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Anti-cancer and Antioxidant Activities of Some New Synthesized Mannich Bases Containing an Imidazo (2, 1-B) Thiazole Moiety

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Abstract

Synthesis and preliminary biological evaluation of imidazo (2, 1-b) Thiazole derivatives is reported. Under Mannich conditions, a series of new imidazo (2, 1-b) Thiazole derivatives were synthesized. Starting from the reaction of 2- amino thiazole with 4- bromo phenyl bromide to produce 5-(4-bromo phenyl) imidazo (2, 1-b) thiazoles, following by introduce the substituted aminomethyl at position 6-by reacting with different aromatic amines under Mannich conditions to afford 6-secondary amine-5-(4-bromo phenyl) imidazo (2,1-b) thiazole in high yields.
FT-IR, 1H NMR, and 13C NMR techniques were used to characterize the synthesized derivatives. In addition, all compounds were tested for their antioxidant activity, and three of them were examined for cytotoxic activity against kidney cancer using an MTT assay. The product (A9) has shown an excellent anti-cancer activity with 69.69 value.

Keywords: Imidazo [2, 1-b] thiazole, One-pot reaction, Mannich bases, Antioxidants, Anti-cancer.

الفعالية المضادة للسرطان والفعالية المضادة للأكسدة لبعض قواعد مانخ الجديدة المحتوية لمكون imidazo(2,1−b) thiazole

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الخلاصة

سلسلة جديدة من قواعد مانخ تم تحضيرها ابتداءاً من تفاعل تكاثف 5−معوض اميدازو (2,1 −b) ثايازول من خلال تفاعل 2−امينو ثايازول مع 4−برومو فنيل بروماید لإنتاج 5−(4−برومو−فينيل) إيميدازو (2،1−ب) ثيازول، ثم ادخال معوض مثيل امين في موضع 6 تحت ظروف مانخ ليعطي 6−امينات ثانوية − 5−معوض اميدازو (2,1 −b)−ثايازول. تم تشخيص هذه المشتقات الجديدة من قواعد مانخ بواسطة اطياف الاشعة تحت الحمراء و وبروتون وكاربون الرنين المغناطيسي النووي بالإضافة الى ذلك تم تقييم فعالية بعض قواعد مانخ كمضادات للأكسدة حيث أظهرت بعضها نتائج جيدة مضادة للأكسدة وفقًا للتركيز المثبط .(IC50) بالإضافة إلى ذلك، تم فحص السمية الخلوية لقواعد مانخ الثلاثة الجديدة في المختبر باستخدام اختبار MTT ضد سرطان الكلى. أظهر المركب ((A 9نشاطا واعدا بقيمة 69.69 (IC50) جم / مل.

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Introduction:

Fused heterocyclics that conations a sulfur and nitrogen as heteroatoms are widely found in many natural products as well as pharmaceutically important synthetic chemicals [1]. One of the most widespread heterocyclics found in pharmaceuticals is the imidazole core [2]. The synthesis of hybrid molecules containing more than one bioactive molecule has sparked attention in medicinal chemistry in recent years due to the advantages of these compounds in terms of efficacy and applicability in a variety of disorders [3]. Imidazo [2, 1-b] thiazole derivatives are significant heterocyclic compounds having antibacterial [4], antiparasitic [5], antifungal [6], antiviral [7], antihelmintic [8], anticancer [9-11], cardiac tonic [12], chemopreventive, and antioxidant characteristics [13]. Furthermore, previous study has shown that imidazo [2, 1-b] thiazoles derivatives had significant antiproliferative activity when evaluated against a panel of cancer cells when compared to well-known anticancer medications [14]. The activity of a series of imidazo [2, 1-b] thiazole guanyl hydrazones against a variety of cancer cell lines has been demonstrated, and levamisole, a well-known antihelminthic and immunomodulatory medication, contains an imidazo [2, 1-b] thiazole core [15].

We demonstrate here the first access to highly functionalized imidazo[2,1-b] thiazoles, as well as anti-infectious screening of these novel compounds, using the Mannich approach. Certain amines, Mannich bases with an imidazo [2, 1-b] thiazole core were applied in this study. Mannich bases are a fascinating family of substances generated by the Mannich reaction for medicinal chemistry [16]. The traditional Mannich reaction, which is a three-component condensation between structurally separate substrates (XeH), produces a series of molecules known as Mannich bases, which include one active hydrogen atom, an aldehyde component (typically R1 -CHO), and an amine reagent [17]. The Mannich reaction has also been widely applied in the discovery of new prospective drug candidates with high activity potential. Furthermore, adding amino alkyl side chains to anticancer treatments such as the camptothecin derivative topotecan and other antibiotics has been shown to improve water solubility, bioavailability, and chemical stability of parent medications (e.g. certain tetracycline, amodiaquine, and pyronaridine) [18-20].

1. Experimental part:

Materials

Sigma/Aldrich, BDH, and Merck chemicals supplied all of the components and solvents, and purchased through CDH and Reagent World. Pre-coated aluminum sheets with silica gel 60, provided by Merck Company, were used in thin-layer chromatography (TLC), and the spots were marked with iodine vapors.

Instrumentation

Uncorrected melting points were recorded using Gallenkamp capillary melting point equipment**.** The Shimadzu Fourier Transform Infrared (FT-IR-8400S) Spectrophotometer was used to record infrared spectra at the University of Baghdad/ College of Science.

Using deuterated dimethyl sulfoxide as a solvent, the Varian model ultra-shield nuclear magnetic resonance spectrometer was utilized to acquire ^{1}H and ^{13}C NMR spectra at 400 and 499.67 MHz and 125 MHz, respectively (DMSO- d^{6}). The chemical changes are measured in parts per million (ppm) in comparison to internal reference tetra-methyl silane (TMS) at Iran's University of Tehran.

Synthesis 6-(4-bromophenyl) imidazo [2, 1-b] thiazole (compound 1A)

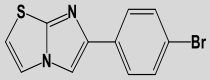
A mixture of 2-aminothiazole (0.01 mol) and 4-bromophenacyl bromide (0.01 mol) was heated in ethyl alcohol (80 mL) under reflux for 18-20 hours. The reaction was monitored by TLC (petroleum ether/ethyl acetate 1:2) until there were no more starting materials. After filtering the reaction mixture, a solution of NaOH (5%) was added to bring the pH of the mixture to around (10-11). The mixture was then allowed for 4 hours to acquire the most quantity of precipitate possible during the digestion process. The precipitate was then filtered, rinsed in distilled water, dried, and re-crystallized with EtOH, yielding yellowish-orange crystals with an Excellent yield (90 %). [21,22], and the physical properties of this compound **1A** are shown in Table 1.

FT-IR (KBr, ν, cm^{-1}):3132, 3050 (C-H) aromatic; 1662 (C=N); 1583, 1535, 1492 (C=C) aromatic; 1276 (C-N); 748 (C-S-C); 723 (C-Br).

^{1}HNMR (ppm): (DMSO- d^6, 500 MHz) δ: 8.14 - 7. 3 (m, 6H, Ar-H).

^{13}CNMR (ppm): (DMSO-d^6, 125 MHz) δ: 145.5 (S-C-N), 140.2-107.4 (2 C=N); 141.5 - 121.4, (4 C=C) aromatic; 137.1 (C=S); (Cm^{-1}) 123.8 (C-Br).

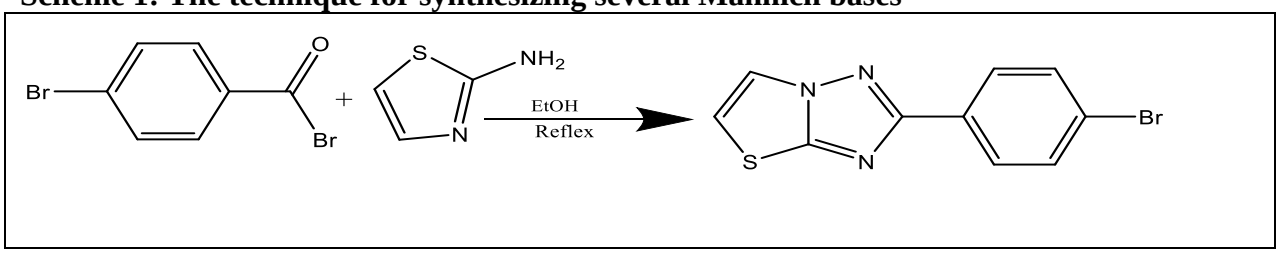
Table 1: The physical properties of the compound (1A)

Comp. No.	Structure	Molecular formula	Molecular weight g/mole	Color	m.p.,°C	Time (hour)	Yield %	Rf.
1A		$C_{11}H_8BrN_2S$	280.16	orange	185-186	18-20	89.7	0.67

Synthesis of Mannich bases compounds (2A-13A)

Drops of conc. HCl solution were added to a combination of compound 1A (1 mmol) and formaldehyde (37 %) (1 mmol) until the pH of the reaction medium reached 5-4, then agitated for 30 minutes. Then one of the different amines (primary or secondary) was added (1 mmol) and the mixture was heated for 4-18 hours. The solid mass was filtered, washed, dried, and purified with EtOH once the reflux was halted and the reaction mixture had cooled to room temperature [23]. A synthetic route of various Mannich bases derivatives is shown in the scheme 1 and the physical properties of these compounds 2-13 A are shown in Table 2. and FT-IR spectra data (cm^{-1}) of Mannich bases derivatives 2-13 A are shown in Table 3, ^{1}HNMR spectra data (δ ppm) of these derivatives 2-13 A are shown in Table 4, ^{13}CNMR spectral data (ppm) was listed in Table 5.

Scheme 1: The technique for synthesizing several Mannich bases



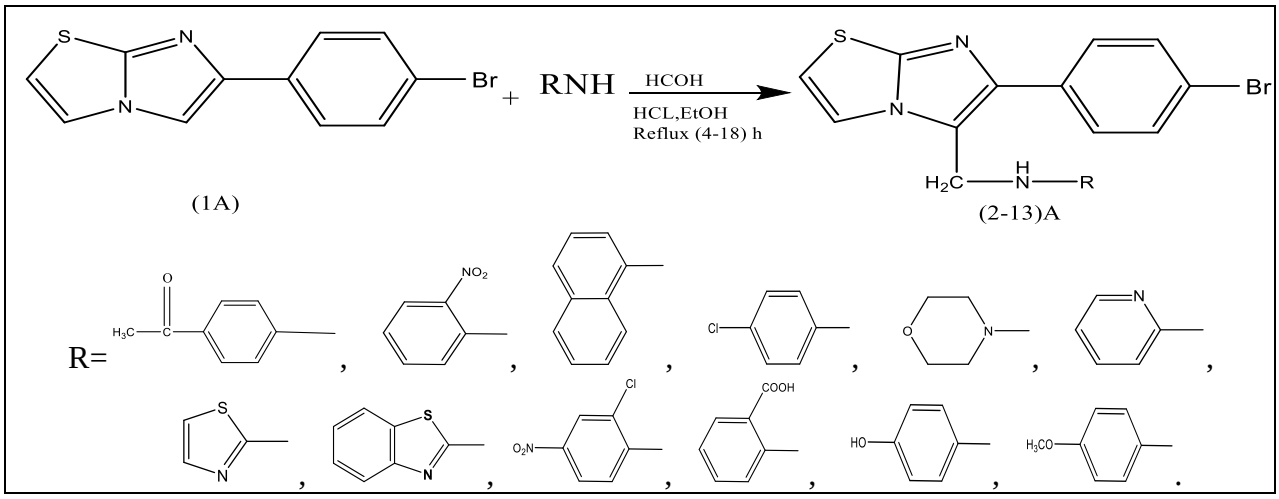
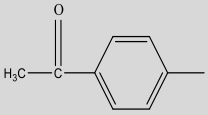
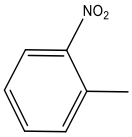
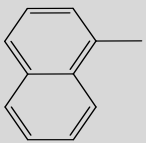
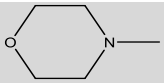
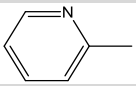
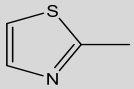
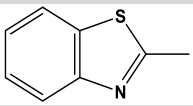
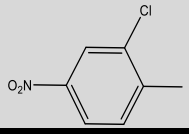
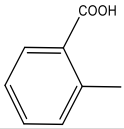
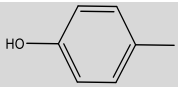
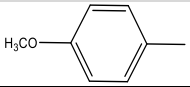


Table 2: Physical characteristics of Mannich bases compounds 2-13 A

Comp. no.	R	Molecular formula	Molecular weight g/mole	Color	m.p.,°C	Time (hour)	Yield, %	Rf.
2A		$C_{20}H_{16}N_3OSBr$	426.33	Yellow	166-168	4	85.6	0.67
3A		$C_{18}H_{13}N_4O_2SBr$	429.29	Red	148-149	7	87	0.78
4A		$C_{22}H_{16}N_3SBr$	434.36	Purple	156-159	11-14	75	0.73
5A		$C_{18}H_{13}N_3SClBr$	418.74	Brown	Oily	16	80	0.42
6A		$C_{16}H_{16}N_3OSBr$	378.29	Pale yellow	202-204	10-12	88	0.66
7A		$C_{17}H_{13}N_4SBr$	385.28	Pale yellow	110-112	9-12	79	0.71
8A		$C_{15}H_{11}N_4S_2Br$	391.31	Pale yellow	141-143	20	85	0.82
9A		$C_{19}H_{13}N_4S_2Br$	441.37	Pale yellow	120-123	20	89	0.88
10A		$C_{18}H_{12}N_4SO_2BrCl$	463.73	Dark yellow	189-191	16	84	0.46

11A		$C_{19}H_{14}N_3SO_2Br$	428.30	Mustard	68-71	9-11	81	0.71
12A		$C_{18}H_{14}N_3OSBr$	400.29	Yellow green	113-115	9-11	79	0.25
13A		$C_{19}H_{16}N_3OSBr$	414.32	Brown	Gum	11-14	75	0.84

1-(4-(((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) amino) phenyl) ethan-1-one) (2A)

Compound 2A: FT-IR (KBr/cm^{-1}): 3138and 3111(NH), 3067(CH) aromatic 2972 and 2924 (CH aliphatic), 1663(C=O), 1597 (C=N) imidazo, 1535 and 1462 (C=C), 773 (C-S-C). ^{1}HNMR (DMSO, 500 MHz) δ: 8.83-6.7(m, 10H, Ar-H), 6.34 (s, 1H, NH), 4.83(s, 2H, CH_2), 1.17 (t, 3H, CH3). ^{13}CNMR (DMSO, 500 MHz) δ: 195.7 (C=O), 152.6-148.8 (C=N), 143-111.7 (m, 8C, C=C), 124 (C-Br), 37.6(CH_2), 26.7(CH_3).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl)-2-nitroaniline (3A)

Compound 3A: FT-IR (KBr/cm^{-1}): 3137 (NH), 3026 (CH aromatic), 2948 (CH aliphatic), 1620 (C=N) imidazo, 1510 and 1342 (NO_2), 1460 and 1423 (C=C), 734 (C-Br). ^{1}H-NMR (DMSO, 500 MHZ) δ: 8.9-6.5 (m, 10H, Ar-H), 6.3 (s, 1H, NH), 4.8 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MHZ) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.9 (C-Br), 36.5 (CH_2).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) - naphthalene-1- amine (4A)

Compound 4A: FT-IR (KBr/cm^{-1}): 3377 (N-H), 3026 (CH aromatic), 2923 (CH aliphatic), 1583 (C=N) imidazo, 1481 and 1465 (C=C), 769 (C-Br). ^{1}H-NMR (DMSO, 500 MHz) δ: 8.9-6.5 (m, 13H, Ar-H), 6.3 (s, 1H, NH), 4.75 (s, 2H, CH_2). ^{13}C-NMR (DMSO, 500 MHz) δ: 168.2-147.3 (C=N), 146.7-109.5(C=C), 36.54 (CH_2).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) - 4-chloro aniline (5A)

Compound 5A: FT-IR (KBr/cm^{-1}): 3231 (N-H), 3112 (CH aromatic), 2931 and 2889 (CH aliphatic), 1539 (C=N), 1492 (C=C) aromatic, 1495 (C-N), 1091 (C-Cl), 771 (C-S), 657 (C-Br). ^{1}H-NMR (DMSO, 500 MHZ) δ: 8.83-6.51 (m, 10H, Ar-H), 6.34 (s, H, NH), 4.79 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MHZ) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.78 (C-Br), 36.54 (CH_2).

4-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) morpholine (6A)

Compound 6A: FT- IR (KBr/cm^{-1}): 3229 (N-H), 3090 (CH aromatic), 2927 (CH aliphatic), 1569 (C=N), 1535 (C=C) aromatic, 1265 (C-O-C), 1473 (C-N), 798 (C-S), 725 (C-Br). ^{1}H-NMR (DMSO, 500 MHZ) δ: 8.17-7.3 (m, 6H, Ar-H), 6.85 (s, H, NH), 3.36 (s, 2H, CH_2). ^{13}C-NMR (DMSO, 500 MHZ) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.78 (C-Br), 36.54 (CH_2).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) pyridine-2-amine (7A)

Compound 7A: FT-IR (KBr/cm^{-1}): 3255 (N-H), 3110 (CH arom.), 2972 (CH aliphatic), 1596 (C=N), 1535 (C=C) arom. , 1460 (C-N), 771 (C-S), 648 (C-Br). ^{1}H-NMR (DMSO, 500 MH_Z) δ: 8.17-7.3(m, 10H, Ar-H), 6.85 (s, 1H, NH), 3.36 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MH_Z) δ: 166.6-149.3(C=N), 146.7-109.5(C=C), 34.5 (CH_2).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) thiazol-2-amine (8A)

Compound 8A: FT-IR (KBr/cm^{-1}): 3233 (N-H), 3110(CH aromatic), 2923 (CH aliphatic), 1533 (C=N), 1460 (C=C) aromatic, 1460 (C-N), 730 (C-S), 646 (C-Br). ^{1}H-NMR (DMSO, 500 MH_Z) δ: 8.17-7.2(m,8H ,Ar-H), 6.92 (H, NH), 4.33 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MH_Z) δ: 163.2 (S-C=N, 2- thiazol), 146.7-123.3 (m,14C , aromatic C), 145.56 (C=N), 137 (CH, 2-thiazole), 123.78 (C-Br), 119.59 (C-H, thiazol), 36.6 (CH_2 aliphatic).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) benzo [d] thiazol-2-amine (9A)

Compound 9A: FT-IR (KBr/cm^{-1}): 3247 (N-H), 3137 (CH aromatic), 2975 (CH aliphatic), 1535 (C=N), 1460 (C=C) aromatic, 1469 (C-N), 730 (C-S), 648 (C-Br); ^{1}H-NMR (DMSO, 500 MH_Z) δ: 8.97-7.15 (m,10H ,Ar-H), 6.56(H, NH), 4.55 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MH_Z) δ: 170.5 (C-N), 146.7-118.3 (m, C aromatic), 123.78 (C-Br), 34.52 (CH_2 aliphatic).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl)-2-chloro-4-nitroaniline (10A)

Compound 10A: FT-IR (KBr/cm^{-1}): 3201 (N-H), 3107 (CH aromatic), 2908 (CH aliphatic), 1587 (C=N), 1523 (NO_2), 1463 (C=C) aromatic, 1470 (C-N), 620 (C-Cl), 746 (C-S), 686 (C-Br). ^{1}H-NMR (DMSO, 500 MHZ) δ: 7.97-6.93 (m, 9H, Ar-H), 6.56 (H, NH), 4.25 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MHZ) δ: 150 (C-NH), 45.5-119.6 (m,12C, aromatic C), 137.5 (C-NO_2), 122.6 (C-Cl), 123.1 (C-Br), 35.52 (CH_2 aliphatic).

2-(((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) amino) benzoic acid (11A)

Compound 11A: FT-IR (KBr/cm^{-1}): 3514 (C-OH), 3221 (N-H), 3110 (CH aromatic), 2972 (CH aliphatic), 1726 (C=O), 1672 (C=N), 1535 (C=C) aromatic, 1475 (C-N), 759 (C-S), 655 (C-Br); ^{1}H-NMR (DMSO, 500 MHz) δ: 13.11(s, 1H, OH), 7.95-6.81 (m, 10H,Ar-H), 6.81(H, NH), 4.33 (s, 2H, CH_2); ^{13}C-NMR (DMSO, 500 MHz) δ: 169.3 (C, carbonyl), 150 (N-C benzene), 150.1-116.4 (m, 10C, aromatic C), 123.1 (C-Br), 36.9 (CH_2 aliphatic).

4-(((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) amino) phenol (12A)

Compound 12A: FT-IR (KBr/cm^{-1}): 33821(C-OH), 3110 (N-H), 3076 (CH aromatic), 2923 (CH aliphatic), 1612 (C=N), 1535 (C=C) aromatic, 1460 (C-N), 717 (C-S), 657 (C-Br); ^{1}H-NMR (DMSO, 500 MHz) δ: 9.44 (s, 1H, OH), 7.95-6.60 (m, 10H, Ar-H), 6.28 (H, NH), 4.28(s, 2H, CH_2). ^{13}C-NMR (DMSO, 500 MHz) δ: 146.9-116.7 (m, 12C, aromatic C), 123.1 (C-Br), 36.9 (CH_2 aliphatic).

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl)-4-methoxy aniline (13A)

Compound 13A: FT-IR (KBr/cm^{-1}): 3225 (N-H), 3110 (CH aromatic), 2972 (CH aliphatic), 1652 (C=N), 1537 (C=C) aromatic, 1466 (C-N), 1135 (-O-CH_3) 771 (C-S), 657 (C-Br); ^{1}H-NMR (DMSO, 500 MHz) δ: 7.95-6.70 (m, 10H ,Ar-H), 6.28 (H, NH), 4.33 (s,2H, CH_2), 3.80 (s, 3H, OCH3); ^{13}C-NMR (DMSO, 500 MHz) δ: 151.7-115.1 (m,14C , aromatic C), 123.1 (C-Br), 55.8 (H_3C-O), 36.5 (CH_2 aliphatic).

Antioxidant activity

Radical Scavenging Activity of DPPH

- The solution was shielded from light by shielding the aluminum foil test tubes, the solution was prepared by dissolving DPPH (1,1-Diphenyl-2-picryl-hydrazyl) (4 mg) in methanol (100 mL). From 1 to 8, different doses (100, 50, 25, 12.5, and 6.25) ppm were generated by dissolving DPPH (1,1-Diphenyl-2-picryl-hydrazyl) (1 mg) in methanol (10 mL). After that,

the concentration was diluted to make the concentration listed below. Similar amounts of ascorbic acid (vitamin C) were used to generate the compounds.

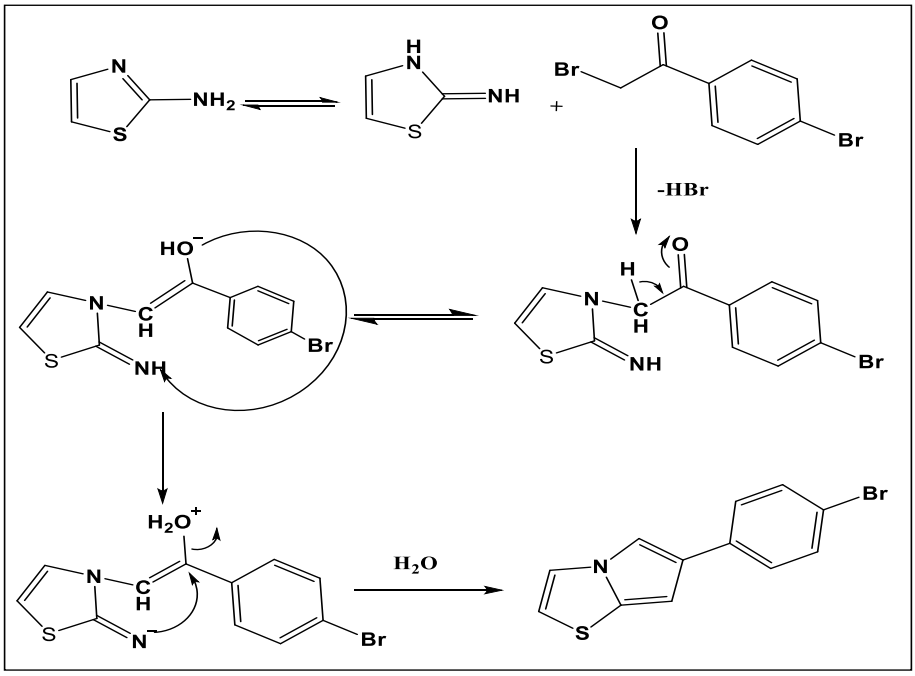
Anticancer Activity

Using 3-(dimethyltiazole-2-yl) 2, 5-diphenyltetrazolium bromide, the cytotoxic effect of the novel imidazo [2, 1-b] thiazole derivatives on the kidney cancer cell line (HEK293) was assessed (MTT). After treating HEK293 cells separately with compounds 4A, 8A, and 9A, the MTT test was used to compute cell viability and inhibition rate on the tumor cell line using varied quantities of compounds, and the parameters of assessment were determined. The viability percentage of treated cells was calculated in contrast to the normal cell line WRL.

Results and Discussion:

Synthesis 6-(4-bromophenyl) imidazo [2, 1-b] thiazole (compound 1A)

The mechanism for the formation of **1A** is shown in Scheme (3).



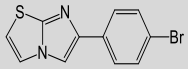
Scheme 3: mechanism of formation compound 1A

The formation of 1A as given approval by FT-IR spectrum data which includes disappearance of NH_2 band at 3200-3300 cm^{-1} and (C=O) band at 1700 cm^{-1} new bands are forming of (C=N) imidazo at (1679-1662) cm^{-1} due to formation of imidazole ring. The FT-IR spectra data (cm-1) of compound (1A) were shown in Table (2a)

The ^{1}HNMR spectrum data of compound [1A] included the appearance of multi signals of aromatic rings protons at (8.14-7.36).

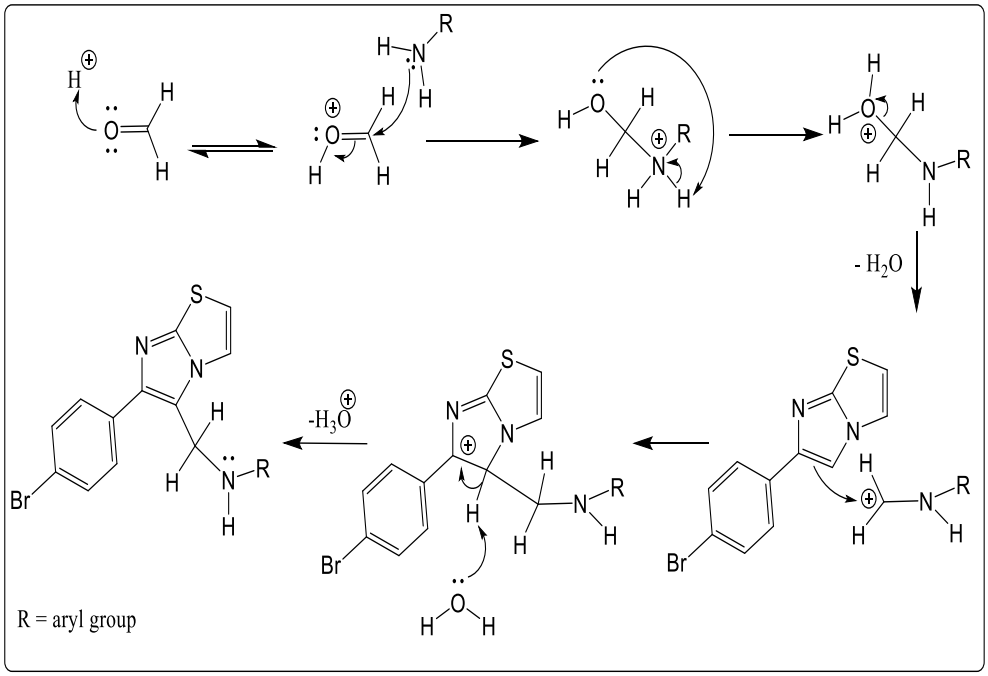
The ^{13}C-MR data of compound [1A] included the appearance of (N-CH=C imidazole) at (108.7) ppm and (C=N) at (146.5) ppm.

Table 2a: FT-IR spectra data (cm^{-1}) of Compound formation (1A)

Com. No.	Compound structure	υ (C-H) Arom.	υ (C=N)	υ (C=C) Arom.	υ (C-N)	υ (C-S-C)	Other Bands
1A		3050	1662	1583, 1535, 1492	1276	748	723 (C-Br)

Synthesis of Mannich derivatives 2-13 A

The mechanism for the formation of new Mannich derivatives as described in Scheme4:



Scheme 4: mechanism of Mannich bases derivatives

The FT-IR of Mannich bases derivatives includes the appearance of (N-H) stretching bands at (3436-3371) cm^{-1}. Also, FT-IR data have shown that the (CH) aliphatic bands at (2995-2927) cm^{-1}. FT-IR characteristic data are reported in Table 3.^{1}H-NMR spectrum data Mannich derivative included appearance of singlet signal of (-CH2) protons at δ= (3.36) ppm, and singlet signal of (-NH) at δ= (9.85) ppm, ^{1}H-NMR spectrum data of Mannich bases derivative as listed in the Table 4.^{13}CNMR data appearance δ at 36.7 of (CH_2) aliphatic. ^{13}CNMR data of Mannich bases derivative as listed in the Table 5.

Table 3: FT-IR spectra data (cm^{-1}) of Mannich bases derivatives 2-13 A

Comp. no.	ν(N-H)	ν(C-H) aromatic	ν(C-H) aliphatic	υ (C-N) aliphatic	ν (C=N)	ν (C=C) aromatic	ν (C-Br)	Others
2A	3137	3066	2972	1465	1596	1604	648	ν (C=O) 1662,ν(C-S-C) 773
3A	3110	3026	2948	1460	1620	1573	734	ν (NO_2) asym1510 sym1342
4A	3219	3110	2923	1481	1583	1460	713	ν (C-S-C) 769
5A	3240	3112	2931	1495	1539	1492	657	ν (C-Cl) 619
6A	3229	3090	2927	1473	1569	1535	725	ν (C-O-C) 1265
7A	3255	3110	2972	1460	1596	1535	648	ν (C-S) 771
8A	3233	3110	2923	1460	1533	1460	646	ν (C-S) 730
9A	3248	3137	2975	1469	1535	1460	648	ν (C-S) 730
10A	3201	3107	2908	1470	1587	1463	686	ν (NO_2) 1523 , ν (C-Cl) 620, ν (C-S) 746
11A	3421	3110	2972	1475	1672	1535	655	ν (C-OH) 3514 ν (C=O) 1726
12A	3110	3076	2923	1456	1612	1535	657	ν (C-OH) 33821
13A	3425	3110	2972	1466	1652	1537	657	ν (-O-CH_3) 1135, ν (C-S) 771

Table 4: ^{1}HNMR spectra data (δ ppm) of Mannich bases derivatives 2-13 A

Com. No.	Chemical shift
2A	^{1}HNMR (DMSO, 500 MHz) δ: 8.83-6.7(m, 10H, Ar-H), 8.57 (s, 1H, NH), 6.34- 5.9 (s, H, NH), 4.83(s, H, CH), 1.17(t, 3H, CH_3).
3A	^{1}HNMR (DMSO, 500 MHz) δ: 8.83-6.51(m, 10H, Ar-H), 6.34 (s, H, NH), 4.79 (s, 2H, CH_2).
4A	^{1}HNMR (DMSO, 500 MHz) δ: ^{1H}-NMR (DMSO, 499.67 MHZ) δ: 8.83-6.51(m, 13A, Ar-H), 6.34 (s, 1H, NH), 4.79 (s, 2H, CH_2).
5A	^{1}HNMR (DMSO, 500 MHz) δ: 8.83-6.51 (m, 10H, Ar-H), 6.34 (s, 1H, NH), 4.79 (s, 2H, CH_2).
6A	^{1}HNMR (DMSO500 MHz) δ: 8.17-7.3(m, 6H, Ar-H), 6.85 (s, H, NH), 3.36 (s, 2H, CH_2).
7A	^{1}HNMR (DMSO, 500 MHz) δ:8.17-7.3(m, 10H, Ar-H), 6.85 (s, H, NH), 3.36 (s, 2H, CH_2)
8A	^{1}HNMR (DMSO, 500 MHz) δ: 8.17-7.2(m, 8H, Ar-H), 6.92 (H, NH), 4.33 (s, 2H, CH_2).
9A	^{1}HNMR (DMSO, 500 MHz) δ: 8.97-7.15 (m, 10H, Ar-H), 6.56(H, NH), 4.56 (s, 2H, CH_2).
10A	^{1}HNMR (DMSO, 500 MHz) δ: 7.97-6.93 (m, 9H, Ar-H), 6.56(H, NH), 4.26(s, 2H, CH_2).
11A	^{1}HNMR (DMSO, 500 MHz) δ: 13.11(s, 1H, OH), 7.95-6.81 (m, 10H, Ar-H), 6.81(H, NH), 4.33 (s, 2H, CH_2).
12A	^{1}HNMR (DMSO, 500 MHz) δ: 9.44 (s, 1H, OH), 7.95-6.60 (m, 10H, Ar-H), 6.28H, NH), 4.28 (s, 2H, CH_2).
13A	^{1}HNMR (DMSO, 500 MHz) δ: 7.95-6.70 (m, 10H, Ar-H), 6.28(H, NH), 4.33 (s, 2H, CH_2), 3.80(s, 3H, OCH3).

Table 5: ^{13}CNMR spectral data (ppm) of Mannich bases derivatives 2-13 A

Com. No.	Chemical shift
2A	^{13}C-NMR (DMSO, 500 MHz) δ: 195.65(C=O), 152.58-148.79 (C=N), 143.33-111.60(m, 8C, C=C), 123.78 (C-Br), 37.58(CH2), 26.65(CH_3).
3A	^{13}C-NMR (DMSO, 500 MHz) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.78 (C-Br), 36.54 (CH2).
4A	^{13}C-NMR (DMSO, 500 MHz) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 36.54 (CH2).
5A	^{13}C-NMR (DMSO, 500 MHz) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.78 (C-Br), 36.54 (CH_2).
6A	^{13}C-NMR (DMSO, 500 MHz) δ: 168.2-147.3(C=N), 146.7-109.5(C=C), 123.78 (C-Br), 36.54 (CH_2).
7A	^{13}C-NMR (DMSO, 500 MHz) δ: 166.6-149.3(C=N), 146.7-109.5(C=C), 34.5 (CH_2).
8A	^{13}C-NMR (DMSO, 500 MHz) δ: 163.2 (S-C=N, 2- thiazol), 146.7-123.3 (m, 14C, aromatic C), 145.56 (C=N), 137 (CH, 2-thiazole), 123.78 (C-Br), 119.59 (C-H, thiazol), 36.6 (CH_2 aliphatic).
9A	^{13}C-NMR (DMSO, 500 MHz) δ: 170.5 (C-N), 146.7-118.3 (m, C aromatic), 123.78 (C-Br), 34.52 (CH_2 aliphatic).
10A	^{13}C-NMR (DMSO, 500 MHz) δ: 150 (C-NH), 45.5-119.6 (m, 12C, aromatic C), 137.5 (C-NO_2), 122.6 (C-Cl), 123.1 (C-Br), 35.52 (CH_2 aliphatic).
11A	^{13}C-NMR (DMSO 500 MHz) δ: 169.3 (C, carbonyl), 150 (N-C benzene), 150.1-116.4 (m, 10C aromatic C), 123.1 (C-Br), 36.9 (CH_2 aliphatic).
12A	^{13}C-NMR (DMSO, 500 MHz) δ: 146.9-116.7 (m, 12C, aromatic C), 123.1 (C-Br), 36.9 (CH_2 aliphatic).
13A	^{13}C-NMR (DMSO, 500 MHz) δ: 151.7-115.1(m, 14C, aromatic C), 123.1 (C-Br), 55.8(H_3C-O), 36.5 (CH_2 aliphatic).

Antioxidant activity

DPPH Radical Scavenging Activity

Antioxidant activity and a significant scavenging percentage against the DPPH free radical were found in some of the newly synthesized compounds. As a result, the compounds that were tested demonstrated antioxidant capabilities and were chosen for future investigation. As a consequence, inhibitory concentrations (IC50) were calculated and recorded in Table 7. In this study, we employed Phongpaichit's antioxidant activity classification system, which is based on IC50 range values. For more information on these ranges, see Table 6.

1- The highest antioxidant activity and the lowest IC_{50} values were found for compound (3A), (12A) and (4A) respectively.

2- As shown in Table 10, the antioxidant activities and IC50 values of the other compounds varied, with a range of high, middle, and low activities.

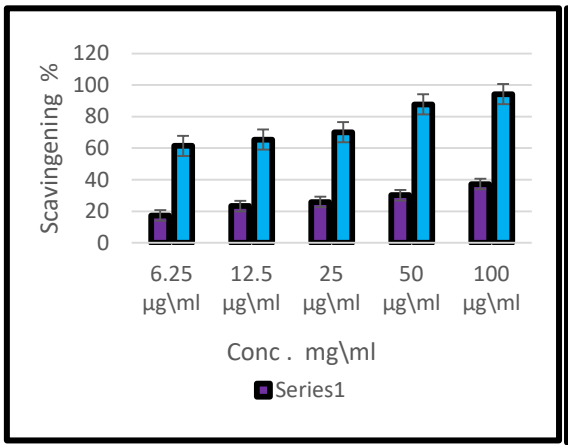
- Compounds (3A), (4A), and (12A) possess strong antioxidant activity.
- Compounds (7A) intermediate antioxidant activity.
- Compounds (2A), (6A), and (8A-11A) possess weak antioxidant activity.

Table 6: Antioxidant Activity (according to Phongpaichit, 2007)

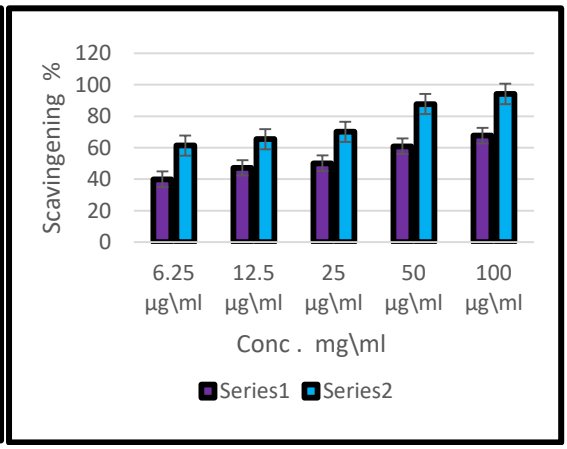
Comp. NO.	Scavenging %					Linear eq.	R^2	Ic50
	6.250 mg\ml	**12.50 mg\ml**	**25.00 mg\ml**	**50.00 mg\ml**	**100.00 mg\ml**			
2A	17.3	23.3	25.8	30.2	37.2	y = 0.1875x + 19.496	R² = 0.9151	162,7
3A	39.9	47.2	50.1	60.9	67.7	y = 0.2765x + 42.446	R² = 0.9151	27.3
4A	14.7	25.9	42.9	68.1	74.3	y = 0.6141x + 21.383	R² = 0.8182	46.6
6A	7.2	9.2	13.5	19.3	38.7	y = 0.3314x + 4.7375	R² = 0.9919	136.5
7A	28.9	33.1	39.4	42.6	50.9	y = 0.2145x + 30.667	R² = 0.9176	90.1
8A	5.9	6.9	13.7	27.2	31.9	y = 0.2922x + 5.7958	R² = 0.8831	151.4
9A	19.5	20.9	28.6	30.3	43.5	y = 0.246x + 19.029	R² = 0.9581	125,9
10A	14.9	15.9	21.6	28.3	34.1	y = 0.2079x + 14.904	R² = 0.9329	168.8
11A	1.2	5.8	13.4	22.5	30.4	y = 0.2993x + 3.0625	R² = 0.9127	156.8
12A	20.1	42.7	54.9	66.4	79.5	y = 0.5312x + 32.138	R² = 0.7909	33.6
Ascorbic acid	39.4	67.4	89.1	97.7	98.2	y = 0.3579x + 61.892	R² = 0.899	-33.22

Table 7: inhibitory concentrations (IC_{50}) values

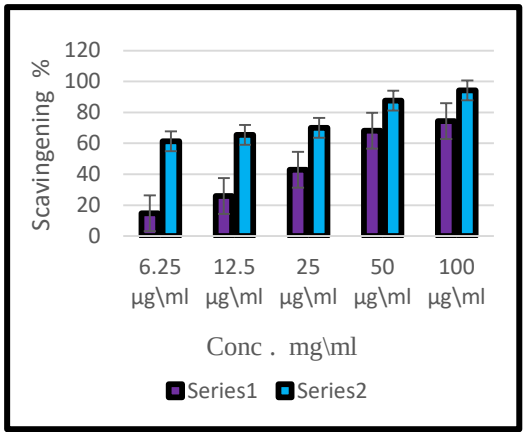
IC50 (mg/mL)	Allusion
10-50 mg/mL	Strong Antioxidant Properties
50-100 mg/mL	Antioxidant Activity in the Intermediate Range
100 mg/mL	Antioxidant Activity Isn't Very Strong



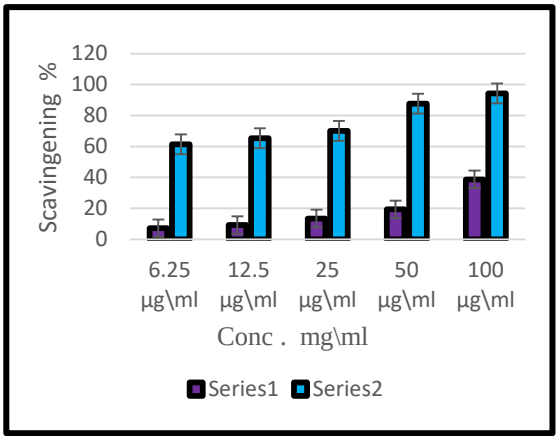
Scavenging % compound No. 2A



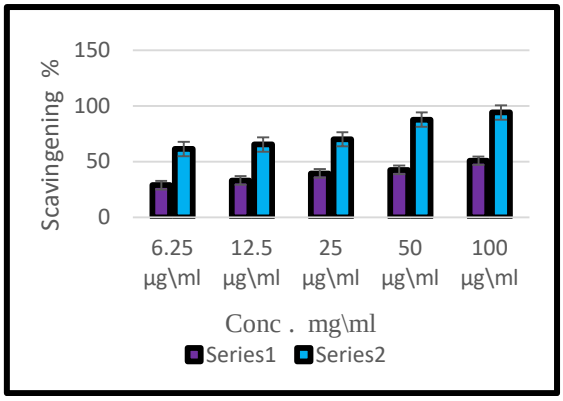
Scavenging % compound No. 3A



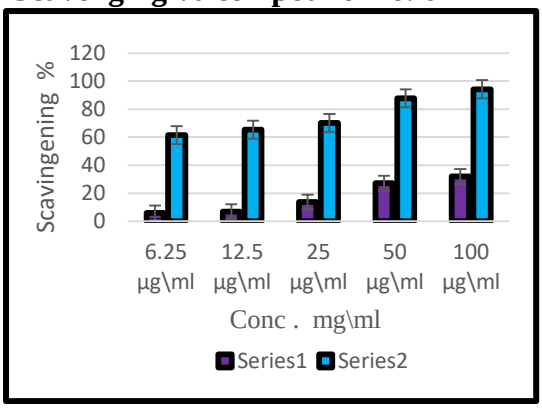
Scavenging % compound No. 4A



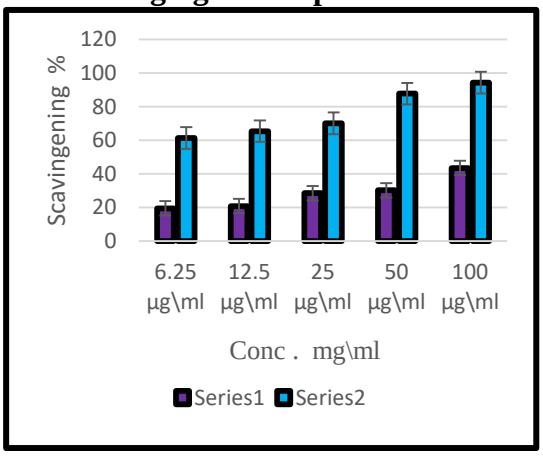
Scavenging % compound No. 6A



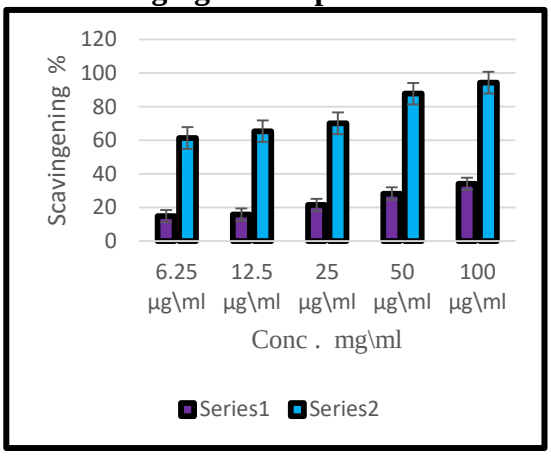
Scavenging % compound No. 7A



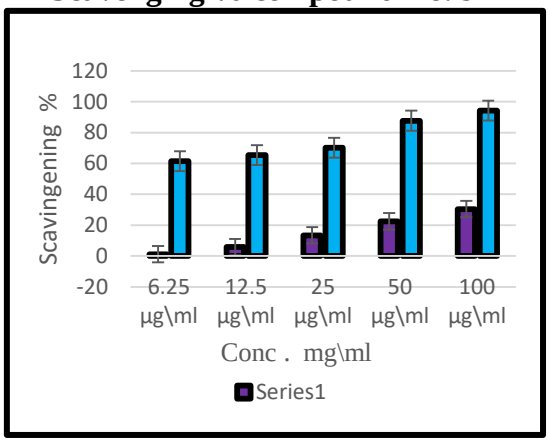
Scavenging % compound No. 8A



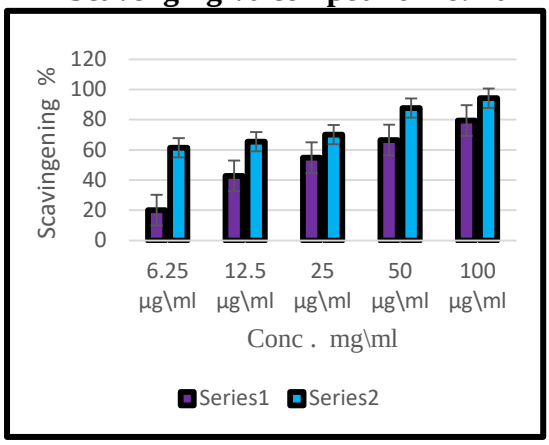
Scavenging % compound No. 9A



Scavenging % compound No. 10A



Scavenging % compound No. 11A



Scavenging % compound No. 12A

Anticancer Activity

The cytotoxicity of three of the novel Manach bases was examined in vitro using the MTT assay against kidney cancer (HEK293 cancer cell line). To determine the cytotoxic effect of 6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl methyl - naphthalene-1-amine 4A on a kidney cancer cell line, the MTT method was applied (HEK293). The MTT Assay was used to evaluate cell viability and inhibition rate on a tumor cell line using different dosages of chemical 4A. In comparison to the normal cell line WRL-68, the proportion of treated cells that survived was calculated. On HEK293 cells, the cytotoxic effect of compound 4A in concentrations ranging from 12.5 to 400 $mg.ml^{-1}$ resulted in a dose-dependent decrease in cell viability (Table 8). By raising the concentration of 4A chemicals, cell viability was lowered. The lowest HEK293 cell viability (percentage) was seen at 400 $mg.ml^{-1}$ and the maximum HEK293 cell viability was observed at 12.5 $mg.ml^{-1}$. With an IC50 value of 101.9 g/ml, compound 4A showed strong cytotoxic action. The effect of compound 4A on WRI-68 cell line normalization yielded an IC50 of 122.6 $mg.ml^{-1}$ (Figure 1).

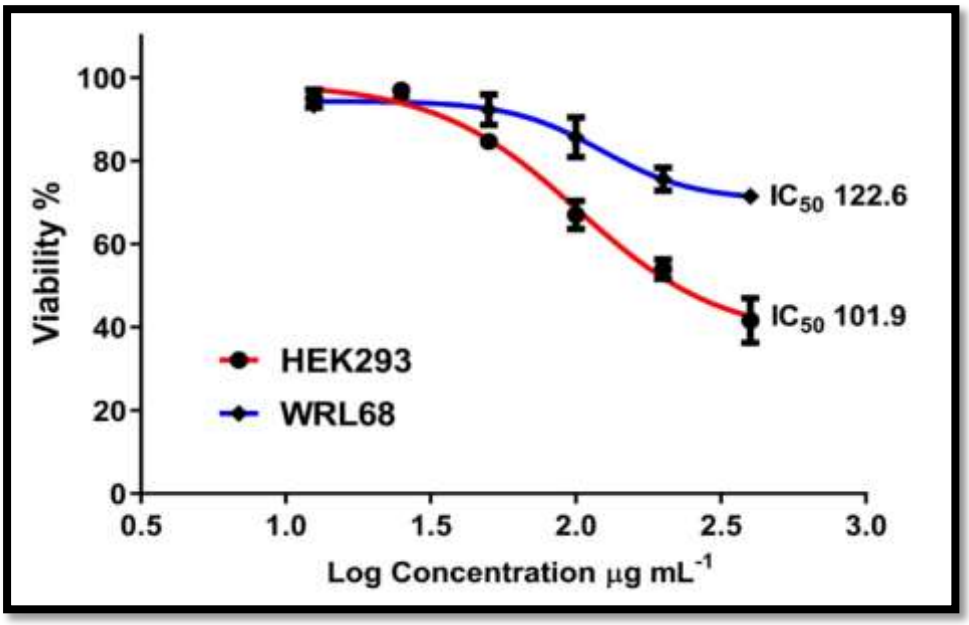


Figure 1: effect of compound (4A) on WRI-68 cell line normalization

Table 8: the cytotoxic effect of compound (4A) in concentrations on cell viability

Concentration $mg.ml^{-1}$	Cell count of HEK293 cell line Mean ± S.D.	Cell count of WRL68 cell line Mean ± S.D.
400	41.59 ± 5.38	71.41 ± 1.56
200	53.90 ± 2.37	75.58 ± 2.77
100	66.94 ± 3.41	85.63 ± 4.74
50	84.61± 1.25	92.28 ± 3.68
25	96.95 ± 1.14	95.25 ± 1.03
12.5	94.91 ± 2.20	93.29 ± 1.82

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) thiazol-2-amine compound (8A)

The cytotoxic properties of compound 8A was examined. The viability of HEK293 cells was reduced in a dose-dependent pattern at concentrations ranging from 12.5 to 400 mg.ml-1, as shown in Table 9. Increased concentrations of the chemical (8A) decrease cell viability. The waning in HEK293 cell viability (%) was noticed at 400 $mg.ml^{-1}$ (47.26 ± 1.41), the maximum cell viability was found in HEK293 cells at 12.5 $mg.ml^{-1}$. With an IC50 of 26.00 mg/ml, compound 8A showed a strong cytotoxic action. The effect of 8A on WRI-68 cell line normalization yielded an IC50 of 61.52 $mg.ml^{-1}$ (Figure 2).

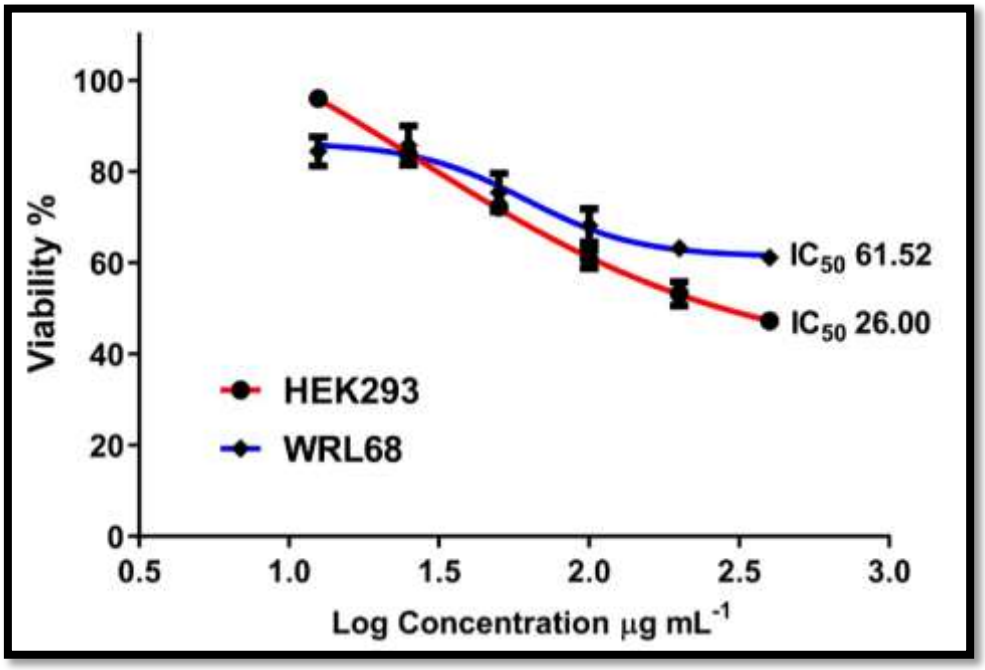


Figure 2: effect of compound (8A) on WRI-68 cell line normalization

Table 9: the cytotoxic effect of compound (8A) in concentrations on cell viability

Concentration $mg.ml^{-1}$	Cell count of HEK293 cell line Mean ± S.D.	Cell count of WRL68 cell line Mean ± S.D.
400	47.26 ± 1.41	61.07 ± 0.75
200	53.16 ± 2.61	63.19 ± 1.62
100	60.80 ± 2.10	68.13 ± 3.66
50	72.18 ± 1.18	75.39 ± 4.22
25	83.64 ± 0.82	85.73 ± 4.31
12.5	95.95 ± 1.03	84.41 ± 3.17

N-((6-(4-bromophenyl) imidazo [2, 1-b] thiazol-5-yl) methyl) benzo [d] thiazol-2-amine compound (9A)

On HEK293 cells, the cytotoxic effects of compound 9A in concentrations ranging from 6.25 to 400 mg/ml have resulted in a dose-dependent decrease in cell viability Table 10. The viability of the cells decreases as the concentration of the chemical 9A increases. The tapering in HEK293 cell viability (%) was noticed at 400 mg/ml (40.90 ± 3.74) while the uppermost cell HEK293 viability at 6.25 mg/ml was marked (96.41 ± 0.93). With an IC50 value of 69.69 mg/ml, compound 9A showed the highest cytotoxic action amongst the synthesized

substances. The effect of 9A on WRI-68 cell line normalization yielded an IC50 of 90.31 $mg.ml^{-1}$ (Figure 3).

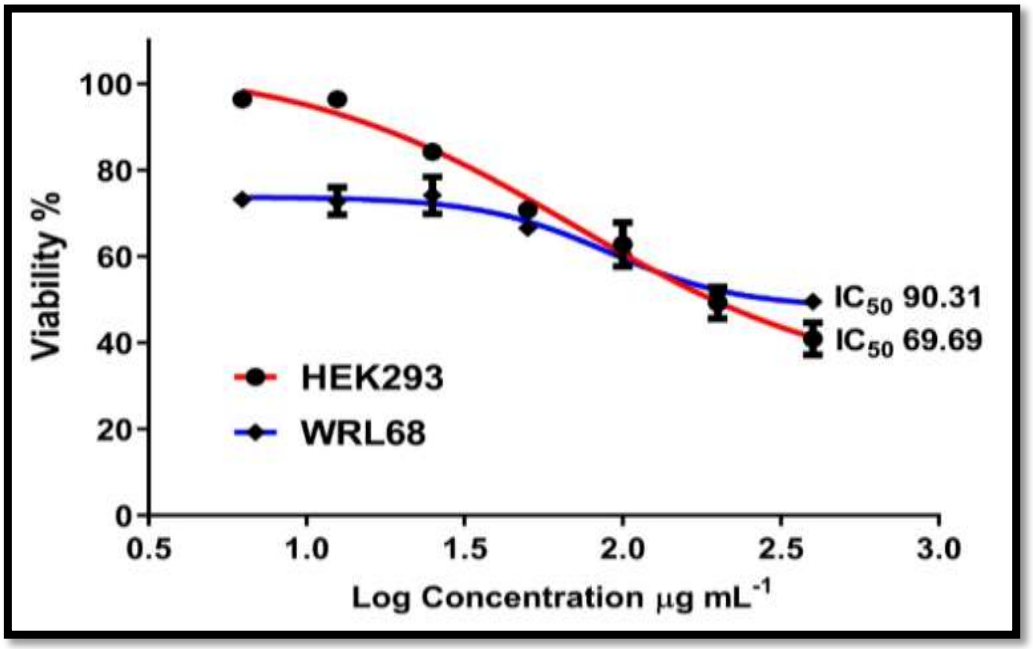


Figure 3: effect of compound (9A) on WRI-68 cell line normalization

Table 10: the cytotoxic effect of compound (9A) in concentrations on cell viability

Concentration $mg.ml^{-1}$	Cell count of HEK293 cell line Mean ± S.D.	Cell count of WRL68 cell line Mean ± S.D.
400	40.90 ± 3.74	49.50 ± 0.75
200	49.27 ± 3.65	51.47 ± 1.40
100	62.77 ± 5.13	60.57 ± 1.16
50	70.76 ± 1.01	66.51 ± 1.76
25	84.18 ± 0.84	74.15 ± 4.31
12.5	96.37 ± 1.77	72.84 ± 3.17
6.25	96.41 ± 0.93	73.19 ±0.41

Conclusion

New Mannich bases were synthesized using a well-known technique in this work. Because of their strong aromaticity, some of these novel bases showed good potential as an antioxidant activity and a high scavenging percentage against the DPPH free radical. Moreover, the applied procedure gets many of advantages such as high amount of yield, reactants and less time reaction. Other bases were also evaluated as cytotoxic agents and found to have a high cytotoxic effect with an IC50 value of 69.69 gm/ml. The antioxidant and anticancer effects of imidazo (2,1-b) thiazole derivatives were found to be in good agreement with other findings in the literature.

Acknowledgment

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