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ARTICLE

Reactions, Anticancer, Antialzheimer and Anti COX-2 Activities of Newly Synthesized 2-Substituted Thienopyridines(II)

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ABSTRACT

3-Amino-4-(2-thienyl or 2-furyl)-6-pyridin-3-ylthieno[2,3-*b*]pyridine-2-carbonitriles and 3-amino-4-(2-thienyl or 2-furyl)-6-pyridin-3-ylthieno[2,3-*b*]pyridine-2-carboxamides) (**1a-d**) were used as the starting materials in the present study. The newly synthesized pyridothieno-pyrimidines, pyridothienotriazines and pyridothien-oxazines were obtained through the reactions of compounds **1a-d** with each of carbon disulfide, formic acid, triethyl orthoformate, acetic anhydride and nitrous acid, respectively. The newly synthesized hetrocyclic compounds were tested as anticancer, antialzheimer and anti COX-2 agents and their structures elucidated by considering the data of IR, ^{1}H NMR, mass spectra as well as that of elemental analyses.

KEYWORDS

Triethylorthoformate, Dimethylformamide-Dimethylacetal, Pyrido-thieno-pyrimidinone, Pyridothienotriazinone, Pyridothienopyrimidine-dithione, Pyridothieno-pyrimidinethione.

INTRODUCTION

The object of this study is a further extension of compounds **1a-d** reactions with laboratory reagents to afford several newly synthesized heterocyclic compounds required for several chemical transformations as well as our medicinal chemistry program. In continuation to previous work [1-21] we interested here to investigate the synthetic potentiality of both 3-amino-4-(2-thienyl or 2-furyl)-6-pyridin-3-ylthieno-[2,3-*b*]pyridine-2-carboxamides and 3-amino-4-(2-thienyl or 2-furyl)-6-pyridin-3-ylthieno-[2,3-*b*]pyridine-2-carbonitriles (**1a-d**) [1].

EXPERIMENTAL

All melting points were uncorrected. IR (KBr discs) spectra were recorded on a Shimadzu FTIR-8201PC Spectrophotometer. ^{1}H NMR spectra were recorded on a Varian Mercury 300 MHz. and a Varian Gemini 200 MHz. spectrometers using TMS as an internal standard and $CDCl_3$, DMSO-d_6 and $(CD_3)_2CO$ as solvents. Chemical shifts were expressed as δ (ppm) units. Mass spectra were recorded on Shimadzu GCMS-QP1000EX using an inlet type at 70 eV. The Micro analytical Center of Cairo University performed the microanalyzes.

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Synthesis of compounds 2a-b

Method A: A solution of each of compounds **1a-d** (0.33 g, 0.32 g, 0.35 g and 0.34 g, 1 mmol) and formic acid (15 mL) was heated under reflux for 6 h. The excess solvent was evaporated and cooled. The solid was collected by filtration, dried and crystallized from the proper solvent to give compounds **2a-b**, respectively.

Method B: A solution of each of compounds **1c-d** (0.35 g and 0.34 g, 1 mmol) and triethylorthoformate (2 mL) in acetic anhydride (10 mL) was refluxed for 4 h. The excess solvent was evaporated and cooled. The solid was collected by filtration, dried and crystallized from the proper solvent to give **2a,b**, respectively.

7-(Pyridin-3-yl)-9-(thiophen-2-yl)pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (2a): Pale yellow crystals crystallization from EtOH (85 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3169 (NH), 3094 (C-H aromatic), 1670 (CO); MS (*m/z*): 362 (M^+, 80.6 % which corresponding to the molecular weight of the molecular formula $C_{18}H_{10}N_4OS_2$ of the assigned structure), 361 (M^+-H, 100 %), 360 (M^+-2H, 3.8 %), 335 (M^+-NCH, 2.1 %), 334 (M^+-CO, 7 %), 306 (M^+-CONHCH, 29.3 %); Anal. for $C_{18}H_{10}N_4OS_2$ (362), Calcd./Found (%): C (59.65/59.68), H (2.78/2.80), N (15.46/15.49), S (17.69/17.71).

9-(Furan-2-yl)-7-(pyridin-3-yl)pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidin-4(3*H*)-one (2b): Orange crystals crystallization from AcOH (71 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3146 (NH), 2990 (C-H aromatic), 1665 (CO); MS (*m/z*): 346 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{18}H_{10}N_4O_2S$ of the assigned structure), 345 (M^+-H, 73.5 %), 318 (M^+-CO, 12.9 %), 290 (M^+CONHCH, 18.2 %); Anal. for $C_{18}H_{10}N_4O_2S$ (346), Calcd./Found (%): C (62.42/62.45), H (2.91/2.93), N (16.18/16.20), S (9.26/9.29).

Synthesis of compounds 3a-b: A solution of each of compounds **1a-d** (0.33 g, 0.32 g, 0.35 g and 0.34 g, 1 mmol) in acetic anhydride (15 mL) was heated under reflux for 4 h. The excess solvents were evaporated and cooled. The solid was collected by filtration, dried and crystallized from the proper solvents to give compounds **3a-b**, respectively.

2-Methyl-7-(pyridin-3-yl)-9-(thiophen-2-yl)pyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3a): Yellow crystals crystallization from EtOH (66 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3459 (NH), 3065 (C-H aromatic), 1666 (CO); MS (*m/z*): 376 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{19}H_{12}N_4OS_2$ of the assigned structure), 375 (M^+-H, 88.2 %), 361 (M^+-CH_3, 21 %); Anal. for $C_{19}H_{12}N_4OS_2$ (376), Calcd./Found (%): C (60.62/60.64), H (3.21/3.24), N (14.88/14.90), S (17.04/17.06).

9-(Furan-2-yl)-2-methyl-7-(pyridin-3-yl)pyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (3b): Yellow crystals, crystallization from dioxan (84 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3141 (NH), 3055 (C-H aromatic), 1656 (CO); ^{1}H NMR (DMSO-d_6) (δ ppm): 1.912 (s, 3H, CH_3); 7.573-8.729 (m, 8H, pyridinyl H's, furyl H's, NH); 9.384 (s, 1H, pyridinyl C_8H); Anal. for $C_{19}H_{12}N_4O_2S$ (360), Calcd./Found (%): C (63.32/63.35), H (3.36/3.38), N (15.55/15.57), S (8.90/8.93).

Synthesis of compounds 4a-b: Sodium nitrite solution 10 % (5 mL) was added to a solution of compounds **1a-b** (0.33 g and 0.32 g, 1 mmol) in concentrated sulphuric acid (5 mL) and glacial acetic acid (5 mL) at 0 °C during 5 min, with stirring. The mixture was allowed to stand at room temperature for 30 min. The solid that precipitated on dilution with water was collected and recrystallized from proper solvents.

7-(Pyridin-3-yl)-9-(thiophen-2-yl)pyrido[3',2':4,5]-thieno[3,2-*d*][1,2,3]triazin-4(3-*H*)-one (4a): Yellow crystals, crystallization from AcOH (75 %); m.p. = 270 °C; IR (KBr, ν_{max}, cm^{-1}): 3356 (NH), 3042 (C-H aromatic), 1683 (CO); MS (*m/z*): Anal. for $C_{17}H_9N_5OS_2$ (363), Calcd./Found (%): C (56.18/56.20), H (2.50/2.53), N (19.27/19.30), S (17.65/17.68).

9-(Furan-2-yl)-7-(pyridin-3-yl)pyrido[3',2':4,5]thieno-[3,2-*d*][1,2,3]-triazin-4(3*H*)-one (4b): Yellow crystals, crystallization from dioxane (80 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3421 (NH), 3075 (C-H aromatic), 1689 (CO); Anal. for $C_{17}H_9N_5O_2S$ (347), Calcd./Found (%): C (58.78/58.80), H (2.61/2.63), N (20.16/20.18), S (9.23/9.25).

Synthesis of compound 5b: A solution of each of compound **1b** (0.32 g, 1 mmol) and carbon disulphide (5 mL) in pyridine (15 mL) was heated under reflux for 6 h, cooled, poured onto ice-cold water and neutralized with drops acetic acid the solid was collected by filtration, dried and crystallized from the dioxane to give **5b**.

9-(Furan-2-yl)-7-(pyridin-3-yl)pyrido[3',2':4,5]thieno-[3,2-*d*]pyrimidine-2,4(1*H*,-3*H*)-dithione (5b): Orange crystals (80 %); m.p. => 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3331 (NH), 3056 (C-H aromatic), 1542 (C=S); MS (*m/z*): 394 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{18}H_{10}N_4OS_3$ of the assigned structure), 378 (M^+-H, NH, 98.4 %), 351 (M^+-CONH, 4.7 %), 307 (M^+-NHCS, 34.4 %), 292 (M^+-CONHCSNH, 15.6 %); Anal. for $C_{18}H_{10}N_4OS_3$ (394), Calcd./Found (%): C (54.80/54.83), H (2.56/2.58), N (14.20/14.23), S (24.38/24.40).

Synthesis of compounds 6a-b: A solution of each of compounds **1a-b** (0.33 g and 0.32 g, 1 mmol) and phenyl isothiocyanate (0.135 g, 1 mmol) in pyridine (15 mL) was heated under reflux for 6 h, cooled, poured onto ice-cold water and neutralized with drops acetic acid. The solid was collected by filtration, dried and crystallized from the dioxane to give compounds **6a-b**, respectively.

4-Imino-3-phenyl-7-(pyridin-3-yl)-9-(thiophen-2-yl)-3,4-dihydro-pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidine-2(1*H*)-thione (6a): Yellow crystals (76 %); m.p. => 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3241, 3188 (two NH), 3033 (C-H aromatic), 1602 (CO); MS (*m/z*): 469 (M^+, 89.9 % which corresponding to the molecular weight of the molecular formula $C_{24}H_{15}N_5S_3$ of the assigned structure), 468 (M^+-H, 30.7 %), 411 (M^+-NCS, 100 %), 410 (M^+-NHCS, 10.5 %), 332 (M^+-$NHCSNC_6H_5$, 11.8 %), 332 (M^+-NHCSN(C_6H_5)=NH 8.6 %); ^{1}H NMR (DMSO-d_6) (δ ppm): 6.934 (s, 1H, NH); 7.080-8.713 (m, 12H, Ar H's, pyridinyl H's, thienyl H's); 9.415 (s, 1H, pyridinyl C_8H), 10.112 (s, 1H, NH); Anal. for $C_{24}H_{15}N_5S_3$ (469), Calcd./Found (%): C (61.38/61.40), H (3.22/3.25), N (14.91/14.94), S (20.48/20.50).

9-(Furan-2-yl)-4-imino-3-phenyl-7-(pyridin-3-yl)-3,4-dihydropyrido-[3',2':4,5]-thieno[3,2-*d*]pyrimidine-2(1*H*)-

thione (6b): Yellow crystals (65 %); m.p. = > 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3337 (NH), 3047 (C-H aromatic), 1545 (C=S); MS (*m/z*): 453 (M^+, 3.8 % which corresponding to the molecular weight of the molecular formula $C_{27}H_{19}N_5OS_2$ of the assigned structure), 394 (M^+-NHCS, 11.3 %), 392 (M^+-2H, NHCS, 100 %); Anal. for $C_{24}H_{15}N_5OS_2$ (453), Calcd./Found (%): C (63.56/63.58), H (3.33/3.35), N (15.44/15.47), S (14.14/14.16).

Synthesis of compound 8b: A solution of each of compounds **7a-b** (0.35 g and 0.34 g, 1 mmol) and acetic anhydride (15 mL) was heated under reflux for 4 h. The excess solvent was evaporated and cooled. The solid was collected by filtration, dried, to give compounds **8a-b**, respectively.

2-Methyl-7-(pyridin-3-yl)-9-(thiophen-2-yl)-4*H*-pyrido-[3',2':4,5]-thieno[3,2-*d*][1,3]-oxazin-4-one (8a): White crystals (73 %); m.p. = 268 °C; IR (KBr, ν_{max}, cm^{-1}): 3083 (C-H aromatic), 1742 (CO); MS (*m/z*): 377 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{19}H_{11}N_3O_2S_2$ of the assigned structure), 376 (M^+-H, 12.8 %), 336 (M^+-$NCCH_3$, 8.4 %), 333 (M^+-COO, 14.1 %), 306 (M^+-$COOCCH_3$, 33.6 %), 292 (M^+-$COOCCH_3N$, 3.6 %); Anal. for $C_{19}H_{11}N_3O_2S_2$ (377), Calcd./Found (%): C (60.46/60.49), H (2.94/2.97), N (11.13/11.15), S (16.99/17.02).

9-(Furan-2-yl)-2-methyl-7-(pyridin-3-yl)-4*H*-pyrido-[3',2':4,5]thieno[3,2-*d*][1,3]-oxazin-4-one (8b): White crystals (73 %); m.p. = 273 °C; IR (KBr, ν_{max}, cm^{-1}): 3045 (C-H aromatic), 1741 (CO); Anal. for $C_{19}H_{11}N_3O_3S$ (361), Calcd./Found (%): C (63.15/63.17), H (3.07/3.09), N (11.63/11.66), S (8.87/8.89).

Synthesis of compounds 9a-b: A solution of each of compounds **8a-b** (0.38 g and 0.36 g, 1 mmol) and aniline (0.09 g, 1 mmol) in acetic acid (10 mL) was heated under reflux for 3 h. The excess solvent was evaporated and cooled. The solid was collected by filtration, dried and crystallized from the acetic acid to give compounds **9a-b**, respectively.

2-Methyl-3-phenyl-7-(pyridin-3-yl)-9-(thiophen-2-yl)pyrido[3',2':4,5]-thieno[3,2-*d*]pyrimidin-4(3*H*)-one (9a): Pale yellow crystals (62 %); m.p. = 320 °C; IR (KBr, ν_{max}, cm^{-1}): 3007 (C-H aromatic), 1657 (CO); MS (*m/z*); 452 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{25}H_{16}N_4OS_2$of the assigned structure), 451 (M^+-H, 42.9 %), 437 (M^+-CH_3, 17.2 %), 424 (M^+-CO, 1.0 %), 333 (M^+-$CONC_6H_5$, 2.6 %); ^{1}H NMR (DMSO-d_6) (δ ppm): 2.113 (s, 3H, CH_3); 7.267-8728 (m, 12H, Ar-H's, pyridinyl H's, thienyl H's), 9.431 (s, 1H, pyridinyl C_8H); Anal. for $C_{25}H_{16}N_4OS_2$ (452), Calcd./Found (%): C (66.35/66.37), H (3.56/3.58), N (12.38/12.40), S (14.17/14.19)

9-(Furan-2-yl)-2-methyl-3-phenyl-7-(pyridin-3-yl)-pyrido[3',2':4,5]-thieno[3,2-*d*]-pyrimidin-4(3*H*)-oneb (9b): Pale yelllow crystals (62 %); m.p. => 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3013 (C-H aromatic), 1674 (CO); Anal. for $C_{25}H_{16}N_4O_2S$ (436), Calcd./Found (%): C (68.79/68.82), H (3.69/3.72), N (12.84/12.87), S (7.35/7.36).

Synthesis of compound 10b: A solution of each of compounds **8a-b** (0.38 g and 0.36 g, 1 mmol) in hydrazine hydrate (5 mL) and ethanol (10 mL) was heated under reflux for 2 h. The excess solvents were evaporated and cooled. The solid was collected by filtration, dried and crystallized from the proper solvents to give compounds **10a-b**, respectively.

3-Amino-2-methyl-7-(pyridin-3-yl)-9-(thiophen-2-yl)pyrido[3',2':4,5]-thieno[3,2-*d*]-pyrimidin-4(3*H*)-one (10a): Orange crystals, crystallization from EtOH (77 %); m.p. =>330 °C; IR (KBr, ν_{max}, cm^{-1}): 3419, 3275 (NH_2), 3094 (C-H aromatic), 1664 (CO); MS (*m/z*): 391 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{19}H_{13}N_5OS_2$ of the assigned structure), 390 (M^+-H, 14.5 %), 376 (M^+-CH_3, 7.5 %), 375 (M^+-NH_2, 4.6 %), 363 (M^+-CO, 11.2 %), 333 (M^+-$CON(NH_2)$, 4.2 %), 306 (M^+-$CON(NH_2)CCH_3$, 17.0 %), 292 (M^+-$CON(NH_2)$ C (CH_3)N, 1.7 %); Anal. for $C_{19}H_{13}N_5OS_2$ (391), Calcd./Found (%): C (58.29/58.31), H (3.35/3.37), N (17.89/17.91), S (16.38/16.40).

3-Amino-9-(furan-2-yl)-2-methyl-7-(pyridin-3-yl)-pyrido[3',2':4,5]thieno[3,2-*d*]pyrimidin-4(3*H*)-one (10b): Pale yellow crystals, crystallization from dioxane (77 %); m.p. => 330 °C; IR (KBr, ν_{max}, cm^{-1}): 3423, 3251, 3159 (NH_2), 2921 (C-H aromatic), 1676 (CO); Anal. for $C_{19}H_{13}N_5O_2S$ (375), Calcd./Found (%): C (60.79/60.81), H (3.49/3.51), N (18.66/18.69), S (8.54/8.57).

Synthesis of compounds 12a-b: A solution of each of compounds **11a-b** (0.38 g and 0.37 g, 1 mmol) and dimethylformamide dimethylacetal (0.24 g, 2 mmol) in dry xylene (20 mL) was heated under reflux for 5 h. The excess solvent was evaporated and cooled. The solid was collected by filtration, dried and crystallized from ethanol to give compounds **12a-b**, respectively.

Ethyl 3-{[(dimethylamino)methylidene]amino}-6-(pyridin-3-yl)-4-(thiophen-2-yl)-thieno[2,3-*b*]pyridine-2-carboxylate (12a): Yellow crystals (90 %); m.p. = 160 °C; IR (KBr, ν_{max}, cm^{-1}): 3042 (C-H aromatic), 1706 (CO); MS (*m/z*): 436 (M^+, 27.3 % which corresponding to the molecular weight of the molecular formula $C_{22}H_{20}N_4O_2S_2$ of the assigned structure), 378 (M^+-$CHN(CH_3)_2$, 8.7 %), 365 (M^+-$N{=}CHN(CH_3)_2$, 31.1 %), 363 (M^+-COOEt, 100 %); Anal. for $C_{22}H_{20}N_4O_2S_2$ (436), Calcd./Found (%): C (60.53/60.56), H (4.62/4.65), N (12.83/12.85), S (14.69/14.71).

Ethyl 3-{[(dimethylamino)methylidene]amino}-4-(furan-2-yl)-6-(pyridin-3-yl)-thieno[2,3-*b*]pyridine-2-carboxylate (12b): Yellow crystals (90 %); m.p. = 198 °C; IR (KBr, ν_{max}, cm^{-1}): 3047 (C-H aromatic), 1673 (CO); **^{1}H NMR** (DMSO-d_6) (δ ppm): 1.272 (t, 3H, J = 6.9 Hz, $COOCH_2\underline{CH_3}$); 2.854 (s, 3H, $N(\underline{CH_3})_2$); 3.006 (s, 3H, $N(\underline{CH_3})_2$); 4.281 (q, 2H, J = 6.9 Hz, $\underline{CH_2}CH_3$); 7.422-8.706 (m, 7H, pyridinyl H's, furyl H's and N=CH-), 9.409 (s, 1H, pyridinyl C_5H); Anal. for $C_{22}H_{20}N_4O_3S$ (420), Calcd./Found (%): C (62.84/62.86), H (4.79/4.81), N (13.32/13.35), S (7.63/7.65).

Synthesis of compounds 13a-b: A solution of each of compounds **12a-b** (0.44 g and 0.42 g, 1 mmol) in hydrazine hydrate (10 mL) and ethanol (20 mL) was heated under reflux for 2 h. The excess solvents were evaporated and cooled. The solid was collected by filtration, dried and crystallized from the dioxane to give compounds **13a-b**, respectively.

3-Amino-7-(pyridin-3-yl)-9-(thiophen-2-yl)pyrido-[3',2':4,5]thieno[3,2-*d*]pyramid-in-4(3*H*)-one (13a): Yellow crystals (90 %); m.p. = 270 °C; IR (KBr, ν_{max}, cm^{-1}): 3463, 3322 (NH_2), 3048 (C-H aromatic), 1673 (CO); MS (*m/z*): 377 (M^+, 100 % which corresponding to the molecular weight of the molecular formula $C_{18}H_{11}N_5OS_2$ of the assigned structure),

376 (M^+-H, 35.2 %), 362 (M^+-NCH, 1.9 %), 361 (M^+-NH_2, 3.8 %), 349 (M^+-CO, 11.7 %), 319 (M^+-$CONNH_2$, 7.9 %), 306 (M^+-$CON(NH_2)CH$, 19.9 %); Anal. for $C_{18}H_{11}N_5OS_2$ (377), Calcd./Found (%): C (57.28/57.30), H (2.94/2.97), N (18.55/18.58), S (16.99/17.01).

3-Amino-9-(furan-2-yl)-7-(pyridin-3-yl)pyrido-[3',2':4,5]thieno[3,2-*d*]pyrimidin-4-(3*H*)-one (13b): Yellow crystals (90 %); m.p. = 310 °C; IR (KBr, ν_{max}, cm^{-1}): 3268, 3150 (NH_2), 2959 (C-H aromatic), 1677 (CO); ^1H NMR (DMSO-d_6) (δ ppm): 6.146 (s, 2H, NH_2); 6.798-8.719 (m, 7H, pyridinyl H's, furyl H's), 9.355 (s, 1H, pyridinyl C_8H); Anal. for $C_{18}H_{11}N_5O_2S$ (361), Calcd./Found (%): C (59.82/59.85), H (3.07/3.09), N (19.38/19.40), S (8.87/8.90).

Aβ42 and Aβ40 assay: Aβ42 and Aβ40 measured in the culture medium of H4 cells, a human neuroglioma cell line expressing the double Swedish mutation (K595N/M596L) of human APP (APPsw). Cells were seeded onto 24-well plates (2×10^5 cell $well^{-1}$) and allowed to grow to confluence for 24 h, in 5 % CO_2/95 % air in a humidified atmosphere. Increasing concentrations (from 3 to 300-400 μM) of the compounds were added to the cells overnight in a final volume of 0.5 mL. *R*-Flurbiprofen was used as positive control (3-1000 μM). DMSO (1 %) was used as negative control. At the end of the incubation, 100 μL of supernatants were removed and treated with a biotinylated mouse monoclonal antibody (4G8, Signet Laboratories Inc., Dedham, MA, USA), specifically recognizing the 17-24 amino acid region of Aβ and two rabbit polyclonal antibodies (C-term 42 and C-term 40, BioSource International, Camarillo, CA, USA), specifically recognizing the C-terminus of Aβ42 and Aβ40, respectively. Antigen-antibodies complexes were recognized by TAG-donkey anti-rabbit IgG (Jackson Immuno Research Laboratories, Soham, UK). Streptavidin coated magnetic beads captured the complexes and the signals were read by an electrochemiluminescence instrument (Origen M8 Analyzer, BioVeris Corporation, Gaithersbur g, MD, USA). The cytotoxicity potential of test compounds was assessed in the same cells of the Aβ assay (H4) with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) assay. MTT is a soluble pale yellow salt that is reduced by mitochondrial succinate dehydrogenase to form an insoluble dark blue formazan product to which the cell membrane is impermeable. The ability of cells to reduce MTT provides an indication of mitochondria integrity and activity and it may be interpreted as a measure of viability and/or cell number. After medium removal for of Aβ 42 and Aβ 40 determination, cells were incubated for 3 h with 500 μL culture medium containing 0.5 mg mL^{-1} MTT, at 37 °C, 5 % CO_2 and saturated humidity. After removal of the medium, 500 μL of 100 % DMSO were added to each well. The amount of formed formazan was determined reading the samples at 570 nm (background 630 nm) using a microplater reader (model 450, Bio-Rad, Hercules, CA, USA).

COX-1 and COX-2 assay: The inhibition of the cyclooxygenase activity was estimated measuring PGE2 production from arachidonic acid according to a modified version of the method [25]. Recombinant human prostaglandin H2 synthase-1 (PGHS-1) and-2 (PGHS-2) were expressed in transfected Spodoptera frugiperda (Sf-9) cells (Invitrogen, San Diego, CA, USA). The microsomal fractions were prepared from the transfected cells and used to assay the enzymatic activities. Briefly, the enzymes (2 g) reconstituted in a buffer (100 mM Tris-HCl, pH 8.0) containing 2mM phenol, were preincubated with vehicle (DMSO) or test compounds in DMSO (1 % DMSO in the final assay) for 20 min at 22 °C. The reaction mixture was completed with 1 M hematin. The reaction was initiated adding arachidonic acid (4 and 2 μ for COX-1 and COX-2, respectively) and the mixture was incubated for 5 min at 22 °C for COX-1 assay, or for 10 min at 25 °C for COX-2 assay. For control measurements, arachidonic acid was omitted from the reaction mixture. The reactions were stopped by the sequential addition of 1 M HCl and 1 M Tris-HCl (pH 8.0), followed by cooling to 4 °C. The amount of PGE2 present in the reaction mixture was quantified using an enzyme-immunoassay.

Studies in Tg2576 transgenic mice: Young male and female transgenic mice (Tg2576) expressing the human APP gene with the Swedish double mutation (K670N/M671L) under the transcriptional control of the hamster prion protein promoter [26] were used for the *in vivo* studies. Male animals were housed singly in individual cages while female animals were placed in groups of 3-5 animals per cage. The experiments were performed in accordance with EEC Guidelines (86/609/ECC) for the use of laboratory animals.

Study 1: Groups of male mice 4-5 months, each group composed of twenty-one male mice of 4–5 months of age were given by oral *gavage* vehicle (Kool-Aid 7.5 mL kg^{-1}) or a suspension of each individual compound (100 or 300 mg kg^{-1} day^{-1} in Kool-Aid) once daily for 5 days. This vehicle was selected to replicate that reported with flurbiprofen in similar studies [27-29]. On day 5, mice were given a final dose of 100 or 300 mg kg^{-1} or vehicle and sacrificed 3 h later, as described below.

Study 2: Groups of female mice of 5-7 months of age, each group composed seventeen female mice of 5-7 months of age were given by oral *gavage* vehicle (Kool-Aid 7.5 mL kg^{-1}) or a suspension of individual compound (100 or 300 mg kg^{-1} day^{-1} in Kool-Aid) once daily for 4 days. On day 4, mice were given a final dose of 100 or 300 mg kg^{-1} or vehicle and sacrificed 3 h later, as described below.

Study 3: Groups of male and female mice of 4–5 months, each group composed of thirty-three male and female mice of 4–5 months were given vehicle or tested compounds or *R*-flurbiprofen-supplemented chow adlibitum for 4 weeks. There were 11 animals in each treatment group. *R*-Flurbiprofen (Sigma, St. Louis, MO, USA) and the tested compounds were formulated into standard, colour-coded, rodent diet by Charles River (Calco, Italy) at a final drug concentration of 375 ppm. The concentration of the drugs in the diet was the same as that used for flurbiprofen in previous studies [30-32]. Body weight and food consumption were monitored every 3-4 days.

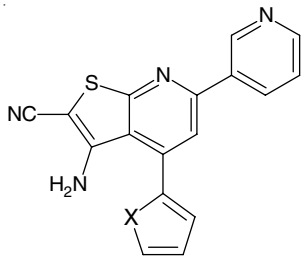
Plasma and brain Aβ measurements: Before 24 h starting treatment, one blood sample was collected by means of retro-bulbar puncture for measurement of baseline plasma, Aβ40 and Aβ42 concentrations. On the last day of treatment, mice were sacrificed by decapitation. Blood samples were collected in EDTA-coated tubes and centrifuged at 800 rpm for 20 min. to separate plasma. Plasma samples were divided into two aliquots of approximately 100 μL each and stored at -80 °C until Aβ and drug assay. The brains were quickly removed

and placed on an ice-cold plate. Cortex and hippocampus were dissected and immediately frozen on dry ice and stored at -80 °C for Aβ assay. The remaining brain was immediately frozen on dry ice and stored at -80 °C for drug level measurements. Plasma was diluted 1:4 for Aβ42 and 1:20 for Aβ40. For measurement of Aβ, brain tissue samples were homogenized in 70 % formic acid at 1:10 (w/v). Homogenates were agitated at 4 °C for 3 h and then centrifuged at 15,000×g for 25 min at 4 °C. The supernatants were collected and neutralized with 1 M Tris, pH 11 at 1:20 (w/v) dilution with 3 × protease inhibitor mixtures (Boehringer Mannheim, Mannheim, Germany). Levels of Aβ40 and Aβ42 in plasma and in brain homogenate supernatants were measured with commercial ELISA kits (The Genetics Company, Zurich, Switzerland). The micro-titre plates were coated with capturing purified monoclonal antibodies specifically recognizing the C-terminus of human Aβ40 (clone G2-10, reactive to amino acid residues 31-40, isotype IgG2b, kappa) or Aβ42 (clone G2-13, reactive to amino acid residues 33-42, isotype IgG1, kappa). As detection antibody, a monoclonal biotin conjugated antibody recognizing the N-terminus of human Aβ (clone W0-2, reactive to amino acid residues 4-10, isotype IgG2a, kappa) was used. The assay was linear in the range 25-500 pg mL^{-1} and the detection limit was 25 pg mL^{-1}.

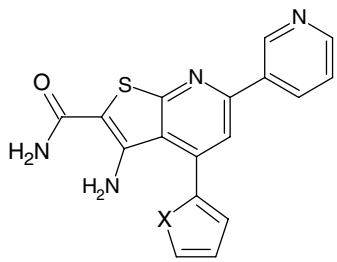
Plasma and brain drug measurements: Drugs levels in plasma and in brain samples were measured by liquid chromatography as previously described [33]. Briefly, samples were prepared by adding 300 µL acetonitrile and 40 µL phosphoric acid 40 % to 100 µL plasma or brain homogenate and placing the mixture in a vortex for 5 s. Plasma and brain samples were then centrifuged at 14,000 rpm for 5 min and the supernatants (15 and 50 µL, respectively) were injected into the HPLC system. Equipment systems with fluorescence (Waters 474, Waters, Guyancourt, France) or mass spectrometry (API 2000, Applied Biosystems, Foster City, CA, USA) detectors were used. The chromatographic conditions were adapted to each compound to obtain good peak separation and detection sensitivity. A mixture of ammonium formate (20 mM) buffer-acetonitrile-methanol was used as mobile phase for the fluorescence detector. For drugs the assay was linear in the range 20–4000 ng g^{-1} in the brain and 5-1000 ng mL^{-1} in plasma with limits of quantitation of 20 ng g^{-1} in the brain and 5 ng mL^{-1} in plasma. For drugs, the assay was linear between 400 and 20,000 ng g^{-1} in the brain and 100-8500 ng mL^{-1} in plasma with limits of quantitation of 400 ng g^{-1} in the brain and 100 ng mL^{-1} in plasma.

RESULTS AND DISCUSSION

The presence and the synthetic potentiality of the -NH_2, -CN and -$CONH_2$ in compounds **1a-d** were investigated in this study. Thus, it has been found that each of compounds **1a-d** were reacted with formic acid to afford the reaction products compounds **2a-b**. The IR (cm^{-1}) of compounds **2a-b** showed the bands of CO and NH groups and their mass spectra gave *m/z* = 362 and 346 which corresponding to their molecular weights, respectively. It is important to report here that the compounds **2a-b** were obtained authentically *via* the reaction of compounds **1c-d** with triethylorthoformate in acetic anhydride.

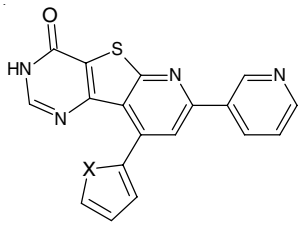


1a,b (X = S, O)

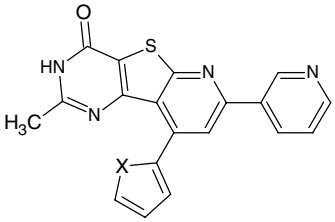


1c,d (X = S, O)

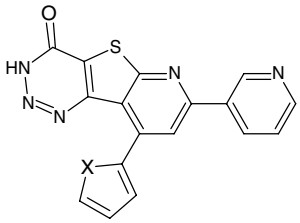
Compounds **1a-d** were reacted also, with acetic anhydride under reflux to afford unexpectedly the reaction products **3a-b**. The reaction of compounds **1a-b** with acetic anhydride most probably proceeded *via* the acylation of the NH_2 group at 3-position, followed by partial hydrolysis of the CN group at 2-position to give $CONH_2$ group which underwent intramolecular cyclization to afford compounds **3a-b**, respectively. The IR (cm^{-1}) of these reactions products showed the bands corresponding to NH and CO groups. Moreover, their mass spectrum gave *m/z* = 376 and 360 which corresponding to their molecular weights, respectively. On the other hand, compounds **3a-b** with the same physical and chemical properties obtained *via* the reaction of compounds **1c-d** with acetic anhydride (*cf.* Exp. part). Compounds **1a-b** reacted also, with nitrous acid to give the corresponding pyridothienotriazines compounds **4a-b**. The structure of compounds **4a-b** elucidated through the absorption bands of both CO and NH groups in IR spectra as well as the peaks at *m/z* = 363 and 347 which corresponding to their molecular weights, respectively (*cf.* Exp. part).



2a,b (X = S, O)



3a,b (X = S, O)

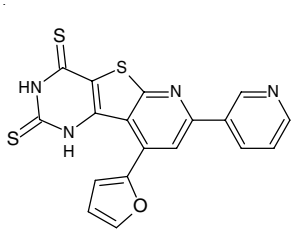


4a,b (X = S, O)

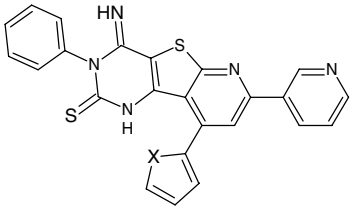
In a further extension compound **1b** reacted with carbon disulphide in pyridine to give a reaction product **5b** whose structure established by considering the data of IR and elemental analysis. Moreover, its mass spectrum gave *m/z* = 394 which corresponding to its molecular weight (*cf.* Exp. part). On the other hand, all attempts to isolate compound **5a** at varieties of experimental condition are failed.

To investigate the nucleophilic behaviour of each of compounds **1a-b** reacted with phenyl isothiocyanate in pyridine under reflux to give the reaction products **6a-b**, respectively.

The IR, elemental analyses and mass spectral data used as the good tools to elucidated the structure of each of compounds **6a-b** (*cf.* Exp. part).

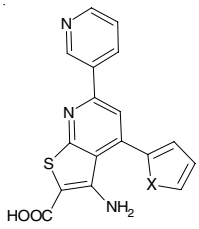


5b

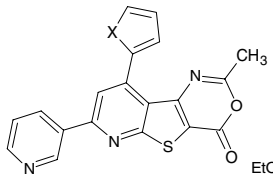


6a,b (X = S, O)

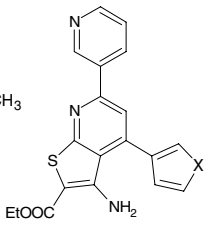
Aiming to obtain the newly synthesized pyrimidinones we used thienopyridines compounds **7a-b** [5] and compounds **11a-b** [5] as the starting materials. Thus it has been found that compounds **7a-b** reacted with acetic anhydride to give the corresponding pyridothienooxazines compounds **8a-b**, respectively. Compounds **8a-b** reacted with aniline and hydrazine hydrate in two separated reactions to give the reaction products **9a-b** and **10a-b**, respectively.



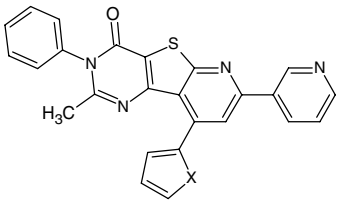
7a,b (X = S, O)



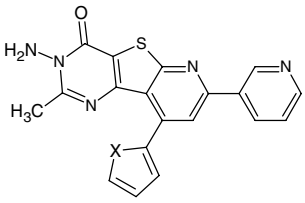
8a,b (X = S, O)



11a,b (X = S, O)



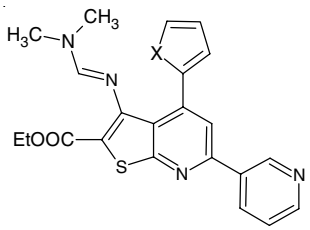
9a,b (X = S, O)



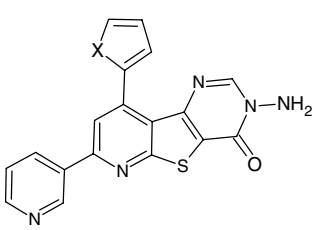
10a,b (X = S, O)

The structures of compounds **8a-b**, **9a-b**, **10a-b** elucidated by considering the data of IR and elemental analyses (*cf.* Exp. part). Moreover, their mass spectra gave *m/z* = 377, 361, 452, 436, 391 and 375, respectively and this corresponding to their molecular weights, respectively (cf, Exp. part 2).

On the other hand, compounds **11a-b** [5] reacted with dimethylformamide-dimethylacetal under reflux in dry xylene to afford the reaction product **12a-b** which reacted with hydrazine hydrate to give the corresponding pyrimidinone derivatives **13a-b**. The mass spectra of compounds **12a** and **13a** as the typical examples gave *m/z* = 436 and 377 which corresponding to their molecular weights, respectively (*cf.* Exp. part).



12a,b (X = S, O)



13a,b (X = S, O)

Antialzheimer activity: Compounds **1a-d, 2a-b, 3a-b, 4a-b, 5b** and **6a-b** with potency as antialzheimer agents relative to flurbiprofen arranged in descending order as follows: compounds **1a, 1c, 2a, 6a, 4a** and **3a** (*cf.* Fig. 1).

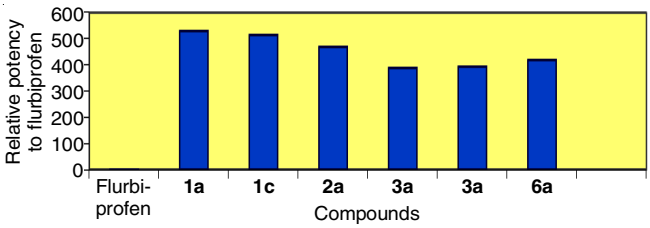


Fig. 1. Antialzheimer relative to flurbiprofen of compounds **1a-6a**

It is worthy to mention that as the activity increases both the pharmacokinetics and pharmacodynamics properties greatly improved to be directed towards a good bioavailability drug profiles (*cf.* Figs. 2 and 3).

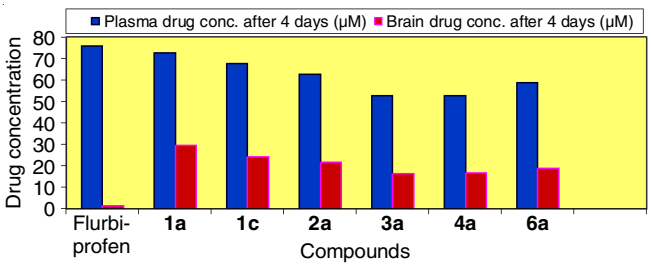


Fig. 2. *in vivo* pharmakinetic of compounds **1a-6a**

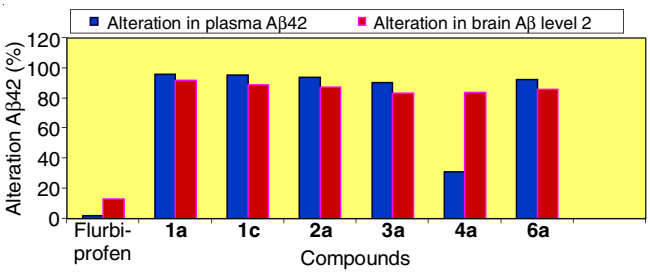


Fig. 3. *in vivo* pharmacodynamic of compounds **1a-6a**

Compounds **7a-b, 8a-b, 9a-b, 10a-b, 11a-b, 12a-b** and **13a-b** with potency as antialzheimer agents relative to flurbiprofen arranged in descending order as follows: compounds **11a, 8b, 10b, 9b, 13b, 12a, 13a, 10a, 9a, 8a** and **7a** (*cf.* Fig. 4).

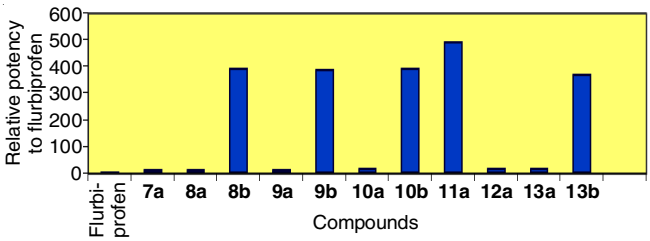


Fig. 4. Antialzheimer relative to flurbiprofen of compounds **7-13**

It is worthy to mention here that as the activity increases both the pharmacokinetics and pharmacodynamics properties greatly improved to be directed towards a good bioavailability drug profiles (*cf.* Fig. 5).

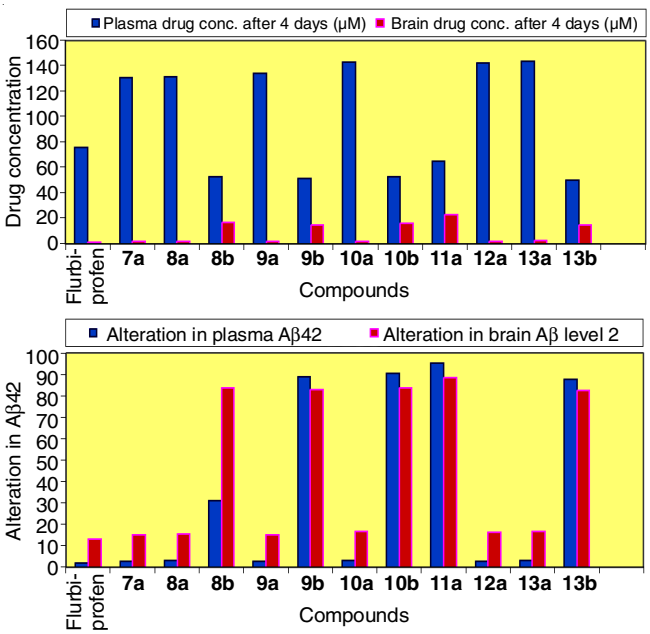


Fig. 5. *in vivo* pharmakinetic and pharmacokinetic of compounds **7-13**

Structural activity relationship of antialzheimer activity: Generally the 2-thienyl moiety for compounds **1a, 1c, 2a, 3a, 4a** and **6a** provides the highest antialzheimer activity than the same compound with the 2-furyl moiety. Thus, we can concluded that the 2-furyl group has no effect.

Anti COX-2 activity: For the compounds **1a, 1c, 2a, 3a, 4a** and **6a** have high potent activities as anti COX-2 activities in descending order on the other hand, the compounds showed moderate potent activities in descending order. Generally the fused pyrimidinone ring onto the thiophene derivatives caused increasing of the activity. Thus, it is concluded that the 2-furyl group has no effect (*cf.* Fig. 6).

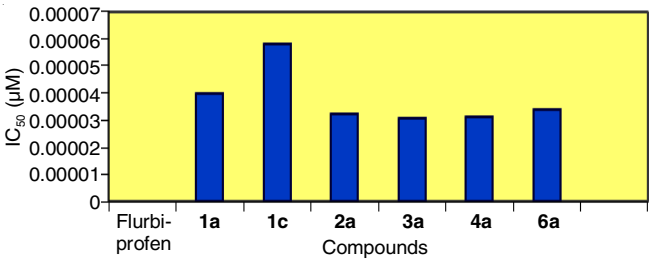


Fig. 6. Effect on COX-2 of compounds **1a-6a**

The compounds **8b, 9b, 10b, 11a** and **13b** have high potent activities as anti COX-2 activities in descending order. Generally the fused pyrimidinone ring onto the thiophene derivatives caused an increasing in the activity (*cf.* Fig. 7).

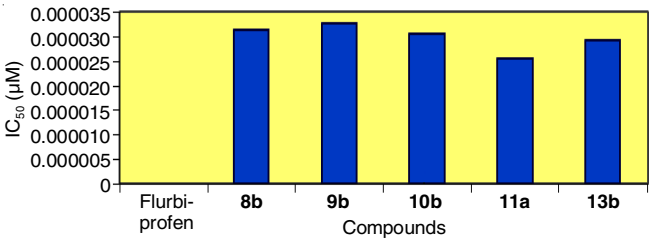


Fig. 7. Effect on COX-2 of compounds **8b-13b**

Structural activity relationship for anti COX-2: The introducing of O atom into the five member ring gave highest activity *i.e.*, compounds **8b, 9b, 10b, 11a** and **13b** exhibit high activity.

Acute toxicity of both compounds **1a, 1c, 2a, 3a, 4a** and **6a** illustrated by Fig. 8. Acute toxicity of both compounds **7a, 8a, 9a, 10a, 11a, 12a** and **13a** illustrated by Fig. 9.

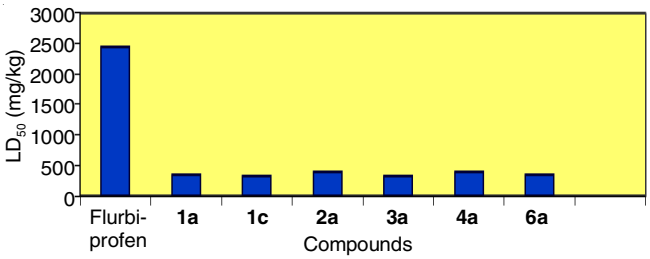


Fig. 8. Acute toxicity of compounds **1a-6a**

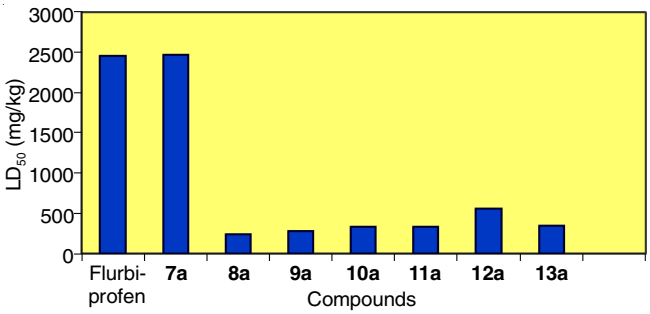


Fig. 9. Acute toxicity of compounds **7a-13a**

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