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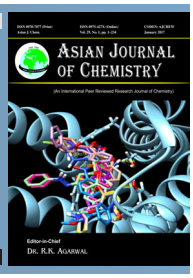

# Synthesis, Characterization and Antiproliferative Activity of Certain Meclofenamic Acid Amides

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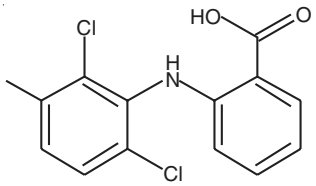
Synthesis of new meclofenamic acid amide derivatives is reported using various amino acid esters and dicyclohexylcarbodiimide as coupling agent. All of the synthesized compounds were characterized by their FT-IR, $^1$H NMR, $^{13}$C NMR and mass spectral data. The antiproliferative activity against human adenocarcinoma cell line HT29 was determined. Compounds **3**, **4**, **6**, **7**, **9**, **10** and **11** displayed excellent inhibitory activity compared to the parent drug. Of these seven initial hits, compound **7** was the most active.

**Keywords: NSAIDs, Meclofenamic acid, Colorectal cancer, Chemoprevention.**

## INTRODUCTION

Colorectal cancer (CRC) is the most common solid tumor worldwide, with its prevalence continues to increase in developing countries [1]. It is considered to be the third leading cause of cancer death in the United States [2]. Experimental, clinical and epidemiological studies demonstrating an inverse relationship between non-steroidal anti-inflammatory drugs (NSAIDs) and colorectal cancer [3]. However, the use of such agents for the purpose of chemoprevention is not a problem free due to the adverse side effects associated with long-term use of such agents [4]. Selective COX-2 inhibitors, such as rofecoxib (Vioxx®) (withdrawn in 2004) and celecoxib (Celebrex®), are the new generation of NSAIDs that exhibit antiproliferative activity similar or superior to conventional NSAIDs. While reducing the prevalence of adverse GI events, recent clinical studies have shown an association between the use of COX-2 selective inhibitors and increased incidence of stroke and myocardial infarction [5]. Recently, many studies have revealed that amide and ester modification of the carboxylic group of existing NSAIDs impart an enhanced potency, selectivity, bioavailability and safety on these drugs as antiproliferative agents as well as utility for treating malignant disease if combined with chemotherapy. This may be due to an enhanced lipophilicity and/or better cell uptake of these derivatives [6-11]. Meclofenamic acid (MCFA) (**1**) is a non-steroidal anti-inflammatory drug that has shown therapeutic potential for different types of cancers [12]. In view of the above facts and in continuation of our interest to synthesize a new NSAIDs derivatives with an enhanced antiproliferative activity against colorectal cancer with GI sparing property [9,13] a series of meclofenamic acid amides of amino acid esters were synthesized and their antiproliferative activity has been investigated against colorectal cancer cell line.




Structure of meclofenamic acid (**1**)

## EXPERIMENTAL

Meclofenamic acid and amino acid esters hydrochloride were obtained from Sigma-Aldrich. Other reagents and solvents used were of analytical grade, the reactions were monitored by TLC on precoated silica G plates using UV lamp. Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were recorded as KBr discs using the Perkin Elmer FT-IR Spectrum BX apparatus located at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). NMR Spectra were scanned in DMSO-$d_6$ on a Bruker NMR spectrometer operating at 500 MHz for $^1$H and 125.76 MHz for $^{13}$C at the Research Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Chemical shifts are expressed in δ-values (ppm)

relative to TMS as an internal standard. Coupling constants (*J*) are expressed in Hz. $D_2O$ was added to confirm the exchangeable protons. Mass spectra were measured on Agilent Triple Quadrupole 6410 QQQ LC/MS with ESI (Electrospray ionization) source.

**General procedure for synthesis of compounds 3-15:** Meclofenamic acid (**1**) (0.01 mol) was dissolved in dichloromethane (20 mL) followed by addition of dicyclohexyl carbodiimide (0.015 mol), the reaction mixture was stirred for 0.5 h and a mixture of appropriate amino acid hydrochloride (0.02 mol) and triethylamine (TEA, 0.2 mL) in dichloromethane (10 mL) was added drop-wise. The reaction mixture was stirred at 0 °C initially for 2 h followed by stirring at room temperature for overnight, the precipitated dicyclohexyl urea was filtered off and the solvent was distilled off under reduced pressure. Ethyl acetate (10 mL) was added to the dried product to remove any remaining dicyclohexyl urea. The ethyl acetate layer was washed with aqueous solution of sodium bicarbonate (10 %) and then with distilled water to remove triethylamine hydrochloride and any traces of alkali. Ethyl acetate layer was dried over anhydrous magnesium sulphate, filtered off and the filtrate was distilled off under reduced pressure to obtain the crude product, which was then purified by column chromatography using ethyl acetate:hexane (mobile phase ratio was adjusted in each case), then they were recrystallized from the appropriate solvent.

**Ethyl 2-[2-(2,6-dichloro-3-methylphenylamino)benzamido]acetate (3):** Compound **3** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3274 (NH), 1753, (C=O, esters), 1628 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.25 (d, 3H, *J* = 7, -$OCH_2C\boldsymbol{H_3}$), 2.32 (s, 3H, Ar-$C\boldsymbol{H_3}$), 4.17-4.21 (m, 4H, $CH_2CO$ and -$OC\boldsymbol{H_2}CH_3$), 6.2 (d, 1H, *J* = 8, H-42), 6.6 (s, 1H, N***H***, $D_2O$ exchangeable), 6.734 (t, 1H, *J* = 7, H-52), 6.98 (d, 1H, *J* = 8, H-5), 7.15 (d, 1H, *J* = 7, H-32), 7.20 (t, 1H, *J* = 8, H-4), 7.48 (d, 1H, *J* = 7, H-62), 9.1 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.18 (-$OCH_2\boldsymbol{C}H_3$), 20.66 (Ar-$\boldsymbol{C}H_3$), 41.74 (NH$\boldsymbol{C}H_2$-), 61.72 (-$O\boldsymbol{C}H_2CH_3$), 114.81 (C-52), 116.49 (C-32), 117.99 (C-12), 127.57 (C-5), 127.71 (C-62), 127.76 (C-4), 130.24 (C-3), 132.39 (C-42),133.34 (C-2), 135.59 (C-1), 136.38 (C-6), 145.60 (C-12), 169.34 (C=O amide), 170.06 (C=O ester). HRMS: Found 381.1329, calculated (381.2531). MS-ESI: *m/z* 381.2 ($M^+$). MS-EI: *m/z* 361.2 (8 %), 359.1 (23 %), 323.1 (1 %), 305 (4 %), 287 (1 %), 281 (3 %), 278 (20 %), 245 (13 %), 244 (37 %), 243.1 (40 %), 242 (100 %), 214 (12 %), 180 (14 %), 62 (6 %), 44 (11 %).

**(R)-Methyl 2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]propanoate (4):** Compound **4** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3321 (NH), 1736, (C=O, ester), 1627 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.56 (d, 3H, *J* = 7, -$CHC\boldsymbol{H_3}$), 2.41 (s, 3H, Ar-$C\boldsymbol{H_3}$), 3.82 (s, 3H, -$OC\boldsymbol{H_3}$), 4.85 (t, 1H, *J* = 8, C***H***NH), 6.38 (d, 1H, *J* = 8, N***H***, $D_2O$ exchangeable), 6.82-7.5 (6H, Ar***H***), 9.1 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 18.75 (-$CH\boldsymbol{C}H_3$), 20.67 (Ar-$\boldsymbol{C}H_3$), 48.30 (NH***C***H-), 52.63 (-$O\boldsymbol{C}H_3$), 114.81, 116.49, 117.99, 127.57, 127.73, 132.31, 148.4 (Ar***C***), 169.2 (C=O amide), 172.90 (C=O ester). MS-ESI: *m/z* 381.2 $[M]^+$. MS-EI: *m/z* 369.2 (1 %), 355.1 (1 %), 333.1 (2 %), 327 (2 %), 305 (11 %), 282 (2 %), 280.9 (3 %), 279.9 (16 %), 278 (27 %), 245 (20 %), 244 (39 %), 243.1 (54 %), 242 (100 %), 214 (16 %), 180 (17 %), 179 (12 %), 92 (7 %), 63 (4 %), 44 (11 %).

**(S)-Methyl 2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]propanoate (5):** Compound **5** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3321 (NH), 1736, (C=O, ester), 1627 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.57 (d, 3H, *J* = 7, -$CHC\boldsymbol{H_3}$), 2.42 (s, 3H, Ar-$C\boldsymbol{H_3}$), 3.83 (s, 3H, -$OC\boldsymbol{H_3}$), 4.85 (t, 1H, *J* = 8, C***H***NH), 6.39 (d, 1H, *J* = 8, N***H***, $D_2O$ exchangeable), 6.82-7.56 (6H, Ar***H***), 9.20 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 18.74 (-$CH\boldsymbol{C}H_3$), 20.65 (Ar-$\boldsymbol{C}H_3$), 48.30 (NH***C***H-), 52.62 (-$O\boldsymbol{C}H_3$), 114.78, 116.68, 117.94, 127.52, 127.71, 132.31, 148.58 (Ar***C***), 169.25 (C=O amide), 172.87 (C=O ester). MS-ESI: *m/z* 382.3 $[M+1]^+$. MS-EI: *m/z* 382.1 $[M]^+$ (7 %), 380 (11 %), 347 (6 %), 345.1 (15 %), 335.1 (1 %), 327 (2 %), 305 (2 %), 281 (1 %), 280.9 (3 %), 279.9 (6 %), 278 (6 %), 245 (9 %), 244 (35 %), 243.1 (27 %), 242 (100 %), 214 (12 %), 180 (12 %), 179 (10 %), 92 (6 %), 63 (4 %).

**(RS)-Methyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]propanoate (6):** Compound **6** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3341 (NH), 1732, (C=O, ester), 1640 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.32 (m, 3H, $OCH_2C\boldsymbol{H_3}$), 1.58 (d, 3H, *J* = 4, -$CHC\boldsymbol{H_3}$), 2.42 (s, 3H, Ar-$C\boldsymbol{H_3}$), 4.27 (d, 2H, *J* = 7 -$OC\boldsymbol{H_2}CH_3$), 4.82 (m, 1H, C***H***NH), 6.38 (d, 1H, *J* = 8, N***H***, $D_2O$ exchangeable), 6.82-7.56 (6H, Ar***H***), 9.30 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.17 (-$OCH_2\boldsymbol{C}H_3$), 18.79 (-$CH\boldsymbol{C}H_3$), 20.66 (Ar-$\boldsymbol{C}H_3$), 48.39 (NH***C***H-), 61.68 (-$O\boldsymbol{C}H_2CH_3$), 114.75, 116.58, 117.93, 127.53, 127.71, 130.23, 132.26, 136.36 148.56 (Ar***C***), 169.50 (C=O amide), 172.91 (C=O ester). MS-ESI: *m/z* 395.2 $[M]^+$.

**Ethyl-3-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]propanoate (7):** Compound **7** was obtained as white crystals. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3336 (NH), 1741, (C=O, ester), 1627 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.31 (t, 3H, *J* = 6, $OCH_2C\boldsymbol{H_3}$), 2.42 (s, 3H, Ar-$C\boldsymbol{H_3}$), 2.70 (s, 2H, -$C\boldsymbol{H_2}CO$), 3.76 (m, 2H, $NHC\boldsymbol{H_2}$-), 4.22 (q, 2H, *J* = 7, -$OC\boldsymbol{H_2}CH_3$), 6.38 (d, 1H, *J* = 8, Ar-***H4***), 6.81 (d, 1H, *J* = 7, Ar***H4***), 6.93 (s, 1H, CON***H***-, $D_2O$ exchangeable), 7.07 (d, 1H, *J* = 8, Ar***H5***), 7.24 (t, 1H, *J* = 7, Ar***H5***), 7.29 (d, 1H, *J* = 8, Ar***H6***), 7.45 (d, 1H, *J* = 7, Ar***H3***), 9.4 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.21 (-$OCH_2\boldsymbol{C}H_3$), 20.66 (Ar-$\boldsymbol{C}H_3$), 34.00 ($\boldsymbol{C}H_2CO$), 35.06 (NH$\boldsymbol{C}H_2$-) 60.86 (-$O\boldsymbol{C}H_2CH_3$), 114.73, 117.03, 117.98, 127.28, 127.63, 127.71, 130.13, 132.06, 133.23, 135.72, 145.49 (Ar***C***), 169.34 (C=O amide), 172.90 (C=O ester). MS-ESI: *m/z* 395.1 $[M]^+$. MS-EI: *m/z* 385.2 (7 %), 284 (2 %), 229 (4 %), 212 (2 %), 187 (5 %), 186 (44 %), 185 (100 %), 183 (5 %), 171.1 (14 %), 169 (12 %), 152.9 (10 %), 141 (12 %), 115.9 (33 %), 98 (6 %), 72 (29 %) 57 (11 %), 55 (6 %).

**(RS)-Methyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]butanoate (8):** Compound **8** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3351 (NH), 1732, (C=O, ester), 1643 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 0.92 (t, 3H, *J* = 7, -$CH_2C\boldsymbol{H_3}$), 1.81 (m, 1H, -$C\boldsymbol{H_2}CH_3$), 1.97 (m, 1H, -$C\boldsymbol{H_2}CH_3$), 2.32 (s, 3H, Ar-$C\boldsymbol{H_3}$), 3.70 (s, 3H, -$OC\boldsymbol{H_3}$), 4.74 (q, 1H, *J* = 6, -C***H***NH), 6.29 (d, 1H, *J* = 8, Ar-***H4***), 6.90 (d, 1H, *J* = 6 CON***H***-, $D_2O$ exchangeable), 6.72-7.46 (5H, Ar***H***), 9.13 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 9.53 (-$CH_2\boldsymbol{C}H_3$), 20.66 (Ar-$\boldsymbol{C}H_3$), 25.80 (-$\boldsymbol{C}H_2CH_3$), 52.84 (-$O\boldsymbol{C}H_3$), 53.41 ($\boldsymbol{C}$HNH-)

114.81, 116.92, 117.99, 127.53, 127.72, 130.22, 132.29, 133.31, 135.63, 136.73, 145.51 (Ar***C***), 169.01 (C=O amide), 173.02 (C=O ester). MS-ESI: *m/z* 395.2 $[M]^+$. MS-EI: *m/z* 387.2 (27 %), 351 (2 %), 313 (3 %), 305 (16 %), 279 (26 %), 278 (40 %), 259 (4 %), 245 (21 %), 244 (42 %), 243 (68 %) 242 (100 %), 214 (16 %), 180 (20 %), 178 (12 %), 151.1 (8 %), 92 (5 %) 77 (8 %).

**(R)-Methyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]-3-methyl butanoate (9):** Compound **9** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3421 (NH), 1734, (C=O, ester), 1639 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 0.98-1.07 (m, 6H, ($CH_3$)$_2$CH), 2.31-2.41 (m, 4H, ($CH_3$)$_2$C***H*** and Ar-C***H₃***), 3.81 (s, 3H, -OC***H₃***), 4.82 (d, 1H, *J* = 4, -C***H***NH), 6.39 (d, 1H, *J* = 8, Ar-***H4***), 6.84 (d, 1H, *J* = 7 CON***H***-, $D_2O$ exchangeable), 6.85-7.58 (5H, Ar***H***), 9.14 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 18.09 (($CH_3$)$_2$CH), 19.00 (($CH_3$)$_2$CH), 20.65 (Ar-***C***$H_3$), 31.61 (($CH_3$)$_2$***C***H)), 52.82 (-O***C***$H_3$), 57.21 (***C***HNH-), 114.85, 116.91, 118.06, 127.53, 127.72, 130.22, 132.27, 133.31, 135.63, 136.71, 145.44 (Ar***C***), 169.14 (C=O amide), 172.60 (C=O ester). MS-ESI: *m/z* 409.2 $[M]^+$. MS-EI: *m/z* 344.2 (2 %), 343 (7 %), 212 (2 %), 187 (2 %), 186 (23 %), 185 (100 %), 171 (9 %), 155 (4 %), 154 (6 %), 153 (8 %) 142 (5 %), 141 (10 %), 114 (7 %), 102 (3 %), 92 (2 %) 74 (3 %), 56 (5 %).

**(S)-Ethyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]-3-methyl butanoate (10):** Compound **10** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3353 (NH), 1742, (C=O, ester), 1628 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 0.97-1.07 (m, 6H, ($CH_3$)$_2$CH), 1.23-1.34 (m, 3H, -OCH$_2$C***H₃***), 2.32-2.41 (m, 4H, ($CH_3$)2C***H-*** and Ar-C***H₃***), 4.25-4.28 (q, 2H, *J* = 7 -OC***H₂***$CH_3$), 4.82 (d, 1H, *J* = 4, -C***H***NH), 6.38 (d, 1H, *J* = 8, Ar-***H4***), 6.77 (s, 1H, CON***H***-, $D_2O$ exchangeable), 6.82-7.58 (5H, Ar***H***), 9.15 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$CNMR: δ 14.27 (OCH$_2$***C***H$_3$)18.06 (($CH_3$)$_2$CH), 18.99 (($CH_3$)$_2$CH), 20.66 (Ar-***C***$H_3$), 31.65 (($CH_3$)$_2$***C***H)), 57.19 (***C***HNH-), 61.41 (-O***C***H$_2$CH$_3$) 114.82, 116.92, 118.05, 127.54, 127.71, 130.21, 132.22, 133.35, 135.65, 136.35, 145.52 (Ar***C***), 169.12 (C=O amide), 172.09 (C=O ester). MS-ESI: *m/z* 423.2 $[M]^+$. MS-EI: *m/z* 384.2 (2 %), 382 (11 %), 380 (17 %), 347.1 (7 %), 345 (20 %), 305 (2 %), 299 (2 %), 280 (4 %), 276.9 (10 %), 271 (3 %) 245 (100 %), 244 (36 %), 243 (29 %), 242 (99 %), 214 (12 %) 180 (12 %), 176 (4 %), 151 (8 %), 92 (7 %), 76 (8 %).

**(S)-Methyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]-4-methyl pentanoate (11):** Compound **11** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3310 (NH), 1761, (C=O, ester), 1626 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 0.91 (d, 6H, *J* = 7.5 ($CH_3$)$_2$CH), 1.48 (m, 1H, ($CH_3$)$_2$C***H***), 1.60-1.72 (m, 2H, -C***H₂***), 2.32 (s, 3H, Ar-C***H₃***), 3.71 (s, 3H, -OC***H₃***), 4.82 (m, 1H, -C***H***NH), 6.28 (d, 1H, *J* = 8, Ar-***H4***), 6.50 (s, 1H, CON***H***-, $D_2O$ exchangeable), 6.71-7.45 (5H, Ar***H***), 9.07 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). **$^{13}$C** NMR: δ 20.65 (Ar-***C***$H_3$), 22.24 (***C***$H_3$)$_2$CH-), 22.81 (***C***$H_3$)$_2$CH-), 25.04 ($CH_3$)$_2$***C***H-), 41.82 (-***C***H$_2$-), 50.96 (***C***HNH-), 52.42 (-O***C***$H_3$) 114.79, 116.85, 117.96, 127.51, 127.78, 130.25, 132.30, 133.35, 135.65, 136.35, 145.56 (Ar***C***), 169.65 (C=O amide), 172.59 (C=O ester). MS-ESI: *m/z* 423.2 $[M]^+$. MS-EI: *m/z* 384.2 (2 %), 380 (17 %), 347.1 (7 %), 345 (18 %), 307 (4 %), 280 (7 %), 278 (10 %), 245 (10 %), 244 (38 %), 243 (38 %), 242 (100 %), 214 (10 %) 180 (15 %), 179 (10 %), 152 (9 %), 92 (6 %), 76 (10 %) 58 (3 %).

**(S)-Ethyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]-4-methyl pentanoate (12):** Compound **12** was obtained as white powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3376 (NH), 1718, (C=O, ester), 1643 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.02 (s, 6H, ($CH_3$)$_2$CH), 1.33 (t, 3H, *J* = 7 -OCH$_2$C***H₃***), 1.71-1.80 (m, 3H, -CH-C***H₂*** and ($CH_3$)$_2$C***H***), 2.42 (s, 3H, Ar-C***H₃***), 4.25-4.26 (m, 2H, -OC***H₂***), 4.86 (m, 1H, -C***H***NH), 6.38 (d, 1H, *J* = 8, Ar-***H4***), 6.60 (d, 1H, *J* = 6.5, Ar***H5***$^-$), 6.82 (d, 1H, *J* = 8 CON***H***-, $D_2O$ exchangeable), 7.07-7.55 (4H, Ar***H***), 9.17 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.19 (-OCH$_2$***C***$H_3$), 20.66 (Ar-***C***$H_3$), 22.30 (***C***$H_3$)$_2$CH-), 22.82 (***C***$H_3$)$_2$CH-), 25.05 ($CH_3$)$_2$***C***H-), 41.86 (-***C***H$_2$-), 51.05 (***C***HNH-), 61.47 (-O***C***H$_2$CH$_3$), 114.76, 117.01, 117.95, 127.50, 127.69, 127.76, 130.34, 132.24, 133.44, 135.65, 136.35, 145.52 (Ar***C***), 169.02 (C=O amide), 173.16 (C=O ester). MS-ESI: *m/z* 437.2 $[M]^+$. MS-EI: *m/z* 384.2 (2 %), 380 (14 %), 380 (17 %), 347.1 (5 %), 345 (17 %), 305 (4 %), 280 (6 %), 278 (9 %), 245 (11 %), 244 (34 %), 243 (36 %), 242 (100 %), 214 (14 %) 180 (11 %), 178.9 (12 %), 152 (7 %), 88 (6 %), 76 (6 %) 63 (5 %).

**(S)-Dimethyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]succinate (13):** Compound **13** was obtained as white crystals. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3378 (NH), 1740,1720 (C=O, esters), 1638 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 2.32 (s, 3H, Ar-C***H₃***), 3.22 (dd, 2H, *J* = 4, 17.5 -C***H₂***CO), 3.65 (s, 3H, -CH$_2$COOC***H₃***), 3.74 (s, 3H, -CHCOOC***H₃***), 5.02 (t, 1H, *J* = 4, -C***H***NH), 6.29 (d, 1H, *J* = 8, -CON***H***, $D_2O$ exchangeable), 6.72-7.47 (6H, ArH), 9.20 (s, 1H, ArNHAr, $D_2O$ exchangeable). $^{13}$CNMR: δ 20.66 (Ar-***C***$H_3$), 36.16 (***C***H$_2$CO), 48.64 (-NH***C***H-), 52.13, (CH$_2$COO***C***$H_3$), 52.98, (CHCOO***C***$H_3$), 114.76, 116.23, 118.02, 127.73, 127.82, 130.28, 132.53, 133.37, 136.39, 145.73 (Ar-***C***), 168.90 (-***C***ONH), 171.33 (CH$_2$***C***OOCH$_3$), 171.74 (-CH***C***OOCH$_3$). MS-ESI: *m/z* 439.2 (M+1). MS-EI: *m/z* 394.1 (12 %), 361 (7 %), 359.1 (20 %), 305 (5 %), 278 (13 %), 245 (13 %), 244 (40 %), 243 (44 %), 242 (100 %), 214 (14 %), 180 (15 %), 179 (11 %), 152 (9 %), 89 (9 %), 77 (8 %), 63 (5 %).

**(S)-Ethyl-2-[2-(2,6-dichloro-3-methylphenylamino)-benzamido]-3-phenyl propanoate (14):** Compound **14** was obtained as yellow powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3421 (NH), 1734 (C=O, ester), 1654 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.21 (t, 3H, *J* = 7 Hz, OCH$_2$C***H₃***), 2.33 (s, 3H, Ar-C***H₃***), 3.17-3.19, 3.24-3.28 (dd, 2H, *J* = 5, 14 Hz, ArC***H₂***-), 4.13-4.16 (q, 2H, *J* = 7 Hz, -OC***H₂***$CH_3$), 5.00 (m, 1H, -C***H***NH,), 6.28 (d, 1H, *J* = 8, Ar***H4***), 6.61 (s, 1H, CON***H***,$D_2O$ exchangeable), 6.86-7.32 (10 Ar***H***), 9.00 (s, 1H, ArN***H***Ar, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.12 (OCH$_2$***C***$H_3$), 20.72 (Ar-***C***$H_3$), 36.91 (Ar***C***H$_2$-), 53.07 (-***C***HNH), 61.36 (O***C***H$_2$CH$_3$), 114.71, 117, 119, 127, 128, 129, 131, 134, 138, 139, 145.68 (Ar***C***), 169.59 (-C=O amide), 171.98 (-C=O ester). MS-ESI: *m/z* 471.4 $[M]^+$. MS-EI: *m/z* 431.2 (3 %), 430 (5 %), 415 (3 %), 355 (13 %), 281 (12 %), 265 (2 %), 221 (18 %), 207 (8 %), 147 (30 %), 83 (2 %), 74 (5 %), 73 (100 %), 56 (2 %).

**(S)-Ethyl-3-(benzylthio)-2-[2-(2,6-dichloro-3-methyl-phenylamino)benzamido]propanoate (15):** Compound **15** was obtained as yellow powder. IR (KBr, $\nu_{max}$, $cm^{-1}$): 3292

(NH), 1728 (C=O, ester), 1626 (C=O, amide). $^1$H NMR: ($CDCl_3$) δ 1.32 (t, 3H, *J* = 7 -$OCH_2CH_3$), 2.42 (s, 3H, Ar$CH_3$) 3.07 (dd, 2H, *J* = 4, 17.5, S-$CH_2$CH), 3.78 (s, 2H, Ar-$CH_2$S), 4.26-4.30 (q, 2H, *J* = 7, O$CH_2CH_3$), 5.03 (m, 1H, -CHNH), 6.39 (d, 1H, *J* = 8, -CONH, $D_2O$ exchangeable), 6.82-6.85 (m, 1H, ArH), 7.02-7.10 (m, 2H, ArH), 7.24-7.31 (m, 7H, ArH), 7.55 (d, 1H, *J* = 8, ArH), 9.26 (s, 1H, ArNHAr, $D_2O$ exchangeable). $^{13}$C NMR: δ 14.17 ($OCH_2CH_3$), 20.67 (Ar$CH_3$), 33.47 (S$CH_2$CH-), 36.88 (Ar$CH_2$S-), 52.13 (CHNH-), 62.02 (-O$CH_2CH_3$), 114.77, 116.46, 118.05, 127.24, 127.73, 127.80, 128.60, 128.96, 130.29, 132.47, 133.38, 135.58, 136.39, 137.66, 145.67 (Ar-C), 168.97 (C=O amide), 170.88 (C=O ester). MS-ESI: *m/z* 517.2 $[M+1]^+$. MS-EI: *m/z* 516.8 (1 %), 475 (1 %), 460 (2 %), 429 (14 %), 355 (13 %), 341 (6 %), 295 (13 %), 280 (15 %), 221 (27 %), 207 (14 %), 147 (56 %), 75 (10 %), 73 (100 %), 57 (3 %).

**Antiproliferative activity against human colon adenocarcinoma HT-29 (cell line and wst-1 cell proliferation assay):** HT-29 colon adeno carcinoma cell line was purchased from the American Type Culture Collection. Cells were maintained in DMEM (Sigma), supplemented with 10 % FBS (Lonza), 100 IU/mL penicillin, 100 mg/mL streptomycin and 2 mmol/L L-glutamine (Sigma) and seeded into 96-well plates at 4 × $10^3$ cells/well and incubated overnight. The medium was replaced with fresh one that containing the desired concentrations of the compounds. After 72 h, 10 µL of the WST-1 reagent was added to each well and the plate was re incubated for 4 h at 37 °C. The amount of formazan was quantified using ELISA reader at 450 nm.

## RESULTS AND DISCUSSION

In this study, meclofenamic acid amide derivatives (**3-15**) were synthesized by coupling of meclofenamic acid (**1**) with the appropriate amino acid ester (**2**) using dicyclohexylcarbdiimide (DCC) in dicholoromethane (DCM) (Fig. 1, Table-1).

The IR spectra of the compounds **3-15** exhibited, a band in the region 1754-1627 $cm^{-1}$ due to the carbonyl absorptions of amide and ester groups whereas the absorption band of NH amide function appeared in the region 3421-3299 $cm^{-1}$, but it was obscured by strong and sharp NH secondary amine absorption at 3421-3292 $cm^{-1}$. In addition, compound **13** containing aspartic acid moiety exhibited an additional carbonyl absorption band within the same above mentioned region, due to

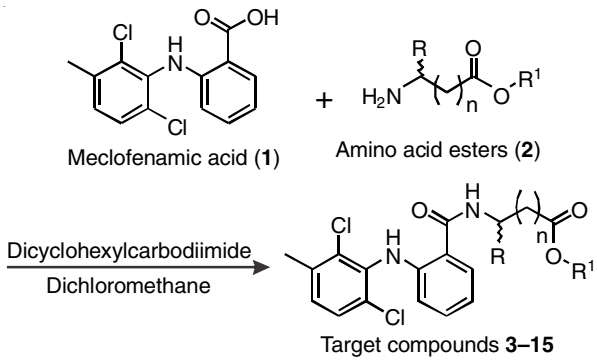



Fig. 1. Synthesis of target amide derivatives (**3-15**)

the additional ester moiety of aspartic acid. The $^1$H NMR spectra of compounds **3-15** revealed the presence of two $D_2O$ exchangeable NH signals in the regions δ 6.1-6.6 and δ 9.10-9.20 ppm due to amidic NH and Ar-NH-Ar, respectively, in addition to the characteristic signals of diphenyl amine (aromatic moietiy) around δ 7.07-7.45 ppm. Furthermore, the amino acid protons were distinguished in each molecule. $^{13}$C NMR spectra of **3-15** exhibited the characteristic $^{13}$C aromatic carbons signals of the diphenylamine moiety at δ 114.71-145.69 ppm, in addition to the distinguished signals of each amino acids esters carbons. Compounds **3-15** provide, in each case molecular ion peaks corresponding to their molecular weights by electron impact (EI-MS), electron spray ionization (ESI-MS) and high resolution mass spectroscopy (HRMS). Lipophilicity is a physico-chemical property of principal importance in drug discovery and development. Consequently, lipophilicity of the synthesized compounds expressed in the term of their C log P values is shown in Table-1. Computation of the log P was based on the fragment method developed by Leo contained in a PC software package since many studies of the most commonly used methods confirm the superiority of the fragmental methods over the atom-based approaches [14].

### Biological investigations

***in vitro* Antiproliferative activity:** In order to appreciate the actual utility of the newly synthesized compounds in colorectal cancer chemoprevntion, *in vitro* antiproliferative activity was carried out by the cell growth inhibition assay. The general *in vitro* antitumor evaluation of the synthesized compounds

TABLE-1
STRUCTURE, m.p., YIELD AND C log P OF COMPOUNDS **3-15**

| Compounds | R | $R^1$ | n | Amino acid ester (**2**) | m.p. (°C) | Yield (%) | log P |
|---|---|---|---|---|---|---|---|
| **3** | H | Et | 0 | Ethyl glycinate | 90-93 | 67 | 5.68 |
| **4** | $-CH_3$ | $-CH_3$ | 0 | D-Alanine methyl ester | 105-108 | 74 | 5.46 |
| **5** | $-CH_3$ | $-CH_3$ | 0 | L-Alanine methyl ester | 101-104 | 63 | 5.46 |
| **6** | $-CH_3$ | Et | 0 | DL-Alanine ethyl ester | 100-103 | 76 | 5.99 |
| **7** | H | Et | 1 | β-Alanine ethyl ester | 82-185 | 73 | 6.02 |
| **8** | Et | $-CH_3$ | 0 | MethylDL-α-amino butyrate | 139-141 | 62 | 5.99 |
| **9** | $-CH(Me)_2$ | $-CH_3$ | 0 | D-Valine methyl ester | 60-62 | 37 | 6.39 |
| **10** | $-CH(Me)_2$ | Et | 0 | L-Valine ethyl ester | 67-70 | 45 | 6.92 |
| **11** | $-CH_2-CH(Me)_2$ | $-CH_3$ | 0 | L-Leucine methyl ester | 64-66 | 67 | 6.92 |
| **12** | $-CH_2-CH(Me)_2$ | Et | 0 | L-Leucine ethyl ester | 86-89 | 52 | 7.45 |
| **13** | $-CH_2-COOMe$ | $-CH_3$ | 0 | L-Aspartate dimethyl ester | 99-101 | 39 | 5.22 |
| **14** | $-CH_2-Ph$ | Et | 0 | L-Phenylalanine ethyl ester | 53-56 | 42 | 7.41 |
| **15** | $-CH_2S-CH_2-Ph$ | Et | 0 | (S)-benzyl-L-cysteine ethyl ester | 60-63 | 51 | 8.16 |

together with their parent meclofenamic acid was conducted by use WST-1 reagent for determination of $IC_{50}$ for each compound according to the protocol mentioned as above. Table-2 provided the $IC_{50}$ for each compound.

TABLE-2
TUMOR CELL GROWTH INHIBITORY ACTIVITY OF MECLOFENAMIC ACID (1) AND ITS AMIDE DERIVATIVES AGAINST HT29 COLORECTAL CANCER CELL LINE

| Compounds | $IC_{50}$[a] (μM) | Compounds | $IC_{50}$ (μM) |
|---|---|---|---|
| **1** | >1000 | **9** | 17.60 |
| **3** | 17.44 | **10** | 17.48 |
| **4** | 40.52 | **11** | 27.87 |
| **5** | IN[b] | **12** | IN |
| **6** | 23.71 | **13** | IN |
| **7** | 13.03 | **14** | IN |
| **8** | IN | **15** | IN |

[a]$IC_{50}$ = Concentration of the compound (μM) producing 50 % cell growth inhibition after 48 h of compound exposure as determined by the WST-1 assay. Each experiment was run at least two times.
[b]Inactive within 40 μM concentration range

The tested compounds showed different antiproliferative effect on the presented colorectal cancer HT29 cell line (Table-2). Meclofenamic acid (**1**) showed low growth inhibitory activity at the tested concentration range, which is in agreement with the tumor cell growth-inhibitory effective concentrations of NSAIDs in various tumor cell types published so far; the 50 % inhibitory concentrations reported usually vary between 0.1 and 5 mM [15]. Generally, all meclofenamic acid active derivatives were more potent than the previously reported naproxen derivatives with similar structure [13]. Compounds **4** and **5** are enantiomers carrying D-alanine methyl ester and L-alanine methyl ester moieties, respectively and it was found that, compound **5** was inactive. Moreover, compound **6** which is racemic mixture (carrying DL-alanine ethyl ester moiety) displayed an enhanced potency that reflected the stereochemical and side-chain extension influences on antiproliferative activity for these derivatives. However, compounds **9** and **10** (carrying D-valine methyl ester and L-valine ethyl ester, respectively) exhibited similar activity. On the other hand, compound **11** (carrying L-leucine methyl ester) displayed an enhanced potency, while compound **12** in which the methyl ester side chain of compound **11** extended to ethyl was found to be inactive. Compounds **13-15** were devoid from antitumor activity against HT29 cell line. Compound **7** (carry β-alanine ethyl ester) is the most active one, with potency that 3-4 times the standard drug approved by FDA for colorectal cancer chemoprevention celecoxib (Celebrex®) ($IC_{50}$ = 50 μM) [16]. It was found that there is no evident relation between the tumor cell growth inhibitory activity and their lipophilicity expressed as C log P. Clearly the lipophilicity has an influence on the activity, but it does not solely determine the antiproliferative activity of these compounds.

## Conclusion

Thirteen amide derivatives of meclofenamic acid and amino acid esters were synthesized, characterized and investigated for activity against colorectal cancer HT29 cell line, most of them are active, especialy compound **7** which is 3-4 times more potent than celecoxib.

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