Anti-Alzheimer Activity of Novel 1, 3, 4-Thiadiazole Derivatives

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Abstract: The reaction of the new enaminone **2** with α-ketohydrazonoyl chlorides **3** afforded 5-(pyrazol-4-yl)-carbonyl-2-benzoylimino-1,3,4-thiadiazoles **5**. Treatment of **5a** with hydrazine hydrate led to formation of pyrazolo[3,4-*d*]pyridazine derivatives **8**. The reaction of enaminone **2** with 6-amino-2-thioxopyrimidin-4-one **9** in acetic acid under reflux produced 2-thioxopyridopyrimidinone derivative **10**. The latter compound or its methylthio derivative **15** reacts with hydrazonoyl chlorides **3** to give pyridotriazolopyrimidine derivatives **14**. The newly synthesized compounds **2**, **5**, **8** and **14** were tested for their anti-alzheimer activity and the results which obtained revealed their potency as drugs for Alzheimer disease.

Key words: Enaminone · 1, 3,4-thiadiazole · pyridotriazolo-pyrimidinones · Alzheimer agent

INTRODUCTION

Alzheimer's disease (AD), the most common form of dementia among the aged, is a fatal neurodegenerative disease characterized by loss of mental ability, cognition deterioration, progressive impairment of daily activities and a variety of neuropsychiatric symptoms and behavioral disturbances [1, 2]. Beta-amyloid is a tiny microscopic protein present in the brain. Scientists believe that the destruction of brain cells seen in Alzheimer's disease is caused by faults in the way beta-amyloid is produced, how it accumulates and how it is got rid of. Scientists are exploring and developing drugs that may block or inhibit the production of beta-amyloid. In continuation of our previous work [3-6] directed to synthesize biologically active compounds, we report herein the synthesis of a variety of heterocyclic compounds incorporating1,3,4-thiadiazolemoiety. Our interest for synthesis of the target compounds is based on the finding that various derivatives of 1,3,4-thiadiazole were expected to have various pharmacological activities. For example, some derivatives of 1,3,4-thiadiazole were reported to have anticancer [7, 8], anti-analgesic and antiulcer activities [9, 10]. The newly synthesized compounds were prepared *via* reaction of enaminone derivatives **2** with each of hydrazonoyl chloride derivatives **3**, 6-amino-2-thioxo-pyrimidin-4-one **9** and methylthio derivative **15**. The regiochemistry of the

reactions studied and the activity of the products isolated as anti-Alzheimer from such reactions have been investigated.

Experimental: Melting points were determined on an electrothermal Gallenkamp apparatus and uncorrected. The IR spectra were recorded in KBr disks using Pye Unicam SP-1000 Spectrophotometer. ^{1}HNMR spectra were recorded in $CDCl_3$ and DMSO-d_6 using a Varian Em-300 MH*z* Spectrometer and TMS as internal reference. Mass spectra were recorded on AEI MS 30 mass spectrometer operating at 70eV was carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **3a-j** [11, 12] and compound **1**[13] were prepared following literature procedures.

2-Benzoylimino-5-(3-dimethylamino-2-propenoyl)-3-phenyl-3*H*-[1, 3,4]thiadiazole (2): A mixture of 1,3,4-thiadiazole derivative **1** [13] (10 mmol) and dimethylformamide-dimethylacetal (DMF-DMA) (2.4 g, 20 mmol) in dry toluene (20 ml) was refluxed for 2 h then left to cool. Methanol was added to the cold mixture and the resulting solid was collected by filtration, washed with methanol, dried and finally crystallized from ethanol to afford the enaminone **2** as orange crystals, (80% yield), mp 268°C, IR (KBr) v_{max} 1641, 1610 (2C=O) cm^{-1}, ^{1}H NMR

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(DMSO-d_6): δ 2.95 (s, 3H, CH$_3$), 3.22 (s, 3H, CH$_3$), 5.83 (d, J = 12 Hz, 1H, CO-CH=), 7.46-7.98 (m, 10H Ar-H), 8.08 (d, J =12 Hz, 1H, =CH-N), MS m/z (%): 378 (M$^+$, 22), 361 (12), 105 (51), 98 (100), 77 (38). Anal. Calcd. for C$_{20}$H$_{18}$N$_4$O$_2$S (378.46): C, 63.47, H, 4.79, N, 14.80. Found: C, 63.29, H, 4.66, N, 14.59%.

Reaction of enaminone 2 with hydrazonoyl chlorides 3. Synthesis of compounds (5a-j) General procedure - To a stirred solution of the enaminone **2** (0.378 g, 1 mmol) and the appropriate hydrazonoyl chloride **3** (1 mmol) in dry dioxane (30 mL), was added triethylamine (0.5 mL) and the mixture was refluxed for 5 h. The precipitated triethylamine hydrochloride was filtered off and the filtrate was evaporated under reduced pressure and the residue was triturated with methanol. The solid product, so formed in each case, was collected by filtration, washed with water, dried and crystallized from ethanol to afford the corresponding 1, 3,4-thiadiazole derivatives **5**. The products **5a-j** prepared are listed below together with their physical constants.

5-[3-Acetyl-1-phenyl-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole(5a): Brown solid, (90% yield), mp 100 - 102°C, IR (KBr) ν_{max} 1683, 1646, 1605 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.49 (s, 3H, CH$_3$), 7.48-8.10 (m, 15H, Ar-H), 9.48 (s, 1H, pyrazole-H), MS m/z (%) 493 (M$^+$, 1), 213 (11), 104 (87), 97 (39), 76 (100). Anal. Calcd. for C$_{27}$H$_{19}$N$_5$O$_3$S (493.55): C, 65.71, H, 3.88, N, 14.19. Found: C, 65.94, H, 3.62, N, 14.35%.

5-[3-Acetyl-1-(4-methylphenyl)-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole (5b): Yellow solid, (80% yield), mp 138 - 140°C, IR (KBr) ν_{max} 1693, 1643, 1618 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.38 (s, 3H, CH$_3$), 2.62 (s, 3H, CH$_3$), 7.39-8.14 (m, 14H, Ar-H), 9.27 (s, 1H, pyrazole-H), MS m/z (%) 507 (M$^+$, 4), 398 (5), 321 (3), 253 (5), 226 (29), 104 (84), 76 (100). Anal. Calcd. for C$_{28}$H$_{21}$N$_5$O$_3$S (507.58): C, 66.26, H, 4.17, N, 13.80:. Found: C, 66.03, H, 4.05, N, 13.97%.

5-[3-Acetyl-1-(4-chlorophenyl)-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole (5c): Yellow solid, (85% yield), mp 225-226°C, IR (KBr) ν_{max} 1694, 1640, 1615 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3H, CH$_3$), 7.46-8.14 (m, 14H, Ar-H), 9.35 (s, 1H, pyrazole H), MS m/z (%) 529 (M$^+$+2, 9), 527 (M$^+$, 30), 111 (22), 105 (79), 98 (35), 77 (100). Anal. Calcd. for C$_{27}$H$_{18}$ClN$_5$O$_3$S (527.99): C, 61.42, H, 3.44, N, 13.26. Found: C, 61.25, H, 3.61, N, 13.16%.

5-[3-Acetyl-1-(4-nitrophenyl)-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole (5d): Orange solid, (85% yield), mp 170 - 172°C, IR (KBr) ν_{max} 1694, 1639, 1620 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ, 2.65 (s, 3H, CH$_3$), 7.49-8.45 (m, 14H, Ar-H), 9.54 (s, 1H, pyrazole H), MS m/z (%) 538 (M$^+$, 1), 253 (2), 219 (2), 152 (8), 137 (11), 104 (79), 98 (83), 76 (100). Anal. Calcd. for C$_{27}$H$_{18}$N$_6$O$_5$S (538.55): C, 60.22, H, 3.37, N, 15.61. Found: C, 60.16., H, 3.23, N, 15.54%.

5-[3-Ethoxycarbonyl-1-phenyl-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole (5e): Yellow solid, (80% yield), mp 215 - 216°C, IR (KBr) ν_{max} 1736, 1642, 1616 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ, 1.16 (t, J = 7 Hz, 3H, CH$_3$), 4.22 (q, J = 7 Hz, 2H, CH$_2$), 7.46-8.15 (m, 15H, Ar-H), 9.37 (s, 1H, Pyrazole-H), MS m/z (%) 523 (M$^+$, 3), 104 (50), 97 (45), 76 (100). Anal. Calcd. for C$_{28}$H$_{21}$N$_5$O$_4$S (523.57): C, 64.23, H, 4.04, N, 13.38. Found: C, 64.18, H, 4.00, N, 13.50%.

5-[3-Ethoxycarbonyl-1-(4-methylphenyl)-1H-pyrazole-4-carbonyl]-2-benzoylimino-3-phenyl-3H-[1,3,4]thiadiazole (5f): Yellow solid, (80% yield), mp 200 - 202°C, IR (KBr) ν_{max}, 1739, 1637, 1620 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 1.17 (t, J = 7Hz, 3H, CH$_3$), 2.49 (s, 3H, CH$_3$), 4.24 (q, J = 7Hz, 2H, CH$_2$), 7.46-8.47 (m, 14H, Ar-H), 9.58 (s 1H, Pyrazole-H), MS m/z (%) 537 (M$^+$, 2), 378 (29), 361 (14), 104 (37), 97 (100), 77 (37). Anal. Calcd. for C$_{29}$H$_{23}$N$_5$O$_4$S (537.60): C, 64.79, H, 4.31, N, 13.03. Found: C, 64.57, H, 4.26, N, 12.97%.

5-[3-Ethoxycarbonyl-1-(4-chlorophenyl)-1H-pyrazole-4-carbonyl]-2-benzoyl-imino-3-phenyl-3H-[1,3,4]thiadiazole (5g): Brown solid, (80% yield), mp 150-152°C, IR (KBr) ν_{max}, 1730, 1641, 1625 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 1.15 (t, J = 7Hz, 3H, CH$_3$), 4.23 (q, J = 7Hz, 2H, CH$_2$), 7.26-8.15 (m, 14H, Ar-H), 9.39 (s, 1H, Pyrazole-H), MS m/z (%) 558 (M$^+$, 1), 269 (16), 167 (34), 141 (27), 126 (100), 98 (38), 89 (25), 77 (16). Anal. Calcd. for C$_{28}$H$_{20}$ClN$_5$O$_4$S (558.02): C, 60.27, H, 3.61, N, 12.55. Found: C, 60.48, H, 3.76, N, 12.34%.

5-[3-Ethoxycarbonyl-1-(4-nitrophenyl)-1H-pyrazole-4-carbonyl]-2-benzoyl-imino-3-phenyl-3H-[1,3,4]thiadiazole (5h): Yellow solid, (80% yield), mp 170-172°C, IR (KBr) ν_{max} 1719, 1644, 1628 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 1.16 (t, J = 7Hz, 3H, CH$_3$), 4.22 (q, J = 7Hz, 2H, CH$_2$), 7.38-8.15 (m, 14H, Ar-H), 9.32 (s, 1H, Pyrazole-H), Anal. Calcd. for C$_{28}$H$_{20}$N$_6$O$_6$S (568.57): C, 59.15, H, 3.55, N, 14.78. Found: C, 59.01, H, 3.29, N, 14.59%.

5-[1-(4-Methylphenyl)-3-phenylcarbamoyl-1*H*-pyrazole-4-carbonyl]-2-benzoyl-imino-3-phenyl-3*H*-[1,3,4] thiadiazole (5i):

Yellow solid, (90% yield), mp 240-242°C, IR (KBr) v_{max} 3298(NH), 1663, 1635, 1619 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.39 (s, 3H, CH$_3$), 7.46-8.12 (m, 19H, Ar-H), 9.34 (s, 1H, Pyrazole-H), 10.46 (s, 1H, NH), MS m/z (%) 584 (M$^+$, 9), 506 (17), 378 (21), 104 (68), 98 (100), 77 (54). Anal. Calcd. for C$_{33}$H$_{24}$N$_6$O$_3$S (584.66): C, 67.79, H, 4.14, N, 14.37. Found: C, 67.67, H, 4.31, N, 14.25%.

5-[1-(4-Chlorophenyl)-3-phenylcarbamoyl-1*H*-pyrazole-4-carbonyl]-2-benzoyl-imino-3-phenyl-3*H*-[1,3,4] thiadiazole (5j):

Yellow solid, (75% yield), mp 185-186°C, IR (KBr) v_{max} 3358 (NH), 1672, 1640, 1604 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ, 7.03-8.12 (m, 19H, Ar-H), 9.34 (s, 1H, Pyrazole-H), 10.65 (s, 1H, NH), Anal. Calcd. for C$_{32}$H$_{21}$ClN$_6$O$_3$S (605.08): C, 63.52, H, 3.50, N, 13.89. Found: C, 63.26, H, 3.28, N, 13.71%.

Synthesis of *N*-[5-(7-methyl-2-phenyl-2H-pyrazolo [3,4-*d*]pyridazin-4-yl)-3-phenyl-3*H*-[1,3,4]thiadiazol-2-ylidene]-benzamide (8):

A mixture of compound **5a** (5 mmol) and hydrazine hydrate (5 mL, 80%) in absolute ethanol (10 mL) was refluxed for 10 h and the reaction mixture was then cooled. The solid that precipitated was filtered off and crystallized from ethanol to give compound **8** as white solid, (70% yield), mp 220°C, IR (KBr) v_{max} 1627 (C=O) cm^{-1}, MS m/z (%), 489 (M$^+$, 12), 481 (1), 235 (54), 158 (11), 102 (100), 89 (25). Anal. Calcd. for C$_{27}$H$_{19}$N$_7$OS (489.56): C, 66.24, H, 3.91, N, 20.03. Found: C, 66.08, H, 3.84, N, 19.93%.

Synthesis of 5-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-2-thioxo-2, 3-dihydro-1*H*-pyrido [2,3-*d*] pyrimidin-4-one (10):

A mixture of enaminone **2** (3.78 g, 10 mmol) and 6-amino-2-thioxo-2,3-dihydropyrimidin-4(1*H*)-one **(9)** (1.43 g, 10 mmol) in acetic acid (40 mL) was refluxed for 6 hrs. The reaction mixture was cooled and diluted with methanol and the solid product was collected by filtration and recrystallized from dioxane to give **10** as yellow solid, (90% yield), mp > 300°C, IR (KBr) v_{max} 3165, 3129 (2NH), 1678, 1612 (2C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 7.48-8.11 (m, 10H, Ar-H), 8.12 (d, J = 6 Hz, 1H, pyridine-H), 8.44 (d, J = 6 Hz, 1H, pyridine-H), 12.70 (s, 1H, NH), 13.33 (s, 1H, NH), MS m/z (%) 458 (M$^+$, 1), 118 (11), 104 (82), 98 (37), 87 (27), 76 (100). Anal. Calcd. for C$_{22}$H$_{14}$N$_6$O$_2$S$_2$ (458.52): C, 57.63, H, 3.08, N, 18.33. Found: C, 57.45, H, 2.94, N, 18.40%.

Synthesis of 2-methylthio-5-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-3*H*-pyrido [2,3*d*]pyrimidin-4-one (15):

To a stirred mixture of thione **(10)** (2.29 g, 5 mmol) in dimethylformamide (20 ml) was added anhydrous potassium carbonate (1.14 g, 7.5 mmol) and methyl iodide (0.71 g, 5 mmol). The reaction mixture was stirred overnight at room temperature then poured into ice-water. The solid formed was filtered off, washed with water, dried and recrystallized from ethanol /dioxane mixture to give compound **15** as yellow solid, yield 90%, mp 218 - 220°C, IR (KBr) v_{max} = 3466 (NH), 1684, 1616 (2 CO) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ = 3.17 (s, 3H, CH$_3$), 7.45 -8.04 (m, 10H, Ar-H), 8.07 (d, J = 6Hz, 1H, pyridine-H), 8.39 (d, J = 6 Hz, 1H, pyridine-H), 12.69 (s, 1H, NH) ppm., MS, *m/z* (%) 472 (M$^+$, 16), 105 (100), 91 (53) 77 (80), Anal. Calcd. for C$_{23}$H$_{16}$N$_6$O$_2$S$_2$ (472.55): C, 58.46, H, 3.41, N, 17.78. Found: C, 58.28, H, 3.51, N, 17.55%.

Synthesis of pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one derivatives (14 a,c,e,f,k)

Method A: To a mixture of equimolar amounts of **10** and the appropriate hydrazonoyl chlorides **3** (1 mmol of each) in dioxane (15 mL) was added triethylamine (0.14 mL, 1 mmol). The reaction mixture was refluxed till all of the starting materials have disappeared and hydrogen sulfide gas ceased to evolve (2h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and recrystallized from dioxane to give products **14**.

Method B: To a mixture of equimolar amounts of **15** and the appropriate hydrazonoyl chloride **3a** (1 mmol) in dioxane (15 mL) was added triethylamine (0.14 mL, 1 mmol). The reaction mixture was refluxed till all methanethiol gas ceased to evolve (2 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered and recrystallized from dioxane to give product identical in all respects (mp, mixed mp and IR) with **14a**.

3-Acetyl-6-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-1-phenyl-1,5-dihydro-pyrido[2,3-*d*][1,2,4] triazolo[4,3-*a*]pyrimidin-5-one (14a):

Yellow solid, (80% yield), mp 300°C, IR (KBr) v_{max} 1719, 1642, 1614 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.78 (s, 3H, CH$_3$), 7.45-8.09 (m, 15H, Ar-H), 8.23 (d, J = 6 Hz, 1H, pyridine-H), 8.69 (d, J = 6 Hz, 1H, pyridine-H), MS m/z (%) 585 (M$^+$+1, 4), 584 (M$^+$, 10), 104 (91), 91 (47), 76 (100). Anal. Calcd. for C$_{31}$H$_{20}$N$_8$O$_3$S (584.62): C, 63.69, H, 3.45, N, 19.17. Found: C, 63.48, H, 3.31, N, 19.05%.

3-Acetyl-6-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-1-(4-chloro-phenyl)-1,5-dihydro-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one(14c):
Yellow solid, (70% yield), mp 120°C, IR (KBr) v_{max} 1708. 1652, 1613 (3 C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 2.79 (s, 3H, CH$_3$), 7.20- 8.21 (m, 14H, Ar-H), 8.25 (d, J = 6 Hz, 1H, pyridine-H), 8.68 (d, J = 6 Hz, 1H, pyridine-H), MS m/z (%) 620 (M$^+$+2, 9), 618 (M$^+$, 30), 585 (36), 508 (21), 330 (11), 104 (100), 91 (49), 77 (79). Anal. Calcd. for C$_{31}$H$_{19}$ClN$_8$O$_3$S (619.07): C, 60.15, H, 3.09, N, 18.10. Found: C, 60.04, H, 3.00, N, 18.03%.

3-Ethoxycarbonyl-6-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-1-phenyl-1,5-dihydro-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one   (14e): Yellow solid, (80% yield), mp 90-92°C, IR (KBr) v_{max} 1708, 1613, 1582 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 1.40 (t, J = 7 Hz, 3H, CH$_3$), 4.55 (q, J = 7 Hz, 2H, CH$_2$), 7.35 -8.14 (m, 15H, Ar-H), 8.20 (d, J = 6 Hz, 1H, pyridine-H), 8.73 (d, J = 6 Hz, 1H, pyridine-H), MS m/z (%) 614 (M$^+$, 9), 570 (24), 104 (100), 90 (24), 76 (64). Anal. Calcd. for C$_{32}$H$_{22}$N$_8$O$_4$S (614.65): C, 62.53, H, 3.61, N, 18.23. Found: C, 62.33, H, 3.52, N, 18.10%.

3-Ethoxycarbonyl-6-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-1-(4-methylphenyl)-1,5-dihydro-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (14f)
Brown solid, (70% yield), mp 120-122°C, IR (KBr) v_{max} 1712, 1678, 1616 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 1.19 (t, J = 7 Hz, 3H, CH$_3$), 2.39 (s, 3H, CH$_3$), 4.45 (q, J = 7 Hz, 2H, CH$_2$), 7.05 (d, 2H, ArH), 7.22 (d, 2H, ArH), 7.45 (d, J = 6 Hz, 1H, pyridine-H), 7.57-8.11 (m, 10H, Ar-H), 8.65 (d, J = 6 Hz, 1H, pyridine-H), MS m/z (%) 628 (M$^+$, 9), 148 (12), 128 (12), 116 (16), 104 (49), 91 (13), 77 (100). Anal. Calcd. for C$_{33}$H$_{24}$N$_8$O$_4$S (628.67): C, 63.05, H, 3.85, N, 17.82. Found: C, 63.12, H, 3.64, N, 17.64%.

3-Phenylcarbamoyl-6-(2-benzoylimino-3-phenyl-3*H*-[1,3,4]-thiadiazol-5-yl)-1-phenyl-1,5-dihydro-pyrido[2,3-*d*][1,2,4]triazolo[4,3-*a*]pyrimidin-5-one (14k): Yellow solid, (80% yield), mp 170-172°C, IR (KBr) v_{max} 3380 (NH), 1660, 1658, 1642 (3C=O) cm^{-1}, ^{1}H NMR (DMSO-d_6) δ 7.29-7.78 (m, 20H, Ar-H), 7.79 (d, J = 6 Hz, 1H, pyridine-H), 8.01 (d, J = 6 Hz, 1H, pyridine-H), 10.19 (s, 1H, NH), MS m/z (%) 661 (M$^+$, 20), 401 (14), 150 (20), 104 (41), 91 (100), 76 (87). Anal. Calcd. for C$_{36}$H$_{23}$N$_9$O$_3$S (661.71): C, 65.35, H, 3.50, N, 19.05. Found: C, 65.53, H, 3.72, N, 19.12%.

Aβ42 and Aβ40 Assay: Aβ42 and Aβ40 were 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 (2x105 cell well-1) and allowed to grow to confluence for 24h, in 5% CO$_2$/95% air in 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-d_6 (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, Gaithersburg, MD, USA). The cytotoxicity potential of test compound was assessed in the same cells of the Aβ assay (H4) with the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazol-iumbromide (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 reduced 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 Aβ42 and Aβ40 determination, cells were incubated for 3h with 500μl culture medium containing 0.5 mg ml^{-1} MTT, at 37°C, 5% CO$_2$ and saturated humidity. After removal of the medium, 500μl of 100% DMSO-d_6 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).

Study 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 [14] 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 compound 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-Acid) once daily for 5 days. This vehicle was selected to replicate that reported with flurbiprofen in similar studies [15-17]. On day 5, mice given a final dose of 100 or 300 mg kg^{-1} or vehicle and sacrificed 3 h later, as described [15-17].

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 each individual compound (100 or 300 mg k^{-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 k^{-1} or vehicle and sacrificed 3 h later, as described below.

Study 3: Groups of male and female mice of 4-5 month, each group composed of thirty-three male mice and female mice of 4-5 months were given vehicle or tested compounds or R-flurbiprofen elemented 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, color-coded, rodent diety 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 continuous studies [18-20]. Weight and food consumption were monitored every 3-4 days.

Plasma and Brain Aβ Measurements: Twenty-four hours before starting treatment, one blood sample was collected by means of retro-par puncture for measurement of baseline plasma, Aβ40 and Aβ42 concentration. On the last of treatment, mice were sacrificed by decapitation. Blood samples were collected in EDTA-tasted tubes and centrifuged at 800 rpm for 20 min. to separate plasma. Plasma samples were divided into two aliquots of

approximately 100μl 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. 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, in tissue samples were homogenized in 70% formic acid at 1:10 (w/v). Homogenates were measured at 4°C for 3 h and then centrifuged at 15,000xg for 25 min at 4°C. The supernatants were collected and neutralized with 1M Tris. PH 11 at 1:20 (w/v) dilution with 3 x 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 cterminus of human Aβ40 (clone G2-10, reactive to amino acid residues 31-40, isotype IgG2b, 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: Drug levels in plasma and in brain samples were measured by liquid chromatography as previously described by Weggen *et al.* [21]. Briefly, samples were prepared by adding 300μl acetonitrile and 40μl phosphoric acid 40% to 100μl 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 system with fluorescence (Water 474, Waters, Guyancourt, France) or mass spectrometry (API2000, 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^{-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 liner 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.

Notch-Mediated Hes1-Luciferase Reporter Assay: The Notch-Hes1 reporter assay was modified from procedures as described in ref.. HEK293 cells were plated in a six-well dish ($\approx$5 x 10^{5} cells) and transiently cotransfected with the following plasmids: luciferase reporter construct Hes1-Luc (0.3 μg), Notch construct pCS2-ΔEMV-6MT (also named Notch ΔE) (3.0μg), which contains the transmembrane and intracellular domains of Notch1 or control vector pCS2 vector (3.0 μg) and pCH110 (0.1μg), which expresses β-galactosidase (Amersham Pharmacia) for normalization of the transfection efficiency and luciferase activity. The cells were transfected with these plasmids for 24 h and then fresh media and inhibitors were added to the cells. The cells were harvested after 24 h and lysed for measuring luciferase activity by using the Luciferase Reporter Gene Assay kit (Roche). The luciferase activity was normalized according to β-galactosidase activity [22].
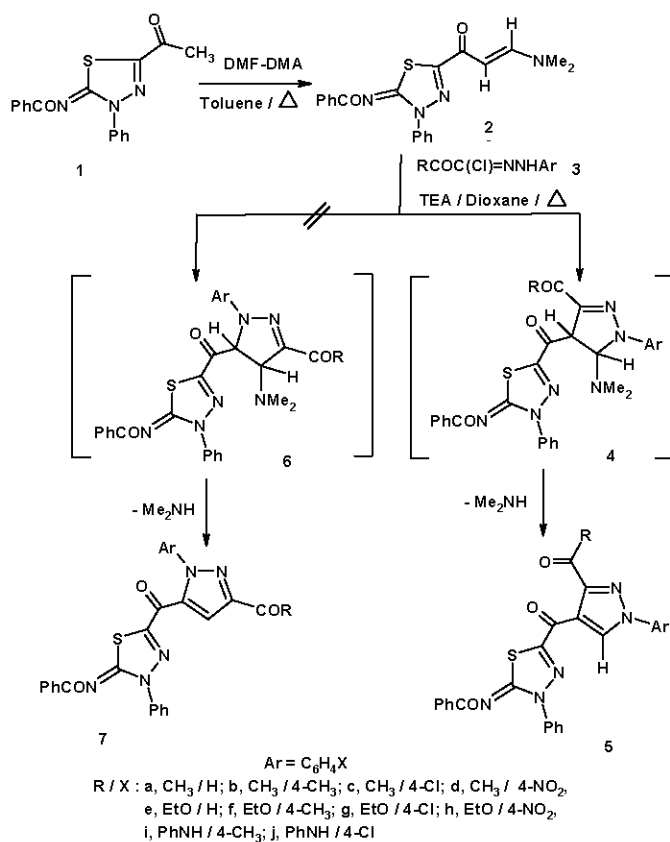
RESULTS AND DISCUSSION

The starting new enaminone **2** was prepared by Heating 5-acetyl-2-benzoylimino-3-phenyl-1,3,4-thiadiazole **1** [13] with dimethylformamide-dimethylacetal (DMF-DMA) in dry toluene (**Scheme 1**). Reaction of enaminone **2** with α-ketohydrazonoyl chlorides **3** in dioxane in the presence of triethylamine yielded in each case, only one product as evidenced by TLC analysis of the crude product. The structure **5** assigned for the obtained products was established by elemental analysis and spectral (IR, ^{1}H NMR and MS) data. The IR spectra revealed in each case, three absorption bands due to the three carbonyl groups in structure **5**. The ^{1}H NMR spectra revealed the absence of the signals characteristic for the protons in enaminone residue of compound **2** and instead revealed the presence of singlet signal at δ 8.6-9.22 attributed to the proton at position 5 of the pyrazole ring [23], in addition to the signals characteristic for the aromatic protons. Based on the data of the ^{1}H NMR, the possible isomeric product **7** was excluded since structure **7** contains a proton in position 4 of the pyrazole ring which appeared at δ5.81-5.89 [24, 25]. The formation of the product **5** was

suggested to be formed *via* 1, 3-dipolar cycloaddition of the enaminone **2** to the nitrilimine generated in *situ* during the course of the reaction *via* base catalyzed dehydrohalogenation of the hydrazonoyl chloride **3**, followed by in *situ* elimination of dimethylamine from the non isolated intermediate **4**. The formation of product **5** and not the possible isomeric product **7** indicates that the addition of the enaminone **2** to the nitrilimine is regiospecific (**Scheme 1**).
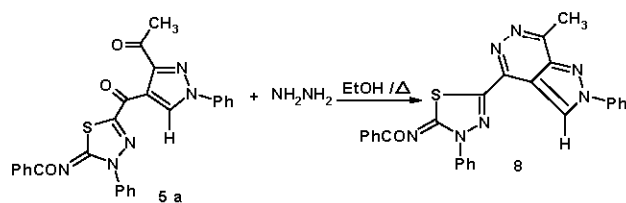
The assigned structure **5** was further established by chemical transformation. Thus, reaction of compound **5a** with hydrazine hydrate in refluxing ethanol for 10 hr led to formation of the expected product **8**. The structure of product **8** was also elucidated by the data obtained from elemental analyses and spectral data (see Experimental). For example, the IR spectrum of **8** revealed the absence of the two absorption bands of the acetyl and the ketonic groups bonded to the pyrazole and the thiadiazole rings (**Scheme 2**).

Also, we studied the reactivity of the enaminone **2** towards some nitrogen nucleophiles. Thus, reaction of enaminone **2** with 6-amino-2-thioxopyrimidin-4-one **9** under reflux in acetic acid led to formation of product which could be formulated as **10** or **11**. The possible isomeric structure **11** was discarded based on the data obtained from ^{1}H NMR spectra. Thus, the ^{1}H NMR spectrum for the product **10** revealed in addition to the signals of the aromatic protons and the 2 NH protons, two doublet signals at δ 7.45-8.25 and 8.01-8.73 ppm attributed to the protons of the pyridine ring. The formation of product **10** and not the possible isomeric product **11** [26] indicated that the reaction starts with initial nucleophilic attack of the amino group of compound **9** to the olefinic carbon attached to the –NMe$_2$ group in enaminone **2**, followed by elimination of dimethylamine and subsequent ring closure *via* elimination of water to give the pyridine ring (**Scheme 3**).
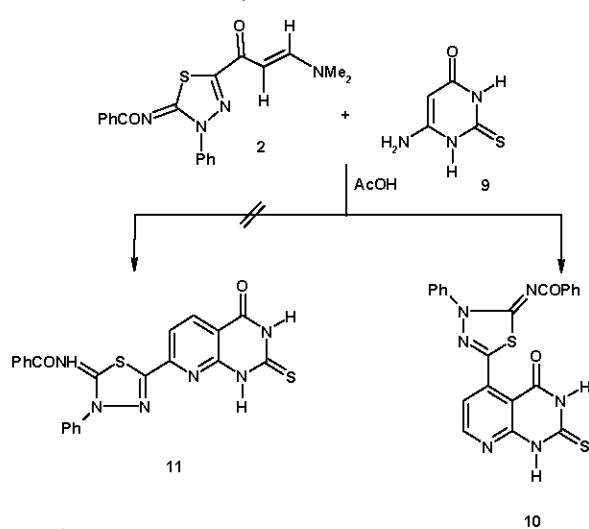
Next, we examined the reactivity of product **10** towards α-keto-hydrazonoyl chlorides **3** since the structure of compound **10** contains a thiourea residue. Thus, reaction of 5-substituted-2-thioxo-pyrido[2,3-*d*]pyrimidin-4-one **10** with hydrazonoyl chlorides **3** in refluxing dioxane in the presence of triethylamine led to the formation of products **14** through the non-isolated intermediates **12** and **13** (**Scheme 4**). 1, 3, 6-Trisubstituted-triazolopyrido-pyrimidinones **14** were also obtained by reaction of 2-methylthio-5-substituted-pyrido [2, 3-*d*] pyrimidin-4-one **15** with hydrazonoyl chlorides

Scheme 1: Synthesis of compounds 5a-j:

Ar = C$_6$H$_4$X
R / X : a, CH$_3$ / H; b, CH$_3$ / 4-CH$_3$; c, CH$_3$ / 4-Cl; d, CH$_3$ / 4-NO$_2$,
e, EtO / H; f, EtO / 4-CH$_3$; g, EtO / 4-Cl; h, EtO / 4-NO$_2$,
i, PhNH / 4-CH$_3$; j, PhNH / 4-Cl

Scheme 2: Synthesis of compound 8

Scheme 3: Synthesis of compound 10
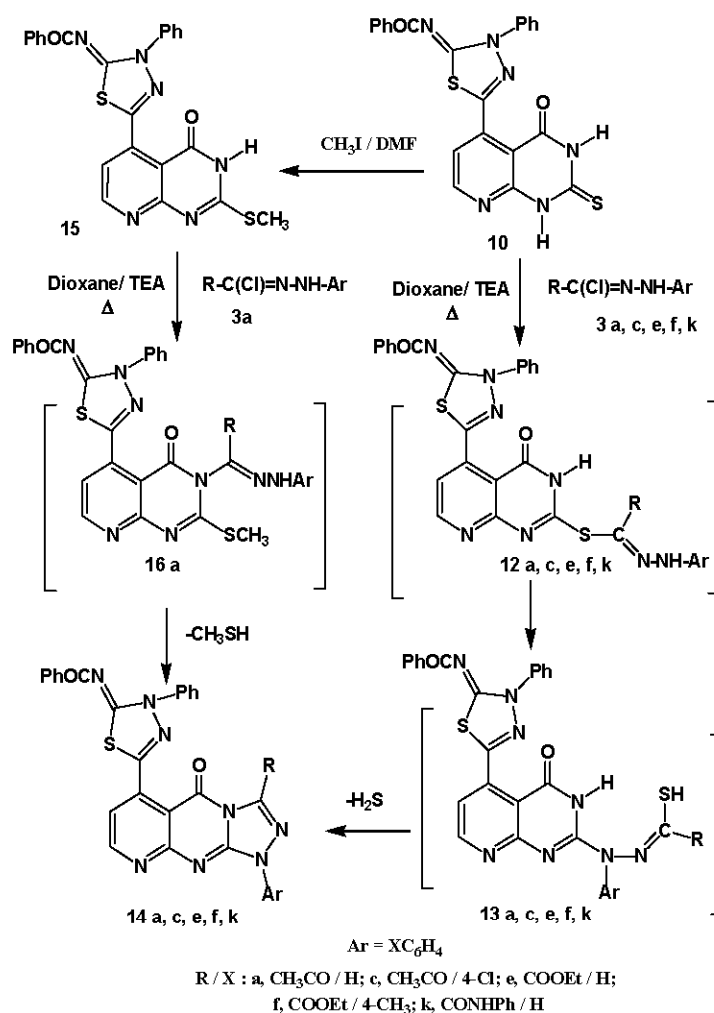
Scheme 4: Synthesis of Compounds **14**

3 under the same reaction conditions (**Scheme 4**). The mechanism of formation of products **14** was previously discussed [11, 27]. The structure assigned for the products **14** was based on both microanalytical and spectral data. For example, the IR spectra showed the absence of the absorption bands assigned for the two NH groups. Also, the ^{1}H NMR revealed the absence of the signals due to the 2NH protons and instead revealed the signals attributed to the protons of the R substituent in the assigned structure **14** (see Experimental).

Anti-Alzheimer Activity: All the newly tested compounds showed potent anti-Alzheimer activity due to its Abeta inhibiting activity. The order of activity is **5e, (5b, 14k), 8, 5f, 5g, 5c, 2, 14f, 5h, 5j, 14c, 14a, 5a, 5d, 5i** and **14e**. It is worth to mention that the tested compounds

showed reasonable therapeutic indexes especially those of high activities, this beside their lower possible side effects due to no action on Notch intracellular domain responsive genes. All the tested drugs showed the same anti-Alzheimer activity *via* the same mechanistic pathway involving lowering Plasma Abet 42 and brain Abet for long period that sustained up to 4 days. The tested compounds pass Blood Brain Barrier (BBB) and showed excellent pharmacokinetics profiles.

Structure-Activity Relationship: Pyrazole derivatives (**5**) are more active than the triazolopyridopyrimidine derivatives (**14**) due to its high affinity binding to Beta amyloid. For pyrazole derivatives (**5**) the order of activity is **5e, 5b, 5f, 5g, 5c, 5j, 5a, 5d, 5i.** So, we can conclude that ethoxy and methyl substituents provide more activity than phenyl amino ones.

Table 1: The *in vitro* Abeta inhibiting activity was evaluated in a human neuroglioma cell line (H4) carrying the double Swedish mutation (K595N/M596L) of the human amyloid precursor protein (APPsw)

Compound No	(IC(50)s {microM}	Relative potency to Flurbi.profen	Effect On Notch intracellular domain responsive genes
2	$0.0512\pm2.13\times10^{-6}$	5292.97	Nil
5a	$0.0731\pm6.8\times10^{-6}$	3707.25	Nil
5b	$0.0382\pm1.23\times10^{-6}$	7094.24	Nil
5c	$0.0511\pm4.22\times10^{-6}$	5303.33	Nil
5d	$0.0765\pm5.6\times10^{-6}$	3542.48	Nil
5e	$0.0372\pm1.33\times10^{-6}$	7284.95	Nil
5f	$0.0421\pm2.16\times10^{-6}$	6437.05	Nil
5g	$0.0473\pm5.14\times10^{-6}$	5729.39	Nil
5h	$0.0534\pm6.12\times10^{-6}$	5074.91	Nil
5i	$0.0873\pm8.9\times10^{-6}$	3104.24	Nil
5j	$0.0542\pm5.6\times10^{-6}$	5000.00	Nil
8	$0.0402\pm2.4\times10^{-6}$	6741.29	Nil
14a	$0.0692\pm6.8\times10^{-6}$	3916.18	Nil
14c	$0.0582\pm5.4\times10^{-6}$	4656.36	Nil
14e	$0.0921\pm3.4\times10^{-6}$	2942.45	Nil
14f	$0.0522\pm2.3\times10^{-6}$	5191.57	Nil
14k	$0.0382\pm2.5\times10^{-6}$	7094.24	Nil

Table 2: *in vivo* pharmacokinetic and pharmacodynamic profiles of some newly synthesized agents were evaluated in young APPsw transgenic mice (Tg2576) after oral gavage (100 or 300 mg kg^{-1} day^{-1} for 4-5 days

Compound No	Plasma drug conc after 4 days {microM}	Brain drug conc after 4 days{microM}	% alteration in plasma Abet 42	% Alteration brain Abeta
level 2				
2	$0.001188\pm1.11\times10^{-12}$	$0.001167\pm0.11\times10^{-12}$	88.942 ± 0.234	75.783 ± 0.234
5a	$0.001882\pm1.23\times10^{-12}$	$0.001311\pm0.35\times10^{-12}$	87.323 ± 0.3423	73.776 ± 0.323
5b	$0.00075\pm1.23\times10^{-12}$	$0.0007\pm0.23\times10^{-12}$	92.367 ± 0.564	76.478 ± 0.444
5c	$0.00101\pm0.98\times10^{-12}$	$0.001\pm2.98\times10^{-12}$	89.765 ± 0.564	76.034 ± 0.322
5d	$0.001982\pm0.87\times10^{-12}$	$0.001443\pm12.89\times10^{-12}$	87.224 ± 0.675	73.687 ± 0.345
5e	$0.00074\pm0.78\times10^{-12}$	$0.0007\pm0.16\times10^{-12}$	92.245 ± 0.4534	76.568 ± 0.432
5f	$0.00094\pm0.54\times10^{-12}$	$0.0009\pm0.12\times10^{-12}$	91.368 ± 0.654	76.124 ± 0.333
5g	$0.00099\pm0.23\times10^{-12}$	$0.0009\pm0.24\times10^{-12}$	90.325 ± 0.456	76.056 ± 0.345
5h	$0.001158\pm0.35\times10^{-12}$	$0.001137\pm0.25\times10^{-12}$	88.971 ± 0.432	75.882 ± 0.345
5i	$0.002111\pm0.12\times10^{-12}$	$0.001522\pm0.12\times10^{-12}$	86.492 ± 0.456	72.229 ± 0.344
5j	$0.001198\pm0.11\times10^{-12}$	$0.001176\pm0.21\times10^{-12}$	98.921 ± 0.443	75.562 ± 0.222
8	$0.00085\pm0.23\times10^{-12}$	$0.0008\pm0.11\times10^{-12}$	91.568 ± 0.221	76.345 ± 0.333
14a	$0.001524\pm0.45\times10^{-12}$	$0.001272\pm0.34\times10^{-12}$	87.632 ± 0.345	74.157 ± 0.444
14c	$0.001217\pm0.26\times10^{-12}$	$0.001195\pm0.34\times10^{-12}$	88.913 ± 0.353	74.483 ± 0.555
14e	$0.002341\pm0.87\times10^{-12}$	$0.001591\pm0.23\times10^{-12}$	86.191 ± 0.332	72.109 ± 0.343
14f	$0.001168\pm0.21\times10^{-12}$	$0.001147\pm0.12\times10^{-12}$	88.961 ± 0.234	75.812 ± 0.222
14k	$0.00082\pm0.11\times10^{-12}$	$0.0008\pm0.1678\times10^{-12}$	91.999 ± 0.222	76.456 ± 0.333

Compound				LD$_{50}$ mg/kg
2				456.23 ± 0.26739
5a				56.32 ± 0.3987
5b				286.345 ± 0.293
5c				987.43 ± 0.3983
5d				34.56 ± 0.2736
5e				563 ± 0.387
5f				$657.567\pm04987.$
5g				867.89 ± 0.498
5h				785.66 ± 0.3987
5i				56.78 ± 0.3876
5j				234.67 ± 0.4876
8				236.345 ± 0.94
14a				67.23 ± 0.0-44
14c				567.34 ± 0.498
14e				155.23 ± 0.444
14f				345.79 ± 0.984
14k				678.43 ± 0.498

Also, the electron donating substituents provide more Beta amyloid activity binding than those of electron withdrawing groups. For triazolopyridopyrimidine derivatives the order of activity is **14k, 14f, 14c, 14a, 14e**. So, we can conclude that amide substituents provide more activity than ester ones.

In conclusion, the novel 1, 3,4-thiadiazole derivatives are prepared *via* reaction of enaminone **2** with a variety of reagents such as hydrazonoyl chlorides and 6-amino-2-thioxo-pyrimidin-4-one. The synthesized compounds seem to be interesting for biological activity studies. They revealed high potency as anti-Alzheimer agents. Also, the results obtained from the testing compounds showed reasonable therapeutic indexes especially those of high activities and this beside their lower possible side effects due to no action on Notch intracellular domain responsive genes.

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