Med Chem Res
DOI 10.1007/s00044-017-2088-2

MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Synthesis of novel acridine-sulfonamide hybrid compounds as acetylcholinesterase inhibitor for the treatment of alzheimer's disease

Ramazan Ulus[1] · İbrahim Esirden[1] · Burak Aday[1] · Gurbet Çelik Turgut[2] · Alaattin Şen[2] · Muharrem Kaya[3]

Received: 2 November 2015 / Accepted: 25 September 2017

Abstract In this study we report that amino acridine intermediates **7** and **8** were obtained from the reduction of nitro acridine derivatives **5** and **6** that were synthesized via the condensation of dimedone, *p*-nitrobenzaldehyde with various amine derivatives, respectively. Then acridine sulfonamide hybrid compounds (**9–18**) were synthesized by the reaction of amino acridine **7, 8** with sulfonyl chlorides. The new hybrid compounds were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, and HRMS analyzes. The evaluation of in vitro anticholinesterase action of the synthesized compounds against AChE showed that some of them are potent inhibitors. Among them, compound **17** showed the most potent activity against AChE with an IC_{50} of 0.14 µM.

Keywords Anticholinesterase activity · Tacrine · Acridine · Sulfonamide

Introduction

Alzheimer's disease (AD), the most common form of dementia among the elderly, is a progressive degenerative disorder of the brain with loss of memory and cognition. Inhibition of human AChE is an effective means for treating memory loss in early stage of Alzheimer's disease. Tacrine molecules (Scheme 1), an acridine derivative, are heterocyclic compounds and these compounds are known as anti-Alzheimer's agent. The first synthesis of tacrine was reported in 1945 by Albert and Gledhill. In 1961, Heilbronn showed that tacrine is a reversible inhibitor of both AChE and another biologically important cholinesterase, butyrylcholinesterase (BChE). Tacrine received FDA approval for AD-related dementia in 1993. This drug may be an effective anti-Alzheimer's agent but these drugs tend to pose tolerability problems in many patients because of reduced adverse side effects. Also, tacrine has been discontinued in the US due to safety concerns in 2013. Thus, it is necessary to develop new compounds and new drugs with better activity and lesser side effects (Marco-Contelles et al. 2009; Anand and Singh 2013; Hamulakovaa et al. 2014)

Acridine molecules are one of the principal classes of heterocyclic compounds that contains nitrogen atom and they are planar tricyclic aromatic molecules. These molecules were discovered in nineteenth century and their synthesis and application have been widely studied until today. Recently, acridine-based compounds have been reported to have many important biological properties such as fungicides (Srivastava and Nizamuddin 2004), antibacterial (Palani et al. 2005), anti-multidrug-resistant (Gallo et al. 2003), cytotoxic (Antonini et al. 1999), antimicrobial (Kaya et al. 2011), β-channel opener in cardiovascular disease (Öcal et al. 2002), and as carbonic anhydrase inhibitor for glaucoma treatment (Kaya et al. 2012, 2013; Yeşildağ et al. 2014).

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00044-017-2088-2) contains supplementary material, which is available to authorized users.

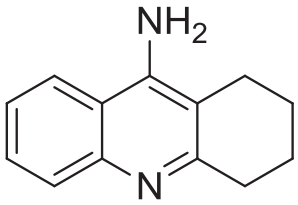
✉ Muharrem Kaya
mhrkaya@yahoo.com

[1] Chemistry Department, Faculty of Arts and Science, Dumlupınar University, 43100 Kütahya, Turkey

[2] Biology Department, Faculty of Arts & Science, Pamukkale University, 20070 Denizli, Turkey

[3] Biochemistry Department, Faculty of Arts and Science, Dumlupınar University, 43100 Kütahya, Turkey

Published online: 13 October 2017



Scheme 1 Tacrine

Acetylcholinesterase (AChE) belongs to the serine hydrolase family that handles acetylcholine breakdown in the body. Although the primary role of AChE is hydrolysis of ACh, it also plays an important part in the expression of amyloid precursor protein, promotion of amyloid assembly, phosphorylation of tau protein, inflammation, cell proliferation, and apoptosis (Soreq and Seidman 2001; Silman and Sussman 2005). Design and synthesis of compounds that can inhibit AChE activities have recently gained higher interest since AChE inhibitors are the key compounds used to treat Alzheimer's disease (AD) and myasthenia gravis.

Sulfonamides are another important compounds family for the medicinal industry and they are now extensively used drugs for the treatment or conservation of different illnesses (Supuran and Scozzafava 2001). In clinical medicine, they have been used as anticancer (Monti et al. 2013), antimicrobial (Kaya et al. 2013), antiobesity (Scozzafava et al. 2013), carbonic anhydrase inhibitors (Zolnowska et al. 2014; Slawinski et al. 2014a, b), and acetylcholinesterase inhibitor agents for Alzheimer's disease (Bag et al. 2015).

For this reason, novel acridine-sulfonamide hybrid compounds were synthesized and characterized by Fourier transform infrared, ^{1}H-nuclear magnetic resonance (NMR), ^{13}C-NMR, and high-resolution mass spectral (HRMS) analyzes. The inhibitory effects of newly synthesized compounds on acetylcholinesterase enzyme were investigated under in vitro conditions using SH-SY5Y cell lines AChE activity.

Methods

General procedure for preparation of acridine derivatives (5 and 6)

A mixture of dimedone **1** (2 mmol), *p*-nitrobenzaldehyde **2** (1 mmol), 4-chloroaniline **3** (1 mmol), and dodecylbenzenesulphonic acid (DBSA) catalyst (10% mmol) in 10 mL H_2O was heated to reflux continuously for 6 h. The reaction progress was monitored by thin layer chromatography (TLC). The solid product was filtered, washed with 500 mL water, and recrystallized from ethanol (88–91%) (Kaya et al. 2012).

*10-(4-Chlorophenyl)-3,3,6,6-tetramethyl-9-(4-nitrophenyl)-3,4,6,7,9,10-hexahydro acridine-1,8(2H,5H)-dione (**5**)*

As yellow solid, (89%), mp 310 °C (ethanol) (Tang et al. 2010).

*10-(4-Bromophenyl)-3,3,6,6-tetramethyl-9-(4-nitrophenyl)-3,4,6,7,9,10-hexahydro acridine-1,8(2H,5H)-dione (**6**)*

Yield 91%; m.p. 315–316 oC; IR (ATR) ν_{max} 3047, 2956, 1631, 1575 cm^{-1}; ^{1}H NMR (300 MHz, dimethyl sulphoxide (DMSO)-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.80 (d, 2H, $J = 17.46$Hz, –CH_2), 2.00 (d, 2H, $J = 15.93$ Hz, –CH_2), 2.18–2.24 (m, 4H, 2x-CH_2), 5.10 (s, 1H, –CH) 7.48 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.58 (d, 2H, $J = 8.7$ Hz, Ar–H), 7.83 (d, 2H, $J = 8.4$ Hz, Ar–H), 8.14 (d, 2H, $J =$ 8.7 Hz, Ar–H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.57, 29.60, 32.47, 33.35, 41.38, 49.86, 112.50, 123.21, 123.75, 129.42, 132.46, 133.50, 137.98, 146.16, 151.25, 154.00, 195.51; HRMS (QTOF-ESI): *m/z* calcd. for $C_{29}H_{29}BrN_2O_4$: 548.1311; found: 571.1209 $[M + Na]^+$.

General procedure for preparation of reduced acridine compounds (7 and 8)

The acridine compounds (**5**) were dissolved in 10 ml ethanol. This solution of sodium poly-sulfur was then added drop wise to a stirred and warm solution of compound **5** (1 mmol) in 50 ml ethanol–water. The progress of the reaction was monitored by TLC. Once the reaction is completed, the mixture was cooled to room temperature and solid filtered off and washed with H_2O. The sulfonamide product was purified and recrystallized from the ethanol (84–87%) (Kaya et al. 2013).

*9-(4-Aminophenyl)-10-(4-chlorophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro acridine-1,8(2H,5H)-dione (**7**)*

Yield 84%; m.p. 254–255 °C; IR (ATR) ν_{max} 3408, 3047, 2956, 1631, 1575 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.40$ Hz, –CH_2), 1.99 (d, 2H, $J = 16.03$ Hz, –CH_2), 2.14–2.20 (m, 4H, 2x-CH_2), 4.79 (s, 2H, –NH_2), 4.86 (s, 1H, –CH) 6.42 (d, 2H, $J = 8.3$ Hz, Ar–H), 6.93 (d, 2H, $J =$ 8.3 Hz, Ar–H), 7.42 (s, 2H, Ar–H), 7.67 (d, 2H, $J = 8.8$ Hz, Ar–H); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.53, 29.80, 30.94, 32.43, 41.34, 50.17, 114.11, 114.24, 128.37, 130.52, 132.08, 134.26, 134.53, 137.99, 146.81, 149.88, 195.58; HRMS (QTOF-ESI): *m/z* calcd. for $C_{29}H_{31}ClN_2O_2$: 474.2074; found: 473.2000 $[M - H]^-$.

*9-(4-Aminophenyl)-10-(4-bromophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro acridine-1,8(2H,5H)-dione (**8**)*

Yield 87%; m.p. 268–269 °C; IR (ATR) ν_{max} 3404, 3063, 2947, 1630, 1578 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.75 (d, 2H, J = 17.32 Hz, –CH_2), 1.99 (d, 2H, J = 16.04 Hz, –CH_2), 2.14–2.20 (m, 4H, 2x-CH_2), 4.80 (s, 2H, –NH_2), 4.90 (s, 1H, –CH) 6.42 (d, 2H, J = 8.3 Hz, Ar–H), 6.94 (d, 2H, J = 8.3 Hz, Ar–H), 7.43 (s, 2H, Ar–H), 7.67 (d, 2H, J = 8.8 Hz, Ar–H); ^{13}C-NMR (75 MHz, DMSO-d_6): 26.53, 29.79, 30.92, 32.44, 41.33, 50.17, 114.07, 114.23, 122.88, 128.35, 132.46, 133.49, 134.46, 138.42, 146.87, 149.81, 195.57; HRMS (QTOF-ESI): *m/z* calcd. for $C_{29}H_{31}BrN_2O_2$: 518.1569; found: 519.1469 $[M + H]^+$.

General procedure for preparation of acridine-sulfonamide hybrid compounds (9–18)

A mixture of the amino acridine compound (0.5 mmol) and the sulphonyl chlorides (0.5 mmol) in dry THF (5 ml) was refluxed for 12 h. After the solvent was removed in vacuo, the crude product was purified by recrystallization from ethanol (72–90%) (Supuran and Scozzafava 2001).

N-*(4-(10-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl) benzenesulfonamide (**9**)*

Yield 80%; m.p. 290–291 °C; IR (ATR) ν_{max} 3203, 3060, 2958, 1632, 1572, 1507, 1363, 1160 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, J = 17.37 Hz, –CH_2), 1.97 (d, 2H, J = 16.01 Hz, –CH_2), 2.12–2.18 (m, 4H, 2x-CH_2), 4.90 (s, 1H, –CH), 6.94 (d, 2H, J = 8.4 Hz, Ar–H), 7.13 (d, 2H, J = 8.4 Hz, Ar–H), 7.43–7.69 (m, 9 H, Ar–H), 10.10 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.40, 29.69, 31.64, 32.42, 41.33, 49.98, 113.37, 120.59, 126.96, 128.55, 129.54, 130.49, 132.13, 133.20, 134.35, 135.70, 137.73, 140.12, 142.62, 150.49, 195.50; HRMS (QTOF-ESI): *m/z* calcd. for $C_{35}H_{35}ClN_2O_4S$: 614.2006; found: 637.1940 $[M + Na]^+$.

N-*(4-(10-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl)-4-methylbenzenesulfonamide (**10**)*

Yield 81%; m.p. 294–295 °C; IR (ATR) ν_{max} 3200, 3054, 2959, 1631, 1571, 1508, 1363, 1162 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, J = 17.36Hz, –CH_2), 1.97 (d, 2H, J = 16.07 Hz, –CH_2), 2.12–2.18 (m, 4H, 2x-CH_2), 2.31 (s, 3 H, –CH_3), 4.90 (s, 1H, –CH), 6.93 (d, 2H, J = 8.4 Hz, Ar–H), 7.13 (d, 2H, J = 8.4 Hz, Ar–H), 7.27 (d, 2H, J = 8.1 Hz, Ar–H), 7.45 (d, 2H, J = 8.1 Hz, Ar–H), 7.57 (d, 2H, J = 8.2 Hz, Ar–H), 7.66 (d, 2H, J = 8.8 Hz, Ar–H), 10.05 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 21.41, 26.40, 29.69, 31.64, 32.42, 41.33, 49.99, 113.40, 120.32, 127.02, 128.54, 130.00, 130.50, 132.16, 134.35, 135.85, 137.34, 137.74, 142.44, 143.51, 150.48, 195.51; HRMS (QTOF-ESI): *m/z* calcd. for $C_{36}H_{37}ClN_2O_4S$: 628.2163; found: 651.2057 $[M + Na]^+$.

N-*(4-(10-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl)-4-methoxybenzenesulfonamide (**11**)*

Yield 82%; m.p. 300–302 °C; IR (ATR) ν_{max} 3242, 3051, 2959, 1637, 1580, 1500, 1362, 1151 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, J = 17.41 Hz, –CH_2), 1.97 (d, 2H, J = 16.14 Hz, –CH_2), 2.12–2.18 (m, 4H, 2x-CH_2), 3.80 (s, 3H, –OCH_3), 4.90 (s, 1H, –CH), 6.92–7.00 (m, 4H, Ar–H), 7.13 (d, 2H, J = 8.4 Hz, Ar–H), 7.45 (d, 2H, J = 7.9 Hz, Ar–H), 7.60–7.68 (m, 4H, Ar–H), 9.95 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.40, 29.69, 31.62, 32.42, 41.32, 49.99, 56.00, 113.41, 114.67, 120.26, 128.51, 129.19, 130.51, 131.80, 132.15, 134.35, 135.98, 137.74, 142.34, 150.48, 162.74, 195.51; HRMS (QTOF-ESI): *m/z* calcd. for $C_{36}H_{37}ClN_2O_5S$: 644.2112; found: 667.2024 $[M + Na]^+$.

4-Bromo-N-*(4-(10-(4-chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl) phenyl)benzenesulfonamide (**12**)*

Yield 85%; m.p. 324–326 °C; IR (ATR) ν_{max} 3191, 3064, 2959, 1638, 1570, 1509, 1362, 1165 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.65 (s, 6H, 2x-CH_3), 0.85 (s, 6H, 2x-CH_3), 1.74 (d, 2H, J = 17.43 Hz, –CH_2), 1.97 (d, 2H, J = 16.03 Hz, –CH_2), 2.16 (d, 4H, J = 16.63 Hz, 2x-CH_2), 4.90 (s, 1H, –CH), 6.94 (d, 2H, J = 8.4 Hz, Ar–H), 7.15 (d, 2H, J = 8.4 Hz, Ar–H), 7.45 (d, 2H, J = 7.9 Hz, Ar–H), 7.57–7.71 (m, 6H, Ar–H), 10.20 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.37, 29.71, 31.69, 32.42, 41.33, 49.98, 113.35, 120.84, 127.08, 128.65, 129.03, 130.50, 132.13, 132.65, 134.36, 135.37, 137.72, 139.30, 142.92, 150.50, 195.48; HRMS (QTOF-ESI): *m/z* calcd. for $C_{35}H_{34}BrClN_2O_4S$: 692.1111; found: 717.0994 $[M + Na]^+$.

N-*(4-(10-(4-Chlorophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl)-2,4,6-trimethylbenzenesulfonamide (**13**)*

Yield 72%; m.p. 308–309 °C; IR (ATR) ν_{max} 3196), 3049, 2957, 1636, 1573, 1507, 1363, 1152 cm^{-1}; ^{1}H NMR (300

MHz, DMSO-d_6): δ 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.45$ Hz, –CH_2), 1.96 (d, 2H, $J = 15.99$ Hz, –CH_2), 2.12–2.20 (m, 7H, 2x-CH_2 and –CH_3), 2.47 (s, 6H, 2x-CH_3), 4.90 (s, 1H, –CH), 6.84 (d, 2H, $J = 7.8$ Hz, Ar–H), 6.95 (s, 2H, Ar–H), 7.12 (d, 2H, $J = 7.9$ Hz, Ar–H), 7.45, (d, 2H, $J = 7.8$ Hz, Ar–H), 7.66 (d, 2H, $J = 7.9$ Hz, Ar–H), 9.90 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.83, 22.90, 26.44, 29.69, 31.72, 32.41, 41.33, 49.98, 113.40, 120.63, 128.55, 130.50, 132.15, 134.35, 134.58, 135.46, 137.74, 138.99, 142.24, 142.51, 150.47, 195.50; HRMS (QTOF-ESI): *m/z* calcd. for $C_{38}H_{41}ClN_2O_4S$: 656.2476; found: 679.2368 $[M + Na]^+$.

N-*(4-(10-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl) benzenesulfonamide (**14**)*

Yield 83%; m.p. 295–296 °C; IR (ATR) ν_{max} 3190, 3059, 2956, 1634, 1572, 1507, 1363, 1160 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.65 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.37$ Hz, –CH_2), 1.97 (d, 2H, $J = 16.05$ Hz, –CH_2), 2.12–2.18 (m, 4H, 2x-CH_2), 4.90 (s, 1H, –CH), 6.94 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.13 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.38 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.45–7.60 (m, 3H, Ar–H), 7.67 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.79 (d, 2H, $J = 8.8$ Hz, Ar–H), 10.10 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.40, 29.69, 31.65, 32.43, 41.33, 49.98, 113.37, 120.59, 123.00, 126.96, 128.55, 129.54, 132.45, 133.21, 133,45, 135.70, 138.16, 140.12, 142.62, 150.43, 195.50; HRMS (QTOF-ESI): *m/z* calcd. for $C_{35}H_{35}BrN_2O_4S$: 658.1501; found: 683.1387 $[M + Na]^+$.

N-*(4-(10-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl)-4-methoxybenzenesulfonamide (**15**)*

Yield 84%; m.p. 304 °C; IR (ATR) ν_{max} 3243, 3047, 2959, 1636, 1578, 1360, 1149 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.65 (s, 6H, 2x-CH_3), 0.85 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.32$ Hz, –CH_2), 1.97 (d, 2H, $J = 16.05$ Hz, –CH_2), 2.12–2.18 (m, 4H, 2x-CH_2), 3.75 (s, 3H, –OCH_3), 4.90 (s, 1H, –CH), 6.91–6.99 (m, 4H, Ar–H), 7.12 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.38 (d, 2H, $J = 7.8$ Hz, Ar–H), 7.59–7.63 (m, 2H, Ar–H), 7.79 (d, 2H, $J = 8.7$ Hz, Ar–H), 9.95 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.41, 29.69, 31.61, 32.43, 41.32, 50.00, 56.00, 113.40, 114.67, 120.26, 122.99, 128.51, 129.19, 131.80, 132.44, 133.45, 135.98, 138.17, 142.34, 150.42, 162.74, 195.51; HRMS (QTOF-ESI): *m/z* calcd. for $C_{36}H_{37}BrN_2O_5S$: 688.1607; found: 713.1487 $[M + Na]^+$.

*4-Bromo-*N*-(4-(10-(4-bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl) phenyl)benzenesulfonamide (**16**)*

Yield 88%; m.p. 330–332 °C; IR (ATR) ν_{max} 3158, 3063, 2955, 1638, 1626, 1571, 1362, 1165 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.65 (s, 6H, 2x-CH_3), 0.85 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.41$ Hz, –CH_2), 1.98 (d, 2H, $J = 16.05$ Hz, –CH_2), 2.13–2.18 (m, 4H, 2x-CH_2), 4.90 (s, 1H, –CH), 6.94 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.15 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.38 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.59 (d, 2H, $J = 8.6$Hz, Ar–H), 7.70 (d, 2H, $J = 8.6$Hz, Ar–H), 7.80 (d, 2H, $J = 8.7$ Hz, Ar–H), 10.20 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 26.36, 29.70, 31.69, 32.43, 41.33, 49.98, 113.34, 120.84, 123.01, 127.08, 128.65, 129.03, 132.65, 133.45, 135.37, 138.15, 139.29, 142.92, 150.45, 195.49; HRMS (QTOF-ESI): *m/z* calcd. for $C_{35}H_{34}Br_2N_2O_4S$: 736.0606; found: 739.0653 $[M + H]^+$.

N-*(4-(10-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl)-2,4,6-trimethylbenzenesulfonamide (**17**)*

Yield 78%; m.p. 312 °C; IR (ATR) ν_{max} 3357, 3052, 2958, 1640, 1578, 1506, 1363, 1158 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): 0.70 (s, 6H, 2x-CH_3), 0.90 (s, 6H, 2x-CH_3), 1.74 (d, 2H, $J = 17.40$ Hz, –CH_2), 1.97 (d, 2H, $J = 15.97$ Hz, –CH_2), 2.12–2.20 (m, 7 H, 2x-CH_2 and –CH_3), 2.45 (s, 6H, 2x-CH_3) 4.90 (s, 1H, –CH), 6.83 (d, 2H, $J = 8.4$ Hz, Ar–H), 6.95 (s, 2H, Ar–H), 7.12 (d, 2H, $J = 8.4$ Hz, Ar–H), 7.38 (d, 2H, $J = 8.1$ Hz, Ar–H), 7.79 (d, 2H, $J = 8.7$ Hz, Ar–H), 9.90 (s, 1H, –NH); ^{13}C-NMR (75 MHz, DMSO-d_6): δ 20.84, 22.91, 26.45, 29.69, 31.72, 32.43, 41.33, 49.99, 113.41, 120.64, 123.00, 128.55, 132.15, 132.44, 133.44 134.59, 135.46, 138.18, 139.00, 142.24, 142.51, 150.41, 195.49; HRMS (QTOF-ESI): *m/z* calcd. for $C_{38}H_{41}BrN_2O_4S$: 700.1970; found: 725.1843 $[M + Na]^+$.

N-*(4-(10-(4-Bromophenyl)-3,3,6,6-tetramethyl-1,8-dioxo-1,2,3,4,5,6,7,8,9,10-decahydro acridin-9-yl)phenyl) naphthalene-2-sulfonamide (**18**)*

Yield 90%; m.p. 320–321 °C; IR (ATR) ν_{max} 3188, 3055, 2956, 1634, 1577, 1506, 1363, 1157 cm^{-1}; ^{1}H NMR (300 MHz, DMSO-d_6): δ 0.60 (s, 6H, 2x-CH_3), 0.80 (s, 6H, 2x-CH_3), 1.70 (d, 2H, $J = 17.33$ Hz, –CH_2), 1.92 (d, 2H, $J = 15.98$ Hz, –CH_2), 2.10–2.15 (m, 4H, 2x-CH_2), 4.90 (s, 1H, –CH), 6.98 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.12 (d, 2H, $J = 8.5$ Hz, Ar–H), 7.35 (d, 2H, $J = 7.6$Hz, Ar–H), 7.59–7.71 (m, 3 H, Ar–H), 7.77 (d, 2H, $J = 8.7$ Hz, Ar–H), 7.96–8.07 (m, 3H, Ar–H), 8.35 (s, 1H, Ar–H), 10.20 (s, 1H, –NH); ^{13}C-

NMR (75 MHz, DMSO-d_6): δ 26.32, 29.64, 31.68, 32.38, 41.29, 49.94, 113.35, 120.66, 122.42, 122.97, 128.02, 128.20, 128.27, 128.59, 129.28, 129.61, 129.72, 131.96, 132.44, 133.41, 134.63, 135.68, 137.17, 138.14, 142.65, 150.36, 195.45; HRMS (QTOF-ESI): *m/z* calcd. for $C_{39}H_{37}BrN_2O_4S$: 708.1657; found: 733.1545 $[M + Na]^+$.

Cell culture

The human neuroblastoma cell line (SH-SY5Y) was obtained from ATCC. The cells were cultured in DMEM: F12 supplemented with 10% FBS and 1% penicillin/streptomycin mixture in a humidified atmosphere of 95% air with 5% CO_2 at 37 °C and were sub-cultured twice a week.

Preparation of cell homogenate

SH-SY5Y cells (1×10^6 cells per 75 cm^2 flask) were washed twice with PBS and scraped from culture dishes in lysis buffer (0.1 M phosphate buffer, pH 7.8, 0.2% Triton X-100, 2 mM EDTA, 0.5 mM PMFS, 0.3 mM EACA, and 1 mM DTT) and homogenized mechanically by sonication. Total cellular protein concentration was determined by Bradford assay using BSA as the standard (Bradford 1976).

Cholinesterase assay

SH-SY5Y cell lysate AChE activity was characterized using acetylthiocholine iodide (ATCh-I) substrate by following the appearance of thiocholine-DTNB conjugate at 412 nm according to the method of Ellman (Ellman et al. 1961). The test compounds were dissolved in a mixture of DMSO and 0.1 M Tris–HCl buffer pH 8.5 (the final concentration of DMSO is 0.02%. For each compound, at least five different concentrations were tested to obtain the range of 10–90% enzyme inhibition. AChE activities were assayed in a medium containing 0.6 DTNB in 80 mM Tris–HCl buffer pH 8.5, 1 mg protein, and test compound. After incubation for 5 min at 25 °C, reaction was initiated by adding 2.5 mM ATCh-I and the reaction rate was monitored spectrophotometrically on an Analytik Jena Specord 200 UV-1601 double-beam spectrophotometer at 412 nm for 150 s at 25 °C. Finally, the IC_{50} values were determined graphically from inhibition curves (log inhibitor concentration vs. percent of inhibition). One unit activity was defined to be the amount of enzyme that catalyzed the hydrolysis of 1 mmol of substrate/min at saturating substrate concentration (Talesa et al. 1993).

Results and discussion

Chemistry

The general synthetic method shown in Scheme 2 was used to prepare the novel acridine sulfonamide hybrid compounds **9–18**.

In the first step, acridine compounds were synthesized in water in a single process through three successive reactions (Aldol condensation, Michael addition, and cyclization) and p-DBSA. Dimedone (**1**) were condensed with 4-nitro benzaldehyde (**2**) and various amine derivatives (**3** and **4**), in the molar range of 2:1:1.

In the second step, synthesized acridine compounds (**5**, **6**) are reduced in the presence of sodium poly-sulfur in ethanol-water mixture. As a result of reduction, compounds 9-(4-aminophenyl)-10-(4-chlorophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydroacridine-1,8(2*H*, 5*H*)-dione (**7**) and 9-(4-aminophenyl)-10-(4-bromophenyl)-3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro acridine-1,8 (2*H*, 5*H*)-dione (**8**) are obtained.

In the last step, novel acridine sulfonamide hybrid compounds (**9–18**) were obtained by interacting various sulfonyl chlorides with the compounds **7** and **8**, which are obtained as a result of the reduction reaction in dry THF refluxed for 12 h.

The infrared (IR) spectra of all the acridine compounds showed sharp peaks for the carbonyl groups in the region between 1640–1626 cm^{-1}. Besides, in the IR spectra of the compounds, aliphatic C–H stretching bands were observed between 2962–2947 cm^{-1} and aromatic C–H stretching bands were observed between 3064–3034 cm^{-1}.

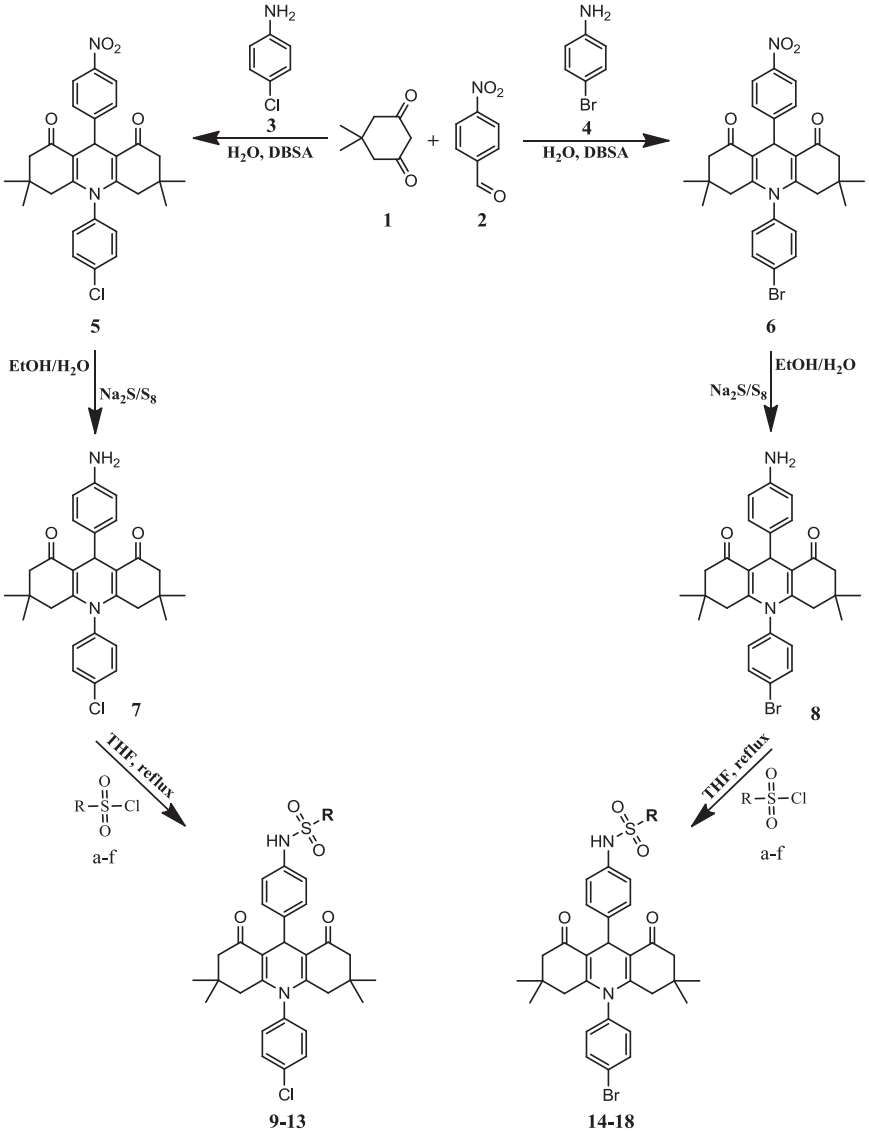
The ^{1}H-NMR spectra of the acridine compounds (**5–18**) belonging to protons of the methyl groups showed singlet peaks in position 3 and 6 between 0.60 and 0.90 ppm. The $-CH_2$ group protons of the cyclohexene rings of the compounds **5–18** showed doublet peaks in the 1.69–2.24 ppm. Signals for the methoxy group of protons for compounds **12** and **16** were shown in the range of 3.75–3.80 ppm. The signals for the –CH protons were observed at 4.86–5.10 ppm and the signals for the aromatic protons were observed in the range of 6.42–8.14 ppm. All compounds found in sulfonamide groups –NH protons were observed between 9.90 and 10.20 ppm.

The ^{13}C-NMR (APT) spectra of all the compounds **5–18** showed carbonyl carbons peaks at 195.45–195.58 ppm (Ulus et al. 2015; Pamuk et al. 2015). Also, when their high-resolution mass spectra (HRMS) are examined, the observed molecule ion peaks are in compliance with the recommended structures.

Anticholinesterase activity

All compounds (**5–18**) reported here and the standard drug tacrine were assayed as inhibitors of human

Scheme 2 The synthesis of novel acridine-sulfonamide hybrid compounds



	a	b	c	d	e	f
R	C_6H_5-	*p*-$CH_3C_6H_4$-	*p*-BrC_6H_4-	*p*-$CH_3OC_6H_4$-	2,4,6-$(CH_3)_3C_6H_2$-	2-Naphthyl-

acetylcholinesterase (AChE) (Table 1). As seen from data of Table 1, IC_{50} analysis values of the compounds revealed that some compounds had potent in vitro anticholinesterase activity. The following structure–activity relationship (SAR) could be observed: The *h*AChE was inhibited with inhibition constants in the micromolar range by most of the newly synthesized amino-acridine compounds (**7** and **8**) and acridine-sulfonamides **7–18**, which showed IC_{50} of 0.14–1.46 μM (Table 1). The compound 14 was less effective as *h*AChE inhibitors with IC_{50} of 1.41 ± 0.045 μM. The novel synthesized compounds **9–18** showed better inhibition effects than tacrine molecule. In addition, the compound **13** (IC_{50} 0.15 ± 0.012), compound **17** (IC_{50} 0.14 ± 0.011 μM), possessing 2-mesitylene sulfonamide substituent as the part of the molecule that is originally present in the amino component, were the best AChE inhibitor.

Conclusions

A series of novel acridine sulfonamide hybrid compounds showed selective AChE inhibitor effects. In order to determine the in vitro anti-acetylcholinesterase activity, SH-SY5Y human neuroblastoma cells were used as AChE source since it is human? and the expression, activity and localization of the AChE are well documented (Thullbery

Table 1 The acetylcholinesterase inhibition activities of novel acridine-sulfonamide hybrid compounds

Entry	−R	IC_{50} (μM)
5	–	0.48 ± 0.021
6	–	0.56 ± 0.015
7	–	0.46 ± 0.023
8	–	0.39 ± 0.018
9	$-C_6H_5$	0.19 ± 0.018
10	p-$CH_3C_6H_4$	0.32 ± 0.022
11	p-$CH_3OC_6H_4$	0.33 ± 0.012
12	p-BrC_6H_4	0.20 ± 0.014
13	2,4,6-$(CH_3)_3C_6H_2$	0.15 ± 0.012
14	$-C_6H_5$	1.41 ± 0.045
15	p-$CH_3OC_6H_4$	0.34 ± 0.021
16	p-BrC_6H_4	0.22 ± 0.018
17	2,4,6-$(CH_3)_3C_6H_2$	0.14 ± 0.011
18	2-Naphthyl	0.22 ± 0.018
Tacrine[a]	–	0.50 ± 0.020

[a] Tacrine was used as a standard inhibitor for all acetylcholinesterase investigated here

et al. 2005). Therefore, it yields much more precise result for inhibition studies since it is known that small amino acid substitutions may strongly affect the stereoselectivity of inhibitors (Bosak et al. 2008). Analysis of IC_{50} values of the synthesized compounds demonstrated that some of them can be potently drug since they are selective inhibitors of AChE. Among them, **17** with IC_{50} value of 0.14 μM was the most potent compound. Further studies such as toxicity and safety evaluations should be carried out to determine its effectiveness as model compounds for potential drugs.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

References

Anand P, Singh B (2013) A review on cholinesterase inhibitors for Alzheimer's disease. Arch Pharmacal Res 36:375–399

Antonini I, Polucci P, Kelland RL, Menta E, Pescalli N, Martelli S (1999) 2,3-dihydro-1H,7H-pyrimido[5,6,1-de]acridine-1,3,7-trione derivatives, a class of cytotoxic agents active on multidrug-resistant cell lines: synthesis, biological evaluation, and structure−activity relationships. J Med Chem 42:2535–2541

Bag S, Tulsan R, Sood A, Cho H, Redjeb H, Zhou W, LeVine H, Török B, Török M (2015) Sulfonamides as multifunctional agents for Alzheimer's disease. Bioorg Med Chem Lett 25:626–630

Bosak A, Gazić I, Vinković V, Kovarik Z (2008) Stereoselective inhibition of human, mouse, and horse cholinesterases by bambuterol enantiomers. Chem Biol Interact 175:192–195

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72:248–254

Ellman GL, Courtney KD, Andres V, Feather-Stone RM (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharm 7:88–95

Gallo S, Atifi S, Mahamoud A, Santelli-Rouvier C, Wolfart K, Molnar J (2003) Synthesis of aza mono, bi and tricyclic compounds. Evaluation of their anti MDR activity. Eur J Med Chem 38:19–26

Hamulakovaa S, Imricha J, Janoveca L, Kristiana P, Danihela I, Holasb O, Pohankac M, Böhmd S, Kozurkovaa M, Kucac K (2014) Novel tacrine/acridine anticholinesterase inhibitors with piperazine and thiourea linkers. Int J Biol Macromol 70:435–439

Kaya M, Basar E, Çakir E, Tunca E, Bulbul M (2012) Synthesis and characterization of novel dioxoacridine sulfonamide derivatives as new carbonic anhydrase inhibitors. J Enzyme Inhib Med Chem 27:509–514

Kaya M, Demir E, Bekci H (2013) Synthesis, characterization and antimicrobial activity of novel xanthene sulfonamide and carboxamide derivatives. J Enzyme Inhib Med Chem 28:885–893

Kaya M, Yıldırır Y, Çelik GY (2011) Synthesis and antimicrobial activities of novel bisacridine-1,8-dione derivatives. Med Chem Res 20:293–299

Marco-Contelles J, León R, Ríos CL, Samadi A, Bartolini M, Andrisano V, Huertas O, Barril X, Luque FJ, Rodríguez-Franco MI, López B, López MG, García AG, Carreiras MC, Villarroya M (2009) Tacripyrines, the first tacrine−dihydropyridine hybrids, as multitarget-directed ligands for the treatment of alzheimer's disease. J Med Chem 52:2724–2732

Monti SM, Supuran CT, Simone GD (2013) Anticancer carbonic anhydrase inhibitors: a patent review. Expert Opin Ther Patents 23:737–749

Öcal B, Saraç B, Simsek R, Yıldırım S, Sarıoğlu Y, Safak C (2002) Vasorelaxing properties of some phenylacridine type potassium channel openers in isolated rabbit thoracic arteries. Eur J Med Chem 37:519–523

Palani K, Thirumalai D, Ambalavanan P, Ponnuswamy MN, Ramakrishnan VT (2005) Synthesis and characterization of 9-(4-nitrophenyl)3,3,6,6-tetramethyl-3,4,6,7,9,10-hexahydro-1,8 (2H,5H) acridinedione and its methoxyphenyl derivative. J Chem Crystallogr 35:751–760

Pamuk H, Aday B, Şen F, Kaya M (2015) Pt NPs@GO as a highly efficient and reusable catalyst for one-pot synthesis of acridinedione derivatives. RSC Adv 5:49295–49300

Scozzafava A, Supuran CT, Carta F (2013) Antiobesity carbonic anhydrase inhibitors: a literature and patent review. Expert Opin Ther Patents 23:725–735

Silman I, Sussman JL (2005) Acetylcholinesterase: 'classical' and 'non-classical' functions and pharmacology. Curr Opin Pharmacol 5:293–302

Slawinski J, Brzozowski Z, Zolnowska B, Szafranski K, Pogorzelska A, Vullo D, Supuran CT (2014a) Synthesis of a new series of N^4-substituted 4-(2-aminoethyl)benzenesulfonamides and their inhibitory effect on human carbonic anhydrase cytosolic isozymes I and II and transmembrane tumor-associated isozymes IX and XII. Eur J Med Chem 84:59–67

Slawinski J, Pogorzelska A, Zolnowska B, Brozewicz K, Vullo D, Supuran CT (2014b) Carbonic anhydrase inhibitors. Synthesis of a novel series of 5-substituted 2,4-dichlorobenzenesulfonamides and their inhibition of human cytosolic isozymes I and II and the transmembrane tumor-associated isozymes IX and XII. Eur J Med Chem 82:47–55

Soreq H, Seidman S (2001) Acetylcholinesterase-new roles for an old actor. Nat Rev Neurosci 2:294–302

Srivastava A, Nizamuddin C (2004) Synthesis and fungicidal activity of some acridine derivatives. Indian J Heterocycl Chem 13:261–264

Supuran CT, Scozzafava A (2001) Carbonic anhydrase inhibitors. Curr Med Chem 1:61–97

Talesa V, Principato E, Di Giovannini MV, Grauso G, Rosi G (1993) Evidence for a molecular polimorphism of cholinesterase in sepia officinalis. Comp Biochem Physiol B 106:557–562

Tang ZQ, Chen Y, Liu CN, Cai KY, Tuc SJ (2010) A green procedure for the synthesis of 1,8-dioxodecahydroacridine derivatives under microwave irradiation in aqueous media without catalyst. J Heterocyclic Chem 47:363–367

Thullbery MD, Cox HD, Schule T, Thompson CM, George KM (2005) Differential localization of acetylcholinesterase in neuronal and non-neuronal cells. J Cell Biochem 96: 599–610

Ulus R, Yeşildağ İ, Elmastaş M, Kaya M (2015) Rapid synthesis of novel 1,8-dioxoacridine carboxylic acid derivatives by microwave irradiation and their free radical scavenging activity. Med Chem Res 24:3752–3759

Ulus R, Yeşildağ İ, Tanc M, Bülbül M, Kaya M, Supuran CT (2013) Synthesis of novel acridine and bis acridine sulfonamides with effective inhibitory activity against the cytosolic carbonic anhydrase isoforms II and VII. Bioorg Med Chem 21:5799–5805

Yeşildağ İ, Ulus R, Basar E, Aslan M, Kaya M, Bülbül M (2014) Facile, highly efficient, and clean one-pot synthesis of acridine sulfonamide derivatives at room temperature and their inhibition of human carbonic anhydrase isoenzymes. Monatsh Chem 145:1027–1034

Zolnowska B, Slawinski J, Pogorzelska A, Chojnacki J, Vullo D, Supuran CT (2014) Carbonic anhydrase inhibitors. Synthesis, and molecular structure of novel series N-substituted N′-(2-arylmethylthio-4-chloro-5-methylbenzenesulfonyl) guanidines and their inhibition of human cytosolic isozymes I and II and the transmembrane tumor-associated isozymes IX and XII. Eur J Med Chem 71:135–147