Novel Estradiol-17α-1,2,4-triazoline Derivatives. Synthesis and *in vitro* Anabolic-catabolic Properties and Binding Affinity to Steroid Receptors

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Several novel estradiol-17α-triazolines were synthesized and tested *in vitro* for anabolic-catabolic acitivity and binding affinity to steroid receptors. While no binding affinity to steroid receptors was detected, due to the presence of a bulky triazoline ring in the 17α-position of the steroidal nucleus, the products showed almost the same catabolic properties exhibited by estrone.

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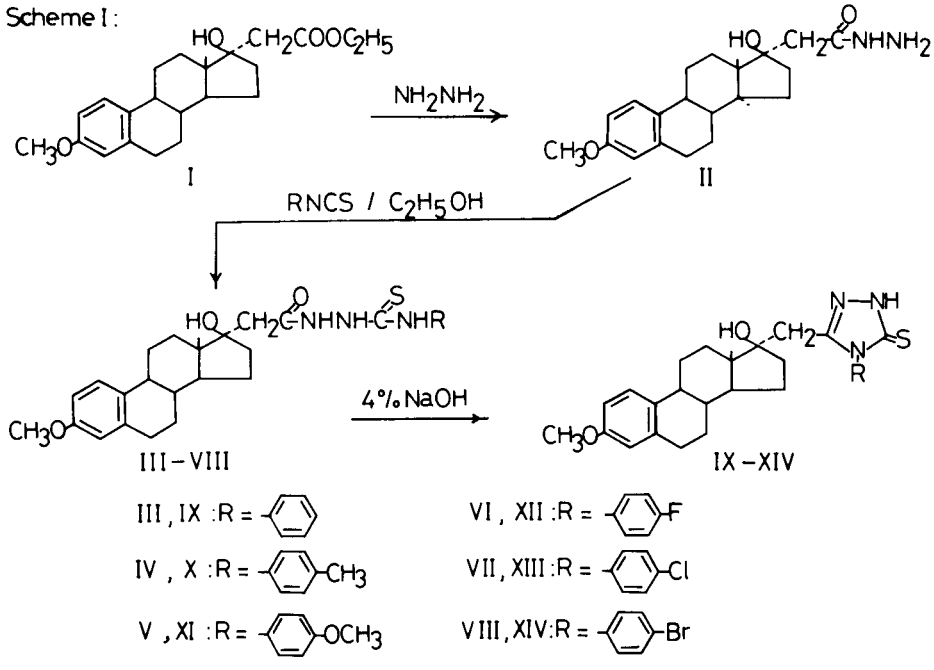
Part of the extensive studies of the structure activity relationship in steroids has been devoted to the introduction of a variety of functional groups [1-5] or heterocyclic rings [6-11] in the various positions of the steroidal nucleus and, as a result, numerous modified steroids possessing diverse pharmacological properties have been developed. Recently, we have described the synthesis of several thiosemicarbazones [12] and acylhydrazones [13-15] derived from androgenic and estrogenic hormones and evaluated the products for anticancer [12,14] and endocrinological [12, 13,15] properties. In addition, various steroidal heterocycles, involving the fusion of differently substituted oxazole [16] and oxazoline [17] rings to the 2,3- or 3,4-positions of estrone, or the attachment of a substituted thiazoline [18] ring to the 2- or 4-position of estrone-3-methyl ether, were prepared and tested *in vitro* for anabolic-catabolic properties.

In pursuing the studies of the effect of structure modification on the biological activity of hormones, the synthesis of the various estradiol-17α-triazoline derivatives IX-XIV, Scheme I, was undertaken. The compounds retain the 3-OCH_3 and the 17β-OH groups fulfilling the spatial requirements for binding to steroid receptors while locating a bulky heterocyclic moiety in the 17α-position of estradiol. The products were tested *in vitro* for anabolic-catabolic activity and binding affinity to steroid receptors.

The required steroidal triazolines IX-XIV were synthesized as shown in Scheme I. Estrone-3-methyl ether was reacted with ethyl bromoacetate and zinc dust, under Reformatzky reaction conditions [19], to give compound I in which the ethoxycarbonylmethyl function was introduced in the 17α-acetylhydrazide derivative II which, on treatment with the equivalent amounts of the appropriate arylisothiocyanates in boiling ethanol, afforded the required 4'-substituted-1'-(3-methoxy-17β-hydroxyestra-1,3,5(10)-trien-17α-yl)acetyl-3'-thiosemicarbazides III-VIII in high yields. The subsequent boiling of the products with an aqueous solution of sodium hydroxide [20,21] con-



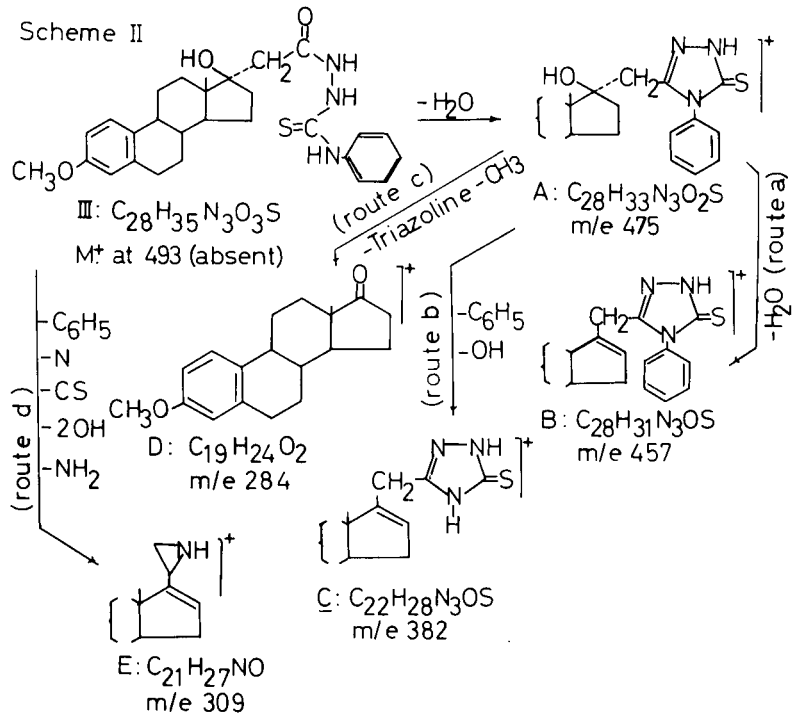


Table I

Synthesized Estradiol-17α-acetylthiosemicarbazides III-VIII and 1,2,4-Triazoline IX-XIV Derivatives

Compound No.	Yield (%)	Mp °C Crystallization Solvent	Molecular Formula	Analysis (%) Calcd./Found C	H	N
III	97	132-134 [a]	$C_{28}H_{35}N_3O_3S$	68.13	7.15	8.51
		A		68.32	7.17	8.24
IV	99	189-191	$C_{29}H_{37}N_3O_3S$	68.61	7.35	8.28
		B		68.24	7.44	8.38
V	96	135-137	$C_{29}H_{37}N_3O_4S$	66.52	7.12	8.03
		A		66.80	7.26	8.10
VI	98	127-129	$C_{28}H_{34}FN_3O_3S$	65.75	6.65	8.21
		C		65.39	6.73	8.26
VII	86	203-205	$C_{28}H_{34}ClN_3O_3S$	63.69	6.44	7.99
		D		63.39	6.45	7.58
VIII	98	205-207	$C_{28}H_{34}BrN_3O_3S$	58.74	5.94	7.34
		B		58.84	6.22	7.58
IX	99	166-168	$C_{28}H_{33}N_3O_2S$	70.71	6.99	8.84
		B		70.40	6.93	8.89
X	88	158-160	$C_{29}H_{35}N_3O_2S$	71.14	7.21	8.58
		C		71.24	7.48	8.57
XI	98	155-157	$C_{29}H_{35}N_3O_3S$	68.89	6.98	8.31
		C		69.05	7.34	8.35
XII	93	225-227	$C_{28}H_{32}FN_3O_2S$	68.15	6.49	8.51
		B		68.33	6.61	8.82
XIII	92	232-234	$C_{28}H_{32}ClN_3O_2S$	65.94	6.28	8.51
		C		66.22	6.43	8.23
XIV	93	242-244	$C_{28}H_{32}BrN_3O_2S$	60.64	5.77	7.58
		B		60.32	6.02	7.74

[a] Crystallization Solvents: A = Ethanol, B = Benzene/ethanol, C = Aqueous ethanol, D = Benzene/light petroleum.

Table II

Some ^{1}H-NMR Data for the Synthesized Steroidal Thiosemicarbazides and Triazolines

	Chemical Shift (δ ppm) in Deuteriochloroform									
	CH_3 (18)	OH (17β)	OCH_3 (3)	H (4) [a]	H (2) [b]	H (1) [c]	Ar-H	Ar-NH	NHCO	NHCS
III	0.93 (s)	3.72 (s)	3.77 (s)	6.61 (d)	6.73 (d)	7.15 (d)	7.39 (m)	7.39 (s)	8.47 (s)	8.47 (s)
IV	0.92 (s)	3.60 (s)	3.77 (s)	6.62 (d)	6.73 (d)	7.17 (d)	7.22 (m)	7.24 (s)	8.27 (s)	8.27 (s)
XII	0.92 (s)	3.20 (s)	3.80 (s)	6.63 (d)	6.73 (d)	7.16 (d)	7.34 (m)	9.26 (s)	8.90 (s)	8.48 (s)
X	0.91 (s)	3.72 (s)	3.76 (s)	6.58 (d)	6.70 (d)	7.12 (d)	7.20 (d) [d]			
							7.36 (d)			
XI	0.91 (s)	3.70 (s)	3.76 (s)	6.60 (d)	6.72 (d)	7.15 (d)	7.06 (d)			
							7.27 (d)			
XIII	0.91 (s)	3.02 (s)	3.76 (s)	6.58 (d)	6.70 (d)	7.12 (d)	7.32 (d)			
							7.54 (d)			

[a] J = 3 Hz. [b] J = 3 Hz. [c] J = 8 Hz. [d] J = 8 Hz.

taining a few drops of ethanol led to their cyclization into the 3-methoxy-17α-(1′*H*-4′-substituted-5′-thio-1′,2′,4′-triazoline-3′-methylene)estra-1,3,5(10)-trien-17β-ol derivatives IX-XIV, Scheme I. The structure of the products was confirmed by elemental analysis (Table I), ir, ^{1}H nmr and, for representative examples, by mass spectra. The ir spectra of the steroidal thiosemicarbazides III-VIII showed the bands characterizing the OH, NH, C=O and NCS functions [22], while those of the triazolines IX-XIV lacked the C=O absorption bands and showed the C=N and C=C mixed absorptions. They have also indicated that the triazolines exist in the thione form, rather than in the thiol form, by the existence of the four bands which characterize the NCS functions (experimental). In the ^{1}H nmr spectra (Table II), the steroidal thiosemicarbazides III, IV and VII showed the protons of the C_{18}-CH_3, 17β-OH, C_4-H, C_2-H and C_1-H at the expected chemical shifts [23]. The N_4-proton of the thiosemicarbazide moiety was found to be resonating at various chemical shifts depending on the nature of the substituent on the aromatic ring. In the phenylthiosemicarbazide III, it was mixed with the aromatic protons at 7.39 ppm while in the *p*-tolyl IV and the *p*-chlorophenyl VII derivatives, it resonated as a singlet at 7.24 and 9.26 ppm respectively. The N_1 and N_2-protons were identified as a one singlet or two separate singlets between 8.27 and 8.9 ppm. The spectra of the steroidal triazolines, X, XI and XIII, on the other hand, showed the signal of the N-H proton included in the multiplet of the aromatic protons.

The mass spectrum of 4′-phenyl-1′-(3-methoxy-17β-hydroxyestra-1,3,5(10)-trien-17α-yl)-3′-thiosemicarbazide (III), under electron impact, did not show the molecular ion peak at m/e 493. It however indicated that the molecule had lost one molecule of water and had undergone cyclization into the triazoline ion **A** at m/e 475 (Scheme II). Further fragmentation of ion **A** through elimination of water (route a), removal of a phenyl and a hydroxyl group (route b), or cleavage of the triazoline ring and a methyl group (route c) produced ion **B**, at m/e 457, ion **C**, at m/e 382, and ion **D** at m/e 284 respectively. The alternative cleavage of a phenyl group, nitrogen, carbon monosulfide, two hydroxyl groups and an amino function successively from the whole molecule (route d) gave the steroidal aziridine ion **E** at m/e 309. The additional ions, including the base peak at m/e 199, due to reported fragments of estrone-3-methyl ether [24] and similar thio functions were identified [25]. The spectrum of the *p*-chlorophenylthiosemicarbazide (VII) was found to be following the same pattern proposed for the fragmentation of compound III. The spectra of the 3-methoxy-17α-(1′*H*-4′-phenyl-5′-thio-1′,2′,4′-triazoline-3′-methylene)estra-1,3,5(10)-trien-17β-ol (IX) and the corresponding 4′-*p*-bromophenyl derivative XIV, on the other hand, showed the molecular ion peaks at m/e 475 and 553 respectively. Their mode of fragmentation was found to be following the same pathways proposed for the fragmentation of ion **A** in the spectrum of compound III, Scheme II. The base peaks appeared at m/e 191 and 269 respectively.

Compounds III, V, XI, XII and XIV showed insignificant catabolic activity when tested *in vitro* for their effects on bovine pancreatic ribonuclease activity [17,26]. The steroidal thiosemicarbazide V and triazoline XII derivatives, on the other hand, were devoid of binding affinity to the steroid receptors. The assays of such binding affinity were performed in accordance with the protocol of the biological screening division, Russel UCLAF, France.

EXPERIMENTAL

All melting points are uncorrected and were measured in open capillaries. The ir spectra were recorded on a Beckman 4210 ir spectrophotometer. The ^{1}H nmr spectra were measured on a Varian EM 360L and ms on Finnigan 3200.

3-Methoxy-17β-hydroxyestra-1,3,5(10)-triene-17α-acetylhydrazide (II).

A mixture of the ester I (1.5 g, 4.03 mmoles) and hydrazine hydrate (0.8 g, 16 mmoles) was heated for 3.5 hours at 115° when a white solid began to separate after 2 hours of heating. Excess hydrazine hydrate was re-

moved by evaporation and the mixture was cooled, treated with water, filtered, washed several times with water (3 × 25 ml) and dried. Crystallization of the product from ethanol gave 1.39 g (97%) of compound II as white crystals melting at 173-174°; ir (nujol): 3400 (OH), 3300 (NH), 1640 (C=O), 1610 and 1500 (C=C, aromatic), 1250 and 1030 cm^{-1} (C-O-C); ^1H nmr (deuteriochloroform): δ 0.92 (s, 3H, C_{18}-CH_3), 3.76 (s, 1H, overlapping with the singlet of OCH_3, exchangeable, C_{17}-β-OH), 3.77 (s, 3H, OCH_3), 6.60 (d, 1H, J = 3 Hz, C_4-H), 6.71 (d, 1H, J = 3 Hz, C_2-H), 7.16 (d, 1H, J = 8 Hz, C_1-H), 7.50 (s, 1H, CONH, exchangeable).

Anal. Calcd. for $C_{21}H_{30}N_2O_3$: C, 70.36; H, 8.44; N, 7.82. Found: C, 70.42; H, 8.59; N, 7.95.

4'-Substituted-1'-(3-methoxy-17β-hydroxyestra-1,3,5(10)-triene-17α-yl)-acetyl-3'-thiosemicarbazides. III-VIII. General Procedure.

A mixture of the acid hydrazide II (150 mg, 0.41 mmole) and the appropriate isothiocyanate derivative (1.2 molar equivalents) in 5 ml of ethanol (absolute) was heated under reflux for 30 minutes. The clear colorless solution was concentrated and allowed to stand at room temperature when it deposited a white solid. This was filtered, washed with light petroleum and weighed to determine the yield. In the case of *p*-bromophenylthiosemicarbazide VIII, the product precipitated from the reaction mixture during the reflux period. The products were crystallized from the proper solvents and identified as shown in Table I; ir (nujol): 3460-3360 (OH), 3320-3090 (NH, three bands), 1680-1655 (C=O), 1610-1600 and 1495-1480 (C=C, aromatic), 1340-1230, 1040-1030 (C-O-C), and in the regions 1555-1530, 1350-1340, 1170-1155 and 970-960 cm^{-1} (NCS amide I, II, III and IV bands respectively); ms: m/e (relative abundance %) compound III $M^{+\cdot}$ at 493 (absent), 475 (3), 457 (9), 456 (20), 438 (7), 414 (3), 400 (14), 383 (10), 382 (14), 366 (6), 340 (19), 325 (24), 309 (23), 299 (6), 286 (29), 285 (71), 284 (94), 269 (17), 268 (31), 267 (38), 258 (8), 257 (22), 256 (20), 251 (26), 242 (22), 240 (33), 233 (8), 228 (48), 227 (63), 226 (29), 214 (35), 213 (27), 200 (40), 199 (100), 191 (17), 189 (22), 186 (65), 185 (55), 184 (49), 178 (13), 177 (10), 174 (67), 173 (78), 172 (87), 160 (85), 159 (78), 147 (44), 145 (74), 141 (47), 134 (59), 131 (52), 130 (58), 128 (47), 121 (56), 118 (23), 115 (74), 107 (33), 106 (41), 103 (33), 102 (27), 100 (19), 99 (30), 97 (50), 93 (34), 91 (65); for compound VII, $M^{+\cdot}$ at 527 and M + 2 at 529 (absent), 456 (7), 400 (7), 348 (3), 340 (9), 325 (10), 309 (12), 285 (67), 284 (93), 269 (13), 268 (31), 258 (5), 257 (21), 256 (15), 251 (17), 242 (17), 241 (21), 240 (19), 233 (5), 228 (28), 227 (70), 214 (23), 213 (31), 200 (40), 199 (100), 190 (6), 189 (9), 186 (65), 185 (53), 184 (42), 178 (52), 173 (67), 172 (71), 171 (83), 160 (85), 159 (81), 158 (55), 147 (46), 145 (43), 141 (45), 139 (16), 134 (60), 132 (23), 130 (49), 129 (57), 128 (58), 127 (52), 121 (43), 118 (30), 115 (62), 107 (47), 103 (33), 102 (30), 101 (23), 99 (26), 96 (47), 93 (30), 91 (70).

3-Methoxy-17α-(1'*H*-4'-substituted-5'-thio-1',2',4'-triazoline-3'-methyl)-estra-1,3,5(10)-trien-17β-ol (IX-XIV). General Procedure.

A mixture of the steroidal thiosemicarbazides III-VIII (200 mg) in 2.5 ml of 4% aqueous sodium hydroxide solution and 0.5 ml of ethanol was heated under reflux for 3 hours. The clear solution was acidified with dilute hydrochloric acid to form a white precipitate which was filtered, washed several times with water, dried and weighed. The products were crystallized from the proper solvents and identified as specified in Table I; ir (nujol): 3500-3300 (OH), 3260-3150 (NH), 1610-1605 (C=N mixed with C=C, aromatic), 1490-1485 (C=C, aromatic), 1250-1230 and 1040-1010 (C-O-C) and in the regions 1575-1570, 1335-1330, 1165-1140 and 970-965 (NCS amide I, II, III and IV bands respectively); ms: m/e (relative abundance %) compound IX, 475 (60) ($M^{+\cdot}$), 457 (21), 442 (17), 425 (5), 424 (7), 411 (4), 385 (4), 341 (4), 325 (9), 317 (5), 308 (6), 304 (9), 298 (5), 285 (53), 267 (34), 258 (6), 256 (13), 251 (16), 246 (37), 242 (13), 233 (41), 229 (15), 227 (29), 221 (15), 218 (20), 214 (13), 200 (14), 199 (31), 191 (100), 186 (16), 184 (15), 177 (10), 174 (17), 173 (33), 171 (33), 160 (26), 159 (28), 158 (29), 147 (33), 145 (16), 141 (16), 134 (13), 132 (22), 130 (15), 128 (23), 121 (21), 118 (24), 115 (24), 107 (16), 102 (15), 97 (13), 93 (16), 91 (26); for compound XIV, 555 (13) (M + 2), 553 (13) ($M^{+\cdot}$), 536 (3), 534 (3), 522 (3), 520 (3), 475 (3), 457 (5), 442 (5), 326 (8), 313 (10), 311 (10), 298 (3), 296 (3), 285 (40), 271 (78), 269 (100), 265 (5), 251 (5), 237 (3), 229 (6), 227 (12), 211 (3), 199 (14), 195 (7), 191 (3), 186 (8), 184 (3), 175 (5), 173 (15), 171 (13), 160 (8), 159 (8), 158 (6), 147 (12), 145 (3), 141 (3), 134 (3), 131 (3), 129 (3), 128 (7), 117 (3), 115 (8), 107 (3), 97 (3), 91 (6).

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