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Design and synthesis of novel 5-(4-chlorophenyl)furan derivatives with inhibitory activity on tubulin polymerization

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Aim: Discovery of novel series of colchicine binding site inhibitors (CBSIs). **Materials & methods:** Isoxazoline **3a–d**, pyrazoline **4a–b**, **7a–f** and **8a–f**, cyclohexenone **9a–b** and **10a–b** or pyridine derivatives **11a–b** were synthesized and evaluated for their inhibition of tubulin polymerization and cytotoxicity. Most of the compounds displayed potent to moderate antitumor activity against leukemia SR cell line. **7c**, **7e** and **11a** were more potent than colchicine with IC₅₀ of 0.09, 0.05 and 0.06 μ M, and percentage inhibition in tubulin polymerization of 95.2, 96.0 and 96.3%, respectively. Compounds **7c** and **11a** showed cell-cycle arrest at G2/M phase and induced apoptosis and were able to bind the colchicine binding site of tubulin with comparable affinity to colchicine. Docking study showed that these compounds may interact with tubulin exploiting a binding cavity not commonly reported in the binding of CBSI. **Conclusion:** Compounds **7c** and **11a** may be considered as promising CBSI based on their excellent activity and favorable drug likeness profile.

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Keywords: 5-(4-chlorophenyl)furan • colchicine • tubulin

Microtubules are globular protein made of closely related subunits called as α -tubulin and β -tubulin that are combined together to form heterodimers [1]. Microtubules are crucial for various cellular processes like cell proliferation, maintenance of cell shape and structure, motility regulation, cell signaling and intracellular transport [2]. Microtubules are attractive target for development of anticancer drugs because of their role in mitotic process [1]. Microtubule-inhibiting agents may be broadly classified into two groups: stabilizing and destabilizing agents [3]. Microtubule-stabilizing agents, including taxanes and epothilones, act by promoting the polymerization of tubulin thus, increasing the mass of microtubule polymer in cells while destabilizing agents, such as the vinca alkaloids and colchicine, act by depolymerization of microtubules and inhibit their polymerization [3,4]. The natural compound colchicine (**I**) was the first identified antimitotic destabilizing agent but was not clinically used as an anticancer drug due to its low therapeutic index [5]. Its toxicity includes neutropenia, gastrointestinal upset, bone marrow damage and anemia [5]. Nevertheless, there have been multiple efforts to develop colchicine binding site inhibitors (CBSI) with clinical application since CBSIs can prevent new blood vessels formation (angiogenesis inhibitors) or destroy the existing tumour vasculature (vascular-disrupting agents) [4]. Colchicine (**I**), which binds at the intradimer interface of β -subunit of tubulin; is a rigid molecule whose rigidity is imparted by the B-ring which anchors rings A and C. Rings A and C fit the hydrophobic pockets in the colchicine binding site created by Leu β 242, Ala β 250 and Leu β 255 for the phenyl A-ring and by Ala β 316, Lys β 352, Ile β 318 and Val β 315 for the tropanone moiety (Ring C) [6]. In addition, a methoxy group in ring A forms a key hydrogen bond with Cys β 241 which is a crucial feature of these inhibitors [6]. In the course of developing novel CBSI with higher activity and lower toxicity, a diverse array of structures (**II–X**) was identified Figure 1 [7–13].

The 7-acetamide moiety of colchicine (**I**) had been modified leading to more potent antitumor derivatives **II** and **III** [7]. More flexible CBSI involve among others, the natural stilbenoid phenol, combretastatin A-4 (**IV**) [5] featuring the replacement of ring B with an olefinic bridge. Insertion of a carbonyl function as in phenastatin (**V**)

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Figure 1. Structures of some colchicine binding site inhibitors.

and the benzofuran derivative (**VI**) [9,10] or a variety of heterocyclic rings handling rings A and C, as exemplified by the isoxazoline derivative (**VII**) [11] or pyrazoline derivative (**VIII**) [12] had been also reported. Alternatively, an alicyclic ring was used as in the cyclohexenone derivative (**IX**) [13]. Alterations adding an extra pendant phenyl to ring C were reported as in compound (**X**) which was based on the benzophenone derivative, phenastatin (**V**) (Figure 1) [14].

Compound (**X**) reported by Romagnoli *et al.* was more active than combretastatin A-4, (**IV**), as inhibitor of tubulin polymerization with superior antiproliferative activity against murine L1210 and human K562/21a leukemia cells [14]. Molecular docking of compound (**X**) suggested the superimposition of its 3,4,5-trimethoxyphenyl moiety

Figure 2. General design of target compounds.

with ring A of colchicine and that its 4-fluorophenyl moiety was directed at the opposite side of the binding pocket to interact with Gln α 11, in a region that has been poorly involved in the binding of CBSI [14].

From these findings, we initiated a research work directed at the design and synthesis of a series of novel CBSIs based on the following, maintaining the hydrophobic center A represented by 4-methoxyphenyl or 3,4,5-trimethoxyphenyl moieties, replacing the phenyl ring C by 4-chlorophenyl furan or 4-chlorophenyl thiophene moieties similar to compound (**X**) and varying the nature of ring B (Figure 2). Design of target compounds **3a–d** was based on modifying ring B into an isoxazoline ring similar to (**VII**) with a 4-chlorophenyl furan (in ring C) in **3a–b** or with a 4-chlorophenyl thiophene (ring C) in **3c–d**. Since the oxygen in furan may have the potential for hydrogen bond formation with the target, furan ring was preferred over thiophene in the design of the rest of the target compounds. Ring B in compounds **4a–b** was modified into a pyrazoline ring as in compound (**VIII**). Analogous to the more potent colchicine derivatives (**II**) and (**III**), tertiary amine terminals were grafted into the pyrazoline ring through an acetyl or propionoyl linker in compounds **7a–f** and **8a–f**, respectively. Finally, ring B in target compounds were modified to a six-membered cyclohexenone ring in compounds **9a–b** and **10a–b** or aminocyanopyridine derivatives **11a–b** for comparative reasons (Figure 2).

A preliminary molecular docking study of the designed compounds into the active site of tubulin in its complex with colchicine (PDB: 3UT5) [15] was performed prior to synthesizing the target compounds and revealed some interesting findings. While colchicine (**I**) was bound at the interface between α and β subunits of tubulin with minor interactions with the residues in α subunit, all the designed molecules were capable of hydrogen bond formation with Cys β 241 by virtue of a methoxy group in ring A, but being larger in size compared with **colchicine (I)**, were able to fit the colchicine binding site more tightly and were differently oriented relative to colchicine skeleton in a manner similar to compound (**X**). Ring B in the designed molecules was more or less occupying the center of the binding site similar to colchicine facing the backbone of Thr α 179 or Val α 181 in which furan/thiophene rings with their 4-chlorophenyl moiety were oriented toward the vacant cavity, close to the α -subunit of tubulin, that was reported to accommodate the 4-fluorophenyl moiety in compound (**X**) (Supplementary Figure 1) [14].

Figure 3. Synthesis of compounds **1a**, **b**, **2a–d**, **3a–d**, **4a**, **b**, **5a**, **b**, **6a**, **b**, **7a–f** and **8a–f**. Reagents and conditions: (i) 4-chloroaniline, HCl, NaNO₂, CuCl₂; (ii) 4-methoxyacetophenone or 3,4,5-trimethoxyacetophenone, 50% NaOH, absolute ethanol, 18 h; (iii) hydroxylamine HCl, 10% KOH, K₂CO₃, absolute ethanol, 8 h; (iv) hydrazine hydrate, absolute ethanol, 24 h; (v) chloroacetyl chloride or chloropropionyl chloride, methylene chloride, room temperature, overnight; (vi) 2ry amine, KI, absolute ethanol, 8 h.

Materials & methods

Chemistry

Starting materials and solvents were purchased from commercial suppliers and were used without further purification. ¹H NMR spectra were recorded on Varian Mercury spectrophotometer (Agilent Technologies, Inc., CA, USA) at 300 MHz. Chemical shift values (δ) are given in parts per million (p.p.m.) downfield from tetramethylsilane as internal reference, coupling constants are given in hertz (Hz) and spin multiplicities are given as s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet) or m (multiplet). ¹³C NMR spectra were recorded on Bruker (MA, USA), 100 MHz NMR spectrophotometer or Varian Mercury spectrophotometer at 75 MHz. Infrared spectra (IR) were recorded as potassium bromide discs on Shimadzu FT-IR 8400S spectrophotometer (Kyoto, Japan) and expressed in wave number (ν_{max}, cm⁻¹). TLC was performed using silica gel/TLC cards DC-Alufolien-Kiesel gel (Vilber GmbH, Germany) with fluorescent indicator UV₂₅₄ using chloroform or hexane: ethyl acetate 8.5 : 1.5 as the eluting system and the spots were visualized using Vilber Lourmet ultraviolet lamp (Vilber GmbH, Germany) at λ = 254 nm. Melting points were determined by open capillary tube method using Electrothermal 9100 melting point apparatus (Staffordshire, UK) and are uncorrected.

General procedure for synthesis of compounds **1a–b**

Compounds **1a**, **b** were prepared adopting previously reported methods [16,17].

5-(4-chlorophenyl)furan-2-carbaldehyde (**1a**)

Compound **1a** was prepared from 2-furaldehyde using water as a solvent. Yield 45.2%, orange crystals, melting point (m.p.) 126–8°C (as reported) [16].

5-(4-chlorophenyl)thiophene-2-carbaldehyde (**1b**)

Compound **1b** was prepared from 2-thiophene aldehyde using DMSO as a solvent. Yield 25%, m.p. 88–90°C (as reported) [17].

General procedure for synthesis of compounds (**2a–d**)

Compounds **2a–d** were prepared adopting previously reported method [18].

3-[5-(4-chlorophenyl)furan-2-yl]-1-(4-methoxyphenyl)prop-2-en-1-one (**2a**)

Compound **2a** was prepared from **1a** and 4-methoxyacetophenone. Yield 80%, yellow crystals, m.p. 179–81°C (reported 175°C) [18].

3-[5-(4-chlorophenyl)furan-2-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (2b)

Compound **2b** was prepared from **1a** and 3,4,5-trimethoxyacetophenone. Yield 80%, yellow crystals, m.p. 150–2°C. IR (ν_{max} , cm^{-1}): 1659 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 3.78 (s, 3H, OCH_3), 3.91 (s, 6 H, 2 OCH_3), 7.25 (d, 1 H, $J = 1.5$, H_{furan}), 7.39 (s, 2 H, Ar-H), 7.55 (d, 2 H, $J = 7.5$, Ar-H), 7.56 (d, 1 H, $J = 1.5$, H_{furan}), 7.61 (d, 1 H, $J = 15.3$, $\text{COCH} =$), 7.74 (d, 1H, $J = 15.6$, = CH-Ar), 7.97 (d, 2 H, $J = 8.7$, Ar-H). ^{13}C NMR (75 MHz, CDCl_3) δ : 56.52 (OCH_3), 61.01 (2 OCH_3), 106.19, 108.67, 118.78, 125.68, 128.31, 129.18, 130.38, 133.65, 134.44, 142.6, 151.42, 153.19, 155.29, 188.63 (C = O). Anal. calcd for $\text{C}_{22}\text{H}_{19}\text{ClO}_5$: C, 66.25; H, 4.77. Found: C, 66.40; H, 4.90.

3-[5-(4-chlorophenyl)thiophen-2-yl]-1-(4-methoxyphenyl)prop-2-en-1-one (2c)

Compound **2c** was prepared from **1b** and 4-methoxyacetophenone. Yield 83%, yellow crystals, m.p. 173–5°C. IR (ν_{max} , cm^{-1}): 1651 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 3.92 (s, 3H, OCH_3), 7.05 (d, 2H, $J = 8.9$, Ar-H), 7.29 (d, 1H, $J = 3.7$, $\text{H}_{\text{thiophene}}$), 7.33 (d, 1H, $J = 3.8$, $\text{H}_{\text{thiophene}}$), 7.38 (d, 1H, $J = 15.2$, $\text{COCH} =$), 7.41 (d, 2 H $J = 8.6$, Ar-H), 7.59 (d, 2H, $J = 8.6$, Ar-H), 7.93 (d, 1 H, $J = 15.2$, $\text{COCH} =$), 8.07 (d, 2 H, $J = 8.8$, Ar-H). ^{13}C NMR (75 MHz, CDCl_3): δ 55.5 (OCH_3), 113.86, 120.56, 128.3, 128.43, 130.72, 131.03, 131.73, 136.21, 136.4, 140.6, 163.44, 188.08 (C = O). Anal. calcd for $\text{C}_{20}\text{H}_{15}\text{ClO}_2\text{S}$: C, 67.69; H, 4.26. Found: C, 67.90; H, 4.31.

3-[5-(4-chlorophenyl)thiophen-2-yl]-1-(3,4,5-trimethoxyphenyl)prop-2-en-1-one (2d)

Compound **2d** was prepared from **1b** and 3,4,5-trimethoxyacetophenone. Yield 82%, yellow crystals, m.p. 140–2°C. IR (ν_{max} , cm^{-1}): 1647 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 3.96 (s, 6 H, 2 OCH_3), 3.99 (s, 3 H, OCH_3), 7.17 (d, 1 H, $J = 5.0$, $\text{H}_{\text{thiophene}}$), 7.26 (s, 2 H, Ar-H), 7.31 (d, 2 H, $J = 8.6$, Ar-H), 7.37 (d, 1 H, $J = 3.6$, $\text{H}_{\text{thiophene}}$), 7.45 (d, 1 H, $J = 15.2$, $\text{COCH} =$), 7.60 (d, 2 H, $J = 8.2$, Ar-H), 7.96 (d, 1H, $J = 15.0$, $\text{COCH} =$). Anal. calcd for $\text{C}_{22}\text{H}_{19}\text{ClO}_4\text{S}$: C, 63.69; H, 4.62. Found: C, 63.84; H, 4.32.

General procedure for synthesis of compounds 3a–d

Compounds **3a–d** were synthesized from **2a–d** adopting the method described in Supplementary Information.

5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3a)

Compound **3a** was prepared from **2a**. Yield 71%, yellow crystals, m.p. 137–140°C. ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 3.28 (dd, 1 H, $J = 8.08$, $J = 16.52$, $\text{H}4_{\text{isoxazole}}$), 3.70 (dd, 1 H, $J = 7.76$, $J = 16.52$, $\text{H}4'_{\text{isoxazole}}$), 3.80 (s, 3 H, OCH_3), 5.75 (t, 1 H, $J = 9.76$, $\text{H}5_{\text{isoxazole}}$), 6.67 (d, 1 H, $J = 3$, H_{furan}), 6.73 (d, 1 H, $J = 3$, H_{furan}), 7.03 (d, 2 H, $J = 8.4$, Ar-H), 7.05 (d, 2 H, $J = 8.7$, Ar-H), 7.49 (d, 2 H, $J = 8.7$, Ar-H), 7.72 (d, 2 H, $J = 8.7$, Ar-H). Anal. calcd for $\text{C}_{20}\text{H}_{16}\text{ClNO}_3$: C, 67.90; H, 4.56; N, 3.96. Found: C, 67.98; H, 4.59; N, 4.12.

5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (3b)

Compound **3b** was prepared from **2b**. Yield 65%, yellow crystals, m.p. 112–5°C. ^1H NMR (300 MHz, CDCl_3) δ : 3.67 (dd, 1 H, $J = 7.76$, $J = 16.68$, $\text{H}4_{\text{isoxazole}}$), 3.92 (dd, 1 H, $J = 7.8$, $J = 16.52$, $\text{H}4'_{\text{isoxazole}}$), 3.93 (s, 6 H, 2 OCH_3), 3.99 (s, 3 H, OCH_3), 5.80 (t, 1 H, $J = 9.72$, $\text{H}5_{\text{isoxazole}}$), 6.53 (d, 1 H, $J = 3.30$, H_{furan}), 6.64 (d, 1 H, $J = 3.3$, H_{furan}), 6.99 (s, 2 H, Ar-H), 7.37 (d, 2 H, $J = 8.5$, Ar-H), 7.60 (d, 2 H, $J = 8.5$, Ar-H). Anal. calcd for $\text{C}_{22}\text{H}_{20}\text{ClNO}_5$: C, 63.85; H, 4.87; N, 3.38. Found: C, 64.03; H, 4.92; N, 3.47.

5-(5-(4-chlorophenyl)thiophen-2-yl)-3-(4-methoxyphenyl)-4,5-dihydroisoxazole (3c)

Compound **3c** was prepared from **2c**. Yield 75%, yellow crystals, m.p. 92–5°C. ^1H NMR (300 MHz, CDCl_3) δ : 3.47 (dd, 1 H, $J = 8.08$, $J = 16.5$, $\text{H}4_{\text{isoxazole}}$), 3.76 (dd, 1 H, $J = 10.48$, $J = 16.52$, $\text{H}4'_{\text{isoxazole}}$), 3.87 (s, 3 H, OCH_3), 5.98 (t, 1 H, $J = 8.08$, $\text{H}5_{\text{isoxazole}}$), 6.97 (d, 2 H, $J = 8.8$, Ar-H), 7.01 (d, 1 H, $J = 3.6$, $\text{H}_{\text{thiophene}}$), 7.03 (d, 2 H, $J = 8.6$, Ar-H), 7.13 (d, 1 H, $J = 3.4$, $\text{H}_{\text{thiophene}}$), 7.33 (d, 2 H, $J = 6.04$, Ar-H), 7.68 (d, 2 H, $J = 8.8$, Ar-H). ^{13}C NMR (75 MHz, CDCl_3) δ : 43.31, 55.38 (OCH_3), 78.17, 114.2, 121.87, 125.38, 125.69, 126.9, 1128.34, 143.61, 155.93, 161.2. Anal. calcd for $\text{C}_{20}\text{H}_{16}\text{ClNO}_2\text{S}$: C, 64.95; H, 4.36; N, 3.79. Found: C, 65.13; H, 4.33; N, 3.93.

5-(5-(4-chlorophenyl)thiophen-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydroisoxazole (3d)

Compound **3d** was prepared from **2d**. Yield 63%, yellow crystals, m.p. 120–3°C. ^1H NMR (300 MHz, CDCl_3) δ : 3.48 (dd, 1 H, $J = 7.76$, $J = 16.68$, $\text{H}4_{\text{isoxazole}}$), 3.70 (dd, 1 H, $J = 8.0$, $J = 16.52$, $\text{H}4'_{\text{isoxazole}}$), 3.87 (s, 3 H,

Figure 4. Synthesis of compounds 9a, b, 10a, b and 11a, b. Reagents and conditions: (i) ethylacetoacetate, 10% KOH, absolute ethanol, 2 h; (ii) 50% KOH, absolute ethanol, 4 h; (iii) acetylacetone, 10% KOH, absolute ethanol, 12 h; (iv) malononitrile, ammonium acetate, absolute ethanol, 10 h.

OCH₃), 3.92 (s, 6 H, 2OCH₃), 5.99 (t, 1 H, $J = 7.72$, H₅_{isoxazole}), 6.97 (s, 2 H, Ar-H), 7.10 (d, 1 H, $J = 3.4$, H_{thiophene}), 7.18 (d, 1 H, $J = 3.5$, H_{thiophene}), 7.38 (d, 2 H, $J = 8.7$, Ar-H), 7.52 (d, 2 H, $J = 8.4$, Ar-H). Anal. calcd for C₂₂H₂₀ClNO₄S: C, 61.46; H, 4.69; N, 3.26. Found: C, 61.55; H, 4.76; N, 3.33.

General procedure for synthesis of compounds 4a, b

Compounds **4a**, **b** were prepared adopting a previously described method [18].

5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1 *H*-pyrazole (**4a**)

Compound **4a** was prepared from **2a**. Yield 80%, white crystals. m.p. 126–8°C (as reported) [18].

5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1 *H*-pyrazole (**4b**)

Compound **4b** was prepared from **2b**. Yield 82%, white crystals, m.p. 112–4°C. ¹H NMR (300 MHz, DMSO-*d*₆) δ: 3.18 (dd, 1 H, $J = 9.6$, $J = 16.5$, H₄_{pyrazole}), 3.43 (dd, 1 H, $J = 10.2$, $J = 15.9$, H₄_{pyrazole}), 3.68 (s, 3 H, OCH₃), 3.81 (s, 6 H, 2OCH₃), 4.93 (t, 1 H, $J = 9.9$, H₅_{pyrazole}), 6.49 (d, 1 H, $J = 3.3$, H_{furan}), 6.88 (d, 1 H, $J = 3.3$, H_{furan}), 6.95 (s, 2 H, Ar-H), 7.49 (d, 2 H, $J = 8.4$, Ar-H), 7.52 (s, 1 H, NH, D₂O exchange), 7.72 (d, 2 H, $J = 8.7$, Ar-H). ¹³C NMR (75 MHz, CDCl₃) δ: 37.99, 56.23 (2OCH₃), 57.48, 60.95 (OCH₃), 103.43, 106.13, 108.33, 124.99, 128.13, 128.89, 129.02, 133.13, 139.17, 151.87, 152.76, 153.33, 154.5. Anal. calcd for C₂₂H₂₁ClN₂O₄: C, 64.00; H, 5.13; N, 6.79. Found: C, 64.08; H, 5.11; N, 6.88.

General procedure for synthesis of compounds 5a, b & 6a, b

Compounds **5a**, **b** and **6a**, **b** were synthesized from **4a** or **4b** adopting the method described in Supplementary Information.

2-Chloro-1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1 *H*-pyrazol-1-yl)ethan-1-one (**5a**)

Compound **5a** was prepared from **4a** and chloroacetyl chloride. Yield 45%, colorless crystals, m.p. 145–8°C. IR (ν_{max}, cm⁻¹): 1680 (C = O); ¹H NMR (300 MHz, DMSO-*d*₆) δ: 3.53 (dd, 1 H, $J = 9.6$, $J = 16.5$, H₄_{pyrazole}),

3.65 (dd, 1H, $J = 10.2, 15.9$, H_4^{pyrazole}), 3.89 (s, 3 H, OCH_3), 4.58 (s, 2 H, $-CH_2Cl$), 5.73 (dd, 1H, $J = 5.1$, $J = 11.2$, H_5^{pyrazole}), 6.47 (d, 1 H, $J = 3.3$, H_{furan}), 6.58 (d, 1 H, $J = 3.3$, H_{furan}), 7.00 (d, 2 H, $J = 8.7$, Ar-H), 7.28 (d, 2H, $J = 8.7$, Ar-H), 7.50 (d, 2 H, $J = 8.1$, Ar-H), 7.75 (d, 2 H, $J = 8.7$, Ar-H). Anal. calcd for $C_{22}H_{18}Cl_2N_2O_3$: C, 61.55; H, 4.23; N, 6.53. Found: C, 61.72; H, 4.21; N, 6.33.

2-Chloro-1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)ethan-1-one (5b)

Compound **5b** was prepared from **4b** and chloroacetyl chloride. Yield 48%, colorless crystals, m.p. 170–3°C. IR (ν_{max} , cm^{-1}): 1680 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 3.6 (dd, 1 H, $J = 7.8, J = 18.0$, H_4^{pyrazole}), 3.72 (s, 3 H, OCH_3), 3.75 (dd, 1 H, $J = 12.0, J = 18.6$, H_4^{pyrazole}), 3.85 (s, 6 H, 2 OCH_3), 4.27 (s, 2 H, $-CH_2Cl$), 5.73 (dd, 1H, $J = 5.1, J = 11.2$, H_5^{pyrazole}), 6.50 (d, 1 H, $J = 2.4$, H_{furan}), 6.95 (d, 1 H, $J = 3.3$, H_{furan}), 7.14 (s, 2 H, Ar-H), 7.47 (d, 2 H, $J = 8.7$, Ar-H), 7.66 (d, 2 H, $J = 9.6$, Ar-H). Anal. calcd for $C_{24}H_{22}Cl_2N_2O_5$: C, 58.91; H, 4.53; N, 5.72. Found: C, 58.97; H, 4.56; N, 5.79.

3-Chloro-1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one (6a)

Compound **6a** was prepared from **4a** and chloropropionyl chloride. Yield 62%, colorless crystals, m.p. 150–3°C. IR (ν_{max} , cm^{-1}): 1651 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 2.87 (t, 2H, $J = 6.9$, COCH_2), 3.29 (dd, 1 H, $J = 6.6, J = 17.3$, H_4^{pyrazole}), 3.67 (dd, 1 H, $J = 11.7, J = 17.3$, H_4^{pyrazole}), 3.78 (t, 2 H, $J = 6.6$, CH_2Cl), 3.88 (s, 3 H, OCH_3), 5.73 (dd, 1 H, $J = 4.7, J = 11.4$, H_5^{pyrazole}), 6.42 (d, 1 H, $J = 3.3$, H_{furan}), 6.57 (d, 1H, $J = 3.3$, H_{furan}), 6.98 (d, 2 H, $J = 8.7$, Ar-H), 7.30 (d, 2 H, $J = 8.4$, Ar-H), 7.50 (d, 2 H, $J = 8.7$, Ar-H), 7.74 (d, 2 H, $J = 8.3$, Ar-H). Anal. calcd for $C_{23}H_{20}Cl_2N_2O_3$: C, 62.31; H, 4.55; N, 6.32. Found: C, 62.40; H, 4.52; N, 6.49.

3-Chloro-1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)propan-1-one (6b)

Compound **6b** was prepared from **4b** and chloropropionyl chloride. Yield 65%, colorless crystals, m.p. 180–3°C. IR (ν_{max} , cm^{-1}): 1659 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 2.88 (t, 2 H, $J = 7.8$, COCH_2), 3.34 (t, 2 H, $J = 6.9$, CH_2Cl), 3.49 (dd, 1 H, $J = 6.6, J = 17.3$, H_4^{pyrazole}), 3.70 (dd, 1 H, $J = 11.60, J = 17.3$, H_4^{pyrazole}), 3.91 (s, 3 H, OCH_3), 3.94 (s, 6 H, 2 OCH_3), 5.73 (dd, 1 H, $J = 4.7, J = 11.4$, H_5^{pyrazole}), 6.43 (d, 1 H, $J = 3.3$, H_{furan}), 6.58 (d, 1 H, $J = 3.0$, H_{furan}), 7.02 (s, 2 H, Ar-H), 7.32 (d, 2 H, $J = 8.7$, Ar-H), 7.52 (d, 2 H, $J = 8.4$, Ar-H). Anal. calcd for $C_{25}H_{24}Cl_2N_2O_5$: C, 59.65; H, 4.81; N, 5.57. Found: C, 59.82; H, 4.79; N, 5.74.

General procedure for synthesis of compounds 7a–f & 8a–f

Compounds **7a–f** and **8a–f** were synthesized from **5a**, **b** or **6a**, **b** adopting the method described in Supplementary Information.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-morpholinoethan-1-one (7a)

Compound **7a** was prepared from **5a** and morpholine. Yield 50%, brown crystals, m.p. 120–3°C. IR (ν_{max} , cm^{-1}): 1667 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 3.20 (dd, 1 H, H_4^{pyrazole}), 3.43 (s, 2 H, $-CH_2\text{C} = \text{O}$), 3.52 (t, 4 H, 2 $-CH_2\text{morpholine}$), 3.70 (dd, 1 H, H_4^{pyrazole}), 3.91 (s, 3 H, OCH_3), 4.13 (t, 4 H, 2 $-CH_2\text{morpholine}$), 5.77 (t, 1 H, H_5^{pyrazole}), 6.48 (d, 1 H, $J = 2.4$, H_{furan}), 6.58 (d, 1 H, $J = 3.3$, H_{furan}), 7.03 (d, 2 H, $J = 8.7$, Ar-H), 7.32 (d, 2H, $J = 8.4$, Ar-H), 7.73 (d, 2 H, $J = 8.4$, Ar-H), 8.10 (d, 2 H, $J = 9.0$, Ar-H). Anal. calcd for $C_{26}H_{26}ClN_3O_4$: C, 65.06; H, 5.46; N, 8.76. Found: C, 65.17; H, 5.43; N, 8.93.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-morpholinoethan-1-one (7b)

Compound **7b** was prepared from **5b** and morpholine. Yield 55%, brown crystals, m.p. 132–5°C. IR (ν_{max} , cm^{-1}): 1655 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 3.26 (dd, 1 H, H_4^{pyrazole}), 3.55 (t, 4 H, 2 $-CH_2\text{morpholine}$), 3.85 (dd, 1 H, H_4^{pyrazole}), 3.94 (s, 3 H, OCH_3), 3.96 (s, 6 H, 2 OCH_3), 4.01 (t, 4 H, 2 $-CH_2\text{morpholine}$), 4.14 (s, 2 H, $-CH_2\text{C} = \text{O}$), 5.84 (t, 1 H, H_5^{pyrazole}), 6.51 (d, 1 H, $J = 2.3$, H_{furan}), 6.60 (d, 1 H, $J = 2.3$, H_{furan}), 7.05 (s, 2 H, Ar-H), 7.34 (d, 2 H, $J = 8.2$, Ar-H), 7.51 (d, 2 H, $J = 8.3$, Ar-H). ^{13}C NMR (100 MHz, CDCl_3) δ : 39.23, 43.21, 52.38, 54.51 (OCH_3), 56.6 (2 OCH_3), 61.03, 63.54, 104.7, 106.45, 110.74, 124.94, 125.37,

128.61, 129.00, 133.49, 141.2, 150.58, 152.86, 153.53, 157.66, 160.61 (C = O). Anal. calcd for $C_{28}H_{30}ClN_3O_6$: C, 62.28; H, 5.60; N, 7.78. Found: C, 62.34; H, 5.67; N, 7.90.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-(piperazin-1-yl)ethan-1-one (7c)

Compound **7c** was prepared from **5a** and piperazine. Yield 45%, yellow crystals, m.p. 110–3°C. IR ($\nu_{\max}$, cm^{-1}): 3422 (NH), 1663 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 1.89 (s, 1 H, NH, D_2O exchangeable), 2.66 (s, 4 H, 2 CH_2 piperazine), 2.88 (s, 2 H, CH_2CO), 2.94 (s, 4 H, 2 CH_2 piperazine), 3.52 (dd, 1 H, $J = 5.0$, $J = 17.64$, $\text{H}^4_{\text{pyrazole}}$), 3.62 (dd, 1 H, $J = 11.44$, $J = 17.44$, $\text{H}^4_{\text{pyrazole}}$), 3.87 (s, 3 H, OCH_3), 5.73 (dd, 1 H, $J = 4.8$, $J = 11.36$, $\text{H}^5_{\text{pyrazole}}$), 6.41 (d, 1 H, $J = 3.3$, H_{furan}), 6.56 (d, 1 H, $J = 3.6$, H_{furan}), 6.98 (d, 2 H, $J = 9.0$, Ar-H), 7.30 (d, 2 H, $J = 9.0$, Ar-H), 7.50 (d, 2 H, $J = 8.7$, Ar-H), 7.73 (d, 2 H, $J = 9.0$, Ar-H). Anal. calcd for $C_{25}H_{27}ClN_4O_3$: C, 65.20; H, 5.68; N, 11.70. Found: C, 65.32; H, 5.74; N, 11.94.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-(piperazin-1-yl)ethan-1-one (7d)

Compound **7d** was prepared from **5b** and piperazine. Yield 43%, yellow crystals, m.p. 183–5°C. IR ($\nu_{\max}$, cm^{-1}): 3429 (NH), 1663 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 1.90 (s, 1 H, NH, D_2O exchangeable), 2.80 (s, 4 H, 2 CH_2 piperazine), 3.50 (s, 4 H, 2 CH_2 piperazine), 3.60 (dd, 1 H, $J = 5.0$, $J = 17.6$, $\text{H}^4_{\text{pyrazole}}$), 3.74 (dd, 1 H, $J = 11.70$, $J = 17.6$, $\text{H}^4_{\text{pyrazole}}$), 3.91 (s, 3 H, OCH_3), 3.93 (s, 6 H, 2 OCH_3), 3.98 (s, 2 H, CH_2CO), 5.70 (dd, 1 H, $J = 4.8$, $J = 11.36$, $\text{H}^5_{\text{pyrazole}}$), 6.42 (d, 1 H, $J = 3.3$, H_{furan}), 6.56 (d, 1 H, $J = 3.3$, H_{furan}), 6.98 (s, 2H, Ar-H), 7.31 (d, 2 H, $J = 8.4$, Ar-H), 7.50 (d, 2 H, $J = 8.4$, Ar-H). Anal. calcd for $C_{28}H_{31}ClN_4O_5$: C, 62.39; H, 5.80; N, 10.39. Found: C, 62.48; H, 5.78; N, 10.52.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-(dimethylamino)ethan-1-one (7e)

Compound **7e** was prepared from **5a** and dimethylamine. Yield 58%, yellow crystals, m.p. 220–3°C. IR ($\nu_{\max}$, cm^{-1}): 1667 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 3.16 (s, 6 H, 2 CH_3), 3.50 (dd, 1 H, $\text{H}^4_{\text{pyrazole}}$), 3.70 (dd, 1 H, $\text{H}^4_{\text{pyrazole}}$), 3.88 (s, 2 H, $-\text{CH}_2\text{CO}$), 3.89 (s, 3 H, OCH_3), 5.70 (t, 1 H, $\text{H}^5_{\text{pyrazole}}$), 6.47 (d, 1 H, $J = 3.3$, H_{furan}), 6.59 (d, 1 H, $J = 3.3$, H_{furan}), 7.00 (d, 2 H, $J = 6$, Ar-H), 7.32 (d, 2 H, $J = 8.1$, Ar-H), 7.58 (d, 2 H, $J = 8.4$, Ar-H), 7.75 (d, 2 H, $J = 6$, Ar-H). Anal. calcd for $C_{24}H_{24}ClN_3O_3$: C, 65.83; H, 5.52; N, 9.60. Found: C, 65.94; H, 5.57; N, 9.78.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-2-(dimethylamino)ethan-1-one (7f)

Compound **7f** was prepared from **5b** and dimethylamine. Yield 56%, yellow crystals, m.p. 200–3°C. IR ($\nu_{\max}$, cm^{-1}): 1666 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 3.04 (s, 6 H, 2 CH_3), 3.56 (dd, 1 H, $J = 5.48$, $J = 18.28$, $\text{H}^4_{\text{pyrazole}}$), 3.78 (dd, 1 H, $J = 11.72$, $J = 18.3$, $\text{H}^4_{\text{pyrazole}}$), 3.95 (s, 3 H, OCH_3), 3.97 (s, 6 H, 2 OCH_3), 4.41 (s, 2 H, $-\text{CH}_2\text{CO}$), 5.81 (dd, 1 H, $J = 4.24$, $J = 10.32$, $\text{H}^5_{\text{pyrazole}}$), 6.52 (d, 1 H, $J = 3.0$, H_{furan}), 6.63 (d, 1 H, $J = 3.3$, H_{furan}), 7.02 (s, 2 H, Ar-H), 7.36 (d, 2 H, $J = 8.6$, Ar-H), 7.53 (d, 2 H, $J = 8.6$, Ar-H). Anal. calcd for $C_{26}H_{28}ClN_3O_5$: C, 62.71; H, 5.67; N, 8.44. Found: C, 62.84; H, 5.72; N, 8.57.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1*H*-pyrazol-1-yl)-3-morpholinopropan-1-one (8a)

Compound **8a** was prepared from **6a** and morpholine. Yield 65%, brown crystals, m.p. 223–5°C. IR ($\nu_{\max}$, cm^{-1}): 1647 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 2.71 (s, 4 H, 2 $-\text{CH}_2$ morpholine), 2.80 (t, 2 H, $J = 7.96$, $-\text{COCH}_2$), 3.49 (dd, 1 H, $J = 4.72$, $J = 17.36$, $\text{H}^4_{\text{pyrazole}}$), 3.64 (dd, 1 H, $J = 11.56$, $J = 17.3$, $\text{H}^4_{\text{pyrazole}}$), 3.70 (s, 4 H, 2 $-\text{CH}_2$ morpholine), 3.89 (s, 3 H, OCH_3), 4.03 (t, 2 H, $J = 7.84$, $-\text{CH}_2$), 5.71 (dd, 1 H, $J = 4.64$, $J = 11.44$, $\text{H}^5_{\text{pyrazole}}$), 6.57 (d, 1 H, $J = 3.3$, H_{furan}), 6.71 (d, 1 H, $J = 3.6$, H_{furan}), 7.42 (d, 2 H, $J = 8.4$, Ar-H), 7.32 (d, 2 H, $J = 8.4$, Ar-H), 7.50 (d, 2 H, $J = 8.4$, Ar-H), 7.76 (d, 2 H, $J = 8.7$, Ar-H). Anal. calcd for $C_{27}H_{28}ClN_3O_4$: C, 65.65; H, 5.71; N, 8.51. Found: C, 65.73; H, 5.74; N, 8.62.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-morpholinopropan-1-one (8b)

Compound **8b** was prepared from **6b** and morpholine. Yield 67%, brown crystals, m.p. 235–7°C. IR ($\nu_{\max}$, cm^{-1}): 1680 (C = O); ^1H NMR (300 MHz, CDCl_3): δ 3.25 (s, 4 H, 2 $-\text{CH}_2$ morpholine), 3.49 (dd, 1 H, $J = 5.04$, $J = 11.00$, $\text{H}^4_{\text{pyrazole}}$), 3.45 (t, 2 H, $J = 7.88$, $-\text{COCH}_2$), 3.54 (t, 2 H, $J = 5.84$, $-\text{CH}_2$), 3.72 (dd, 1 H, $J = 11.76$, $J = 17.56$, $\text{H}^4_{\text{pyrazole}}$), 3.94 (s, 3 H, OCH_3), 3.97 (s, 6 H, 2 OCH_3), 4.01 (s, 4 H, 2 $-\text{CH}_2$ morpholine), 5.70 (dd, 1 H, $J = 5.00$, $J = 11.64$, $\text{H}^5_{\text{pyrazole}}$), 6.45 (d, 1 H, $J = 3.4$, H_{furan}), 6.60 (d, 1 H, $J = 3.4$, H_{furan}), 7.04 (s, 2 H, Ar-H), 7.34 (d, 2 H, $J = 8.6$, Ar-H), 7.52 (d, 2 H, $J = 8.6$, Ar-H). ^{13}C NMR (100 MHz, CDCl_3): δ : 29.05, 38.93, 43.34, 52.03, 52.56, 52.82, 54.2 (OCH_3), 56.64 (2OCH_3), 61.02, 63.67, 104.49, 106.32, 110.19, 124.94, 125.98, 128.96, 133.37, 140.84, 151.32, 152.68, 153.51, 155.71, 166.91 (C = O). Anal. calcd for $\text{C}_{29}\text{H}_{32}\text{ClN}_3\text{O}_6$: C, 62.87; H, 5.82; N, 7.58. Found: C, 62.96; H, 5.85; N, 7.69.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(piperazin-1-yl)propan-1-one (8c)

Compound **8c** was prepared from **6a** and piperazine. Yield 63 %, yellow crystals, m.p. 137–40°C. IR ($\nu_{\max}$, cm^{-1}): 3426 (NH), 1670 (C = O); ^1H NMR (300 MHz, CDCl_3): δ : 1.80 (s, 1 H, NH, D_2O exchangeable), 2.50 (t, 4 H, 2 CH_2 piperazine), 2.80 (t, 2 H, CH_2), 2.90 (t, 4 H, 2 CH_2 piperazine), 3.06 (dd, 1 H, $\text{H}^4_{\text{pyrazole}}$), 3.50 (t, 2 H, CH_2), 3.67 (dd, 1 H, $\text{H}^4_{\text{pyrazole}}$), 3.89 (s, 3 H, OCH_3), 5.74 (dd, 1 H, $\text{H}^5_{\text{pyrazole}}$), 6.46 (d, 1 H, $J = 3.4$, H_{furan}), 6.58 (d, 1 H, $J = 3.4$, H_{furan}), 6.99 (d, 2 H, $J = 8.9$, Ar-H), 7.32 (d, 2 H, $J = 8.6$, Ar-H), 7.52 (d, 2 H, $J = 8.6$, Ar-H), 7.74 (d, 2 H, $J = 8.8$, Ar-H). ^{13}C NMR (100 MHz, CDCl_3): δ 31.74, 38.45, 45.39, 53.2, 53.62, 54.17, 55.44 (OCH_3), 106.41, 109.75, 114.25, 123.84, 124.88, 128.23, 128.83, 129.1, 132.97, 152.08, 152.3, 153.95, 161.49, 170.03 (C = O). Anal. calcd for $\text{C}_{27}\text{H}_{29}\text{ClN}_4\text{O}_3$: C, 65.78; H, 5.93; N, 11.36. Found: C, 65.91; H, 6.01; N, 11.52.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(piperazin-1-yl)propan-1-one (8d)

Compound **8d** was prepared from **6b** and piperazine. Yield 65%, yellow crystals, m.p. 170–2°C. IR ($\nu_{\max}$, cm^{-1}): 3412 (NH), 1661 (C = O); ^1H NMR (300 MHz, CDCl_3): δ : 1.98 (s, 1 H, NH, D_2O exchangeable), 2.86 (s, 4 H, 2 CH_2 piperazine), 2.94 (t, 2 H, $J = 4.72$, COCH_2), 2.99 (t, 2 H, $J = 5.84$, CH_2), 3.16 (s, 4 H, 2 CH_2 piperazine), 3.49, 3.52 (dd, 1 H, $J = 4.04$, $J = 17.24$, $\text{H}^4_{\text{pyrazole}}$), 3.71 (dd, 1 H, $J = 11.48$, $J = 17.08$, $\text{H}^4_{\text{pyrazole}}$), 3.93 (s, 3 H, OCH_3), 3.94 (s, 6 H, 2 OCH_3), 5.73 (dd, 1 H, $J = 4.04$, $J = 11.12$, $\text{H}^5_{\text{pyrazole}}$), 6.42 (d, 1 H, $J = 3.6$, H_{furan}), 6.60 (d, 1 H, $J = 3.6$, H_{furan}), 6.99 (s, 2 H, Ar-H), 7.33 (d, 2 H, $J = 8.0$, Ar-H), 7.52 (d, 2 H, $J = 8.0$, Ar-H). Anal. calcd for $\text{C}_{29}\text{H}_{33}\text{ClN}_4\text{O}_5$: C, 62.98; H, 6.01; N, 10.13. Found: C, 63.06; H, 6.01; N, 10.24.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(dimethylamino)propan-1-one (8e)

Compound **8e** was prepared from **6a** and dimethylamine. Yield 65%, yellow crystals, m.p. 123–5°C. IR ($\nu_{\max}$, cm^{-1}): 1655 (C = O); ^1H NMR (300 MHz, CDCl_3): δ : 2.52 (s, 6 H, 2 CH_3), 3.19 (t, 2 H, $J = 6.90$, COCH_2), 3.75 (t, 2 H, $J = 6.90$, CH_2), 3.49 (dd, 1 H, $J = 5.1$, $J = 11.52$, $\text{H}^4_{\text{pyrazole}}$), 3.68 (dd, 1 H, $J = 11.40$, $J = 15.0$, $\text{H}^4_{\text{pyrazole}}$), 3.88 (s, 3 H, OCH_3), 5.75 (dd, 1 H, $J = 6.30$, $J = 12$, $\text{H}^5_{\text{pyrazole}}$), 6.47 (d, 1 H, $J = 3.3$, H_{furan}), 6.59 (d, 1 H, $J = 3.3$, H_{furan}), 7.00 (d, 2 H, $J = 6$, Ar-H), 7.32 (d, 2 H, $J = 8.1$, Ar-H), 7.58 (d, 2 H, $J = 8.4$, Ar-H), 7.75 (d, 2 H, $J = 6$, Ar-H). Anal. calcd for $\text{C}_{25}\text{H}_{26}\text{ClN}_3\text{O}_3$: C, 66.44; H, 5.80; N, 9.30. Found: C, 66.57; H, 5.83; N, 9.52.

1-(5-(5-(4-chlorophenyl)furan-2-yl)-3-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-3-(dimethylamino)propan-1-one (8f)

Compound **8f** was prepared from **6b** and dimethylamine. Yield 61%, yellow crystals, m.p. 102–3°C. IR ($\nu_{\max}$, cm^{-1}): 1647 (C = O); ^1H NMR (300 MHz, CDCl_3): δ : 2.83 (s, 6 H, 2 CH_3), 3.51 (dd, 1 H, $J = 4.84$, $J = 12.0$, $\text{H}^4_{\text{pyrazole}}$), 3.49 (t, 2 H, $J = 6.90$, COCH_2), 3.5 (t, 2 H, $J = 6.90$, CH_2Cl), 3.77 (dd, 1 H, $J = 11.84$, $J = 17.64$, $\text{H}^4_{\text{pyrazole}}$), 3.93 (s, 3 H, OCH_3), 3.96 (s, 6 H, 2 OCH_3), 5.72 (dd, 1 H, $J = 4.96$, $J = 11.64$, $\text{H}^5_{\text{pyrazole}}$), 6.44 (d, 1 H, $J = 3.3$, H_{furan}), 6.59 (d, 1 H, $J = 3.3$, H_{furan}), 7.03 (s, 2 H, Ar-H), 7.33 (d, 2 H, $J = 8.5$, Ar-H), 7.51 (d, 2 H, $J = 8.5$, Ar-H). Anal. calcd for $\text{C}_{27}\text{H}_{30}\text{ClN}_3\text{O}_5$: C, 63.34; H, 5.91; N, 8.21. Found: C, 63.49; H, 6.02; N, 8.45.

General procedure for synthesis of compounds 9a, b

Compounds **9a, b** were synthesized from **2a** or **2b** adopting the method described in Supplementary Information.

Ethyl 6-[5-(4-chlorophenyl)furan-2-yl]-2-oxo-4-(4-methoxyphenyl) cyclohex-3-enecarboxylate (9a)

Compound **9a** was prepared from **2a**. Yield 66%, yellow crystals, m.p. 128–130°C. IR ($\nu_{\max}$, cm^{-1}): 1742, 1655 (2 C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 1.21 (t, 3 H, $J = 7.2$, CH_3 ethyl), 3.06 (dd, 1 H, $J = 4.8$, $J = 17.8$, $\text{H}5_{\text{cyclohexenone}}$), 3.26 (dd, 1 H, $J = 4.5$, $J = 17.7$, $\text{H}5'_{\text{cyclohexenone}}$), 3.8 (d, 1 H, $J = 4.8$, $\text{H}1_{\text{cyclohexenone}}$), 3.86 (s, 3 H, OCH_3), 4.00 (m, 1 H, $\text{H}6_{\text{cyclohexenone}}$), 4.23 (q, 2 H, $J = 7.2$, $-\text{CH}_2$ ethyl), 6.26 (d, 1 H, $J = 3.3$, H_{furan}), 6.53 (s, 1 H, $\text{H}3_{\text{cyclohexenone}}$), 6.56 (d, 1 H, $J = 3.6$, H_{furan}), 6.97 (d, 2 H, $J = 9$, Ar-H), 7.36 (d, 2 H, $J = 8.7$, Ar-H), 7.57 (d, 2 H, $J = 8.4$, Ar-H), 7.58 (d, 2 H, $J = 9$, Ar-H). ^{13}C NMR (100 MHz, CDCl_3): δ 14.13 (CH_3), 32.37, 37.33, 55.46 (OCH_3), 57.27 (CH_2), 61.34, 106.14, 108.33, 114.1, 122.34, 125.1, 127.81, 127.91, 129.14, 129.66, 133, 152.26, 154.4, 157.4, 161.44, 169.32 (C = O), 192.98 (C = O). Anal. calcd for $\text{C}_{26}\text{H}_{23}\text{ClO}_5$: C, 69.25; H, 5.14. Found: C, 69.42; H, 5.22.

Ethyl 6-[5-(4-chlorophenyl)furan-2-yl]-2-oxo-4-(3,4,5-trimethoxyphenyl) cyclohex-3-enecarboxylate (9b)

Compound **9b** was prepared from **2b**. Yield 70%, yellow crystals, m.p. 100–5°C. IR ($\nu_{\max}$, cm^{-1}): 1740, 1663 (2 C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 1.20 (t, 3 H, $J = 6.9$, CH_3 ethyl), 3.11 (dd, 1 H, $J = 4.8$, $J = 15.8$, $\text{H}5_{\text{cyclohexenone}}$), 3.22 (dd, 1 H, $J = 4.8$, $J = 15.8$, $\text{H}5'_{\text{cyclohexenone}}$), 3.80 (d, 1 H, $J = 4.5$, $\text{H}1_{\text{cyclohexenone}}$), 3.85 (s, 3 H, OCH_3), 3.90 (s, 6 H, 2 OCH_3), 4.04 (m, 1 H, $\text{H}6_{\text{cyclohexenone}}$), 4.23 (q, 2 H, $J = 7.2$, $-\text{CH}_2$ ethyl), 6.27 (d, 1 H, $J = 3.3$, H_{furan}), 6.53 (s, 1 H, $\text{H}3_{\text{cyclohexenone}}$), 6.58 (d, 1 H, $J = 3.6$, H_{furan}), 6.80 (s, 2 H, Ar-H), 7.37 (d, 2 H, $J = 8.1$, Ar-H), 7.58 (d, 2 H, $J = 8.1$, Ar-H). Anal. calcd for $\text{C}_{28}\text{H}_{27}\text{ClO}_7$: C, 65.82; H, 5.33. Found: C, 65.94; H, 5.38.

General procedures for synthesis of compounds 10a, b

Compounds **10a, b** were prepared following either method A or method B described in Supplementary Information.

5-[5-(4-chlorophenyl)furan-2-yl]-3-(4-methoxyphenyl)-cyclohex-2-enone (10a)

Compound **10a** was prepared from **9a** or **2a**. Yield 66 and 62%, respectively, yellow crystals, m.p. (117–20). IR ($\nu_{\max}$, cm^{-1}): 1651 (C = O); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 2.66 (dd, 1 H, $J = 4.4$, $J = 17.76$, $\text{H}4_{\text{cyclohexenone}}$), 2.72 (dd, 1 H, $J = 4.4$, $J = 17.8$, $\text{H}4'_{\text{cyclohexenone}}$), 2.99 (dd, 1 H, $J = 3.9$, $J = 16.56$, $\text{H}6_{\text{cyclohexenone}}$), 3.19 (dd, 1 H, $J = 3.9$, $J = 16.7$, $\text{H}6'_{\text{cyclohexenone}}$), 3.65 (m, 1 H, $\text{H}5_{\text{cyclohexenone}}$), 3.80 (s, 3 H, OCH_3), 6.38 (d, 1 H, $J = 3.6$, H_{furan}), 6.41 (s, 1 H, $\text{H}2_{\text{cyclohexenone}}$), 6.93 (d, 1 H, $J = 3$, H_{furan}), 7.02 (d, 2 H, $J = 9$, Ar-H), 7.47 (d, 2 H, $J = 9$, Ar-H), 7.71 (d, 2 H, $J = 7.5$, Ar-H), 7.73 (d, 2 H, $J = 7.5$, Ar-H). ^{13}C NMR (100 MHz, CDCl_3) δ : 32.77, 34.42, 41.31, 55.51 (OCH_3), 106.1, 106.91, 114.27, 126.65, 127.81, 128.4, 128.89, 129.69, 132.86, 134.27, 151.6, 151.97, 154.99, 198.09 (C = O). Anal. calcd for $\text{C}_{23}\text{H}_{19}\text{ClO}_3$: C, 72.92; H, 5.06. Found: C, 72.96; H, 5.00.

5-[5-(4-chlorophenyl)furan-2-yl]-3-(4-methoxyphenyl)-cyclohex-2-enone (10b)

Compound **10b** was prepared from **9b** or **2b**. Yield 63 and 60%, respectively, yellow crystals, m.p. 135–7°C. IR ($\nu_{\max}$, cm^{-1}): 1650 (C = O); ^1H NMR (300 MHz, CDCl_3) δ : 2.62 (dd, 1 H, $J = 4.4$, $J = 17.7$, $\text{H}4_{\text{cyclohexenone}}$), 2.82 (dd, 1 H, $J = 4.4$, $J = 17.8$, $\text{H}4'_{\text{cyclohexenone}}$), 3.05 (dd, 1 H, $J = 3.9$, $J = 16.56$, $\text{H}6_{\text{cyclohexenone}}$), 3.18, 3.24 (dd, 1 H, $J = 3.9$, $J = 15.72$, $\text{H}6'_{\text{cyclohexenone}}$), 3.74 (m, 1 H, $\text{H}5_{\text{cyclohexenone}}$), 3.90 (s, 6 H, 2 OCH_3), 3.95 (s, 3 H, OCH_3), 6.23 (d, 1 H, $J = 3.3$, H_{furan}), 6.47 (s, 1 H, $\text{H}2_{\text{cyclohexenone}}$), 6.60 (d, 1 H, $J = 3.3$, H_{furan}), 6.79 (s, 2 H, Ar-H), 7.37 (d, 2 H, $J = 8.7$, Ar-H), 7.58 (d, 2 H, $J = 8.4$, Ar-H). Anal. calcd for $\text{C}_{25}\text{H}_{23}\text{ClO}_5$: C, 68.41; H, 5.28. Found: C, 67.48; H, 5.31.

General procedure for synthesis of compounds 11a, b

Compounds **11a, b** were prepared following the method described in Supplementary Information.

2-amino-4-(5-(4-chlorophenyl)furan-2-yl)-6-(4-methoxyphenyl)nicotinonitrile (11a)

Compound **11a** was prepared from **2a**. Yield 80%, yellow crystals, m.p. 220–3. IR : ($\nu_{\max}$, cm^{-1}): 3307, 3177 (NH_2), 2200 (CN); ^1H NMR (300 MHz, $\text{DMSO}-d_6$) δ : 3.84 (s, 3 H, OCH_3), 6.90 (s, 2 H, NH_2 , D_2O exchangeable), 7.09 (d, 2 H, $J = 9$, Ar-H), 7.33 (d, 1 H, $J = 3.9$, H_{furan}), 7.59 (d, 2 H, $J = 8.4$, Ar-H), 7.62 (s,

Table 1. IC₅₀ (μM) of target compounds against leukemia SR cell line and percentage inhibition of tubulin polymerization.

Compound ID	IC ₅₀ (μM) [†]	Percentage inhibition of tubulin polymerization
3a	0.80 ± 0.042	89.8
3b	2.51 ± 0.131	84.2
3c	1.78 ± 0.090	85.0
3d	7.90 ± 0.340	62.6
4a	0.56 ± 0.035	86.1
4b	10.70 ± 0.51	31.0
7a	0.15 ± 0.013	93.6
7b	15.80 ± 0.91	25.4
7c	0.09 ± 0.005	95.2
7d	0.79 ± 0.030	88.2
7e	0.05 ± 0.006	96.0
7f	2.50 ± 0.140	82.9
8a	0.40 ± 0.023	86.1
8b	0.15 ± 0.010	94.7
8c	0.32 ± 0.017	93.3
8d	0.63 ± 0.030	92.2
8e	0.40 ± 0.028	91.7
8f	0.15 ± 0.011	94.9
9a	1.12 ± 0.072	67.4
9b	0.95 ± 0.047	78.1
10a	0.79 ± 0.036	77.0
10b	0.71 ± 0.042	82.6
11a	0.06 ± 0.004	96.3
11b	0.28 ± 0.015	94.1
Colchicine	0.10 ± 0.004	ND

[†] Results are the mean of three experiments ± SD.
ND: Not determined.

1 H, pyridine-H), 7.70 (d, 1 H, J = 3.9, H_{furan}), 7.98 (d, 2 H, J = 8.4, Ar-H), 8.18 (d, 2 H, J = 8.7, Ar-H). Anal. calcd for C₂₃H₁₆ClN₃O₂: C, 68.74; H, 4.01; N, 10.46. Found: C, 68.79; H, 4.02; N, 10.59.

2-amino-4-(5-(4-chlorophenyl)furan-2-yl)-6-(3,4,5-trimethoxyphenyl)nicotinonitrile (11a)

Compound **11a** was prepared from **2b**. Yield 77%, yellow crystals, m.p. 242–5°C. IR (ν_{max} , cm⁻¹): 3475, 3319 (NH₂), 2204 (CN); ¹H NMR (300 MHz, DMSO-*d*₆) δ : 3.95 (s, 3H, OCH₃), 4.02 (s, 6 H, 2 OCH₃), 5.45 (s, 2 H, NH₂, D₂O exchangeable), 6.81 (d, 1 H, J = 3.6, H_{furan}), 6.92 (d, 1 H, J = 3, H_{furan}), 7.29 (s, 2 H, Ar-H), 7.45 (s, 1 H, pyridine-H), 7.48 (d, 2 H, J = 8.7, Ar-H), 7.80 (d, 2 H, J = 8.4, Ar-H). Anal. calcd for C₂₅H₂₀ClN₃O₄: C, 65.01; H, 4.36; N, 9.10. Found: C, 65.08; H, 4.41; N, 9.22.

Biological evaluation

In vitro antitumor evaluation (NCI-60 human tumor cell lines screen)

Eight compounds, **3a**, **4a**, **7a–d** and **11a**, **b**, were selected by the National Cancer Institute (NCI), MD, USA, to be tested for their *in vitro* growth inhibitory activity against a panel of 60 human cancer cell lines. The compounds were tested at a single concentration of 10 μM, and the percentages of growth inhibitions were determined. The IC₅₀ of compound **3a** against the panel was determined using the five-dose testing procedure.

Antiproliferative activity against leukemia SR cell line by MTT assay

Antiproliferative activity of the target compounds in leukemia SR cell line was determined using MTT assay. See Supplementary Information.

Table 2. Molecular properties of compounds **7c**, **7e** and **11a** predicted using SwissADME server.

Property	7c	7e	11a	Colchicine
MW	437.92	478.97	400.86	399.44
XLogP _{3o/w}	4.13	3.48	5.42	2.34
#Rotatable bonds	7	7	4	6
#H-bond acceptors	5	6	3	6
#H-bond donors	0	1	1	1
MR	128.74	146.88	115.64	109.36
TPSA	58.28	70.31	72.18	83.09
Lipinski violations	0	0	0	0
Ghose violations	0	1 (MR >130)	1 (MR >130)	0
Veber violations	0	0	0	0
Egan violations	0	0	1 (WLOGP >5.88)	0
Muegge violations	0	0	1 (XLOGP3 >5)	0
PAINS alerts	0	0	0	0
Brenk alerts	0	0	1 (aniline)	0
Leadlikeness violations	2 MW >350, XLOGP3 >3.5	1 MW >350	2 MW >350, XLOGP3 >3.5	1 MW >350
GI absorption	High	High	High	High
BBB permeant	Yes	Yes	No	No
P-gp substrate	No	Yes	Yes	Yes
CYP1A2 inhibitor	No	No	Yes	No
CYP2C19 inhibitor	Yes	Yes	Yes	No
CYP2C9 inhibitor	Yes	Yes	Yes	No
CYP2D6 inhibitor	Yes	Yes	Yes	Yes
CYP3A4 inhibitor	Yes	Yes	Yes	Yes

BBB: Blood–brain barrier; GI: Gastrointestinal; MR: Molar refractivity; P-gp: P-glycoprotein; PAINS: Pan Assay Interfering Substances; TPSA: Total polar surface area.

Inhibition of tubulin polymerization

The effect of the target compounds on tubulin polymerization was determined employing a sandwich enzyme immunoassay using ELISA kit for Tubulin β (TUBb), SEB870Hu (Cloud-Clone Corp., TX, USA) according to the procedure of the manufacturer. See Supplementary Information.

Drug-likeness profiles

SwissADME server [19] was used to calculate the drug-likeness profiles of compounds **7c**, **7e**, **11a** and colchicine.

Cell-cycle analysis

The effect of compounds **7c** and **11a** on the phases of the cell cycle in leukemia SR cells was determined using Propidium Iodide Flow Cytometry Kit/BD (ab139418) and BD FACS Calibur cell analyzer, BD Biosciences Propidium Iodide Flow Cytometry Kit (ab139418; Abcam, MA, USA). BD FACS Calibur cell analyzer (BD Biosciences, CA, USA) applying the standard procedure of the manufacturer.

Colchicine site competitive binding assay

The affinity of compounds **7c** and **11a** to colchicine binding site was determined using Colchicine Site Competitive Assay kit CytoDYNAMIX Screen15 (Cytoskeleton, Inc., CO, USA) using the standard protocol of the manufacturer to determine K_i values (μM). See Supplementary Information.

Molecular docking study

Molecular docking of the compounds was performed using BIOVIA Discovery Studio software v4.0.0 [20]. X-ray crystal structure of colchicine in complex with tubulin was downloaded from <http://www.rcsb.org/pdb> (PDB ID: 3UT5) [15]. See Supplementary Information.

Figure 5. The effect of compounds 7c and 11a on the phases of the cell cycle in leukemia SR cell line.

Results & discussion

Chemistry

The starting compounds **1a, b** were prepared using the conditions of Meerwein reaction [16,21]. Claisen–Schmidt condensation of the aldehyde derivatives **1a, b** with 4-methoxyacetophenone or 3,4,5-trimethoxyacetophenone was performed in basic medium [18] to afford chalcones **2a–d** (Figure 3). The $^1\text{H-NMR}$ spectra of compounds **2a–d** revealed two doublet signals around 7.4 and 7.9 p.p.m. with coupling constant about 15 Hz corresponding to the two chalcone protons. Reaction of chalcones derivatives **2a–d** with hydroxylamine hydrochloride or hydrazine hydrate afforded the isoxazoline derivatives **3a–d** and the pyrazoline derivatives **4a, b**, respectively (Figure 3). Reaction of **4a, b** with chloroacetyl chloride or chloropropionyl chloride in methylene chloride afforded compounds **5a, b** and **6a, b**, respectively. **5a, b** and **6a, b** are considered the key intermediates for the synthesis of a variety of amine derivatives **7a–f** and **8a–f** via reaction with appropriate secondary amines in the presence of anhydrous potassium carbonate (Figure 3). $^1\text{H NMR}$ of compounds **3a–d** and **4a, b** showed the disappearance of the two doublets of chalcone protons and the appearance of isoxazoline or pyrazoline protons signals while compounds **5a, b** and **6a, b** showed the disappearance of the pyrazoline NH signal and appearance of a singlet signal around 4.40 p.p.m. correlating with the methylene protons in compounds **5a, b** and two triplets at almost equal to 2.90 and 3.50 p.p.m. corresponding to the adjacent methylene groups in compounds **6a, b**. The structures of compounds **7a–f** and **8a–f** were in agreement with their spectral data since $^1\text{H-NMR}$ spectra of these compounds revealed additional aliphatic signals correlating with their amine terminals (See Materials & methods).

Chalcone derivatives can readily undergo Michael addition with active methylene compounds in the presence of basic catalyst [22,23]. Accordingly, reacting the chalcones **2a, b** with ethyl acetoacetate in the presence of KOH under reflux afforded **9a, b**. The labile ester moiety in **9a, b** was easily decarboxylated by alkali in aqueous ethanol under reflux to afford derivatives **10a, b**. Attempts to react the chalcone derivatives **2a, b** with acetyl acetone in the presence of KOH, to obtain the ethoxycarbonyl-substituted cyclohexenone derivative, surprisingly resulted in the formation of compounds **10a, b**. Furthermore, substituted cyanopyridines **11a, b** were synthesized by the condensation of chalcones **2a, b** with malononitrile in presence of ammonium acetate (Figure 4). Spectral data of compound **9a, b**, **10a, b** and **11a, b** were in agreement with their structures (See Materials and Methods).

Biological evaluation

In vitro antitumor evaluation (NCI-60 Human Tumor Cell Lines Screen)

Compounds **3a, 4a, 7a–d** and **11a, b** were tested by the NCI in an *in vitro* anticancer assay against a panel of 60 human cancer cell lines from nine different organs (lung, colon, breast, ovary, blood, kidney, skin, prostate and

Figure 6. Top docking poses of compounds **7e** (magenta) and **11a** (yellow) overlaid on colchicine (green) in tubulin binding site (PDB:3UT5).

For color figures please see online at: <http://www.future-science.com/doi/pdf/10.4155/fmc-2018-0036>

brain) at a concentration of 10 μM . The tested compounds displayed potent to moderate antiproliferative activity against different cell lines and leukemia cell lines were among the most sensitive cell lines. The oxazoline derivative **3a** showed the highest activity and was promoted to the five-dose assay to determine its GI_{50} concentration. The oxazoline derivative **3a** displayed potent broad spectrum antiproliferative activity against the entire panel with GI_{50} ranging from 0.0756 to 28.7 μM . Compound **3a** showed high antiproliferative effect at sub-micro molar level against, leukemia (HL-60[TB], K-562, SR) with GI_{50} = 0.076, 0.457 and 0.9163 μM , respectively, melanoma (MDA-MB-435) GI_{50} = 0.302 μM , lung (NCI-H522) GI_{50} = 0.450 μM , CNS (SF-295) GI_{50} = 0.563 μM , renal (A498) GI_{50} = 0.884 μM and ovarian cancer (NCI/ADR-RES) GI_{50} = 0.947 μM . (Supplementary Table 1).

Antiproliferative activity & inhibition of tubulin polymerization in leukemia SR cell line

In order to gain a more clear insight to the structure–activity relationship, all final compounds were evaluated for their antiproliferative activity against an in-house leukemia SR cell line and for their inhibitory effect on tubulin polymerization. Most of the tested compounds displayed potent to moderate antitumor activity (IC_{50} : 0.05–15.80 μM) (Table 1). A good correlation between the *in vitro* antiproliferative activity against leukemia SR cell line and percentage inhibition of tubulin polymerization was observed. Compounds **7c**, **7e** and **11a** showed superior potent antiproliferative activity compared with colchicine against leukemia SR cell line with IC_{50} 0.09, 0.05 and 0.06 μM , respectively and percentage inhibition of tubulin polymerization of 95.2, 96.0 and 96.3%, respectively, while compounds **3d**, **4b** and **7b** with the least activity in leukemia SR cell line (IC_{50} = 7.90, 10.70 and 15.80 μM , respectively) showed the lowest percentage of inhibition in tubulin polymerization assay (62.6, 31.0 and 25.4%, respectively).

The oxazoline series **3a–d** showed potent to moderate activity with IC_{50} ranging from 0.80 to 7.90 μM . The highest activity was observed with the 4-methoxy derivative of the furan analog **3a** (IC_{50} = 0.80 μM and 89.8% inhibition). Replacement of furan with thiophene reduces the activity of compounds **3c** and **3d**. The 4-methoxy derivatives **3a**, and **3c** were more active than the 3,4,5-trimethoxy congeners **3b** and **3d** with lower percentage of inhibition. The superior activity of the 4-methoxy derivatives over the 3,4,5-methoxy was maintained in most of the pyrazoline derivatives **4a**, **b** and **7a–f**. Moreover, the replacement of isoxazoline in **3a**, **b** by pyrazoline in **4a**, **b** slightly increased the activity. In series **7a–f**, the incorporation of different secondary amines into pyrazoline

NH through an acetyl linker enhances the potency, and the highest activity ($IC_{50} = 0.05 \mu M$) was observed with the noncyclic dimethyl amine derivative, **7e**. Generally, the order of activity was dimethyl amine > piperazine > morpholine. Interestingly, elongation of carbon spacer from acetyl to propionyl enhanced the activity of the 3,4,5-trimethoxy phenyl derivatives (e.g., **7f** and **8f**) but reduced that of the 4-methoxy phenyl counterparts, series (e.g., **7c** versus **8c** and **7e** versus **8e**).

The cyclohexenones **9a, b**, **10a, b** displayed IC_{50} ranges from 0.71 to 1.12 μM . Regarding the effect of substitution pattern on the phenyl ring, the 4-methoxy derivatives **9a** and **10a** and the 3,4,5-trimethoxy derivatives **9b** and **10b** are almost equivalent. However, substitution on the cyclohexenone ring with ethyl carboxylate in **9a, b** slightly decreases the activity compared with **10a, b**. Interestingly, replacement of cyclohexenone in **10a, b** by amino cyanopyridine in **11a, b** results in up to 15-fold increase in the antitumor potency ($IC_{50} = 0.06$ – $0.28 \mu M$).

Drug-likeness properties

The drug-likeness profiles for colchicine (**I**) and the most active compounds **7c**, **7e**, **11a** were predicted using SwissADME server [19]. The results of the Swiss ADME prediction of drug likeness of these compounds are shown in Table 2. Compounds **7c**, **7e** and **11a** showed no violation to Li, Pinski and other pharmacokinetics filters, and contained no alerts for Pan Assay Interfering Substances (PAINS). The pyrazoline derivative **7c** was predicted not to be a P-glycoprotein (P-gp) substrate and this is of special importance since colchicine (**I**) was reported to be a P-gp substrate [24] and expression inducer [25]. P-gp is one of the best-characterized mechanisms of cancer multidrug resistance [26] and pharmacokinetic drug–drug interactions as well [27].

Cell-cycle analysis

Compounds **7c** and **11a** were selected as representative examples to study their effect on the phases of the cell cycle of leukemia SR cells. Treatment with compounds **7c** or **11a** induced a clear accumulation of cells in the G2/M phase with a corresponding depletion of the G0/G1 phase compared with the untreated control cells correlating with the inhibition of tubulin polymerization and induction of apoptosis in the cells as evident from the increase in the percent of cells in the pre-G1 phase (Figure 5) (For details see Supplementary Table 2 & Supplementary Figure 2).

Colchicine site competitive binding assay

The affinity of compounds **7c** and **11a** to colchicine binding site was determined using colchicine site competitive assay kit. The K_i values of compounds **7c** and **11a** were 2.227 1.935 μM compared with 2.1 μM for colchicine confirming their affinity to the colchicine binding site.

Molecular docking

Docking of the designed compounds was performed prior to their synthesis and as mentioned before, they were capable of hydrogen bond formation with Cys $\beta 241$ by virtue of a methoxy group in ring A, (Supplementary Figure 1). Ring B in the designed molecules was more or less occupying the center of the binding site similar to colchicine (**I**) facing the backbone of Thr $\alpha 179$ in which furan/thiophene rings with its 4-chlorophenyl moiety were oriented toward a vacant cavity close to the α -subunit of tubulin not occupied by the tropane ring in colchicine (**I**). The furan ring was close enough to form hydrogen bond with Asn $\alpha 101$ which was in agreement with our hypothesis to focus on furan derivatives. The 4-chlorophenyl furan moiety was also interacting with Tyr $\alpha 224$ by π – π interaction and/or Lys $\beta 254$ by cation– π interaction. The excellent inhibition of tubulin polymerization of compounds **7c**, **7e** and **11a** suggested a detailed analysis of their possible binding modes in the colchicine binding site of tubulin. The docking results of compounds **7c**, **7e**, **11a** and colchicine (**I**) with amino acids of the active site are summarized in Supplementary Table 2. The pyrazolines **7c** and **7e** showed comparable or even higher binding to tubulin while compound **11a** showed slightly lower binding CDOKER interaction energy compared with colchicine. Compounds **7c**, **7e** and **11a** form an additional hydrogen bond to Asn $\beta 101$ via the oxygen of furan in **7c** and to Thr $\alpha 179$ for **11a** by virtue of its amino group. The inability of compound **7e** to form hydrogen bond with Asn $\beta 101$ may be attributed to the bulk of its piperazinyl moiety compared with the dimethyl moiety in **7c**. Compounds **7c**, **7e** and **11a** form more abundant hydrophobic interactions with various amino acids in the β subunit compared with colchicine, and this may in part account for their high CDOKER interaction energy despite the lower number of hydrogen bonds. In addition, Lys $\beta 254$ was engaged in cation– π interaction with the

furan ring in **7c** and **7e** or the chlorophenyl moiety in **11a** (Figure 6). In summary, the results of the molecular docking study are consistent with the high inhibitory activity observed for compounds **7c** and **11a**.

Conclusion & future perspective

Tubulin is a major player in the process of mitosis. Colchicine binding site inhibitors CBSI represent a promising class against cancer. More and more inhibitors are being discovered with higher activity compared with colchicine. This research work aimed at discovering some novel CBSI. The cytotoxic activity of the newly synthesized compounds is very promising and may represent a starting point for further study of structure–activity relationship and optimization.

Summary points

- Isoxazoline **3a–d**, pyrazoline **4a, b**, **7a–f** and **8a–f**, cyclohexenone **9a, b** and **10a, b** or pyridine derivatives **11a, b** were synthesized and evaluated for their inhibition of tubulin polymerization and cytotoxicity.
- Docking of these compounds at colchicine binding site showed that these compounds may interact with tubulin exploiting a binding cavity not commonly reported in the binding of colchicine binding site inhibitors.
- The tested compounds showed variable activity on the leukemia SR cell line with IC₅₀ ranges from 0.05 to 15.8 μ M. Within series **3a–d**, the highest activity is observed with the 4-methoxy derivative of furan analog **3a**.
- Replacement of furan with thiophene reduces the activity. Moreover, the replacement of isoxazoline by pyrazoline in compounds **4a, b** slightly increases the activity.
- Incorporation of different secondary amines into pyrazoline NH through an acetyl linker **7a–f** enhances the potency, and the highest activity was observed with the dimethyl amine **7e**.
- Elongation of carbon spacer from acetyl to propionyl enhanced the activity of the 3,4,5-trimethoxy phenyl derivatives but reduced that of the 4-methoxy phenyl counterparts **8a–f**.
- The cyclohexenones **9a, b** and **10a, b** displayed IC₅₀ ranges from 0.71 to 1.12 μ M. Substitution on the cyclohexenone ring with carboxylate **9a, b** slightly decreased the activity.
- Replacement of cyclohexenone by aminocyanopyridine **11a, b** results in up to 15-fold increase in the antitumor potency.
- The new compounds have the potential to exhibit antitumor activity through inhibition of tubulin polymerization.
- Compounds **7c**, **7e** and **11a** were the most potent colchicine binding site inhibitors against leukemia SR cell line with its IC₅₀ 0.09, 0.05 and 0.06 μ M, and the percentage inhibition of tubulin polymerization 95.2, 96.0 and 96.3%, respectively.
- Compounds **7c** and **11a** showed cell-cycle arrest in G2/M phase in SR leukemia cells and competitive binding to colchicine site of tubulin.
- Compounds **7c** and **11a** may be considered a promising lead compounds based on their excellent activity and their favorable drug-likeness profiles.

Supplementary data

See online at: <https://www.future-science.com/doi/10.4155/fmc-2018-0036>

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Ethical conduct of research

The authors state that they have obtained appropriate institutional review board approval or have followed the principles outlined in the Declaration of Helsinki for all human or animal experimental investigations. In addition, for investigations involving human subjects, informed consent has been obtained from the participants involved.

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