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Synthesis and Antitumor Activity Evaluation of New 2-(4-aminophenyl) benzothiazole/oxazole/imidazole Derivatives

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A NEW series of 2-(4-aminophenyl)benzothiazole and related structure compounds, bearing thiocyanate, thiol in the 3'postion as well as new condensed benzothiazoles and related structure compounds having thiazolones ,tetrazole, oxadiazole have been synthesized . All the newly synthesized compounds were screened for their anti-tumor activities. The results revealed promising effects against most of the cancer cell lines.

Keywords: 2-(4 aminophenyl) benzothiazole/ benzoxazole/ benzimidazole, Tetrazole, oxadiazole, Thiazole, Oxobutanoate, Tolyiformamidine, Chlorophenyl-formamidine, Anticancer agent.

The relationship between chemical and biological activity was found of interest to pharmacologists and medicinal chemists , it was the basic of attempts to design compounds of therapeutic value. The structural simplicity and synthetic accessibility of benzothiazole[(1-4)],benzoxazole and benzimidazole series show remarkable antitumor properties. A series of potent and selective agents derived from 2-(4-Aminophenyl)benzothiazole structure was extensively examined and developed during recent years to have antitumor activity since 1996[(5-19)], Unexpectedly, it was found that , 2-(4-aminophenyl) benzothiazole and related structure derivatives inhibit cancer cell growth with nanomolar scale against a large panel of human cancer cell lines particularly against breast , colon and ovarian cell lines in *in-vitro* anticancer screening program of the National Cancer Institute (NCI) with a characteristic biphasic dose–response relationship. The original (unsubstituted) member of this series, 2-(4-aminophenyl) benzothiazole was considered to be "Lead "approach to drug discovery of new anticancer agents. They are, therefore, used as important intermediates for synthesis of a variety of potent commercial drugs[(20-22)] .For example, chemical structures of these drugs here pimobendan (ionodilator), mebendazole (anthemintic) , phortress, Albendazole and

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Omeprazole. Phortress8 (NSC 710305) as shown in Fig.1. Motivated by the above observations and as extension of on our previous study[23-24], we planned to synthesize new series of 2-(4-aminophenyl) benzothiazole derivatives including (benzimidazole / benzoxazole/ benzothiazole substituted in the 3-position of the phenyl ring by thiocyanate or thiol substituents. All the newly synthesized compounds were evaluated for their antitumor activity against nine cancer types comprised of approximately 60 cell lines at NCI at USA.

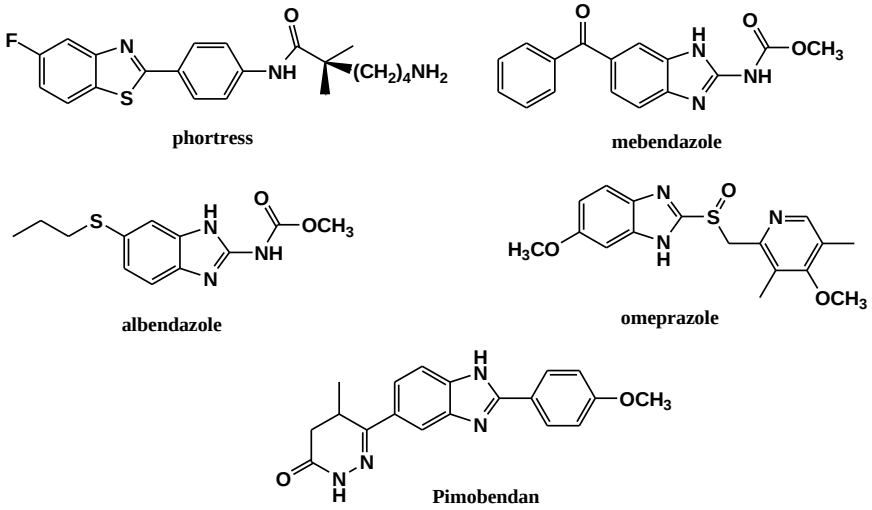


Fig.1.

Results and Discussion

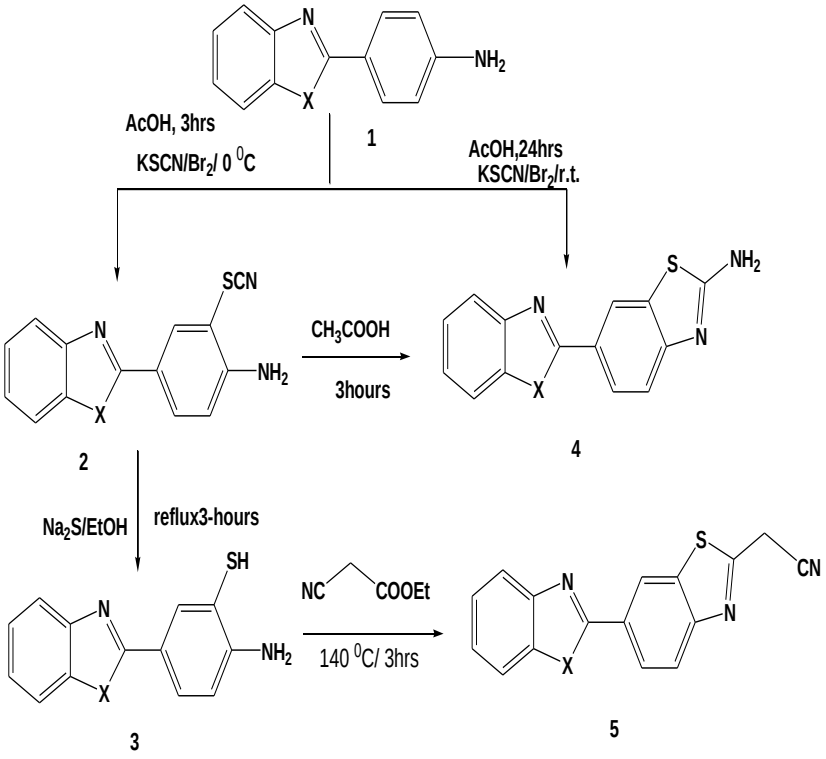
Chemistry

The key starting material 2-(4-aminophenyl) benzothiazole and its structures (1a-c) were prepared as previously reported[25] by the condensation of 4-aminobenzoic acid with 2-aminothiophenol, 2-aminophenol or 1, 2-phenylenediamine, respectively, in presence of polyphosphoric acid at high temperature.

One-pot reaction of appropriate 1a-c with thiocyanogen generated from bromine and alkaline thiocyanate in acetic acid medium afforded different compounds. So, when the reaction was carried out at zero temps for 3 hr. The compounds 2a-c thiocyanate benzeneamine were formed.

On the other hand, new amino thiazole derivatives 4a-c were formed when the reaction was carried out with stirring at room temperature for 24 hr. Also, it were formed when compounds 2a-c was heated in acetic acid at 60^0C for 3hr to give 4a-c. When compound 2a-c was reacted with sodium sulfide in ethyl alcohol, substitution of the thiocyanate by thiol was occurred at 3' -postion of 2-phenyl group to afforded 3a-c derivatives. The latter compounds

were reacted with ethyl cyanoacetate at 140^{0}C for 3 hours to give thiazol-2-acetonitrile derivatives 5a, b as shown in Scheme1.

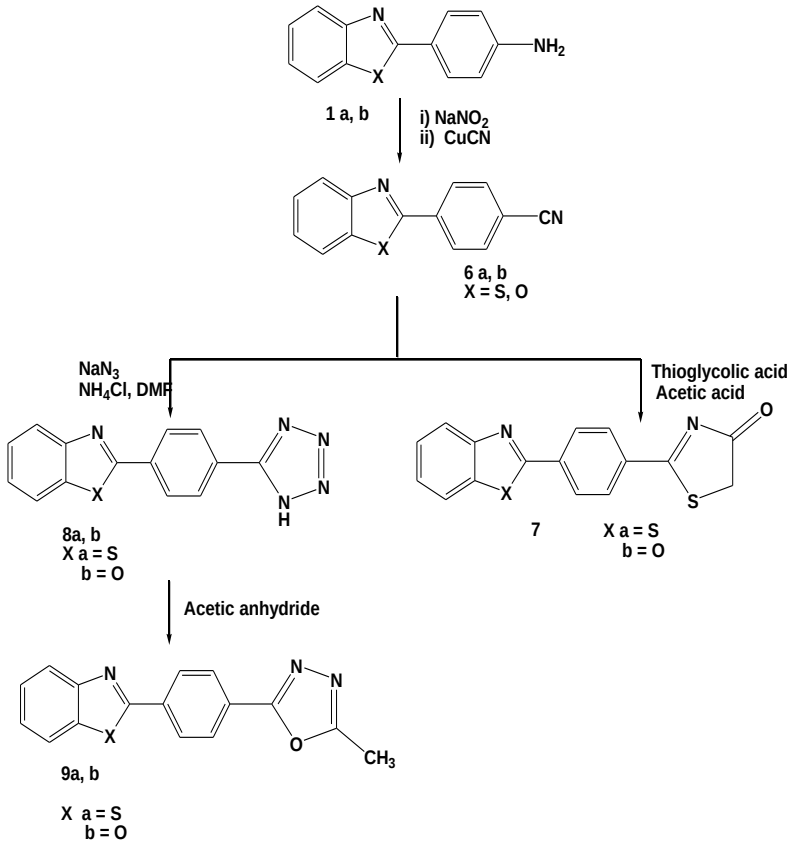


Scheme 1.

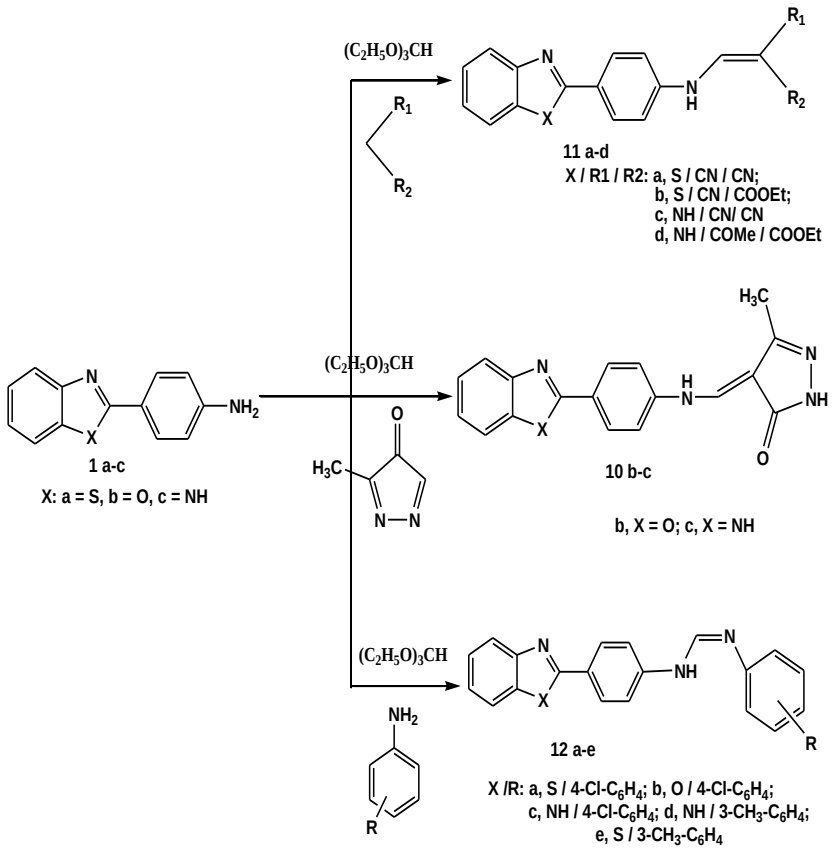
Moreover, the action of copper (I) cyanide on diazonium salts of compounds 1a-c at 60-70^{0}C for 15 min furnished compounds 6a, b through the Sandmeyer reaction. Cyclocondensation occurred between the latter compounds with thioglycolic acid in boiling glacial acetic acid to form thiazolidin-4-one derivatives 7a-c as shown in (Scheme2).

On the other hand, reaction of 6a, b with sodium azide and ammonium chloride in DMF gave tetrazoles analogs of benzothiazole/ benzoxazole structures of compounds 8a, b. 2-Methyl -1, 3, 4- oxadiazoles derivatives 9a,b were prepared through thermal rearrangement of compound 8 by heating with acetic anhydride . The reaction was represented as ring openings of tetrazoles to give intermediates which loss nitrogen to give new oxdiazoles rings[26]. When 2-(4-aminophenyl) benzothiazole and its derivatives 1b,c

reacted with 3- methylpyrazolone and triethyl orthoformate gave methylene -3 - 1 H - pyrazolo- 5 - (4H)- one compounds 10b,c . Also,1a, b reacted with triethyl orthoformate and active methylene aliphatic compounds as malononitrile , ethyl cyanoacetate , ethyl acetoacetate and afforded methylenecyanoacrylate (oxobutanoate) derivatives 11a-d. As well as 1a,b reacted with aromatic amino compounds and triethylorthoformate and gave formamidine derivatives 12a-e as shown in Scheme 3. The structures of the synthesized compounds were assigned on the basis of elemental analysis ,IR,^{1}H-NMR and mass spectra data .The compounds were screened for their *in-vitro* antitumor activity.



Scheme 2



Scheme 3.

Biological screening (antitumor activity)

The hetero cyclic compounds, prepared in this study, were evaluated according to their cytotoxic and /or growth inhibitory effects of the compounds were evaluate *in vitro* against approximately 60 human tumor cell lines derived from nine neoplastic diseases ,namely: leukemia(L), non-small cell lung cancer(NSCLC),colon cancer (CC),central nervous system cancer (CNSC), melanoma(M), ovarian cancer(OC), renal cancer (RC),prostate cancer(PC) and breast cancer (BC) at NCI, Bethesdas, USA. The *in vitro* screening program was based upon the use of multiple panels of 60 human tumor cell lines, against which the compounds were tested at tenfold dilutions of five concentrations ranging from 10^{-4} to 10^{-8} M. After incubation of 48-h continuous drug exposure protocol was followed, and a sulforhodamine B protein assay was used to estimate cell growth[27-29]. All the newly synthesized compounds were offered for testing their anticancer activity according to *in-vitro* drugs screening

protocol of the institute. Compounds 2b,3b,7a,c,9b,10c,12a,b and 12c were carried out against 60 human tumor cell lines drived from nine cancer types L,NSCLC, CC,CNS, RC, PC and BC.

The results were reported both as dose response curves and pictographically as "mean graphs" compounds were consider to be active only if their $LogGI_{50}$ values less than -4.5 compared to 5-Fluorouracil(5-Flu) the NCI standard anticancer agent.

The obtained data revealed that most of the newly synthesized compounds showed potent antitumor activity as shown in Table 1.

Both compounds possess Log GI_{50} values lawer than -4.5 or-5,showing a notable activity level. Among the tested compounds , compound 10c demonstrate higher activity than standard against the following cell lines L and BC $LogGI_{50}$ -7.18 and -8.0 respectively as shown in Fig.2.

Also, Compound 2b showed higher activity than standard against the following cell line CNS and L. $LogGI_{50}$-7.16 and -6.6, respectively as shown in Fig.3.

TABLE 1. *In-vitro* disease-oriented anti-tumor screening.

***No. of Comp.**	**L**	**CNS**	**NSCL**	**BC**	**MC**	**CC**	**RC**	**OC**	**PC**
2b	-6.66	-7.16	-5.42	-4.86	NT	-4.87	-4.91	NT	-5.06
3b	-5.11	-4.97	-4.93	-5.21	NT	-5.09	-4.94	-5.12	-5.16
7a	-4.88	-4.55	-5.74	-5.12	-5.13	-5.51	NT	-4.80	NT
7c	-5.24	-4.53	-5.49	5.05	-4.80	-4.78	-4.76	-4.64	-4.50
9b	-4.59	-4.79	-4.58	NT	-4.81	NT	NT	NT	NT
10c	-7.18	-4.84	-4.70	-8.0	-NT	-4.74	NT	-4.83	NT
12a	-5.02	-5.11	-5.40	-5.03	-6.84	-5.02	-4.93	-5.03	-4.78
12b	-4.63	-4.98	-5.40	-5.03	-5.02	-4.96	-4.93	-4.95	-5.72
12c	-6.20	-4.68	-6.32	-5.58	-6.41	-6.16	-6.08	-5.27	NT
5-FU[a]	-6.25	-5.87	-5.70	-5.47	-6.03	-6.73	-6.24	-5.74	-6.03

$LogGI_{50}$:Log concentration which able reduce cell growth to 50% (a).NCI's data for 5-Flourouracil NSC 19893(NCI standard compound.).

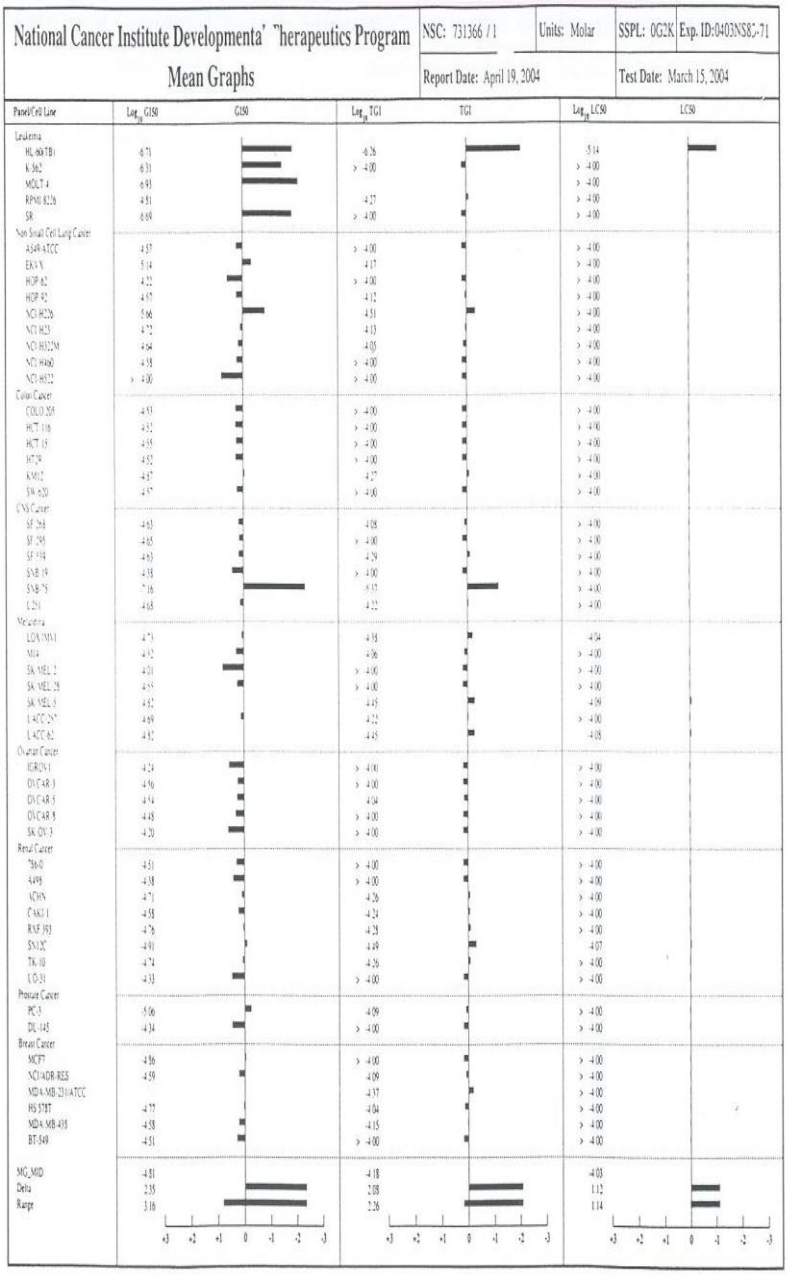


Fig. 2. It represent higher activity for compound 10c than standard against the following cell lines L and BC LogGI50 -7.18 and -8.0, respectively .

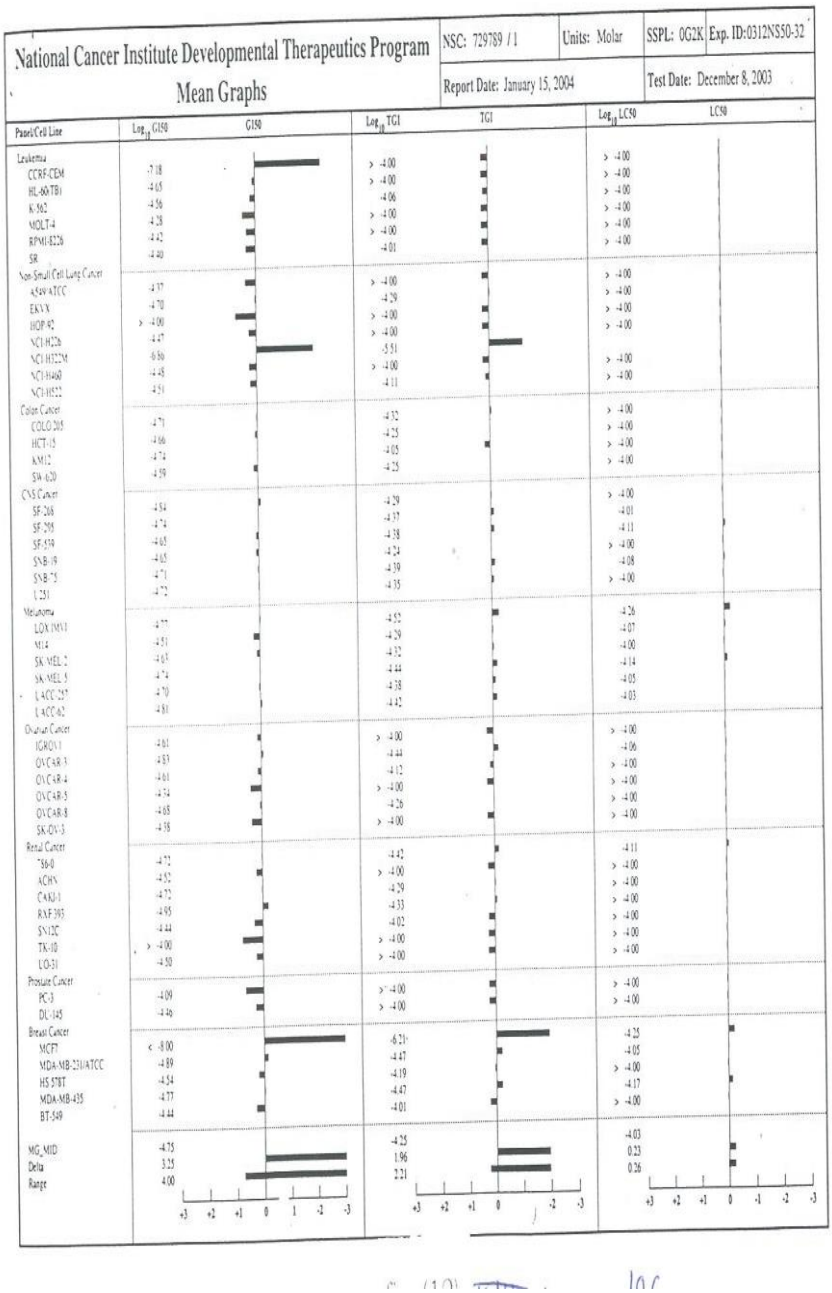


Fig.3. Compound 2b showed higher activity than standard against the following cell line CNS and L. LogGI50-7.16 and -6.6, respectively.

Experiment

Experimental procedure

The structure of all the synthesized compounds are confirmed by various spectral studies. All melting points are uncorrected and determined by the open capillary method using Gallen Kamp melting point apparatus. Microanalysis were carried out by the Micro Analytical unit at Cairo University. IR spectra KBr disk were recorded on FTIR-300E Jasco spectro- photometer. ^{1}HNMR spectra were recorded on a Varian EM 200 MHz using (TMS as internal reference). Mass spectra were taken on Cairo University GC MS-QP/1000 EX(Shimadzu). All the results were in an acceptable range. Compounds 1a-c were synthesized according to the reported method[25].

General method for the synthesis of compounds (2a-c)

A mixture of appropriate 2-(4-aminophenyl) benzothiazol, benzoxazol, benzimidazole 1a-c (0.01mol), potassium thiocyanate (0.01 mol) and bromine (0.01mol) in glacial acetic acid (40ml) was stirred for 3 hr at 0^0C then the reaction was allowed to cool to room temp. and poured into water. The precipitate was collected and washed several times with water and re-crystallized from ethanol to give 2a-c.

4-(benzo[d]thiazol-2-yl)-2-thiocyanatobenzenamine (2a)

Yellow solid, (80% yield), m.p. 88-90 °C; IR (KBr) ν_{max} 3449,3410 (NH_2),3053(aromaticC-H), 2212 (CN), 1600 , 1575, 1445 (CH-aromatic) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ: 3.56 (s, 2H, NH_2), 6.36 (s,1H, aromatic-H6), 7.3-8.12 (m, 6H, ArH's); Anal. Calcd. for $C_{14}H_9N_3S_2$ (283.37): C, 59.34; H, 3.20. Found: C, 59.04; H, 3.53% .

4-(benzo[d]oxazol-2-yl)-2-thiocyanatobenzenamine (2b)

White solid, (65% yield), mp 129-130 °C; IR (KBr) ν_{max} 3443, 3400 (NH_2), 3067(aromatic-CH) ,2155 (CN), 1600 , 1575, 1445 (CH-aromatic) cm^{-1}; ^{1}H NMR (DMSO-d_6) δ:5.6(s,2H,NH_2),6.36(s,1H,aromatic-H5),7.01-7-15(m,2H,ArH),7.26-7.20(m,4H,ArH); MS; m/z (%) 267 (M^+,100) , 269 (M^{+2}, 7.7) , (209, 1.3) , (119, 1.6), (93, 2.0) Anal. Calcd. for $C_{14}H_9N_3OS$ (267.31) C, 62.91; H, 3.39; Found: C, 59.04; H, 3.53%.

4-(1H-benzo[d]imidazol-2-yl)-2-thiocyanatobenzenamine (2c)

Yellow solid, (68% yield), mp 213-215°C; IR (KBr) ν_{max} 3422,3410 (NH_2), 3321 (NH),3174(aromatic- CH) , 2218 (CN), 1600 , 1575, 1445 (CH-aromatic) cm^{-1} ; ^{1}H NMR (DMSO-d_6) δ: 4.3 (s,2H,NH_2) , 6.5-7.2(m, 3H, ArH), 7.25-7.50 (m , 4H, benzimidazol-H), 10.73(s,1H,NH) ; Anal. Calcd. for $C_{14}H_{10}N_4S$ (266.32): C, 63.14; H, 3.78; Found: C, 63.44; H, 3.53%.

General method for synthesis of compounds (3a-c).

A solution of 2a-c (0.01 mol) in ethyl alcohol (50 ml) and sodium sulphide (0.01mol) in (20 ml) water was heated on water bath for one hour, then allowed

to cool at room temperature, and poured into water, acidified with acetic acid. The precipitate was collected and washed several times with water then with ether and re-crystallized from ethyl alcohol to give 3a-c.

2-amino-5-(benzo [d] thiazol-2-yl) benzenethiol (3a)

Yellow solid, (50% yield), mp 283-285 °C; IR (KBr) ν_{max} 3366 (NH_2), 3053(aromatic-CH) ,2368 (SH), 1600 , 1575, 1445 (CH-aromatic) cm^{-1}; ^1H NMR (DMSO-d_6) δ :2.5(s,1H,SH),5.20(s, 2H, NH_2), 6.50 (s,1H, aromatic-H5), 7.40-7.48 (m, 6H, Ar-H); Anal. Calcd. for $C_{13}H_{10}N_2S_2$ (258.03): C, 60.43; H, 3.90. Found: C, 60.75; H, 3.55.%.

2-amino-5-(benzo[d]oxazol-2-yl) benzenethiol(3b)

Yellow solid, (55% yield), mp 283-285 °C; IR (KBr) ν_{max} 3326 (NH_2),3174(aromatic-CH), 2212 (SH) cm^{-1}; ^1H NMR (DMSO-d_6) δ: 3.0(s,1H,SH),6.41 (d, 2H, NH_2), 6.44 (s,1H, aromatic-H5), 6.95-7.95 (m, 6H, Ar-H); Anal. Calcd. for $C_{13}H_{10}N_2OS$ (242.05): C, 64.44; H, 4.16 Found: C, 64.75; H,4.74%.

2-amino-5-(1H-benzo[d]imidazol-2-yl)benzenethiol(3c)

White solid, (50% yield), mp 172-175 °C; IR (KBr) ν_{max} 3316 (NH_2),3049(aromatic-CH), 2216 (SH), 1600 , 1575, 1445 (CH-aromatic) cm^{-1}; ^1H NMR (DMSO-d_6) δ 2.8(s,1H,SH),5.91(s, 2H, NH_2), 6.68 (s,1H, aromatic-H5), 7.40-7.48 (m, 6H, Ar-H),10.73(s,1H,NH); Anal. Calcd. for $C_{13}H_{11}N_3S$ (241.07): C, 64.70; H, 4.59; Found: C, 65.02; H, 4.29%.

General method for synthesis of (4a,b)

A mixture of 1a ,b (0.01 mol), potassium thiocyanate (0.01 mol) and bromine (0.01) in glacial acetic acid (40 ml) was stirred at room temperature for 21 hr, then poured into water, the precipitate was collected and washed several times with water then with ether and recrystallized from ethanol to give 4a,b.

6-(benzo[d]thiazol-2-yl)benzo[d]thiazol-2-amine(4a)

Yellow solid, (65% yield), m.p. 199-200 °C; IR (KBr) ν_{max} 3366 (NH_2),3067(aromatic-CH) 1600 , 1575, 1445 (CH-aromatic) cm^{-1}; ^{1}HNMR (DMSO-d_6) δ :5.91(s, 2H, NH_2), 7.48(m,6H,Ar-H),8.29(d,1H,aromatic-H5); MS: m/z's 283(M^+,100%) , 284(M^{+1},18.6%),285(M^{+2},10.0%),(267,1.9%); Anal. Calcd. for $C_{14}H_9N_3S_2$ (283.37.37): C, 59.34; H, 3.20. Found: C, 60.75; H, 3.55%.

6-(benzo[d]oxazol-2-yl)benzo[d]thiazol-2-amine(4b)

Yellow solid, (60% yield), m.p. 209-210°C; IR (KBr) cm^{-1}; 3366 (NH_2), 3067(aromatic-CH), 1575(aromatic ring); ^1H NMR(DMSO-d6) δ : 6.4 (s, 2H, NH_2), 7.13-8.30 (m, 7H, ArH),8.30(d,1H,aromatic-H-5). Ms:m/ z's (M^+, 100%), 268(M^{+1}, 14.1%) , (192, 15.8%), (118, 4.5%), (92, 11.3) ; Anal .

Calculated for $C_{14}H_9N_3OS$ (267.31) : C,62.92; H,3.40. Found: C,62.93; H,3.38 %.

General method for compounds of 5a,b

A mixture of 3a,b (0.01 mol) and ethyl cyanoacetate (0.015 mol) was heated at 130-140 ^{0}C in dimethylformamide (10ml) for 4 hr, the reaction mixture was then 1 cooled to room temperature and ether (30ml) was added. the solid precipitate was collected and washed several times with water and re-crystallized from a suitable solvent to give 5a,b.

2-(6-(benzo[d]thiazol-2-yl)-2,3-dihydrobenzo[d]thiazol-2-yl) acetonitrile(5a)

Yellow solid (71% yield) m.p. 147-150°C IR (KBr) cm^{-1};3067(aromatic-CH), 2368(CN), 1600, 1455(CH-aromatic); ^{1}H NMR (DMSO-d6)δ:4.32 (d, 2H, CH_2), 6.39-8.23 (m, 7H, ArH) . Anal. Calculated for $C_{16}H_9N_3S_2$ (307.39) :C,62.56, H,2.94. Found: C, 62.56; H,2.94.%

2-(6-(benzo[d]oxazol-2-yl)benzo[d]thiazol-2-yl)acetonitrile(5b)

Yellow solid, (50% yield) m.p.290-292°C; IR (KBr) cm^{-1} :3053(aromatic-CH),2368 (CN), 1600, 1455(aromatic ring system); ^{1}H NMR; δ:4.00(s, 2H, CH_2), 7.5-7.7(m, 7H, ArH), Anal. Calculated For $C_{16}H_9N_3OS$(291.33) :C,65.96 ;H,3.11 .Found:C,65.98; H,3.09 %.

General method for synthesis of compounds 6a,b

To a cold solution 1a,b (0.03 mol) in concentrated hydrochloric acid (7.98 ml), diluted with 30 ml water with stirring, was added a solution of sodium nitrite (0.02 mol) dissolved in (80 ml) of water maintaining the temperature below 5^{0}C.The mixture was stirred for 30 min then added portion wise to a solution of copper (I) cyanide (0.26mol) with stirring at 60-70^{0}C for 15 min, then poured into water. The precipitate that formed was filtered and washed with water and re-crystallized from ethanol to give 6a,b.

4-(benzo[d]thiazol-2-yl)benzonitrile(6a)

Orang solid, (50% yield), m.p. 119-220°C; IR (KBr) cm^{-1}; 3053(aromatic-CH), 2230 (CN); ^{1}H NMR ; δ=7.57-8.23 ppm (m, 8H, ArH) ; Anal. Calculated For $C_{14}H_8N_2S$ (236.29): C, 71.16; H, 3.41. Found: C, 71.45; H, 3.11% .

4-(benzo[d]oxazol-2-yl)benzonitrile(6b)

Yellow solid (60% yield), m.p. 223-225°C; IR (KBr) cm^{-1}, 3029 (aromatic-CH), 2250 (CN); 1H NMR δ:7.75-8.23 ppm (m, 8H, ArH);. MS; m/z (%) 221 (M^+, 100%), , (192 ,15%), (118 ,5%), (92 ,11%), (64 ,53%). Anal. Calculated For $C_{14}H_8N_2O$ (220.06): C, 73.50; H, 4.10, Found: C, 73.81; H, 4.61% .

General method for synthesis of compounds 7 a and b

A mixture of 6 a,c (0.01mol) and thioglycolic acid (0.01mol) in 20ml glacial acetic acid was refluxed for 5 hr , allowed the reaction mixture to cool then

poured into water. The precipitate was collected and washed several times with water and re-crystallized from ethanol to give 7a,c.

2-(4-(benzo[d]thiazol-2-yl) phenyl) thiazol-4(5H)-one (7a)

Orang solid, (65% yield), m.p 128-130°C; IR (KBr) cm^{-1}: 3045 (CH-aromatic), 1645 (C=O); ^{1}HNMR (DMSO-d_6) δ: 3.81 (s, 2H, CH_2), 7.6-8.4 (m, 8H, aromatic protons). Anal. Calculated For $C_{16}H_{10}N_2OS_2$: (310.02), C,61.91; H, 3.25. Found: C,62.14; H,2.90% .

2-(4-(benzo[d]oxazol-2-yl) phenyl) thiazol-4(5H)-one (7b)

Yellow solid, (60% yield), m.p 278-280°C; IR (KBr) cm^{-1} :3225(NH), 3045 (CH aromatic), 1645(C=O); ^{1}H NMR(DMSO-d6)δ=3.98(s, 2H, CH2), 7.60-8.4ppm (m, 8H, aromatic protons Anal. Calculated for $C_{16}H_{10}N_2O_2S$(294.33):C,65.29;H,3.42.Found:C,65.3;H,3.44%.

General method for synthesis of componds of 8a and b

A mixture of 4-(1,3-benzoazol-2-yl) benzonitrile 6a,b (0.006 mol) sodium azide (0.007 mol) and ammonium chloride (0.007 mol) dissolved in dimethylformamide, was refluxed in an oil bath at 120-130^{0}C for about 24 hr , then cooled, poured into water and the precipitate was collected and re-crystallized from dimethylformamide to give 8a,b.

2-(4-(1H-tetrazol-5-yl) phenyl)benzo[d]thiazole(8a)

Orang solid, (60% yield), m.p. 293-295°C; IR (KBr) cm^{-1}; 3193, 3064(NH),3049(aromatic-CH),1332-1115(C-N), 1108, 1138 (C=H- aromatic); ^{1}HNMR; δ=7.54- 8.21 (m, 8H, Ar-H) , 8.52 (s, 1H, NH) . Anal. Calculated For $C_{14}H_9N_5S$ (279.32): C, 60.20; H, 3.25. Found: C, 60.50; H, 3.02%.

2-(4-(1H-tetrazol-5-yl)phenyl)benzo[d]oxazole(8b)

Orang solid, (65% yield), m.p. 297-299°C; IR (KBr) cm^{-1}: 3193 (NH), 3067(aromatic-CH),13423-1032(C-N)1108,1138(C=H-Aromatic); H^{1}NMR; δ=7.54-8.12 (m, 8H, Ar-H), 4.3 (s, 1H, NH) ; MS m/z (%) Anal. Calcd. for $C_{14}H_9N_5O$ (263.08): C, 63.87; H, 3.45. Found: C, 64.18; H, 3.14%.

General method for synthesis of 9a and b

A solution of 2-(4-(1H-tetrazol-5-yl)phenyl)benzo[d]X hydrochloride 8a,b (0.01mol) and acetic anhydride (0.5 mol) was refluxed at 150^0 in an oil bath for 3 hr , then cooled and poured into water the precipitate was collected and recrystallized from ethanol to give 9a,b.

2-(4-(5-methyl-1, 3, 4-oxadiazol-2-yl) phenyl) benzo[d]thiazole (9a)

Orang solid, (75% yield), m.p. 135-136 °C; IR (KBr) cm^{-1} , 3053(aromatic-C-H);^{1}HNMR; δ = 3.5 (s, 3H, CH_3), 7.3-8.5 (m, 8H, ArH). MS m/z (%), M^+(293,100%), M^{+1}(294,10.8%), M^{+2}(295,1.4%), Anal. Calculated For $C_{16}H_{11}N_3OS$ (293.34) : C, 65.51; H,3.78. Found: C, 65.53; H, 3.88%.

2-(4-(5-methyl-1, 3, 4-oxadiazol-2-yl) phenyl) benzo[d]oxazole (9b)

Orang solid, (75% yield), m.p 294-295 °C; IR (KBr) cm^{-1}, 3067(aromatic-CH);^{1}HNMR; δ= 2.9 (s, 3H, CH_3), 7.3-8.5 (m, 8H, ArH). MS m/z (%),M(277,100%),M^{+1}(278,1.9%) ,Anal.Calculated For $C_{16}H_{11}N_3O_2$ (277.28): C, 69.31; H, 4.00. Found: C, 69.32; H, 3.98 %.

General method for synthesis of 10 b and c

A solution of (1b, c) (0.2mol), triethylorthoformate (0.2mol) and 4-methyl pyrazolone, (0.2 mol) in dimethylformamide in presence of triethylamine were heated under reflux in an oil bath at 140^0C for about 2.5 hr. The alcohol was permitted to distill off from the reaction mixture and the solid residue was cooled and treated with petroleum ether $(40\text{-}60)^0$C to give 10b,c.

4-((4-(benzo[d]oxazol-2-yl)phenylamino)methylene)-3-methyl-1H-pyrazol-5(4H)- one (10b)

White solid (75% yield), m.p. 278-280 °C; IR (KBr) cm^{-1}, 3265 (NH),3050(Ar-H), 1648.6 (CO), ^{1}H NMR; δ = 3.4 (s, 3H, CH_3), 6.0 (s, 1H, C=H), 6.5 (s, 1H, NH-CH), 6.52-7.26 (m, 8H,ArH) 8.5 (s, 1H, NH) D_2O exchangeable. MS m/z(%)Anal. Calculated For $C_{18}H_{14}N_4O_2$ (318.33): C, 67.91; H, 4.43; Found: C, 67.80; H, 4.40.%

4-((4-(benzo[d]Imidazol-2-yl)phenylamino)methylene)-3-methyl-1H-pyrazol-5(4H)-one(10c).

White solid, (70% yield), m.p. 258-260°C; IR (KBr) cm^{-1}: 3215(NH), 3040(aromaticC-H),1700 (CO), ^{1}H NMR; δ = 2.9 (s, 3H, CH_3), 5.86 (d, 1H, =CH). 6.31 (d, 1H, NH) D_2O exchangeable, 6.7-7.8 (m, 8H, ArH+1H,NH,D_2O exchangeable), 8.1 (s, 1H, NH)D_2O exchangeable , Anal .Calculated For $C_{18}H_{15}N_5O$(317.34): C, 68.13; H, 4.76; Found: C, 68.14; H, 4.72.% .

General method for synthesis of 11a-d.

A solution of the appropriate compounds 1a-c (0.2 mol), triethyl orthoformate (0.2 mol) and active methylene compounds (0.2 mol) in dimethylformamide was heated under reflux for 2.5 hr, The alcohol was permitted to distil off from the reaction mixture. The solid formed was re-crystallized from petroleum ether (60-80 ^{0}C) to give 11a-d.

2-((4-(benzo[d]thiazol-2-yl)phenylamino)methylene)malononitrile(11a)

Brown solid, (65 % yield), m.p 296°-299°C; IR(KBr) cm^{-1}, 3215 (NH),3067(aromatic-CH), 2220(CN). ^{1}H NMR; δ = 4.09 (s, 1H, NH), 6.8 (s, 1H,C=H), 7.30-7.70. (m, 8H, ArH) .Anal. Calculated For $C_{17}H_{10}N_4S$ (302.53): C, 67.53; H, 3.33;N,18.53. Found: C, 67.45; H,3.1% .

Ethyl 3-(4-(benzo[d]thiazol-2-yl)phenylamino)-2-cyanoacrylate(11b)

Black solid, (50 % yield), m.p 159-160 °C; IR(KBr) cm^{-1} 3122(NH), 3090(aromatic-CH),2120(CN),1746(COO),1648.6 (CO); ^{1}H NMR; δ = 2.36 (t, 3H, CH_3), 3.80 (q, 2H, CH_2), 7.12 (s, 1H ethylene), 6.52-8.23 (m, 8H, ArH),

11.38 (s, 1H, NH)D_2O exchangeable. Anal. Calculated For $C_{19}H_{15}N_3O_2S$ (349.41): C, 65,31; H, 4.33 Found: C, 65.29; H, 4.28% .

2 - (4-(1H-benzo[d]imidazol-2-yl)phenylamino) methylene) malononitrile (11c)

Brown solid, (65% yield), m.p. 210-212 °C; IR (KBr) cm^{-1}: 3058, (NH),3174(aromatic-CH),*)*,2200(CN) ,1648.6 (CO); ^{1}HNMR; δ= 4.09 (s, 1H, NH), 5.8 (s, 1H, benzimidazole-H), 7.7 *(s*, 1H, C=H), 7.30-7.70 (m, 8H, ArH); MS: m/z % 285(M^+ , 100), 259 (15.4), 234 (4.8), 208 (1.3), 181 (3.3), 117 (1.3), 90 (9.2), 63 (28.6). Anal .Calculated For $C_{17}H_{11}N_5$ (285.3): C, 71.57; H, 3.89; Found : C, 71.61; H, 4.00%.

Ethyl-2-((4-(1H-benzo[d]imidazol-2-yl)phenylamino)methylene)-3-oxobutanoate(11d)

Orang solid, (75% yield); m.p. 280-285 °C; IR (KBr) cm^{-1}: 3058, (NH),3000(aromatic-CH), 1648 (CO); ^{1}HNMR; δ= 1.31 (t, 3H, CH_3), 2.0 (s, 3H,CH_3), 4.2 (q, 2H, CH_2), 5.0 (s, 1H, NH)D_2O exchangeable, 6.49-7.21 (m, 4H, ArH), 7.12(s,1H,C=H),7.26-7.70 (m, 4H, ArH), 10.7 (s, 1H, NH)D_2O exchangeable. Anal. Calculated for $C_{20}H_{19}N_3O_3$ (349.38): C, 68.75; H, 5.48 Found: C, 68.81; H, 5.41%

General method for synthesis of 12a-e

A solution of 1a-c (0.2 mol) ,triethylorthoformate (0.2 mol) and aromatic amine compounds (0.2 mol) in dimethylformamide were heated under reflux for about two and half hours the alcohol was permitted to distal off from the reaction mixture. The solution solidified and re-crystallized from petroleum ether 60-80 ^{0}C to give 12a-e.

N-(4-(benzo[d]thiazol-2-yl)phenyl)-N'-(4-chlorophenyl)formamidine 12a.

Black solid, (60 % yield), m.p.96- 99°C; IR(KBr) cm^{-1}; 3194(NH),3050(aromatic-CH) 1562(C=N),835(C-CL); ^{1}HNMR; δ = 4.8 (s, 1H, NH), 6.4-7.2 (m, 8H, ArH),7.5(s,1H,methylene), 7.7-8.3 (m, 4H, ArH), . Anal. Calculated for $C_{20}H_{14}ClN_3S$(363.86): C, 66.02 ; H, 3.88 . Found: C, 66.06 ; H, 4.02% (-N-(4-(benzo[d]oxazol-2-yl)phenyl)-N'-(4-chlorophenyl)formamidine 12b. Orang Solid, (65 % yield), m.p.220- 223 °C; IR (KBr) cm^{-1}, 3057 (NH),3050(aromatic-CH), 1602 (C=N),830(C-CL); ^{1}HNMR; δ=4.3 (s, 1H, NH), 6.5-7.2 (m,8H, ArH's), 7.3-7.4 (m, 4H, ArH) 7.80 (s, 1H, CH).Anal. Calculated for $C_{20}H_{14}ClN_3O$(347.8): C, 69.07; H, 4.06. Found: C, 69.05; H, 4.03%.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-N'-(4-chlorophenyl)formamidine 12c

Orang solid, (70 % yield), m.p. 110-115° C; IR (KBr) cm^{-1}, 3194, 3057 (NH), 1602 (CN),789(C-Cl); ^{1}HNMR δ=5.59 (s, 1H, imidazole-H), 6.64-7.5(q, 4H, phenyl protons),7.50(s,1H,CH=N),7.75-8.1(m,8H, ArH) and 12.83

(d, 1H, NH)D_2O. Anal. Calculated for $C_{20}H_{15}ClN_4$(346.81) : C, 69.26; H, 4.32. Found: C, 69.27; H, 4.31%.

N-(4-(1H-benzo[d]imidazol-2-yl)phenyl)-N'-m-tolylformamidine 12d.

Red solid, (50 % yield), m.p.103-105°C; IR: 3194, 3057 (NH), 1602 (C=N), ^{1}HNMR;δ= 3.4 (s, 3H, CH_3), 5.59 (s, 1H, NH), 6.64-7.5 (q, 4H, ArH's), 7.75-8.1 (m, 8H, Ar-H's), 10.37 (d, 1H,CH), 12.83 (d, 1H, NH)D_2O exchangeable. Anal. Calculated for $C_{21}H_{18}N_4$(326.39) :C, 77.30; H, 5.52. Found: C, 77.29; H, 5.51.%

N-(4-(benzo[d]thiazol-2-yl)phenyl)-N'-m-tolylformamidine12e

Yellow solid, (50 % yield), m.p.200-210 °C ; IR: 3194, 3057 (NH), 1602 (C=N), ^{1}HNMR, δ= 3.2 (s, 3H, CH_3), 6.68 (d, 1H, CH), 7.5-7.7 (q, 4H, ArH), 8.01-8.25 (m, 8H, ArH), 8.22 (d, 1H, NH)D_2O exchangeable. Anal. Calculated for $C_{21}H_{17}N_3S$(343.44) :C, 73.44; H, 4.96. Found: C, 73.46; H, 4.95.%

Conclusion

The synthesis of new 2-(4aminophenyl) benzothiazole/ benzoxazole/ benzimidazole heterocyclic ring systems was accomplished through simple routes and their antitumor activity have been investigated and reported ,in this study.

Pharmacological evaluation of compounds 2-12 against nine cell lines revealed that these compounds possess high or moderate anti-tumor activities .

From the above antitumor screening results some structure activity relationships can be suggested .The presence of pyrazole moiety as in compound 10c named -4-((4-(benzo[d]Imidazol-2-yl)phenylamino)methylene)-3-methyl-1H-pyrazol-5(4H)-one enhance the cytotoxic activity.

Also, the introduction of thiocyanate in benzenamine moiety as in compound 2b named 4-(benzo[d]oxazol-2-yl)-2-thiocyanatobenzenamine exhibited strong antitumor activity against various cancer diseases.

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تشييد وتقييم الفاعلية المضادة للاورام لمركبات جديدة من مشتقات ٢(٤- امينوفينيل) بينزوثيازول/اوكسازول/ايميدازول

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قد تم تشييد مركبات متعددة من ٢-(٤-أمينو فينيل) بينزوثيازول وكذلك مركبات الشبيهة التركيب الجزيئى والتى مرتبطة بمجموعات مثل الثيوسينات وثايول فى موقع ٣ من الحلقة .

وقد تم تشييد مركبات مدموجة الحلقات البنزوثايزول ومركباتها المتشابهة التركيب الجزيئى وتحتوى على الثيازولونز وتيترازولوا والاكسيدايزول.

وقد تم تقييم واختبار تلك المركبات الجديدة من حيث فاعليها ضد السرطان وقد أظهرت النتائج تاثيرات متفائلة ضد الخلايا السرطانية.