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Synthesis and biological activity of new steroidal heterocyclic compounds

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ABSTRACT

Preparation of two new steroidal heterocyclic compounds derived from testosterone and pregnenalone was described. The structure of new products was elucidated by spectroscopic methods. The products were examined for their biological activity against bacteria and fungi and their results were compared with standard antibiotics.

Keywords: Steroidal heterocyclic, biological activity, standard antibiotics.

INTRODUCTION

It is known from the literature that both steroids and heterocyclic compounds have medicinal and biological activity. They are also used as drugs, antibacterial and antiviral agents. The presence of heterocycles in all kinds of organic compounds of interest in biology, pharmacology, optics, electronics and material sciences and so on is very well known[2]. Among them sulfur and nitrogen-containing heterocyclic compounds have maintained the interest of researchers through decades of historical development of organic synthesis. The ground of this interest was their biological activity and unique structure that led to several applications in different areas of pharmaceutical and agrochemical research, ormore recently in material sciences[3].

On the other hand steroids have great effects as anti-inflammatory, antibacterial and antiviral drugs[4].

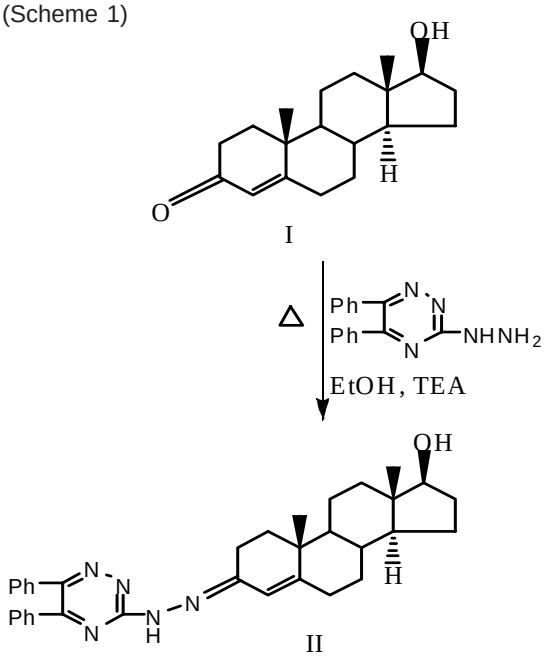
Nonsteroidal anti-inflammatory drugs (NSIDs) are an inhomogeneous family of pharmacologically active compounds which are widely used in treatment of acute and chronic inflammation, pain and fever, However, long-term clinical employment of NSIDs is associated with significant side effects. Therefore, the discovery of new safer drugs is a challenging goal for research[5].

Attention has been devoted in the literature to synthesis of several steroidal heterocyclic derivatives that exhibit marked medicinal activity[6,7,8]. In recent years, heterocyclic pregnane derivatives have been found to possess a variety of interesting pharmacological and biological activities[9]. Recently, reactivity of some steroidal hormones towards lawessons's reagent as sulfur moiety produced acyclic and cyclic sulfur compounds containing a steroidal unit which have an antibacterial and antifungal activities[10].

These observations led us to attempt to make new heterocyclic systems bearing steroidal moieties.

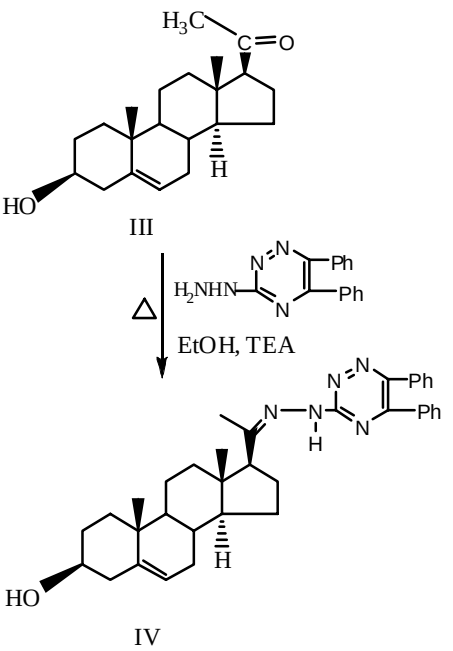
RESULTS AND DISCUSSION

17a-Hydroxyandrost-4-en-3-one (I) was refluxed with hydrazinotriazine to afford 17a-hydroxyandrost-4-en-3-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (II). The IR spectrum showed a signal at 3301 cm^{-1} (NH) and the absence of the carbonyl group. The ^1H NMR spectrum of the product contained new signals at 7.4-7.8 (10H, m, phenyl protons), 10.75, (1H, brs, NH). The ^{13}C NMR revealed a signal at d 157.65 which was confirmed the presence of (C=N) group. These results confirmed that the product was 17a-hydroxyandrost-4-en-3-(5,6-diphenyl-1,2,4-triazin-3-hydrazone(II). (Scheme 1)



Scheme1

3b-Hydroxypregn-5-en-20-one (III) was refluxed with hydrazinotriazine to give 3b-hydroxypregn-5-en-20-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (IV). The IR spectrum showed a signal at 3311 cm^{-1} (NH) and the absence of the signal of carbonyl group. The 1H NMR spectrum of the product contained new signals at 7.4-7.8 (10H, m, phenyl protons) and 10.61,(1H, brs, NH). The ^{13}C NMR revealed signal at d 165.6 which confirmed the presence of (C=N) group. These results confirmed that the product was 3b-hydroxypregn-5-en-20-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (XII). (Scheme 2)



Scheme 2

EXPERIMENTAL

Melting points were determined by Thomas-Hoover capillary melting point apparatus and are uncorrected. IR spectra were recorded using KBr disks on a Nicolet Magna 520 Fourier transform spectrometer. ^1H NMR spectra were determined in deuteriochloroform with TMS as an internal standard reference at 600 MHz on a Brucker Avance DPX 600 spectrometer while ^{13}C NMR spectra were recorded in deuteriochloroform at 150 MHz with a Brucker Avance DPX 400 spectrometer. Mass spectra were recorded on a VG Autospec. Micro-analysis were carried out using Perkin Elmar.

1. 17b-Hydroxyandrost-4-en-3-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (II)

A mixture of 17b-hydroxyandrost-4-en-3-one (I) (0.01 mol), hydrazinotriazine (0.01mol) in absolute ethanol (50ml) with few drops of triethyl amine was refluxed for 4 hrs, cooled and poured onto ice. The solid product was filtered off to give 17β-hydroxyandrost-4-en-3-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (II) (1.2 g, 65%) which was

recrystallized from ethyl acetate as yellow plates.
m.p. 203-205°C
HRMS found 533.3143 $C_{34}H_{39}N_5O$, Calculated 533.3155
FTIR ν_{max}/cm^{-1} 3382 (OH), 3401 (NH) and 1616 (C=N)
^1H NMR ($CDCl_3$, 600 MHz) δ 0.85 (3H, s, 18-H), 1.85 (3H, s, 19-H), 3.69 (1H, t, J= 8.4 Hz, 17a-H), 5.85 (1H, s, 4-H), 7.4-7.8 (10H, m, phenyl protons), 10.75,(1H, brs, NH).

2. 3b-Hydroxypregn-5-en-20-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (IV)

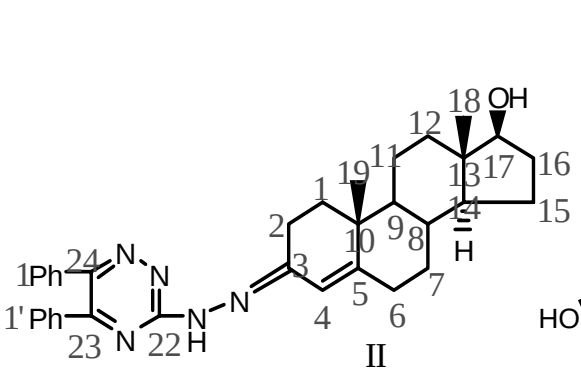
A mixture of 3b-hydroxypregn-5-en-20-one (III) (0.01 mol), hydrazinotriazine (0.01mol) in absolute ethanol (50ml) with few drops of triethyl amine was refluxed for 4 hrs, cooled and poured onto ice. The solid product was filtered off to give 3β-hydroxypregn-5-en-20-(5,6-diphenyl-1,2,4-triazin-3-hydrazone (IV) (1.3 g, 73%) which was recrystallized from ethyl acetate as yellow cubes.
m.p. 189-192°C
HRMS found 561. 3460 $C_{36}H_{43}N_5O$, Calculated 561.3468
FTIR ν_{max}/cm^{-1} 3402 (OH), 3311 (NH) and 1621 (C=N)
^1H NMR ($CDCl_3$, 400 MHz) δ 1.06 (3H, s, 18-H), 1.10 (3H, s, 19-H), 3.64 (1H, tt, J= 8.4, 11.0 Hz, 3b-H), 5.77 (1H, s, 6-H) , 7.4-7.8 (10H, m, phenyl protons), 10.61 (1H, brs, NH).

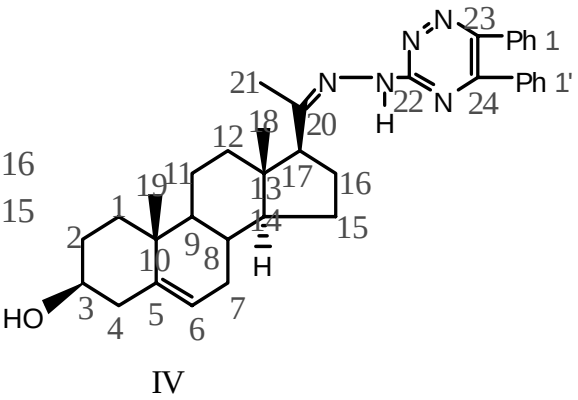
Biological effects

Upon the irradiation of visible light with appropriate wavelength, the photosensitizer can derive molecular oxygen into excited triplet state, transferring energy into ground state molecular

^{13}C NMR data determined in $CDCl_3$ at 150 MHz of new compounds II and IV

Carbon no. / Compound no.	II	IV
C-1	39.35	38.03
C-2	20.21	30.98
C-3	157.65	72.05
C-4	113.35	42.35
C-5	163.35	143.05
C-6	34.11	123.12
C-7	31.91	31.91
C-8	35.93	32.25
C-9	54.59	53.32
C-10	41.09	38.19
C-11	21.39	21.05
C-12	37.96	39.26
C-13	44.01	42.41
C-14	51.82	56.82
C-15	24.21	27.21
C-16	31.03	33.93
C-17	81.38	31.92
C-18	12.23	13.72
C-19	18.71	19.41
C-20	---------	165.36
C-21	---------	26.91
C-22	167.65	165.43
C-23	149.38	152.38
C-24	155.96	158.32
1' Ph-6 carbons	126.51, 128.67, 129.74,134.65	127.01, 129.12,130.14, 135.05
1Ph-6 carbons	127.38, 128.45, 129.55,137.23	128.03, 129.52 130.15,139.11





oxygen to produce singlet molecular oxygen. Activated singlet oxygen, or reactive oxygen species (ROS) in general, plays an important role in cytotoxic effects on affected tissues. A variety of attractive pharmacological effects were attributed to sulfur mono- and polycyclic heterocyclic systems. Thus we aimed to investigate the biological effects of the new prepared compounds towards some microorganisms and use as photochemical probe agents, as well as strong bacterial and fugicidal agents.

The bacterial isolates [*E. coli, P. aeuroginosa* and *K.pneumonia*; *Gram-negative* bacteria], [*B. subtilis, S. aureus*, *Gram-positive* bacteria] and fungi [*A. fumigatus; C. albicans*] were recovered on Nutrient and Mac Lonky agar, and on Sabouraud Dextrose agar (oxoid) (BioMeriieux). The fungi were obtained from Assuit University collection center, Egypt.

A- Antimicrobial assays

Some new synthesized compounds were tested in-vitro using the agar diffusion disk method[3,4]. The antimicrobial potentialities of the tested compounds were estimated by placing the presterilized filter paper disks (6 mm in diameter) impregnated with 50 mg/disk. DMF which showed no inhibition zone, was used as solvent for dissolving the tested compounds. Inhibition zone (IZ) of the tested compounds (mm) were measured after 24-28 h incubation at 37°C for bacteria and after 5 days incubation period at 28°C for fungi (Table 1).

The minimal inhibitory concentration (MIC) (Table 2,3) method of the biologically active compounds was applied using different concentrations per disks against bacteria and fungi using Naliolixic acid and Nystalin as reference drugs, The sensitivity of microorganisms to the tested compounds is defined in the following manners:

Highly active : inhibition zone = 12 mm
Moderately active: inhibition zone 9 - 12 mm
Slightly active: inhibition zone 6 - 9 mm
Not sensitive: inhibition zone 6 mm

Table 1: The preliminary screening antimicrobial activity of some synthetic compounds

Compound No	*Gram+ve* Bacteria		Gram-ve Bacteria			Fungi	
	B	S	E	P	K	C	A
XI	16	20	14	22	24	12	6
XII	15	20	14	20	24	11	6
Na.	32	30	30	12	22	6	6
Ny.	6	6	6	6	10	10	32

+ve Bacteria : B : *Bacillus subtilis*; S: *Staphylococcus aureus*
-ve Bacteria : E : *Escherichia coli*; P: *Pesudomonas aeuruginosa*. K: *Klebsiella Pneumonia.*
Fungi: C: *Candida albicans* (Aucc 1720), A : *Aspergillus fumigatus* (Aucc 1924).
Na: Nalidixic acid, 30 mg/disk, Bioanalize, Egypt.
Ny: Nystatin: Manufactured by Pasteur Lab. Egypt, NS 100 Units.

Table 2: MIC of the biological active compounds towards Gram -ve bacteria

Compound No.	Inhibition zone (nm)														
	E. coli					*P. aeuruginosa*					*K. pneumonia*				
	50	40	30	20	10	50	40	30	20	10	50	40	30	20	10
XI	14	10	10	8	6	22	18	14	8	6	24	18	14	8	6
XII	14	14	11	8	6	20	13	11	8	6	24	18	12	8	6

Table 3: MIC of the biological active compounds towards Gram +ve bacteria

Compound No.	Inhibition zone (nm)									
	B. subtilis					*S. aureus*				
	50	**40**	**30**	**20**	**10**	**50**	**40**	**30**	**20**	**10**
XI	16	14	10	8	6	20	14	6	6	6
XII	15	13	10	8	6	20	15	8	6	6

*Concentration in mg/disk. **MIC: Minimal Inhibitory Concentration.

CONCLUSION

From above results (Table 1-3) we can concluded that:

1- The tested compounds were very active towards the tested microorganisms.
2- The tested compounds were more effective than standard antibiotics at MIC evaluations.
3- After using uv-visible light, the tested compounds showed a high effect especially towards *Gram-ve* bacteria (*Pseudomonas aeuruginosa*) and also fungi (*Candida albicans* and *Asperigllus fumigatus*).
4- Only compounds a3 and a4 showed a very highly effect than other compounds towards all the tested compounds.
5- All the tested compounds showed a high effect whene compared with the antibiotic Nystatin.

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