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Synthesis and *In Vitro* Anticancer Evaluation of Chrysin Containing Hybrids and Other Chrysin Derivatives

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Abstract

Chrysin, a well-known naturally occurring flavonoid having several biological effects including antiproliferative activity, was coupled with different pharmacophore structures. Coupling was carried out with spacers of different lengths and types. Structures selected for hybrid formation were amines, cyclic amino acid esters, and (hetero)aromatic compounds. In addition, vindoline, which is a *Vinca* alkaloid containing an indole skeleton, was also used. The alkylation of amines in the presence of carbonate base resulted in an interesting carbamate side product formation beside the expected amine. We also present the detailed structure elucidation of the carbamates. The *in vitro* anticancer activities of the synthesized derivatives were examined against 60 human tumor cell lines in National Cancer Institute (NCI, USA).

Keywords

chrysin, flavonoid, vindoline, heterocycles, anticancer effect

1 Introduction

Recently, extensive pharmacochemical efforts have been carried out to synthesize new and more potent derivatives of the classical anticancer agents, e.g. vinblastine [1–3], with decreased adverse effects. Moreover, one of the new trends in modern cancer research is the synthesis and investigation of hybrid molecules [4–7]. In addition, studies are in progress to avoid anticancer drug resistance using hybrid structures [8]. One group of the important agents is the hybrid molecule family of *Vinca* alkaloids [9]. Among the natural substances, another large group is flavonoids, which have a number of biological effects including anticancer activity [10–12]. One of the most widely known and used flavonoids is chrysin (**1**) (Fig. 1) [13].

After the elaboration of the syntheses of several hybrid molecules in the field of *Vinca* alkaloids, especially with vindoline (**2**) [14–16], we attempted to extend our research work to flavonoids, namely the synthesis of chrysin hybrids [17, 18].

Continuing the synthesis of chrysin hybrids with potentially antiproliferative activity, further coupling agents were used as synthetic components for the preparation of

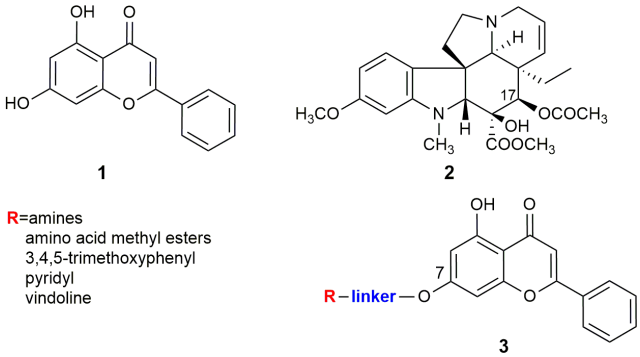

Fig. 1 Chrysin (**1**); vindoline (**2**); and the title compounds (**3**)

the designed structures (**3**). The connection between the two components was performed with different types of spacers, namely with chloroacetyl chloride, 5-bromovaleric acid, and 1,4-dibromobutane.

Pharmacophores first used were amines and amino acid esters (Fig. 1), which were selected on the basis of previous results [16, 19]. An important component for the synthesis of chrysin hybrids was 1,2,3,4-tetrahydroisoquinoline, because hybrids containing this type of heterocycle showed MDR resistance reverting activity [20] and proved to be P-gp inhibitors [21]. Aromatic compounds [17] and

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some heteroaromatics, e.g., pyridine derivatives [22–24], are often parts of hybrid molecules. They were also used as further pharmacophores, in spite of the fact that some of the latter are already known, although examining other indication. Vindoline (2) proved to be an excellent coupling component taking part in the synthesis of new hybrids having antiproliferative effect [9]. The previous results have shown that coupling vindoline with chrysin could result in a hybrid having prominent antineoplastic effect [18], therefore, we aimed to study the effect of the spacer length between the alkaloid and the flavonoid part on cell growth inhibition.

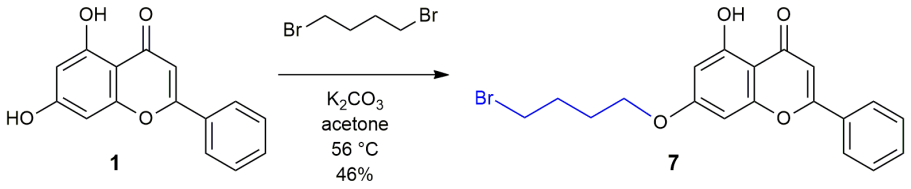
2 Results and discussion

2.1 Chemistry

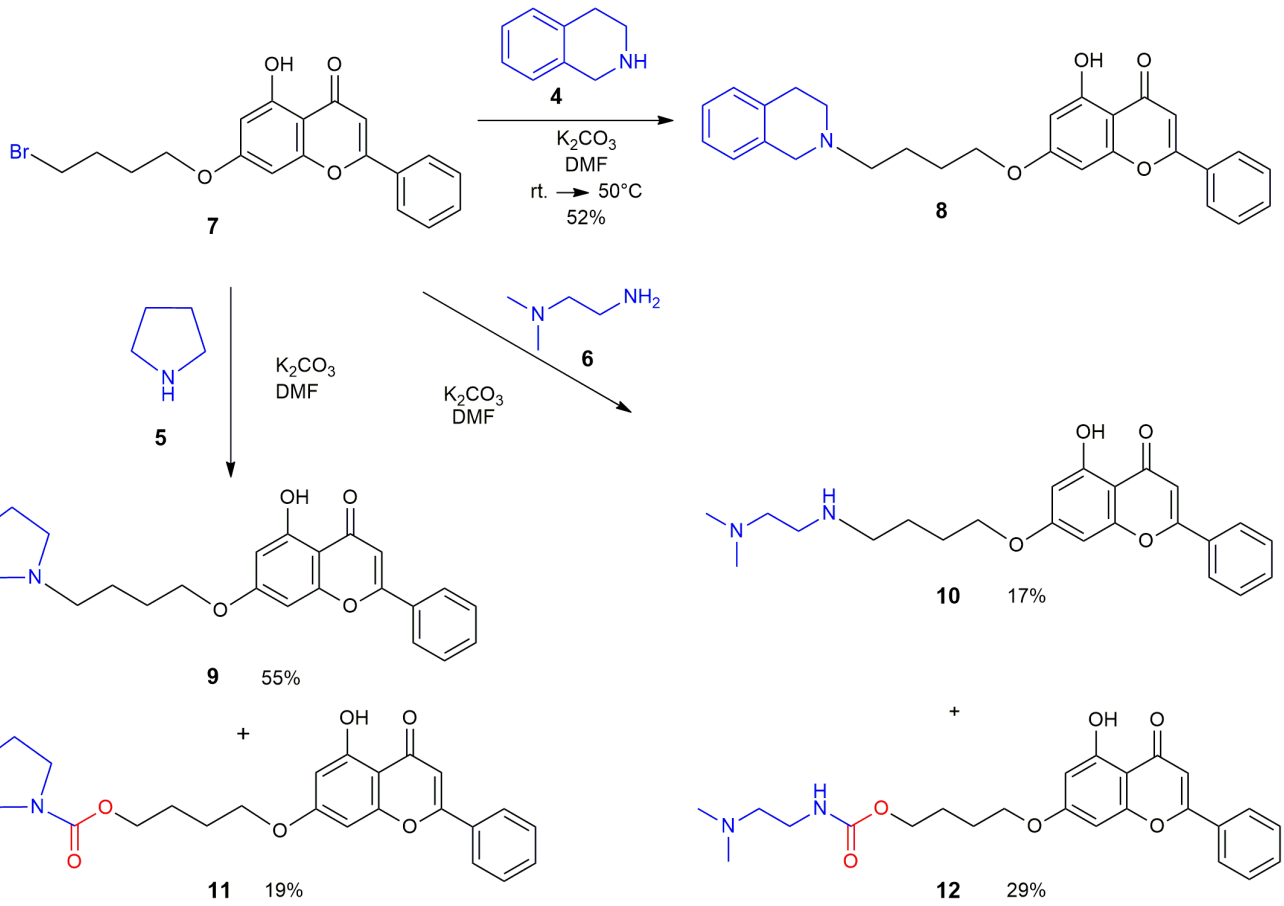
Firstly, amines were coupled with chrysin (**1**) using 1,2,3,4-tetrahydroisoquinoline (**4**), pyrrolidine (**5**) and *N,N*-dimethylethylenediamine (**6**). Despite the fact that *N,N*-dimethylethylenediamine (**6**) is not counted as a pharmacophore, it was able to increase the *in vitro* efficiency of some anticancer compounds [25]. The linker was formed on the chrysin (**1**) moiety utilizing the method of Babu et al. (Scheme 1) [19]. The intermediate (**7**) was then coupled with the corresponding amines (**4-6**) using the common method in every case (Scheme 2).

An interesting side product formation was observed. Beside the desired alkylated compounds (**8-10**), carbamate derivatives were isolated (**11**, **12**). Similar compounds were synthesized by Kong et al. [26], although carbon dioxide atmosphere was used in their experiments. In our case, the thermal decomposition of the forming carbonic acid may be the source of the inserted carbon dioxide.

Amino acid – flavonoid conjugates are known in the literature [27, 28]. We aimed to synthesize cyclic amino acid – chrysin hybrids with different linker lengths. In this



Scheme 1 Linker formation with 1,4-dibromobutane on chrysin (**1**)



Scheme 2 Synthesis of amine derivatives (**8-10**) and carbamate compounds (**11**, **12**)

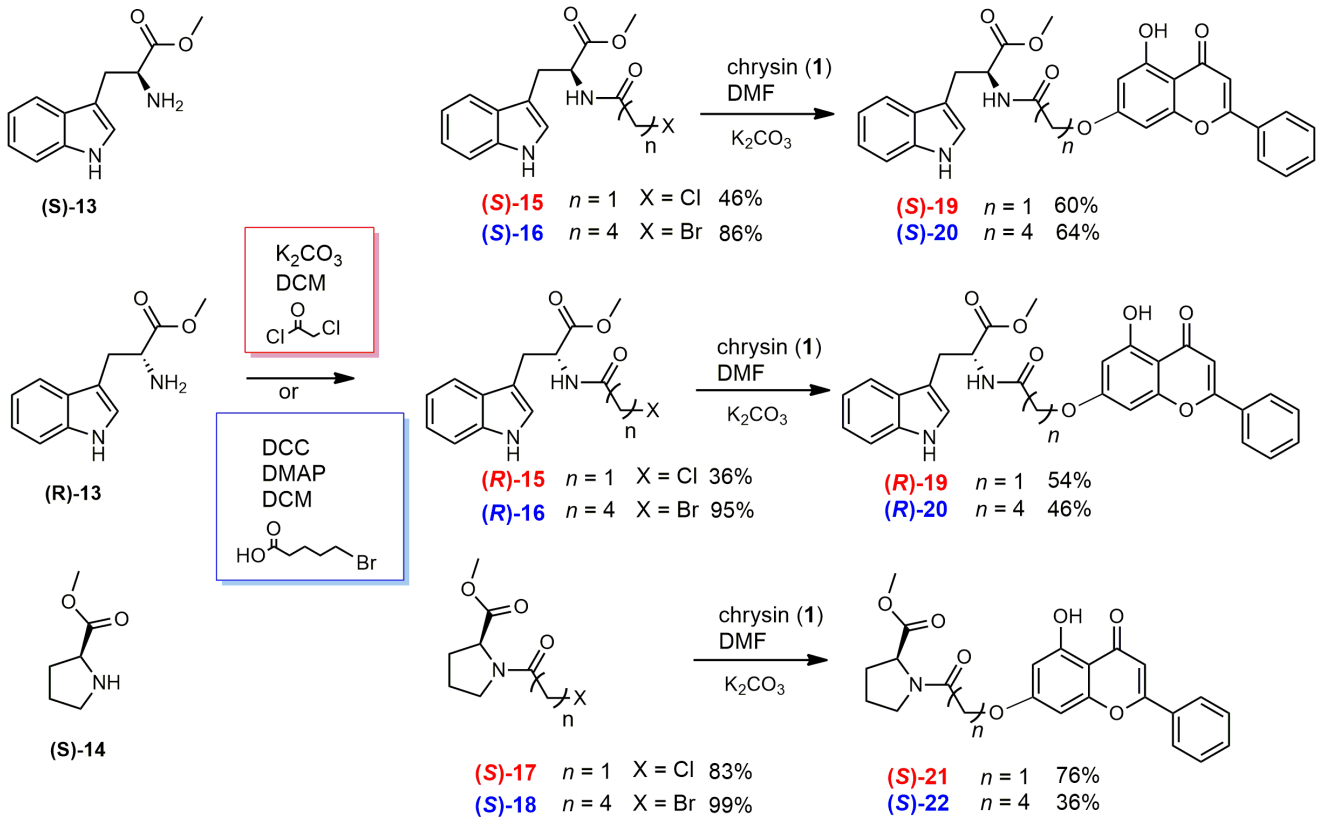
case, the linkers were formed on the chosen amino acids (L-, D-tryptophan- and L-proline methyl ester) (Scheme 3). Then the coupling reactions were carried out using chrysin (**1**). Two different linkers were used. Amino acid esters were allowed to react with chloroacetyl chloride in dry dichloromethane (DCM) using K_2CO_3 as a base at room temperature [29, 30], and with 5-bromovaleric acid in dry DCM using *N,N'*-Dicyclohexylcarbodiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) as coupling agents. Intermediates (**15-18**) were allowed to react with chrysin (**1**) using a general method.

Previous research of the modified chrysin derivatives at position 7 showed that hybrids containing an aryloxy acetamide or a diphenylamine-type structural unit are potentially good anticancer agents [17, 22]. Therefore, a series of similar chrysin derivatives (**27-32**) were synthesized during our experiments (Scheme 4). Earlier we developed a method using mild conditions to obtain diphenylamine-type 7-aminochrysin derivatives [17], which procedure was used to synthesize compound **31**. The synthesis of compounds **28** and **30** was reported previously by Choe et al. [22] and Khanapur et al. [23], but the compounds were examined in a different indication. Here we used a reverse order synthesis to obtain the products and the synthesized compounds were tested in antiproliferation assays *in vitro*.

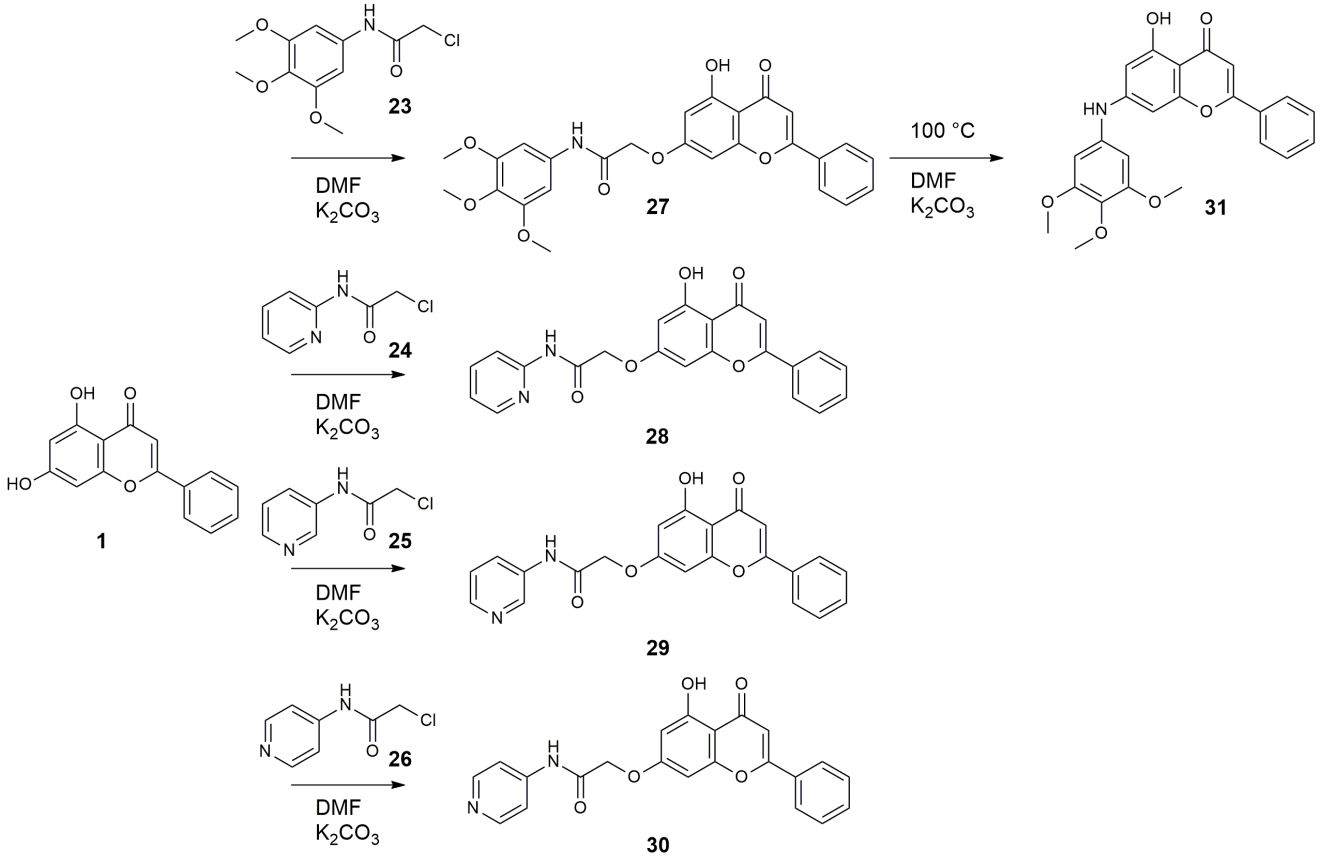
Furthermore, chrysin (**1**) was coupled with vindoline (**2**) which is the monomeric building block of some of the well-known antimitotic *Vinca* alkaloids (e.g. vinblastine, vinorelbine and vinflunine). Previously we described two vindoline – chrysin hybrids, one of which showed prominent antineoplastic effect [18]. Therefore, we aimed to synthesize hybrids with different spacer lengths. The first step is the elimination of the acetyl group located at position 17 using the method described by Passarella et al. [31]. In the following step, the linkers were formed on the 17-OH utilizing the methods mentioned above and described previously [14]. The resulting intermediates (**33, 34**) were coupled with chrysin (**1**) (Scheme 5).

2.1.1 Structure elucidation of carbamates 11 and 12

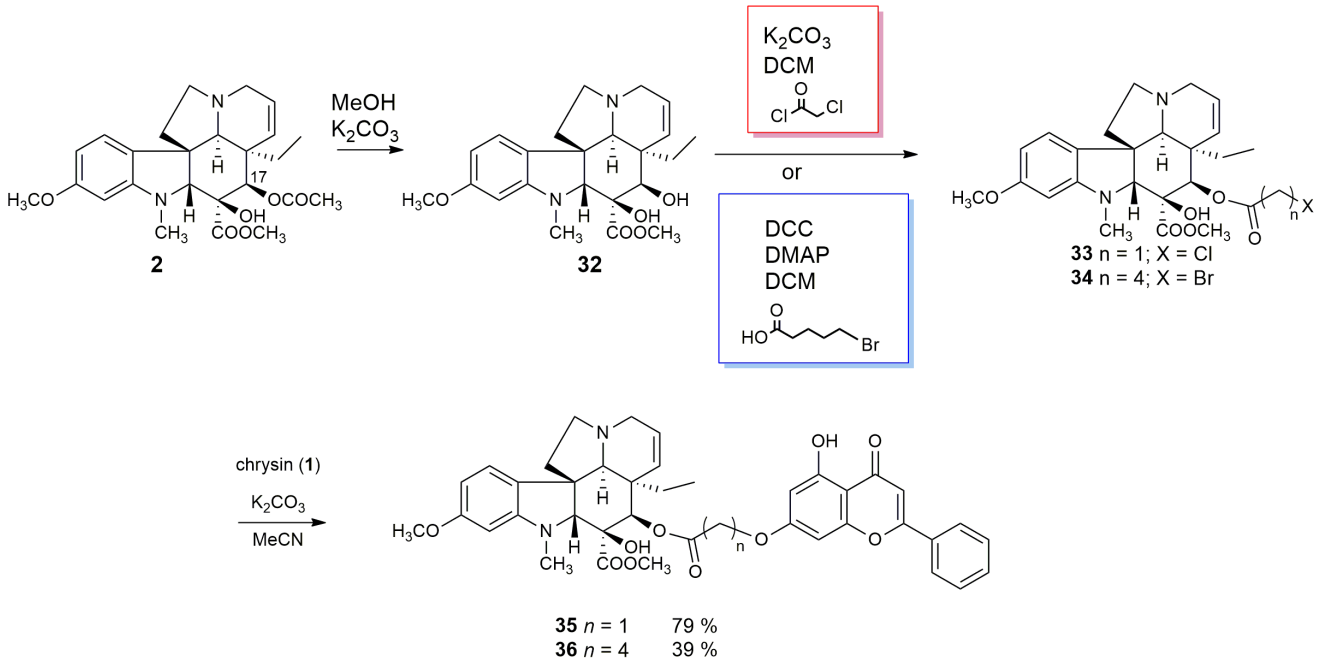
The elemental composition of the side product of chrysin – *N,N*-dimethylethylenediamine hybrid (Fig. 2, compound **12**) is $C_{24}H_{29}O_6N_2$, which is larger by a CO_2 unit than the expected structure. The fragmentation behavior of this protonated molecular ion suggests the presence of an oxygen between the alkyl chain and the *N,N*-dimethylethylenediamine ($C_{19}H_{19}O_5$; *m/z* 327). Taking into account a minor fragment ($C_9H_{19}O_2N_2$; *m/z* 187), an "oxy-carbonyl" group can be found at the alkyl chain.



Scheme 3 Synthesis of amino acid-chrysin hybrids



Scheme 4 Aryloxy acetamides and diphenylamine-type chrysin derivatives



Scheme 5 Synthesis of vindoline – chrysin hybrids (**35, 36**)

The elemental composition of the side product of chrysin – pyrrolidine hybrid (Fig. 2, compound **11**) is $C_{24}H_{26}O_6N$, which is larger by a CO_2 unit than the expected structure. The fragmentation behavior of this protonated molecular ion proves that there is an "oxy-carbonyl", rather than a "carbonyl-oxy" group, between the alkyl chain and the

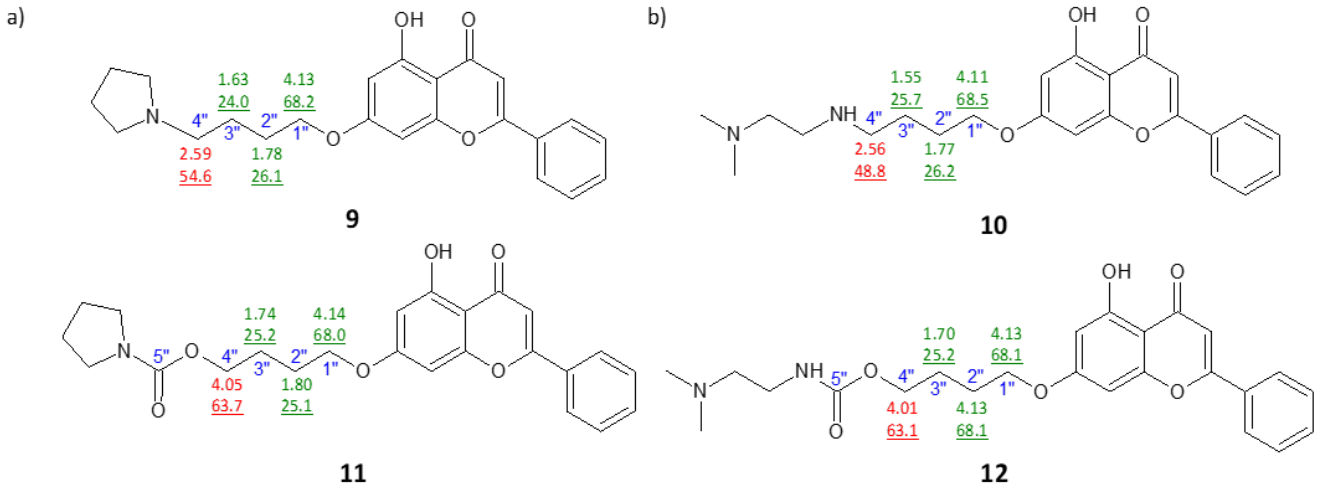


Fig. 2 Chemical shifts of linker methylene groups of compounds; a) **9** and **11**; b) **10** and **12**. Labels show the ^{1}H and ^{13}C (underlined) NMR chemical shifts in ppm. Green labels show the similar chemical shifts between the desired (compounds **9** and **10**) and the side products (compounds **11** and **12**). Red labels show the methylene groups with significantly different chemical shifts. Atom numbering is labeled in blue

pyrrolidine ring (on the basis of these two fragment ions: $C_{20}H_{17}O_6$ – *m/z* 353 and $C_{19}H_{17}O_4$ – *m/z* 309).

In accordance with this, the IR data shows that in both cases a new absorption peak can be observed (**9**: 1661, 1622 cm^{-1} vs **11**: 1683, 1657, 1612 cm^{-1} and **10**: 1660, 1615 cm^{-1} vs **12**: 1693, 1662, 1615 cm^{-1}) with a wavenumber of a carbonyl >C=O stretch.

The NMR data (^{1}H and ^{13}C NMR chemical shifts) confirmed the structure of the C(7)–*O*-alkylated chrysin moiety of **11** and **12**. Furthermore, the chemical shifts of the linker methylene groups 1"-3" had fairly similar values for compounds **9-12** (Fig. 2, groups with green labels). However, in both cases we observed suspiciously high chemical shifts for methylene group 4" (Fig. 2, groups highlighted in red). The ^{1}H NMR chemical shift of a methylene group that is covalently connected to a nitrogen atom is usually between 1.8 and 3.1 ppm (^{13}C NMR chemical shifts are between 30 and 60 ppm). The observed high ^{1}H NMR chemical shifts are typical for groups neighboring an oxygen atom (the usual values are between 3.0 and 5.0 ppm).

As compared to the target compounds **9-10**, we found an additional ^{13}C NMR resonance with a high chemical shift, corresponding to a carbonic acid derivative (154.0 ppm for compound **11** and 156.2 ppm for **12**).

To elucidate structure **11**, we used the Heteronuclear Multiple Bond Correlation (HMBC) measurement for detecting carbon and hydrogen atom pairings that are connected via two or three bonds. Therefore, we could determine the connectivity of the additional carbon atom to the rest of the molecule. The observed HMBC correlations are shown in Fig. 3 (a). On the other hand, for compound **9**, we would have probably detected cross-peaks indicating multiple bond correlations between CH_2(4") and CH_2(2') or CH_2(5') (Fig. 3 (b)).

Structure **11** is further supported by the fact that the two methylene groups of the symmetric pyrrolidine moiety do not share the same ^{13}C NMR chemical shifts; the hindered rotation of the amide group prevents the chemical environments of CH_2(2') and CH_2(5') from being averaged out.

In the ^{1}H NMR spectrum of compound **12**, we found an additional triplet signal at 6.98 ppm (Fig. 4 (a)), indicating an amide NH adjacent to a CH_2 group. Secondary amine hydrogen atoms do not give sharp signals (they give broad or undetectable signals, instead) in ^{1}H NMR spectra due to fast chemical exchange with the residual water content of the NMR solvent. Amide hydrogen atoms, however, do not take part in that kind of chemical exchange; these atoms usually give sharp ^{1}H NMR signals in dimethylsulfoxide-d_6. Considering the 156.2 ppm chemical shift of the additional carbon atom [C(5")], the amide NH group belongs to a carbamate group. The HMBC correlation between C(5") and the amide hydrogen atom (Fig. 4 (a)) supports this further. The connection of the carbamate group is based on the HMBC correlations between C(5") and CH_2(1') or CH_2(4") (Fig. 4 (a)). On the contrary, in the HMBC spectrum of compound **10** we would have observed correlations between methylene groups 1' and 4".

2.1.2 Amide-rotamers of compounds 21 and 22

The ^{1}H NMR spectra of compounds 21 and 22 shown two sets of signals in a ratio of 4:1 (cf. Section 4.2). This could indicate the presence of some kind of impurity; however, this is not the case. The hindered rotation of the amide group results in two favorable conformers in

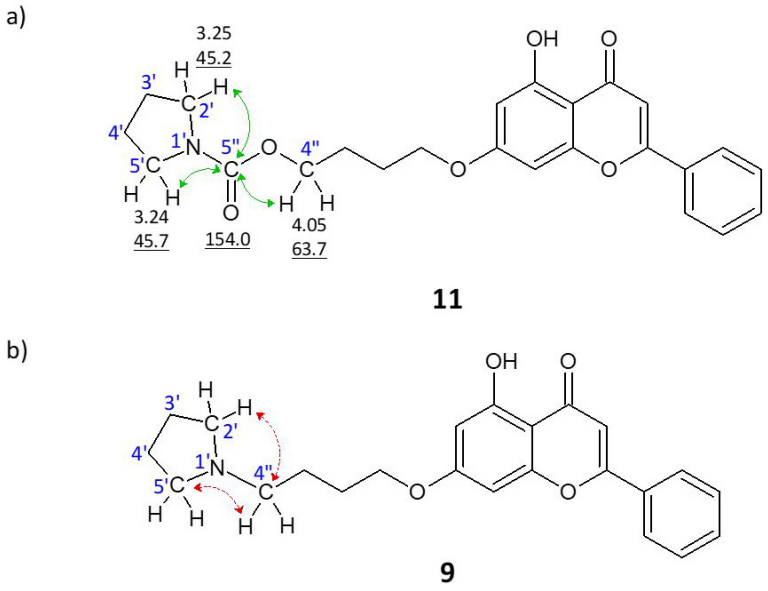


Fig. 3 a) Observed HMBC correlations to the additional carbon atom (154.0 ppm) of compound **11**; b) expected HMBC correlations for the expected structure **9**. Atom numbering is labeled with blue

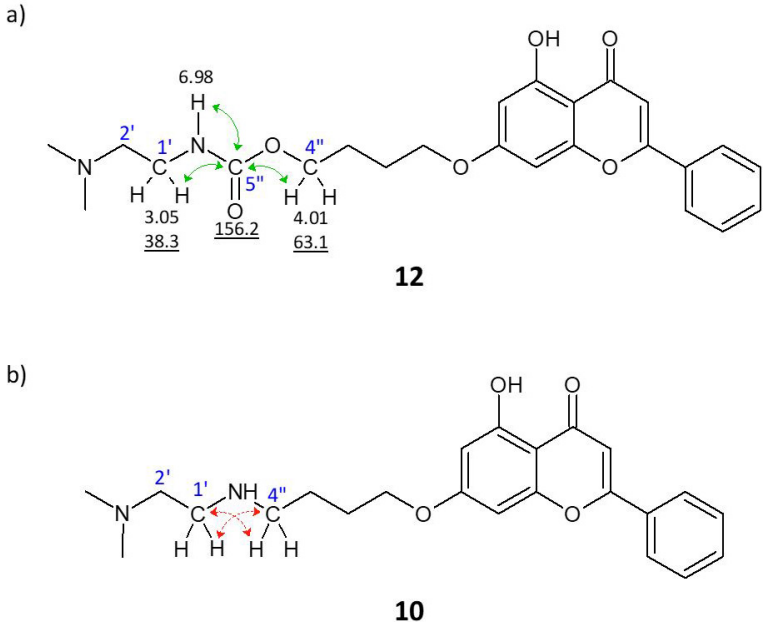


Fig. 4 a) Observed HMBC correlations to the additional carbon atom (C-5") of compound **12**; b) Expected HMBC correlations for the expected structure **10**. Atom numbering is labeled with blue

the 1-acyl-pyrrolidine moiety (Fig. 5). The same hydrogen atoms in the two conformers sense different chemical environments, hence they give rise to distinct NMR signal sets. One of these conformers is more favorable than the other (meaning that it has a higher concentration in the solution). Therefore, the two signals are not equally intense, rather, showing the above mentioned 4:1 ratio.

To prove that the two sets of signals in fact belong to a single compound, we should observe the slow exchange between the two conformers. This can be done through Rotating-frame Nuclear Overhauser Effect Spectroscopy (ROESY) measurements. This homonuclear experiment detects hydrogen-hydrogen correlations via spatial proximity. Normally the respective signals have a phase opposite to the main diagonal of the spectrum (e.g. Fig. 6, cross-peak highlighted in red). However, if two hydrogen atoms are in a slow exchange with each other, we can observe a cross-peak with the same phase as the main diagonal in the ROESY spectrum between them. We found such signals for both **21** and **22**. An example is shown in Fig. 6. Such signals prove that the minor signal set is not due to an impurity, but the presence of an amide rotamer.

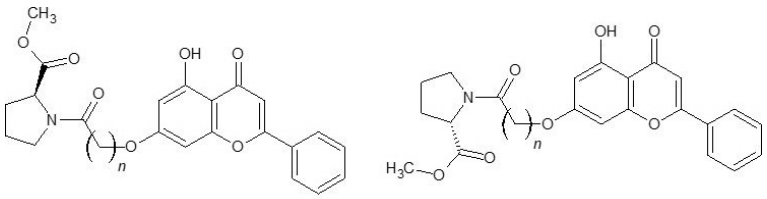


Fig. 5 Amide rotamers of compounds **21** and **22**

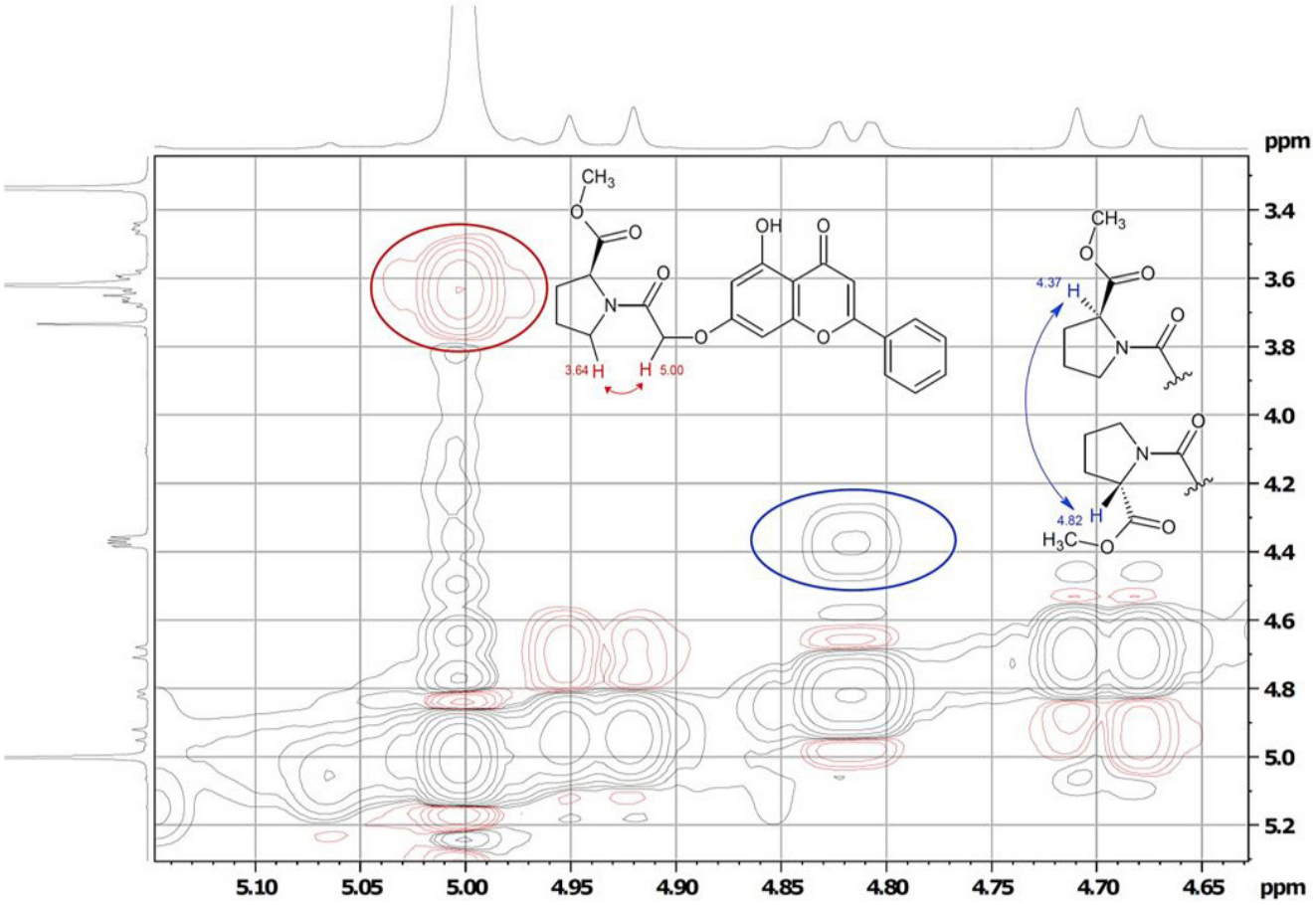


Fig. 6 Portion of the ROESY spectrum of compound **21**; the axes show ^{1}H NMR chemical shifts. The cross-peak highlighted in red indicates spatial proximity between the hydrogen atoms highlighted in red. The signal highlighted in blue indicates slow chemical exchange between the two conformers (see the hydrogen atoms highlighted in blue for the two rotamers)

2.2 Biological results

The antiproliferative activities of the synthesized compounds were examined against 60 human tumor cell lines (NCI60), representing leukemia, non-small cell lung cancer, colon cancer, CNS cancer, melanoma, ovarian cancer, renal cancer, prostate cancer, and breast cancer, respectively, at the National Cancer Institute (NCI, USA) [32–36].

2.2.1 One-dose screen

All the molecules were screened initially at a single dose of 10^{-5} M on 60 cancer (NCI60) cell lines. The resulting numbers for the one-dose assay are percentages of growth relative to an untreated control, after the 48 h of incubation. This allows the observation of both growth inhibition (values between 0 and 100) and lethality (values less than 0). For instance, a result of 100 means zero inhibition in the terms of cell growth. A value of 30 means 70% growth inhibition. A value of 0 means no net growth during the experiment. A value of −65 means that after the 48 h of incubation the starting cell number has decreased by 65%, which means not only growth inhibition, but the loss of cells indicates lethality, too. A value of −100 means all of the starting cells are dead after the experiment. The results of the one-dose screen for the tested

compounds are given in Table 1 where the results stronger than −50% are highlighted in red. Compounds can be seen against the control chrysin (**1**) and 17-deacetylvindoline (**32**) which are not shown any effect in terms of cell division. Amongst the synthesized compounds **10** and **12** show a prominent antineoplastic effect. These chrysin derivatives coupled with *N,N*-dimethylethylenediamine differs only in the linker part of the molecule. While **10** contains only an alkane chain compound **12** is a carbamate derivative. The introduction of a "carbon dioxide" to compound **10** resulting compound **12** increased the *in vitro* activity greatly. The latter two derivatives were found to be highly effective against melanoma cell lines. At a dose of 10 μM most of the growth inhibition percentages are under −50, which means not only the inhibition of cell growth over the incubation time but the loss of the starting cell number by more than 50%. Furthermore, these compounds were highly active in the case of leukemia and compound **12** was active against colon cancer, CNS cancer, renal cancer, and breast cancer. The outstanding effect of derivative **12** can either be seen in the mean cell growth inhibition data which is −39% indicating that the average growth inhibition against the NCI 60 cell palette shows not even just inhibition but a great loss of the starting cell number over the course of the assay.

3 Conclusion

In the course of our research work, many hybrids and hybrid-like derivatives of chrysin were prepared. Different coupling components were used, e.g. amino acid esters, (cyclic) amines, substituted aromatic and heteroaromatic compounds as well as the *Vinca* alkaloid vindoline. The compounds prepared were investigated on 60 cell lines of 9 types of cancer. According to the results presented exclusively the *N,N*-dimethylethylenediamine containing chrysin derivatives (**10**, **12**) showed important activity, especially in the case of melanoma.

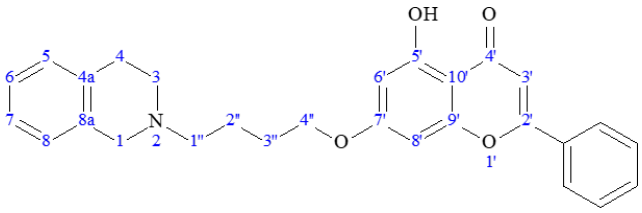
4 Experimental

4.1 General materials and methods

All chemicals were purchased from Sigma-Aldrich (Budapest, Hungary) and were used as received. Melting points were measured on a VEB Analytik Dresden PHMK-77/1328 apparatus (Dresden, Germany) and are uncorrected. IR spectra were recorded on Zeiss IR 75 and 80 instruments (Thornwood, NY, USA). NMR measurements were performed on a Bruker Avance III HDX 400 MHz NMR spectrometer equipped with a $^{31}P–^{15}N\{^{1}H–^{19}F\}$ 5 mm CryoProbe Prodigy BBO probe, a Bruker Avance III HDX 500 MHz NMR spectrometer equipped with a $^{1}H\{^{13}C/^{15}N\}$ 5 mm TCI CryoProbe, and a Bruker Avance III HDX 800 MHz NMR spectrometer equipped with a $^{1}H–\ ^{19}F\{^{13}C/^{15}N\}$ 5 mm TCI CryoProbe (Bruker Corporation, Billerica, MA, USA). ^{1}H And ^{13}C chemical shifts are given on the delta scale as parts per million (ppm) relative to tetramethyl silane. One-dimensional ^{1}H, and ^{13}C spectra and two-dimensional $^{1}H–^{1}H$ COSY, $^{1}H–^{1}H$ NOESY, $^{1}H–^{13}C$ HSQC, and $^{1}H–^{13}C$ HMBC spectra were acquired using pulse sequences included in the standard spectrometer software package (Bruker TopSpin 3.5, Bruker Corporation). ESI-HRMS and MS-MS analyses were performed on a Thermo Velos Pro Orbitrap Elite (Thermo Fisher Scientific, Bremen, Germany) system. The ionization method was ESI, operated in positive ion mode. The protonated molecular ion peaks were fragmented by CID (collision-induced dissociation) at a normalized collision energy of 35–65%. For the CID experiment, helium was used as the collision gas. The samples were dissolved in methanol. EI-HRMS analyses were performed on a Thermo Q Exactive GC Orbitrap (Thermo Fisher Scientific, Bremen, Germany) system. The ionization method was EI and operated in positive ion mode. Electron energy was 70 eV and the source temperature was set at 250 °C. Data acquisition and analysis were accomplished with Xcalibur software version 4.0 (Thermo Fisher Scientific). Thin-layer chromatography (TLC) was carried out using DC-Alufolien Kieselgel 60 F_{254} (Merck, Budapest, Hungary) plates. Preparative TLC analyses were performed on silica gel 60 $PF_{254+366}$ (Merck) glass plates.

4.2 Chemistry

4.2.1 Chrysin – 1,2,3,4-tetrahydroisoquinoline hybrid (8)



50 mg (0.128 mmol) 7-(bromobutoxy)chrysin (**7**) was dissolved in 2 ml *N,N*-dimethylformamide (DMF). Then 34 mg K_2CO_3 (0.244 mmol) and 16 μL (0.122 mmol) 1,2,3,4-tetrahydroisoquinoline (4) were added. The mixture was stirred overnight at 23 °C and a further 1.5 h at a temperature of 50 °C. The reaction mixture was poured into cold water (20 ml). The forming suspension was extracted with DCM (2 × 15 ml). The combined organic layer was dried

Table 1 Growth percent rate results of the one-dose assay at 10 μM (%)

	1	32	8	10	12	(*R*)-19	(*S*)-19	(*R*)-20	(*S*)-20	(*S*)-21	(*S*)-22	27	28	29	31	36
Leukemia																
CCRF-CEM	102.24	88.46	87.73	−3.67	−5.67	86.02	79.82	83.12	93.79	90.92	101.11	113.16	106.97	99.98	101.72	74.03
HL-60(TB)	116.20	107.24	78.36	**−51.40**	**−61.65**	99.10	65.67	84.09	97.26	87.76	99.83	99.06	130.99	98.04	114.81	89.06
K-562	96.80	105.03	47.79	**−72.42**	8.72	64.24	72.82	83.62	67.28	83.23	103.05	105.09	113.45	86.47	84.29	68.61
MOLT-4	105.93	95.75	78.15	−33.02	−42.05	63.84	0.72	65.14	68.99	66.74	103.99	105.12	117.76	99.25	108.84	65.67
RPMI-8226	101.04	99.27	66.81	−19.36	−9.41	75.51	82.23	71.76	78.42	98.47	104.77	108.80	114.88	100.98	90.59	75.62
SR	77.87	53.35	82.75	**−69.97**	−28.85	78.87	21.92	72.27	78.01	11.10	95.69	117.44	95.36	93.96	77.62	35.24
Non-small cell lung cancer																
A549/ATCC	98.46	94.87	92.86	12.85	4.88	94.38	87.51	86.74	94.74	101.26	106.43	101.77	93.95	93.80	100.39	51.94
EKVX	89.35	98.43	78.29	57.29	11.67	90.06	78.19	86.83	84.01	95.49	94.83	106.24	93.88	98.39	99.51	81.45
HOP-62	113.09	105.06	110.28	58.66	2.14	113.53	104.54	96.19	106.46	106.79	113.44	105.58	96.19	83.75	93.91	80.90
HOP-92	77.93	82.56	67.31	50.84	−42.55	94.55	58.34	60.61	109.47	111.33	129.40	n.d.	90.21	98.21	78.70	106.77
NCI-H226	86.84	101.27	92.28	44.05	12.07	80.74	94.06	88.76	78.75	99.03	98.90	99.39	102.71	59.56	112.64	74.89
NCI-H23	92.57	106.42	82.80	56.25	−2.08	85.82	91.63	35.17	84.57	94.60	99.26	102.14	91.76	84.67	84.04	86.98
NCI-H322M	98.30	93.24	99.94	51.27	−31.25	110.31	107.56	95.85	108.42	100.77	102.38	105.96	96.05	98.07	100.08	96.23
NCI-H460	98.34	100.04	90.93	18.79	−33.92	110.92	102.87	94.45	104.44	96.90	115.05	109.09	85.54	103.26	81.31	27.01
NCI-H552	88.95	90.50	82.53	−1.13	**−76.96**	87.55	76.94	86.66	87.91	93.28	103.53	97.40	92.71	70.67	90.56	76.31
Colon cancer																
COLO 205	104.94	112.59	69.61	**−76.30**	−46.42	116.28	86.23	90.39	147.98	136.72	156.59	110.70	119.39	151.27	108.73	86.48
HCC-2998	102.88	109.05	102.00	29.78	**−50.76**	109.88	95.60	96.54	105.46	93.08	102.78	113.32	105.58	117.08	99.57	100.38
HCT-116	82.69	91.69	70.56	5.38	**−68.94**	74.11	87.63	92.08	81.60	71.48	110.55	108.58	72.40	95.04	75.21	59.70
HCT-15	90.99	95.33	58.17	−16.16	**−51.79**	72.03	85.74	80.78	81.73	94.47	100.66	103.17	97.75	91.13	86.37	72.89
HT29	102.89	106.94	74.22	1.75	**−54.41**	96.92	102.80	107.35	106.70	108.99	116.34	104.82	108.24	108.97	110.12	87.63
KM12	92.93	98.52	98.47	66.08	−49.82	97.74	98.75	95.30	99.92	86.74	106.97	104.91	93.78	108.12	97.69	85.32
SW-620	101.60	105.96	86.70	8.47	−31.68	108.19	101.22	95.77	113.97	96.11	115.96	97.80	103.78	124.00	92.95	61.51
CNS cancer																
SF-268	101.55	104.14	82.14	56.89	−44.93	83.01	88.55	84.24	80.37	86.35	98.57	112.33	92.50	116.81	101.02	79.69
SF-295	99.86	102.19	102.90	33.09	**−90.53**	94.24	100.45	91.17	99.62	103.09	97.99	111.31	96.04	95.07	96.67	73.55
SF-539	92.17	94.51	82.95	**−98.03**	**−100.00**	97.26	89.23	90.28	93.89	91.51	123.24	102.95	79.95	116.18	67.94	64.59
SNB-19	86.04	90.73	94.93	n.d.	−32.73	83.39	89.16	89.43	87.30	94.00	96.23	97.63	82.48	68.90	86.53	75.20
SNB-75	88.98	n.d.	n.d.	34.94	102.83	110.86	n.d.	72.33	102.55	104.71	102.80	112.97	n.d.	99.04	n.d.	82.28
U251	80.67	101.62	85.01	0.05	**−64.09**	88.81	92.93	92.23	95.62	83.64	102.61	105.17	87.98	74.17	102.61	65.26

Table 1 Growth percent rate results of the one-dose assay at 10 μM (%) (Continued)

	1	32	8	10	12	(*R*)-19	(*S*)-19	(*R*)-20	(*S*)-20	(*S*)-21	(*S*)-22	27	28	29	31	36
Melanoma																
LOX IMVI	85.08	102.29	83.39	**−85.21**	**−100.00**	89.05	101.25	54.69	91.19	67.46	98.05	100.13	99.11	93.45	91.26	58.00
MALME-3M	101.76	117.60	76.70	**−68.64**	**−96.78**	100.75	90.13	94.30	97.77	85.10	95.35	n.d.	103.80	92.02	71.20	77.92
M14	106.78	103.27	73.99	**−67.92**	**−75.07**	96.91	89.74	97.20	107.53	82.23	105.91	110.31	102.67	112.90	101.41	95.68
MDA-MB-435	99.53	95.22	92.02	14.07	**−50.99**	105.60	99.10	91.62	109.24	96.56	111.57	105.95	101.53	108.29	83.38	95.11
SK-MEL-2	109.90	112.76	n.d.	n.d.	**−59.74**	111.78	n.d.	90.52	114.13	108.29	114.95	107.05	107.47	111.17	114.21	131.13
SK-MEL-28	101.70	105.45	86.45	**−70.37**	**−91.55**	155.49	99.40	101.24	148.95	144.43	155.64	109.12	108.06	110.00	92.38	101.86
SK-MEL-5	92.85	87.00	87.00	89.00	**−100.00**	72.62	100.65	**−97.81**	84.96	97.17	97.64	98.93	101.61	97.17	94.11	82.21
UACC-257	118.94	111.93	82.47	−49.60	**−79.74**	105.39	79.57	97.06	103.80	91.60	100.81	100.69	99.83	104.48	98.94	93.68
UACC-62	82.24	79.09	60.71	**−55.98**	**−88.37**	81.77	86.71	66.30	66.80	79.58	95.21	97.69	85.75	95.21	85.06	44.84
Ovarian cancer																
IGROV1	95.22	96.04	99.67	74.65	−9.88	94.89	108.43	87.55	99.74	99.06	106.78	110.80	93.51	100.79	100.51	74.87
OVCAR-3	97.60	107.87	96.30	75.76	**−77.10**	102.75	103.12	n.d.	98.23	80.87	101.66	118.67	114.68	90.76	113.01	93.83
OVCAR-4	112.07	99.15	82.45	67.64	−16.21	86.67	90.41	80.03	80.03	97.42	103.86	104.82	102.81	92.06	101.10	90.71
OVCAR-5	99.07	102.99	84.00	55.06	−4.32	181.77	106.43	103.74	176.10	126.46	172.33	105.63	100.41	178.59	102.43	95.15
OVCAR-8	95.19	103.94	88.48	20.58	3.84	94.66	89.60	87.52	96.80	93.07	106.22	100.92	84.55	80.75	100.70	84.91
NCI/ADR-RES	92.84	102.43	88.99	37.07	27.94	81.17	82.90	80.88	79.75	101.11	101.01	99.08	80.92	84.64	91.15	94.35
SK-OV-3	128.15	119.13	103.04	101.07	99.14	130.99	102.09	97.75	116.53	110.62	126.96	100.56	110.43	100.16	110.44	107.33
Renal cancer																
786-0	99.20	89.92	82.73	9.47	−34.48	88.02	100.44	96.97	80.58	98.19	103.00	101.71	95.86	69.60	87.19	78.07
A498	86.62	91.37	120.75	119.00	**−76.37**	147.75	105.40	69.97	104.88	116.97	113.42	125.27	113.67	135.50	100.55	99.32
ACHN	85.03	93.97	96.88	31.22	**−97.48**	108.33	88.79	91.83	105.83	112.26	113.84	104.96	82.39	100.88	76.73	46.78
CAKI-1	83.56	83.35	84.68	86.55	−39.15	102.57	87.77	71.92	95.06	99.16	101.41	103.50	85.80	98.63	86.05	67.70
RXF 393	91.19	115.84	84.78	**−78.22**	n.d.	n.d.	77.31	76.76	n.d.	n.d.	n.d.	102.91	121.17	n.d.	103.60	n.d.
SN12C	85.77	90.88	84.42	53.19	−9.43	87.05	88.46	95.23	86.56	92.35	98.26	103.98	82.88	93.33	82.39	79.53
TK-10	107.21	97.05	103.38	18.81	0.63	131.31	111.34	131.50	137.41	127.43	118.38	105.93	109.34	106.95	110.65	94.64
UO-31	89.42	74.17	n.d.	n.d.	**−91.17**	59.57	n.d.	68.80	80.70	79.79	83.56	100.93	88.39	88.76	100.39	65.49

Table 1 Growth percent rate results of the one-dose assay at 10 μM (%) (Continued)

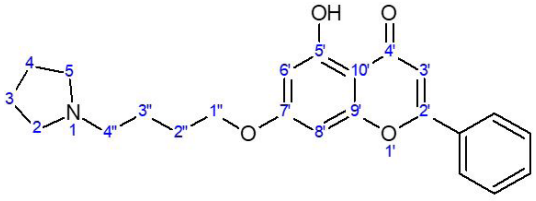
	1	32	8	10	12	(*R*)-19	(*S*)-19	(*R*)-20	(*S*)-20	(*S*)-21	(*S*)-22	27	28	29	31	36
Prostate cancer																
PC-3	93.17	97.87	78.43	47.93	10.27	66.79	85.57	73.63	85.33	86.96	104.12	108.10	95.85	104.31	86.14	62.36
DU-145	92.00	98.73	89.60	43.52	−26.96	101.68	95.80	93.30	88.24	92.88	107.42	105.38	99.19	93.05	107.60	88.85
Breast cancer																
MCF7	103.05	88.91	74.86	−16.21	**−89.83**	68.12	76.40	94.85	73.59	71.05	88.68	103.17	92.02	84.42	64.67	41.56
MDA-MB-231/ATCC	82.64	99.28	n.d.	n.d.	**−77.79**	102.11	n.d.	81.67	95.42	91.13	109.67	100.56	82.02	94.52	90.09	102.06
HS 578T	92.51	98.35	89.09	72.49	40.74	89.23	82.87	80.86	81.05	111.45	108.95	121.13	91.10	80.21	90.78	85.69
BT-549	91.01	99.37	103.45	88.23	**−59.92**	85.31	110.60	90.75	75.57	100.67	111.27	108.35	78.72	75.39	100.60	93.61
T-47D	101.44	89.75	75.35	−12.70	**−65.79**	62.72	34.63	59.69	51.60	97.66	100.40	106.67	107.31	96.56	88.62	89.57
MDA-MB-468	91.09	107.74	70.83	53.98	**−81.15**	67.70	44.25	71.29	54.57	66.53	100.70	91.49	99.34	94.88	100.46	103.96
Summary																
Mean	96.10	98.26	85.20	14.24	−38.90	95.40	87.31	82.22	95.04	94.82	107.80	105.63	98.04	98.38	94.44	79.76

over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* preparative TLC (ethyl acetate (EtOAc)) resulting 21 mg (52%) of the product (8). M.p.: 108–110 °C. TLC: EtOAc: R_f = 0.46. IR (KBr): 2953, 2922, 2852, 1611, 1349, 1158, 796 cm^{-1}. ^1H NMR (799.7 MHz; DMSO-d_6) δ (ppm) 1.66–1.71 (2H; m; H_2-2"); 1.79–1.83 (2H; m; H_2-3"); 2.50–2.53 (2H; m; H_2-1"); 2.65 (2H; t; J = 5.9 Hz; H_2-3); 2.80 (2H; t; J = 5.9 Hz; H_2-4); 3.54 (2H; s; H_2-1); 4.16 (2H; t; J = 6.6 Hz; H_2-4"); 6.40 (1H; d; J = 2.1 Hz; H-6'); 6.82 (1H; d; J = 2.1 Hz; H-8'); 7.02–7.04 (1H; m; H-8); 7.04 (1H; s; H-3'); 7.06–7.10 (3H; m; H-5. H-6. H-7); 7.58–7.65 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.09–8.12 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 12.80 (1H; wide s; C(5')-O$\underline{H}$). ^{13}C NMR (201.1 MHz; DMSO-d_6) δ (ppm) 22.7 (C-2"); 26.2 (C-3"); 28.6 (C-4); 50.4 (C-3); 55.4 (C-1); 57.0 (C-1"); 68.3 (C-4"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.2 (C-3'); 125.3 (C-7); 125.8 (C-6); 126.3 (C-8); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 128.2 (C-5); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 134.1 (C-4a); 134.8 (C-8a); 157.3 (C-9'); 161.1 (C-5'); 163.3 (C-2'); 164.7 (C-7'); 182.0 (C-4'). HRMS: M + H = 442.20050 (delta = −1.8 ppm; $C_{28}H_{28}O_4N$). HR-ESI-MS-MS (CID=60%; rel. int. %): 188(100).

4.2.2 Chrysin – pyrrolidine hybrid (9) and carbamate side product (11)

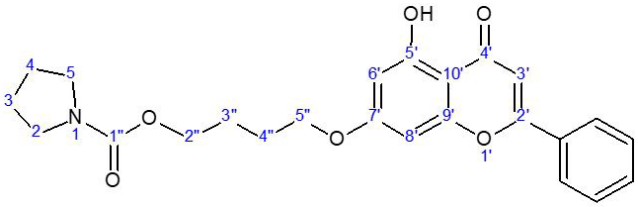
87 mg (0.224 mmol) 7-(bromobutoxy)chrysin (**7**) was dissolved in 5 ml DMF. Then 62 mg K_2CO_3 (0.447 mmol) and 19 µL (0.224 mmol) pyrrolidine (**5**) were added. The mixture was stirred for 30 h at a temperature of 23 °C. The reaction mixture was poured into cold water (20 ml). The forming suspension was extracted with DCM (2 × 20 ml). The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* preparative TLC (DCM/ methanol (MeOH) = 5/1) resulting 47 mg (55%) of the product (**9**) and 16 mg (19%) of the side product (**11**).

*Chrysin – pyrrolidine hybrid (**9**)*



M.p.: 157–159 °C. TLC: DCM:MeOH = 5:1; R_f = 0.43. IR (KBr): 2929, 1661, 1622, 1380, 1169, 823, 676 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.56–1.67 (2H; m; H_2-2"); 1.67–1.83 (6H; m; H_2-2. H_2-3. H_2-3"); 2.53–2.69 (4H; wide m; H_2-1; H_2-1"); 4.13 (2H; t; J = 6.5 Hz; H_2-4"); 6.40 (1H; d; J = 2.2 Hz; H-6'); 6.83 (1H; d; J = 2.2 Hz; H-8'); 7.05 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.07–8.14 (2H; m; C(2')-Ph: H_{ortho}); 12.80 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 22.9 (C-3. C-4); 24.0 (C-3"); 26.1 (C-2"); 53.3 (C-2. C-5); 54.6 (C-4"); 68.2 (C-1"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.2 (C-3'); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 157.3 (C-9'); 161.1 (C-5'); 163.4 (C-2'); 164.6 (C-7'); 182.0 (C-4'). HRMS: M + H = 380.18339 (delta = −5.9 ppm; $C_{23}H_{26}O_4N$). HR-ESI-MS-MS (CID=40%; rel. int. %): 126(100).

*Side product (**11**)*



M.p.: – (oil). TLC: DCM:MeOH = 5:1; R_f = 0.81. IR (KBr): 2952, 1683, 1657, 1612, 1347, 1161, 764 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.68–1.85 (8H; m; H_2-3. H_2-4. H_2-3". H_2-4"); 3.24 (4H; m; H_2-2. H_2-5); 4.05 (2H; t; J = 6.4 Hz; H_2-2"); 4.14 (2H; t; J = 6.2 Hz; H_2-5"); 6.38 (1H; ~s; H-6'); 6.80 (1H; ~s; H-8'); 7.03 (1H; s; H-3'); 7.48–7.65 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.07–8.12 (2H; m; C(2')-Ph: H_{ortho}); 12.78 (1H; s; C(5')-O$\underline{H}$); ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 24.3. 24.9 (C-3. C-4); 25.1 (C-4"); 25.2 (C-3"); 45.2. 45.7 (C-2. C-5); 63.7 (C-2"); 68.0 (C-5"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.2 (C-3'); 126.3 (C(2')-Ph: C_{ortho}); 129.0 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.0 (C(2')-Ph: C_{para}); 154.0 (C-1"); 157.3 (C-9'); 161.1 (C-5'); 163.3 (C-2'); 164.5 (C-7'); 181.9 (C-4'). HRMS: M + H = 424.17337 (delta= −4.9 ppm; $C_{24}H_{26}O_6N$). HR-ESI-MS-MS (CID=40%; rel. int. %): 353(17); 309(100); 267(4); 255(4); 170(5).

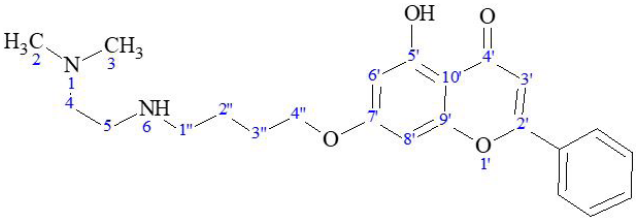
4.2.3 Chrysin – *N,N*-dimethylethylenediamine hybrid (10) and side product (12)

100 mg (0.257 mmol) 7-(bromobutoxy)chrysin (**7**) was dissolved in 5 ml DMF, then 71 mg (0.514 mmol) K_2CO_3 and 0.03 ml (0.257 mmol) *N,N*-dimethylethylenediamine (**6**) were added. The mixture was stirred for 26 h at a temperature of 23 °C. The reaction mixture was poured into cold water (30 ml), and the forming suspension was extracted with DCM (2 × 15 ml). The combined organic layer was dried

over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* preparative TLC (DCM/MeOH = 1/1 + a few drops of $_{aq.}NH_3$ (25%)) resulting 18 mg (17%) of the product (**10**) and 30 mg (29%) of the side product (**12**).

*Chrysin – N,N-dimethylethylenediamine hybrid (**10**)*

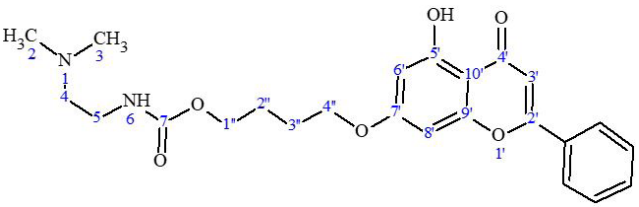
M.p.: – (oil). TLC: DCM:MeOH = 1:1 + 1 drop NH_4OH;



R_f = 0.27. IR (KBr): 3255, 3077, 2922, 2761, 2301, 1660, 1615, 823 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.50–1.58 (2H; m; H_2-2"); 1.72–1.81 (2H; m; H_2-3"); 2.12 (6H; s; H_3-2. H_3-3); 2.29 (2H; t; J = 6.4 Hz; H_2-4); 2.54–2.60 (4H; m; H_2-5. H_2-1"); 4.11 (2H; t; J = 6.5 Hz; H_2-4"); 6.39 (1H; d; J = 2.0 Hz; H-6'); 6.82 (1H; d; J = 2.0 Hz; H-8'); 7.04 (1H; s; H-3'); 7.55–7.67 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.07–8.13 (2H; m; C(2')-Ph: H_{ortho}). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 25.7 (C-2"); 26.2 (C-3"); 45.2 (C-2. C-3); 46.9 (C-5); 48.8 (C-1"); 58.8 (C-4); 68.4 (C-4"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.2 (C-3'); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 157.3 (C-9'); 161.1 (C-5'); 163.3 (C-2'); 164.7 (C-7'); 182.0 (C-4'). HRMS: M + H = 397.21130 (delta = −2.2 ppm; $C_{23}H_{29}O_4N_2$). HR-ESI-MS-MS (CID=40%; rel. int. %): 352(100); 255(32); 143(22).

*Side product (**12**)*

M.p.: 109−111 °C. TLC: DCM:MeOH = 1:1 + 1 drop NH_4OH;



R_f = 0.73. IR (KBr): 3324, 2952, 2854, 1693, 1662, 1615, 1333, 824 cm^{-1}. NMR: ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.66–1.74 (2H; m; H_2-2"); 1.75–1.84 (2H; m; H_2-3"); 2.11 (6H; s; H_3-2. H_3-3); 2.25 (2H; t; J = 6.8 Hz; H_2-4); 3.05 (2H; td; J = 6.8. 5.6 Hz; H_2-5); 4.01 (2H; t; J = 6.3 Hz; H_2-1"); 4.13 (2H; t; J = 6.2 Hz; H_2-4"); 6.39 (1H; wide d; $J \approx$ 2.0 Hz; H-6'); 6.82 (1H; wide d; $J \approx$ 2.0 Hz; H-8'); 6.98 (1H; wide t; $J \approx$ 5.6 Hz; NH-6); 7.04 (1H; s; H-3'); 7.57–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.08–8.13 (2H; m; C(2')-Ph: H_{ortho}). 12.80 (1H; wide s; C(5')-OH). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 25.0 (C-3"); 25.2 (C-2"); 38.3 (C-5); 45.1 (C-2. C-3); 58.4 (C-4); 63.1 (C-1"); 68.1 (C-4"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.3 (C-3'); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 156.2 (C-7); 157.3 (C-9'); 161.1 (C-5'); 163.3 (C-2'); 164.6 (C-7'); 182.0 (C-4'). HRMS: M + H = 441.20088 (delta = −2.6 ppm; $C_{24}H_{29}O_6N_2$). HR-ESI-MS-MS (CID=40%; rel. int. %): 327(4); 309(100); 267(10); 255(9).

4.2.4 General procedure for preparation of amino acid esters substituted with 5-bromovaleric acid ((*S*)-16, (*R*)-16, (*S*)-18)

Amino acid hydrochloric salt was neutralized in a two-phase mixture of DCM and 10% $NaHCO_3$ solution. The resulting amino acid esters were solved in dry DCM (5 ml/mmol). 5-Bromovaleric acid (1.1 equiv.) was added, and the mixture was stirred for 10 min under an argon atmosphere. A solution of DCC (1.1 equiv.) in dry DCM (5 ml/mmol) was added dropwise. After completion, the mixture was filtered and concentrated *in vacuo*. Products (**(*S*)-16:** 86%, **(*R*)-16:** 95%, **(*S*)-18:** 99%) were used immediately in the following reactions due to the observed instability during storage at 4 °C.

(S)-16, ***(R)-16***: TLC: DCM:MeOH = 20:1; R_f = 0.55.

(S)-18: TLC: DCM/MeOH = 5/1 (ninhydrin); R_f = 0.50.

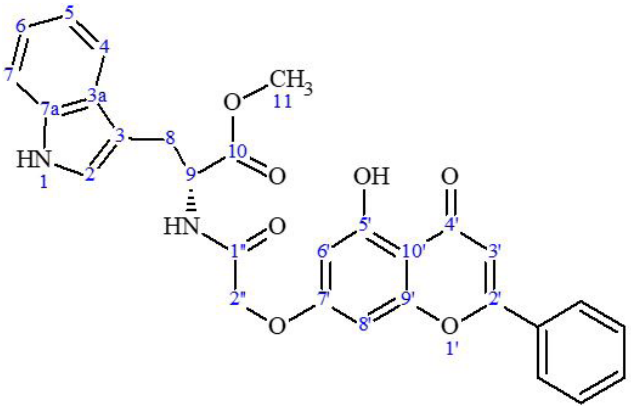
4.2.5 General procedure for preparing chrysin – amino acid hybrids ((*S*)-19, (*R*)-19, (*S*)-20, (*R*)-20, (*S*)-21, (*S*)-22)

Chrysin (**1**) was dissolved in DMF (15 ml/mmol) then the amino acid derivative (**(*R/S*)-15**, **(*R*/*S*)-16**, **(*S*)-17**, **(*S*)-18**) (1,1 equiv.) and K_2CO_3 (2 equiv.) were added. The applied temperatures differ and are shown in Table 2. The mixtures were poured into water and extracted with DCM. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The crude products were purified *via* preparative TLC (heptane/EtOAc = 1/1) to obtain the title compounds (**(*S*)-19**: 60%, **(*R*)-19**: 54%, **(*S*)-20**: 64%, **(*R*)-20**: 46%, **(*S*)-21**: 76%, **(*S*)-22**: 36%).

Table 2 Reaction conditions for producing chrysin – amino acid hybrids

Reaction	Temperature
(*S*)-15 → (*S*)-19	80 °C
(*S*)-15 → (*S*)-19	60 °C
(*S*)-15 → (*S*)-19	80 °C
(*S*)-15 → (*S*)-19	60 °C
(*S*)-15 → (*S*)-19	80 °C
(*S*)-15 → (*S*)-19	60 °C

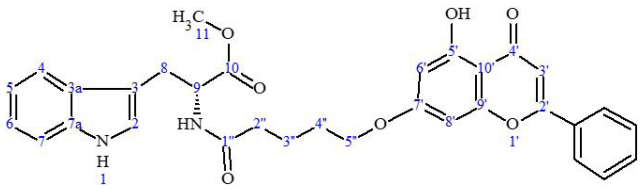
(S)-19



M.p.: 74-75 °C. TLC: DCM:MeOH = 5:1; R_f = 0.88. IR (KBr): 3391, 3306, 2922, 2851, 1735, 1609, 1166, 741 cm^{-1}. ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 3.18 (1H; ~dd; J = 14.6. 8.3 Hz; H_x-8); 3.24 (1H; ~dd; J = 14.6. 5.5 Hz; H_y-8); 3.62 (3H; s; H_3-11); 4.62 (1H; ddd; J = 8.3. 7.7. 5.5 Hz; H-9); 4.65. 4.68 (2H; AB; J = 14.9 Hz; H-2"); 6.41 (1H; d; J = 2.2 Hz; H-6'); 6.76 (1H; d; J = 2.2 Hz; H-8'); 6.97 (1H; ddd; J = 7.9. 7.0. 1.0 Hz; H-5); 7.03–7.07 (1H; m; H-6); 7.07 (1H; s; H-3'); 7.18 (1H; d; J = 2.3 Hz; H-2); 7.32 (1H; ~d; J = 8.1 Hz; H-7); 7.51 (1H; ~d; J = 7.9 Hz; H-4); 7.57–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.08–8.12 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 8.50 (1H; d; J = 7.7 Hz; C(9)-N$\underline{H}$); 10.89 (1H; wide s; NH-1); 12.81 (1H; wide s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 26.6 (C-8); 51.9 (C-11); 52.8 (C-9); 66.8 (C-2"); 93.5 (C-8'); 98.6 (C-6'); 105.2 (C-10'); 105.4 (C-3'); 109.1 (C-3); 111.4 (C-7); 117.8 (C-4); 118.3 (C-5); 120.9 (C-6); 123.7 (C-2); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 127.0 (C-3a); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 136.0 (C-7a); 157.1 (C-9'); 161.0 (C-5'); 163.47. 163.48 (C-2'. C-7'); 166.9 (C-1"); 171.9 (C-10); 182.0 (C-4'). HRMS: M-H = 511.15062 (delta = 1.3 ppm; $C_{29}H_{23}O_7N_2$).

(R)-19: M.p.: 77-79 °C. TLC: DCM:MeOH = 5:1; R_f = 0.80. IR (KBr): 3394, 3076, 2922, 2852, 1610, 1167, 741 cm^{-1}. ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 3.18 (1H; ~dd; J = 14.6. 8.3 Hz; H_x-8); 3.24 (1H; ~dd; J = 14.6. 5.4 Hz; H_y-8); 3.62 (3H; s; H_3-11); 4.62 (1H; ddd; J = 8.3. 7.7. 5.4 Hz; H-9); 4.65. 4.69 (2H; AB; J = 14.9 Hz; H-2"); 6.41 (1H; d; J = 2.0 Hz; H-6'); 6.76 (1H; d; J = 2.0 Hz; H-8'); 6.95–7.00 (1H; m; H-5); 7.03–7.08 (2H; m; H-6. H-3'); 7.18 (1H; d; J = 2.1 Hz; H-2); 7.33 (1H; ~d; J = 8.1 Hz; H-7); 7.51 (1H; ~d; J = 7.9 Hz; H-4); 7.56–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.07–8.12 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 8.50 (1H; d; J = 7.7 Hz; C(9)-N$\underline{H}$); 10.89 (1H; wide s; NH-1); 12.82 (1H; wide s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 26.7 (C-8); 51.9 (C-11); 52.8 (C-9); 66.8 (C-2"); 93.5 (C-8'); 98.6 (C-6'); 105.2 (C-10'); 105.4 (C-3'); 109.1 (C-3); 111.4 (C-7); 117.8 (C-4); 118.4 (C-5); 120.9 (C-6); 123.7 (C-2); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 127.0 (C-3a); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 136.0 (C-7a); 157.1 (C-9'); 161.0 (C-5'); 163.47. 163.49 (C-2'. C-7'); 166.9 (C-1"); 171.9 (C-10); 182.0 (C-4'). HRMS: M+H=511.15072 (delta= 1.5 ppm; $C_{29}H_{23}O_7N_2$). HR-ESI-MS-MS (CID=40%; rel. int. %): 479(4); 435(4); 382(100); 352(14); 350(74); 322(8); 310(14); 306(5); 253(71).

(S)-20

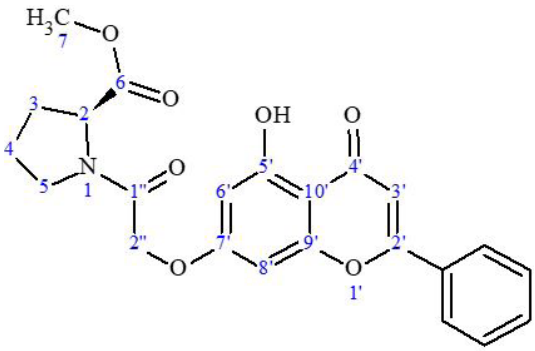


M.p.: 182-184 °C. TLC: DCM:MeOH = 20:1; R_f = 0.36 IR (KBr): 3270, 2923, 2853, 1736, 1648, 1162 cm^{-1}. ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.55–1.68 (4H; m; H_2-3". H_2-4"); 2.12–2.23 (2H; m; H_2-2"); 3.03 (1H; dd; J = 14.5. 8.8 Hz; H_x-8); 3.15 (1H; dd; J = 14.5. 5.4 Hz; H_y-8); 3.58 (3H; s; H_3-11); 4.02–4.07 (2H; wide t; J = 6.0 Hz; H_2-5"); 4.49–4.56 (1H; m; H-9); 6.37 (1H; wide s; H-6'); 6.78 (1H; wide s; H-8'); 6.98 (1H; wide t; $J \approx$ 7.6 Hz; H-5); 7.03 (1H; wide s; H-3'); 7.06 (1H; wide t; $J \approx$ 7.5 Hz; H-6); 7.15 (1H; wide d; J = 2.0 Hz; H-2); 7.34 (1H; d; J = 8.1 Hz; H-7); 7.49 (1H; d; J = 7.8 Hz; H-4); 7.52–7.65 (3H; m; C(2')-Ph: $\underline{H}_{meta}$.$\underline{H}_{para}$); 8.07–8.13 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 8.32 (1H; wide d; J = 7.6 Hz; C(9)-N$\underline{H}$); 10.93 (1H; wide s; NH-1); 11.5–13.9 (1H; wide; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 21.5 (C-3"); 27.0 (C-8); 27.6 (C-4"); 34.3 (C-2"); 51.7 (C-11); 52.9 (C-9); 68.0 (C-5"); 93.0 (C-8'); 98.5 (C-6'); 104.9 (C-10'); 105.3 (C-3'); 109.4 (C-3); 111.3 (C-7); 117.9 (C-4); 118.3 (C-5); 120.8 (C-6); 123.6 (C-2); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 127.0 (C-3a); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.0 (C(2')-Ph: $\underline{C}_{para}$); 136.0 (C-7a); 157.4 (C-9'); 161.2 (C-5'); 163.3 (C-2'); 164.6 (C-7'); 172.0 (C-1"); 172.5 (C-10); 181.9 (C-4'). HRMS: M + H = 555.21254 (delta = −0.1 ppm; $C_{32}H_{31}O_7N_2$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 523(12); 495(10); 301(100); 202(7).

(R)-20: M.p.: 172-174 °C. TLC: DCM:MeOH = 30:1; R_f = 0.33. IR (KBr): 3309, 2927, 2850, 1736, 1615, 1172 cm^{-1}. ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.55–1.68 (4H; m; H_2-3". H_2-4"); 2.12–2.22 (2H; m;

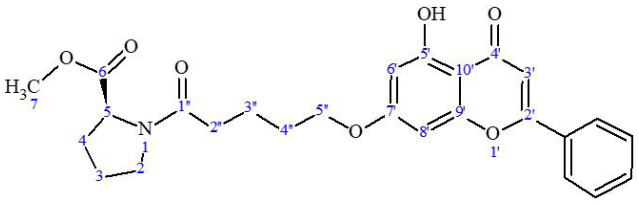
H_2-2"); 3.03 (1H; dd; J = 14.6. 8.8 Hz; H_x-8); 3.15 (1H; dd; J = 14.6. 5.6 Hz; H_y-8); 3.58 (3H; s; H_3-11); 4.06 (2H; ~t; J = 6.1 Hz; H_2-5"); 4.52 (1H; ddd; J = 8.8. 7.6. 5.6 Hz; H-9); 6.39 (1H; d; J = 2.2 Hz; H-6');6.81 (1H; d; J = 2.2 Hz; H-8'); 6.98 (1H; ddd; J = 8.0. 7.0. 1.0 Hz; H-5); 7.05 (1H; s; H-3'); 7.06 (1H; ddd; J = 8.0. 7.0. 1.1 Hz; H-6); 7.14 (1H; d; J = 2.3 Hz; H-2); 7.33 (1H; ~d; J = 8.0 Hz; H-7); 7.49 (1H; ~d; J = 8.0 Hz; H-4); 7.57–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.09–8.13 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 8.29 (1H; d; J = 7.6 Hz; C(9)-N$\underline{H}$); 10.86 (1H; wide d; $J \approx$ 2.3 Hz; NH-1); 12.80 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm)21.5 (C-3"); 27.0 (C-8); 27.6 (C-4"); 34.3 (C-2"); 51.7 (C-11); 52.9 (C-9); 68.0 (C-5"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.3 (C-3'); 109.5 (C-3); 111.3 (C-7); 117.9 (C-4); 118.3 (C-5); 120.8 (C-6); 123.5 (C-2); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 127.0 (C-3a); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 136.0 (C-7a); 157.3 (C-9'); 161.1 (C-5'); 163.4 (C-2'); 164.7 (C-7'); 172.0 (C-1"); 172.5 (C-10); 182.0 (C-4'). HRMS: M + H = 555.21224 (delta= −0.6 ppm; $C_{32}H_{31}O_7N_2$). HR-ESI-MS-MS (CID=40%; rel. int. %): 523(12); 495(10); 337(2); 301(100); 202(6).

(S)-21



M.p.: 142-144 °C. TLC: EtOAc; R_f = 0.69. IR (KBr): 3437, 3067, 2952, 1740, 1609, 1156, 765 cm^{-1}. NMR: Amide-rotamers in a ratio of 4:1, major conformer: ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.82–1.92 (1H; m; H_x-3); 1.93–2.02 (2H; m; H_2-4); 2.14–2.27 (1H; m; H_y-3); 3.55–3.69 (2H; m; H_2-5); 3.62 (3H; s; H_3-7); 4.37 (1H; dd; J = 8.6. 4.6 Hz; H-2); 5.00 (2H; ~s; H_2-2"); 6.41 (1H; d; J = 2.0 Hz; H-6'); 6.78 (1H; d; J = 2.0 Hz; H-8'); 7.05 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.06–8.14 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 12.81 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 24.4 (C-4); 28.4 (C-3); 45.2 (C-5); 51.7 (C-7); 58.5 (C-2); 66.0 (C-2"); 93.7 (C-8'); 98.6 (C-6'); 105.0 (C-10'); 105.3 (C-3'); 126.3 (C(2')-Ph: $\underline{C}_{ortho}$); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 157.0 (C-9'); 161.0 (C-5'); 163.4 (C-2'); 164.1 (C-7'); 165.3 (C-1"); 172.1 (C-6); 182.0 (C-4'). Minor conformer: ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.66–1.77 (1H; m; H_x-4); 1.84–1.93 (1H; m; H_y-4); 2.12–2.29 (2H; m; H_2-3); 3.42–3.49 (2H; m; H_2-5);3.73 (3H; s; H_3-7); 4.69 (1H; d; J = 15.2 Hz; H_x-2"); 4.82 (1H; dd; J = 8.5. 1.8 Hz; H-2); 4.94 (1H; d; J = 15.2 Hz; H_y-2"); 6.37 (1H; d; J = 2.0 Hz; H-6'); 6.74 (1H; d; J = 2.0 Hz; H-8'); 7.05 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.06–8.14 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 12.81 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 21.4 (C-4); 30.7 (C-3); 46.2 (C-5); 52.4 (C-7); 57.8 (C-2); 66.5 (C-2"); 93.5 (C-8'); 98.4 (C-6'); 105.0 (C-10'); 105.3 (C-3'); 126.3 (C(2')-Ph: $\underline{C}_{ortho}$); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 157.0 (C-9'); 161.0 (C-5'); 163.5 (C-2'); 163.9 (C-7'); 165.4 (C-1"); 172.2 (C-6); 182.0 (C-4'). HRMS: M + H = 424.13869 (delta = −0.9 ppm; $C_{23}H_{22}O_7N$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 364(70); 295(100); 267(10); 255(4); 130(5).

(S)-22



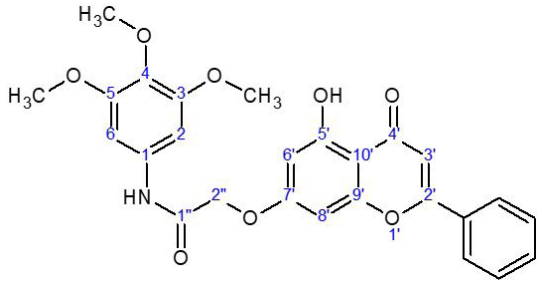
M.p.: – (oil). TLC: DCM:MeOH = 20:1; R_f = 0.50. IR (KBr): 3326, 2925, 2873, 2851, 2101, 1956, 1741, 1159 cm^{-1}. NMR: Amide-rotamers in a ratio of 4:1, major conformer: ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.60–1.70 (2H; m; H_2-3"); 1.73–1.86 (3H; m; H_2-4". H_x-4); 1.87–1.95 (2H; m; H_2-3); 2.11–2.20 (1H; m; H_y-4); 2.32–2.43 (2H; m; H_2-2"); 3.50–3.58 (2H; m; H_2-2); 3.61 (3H; s; H_3-7); 4.12 (2H; t; J = 6.4 Hz; H_2-5"); 4.29 (1H; dd; J = 8.7. 4.3 Hz; H-5); 6.39 (1H; d; J = 2.0 Hz; H-6'); 6.82 (1H; d; J = 2.0 Hz; H-8'); 7.04 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: $\underline{H}_{meta}$. $\underline{H}_{para}$); 8.07–8.13 (2H; m; C(2')-Ph: $\underline{H}_{ortho}$); 12.80 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 20.6 (C-3"); 24.3 (C-3); 27.8 (C-4"); 28.7 (C-4); 32.8 (C-2"); 46.4 (C-2); 51.6 (C-7); 58.1 (C-5); 68.2 (C-5"); 93.1 (C-8'); 98.4 (C-6'); 104.8 (C-10'); 105.2 (C-3"); 126.4 (C(2')-Ph: $\underline{C}_{ortho}$); 129.1 (C(2')-Ph: $\underline{C}_{meta}$); 130.5 (C(2')-Ph: $\underline{C}_{ipso}$); 132.1 (C(2')-Ph: $\underline{C}_{para}$); 157.3 (C-9'); 161.1 (C-5'); 163.3 (C-2');

164.7 (C-7'); 170.5 (C-1"); 172.5 (C-6); 182.0 (C-4'). Minor conformer: ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 1.60–1.70 (2H; m; H_2-3"); 1.70–1.73 (1H; m; H_x-3); 1.73–1.81 (2H; m; H_2-4"); 1.81–1.86 (1H; m; H_y-3); 2.02–2.09 (1H; m; H_x-4). 2.19–2.26 (1H; m; H_y-4); 2.32–2.43 (2H; m; H_2-2"); 3.34–3.44 (2H; m; H_2-2); 3.69 (3H; s; H_3-7); 4.12 (2H; t; J = 6.4 Hz; H_2-5"); 4.65 (1H; dd; J = 8.7. 2.1 Hz; H-5); 6.38 (1H; d; J = 2.0 Hz; H-6'); 6.80 (1H; d; J = 2.0 Hz; H-8'); 7.04 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.07–8.13 (2H; m; C(2')-Ph: H_{ortho}); 12.80 (1H; s; C(5')-OH). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 20.7 (C-3"); 22.0 (C-3); 27.8 (C-4"); 30.7 (C-4); 32.6 (C-2"); 45.8 (C-2); 52.2 (C-7); 58.4 (C-5); 68.2 (C-5"); 93.1 (C-8'); 98.4 (C-6'); 104.8 C-10'); 105.2 (C-3'); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 157.3 (C-9'); 161.1 (C-5"); 163.3 (C-2'); 164.7 (C-7'); 170.6 (C-1"); 172.8 (C-6); 182.0 (C-4'). HRMS: M + H = 466.18535 (delta = −1.5 ppm; $C_{26}H_{28}O_7N$). HR-ESI-MS-MS (CID=40%; rel. int. %): 406(3); 212(100).

4.2.6 General procedure for synthesizing aryloxy acetamides (27-30)

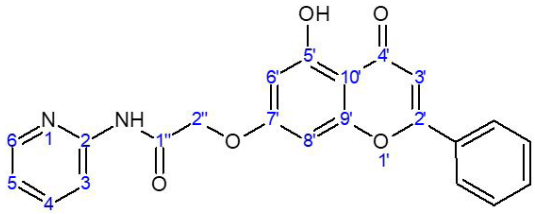
Chrysin (**1**) was dissolved in DMF (5 ml/mmol). Acetamide (1.1 equiv.) and K_2CO_3 (1,5 equiv.) were added to the solution. The reaction mixture was stirred at a temperature of 40 °C. After completion, the mixture was poured into water. The precipitate was filtered out, and the aqueous phase was extracted with DCM. The filtered and the extracted crudes were combined and purified *via* preparative TLC (DCM/MeOH = 20/1).

*2-((Chrysin-7-yl)oxy)-N-(3,4,5-trimethoxyphenyl) acetamide (**27**)*



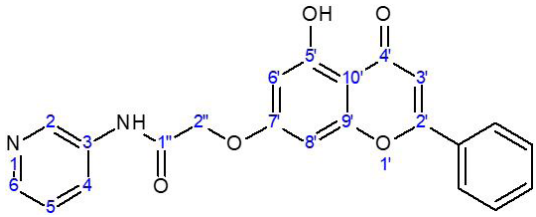
M.p.: 202–204 °C. TLC: DCM:MeOH = 20:1; R_f = 0.57. IR (KBr): 3273, 2836, 1677, 1607, 1355, 1130, 833 cm^{-1}. ^{1}H NMR (799.9 MHz; DMSO-d_6) δ (ppm) 3.62 (3H; s; C(4)-OCH_3); 3.74 (6H; s; C(3)-OCH_3. C(5)-OCH_3); 4.86 (2H; s; H_2-2"); 6.51 (1H; d; J = 2.2 Hz; H-6'); 6.89 (1H; d; J = 2.2 Hz; H-8'); 7.05 (2H; s; H-2. H-6); 7.07 (1H; s; H-3'); 7.58-7.60 (2H; m; C(2')-Ph: H_{meta}); 7.62-7.64 (1H; m; C(2')-Ph: H_{para}); 8.09-8.12 (2H; m; C(2')-Ph: H_{ortho}) 10.07 (1H; s; C(1)-NH); 12.84 (1H; s; C(5)-OH). ^{13}C NMR (201.1 MHz; DMSO) δ (ppm) 55.6 (C(3)-OCH_3. C(5)-OCH_3); 60.0 (C(4)-OCH_3); 67.1 (C-2"); 93.6 (C-8'); 97.2 (C-2. C-6); 98.6 (C-6'); 105.2 (C-10'); 105.3 (C-3'); 126.4 (C(2')-Ph: C_{ortho}); 129.7 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 133.6 (C-4); 134.3 (C-1); 152.6 (C-3. C-5); 157.1 (C-9'); 161.1 (C-5'); 163.5 (C-2'); 163.7 (C-7'); 165.4 (C-1"); 182.0 (C-4'). HRMS: M + H = 478.14941 (delta = −0.5 ppm; $C_{26}H_{24}O_8N$). HR-ESI-MS-MS (CID = 35%; rel. int. %): 460(2); 295(100); 267(12); 255(9); 224(2); 196(3); 193(2); 184(5) 153(1).

*2-((Chrysin-7-yl)oxy)-N-(pyridin-2-yl)acetamide (**28**)*



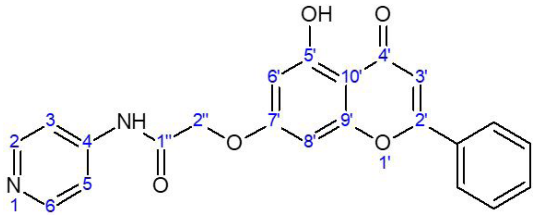
M.p.: 221-222°C. TLC: DCM:MeOH = 20:1; R_f = 0.72. IR (KBr): 1730, 1612, 1496, 1173, 766, 673 cm^{-1}. ^{1}H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 4.98 (2H; s; H_2-2"); 6.48 (1H; d; J = 2.3 Hz; H-6'); 6.88 (1H; d; J = 2.3 Hz; H-8'); 7.06 (1H; s; H-3'); 7.15 (1H; ddd; J = 7.3. 4.9 1.0 Hz; H-5); 7.56–7.65 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 7.81 (1H; ddd; J = 8.2. 7.5. 1.9 Hz; H-4); 8.05 (1H; d; J = 8.2 Hz; H-3); 8.08–8.12 (2H; m; C(2')-Ph: H_{ortho}); 8.36 (1H; ddd; J = 4.9. 1.9. 0.9 Hz; H-6); 10.66 (1H; s; C(2)-NH); 12.84 (1H; s; C(5')-OH). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 66.8 (C-2"); 93.5 (C-8'); 98.5 (C-6'); 105.2 (C-10'); 105.3 (C-3'); 113.5 (C-3); 119.7 (C-5); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 138.3 (C-4); 148.0 (C-6); 151.2 (C-2); 157.1 (C-9'); 161.1 (C-5'); 163.5 (C-2'); 163.9 (C-7'); 166.3 (C-1"); 182.0 (C-4'). HRMS: M + H = 389.11162 (delta = −4.1 ppm; $C_{22}H_{17}O_5N_2$). HR-ESI-MS-MS (CID = 50%; rel. int. %): 371(100); 295(61); 255(3); 135(33).

*2-((Chrysin-7-yl)oxy)-N-(pyridin-3-yl)acetamide (**29**)*



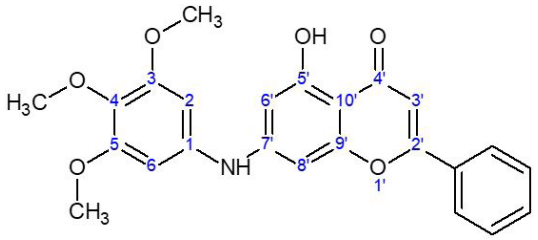
M.p.: 269–271 °C. TLC: DCM:MeOH = 20:1; R_f = 0.25. IR (KBr): 1643, 1597, 1172, 834, 677, 506 cm^{-1}. NMR: ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 4.93 (2H; s; H_2-2"); 6.53 (1H; d; J = 2.0 Hz; H-6'); 6.92 (1H; d; J = 2.0 Hz; H-8'); 7.08 (1H; s; H-3'); 7.38 (1H; dd; J = 8.3. 4.7 Hz; H-5); 7.53–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.08 (1H; ~d; J = 8.3 Hz; H-4); 8.09–8.13 (2H; m; C(2')-Ph: H_{ortho}); 8.31 (1H; ~d; J = 4.7 Hz; H-6); 8.80 (1H; d; J = 2.2 Hz; H-2); 10.38 (1H; s; C(3)-N<u>H</u>); 12.84 (1H; s; C(5')-O<u>H</u>). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 67.0 (C-2"); 93.6 (C-8'); 98.7 (C-6'); 105.2 (C-10'); 105.4 (C-3'); 123.6 (C-5); 126.4 (C(2')-Ph: C_{ortho}); 126.7 (C-4); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 134.9 (C-3); 141.2 (C-2); 144.7 (C-6); 157.1 (C-9'); 160.1 (C-5'); 163.5 (C-2'); 163.7 (C-7'); 166.2 (C-1"); 182.0 (C-4'). HRMS: M + H = 389.11328 (delta = −2.6 ppm; $C_{22}H_{17}O_5N_2$). HR-ESI-MS-MS (CID=40%; rel. int. %): 361(7); 295(20); 267(100); 255(52); 237(11); 135(3).

*2-((Chrysin-7-yl)oxy)-N-(pyridin-4-yl)acetamide (**30**)*



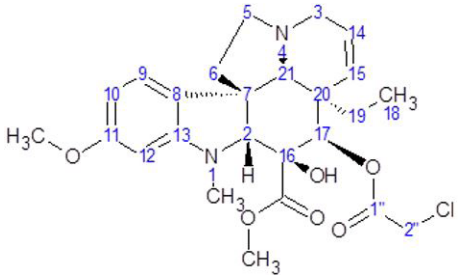
M.p.: 238-240 °C. TLC: DCM:MeOH = 10:1; R_f = 0.61. IR (KBr): 3392, 1658, 1583, 1505, 1170, 809, 500 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 4.95 (2H; s; H_2-2"); 6.51 (1H; d; J = 2.3 Hz; H-6'); 6.90 (1H; d; J = 2.3 Hz; H-8'); 7.07 (1H; s; H-3'); 7.56-7.66 (5H; m; C(2')-Ph: H_{meta}. H_{para}; H-3. H-5); 8.09-8.13 (2H; m; C(2')-Ph: H_{ortho}); 8.44-8.48 (2H; m; H-2. H-6); 10.58 (1H; s; C(4)-N<u>H</u>); 12.84 (1H; wide; C(5')-O<u>H</u>). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 67.0 (C-2"); 93.6 (C-8'); 98.6 (C-6'); 105.2 (C-10'); 105.4 (C-3'); 113.4 (C-3. C-5); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 144.9 (C-4); 150.4 (C-2. C-6); 157.1 (C-9'); 160.1 (C-5'); 163.5 (C-2'); 163.7 (C-7"); 166.9 (C-1"); 182.0 (C-4'). HRMS: M + H = 389.11295 (delta = −0.6 ppm; $C_{22}H_{17}O_5N_2$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 361(4); 343(4); 295(63); 267(100); 255(18); 237(5); 135(10).

4.2.7 7-((3,4,5-Trimethoxyphenyl)amino)chrysin (31)



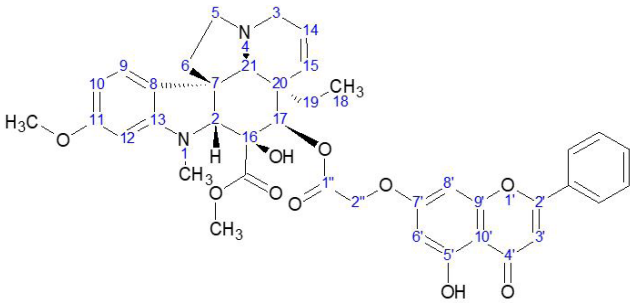
200 mg (0.419 mmol) 2-((chrysin-7-yl)oxy)-*N*-(3,4,5-trimethoxyphenyl)acetamide (**27**) was dissolved in 4 ml DMF, 115 mg (0.838 mmol) K_2CO_3 was added, and the mixture was stirred for 48 h at a temperature of 100 °C. The reaction mixture was poured into water (30 ml) and was extracted with DCM (3 × 30 ml). The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified *via* preparative TLC (DCM/MeOH = 20/1) to give 77 mg (44%) of the title compound (**31**). M.p.: 258–259 °C. TLC: DCM:MeOH = 20:1; R_f = 0.73. IR (KBr): 3282, 1661, 1616, 1380, 1229, 905, 490 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 3.67 (3H; s; C(4)-OC<u>H</u>$_3$); 3.78 (6H; s; C(3)-OC<u>H</u>$_3$. C(5)-OC<u>H</u>$_3$); 6.29 (1H; d; J = 1.9 Hz; H-6'); 6.54 (2H; s; H-2. H-6); 6.63 (1H; d; J = 1.9 Hz; H-8'); 6.92 (1H; s; H-3'); 7.53–7.62 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.05-8.09 (2H; m; C(2')-Ph: H_{ortho}); 8.98 (1H; s; C(1)-N<u>H</u>); 12.81 (1H; s; C(5')-O<u>H</u>). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 55.8 (C(3)-O<u>C</u>H$_3$; C(5)-O<u>C</u>H$_3$); 60.0 (C(4)-OC<u>H</u>$_3$); 90.8 (C-8'); 96.8 (C-6'); 99.6 (C-2. C-6); 103.0 (C-10'); 105.1 (C-3'); 126.3 (C(2')-Ph: C_{ortho}); 129.0 (C(2')-Ph: C_{meta}); 130.7 (C(2')-Ph: C_{ipso}); 131.8 (C(2')-Ph: C_{para}); 133.8 (C-4); 135.9 (C-1); 151.8 (C-7'); 153.3 (C-3. C-5); 157.7 (C-9'); 160.9 (C-5'); 162.6 (C-2'); 180.9 (C-4'). HRMS: M + H = 420.14252 (delta = −3.9 ppm; $C_{24}H_{22}O_6N$). HR-ESI-MS-MS (CID = 50%; rel. int. %): 404(20); 390(41); 387(25); 359(100).

4.2.8 17-(2-Chloroacetyl)vindoline (33)



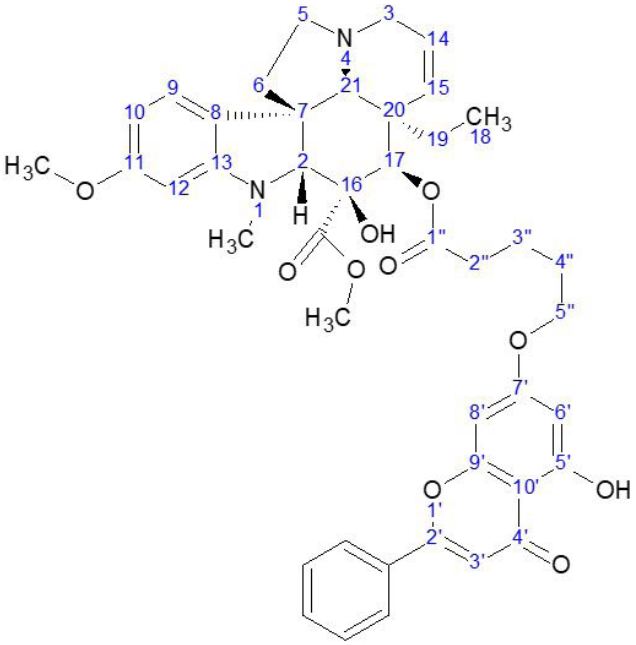
200 mg (0.483 mmol) 17-deacetylvindoline (**31**) was dissolved in dry DCM (10 ml). 128 mg (1.21 mmol) K_2CO_3 was added, and the mixture was cooled to 0 °C. 40 µl (0.507 mmol) 2-chloroacetyl chloride was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 70 h. The mixture was filtered, and the solution was washed with 25 ml water. After phase separation, the aqueous phase was extracted with DCM. The combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified *via* preparative TLC (DCM/MeOH = 20/1) resulting 126 mg (53%) product. M.p.: 55–57 °C. TLC: DCM/MeOH = 20/1; R_f = 0.72. IR (KBr): 2953, 1735, 1598, 1502, 1226, 1158, 786 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6): δ (ppm) 0.46 (3H; t; J = 7.3 Hz; H_3-18); 0.98 (1H; dq; J = 14.1, 7.3 Hz; H_x-19); 1.47 (1H; dq; J = 14.1, 7.3 Hz; H_y-19); 2.19-2.36 (2H; m; H_2-6); 2.59 (3H; N(1)-C$\underline{H}_3$); 2.78-3.16 (3H; br m; H-3$_x$, H-5$_x$, H-21); 3.41-3.52 (1H; br m; H_y-5); 3.54–3.64 (2H; m; H-2, H_y-3); 3.68 (3H; s; C(16)-COOC$\underline{H}_3$); 3.72 (3H; s; C(11)-OC$\underline{H}_3$); 4.27 (2H; s; H_2-2'); 5.19-5.33 (2H; m; H-15, H-17); 5.87 (1H; ddd; J = 10.3, 5.0, 1.2 Hz; H-14); 6.24 (1H; d; J = 2.2 Hz; H-12); 6.33 (1H; dd; J = 8.2, 2.2 Hz; H-10); 7.13 (1H; d; J = 8.2 Hz; H-9); 8.92 (1H; br s; C(16)-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6): δ (ppm) 7.3 (C-18); 30.6 (C-19); 38.1 (N(1)-$\underline{C}H_3$); 41.0 (C-2'); 42.2 (C-20); 43.0 (C-6); 49.8 (C-3); 50.7 (C-5); 51.7 (C-7); 52.0 (C(16)-COO$\underline{C}H_3$); 55.0 (C(11)-O$\underline{C}H_3$); 66.2 (C-21); 77.2 (C-17); 78.5 (C-16); 81.8 (C-2); 95.6 (C-12); 104.8 (C-10); 123.1 (C-9); 123.9 (C-14); 124.6 (C-8); 129.5 (C-15); 153.2 (C-13); 160.7 (C-11); 167.0 (C-1'); 171.2 (C(16)-$\underline{C}OOCH_3$). HRMS: M + H = 491.19391 (delta = −0.9 ppm; $C_{25}H_{32}O_6N_2Cl$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 473(15); 431(23); 413(2); 397(100); 365(4); 337(2); 188(27); 162(2).

4.2.9 Chrysin – vindoline hybrid *via* a linker containing 2 carbon atoms (35)



60 mg (0.236 mmol) chrysin (**1**) and the linker containing vindoline derivative (**33**) were dissolved in acetonitrile (5 ml). 32 mg (0.236 mmol) K_2CO_3 and catalytic amount of KI were added, and stirred at 50 °C. The solvent was evaporated, and the residue was dissolved in DCM and was washed with 10% Na_2CO_3 solution. After the phase separation, the aqueous phase was washed with DCM and the combined organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The crude material was purified *via* preparative TLC (EtOAc/MeOH = 15/1) resulting 132 mg (79%) product. M.p.: 114-116 °C. TLC: DCM:MeOH = 20:1; R_f = 0.70. IR (KBr): 2952, 1736, 1613, 1500, 1450, 1245, 821, 767 cm^{-1}. ^1H NMR (399.8 MHz; DMSO-d_6) δ (ppm) 0.45 (3H; t; J = 7.4 Hz; H_3-18); 0.97 (1H; dq; J = 13.9. 7.4 Hz; H_x-19); 1.44 (1H; dq; J = 13.9. 7.4 Hz; H_y-19); 2.16–2.25 (2H; m; H_2-6); 2.54–2.64 (4H; m; N(1)-C$\underline{H}_3$. H_x-5); 2.73 (1H; s; H-21); 2.83 (1H; wide d; J = 16.4 Hz; H_x-3); 3.23–3.34 (1H; m; H_y-5); 3.42 (1H; wide dd; J = 16.1. 5.0 Hz; H_y-3); 3.61 (1H; s; H-2); 3.68 (3H; s; C(11)-OC$\underline{H}_3$); 3.74 (3H; s; C(16)-COOC$\underline{H}_3$); 4.84 (1H; d; J = 16.9 Hz; H_x-2"); 4.99 (1H; d; J = 16.9 Hz; H_y-2"); 5.23 (1H; wided d; J = 10.2 Hz; H-14); 5.27 (1H; s; H-17); 5.86 (1H; ddd; J = 10.2. 4.8. 1.2 Hz; H-15); 6.19 (1H; d; J = 2.2 Hz; H-12); 6.28 (1H; dd; J = 8.2. 2.2 Hz; H-10); 6.40 (1H; d; J = 2.3 Hz; H-6'); 6.78 (1H; d; J = 2.3 Hz; H-8'); 7.05 (1H; d; J = 8.2 Hz; H-9); 7.07 (1H; s; H-3'); 7.56–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.08–8.13 (2H; m; C(2')-Ph: H_{ortho}); 8.91 (s; 1H; C(16)-O$\underline{H}$); 12.78 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (100.5 MHz; DMSO-d_6) δ (ppm) 7.4 (C-18); 30.3 (C-19); 38.1 (N(1)-$\underline{C}H_3$); 42.3 (C-20); 43.6 (C-6); 50.2 (C-3); 50.8 (C-5); 51.7 (C(16)-COO$\underline{C}H_3$); 52.1 (C-7); 55.0 (C(11)-O$\underline{C}H_3$); 64.8 (C-2"); 65.8 (C-21); 77.0 (C-17); 78.7 (C-16); 82.7 (C-2); 93.4 (C-8'); 95.5 (C-12); 98.6 (C-6'); 104.6 (C-10); 105.3 (C-10'); 105.4 (C-3'); 123.0 (C-9); 124.6 (C-14); 124.9 (C-8); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 129.6 (C-15); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 153.3 (C-13); 157.0 (C-9'); 160.5 (C-11); 161.0 (C-5'); 163.41. 163.45 (C-2'. C-7'); 167.8 (C-1"); 171.5 (C(16)-$\underline{C}OOCH_3$); 182.0 (C-4'). HRMS: M + H = 709.27376 (delta = −2.6 pm; $C_{40}H_{41}O_{10}N_2$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 691(35); 649(61); 631(4); 397(100); 365(8); 337(4).

4.2.10 Chrysin – vindoline hybrid *via* a linker containing 5 carbon atoms (36)



68 mg (0.236 mmol) chrysin (**1**) and 150 mg (0.260 mmol) linker containing vindoline derivative (**34**) were dissolved in acetonitrile (4 ml). 55 mg (0.399 mmol) K_2CO_3 was added and stirred at 60 °C for 16 h. The solvent was evaporated, and the crude material was purified *via* preparative TLC (DCM/MeOH = 20/1) resulting 76 mg (39%) product. M.p.: 107–109 °C. TLC: DCM:MeOH = 20:1; R_f = 0.58. IR (KBr): 2874, 1734, 1657, 1611, 1500, 1159, 818, 766 cm^{-1}. ^1H NMR (499.9 MHz; DMSO-d_6) δ (ppm) 0.41 (3H; t; J = 7.3 Hz; H_3-18); 0.97 (1H; dq; J = 14.0. 7.3 Hz; H_x-19); 1.49 (1H; dq; J = 14.0. 7.3 Hz; H_y-19); 1.60–1.69 (2H; m; H_2-3"); 1.71–1.81 (2H; m; H_2-4"); 2.18–2.23 (2H; m; H_2-6); 2.24–2.38 (2H; m; H-2"); 2.53–2.60 (4H; m; N(1)-C$\underline{H}_3$. H_x-5); 2.65 (1H; s; H-21); 2.79 (1H; wide d; J = 16.4 Hz; H_x-3); 3.24–3.31 (1H; m; H_y-5); 3.41 (1H; wide dd; J = 16.5. 5.0 Hz; H_y-3); 3.55 (1H; s; H-2); 3.64 (3H; s; C(16)-COOC$\underline{H}_3$); 3.71 (3H; s; C(11)-OC$\underline{H}_3$); 4.11 (2H; t; J = 6.3 Hz; H_2-5"); 5.07 (1H; wide d; J = 10.1 Hz; H-14); 5.22 (1H; s; H-17); 5.82 (1H; ddd; J = 10.2. 4.9. 1.3 Hz; H-15); 6.20 (1H; d; J = 2.2 Hz; H-12); 6.28 (1H; dd; J = 8.2. 2.2 Hz; H-10); 6.40 (1H; d; J = 2.2 Hz; H-6'); 6.83 (1H; d; J = 2.2 Hz; H-8'); 7.04 (1H; s; H-3'); 7.05 (1H; d; J = 7.6 Hz; H-9); 7.57–7.66 (3H; m; C(2')-Ph: H_{meta}. H_{para}); 8.08–8.13 (2H; m; C(2')-Ph: H_{ortho}); 8.78 (s; 1H; C(16)-O$\underline{H}$); 12.80 (1H; s; C(5')-O$\underline{H}$). ^{13}C NMR (125.7 MHz; DMSO-d_6) δ (ppm) 7.5 (C-18); 21.0 (C-3"); 27.6 (C-4"); 30.4 (C-19); 33.0 (C-2"); 38.0 (N(1)-$\underline{C}H_3$); 42.4 (C-20); 43.6 (C-6); 50.4 (C-3); 51.1 (C-5); 51.6 (C(16)-COO$\underline{C}H_3$); 52.0 (C-7); 55.0 (C(11)-O$\underline{C}H_3$); 66.1 (C-21); 68.1 (C-5"); 75.7 (C-17); 78.6 (C-16); 82.8 (C-2); 93.1 (C-8'); 95.4 (C-12); 98.4 (C-6'); 104.5 (C-10); 104.8 (C-10'); 105.2 (C-3'); 123.0 (C-9); 124.4 (C-15); 125.3 (C-8); 126.4 (C(2')-Ph: C_{ortho}); 129.1 (C(2')-Ph: C_{meta}); 129.8 (C-14); 130.5 (C(2')-Ph: C_{ipso}); 132.1 (C(2')-Ph: C_{para}); 153.4 (C-13); 157.3 (C-9'); 160.4 (C-11); 161.1 (C-5'); 163.4 (C-2'); 164.6 (C-7'); 171.6 (C(16)-$\underline{C}OOCH_3$); 172.3 (C-1"); 182.0 (C-4'). HRMS: M + H = 751.31836 (delta = −5.5 ppm; $C_{43}H_{47}O_{10}N_2$). HR-ESI-MS-MS (CID = 40%; rel. int. %): 733(38); 691(35); 397(100); 365(4).

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