; N 12.15. $C_{33}H_{38}N_6O_{11}$. Calculated, %: C 57.05; H 5.51; N 12.10.

4-Acetyl-5-(1,2,3,4,5-penta-*O*-acetyl-D-mannopentitolyl-{2-[3-(benzylideneamino)-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-1-yl]methyl}-2,3-dihydro-1,3,4-oxadiazoline (11). A solution of **9** (0.48 g, 1 mmol) and $(CH_3CO)_2O$ (10 mL) was refluxed for 2 h. The r.m. was colled then poured onto cold crushed ice with swirling until the ppt. was obtained and collected by filtration, washed by a solution of Na_2CO_3 then H_2O and recrystalized from methanol alcohol to afford product **11** as oil. Yield: 40%. IR (KBr) cm^{-1}, *v*: 2925-2919 (CH aliphatic), 1740 (OAc), 1675-1630 (C=N), 1618 (C=O). ^{1}H NMR spectrum, *δ*, ppm: 1.83, 1.89, 1.92, 1.96, 1.98, 2.02 s (18H, 6 $OCOCH_3$), 2.37 s (3H, CH_3), 2.48 s (3H, CH_3), 4.15 m (2H, CH_2), 4.33 s (2H, NCH_2), 5.01-5.64 m (4H, alditoyl protons), 5.88 d (1H, oxadiazole), 6.87 s (1H, H-5, ArH), 7.01-7.45 m (5H, Ar-H), 8.89 s (1H, N=CH). ^{13}C NMR spectrum, *δ*c, ppm: 18.46, 21.11, 22.55, 23.14, 24.89 (8xCH_3), 54.31 (CH_2), 61.09, 62.25, 66.54, 68.12, 74.29, 76.78 (CH aliphatic), 102.72, 122.65, 128.47, 132.91, 135.42, 145.64, 149.57, 153.49, (Ar-Carbons), 159.14 (CH=N), 168.11, 168.48, 169.44, 170.16 (6xCOAc). Found, %: C 57.16; H 5.56; N 11.33. $C_{35}H_{40}N_6O_{12}$. Calculated, %: C 57.06; H 5.47; N 11.41.

Benzylidene-[1-(2,2-dimethyl-[1,3]dioxolan-4-ylmethyl)-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-yl]amine (12). To a stirred solution of **6** (2.4 g, 10 mmol) in dimethylformamide (10 mL) was inserted sodium hydride (0.4 g, 10 mmol). After exactly full evolution of hydrogen gas, the r.m. was warmed to 100ºC for 1 h. then isopropylidene tosylate derivative (10 mmol) was inserted. The r.m. was stirred for 3 h. at 100ºC, iced to r.t. and filtered off. The solvent was vaporized to dehydration, coevaporated with toluene (3 x 10 mL) and refined with silica gel chromatography using 2% methanol in Dichloromethane to give **12** in 70% yield as a pale brown powder. Yield: 70%, mp 170-172°C. IR (KBr) cm^{-1}, *v*: 2943-2920 (CH aliphatic), 1641-1567 (C=N). ^{1}H NMR spectrum, *δ*, ppm: 1.18 s (6H, 2×CH_3), 2.37 s (3H, CH_3), 2.67 s (3H, CH_3), 3.88 q (2H, NCH_2), 4.03 q (2H, OCH_2), 4.65 s (1H, OCH), 6.88 s (1H, H-5, ArH), 7.43-8.00 m (5H, Ar-H), 9.06 s (1H, N=CH). ^{13}C NMR spectrum, *δ*c, ppm: 17.99, 21.04, 24.30, 26,32 (4 CH_3), 59.80 (CH_2), 63.86,

64.79, 70.22, 72.68, 108.72, 118.42, 127.61, 128.90, 130.10, 131.71, 135.89, 142.96, 148.78, 151.52, 158.24, 159,14 (Ar-Carbons). Found, %: C 69.12; H 6.69; N 15.47. $C_{21}H_{24}N_4O_2$. Calculated, %: C 69.21; H 6.64; N 15.37.

3-[3-(Benzylideneamino)-4,6-dimethyl-pyrazolo[3,4-*b*]pyridin-1-yl]-propane-1,2-diol (13). The isopropylidines **12** (1.8 g, 5 mmol) was dispersed in 70% AcOH (5 mL). The r.m. was refluxed for 2h. The solvent was vaporized and the remainder was coevaporated with water (2x3 mL) and C_2H_5OH (2x3 mL). The remainder oil was cleaned by column chromatography using 5% methanol in chloroform to afford **13** in 73% yield as a dark brown powder. Yield: 73%, mp 160-162°C. IR (KBr) cm^{-1}, *v*: 3359 (OH), 2933-2922 (CH-aliph.), 1643-1577 (C=N). 1H NMR spectrum, δ, ppm: 2.36 s (3H, CH3), 2.57 s (3H, CH3), 3.89 q (2H, NCH2), 4.13 q (2H, OCH2), 4.35 m (1H, OH), 4.68 s (1H, OCH), 5.58 m (1H, OH), 6.89 s (1H, H-5, ArH), 7.33-8.00 m (5H, Ar-H), 8.99 s (1H, N=CH). ^{13}C NMR spectrum, *δ*c, ppm: 18.31, 24.23 (2xCH_3), 61.51, 63.56, 92.18, 102.16, 123.41, 128.11, 129.78, 132.54, 135.47, 147.70, 148.80, 151.49, 153.82, 158.19, 159.81 (Ar-Carbons). Found, %: C 66.59; H 6.26; N 17.18. $C_{18}H_{20}N_4O_2$. Calculated, %: C 66.65; H 6.21; N 17.27.

Cytotoxic Activity

Material; ATCC through Vacsera tissue culture laboratories provided us with thr cell lines, Lonza, Belgium, serum from Gibco, trypsin provided us with All media, and Biobasic Canada provided us with MTT.

Viability test; After 24h of preparing 20000 cells per well (in 96-well plates), as cells became 60-70%, the medium turned to serum-free medium exhibiting an ending count of the established samples of 100 μM in triplicates. Then, they were cured for 72 h and Doxorubicin (100μM) was exhausted as a positive control and serum-free medium was exhausted as a negative control.

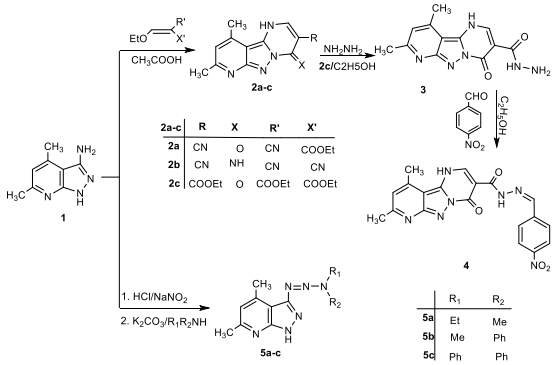
IC50 control was tested on samples which gave high cytotoxicity percentage via applying the SPSS leading to non-linear regression analysis to get the IC50 values. Cell viability was finalized using the MTT assay [28].

3. RESULTS AND DISCUSSION

Chemistry

The amino-1*H*-pyrazolo[3,4-*b*]pyridine **1** was obtained by the published procedure [26]. In this investigation, when the pyrazolopyridine **1** was reacted with ethyl 2-cyano-3-ethoxyacrylate, 2-(ethoxymethylene)malononitrile and diethyl 2-(ethoxymethylene)malonate and catalytic amount of acetic acid gave the fluorene derivatives **2a-c**, respectively in 40-55% yields after purification via column chromatography using 5% MeOH in CH_2Cl_2 as eluent. The mechanism of formation of the tricyclic compounds **2a-c** starts by nucleophilic attack of the pyrazolopyridine-N^2. Heterocyclization ring via another attack of the NH_2 nitrogen on the nitrile carbon and rearrangement lead to the fused pyrimidine ring affording compounds **2a-c**. Another possible rout involved the first nucleophilic reaction by the amino nitrogen followed by the attack of the N^2 affording the tricyclic system after rearrangement. The absence of NH_2 group and appearance of CN, C=O, COOEt groups in IR spectra and ^{1}H NMR analysis confirm the new compounds. A mixture of **2c** and $N_2H_4.H_2O$ in ethanol was heated under reflux to give compound **3** as white crystals (75%) which was condensed with *p*-nitrobenzaldehyde with 1-3 drops of glacial acetic acid to give a white powder of **4** as a yellow powder (86%). Also absence of NH_2 group in IR spectra,^{1}H NMR analysis and appear of benzine ring confirm compound **4**.

Diazotization of pyrazolpyridine **1** by using conc. HCl, H_2O, and $NaNO_2$ followed by addition of the appropriate amines gave the substituted triaza-pyrazolpyridine products **5a-5c** in 50-70% yields (**Scheme1**).

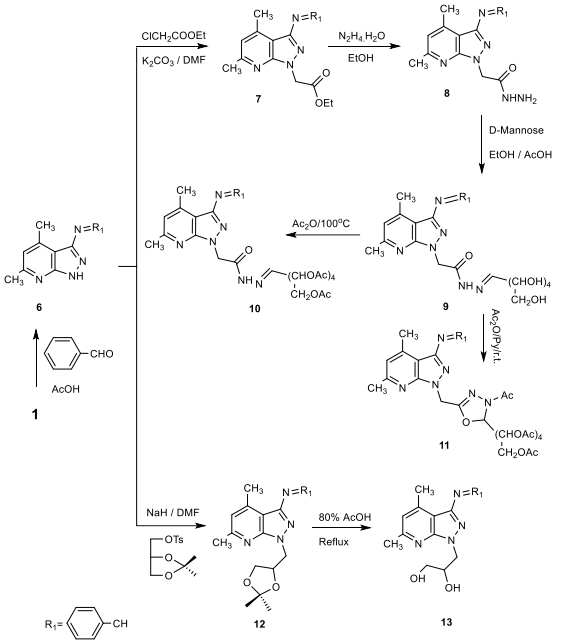


Scheme 1: Synthesis of tetraazafluorene and substituted pyrazolopyridine derivatives

On the other hand, (*E*)-*N*-benzylidene-4,6-dimethyl-1*H*-pyrazolo[3,4-*b*]pyridin-3-amine (**6**) was obtained by condensation of **1** and benzaldehyde with few drops of acetic acid according to the published procedure [15]. The potassium salt of **6** in dry DMF was stirring with ethyl chloroacetate to give the white crystals of **7** (91%) which was refluxed with $N_2H_4.H_2O$ in ethanol to give a white powder of **8** (92 %). D-Mannose was treated with a solution of **8** in ethanol (75 mL) and 1-3 drops of acetic acid to give the corresponding sugar hydrazone **9** as a white powder (65 %). sp^2 character of the methine proton (originally H1 in the sugar part) at 7.49 ppm was detected with high chemical shifts at 7.49 ppm prove the acyclic conformation of the sugar moiety in hydrazones **9**.

Sugar hydrazone **9** was reacted with acetic anhydride in two routes, the first involved the reaction in dry pyridine at r.t. giving the corresponding acetylated sugar hydrazone **10** in 51% yield. In the other route the reaction was carried out at reflux temperature for 2 h in an excess of acetic anhydride and afforded compound **11**, in which the 1,3,4-oxadiazoline ring is substituted with acetylated sugar chain, as an oily substance in 40% yield. The ^{1}H NMR of oxadiazoline sugar derivatives **11** explained the oxadiazoline-H^2 signals (originally H^1 in the sugar part) at 5.88 ppm indicating its sp^3 nature.

At last, the sodium salt of **6** (10 mmol) in dry Dimethylformamide was treated with isopropylidene tosylate derivative and the reaction mixture was filtered off. The excess solvent was vaporized, coevaporated with toluene (3 x 10 mL) and cleaned with silica gel chromatography using 2% MeOH in CH_2Cl_2 to give **12** in 70% yield as a pale brown powder. Deisopropylidenation of **12** was carried out by refluxing in 70% AcOH to give **13** in 73% yield, the structures was elucidated by analysis IR and NMR.(**Scheme 2**).



Scheme 2: Synthesis of pyrazolopyridine sugar hydrazones and acyclic nucleoside analogs

Anticancer screening

The new products were scanned in vitro for their cytotoxic activity against HCT-116 and MCF-7 cancer cells utilized the MTT assay. The results were outlined as the percentage cytotoxicity of the new compounds on both cancer cell lines (**Table 1, Figure** 2) and IC50 values for the most potent compounds (**Table 2**). Doxorubicin was utilized as a reference drug for comparison with the obtained results of the screened compounds in the current investigation. The percentages of healthy cells and the control group were compared.

The results outlined in tables 1 and 2 showed that compounds **11**, **12** and **13** showed the highest activities against the two cancer cells with IC_{50} 41±6, 39±4 and 35±4.1 µM, respectively. Other compounds such as **3**, **4**, **7** and **9** revealed a type of selectivity toward one cancer cell while the activity was relatively lost against the other cancer cell line. Thus, according table 1, in terms of the percentage cytotoxicity results, compound **9** was moderately active against HCT116 cell while the its activity was markedly lowered with respect to MCF-7 cell. On the other hand, compound **4** showed a different behavior since its activity against MCF7 cell was, obviously higher than its activity against the HCT116 cell line. The same behavior was also revealed by compounds **3** and **7**.

Correlation of the obtained activity outcomes with the characteristic structure features of the active products and their related structural analogs has revealed that incorporation of a 1,3,4-oxadiazole ring substituted with an acetylated sugar moiety to the pyrazolopyridine system lead to increased activity against both of the cancer cells. The activity of the acetylated sugar hydrazone **10** which is a structural analog of the later derivative but lacked for the 1,3,4-oxadiazole ring (**Table 1,2**) and (**Figure 2**) was found markedly lower than that of the oxadiazole incorporating derivative. Furthermore, the pyrazolopyridine nucleoside analog **12** and its derived acyclic nucleoside analog **13** showed raised cytotoxic activities against the two cancer cells. The sugar hydrazones possessing the acyclic sugar parts linked to the pyrazolpyridine system via a hydrazonyl linkage showed low activities compared to the later nucleoside analogs in which the sugar part was directly attached to the pyrazole ring system. In addition, the results showed that the deacetylated sugar hydrazone with free hydroxyl groups showed higher activity against the human colorectal carcinoma cell than its per-*O*-acetylated derivative. On the other hand, the attachment of the aryl system to the free hydrazide group (compound 4) resulted in loss of activity against the HCT116 cancer cell.

Table 1. Percentage cytotoxicity on HCT116 and MCF7 cancer cell lines at 100 µM[a]

[a]The data are presented as average cytotoxicity of 3 results ±standard deviation.

Compound	HCT116	MCF7
2a	34.97±0.65	35.37±7.09
2b	27.76±3.53	16.21±3.17
2c	56.01±0.1	33.43±0.23
3	68.38±0.91	46.27±0.19
4	17.79±5.25	70.7±1.76
5a	8.04±0.21	15.81±0.22
5b	31.59±5.73	34.9±3.18
5c	19.03±3.92	45.87±2.46
6	21.46±5.8	8.56±5.17
7	37.69±0.42	63.09±0.8
8	52.08±2.01	49.19±2.76
9	64.54±3.94	27.48±8.37
10	15.46±7.35	47.53±2.38
11	97.33±0.81	96.17±1.11
12	92.33±1.18	90.20±0.92
13	90.33±1.91	88.97±0.55
Doxorubicin	100.00	100.00

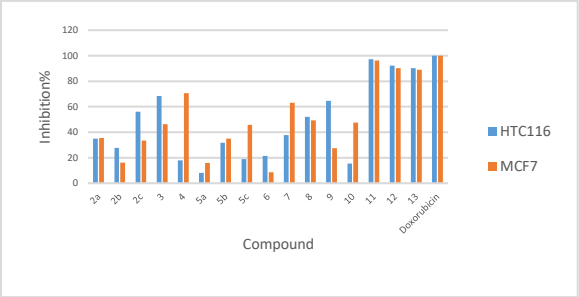


Figure 2. Inhibition percentage of compounds

Table 2. IC_{50} values for the more active compounds

Compound	IC_{50},µM	
	HCT116	**MCF7**
11	41±6, r^2 = 0.96	26.79±2.91, r^2 = 0.93
12	39±4, r^2 = 0.98	17.17±2.93, r^2 = 0.98
13	35±4.1, r^2 = 0.97	28.34±3.91, r^2 = 0.92
Doxorubicin	2.2±3.1, r^2 = 0.99	12.8±1, r^2 = 0.96

[a]The results are presented as average IC_{50} ±standard deviation, (r_2) coefficient of determination.

4. CONCLUSION

The factionalized pyrazolopyridine system could be a useful precursor for new hybrid molecules with good cytotoxic activities against cancer cells. A number of the functionalized synthesized compounds showed varied behavior towards the two cancer cell lines revealing a type of selectivity for one cell compared to the other. The attachment of a sugar substituted 1,3,4-oxadiazole ring to the pyrazole ring system resulted in increased anticancer activities against HCT116 and MCF-7 human cancer cells. The attachment of a modified sugar moiety weather cyclic or free hydroxyl acyclic lead to more active pyrazolopyridine nucleoside analogs.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

FUNDING

This research received no external funding.

ACKNOWLEDGEMENTS

Declared None.

REFERENCES

1. Khalaf H.S., Tolan H.E.M., El-Bayaa M.N., Radwan M.A.A., El-Manawaty M. and El-Sayed W.A. Synthesis and Anticancer Activity of New Pyridine-Thiophene and Pyridine-Furan Hybrid Compounds, Their Sugar Hydrazone, and Glycosyl Derivatives. *Russ. J. Gen. Chem.*, **90**, 1706–1715(2020).
2. El-Essawy F.A., El-Sayed W.A., El-Etrawy A.S. and El-Bayaa M.N., Synthesis of new isolated and fused tri-and tetracyclic pyridine derivatives. *Chem. Heterocycl. Compd.*, **48**(12), 1853-1862(2013).
3. Liu X.H., Cui P., Song B.A., Bhadury P.S., Zhu H.L. and Wang S.F., Synthesis, Structure and Antibacterial Activity of Novel 1-(5-Substituted-3-substituted-4,5-dihydropyrazol-1-yl)Ethanone Oxime Ester Derivatives. *Bioorg. Med. Chem.*, **16**, 4075(2008).
4. Ouyang G., Chen Z., Cai X.J., Song B.A., Bhadury P.S., Yang S., Jin L.H., Xue W., Hu D.Y. and Zeng S., Synthesis and antiviral activity of novel pyrazole derivatives containing oxime esters group. *Bioorg. Med. Chem.*, **16**, 9699(2008).
5. Abdel-Hafez E.M.N., Rahma G.E.A.A., Aziz M.A., Radwan M.F. and Farag H.H., synthesis and biological investigation of certain pyrazole- 3-carboxylic acid derivatives as novel carriers for nitric oxide. *Bioorg. Med. Chem.*, **17**, 3829(2009).
6. Park H.-J., Lee K., Park S.-J., Ahn B., Lee J.C., Cho H. and Lee K.-I., *Bioorg. Med. Chem. Lett.*, Identification of antitumor activity of pyrazole oxime ethers. **15**, 3307(2005).
7. Ouyang G., Cai X. J., Chen Z., Song B. A., Bhadury P. S., Yang S., Jin L-H., Xue W., Hu D-Y. and Zeng S., Synthesis and antiviral activities of pyrazole derivatives containing an oxime moiety. *J. Agric. Food Chem.*, **56**, 101601(2008).
8. Dai H., Li Y.Q., Du D., Qin X., Zhang X., Yu H.B. and Fang J.X., Synthesis and biological activities of novel pyrazole oxime derivatives containing a 2-chloro-5-thiazolyl moiety. *Food Chem.*, **56**, 10805(2008).
9. Riyadh S.M., Farghaly T.A., Abdallah M.A., Abdalla M.M. and El-Aziz M.R.A., New pyrazoles incorporating pyrazolylpyrazole moiety: Synthesis, anti-HCV and antitumor activity *Eur. J. Med. Chem.*, **45**, 1042(2010).
10. Anzaldi M., Maccio C., Mazzei M., Bertolotto M., Ottonello L., Dallegri F. and Balbi A., Antiproliferative and Proapoptotic Activities of a New Class of Pyrazole Derivatives in HL-60 Cells. *Chem. Biodivers.*, **6**, 1674(2009).
11. El-Shafei A., Fadda A.A., Khalil A.M., Ameen T.A.E. and Badria F.A., Synthesis, Antitumor Evaluation, Molecular Modeling and Quantitative Structure-Activity Relationship (QSAR) of Some Novel Arylazopyrazolodiazine and Triazine Analogs. *Bioorgan. Med. Chem.*, **17**, 5096(2009).
12. Elnagdi M.H, Al-Awadi N. and Erian A.W., Bicyclic 5-6 Systems: Other Four Heteroatoms 2: 2. *Comprehensive Heterocyclic Chem II, 4th ed. Pergamon: Oxford*, 431(1996).
13. Desenko S.M., Komykhov S.A., Orlov V.D. and Meier H., Cyclocondensation of 6-acetyl-4, 7-dihydro-5-methyl-7-phenyl [1, 2, 4] triazolo [1, 5-a] pyrimidine with hydroxylamine and hydrazine. *J. Heterocycl. Chem.*, **35**(4), 989-990(1998).
14. Quiroga J, Insuasty B, Cruz S, Hernandez P., Bolaños A., Moreno R., Hormaza A. and S.de Almeida R., H., Reaction of 5-aminopyrazoles with β-dimethylaminopropiophenones. Synthesis of new pyrazolo [3, 4-b] pyridines. *J. Heterocycl. Chem.*, **35**, 333-338(1998).
15. Frantz S., Drug discovery: playing dirty. *Nature*, **437**, 942-43(2005).
16. Jorda R., Havlícek L., McNae I.W., Walkinshaw M.D., Voller J., Sturc A., Navrátilová J., Kuzma M., Mistrík M., Bártek J. Strnad M. and Kryštof V., Pyrazolo [4, 3-d] pyrimidine bioisostere of roscovitine: evaluation of a novel selective inhibitor of cyclin-dependent kinases with antiproliferative activity. *J. Med. Chem.*, **54**(8), 2980-2993(2011).
17. Braña M.F., Cacho M., Garcı´a M.L., Mayoral E.P., Lo'pez B, de Pascual-Teresa B., Ramos A., Acero N., Llinares F., Muñoz-Mingarro D., Lozach O. and Meijer L., Pyrazolo[3,4-c]pyridazines as Novel and Selective Inhibitors of Cyclin-Dependent Kinases. *J. Med. Chem.*, **48**(22), 6843-6854(2005).
18. Mohamed M.S., Awad Y.E.E.D., El-Hallouty S.M. and El-Araby M., Design, Synthesis and Cancer Cell Line Activities of Pyrazolo[3,4-b]pyridine Derivatives. *Open J. Med. Chem.*, **2**, 78-88(2012).
19. Imaizumi K. and Sado T., Bone metabolism

improvers containing pyrazolopyrazine. *Chem. Abstr.*, **121**, 91797(1994).
20. Akahane A. and Tanaka A., Pyrazolopyrazine Compound and Pharmaceutical Use Thereof. *Chem. Abstr.*, **138**, 24732a(2003).
21. Kumar G.V.S. and Prasad Y.R., Synthesis and pharmacological evaluation of some novel 4-isopropyl thiazole-based sulfonyl derivatives as potent antimicrobial and antitubercular agents. *Med. Chem. Res.*, **22**, 4239(2013).
22. Soliman H.A., Kotb E.R., El-Bayaa M.N., Kutkat O.M. and Abdel-Magied F.M.E., Synthesis and Anti-H5N1 Activity of Substituted Pyridine Glycosides and (Oxadiazolyl) oxymethylpyridine Acyclic C-Nucleoside Analogues. *Russ. J. Gen. Chem.*, **88**, 815-824(2018).
23. Alminderej F.M., Elganzory H.H., El-Bayaa M.N., Awad H.M. and El-Sayed W.A., Synthesis and cytotoxic activity of new 1, 3, 4-thiadiazole thioglycosides and 1, 2, 3-triazolyl-1, 3, 4-thiadiazole N-glycosides. *Molecules*, **24**, 3738(2019).
24. Tolan H.E.M., Radwan M.A.A., Khalaf H.S., El-Bayaa M.N., Awad H.M. and El-Sayed W.A., Synthesis and Cytotoxic Activity of New 1, 4-Dithiazolyl-5-oxopyrrole Derivatives, Their 1, 2, 4-Triazoles and Nucleoside Analogs. *Russ. J. Gen. Chem.*, **90**(8), 1544-1552(2020).
25. Srour A.M., El-Bayaa M.N., Omran M.M., Sharaky M.M. and El-Sayed W.A., Synthesis and Cytotoxic Properties of New Substituted Glycosides-Indole Conjugates as Apoptosis Inducers in Cancer Cells. *Anticancer Agents Med Chem.*, **21**(10), 1323-1333(2021).
26. Kalme Z.A., Roloff B., Pelcher Y.E., Popelis Y.Y., Khagen F. and Dubur G.Y., Nucleophilic substitution reactions in 2-chloropyridines and 2,5-dioxo-1,2,5,7-tetrahydro-1H-furo[3,4-b] pyridines. *Chem Heterocycl Compd*, **28**, 1031-1035(1992).
27. El-Essawy F.A., Synthesis of tetrahetrocyclic systems including pyrido [2′, 3′: 3, 4] pyrazolo [1, 5-a] pyrimidine fused with pyrazole derivatives and isolated with 1, 3, 4-oxa-, thiadiazole, and 1, 2, 4-tetrazole derivatives. *J. Heterocycl. Chem*, **47**, 318-323(2010).
28. Mosmann T., Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods*, **65**, 55(1983).