Chemistry Research Journal, 2024, 9(4):15-25
Available online www.chemrj.org

Research Article

ISSN: 2455-8990
CODEN(USA): CRJHA5

Antiproliferative and Antimicrobial Activities of Novel heterocyclic Derivatives

Abdel Mohsen M. Soliman*[1], Salwa S. Soliman[2]

[1]Theraputic Chemistry Department, National Research Center, 33 elBehouth St., Dokki, Giza, Egypt.
[2]Nutrition and Food Science Department, National Research Centre, 33 elBehouth St., Dokki, Giza, Egypt.
*e-mail: amsolimannrc@gmail.com

Abstract
Novel heterocyclic Derivatives were obtained by cyclization reactions of diaminophenyl derivative **1** with oxalic acid dihydrate. Quinoxline derivative **3** was synthesised by reaction with hydrazine hydrate then a series of new Quinoxline derivatives **4 a, b, 5a-c,6a, b,7a-c** were synthesized with various reagents. Novel synthesized **1,3,4a, b,5a-c, 6a, b,7a-c**compounds were tested for their antiproliferative potential in vitro towards different human cell lines, including: breast (MCF7) and colon (HCT116) cancer cell lines. Results indicated that compounds **5b, 5c and 7a** were the most potent against tested cancer cell lines. Moreover, compounds **4b, 6a** and **7c** indicated valuable antimicrobial activity against all the pathogenic tested microorganisms.

Keywords: Quinoxline derivatives, antiproliferative, Antibacterial activity, Antifungal activity

1. Introduction

Microbial drug resistance motivates researchers to seek out new compounds to treat these deadly pathogenic microbes **[1]**.This quest is especially crucial in view of the growing number of antibiotic-resistant strains and the increased challenges associated with infection treatment. The Quinoxline pharmacophoric, which is isosteric to purine antimetabolites, has offered an intriguing platform for the creation of various antibiotics **[2]**. Bicyclical Quinoxline antibiotics have been shown to be effective against gram-positive bacteria **[3,4]** as well as certain animal tumors **[5, 6].** The method of action involves their attaching to DNA and acting as bi-functional intercalating agents. The antibiotic Echinomycin(1)and the Trios tins are two well-known antibiotic families. Both series have a similar composition, including two quinoxaline-2-carboxylic acid moieties **[5].**

A Quinoxline ring is also found in commercially marketed drugs such as Levomycin, Actinoleutin, and Quinacillin **[6,7]**. Quinoxaline is a bioactive precursor with anti-inflammatory **[8,9]**, antiviral **[10,11]**, ant diabetic **[12,13]**, antidepressant **[14]**, anthermitic **[2]**, antituberculosis **[15]**, and antiprotozoal **[16]** properties. As a result of the wide range of useful applications for quinoxaline, scientists devote significant attention to traditional synthetic methods, their modification, and the development of new methods for synthesizing Quinoxline in order to ensure the availability of more functionalized Quinoxline.

The pyrazoline moiety is a versatile lead molecule in the pharmaceutical development and has a wide range of biological activities **[17–19]**.

The combination of Quinoxline and pyrazoline results in the production of compounds with remarkable biological properties, which could be attractive alternatives to present treatments. The present goal is to develop a new synergic

medication by combining a bio-active quinoxaline moiety with an effective chemical ligand such as pyrazoline. The target complexes' spectrum and structural analyses were carried out utilizing single FTIR, mass spectrometry, and ^{1}H NMR spectroscopic methods. Furthermore, assessing the antibacterial capability of the complexes as zone of inhibition and MIC (minimum inhibitory concentration) allowed us to compare their activity to that of commercially known antibiotics. The antitumor potential of the new Quinoxline-Pyrazole complexes is also explored.

2. Experimental sections

i. *Chemistry*

All chemicals were provided by Fluka or Aldrich companies and were used without additional purification. Elemental microanalyses were carried out at Micro analytical Unit, Central Services Laboratory, National Research Centre, Dokki, Giza, Egypt, using Vario Elementary and were found within ± 0.4% of the theoretical values. All melting points were uncorrected and were taken in open capillary tubes using electro thermal apparatus 9100. FT-IR spectra were recorded with a Perkin-Elmer Frontier. ^{1}H-NMR, ^{13}C-NMR spectra were determined on a Varian-Gemini-300 MHz and Jeol-Ex-300 MHz NMR spectrometer using TMS as an internal standard with (chemical shift δ= 0 ppm). The mass spectra were measured with a GC Finnigan MAT SSQ-7000 mass spectrometer. The reactions were followed, and the purity of the compounds was checked using TLC on silica gel-precoated aluminum sheets (Type 60, F 254, Merck, Darmstadt, Germany) and the spots were detected by exposure to UV lamp at λ_{254nm}. The chemical names given for the prepared compounds are according to the IUPAC system. The reported yields are based upon pure materials isolated by Solvents were dried/purified according to conventional procedures

1-(phenyl)methanone-(1,2,3,4-Tetrahydroquinoxline-2,3-dione) 2:

Compound **2 was** prepared from (3,4-diaminophenyl) (phenyl)methanone**1** and oxalic acid dihydrate: A soln. of oxalic acid dehydrate (0.01 mol) in H_2O (100 ml) was heated and conc. HC1 soln. (4 ml) was added followed by 3,4-diaminophenyl) (phenyl) methanone (0.01mol) with continuous stirringl00^{0} for 20 min. The mixture was cooled by addition of ice and the colorless precipitate filtered off, washed with H_2O, and dried at 90°:

1-(phenyl)methanone- quinoxline-2,3(1H,4H)-dione (2):

Product **2** was separated as reddish brown, ppt yield 78.7%, m.p. 172-4 ^{0}C. IR (KBr, cm^{-1}): 2451, 2343(2NH Quinoxline), 1680, 1671(3C=O). ^{1}HNMR (DMSO-d6, δ, ppm): 8.34, 8.35 (ss, 2H, 2NH Quinoxline**,** D_2O exchangeable); 7.59-7.60 (m, 8H, aromatic). MS: m/z (%) = M+, 266.1 m/e: 266.1 (22%), 267.1 (33.3%), 268.1 (11.4%). Anal. Calcd for $C_{15}H_{10}N_2O_3$ (Mol. Wt.: 266.3): C, 67.67; H, 3.79; N, 10.52. Found: C, 67.65; H, 3.78; N, 10.50.

1-(phenyl)methanone- Hydrazinylquinoxlin-2(1H)-one (phenyl)methanone (3):

Compound **3 was** prepared ay refluxing (0.01 mole) compound (2) in (10 ml) of ethanol, and (0.01 mole) hydrazine hydrate in 10 ml ethanol was added in 50 ml round bottom flask. A few drops of glacial acetic acid were added to adjust pH of solution. The reaction mixture was reflux in 5 hr. After reflux add cool water, the obtained precipitate as collected by filtration. Dry it well and recrystallized from ethanol and dried at room temperature.

Product **3** was separated as pale grey ppt., yield 64.3%, m.p. 248-251 decomposition. IR (cm-1): 3456 (NH_2, Hydrazinyl), 3274 (NH, Hydrazinyl), 2334(NH Quinoxline), 1666, 1625 (2C=O), 1605(C=N). ^{1}HNMR (DMSO-d6, δ, ppm): 12.8. 12.9 (ss, 2H, NH_2 Hydrazinyl, D_2O exchangeable);9.92 (s, 1H, NH Hydrazinyl, D_2O exchangeable); 7.59 (s, 1H, NH Quinoxline**,** D_2O exchangeable); 7.67-7.55 (m, 8H, arom.). MS: m/z (%) = M+, 280.1 (15%), 281.1 (34.6%), 282.1 (12.1%). Anal. Calcd for $C_{15}H_{12}N_4O_2$ (Mol. Wt.: 280.3): C, 64.28; H, 4.32; N, 19.99. Found: C, 64.26; H, 4.31; N, 19.97.

Preparation of compounds 4a,b

A mixture of (0.01mole) compound **3** in (50ml) absolute ethanol and (25ml) acetone, 3 drops of glacial acetic acid & (0,01 mole) 4-chlorobenzaldehyde /or 2,4,6-trimethoxybenzaldehyde, respectively was refluxed for 7-9 h. After reaction completion, the solvent was evaporated under vacuum and the solid residue obtained was washed with water, filtered off and recrystallized from ethanol.

1-(phenyl)methanone- 3-(2-(4-(chloro)benzylidene)hydrazinyl)quinoxlin-2(1H) one (4a).

Product **4a** was separated as red ppt., yield 51.1%, m.p.141-3^{0}C IR (KBr, cm^{-1}): 3206, 3205 (2NH), 1678, 1624(2C=O), 1593(C=N). ^{1}HNMR (DMSO-d6, δ, ppm): 8.56 (s, 1H, CH=N); 8.67, 8.68. (ss., 2H, 2NH**,** D_2O exchangeable); 7.86-7.48 (m, 12H, arom.). MS: m/z (%) = M+, 402.1(65.0%), 403.1 (45.5%), 404.1 (22.5%). Anal. Calcd for $C_{22}H_{15}ClN_4O_2$ (402.8): C, 65.59; H, 3.75; Cl, 8.80; N, 13.91. Found: C, 65.57; H, 3.73; Cl, 8.80; N, 13.89

1-(phenyl)methanone-3-(2-(2,4,6-trimethoxybenzylidene)hydrazinyl)quinoxlin-2(1H)one(4b):

Product **4b** was separated as; yield, 45.9%, m.p. 229-231^{0}C. IR (KBr, cm^{-1}): 3216, 3215 (2NH); 1676, 1631(1C=O), 1608 (C=N). 1H NMR (DMSO-d6, δ, ppm): 8.53 (s, 1H, CH=N); 8.78,8.82 (ss, 2H, 2NH**,** D_2O exchangeable); 7.77-7.43 (m, 10H, arom.); 3.68, 3.71, 3.72 (3s, 9H, 3OCH_3). MS: m/z (%) = M+, 458.2 (73.0%), 459.2 (43.9%), 460.2 (32.1%). Anal. Calcd for $C_{25}H_{22}N_4O_5$ (458.5): C, 65.49; H, 4.84; N, 12.22. Found: C, 65.49; H, 4.84; N, 12.22.

Preparation of compounds 5a-c

A mixture of (0.01mole) compound **3** and (0.01mole) ethyl bromoacetate/or chloroacetyl chloride /or triethylorthoformate was dissolved in (30 ml) DMF. The reaction mixture was refluxed at 90 ^{0}C for 8 h. After reaction completion, the formed solid was filtered off. The crude product obtained was washed with methanol and purified by recrystallization from DMF/ methanol (1:2) mixture.

1-(phenyl)methanone -5-Hydroxy-3H-[1,2,4]triazino[4,3-a]quinoxlin-5(6H)-one (5a):

Product **5a** was separated as; yellow ppt., yield 66.3%, m.p142-3^{0}C. IR (KBr, cm^{-1}): 3453 (OH), 3329, 3220 (2NH), 1669, 1623 (2C=O), 1465 (C=N). ^{1}HNMR (DMSO-d6, δ, ppm): 11.77 (s**,** OH, D_2O exchangeable); 9.78, 8.80 (ss, 2H, 2NH, D_2O exchangeable); 7.55-7.27 (m, 8H, arom.); 6.49 (s, 1H, C=CH). MS: m/z (%) = M+, 326.1 (54.1%), 327.1 (54.8%), 328.1 (11.0%). Anal. Calcd for $C_{17}H_{18}N_4O_3$ (Mol. Wt.: 326.3): C, 62.75; H, 5.65; N, 17.17. Found: C, 62.73; H, 5.63; N, 17.15

1-(phenyl)methanone -1H-[1,2,4]triazino[4,3-a]quinoxaline-2,5(3H,6H)-dione (5b):

Product **5b** was separated as; grey ppt., yield 65%, m.p.162-4°C. IR (KBr, cm^{-1}): 3329, 3284(2NH); 1671, 1625 (2C=O). ^{1}HNMR (DMSO-d6, δ, ppm): 10.2, 9.0 (ss, 2H, 2NH, D_2O exchangeable); 7.56-7.30 (m, 8H, arom.); 3.9(s, 1H, CH). MS: m/z (%) = M+, 331.2 (65.1%), 332.2 (42.1%), 333.2 (23.2%). Anal. Calcd for $C_{17}H_{23}N_4O_3$ (331.4): C, 61.61; H, 7.00; N, 16.91. Found: C, 61.59; H, 6.98; N, 16.89.

1-(phenyl)methanone- [1,2,4]triazolo[4,3-a]quinoxlin-4(5H)-one (5c):

Product **5c** was separated as yellow ppt; yield 78%, m.p. 206-8°C. IR (KBr, cm^{-1}): 3302(NH), 1681, 1668 (2C=O). ^{1}H NMR (DMSO-d6, δ, ppm): 9.76 (s, 1H, CH=N); 8.65(s, 1H, NH, D_2O exchangeable); 7.65-7.34 (m, 8H, arom.. MS: m/z (%) = M+, 295.1 (76.1%), 296.1 (54.1%), 297.1 (21.1%). Anal. Calcd for $C_{16}H_{15}N_4O_2$ (Mol. Wt.: 295.3**)**: C, 65.07; H, 5.12; N, 18.97. Found: C, 65.05; H, 5.10; N, 18.95.

Preparation of compounds 6a,b

A solution of (0.01mole) methyl isothiocyanate /or phenyl isothiocyanate in (15ml) toluene was added to a solution of (0.01mole) compound **3** in (15ml) toluene. The reaction mixture was refluxed for 12 h. The solvent was evaporated under reduced pressure. The solid was washed with cold water and recrystallized from ethanol.

1-(phenyl)methanone -4'-methyl-5'-thioxo-1H-spiro[quinoxline-2,3'[1,2,4]triazolidin]-3(4H)-one (6a):

Product **6a** was separated as; Reddish brown ppt., yield 58%, m.p.265-7°C. (KBr, cm^{-1}): 3300-3219 (4NH), 1711, 1661(2C=O). ^{1}H NMR (DMSO-d6, δ, ppm): 8.56-9.2 (br., 2NH, D_2O exchangeable); 7.66-7.40 (m, 9H, arom.); 3.6, 3.1 (ss, 2H, 2NH, D_2O exchangeable); 2.67(s, 3H, CH_3). ^{13}C NMR (270 MHz, DMSO-*d6*): 216.45, 190.98, 141.53, 138.84, 130.74, 130.40, 128.94, 128.25, 56.59. MS: m/z (%) = M+, 353.1 (77.1%), 354.1 (44.8%), 355.1 (22.8%). Anal. Calcd for $C_{17}H_{15}N_5O_2S$ (353.4): C, 57.78; H, 4.28; N, 19.82; S, 9.07. Found: C, 57.76; H, 4.26; N, 19.80; S, 9.05.

1-(phenyl)methanone -4'-phenyl-5'-thioxo-1H-spiro[quinoxline-2,3'[1,2,4]triazolidin]-3(4H)-one (6b):

Product **6b** was separated as; pale yellow ppt., yield 55%, m.p.345-7°C. IR (KBr, cm^{-1}): 3378- 3222(4NH), 1698, 1663(2C=O). ^{1}H NMR (DMSO-d6, δ, ppm): 10.05, 11.00 (ss, 2NH, D_2O exchangeable); 4.71, 4.77(ss., 2H, 2NH, D_2O exchangeable); 7.54-7.13 (m, 13H, arom.). ^{13}CNMR (270 MHz, DMSO-*d6*): 194.10, 175.46, 165.37, 164.77, 161.73, 161.56, 158.94, 158.77, 151.20, 139.18, 138.65, 130.35, 129.67, 125.45, 122.42, 112.68, 111.53, 111.39,

110.67, 100.74, 99.99, and 39.88. MS: m/z (%) = M+, 403.1 (77.8.0%), 404.1 (65.00%), 405.1 (21/0%). Anal. Calcd for $C_{21}H_{17}N_5O_2S$ (403.5): C, 62.52; H, 4.25; N, 7.93; S, 7.95. Found: C, 62.50; H, 4.23; N, 17.34; S, 7.93.

Preparation of compounds 7a-c

A mixture of (0.01 mol) compound **3** and (0.01 mol) diethyl malonate /or diethyl succinate /or malononitrile was dissolved in (30 ml) DMF and few drops of triethyl amine. The reaction mixture was stirred at 90 °C for 12 hr. After reaction completion, the formed solid was filtered off. The crude product obtained was washed with methanol and finally purified by recrystallization from DMF / methanol (1:2) mixture.

1-(phenyl)methanone- 1-(3-oxo-3,4-dihydroquinoxlin-2-yl)pyrazolidine-3,5-dione (7a):

Product **7a** was separated as; orange, yield: 55%, m.p. 168-200^{0}C. IR (KBr, cm^{-1}): 3265, 3254 (2NH), 1676, 1661, 1658 (4C=O), 1609 (C=N). $_1$H NMR (DMSO-d6, δ, ppm): 8.98 (s., 1H, NH, D_2O exchangeable); 7.11-7.67 (m, 9H, arom.); 4.21(s., 1H, NH, D_2O exchangeable); 4.11(s, 2H, CH_2). ^{13}CNMR (270 MHz, DMSO-*d6*):194.10, 175.46, 165.38, 164.78, 163.39, 161.95, 161.75, 151.57, 139.10, 129.22, 128.73, 125.16, 123.06, 113.32, 111.56, 111.42, 110.74.105.41, 100.77 and 39.95. MS: m/z (%) = M+, 348.1 (45.0%), 349.1 (66.9 %), 350.1 (54.6%) .Anal.Calcd for $C_{18}H_{12}N_4O_4$ (Mol. Wt.: 348.3): C, 62.07; H, 3.47; N, 16.09. Found: C, 62.05; H, 3.46; N, 16.07.

1-(phenyl)methanone- 1-(3-Oxo-3,4-dihydro-quinoxlin-2-yl)-tetrahydro-pyridazine-3,6 dione (7b).

Product **7b** was separated as; brown ppt., Yield: 65%, m.p. 246-248^{0}C. IR (KBr, cm^{-1}): 3335, 3254(2NH), 1698, 1678, 1664, 1627 (4C=O), 1613(C=N). ^{1}H NMR (DMSO-d6, δ, ppm): 8.66 (s, 1H, NH, D_2O exchangeable); 7.27-7.67 (m, 9H, arom.); 4.15(m, 2H, CH_2), 4.11(s, 1H, NH, D_2O exchangeable);. MS: m/z (%) = M+, 362.1 (76.0%), 363.1 (43.5%), 364.1 (31.8%). Anal.Calcd for $C_{19}H_{14}N_4O_4$ (Mol. Wt.: 362.3): C, 62.98; H, 3.89; N, 15.46. Found: C, 62.96; H, 3.87; N, 15.44.

1-(phenyl)methanone- 1-(3,4-dihydroquinoxlin-2-yl) -pyrazole-3,5-diamine (7c).

Product **7c** was separated as; reddish brown, yield: 54%, m.p. 197-200 ^{0}C. IR (KBr, cm^{-1}): 3356, 3332 (2NH_2), 3276 (NH, quinoxaline), 1676, 1656 (2C=O), 1605(C=N). ^{1}HNMR (DMSO-d6, δ, ppm): 11.90, 12.00 (dd., 4H, 2NH_2 D_2O exchangeable); 8.00 (s, 1H, NH, D_2O exchangeable); 7.00-7.23 (m, 9H, arom.); 6.49(s, 1H, CH=C). MS: m/z: 362.1 (M+, 20%), 363.1 (65.5%), 364.1 (43.4%), 348.1 (12.4%). Anal.Calcd for $C_{19}H_{18}N_6O_2$ (362.4): C, 62.97; H, 5.01; N, 23.19; Found: C, 62,95; H, 4.99; N, 23.17.

ii. ***Antiproliferative activity of newly synthesized derivatives***

Material and methods

Cells

Determination of in vitro anticancer activity

Cell lines: Human breast **(MCF7)** and human colon **(HCT116)** cancer cell lines were obtained from American Type Culture Collection (Rockville, Maryland, USA). Cells were cultured in Eagle medium (IIET, Wroclaw, Poland) supplemented with 2 mM L-glutamine, 10% fetal bovine serum, 8 ug/mL of insulin and 1% mem non-essential amino acid solution 100x (all from Sigma–Aldrich Chemie GmbH, Steinheim, Germany). Murine fibroblast normal cell line (BALB/3T3) was cultured in DMEM (Gibco, UK) supplemented with 2 mM L-glutamine, 10% fetal bovine serum (GE Healthcare, Logan, UT, USA). All culture media were also supplemented with antibiotics: 100 µg/ml streptomycin (Sigma–Aldrich Chemie GmbH, Steinheim, Germany) and 100 units/ml penicillin (Polfa Tarchomin SA, Warsaw, Poland). All cell lines were grown at 37 °C with 5% CO_2 humidified atmosphere.

Compounds

Prior to usage, the compounds were dissolved in DMSO (stock solution 10 mg/ml) and culture medium (1:9) to the concentration of 1 mg/ml and subsequently diluted in culture medium to reach the required concentrations (ranging from 100 to 0,1 µg/ml, only one compound 3a was tested in different range of concentrations from 10 to 0,01 µg/ml, because of small amount of tested compound).

An anti-proliferative assay in vitro

24 hours before addition of the tested compounds, the cells were plated in 96-well plates (Sarstedt, Germany) at density of 1x10^4 cells per well. The assay was performed after 72 hours exposure to varying concentrations of the tested compounds. The *in vitro* cytotoxic effect of all compounds was examined using the SRB assay.

Cytotoxic test SRB

The details of this technique were described by Skehan et al [29]. The cells were attached to the bottom of plastic wells by fixing them with cold 50% TCA (trichloroacetic acid, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) on the top of the culture medium in each well. The plates were incubated

at 4°C for 1 hour and then washed five times with tap water. The cellular material fixed with TCA was stained with 0.4% sulphorhodamine B (SRB, Sigma-Aldrich Chemie GmbH, Steinheim, Germany) dissolved in 1% acetic acid (POCH, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (fife times) in 1% acetic acid. The protein-bound dye was extracted with 10 mM unbuffered Tris base (POCH, Gliwice, Poland) for determination of the optical density (λ = 540 nm) in Synergy H4 multi-mode microplate reader (BioTek Instruments USA).

The relation between surviving fraction and drug concentration is plotted to get the survival curve for each cell line after the specified time. The concentration required for 50% inhibition of cell viability (IC_{50}) was calculated and the results are given in Table 1. The results were compared to the antiproliferative effects of the reference control doxorubicin.

iii-*Antibacterial & antifungal activity of newly synthesized derivatives*:

Materials and Methods

1.1 Agar Disc Diffusion Test (ADDT)

Antimicrobial tests were carried out using the agar disc diffusion method. Sterile plates of Muller Hinton agar (Oxoid), and potato dextrose agar were prepared under aseptic conditions. Then the media stand for 15 mins to cool and solidify.

Bacterial and mycotic freshly prepared suspension was prepared using reference strains/ isolates. Then using 100 µL of suspension containing 1×10^8 CFU/mL of pathological tested bacteria/ mycotic strains /isolates were spread onto the surface of the Muller Hinton Agar plates and potato dextrose agar. Then the material was cut under sterile conditions into squares of 1cm dimensions and after spreading of the reference strain the material is place onto the surface and pressed well to allow spread of active ingredient under study. The inoculated plates were then incubated for 24hrs at 37˚C for bacterial growth and for 25˚C for fungal growth. After incubation time, antimicrobial activities were expressed as inhibition diameter zones in millimeters (mm). The experiment was carried out in triplicate and the average zone of inhibition was calculated. Zone of inhibition is measured using measuring caliber. In case of negative inhibition, results are given as "zero" ZOI. Results were expressed as well with scale of –ve and +ve scoring as defined as follows; 0-6mm (-), 7-12 mm (+), 13-18mm (++), 19-24 mm (+++), 25-30 mm (++++), 31-36 mm (+++++).

Statistical analysis

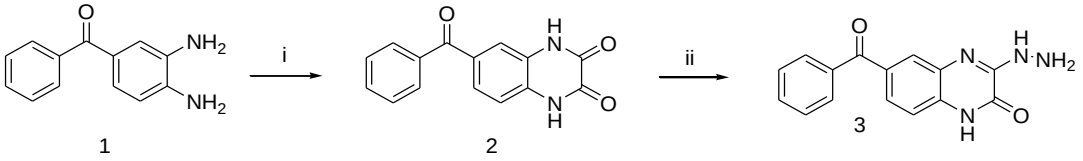
The results are reported as Mean ± Standard error (S.E.) for at least six times experiments.

3. Results and discussion

i. Chemistry

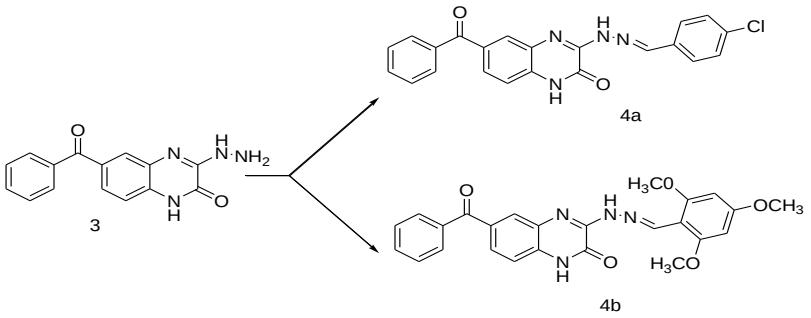
Quinoxline derivatives were of an important heterocycle had a broad spectrum of pharmacological activities and medicinal applications. In this work, Quinoxline derivatives were prepared according to (Shadia et al) [20]. Compound 2 was synthesized by the refluxing of Compound**1** with oxalic acid dihydrate at $l00^0$ for 20 min. **(Scheme 1)**. This was indicated by the disappearance of 2 NH_2incompound **1**in both IR and ^{1}H-NMR spectra and appearance of ν (3C=O) peaks from 1680, 1671(3C=O) cm^{-1} in compound **2**.

Compound**3**[21,22]can be obtained by reaction of compound **2** with hydrazine hydrate in ethanol **(Scheme1).**IR and ^{1}H-NMR spectra were showed the appearance of NH, NH_2 peaks. It was observed that compounds containing the azomethine (–NHN=CH-) protons, have been possess anticancer activity **[23.24]** and these compounds were an important class of drug development **[25].**



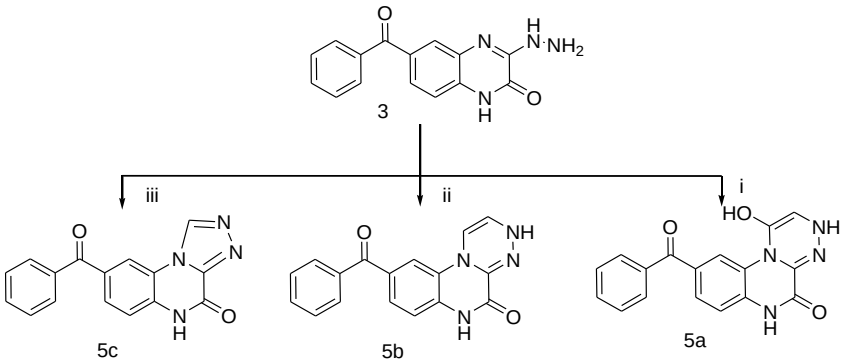
Scheme 1: *Synthesis of compounds* ***2,3***

Reagents and conditions: (i) oxalic acid, 4N HCl, reflux. (ii) Hydrazine hydrates 98%, ethanol /Acetone, reflux.
Compound**3** was used as starting agent to prepare: quinoxalinone compounds **4a,b** were obtained at refluxing of compound **3** with4-chlorobenzaldehyde or 2,4,6-trimethoxybenzaldehyde respectively **(Scheme2)**in ethanol/ acetone. IR and ^{1}H-NMR spectra were showed the disappearance of NH_2peaksin compound **3** and appearance (**N=CH**) peaks in compound **4a,b**. [26]



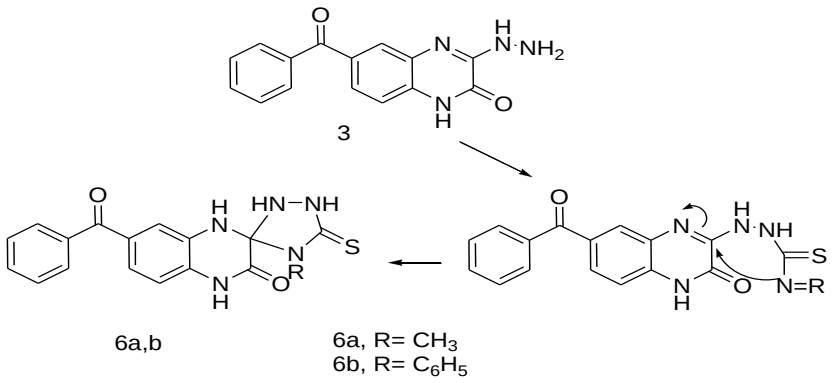
Scheme 2: *Synthesis of compounds* ***4a, b***

Reagent and conditions: 4-chlorobenzaldehyde /or 2,4,6-trimethoxybenzaldehyde, DMF, stirring.
Derivatives**5a,b** and **5c** were prepared by treating compound **3** with ethyl bromoacetate/or chloroacetyl chloride/or triethyl orthoformate, respectively. **(scheme3)**. IR and ^{1}H-NMR spectra were showed the disappearance of NH_2peak in compound **3**.[27]



Scheme 3: *Synthesis of compounds* ***5a-c***

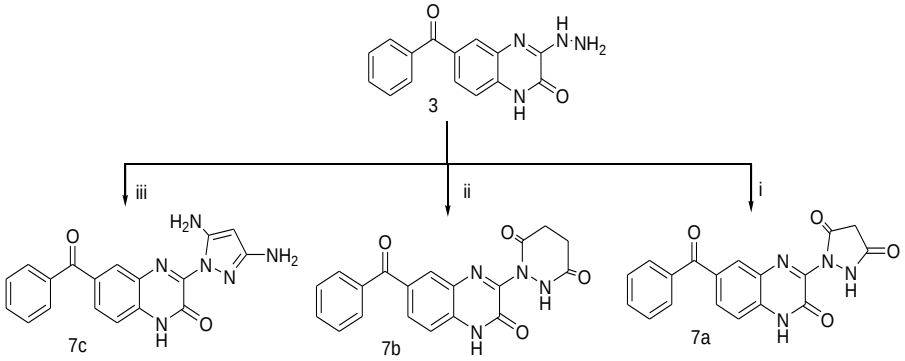
Reagents and conditions: i) Ethyl bromoacetate, DMF, reflux. ii) Chloroacetyl chloride DMF, reflux. iii) Triethyl orthoformate DMF, reflux.
Products **6 a, b (Scheme 4)** were prepared by reactions of methyl isothiocyanate /or phenyl isothiocyanate with compound**3.**The spiro compounds explained by the mechanism involving C=N atom of quinoxaline in the reaction, the **N** becoming electron donating amine nitrogen. The formation of products **6a,b** were was formed according [Mamedov etal.] **[28]**



Scheme 4: *Synthesis of compounds* ***6a, b***

Reagents and conditions: methyl isothiocyanate /or phenyl isothiocyanate, toluene reflux, 12h.

Treatment of compound**3** with diethyl malonate/or diethyl succinate /or malononitrile/or dimethylformamide yielded quinoxalinyl pyrazolidine derivative **7a, c** and **7b** respectively **(Scheme 5)**. The IR spectrum of **7a, b** confirmed the presence of (C=O) absorption, while IR spectrum of **7c** confirmed the presence of NH_2 absorption, The ^{1}H-NMR spectrum of **7a** exhibited (s) signal of (CH_2) protons, **7b**exhibited triplet triplet(tt) signal of (CH_2CH_2) protons, **7c**exhibited singlet (s) signal of (CH=C) proton of pyrazolidine-3,5-diamino.



Scheme 5: *Synthesis of compounds* ***7a-c***

Reagents and conditions: Diethyl malonate, DMF, Et_3N, reflux, 10 h. ii) Diethyl succinate, DMF, reflux, 12h. iii) malonate, DMF, Et_3N, reflux, 11 h

The synthetic Quinoxline compounds of **2-7**were fully characterized by different spectroscopic techniques elemental analyses, IR, ^{1}HNMR and ^{13}C NMR data. Their mass spectra displayed molecular ion peaks at appropriate m/z values.

All the reactions were followed by TLC. All products were characterized and identified with elemental and spectroscopic analyses (cf. Experimental).

ii. Antiproliferative activity of the tested compounds

The antiproliferative activities were expressed by half median growth inhibitory concentration (IC_{50}). As shown in table 1, in vitro antiproliferative activity towards human breast **(MCF7)** and human colon **(HCT116)** cancer cell lines, were evaluated using SRB assay, in comparison with doxorubicin as reference drug.

The results revealed that most compounds showed variable activity against tested cancer cell lines. The tumor cell line showed normal growth in our culture system and DMSO did not seem to have any noticeable effect on cellular

growth. A gradual decrease in viability of cancer cells was observed with increasing concentration of the tested compounds, in a dose-dependent inhibitory effect.

Evaluation of the antitumor effect of the tested compounds towards human breast **(MCF7)** cancer cells revealed that compounds **3, 5a, 4b** and **6a** had no effect on this cell line, while compounds **7c**and**4a** showed weak potency and compounds **6b** and **1**gave moderate activity. On the other hand, compounds**: 5b, 5c, 7a** and **7b**were found to be the most potent derivatives towards breast **(MCF7)** cancer cells compared to doxorubicin the standard anticancer drug, with IC_{50} values: 9.3 ± 1.1, 11.2 ± 1.7, 16.3 ± 1.9and 23.7 ± 2.7µg/ml versus 5.03 ± 0.7µg/ml for doxorubicin. Moreover, data in Table 1 indicated that only compound **1** showed a strong antiproliferative activity towards human colon cancer cell line (HCT116) with IC_{50} value of 19.1 ± 2.3µg/ml versus 4.8 ± 0.6µg/ml for doxorubicin.

Table 1: *In vitro* cytotoxic activity of the newly synthesized compounds towards human colon (HCT116) and Breast (MCF7) cancer cell line

Compounds	Colon (HCT116) IC_{50} [µg/ml]	Breast (MCF7) IC_{50} [µg/ml]
1	19.1 ± 2.3	42.3 ± 3.1
3	27.6 ± 3.2	N.A.
4a	40.7 ± 5.1	56.54 ± 5.3
4b	60.2 ± 8.7	N.A.
5a	N.A.	N.A.
5b	90.3 ± 16.2	9.3 ± 1.1
5c	94.5 ± 11.6	11.2 ± 1.7
6a	44.6 ± 10.5	N.A.
6b	76.9 ± 12.6	31.2 ± 3.1
7a	32.4 ± 8.9	16.3 ± 1.9
7b	81.7 ± 9.3	23.7 ± 2.7
7c	N.A.	53.2 ± 4.6
Dox	4.8 ± 0.6	5.03 ± 0.7
DMSO	N.A.	N.A.

Data were expressed as Mean ± SD of three independent experiments.

IC50 (µg/mL): 1–10 (very strong), 11–25 (strong), 26–50 (moderate), 51–100 (weak).

DOX: Doxorubicin is the drug reference.

N.A.: is no activity

iii. Antibacterial & antifungal activities of the tested compounds

Antibacterial activity was achieved by sample No. **8** followed by samples **11** and **12**. Sample **8** exhibited inhibition power exceeded the reference antibiotic against both gram negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa* while samples **11** and **12**. exerted inhibition activity reached the reference antibiotic against the same gram-negative bacteria. The same samples**8, 11** and **12** gave antibacterial effect against the gram-positive bacteria *Bacillus cereus* and Staphylococcus aurous in the range between 50% and 66 %. With respect to *Candida albicans*, it was inhibited by sample 8 in addition to samples **7, 11** and **12**. Samples **1 - 4** showed no antibacterial nor anti yeast activity. Antifungal activity was tested against *Rhizopus* sp and *Mucor* sp. *Rhizopus* sp was inhibited by the majority of the tested samples included samples **1 – 4** which were inert against bacteria and pathogen yeast *Candida albicans*. Samples **3, 7, 8**, and **13** exhibited high antifungal activity against *Rhizopus* sp. reaching the inhibition power of Ampicillin the reference antibiotic. Only samples **3, 4**, and **6** gave antifungal effect against *Mucor* sp **[30–32].** The obtained results (Table 2) revealed that the tested compounds had different antimicrobial responses.

Table 2: Antibacterial and antifungal activities of the tested compounds

Microorganism	**Gram stain**	1	2	3	4b	5a	5b	6a	6b	7a	7b	7c	Ampicillin **Ref. antibiotic**
Bacillus cereus	Positive	0	0	0	17	0	14	15	12	14	10	16	30
Escherichia coli	Negative	0	0	0	15	0	0	20	10	14	10	14	15
Pseudomonas aeruginosa	Negative	0	0	0	15	0	15	28	15	18	22	14	20
Staphylococcus aureus	Positive	0	0	0	20	0	19	18	12	15	14	20	30
Candida albicans	Yeast	0	0	0	18	0	15	20	15	18	0	18	40
*Rhizopus*sp	Fungus	25	20	35	25	25	0	30	0	30	20	20	30
*Mucor*sp	Fungus	0	0	30	0	25	0	0	0	0	20	0	25

Conclusions

In conclusion, some of the tested compounds (**1, 4b, 6a, 7a,** and **7c**), exert significant anti-proliferative potency towards tested human breast **(MCF7)** and colon (HCT116) cancer cell linesthrough reducing cell proliferation and resulted in reasonable significant growth inhibitory effect. On the other hand, compounds (**3, 4a, 6a**, and **7a**) showed moderate activity on colon (HCT116) cancer cell line and also two compounds (10 and 1) indicated moderate potency towards breast (MCF7) cancer cell line. So, the present study revealed that human breast (MCF7) and colon (HCT116) cancer cell lines are more sensitive to some of the tested compounds, while the rest of compounds gave no or weak activities towards the investigated cell lines, which indicate a specific anti-proliferative potency of some of these newly synthesized compounds towards different cancer cell lines. Also, Antibacterial activity was showed effective inhibition by samples **4b, 6a** and **7c** against gram-negative, gram-positive bacteria and pathogen yeast Candida albicans. Antifungal activity was achieved against Rhizopus sp., by the majority of the examined samples while only samples **3, 5a** and **5c** could inhibit Mucor sp. Further pharmacological investigations are needed to study the efficacy of these compounds.

References

[1]. Ventola CL. The Antibiotic Resistance Crisis: Part 1: Causes and Threats. Pharm Ther [Internet]. 2015 [cited 2023 Feb 5];40(4):277. Available from: /pmc/articles/PMC4378521/

[2]. Kim YB, Kim YH, Park JY, Kim SK. Synthesis and biological activity of new quinoxaline antibiotics of echinomycin analogues. Bioorganic Med Chem Lett [Internet]. 2004 [cited 2023 Feb 5];14(2):541–4. Available from: https://pubmed.ncbi.nlm.nih.gov/14698199/

[3]. Bough RG, Everest RP, Hale LJ, Lessel B, Mason CG, Spooner DF. Chemotherapeutic and Toxicological Properties of Quinacillin. Chemotherapy [Internet]. 1971 [cited 2023 Apr 30];16(3):183–95. Available from: https://www.karger.com/Article/FullText/220726

[4]. Christie AB, Mitchell AAB, Walker RS. Quinacillin. A study of a new penicillin. Scott Med J [Internet]. 1966 [cited 2023 Apr 30];11(5):176–81. Available from: https://pubmed.ncbi.nlm.nih.gov/5941626/

[5]. El-Atawy MA, Hamed EA, Alhadi M, Omar AZ. Synthesis and antimicrobial activity of some new substituted quinoxalines. Molecules. 2019 Nov 19;24(22).

[6]. El-Sabbagh OI, El-Sadek ME, Lashine SM, Yassin SH, El-Nabtity SM. Synthesis of new 2(1H)-quinoxalinone derivatives for antimicrobial and antiinflammatory evaluation. Med Chem Res [Internet]. 2009 [cited 2023 Apr 30];18(9):782–97. Available from: https://www.academia.edu/10706465/Synthesis_of_new_2_1H_quinoxalinone_derivatives_for_antimicrobial_and_antiinflammatory_evaluation

[7]. Fonseca T, Gigante B, Marques MM, Gilchrist TL, De Clercq E. Synthesis and antiviral evaluation of benzimidazoles, quinoxalines and indoles from dehydroabietic acid. Bioorganic Med Chem [Internet]. 2004 Jan 2 [cited 2023 Apr 30];12(1):103–12. Available from: https://pubmed.ncbi.nlm.nih.gov/14697775/

[8]. Ali IAI, Al-Masoudi IA, Hassan HG, Al-Masoudi NA. Synthesis and anti-HIV activity of new homo acyclic nucleosides, 1-(pent-4-enyl)quinoxalin-2-ones and 2-(pent-4-enyloxy)quinoxalines. Chem Heterocycl Compd [Internet]. 2007 Aug [cited 2023 Apr 30];43(8):1052–9. Available from: https://link.springer.com/article/10.1007/s10593-007-0164-0

[9]. Gupta D, Ghosh NN, Chandra R. Synthesis and pharmacological evaluation of substituted 5-[4-[2-(6,7-dimethyl-1,2,3,4-tetrahydro-2-oxo-4-quinoxalinyl)ethoxy]phenyl]methylene]thiazolidine-2,4-dione derivatives as potent euglycemic and hypolipidemic agents. Bioorg Med Chem Lett [Internet]. 2005 Feb 15 [cited 2023 Apr 30];15(4):1019–22. Available from: https://pubmed.ncbi.nlm.nih.gov/15686904/

[10]. Yang Y, Zhang S, Wu B, Ma M, Chen X, Qin X, et al. An efficient synthesis of quinoxalinone derivatives as potent inhibitors of aldose reductase. ChemMedChem [Internet]. 2012 May [cited 2023 Apr 30];7(5):823–35. Available from: https://pubmed.ncbi.nlm.nih.gov/22416050/

[11]. Sarges R, Howard HR, Browne RG, Lebel LA, Seymour PA, Koe BK. 4-Amino[1,2,4]triazolo[4,3-a]quinoxalines. A novel class of potent adenosine receptor antagonists and potential rapid-onset antidepressants. J Med Chem [Internet]. 1990 [cited 2023 Apr 30];33(8):2240–54. Available from: https://pubmed.ncbi.nlm.nih.gov/2374150/

[12]. Ancizu S, Moreno E, Solano B, Villar R, Burguete A, Torres E, et al. New 3-methylquinoxaline-2-carboxamide 1,4-di-N-oxide derivatives as anti-Mycobacterium tuberculosis agents. Bioorganic Med Chem. 2010 Apr 1;18(7):2713–9.

[13]. Hui X, Desrivot J, Bories C, Loiseau PM, Franck X, Hocquemiller R, et al. Synthesis and antiprotozoal activity of some new synthetic substituted quinoxalines. Bioorg Med Chem Lett [Internet]. 2006 Feb 15 [cited 2023 Apr 30];16(4):815–20. Available from: https://pubmed.ncbi.nlm.nih.gov/16309903/

[14]. Goda FE, Maarouf AR, El-Bendary ER. Synthesis and antimicrobial evaluation of the new Isoxazole and Pyrazole derivatives. Saudi Pharm J [Internet]. 2003 [cited 2023 Apr 30];11(3):111–7. Available from: http://inis.iaea.org/Search/search.aspx?orig_q=RN:35071143

[15]. El-Emary TI. Synthesis and Biological Activity of Some New Pyrazolo[3,4-b]Pyrazines. J Chinese Chem Soc. 2006;53(2):391–401.

[16]. Mansour AK, Eid MM, Khalil NSAM. Synthesis and Reactions of Some New Heterocyclic Carbohydrazides and Related Compounds as Potential Anticancer Agents. Mol 2003, Vol 8, Pages 744-755 [Internet]. 2003 Oct 31 [cited 2023 Apr 30];8(10):744–55. Available from: https://www.mdpi.com/1420-3049/8/10/744/htm

[17]. Cheeseman GWH. 223. Quinoxalines and related compounds. Part VI. Substitution of 2,3-dihydroxyquinoxaline and its 1,4-dimethyl derivative. J Chem Soc [Internet]. 1962 Jan 1 [cited 2023 Feb 5];79(0):1170–6. Available from: https://pubs.rsc.org/en/content/articlehtml/1962/jr/jr9620001170

[18]. Ajani OO, Obafemi CA, Nwinyi OC, Akinpelu DA. Microwave assisted synthesis and antimicrobial activity of 2-quinoxalinone-3-hydrazone derivatives. Bioorg Med Chem [Internet]. 2010 Jan 1 [cited 2023 Feb 5];18(1):214–21. Available from: https://pubmed.ncbi.nlm.nih.gov/19948407/

[19]. Jadhav S, Kapadnis K. Synthesis and Characterization of Schiff bases of Benzaldehyde with Nitroanilines and their Cobalt, Nickel and Copper metal Complexes. 2018.

[20]. Hameed S, Kanwal, Seraj F, Rafique R, Chigurupati S, Wadood A, et al. Synthesis of benzotriazoles derivatives and their dual potential as α-amylase and α-glucosidase inhibitors in vitro: Structure-activity relationship, molecular docking, and kinetic studies. Eur J Med Chem [Internet]. 2019 Dec 1 [cited 2023 Apr 30];183. Available from: https://pubmed.ncbi.nlm.nih.gov/31514061/

[21]. Mamedov VA, Murtazina AM, Gubaidullin AT, Hafizova EA, Rizvanov IK. Efficient synthesis of 2-(pyrazol-3-yl)benzimidazoles from 3-arylacylidene-3,4-dihydroquinoxalin-2(1H)-ones and hydrazine hydrate via a novel rearrangement. Tetrahedron Lett. 2009 Sep 16;50(37):5186–9.

[22]. Maigali SS, Tawfik HA, Abd-El-Maksoud MA, Soliman FM, Moharam ME, Dondeti MF. Synthesis, Molecular Docking and Antimicrobial Activities of 3-Formyl-2- (1H)quinolinone Schiff Base Derivatives and 3-(((3-Acetylphenyl)imino)- methyl)quinolin-2-(1H)-one Chalcone Derivatives. Egypt J Chem

[Internet]. 2020 Oct 1 [cited 2023 Apr 30];63(10):3903–14. Available from: https://ejchem.journals.ekb.eg/article_88183.html

[23]. Abdel Rehim MH, Yassin MA, Zahran H, Kamel S, Moharam ME, Turky G. Rational design of active packaging films based on polyaniline-coated polymethyl methacrylate/nanocellulose composites. Polym Bull [Internet]. 2020 May 1 [cited 2023 Apr 30];77(5):2485–99. Available from: https://www.researchgate.net/publication/334148731_Rational_design_of_active_packaging_films_based_on_polyaniline-coated_polymethyl_methacrylatenanocellulose_composites

[24]. Bassyouni F, Tarek M, Salama A, Ibrahim B, Dine SS El, Yassin N, et al. Promising Antidiabetic and Antimicrobial Agents Based on Fused Pyrimidine Derivatives: Molecular Modeling and Biological Evaluation with Histopathological Effect. Molecules [Internet]. 2021 Apr 2 [cited 2023 Apr 30];26(8). Available from: /pmc/articles/PMC8072832/

[25]. Mirzaei S, Eisvand F, Hadizadeh F, Mosaffa F, Ghasemi A, Ghodsi R. Design, synthesis and biological evaluation of novel 5,6,7-trimethoxy-N-aryl-2-styrylquinolin-4-amines as potential anticancer agents and tubulin polymerization inhibitors. Bioorg Chem. 2020 May 1;98.

[26]. Hasanin M, Hashem AH, El-Rashedy AA, Kamel S. Synthesis of novel heterocyclic compounds based on dialdehyde cellulose: characterization, antimicrobial, antitumor activity, molecular dynamics simulation and target identification. Cellulose. 2021 Sep 1;28(13):8355–74.

[27]. Machaba KE, Mhlongo NN, Soliman MES. Induced Mutation Proves a Potential Target for TB Therapy: A Molecular Dynamics Study on LprG. Cell Biochem Biophys. 2018 Sep;76(3):345–56.

[28]. Cournia Z, Allen B, Sherman W. Relative Binding Free Energy Calculations in Drug Discovery: Recent Advances and Practical Considerations. Vol. 57, Journal of Chemical Information and Modeling. American Chemical Society; 2017. p. 2911–37.

[29]. Skehan P, Storeng R, Scudiero D, Monks A, Mcmahon J, Vistica D, et al. New Colorimetric Cytotoxicity Assay for Anticancer-Drug Screening. JNCI J Natl Cancer Inst [Internet]. 1990 Jul 4 [cited 2023 Apr 30];82(13):1107–12. Available from: https://academic.oup.com/jnci/article/82/13/1107/970741

[30]. Liu X, Ouyang S, Yu B, Liu Y, Huang K, Gong J, et al. PharmMapper server: a web server for potential drug target identification using pharmacophore mapping approach. Nucleic Acids Res [Internet]. 2010 Apr 29 [cited 2023 Oct 4];38(Web Server issue). Available from: https://pubmed.ncbi.nlm.nih.gov/20430828/

[31]. PharmMapper [Internet]. [cited 2023 Oct 4]. Available from: https://www.lilab-ecust.cn/pharmmapper/.

[32]. Narramore S, Stevenson CEM, Maxwell A, Lawson DM, Fishwick CWG. New insights into the binding mode of pyridine-3-carboxamide inhibitors of E. coli DNA gyrase. Bioorg Med Chem [Internet]. 2019 Aug 15 [cited 2022 Dec 5];27(16):3546–50.