ChemComm

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

rsc.li/chemcomm

View Article Online
DOI: 10.1039/C8CC05643A

ChemComm

COMMUNICATION

Exploring Physicochemical Space via a Bioisostere of the Trifluoromethyl and Ethyl Groups (BITE): Attenuating Lipophilicity in Fluorinated Analogues of Gilenya® for Multiple Sclerosis

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Nathalie Erdeljac,[a] Gerald Kehr,[a] Marie Ahlqvist,[b] Laurent Knerr*[c] and Ryan Gilmour*[a,d]

The direct, catalytic *vicinal* difluorination of terminal alkenes via an I(I)/I(III) manifold was exploited to install a chiral, hybrid bioisostere of the CF_3 and Et groups (BITE) in Gilenya®; the first orally available drug for the clinical management of Multiple Sclerosis (MS). This subtle fluorination pattern allows lipophilicity (log D) to be tempered compared to the corresponding CF_3 and Et derivatives (CH_2CH_3>CH_2CF_3>$CHFCH_2F$).

Structural editing with fluorine is a ubiquitous strategy to modulate the physicochemical and pharmacological properties of small molecule drug candidates. Parameters ranging from lipophilicity, intrinsic potency, metabolic stability through to the pK_a of proximal functional groups underscore the strategic value of fluorination in contemporary medicinal chemistry.[1] Often, the electronic factors that govern these physicochemical characteristics manifest themselves in molecular topology and can thus be rationalised at the structural level. This is particularly pronounced in acyclic systems when fluorine is situated *vicinal* to an electron deficient motif (X = F, O, N, S).[2,3] In such scenarios, the synergistic interplay of stereoelectronic ($\sigma_{C\text{-}H} \rightarrow \sigma_{C\text{-}F}^*$) and electrostatic ($R^{+\cdots}F^{\delta-}$) interactions ensures that *syn-clinal* conformers are significantly populated (the *Gauche Effect*). Predicated on a donor acceptor model, F-C-C-(X) dihedral angles approaching 60° ensure maximum orbital overlap and also allow the fluorine atom to engage in stabilising Coulombic interactions with proximal cations. Consequently, judicious aliphatic fluorination provides an expansive platform to steer conformation in a manner antipodal to classic steric approaches. These subtle changes in fluorination pattern can also be harnessed to fine-tune the physicochemical profiles of small molecule drug candidates: This has been elegantly demonstrated by Müller and Carreira.[4]

Inspired by the structure-function interplay of fluorination patterns in medicinal chemistry, and in response to a deficiency in the synthesis repertoire, we recently reported a direct, catalytic, *vicinal* difluorination of terminal alkenes.[5] Catalysis proceeds via an I(I)/I(III) manifold that exploits Selectfluor® as the terminal oxidant, whilst HF•amine serves as both an inexpensive fluoride source and Brønsted acid. Since the resulting *vicinal* difluoro-motif is a chiral, hybrid bioisostere of the trifluoromethyl and ethyl groups (BITE group),[2e,6] a suitable platform was sought to explore the physicochemical profile of this unit (Figure 1). Specifically, lipophilicity was of interest due to its importance in the early stages of drug development and associated requirement to generate molecular "fragments" to tailor the overall quality and "*drug-likeness*" of a candidate.[7]

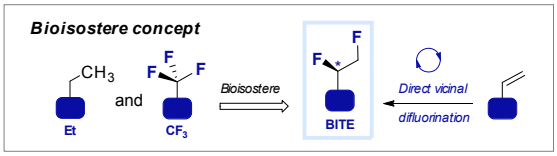


Fig. 1 The BITE group: A chiral, hybrid bioisostere of the trifluoromethyl and ethyl groups.

Lipinski highlighted the significance of this parameter in his "*rule of five*", to lower attrition rates during drug discovery, which suggests that the desired clogP for orally administered drugs should not exceed 5.[8] While the influence of fluorine substitution on aryl ring systems generally leads to a moderate increase in lipophilicity, the effects on aliphatic systems are more complex.[4,9] To that end, we sought to explore the effect of the BITE group on the lipophilicity of fluorinated analogues of the multiple sclerosis drug Fingolimod / Gilenya® (Figure 2).

[a] *Organisch-Chemisches Institut*
Westfälische Wilhelms-Universität Münster
Corrensstraße 40, 48149 Münster (Germany)
E-mail: ryan.gilmour@uni-muenster.de
Homepage: http://www.uni-muenster.de/Chemie.oc/gilmour/index.html
[b] *Drug Metabolism and Pharmacokinetics, Cardiovascular, Renal and Metabolism*
IMED Biotech Unit, Astrazeneca, Gothenburg (Sweden)
[c] *Medicinal Chemistry, Cardiovascular, Renal and Metabolism*
IMED Biotech Unit, Astrazeneca, Gothenburg (Sweden)
E-mail: Laurent.Knerr@astrazeneca.com
[d] *Excellence Cluster EXC 1003, Cells in Motion*
Westfälische Wilhems-Universität Münster
Münster (Germany)
† Electronic Supplementary Information (ESI) available. See DOI: 10.1039/x0xx00000x

View Article Online
DOI: 10.1039/C8CC05643A

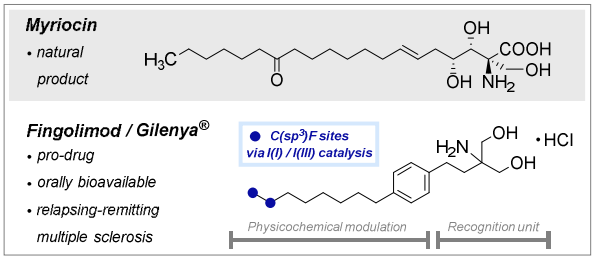


Fig. 2 The natural product *Myriocin* and the relapsing-remitting multiple sclerosis drug Fingolimod / *Gilenya*®.

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) in which demyelination and axon damage leads to the dysfunction and death of neural cells.[10] With a usual age of onset around 30 years, MS is considered to be the most prevailing cause of disability among young adults and is estimated to affect around 2.1 million people worldwide.[11] So far, there is no cure for MS and current treatment strategies rely on various disease modifying drugs (DMO) to reduce relapse rates and slow down the progression of the disease.[12] First-line therapy continues to consist of intramuscular (IM) or subcutaneous (SC) administration of interferon betas (Avonex®, Biogen Idec; Betaferon®, Bayer; Extavia®, Novartis Pharma) or glatiramer acetate (Copaxone®, Teva Pharmaceutical Industries).[13] In 2010, Fingolimod (Gilenya®, Novartis Pharma) was approved by the US Food and Drug Administration (FDA) as the first orally bioavailable drug for the treatment of relapsing-remitting multiple sclerosis (RRMS).[14] As a structural analogue of the naturally occurring sphingosine myriocin (Figure 2), fingolimod is phosphorylated *in vivo* by sphingosine kinase 2 to its active metabolite *S*-fingolimod-phosphate.[15] Its immunomodulatory effects are mediated *via* initial activation of sphingosine 1-phosphate (S1P) receptors on lymphocytes, which obstructs their egress from the lymph nodes into the peripheral circulation and thereby reducing their infiltration into the CNS.[16]

As a platform to validate the BITE group as a physicochemical handle to modulate lipophilicity in small molecule drug candidates, fluorinated analogues of Fingolimod (Gilenya®) were investigated. The direct, catalytic difluorination of terminal alkenes[5] accelerated this investigation by allowing hydrocarbon tails with 1,2-difluorinated alkyl chains of varying length to be rapidly prepared.

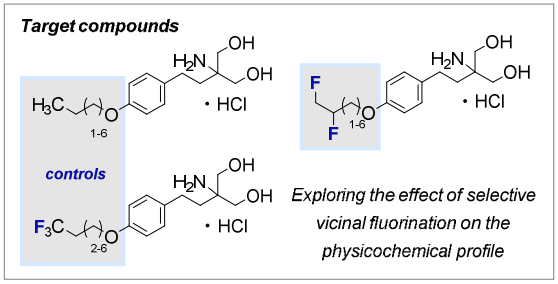
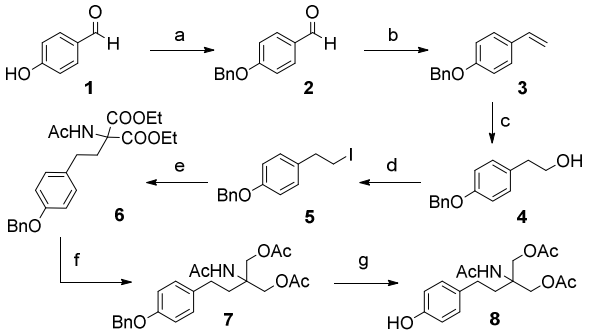


Fig. 3 The *Gilenya*® analogues for comparative physicochemical profiling.

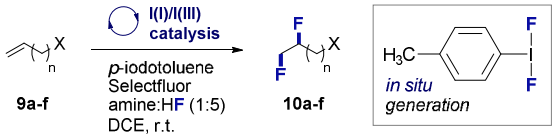
In addition, the synthesis of the non-fluorinated (Et) and trifluoromethylated (CF_3) counterparts was performed to allow for a comparative physicochemical profiling of the three substituents (Figure 3: Target scaffolds).



Scheme 1 Synthesis of the aromatic core. *Reagents and conditions [yield]:* (a) BnBr, K_2CO_3, DMF, r.t. [99%]; (b) H_3CPPh_3I, NaH, THF, r.t. [96%]; (c) (i) BH_3/THF, 0 °C to r.t. and (ii) NaOH, H_2O_2, EtOH/H_2O, 0 °C to r.t. [82%]; (d) I_2, PPh_3, imidazole, CH_2Cl_2, 0 °C to r.t. [99%]; (e) NaH, AcNH(COOEt)$_2$, DMF, 95 °C [63%]; (f) (i) LiCl, $NaBH_4$, THF/EtOH, 0 °C to r.t. and (ii) Ac_2O, Et_3N, DMAP, THF, r.t. [75%]; (g) H_2, Pd/C, EtOH, r.t. [92%].

Synthesis: The small library of compounds was prepared from a common precursor **8** in a highly concise manner (Scheme 1). Commercially available *p*-hydroxy benzaldehyde **1** was processed to the corresponding styrene **3** via a benzylation / methylenation sequence. Subsequent hydroboration / oxidation furnished alcohol **4**, which was converted to the iodide **5**. The drug head group was installed by displacement using diethyl acetamidomalonate to furnish **6** prior to double reduction of the esters and acetate protection (**7**). Finally, debenzylation liberated phenol **8** as a handle to install the alkyl spacer for physicochemical modulation.

Table 1 Catalytic, *vicinal* difluorination of alkenes via an I(I)/I(III) manifold.



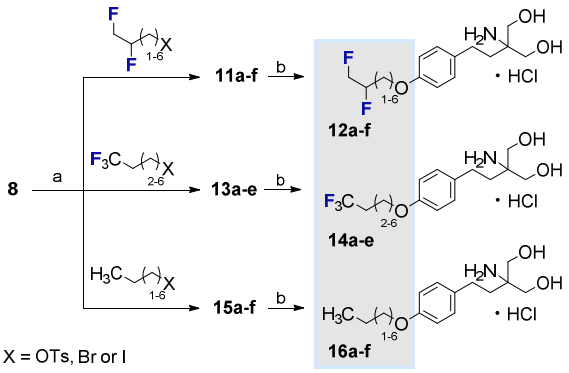
10	X	n	Yield [%]
a	OTs	1	48
b	OTs	2	46
c	OTs	3	72
d	OTs	4	79
e	OTs	5	55
f	Br	6	72

The alkyl chains containing the *vicinal* BITE motif required for coupling with **8** were prepared directly from the corresponding terminal alkenes (Table 1, **9a-f** → **10a-f**). To that end, *p*-iodotoluene was employed as a catalyst to generate the requisite *p*-TolIF$_2$ species *in situ*. This was enabled by utilizing Selectfluor® as oxidant,

View Article Online
DOI: 10.1039/C8CC05643A

and a mixture of Olah's reagent and $Et_3N•5HF$ as both the HF source and Brønsted acid activator (Table 1).[5] The desired 1,2-difluorinated products were obtained in racemic mixture in moderate to good yields (up to 79%). The corresponding trifluoromethylated and non-fluorinated hydrocarbon chains were either obtained commercially or prepared from available starting materials (full details are provided in the Supporting Information).

Finally, the aliphatic chains were coupled to the Gilenya® head group **8** *via* displacement chemistry (**11a-f**, **13a-e** and **15a-f**, BITE group, CF_3 and Et, respectively) and the target molecules were globally deprotected and converted to their respective hydrochloride salts (Scheme 2, **12a-f**, **14a-e** and **15a-f**, BITE group, CF_3 and Et respectively). For the comparative physicochemical analysis, the parent drug Gilenya® was also synthesised as a reference (full details are provided in the Supporting Information).[17]



Scheme 2 Coupling of the aliphatic chains to **8**, deprotection and hydrochloride salt formation. *Reagents and conditions [yield]:* (a) K_2CO_3, DMF, r.t. [**11a-f** 36-94%, **13a-e** 66-88%, **15a-f** 72-97%]; (b) (i) aq. LiOH (2M), MeOH, reflux and (ii) HCl in dioxane (4M), r.t. [**12a-f** 39-90%, **14a-e** 48-73%, **16a-f** 47-99%].

Physicochemical Profiling: Compounds **12a-f**, **14a-e** and **16a-f** were subjected to *in vitro* profiling to assess the impact of BITE fluorination on lipophilicity, solubility, passive permeability and metabolic stability (Table 2). Predictably, the introduction of the trifluoromethyl (CF_3) group had a clear effect on lipophilicity compared to the alkyl series (average $\Delta logD_{7.4}$ = -0.4) (Figure 4). As expected, and in line with previously published data,[4] the effect of *vicinal* difluoroethyl BITE group was significantly more pronounced (average $\Delta logD_{7.4}$ = -1.5 and -1.2 *versus* the alkyl and trifluoromethyl series, respectively). Furthermore, the *vicinal* difluoroethyl BITE series performed better in terms of solubility compared to the roughly comparable trifluoromethyl and alkyl series: This was especially apparent for larger alkyl groups (5 to 10 fold better solubility for n=3 and above) (Figure S1). It is interesting to note that, despite an average lower lipophilicity, the trifluoromethyl series performed poorer in that regard compared to the alkyl series. As expected, the passive permeability measured using a Caco-2 assay (Figure S2) showed a clear relationship with the lipophilicity (Table 2).

Fig. 4 Measured $logD_{7.4}$ profile of the compounds in the different series as a function of the methylene spacer length: alkyl (grey), trifluoromethyl (green), 1,2-difluoroethyl (blue). Fingolimod has been added as reference (red). The labels indicate the individual measured $logD_{7.4}$ values. For a detailed description of the assay see the supporting information.

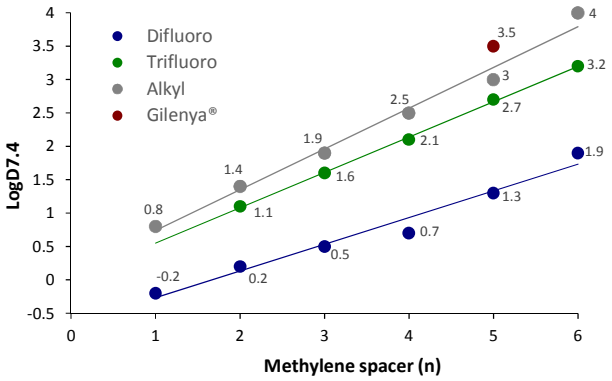


Table 2 Lipophilicity, solubility, apparent passive permeability and *in vitro* metabolic stability comparison of compounds described.[a] See supporting information for assay details.[b] All compounds were stable in the presence of human microsomes (Cl_{int} (µM/min/mg) <4.5).

Entry	n	$LogD_{7.4}$[a]	Solubility (pH7.4, µM)[a]	Caco-2 Papp (10^{-6} cm/s)[a]	Rat/Human Hepatocytes Cl_{int} (µL/min/10^6 cells)[a,b]
Fingolimod	5	3.5	2	-	12.8/-
12a	1	-0.2	968	-	1.53/-
12b	2	0.2	875	1.19	2.67/-
12c	3	0.5	874	0.84	9.91/<1
12d	4	0.7	585	-	10.6/-
12e	5	1.3	133	-	20.3/-
12f	6	1.9	59	-	47.3/-
14a	2	1.1	152	2.61	8.28/-
14b		1.6	88	2.95	11.6/<1
14c	3	2.1	65.1	-	15.8/-
14d	4	2.7	16	-	37.3/-
14e	5	3.2	5.8	-	12.3/-
16a	6	0.8	1000	-	15.9/-
16b	1	1.4	796	5.09	11.9/-
16c	2	1.9	175	5.02	12.3/3.34
16d	3	2.5	58	-	9.90/-
16e	4	3	5	-	13.1/-
16f	5	4	4	-	11.5/-
	6				

Indeed, the more polar *vicinal* difluoroethyl BITE derivatives studied demonstrated low permeability compared to their trifluoromethyl analogues. This was even more pronounced in comparison to the alkyl systems, which showed moderate to high permeability (Table 2). The metabolic stability measured in rat and human hepatocytes

View Article Online
DOI: 10.1039/C8CC05643A

(see Supporting Information for assay details) revealed a clear correlation between Rat Cl_{int} (unbound) versus logD and demonstrated a positive impact of fluorine introduction in that respect (Figure S3). There is, however, a disconnect between Clint in human and rat hepatocytes (**12c** and **14b**), which could be due to differences in metabolism in these two species. All compounds reported here were however stable in the presence of human liver microsomes (Cl_{int} (µM/min/mg) <4.5).

Conclusions

Contemporary medicinal chemistry requires new areas of chemical space to be explored to place physicochemical parameters on a structural foundation. The hybrid bioisostere of the CF_3 and Et groups (BITE group) has been found to attenuate lipophilicity in a series of fluorinated analogues of the Multiple Sclerosis drug Gilenya®. Comparative physicochemical profiling with the corresponding CF_3 and Et derivatives demonstrated that lipophilicity (log D) can be tempered in the order CH_2CH_3>CH_2CF_3>$CHFCH_2F$. In addition, a comparison of the three groups on solubility, apparent passive permeability and *in vitro* metabolic stability has been established. Efficient installation of the BITE group was achieved by direct, catalytic *vicinal* difluorination of α-olefins without the need for substrate pre-functionalisation.

We gratefully acknowledge generous financial support from the WWU Münster, the DFG ("*Cells in Motion, Cluster of Excellence*" CiM, and SFB 858), and the European Research Council (ERC-2013-StG Starter Grant 336376-ChMiFluorS to RG).

Conflicts of interest

There are no conflicts of interest to declare.

Notes and references

1 (a) H.-J. Böhm, D. Banner, S. Bendels, M. Kansy, B. Kuhn, K. Müller, U. Obst-Sander, M. Stahl, *ChemBioChem*, 2004, **5**, 637-643; (b) P. Shah, A. D. Westwell, *J. Enzyme Inhib. Med. Chem.,* 2007, **22**, 527-540; (c) K. Müller, C. Faeh, F. Diederich, *Science*, 2007, **317**, 1881-1886; (d) E. P. Gillis, K. J. Eastman, M. D. Hill, D. J. Donnelly, N. A. Meanwell, *J. Med. Chem*., 2015, **58**, 8315−8359; (e) M. Salwiczek, E. K. Nyakatura, U. I. M. Gerling, S. Ye, B. Koksch, *Chem. Soc. Rev.,* 2012, **41**, 2135-2171; (f) A. Harsanyi, G. Sandford, *Green Chem.,* 2015, **17**, 2081-2086; (g) N. A. Meanwell, *J. Med. Chem.,* 2018, doi: 10.1021/acs.jmedchem.7b01788.

2 (a) D. O'Hagan, *Chem. Soc. Rev.,* 2008, **37**, 308-319; (b) L. Hunter, *Beilstein J. Org. Chem.,* 2010, **6**, No. 38. doi: 10.3762/bjoc.6.38; (c) L. E. Zimmer, C. Sparr, R. Gilmour, *Angew. Chem. Int. Ed.,* 2011, **50**, 11860-11871; (d) *Angew. Chem.,* 2011, **123**, 12062-12074; (e) I. G. Molnár, C. Thiehoff, M. C. Holland, R. Gilmour, *ACS Catal.,* 2016, **6**, 7167-7173; (f) F. Scheidt, P. Selter, N. Santschi, M. C. Holland, D. V. Dudenko, C. Daniliuc, C. Mück-Lichtenfeld, M. R. Hansen, R. Gilmour, *Chem. Eur. J.*, 2016, **22**, 1 – 9; (g) C. Thiehoff, Y. P. Rey, R. Gilmour, *Isr. J. Chem.,* 2017, **57**, 92-100; (h) M. Aufiero, R. Gilmour, *Acc. Chem. Res*., 2018, **51**, 1701-1710.

3 (a) C. Thiehoff, M. C. Holland, C. Daniliuc, K. N. Houk, R. Gilmour, *Chem. Sci.,* 2015, ***6***, 3565-3571; (b) C. Thiehoff, L. Schifferer, C. G. Daniliuc, N. Santschi, R. Gilmour, *J. Fluorine Chem*., 2016, **182**, 121-126; (c) N. Santschi, C. Thiehoff, M. C. Holland, C. G. Daniliuc, K. N. Houk and R. Gilmour, *Organometallics,* 2016, **35**, 3040–3044.

4 (a) Q. A. Huchet, B. Kuhn, B. Wagner, H. Fischer, M. Kansy, D. Zimmerli, E. M. Carreira, K. Müller, *J. Fluorine Chem.,* 2013, **152**, 119-128; (b) K. Müller, *Chimia,* 2014, **68**, 356-362; (c) Q. A. Huchet, N. Trapp, B. Kuhn, B. Wagner, H. Fischer, N. A. Kratochwil, E. M. Carreira, K. Müller, *J. Fluorine Chem.,* 2017, **198**, 34-46; (d) Q. A. Huchet, B. Kuhn, B. Wagner, N. A. Kratochwil, H. Fischer, M. Kansy, D. Zimmerli, E. M. Carreira, K. Müller, *J. Med. Chem.,* 2015, **58**, 9041-9060.

5 (a) I. G. Molnár, R. Gilmour, *J. Am. Chem. Soc.,* 2016, **138**, 5004-5007. (b) For a contemporaneous report see: S. M. Banik, J. W. Medley, E. N. Jacobsen, *J. Am. Chem. Soc.,* 2016, **138**, 5000-5003. (c) J. C. Sarie, C. Thiehoff, R. J. Mudd, C. G. Daniliuc, G. Kehr, R. Gilmour, *J. Org. Chem*., *2017*, **82**, 11792-11798.

6 M. Jagodzinska, F. Huguenot, G. Candiani, M. Zanda, *ChemMedChem,* 2009, **4**, 49-51.

7 (a) T. W. Johnson, K. R. Dress, M. Edwards, *Bioorg. Med. Chem. Lett.,* 2009, **19**, 5560–5564; (b) M. J. Waring, *Expert Opin. Drug Discov.,* 2010, **5**, 235-24.

8 C. A. Lipinski, F. Lombardo, B. W. Dominy, P. J. Feeney, *Adv. Drug Deliv. Rev.,* 1997, **23**, 3-25.

9 (a) B. E. Smart, *J. Fluorine Chem.,* 2001, **109**, 3-11; (b) B. Linclau, Z. Wang, G. Compain, V. Paumelle, C. Q. Fontenelle, N. Wells, A. Weymouth-Wilson, *Angew. Chem. Int. Ed.,* 2016, **55**, 674-678; (c) D. O'Hagan, R. J. Young, *Angew. Chem. Int. Ed.,* 2016, **55**, 3858-3860.

10 A. Compston, A. Coles, *Lancet*, 2008, **372***,* 1502-1517.

11 (a) Atlas Multiple Sclerosis Resources in the World 2008, WHO Press, Geneva, p. 56; (b) H. L. Zwibel, J. Smrtka, *Am J Manag Care.,* 2011, **17**, 139-45; (c) E. Grebenciucova, A. T. Reder, J. T. Bernard, *Neurology*, 2016, **86**, 1014-1021; (d) J. Raffel, B. Wakerley, R. Nicholas, *Medicine,* 2016, **44**, 537-541.

12 (a) V. Brinkmann, A. Billich, T. Baumruker, P. Heining, R. Schmouder, G. Francis, S. Aradhye, P. Burtin, *Nat. Rev. Drug. Discov.,* 2010, **9**, 883-897; (b) N. Mulakayala, P. Rao, J. Iqbal, R. Bandichhor, S. Oruganti, *Eur. J. Med. Chem*., 2013, **60**, 170-186.

13 (a) F. Lublin, *J. Neurol*., 2005, **252**, III/3–III/9; (b) D. Buck, B. Hemmer, *J. Neurol*., 2011, **258**, 1747–1762; (c) M. Marziniak, S. Meuth, *Adv. Ther*., 2014, **31**, 915–931; (d) A. H. Cross, R. T. Naismith, *J. Intern. Med*., 2014, **275**, 350-363; (e) K. Harrison, *Br. J. Nurs.,* 2014, **23**, 582-589.

14 (a) FDA approves first oral drug to reduce MS relapses. Available from: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm226755.htm (14.20 pm, 27.02.2017); (b) S. Sharma, A. G. Mathur, S. Pradhan, D. B. Singh, S. Gupta, J. *Pharmacol. Pharmacother.*, 2011, **2**, 49-51.

15 (a) S. Kim, H. Lee, M. Lee, T. Lee, *Synthesis*, 2006, **5**, 753-755; (b) C. R. Strader, C. J. Pearce, N. H. Oberlies, *J. Nat. Prod*., 2011, **74**, 900-907.

16 L. J. Scott, *CNS Drugs.,* 2011, **25**, 673-698.

17 R. S. Shaikh, S. S. Schilson, S. Wagner, S. Hermann, P. Keul, B. Levkau, M. Schäfers, G. Haufe, *J. Med. Chem.* 2015, **58**, 3471-3484.