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SYNTHESIS, CHARACTERIZATION AND CYTOTOXICITY EVALUATION OF SOME NOVEL PYRAZOLE, PYRIMIDINE AND ISOXAZOLE DRIVATIVES CONTAINING BENZOTHIAZOLE MOIETY

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Abstract - The 2-(2-benzothiazolyl)-3-(2-methoxy-1-naphthyl)acrylonitrile **(3)** was used as precursor for the synthesis of some novel pyrazole, isoxazole, pyrimidine derivatives and other related products containing benzothiazole moiety *via* the reaction of compound **3** with appropriate chemical reagents. The structures of the newly synthesized products were confirmed by elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR and mass spectral data. These compounds were screened for their antitumor activities. Compound **7** displayed promising *in vitro* antitumor activity in the four cell lines assay.

INTRODUCTION

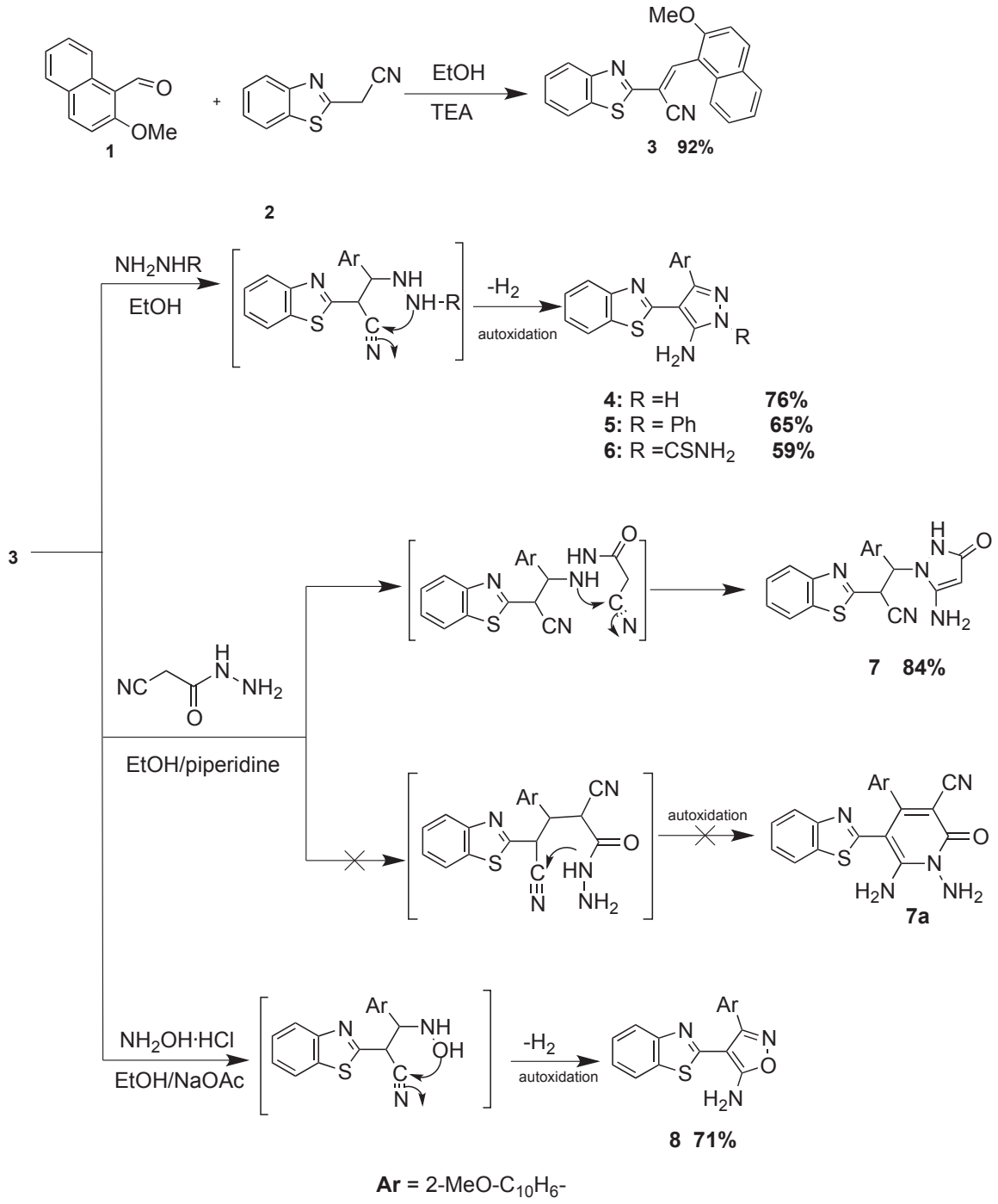
Benzothiazole derivatives constitute a subject of great interest because of diverse biological and pharmacological activities. They have been investigated for their anti-inflammatory,[1,2] antiallergic,[1] antitumor[3–7] and analgesic[8,9] activity. Considering the mechanism of action, it was shown that benzothiazole derivatives act as tyrosine kinase[10–13] and topoisomerase I and II inhibitors.[14,15] It was reported that incorporation of alkoxy substituents results in significant enhancement of antitumor activity due to intensification of compounds' lipophilicity.[16,17] Therefore, the target compounds were designed so as to comprise 2-methoxy-1-naphthyl group as a substituent. In addition, incorporation of heterocyclic groups in position 2 of benzothiazole moiety such as pyrazoles, isoxazole and pyrimidine was considered as an interesting structure variation that might impose an impact on the potential biological activities owing to their documented chemotherapeutic activity.[18-24]

RESULTS AND DISCUSSION

(*E*)-2-(Benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)acrylonitrile (**3**) was prepared in analogy to reported literatures[25-27] in excellent yield by refluxing equimolar amounts of 2-methoxy-1-naphthaldehyde (**1)** and 2-cyanomethylbenzothiazole (**2**) in EtOH containing a catalytic amount of triethylamine (Scheme 1**)**. The configuration of the acrylonitrile double bond could not be established by NMR methods. However, the steric repulsions between the 2-methoxy-1-naphthyl group and benzothiazole moiety showed that the *E*-isomer is more stable than *Z*-isomer.[25,26] The assignment of structure **3** was supported by elemental analysis and spectroscopic data. Its IR spectrum showed characteristic absorption bands at 2227 and 1620 cm^{-1} attributable to C≡N and C=N groups, respectively. Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of two singlet signals at 4.09 and 8.79 ppm assignable for MeO and a vinylic proton, respectively. The mass spectrum showed the molecular ion peak at *m/z* 342 which coincide with the molecular weight of proposed structure.

Then, the reactivity of arylidene derivative **3** towards some *N*-nucleophiles was investigated. Thus, reaction of **3** with hydrazine hydrate or phenylhydrazine in refluxing EtOH furnished the pyrazole derivatives **4** and **5**, respectively[28] *via* aza-Michael addition followed by cycloaddition to a cyano function and spontaneous dehydrogenation (Scheme 1). Structures of compounds **4** and **5** were established on the basis of its elemental analyses and spectroscopic data. The IR spectra of pyrazoles **4** and **5** are devoid of an absorption band for the cyano group but showed stretching absorption bands in the region of 3450-3300 cm^{-1} due to amino group.

On the other hand, treatment of compound **3** with thiosemicarbazide in refluxing pyridine afforded 5-amino-4-(benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)-1*H*-pyrazole-1-carbothioamide (**6**) (Scheme 1). The assignment of structure **6** was supported by elemental analysis and spectroscopic data. Its IR spectrum lacked any absorption bands of the nitrile group, which confirm that the nitrile group was involved in the cyclization reaction. Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of singlet signals at 4.05, 6.21 and 6.64 ppm assignable for MeO, NH_2 and $CSNH_2$ groups, respectively. Its ^{13}C-NMR spectrum was characterized by a signal at 178.6 ppm assignable to the C=S group.



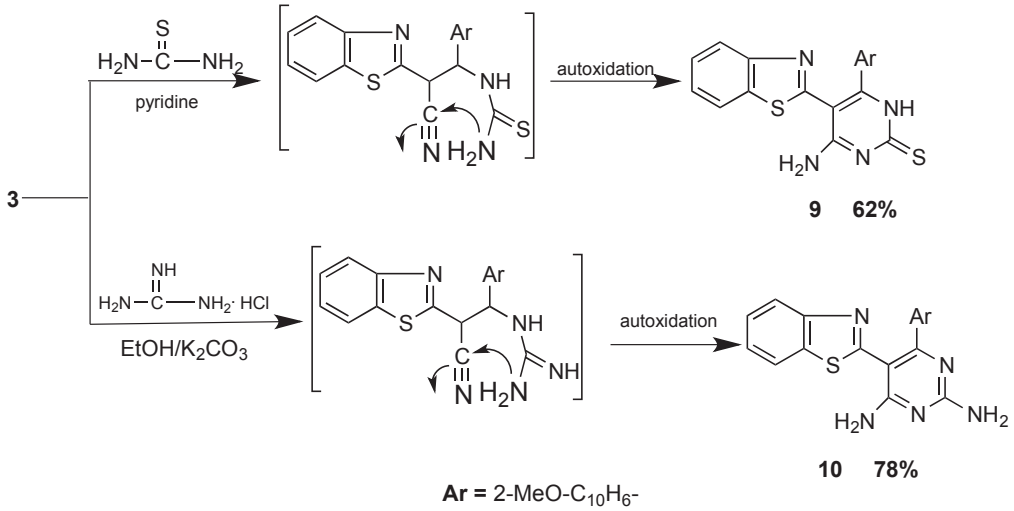
Scheme 1. Synthesis of azoles derivatives **4-8**

Thus, when **3** was treated with 2-cyanoacetohydrazide in refluxing EtOH containing a catalytic amount of piperidine, it furnished a single product for which two possible structures; the pyrazolone derivative structure **7** *via* aza-Michael addition[29] or the 2-pyridone derivative structure **7a** *via* Michael addition[30] (Scheme 1). Based on the spectral data, structure **7** was assigned to the structure product. For example, its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of four singlet signals at 3.98, 4.82, 5.89 and 9.97

ppm assignable for MeO, C_4-H of pyrazole, NH_2 and NH, respectively. In addition, two doublet signals appear at 5.32 and 5.45 ppm due to two vicinal CH groups. Also, ^{13}C-NMR spectrum revealed four signals in the region of 40-80 ppm corresponding to methoxy group, C_2, C_3 of propanenitrile and C_4 of pyrazole. The synthesis of isoxazole derivatives from α, β-unsaurated nitriles was described in the literature[31] *via* multistep reaction. Herein, we synthesized the isoxazole derivative **8** by the one-pot reaction of compound **3** with hydroxylamine hydrochloride in refluxing EtOH containing anhydrous NaOAc (Scheme 1). The structure of the product **8** was supported by its elemental analysis and spectroscopic data. Its IR spectrum displayed stretching vibration bands at 3418, 3308 cm^{-1} corresponding to NH_2 group. Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of singlet signals at δ 3.96 and 6.15 ppm assignable for MeO and NH_2 groups, respectively.

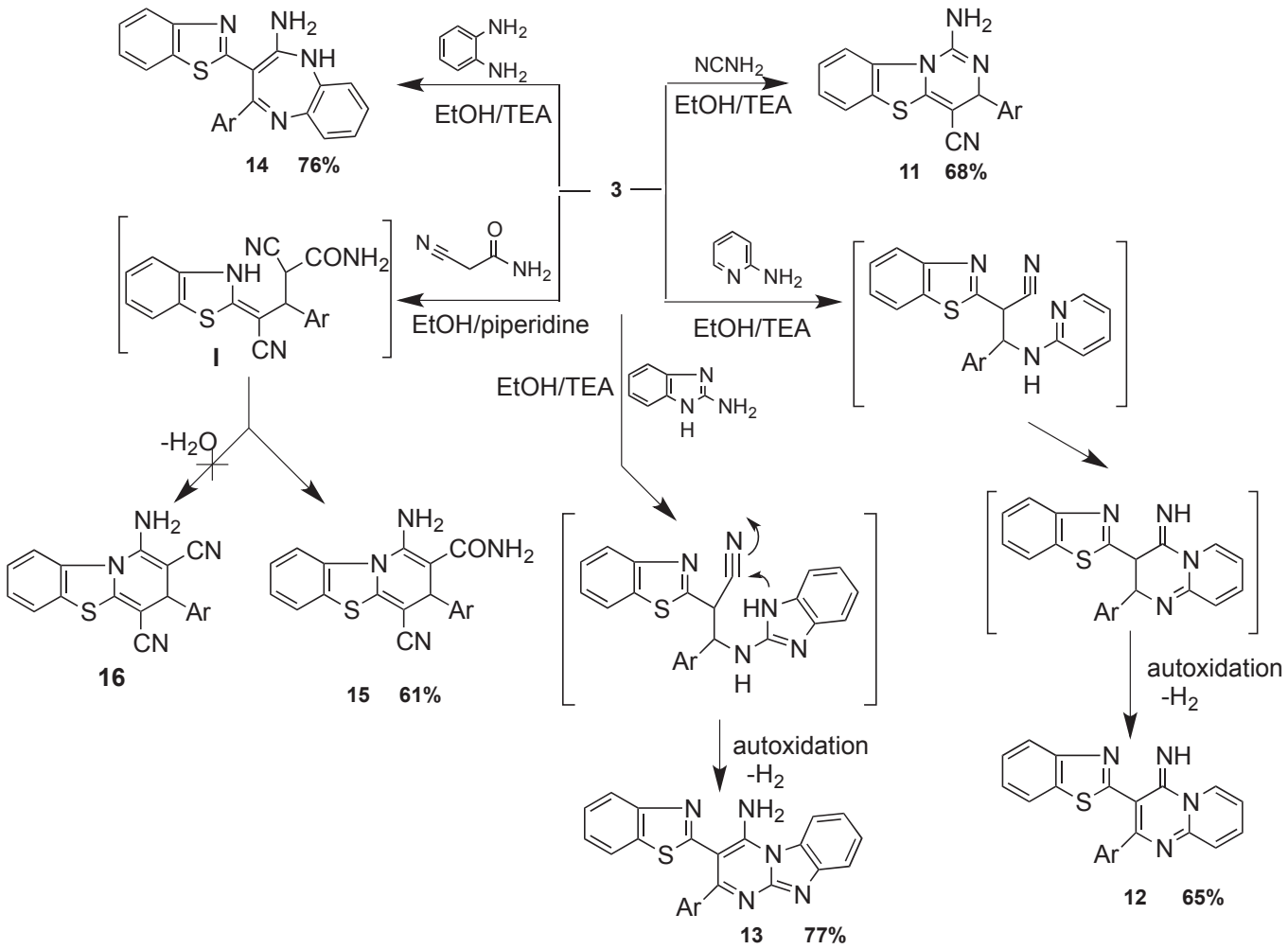
We have also investigated the reactivity of **3** towards 1,3-bifunctional nucleophiles.[32] Thus, treatment of **3** with thiourea in boiling pyridine afforded 4-amino-5-(benzo[*d*]thiazol-2-yl)-6-(2-methoxynaphthalen-1-yl)-pyrimidine-2(1*H*)-thione (**9**) (Scheme 2). Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of singlet signals at 4.04, 5.12 and 10.71 ppm assignable for MeO, NH_2 and NH groups, respectively. The ^{13}C-NMR spectrum revealed a signal at 180.3 ppm due to C=S group.

Similarly, when **3** reacted with guanidine hydrochloride in EtOH containing anhydrous K_2CO_3, 5-(benzo[*d*]thiazol-2-yl)-6-(2-methoxynaphthalen-1-yl)pyrimidine-2,4-diamine (**10**) was obtained (Scheme 2). Its IR spectrum displayed four stretching vibration bands from 3454 - 3345 cm^{-1} corresponding to two NH_2 groups. Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of three singlet signals at 3.98, 5.65 and 5.84 ppm assignable for MeO and two NH_2 groups. In addition, the structure of compound **10** was confirmed by its spectroscopic measurement.



Scheme 2. Synthesis of pyrimidine derivatives **9-10**

The foregoing results prompted us to investigate the applicability and synthetic potency of compound **3** to develop a facile and convenient route to bridgehead *N*-heterocyclic systems namely 3*H*-pyrimido[4,3-*b*]benzothiazole derivative (**11**), pyrido[1,2-*a*]pyrimidine derivative (**12**) and pyrimido[1,2-*a*]benzimidazole derivative (**13**) of an expected pharmaceutical interest.[33,34] Thus, reaction of **3** with equimolar amounts of cyanamide, 2-aminopyridine and 2-aminobenzimidazole, respectively, in refluxing EtOH containing a catalytic amount of TEA afforded the corresponding bridgehead heterocyclic *N*-compounds **11-13** (Scheme 3). The formation of compounds **12** and **13** was assumed to proceed *via* nucleophilic addition of amino group to α, β-unsaturated nitrile **3** followed by cycloaddition of NH to nitrile group and finally autoxidation (Scheme 3). Analytical and spectroscopic data for the later compounds were in agreement with the proposed structures. The mass spectra of compounds **11, 12** and **13** showed the molecular ion peaks at *m/z* 384, 434 and 473, respectively, which are in agreement with their proposed structures.



Scheme 3. Synthesis of 1,3-diazine derivatives **11-13,** 1,4-diazepine derivative **14** and pyridobezothiazole derivative **15**

On the other hand, 3-(benzo[*d*]thiazol-2-yl)-4-(2-methoxynaphthalen-1-yl)-1*H*-benzo[*b*][1,4]diazepin-2-amine **(14)** could be achieved by the reaction of **3** with *o*-phenylenediamine in EtOH and a catalytic amount of TEA under reflux rather than benzimidazole derivative reported in the literature[35] (Scheme 3). The formation of compound **14** was assumed to proceed *via* nucleophilic addition of amino group to acrylonitrile derivative **3** followed by cyclization through intramolecular nucleophilic addition of other amino group of *o*-phenylenediamine to the cyano moiety and finally autoxidation. The assignment of structure **14** was based on analytical and spectroscopic data. Its IR spectrum displayed stretching vibration bands at 3442, 3345 and 3183 cm^{-1} corresponding to NH_2 and NH groups. Its ^{1}H-NMR spectrum (DMSO-d_6) revealed the presence of singlet signals at 4.04, 6.42 and 10.26 ppm assignable for MeO, NH_2 and NH groups, respectively. Finally, reaction of **3** with a *C*-nucleophile was also studied under basic condition. Thus, treatment of **3** with 2-cyanoacetamide in EtOH containing a catalytic amount of piperidine afforded Michael adduct intermediate **I,** which can undergo an intramolecular cyclization into **15** or **16**[36] (Scheme 3). Mass spectrum gave molecular ion peak at *m/z* 426 which coincide with the molecular weight of structure **15** and not **16**. Also, IR spectrum displayed stretching vibration bands at 3434, 3422, 3417, 3387 and 2211 cm^{-1} corresponding to two NH_2 and a C≡N groups, in addition to the stretching vibration of a C=O group at 1666 cm^{-1}. ^{1}H-NMR spectrum (DMSO-d_6) of **15** revealed the presence of singlet signals at 4.10, 4.66, 6.62 and 8.50 ppm assignable for MeO, C_4-H pyridine, NH_2 and $CONH_2$ groups, respectively.

CYTOTOXICITY ACTIVITY

The newly synthesized compounds were tested for their *in-vitro* anticancer effect via the standard MTT method[37] against a panel of four human tumor cell lines namely; hepatocellular carcinoma (liver) HepG-2, colorectal carcinoma (colon) HCT-116, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2. 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. Cytotoxicity (IC_{50}) of tested compounds on different cell lines

Compounds	*in vitro* Cytotoxicity IC_{50} (μg/mL)			
	HePG2	HCT-116	MCF-7	Hep-2
5-Fu	2.3±0.09	5.0±0.23	6.6±0.37	3.5±0.21
3	3.3±0.26	5.9±0.25	7.8±0.28	4.2±0.22
4	8.5±0.43	9.3±0.55	11.5±0.64	7.9±0.47
5	5.5±0.23	8.0±0.38	7.6±0.33	6.8±0.31
6	6.3±0.29	7.7±0.32	7.3±0.27	6.2±0.29

7	2.2±0.17	5.1±0.27	6.4±0.23	3.2±0.17
8	3.8±0.19	6.5±0.31	8.4±0.37	5.1±0.23
9	4.9±0.20	7.8±0.29	9.8±0.42	5.8±0.27
10	4.3±0.18	7.1±0.26	9.1±0.34	5.6±0.26
11	3.8±0.11	6.3±0.24	7.9±0.28	5.1±0.22
12	4.4±0.24	7.6±0.33	9.6±0.35	5.7±0.24
13	7.2±0.34	8.6±0.37	10.8±0.52	7.4±0.42
14	8.1±0.47	9.0±0.42	11.0±0.57	7.7±0.48
15	7.2±0.27	8.5±0.29	10.8±0.49	7.4±0.39

IC_{50} (μmol/L): (1-10) very strong, 11-25 (strong), 26-50 (moderate), 51-100 (very weak), 200 (non-cytotoxicity), 5-Fu = 5-Fluorouracil.

In general, activity was observed by all of these molecules ranged from very strong to strong cytotoxic. The obtained results revealed that compound **7** are more potent and efficacious than 5-fluorouracil as reference drug towards hepatocellular carcinoma (liver) HepG-2, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2. As for activity against hepatocellular carcinoma HepG-2, the highest cytotoxic activity was displayed by compounds **3**, **7**, **8** and **11**, which showed the percentage viability IC_{50} at 3.3, 2.2, 3.8 and 3.8 μg/mL, respectively.

Colorectal carcinoma (colon) HCT-116 cell line showed the highest sensitivity towards the tested compounds, as its growth was found to be initiated by five compounds. The best activity was demonstrated by compounds **3**, **7**, **8** and **11**, which have IC_{50} at 5.9, 5.1, 6.5 and 6.3 μg/mL, respectively. On the other hand, mammary gland (breast) MCF-7 cell line showed highest sensitivity towards the tested compounds, as its growth was found to be initiated by five compounds. The best activity was demonstrated by compounds **3**, **5**, **6**, **7** and **11**, which have IC_{50} at 7.8, 7.6, 7.3, 6.4 and 7.9 μg/mL, respectively. Further interpretation of the results revealed that compounds **3** and **7** showed high cytotoxic activity against larynx cancer Hep-2 with IC_{50} at 4.2 and 3.2 μg/mL.

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, compound **7** showed the highest cytotoxic activity towards four line cells.
- The activity of compound **7** cannot compared with the activity of other novel compounds because in compound **7**, the pyrazolone moiety linked with benzothiazole via ethyl linkage, while in other

novel compounds, heterocyclic moiety (pyrazole, pyrimidine or isoxazole) linked directly with benzothiazole ring.

- The activity of compound **7** may be attributed to the presence of the electron withdrawing carbonyl group in pyrazole ring, which may enhance the reactivity of pyrazole compounds.
- The results revealed that compound **6** exhibited the best degrees of inhibitory activity towards colorectal carcinoma (colon) HCT-116, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2 compared with pyrazole derivatives **4** and 5, that may be attributed to the presence of carbothioamide group.
- Significant activities against four cell lines were noted with the attachment of isoxaozole derivative to benzothiazole nucleus as in compound **8** than compounds containing linking pyrimidine-benzothiazole as in compounds **9, 10.**
- Fused pyrimido[6,1-*b*]benzothiazole derivative **11** displayed very strong activity towards four line cells, while introducing of other fused heterocyclic system ring to benzothiazole derivative diminishes the activity against all cell lines, may be due its bulky size as in compounds **12-15**.

CONCLUSION

The pyrazole, pyrimidine and isoxazole derivatives incorporating benzothiazole moiety were prepared by simple and efficient synthetic methodology. All compounds showed prominent cytotoxic activity against four human tumor cell lines. Compound **7** is the most active member in this study with special effective against the human HepG-2, HCT-116, MCF-7 and Hep-2.

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 1H 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 *(E)*-2-(benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)acrylonitrile (3). A mixture of **1** (1.86 g, 0.01 mol) and 2-cyanomethylbenzothiazole (1.74 g, 0.01 mol) in EtOH (20 mL) containing four

drops of TEA was heated under reflux for 2 h, then left to cool to room temperature. The yellow precipitate was filtered off and recrystallized from EtOH to give **3**. Yellow crystals; yield (3.14 g, 92%); mp 185 ºC (EtOH); IR (KBr) ν/cm^{-1} = 2227 (C≡N), 1620 (C=N), 1602 (C=C); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.09 (s, 3H, MeO), 7.49-8.27 (m, 10H, Ar-H), 8.79 (s, 1H, vinylic-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.5, 106.4, 114.3, 117.5, 119.2, 122.3, 123.1, 123.7, 124.2 124.5, 125.4, 126.8, 128.4, 129.1, 129.9, 130.2, 136.5, 150.5, 153.4, 153.7, 161.9; MS (EI, 70 eV) *m/z* (%): 342 (M$^+$, 2.7). Anal. Calcd for $C_{21}H_{14}N_2OS$ (342.42): C, 73.66; H, 4.12; N, 8.18. Found: C, 73.59; H, 4.07; N, 8.21.

General procedure for the reaction of 3 with hydrazines. To a solution of **3** (0.68 g, 2 mmol) in EtOH (20 mL), $NH_2NH_2{\cdot}H_2O$ (80%, 0.2 mL) or phenylhydrazine (0.2 mL, 2 mmol) was added. The mixture was heated under reflux for 8 h, and then cooled. The solid product so formed was filtered off, washed with EtOH, dried and recrystallized from a mixture of DMF/EtOH (1:2) to give compounds **4** and **5,** respectively.

4-(Benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)-1*H*-pyrazol-5-amine (4). Yellow powder; yield (0.56 g, 76%); mp 266 ºC (DMF/EtOH); IR (KBr) ν/cm^{-1} = 3435, 3414 (NH_2), 3125 (NH), 1628 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.06 (s, 3H, MeO), 6.21 (s, 2H, NH_2, D_2O exchangeable), 7.50-8.07 (m, 10H, Ar-H), 9.86 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.4, 90.2, 107.6, 119.8, 121.6, 122.8, 123.7, 124.1, 124.4, 125.3, 126.6, 128.2, 128.7, 129.8, 130.6, 136.5, 144.3, 150.6, 153.5, 154.2, 157.6; MS (EI, 70 eV) *m/z* (%): 372 (M$^+$, 5.9). Anal. Calcd for $C_{21}H_{16}N_4OS$ (372.45): C, 67.72; H, 4.33; N, 15.04. Found: C, 67.66; H, 4.26; N, 14.99.

4-(Benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)-1-phenyl-1*H*-pyrazol-5-amine (5).
Yellowish brown powder; yield (0.58 g, 65%); mp 152 ºC (DMF/EtOH); IR (KBr) ν/cm^{-1} = 3407, 3382 (NH_2), 1626 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, MeO), 6.28 (s, 2H, NH_2), 7.30-8.20 (m, 15H, Ar-H); ^{13}C-NMR (75 MHz, (DMSO-d_6) δ (ppm): 56.3, 90.2, 107.6, 119.5, 121.5, 122.1, 123.6 (2C), 124.7, 124.9, 125.1, 125.4, 126.2, 126.9, 128.6, 129.1 (2C), 129.7, 130.0, 130.4, 133.4, 139.4, 142.2, 144.5, 150.5, 153.4, 157.5; MS (EI, 70 eV) *m/z* (%): 448 (M$^+$, 52.3). Anal. Calcd for $C_{27}H_{20}N_4OS$ (448.54): C, 72.30; H, 4.49; N, 12.49. Found: C, 72.26; H, 4.42; N, 12.51.

Synthesis of 5-amino-4-(benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)-1*H*-pyrazole-1-carbothioamide (6). A mixture of **3** (0.68 g, 2 mmol) and thiosemicarbazide (0.18 g, 2 mmol) in pyridine (15 mL) was heated under reflux for 9 h, then allowed to cool to room temperature. The solid product so formed was filtered off and recrystallized from EtOH to afford **6**. Yellowish brown crystals; yield (0.50 g, 59%); mp 222 ºC (EtOH); IR (KBr) ν/cm^{-1}= 3437, 3422, 3401, 3397 ($2NH_2$), 1625 (C=N), 1238 (C=S);

^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.05 (s, 3H, MeO), 6.21 (s, 2H, NH_2), 6.64 (s, 2H, $CSNH_2$), 7.40-8.10 (m, 10H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.2, 87.2, 107.4, 119.6, 121.5, 121.8, 122.8, 123.5, 124.3, 125.3, 126.6, 128.4, 129.8, 131.3, 132.8, 133.2, 137.3, 146.2, 150.5, 153.4, 157.5, 178.6; MS (EI, 70 eV) *m/z* (%): 431 (M^+, 50.0). Anal. Calcd for $C_{22}H_{17}N_5OS_2$ (431.53): C, 61.23; H, 3.97; N, 16.23. Found: C, 61.17; H, 3.91; N, 16.19.

Synthesis of 3-(5-amino-3-oxo-2,3-dihydro-1*H*-pyrazol-1-yl)-2-(benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)propanenitrile (7). A mixture of **3** (0.68 g, 2 mmol) and 2-cyanoacetohydrazide (0.198 g, 2 mmol) in EtOH (20 mL) containing four drops of piperidine, was heated under refluxed for 3 h. The solid product so formed was filtered off and recrystallized from EtOH to give compound **7**. White crystals; yield (0.74 g, 84%); mp 208-210 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3407, 3349 (NH_2), 3222 (NH), 2254 (C≡N), 1692 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.98 (s, 3H, MeO), 4.82 (s, 1H, C_4-H pyrazole), 5.32 (d, 1H, J = 6.8 Hz, C_2-H propanenitrile), 5.45 (d, 1H, J = 6.9 Hz, C_3-H propanenitrile), 5.89 (s, 2H, NH_2), 7.40-8.12 (m, 10H, Ar-H), 9.97 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 40.2, 55.9, 56.5, 76.3, 107.2, 119.6, 120.5, 121.4, 122.4, 122.8, 123.5, 124.3, 125.5, 126.8, 128.3, 128.8, 129.5, 133.2, 135.5, 153.4, 154.2, 168.2, 171.4, 172.6; MS (EI, 70 eV) *m/z* (%): 441 (M^+, 43.3). Anal. Calcd for $C_{24}H_{19}N_5O_2S$ (441.51): C, 65.29; H, 4.34; N, 15.86. Found: C, 65.25; H, 4.28; N, 15.89.

Synthesis of 4-(benzo[*d*]thiazol-2-yl)-3-(2-methoxynaphthalen-1-yl)isoxazol-5-amine (8). A mixture of **3** (0.68 g, 2 mmol) and $NH_2OH{\cdot}HCl$ (0.16 g, 2.3 mmol) in EtOH (20 mL) containing anhydrous NaOAc (0.9 g, 11 mmol) was heated under reflux for 6 h, then allowed to cool to room temperature and diluted with ice cold H_2O (30 mL). The solid product so formed was filtered off, washed with H_2O and recrystallised from EtOH to afford **8**. Yellow crystals; yield (0.53 g, 71%); mp 147 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3418, 3308 (NH_2), 1623 (C=N);^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.96 (s, 3H, MeO), 6.15 (s, 2H, NH_2, D_2O exchangeable), 7.41-8.16 (m, 10H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.2, 98.6, 107.6, 117.5, 119.2, 121.5, 123.3, 124.0, 124.2, 125.3, 126.6, 128.0, 129.5, 129.7, 133.2, 133.4, 150.5, 153.8, 154.1, 157.4, 160.1; MS (EI, 70 eV) *m/z* (%): 373 (M^+, 30.1). Anal. Calcd for $C_{21}H_{15}N_3O_2S$ (373.43): C, 67.54; H, 4.05; N, 11.25. Found: C, 67.49; H, 3.98; N, 11.20.

Synthesis of 4-amino-5-(benzo[*d*]thiazol-2-yl)-6-(2-methoxynaphthalen-1-yl)pyrimidine-2(1*H*)-thione (9). A mixture of **3** (0.68 g, 2 mmol) and thiourea (0.15 g, 2 mmol) in pyridine (15 mL) was heated under reflux for 10 h, then allowed to cool to room temperature. The solid product was collected by filtration and recrystallized from EtOH to afford compound **9**. Yellowish brown crystals; yield (0.51 g, 62%); mp 171 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3453, 3417 (NH_2), 3227 (NH), 1623 (C=N); ^{1}H-NMR (300

MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, MeO), 5.12 (s, 2H, NH_2), 7.40-8.10 (m, 10 H, Ar-H), 10.71 (s, 1H, NH); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.2, 100.2, 114.3, 119.6, 121.6, 121.9, 123.8, 124.5, 125.1, 125.3, 126.6, 128.5, 129.8, 130.3, 132.8, 133.1, 136.4, 150.4, 151.6, 158.5, 160.5, 180.3; MS (EI, 70 eV) *m/z* (%): 416 (M^+, 9.9). Anal. Calcd for $C_{22}H_{16}N_4OS_2$ (416.52): C, 63.44; H, 3.87; N, 13.45. Found: C, 63.40; H, 3.81; N, 13.39.

Synthesis of 5-(benzo[*d*]thiazol-2-yl)-6-(2-methoxynaphthalen-1-yl)pyrimidine-2,4-diamine (10). A mixture of **3** (0.68 g, 2 mmol) and guanidine hydrochloride (0.22 g, 2.3 mmol) in EtOH (20 mL) containing an anhydrous K_2CO_3 (0.55 g, 4 mmol) was heated under reflux for 8 h. The reaction mixture was allowed to cool to room temperature, and diluted with ice-cold H_2O (30 mL) containing few drops with HCl. The solid product so formed was filtered off, washed with H_2O and recrystallized from EtOH to afford **10**. Yellowish brown crystals; yield (0.62 g, 78%); mp 157 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3454, 3419, 3389, 3345 ($2NH_2$), 1622 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.98 (s, 3H, MeO), 5.65 (s, 2H, NH_2), 5.84 (s, 2H, NH_2), 7.33-8.10 (m, 10 H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.2, 104.1, 107.3, 119.6, 121.5, 122.2, 123.8, 124.1, 124.4, 125.6, 126.6, 128.4, 129.6, 129.8, 133.6, 134.2, 150.5, 153.4, 157.6, 160.2, 162.4, 164.1; MS (EI, 70 eV) *m/z* (%): 399 (M^+, 70.5). Anal. Calcd for $C_{22}H_{17}N_5OS$ (399.47): C, 66.15; H, 4.29; N, 17.53. Found: C, 66.16; H, 4.26; N, 17.48.

General procedure for the reaction of 3 with different amines. To a solution of **3** (0.68 g, 2 mmol) in EtOH (20 mL) containing four drops of Et_3N, an equimolar amount of the appropriate amines (cyanamide, 2-aminopyridine and 2-aminobenzimidazole) was added and the mixture was heated under reflux for 8 h, then allowed to cool. The precipitate product was filtered off and recrystallized from EtOH to give compounds **11-13**.

1-Amino-3-(2-methoxynaphthalen-1-yl)-3*H*-pyrimido[4,3-*b*]benzothiazole-4-carbonitrile (11). Yellowish brown powder; yield (0.52 g, 68%); mp 183-185 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3445, 3345 (NH_2), 2234 (C≡N), 1620 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 3.82 (s, 3H, MeO), 4.28 (s, 1H, C_3-H), 6.61 (s, 2H, NH_2), 7.35-8.10 (m, 10 H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 45.2, 56.5, 70.2, 107.3, 117.4, 119.5, 120.8, 121.2, 122.7, 123.2, 123.5, 124.6, 126.4, 126.6, 128.2, 128.3, 129.2, 133.3, 145.5, 153.2, 153.9, 160.3; MS (EI, 70 eV) *m/z* (%): 384 (M^+, 17.8). Anal. Calcd for $C_{22}H_{16}N_4OS$ (384.46): C, 68.73; H, 4.20; N, 14.57. Found: C, 68.68; H, 4.18; N, 14.51.

3-(Benzo[*d*]thiazol-2-yl)-2-(2-methoxynaphthalen-1-yl)-4*H*-pyrido[1,2-*a*]pyrimidin-4-imine (12). Yellowish green powder; yield (0.56 g, 65%); mp 195 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3228 (NH), 1623 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, MeO), 7.47-8.22 (m, 14H, Ar-H), 9.81 (s, 1H,

NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.5, 107.3, 114.2, 119.3, 121.4, 121.6, 123.6, 124.7, 124.9, 125.1, 125.6, 125.8, 126.6, 128.2, 129.8, 130.2, 133.2, 136.3, 136.5, 138.1, 145.5, 150.4, 151.0, 153.2, 160.2, 162.3; MS (EI, 70 eV) *m/z* (%): 434 (M^+, 82.1). Anal. Calcd for $C_{26}H_{18}N_4OS$ (434.52): C, 71.87; H, 4.18; N, 12.89. Found: C, 71.82; H, 4.11; N, 12.81.

3-(Benzo[*d*]thiazol-2-yl)-2-(2-methoxynaphthalen-1-yl)pyrimido[1,2-*a*]benzimidazole-4-amine (13). Yellowish green powder; yield (0.72 g, 77%); mp 180 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3443, 3345 (NH_2), 1620 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, MeO), 6.42 (s, 2H, NH_2), 7.47-8.22 (m, 14H, Ar-H); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.5, 78.2, 95.5, 114.2, 117.6, 117.8, 119.0, 119.4, 119.7, 121.6, 124.4, 124.7, 125.1, 125.6, 126.8, 127.3, 128.3, 129.5, 133.4, 133.7, 136.2, 136.5, 138.4, 150.2, 153.3, 155.6, 160.4, 163.1; MS (EI, 70 eV) *m/z* (%): 473 (M^+, 32.5). Anal. Calcd for $C_{28}H_{19}N_5OS$ (473.55): C, 71.02; H, 4.04; N, 14.79. Found: C, 70.95; H, 3.98; N, 14.74.

Synthesis of 3-(benzo[*d*]thiazol-2-yl)-4-(2-methoxynaphthalen-1-yl)-1*H*-benzo[*b*][1,4]diazepin-2-amine (14). A mixture of **3** (0.68 g, 2 mmol) and *o*-phenylenediamine (0.21 g, 2 mmol) in EtOH (20 mL) containing two drops of Et_3N was heated under reflux for 8 h, then allowed to cool to room temperature. The solid product so formed was filtered off and recrystallized from EtOH to afford **14**. Yellow powder; yield (0.68 g, 76%); mp 245 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3442, 3345 (NH_2), 3183 (NH), 1620 (C=N); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.04 (s, 3H, MeO), 6.42 (s, 2H, NH_2), 7.33-8.20 (m, 14H, Ar-H), 10.26 (s, 1H, NH, D_2O exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 56.5, 76.2, 108.3, 113.4, 119.5, 121.4, 123.6, 123.8, 124.0, 124.2, 124.5, 125.1, 126.6, 126.9, 127.6, 128.5, 129.6, 133.2, 136.4, 137.1, 138.8, 141.2, 150.3, 153.6, 154.2, 160.1, 166.4; MS (EI, 70 eV) *m/z* (%): 448 (M^+, 16.0). Anal. Calcd for $C_{27}H_{20}N_4OS$ (448.54): C, 72.30; H, 4.49; N, 12.49. Found: C, 72.24; H, 4.42; N, 12.41.

Synthesis of 1-amino-4-cyano-3-(2-methoxy-1-naphthyl)-3*H*-pyrido[2,1-*b*]benzothiazole-2-carboxamide (15). A mixture of **3** (0.68 g, 2 mmol) and 2-cyanoacetamide (0.17 g, 2 mmol) in EtOH (20 mL) containing two drops of piperidine was heated under reflux for 8 h, then allowed to cool to room temperature. The solid product so formed was filtered off and recrystallized from EtOH to afford compound **15**. Yellow powder; yield (0.52 g, 61%); mp 191 ºC (EtOH); IR (KBr) ν/cm^{-1} = 3434, 3422, 3417, 3387 ($2NH_2$), 2211 (C≡N), 1666 (C=O); ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 4.10 (s, 3H, MeO), 4.66 (s, 1H, C_3-H), 6.62 (s, 2H, NH_2), 7.30-8.28 (m, 14H,Ar-H), 8.50 (s, 2H, $CONH_2$); ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 38.1, 56.2, 74.3, 81.4, 107.5, 117.6, 119.4, 120.3, 121.2, 112.9, 123.5, 123.7, 124.2, 126.6, 126.7, 128.0, 128.5, 129.1, 133.6, 145.5, 153.5, 157.6, 160.2, 172.5; MS (EI, 70 eV) *m/z* (%): 426 (M^+, 1.0). Anal. Calcd for $C_{24}H_{18}N_4O_2S$ (426.49): C, 67.59; H, 4.25; N, 13.14. Found: C, 67.55; H, 4.28; N, 13.11.

ANTITUMOR EVALUATION

The synthesized compounds were evaluated for their *in vitro* anticancer effect *via* the standard MTT method,[30] against a panel of four human tumor cell lines namely; hepatocellular carcinoma (liver) HepG-2, colorectal carcinoma (colon) HCT-116, mammary gland (breast) MCF-7 and epidermoid carcinoma (larynx) Hep-2. The cell lines were obtained from ATCC *via* the Holding company for biological products and vaccines (VACSERA), Cairo, Egypt. 5-Fluorouracil (5-Fu) was used as a standard anticancer drug for comparison.

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