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Synthesis of some new heterocycles containing 1-(2-benzothiazolyl) pyrazolone moiety and their cytotoxicity evaluation

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Abstract The arylidene derivatives of 2-(benzo[d]thiazol-2-yl)-5-methyl-2,4-dihydro-3H-pyrazol-3-one **(2a,b)** was a versatile material for the synthesis of some new fused heterocyclies containing benzothiazalyl moiety by reaction with certain bifunctional nucleophilic reagents namely (hydroxylamine hydrochloride, guandine nitrate, thioactamide, cyanoacetamide, acetylacetone and ethyl acetoacetate) to yield pyrazoloisoxazole, pyrazolopyrimidine, pyrazolothiazine, pyrazolopyridine and pyranopyrazole derivatives, respectively. All the newly synthesized compounds were tested against hepatocellular carcinoma (liver) HePG-2, mammary gland (breast) MCF-7, Human (prostate) cancer cell line PC3 and Colorectal carcinoma (colon) HCT-116. The structures of all products were confirmed by analytical and spectral analyses.

Introduction

Cancer still remains a main threat to human health, representing the second leading cause of death worldwide (Caleta, et al.; 2009). It is estimated that 13.1 million people will die from cancer in 2030 (WHO). Recently, many efforts have been made to develop safe and effective ways of treating this disease and to search for novel chemotherapeutic agents with minimal side effects (Caleta, et al.; 2009). In this context, the major challenge is the development of more effective and safe drugs for the treatment of cancer. Through searching in the literature, it was found that benzothiazole derivatives play an important role on designing of new drugs, since they present an interesting pharmacological profile (Rana, et al.; 2007), including anti-allergic (Ban, et al.;1998), anti-inflammatory (Oketani, et al.; 2001), antitumor (Yoshida, et al.; 2005), analgesic (Westaway, et al.; 2008), antimicrobial (Sharma & Sharma, 2009 and Sigmundova, et al.; 2008), anthelmintic (Bhusari, et al.; 2000), antileishmanial (Delmas, et al.; 2004), anticonvulsant (Jimonet, et al.; 1991) activities, also with considerable efficacy as kinase, topoisomerase I/II and transretinoic acid metabolism inhibitors (Caleta, et al.; 2009). In addition, pyrazole nucleus is an important pharmacophore with a wide range of therapeutically activities such as antitumor (Wentland, et al.; 1995), antibacterial, anti-inflammatory (Paul, et al.; 2001) and hypotensive (Paul, et al.; 2001) efficacies and also acts as ligands for benzodiazepine receptors (Fryer, et al.; 1993).

It was aimed in this work to synthesize a new series of heterocyclic compounds bearing pyrazole nucleus linked to benzothiazole to assess their anti-carcinogenic effects against hepatocellular carcinoma (liver) HePG-2, mammary gland (breast) MCF-7,

Human (prostate) cancer cell line PC3 and Colorectal carcinoma (colon) HCT-116.

Results and Discussion

Now, we describe the synthesis of 2-(benzo[d]thiazol-2-yl)-5-methyl-4(aryl-4-ylmethylene)-2,4dihydro -3H -pyrazol-3-one **(2a,b)** by Knoevenagel condensation of the active methylene pyrazolinone **1** with furfural in boiling glacial acetic acid containing a catalytic amount of freshly fused sodium acetate led to formation of **2a,** while **2b** was prepared by reaction of **1** with 4-formyl pyridine in refluxing ethanol containing few drops of pipreidine (**Scheme 1**). Condensation addition cyclization reaction of the compound **2a,b** with hydroxylamine hydrochloride, involving exo-cyclic enone grouping, led to the formation of the fused five-membered pyrazoloisoxazole derivatives **3a,b** (**Scheme 1**). On the other hand, six-membered heterocyclo-pyrazoles were achieved via similar addition-condensation cyclization reactions with different 1,3-bidentate nucleophiles. Thus, treatment of compound **2a,b** with guanidine nitrate gave the pyrazolopyrimidine **4a,b** (**Scheme 1**). For example, the possibility of structure of **4b** was established derivatives based on the elemental analysis and spectral data. Thus, the IR spectrum of reaction product **4b** showed the presence of a (NH_2) group at 3442 and 3420 cm^{-1}, (C=N) and (C=C) at 1663 and 1636 cm^{-1}, respectively. The ^{1}H- NMR spectrum showed the presence of singlet signal at δ 1.79 ppm corresponding to (CH_3) protons, singlet signal (D_2O-exchangeable) at δ 5.43 ppm owing to (NH_2) protons, in addition to aromatic protons at δ 7.27-8.02 ppm, doublet signal at δ 8.90 ppm due to (C_2-H, C_6-H pyridine) protons. Also the structure of compound **4b** was confirmed by mass spectrometric measurement which gave molecular ion peak at m/z =361 corresponding to its correct molecular weight of the molecular formulae $C_{18}H_{13}N_7S$.

Reaction of the compound **2a,b** with thioacetamide, in the presence of piperidine as catalyst, afforded pyrazolothiazine **5a,b** (**Scheme 1**). The structure of **5a** was established as reaction product based on analytical and spectral data. IR spectrum of **5a** showed absorption band at υ 1655 and 1621 cm^{-1} due to of (C=N), (C=C), respectively. ^{1}H-NMR spectrum of **5a** displayed two singlet signals at δ 1.10 and 1.17 ppm owing to (two CH_3) protons, singlet signal at δ 5.20 ppm due to (C_6-H thiazine) proton, in addition to multiplet signals at δ 6.80-8.20 ppm due to aromatic protons. The structure of compound **5a** was confirmed also by mass spectrum which showed molecular ion peak at m/z =367 corresponding to its correct molecular weight of the molecular formulae $C_{18}H_{14}N_4OS_2$.

These results along with IR spectrum and elemental data emphasis that an addition of S-thioacetamide to the methylene of enone moiety took place and followed by condensation of the amino group with the oxo-pyrazoline. The reactivity of compound **2a, b** towards Michael reaction with some C-nucleophiles was investigated by reaction with certain active methylene compounds the presences of suitable basic catalyst. It is well known that Michael addition reactions of activated alkenes with C-nucleophiles lead to the formation of (C–C) extra bond at the position of addition; hence to affect a consequent cyclization it is worthwhile to use the proper reagent of general formula (X–CH_2–Y). These selected reagents must contain appropriate X and Y groups which are capable for a further intramolecular reaction with the tautomeric (NH–C=O↔N=C–OH) of the pyrazoline moiety. Thus, the reaction of compound **2a,b** with cyanoacetamide was considered and the product was characterized as pyrazolopyridone **6a,b** (**Scheme 1**). IR spectrum of **6a** showed absorption band for NH at υ 3440 cm^{-1} , in addition to absorption band at 2205 cm^{-1} due to (CN) group, stretching frequency at υ 1637 cm^{-1} due to (C=O). ^{1}H- NMR spectrum of **6a** displayed a singlet signal at δ 2.11 ppm due to (CH_3) protons, in addition to multiplet signals at δ 6.80-8.20 ppm due to aromatic protons, singlet signal (D_2O-exchangeable) at δ 10.95 ppm owing to NH protons. The structure of compound **6a** was confirmed by mass spectrometric which showed molecular ion peak at m/z =373 corresponding to its correct

molecular weight of the molecular formulae $C_{19}H_{11}N_5O_2S$. The IR spectrum of compound **6b** showed absorption bands at 3223 cm^{-1} (NH), 2215 cm^{-1} (CN) and 1637 cm^{-1}. ^{1}H-NMR spectrum of **6b** displayed a singlet signal at δ 2.12 ppm owing to (CH_3) protons, in addition to multiplet signals at δ 6.80-8.20 ppm due to aromatic protons, doublet signal at δ 8.50 ppm due to (C_2-H, C_6-H pyridine) proton, singlet signal (D_2O-exchangeable) at δ 11.05 ppm owing to NH protons. The structure of compound **6b** was confirmed also by mass spectrum which gave molecular ion peak at m/z =384 corresponding to its correct molecular weight of the molecular formulae $C_{20}H_{12}N_6OS$.

Also, the multi-functional pyranopyrazoles **7a, b** and **8a, b** (**Scheme 1**) were prepared via similar cyclization process with certain acyclic active methylene compounds, which always leads to 5,6-disubstiuted pyranopyrazoles. Thus, the reaction of compound **2a,b** with acetylacetone in refluxing DMF containing a catalytic amount of pipreridine yielded 5-acetyl-6-methylpyranopyrazole **7a,b** , while addition-condensation reaction of compound **2a,b** with ethyl acetoacetate **8a,b** under similar reaction condition gave the ethyl 6-methyl-3-aryl-pyranopyrazole-5-carboxylates **8a,b** (**Scheme 1**). IR spectra of these esters evidently revealed the characteristic carbonyl absorption vibrational bands due to carboxylate groups. Also, ^{1}H NMR spectra of the esters **8a, b** which showed the triplet-quartet pattern of ethyl group protons. These results arose the conclusion that without any doubt carboxylate group is not involved in the intramolecular condensation reaction but the keto-group does.

Biological Evaluation

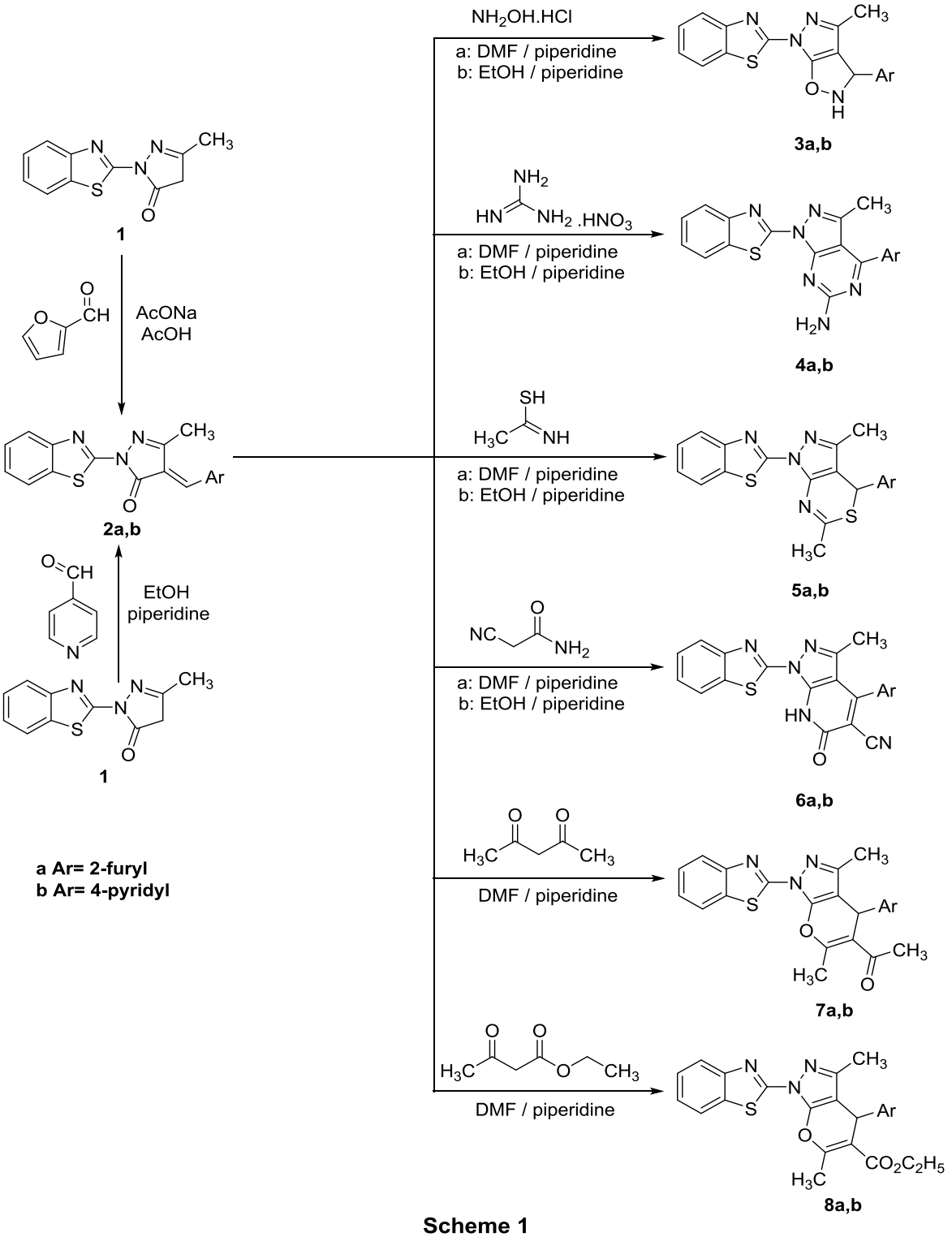
Antioxidant activity screening assay ABTS method

Total antioxidant activities of compounds **(2a, b- 8a, b)** were evaluated using free radical scavenging activity determined by **Antioxidant activity screening assay** ABTS method. The inhibition percentage equation of radical scavenging activity was calculated by using the equation:

$$\textbf{Inhibition } (\%) = [(A_0 - A_S)/A_0] \times 100$$

Where: A_0 is absorbance of the blank, A_S is absorbance of the sample at 734 nm.

L- Ascorbic acid was used as the positive control; negative control was run using ABTS. From the results of the screening studies displayed in table 1.


NH2OH.HCl
a: DMF / piperidine
b: EtOH / piperidine
3a,b
NH2
HN
NH2 .HNO3
a: DMF / piperidine
b: EtOH / piperidine
4a,b
SH
H3C
NH
a: DMF / piperidine
b: EtOH / piperidine
5a,b
NC
NH2
a: DMF / piperidine
b: EtOH / piperidine
6a,b
H3C
CH3
DMF / piperidine
7a,b
H3C
CO2C2H5
DMF / piperidine
8a,b
1
AcONa
AcOH
2a,b
EtOH
piperidine
1
a Ar= 2-furyl
b Ar= 4-pyridyl

Scheme 1

Table 1: Anti-Oxidant Assay by ABTS Method

No.	Method	ABTS Abs(control)-Abs(test)/Abs(control)X100	
	Compounds	Absorbance of samples	% inhibition
	Control of ABTS	0.510	0%
	Ascorbic-acid	0.064	87.45%
1	2a	0.451	11.56%
2	2b	0.062	87.84%
3	3a	0.391	23.33%
4	3b	0.065	87.25%
5	4a	0.350	31.37%
6	4b	0.322	36.86%
7	5a	0.174	65.88%
8	5b	0.452	11.37%
9	6a	0.167	67.25%
10	6b	0.444	12.94%
11	7a	0.170	66.66%
12	7b	0.120	76.47%
13	8a	0.069	86.47%
14	8b	0.358	29.80%

From Table 1, it can be suggested that all compounds gave moderate to excellent antioxidant activity except **2a, 5b** and **6b**. The compounds **2b, 3b, 7b** and **8a** gave very high antioxidant activity. Compound **2b** displayed the best antioxidant property (87.84%) even more than the standard L-Ascorbic acid (87.45%). The other compounds **3b**

Cytotoxic Screening

The In vitro cytotoxicity IC_{50} (µmol/L) of the newly synthesized compounds were studied using the 5-fluorouracil as reference drug, including hepatocellular carcinoma (liver) **HePG-2**, mammary gland (breast) **MCF-7**, Human (prostate) cancer cell line **PC3** and Colorectal carcinoma (colon) **HCT-116 Table 2** .

All compounds showed cytotoxicity against hepatocellular carcinoma (liver) **HePG-2**, mammary gland (breast) **MCF-7**, human (prostate) cancer cell line **PC3** and colorectal carcinoma (colon) **HCT-116**, except compounds **2a, 5b** and **6b**, showed no activity.

Table 2: Cytotoxic activity of some compounds against human tumor cells

Comp.	In vitro Cytotoxicity IC50 (µg/ml)•			
	HePG2	PC3	HCT-116	MCF-7
5-FU	7.53± 0.22	5.13± 0.22	6.25± 0.34	4.05± 0.15
2a	>100	>100	>100	>100
2b	6.11± 0.16	5.04± 0.08	6.25± 0.35	4.17± 0.04
3a	96.76± 2.11	88.24± 3.14	95.23± 2.06	>100
3b	7.50± 0.14	6.79± 0.30	7.83± 0.21	4.06± 0.11
4a	>100	>100	90.15± 3.24	>100
4b	78.92± 2.87	84.82± 4.31	79.55± 1.63	85.41± 2.20
5a	39.05± 0.66	46.51± 1.52	41.35± 0.69	49.77± 0.89
5b	>100	>100	>100	>100
6a	22.76± 0.09	27.38± 0.36	28.73± 0.86	25.53± 0.39
6b	>100	>100	>100	>100
7a	28.65± 0.24	31.76± 0.45	37.44± 0.74	29.71± 0.61
7b	20.12± 0.84	24.62± 1.05	18.76± 0.93	15.99± 0.67
8a	8.13± 0.13	9.94± 0.35	8.48± 0.17	6.50± 0.10
8b	93.45± 2.39	>100	92.60± 1.86	>100

• **IC_{50} (µg/ml)**: 1 – 10 (very strong). 11 – 20 (strong). 21 – 50 (moderate). 51 – 100 (weak) and above 100 (non-cytotoxic).

(87.25%), **8a** (84.64 %) and **7b** (76.47%) gave excellent antioxidant activity.

It was also found that, results obtained mainly in case of 2-pyrdiyl substituent are better than the furul substituent.

Compounds **2b, 3b** and **8a** showed very strong cytotoxicity against the four cell lines. Compound **2b** showed cytotoxicity activity more strong than standard drug **5-FU** against **HePG-2**, **HCT-116**, **PC3** and more than **MCF-7.** In addition, compound **7b** has strong cytotoxicity activity against **HePG-2**, **HCT-116**, **MCF-7** and moderate against **PC3**, while **5a, 6a** and **7a** have moderate cytotoxicity activity against **HePG-2**, **HCT-116**, **MCF-7** and **PC3**. While **4b** exhibit weakly cytotoxicity activity against **HePG-2**, **HCT-116**, **MCF-7**

and **PC3**. Compounds **3a, 4a** and **8b** showed activities between weakly and non-cytotoxic against all the cell lines.

Experimental

Melting points are uncorrected and were determined in open capillary tubes on a digital Gallen-Kamp MFB-595. IR spectra were taken on a Perkin-Elmer FT-IR 1650 spectrophotometer (ν, cm^{-1}), using samples in KBr disks. ^{1}H NMR spectra were recorded on a Bruker AC200 (200 MHz) spectrometer (δ, ppm), using DMSO-d_6 as solvent and TMS as internal standard.

Synthesis of 2-(benzo[d]thiazol-2-yl)-4-(furan-2-ylmethylene)-5-methyl-2,4-dihydro-3H-pyrazol-3- one (2a)

To a solution of compound **1** (2.31 g, 0.01 mol) in glacial acetic acid (20 ml), furfural (0.96 g, 0.01 mol) and sodium acetate (0.82 g, 0.01 mol) were added and the reaction mixture was heated under reflux for 3 hours (TLC controlled). Then the reaction mixture was filtered off and the solid product was recrystallized from DMF-Ethanol to give compound **2a**.

2a: Dark brown solid; Yield= 75 %, m.p. >350 °C. IR *ν* (KBr) cm^{-1}= 1645 (C=O), 1605 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm= 2.24 (s, 3H, CH_3), 6.73 (s, 1H, Vinylic H), 6.90-8.30 (m, 7H, Ar-H). MS (*m/z, %)*: 309, 47.12%, 266, 46.60%, 119, 59.16%, 64, 100%. Anal. Calcd. For $C_{16}H_{11}N_3O_2S$ (309.34): C, 62.12; H, 3.58; N, 13.58; S, 10.36 %. Found: C, 62.07; H, 3.63; N, 13.52; S, 10.42 %.

Synthesis of 2-(benzo[d]thiazol-2-yl)-5-methyl-4-(pyridin-4-ylmethylene)-2,4-dihydro-3H-pyrazol-3-one (2b)

To a solution of compound **1** (2.31 g, 0.01 mol) in ethanol (20 ml), 4-formyl pyridine (0.95 ml, 0.01 mol) and catalytic amount of piperidine where added and the mixture was heated under refluxed for 4 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **2b**.

2b: Dark brown solid; Yield= 33 %, m.p.= 244 °C. IR *ν* (KBr) cm^{-1}=1652(C=O), 1599 (C=C), 1519 (CH_2); ^{1}H NMR (DMSO-d_6): δ ppm=2.23 (s, 3H, CH_3), 6.80-8.20 (m, 7H, Ar-H + vinylic H), 8.55 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z, %)*: 322(M^+ +2) 52.46%, 320, 83.59%, 231, 100%, 135, 35.66%. 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 6-(benzo[d]thiazol-2-yl)-3-(furan-2-yl)-4-methyl-3,6-dihydro-2H-pyrazolo[4,3-d]isoxazole (3a)

A mixture of compound **2a** (3.09 g, 0.01 mol) and hydroxylamine hydrochloride (0.96 g, 0.01 mol), in DMF (20 ml), was treated with catalytic amount of piperidine and heated under refluxed for 3hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **3a**.

3a: Dark brown solid; Yield= 80 %, m.p. >350 °C. IR *ν* (KBr) cm^{-1}= 3185 (NH), 1647 (C=N), 1598 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm= 2.10 (s, 3H, CH_3), 5.63 (s, 1H, C_3-H isoxazole), 6.45 (s, 1H, NH, D_2O exchangeable), 6.8-8.30 (m, 7H, Ar-H). MS (*m/z, %)*: 324, 75.53%, 220, 100%, 170, 89.36%, 57, and 100%. Anal. Calcd. for $C_{16}H_{12}N_4O_2S$ (324.36) : C, 59.25; H, 3.73; N, 17.27; S, 9.88%. Found: C, 59.18; H, 3.79; N, 17.22; S, 9.94%.

Synthesis of 6-(benzo[d]thiazol-2-yl)-4-methyl-3-(pyridin-4-yl)-3,6-dihydro-2H-pyrazolo[4,3-d]isoxazole 3b

A mixture of compound **2b** (3.20 g, 0.01 mol) and hydroxylamine hydrochloride (0.96 g, 0.01 mol) in ethanol (20 ml) was treated with catalytic amount of piperidine and heated under refluxed for 15 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **3b**.

3b: Dark red solid; Yield= 55 %, m.p.= 285-288 °C. IR *ν* (KBr) cm^{-1}= 3437 (NH), 1636 (C=N), 1611 (C=C); ^{1}H NMR (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), 6.80-8.20 (m, 6H, Ar-H), 8.59 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z, %)*: 335, 9.80%, 180, 87.00%, 150, 51.10%, 91,

99.90%.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)-4-(furan-2-yl)-3-methyl-1H-pyrazolo[3,4-d]pyrimidin-6-amine (4a)

A mixture of compound **2a** (3.09 g, 0.01 mol) and guanidine nitrate (1.22 g, 0.01mol), in DMF (20 ml), was treated with catalytic amount of piperidine and the mixture was heated under refluxed for 4 hours (TLC controlled). Afterwards the reaction mixture was left to cool then filtered off and recrystallized from DMF-Ethanol to give compound **4a**.

4a: Dark brown solid; Yield= 80 %, m.p. >350 °C. IR *v* (KBr) cm^{-1}= 3429, 3403 (NH_2), 1658 (C=N); ^{1}H NMR (DMSO-d_6): δ ppm= 2.39 (s, 3H, CH_3), 6.68 (s, 2H, NH_2, D_2O exchangeable), 6.80- 8.20 (m, 7H, Ar-H). MS (*m/z, %)*: 351, 37.14%, 311, 42.29%, 173, 41.71%, 80, 100%. Anal. Calcd. for $C_{17}H_{12}N_6OS$ (348.38) : C, 58.61; H, 3.47; N, 24.12; S, 9.20%. Found: C, 58.55; H, 3.54; N, 24.05; S, 9.22%.

Synthesis of 1-(benzo[d]thiazol-2-yl)-3-methyl-4-(pyridin-4-yl)-1H-pyrazolo [3,4-d]pyrimidin-6-amine (4b)

A mixture of compound **2b** (3.20 g, 0.01mol) and guanidine nitrate (1.22 g, 0.01mol) in ethanol (20 ml) was treated with catalytic amount of piperidine and heated under refluxed for 12 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **4b**.

4b: Dark red solid; Yield= 40 %, m.p.= 295-298 °C. IR *v* (KBr) cm^{-1}=3442, 3420 (NH_2), 1663 (C=N), 1636 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm=1.79 (s, 3H, CH_3), 5.43 (s, 2H, NH_2), 7.27-8.02 (m, 6H, Ar-H), 8.90 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z, %)*: 361, 1.20%, 215, 25.50%, 103, 31.60%, 43, 99.90%. 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)-4-(furan-2-yl)-3,6-dimethyl-1,4-dihydropyrazolo[3,4-d][1,3]thiazine (5a)

To a mixture of compound **2a** (3.09 g, 0.01 mol) and thioacetamide (0.75 g, 0.01mol), in DMF (20 ml), was treated with catalytic amount of piperidine and the mixture was heated under refluxed for 4 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **5a**.

5a: Dark brown solid; Yield= 80 %, m.p. >350 °C. IR *v* (KBr) cm^{-1}= 1655 (C=N), 1621 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm= 1.10 (s, 3H, CH_3), 1.17 (s, 3H, CH_3), 5.20 (s, 1H, C_6-H 1,3-thiazine), 6.80-8.20 (m, 7H, Ar-H). MS (*m/z, %)*: 367, 74.00%, 276, 84.00%, 210, 81.00%, 57, 100%. Anal. Calcd. for $C_{18}H_{14}N_4OS_2$ (366.46) : C, 59.00; H, 3.85; N, 15.29; S, 17.50%. Found: C, 58.94; H, 3.90; N, 15.32; S, 17.45%.

Synthesis of 1-(benzo[d]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrazolo[3,4-d][1,3]thiazine (5b)

To a mixture of compound **2b** (3.20 g, 0.01 mol) and thioacetamide (0.75 g, 0.01 mol) in ethanol (20 ml) was treated with catalytic amount of piperidine then heated under refluxed for 12 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **5b**.

5b: Red solid; Yield= 40 %, m.p.= 253-258 °C. IR *v* (KBr) cm^{-1}= 1651 (C=N), 1599 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm=1.85 (s, 3H, CH_3), 2.50 (s, 3H, CH_3), 4.56 (s, 1H, C_6-H 1,3-thiazine), 7.30-8.15 (m, 6H, Ar-H), 8.99 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z, %)*: 377, 90.30%, 289, 99.90%, 197, 33.90%, 43, 61.10%. Anal. Calcd. for $C_{19}H_{15}N_5S_2$ (377.48) : C, 60.46; H, 4.01; N, 18.55; S, 16.99%. Found: C, 60.42; H, 4.04; N, 18.50; S, 16.96%

Synthesis of 1-(benzo[d]thiazol-2-yl)-4-(furan-2-yl)-3-methyl-6-oxo-6,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (6a)

To a solution of compound **2a** (3.09 g, 0.01 mol) in DMF (20 ml), and cyanoacetamide (1.00 g, 0.01mol) and catalytic amount of

piperidine where added and the mixture was heated under refluxed for 4 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **6a**.

6a: Dark brown solid; Yield= 80 %, m.p. >350 °C. IR *v* (KBr) cm^{-1}= 3440 (NH), 2205 (CN), 1665 (C=O); ^{1}H NMR (DMSO-d_6): δ ppm= 2.11 (s, 3H, CH_3), 6.80-8.20 (m, 7H, Ar-H), 10.95 (s, 1H, NH, D_2O exchangeable). MS (*m/z, %)*: 375, 29.19%, 326, 43.78%, 64, 100%, 58, 94.59%. Anal. Calcd. for $C_{19}H_{11}N_5O_2S$ (373.39) : C, 61.12; H, 2.97; N, 18.76; S, 8.59%. Found: C, 61.04; H, 2.94; N, 18.81; S, 8.54%.

Synthesis of 1-(benzo[d]thiazol-2-yl)-3-methyl-6-oxo-4-(pyridin-4-yl)-6,7-dihydro-1H-pyrazolo[3,4-b]pyridine-5-carbonitrile (6b)

To a solution of compound **2b** (3.20 g, 0.01 mol) and cyanoacetamide (1.00 g, 0.01mol) in ethanol (20 ml) and catalytic amount of piperidine where added and the mixture was heated under refluxed for 12 hours (TLC controlled). Then the reaction mixture was filtered off and recrystallized from DMF-Ethanol to give compound **6b**.

6b: Dark red solid; Yield= 43 %, m.p.= 344-346 °C. IR *v* (KBr) cm^{-1}= 3223 (NH), 1730 (C=O), 1637 (C=O); ^{1}H NMR (DMSO-d_6): δ ppm= 2.12 (s, 3H, CH_3), 6.80-8.20 (m, 6H, Ar-H), 8.5 (d, 2H, C_2-H, C_6-H pyridine), 11.05 (s, 1H, NH,D_2O exchangeable). MS (*m/z, %)*: 386, 20.80%, 169, 73.30%, 127, 63.00%, 43, 99.90%. Anal. Calcd. for $C_{20}H_{12}N_6OS$ (384.42) : C, 62.49; H, 3.15; N, 21.86; S, 8.34%. Found: C62.44; H, 3.17; N, 21.81; S, 8.38%.

Reaction of Active Methylene Compounds with Pyrazolinone 2a,b. General Procedure

To a mixture of compound **2a,b** ((a;3.09 g), (b;3.20 g), 0.01 mol) and the proper active methylene compound (0.01 mol), in DMF (20 ml), catalytic amount of piperidine was added and the mixture was heated under refluxed for 9-13 hours (TLC controlled). Afterwards the reaction mixture was left to cool and acidified using diluted acetic acid till complete precipitation. The precipitate so obtained off. Then the product was recrystallized from DMF-Ethanol to give compounds **7a, b** and **8a, b**.

Synthesis of 1-(1-(benzo[d]thiazol-2-yl)-4-(furan-2-yl)-3,6-dimethyl-1,4-dihydropyrano[2,3-c]pyrazol-5-yl)ethan-1-one (7a)

This compound was prepared from compound **2a** and acetylacetone (1.02 ml). **7a:** Dark brown solid; Yield= 75 %, m.p. >350 °C. IR *v* (KBr) cm^{-1}=1693 (C=O); ^{1}H NMR (DMSO-d_6): δ ppm= 2.06 (s, 3H, CH_3 at C_3), 2.18 (s, 3H, CH_3C=O), 2.34 (s, 3H, CH_3 at C_6), 5.27 (s, 1H, C_4-H pyran), 6.80-8.20 (m, 7H, Ar-H). MS (*m/z, %)*: 391, 41.46%, 170, 50.00%, 84, 82.32%, 56, 100%. Anal. Calcd. for $C_{21}H_{17}N_3O_3S$ (391.45) : C, 64.44; H, 4.38; N, 10.73; S, 8.19%. Found: C, 64.38; H, 4.33; N, 10.77; S, 8.15%.

Synthesis of 1-(1-(benzo[d]thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydro pyrano[2,3-c]pyrazol-5-yl)ethan-1-one (7b)

This compound was prepared from compound **2b** and acetylacetone (1.02 ml). **7b:** Dark brown solid; Yield= 40 %, m.p.= 240-243 °C. IR *v* (KBr) cm^{-1}= 1663 (C=O); ^{1}H NMR (DMSO-d_6): δ ppm= 2.12 (s, 3H, CH_3 at C_3), 2.27 (s, 3H, CH_3C=O), 2.37 (s, 3H, CH_3 at C_6), 5.06 (s, 1H, C_4-H pyran), 6.80-8.20 (m, 6H, Ar-H), 8.66 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z %)*: 402, 45.10%, 342, 76.80%, 122, 99.90%, 43, 87.00%. 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%.

Synthesis of ethyl 1-(benzo[d]thiazol-2-yl)-4-(furan-2-yl)-3,6-dimethyl-1,4-dihydropyrano[2,3-c]pyrazole-5-carboxylate (8a)

This ester was formed from compound **2a** and ethyl acetoacetate (1.27 ml). **8a:** Dark brown solid; Yield= 75 %, m.p. >350 °C. IR *v* (KBr) cm^{-1}=1710 (C=O), 1651 (C=N); ^{1}H NMR (DMSO-d_6): δ ppm= 1.10 (t, 3H, CH_3), 2.03 (s, 3H, CH_3 in C_3), 2.23 (s, 3H, CH_3 in C_3), 3.98 (q, 2H, OCH_2), 5.33 (s, 1H, C_4-H pyran), 6.80-

8.20 (m, 7H, Ar-H). MS (*m/z, %)*: 423, 67.35%, 373, 82.65%, 244, 100%, 73, 100%. Anal. Calcd. For $C_{22}H_{19}N_3O_4S$ (421.47): C, 62.70; H, 4.54; N, 9.97; S, 7.61%. Found: C, 62.62; H, 4.57; N, 9.92; S, 7.67%.

Synthesis of ethyl 1-(benzo[d] thiazol-2-yl)-3,6-dimethyl-4-(pyridin-4-yl)-1,4-dihydropyrano[2,3-c]pyrazole-5-carboxylate (8b)

This ester was formed from compound **2b** and ethyl acetoacetate (1.27 ml). **8b:** Light red solid; Yield= 40 %, mp=247-250 °C. IR *v* (KBr) cm^{-1}= 1714 (C=O), 1631 (C=N), 1598 (C=C); ^{1}H NMR (DMSO-d_6): δ ppm= 1.06 (t, 3H, CH_3), 1.98 (s, 3H, CH_3 in C_3), 2.34 (s, 3H, CH_3 in C_6), 3.98 (q, 2H, OCH_2), 4.93 (s, 1H, C_4-H pyran), 6.80-8.20 (m, 6H, Ar-H), 8.67 (d, 2H, C_2-H, C_6-H pyridine). MS (*m/z, %)*: 432, 2.30%, 378, 21.20%, 283, 99.90%, 165, 31.80. 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%.

Antioxidant Activity Screening Assay Abts Method

For each of the investigated compounds (2 mL) of ABTS solution (60 µM) was added to 3 mL MnO2 solution (25mg/mL), all prepared in (5 mL) aqueous phosphate buffer solution (pH 7, 0.1 M). The mixture was shaken, centrifuged, filtered and the absorbance of the resulting green blue solution (ABTS radical solution) at 734 nm was adjusted to approx. ca. 0.5. Then, 50 µl of (2 mM) solution of the tested compound in spectroscopic grade MeOH/phosphate buffer (1:1) was added. The absorbance was measured and the reduction in color intensity was expressed as inhibition perecentage. L – ascorbic acid was used as standard antioxidant (Positive control). Blank sample was run without ABTS and using MeOH/phosphate buffer (1:1) instead of tested compounds. Negative control was run with ABTS and MeOH/phosphate buffer (1:1) only.

Cytotoxicity Assay

Materials and methods: Cell line

Four human tumor cell lines namely ; hepatocellular carcinoma (liver) HePG-2, mammary gland (breast) MCF-7, Human (prostate) cancer cell line PC3 and Colorectal carcinoma (colon) HCT-116. The cell lines were obtained from ATCC via Holding company for biological products and vaccines (VACSERA), Cairo, Egypt.

Chemical reagents

The reagents RPMI-1640 medium,MTT and DMSO and 5-fluorouracil (sigma co., St. Louis, USA), Fetal Bovine serum (GIBCO, UK) . 5-fluorouracil was used as a standard anticancer drug for comparison.

MTT assay.

The different 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. The cells were cultured in RPMI-1640 medium with 10% fetal bovine serum. Antibiotics added were 100 units/ml penicillin and 100µg/ml streptomycin at 37 C in a 5% Co_2 incubator. The cells were seedes in a 96-well plate at a density of 1.0x 104 cells/ well.

At 37 °C for 48 h under 5% CO_2. After incubation the cells were treated with different concentration 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). The relative cell viability in percentage was calculated as (A570 of treated samples/A570 of untreated sample) X 100.

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تخليق بعض حلقات الغير متجانسة جديدة تحتوي على نواة ١-(٢-بنزوثيازوليل) بيرازولون والتقيم نشاطها سمية لخلايا

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٢-(بنزو[d]ثيازول-٢-يل)-٥-ميثيل-٢,٤-ثنائي هيدرو-3H-بيرازول-٣- و(**2a, b**) من المشتقات اريليدين كان مادة متعددة الاستعمالات لتحضير بعض حلقات الغير متجانسة جديدة مندمجة تحتوي على نواة بنزوثيازوليل من خلال التفاعل مع بعض الكواشف ثنائية وظيفية أليفة النواة وهي (هيدروكلوريد هيدروكسيل و جواندين نترات و ثيواسيتمايد و سيانو اسيتمايد و اسيتايل اسيتون و ايثيل اسيتواسيتيد) لتسفر عن المشتقلت بيرازولوايسوكسازول و بيرازولوبيريمدين و بيرازولوسايزين و بيرازولوبيريدين و بيرانوبيرازول على التوالي. تم اختبار جميع المركبات المحضر حديثا ضد سرطان الكبد (الكبد) HePG-2 و الغدة الثديية (الثدي) MCF-7 و خلية سرطان سلالة البشري (البروستات)PC3 و ورم السرطان القولون والمستقيم (القولون)HCT-116. وقد أكد هياكل جميع المنتجات من خلال طرق التحليل والتحليلات الطيفية.