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Graphical Abstract

New Inhibitors of Tyrosyl-DNA Phosphodiesterase I (Tdp 1) Combining 7-Hydroxycoumarin and Monoterpenoid Moieties

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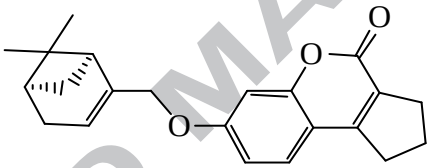

Compound **(+)-25c**. IC_{50} against Tdp1 0.675±0.007μM. **(+)-25c** enhances the cytotoxic effect of camptothecin in 8 times

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ABSTRACT

A number of derivatives of 7-hydroxycoumarins containing aromatic or monoterpene substituents at hydroxy-group were synthesized based on a hit compound from a virtual screen. The ability of these compounds to inhibit tyrosyl-DNA phosphodiesterase I (Tdp 1), important target for anti-cancer therapy, was studied for the first time. It was found that the 7-hydroxycoumarin derivatives with monoterpene pinene moiety are effective inhibitors of Tdp 1 with the most active derivative **(+)-25c** with IC_{50} value of 0.675 μM. This compound has low cytotoxicity (CC_{50} > 100 μM) when tested against human cancer cells which is crucial for presupposed application in combination with clinically established anticancer drugs. The ability of the new compounds to enhance the cytotoxicity of camptothecin, an established topoisomerase 1 poison, was demonstrated.

1. Introduction

Human tyrosyl-DNA phosphodiesterase (Tdp1) is a member of the phospholipase D superfamily.[1] Tdp1 hydrolyses the phosphodiester bond between a catalytic tyrosine Tyr723 (human) of topoisomerase 1 (Topo1) and DNA 3′-phosphate.[2] Tdp1 plays a key role in the removal of DNA damage, resulting from Topo1 inhibition with camptothecin and its derivatives irinotecan and topotecan. Furthermore, Tdp1 is also involved in the removal of DNA damage caused by other anticancer drugs commonly used in clinical practice, e.g., temozolomide, bleomycin, etoposide, etc.[3] The mechanisms of action are different for these drugs, as are the various repair ensembles of proteins that remove DNA damage. It is believed that Tdp1 is responsible for the drug resistance of some cancers,[4] making it a promising target to enhance anticancer treatment in conjunction with DNA damaging therapies.

The literature describes relatively few Tdp1 inhibitors,[5-12] with potency varying from submicromolar to millimolar values. Structures of some of these Tdp1 inhibitors (compounds **1-4**) are presented in Fig.1. Some were shown to be dual Tdp1/Topo1 inhibitors that stabilize the Topo1-DNA covalent complex and catalytically inhibit Tdp1, for example compound **3**.[7,8,9,10] Recently we have found that benzopentathiepine derivatives are effective inhibitors of Tdp1,[13] with compound **5** (Fig. 1) being the most active (IC_{50} = 0.2 μM). Compound **5** causes apoptotic cell death in MCF-7 and Hep G2 tumor cells.[13] Specific inhibition of Tdp1 is not expected be cytotoxic in itself because Tdp1-/- mice are indistinguishable from wild-type mice, physically, histologically, behaviorally, and electrophysiologically.[14] Compared to wild-type mice, Tdp1-/- mice are hypersensitive to camptothecin and bleomycin.[14] The absence of increased incidence of cancer or other health problems in Tdp1-/- mice suggests that compound **5** toxicity is associated with other cellular targets.

The combination of effective Tdp1 inhibition with general cytotoxic potential opens the possibility for an antitumor monotherapy based on derivative **5**.

At the same time, it is highly desirable to develop Tdp1 inhibitors with little or no inherent cytotoxicity for application in combination with clinically established anticancer drugs such as camptothecin.

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The aim of this work is to identify and develop a new class of potent inhibitors against Tdp1 capable of enhancing the effect of camptothecin on the tumor cells.

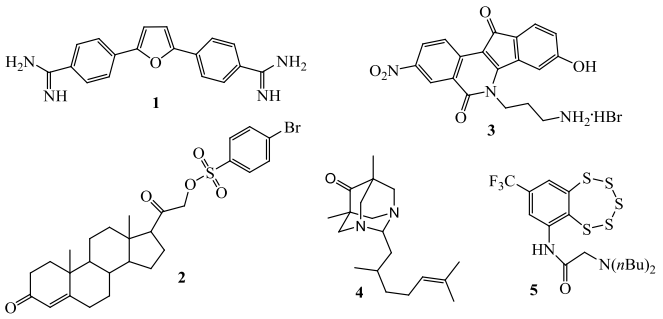


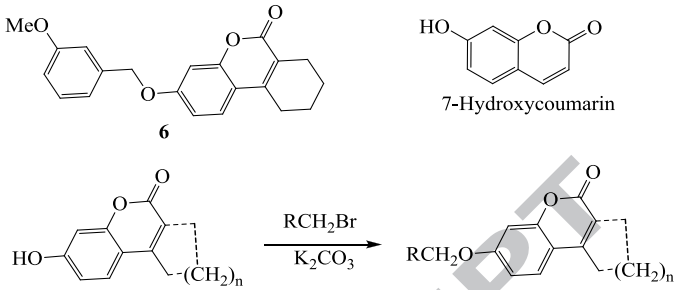
Figure 1. Structures of reported Tdp1 inhibitors.

2. Results and Discussion

2.1. Virtual Screen

One of the problems encountered during the search for new biologically active compounds by screening commercially available libraries of small molecules is their lack of chemical diversity, combined with a low proportion of biologically relevant compounds.[15] To sidestep this problem, we used the library of natural products and their derivatives (42,000 compounds, InterBioScreen). Interestingly, many antitumor agents used for chemotherapy are natural products, or their analogues.[16-18] Furthermore, many researchers are interested in developing new cytotoxic agents from this class of compounds.[19-22]

The molecules of the library were screened to the binding pocket of the crystal structure of Tdp1 (PDB ID: 1MU7, resolution 2.0 Å).[23] From the InterBioScreen natural product library 10179 compounds were used. The GoldScore (GS),[23] ChemScore (CS),[25,26] ChemPLP[27] and ASP[28] scoring functions were used to assess the binding of the ligands. The virtual screen was done in two phases using first relatively low screening efficiency weeding out compounds that were unlikely to fit the binding pocket followed by a more robust search for the remaining ligands. First, all ligands were screened and those with no or weak predicted hydrogen bonding (<1) were eliminated as well as those with low predicted binding energies (GS<43, CS<28, ChemPLP<78 and ASP<38). This left 470 candidates, which were screened again with high search efficiency and again were eliminated based on their binding energies (GS<54, CS<32, ChemPLP<85 and ASP<43) and poor hydrogen bonding (<1) resulting in 111 candidates. They were inspected visually for consensus of the best predicted configuration of the ligands between the four scoring functions, that the ligands had plausible configurations, i.e., not strained, for lipophilic moieties pointing into the water environment and, finally for undesirable moieties linked to cell toxicity and chemical reactivity.[29] The screening approach used has been previously successfully applied to find active ligands against the Phosphoinositide specific-phospholipase C-γ2 enzyme and Autophagy.[30,31] A detailed description of the virtual screen is given in the Methodology section. Sixteen compounds were selected for experimental testing and compound **6**, which is 3-methoxybenzyl derivative of 7-hydroxycoumarin annelated with the cyclohexane ring (Scheme 1), was identified as a promising inhibitor with an IC_{50} value of 5 μM using our oligonucleotide-based fluorescence assay.[13] Based on this result, we decided to synthesize structural analogs of compound **6** modified at the coumarin skeleton as well as containing other substituents at a hydroxyl group as shown in Scheme 1.



Scheme 1. General scheme of preparation of proposed inhibitors.

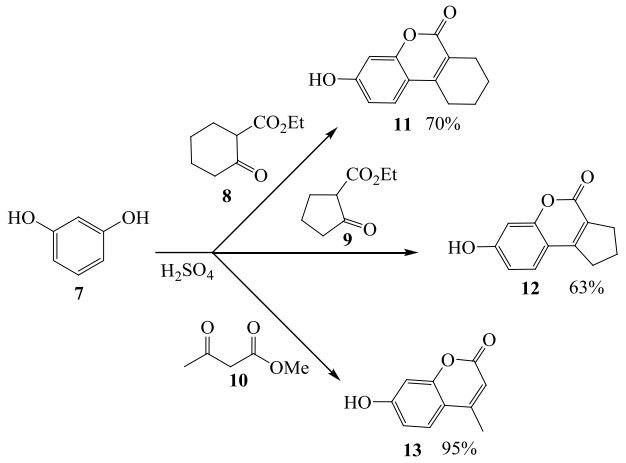
Preliminary molecular modeling against the Tdp1 binding pocket indicated that the replacement of the aromatic substituent by bulky aliphatic substituents, for example with bicyclo[3.1.1]heptane framework, could improve the efficacy of the inhibitors.

2.2. Chemistry

Synthesis of the unsubstituted at hydroxy-group coumarins was carried out by the reaction of resorcinol 7 with esters of corresponding β-keto acids **8-10** (Scheme 2), the yields of products **11-13** amounted to 63%-95%.

Bromides required for reaction with the phenolic group of coumarins were synthesized by reduction of the corresponding aldehydes to alcohols which were then reacted with PBr3 (Scheme 3). As the starting compound for the preparation of bulk aliphatic substituents we chose monoterpenoid myrtenal **18** having a bicyclo[3.1.1]heptane framework, which is accessible as both enantiomers. (-)-Enantiomer **(-)-19** was synthesized from commercially available (-)-myrtenal **(-)-18**, and for its (+)-enantiomer **(+)-19** we first obtained (+)-myrtenal **(+)-18** from (+)-α-pinene **20** (Scheme 3).

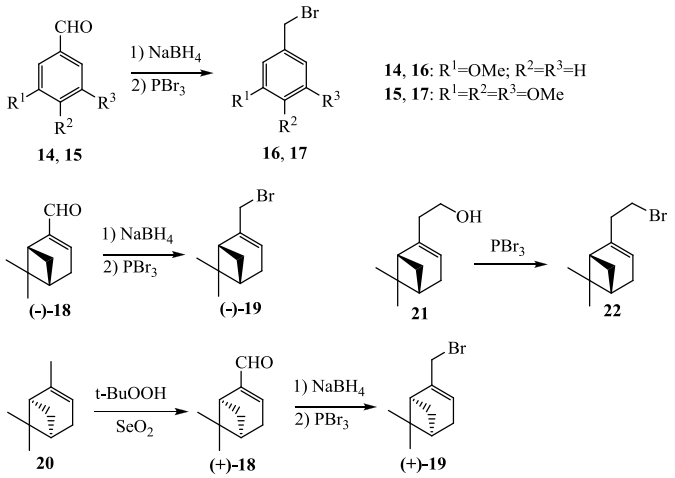
Compound **22** which is an analogue of (-)-myrtenal **(-)-18** with additional methylene group was synthesized based on nopol **21**.



Scheme 2. Synthesis of substituted 7-hydroxycoumarins.

Target compounds **6, 24b-e, 25a-e** and **26a-d** were prepared by reaction of coumarins 11-13 with the synthesized bromides **16, 17, (-)-** and **(+)-19**, and **22** as well as with benzyl bromide **23** according to the procedure shown in Scheme 1. The products

were purified by recrystallization or column chromatography. The structures of the compounds obtained are given in Table 1.



Scheme 3. Synthesis of bromides.

We did not succeed in the reaction of bromide **22** with methylcoumarin **13** due to the formation of complex reaction mixture with high level of resinification.

2.3. Biology

Tdp1 activity

Previously, we designed real-time oligonucleotide biosensor based on the capability of Tdp1 to remove fluorophore quenchers from the 3'-end of DNA.[32] Hexadecameric oligonucleotide were chosen as Tdp1 substrate, which had 5(6)-carboxyfluorescein (FAM) at the 5' end and fluorophore quencher BHQ1 (Black Hole Quencher-1) at the 3'-end.[13] This approach was used to measure Tdp1 activity and to determine inhibitory properties of the synthesized compounds.

The results of the Tdp1 assay for the coumarin derivatives are shown in Table 1. The removal of the methoxy group, **24b** compared to **6**, did not result in a significant change in activity. However, adding two additional methoxy groups to the phenyl group led to loss of activity (**24d**). Replacement of aromatic substituents by monoterpene residue (compounds **(-)-** and **(+)-24c**) quadrupled the inhibitor potency, both enantiomers were equally active. The introduction of an additional methylene link in the aliphatic chain did not affect the inhibition (see **(-)-24c** and **24e**).

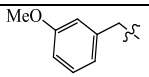
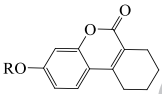
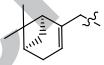
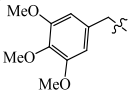
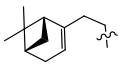
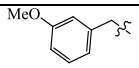
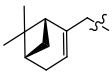
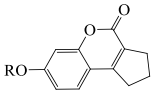
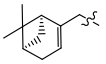
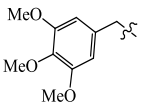
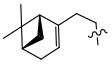
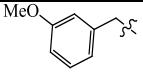
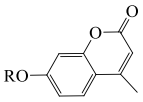
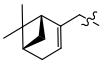
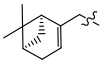
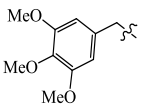
We observed decrease in efficacy of compounds **25a** and **25b**, containing aromatic substituents, when replacing the cyclohexane ring annelated with the coumarin scaffold with a cyclopentane moiety (Table 1). However, the efficiency of compounds **(-)-25c** and **25e** is similar as for the type **24** analogues (Table 1). Enantiomer **(+)-25c** was twice as active as its **(-)-25c** counterpart.

Compound **26b** was as active as its analogue **24b**, while **26a** and **26d** were inactive. The inhibitory effectiveness of compounds **(-)-** and **(+)-26c** is close to the efficiency of its **24** and **25**, counterparts. Thus the most active compounds from these series is **(+)-25c** with the impressive IC_{50} of 0.675 μM.

Cell growth and viability

To investigate the cytotoxic effect of Tdp1 inhibitors we chose the cell line MCF- 7 (human breast adenocarcinoma) with higher than the average level of Tdp1 gene expression according to BioGPS data base http://biogps.org/#goto=welcome. To compare, we chose cell line RPMI 8226 (human multiple myeloma) with Tdp1 expression level 10 times lower.

Table 1.
Inhibitory activities of compounds **6, 24b-e, 25a-e** and **26a-d** against Tdp1.

Compound	R	IC_{50}, μM
6		4.93 ± 0.49
24b		5.62 ± 0.15
(-)-24c		1.23 ± 0.34
(+)-24c	 	1.20 ± 0.53
24d		> 10
24e		1.45 ± 0.15
25a		> 10
25b		9.17 ± 0.69
(-)-25c		1.37 ± 0.29
(+)-25c	 	0.675±0.007
25d		> 10
25e		1.09 ± 0.24
26a		>10
26b		5.28 ± 0.25
(-)-26c	 	1.56 ± 0.50
(+)-26c		4.31 ± 0.46
26d		> 10

The cells MCF-7 and RPMI 8226 were exposed to the inhibitors in concentrations up to 100 μM. None of the four

tested compounds (**(+)-24c, (+)-25c, (+)-26c** and **(-)-26c**) had effect on RPMI 8226 cells viability (data not shown) Part of them do not show any antiproliferative effect for concentration up to 100 μM and are therefore not cytotoxic for MCF-7 cells (Fig. 2, **(+)-24c**, black, **(-)-24c**, red, **(-)-25c**, pink).

Compounds **(+)-26c** and **(-)-26c** have some influence on MCF-7 cells (Fig. 2, dark blue and purple), but no concentration dependence was observed. Derivatives **24e** and **25e** (Fig. 2, blue and green) demonstrated weak concentration-dependence on MCF-7 cells as evaluated by the MTT cytotoxicity assay, but it was not possible to determine the 50% cytotoxic concentration (CC_{50} value) in the concentration range used.

Synergistic activity with camptothecin against tumor cells

Camptothecin (CPT) and its derivatives are important Topo1 inhibitors, recognized for their anticancer activities. For instance, irinotecan is key chemotherapeutic drug for metastatic colorectal cancer and other types of tumors.[33] Topotecan is used to treat cancer of the ovaries when other treatments have failed and may also be used in certain types of lung cancer (small cell lung cancer). Since Tdp1 is involved in the removal of DNA damage caused by CPT, it is believed that Tdp1 is responsible for drug resistance of some cancers. Thus, a combination of these anticancer drugs and Tdp1 inhibitors could significantly improve the effectiveness of chemotherapy.

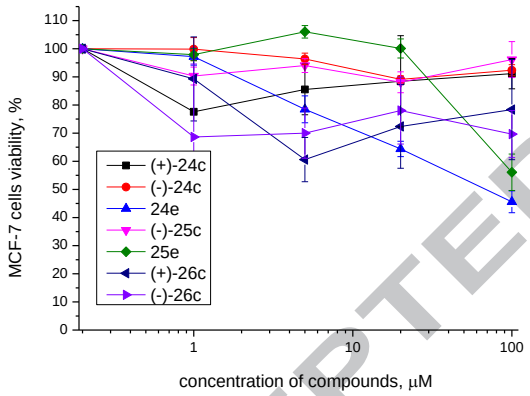


Figure 2. Dose-dependent action of the coumarins on MCF-7 cells viability measured with the MTT assay.

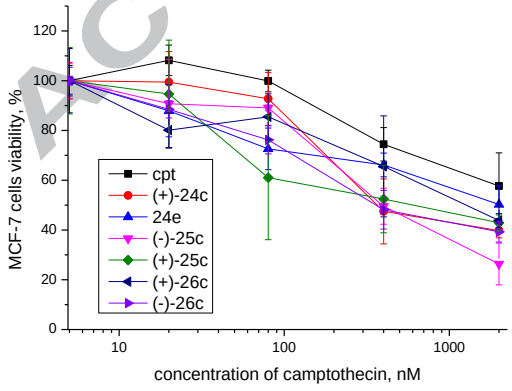
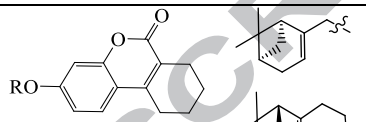
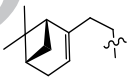
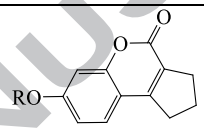
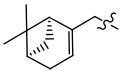
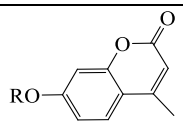
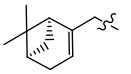


Figure 3. Dose-dependent action of CPT in combination with the coumarins on MCF-7 cell viability.

To check this hypothesis, we evaluated the cellular effect of CPT in the presence of the Tdp1 inhibitors. We used nontoxic concentrations of coumarins (5 μM) and different concentrations of CPT to estimate CC_{50} values for MCF-7 and RPMI 8226 cells. MTT tests showed marked reduction of CC_{50} values for MCF-7 cells in the presence of the coumarine derivatives as shown in Fig. 3 and Table 2.

Table 2.
CC_{50} values for MCF-7 cells in the presence of coumarine derivatives in combination with CPT.

Compound	R	CC_{50} for CPT, nM
CPT		1430 ± 430
(+)-24c		410 ± 165
24e		2300 ± 840
(-)-25c		330 ± 20
(+)-25c		180 ± 70
(-)-26c		340 ± 80
(+)-26c		320 ± 95

It is clear that most of the coumarins enhance the cytotoxicity of camptothecin on MCF-7 cells. Only compound **24e** did not enhance the CPT activity. The best results were again obtained for compound **(+)-25c**, which was able to reduce the CC_{50} of camptothecin eight fold.

In contrast to MCF-7 cells, RPMI 8226 cells did not show increased sensitivity to camptothecin in the presence of inhibitor **(+)-25c** (Fig. 4). Most likely, such differences can be explained by the different levels of Tdp1 gene expression in these cell lines. It is indirect proof that the obtained growth inhibition occurs due to specific targeting of Tdp1.

Thus, coumarins are a promising class of compounds to develop as sensitizers of Topo1 inhibitors for an enhanced therapeutic effect.

2.4. Molecular modelling

All of the derivatives were docked against the binding pocket of the Tdp1 crystal structure (PDB ID: 1MU7, resolution 2.0 Å),[23] and the results of the scoring functions used are given in Table S1 in the SI. The docked configuration of the most active derivative **(+)-25c** is shown in Fig. 5. As can be seen, three hydrogen bonding interactions are predicted with Asparagine (Asn) 283, Histidines (His) 493 and 263, respectively.

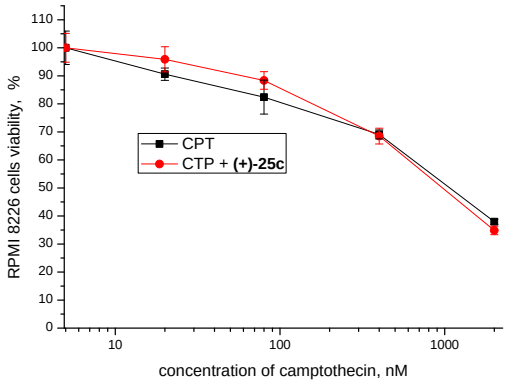


Figure 4. Dose-dependent action of CPT in combination with the coumarin (+)-25c on RPMI 8226 cell viability.

Previous molecular modelling studies strongly indicate that hydrogen bonding to Histidine 263 is required for effective inhibition.[5,12] Also, the hydrophobic pocket in the binding site is occupied by the terpene moiety. It can therefore be stated that a plausible binding mode is predicted.

Considering the difference of the **24, 25** and **26** series it is clear from the modelling that the aliphatic ring systems and the methyl group are accommodated by a lipophilic cleft. Regarding, the analogues containing the 3,4,5-trimethoxy substituted phenyl ring (**24d, 25d** and **26d**) a good fit was not predicted to the binding site, the phenyl moiety was not inserted into the lipophilic pocket, which can explain their inactivity.

The mainstream molecular descriptors are given in Table S2 in the Supplementary Information. The coumarins are relatively small all with MW < 400 g mol^{-1}. The log P values range from 3.5 to 5.5 with only three derivatives exceeding 5. None of them have hydrogen bond donors but have three to six hydrogen bond acceptors. In general, the coumarins are within drug-like chemical space.

2.5. Conclusions

It was found that the 7-hydroxycoumarin derivative **(+)-25c** with monoterpene pinene moiety is an effective inhibitor of tyrosyl-DNA phosphodiesterase I (Tdp 1) with IC_{50} value 675 nM. Furthermore, it has low cytotoxicity (CC_{50} > 100 µM) when tested against human cancer cells. Finally, the ability of the coumarin derivatives to substantially enhance the cytotoxicity of camptothecin was demonstrated, with **(+)-25c** by an order of a magnitude.

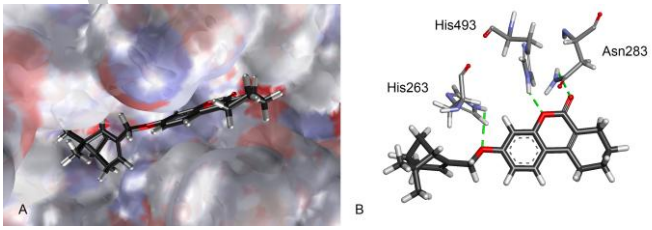


Figure 4. The docked configuration of **(+)-25c** to the binding site of Tdp1 using the ASP scoring function. (A) The protein surface is rendered. The terpene group is occupying a lipophilic pocket and the coumarin is in a cleft. Red depicts a positive partial charge on the surface, blue depicts negative partial charge and grey shows neutral/lipophilic areas. (B) Hydrogen bonds are depicted as green lines between the ligand and the amino acids His263, Asn282 and His493.

3. Experimental section

3.1. General chemical methods

Reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich, Acros) and used as received. GC-MS: *Agilent 7890A* gas chromatograph equipped with a quadrupole mass spectrometer *Agilent 5975C* as a detector; quartz column HP-5MS (copolymer 5%–diphenyl–95%–dimethylsiloxane) of length 30 m, internal diameter 0.25 mm and stationary phase film thickness 0.25 µm. Optical rotation: polAAr 3005 spectrometer; $CHCl_3$ soln. ^1H and ^{13}C NMR: *Bruker DRX-500* apparatus at 500.13 MHz (^1H) and 125.76 MHz (^{13}C), *J* in Hz; structure determinations by analyzing the ^1H NMR spectra, including ^1H – ^1H double resonance spectra and ^1H – ^1H 2D homonuclear correlation, *J*-modulated ^{13}C NMR spectra (JMOD), and ^{13}C – ^1H 2D heteronuclear correlation with one-bond and long-range spin-spin coupling constants (C – H COSY, ^1J(C,H) = 160 Hz, COLOC, $^{2,3}J$(C,H) = 10 Hz). HR-MS: DFS Thermo Scientific spectrometer in a full scan mode (15-500 m/z, 70 eV electron impact ionization, direct sample administration).

Spectral and analytical investigations were carried out at Collective Chemical Service center of Siberian Branch of Russian Academy of Sciences. All product yields are given for pure compounds purified by recrystallization or isolated by column chromatography. Column chromatography (CC): silica gel (SiO_2; 60-200 µ; *Macherey-Nagel*); hexane, solution containing from 25 to 100% chloroform in hexane, ethanol. The purity of the target compounds was determined by GC-MS methods. All of the target compounds reported in this paper have a purity of no less than 95%.

Synthesis of (1*S*,5*R*)-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-carbaldehyde (+)-18.

(+)-Myrtenal (+)-**18** was synthesized according to the procedure[33] by oxidation of (+)-α-pinene (**20**) ($[\alpha]_{589}^{30.8}$=50.4 (neat)) using *t*-BuOOH/SeO_2 system with 57% yield.

Synthesis of compounds 11-13.

Syntheses were carried out from resorcinol **7** (45 mmol) and appropriate β-keto esters (**8** -**10**) in accordance with article.[35] Conc. H_2SO_4 (5 mL, 94 mmol) was added dropwise to cooled (0-5 °C) solution of resorcinol **7** (45 mmol) and appropriate β-keto esters (**8-10**) (45 mmol) in dry ethanol (15 mL) with vigorously stirring. The mixture was stirred until to be congealed, left overnight at r.t., and poured into ice water (150 mL). The resulting solid was filtered off and crystallized from ethanol-water (75%). The yields of **11**, **12** and **13** were 70%, 64% and 95% respectively.

Synthesis of compounds 16, 17, (+)-19, (-)-19, 22.

Bromides **16**, **17**, (+)- and (-)-**19**, **22** were synthesized from the corresponding monoterpene or methoxyphenyl aldehydes (**14**, **15**, (+)- and (-)-**18**) via reduction to alcohols with $NaBH_4$,[34] followed by the reaction with PBr_3.[36]

$NaBH_4$ (10.3 mmol) was added to cooled (0-5°C) solution of 10.3 mmol of appropriate aldehyde in methanol (20 ml) and the reaction mixture was stirred for 3 h at r.t. Then 5% aqueous HCl was added to reach pH 4-5. The solvent was distilled off and the product was extracted by ether, dried with Na_2SO_4. The solvent was evaporated; resulting alcohols were used in the synthesis without purification (yields of alcohols– 34-73%).

PBr_3 (2.5 mmol) was added to cooled (0-5°C) solution of 7.5 mmol of appropriate alcohol in dry ether (10 ml) and the reaction mixture was stirred for 2 h at r.t. Saturated aqueous $NaHCO_3$ was added and the product was extracted with ether. The extracts were washed with brine, dried with Na_2SO_4 and evaporated. Compounds **16**, **17**, (+)- and (-)-**19** (the yields 65%, 61%, 55% and 60%, respectively) were sufficiently pure and used for the next step without purification. The compound **22** was purified by column chromatography on SiO_2 (yield 24%).

Synthesis of compounds 6, **24b-e**, **25a-e and 26a-d.**

Compounds **6**, **24b-e**, **25a-e** and **26a-d** were synthesized according to the procedure.[37] To 0.5 mmol of corresponding compound **11**, **12** or **13** in dry ethanol (5 mL) 0.75 mmol of K_2CO_3, and 0.75 mmol of bromide (**16**, **17**, (-)-**19**, (+)-**19**, **22**) were added at r.t. under stirring. The reaction mixture was stirred at r.t. for 15 minutes, and then heated at 60 °C for 5 hours. The hot solution was filtered; the filtrate was kept at -18 °C for 48 hours. The products were isolated in the individual form a) by recrystallization from ethanol; or b) by column chromatography on silica gel, eluent - solution containing from 25 to 100% chloroform in hexane.

*3-((3-Methoxybenzyl)oxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**6**).* Yield 56%, method **a**. M.p. 90 °C. ^{1}H-NMR ($CDCl_3$, δ_H):1.74-1.86 (m, 4H, 2H-C(11), 2H-C(12)); 2.53 (tm, 2H, *J*(10,11)=6.2, 2H-C(10)); 2.72 (tm, 2H, *J*(13,12)=6.2, 2H-C(13)); 3.80 (s, 3H-C(21)); 5.07 (s, 2H, 2H-C(14)); 6.84 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.85 (ddd, 1H, *J*(18,19)=8.3, *J*(18,16)=2.6, *J*(18,20)=0.9, H-C(18)); 6.88 (dd, 1H, *J*(7,6)=8.8, J(7,9)=2.5, H-C(7)); 6.95 (dd, 1H, *J*(16,18)=2.6, *J*(16,20)=1.6, H-C(16)); 6.98 (ddd, 1H, *J*(20,19)=7.5, *J*(20,16)=1.6, *J*(20,18)=0.9, H-C(20)); 7.28 (dd, 1H, *J*(19,18)=8.3, *J*(19,20)=7.5, H-C(19)); 7.43 (d, 1H, J(6,7)=8.8, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 153.33 (s, C(1)); 161.97 (s, C(2)); 120.58 (s, C(3)); 147.07 (s, C(4)); 113.89 (s, C(5)); 124.01 (d, C(6)); 112.48 (d, C(7)); 160.31 (d, C(8)); 101.65 (d, C(9)); 23.73 (t, C(10)); 21.57 (t, C(11)); 21.28 (t, C(12)); 25.10 (t, C(13)); 70.13 (t, C(14)); 137.53 (s, C(15)); 112.83 (d, C(16)); 159.81 (s, C(17)); 113.59 (d, C(18)); 129.66 (d, C(19)); 119.49 (d, C(20)); 55.14 (q, C(21)). HRMS: 336.1363 ([M]$^+$, m/z calcd for $C_{21}H_{20}O_4$ 336.1356).

*3-(Benzyloxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**24b**).* Yield 84%, method **a**. M.p. 140 °C. ^{1}H-NMR ($CDCl_3$, δ_H):1.74-1.85 (m, 4H, 2H-C(11), 2H-C(12)); 2.53 (tm, 2H, *J*(10,11)=6.2, 2H-C(10)); 2.71 (tm, 2H, *J*(13,12)=6.2, 2H-C(13)); 5.09 (s, 2H, 2H-C(14)); 6.84 (d, 1H, *J*(9,7) = 2.5, H-C(9)); 6.88 (dd, 1H, *J*(7,6)= 8.8, *J*(7,9) =2.5, H-C(7)); 7.32 (tt, 1H, *J*(18,17(19))=7.1, *J*(18,16(20))=1.5, H-C(18)); 7.37 (tm, 2H, *J*=7.1, H-C(17), H-C(19)); 7.41 (br. d, 2H, *J*(16,17)=*J*(20,19)=7.1, H-C(16), H-C(20)). ^{13}C-NMR ($CDCl_3$, δ_C): 153.31 (s, C(1)); 161.95 (s, C(2)); 120.54 (s, C(3)); 147.05 (s, C(4)); 113.85 (s, C(5)); 124.00 (d, C(6)); 112.46 (d, C(7)); 160.33 (s, C(8)); 101.60 (d, C(9)); 23.71 (t, C(10)); 21.56 (t, C(11)); 21.26 (t, C(12)); 25.08 (t, C(13)); 70.24 (t, C(14)); 135.93 (s, C(15)); 127.36 (d, C(16), C(20)); 128.57 (d, C(17), C(19)); 128.13 (d, C(18)). HRMS: 306.1249 ([M]$^+$, m/z calcd for $C_{20}H_{18}O_3$ 306.1251).

*3-(((1R,5S)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one ((-)-**24c**).* Yield 38%, method **a**. M.p. 110 °C, $[\alpha]_{589}^{30.8}$=-25 (*c*=0.75, EtOH). ^{1}H-NMR ($CDCl_3$, δ_H): 0.80 (s, 3H-C(23)); 1.16 (d, 1H, ^{2}*J*=8.7, H-C(21a)); 1.27 (d, 3H-C(22)); 1.74-1.85 (m, 4H, 2H-C(11), 2H-C(12)); 2.09 (ddddd, 1H, *J*(18,20)=*J*(18,21s)=5.6, *J*(18,17a)=*J*(18,17s)=2.8, *J*(18,16)=1.3, H-C(18)); 2.20 (ddd, 1H, J(20,18)=J(20,21s)=5.6, J(20,16)=1.4, H-C(20)); 2.24 (dm, 1H, ^{2}*J*=18.0, H-C(17a)); 2.32 (dm, 1H, ^{2}*J*=18.0, H-C(17s)); 2.39(ddd, 1H, ^{2}*J*=8.7, *J*(21s,18)=*J*(21s,20)=5.6, H-C(21s)); 2.53 (tm, 2H, *J*(10,11)=6.3, 2H-C(10)); 2.72 (tm, 2H, *J*(13,12)=6.3, 2H-C(13)); 4.41 (dm, 1H, ^{2}*J*=12.4, other *J*≤2.0, H-C(14)); 4.43 (dm, 1H, ^{2}*J*=12.4, other *J*≤2.0, H-C(14')); 5.59-5.62 (m, 1H, H-C(16)), 6.79 (d, 1H, *J*(9,7)=2.4, H-C(9)); 6.81 (dd, 1H, *J*(7,6)=8.7, *J*(7,9)=2.4, H-C(7)); 7.40 (d, 1H, *J*(6,7)=8.7, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 153.30 (s, C(1)); 162.12 (s, C(2)); 120.29 (s, C(3)); 147.17 (s, C(4)); 113.54 (s, C(5)); 123.79 (d, C(6)); 112.62 (d, C(7)); 160.69 (s, C(8)); 101.49 (d, C(9)); 23.72 (t, C(10)); 21.60 (t, C(11)); 21.29 (t, C(12)); 25.10 (t, C(13)); 70.93 (t, C(14)); 143.12 (s, C(15)); 121.12 (d, C(16)); 31.16 (t, C(17)); 40.69 (d, C(18)); 37.98 (s, C(19)); 43.06 (d, C(20)); 31.38 (t, C(21)); 26.01 (q, C(22)), 20.96 (q, C(23)). HRMS 350.1875 ([M]$^+$, m/z calcd for $C_{23}H_{26}O_3$ 350.1877).

*3-(((1S,5R)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one ((+)-**24c**).* Yield 38%, the method **a**. M.p. 105°C. $[\alpha]_{589}^{31.7}$ =+32.6 (*c*=0.65. EtOH). ^{1}H and ^{13}C NMR spectra of **((+)-24c)** correspond to the spectra of **((-)-24c).** HRMS 350.1872 ([M]$^+$, m/z calcd for $C_{23}H_{26}O_3$ 350.1876).

*3-((3,4,5-Trimethoxybenzyl)oxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**24d**).* Yield 44%, method **b**. M.p. 182 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 1.74-1.86 (m, 4H, 2H-C(11), 2H-C(12)); 2.54 (tm, 2H, *J*(10,11)=6.2, 2H-C(10)); 2.73 (tm, 2H, *J*(13,12)=6.2, 2H-C(13)); 3.83 (s, 3H-C(22)); 3.85 (s, 6H-C(23), -C(21)); 5.01 (s, 2H, 2H-C(14)); 6.63 (s, 2H, H-C(16), H-C(20)); 6.85 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.89 (dd, 1H, *J*(7,6)=8.8, *J*(7,9)=2.5, H-C(7)); 7.44 (d, 1H, *J*(6,7)=8.8, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 153.32 (s, C(1)); 162.00 (s, C(2)); 120.64 (s, C(3)); 147.12 (s, C(4));113.95 (s, C(5)); 124.05 (d, C(6)); 112.50 (d, C(7)); 160.27 (s, C(8)); 101.54 (d, C(9)); 23.72 (t, C(10)); 21.55 (t, C(11)); 21.26 (t, C(12)); 25.11 (t, C(13)); 70.53 (t, C(14)); 131.44 (s, C(15)); 104.52 (d, C(16), C(20)); 153.42 (s, C(17), C(19)); 137.84 (s, C(18)); 56.05 (q, C(21), C(23)); 60.72 (q, C(22)). HRMS 396.1563 ([M]$^+$ m/z calcd for $C_{23}H_{26}O_3$ 396.1567).

*3-(2-((1R,5S)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethoxy)-7,8,9,10-tetrahydro-6H-benzo[c]chromen-6-one (**24e**).* Yield 32%, method **a**. M.p. 120 °C. $[\alpha]_{589}^{27.1}$=-12.7 (*c*=0.55, EtOH). ^{1}H-NMR ($CDCl_3$, δ_H): 0.80 (s, 3H-C(24)); 1.15 (d, 1H, ^{2}*J*=8.5, H-C(22a)); 1.25 (s, 3H-C(23)); 1.73-1.86 (m, 4H, 2H-C(11), 2H-C(12)); 2.05-2.10 (m, 2H, H-C(19), H-C(21)); 2.19 (dm, 1H, ^{2}*J*=17.7, H-C(18)); 2.26 (dm, 1H, ^{2}*J*=17.7, H-C(18')); 2.36 (ddd, 1H, ^{2}*J*=8.5, *J*(22s,19)=*J*(22s,21)=5.6, H-C(22s)); 2.39-2.50 (m, 2H-C(15)); 2.50-2.55 (m, 2H-C(10)); 2.69-2.75 (m, 2H-C(13)); 3.95-4.00 (m, 2H-C(14)); 5.32-5.35 (m, 1H, H-C(17)); 6.75 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.79 (dd, 1H, *J*(7,6)=8.8, *J*(7,9)=2.5, H-C(7)); 7.41 (d, 1H, *J*(6,7)=8.8, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 153.36 (s, C(1)); 162.12 (s, C(2)); 120.24 (s, C(3)); 147.24 (s, C(4)); 113.49 (c, C(5)); 123.93 (d, C(6)); 112.32 (d, C(7)); 160.64 (s, C(8)); 100.94 (d, C(9)); 23.69 (t, C(10)); 21.27 (t, C(11)); 21.58 (t, C(12)); 25.10 (t, C(13); 66.68 (t, C(14)); 36.11 (t, C(15)); 143.95 (s, C(16)); 118.89 (d, C(17)); 31.24 (t, C(18)); 40.56 (d, C(19)); 37.96 (s, C(20)); 45.69 (d, C(21)); 31.52 (t, C(22)); 26.15 (q, C(23)); 21.08 (q, C(24)). HRMS: 363.1947 ([M]]$^+$, m/z calcd for $C_{24}H_{27}O_3$ 363.1955).

*7-((3-Methoxybenzyl)oxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one (**25a**).* Yield 40%, method **a**. M.p. 122 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 2.14-2.22 (m, 2H, 2H-C(11)); 2.84-2.89 (m, 2H, 2H-C(10)); 2.99-3.04 (m, 2H, 2H-C(12)); 3.80 (s, 3H-C(20)); 5.08 (s, 2H, 2H-C(13)); 6.86 (ddd, 1H, *J*(17,18)=8.3, *J*(17,15)=2.6, *J*(17,19)=1.0, H-C(17)); 6.89 (dd, 1H, *J*(7,6)=9.2,

J(7,9)=2.5, H-C(7)); 6.90 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.95 (dd, 1H, *J*(15,17)=2.6, *J*(15,19)=1.6, H-C(15)); 6.98 (ddd, 1H, *J*(19,18)=7.6, *J*(19,15)=1.6, *J*(19,17)=1.0, H-C(19)); 7.29 (dd, 1H, *J*(18,17)=8.3, *J*(18,19)=7.6, H-C(18)); 7.32 (d, 1H, *J*(6,7)=9.2, H-C(6)).^{13}C-NMR ($CDCl_3$, δ_C): 155.60 (s, C(1)); 160.32 (s, C(2)); 124.57 (s, C(3)); 156.10 (s, C(4)); 112.55 (s, C(5)); 125.49 (d, C(6)); 112.70 (d, C(7)); 160.89 (s, C(8)); 101.81 (d, C(9)); 30.26 (t, C(10)); 22.45 (t, C(11)); 31.93 (t, C(12)); 70.19 (t, C(13)); 137.45 (s, C(14)); 112.85 (d, C(15)); 159.83 (s, C(16)); 113.62 (d, C(17)); 129.68 (d, C(18)); 119.50 (d, C(19)); 55.15 (q, C(20)). HRMS 322.1203 ([M]$^+$, m/z calcd for $C_{20}H_{18}O_4$ 322.1200).

*7-(Benzyloxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one **(25b)***. Yield 35%, method **a**. M.p. 223 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 2.13-2.21 (m, 2H, 2H-C(11)); 2.85-2.90 (m, 2H, 2H-C(10)); 2.99-3.04 (m, 2H, 2H-C(12)); 5.11 (s, 2H, 2H-C(13)); 6.90 (dd, 1H, *J*(7,6) = 8.4, *J*(7,9) = 2.5, H-C(7)); 6.92 (d, 1H, *J*(9,7)= 2.5, H-C(9)); 7.28-7.35 (m, 2H, H-C(6), H-C(17)); 7.38 (br.t, 2H, *J*=7.5, H-C(16), H-C(18)); 7.41 (br.d, 2H, *J*(15,16)=*J*(19,18)=7.5, H-C(15), H-C(19)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.64 (s, C(1)); 160.36 (s, C(2)); 124.59 (s, C(3)); 156.13 (s, C(4)); 112.55 (s, C(5)); 125.50 (d, C(6)); 112.75 (d, C(7)); 160.96 (s, C(8)); 101.80 (d, C(9)); 30.28 (t, C(10)); 22.48 (t, C(11)); 31.95 (t, C(12)); 70.35 (t, C(13)); 135.88 (s, C(14)); 127.41 (d, C(15), C(19)); 128.63 (d, C(16), C(18)); 128.21 (d, C(17)). HRMS: 292.1098 ([M]$^+$, m/z calcd for $C_{19}H_{16}O_3$ 292.1094).

*7-(((1R,5S)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one ((-)-**25c**)*. Yield 40%, method **a**. M.p. 145 °C. $[\alpha]_{589}^{30.8}$ =-20.5, (*c*=0.5, EtOH). ^{1}H-NMR ($CDCl_3$, δ_H): 0.80 (s, 3H-C(22)); 1.16 (d, 1H, ^2J=8.6, H-C(20a)); 1.28 (s, 3H-C(21)); 2.10 (ddddd, 1H, *J*(17,19)=*J*(17,20s)=5.6, *J*(17,16a)=*J*(17,16s)=2.9, *J*(17,15)=1.3, H-C(17)); 2.13-2.20 (m, 2H, 2H-C(11)); 2.20 (ddd, 1H, *J*(19,17)=*J*(19,20s)=5.6, *J*(19,15)=1.4, H-C(19)); 2.25 (dm, 1H, ^2J=18.0, H-C(16a)); 2.32 (d m, 1H, ^2J=18.0, H-C(16s)); 2.40 (ddd, 1H, ^2J=8.6, *J*(20s,17)=*J*(20s,19)=5.6, H-C(20s)); 2.84-2.88 (m, 2H, 2H-C(10)); 2.99-3.03 (m, 2H, 2H-C(12)); 4.42 (dm, 1H, ^2J=12.4, other *J*≤2.0, H-C(13)); 4.44 (dm, 1H, ^2J=12.4, other *J*≤2.0, H-C(13')); 5.60-5.63 (m, 1H, H-C(15)), 6.82 (dd, 1H, *J*(7,6)=8.6, *J*(7,9)=2.4, H-C(7)); 6.85 (d, 1H, *J*(9,7)=2.4, H-C(9)); 7.29 (d, 1H, *J*(6,7)=8.6, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.60 (s, C(1)); 160.48 (s, C(2)); 124.28 (s, C(3)); 156.23 (s, C(4)); 112.21 (s, C(5)); 125.28 (d, C(6)); 112.85 (d, C(7)); 161.29 (s, C(8)); 101.67 (d, C(9)); 30.24 (t, C(10)); 22.47 (t, C(11)); 31.93 (t, C(12)); 71.00 (t, C(13)); 143.07 (s, C(14)); 121.22 (d, C(15)); 31.17 (t, C(16)); 40.69 (d, C(17)); 37.99 (s, C(18)); 43.07 (d, C(19)); 31.39 (t, C(20)); 26.01 (q, C(21)); 20.97 (q, C(22)). HRMS: 336.1722 ([M]$^+$, m/z calcd for $C_{22}H_{24}O_3$ 336.1720).

*7-(((1S,5R)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one ((+)-**25c**)*. Yield 55%, method **a**. M.p. 140 °C. $[\alpha]_{589}^{30.7}$ =+27.6 (*c*=0.65, EtOH). The ^{1}H and ^{13}C NMR spectra of **((+)-25c)** correspond to the spectra of the enantiomer **((-)-25c)**. HRMS 336.1718 ([M]$^+$, m/z calcd for $C_{22}H_{24}O_3$ 336.1720).

*7-((3,4,5-Trimethoxybenzyl)oxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one **(25d)***. Yield 40%, method **b**. M.p. 179 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 2.14-2.22 (m, 2H, H-C(11)); 2.85-2.90 (m, 2H, H-C(10)); 3.00-3.05 (m, 2H, H-C(12)); 3.84 (s, 3H, H-C(21)); 3.86 (s, 6H, 3H-C(20), 3H-C(22)); 5.02 (s, 2H, H-C(13)); 6.64 (s, 2H, H-C(15), H-C(19)); 6.91 (dd, *J*(7,6)=8.8, J(7,9)=2.5, 1H, H-C(7); 6.92 (d, *J*(9,7)=2.5, 1H, H-C(9)); 7.34 (br. d, *J*(6,7)=8.8, 1H, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.63 (s, C(1)); 160.35 (s, C(2)); 124.68 (s, C(3)); 156.14 (s, C(4)); 112.64 (s, C(5)); 125.54 (d, C(6)); 112.73 (d, C(7)); 160.87 (s, C(8)); 101.73 (d, C(9)); 30.28 (t, C(10)); 22.47 (t, C(11)); 31.96 (t, C(12)); 70.62 (t, C(13)); 131.37 (s, C(14)); 104.56 (d, C(15), C(19)); 153.46 (c, C(16), C(18)); 137.91 (s, C(17)); 56.08 (q, C(20), C(22)); 60.75 (q, C(21)). HRMS: 382.1410 ([M]$^+$, m/z calcd for $C_{22}H_{22}O_6$ 382.1411).

*7-(2-((1R,5S)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)ethoxy)-2,3-dihydrocyclopenta[c]chromen-4(1H)-one **(25e)***. Yield 29%, method **b**. M.p. 149 °C. $[\alpha]_{589}^{28.3}$ =-9.7 (*c*=0.31, MeOH). ^{1}H-NMR ($CDCl_3$, δ_H): 0.81 (s, 3H, H-C(23)); 1.16 (d, 1H, ^2J=8.5, H-C(21a)); 1.26 (s, 3H, H-C(22)); 2.06-2.10 (m, 2H, H-C(18), H-C(20)); 2.13-2.23 (m, 3H, 2H-C(11), H-C(17)); 2.27 (dm, 1H, ^2J=17.6, H-C(17')); 2.36 (ddd, 1H, ^2J=8.5, *J*(21s,18)=*J*(21s,20)=5.6, H-C(21s)); 2.40-2.51 (m, 2H, H-C(14)); 2.84-2.90 (m, 2H, H-C(10)); 2.99-3.04 (m, 2H, H-C(12)); 4.00 (td, 2H, *J*(13,14)=7.0, *J*(13,9)=0.7; H-C(13)); 5.32-5.36 (m, 1H, H-C(16)); 6.80 (dd, 1H, *J*(7,6)=8.5, *J*(7,9)=2.4, H-C(7)); 6.82 (br.d, 1H, *J*(9,7)=2.4, H-C(9)); 7.30 (d, 1H, *J*(6,7)=8.5, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.72 (s, C(1)); 160.44 (s, C(2)); 124.29 (s, C(3)); 156.23 (s, C(4)); 112.21 (s, C(5)); 125.40 (d, C(6)); 112.56 (d, C(7)); 161.29 (s, C(8)); 101.20 (d, C(9)); 30.25 (t, C(10)); 22.49 (t, C(11)); 31.94 (t, C(12)); 66.80 (t, C(13); 36.14 (t, C(14)); 143.99 (s, C(15)); 118.93 (d, C(16)); 31.29 (t, C(17)); 40.66 (d, C(18)); 37.99 (s, C(19)); 45.81 (d, C(20)); 31.55 (t, C(21)); 26.19 (q, C(22)); 21.09 (q, C(23)). HRMS: 349.1797 ([M-H]$^+$, m/z calcd for $C_{23}H_{25}O_3$ 349.1798).

*7-((3- Methoxybenzyl) oxy)-4-methyl-2H-chromen-2-on **(26a)***. Yield 46%, method **a**. M.p. 87 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 2.36 (d, *J*(10,3)=1.2, 3H-C(10)); 3.80 (s, 3H-C(18)); 5.08 (s, 2H-C(11)); 6.10 (q, 1H, *J*(3,10)=1.2, H-C(3)); 6.847 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.854 (ddd, 1H, *J*(15,16)=8.0, *J*(15,13)=2.6, *J*(15,17)=1.0, 1H-C(15)); 6.91 (dd, *J*(7,6)=8.8, J(7,9)=2.5, H-C(7)), 6.95 (dd, 1H, *J*(13,15)=2.6, *J*(13,17)=1.6, H-C(13)); 6.98(ddd, 1H, *J*(17,16)=7.5, *J*(17,13)=1.6, *J*(17,15)=1.0, H-C(17)), 7.28 (dd, 1H, *J*(16,15)=8.0, *J*(16,17)=7.5, H-C(16)); 7.47 (d, 1H, *J*(6,7)=8.8, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.05 (s, C(1)); 161.07 (s, C(2)); 111.91 (d, C(3)); 152.35 (s, C(4)); 113.65 (s, C(5)); 125.42 (d, C(6)); 112.74 (d, C(7)); 161.51 (s, C(8)); 101.84 (d, C(9)); 18.46 (q, C(10)); 70.18 (t, C(11)); 137.29 (s, C(12)); 112.84(d, C(13)); 159.80 (s, C(14)); 113.59 (d, C(15)); 129.67 (d, C(16)); 119.44 (d, C(17)); 55.11 (q, C(18)). HRMS 296.1045 ([M]$^+$, m/z calcd for $C_{18}H_{16}O_4$ 296.1043).

*7-(Benzyloxy)-4-methyl-2H-chromen-2-on **(26b)***. Yield 78%, method **b**. M.p. 115°C. The ^{1}H NMR spectrum of **26b** is coincided with the corresponding spectra published in literature.[38]

*7-(((1R,5S)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-4-methyl-2H-chromen-2-one ((-)-**26c**)*. Yield 54%, method **b**. M.p. 70 °C. $[\alpha]_{589}^{31.7}$ =-36 (*c*=0.8, EtOH). ^{1}H-NMR ($CDCl_3$, δ_H): 0.80 (s, 3H-C(20)); 1.17 (d, 1H, ^2J=8.7, H-C(18a)); 1.28 (s, 3H-C(19)); 2.08-2.13 (m, 1H, H-C(15)); 2.20 (ddd, 1H, *J*(17,15)=*J*(17,18s)=5.6, *J*(17,13)=1.4, H-C(17)); 2.25 (dm, 1H, ^2J=18.0, H-C(14a)); 2.33 (dm, 1H, ^2J=18.0, H-C(14s)); 2.35 (d, 3H, *J*(10,3)=1.2, H-C(10)); 2.40 (ddd, 1H, ^2J=8.7, *J*(18s,15)=*J*(18s,17)=5.6, H-C(18s)); 4.43 (dm, 1H, ^2J=12.4, other *J*≤2.0, H-C(11)); 4.45 (dm, 1H, ^2J=12.4, other *J*≤2.0, H-C(11')); 5.60-5.63 (m, 1H, H-C(13)), 6.10 (q, 1H, *J*(3,10)=1.2, H-C(3)); 6.80 (d, 1H, *J*(9,7)=2.4, H-C(9)); 6.83 (dd, 1H, *J*(7,6)=8.7, *J*(7,9)=2.4, H-C(7)); 7.44 (d, 1H, *J*(6,7)=8.7, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.10 (s, C(1)); 161.28 (s, C(2)); 111.74 (d, C(3)); 152.44 (s, C(4)); 113.37 (s, C(5)); 125.21 (d, C(6)); 112.91 (d, C(7)); 161.96 (s, C(8)); 101.75 (d, C(9)); 18.51 (q, C(10)); 71.06 (t, C(11)); 142.96 (s, C(12)); 121.30 (d, C(13)); 31.18 (t, C(14)); 40.70 (d, C(15)); 37.99 (s, C(16)); 43.10 (d,

C(17)); 31.39 (t, C(18)); 26.01 (q, C(19)); 20.96 (q, C(20)). HRMS: 310.1564 ([M]$^+$, m/z calcd for $C_{20}H_{22}O_3$ 310.1563).

*7-(((1S,5R)-6,6-Dimethylbicyclo[3.1.1]hept-2-en-2-yl)methoxy)-4-methyl-2H-chromen-2-one ((+)-**26c**).* Yield 30%, method **b**. M.p. 85 °C. $[\alpha]_{589}^{30.7}$ =+36 (*c*=0.8, EtOH). The ^{1}H and ^{13}C NMR spectra of ((+)-**26c**) correspond to the spectra of the enantiomer (-)-**26c**. HRMS: 310.1559 ([M]$^+$, m/z calcd for $C_{20}H_{22}O_3$ 310.1563).

*4-Methyl-7-((3,4,5-trimethoxybenzyl)oxy)-2H-chromen-2-one (**26d**).* Yield 34%, method **a**. M.p. 145 °C. ^{1}H-NMR ($CDCl_3$, δ_H): 2.38 (d, 3H, *J*(10,3)=1.2, H-C(10)); 3.83 (s, 3H, H-C(19)); 3.86 (s, 6H, 3H-C(18), 3H-C(20)); 5.03 (s, 2H, H-C(11)); 6.12 (q, 1H, *J*(3,10)=1.2, H-C(3)); 6.63 (s, 2H, H-C(13), H-C(17)); 6.87 (d, 1H, *J*(9,7)=2.5, H-C(9)); 6.92 (dd, 1H, *J*(7,6)=8.8, *J*(7,9)=2.5, H-C(7)); 7.49 (d, 1H, *J*(6,7)=8.8, H-C(6)). ^{13}C-NMR ($CDCl_3$, δ_C): 155.11 (s, C(1)); 161.06 (s, C(2)); 112.04 (d, C(3)); 152.33 (s, C(4)); 113.76 (s, C(5)); 125.47 (d, C(6)); 112.78 (d, C(7)); 161.50 (s, C(8)); 101.80 (d, C(9)); 18.52 (q, C(10)); 70.64 (t, C(11)); 131.22 (s, C(12)); 104.57 (d, C(13), C(17)); 153.46 (s, C(14), C(16)); 137.96 (s, C(15)); 56.07 (q, C(18), C(20)); 60.72 (q, C(19)). HRMS 353.1363 ([M]$^+$ m/z calcd for $C_{20}H_{20}O_6$ 353.1365).

3.2. General biological methods

The recombinant Tdp1 was purified to homogeneity by the chromatography on Ni-chelating resin and phosphocellulose P11 as described[1,39] using plasmid pET 16B-Tdp1 kindly provided by Dr. K.W. Caldecott (University of Sussex, United Kingdom).

Tdp1-biosensor 5'-(5,6 FAM-aac gtc agg gtc ttc c-BHQ1)-3' was synthesized in Laboratory of Medicinal Chemistry, Institute of Chemical Biology and Fundamental Medicine, Novosibirsk, Russia.

Real-time detection of Tdp1 activity

The Tdp1 activity measurements were carried out as described.[13] Briefly, Tdp1-biosensor with final concentration of 50 nM was incubated in a volume of 200 uL containing Tdp1 buffer (50 mM Tris–HCl pH 8.0, 50 mM NaCl, 7 mM β-mercaptoethanol) supplemented with a purified 1.3 nM Tdp1. The reaction mixtures were incubated at a constant temperature of 26 °C in a POLARstar OPTIMA fluorimeter, BMG LABTECH, GmbH, to measure fluorescence every 1 min (Ex_{485}/Em_{520} nm). The efficiency of Tdp1 inhibition was calculated by comparing the rate of increase in fluorescence of biosensor in the presence of compound to that of DMSO control wells. Values IC_{50} were determined using an eleven-point concentration response curve. The data were imported into MARS Data Analysis 2.0 program (BMG LABTECH) and the slope during the linear phase (here data from 0-7 min) was calculated.

Cell Culture Assays

Tumor cell lines from human myeloma RPMI 8226 were plated into the wells of 96-well plate at a density ~2000 cells per wells, treated with coumarins and incubated at 37 ºC in RPMI 1640 medium (5% CO_2). Tumor cells from human mammary adenocarcinoma MCF-7 (~2000 cells per well) were incubated for 24 h at 37º C in IMDM medium (5% CO_2) and then they were treated with the coumarine derivatives or camptothecin. After 72 h of cell incubation, the relative amount of live cells was determined using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (a standard colorimetric MTT-test).[40]

3.3. Molecular Modelling and virtual screening

The compounds were docked to the crystal structure of Tdp1 (PDB ID: 1MU7, resolution 2.0 Å),[23] which was obtained from the Protein Data Bank (PDB).[41,42] This structure can be found here: http://www.rcsb.org/pdb/explore.do?structureId=1mu7. The Scigress Ultra version 7.7.0.47 program[43] was used to prepare the crystal structure for docking, i.e., hydrogen atoms were added, the co-crystallised tungsten(VI)ion was removed as well as crystallographic water molecules. The Scigress software suite was also used to build the inhibitors and the MM2[44] force field was used to optimise the structures. The centre of the binding pocket was defined as the position of the hydrogen atom of HIS263, which nitrogen formed a coordination bond with the tungsten ion (x = 8.312, y = 12.660, z = 35.452) with 10 Å radius. For the initial screen 30% search efficiency was used (virtual screen) with ten runs per compound. For the second phase (re-dock) and the molecular modelling 100% efficiency was used in conjunction with fifty docking runs. The basic amino acids lysine and arginine were defined as protonated. Furthermore, aspartic and glutamic acids were assumed to be deprotonated. The GoldScore (GS),[24] ChemScore (CS),[25,26] ChemPLP[27] and ASP[28] scoring functions were implemented to validate the predicted binding modes and relative energies of the ligands using the GOLD v5.2 software suite. The virtual screen was conducted with the InterBioScreen natural product collection.

The QikProp 3.2[45] software package was used to calculate the molecular descriptors of the compounds. The reliability of QikProp is established for the molecular descriptors.[46]

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Supplementary Material

Supplementary information (the results of the scoring functions for compounds **6, 24b-e, 25a-e, 26a-d**; the calculated molecular descriptors for compounds **6, 24b-e, 25a-e, 26a-d**; and NMR ^{1}H and ^{13}C spectra) associated with this article can be found, in the online version.

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