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Synthesis and Antitumor Activities of Some New *N*1-(Flavon-6-yl)amidrazone Derivatives

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A new series of *N*1-(flavon-6-yl)amidrazones were synthesized by reacting the hydrazonoyl chloride derived from 6-aminoflavone with the appropriate *sec*-cyclic amines. The antitumor activities of these compounds were evaluated on breast cancer (MCF-7) and leukemic (K562) cell lines. Among the compounds tested, the *N*-morpholine derivative was the most active against the MCF-7 and K562 cell lines, with IC_{50} values of 5.18 and 2.89 μM, respectively. Our docking studies showed that the *N*-morpholino derivative fits and blocks the oncogenic tyrosine kinases bcr/abl and epidermal growth factor receptor (EGFR) in a similar fashion to that of the potent anticancer agent imatinib.

Keywords: Antitumor activity / *N*1-(Flavon-6-yl)amidrazones / Oncogenic tyrosine kinases bcr/abl / 4-Oxo-*N*-(chromen-6-yl)hydrazonoyl chloride

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Introduction

Flavonoids are naturally occurring compounds [1, 2] that have shown multiple biological activities [3] such as immune-stimulatory [4], anti-estrogenic [5], anti-allergic [6], anti-inflammatory [7], anti-viral [8], anti-HIV [9], anti-ulcer [10], and antitumor activities [11]. The antitumor activity spurred research interest that led to the discovery of flavonoids having preventive and apoptosis properties in cancer treatment. Examples included kaempferol that was shown to inhibit the growth of ovarian cancer and breast cancer cell lines (Fig. 1) [12], epigallocatechin-3-gallate that proved high potency against the NBT-II bladder tumor and breast cancer cell lines (Fig. 1) [13], and quercetin that inhibits cancer cell proliferation and migration (Fig. 1) [14]. Furthermore, using ultrasound application with quercetin significantly improves the inhibition of skin and prostate cancer [15].

The anticancer activity of flavonoids, as polyphenolic compounds, is due to their antioxidant properties. These antioxidant features are mediated by different mechanisms including scavenging radical species (e.g., nitric oxide synthase) and suppressing some enzymes or chelating trace metals involved in free radical production (e.g., protein kinase C, cyclooxygenase, lipoxygenase, microsomal succinoxidase, and NADH oxidase) [3].

Flavones were the core of several synthetic and structural modification studies in order to improve their biological activities [16]. Based on flavone structure–activity relationship studies, it has been reported that the double bond between C2 and C3, the carbonyl group at position 4, hydroxy, methoxy, or amino groups appended at the 5 and 7 positions frequently improve the biological activity [17]. Such flavone derivatives showed high potency as antineoplastic agents and/or inhibitors for some selective kinase enzymes, such as cyclin-dependent kinase, several protein-tyrosine kinases, aromatase, topoisomerase, or protein kinase C [18].

Piperazinyl benzoamidrazones **1** (Fig. 2) were found to exhibit substantial antitumor activity against a number of cell lines ($IC_{50} = 4$ μM) [19]. Noteworthy, lower antitumor potency was reported when the *N*-phenyl group was replaced by coumarin **2** [20] or 2-methylchromone **3** [21] (Fig. 2). However, the 7-flavonyl amidrazone derivatives **4** (Fig. 2) displayed excellent cytotoxicity [21], in particular, the piperazine-substituted amidrazone (**4**: X = NH). On the other hand, 6-aminoflavone **5** (Scheme 1) has been

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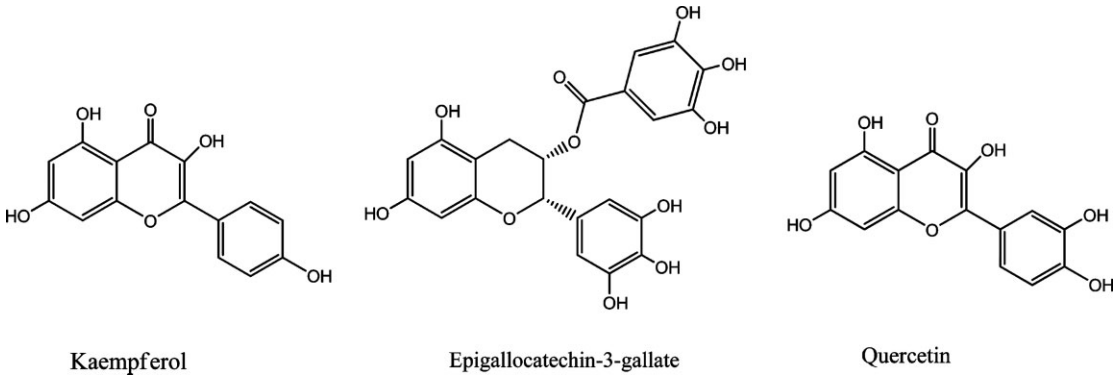


Figure 1. Some polyphenolic flavonoids.

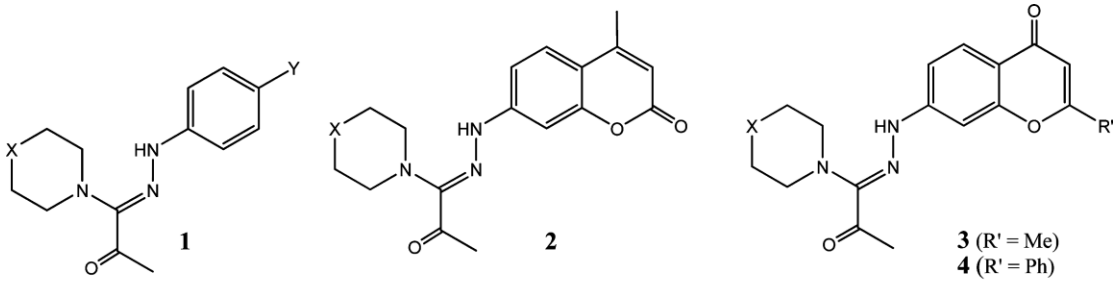


Figure 2. Cyclic amine-substituted amidrazones.

reported to show weak activity against breast cancer cell line ($IC_{50} = 60\ \mu M$) [22].

With the preceding information in mind, we anticipated that a hybrid structure based on amidrazone-flavone pharmacophores would possess high cytotoxicity. Accordingly, this class of compounds has become a lead target structure in order to improve its efficacy as antitumor agent. Herein, we report on the synthesis and potency of a selected set of new 6-flavone-substituted amidrazone analogs (**7a–e**/Scheme 1) against a panel of cancer cell lines. Interestingly, the *N*-morpholino derivative of the new series docks into the binding pocket of oncogenic tyrosine kinase bcr/abl in a comparable fashion to the potent anticancer agent imitanib (*vide infra*).

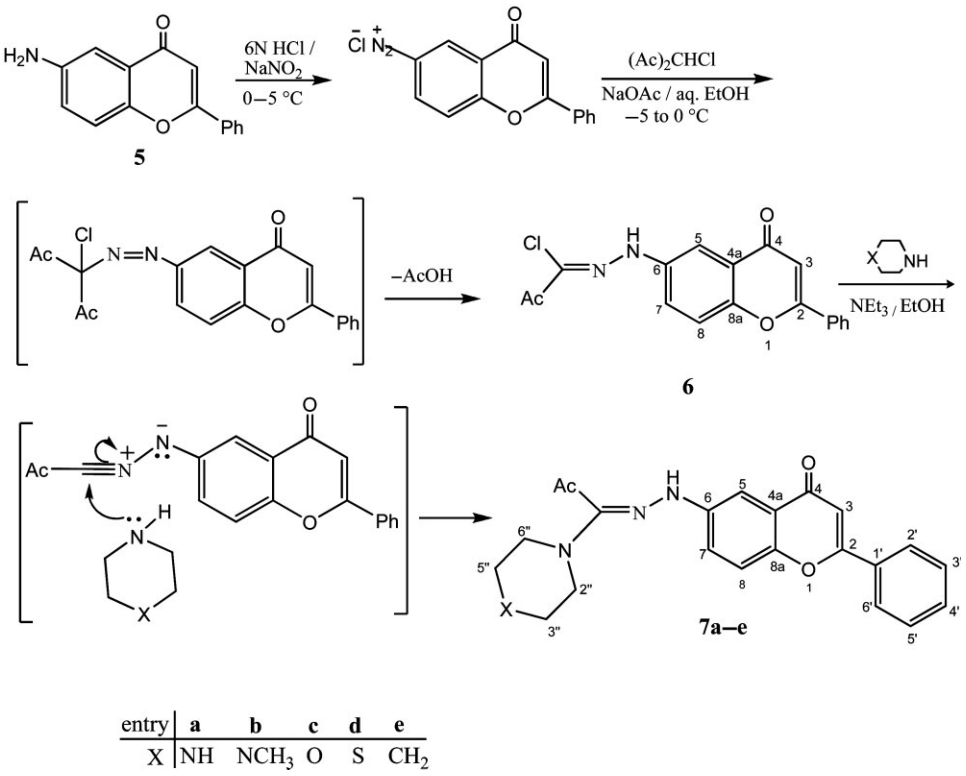
Results and discussion

Chemistry

The hydrazonoyl chloride **6**, required in this study, was prepared via direct coupling of the respective flavon-6-diazonium chloride with 3-chloropentane-2,4-dione in aqueous alcoholic sodium acetate solution (Japp–Klingemann reaction) [23] (Scheme 1). The flavon-6-diazonium chloride is freshly prepared by diazotization of the respective 6-aminoflavone **5** (suspended in 6 N aq. HCl).

Piperazines, morpholine, thiomorpholine, and piperidine, acting as nitrogen nucleophiles, are expected to add readily onto *N*-(flavon-6-yl)nitrile imine (the reactive 1,3-dipolar species generated *in situ* from its corresponding hydrazonoyl chloride precursor **6** in the presence of triethylamine) to give the respective amidrazone adducts **7a–e** (Scheme 1). This mode of nucleophilic addition reaction of various nucleophiles onto 1,3-dipoles is well documented [24–26].

The newly synthesized compounds **7a–e** were characterized by MS and NMR spectral data. These data, detailed in the Experimental section, are consistent with the suggested structures. Thus, the mass spectra display the correct molecular ion peaks for which the measured high resolution mass spectral (HRMS) data are in good agreement with the calculated values. DEPT and 2D (COSY, HMQC, and HMBC)



Scheme 1. Synthesis of the *N*1-(flavon-6-yl)amidrazone derivatives (**7a**–**e**).

experiments showed correlations that helped in the ^{1}H- and ^{13}C-signal assignments to the different carbons and their attached and/or neighboring hydrogens.

Biology

The antitumor activity of the newly synthesized 6-flavone-substituted amidrazones (**7a**–**e**/Scheme 1) was characterized by conducting cell viability assay using tetrazolium dye 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT). Cultures of the breast cancer cell line MCF-7 and the leukemic cell line K562 were treated with different concentrations and the resultant IC_{50} values are listed in Table 1. From the structure–activity relationship point of view, the nature of substituent appended to the 6-position seems to play a critical role in determining the anticancer activity. Clearly, different substitutions at position 6 result in remarkable variations in the determined IC_{50}. For example, in case of compound **7d**, no activity was observed under 50 μM in both cell lines. This weak activity was also observed with compound **7a** against K562 cell line. Interestingly, compound **7c** was the most potent at both cell lines scoring a relatively low IC_{50}. These low IC_{50} values reveal the importance of the morpholino moiety for the anticancer activity. This efficient anticancer activity may result from the high reactivity of the oxygen atom in the morpholino ring and its high ability toward forming more hydrogen bonds with its cellular target.

Molecular modeling

The potent inhibitory effects of the new compounds **7a**–**e** against K562 and MCF-7 cancer cell lines, which over-express bcr/abl and epidermal growth factor receptor (EGFR) tyrosine kinases, respectively, combined with the apparent pharmacophoric commonalities between these compounds and the anticancer agent imatinib, prompted us to anticipate that their observed anticancer properties are attributable to their

Table 1. The IC_{50} values for the five tested compounds against MCF-7 and K562 cell lines.

Compound	IC_{50} MCF-7 (μM) ± SD	IC_{50} K562 (μM) ± SD
Doxorubicin	0.31 ± 0.01	1.41 ± 0.31
7a	16.77 ± .88	>50
7b	11.75 ± 0.59	12.89 ± 0.41
7c	5.18 ± 1.20	2.89 ± 1.27
7d	>50	>50
7e	11.76 ± 3.10	13.76 ± 2.87

Doxorubicin was used as a positive control.

abilities to effectively bind and block oncogenic tyrosine kinases, particularly bcr/abl.

Figure 3 compares how imatinib binds within the ATP binding pocket of bcr/abl (PDB code: 1IEP, resolution 2.1 Å) with the way **7c** (the most active analog, as in Table 1) docks into the binding pocket of the same protein. Clearly from the figure, positioning the pyridinylpyrimidine fragment of imatinib within the aromatic hydrophobic pocket of the side chains of Phe382, Tyr253, and Phe317 (Fig. 3A) compares to fitting the phenyl substituent of **7c** into the same pocket (Fig. 3C), i.e., via π-stacking interactions. Similarly, hydrogen-bonding interactions connecting the amidic linker of imatinib with the carboxylic acid side chain of Glu286 and the peptidic NH of Asp381 correlate well with hydrogen-bonding interactions connecting the amidrazone NH and the morpholino ether oxygen of **7c** with the same amino-acid residues, respectively. The latter interaction, i.e., tying the morpholino oxygen of **7c** and the peptidic NH of Asp381, is specific for **7c** compared to other analogs (**7a**, **7b**, **7d**, and **7e** as in Fig. 4). Similar analogy can be noticed between hydrogen-bonding interactions connecting the hydroxyl of Thr315 with the aromatic NH of imatinib (Fig. 3A) and the flavone oxygen of **7c** (Fig. 3C).

Furthermore, hydrophobic stacking of the methylbenzene linker of imatinib close to the $(CH_2)_4$ of Lys271 (Fig. 3A) compares to fitting the flavone ring of **7c** close to the same side chain (Fig. 3B) allowing similar hydrophobic interactions. Finally, the apparent electrostatic attraction connecting the piperazine ring of imatinib with the carboxylate side chain of Asp381 (Fig. 3A) corresponds to electrostatic attraction connecting the morpholino nitrogen of **7c** with the same carboxylate group in the binding pocket.

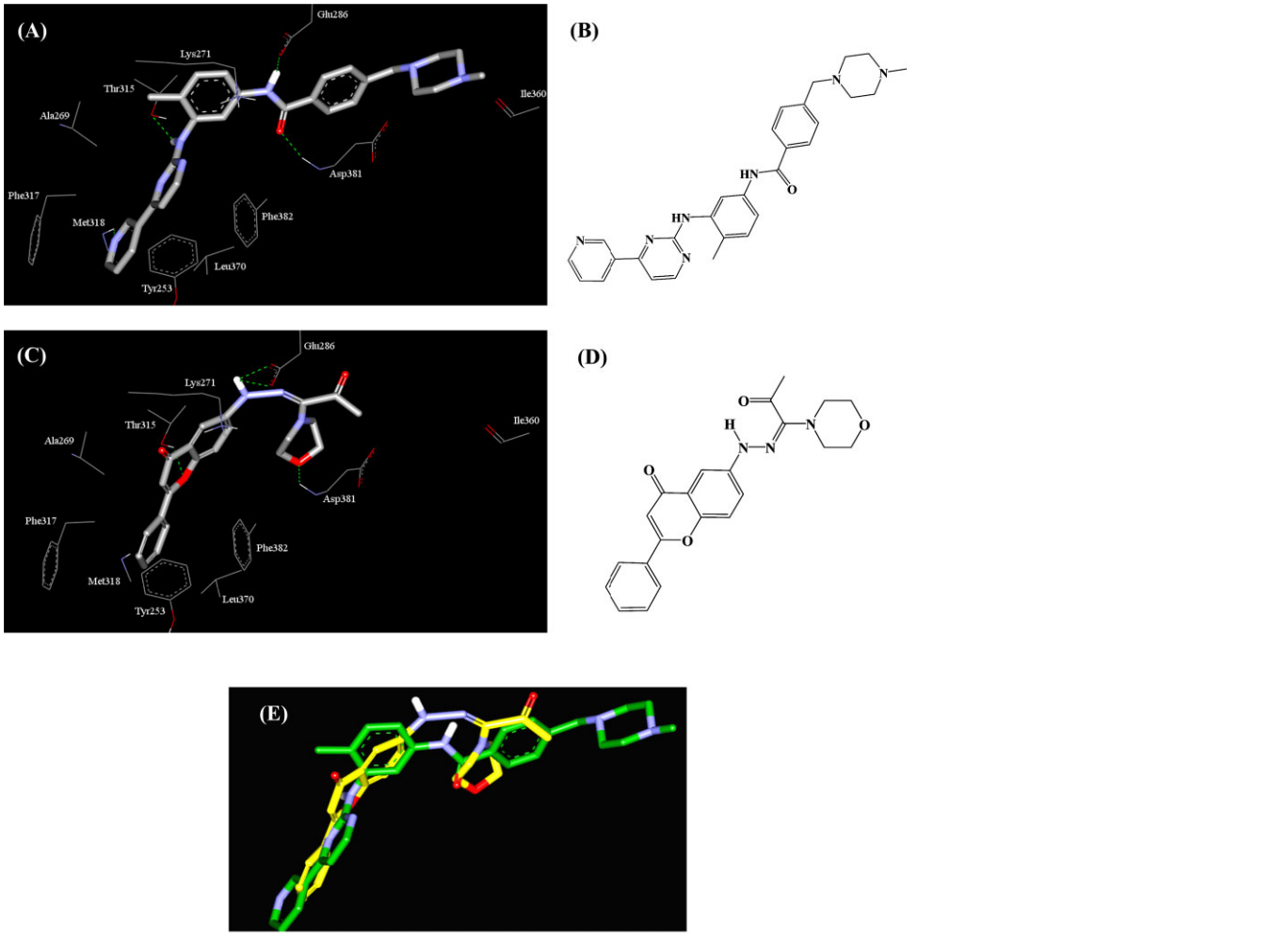
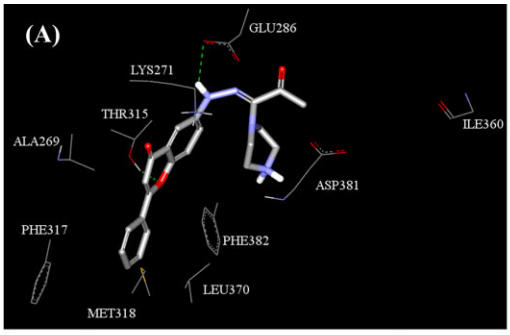
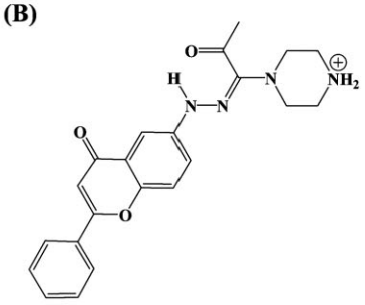
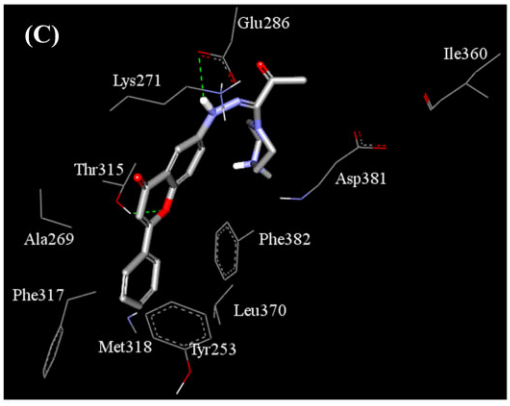
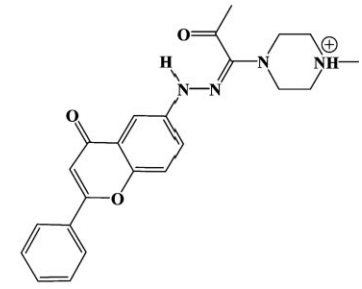


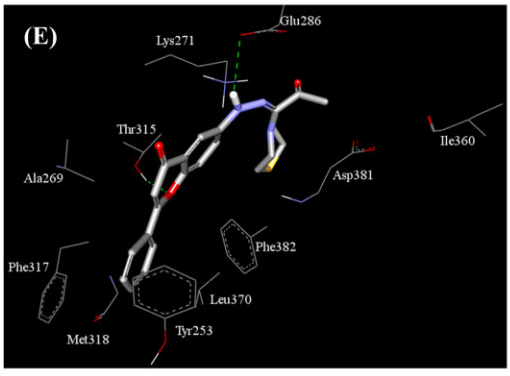
Figure 3. (A) X-ray crystallographic structure of imatinib co-crystallized within bcr/abl kinase domain (PDB code: 1IEP, resolution 2.1 Å), (B) chemical structure of imatinib, (C) compound **7c** docked within the same binding pocket, (D) chemical structure of **7c**, (E) superposition of the co-crystallized structure of imatinib over the docked structure of **7c**.

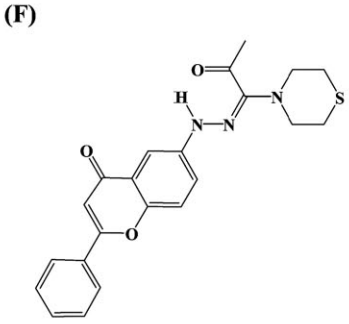


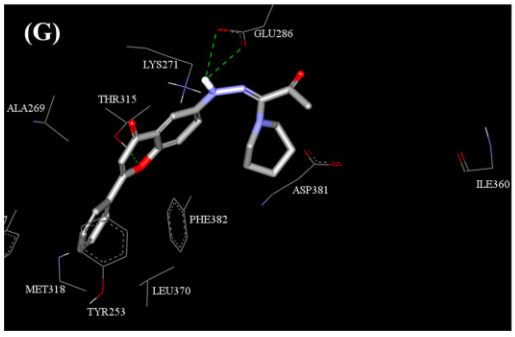












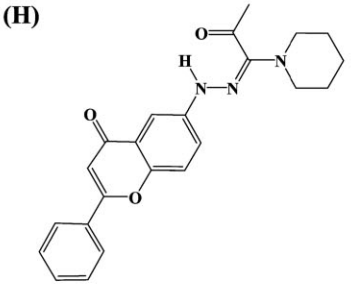


Figure 4. Docked poses of **7a** (A), **7b** (C), **7d** (E), and **7e** (G) within the same binding pocket (PDB code: 1IEP, resolution 2.1 Å); (B), (D), (F), and (H) show the chemical structures of **7a**, **7b**, **7d**, and **7e**, respectively.

Interestingly, the docked poses of the other less active analogs, i.e., **7a**, **7b**, **7d**, and **7e**, closely resemble that of **7c** (Fig. 4) albeit their bound poses lack hydrogen bonding interaction with the peptidic NH of Asp381. This seems to be due to the lack of a suitably positioned a hydrogen-bond acceptor corresponding to the morpholino oxygen in the cases of **7c** as in **7d** (sulfur is replacing oxygen) and **7e** (methylene is replacing oxygen). On the other hand, although **7a** and **7b** have hydrogen bond acceptors corresponding to the morpholino oxygen of **7c**, i.e., their piperazine nitrogens, the fact that amine nitrogens are ionized under physiological conditions deprive them from their hydrogen-bond acceptor properties and hinder formation of corresponding hydrogen bonds with the peptidic NH of Asp381.

We believe that the inferior potencies of **7a**, **7b**, **7d**, and **7e** can be partially explained by the loss of hydrogen-bonding interaction with peptidic NH of Asp381. However, the sheer size of sulfur atom in **7d** seems to sterically clash with binding pocket residues, thus causing significant reduction in the affinity of **7d** compared to other analogs.

Conclusion

In summary, a new series of *N*1-(flavon-6-yl)amidrazones **7a–e** incorporating *N*-morpholine, *N*-piperazine, and related congeners were prepared by direct reaction of the corresponding hydrazonoyl chloride with the particular secondary cyclic amine. These amidrazones were tested *in vitro* for their antitumor activity against breast cancer (MCF-7) and leukemic (K562) cell lines. The results revealed that the morpholino derivative **7c** exhibited significant antiproliferative activity against breast cancer (MCF-7) and leukemic (K562) cell lines with IC_{50} values of 5.18 and 2.89 μM, respectively. This indicates that the nature of substituent, located at the 6-position, seems to play a critical role in determining the anticancer activity.

Experimental

The following chemicals, used in this study, were purchased from Aldrich and were used as received: 6-aminoflavone, 3-chloropentane-2,4-dione, piperazine, *N*-methylpiperazine, morpholine, thiomorpholine, and piperidine. Melting points were determined on a Stuart scientific melting point apparatus in open capillary tubes. ^{1}H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer (Bruker AVANCE-III). Chemical shifts are expressed in δ units; ^{1}H–^{1}H coupling constants are given in Hertz. High-resolution mass spectra (HRMS) were acquired by electrospray ionization (ESI) technique with the aid of Bruker APEX-2 instrument. The samples were dissolved in acetonitrile, diluted in spray solution (methanol/water 1:1 v/v + 0.1% formic acid), and infused using a syringe pump with a flow rate of 2 μL/min. External calibration was conducted using arginine cluster in the mass range *m*/*z* 175–871.

Experimental procedure for the synthesis of *N*-2-oxo-(4-oxo-2-phenyl-4*H*-chromen-6-yl)-propanehydrazonoyl chloride (6)

The title compound was prepared by the following procedure:

Step (i). 6-Aminoflavone **5** (0.10 mol) was dissolved in 6 N aqueous hydrochloric acid (160 mL). To this solution, a solution of sodium nitrite (7.6 g, 0.11 mol) in water (15 mL) was added dropwise with efficient stirring at 0–5°C. Stirring was continued for 20–30 min, and the resulting fresh cold, flavon-6-yl diazonium chloride solution was used immediately as such for the following coupling reaction.

Step (ii). A cold (0–5°C) freshly prepared solution of 2-phenyl-4-oxo-4*H*-chromen-6-yl diazonium chloride (0.1 mol) was poured onto a cold solution (−5 to 0°C, ice-salt bath) of 3-chloropentane-2,4-dione (13.5 g, 0.1 mol) in ethanol/water (160 mL, 1:1 v/v) containing 30 g of sodium acetate with vigorous stirring. The resulting orange-colored mixture was further stirred until a solid precipitate was formed (5–10 min). The reaction mixture was then diluted with cold water (200 mL), and the solid product was collected by suction filtration, washed several times with cold water, dried, and recrystallized from $CHCl_3$/pet. ether. Yield: 92%; m.p.: >300°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3–C=O), 7.02 (s, 1H, H-3), 7.61 (m, 3H, H-3′ + H-5′ + H-4′), 7.83 (d, J = 9.0 Hz, 1H, H-8), 7.93 (dd, J = 9.0, 2.7 Hz, 1H, H-7), 8.01 (d, J = 2.7 Hz, 1H, H-5), 8.10 (dd, J = 7.6, 2.0 Hz, 2H, H-2′ + H-6′), 10.97 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.9 (CH_3–C=O), 106.8 (C-3), 108.1 (C-7), 120.4 (C-8), 122.1 (C-5), 124.4 (–C=N), 124.5 (C-4a), 126.8 (C-2′/C-6′), 129.6 (C-3′/C-5′), 131.6 (C-1′), 132.3 (C-4′), 140.6 (C-6), 152.1 (C-8a), 162.9 (C-2), 177.3 (C-4), 188.4 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{18}H_{13}ClN_2O_3Na$ $[M+Na]^+$ 363.05124; found 363.05069.

Experimental procedure for the synthesis of the target amidrazones (7a–e)

To a cold suspension (−10 to 0°C) of **6** (0.20 g, 0.57 mmol) in 10 mL of ethanol, a solution of the appropriate secondary amine (0.7 mmol) and triethylamine (2 mL) in ethanol (5 mL) was added, with stirring. Stirring was continued at 0–5°C for 2–4 h, and then at ambient temperature for additional 2 h. The reaction mixture was then poured into water (50 mL), and the resulting crude solid product was collected by suction filtration, washed with water, dried, and purified on preparative silica gel TLC plates. Using this same general procedure, the following compounds were prepared.

*6-{2-[2-Oxo-1-(piperazin-1-yl)propylidene]hydrazinyl}-2-phenyl-4H-chromen-4-one (**7a**)*

Piperazine (5.7 mmol) and 0.57 mmol of **6** were used and the reaction mixture was stirred for 24 h. Yield: 0.12 g, 52%; m.p.: >300°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.75 (s, 1H, N(4″)-H), 2.48 (s, 3H, CH_3–C=O), 3.0 (m, 4H, H_2-2″ + H_2-6″), (m, 4H, H_2-3″ + H_2-5″), 6.85 (s, 1H, H-3), 7.54 (m, 3H, H-3′ + H-5′ + H-4′), 7.62 (d, J = 9.0 Hz, 1H, H-8), 7.75 (dd, J = 9.0, 2.6 Hz, 1H, H-7), 7.82 (d, J = 2.6 Hz, 1H, H-5), 7.93 (dd, J = 7.5, 2.3 Hz, 2H, H-2′ + H-6′), 9.43 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.9 (H_3C–C=O), 46.7 (N–CH_3), 49.2 (C-2″/C-6″), 55.8 (C-3″/C-5″), 106.9 (C-3), 108.3 (C-7), 119.6 (C-8), 120.5 (C-5), 124.7 (C-4a), 126 (C-2′/C-6′), 129.1 (C-3′/C-5′), 131.6 (C-4′), 131.8 (C-1′), 140.3 (C-6), 144.3 (–C=N), 151.8 (C-8a), 163.5 (C-2), 178.3 (C-4), 195.0 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{22}H_{23}N_4O_3$ $[M+H]^+$ 391.17702; found 391.17647.

*6-{2-[1-(4-Methylpiperazin-1-yl)-2-oxopropylidene]-hydrazinyl}-2-phenyl-4H-chromen-4-one (**7b**)*

Yield: 0.18 g, 76%; m.p.: 268–269°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.37 (s, 3H, CH_3–N), 2.43 (s, 3H, CH_3–C=O), 2.54 (m, 4H, H_2-3″ + H_2-5″), 3.10 (m, 4H, H_2-2″ + H_2-6″), 6.82 (s, 1H, H-3), 7.53 (m, 3H, H-3′ + H-5′ + H-4′), 7.62 (d, J = 9.0, 2.1 Hz 1H, H-8), 7.76 (m, 2H, H-5 + H-7), 7.92 (dd, J = 7.9, 2.1 Hz 2H, H-2′ + H-6′), 9.31 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.8 (CH_3–C=O), 46.3 (H_3C–N), 47.8 (C-2″/C-6″), 55.8 (C-3″/C-5″), 106.9 (C-3), 108.3 (C-7), 119.6 (C-8), 120.5 (C-5), 124.7 (C-4a), 126.1 (C-2′/C-6′), 128.5 (C-3′/C-5′), 131.6 (C-4′), 131.8 (C-1′), 140.3 (C-6), 144.2 (–C=N), 151.8 (C-8a), 163.5 (C-2), 178.2 (C-4), 194.9 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{23}H_{25}N_4O_3$ $[M+H]^+$ 405.19267; found 405.19212.

*6-[2-(1-Morpholino-2-oxopropylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**7c**)*

Yield: 0.20 g, 87%; m.p.: 291–292°C. ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.45 (s, 3H, CH_3–C=O), 3.07 (m, 4H, H_2-2″ + H_2-6″), 3.83 (m, 4H, H_2-3″ + H_2-5″), 6.82 (s, 1H, H-3), 7.55 (m, 3H, H-3′ + H-5′ + H-4′), 7.62 (d, J = 9.0 Hz, 1H, H-8), 7.77 (dd, J = 9.0, 2.7 Hz, 1H, H-7), 7.83 (d, J = 2.7 Hz, 1H, H-5), 7.96 (dd, J = 7.6, 2.3 Hz, 2H, H-2′ + H-6′), 9.43 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.9 (CH_3–C=O), 48.2 (C-2″/*C*-6″), 67.5 (C-3″/*C*-5″), 106.9 (*C*-3), 108.1 (C-7), 119.6 (C-8), 120.6 (*C*-5), 124.7 (C-4a), 126.3 (C-2′/*C*-6′), 129.1 (C-3′/*C*-5′), 131.7 (C-4′), 131.8 (C-1′), 140.1 (*C*-6), 143.5 (–C=N), 151.9 (C-8a), 163.5 (*C*-2), 178.2 (C-4), 194.9 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{22}H_{22}N_3O_4$ $[M+H]^+$ 392.16103; found 392.16048.

*6-[2-(2-Oxo-1-thiomorpholinopropylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**7d**)*

Yield: 0.20 g, 84%; m.p.: >300°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.47 (s, 3H, CH_3–C=O), 2.79 (m, 4H, H_2-3″ + H_2-5″), 3.30 (m, 4H, H_2-2″ + H_2-6″), 6.82 (s, 1H, H-3), 7.56 (m, 3H, H-3′ + H-5′ + H-4′), 7.62 (d, J = 9.0 Hz, 1H, H-8), 7.75 (dd, J = 9.0, 2.5 Hz, 1H, H-7), 7.79 (d, J = 2.5 Hz, 1H, H-5), 7.96 (dd, J = 7.7, 2.2 Hz, 2H, H-2′ + H-6′), 9.26 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.8 (CH_3–C=O), 28.6 (C-3″/C-5′), 50.3 (C-2″/C-6″), 107.0 (C-3), 108.5 (C-7), 119.6 (C-8), 120.5 (C-5), 124.7 (C-4a), 126.3 (C-2′/C-6′), 129.1 (C-3′/C-5′), 131.6 (C-4′), 131.8 (C-1′), 140.1 (C-6), 144.7 (–C=N), 151.9 (C-8a), 163.5 (C-2), 178.2 (C-4), 194.9 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{22}H_{22}N_3O_3S$ $[M+H]^+$ 408.13819; found 408.13764.

*6-[2-(2-Oxo-1-(piperidin-1-yl)propylidene)hydrazinyl]-2-phenyl-4H-chromen-4-one (**7e**)*

Yield: 0.18 g, 79%; m.p.: 264–265°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.86 (m, 2H, H_2-4″), 1.72 (m, 4H, H_2-3″ + H_2-5″), 2.47 (s, 3H, CH_3–C=O), 3.0 (m, 4H, H_2-2″ + H_2-6″), 6.86 (s, 1H, H-3), 7.57 (m, 3H, H-3′ + H-5′ + H-4′), 7.62 (d, J = 9.0 Hz, 1H, H-8), 7.78 (dd, J = 9.0, 2.5 Hz, 1H, H-7), 7.81 (d, J = 2.5 Hz, 1H, H-5), 7.96 (dd, J = 7.7, 2.2 Hz, 2H, H-2′ + H-6′), 9.35 (s, 1H, N–H, exchangeable with D_2O). ^{13}C NMR (75 MHz, $CDCl_3$): δ 24.0 (C-4″), 25.9 (CH_3–C=O), 26.7 (C-3″/C-5″), 49.2 (C-2″/C-6″), 106.9 (C-3), 108.2 (C-7), 119.5 (C-8), 120.5 (C-5), 124.7 (C-4a), 126.3 (C-2′/C-6′), 129.1 (C-3′/C-5′), 131.6 (C-4′), 131.9 (C-1′), 140.5 (C-6), 145.6 (–C=N), 151.7 (C-8a), 163.4 (C-2), 178.3 (C-4), 195.2 (O=*C*-Me). HRMS (ESI) *m*/*z*: calcd. for $C_{23}H_{24}N_3O_3$ $[M+H]^+$ 390.18177; found 390.18122.

Cell lines and cell culture

The K562 leukemia cell line was obtained from Dr. Mona Hassona (Faculty of Science, The University of Jordan) and cultured in RPMI, while the MCF-7 breast cancer cells were obtained from American Type Culture Collections (ATCC) and cultured in DMEM/F12. All media were supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco Invitrogen), 1% of 2 mM L-glutamine (Lonza), 50 IU/mL penicillin (Lonza), and 50 μg/mL streptomycin (Lonza) and cells were maintained at 37°C, 5% CO_2 humidified incubator.

Cell proliferation assay

MCF-7 and K562 cells were seeded at a density of 1×10^4, 1×10^4, and 4×10^4 cells per well in 96-well plates in appropriate medium. For anti-MCF7 and anti-K562 screening, the cells were treated with 50 μM concentrations of the tested compounds. For the IC_{50} determination, the cells were treated with increasing concentrations of the tested compound (1.56–100 μM). In all assays, the drugs were dissolved in DMSO immediately before the addition to cell cultures and equal amounts of the solvent were added to control cells. Cell viability was assessed, after 3 days of treatment, with tetrazolium dye MTT, obtained from Sigma (Dorset, UK). IC_{50} concentrations were obtained from the dose–response curves using Graph Pad Prism Software 5 (San Diego, California, USA, www.graphpad.com).

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