Full Paper

Hydrazonoyl Halides As Precursors For New Fused Heterocycles of 5α-Reductase Inhibitors

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A new series of benzo[6,7]cyclohepta[1,2-*d*]triazolo[4,3-*a*]pyrimidines **8a–l** was synthesized *via* reaction of heterocyclic thione **4** or its methyl derivatives **10** with hydrazonoyl halides **5a–l**. Also, reaction of compound **4** with a mixture of chloroacetic acid and aromatic aldehyde derivatives gave benzo[6,7]cyclohepta[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3-ones **12–14**. The microanalyses and spectral data of the synthesized compounds are in full agreement with their molecular structure. All the newly synthesized products were screened against 5α-reductase and showed activities with good ED_{50} for all compounds.

Keywords: 5α-Reductase inhibitors / Benzo[6,7]cyclohepta[1,2-*d*]triazolo[4,3-*a*]pyrimidines / Benzo[6,7]cyclohepta[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3-ones / Hydrazonoyl halides

Received: June 20, 2011; Revised: August 26, 2011; Accepted: September 1, 2011

DOI 10.1002/ardp.201100212

Introduction

In a previous work we reported that some of our newly substituted heterocyclic compounds exhibited antitumor [1–5], antimicrobial [3, 4], 5α-reductase inhibitor [6] and anti-HCV [1, 2, 4] activities. Benzosuberone ring system and its fused systems present interesting pharmacological and biological activities as blood platelet aggregation inhibitors [7], antidepressants [8], analgesic [9], anti-inflammatory [9], antipyretic [10], and antirheumatic activities [11]. In addition, the structure of anastrozole (a drug used to treat breast cancer after surgery and for metastases in both pre- and post-menopausal women) is 1-substituted-1,2-4-triazole. In view of these reports and in continuation of our previous works in synthesis of bioactive heterocyclic compounds [1–6], we have herein been interested in synthesis of fused triazoles with pyrimidine and benzosuberone moieties and investigated their activity as 5α-reductase inhibitors compared with *anastrozole* drug.

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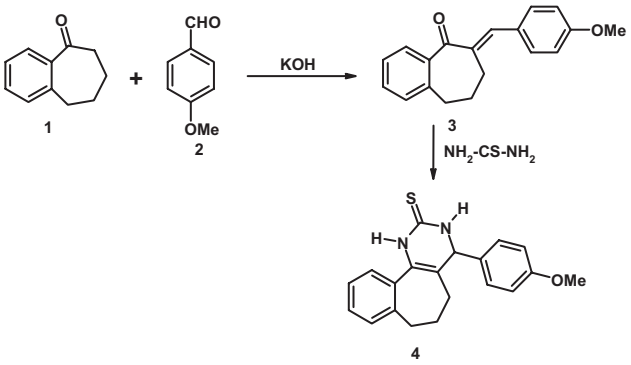
Results and discussion

Chemistry

The required 4-(4-methoxyphenyl)-1,3,4,5,6,7-hexahydro-2-thioxo-benzo[6,7]cyclohepta[1,2–d]pyrimidine (**4**) was prepared as outlined in Scheme 1. Thus, reaction of thiourea with 2-(4-methoxybenzylidene)-benzo[1,2]cycloheptan-1-one (**3**) in absolute ethanol in presence of potassium hydroxide led to formation of compound **4**. The structure of compound **4** was confirmed by elemental analyses and spectral data (see Experimental).

Refluxing equimolar quantities of each of hydrazonoyl halides **5a–l** with 4-(4-methoxyphenyl)-1,3,4,5,6,7-hexahydro-2-thioxo-benzo[6,7]cyclohepta[1,2-*d*]pyrimidine (**4**) in dioxane in presence of triethylamine gave in each case a single product as indicated by TLC analysis of the crude product. The structure of the product was identified as benzo[6,7]cyclohepta[1,2-*d*]triazolo[4,3-a]pyrimidine **8** (Scheme 2) based on its elemental analysis and spectral (IR, ^{1}H NMR and MS) data. The other possible isomeric structure, namely, benzo[6,7]cyclohepta[2,1-*e*]triazolo[4,3-a]pyrimidine **9** was discarded. For example, the ^{1}H NMR spectra of the isolated products showed in each case a singlet signal in the region δ 5.86–6.51 ppm, assigned to H-4 of the pyrimidine ring residue in the products **8**. This assignment is based on the

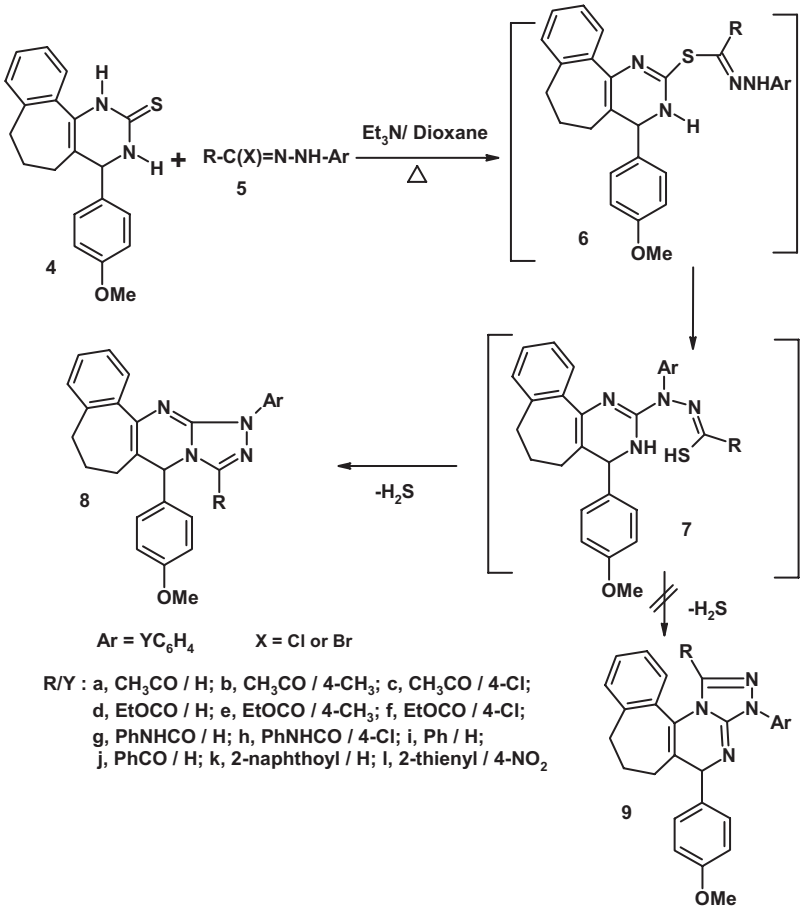


Scheme 1. Synthesis of compound **4**.

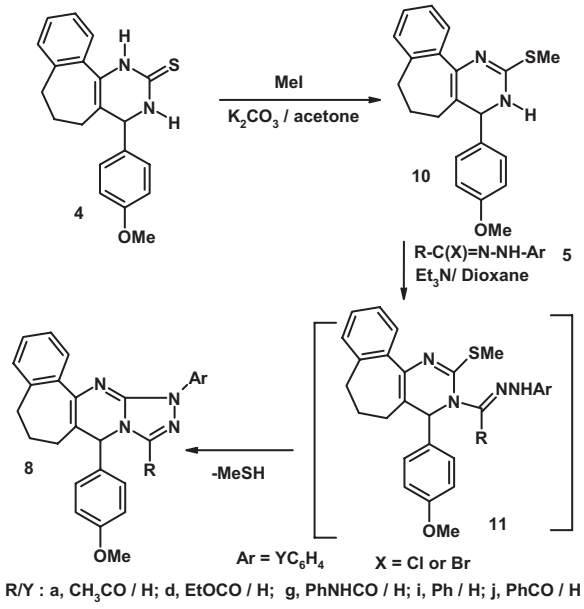
fact that the H-4 in the pyrimidine ring is markedly affected by the nature of the adjacent nitrogen (N3) (pyrrole type in structure **8** and pyridine type in structure **9**). The carbon atom near N-pyrrole type is electron rich carbon and so H-4 is expected to be more shielded than the carbon atom near N-pyridine type. Typically the signal of H-4 in similar compounds to **8** usually appears at δ 5.6 – 6.3 [6, 12–14] whereas that similar to compound **9** appears at δ 3.95–4.15 [15].

Alternatively, the formation of **8** from 2-methylthio derivative **10** (prepared from the reaction of thione **4** with methyl iodide in acetone in presence of anhydrous potassium carbonate) and hydrazonoyl halides **5** can be accomplished by cyclization *in situ* of the non isolated intermediate amidrazone **11** with concurrent elimination of methanethiol (Scheme 3).

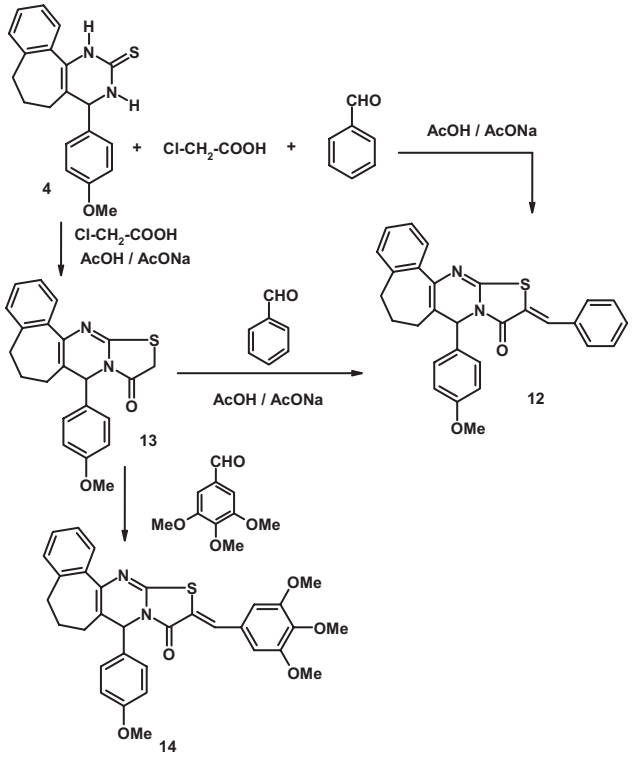
Finally, compound **4** reacted with chloroacetic acid in a mixture of acetic acid and acetic anhydride and in presence of fused sodium acetate to give 5-(4-methoxyphenyl)-2,5,6,7,8-pentahydro-benzo[6,7]cyclohepta[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3-one (**13**). Treatment of the latter product **13** with benzaldehyde or 3,4,5-trimethoxybenzaldehyde in acetic acid and in presence of fused sodium acetate afforded 5-(4-methoxyphenyl)-2-(phenylmethylene)-5,6,7,8-tetrahydro-benzo[6,7] cyclohepta[2,1-*d*]thiazolo[3,2-*a*] pyrimidin-3-one (**12**) or 5-(4-methoxyphenyl)-2-(3,4,5-trimethoxy-phenylmethylene)-5,6,7, 8-tetrahydro-benzo[6,7]cyclohepta[1,2-*d*]thiazolo[3,2-*a*]pyrimidin-3-one (**14**), respectively. Compound **12** was also prepared by one step reaction *via* chloroacetic acid and benzaldehyde under the same reaction conditions (Scheme 4). The structure of compounds **12–14** was confirmed by elemental analyses and spectral data (see Experimental).



Scheme 2. Synthesis of compound **8**.



Scheme 3. Alternative synthesis of compound **8**.



Scheme 4. Synthesis of compounds **12** and **14**.

Pharmacological screening

Circulating testosterone and dihydrotestosterone hormone levels or tissue concentrations were measured after administration of 5α-reductase inhibitor radioimmuno assays. All synthesized compounds were tested for their 5α-reductase inhibitor activity *in vivo;* the ED_{50} data that indicate efficacy were determined and are given in Table 1. (We determined ED_{50} not IC_{50} according to the adopted procedure where we measure ED_{50} mg/kg not the IC_{50} that involve molar measurements.)

Table 1. Evaluation of ED_{50} and *5α*-reductase inhibitor activities relative to anastrozole

Compd. No.	ED_{50}[a] (mg kg^{-1})	Potency relative to anastrozole
8k	0.0123 ± 000046	88.61789
8b	0.0156 ± 000043	69.87179
8e	0.0188 ± 000022	57.97872
8f	0.0196 ± 000023	556.1224
13	0.0256 ± 000021	42.57813
14	0.0278 ± 000023	39.20863
10	0.0311 ± 000045	35.04823
8c	0.0411 ± 000067	26.52068
8d	0.0512 ± 000054	21.28906
8l	0.0678 ± 000033	16.0767
8h	0.0689 ± 000046	15.82003
8j	0.0714 ± 000067	15.26611
8i	0.0723 ± 000065	15.07607
4	0.0744 ± 000044	14.65054
12	0.0754 ± 000011	14.45623
Anastrozole	1.09	1.00

[a]ED_{50}: Dose caused 50% of pharmacological response in the test.

Some selected derivatives were screened for their 5α-reductase inhibitor activities and it is worth to mention that all are more active than the reference drug anstrozole and they arranged as follow in descending order of potency **8k**, **8b**, **8e**, **8f**, **13**, **14**, **10**, **8c**, **8d**, **8I**, **8h**, **8j**, **8i**, **4**, **12**.

Experimental

All melting points were determined on an electrothermal Gallenkamp apparatus. Solvents were distilled and dried by standard literature procedures prior to use. The IR spectra were measured on a Pye-Unicam SP300 instrument in potassium bromide discs. The ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury (300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C NMR) and the chemical shifts were related to that of the solvent DMSO-d_6. The mass spectra were recorded on GCMS-Q1000-EX Shimadzu and GCMS 5988-A HP spectrometers, the ionizing voltage was 70 eV. Elemental analyses were carried out by the Microanalytical Center of Cairo University, Giza, Egypt. Hydrazonoyl halides **5a–l** were prepared following literature methods [16].

*Preparation of 4-(4-methoxyphenyl)-1,3,4,5,6,7-hexahydro-2-thioxo-benzo[6,7]cyclohepta[1,2-d]-pyrimidine (**4**)*

To a boiling mixture of compound **3** (2.78 g, 0.01 mol) in ethanol (100 mL) containing potassium hydroxide (1 g) in water (0.5 mL) was added thiourea (0.76 g, 0.01 mol) and the reaction mixture

was refluxed for 3h, allowed to cool and the solid formed was filtered off, dissolved in water and precipitated by HCl. The solid formed was filtered off and crystallized from ethanol to afford compound **4** as yellow crystals. Yield (96%) mp 210°C. IR (KBr, cm^{-1}) 3298, 3201 (2NH), ^{1}H NMR (DMSO-d_6) at δ = 1.40–2.42 (m, 6H, $3CH_2$), 3.76 (s, 3H, OCH_3), 4.87 (s, 1H, pyrimidine–H), 6.95 (d, *J* = 8 *Hz*, 2H, Ar-H), 7.20–7.26 (m, 4H, ArH), 7.28 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.83 (s, 1H, NH, exchangeable with D_2O), 9.66 (s, 1H, NH, exchangeable with D_2O), MS *m/z* (%) 336 (M^+, 100), 335 (77), 334 (34), 307 (66), 275 (24), 229 (92), 214 (11), 154 (18), 128 (20), 115 (46), 91 (14), 77 (23), Anal. Calcd. for $C_{20}H_{20}N_2OS$ (336.46): C, 71.40; H, 5.99; N, 8.33. Found C, 71.21; H, 5.82; N, 8.15%.

*Preparation of 4-(4-methoxyphenyl)-3,4,5,6,7-pentahydro-2-methylthio-benzo[6,7]cyclohepta[1,2-d]pyrimidine (**10**)*

To a stirred solution of compound **4** (1.68 g, 5 mmol) in acetone (20 mL) was added anhydrous potassium carbonate (0.70 g, 5 mmol) and methyl iodide (0.71 g, 5 mmol). The reaction mixture was stirred overnight at room temperature, then the solvent was evaporated. The solid formed was filtered off, washed with water, dried and crystallized from ethanol to give compound **10** as yellow solid. Yield (81%) mp 200°C. IR (KBr, cm^{-1}) 3245 (NH). ^{1}H NMR (DMSO-d_6) at δ = 1.23–2.42 (m, 6H, $3CH_2$), 2.67 (s, 3H, SCH_3), 3.77 (s, 3H, OCH_3), 4.73 (s, 1H, pyrimidine–H), 6.95 (d, *J* = 9 *Hz*, 2H, Ar-H), 7.19–7.29 (m, 4H, ArH), 7.31 (d, *J* = 9 *Hz*, 2H, Ar-H), 9.41 (s, 1H, NH, exchangeable with D_2O). MS *m/z* (%) 350 (M^+, 21), 348 (44), 320 (13), 241 (100), 168 (11), 128 (28), 115 (30), 91 (24), 77 (41). Anal. Calcd. for $C_{21}H_{22}N_2OS$ (350.49): C, 71.97; H, 6.33; N, 7.99. Found C, 71.76; H, 6.25; N, 7.84%.

*Preparation of benzo[6,7]cyclohepta[1,2-d]triazolo[4,3-a]pyrimidines (**8a–l**)*

Method A: To a mixture of equimolar amounts of **4** and the appropriate hydrazonoyl halides **5a–l** (2.5 mmol of each) in dioxane (20 mL) was added triethylamine (0.35 mL, 2.5 mmol). The reaction mixture was refluxed till all of the starting materials have disappeared and hydrogen sulfide gas ceased to evolve (10 h monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and crystallized from the appropriate solvent to give compounds **8a–l**.

Method B: To a mixture of equimolar amounts of **10** and the appropriate hydrazonoyl halides **5a**,**d**,**g**,**l**,**h** (2.5 mmol of each) in dioxane (20 mL) was added triethylamine (0.35 mL, 2.5 mmol). The reaction mixture was refluxed till all methanethiol gas ceased to evolve (20 h, monitored by TLC). The solvent was evaporated and the residue was treated with methanol. The solid that formed was filtered off and crystallized from the appropriate solvent to give products identical in all respects (mp, mixed mp and IR) with that formed by Method A.

*3-Acetyl-1,5,6,7,8-pentahydro-1-phenyl-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8a**)*

Yellow solid, yield (80%) mp 120–122°C (ethanol), IR (KBr, cm^{-1}) 1692 (CO), ^{1}H NMR (DMSO-d_6) δ = 1.20–2.43 (m, 6H, $3CH_2$), 2.51 (s, 3H, $COCH_3$), 3.79 (s, 3H, OCH_3), 6.46 (s, 1H, pyrimidine-H), 7.01–8.03 (m, 13H, Ar-H). MS *m/z* (%) 462 (M^+, 10), 109 (35), 91 (80), 77 (100), Anal. Calcd. for $C_{29}H_{26}N_4O_2$ (462.56): C, 75.30; H, 5.67; N, 12.11. Found C, 75.21; H, 5.48; N, 12.05%.

*3-Acetyl-1,5,6,7,8-pentahydro-1-(4-methylphenyl)-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[2,1-d]-triazolo[4,3-a]pyrimidine (**8b**)*

Dark orange solid, yield (90%), mp 82–84°C (ethanol), IR (KBr, cm^{-1}) 1693 (CO). ^{1}H NMR (DMSO-d_6) δ = 1.25–2.0 (m, 6H, $3CH_2$), 2.35 (s, 3H, CH_3), 2.51 (s, 3H, $COCH_3$), 3.81 (s, 3H, OCH_3), 6.28 (s, 1H, pyrimidine-H), 6.88 (d, *J* = 8 *Hz*, 2H, Ar-H), 7.13–7.59 (m, 8H, Ar-H), 8.14 (d, *J* = 8 *Hz*, 2H, Ar-H). MS *m/z* (%) 477 (M^++1, 17), 476 (M^+ , 66), 368 (100), 336 (37), 242 (49), 202 (34), 178 (57), 154 (69), 127 (63), 104 (37), 92 (49), 77 (97). Anal. Calcd. for $C_{30}H_{28}N_4O_2$ (476.58): C, 75.61; H, 5.92; N, 11.76. Found C, 75.88; H, 5.83; N, 11.54%.

*3-Acetyl-1,5,6,7,8-pentahydro-1-(4-chlorophenyl)-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8c**)*

Orange crystal, yield (81%), mp 120°C (ethanol), IR (KBr, cm^{-1}) 1697 (CO). ^{1}H NMR (DMSO-d_6) δ = 1.15–2.46 (m, 6H, $3CH_2$), 2.58 (s, 3H, $COCH_3$), 3.76 (s, 3H, OCH_3), 6.26 (s, 1H, pyrimidine–H), 6.88–7.61 (m, 12H, ArH). MS *m/z* (%) 498 (M^++2, 15), 497 (M^++1, 41), 496 (M^+, 66), 389 (100), 336 (95), 229 (59), 190 (46), 168 (39), 153 (42), 121 (75), 115 (46), 111 (22), 91 (53), 76 (19). Anal. Calcd. for $C_{29}H_{25}ClN_4O_2$ (497.0): C, 70.09; H, 5.07; N, 7.13. Found C, 70.10; H, 5.32; N, 7.09%.

*3-Ethoxycarbonyl-1,5,6,7,8-pentahydro-1-phenyl-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8d**)*

Yellow solid, yield (79%), mp 146–148°C (ethanol), IR (KBr, cm^{-1}) 1724 (CO).^{1}H NMR ($CDCl_3$) δ = 1.27 (t, *J* = 7 *Hz*, 3H, CH_3), 1.73–2.55 (m, 6H, $3CH_2$), 3.80 (s, 3H, OCH_3), 4.34 (q, *J* = 7 *Hz*, 2H, CH_2), 6.32 (s, 1H, pyrimidine–H), 6.84 (d, *J* = 8 *Hz*, 2H, Ar-H), 6.98–7.77 (m, 9H, ArH), 8.27 (d, *J* = 8 *Hz*, 2H, Ar-H). MS *m/z* (%) 493 (M^++1, 7), 492 (M^+, 29), 385 (48), 307 (17), 278 (20), 135 (33), 121 (31), 115 (30), 105 (22), 103 (29), 91 (96), 77 (100). Anal. Calcd. for $C_{30}H_{28}N_4O_3$ (492.58): C, 73.15; H, 5.73; N, 11.37. Found C, 73.34; H, 5.54; N, 11.49%.

*3-Ethoxycarbonyl-1,5,6,7,8-pentahydro-1-(4-methylphenyl)-5-(4-methoxyphenyl)-benzo[6,7]-cyclohepta[1,2-d]triazolo[4,3-a]pyrimidine (**8e**)*

Yellow solid, yield (78%), mp 160°C (ethanol/dioxane), IR (KBr, cm^{-1}) 1724 (CO).^{1}H NMR ($CDCl_3$) δ = 1.23 (t, *J* = 7 *Hz*, 3H, CH_3), 1.60–2.0 and 2.39–2.49 (m, 6H, $3CH_2$), 2.33 (s, 3H, CH_3), 3.73 (s, 3H, OCH_3), 4.27 (q, *J* = 7 *Hz*, 2H, CH_2), 6.25 (s, 1H, pyrimidine–H), 6.90 (d, *J* = 9 *Hz*, 2H, Ar-H), 7.15–7.32 (m, 4H, ArH), 7.35 (d, *J* = 8 *Hz*, 2H, Ar-H), 7.57 (d, *J* = 8 *Hz*, 2H, Ar-H), 8.08 (d, *J* = 9 *Hz*, 2H, Ar-H). MS *m/z* (%) 507 (M^++1, 27), 506 (M^+, 71), 399 (99), 327 (36), 121 (56), 115 (28), 104 (42), 91 (100), 77 (54). Anal. Calcd. for $C_{31}H_{30}N_4O_3$ (506.61): C, 73.50; H, 5.97; N, 11.06. Found C, 73.38; H, 5.82; N, 11.00%.

*3-Ethoxycarbonyl-1,5,6,7,8-pentahydro-1-(4-chlorophenyl)-5-(4-methoxyphenyl)-benzo[6,7]-cyclohepta[1,2-d]triazolo[4,3-a]pyrimidine (**8f**)*

Yellow solid, yield (85%), mp 100–102°C (ethanol/dioxane), IR (KBr, cm^{-1}) 1728 (CO).^{1}H NMR ($CDCl_3$) δ = 1.24 (t, *J* = 7 *Hz*, 3H, CH_3), 1.60–2.40 (m, 6H, $3CH_2$), 3.73 (s, 3H, OCH_3), 4.28 (q, *J* = 7 *Hz*, 2H, CH_2), 6.26 (s, 1H, pyrimidine–H), 6.90

(d, $J = 9$ *Hz*, 2H, Ar-H), 7.18–7.32 (m, 4H, ArH), 7.36 (d, $J = 9$ *Hz*, 2H, Ar-H), 7.56 (d, $J = 9$ *Hz*, 2H, Ar-H), 8.26 (d, $J = 9$ *Hz*, 2H, Ar-H). MS *m/z* (%) 528 (M^++2, 6), 527 (M^++1, 17), 526 (M^+, 49), 419 (70), 126 (79), 125 (87), 111 (100), 90 (72), 77 (45). Anal. Calcd. for $C_{30}H_{27}ClN_4O_3$ (527.03) C, 68.37; H, 5.16; N, 10.63. Found C, 68.21; H, 5.09; N, 10.58%.

*3-Phenylcarbamoyl-1,5,6,7,8-pentahydro-1-phenyl-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8g**)*

Dark red solid, yield (70%), mp 130°C (ethanol), IR (KBr, cm^{-1}) 3421 (NH), 1693 (CO), MS *m/z* (%) 540 (M^++1, 20), 539 (M^+, 42), 432 (43), 418 (37), 280 (31), 170 (31), 159 (43), 119 (51), 105 (17), 91 (86), 77 (100). Anal. Calcd. for $C_{34}H_{29}N_5O_2$ (539.64): C, 75.68; H, 5.42; N, 12.98. Found C, 75.92; H, 5.27; N, 12.78%.

*3-Phenylcarbamoyl-1,5,6,7,8-pentahydro-1-(4-chlorophenyl)-5-(4-methoxy-phenyl)-benzo[6,7]-cyclohepta[1,2-d]triazolo[4,3-a]pyrimidine (**8h**)*

Yellow solid, yield (73%), mp. 85°C (ethanol/dioxane), IR (KBr, cm^{-1}) 3305 (NH), 1685 (CO).^{1}H-NMR ($CDCl_3$) δ = 1.27–2.63 (m, 6H, $3CH_2$), 3.77 (s, 3H, OCH_3), 6.51 (s, 1H, pyrimidine–H), 6.83–8.24 (m, 17H, ArH), 11.42 (s, 1H, NH, exchangeable with D_2O). MS *m/z* (%) 576 (M^++2, 14), 574 (M^+, 42), 468 (51), 466 (100), 426 (54), 316 (51), 225 (30), 151 (74), 147 (42), 121 (58), 111 (28), 105 (35), 91 (91), 77 (95). Anal. Calcd. for $C_{34}H_{28}ClN_5O_2$ (574.09): C, 71.14; H, 4.92; N, 12.20. Found C, 71.32; H, 5.04; N, 12.25%.

*1,3-Diphenyl-1,5,6,7,8-pentahydro-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]triazolo[4,3-a]pyrimidine (**8i**)*

Yellow solid, yield (79%), mp. 100°C (ethanol), ^{1}H-NMR (DMSO-d_6) δ = 1.39–2.62 (m, 6H, $3CH_2$), 3.72 (s, 3H, OCH_3), 5.86 (s, 1H, pyrimidine–H), 6.62–8.40 (m, 18H, ArH). MS *m/z* (%) 496 (M^+, 39), 495 (13), 392 (28), 389 (41), 140 (20), 121 (47), 115 (25), 105 (17), 103 (31), 91 (93), 77 (100). Anal. Calcd. for $C_{33}H_{28}N_4O$(496.62): C, 79.81; H, 5.68; N, 11.28. Found C, 79.73; H, 5.43; N, 11.20%.

*3-Benzoyl-1,5,6,7,8-pentahydro-1-phenyl-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8j**)*

Red solid, yield (76%), mp. 158°C (ethanol/dioxane), IR (KBr, cm^{-1}) 1680 (CO), ^{1}H-NMR (DMSO-d_6) δ = 1.20–2.43 (m, 6H, $2CH_2$), 3.66 (s, 3H, OCH_3), 6.42 (s, 1H, pyrimidine–H), 6.83 (d, $J = 9$ *Hz*, 2H, Ar-H), 7.17–8.02 (m, 14H, ArH), 8.25 (d, $J = 9$ *Hz*, 2H, Ar-H). MS *m/z* (%) 524 (M^+, 6), 394 (18), 393 (23), 392 (36), 336 (24), 339 (15), 141 (21), 130 (18), 117 (18), 115 (33), 105 (68), 91 (44), 89 (19), 77 (100). Anal. Calcd. for $C_{34}H_{28}N_4O_2$ (524.63): C, 77.84; H, 5.38; N, 10.68. Found C, 77.82; H, 5.43; N, 10.48%.

*3-(2-Naphthoyl)-1,5,6,7,8-pentahydro-1-phenyl-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8k**)*

Orange solid, yield (82%), mp. 130°C (ethanol/dioxane), IR (KBr, cm^{-1}) 1693 (CO). ^{1}H-NMR (DMSO-d_6) δ = 1.20–2.41 (m, 6H, $2CH_2$), 3.72 (s, 3H, OCH_3), 6.27 (s, 1H, pyrimidine–H), 6.88 (d, $J = 9$ *Hz*, 2H, Ar-H), 7.16–7.28 (m, 13H, ArH), 7.37 (d, $J = 8$ *Hz*, 2H, naphthyl-H), 7.45 (s, 1H, naphthyl-H), 7.59 (d, $J = 8$ *Hz*, 1H, naphthyl-H), 8.32 (d, $J = 9$ *Hz*, 2H, Ar-H). MS *m/z* (%) 575 (M^++1, 11), 574 (M^+, 36), 467 (32), 452 (30), 279 (23), 155 (100), 129 (27), 127 (93), 126 (59), 121 (48), 115 (39), 92 (30), 77 (86). Anal. Calcd. for $C_{38}H_{30}N_4O_2$(574.68): C, 79.42; H, 5.26; N, 9.75. Found: C, 79.52; H, 5.43; N, 9.59%.

*3-(2-Thienyl)-1,5,6,7,8-pentahydro-1-(4-nitrophenyl)-5-(4-methoxyphenyl)-benzo[6,7]cyclohepta[1,2-d]-triazolo[4,3-a]pyrimidine (**8l**)*

Yellow solid, yield (74%), mp. 138°C (ethanol/dioxane); MS *m/z* (%) 548 (M^++1, 26), 547 (M^+, 57), 437 (61), 228 (30), 217 (61), 167 (44), 131 (65), 127 (57), 121 (91), 115 (100), 111 (22), 106 (70), 91 (78), 77 (61), 76 (91)105 (46), 91 (100). Anal. Calcd. for $C_{31}H_{25}N_5O_3S$ (547.64): C, 67.99; H, 4.60; N, 12.79. Found: C, 67.86; H, 4.65; N, 12.58%.

*Preparation of 5-(4-methoxyphenyl)-2,5,6,7,8-pentahydro-benzo[6,7]cyclohepta [1,2-d]thiazolo[3,2-a]pyrimidine-3-one (**13**)*

A mixture of compound **4**, chloroacetic acid (0.005 mol each), and fused sodium acetate (3 g) in glacial acetic acid (15 mL) and acetic anhydride (5 mL) was refluxed for 3h, left to cool, then poured gradually with stirring onto cold water. The solid formed was filtered off, washed with water and crystallized from ethanol to give compound **13** as rose solid, yield (71%), mp. 200–202°C (ethanol), IR (KBr, cm^{-1}) 1695 (CO). ^{1}H NMR (DMSO-d_6) at δ = 1.37–2.25 (m, 6H, $3CH_2$), 3.75 (s, 3H, OCH_3), 5.69 (s, 2H, CH_2), 6.93–7.46 (m, 9H, ArH + pyrimidinyl-H). MS *m/z* (%) 376 (M^+,12), 346 (26), 332 (100), 121 (48), 104 (16), 84 (25), 77 (31). Anal. Calcd. for $C_{22}H_{20}N_2O_2S$ (376.48): C, 70.19; H, 5.35; N, 7.44. Found: C, 70.22; H, 5.25; N, 7.29%.

*Preparation of 2-arylidine-benzo[6,7]cyclohepta[1,2-d]-thiazolo[3,2-a]pyrimidine-3-one (**12** and **14**)*

Method A: A mixture of compound **4** (0.34 g, 0.001 mol), chloroacetic acid (0.10 g, 0.001 mol) and fused sodium acetate (0.6 g) in glacial acetic acid (10 mL), acetic anhydride (5 mL) and the appropriate aromatic aldehyde (0.001 mole) was refluxed for 3h. The reaction mixture was cooled, poured onto cold water and the solid formed was collected and crystallized from ethanol to give compound **12** or **14**.

Method B: A mixture of compound **13** (0.38 g, 0.001 mol), and fused sodium acetate (0.6 g) in glacial acetic acid (10 mL), acetic anhydride (5 mL) and benzaldehyde (0.11 g, 0.001 mole) was refluxed for 3h. The reaction mixture was cooled, poured onto cold water and the solid formed was collected and crystallized from ethanol to give compound **12**.

5-(4-Methoxyphenyl)-2-(phenylmethylene)-5,6,7,8-tetrahydro-benzo[6,7]cyclohepta [1,2-d]thiazolo[3,2-a]pyrimidine-3-one (**12**) as yellow solid, yield (65%), mp. 155°C (EtOH). MS *m/z* (%) 465 (M^++1, 19), 464 (M^+, 48), 343 (51), 329 (30), 232 (16), 134 (74), 133 (46), 121 (26), 92 (17), 76 (14). Anal. Calcd. for $C_{29}H_{24}N_2O_2S$ (464.59): C, 74.97; H, 5.21; N, 6.03. Found: C, 74.85; H, 5.19; N, 6.27%.

5-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenylmethylene)-5,6,7,8-tetrahydro-benzo [6,7]cyclohepta[1,2-d]thiazolo[3,2-a]pyrimidine-3-one (**14**) as yellow solid, yield (62%), mp. 179°C (EtOH). ^{1}H-NMR (DMSO-d_6) at δ = 1.25–2.41 (m, 6H, $2CH_2$), 3.72, 3.80, 3.83, 3.87 (4s, 12H, $4OCH_3$), 5.79 (s, 1H, pyrimidine–H), 6.94 (d, $J = 9$ *Hz*, 2H, Ar-H), 7.11–7.34 (m, 6H, ArH), 7.37 (d, $J = 9$ *Hz*, 2H, ArH), 7.67 (s, 1H, =CH). MS *m/z* (%) 554 (M^+, 36), 553 (20), 448 (31), 447 (100), 446

(45), 433 (28), 224 (17), 209 (30), 120 (15), 77 (8). Anal. Calcd. for $C_{32}H_{30}N_2O_5S$ (554.67): C, 69.29; H, 5.45; N, 5.05. Found: C, 69.35; H, 5.28; N, 5.27%.

Pharmacology

Biological assay

Treatment of animals

Animals were obtained from the animal house colony of the National Research Center, Cairo, Egypt. All animals were allowed free access to water and were kept on a constant standard diet. Twenty three groups, each of 12 male Sprague-Dawley rats in the postnatal third days, were treated subcutaneously with the 5α-reductase inhibitor (tested compound or reference standard). The tested compounds were dissolved in 5% Tween 80 in water. The solvent was used for both standard and negative control group, beginning on the postnatal third day until the age of seven weeks.

Twenty-one groups were used to test the activities, of which one was used as the positive control for anastrozole and another served as the negative control group. After scarifying, blood was withdrawn for testosterone and dihydrotestosterone (DHT) determination [17]. Moreover, intraprostatic concentrations of testosterone and DHT were determined [18].

The biological experiments were performed according to the official standards.

Radioimmuno assay for testosterone and dihydrotestosterone

Serum testosterone and dihydrotestosterone were measured by radioimmuno assay in serum extracts using specific antisera without prior chromatography. Serum samples of 0.5 mL were extracted with 2 mL of freshly purified peroxide-free diethyl ether by shaking for 60 s on a Vortex mixer. The aqueous phase was frozen at −70°C, the ether phase containing steroids was transferred to a conical test tube and evaporated in BSA/phosphate buffer (pH = 7.4) containing (1,2,6,7-3H)-testosterone or (1,2,6,7-3H)-dihydrotestosterone and then specific antisera were added and incubated over a period of 24 h at 4°C under non-equilibrium conditions. Bound hormone and free hormone were separated by adsorption on dextran-coated charcoal. The activity of each sample was determined in a Beckman-counter (USA) using a commercially available scintillation cocktail (Mini-RIA, Zinsser, Spain). As for other steroid hormones, commercially available KIA-kits, *e.g.*, Biermann GmbH, Germany, can be used. The hormone level in the sample was calculated from a standard curve by means of a computer program (KIA-Calc, LKB, Canada), using appropriate control sera. Steroid levels of rats treated with different doses of 5α-reductase inhibitors were compared with vehicle-treated controls (Table 1). The relative potency was calculated by dividing the ED_{50} (dose that causes 50% of pharmacological response in the test) of anastrozole by that of a tested compound.

Determination of acute toxicity

ED_{50} was determined by using male albino rats and injecting them with different increasing doses of agents. Doses that killed 50% of the tested animals, was calculated according to Austen *et al*. [19] (Table 1).

The authors have declared no conflict of interest.

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