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Alkynyl–coumarinyl ethers as MAO-B inhibitors

Matthias D. Mertens, Sonja Hinz, Christa E. Müller *, Michael Gütschow *

Pharmaceutical Institute, Pharmaceutical Chemistry I, University of Bonn, An der Immenburg 4, D-53121 Bonn, Germany

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ABSTRACT

In this study, alkynyl–coumarinyl ethers were developed as inhibitors of human monoamine oxidase B (MAO-B). A series of 31 new, ether-connected coumarin derivatives was synthesized via hydroxycoumarins, whose phenolic group at position 6, 7 or 8 was converted by means of the Mitsunobu reaction. The majority of the final products were produced from primary alcohols with a terminal alkyne group. The inhibitors were optimized with respect to the structure of the alkynyloxy chain and its position at the fused benzene ring as well as the residue at position 3 of the pyran-2*H*-one part. A hex-5-ynyloxy chain at position 7 was found to be particular advantageous. Among the 7-hex-5-ynyloxy-coumarins, the 3-methoxycarbonyl derivative **36** was characterized as a dual-acting inhibitor with IC_{50} values of less than 10 nM towards MAO-A and MAO-B, and the 3-(4-methoxy)phenyl derivative **44** was shown to combine strong anti-MAO-B potency (IC_{50} = 3.0 nM) and selectivity for MAO-B over MAO-A (selectivity >3400-fold).

1. Introduction

The degradation of certain neurotransmitters as well as dietary and xenobiotic amines is catalyzed by monoamine oxidase (MAO, EC 1.4.3.4). MAO is a flavin-dependent enzyme found in the outer mitochondrial membrane of neuronal, glial and all other mammalian cells. Two isoforms of monoamine oxidase are known, MAO-A and MAO-B. Both isoforms share about 70% of their amino acid identity, but they differ in their tissue distribution, substrate and inhibitor preferences.[1] While MAO-A is ubiquitously present in human tissues, MAO-B predominantly resides in the brain, in particular in the basal ganglia. MAO-B preferentially catalyzes the deamination of 2-phenylethylamine, and MAO-A favorably oxidizes serotonin; while dopamine is a substrate for both isoforms.[2] During aging, the expression of MAO-B increases, and MAO-B is believed to be involved in aging-related neurodegenerative diseases. The increased expression is connected with an enhanced dopamine metabolism. Hydrogen peroxide is produced during the MAO-catalyzed deamination reaction, resulting in oxidative damage and apoptotic signaling events.[3] X-ray crystal structures of MAO-A and MAO-B from different species and cocrystallized with various ligands are available and provided a closer insight into the active site of the two enzymes.[1]

Both isoforms of MAO are important targets for drug discovery and development. Tranylcypromine represents an irreversible inhibitor for both isoforms. Its lack of selectivity and the undesired 'cheese effect' have led to a limited use, restricted to the treatment of therapy-resistant depression.[4] Fluorinated analogues of this drug showed better selectivity and expectedly fewer side effects.[5,6] Moclobemide, a reversible MAO-A inhibitor, is currently used in the clinic against depression and anxiety. MAO-B inhibitors are frequently administered in the therapy of neurodegenerative disorders such as Alzheimer's and Parkinsons diseases. Two irreversible MAO-B inhibitors, that is, rasagiline and selegiline (**1**, Fig. 1) are successfully applied in the treatment of Parkinson's disease.[7] Another promising MAO-B inhibitor, safinamide (**2**), has been evaluated in phase III clinical trials with a positive outcome.[8] This reversible inhibitor additionally blocks dopamine reuptake.[9] Several dual-acting MAO inhibitors have been developed which address a second target. Inhibition of acetylcholinesterase, for example, leads to a prolonged action of acetylcholine in the synaptic cleft and proved to be beneficial in Alzheimer's disease.[10–14] For the treatment of Parkinson's disease, xanthine and benzothiazinone derivatives have been described combining MAO-B inhibition and adenosine A_{2A} receptor antagonism.[15,16]

The simple molecular scaffold of the coumarin (benzopyran-2-one) has attracted much attention in drug research because of the variety of biological activities of its representatives. Coumarins are known to act as inhibitors or modulators on various target

* Corresponding authors. Tel.: +49 228 732301; fax: +49 228 732567 (C.E.M.); tel.: +49 228 732317; fax: +49 228 732567 (M.G.).
E-mail addresses: christa.mueller@uni-bonn.de (C.E. Müller), guetschow@uni-bonn.de (M. Gütschow).

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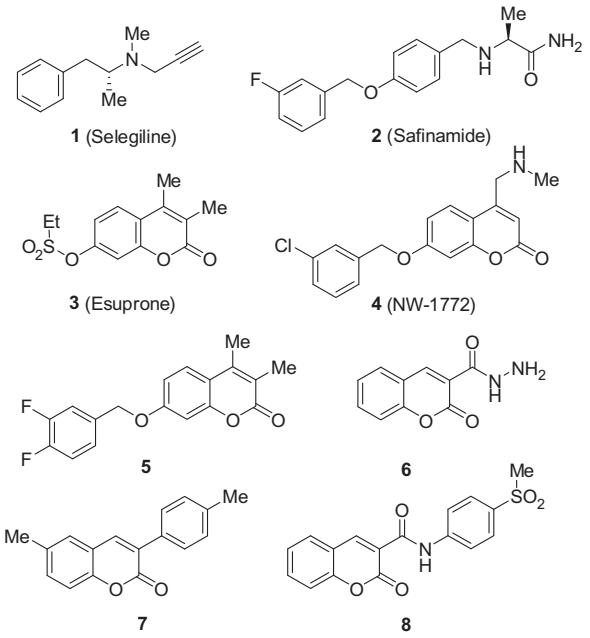


Figure 1. Structures of known MAO inhibitors including those with coumarin scaffold.

structures; for example, derivatives have been developed that possess anticoagulant or antiproliferative activities.[17,18] Certain coumarins act as inhibitors of aromatase,[19] 17β-hydroxysteroid dehydrogenase 1,[20] or acetylcholinesterase.[21,22] Furthermore, coumarins have been described as $GABA_A$ receptor modulators,[23] as cannabinoid receptor ligands,[24,25] or as ligands at other G protein-coupled receptors.[26] Thus, the coumarin scaffold can be regarded as a privileged structure in medicinal chemistry.[25] The facile synthetic accessibility and the versatile possibilities to modify the coumarin core have contributed to the wide utilization of this heterocycle. Noteworthy, coumarins or coumarin-like structures have been utilized for the design of activity-based probes for MAO enzymes,[27–30] and differently substituted coumarins have been synthesized or were isolated from natural sources and evaluated for their potential as MAO inhibitors, showing selective inhibition of either MAO-A or MAO-B isoforms from the micromolar to the picomolar range (Fig. 1).[31,32] Depending on their substitution pattern, the selectivity towards the two isoforms can be modulated. For example, coumarins with a sulfonic ester moiety attached to the 7-position exhibit a preferred affinity for MAO-A, as can be seen in esuprone (**3**).[33] For MAO-B, halo-substituted benzyloxy residues in position 7 of the coumarin core, as in **4** and **5**, are well accepted.[34,35] Such a substitution pattern is, however, not essential, as the carbohydrazide **6** revealed high potency and MAO-B selectivity.[36] Aryl substituents at position 3, either directly connected to the coumarin core (**7**)[37] or via amide linker (**8**)[38] caused strong activity against MAO-B.

The present study was performed to develop alkynyl–coumarinyl ethers as MAO-B inhibitors. Thus, coumarins bearing an alkynyloxy substituent with a terminal triple bond have been synthesized and evaluated for their inhibitory profile towards both human MAO isoforms. We attempted to optimize the inhibitors with respect to the structure of the alkynyloxy chain and its position at the fused benzene ring as well as the residue at position 3 of the pyran-2*H*-one part. Among the 31 new coumarin derivatives, several exhibited strong affinity to the MAO enzymes. Compound **44** was identified to be a highly potent and particularly selective inhibitor of MAO-B.

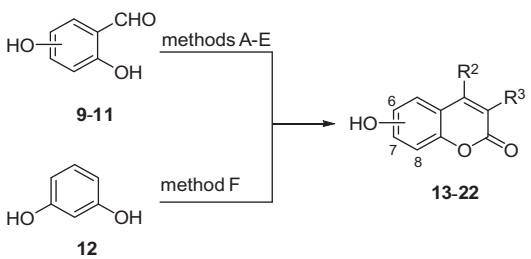
2. Results and discussion

A small library of substituted hydroxycoumarins (**13**–**22**) was prepared as outlined in Scheme 1 and Table 1. Different established methods were applied to convert salicylaldehydes or resorcinol to coumarins. Starting from commercially available salicylaldehydes **9**–**11**, Knoevenagel condensation with either dimethyl malonate or ethyl 3-(4-methoxyphenyl)-3-oxopropanoate as reactive methylene compounds and a catalytic amount of piperidine afforded coumarins **13**–**17** in good yields. Perkin condensation with *N*-acetylglycine or 4-methoxyphenylacetic acid in acetic anhydride gave the corresponding acetoxy precursors, whose deprotection under basic conditions furnished the hydroxycoumarins **18**–**20**. Umbelliferone (**21**) was available through a one-pot Wittig protocol. Pechmann condensation of ethyl acetoacetate and resorcinol (**12**) in the presence of concentrated sulfuric acid resulted in 4-methylumbelliferone (**22**) in good yield.

With these substituted hydroxycoumarins in hand, several final products (**24**–**52**) were synthesized (Scheme 2 and Table 2). Four different primary alcohols with a terminal alkyne group (to produce **24**–**29**, **33**–**44**) and five other alcohols (to produce **45**–**52**) were subjected to the Mitsunobu reaction using PPh_3 and diethyl azodicarboxylate (DEAD) or diisopropyl azodicarboxylate (DIAD) in tetrahydrofuran (THF). Most of the reactions proceeded smoothly within two hours at room temperature, and recrystallisation furnished the coumarinyl ethers in high purity and moderate to good yields. Similarly, **55** was prepared via Mitsunobu reaction with the non-coumarin phenol **23**. Cleavage of the methyl ester of **26** with lithium hydroxide gave the free carboxylic acid **30**, which was coupled to 4-fluoroaniline or Boc-piperazine with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate N-oxide (HATU) in the presence of diisopropylethylamine (DIPEA), affording the amide-type coumarins **31** and **32** (Scheme 2).

The synthesis of two aza-analogues **53** and **54**, in which a methylene unit of the alkynyloxy substituent of **36** and **42** is replaced by NH, is depicted in Scheme 3. The route started again with a Mitsunobu reaction of hydroxycoumarins (**13** and **21**) and 2-bromoethanol, followed by alkylation of 2-nitro-*N*-(prop-2-ynyl)benzenesulfonamide in the presence of potassium carbonate. Thiophenol-mediated nosyl deprotection furnished the free amines (**53** and **54**) in good yields.

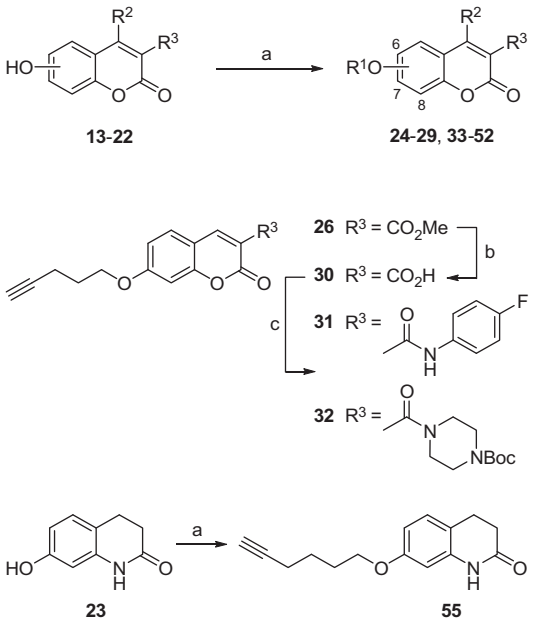
Coumarins **24**–**55** were investigated for inhibition of MAO-A and MAO-B using human recombinant enzymes. The Amplex Red monoamine oxidase assay was performed. The inhibitory potencies of the coumarins are listed in Table 2. Except for the inhibitor **24**, all compounds, if active at all, displayed a higher affinity for MAO-B than for MAO-A. First, the impact of the chain length on biological activity was examined on the basis of compounds with



Scheme 1. Synthesis of hydroxycoumarins **13**–**22** (for structures see Table 1). Reagents and conditions: method A, dimethyl malonate, piperidine, MeOH; method B, ethyl 3-(4-methoxyphenyl)-3-oxopronoate, piperidine, MeOH; method C, (i) *N*-acetylglycine, NaOAc, Ac_2O; (ii) K_2CO_3, MeOH; method D, (i) 4-methoxyphenylacetic acid, pyridine, Ac_2O; (ii) K_2CO_3, MeOH; method E, PPh_3, ethyl bromoacetate, EtOH, KOH; method F, ethyl acetoacetate, H_2SO_4.

Table 1
Synthesized hydroxycoumarins **13–22**

Compd	Hydroxy subst.	R^2	R^3	Method
13	7-	H	CO_2Me	A
14	6-	H	CO_2Me	A
15	8-	H	CO_2Me	A
16	7-	H	COC_6H_4-4-OMe	B
17	6-	H	COC_6H_4-4-OMe	B
18	7-	H	NHCOMe	C
19	6-	H	NHCOMe	C
20	7-	H	C_6H_4-4-OMe	D
21	7-	H	H	E
22	7-	Me	H	F



Scheme 2. Synthesis of alkynyloxy-substituted coumarins **24–52** and **55** (for structures see Table 2). Reagents and conditions: (a) R^1OH, DEAD or DIAD, PPh_3, THF; (b) LiOH, THF/H_2O; (c) 4-fluoroaniline or 1-Boc-piperazine, HATU, DIPEA, DMF.

a fixed pyran-2*H*-one part (R^2 = H, R^3= CO_2Me). The 7-propargyloxy-substituted compound **24** inhibited both isoforms to the same extent (IC_{50} values of ~2 μM). The elongation of the 7-alkynyloxy chain by methylene units led to a stepwise improvement of the inhibitory potency (**24** vs **25** vs **26** vs **36**). This correlation was particularly impressive for our main target enzyme, MAO-B, where the exchange of the 3-carbon chain (**24**) by a 6-carbon chain (**36**) increased the affinity by three orders of magnitude. The coumarin ester **36** already represented the most potent dual-acting inhibitor of this study, exhibiting IC_{50} values of 1.4 and 9.6 nM for MAO-B and MAO-A, respectively.

Using the example of the 5-carbon chain, its linkage to the benzene ring was analyzed. Whereas the attachment at the 8-position (**28**) resulted in a loss of activity, the 7- and 6-substituted isomers were both slightly selective for MAO-B, but the former was 17-fold more active at MAO-B (**26** vs **27**). Thus, in the following inhibitor design, we focused on an ether-attachment to position 7 of the coumarin. Further isomeric pairs of 6- and 7-substituted coumarins, provided in a later stage of this study, revealed a trend in that 6-isomers showed a better MAO-B selectivity, but 7-isomers were more potent (**36** vs **37**; **38** vs **39**; **40** vs **41**; **47** vs **48**; **50** vs **51**).

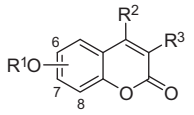
Furthermore, the hex-5-ynyloxy chain of **36** was replaced by other ether-connected moieties, such as substituted benzyl and phenethyl, geranyl, hexyl and propargylaminoethyl. The resulting compounds were included in the study for comparative evaluation. Their design was based on known coumarin-type MAO inhibitors, for example, coumarins with halo-substituted arylalkoxy chains,[10,34–36,38] as realized in **45** and **47**. The preparation of **50** was prompted by the geranyl structure of auraptene, a coumarin derivative from *Dictamnus albus* and the related natural product geiparvarin from *Geijera parviflora*, both processing anti-MAO activities.[39,40] The coumarin core was decorated with a hexyloxy chain (in **52**) in order to verify the impact of the triple bond by comparison with the hex-5-ynyloxy derivative **36**. Compound **53** represents an aza-analogous structure of **36** with improved water solubility. Among this subseries of six 7-substituted coumarin 3-methyl esters, inhibitors **36**, **45**, **47** and **52** exhibited IC_{50} values of less than 2 nM. Compounds **45**, **47**, **50** and **52** showed remarkable MAO-B selectivity. Thus, to achieve potent and selective coumarin-type MAO-B inhibitors, the 7-alkynyloxy substituent is not necessary.

In addition to the synthetic work and the enzyme inhibition assays, the lipophilicity of representative coumarins was examined. The log $D_{7.4}$ values (Table 2) were experimentally determined by an HPLC-based procedure.[41] As expected, a replacement of the CH_2 unit in **36** (log $D_{7.4}$ = 2.5) by NH decreased the lipophilicity of the aza analogue **53** (log $D_{7.4}$ = 0.5), which was only moderately active. The aforementioned potent compounds, **36**, **45**, **47** and **52**, turned out to be relatively lipophilic with log $D_{7.4}$ values between 2.5 and 4.2. An undesired high log $D_{7.4}$ value resulted from the exchange of the C≡CH terminus (**36**, log $D_{7.4}$ = 2.5) by CH_2CH_3 (**52**, log $D_{7.4}$ = 4.2). Although both 6-carbon chains led to highly potent MAO-B inhibitors, the 7-hex-5-ynyloxy substitution pattern of **36** was maintained for further structural optimization.

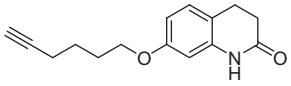
To produce molecular diversity at the pyran-2*H*-one part, compounds **38**, **40**, **42**, **43** have been synthesized, considering structures of known MAO inhibiting coumarins.[14,35,42] Introduction of the bulky aroyl residue in **38** resulted in a reduction of MAO-B inhibitory activity but an enhanced selectivity. The acetylamino derivative **40** was neither potent nor selective. On the other hand, removal of the methoxycarbonyl residue improved the selectivity of the corresponding inhibitors **42** and **43**. These trends for 7-hex-5-ynyloxy compounds were confirmed for the corresponding 7-pent-4-ynyloxy analogues (**38** and **29** vs **36** and **26**; **40** and **33** vs **36** and **26**, **42** and **34** vs **36** and **26**, **43** and **35** vs **36** and **26**). Comparison of pairs of 6-carbon chain and 5-carbon chain homologues revealed a more advantageous profile of the former ones (**38** vs **29**, **40** vs **33**, **42** vs **34**, **43** vs **35**). A final coumarin modification in the course of this study was carried out by excision of the exocyclic carbonyl group in **38**, leading to **44**. Otherwise, **44** can be regarded to result from a replacement of the CO_2 unit in **36** by a phenylene spacer. In recent years, 3-aryl substitution in coumarins was established to be suitable for MAO-B inhibition. In particular, the 4-methoxyphenyl moiety at 3-position was combined with small substituents at positions 6 and 8 to achieve potent and MAO-B selective coumarin inhibitors.[31,37,42–45] Compound **44** inhibited MAO-B with an IC_{50} value of 3.0 nM and showed a more than 3400-fold selectivity over MAO-A. Thus, in future studies it would be interesting to decorate the 3-(4-methoxyphenyl)coumarin with other elongated substituents at 7-position (as present in **45–52**). However, the lipophilicity of **44** (log $D_{7.4}$ = 4.3) was increased compared to **36**. The hex-5-ynyloxy chain was also placed at the corresponding position of an appropriate non-coumarin scaffold[46] demonstrating the feasibility of obtaining selective MAO-B inhibitors that way. The resulting dihydroquinolin-2(1*H*)-one **55** (log $D_{7.4}$ = 1.6) had an IC_{50} value of 53 nM.

Currently used MAO-B inhibitors display an irreversible mode of action. However, reversible inhibition may have advantages including better controllability. Two selected potent MAO-B

Table 2
MAO-A and MAO-B inhibitory activities of compounds **24**–**55**



24–**54**

Compd	Alkoxy subst.	R^1	R^2	R^3	MAO-A IC_{50} ± SEM[a] (nM)	MAO-B IC_{50} ± SEM[a] (nM)	MAO-B selectivity[b]
24	7-	$CH_2C{\equiv}CH$	H	CO_2Me	1570 ± 80	1790 ± 110	1
25	7-	$(CH_2)_2C{\equiv}CH$	H	CO_2Me	818 ± 36	258 ± 27	3
26	7-	$(CH_2)_3C{\equiv}CH$	H	CO_2Me	953 ± 78	82.4 ± 1.1	12
27	6-	$(CH_2)_3C{\equiv}CH$	H	CO_2Me	>10,000	1440 ± 10	>7
28	8-	$(CH_2)_3C{\equiv}CH$	H	CO_2Me	>10,000	>10,000	—
29[c]	7-	$(CH_2)_3C{\equiv}CH$	H	COC_6H_4-4-OMe	>10,000	535 ± 60	>19
30	7-	$(CH_2)_3C{\equiv}CH$	H	CO_2H	>10,000	>10,000	—
31	7-	$(CH_2)_3C{\equiv}CH$	H	$CONHC_6H_4$-4-F	>10,000	>10,000	—
32	7-	$(CH_2)_3C{\equiv}CH$	H	$CON(C_2H_4)_2N$-Boc	>10,000	≈10,000	—
33	7-	$(CH_2)_3C{\equiv}CH$	H	NHCOMe	930 ± 82	43.6 ± 1.9	21
34	7-	$(CH_2)_3C{\equiv}CH$	H	H	3520 ± 270	91.8 ± 9.1	38
35	7-	$(CH_2)_3C{\equiv}CH$	Me	H	553 ± 19	18.2 ± 2.0	30
36[c]	7-	$(CH_2)_4C{\equiv}CH$	H	CO_2Me	9.64 ± 0.84	1.41 ± 0.15	7
37	6-	$(CH_2)_4C{\equiv}CH$	H	CO_2Me	>10,000	123 ± 12	>81
38[c]	7-	$(CH_2)_4C{\equiv}CH$	H	COC_6H_4-4-OMe	>10,000	67.1 ± 7.2	>150
39	6-	$(CH_2)_4C{\equiv}CH$	H	COC_6H_4-4-OMe	>10,000	>10,000	—
40	7-	$(CH_2)_4C{\equiv}CH$	H	NHCOMe	66.8 ± 2.5	41.7 ± 2.5	2
41[c]	6-	$(CH_2)_4C{\equiv}CH$	H	NHCOMe	>10,000	24.7 ± 2.0	>400
42	7-	$(CH_2)_4C{\equiv}CH$	H	H	367 ± 21	2.62 ± 0.40	140
43	7-	$(CH_2)_4C{\equiv}CH$	Me	H	59.9 ± 2.3	0.770 ± 0.074	78
44[c]	7-	$(CH_2)_4C{\equiv}CH$	H	C_6H_4-4-OMe	>10,000	2.96 ± 0.10	>3400
45[c]	7-	$CH_2C_6H_4$-3-F	H	CO_2Me	77.2 ± 7.7	0.588 ± 0.054	130
46	6-	$CH_2C_6H_4$-3-Cl	H	CO_2Me	>10,000	31.5 ± 1.9	>320
47[c]	7-	$(CH_2)_2C_6H_4$-4-Cl	H	CO_2Me	74.8 ± 2.4	1.41 ± 0.02	53
48	6-	$(CH_2)_2C_6H_4$-4-Cl	H	CO_2Me	>10,000	120 ± 3	>83
49	7-	$CH_2C_6H_4$-3-Cl	H	NHCOMe	62.7 ± 6.7	13.5 ± 1.2	5
50	7-	Geranyl	H	CO_2Me	≈10,000	78.9 ± 1.9	130
51	6-	Geranyl	H	CO_2Me	>10,000	325 ± 53	>31
52[c]	7-	Hexyl	H	CO_2Me	66.1 ± 1.7	0.875 ± 0.047	75
53[c]	7-	$(CH_2)_2NHCH_2C{\equiv}CH$	H	CO_2Me	2680 ± 160	377 ± 78	7
54	7-	$(CH_2)_2NHCH_2C{\equiv}CH$	H	H	≈10,000	852 ± 27	12
55[c]					>10,000	52.6 ± 1.5	>190

[a] IC_{50} value are means from three experiments.
[b] IC_{50} (MAO-A) divided by IC_{50} (MAO-B).
[c] Log$D_{7.4}$ values were determined as follows, 3.1 (**29**), 2.5 (**36**), 3.5 (**38**), 2.4 (**41**), 4.3 (**44**), 3.2 (**45**), 3.9 (**47**), 4.2 (**52**), 0.5 (**53**), 1.6 (**55**).

inhibitors of the present series were investigated for reversibility of the inhibitory effect. Thus, MAO-B was preincubated with inhibitor, subsequently the substrate *p*-tyramine (10 μM) was added, and after 22 min the substrate concentration was increased to 1 mM (Fig. 2). Inhibitors **36** and **44** were studied at concentrations roughly correlating to their IC_{80} values. For comparison, the reversible inhibitor safinamide (**2**), and the irreversible inhibitor selegiline (**1**) were investigated under the same conditions. The large increase in substrate concentration leads to a displacement of reversible inhibitors, while no significant change is observed for irreversible inhibitors. In fact, **36** and **44** behaved like the reversible inhibitor safinamide. Therefore it can be concluded that the triple bond in the new inhibitors **24**–**44** and **55** does not lead to irreversible enzyme inhibition in contrast to the propargyl group present in the irreversible inhibitor selegiline.

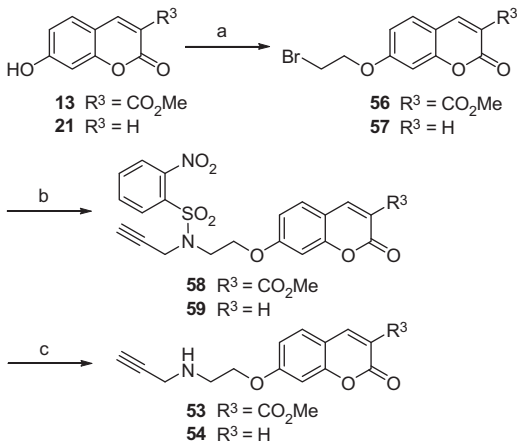
3. Conclusion

In this study, a stepwise approach to structurally optimized coumarins as MAO-B inhibitors has been undertaken. A comparison of isomeric coumarin pairs with the same ether-connected chain showed that the 7-isomers tend to be more potent at MAO-B, and the 6-isomers to be more selective for MAO-B over MAO-A. It was found that a hex-5-ynyloxy chain at position 7 was suitable to receive strong MAO-B inhibition. For example, the dual-acting compound **36** exhibited high affinity towards both MAO isoforms (IC_{50} = 1.4 and 9.6 nM for MAO-B and MAO-A), whereas **44** was the most promising candidate of this series with respect to both potency and selectivity (MAO-B IC_{50} = 3.0 nM, selectivity >3400-fold). Experimentally determined log$D_{7.4}$ values indicated that the introduction of the triple bond (as present in **36**) significantly reduced the lipophilicity (in comparison to the saturated analogue **52**). When appropriately placed at various bicyclic scaffolds, the hex-5-ynyloxy chain is considered to be a valuable substructure for the development of MAO-B inhibitors.

4. Experimental section

4.1. General methods and materials

Thin-layer chromatography was carried out on Merck aluminum sheets, silica gel 60 F_{254}. Detection was performed with UV light at 254 nm. Preparative column chromatography was performed on Merck silica gel 60 (70–230 mesh). Melting points



Scheme 3. Synthesis of propargylaminoethoxy-substituted coumarins **53** and **54**. Reagents and conditions: (a) 2-bromoethanol, DEAD, PPh_3, THF; (b) 2-nitro-*N*-(prop-2-ynyl)benzenesulfonamide, K_2CO_3, DMF; (c) thiophenol, K_2CO_3, DMF.

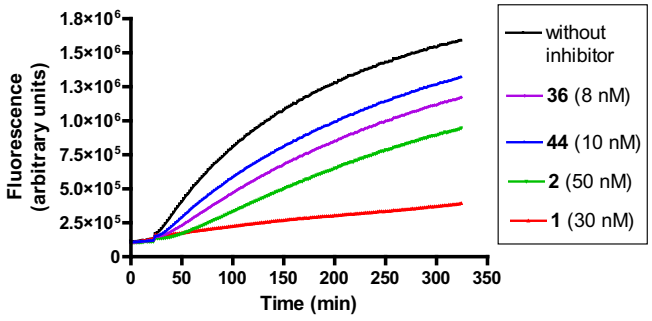


Figure 2. Human MAO-B was preincubated for 1 h with several inhibitors each at the concentration that approximately represents its IC_{80} value. The enzyme reaction was started by the addition of *p*-tyramine. After 22 min, the substrate concentration was increased from 10 μM to 1 mM, and fluorescence was measured over a time period of 5 h.

were determined by a Boëtius melting point apparatus from VEB Wägetechnik Rapido PHMK and are uncorrected. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded on a Bruker Avance DRX 500. Chemical shifts δ are given in ppm referring to the signal center using the solvent peaks for reference: DMSO-d_6 2.49/39.7 ppm. Elemental analyses were performed with a Vario EL apparatus. LC-DAD chromatograms and ESI-MS spectra were recorded on an Agilent 1100 HPLC system with Applied Biosystems API-2000 mass spectrometer. HRMS-ESI spectra were recorded on a micrOTOF-Q spectrometer. Solvents and reagents were obtained from Acros (Geel, Belgium), Fluka (Taufkirchen, Germany), Alfa Aesar (Karlsruhe, Germany), Activate Scientific (Prien, Germany) or Sigma–Aldrich (Steinheim, Germany). Hydroxycoumarins **13** and **20** were prepared as described elsewhere.[47] Compound **23** was purchased from Activate Scientific (Prien, Germany).

4.2. Preparation of hydroxycoumarins 14–19, 21, 22

4.2.1. Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (14)

2,5-Dihydroxy-benzaldehyde (2.76 g, 20 mmol) was dissolved in absolute MeOH (30 mL). Dimethyl malonate (2.90 g, 2.51 mL, 22 mmol) and piperidine (0.17 g, 0.2 mL, 2.0 mmol) were added and the mixture was refluxed for 2 h. The reaction mixture was cooled to 0 °C. The product was filtered off and washed with cold EtOH (10 mL) and dried to yield a light brown solid (2.62 g, 60%): mp 206–208 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.81 (s, 3H, CH_3), 7.15 (dd, 1H, ^3J = 8.8 Hz, ^4J = 2.9 Hz, 7-H), 7.19 (d, 1H, ^4J = 2.9 Hz, 5-H), 7.27 (d, 1H, 8-H, ^3J = 8.8 Hz), 8.66 (s, 1H, 4-H), 9.86 (br s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 53.2, 114.7, 118.0, 118.4, 119.1, 123.6, 148.8, 149.7, 154.9, 157.1, 164.2. Anal. Calcd for $C_{11}H_8O_5$: C, 60.00; H, 3.66. Found: C, 59.87; H, 3.71. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.9% purity, m/z = 221.20 ([M+H]$^+$).

4.2.2. Methyl 8-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (15)

2,3-Dihydroxy-benzaldehyde (2.76 g, 20 mmol) was dissolved in MeOH (30 mL). Dimethylmalonate (2.90 g, 2.51 mL, 22 mmol) and piperidine (0.17 g, 0.2 mL, 2.0 mmol) were added. The solution was refluxed for 2 h. The reaction mixture was cooled to 0 °C. The product crystallized from the reaction mixture was filtered off, washed with MeOH (20 mL) and dried to give a light yellow solid (2.71 g, 62%): mp 203–204 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.82 (s, 3H, CH_3), 7.17–7.21 (m, 2H, arom.H), 7.30–7.32 (m, 1H, arom.H), 8.69 (s, 1H, 4-H), 10.33 (s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.5, 117.4, 118.8, 120.3, 120.8, 125.0, 143.4, 144.6, 149.5, 156.0, 163.4. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{11}H_8O_5Na$: 243.0264, found: 243.0269. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 221.20 ([M+H]$^+$).

4.2.3. 7-Hydroxy-3-(4-methoxybenzoyl)-2*H*-chromen-2-one (16)

2,4-Dihydroxybenzaldehyde (2.76 g, 20.0 mmol), ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (4.44 g, 20.0 mmol) and piperidine (0.43 g, 0.5 mL, 5.0 mmol) were refluxed in MeOH (50 mL) for 4 h. A yellow solid precipitated and was filtered off, washed with MeOH (10 mL) to give a yellow powder (3.92 g, 66%): mp 190–191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 3H, CH_3), 6.71 (d, 1H, ^4J = 2.2 Hz, 8-H), 6.79 (dd, 2H, ^4J = 2.2 Hz, ^3J = 8.5 Hz, 6-H), 7.03 (d, 2H, ^3J = 8.9 Hz, 2′,6′-H), 7.62 (d, 1H, ^3J = 8.5 Hz, 5-H), 7.83 (d, 2H, ^3J = 9.2 Hz, 3′,5′-H), 8.22 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C) δ 55.7, 102.3, 110.3, 114.0, 114.6, 120.8, 129.7, 131.4, 132.0, 146.0, 156.9, 158.7, 163.6, 164.7, 190.5. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{17}H_{12}O_5Na$: 319.0577, found: 319.0580. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–450 nm), 99.8% purity, m/z = 297.38 ([M+H]$^+$).

4.2.4. 6-Hydroxy-3-(4-methoxybenzoyl)-2*H*-chromen-2-one (17)

2,5-Dihydroxybenzaldehyde (0.69 g, 5.0 mmol), ethyl 3-(4-methoxyphenyl)-3-oxopropanoate (1.33 g, 6.0 mmol) and piperidine (0.17 g, 0.2 mL, 2.0 mmol) were refluxed in MeOH (20 mL) for 2 h. A yellow precipitate was filtered off and washed with MeOH (10 mL) to give the product as yellow powder (1.34 g, 91%): mp 239–241 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.84 (s, 3H, CH_3), 6.71 (d, 1H, ^4J = 2.2 Hz, 8-H), 6.79 (dd, 2H, ^4J = 2.2 Hz, ^3J= 8.5 Hz, 6-H), 7.03 (d, 2H, ^3J = 8.9 Hz, 2′,6′-H), 7.62 (d, 1H, ^3J = 8.5 Hz, 5-H), 7.83 (d, 2H, ^3J = 9.2 Hz, 3′,5′-H), 8.22 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 55.8, 113.5, 114.2, 117.3, 118.9, 121.5, 127.1, 129.0, 132.2, 144.2, 147.5, 154.2, 158.5, 164.0, 190.3. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{17}H_{12}O_5Na$: 319.0577, found: 319.0581. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 297.25 ([M+H]$^+$).

4.2.5. *N*-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (18)

2,4-Dihydroxy-benzaldehyde (2.76 g, 20.0 mmol), *N*-acetylglycine (2.34 g, 20.0 mmol) and anhydrous sodium acetate (4.92 g, 60.0 mmol) were refluxed in Ac_2O (100 mL) for 4 h. The reaction mixture was evaporated in vacuo and the residue was recrystallized from EtOH (100 mL). The product was filtered off and washed with cold EtOH (10 mL) to yield 3-acetamido-2-oxo-2*H*-chromen-7-yl

acetate as a light brown solid (1.77 g, 34%): mp 233–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 7.12 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.6 Hz, 6-H), 7.25 (d, 1H, ^4J = 2.3 Hz, 8-H), 7.72 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.60 (s, 1H, 4-H), 9.71 (br s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.0, 24.1, 109.8, 117.6, 119.1, 123.3, 124.2, 128.6, 150.0, 151.1, 157.4, 169.0, 170.3. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.1% purity, m/z = 262.23 ([M+H]$^+$). 3-Acetamide-2-oxo-2*H*-chromen-7-yl acetate (1.31 g, 5.0 mmol), K_2CO_3 (1.38 g, 20.0 mmol) were refluxed in MeOH (25 mL) for 1.5 h The reaction mixture was evaporated and solved in water (25 mL). After acidification with 2 N HCl to pH ~3, the resulting precipitate was filtered off to yield a light brown solid (0.42 g, 38%): mp 303–304 °C (lit. mp 305 °C);[48] ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 7.12 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.6 Hz, 6-H), 7.25 (d, 1H, ^4J = 2.3 Hz, 8-H), 7.72 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.60 (s, 1H, 4-H), 9.71 (br s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.0, 24.1, 109.8, 117.6, 119.1, 123.3, 124.2, 128.6, 150.0, 151.1, 157.4, 169.0, 170.3. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 200–400 nm), 99.1% purity, m/z = 220.09 ([M+H]$^+$).

4.2.6. *N*-(6-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (19)

2,5-Dihydroxy-benzaldehyde (2.76 g, 20 mmol), *N*-acetylglycine (2.34 g, 20 mmol) and anhydrous sodium acetate (4.92 g, 60 mmol) were refluxed in Ac_2O (40 mL) overnight. The reaction mixture was allowed to cool to room temperature. The product was filtered off, washed with MeOH (30 mL) and dried to yield 3-acetamido-2-oxo-2*H*-chromen-6-yl acetate as a light grey solid (1.23 g, 24%): mp 222–223 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.16 (s, 3H, CH_3), 2.29 (s, 3H, CH_3), 7.12 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.6 Hz, 6-H), 7.25 (d, 1H, ^4J = 2.3 Hz, 8-H), 7.72 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.60 (s, 1H, 4-H), 9.71 (br s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.0, 24.1, 109.8, 117.6, 119.1, 123.3, 124.2, 128.6, 150.0, 151.1, 157.4, 169.0, 170.3. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.5% purity, m/z = 262.15 ([M+H]$^+$). 3-Acetamide-2-oxo-2*H*-chromen-6-yl acetate (1.04 g, 4.0 mmol), K_2CO_3 (1.10 g, 8.0 mmol) were refluxed in MeOH (20 mL) for 1.5 h. The reaction mixture was evaporated and dissolved in water (50 mL). After acidification with 2 N HCl to pH ~3, the resulting precipitate was filtered off and recrystallized from MeOH (80 mL) to yield a light brown solid (0.22 g, 25%): mp 217-218 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.15 (s, 3H, CH_3), 6.90 (dd, 1H, ^4J = 2.9 Hz, ^3J = 8.8 Hz, 7-H), 6.94 (d, 1H, ^4J = 2.9 Hz, 5-H), 7.20 (d, 1H, ^3J = 8.9 Hz, 8-H), 9.63-9.64 (2s overlapping, 2H, NH and OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.1, 112.0, 116.8, 117.6, 120.3, 123.7, 124.7, 143.2, 154.3, 157.8, 170.3. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{11}H_{19}NO_4Na$: 242.0424, found: 242.0420. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 220.14 ([M+H]$^+$).

4.2.7. 7-Hydroxy-2*H*-chromen-2-one (21)

Ethyl bromoacetate (1.67 g, 1.11 mL, 10 mmol) and PPh_3 (3.15 g, 12 mmol) were solved in dry EtOH (40 mL) and refluxed for 4 h. The mixture was cooled to room temperature, KOH (0.90 g, 16 mmol) and 2,4-dihydroxybenzaldehyde (1.31 g, 9.5 mmol) were added and the mixture was refluxed for 1.5 h. After evaporation in vacuo, the residue was suspended in water (50 mL) and acidified to pH 2. The aqueous phase was extracted with EtOAc (3 × 50 mL). The organic phase was dried, filtered and purified by column chromatography using CH_2Cl_2/MeOH (29:1) as eluent. The product was obtained as a white solid (0.42 g, 27%): mp 227–228 °C (lit. mp 226.5–228 °C);[49] ^{1}H NMR (500 MHz, DMSO-d_6) δ 6.18 (d, 1H, ^3J = 9.5 Hz, 3-H), 6.69 (d, 1H, ^4J = 2.2 Hz, 8-H), 6.77 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.5 Hz, 6-H), 7.50 (d, 1H, ^3J = 8.5 Hz, 5-H), 7.91 (d, 1H, ^3J = 9.2 Hz, 4-H), 10.52 (br s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 102.3, 111.4, 111.5, 113.3, 129.8, 144.6, 155.7, 160.6, 161.4. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.1% purity, m/z = 163.30 ([M+H]$^+$).

4.2.8. 7-Hydroxy-4-methyl-2*H*-chromen-2-one (22)

Resorcinol (2.20 g, 20.0 mmol) and ethyl acetoacetate (2.60 g, 2.53 mL, 20.0 mmol) were stirred for 10 min at 90 °C. Concentrated H_2SO_4 (0.5 mL) was added to the mixture and stirring was continued for 1 h. The mixture was then poured onto icewater (50 mL) and extracted with EtOAc (3 × 50 mL). The organic phase was dried over Na_2SO_4, filtrated and evaporated in vacuo. The residue was recrystallized from H_2O/EtOH (1:1, 30 mL) to give light brown crystals (2.09 g, 11.9 mmol, 59%): mp 180–182 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.34 (d, 3H, ^4J = 1.3 Hz, CH_3), 6.10 (d, 1H, ^4J = 1.3 Hz, 3-H), 6.68 (d, 1H, ^3J = 2.2 Hz, 8-H), 6.78 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.7 Hz, 6-H) 7.56 (d, 1H, ^3J = 8.5 Hz, 5-H), 10.46 (br s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.2, 102.3, 110.4, 112.2, 113.0, 126.7, 153.6, 155.0, 160.4, 161.3. Anal. Calcd for $C_{10}H_8O_3$ × 0.15 H_2O: C, 67.15; H, 4.68. Found: C, 67.10, H, 4.85. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 96.7% purity, m/z = 177.30 ([M+H]$^+$).

4.2.9. Preparation of alkynyloxy-substituted coumarins 24–52 and 55–57

4.2.9.1. General procedure for the Mitsunobu reaction to obtain 24–52 and 55–57. A mixture of the appropriate hydroxycoumarin (**13**–**22**; 2.0 mmol), triphenyl phosphine (1.04 g, 4.0 mmol), the corresponding alcohol (3.0 mmol) and THF (30 ml) was cooled to 0 °C. DEAD (0.52 g, 0.49 mL, 3.0 mmol) or DIAD (0.61 g, 0.59 mL, 3.0 mmol) was added dropwise and the solution was stirred overnight at room temperature. The volume was reduced in vacuo the residue was diluted with EtOAc (100 mL), washed with 1 N NaOH (3 × 100 mL) and brine (80 mL), dried over Na_2SO_4 and evaporated to dryness. The products were obtained either after recrystallization or column chromatography.

4.2.9.2. Methyl 2-oxo-7-(prop-2-ynyloxy)-2*H*-chromene-3-carboxylate (24). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with propagyl alcohol (168 mg) and DIAD. The residue was recrystallized from MeOH (90 mL) to give the product as light green solid (279 mg, 54%): mp 188–189 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.65 (t, 1H, ^4J = 2.5 Hz, CH), 3.80 (s, 3H, CH_3), 4.97 (d, 2H, ^4J = 2.3 Hz, CH_2–O), 7.04 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.7 Hz, 6-H), 7.07 (d, 1H, ^4J = 2.5 Hz, 8-H), 7.85 (d, H, ^3J = 8.8 Hz, 5-H), 8.75 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.4, 56.6, 78.4, 79.3, 101.5, 112.1, 113.7, 113.8, 131.8, 149.4, 156.2, 156.8, 162.7, 163.4. Anal. Calcd for $C_{14}H_{10}O_5$: C, 65.12; H, 3.90. Found: C, 64.80; H, 4.09. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.2% purity, m/z = 259.34 ([M+H]$^+$).

4.2.9.3. Methyl 2-oxo-7-(but-3-ynyloxy)-2*H*-chromene-3-carboxylate (25). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with 3-butyn-1-ol (210 mg) and DEAD. The residue was recrystallized from MeOH (75 mL) to give the product as colorless needles (323 mg, 60%): mp 167–168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (app quint, 2H, ^3J = 6.3 Hz, CH_2), 2.33 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.0 Hz, CH_2–CCH), 2.88 (t, 1H, ^4J = 2.5 Hz, CH), 3.77 (s, 3H, CH_3), 4.18 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.00 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.7 Hz, 6-H), 7.03 (d, 1H, ^4J = 2.6 Hz, 8-H), 7.82 (d, H, ^3J = 8.5 Hz, 5-H), 8.73 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.8, 52.3, 66.9, 72.7, 81.2, 101.0, 111.7, 113.3, 113.7, 131.9, 149.5, 156.3, 157.1, 163.5, 163.8. Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 65.93; H, 4.64. LC–MS

(ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 96.3% purity, m/z = 273.21 ($[M+H]^+$).

4.2.9.4. Methyl 2-oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carboxylate (26). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with 4-pentyn-1-ol (252 mg) and DIAD. The residue was recrystallized from MeOH (50 mL) to give the product as colorless needles (403 mg, 70%): mp 130–132 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (app quint, 2H, ^3J = 6.3 Hz, CH_2), 2.33 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.0 Hz, CH_2–CCH), 2.88 (t, 1H, ^4J = 2.5 Hz, CH), 3.77 (s, 3H, CH_3), 4.18 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.99–7.02 (m, 2H, 6-H, 8-H), 7.82 (d, H, ^3J = 8.6 Hz, 5-H), 8.73 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6, 25 °C) δ 14.5, 27.5, 52.3, 67.3, 71.9, 83.6, 100.9, 111.6, 113.2, 113.7, 131.9, 149.6, 156.4, 157.1, 163.5, 164.2. Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.13; H, 4.93. Found: C, 67.10; H, 5.01. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.1% purity, m/z = 287.19 ($[M+H]^+$).

4.2.9.5. 2-Oxo-6-(pent-4-ynyloxy)-2*H*-chromene-3-carboxylic acid (27). Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**14**, 2.0 mmol) weas reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was recrystallized from MeOH (35 mL) to give the product as green solid (471 mg, 82%): mp 133–134 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (app quint, 2H, ^3J = 6.6 Hz, 2-CH_2), 2.33 (dt, 2H, ^3J = 7.3 Hz, ^4J = 2.6 Hz, 3-CH_2), 2.80 (t, 1H, ^4J = 2.5 Hz, CH), 4.08 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.33 (dd, 1H, ^3J = 9.0 Hz, ^4J = 2.9 Hz, 7-H), 7.36 (d, H, ^3J = 8.9 Hz, 8-H), 7.48 (d, 1H, ^4J = 2.9 Hz, 5-H), 8.71 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 27.7, 52.6, 66.9, 71.8, 83.7, 112.8, 117.4, 117.8, 118.4, 122.9, 148.9, 149.2, 155.1, 156.2, 163.3. Anal. Calcd for $C_{15}H_{12}O_5$: C, 67.13; H, 4.93. Found: C, 66.73; H, 5.01. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.5% purity, m/z = 287.16 ($[M+H]^+$).

4.2.9.6. Methyl 2-oxo-8-(pent-4-ynyloxy)-2*H*-chromene-3-carboxylate (28). Methyl 8-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**15**, 440 mg) was reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was purified by column chromatography using petroleum ether/EtOAc (3:2) as eluent. The product was obtained as pale green solid (411 mg, 72%): mp 98–99 °C; ^{1}H NMR (500 MHz, DMSO-d_6,) δ 1.93 (app quint, 2H, ^3J = 7.0 Hz, 2-CH_2), 2.37 (dt, 2H, ^3J = 7.1 Hz, ^4J = 2.5 Hz, 3-CH_2), 2.80 (t, 1H, ^4J = 2.6 Hz, CH), 3.83 (s, 3H, CH_3), 4.19 (t, 2H, ^3J = 6.0 Hz, CH_2–O), 7.31 (app. d, 1H, ^3J = 8.2 Hz, ^3J = 7.9 Hz, 6-H), 7.42–7.46 (m, 2H, 5-H, 7-H), 8.74 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 27.8, 52.6, 67.6, 71.9, 83.6, 117.7, 117.8, 118.6, 121.6, 124.9, 144.3, 145.6, 149.3, 155.8, 163.2. HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{16}H_{15}O_5Na$: 309.0733, found: 309.0736. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.6% purity, m/z = 287.17 ($[M+H]^+$).

4.2.9.7. 3-(4-Methoxybenzoyl)-7-(pent-4-ynyloxy)-2*H*-chromen-2-one (29). 7-Hydroxy-3-(4-methoxybenzoyl)-2*H*-chromen-2-one (**16**, 594 mg) was reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was recrystallized from MeOH (120 mL) to give the product as yellow crystals (550 mg, 1.52 mmol, 76%): mp 151–152 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.93 (app quint, 2H, ^3J = 6.3 Hz, CH_2), 2.35 (dt, 2H, ^4J = 2.8 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.81 (t, 1H, ^4J = 2.9 Hz, CH), 3.85 (s, 3H, CH_3), 4.19 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.02 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.8 Hz, 6-H), 7.05 (d, 2H, ^3J = 8.9 Hz, 2′,6′-H), 7.07 (d, 1H, ^4J = 2.5 Hz, 8-H), 7.76 (d, 1H, ^3J = 8.8 Hz, 5-H), 7.87 (d, 2H, ^3J = 8.9 Hz, 3′,5′-H), 8.28 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 27.6, 55.8, 67.2, 71.9, 83.6, 101.2, 112.0, 113.5, 114.1, 123.1, 129.3, 131.1, 132.1, 145.3, 156.4, 156.4, 158.5, 163.2, 163.8, 190.3. Anal. Calcd for $C_{22}H_{18}O_5$: C, 72.92; H, 5.01. Found: C, 72.56; H, 5.11. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.9% purity, m/z = 363.37 ($[M+H]^+$).

4.2.9.8. *N*-(2-Oxo-7-(pent-4-ynyloxy)-2*H*-chromen-3-yl)acetamide (33). *N*-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (**18**, 438 mg) was reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was recrystallized from MeOH (70 mL) to give the product as pale brown needles (294 mg, 52%): mp 172–173 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.90 (app quint, 2H, ^3J = 6.6 Hz, CH_2), 2.13 (s, 3H, CH_3), 2.33 (dt, 2H, ^4J = 2.5 Hz, ^3J = 5.8 Hz, CH_2–CCH), 2.80 (t, 1H, ^4J = 3.1 Hz, CH), 4.11 (t, 2H, ^3J = 6.0 Hz, CH_2–O), 6.94 (dd, 2H, ^4J = 2.2 Hz, ^3J = 8.7 Hz, 6-H), 6.98 (d, H, ^4J = 2.6 Hz, 8-H), 7.59 (d, 1H, ^3J = 8.9 Hz, 5-H), 8.52 (s, 1H, 4-H), 9.57 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 23.9, 27.7, 66.8, 71.8, 83.7, 101.2, 112.9, 113.2, 122.0, 125.1, 128.9, 151.4, 157.9, 160.1, 170.0. Anal. Calcd for $C_{16}H_{15}NO_4$: C, 67.36; H, 5.30; N, 4.91. Found: C, 67.11; H, 5.41; N, 4.98. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.3% purity, m/z = 286.19 ($[M+H]^+$).

4.2.9.9. 7-(Pent-4-ynyloxy)-2*H*-chromen-2-one (34). Umbelliferone (**21**, 324 mg) was reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was recrystallized from MeOH (20 mL) to give the product as colorless needles (387 mg, 85%): mp 97–98 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (app quint, 2H, ^3J = 6.3 Hz, CH_2), 2.33 (dt, 2H, ^4J = 2.6 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.80 (t, 1H, ^4J = 2.5 Hz, CH), 4.18 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.27 (d, 1H, ^3J = 6.3 Hz, 3-H), 6.94 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.5 Hz, 6.97 (d, H, ^4J = 2.6 Hz, 5-H), 7.61 (d, 1H, ^3J = 8.5 Hz, 6-H), 7.97 (d, 1H, ^3J = 9.2 Hz, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 27.6, 66.9, 71.8, 83.6, 101.4, 112.5, 112.6, 112.8, 129.7, 144.4, 155.5, 160.4, 161.8. Anal. Calcd for $C_{14}H_{12}O_3$: C, 73.67; H, 5.30. Found: C, 73.62; H, 5.35. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.5% purity, m/z = 229.36 ($[M+H]^+$).

4.2.9.10. 4-Methyl-7-(pent-4-ynyloxy)-2*H*-chromen-2-one (35). 7-Hydroxy-4-methyl-2*H*-chromen-2-one (**22**, 352 mg) was reacted with 4-pentyn-1-ol (252 mg) and DEAD. The residue was recrystallized from MeOH (15 mL) to give the product as colorless needles (200 mg, 0.83 mmol, 41%): mp 102–103 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.91 (app quint, 2H, ^3J = 6.0 Hz, CH_2), 2.33 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.38 (d, 3H, ^4J = 1.3 Hz, CH_3), 2.80 (t, 1H, ^4J = 2.9 Hz, CH), 4.14 (t, 2H, ^3J = 6.4 Hz, CH_2–O), 6.19 (d, 1H, ^4J = 1.3 Hz, 3-H), 6.94–6.97 (m, 2H, 6-H, 8-H), 7.65–7.67 (d, H, ^3J = 8.6 Hz, 5-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.6, 18.2, 27.6, 66.9, 71.8, 83.6, 101.4, 111.3, 112.5, 113.3, 126.6, 153.5, 154.9, 160.2, 161.7. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.32; H, 5.87. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.0% purity, m/z = 243.46 ($[M+H]^+$).

4.2.9.11. Methyl 2-oxo-7-(hex-5-ynyloxy)-2*H*-chromene-3-carboxylate (36). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was recrystallized from MeOH (50 mL) to give the product as colorless needles (374 mg, 62%): mp 104–105 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, ^3J = 6.9 Hz, CH_2), 1.83 (quint, 2H, ^3J = 6.3 Hz, CH_2), 2.23 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2CCH), 2.76 (t, 1H, ^4J = 2.5 Hz, CH), 3.80 (s, 3H, CH_3), 4.13 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.98–7.01 (m, 2H, 6-H, 8-H), 7.81 (d, H, ^3J = 8.5 Hz, 5-H), 8.72 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.6, 27.6, 52.3, 68.3, 71.6, 84.3, 100.8, 111.5, 113.1, 113.8, 131.8, 149.6, 156.4, 157.2, 163.5, 164.4. Anal. Calcd for $C_{17}H_{16}O_5$: C, 67.99; H, 5.37. Found:

C, 67.90; H, 5.65. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.7% purity, m/z = 301.17 ($[M+H]^+$).

4.2.9.12. Methyl 6-(hex-5-ynyloxy)-2-oxo-2*H*-chromene-3-carboxylate (37). Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**14**, 440 mg) was reacted with 5-hexyn-1-ol (294 mg) and DEAD. The residue was recrystallized from MeOH (30 mL) to give the product as pale green needles (442 mg, 74%): mp 124–125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, ^3J = 7.3 Hz, CH_2), 1.82 (m, 2H, CH_2), 2.23 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.1 Hz, CH_2CCH), 2.76 (t, 1H, ^4J = 2.6 Hz, CH), 3.82 (s, 3H, CH_3), 4.03 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.32 (dd, 1H, ^4J = 2.8 Hz, ^3J = 8.8 Hz, 7-H), 7.36 (d, H, ^3J = 9.2 Hz, 8-H), 7.46 (d, H, ^4J = 2.9 Hz, 5-H), 8.69 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.7, 27.8, 52.5, 67.9, 71.5, 84.4, 112.8, 117.4, 117.7, 118.3, 122.8, 148.9, 149.1, 155.2, 156.2, 163.3. Anal. Calcd for $C_{16}H_{14}O_5$: C, 67.99; H, 5.37. Found: C, 67.52; H, 5.56. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.5% purity, m/z = 301.22 ($[M+H]^+$).

4.2.9.13. 3-(4-Methoxybenzoyl)-7-(hex-5-ynyloxy)-2*H*-chromen-2-one (38). 7-Hydroxy-3-(4-methoxybenzoyl)-2*H*-chromen-2-one (**16**, 592 mg) was reacted with 5-hexyn-1-ol (294 mg) and DEAD. The residue was recrystallized from MeOH (100 mL) to give the product as yellow crystals (475 mg, 63%): mp 145–146 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.62 (app quint, 2H, CH_2), 1.84 (m, 2H, CH_2), 2.25 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.77 (t, 1H, ^4J = 2.9 Hz, CH), 3.85 (s, 3H, CH_3), 4.15 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.00 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.7 Hz, 6-H), 7.04 (d, 2H, ^3J = 8.9 Hz, 2′,6′-H), 7.06 (d, 1H, ^4J = 2.2 Hz, 8-H), 7.74 (d, 1H, ^3J = 8.9 Hz, 5-H), 7.86 (d, 2H, ^3J = 8.9 Hz, 3′,5′-H), 8.28 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.6, 27.6, 55.8, 68.2, 71.5, 84.4, 101.2, 111.9, 113.5, 114.1, 123.0, 129.3, 131.0, 132.1, 145.4, 156.4, 158.5, 163.3, 163.8, 190.3. Anal. Calcd for $C_{23}H_{20}O_5$: C, 73.39; H, 5.36. Found: C, 73.24; H, 5.56. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.9% purity, m/z = 377.30 ($[M+H]^+$).

4.2.9.14. 3-(4-Methoxybenzoyl)-6-(hex-5-ynyloxy)-2*H*-chromen-2-one (39). 6-Hydroxy-3-(4-methoxybenzoyl)-2*H*-chromen-2-one (**17**, 592 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was recrystallized from MeOH (30 mL) to give the product as yellow crystals (348 mg, 46%): mp 117–118 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.61 (app quint, 2H, ^3J = 7.2 Hz, CH_2), 1.82 (m, 2H, CH_2), 2.23 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.76 (t, 1H, ^4J = 2.5 Hz, CH), 3.86 (s, 3H, CH_3), 4.04 (t, 2H, ^3J = 6.4 Hz, CH_2–O), 7.05 (d, 2H, ^3J = 9.2 Hz, arom.H), 7.30 (dd, 1H, ^4J = 2.9 Hz, ^3J = 9.0 Hz, 7-H), 7.38 (d, 2H, ^4J = 2.8 Hz, 5-H), 7.41 (d, 1H, ^3J = 9.1 Hz, 8-H), 7.90 (d, 1H, ^3J = 9.2 Hz, arom.H), 8.24 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.6, 24.7, 27.8, 55.8, 68.9, 71.5, 84.4, 112.4, 114.2, 117.5, 118.8, 121.5, 127.2, 128.9, 132.2, 144.2, 148.5, 155.3, 158.3, 164.0, 190.2. Anal. Calcd for $C_{22}H_{20}O_5 \times 0.5$ MeOH: C, 71.93; H, 5.65. Found: C, 71.85; H, 5.65. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 95.9% purity, m/z = 377.23 ($[M+H]^+$).

4.2.9.15. *N*-(2-Oxo-7-(hex-5-ynyloxy)-2*H*-chromen-3-yl)acetamide (40). *N*-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (**18**, 438 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was recrystallized from MeOH (20 mL) to give the product as pale brown needles (346 mg, 58%): mp 146–147 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, CH_2), 1.81 (m, 2H, CH_2), 2.13 (s, 3H, CH_3), 2.23 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.75 (t, 1H, ^4J = 2.5 Hz, CH), 4.06 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.93 (dd, 2H, ^4J = 2.6 Hz, ^3J = 8.7 Hz, 6-H), 6.96 (d, H, ^4J = 2.6 Hz, 8-H), 7.58 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.52 (s, 1H, 4-H), 9.56 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 23.9, 24.7, 27.7, 67.8, 71.5, 84.4, 101.1, 112.7, 113.2, 121.9, 125.2, 128.9, 151.4, 157.9, 160.2, 170.0. Anal. Calcd for $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.34; H, 5.78; N, 4.78. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.1% purity, m/z = 300.24 ($[M+H]^+$).

4.2.9.16. *N*-(2-Oxo-6-(hex-5-ynyloxy)-2*H*-chromen-3-yl)acetamide (41). *N*-(6-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (**19**, 438 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was recrystallized from MeOH (40 mL) to give the product as white solid (468 mg, 78%): mp 158–160 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, CH_2), 1.81 (m, 2H, CH_2), 2.13 (s, 3H, CH_3), 2.23 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.75 (t, 1H, ^4J = 2.5 Hz, CH), 4.02 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 7.05 (dd, 2H, ^4J = 2.8 Hz, ^3J = 9.0 Hz, 7-H), 7.27 (d, H, ^4J = 2.9 Hz, 5-H), 7.28 (d, 1H, ^3J = 9.1 Hz, 8-H), 8.57 (s, 1H, 4-H), 9.67 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.1, 24.7, 27.8, 67.7, 71.5, 84.4, 111.0, 116.9, 117.6, 120.3, 123.6, 124.9, 144.0, 155.5, 157.7, 170.3. Anal. Calcd. For $C_{17}H_{17}NO_4$: C, 68.21; H, 5.72; N, 4.68. Found: C, 68.06; H, 5.66; N, 4.79. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.4% purity, m/z = 300.19 ($[M+H]^+$).

4.2.9.17. 7-(Hex-5-ynyloxy)-2*H*-chromen-2-one (42). Umbelliferone (**21**, 324 mg) was reacted with 5-hexyn-1-ol (294 mg) and DEAD. The residue was recrystallized from EtOAc/*n*-hexane (10/30 mL) to give the product as colorless solid (349 mg, 71%): mp 82–83 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, CH_2), 1.82 (m, 2H, CH_2), 2.23 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.76 (t, 1H, ^4J = 2.5 Hz, CH), 4.08 (t, 2H, ^3J = 6.4 Hz, CH_2–O), 6.26 (d, 1H, ^3J = 9.5 Hz, 3-H), 6.93 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.7 Hz), 6.97 (d, H, ^4J = 2.2 Hz, 5-H), 7.60 (d, 1H, ^3J = 8.6 Hz, 6-H), 7.96 (d, 1H, ^3J = 9.5 Hz, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.7, 27.7, 67.9, 71.5, 84.4, 101.3, 112.4, 112.5, 112.8, 129.6, 144.4, 155.6, 160.4, 162.0. Anal. Calcd for $C_{15}H_{14}O_3$: C, 74.36; H, 5.82. Found: C, 74.36; H, 6.04. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 243.31 ($[M+H]^+$).

4.2.9.18. 4-Methyl-7-(hex-5-ynyloxy)-2*H*-chromen-2-one (43). 7-Hydroxy-4-methyl-2*H*-chromen-2-one (**22**, 352 mg) was reacted with 5-hexyn-1-ol (294 mg) and DEAD. The residue was recrystallized from MeOH (15 mL) to give the product as colorless needles (350 mg, 68%): mp 75–76 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.60 (app quint, 2H, CH_2), 1.82 (m, 2H, CH_2), 2.23 (dt, 2H, ^4J = 2.9 Hz, ^3J = 7.1 Hz, CH_2–CCH), 2.38 (d, 3H, ^4J = 1.3 Hz, CH_3), 2.76 (t, 1H, ^4J = 2.5 Hz, CH), 4.10 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.18 (d, 1H, ^4J = 1.3 Hz, 3-H), 6.93–6.95 (m, 2H, 6-H, 8-H), 7.65–7.67 (d, H, ^3J = 7.3 Hz, 5-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 18.2, 24.7, 27.7, 67.9, 71.5, 84.4, 101.3, 111.2, 112.6, 113.2, 126.6, 153.5, 154.9, 160.3, 161.9. Anal. Calcd for $C_{16}H_{16}O_3$: C, 74.98, H, 6.29. Found: C, 74.94; H, 6.51. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.0% purity, m/z = 257.39 ($[M+H]^+$).

4.2.9.19. 7-(Hex-5-ynyloxy)-3-(4-methoxyphenyl)-2*H*-chromen-2-one (44). 7-Hydroxy-3-(4-methoxyphenyl)-2*H*-chromen-2-one (**20**, 536 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was recrystallized from MeOH (30 mL) to give to product a pale green platelets (552 mg, 79%): mp 127–128 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.58–1.64 (m, 2H, CH_2), 1.83 (m, 2H, CH_2), 2.24 (dt, 2H, ^4J = 2.6 Hz, ^3J = 6.9 Hz, CH_2), 2.76 (t, 1H, ^4J = 2.5 Hz, CH), 3.79 (s, 3H, CH_3), 4.10 (t, 2H, ^3J = 6.3 Hz, OCH_2),

6.96 (dd, H, ^4J = 2.5 Hz, ^3J = 8.5 Hz, 6-H), 6.99–7.00 (m, 3H, arom.H), 7.64–7.67 (m, 3H, arom.H), 8.11 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.5, 24.7, 27.7, 55.3, 67.9, 71.5, 84.4, 100.8, 113.0, 113.3, 113.8, 122.9, 127.3, 129.6, 129.7, 139.5, 154.7, 159.4, 160.2, 161.6. Anal. Calcd for $C_{22}H_{20}O_4$: C, 75.84; H, 5.79. Found: C, 76.07; H, 5.83. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.5% purity, m/z = 349.13 ([M+H]$^+$).

4.2.9.20. Methyl 7-(3-fluorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxylate (45). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with (3-fluorophenyl)methanol (252 mg, 2.0 mmol) and DEAD. The residue was recrystallized from MeOH (80 mL) to give the product as white needles (456 mg, 70%): mp 188–189 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.78 (s, 3H, CH_3), 5.26 (s, 2H, CH_2), 7.06–7.09 (m, 2H), 7.14–7.18 (m, H), 7.29–7.30 (m, 1H), 7.41–7.46 (m, 1H), 7.83 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.71 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.3, 69.4, 101.4, 111.9, 113.4, 113.9, 114.6 (d, $^2J_{C,F}$ = 21.8 Hz), 115.1 (d, $^2J_{C,F}$ = 21.8 Hz), 123.9, 130.7 (d, $^3J_{C,F}$ = 8.2 Hz), 131.9, 139.0 (d, $^3J_{C,F}$ = 7.4 Hz), 149.5, 156.3, 157.0, 162.3 (d, $^1J_{C,F}$ = 243 Hz), 163.5, 163.7. Anal. Calcd for $C_{18}H_{13}FO_5$: C, 65.85; H, 3.99. Found: C, 65.53; H, 4.04. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.7% purity, m/z = 329.36 ([M+H]$^+$).

4.2.9.21. Methyl 6-(3-chlorobenzyloxy)-2-oxo-2*H*-chromene-3-carboxylate (46). Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**14**, 440 mg) was reacted with (3-chlorophenyl)methanol (426 mg) and DIAD. The residue was recrystallized from MeOH (90 mL) to give the product as light green needles (350 mg, 51%): mp 167–168 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.82 (s, 3H, CH_3), 5.16 (s, 2H, CH_2), 7.37–7.43 (m, 5H, arom.H), 7.53 (br s, 1H, arom.H), 7.55 (d, 1H, ^4J = 2.6 Hz, 5-H), 8.69 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 52.5, 69.1, 113.4, 117.5, 117.9, 118.3, 123.0, 126.5, 127.6, 128.1, 130.5, 133.3, 139.2, 148.7, 149.4, 154.6, 156.1, 163.3. Anal. Calcd for $C_{18}H_{13}ClO_5$: C, 62.71; H, 3.80. Found: C, 62.43; H, 3.82. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 345.27 ([M+H]$^+$).

4.2.9.22. Methyl 7-(4-chlorophenethoxy)-2-oxo-2*H*-chromene-3-carboxylate (47). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with 2-(4-chlorophenyl)ethanol (470 mg) and DIAD. The residue was recrystallized from MeOH (35 mL) to give the product as white needles (496 mg, 69%): mp 143–145 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.06 (t, 2H, ^3J = 6.6 Hz, CH_2), 3.80 (s, 3H, CH_3), 4.34 (t, 2H, ^3J = 7.0 Hz, O-CH_2), 6.97 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.8 Hz, 6-H), 7.02 (d, 1H, ^4J = 2.6 Hz, 8-H), 7.36 (m, 4H, arom.H), 7.80 (d, 1H, ^3J = 8.9 Hz, 5-H), 8.72 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 33.9, 52.3, 68.9, 101.0, 111.6, 113.2, 113.7, 128.4, 131.0, 131.2, 131.8, 137.1, 149.5, 156.3, 157.1, 163.5, 164.0. Anal. Calcd for $C_{19}H_{15}ClO_5$: C, 63.61; H, 4.21. Found: C, 63.26; H, 4.37. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.8% purity, m/z = 359.13 ([M+H]$^+$).

4.2.9.23. Methyl 6-(4-chlorophenethoxy)-2-oxo-2*H*-chromene-3-carboxylate (48). Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**14**, 440 mg) was reacted with 2-(4-chlorophenyl)ethanol (470 mg) and DIAD. The residue was recrystallized from MeOH (30 mL) to give the product as light green needles (249 mg, 35%): mp 142–143 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.05 (t, 2H, ^3J = 7.0 Hz, CH_2), 3.82 (s, 3H, CH_3), 4.22 (t, 2H, ^3J = 7.0 Hz, O–CH_2), 7.30 (dd, 1H, ^4J = 2.9 Hz, ^3J = 8.8 Hz, 6-H), 7.36 (m, 5H, arom.H), 7.48 (d, 1H, ^4J = 2.9 Hz, 5-H), 8.68 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 34.1, 52.5, 68.7, 112.9, 117.4, 117.8, 118.3, 122.9, 128.4, 131.0, 131.2, 137.3, 148.9, 149.2, 154.9, 156.2, 163.3. Anal. Calcd for $C_{19}H_{15}ClO_5$: C, 63.61; H, 4.21. Found: C, 63.27; H, 4.46. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 359.12 ([M+H]$^+$).

4.2.9.24. *N*-(7-(3-Chlorobenzyloxy)-2-oxo-2*H*-chromen-3-yl)acetamide (49). *N*-(7-Hydroxy-2-oxo-2*H*-chromen-3-yl)acetamide (**18**, 441 mg, 2 mmol) was reacted with 3-chloro benzylalcohol (427 mg) and DIAD. The residue was recrystallized from MeOH/H_2O (100 mL, 9/1) to give the product as pale brown needles (136 mg, 20%): mp 218–219 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.13 (s, 3H, CH_3), 5.21 (s, 2H, CH_2), 7.02 (dd, 2H, ^4J = 2.5 Hz, ^3J = 8.7 Hz, 6-H), 7.07 (d, H, ^4J = 2.5 Hz, 8-H), 7.39–7.44 (m, 3H, arom.H), 7.54 (s, 1H, arom.H), 7.62 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.53 (s, 1H, 4-H), 9.58 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 24.0, 69.0, 101.7, 113.2, 113.5, 122.2, 124.9, 126.5, 127.6, 128.1, 129.0, 130.6, 133.3, 139.1, 151.3, 157.8, 159.6, 170.0. Anal. Calcd for $C_{18}H_{14}ClNO_4 \times 0.6\ H_2O$: C, 60.97; H, 4.32; N, 3.95. Found: C, 60.98; H, 4.16; N, 3.91. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 95.7% purity, m/z = 344.18 ([M+H]$^+$).

4.2.9.25. (*E*)-Methyl 7-(3,7-dimethylocta-2,6-dienyloxy)-2-oxo-2*H*-chromene-3-carboxylate (50). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with geraniol (463 mg) and DEAD. The residue was purified by column chromatography using petroleum ether/EtOAc (3:1) as eluent. The product was obtained colorless powder (232 mg, 33%): mp 81–82 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.55 (d, 3H, ^4J = 0.6 Hz, CH_3), 1.59 (d, 3H, ^4J = 1.0 Hz, CH_3), 1.72 (d, 3H, ^4J = 1.3 Hz, CH_3), 2.05 (m, 4H, 2 × CH_2) 3.80 (s, 3H, OCH_3), 4.69 (d, 2H, ^3J = 6.6 Hz, OCH_2), 5.04 (m, 1H, CH), 5.44 (m, 1H, CH), 6.98 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.5 Hz, 6-H), 7.00 (d, 1H, ^4J = 2.6 Hz, 8-Hv), 7.82 (d, H, ^3J = 8.5 Hz, 5-H), 8.72 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 16.5, 17.7, 25.5, 25.9, 52.3, 65.6, 101.0, 111.5, 113.0, 114.0, 118.8, 123.8, 131.2, 131.7, 141.8, 149.6, 156.4, 157.1, 163.5, 164.2. Anal. Calcd for $C_{14}H_{14}O_6$: C, 70.77; H, 6.79. Found: C, 70.45; H, 6.88. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.9% purity, m/z = 357.40 ([M+H]$^+$).

4.2.9.26. (E)-Methyl 6-(3,7-dimethylocta-2,6-dienyloxy)-2-oxo-2*H*-chromene-3-carboxylate (51). Methyl 6-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**14**, 440 mg) was reacted with geraniol (463 mg) and DEAD. The residue was purified by column chromatography using petroleum ether/EtOAc (3:1) as eluent. The product was obtained yellow syrup (388 mg, 54%): ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.55 (s, 3H, CH_3), 1.60 (d, 3H, ^4J = 1.0 Hz, CH_3), 1.71 (d, 3H, ^4J = 1.3 Hz, CH_3), 2.03–2.08 (m, 4H, 2 × CH_2) 3.82 (s, 3H, OCH_3), 4.58 (d, 2H, ^3J = 6.6 Hz, OCH_2), 5.03–5.06 (m, 1H, CH), 5.42–5.45 (m, 1H, CH), 7.32 (dd, 1H, ^4J = 2.9 Hz, ^3J = 9.2 Hz, 7-H), 7.36 (d, 1H, ^3J = 8.8 Hz, 8-H), 7.46 (d, H, ^4J = 2.9 Hz, 5-H), 8.69 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 16.5, 17.7, 25.5, 25.9, 52.5, 65.2, 113.2, 117.3, 117.7, 118.3, 119.3, 123.0, 123.8, 131.1, 141.1, 148.9, 149.1, 155.0, 156.2, 163.3. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{21}H_{24}O_5Na$: 379.1516, found: 379.1522. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.4% purity, m/z = 356.99 ([M+H]$^+$).

4.2.9.27. Methyl 2-oxo-7-(hexyloxy)-2*H*-chromene-3-carboxylate (52). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with hexan-1-ol (252 mg) and DIAD. The residue was recrystallized from MeOH (20 mL) to give the product as colorless needles (434 mg, 71%): mp 104–105 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.86 (t, 3H, ^3J = 7.0 Hz, CH_3),

1.28–1.31 (m, 4H, CH_2), 139–1.42 (m, 2H, CH_2), 1.72 (q, 2H, ^3J = 6.6 Hz, CH_2), 3.80 (s, 3H, CH_3), 4.09 (t, 2H, ^3J = 6.6 Hz, CH_2–O), 6.96–6.98 (m, 2H, 6-H, 8-H), 7.80 (d, H, ^3J = 8.9 Hz, 5-H), 8.71 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.0, 22.1, 25.1, 28.4, 31.0, 52.3, 68.8, 100.8, 111.4, 113.0, 113.7, 131.8, 149.6, 156.3, 157.2, 163.5, 164.4. Anal. Calcd for ($C_{17}H_{20}O_5$) C, 67.09; H, 6.62. Found C, 67.00; H, 6.56. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 98.7% purity, m/z = 305.21 ([M+H]$^+$).

4.2.9.28. 7-(Hex-5-ynyloxy)-3,4-dihydroquinolin-2(1*H*)-one (55). 7-Hydroxy-3,4-dihydroquinolin-2(1*H*)-one (**23**, 326 mg) was reacted with 5-hexyn-1-ol (294 mg) and DIAD. The residue was purified by column chromatography with petroleum ether/EtOAc (1:1) to obtain a white solid (182 mg, 37%): mp 84–86 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.54–1.60 (m, 2H, CH_2), 1.72–1.79 (m, 2H, CH_2), 2.21 (dt, 2H, ^4J = 2.5 Hz, ^3J = 7.3 Hz, CH_2), 2.38–2.41 (m, 2H, CH_2), 2.75 (t, 1H, ^4J = 7.3 Hz, CH), 2.77.278 (m, 2H, CH_2), 3,89 (t, 2H, ^3J = 6.3 Hz, CH_2), 6.42 (d, 1H, ^4J = 2.5 Hz, 8-H), 6.46 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.3 Hz, 6-H), 7.02 (d, 1H, ^3J = 8.2 Hz, 8-H), 9.92 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 17.6, 24.1, 24.8, 27.9, 30.9, 67.1, 71.5, 84.4, 101.9, 107.7, 115.7, 128.5, 139.3, 158.0, 170.4. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{15}H_{17}NO_2Na$: 266.1151, found: 266.1159. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 95.7% purity, m/z = 244.32 ([M+H]$^+$).

4.2.9.29. Methyl 7-(2-bromoethoxy)-2-oxo-2*H*-chromene-3-carboxylate (56). Methyl 7-hydroxy-2-oxo-2*H*-chromene-3-carboxylate (**13**, 440 mg) was reacted with 2-bromoethanol (0.38 g, 0.21 mL) and DEAD. The residue was recrystallized from MeOH (40 mL) and obtained as white needles (535 mg, 82%): mp 169–170 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.80 (s, 3H, CH_3), 3.84 (t, 2H, ^3J = 5.4 Hz, CH_2), 4.48 (t, 2H, ^3J = 5.4 Hz, CH_2), 7.03 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.5 Hz, 6-H), 7.05 (d, 1H, ^4J = 2.5 Hz, 8-H), 7.84 (d, H, ^3J = 8.5 Hz, 5-H), 8.74 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6, 30 °C) δ 31.0, 52.3, 68.8, 101.1, 111.9, 113.5, 113.7, 131.9, 149.5, 156.3, 157.0, 163.5, 163.5. Anal. Calcd for $C_{13}H_{11}BrO_5$: C, 47.73; H, 3.39. Found: C, 48.10; H, 3.48. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.5% purity, m/z = 329.14 ([M+H]$^+$).

4.2.9.30. 7-(2-Bromoethoxy)-2*H*-chromen-2-one (57). Umbelliferone (**21**, 324 mg) was reacted with 2-bromoethanol (0.38 g, 0.218 mL) and DEAD. The residue was purified by column chromatography (petroleum ether/EtOAc 3:1) followed by recrystallisation from MeOH (20 mL) to obtain colorless needles (330 mg, 62%): mp 130–132 °C (lit. mp 130.5 °C);[50] ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.82 (t, 2H, ^3J = 5.7 Hz, CH_2), 4.43 (t, 2H, ^3J = 5.4 Hz, CH_2), 6.29 (d, 1H, ^3J = 9.5 Hz, 3-H), 6.97 (dd, 1H, ^4J = 2.6 Hz, ^3J = 8.5 Hz, 6-H), 7.01 (d, 1H, ^4J = 2.6 Hz, 8-H), 7.84 (d, H, ^3J = 8.5 Hz, 5-H), 7.98 (d, 1H, ^3J = 9.5 Hz, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 31.1, 68.5, 101.6, 112.9, 112.9, 129.7, 144.4, 155.5, 160.3, 161.1. Anal. Calcd for $C_{11}H_9BrO_3$: C, 49.10; H, 3.37. Found: C, 49.08; H, 3.45. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 268.98 ([M+H]$^+$).

4.2.10. Preparation of compounds 30–32

4.2.10.1. 2-Oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carboxylic acid (30). Methyl 2-oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carboxylate (**26**, 286 mg, 1.0 mmol) was dissolved in THF (12 mL) and water (18 mL). Lithium hydroxide monohydrate (252 mg, 6.0 mmol) was added and the solution was stirred at room temperature for 2 h. The solution was filtrated and the filtrate was cooled on an ice bath before 10% HCl (10 mL) was added. The product precipitated and was filtered off, washed with H_2O (10 mL) to give a light green solid (252 mg, 93%): mp 196–198 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.92 (app quint, 2H, ^3J = 6.6 Hz, 2-CH_2), 2.33 (dt, 2H, ^3J = 7.1 Hz, ^4J = 2.9 Hz, 3-CH_2), 2.80 (t, 1H, ^4J = 2.9 Hz, CH), 4.18 (t, 2H, ^3J = 6.3 Hz, CH_2–O), 6.99 (dd, 1H, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 6-H), 7.02 (d, H, ^4J = 2.6 Hz, 8-H), 7.81 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.70 (s, 1H, 4-H), 12.94 (br s, 1H, OH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.5, 27.6, 67.3, 71.9, 83.6, 100.9, 111.8, 113.7, 114.0, 131.7, 149.1, 157.0, 157.4, 164.0, 164.2. Anal. Calcd for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44. Found: C, 65.86; H, 4.47. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.0% purity, m/z = 273.21 ([M+H]$^+$).

4.2.10.2. *N*-(4-Fluorophenyl)-2-oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carboxamide (31). 2-Oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carboxilic acid (**30**, 272 mg, 1.0 mmol), 4-Fluoro-aniline (111 mg, 1.0 mmol) and DIPEA (0.26 g, 0.36 mL, 2.0 mmol) were dissolved in DMF (10 mL). HATU (456 mg, 1.2 mmol) was added and the solution was stirred at room temperature. After 1 h a precipitate occurred and the solution was stirred for an additional hour. After evaporation in vacuo the residue was suspended in EtOAc (100 mL). The organic phase was washed with 10% citric acid solution, saturated $NaHCO_3$ and brine. After drying (Na_2SO_4) the solvent was removed under reduced pressure. The residue was recrystallized from MeOH (30 mL) to give the product as a light green solid (135 mg, 37%): mp 189–191 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.93 (app quint, 2H, ^3J = 6.3 Hz, 2-CH_2), 2.33 (dt, 2H, ^3J = 7.1 Hz, ^4J = 2.5 Hz, 3-CH_2), 2.81 (t, 1H, ^4J = 2.8 Hz, CH), 4.21 (t, 2H, ^3J = 6.0 Hz, CH_2–O), 7.07 (dd, 1H, ^3J = 8.7 Hz, ^4J = 2.2 Hz, 6-H), 7.15 (d, H, ^4J = 2.6 Hz, 8-H), 7.19–7.22 (m, 2H, arom.H), 7.73–7.76 (m, 2H, arom.H), 7.92 (d, 1H, ^3J = 8.5 Hz, 5-H), 8.89 (s, 1H, 4-H), 10.62 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.5, 27.5, 67.4, 71.9, 83.6, 101.0, 112.4, 114.2, 115.6, 115.6, 115.8, 121.9, 122.0, 131.9, 134.6, 148.2, 156.4, 157.7, 159.6, 160.2, 161.1, 164.0. HRMS-ESI m/z [M+Na]$^+$ calcd for $C_{21}H_{16}FNO_4$Na: 388.0956, found: 388.0952. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.7% purity, m/z = 366.19 ([M+H]$^+$).

4.2.10.3. *tert*-Butyl 4-(2-oxo-7-(pent-4-ynyloxy)-2*H*-chromene-3-carbonyl)piperazine-1-carboxylate (32). 2-Oxo-7-(pent-4-ynyloxy)-2H-chromene-3-carboxylic acid (**30**, 136 mg, 0.5 mmol) was dissolved in DMF (10 mL) and cooled to 0 °C. 1-Boc-piperazine (95 mg, 0.6 mmol) and DIPEA (0.13 g, 0.18 mL, 1.0 mmol) were added. HATU (228 mg, 0.6 mmol) was added to the mixture and the solution was stirred for 3 h at room temperature. After evaporation, the residue was dissolved in EtOAc (50 mL). The organic phase was washed with 10% citric acid (3 × 50 mL), saturated $NaHCO_3$ (3 × 50 mL) and brine (50 mL). The organic extract was dried over Na_2SO_4, filtrated and purified by column chromatography with petroleum ether/EtOAc 1:1 to obtain a white solid (179 mg, 81%): mp 117–119 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.40 (s, 9H, $(CH_3)_3$), 1.92 (app. quint, 2H, ^3J = 6.6 Hz, 2-CH_2), 2.34 (dt, 2H, ^3J = 7.3 Hz, ^4J = 2.5 Hz, 3-CH_2), 2.81 (t, 1H, ^4J = 2.6 Hz, 5-CH), 3.31–3.37 (m, 6H, CH_2), 3.56 (br s, 2H, CH_2), 4.17 (t, 2H,, ^3J = 6.0 Hz, 1-CH_2), 7.00 (d, 1H, ^3J = 8.7 Hz, ^4J = 2.5 Hz, 6-H), 7.04 (dd, 1H, ^4J = 2.5 Hz, 8-H), 7.68 (d, H, ^3J = 8.5 Hz, 5-H), 8.12 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.5, 27.6, 28.2, 41.4, 46.3, 67.1, 71.9, 79.4, 83.6, 101.3, 112.1, 113.3, 120.9, 130.3, 143.1, 154.0, 155.7, 158.0, 162.5, 163.6. Anal. Calcd for $C_{24}H_{28}N_2O_6$: C, 65.44; H, 6.41; N, 6.36. Found: C, 65.05; H, 6.44; N, 6.17. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 97.7% purity, m/z = 441.50 ([M+H]$^+$).

4.2.11. Preparation of compounds 58, 59, 53, 54

4.2.11.1. Methyl 7-(2-(-2-nitro-*N*-(prop-2-ynyl)phenylsulfonamido)ethoxy)-2-oxo-2*H*-chromene-3-carboxylate (58). Propargylamine (1.65 g, 1.92 mL, 30 mmol) was dissolved in CH_2Cl_2 (50 mL). Triethylamine (6.06 g, 8.35 mL, 60 mmol) was added. A solution of 2-nitrobenzenesulfonyl chloride (6.65 g, 30 mmol) in CH_2Cl_2 (50 mL) was added dropwise over 30 min. After stirring for 4 h at room temperature, the solution was washed with 1 N HCl solution (100 mL), saturated $NaHCO_3$ (100 mL) and brine (100 mL). The organic phase was dried over Na_2SO_4, filtrated and evaporated in vacuo. The residue was purified by column chromatography using petroleum ether/EtOAc (3:1) as eluent. 2-Nitro-*N*-(prop-2-ynyl)benzenesulfonamide was obtained as a pale green solid (4.80 g, 67%): mp 95–96 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.03 (t, 1H, ^4J = 2.5 Hz, CH), 3.84 (d, 2H, ^4J = 2.5 Hz, CH_2), 7.84–7.87 (m, 2H, arom.H), 7.95–7.98 (m, 1H, arom.H), 8.02–8.06 (m, 1H, arom.H), 8.51 (br s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6) δ 32.2, 75.0, 79.2, 124.5, 130.1, 132.7, 133.1, 134.3, 147.8. Anal. Calcd for $C_9H_8N_2O_7S$: C, 45.00; H, 3.36; N, 11.66. Found: C, 44.90; H, 3.50; N, 11.59. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.0% purity, m/z = 258.38 ($[M+NH_4]^+$). 2-Nitro-*N*-(prop-2-ynyl)benzenesulfonamide (240 mg, 1.0 mmol), K_2CO_3 (276 mg, 2.0 mmol) and methyl 7-(2-bromoethoxy)-2-oxo-2*H*-chromene-3-carboxylate (**56**, 325 mg, 1.0 mmol) were suspended in DMF (10 mL). The reaction mixture was stirred for 2 d at room temperature. After evaporation in vacuo, the residue was dissolved in H_2O (50 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were dried over Na_2SO_4 and evaporated. The residue was purified by column chromatography using petroleum ether/EtOAc (1:1). The product was obtained as a white solid (300 mg, 62%): mp 169–172 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.23 (t, 1H, ^4J = 2.2 Hz, CH), 3.77 (t, 2H, ^3J = 5.4 Hz, CH_2), 3.80 (s, 3H, OCH_3), 4.31–4.33 (m, 4H, CH_2, CH_2), 6.87 (dd, 1H, ^4J = 2.2 Hz, ^3J = 8.7 Hz, 6-H), 6.95 (d, 1H, ^4J = 2.2 Hz, 8-H), 7.80 (d, 1H, ^3J = 8.8 Hz, 5-H), 7.81–7.84 (m, 1H, arom.H), 7.87–7.90 (m, 1H, arom.H), 7.96–7.98 (m, 1H, arom.H), 8.09–8.11 (m, 1H, arom.H), 8.72 (s, 1H, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.5, 46.0, 52.3, 66.3, 76.6, 77.6, 101.0, 111.8, 113.5, 113.6, 124.4, 130.4, 131.3, 131.8, 132.6, 135.0, 147.8, 149.5, 156.3, 157.0, 163.5. HRMS-ESI m/z $[M+Na]^+$ calcd for $C_{22}H_{18}N_2O_9SNa$: 509.0625, found: 509.0630. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 487.26 ($[M+H]^+$), 504.44 ($[M+NH_4]^+$).

4.2.11.2. 2-Nitro-*N*-(2-(2-oxo-2*H*-chromen-7-yloxy)ethyl)-*N*-(prop-2-ynyl)benzenesulfonamide (59). 2-Nitro-*N*-(prop-2-ynyl)benzenesulfonamide (240 mg, 1.0 mmol, prepared as noted in the aforementioned procedure), K_2CO_3 (553 mg, 4.0 mmol) and 7-(2-bromoethoxy)-2*H*-chromen-2-one (**57**, 267 mg, 1.0 mmol) were suspended in DMF (10 mL). The mixture was stirred for 4 d at room temperature. After evaporation in vacuo, the residue was dissolved in H_2O (50 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The organic layer was dried over Na_2SO_4, filtrated and evaporated. The residue was purified by column chromatography using petroleum ether/EtOAc (1:1). The product was obtained as a white solid (320 mg, 75%): mp 104–105 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.23 (t, 1H, ^4J = 2.6 Hz, CH), 3.76 (t, 2H, ^3J = 5.4 Hz, CH_2), 4.28 (t, 2H, ^3J = 5.4 Hz, CH_2), 4.33 (d, 2H, ^4J = 2.6 Hz, CH_2CCH), 6.28 (d, 1H, ^3J = 9.5 Hz, 3-H), 6.91 (d, 1H, ^4J = 2.2 Hz, 8-H), 7.06 (d, 2H, ^3J = 8.5 Hz, 5-H), 7.81–7.84 (m, 1H, arom.H), 7.87–7.90 (m, 1H, arom.H), 7.96 (d, 1H, ^3J = 9.5 Hz, 4-H), 7.96–7.98 (m, 1H, arom.H), 8.09–8.11 (m, 1H, arom.H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.5, 46.0, 66.0, 76.6, 77.6, 101.4, 112.7, 112.8, 124.4, 129.6, 130.4, 131.4, 132.5, 134.9, 144.4, 147.8, 155.4, 160.3, 161.1. HRMS-ESI m/z $[M+H]^+$ calcd for $C_{20}H_{17}N_2O_7S$: 429.0751, found: 429.0749. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 429.16 ($[M+H]^+$), 446.18 ($[M+NH_4]^+$).

4.2.11.3. Methyl 2-oxo-7-(2-(prop-2-ynylamino)ethoxy)-2*H*-chromene-3-carboxylate (53). Methyl 7-(2-(-2-nitro-*N*-(prop-2-ynyl)phenylsulfonamido)ethoxy)-2-oxo-2*H*-chromene-3-carboxylate (**58**, 260 mg, 0.53 mmol), K_2CO_3 (221 mg, 1.59 mmol) and thiophenol (0.12 g, 0.10 mL, 1.06 mmol) were stirred for 3 h in DMF (10 mL). After evaporation in vacuo, the residue was dissolved in H_2O (50 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtrated and evaporated. The residue was purified by column chromatography using EtOAc + 1% Et_3N as eluent. The product was obtained as white solid (109 mg, 68%). The product was finally lyophilized with HCl to obtain the salt: mp 233–234 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 3.42 (t, 2H, ^3J = 5.1 Hz, NCH_2), 3.71 (t, 1H, ^4J = 2.6 Hz, CH), 3.81 (s, 3H, CH_3), 3.98 (d, 2H, ^4J = 2.6 Hz, CH_2), 4.43 (t, 2H, ^3J = 5.4 Hz, CCH_2), 7.06 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.7 Hz, 6-H), 7.09 (d, 1H, ^4J = 2.2 Hz, 8-H), 7.88 (d, 1H, ^3J = 8.8 Hz, 5-H), 8.76 (s, 1H, 4-H), 9.61 (br s, 2H, NH_2); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.1, 45.0, 52.4, 64.4, 75.2, 79.7, 101.2, 112.1, 113.7, 131.9, 149.5, 156.3, 156.9, 163.2, 163.4. Anal. Calcd for $C_{16}H_{16}ClNO_5$: C, 56.90; H, 4.77; N, 4.15. Found: C, 56.71; H, 4.82; N, 4.06. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 100% purity, m/z = 302.17 ($[M+H]^+$).

4.2.11.4. 7-(2-(Prop-2-ynylamino)ethoxy)-2*H*-chromen-2-one (54). 2-Nitro-*N*-(2-(2-oxo-2*H*-chromen-7-yloxy)ethyl)-*N*-(prop-2-ynyl)benzenesulfonamide (**59**, 300 mg, 0.7 mmol), K_2CO_3 (290 mg, 2.1 mmol) and thiophenol (0.15 g, 0.16 mL, 1.4 mmol) were stirred for 6 h in DMF (10 mL). After evaporation in vacuo, the residue was solved in H_2O (50 mL) and the aqueous phase was extracted with EtOAc (3 × 50 mL). The organic layers were washed with brine (50 mL), dried over Na_2SO_4, filtrated and evaporated. The residue was purified by column chromatography using EtOAc + 1% Et_3N as eluent. The product was obtained as white solid (92 mg, 54%): mp 103–104 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.25 (br s, 1H, NH), 2.94 (t, 2H, ^3J = 5.4 Hz, CH_2), 3.05 (t, 1H, ^4J = 2.5 Hz, CH), 3.37 (d, 2H, ^4J = 2.5 Hz, CH_2), 4.13 (t, 2H, ^3J = 5.4 Hz, CH_2), 4.33 (d, 2H, ^4J = 2.6 Hz, CH_2CCH), 6.26 (d, 1H, ^3J = 9.8 Hz, 3-H), 6.94 (dd, 1H, ^4J = 2.5 Hz, ^3J = 8.7 Hz, 6-H), 6.97 (d, 2H, ^4J = 2.2 Hz, 8-H), 7.61 (d, 1H, ^3J = 8.5 Hz, 5-H), 7.97 (d, 1H, ^3J = 9.5 Hz, 4-H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 37.5, 46.7, 68.2, 73.8, 83.1, 101.4, 112.5, 112.6, 112.8, 129.6, 144.4, 155.5, 160.4, 161.9. Anal. Calcd for $C_{14}H_{13}NO_3$: C, 69.13; H, 5.39; N, 5.76. Found C, 68.89; H, 5.31; N, 5.58. LC–MS (ESI) (90% H_2O to 100% MeOH in 10 min, then 100% MeOH to 20 min, DAD 220–400 nm), 99.6% purity, m/z = 244.34 ($[M+H]^+$).

4.3. Procedure for log$D_{7.4}$ estimation

Log$D_{7.4}$ estimation was performed by an HPLC method as described.[41] HPLC grade acetonitrile was from VWR international (Leuven, Belgium). Atenolol was from Fagron (Barsbüttel, Germany), metoprolol tartrate from Synopharm (Barsbüttel, Germany), diltiazem hydrochloride from Hexal (Munich, Germany), labetalol hydrochloride from the EDQM (Strasbourg, France) and triphenylene from Alfa Aesar (Karlsruhe, Germany). Analytical HPLC was performed on a Jasco HPLC 2000 instrument with a Polaris C18-A column (2 mm I.D., 50 mm length, 3 μm particle size) from Varian Inc. (Lake Forest, CA, USA). A linear mobile phase gradient was used with 10 mM ammonium acetate (adjusted to pH

7.4 with ammonium hydroxide and acetic acid) as mobile phase A and acetonitrile as mobile phase B. The gradient table was as follows, 0 min/0% B, 2.5 min/95% B, 4.0 min/95% B, 4.1 min/0% B, 5.5 min/0% B. A flow rate of 0.8 mL/min and a column temperature of 40 °C were applied. UV detection wavelength was 254 nm. Solutions of test compounds in DMSO (0.5 mg/mL) were injected and measurements were performed in duplicate.

4.4. MAO inhibition assays

Recombinant human MAO-A and MAO-B enzymes expressed in baculovirus-infected insect cells were purchased from Sigma Aldrich (M7441, M7316). The assays were carried out at room temperature in 96-well plates in a final volume of 200 μL. Test compound (2 μL) dissolved in DMSO was pipetted into the 96-well plates. Enzyme solution (90 μL) in sodium phosphate buffer (50 mM, pH 7.4) was subsequently added, and the mixture was preincubated for 30 min at room temperature. To each well, 0.3 μg of recombinant human MAO-A, or 0.9 μg MAO-B, respectively, was added, followed by 90 μL of freshly prepared Amplex Red reagent (Invitrogen A12214), prepared according to the manufacturer's recommendation as follows: for each plate, 1 mg of Amplex Red, dissolved in 200 μL of DMSO and 100 μL of reconstituted horseradish peroxidase (HRP 200 U/mL, Sigma Aldrich P6782) were added to 9700 μL of sodium phosphate buffer (50 mM, pH 7.4). To each well, 20 μL of an aqueous solution (final concentration 150 μM) of *p*-tyramine (Alfa Aesar A12220) was added to start the enzymatic reaction, which was kept in the dark. The production of hydrogen peroxide produced resorufin which was monitored and quantified by a microplate fluorescence reader (PHERAstar BMG Labtech, excitation 544 nm, emission 590 nm) over 45 min. Data were analyzed using GRAPH PAD PRISM Version 4 (San Diego, CA, USA).

4.5. MAO-B reactivation experiment

Time-dependent activity measurements were performed using human MAO-B under assay conditions. Inhibitors **36** and **44** as well as two reference compounds were examined at concentrations approximately corresponding to their IC_{80} value. The enzyme/inhibitor mixture was preincubated for 1 h. The enzyme reaction was started by the addition of 10 μM *p*-tyramine. After 22 min, the substrate concentration was increased to a final concentration of 1 mM of *p*-tyramine. The reactivation of the enzyme was monitored by fluorescence measurements over a period of 5 h.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2014.01.046.

References and notes

1. Youdim, M. B.; Edmondson, D.; Tipton, K. F. *Nat. Neurosci.* **2006**, *7*, 295.
2. Geha, R. M.; Rebrin, I.; Chen, K.; Shih, J. C. *J. Biol. Chem.* **2001**, *276*, 9877.
3. Mallajosyula, J. K.; Kaur, D.; Chinta, S. J.; Rajagopalan, S.; Rane, A.; Nicholls, D. G.; Di Monte, D. A.; MacArthur, H.; Andersen, J. K. *PLoS ONE* **2008**, *3*, e1616.
4. Riederer, P.; Laux, G. *Exp. Neurobiol.* **2011**, *20*, 1.
5. Yoshida, S.; Rosen, T. C.; Meyer, O. G.; Sloan, M. J.; Ye, S.; Haufe, G.; Kirk, K. L. *Bioorg. Med. Chem.* **2004**, *12*, 2645–2652.
6. Hruschka, S.; Rosen, T. C.; Yoshida, S.; Kirk, K. L.; Fröhlich, R.; Wibbeling, B.; Haufe, G. *Bioorg. Med. Chem.* **2008**, *16*, 7148.
7. Yamada, M.; Yasuhara, H. *Neurotoxicology* **2004**, *25*, 215.
8. Müller, T. *Expert Rev. Neurother.* **2013**, *13*, 969.
9. Schapira, A. H. *CNS Drugs* **2011**, *1061*, 25.
10. Brühlmann, C.; Ooms, F.; Carrupt, P. A.; Testa, B.; Catto, M.; Leonetti, F.; Altomare, C.; Carotti, A. *J. Med. Chem.* **2001**, *44*, 3195.
11. Sterling, J.; Herzig, Y.; Goren, T.; Finkelstein, N.; Lerner, D.; Goldenberg, W.; Miskolczi, I.; Molnar, S.; Rantal, F.; Tamas, T.; Toth, G.; Zagyva, A.; Zekany, A.; Finberg, J.; Lavian, G.; Gross, A.; Friedman, R.; Razin, M.; Huang, W.; Krais, B.; Chorev, M.; Youdim, M. B.; Weinstock, M. *J. Med. Chem.* **2002**, *45*, 5260.
12. Bolea, I.; Juárez-Jiménez, J.; de Los Ríos, C.; Chioua, M.; Pouplana, R.; Luque, F. J.; Unzeta, M.; Marco-Contelles, J.; Samadi, A. *J. Med. Chem.* **2011**, *54*, 8251.
13. Samadi, A.; de Los Ríos, C.; Bolea, I.; Chioua, M.; Iriepa, I.; Moraleda, I.; Bartolini, M.; Andrisano, V.; Gálvez, E.; Valderas, C.; Unzeta, M.; Marco-Contelles, J. *Eur. J. Med. Chem.* **2012**, *52*, 251.
14. Viña, D.; Matos, M. J.; Yáñez, M.; Santana, L.; Uriarte, E. *Med. Chem. Commun.* **2012**, *3*, 213.
15. Petzer, J. P.; Steyn, S.; Castagnoli, K. P.; Chen, J. F.; Schwarzschild, M. A.; van der Schyf, C. J.; Castagnoli, N. *Bioorg. Med. Chem.* **2003**, *11*, 1299.
16. Stößel, A.; Schlenk, M.; Hinz, S.; Küppers, P.; Heer, J.; Gütschow, M.; Müller, C. E. *J. Med. Chem.* **2013**, *56*, 4580.
17. Frederick, R.; Robert, S.; Charlier, C.; de Ruyck, J.; Wouters, J.; Pirotte, B.; Masereel, B.; Pochet, L. *J. Med. Chem.* **2005**, *48*, 7592.
18. Zhao, H.; Donnelly, A. C.; Kusuma, B. R.; Brandt, G. E.; Brown, D.; Rajewski, R. A.; Vielhauer, G.; Holzbeierlein, J.; Cohen, M. S.; Blagg, B. S. *J. Med. Chem.* **2011**, *54*, 3839.
19. Stefanachi, A.; Favia, A. D.; Nicolotti, O.; Leonetti, F.; Pisani, L.; Catto, M.; Zimmer, C.; Hartmann, R. W.; Carotti, A. *J. Med. Chem.* **2011**, *54*, 1613.
20. Starcević, S.; Brozic, P.; Turk, S.; Cesar, J.; Rizner, T. L.; Gobec, S. *J. Med. Chem.* **2011**, *54*, 248.
21. Catto, M.; Pisani, L.; Leonetti, F.; Nicolotti, O.; Pesce, P.; Stefanachi, A.; Cellamare, S.; Carotti, A. *Bioorg. Med. Chem.* **2013**, *21*, 146.
22. Elsinghorst, P. W.; Härtig, W.; Goldhammer, S.; Grosche, J.; Gütschow, M. *Org. Biomol. Chem.* **2009**, *7*, 3940.
23. Cervi, A.; Aillard, P.; Hazeri, N.; Petit, L.; Chai, C. L.; Willis, A. C.; Banwell, M. G. *J. Org. Chem.* **2013**, *78*, 9876.
24. Behrenswerth, A.; Volz, N.; Toräng, J.; Hinz, S.; Bräse, S.; Müller, C. E. *Bioorg. Med. Chem.* **2009**, *17*, 2842.
25. Rempel, V.; Volz, N.; Hinz, S.; Karcz, T.; Meliciani, I.; Nieger, M.; Wenzel, W.; Bräse, S.; Müller, C. E. *J. Med. Chem.* **2012**, *55*, 7967.
26. Rempel, V.; Volz, N.; Gläser, F.; Nieger, M.; Bräse, S.; Müller, C. E. *J. Med. Chem.* **2013**, *56*, 4798.
27. Chen, G.; Yee, D. J.; Gubernator, N. G.; Sames, D. *J. Am. Chem. Soc.* **2005**, *127*, 4544.
28. Krysiak, J. M.; Kreuzer, J.; Macheroux, P.; Hermetter, A.; Sieber, S. A.; Breinbauer, R. *Angew. Chem., Int. Ed.* **2012**, *51*, 7035.
29. Albers, A. E.; Rawls, K. A.; Chang, C. J. *Chem. Commun.* **2007**, 4647.
30. Long, S.; Chen, L.; Xiang, Y.; Song, M.; Zheng, Y.; Zhu, Q. *Chem. Commun.* **2012**, 7164.
31. Matos, M. J.; Viña, D.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L. *Curr. Top. Med. Chem.* **2012**, *12*, 2210.
32. Patil, P. O.; Bari, S. B.; Firke, S. D.; Deshmukh, P. K.; Donda, S. T.; Patil, D. A. *Bioorg. Med. Chem.* **2013**, *21*, 2434.
33. Rendenbach-Müller, B.; Schlecker, R.; Traut, M.; Weifenbach, H. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1195.
34. Pisani, L.; Muncipinto, G.; Miscioscia, T. F.; Nicolotti, O.; Leonetti, F.; Catto, M.; Caccia, C.; Salvati, P.; Soto-Otero, R.; Mendez-Alvarez, E.; Passeleu, C.; Carotti, A. *J. Med. Chem.* **2009**, *52*, 6685.
35. Gnerre, C.; Catto, M.; Leonetti, F.; Weber, P.; Carrupt, P. A.; Altomare, C.; Carotti, A.; Testa, B. *J. Med. Chem.* **2000**, *43*, 4747.
36. Secci, D.; Carradori, S.; Bolasco, A.; Chimenti, P.; Yáñez, M.; Ortuso, F.; Alcaro, S. *Eur. J. Med. Chem.* **2011**, *46*, 4846.
37. Matos, M. J.; Terán, C.; Pérez-Castillo, Y.; Uriarte, E.; Santana, L.; Viña, D. *J. Med. Chem.* **2011**, *54*, 7127.
38. Chimenti, F.; Secci, D.; Bolasco, A.; Chimenti, P.; Bizzarri, B.; Granese, A.; Carradori, S.; Yáñez, M.; Orallo, F.; Ortuso, F.; Alcaro, S. *J. Med. Chem.* **2009**, *52*, 1935.
39. Jeong, S. H.; Han, X. H.; Hong, S. S.; Hwang, J. S.; Hwang, J. H.; Lee, D.; Lee, M. K.; Ro, J. S.; Hwang, B. Y. *Arch. Pharm. Res.* **2006**, *29*, 1119.
40. Carotti, A.; Carrieri, A.; Chimichi, S.; Boccalini, M.; Cosimelli, B.; Gnerre, C.; Carotti, A.; Carrupt, P. A.; Testa, B. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 3551.
41. Blättermann, S.; Peters, L.; Ottersbach, P. A.; Bock, A.; Konya, V.; Weaver, C. D.; Gonzalez, A.; Schröder, R.; Tyagi, R.; Luschnig, P.; Gäb, J.; Hennen, S.; Ulven, T.; Pardo, L.; Mohr, K.; Gütschow, M.; Heinemann, A.; Kostenis, E. *Nat. Chem. Biol.* **2012**, *8*, 631.
42. Matos, M. J.; Vazquez-Rodriguez, S.; Uriarte, E.; Santana, L.; Viña, D. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 4224.
43. Matos, M. J.; Viña, D.; Quezada, E.; Picciau, C.; Delogu, G.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 3268.
44. Matos, M. J.; Viña, D.; Picciau, C.; Orallo, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5053.

45. Matos, M. J.; Viña, D.; Janeiro, P.; Borges, F.; Santana, L.; Uriarte, E. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 5157.
46. Meiring, L.; Petzer, J. P.; Petzer, A. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5498.
47. Mertens, M. D.; Gütschow, M. *Chirality* **2013**, *25*, 957.
48. Kralj, L.; Hvala, A.; Jurij, S.; Golič, L.; Stanovnik, B. *J. Heterocycl. Chem.* **1997**, *34*, 247.
49. Kutubi, Md. S.; Hashimoto, T.; Kitamura, T. *Synthesis* **2011**, 1283.
50. Singh, H.; Warmuth, R. *Tetrahedron* **2002**, *58*, 1257.