JOURNAL OF PURE & APPLIED MICROBIOLOGY, Nov. 2014. Vol. **8**(Spl. Edn. 2), p. 447-454

Androgenic Anabolic Activities of Some Substituted Androstan[16,17-c]pyrazoline and Androstan [17,16-c]isoxazole Derivatives

Mohamed M. Abdulla[1], Abd El-Galil E. Amr[2,3], Mohamed A. Al-Omar[2], Azza A. Hussain[4]* and Mohamed S. Amer[4]

[1]Research Unit, Saco Pharm. Co., 6th October City 11632, Egypt.
[2]Pharmaceutical Chemistry Department, Drug Exploration & Development Chair (DEDC), College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia.
[3]Department of Applied Organic Chemistry, National Research Center, Dokki, Cairo, Egypt.
[4]Department of Chemistry, Faculty of Science, Zagazige University, Zagazige Egypt.

(Received: 21 August 2014; accepted: 30 October 2014)

We herein report the androgenic-anabolic activities of some synthesized substituted androstano[17,16-c]pyrazoline (steroidal structure). Twenty six steroid derivatives 1-11 containing acetyl, ester, methoxy and oxazole moieties attached to a steroid moiety were conveniently synthesized and screened for their androgenic anabolic activities. Synthetic steroid structures linked to a different function groups seem to be a promising approach in the search for novel leads for potent androgenic agents. The detailed synthetic pathways of obtained compounds and androgenic activities were reported.

Key words: Steroid derivatives, androstanopyrazolines, androstanoisoxazoles, androgenic anabolic activities.

In our previous work, we found that certain of substituted steroidal and terpenoidal derivatives showed antiandrogenic, anabolic, and antioxidant activities[1-3]. Some new heterocyclic compounds containing steroid moieties have been synthesized and used as 5α-reductase and aromatase inhibitors, anti-inflammatory, anti-alzheimer, Anti-parkinsonism and anti-arthritic and immunosuppressive[4-10] agents. Androgenic hormone is natural or synthetic compound, usually a steroid hormone, which stimulates or controls the development and maintenance of male characteristics in vertebrates by binding to androgen receptors. This includes the activity of the accessory male sex organs and development of male secondary sex characteristics. The androgen receptor (AR), also known as NR3C4 (nuclear receptor subfamily 3, group C, member 4), is a type of nuclear receptor[11] that is activated by binding of either of the androgenic hormones testosterone or dihydrotestosterone[12] in the cytoplasm and then translocating into the nucleus. The androgen receptor is most closely related to the progesterone receptor, and progestins in higher dosages can block the androgen receptor[13,14]. The main function of the androgen receptor is as a DNA-binding transcription factor that regulates gene expression[15], however, the androgen receptor has other functions as well[16]. Androgen regulated genes are critical for the development and maintenance of the male sexual phenotype. The

* To whom all correspondence should be addressed.
E-mail: hussainazza2014@yahoo.com

primary mechanism of action for androgen receptors is direct regulation of gene transcription. The binding of an androgen to the androgen receptor results in a conformational change in the receptor that, in turn, causes dissociation of heat shock proteins, transport from the cytosol into the cell nucleus, and dimerization. The androgen receptor dimer binds to a specific sequence of DNA known as a hormone response element. Androgen receptors interact with other proteins in the nucleus, resulting in up- or down-regulation of specific gene transcription[17]. Up-regulation or activation of transcription results in increased synthesis of messenger RNA, which, in turn, is translated by ribosomes to produce specific proteins. One of the known target genes of androgen receptor activation is insulin-like growth factor I (IGF-1)[18]. Thus, changes in levels of specific proteins in cells are one way that androgen receptors control cell behavior. One function of androgen receptor that is independent of direct binding to its target DNA sequence is facilitated by recruitment via other DNA-binding proteins. One example is serum response factor, a protein that activates several genes that cause muscle growth[19]. Androgen receptor is modified by acetylation, which directly promotes contact independent growth of prostate cancer cells[20]. More recently, androgen receptors have been shown to have a second mode of action. As has been also found for other steroid hormone receptors such as estrogen receptors, androgen receptors can have actions that are independent of their interactions with DNA[21]. In view of these observations and as continuation of our previous works in steroidal chemistry, we have herein screened some of steroidal derivatives fused with pyrazole and isoxazole rings as Androgenic anabolic activities in comparison to Testosterone as positive controls.

EXPERIMENTAL

Chemistry

All the tested compounds were confirmed according to the previously reported procedures[22].

Pharmacological screening

Animals

Biological experiments were made according to the ethical rules and 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. Prepubertal Sprague-Dawley male albino rats 21 day old (45-50 g), were used to investigate the effect of the tested compounds on the development of male sex organs. Adult male albino rats (150 days) weighting (150-200 g) were used in the present work to evaluate the androgenic-anabolic activity of all newly synthesized derivatives.

Androgenic-anabolic activity

Groups of immature male albino rats (each group contains 8 animals), 21 days old, received subcutaneously particular target compounds and testosterone as reference standard at a total dose of 0.7 mg kg^{-1} according to the following design: group 1 received the vehicle (DMSO); group 2 received the testosterone reference standard in vehicle (DMSO) (at a dose of 0.1 mg kg^{-1} daily for 7 days); group 3 was subdivided into 22 subgroups, each received individually one of the tested compounds (at a dose of 0.1 mg kg^{-1} daily for 7 days). Androgenic-anabolic activity of all newly synthesized compounds was measured according to the reported procedure[23,24]. Groups of prepubertal albino rats (n = 8) 21 days old, were kept on a constant diet and tap water. Each animal was given daily a subcutaneous injection of the tested compound as well as testosterone as reference standard at a dose of 0.1 mg kg^{-1} for seven days. On day eight (22-26 hours after the last injection), the animals were killed, dissection of the levator ani muscle, the ventral prostate gland, testis, seminal vesicles, vas deference and epididymis were carried out and were weighed. The ratio of the mass gain of the levator ani-muscle to the mass gain of the ventral prostate gland was calculated where the mass gain of the levator ani muscle indicates

Acute toxicity (LD_{50})

Male rats were used to determine intraperitoneal LD_{50} of the tested compounds. Prior to determination of the LD_{50} value, a range finding screen were conducted using 20 rats each treat with a tested compound at a dose ranging from 3-2000 mg kg^{-1}. Based on the mortality observed within 14 days, the doses used for the LD_{50} determination were 3, 10, 30, 100, 300, 1000, 2000 mg kg^{-1} for compounds administered by

intraperitoneal injection as a 10% solution in dimethyl sulphoxide (DMSO). Control animals received intraperitoneal injections of DMSO. For each concentration and control, ten male rats were injected with the tested compounds twice daily for two weeks to kill any swirved animals. From the mortality data of all tested animals, the intraperitoneal LD_{50} values for each agent were determined according to Austen *et al*.[25].

RESULTS AND DISCUSSION

Chemistry

In continuation of our previous work, a series of some substituted steroidal derivatives and fused with pyrazoline or isooxazole moities 1-11 (Figures 1 and 2) were synthesized and reported before[22]. Herein, we used these compounds for evaluation as androgenic-anabolic agents.

Pharmacological activities

Androgenic-anabolic activity

From Tables 1 and 2, the ratio of the mass gained by the levator ani-muscle to the mass gained by the prostate gland was calculated, where the former indicates the anabolic activity and the latter shows the androgenic effect of the tested compounds. It was revealed that all the tested compounds have significant androgenic as well as anabolic effects. All the tested compounds showed potent anabolic activities where the ratio of mass gained by the levator ani-muscle (anabolic) to the mass gained by the ventral prostate were high (Table 1).

The 1‘-acetyl-1‘H-5‘-substituted phenyl-5α-androstan[17,16-c]pyrazoline-3β-yl-trifluoroacetate derivatives 1a-c only showed potent anabolic activities due to the presence of

Table 1. Androgenic-anabolic activities of the tested compounds 1-11

Comp.No.	Mass of prostate gland (g)[a]	Mass of levator ani-muscle (g)[a]	Ratio[b]
Testosterone	0.760±0.004	0.204±0.005	0.27
Control	0.20±0.005	0.18±0.006	-
1a	0.234±0.005	0.276±0.006	1.18
1b	0.345±0.005	0.400±0.007	1.16
1c	0.345±0.004	0.386±0.007	1.12
2a	0.789±0.004	0.860±0.006	1.09
2b	0.987±0.004	0.197±0.005	0.20
2c	0.789±0.005	0.174±0.006	0.22
3a	0.786±0.006	0.197±0.007	0.25
3b	0.567±0.007	0.150±0.006	0.27
3c	0.449±0.008	0.125±0.005	0.28
4a	0.953±0.009	0.276±0.004	0.29
4b	0.675±0.008	0.203±0.005	0.30
5a	0.768±0.007	0.238±0.006	0.31
5b	0.564±0.006	0.192±0.007	0.34
6a	0.365±0.005	0.128±0.006	0.35
6b	0.365±0.006	0.131±0.005	0.36
7a	0.587±0.007	0.217±0.005	0.37
7b	0.588±0.006	0.229±0.004	0.39
7c	0.589±0.005	0.241±0.005	0.41
8a	0.465±0.004	0.205±0.006	0.44
8b	0.324±0.005	0.152±0.007	0.47
9a	0.356±0.006	0.171±0.007	0.48
9b	0.574±0.006	0.293±0.007	0.51
10a	0.465±0.007	0.293±0.006	0.63
10b	0.475±0.005	0.342±0.007	0.72
11a	0.564±0.006	0.412±0.006	0.73
11b	0.578±0.007	0.428±0.005	0.74

[a] Mean ± SEM (n = 8).

[b] Ratio of the mass gained by the levator ani-muscle to the mass gained by the prostate gland.

acetyl pyrazoline groups that permit hydrogen bonding with the unbound receptor site for anabolic activities.

The 1‘-Phenyl-1‘H-5‘-(4-bromophenyl)-5α-androstan[17,16-c]pyrazoline-3β-yl-trifluoroacetate (2a) showed nearly equal anabolic androgenic activities which are the margine line and one of the derivatives with long-sought property binds equally to NR3C4 and the unbound receptor site for anabolic activities. Both the phenylylpyrazoline 1‘-phenyl-1‘H-5‘-(4-fluorophenyl)-5α-androstan [17,16-c]pyrazol-ine-3β-yl-trifluoroacetate (2b) and 1‘-phenyl-1‘H-5‘-(4-methylphenyl)-5α-androstan [17,16-c]pyrazol-ine-3β-yl-trifluoroacetate (2c) showed dramatically abnormally the highest androgenic activities due to high affinity to NR3C4. The 1‘-methyl-1‘H-5‘-substituted phenyl-5α-androstan[17,16-c] pyrazoline-3β-yl-trifluoroacetate derivatives 3a, 3b and 3c showed high androgenic activities but lower than that of the phenyl ones because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 5‘-(substituted phenyl-5α-androstan[17,16-c]isoxazole-3²-ol derivatives 4a,b showed lower androgenic activities than that of the methylpyrazoline 3a, 3b and 3c because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 3β-acetoxy isoxazole derivatives 5a and 5b showed lower androgenic activities than that of the isoxazole derivatives 4a and 4b because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 3β-trifluoro acetoxy isoxazole derivatives 6a and 6b showed lower androgenic activities than of the

Table 2. Effect of the tested compounds on androgenic organs 1-11

Comp. No.	Mass (g)[a]			
	Epididymis	Testis	Seminal vesicles	Vasdifference
Testosterone	0.360±0.005	0.700±0.002	0.630±0.007	0.190±0.002
1a	0.111±0.003	0.216±0.005	0.194±0.004	0.257±0.005
1b	0.163±0.002	0.318±0.006	0.286±0.003	0.373±0.004
1c	0.163±0.003	0.318±0.005	0.286±0.004	0.360±0.003
2a	0.374±0.003	0.727±0.004	0.654±0.003	0.801±0.004
2b	0.468±0.003	0.909±0.003	0.818±0.004	0.183±0.005
2c	0.374±0.003	0.727±0.004	0.654±0.003	0.162±0.006
3a	0.372±0.002	0.724±0.004	0.652±0.005	0.183±0.005
3b	0.269±0.003	0.522±0.003	0.470±0.004	0.140±0.004
3c	0.213±0.003	0.414±0.003	0.372±0.004	0.116±0.005
4a	0.451±0.004	0.878±0.003	0.790±0.003	0.257±0.006
4b	0.320±0.003	0.622±0.002	0.560±0.004	0.189±0.006
5a	0.364±0.003	0.707±0.007	0.637±0.004	0.222±0.005
5b	0.267±0.002	0.519±0.008	0.468±0.003	0.179±0.004
6a	0.173±0.003	0.336±0.007	0.303±0.003	0.119±0.004
6b	0.173±0.002	0.336±0.007	0.303±0.003	0.122±0.003
7a	0.278±0.002	0.541±0.006	0.487±0.004	0.202±0.004
7b	0.279±0.002	0.542±0.005	0.487±0.004	0.213±0.005
7c	0.279±0.002	0.543±0.004	0.488±0.003	0.224±0.006
8a	0.220±0.003	0.428±0.004	0.385±0.004	0.191±0.006
8b	0.153±0.002	0.298±0.005	0.269±0.005	0.142±0.005
9a	0.169±0.003	0.328±0.004	0.295±0.004	0.159±0.004
9b	0.272±0.002	0.529±0.003	0.476±0.005	0.273±0.005
10	0.220±0.003	0.428±0.003	0.385±0.004	0.273±0.004
10b	0.225±0.002	0.438±0.004	0.394±0.004	0.319±0.004
11a	0.267±0.003	0.519±0.005	0.468±0.004	0.384±0.003
11b	0.274±0.002	0.532±0.004	0.479±0.003	0.399±0.004

Mean _±SE (n = 8).
All results were significantly different from normal control value (p ≤0.05).
All results were significantly different from testosterone value (p ≤0.05).

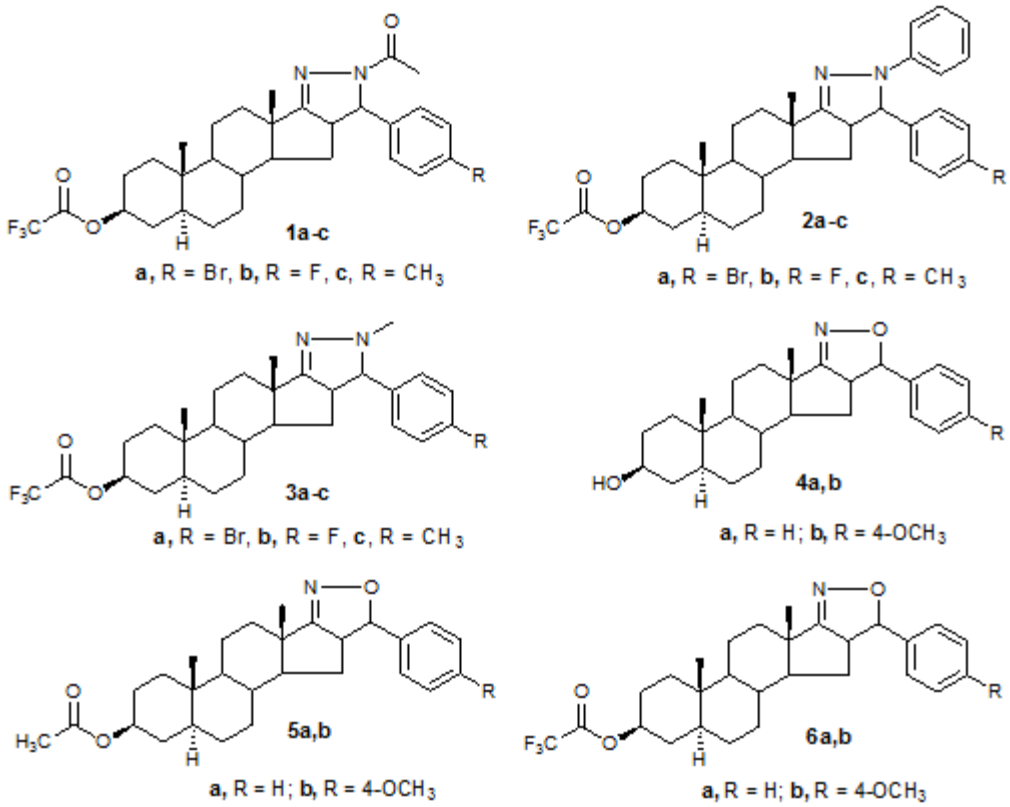

Fig. 1. Chemical structure for compounds **1-6**

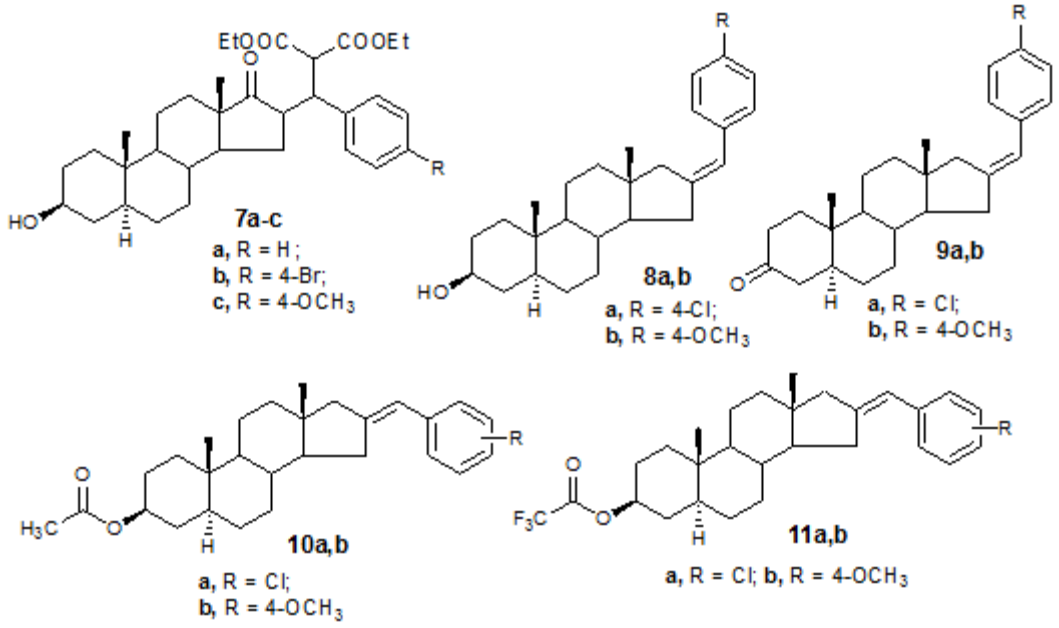

Fig. 2. Chemical structure for compounds **7-11**

3β-acetoxy isoxazole derivatives 5a and 5b.

The 16-[(±-diethyl)malonyl)-substituted phenyl]-3β-hydroxyl-androstan-17-one **7a-c** showed lower androgenic activities than that of the 3β-trifluoro acetoxy isoxazole derivatives **6a,b** because the molecule lipophilic character decreased and affinity to NR3C4 decreased because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 16-[substituted phenyl]-methylene-5α-androstan-3²-ols **8a,b** showed lower androgenic activities than that of the 16-[(α-diethyl)malonyl)-substituted phenyl]-3β-hydroxyl-androstan-17-one 7a-c because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 16-[substituted phenyl]-methylene-5α-androstan-3-one 9a,b showed lower androgenic activities than that of the 16-[substituted phenyl]-methylene-5α-androstan-3β-ol 8a,b, The 16-[substituted phenyl]-methylene-5α-androstan-3β-yl-acetate 10a,b showed lower androgenic activities than that of the 16-[substituted phenyl]-methylene-5α-androstan-3-one 9a,b because the molecule lipophilic character decreased and affinity to NR3C4 decreased. The 16-[substituted phenyl]-methylene-5α-androstan-3β-yl-acetate 11a,b showed lower androgenic activities than that of the 16-[substituted phenyl]-methylene-5α-androstan-3β-yl-acetate 10a,b because the molecule lipophilic character decreased and affinity to NR3C4 decreased.

Table 3. Evaluation of LD_{50} of the tested compounds 1-11

Comp. No	LD_{50} (mg kg^{-1})[a]
Testosterone	2751.00±7.7
Dehydroepiandrosterone	3411.00±5.77
1a	4956.29±8.66
1b	4961.24±5.55
1c	4966.21±9.56
2a	4971.17±5.67
2b	4976.14±8.78
2c	4981.12±5.87
3a	4986.10±8.76
3b	4991.09±5.64
3c	4996.08±8.65
4a	5001.07±5.66
4b	5006.07±5.55
5a	5011.08±5.55
5b	5026.11±8.66
6a	5041.19±5.78
6b	5056.32±8.77
7a	5071.48±8.76
7b	5086.70±5.85
7c	5101.96±7.76
8a	5117.27±5.67
8b	5132.62±5.56
9a	5148.02±8.58
9b	5163.46±5.47
10a	5178.95±8.57
10b	5194.49±9.69
11a	5210.07±9.78
11b	5225.70±9.67

[a] Mean ± SD, n = 24

Acute toxicity (LD_{50})

Initially, acute toxicity of the synthesized compounds was assayed by determining their LD_{50}. Interestingly, most compounds were less toxic than the reference drug, The LD_{50} (rats) was determined by injecting different increasing doses and calculating the dose that killed 50% of the animals (Table 3).

Structure activity relationship (SAR)

N-acetyl of pyrazoline showed potent anabolic activities. Replacing the N-acetyl of pyrazoline with phenyl one nearly equalizes anabolic androgenic activities. 1 *N*-Acetyl of pyrazoline showed potent showed high androgenic activities but lower than that of the phenyl ones. The isoxazole derivatives showed lower androgenic activities than that of the methylpyrazoline. The 3β-acetoxy isoxazole derivatives decrease the androgenic activities. The 3²-trifluoroacetoxy isoxazole derivatives showed lower androgenic activities than of the 3β-acetoxyisoxazole derivatives. 16-[(α-Diethyl)-malonyl) substituted phenyl]-3β-hydroxylandrostane decreases the androgenic activities than that of the isoxazole derivatives.

CONCLUSION

All the tested compounds showed potent anabolic activities where the ratio of mass gained by the levator ani-muscle (anabolic) to the mass gained by the ventral prostate were high (Table 1). NR3C4, careful examination of the structure of these agents in correlation with their activities leads to the following assumptions of SAR. The descending order of androgenic activities were as following (3a-c) > (4a,b) > (5a,b) > (6a,b) > (7a-c) > (8a,b) > (9a,b) >(10a,b) > (11a,b).

ACKNOWLEDGMENTS

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project No. RG-172.

REFERENCES

1. Amr, A. E. and Abdulla, M. M. Synthesis and pharmacological screening of some new pyrimidines and cyclohexenone fused steroidal derivatives. *Ind. J. Heterocycl. Chem*., 2002; **12**: 129-134.
2. Amr, A. E., Abdel-Latif, N. A. and Abdalla, M. M. Synthesis and antiandrogenic activity of some new 3-substituted androstano[17,16-c]-5‘-aryl-pyrazoline and their derivatives. *Bioorg. Med. Chem.* 2006; **14**: 373-384.
3. Amr, A. E., Ali, K. E. and Abdalla, M. M. Cytotoxic, antioxidant activities and structure activity relationship of some newly synthesized terpenoidal oxaliplatin analogs. *Eur. J. Med. Chem.*, 2009; **44**: 901-907.
4. Al-Mohizea, A. M., Al-Omar, M. A., Abdalla, M. M. and Amr, A. E. 5±-Reductase inhibitors, antiviral and anti-tumor activities of some steroidal cyanopyridinone derivatives. *Int. J. Biol. Macromol*., 2012; **50**: 171-179.
5. Abdalla, M. M., Al-Omar, M. A., Bhat, M. A., Amr, A. E. and Al-Mohizea, A. M. Steroidal pyrazolines evaluated as aromatase and quinone reductase-2-inhibitors for chemoprevention of cancer. *Int. J. Biol. Macromol.*, 2012; **50**: 1127-1132.
6. Abdalla, M. M., Al-Omar, M. A., Al-Salahi, R. A., Amr, A. E. and Sabry, N. M. A new investigation for some steroidal derivatives as anti-alzheimer agents. *Int. J. Biol. Macromol*., 2012; **51**: 56-63.
7. Bahashwan, S. A., Al-Harbi, N. O., Fayed, A. A., Amr, A. E., Shadid, K. A., Alalawi, A. M. and Bassati, I. M. S. Synthesis and pharmacological evaluation of novel triazolo[4,3-b]pyrazolo[3,4-c]pyridazine derivatives. *Int. J. Biol. Macromol*., 2012; **51**: 7-17.
8. Khalifa, N. M., Al-Omar, M. A., Amr, A. E. and Haiba, M. E. Antiviral activity of some new polycyclic nucleoside pyrene candidate against HIV-1 and HSV-1 virus. *Int. J. Biol. Macromol.*, 2013; **54**: 51-56.
9. Al-Harbi, N. O., Bahashwan, S. A., Fayed, A. A., Aboonq, M. S. and Amr, A. E. Anti-parkinsonism, hypoglycemic and anti-microbial activities of some new poly ring heterocyclic candidates. *Int. J. Biol. Macromol*., 2013; **57**: 165-173.
10. Alanazi, A. M., Al-Omar, M. A., Abdulla, M. M. and Amr, A. E. Anti-arthritic and immune-suppressive activities of substituted triterpenoidal candidates. *Int. J. Biol. Macromol*., 2013, **58**: 245-252.
11. Wardell, S. E., Burnstein, K.L., Defranco, D., Fuller, P.J., Giguere, V., Hochberg, R.B., McKay, L., Renoir, J. M., Weigel, N.L., Wilson, E.M., McDonnell, D.P. and Cidlowski, J.A. International Union of Pharmacology. LXV. The pharmacology and classification of the nuclear receptor superfamily: glucocorticoid, mineralocorticoid, progesterone, and androgen receptors. *Pharmacol. Rev*., 2006; **58**: 782-797.
12. Roy, A.K., Lavrovsky, Y., Song, C.S., Chen, S., Jung, M.H., Velu, N.K., Bi, B. and Chatterjee, B. Regulation of androgen action. *Vitam Horm*., 1999; **55**: 309-352.
13. Bardin, C.W., Brown, T., Isomaa, V.V. and Jänne, O.A. Progestins can mimic, inhibit and potentiate the actions of androgens. *Pharmacol. Ther.*, 1983; **23**: 443-459.
14. Raudrant, D. and Rabe, T. Progestogens with antiandrogenic properties. *Drugs* 2003; **63**: 463-492.
15. Mooradian, A.D., Morley, J.E. and Korenman, S.G. Biological actions of androgens. *Endocr. Rev.*, 1987; **8**: 1-28.
16. Heinlein, C.A. and Chang, C. The roles of androgen receptors and androgen-binding proteins in nongenomic androgen actions. *Mol. Endocrinol.*, 2002; **16**: 2181- 2187.
17. Heemers, H.V. and Tindall, D.J. Androgen receptor (AR) coregulators: a diversity of functions converging on and regulating the AR transcriptional complex. *Endocr. Rev*., 2007; **28**: 778- 808.
18. Pandini, G., Mineo, R., Frasca, F., Jr Roberts, C.T., Marcelli, M., Vigneri, R. and Belfiore, A. Androgens up-regulate the insulin-like growth factor-I receptor in prostate cancer cells. *Cancer Res.*, 2005; **65**: 1849-1857.
19. Vlahopoulos, S., Zimmer, W.E., Jenster, G., Belaguli, N.S., Balk, S.P., Brinkmann, A.O., Lanz, R. B., Zoumpourlis, V.C. and Schwartz, R.J. Recruitment of the androgen receptor via serum response factor facilitates expression of a myogenic gene. *J. Biol. Chem.*, 2005; **280**: 7786-7792.
20. Fu, M., Wang, C., Reutens, A.T., Wang, J., Angeletti, R.H., Siconolfi-Baez, L., Ogryzko, V., Avantaggiati, M.L. and Pestell, R.G. p300 and p300/cAMP-response element-binding

protein-associated factor acetylate the androgen receptor at sites governing hormone-dependent transactivation. *J. Biol. Chem.*, 2000; **275**: 20853-20860.

21. Fix, C., Jordan, C., Cano, P. and Walker, W. H. Testosterone activates mitogen-activated protein kinase and the cAMP response element binding protein transcription factor in Sertoli cells. *Proc. Natl. Acad. Sci.* USA, 2004; **101**: 10919-10924.
22. Abdulla, M. M., Amr, A. E., Al-Omar, M.A., Hussain, A. A. and Amer, M. S. Synthesis and reactions of some new substituted androstano-pyrazoline and androstanoisoxazole derivatives using their arylmethylene as starting materials. *Life Sci. J.*, 2013; **10**: 599-607.
23. Hershberger, L. G., Shipley, E. G. and Meyer, R. K. Myotrophic activity of 19-nortestosterone and other steroids determined by modified levator ani muscle method. *Proc. Soc. Exp. Biol. Med*., 1953; **83**: 175-180.
24. Kuhnz, W. and Beier, S. Comparative progestational and androgenic activity of norgestimate and levonorgestrel in the rat. *Contraception* 1994; **49**: 275-289.
25. Austen, K. F. and Brocklehurst, W. E. Anaphylaxis in chopped guinea pig lung. I. Effect of peptidase substrates and inhibitors. *J. Exp. Med*., 1961; **113**: 521-539.