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Original article

Synthesis, photochemical synthesis, DNA binding and antitumor evaluation of novel cyano- and amidino-substituted derivatives of naphtho-furans, naphtho-thiophenes, thieno-benzofurans, benzo-dithiophenes and their acyclic precursors

Kristina Starčević [a], Marijeta Kralj [b,*], Ivo Piantanida [c], Lidija Šuman [b], Krešimir Pavelić [b], Grace Karminski-Zamola [a,*]

[a] *Department of Organic Chemistry, Faculty of Chemical Engineering and Technology, University of Zagreb, Marulićev trg 20, P.O. Box 177, HR-10000 Zagreb, Croatia*
[b] *Division of Molecular Medicine, Laboratory of Functional Genomics, Ruđer Bošković Institute, Bijenička cesta 54, P.O. Box 180, HR-10000 Zagreb, Croatia*
[c] *Division of Organic Chemistry and Biochemistry, Laboratory of Supramolecular and Nucleoside Chemistry, Rudjer Boskovic Institute, P.O. Box 180, HR-10002 Zagreb, Croatia*

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Abstract

As a part of the research on the improvement of an alternative to conventional photodynamic therapy by light-induced formation of intercalators, we synthesized a series of novel heterocyclic compounds and their acyclic precursors. We now report details about their synthesis/characterization in respect to their potential of photoinduced cyclization, interactions with DNA and inhibition of the tumor cell growth in vitro. Among studied compounds only amidino-furyl-substituted phenyl acrylates were efficiently converted to the corresponding naphthofuranes, while their thiophene analogues, all non-charged derivatives and amidino-phenyl-substituted analogues didn't show acceptable photoconversion. The significantly stronger antiproliferative activity of cyclic analogues could be correlated to the property of these molecules to intercalate into DNA. The acyclic molecules did not show any interaction with DNA, correlating with the inferior biological activity, except for one cyano-bearing molecule.

Keywords: Antitumor substances; Intercalators; Photocyclization; Tumor cell lines

1. Introduction

The discovery of new compounds with antitumoral activity has become one of the most important goals in medicinal chemistry. One of the most often used classes of chemotherapeutic agents in cancer therapy comprises molecules that interact with DNA, such as groove binders, DNA alkylating substances and intercalators. Moreover, the study of the exact mechanisms of action of these agents, as well as, DNA damage response of the tumor cells are of high interest for medicinal chemists and molecular biologists. For example, the intercalation of planar aromatic molecules into the DNA double helix and poisoning of DNA topoisomerases I and/or II are considered to be important in the therapeutic action of many antitumor agents [1,2]. The best examples are anthracyclines (e.g. doxorubicin), anthracenediones (e. g. mitoxantrone), some acridines (e.g. acridine-4-carboxamide) and ellipticines [3].

In our previous paper [4] we reported about the photoinduced switch of DNA/RNA inactive amidino-substituted 3-(2-furyl)-2-phenyl-acrylate into a classical intercalator as amidino-substituted naphthofuran carboxylate. Acyclic precursors, as well as cyclized compounds were also tested for the antiproliferative effect on a panel of six human cell lines, five of which

* Corresponding author.
E-mail addresses: kstarcev@fkit.hr (K. Starčević), mhorvat@irb.hr (M. Kralj), pianta@irb.hr (I. Piantanida), suman@irb.hr (L. Šuman), pavelic@irb.hr (K. Pavelić), gzamola@pierre.fkit.hr (G. Karminski-Zamola).

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were derived from five cancer types. The observed results clearly demonstrate one order of magnitude superior action of cyclic compound to its acyclic precursor. These experiments present the conceptual basis for development of new photoinduced anticancer therapy based on the photochemical transformation on a non intercalating molecule into the intercalating one, opposed the conventional photodynamic therapy, as a novel approach to selective antitumor therapy.

Searching for another planar condensed heterocyclic compounds, related to naphthofurans, naphthothiophenes, thienobenzofurans and benzodithiophenes which could serve as DNA intercalators and, as a consequence, could exert antitumor activity, we have prepared a new group of cyano- and amidino-substituted naphtho[2,1-*b*]furans (**6a, 6b, 7b, 8a**), naphtho[2,1-*b*]thiophenes (**6c**, **7a**), benzothienofurans (**11a, 12a, 12b, 15** and **16**) and benzodithiophenes (**11b**, **12c**) from the corresponding acyclic precursors, of cyano- or amidino-substituted-furyl-phenyl-acrylates, thienyl-phenyl-acrylates, furyl-thienyl acrylates and dithienyl-acrylates (**2a**, **2b**, **3a**, **3b**, **3c**, **4a**, **4b**, **4c**, **5a**, **5b**, **5c**, **10a, 10b** and **14**).

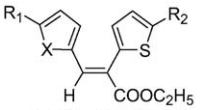
The constant and growing interest in the development of new efficient and general synthetic methods for the preparation of fused heterocyclic systems involving furan and thiophene subunits is justified by their well-established valuable physiological and pharmacological properties [5]; however, there is little new literature data describing the synthesis and biological activity of these systems [1,6]. Recent technical applications of polysubstituted benzofurans and benzothiophenes, including numerous fluorescent dyes used as retrograde tracers, and Ca^{2+} and Mg^{2+} fluorescent indicator conjugates, etc. [5] increase their significance. The biological activity of many arenofurane derivatives was improved by addition of positively charged aliphatic substituents. For example, amidine-substituted heterocycles based on furan moiety have shown a number of intriguing modes of biological activity, like antimicrobial [7], hallucinogenic agents [8], and some furamidines were found to be active against diverse, highly infectious parasites [9]; the best being selected for currently undergoing phase II clinical trials [10]. The results suggest that the presence of amidine terminal groups plays a role in facilitating nuclear accumulation into cells, probably as a result of nucleic acid binding [11]. For example, series of pyrrolo[2,1-*c*][1,4]benzodiazepine amidines has been evaluated for in vitro DNA binding through thermal denaturation studies. Some of these compounds cause a significant increase in melting of calf thymus DNA [12]. Inhibition of tumor growth and polyamine uptake by tetracyclic amidines were described [13]. DNA binding properties and cytotoxic activity of novel aromatic amidines in cultured human skin fibroblasts were examined [14]. The synthesis and antitumor evaluation of some new substituted amidino benzimidazolyl naphtho[2,1-b]furan carboxylates was described recently [15].

In this paper we present the synthesis of a number of new acyclic substances, describe their potential of photoinduced conversion into condensed heterocyclic compounds under biologically relevant conditions, the study of their interactions with DNA and tested their antitumor activity on several human tumor cell lines.

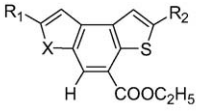
2. Results and discussion

2.1. Chemistry

All compounds (**2a**–**16**) shown in the Figs. 1–3 were prepared according to the Schemes 1–3. Acyclic cyano compounds (**2a** and **2b**) were prepared by aldol condensation from

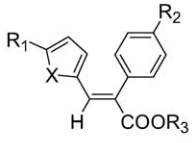


10a R_1, R_2=CN, X=O
10b R_1=H, R_2=CN, X=S

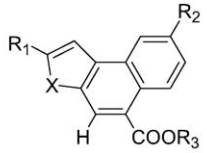


11a R_1, R_2=CN, X=O
11b R_1=H, R_2=CN, X=S
12a R_1=*iso*-pr-am, R_2=*iso*-pr-am, X=O
12b R_1=morph-amd, R_2=morph-am.,X=O
12c R_1=H, R_2=*iso*-pr-am, X=S

Fig. 2. Substituted thieno[3,2-*e*]benzo[*b*]furans, thieno[2,3-*e*]benzo[*b*]thiophenes and their acyclic precursors (**10a–b, 11a–b, 12a–c**).

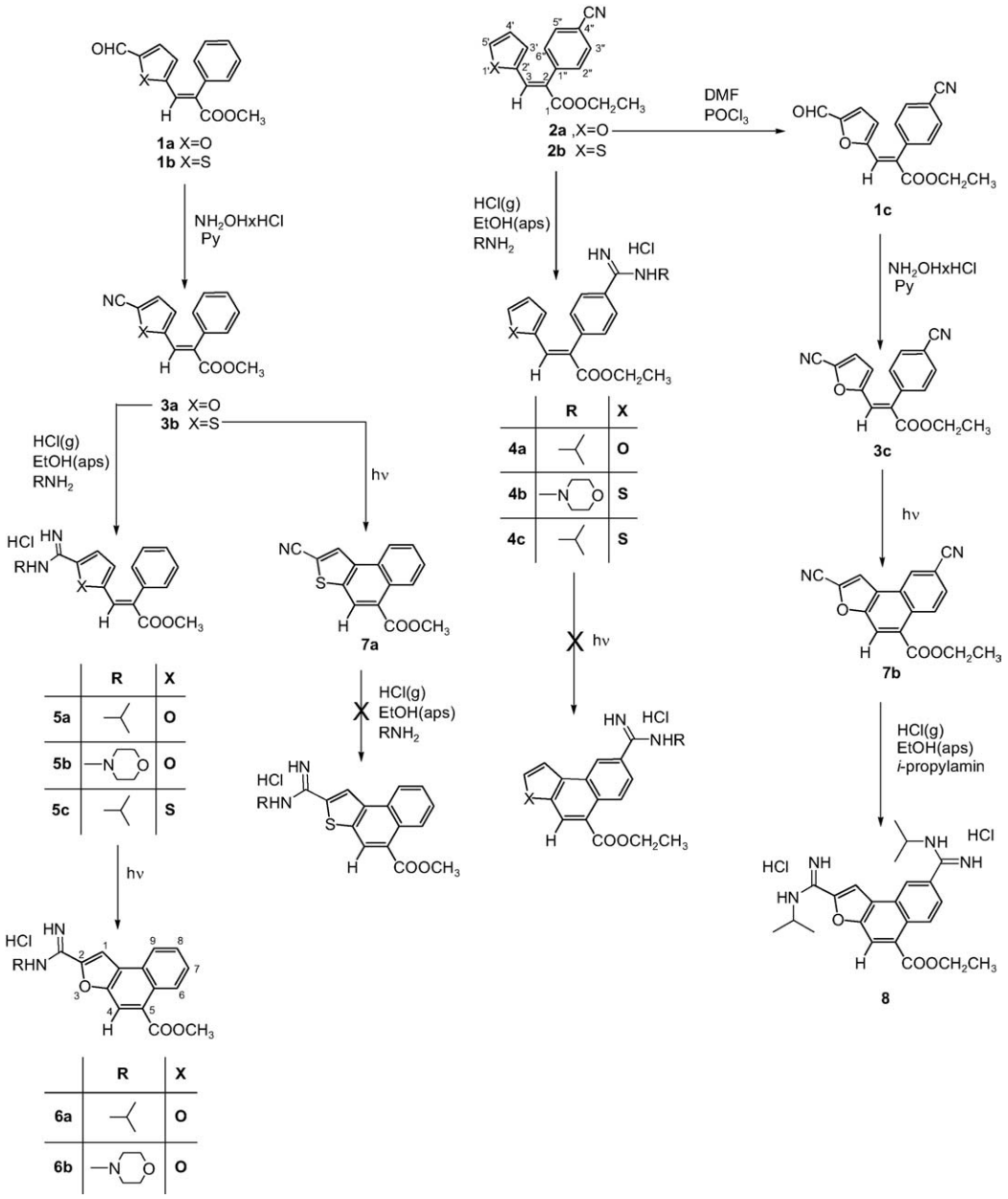


2a R_1=H, R_2=CN, R_3=ethyl X=O
2b R_1=H, R_2=CN, R_3=ethyl X=S
3b R_1=CN, R_2=H, R_3=methyl X=O
3a R_1=CN, R_2=H, R_3=methyl X=S
3c R_1=CN, R_2=CN, R_3=ethyl X=O
4a R_1=H, R_2=*iso*-pr-am, R_3=ethyl, X=O
4b R_1=H, R_2=morph-am, R_3=ethyl, X=S
4c R_1=H, R_2=*iso*-pr-am, R_3=ethyl, X=S
5a R_1=*iso*-pr-am, R_2=H, R_3=methyl, X=O
5b R_1=morph-am, R_2=H, R_3=methyl, X=O
5c R_1=*iso*-pr-am, R_2=H, R_3=methyl, X=S



6a R_1=*iso*-pr-am, R_2=H, R_3=methyl, X=O
6b R_1=morph-am, R_2=H, R_3=methyl, X=O
7a R_1=CN, R_2=H, R_3=methyl, X=S
7b R_1=CN, R_2=CN, R_3=ethyl, X=O
8 R_1=*iso*-pr-am, R_2=*iso*-pr-am, R_3=ethyl, X=O

Fig. 1. Substituted naphtho[2,1-b]furans, naphtho[2,1-b]thiophenes and their acyclic precursors (**2a–b, 3a–c, 4a–c, 5a–c, 6a–b, 7a–b, 8**).



Scheme 1. Synthesis of cyano- and amidino-substituted derivatives of naphtho[2,1-b]furans, naphtho[2, 1-b]thiophenes and their acyclic precursors.

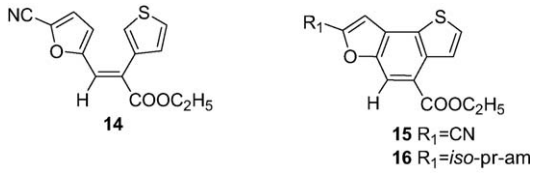
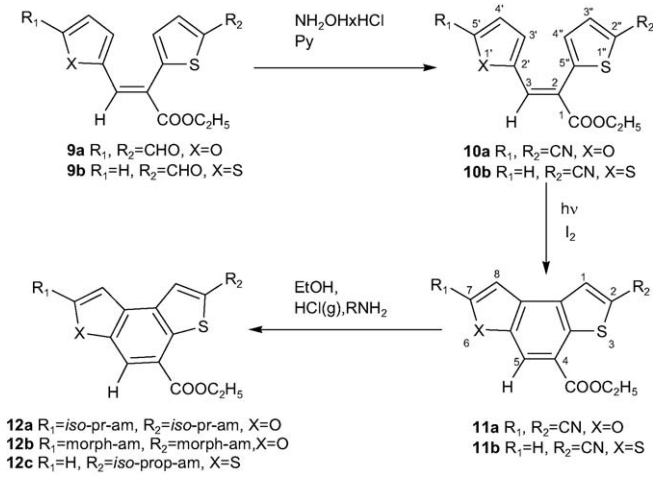


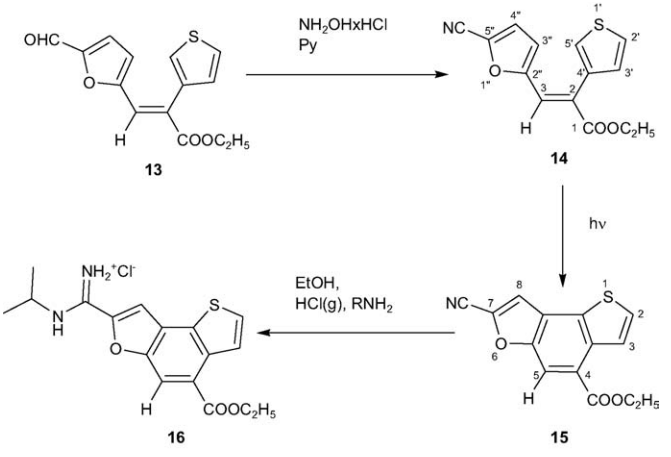
Fig. 3. Substituted thieno[2,3-*e*]benzo[*b*]furans and their acyclic precursors (**14, 15, 16**).

cyanophenylacetic acid and corresponding heterocyclic aldehydes in the yield about 30%, while compounds **3a**, **3b**, **3c**, **10** and **14** were prepared from corresponding aldehydes (**1a**, **1b**, **1c**, **9a, 9b** and **13**) [16–18] in the reaction with hydroxylamonium hydrochloride in the yield from about 50–70%. Novel isopropylamidino-substituted acyclic compounds (**4a**, **4c**, **5a**, **5c**) were prepared in the Pinner reaction [19] from cyano-derivatives **2a**, **2b**, **3a**, **3b** and isopropylamine in the second phase of the reaction, while morpholino-substituted amidines **4b**, **5b** were prepared in the same manner in the reaction with morpholinoamine.

Cyclized amidino-substituted naphthofurans (**6a** and **6b**) were prepared in the photochemical dehydrocyclization reaction in ethanol and water from **5a** and **5b** in the yields about 35%, while **4a**, **4b**, **4c** and **5c** didn't give corresponding photochemical cyclic product. Isopropylamidino-substituted naphthofuran (**8**) was prepared in Pinner reaction from corresponding dicyano-naphthofuran (**7b**) in the yields about 10–20%. Cyano-substituted cyclized derivatives **(7a** ,**7b**),(Fig. 1), as well as **11a, 11b** and **15** (Figs. 2 and 3), were prepared in the photochemical dehydrocyclization reaction from corresponding



Scheme 2. Synthesis of cyano- and amidino-substituted derivatives of thieno [3,2-*e*] benzo[*b*]furans and thieno[2,3-*e*]benzo[*b*]thiophenes and their acyclic precursors.



Scheme 3. Synthesis of cyano- and amidino-substituted derivatives of thieno [2,3-*e*] benzo[*b*]furans and their acyclic precursors.

cyano-substituted acyclic compounds (**3b**, **3c**, **10a, 10b** and **14**) in the toluene solution and in the yields about 40–70%. Finally, isopropyl- and morpholino-substituted amidino-thieno-benzofurans (**12a** and **12b)** and benzo-dithiophene derivative **12c** were prepared from **11a** and **11b** in the Pinner reaction. Compound **16** was prepared in the same reaction from **15.**

It is very important to mention that only amidino-compounds **5a** and **5b** from the furan series bearing the amidino-substituent on the position 5 of the furan ring are successfully photochemically cyclized into the corresponding cyclic amidines **6a** and **6b**. The reaction was introduced in ethanol, as well as, in water, with or without the bubbling of air through the solution. The time of the cyclization (about one hour) and the yields in both cases were the same. All other furan acyclic compounds with the amidino-substituent on the benzene nuclei couldn't be photochemically cyclized at any conditions, while all amidino-substituted acyclic thiophene compounds decomposed in attempt of photochemical reaction. Acyclic neutral cyano-compounds were photochemically cyclized successfully into the cyclic compounds in toluene solution.

2.2. Spectroscopic properties

The UV–vis spectra of all studied compounds didn't show any pH dependent changes in the physiologically relevant range (pH = 3.5–8.0), which is also in agreement with the pK_a values of ~8–9 reported for aromatic amidines [20]. Therefore, we have performed our studies at pH = 7. Stock solutions of positively charged compounds (**5a**, **6a–b**, **12a**, **16**) were prepared in water, while stock solutions of cyano-substituted compounds (**2a–b, 3a–c**, **10a**, **14**) due to their low solubility in water were prepared in DMSO. Before experiment respective aliquots were added to the aqueous buffer, partial volume of DMSO in water not exceeding 0.1%. We were not able to study compounds (**7a–b**, **11a**, **15**) because of their insolubility in water. Stock solutions (both aqueous and DMSO) of all studied compounds were stable over long period.

Absorbancies of aqueous solutions of compounds are proportional to their concentrations up to 6×10^{-5} mol dm^{-3} (**2a–b, 3a–c**, **10a**, **14)**, 8×10^{-5} mol dm^{-3} (**5a**, **6a–b**, **12a**), 2×10^{-4} (**16**) mol dm^{-3}, indicating that there is no significant intermolecular stacking which should give rise to hypochromicity effects. Obtained electronic absorption data are presented in the Section 4.

2.2.1. Interactions of cyano (*2a–b, 3a–c, 10a, 14)* and amidino (*5a–b)* acyclic derivatives and their cyclic analogues (*6a–b 12a, 16)* with double stranded ct-DNA

The UV–vis titrations of buffered aqueous solutions of compounds **6b**, **12a** and **16** ($c = 10^{-5}$ mol dm^{-3}) with *ct*-DNA resulted by significant batochromic ($\Delta\lambda_{max}$ = 1–9 nm) and hypochromic effects (30–50%) in the range of $\lambda > 300$ nm at which *ct*-DNA does not absorb light. The appearance of isosbestic points in all UV–vis titrations at $\lambda > 300$ nm strongly supported the presence of only two spectroscopically active species, namely the free compounds (**6b**, **12a**, **16**) and only one type of the complex with *ct*-DNA (Fig. 4).

Binding constants (K_s) and ratios n calculated according to the Scatchard equation [21] from titrations of **6b**, **12a** and **16** with *ct*-DNA ($\log K_s$ = 4.6–5.3; $n \approx 0.2$) were found to be in good agreement with values previously reported for their close analogue **6a** [4] and also are in accordance with values obtained for a number of heteroaromatic molecules, which bind to ds-DNA by intercalation [2].

Thermal denaturation experiments have shown that addition of cyclic amidino derivatives **6b**, **12a** and **16** weakly stabilized the double helix of *ct*-DNA at all examined ratios $r_{[\text{compound}]/[ct\text{-DNA}]}$ = 0.1–0.3 at highest ΔT_m(**12a**) = 4.0 °C, all values comparable with previously obtained for **6a** [4]. Thermal denaturation transitions were monophasic, pointing toward only one dominant binding mode.

Titrations of buffered aqueous solutions of acyclic derivatives **2a–b**, **3a–c**, **10a** and **14** with *ct*-DNA didn't yield any measurable changes in the UV–vis spectra of studied compounds. In line with that, addition of same series of compounds didn't result in measurable stabilization of *ct*-DNA double helix in the thermal denaturation experiments even at the ratio

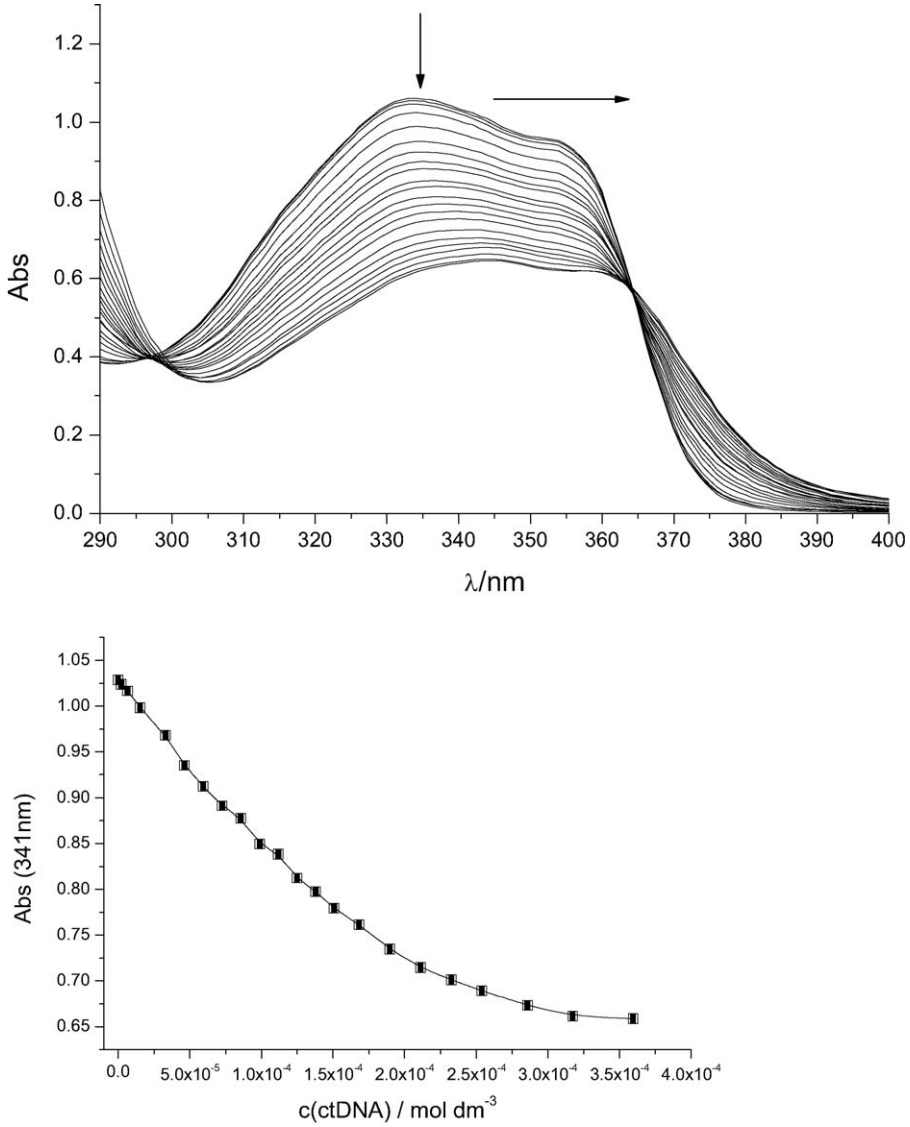


Fig. 4. UV–vis spectral changes **12a** ($c = 8.\ 7 \times 10^{-5}$ mol dm^{-3}); upon the addition of *ct*- DNA at pH = 7, (buffer sodium cacodylate, $I = 0.05$ mol dm^{-3}).

$r_{[\text{compound}]/[ct\text{-DNA}]} = 0.3$ (compounds in excess over the intercalation binding sites). All aforementioned suggested that studied acyclic derivatives do not bind to the *ct*-DNA, what is in accordance with previously presented results for analogous **5a** [4].

The above presented results support main idea presented for **5a**–**6a** system, former (acyclic) compound showing no interactions with ds-DNA, while latter (cyclic) compound forming stable, intercalative complex with *ds*-polynucleotides [4].

2.3. Biological results

Compounds **1c**–**16** were tested for their potential antiproliferative effect on a panel of 6 human cell lines, five of which derived from five cancer types: HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma) and WI 38 (diploid fibroblasts).

All tested compounds showed a certain antiproliferative effect (Tables 1a and 1b). The compounds could be divided, for comparison reasons, into two major subclasses: positively charged molecules (compounds **4, 5, 6, 8, 12** and **16**) and neutral molecules (**1**, **2**, **3**, **7**, **10**, **11**, **14** and **15**). The IC_{50} values showing the cell growth inhibiting activity of positively charged compounds (Table 1a) reveal that among these molecules the "acyclic" ones in general demonstrate significantly lower effect (Fig. 5a) than their cyclic analogues (Fig. 5b). Within the cyclic analogues the activity of mono-substituted isopropylamidino derivatives (**6a**, **16**) show more pronounced inhibitory effect (within a micromolar range) than the morpholinoamindino-substituted one (**6b**), most likely due to the steric hindrances of positive charge by more bulky substituent of the latter. This difference is not observed for their bis-substituted analogues (**8**, **12a, b**).

On the other hand, regarding the series of neutral molecules direct comparison of cyclic and acyclic analogues is not suitable because of rather low water solubility of the former mole-

Table 1a
In vitro growth inhibition of tumor cells and normal human fibroblasts (WI 38) by positively charged molecules and reference compounds cisplatin, doxorubicin and etoposide

Compound	IC_{50}[a] (μM)					
	Cell lines					
	Hep-2	HeLa	MiaPaCa-2	SW 620	MCF-7	WI 38
4a	≥ 100	≥ 100	> 100	> 100	69 ± 22	83 ± 46
4b	71 ± 37	≥ 100	89 ± 0.5	> 100	78 ± 29	53 ± 27
4c	43 ± 26	52 ± 10	70 ± 8	76 ± 26	73 ± 35	37 ± 35
5a	43 ± 3	78 ± 21	53 ± 0.9	60 ± 27	43 ± 10	50 ± 7
5b	61 ± 32	85 ± 18	≥ 100	> 100	75 ± 26	> 100
5c	53 ± 6	> 100	79 ± 26	> 100	≥ 100	56 ± 39
6a	4.6 ± 0.7	5 ± 0.008	6 ± 0.3	5 ± 0.7	4 ± 0.2	7 ± 2
6b	39 ± 15	31 ± 14	41 ± 5	16 ± 22	44 ± 17	41 ± 9
8	54 ± 11	33 ± 9	20 ± 7	36 ± 0.7	31 ± 9	38 ± 2
12a	6 ± 3	3 ± 1.5	4.3 ± 0.3	4 ± 2	7 ± 1	5 ± 3
12b	7 ± 1	8 ± 3	11 ± 3	6 ± 3	19 ± 17	20 ± 14
16	19 ± 11	11 ± 0.1	29 ± 1	22 ± 16	39 ± 10	40 ± 1
Cis[b]	2 ± 0.3	3 ± 0.6	5 ± 2	4 ± 2	12 ± 6	19 ± 20
Dox[b]	0.04 ± 0.01	0.04 ± 0.01	0.02 ± 0.01	0.02 ± 0.02	0.04 ± 0.01	0.1 ± 0.01
Eto[b]	N.T.[c]	3 ± 1	15.4 ± 14	20 ± 3.4	50 ± 30	N.T.

[a] IC_{50}: the concentration that causes a 50% reduction of the cell growth.
[b] Cis: cisplatin; Dox: doxorubicin; Eto: etoposide.
[c] N.T.: not tested.

Table 1b
In vitro growth inhibition of tumor cells and normal human fibroblasts (WI 38) by neutral molecules

Compound	IC_{50}[a] (μM)					
	Cell lines					
	Hep-2	HeLa	MiaPaCa-2	SW 620	MCF-7	WI 38
1c	62 ± 16	44 ± 4	35 ± 7	7 ± 2	33 ± 5	43 ± 2
2a	96 ± 60	73 ± 36	≥ 100	>100	31 ± 44	50 ± 20
2b	91 ± 21	68 ± 12	33 ± 47	68 ± 8	67 ± 7	46 ± 6
3a	64 ± 30	60 ± 5	62 ± 4	66 ± 12	≥ 100	>100
3b	71 ± 19	87 ± 10	79 ± 8	>100	83 ± 2	>100
3c	78 ± 21	62 ± 18	46 ± 12	48 ± 14	57 ± 25	32 ± 16
7a[b]	2 ± 1	0.6 ± 0.1	18 ± 12	≥ 100	≥ 100	20 ± 6
10a	4 ± 3	6 ± 1	6 ± 2	6 ± 2	23±5	43 ± 23
10b	74 ± 8	54 ± 24	74 ± 10	78 ± 23	86 ± 12	≥ 100
11a[b]	≥ 100	> 100	> 100	> 100	≥ 100	≥ 100
11b[b]	97 ± 19	17 ± 12	13 ± 10	4 ± 0.5	> 100	6 ± 4
14	81 ± 1	39 ± 1	43 ± 6	52 ± 14	86 ± 17	> 100
15[b]	8 ± 1	6 ± 0.3	57 ± 5	71 ± 17	≥ 100	92 ± 17

[a] IC_{50}: the concentration that causes a 50% reduction of the cell growth.
[b] Because of low water solubility, the suspensions were used.

cules, which is possibly the reason of a relatively low biological activity (**7a–b**, **11a–b** and **15**). Nevertheless, rather pronounced and selective activity of cyano-substituted **7a** and **15** was observed, especially on HeLa and Hep-2 cell lines (Fig. 5c, d), regardless of low water solubility, which actually point to even lower inhibitory concentrations sufficient for the growth inhibition of these two cell lines and their extreme cell-type selectivity. Moreover, similar selective effect towards HeLa and Hep-2 cells was reported by Jarak et al. [22] using carboxanilides bearing cyano substituent either on anilide or benzothiophene part of the molecule, as well as "acyclic" cyano derivatives of thiophene-2-carboxamides [23]. Unfortunately, the reason for this interesting selectivity on HeLa and Hep-2 cell lines are not completely clear and further studies aimed to provide the indication of what genetic and/or structural background is responsible for this motivating correlation are underway. What is known so far is that these two cell lines have similar genetic backgrounds, both having human papilloma virus (HPV) type 18 DNA integrated into their genomes. Since it is known that the high-risk HPV E6 and E7 genes are major viral oncogenes, responsible for the initiation of tumorigenesis (e.g. cervical carcinoma) [24], it could be possible that these compounds specifically inhibit the E6/E7 expression. Namely, repression of E6 and E7 expression results in acquisition of the senescent phenotype and in the rescue of functional p53 and $p105^{Rb}$ pathways; therefore, therapies directed against either viral protein may be beneficial [25].

Interestingly, the acyclic cyano-substituted neutral molecules (**10a, b**) (Fig. 5e, f), show pronounced and selective activity; **10a** being an order of magnitude more active than **10b**, most probably due to two cyano-substituents. However, both molecules do not interact with DNA (Section 2.2.1.), and the target(s) of cyano-substituted heteroaromatic systems are not apparent, neither is the mechanism of antiproliferative action.

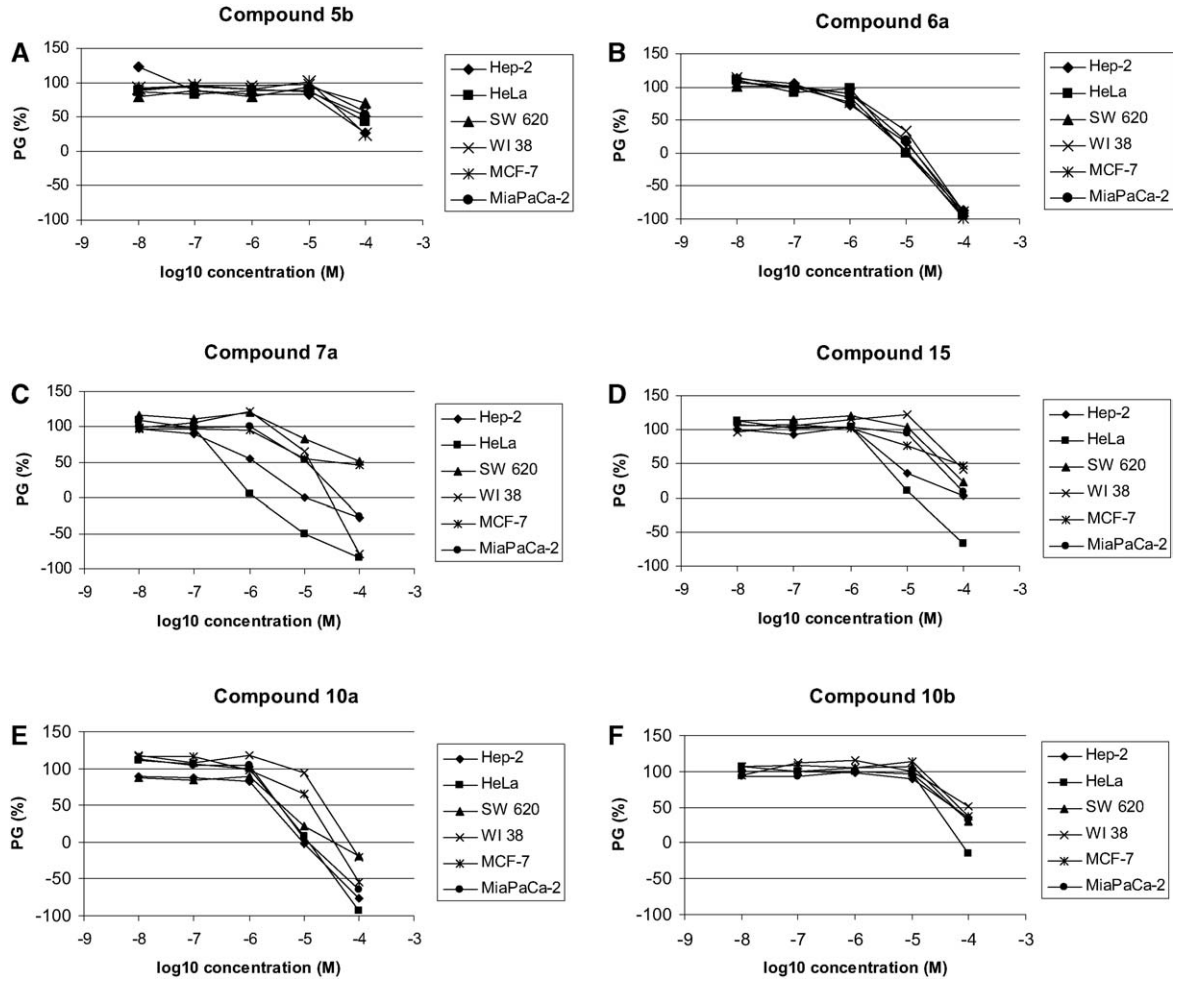


Fig. 5. Dose–response profiles for the representative acyclic **(5b, A; 10a, E; 10b, F)** and cyclic compounds **(6a, B; 7a, C; 15, D)** tested on various human cell lines in vitro. The cells were treated with the compounds at different concentrations, and percentage of growth (PG) was calculated. Each point represents a mean value of four parallel samples in three individual experiments.

Nevertheless, comparable interesting in vitro activity of cyano-substituted heteroaromatic system was reported by Bénéteau et al. [26], indicating together with our observations that cyano-substituted heteroaromatic systems and their correlation with the potential biomolecular targets are of highest interest for more detailed systematic investigations.

3. Conclusions

The main aim of the presented study was to convert acyclic derivatives into their cyclic analogues by photodehydrocyclization in aqueous, biologically relevant conditions, which could serve as DNA intercalators and efficient possible antitumor substances. Among all studied compounds only amidino-furyl-substituted phenyl acrylates were efficiently converted to the corresponding naphthofuranes. The replacement of furan by thiophene moiety did not enable the photochemical reaction, pointing to the high sensitivity of photodehydrocyclization on the electronic properties of starting material. Also, changing the position of the amidino substituent from furyl to phenyl moiety inhibited aforementioned photochemical reaction. Although the study of furo-thiophene series was initiated with the same idea, the preparation of amidino-substituted acyclic-precursors was possible, but the pure product could not be obtained, thus not allowing the study of the photoinduced dehydrocyclization in water.

In conclusion, here presented synthetic part of the research clearly leads to the amidine substituted furyl-phenyl acrylate system as only efficient in photoinduced dehydrocyclization in water. Studies of new systems capable of undergoing photoinduced dehydrocyclizations in water and by visible light irradiation, for in vivo application are necessary.

Apparently the significantly stronger antiproliferative activity of cyclic, positively charged analogues (IC_{50} concentrations are within a micromolar range) is to be correlated to the property of these molecules to intercalate into DNA. Although the cyclic neutral molecules could not be adequately tested on DNA binding due to their low solubility in water, cyano-substituted **7a** and **15** compounds show intriguing strong and selective antiproliferative activity on tumor cell lines. On the

other hand, the acyclic, either positively charged or neutral molecules, except **10a,** did not show any interaction with DNA, correlating with the inferior biological activity.

4. Experimental protocols

4.1. Chemistry

Melting points were determined on a Kofler hot stage microscope and are uncorrected. IR spectra were recorded on a Nicolet Magna 760 spectrophotometer in KBr discs. ^{1}H and ^{13}C NMR spectra were recorded on either a Varian Gemini 300 or a Bruker Avance DPX 300 i 500 spectrometer using TMS as an internal standard in DMSO-d_6. Elemental analysis for carbon, hydrogen and nitrogen were performed on a Perkin-Elmer 2400 elemental analyzer. Where analyses are indicated only as symbols of elements, analytical results obtained are within 0.4% of the theoretical value. Irradiation was performed at room temperature with a water cooled immersion well fitted with a 400 W high pressure mercury arc lamp using Pyrex filter. All compounds were routinely checked by TLC with Merck silica gel 60F-254 glass plates.

4.1.1. Methyl-E-3-(5-formyl-2-furyl)-2-phenylacrylate (*1a*)

Corresponding methyl-*E*-3-(2-furyl)-2-phenylacrylate was formylated by Vilsmeier formylation. Phosphorus oxychloride (22.8 ml, 250 mmol) was added drop-wise with cooling to DMF (20 ml, 250 mmol). The reaction mixture was stirred for 0.5 h by cooling on ice, the apparatus being protected with a calcium chloride tube. A solution of methyl-*E*-3-(2-furyl)-2-phenylacrylate (13g, 57 mmol) in DMF (10 ml) was added drop-wise to the mixture. After the addition was completed, the mixture was stirred for 1 h at room temperature, then heated at 90 °C for 3 h, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left over-night on ice. The solid was filtered off, washed with water and recrystallized from methanol. Orange crystals 12.2 g (83.5%) are obtained, m.p. 102–104 °C (Ref. [18a], m.p. 103–105 °C). IR (KBr) (υ_{max}/cm^{-1}):1710, 1665, 1630; ^{1}H NMR (300 MHz, DMSO) δ: 9.57 (s, 1H, CHO), 7.65 (s, 1H, H_3), 7.50–7.17 (m, 5H), 7.00 (d, $J_{4',3'}$ = 3.5 Hz, 1H), 5.76 (d, $J_{3',4'}$ = 3.5 Hz, 1H), 3.78 (s, OCH_3, 3H).

4.1.2. Methyl-E-3-(5-formyl-2-thienyl)-2-phenylacrylate (*1b*)

Compound **1b** was prepared using the method described for preparation of compound **1a,** from phosphorus oxychloride (36.8 ml, 0.21 mol), DMF (42 ml, 0.21 mol) and methyl-*E*-3-(2-thienyl)-2-phenylacrylate (21 g, 86 mmol) in DMF (10 ml). Orange crystals 20.3 g (87%) are obtained, mp 121–122 °C (Ref. [18b], m.p. 120–121 °C). IR (KBr) (υ_{max}/cm^{-1}): 1700, 1660, 1610; ^{1}H NMR (300 MHz, DMSO) δ: 9.79 (s, CHO, 1H), 8.09 (s, H_3, 1H), 7.88 (d, $J_{4',3'}$ = 4.1 Hz, 1H), 7.64–7.47 (m, 3H), 7.49 (d, $J_{3',4'}$ = 4.1 Hz, 1H), 7.29–7.19 (m, 2H) 3.75 (s, OCH_3, 3H).

4.1.3. Ethyl-E-3-(5-formyl-2-furyl)-2-(4-cyano)-phenylacrylate (*1c*)

Compound **1c** was prepared using the method described for preparation of compound **1a,** from phosphorus oxychloride (1.38 ml, 8 mmol), DMF (1.2 ml, 6 mmol) and methyl-*E*-3-(2-furyl)-2-(4-cyano)-phenylacrylate (3.45 mmol) in DMF (10 ml). Orange crystals 0.168 g (53%) are obtained, m.p. 111–113 °C; IR (KBr) (υ_{max}/cm^{-1}): 2230, 1716, 1687, 1606; ^{1}H NMR (500 MHz, DMSO) δ: 9.46 (s, 1H) 7.9 (d, $J_{3'',2''}$ = $J_{5'',6''}$ = 7.5 Hz, 2H), 7.70 (s, H_3, 1H), 7.50 (d, $J_{2'',3}$ = $J_{6'',5''}$ = 7.5 Hz, 2H), 7.42 (d, $J_{4',3'}$ = 3.7 Hz, 1H), 6.46 (d, $J_{3',4'}$ = 3.7 Hz, 1H) 4.21 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H), 1.22 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H); ^{13}C NMR (125 MHz; DMSO) δ:179.3, 166.0, 154.4, 153.3, 140.8, 133.2, 133.2 (2C), 131.1 (2C), 127.3, 124.1, 119.6, 118.8, 62.2, 14.8; elemental analysis calcd (%) for $C_{16}H_{11}O_4N$: C 69.15; H 4.41; N 4.75; found C 68.9; H; 4.52; N 4.91.

4.1.4. Ethyl-E-3-(2-furyl)-2-(4-cyano)-phenylacrylate (*2a*)

Ethyl ester of *p*-cyano phenylacetic acid (5.00 g, 26 mmol) and furfural (2.998 g, 31.2 mmol) in a mixture of triethylamine (4.2 ml) and acetic acid anhydride (4.2 ml) were refluxed for 3 hours. After the reaction was completed, the mixture was cooled, acidified with hydrochloric acid and extracted with ether. The organic layer was washed with water and dried over anhydrous $MgSO_4$ and evaporated in vacuum. The dark residue was recrystallized from petrol ether and gave yellow crystals (3.170 g, 45.6%), m.p. 125–127 °C. IR (KBr) (υ_{max}/cm^{-1}): 2229, 1693, 1621, 1604; ^{1}H NMR (300 MHz DMSO) δ: 7.88 (dd, $J_{3'',2''}$ = $J_{5'',6''}$ = 8.1 Hz, $J_{3'',5''}$ = 1.5 Hz, 2H), 7.68 (d, $J_{5',4'}$ = 1.8 Hz, 1H), 7.66 (s, 1H$_3$), 7.46 (dd, $J_{2'',3''}$ = $J_{6'',5''}$ = 8.1 Hz, $J_{2'',6''}$ = 1.5 Hz, 2H), 6.52 (dd, $J_{4',3'}$ = 3.3 Hz; $J_{4',5'}$ = 1.8 Hz, 1H), 6.44 (d, $J_{3',4'}$ = 3.3 Hz, 1H), 4.17 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H), 1.24 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.77, 149.54, 146.26, 141.01, 132.01, 130.72, 127.30, 126.63, 118.82, 117.367, 112.63, 110.55, 60.95, 14.05; elemental analysis calcd (%) for $C_{16}H_{13}NO_3$: C 71.91; H 4.87; N 4.71; found C 71.99; H 4.90; N 5.13.

4.1.5. Ethyl-E-3-(2-thienyl)-2-(4-cyano)-phenylacrylate (*2b*)

The compound **2b** was prepared in a manner similar to the preparation of **2a,** from ethyl ester of *p*-cyano phenylacetic acid (5.00 g, 26 mmol) and 2-thiophene aldehyde (2.998 g, 31.2 mmol) in a mixture of triethylamine (4.2 ml) and acetic acid anhydride (4.2 ml). Yellow crystals (3.170 g, 15%) were obtained; m.p. 134–135 °C; IR (KBr) (υ_{max}/cm^{-1}): 2223, 1703, 1621, 1602; ^{1}H NMR (300 MHz DMSO) δ: 8.07 (s, 1H$_3$), 7.93 (dd, $J_{3'',2''}$ = $J_{5'',6''}$ = 8.5 Hz, $J_{3'',5''}$ = 1.4 Hz, 2H), 7.59 (d, $J_{3',4'}$ = 5.1 Hz, 1H), 7.50–7.46 (m, 2H$_{Ph}$, 1H$_{thioph.}$), 7.06 (dd, $J_{4',3'}$ = 3.6 Hz, $J_{4',5'}$ = 5.1 Hz, 1H), 4.18 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H) 1.20 (t, J_1 = 7.1 Hz, COOCH$_2$$CH_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.7, 140.5, 137.02, 135.4, 133.9, 132.8 (2C), 132.3, 131.2 (2C), 127.3, 127.1, 118.7, 111.2, 60.8, 14.0; elemental analysis calcd (%) for $C_{16}H_{13}O_2SN$: C 67.84; H 4.59; N 4.95; found C 67.80; 4.61; N 5.02.

*4.1.6. Methyl-E-3-(5-cyano-2-furyl)-2-phenylacrylate (**3a**)*

Compound **1a** (2.00 g, 8.33 mmol) and hydroxyl ammonium hydrochloride (0.995g, 14.3 mmol) were heated in pyridine (6 ml) at 60 °C for 30 min, then acetic anhydride (4 ml) was successively added at a temperature not exceeding 95 °C, which was being kept for additional 2 h. The mixture was cooled to 20 °C, poured into water (37.5 ml) and the separated nitrile was stirred for 1 h, filtered, washed with water, dried, and crystallized from petrol ether gave **3a** (1.745 g, 82.8% yield); m.p. 85–86 °C; IR (KBr) (υ_{max}/cm^{-1}): 3120, 3100, 2200, 1700, 1610, 1475, 1425, 1336, 1238, 1185, 1160, 1060, 1015; UV–vis (EtOH): λ_{max} (lgε) = 307 (32179); ^{1}H NMR (300 MHz DMSO) δ: 7.61 (s, 1H$_3$), 7.49 (d, $J_{4',3'}$ = 3.9 Hz, 1H), 7.46–7.45 (m, 3H), 7.25 (dd, $J_{2'',3''} = J_{6'',5''}$ = 8.7 Hz, $J_{2'',6''}$ = 1.3 Hz, 2H), 6.16 (d, $J_{3',4'}$ = 3.9 Hz, 1H.), 3.73 (s, COO*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 52.5, 111.3, 115.5, 124.8, 125.1, 125.2, 128.3, 128.4, 128.8, 134.4, 134.6, 154.5, 166.0; elemental analysis calcd (%) for $C_{15}H_{11}O_3N$: C 71.14; H 4.34; N 5.53; found C 71.45; H 4.20; N 5.23.

*4.1.7. Methyl-E-3-(5-cyano-2-thienyl)-2-phenylacrylate (**3b**)*

Compound **3b** was prepared using the method described for preparation of compound **3a,** from **1b** (2.00 g, 7.33 mmol) and hydroxylammonium hydrochloride (0.0.878g, 14.3 mmol). Yellow-orange crystals of **3b** 0.998 g (50.6%) were obtained. m.p. 120–121 °C; IR (KBr) (υ_{max}/cm^{-1}): 2217, 1712, 1616, 1599; ^{1}H NMR (300 MHz DMSO) δ: 8.12 (s, 1H), 7.89 (d, $J_{4',3'}$ = 3.9, 1H), 7.64 (d, $J_{3',4'}$ = 3.9, 1H), 7.59 (m, 3H), 7.26 (m, 2H) 3.72 (s, COO*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 166.8, 144.9, 138.4, 134.9, 134.3, 133.7, 132.3, 130.2 (2C), 129.9, 129.7 (2C), 114.4, 111.7, 52.9; elemental analysis calcd (%) for $C_{15}H_{11}O_2SN$: C 66.91; H 4.09; N 5.20; found C 66.84; H 4.21; N 5.02.

4.1.8. Ethyl-E-3-(5-cyano-2-furyl)-2-(4-cyano)-phenylacrylate (3c)

Compound **3c** was prepared using the method described for preparation of compound **3a,** from **1c** (0.317 g, 1.07 mmol) and hydroxylammonium hydrochloride (0.09 g, 1.3 mmol). Yellow crystals of **3c** 0.225 g (72%) were obtained; mp 128–129 °C; ^{1}H NMR (300 MHz DMSO) δ: 7.93 (d, $J_{3'',2''} = J_{5'',6''}$ = 8.4 Hz, 2H), 7.69 (s, H$_3$ 1H), 7.55 (d, $J_{4',3'}$ = 3.8 Hz, 1H), 7.53 (d, $J_{2'',3''} = J_{6'',5''}$ = 8.4 Hz, 2H) 6.59 (d, $J_{3',4'}$ = 3.8 Hz, 1H), 4.21 (q, J = 7.1 Hz, COO*CH*$_2$CH$_3$, 2H), 1.21 (t, J = 7.1 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.26, 153.99, 140.04, 132.22, 132.12, 130.50, 125.83, 125.75, 125.02, 118.79, 117.24, 111.31, 111.15, 61.56, 14.08; elemental analysis calcd (%) for $C_{17}H_{12}O_3N_2$: C 69.86; H 4.11; N 9.59; found C 69.90; H; 4.15; N 9.56.

4.1.9. Ethyl-E-3-(2-furyl)-2-(4-(N-isopropylamidino)-phenylacrylate hydrochloride (4a)

A stirred suspension of **2a** (0.578 g, 2.16 mmol) in anhydrous EtOH (5 ml) was cooled in an ice-salt bath and was saturated with HCl gas. The flask was then tightly stoppered and the mixture was maintained at room temperature for 2 days, until nitrile band disappeared (monitored by IR analysis at 2200 cm^{-1}). The reaction mixture was purged with N_2 gas and diluted with ether (50 ml). The crude imidate was filtered off and was immediately suspended in anhydrous ethanol (5 ml). The isopropylamine (0.8 ml, 9.85 mmol) was added and the mixture was stirred for one day at room temperature. The reaction mixture was diluted with ether (50 ml) to give a precipitate. The precipitate was collected and recrystallized from ethanol ether to give pale yellow powder 0.299 g (42.8%). m.p. 225.8–226.5 °C; IR (KBr) (υ_{max}/cm^{-1}): 3201, 3042, 2980, 1703, 1676, 1620; ^{1}H NMR (300 MHz DMSO) δ : 9.42 (bs, 3H), 7.78 (d, $J_{3'',2''} = J_{5'',6''}$ = 8.3 Hz, 2H), 7.69 (s, 2H), 7.48 (d, $J_{2''3''} = J_{6'',5''}$ = 8.3 Hz, 2H) 6.53 (dd, $J_{4',3'}$ = 3.4 Hz, $J_{4',5'}$ = 1.6 Hz, 1H.), 6.31 (d, $J_{3',4'}$ = 3.4 Hz, 1H), 4.19 (q, J = 7.1 Hz, COO*CH*$_2$CH$_3$, 2H), 4.08–4.03 (m, 1H, *CH*(CH$_3$)$_2$, 1H) 1.29 (d, J = 6.3 Hz, –CH(*CH*$_3$)$_2$, 6H), 1.20 (t, J = 7.1 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.26, 153.99, 140.04, 132.22, 132.12, 130.50, 125.83, 125.75, 125.02, 118.79, 117.24, 111.31, 111.15, 61.56, 14.08; elemental analysis calcd (%) for $C_{19}H_{23}O_3N_2Cl$: C 62.83; H 6.33; N 7.71; found C 62.61; H; 6.58; N 7.96.

*4.1.10. Ethyl-E-3-(2-thienyl)-2-(4-morpholinamidino)-phenylacrylate hydrochloride (**4b**)*

Compound **4b** was prepared using the general method described for the preparation of **4a**, from compound **2a** (0.49 g, 1.84 mmol) in anhydrous EtOH (4 ml) and aminomorpholine (0.92 ml, 9.12 mmol). Precipitate was collected and recrystallized from ethanol-ether to give yellow powder (0.290 g, 38.8%). m.p. 129–130 °C; IR (KBr) (υ_{max}/cm^{-1}): 3378, 3092, 2979, 2927, 2860, 1703, 1654, 1603; ^{1}H NMR (300 MHz DMSO) δ: 11.32 (s, 1H), 9.89 (s, 1H), 9.23 (s, 1H), 8.12 (s, 1H$_3$), 7.93 (d, $J_{3'',2''} = J_{5'',6''}$ = 8.16 Hz, 2H), 7.63 (d, $J_{5',4'}$ = 5.0 Hz, 1H.), 7.52–7.50 (m, 3H), 7.07 (d, $J_{4',5'}$ = 5.0 Hz, 1H), 4.17 (q, J = 7.1 Hz, COO*CH*$_2$CH$_3$, 2H), 3.79 (bs, 4H), 2.97 (bs, 4H), 1.19 (t, J = 7.1 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 166.38, 162.08, 141.45, 137.69, 135.82, 134.33, 132.56, 131.14, 129.59, 128.0, 127.79, 126.62, 66.01, 61.32, 54.33, 14.67; elemental analysis calcd (%) for $C_{20}H_{24}O_3N_3SCl$: C 56.94; H 5.69; N 9.96; found C 56.31; H 5.45; N 10.05.

*4.1.11. Ethyl-E-3-(2-thienyl)-2-(4-(N-isopropyl)amidino)-phenylacrylate hydrochloride (**4c**)*

Compound **4c** was prepared using the general method described for the preparation of **4a**, from **2b** (1.0 g, 3.53 mmol) in anhydrous EtOH (10 ml) and isopropylamine (50 ml). The precipitate was collected and recrystallized from ethanol ether to give white powder (0.250 g 18.6%); IR (KBr) (υ_{max}/cm^{-1}): 3203, 3031, 2973, 1698, 1679, 1614; ^{1}H NMR (300 MHz DMSO) δ: 9.33 (bs, 3H), 8.11 (s, 1H$_3$), 7.85 (d, $J_{3'',2''} = J_{5'',6''}$ = 8.0 Hz, 2H), 7.63 (d, $J_{5',4'}$ = 4.9 Hz, 1H), 7.51–7.48 (m, 3H) 7.07 (dd, $J_{4,,5,}$ = 4.9 Hz, $J_{4,,3'}$ = 3.9 Hz, 1H), 4.21–4.10 (m, COO*CH*$_2$CH$_3$, *CH*(CH)$_3$, 3H), 1.30 (d, J = 6.3 Hz, –CH(*CH*$_3$)$_2$, 6H), 1.20 (t, 3H, J_1 = 7.0 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 166.4, 161.9, 154.42, 140.8, 137.7, 135.8, 134.3, 132.6, 130.9 (2C), 129.5 (2C), 128.0, 127.8, 61.3, 45.6, 45.6, 21.7, 14.7; elemental analysis calcd

(%) for $C_{18}H_{23}O_2N_2SClx1/2H_2O$: C 58.85; H 6.19; N 7.20; found C 58.55; H 6.33; N 7.44.

*4.1.12. Methyl-E-3-(5-(N-isopropylamidino)-2-furyl)-2-phenylacrylate hydrochloride (**5a**)*

Compound **5a** was prepared using the general method described for the preparation of **4a**, from **3a** (0.499 g, 1.97 mmol) and the isopropylamine (1 ml, 9.85mmol). The precipitate was collected and recrystallized from ethanol ether to give white powder 0.294 g (42.8%). m.p. 189–191 °C; IR (KBr) (υ_{max}/cm^{-1}): 3150, 3000, 1700, 1650, 1600, 1420, 1330, 1245, 1155, 1115; ^{1}H NMR (300 MHz, DMSO) δ: 9.46 (brs, 2H), 9.29 (brs, 1H), 7.68 (s, 1H$_3$), 7.67 (d, $J_{4,3'}$ = 3.9 Hz, 1H), 7.50–7.48 (m, 3H), 7.28 (dd, $J_{2'',3''} = J_{6'',5''}$ = 7.5; $J_{2'',6''}$ = 2.1 Hz, 2H), 5.77 (d, $J_{3',4'}$ = 3.3 Hz, 1H), 4.20 (m, *CH*(CH$_3$)$_2$, 1H), 3.73 (s, COO*CH$_3$*, 3H) 1.23 (d, *J* = 6.3 Hz, CH(*CH$_3$*)$_2$, 6H); elemental analysis calcd (%) for $C_{18}H_{21}O_3N_2Clx7H_2O$: C, 45.52; H, 7.37; N, 5.90 found C, 45.49; H 7.63; N 5.51.

*4.1.13. Methyl-E-3-(5-morpholinamidino-2-furyl)-2-phenylacrylate hydrochloride (**5b**)*

Compound **5b** was prepared using the general method described for the preparation of **4a,** from **3a** (0.467 g, 1.8 mmol) and the 4-aminomorpholin (0.9 ml, 9.2 mmol). The precipitate was collected and recrystallized from ethanol-ether to give light yellow powder (0.38 g, 63%); m.p. 194–195 °C; IR (KBr) (υ_{max}/cm^{-1}): 3120, 2990, 1710, 1680, 1600; ^{1}H NMR (300 MHz DMSO) δ: 11.23 (s, 1H), 9.70 (s, 1H), 9.14 (s, 1H), 7.68 (s, 1H$_3$), 7.61 (d, $J_{4',3'}$ = 3.9 Hz, 1H), 7.52–7.49 (m, 3H), 7.30–7.27 (m, 2H), 5.73 (d, $J_{3',4'}$ = 3.9 Hz, 1H), 3.74 (s, 3H, COO*CH$_3$*), 3.62 (s, 4H), 2.88 (s, 4H); ^{13}C NMR (300 MHz DMSO) δ: 166.08, 153.93, 150.29, 139.81, 134.55, 129.01, 128.75, 125.96, 120.40, 114.90, 65.50, 53.52, 52.64; elemental analysis calcd (%) for $C_{19}H_{22}O_4N_4Cl$: C 69.63, H 6.72, N 12.83 found C 69.45; H 6.89; N 13.01.

*4.1.14. Methyl-E-3-(5-(N-isopropylamidino)-2-thienyl)-2-phenylacrylate hydrochloride (**5c**)*

Compound **5c** was prepared using the general method described for the preparation of **4a,** from **3b** (0.968 g, 3.6 mmol) and the isopropylamine (1.5 ml, 18 mmol) The precipitate was collected and recrystallized from ethanol-ether to give white powder (0.396 g, 30.2%); m.p. 179–180 °C; .IR (KBr) (υ_{max}/cm^{-1}): 3201, 3042, 2980, 1703, 1676, 1620; ^{1}H NMR (300 MHz DMSO) δ: 9.63 (bs, 1H), 9.61 (bs, 1H), 9.16 (bs, 1H), 8.07 (s, H$_3$, 1H), 7.73 (d, $J_{4',3'}$ = 3.9 Hz, 1H), 7.55–7.54 (m, 3H), 7.27–7.25 (m, 3H), 4.02–4.0 (m, *CH*(CH$_3$)$_2$, 1H), 3.94 (s, COO*CH$_3$*, 3H), 1.18 (d, *J* = 6.4 Hz, –CH(*CH$_3$*)$_2$, 6H); ^{13}C NMR (300 MHz DMSO) δ: elemental analysis calcd (%) for $C_{18}H_{21}O_2N_2SCl$: C 59.25, H 5.80, N 7.68; found C 59.01, H 5.96, N 7.55.

*4.1.15. Methyl-2-(N-isopropylamidino)-naphtho[2,1-b]furan-5-carboxylate hydrochloride (**6a**)*

4.1.15.1. Method A. To ethanolic solution of **5a** (0.10 g, 0.287 mmol in 250 ml) I_2 was added and the air was bubbled through. Prepared solution was then irradiated with high pressure mercury arc lamp during 2 h. The solvent was evaporated and recrystallization from ethanol-ether gave **6a** (0.024 g, 24.1%).

4.1.15.2. Method B. To aqueous solution of **5a** (0.134 g, 0.384 mmol in 250 ml) I_2 was added and the air was bubbled through. Prepared solution was then irradiated with high pressure mercury arc lamp during 2 h. The water was evaporated and recrystallization from ethanol-ether gave **6a** (0.040 g, 30.05%). m.p. 221–223 °C; IR (KBr) (υ_{max}/cm^{-1}) 3010, 2930, 1705, 1650, 1610, 1435, 1420, 1325, 1260, 1190, 1115, 1080, 1010; ^{1}HNMR (300 MHz DMSO) δ: 10.1 (bs, 1H), 9.90 (bs, 1H), 9.41 (bs, 1H), 8.80 (d, $J_{6,7}$ = 8.6 Hz, 1H), 8.79 (s, H-4, 1H), 8.41–8.39 (m, H-9, H-1, 2H), 7.88 (dd, $J_{7,8}$ = 8.4 Hz, $J_{7,9}$ = 1.3 Hz, 1H), 7.79 (dd, $J_{8,7}$ = 8.4 Hz, $J_{8,6}$ = 1.3 Hz, 1H), 4.15 (m, *CH*(CH$_3$)$_2$, 1H), 4.0 (s, COO*CH$_3$*, 3H), 1.34 (d, *J* = 6.4 Hz, CH(*CH$_3$*)$_2$, 6H); ^{13}C (300 MHz DMSO) δ: 21.1 (2C), 45.1, 52.8, 112.5, 115.3 , 123.9, 125.9, 126.6, 127.3, 127.4, 127.5, 128.3, 128.4, 144.6, 150.7, 151.1, 166.7; elemental analysis calcd (%) for ($C_{18}H_{19}O_3N_2$Ì5H_2O): C, 49.60; H, 6.66; N, 6.43 found C, 49.22; H 7.60; N 6.32.

*4.1.16. Methyl-(2-morpholinamidino)-naphtho[2,1-b]furan-5-carboxylate hydrochloride (**6b**)*

Compound **6b** was prepared from **5b** (0.114 g, 0.35 mmol) which was dissolved in ethanol (250 ml) and irradiated with high pressure mercury arc 400 W lamp during 2 h. I_2 was added into the solution and the air was bubbled through. The solvent was evaporated recrystallization from ethanol-ether gave **6b** 0.05 g (44%); m.p.165–166 °C; IR (KBr) (υ_{max}/cm^{-1}) ^{1}H NMR (300 MHz DMSO) δ: 11.5 (bs, 1H), 10.25 (bs, 1H), 9.43 (bs, 1H), 8.89 (s, H-4, 1H), 8.79 (d, $J_{6,7}$ = 8.4 Hz, 1H), 8.39–8.37 (m, H-9, H-1, 2H), 7.87 (dd, $J_{7,8}$ = 8.3 Hz, $J_{7,9}$ = 1.3 Hz, 1H), 7.78 (dd, $J_{8,7}$ = 8.3 Hz, $J_{8,6}$ = 1.3 Hz, 1H), 4.01 (s, COO*CH$_3$* ,3H), 4.01 (bs, 4H), 2.99 (bs, 4H); ^{13}C (300 MHz DMSO) δ: 168.07, 152.67, 144.23, 130.03, 129.71, 128.79, 128.68, 127.93, 127.09, 125.32, 116.69, 114.59, 66.81, 55.16, 54.12; elemental analysis calcd (%) for ($C_{19}H_{20}O_4N_3Cl$): C 70.05; H 6.15; N 12.90 found C, 70.31; H 6.25; N 13.1.

*4.1.17. Methyl-2-cyano-naphtho[2,1-b]thiophen-5-carboxylate (**7a**)*

Compound **7a** was prepared from **3b** (0.303 g, 1.3 mmol) in 250 ml toluene by irradiation for 3 hours. The air was bubbled through the solution and I_2 was added. After solvent was evaporated the crystals were recrystallized from petrol ether. Light yellow crystals of **7a** (0.046 g, 15.3%) were obtained, m.p. 179–180 °C; IR (KBr) (υ_{max}/cm^{-1}) 2216, 1728, 1509; ^{1}H NMR (300 MHz DMSO) δ: 9.26 (s, 1H), 8.84 (s, 1H), 8.78 (d, $J_{6,7}$ = 7.8 Hz, 1H), 8.67 (d, $J_{9,8}$ = 7.8 Hz, 1H) 7.83–7.76 (m, H-7, H-8, 2H), 3.99 (s, COO*CH$_3$,* 3H); ^{13}C NMR (300 MHz DMSO) δ: 167.34, 138.86, 137.64, 135.26, 129.67, 128.51, 128.31, 128.22, 128.11, 126.69, 125.18, 125.07, 114.96, 111.97, 53.13; elemental analysis calcd (%) for ($C_{15}H_9O_2SN$): C 67.42; H 3.37; N 5.24 found C 67.53; H; 3.41 N 5.40.

*4.1.18. Ethyl-2,4-dicyano-naphto[2,1-b]furan-5-carboxylate (**7b**)*

Compound **7b** was prepared using the method described for the preparation of **7a**, from **7b** (0.102 g, 0.35 mmol). White crystals of **7a** 0.029 g (28.6%) were obtained, m.p. 247–250 °C; IR (KBr)/cm^{-1} 2220, 1710, 1650, 1606; ^{1}H NMR (300 MHz DMSO) δ: 9.16 (s, H-4, 1H), 8.95 (d, $J_{6,7}$ = 8.91 Hz, 1H), 8.89 (s, H-1, 1H), 8.67 (s, H-9, 1H), 8.08 (d, $J_{7,6}$ = 8.9 Hz, 1H), 4.35 (m, COOCH_2CH$_3$, 2H), 1.50 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H) elemental analysis calcd (%) for $C_{17}H_8O_3N_2$: C 70.28; H 2.75; N 9.64 found C 70.51; H 3.01; N 9.6.

*4.1.19. Ethyl-2,4-(N-isopropylamidino)-naphtho[2,1-b]furan-5-carboxylate dihydrochloride (**8**)*

Compound **8** was prepared using the general method described for the preparation of **4a,** from **7b** (0.100 g, 0.34 mmol) and the isopropylamine (0.15 ml, 1.6 mmol) The precipitate was collected and recrystallized from ethanol-ether to give white powder (0.03 g, 20.7%); m.p. 190–191 °C; ^{1}H NMR (300 MHz; DMSO) δ: 9.6 (brs, 3H), 9.17 (s, H-4, 1H), 8.97 (d, $J_{6,7}$ = 8.8 Hz, 1H), 8.89 (s, H-1, 1H), 8.68 (s, H-9, 1H), 8.1 (d, $J_{7,6}$ = 8.9 Hz, 1H), 4.45 (m, COOCH_2CH$_3$, 2H), 4.13 (m, CH(CH$_3$)$_2$, 2H), 1.46 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H), 1.35 (d, J = 7.1 Hz, CH(CH_3)$_2$, 12H); ^{13}C NMR (125 MHz; DMSO) δ: 165.6, 152.2, 130.2, 129.3, 128.6, 128.5, 128.1, 127.8, 126.7, 125.1, 118.9, 118.5, 118.4, 111.5, 110.3, 61.8, 45.1, 42.9, 21.1, 20.3, 13.9; elemental analysis calcd (%) for $C_{23}H_{30}O_3N_4Cl_2$: C 57.38; H 6.28; N 11.64; found C 57.62; H 6.12; N 11.48.

*4.1.20. Ethyl-Z-3-(5-formyl-2-furyl)-2-(5-formyl-2-thienyl) acrylate (**9a**)*

Compound **9a** was prepared using the method described for preparation of compound **1a–c,** Phosphorus oxychloride (11.1 ml, 11 mmol) was added drop-wise with cooling to DMF (10.6 ml, 12.6 mol) and the reaction mixture was stirred for 0.5 h. A solution of *Z*-ethyl-3-(2-furyl)-2-(2-thienyl)acrylate (5 g, 24 mmol) in DMF (5 ml) was added drop-wise to the mixture and stirred for 1 h at room temperature, then heated at 90 °C for 45 min, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left over-night on ice. The solid was filtered off, washed with water and recrystallized from methanol. Orange crystals 2.76 g (38%) are obtained, mp 120–123 °C (Ref. [16], m.p. 120–123 °C).

*4.1.21. Ethyl-Z-2-(5-formyl-2-thienyl)-3-(2-thienyl)acrylate (**9b**)*

Compound **9b** was prepared using the method described for preparation of compound **1a–c**, **9a.** Phosphorus oxychloride (1.8 ml, 12 mmol) was added drop-wise with cooling to DMF (1.8 ml, 9 mmol) and the reaction mixture was stirred for 0.5 h by. A solution of *Z*-ethyl-2-(2-thienyl)-3-(2-thienyl) acrylate (1.0 g, 3.78 mmol) in DMF (1 ml) was added drop-wise to the mixture and stirred for 1 h at room temperature, then heated at 90 °C for 75 min, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left over-night on ice. The solid was filtered off, washed with water and recrystallized from methanol. Yellow crystals 0.4125 g (37.3%) was obtained, m.p. 87–88 °C; IR (KBr) (υ_{max}/cm^{-1}): 1705, 1668, 1605, 1508; ^{1}H NMR (300 MHz, DMSO) δ: 9.96 (s, *CHO*, 1H), 8.19 (s, 1H$_3$), 7.80 (d, $J_{4',3'}$ = 3.9 Hz, 1H), 7.38 (d, $J_{2'',3''}$ = 5.06 Hz, 1H), 7.27 (d, $J_{3',4'}$ = 3.9 Hz, 1H), 7.12 (d, $J_{4'',3''}$ = 3.4 Hz, 1H), 7.01 (dd, $J_{3'',4''}$ = 3.4 Hz $J_{4',5'}$ = 5.06 Hz, 1H), 4.30 (q, J = 6.9 Hz, COO CH_2CH$_3$, 2H), 1.21 (t, J = 6.9 Hz, COOCH$_2$$CH_3$, 3H); elemental analysis calcd (%) for C 57.51; H 4.14, found C 57.45; H 4.21.

*4.1.22. Ethyl-Z-3-(5-cyano-2-furyl)-2-(5-cyano-2-thienyl) acrylate (**10a**)*

Compound **10a** was prepared in similar manner as compound **3a–c;** compound **9a** (1.5 g, 5 mmol) and hydroxylammonium hydrochloride (1.2 g, 17 mmol) were heated in pyridine (4 ml) at 60°Cfor 30 min, then acetic anhydride (5.5 ml) was successively added at a temperature not exceeding 95 °C, which was being kept for additional 2 h. The mixture was cooled to 20 °C, poured into water (25 ml) and the separated nitrile was stirred for 1 h, filtered, washed with water, dried and crystallized from petrol ether gave **10a** 0.500 g (33%). m.p. 59–60 °C; IR (KBr) (υ_{max}/cm^{-1}) 2200, 1690, 1600; ^{1}H NMR (300 MHz, DMSO) δ: 8.0 (d, $J_{4',3'}$ = 3.9 Hz, 1H), 7.78 (s, H$_3$ 1H), 7.62 (d, $J_{4'',3''}$ = 3.6 Hz, 1H), 7.32 (d, $J_{3'',4''}$ = 3.6 Hz, 1H), 6.08 (d, $J_{3',4'}$ = 3.9 Hz, 1H), 4.21 (q, 2H, J = 7.1 Hz, COOCH_2CH$_3$), 1.22 (t, 3H, J = 7.1 Hz, COOCH$_2$$CH_3$); ^{13}C NMR (300 MHz, DMSO) δ: 164.77, 153.40, 142.37, 138.62, 130.39, 128.27, 126.38, 125.19, 123.80, 118.82, 114.22, 111.39, 109.95, 62.05, 14.09; elemental analysis calcd (%) for $C_{15}H_{10}N_2O_3S$: C 60.39; H, 3.38; N, 9.39 found C 60.40; H 3.35; N 9.59.

*4.1.23. Ethyl-Z-2-(5-cyano-2-thienyl)-3-(2-thienyl)acrylate (**10b**)*

Compound **10b** was prepared in similar manner as compound **3a–c;** from compound **9b** (0.4 g, 1.4 mmol)) and hydroxylammonium hydrochloride (0.176 g, 2.7 mmol). Yellow crystals **10b** 0.339 g (85.8%) were obtained, m.p. 135–136 °C; IR (KBr) (υ_{max}/cm^{-1}) 2200, 1690, 1600; ^{1}H NMR (300 MHz, DMSO) δ: 8.25 (s, H$_3$, 1H), 8.05 (d, $J_{4',3'}$ = 3.8 Hz, 1H), 7.75 (d, $J_{3'',2''}$ = 4.9 Hz, 1H), 7.65 (d, $J_{4',3'}$ = 3.8 Hz, 1H), 7.28 (d, $J_{3,4}$ = 3.8 Hz, 1H), 7.13 (dd, $J_{3'',4''}$ = 3.8 Hz, $J_{3,2'}$ = 4.9 Hz, 1H), 4.20 (q, 2H, J = 7.1 Hz, COO CH_2CH$_3$), 1.22 (t, 3H, J = 7.1 Hz, COOCH$_2$$CH_3$); ^{13}C NMR (300 MHz, DMSO) δ: 165.58, 143.87, 140.02, 138.66, 137.49, 137.08, 134.36, 131.06, 127.91, 118.96, 114.50, 110.82, 61.69, 14.58; elemental analysis calcd (%) for $C_{14}H_{11}NO_2S_2$: C 58.11; H, 3.83; N, 4.84 found C 58.45; H 4.01; N 4.69.

*4.1.24. 4-Ethyl-2,7-dicyano-thieno[3,2-e]benzo[b]furan (**11**)*

Compound **11a** was prepared from **10a** (0.298 g, 1.0 mmol) in 400 ml toluene by irradiation for 2.5 hours. The air was bubbled through the solution and I_2 was added. After solvent

was evaporated the crystals were recrystallized from petrol ether. Light yellow crystals of **7a** 0.140 g (47%) were obtained. m.p. 196–198 °C; IR (KBr) (υ_{max}/cm^{-1}) 2200, 2190, 1695; ^{1}H NMR (300 MHz, DMSO) δ: 8.81 (s, H_5, 1H), 8.61 (s, H_1, 1H), 8.55 (s, H_8, 1H), 4.49 (q, 2H, J = 7.1 Hz, COO*CH*$_2$CH$_3$), 1.43 (t, 3H, J = 7.1 Hz, COOCH$_2$*CH*$_3$); ^{13}C NMR (300 MHz, DMSO) δ: 164.66, 153.28, 136.90, 133.54, 131.75, 129.59, 125.44, 123.56, 118.60, 114.87, 114.27, 113.64, 111.50, 62.57, 14.17; elemental analysis calcd (%) for $C_{15}H_8O_3N_2S$: C 60.81 H 2.70 N 9.46 found C 61.13 H 2.85 N 9.33.

*4.1.25. 4-Ethyl-2-cyano-thieno[2,3-e]benzo[b]thiophene (**11b**)*

Compound **11b** was prepared from **10b** (0.298 g, 1.1 mmol) in 250 ml toluene by irradiation for 2 hours. The air was bubbled through the solution and I_2 was added. After solvent was evaporated the crystals were recrystallized from petrol ether. Light yellow crystals of **7a** 0.230 g (80%) were obtained; m.p. 205–206 °C; IR (KBr) (υ_{max}/cm^{-1}) 2200, 1695; ^{1}H NMR (DMSO) δ: 8.98 (s, 2H), 8.34 (d, $J_{7,8}$ = 4.9 Hz, 1H), 8.13 (d, $J_{8,7}$ = 4.9 Hz, 1H), 4.40 (q, J = 7.1 Hz, COO*CH*$_2$CH$_3$, 2H), 1.41 (t, J = 7.1 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (DMSO) δ: 162.5, 139.19, 137.38, 135.77, 134.79, 133.67, 128.70 126.44, 123.06, 119.98, 115.20, , 111.34, 62.37, 14.64; elemental analysis calcd (%) for C 58.54; H 3.14; N 4.88; found C 58.71; H 3.21; N 5.02.

*4.1.26. 4-Ethyl-2,7-bis-(N-isopropylamidino)-thieno[3,2-e] benzo[b]furan dihydrochloride (**12a**)*

Compound **12a** was prepared using the method described for the preparation of **4a**; from **11a** (0.103 g, 0.34 mmol) and the isopropyl amine (0.103 g, 0.17 mmol). The precipitate was collected and recrystallized from ethanol-ether to give light yellow crystals 0.030 g (17.8%). m.p. 251–253 °C. IR(KBr) (υ_{max}/cm^{-1}): 3460, 3396, 3176, 2981, 1702, 1675, 1633; ^{1}H NMR (DMSO) δ: 10.42 (d, J = 7.9 Hz, 1H), 10.25 (s, 1H), 10.15 (d, J = 7.9 Hz, 1H), 9.93 (s, 1H), 9.66 (s, 1H), 9.47 (s, 1H), 8.83 (s, H_5, 1H), 8.78 (s, H_1, 1H), 8.55 (s, H_8, 1H), 4.52 (q, J = 7.2 Hz, COO*CH*$_2$CH$_3$, 2H), 4.26–4.16 (m, 2H, *CH*(CH$_3$)$_2$), 1.46–1.42 (t, J = 7.2 Hz, COOCH$_2$*CH*$_3$, 3H), 1.35 (d, 6H, J = 6.3 Hz, CH(*CH*$_3$)$_2$); ^{13}C NMR (DMSO) δ: 164.54, 155.36, 152.28, 150.26, 146.25, 135.81, 134.49, 132.18, 126.98, 126.49, 126.44, 22.90, 113.81, 112.04, 62.23, 45.57, 45.26, 21.12, 21.08, 13.97; elemental analysis calcd (%) for ($C_{21}H_{30}N_4O_4SCl_2x2H_2O$): C 48.18, H 6.10, N 10.70 found C 48.51, H 6.07, N 10.52.

*4.1.27. 4-Ethyl-2,7-bis-(morpholinamidino)-thieno[3,2-e] benzo[b]furan dihydrochloride (**12b**)*

Compound **12b** was prepared using the method described for the preparation of **4a**; from **11a** (0.143 g, 0.48 mmol) and the aminomorpholin (0.6 ml, 5 mmol). The precipitate was collected and recrystallized from ethanol-ether to give light yellow crystals 0.124 g (51.3%), m.p. 231–232 °C. IR(KBr) (υ_{max}/cm^{-1}): 2821, 1701, 1616, 1581; ^{1}H NMR (DMSO) δ: 12.21 (s, 1H), 11.81 (s, 1H), 10.55 (s, 1H), 10.27 (s, 1H), 9.47 (s, 1H), 9.37 (s, 1H), 8.91(s, H_5, 1H), 8.85 (s, H_1, 1H), 8.45 (s, H_8, 1H), 4.53–4.48 (q, J = 7.2 Hz, COO*CH*$_2$CH$_3$, 2H), 3.85 (bs, 4H), 3.67 (bs, 4H), 3.03 (bs, 4H), 2.93 (bs, 4H), 1.40 (t, J = 7.2 Hz, COOCH$_2$*CH*$_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.7 153.83, 152.35, 151.7, 148.52, 143.40, 135.36, 133.26, 127.44, 120.61, 120.24, 112.08, 104.93, 66.52, 66.18, 61.88, 59.64, 54.81, 54.71, 14.67; 3449, 3397, 3297, 2963; elemental analysis calcd (%) for $C_{23}H_{30}N_6O_5SCl_2$: C 55.19 H 5.64 N 16.79 found C 55.94 H 5.57 N 16.52; MS (ESI, *m*/*z*, -HCl): 501.4 (MH^+), 251.4 $(M2H^+)^{2+}$.

*4.1.28. 4-Ethyl-2-(N-isopropylamidino)-thieno[2,3-e]benzo[b] thiophene hydrochloride (**12c**)*

Compound **12c** was prepared using the method described for the preparation of **4a**; from **11b** (0.224 g, 0.78 mmol) and the isopropyl amine (0.231g, 3.9 mmol). The precipitate was collected and recrystallized from ethanol–ether to give light yellow crystals 0.10 g (33.4%), m.p. 210–212 °C. ^{1}H NMR (300 MHz DMSO) δ: 9.1 (bs, 3H), 8.81 (s, H_4, 1H), 8.69 (s, H_1 1H), 8.26 (d, $J_{7,8}$ = 5.4 Hz, 1H), 7.95 (d, $J_{8,7}$ = 5.4 Hz, 1H), 4.47 (q, 2H, J = 7.1 Hz, COO*CH*$_2$CH$_3$), 4.33 (m, 1H, *CH*(CH$_3$), 1.42 (t, 3H, J = 7.1 Hz, COOCH$_2$*CH*$_3$.), 1.35 (d, 6H, J = 6.3 Hz, CH(*CH*$_3$); elemental analysis calcd (%) for $C_{17}H_{19}N_2O_2S_2Cl$: C53.27 H 4.96 N 7.3 found C 53.41 H 5.11 N 7.12.

*4.1.29. Ethyl-Z-3-(5-formyl-2-furyl)-2-(3-thienyl)acrylate (**13**)*

Compound **13** was prepared using the method described for preparation of compound **1a,** from phosphorus oxychloride (2.4 ml, 26 mmol), DMF (2.3 ml, 26 mmol) and the ethyl-*Z*-3-(2-furyl)-2-(3-thienyl)acrylate (4.4 mmol) in DMF (2 ml) was added drop-wise to the mixture and stirred for 1 h at room temperature, then heated at 90 °C for 45 min, cooled and poured onto crushed ice and made weakly alkaline with sodium carbonate solution, and left over-night on ice. The solid was filtered off, washed with water and recrystallized from methanol. Yellow orange crystals 1.159 g (96%) was obtained, mp 65–67 °C. IR (KBr) (υ_{max}/cm^{-1}) 1680, 1650, 1600; ^{1}H NMR (300 MHz DMSO) δ: 9.5 (s, CHO, 1H); 7.63 (s, H_3, 1H); 7.63 (d, $J_{5',4'}$ = 5.0 Hz, 1H); 7.56 (s, H-2', 1H); 7.43 (d, $J_{4'',3''}$ = 3.5 Hz, 1H); 7.03 (d, $J_{4'',5'}$ = 5.0 Hz, 1H); 6.21 (d, $J_{3'',4''}$ = 3.5 Hz, 1H); 4.20 (q, J = 7.0 Hz, COO*CH*$_2$CH$_3$, 2H); 1.22 (t, J = 7.0 Hz, COOCH$_2$*CH*$_3$, 3H); elemental analysis calcd (%) for $C_{14}H_{12}O_4S$: C60.86 H 4.38 found C 60.61 H 4.11

*4.1.30. Ethyl-Z-3-(5-cyano-2-furyl)-2-(3-thienyl)acrylate (**14**)*

Compound **14** was prepared using the method described for the preparation of **3a**, from **13** (1 g, 3.6 mmol) and hydroxylammonium hydrochloride (0.439 g) were heated in pyridine (3 ml) at 60 °C for 30 min, then acetic anhydride (2 ml) was successively added at a temperature not exceeding 95 °C, which was being kept for additional 2 h. The mixture was cooled to 20 °C, poured into water (25 ml) and the separated nitrile was stirred for 1 h, filtered, washed with water, dried and crystallized from petrol ether gave **14** 0.623 g (63.4%). m.p. 105–106 °C . IR (KBr) (υ_{max}/cm^{-1}): 2235 , 1710, 1626.;

^{1}H NMR (300 MHz DMSO) δ: 7.64 (d, $J_{5',4'}$ = 4.9 Hz, 1H), 7.57–7.56 (m, H-3, H-2',H-4', 3H), 7.05 (d, $J_{4'',3''}$ = 3.7 Hz, 1H), 6.39 (d, $J_{3'',4''}$ = 3.7 Hz, 1H), 4.2 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H), 1.23 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.64, 154.57, 129.45, 125.99, 125.85, 125.09, 124.84, 115.3, 111.39, 61.23, 13.99; elemental analysis calcd (%) for ($C_{14}H_{11}O_3NS$): C 61.47, H 4.02, N 5.12 found C 61.15, H 3.91, N 4.88.

4.1.31. 4-ethyl-7-cyano-thieno[2,3-e]-benzo[b]furan (15)

Compound **15** was prepared using the method described for the preparation of **7a**; from **13** (304 mg, 1.1 mmol). The residue was recrystallized from petrol ether afforded white crystals **15** 0.144 g (47%), m.p. 113–114 °C. IR(KBr) (υ_{max}/cm^{-1}): 2210, 1705, 1675;^{1}H NMR (300 MHz DMSO) δ: 8.79 (s, H$_5$, 1H), 8.52 (s, H$_8$, 1H), 8.20 (d, $J_{3,2}$ = 5.5 Hz, 1H), 8.02 (d, $J_{2,3}$ = 5.5 Hz, 1H), 4.42 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H), 1.40 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H); ^{13}C NMR (300 MHz DMSO) δ: 165.26, 151.87, 135.09, 133.49, 128.72, 128.64, 124.72, 124.44, 123.53, 118.14, 112.16, 111.56, 61.44, 14.08; elemental analysis calcd (%) for ($C_{14}H_9O_3NS$): C 61.92 H 3.32; N 5.16. found C 62.05 H 3.61 N 4.98.

4.1.32. 4-Ethyl-7-(N-isopropylamidino)-thieno[2,3-e]-benzo[b]furan hydrochloride (16)

Compound **16** was prepared in similar manner as **4a,** from **15** (0.116 g, 0.43 mmol) and the isopropylamine (0.103 g, 2.1 mmol). The precipitate was collected and recrystallized from ethanol-ether afforded light yellow crystals 0.134 g (85.3%). m.p. 180–182 °C IR (KBr) (υ_{max}/cm^{-1}): 3360, 3296, 3076, 1675, 1633; ^{1}H NMR (DMSO) δ: 9.8 (bs, 3H), 8.64 (s, H$_5$, 1H), 8.31 (s, H$_8$, 1H), 8.26 (d, $J_{3,2}$ = 5.5 Hz, 1H), 8.02 (d, $J_{2,3}$ = 5.5 Hz, 1H), 4.45 (q, J = 7.1 Hz, COOCH_2CH$_3$, 2H), 4.2 (m, CH(CH$_3$)$_2$, 1H 1.40 (t, J = 7.1 Hz, COOCH$_2$$CH_3$, 3H), 1.19 (d, J = 6.5 Hz, CH(CH_3)$_2$, 6H); ^{13}C NMR (DMSO) δ: 165.30, 151.28, 150.40, 145.54, 135.0, 133.68, 128.72, 128.57, 124.73, 124.71, 124.65, 124.34, 124.33, 112.05, 111.85, 61.45, 45.29, 42.89, 21.18, 20.34; elemental analysis calcd (%) for $C_{17}H_{21}O_3N_2Cl$: C 56.21; H 5.78; N 7.72; found C 56.54; H 5.91; N 7.55.

4.2. Interactions with DNA

The electronic absorption spectra were obtained on Varian Cary 100 Bio spectrometer using quartz cuvettes (1 cm) (Table 2).

Calf thymus (*ct*-) DNA was purchased from Fluka, dissolved in the sodium cacodylate buffer, I = 0.05 mol dm^{-3}, pH 7 and additionally sonicated and filtered through the 0.45 μm filter. The final concentration of the calf thymus (*ct*-) DNA solution expressed as the concentration of phosphates was determined spectroscopically [27,28] by measuring the absorbance of buffered solution at λ = 260 nm for at least five independent additions of the DNA stock solution aliquots and dividing the averaged absorbance value by molar extinction

Table 2
Electronic absorption data of studied compounds[a]

	λ_{max} (nm)	ε (dm^3 mol^{-1} cm^{-1})
2a	323	4797
2b	321	6316
3a	321	7680
3b	324	9862
3c	319	9910
[b]**5a**	327	15489
	258	16853
[b]**6a**	321	15571
	341	14836
	255	15116
6b	321	11572
	344	13192
10a	268	5498
	343	10609
	357	10702
12a	256	2572
	333	2975
	352	2686
14	318	4797
16	251	5121
	325	3437

[a] Sodium cacodylate buffer, I = 0.05 mol dm^{-3}, pH = 7.
[b] Previously published results added for comparison [4].

coefficient ε(ct-DNA) = 6600 mmol^{-1} cm^{-2}. The measurements were performed in aqueous buffer solution (pH = 7; sodium cacodylate buffer, I = 0.05 mol dm^{-3}). Under the experimental conditions used, the absorbencies of studied compounds were proportional to their concentrations. The binding constants (K_S) and [bound compound]/[polynucleotide phosphate] ratio (n) were calculated according to the Scatchard equation by non-linear least-squares fitting [21,29], all having satisfying correlation coefficients (> 0.999). The thermal denaturation experiments were performed by following the absorption change at 260 nm as a function of temperature. The absorbance scale was normalized; T_m values are the midpoints of the transition curves, determined from the maximum of the first derivative or graphically by a tangent method. ΔT_m values were calculated by subtracting T_m of the free nucleic acid from T_m of the complex. Every ΔT_m value here reported was the average of at least two measurements, the error in ΔT_m is ± 0.5 °C.

4.3. Antitumor activity assay

The HeLa (cervical carcinoma), MCF-7 (breast carcinoma), SW 620 (colon carcinoma), MiaPaCa-2 (pancreatic carcinoma), Hep-2 (laryngeal carcinoma) and WI 38 (diploid fibroblasts) cells were cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U ml^{-1} penicillin and 100 μg ml^{-1} streptomycin in a humidified atmosphere with 5% CO_2 at 37 °C.

The growth inhibition activity was assessed according to the slightly modified procedure performed at the National Cancer Institute, Developmental Therapeutics Program [30]. The cells were inoculated onto standard 96-well microtiter plates on day

0. The cell concentrations were adjusted according to the cell population doubling time (PDT): 1×10^4 ml^{-1} for HeLa, Hep-2, MiaPaCa-2 and SW 620 cell lines (PDT = 20–24 hours) 2×10^4 ml^{-1} for MCF-7 cell lines (PDT = 33 hours) and 3×10^4 ml^{-1} for WI 38 (PDT = 47 hours). Test agents were then added in five, 10-fold dilutions (10^{-8}–10^{-4} mol l^{-1}) and incubated for further 72 hours. Working dilutions were freshly prepared on the day of testing. The solvent (DMSO) was also tested for eventual inhibitory activity by adjusting its concentration to be the same as in working concentrations. After 72 hours of incubation, the cell growth rate was evaluated by performing the MTT assay [31], which detects dehydrogenase activity in viable cells. The absorbance (OD, optical density) was measured on a microplate reader at 570 nm. The percentage of growth (PG) of the cell lines was calculated according to one or the other of the following two expressions:

If (mean OD_{test} – mean OD_{tzero}) ≥ 0 then

$$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero})/(\text{mean } OD_{ctrl} - \text{mean } OD_{tzero})$$

If (mean OD_{test} – mean OD_{tzero}) < 0 then

$$PG = 100 \times (\text{mean } OD_{test} - \text{mean } OD_{tzero})/OD_{tzero}$$

Where

Mean OD_{tzero} = the average of optical density measurements before exposure of cells to the test compound.

Mean OD_{test} = the average of optical density measurements after the desired period of time.

Mean OD_{ctrl} = the average of optical density measurements after the desired period of time with no exposure of cells to the test compound.

Each test point was performed in quadruplicate in three individual experiments. The results are expressed as IC_{50}, which is the concentration necessary for 50% of inhibition. The IC_{50} values for each compound are calculated from dose–response curves using linear regression analysis by fitting the test concentrations that give PG values above and below the reference value (i.e. 50%). If however, for a given cell line all of the tested concentrations produce PGs exceeding the respective reference level of effect (e.g. PG value of 50), then the highest tested concentration is assigned as the default value, which is preceded by a ">" sign. Each result is a mean value from three separate experiments.

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References

[1] C. Bailly, Curr. Med. Chem. 7 (2000) 39–58.

[2] M. Demeunynck, C. Bailly, W.D. Wilson, D.N.A. In, R.N.A. Binders, Wiley-VCH, Weinheim, 2002.

[3] a) J.M. Fortune, L. Velea, D.E. Graves, T. Utsugi, Y. Yamada, N. Oshero, Biochemistry 38 (1999) 15580–15586.; b) C. Bailly, X.Qu, J. B.Chaires, P.Colson, C. Houssier, M. Ohkubo, S.Nishimura, T. Yoshinari, J. Med. Chem. 42 (1999) 2927–2935.; c) J.A.Wilson-Byl, J.M. Fortune, A. Burden, J.L. Nitiss, T. Utsugi, Y. Yamada; N. Oshero, Biochemistry 38 (1999) 15573–15579; d) H. Minderman, C. Wrzosek, S.Cao, T. Utsugi, T. Kobunai, Y. Yamada, Y. M. Rustum, Cancer Chemother. Pharmacol. 45 (2000) 78–84.

[4] K. Starčević, G. Karminski-Zamola, I. Piantanida, L. Šuman, M. Kralj, J. Am. Chem. Soc. 127 (2005) 1074–1075.

[5] a) P. Cagniant, D. Cagniant, in: A.R. Katritzky, A.J. Boulton, (Eds.), Advances in Heterocyclic Chemistry, vol. 18, Academic Press, New York, 1975, pp. 338.; b) E. Campaigne, in: C.W. Bird, Cheeseman (Eds.), Comprehensive Heterocyclic Chemistry, vol 4, G.W.H. Pergamon, Oxford, 1984, pp. 910; c) B.A. Keay, P.W. Dibble, in: A.R. C.W. Katritzky, Rees, E. F. V. Scriven (Eds.), Comprehensive Heterocyclic Chemistry II, vol 2, Pergamon, Oxford, 1996, pp. 395; d) R.K. Russel, in: A.R. Katritzky; C.W. Rees, E.F.V. Scriven (Eds.), Comprehensive Heterocyclic Chemistry II, vol 2, Pergamon, Oxford, 1996, pp 679; e) R.P. Haugland, in: M.T.Z. Spence (Eds.), Handbook of Fluorescent Probes and Research Chemicals, Molecular Probes, Inc. O.R. Eugene, 1996.

[6] L. Benameur, Z. Bouaziz, P. Nebois, M.H. Bartoli, M. Boitard, H. Fillion, Chem. Pharm. Bull. (Tokyo) 44 (1996) 605–608.

[7] a) S. Wang, J.E. Hall, F.A. Tanious, W.D. Wilson, D.A. Patrick, D.R. McCurdy, B.C. Bender and R.R. Tidwell, Eur. J. Med. Chem., 34 (1999) 215–224.; b) C. Bilger, P. Demerseman; J.-P. Buisson, R. Royer, P. Gayral, J. Fourniat, Eur. J. Med. Chem. Chim. Ther. 22 (1987) 213–220.

[8] A.P. Monte, D. Marona-Lewicka, M.M. Lewis, R.B. Mailman, D.B. Wainscott, D.L. Nelson, D.E. Nichols, J. Med. Chem. 41 (1998) 2134–2145.

[9] a) B. P. Das, D. W. Boykin, J. Med. Chem. 20 (1977) 531–536; b) E. A. Steck, K. K. Kinnamon, D. E. Davidson, R. E. Duxbury, A. J. Johnson, R. E. Masters, Exp. Parasitol. 53 (1982) 133–144.

[10] D.W. Boykin, A. Kumar, B.K. Bender, J.E. Hall, R.R. Tidwell, Bioorg. Med. Chem. Lett. 6 (1996) 3017–3021.

[11] A. Lansiaux, L. Dassonneville, M. Facompré, A. Kumar, C.E. Stephens, M. Bajić, F. Tanious, W.D. Wilson, D.W. Boykin, C. Bailly, J. Med. Chem. 45 (2002) 1994–2002.

[12] a) G. Feriotto, C. Nastruzzi, R. Cortesi, A. Ciucci, C. Mischiati, R. Gambari, Anti-Cancer Drug Des. 1994, 9, 449–469; b) M.P. Foloppe, S. Rault, D.E. Thurston, T.C. Jenkins, M. Robba, Eur. J. Med. Chem. 31 (1996) 407–410.

[13] T. Mens, S. Tomasi, V.L. Eiflerlima, P. Uriac, J. Huet, V. Catrosquemener, Anticancer Res. 17 (19979 4327–4332.

[14] A. Bielawska, K. Bielawski, S. Wolczynski, Pharmazie 56 (2001) 428–429.

[15] M. Hranjec, M. Grdiša, K. Pavelić, D.W. Boykin, G. Karminski-Zamola, Il Farmaco 58 (2003) 1319–1324.

[16] K. Starčević, D.W. Boykin, G. Karminski-Zamola, Heterocycl. Comm. 8 (2002) 221–226.

[17] K. Starčević, D.W. Boykin, G. Karminski-Zamola, Heteroat. Chem. 14 (2003) 218–222.

[18] a) M. Bajić, G. Karminski-Zamola, Croat. Chem. Acta 65 (1992) 835–846 b) G. Karminski-Zamola, Pavličić, M. Bajić, N, Blažević, Heterocycles 32 (1991) 2323.

[19] T.A. Fairley, R.R. Tidwell, I. Donkor, N.A. Naiman, K.A. Ohemeng, R.J. Lombardy, J.A. Bentley, J. Med. Chem. 36 (1993) 1746–1753.

[20] J. Oszczapowicz, in: S. Patai, Z. Rappoport (Eds.), The chemistry of Amidines and Imidates, vol. 2, John Wiley and Sons, London, New York, 1991, p. 623.

[21] a) G. Scatchard, Ann. N.Y. Acad. Sci. 51 (1949) 660–664; b) J.D. McGhee; P.H. von Hippel, J. Mol. Biol. 86 (1974) 469–489.

[22] I. Jarak, M. Kralj, L. Šuman, L.G. Pavlović, J. Dogan, I. Piantanida, M. Žinić, K. Pavelić, G. Karminski-Zamola, J. Med. Chem. 48 (2005) 2346–2360.
[23] I. Jarak, M. Kralj, I. Piantanida, L. Šuman, M. Žinić, K. Pavelić, G. Karminski-Zamola, Bioorg. Med. Chem. 14 (2006) 2859–2868.
[24] E.C. Goodwin, D. DiMaio, Proc. Natl. Acad. Sci. USA 97 (2000) 12513–12518.
[25] D. Lembo, M. Donalisio, M. De Andrea, M. Cornaglia, S. Scutera, T. Musso, S. Landolfo, FASEB J. (2005) (published Oct 27, 2005, doi: 10.1096/fj.05-3904fje).
[26] V. Bénéteau, T. Besson, J. Guillard, S. Léonce, B. Pfeiffer, Eur. J. Med. Chem. 34 (1999) 1053.
[27] J.B. Chaires, N. Dattagupta, D.M. Crothers, Biochemistry 21 (1982) 3933–3940.
[28] B.S. Palm, I. Piantanida, M. Žinić, H.J. Schneider, J. Chem. Soc., Perkin Trans. 2 (2000) 385–392.
[29] I. Piantanida, B.S. Palm, M. Žinić, H.-J. Schneider, J. Chem. Soc., Perkin Trans. 2 (2001) 1808–1816.
[30] M.R. Boyd, K.D. Paull, Drug Dev. Res. 34 (1995) 91–109.
[31] T. Mossman, J. Immunol. Methods 65 (1983) 55–61.