Development of terphenyl-2-methyloxazol-5(4H)-one derivatives as selective reversible MAGL inhibitors

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ABSTRACT

Monoacylglycerol lipase is a serine hydrolase that plays a major role in the degradation of the endocannabinoid neurotransmitter 2-arachidonoylglycerol. A wide number of MAGL inhibitors are reported in literature; however, many of them are characterised by an irreversible mechanism of action and this behavior determines an unwanted chronic MAGL inactivation, which acquires a functional antagonism of the endocannabinoid system. The possible use of reversible MAGL inhibitors has only recently been explored, due to the lack of known compounds possessing efficient reversible inhibitory activities. In this work, we report a new series of terphenyl-2-methyloxazol-5(4H)-one derivatives characterised by a reversible MAGL-inhibition mechanism. Among them, compound 20b showed to be a potent MAGL reversible inhibitor (IC50 = 348 nM) with a good MAGL/FAAH selectivity. Furthermore, this compound showed antiproliferative activities against two different cancer cell lines that overexpress MAGL.

Introduction

Endocannabinoids are lipid transmitters that act as endogenous ligands of the CB1 and CB2 cannabinoid receptors. The endogenous ligands 2-arachidonoylglycerol (2-AG) and N-arachidonoyl ethanolamine (AEA) are considered as the two major endocannabinoids and modulate multiple physiological processes including pain, inflammation, appetite, memory and emotion. Their signaling activity is terminated by enzymatic hydrolysis, which is mainly mediated by serine hydrolase monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. Because of its key role in 2-AG catabolism, selective inactivation of MAGL represents an interesting approach for obtaining the desirable effects of endocannabinoids and modulate multiple physiological processes including pain, inflammation, appetite, memory and emotion. Their signaling activity is terminated by enzymatic hydrolysis, which is mainly mediated by serine hydrolase monoacylglycerol lipase (MAGL) and fatty acid amide hydrolase (FAAH), respectively. Because of its key role in 2-AG catabolism, selective inactivation of MAGL represents an interesting approach for obtaining the desirable effects of endocannabinoids.
General procedure for the formation of terphenyl derivatives 6, 12, 14 and 19a–h
A solution of Pd(OAc)₂ (0.06 eq) and triphenylphosphine (0.30 eq) in absolute ethanol (6 ml/2.7 mmol halogenated derivative) and toluene (6 ml/2.7 mmol halogenated derivative) was stirred at room temperature (RT) under nitrogen for 10 min. After that period, commercially available dibromo- or dichloro-substituted aldehydes 2, 10 or 11 (1 eq), 2 M aqueous Na₂CO₃ (6 ml/2.7 mmol halogenated derivative), and opportunistically substituted phenylboronic acid (3.2 eq) were sequentially added. The resulting mixture was heated at 100 °C in a sealed vial under nitrogen for 24 h. After being cooled to RT, it was checked by TLC and if starting material was still present or it was visible the presence of two close spots (probable mono- and di-substitution products), it was added Pd(OAc)₂ (0.03 eq), triphenylphosphine (0.15 eq) and phenylboronic acid (1.6 eq). The mixture was heated again at 100 °C for further 24 h. Finally, the mixture was cooled to RT, diluted with water and extracted with EtOAc. The combined organic phase was dried and concentrated. The crude product was purified by flash chromatography using the indicated eluent and pure fractions containing the desired compound were evaporated to dryness affording the desired product.

1(1,1’-3’,1’’-Terphenyl)-4’-carbaldehyde (6)
Yellow crystalline solid, yield: 94% (277.4 mg) from 2 and phenylboronic acid. Rᵢ = 0.11 (n-hexane/EtOAc 98:2). ¹H-NMR (CDCl₃ 400 MHz) δ (ppm): 7.39–7.53 (m, 8H), 7.65–7.70 (m, 3H), 7.73 (dd, 1H, J = 8.2, 1.0 Hz), 8.12 (d, 1H, J = 8.0 Hz), 10.02 (s, 1H).

1(1,1’-3’,1’’-Terphenyl)-5’-carbaldehyde (12)
White solid, yield: 93% (274.0 mg) from 10 and phenylboronic acid. Rᵢ = 0.08 (n-hexane/EtOAc 98:2). ¹H-NMR (CDCl₃ 400 MHz) δ (ppm): 7.43 (tt, 2H, J = 7.4, 1.7 Hz), 7.48–7.53 (m, 4H), 7.67–7.71 (m, 4H), 8.06–8.10 (m, 3H), 10.16 (s, 1H).

1(1,1’-4’,1’’-Terphenyl)-2’-carbaldehyde (14)
White solid, yield: 80% (236.0 mg) from 11 and phenylboronic acid. Rᵢ = 0.17 (n-hexane/EtOAc 98:2). ¹H-NMR (CDCl₃ 400 MHz) δ (ppm): 7.38–7.57 (m, 9H), 7.67–7.71 (m, 2H), 7.89 (dd, 1H, J = 8.0, 2.1 Hz), 8.28 (d, 1H, J = 2.0 Hz), 10.05 (s, 1H).

4,4’-Difluoro-(1,1’-4’,1’’-terphenyl)-2’-carbaldehyde (19a)
White solid, yield: 97% (325.5 mg) from 11 and 4-fluorophenylboronic acid. Rᵢ = 0.18 (n-hexane/EtOAc 98:2). ¹H-NMR (CDCl₃ 400 MHz) δ (ppm): 7.14–7.22 (m, 4H), 7.39 (double AA’XX’, 2H, J_{AA/XX} = 5.3 Hz, J_{AX} = 8.8 Hz, J_{AX/XX} = 2.5 Hz), 7.50 (d, 1H, J = 7.9 Hz), 7.64 (double AA’XX’, 2H, J_{AA/XX} = 5.2 Hz, J_{AX} = 8.8 Hz, J_{AX/XX} = 2.6 Hz), 7.83 (dd, 1H, J = 8.0, 2.1 Hz), 8.20 (d, 1H, J = 2.0 Hz), 10.02 (s, 1H).
2,5-Bis[benzo(d)(1,3)dioxol-5-yl]benzaldehyde (19f)

White solid, yield: 99% (363.5 mg) from 11 and 4-methoxyphenylboronic acid. \( R_f = 0.25 \) (n-hexane/EtOAc 9:1). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 3.87 (3H, s, 3H), 3.89 (3H, s, 3H), 6.99–7.05 (m, 4H), 7.35 (AA’X’’, 2H, \( J_{AA} = 8.8\) Hz, \( J_{AX} = 2.1\) Hz), 7.49 (d, 1H, \( J = 8.0\) Hz), 7.62 (AA’X’’, 2H, \( J_{AA} = 8.9\) Hz, \( J_{AX} = 2.2\) Hz), 7.82 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.20 (d, 1H, \( J = 2.0\) Hz), 10.05 (s, 1H).

4,4’-Bis(trifluoromethoxy)-(1,1’4,1’-terphenyl)-2’-carbaldehyde (19c)

Colorless oil, yield: 89% (432.4 mg) from 11 and 4-trifluoromethoxyphenylboronic acid. \( R_f = 0.13 \) (n-hexane/EtOAc 99:1). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 7.32–7.38 (m, 4H), 7.46 (AA’XX’, 2H, \( J_{AX} = 8.8\) Hz, \( J_{AA} = 2.4\) Hz), 7.52 (d, 1H, \( J = 8.0\) Hz), 7.69 (AA’X’’, 2H, \( J_{AA} = 8.9\) Hz, \( J_{AX} = 2.5\) Hz), 7.86 (dd, 1H, \( J = 8.0, 2.2\) Hz), 8.23 (d, 1H, \( J = 1.8\) Hz), 10.03 (s, 1H).

4,4’-Bis(trifluoromethyl)-(1,1’4,1’-terphenyl)-2’-carbaldehyde (19d)

White solid, yield: 95% (424.8 mg) from 11 and 4-trifluoromethylphenylboronic acid. \( R_f = 0.15 \) (n-hexane/EtOAc 99:1). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 7.54–7.59 (m, 3H), 7.74–7.81 (m, 6H), 7.92 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.30 (d, 1H, \( J = 1.8\) Hz), 10.03 (s, 1H).

3,3’-Difluoro-(1,1’4,1’-terphenyl)-2’-carbaldehyde (19e)

White solid, yield: 94% (316.0 mg) from 11 and 3-fluorophenylboronic acid. \( R_f = 0.10 \) (n-hexane/EtOAc 98:2). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 7.07–7.14 (m, 1H), 7.14–7.21 (m, 3H), 7.35–7.40 (m, 1H), 7.42–7.50 (m, 3H), 7.53 (d, 1H, \( J = 8.0\) Hz), 7.87 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.25 (d, 1H, \( J = 2.0\) Hz), 10.04 (s, 1H).

2,5-Bis[benzo(d)(1,3)dioxol-5-yl]benzaldehyde (19f)

Dark yellow solid, yield: 75% (295.6 mg) from 11 and 3,4-methylenedioxyphenylboronic acid. \( R_f = 0.18 \) (n-hexane/EtOAc 9:1). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 6.03 (s, 2H), 6.06 (s, 2H), 6.83 (dd, 1H, \( J = 7.8, 1.9\) Hz), 6.89–6.94 (m, 3H), 7.12–7.16 (m, 2H), 7.47 (d, 1H, \( J = 8.0\) Hz), 7.77 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.15 (d, 1H, \( J = 2.0\) Hz), 10.05 (s, 1H).

3,3’-Difluoro-4,4’-dimethoxy-(1,1’4,1’-terphenyl)-2’-carbaldehyde (19g)

Pearly white solid, yield: 82% (329.5 mg) from 11 and 3-fluoro-4-methoxyphenylboronic acid. \( R_f = 0.13 \) (n-hexane/EtOAc 9:1). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 3.96 (s, 3H), 3.97 (s, 3H), 7.04–7.12 (m, 3H), 7.19 (dd, 1H, \( J = 11.6, 2.0\) Hz), 7.38–7.44 (m, 2H), 7.48 (d, 1H, \( J = 8.0\) Hz), 7.80 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.18 (d, 1H, \( J = 2.0\) Hz), 10.05 (s, 1H).

4,4’-Dichloro-(1,1’4,1’-terphenyl)-2’-carbaldehyde (19h)

White solid, yield: 61% (143.0 mg) from 11 and 4-chlorophenylboronic acid. \( R_f = 0.40 \) (n-hexane/EtOAc 98:2). \(^1\)H-NMR (CDCl\(_3\), 400 MHz) \( \delta \) (ppm): 7.36 (AA’X’’, 2H, \( J_{AX} = 8.6\) Hz, \( J_{AA} = 2.3\) Hz), 7.44–7.52 (m, 5H), 7.61 (AA’XX’, 2H, \( J_{AX} = 8.7\) Hz, \( J_{AA} = 2.3\) Hz), 7.85 (dd, 1H, \( J = 8.0, 2.1\) Hz), 8.22 (d, 1H, \( J = 2.1\) Hz), 10.03 (s, 1H).

Procedure for the synthesis of (1,1’2,1’-terphenyl)-4’-carbaldehyde (4)

3,4-Dichlorobenzaldehyde 1 (500 mg, 2.86 mmol, 1 eq) was placed in a vial together with phenylboronic acid (1.39 g, 11.4 mmol), potassium phosphate (2.67 g, 12.6 mmol), Pd(OAc)\(_2\) (57.8 mg, 0.0858 mmol), tetrabutylammonium bromide (TBAB) (14.3 g, 44.3 mmol) and water (6.4 ml). The vial was sealed and heated under stirring at 125 \( ^\circ\)C for 48 h. The reaction mixture was cooled to RT and then diluted with water. The water phase was acidified with 1 N aqueous HCl and repeatedly extracted with EtOAc. The combined organic phase was washed with brine, dried over anhydrous sodium sulfate and evaporated to afford a crude residue that was purified by column chromatography over silica gel using n-hexane/EtOAc 98:2 (\( R_f = 0.16 \) as the eluent, to give pure 4 as a yellow oily compound (157.9 mg, 21% yield). \(^1\)H-NMR (DMSO-d\(_6\), 400 MHz) \( \delta \) (ppm): 7.14–7.20 (m, 4H), 7.25–7.32 (m, 6H), 7.65 (d, 1H, \( J = 8.0\) Hz), 7.94 (d, 1H, \( J = 1.2\) Hz), 7.98 (dd, 1H, \( J = 7.6, 1.6\) Hz), 10.11 (s, 1H).

General procedure for the preparation of the 2-methyloxazol-5(4H)-one derivatives 5, 7, 9, 13, 15

A mixture of diphenyl-substituted benzaldehydes 4, 6, 8, 12 or 14 (1 eq), N-acetylglucose (1 eq) and sodium acetate (1 eq) in acetic
anhydride (5 ml/5 mmol aldehyde) was stirred at reflux for 5 h and then warmed to RT. The reaction was quenched with water and extracted with AcOEt. The organic layer was washed sequentially with water and saturated brine, dried over Na2SO4 and the solvent was removed under reduced pressure. The residue was purified with a flash column chromatography using the indicated eluent and pure fractions containing the desired compound were evaporated to dryness affording the desired product.

(Z)-4-[(1′,2′,1′′-Terphenyl)-4′-ylmethylene]-2-methoxyazol-5(4H)-one (15)
Yellow solid, yield: 15% (43.0 mg) from 14. \( R_f = 0.18 \) (n-hexane/EtOAc 95:5). 1H-NMR (CDCl3, 400 MHz) \( \delta \) (ppm): 2.42 (s, 3H), 7.11 (s, 1H), 7.41–7.57 (m, 9H), 7.75–7.79 (m, 2H), 7.84 (dd, 1H, \( J = 8.0 \), 2.0 Hz), 9.10 (d, 1H, \( J = 2.0 \) Hz). 13C-NMR (CDCl3, 100 MHz) \( \delta \) (ppm): 15.74, 127.82 (3 C), 128.67, 128.70, 128.86, 129.44 (2 C), 129.92, 129.97 (2 C), 130.80 (2 C), 131.16, 131.80, 134.52, 140.52, 141.24, 144.54, 168.16, 168.23. HPLC analysis: retention time = 11.425 min; peak area, 95%. Elemental analysis for \( C_{23}H_{23}NO_2 \) calculated: % C, 81.40; % H, 5.05; % N, 4.13; found: % C, 81.65; % H, 5.06; % N, 4.15.

General procedure for the preparation of the 2-methoxyazol-5(4H)-one derivatives 20a–h
The procedure for the synthesis of these compounds is similar to that used for previous analog final products, with the exception of the used equivalents of N-acetylglycine (2 eq) and sodium acetate (2 eq).

(Z)-4-[(4,4′-difluoro-(1′,1′′)-terphenyl)-2′-ylmethylene]-2-methoxyazol-5(4H)-one (20a)
Yellow solid, yield: 35% (46.5 mg) from 19a. \( R_f = 0.20 \) (n-hexane/EtOAc 95:5). 1H-NMR (acetone-d6, 400 MHz) \( \delta \) (ppm): 2.43 (s, 3H), 7.06 (s, 1H), 7.28–7.34 (m, 4H), 7.48 (double AA'X'X, 2H), 4JHF \( m = 5.4 \) Hz, \( J_{AA} = 8.9 \) Hz, \( J_{AX/XX} = 2.2 \) Hz, 7.55 (d, 1H, \( J = 8.0 \) Hz), 7.78–7.84 (m, 3H), 9.04 (d, 1H, \( J = 1.9 \) Hz). 13C-NMR (acetone-d6, 100 MHz) \( \delta \) (ppm): 15.71, 116.25 (d, 2 C, \( J = 21.4 \) Hz), 116.69 (d, 2 C, \( J = 21.8 \) Hz), 128.23, 129.75 (d, 2 C, \( J = 8.1 \) Hz), 129.81, 131.03, 131.86, 132.32, 132.74 (d, 2 C, \( J = 8.1 \) Hz), 134.75, 136.68 (d, 13JHF \( m = 3.5 \) Hz), 137.27 (d, \( J = 3.2 \) Hz), 140.29, 143.29, 163.60 (d, \( J = 234.1 \) Hz), 163.60 (d, \( J = 257.3 \) Hz), 168.07, 168.42. HPLC analysis: retention time = 11.735 min; peak area, 96%. Elemental analysis for \( C_{23}H_{13}F_4NO_2 \) calculated: % C, 73.59; % H, 4.03; % N, 3.73; found: % C, 73.35; % H, 4.01; % N, 3.72.

(Z)-4-[(1′,2′,1′′-Terphenyl)-3′-ylmethylene]-2-methoxyazol-5(4H)-one (19)
Yellow solid, yield: 13% (39.4 mg) from 8. \( R_f = 0.14 \) (n-hexane/EtOAc 95:5). 1H-NMR (CDCl3, 400 MHz) \( \delta \) (ppm): 2.41 (s, 3H), 6.90–7.05 (m, 5H), 7.12–7.17 (m, 3H), 7.20–7.25 (m, 3H), 7.48 (dd, 1H, \( J = 7.6, 1.5 \) Hz), 7.54 (d, 1H, \( J = 7.7 \) Hz), 8.65 (dd, 1H, \( J = 7.8, 1.4 \) Hz). 13C-NMR (CDCl3, 100 MHz) \( \delta \) (ppm): 15.86, 126.64, 127.57, 127.81 (3 C), 128.01 (2 C), 129.82 (2 C), 130.97, 131.03, 131.29 (2 C), 132.23, 132.74, 133.87, 138.12, 141.21, 142.40, 143.29, 166.41, 167.56. HPLC analysis: retention time = 10.983 min; peak area, 94%. Elemental analysis for \( C_{23}H_{17}NO_2 \) calculated: % C, 81.40; % H, 5.05; % N, 4.13; found: % C, 81.07; % H, 5.03; % N, 4.11.

(Z)-4-[(1′,3′,1′′-Terphenyl)-3′-ylmethylene]-2-methoxyazol-5(4H)-one (13)
Yellow solid, yield: 41% (142.0 mg) from 12. \( R_f = 0.17 \) (n-hexane/EtOAc 95:5). 1H-NMR (CDCl3, 400 MHz) \( \delta \) (ppm): 2.43 (s, 3H), 7.27 (s, 1H), 7.41 (tt, 2H, \( J = 7.3, 1.5 \) Hz), 7.47–7.53 (m, 4H), 7.66–7.70 (m, 4H), 7.86 (t, 1H, \( J = 1.7 \) Hz), 8.30 (d, 2H, \( J = 1.6 \) Hz). 13C-NMR (CDCl3, 100 MHz) \( \delta \) (ppm): 15.73, 128.06, 128.76, 128.84, 129.89 (11 C), 130.36, 130.58, 134.59, 135.60, 141.13, 143.16, 168.14, 168.21. HPLC analysis: retention time = 11.496 min; peak area, 97%. Elemental analysis for \( C_{23}H_{17}NO_2 \) calculated: % C, 81.40; % H, 5.05; % N, 4.13; found: % C, 81.68; % H, 5.06; % N, 4.14.
140.04, 140.15, 143.19, 149.83, 149.84, 168.00, 168.66. HPLC analysis: retention time = 13.106 min; peak area, 94%. Elemental analysis for C_{25}H_{21}F_{2}NO_{2} calculated: % C, 70.25; % H, 4.01; % N, 3.28; found: % C, 59.40; % H, 2.99; % N, 2.77.

(Z)-4-[(4,4′-Bis[trifluoromethyl]-(1′,1′,4′,1′-terphenyl)-2′-yl)methylen]-2-methyloxazol-5(4H)-one (20d)

Yellow solid, yield: 54% (260.3 mg) from 19d. \( R_f = 0.18 \) (n-hexane/ EtOAc 9:1). \(^{1}H-\)NMR (acetone-d\(_6\), 400 MHz) \( \delta \) (ppm): 2.44 (s, 3H), 7.04 (s, 1H), 7.65 (d, 1H, \( J = 8.00 \) Hz), 7.68–7.73 (m, 2H), 7.87–7.93 (m, 4H), 7.96 (dd, 1H, \( J = 8.10, 2.00 \) Hz), 7.99–8.04 (m, 2H), 9.16 (d, 1H, \( J = 2.00 \) Hz). 13C-NMR (acetone-d\(_6\), 100 MHz) \( \delta \) (ppm): 15.75, 125.36 (q, \( J = 271.5 \) Hz), 126.45 (q, \( J = 271.2 \) Hz), 126.37 (q, 2C, \( J = 3.70 \) Hz), 126.91 (q, 2C, \( J = 3.96 \) Hz), 124.68, 128.60, 131.14, 130.27 (q, 1C, \( J = 132.30 \) Hz), 132.07 (2C, \( J = 131.57 \) Hz), 132.02, 132.51, 135.34, 140.33, 143.55, 144.41 (q, 2C, \( J = 14.30 \) Hz), 144.70 (q, 2C, \( J = 14.70 \) Hz), 167.93, 168.87. HPLC analysis: retention time = 12.638 min; peak area, 97%. Elemental analysis for C_{25}H_{21}F_{2}NO_{2} calculated: % C, 69.98; % H, 3.99; % N, 3.27.

(Z)-4-[(3,3′-Difluoro-(1′,1′,4′,1′-terphenyl)-2′-yl)methylen]-2-methyloxazol-5(4H)-one (20e)

Yellow solid, yield: 45% (89.5 mg) from 19h. \( R_f = 0.18 \) (n-hexane/ EtOAc 95:5). \(^{1}H-\)NMR (acetone-d\(_6\), 400 MHz) \( \delta \) (ppm): 2.43 (s, 3H), 7.05 (s, 1H), 7.46 (AA′′′, 2H, \( J_{XX} = 8.70 \) Hz, \( J_{AA/XX} = 2.30 \) Hz), 7.54–7.59 (m, 5H), 7.79 (AA′′′, 2H, \( J_{XX} = 8.80 \) Hz, \( J_{AA/XX} = 2.40 \) Hz), 7.85 (dd, 1H, \( J = 8.10, 2.00 \) Hz), 9.06 (d, 1H, \( J = 1.00 \) Hz). 13C-NMR (acetone-d\(_6\), 100 MHz) \( \delta \) (ppm): 15.73, 129.95, 129.45 (2C, 129.54 (2C), 129.80, 130.03 (2C, 131.10, 131.83, 132.34, 132.24 (2C, 134.42, 134.65, 134.95, 136.91, 140.20, 143.32, 168.01, 168.57. HPLC analysis: retention time = 12.830 min; peak area, 95%. Elemental analysis for C_{25}H_{21}Cl_{2}NO_{2} calculated: % C, 67.66; % H, 3.70; % N, 3.43; found: % C, 67.90; % H, 3.71; % N, 3.44.

Biological evaluation

Human recombinant MAGL, and 4-nitrophenylacetate substrate (4-NPA) were from Cayman Chemical. The IC_{50} values for compounds were generated in 96-well microtiter plates. The MAGL reaction was conducted at RT at final volume of 200 \( \mu L \) in 10mM Tris buffer, pH 7.2, containing 1 mM EDTA. A total of 150 \( \mu L \) of 4-NPA 133.3 \( \mu M \) (final concentration = 100 \( \mu M \)) was added to 10 \( \mu L \) of DMSO containing the appropriate amount of compound. The reaction was initiated by the addition of 40 \( \mu L \) of MAGL (11 ng/well) in such a way that the assay was linear over 30 min. The final concentration of the analyzed compounds ranged for CAY10499 and JZL-184 from 10 to 0.00001 \( \mu M \) and for the synthesised compounds from 200 to 0.0128 \( \mu M \). After the reaction had proceeded for 30 min, absorbance values were then measured by using a VictorX3 PerkinElmer instrument at 405 nm. Two reactions were also run: one reaction containing no compounds and the second one containing neither inhibitor nor enzyme. IC_{50} values were derived from experimental data using the Sigmoidal dose–response fitting of GraphPad Prism software as reported earlier. To remove possible false positive results, for each compound concentration a blank analysis was carried out, and the final concentration results were obtained detracting the absorbance produced by the presence of all the components except MAGL in the same conditions.

MAGL preincubation assay

The MAGL reaction was conducted at RT at a final volume of 200 \( \mu L \) in 10mM Tris buffer, pH 7.2, containing 1mM EDTA. A total of 150 \( \mu L \) of MAGL (11 ng/well) was added to 10 \( \mu L \) of DMSO containing the appropriate amount of compound. After 0, 30, and 60 min of incubation time the reaction was initiated by the addition of 40 \( \mu L \) of 4-NPA 500 \( \mu M \). The enzyme activity was then measured according to the procedure described above.

MAGL dilution assay

The enzyme (880 ng in 75 \( \mu L \) of Tris buffer, pH 7.2) was incubated during 60 min at RT with 5 \( \mu L \) of compound 20b (concentration of 10 \( \mu M \) in the mixture) dissolved in DMSO. The MAGL-inhibitor
mixture was then diluted 40-fold with the buffer. After 15 min of incubation, the reaction was initiated on a 160 µL aliquot by the addition of 40 µL of 4-NPA 500 µM and the enzyme activity was measured according to the procedure described above.

**FAAH inhibition assay**

The IC₅₀ values for compounds were generated in 96-well microtiter plates. The FAAH reaction was conducted at RT at a final volume of 200 µL in 125 mM Tris buffer, pH 9.0, containing 1 mM EDTA. A total of 150 µL of AMC arachidonoyl amide 13.3 µM (final concentration = 10 µM) was added to 10 µL of DMSO containing the appropriate amount of compound. The reaction was initiated by the addition of 40 µL of FAAH (0.9 µg/well) in such a way that the assay was linear over 30 min. After the reaction had proceeded for 30 min, fluorescence values were then measured by using a VictorX3 PerkinElmer instrument at an excitation wavelength of 340 nm and an emission of 460 nm. Two reactions were also run: one reaction containing no compounds and the second one containing neither inhibitor nor enzyme. IC₅₀ values were derived from experimental data using the Sigmoidal dose–response fitting of GraphPad Prism software as reported earlier. To remove possible false-positive results, for each compound concentration, a blank analysis was carried out, and the final fluorescence results were obtained detracting the fluorescence produced by the presence of all the components except FAAH in the same conditions.

**Cell viability assay**

COV318, OVCAR-3 (from ATCC) and hMSC (from AB Cell-Bio) were maintained at 37°C in a humidified atmosphere containing 5% CO₂ accordingly to the supplier. Normal (1.5 × 10⁵) and tumor (5 × 10⁴) cells were plated in 96-well culture plates. The day after seeding, vehicle or compounds were added at different concentrations to the medium. Compounds were added to the cell culture at a concentration ranging from 200 to 0.02 µM. Cell viability was measured after 96 h according to the supplier (Promega, G7571) with a Tecan F200 instrument. IC₅₀ values were calculated from logistical dose–response curves. Averages were obtained from three independent experiments, and error bars are standard deviations (n = 3).

**Molecular modeling**

**Consensus docking studies**

The ligand was built by means of Maestro and was then minimised in a water environment (using the Generalised Born/surface area model) by means of Macromodel. It was minimised using the conjugate gradient (CG), the MMFFs force field, and a distance-dependent dielectric constant of 1.0 until they reached a convergence value of 0.05 kcal Å⁻¹ mol⁻¹. Nine different docking procedures were applied and for each docking calculation only the best scored pose was taken into account. The ligand was docked in the human MAGL (3JWE PDB code) and the humanised-rat FAAH (3LJ7 PDB code). The ligand was docked into the two proteins by using the different docking procedures, then the root mean square deviation (RMSD) of each of these docking poses against the remaining docking results was evaluated by using the rms_analysis software of the GOLD suite. The most populated cluster was then considered and subjected to molecular dynamic (MD) simulations.

**AUTODOCK 4.2.3**

AUTODOCK Tools utilities were used in order to identify the torsion angles in the ligand, to add the solvent model and assign the Gasteiger atomic charges to proteins and ligand. The regions of interest used by AUTODOCK were defined by considering the reference ligand as the central group of a grid box of 10 Å in the x, y, and z directions. A grid spacing of 0.375 Å and a distance-dependent function of the dielectric constant were used for the energetic map calculations. By using the Lamarckian genetic algorithm, the docked compounds were subjected to 20 runs of the AUTODOCK search using 2,500,000 steps of energy evaluation and the default values of the other parameters.

**DOCK 6.7**

The molecular surface of the binding site was calculated by means of the MS program, generating the Connolly surface with a probe with a radius of 1.4 Å. The points of the surface and the vectors normal to it were used by the Sphgen program in order to build a set of spheres, with radii varying from 1.4 to 4.0 Å that describe, from a stereoelectronic point of view, the negative image of the site. Spheres within a radius of 10 Å from the reference ligand were used to represent the site. For each docking calculation, DOCK 6.7 calculated 1000 orientations; of these, the best grid scored was taken into consideration. The ligand charge was calculated using the AM1-BCC method, as implemented in the MOLCHARGE program.

**FRED 3.0**

FRED requires a set of input conformers for each ligand. The conformers were generated by OMEGA. The following modifications to the default settings of OMEGA were applied: the energy window was set at 50.0, the maximum number of output conformers was set at 10,000, the time limit was set at 1200, and the RMSD value below which two conformations were considered to be similar was set at 0.3 Å. The region of interest for the docking studies was defined in such a manner that it contained all residues which stayed within 10 Å from the ligand in the X-ray structures. FRED default parameters were used setting the high dock_resolution.

**GLIDE 5.0**

The binding site was defined by a rectangular box of 10 Å in the x, y, and z directions centered on the ligand. The option allowing only the docking of ligands containing a defined range of atoms was deactivated, whereas the GLIDE defaults were used for all other parameters. Docking calculations were carried out using the standard precision (SP) method.

**GOLD 5.1**

The region of interest for the docking studies was defined in such a manner that it contained all residues which stayed within 10 Å from the ligand in the X-ray structures; the “allow early termination” command was deactivated, while the possibility for the ligand to flip ring corners was activated. For all other parameters, GOLD defaults were used and the ligands were subjected to 30 genetic algorithm runs. Three docking analyzes were carried out by using three fitness functions implemented in GOLD, i.e., GoldScore (GS), ChemScore (CS) and Astex Statistical Potential (ASP).

**AUTODOCK VINA 1.1**

The input files for the ligand and proteins originated from the AUTODOCK Tools utilities for the AUTODOCK calculations were also used for the AUTODOCK VINA calculations, including the grid box dimensions. The exhaustiveness parameter was set to 10
and the Energy_range to 1, whereas for all other parameters, AUTODOCK VINA defaults were used.

**PLANTS**

This docking software uses Ant Colony Optimisation, a state-of-the-art global optimisation algorithm to find minima of a scoring function representing favorable complex structures\(^{37}\). ChemPLP scoring function was employed to score protein–ligand interactions as well as intra-ligands clash terms. Standard settings for all parameters were used for the scoring function as well as the optimisation algorithm (search speed setting: “speed1”). The regions of interest used by PLANTS\(^ {37} \) were defined by considering the reference ligand as the central group of a grid box of 10 Å in the x, y, and z directions.

**MD simulations**

All simulations were performed using AMBER, version 14\(^ {38} \). MD simulations were carried out using the ff14SB force field at 300 K. The complex was placed in a rectangular parallelepiped water box. An explicit solvent model for water, TIP3P, was used, and the complexes were solvated with a 20 Å water cap. Chlorine or Sodium ions and were added as counter ions to neutralise the system. Prior to MD simulations, two steps of minimisation were carried out using the same procedure described above. Particle mesh Ewald (PME) electrostatics and periodic boundary conditions were used in the simulation\(^ {39} \). The MD trajectory was run using the minimised structure as the starting conformation. The time step of the simulations was 2.0 fs with a cutoff of 10 Å for the nonbonded interaction, and SHAKE was employed to keep all bonds involving hydrogen atoms rigid. Constant-volume periodic boundary MD was carried out for 1.0 ns, during which the temperature was raised from 0 to 300 K. Then, 50 ns of constant-pressure periodic boundary MD was carried out at 300 K using the Langevin thermostat to maintain constant the temperature of our system.

**Binding energy evaluation**

The evaluation of the binding energy associated to the two ligand–protein complexes analyzed through MD simulations was carried out using AMBER 14. The trajectories relative to the last 50 ns of each simulation were extracted and used for the calculation, for a total of 50 snapshots (at time intervals of 1 ns). Van der Waals, electrostatic and internal interactions were calculated with the SANDER module of AMBER 14, whereas polar energies were calculated using both the Generalised Born and the Poisson–Boltzman methods with the MM-PBSA module of AMBER 14. Dielectric constants of 1 and 80 were used to represent the gas and water phases, respectively, while the MOLSURF program was employed to estimate the nonpolar energies. The entropic term was considered as approximately constant in the comparison of the ligand–protein energetic interactions.

**Results and discussion**

**Chemistry**

The terphenyl compounds were synthesised following the same synthetic strategy applied for the previous series of methyleneoxazol-5(4\(^{\text{H}}\))-one derivatives, differing only for the first step, in which a double cross-coupling reaction was necessary to replace both the halogen atoms by two phenyl rings in each appropriate dihalo-substituted precursor (Schemes 1 and 2). All the possible combinations of substitutions with phenyl rings in the central aromatic scaffold were explored, with the exception of the 2,6-diphenyl derivative (Scheme 3, see the following chapter for

**Scheme 1.** Reagents and conditions: (a) for compound 4: phenylboronic acid, Pd(OAc)\(_{2}\), K\(_{3}\)PO\(_{4}\), TBAB, H\(_{2}\)O, 125 °C; for compound 6: phenylboronic acid, Pd(OAc)\(_{2}\), PPh\(_{3}\), aq. 2 M Na\(_{2}\)CO\(_{3}\), toluene, Et\(_{2}\)O, 100 °C; for compound 8: phenylboronic acid, Pd(OAc)\(_{2}\), PPh\(_{3}\), aq. 2 M Na\(_{2}\)CO\(_{3}\), toluene, Et\(_{2}\)O, 100 °C, then phenylboronic acid, Pd\(_{2}(\text{dba})_{3}\), Cs\(_{2}\)CO\(_{3}\), Cy\(_{3}\)P 29% toluene, dioxane, 100 °C; (b) N-acetylglycine, Ac\(_{2}\)O, CH\(_{3}\)COONa, reflux.
According to the availability in our laboratory of the starting aldehydes, the coupling reactions were performed on dichloro- or dibromo-substituted compounds and different conditions were chosen on the basis of a preliminary prevision of the reaction outcome, which was highly dependent on the precursor structure. 3,4-Dichlorobenzaldehyde 10 was subjected to a Pd-catalyzed cross-coupling reaction under phosphine-free conditions in water, by using TBAB as the phase-transfer agent, in the presence of potassium phosphate, upon prolonged thermal heating, to give the diaryl derivative 13. An Erlenmeyer–Plöchl condensation of the substituted aromatic aldehyde 13 with N-acetylglycine and sodium acetate in refluxing acetic anhydride gave the final product 15 as the (Z)-isomer (Scheme 1). In the case of 2,4-dichlorobenzaldehyde 11, the classical thermal Pd(PPh₃)₄-catalyzed Suzuki
conditions gave the desired product 6. This may appear as a surprising outcome, because the starting material is an aryl chloride which generally is less reactive under these “mild” cross-coupling conditions that are commonly suitable for aryl bromides 41. Nevertheless, the presence of the aldehyde group in para or ortho-position to the chloro atoms makes this compound more reactive toward the cross-coupling reaction (Scheme 1).

Differently, in the case of 2,3-dichlorobenzaldehyde 3 we failed to perform the double cross-coupling by using simple Suzuki conditions, since 1H-NMR analysis revealed the formation of only a mono-substitution product, probably due to the steric hindrance caused by the two chlorine atoms in adjacent positions to the aldehyde group. Therefore, the crude product of the reaction was subjected again to a second cycle of cross-coupling, adopting the Fu-type conditions42, which consists in using the more reactive catalytic system comprised of Pd2(dba)3, together with tricyclohexylphosphine as the catalyst ligand and cesium carbonate as the base (Scheme 1). Both compounds 6 and 8 were then transformed into the corresponding methyloxazol-5(4H)-one derivatives 7 and 9 as seen before for the preparation of 5 (Scheme 1).

The synthesis of terphenyl-methyloxazol-5(4H)-one compounds 13 and 15 started from bromo-aryl precursors 10 and 11 and cross-coupling, which were performed by adopting Suzuki conditions, allowed the formation of the diphenyl-substituted intermediates 12 and 14 with good yields, respectively (Scheme 2). Finally, we tried to obtain the last derivative of this series of compounds, which derives from the combination of the two phenyl rings in both of the ortho-positions to the oxazolone ring (compound 18, Scheme 3), starting from the 2,6-dichlorobenzaldehyde 16. Unfortunately intermediate 17, which was obtained in high yield from 16 by a Fu-type coupling, did not react under the classical Erlenmeyer–Plöchl conditions, neither by increasing the equivalents of the reagents (N-acetylglycine and acetic anhydride: two equivalents) or by extending the reaction time (24 or 48 h). This problem could be ascribed to the steric hindrance of the structure bearing two phenyl rings close to the aldehyde moiety, which hampered the formation of the additional five-membered cycle.

Considering the promising biological activity of compound 15 (see “Biological evaluation” section), which proved to be the most potent hMAGL inhibitor among all the possible combinations of terphenyl derivatives synthesised, a series of similar compounds variously substituted on the two peripheral aromatic rings were prepared, in order to investigate the effects of the additional substituents on the enzyme inhibition potency. All these compounds were obtained following the same synthetic pathway adopted for compound 15 (Scheme 2). 2,5-Dibromobenzaldehyde 11 was subjected to a double cross-coupling reaction using the Suzuki conditions with the appropriate boronic acid. Then, intermediates 19a–h were reacted with N-acetylglycine and sodium acetate in

| #  | Ar1  | Ar2  | Ar3  | Ar4  | MAGL IC50 (nM) | FAAH IC50 (nM) | MAGL/FAAH selectivity |
|----|------|------|------|------|----------------|-----------------|-----------------------|
| 5  | H    | Ph   | Ph   | H    | 837 ± 18       | 2331 ± 51       | 3                     |
| 7  | Ph   | H    | Ph   | H    | 546 ± 20       | 18161 ± 904     | 32                    |
| 9  | Ph   | Ph   | H    | H    | 2558 ± 172     | 20055 ± 1438    | 8                     |
| 13 | H    | Ph   | H    | Ph   | 457 ± 10       | 14763 ± 1176    | 32                    |
| 15 | Ph   | H    | H    | Ph   | 320 ± 10       | 10860 ± 161     | 34                    |

| #  | R1   | R2   | MAGL IC50 (nM) | FAAH IC50 (nM) | MAGL/FAAH selectivity |
|----|------|------|----------------|-----------------|-----------------------|
| 20a| F    | H    | 683 ± 50       | 27989 ± 1306    | 41                    |
| 20b| OCH3 | H    | 348 ± 38       | 36118 ± 1123    | 104                   |
| 20c| OCF3 | H    | 4194 ± 299     | 17728 ± 1198    | 4                     |
| 20d| CF3  | H    | 6763 ± 1125    | 28992 ± 1157    | 4                     |
| 20e| H    | F    | 628 ± 32       | 11713 ± 895     | 19                    |
| 20f| –OCH2O– | 673 ± 23 | 23712 ± 1348 | 35                |
| 20g| OCH3 | F    | 335 ± 22       | 22311 ± 1239    | 67                    |
| 20h| Cl   | H    | 476 ± 39       | 11487 ± 998     | 24                    |
| CAY10499 |      |      | 144 ± 4       | 14.7 ± 0.2      | 0.1                   |
| JZL-184  |      |      | 49.8 ± 4.2    | 3301 ± 205      | 66                    |
refluxing acetic anhydride to give the final compounds 20a–h (Scheme 4).

**Biological evaluation**

The inhibitory effects of the newly synthesised compounds on human isoforms of MAGL and FAAH are reported in Table 1, together with those of reference inhibitors CAY10499 and JZL-184.

Given the wide range of biological processes regulated by hydrolases, new MAGL inhibitors with a very high level of specificity should be required to minimise mechanism-based toxicities. Dual FAAH/MAGL inhibitors promote cataleptic and drug dependence behaviors in mice that are more reminiscent of direct CB1 agonists, underscoring the importance of maintaining high levels of selectivity to avoid simultaneous blockade of both FAAH and MAGL.

The series of diphenyl-substituted derivatives (5, 7, 9, 13, and 15) revealed that the presence of adjacent phenyl rings in the central scaffold is not ideal, since compound 5 (3,4-diphenyl) and, in particular, 9 (2,3-diphenyl) show the weakest potencies of this initial class. The introduction of further space between the two phenyl rings progressively improves the inhibition abilities of these compounds. In fact, when these substituents are placed in respective meta-positions (7 and 13), we can observe a significant improvement of the IC50 values obtained. This effect is further enhanced when the two phenyl rings are placed in para-positions to each other, since compound 15 (2,5-diphenyl) displays the highest MAGL-inhibition potency (IC50 = 320 nM) and MAGL/FAAH selectivity (34-fold) of this initial miniseries.

Therefore, we decided to further decorate the phenyl substituents of 15, and extend the series of 2,5-diamyl-substituted methyleneoxazol-5(4H)-one derivatives (20a–h). The data reported in Table 1 show that relatively large substituents in the para-positions, such as OCF3 (20c) and CF3 (20d), do not seem to fit nicely in the enzyme active site since the MAGL-inhibitory activities associated to these compounds are very poor (IC50 values of 4–6 μM). The introduction of small halogens, such as fluorine (20e) or chlorine (20h) atoms, or of a dioxolane portion (20f) is better tolerated, although the IC50 values obtained with these compounds are always higher than that of 15. Instead, the introduction of para-methoxy groups in the peripheral aryl rings, although they do not significantly improve the MAGL-inhibition potency of the resulting compounds (20b, g) when compared to their unsubstituted counterpart 15, cause remarkable reductions of their FAAH-inhibitory abilities, thus resulting in a substantial increase in their MAGL/FAAH selectivity. This is particularly evident in 20b: this compound displays an IC50 values of 348 nM against MAGL (similar to that of 15), together with a noticeable 104-fold selectivity for MAGL over FAAH, which is substantially higher than that shown by both its unsubstituted analog 15 and by reference inhibitor JZL-184. Comparing these results with those previously obtained for the monophenyl-substituted derivatives, we can highlight an improvement in terms of MAGL activity and MAGL/FAAH selectivity. In fact, the previously reported compounds showed a MAGL activity in the low micromolar range (IC50 = 1.0–2.2 μM) and a MAGL/FAAH selectivity from 15 up to 69-fold. Conversely, the most active compounds of this series (15, 20b, 20g) displayed IC50 values ranging from 320 to 348 nM, thus the presence of two phenyl rings placed in para-positions to each other markedly increased the inhibitory potency on MAGL. Moreover, the presence of a methoxy group in para-position on both phenyl rings allowed an increase in the MAGL/FAAH selectivity up to 104-fold, as for compound 20b. Therefore, this compound can be considered as the most promising inhibitor of the present series of methyleneoxazol-5(4H)-one derivatives.

In order to study the inhibition mechanism of the new reported compounds, the effects of preincubation and dilution in the inhibitory ability of compound 20b were evaluated. In the preincubation experiments, an irreversible inhibitor will increase its capacity to block the enzyme with increasingly longer incubation times in the presence of enzyme prior to addition of substrate; a constant IC50, conversely, supports a reversible mechanism. As expected, compound 20b did not show any significant increase in its ability to block MAGL activity after 30 and 60 min (Figure 3(A)), supporting that it should be a reversible inhibitor. In the dilution experiments, if 20b is an irreversible inhibitor, then its inhibition potency should not drop upon dilution, whereas inhibition levels should be substantially reduced upon dilution in presence of a reversible compound. As shown in Figure 3(B), 20b showed reversible inhibition, since the inhibition produced by 10 μM of the compound is significantly higher compared with the inhibition observed upon 40× dilution, which appears similar to that produced by a 0.25 μM concentration of the compound.

![Figure 3](image-url)  
Figure 3. Compound 20b-MAGL inhibition analysis. (A) IC50 (μM) values of 20b at different preincubation times with hMAGL (0, 30 and 60 min). (B) Dilution assay: the first two columns indicate the inhibition percentage of compound 20b at a concentration of 10 and 0.25 μM. The third column indicates the inhibition percentage of compound 20b after dilution (final concentration = 0.25 μM).
Compounds 20b was also selected for in vitro experiments to evaluate its antiproliferative potency on cancer cells. Two human ovarian cancer cell lines, OVCAR3 and CAOV3, were chosen because western blot analysis highlighted an overexpression of MAGL in these two cell lines. The compound produced appreciable inhibition of cell viability for both cell lines, with IC50 values of 41.6 μM for OVCAR3 and 23.8 μM for CAOV3. Furthermore, it showed negligible potency against noncancerous human mesenchymal stem cells (hMSC, IC50 > 100 μM).

Molecular modeling studies

To suggest a possible binding mode for this class of derivatives, the interaction of compound 20b with MAGL and FAAH was analyzed by means of docking and MD simulations. This docking analysis helps us to identify the most significant interactions of the compound before the formation of the covalent bond with the catalytic serine, thus highlighting the key points for the ligand recognition. As a first step, a consensus docking method was applied as it is shown to predict the ligand-binding pose better than the single docking programs. By using this kind of approach, one ligand is docked into the target protein by means of different docking procedures. Then, among the different best-ranked poses (originated by the different docking procedures) the pose in common with the largest number of docking procedures is considered as the best docking pose. The 20b-MAGL and 20b-FAAH complexes obtained by means of this docking strategy were then subjected to 51 ns of MD simulation with explicit water molecules, as described in the “Material and methods” section. Figure 4(A) shows the main interactions of 20b with MAGL. The dimethoxyterphenyl fragment occupies the central core of the binding site showing a large number of lipophilic interactions such as those with L148, A151, P178, I179, L184, L205, L213 and L241; whereas the 2-methyloxazolone ring is placed near the catalytic S122 and forms two H-bonds with the nitrogen backbone of A51 (HB1, Figure 4(A)) and M123 (HB2, Figure 4(A)). Interestingly, as shown in Figure 4(B) the H-bonds interactions between the ligand and A51 and M123 displayed a high stability, as they were maintained for the whole MD simulation.

As shown in Figure 5, the binding site shape of FAAH does not seem to allow an interaction of the 2-methyloxazolone ring of 20b in proximity to the catalytic region of the enzyme. The ligand shows a binding disposition that is completely different from that observed in the MAGL binding site, and the 2-methyloxazolone ring is ∼10 Å away from the catalytic S241. Furthermore, the compound does not form any H-bonds with the protein and the ligand is stabilised only by lipophilic interactions with F192, I238, L380, L404 and F432.

In order to further analyze the interaction of 20b into MAGL and FAAH, the two MD trajectories were further analyzed through the MM-PBSA method, which has shown to accurately estimate the ligand–protein energy interaction. This approach averages contributions of gas-phase energies, solvation free energies, and solute entropies calculated for snapshots of the complex molecule as well as the unbound components extracted from MD trajectories, according to the procedure fully described in “Material and methods” section. The MM-PBSA results (Table 2) suggested that the interaction of 20b with the MAGL binding cavity was more stable by ∼8 kcal/mol than its interaction with FAAH and this energy difference was mainly determined by the lack of strong electrostatic interactions into the FAAH-binding site.

Table 2. MM-PBSA results for compound 20b docked into MAGL and FAAH.

| Protein | Ele | VdW | PBsur | PB | ΔPBSA |
|---------|-----|-----|-------|----|-------|
| MAGL    | −12.5 | −52.2 | 43.4 | −5.2 | −26.5 |
| FAAH    | −3.2 | −50.1 | 40.2 | −5.3 | −18.5 |

ΔPBSA is the sum of the electrostatic (Ele), van der Waals (VdW), polar (PB) and non-polar (PBsur) solvation free energy. Data are expressed as kcal/mol.

Conclusions

In summary, we designed and synthesised a new class of terphenyl-2-methyloxazol-5(4H)-one derivatives by optimising the benzylidene-2-methyloxazol-5(4H)-one scaffold, which was
previously identified as a suitable moiety able to efficiently interact with the MAGL-binding site. The reported structural optimisation led to the identification of compound 20b, which displayed a high MAGL-inhibition activity with an IC₅₀ value of 348 nM together with a very good MAGL/FAAH selectivity ratio. Moreover, the biochemical experiments confirmed the reversible properties of this compound and, finally, cell-based assays showed promising cell growth inhibitory activities in the OVCAR-3 and CAOV3 cell lines which overexpress MAGL. Since the in vivo possible application of reversible MAGL inhibitors has only recently been explored, mainly due to the deficiency of known compounds possessing efficient reversible inhibitory activities, the present findings constitute an interesting extension to the knowledge of the MAGL inhibition.

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