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Synthesis and biological evaluation of some new 2-pyridylquinazoline derivatives

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2-pyridyl [3*H*]-quinazolin-4-one derivatives fused or substituted with different oxygen or nitrogen heterocycle moieties as potential anti-tumor and anti-microbial agents were prepared, characterized and biologically screened. The synthesis process started from 5-bromo-2-[pyridin-4-ylcarbonyl]amino]benzoic acid which was converted to the crucial building block 6-Bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one via two alternative routes. Compound **3** underwent halogenation reaction $POCl_3$ and PCl_5 to afford compound 6-Bromo-4-chloro-2-(pyridin-4-yl)quinazoline **4.** The novel cyclized products **5a,b-10** were subsequently prepared. Some of the newly synthesized compounds **5a**, **5b**, **6**, **7**, **8**, **9** and **10** were screened for their antiproliferative and antimicrobial activities against various eukaryotic and prokaryotic cells. Compound **9** showed selective antibacterial activity against Gram-positive bacteria *S. aureus* (IZ = 26 mm, MIC = 256 µg/ml) and may serve as a good candidate for further developmental studies.

1. Introduction

3*H*-quinazolin-4-ones and their derivatives are among the most investigated scaffolds in the field of synthetic medicinal chemistry because of their proven widespread pharmacological activities[1-4]. 3H-Quinazolin-4-one moiety is a frequently encountered scaffold in many biologically active natural products[5] such as the alkaloid (+)-Febrifugine[6], Methylisatoid[7], Tryptanthrin[8] and Rutecarpine[9] (**Fig. 1**).

Quinazoline moiety is also found in many commercially available FDA approved drugs, such as the antihypertensive Prazosin[10], the anti-glaucoma Bunazosin[11], Alfuzosin for treatment of benign prostatic hypertrophy[12]and the antifungal Albaconazole[13] (**Fig. 2**).

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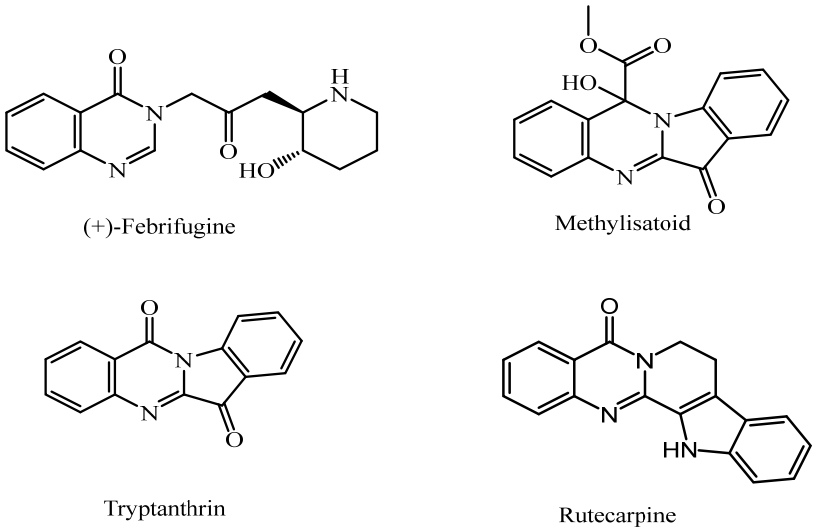


Fig. 1. Biologically active quinazolinone natural products.

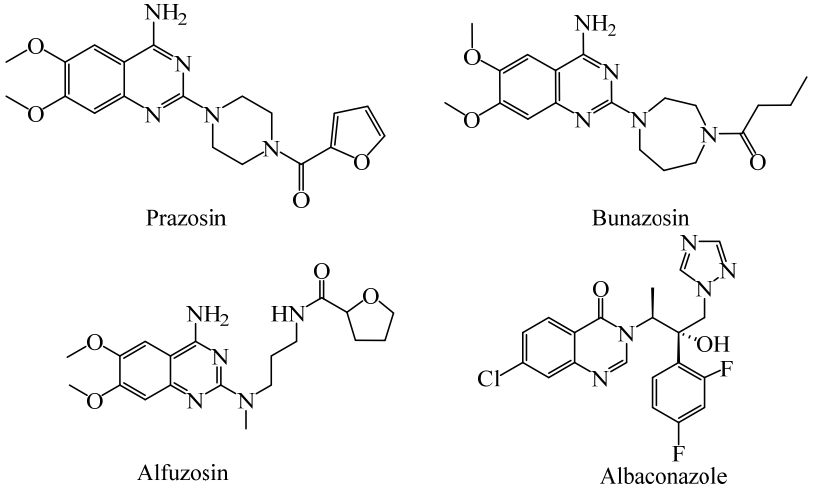


Figure 2. quinazoline FDA approved drugs

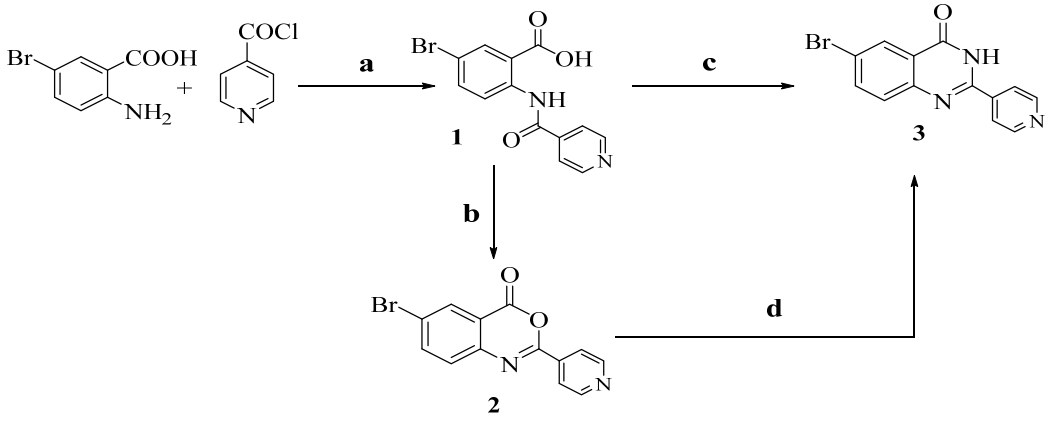
Any different quinazolinone derivatives possess a multitude of interesting pharmacological activities including anti-convulsant , anti-diabetic[14, 15], analgesic[16], sedative[17] and anti-inflammatory activities[18, 19]. Quinazoline derivatives are also well known by its wide diversity of enzyme inhibitory activity[20, 21]. Moreover, multiple publications have reported quinazoline derivatives to poses anti-HIV activity[22]. In addition, quinazoline derivatives play an important role in cancer chemotherapy to the extent that there are many quinazoline derivatives were found in FDA approved anticancer drugs[23, 24] . Based on the aforementioned data and in continuation of previous publication on the synthesis of biologically active quinazoline derivatives[16] it is rationalized to synthesize a novel series of 6-bromoquinazolin-4[3H]-ones linked or fused to other heterocyclic moieties such as pyrazolone, triazole, tetrazole ring systems to be evaluated for their antimicrobial and antiproliferative activity.

2. Results and Discussion

2.1 Chemistry

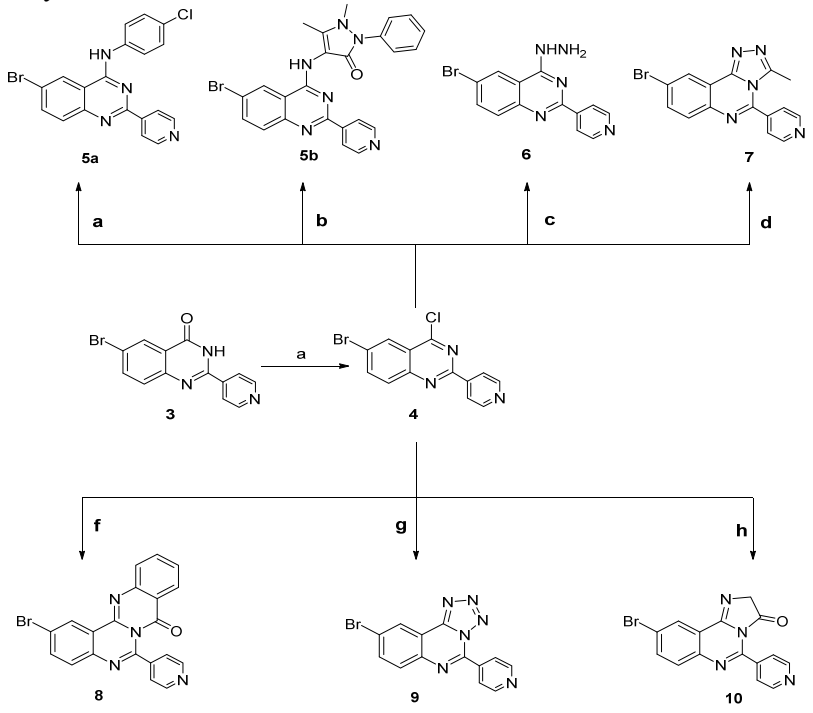
The study started with the synthesis of compound **1** which was achieved by the reaction of 5-bromoanthranilic acid and isonicotinoyl chloride in pyridine. Compound **1** was converted to the crucial starting material compound **3** via two different routes. The first route started by treatment of compound **1** with acetic anhydride to afford the benzoxazin-4-one **2** which then undergo reaction with ammonium

acetate under fusion conditions to afford compound **3**. The second route to prepare **3** was achieved by reacting compound **1** with ammonium acetate in pyridine in the presence of ammonium hydroxide and sodium hydroxide under reflux conditions (Scheme1).



Scheme 1: Reagents and conditions: *a:* isonicotinoyl chloride/Pyridine/ stirring, ambient 24 h; *b:* acetic anhydride, reflux; *c:* pyridine, CH_4COONH_4, NH_4OH, NaOH; *d:* CH_4COONH_4, fusion

Compound **3** was converted to 6-Bromo-4-chloro-2-(pyridin-4-yl)quinazoline **4** by treatment with phosphorus oxychloride in the presence of a catalytic amount of PCl_5 (Scheme 2). Compound **4** was used as a building block as it underwent various substitution/cyclization reactions to afford compounds **5a,b-10** as follows.: a) upon refluxing compound **4** with *p*-chloraoaniline or 4-aminoantipyrine, in methanol in presence few drops of pyridine afforded N-substituted 4-amino quinazoline products **5a,b**.; b) on the other hand, when compound **4** reacted with hydrazine hydrate it gave the corresponding 4-hydazino derivative **6** (Scheme 2). Compound **4** was also, allowed to react with acetyl hydazide, anthranilic acid, sodium azide and/or glycine in n-butanol to give the corresponding products **7**, **8**, **9** and/or **10** respectively.



Scheme 2 Reagents and conditions: *a:* $POCl_3$/ PCl_5, steam bath, 8h; *b:* p-chloroaniline, CH_3OH, Pyridine, reflux 10h; *c*: 4-aminoantipyrine, CH_3OH, Pyridine, reflux 10h; *d:* NH_2NH_2, Methanol, stir 8 h; *e:* acetyl hydrazide, n-butanol, reflux 8h; *f:* anthranilic acid, n-butanol, reflux 18h; *g:* sodium azide, n-butanol, reflux 18h; *h:* glycine, n-butanol, reflux 8h.

2.2 Antimicrobial activity

Antibacterial drugs have selective toxicity for the prokaryotic cell, causing toxicity to a bacterial cell, not the eukaryotic cell. Based on the previous fact the compounds **5a**, **5b**, **6**, **7**, **8**, **9** and **10** were subjected to cytotoxic study which revealed that all the tested compounds did not induce cytotoxic activity to fibroblast cells nor to cancer cell lines (**Table 2**) even at high concentration. Interestingly, among the previously mentioned compounds, only compound **9** was found to have selective antibacterial activity as it inhibited the growth of Gram-positive bacteria (*Staphylococcus aureus*). The structure of compound **9** is closely related to suggesting that DNA gyrase and DNA topoisomerases might be potential targets[25]. Some isoquinoline derivatives have also been reported to induce antibacterial effects via FtsZ disruption of FtsZ protein polymerization. For instance, 3-Phenyl substituted 6,7-dimethoxyisoquinoline derivatives exhibited potent antibacterial activity against *Staphylococcus aureus* and such activity via disruptive effects on FtsZ function[26]. However, the antibacterial activity of **9** was observed solely against Gram-positive bacteria indicating a narrow spectrum of compound **9**

Table 1. Antimicrobial screening of quinazoline derivatives

Compound	***S. aureus*** **ATCC 29213**		***E. coli*** **ATCC 25922**		***K. pneumoniae*** **ATCC 700603**		***P. mirabilis*** **ATCC 14153**		***P. aeruginosa*** **ATCC 27853**		***A. baumannii*** **Clinical Isolate**		***C. albicans*** **ATCC 10231**	
	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)	IZ (mm)	MIC (µg/ml)
5a	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
5b	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
6	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
7	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
8	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
9*	26	256	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512
10	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512	0.00	>512

*The MBC value for compound **9** was >1024µg/ml

2.3 Antiproliferative activity

The ability of test compounds to induce growth inhibition was tested against a range of human cell lines (from normal and cancerous tissue origin). The cell lines were selected from different tissue origin in order to explore any possible selectivity. Table 2 below shows that none of the tested quinazoline derivatives has exhibited cytotoxicity on any of the cell lines (IC_{50} > 100 µM). These results indicate that indirect toxicity is also not likely. Exposure to high concentrations of the tested compounds for 96 h should have been evident in case cellular activating mechanisms are required to render the tested quinazoline derivatives into antiproliferative agents. The structures of all prepared compounds were fully characterized by their correct CHN elemental analysis, IR spectrophotometry, proton NMR and Mass. Structural data of compounds **3** and **4** were in complete agreement with the literature values. The newly prepared compounds underwent antimicrobial screening as well as cytotoxicity evaluation against five different cell lines. Only compound **9** was found to possess selective activity against *Staphylococcus aureus*. Finally, a toxicological assessment of compound **9** revealed no human

cytotoxicity on tested normal and cancer cell lines. Therefore, **9** as a selective antibacterial compound may serve as a good candidate for further development studies by structure modification in order to optimize its potency. Further studies are also recommended to explore the antibacterial mechanism of action.

Table 2. Antiproliferative screening of quinazoline derivatives

Compound	MRC5 IC_{50} ± SD	MCF7 IC_{50} ± SD	A2780 IC_{50} ± SD	RKO IC_{50} ± SD	SW480 IC_{50} ± SD
Doxorubicin	106.2 ± 23.3 nM	76.2 ± 19.5 nM	95.5 ± 29.8 nM	31.2 ± 11.1 nM	128.4 ± 31.8 nM
5a	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
5b	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
6	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
7	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
8	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
9	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM
10	> 100 µM	> 100 µM	> 100 µM	> 100 µM	> 100 µM

SD: Standard deviation, calculated from three independent experiments.

3. Conclusions

A new series of 2-pyridyl [3H]-quinazolin-4-one derivatives incorporated into diverse N and O heterocyclic moieties as potential anti-microbial and anti-tumor agents, were synthesized, characterized and screened for their anti-microbial and cytotoxic activity. Among all new compounds only compound **9** was found to process selective antibacterial activity against Gram-positive bacteria *S. aureus* (IZ = 26 mm, MIC = 256 µg/ml).

4. Experimental

4.1. Materials and Methods

Chemistry

All chemicals were purchased from common commercial suppliers and used without further purification. All reactions were carried out under argon with dry solvents. Also, all reactions were monitored by TLC carried out on Merck silica gel-coated plastic sheets (60 F254) by using UV light as visualizing agent. Melting points (m.p.) were determined on an Electro-thermal IA 9100 melting point apparatus and were uncorrected. IR spectra (KBr disks) were recorded on Schimadzu 435 IR Spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Gemini 300 MHz Spectrophotometer in $CDCl_3$ or DMSO as a solvent and TMS as an internal standard. Chemical shift values (δ) are given using parts per million scale (ppm). Mass spectra were recorded on Hewlett Packard 5988 Spectrometer using CI, EI or FAB ionization techniques. Microanalyses were performed at the Micro analytical Centre of Cairo University. Chemical nomenclature and calculation of molecular weight (Mwt.) of new compounds were performed by ChemDraw 15 software.

Pharmacology

Microorganisms, cell lines, media and chemicals

All microorganisms used in this study were provided by the Department of Microbiology, College of Pharmacy, Taif University, Taif, KSA. All broth media and agars used for microorganism growth were purchased from Difco Laboratories (USA). All other chemicals were purchased from Sigma Aldrich (NY, USA) unless specified otherwise.

Cell lines and Medium conditions

Human lung fibroblast (MRC5) and Human colorectal adenocarcinoma (SW480) cell lines were a kind gift from Dr. A. N. Abdalla (Department of Pharmacology and Toxicology, College of Pharmacy, Umm Al-Qura University, Makkah, KSA). Human colorectal adenocarcinoma (RKO) and ovarian cancer (A2780) cell lines were obtained as a kind gift from Dr. A. Aljada (Department of Basic Medical Sciences, College of Medicine, King Saud Bin Abdulaziz University for Health Sciences, Riyadh, KSA). Breast cancer cell line (MCF7) obtained from the American type culture collection (ATCC, USA). Cell culture media and supplements were obtained from Gibco (Thermo-Fisher scientific Inc., Waltham, MA, USA) and all sterile plastics used in cell culture and experiments were obtained from Corning (NY, USA) unless specified otherwise.

4.2. General procedure

Chemistry

5-Bromo-2-[(pyridin-4-ylcarbonyl)amino]benzoic acid (1)

A mixture of 5-bromoanthranilic acid (0.01 mol) and isonicotinylchloride (0.01 mol) in dry pyridine (30ml) was stirred at room temperature for 24 h. The reaction mixture was then poured into ice/water, the produced precipitate was filtered off and air dried. The product was crystallized from acetic acid to give compound **1**. Yield 82%, m.p. 250-252 ^{O}C

6-Bromo-2-(pyridin-4-yl)-4*H*-3,1-benzoxazin-4-one (2)

A mixture of compound **1** (3.2 g, 0.01 mol) and acetic anhydride (5ml) was heated under fusion at 150^{O}C over sand bath for 2 h. The reaction was allowed to cool down to ambient temperature, the crude product was crystallized from ethanol twice to afford dark brown crystals of **2.** Yield 82%; m.p. 275-277 ^{O}C

6-Bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (3)

Method A

A mixture of compound **2** (3g, 0.01 mol, hydrazine hydrate (3ml) and ammonium acetate were heated under fusion condition at 150^{O}C over sand bath for 2 h. After cooling down to ambient temperature, the crude product was crystallized twice from ethanol to afford dark brown crystals of **3.**

Method B

Compound (**3**) was also prepared by refluxing of compound **1** with an excess of ammonium acetate, ammonium hydroxide and sodium hydroxide in pyridine. Yield 28% m.p.200-203^{O}C

6-Bromo-4-chloro-2-(pyridin-4-yl)quinazoline (4)

A mixture (0.01 mol) of **3** and (0.015 mol) phosphorus pentachloride in phosphorus oxychloride as a solvent (20 ml) was heated over a steam bath for 8h. The reaction mixture gradually poured into crashed ice. The precipitate was filtered off, air dried then crystallized from acetic acid to afford compound **4**. Yield 82% m.p.178-181 ^{O}C

General procedure for preparation of **5a,b**

A mixture of **4** (0.01 mol) and two different aromatic amines namely, 4-chloroaniline and 4-amino-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (0.01 mol) and few drops of pyridine as a catalyst in methanol (25 ml) was heated under reflux for 8-10hr. The formed crude precipitate was filtered off, vacuum dried then recrystallized from methanol to afford compounds **5a,b**. **(5a)** Yield 80 % m.p.256^{O}C; **(5b)** Yield 75% m.p.287-289^{O}C.

6-Bromo-4-hydrazinyl-2-(pyridin-4-yl)quinazoline (6)

A mixture of (0.01 mol) 4 and (0.015 mol) hydrazine hydrate in methanol (15 ml) was allowed to stir at room temperature for 8 hr. The formed crude solid was filtered off, dried under vacuum then recrystallized from acetic acid to give compound **6.** Yield 73% m.p. >300^{O}C.

9-Bromo-3-methyl-5-(pyridin-4-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (7)

A solution of compound **4** (0.001 mol) and acetyl hydrazide (0.0015 mol) in (30 ml) n-butanol was heated under reflux for 18 hr., the crude product formed after cooling was dried, recrystallized from DMF/water to afford compound **7.** Yield 60%; m.p. >300 ^{O}C.

2-bromo-6-(pyridin-4-yl)-8H-quinazolino[4,3-b]quinazolin-8-one (8)

A solution of compound **4** (0.001 mol) and anthranilic acid (0.015 mol) in (30 ml) n-butanol was heated under reflux for 10 hr., the product obtained after cooling was dried under suction and crystallized from DMF/water to give compound **8**. Yield 65%; m.p.>300^{O}C.

9-Bromo-5-(pyridin-4-yl)tetrazolo[1,5-*c*]quinazoline (9)

A solution of compound **4** (0.001 mol) and sodium azide (0.005 mol) in (30 ml) n-butanol was heated under reflux for 18 hrs., the product obtained after cooling was filtered off, dried under suction and crystallized from DMF/water to give compound **9**. Yield 66%; m.p.132-133^{O}C.

9-Bromo-5-(pyridin-4-yl)imidazo[1,2-*c*]quinazolin-3(2*H*)-one (10)

A mixture of **4** (0.01 mol) and glycine (0.01 mol) in n-butanol (30 ml) was heated under reflux for 8 h; the crude solid produced was refluxed with glacial acetic acid (5 ml) for 5 hrs. The crude solid product was filtered off, dried under vacuum and recrystallized from methanol to afford orange crystals of compound **10.** Yield 64%; m.p118-120^{O}C.

Pharmacology

Cell lines and Medium conditions

All cancer cell lines were routinely maintained in advanced RPMI media supplemented with 4 mM L-Glutamine, 4% Fetal Bovine Serum (FBS) and 100 U/mL Penicillin-Streptomycin. MRC5 cells were routinely maintained in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 1mM Sodium Pyruvate, 2 mM L-Glutamine, 10% FBS, and 100 U/mL Penicillin-Streptomycin. All cells were propagated in a humidified atmosphere at 37 °C supplemented with 5% CO_2.

Antiproliferative activity

All compounds (100 mM in DMSO), stored at -20 °C until required. The antiproliferative activities were determined by the MTT colorimetric assay as previously reported[27]. Briefly, cancer cells at 2000 cells/well were seeded into a 96-well plate and allowed to adhere overnight at 37 °C in a humidified incubator with 5% CO_2. Cells were then exposed to a range of drug concentration for 96 h. The final concentration of DMSO was ≤ 0.1%. Wells containing no drug or no cells were included in each plate as negative and positive control, respectively. the medium was then replaced with 200 µl/well of cell culture medium containing 0.5 mg/ml MTT and incubated for 4 h at 37 °C in a humidified incubator with 5% CO2. The insoluble formazan was dissolved by 150 µl DMSO per well, and absorbances were measured at 540 nm wavelength (Spectramax® plus 384, Molecular Devices, CA, USA). The IC_{50} values were defined as the drug concentrations that inhibited the cell growth by 50% after 96 h drug exposure. All experiments have been performed in triplicates.

Direct colony suspension method

Inoculum preparation for antimicrobial testing by disc diffusion method was performed using direct colony suspension method described elsewhere [28]. Briefly, a few colonies of tested isolate were resuspended in sterile normal saline and adjusted to achieve turbidity equivalent to 0.5 McFarland standard which approximately equivalent to 1-2 x 10^8 CFU/ml. For broth micro dilution method, the 0.5 McFarland adjusted inoculum was further diluted in double-strength nutrient broth (DSNB) to obtain a final inoculum concentration of 5 x 10^5 CFU/ml. The inoculated broth was used directly within 15 minutes of preparation.

Disc Diffusion Method

Antimicrobial susceptibility testing was primarily done for tested compounds by a modified Kirby-Bauer method following a procedure published elsewhere [29]. Briefly, a sterile cotton swab was dipped into the bacterial suspension (adjusted to 0.5 McFarland) of the tested isolate, and the excess suspension was removed by pressing the cotton swab against the wall of a test tube containing the bacterial suspension. The swab was streaked all over the surface of the Muller-Hinton agar (MHA) plate three times by rotating the plate at an angle of 60° after each application. Finally, the swab was passed around the edge of the agar surface. The inoculated MHA plates were left to dry for a few minutes at room temperature with the lid closed. The compound-loaded discs (380µg/disc) were placed on the inoculated plates. The plates were then incubated overnight at 37°C. On the next day, the diameter of inhibition zones (including the diameter of the discs) were measured with a ruler under the surface of the plate, and the results were recorded in mm.

Determination of Minimum Inhibitory Concentration (MIC) by Broth Microdilution Method

Broth micro dilution method was performed as described previously by Qaiyumi [30]. Inoculum (100 µl/well) in DSNB containing a selected microorganism (5 x 10^5 CFU/ml) were seeded into 96-well plate. Inoculum in each well was diluted with 100 µl of sterile distilled water containing tested compound at a suitable range of concentration. Wells containing no drug or no microorganism were included in each plate as negative and positive control, respectively. The plates were incubated at 37°C for 18 hours. MIC values were then determined by observing the wells with no visible growth.

Determination of Minimum Bactericidal Concentration (MBC)

After the determination of MIC values, 5 µL from each well that showed no visible growth were inoculated onto Muller Hinton agar plate and incubated overnight at 37 °C. MBC value was considered as the compound concentration resulted in no microorganism growth in an agar plate.

4.3 Physical and Spectral Data

5-Bromo-2-[(pyridin-4-ylcarbonyl)amino]benzoic acid (1)

Anal. Calc. for $C_{13}H_9BrN_2O_3$ (321.13): C 48.62 H 2.82 N 8.72, found: C 48.60 H 2.80 N 8.70, IR (KBr, cm^{-1}): 3450 (OH), 3125 (NH), 3030, 3019 (Ar-H), 1680 (CO of benzoic acid), 1645 (C=C), ^{1}H NMR (DMSO-d_6,): δ = 7.1-7.5 (7H, m, Ar-H), 9.7, 12.1 (2H, s, NH, OH exchangeable with D_2O) ppm, MS:(m/z) M^+ at m/z ≈ 321 (45%), 323 (44%),77 (62%), 138 (45%), 159 (100%), 201 (48%), 248 (46%), 382 (56%), 321 (45%), 323 (44%).

6-Bromo-2-(pyridin-4-yl)-4*H*-3,1-benzoxazin-4-one (2)

Anal. Calc. for $C_{13}H_7BrN_2O_2$ (303.11): C 51.51 H 2.33 N 9.24 found: C 51.49 H 2.31 N 9.22, IR (KBr, cm^{-1}): 3125 (NH), 3030, 3019 (Ar-H), 1685 (CO of benzoxain-4one),1626 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 7.1-7.5 (7H, m, Ar-H), MS: (m/z) M^+ at m/z ≈ 303 (13%), 305 (12%),90 (45%),171 (31%), 198 (78%), 214 (38%).

6-Bromo-2-(pyridin-4-yl)quinazolin-4(3*H*)-one (3)

Anal. Calc. for $C_{13}H_8BrN_3O$ (302.13): C 51.68 H 2.67 N 13.91 found: C 51.66 H 2.65 N 13.89, IR (KBr, cm-1): 3125 (NH), 3030, 3019 (Ar-H),1687 (CO of quinoxalone), 1631 (C=N),1H NMR (DMSO-d6, δ ppm) 6.9-7.5 (7H, m, Ar-H), 10.1 (1H, s, 1 NH exchangeable with D2O), MS: (m/z) M+ at m/z ≈ 302 (23%), 304 (22%),90 (45%),171 (31%), 198 (78%), 214 (38%).

6-Bromo-4-chloro-2-(pyridin-4-yl)quinazoline (4)

Anal. Calc. for $C_{13}H_7BrClN_3$ (320.58): C 48.71 H 2.20 N 13.11 found: C 48.69 H 2.18 N 13.09, IR (KBr, cm^{-1}): 3030, 3019 (Ar-H), 1625 (C=N),^{1}H NMR (DMSO-d_6, δ ppm) 7.1-7.5 (7H, m, Ar-H), MS: (m/z) M^+ at m/z ≈ 319 (45.1%), 321 (44.7%),108 (100%),179 (21.5 %), 225 (14.1%), 270 (45.2%).

6-Bromo-*N*-(4-chlorophenyl)-2-(pyridin-4-yl)quinazolin-4-amine (5a)

Anal. Calc. for $C_{19}H_{12}BrClN_4$ (411.69): C 55.43 H 2.94 N 13.61 found: C 55.41 H 2.92 N 13.59, IR (KBr, cm^{-1}): 3125 (NH), 3030, 3019 (Ar-H), 1627 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 7.1-7.9 (7H, m, Ar-H), 10.1 (1H, s, 1 NH exchangeable with D_2O), MS: (m/z) M^+ at m/z ≈ 411 (11%), 413 (10%).

4-((6-bromo-2-(pyridin-4-yl)quinazolin-4-yl)amino)-1,5-dimethyl-2-phenyl-1,2-dihydro-3H-pyrazol-3-one (5b)

Anal. Calc. for $C_{24}H_{19}BrN_6O$ (487.36): C 59.15 H 3.93 N 17.24 found: C 59.13 H 3.91 N 17.22, IR (KBr, cm^{-1}): 3125 (NH), 3030, 3019 (Ar-H), 1665 (CO of Pyrazolone), 1628 (C=N),^{1}H NMR (DMSO-d_6, δ ppm) 2.4 (3H, s, C-CH_3), 3.7 (3H, s, N-CH_3), 6.8-7.9 (12H, m, Ar-H), 9.7(1H, s, 1 NH exchangeable with D_2O), MS:(m/z) M^+ at m/z = 119 (14%) ,165 (100%), 181(32.1 %), 207(85.1), 237 (37.1%), 278 (3 6%), 320 (35%), 390 (8.6%), 432 (6.7%), 486 (4%), 488 (3%).

6-Bromo-4-hydrazinyl-2-(pyridin-4-yl)quinazoline (6)

Anal. Calc. for $C_{13}H_{10}BrN_5$ (316.16): C 49.39 H, 3.19 N 22.15 found: C 49.37 H 3.17 N 22.13, IR (KBr, cm^{-1}): 3450,3380 (NH_2),3125 (NH), 3030, 3019 (Ar-H), 1620 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 6.9-7.5 (7H, m, Ar-H), 10.4,10.7,11.2 (3H, s, of NH_2, 1 NH exchangeable with D_2O), MS: (m/z) M^+ at m/z = 316 (23%), 318 (21%).

9-Bromo-3-methyl-5-(pyridin-4-yl)[1,2,4]triazolo[4,3-*c*]quinazoline (7)

Anal. Calc. for $C_{15}H_{10}BrN_5$ (340.18): C 52.96 H 2.96 N 20.59 found: C 52.94 H 2.94 N 20.57, IR (KBr, cm^{-1}): 3030, 3019 (Ar-H), 1620 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 2.5 (3H, s, C-CH_3), 6.8-7.7 (7H, m, Ar-H), 8.6 (1H, s, N=CH). MS: (m/z) M^+ at m/z ≈ 340 (15%), 342 (14%).

2-bromo-6-(pyridin-4-yl)-8H-quinazolino[4,3-b]quinazolin-8-one (8)

Anal. Calc. for $C_{20}H_{11}BrN_4O$ (403.23): C 59.57 H 2.75 N 13.89 found: C 59.55 H 2.73 N 13.87, IR (KBr, cm-1): 3030, 3019 (Ar-H), 1712, (CO), 1620 (C=N). 1H NMR (DMSO-d6, δ ppm) 7.5-8.2 (11H, m, Ar-H), MS: (m/z) M+ at m/z ≈ 403 (15%), 405 (14%),106 (78%), 197 (69%), 229 (53%), 341 (24%).

9-Bromo-5-(pyridin-4-yl)tetrazolo[1,5-*c*]quinazoline (9)

Anal. Calc. For $C_{13}H_7BrN_6$ (327.14): C 47.73 H 2.16 N 25.69 found: C 47.71 H 2.14 N 25.67, IR (KBr, cm^{-1}): 3030, 3019 (Ar-H), 1654 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 7.4-8.1 (7H, m, Ar-H), MS: (m/z) M^+ at m/z ≈ 325.99 (33.9%),327 (32%),76 (7.7%),149 (100%), 177 (19.1%), 281(11%).

9-Bromo-5-(pyridin-4-yl)imidazo[1,2-*c*]quinazolin-3(2*H*)-one (10)

Anal. Calc. for $C_{15}H_9BrN_4O$ (341.16): C 52.81 H 2.66 N 16.42 found: C 52.79 H 2.64 N 16.40, IR (KBr, cm^{-1}): 3030, 3019 (Ar-H), 1687 (CO), 1620 (C=N), ^{1}H NMR (DMSO-d_6, δ ppm) 4.5 (2H, s, CH_2),7.6-8.5 (7H, m, Ar-H), MS: (m/z) M^+ at m/z ≈ 341 (12%), 343 (11%).

Conflict of Interest

No conflict of interest associated with this work

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