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Research paper

New 3′-*O*-aromatic acyl-5-fluoro-2′-deoxyuridine derivatives as potential anticancer agents

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ABSTRACT

New aromatic and aliphatic 3′-*O*-acyl-5-fluoro-2′-deoxyuridine derivatives were synthesized and evaluated as candidates for prodrugs against various cancer cell lines. As the most promising candidate for antimalignant therapeutics was found a dual-acting acyl derivative **7h**, which apparently released not only the known anticancer nucleoside, 5-fluoro-2′-deoxyuridine (FdU), but also an additional active metabolite, acetylsalicylic acid, reinforcing thus therapeutic effect of FdU. Promising therapeutic indices showed also some aromatic dicarboxylic acids derivatives decorated with FdU esters (**11** and **12**).

1. Introduction

5-Fluoro-2′-deoxyuridine (FdU) as an antimetabolite has been discovered nearly 60 years ago [1] and after approval by FDA in 1970, it has been widely used in anticancer therapy [2]. Cytostatic activity of FdU is due to depletion of thymidine 5′-triphosphate (TTP) concentration as a result of thymidylate synthase inhibition. To acquire this activity, FdU has to be first phosphorylated to 5-fluoro-2′-deoxyuridine-5′-phosphate (FdUMP) that is a true and the specific enzyme inhibitor [3]. Unfortunately, efficiency of the FdU transformation into FdUMP in cell is hampered by a simultaneous cleavage of the glycosyl bond of the nucleoside by thymidine phosphorylase that releases nucleobase 5-fluorouracil (FUra). To regain its anticancer activity FUra has to go again through the whole biosynthetic cycle from nucleobase via FdU to FdUMP, and this diminished anticancer potency of the original FdU drug [4].

Although clinically valuable and effective, FdU discloses off target side-effects in the gastrointestinal tract and bone marrow that may pose problems during therapy [5–7]. In addition, due to low oral absorption, FdU requires expensive and often harmful intravenous infusion [5]. To improve bioavailability of FdU, the prodrug approach was recently explored for its delivery [8–10], notably by using 3′, 5′-*O*-di-acyl and 3-*N*, 3′-, 5′-*O*-acyl esters, that in several instances improved anticancer potency and therapeutic value of FdU. The most important feature of these prodrugs was an ability to modulate their conversion into a biologically active drug in physiological milieu (via chemical and enzymatic hydrolysis [11,12]), solubility and physicochemical properties in aqueous environment, and cellular uptake (cellular membrane interaction and transmembrane transport). Recent papers have shown [10,13] that simple acyl esters of FdU possess the presupposed features in terms of lipophilicity, resistance to chemical hydrolysis, and variation in susceptibility to enzymatic hydrolysis. Although none of the newly synthesized compounds delivered outstanding anticancer properties, several valuable conclusions could be drawn from those studies. The most interesting observation was that lipophilicity, commonly accepted to be responsible for the transmembrane transport of a compound, although important, can't be correlated directly with the observed cytostatic activity. Despite of

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[1] Contribution of both authors was equal.

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intensive studies on FdU acyl esters [8,11,12,14–16], none of the 3′-*O*- or 5′-*O*-acyl-FdU derivatives found application in anticancer therapy yet. However, several trends in modulation of cytostatic activity of acyl-FdU as function of the acyl group used could be distinguished. In general, presence of an acyl group usually increases total lipophilicity of the compound and often improves its IC_{50}, and thus can be used for tuning stability in physiological-like media (RPMI/FBS). The same seems to be true for amino acid derivatives of FdU [10,13,17] but in these cases, active intracellular transport emerged as an additional factor strongly affecting cytostatic activity. It should be also mentioned that 3′-carboxylic ester of 5-fluoro-2′-deoxyuridine bearing tumor-homing cyclic peptides CNGRC [18] or plasminogen activator inhibitor of type II (PAI-2) [19] disclose very selective cytostatic activity when targeting the tumor marker APN/CD13 of HT-1080 tumor cell lines and urokinase overexpressing malignant cells.

Inspired by the studies on 3′-*O*-retinoyl-5-fluoro-2′-deoxyuridine [20,21] as a dual-acting anticancer nucleosidic compound, we designed 3′-*O*-acyl-FdU bearing esters of carboxylic acids, that by simple chemical or carboxylesterase-assisted hydrolysis can generate additional active metabolites, e.g. lipoic acid [22–24], acetylsalicylic acid [25–29], and indazole-3-carboxylic acid derivative (lonidamine [30,31]) reinforcing thus therapeutic effect of FdU. Modulation of FdU anticancer potency of the compounds designed herein was empowered by a set of aromatic multi carboxylic acid esterified with FdU that supposedly may serve as an effective vehicle delivering in a form of one conjugated molecule more than one of cytostatic metabolite.

2. Results and discussion

2.1. Chemistry

2.1.1. 3′-O-aromatic acyls-5-fluoro-2′-deoxyuridines

The starting material for the synthesis of all 3′-*O*-acyl-5-fluoro-2′-deoxyuridines of type **7** (Scheme 1) was 5′-*O*-(4,4′-dimethoxytrityl)-5-fluoro-2′-deoxyuridine, obtained by a classical Khorana's procedure [32,33]. The choice of methods for the introduction of acyl groups into the 3′-*O*-position of 5-fluoro-2′-deoxyuridine based exclusively on simplicity of the procedure and commercial availability of the acylating reagents. Thus, 3′-*O*-acylated nucleosides **7a**–**h** bearing aromatic acyls were obtained by acylation of suitably protected 5-fluoro-2′-deoxyuridine derivative **1** with acyl chlorides **2**. To this end 5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine **1** was dissolved in pyridine and treated with the respective acylating agent of type **2** (2 M equiv.) until the nucleosidic substrate reacted completely (ca 2 h at room temp.). Inspection of the reaction mixtures by TLC analysis usually revealed the presence of two acylated compounds, 3′-*O*-monoacyl nucleoside of type **5** and, most likely, 3′-*O*, 3-*N*-bisacyl nucleoside of type **6** (^{1}H and ^{13}C NMR spectroscopy analysis).

Formation of the bisacylated by-products **6** was studied in detail using benzoyl chloride **2a** as an acylating agent in pyridine [34]. The structure of the isolated **6a** was unambiguously assigned as a 3′-*O*, 3-*N*-bisacyl derivative on the basis of comparative ^{13}C NMR spectroscopic analysis of compounds **5a**, **6a** and **7a** [35,36]. The use of a stoichiometric amount of acylating reagent **2a** reduced significantly formation of the undesired *N*-acylation products, but at the same time a considerable portion of the starting nucleoside **1** remained unreacted.

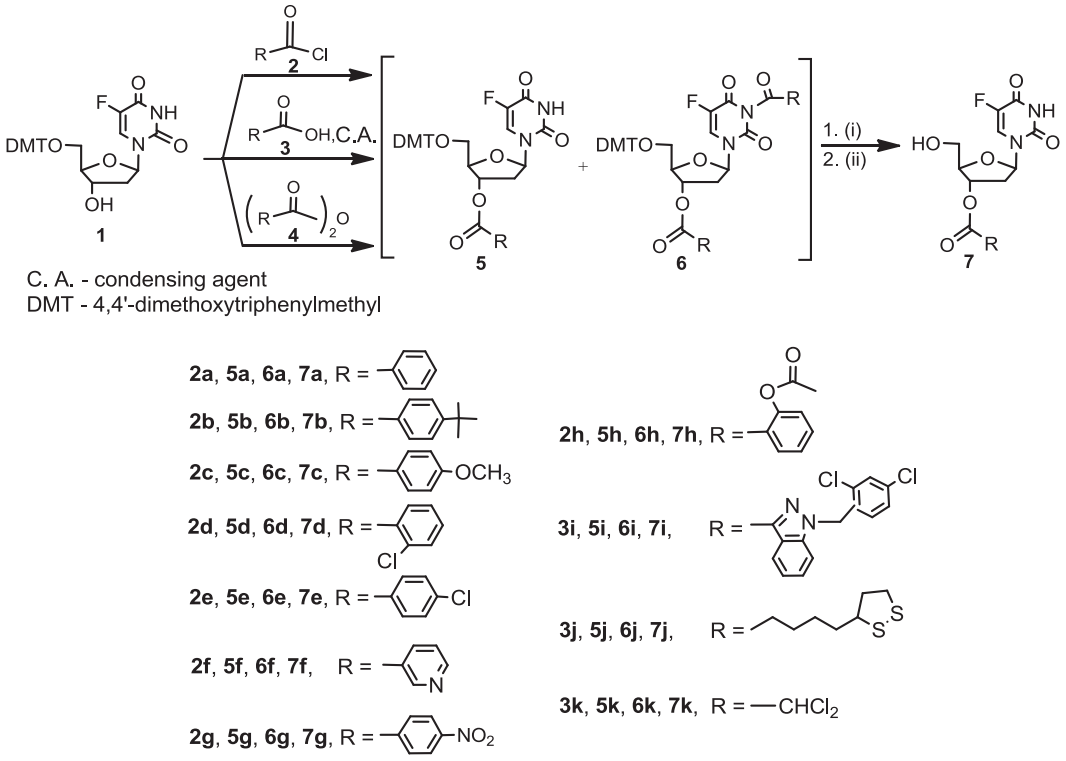
Considering economy of the procedure and rather high costs of 5-fluoro-2′-deoxyuridine, we chose an approach in which the use of higher excess of benzoyl chloride (2 M equiv.) resulted in complete consumption of the starting nucleoside **1**, and then the formed *N*-acylated by-products **6** were attempted to convert into the desired products **5**. To this end, the crude reaction mixture containing products **5a** and **6a** was treated for 30 min with 3 M excess of morpholine in methylene chloride (DCM). It was rewarding to observe that under these conditions 3-*N*-benzoyl group was removed selectively from bisacylated product **6a** without affecting 3′-*O*-benzoyl ester group in **5a**. This procedure was found to be also effective for other *N*-acylated products **6a-g** having different aromatic acyl groups, and allowed to obtain the desired 3′-*O*-acyl-5-fluoro-2′-deoxyuridines **7a-g** in satisfactory overall yields (>70%, vide Experimental) [37,38].

During synthesis of **7h**, however, somewhat unexpectedly acylation of nucleoside **1** with 2-acetylsalicylyl chloride **2h** in pyridine afforded several products (TLC analysis). Treatment of such reaction mixture with morpholine (to remove possible *N*-acyl groups), followed by 80% acetic acid aq. (to remove the 5′-*O*-DMT group) produced two compounds (ratio ca 1:1), which after purification were identified (^{1}H, ^{13}C NMR, and HRMS analyses) as target product **7h** and 3′-*O*-(2-acetylsalicylyl)salicylyl derivative **8** (Fig. 1). To explain formation of compound **8** we considered two scenarios, with a key role played by pyridine. In the first one, the initially formed product **5h** was assumed to undergo a pyridine-catalyzed transacylation of the acetyl group in the 2-acetylsalicyl moiety by excess of acyl chloride **2h** to produce **8** [39], and in the second one, a similar phenomenon (a pyridin-catalyzed transacylation of the acetyl group) could occur in 2-acetylsalicylyl chloride **2h** itself, prior to acylation of nucleoside **1** [40]. Since the second scenario appeared to be more likely, in order to avoid formation of salicylylsalicylated products of type **8** the reactions of **1** with **2h** was carried out in methylene chloride (DCM), in the absence of pyridine. The acylation was slow (completion overnight) and at the end of the reaction, the TLC analysis revealed, apart from 3′-*O*-acylated-, and 3′-*O*,3-*N*-bisacylated products, **5h** and **6h**, formation of some side products due to instability of the DMT group under the reaction conditions. To remedy this additional problem we added to the reaction mixture 2,6-lutidine as moderately strong (pK_a 6.60) [41] non-nucleophilic base (6 M equiv.). This secured fast (3 h), clean, and quantitative conversion of nucleoside **1** in the reaction with **2h** into 2-acetylsalicylated products **5h** and **6h**. These, after a successive treatment with morpholine and 80% acetic acid, afforded after purification the desired 3′-*O*-(2-acetylsalicylyl)-5-fluoro-2′-deoxyuridine **7h** in 68% yield. The only disadvantage of this approach was a tedious procedure for the removal of lutidine before the deprotection of the DMT group (at least five extractions with phosphoric buffer pH 4.0). This inconvenience could be alleviated by using stronger, non-nucleophilic base, namely diisopropylethylamine (DIPEA, pK_a 11.44) [42] (3 M equiv.) and this resulted also in higher isolated yield of **7h** (79%).

2.1.2. Double-barrelled 3′-O-acyl-5-fluoro-2′-deoxyuridines

Compound **7h** was first in the series of double barrelled compounds (**7h-k**) bearing additional to FdU pharmacophore such as lonidamine, lipoic acid, and dichloroacetic acid. With the exception of dichloroacetyl derivative **7k**, for the synthesis of the other compounds we chose an approach in which dimethoxytritylated fluorodeoxyuridine **1** was acylated with the corresponding carboxylic acid (**3i** and **3j** respectively) activated with *N*-ethyl-*N*′-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) in the presence of 4-(dimethylamino)pyridine (DMAP) (Scheme 1). The choice of EDC over other carbodiimides [e.g. *N*,*N*′-dicyclohexylcarbodiimide [43] (DCC), *N*,*N*′-diisopropylcarbodiimide [44] (DIC) or unsymmetrical *N*-cyclohexyl-*N*′-isopropylcarbodiimide [45]] as carboxylic acids activator was based on its proven efficacy in similar reactions of lonidamine with various carbohydrates, trouble-free work-up, and simple isolation procedure of the final products [46].

In a typical procedure 5′-protected nucleoside **1** and the desired



Reagents and conditions: (i) morpholine, 3 molar equiv.; (ii) 80% acetic acid aq.

Scheme 1. Synthesis of 3′-*O*-acyl 5-fluoro-2′-deoxyuridines.

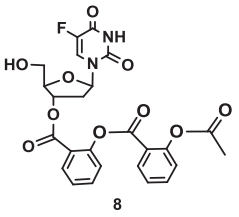


Fig. 1. A side product formed during acylation of **1** with 2-acetylsalicyl chloride.

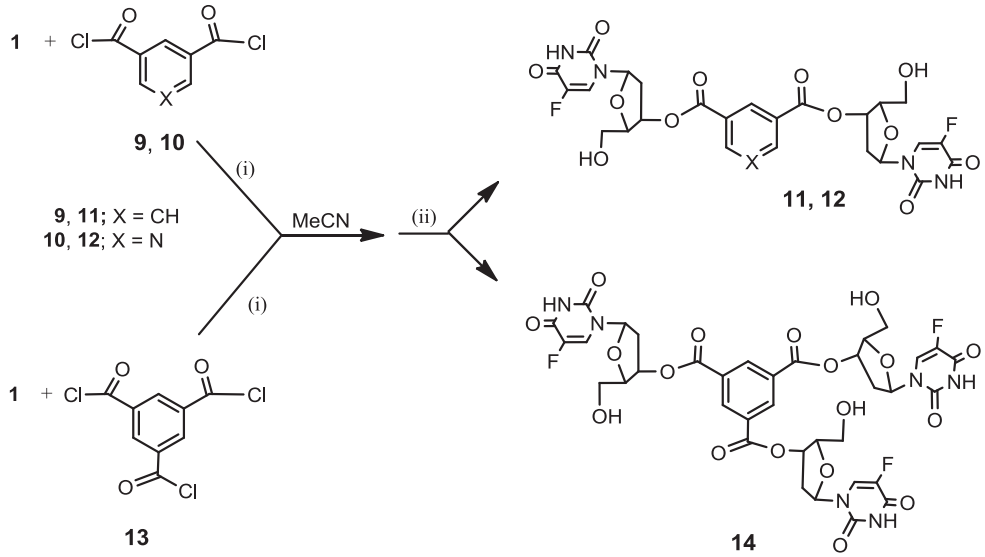
carboxylic acid **3** (1.2 M equiv.) were dissolved in acetonitrile and treated with EDC (1.7 M equiv.) in the presence of DMAP (2.2 M equiv.) at room temperature. After 20 h (usually overnight) the reactions were complete and TLC analysis of the reaction mixtures usually revealed presence of a major product of higher R_f value (acylated nucleosides of type **5**). After evaporation of acetonitrile and removal of the DMT group with 80% acetic acid, the final products **7i,j** were isolated by the silica gel column chromatography (yields 64–88%). In none of the instances the formation of possible *N*-acylated products (vide supra) was observed, apparently, due to lower acylating potency of the in situ activated carboxylic acid vs acyl chlorides.

For the synthesis of dichloroacetyl derivative **7k**, the last in the series of double barrelled compounds, dichloroacetic anhydride **4** (2 M excess) was used as an acylating agent. The reaction was carried out in pyridine in the presence of DMAP (2.5 M equiv.) to afford 3′-*O*-acylated nucleoside **5k** (TLC analysis), which upon treatment with 80% acetic acid aq., furnished 3′-*O*-dichloroacetyl-5-fluoro-2′-deoxyuridine **7k**. After standard work-up and the silica gel column purification, the final product **7k** was obtained in high yield (72%). No *N*-acylation of the nucleobase was observed under the reaction conditions.

2.1.3. *Multi carboxylic aromatic acids esterified with 3′-O-5-fluoro-2′-deoxyuridine*

As a conceptual extension of gathering anticancer potency in one molecule, we have synthesized and studied 5-fluro-2′-deoxyuridine esters of di- and tricarboxylic aromatic acids i.e. isophthalic acid, pyridine 3,5-dicarboxylic acid and benzene-1,3,5-tricarboxylic acid (compounds **11**, **12** and **14** respectively, Scheme 2).

Di-(5-fluoro-2′-deoxyuridin-3′-yl) benzene-1,3-dicarboxylate and di-(5-fluoro-2′-deoxyuridin-3′-yl) pyridine-3,5-dicarboxylate **11** and **12** respectively, and tri-(5-fluoro-2′-deoxyuridin-3′-yl)-benzene-1,3,5-tricarboxylate **14**, were obtained in the reaction of dicarboxylic acid chlorides **9** or **10** or benzene-1,3,5-tricarboxylic acid chloride **13** with a slight excess of 5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine **1** (1.05 M equiv. per one acid chloride function) in the presence of excess of DMAP (2.5 M equiv. per one acid chloride function) (Scheme 2). The reactions were carried out in MeCN and the molar ratio of reagents used ensured completion of the reaction in 2 h. After excess of DMAP was shed off the reaction mixture was treated with acetic acid (80% aq.) to obtain the unprotected compound. Such a two-step one-pot approach was possible to apply since both reactions i.e. esterification of **9**, **10** and **13** as well as dimethoxytrityl group removal, proceeded practically quantitatively. After acetic acid was evaporated, final products **11, 12** and **14** were isolated by the silica gel column chromatography using a stepwise gradient (0–20% v/v) of propan-2-ol in dichloromethane. Pure products were solidified by freeze-drying from benzene/methanol and were obtained as white powders in high



Reagents and conditions: (i) DMAP 7.5 equiv., in MeCN. (ii) 80 % AcOH aq., 30 min.

Scheme 2. Synthesis of FdU esterified aromatic di- and tricarboxylic acids.

yields (**11**, 76%; **12**, 72%, **14**, 68%). Their structure were unambiguously confirmed with ^{1}H, ^{13}C NMR and HRMS analysis.

2.2. Physicochemical and chemical data

To evaluate drug-likeness of chemical compounds at the early stages of a drug discovery process, several descriptors have been devised. For example, Lipinski et al., proposed "rule of 5" [47–50], M. Congreve et al., "rule of 3" [51], and A. Rayna et al., a set of rules for prediction of oral drug-likeness [52]. At the moment these "rules" are unquestionably useful in introductory considerations of drug candidate's structure but are constantly discussed and many modifications and extensions were postulated [53–64]. In our studies we chose a common set of physicochemical and chemical parameters, which would provide the most useful information on structure relation activity (SAR) and biological potency of the studied compounds. Additionally, because our compounds contain aromatic rings we also included aromatic ring count which appeared to be an important parameter defining aqueous solubility, lipophilicity, or protein binding [65]. The collective physicochemical data for all the compounds investigated in this paper are listed in Tables 1 and 2.

2.2.1. Drug likeness

Except of multicarboxylic acids derivatives **11**, **12** and **14** (vide infra) the compounds investigated in this paper obey, within acceptable tolerance, all the criteria of the Lipinski drug-likeness "rule of 5" [47] (Table 1). In majority of cases physicochemical parameters of compounds **7a-k** argue against the "rule of 3" [51] but this disagreement seems to be rather apparent because this rule is dedicated to leads and not to initial screenings presented herein. The other essential for cellular up-take and blood–brain barrier penetration parameter, namely polar surface area (PSA), in most of the cases do not exceed value of 90 Å^2 and should favour effective cellular internalisation of a given molecule [66]. Considering analysis by T. J. Ritchie and J. F. Macdonald [65] two aromatic rings present in compounds **7a-h** should not affect their drug developability. Thus, all physicochemical parameters of compounds **7a-k** are within limits of values for high drug-likeness of potential drug candidates.

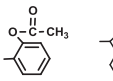
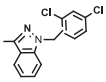
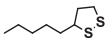
Considering drug-likeness parameters alone i.e., M.W., logP^a, PSA, HBD, HBA, tPapp, similar conclusions can be drawn for double barrelled FdU derivatives **7i-k** (Scheme 1, Table 1), although in these instances the SAR might not be as straightforward as for compounds **7a-h**. For more complex and conceptually different molecules **11**, **12** and **14** (Scheme 2, Table 2) which do not obey standard the Lipinski "rule of 5" [47–50], (MW > 500, higher number of HBD and particularly HBA), drug-likeness prediction needs probably other type of evaluation regime (for instance, kinetics of liberation of biologically active FdU, vide infra).

2.2.2. Stability in cell culture media

By checking stability of compounds **7a-h** in neat RPMI, RPMI/FBS 9:1 (v/v), and human blood plasma (HBP) one can get rough information what can happen with the investigated compounds under physiological conditions. Using linear regression method, the concentrations of substrates **7a-h** and their metabolites were calculated on the basis of integration of peaks area obtained by HPLC analysis. As the neat RPMI is free from enzymatic activities, half-life times in this media reflect susceptibility of the investigated compounds to chemical degradation (hydrolysis), while in RPMI/FBS, and particularly in HBP, in which enzymatic activities are expected (e.g. carboxyesterases), both chemical and enzymatic factors can be responsible for stability. As it is apparent from data in Table 1, all the analysed compounds were rather stable under the investigated reaction conditions and this could be correlated with pK_a values of the respective carboxylic acids [67,68] (the stronger the acid, the less stable the ester). A potential carboxyesterases activity expected for RPMI/FBS and HBP had no pronounced effect along the series **7a-h**. Similar $t_{1/2}$ values in RPMI, RPMI/FBS, and HBP supports the assumption that in these media contribution of enzymatic transformation of **7a-h** is not essential. In addition, considering high half-times it might be presumed that during 48 h the experimental cells are exposed mainly to unchanged compounds **7a-h** what should facilitate an effective cellular up-take of the intact drug molecules.

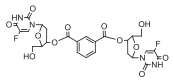
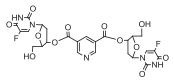
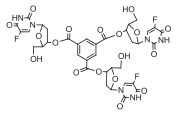
Stabilities in cell culture media of potentially dual-acting compounds **7i-k** derived from FdU, bearing lonidaminyl, lipoyl and dichloroacetyl moieties (Scheme 1) differed significantly. The FdU-lonidaminyl conjugate **7i** was stable in all the investigated media

Table 1
Selected physicochemical data of 3′-*O*-aryl acyl-5-fluoro-2′-deoxyuridines **7a-k**.

Cpd	FdU	**7a**	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7j**	**7k**
R	–											— $CHCl_2$
LogP[a]	−1.28	0.72	2.27	0.61	1.21	1.25	−0.45	0.64	0.54	3.24	1.45	0.06
PSA [Å^2]	153.7	88.7	82.7	78.1	83.4	84.7	92.8	143.6	86.0	78.1	143.9	153.7
aPSA [Å^2]	119.3	123.7	134.7	136.2	129.0	125.3	113.8	87.3	128.9	167.9	78.4	160.8
HBD	3	2	2	2	2	2	2	2	2	2	2	2
HBA	7	8	8	9	8	8	9	11	10	10	8	8
MW	246.19	350.30	406.41	380.33	384.74	384.74	351.29	395.30	408.33	549.34	434.50	357.12
tP_{app} (nm/s)	20.62	19.74	21.42	18.49	20.47	18.37	19.65	11.38	18.73	20.68	14.72	20.11
$t_{1/2}$ [h] RPMI		>240	>240	>240	77	82.5	21.5	12.7	21.4	>240	72.2	<0.1
$t_{1/2}$ [h] RPMI/FBS		228	>240	>240	81.6	144.4	23	28.8	41.3	>240	105	<0.1
$t_{1/2}$ [h] HBP		144	>240	>240	105	58.5	105	15	21.8	>240	14.5	<0.1
No of Ar	0	2	2	2	2	2	2	2	2	4	0	0

LogP[a] – ALOGPS 2.1 software (vide Experimental). PSA – polar surface area, HBD – hydrogen bonds donors, HBA – hydrogen bonds acceptors, tPapp – apparent theoretical permeability; RPMI – RPMI 1640 media; FBS – foetal bovine serum, HBP – human blood plasma, MRC-5 – normal human foetal lung fibroblast, SI – IC_{50} non-tumor cell line/ IC_{50} tumor cell line.

Table 2
[a]Selected physicochemical and pharmacokinetic data of multicarboxylic acid esters decorated with 3′-*O*-5-fluoro-2′-deoxyuridine, **11**,**12** and **14**.

Cpds	FdU	**11**	**12**	**14**
Structure				
t $_{1/2}$ RPMI		ND	6.3 h	ND
t $_{1/2}$ RPMI/FBS		ND	2.8 h	ND
t $_{1/2}$ BP		ND	5.2 h	ND
LogP	−1.28	−0.46	−1.64	−1.61
PSA (Å^2)	153.7	107.9	111.3	105.8
aPSA (Å^2)	119.3	159.5	155.8	203.9
HBD	3	4	4	6
HBA	7	16	17	24
MW	246.19	622.49	623.47	894.68
Absorption				
tP_{app} (nm/s)	20.62	17.25	17.33	15.25
IC_{50} [μM]/SI				
HeLa	5.31/4	2.57/17	8.18/10	7.68/4
Caco-2	12.85/2	>100/<1	>100/<1	31.16/1
T-47D	5.61/4	3.83/12	14.73/6	8.17/4
T98G	5.57/4	17.97/2	6.45/13	7.03/4
U-118 MG	23.40/1	31.16/1	18.86/4	29.71/1
U-87 MG	10.37/2	26.03/1	10.63/8	10.49/3
MRC-5	22.46	44.15	84.50	30.74

[a] Abbreviations as in Table 1.

($t_{1/2}$ > 240 h), while 3′-*O*-dichloroacetyl-FdU **7k** under the same conditions hydrolysed readily ($t_{1/2}$ < 0.1 h). Lipoyl-FdU **7j** conjugate showed moderate stability in neat RPMI (chemical stability), but in the RPMI/FBS and HBP media, which contain enzymatic activity, $t_{1/2}$ value of **7j** changed somewhat unpredictable, possibly as a result of different substrate affinity towards enzyme present in these media (Table 1).

It seems that stability of FdU multicarboxylates in cell culture media as well as in the cell might be crucial for their cytostatic activity because the active part after hydrolysis of multiester is free nucleoside – FdU. It might be presumed then, that their activity should correlate straightforward with unleashing of FdU. Unfortunately, we were able to measure half-time only for diFdU ester of 3,5-dicarboxy pyridinic acid **12** because of solubility of compound **11** and **14** in aqueous environment (RPMI or HBP) was well below 2 mM and was insufficient for monitoring of their metabolism by HPLC under our standard analytical conditions (initial concentration 2 mM). Stability of compound **12** in the discussed media followed the order RPMI > HBP > RPMI/FBS (Table 2). This stability order points to involvement of enzymatic hydrolysis of the investigated compounds. In addition, what potentially might be beneficial for pharmacodynamics of compounds of type **12** is that hydrolysis of the second carboxylic ester was much slower even in RPMI/FBS (see Supplementary data). This is in agreement with known features of carboxyestrases which disclose much lower reactivity towards polar or charged substrates [69].

Although stability of compounds **11** and **14** could not be measured, it is likely that all rules governing their kinetics of hydrolysis remain similar as those for compound **12** described above.

2.2.3. Hydrodynamic diameters

Since most of the compounds studied herein are amphiphilic and consist of a polar part i.e. 5-fluorouracil-1-yl and 2′-deoxyribose residue at one side, and a lipophilic aromatic substituent at the 3′-position of nucleoside at the other one, one can suspect formation of microstructures (e.g. micromicelles) in aqueous

environment. This might reinforced their drug-likeness and facilitate the cellular and/or intestinal up-take [70,71]. For this reason we attempted to measure hydrodynamic diameters using a dynamic light scattering (DLS) method, expecting that if any microstructures will be formed they should be detected by this method. The obtained results for **7a** indicated that this type of compounds may form stable, DLS-detectable aggregates of size close to 200–400 nm. After dilution by pure water to the final concentration in the range of 1–0.25 mg/mL, the values of hydrodynamic diameter remains unchanged, what may suggests that the investigated compounds under the experimental conditions did not form micelle-like structures.

2.3. In vitro cytostatic activity

Glioblastoma multiforme (GBM) is the most common, most aggressive, and lethal type of primary brain tumor. Current therapies for GBM (surgical resection, radiotherapy and chemotherapy) are weakly successful [72] with five-year survival rates less than 5% (according to The Central Brain Tumor Registry of the United States, 2012). Therefore, novel effective therapeutic agents for treating GBM are urgently needed. It has been shown recently [73] that the anticancer drugs from distinct pharmacological classes (other than temozolomide – widely used drug in GBM treatment) exert inhibitory effects on GBM cell growth and invasion.

Few years ago Barciszewski et al., from our institute, reported on a successful application of a sequence specific iRNA in brain tumor therapy [74,75]. Since FdU was hardly explored as drug against GBM, in this studies we focused on law molecular weight, novel FdU derivatives **7a-7k**, **11**, **12** and **14** which were evaluated for their antiproliferative activity against six human cancer cell lines, including three glioma cell lines i.e. T98G, U-118 MG, U-87 MG. Similar investigations were performed also on the cervical (HeLa), breast (T-47D) and colon (Caco-2) cancer cell lines because of slightly different reasons. HeLa cells were used as commonly accepted standard of human malignant cells, T47D cells were used because 5-fluorouracil (FU) is often one of the components in breast cancer multi-drug therapy [76,77], and Caco-2 cells were used for preliminary evaluation of intestinal up-take potency of the examined compounds when administrated orally [78]. Finally, non-cancerous lung fibroblasts (MRC-5) were used as the reference for estimation of therapeutic value of the examined compounds for malignant vs normal cells. The results of antiproliferative activity of FdU derivatives **7a-k**, **11**, **12** and **14** are listed in Table 3 and Table 2, respectively.

2.3.1. 3′-O-acyl- FdU derivatives of aromatic carboxylic acids

The obtained results indicate that, with a few exceptions, all 3′-*O*-acyl-5-fluoro-2′-deoxyuridines **7a-h** are more cytotoxic than the parent FdU in the investigated cancer cell lines and in the reference normal human foetal lung fibroblasts (MRC-5) (Table 3). Considering character of the 3′-*O*-acyl groups, it seems reasonable to postulate that the observed higher antimalignant potency of **7a-h** vs FdU might be attributed to their higher lipophilicity (as is apparent from the corresponding logP, PSA and aPSA values, Table 1), that should facilitate cell membrane penetration and cellular up-take.

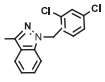
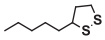
Regardless of a higher cytotoxicity (IC_{50}) of **7a-g** in all examined carcinoma cells, their potential therapeutic value as measured by a selectivity index (SI), is lower than that of the parent FdU (in most of the cases SI < 1) and thus do not justify their further development as potential anticancer drugs. The only exception in this subseries of the aromatic acyl derivatives, was a double barrelled 3′-*O*-(2-acetylsalicyl)-5-fluoro-2′-deoxyuridine (**7h**) whose cytotoxicity in each cancer cell lines investigated was distinctly higher than FdU (in some cases well above ten times) with superior selectivity indices (SI), e.g. for HeLa and Caco-2 cell lines (SI 18 and 33 respectively), and for the series of glioma cell lines T98G, U-118 MG and U-87MG (SI 5, 6 and 14 respectively). Considering that apart from FdU, the other metabolite generated form **7h** is aspirin or salicylic acid, the observed phenomenon is in line with the finding that both compounds can induce growth inhibition of various malignancies while leaving normal cells unaffected [25–27,29]. The pronounced differences in SI values of **7a-g** vs **7h** were rather unexpected but it might be speculated that kinetics of internalization and intracellular metabolism of **7h** towards biologically active FdU and aspirin or salicylic acid is so far distinct from the analogous decomposition of 7a-g that may result in much higher selectively of growth inhibition of cancer cells vs normal cells (MRC-5).

2.3.2. Double barrelled FdU derivatives

In comparison to the parent FdU, with a few exceptions (i.e. **7a**, **7f** and Caco-2 cells, **7a**, **7d**, **7f**, **7h**, **7k** and T-47D cells, **7h** and T98G cell line, **7d**, **7e** and U-118MG cells), all investigated compounds disclosed higher (up to 90 times, vide U-118G cells and compound **7a**) cytostatic activity in cancer cell lines but with exception of **7h**, also clearly higher cytotoxicity for referential normal MRC-5 cells (Table 3). This makes application of these compounds rather doubtful in cancer therapy. Unfortunately, this tentative conclusion concerns also to compounds **7i-k** armed with two type of anticancer activities (FdU and second metabolite lonidamine, lipoic acid, dichloroacetic acid, respectively). Although some of them showed antiproliferating activity but none of them disclosed anticancer potency advantageous over the parent FdU.

However, selectivity indices SI of 3′-*O*-benzoyl- and 3′-*O*-

Table 3
[a]Cytostatic and SI values [IC_{50} (μM)/SI] of 3′-*O*-aryl acyl-5-fluoro-2′-deoxyuridines **7a-k**.

Cpd	FdU	7a	7b	7c	7d	7e	7f	7g	7h	7i	7j	7k
R	–											
HeLa	5.31/4	0.57/3	0.39/<1	0.55/1	6.46/<1	1.75/<1	0.94/4	0.87/1	2.75/18	1.12/4	0.86/1	6.95/<1
Caco-2	12.85/2	36.96/<1	0.18/<1	0.38/2	1.26/2	0.42/1	70.55/<1	2.12/<1	1.53/33	1.03/4	6.92/<1	12.23/<1
T-47D	5.61/4	7.52/<1	2.98/<1	2.06/<1	13.77/<1	0.68/1	16.35/<1	1.06/1	15.04/3	4.41/1	7.23/<1	7.02/<1
T98G	5.57/4	0.44/<3	1.11/<1	0.39/2	1.50/2	0.67/1	0.66/<1	0.40/3	9.69/5	3.97/1	0.33/2	5.09/<1
U-118 MG	23.40/1	0.26/<6	3.88/<1	19.26/<1	41.35/<1	46.27/<1	5.48/<1	0.65/2	8.79/6	9.21/1	6.19/<1	2.71/1
U-87 MG	10.37/2	0.53/<3	0.34/<1	0.52/1	0.64/4	0.42/1	13.35/<1	0.88/1	3.58/14	5.65/1	0.56/1	n.d*.
MRC-5	22.46	1.48	0.07	0.66	2.46	0.60	3.30	1.14	50.24	4.15	0.59	3.75

*n.d. – not determined.
[a] Abbreviations as in Table 1.

acetylsalicylyl derivatives of FdU (**7a** and **7h** respectively), stand out from the others due to their SI parameter for GMB cell lines that are in each case exceeding those of FdU (Table 3). It is worthy to notice that in the case of **7a**, favorably values of SI parameters were due to its high cytostatic activity (low IC_{50}) while in the case of compound **7h** the high SI values were related to its low toxicity towards non-malignant MRC-5 cells. It is also worth to notice that **7h** disclosed highest therapeutic potency in Caco-2 and HeLa cells (SI = 33 and 18, respectively, Table 3) what makes this compound therapeutically promising in fighting malignancy of other tissues. To lesser extend similar conclusions concern also compound **7g**.

Some comments deserve results of experiments performed on Caco-2 cells in which with exception of compounds **7a** and **7f** all other investigated compounds showed much higher anticancer activity (IC_{50} values in the range 0.18–6.92 μM) than FdU (IC_{50} = 12.85 μM). Considering that monolayer Caco-2 cells are widely used as a *in vitro* model for prediction of drug absorption in humans [79–81], it might be concluded that compounds **7b-e** and **7g-l** should be well absorbed from intestinal fluid. Derivatives **7a** and **7f** proved to be poorly active in Caco-2 cells (IC_{50} 36.96 and 70.55 μM respectively) and their low antiproliferative activity may suggests that they are substrates for P-glycoprotein, a protein belonging to the ATP-binding cassette (ABC) transporters superfamily, that has clinical relevance due to its role in drug metabolism and multidrug resistance (MDR). The last partially argues against calculated tP_{app} value because all of the compounds including **7a** and **7f** (19.74 nm/s and 19.65 nm/s respectively) belong to class of medium permeability [82] with tP_{app} in the range of 11.38 nm/s (for **7g**) and 21.42 nm/s (for **7b**). Apparently, low antiprolifeartive potency of **7a** and **7f** in Caco-2 cells was an effect of unrecognized yet intracellular and not outer membrane "molecular" events.

Considering therapeutic potency of the examined derivatives of FdU **7a-h** and the double barreled **7i-k** (Table 3) in fighting breast cancer cell line T-47D, their parameters IC_{50} and SI clearly point out that these compounds (with exception of **7h**) did not exceed therapeutic potency of FdU. Although antiproliferative activities (IC_{50}) of **7b**, **7c**, **7e**, **7g**, **7i** were higher than that of FdU but at the same time their cytoxicity towards non-malignant cells was also higher (Table 3), the SI values for these compounds did not exceed 1 (except for **7h**, SI = 3).

2.3.3. FdU esters of multi carboxylic acids

Biological activity found for FdU esters of multi carboxylic acids **11**, **12** and **14** are somehow slightly difficult to rationalise. In comparison to FdU, all these compounds disclosed comparable antiproliferative activity but at the same time were less toxic (up to four times, compound **12**) for normal MRC-5 cells (Table 2). As a result their SI value in several cases were essentially improved as compare to those of FdU. For example, di-(5-fluoro-2′-deoxyuridin-3′-yl)-3,5-dicarboxypyridinylate **12** showed higher therapeutic potency in nearly all the examined cell lines (with the exception of Caco-2 cells) and the SI exceeded even six times (for T98G cells) that of FdU. This apparent increase in value of SI parameter of compound **12** vs FdU, might points to this compound as a good candidate for a new antimalignant pro-drug. As such conclusion might be premature, the results obtained for dicarboxylate **12** should certainly warrant further studies on development of anticancer compounds based on FdU skeleton. The second noteworthy in this series compound is di-(5-fluoro-2′-deoxyuridin-3′-yl)-1,3-dicarboxybenzoate **11** that showed much higher antiproliferative potency than FdU in two cell lines, i.e. HeLa (four times) and breast cancer T-47D cells (three times). The last finding can be of interest because none of the investigated herein compounds disclosed advantageous over the parent FdU therapeutic potency in this cell line. Finally, it was slightly surprising that tri-(5-fluoro-2′-deoxyuridin-3′-yl)-1,3,5-tricarboxybenzoate **14** did not showed any advantages over 5-fluoro-2′-deoxyuridine in all of the investigated cell lines.

3. Conclusions

We have synthesised new aromatic and aliphatic 3′-*O*-acyl-5-fluoro-2′-deoxyuridine derivatives **7a-k**, **11**, **12** and **14** as potential anticancer agents. The chemistry involved acylation of 5′-*O*-dimetoxytrityl-5-fluoro-2′-deoxyuridine with various acylating reagents (acyl chlorides, carboxylic acid anhydrides) or reagent systems (carboxylic acid in combination with condensing agents) that afforded the corresponding 3′-*O*-acylated FdU derivatives in good yield. In some cases the formation of *O*,*N*-bisacylated nucleosides was observed, but this problem was remedied by a selective removal of the *N*-acyl groups by a short treatment with morpholine.

For evaluation of antimalignant potency of the synthesised compounds three lines of glioblastoma cells (T98G, U-118 MG and U-87 MG), HeLa cells, breast cancer cells (T-47D), and Caco-2 cells were chose. The first series of the investigated compounds, **7a-g**, consisted of FdU acylated with electronically and structurally different aromatic groups, and did not provide any significant improvements in terms of SI over the parent FdU. In contrast to this, for the double-barrelled 3′-*O*-(2-acetylsalicylyl)-5-fluoro-2′-deoxyuridine **7h**, the therapeutic indices were notably higher than those of FdU (except for breast cancer cells, T-47D). Compound **7i-k**, bearing potentially active additional metabolites (lipoic acid, **7i**; lonidamine, **7j**; dichloroacetic acid, **7k**) turned out to be disappointing as anticancer agents in the investigated cell lines.

Finally, in the third series of compounds investigated, multi-FdU esters of di- and tri-carboxylic aromatic acids i.e. **11**, **12** and **14**, di-FdU 1,3-dicarboxybenzoate **11** appeared to be active in two cell lines (HeLa and breast cancer cells T-47D), with the SI indices ca three times higher than that of FdU. Compound **12** had therapeutic parameters evidently superior over the parent nucleoside (FdU) in glioblastoma cell lines T98G, U-118 MG and U-87 MG, mainly due to its much lower toxicity. The IC_{50} and SI parameters of benzene-tricarboxylic acid derivative **14** in all the investigated cell lines were similar to those of FdU.

Although not impressive in terms of biological activity, some of the investigated compounds (for instance **7h** and **12**) can constitute a launch pad for further studies on double-barrelled and multi loaded antimalignant potential drugs.

4. Experimental

4.1. Material and methods

^{1}H, ^{13}C NMR spectra were recorded on Bruker Avance II 400 or 500 MHz machines. Mass spectra were recorded with the ESI technique with negative or positive ionization with accuracy below 5 ppm. Amount of water in anhydrous solvents was controlled using Karl Fischer coulometric titration (Metrohm 684 KF coulometer). Thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates and visualized with UV. For column chromatography Kieselgel 60 Merck was used. HPLC analyses were performed on a Nucleosil 100-5C18 column (5.0 μm, 4.6 mm × 150 mm) using and Waters Breeze HPLC systems with A + B solvent systems (A, 0.01 M aqueous triethylammonium acetate pH 7.4; B, A/acetonitrile, 1:4, v/v) at 35 °C, flow rate 1.5 mL/min; events: 5 min A 100%, linear gradient of B 0–100% in 20 min, 5 min B 100% and A 100% 10 min wash. Lonidamine was obtained as described previously by I.G. George et al. [83]. Aromatic di-carboxylic acid dichlorides were obtained according to R. Martin et al. [84]. Hydrodynamic

diameters were measured with dynamic light scattering (DLS) method using Zetasizer Nano ZS Malvern. Samples were prepared in DMSO at the following concentrations: 7 mg/mL, 2 mg/mL and 1 mg/mL. Next, the samples were diluted by pure water and the hydrodynamic size of compounds was measured using the glass cuvette. To check stability of prepared solutions the measurements were performed in intervals of time during 12 h.

4.1.1. General procedure for the synthesis of 3′-O-acyl-5-fluoro-2′-deoxyuridines of type ***7***

Method A (for compounds ***7a-h****).* 5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine **1** (1 mmol) was rendered anhydrous by evaporation of the added pyridine and then dissolved in pyridine (10 mL) (or in DCM with 3 M equiv. excess of DIPEA for compound **7 h**). To this, the respective acyl chloride of type **2** (2 mmol) was added, and the reaction mixture was left for 2–3 h at room temp yielding two acylated compounds of type **5** and **6.** Then the solvent was evaporated, the crude mixture dissolved in DCM (10 mL) was treated for 30 min with 3 M excess of morpholine. After this time the resulting mixture was washed twice with water (2 × 5 mL), the organic layer separated, evaporated and treated with 80% acetic acid aq. (5 mL) for 30 min. After deprotection with AcOH, the mixtures were concentrated to an oil under reduced pressure. 3′-*O*-acyl-5-fluoro-2′-deoxyuridines **7** were isolated by a silica gel 60 column chromatography using a stepwise gradient (0–10%) of propan-2-ol in methylene chloride. The fractions containing pure products were collected and evaporated yielding non-hygroscopic foams. After freeze-drying from benzene, compounds **7** were obtained as amorphous solids.

Method B (for compounds ***7i-k****).* 5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine **1** (1 mmol) and the respective carboxylic acids **3** (1.2 mmol) were rendered anhydrous by the evaporation of the added pyridine and then were dissolved in acetonitrile (10 mL) and treated by EDC (1.7 mmol) in the presence of DMAP (2.2 mmol) at room temperature. After 20 h the reaction was complete (TLC analysis), acetonitrile was evaporated, and the residue was treated with 80% acetic acid aq., (5 mL) for 30 min. Further work-up as for Method A.

Method C (for compound ***7l****).* 5′-*O*-dimethoxytrityl-5-fluoro-2′-deoxyuridine **1** (1 mmol) was rendered anhydrous by the evaporation of the added pyridine and then dissolved in pyridine (10 mL). To this, 2 M excess of dichloroacetic anhydride **4** in the presence of 2.5 M equiv. of DMAP was added. After 4 h the reaction was complete (TLC analysis), solvent was evaporated, and the residue was treated with 80% acetic acid (5 mL). Further work-up as for Method A.

*3′-O-benzoyl-5-fluoro-2′-deoxyuridine (****7a****).* White solid; yield 0.29 g, 83%; RP HPLC Rt 13.96 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.88 (s, 1H, NH), 8.27 (d, 1H, J $_{6\text{-}F5}$ = 7.2 Hz, H-6), 8.03–8.01 (m, 2H, *o*-H of Ar), 7.71–7.67 (m, 1H, *p*-H of Ar), 7.58–7.54 (m, 2H, *m*-H of Ar), 6.24 (t, J = 6.6 Hz, 1H, H-1′), 5.48 (d, J = 5.6 Hz, 1H, H-3′), 4.20–4.21 (m, 1H, H-4′), 3.70–3.74 (m, 2H, H-5′, H-5″), 2.40–2.46 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$): δ_C 165.65 (C═O), 157.40 (d, J = 26 Hz, C-4), 149.47 (C-2), 141.53 (d, J = 230 Hz, C-5), 134.03, 129.75, 129.20 (C of Ar), 124.93 (d, J = 34 Hz, C-6), 85.33 (C-4′), 84.95 (C-1′), 76.07 (C-3′), 61.74 (C-5′), 37.43 (C-2′) ppm; HRMS (ESI): calcd for $C_{16}H_{14}N_2O_6F$ [M-H]$^-$: 349.08359, found: 349.08459.

*3′-O-(4-tert-butylbenzoyl)-5-fluoro-2′-deoxyuridine (****7b****).* White solid; yield 0.30 g, 73%; RP HPLC Rt 17.56 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.89 (s, 1H, NH), 8.26 (d, 1H, J $_{6\text{-}F5}$ = 7.2 Hz, H-6), 7.94 (d, J = 8.4 Hz, 2H, *o*-H of Ar), 7.57 (d, J = 8.4 Hz, 2H, *m*-H of Ar), 6.26 (t, J = 6.8 Hz, 1H, H-1′), 5.48–5.49 (m, 1H, H-3′), 4.20–4.21 (m, 1H, H-4′), 3.71–3.72 (m, 2H, H-5′, H-5″), 2.39–2.43 (m, 2H, H-2′, H-2″), 1.31 (s, 9H, CH_3 of *t*-Bu) ppm, ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C 165.56 (C═O), 157.40 (d, J = 26 Hz, C-4), 157.12 (C of Ar), 149.47 (C-2), 140.53 (d, J = 230 Hz, C-5), 129.68, 127.045 & 126.013 (C of Ar), 124.91 (d, J = 34 Hz, C-6), 85.35 (C-4′), 84.93 (C-1′), 75.84 (C-3′), 61.74 (C-5′), 37.47 (C-2′), 35.28 [**C**$(CH_3)_3$], 31.19 [C(**C**$H_3)_3$] ppm; HRMS (ESI): calcd for $C_{20}H_{22}N_2O_6F$ [M-H]$^-$: 405.14619, found: 405.14736.

*3′-O-(4-methoxybenzoyl)-5-fluoro-2′-deoxyuridine (****7c****).* White solid; yield 0.28 g, 75%; RP HPLC Rt 14.14 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.88 (s, 1H, NH), 8.26 (d, J $_{6\text{-}F5}$ = 7.2 Hz, 1H, H-6), 7.96 (d, J = 8.8 Hz, 2H, *o*-H of Ph), 7.06 (d, J = 8.8 Hz, 2H, *m*-H of Ar), 6.26 (t, J = 6.8 Hz, 1H, H-1′), 5.43–5.45 (m, 1H, H-3′), 4.17–4.18 (m, 1H, H-4′), 3.84 (s, 3H, OCH_3), 3.70–3.73 (m, 2H, H-5′, H-5″), 2.38–2.43 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$) δ_C 165.31 (C═O), 163.81 (C of Ar), 157.40 (d, J = 26 Hz, C-4), 149.47 (C-2), 140.52 (d, J = 230 Hz, C-5), 131.91 (C of Ar), 124.92 (d, J = 34 Hz, C-6), 121.90, 114.48 (C of Ar), 85.40 (C-4′), 84.94 (C-1′), 75.67 (C-3′), 61.73 (C-5′), 55.73 (OCH_3), 37.27 (C-2′) ppm; HRMS (ESI): calcd for $C_{17}H_{16}N_2O_7F$ [M-H]$^-$: 379.09415, found: 379.09524.

*3′-O-(2-chlorobenzoyl)-5-fluoro-2′-deoxyuridine (****7d****).* White solid; yield 0.33 g, 85%; RP HPLC Rt 14.52 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.88 (s, 1H, NH), 8.25 (d, 1H, J $_{6\text{-}F5}$ = 7.2 Hz, H-6), 7.88–7.91 (m, 1H, *o*-H of Ar), 7.59–7.62 (m, 2H, *m*-H of Ar), 7.48–7.52 (m, 1H, *p*-H of Ar), 6.23–6.26 (m, 1H, H-1′), 5.49–5.51 (m, 1H, H-3′), 4.19–4.21 (m, 1H, H-4′), 3.71–3.73 (m, 2H, H-5′, H-5″), 2.41–2.45 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (100 MHz, DMSO-d$_6$): δ_C 164.90 (C═O), 157.42 (d, J = 27 Hz, C-4), 149.47 (C-2), 141.54 (d, 230 Hz, C-5), 133.94, 132.36, 131.80, 131.27, 129.98, 127.89 (C of Ar), 124.91 (d, J = 35 Hz, C-6), 85.16 (C-4′), 84.94 (C-1′), 76.73 (C-3′), 61.73 (C-5′), 37.27 (C-2′) ppm; HRMS (ESI): calcd for $C_{16}H_{13}N_2O_6ClF$ [M-H]$^-$: 383.04462, found: 383.04573.

*3′-O-(4-chlorobenzoyl)-5-fluoro-2′-deoxyuridine (****7e****).* White solid; yield 0.29 g, 75%; RP HPLC Rt 15.32 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.88 (s, 1H, NH), 8.26 (d, 1H, J $_{6\text{-}F5}$ = 7.2 Hz, H-6), 8.02 (d, J = 8.4 Hz, 2H, *o*-H of Ar), 7.62 (d, J = 8.4 Hz, 2H, *m*-H of Ar), 6.27 (t, J = 7.2 Hz, 1H, H-1′), 5.47–5.48 (m, 1H, H-3′), 4.19–4.21 (m, 1H, H-4′), 3.66–3.76 (m, 2H, H-5′, H-5″), 2.32–2.50 (m, 2H, H-2′, H-2″); ^{13}C NMR (100 MHz, DMSO-d$_6$): δ_C 164.43 (C═O), 157.13 (d, J = 26 Hz, C-4), 149.47 (C-2), 140.11 (d, J = 229 Hz, C-5), 138.53, 131.24, 128.96, 128.21 (C of Ar), 124.17 (d, J = 34 Hz, C-6), 84.86 (C-4′), 84.52 (C-1′), 75.97 (C-3′), 61.32 (C-5′), 36.96 (C-2′) ppm; HRMS (ESI): calcd for $C_{16}H_{13}N_2O_6ClF$ [M-H]$^-$: 383.04407, found: 383.04562.

*3′-O-(nicotinoyl)-5-fluoro-2′-deoxyuridine (****7f****).* White solid; yield 0.27 g, 78%; RP HPLC Rt 11.75 min; ^{1}H NMR (400 MHz, DMSO-d$_6$): δ_H 11.88 (s, 1H, NH), 9.15–9.16 (m, 1H, *o*-H of Ar), 8.83–8.85 (m, 1H, *p*-H of Ar), 8.34–8.37 (m, 1H, *o*-H of Ar), 8.26 (d, 1H, J $_{6\text{-}F5}$ = 7.2 Hz, H-6), 7.58–7.61 (m, 1H, *m*-H of Ar), 6.29 (t, J = 7.2 Hz, 1H, H-1′), 5.50–5.51 (m, 1H, H-3′), 4.24 (br s, 1H, H-4′), 3.68–3.77 (m, 2H, H-5′, H-5″), 2.39–2.44 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (125 MHz, DMSO-d$_6$): δ_C 164.77 (C═O), 157.47 (d, J = 27 Hz, C-4), 154.34, 150.70 (C of Ar), 149.53 (C-2), 141.59 (d, J = 228 Hz, C-5), 137.56, 125.91 (C of Ar), 125.03 (d, J = 34 Hz, C-6), 124.39 (C of Ar), 85.24 (C-4′), 85.03 (C-1′), 76.55 (C-3′), 61.78 (C-5′), 37.4 (C-2′) ppm; HRMS (ESI): calcd for $C_{15}H_{13}N_3O_6F$ [M-H]$^-$: 350.07884, found: 350.07959.

*3′-O-(4-nitrobenzoyl)-5-fluoro-2′-deoxyuridine (****7g****).* Yellow solid; yield 0.28 g, 71%; RP HPLC Rt 15.54 min; ^{1}H NMR (400 MHz, DMSO-

d_6): δ_H 11.89 (s, 1H, NH), 8.35–8.38 (m, 2H, *o*-H of Ar), 8.24–8.28 (m, 3H, *m*-H of Ar and H-6), 6.28–6.32 (m, 1H, H-1′), 5.52–5.53 (m, 1H, H-3′), 4.25 (br s, 1H, H-4′), 3.68–3.78 (m, 2H, H-5′, H-5″), 2.40–2.55 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 164.30 (C═O), 157.46 (d, J = 27 Hz, C-4), 150.84 (C of Ar), 149.55 (C-2), 141.61 (d, J = 230 Hz, C-5), 135.35, 131.38 (C of Ar), 125.49 (d, J = 34 Hz, C-6), 124.34 (C of Ar), 85.24 (C-4′), 85.01 (C-1′), 77.08 (C-3′), 61.82 (C-5′), 37.38 (C-2′) ppm; HRMS (ESI): calcd for $C_{16}H_{13}N_3O_8F$ [M-H]$^-$: 394.06867, found: 394.06964.

*3′-O-(2-acetylsalicylyl)-5-fluoro-2′-deoxyuridine (**7h**).* White solid; yield 0.32 g, 79%; RP HPLC Rt 14.59 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.88 (s, 1H, NH), 8.26 (d, 1H, $J_{6\text{-}F5}$ = 7.2 Hz, H-6), 8.01 (d, 1H, J = 8.0 Hz *o*-H of Ar), 7.71 (t, 1H, J = 8.0 Hz *p*-H of Ar), 7.44 (m, J = 7.8 Hz, 1H, *m*-H of Ar), 7.28 (d, J = 8.0 Hz, 1H, *m*-H of Ar), 6.24 (t, J = 7.6 Hz, 1H, H-1′), 5.45 (br s, 1H, H-3′), 4.13 (br s, 1H, H-4′), 3.70 (m, 2H, H-5′, H-5″), 2.38–2.41 (m, 2H, H-2′, H-2″), 2.31 (s, 3H, CH_3) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 169.60 (C═O), 164.07 (C═O), 157.48 (d, J = 26 Hz, C-4), 150.39 (C of Ar), 149.54 (C-2), 140.59 (d, J = 228 Hz, C-5), 135.07, 131.95, 128.68, 126.85, 129.98, 127.89 (C of Ar), 124.66 (d, J = 35 Hz, C-6), 124.52, 123.28 (C of Ar), 85.26 (C-4′), 84.94 (C-1′), 76.33 (C-3′), 61.76 (C-5′), 37.35 (C-2′) ppm; HRMS (ESI): calcd for $C_{18}H_{16}N_2O_8F$ [M-H]$^-$: 407.08907, found: 407.09018.

*3′-O- [1-(2,4-dichlorobenzyl)-1H-indazole-3-carbonyl]-5-fluoro-2′-deoxyuridine (**7i**).* White solid; yield 0.35 g, 64%; RP HPLC Rt 20.97 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.90 (s, 1H, NH), 8.29 (d, 1H, $J_{6\text{-}F5}$ = 7.2 Hz, H-6), 8.12 (d, 1H, J = 8.0 Hz, H of Ar), 7.81 (d, 1H, J = 8.8 Hz, H of Ar), 7.64 (d, 1H, J = 2.0 Hz, H of Ar), 7.52 (t, 1H, J = 7.8 Hz, H of Ar), 7.39 (t, J = 7.4 Hz, 1H, H of Ar), 7.35 (dd, J = 8.4 Hz, J = 2.0 Hz, 1H, H of Ar), 6.92 (d, J = 8.4 Hz, 1H, H of Ar), 6.30 (t, J = 6.4 Hz, 1H, H-1′), 5.88 (s, 2H, CH_2), 5.59–5.61 (m, 1H, H-3′), 4.29 (br s, 1H, H-4′), 3.72–3.80 (m, 2H, H-5′, H-5″), 2.50–2.57 (m, 2H, H-2′, H-2″); ^{13}C NMR (100 MHz, DMSO-d_6): δ 161.13 (C═O), 157.02(d, J = 27 Hz, C-4), 149.10 (C-2), 140.89 (C of LND), 140.15 (d, 229.5 Hz, C-5), 134.50, 133.47, 133.19, 133.00, 130.76, 129.08, 127.76, 127.35 (C of LND), 124.52 (d, J = 35 Hz, C-6), 123.68, 122.89, 121.37, 110.86 (C of LND), 84.94 (C-4′), 84.58 (C-1′), 75.59 (C-3′), 61.34 (C-5′), 49.98 (C of LND), 37.10 (C-2′); HRMS (ESI): calcd for $C_{24}H_{18}N_4O_6Cl_2F$ [M-H]$^+$: 547.05874, found: 547.06033.

*3′-O- [(R)-5-(1,2-Dithiolan-3-yl)-pentanoyl]-5-fluoro-2′-deoxyuridine (**7j**).* Yellow solid; yield 0.38 g, 88%; RP HPLC Rt 17.83 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.87 (s, 1H, NH), 8.21 (d, 1H, $J_{6\text{-}F5}$ = 7.2 Hz, H-6), 6.15 (t, J = 7.6 Hz, 1H, H-1′), 5.31 (t, J = 5.2 Hz, 1H, H-3′), 4.00 (br s, 1H, H-4′), 3.62–3.65 [m, 3H, H-5′, H-5″, H of lipoic acid (LA)], 3.08–3.22 (m, 2H, H of LA), 2.31–2.45 (m, 3H, H-2′, H-2″ & H of LA), 2.22–2.30 (m, 2H, H of LA), 1.34–1.91 (m, 7H, H of LA) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 172.44 (C═O), 156.97 (d, J = 26 Hz, C-4), 149.02 (C-2), 140.08 (d, J = 229 Hz, C-5), 124.44 (d, J = 34 Hz, C-6), 84.94 (C-4′), 84.40 (C-1′), 74.60 (C-3′), 61.24 (C-5′), 56.00, 40.17, 38.08 (C of LA), 36.88 (C-2′), 34.76, 33.23, 28.00, 24.04 (C of LA) ppm; HRMS (ESI): calcd for $C_{17}H_{22}N_2S_2O_6F$ [M-H]$^-$: 433.08978, found: 433.09117.

*3′-O-dichloroacetyl-5-fluoro-2′-deoxyuridine (**7k**).* Yellow solid; yield 0.26 g, 72%; RP HPLC Rt 13.88 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.89 (s, 1H, NH), 8.20 (d, 1H, $J_{6\text{-}F5}$ = 7.2 Hz, H-6), 6.92 (s, 1H, CH), 6.18 (t, J = 7.6 Hz, 1H, H-1′), 5.38–5.40 (m, 1H, H-3′), 4.08 (br s, 1H, H-4′), 3.66 (t, J = 4 Hz, 2H, H-5′, H-5″), 2.33–2.42 (m, 2H, H-2′, H-2″); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 163.94 (C═O), 156.96 (d, J = 26 Hz, C-4), 149.04 (C-2), 140.12 (d, J = 229 Hz, C-5), 124.39 (d, J = 34 Hz, C-6), 84.28 (C-4′ & C-1′), 78.38 (C-3′), 66.34 (CH), 61.21 (C-5′), 36.39 (C-2′) ppm; HRMS (ESI): calcd for $C_{11}H_{10}N_2Cl_2O_6F$ [M-H]$^-$: 354.98999, found: 354.99103.

4.1.2. General procedure for the synthesis of 3′-O-esters of 5-fluoro-2′-deoxyuridines with aromatic multi carboxylic acids

Nucleoside **1** (1.05 equiv. per one acid chloride function) was rendered anhydrous by evaporation with dry pyridine and dissolved in acetonitrile (10 mL/1 mmol of nucleoside **1**). To this solution DMAP (2.5 equiv per one acid chloride function) and the respective carboxylic acid chloride (1 mmol) were added and the reaction mixture was left for 2 h at room temperature (TLC analysis). The reaction was quenched by addition of water (1 mL) and the solution concentrated to a viscous oil. The residue was dissolved in methylene chloride (10 mL/1 mmol) and washed three times with water (3 × 5 mL), the organic layer separated, and after drying (Na_2SO_4 anhyd.) it was evaporated under vacuum. After dimethoxytrityl group was removed (80% acetic acid, 5 mL, 30 min) and the final product was isolated by a silica gel 60 column chromatography using a stepwise gradient 0 → 20% (v/v) propane-2-ol in dichloromethane. The fractions containing pure products were combined, evaporated and solidified by freeze-drying from benzene/methanol.

*Di-(5-fluoro-2′-deoxyuridin-3′-yl)-benzene-1,3-dicarboxylate (**11**).* White solid 0.47 g, yield: 76%; RP HPLC Rt 13.97 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.87 (s, 2H, NH), 8.53 (t, J = 2 Hz, 2H, H of Ar), 8.30 (dd, J = 1.8 Hz, J = 7.8 Hz, 4H, H of Ar), 8.27 (d, $J_{6\text{-}F5}$ = 7.2 Hz, 2H, H-6), 7.78 (t, J = 2 Hz, 1H, H of Ar), 6.26–6.30 (m, 2H, H-1′), 5.40 (t, J = 5.2 Hz, 2H, H-3′), 4.21–4.24 (m, 2H, H-4′), 3.68–3.73 (m, 4H, H-5′, H-5″), 2.38–2.53 (m, 4H, H-2′, H-2″); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 164.46 (C═O), 157.01 (d, J = 26 Hz, C-4), 149.07 (C-2), 140.12 (d, J = 229 Hz, C-5), 134.13, 134.01, 130.02, 129.62 (C of Ar), 124.49, 124.44 (2xd, J = 34 Hz, C-6), 84.78 (C-4′), 84.51 (C-1′), 76.17 (C-3′), 61.32 (C-5′), 36.93 (C-2′) ppm; HRMS (ESI): calcd for $C_{26}H_{23}N_4O_{12}F_2$ [M−H]$^-$: 621.12805 found: 621.12904.

*Di-(5-fluoro-2′-deoxyuridin-3′-yl)-pyridine-3,5-dicarboxylate (**12**).* White solid; yield 0.45 g, 72%; RP HPLC Rt 12.68 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.87 (s, 2H, NH), 9.38 (d, J = 2 Hz, 2H, *o*-H of Ar), 8.72 (t, 1H, J = 2 Hz, 1H, *p*-H of Ar), 8.28 (d, 2H, $J_{6\text{-}F5}$ = 6.8 Hz, H-6), 6.29–6.33 (m, 2H, H-1′), 5.39 (t, J = 5.0 Hz, 2H, H-3′), 4.28–4.29 (m, 2H, H-4′), 3.69–3.78 (m, 4H, H-5′, H-5″), 2.40–2.58 (m, 4H, H-2′, H-2″); ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 163.59 (C═O), 157.07 (d, J = 26 Hz, C-4), 153.99(C of Ar), 149.12 (C-2), 140.17 (d, J = 229 Hz, C-5), 137.42, 125.70 (C of Ar), 124.57 (d, J = 34 Hz, C-6), 84.69 (C-4′), 84.60 (C-1′), 76.65 (C-3′), 61.35 (C-5′), 36.92 (C-2′) ppm; HRMS (ESI): calcd for $C_{25}H_{23}N_5O_{12}F_2$ [M-H]$^-$: 622.12330, found: 622.12475.

*Tri-(5-fluoro-2′-deoxyuridin-3′-yl)-benzene-1,3,5-tricarboxylate (**14**).* White solid; yield 0.61 g, 68%; RP HPLC Rt 13.52 min; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.86 (s, 3H, NH), 8.72 (s, 3H, H of Ar), 8.26 (d, $J_{6\text{-}F5}$ = 7.2 Hz, 2H, H-6), 6.29 (t, J = 6.6 Hz, 3H, H-1′), 5.54–5.56 (m, 3H, H-3′), 4.24–4.29 (m, 3H, H-4′), 3.58–3.60 (m, 6H, H-5′, H-5″), 2.40–2.58 (m, 6H, H-2′, H-2″); ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 163.73 (C═O), 157.18 (d, J = 25.75 Hz, C-4), 149.21 (C-2), 140.16 (d, J = 229 Hz, C-5), 133.93, 130.94, 128.30 (C of Ar), 124.44 (d, J = 35 Hz, C-6), 84.61 (C-4′), 84.49 (C-1′), 76.70 (C-3′), 61.31 (C-5′), 36.84 (C-2′) ppm; HRMS (ESI): calcd for $C_{36}H_{32}N_6O_{18}F_3$ [M-H]$^-$: 893.17197, found: 893.17464.

*3′-O- [2-(2-acetylysalicylyl)-salicylyl)-5-fluoro-2′-deoxyuridine (**8**).* White solid; ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.87 (s, 1H, NH), 8.23 (dd, J = 1.6 Hz, J = 8 Hz, 1H, H of Ar), 8.19 (d, $J_{6\text{-}F5}$ = 6.8 Hz, 1H, H-6), 8.06 (dd, J = 1.6 Hz, J = 7.6 Hz, 1H, H of Ar), 7.75–7.81 (m, 2H, H of Ar), 7.51 (t, J = 7.6 Hz, 2H, H of Ar), 7.28–7.37 (m, 2H, H of Ar), 6.11 (t, J = 6.4 Hz, 1H, H-1′), 5.37–5.38 (m, 1H, H-3′), 3.99 (br s, 1H, H-4′),

3.60 (m, 2H, H-5′, H-5″), 2.22–2.31 (m, 2H, H-2′, H-2″), 2.21 (s, 3H, CH_3) ppm; ^{13}C NMR (125 MHz, DMSO-d_6): δ_C 169.07 (C═O), 163,44, (C═O), 162.22 (C═O), 156.95 (d, J = 26 Hz, C-4), 150.77, 149.52 (C of Ar), 148.93 (C-2), 140.04 (d, J = 230 Hz, C-5), 135.27, 134.75, 131.93, 131.54, 126.72, 126.50 (C of Ar), 124.46 (d, J = 34 Hz, C-6), 122.94, 121.67 (C of Ar), 84.53 (C-4′), 84.35 (C-1′), 75.66 (C-3′), 61.07 (C-5′), 36.87 (C-2′), 20.64 (CH_3) ppm; HRMS (ESI): calcd for $C_{25}H_{21}N_2O_{10}FNa$ $[M+Na]^+$: 551.1072, found: 551.1091.

*5′-O-(4,4′-dimethoxytrityl)-3′-O-benzoyl-5-fluoro-2′-deoxyuridine (**5a**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 11.93 (s, 1H, NH), 7.98–8.01 (m, 3H, H-6, *o*-H of Bz), 7.67–7.71 (m, 1H, *p*-H of Bz), 7.53–7.57 (m, 2H, *m*-H of Bz), 7.39–7.41 (m, 2H, H of DMTr), 7.20–7.30 (m, 7H, H of DMTr), 6.86 (d, J = 8.8 Hz, 4H, H of DMTr), 6.26 (t, J = 6.8 Hz, 1H, H-1′), 5.51 (t, J = 3.2 Hz, 1H, H-3′), 4.26–4.27 (m, 1H, H-4′), 3.72 (s, 6H, OCH_3), 3.28–3.48 (m, H-5′, H-5″), 2.53–2.64 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 165.58 (C═O), 158.56 (C of DMTr), 157.40 (d, J = 26 Hz, C-4), 149.40 (C-2), 145.05 (C of DMTr), 140.55 (d, J = 230 Hz, C-5), 135.72, 135.61 (C of DMTr), 134.04 (C of Bz), 130.11 (C of DMTr), 130.09, 129.63 (C of Bz), 128.39, 128.01, 127.19 (C of DMTr), 125.01 (d, J = 34 Hz, C-6), 113.64 (C of DMTr), 86.46 (C-4′), 85.03 (C-1′), 83.22 (C of DMTr), 75.13 (C-3′), 66.76 (OCH_3), 64.03 (C-5′), 36.86 (C-2′) ppm.

*5′-O-(4,4′-dimethoxytrityl)-3′-O-benzoyl-5-fluoro-3-N-benzoyl-2′-deoxyuridine (**6a**).* ^{1}H NMR (400 MHz, DMSO-d_6): δ_H 8.24 (d, $J_{6\text{-}F5}$ = 6.8 Hz, 1H, H-6), 8.12 (d, J = 7.6 Hz, 2H, *o*-H of *N*-Bz), 7.99 (d, J = 7.2 Hz, 2H, *o*-H of *O*-Bz), 7.81 (t, J = 7.3 Hz, 1H, *p*-H of *N*-Bz), 7.68 (t, J = 7.3 Hz, 1H, *p*-H of *O*-Bz), 7.61 (t, J = 7.1 Hz, 2H, *m*-H of *N*-Bz), 7.54 (t, J = 7.1 Hz, 2H, *m*-H of *O*-Bz), 7.41–7.43 (m, 2H, H of DMTr), 7.21–7.30 (m, 7H, H of DMTr), 6.88 (d, J = 7.92 Hz, 4H, H of DMTr), 6.26–6.27 (m, 1H, H-1′), 5.54 (br s, 1H, H-3′), 4.3 (br s, 1H, H-4′), 3.72 (s, 6H, OCH_3), 3.47–3.61 (m, H-5′, H-5″), 2.60–2.76 (m, 2H, H-2′, H-2″) ppm; ^{13}C NMR (100 MHz, DMSO-d_6): δ_C 168.49 (C of Bz), 165.57 (C═O), 158.57 (C of DMTr), 156.28 (d, J = 26 Hz, C-4), 147.99 (C-2), 145.03 (C of DMTr), 140.22 (d, J = 230 Hz, C-5), 136.33 (C of Bz), 135.71, 135.59 (C of DMTr), 134.06 (C of Bz), 131.16, 131.08 (C of Bz), 130.12 (C of DMTr), 129.93, 129.93, 129.60, 129.17 (C of Bz), 128.32, 128.03, 127.21 (C of DMTr), 126.14 (d, J = 34 Hz, C-6), 113.67 (C of DMTr), 86.55 (C-4′), 85.67 (C-1′), 83.43 (C of DMTr), 74.71 (C-3′), 66.76 (OCH_3), 63.84 (C-5′), 37.06 (C-2′) ppm.

4.2. Biological assays

4.2.1. Cell line and culture conditions

GBM cell lines (T98G, U-118 MG, U-87 MG), HeLa (cervical cancer cell line), T-47D (breast cancer cell line), Caco-2 (colon cancer cell line), and non-cancerous lung fibroblast cell line (MRC-5) were purchased from ATCC (Manassas, USA). All cell lines were from human origin. HeLa and T-47D were cultured in RPMI 1640 medium. Caco-2 and U-118 MG were cultured in DMEM medium. T98G, U-87 MG and MRC-5 were cultured in EMEM medium. Each medium was supplemented with 10% fetal bovine serum (FBS) and 10 mg/mL antibiotics (penicillin and streptomycin). Cells were cultured at 37 °C with 5% CO_2 in humidified air. Cell media (RPMI 1640, DMEM, EMEM), Human Blood Plasma (HBP) were obtained from Sigma–Aldrich Chemie GmbH (Steinheim, Germany) and ATCC. Cell concentrations in culture were adjusted to allow for exponential growth.

4.2.2. Cell viability/proliferation assay

Cell viability/proliferation was evaluated by a dye staining method using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT). The protocol was adapted from the literature methods [85]. The monolayer cell culture was trypsinized and counted. To each well of the 96-well plate, 100 μL of the diluted cell suspension (1×10^4 cells) was added. After 24 h, when a partial monolayer was formed, 100 μL of fresh medium with different compound concentrations (7.81, 15.625, 31.25, 62.5, 125, 250, 500 and 1000 μg/mL) were added to the cells. After 48 h, the supernatant was washed out and 100 μL of MTT solution in medium (final concentration 0.5 mg/mL) were added to each well for 2 h. After the incubation time was complete, unreacted dye was removed by aspiration. The formazan crystals were dissolved in 100 μL/well DMSO and measured spectrophotometrically in a multi-well Synergy2 plate reader (BioTek Instruments, USA) at a test wavelength of 492 nm and a reference wavelength of 690 nm. The results were calculated as an IC_{50} (inhibitory concentration 50) – the IC_{50} corresponds to the concentration of tested compound that inhibits cell viability/proliferation by 50%. Results are presented as mean of at least three independent experiments.

4.2.3. In silico pharmacokinetic prediction

Calculations of pharmacokinetic profile descriptors of the synthesized compounds were performed by various software solutions accessible on-line. The transformation of the stoichiometric formulas of the compounds into a SMILES code (Simplified Molecular Input Line Entry System) was carried out by ChemBioDraw Ultra version 12.0 program (Cambridge Software). The SMILES code was applied to calculate logP values with ALOGPS 2.1 software [86] (mean value obtained from ALOGPs, AC_logP, miLogP, ALOGP, MLOGP, LogKOWWIN, XLOGP2, XLOGP3 methods), PSA (topological polar surface area) and aPSA (apolar surface area) values. PSA and aPSA descriptors were calculated using the VEGA ZZ program [87]. The pharmacokinetic profile was evaluated according to the Lipinski "rule of five" [48] by using Molinspiration application (http://www.molinspiration.com), which includes also analyses of molecular weight (MW), number of hydrogen-bond acceptors (HBA) and number of hydrogen-bond donors (HBD). The Caco-2 prediction model based on descriptors generated by preADMET (http://preadmet.bmdrc.org) was used to compute Caco-2 apparent permeability (tP_{app}). In this model a number of hydrogen bond donors and three molecular surface area properties determine membrane permeability of compounds.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in the online version, at http://dx.doi.org/10.1016/j.ejmech.2016.03.010. These data include MOL files and InChiKeys of the most important compounds described in this article.

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[40] This scenario was checked by keepieng *O*-acetylsalicyl chloride **2h** in neat pyridine-d5 and recording periodically the ^{1}H NMR spectra of the reaction mixture. After 10 min in the region of chemical shifts δ_H 2.3 – 2.5 ppm typical for resonance frequency of the methyl group of the acetyl moiety in **2h**, several additional signals appeared, and their number and intensities were increasing in time. After 3 h intensity of the signal of the methyl group of **2h** became comparable to those of the new signals (data not shown). This seemed to indicate that *O*-acetylsalicyl chloride **2h** when left in pyridine underwent deacetylation and produced dimeric (and most likely also polymeric) acylating species, which in the reaction with nucleoside **1** could produce *O*-salicyl-salicylated 5-fluoro-2′-deoxyuridine **8** as a predominant product.
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