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Synthesis, docking and *in vitro* anticancer evaluation of some new benzopyrone derivatives

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ABSTRACT

The synthesis of some new 3-alkyl-7-hydroxy-4-methyl-8-substituted-1*H*-benzopyran-2-ones, 6-alkyl-7-methyl-2-substituted amino-5*H*-pyrano[6,5-e] benzoxazol-5-ones, 7-alkyl-8-methyl-3-substituted-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-ones, 7,8-disubstituted-3-ethyl-4-methyl-1*H*-benzopyran-2-ones and 3-alkyl-4-methyl-7-substituted-1*H*-benzopyran-2-ones were described. Fourteen compounds were selected by National Cancer Institute (NCI), Bethesda, and evaluated for their *in vitro* anticancer activity in the full NCI 60 cell lines panel assay by a single dose test. Compounds **4a**, **18a**, **18b** and **23a** were found to be broad-spectrum antitumors showing effectiveness toward numerous cell lines that belong to different tumor subpanels. Furthermore, docking studies were undertaken to gain insight into the possible binding mode of these compounds with the binding site of the casein kinase II (CK2) enzyme which is involved in cell survival and proliferation through a number of downstream effectors.

1. Introduction

Cancer is a leading cause of death worldwide and accounted for 7.6 million deaths (around 13% of all deaths) in 2008 and deaths from cancer worldwide are projected to continue to rise to over 13.1 million in 2030 [1]. Cancer cells develop a degree of autonomy and grow uncontrollably disregarding the normal rules of cell division, resulting in uncontrolled growth and proliferation. In fact, almost 90% of cancer- related deaths are due to tumor spreading or dissemination [2]. Several techniques involving surgery, radiation, immunotherapy and chemotherapy were adopted for eradication of cancerous cells. Unfortunately, no currently available anticancer drugs would eradicate cancer cells without harming normal tissues [3]. Accordingly, continued research is needed to develop new and efficient antitumor agents.

Benzopyran-2-one comprises a group of natural compounds found in a variety of plant sources. Benzopyran-2-ones are recognized to possess a wide variety of biological activities against bacteria [4,5], fungi [6] and protozoa [7]. In addition, they are also reported to possess anti-inflammatory [8], antioxidant [6,9], antiallergic [10], antithrombotic [11], antiHIV [12], antidepressant [13–15], photosensitizing [16,17], estrogenic like [18] and anticancer activities [19–22]. Warfarin **A** (Fig. 1) reduced metastases from intestinal carcinomas to a great extent [23] and also used as an adjunct to the surgical treatment of malignant tumors [24].

The inhibition activity of benzopyran-2-one derivative **B** (Fig. 1) against different cancer cell lines showed a high selectivity for HUVEC that can be potentially utilized as lead compound to develop non toxic angiogenesis inhibitors and small molecular ligands to target HUVEC [25].

In addition, daphnetin **C** (Fig. 1) was proven to act as tyrosine kinase inhibitor. Daphnetin inhibited tyrosine kinase, epidermal growth factor receptor, serine/threonine- specific protein kinase, and protein kinase C *in vitro* [26]. Also, benzopyran-2-one derivative **D** (Fig. 1) was identified as a novel class of MEK 1 kinase inhibitors [27].

Furthermore, some heterocycles such as oxathiazolidine [28], triazole [29], oxazole [30] and thiadiazole [31] were found to possess potential antitumor activity.

These findings have encouraged us to prepare compounds containing the benzopyran-2-one nucleus substituted at 7-position with different bioisosteric moieties as triazole, thiadiazole, thiazolidinone, thiazole, hydrazone, oxathiazolidine, dihydropyrazole, dihydropyrrole and dioxopyrrolidine. Also, 8-substituted derivatives as chalcones, dihydropyridines, ureas and imidazolidinetriones in addition to oxazolo and oxazinobenzopyran-2-one derivatives were prepared. Fourteen compounds of the synthesized compounds were selected by National Cancer Institute (NCI),

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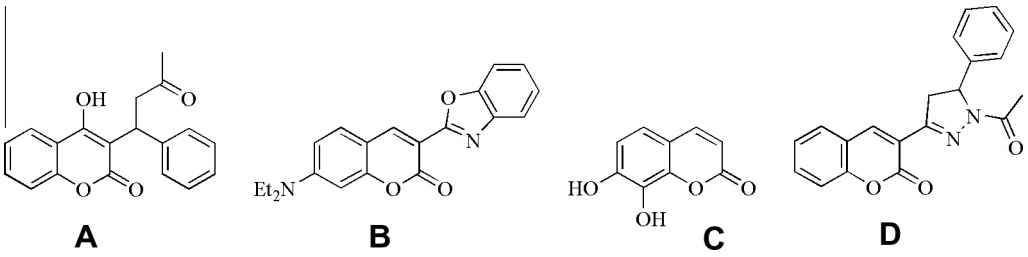


Fig. 1. Anticancer, anti-angiogenic and kinase inhibitors benzopyrone derivatives.

Bethesda, MD, U.S.A., for *in vitro* one dose testing in the full NCI 60 cell lines panel assay. In addition, attempt to elucidate a molecular target for activity was achieved via molecular docking of the prepared compounds in the active site of casein kinase II enzyme (CK2) using Molsoft ICM 3.4–8C program.

2. Experimental

2.1. Chemistry

Melting points were determined by open capillary tube method using Stuart SMP10 melting point apparatus and were uncorrected. Microanalysis was carried out at The Regional Center for Mycology and Biotechnology, Al-Azhar University. Infrared Spectra were recorded as potassium bromide discs on Schimadzu FT-IR 8400S spectrophotometer (Shimadzu, Kyoto, Japan) and Bruker FT-IR spectrophotometer and expressed in wave number υ_{max} (cm^{-1}). The ^1H NMR spectra were recorded on a Varian Mercury VX-300 NMR spectrometer at 300 MHz and *JEOL-ECA500 NMR spectrometer at 500 MHz in chloroform ($CDCl_3$) or dimethylsulfoxide (DMSO-d_6). Chemical Shifts are quoted in δ as parts per million (ppm) downfield from tetramethylsilane (TMS) as internal standard and J values are reported in Hz. Mass spectra were performed as EI at 70 eV on Hewlett Packard Varian (Varian, Polo, USA) and Shimadzu Gas Chromatograph Mass spectrometer-QP 1000 EX and direct inlet unit of Shimadzu GC/MS-QP5050A. TLC were carried out using Macherey–Nagel Alugram Sil G/UV_{254} silica gel plates with fluorescent indicator UV_{254} and acetonitrile:methanol (9:1) as the eluting system and the spots were visualized at 366, 254 nm by UV Vilber Lourmat 77202 (Vilber, Marne La Vallee, France).

2.1.1. 3,4-Dimethyl-7-hydroxy-2H-1-benzopyran-2-one and 3-ethyl-7-hydroxy-4-methyl-2H-1-benzopyran-2-one ***1a,b***

were prepared as reported in literature [32].

2.1.2. General procedure for synthesis of 3-alkyl-7-hydroxy-4-methyl-8-phenylazo-2H-1-benzopyran-2-ones ***2a,b*** *(Scheme 1)*

Phenyl diazonium chloride {freshly prepared by addition of sodium nitrite solution (4 g, 0.06 mol) in water (20 ml) to a mixture of aniline (3.81 g, 0.041 mol) and hydrochloric acid (16 ml) dropwise while cooling in an ice bath 0–5 °C} was added slowly to a cooled solution of compound **1a,b** (0.041 mol) in 5% sodium hydroxide solution (45 ml). The reaction mixture was stirred for 1 h, filtered, washed and dried.

2.1.2.1. 3,4-Dimethyl-7-hydroxy-8-phenylazo-2H-1-benzopyran-2-one ***2a***. The crude product was crystallized from methanol. Yield 50%. mp 140–141 °C. IR υ_{max}/cm^{-1}: 3192 (OH), 2924, 2850 (CH aliphatic), 1670 (C=O), 1620, 1612, 1566, 1510 (N=N, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.10 (s, 3H, CH_3 at C4), 2.38 (s, 3H, CH_3 at C3), 6.97 (d, 1H, J = 9.3 Hz, H-6 Ar), 7.60–7.67 (m, 3H, H-3′,4′,5′Ar), 7.82 (d, 1H, J = 9.3 Hz, H-5 Ar), 7.98 (d, 2H, J = 8.4 Hz, H-2′,6′ Ar), 13.37 (s, 1H, OH). MS m/z (%): 296, $M^+ + 2$ (16.49%). Anal. Calcd. for $C_{17}H_{14}N_2O_3$ (294.30): C, 69.38; H, 4.79; N 9.52. Found: C, 69.44; H, 4.82; N, 9.60.

2.1.2.2. 3-Ethyl-7-hydroxy-4-methyl-8-phenylazo-2H-1-benzopyran-2-one ***2b***. The crude product was crystallized from methanol. Yield 66%. mp 130–133 °C. IR υ_{max}/cm^{-1}: 3460 (OH), 3047 (CH Ar), 2968, 2910 (CH aliphatic), 1708 (C=O), 1629, 1593, 1550 (N=N, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.07 (t, 3H, $CH_2C\underline{H_3}$), 2.42 (s, 3H, CH_3), 2.59 (q, 2H, $C\underline{H_2}CH_3$), 6.99 (d, 1H, J = 9.0 Hz, H-6 Ar), 7.57–7.67 (m, 3H, H-3′,4′,5′ Ar), 7.83 (d, 1H, J = 9.3 Hz, H-5 Ar), 7.98 (d, 2H, J = 7.8 Hz, H-2′,6′ Ar), 13.37 (s, 1H, OH). MS m/z (%): 308, M^+ (0.71%). Anal. Calcd. for $C_{18}H_{16}N_2O_3$ (308.33): C, 70.12; H, 5.23; N, 9.09. Found: C, 70.14; H, 5.27; N, 9.14.

2.1.3. General procedure for synthesis of 3-alkyl-8-amino-7-hydroxy-4-methyl-2H-1-benzopyran-2-ones ***3a,b*** *(Scheme 1)*

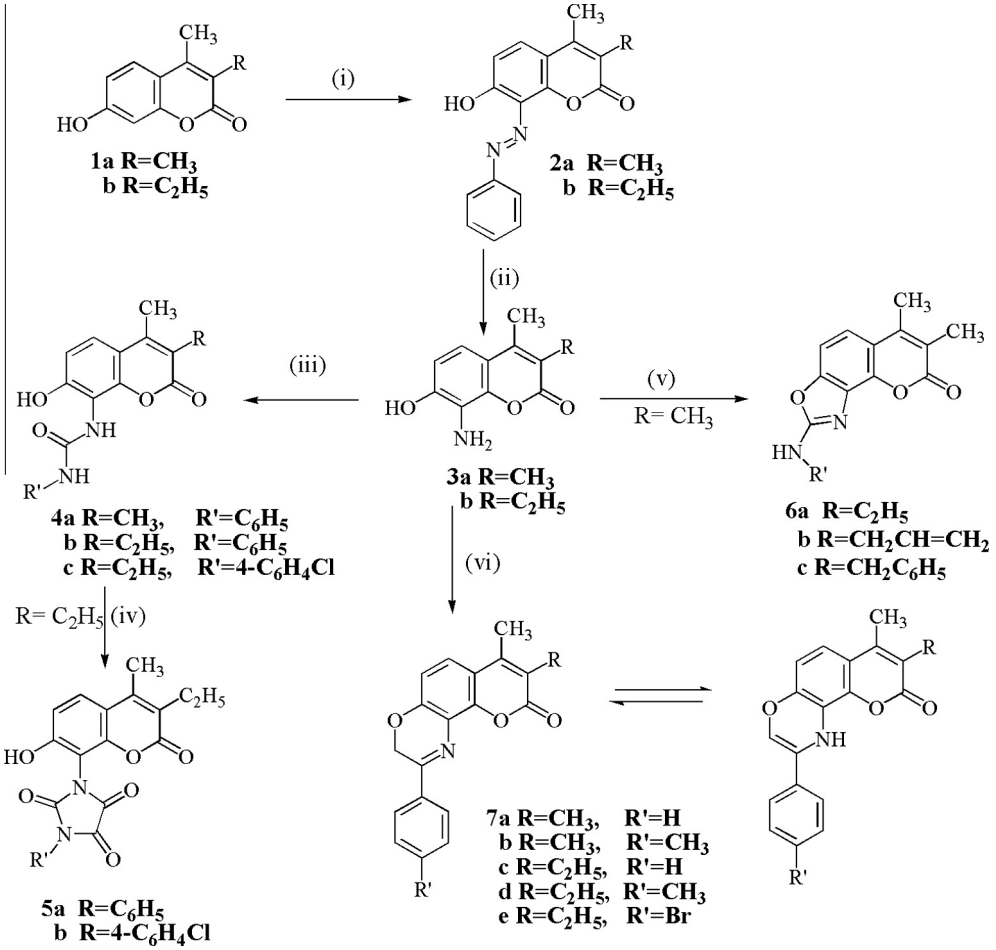
A solution of sodium dithionite (7 g, 0.04 mol) in water (30 ml) was quickly added to a solution of the azo compound **2a,b** (0.01 mol) in 30% ammonium hydroxide solution (20 ml) and the reaction mixture was refluxed for 15 min. After cooling, the crude product was filtered off, washed and dried.

2.1.3.1. 8-Amino-3,4-dimethyl-7-hydroxy-2H-1-benzopyran-2-one ***3a***. The crude product was crystallized from isopropanol. Yield 69%. mp 228–230 °C. IR υ_{max}/cm^{-1}: 3444, 3365 (NH_2), 3199 (OH), 2927, 2856 (CH aliphatic), 1680 (C=O), 1616, 1568, 1510 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.05 (s, 3H, CH_3 at C4), 2.30 (s, 3H, CH_3 at C3), 6.67 (s, 2H, NH_2), 6.89 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.59 (d, 1H, J = 8.7 Hz, H-5 Ar), 10.31 (s, 1H, OH). MS m/z (%): 205, M^+ (0.54%). Anal. Calcd. for $C_{11}H_{11}NO_3$ (205.21): C, 64.38; H, 5.40; N, 6.83. Found: C, 64.37; H, 5.39; N, 6.91.

2.1.3.2. 8-Amino-3-ethyl-7-hydroxy-4-methyl-2H-1-benzopyran-2-one ***3b***. The crude product was crystallized from isopropanol. Yield 58%. mp 176–178 °C. IR υ_{max}/cm^{-1}: 3325, 3296 (NH_2, OH), 3074 (CH Ar), 2970, 2872 (CH aliphatic), 1708 (C=O), 1620, 1610, 1602, 1566, 1510 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.02 (t, 3H, $CH_2C\underline{H_3}$), 2.34 (s, 3H, CH_3), 2.55 (q, 2H, $C\underline{H_2}CH_3$), 3.80 (br s, 2H, NH_2), 6.78 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.59 (d, 1H, J = 9.0 Hz, H-5 Ar), 9.98 (s, 1H, OH). MS m/z (%): 219, M^+ (0.06%). Anal. Calcd. for $C_{12}H_{13}NO_3$ (219.24): C, 65.74; H, 5.98; N, 6.39. Found: C, 65.78; H, 6.02; N, 6.46.

2.1.4. General procedure for synthesis of 1-(3-alkyl-4-methyl-7-hydroxy-2-oxo-2H-1-benzopyran-8-yl)-3-(4-(un)substituted phenyl) ureas ***4a–c*** *(Scheme 1)*

A mixture of the amino compound **3a,b** (0.01 mol), the appropriate isocyanate (0.01 mol) in dichloromethane (5 ml) was refluxed with stirring for 6 h. The obtained solid product was filtered off, washed with ether and dried.



Scheme 1. Reagents and conditions: (i) phenyl diazonium chloride, 5% NaOH, r.t., 1 h, (ii) sodium dithionite, 30% NH_4OH, reflux, 15 min., (iii) appropriate isocyanate, CH_2Cl_2, reflux, 6 h, (iv) oxalyl chloride, benzene, 60–65 °C, 8–10 h, (v) appropriate isothiocyanate, ethanol, triethylamine, reflux, 15 h, and (vi) appropriate phenacyl bromide, sodium ethoxide, ethanol, reflux, 2 h.

2.1.4.1. 1-(3,4-Dimethyl-7-hydroxy-2-oxo-2H-1-benzopyran-8-yl)-3-phenylurea ***4a****.* The crude product was crystallized from isopropanol. Yield 76%. mp 312–314 °C. IR υ_{max}/cm^{-1}: 3300, 3230 (NH, OH), 3057 (CH Ar), 2965, 2875 (CH aliphatic), 1697, 1685 (2C=O), 1622, 1608, 1564, 1539, 1512 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.04 (s, 3H, CH_3 at C4), 2.32 (s, 3H, CH_3 at C3), 6.78 (d, 1H, J = 8.1 Hz, H-6 Ar), 6.96 (t, 1H, H-4′ Ar), 7.27 (t, 2H, H-3′,5′ Ar), 7.44 (d, 1H, J = 7.8 Hz, H-5 Ar), 7.59 (d, 2H, J = 9.0 Hz, H-2′,6′ Ar), 8.66 (s, 1H, NH), 10.37 (s, 2H, NH, OH). MS m/z (%): 324, M^+ (0.08%). Anal. Calcd. for $C_{18}H_{16}N_2O_4$ (324.33): C, 66.66; H, 4.97; N, 8.64. Found: C, 66.72; H, 5.03; N, 8.73.

2.1.4.2. 1-(3-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)-3-phenylurea ***4b****.* The crude product was crystallized from isopropanol. Yield 88%. mp 159–160 °C. IR υ_{max}/cm^{-1}: 3263, 3136 (2NH, OH), 3082 (CH Ar), 2966, 2873 (CH aliphatic), 1743, 1678 (2C=O), 1604, 1554, 1500 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.01 (t, 3H, $CH_2C\underline{H_3}$), 2.33 (s, 3H, CH_3), 2.52 (q, 2H, $C\underline{H_2}CH_3$), 4.99 (s, 1H, NH), 6.47 (d, 1H, J = 7.5 Hz, H-6 Ar), 6.77 (d, 1H, J = 8.7 Hz, H-5 Ar), 6.98 (t, 2H, H-3′,5′ Ar), 7.30 (t, 1H, H-4′ Ar), 7.58 (d, 2H, J = 8.7 Hz, H-2′,6′ Ar), 8.65 (s, 1H, NH), 10.37 (s, 1H, OH). MS m/z (%): 338, M^+ (0.05%). Anal. Calcd. for $C_{19}H_{18}N_2O_4$ (338.38): C, 67.44; H, 5.36; N, 8.28. Found: C, 67.48; H, 5.34; N, 8.36.

2.1.4.3. 1-(3-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)-3-(4-chlorophenyl)urea ***4c****.* The crude product was crystallized from isopropanol. Yield 57%. mp 197–198 °C. IR υ_{max}/cm^{-1}: 3300, 3230 (NH, OH), 2950, 2860 (CH aliphatic) 1697, 1676 (2C=O), 1614, 1560, 1535, 1512 (NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.01 (t, 3H, $CH_2C\underline{H_3}$), 2.34 (s, 3H, CH_3), 2.52 (q, 2H, $C\underline{H_2}CH_3$), 5.22 (s, 1H, NH), 6.53 (d, 1H, J = 8.7 Hz, H-6 Ar), 6.77 (d, 1H, J = 8.4 Hz, H-5 Ar), 7.31 (d, 2H, J = 9.0 Hz, H-3′,5′ Ar), 7.48 (d, 2H, J = 9.0 Hz, H-2′,6′ Ar), 8.84 (s, 1H, NH), 10.36 (s, 1H, OH). MS m/z (%): 374, $M^+ + 1$ (11.76%). Anal. Calcd. for $C_{19}H_{17}ClN_2O_4$ (373.80): C, 61.21; H, 4.60; Cl, 9.51; N, 7.51. Found: C, 61.24; H, 4.67; Cl, 9.62; N, 7.56.

2.1.5. General procedure for synthesis of 1-(3-ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)-3-(4-(un)substituted phenyl) imidazolidin-2,4,5-triones ***5a,b*** *(Scheme 1)*

To a solution of urea derivatives **4a,b** (0.001 mol) in dry benzene (5 ml), oxalyl chloride (0.25 g, 0.002 mol) was added dropwise while stirring. The reaction mixture was heated at 60–65 °C for 8–10 h, cooled, filtered and washed with ether.

2.1.5.1. 1-(3-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)-3-phenylimidazolidin-2,4,5-trione ***5a****.* The crude product was crystallized from ethanol. Yield 62%. mp 205–208 °C. IR υ_{max}/cm^{-1}: 3267 (OH), 3082 (CH Ar), 2974, 2873 (CH aliphatic), 1747, 1685 (4C=O), 1600, 1550, 1500 (C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H_3}$), 2.36 (s, 3H, CH_3), 2.56 (q, 2H, $C\underline{H_2}CH_3$), 6.53 (d, 1H, J = 7.2 Hz, H-6 Ar), 6.97 (d, 1H, J = 7.2 Hz, H-5 Ar), 7.25–7.61 (m, 3H, H-3′,4′,5′ Ar), 7.84 (d, 2H,

J = 9.0 Hz, H-2′,6′ Ar), 10.37 (s, 1H, OH). MS m/z (%): 394, M^+ + 2 (0.04%). Anal. Calcd. for $C_{21}H_{16}N_2O_6$ (392.36): C, 64.28; H, 4.11; N, 7.14. Found: C, 64.31; H, 4.10; N, 7.19.

2.1.5.2. 3-(4-Chlorophenyl)-1-(3-ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)imidazolidin-2,4,5-trione ***5b****.* The crude product was crystallized from ethanol. Yield 89%. mp 230–232 °C. IR υ_{max}/cm^{-1}: 3284 (OH), 3053 (CH Ar), 2966, 2873 (CH aliphatic), 1759, 1708, 1689 (4C=O), 1614, 1597, 1543, 1504 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.02 (t, 3H, $CH_2C\underline{H_3}$), 2.34 (s, 3H, CH_3), 2.52 (q, 2H, $C\underline{H_2}CH_3$), 6.78 (d, 1H, J = 8.7, H-6 Ar), 7.31 (d, 1H, J = 9.0 Hz, H-5 Ar), 7.47 (d, 2H, J = 8.7 Hz, H-3′,5′ Ar), 7.59 (d, 2H, J = 8.7 Hz, H-2′,6′ Ar), 10.39 (s, 1H, OH). MS m/z (%): 426, M^+ (0.03%). Anal. Calcd. for $C_{21}H_{15}ClN_2O_6$ (426.81): C, 59.10; H, 3.54; Cl, 8.31; N, 6.56. Found: C, 59.13; H, 3.59; Cl, 8.39; N, 6.62.

2.1.6. General procedure for synthesis of 6,7-dimethyl-2-substituted amino-5H-pyrano[6,5-e] benzoxazol-5-ones ***6a–c*** *(Scheme 1)*

To a solution of amino compound **3b** (2.05 g, 0.01 mol) in ethanol (75 ml) containing few drops of triethylamine, the appropriate isothiocyanate derivative (0.01 mol) was added. The solution was refluxed for 15 h or till the evolution of hydrogen sulfide gas ceases. The solvent was distilled under reduced pressure and the residue was crystallized from the appropriate solvent.

2.1.6.1. 6,7-Dimethyl-2-ethylamino-5H-pyrano[6,5-e] benzoxazol-5-one ***6a****.* The crude product was crystallized from ethanol. Yield 50%. mp 199–203 °C. IR υ_{max}/cm^{-1}: 3263 (NH), 3062 (CH Ar), 2966, 2873 (CH aliphatic), 1678 (C=O), 1604, 1554, 1500 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.21 (t, 3H, $CH_2C\underline{H_3}$), 2.04 (s, 3H, CH_3 at C4), 2.32 (s, 3H, CH_3 at C3), 3.39 (q, 2H, $C\underline{H_2}CH_3$), 6.77 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.59 (d, 1H, J = 9.0 Hz, H-5 Ar), 8.16 (t, 1H, NH). MS m/z (%): 258, M^+ (21.41)%. Anal. Calcd. for $C_{14}H_{14}N_2O_3$ (258.27): C, 65.11; H, 5.46; N, 10.85. Found: C, 65.17; H, 5.50; N, 11.04.

2.1.6.2. 2-Allylamino-6,7-dimethyl-5H-pyrano[6,5-e] benzoxazol-5-one ***6b****.* The crude product was crystallized from isopropanol. Yield 45%. mp 278–281 °C. IR υ_{max}/cm^{-1}: 3267 (NH), 3062 (CH Ar), 2970, 2830 (CH aliphatic), 1690 (C=O), 1600, 1550, 1500 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.11 (s, 3H, CH_3 at C4), 2.41 (s, 3H, CH_3 at C3), 3.97–4.02 (m, 2H, $C\underline{H_2}CH{=}CH_2$), 5.12–5.31 (m, 2H, $CH_2CH{=}C\underline{H_2}$), 5.92–6.31 (m, 1H, $CH_2C\underline{H}{=}CH_2$), 7.37 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.41 (d, 1H, J = 8.4 Hz, H-5 Ar), 8.40 (t, 1H, NH). MS m/z (%): 270, M^+ (100%). Anal. Calcd. for $C_{15}H_{14}N_2O_3$ (270.28): C, 66.66; H, 5.22; N, 10.36. Found: C, 66.71; H, 5.24; N, 10.49.

2.1.6.3. 2-Benzylamino-6,7-dimethyl-5H-pyrano[6,5-e] benzoxazol-5-one ***6c****.* The crude product was crystallized from ethanol. Yield 33%. mp 241–243 °C. IR υ_{max}/cm^{-1}: 3398 (NH), 2954, 2854 (CH aliphatic), 1701 (C=O), 1612, 1566, 1508 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.05 (s, 3H, CH_3 at C4), 2.33 (s, 3H, CH_3 at C3), 4.57 (d, 2H, J = 6.3 Hz, $C\underline{H_2}C_6H_5$), 6.78 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.33–7.42 (m, 5H, Ar-H), 7.60 (d, 1H, J = 8.7 Hz, H-5 Ar), 8.75 (t, 1H, NH). MS m/z (%): 320, M^+ (0.25%). Anal. Calcd. for $C_{19}H_{16}N_2O_3$ (320.34): C, 71.24; H, 5.03; N, 8.74. Found: C, 71.31; H, 5.06; N, 8.85.

2.1.7. General procedure for synthesis of 7-alkyl-8-methyl-3-(4-(un) substituted phenyl)-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-ones ***7a–e*** *(Scheme 1)*

To a solution of compound **3a,b** (0.01 mol) and sodium ethoxide (0.01 mol) in ethanol (50 ml), the appropriate phenacyl bromide derivative (0.02 mol) was added and the solution was refluxed for 2 h. The reaction mixture was filtered and the filtrate was concentrated then left to cool. The formed precipitate was filtered, washed and dried.

2.1.7.1. 7,8-Dimethyl-3-phenyl-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-one ***7a****.* The crude product was crystallized from isopropanol. Yield 66%. mp 366–367 °C. IR υ_{max}/cm^{-1}: 2927, 2852 (CH aliphatic), 1680 (C=O), 1616, 1566, 1510 (C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 5.73 (s, 2H, CH_2 Oxazine), 6.99–7.05 (m, 2H, H-6,4′ Ar), 7.58 (t, 2H, H-3′,5′ Ar), 7.70 (d, 1H, J = 8.7 Hz, H-5 Ar), 8.04 (d, 2H, J = 8.4 Hz, H-2′,6′ Ar). MS m/z (%): 305, M^+ (57.44%). Anal. Calcd. for $C_{19}H_{15}NO_3$ (305.33): C, 74.74; H, 4.95; N, 4.59. Found: C, 74.81; H, 5.02; N, 4.67.

2.1.7.2. 7,8-Dimethyl-3-(4-methylphenyl)-4,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-one ***7b****.* The crude product was crystallized from isopropanol. Yield 55%. mp 267–269 °C. IR υ_{max}/cm^{-1}: 3258 (NH), 3034 (CH Ar), 2969, 2873 (CH aliphatic), 1702 (C=O), 1609, 1581, 1569, 1505 (NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 2.03 (s, 3H, CH_3 at C4), 2.31 (s, 3H, CH_3 at C3), 2.36 (s, 3H, CH_3 at C4′), 5.64 (s, 1H, CH Oxazine), 6.63 (d, 1H, J = 6.0 Hz, H-6 Ar), 6.74 (d, 2H, J = 6.9 Hz, H-3′,5′ Ar), 7.35 (d, 1H, J = 7.7 Hz, H-5 Ar), 7.55 (d, 2H, J = 8.4 Hz, H-2′,6′ Ar), 10.33 (s, 1H, NH). MS m/z (%): 321, M^+ + 2 (0.03%). Anal. Calcd. for $C_{20}H_{17}NO_3$ (319.35): C, 75.22; H, 5.37; N, 4.39. Found: C, 75.19; H, 5.41; N, 4.52.

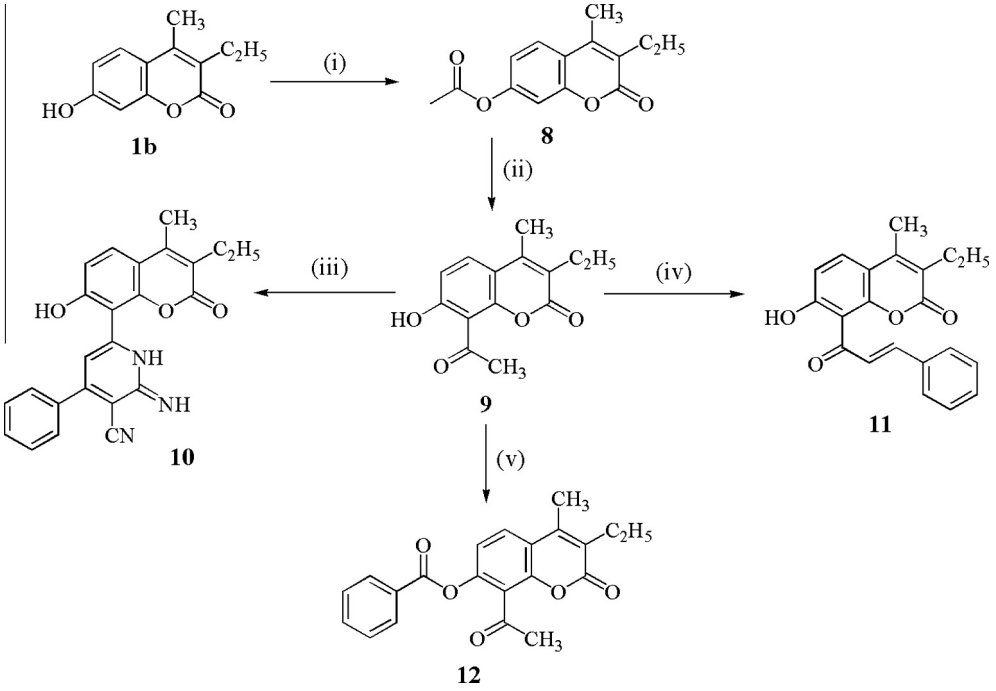
2.1.7.3. 7-Ethyl-8-methyl-3-phenyl-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-one ***7c****.* The crude product was crystallized from isopropanol. Yield 71%. mp 130–133 °C. IR υ_{max}/cm^{-1}: 2927 (CH aliphatic), 1678 (C=O), 1616, 1562, 1508 (C=N, C=C). ^{*1}H NMR (500 MHz, $CDCl_3$) δ ppm: 1.11 (t, 3H, $CH_2C\underline{H_3}$), 2.35 (s, 3H, CH_3), 2.64 (q, 2H, $C\underline{H_2}CH_3$), 5.35 (s, 2H, CH_2 Oxazine), 6.75 (d, 1H, J = 8.4 Hz, H-6 Ar), 6.91 (d, 2H, J = 8.4 Hz, H-5 Ar), 7.49 (t, 2H, H-3′,5′ Ar), 7.63 (t, 1H, H-4′Ar), 7.97 (d, 2H, J = 7.7 Hz, H-2′,6′ Ar). MS m/z (%): 319, M^+ (50.00%). Anal. Calcd. for $C_{20}H_{17}NO_3$ (319.35): C, 75.22; H, 5.37; N, 4.39. Found: C, 75.21; H, 5.39; N, 4.51.

2.1.7.4. 7-Ethyl-8-methyl-3-(4-methylphenyl)-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-one ***7d****.* The crude product was crystallized from isopropanol. Yield 88%. mp 110–112 °C. IR υ_{max}/cm^{-1}: 2927, 2854 (CH aliphatic), 1678 (C=O), 1616, 1566, 1512 (C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H_3}$), 2.38 (s, 3H, CH_3 at C4), 2.40 (s, 3H, CH_3 at C4′), 2.50 (q, 2H, $C\underline{H_2}CH_3$), 5.68 (s, 2H, CH_2 Oxazine), 6.99 (d, 1H, J = 9.0 Hz, H-6 Ar), 7.38 (d, 2H, J = 7.8 Hz, H-3′,5′ Ar), 7.69 (d, 1H, J = 8.7 Hz, H-5 Ar), 7.93 (d, 2H, J = 8.4 Hz, H-2′,6′ Ar). MS m/z (%): 333, M^+ (0.06%). Anal. Calcd. for $C_{21}H_{19}NO_3$ (333.38): C, 75.66; H, 5.74; N, 4.20. Found: C, 75.69; H, 5.72; N, 4.28.

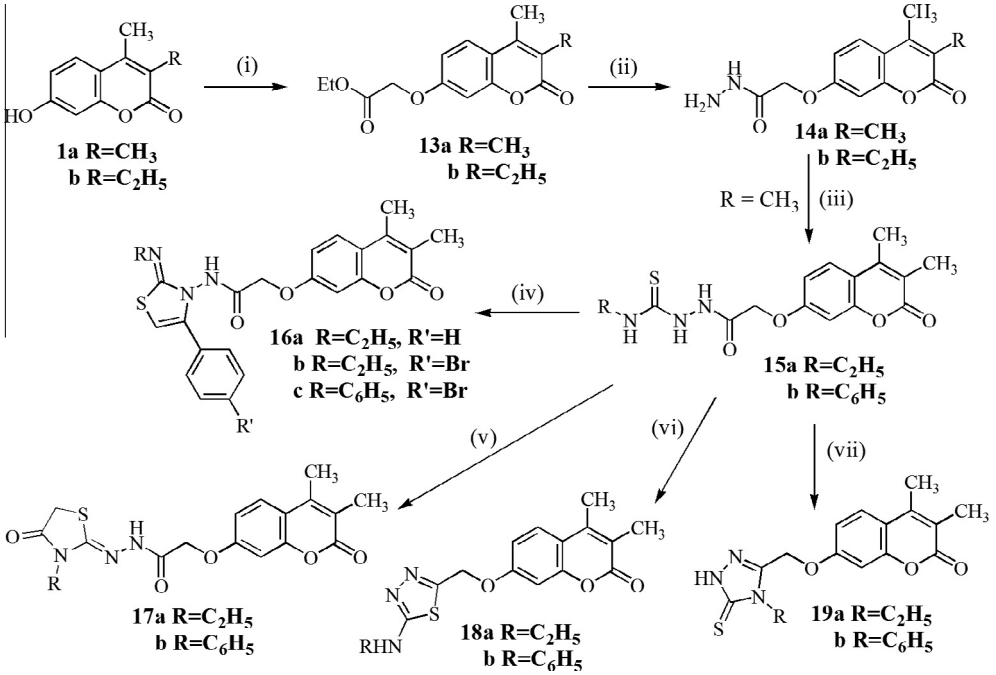
2.1.7.5. 3-(4-Bromophenyl)-7-ethyl-8-methyl-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-one ***7e****.* The crude product was crystallized from isopropanol. Yield 65%. mp 146–149 °C. IR υ_{max}/cm^{-1}: 3072 (CH Ar), 2926 (CH aliphatic), 1699 (C=O), 1618, 1608, 1566, 1510 (C=N, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H_3}$), 2.38 (s, 3H, CH_3), 2.52 (q, 2H, $C\underline{H_2}CH_3$), 5.70 (s, 2H, CH_2 Oxazine), 7.00 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.69 (d, 1H, J = 8.7 Hz, H-5 Ar), 7.80 (d, 2H, J = 8.7 Hz, H-3′,5′ Ar), 7.96 (d, 2H, J = 8.1 Hz, H-2′,6′ Ar). MS m/z (%): 398, M^+ (0.11%). Anal. Calcd. for $C_{20}H_{16}BrNO_3$ (398.25): C, 60.32; H, 4.05; Br, 20.06; N, 3.52. Found: C, 60.38; H, 4.11; Br, 20.12; N, 3.63.

2.1.8. (3-Ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yl)acetate ***8*** *(Scheme 2)*

was prepared as reported in literature [33].



Scheme 2. Reagents and conditions: (i) acetic anhydride reflux, 2 h, (ii) anhydrous $AlCl_3$, 125–155 °C, 2 h, (iii) malononitrile, benzaldehyde, ammonium acetate, ethanol, reflux, 8 h, (iv) benzaldehyde, 2.5% NaOH, ethanol, r.t., 3 h, and (v) benzoyl chloride, 160–170 °C, 1.5 h.



Scheme 3. Reagents and conditions: (i) ethyl chloroacetate, anhydrous K_2CO_3, acetone, reflux, 24 h, (ii) hydrazine hydrate, ethanol, reflux, 24 h, (iii) appropriate isothiocyanate, ethanol, reflux, 12 h, (iv) appropriate phenacyl bromide, ethanol/chloroform 1:3, reflux, 3 h, (v) chloroacetic acid, anhydrous sodium acetate, glacial acetic acid, reflux, 4 h, (vi) conc. H_2SO_4, r.t. overnight, and (vii) 2N NaOH, reflux, 2 h.

2.1.9. 8-Acetyl-3-ethyl-7-hydroxy-4-methyl-2H-1-benzopyran-2-one ***9*** *(Scheme 2)*

was prepared as reported in literature [33].

2.1.10. 6-(3-Ethyl-7-hydroxy-4-methyl-2-oxo-2H-1-benzopyran-8-yl)-2-imino-4-phenyl-1,2-dihydropyridine-3-carbonitrile ***10*** *(Scheme 2)*

A mixture comprised of acetyl compound **9** (2.46 g, 0.01 mol), malononitrile (0.66 g, 0.01 mol), ammonium acetate (6.16 g, 0.08 mol) and benzaldehyde (1.06 g, 0.01 mol) in ethanol (50 ml) was refluxed and stirred for 8 h. The reaction mixture was cooled, the formed solid precipitate was filtered and dried. The crude product was crystallized from ethanol. Yield 92%. mp 151–152 °C. IR υ_{max}/cm^{-1}: 3442 broad (2NH, OH), 3030 (CH Ar), 2933, 2835 (CH aliphatic), 2210 (CN), 1716 (C=O), 1620, 1597, 1546 (C=N, NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.01 (t, 3H, $CH_2\underline{CH_3}$), 2.31 (s, 3H, CH_3), 2.52 (q, 2H, $\underline{CH_2}CH_3$), 6.78 (s, 1H, C5-H dihydropyridine), 6.93 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.24–8.20 (m, 6H, Ar-H, H-5 Ar), 8.26 (s, 1H, NH exchanged with D_2O), 8.90 (s, 1H, NH ex-

changed with D_2O), 12.60 (s, 1H, OH exchanged with D_2O) MS *m/z* (%): 397, M^+ (12.69%). Anal. Calcd. for $C_{24}H_{19}N_3O_3$ (397.43): C, 72.53; H, 4.82; N, 10.57. Found: C, 72.51; H, 4.88; N, 10.71.

2.1.11. 8-Cinnamoyl-3-ethyl-7-hydroxy-4-methyl-2H-1-benzopyran-2-one ***11*** *(Scheme 2)*

A solution of benzaldehyde (1.06 g, 0.01 mol) in ethanol (30 ml) was added while stirring and cooling to a solution of the acetyl compound **9** (2.46 g, 0.01 mol) in 2.5% solution of sodium hydroxide (12 ml). The reaction mixture was stirred at room temperature for 3 h and left overnight. It was poured onto ice and the separated precipitate was filtered and dried. It was crystallized from ethanol. Yield 89%. mp 187–189 °C. IR υ_{max}/cm^{-1}: 3446 (OH), 3032 (CH Ar), 2966, 2873 (CH aliphatic), 1716, 1678 (2C=O), 1618, 1604, 1564 (C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.04 (t, 3H, $CH_2C\underline{H_3}$), 2.39 (s, 3H, CH_3), 2.86 (q, 2H, $C\underline{H_2}CH_3$), 6.93 (d, 1H, *J* = 9.0 Hz, H-6 Ar), 7.08–7.99 (m, 8H, CH=CH, Ar-H, H-5 Ar), 10.85 (s, 1H, OH). MS *m/z* (%): 334, M^+ (41.97%). Anal. Calcd. for $C_{21}H_{18}O_4$ (334.37): C, 75.43; H, 5.43. Found: C, 75.47; H, 5.42.

2.1.12. (8-Acetyl-3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yl) benzoate ***12*** *(Scheme 2)*

A mixture of compound **9** (2.62 g, 0.01 mol) and benzoyl chloride (2.81 g, 0.02 mol) was refluxed for 1.5 h at 160–170 °C in an oil bath. The mixture was poured onto ice-water. The obtained sticky mass was suspended in the least amount of ethanol and filtered off. The residue was washed several times with petroleum ether and dried. It was crystallized from isopropanol. Yield 95%. mp 195–196 °C. IR υ_{max}/cm^{-1}: 3085 (CH Ar), 2970, 2887 (CH aliphatic), 1723 (3C=O), 1594 (C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.08 (t, 3H, $CH_2C\underline{H_3}$), 2.47 (s, 3H, CH_3), 2.58 (s, 3H, $COCH_3$), 2.62 (q, 2H, $C\underline{H_2}CH_3$), 7.42 (d, 1H, *J* = 8.7 Hz, H-6 Ar), 7.63 (t, 2H, H-3′,5′ Ar), 7.78 (t, 1H, H-4′ Ar), 7.98 (d, 1H, *J* = 8.7 Hz, H-5 Ar), 8.08 (d, 2H, *J* = 8.4 Hz, H-2′,6′Ar). MS *m/z* (%): 350, M^+ (0.07%). Anal. Calcd. for $C_{21}H_{18}O_5$ (350.36): C, 71.99; H, 5.18. Found: C, 72.08; H, 5.18.

2.1.13. General procedure for synthesis of ethyl (3-alkyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetates ***13a*** *[34],* ***b*** *(Scheme 3)*

A mixture of the hydroxy coumarin derivatives **1a,b** (0.1 mol), anhydrous potassium carbonate (2.76 g, 0.2 mol) and ethyl chloroacetate (14.64 g, 0.12 mol) in dry acetone (50 ml) was refluxed with continuous stirring for 24 h. After completion of the reaction, reaction mixture was cooled, filtered and washed with acetone. The washing and filtrate was evaporated and residue obtained was dried.

2.1.13.1. Ethyl (3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetate ***13b****.* The crude product was crystallized from ethanol. Yield 71%. mp 99–101 °C. IR υ_{max}/cm^{-1}: 3030 (CH Ar), 2970, 2873 (CH aliphatic), 1757, 1707 (2C=O), 1608, 1566, 1508 (C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.03 (t, 3H, $CH_2C\underline{H_3}$), 1.21 (t, 3H, $OCH_2C\underline{H_3}$), 2.38 (s, 3H, CH_3), 2.55 (q, 2H, $C\underline{H_2}CH_3$), 4.18 (q, 2H, $OC\underline{H_2}CH_3$), 4.90 (s, 2H, OCH_2), 6.95 (d, 2H, *J* = 9.6 Hz, H-6, 8 Ar), 7.69 (d, 1H, *J* = 8.3 Hz, H-5 Ar). MS *m/z* (%): 290, M^+ (0.14%). Anal. Calcd. for $C_{16}H_{18}O_5$ (290.31): C 66.19; H 6.25. Found: C 66.21; H 6.28.

2.1.14. General procedure for synthesis of (3-alkyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetic acid hydrazides ***14a*** *[34],* ***b*** *(Scheme 3)*

A solution of **14a,b** (0.01 mol) and hydrazine hydrate 99% (1 g, 0.02 mol) in absolute ethanol (10 ml) was refluxed for 2 h. The precipitate **14a** or **14b** was filtered and dried.

2.1.14.1. (3-Ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetic acid hydrazide ***14b****.* The crude product was crystallized from acetic acid. Yield 90%. mp 175–177 °C. IR υ_{max}/cm^{-1}: 3446, 3213 (NH, NH_2), 3062 (CH Ar), 2962, 2870 (CH aliphatic), 1695, (2 C=O), 1625, 1612, 1568, 1510, (NH, C=C). $^{*1}H$ NMR (500 MHz, DMSO-d_6) δ ppm: 1.00 (t, 3H, $CH_2C\underline{H_3}$), 2.34 (s, 3H, CH_3), 2.51 (q, 2H, $C\underline{H_2}$-CH_3), 4.32 (s, 2H, NH_2), 4.56 (s, 2H, OCH_2), 6.89 (s, 1H, H-8 Ar), 6.94 (d, 1H, *J* = 8.4 Hz, H-6 Ar), 7.66 (d, 1H, *J* = 8.4 Hz, H-5 Ar), 9.37 (s, 1H, NH). MS *m/z* (%): 276, M^+ (36.51%). Anal. Calcd. for $C_{14}H_{16}N_2O_4$ (276.29): C, 60.86; H, 5.84; N, 10.14. Found: C, 61.59; H, 5.85; N, 9.35.

2.1.15. General procedure for synthesis of 1-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetyl-4-substituted thiosemicarbazides ***15a,b*** *(Scheme 3)*

To a solution of compound **14a** (2.62 g, 0.01 mol) in ethanol (30 ml), the appropriate substituted isothiocyanate (0.01 mol) was added and the mixture was refluxed while stirring for 12 h. The separated solid was filtered, washed with diethyl ether and dried.

2.1.15.1. 1-(3,4-Dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetyl-4-ethylthiosemicarbazide ***15a****.* The crude product was crystallized from ethanol. Yield 76%. mp 221–223 °C. IR υ_{max}/cm^{-1}: 3392, 3365, 3246 (3NH), 3061 (CH Ar), 2980, 2873 (CH aliphatic), 1708, 1691 (2C=O), 1627, 1600, 1568, 1548 (NH, C=C), 1282 (C=S). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.06 (t, 3H, $CH_2C\underline{H_3}$), 2.08 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 3.45 (q, 2H, $C\underline{H_2}CH_3$), 4.70 (s, 2H, OCH_2), 6.63 (s, 1H, NH exchanged with D_2O), 6.97 (s, 1H, H-8 Ar), 7.01 (d, 1H, *J* = 8.4 Hz, H-6 Ar), 7.18 (s, 1H, NH, exchanged with D_2O), 7.72 (d, 1H, *J* = 9.0 Hz, H-5 Ar), 8.03 (t, 1H, NH exchanged with D_2O). MS *m/z* (%): 349, M^+ (0.58%). Anal. Calcd. for $C_{16}H_{19}N_3O_4S$ (349.40): C, 55.00; H, 5.48; N, 12.03; S, 9.18. Found: C, 55.05; H, 5.52; N, 11.98; S, 9.22.

2.1.15.2. 1-(3,4-Dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetyl-4-phenylthiosemicarbazide ***15b****.* The crude product was crystallized from ethanol. Yield 88%. mp 200–204 °C. IR υ_{max}/cm^{-1}: 3332, 3192, 3126 (3NH), 3070 (CH Ar), 2927, 2856 (CH aliphatic), 1740, 1710 (2C=O), 1606, 1546 (NH, C=C), 1251 (C=S). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 2.06 (s, 3H, CH_3 at C4), 2.33 (s, 3H, CH_3 at C3), 4.75 (s, 2H, OCH_2), 6.90–7.74 (m, 8H, Ar-H), 10.26 (s, 1H, NH), 11.00 (s, 1H, NH), 14.08 (s, 1H, NH). MS *m/z* (%): 397, M^+ (16.48%). Anal. Calcd. for $C_{20}H_{19}N_3O_4S$ (397.45): C, 60.44; H, 4.82; N, 10.57; S, 8.07. Found: C, 60.48; H, 4.86; N, 10.65; S, 8.04.

2.1.16. General procedure for synthesis of 2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxyl)-N-[2-(substituted imino)-4-(4-(un)substituted phenyl)thiazol-3(2H)-yl] acetamides ***16a–c*** *(Scheme 3)*

A mixture of compound **15a,b** (0.002 mol) and the appropriate phenacyl bromide (0.002 mol) in ethanol/chloroform 1:3 mixture (100 ml) was refluxed for 3 h, concentrated, left to cool, filtered, washed with water and dried.

2.1.16.1. 2-(3,4-Dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)-N-(2-ethylimino-4-phenylthiazol-3(2H)-yl)- acetamide ***16a****.* The crude product was crystallized from glacial acetic acid. Yield 56%. mp 160–162 °C. IR υ_{max}/cm^{-1}: 3435 (NH), 3061 (CH Ar), 2943, 2895 (CH aliphatic), 1730, 1708 (2C=O), 1620, 1571, 1506 (C=N, NH, C=C). ^1H NMR (300 MHz, DMSO-d_6) δ ppm: 1.31 (t, 3H, $CH_2C\underline{H_3}$), 2.08 (s, 3H, CH_3 at C4), 2.36 (s, 3H, CH_3 at C3), 4.07 (q, 2H, $C\underline{H_2}CH_3$), 4.98 (s, 1H, NH, exchanged with D_2O), 5.40 (s, 2H, OCH_2), 7.04 (d, 1H, *J* = 9.0 Hz, H-6 Ar), 7.14 (s, 2H, H-8 Ar, C5-H thiazoline), 7.55 (t, 1H, H-4′ Ar), 7.65–7.74 (m, 3H, H-5 Ar, H-3′,5′ Ar), 8.01 (d, 2H, *J* = 8.1 Hz, H-2′,6′ Ar). MS *m/z* (%): 449, M^+ (2.35%). Anal. Calcd. for $C_{24}H_{23}N_3O_4S$ (449.52): C, 64.13; H, 5.16; N, 9.35; S, 7.13. Found: C, 64.21; H, 5.14; N, 9.47; S, 7.22.

2.1.16.2. N-[4-(4-bromophenyl)-2-ethyliminothiazol-3(2H)-yl]-2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetamide ***16b***. The crude product was crystallized from glacial acetic acid. Yield 46%. mp 140–142 °C. IR υ_{max}/cm^{-1}: 3431 (NH), 3070 (CH Ar), 2976, 2872 (CH aliphatic), 1708, 1695 (2C=O), 1612, 1583, 1568, 1508 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.18 (t, 3H, $CH_2C\underline{H_3}$), 2.04 (s, 3H, CH_3 at C4), 2.32 (s, 3H, CH_3 at C3), 3.99 (q, 2H, $C\underline{H_2}CH_3$), 4.93 (s, 1H, NH), 5.39 (s, 2H, OCH_2), 7.00 (s, 2H, H-8 Ar, C5-H thiazoline), 7.10 (d, 1H, J = 6.9 Hz, H-6 Ar), 7.68 (d, 2H, J = 9.2 Hz, H-2′,6′ Ar), 7.73 (d, 1H, J = 6.1 Hz, H-5 Ar), 7.97 (d, 2H, J = 9.2 Hz, H-3′,5′ Ar). MS m/z (%): 528, M^+ (4.51%). Anal. Calcd. for $C_{24}H_{22}BrN_3O_4S$ (528.42): C, 54.55; H, 4.20; Br, 15.12; N, 7.95; S, 6.07. Found: C, 54.58; H, 4.27; Br, 15.23; N, 8.11; S, 6.10.

2.1.16.3. N-[4-(4-bromophenyl)-2-phenyliminothiazol-3(2H)-yl]-2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetamide ***16c***. The crude product was crystallized from glacial acetic acid. Yield 32%. mp 180–182 °C. IR υ_{max}/cm^{-1}: 3406 (NH), 3059 (CH Ar), 2924, 2852 (CH aliphatic), 1710 (2C=O), 1664, 1612, 1587, 1566, 1548 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.06 (s, 3H, CH_3 at C4), 2.34 (s, 3H, CH_3 at C3), 4.94 (s, 1H, NH), 5.20 (s, 2H, OCH_2), 6.74 (s, 1H, H-8 Ar), 7.06 (d, 1H, J = 8.4 Hz, H-6 Ar), 7.09–7.47 (m, 11H, Ar H, H-5 Ar, C5-H thiazoline). MS m/z (%): 578, M^++2 (0.02%). Anal. Calcd. for $C_{28}H_{22}BrN_3O_4S$ (576.46): C, 58.34; H, 3.85; Br, 13.86; N, 7.29. Found: C, 58.41; H, 3.93; Br, 14.01; N, 7.35.

2.1.17. General procedure for synthesis of N′-(4-Oxo-3-substituted thiazolidin-2-yl)-2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetohydrazides ***17a,b*** *(Scheme 3)*

To a suspension of substituted thiosemicarbazide derivatives **15a,b** (0.01 mol) in glacial acetic acid (5 ml), anhydrous sodium acetate (1.64 g, 0.02 mol) and chloroacetic acid (1.89 g, 0.02 mol) were added. The reaction mixture was refluxed for 4 h then cooled, diluted with water and allowed to stand overnight. The product was filtered off, washed and dried.

2.1.17.1. N′-(3-Ethyl-4-oxothiazolidin-2-yl)-2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetohydrazide ***17a***. The crude product was crystallized from DMF/ H_2O. Yield 87%. mp 232–235 °C. IR υ_{max}/cm^{-1}: 3431 (NH), 3047 (CH Ar), 2980, 2875 (CH aliphatic), 1735, 1722, 1703 (3C=O), 1624, 1595, 1570, 1539 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.25 (t, 3H, $CH_2C\underline{H_3}$), 2.04 (s, 3H, CH_3 at C4), 2.33 (s, 3H, CH_3 at C3), 3.65 (q, 2H, $C\underline{H_2}CH_3$), 4.71 (s, 2H, CH_2 thiazolidine), 5.38 (s, 2H, OCH_2), 7.01 (d, 1H, J = 8.4, H-6 Ar), 7.11 (s, 1H, H-8 Ar), 7.60 (d, 1H, J = 8.3 Hz, H-5 Ar), 10.53 (s, 1H, NH exchanged with D_2O). MS m/z (%): 389, M^+ (79.95%). Anal. Calcd. for $C_{18}H_{19}N_3O_5S$ (389.43): C, 55.52; H, 4.92; N, 10.79; S, 8.23. Found: C, 55.57; H, 4.88; N, 10.92; S, 8.19.

2.1.17.2. N′-(4-Oxo-3-phenylthiazolidin-2-yl)-2-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)acetohydrazide ***17b***. The crude product was crystallized from DMF/ H_2O. Yield 83%. mp 347–350 °C. IR υ_{max}/cm^{-1}: 3192 (NH), 3070 (CH Ar), 2916, 2862 (CH aliphatic), 1703, 1687, 1680 (3C=O), 1624, 1606, 1566 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.06 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 4.72 (s, 2H, CH_2 thiazolidine), 5.19 (s, 2H, OCH_2), 6.84 (d, 1H, J = 9.0 Hz, H-6 Ar), 6.94 (s, 1H, H-8 Ar), 7.01 (d, 1H, J = 9.0 Hz, H-5 Ar), 7.48–7.73 (m, 5H, Ar-H), 10.16 (s, 1H, NH exchanged with D_2O). MS m/z (%): 439, M^+ + 2 (0.01%). Anal. Calcd. $C_{22}H_{19}N_3O_5S$ for (437.47): C, 60.40; H, 4.38; N, 9.61; S, 7.33. Found: C, 60.38; H, 4.43; N, 9.70; S, 7.41.

2.1.18. General procedure for synthesis of 7-(5-Substituted amino-1,3,4-thiadiazol-2-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-one ***18a,b*** *(Scheme 3)*

A solution of thiosemicarbazides **15a,b** (0.01 mol) in concentrated sulfuric acid (10 ml) was cooled and allowed to stand overnight. The reaction mixture was cooled then poured onto ice-water and neutralized with ammonium hydroxide solution to pH 7. The produced precipitate was filtered off, washed and dried.

2.1.18.1. 7-(5-Ethylamino-1,3,4-thiadiazol-2-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-one ***18a***. The crude product was crystallized from glacial acetic acid. Yield 91%. mp 247–249 °C. IR υ_{max}/cm^{-1}: 3157 (NH), 3030 (CH Ar), 1708 (C=O), 1660, 1612, 1566 (C=N, NH, C=C). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.22 (t, 3H, $CH_2C\underline{H_3}$), 2.04 (s, 3H, CH_3 at C4), 2.33 (s, 3H, CH_3 at C3), 4.00 (q, 2H, $C\underline{H_2}CH_3$), 5.33 (s, 2H, OCH_2), 7.01 (d, 1H, J = 10.7 Hz, H-6 Ar), 7.10 (s, 1H, H-8 Ar), 7.70 (d, 1H, J = 9.2 Hz, H-5 Ar), 13.87 (s, 1H, NH). MS m/z (%): 331, M^+ (78.89%). Anal. Calcd. for $C_{16}H_{17}N_3O_3S$ (331.39): C, 57.99; H, 5.17; N, 12.68; S, 9.68. Found: C, 58.06; H, 5.22; N, 12.83; S, 9.64.

2.1.18.2. 7-(5-Phenylamino-1,3,4-thiadiazol-2-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-one ***18b***. The crude product was crystallized from glacial acetic acid. Yield 96%. mp 305–306 °C. IR υ_{max}/cm^{-1}: 3305 (NH), 3041 (CH Ar), 2924, 2854 (CH aliphatic), 1693 (C=O), 1606, 1573, 1504 (C=N, NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.08 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 5.53 (s, 2H, OCH_2), 7.05–7.58 (m, 7H, H-6, 8 Ar, Ar-H), 7.74 (d, 1H, J = 8.7 Hz, H-5 Ar), 10.50 (s, 1H, NH). MS m/z (%): 379, M^+ (64.41%). Anal. Calcd. for $C_{20}H_{17}N_3O_3S$ (379.43): C, 63.31; H, 4.52; N, 11.07; S, 8.45. Found: C, 63.35; H, 4.56; N, 11.13; S, 8.53.

2.1.19. General procedure for synthesis of 7-(4-Substituted-5-thioxo-1,2,4-triazol-3-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-ones ***19a,b*** *(Scheme 3)*

A solution of compound **15a,b** (0.04 mol) in 2 N sodium hydroxide (12 ml) was refluxed for 2 h. The reaction mixture was cooled and acidified with 10% hydrochloric acid to pH 5. The produced precipitate was filtered, washed and dried.

2.1.19.1. 7-(4-Ethyl-5-thioxo-1,2,4-triazol-3-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-one ***19a***. The crude product was crystallized from methanol. Yield 69%. mp 198–200 °C. IR υ_{max}/cm^{-1}: 3390 (NH), 3057 (CH Ar), 2920, 2850 (CH aliphatic), 1689 (C=O), 1612, 1573, 1506 (C=N, NH, C=C), 1274 (C=S). ^{*1}H NMR (500 MHz, DMSO-d_6) δ ppm: 1.20 (t, 3H, $CH_2C\underline{H_3}$), 2.02 (s, 3H, CH_3 at C4), 2.30 (s, 3H, CH_3 at C3), 4.06 (q, 2H, $C\underline{H_2}CH_3$), 4.70 (br s, 1H, NH), 5.30 (s, 2H, OCH_2), 7.00 (d, 1H, J = 8.4 Hz, H-6 Ar), 7.09 (s, 1H, H-8 Ar), 7.67 (d, 1H, J = 8.4 Hz, H-5 Ar). MS m/z (%): 331, M^+ (0.80%). Anal. Calcd. for $C_{16}H_{17}N_3O_3S$ (331.39): C, 57.99; H, 5.17; N, 12.68; S, 9.68. Found: C, 58.04; H, 5.19; N, 12.79; S, 9.74.

2.1.19.2. 7-(4-Phenyl-5-thioxo-1,2,4-triazol-3-yl)methoxy-3,4-dimethyl-2H-1-benzopyran-2-one ***19b***. The crude product was crystallized from methanol. Yield 38%. mp 191–194 °C. IR υ_{max}/cm^{-1}: 3415 (NH), 3039 (CH Ar), 2924, 2856 (CH aliphatic), 1700 (C=O), 1643, 1602, 1568, 1502 (C=N, NH, C=C), 1286 (C=S). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.05 (s, 3H, CH_3 at C4), 2.33 (s, 3H, CH_3 at C3), 5.09 (s, 2H, OCH_2), 6.80 (d, 1H, J = 8.7 Hz, H-6 Ar), 6.90 (s, 1H, H-8 Ar), 7.43–7.55 (m, 5H, Ar-H), 7.64 (d, 1H, J = 8.7 Hz, H-5 Ar), 14.06 (s, 1H, NH exchanged with D_2O). MS m/z (%): 380, M^+ + 1 (57.34%). Anal. Calcd. for $C_{20}H_{17}N_3O_3S$ (379.43): C, 63.31; H, 4.52; N, 11.07; S, 8.45. Found: C, 63.36; H, 4.57; N, 11.18; S, 8.51.

*2.1.20. 4-Benzyl-1-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yloxy-acetyl)semicarbazide **20** (Scheme 4)*

A mixture of **14a** (2.62 g, 0.01 mol) and benzyl isocyanate (1.59 g, 0.01 mol) in dichloroethane was stirred and refluxed for 3 h. The formed precipitate was filtered, washed with diethylether and dried. The crude product was crystallized from isopropanol. Yield 98%. mp 272–273 °C. IR υ_{max}/cm^{-1}: 3460, 3327, 3294 (3 NH), 3057 (CH Ar), 2912, 2877 (CH aliphatic), 1722, 1693 (3C=O), 1649, 1624, 1566, 1537, 1500 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3 at C4), 2.36 (s, 3H, CH_3 at C3), 4.24 (d, 2H, J = 6.0 Hz, $C\underline{H_2}C_6H_5$), 4.69 (s, 2H, OCH_2), 6.96–7.28 (m, 7H, H-6, 8 Ar, Ar-H), 7.71 (d, 1H, J = 8.7 Hz, H-5 Ar), 7.95 (s, 1H, NH), 8.14 (s, 1H, NH), 9.90 (s, 1H, NH). MS m/z (%): 395, M^+ (1.42%). Anal. Calcd. for $C_{21}H_{21}N_3O_5$ (395.41): C, 63.79; H, 5.35; N, 10.63. Found: C, 63.81; H, 5.39; N, 10.76.

*2.1.21. 3-Benzyl-1-(3,4-dimethyl-2-oxo-2H-1-benzopyran-7-yl-oxy) acetamidoimidazolidin-2,4,5-trione **21** (Scheme 4)*

To a solution of compound **20** (3.95 g, 0.001 mol) in benzene (5 ml), oxalyl chloride (0.25 g, 0.002 mol) was added dropwise while stirring, then refluxed at 60–65 °C for 2 h. The solvent was distilled under reduced pressure and the obtained residue was dried. The crude product was crystallized from ethanol. Yield 88%. mp 207–208 °C. IR υ_{max}/cm^{-1}: 3415 (NH), 3084 (CH Ar), 2972, 2873 (CH aliphatic), 1749, 1728, 1707, 1699 (5C=O), 1620, 1597, 1558 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3 at C4), 2.37 (s, 3H, CH_3 at C3), 4.78 (s, 2H, $C\underline{H_2}C_6H_5$), 4.96 (s, 2H, OCH_2), 6.98 (s, 1H, H-8 Ar), 7.01 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.32–7.36 (m, 5H, Ar-H), 7.74 (d, 1H, J = 9.0 Hz, H-5 Ar), 11.44 (s, 1H, NH). MS m/z (%): 449, M^+ (17.70%). Anal. Calcd. for $C_{23}H_{19}N_3O_7$ (449.41): C, 61.47; H, 4.26; N, 9.35. Found: C, 61.49; H, 4.31; N, 9.43.

*2.1.22. General procedure for synthesis of 2-(3-Alkyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)-N′-(4-substituted benzylidene)acetohydrazides **22a,b** (Scheme 4)*

A solution of the acid hydrazides **14a,b** (0.01 mol) and the appropriate aromatic aldehyde (0.01 mol) in glacial acetic acid (20 ml) was refluxed for 6 h. The solvent was distilled under vacuum.

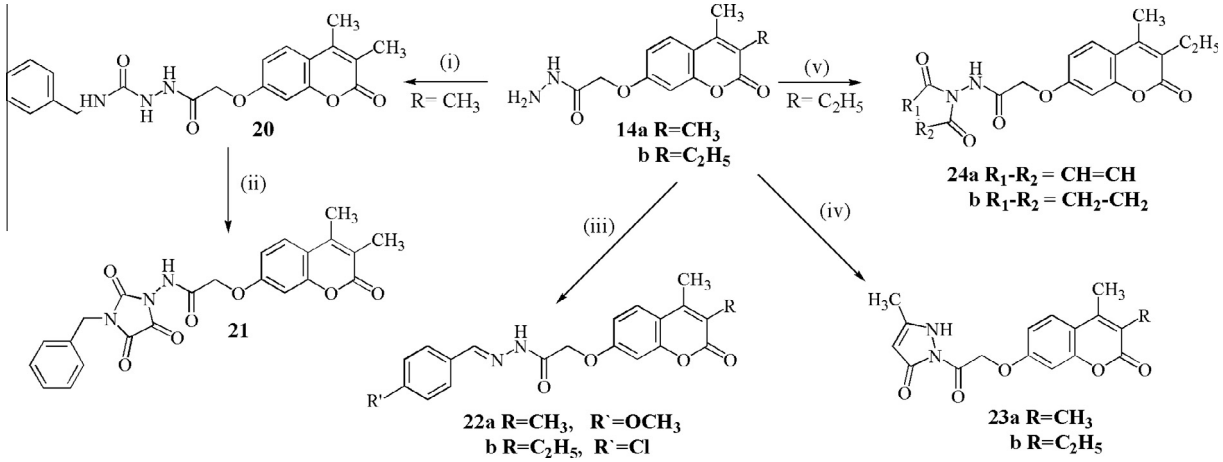
*2.1.22.1. 2-(3,4-Dimethyl-2-oxo-2H-1-benzopyran-7-yloxy)-N′-(4-methoxybenzylidene)acetohydrazide **22a**.* The crude product was crystallized from ethanol. Yield 69%. mp 225–226 °C. IR υ_{max}/cm^{-1}: 3427 (NH), 2932 (CH aliphatic), 1685 (2C=O), 1612 (C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 2.07 (s, 3H, CH_3 at C4), 2.36 (s, 3H, CH_3 at C3), 3.80 (s, 3H, OCH_3), 5.24 (s, 2H, OCH_2), 6.93 (d, 1H, J = 6.9 Hz, H-6 Ar), 7.00 (d, 2H, J = 8.4 Hz, H-3′,5′ Ar), 7.62–7.74 (m, 4H, H-5, 8 Ar, H-2′,6′ Ar), 8.27 (s, 1H, N=CH), 11.49 (s, 1H, NH, exchanged with D_2O). MS m/z (%): 380, M^+ (73.18%). Anal. Calcd. for $C_{21}H_{20}N_2O_5$ (380.39): C, 66.31; H, 5.30; N, 7.36. Found: C, 66.37; H, 5.34; N, 7.49.

*2.1.22.2. N′-(4-Chlorobenzylidene)-2-(3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetohydrazide **22b**.* The crude product was crystallized from ethanol. Yield 53%. mp 252–254 °C. IR υ_{max}/cm^{-1}: 3446 (NH), 3030 (CH Ar), 2976, 2875 (CH aliphatic), 1732, 1716 (2C=O), 1624, 1610, 1595, 1558, 1541, 1505 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.04 (t, 3H, $CH_2C\underline{H_3}$), 2.39 (s, 3H, CH_3), 2.56 (q, 2H, $C\underline{H_2}CH_3$), 5.27 (s, 2H, OCH_2), 6.94 (s, 1H, H-8), 6.98 (d, 1H, J = 8.4 Hz, H-6 Ar), 7.50 (d, 1H, J = 8.4 Hz, H-5 Ar), 7.58 (d, 2H, J = 8.4 Hz, H-3′,5′ Ar), 7.90 (d, 2H, J = 8.7 Hz, H-2′,6′ Ar), 8.01 (s, 1H, N=CH), 11.70 (s, 1H, NH). MS m/z (%): 398, M^+ (0.39%). Anal. Calcd. for $C_{21}H_{19}ClN_2O_4$ (398.84): C, 63.24; H, 4.80; N, 7.02. Found: C, 63.25; H, 4.83; N, 7.14.

*2.1.23. General procedure for synthesis of 1-[2-(3-Alkyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetyl]-3-methyl-1,2-dihydropyrazol-5-one **23a** [13], b (Scheme 4)*

A mixture of the acid hydrazide **14a,b** (0.01 mol) and ethyl acetoacetate (2.6 g, 0.02 mol) in glacial acetic acid (5 ml) was refluxed for 6 h then diluted with water. The precipitated solid **23a,b** was filtered and dried.

*2.1.23.1. 1-[2-(3-Ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy) acetyl]-3-methyl-1,2-dihydropyrazol-5-one **23b**.* The crude product was crystallized from glacial acetic acid. Yield 35%. mp 178–179 °C. IR υ_{max}/cm^{-1}: 3257 (NH), 3034 (CH Ar), 2987, 2856 (CH aliphatic), 1747, 1732, 1705 (3C=O), 1612, 1570, 1543, 1500 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.05 (t, 3H, $CH_2C\underline{H_3}$), 1.87 (s, 3H, CH_3 at C3 pyrazolone), 2.38 (s, 3H, CH_3 at C4), 2.56 (q, 2H, $C\underline{H_2}CH_3$), 4.71 (s, 2H, OCH_2), 4.79 (s, 1H, CH pyrazolone), 6.94 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.01 (s, 1H, H-8 Ar), 7.69 (d, 1H, J = 8.7 Hz, H-5 Ar), 10.08 (s, 1H, NH). MS m/z (%): 343, $M^+ + 1$ (5.41%). Anal. Calcd. for $C_{18}H_{18}N_2O_5$ (342.35): C, 63.15; H, 5.30; N, 8.18. Found: C, 63.19; H, 5.28; N, 8.29.



Scheme 4. Reagents and conditions: (i) benzyl isocyanate, dichloroethane, reflux, 3 h, (ii) oxalyl chloride, benzene, reflux, 2 h, (iii) appropriate aromatic aldehyde, glacial acetic acid, reflux, 6 h, (iv) ethyl acetoacetata, glacial acetic acid, reflux, 6 h, and (v) appropriate cyclic acid anhydride, glacial acetic acid, reflux, 9 h.

*2.1.24. General procedure for synthesis of N-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-(3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy) acetamide **24a** and N-(2,5-Dioxopyrrolidin-1-yl)-2-(3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetamide **24b** (Scheme 4)*

A mixture of compound (**14b**) (0.01 mol) and the appropriate cyclic acid anhydride (0.01 mol) in glacial acetic acid (10 ml) was refluxed for 9 h. The solvent was concentrated then poured onto ice-water, the precipitated product was filtered, dried and crystallized from isopropanol.

*2.1.24.1. N-(2,5-Dioxo-2,5-dihydro-1H-pyrrol-1-yl)-2-(3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetamide **24a***. The crude product was crystallized from isopropanol. Yield 29%. mp 208–210 °C. IR υ_{max}/cm^{-1}: 3446 (NH), 3093 (CH Ar), 2968, 2873 (CH aliphatic), 1751, 1703, 1691, 1678 (4C=O), 1622, 1604, 1568, 1506 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm: 1.04 (t, 3H, CH_2-$C\underline{H}_3$), 2.40 (s, 3H, CH_3), 2.57 (q, 2H, $C\underline{H}_2CH_3$), 4.90 (s, 2H, OCH_2), 6.99–7.05 (m, 3H, $C\underline{H}$=$C\underline{H}$, H-6 Ar), 7.20 (s, 1H, H-8), 7.74 (d, 1H, J = 9.0 Hz, H-5 Ar), 10.80 (s,1H, NH). MS m/z (%): 356, M^+ (7.59%). Anal. Calcd. for $C_{18}H_{16}N_2O_6$ (356.33): C, 60.67; H, 4.53; N, 7.86. Found: C, 60.70; H, 4.52; N, 7.98.

*2.1.24.2. N-(2,5-Dioxopyrrolidin-1-yl)-2-(3-ethyl-4-methyl-2-oxo-2H-1-benzopyran-7-yloxy)acetamide **24b***. The crude product was crystallized from isopropanol. Yield 69%. mp 238–239 °C. IR υ_{max}/cm^{-1}: 3446 (NH), 3070 (CH Ar), 2981, 2833 (CH aliphatic), 1707, 1691, 1676 (4C=O), 1602, 1583, 1558, 1525 (NH, C=C). ^{1}H NMR (300 MHz, DMSO-d_6) δ ppm:1.04 (t, 3H, $CH_2C\underline{H}_3$), 2.39 (s, 3H, CH_3), 2.57 (q, 2H, $C\underline{H}_2CH_3$), 2.80 (t, 4H, $C\underline{H}_2C\underline{H}_2$), 4.88 (s, 2H, OCH_2), 6.99 (s, 1H, H-8 Ar), 7.03 (d, 1H, J = 8.7 Hz, H-6 Ar), 7.74 (d, 1H, J = 8.7 Hz, H-5 Ar), 10.80 (s, 1H, NH). MS m/z (%): 358, M^+ (11.72%). Anal. Calcd. for $C_{18}H_{18}N_2O_6$ (358.35): C, 60.33; H, 5.06; N, 7.82. Found: C, 60.39; H, 5.10; N, 7.97.

2.2. Antitumor screening

The human tumor cell lines of the cancer screening panel were grown in RPMI 1640 medium containing 5% fetal bovine serum and 2 mM L-glutamine. For a typical screening experiment, cells were inoculated into 96 well microtiter plates in 100 μl at plating densities ranging from 5000 to 40,000 cells/well depending on the doubling time of individual cell lines. After cell inoculation, the microtiter plates were incubated at 37 °C, 5% CO_2, 95% air and 100% relative humidity for 24 h prior to addition of experimental drugs.

After 24 h, two plates of each cell line were fixed *in situ* with trichloroacetic acid (TCA), to represent a measurement of the cell population for each cell line at the time of drug addition (Tz). Experimental drugs were solubilized in dimethyl sulfoxide (DMSO) at 400-fold the desired final maximum test concentration and stored frozen prior to use. At the time of drug addition, an aliquot of frozen concentrate was thawed and diluted to twice the desired final test concentration (10^{-5} M) with complete medium containing 50 μg/ml gentamicin. Aliquots of 100 μl of these drug dilutions were added to the appropriate microtiter wells already containing 100 μl of medium, resulting in the required final drug concentrations.

Following drug addition, the plates were incubated for an additional 48 h at 37 °C, 5% CO_2, 95% air, and 100% relative humidity. For adherent cells, the assay was terminated by the addition of cold TCA. Cells were fixed *in situ* by the gentle addition of 50 μl of cold 50% (w/v) TCA (final concentration, 10% TCA) and incubated for 60 min. at 4 °C. The supernatant was discarded, and the plates were washed five times with tap water and air dried. Sulforhodamine B (SRB) solution (100 μl) at 0.4% (w/v) in 1% acetic acid was added to each well, and plates were incubated for 10 min. at room temperature. After staining, unbound dye was removed by washing five times with 1% acetic acid and the plates were air dried. Bound stain was subsequently solubilized with 10 mM trizma base, and the absorbance was read on an automated plate reader at a wavelength of 515 nm [35–38].

Using the seven absorbance measurements [time zero, (Tz), control growth, (C), and test growth in the presence of drug at the 10^{-5} M concentration level (Ti)], the percentage growth was calculated. Percentage growth inhibition was calculated as:

$$[(Ti - Tz)/(C - Tz)] \times 100 \text{ for concentrations for which } Ti \geqslant Tz$$

$$[(Ti - Tz)/Tz] \times 100 \text{ for concentrations for which } Ti < Tz$$

Mean graph is the mean of presenting the *in vitro* test results to emphasize differential effects of test compounds on various human tumor cell lines. It plots the growth relative to no drug control and relative to time zero number of cells. The mean is the average of growth across the tested cell lines, while delta is the maximum difference from the mean.

2.3. Molecular docking

Docking studies of fourteen screened compounds (**4a–c, 5a, 6c, 7a–e, 10, 18a,b, 23a**) compounds performed by Molsoft ICM 3.4-8C program.

2.3.1. Preparation of the target enzyme

Convert PDB file into an ICM object:

The X-ray crystal structure of the enzyme with coumarin ligand DBC, 3,8-dibromo-7-hydroxy-4-methylchromen-2-one, (PDB code: 2QC6) [39] was obtained from the protein data bank in PDB format. This conversion involves addition of hydrogen bonds, assignment of atom types, and charges from the residue templates. Click on MolMechanics/convert/protein, and then delete water molecules.

To perform ICM small molecule docking:

Setup Docking Project:

(1) Set Project Name: Click on Docking/set project name, press OK.
(2) Setup the Receptor: Click on Docking/Receptor setup, enter the receptor molecule in the receptor molecule data entry box (a_*) will do, then click on identify the binding sites button to identify the potential ligand binding pockets, press OK. After the receptor setup is complete, the program normally displays the receptor with selected binding site residues highlighted in yellow Xstick presentation.
(3) Review and Adjust Binding Site: ICM makes a box around the ligand binding site based on the information entered in the receptor setup section. The position of the box encompasses the residue expected to be involved in ligand binding. Click on the menu Docking/Review/Adjust ligand/Box.
(4) Make Receptor Maps: The step now is to construct energy maps of the environment within the docking box. Click on menu Docking/Make Receptor Maps, select the resolution of the map by entering a value into the grid cell size data entry box which is 0.5, this step takes few min.

2.3.2. Preparation of compounds for docking

The target compounds stated earlier were built using ChemDraw ultra version 9.0.3 and their energy were minimized through Chem3D ultra version 9.0.3/MOPAC, Jop Type: Minimum RMS Gradient of 0.010 kcal/mol and RMS distance of 0.1 Å, and saved as MDL MolFile (*mol).

2.3.3. Docking running

Use interactive docking to dock one ligand at a time. Click on menu Docking/Interactive docking/Mol Table Ligand, use the drop down arrow to find the table of ligand and/or compounds to be docked, and then enter the thoroughness which represents the length of simulation. Generally 1 is a reasonable value, select Calc. ICM Score, then select Display run which display the ligand sampling the energy in the ligand binding project.

2.3.4. Display the result

*Click Docking/Browse/Stack Conformations.

*ICM stochastic global optimization algorithm attempts to find the global minimum of the energy function that include five grid potentials describing interaction of the flexible ligand with receptor and internal conformational energy of the ligand, during this process a stack of alternative low energy conformations is saved.

3. Results and discussion

3.1. Chemistry

The procedure for the preparation of target compounds **4a–c**–**7a–e, 10**–**12,** and **15a,b**–**24a,b** were summarized in Schemes 1–4. The starting compounds **1a,b** were prepared as reported in literature [32].

The key intermediates, 8-aminobenzopyrone **3a,b** (Scheme 1) were prepared in two steps from starting compounds **1a,b** by the formation of 8-azo derivatives **2a,b** followed by its reduction. Azo coupling reaction [40] followed by reduction [41] was chosen instead of nitration followed by reduction due to the facile work up and purity of the products. Compounds **2a,b** were obtained through diazo-coupling of 3-alkyl-7-hydroxy-4-methyl-2*H*-1-benzopyran-2-one **1a,b** by dropwise addition of freshly prepared phenyl diazonium chloride. The structures of **2a,b** were confirmed by microanalyses and spectral data. ^{1}H NMR spectra revealed the disappearance of singlet signal corresponding to H-8 and appearance of multiplet signal at δ 7.57–7.67 ppm corresponding to the H-3′,4′,5′ and a doublet signal at 7.98 ppm assigned to H-2′,6′ of added phenyl protons. Mass spectra revealed their molecular ion peaks. 8-Amino compounds **3a,b** were synthesized by reducing the azo derivatives **2a,b** using sodium dithionite in ammonia for 15 min. The structures of **3a,b** were confirmed by elemental analyses and spectral data. IR spectra showed 2 bands at 3444–3296 cm^{-1} corresponding to NH_2 group. ^{1}H NMR spectrum revealed the appearance of a singlet signal at δ 3.80–6.67 ppm corresponding to NH_2 and the disappearance of multiplet signals corresponding to the aromatic protons of the phenyl azo moiety. MS spectra showed their molecular ion peaks at 205 for **3a** and at 219 for **3b**.

Reaction of amino compounds **3a,b** with different isocyanate derivatives in dichloromethane yielded the corresponding substituted ureas **4a–c** (Scheme 1). The structures of **4a–c** were elucidated with the aid of elemental analyses and spectral data. IR spectra revealed bands at 3300–3136 cm^{-1} corresponding to 2 NH in addition to 2 carbonyl bands at 1743–1676 cm^{-1}. ^{1}H NMR showed 2 singlet signals for compound **4a** and 3 singlet signals for compounds **4b,c** at δ 4.99–10.37 ppm assigned to 2 NH and OH, in addition to signals at 6.98–7.59 ppm corresponding to additional aromatic protons. MS spectrum revealed their molecular ion peaks.

Cyclization of substituted ureas **4b,c** with oxalyl chloride in dry benzene provided the imidazolidin-2,4,5-trione derivatives **5a,b** (Scheme 1). The structures of **5a,b** were deduced by microanalytical and spectral data. The IR spectra revealed the disappearance of NH bands and an additional C=O bands at 1759–1685. ^{1}H NMR spectra revealed the disappearance of signals corresponding to the 2 NH protons of the urea group. MS spectra showed their molecular ion peaks.

Reaction of amino group of *o*-aminophenol **3b** with appropriate isothiocyanates in ethanol formed thioureas which were spontaneously cyclized with neighboring OH giving the 2-substituted aminoxazole compounds **6a–c** (Scheme 1) in good yields with the evolution of hydrogen sulfide gas [42,43]. The termination of the reaction was detected and monitored by lead acetate paper in addition to TLC. In addition to the elemental microanalyses, IR spectra showed disappearance of bands corresponding to NH_2 and OH group and appearance of band at 3398–3263 cm^{-1} corresponding to the newly formed NH group. ^{1}H NMR showed triplet signal at δ = 8.16–8.75 ppm corresponding to NH group with the disappearance of signal corresponding to NH_2 and OH. Appearance of triplet signal at 1.21 ppm and a quartet signal at 3.39 ppm were assigned for ethyl protons in spectra of compound **6a**. ^{1}H NMR of compound **6b** showed three multiples at 3.79–4.02, 5.12–5.31 and 5.92–6.31 ppm assigned to allyl protons. Compound **6c** revealed a singlet at δ = 4.56 ppm and a multiplet at 7.33–7.42 ppm corresponding to CH_2 and the phenyl protons of the benzyl moiety, respectively.

1,4-Benzoxazines **7a–e** were achieved starting from *o*-aminophenol **3b** via reaction with appropriate phenacyl bromide derivative in presence of sodium ethoxide (Scheme 1). The structures of **7a–e** were proved by the elemental analyses and spectral data. IR spectra revealed disappearance of bands corresponding to OH and NH_2 group except for only spectra of compound **7b** which showed band at 3258 corresponding to NH group which confirmed the formation of oxazine ring. ^{1}H NMR spectra of **7a,c–e** revealed disappearance of signals corresponding to OH, NH and appearance of a singlet signal at δ = 5.35–5.73 ppm corresponding to two protons of CH_2 of oxazine ring, added aromatic protons at δ = 6.99–8.04 ppm corresponding to the phenyl or *p*-substituted phenyl ring. ^{1}H-NMR spectrum of **7b** showed three singlet signals at δ = 2.36, 5.64 and 10.33 ppm assigned to protons of methyl group at C-4′, CH oxazine and NH, respectively in addition to two doublets at 6.74 and 7.55 ppm assigned to aromatic protons of *p*-substituted phenyl ring. MS spectra showed their molecular ion peaks.

The key intermediate 8-acetyl-7-hydroxybenzopyrone **9** (Scheme 2) [33] were prepared starting from compound **1b** in two steps by formation of acetate ester compound **8** followed by Fries rearrangement reaction. Fries rearrangement catalyzed with Lewis acid such as $AlCl_3$ [44] is the preferred method for rearrangement of phenyl esters into *o*-ketophenols.

One pot reaction of acetyl derivative **9**, malononitrile and benzaldehyde in presence of ammonium acetate yielded 2-iminodihydropyridine-3-carbonitrile derivative **10** (Scheme 2). The structure of compound **10** was confirmed by the elemental analysis and spectral data. IR spectrum showed broad band at 3442 cm^{-1} corresponding to 2NH and OH groups and a band at 2210 cm^{-1} assigned to the nitrile group. ^{1}H NMR showed appearance of singlet signal at δ = 6.78 ppm assigned to C5-H of dihydropyridine, multiplet signal at δ = 7.24–8.20 ppm corresponding to the added phenyl protons and two singlet signals at δ = 8.26 and 8.90 ppm that were exchanged with D_2O corresponding to two NH groups. MS spectrum showed molecular ion peak at 397.

Base catalyzed Claisen–Schmidt condensation of 8-acetyl derivative **9** with benzaldehyde in ethanol in presence of sodium hydroxide afforded chalcone derivative **11** (Scheme 2). The structure of **11** was elucidated by analytical and spectral data. ^{1}H NMR revealed disappearance of methyl protons of acetyl group and appearance of multiplet signal at δ = 7.08–7.99 ppm corresponding CH=CH and phenyl protons.

Acylation of 8-acetyl-7-hydroxybenzopyrone **9** with benzoyl chloride provided compound **12** (Scheme 2). Negative $FeCl_3$ test indicated the absence of the hydroxyl group and verified the produced ester. The structure of **12** was confirmed by elemental analysis which was in accordance with the spectral data. IR spectrum showed disappearance of band corresponding to OH group. ^1H NMR showed disappearance of signal assigned to OH group and presence of two triplets and one doublet signals at δ = 7.63, 7.78 and 8.08 ppm corresponding to the added phenyl ring protons. MS spectra showed molecular ion peak at 350.

The key intermediates (3-alkyl-4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy) acetic acid hydrazides **14a** [34], **b** were synthesized from ethyl (3-alkyl-4-methyl-2-oxo-2*H*-1-benzopyran-7-yloxy)acetate **13a** [34], **b** starting from hydroxycoumarin derivatives **1a,b** by reaction with ethyl chloroacetate in presence of anhydrous potassium carbonate followed by hydrazinolysis (Scheme 3). The structure of **13b** was confirmed by IR spectrum that revealed the disappearance of band corresponding to OH group and appearance of two bands at 1757, 1707 cm^{-1} corresponding to 2 C=O groups due to additional ester group. ^1H NMR elicited the appearance of a singlet signal at δ = 4.90 ppm corresponding to OCH_2CO protons, in addition to triplet and a quartet signals at δ = 1.21 and 4.18 ppm, respectively corresponding to ethyl protons of ester moiety. MS spectrum showed the molecular ion peak at 290. In addition, structure of **14b** was deduced by IR spectrum that showed bands at 3446, 3213 cm^{-1} corresponding to NH and NH_2 groups. ^1H NMR spectrum revealed 2 singlet signals at 4.32 and 9.37 ppm corresponding to NH_2 and NH protons, respectively and disappearance of the triplet and quartet signals corresponding to ethyl protons of ester moiety.

Acylthiosemicarbazides **15a,b** were achieved via reaction of acid hydrazide **14a** with the appropriate isothiocyanate in ethanol (Scheme 3). The structure of compounds **15a,b** were elucidated by their elemental analyses and spectral data. IR spectra showed three bands at 3392–3126 cm^{-1} corresponding to 3NH, a band at 1282–1251 cm^{-1} corresponding to C=S. ^1H NMR spectrum revealed three signals at δ = 6.63–14.08 ppm corresponding to the 3NH groups. Appearance of a triplet signal at δ = 1.06 ppm and a quartet signal at δ = 3.45 ppm corresponding to the ethyl protons in **15a** spectrum and a multiplet at δ = 6.90–7.74 ppm corresponding to the phenyl protons in compound **15b** spectra. Ms. showed their molecular ion peaks at 349 for **15a** and at 397 for **15b**.

Cyclization of acylthiosemicarbazides **15a,b** with substituted phenacyl bromide derivatives in ethanol/chloroform mixture gave 2-(substituted imino)-4-(substituted phenyl)thiazoline derivatives **16a–c** (Scheme 3). The structures of compounds **16a–c** were confirmed with elemental analyses and spectral data. IR spectra revealed disappearance of a band at 1282–1251 cm^{-1} corresponding to C=S and only one band at 3435–3406 cm^{-1} corresponding to one NH group. ^1H NMR revealed a singlet signal at δ = 4.93–4.98 ppm assigned to one NH group. In addition, a singlet signal at δ = 7.00–7.14 ppm assigned to C5-H thiazoline and H-8 Ar protons for compounds **16a,b** while in compound **16c**, a multiplet signal at δ = 7.09–7.47 ppm assigned to C5-H thiazoline proton and 10 aromatic protons. MS spectra showed their molecular ion peaks.

2-Substituted iminothiazolidin-4-ones **17a,b** were obtained by the reaction of acylthiosemicarbazides **15a,b** with chloroacetic acid, anhydrous sodium acetate in glacial acetic acid (Scheme 3). The form (I) not (II) was assigned to the resulting thiazolidin-4-one (Fig. 2). This was attributed to previous literature that reported cyclization of 1-acyl-4-substituted thiosemicarbazide with ethyl bromoacetate yielded 2-substituted iminothiazolidin-4-one in case of aliphatic substituted thiosemicarbazide and 1,3,4-oxadiazole derivatives in case of aryl substituted thiosemicarbazide [45]. The structures of **17a,b** were elucidated by microanalyses and spectral data. IR spectra showed band at 3431–3192 cm^{-1} corresponding to NH group and three bands at 1735–1680 cm^{-1} assigned to 3 C=O of formed thiazolidin-4-one. ^1H NMR revealed two singlet signals at δ = 4.71–4.72 ppm corresponding to CH_2 group of the thiazolidinone moiety and 10.16–10.53 ppm exchanged with D_2O assigned to NH. MS spectra showed their molecular ion peaks.

Cyclization of acylthiosemicarbazides **15a,b** with concentrated sulfuric acid on cold yielded 1,3,4-thiadiazole derivatives **18a,b** (Scheme 3). The structures of **18a,b** were deduced by elemental analyses and spectral data. IR spectra showed disappearance of C=S band at 1282–1251 cm^{-1} and only one NH band at 3305–3157 cm^{-1}. ^1H NMR revealed one singlet signal at δ = 10.50–13.87 ppm corresponding to NH proton. MS spectra showed their molecular ion peaks at 331 and 379 for **18a** and **18b**, respectively.

4-Substituted-1,2,4-triazol-5-thione derivatives **19a,b** were obtained upon reaction of acylthiosemicarbazide derivatives **15a,b** with a 2 N NaOH (Scheme 3). The structures of **19a,b** were confirmed by the elemental analyses and spectral data. IR spectra revealed a band at 3415–3390 cm^{-1} corresponding to NH group and a band at 1286–1274 cm^{-1} corresponding to C=S. ^1H NMR of **19a** revealed a broad singlet signal at 4.70 ppm corresponding to NH proton while compound **19b** showed a singlet signal at

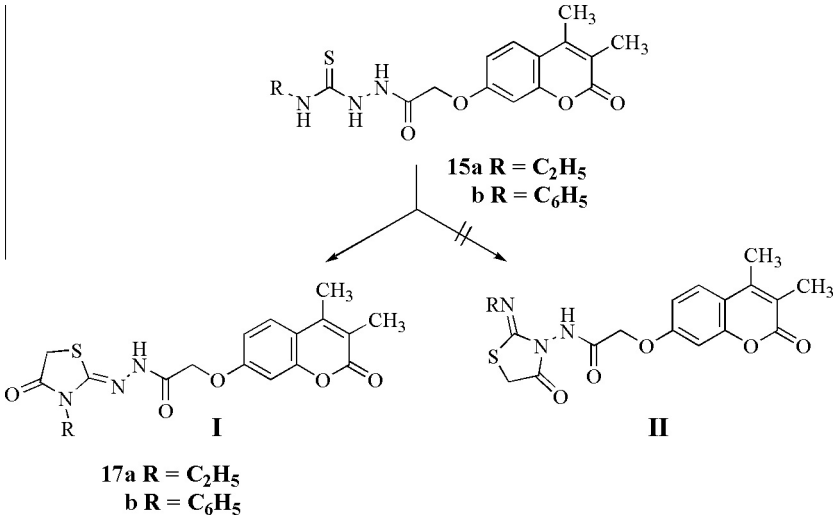


Fig. 2. The proposed structure of compounds **17a,b.**

δ = 14.06 ppm exchanged with D_2O corresponding to one NH proton.

4-Benzyl-1-acylsemicarbazide **20** was achieved via refluxing acid hydrazide **14a** with benzyl isocyanate in dichloroethane (Scheme 4). The structure of **20** was confirmed by elemental analysis and spectral data. IR spectrum showed bands at 3460–3294 cm^{-1} corresponding to 3NH. ^1H NMR spectrum revealed a singlet signal at δ = 4.23 ppm and a multiplet signal at 6.96–7.28 ppm corresponding to CH_2 and phenyl protons of the benzyl moiety in addition to three a singlet signals at 7.95, 8.14 and 9.90 ppm corresponding to 3NH protons. MS spectrum showed the molecular ion peak at 395.

Cyclization of acylsemicarbazide **20** using oxalyl chloride in dry benzene produced imidazolidin-2,4,5-trione **21** (Scheme 4). The structure of **21** was proved by microanalysis and spectral data. IR spectrum showed a band at 3415 cm^{-1} corresponding to NH group and bands at 1749–1699 cm^{-1} corresponding to 5C=O groups. ^1H NMR spectrum revealed a singlet signal at δ = 11.44 ppm corresponding to one NH group. MS spectrum showed molecular ion peak at 449.

Benzylidene acetohydrazide derivatives **22a,b** were achieved via refluxing acid hydrazide **14a,b** with appropriate aromatic aldehyde in glacial acetic acid (Scheme 4). The structures of **22a,b** were confirmed with elemental analyses and spectral data. IR spectrum elicited a band at 3446–3427 cm^{-1} corresponding to NH group. ^1H NMR displayed a singlet at δ = 8.01–8.27 ppm corresponding to benzylidene proton and another singlet at 11.49–11.70 ppm assigned to NH. MS spectra revealed their molecular ion peaks.

Condensation of acid hydrazide **14a,b** with ethyl acetoacetate in glacial acetic acid afforded the corresponding pyrazol-5-one derivatives **23a** [13], **b** (Scheme 4). The structure of **23b** was deduced from microanalytical and spectral data. IR spectra displayed NH band at 3257 cm^{-1} and 3C=O bands at 1747, 1732, 1705 cm^{-1}. ^1H NMR spectra showed three singlet signals at 1.87 ppm assigned to CH_3 at C3 pyrazolone, 4.79 ppm corresponding to CH pyrazolone and 10.08 ppm exchanged with D_2O assigned to NH.

Upon reacting acid hydrazide **14b** with cyclic anhydrides in glacial acetic acid the corresponding 2,5-dihydro-1*H*-pyrrol-2,5-dione **24a** or pyrrolidin-2,5-dione **24b** was obtained (Scheme 4). The IR spectra of **24a,b** showed bands at 1751–1676 cm^{-1} assigned to 4C=O groups. ^1H NMR spectra of **24a,b** displayed disappearance of NH_2 and NH singlet signals at δ = 4.32 and 9.37 ppm of parent acid hydrazide **14b** and presence of a singlet signal at 10.80 ppm corresponding to NH group. ^1H NMR spectrum of **24a** showed presence of multiplet signal at 6.99–7.05 ppm assigned to CH=CH protons while ^1H NMR spectrum of **24b** revealed a triplet signal at δ = 2.80 ppm corresponding to four protons of CH_2CH_2. MS spectra revealed the molecular ion peaks 356 and 358, respectively.

3.2. Antitumor screening

3.2.1. Preliminary in vitro antitumor screening

Fourteen newly synthesized compounds (**4a–c, 5a, 6c, 7a–e, 10, 18a,b, 23a**) were selected by National Cancer Institute (NCI) Developmental Therapeutic Program (http://www.dtp.nci.nih.gov), Bethesda, MD, U.S.A. The synthesized compounds were subjected to the NCI's disease-oriented human cell lines screening assay to be evaluated for their *in vitro* antitumor activity. The anticancer assays were performed in accordance with the protocol of the Drug Evaluation Branch, NCI, Bethesda [35–37]. A single dose (10 μM) of the test compounds were used in the full NCI 60 cell lines panel assay which includes nine tumor subpanels namely: leukemia, non-small cell lung, colon, CNS, melanoma, ovarian, renal, prostate and breast cancer cells. A 48 h drug exposure protocol was used and sulforhodamine B (SRB) protein assay was applied to estimate the cell viability and growth [38]. The results were reported as mean graph of the percent growth of the treated cells and presented as percentage growth inhibition (GI%). The obtained results of the tested benzopyrone analogs showed distinctive potential pattern of selectivity, as well as broad-spectrum antitumor activity (Table 1).

Regarding the activity towards individual cell lines, urea derivative as compound **4a** showed a promising activity towards most of the cell lines, compared to compounds **4b,c.** It revealed a potent activity against the leukemia cell line RPMI-8226 with GI value of 69.50%, while recording a good overall activity against the other leukemia subpanels CCRF-CEM, HL-60 (TB) and K-562, with GI values of 34.32, 41.78 and 49.57%, respectively. It also exhibited activity towards the non-small cell lung cancer HOP-92 and NCI-H522 with GI values of 38.58 and 37.45%, respectively. Moreover it revealed activity against the colon subpanel KM12 with GI values of 37.30%, the CNS cell line SF-295 and U251 with GI value of 45.07 and 31.24%, respectively. The melanoma cell lines LOX IMVI, MALME-3M, SK-MEI-5 and UACC-62 with GI values of 31.09, 47.65, 35.35 and 52.02%, respectively, while it showed activity against the ovarian subpanel OVCAR-3, renal cell lines ACHN and SN12C, prostate PC-3 and the breast T-47D and MDA-MB-468 with GI values of 55.39, 39.40, 32.73, 51.41, 38.34 and 56.53% respectively.

On the other hand, compound **5a** showed activity towards the leukemia cell lines SR with GI values of 35.68% while compound **6c** exhibited activity towards renal cancer subpanel UO-31 with GI value of 32.71%. The oxazole ring had reported selective toxicity against renal cell line UO-31, which may account for **6c** effect on renal cancer [46].

Concerning pyranobenzoxazines **7a–e**, they revealed a similar pattern of antitumor activity. Compound **7a** showed GI values 22.62, 27.55 and 20.24% against non-small cell lung cancer HOP-92, colon HCT-15 and prostate PC-3, respectively. Compound **7c** showed GI values 25.04 and 20.10% against leukemia subpanel SR and renal UO-31, respectively. Compound **7d** showed activity towards the renal cancer subpanel A498 with GI values of 21.69% while compound **7e** exhibited activity towards non-small cell lung cancer HOP-92 with GI value of 25.07%.

Dihydropyridine carbonitrile compound **10** revealed a considerable broad antitumor activity against most of the cell lines tested. It showed activity against leukemia cancer HL-60 (TB), MOLT-4, SR, CNS cancer SNB-75, melanoma SK-MEL-5, renal A498, CAKI-1, UO-31, prostate PC-3, breast MDA-MB-231/ATCC and T-47D with GI values ranging from 20 to 30%. Good activity was reported against the non-small cell lung cancer HOP-92 with GI value of 53.04%. This effect on HOP-92 may be due to the pyridine ring which is reported to have a selective effect on non-small cell lung cancer [47] while broad antitumor activity against various cell lines may be due to the presence of the pyridine carbonitrile that was reported to possess a cytotoxic effect against different types of cancers [48].

Thiadiazole compounds **18a,b** showed excellent activity on leukemia cancer where **18a** and **18b** caused GI values of 56.58 and 71.00% against RPMI-8226, respectively while revealed good activity upon K-562 cell lines with GI values of 34.27% and 40.43%, respectively. This may be due to the thiadiazole ring that is reported to have an excellent inhibitory effect upon leukemia [49]. Compound **18a** showed moderate activity against ovarian cancer OVCAR-3, prostate PC-3 and breast cancer MDA-MB-468 with GI value of ≈30–45%. In addition, **18a** showed activity upon non small cell lung cancer HOP-92 and NCI-H522, CNS cancer SF-295, melanoma MALME-3M and UACC-62 and breast T-47D with GI value of ≈20–30%. Also compound **18b** elicited a high activity upon CNS cancer SF-295, melanoma MALME-3M, ovarian OVAR-3 and breast cancer MDA-MB-468 with GI value of 50.46%, 56.89%, 58.37% and 55.31%, respectively. **18b** revealed GI values of ≈30–45% against leukemia K-562, colon KM12, melanoma SK-MEL-5 and UACC-62, renal ACHN, prostate PC-3 and breast cancer

Table 1
Growth inhibition percent of tested compounds against 60 different cell lines.

Panel/cell line	Test compounds and growth inhibition percent of cell line													
	4a	**4b**	**4c**	**5a**	**6c**	**7a**	**7b**	**7c**	**7d**	**7e**	**10**	**18a**	**18b**	**23a**
Leukemia														
CCRF-CEM	34.32	nt	nt	nt	nt	nt	nt	nt	nt	nt	nt	13.42	27.41	27.69
HL-60 (TB)	41.78	–	–	16.58	–	13.71	–	11.33	–	10.92	20.83	18.20	19.42	28.91
K-562	49.57	–	–	–	–	–	–	14.72	–	11.60	–	34.27	40.43	42.61
MOLT-4	24.27	–	–	12.12	–	–	–	11.36	–	11.25	23.61	–	–	14.39
RPMI-8226	69.50	–	–	–	–	–	–	–	12.48	–	18.34	56.58	71.00	74.04
SR	21.76	–	–	35.69	–	15.66	–	25.04	12.30	19.88	23.88	–	–	–
Non-small cell lung cancer														
A549/ATCC	25.75	–	–	–	–	–	–	–	–	–	10.73	–	nt	13.41
EKVX	nt	nt	nt	nt	nt	nt	nt	nt	–	nt	19.87	nt	nt	nt
HOP-62	–	–	–	–	14.79	–	–	–	–	–	–	–	–	–
HOP-92	38.58	–	21.61	20.07	13.23	22.62	12.90	18.46	–	25.07	53.04	28.67	–	47.63
NCI-H226	–	14.43	–	–	–	–	–	–	–	–	12.62	–	–	13.77
NCI-H23	13.22	–	–	–	–	–	–	–	12.35	12.35	14.67	–	–	13.74
NCI-H322M	14.18	–	–	–	–	15.26	–	–	–	–	–	–	–	–
NCI-H460	27.42	–	–	–	–	–	–	–	–	–	13.22	–	10.68	10.43
NCI-H522	37.45	–	nt	nt	nt	nt	nt	nt	–	nt	12.60	28.31	27.45	35.06
Colon cancer														
COLO 205	11.84	–	–	–	–	–	–	–	–	–	–	10.25	18.91	–
HCC-2998	–	–	–	–	–	–	–	–	–	–	–	–	–	–
HCT-116	26.99	–	–	–	–	–	–	–	–	–	19.27	14.14	21.17	18.83
HCT-15	15.26	–	–	–	–	27.55	–	–	–	–	–	–	–	–
HT29	28.65	–	–	–	–	–	–	–	–	–	–	22.32	20.99	34.96
KM12	37.30	–	–	–	–	–	–	–	–	–	10.46	12.69	30.29	24.81
SW-620	21.28	–	–	–	–	–	–	–	–	–	–	–	–	15.03
CNS cancer														
SF-268	11.08	–	–	–	–	–	–	–	–	–	–	–	–	–
SF-295	45.07	–	–	11.67	–	–	–	–	–	–	13.39	27.34	50.46	39.73
SF-539	–	–	–	–	18.84	–	nt	–	–	–	–	–	–	–
SNB-19	13.10	–	–	–	–	–	–	11.16	–	–	–	–	12.52	13.93
SNB-75	14.63	13.48	21.35	22.91	25.21	10.52	–	–	–	–	25.72	10.79	–	–
U251	31.24	–	nt	nt	nt	nt	nt	nt	–	nt	–	15.12	nt	25.80
Melanoma														
LOX IMVI	31.09	–	–	–	–	–	–	–	–	–	18.21	–	13.12	–
MALME-3M	47.65	–	–	–	–	–	–	–	–	–	–	26.89	56.89	49.40
M14	28.88	–	–	–	–	–	–	–	–	–	–	11.48	27.27	20.32
MDA-MB-435	18.35	–	–	–	–	–	–	–	–	–	–	–	22.21	–
SK-MEL-2	21.84	–	–	–	–	–	–	–	–	–	–	–	–	–
SK-MEL-28	18.82	–	–	–	–	–	–	–	–	–	–	–	22.19	–
SK-MEL-5	35.35	–	–	–	–	–	–	–	–	–	28.56	18.61	32.52	32.53
UACC-257	20.10	–	nt	nt	nt	nt	nt	nt	–	nt	–	–	nt	12.43
UACC-62	52.02	–	–	–	–	–	–	–	–	–	13.36	21.97	39.83	28.42
Ovarian cancer														
IGROV1	22.76	–	–	–	–	–	–	–	–	–	–	–	15.77	–
OVCAR-3	55.39	–	–	–	–	–	–	–	–	–	–	33.31	58.37	49.50
OVCAR-4	24.32	nt	nt	nt	–	–	–	nt	nt	–	nt	–	16.31	18.56
OVCAR-5	–	–	–	–	–	–	–	–	–	–	–	–	–	–
OVCAR-8	21.97	–	–	–	–	–	–	–	–	–	–	19.68	–	18.56
NCI/ADR-RES	–	–	–	–	–	–	–	–	–	–	13.84	–	–	–
SK-OV-3	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Renal cancer														
786-0	–	16.01	–	–	–	–	–	–	19.18	–	–	–	–	–
A498	–	10.22	–	–	–	–	–	–	21.69	–	23.37	–	–	22.69
ACHN	39.40	–	–	–	–	–	–	–	–	–	10.38	15.63	34.84	27.73
CAKI-1	12.69	–	12.28	–	17.49	–	–	–	–	–	23.77	–	–	–
RXF 393	–	–	–	–	–	–	–	–	–	–	–	–	–	–
SN12C	32.73	–	–	–	–	–	–	–	–	–	–	–	19.66	19.18
TK-10	26.67	–	–	14.06	–	–	–	–	–	–	–	–	–	13.06
UO-31	26.86	–	19.03	16.70	32.71	19.53	17.38	20.1	–	12.47	29.88	10.51	17.41	27.89
Prostate cancer														
PC-3	51.41	–	14.25	10.71	10.23	20.24	11.14	11.38	–	–	20.70	39.41	44.70	51.25
DU-145	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Breast cancer														
MCF7	–	–	–	–	–	–	–	–	–	–	16.55	–	22.03	22.26
MDA-MB-231/ATCC	–	–	–	–	–	–	–	10.59	–	–	28.25	–	–	–
HS 578T	–	–	–	–	–	–	–	–	–	–	–	–	–	–
BT-549	19.29	–	12.08	–	–	–	–	–	–	–	13.48	–	–	nt
T-47D	38.34	–	–	11.16	–	–	10.19	–	–	–	26.21	24.05	32.63	27.89
MDA-MB-468	56.53	–	–	–	–	–	–	–	–	–	–	41.28	55.31	56.98

–, GI < 10%; nt, not tested.

MDA-MB-468. In addition, **18b** showed activity upon leukemia CCRF-CEM, non small cell lung cancer NCI-H522, colon HCT-116 and HT-29, melanoma M14, MDA-MB-435, SK-MEL-28 and breast MCF-7 with GI value of ≈20–30%. Overall, **18b** seemed to have improved activity compared to **18a**.

Dihydropyrazol-5-one compound **23a** revealed the most potent effect upon various cell lines with the highest effect on leukemia cancer K-562 and RPMI-8226 with GI values of 42.61% and 74.04%, respectively. GI values of 47.63% and 35.06% was also observed on non small cell lung cancer HOP-92 and NCI-H522, GI values of 34.96% and 24.81% on colon cancer HT29, KM12, 39.73% and 25.80% on CNS cancer SF-259, U251, respectively. Compound **23a** also showed activity on melanoma subpanel MALME-3M, SK-MEL-5 and UACC-62 with GI values of 49.40%, 32.53% and 28.42%, respectively. At last, it revealed GI value of 49.50%, 51.25% and 56.98% on ovarian cancer OVCAR-3, prostate PC-3 and breast MDA-MB-468 cell lines respectively. This was attributed to presence of the dihydropyrazole ring that was reported to have broad spectrum antitumor effect over various types of cancers [50,51].

Close examination of the data presented in Table 1, revealed that compounds **4a**, **18a**, **18b** and **23a** were the most active members of this study, showing effectiveness toward numerous cell lines belong to different tumor subpanels. They exhibited high GI values against leukemia cell line RPMI-8226, CNS cell line SF-295, melanoma cell line MALME-3M, ovarian subpanel OVCAR-3, prostate cell line PC-3 and breast cell line MDA-MB-468. Compound **10** revealed a considerable broad antitumor activity against most of the cell lines tested. On the other hand, compounds **5a, 7a, 7c, 7d** and **7e** possessed moderate antitumor activity; while compounds **4b, 4c, 6c** and **7b** were the least active antitumors in the present investigation.

3.2.2. Structure–activity correlation

Examination of the bioactivity results revealed that combination of benzopyrone scaffold with dihydropyrazole ring, compound **23a** or thiadiazole ring compounds **18a,b** afforded broad antitumor activity with high GI values. This was attributed to previous knowledge of antitumor activity of both dihydropyrazole and thiadiazole rings. In addition, selective effect of pyridine ring on non-small cell lung cancer was expressed by high GI value of compound **10** against non small cell lung cancer HOP-92.

Disubstituted urea derivative with 3-methyl or 3-ethyl substituted benzopyrone and aryl substituent varied in activity. Substitution of benzopyrone scaffold with methyl group at C3, compound **4a,** exhibited broad antitumor activity with high GI values while substitution of with ethyl group at C3 lowered activity, compounds **4b,c**. Cyclization of aryl substituted urea derivative, compound **4b,** to imidazolidin-2,4,5-trione, compound **5a,** slightly improved activity.

Fused ring system of benzopyrone with oxazine ring, compounds **7a,c–e**, exhibited moderate activity while in case of oxazole ring, compound **6c,** activity was low.

3.3. Molecular docking

Casein Kinase II enzyme (CK2) represents one of most exceptional protein Kinase. Several experimental data support the CK2 importance for cancer transformation and development. CK2 has been found to be over expressed in tumors in the head and neck [52], in prostate [53], in the kidney [54], in mammary gland [55] and lung [56]. The final effect of CK2 seems linked to an action on both oncogenes and tumor suppressor proteins. It promotes a number of proto-oncogenic products stimulating cell proliferation and differentiation and inhibiting apoptosis. In humans, CK2 exists in a tetrameric form composed by two catalytic units, CK2α with three possible isoforms and two regulatory units, CK2β [57]. In the majority of the case, the catalytic unit has demonstrated to phosphorylate a particular consensus sequence different from other protein kinase known. This is composed by 4 aminoacids: Ser-X-X-Acidic, where the acidic residues can be Glu, Asp, pSer or pTyr [58]. On the other hand the β units are able to stabilize the tetrameric complex and at the same time to enhance and to modulate the activity thanks to a crucial role in substrates recruitment [59,60].

The binding affinities of the ligand was evaluated with energy score ICM (kcal/mol). The compound which revealed the highest binding affinity, minimum dock score, is the one forming the most stable ligand enzyme complex. Number and length of the hydrogen bond were used to assess the binding models. The results of the docking studies, ICM scores, and involved amino acids interacted ligand moieties and hydrogen bond length for each compound and the reference native ligand are listed in Table 2, Fig. 3–5.

Analysis of docking results revealed that:

(1) The inhibitor DBC, 3,8-dibromo-7-hydroxy-4-methylchromen-2-one, nearly fits in the active site of CK2 and has ICM score (−53.11 kcal/mol, Table 2), and form two hydrogen bonds between O of the 7-OH group with Lys 68 and Asp 175 of distance 2.16 Å and 2.63 Å respectively (Fig. 3).
(2) For urea derivatives **4a–c** (dock scores, −66.61 to −79.17 kcal/mol) a high negative score was estimated to the 3-methyl substituent, compound **4a**, the most active compound in the series, while the other two derivatives with 3-ethyl substituent that revealed a weak antitumor activity were found to have less negative dock scores.

Imidazolidin-2,4,5-trione, compound **5a** revealed a high dock score of (−76.13 kcal/mol), this value was not correlated to the activity of **5a** that had a moderate antitumor activity.

Concerning the oxazole derivative **6c** the docking scores was (−66.67 kcal/mol) which is consistent with the weak antitumor activity it had over various cell lines.

The oxazine compounds **7a–e** had different ICM scores in the range between (−47.79 to −69.20 kcal/mol) and this was correlated to the antitumor screening where compounds **7a,c–e** revealed moderate activity while **7b** with the lowest dock score (−47.79 kcal/mol) exhibited an overall weak activity against various cell lines.

Pyridine carbonitrile derivative **10** (dock score, −73.83 kcal/mol), this high dock score was consistent with the considerable activity it revealed in the one dose assay.

In case of thiadiazole derivatives **18a,b,** both had high ICM scores of −83.22 and −85.31 kcal/mol respectively. These scores are correlated to the high antitumor activity observed with compound **18b** having a better score and a better biological activity compared to **18a**.

Finally compound **23a** had ICM score of −80.25 kcal/mol. The high score of **23a** is related to the potent antitumor activity it revealed.

(3) Inspection of the binding mode also demonstrated that all compounds show from one to six hydrogen bonds with the enzyme active site residue. Tyr 307, Arg 278, Leu 249, Asp 175, Arg 172, Lys 170, Asn 118, Lys 68, Ser 51, Tyr 50 and Asp 14 are the amino acid residues involved in these interactions. In common with DBC, CK2 inhibitor, most of the docked compound, that exhibited some kind of activity, interacted with at least one amino acid residues Asp 175 and Lys 68.

Table 2
Docking results.

Compound	ICM scores (kcal/mol)	No of H bonds	Involved group of amino acid	Atom of ligand involved	Length of H-bond (Å)
DBC	−53.11	2	Lys 68···HH	O of 7-OH	2.16
			Asp175···HH	O of 7-OH	2.63
4a	−79.17	5	Ser 51···HH	O of CO of urea	1.94
			Ser 51···O	H of NH	2.29
			Lys 68···HH	O of 7-OH	2.22
			Lys 68···HH	O of CO of pyrone	2.65
			Asp 175···HH	O of 7-OH	2.68
4b	−67.28	4	Leu 249···O	H of NH	2.55
			Leu 249···O	H of NH	1.38
			Leu 249···O	H of NH	2.20
			Arg 278···HH	O of CO of urea	2.31
4c	−66.61	3	Asp 14···HH	O of 7-OH	2.36
			Asp 14···O	H of NH	2.06
			Asp 14···O	H of NH	2.34
5a	−76.13	1	Asn 118···HH	O of imidazolidintrione	1.52
6c	−66.67	1	Tyr 307···HH	O of oxazole	2.21
7a	−55.49	3	Lys 170···HH	O of oxazine	2.15
			Lys 170···HH	O of CO of pyrone	2.52
			Arg 172···HH	O of CO of pyrone	2.13
7b	−47.79	3	Arg 278···HH	O of CO of pyrone	1.65
			Arg 278···HH	O of oxazine	2.37
			Arg 278···HH	O of CO of pyrone	2.39
7c	−66.18	1	Lys 68···HH	O of oxazine	2.32
7d	−69.20	1	Lys 68···HH	O of oxazine	2.20
7e	−62.15	1	Lys 68···HH	O of oxazine	2.29
10	−73.83	5	Ser 51···HH	N of Pyridine	1.85
			Ser 51···HH	N of Pyridine	2.59
			Lys 68···HH	N of Pyridine	2.74
			Lys 68···HH	O of 7-OH	2.31
			Asp 175···HH	O of 7-OH	2.58
18a	−83.22	4	Ser 51···HH	N-3 of Thiadiazole	2.14
			Lys 68···HH	O of CO of pyrone	2.40
			Lys 68···HH	O of OCH_2	2.17
			Asp 175···HH	O of CO of pyrone	2.66
18b	−85.31	2	Asn 118···HH	O of CO of pyrone	2.54
			Asp 175···O	H of NH	2.10
23a	−80.25	6	Tyr 50···HH	O of Pyrazolone	2.26
			Ser 51···HH	O of Pyrazolone	2.47
			Lys 68···HH	O of CO of pyrone	2.36
			Lys 68···HH	O of pyrone	2.22
			Asp 175···HH	O of CO of pyrone	2.55
			Asp 175···O	H of NH	2.75

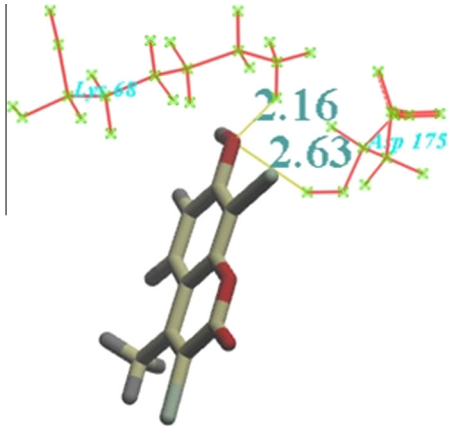


Fig. 3. Binding mode of DBC into the binding site of CK2 enzyme.

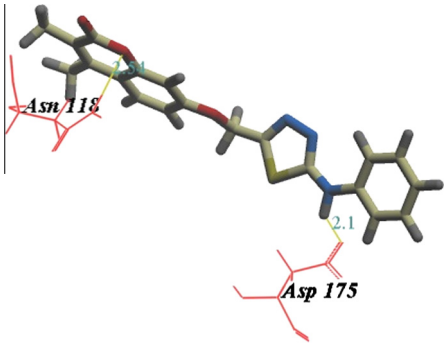


Fig. 4. Binding mode of **18b** into the binding site of CK2 enzyme.

For example, binding mode of **4a** into the active site of the enzyme mediated five hydrogen bonds, two hydrogen bonds between Ser 51 and O of CO of urea and H of NH, two hydrogen bonds between Lys 68 and O of 7-OH and O of CO of pyrone, one hydrogen bond between Asp 175 with O of 7-OH with a distance of 1.94, 2.29, 2.22, 2.65 and 2.68 Å respectively.

The binding mode of **10** show five hydrogen bonds, two hydrogen bonds between Ser 51 with N of pyridine moiety, two hydrogen bonds between Lys 68 with N of pyridine and O of 7-OH and

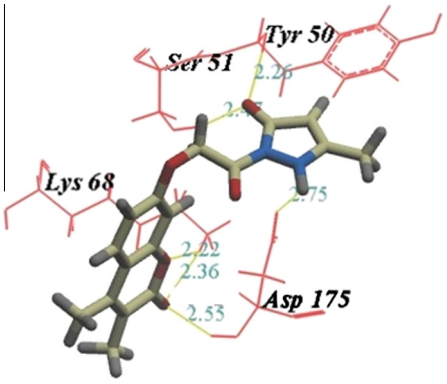


Fig. 5. Binding mode of **23a** into the binding site of CK2 enzyme.

finally one hydrogen bond between Asp 175 and O of 7-OH with a distances of 1.85, 2.59, 2.74, 2.31 and 2.58 Å, respectively.

Compound **18b** formed two hydrogen bonds, one hydrogen bond between Asn 118 with O of CO of pyrone and another one between Asp 175 with H of NH with a distance of 2.54 and 2.10 Å, respectively (Fig. 4).

Finally the binding mode of **23a** into the active site of CK2 enzyme revealed six hydrogen bonds, one hydrogen bond between Tyr 50 and O of pyrazolone, one between Ser 51 and O of pyrazolone, two hydrogen bonds between Lys 68 with O of CO of pyrone and O of pyrone moiety, and another two between Asp 175 with O of CO of pyrone and H of NH with a distance of 2.26, 2.47, 2.36, 2.22, 2.55 and 2.75 Å, respectively (Fig. 5).

4. Conclusion

New benzopyrone derivatives comprised of substituted-1*H*-benzopyran-2-ones, substituted amino-5*H*-pyrano[6,5-e]benzooxazol-5-ones and substituted-2,6-dihydropyrano[6,5-f]-1,4-benzoxazin-6-ones were synthesized. Fourteen compounds were selected by National Cancer Institute (NCI), Bethesda, and evaluated for their *in vitro* anticancer activity in the full NCI 60 cell lines panel assay by a single dose test (10 μM). Results showed that, combination of benzopyrone scaffold with dihydropyrazole ring (compound **23a**) or thiadiazole ring (compounds **18a**,**b**) afforded broad antitumor activity toward various cell lines that belong to different tumor subpanels while combination with pyridine ring (compound **10**) expressed selective effect on non-small cell lung cancer. In addition, disubstituted urea derivatives varied in activity (compound **4a** showed broad-spectrum antitumor activity) and cyclization of disubstituted urea derivative slightly improved activity (cyclized compound **5a** compared with urea derivative **4b**). Moreover, fused ring system of benzopyrone with oxazine ring exhibited moderate activity while in case of oxazole ring activity was low. Docking studies of biological evaluated compounds were performed with casein kinase II (CK2) enzyme in order to gain insight into their possible binding mode. Although a correlation between dock score and observed *in vitro* antitumor activity expressed as GI value by the compounds was routinely observed, most of the docked compounds shared some binding interactions with CK2 similar to those of the native ligand inhibitor. This suggests that these compounds might possibly act as CK2 inhibitors, and this may contribute at least in part to their antitumor activity.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bioorg.2014.02.003.

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