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SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY EVALUATION OF SOME NOVEL PYRAZOLE AND PYRROLE DERIVATIVES CONTAINING BENZOTHIAZOLE MOIETY

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Abstract – The 1-(2-benzothiazolyl)-1-cyano-3-chloroacetone (**1**) was used as a key intermediate for the synthesis of pyrazole derivatives (**3a-d**) and pyrrole derivatives (**5a-e**) by its reaction with arylhydrazonomalononitrile and primary arylamines, respectively. Moreover, the reaction of 2-(benzo[*d*]thiazol-2-yl)-5-methyl-4-(pyridin-4-ylmethylene)-2,4-dihydro-3*H*-pyrazol-3-one (**7**) with a variety of reagents have been investigated aiming to explore its synthetic potentialities in synthesis of some novel pyrazole fused heterocyclic derivatives containing benzothiazole moiety. The structures of newly synthesized compounds have been established on the basis of their IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. New compounds were tested for in *vitro* cytotoxicity against hepatocellular carcinoma (HepG-2) and breast cancer (MCF-7).

INTRODUCTION

The benzothiazole ring system is a core structure of a variety of natural and synthetic compounds with a broad range of biological activity[1–7] including anticancer activity.[8–13] During the past few years, several attempts have been made to modify their benzothiazole nucleus to improve their antitumor activity.

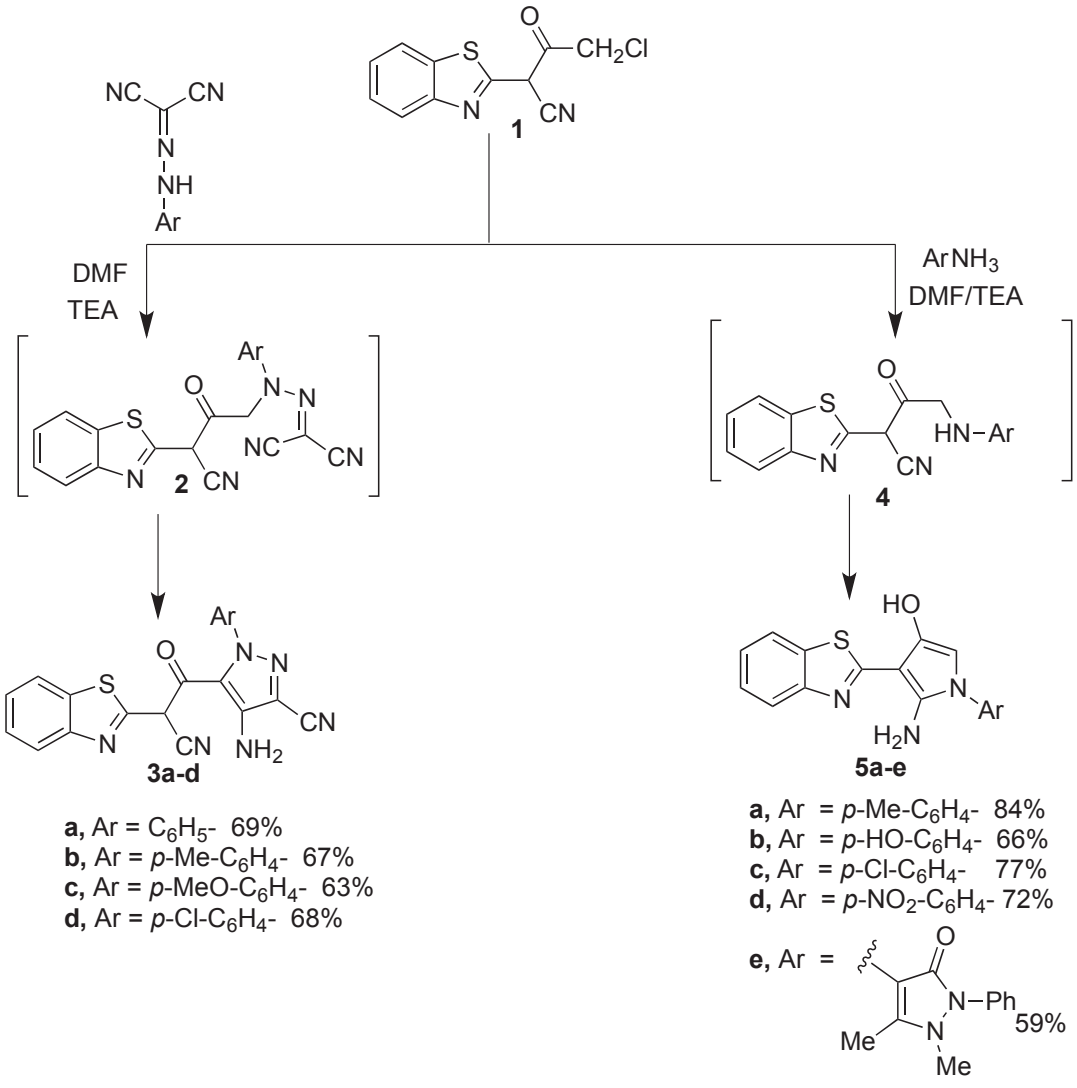
Such modification has resulted in identification of a variety of promising benzothiazole derivatives with remarkable anticancer activity against malignant cell lines. Pyrazole derivatives have also attracted the attention of organic chemists because of their biological and chemotherapeutic importance. They are known to have such biological activity as antitumor,[14] antileukemic,[15] anti-inflammatory,[16] analgesic,[17] anticoagulant,[18] and antimicrobial[19] activity. On the other hand, benzothiazole-pyrrole derivatives have been reported for cytotoxic activity.[20]

Stimulated by these observations, we report here the synthesis and cytotoxicity evaluation of some new pyrazole and pyrrole derivatives containing benzothiazole moiety.

RESULTS AND DISCUSSION

As a part of our continued interest on benzothiazole derivatives and synthesis of diverse heterocyclic compounds of biological significance,[21-26] we have investigated the possible utility of 1-(2-benzothiazolyl)-1- cyano-3-chloroacetone (**1**)[27] for the synthesis of some novel pyrazole and pyrrole derivatives containing benzothiazole moiety.

It has been found that the reaction of **1** with arylhydrazonomalononitrile in refluxing DMF containing a catalytic amount of TEA afforded 4-amino-1-aryl-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1*H*-pyrazole-3-carbonitrile (**3a-d**) **(Scheme 1)**.



Scheme 1

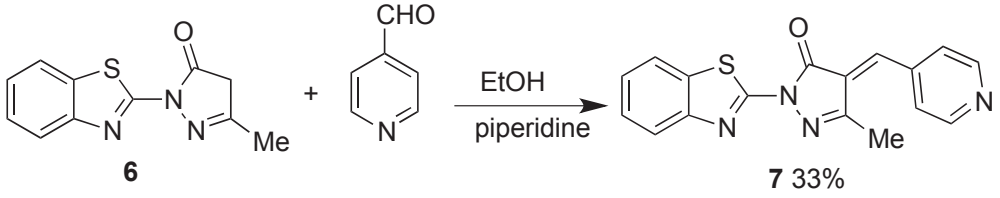
The structures of the products **3a-d** were indicated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and

elemental analyses. The IR spectra of compounds **3a-d** showed characteristic absorption bands in the region of 2198-2230 cm^{-1} corresponding to the stretching vibration of the two cyano groups. The high frequency region of the spectra showed two strong absorption bands at 3300-3400 cm^{-1} due to the stretching vibrations of the NH_2 group. The ^{1}H-NMR spectra of **3a-d** showed the presence of a singlet signal in the region of δ 4.68-4.72 ppm due to the CH proton and a singlet signal (D_2O exchangeable) in the region of δ 6.20-6.30 ppm due to an amino group.

In addition, the structures of compounds **3a-d** were confirmed by their mass spectroscopic measurement. The corresponding compounds 5-amino-1-aryl-4-(benzo[*d*]thiazol-2-yl)-1*H*-pyrrol-3-ol (**5a-e**) were synthesized by reacting **1** with appropriate primary arylamines in refluxing DMF containing a catalytic amount of TEA **(Scheme 1)**.

The structures of the compounds **5a-e** were ascertained from the elemental analyses and spectroscopic data. The IR spectra showed absence of any absorption peaks in the region of 2000-2250 cm^{-1}, thus clearly indicating the cyano moiety was involved in the cyclization reaction. The ^{1}H NMR spectra showed the presence of a singlet signal (D_2O exchangeable) in the region of δ 4.58-4.87 ppm due to NH_2 group, in addition to a singlet signal in the region of δ 8.85-10.10 ppm assignable to OH group. The mass spectra of compounds **5a-e** gave an additional evidence for proposed structures.

The incorporation of another heterocyclic moiety in pyrazole, as a fused component, changes its properties and converts it into an altogether new and important heterocyclic derivative. Thus, according to the previously reported method,[28] we synthesized the starting material 2-(benzo[*d*]thiazol-2-yl)-5-methyl-2,4-dihydro-3*H*-pyrazol-3-one (**6**) and we subjected it to a reaction with isonicotinaldehyde in refluxing ethanol catalyzed by piperidine to give 2-(benzo[*d*]thiazol-2-yl)-5-methyl-4-(pyridin-4-ylmethylene)-2,4-dihydro-3*H*-pyrazol-3-one (**7**) (**Scheme 2**).

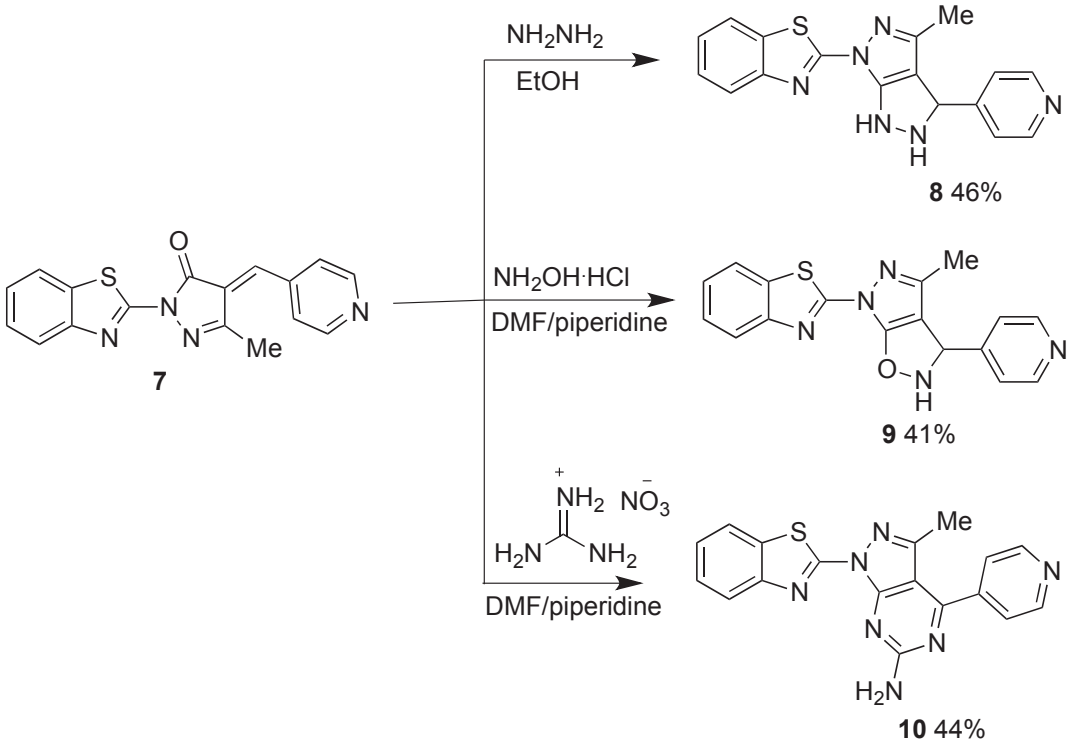


Scheme 2

The structure of the product **7** was indicated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analyses. Its ^{1}H-NMR spectrum revealed the presence of two singlet signals at δ 2.23 and 7.71 ppm assignable to methyl and vinylic proton, respectively. The mass spectrum showed the molecular ion peak at *m/z* 320 corresponding to the molecular formula of the proposed structure.

In this context, we used compound **7** as synthon for synthesis of some novel benzothiazole containing fused pyrazole heterocyclic compounds. Addition-condensation cyclization of the compound **7** with

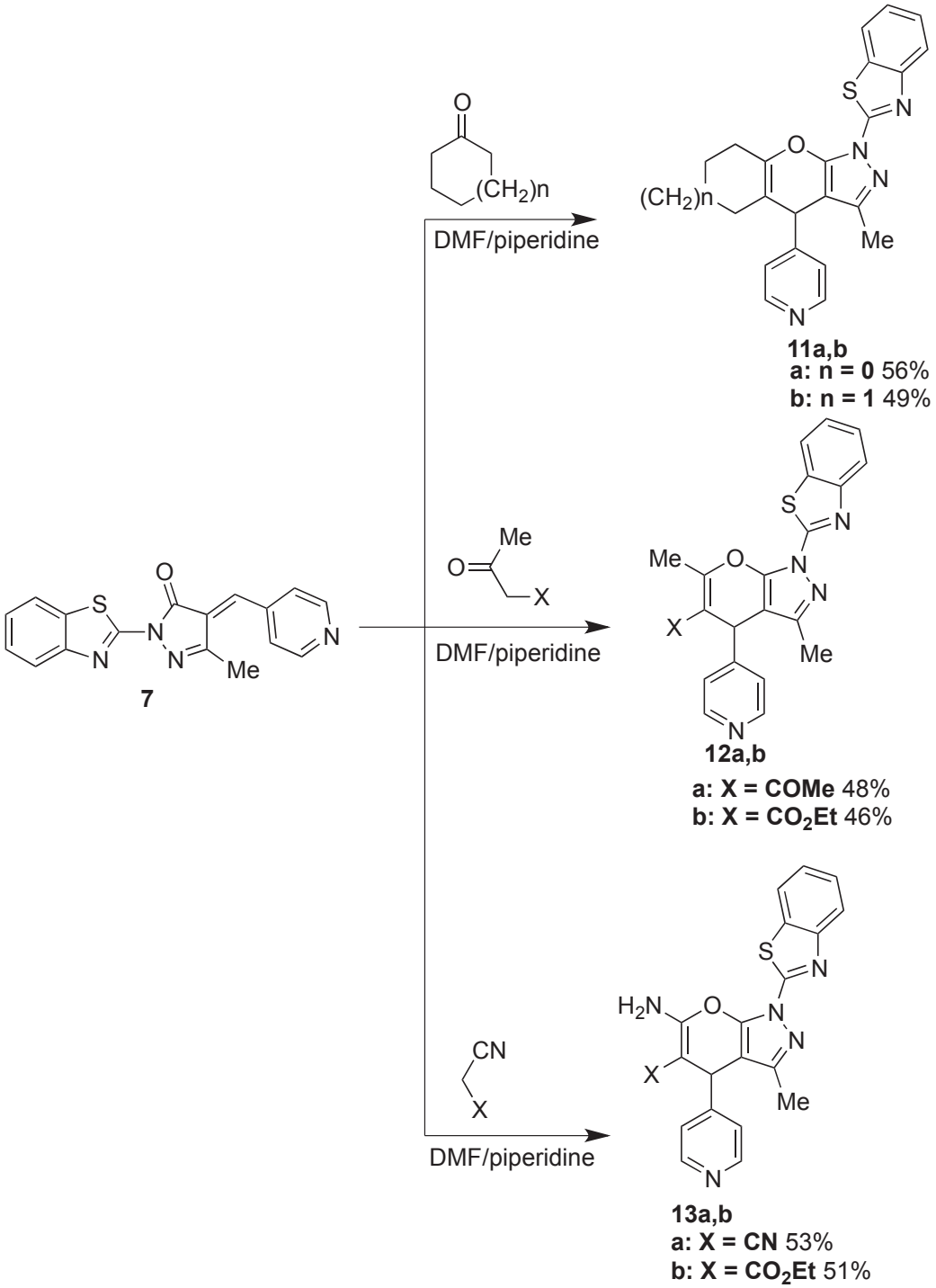
hydrazine hydrate and/or hydroxylamine hydrochloride, led to the formation of the five-membered rings pyrazolopyrazole **8** and pyrazoloisoxazole **9** derivatives, respectively (**Scheme 3**). On the other hand, pyrazolopyrimidine **10** was achieved *via* similar addition-condensation cyclization reaction of **7** with guanidine nitrate followed by auto-oxidation (**Scheme 3**). The assignment of structures **8**, **9** and **10** were supported by elemental analyses and spectral data. The IR spectra of **8**, **9** and **10** lacked any absorption bands of carbonyl group, which confirm that carbonyl group, was involved in the condensation cyclization reaction. In addition, ^{1}H NMR spectrum of **8** showed four singlet signals at δ 2.21, 4.48, 5.34 and 6.33 ppm assignable to Me, CH and two NH protons, respectively. On the other hand, ^{1}H NMR spectrum of **9** showed three singlet signals at δ 2.14, 5.44 and 6.49 ppm attributable to Me, CH and NH protons. The mass spectra of compounds **8, 9** and **10** gave additional evidences for the proposed structures.



Scheme 3

A considerable approach for the synthesis of novel benzothiazole containing pyrano[2,3-*c*]pyrazole moiety derivatives was fulfilled through interaction of compound **7** with some carbon nucleophiles by its reaction with certain active methylene compounds in the presences of piperidine catalyst (**Scheme 4**).

Formation of pyranopyrazole derivatives occur *via* Michael addition reaction of active methylene compounds to α,β-unsaturated ketones **7** followed by condensation cyclization reaction as in **11a,b** and **12a,b** or nucleophilic addition of enolic OH of pyrazolone carbonyl group to nitrile group as in **13a,b.**

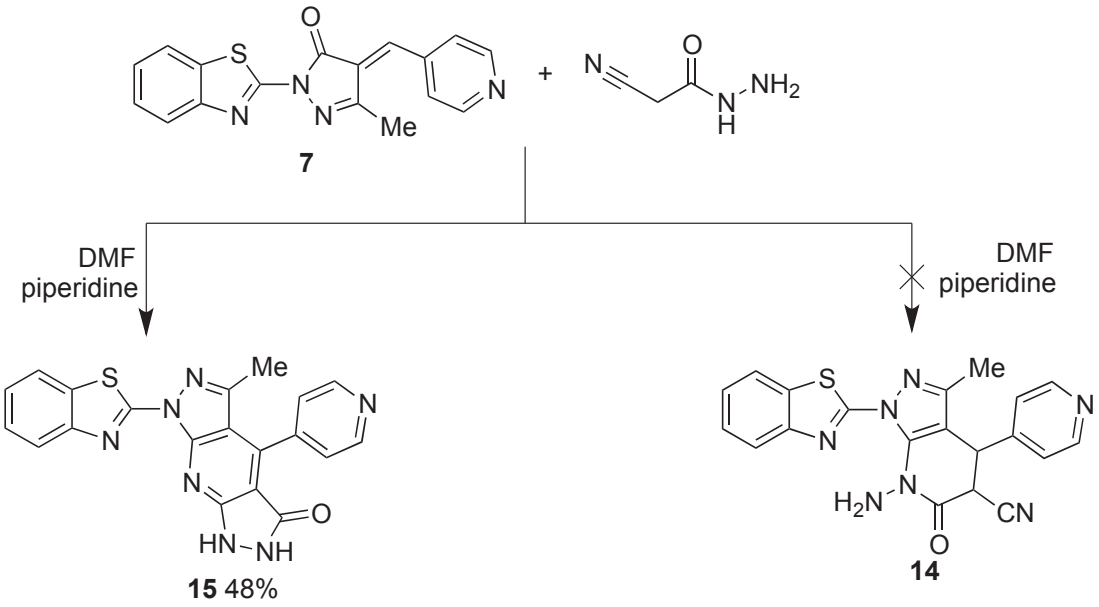


Scheme 4

Thus, the reaction of compound **7** with cyclopentanone yielded 1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-4,5,6,7-tetrahydro-1*H*-cyclopenta[5,6]pyrano[2,3-*c*]pyrazole (**11a**), while addition-condensation reaction of compound **7** with cyclohexanone afforded 1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4,5,6,7,8-hexahydrochromeno[2,3-*c*]pyrazole (**11b**). The structures of the products **11a,b** were indicated by IR, ^{1}H-NMR, ^{13}C-NMR, mass spectroscopy and elemental analyses. ^{1}H-NMR spectra

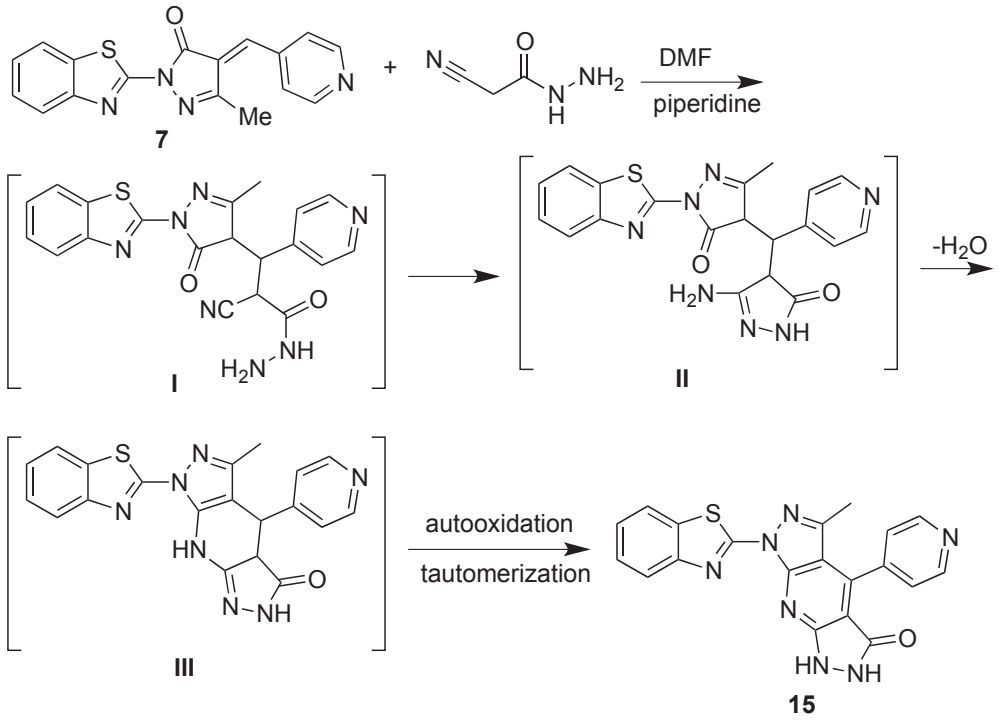
of **11a,b** exhibited a singlet signal in the region of δ 4.95-4.98 ppm due to C_4-H pyran. In addition, ^{1}H-NMR spectrum of **11a** showed two multiplet signals at δ 2.09 and 2.31-2.35 ppm due to three methylene groups of cyclopentane ring. In a similar manner, ^{1}H-NMR spectrum of **11b** showed two multiplet signals at δ 1.66-1.70 and 1.92-1.97 ppm assignable to four methylene groups in cyclohexane ring. In a similar way, the addition condensation reaction of compound **7** with acetylacetone yielded 1-(1-(benzo[*d*]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazol-5-yl)ethan-1-one (**12a**), while addition-condensation reaction of compound **7** with ethyl acetoacetate afforded ethyl 1-(benzo[*d*]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxylate (**12b**). The structures of compounds **12a** and **12b** were based on analytical and spectral data. On the other hand, reaction of **7** with malononitrile or ethyl cyanoacetate in refluxing DMF containing a catalytic amount of piperidine gave 6-amino-1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazole derivatives **(13a,b).** The structures of compounds **13a,b** were confirmed through IR, ^{1}H-NMR, ^{13}C-NMR spectral and elemental analyses. The IR spectra of **13a,b** exhibited characteristic absorption bands at 3360-3420 cm^{-1} , which indicated the presence of NH_2 group. ^{1}H NMR spectra of compounds **13a,b** displayed a singlet signal at δ 2.10-2.25 ppm for three protons of methyl group, it also exhibited a singlet signal (D_2O exchangeable) at δ 6.70-6.80 ppm due to NH_2 group. ^{1}H NMR spectrum of compound **13b** showed triplet-quartet pattern at 1.10 and 4.08 ppm attributable to ethoxy group. In addition, the structures of compounds **13a,b** were confirmed by their mass spectroscopic measurement.

Treatment of compound **7** with 2-cyanoacetohydrazide in refluxing DMF containing a catalytic amount of piperidine afforded 7-(benzo[*d*]thiazol-2-yl)-5-methyl-4-(pyridin-4-yl)-1,7-dihydrodipyrazolo[3,4-*b*:4',3'-*e*]pyridin-3(2*H*)-one (**15)** instead of the expected product 7-amino-1-(benzo[*d*]thiazol-2-yl)-3-methyl-6-oxo-4-(pyridin-4-yl)-4,5,6,7-tetrahydro-1*H*-pyrazolo[3,4-*b*]pyridine-5-carbonitrile (**14**) (**Scheme 5**). The structure of compound **14** was ruled out on the basis of spectral data. IR spectrum showed absence of any stretching bands in the region of 2000-2250 cm^{-1} due to nitrile group, in addition its ^{1}H NMR spectrum devoid of any signals in the region of δ 3.50-5.50 ppm due to two CH protons signals. The structure of compound **15** was confirmed by elemental analysis and spectral data. The IR spectrum gave two stretching frequencies at 3344 and 3259 cm^{-1} due to two NH groups. Its ^{1}H NMR spectrum showed three singlet signals at *δ* 2.09, 6.31 and 9.73 ppm assignable to methyl and two NH groups, respectively.



Scheme 5

The formation of compound **15** was assumed to proceed *via* Michael addition of the active methylene nitrile of 2- cyanoacetohydrazide to α,β-unsaturated ketone **7** to yield the corresponding intermediate **I**, which cyclized *via* nucleophilic addition of NH_2 group on the cyano moiety to produce the intermediate **II** . Then, the intermediate **II** underwent cyclodehydration followed by autoxidation to give final isolable product **15** (**Scheme 6**).



Scheme 6

CYTOTOXICITY ACTIVITY

All the newly synthesized compounds, were selected to evaluate for their *in-vitro* anticancer effect *via* the standard MTT method,[29] against two human tumor cell lines namely; hepatocellular carcinoma (liver) HepG-2 and mammary gland (breast) MCF-7. 5-Fluorouracil (5-Fu) was used as a standard anticancer drug for comparison. The results of cytotoxic activity are reported in **Table 1**.

TABLE 1. CYTOTOXIC ACTIVITY OF THE NEWLY SYNTHESIZED COMPOUNDS

COMPOUNDS	IN VITRO CYTOTOXICITY IC50 (µG/ML)	
	HepG-2	MCF-7
5-FU	7.53±22	4.05±0.15
3a	11.35±0.18	10.45±0.34
3b	8.34±0.14	9.81±0.35
3c	18.10±0.27	11.37±0.22
3d	6.31±0.05	5.30±0.07
5a	16.98±0.35	16.32±0.37
5b	90.60±1.36	83.67±1.11
5c	9.65±0.34	12.55±0.23
5d	39.71±0.50	47.67±0.91
5e	9.57±0.03	8.96±0.08
7	6.11±0.16	4.17±0.04
8	6.32±0.05	3.87±0.12
9	7.50±0.14	4.06±0.11
10	78.92±2.87	85.41±2.20
11a	23.41±0.95	19.84±0.79
11b	86.62±2.22	79.43±3.20
12a	20.12±0.84	15.99±0.67
12b	93.45±2.39	93.45±2.39
13a	48.54±2.35	58.65±2.34
13b	73.71±2.13	83.06±2.31
15	16.63±0.62	16.87±0.18

IC50 (µG/ML) : 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic), 5-fu= 5- fluorouracil.

The obtained results revealed that compound **8** is more potent and efficacious than 5-fluorouracil as reference drug towards the two tested human tumor cell lines. As for the activity against hepatocellular

carcinoma HepG-2, compounds **3d**, **7** and **9** displayed the highest cytotoxic activity even more than the reference drug. On the other hand, mammary gland (breast) MCF-7 cell line showed highest sensitivity towards five of the tested compounds. Compounds **3d**, **5e**, **7**, **8** and **9** demonstrated the best activity. Further interpretation of the results revealed that compounds **7, 8** and **9** showed high anticancer activity against both two tested human tumor cell lines.

STRUCTURE ACTIVITY RELATIONSHIP

By comparing the experimental cytotoxicity of the compounds reported in this study to their structures, the following structure activity relationships (SAR) were postulated.

- Based on the data obtained, the linking pyrazole derivatives to benzothiazole **3b, 3d, 7, 8** and **9** showed higher cytotoxicity activity towards two line cells than obtained by pyrrole derivatives linked benzothiazole **5a-e**.
- Both compounds **5e** and **7** showed very strong cytotoxicity activity towards the two tested human tumor cell lines. This activity may be attributed to the presence of the electron withdrawing carbonyl group in pyrazole ring, which may enhance the reactivity of pyrazole compounds
- Compounds **3d** and **5c** gave very strong cytotoxicity activity towards HepG-2 that may be attributed to the presence of chlorine atom.
- Significant activities against two cell lines were noted with the attachment of pyrazolopyrazole or pyrazoloisoxazole derivatives to benzothiazole nucleus as in compounds **8** and **9.**
- Presence of pyranopyrazole or pyrazolopyrimidine moiety in benzothiazole nucleus diminished the activity against two cell lines.

CONCLUSION

The objective of the present study was to synthesize and investigate the anticancer activity of some novel pyrazole or pyrrole derivatives bearing benzothiazole moiety with the hope of discovering new structures lead serving as anticancer agents. The results of the anticancer screening revealed that compound **8** exhibited the highest *in vitro* cytotoxic activity towards two different cell lines when compared with the other tested compounds and 5-fluorouracil as a reference drug. In addition, compounds **3d**, **7** and **9** displayed the highest cytotoxic activity against the human HepG-2 compared with reference drug.

EXPERIMENTAL

Melting points were recorded on Gallenkamp electric melting point apparatus (Electronic Melting Point Apparatus, Great Britain, London) and are uncorrected. Precoated Merck silica gel 60F-254 plates were used for thin-layer chromatography (TLC) and the spots were detected under UV light (254 nm). The

infrared spectra were obtained from potassium bromide triturate containing 0.5% of the product on Pye Unicam SP 1000 IR spectrophotometer (Thermoelectron Co. Egelsbach, Germany. The ^{1}H-NMR spectra were determined on Varian Gemini 300 MHz (Varian Co., Cairo university, Egypt), ^{13}C-NMR = 75 MHz. Deuterated DMSO-d_6 was used as a solvent, tetramethylsilane (TMS) was used as an internal standard and chemical shifts were measured in δ ppm. Mass spectra were determined on a GC-MS.QP-100 EX Shimadzu (Japan). Elemental analyses were recorded on Perkin-Elmer 2400 Elemental analyzer at the Micro-analytical Center at Cairo University, Cairo, Egypt.

Synthesis of 4-amino-1-aryl-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1*H*-pyrazole-3-carbonitrile derivatives (3a-d). A mixture of **1** (2.5 g, 0.01 mol) and appropriate arylhydrazonomalononitrile (0.01 mol) was refluxed in DMF containing a catalytic amount of TEA (four drops) for 8-12 h (monitored by TLC). The reaction mixture was left to cool at room temperature and poured onto ice cold water (100 mL). The solid product was collected by filtration and recrystallized from DMF-EtOH to give **3a-d**.

4-Amino-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1-phenyl-1*H*-pyrazole-3-carbonitrile (3a). Yellow solid; yield (69%); mp 200 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3356, 3308 (NH_2), 2223, 2205 (two CN), 1662 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 4.71 (s, 1H, CH), 6.22 (s, 2H, NH_2), 7.45-7.70 (m, 7H, Ar-H), 8.01-8.06 (m, 2H, Ar-H); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 46.2, 115.1, 115.9, 116.4, 119.2, 120.8, 121.4, 122.3, 123.9, 125.1, 126.8 (2C), 127.1, 129.4 (2C), 134.5, 138.2, 153.8, 166.7, 192.1; MS (EI, 70 eV) *m/z* 384 (M^+). Anal. Calcd for $C_{20}H_{12}N_6OS$ (384.42): C, 62.49; H, 3.15; N, 21.86; S, 8.34. Found: C, 62.52; H, 3.11; N, 21.85; S, 8.31.

4-Amino-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1-(*p*-tolyl)-1*H*-pyrazole-3-carbonitrile (3b). Yellow solid; yield (67%); mp 183 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3382, 3351 (NH_2), 2228, 2211 (two CN), 1662 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.39 (s, 3H, CH_3), 4.70 (s, 1H, CH), 6.20 (s, 2H, NH_2), 7.19 (d, J = 6.9 Hz, 2H, Ar-H), 7.50-7.58 (m, 4H, Ar-H), 7.99-8.08 (m, 2H, Ar-H); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 23.2, 46.0, 115.2, 115.7, 116.8, 119.2, 121.4, 122.5, 124.2, 125.0, 126.1 (2C), 127.4, 128.8 (2C), 135.3, 136.4, 138.2, 153.7, 166.5, 192.5; MS (EI, 70 eV) *m/z* 398 (M^+). Anal. Calcd for $C_{21}H_{14}N_6OS$ (398.44): C, 63.30; H, 3.54; N, 21.09; S, 8.05. Found: C, 63.35; H, 3.57; N, 21.11; S, 8.09**.**

4-Amino-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1-(*p*-anisyl)-1*H*-pyrazole-3-carbonitrile (3c). Yellow solid; yield (63%); mp 229 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3358, 3329 (NH_2), 2203, 2198 (two CN), 1660 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 3.87 (s, 3H, CH_3), 4.68 (s, 1H, CH), 6.24 (s, 2H, NH_2), 7.03 (d, 2H, J = 7.1 Hz, CH-Ar), 7.45-7.65 (m, 4H, Ar-H), 7.98-8.08 (m, 2H, Ar-H); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 45.9, 61.2, 113.1 (2C), 115.1 (2C), 115.3, 115.8, 116.4, 118.9, 121.0, 121.4, 124.2, 125.4, 126.2, 133.7, 138.1,153.1, 158.1, 166.8, 192.4; MS (EI, 70 eV) *m/z* 414 (M^+).

Anal. Calcd for $C_{21}H_{14}N_6O_2S$ (414.44): C, 60.86; H, 3.41; N, 20.28; S, 7.74. Found: C, 60.84; H, 3.44; N, 20.30; S, 7.75**.**

4-Amino-5-(2-(benzo[*d*]thiazol-2-yl)-2-cyanoacetyl)-1-(4-chlorophenyl)-1*H*-pyrazole-3-carbonitrile (3d). Yellow solid; yield (68%); mp 217 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3334, 3309 (NH_2), 2221, 2001 (two CN), 1664 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 4.70 (s, 1H, CH), 6.28 (s, 2H, NH_2), 7.42-7.58 (m, 6H, Ar-H), 8.02-8.10 (m, 2H, Ar-H); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 46.1, 115.2, 115.9, 116.3, 118.2, 119.4 (2C), 121.1, 121.4, 124.2, 125.2, 125.9, 130.2 (2C), 130.9, 133.7, 138.2, 153.1, 166.5, 192.3; MS (EI, 70 eV) *m/z* 420 (M^++ 2), 418 (M^+). Anal. Calcd for $C_{20}H_{11}ClN_6OS$ (418.86): C, 57.35; H, 2.65; Cl, 8.46; N, 20.06; S, 7.65. Found: C, 57.35; H, 2.65; Cl, 8.46; N, 20.06; S, 7.65.

Synthesis of 5-amino-1-aryl-4-(benzo[*d*]thiazol-2-yl)-1*H*-pyrrol-3-ol derivatives (5a-e). A mixture of **1** (2.5 g, 0.01 mol) and appropriate aromatic amines (0.01 mol) was refluxed in DMF containing a catalytic amount of TEA for 5-7 h (monitored by TLC). The reaction mixture was left to cool at room temperature and poured onto ice cold water (100 mL). The solid product was collected by filtration and recrystallized from DMF-EtOH to give **5a-e.**

5-Amino-4-(benzo[*d*]thiazol-2-yl)-1-(*p*-tolyl)-1*H*-pyrrol-3-ol (5a). Yellow solid; yield (84%); mp 245 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3412 (OH), 3375, 3346 (NH_2); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm); 2.32 (s, 3H, CH_3), 4.59 (s, 2H, NH_2), 7.11 (d, J = 7.4 Hz, 2H, Ar-H), 7.40-7.58 (m, 5H, Ar-H), 7.97-8.07 (m, 2H, Ar-H), 8.85 (s, 1H, OH); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 20.9, 105.2, 111.3, 120.9, 121.2, 122.4, 122.9 (2C), 124.7, 125.9, 128.4 (2C), 133.5, 134.8, 136.3, 139.7, 151.1, 154.2; MS (EI, 70 eV) *m/z* 321 (M^+). Anal. Calcd for $C_{18}H_{15}N_3OS$ (321.40): C, 67.27; H, 4.70; N, 13.07; S, 9.98. Found: C, 67.25; H, 4.71; N, 13.10; S, 9.99.

5-Amino-4-(benzo[*d*]thiazol-2-yl)-1-(4-hydroxyphenyl)-1*H*-pyrrol-3-ol (5b). Yellow solid; yield (66%); mp > 300 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3433, 3408 (2 OH), 3366, 3349 (NH_2); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 4.58 (s, 2H, NH_2), 7.06 (d, J = 7.4 Hz, 2H, Ar-H), 7.44-7.58 (m, 5H, Ar-H), 7.97-8.07 (m, 2H, Ar-H), 8.92 (s, 1H, OH), 10.02 (s, 1H, OH); ^{13}C- NMR (75 MHz, DMSO-d_6) δ (ppm): 105.1, 111.3, 117.4 (2C), 120.9, 121.1, 122.4, 124.6, 125.9, 127.3 (2C), 133.4, 134.8, 135.3, 151.2, 154.2, 156.7; MS (EI, 70 eV) *m/z* 323 (M^+). Anal. Calcd for $C_{17}H_{13}N_3O_2S$ (323.37): C, 63.14; H, 4.05; N, 12.99; S, 9.91. Found: C, 63.11; H, 4.07; N, 13.02; S, 9.90.

5-Amino-4-(benzo[*d*]thiazol-2-yl)-1-(4-chlorophenyl)-1*H*-pyrrol-3-ol (5c).Yellow solid; yield (77%); mp 278 °C (EtOH-DMF); IR (KBr): ν/cm^{-1} = 3425 (OH), 3383, 3342 (NH_2); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 4.62 (s, 2H, NH_2), 7.40-7.58 (m, 7H, Ar-H), 7.99-8.07 (m, 2H, Ar-H), 8.88 (s, 1H, OH); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 105.2, 111.4, 120.9, 121.0 (2C), 121.2, 122.3, 124.7,

126.0, 129.6 (2C), 131.8, 133.5, 134.8, 141.5, 151.1, 154.3; MS (EI, 70 eV) *m/z* 343 (M^++2), 342 (M^++1), 341 (M^+). Anal. Calcd for $C_{17}H_{12}ClN_3OS$ (341): C, 59.74; H, 3.54; Cl, 10.37; N, 12.29; S, 9.38. Found: C, 59.71; H, 3.55; Cl, 10.41; N, 12.30; S, 9.36.

5-Amino-4-(benzo[*d*]thiazol-2-yl)-1-(4-nitrophenyl)-1*H*-pyrrol-3-ol (5d). Pale red solid; yield (72%); mp 215 °C (EtOH-DMF); IR (KBr): *v*/cm^{-1} = 3427 (OH), 3366, 3352 (NH_2); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 4.67 (s, 2H, NH_2), 7.47-7.75 (m, 5H, Ar-H), 8.02-8.08 (m, 2H, Ar-H), 8.25 (d, *J* = 7.9 Hz, 2H, CH-Ar), 9.12 (s, 1H, OH); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 105.4, 111.3, 121.1, 121.4, 121.8 (2C), 122.5, 123.9 (2C), 124.7, 126.0, 133.5, 134.9, 145.6, 148.9, 151.3, 154.5; MS (EI, 70 eV) *m/z* 352 (M^+). Anal. Calcd for $C_{17}H_{12}N_4O_3S$ (352.37): C, 57.95; H, 3.43; N, 15.90; S, 9.10. Found: C, 57.97; H, 3.40; N, 15.91; S, 9.13.

4-(2-Amino-3-(benzo[*d*]thiazol-2-yl)-4-hydroxy-1*H*-pyrrol-1-yl)-1,5-dimethyl-2-phenyl-1,2-dihydro-3*H*-pyrazol-3-one (5e). Yellow solid; yield (59%); mp > 300 °C (EtOH-DMF); IR (KBr): *v*/cm^{-1} = 3399 (OH), 3354, 3339 (NH_2), 1654 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 3.29 (s, 3H, CH_3), 4.87 (s, 2H, NH_2), 7.30-7.60 (m, 8H, Ar-H), 8.00-8.08 (m, 2H, Ar-H), 8.92 (s, 1H, OH); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.2, 36.1, 103.4, 104.1, 108.2, 119.5, 121.2, 122.2, 122.7, 123.8 (2C), 125.2, 125.9, 130.4 (2C), 133.6, 134.1, 134.7, 137.5, 151.7, 154.9, 164.2; MS (EI, 70 eV) *m/z* 417 (M^+). Anal. Calcd for $C_{22}H_{19}N_5O_2S$ (417): C, 63.29; H, 4.59; N, 16.78; S, 7.68. Found: C, 63.30; H, 4.62; N, 16.78; S, 7.66.

Synthesis of 2-(benzo[*d*]thiazol-2-yl)-5-methyl-4-(pyridin-4-ylmethylene)-2,4-dihydro-3*H*-pyrazol-3-one (7). To a solution of compound **6** (2.31 g, 0.01 mol) in EtOH (20 mL), isonicotinaldehyde (0.95 mL, 0.01 mol) and a catalytic amount of piperidine were added. The reaction mixture was heated under reflux for 4 h (TLC controlled), then the reaction mixture was then cooled and allowed to stand overnight. The solids thus separated were filtered, washed with cold EtOH, dried, and recrystallized from DMF-EtOH to give compound **7.** Brown solid; yield (33%); mp 244℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 1652 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 7.48-7.56 (m, 4H, Ar-H), 7.71 (s, 1H, CH), 7.98-8.07 (m, 2H, Ar-H), 8.51 (d, *J* = 8.1 Hz, 2H, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 18.2, 118.3, 118.9, 121.8, 122.2 (2C), 123.9, 130.8, 132.8, 124.9, 144.6, 147.8, 149.9 (2C), 153.2, 165.5, 174.5; MS (EI, 70 eV) *m/z* 320 (M^+). Anal. Calcd for $C_{17}H_{12}N_4OS$ (320.37): C, 63.73; H, 3.78; N, 17.49; S, 10.01 %. Found: C, 63.62; H, 3.74; N, 17.43; S, 10.09.

Synthesis of 2-(3-methyl-4-(pyridin-4-yl)-5,6-dihydropyrazolo[3,4-*c*]pyrazol-1(4*H*)-yl)-benzo[*d*]thiazole (8). A mixture of compound **7** (3.20 g, 0.01 mol) and hydrazine hydrate (0.49 mL, 0.01 mol) was refluxed in absolute EtOH (20 mL) for 6 h (TLC controlled). The reaction mixture was filtered

off and recrystallized from DMF- EtOH to give compound **8**. Brown solid; yield (46%); mp 223-225 ℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 3317, 3212 (two NH); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.21 (s, 3H, CH_3), 4.48 (s, 1H, CH), 5.34 (s, 1H, NH, D_2O exchangeable), 6.33 (s, 1H, NH,D_2O exchangeable), 7.48-7.56 (m, 4H, Ar-H), 8.01-8.07 (m, 2H, Ar-H), 8.50 (d, *J* = 7.8 Hz , 2H, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 16.8, 67.3, 117.8, 119.8, 122.6 (2C), 124.1, 124.7, 125.8, 132.9, 138.4, 139.1, 150.4 (2C), 151.2, 151.9, 159.9; MS (EI, 70 eV) *m/z* 334 (M^+). Anal. Calcd for $C_{17}H_{14}N_6S$ (334.40) : C, 61.06; H, 4.22; N, 25.13 ; S, 9.59. Found: C, 61.01; H, 4.25; N, 25.18; S, 9.51.

Synthesis of 6-(benzo[*d*]thiazol-2-yl)-4-methyl-3-(pyridin-4-yl)-3,6-dihydro-2*H*-pyrazolo[4,3-*d*]-isoxazole (9). A mixture of compound **7** (3.20 g, 0.01 mol) and hydroxylamine hydrochloride (0.69 g, 0.01 mol) in DMF (15 mL) containing a catalytic amount of piperidine was refluxed for 8 h. The reaction mixture was left to cool and poured into ice cold water. The precipitated solid was filtered off, dried and recrystallized from DMF- EtOH to give compound **9**. Dark red solid; yield (41%); mp 288℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 3299 (NH); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.14 (s, 3H, CH_3), 5.44 (s, 1H, C_3-H isoxazole), 6.49 (s, 1H, NH,D_2O exchangeable), 7.48-7.56 (m, 4H, Ar-H), 7.99-8.07 (m, 2H, Ar-H), 8.59 (d, *J* = 7.8 Hz, 2H, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 16.1, 67.5, 118.5, 120.7, 122.4 (2C), 124.3, 124.7, 126.2, 133.0, 138.7, 139.0, 150.5 (2C), 151.7, 152.6, 160.8; MS (EI, 70 eV) *m/z* 335 (M^+). Anal. Calcd for $C_{17}H_{13}N_5OS$ (335.39) : C, 60.88; H, 3.91; N, 20.88; S, 9.56. Found: C, 60.80; H, 3.85; N, 20.91; S, 9.51.

Synthesis of 1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1*H*-pyrazolo[3,4-*d*]pyrimidin-6-amine (10). A mixture of compound **7** (3.20 g, 0.01mol) and guanidine nitrate (1.22 g, 0.01 mol) in DMF (20 mL) containing a catalytic amount of piperidine was refluxed for 6 h. The reaction mixture was left to cool and poured into ice cold water. The precipitated solid was filtered off, dried and recrystallized from DMF- EtOH to give compound **10**.

10: Red solid; yield (44%); mp 297℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 3442, 3420 (NH_2); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 2.20 (s, 3H, CH_3), 6.67 (s, 2H, NH_2 , D_2O exchangeable), 7.49-7.55 (m, 4H, Ar-H), 7.99-8.08 (m, 2H, Ar-H), 8.54 (d, 2H, *J* =7.8 Hz, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.4, 114.5, 120.8, 121.7, 123.6 (2C), 124.0, 124.4, 131.6, 139.5, 142.2, 151.3 (2C), 152.0, 158.1, 161.2, 163.4, 165.2; MS (EI, 70 eV) *m/z* 359 (M^+). Anal. Calcd for $C_{18}H_{13}N_7S$ (359.41) : C, 60.15; H, 3.65; N, 27.28; S, 8.92. Found: C, 60.07; H, 3.72; N, 27.22; S, 8.85.

Synthesis of 1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-pyrano[2,3-*c*]pyrazole derivatives

11a,b. To a mixture of compound **7** (3.2 g, 0.01 mol) and cyclopentanone (0.9 mL, 0.01 mol) or cyclohexanone (1 mL, 0.01 mol), in DMF (20 mL), a catalytic amount of piperidine (0.4 mL) was added and the reaction mixture was heated under reflux for 9-10 h. Afterwards, the reaction mixture was left to cool and acidified using dilute HCl until complete precipitation. The precipitate so obtained was filtered off, washed thoroughly with cold EtOH (10 mL) and crystallized from EtOH-DMF.

1-(Benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-4,5,6,7-tetrahydro-1*H*-cyclopenta[5,6]pyrano[2,3-*c*]pyrazole (11a). Yellowish solid; yield (56%); mp 293℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 2899-2994 (CH-Aliphatic); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.09 (m, 2H, CH_2-6), 2.10 (s, 3H, CH_3), 2.31-2.35 (m, 4H, 2 CH_2), 4.95 (s, 1H, C_4-H pyran), 7.48-7.55 (m, 4H, Ar-H), 7.98-8.07 (m, 2H, Ar-H), 8.62 (d, 2H, *J* = 7.8 Hz C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.6, 19.2, 27.2, 31.5, 44.3, 111.4, 117.2, 119.3, 120.4, 122.2, 122.8, 124.1, 124.3 (2C), 126.1, 131.8, 145.9, 151.3 (2C), 153.4, 161.1, 162.3; MS (EI, 70 eV) *m/z* 386 (M^+). Anal. Calcd for $C_{22}H_{18}N_4OS$ (386.47): C, 68.37; H, 4.69; N, 14.50; S, 8.30. Found: C, 68.31; H, 4.65; N, 14.53; S, 8.34.

1-(Benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4,5,6,7,8-hexahydrochromeno[2,3-*c*]pyrazole (11b). Pale brown solid; yield (49%); mp 244℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 2857-2998 (CH-Aliphatic); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 1.66-1.70 (m, 4H, CH_2-6, CH_2-7), 1.92-1.97 (m, 4H, CH_2-5, CH_2-8), 2.12 (s, 3H, CH_3), 4.98 (s, 1H, C_4-H pyran), 7.50-7.56 (m, 4H, Ar-H), 7.98-8.07 (m, 2H, Ar-H), 8.62 (d, 2H, *J*= 7.7 Hz, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.6, 21.7, 22.5, 23.9, 27.6, 43.1, 111.9, 117.2, 120.8, 121.4, 123.2, 124.0, 124.3 (2C), 131.8, 140.8, 145.9, 151.3 (2C), 152.6, 153.4, 160.9, 162.1; MS (EI, 70 eV) *m/z* 400 (M^+). Anal. Calcd for $C_{23}H_{20}N_4OS$ (400.50) : C, 68.98; H, 5.03; N, 13.99; S, 8.00. Found: C, 68.91; H, 5.06; N, 13.94; S, 8.04.

Synthesis of 1-(benzo[*d*]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]-pyrazole derivatives 12a,b. To a mixture of compound **7** (3.2 g, 0.01 mol) and acetylacetone (1 mL, 0.01 mol), or ethyl acetoacetate (1.3 mL, 0.01 mol) in DMF (20 mL), a catalytic amount of piperidine (0.4 mL) was added and the reaction mixture was heated under reflux for 6-7 h. Afterwards, the reaction mixture was left to cool and acidified using dilute HCl until complete precipitation. The precipitate so obtained was filtered off, washed thoroughly with cold EtOH (10 mL) and crystallized from EtOH-DMF.

1-(1-(Benzo[*d*]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazol-5-yl)-ethan-1-one (12a). Dark brown solid; yield (48%); mp 240-242 ℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 1663 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.12 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.37 (s, 3H, CH_3), 5.06 (s, 1H, C_4-H pyran), 7.49-7.55 (m, 4H, Ar-H), 7.99-8.07 (m, 2H, Ar-H), 8.66 (d, 2H, *J*= 7.8

Hz, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.2, 14.8, 28.1, 39.3, 115.5, 117.9, 120.8, 121.4, 123.3, 124.0, 124.3 (2C), 131.5, 140.8, 145.7, 151.4 (2C), 152.6, 153.4, 161.1, 161.9, 189.8; MS (EI, 70 eV) *m/z* 402 (M^+). Anal. Calcd for $C_{22}H_{18}N_4O_2S$ (402.47): C, 65.65; H, 4.51; N, 13.92; S, 7.97. Found: C, 65.58; H, 4.54; N, 13.97; S, 7.91.

Ethyl 1-(benzo[*d*]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carboxylate (12b). Pale red solid; yield (46%); mp 249℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 1709 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 1.06 (t, 3H, CH_3), 1.98 (s, 3H, CH_3), 2.34 (s, 3H,), 3.98 (q, 2H, OCH_2), 4.93 (s, 1H, C_4-H pyran), 7.51-7.55 (m, 4H, Ar-H), 8.02-8.09 (m, 2H, Ar-H), 8.67 (d, *J*= 7.8 Hz, 2H, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.1, 14.8, 15.6, 39.3, 63.4, 105.8, 117.8, 120.8, 121.4, 123.3, 124.1, 124.4 (2C), 130.9, 141.1, 145.7, 151.4 (2C), 151.9, 153.4, 160.8, 161.4, 166.7; MS (EI, 70 eV) *m/z* 432 (M^+). Anal. Calcd for $C_{23}H_{20}N_4O_3S$ (432.50) : C, 63.87; H, 4.66; N, 12.95; S, 7.41. Found: C, 63.80; H, 4.59; N, 1303; S, 7.49.

Synthesis of 6-amino-1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]-pyrazole derivatives 13a,b. A mixture of equimolar amounts (0.01 mol) of compound **7** (3.20 g) and malononitrile (0.66 g), or ethyl cyanoacetate (1 mL) in DMF (20 mL), was treated with catalytic amount of piperidine and the reaction mixture was heated under reflux for 8 h (TLC controlled). The reaction mixture was left to cool at room temperature, and poured onto ice cold water (100 mL). The solid product was collected by filtration and recrystallized from DMF-EtOH to give **13a,b.**

6-Amino-1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]pyrazole-5-carbonitrile (13a). Brown solid; yield (53%); mp 214-215 ℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 3417, 3378 (NH_2), 2197 (CN); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.23 (s, 3H, CH_3), 5.09 (s, 1H, C_4-H pyran), 6.74 (s, 2H, NH_2, D_2O exchangeable), 7.49-7.55 (m, 4H, Ar-H), 8.02-8.08 (m, 2H, Ar-H), 8.65 (d, *J* = 7.8 Hz, 2H, C_2-H, C_6-H pyridine); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 14.1, 34.2, 60.3, 115.5, 118.2, 120.9, 121.7, 123.0, 124.2, 124.4 (2C), 133.1, 139.6, 145.8, 151.1 (2C), 151.9, 160.9, 161.3, 174.2; MS (EI, 70 eV) *m/z* 386 (M^+). Anal. Calcd for $C_{20}H_{14}N_6OS$ (386.43): C, 62.16; H, 3.65; N, 21.75; S, 8.30. Found: C, 62.11; H, 3.60; N, 21.79; S, 8.33.

Ethyl 6-amino-1-(benzo[*d*]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-*c*]-pyrazole-5-carboxylate (13b). Brown solid; yield (51%); mp 229℃ (EtOH-DMF); IR (KBr): *v*/ cm^{-1} = 3404, 3369 (NH_2), 1698 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 1.10 (t, 3H, CH_3), 2.12 (s, 3H, CH_3), 4.08 (q, 2H, OCH_2), 4.96 (s, 1H, C_4-H pyran), 6.79 (s, 2H, NH_2, D_2O exchangeable), 7.49-7.54 (m, 4H, Ar-H), 8.00-8.06 (m, 2H, Ar-H), 8.67 (d, *J*= 7.8 Hz, 2H, C_2-H, C_6-H pyridine); ^{13}C-NMR (75 MHz , DMSO-d_6) δ (ppm): 14.1, 14.7, 39.5, 63.2, 80.1, 116.4, 120.8, 121.4, 123.1, 124.0, 124.2 (2C), 131.4, 141.0, 145.7, 151.3 (2C), 151.9, 153.2, 161.1, 161.5, 167.4; MS (EI, 70 eV) *m/z* 433 (.M^+).

Anal. Calcd for $C_{22}H_{19}N_5O_3S$ (433.49) : C, 60.96; H, 4.42; N, 16.16; S, 7.40. Found: C, 60.88; H, 4.49; N, 16.09; S, 7.49.

Synthesis of 7-(benzo[*d*]thiazol-2-yl)-5-methyl-4-(pyridin-4-yl)-1,7-dihydrodipyrazolo[3,4-*b*:4',3'-*e*]-pyridin-3(2*H*)-one (15). A mixture of compound **7** (3.20 g, 0.01mol) and cyanoacetohydrazide (0.99 g, 0.01mol) in DMF (20 mL) containing a catalytic amount of piperidine was refluxed for 12 h. The reaction mixture was allowed to cool and poured into ice cold water. The precipitated solid obtained was filtered off, dried and recrystallized from EtOH-DMF to furnish **15.** Pink solid; Yield (48%); mp > 300℃ (EtOH-DMF); IR (KBr): ν/ cm^{-1} = 3344, 3259 (two NH), 1643 (C=O); ^{1}H-NMR (300 MHz , DMSO-d_6) δ (ppm): 2.09 (s, 3H, CH_3), 6.31 (s, 1H, NH pyrazole, D_2O exchangeable), 7.51-7.57 (m, 4H, Ar-H), 7.99-8.06 (m, 2H, Ar-H), 8.51 (d, J= 7.8 Hz, 2H, C_2-H, C_6-H pyridine), 9.73 (s, 1H, NHCO, D_2O exchangeable); ^{13}C- NMR (75 MHz , DMSO-d_6) δ (ppm): 13.9, 108.6, 112.1, 120.9, 121.6, 123.1, 123.8, 124.3 (2C), 131.6, 139.5, 145.2, 149.8, 150.7, 151.3 (2C), 157.4, 159.9, 165.1, 169.2; MS (EI, 70 eV) *m/z* 399 (M^+). Anal. Calcd for $C_{20}H_{13}N_7OS$ (399.43): C, 60.14; H, 3.28; N, 24.55; S, 8.03. Found: C, 60.18; H, 3.31; N, 24.52; S, 8.06.

ANTITUMOR EVALUATION

Hepatocellular carcinoma (HepG-2) and mammary gland breast cancer(MCF-7) were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil was used as a standard anticancer drug for comparison.

The cell lines mentioned above were used to determine the inhibitory effects of compounds on cell growth using the MTT assay. This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to a purple formazan derivative by mitochondrial succinate dehydrogenase in viable cells. Cell lines were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/mL penicillin and 100 µg/mL streptomycin at 37 C in a 5% CO_2 incubator. The cell lines were seeded in a 96-well plate at a density of 1.0 x10^4 cells/well at 37 ^oC for 48 h under 5% CO_2. After incubation, the cells were treated with different concentrations of compounds and incubated for 24 h. After 24 h of drug treatment, 20 µl of MTT solution at 5mg/ml was added and incubated for 4 h. Dimethyl sulfoxide (DMSO) in volume of 100 µl is added into each well to dissolve the purple formazan formed. The colorimetric assay is measured and recorded at absorbance of 570 nm using a plate reader (EXL 800).

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