Satisfactory access to attain pyrimidoquinoxaline 1,4-dioxide framework of prospective antitumor and antimicrobial activities.

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Abstract

We exploited the utilization of 2-amino-3-cyanoquinoxaline1,4-dioxide 1 to build new series of pyrimidoquinoxaline 1, 4-dioxide derivatives *via* multicomponent reactions of 1 with DMF-DMA, n-butyl amine, morpholine, Lawesson's reagent, benzoyl chloride. Furthermore, the new synthesized compounds were biologically evaluated for antitumor and antimicrobial activities.

Keywords: Quinoxaline 1 4-dioxide, Fused pyrimidines, Antitumor activity, Antimicrobial activity.

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Introduction

Quinoxaline 1,4-dioxide derivatives have numerous interests in medicinal chemistry and constitute many of pharmacological active compounds which compounds possess anticancer especially in solid tumor treatment, antibacterial, antifungal, hypoxia-selective activity and antimycobacterial. Quinoxaline 1,4-dioxides have the ability to make inhibition to the synthesis of Deoxyribonucleic Acid (DNA). The presence of the N-oxide moiety in quinoxaline ring, make parasitic damage and produce radical species which make significant effect on the redox metabolism. Indeed, we can use quinoxaline derivatives for the purpose of imaging or therapeutic by making labeling for some, by using many types of radioisotopes as technetium-99 m. In order to make imaging for tumor facile, 125I-AVQD has been used in tumor sites and this appropriate vector to carry I2-125 to tumor cell molecules. Extensive studies indicate that compound a have a strong affinity toward binding to protein tyrosine kinase and this ability was attributed to the presence of the polar N-Oxide moiety. Also it was used for the treatment of solid tumors, non-solid tumor and non-small cell lung Cancer. The mechanism of its action based on In situ mixing of compound a with the activator will damage the DNA in the cancer cells without making any damage to normal cell. As known, one of the greatest need of Photodynamic Therapy (PDT), is to prepare drugs which can be activated at longer wavelength, to increase the penetration of the tumor by photodynamic therapy. The pyrimidoquinoxalines b, c with some analogs was prepared before their antitumor activity had been approved in primary treatment. Encouraged by these reported activities and with the aim of searching for new, broad spectrum and more potent antimicrobial, antitumor compounds [1].

In view of the remarkable importance of this class of heterocyclic compounds, a great deal of effort has been drawn to develop efficient synthetic routes to, synthesize new pyrimido quinoxaline-5,10-dioxide and its analogs having methyl group in position 6 of the quinoxaline ring, for the sake of enhancing their biological properties as antitumor and antimicrobial efficiency. In addition, these frameworks could absorb radiation at longer wavelength (around λ max $\geq$ 400 nm) with a significant high emission wavelength. Therefore, they could be applied as fluorescent sensors, stains for microscopy and diagnosis in medicine or in optoelectronic devices. Their syntheses will be based on the 2-amino-3-cyano-6-methylquinoxaline1, 4-dioxide 1 as a base moiety.

Results and Discussion

Chemistry

In view of the aforementioned importance of 2-amino-3- cyano-6-methyl quinoxaline 1,4-dioxide 1 framework, we utilized a practical simple synthetic methodology to access the target compounds *via* heterocyclization reactions. Therefore, refluxing of compound 1 with an excess amount of N,N-Dimethylformamide-Dimethyl Acetal (DMF-DMA) gave the amidine derivative 2 (Figure 1). Consequently, transamination of (dimethylamino)vinyl]quinoxaline 1,4-dioxide derivative 2 with secondary amines as morpholine in the presence of catalyst as p-toluenesulfonic acid yielded the morpholino-enamine 3 derivative.

Experimental UV–Vis data of amidine derivative 2 showed high absorption maximum in DMF at 329 nm and at 327 nm in (CH_2Cl_2) and also showed an high emission spectra at 462 nm at DMF and 491 nm at (CH_2Cl_2) (Figures 1 and 2).

The λ max for compound 2 in DMF is lower in its value than that for compound 1 in spite of compound 2 is highly conjugated than compound 1. This phenomenon was attributed to that the ability of compound 1 could form hydrogen bonding with DMF while compound 2 isn't. But in CH_2Cl_2 compound 2 have λ max high in its value than compound 1 in the same solvent due to its conjugation [2].

Also, IR spectrum of compound 3 shows the presence of cyano group at 2366 cm^{-1}. The 1H NMR data for compound 3 showed the presence of vinylic proton at 10.02 ppm and the

morpholine protons appeared at 3.12, 3.22 ppm. Experimental UV–Vis data of compound 3 showed high absorption maximum in DMF at 315 and at 311 nm in (CH_2Cl_2) and also showed an enhancement in emission spectra at 506 nm at DMF and 496 nm at (CH_2Cl_2) (Figures 1 and 2).

While making transamination reactions of the amidine derivative 2, under the same previous condition, with primary aliphatic amines (i.e. reaction with n-butyl amine) gave pyrimido [3,4-b] quinoxaline derivatives 4 (Figure 1). The proposed mechanism for this transformation was postulated to undergo through two subsequent steps. The first one includes transamination of compound 2 with the n-butyl amine in the presence of p-toluenesulfonic acid to give amidine intermediate 4a.

Whileas, the second step included the intramolecular reaction of sec. amine of amidine to the cyano group to give 4 *via* intermediate 4b. The structure of compound 4 was assured through its spectral data.

Thus, the IR spectrum of this compound indicates the disappearance of the cyano group and shown the presence of the carbonyl group for cyclic amide at 1695 $cm^{-1.}$

The 1H NMR spectrum of compound 4 indicated the presence of the butyl protons at 1.26, 2.05, 3.1 and 3.6 ppm. While, its 13C NMR data indicated the presence of the amidic carbonyl carbon signal at .156.99 ppm

Furthermore, pyrimidoquinoxaline ring was constructed by annulation of the pyrimidine ring onto the quinoxaline amide derivative 5. The amide 5 was formed by hydrolysis of the aminonitrile derivative 1 by H2SO4 (98%).

The structure of compound 5 was detected by IR spectrum which showed the disappearance of the cyano group and the presence of amidic carbonyl group at 1672 cm^{-1}. Intermolecular cyclization of compound 5 with p-chlorobenzaldehyde in the presence of iodine gave compounds 6.

The IR data showed the presence of carbonyl absorption at 1672 cm^{-1} and its 1H NMR gave signal for amidic NH protons at 10.72 ppm. The 13C NMR data referred to the presence of carbonyl carbon absorption at 163.23 ppm

Experimental UV–Vis data of compounds 6 showed high absorption maximum in DMF at 315 nm and at 306 nm in (CH_2Cl_2) and also showed high emission spectra at 496 nm at DMF and 436 nm at (CH_2Cl_2) (Figures 1 and 2).

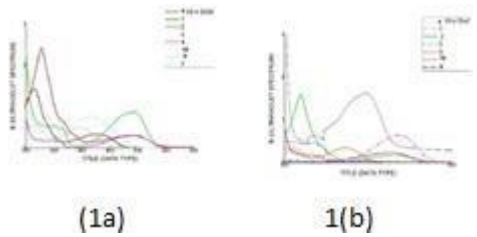

Figure 1. *1a: Electronic absorption spectra of selected quinoxaline; 1,4; 1b: Dioxide derivatives in DMF, CH_2Cl_2.*

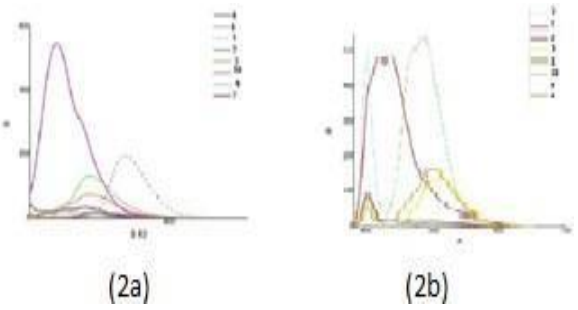

Figure 2. *2a: Fluorescence emission spectra of selected quinoxaline 1,4; 2b: dioxide derivatives in DMF, CH_2Cl_2.*

Also another one pot method for the synthesis of pyrimidoquinoxaline moiety was implemented through refluxing a mixture of aminonitrile derivative 1 with excess of benzoyl chloride, where compound 7 were produced. Which proved by evaluation of its spectral data [3]. The mechanism of this reaction proceeded through three steps. The first step, the transformation of benzoyl chloride with aminonitrile (benzoylation the amino group) to form inseparable intermediate 7a. While in the second step, compound 7a was cyclized in situ to inseparable oxazinoquinoxaline intermediate 7b by using excess of benzoyl chloride. The final step was represented in the intramolecular rearrangement of the oxazinoquinoxaline 7b in presence of acidic medium to the pyrimidoquinoxaline 7. The spectral data are consisted with that for structure 7. The IR data showed the presence of carbonyl absorption at 1672 cm^{-1} and the 1H NMR referred to the presence of amidic NH proton at 0.71 ppm. While, The 13C NMR confirmed these data and indicates the presence of carbonyl carbon at 166.70 ppm [4].

Moreover, the synthesis of diaza-phosphinino quinoxaline ring was performed by the refluxing of aminonitrile 1 with Lawesson's reagent in toluene to form compound 8. The structure of compound 8 was verified by IR spectrum which showed that the cyano group disappeared [5]. The 1H NMR spectrum for this compound showed a signal at 10.07 ppm which characterized for the HN-1 protons and another one at 10.37 ppm of HN-3 protons. Both of these two protons was ppm exchanged on treatment with D_2O and are upper field shifted. The 13CNMR data gave the C=S carbon signal at 195.52 ppm. Experimental UV–Vis data of compound 8 showed high absorption maximum in DMF at 411 nm and at 312 nm in (CH_2Cl_2) and also showed high emission spectra at 486 nm at DMF and 453 nm at (CH_2Cl_2) (Figures 1 and 2).

Biological Evaluation

Determination of SOD-like activity

Both of Compounds 1, 5 and 7 exhibit the strongest Superoxide Dismutase (SOD)-like activity as represented in Table 1 with inhibition percent of 56.4% and 59.6% and 43.6 %. Respectively. From all the previous data, both of Compounds 3 and 5 and 7 can be used as antioxidants to make protection to the cells from the deleterious effect of super oxide radicals [6].

Table 1. *Superoxide (SOD)-like activity of the synthetizedcompounds as antioxidative enzyme.*

Sample	Δ through 5 min	% inhibition
Control	0.468	0
L-Ascorbic acid	0.101	0.784
1	0.204	0.564
2	0.31	0.338
3	0.357	0.237
4	0.438	0.064
5	0.189	0.596
6	0.416	0.111
7	0.264	0.436
8	0.392	0.162

Antibacterial evaluation

All the newly synthesized compounds 1, 3, 4, 5, 6, 7, 8 were initially evaluated for *in vitro* antibacterial activity against Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) and fungal (*Candida albicans*) using conventional Broth dilution method. Ampicillinand Clotrimazole were used as reference drugs.

The inhibition zone diameters and MIC (mg/mL) values are recorded in Tables 2 and 3. It was observed that compounds 5 and 2 exhibited the highest activity against *E. coli* with inhabitation zones growth (13 mm, 24 mm) and with MIC ¼ (125 mg/mL, 50 mg/mL) respectively. And compounds 1, 7 and 5 exhibited the highest activity against S. aureus with inhibition zones growth (18 mm, 10 mm, 27 mm) and with MIC ¼ (93.7 mg/mL,125 mg/mL, 62.5 mg / mL) respectively.

All Compounds 1-8 were also evaluated for their *in vitro* antifungal activity against C. albicans. It was observed that compounds 5, 1 and 2 exhibited the highest activity against C. albicans with inhibition zones growth (21mm, 18 mm, 22 mm) respectively and with MIC ¼ (11.7 mg/mL, 5.6 mg/mL, 6.9 mg / mL) respectively.

Table 2. *Diameter of inhibition zone (mm) of the newly synthesized compounds.*

Compound	*E. coli* (mg/ml)		*S. aureus* (mg/ml)		*C. Albicans* (mg/ml)	
	Diameterof inhibitionzone (in mm)	% Activityindex	Diameterof inhibitionzone (in mm	% Activityindex	Diameterof inhibitionzone (in mm)	% Activityindex
1	9	36	14	60.9	18	69.2
2	24	96	27	117	21	80
3	NA	----	NA	----	6	23.1
4	NA	----	NA	----	NA	----
5	13	52	18	78.3	22	84.6
6	NA	----	NA	----	4	15.4
7	7	28	10	43.5	13	50
8	NA	----	3	13	9	34.6
Ampicillin	25	100	23	100	NA	----
Colitrimazole	NA	----	NA	----	26	100

Table 3. *Minimal inhibitory concentration (MIC, mg/mL) of some of the newly synthesized Compounds.*

Compound	*E. coli*	S. aureus	C. Albicans
1	115	93.7	5.6
2	50	37	6.9
3	NA	NA	125
4	NA	NA	NA
5	125	62.5	11.7
6	NA	NA	250
7	375	125	23.4
8	NA	500	62.5
Ampicillin	125	93.7	----
Colitrimazole	----	----	7.8

Cytotoxicity and in-vitro anticancer evaluation

Eight compounds 1,2,3,4,5,6,7 and 8 were tested for their anticancer activity *in vitro* effect *via* the standard MTT method, against a panel of Four human tumor cell lines ; hepatocellular carcinoma $HePG^{-2}$, mammary gland breast cancer MCF^{-7}, Human skin cancer HFB4 and Colorectal carcinoma HCT-116 [7]. The results were represented. As for activity against HepG2 cell line, the very highest cytotoxic activity was displayed by compound 5 which showed the percentage viability IC50 at 10.95 mg/mL, whereas, the highest cytotoxic activity order of the other compounds was that 1>7>2>3>8>6>4 showing percentage viability IC50 at 19.61, 22.87, 33.66, 42.80, 47.60, 74.26 and 83.99 mg/mL, respectively. Showing that the amide moiety is important for the activity. Yet the introduction of an amide group at position 2 of the quinoxaline 1,4-dioxide ring favored the antitumor activity (IC5010.95 mg/mL) rather than the tricyclic quinoxaline ring. The HCT-116 cell line the very highest cytotoxic activity was displayed by compound 5 which showed the percentage viability IC50 at 9.05 mg/mL, respectively whereas, activity order of the other compounds was that 1>7>2>3>8>6>4 showing percentage viability IC50 at 11.11, 17.85, 25.73, 30.43, 55.10, 61.50and 70.78 mg/mL respectively The HFB4 cell line the very highest cytotoxic activity was displayed by compound 5 which showed the percentage viability IC50 at 7.54 mg/mL. Experimental potential antitumor activity of the tested compounds reported in this study related to their structures, the following structure

activity relationships were postulated: (a) compound 5 reveals the highest cytotoxic activity, contains N-oxide and amide moiety, which is in agreement with that reported. (b) In addition, in case of compounds 9 show high cytotoxic activity may be attributed to the presence of pyrimido moiety [8].

Antioxidant activity assays

A variety of synthetized compounds were biologically assayed as antioxidants. They displayed an excellent antioxidant activity compared with that of the standard ascorbic acid. Thus, the results showed that all tested compounds, 1, 2, 3, 6, 7 and 8 displayed excellent antioxidant activity.

Bleomycin-dependent DNA damage

The synthetized compounds protective activity against DNA damage induced by the bleomycin iron complex were examined for the applied compounds (1, 2, 3, 4, 5, 6, 7 and 8) The compounds 1, 5, 7 exhibited high protection against DNA damage induced by the bleomycin iron complex this diminishing was attributed to the chromogen formation between the damaged DNA and TBA molecules.

Discussion

The objective of the present study was to synthesize and evaluate the new pyrimidoquinoxaline systems for discovering new structures serving as anti-tumor and antibacterial agents. The data showed that most of these compound show significant excellent activity towards human cancer cells and reveals good activity as antibacterial agents and show absorption and emission spectra at high wavelength.

Experimental section

Chemistry: Instruments. All melting points are given in degree Celsius and were determined on a Gallenkamp electric melting point apparatus. The IR spectra were recorded (KBr) on a Mattson 5000 FTIR spectrophotometer (ν,cm^{-1}) at Micro Analytical Unit, Faculty of Science, Mansoura University. The 1H NMR spectra were carried out on a Varian spectrophotometer at 300 MHz using tetramethylsilane as internal reference and hexadeuterated- dimethylsulfoxide (DMSO-d6) and deuterated chloroform ($CDCl_3$) as solvents. 13C NMR spectra were measured at 75MHz using DMSO as a solvent and internal reference, at Micro Analytical Center, Cairo University. The mass spectra (EI) were recorded on Kratos (70 eV) MS equipment and or a Varian MAT 311A Spectrometer, at Micro Analytical Center, Faculty of Science, and Cairo University. Elemental analyses (C,H,and N) were carried out at Micro Analytical Center, Cairo University, Giza, Egypt. The results of the elemental analysis were found to agree favorably with the calculated values. Biological testing was carried out at the Drug Department, Faculty of Pharmacy, Mansoura University, Mansoura, *Egypt. Silicagel* GF254 were used for preparative TLC and detected under UV light at 254/365 nm [9].

2- Amino-3-cyano-6-methylquinoxaline 1, 4-dioxide (1)

To the solution of a mixture of 6-methylbenzo[c] oxadiazole 1-oxide (1.5 g, 1 mmol) and malononitrile (0.66 g, 1mmol) in DMF (4 ml), a solution of TEA (1 g, 1 mmol) in DMF(3 ml) was added dropwise in ice path [10]. The reaction mixture was stirred overnight. The organic product was filtered off and washed with diethyl ether more (3 × 10 ml). The crude product was recrystallized from aquous DMF to give pure product of 1.

Yield, 75 % (1.5 g); Red powder; m.p: 220-222 oC ; IR (KBr) γ max ,cm^{-1} : 2229.31 (CN), 3412 (NH sharp), 1351(NO). 1H NMR; (ppm) : 2.42 (3H, s, CH_3), 4.70 (2H, m, NH_2, exchangeable with D_2O), 7.12-7.36 (3H, m, HAr): 13C NMR ; (ppm) = 21.30, 117, 125.37, 125.58, 128.30, 129.44, 132.22, 138.57, 141.88 and 151.45 (EIMS) m/z (%): 217 (M++1, 14.17), 216 (M+, 100, base peak), 200.15 (11.50), 183.14 (4.12), 172.12 (8.88), 156.11 (9.83), 145.12 (5.85), 131.11 (4.77), 129.11 (6.05), 119.12 (32.39), 104.12 (17.04), 92.12 (23.8), 89.11 (22.11), 77.11 (20.03), 65.10 (16.98); Anal. Calcd. For $C_{10}H_8N_4O_2$ (216.06): C: 55.56; H: 3.73; N: 25.91. Found: C, 55.78; H, 3.75; N, 26.04.

3- Cyano–2–[{(dimethyl amino) methylene} amino]–6-methyl quinoxaline 1, 4-dioxide (2)

A mixture of o-aminonitrile 1 (0.216 g, 1 mmol) and DMF-DMA (1.79 g, 15 mmol) in benzene (20 ml) wasrefluxed for 10 h. The solvent was removed under reduced pressure in vacuum, and the red precipitate was triturated with diethyl ether to give 2.

Yield, 99%; Red powder; m.p=196-198°C; IR (KBr) γ max, cm^{-1}: 2366.23(CN), 1515(NO) $cm^{-1.}$ 1H NMR; (ppm) :2.53 (3H,s,CH_3) , 3.12 (3H,s,CH_3), 3.22 (3H, s, CH_3), 7.78 (1H, d, HAr), 8.12 (1H,s,HAr), 8.23 (1H,d,HAr), 10.09 (1H,s, HC=N). 13C NMR; (ppm): 21.26, 40.02, 30.03, 112.01, 117.56, 118.95, 119.00, 133.22, 136.02, 136.83, 139.99, 144.82 and157.94. (EIMS) m/z (%): 272 (M++1, 9.99), 271 (M+, 32.08), 254.16 (100, basepeak), 238.18 (15.91), 238.18 (15.91), 222.15 (5.08), 184.13 (4.57), 168.11 (7.02), 156.12 (6.14), 127.14 (4.62), 116.11 (10.34), 104.12 (9.57), 89.11 (15.41); Anal. Calcd. For C13H13N5O2 (271.28): C: 57.56; H: 4.83; N: 25.82. Found: C, 57.36; H, 4.80; N, 25.92;

General procedure for transamination reaction

A solution of mixture of the amidine derivative 2 (0.5 g, 2 mmol,) and amine (0.172 g, 2 mmol) in DMF (10 ml) in the presence of p-toluenesulphonic acid (0.34 g, 2 mmol) was refluxed for 6 h. The mixture was then poured onto cold water and leave a brown solid residue. The solid was filtered off and was triturated with cold ether, then filtered off. The crud product was purified using preparative thin layer chromatography using silica gel and a mixture of ethyl acetate: petroleum Ether (1:3) as eluent to give a pure product.

3-Cyano-6-methyl-2-((morpholino methylene) amino) quinoxaline 1, 4-dioxide (3)

Yield,(0.3 g, 50%) ; yellow crystals; m.p: 240-242°C; IR (KBr) γmax, cm^{-1} 2366.23 (CN) and 1515 (N-O) cm^{-1}. 1H NMR; (ppm): 2.49 (3H, s, CH_3), 3.12 (4H ,t , $2CH_2$), 3.22 (4H, t, $2CH_2$), 7.68 (1H, d , HAr), 8.03 (1H, s, HAr), 8.12 (1H, d, HAr), 8.83 (1H,S,CH=N);13C NMR (ppm): 21.27, 38.92(2C), 39.99(2C) , 118.04 , 126.76, 127.63, 133.13, 135.90, 136, 137.48, 142.03, 156.99 ,158.75. (EIMS) m/z (%): 313.13(M+, 1.95), 310.08 (9.48), 292.04 (8.97), 286.63 (11.41), 280.34 (21.85), 253.23 (10.83), 216.18 (5.51), 195.71 (7.10), 191.22 (24.02), 174.85 (13.60), 125.27 (15.78), 113.92 (12.19), 106.22 (16.39), 98.31 (20.53), 91.20 (34.6), 81.13 (29.5), 77.23 (27.33). Anal. Calcd. For $C_{15}H_{15}N_5O_3$ (313.13): C, 57.50; H, 4.83; N, 22.35; Found C, 57.43; H, 4.71; N, 22.11.

3-Butyl-7-methyl-4-oxo-3,4-dihydrobenzo[g]pteridine5,10-dioxide (4)

Yield (0.2 g, 60%); yellow crystals; m.p : 152 °C; IR (KBr) γ max, cm^{-1}: 1680 (CO), 1515 (N-O) cm^{-1}.1H NMR; (ppm): 1.26 (3H, s, CH_3), 2.05 (2H, t ,CH_3), 2.98 (3H, s, CH_3) ,3.19 (2H, t, CH_2) ,3.6 (2H,t, CH_2),7.7 (1H, d, HAr), 8.11 (1H, s, HAr), 8.21 (1H, d, HAr), 10.02 (1H, S, CH=N); 13C NMR; (ppm): 20.9, 21.13, 28.3, 33.63, 50.8, 128.67 ,129.62, 129.75, 130.98, 134.76,136.29, 139.19, 142.03, 144.33, 157.05. Anal. Calcd. For $C_{15}H_{16}N_4O_3$ (300.12): C, 59.99; H, 5.37; N, 18.66; Found C, 59.87; H, 5.66; N, 18.61.

Biological evaluation

Determination of SOD-like activity: By making investigation for Superoxide Dismutase (SOD)-like activity using Bridges and Saline method. Basically this method depend on the inhibition effect of SOD on the reduction of Nitrobluetetrazolium (NBT). Using superoxide anion which is generated by the system of xanthine/xanthine oxidase. After making solution of the synthetized compounds in Dimethylsulphoxide (DMSO). Determined the activity of native Horseradish Superoxide Dismutase (HR SOD) for comparative purposes.

Antibacterial evaluation

We prepared disks of Whitman filter paper with size (5.0 mm diameter) and kept into 1.0 Oz screw capped wide mouthed containers for sterilization. Keeping these bottles into oven at temperature of 150°C. Then, the filter paper disks were impregnated with a solution of the test compound dissolved in DMSO (1 mg/mL), put them on nutrient agar plate which is seeded with the tested organism in triplicates. Using of 106 CFU/mL (Colony Forming U/mL) and 104 CFU/mL in Standard conditions for both of antibacterial and antifungal assay. Using of Pyrex glass Petri dishes with 9 cm in diameter and inoculate two disks of filter paper in each plate. Use for the tested organisms Gram positive bacteria as S. aureus and Gram negative bacteria as *E. coli*. They were evaluated for *in vitro* antifungal potential against C. albicans fungal strain. Using of Ampicillin and Clotrimazole as standard antibacterial and antifungal agents. The control used was DMSO at the same concentration as mentioned above. Making incubation for plates for 24 h at 36°C for bacteria and also 48 h for fungi. Any Compounds with significant growth inhibition zones (>10 mm) also evaluated for Minimal Inhibitory Concentrations (MICs).

Minimal Inhibitory Concentrations (MIC) measurement

For the determination of the antibacterial and antifungal activity, we used the microdilution susceptibility test in Müller-Hinton Broth (Oxoid) and Sabouraud Liquid Medium (Oxoid) respectively. The solution of the tested compound, Ampicillin and Clotrimazole were prepared in DMSO with concentration of 1000 mg/mL. Making dilution for each stock solutions with standard method broth (Difco).As for preparing two dilution at concentrations of (500, 250, 3.125 mg/mL) respectively, Add 10 mL of the broth which contain about 106 CFU/mL of test bacteria to each well of 96-well microliter plate. Then make incubation for sealed microplates in a humid chamber at 36°C for 24 h this for the antibacterial activity and at 36°C for 48 h for antifungal activity. After the end of the incubation time, Record the Minimal Inhibitory Concentrations (MICs) value as it is the lowest concentrations of the substance which hadn't any visible turbidity Control in DMSO and also UN inoculated media were run as the test compounds under the same conditions.

Materials and Methods

The reagents are DMSO, RPMI-1640 medium, MTT, and 5-fluorouracil, Fetal Bovine serum (GIBCO, UK). We obtain the cell line from ATCC at Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Procedure

Making dilution for the stock samples with RPMI-1640 medium to obtain concentrations from 10 to 1000 mg/mL. The final concentration of Dimethylsulfoxide (DMSO), did not exceed 1% v/v. making testing for the cytotoxic activity of the compounds against HepG2, MCF^{-7}, HCT-116 and HFB4. The percentage of viability of cell made visually. Furthermore, 5 Fluorouracil is the standard for the anticancer drug, which is used for comparison. cell were batch cultured for 10 d, after that let them seeded in 96-well plates of 10-103 cells/well in fresh complete growth medium in 96-well microtiter plastic plates at 37°C for 24 h under 5% CO_2 using a water jacketed carbon dioxide incubator (Shedon. TC2323. Cornelius, OR, USA). Adding the medium without serum and incubating cells either alone (negative control) or with different concentrations of sample to give a final concentrations of (1000, 500, 200, 100, 50, 20, 10 mg/ mL). Cells were suspended in RPMI-1640 medium, 1% antibiotic antimycotic mixture (104 mg/mL potassium penicillin, 104 mg/mL streptomycin sulfate and 25 mg/mL Amphotericin B) and 1% L-glutamine in 96-well flat

bottom microplates at 37°C under 5% CO_2. After 96 h of incubation, the medium was again aspirated, trays were inverted onto a pad of paper towels, the remaining cells rinsed carefully with medium, and fixed with 3.7% (v/v) formaldehyde in saline for at least 20 min. The fixed cells were rinsed with water, and examined. The cytotoxic activity was identified as confluent, relatively unaltered mono-layers of stained cells treated with compound.

Calculation of the IC50 for each compound

The estimation of Cytotoxicity is by measuring the concentration which cause losing of monolayer by 50% relatively. Using 5-Fluorouracil as a positive control to calculate IC50, and would need a series of dose response data (e.g., drug concentrations x^1, x^2, x^n and growth inhibition y1, y^2, y^n). The values of y are in the range of 0-1.

Bleomycin-dependent DNA damage assay

By adding the following reagents: 0.05 mg/mL of bleomycin, DNA (0.2 mg/mL), FeCl3 (0.025 mM), magnesium chloride (5 mM), KH_2PO_4 / KOH buffer with pH 7.0 (30 mM) and ascorbic acid (0.24 mM) or adding the test fractions diluted in MeOH in concentration of (0.1 mg/mL). And then make incubation for the reaction mixtures in a water bath at 37°C for 1 h. At the end of the incubation period, we stop the reaction by adding 0.1 mL of Ethylenediaminetetraacetic Acid (EDTA) (0.1). (The iron EDTA complex is unreactive in the bleomycin assay). We made assessing for DNA damage by adding 1 mL 1% (w/v) Thiobarbituric Acid (TBA) and 1 mL of 25% (v/v) H ydrochloric acid (HCl).then heating in a water-bath at 80°C for 15 min. Then make extraction for the chromogen formed with 1-butanol, and measure absorbance at 532 nm.

Conclusion

The objective of the present study was to synthesize and evaluate the new pyrimidoquinoxaline systems for discovering new structures serving as anti-tumor and antibacterial agents. The data showed that most of these compound show significant excellent activity towards human cancer cells and reveals good activity as antibacterial agents and show absorption and emission spectra at high wavelength.

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