 https://doi.org/10.3311/PPch.20662


**Periodica Polytechnica Chemical Engineering**, 66(4), pp. 536–540, 2022

# Assignment of Absolute Configuration to Enantiomers of Anti-Alzheimer Drug Candidate Blarcamesine

Zsuzsanna Szokol[1], Károly Lozsi[1], Attila Virág[2], András Dancsó[1], László Szlávik[1], Gyula Simig[1], Balázs Volk[1*]

[1] Directorate of Drug Substance Development, Egis Pharmaceuticals Plc., H-1475 Budapest, P. O. B. 100, Hungary
[2] Directorate of Finished Product Development, Egis Pharmaceuticals Plc., H-1475 Budapest, P. O. B. 100, Hungary
* Corresponding author, e-mail: volk.balazs@egis.hu

Received: 16 June 2022, Accepted: 19 July 2022, Published online: 30 August 2022

**Abstract**

Blarcamesine is a promising investigational drug for the treatment of Alzheimer's disease. The international nonproprietary name blarcamesine refers to a racemic compound, although it seems likely that it will be marketed in an enantiopure form. A resolution process has been described in the literature, but the absolute configurations of the enantiomers have not yet been disclosed. In the present study, crystals of (*R*)-(-)- and (*S*)-(+)-mandelate salts of (+)- and (-)-blarcamesine and also that of (*R*)-(+)-blarcamesine itself, suitable for single-crystal X-ray diffraction measurement were prepared and the absolute configurations of (+)- and (-)-blarcamesine have been determined.

**Keywords**

blarcamesine, resolution, crystallization, absolute configuration, single-crystal X-ray diffraction

## 1 Introduction

Blarcamesine (ANAVEX2-73, **1**, Fig. 1) is an experimental drug of Anavex Life Sciences acting as a $\sigma_1$ receptor agonist and muscarinic receptor modulator. It is in human Phase III clinical stage, under development for the treatment of Alzheimer's disease, and neuroprotective and neurodevelopmental disorders [1–14].

Blarcamesine is a racemic compound. In a patent application [15] the originator disclosed the advantageous pharmacological properties of the (-)-enantiomer over the (+)-enantiomer therefore launch of an enantiopure drug can be anticipated. Several methods for the preparation of enantiomers of blarcamesine (**1**) are known from the literature [16]. The resolution of compound **1** (Scheme 1) was carried out with mandelic acid enantiomers [(*R*)-(-)-**2** and (*S*)-(+)-**2**] resulting in diastereomeric salts (+)- and (-)-**3**. The enantiomers of **1** were liberated from the diastereomeric salts **3** and obtained as solids after evaporation of ethereal solutions to dryness.

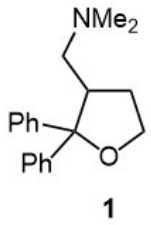
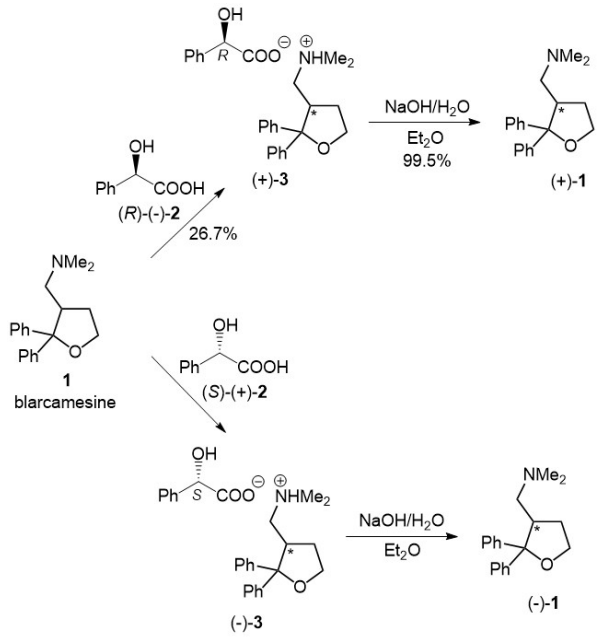



**Fig. 1** Structure of blarcamesine




*the asterisk indicates that the substance is one of the enantiomers

**Scheme 1** Resolution of blarcamesine (**1**) according to the literature

**Cite this article as:** Szokol, Z., Lozsi, K., Virág, A., Dancsó, A., Szlávik, L., Simig, G., Volk, B. "Assignment of Absolute Configuration to Enantiomers of Anti-Alzheimer Drug Candidate Blarcamesine", Periodica Polytechnica Chemical Engineering, 66(4), pp. 536–540, 2022. https://doi.org/10.3311/PPch.20662

A method for the synthesis of blarcamesine enantiomers via separation of covalent diastereomeric derivatives of a racemic intermediate was also described [16]. Racemic alcohol **4** (Scheme 2) was acylated with (*S*)-(-)-camphanic chloride [(*S*)-**5**] and diastereomeric ester (-)-**6** was isolated. Hydrolysis of the ester afforded enantiomerically pure (-)-**4** which was transformed to (-)-**1** by conventional methods. A similar procedure was carried out for the synthesis of (+)-**1** using (*R*)-**5** as the acylating agent. The enantiomeric purities were characterized by optical rotation measurements, *ee* values were not determined.

For the sake of completeness, it has to be mentioned that an enantioselective chemoenzymatic desymmetrization of one of the early intermediates of blarcamesine (**1**) using Amano Lipase PS30 enzyme has also been described [16]. However, the yields and the information disclosed suggest that the procedure is inappropriate for an economical scaled up implementation.

## 2 Results and discussion

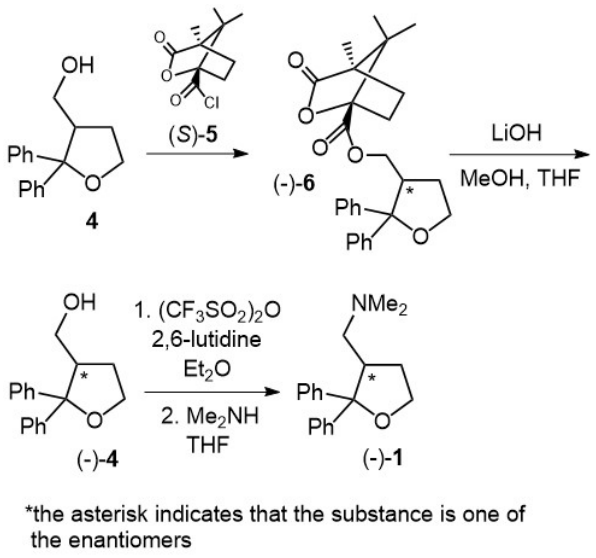
In the course of our work on the process development of blarcamesine drug substance (**1**) it appeared to us that the absolute configuration of its enantiomers had not been disclosed in the literature. Therefore, we aimed to improve the resolution process and to obtain crystals suitable for evaluation of the absolute configurations by single-crystal X-ray diffraction (SCXRD) measurements.

The crude salt (-)-**3** obtained by treatment of racemic blarcamesine (**1**) with 0.52 equivalent of (*S*)-(+)-mandelic acid [(*S*)-(+)-**2**] recrystallized from propan-2-ol resulted in salt (-)-**3** with good yield (39%) and excellent enantiomeric purity (99.88% *ee*, Scheme 3). The absolute configuration of the chiral center in the furan ring, determined by SCXRD measurement of salt (-)-**3**, proved to be *S* (Fig. 2 (a)).

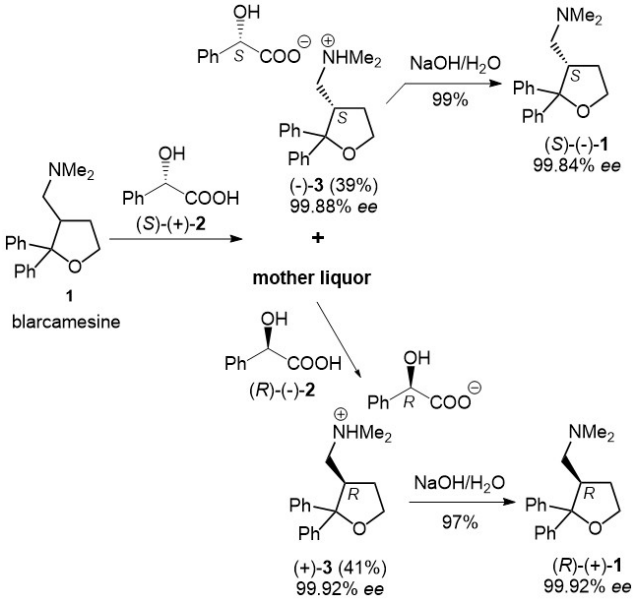
This result was further confirmed by the preparation and SCXRD measurement of salt (+)-**3**. The mother liquor of salt (-)-**3** was treated with (*R*)-(-)-mandelic acid [(*R*)-(-)-**2**] to give salt (+)-**3** (99.92% *ee*). SCXRD measurement of the latter supported the expected *R* absolute configuration of the chiral center in the furan ring (Fig. 2 (b)). The absolute configuration of blarcamesine in salts (-)-**3** and (+)-**3** was determined relative to the known configuration of mandelic acid and supported also by anomalous dispersion, which latter was weak due to the lack of heavy atoms.

Liberation of the bases from salts (-)-**3** and (+)-**3** with aqueous NaOH solution resulted in (*S*)-(-)-**1** (99.84% *ee*) and (*R*)-(+)-**1** (99.92% *ee*) enantiomers of blarcamesine (**1**), respectively, in nearly quantitative yields. In the latter case, our effort to obtain crystals appropriate for SCXRD structure determination was crowned with success (Fig. 2 (c)). The synthetic evidence for the absolute configuration was further supported by the measured anomalous dispersion.

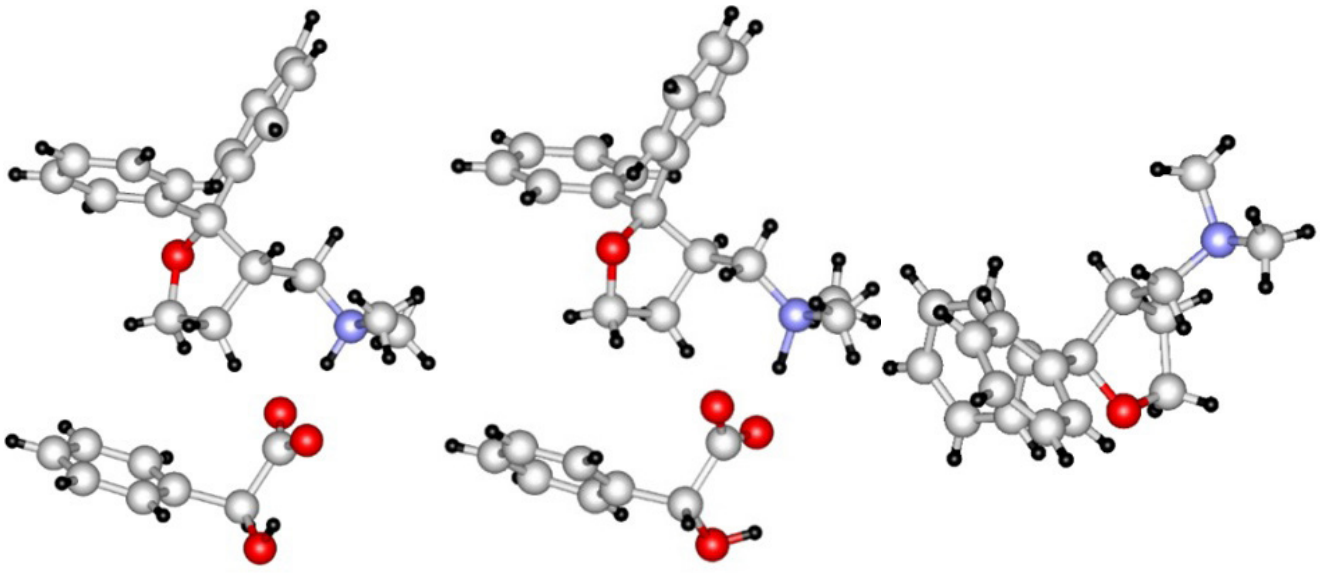
The resolution of **1** was also carried out by changing the sequence of addition of the resolving agents with a smaller batch size (starting from 5.30 g, 18.7 mmol blarcamesine). In this case we first prepared mandelate (+)-**3** (38%, 99.72% *ee*) and then, from the mother liquor the diastereomeric salt (-)-**3** (38%, 99.74% *ee*).




**Scheme 2** Resolution of blarcamesine intermediate (**4**) via covalent diastereomeric derivatives




**Scheme 3** Improved process for the resolution of blarcamesine (**1**)



(a) (b) (c)

**Fig. 2** Single-crystal X-ray structures of (-)-**3** (a), (+)-**3** (b) and (*R*)-(+)-**1** (c)

## 3 Conclusion

The resolution procedure of blarcamesine with (*R*)- and (*S*)-mandelic acid has been improved by recrystallization of the corresponding salts from propan-2-ol resulting in excellent *ee* values and yields. Crystals suitable for SCXRD measurement were grown from (-)-blarcamesine (*S*)-(+)-mandelate, (+)-blarcamesine (*R*)-(-)-mandelate, and also from (*R*)-(+)-blarcamesine, and the absolute configuration of blarcamesine enantiomers was determined.

## 4 Supporting information

The "Supporting Information" contains the ORTEP diagrams, cif, and checkcif files, and structure report files of (-)-**3**, (+)-**3** and (*R*)-(+)-**1** as a zip file and is available on the journal website.

## 5 Experimental section

All melting points were determined on a Büchi Melting Point B-540 melting point apparatus and are uncorrected. IR spectra were obtained on a Bruker ALPHA FT-IR spectrometer operating in transmission mode, in KBr pellets. NMR spectra were recorded at 295 K on a Bruker Avance III HD 600 (600 and 150 MHz for $^{1}$H and $^{13}$C NMR spectra, respectively) spectrometer equipped with a Prodigy cryo-probehead. $^{1}$H assignments were accomplished using general knowledge of chemical shift dispersion with the aid of the $^{1}$H-$^{1}$H coupling pattern. $CDCl_3$ was used as the solvent and tetramethylsilane (TMS) as the internal standard. Chemical shifts ($\delta$) and coupling constants ($J$) are given in ppm and in Hz, respectively. All reagents were purchased from commercial sources and were used without further purification. Reactions were followed by HPLC-MS on a Shimadzu LC-30 HPLC equipment (Kyoto, Japan) equipped with a quaternary pump, degasser, autosampler, column oven, diode array detector and an LCMS-2020 quadrupole mass spectrometer. Elemental analyses were performed on an Elementar Vario MICRO cube CHNS elemental analyzer. Single-crystal X-ray diffraction (SCXRD) measurements were carried out on a Rigaku R-Axis Spider diffractometer with imaging plate area detector using graphite monochromatic Cu-$K_\alpha$ radiation. Single-crystal X-ray structures were deposited at the Cambridge Crystallographic Data Centre under the following numbers: CCDC 2177899 [(-)-**3**], CCDC 2177900 [(+)-**3**], CCDC 2177901 [(*R*)-(+)-**1**].

*Salt of {[(3S)-2,2-diphenyloxolan-3-yl]methyl}dimethylamine formed with (S)-(+)-mandelic acid [(-)-3]:* Blarcamesine (**1**, 60.0 g, 213 mmol) was dissolved in *tert*-butyl methyl ether (MTBE, 180 mL) and (*S*)-(+)-mandelic acid [(*S*)-(+)-**2**, 16.9 g, 110.9 mmol] dissolved in warm propan-2-ol (IPA, 50 mL) was added. The mixture was stirred at room temperature for 2 h. The crude crystalline product separated was filtered, washed with MTBE, dried to give (-)-**3** (40.5 g, 44%, 85.12% *ee*). Recrystallization from IPA resulted in pure (-)-**3** (35.6 g, 39%, 99.88% *ee*). Mp 139–141 °C (IPA). IR (KBr, cm$^{-1}$): 3410, 2893, 2419, 1626, 1347, 1325, 1050, 1020, 730, 694. $^{1}$H NMR ($CDCl_3$, 600 MHz): $\delta$ 7.49 (m, 2H), 7.45 (m, 2H), 7.32 (m, 2H), 7.29 (m, 2H), 7.26 (m, 2H), 7.24 (m, 2H), 7.24 (m, 1H), 7.20 (m, 1H), 7.17 (m, 1H), 4.97 (s, 1H), 4.03 (m, 1H),

3.71 (m, 1H), 3.28 (m, 1H), 2.57 (m, 1H), 2.56 (s, 6H), 2.39 (dd, $J_1$ = 2.8 Hz, $J_2$ = 13.0 Hz, 1H), 2.04 (m, 1H), 1.93 (m, 1H) ppm. $^{13}$C NMR ($CDCl_3$, 150 MHz): 178.25, 144.51, 142.29, 141.98, 128.54, 128.34, 127.96, 127.30, 127.04, 126.37, 125.92, 125.50, 89.31, 74.01, 64.85, 59.58, 43.56, 41.91, 29.28 ppm. Elemental analysis calculated for $C_{27}H_{31}NO_4$ (433.55): C 74.80, H 7.21, N 3.23%, found C 74.79, H 7.47, N 3.19%. $[\alpha]^{25}_D = -128°$ ($c$ = 1, MeOH).

*Salt of {[(3R)-2,2-diphenyloxolan-3-yl]methyl}dimethylamine formed with (R)-(-)-mandelic acid [(+)-**3**]:* (*R*)-(-)-Mandelic acid [(*R*)-(-)-**2**, 16.9 g, 111 mmol] was added to the mother liquor of the crude product (-)-**3**, and the mixture was stirred at room temperature for 2 h. The crystalline product separated was filtered, washed with MTBE and dried to give (+)-**3** (42.8 g, 46%, 91.68% *ee*). Recrystallization from IPA resulted in pure (+)-**3** (38.0 g, 41%, 99.92% *ee*). Mp 139–141 °C (IPA). IR (KBr, $cm^{-1}$): 3410, 2893, 2419, 1626, 1347, 1326, 1050, 1020, 731, 694. $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 7.49 (m, 2H), 7.45 (m, 2H), 7.32 (m, 2H), 7.29 (m, 2H), 7.26 (m, 2H), 7.24 (m, 2H), 7.24 (m, 1H), 7.20 (m, 1H), 7.17 (m, 1H), 4.97 (s, 1H), 4.03 (m, 1H), 3.71 (m, 1H), 3.28 (m, 1H), 2.57 (m, 1H), 2.56 (s, 6H), 2.39 (dd, $J_1$ = 2.6 Hz, $J_2$ = 12.9 Hz, 1H), 2.05 (m, 1H), 1.94 (m, 1H) ppm. $^{13}$C NMR ($CDCl_3$, 150 MHz): 178.27, 144.52, 142.31, 141.97, 128.56, 128.35, 127.97, 127.31, 127.04, 126.38, 125.93, 125.52, 89.32, 74.01, 64.86, 59.61, 43.58, 41.93, 29.30 ppm. Elemental analysis calculated for $C_{27}H_{31}NO_4$ (433.55): C 74.80, H 7.21, N 3.23%, found C 74.78, H 7.52, N 3.18%. $[\alpha]^{25}_D = +128°$ ($c$ = 1, MeOH).

*{[(3S)-2,2-Diphenyloxolan-3-yl]methyl}dimethylamine [(S)-(-)-**1**]:* (*S*)-Mandelate salt (-)-**3** (32.0 g, 73.8 mmol, 99.88% *ee*) was dissolved in water (220 mL), then aqueous NaOH solution (10%, 120 mL) and EtOAc (240 mL) were added, and the mixture was intensively stirred for 20 min. The phases were separated, the aqueous phase was extracted with EtOAc (80 mL). The combined organic phases were extracted with water and brine, dried ($MgSO_4$) and evaporated to give (*S*)-(-)-**1** (20.6 g, 99%, 99.84% *ee*). Mp 73–74 °C. IR (KBr, $cm^{-1}$): 2966, 2761, 1490, 1445, 1050, 748, 706. $^1$H NMR ($CDCl_3$, 600 MHz): δ 7.53 (m, 2H), 7.32 (m, 2H), 7.29 (m, 2H), 7.24 (m, 2H), 7.23 (m, 1H), 7.16 (m, 1H), 4.18 (m, 1H), 3.83 (m, 1H), 3.10 (m, 1H), 2.19 (s, 6H), 2.09 (m, 1H), 2.03 (m, 1H), 1.93 (m, 2H) ppm. $^{13}$C NMR ($CDCl_3$, 150 MHz): $\delta$ 146.43, 143.72, 128.20, 127.68, 126.83, 126.50, 126.38, 126.14, 89.15, 65.61, 61.11, 45.92, 44.43, 29.93 ppm. Elemental analysis calculated for $C_{19}H_{23}NO$ (281.39): C 81.10, H 8.24, N 4.98%, found C 81.24, H 8.31, N 5.00%. $[\alpha]^{25}_D = -243°$ ($c$ = 1, MeOH).

*{[(3R)-2,2-Diphenyloxolan-3-yl]methyl}dimethylamine [(R)-(+)-**1**]:* Starting from (*R*)-mandelate salt (+)-**3** (32.0 g, 73.8 mmol, 99.92% *ee*) and by following the same procedure as for (-)-**3** above, (*R*)-(+)-**1** (20.2 g, 97%, 99.92% *ee*) was prepared. Mp 73–74 °C. IR (KBr, $cm^{-1}$): 2969, 2766, 1491, 1446, 1047, 753, 700. $^1$H NMR ($CDCl_3$, 600 MHz): $\delta$ 7.53 (m, 2H), 7.32 (m, 2H), 7.29 (m, 2H), 7.24 (m, 2H), 7.23 (m, 1H), 7.16 (m, 1H), 4.18 (m, 1H), 3.83 (m, 1H), 3.10 (m, 1H), 2.19 (s, 6H), 2.09 (m, 1H), 2.03 (m, 1H), 1.93 (m, 2H) ppm. $^{13}$C NMR ($CDCl_3$, 150 MHz): 146.43, 143.71, 128.20, 127.67, 126.83, 126.50, 126.37, 126.14, 89.15, 65.61, 61.11, 45.92, 44.43, 29.92 ppm. Elemental analysis calculated for $C_{19}H_{23}NO$ (281.39): C 81.10, H 8.24, N 4.98%, found C 81.12, H 7.92, N 4.96%. $[\alpha]^{25}_D = +243°$ ($c$ = 1, MeOH).

## References

[1] Vamvakides, A., Colocouris, N., Foscolos, G., Fytas, G., Papadopoulou-Daifoti, Z., Pouli, N. "Tetrahydro-*N*,*N*-dimethyl-2,2-diphenyl-3-furanemethanamine, its enantiomers, and their pharmaceutically acceptable acid addition salts", WO 97030983A1, 1997.

[2] Foscolos, G. B., Kolocouris, N., Fytas, G., Marakos, P., Pouli, N., Vamvakides, A. "Synthesis and pharmacological study of some new beta-(dialkylaminomethyl)-gamma-butyrolactones and their tetrahydrofuran analogues", Farmaco, 51(1), pp. 19–26, 1996.

[3] Vamvakides, A. "New sigma(σ)-receptor ligands with anti-apoptotic and/or pro-apoptotic properties over cellular biochemical mechanisms, with neuroprotective, anticancer, anti-metastatic and anti-(chronic) inflammatory action", WO 2008087458A2, 2008.

[4] Villard, V., Espallergues, J., Keller, E., Vamvakides, A., Maurice, T. "Anti-amnesic and neuroprotective potentials of the mixed muscarinic receptor/sigma$_1$ ($\sigma_1$) ligand ANAVEX2-73, a novel aminotetrahydrofuran derivative", Journal of Psychopharmacology, 25(8), pp. 1101–1117, 2011. https://doi.org/10.1177/0269881110379286

[5] Lahmy, V., Meunier, J., Malmström, S., Naert, G., Givalois, L., Kim, S. H., Villard, V., Vamvakides, A., Maurice, T. "Blockade of Tau Hyperphosphorylation and A$\beta_{1-42}$ Generation by the Aminotetrahydrofuran Derivative ANAVEX2-73, a Mixed Muscarinic and $\sigma_1$ Receptor Agonist, in a Nontransgenic Mouse Model of Alzheimer's Disease", Neuropsychopharmacology, 38(9), pp. 1706–1723, 2013. https://doi.org/10.1038/npp.2013.70

[6] Vamvakides, A. "Optimization and therapeutic valorization of the symptomatic treatment of Alzheimer's disease with Rivastigmine, Galantamine or Donepezil, by selected aminotetrahydrofurans acting as mixed sigma-1 muscarinic ligands", WO 2014155138A1, 2014.

[7] Missling, C. U. "Neurodevelopmental disorder therapy", WO 2017132127A1, 2017.

[8] Missling, C. "A2-73 as a therapeutic for insomnia, anxiety, and agitation", WO 2018022848A1, 2018.

[9] Cecchi, M. "Anavex2-73 for the treatment of Alzheimer's disease", WO 2018231216A1, 2018.

[10] Kaufmann, W. E., Sprouse, J., Rebowe, N., Hanania, T., Klamer, D., Missling, C. U. "ANAVEX®2-73 (blarcamesine), a Sigma-1 receptor agonist, ameliorates neurologic impairments in a mouse model of Rett syndrome", Pharmacology Biochemistry and Behavior, 187, 172796, 2019. https://doi.org/10.1016/j.pbb.2019.172796

[11] Missling, C. U., Afshar, M. M., Parmentier, F. "Optimized sigma-1 agonist method of responder selection and treatment", WO 2019222754A1, 2019.

[12] Lisak, R. P., Nedelkoska, L., Benjamins, J. A. "Sigma-1 receptor agonists as potential protective therapies in multiple sclerosis", Journal of Neuroimmunology, 342, 577188, 2020. https://doi.org/10.1016/j.jneuroim.2020.577188

[13] Missling, C. U., Selvey, A. "Anavex2-73 for the treatment of genetic neurodevelopmental disorders", WO 2021016314A1, 2021.

[14] Reyes, S. T., Deacon, R. M. J., Guo, S. G., Altimiras, F. J., Castillo, J. B., van der Wildt, B., Morales, A. P., Park, J. H., Klamer, D., Rosenberg, J., Oberman, L. M., Rebowe, N., Sprouse, J., Missling, C. U., McCurdy, C. R., Cogram, P., Kaufmann, W. E., Chin, F. T. "Effects of the sigma-1 receptor agonist blarcamesine in a murine model of fragile X syndrome: neurobehavioral phenotypes and receptor occupancy", Scientific Reports, 11(1), 17150, 2021. https://doi.org/10.1038/s41598-021-94079-7

[15] Neliat, G. "Enantiomers of tetrahydro-N,N-dimethyl-2,2-diphenyl-3-furanmethanamine (ANAVEX2-73) and use thereof in the treatment of Alzheimer's disease and other disorders modulated by the sigma 1 receptor", WO 2017013496A1, 2017.

[16] Wamvakides, A., Moutsos, V., Schmitt, M. "Synthesis of (+) and (-) 1-(5,5-diphenyltetrahydrofuran-3-yl)-*N*,*N*-dimethylmethanamine, (+) and (-) 1-(2,2-diphenyltetrahydrofuran-3-yl)-*N*,*N*-dimethylmethanamine and (+) and (-) 1-(2,2-diphenyltetrahydrofuran-3-yl)-*N*-methylmethanamine", WO 2013008044A1, 2013.