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RESEARCH ARTICLE

Antitumer evaluation of some newly synthesized Mannich bases derived from benzofuran derivatives

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Abstract

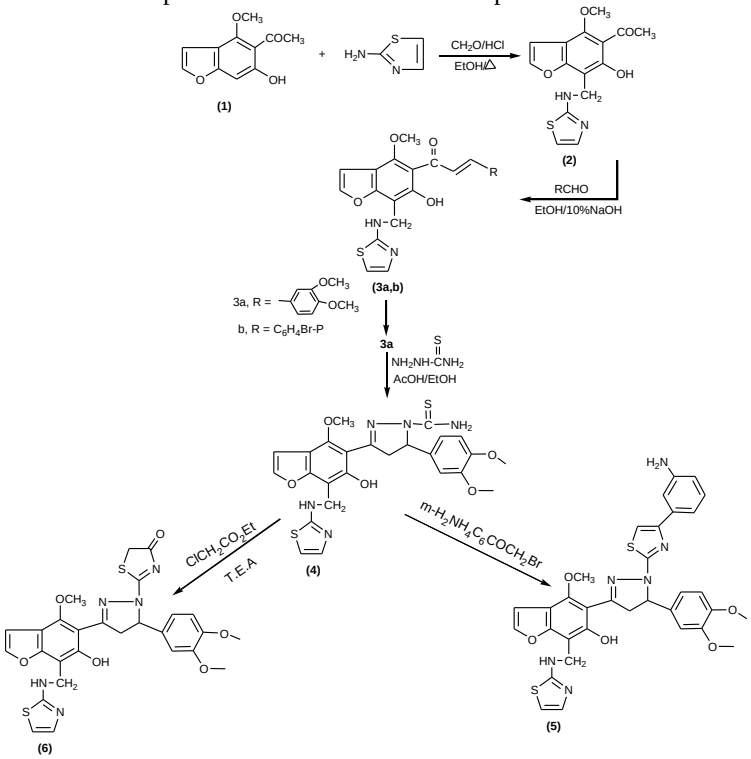
Treatment of [4-methoxy-6-hydroxybenzofuran-5-yl]methyl ketone **(1)** with 2-aminothiazol in presence of formaldehyde gave [4-methoxy-5-acetyl-6-hydroxy-7-(thiazol-2-ylamino)methyl)]benzofuran**(2)** which reacted with 3,4-dimethoxybenzaldehyde or p-bromobenzaldehyde in ethanolic sodium hydroxide to give the corresponding chalcone **(3a,b)** respectively. Cyclization of chalcone **3a** with thiosemicarbazide in ethanolic acetic acid mixture yielded thioamidopyrazoline derivative **(4)** which reacted with each one of 3-aminophenthylbromide or ethylchloroacetate to produce **5,6** respectively. In addition, compound **3a,b** reacted with thiourea in alcoholic potassium hydroxide to give 4,6-disubstituted pyrimidin-2-thione **(7a,b).** Methylation of **7b** with methyliodide led to the formation of 2-methylthiopyrimidine derivative **(8)**. Also, treatment of **7b** with C-ethoxycarbonyl-N-(p-tolyl) hydrazonyl-chloride gave triazolino[4,3-a]pyrimidin-3-carboxylate **(12)**. Moreover, interaction of **3a** with acetylacetone in boiling ethanolic sodium ethoxide gave 6-acetyl-5-(3,4-dimethoxy phenyl)-3-[4-methoxy-6-hydroxy-7-((thiazol-2-ylamino)methyl) benzofuran-5-yl] cyclohex-2-en-1-one**(13)** which reacted with cyanoacetohydrazide in acid medium or base medium to give indazole derivative **(15)** and oxaquinoline derivative **(17)** respectively. On the other hand, when compound **2** treated with phosphorus oxychloride under Vilsmier-Haak reaction condition gave 4-methoxy-9-((thiazol-2-yl amino) methyl)-5H-furo[3,2-g]benzopyran-6-carboxaldehyd-5-one**(18)**.
Condensation of **18** with thiosemicarbazide gave thiocarbazone derivative **(20)**. Similarly, treatment of **18** with cyanoacetohydrazide yielded cyanoacetohydrazone **21**. Cyclization of the latter compound **21** with p-fluorobenzylidinmalononitrile gave 1,2,4-triazolopyridine derivative **(23).**
Evaluation of some new compounds revealed remarkable antitumor activity against HEPG2(Hepatocellular carcinoma cells).

INTRODUCTION

The synthetic Mannich bases are class of compounds having interesting pharmacological activity such as antimalarials, a modiaquine, bialamicol[1], antibacterial[2], antioxidant and anticoagulant[3]. Some Mannich bases were found to be active as local anaesthetics, analgesics[4], parasympatholytics[5], sympathetics and parasympathetics[6]. Moreover, some of benzofuran moieties possesses wide range of biological activities[7-13] and acts as analgesic[14] with low gastric irritancy, anticancer[15] and antioxidant[16]. Also, it used in treatment of asthama, rheumatism and ulcers[17]. Therefore, the aim of the present work is to synthesis some Mannich bases derived from benzofuran in order to test their biological activity.

Results and Discussion:

Interaction of [4-methoxy-6-hydroxybenzofuran-5-yl]methyl ketone **(1)**[18] with 2-aminothiazole in presence of formaldehyde gave [4-methoxy-5-acetyl-6-hydroxy-7((thiazol-2-ylamino)methyl)] benzofuran- **(2)** scheme (1). The structure of **2** was confirmed by elemental analysis and spectral data. The IR spectrum of **2** showed the absorption bands at 1705cm^{-1}, 3310 for (>C=O) and NH group respectively, ^{1}H NMR spectrum of its compound exhibited signals at δ 4.24 ppm. (s, 2H, CH_2), 6.51(d, 1H,CH_{-4}, thiazol), and 7.17 (d, 2H, 1H, CH_{-5},thiazol + 1H, C-3 furan). This was analogy with previous work[19]. Condensation of compound **2** with 3,4-dimethoxy benzaldehyde or p-bromobenzaldehyde in ethanolic sodium hydroxide yielded phenyl prop-2-en-1-one derivative **(3a,b)** scheme (1). The structure of **3a,b** were established from correct elemental analysis and spectral data, IR spectrum of **3a** showed the disappearance of –$COCH_3$ and appeared bands at 1696 cm^{-1} for –CO–CH=CH, 3292 and 3397 for NH and OH groups respectively. ^{1}H NMR spectrum of its compound revealed signals at δ 3.71-3.96 ppm (m, 9H, $3OCH_3$), 6.58-7.88 (m, 8H, 2H, 2CH thiazol + 2H, CH=CH + 3H, Ar–H + 1H, C_{-3} of furan). Cyclization of chalcone **3a** with thiosemicarbazide in ethanolic acetic acid mixture formed thioamido-pyrazoline derivative **(4)** scheme (1). The IR spectrum of **4** exhibited the disappearance of (>C=O) present in the parent compound and appeared bands at 3221 & 3266cm^{-1} for (br, NH_2/NH) group. ^{1}H NMR spectrum of its compound showed the disappearance signal of $-\overset{O}{\overset{\|}{C}}-CH=CH$ and appeared signal at δ 5.52 ppm due to $-\overset{S}{\overset{\|}{C}}-NH_2$. Compound **4** was proved to be a versatile material for the synthesis of some novel 2,4-disubstituted thiazole derivatives. Thus, cyclization of thioamido **(4)** with each one of 3-aminophenthylbromide or ethylcholoroacetate afforded 5-[4,5-dihydro-5-(3,4-dimethoxyphenyl)-1-[4-(3-aminophenylthiazol-2-yl)-1H pyrazlino-3-yl]-4-methoxy-6-hydroxy-7-((thiazol-2ylamino)methyl) benzofuran derivative (5) and thiazolidin-4-one derivative (6) respectively scheme (1). Compounds 5,6 were established according to elemental analysis and spectral data, MS of **5** showed the molecular ion peak M^+ at m/z 653 (M^+-1, 57.11%). The IR spectrum of **6** showed the disappearance of amino group found in the parent and appeared bands at 1677 cm^{-1} (>C=O) for thiazolidinone moiety and 3191 & 3411 due to NH and OH groups respectively. ^{1}H NMR spectrum of **6** showed signals at δ 3.41 ppm (d, 2H, CH_2, pyrazoline) 3.81 (t, 1H, CH, pyrazoline), 4.81 (s, 2H, CH_2-thiazolidinone). All of these compounds are in accordance with previous work[20,21].



Scheme (1)

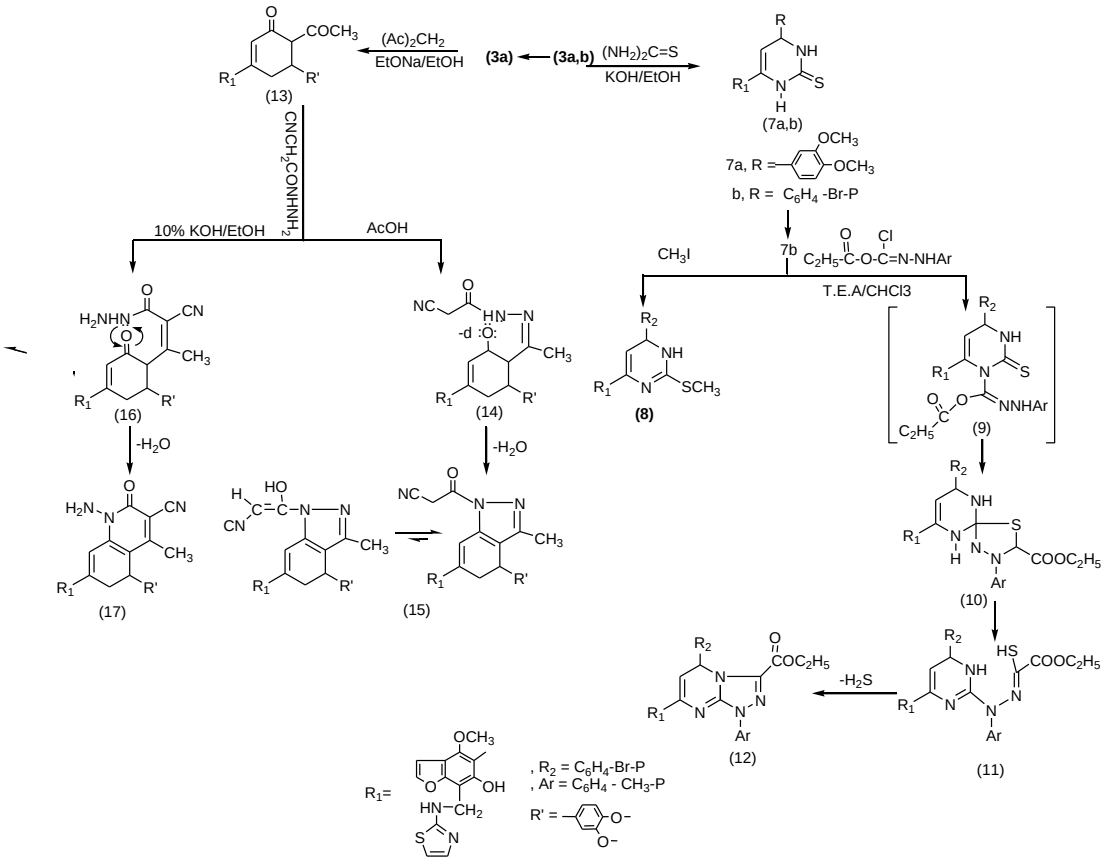
On the other hand, chalcone **(3a,b)** reacted with thiourea in ethanolic potassium hydroxide to yield dihydropyrimidin-2-thione derivatives **(7a,b)** Scheme (2). The IR spectrum of **7a** showed the disappearance of ($\diagdown$C=O) which found in the parent and revealed bands at 3162 , 3311, 3336 cm^{-1}, (3NH) and 3394 for OH group. ^{1}H NMR spectrum of **7b** showed signals due to C_{-4} & C_{-5} of pyrimidine, 3NH, Ar-H and thiazole protons.

Methylation of **7b** with methyliodide in the presence of sodium methoxide led to the formation of 2-methylthio-1,6-dihydropyrimidine derivative **(8)** Scheme (2). MS of **8** afforded a molecular ion peak M^+ at m/z 557 (43.01%). Also, treatment of compound **7b** with C-ethoxy carbonyl-N-(p-tolyl)hydrazonyl chloride in chloroform in presence of triethylamine yielded ethyl-6-(4-methoxy-6-hydorxy-7-((thiazol-2-yl amino)methyl)benzofuran-5-yl]-1-(p-tolyl)-4-(4-bromophenyl)[4,3-a] triazolino[4,3-a] pyrimidin-3-carboxylate **(12)** Scheme (2). The formation of **12** can be obtained via cycloaddition of nitrileimide (generated from hydrazononylhalide with triethylamine) to C=S of pyrimidin-2-thione to produce intermediate **9** which cyclized to intermediate **10** with ring opening to **11** and ring closure to afford **12** through elimination of hydrogen sulfide Scheme (2).

^{1}H NMR spectrum of **12** showed signals at δ 2.21ppm (s, 3H, CH_3), 2.37 (t, 3H, CH_3 of C_2H_5) 4.43 (q, 2H, CH_2 of C_2H_5). All of these compounds in accordance with previous work[22].

Moreover chalcone **(3a)** reacted with acetylacetone in presence of sodium ethoxide under Michael reaction condition to give the corresponding 6-acetyl-3,5-disubstituted-2-cyclohexen-1-one**(13)** Scheme (2). The IR spectrum of **13** showed strong absorption bands at 1668cm^{-1} and 1713 due to 2 $\diagdown$C=O of cyclohexenone and acetyl groups.

Compound **13** used as a starting material for building of fused heterocyclic systems through ring closure reactions with cyanoacetohydrazide. Thus, cyclization of **13** with cyanoacetohydrazide in acid medium afforded 1-cyanoacetyl-4,6-disubstituted-4,5-dihydro-3-methylindazole **(15)** Scheme (2). While, the interaction of **13** with cyanoacetohydrazide in basic medium gave 1-amino-3-cyano-5,7-disubstituted-4-methyl-5,6-dihydro-2-oxaquinoline**(17)** Scheme (2). Compounds **15,17** were established from correct elemental analysis and spectral data, the IR spectrum of **17** showed bands at 2221 cm^{-1} for (C≡N), 3211 & 3324(NH_2/NH) and 3401 (OH) groups and its MS afforded a molecular ion peak M^+ at m/z 611 (16.11%).

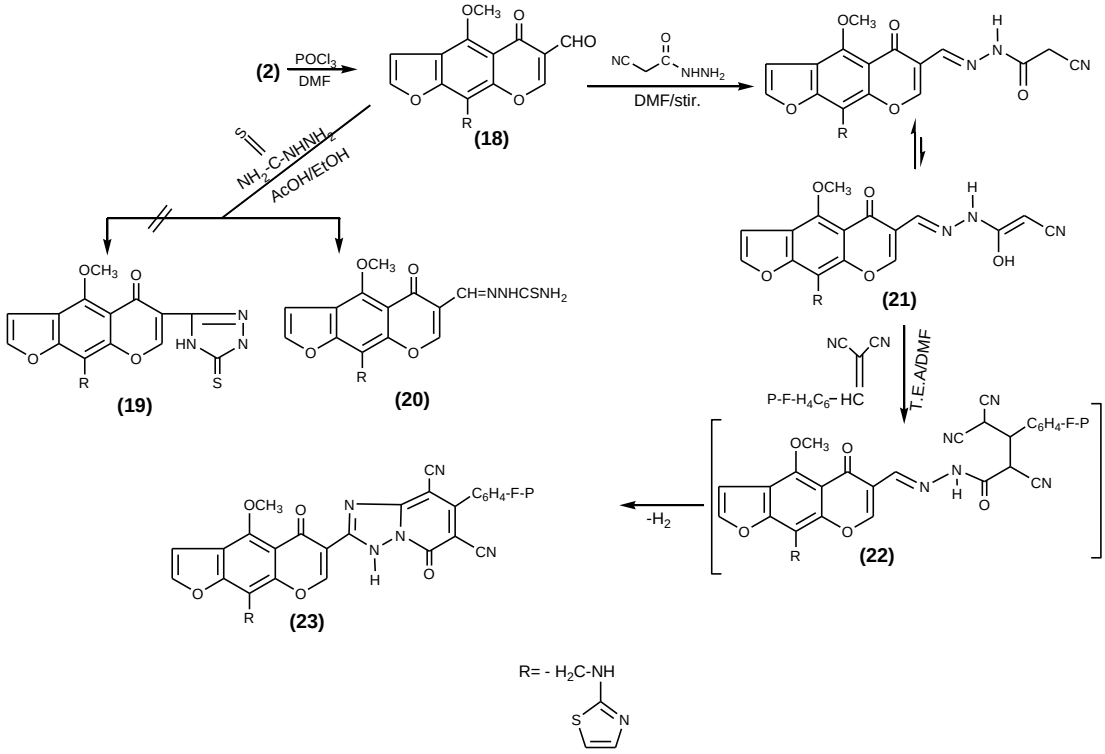


Scheme (2)

On the other hand, when compound **2** was subjected to Vilsmier-Haak reaction gave 6-formyl furochromone derivative **(18)** Scheme (3). The IR spectrum of its compound should the disappearance of hydroxy group found in the parent and revealed bands for 2 C=O and NH groups and its MS showed the molecular ion peak M^+ at m/z 356 (26.12%). The reactivity of **18** towards neucleophilic reagents was investigated. Thus condensation of **18** with thiosemicarbazide in ethanolic acetic acid mixture hoping to obtain triazolo derivative **(19)** but thiocarbazone derivative **(20)** was obtained Scheme (3). In the basis of elemental analysis and spectral data, compound **19** was eliminated and thio- carbazone **(20)** was deduced. The IR spectrum of **20** exhibited absorption bands at 1602cm^{-1} for($\diagdown$C=N), 3137, 3161,3301 (NH_2/2NH), ^{1}H NMR spectrum of its compound showed signals at δ 4.81ppm (br, 2H, NH_2), 6.53-8.05(m, 6H, C_{-7} benzopyran+2H, 2CH, thiazole+2H of furan protons + 1H, CH=N). Similarly, compound **18** condensed with cyanoacetohydrazide to give cyanoacetohydrazone derivative **(21)** Scheme (3)**.**

The IR spectrum of **21** revealed absorption bands at 1669 cm^{-1} for $\diagdown$C=O of γ-pyrone, 1711 for $\diagdown$C=O , 2214 (C≡N), 3282 & 3319 (2NH), ^{1}H NMR spectrum of its compound exhibited signals at δ 3.37 ppm due to $-\overset{O}{\overset{\|}{C}}-CH_2CN$, 6.55 -8.18 (m, 6H, 1H, CH =N + 2H, 2CH-thiazole + 1H, H_{-7} pyrone + 2H, furan protons).

Heterocyclization of compound **21** with p-fluorobenzyliden- malononitrile gave 1,2,4-triazolopyridine derivative **(23)** Scheme (3). The formation of cyclized product **23** was assumed to produce via an initial addition of the active methylene of cyanoacetohydrazone **21** to the activated double bond of arylidinmalononitrile linkage to yield Michael adduct **(22)** which subsequently cyclized between the neucleophilic and electrophilic centers followed by oxidation to afford **23.** The IR spectrum of **23** revealed absorption bands for 2C=O, 2C≡N, 2NH groups, the mass spectrum exhibited the molecular ion peak M^+ at m/z 605 (M^+, 37.11%) and this was analogy[23].



Scheme (3)

Pharmacology :

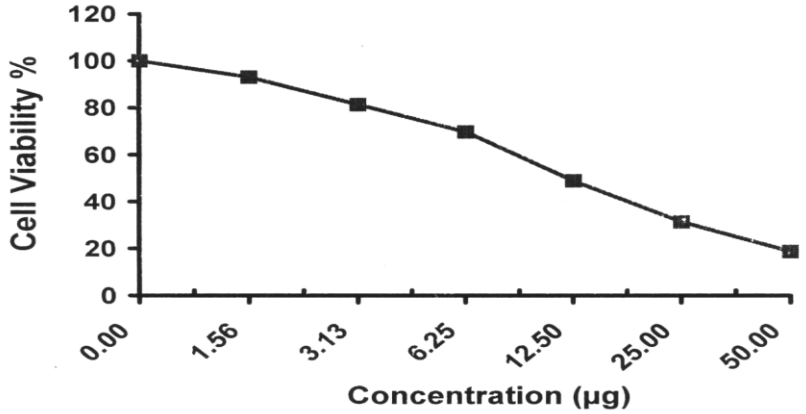
Antitumor screening:

Conclusion :

The newly synthesized compounds **15** and **23** were tested for antitumor activity and exhibited a high significant anticancer activity against $HEpG_2$ (Hepatocellular carcinoma cells) the results are summarized in figures 1,2.

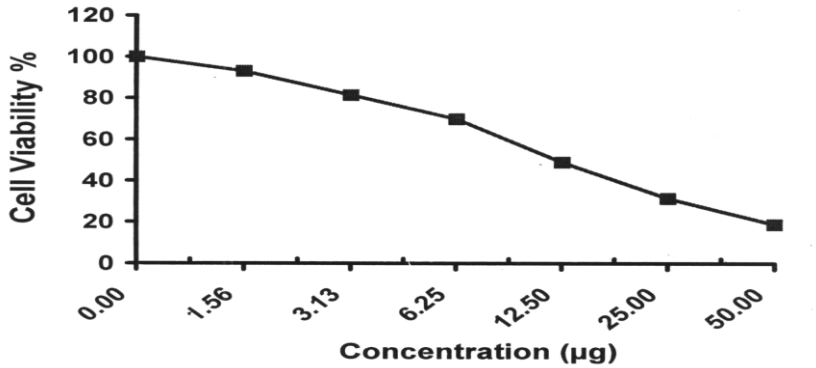
Evaluation of cytotoxicity Effect OF 1,2,4-Triazolopyridine-6,8-dicarbonitriles (23) against HepG-2 cell line.

Samples conc.	Viability %
50	55.74
25	70.38
12.5	84.62
6.25	91.87
3.125	98.69
1.56	100
0	100.00



Evaluation of cytotoxicity Effect OF 1,2,4-Triazolopyridine-6,8-dicarbonitriles (23) against HepG-2 cell line.

Samples conc.	Viability %
50	32.49
25	47.98
12.5	71.52
6.25	86.92
3.125	95.17
1.56	98.83
0	100.00.



Experimental

All melting points were determined in open capillaries and are uncorrected. IR spectra were measured using (KBr) discs and a pye Unicom SP-1000 spectro-photometer. ^{1}H NMR spectra were measured on a Varian EM-390-200 MHz instrument in DMSO-d_6) as solvent using TMS as internal started and chemical shifts are expressed as δ ppm. Mass spectra were measured and a shimadzu G CMSQP-100 Ex mass spectrometer at 70 eV.

Synthesis of [4-methoxy-5-acetyl-6-hydroxy-7-((thiazol-2-ylamino) methyl)] benzofuran] (2),

To a solution of visnaginone (1) (0.01mol) in ethanol (30 ml), 2-aminothiazole (0.01 mol) and formaldehyde (0.015 mol) were added. The reaction mixture was refluxed for about 6 hrs. The solid so formed was collected and recrystallized from ethanol as yellow crystals in 87% yield, m.p 175°C, IR(KBr, cm^{-1}): 1705 (>C=O), 3310 (NH) and 3424 (OH) group ^{1}H NMR (DMSO-d_6: δppm) 2.49 (s, 3H, $COCH_3$), 3.83 (s, 3H, OCH_3), 4.24 (s, 2H, CH_2), 6.51 (d, 1H, J = 2.01Hz, CH_{-4}, thiazole) 7.17 (d, 2H, J = 2.01Hz, CH_{-5} thiazole + 1H, C_{-3} furan), 7.82 (d, 1H, J = 2.2 Hz, C_{-2} furan) 9.11 (s, 1H, NH, D_2O exchangeable), 11.21 (s, 1H, OH, D_2O exchangeable). Anal.calcd. for $C_{15}H_{14}N_2O_4S$ (319.83). C, 56.79: H, 4.41. N, 8.75: S, 10.02% found: C, 56.61: H, 4.39: N, 8.67: S, 10.3

Synthesis of 1-[4-methoxy-6-hydroxy-7-((thiazol-2-ylamino) methyl) benzofuran-5-yl]-3-(3,4-dimethoxyphenyl)/(4-bromophenyl)prop-2-en-1-one (3a,b)

To a solution of **2** (0.01 mol) in ethanol (20 ml) containing sodium hydroxide (10ml,10%) 3,4-dimethoxybenzaldehyde or 4-bromo-benzaldehyde (0.01 mol) was added. The reaction mixture was stirred at room temperature for 2 hrs. The reaction mixture was poured into crushed ice and acidified with dilute hydrochloric acid. The solid product was filtered, washed with water, dried and crystallized. The solid **3a** was recrystallized from ethanol as orange crystals in 86% yield, m.p. 208-210°C, IR (KBr, cm^{-1}): 1696 (>C=O), 3292 (NH) and 3397 (OH) group. ^{1}H NMR (DMSO-d_6: δ, ppm) 3.71-3.96 (m, 9H, $3OCH_3$), 4.07 (s, 2H, CH_2), 6.58-7.88 (m, 8H, 2H, 2CH-thiazole + 2H, CH=CH + 3H, Ar–H + 1H, C_{-3} furan), 8.01 (d, 1H, C_{-2} furan), 9.8 (s, 1H, J= 2.21Hz, NH, D_2O exchangeable), 10.91 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. for $C_{24}H_{22}N_2O_6S$ (466.51): calcd. C, 61.79: H, 4.75: N, 6.00: S, 6.87% Found: C, 61.77: H, 4.77: N, 6.03: S, 6.85.

The solid **3b**, was recrystallized from ethanol as dark red crystals in 80% yield, m.p. 132-134°C, IR(KBr, cm^{-1}) 1691(>C=O), 3401 (br, NH/OH) group. Anal. Calcd. for $C_{22}H_{17}O_4N_2SBr$ (485.357), C, 54.44: H, 3.53: N, 5.77: S, 6.60 : Br, 16.46% found: C, 54.41: H, 3.50: N, 5.76 : S, 6.58: Br, 16.43.

Synthesis of 4,5-dihydro-3[4-methoxy-6-hydroxy-7-((thiazol-2-yl amino) methyl)benzofuran-5-yl]-5-(3,4-dimethoxyphenyl)pyrazolino-1-carbothioamide (4) and 4-methoxy-9-((thiazol-2-yl amino)methyl)-5H-furo[3,2-g] benzopyran-6-carbaldehydthiosemicarbazon-5-one(20).

A mixture of compounds **3a** (0.01 mol) or compound **18** (0.01 mol) and thiosemicarbazide (0.01 mol) in ethanol (20 ml) containing 2 ml acetic acid was refluxed for 6 hrs. The formed precipitate was filtered off, dried and crystallized. The solid **4** recrystallized from ethanol as yellow crystals in 76% yield, m.p. 140-141°C, IR (KBr, cm^{-1}): 1346 (C=S), 3221&3266 (br, NH_2/NH), 3423 (OH) group. ^{1}H NMR (DMSO-d_6. δ, ppm) δ 3.38 (d, 2H, J= 8Hz, CH_2, pyrazoline), 3.71(t, 1H, CH, pyrazoline), 3.81-3.93(m, 9H, $3OCH_3$), 4.14 (s, 2H, CH_2), 5.52(s, 2H, —C(=S)-NH_2), 6.37 (d, 1H, J= 7.1Hz, CH_{-4} thiazole), 6.8-8.12 (m, 6H, 3H, Ar–H + 1H, CH_{-5} thiazole + 2H, 2CH-furan). 10.01 (s, 1H, NH, D_2O exchangeable). 11.29 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. for $C_{25}H_{25}N_5O_5S_2$ (539.63): C, 55.64: H, 4.66: N, 12.97: S,11.88%: found C, 55.68: H, 4.58: N, 13.00: S,11.83.

The solid **20** recrystallized from petroleum ether 40-60 as brownish red crystals in 57% yield, m.p. 68-70°C: IR(KBr, cm^{-1}): 1351 (C=S) 1602(>C=N), 1666(>C=O) 3137, 3161, 3301 (NH_2/2NH) groups. ^{1}H NMR(DMSO-d_6: δ, ppm) 3.97 (s, 3H, OCH_3), 4.09 (s, 2H, CH_2), 4.81 (br, 2H, NH_2) 6.53-8.05 (m, 6H, 1H, C_{-7} of benzopyran+2H, 2CH-thiazole + 2H of furan protons + 1H, CH=N), 9.01, 10.24 (2s, 2H, 2NH, D_2O exchangeable) MS: m/z (%), (429) (M^+, 47%), 339 (12), 296(21), 268 (7.11) with a base peak at 77. Anal. Calcd. For $C_{18}H_{15}N_5O_4S_2$ (429.47): C, 50.34: H, 3.52: N, 16.31: S, 14.93% : Found: C, 50.37: H, 3.44: N, 16.32: S, 14.89.

Synthesis of 5-[4,5-dihydro-5-(3,4-dimethoxyphenyl)–1-(4-(3-amino-phenyl)thiazol-2-yl)(-1H-pyrazolino-3-yl]-4-methoxy-6-hydroxy-7-(thiazol-2yl amino) methyl) benzofuran (5).

A mixture of compound **4** (0.01 mol) with 3-aminophenthylbromide (0.01 mol) in ethanol (30 ml) was refluxed for 3hrs. The resulting solid was filtered and dried. The solid recrystallized from ethanol as brown crystals in 65%

yield, m.p. 118-120°C, IR (KBr, cm^{-1}): 3151 & 3287 (br, NH_2/NH), 3405(OH) groups. MS: m/z (%) 653 (M^+–1, 57.11%) with a base peak at 73. Anal.calcd. for $C_{33}H_{30}N_6O_5S_2$ (654.77): C, 60.53: H, 4.61: N, 12.83: S, 9.79: found C, 60.70: H, 4.34: N, 12.85: S, 9.87.

Synthesis of 5-[4,5-dihydro-5-(3,4-dimethoxyphenyl)-1-(4-oxothiazoli-din-5H-2-yl)-1H pyrazolino-3-yl]-4-methoxy-6-hydroxy-7-((thiazol-2-yl amino) methyl)benzofuran (6)

To a solution of **4** (0.1 mol) in acetone (20 ml), ethylcholoroacetate (0.01 mol) and triethylamine (0.5 ml) were added. The reaction mixture was refluxed for 6hrs. The solid obtained after cooling was filtered, dried and recrystallized from benzene as off white crystals in 57% yield, m.p. 79-81°C. IR (KBr, cm^{-1}): 1677 ($\diagdown\!\!\diagup C{=}O$), 3191 (NH), 3411(OH) group. ^{1}H NMR (DMSO–d_6 δppm): 3.41 (d, 2H, J = 8.01 Hz, CH_2-pyrazoline), 3.81 (t, 1H, CH–pyrazoline), 3.72-3.88 (m, 9H, OCH_3), 4.22 (s, 2H, CH_2), 4.81 (s, 2H,–CH_2-thiazolidinone) 6.46 (d, 1H, J = 7.17 Hz, CH_{-4} thiazol), 6.52-8.01 (m, 6H, 3H, Ar–H, 1H, CH_{-5} thiazole + 2H, furan protons) 8.93(s, 1H, NH, D_2O exchangeable), 10.66 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. For $C_{26}H_{25}N_5O_6S_2$ (567.64): C, 55.01 : H, 4.43: N, 12.33: S, 11.29% found: C, 55.12: H, 4.41: N, 12.36: S, 11.3.

Synthesis of 4-(3,4-dimethoxyphenyl)/(4-bromophenyl)-6-[4-methoxy-6-hydroxy-7-((thiazol-2-ylamino)methyl)benzofuran-5-yl)-3,4-dihy-dropyrimidin-2(1H)-thione (7a,b).

To a solution of **3a or 3b** (0.01 mol) in ethanol 30 ml) was added thiourea (0.01 mol) in presence of potassium hydroxide (0.5 gm) and then refluxed 6hrs. the solvent was removed and the resulting solid crystallized from a suitable solvent . The solid **7a** was recrystallized from ethanol as red crystals in 78% yield, m.p. 122-130°C, IR (KBr, cm^{-1}): 1335(C=S) 3161, 3311, 3336 (3NH), 3394(OH) groups. MS: m/z (%) 528 (M^++1, 62) with a base peak at 102. Anal. calcd. for $C_{29}H_{24}N_4O_5S_2$ (527.66): C, 66.01: H, 4.58: N, 10.61: S, 12.15% found C, 66.03 : H, 4.53: N, 10.58: S, 12.1**.**

The solid **7b** was recrystallized from acetone as red crystals in 91% yield, m.p. 178-80°C, IR(KBr, cm^{-1}): 1344 (C=S), 3166, 3271, 3368 (3NH), 3401(OH). ^{1}H NMR (DMSO-d_6: δppm): 3.98 (s, 3H, OCH_3), 4.26(s, 2H, CH_2), 5.81 (d, 1H, J= 7.1 Hz, CH_{-5} pyrimidine), 6.24 (d, 1H, CH_{-4}pyrimidine), 6.61-8.15 (m, 8H, 2H, 2CH-thiazole + 4H, Ar–H +2H, 2CH–furan), 8.52, 9.81, 10. 62 (3s, 3H, 3NH, D_2O, exchangeable), 10. 69 (s, 1H, OH, D_2O exchangeable). Anal. Calcd. for $C_{23}H_{19}N_4O_3S_2Br$ (543.46) C, 50.83: H, 3.52: N, 10.3: S, 11.8: Br, 14.7% found C, 50.8: H, 3.51: N, 10.01: S, 11.2: Br, 14.5.

Synthesis of 4-(4-bromophenyl)-6-[4-methoxy-6-hydroxy-7-((thiazol-2-yl amino)methyl)benzofuran-5-yl)2-methylthio-3,4-dihydro-pyrimidine (8).

A mixture of **7b** (0.01 mol) and methyl iodide (0.01 mol) in ethanolic sodium ethoxide (30 ml) was refluxed 4hrs. the resulting solid was collected and recrystallized from ethanol as red crystals in 87% yield, m.p. 78-80°C, IR (KBr, cm^{-1}): 3182, 3311 (2NH), 3414 (OH) groups respectively. MS: m/z (%), 557 (M^+, 43.01%) ^{1}H NMR (DMSO-d_6: δ ppm): 2.33 (s, 3H, CH_3), 3.92 (s, 3H, OCH_3), 4.28 (s, 2H, CH_2), 5.55 (d, 1H, J= 7.01 Hz, CH_{-5}, pyrimidine), 6.36-8.11 (m, 9H, 1H, CH_{-4} , pyrimidine + 2H, 2CH, thiazole + 4H,Ar–H + 2H, 2CH, furan), 9.18, 9.61 (2s, 2H, 2NH, D_2O exchangeable) and 10.51 (s, 1H, OH, D_2O exchangeable) Anal. calcd for $C_{24}H_{21}N_4O_3S_2Br$ (557.49): C, 51.70: H, 3.79: N, 10.05: S, 11.5: Br, 14.33% found: C, 51.66: H, 3.60: N, 10.0: S, 11.49: Br, 14.15.

Synthesis of ethyl-6-[4-methoxy-6-hydroxy-7-(thiazol-2-ylamino) methyl)benzofuran-5-yl]-1-(p-tolyl)-4-(4-bromophenyl)[4,3-a] triazolino[4,3-a] pyrimidin-3-carboxylate(12).

A mixture of **7b** (0.01 mol), C-ethoxy carbonyl-N-(p-tolyl) hydrazonylchloride (0.01 mol) and trimethylamine (0.05 ml) in chloroform (20 ml) was refluxed for 10 hrs. the solvent was evaporated and the resulting solid was dried and recrystallized from acetone as brown crystals in 72% yield. M.p. 210-212°C, IR (KBr, cm^{-1}): 1748 ($\diagdown\!\!\diagup C{=}O$), 3292 (NH), 3421 (OH) group. ^{1}H NMR (DMSO-d_6: δ ppm): 2.21 (s, 3H, CH_3), 2.37 (t, 3H, CH_3 of C_2H_5), 3.83 (s, 3H,OCH_3), 4.18 (s, 2H, CH_2), 4.43 (q, 2H, CH_2 of C_2H_5), 5.57-6.31 (m, 2H, 2CH–pyrimidine), 6.51-8.12 (m, 12H, 8H, Ar–H + 2H, 2CH–thiazole +2H, furan protons), 9.83 (s,1H, NH, D_2O exchangeable), 10.99 (s, 1H, OH, D_2O exchangeable). Anal. calcd. For $C_{34}H_{29}N_6O_5SBr$ (713.612): C, 57.22: H, 4.09: N, 11.77: S, 4.49: Br, 11.19%: found : C, 57.01: H, 3.88: N, 11.76: S, 4.43: Br, 11.02.

Synthesis of 6-acetyl-5-(3,4-dimethoxyphenyl)-3-[4-methoxy-6-hydroxy-7-((thiazol-2-yl amino) methyl)benzofuran-5-yl] cyclohex-2-en-1-one (13).

To a solution of **3a**(0.01 mol) in 50 ml of sodium ethoxide solution and acetylacetone (0.01 mol) was added. The reaction mixture was refluxed for 12 hrs., then left a side overnight. The product was collected and recrystallized from acetone as brownish red crystals in 81% yield, m.p. 169-170°C, IR (KBr, cm^{-1}): 1668, 1713 (2 >C=O), 3418 (br, NH /OH) groups. ^{1}H NMR (DMSO–d_6: δ, ppm), 1.81-2.14 (m, 3H, C_{-4}, C_{-5}, cyclohexanone) 2.85(s, 3H, $COCH_3$) 3.44(d, 1H, C_{-6}, cyclohexanone), 3.81-3.91 (m, 9H, $3OCH_3$), 4.33 (s, 2H, CH_2), 6.42-7.78 (m, 8H, 1H, olfinic + 2H, 2CH thiazole + 3H, Ar–H + 2H, furan protons), 10.13 (s, 1H, NH, D_2O exchangeable). 11.01 (s, 1H, OH, D_2O exchangeable), Anal calcd. for $C_{29}H_{28}N_2O_7S$(548.61) C,63.49:H, 5.14: N, 5.10: S, 5.84%: found: C,63.44:H, 5.15: N, 5.11: S, 5.82**.**

Synthesis of 1-cyanoacetyl-4-(3,4-dimethoxyphenyl)-6-(4-methoxy-6-hydroxy-7-((thiazol-2-yl amino) methyl) benzofuran-5-yl]-4,5-dihydro-3-methyl indazole (15).

A mixture of **13** (0.01 mol) and cyanoacetohydrazide (0.01 mol) in acetic acid (20 ml) was refluxed for 20 hrs. After cooling, the product that separated was recrystallized from acetone as brown crystals in 85% yield, m.p. 180°C , IR, (KBr, cm^{-1}): 1664 (>C=O), 2214 (C≡N), 3411(br, NH/OH). ^{1}H NMR (DMSO-d_6: δ, ppm), 2.31(s, 3H, CH_3), 2.72 (d, 2H, CH_2, indazole), 3.61, (s, 2H,CH_2–cyanoacetyl) 3.81-3.99 (s, 9H, $3OCH_3$), 4.26 (s, 2H, CH_2), 4.84(t, 1H, C_{-4}, indazole) 6.47-8.08 (m, 8H, 1H, CH_{-7} indazole, 2H, 2CH, thiazole, 3H, Ar–H+2H, furan) 9.88 (s, 1H, NH, D_2O exchangeable), 11.12 (s, 1H, OH, D_2O exchangeable), Anal. calcd. for $C_{32}H_{29}N_5O_6S$(611.67): C, 62.83: H, 4.77: N, 11.03: S, 5.24% found: C, 62.81: H, 4.74: N, 11.06: S, 5.22.

Synthesis of 1-amino-3-cyano-4-methyl-5-(3,4-dimethoxyphenyl) 7-[4-methoxy-6-hydroxy-7-((thiazol-2-ylamino) methyl)benzofuran-5-yl]-5,6-dihydro-2-oxaquinoline (17).

A mixture of **13** (0.01 mol) and cyanoacetohydrazide (0.01 mol) in absolute ethanol containing potassium hydroxide (5 ml, 10%) was refluxed for 20 hrs. After cooling, it was neutralized with dilute hydrochloric acid. The product was washed well with water, dried then recrystallized from acetone as brown crystals in 70% yield, m.p.> 300°C, IR (KBr, cm^{-1}): 1666(>C=O), 2221(C≡N), 3211 & 3324 (br, NH_2/NH), 3401 (OH) group, MS: m/z (%) 611(M^+, 26.11%): with a base peak at 78 Anal. calcd. for $C_{32}H_{29}N_5O_6S$(611.67): C, 62.83; H, 4.77: N: 11.03: s, 5.24% found: C,62.80: H, 4.74: N, 11.08: S, 5.21.

Synthesis of 4-methoxy-9-((thiazol-2-yl amino) methyl)-5H-furo[3,2-g]benzopyran-6-carboxyladehyd-5-one(18).

To a solution of 2(0.01 mol) in dimethylformamide (30 ml), phosphorus oxychloride (3ml) was added. The reaction mixture was refluxed in water bath for 6 hrs, then the reaction mixture was poured into crushed ice. The solid product was filtered, dried and crystallized from dimethyl formamide as brown crystals in 93% yield, m.p. > 300, IR (KBr, cm^{-1}): 1667(br, 2 >C=O), 3293 (NH) groups, MS: m/z (%) 356 (M^+ , 26.12) with a base peak at 96 ^{1}H NMR (DMSO-d_6: δ, ppm) 3.91 (s, 3H, OCH_3), 4.14 (s, 2H,CH_2), 6.61-8.03 (m, 6H, 1H, CH, formyl, 2H, 2CH-thiazole + 1H, C_{-7} pyrone + 2H, 2CH-furan) 9.03 (s, 1H, NH, D_2O exchangeable), Anal.calcd. for $C_{17}H_{12}N_2SO_5$(356.35): C, 57.94: H, 3.39: N, 7.86: S, 8.99% found: C, 57.91: H, 3.37: N, 7.84: S, 8.96.

Synthesis of 4-methoxy-9-((thiazol-2-yl amino) methyl)-5H-furo[3,2-g] benzopyran-6-cyanoacetylhydrazomethin-5-one(21).

To a solution of **18** (0.01 mol) in dimethylformamide (20ml), cyanoacetohydrazide (0.01 mol) was added. The reaction mixture was stirred at room temperature for 2hrs. the solid product was collected and recrystallized from chloroform as gray crystals in 75% yield, m.p. decomposed at 270°C, IR(KBr, cm^{-1}): 1669 (>C=O) of γ-pyrone, 1711 (>C=O) of cyanoacetyl, 2214(C≡N) 3282 & 3319 (2NH). ^{1}H NMR (DMSO–d_6: δ, ppm.) 3.37(s, 2H, –C(=O)-CH_2CN), 3.86 (s, 3H, OCH_3), 4.31 (s, 2H, CH_2), 6.55-8.18 (m, 6H, 1H, CH=N + 2H, 2CH thiazol + 1H, H_{-7} pyrone + 2H, furan protons), 9.11, 10.06 (2s, 2H, NH, D_2O exchangeable), Anal. calcd. for $C_{20}H_{15}N_5O_5S$ (437.43): C, 54.91: H, 3.45: N, 16.01: S, 7.33% found : C, 54.88: H, 3.43: N, 16.3: S, 7.31.

Synthesis of 5-oxo-2-[4-methoxy-9-((thiazol-2-ylamino)methyl)-5-oxo-5H-furo[3,2-g]benzopyran]-7-(p-fluorophenyl)-3,5-dihydro [1,2,4]-triazolo[1,5-a] pyridin-6,8-dicarbonitrile (23).

To a solution of **21** (0.01 mol) in ethanol (20 ml) in presence of pipridine (0.5 ml) added p-fluorobenzylidenmalononitrile (0.01 mol). The reaction mixture was refluxed for 3hrs. then the solid obtained dried and recrystallized from acetone as orange colour in 66% yield, m.p. 178-180°C, IR (KBr, cm^{-1}) 1672 (br, 2 >C=O), 2198, 2237 (2C≡N), 3314 (br, 2NH), MS: m/z (%) 605, (M^+, 37.11) with a base peak at 55, Anal. calcd. for $C_{30}H_{16}N_7O_5SF$ (605.56): C, 59.50: H, 2.66: N, 16.19: S, 5.29: F, 3.13% found: C, 59.8: H, 2.58: N, 16.22: S, 5.25: F, 3.08.

Cytotoxicity assay:

The cells were propagated in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, HEPES buffer and 50ug/ml gentamycin. All cells were maintained at 37°C in a humidified atmosphere with 5% CO_2 and were subcultured two times a week.

Cell toxicity was monitored by determining the effect of the test samples on cell morphology and cell viability.

Cytotoxicity evaluation using viability assay: For cytotoxicity assay, the cells were seeded in 96-well plate at a cell concentration of 1x 10^4 cells per well in 100µl of growth medium. Fresh medium containing different concentrations of the test sample was added after 24 h of seeding. Serial two-fold dilutions of the tested chemical compound were added to confluent cell monolayers dispensed into 96-well, flat-bottomed microtiter plates (Falcon, NJ, USA) using a multichannel pipette. The microtiter plates were incubated at 37°C in a humidified incubator with 5% CO_2 for a period of 48 h. Three wells were used for each concentration of the test sample. Control cells were incubated without test sample and with or without DMSO. The little percentage of DMSO present in the wells (maximal 0.1%) was found not to affect the experiment. After incubation of the cells for 24 h at 37°C, various concentrations of sample (50, 25, 12.5, 6.25, 3.125 & 1.56 *ig)* were added, and the incubation was continued for 48 h and viable cells yield was determined by a colorimetric method.

In brief, after the end of the incubation period, media were aspirated and the crystal violet solution (1%) was added to each well for at least 30 minutes. The stain was removed and the plates were rinsed using tap water until all excess stain is removed. Glacial acetic acid (30%) was then added to all wells and mixed thoroughly, and then the absorbance of the plates were measured after gently shaken on Microplate reader (TECAN, Inc.), using a test wavelength of 490 nm. All results were corrected for background absorbance detected in wells without added stain. Treated samples were compared with the cell control in the absence of the tested compounds. All experiments were carried out in triplicate. The cell cytotoxic effect of each tested compound was calculated[24,25].

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