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Synthesis, structure elucidation, determination of the lipophilicity and identification of antitumour activities in vitro of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones with a low cytotoxicity towards normal human skin fibroblast cells

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ABSTRACT

Eleven novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12**–**22**) were designed and obtained from appropriate 1-aryl-2-hydrazonoimidazolidines (**1**–**11**) by condensation reaction with 2-oxo-2-furanacetic acid and subsequent cyclocondensation of intermediate chain derivatives. IR, ^{1}H NMR and ^{13}C NMR spectra and elemental analyses confirmed the chemical structure of all the synthesized compounds. The reversed-phase HPLC method was optimized and proved to be applicable and reliable for the analysis of these unknown small molecules (**12**–**22**). These compounds were chromatographed on octadecyl silica (ODS) stationary phase and their hydrophobic parameters expressed as the log k_w values were determined by RP-HPLC, using mixtures of methanol and water as mobile phases with different methanol concentrations. Octane-1-sulfonic acid sodium salt (OSA-Na) and 20% acetate buffer (pH 3.5) was added to the mobile phase (eluent containing 0.01 M/L OSA-Na in organic modifier (MeOH)—buffered mobile phase). The high values of regression coefficients (r >0.9841) for all the compounds investigated proved the excellent fit between experimental data and the Snyder–Soczewiński equation. Results obtained from the reversed-phase HPLC were compared both with those theoretically calculated and with those obtained from an ALOGPS 2.1. software by the use of nine different computational methods for estimation of log *P*. The predicted values of log *P* by use of AB log *P* algorithm revealed the best correlation with the experimental log k_w values for the investigated solutes, since a good correlation (r = 0.7760) between these quantities was found. The majority of novel imidazotriazinones were found to be evidently effective in vitro against human cancerous cells (HeLa and T47D) in an effective concentration of 50 μg/mL. Five compounds (**13**, **15**, **16**, **18** and **22**) revealed remarkable antiproliferative activities and selective cytotoxicities for cancer cells over normal HSF cells. Therefore these ones may be considered as a basis for the design of novel useful non-toxic (**13**, **15** and **16**) and low toxic (**18** and **22**) anticancer agents.

1. Introduction

Cancer is still considered to be the largest cause of mortality (after heart disease) because about 15% of all human deaths in the developed world are evoked by this severe disease.[1] In spite of meaningful progress in cancer chemotherapy there is still a lack of clinically useful cytotoxic drugs selective for cancerous cells. Now then the possibility of application in cancer treatment of known antiproliferative agents is limited by the damage they also evoke to the normal rapidly dividing cells (i.e., the bone marrow cells, the epithelial cells in the gastrointestinal tract) and by side effects characteristic for given anticancer drugs.[2] Therefore, the development of novel synthetic anticancer agents that show a selective cytotoxicity for cancer cells over normal cells seems to be of great urgency. Furthermore, the examination of cytotoxicity profile still remains especially important in the continuous search for novel cytotoxic structures.

Our findings suggest that several polynitrogenated small molecules based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (shown in Table 1) are able to selectively inhibit the growth of tumour cells, and for this reason in the future these

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Table 1
Previously reported[3–5] thesis structures based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template and a brief summary of the most important assay data

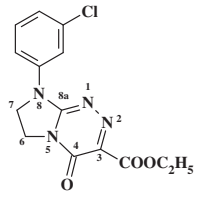
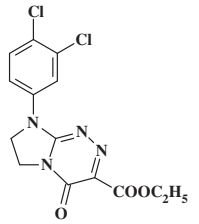
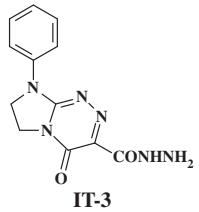
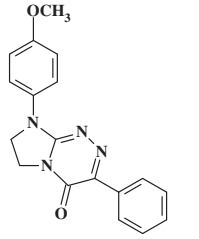
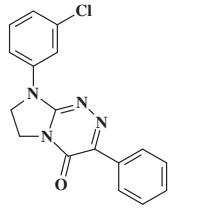
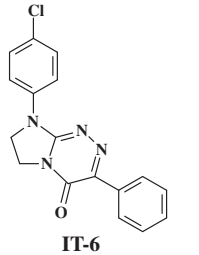
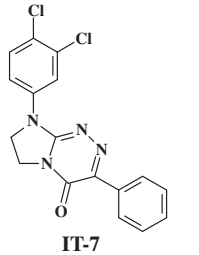
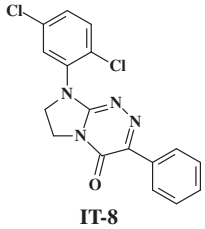
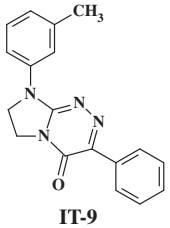
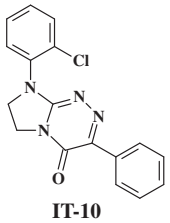
Structure	A brief summary of the most important assay data
 **IT-1**	Selective for cancer cells over normal cells. Almost twofold more cytotoxic against cancer cell lines (mostly against LS180, SiHa and T47D cells) than towards normal HSF cells at concentrations of 10 μg/mL (0.031 mM) and 50 μg/mL (0.156 mM) after 72 h of incubation in the 5-bromo-2′-deoxyuridine (BrdU) cell proliferation assay. Has been proved to inhibit the growth of tumour cells in a concentration-dependent manner.[3] LD_{50} i.p. in male Albino-Swiss mice: over 1000 mg/kg.[4]
 **IT-2**	Reported to be selective for cancer cells over normal cells. Over twofold more cytotoxic against cancer cells (LS180 and SiHa cell lines) than towards normal HSF cells at concentrations of 10 μg/mL (0.028 mM) and 50 μg/mL (0.141 mM) after 72 h of incubation. Has been shown to evoke a dose-dependent antitumour effects in the BrdU assay.[3] Evidently effective against leukaemic Jurkat cells with the IC_{50} value of 0.032 mM and low cytotoxic towards the primary cell line of normal human skin fibroblasts after 24 h of incubation in the assay MTT based (the succinate dehydrogenase inhibition, SDI test)[4]
 **IT-3**	Reported to be effective against leukaemic Jurkat cells with the IC_{50} value of 0.085 mM and simultaneously low cytotoxic towards the primary cell line of normal HSF cells after 24 h of incubation in the MTT assay.[4] Completely non-toxic towards normal GMK cells at concentrations of 10 μg/mL (0.037 mM) and 50 μg/mL (0.184 mM) after 72 h of incubation in a colorimetric BrdU cell proliferation ELISA kit.[4]LD_{50} i.p. in male Albino-Swiss mice: over 1000 mg/kg[4]
 **IT-4**	Has been demonstrated remarkable viability decreases (to 57% and 43% in response to treatment with **IT-4**) in human peripheral blood myeloma cells (RPMI 8226) both at a concentration of 0.050 and 0.100 mM, respectively after exposure for 24 h. Has also been reported to be almost 1.7-times more cytotoxic against RPMI 8226 myeloma cells than towards normal HSF cells at concentration of 0.100 mM in the MTT assay.[5] At a concentration of 0.025 mM was able to inhibit migration of human cervical epithelial carcinoma cells (HeLa B) (41% inhibition of cell migration in response to treatment with **IT-4** versus untreated controls) in the monolayer scratch assay.[5] LD_{50} i.p. in male Albino-Swiss mice: over 2000 mg/kg
 **IT-5**	Has been proven to evoke viability decreases (to 70% and 60% in response to treatment with **IT-5**) both at a concentration of 0.050 and 0.100 mM, respectively, in RPMI 8226 myeloma cells after exposure for 24 h. Has been proven to be almost 1.5-times more cytotoxic for RPMI 8226 cancer cells than towards normal HSF cells at a concentration of 0.100 mM in the MTT assay.[5] At a concentration of 0.025 mM was able to inhibit explicitly migration of HeLa B cancerous cells (48% inhibition of cell migration in response to treatment with **IT-5** versus untreated controls) in the monolayer scratch assay. Has been suggested as a preventive agent against cancer metastasis[5]
 **IT-6**	Selective for cancer cells over normal cells. Has been shown to evoke a dose-dependent viability decreases (to 56% and 36% in response to treatment with **IT-6**) both at a concentration of 0.050 and 0.100 mM, respectively in RPMI 8226 myeloma cells after 24-h incubation period. Completely non-toxic for the primary cell line of normal HSF cells in the MTT assay.[5] At a concentration of 0.025 mM was able to significantly inhibit migration of HeLa B cancer cells (50% inhibition of cell migration in response to treatment with **IT-6** versus untreated controls) in the monolayer scratch assay. Has been proposed as a preventive agent against cancer metastasis.[5] LD_{50} i.p. in Albino-Swiss mice: over 2000 mg/kg
 **IT-7**	Has been reported to evoke viability decreases (to 69 and 58% in response to treatment with **IT-7**) in human peripheral blood myeloma (RPMI 8226) cells both at a concentration of 0.050 and 0.100 mM, respectively after exposure for 24 h in the MTT assay[5]

Table 1 (*continued*)

Structure	A brief summary of the most important assay data
 **IT-8**	Has been demonstrated remarkable viability decreases (to 43% and 41% in response to treatment with **IT-8**) in RPMI 8226 human myeloma cells both at a concentration of 0.050 and 0.100 mM, respectively after 24-h incubation period in the MTT assay[5]
 **IT-9**	Has been demonstrated anti-migratory effects in vitro. At a single concentration of 0.025 mM was able to inhibit migration ability of human cervical epithelial cancer cells (HeLa B cells) (32% inhibition of cell migration in response to treatment with **IT-9** versus untreated controls) in the monolayer scratch assay. Has been suggested as a preventive agent against cancer metastasis[5]
 **IT-10**	Has been proven to exhibit anti-migratory effects in vitro. At a single concentration of 0.025 mM was able to inhibit explicitly migration of HeLa B cancerous cells (41% inhibition of cell migration in response to treatment with **IT-10** versus untreated controls) in the monolayer scratch assay. Has been suggested as a preventive agent against cancer metastasis[5]

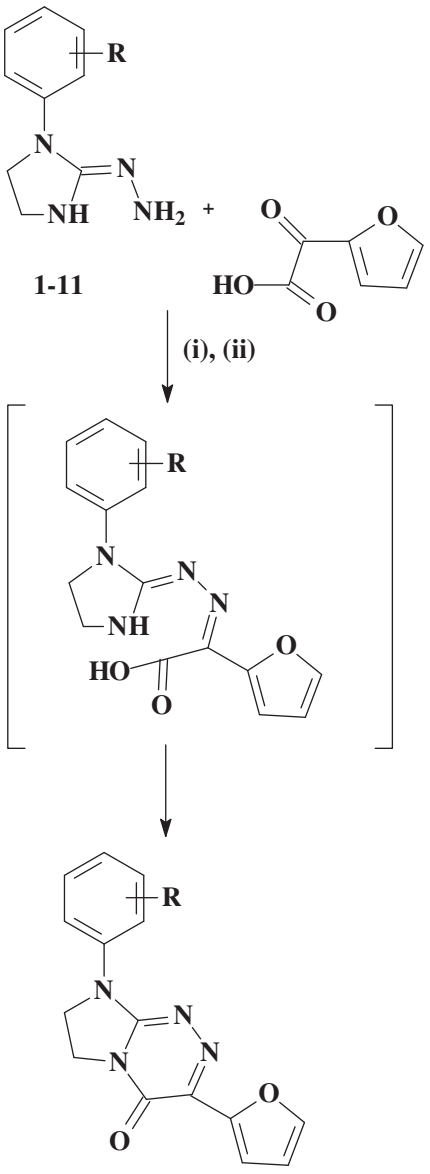
heterocycles might be powerful drug candidates having a beneficial side-effect profile.[3–5] In our recent studies the two successful derivatives of the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one, possessing remarkable dose-dependent anticancer activities and distinctly marked lower cytotoxicities towards normal cells, were discovered. These potent small molecules (namely **IT-1** and **IT-2**), containing the ethoxycarbonyl formation at the C-3 and a 3-chlorophenyl or a 3,4-dichlorophenyl substituent attached to the N-8 of the heterobicylic template,[3] acquired considerable interest because of their evidenced profitable cytotoxicity profile. The 3-carbohydrazide derivative of 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (**IT-3**), with the unsubstituted phenyl ring as the best choice of substitution pattern at the N-8, revealing an antileukaemic activity was also designed in our laboratory and previously reported.[4] This antitumour agent was found to possess a profitable toxicity profile, since it revealed only low cytotoxicity towards normal human skin fibroblast (HSF) cells and a complete lack of cytotoxicity towards normal green monkey kidney (GMK) cells. In addition, its acute toxicity, assessed in mice according to Litchfield and Wilcoxon method,[6] and expressed as LD_{50} value, was found to be relatively low (over 1000 mg kg^{-1} when injected intraperitoneally).[4] We recently reported several successful 3-phenyl-8-substituted derivatives based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template with remarkable antileukaemic and antimetastatic activities and discussed the preliminary structure–activity relationships in this bioactive set of substances.[5] Substituents at the N-8 of heterobicyclic template such as: the 4-methoxyphenyl in **IT-4**, monochloro-substituted phenyl (mostly *meta*-chloro- and *para*-chlorophenyl in **IT-5** and **IT-6**, respectively) or dichloro-substituted phenyl (mostly 3,4-dichloro- and 2,5-dichlorophenyl in **IT-7** and **IT-8**, respectively) proved to be necessary for the antileukaemic potency in this set of compounds. Furthermore, 3-phenyl substituted derivatives based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template, containing the 4-methoxyphenyl (**IT-4**), a 3-methylphenyl (**IT-9**) and monochloro-substituted phenyl (primarily *ortho*-chloro-, *meta*-chloro- and *para*-chlorophenyl (**IT-10**, **IT-5** and **IT-6**, respectively)) at the N-8, revealed significant antimetastatic properties in human cervix epitheloid carcinoma cells. Among them 3-phenyl-8-(4-chlorophenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (**IT-6**), revealing remarkable dose dependent viability decreases in human myeloma cells, and exerting significant antimetastatic properties in human cervical epithelial carcinoma cells, proved to be completely non-toxic towards normal HSF cells.[5]

Prompted by these facts and continuing our attempt to achieve medicinally important agents,[3–5,7–12] we report herein an effective and straightforward route for the synthesis, structure elucidation, determination of the lipophilicity, comparison with results obtained by use of hydrophobic substituent constants π, computational algorithms, and anticancer activities in vitro of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**) with a low cytotoxicity towards normal human skin fibroblast cells. Cytotoxic effects of these solutes against two cancerous cells: human cervix epitheloid carcinoma cell line (HeLa) and human breast cancer cell line (T47D) are discussed considering the substitution pattern of the compounds, their selectivity and drug-likeness.

2. Results and discussion

2.1. Synthesis of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22)

The reported herein biologically important polyazaheterobicycles are based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12**–**22**). The straightforward synthetic route used in our laboratory that is applicable to a wide range of derivatives is shown in Scheme 1. The tautomeric biologically active 1-arylimidazolidin-2-one hydrazones (1-aryl-2-hydrazinoimidazolines) (**1–11**)[3,7,13] were used as excellent key building blocks for the synthesis of the desired heterobicycles. These ones were prepared in good yields (62–76%) under mild reaction conditions by a



12-22

1,12: R = H; **2,13**: R = 2-CH_3; **3,14**: R = 4-CH_3; **4,15**: R = 2,3-$(CH_3)_2$; **5,16**: R = 2-CH_3O; **6,17**: R = 4-CH_3O; **7,18**: R = 2-Cl; **8,19**: R = 3-Cl, **9,20**: R = 4-Cl; **10,21**: R = 3,4-Cl_2; **11,22**: R = 2,6-Cl_2

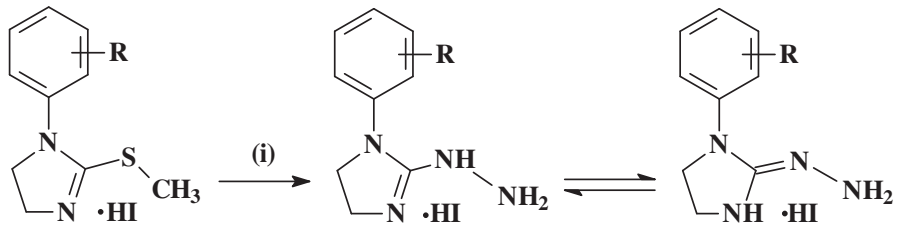
Scheme 1. Synthesis of 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**) considered in the present paper. Reagents and conditions: (i) = (**1–11**)·HI, *n*-BuOH, Et_3N, reflux, 15–55 min; (ii) *n*-BuOH/DMF mixture, reflux, 4–7 h.

nucleophilic displacement of the methylsulfanyl group (attached to the endocyclic C-2 atom in the 1-arylimidazoline structures) on treatment with hydrazine monohydrate, with concomitant loss of methyl mercaptan, using patent pending methodology[11] (see Scheme 2).

All the reported herein compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12–22**) were generated by the annulation of a 1,2,4-triazine ring to an imidazolidine core according to [4+2] pattern.[14] In this case the N–C–N–N fragment of the 1,2,4-triazine scaffold is derived from the imidazolidine moiety, whereas the C–C fragment is provided by the second α-oxoacid reactant used, that is, 2-oxo-2-furanacetic acid.

Equimolar quantities of the available 1-arylimidazolidin-2-one hydrazone hydroiodide salts of the type **1–11** were treated with 2-oxo-2-furanacetic acid in refluxing *n*-butanol/DMF mixture in the presence of triethylamine to afford the corresponding novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**). The reaction conditions were established experimentally. The synthesis proceeded under mild conditions with relatively good overall yields (50–66%). Reaction of 1-aryl-2-hydrazonoimidazolidine derivatives with 2-oxo-2-furanacetic acid has not been reported in the literature yet. Thus, this reaction represents a novel method for the synthesis of the compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template.

The formation of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones has been proposed to proceed by a stepwise mechanism. In the first step of reaction, the



1: R = H; **2**: R = 2-CH_3; **3**: R = 4-CH_3; **4**: R = 2,3-$(CH_3)_2$; **5**: R = 2-CH_3O; **6**: R = 4-CH_3O; **7**: R = 2-Cl; **8**: R = 3-Cl, **9**: R = 4-Cl; **10**: R = 3,4-Cl_2; **11**: R = 2,6-Cl_2

Scheme 2. Synthetic pathway for the preparation of tautomeric 1-arylimidazolidin-2-one hydrazones (1-aryl-2-hydrazinoimidazolines) (**1**–**11**), employed as key building blocks for the synthesis of the title compounds. Reagents and conditions: (i) = hydrazine hydrate/MeOH, reflux, 24–30 h.

intermediate chain derivatives (depicted on Scheme 1 in the square bracket) appeared as a result of condensation of the appropriate 1-aryl-2-hydrazonoimidazolidines (**1–11**) with 2-oxo-2-furanacetic acid and concomitant loss of water molecule. Because of the presence of the hydrogen atom on the endocyclic *N*-3 nitrogen in structures of these intermediates, their subsequent cyclocondensation followed with concomitant loss of water molecule and finally afforded compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12–22**). The scrutiny of ^{1}H NMR, ^{13}C NMR and infrared (IR) data confirmed that under the reaction conditions, formation of the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one system of type **12–22** is accompanied with loss of water molecule. Additionally the concurrent course of cyclization reaction to the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazole derivatives (with concomitant loss of formic acid molecule) was excluded because no traces of these derivatives were detected.

In view of continuous search for novel and more efficient cytotoxic agents that show a profitable toxicity profile, the reported herein preparation route, leading to the formation of the desired compounds (**12–22**), might be considered as a useful synthetic method because of the affordability of the starting reagents and reactants, good yields obtained and straightforward product isolation. Furthermore, this efficient synthetic route allows one to obtain a large number of imidazotriazinone analogues.

2.2. Spectral characteristic of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22)

General structure for unknown imidazotriazinones (**12–22**) with atom numbering is shown in Figure 1. The numbering of the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one scaffold starts with a triazine N-1 nitrogen.

In the infrared (IR) spectra of the title compounds **12–22**, the presence of absorption bands in the region 1676–1691 cm^{-1} and 1548–1557 cm^{-1}, attainable to the triazine-C=O group and the C=N bond at the ring junction, respectively confirmed the formation of cyclic products, that is bicyclic structures with fully conjugated system. In the IR spectra of **12–22**, the difference between the frequency of the ring carbonyl group C=O and the frequency of the C=N bond at the ring junction was found to be in the range 128–140 cm^{-1}. These data are consistent with those previously reported by Le Count and Taylor,[15] since a similar difference between these frequencies in the infrared spectra of a wide range of fused 1,2,4-triazin-5-ones was observed strictly for fully conjugated system, where the C=N bond is contained within the triazinone ring. In addition, in the IR spectra of imidazotriazinones of the type **12–22**, the absorption band at around 1219 cm^{-1} was seen, due to the C–O–CH stretching vibration of the furanyl ring.

NMR spectral characteristic of the title compounds (**12–22**) revealed in their ^{1}H NMR spectra signals of the H-7 and H-6 (of both endocyclic methylene groups) as a multiplet in the region 3.98–4.36 ppm (compounds **12–18**, **20** and **22**), or as a singlet at δ 4.21 ppm (compounds **19** and **21**), integrating for four protons and four protons, respectively. Moreover, in the ^{1}H NMR spectra of all the investigated imidazotriazinones (**12–22**), the additional signals derived from the three-furanyl protons (H-4, H-3, H-5) were observed. The signals of the H-4 hydrogens of the furanyl ring were seen at δ values in the region 6.61–6.68 ppm, increasing in the order of substituents: 2,3-$(CH_3)_2$ < 2-CH_3 = 2-CH_3O < 2-Cl < 4-CH_3 = 4-CH_3O = 2,6-Cl_2 < H < 3-Cl < 4-Cl < 3,4-Cl_2. The signals of the H-3 hydrogens of the furanyl were found to be in the region 7.30–7.43 ppm, increasing in the order of R: 2,3-$(CH_3)_2$ = 2-CH_3O < 2-CH_3 < 4-CH_3O = 2-Cl < 4-CH_3 < 2,6-Cl_2 < H < 4-Cl < 3-Cl < 3,4-Cl_2. In turn, the δ values of the H-5 hydrogens of the furanyl were observed in the region 7.78–7.90 ppm, increasing in the order of R: 2,3$(CH_3)_2$ < 2-CH_3 = 2-CH_3O < 2-Cl < 2,6-Cl_2 < 4-CH_3O < 4-CH_3 < H = <3-Cl < 4-Cl < 3,4-Cl_2.

In the ^{13}C NMR spectra of the title compounds **12–22**, the chemical shift values of the C-7 and C-6 carbon atoms confirmed unequal character of both methylene groups as well (δ values in the region 44.8–47.6 ppm for C-7, and δ values in the region 40.0–41.3 ppm for C-6). In DEPT-135 experiment both methylene carbon atoms were seen as negative signals. The C-4, C-3 and C-5 tertiary carbons of the furanyl ring were seen at δ values ca. 111.2, 112.7 and 143.7 ppm, respectively whereas the C-2 quaternary carbons of the furanyl were observed at δ ca. 146.8 ppm. Additionally, the endocyclic triazine-C=O (triazine-C-4), triazine-C-3 and C-8a signals were seen at δ values ca. 140.2, 149.8 and 151.0 ppm, respectively.

Full spectral characteristic of the title compounds (**12–22**) is given in experimental protocols.

2.3. Determination of the lipophilicity and comparison between experimental and computational approaches

2.3.1. Lipophilicity of novel 3-(2-furanyl)-8-aryl-7,8-dihydro imidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22), expressed as their log k_w values

Lipophilicity as one of the most important physicochemical properties of the bioactive molecule is of high significance in the drug design, quantitative structure–activity relationship (QSAR)[16,17] and quantitative structure retention relationship (QSRR) studies. This molecular property affects penetration of cell membranes, blood–brain barrier distribution, drug-receptor interaction, drug absorption, excretion, metabolism, toxicity, etc.[18–26] The increasing hydrophobicity of a drug-like compound can be empirically correlated with the increase in biological activity.

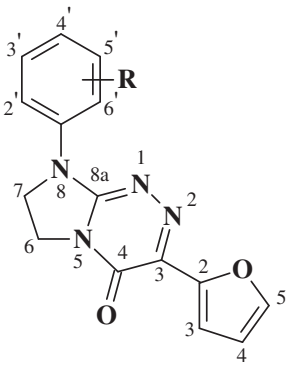


Figure 1. General structure for 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12**–**22**) with atom numbering.

Table 2
Terms of the equation $\log k = \log k_w - S\varphi$ for the investigated 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12**–**22**) on an octadecyl silica column (LC-18) with reversed mobile phases (methanol–water—20% acetate buffer pH 3.5—octane-1-sulfonic acid sodium salt (0.01 M L^{-1}))

Compd	*S* (slope)	log k_w	*r*	*F*	*s*	*n*
12	−4.01 (±0.33)	2.64 (±0.17)	0.9898	144.57	0.1055	5
13	−3.99 (±0.38)	2.48 (±0.20)	0.9869	112.21	0.1190	5
14	−4.58 (±0.38)	3.27 (±0.20)	0.9898	144.33	0.1207	5
15	−4.47 (±0.42)	3.00 (±0.22)	0.9869	112.04	0.1337	5
16	−4.17 (±0.43)	2.54 (±0.23)	0.9841	92.27	0.1372	5
17	−4.44 (±0.41)	2.89 (±0.21)	0.9873	115.54	0.1305	5
18	−4.30 (±0.38)	2.78 (±0.20)	0.9883	126.26	0.1209	5
19	−4.65 (±0.24)	3.45 (±0.14)	0.9947	377.68	0.1001	6
20	−4.66 (±0.25)	3.45 (±0.14)	0.9944	355.86	0.1033	6
21	−5.03 (±0.30)	4.01 (±0.19)	0.9946	276.65	0.0957	5
22	−4.27 (±0.25)	2.97 (±0.15)	0.9931	286.45	0.1055	6

The chromatographic techniques, in particular the reversed phase-high performance liquid chromatography (RP-HPLC) and the reversed phase-liquid chromatography (RP-TLC), that simulate the octanol–water partitioning,[27] are widely used for determination of the lipophilicity of various bioactive molecules.[18,20,26–38] These chromatographic approaches correlate the hydrophobicity of solutes with their retention parameters,[19,39,40] and have the extended measurable lipophilicity range of the partition coefficient (*P*) to 10^6 (in comparison to the traditional shake-flask method, having a limited measurable lipophilicity range of *P* from 10^{-2} to 10^4).[34] The partition coefficient between an aqueous and a stationary phase in reversed-phase liquid chromatography is strictly correlated with the lipophilicity of a solute investigated.[41,42]

In the HPLC series of experiments the newly synthesized 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**) were chromatographed on the octadecyl silica (ODS) stationary phase, using mixtures of water (H_2O) and the organic modifier-methanol (MeOH) as mobile phases. Methanol concentrations in the mobile phase were from 30% to 80%. Octane-1-sulfonic acid sodium salt (OSA-Na) and 20% acetate buffer (pH 3.5) was added to the mobile phase (eluent containing 0.01 M L^{-1} OSA-Na in organic modifier (MeOH)—buffered mobile phase).

The investigated compounds, such as heterocyclic bases are difficult for the chromatographic analysis. In the analysis of basic compounds anionic ion-pairing (IP) reagents, such as octane-1-sulfonic acid sodium salt, pentane-1-sulfonic acid sodium salt (PSA-Na), hexane-1-sulfonic acid (HSA), sodium dodecyl sulfate, alkyl sulfonates, forming neutral associates are widely used.[43–49] The neutral analyte form possesses higher affinity to the organic component of the mobile phase. Better correlation between log k_w and log *P* values are obtained in systems with ion-pairs, thereupon the easier movement of neutral associates (ion-pairs) to the organic phase. In mobile phases of a proper pH with addition of the pair-creative reagent the ion-pair between analyte ionized forms and the pair-creative reagent is appeared. Additional advantages of systems with ion-pairs are found to be both the improvements in peak shape for analytes and their symmetry. In eluent systems containing octane-1-sulfonic acid sodium salt, peaks of most of investigated analytes were found to be symmetric.

The experimental data obtained for reversed-phase HPLC system: the intercepts of regression curve (log k_w), slopes (*S*), regression coefficients (*r*), *F*-test values (of the Fisher test of significance), standard errors of estimate (*s*) and the number of points of concentration of modifier in eluent systems (*n*) calculated for the 95% confidence level are listed in Table 2.

The high values of regression coefficients ($r > 0.9841$) for all eleven solutes based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template proved the excellent fit between experimental data and the Soczewiński–Wachtmeister equation[50,51] (expressed in the form of the Snyder–Soczewiński relationship[52]) (Eq. 1):

$$\log k = \log k_w - S\varphi \quad (1)$$

where the log k_w corresponds to the capacity factor *k* for pure water as mobile phase, that is, $\varphi = 0$; *S* denotes the slope of regression plot and φ is the volume fraction of organic modifier in the mobile phase.

The values of the Fishers statistical significance test were always higher than the critical value *F*, thus proving additionally a good fit of the data with the examined model.

The retention data of presented herein-unknown imidazotriazinone derivatives have not been described in the literature yet and are the first time presented in this paper. The lipophilicity of all the solutes (**12–22**) determined by reversed-phase HPLC, and being based on extrapolation to $\varphi = 0$, is expressed as their log k_w values. The log k_w value, defined as the logarithm of capacity factor for pure water, is widely known as a lipophilicity parameter and is often used in QSAR studies.[37,53] The log k_w value proved to be useful when investigating a series of compounds or drugs covering a wide lipophilicity range[21,35] and helpful in the standardisation of the retention data. Furthermore, the correlation between the log k_w and the log $P_{octanol}$ values are considered as good within a series of closely related compounds.[21,54,55]

The determined log k_w values of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**) were found to be in the range 2.48–4.01. The relatively high values of the investigated solutes can be empirically correlated with their increased permeability through cell membranes, and therefore can play a crucial role in their antitumour activity.

In this series of closely related solutes (analogues), the lipophilicity was dependent both on the character of substituent and on the position of the substituent on the phenyl ring. The most lipophilic imidazotriazinone (**21**), containing the 3,4-dichloro substituent as *R* (with the two electron-withdrawing chlorine atoms), had the highest log k_w value, that is, 4.01. On the contrary, the compound **13** with *ortho*-CH_3 group at the phenyl ring, revealed the lowest lipophilicity (log $k_w = 2.48$). It was proved that the values of the log k_w for the investigated compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template increased in the following order of substituents: 2-CH_3 < 2-CH_3O < H < 2-Cl < 4-OCH_3 < 2,6-Cl_2 < 2,3-$(CH_3)_2$ < 4CH_3 < 3-Cl = 4-Cl < 3,4-Cl_2.

Lipophilicity was affected by the chloro substituent on the phenyl ring, and then the values of the log k_w increased in the order: 2-Cl < 2,6-Cl_2 < 3-Cl = 4-Cl < 3,4-Cl_2. Lipophilicity was also exerted by the methyl substituent as R, and then increased in the order: 2-CH_3 < 2,3-$(CH_3)_2$ < 4-CH_3. In case of the two compounds

(**16** and **17**), with the electron-donating methoxy group attached to the phenyl ring, the analysis of RP-HPLC results confirmed the higher lipophilicity of the *para*-methoxy derivative (log k_w = 2.89), comparatively to the *ortho*-methoxy analogue (log k_w = 2.54).

Generally, the determined values of the log k_w of the *para*- and the *meta*-substituted solutes were found to be higher than those of the *ortho*-substituted derivatives. Thus, the decreased lipophilicity values of the *ortho*-substituted compounds can be attributed to the steric effect. Presumably, the substituents in the *ortho* position at the phenyl ring display the steric hindrance forcing-out-of-plane free rotation, which decreases lipophilicity[53–56] of molecules (**13**, **15**, **16**, **18** and **22**).

The examined solutes: **19**, **20** and **21**, with the electron-withdrawing monochloro and dichloro substituents, revealed the largest log k_w values: 3.45 for both *meta*-chloro- and *para*-chloro-substituted derivatives and 4.01 for the 3,4-dichloro-substituted compound.

It was proved that adding the second chloro substituent to the *meta*-chloro- or the *para*-chloro-substituted derivative (**19** or **20**) resulted in noticeable lipophilicity increase. It is seen for the 3,4-dichloro compound (**21**), having the highest lipophilicity value (log k_w = 4.01). However, adding the second *ortho*-chloro group to the *ortho*-chloro-substituted derivative (**18**) resulted only in slight increase in lipophilicity. It is clearly seen in the case of the 2,6-dichloro-substituted analogue (**22**), that has subtly increased lipophilicity (log k_w value = 2.97) in comparison to the *ortho*-chloro analogue (log k_w = 2.78), supposedly due to the steric hindrance forcing-out-of-plane rotation of molecule, which decreases lipophilicity.

In comparing the influence of the 3,4-dichloro with a 2,6-dichloro substitution on the lipophilicity, the two *ortho*-substitutions decreased significantly the log k_w value. It has been proved for the 2,6-dichloro compound (**22**), revealing the decrease in lipophilicity by 1.04 (log k_w = 2.97), in comparison to the 3,4-dichloro analogue (**21**), with the log k_w of 4.01. The observed remarkable decrease in the lipophilicity may be explained due to the steric effect forcing out-of-plane rotation,[56–59] that is caused by the *ortho*–*ortho* substitution of the bulky halogen atoms in compound **22**.

Analysing the impact of the electron-donating methyl group on the retention indices, we found that *ortho*-methyl substitution (compound **13**) evoked only slight drop in lipophilicity (log k_w = 2.48), whereas *para*- methyl substitution (solute **14**) resulted in significant lipophilicity increase (log k_w = 3.27) in relation to parent compound **12**, revealing the log k_w of 2.64. Introducing the additional methyl substituent to the *ortho*-methyl derivative (**13**), having the log k_w of 2.48, resulted in noticeable lipophilicity increase. It is clearly seen for the 2,3-dimethyl compound (**15**), having the log k_w = 3.00.

Introducing the strong electron-donating methoxy substituent to the phenyl ring resulted in slight lipophilicity decrease in case of the *ortho*-CH_3O analogue (**16**) (log k_w = 2.54), whereas caused the significant lipophilicity increase in case of the *para*-CH_3O compound (**17**) (log k_w = 2.89), in relation to the parent compound (**12**), having a log k_w value of 2.64.

2.3.2. Comparison of determined log k_w values and calculated (log $k_{w\ (theoretical)}$) values

In view of Hansch and Fujita approach, based on the concept of hydrophobic substituent constants π, the trial was carried out with the purpose to derive the theoretical log k_w values for imidazotriazinone analogues that have substituents on the phenyl ring to compare them with those determined experimentally.

The above-mentioned Hansch and Fujita approach, allows one to calculate the partition coefficient for a structural analogue of the parent compound theoretically by knowing the substituent hydrophobicity constants π,[16,56,60–63] that is, the contribution that a given substituent makes to hydrophobicity, relative to hydrogen and having the determined experimentally value of the partition coefficient for the parent compound without substituent.[62] So calculated value of the partition coefficient for an analogue that have a given substituent can be mostly considered as the first approximation of that determined experimentally. For example, the calculated value of the logarithm of partition coefficient for such a simple structure as *para*-nitrotoluene is 2.41, whereas the experimentally determined log *P* value for this compound is found to be 2.37. The theoretical log *P* value for *para*-nitrotoluene is the sum of log *P* for benzene (being the parent compound) and the substituent constant values for CH_3 (0.56) and NO_2 (−0.28).[56]

We were able to calculate the theoretical log k_w values for the following substituted analogues of the examined imidazotriazinones: **13**, **14**, **15**, **16**, **17**, **18**, **19**, **20**, **21**, **22** (see Table 3), having the determined log k_w values for the parent compound, that is, the phenyl analogue (**12**) and known values of hydrophobicity constants π for CH_3, CH_3O and Cl for aromatic substituents (0.56, −0.02 and 0.71[56], respectively), using the Eq. 2:

$$\log k_{w(\text{theoretical})} = \log k_{w(\text{parent compound})} + \pi_{(\text{substituent})} \quad (2)$$

where the log $k_{w\ (theoretical)}$ is the theoretically calculated logarithm of partition coefficient for the compound with a substituent, log $k_{w\ (parent\ compound)}$ is the logarithm of partition coefficient for the parent compound and $\pi_{(substituent)}$ denotes the hydrophobicity constant π for a methyl, methoxy and chloro group (attached to phenyl ring).

Attempts were made to correlate both determined and theoretically calculated log k_w values.

Relationships between determined log k_w values and those theoretically calculated are shown in Figures 2 and 3. We were unable to derive a good correlation, in respect thereof all the substituted compounds (**13–22**), since then the regression coefficient (*r*) value is equal 0.5387 (see Fig. 2), due to the presence of four outliers

Table 3
Comparison of determined log k_w values and those theoretically calculated (log $k_{w\ (theoretical)}$ values)

Parent compd	Compd with substituent	log k_w for parent compd	log k_w for compd with substituent	Theoretical log k_w for compd with substituent
12	**13**	2.64	2.48	2.64 + 0.56 = 3.20
12	**14**	2.64	3.27	2.64 + 0.56 = 3.20
12	**15**	2.64	3.00	2.48 + 2 × 0.56 = 3.76
12	**16**	2.64	2.54	2.64 + (−0.02) = 2.62
12	**17**	2.64	2.89	2.64 + (−0.02) = 2.62
12	**18**	2.64	2.78	2.64 + 0.71 = 3.35
12	**19**	2.64	3.45	2.64 + 0.71 = 3.35
12	**20**	2.64	3.45	2.64 + 0.71 = 3.35
12	**21**	2.64	4.01	2.64 + 2 × 0.71 = 4.06
12	**22**	2.64	2.97	2.64 + 2 × 0.71 = 4.06

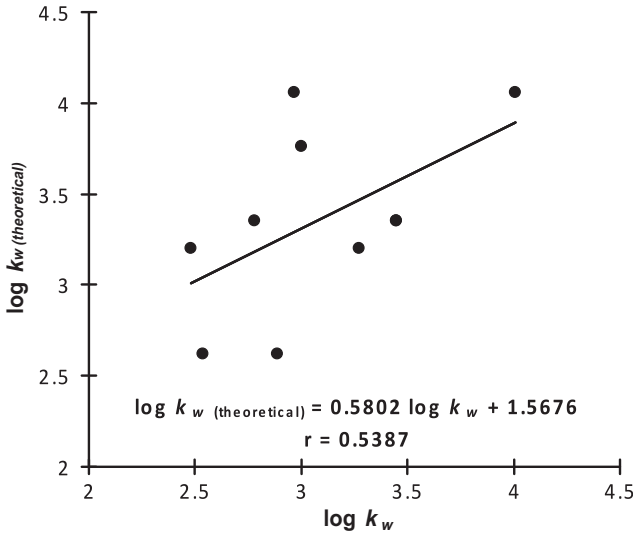


Figure 2. Correlation between measured log k_w values and those theoretically calculated (log $k_{w\ (theoretical)}$) for all the imidazotriazinone analogues that have substituents at the phenyl ring.

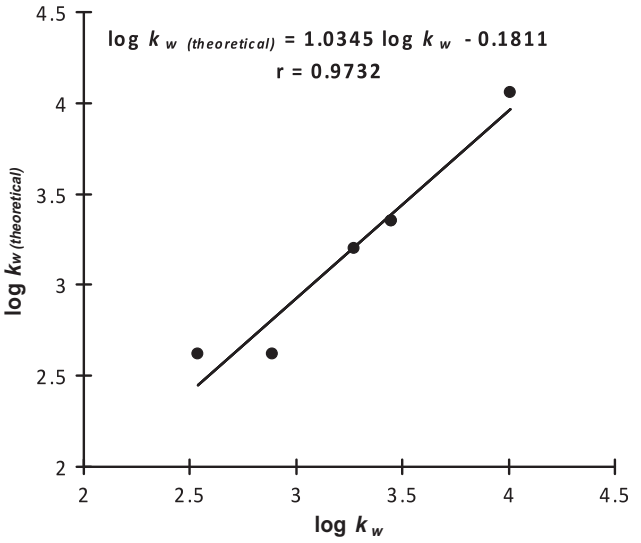


Figure 3. Correlation between experimental log k_w values and those theoretically calculated (log $k_{w\ (theoretical)}$) for substituted imidazotriazinone analogues after omission of four outliers (*ortho*-substituted derivatives: **13**, **15**, **18** and **22** for which the *ortho*-effect was distinctly marked).

(*ortho*-substituted derivatives: **13**, **15**, **18** and **22** for which the *ortho*-effect was distinctly marked). For these *ortho*-substituted compounds the determined log k_w values were found to be distinctly lower than those theoretically calculated using the substituent constants π of Hansch and Fujita.[56] Previously published examples suggest that substituents in the *ortho* position hinder the rotational freedom of the aromatic phenyl ring, which may decrease lipophilicity.[37,56–59] The regression equation obtained after omission of these four outliers was found to be: log $k_{w\ (theoretical)}$ = 1.0345 log $k_{w\ (experimental)}$ − 0.1811 and then the correlation coefficient was very good, r = 0.9732, that is illustrated in Figure 3.

2.3.3. Correlation between log k_w values (for methanol–water mobile phases on the octadecyl silica column (LC-18)) for the investigated imidazotriazinones and calculated log *P* values

The relationships between log k_w and log *P* values were also studied. Log *P* values were obtained from an ALOGPS 2.1. software[64–66] by the use of nine different computational methods for estimation of log *P*: MLOGP, ALOGPs, AClogP, ABlogP, miLogP, ALOGP, KOWWIN, XLOGP2 and XLOGP3. The graphs are drawn by plotting log *P* calculated values versus log k_w determined values. The correlation coefficients (r) are provided in Figure 4 and in Supplementary data (see Figs. 1–8). Results obtained from the reversed-phase HPLC were found to be the most different from those obtained by use of MLOGP (see Fig. 8 in Supplementary data). The predicted values of log *P* by use of AB log *P* algorithm revealed the best correlation with the experimental log k_w values for the investigated solutes, since a good correlation (r = 0.7760) between these quantities was found and shown in Figure 4. Lower correlations were observed between log *P* predicted by other computational methods and the chromatographically determined hydrophobicity parameter, log k_w. All these correlations are shown in Figures 1–8 in Supplementary data.

Both retention data and software calculated retention parameters indicated the 3,4-dichloro-substituted compound (**21**) as solute of the highest lipophilicity. For compounds **19**, **20**, **21** and **22**, that have 3-Cl, 4-Cl, 3,4-Cl_2 and 2,6-Cl_2 substituents, the determined log k_w values were found to be higher by 0.78, 0.88, 0.73 and 0.21, respectively, than those predicted on the basis of ABlogP method of calculation. Besides, for compounds **13**, **14** and **15**, with 2-CH_3, 4-CH_3 and 2,3-$(CH_3)_2$ substituents at the phenyl ring, the

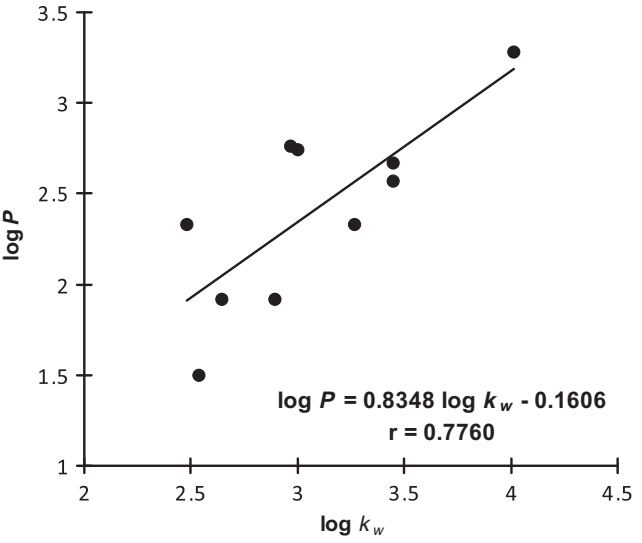
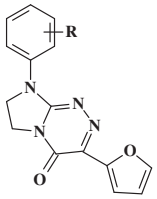


Figure 4. Correlation between log k_w values for reversed mobile phases (methanol–water—20% acetate buffer pH 3.5—octane-1-sulfonic acid sodium salt (0.01 M L^{-1})) on the octadecyl silica column (LC-18)) and calculated log *P* (ABlogP) values for the investigated imidazotriazinones.

determined log k_w values were higher by 0.15, 0.94 and 0.26, respectively than those from ABlogP method of calculation. In turn, for compounds **16** and **17**, with the *ortho*-CH_3O and the *para*-CH_3O substituents, the determined log k_w values were higher by 1.04 and 0.97, respectively, than those predicted on the basis of ABlog *P* method of calculation. The parent compound (**12**) that has unsubstituted phenyl ring revealed the log k_w value higher by 0.78 than that predicted on the basis of ABlogP method of calculation.

The lipophilicity of drug candidates should be determined experimentally, because of the imperfections in lipophilicity prediction.[26] A comparison between experimental log k_w values and those obtained by use of computational algorithms showed that none computational method was able to predict correctly the log *P* of these basic compounds. Therefore the presented herein reversed-phase HPLC technique can be regarded as the better

Table 4
Growth inhibition (GI) ratio of normal and tumour cells by the examined compounds in an effective concentration of 50 μg mL^{-1}



Compound	R	Incubation time (h)	Growth inhibition (%) in cell lines		
			HSF	HeLa	T47D
12	H	24	50	50	50
		48	50	75	85
		72	60	100	95
13	2-CH_3	24	0	25	25
		48	0	25	50
		72	0	50	75
14	4-CH_3	24	30	50	40
		48	50	50	60
		72	50	80	80
15	2,3-$(CH_3)_2$	24	0	60	0
		48	0	75	15
		72	0	90	25
16	2-OCH_3	24	0	25	0
		48	0	35	30
		72	5	80	70
17	4-OCH_3	24	10	80	50
		48	50	80	70
		72	60	95	80
18	2-Cl	24	10	80	5
		48	20	80	15
		72	20	100	25
19	3-Cl	24	25	80	20
		48	60	80	50
		72	75	95	80
20	4-Cl	24	50	50	0
		48	80	75	15
		72	90	90	15
21	3,4-Cl_2	24	0	50	10
		48	50	50	20
		72	80	95	40
22	2,6-Cl_2	24	10	80	10
		48	15	80	20
		72	15	95	25

Normal cell line: HSF, human skin fibroblast cells.
Cancer cell lines: HeLa (ECACC 93021013), human Negroid cervix epitheloid carcinoma cells; T47D (ECACC 85102201), human breast carcinoma cells.
An effective concentration of 50 μg mL^{-1} corresponds to following concentration values: 0.18 mM (**12**), 0.17 mM (**13**, **14**), 0.16 mM (**15**), 0.16 mM (**16**, **17**), 0.16 mM (**18**–**20**), 0.14 mM (**21**, **22**).

alternative in comparison to computational methods of lipophilicity prediction. The determined log k_w values for biologically active compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template can be regarded as their hydrophobic parameters. These ones are the first time presented and can be used in further QSAR studies.

2.4. Anticancer activities in vitro of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22)

The newly synthesized compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12–22**) were screened for their cytotoxicity against two tumour cell lines: HeLa (ECACC 93021013, human Negroid cervix epitheloid carcinoma cells) and T47D (ECACC 85102201, human breast carcinoma cells) in vitro. Furthermore, the primary cell line of normal human skin fibroblast (HSF) cells was included in the cytotoxicity study to estimate the selectivity profile of tested compounds. The obtained results are presented as growth inhibition (GI) ratio of normal and tumour cells by the examined compounds given in an effective concentration of 50 μg mL^{-1} after 24-, 48- and 72-h periods of incubation (see Table 4).

The majority of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones were found to be evidently effective in vitro against human cervix epitheloid carcinoma (HeLa) and human breast carcinoma (T47D) cells.

The highest cytotoxic potency against HeLa cancer cells was found for compound **18**, bearing the electron-withdrawing chloro substituent at *ortho*- position of the phenyl ring. This imidazotriazinone showed 80%, 80% and 100% growth inhibition against HeLa cells after 24, 48 and 72 h of incubation, respectively. Simultaneously, this evidently effective compound (**18**) revealed the distinctly marked lower cytotoxicity towards normal cell line—human skin fibroblast (HSF) cells and several times higher

against HeLa cancer cells. Therefore, imidazotriazinone of the type **18** might be considered as a basis for the design of novel low toxic anticancer agents.

In the case of the most potent 2-Cl derivative (**18**), adding a further chloro substituent at position 6 of the phenyl resulted in still evidently active and selective 2,6-Cl_2 compound (**22**). This one revealed the remarkable antitumour activity against HeLa cancer cells (80%, 80% and 95% growth inhibition after 24, 48 and 72 h of incubation, respectively), slightly improved activity against T47D cancer cells and slightly improved selectivity for cancer cells over normal cells, comparatively to **18**. In conclusion, the two promising cytotoxic agents of the type **18** and **22** demonstrated selective cytotoxicity for HeLa cancer cells over normal HSF cells. Therefore these potent compounds can be viewed as the lead structures for the design of novel low-toxic anticancer drugs.

Replacing the 2,6-Cl_2 substituent with a 3,4-Cl_2 group decreased the activity against HeLa cancer cells (50%, 50% and 95% growth inhibition after 24, 48 and 72 h of incubation, respectively) and resulted in increase of the cytotoxicity against T47D cancer cells after 72 h of incubation. It is clearly seen that this structural change was also responsible for the unwanted cytotoxicity increase towards normal HSF cells after 48- and 72-h periods of incubation. Thus, it was demonstrated that the two-chloro groups had to be retained in *ortho–ortho* positions of the phenyl ring rather than in *meta–para* positions in order to retain the selective action of the structure. Therefore, in this case, the 2,6-dichloro substitution in comparison to 3,4-dichloro substitution is found to be the better substitution pattern for the phenyl ring in this set of compounds.

Moving the chloro substituent to the *meta* position of the phenyl ring resulted in still evidently effective compound (**19**) against HeLa carcinoma cells (80%, 80%, 95% growth inhibition after 24, 48, 72 h of incubation, respectively). Furthermore, this structural change led to an increase in cytotoxicity for T47D cancer cells (20%, 50%, 80% growth inhibition after 24, 48, 72 h of incubation, respectively), comparatively to the 2-Cl analogue (**18**). Unfortunately, this structural change led to a fall in selectivity, demonstrating a significant cytotoxicity increase towards normal HSF cells. A chloro substituent *para* to the phenyl ring led to a slight drop in the potency against HeLa cancer cells (50%, 75%, 90% growth inhibition after 24-, 48-, 72-h periods of incubation, respectively) and to the cytotoxicity decrease against T47D cancer cells but also evoked a significant cytotoxicity increase towards normal HSF cells, as evidenced by the *para*-Cl compound (**20**), comparatively to **19**. In conclusion, it was demonstrated that the monochloro group had to be retained in the *ortho* position of the aromatic phenyl ring rather than *meta* or *para* positions in order to retain the selective action of the structure.

Three compounds: **17**, **19** and **22** proved to be evidently effective in vitro. All these derivatives revealed the same strong antiproliferative effects (80%, 80% and 95% growth inhibition against HeLa cancer cells after 24, 48 and 72 h of incubation, respectively). Furthermore the *para*-CH_3O derivative (**17**) and the *meta*-Cl analogue (**19**) exerted remarkable cytotoxic activities against T47D cancer cells (50%, 70%, 80% growth inhibition, and 20%, 50%, 80% growth inhibition after 24-, 48-, 72-h periods of incubation, respectively). Placing the methoxy group *ortho* to the phenyl ring led to decrease of the antiproliferative activity against HeLa and T47D cancer cells (25%, 35%, 80% growth inhibition and 0%, 30%, 70% growth inhibition after 24, 48, 72 h of incubation, respectively) but also resulted in a nearly complete loss of the cytotoxicity towards normal HSF cells as evidenced by the 2-CH_3O compound (**16**), comparatively to the 4-CH_3O analogue (**17**).

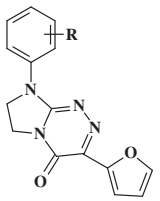
In turn, the highest cytotoxicity against human breast carcinoma (T47D) cells in the set of the tested novel 3-(2-furanyl) -8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones was found for compound **12**, bearing an unsubstituted, electron-rich phenyl ring, which seemed to be beneficial for activity. This potent small molecule proved to be effective both against T47D cancer cells (50%, 85% and 95% growth inhibition) and against HeLa cancer cells (50%, 75% and 100% growth inhibition) after 24, 48 and 72 h of incubation, respectively.

Compound **14** revealed noticeable inhibitory activities both in T47D cancer cells (40%, 60% and 80% growth inhibition) and in HeLa cancer cells (50%, 50% and 80% growth inhibition) after 24-, 48- and 72-h periods of incubation, respectively. Placing a methyl group *ortho* to the phenyl ring significantly decreased the potency against HeLa cancer cells but fortunately resulted in the completely loss of cytotoxicity towards normal HSF cells as evidenced by the *ortho*-CH_3 derivative (**13**). Introducing a further methyl substituent to the phenyl ring to give the 2,3-$(CH_3)_2$ derivative (**15**) was also tried. This resulted in a remarkable increase of the potency against HeLa cancer cells (60%, 75% and 90% growth inhibition after 24, 48 and 72 h of incubation, respectively) and simultaneously led to decrease of the activity against T47D cancer cells, comparatively to the 2-CH_3 analogue (**13**). It is worth noting that this structural change had no influence on the selectivity profile because compound **15** (so as **13**) was found to be completely non-toxic towards normal HSF cells.

Structure–activity relationships in the bioactive set of the compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template, led to heterocycles with remarkable antitumour activities in vitro. It is noteworthy that 2-chloro and 2,6-dichloro substitution was exactly the best choice of substitution pattern in the series of mono- and dichloro-substituted-phenyl analogues of 3-(2-furanyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one, as evidenced by the highly potent and low toxic compounds (**18** and **22**). In addition, it was proved that 2-methyl, 2,3-dimethyl and 2-methoxy substitution at the phenyl ring was essential for the selectivity of the examined structural analogues, as evidenced by imidazotriazinones of the type **13**, **15** and **16**.

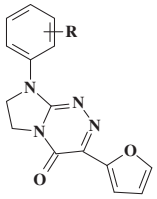
Two bioisosteric replacements of the methyl group that is placed both in the *ortho* and in the *para* position of the phenyl ring (compounds **13** and **14**, respectively) with a chloro substituent, of comparable size and lipophilicity, in place of the methyl, led to the active bioisosters (**18** and **20**, respectively) in vitro. The best balance of cytotoxicity versus more selectivity was achiewed with a methyl group. The *para*-methyl and the *ortho*-methyl substituents on the phenyl ring were found to be better than the *para*-chloro and the *ortho*-chloro groups for activity against T47D cells and

Table 5
The IC_{50} values against HeLa and T47D cancer cells for the most active imidazotriazinone **18** and for the parent compound **12**, calculated from the dose–response curves obtained after 24, 48 and 72 h of incubation



Compound	R	Incubation time (h)	IC_{50} values (in mM) in tumour cell lines	
			HeLa	T47D
12	H	24	0.18	0.18
		48	0.13	0.11
		72	0.10	0.10
18	2-Cl	24	0.11	>0.16
		48	0.11	>0.16
		72	0.09	>0.16

Table 6
Chemical structures and drug-likeness[68] of novel 3-(2-furanyl)-8-(phenyl or substituted-phenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12**–**22**) those lipophilicities were determined



Compd	R	Formula	log k_w[a] (<5)	H-bond donors[b] (<5)	H-bond acceptors[c] (<10)	MWT (<500)
12	H	$C_{15}H_{12}N_4O_2$	2.64	0	6	280.28
13	2-CH_3	$C_{16}H_{14}N_4O_2$	2.48	0	6	294.31
14	4-CH_3	$C_{16}H_{14}N_4O_2$	3.27	0	6	294.31
15	2,3-$(CH_3)_2$	$C_{17}H_{16}N_4O_2$	3.00	0	6	308.34
16	2-OCH_3	$C_{16}H_{14}N_4O_3$	2.54	0	7	310.31
17	4-OCH_3	$C_{16}H_{14}N_4O_3$	2.89	0	7	310.31
18	2-Cl	$C_{15}H_{11}ClN_4O_2$	2.78	0	6	314.73
19	3-Cl	$C_{15}H_{11}ClN_4O_2$	3.45	0	6	314.73
20	4-Cl	$C_{15}H_{11}ClN_4O_2$	3.45	0	6	314.73
21	3,4-Cl_2	$C_{15}H_{10}Cl_2N_4O_2$	4.01	0	6	349.17
22	2,6-Cl_2	$C_{15}H_{10}Cl_2N_4O_2$	2.97	0	6	349.17

[a] log k_w values (HPLC) determined on an octadecyl silica column (LC-18) with mobile phase of the type MeOH–H_2O according to the procedure reported in this paper (see Table 2).
[b] H-bond donors expressed as the sum of NHs and OHs.
[c] H-bond acceptors expressed as the sum of Ns and Os.

for decreased cytoxicity towards HSF cells. However, the *para*-chloro and the *ortho*-chloro substituents on the phenyl ring were better than a methyl group for activity against HeLa cells. From the point of view of further studies, the chloro substituent can be considered as resistant to oxidative biotransformation in vivo, whereas a methyl group as liable to oxidation.[67]

The IC_{50} values against HeLa and T47D carcinoma cells for the most active imidazotriazinone **18** and for the parent compound **12** calculated from the dose-response curves obtained after 24, 48 and 72 h of incubation from 5-bromo-2′-deoxyuridine (BrdU) assay were determined. These are listed in Table 5.

In conclusion, in particular, five novel compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template such as **13**, **15**, **16**, **18** and **22** demonstrate remarkable antiproliferative activities and selectivities, and therefore these may be considered as lead structures for the design of novel useful non-toxic (**13**, **15** and **16**) and low toxic (**18** and **22**) antitumour agents.

2.5. Drug-likeness of the investigated 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22)

Based on Lipinski's rule of five,[68] derived from an analysis of drug-like compounds from the World Drug Index database,[69] all the reported herein unknown small molecules based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12–22**) have all four parameters important for the favourable oral bioavailability: the determined log k_w values are less than 5, the molecular weight of all the compounds is under 500 Da, all the solutes have not more than 5 H-bond donors (expressed as the sum of NHs and OHs) and not more than 10 H-bond acceptors (expressed as the number of Ns and Os) (see Table 6), that would make them likely to be orally active agents. It is worth noting that all eleven solutes studied fulfil all the criteria described by rule of five. Thereby, the increased chances of good bioavailability in this unknown set of compounds make them promising biologically active agents.

3. Conclusion

In conclusion, by annulation of a 1,2,4-triazine ring to an imidazolidine moiety according to [4+2] pattern novel antitumour agents with selective toxicity for cancer cells over normal HSF cells were assembled. In view of continuous search for novel heterocycles with selective cytotoxicity for cancer cells over normal cells, the synthetic approach leading to the formation of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones, described in this paper, might be considered as a useful method for the preparation of these biologically active compounds because of the affordability of the starting materials, straightforward product isolation and good yields obtained. Furthermore, this efficient synthetic route allows one to produce a large number of imidazotriazinone analogues. The reversed-phase HPLC method was optimized and proved to be applicable and reliable for the analysis of eleven novel compounds based on the 7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one template (**12–22**). The high values of regression coefficients (r >0.9841) proved the excellent fit between experimental data and the equation: $\log k = \log k_w - S\varphi$. The obtained log k_w values can be regarded as hydrophobic parameters of the investigated solutes and these were found to be in the range of 2.48–4.01. The majority of novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones were found to be evidently effective in vitro against cancerous cells (HeLa and T47D). Among them, compounds **13**, **15**, **16**, **18** and **22** demonstrate the remarkable antiproliferative activity and selective cytotoxicity for cancer cells over normal human skin fibroblast cells. Therefore these ones may be considered as lead structures for the design of novel useful non-toxic (**13**, **15** and **16**) and low toxic (**18** and **22**) antitumour agents. The drug-likeness of all the compounds investigated was assessed on the basis of their structural properties by applying the Lipniski's rule of five. Fortunately, all the solutes investigated have all four parameters important for the favourable bioavailability.

4. Experimental protocols

4.1. Instrumentations and general materials

Chemicals (2-oxo-2-furanacetic acid, triethylamine) were purchased from Fluka as 'for synthesis grade' and used without further purification. Solvents (*n*-butanol, *N*,*N*-dimethylformamide) were purchased from E. Merck (Darmstadt, Germany). Melting points (mp) were determined on a Boetius apparatus and given uncor-

rected. The purity of each compound was established by thin-layer chromatography. TLC was carried out on commercial Merck SiO_2 60 F_{254} plates having fluorescence indicator. The spots were visualized in UV light $\lambda = 254$ nm and 355 nm. ^{1}H NMR and ^{13}C NMR spectra for compounds **12–22** were recorded on a Bruker Avance 300 MHz spectrometer in DMSO-d_6 with TMS as an external standard at 295 K. The infrared (IR) spectra for compounds **12–22** were determined in KBr disk using a Nicolet 8700 A FT-IR spectrometer (Thermo Scientific).

4.1.1. Synthesis of 1-aryl-2-hydrazinoimidazoline (1-aryl-2-hydrazonoimidazolidine) hydroiodides (1–11)

Compounds **2** and **4** have been synthesized and characterized, as previously described.[13] According to the same synthetic procedure compound **7** has been prepared that spectral characterization has been previously reported.[3] Applying the same synthetic approach compounds **1**, **3**, **5**, **6**, **8–11** have also been obtained and characterized as earlier described.[7]

4.1.2. General procedure for synthesis of 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (12–22)

A mixture of 1-aryl-2-hydrazonoimidazolidine hydroiodide (0.01 mol), 2-oxo-2-furanacetic acid (1.4 g, 0.01 mol) and triethylamine (1.4 mL) in *n*-butanol (20 mL) was heated under reflux with vigorously stirring for 15–55 minutes. After that time the precipitate appeared, while the solution was still boiling. Then appropriate amount of DMF was added until complete solid dissolution was achieved and the liquid reaction solution was still refluxed with stirring for 4–7 h. The reaction mixture was concentrated to ca. two-thirds its original volume by evaporation under reduced pressure. After cooling the mixture was refrigerated overnight, and during that time precipitation of the solid started. Then the formed crude product was filtered off, washed with cold methanol and finally purified by recrystallization from DMF/methanol mixture in the proportion indicated.

4.1.2.1. 3-(2-Furanyl)-8-phenyl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (12). Recrystallization from DMF/MeOH (4:1) mixture; yield 66%, mp 259–261 °C. Anal. Calcd for $C_{15}H_{12}N_4O_2$: C, 64.28; H, 4.32; N, 19.99. Found: C, 64.35; H, 4.30; N, 19.91. IR (KBr) (ν, cm^{-1}): 3135.23–3007.32 (CH), 2972.65–2888.72 ($2CH_2$), 1685.51 (triazine–C=O), 1602.25, 1584.28, 1504.76, 1494.32 (aromatic skeleton), 1552.57 (C=N), 1481.71–1453.94 ($2CH_2$), 1219.17 (furanyl C–O–C), 889.67, 753.82, 688.13 (monosubstituted benzene ring); ^{1}H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.18–4.25 (m, 4H, $2CH_2$), 6.65, 7.36, 7.85 (3H, furanyl H-4, H-3 and H-5), 7.14–7.89 (m, 5H, phenyl H); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 40.0 (C-6, CH_2), 44.8 (C-7, CH_2), 111.2 (CH, furanyl C-4), 113.1 (CH, furanyl C-3), 118.7 (CH), 123.2 (2CH), 128.3 (2CH), 138.5 (C), 140.4 (C-4), 143.8 (CH, furanyl C-5), 146.8 (C, furanyl C-2), 149.7 (C-3), 150.5 (C-8a).

4.1.2.2. 3-(2-Furanyl)-8-(2-methylphenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (13). Recrystallization from DMF/MeOH (4:1) mixture; yield 61%, mp 281–282 °C. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.40; H, 4.77; N, 19.11. IR (KBr) (ν, cm^{-1}): 3148.40–2988.51 (CH), 2958.93–2853.69 (CH_3, $2CH_2$), 1686.06 (triazine –C=O), 1604.43, 1499.69 (aromatic skeleton), 1552.47 (C=N), 1458.10–1380.52 ($2CH_2$, CH_3), 1218.84 (furanyl C–O–C), 761.69 (1,2-disubstituted benzene ring); ^{1}H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 2.28 (s, 3H, CH_3), 4.02–4.33 (m, 4H, $2CH_2$), 6.62, 7.31, 7.79 (m, 3H, furanyl H-4, H-3 and H-5), 7.34–7.46 (m, 4H, phenyl H); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 17.0 (CH_3), 41.0 (C-6, CH_2), 47.3 (C-7, CH_2), 111.1 (CH, furanyl C-4), 112.3 (CH, furanyl C-3), 126.4 (CH), 126.6 (CH), 127.6 (CH), 130.5 (CH), 135.6 (C), 136.2 (C), 139.6 (C-4), 143.4 (CH, furanyl C-5), 147.0 (C, furanyl C-2), 150.0 (C-3), 151.6 (C-8a).

4.1.2.3. 3-(2-Furanyl)-8-(4-methylphenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (14). Recrystallization from DMF/MeOH (5:1) mixture; yield 64%, mp 322–323 °C. Anal. Calcd for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.21; H, 4.80; N, 18.98. IR (KBr) (ν, cm^{-1}): 3152.29–3012.16 (CH), 2956.38–2858.23 (CH_3, $2CH_2$), 1687.08 (triazine –C=O), 1614.71, 1584.72, 1519.29, 1502.19, (aromatic skeleton), 1552.65 (C=N), 1479.40–1379.71 ($2CH_2$, CH_3), 1215.18 (furanyl C–O–C), 819.58 (1,4-disubstituted benzene ring); ^{1}H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 2.32 (s, 3H, CH_3), 4.13–4.24 (m, 4H, $2CH_2$), 6.64 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,4} = 3.38$ Hz, 1H, furanyl H-4), 7.26 (dd, $J = 8.5$ Hz, 2H, phenyl H-2′ and H-6′), 7.34 (dd, $J_{3,4} = 3.4$ Hz, $J_{3,5} = 0.7$ Hz, 1H, furanyl H-3), 7.75 (dd, $J = 8.6$ Hz, 2H phenyl H-3′ and H-5′), 7.84 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,5} = 0.8$ Hz, 1H, furanyl H-5), ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 19.8 (CH_3), 39.9 (C-6, CH_2), 44.9 (C-7, CH_2), 111.2 (CH, furanyl C-4), 112.9 (CH, furanyl C-3), 118.8 (2CH), 128.8 (2CH), 132.6 (C), 136.0 (C), 140.1 (C-4), 143.7 (CH, furanyl C-5), 146.8 (C, furanyl C-2), 149.7 (C-3), 150.5 (C-8a).

4.1.2.4. 3-(2-Furanyl)-8-(2,3-dimethylphenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (15). Recrystallization from DMF/MeOH (3:1) mixture; yield 57%, mp 236–238 °C. Anal. Calcd for $C_{17}H_{16}N_4O_2$: C, 66.22; H, 5.23; N, 18.17. Found: C, 66.13; H, 5.21; N, 18.23. IR (KBr) (ν, cm^{-1}): 3143.50–3012.04 (CH), 2952.53–2849.75 ($2CH_3$, $2CH_2$), 1675.95 (triazine –C=O), 1598.31, 1499.60 (aromatic skeleton), 1548.48 (C=N), 1475.03–1384.67 ($2CH_2$, CH_3), 1221.54 (furanyl C–O–C), 779.04, 764.37, 692.55 (1,2,3-trisubstituted benzene ring); ^{1}H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 2.16 (s, 3H, CH_3), 2.31 (s, 3H, CH_3), 3.98–4.33 (m, 4H, $2CH_2$), 6.61 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,4} = 3.4$ Hz, 1H, furanyl H-4), 7.17–7.28 (m, 3H, phenyl H), 7.30 (dd, $J_{3,4} = 3.4$ Hz, $J_{3,5} = 0.7$ Hz, 1H, furanyl H-3), 7.78 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,5} = 0.8$ Hz, 1H, furanyl H-5); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 13.5 (CH_3), 19.2 (CH_3), 40.9 (C-6, CH_2), 47.6 (C-7, CH_2), 111.1 (CH, furanyl C-4), 112.2 (CH, furanyl C-3), 124.2 (CH), 125.7 (CH), 128.9 (CH), 134.3 (C), 136.3 (C), 137.5 (C), 139.6 (C-4), 143.3 (CH, furanyl C-5), 147.1 (C, furanyl C-2), 150.0 (C-3), 151.8 (C-8a).

4.1.2.5. 3-(2-Furanyl)-8-(2-methoxyphenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (16). Recrystallization from DMF/MeOH (2:1) mixture; yield 58%, mp 248–250 °C. Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.80; H, 4.53; N, 18.12. IR (KBr) (ν, cm^{-1}): 3145.41–2979.62 (CH), 2943.53–2834.73 (CH_3O, $2CH_2$), 1679.60 (triazine –C=O), 1602.79, 1507.62 (aromatic skeleton), 1549.14 (C=N), 1464.15–1435.41 ($2CH_2$, CH_3O), 1223.35 (furanyl C–O–C), 746.91 (1,2-disubstituted benzene ring); ^{1}H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 3.83 (s, 3H, OCH_3), 4.00–4.30 (m, 4H, $2CH_2$), 6.62 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,4} = 3.4$ Hz, 1H, furanyl H-4), 7.01–7.52 (m, 4H, phenyl H), 7.30 (dd, $J_{3,4} = 3.4$ Hz, $J_{3,5} = 0.7$ Hz, 1H, furanyl H-3), 7.79 (dd, $J_{4,5} = 1.8$ Hz, $J_{3,5} = 0.8$ Hz, 1H, furanyl H-5); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 40.9 (C-6, CH_2), 46.7 (C-7, CH_2), 55.6 (CH_3O), 111.1 (CH, furanyl C-4), 112.3 (CH, furanyl C-3), 112.8 (CH), 120.3 (CH), 126.1 (C), 127.9 (CH), 128.5 (CH), 139.8 (C-4), 143.3 (CH, furanyl C-5), 147.1 (C, furanyl C-2), 149.9 (C-3), 152.0 (C-8a), 154.6 (C).

4.1.2.6. 3-(2-Furanyl)-8-(4-methoxyphenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (17). Recrystallization from DMF/MeOH (5:1) mixture; yield 60%, mp 275–277 °C. Anal. Calcd for $C_{16}H_{14}N_4O_3$: C, 61.93; H, 4.55; N, 18.06. Found: C, 61.85; H,

4.57; N, 17.99. IR (KBr) (ν, cm^{-1}): 3119.32–2985.17 (CH), 2934.32–2835.58 (CH_3O, $2CH_2$), 1690.83 (triazine –C=O), 1614.86, 1589.27, 1513.05 (aromatic skeleton), 1556.07 (C=N), 1481.55–1438.09 ($2CH_2$, CH_3O), 1219.27 (furanyl C–O–C), 832.40 (1,4-disubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 3.78 (s, 3H, OCH_3), 4.12–4.25 (m, 4H, $2CH_2$), 6.64, 7.33, 7.83 (3H, furanyl H-4, H-3 and H-5), 7.02 (dd, J = 9.1 Hz, 2H, phenyl H-2′ and H-6′, 7.75 (dd, J = 9.1 Hz, 2H, phenyl H-3′ and H-5′); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 40.0 (C-6, CH_2), 45.4 (C-7, CH_2), 55.0 (CH_3O), 111.2 (CH, furanyl C-4), 112.7 (CH, furanyl C-3), 113.9 (2CH), 120.9 (2CH), 131.6 (C), 140.0 (C-4), 143.6 (CH, furanyl C-5), 146.9 (C, furanyl C-2), 149.8 (C-3), 150.6 (C-8a), 155.7 (C).

4.1.2.7. 3-(2-Furanyl)-8-(2-chlorophenyl)-7,8-dihydroimidazo [2,1-*c*][1,2,4]triazin-4(6*H*)-one (18). Recrystallization from DMF/MeOH (6:1) mixture; yield 59%, mp 286–288 °C. Anal. Calcd for $C_{15}H_{11}ClN_4O_2$: C, 57.24; H, 3.52; Cl, 11.26; N, 17.80. Found: C, 57.15; H, 3.50; Cl, 11.31; N, 17.88. IR (KBr) (ν, cm^{-1}): 3148.12–2993.92 (CH), 2962.16–2904.93 ($2CH_2$), 1686.07 (triazine –C=O), 1591.45, 1499.86 (aromatic skeleton), 1552.54 (C=N), 1458.15–1441.63 ($2CH_2$), 1217.75 (furanyl C–O–C), 1080.57 (aromatic C–Cl), 760.51 (1,2-disubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.05–4.36 (m, 4H, $2CH_2$), 6.63, 7.33, 7.81 (3H, furanyl H-4, H-3 and H-5), 7.43–7.70 (m, 4H, phenyl H; ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 41.1 (C-6), 47.0 (C-7), 111.2 (CH, furanyl C-4), 112.7 (CH, furanyl C-3), 127.8 (CH), 129.3 (CH), 129.4 (CH), 129.8 (CH), 131.2 (C), 135.0 (C), 140.2 (C-4), 143.6 (CH, furanyl C-5), 146.8 (C, furanyl C-2), 149.8 (C-3), 151.7 (C-8a).

4.1.2.8. 3-(2-Furanyl)-8-(3-chlorophenyl)-7,8-dihydroimidazo [2,1-*c*][1,2,4]triazin-4(6*H*)-one (19). Recrystallization from DMF/MeOH (4:1) mixture; yield 54%, mp 277–279 °C. Anal. Calcd for $C_{15}H_{11}ClN_4O_2$: C, 57.24; H, 3.52; Cl, 11.26; N, 17.80. Found: C, 57.39; H, 3.48; Cl, 11.22; N, 17.73. IR (KBr) (ν, cm^{-1}): 3159.92–3001.80 (CH), 2968.44–2899.90 ($2CH_2$), 1694.03 (triazine –C=O), 1596.57, 1576.00, 1496.66 (aromatic skeleton), 1556.69 (C=N), 1445.80–1421.13 ($2CH_2$), 1219.14 (furanyl C–O–C), 1096.49 (aromatic C–Cl), 883.90, 742.72, 698.37 (1,3-disubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.21 (s, 4H, $2CH_2$), 6.66, 7.39, 7.87 (3H, furanyl H-4, H-3 and H-5), 7.19- 8.19 (m, 4H, phenyl H); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 40.0 (C-6, CH_2), 44.7 (C-7, CH_2), 111.3 (CH, furanyl C-4), 113.5 (CH, furanyl C-3), 116.7 (CH), 118.2 (CH), 122.7 (CH), 129.9 (CH), 133.0 (C), 139.8 (C), 140.7 (C-4), 144.0 (CH, furanyl C-5), 146.6 (C, furanyl C-2), 149.6 (C-3), 150.3 (C-8a).

4.1.2.9. 3-(2-Furanyl)-8-(4-chlorophenyl)-7,8-dihydroimidazo [2,1-*c*][1,2,4]triazin-4(6*H*)-one (20). Recrystallization from DMF/MeOH (5:1) mixture; yield 60%, mp 319–320 °C. Anal. Calcd for $C_{15}H_{11}ClN_4O_2$: C, 57.24; H, 3.52; Cl, 11.26; N, 17.80. Found: C, 57.44; H, 3.54; Cl, 11.29; N, 17.77. IR (KBr) (ν, cm^{-1}): 3157.09–2996.01 (CH), 2926.80–2852.55 ($2CH_2$), 1690.33 (triazine –C=O), 1581.33, 1497.17 (aromatic skeleton), 1551.61 (C=N), 1414.43–1395.80 ($2CH_2$), 1217.66 (furanyl C–O–C), 1091.23 (aromatic C–Cl), 829.03 (1,4-disubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.17–4.25 (m, 4H, $2CH_2$), 6.67 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-4 furanyl), 7.38 (dd, $J_{3,4}$ = 3.4 Hz, $J_{3,5}$ = 0.8 Hz, 1H, H-3 furanyl), 7.52 (dd, J = 9.1 Hz, 2H, ar.: H-2′ and H-6′), 7.88 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,5}$ = 0.8 Hz, 1H, H-5 furanyl), 7.93 (dd, J = 9.1 Hz, 2H ar: H-3′ and H-5′); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 40.0 (C-6, CH_2), 44.8 (C-7, CH_2), 111.3 (CH, furanyl C-4), 113.3 (CH, furanyl C-3), 120.2 (2CH), 127.2 (C), 128.2 (2CH), 137.4 (C), 140.6 (C-4), 143.9 (CH, furanyl C-5), 146.7 (C, furanyl C-2), 149.6 (C-3), 150.3 (C-8a).

4.1.2.10. 3-(2-Furanyl)-8-(3,4-dichlorophenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (21). Recrystallization from DMF/MeOH (8:1) mixture; yield 63%, mp 343–344 °C. Analysis Calcd for $C_{15}H_{10}Cl_2N_4O_2$: C, 51.60; H, 2.89; Cl, 20.31; N, 16.05. Found: C, 51.46; H, 2.90; Cl, 20.23; N, 15.99. IR (KBr) (ν, cm^{-1}): 3145.86–2961.62 (CH), 2923.95–2852.49 ($2CH_2$), 1690.99 (triazine –C=O), 1595.01, 1570.83, 1497.49 (aromatic skeleton), 1551.49 (C=N), 1481.29–1395.47 ($2CH_2$), 1219.64 (furanyl C–O–C), 1072.87 (aromatic C–Cl), 884.54, 838.02, 698.65 (1,2,4-trisubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.21 (s, 4H, $2CH_2$), 6.68 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, H-4 furanyl), 7.40 (dd, $J_{3,4}$ = 3.4 Hz, $J_{3,5}$ = 0.8 Hz, 1H, H-3 furanyl), 7.89 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,5}$ = 0.8 Hz, 1H, H-5 furanyl), 7.70–8.36 (m, 3H, phenyl H); ^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 40.0 (C-6, CH_2), 44.7 (C-7, CH_2), 111.3 (CH, furanyl C-4), 113.7 (CH, furanyl C-3), 118.3 (C), 120.0 (CH), 124.9 (CH), 130.1 (CH), 131.0 (C), 138.5 (C), 140.9 (C-4), 144.1 (CH, furanyl C-5), 146.6 (C, furanyl C-2), 149.5 (C-3), 150.2 (C-8a).

4.1.2.11. 3-(2-Furanyl)-8-(2,6-dichlorophenyl)-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-one (22). Recrystallization from DMF/MeOH (4:1) mixture; yield 50%, mp 279–281 °C. Anal. Calcd for $C_{15}H_{10}Cl_2N_4O_2$: C, 51.60; H, 2.89; Cl, 20.31; N, 16.05. Found: C, 51.68; H, 2.87; Cl, 20.26; N, 16.11. IR (KBr) (ν, cm^{-1}): 3154.64–3016.26 (CH), 2981.97–2895.54 ($2CH_2$), 1686.61 (triazine –C=O), 1590.05, 1504.37 (aromatic skeleton), 1552.58 (C=N), 1481.04–1456.43 ($2CH_2$), 1220.02 (furanyl C–O–C), 1102.40 (aromatic C–Cl), 782.46, 758.15, 697.19 (1,2,3-trisubstituted benzene ring); ^1H NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz): 4.06–4.35 (m, 4H, $2CH_2$), 6.64 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,4}$ = 3.4 Hz, 1H, furanyl H-4), 7.35 (dd, $J_{3,4}$ = 3.4 Hz, $J_{3,5}$ = 0.70 Hz, 1H, furanyl H-3), 7.51–7.85 (m, 3H: phenyl 3H); 7.82 (dd, $J_{4,5}$ = 1.8 Hz, $J_{3,5}$ = 0.7 Hz, 1H, furanyl H-5);^{13}C NMR (δ, ppm, DMSO-d_6, TMS, 300 MHz) 41.3 (C-6, CH_2), 46.9 (C-7, CH_2), 111.2 (CH, furanyl C-4), 113.0 (CH, furanyl C-3), 129.0 (CH), 129.2 (CH), 129.9 (C), 131.2 (CH), 131.7 (C), 136.4 (C), 140.5 (C-4), 143.7 (CH, furanyl C-5), 146.8 (C, furanyl C-2), 149.7 (C-3), 151.7 (C-8a).

4.1.3. HPLC experimental

Novel 3-(2-furanyl)-8-aryl-7,8-dihydroimidazo[2,1-*c*][1,2,4]triazin-4(6*H*)-ones (**12–22**) were analyzed with methanol (from 30% to 80%)—20% acetate buffer pH 3.5—octane-1-sulfonic acid sodium salt (0.01 M L^{-1}) (v/v/v) as mobile phases at 22 °C, using an Agilent Technologies 1200 series chromatograph equipped with a quaternary gradient pump with a degasser set at a flow rate of 1 mL/min and with a diode array detector (DAD). Eluates were injected into the eluent with a Rheodyne 20 μL injector. The HPLC apparatus was equipped with a ZORBAX Eclipse XDB-C18 150 mm × 4.6 mm column, 5-μm particle size (Agilent Technologies, Wilmington, DE, USA). Methanol used as the mobile phase modifier was of HPLC grade from C. Roth (Karlsruhe, Germany). Octane-1-sulfonic acid sodium salt (OSA-Na) and acetate buffer were from Merck (Darmstadt, Germany). Double distilled water purified with the Millipore system was used. The pH of buffer used in experiments, that is, 0.2 M acetate was measured for the aqueous solution.

4.1.4. Inhibition of tumour cell growth assay

All newly synthesized imidazotriazinones of the type **12–22**, were evaluated for their cytotoxic properties in vitro against two human tumour cell lines: HeLa (ECACC 93021013, human Negroid cervix epitheloid carcinoma cells) and T47D (ECACC 85102201, human breast carcinoma cells) in vitro. Furthermore, the primary cell line of normal human skin fibroblasts was included in the cytotoxicity study.

In the current protocol each cell line was inoculated at 10^4 cells per mL density on microtiter plates. Test agents were then added at the examined concentration 50 μg mL^{-1} and cultures were incubated at standard conditions (37 °C, 5% CO_2, 90% humidity) for 24, 48 and 72 h. End-point determinations were made with 5-bromo-2′-deoxyuridine (BrdU) labeling and detection kit III[70–73] on Elisa reader (BIO-TEC Instruments USA).

The growth percentage of normal and carcinoma cells was evaluated spectrophotometrically versus untreated controls. Results for each spectrophotometrical measure were noticed as per cent of growth inhibition. All the investigated compounds were dissolved in DMSO prior to dilution into the biological assay. The solvent (DMSO) in the highest concentration used in the bioassay did not reveal any cytotoxic activity. The obtained results are presented as growth inhibition (GI) ratio of normal and tumour cells by the examined compounds given in an effective concentration of 50 μg mL^{-1} after 24-, 48- and 72-h periods of incubation. All experiments were done in triplicates.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.07.027.

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