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Thienochromene derivatives inhibit pSTAT1 and pSTAT5 signaling induced by cytokines

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ABSTRACT

Furanocoumarins, particularly furo[3,2-*c*]coumarins, are found in many natural products. However, coumarins annulated to a thiophene ring have received scarce attention to date in the literature. Therefore, we synthesized 4-oxo-4*H*-thieno[3,2-*c*]chromene derivatives and tested *in vitro* their anti-inflammatory activity. Anti-inflammatory potential of the synthesized compounds (**1**, **2**, **6–8**, **9a–e** and **10a–c**) has been evaluated by measuring various pSTAT (signal transducer and activator of transcription) inhibition within the JAK (Janus-activated family kinase)/STAT signaling pathway. Ethyl 7-hydroxy-4-oxo-4*H*-thieno[3,2-*c*]chromene-2-carboxylate (**7**) showed best inhibition properties on pSTAT5 in GM-CSF (Granulocyte-macrophage colony-stimulating factor)-triggered PBMC assay, with IC_{50} value of 5.0 μM.

JAK/STAT is one of the major pathways through which many cytokines exert and integrate their function. JAK is a family of non-receptor cytoplasmic tyrosine kinases, consisting of four members in mammals: JAK1, JAK2, JAK3 and TYK2.[1] When JAK kinases are activated by their respective effector molecules (ILs, IFNs, colony-stimulating factors, growth factors and hormones) they phosphorylate themselves (auto-phosphorylation) and/or adjacent molecules (*trans*-phosphorylation) including STAT which consists of seven members in mammals (STAT 1–4, 5a, 5b and 6), facilitating their translocation to the nucleus, direct binding to DNA and activation of the transcription process of target genes.[2] Although JAK family has only four members, different cytokines may act through the same JAK family member. Additionally, each cytokine may engage with more than one JAK family member to a varying degree facilitating different duration and signal intensity of STAT.[3] Exaggerated or protracted cytokine signaling has been implicated in many inflammatory and autoimmune diseases. Impaired delicate balance comes with a steep price of inflicting great bodily harm. Therefore, targeting cytokines or their receptors presents a favorable approach to treat diseases related to disturbed cytokine balance.

Abbreviations: DMSO, dimethyl sulfoxide; ELISA, enzyme-linked immunosorbent assay; FBS, fetal bovine serum; GM-CSF, granulocyte-macrophage colony-stimulating factor; IFNα, inerferon alpha; IL-2, interleukine-2; JAK, Janus kinase; PathScan, Intracellular Signaling Array Kit; PBMC, peripheral blood mononuclear cell; PBS, phosphate buffered saline; STAT, signal transducer and activator of transcription

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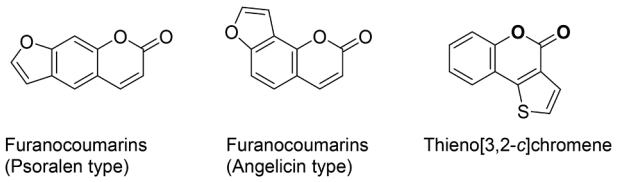


Fig. 1. Furano- and [2,3-*c*]thieno-coumarins.

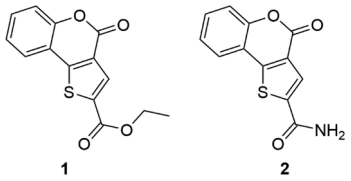


Fig. 2. Ethyl 4-oxo-4*H*-thieno[3,2-*c*]chromene-2-carboxylate (**1**) and 4-oxo-4*H*-thieno[3,2-*c*]chromene-2-carboxamide (**2**).

Derivatives of coumarin have attracted considerable attention due to their diverse range of biological activities[4] which include anti-inflammatory,[5] antimicrobial,[6] antiangiogenic,[7] antinociceptive,[8] anti-HIV,[9] antitumour,[10–11] and anticoagulant.[12] The last decade resulted in over 5000 publications on coumarin derivatives in PubMed database with a currently total of more than eleven thousand publications.

Furanocoumarins, particularly containing psorlen or angelicin scaffold (Fig. 1), are found in many natural products and have been seen to possess antifungicidal, insecticidal, insect antifeedant, anti-HIV and anticancer activities,[13–18] as well as anti-inflammatory properties through their ability to modulate inflammatory cells.[19]

In contrast, coumarins annulated to a thiophene ring, known as thieno[*c*]chromen-4-ones, have received scarce attention to date in the literature (Fig. 1).

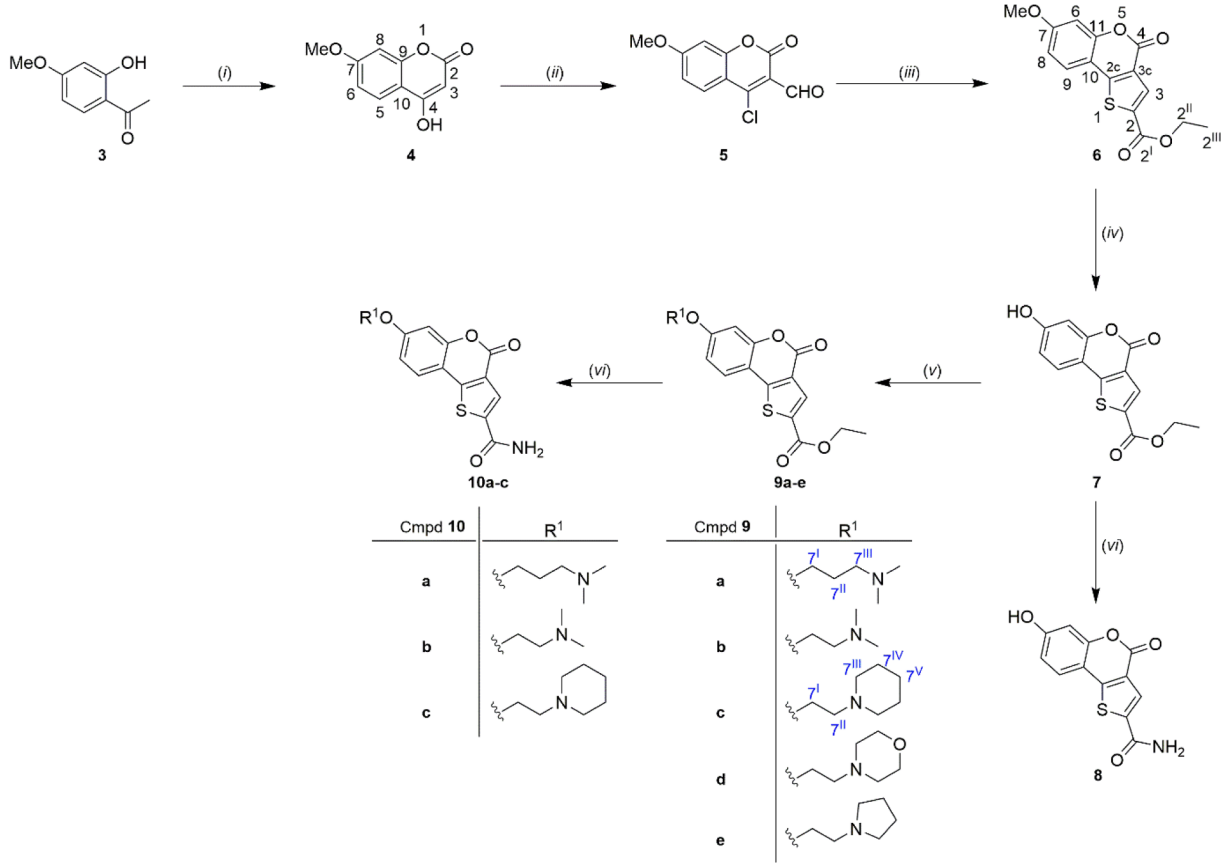
Thiophene and its derivatives represent a prevalent structural motif in many natural products (Gronowitz et al., 1985) and pharmaceutically active agents[20–29] with some of the top selling marketed drugs including thiophene as part of their structure (including clopidogrel, raloxifene, zileuton, and tiotropium bromide),[30] as well as many others, such as duloxetine (antidepressant), eprosartan (anti-hypertensive), rivaroxaban (oral anticoagulant) and olanzapine (antipsyhotic). An overview of the sulfur-containing FDA-approved drugs and their structures was recently published.[31]

Coumarins with additional rings condensed at the 3,4-position, have long been reported to exhibit potent biological activity.[32–33] Most of the literature concerning thieno[3,2-*c*]coumarin derivatives focusses on different protocols for their synthesis[34–48] with only a small portion of publications describing biological activities such as anticancer,[49–51] anticancer and antimicrobial,[52] antifungal activities[53,54] or those related to psoralen[55,56].

A number of thieno[3,2-*c*][1]benzopyran-4-ones synthesized by thermal thio-Claisen rearrangement demonstrated anti-inflammatory, antipyretic, and antiallergic potential[57–61].

In searching for novel anti-inflammatory compounds, we synthesized 4-oxo-4*H*-thieno[3,2-*c*]chromene derivatives with an aminoalkoxy chain linked at the benzene ring of the chromene scaffold.

To test the anti-inflammatory effect on various cytokine-triggered production of pSTAT within the JAK/STAT signaling in cells, PBMCs (peripheral blood mononuclear cells) stimulated with IFNα, IL-2 and GM-CSF were utilized, with levels of pSTAT1 and pSTAT5 production used as a readout in ELISA-based method.[62]



Scheme 1. Reagents and conditions: (a) NaH, Et_2CO_3, 100 °C, 3 h; (b) DMF, $POCl_3$, 90 °C, 12 h; (c) ethyl-2-mercaptoacetate, pyridine, Et_3N, 70 °C, 4 h; (d) 1 M BBr_3, CH_2Cl_2, rt, 5 h; (e) K_2CO_3, DMF, 100 °C, overnight; (f) NH_3, methanol, r.t., overnight.

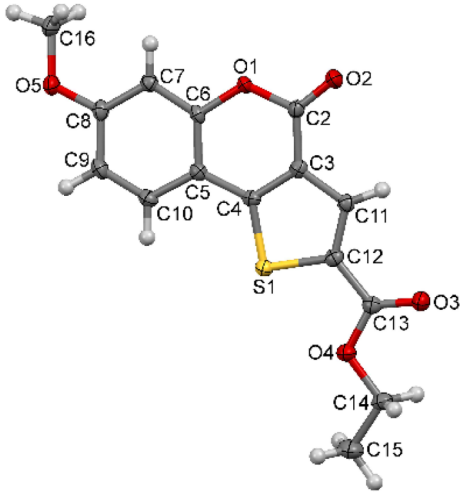


Fig. 3. Molecular structure of **6**, with the atom-numbering scheme. Displacement ellipsoids for non-hydrogen atoms are drawn at the 50% probability level.

Previously synthesized derivatives (**1** and **2**)[50] are shown in Fig 2. Inhibitory potential of these compounds on pSTAT1 and pSTAT5 production was also evaluated together with a newly synthesized compounds **6–8**, **9a-e** and **10a-c**.

Synthetic route to 4-oxo-4*H*-thieno[3,2-*c*]chromene scaffold and its aminoalkoxy derivatives of thiophene esters (**9a-e**) and thiophene amides (**10a-c**) is depicted in Scheme 1.

The parent compound (**3**) (1-(2-hydroxy-4-methoxyphenyl)ethan-1-one) is commercially available and prone to base-mediated cyclization (ring closure) with diethylcarbonate. Reaction of 4-hydroxycoumarin (**4**) under Vilsmeier–Haack reaction conditions using $POCl_3$/DMF afforded *β*-chlorovinyl aldehyde (4-chloro-3-formylcoumarin; 4-chlorocoumarin carboxaldehyde) (**5**), that is essentially a *gem*-activated alkene with a good leaving group (chlorine). In the consecutive step annulation of thiophene ring is achieved according to the well-established process[63–67] by reaction of *β*-chlorovinyl aldeyde **5** with ethyl 2-mercaptoacetate in the presence of triethylamine and pyridine (**6**). Fiesselmann thiophene synthesis has been well studied and it is known that the formation of thiophene to take place via a domino process involving Michael addition, followed by intramolecular Knoevenagel reaction in the presence of different bases. This mechanism has been well studied on ethyl thioglyconate and β-halo-α,β-unsaturated aldehydes.[68] Deprotection of methoxy group with 1 M BBr_3 in dichloromethane yielded **7** with a hydroxyl group in the 7-position. Five different aminoalkoxy chains were introduced to thieno[3,2-*c*]chromene-2-carboxylate (**9a-e**) by Williamson ether synthesis using K_2CO_3 as a base. An important beneficial feature of introducing an alkoxyamino side chain is improved solubility of compounds in polar solvents (methanol, ethanol, water). Amide derivatives **10a-c** were readily obtained by ammonolysis using gaseous ammonia in methanol.

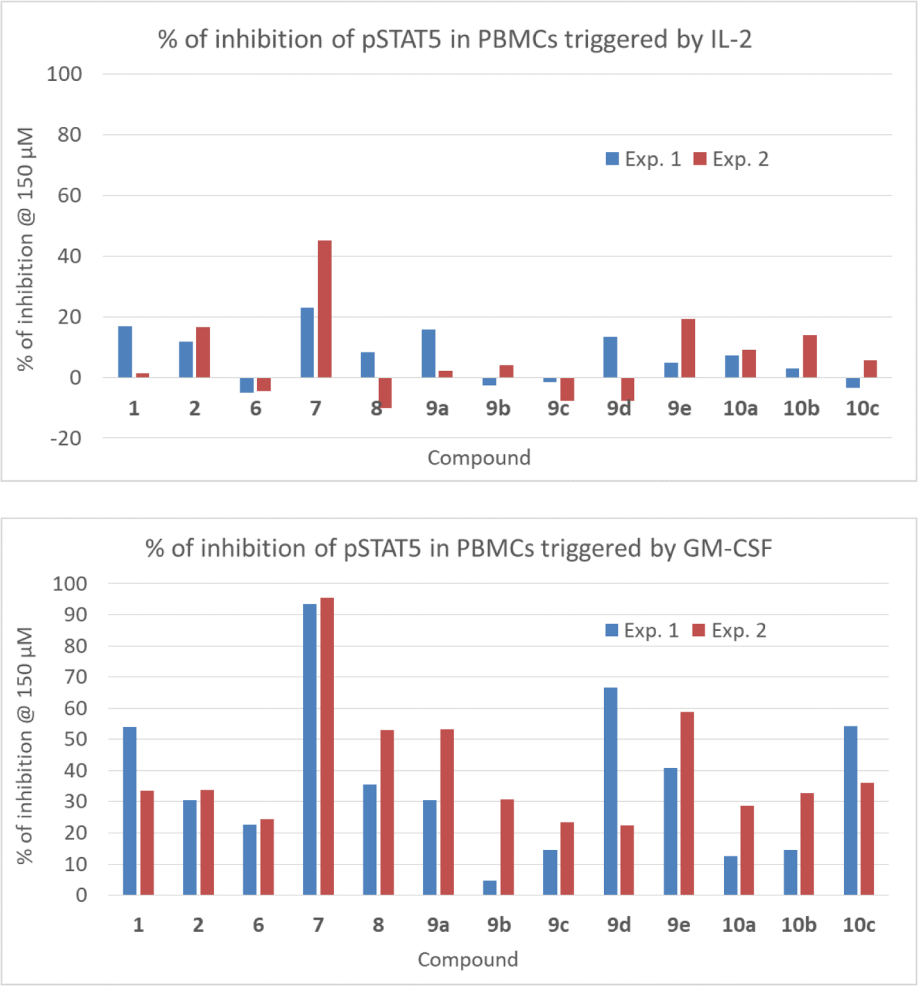


Fig. 4. Inhibition of pSTAT5 production in PBMCs triggered with IL-2 or GM-CSF with tested compounds in one testing concentration (150 µM). Results are presented as average values of duplicates in two separate experiments.

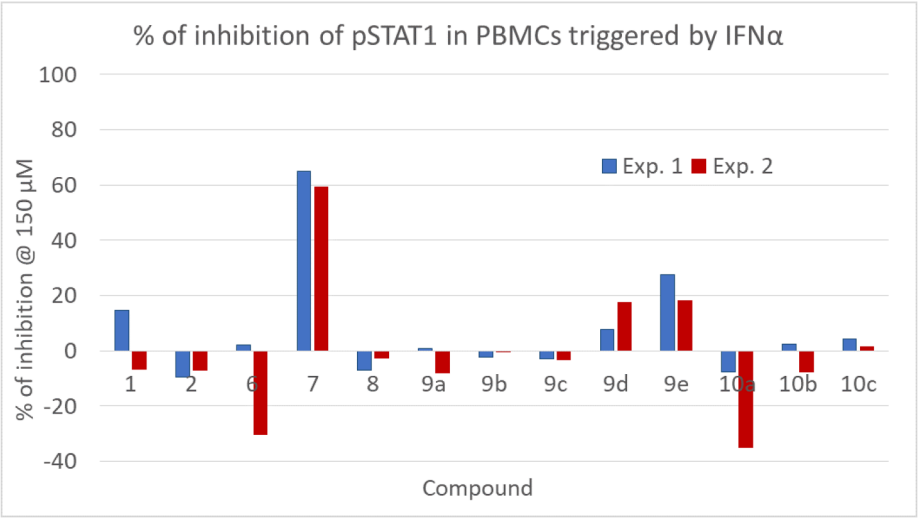


Fig. 5. Inhibition of pSTAT1 production in PBMCs triggered with IFNα with tested compounds in one testing concentration (150 μM). Results are presented as average values of duplicates in two separate experiments.

In **6**[69], ethyl carboxylate and methoxy groups are bonded to the thienyl and phenyl rings, respectively, of thieno[3,2-*c*]chromene moiety (Fig. 3).

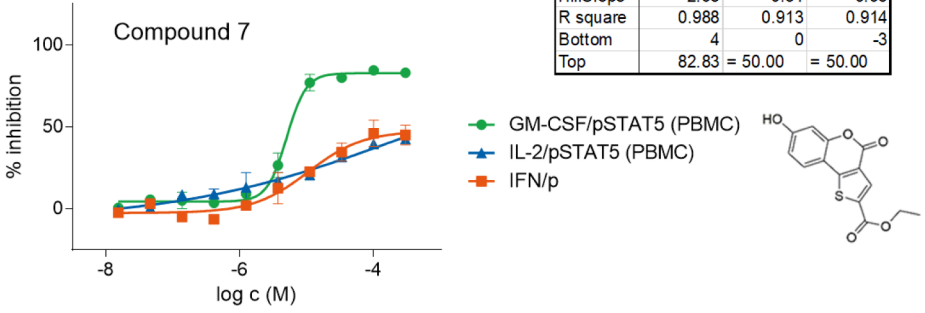
The whole molecule is essentially planar. The biggest deviation one of non-hydrogen atoms from mean plane is 0.215(2) Å, for the terminal C16 atom of the methoxy group. Only one similar structure has been published until now, in which methyl carboxylate and trifluoromethyl groups are bonded to the thienyl ring carbon atoms.[44] Bond lengths and angles in **6** show no unexpected features, and in common parts of the molecule agree well with equivalent ones in structurally related compound.[44] The sulphur S1 atom of the thienyl ring adopts an *anti-periplanar* conformation with respect to the oxygen O3 atom of the ethyl carboxylate group, as defined by the S1–C12–C13–O3 torsion angle of −179.86(14)°.

All compounds[70] were tested on IFNα-triggered measurement of pSTAT1 production, and IL-2 or GM-CSF-triggered measurement of pSTAT5 production in PBMCs[71] in one single concentration (150 μM) in two separate experiments for each of the assay.[72] Results are presented in Figs. 5 and 6. Tofacitinib and Baricitinib, marketed potent anti-inflammatory drugs (low nanomolar JAK inhibitors which interferes with the JAK/STAT signaling pathways), were used as control compounds for inhibition of JAK/STAT signaling, and their obtained activities were in correspondence with internal historical data values in all cell-based assays (Fig. S2, Supporting information).

Taken all together, only compound **7** showed more significant inhibition of pSTAT5 production in both IL-2 or GM-CSF triggered assay in PBMCs (Fig. 4), as well as inhibition of pSTAT1 production in IFNα assay (Fig. 5).

Since compound **7** showed more significant inhibition in all above mentioned tests, its activity was additionally analysed in secondary screening (10-concentration dose–response inhibition profile, 300 μM final starting concentration, 1:3 dilution factor), in order to calculate IC_{50} value for every assay and to estimate its full inhibitory profile and potency. Compound **7** showed best activity properties on inhibition of pSTAT5 production in GM-CSF-triggered assay, with IC_{50} value of 5.0 μM. Inhibition of pSTAT5 in IL-2-triggered assay showed IC_{50} value of 12.1 μM, while inhibition of pSTAT1 in IFNα-triggered assay showed IC_{50} value of 11.9 μM, with lower top values in both of those assays (Fig. 6).

In summary, a series of 4-oxo-4*H*-thieno[3,2-*c*]chromene derivatives with an aminoalkoxy side chain have been synthesized and their anti-inflammatory potential on JAK/STAT signaling pathway evaluated. Among the three different JAK/STAT cellular assays in PBMCs (pSTAT5 in GM-CSF-triggered PBMCs, pSTAT5 in IL-2-triggered PBMCs, and pSTAT1 in IFNα-triggered PBMCs, it is observed that tested compounds inhibit much stronger production of pSTAT5 induced by GM-CSF, in comparison to the production of pSTAT5 triggered by IL-2 in PBMCs, which could point to the conclusion that compounds are more selective toward signaling pathways which goes over JAK2, in comparison to pathways which are more selective to JAK1/3. Although most of the tested compounds showed some level of activity on the production of the pSTAT5 and pSTAT1 in PBMCs, only compound **7** showed more significant inhibition of pSTAT5 production in both, IL-2 and GM-CSF triggered assay, as well as inhibition of pSTAT1 induced by



	GM-CSF	IL-2	IFNα
IC_{50}/μM	5.0	12.1	11.9
HillSlope	2.98	0.51	0.96
R square	0.988	0.913	0.914
Bottom	4	0	-3
Top	82.83	= 50.00	= 50.00

Fig. 6. Inhibition of pSTAT5 production in PBMCs triggered with IL-2 or GM-CSF, or pSTAT1 production in PBMCs triggered with IFNα with Compound **7** (10-concentration dose–response inhibition profile, 300 μM final starting concentration, 1:3 dilution factor). Results are presented as average values of duplicates. Top values were fixed for IL-2 and IFNα-triggered assay, for more precise calculation of IC_{50} values.

IFNα in PBMCs. Compared to compound **1**, pSTAT inhibitory activity of thienochroemene derivative **7** having OH-7 group was increased. Hydroxyl group is generally known to bring favorable features to many biologically-active coumarins. Therefore, compound **7** is a good candidate for further structural modifications to improve detected anti-inflammatory potential. Nevertheless, it should be noted that the rules linking the structural features of coumarin derivatives and their mechanisms of action are unfortunately still lacking.

We are already working on a series of analogue compounds replacing α-pyrone ring with an oxepine ring with a goal to use SAR to clearly discuss on the activities of these class of compounds. Furthermore, we will test the ability of compounds to inhibit NF-κB pathway which is deeply involved in the onset of various inflammatory-related autoimmune disorders.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bmcl.2020.127415. These data include MOL files and InChiKeys of the most important compounds described in this article.

References

1. O'Shea JJ, Kontzias A, Yamaoka K, et al. Kinase inhibitors in autoimmune diseases. *Ann Rheum Dis.* 2013;72(suppl 2):ii111–ii115. https://doi.org/10.1136/annrheumdis-2012-202576.
2. Banerjee S, Biehl A, Gadina M, Hasni S, Schwartz DM. JAK-STAT signaling as a target for inflammatory and autoimmune diseases: current and future prospects. *Drugs.* 2017;77:521–546. https://doi.org/10.1007/s40265-017-0701-9.
3. Fragoulis GE, McInnes IB, Siebert S. JAK-inhibitors. New players in the field of immune-mediated diseases, beyond rheumatoid arthritis. *Rheumatology (Oxford).* 2019;58(Suppl 1):i43–i54. https://doi.org/10.1093/rheumatology/key276.
4. Medina FG, Marrero JG, Macías-Alonso M, et al. Coumarin heterocyclic derivatives: chemical synthesis and biological activity. *Nat Products Report.* 2015;32(10):1472–1507. https://doi.org/10.1039/C4NP00162A.
5. Lee SJ, Lee US, Kim WJ, Moon SK. Inhibitory effect of esculetin on migration, invasion and matrix metalloproteinase-9 expression in TNF-α-induced vascular smooth muscle cells. *Mol Med Rep.* 2011;4(2):337–341. https://doi.org/10.3892/mmr.2011.420.
6. Laurin P, Ferroud D, Klich M, et al. Synthesis and in vitro evaluation of novel highly potent coumarin inhibitors of gyrase B. *Bioorg Med Chem Lett.* 1999;9(14):2079–2084. https://doi.org/10.1016/s0960-894x(99)00329-7.
7. Nam NH, Kim Y, You YJ, Hong DH, Kim HM, Ahn BZ. Preliminary structure-anti-angiogenic activity relationships of 4-senecioyloxymethyl-6,7-dimethoxycoumarin. *Bioorg Med Chem Lett.* 2002;12(17):2345–2348. https://doi.org/10.1016/s0960-894x(02)00392-x.
8. Anand P, Singh B, Singh N. A review on coumarins as acetylcholinesterase inhibitors for Alzheimer's disease. *Bioorg Med Chem.* 2012;20(3):1175–1180. https://doi.org/10.1016/j.bmc.2011.12.042.
9. Kudo E, Taura M, Matsuda K, et al. Inhibition of HIV-1 replication by a tricyclic coumarin GUT-70 in acutely and chronically infected cells. *Bioorg Med Chem Lett.* 2013;23(3):606–609. https://doi.org/10.1016/j.bmcl.2012.12.034.
10. Riveiro ME, De Kimpe N, Moglioni A, et al. Coumarins: old compounds with novel promising therapeutic perspectives. *Curr Med Chem.* 2010;17(13):1325–1338. https://doi.org/10.2174/092986710790936284.
11. Huang XY, Shan ZJ, Zhai HL, Su L, Zhang XY. Study on the anticancer activity of coumarin derivatives by molecular modeling. *Chem Biol Drug Des.* 2011;78(4):651–658. https://doi.org/10.1111/j.1747-0285.2011.01195.x.
12. Hogg K, Weitz JI. *The Pharmacological basis of therapeutics. Chapter 32. Blood coagulation and anti-coagulant, thrombolytic and anti-platelet drugs.* Goodman & Gilman's; 2006:1325–1328.
13. Escoubas P, Fukushi Y, Lajide L, Mizutani J. A new method for fast isolation of insect antifeedant compounds from complex mixtures. *J Chem Ecol.* 1992;18(10):1819–1832. https://doi.org/10.1007/BF02751106.
14. Hadaček F, Müller C, Werner A, Greger H, Proksch P. Analysis, isolation and insecticidal activity of linear furanocoumarins and other coumarin derivatives from Peucedanum(Apiaceae: Apioideae). *J Chem Ecol.* 1994;20(8):2035–2054. https://doi.org/10.1007/BF02066241.
15. Sardari S, Mori Y, Horita K, Micetich RG, Nishibe S, Daneshtalab M. Synthesis and antifungal activity of coumarins and angular furanocoumarins. *Bioorg Med Chem.* 1999;7(9):1933–1940. https://doi.org/10.1016/s0968-0896(99)00138-8.
16. Wang X, Bastow KF, Sun CM, et al. Antitumor Agents. 239. Isolation, structure elucidation, total synthesis, and anti-breast cancer activity of neo-tanshinlactone from Salvia miltiorrhiza. *J Med Chem.* 2004;47(23):5816–5819. https://doi.org/10.1021/jm040112r.
17. Oliveira AM, Raposo MM, Oliveira-Campos AM, et al. Psoralen analogues: synthesis, inhibitory activity of growth of human tumor cell lines and computational studies. *Eur J Med Chem.* 2006;41(3):367–372. https://doi.org/10.1016/j.ejmech.2005.10.016.
18. Rajabi M, Hossaini Z, Khalilzadeh MA, Datta S, Halder M, Mousa SA. Synthesis of a new class of furo[3,2-c]coumarins and its anticancer activity. *J Photochem Photobiol, B.* 2015;148:66–72. https://doi.org/10.1016/j.jphotobiol.2015.03.027.
19. Murakami A, Nakamura Y, Torikai K, et al. Inhibitory effect of citrus nobiletin on phorbol ester-induced skin inflammation, oxidative stress, and tumor promotion in mice. *Cancer Res.* 2000;60(18):5059–5066.
20. Bohlmann F, Zdero C. *Naturally Occuring Thiophenes. Chemistry of Heterocyclic Compounds.* 1985;vol. 44:261.
21. Russell RK, Press JB. *Comprehensive heterocyclic chemistry II.* New York: Pergamon; 1996:6790.
22. Hirbec H, Gaviria M, Vignon J. Gacyclidine: a new neuroprotective agent acting at the N-methyl-D-aspartate receptor. *CNS Drug Rev.* 2001;7(2):172–198. https://doi.org/10.1111/j.1527-3458.2001.tb00194.x.
23. Eicher T, Hauptmann S, Speicher A. *The chemistry of heterocycles.* New York: Wiley-VCH; 2003 Chapter 5, section 5.6.
24. Wu C, Decker ER, Blok N, et al. Discovery, modeling, and human pharmacokinetics of *N*-(2-acetyl-4,6-dimethylphenyl)-3-(3,4-dimethylisoxazol-5-ylsulfamoyl)thiophene-2-carboxamide (TBC3711), a second generation, ETA selective, and orally bioavailable endothelin antagonist. *J Med Chem.* 2004;47(8):1969–1986. https://doi.org/10.1021/jm030528p.
25. Doré K, Dubus S, Ho HA, et al. Fluorescent polymeric ttransducer for the rapid, simple, and specific detection of nucleic acids at the zeptomole level. *J Am Chem Soc.* 2004;126(13):4240–4244. https://doi.org/10.1021/ja038900d.
26. Petroski RE, Pomeroy JE, Das R, et al. Indiplon is a high-affinity positive allosteric modulator with selectivity for alpha1 subunit-containing GABAA receptors. *J Pharmacol Experiment Ther.* 2006;317(1):369–377. https://doi.org/10.1124/jpet.105.096701.
27. Bertaina-Anglade V, La Rochelle CD, Scheller DK. Antidepressant properties of rotigotine in experimental models of depression. *Eur J Pharmacol.* 2006;548(1–3):106–114. https://doi.org/10.1016/j.ejphar.2006.07.022.
28. Chao EC. Dapagliflozin: an evidence-based review of its potential in the treatment of type 2 diabetes. *Drugs of the Future.* 2011;36(5):351–357. https://doi.org/10.2147/CE.S16359.
29. Ruilope L, Jäger B, Prichard B. Eprosartan versus enalapril in elderly patients with hypertension: a double-blind, randomized trial. *Blood Press.* 2001;10(4):223–229. https://doi.org/10.1080/08037050152669747.
30. Acharya A, Parameshwarappa G, Saraiah B, Ila H. Sequential one-pot synthesis of tri- and tetrasubstituted thiophenes and fluorescent push-pull thiophene acrylates involving (het)aryl dithioesters as thiocarbonyl precursors. *J Org Chem.* 2014;80(1):414–427. https://doi.org/10.1021/jo502429c.
31. Scott KA, Njardarson JT. Analysis of US FDA-approved drugs containing sulfur atoms. *Top Curr Chem.* 2018;376(1):5. https://doi.org/10.1007/s41061-018-0184-5.
32. Simonitsch Z, Eisenhuth W, Stamm OA, Schmid H. Über die Struktur des Chartreusins. *Helv Chim Acta.* 1960;43:58–63. https://doi.org/10.1002/hlca.19640470609.
33. Wattenberg LW, Lam LKT, Fladmoe AV. Inhibition of chemical carcinogen-induced neoplasia by coumarins and α-angelicalactone. *Cancer Res.* 1979;39:1651–1654.
34. Darbarwar M, Sundaramurthy V. Synthesis of coumarins with 3:4-fused ring systems and their physiological activity. *Synthesis.* 1982:337–388. https://doi.org/10.1055/s-1982-29806.
35. Trkovnik M. Syntheses of furo-, pyrrolo- and thieno[3,2-*c*]coumarins, organic preparations and procedures international. *New J Org Synthesis.* 1982;14(1–2):21–29. https://doi.org/10.1080/00304948209354892.
36. Weissenfels MZ. Synthese und Eigenschaften von 7-substituierten Thieno[3,2-c]cumarin-3-carbonsäureestern. Chemistry, 1989;29 Jg, Heft 5. doi: 10.1002/zfch.19890290503.
37. Sosnovskikh VY, Usachev BI, Sevenard DV, Lork E, Röschenthaler G-V. Unexpected synthesis of dihydrothienocoumarin derivatives from 2 trifluoromethylchromones and ethyl mercaptoacetate. *Tetrahedron Lett.* 2001;42(30):5117–5119. https://doi.org/10.1016/S0040-4039(01)00946-7.
38. Sosnovskikh VY, Usachev BI. Reactions of 7-polyfluoroalkylnorkhellins with alkyl mercaptoacetates: a simple synthesis of dihydrothienopsoralens and benzofuran derivatives of 2-oxa-7-thiabicyclo[3.2.1]octane. *Tetrahedron Lett.* 2001;42(30):5121–5122. https://doi.org/10.1016/S0040-4039(01)00948-0.

39. Sosnovskikh VY, Usachev BI, Sevenard DV, Röschenthaler G-V. Synthesis of 2-(trifluoromethyl)-1,2-dihydro-4*H*-thieno[2,3-*c*]chromen-4-ones and 2-(trifluoromethyl)-4*H*-thieno[2,3-*c*]chromen-4-ones from 2-trifluoromethylchromones and ethyl mercaptoacetate. *Tetrahedron.* 2003;59(15):2625–2630. https://doi.org/10.1016/S0040-4020(03)00329-6.
40. Sosnovskikh VY, Usachev BI, Vorontsov II. Unusual reaction of 2-(trifluoromethyl)-1,2-dihydro-3lambda(6)-thieno-[2,3-c]chromen-3,3,4-triones with hydrazine as a new route to 3-hydrazinopyridazine derivatives. *J Org Chem.* 2002;67(19):6738–6742. https://doi.org/10.1021/jo0258406.
41. Navarro RA, Bleye LC, Gonzalez-Ortega A, Ruiz MCS. Synthesis of 1*H*-[1]Benzopyrano[4,3-*b*]pyrrole and 4*H*-Thieno[3,2-*c*][1]benzopyran derivatives. Functionalisation by aromatic electrophilic substitution. *Heterocycles.* 2001;55(12):2369–2386. https://doi.org/10.3987/COM-01-9355.
42. Majumdar KC, Ghosh SK. Studies on sulfoxide rearrangements: regioselective synthesis of 3-(aryloxyacetyl)-2,3-dihydrothieno[3,2-*c*][1]benzopyran-4-ones. *Tetrahedron Lett.* 2002;43(11):2123–2125. https://doi.org/10.1016/S0040-4039(02)00200-9.
43. Katsiel AL, Sharipova AN, Fisyuk AS. Synthesis of 4*H*-thieno[3,2-c][1]benzopyran-2-carboxaldehydes. *Mendeleev Commun.* 2008;18(3):169–170. https://doi.org/10.1016/j.mencom.2008.05.020.
44. Iaroshenko VO, Erben F, Mkrtchyan S, et al. 4-Chloro-3-(trifluoroacetyl)- and 4-chloro-3-(methoxalyl)coumarins as novel and efficient building blocks for the regioselective synthesis of 3,4-fused coumarins. *Tetrahedron.* 2011;67(41):7946–7955. https://doi.org/10.1016/j.tet.2011.08.030.
45. Iaroshenko VO, Ali S, Mkrtchyan S, et al. Design and synthesis of condensed thienocoumarins by Suzuki-Miyaura reaction/lactonization tandem protocol. *Tetrahedron Lett.* 2012;53(52):7135–7139. https://doi.org/10.1016/j.tetlet.2012.10.096.
46. Fisyuk AS, Bogza YP, Belyaeva LV, Belyaev VB. Synthesis of 4*H*-thieno[3,2-c]chromenes by intramolecular arylation of 4-aryloxy-methyl-5-iodothiophene-2-carbaldehydes. *Chem Heterocycl Compd.* 2012;48:1078–1084. https://doi.org/10.1007/s10593-012-1102-3.
47. Reddy CR, Valleti RR, Reddy MD. A Thioannulation Approach to Substituted Thiophenes from Morita–Baylis–Hillman Acetates of Acetylenic Aldehydes. *J Org Chem.* 2013;78(13):6495–6502. https://doi.org/10.1021/jo400567h.
48. Shyka OY, Martyak RL, Tupchyak MA, Pokhodylo NT, Obushak MD. Facile synthetic route to benzo[*c*]chromenones and thieno[2,3-*c*]chromenones. *Synth Commun.* 2017;47(24):2399–2405. https://doi.org/10.1080/00397911.2017.1380833.
49. Raju S, Batchu VR, Swamy NK, et al. Palladium-mediated synthesis of 5-substituted 4-alkynylthieno[2,3-*c*]pyran-7-ones. *Tetrahedron Lett.* 2006;47(1):83–88. https://doi.org/10.1016/j.tetlet.2005.10.135.
50. Wittine K, Ratkaj I, Benci K, et al. The novel coumarin[3,2-c]thiophene and its hydroxamic acid and ureido derivatives: synthesis and cytostatic activity evaluations. *Med Chem Res.* 2016;25(4):728–737. https://doi.org/10.1007/s00044-016-1523-0.
51. Huchang Y, Yan L, Zhiuyan F, et al. Synthesis, crystal structure and antitumour activity evaluation of 1*H*-thieno[2,3-*c*]chromen-4(2*H*)-one derivatives. *J Chem Res.* 2017;41(1):36–41. https://doi.org/10.3184/174751917X14837116219573.
52. Mazimba O. Synthetic Protocols on 6*H*-Benzo[c]Chromen-6-Ones: A Review. *Turk J Chem.* 2016;40:1–27. https://doi.org/10.3906/kim-1504-67.
53. Ram VJ, Goel A, Shukla PK, Kapil A. Synthesis of thiophenes and thieno[3,2-c]pyran-4-ones as antileishmanial and antifungal agents. *Bioorg Med Chem Lett.* 1997;7(24):3101–3106. https://doi.org/10.1016/S0960-894X(97)10153-6.
54. El-Dean AMK, Zaki RM, Geies AA, Radwan SM, Tolba MS. Synthesis and antimicrobial activity of new heterocyclic compounds containing thieno[3,2-c]coumarin and pyrazolo[4,3-*c*]coumarin frameworks. *Russ J Bioorg Chem.* 2013;39(5):553–564. https://doi.org/10.7868/S0132342313040088.
55. Rodighiero P, Pastorini G, Chilin A, Marotto A. Synthesis of methyl derivatives of linear and angular thienocoumarins and thiopyranocoumarins. *J Heterocycl Chem.* 1998;35(4):847–852. https://doi.org/10.1002/jhet.5570350411.
56. Rodighiero P, Pastorini G, Dalla Via L, Gia O, Magno M. Synthesis and biological activity of linear and angular 4-methoxymethylthienocoumarins and 4 acetoxymethylthienocoumarins. *Il Farmaco.* 1998;53:313–319. https://doi.org/10.1016/s0014-827x(98)00007-x.
57. Makisumi Y. *JP Pat. Appl.* 1972:48000597.
58. Makisumi Y. *JP Pat. Appl.* 1973:48000596.
59. Majumdar KC, Biswas A. Regioselective synthesis of thieno[3,2-c][1]benzopyran-4-ones by thio-Claisen rearrangement. *Monatsh Chem.* 2004;135:1001–1007. https://doi.org/10.1007/s00706-003-0164-4.
60. Majumdar KC, Pal AK, Ghosh M. Regioselective Synthesis of Thiopyrano[3,2-c][1]benzothiopyran-5(2*H*)-one and Thieno[3,2-*c*][1]benzothiopyran-4(2*H*)-one. *Synth Commun.* 2007;37(9):1525–1534. https://doi.org/10.1080/00397910701229925.
61. Hegab MI, Abdulla MM. 4-Chloro-2,2-disubstituted chromene-3-carboxaldehyde: Synthesis of some fused polycyclic heterocycles as anti-inflammatory, analgesic, anticonvulsant, and antiparkinsonian agents. *Archiv der Pharmazie – Chem Life Sci.* 2006;339:41–47. https://doi.org/10.1002/ardp.200500124.
62. Laurence A, Pesu M, Silvennoinen O, O'Shea J. JAK Kinases in Health and Disease: An Update. *Open Rheumatol J.* 2012;6:232–244. https://doi.org/10.2174/1874312901206010232.
63. Beugelmans R, Bois-Choussy M, Boudet B. Etude des reactions de $s_{rn}1$—partie 10: Action de sulfanions sur les halogenures d'aryle fonctionnalises. Synthese directe de benzothiophenes et thienopyridines. *Tetrahedron.* 1983;39(24):4153–4161. https://doi.org/10.1016/S0040-4020(01)88635-X.
64. Rahman LKA, Scrowston RM. 7-Substituted benzo[*b*]thiophenes and 1,2-benzisothiazoles. Part 2. Chloro and nitro derivatives. *J Chem Soc Perkin Trans.* 1984;1:385. https://doi.org/10.1039/p19840000385.
65. Gronowitz S. *Thiophene and its derivatives, Part 1.* New York: Wiley & Sons; 1985:88.
66. Gronowitz S. Thiophene and its derivatives: Preparation of thiophenes by ring-closure reactions and from other ring systems. In: Weissberger A, Taylor EJ, eds. *The Chemistry of Heterocyclic Compounds.* New York: John Wiley & Sons, Inc.; 1985:1–213.
67. Mullins RJ, Williams DR. Fiesselmann thiophene synthesis. In: Li J-J, Corey EJ, eds. *Name reactions in heterocyclic chemistry.* New Jersey: John Wiley & Sons, Inc.; 2005:184–192.
68. Li JJ, ed. *Name reactions in heterocyclic chemistry.* New York: John Wiley; 2005.
69. The CCDC 1891418 contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.
70. Mother plates with compounds in pure DMSO were prepared from 10 mM DMSO stock solutions. 96-well V-bottom plate (Greiner) were used for mother plate preparation. An aliquot of 600 nL of compound solution was transferred from the mother plate to the test plate with a Mosquito nanoliter liquid handling system (TTP labtech). The assay was performed in 96-well cell culture plate (Greiner) using a 200 μL assay volume. The total percentage of DMSO was 0.3%. Compounds were tested at 150 μM final concentrations in duplicate (primary screening), and as 10-concentration dose-response (300 μM final starting concentration, 1:3 dilution factor) as secondary screening. Control compounds were tested together with tested compound.
71. Human PBMCs was isolated from buffy coat (Croatian Institute for Transfusion Medicine, Zagreb) diluted 1:1 with PBS (Sigma) by using Lymphoprep™ (Axis-Shield). Diluted buffy-coat (25 mL) on top of Lymphoprep was centrifuged for 35 min at 400g and the upper plasma layer was removed. White layer containing the mononuclear cells was isolated and diluted with PBS and centrifuged at 300g, 10 min at 25°C. Cell pellet was resuspended again in PBS, and centrifuged again at 200g, 10 min, 25°C, repeating washing step 2 times until a clear supernatant is obtained. PBMCs was resuspended in RPMI1640 (Lonza) + 10% FBS (Sigma) +1x pen/strep (Gibco) and cell concentration was determined with the cell counter (Countess II, Life Technologies).
72. 10^6 PBMCs was seeded per well of a 96-well plate in 180 μL, and 0.6 μL compound and vehicle control are added to the plate by using Mosquito (TTP labtech). After 30 min incubation at 37°C, 5% CO2, 20 μL IL-2 (final 100 ng/mL), GM-CSF (final 0.05 ng/mL), IFNα (final 5 ng/mL), or 20 μL medium for non-triggers was added, which is than incubated for 30 min at 37°C, 5% CO2. Cell suspension was transfered from 96-well plate into a V-bottom plate, centrifuged 5 min at 1,000rpm, 4°C and supernatant was discarded. Adherent cells were lysed by adding 100 μL of lysis buffer (Cell signalling). Lysate was transfered from 96-well plate to the cells on the V-bottom plate and the plate was stored at -80°C overnight. Plate was thawed on ice, and samples/standards were diluted 1:1 in ELISA Sample Diluent, and incubated overnight at 4°C. Detection ELISA was done with instructions from the kit (PathScan phospho-stat5 (Tyr694) and phospho-stat1 (Tyr701) sandwich ELISA kits, Cell signalling), and readout is done on the EnVision (Perkin Elmer). Calculation of IC50 data, curves and QC analysis was made by using Excel tools and GraphPadPrism software.