JOURNAL OF PHARMACEUTICAL SCIENCE AND BIOSCIENTIFIC RESEARCH (JPSBR)

(An International Peer Reviewed Pharmaceutical Journal that Encourages Innovation and Creativities)

Synthesis and Antibacterial Evaluation of Novel Heterocyclic Compound Containing a Testosterone Moiety

Mrunalini D Kulkarni*[a] Vinay A Joshi[b]

(a) Associate Professor,Chemistry Department, Maharshi Dayanand College of Arts Science and commerce, Parel Mumbai-400012, India
(b) Research Scholar, J.J.T University, Jhunjhunu, Rajasthan

ABSTRACT:

Aiming for the synthesis of new heterocyclic compound containing a testosterone moiety suitable for use as antibacterial agent. From Ethisterone, a testosterone, Danazol was synthesised. Danazole is a first drug to especially treat endometriossis in the early 1970. There were several publication available so far on Danazole for its synthesis, activity, derivatives of Danazole and its physical and chemical properties inorder to further study. Synthesized Danazol then subjected to Glaser Hay coupling reaction to yield a Dimer compound. The newly synthesized compound was tested for antibacterial activity.

KEY WORDS: Glaser Hay coupling, Danazol, endometriossis, tetramethyl-ethylenediamine, antibacterial activity.

Article history:

Received 18 April 2016
Accepted 29 May 2016
Available online 01 July 2016

Citation:
Kulkarni M. D., Joshi V. A. Synthesis and Antibacterial Evaluation of Novel Heterocyclic Compound Containing a Testosterone Moiety. **J Pharm Sci Bioscientific Res**. 2016 6(4):573-576

For Correspondence:
Mrunalini D Kulkarni

Associate Professor,Chemistry Department, Maharshi Dayanand College of Arts Science and commerce, Parel Mumbai-400012, India.

(www.jpsbr.org)

INTRODUCTION:

The Glaser-Hay reaction involves the coupling of terminal alkynes to afford polyynes in good to excellent yields [1–4]. These polyynes are found in various applications ranging from biologically active natural products to optical materials [5–8]. The Glaser-Hay reaction results in a mixture of homodimer and heterodimer products .

Danazol [9], 17α-ethynyl-17β-hydroxy-4-androsteno [2,3-d] isoxazole. It is a derivative of the synthetic steroid Ethisterone [10], a modified testosterone, also known as 17α- ethynyl testosterone. It is used for the treatment of endometriosis. In this research study, it was aimed to synthesize a Dimer of Danazol testosterone using Glaser hay coupling reaction and to study the antibacterial activity of this Dimer.

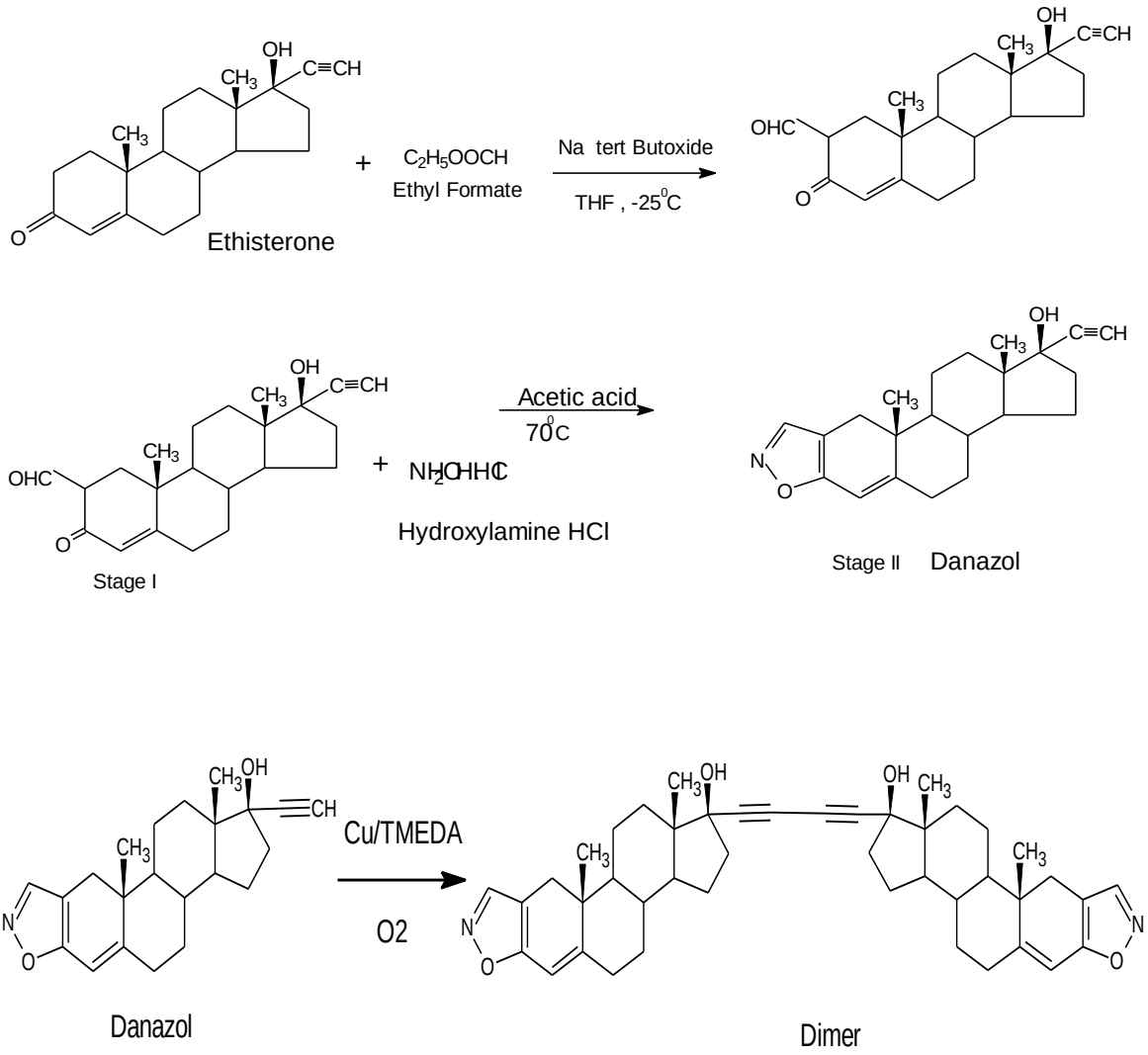
Synthesis of the precursor Danazol and Dimer was achieved by following steps,

1. Formylation of Ethisterone in the presence of ethyl formate and base sodium tertiary butoxide. THF was used as solvent.
2. The formyl Ethisterone then cyclised using hydroxyl amine hydrochloride and heating in acetic acid.
3. The precursor Danazol was then subjected to Glaser Hay coupling using copper as catalyst and tetramethyl ethylenediamine base. (Scheme 1)

The spectral data revealed that this compound exists in Dimer. The MS indicated the molecular ion peak at 674, which is in accordance with the molecular formula. The NMR spectrum is in accordance with the structure. In Danazol IR spectrum -C≡C-H stretching is observed at 3261 cm^{-1} which is

absent in Dimer IR spectrum. This indicates Glaser Hay coupling at acetylene group. Further in Danazol IR spectrum -C≡C stretching observed at 2099 cm-1 which is absent in Dimer IR spectrum due to symmetrical coupling.

SCHEME 1



EXPERIMENTAL SECTION

All the chemicals were used of laboratory grade and were used without further purification. IR spectra were obtained on Agilent FT-IR spectrometer. The proton NMR spectra were recorded with a BRUKER spectrometer with Me_4Si as an internal standard. Elemental analyses were performed by Mettler CHN analyzer. Molecular weight determination was done using Waters Mass spectrometer. Melting points are uncorrected.

Synthesis of Danazol:

In a dry flask added 50ml of THF, cooled to 10^0C and to this was added 3.5 gms of Sodium tert. Butoxide. It was stirred for 30 minutes. Then to this solution was added Ethyl formate solution (3gms in 10ml THF). Further, added 5gms of Ethisterone in lots for 20 minutes. After 1 hour, the reaction was monitored by TLC (Mobile phase:- Hexane: Ethyl Acetate / 80:20).Then 30ml of water and 3 gms of KOH are added to the reaction mixture. It was then filtered. The filtrate was acidified by dilute HCl. The solid obtained was filtered and washed with water. The solid was then dried for 3 hours at 80^0 C.

In a dry flask taken this dried solid and added 50ml of Acetic acid, the mass was heated at 70^0 C. It was stirred for 30 minutes. Then to this solution was added about 2 gms of sodium acetate and stirred again for 15 minutes. Further, added 3gms of hydroxyl amine hydrochloride in

lots for the duration of 20 minutes. After 1 hour of stirring, the reaction was monitored by TLC (Mobile phase :- Hexane: Ethanol / 90:10). Then the reaction mass was cooled and quenched on iced water. It was then filtered. The solid was washed with cold water.

Off white solid , 3.8gms, yield 76%, m.p >224^{0}C, IR (KBr) (ῡmax ,cm-1): 3515 (OH stretching), 3261 (-C≡C-H stretching), 2099 (-C≡C stretching).

1H NMR ($CDCl_3$) δ: 8.03 (1H, -CH=N), 6.14 (1H, -CH=C), 2.70 (1H, -CH), 2.53(1H, -CH≡C) 2.51 (1H, -CH), 2.41(1H, -CH), 2.35 (1H, -CH), 2.28 (1H, -CH), 1.95 (1H, -CH), 1.76 (1H, -CH), 1.74 (1H, -CH), 1.72 (1H, -CH), 1.65 (1H, -CH), 1.60 (1H, -CH), 1.51 (1H, -CH), 1.49 (1H, -CH) , 1.46 (1H, -CH), 1.35 (1H, -CH) , 1.17 (1H, -CH), 1.03 (1H, -CH), 1.00 (3H, $-CH_3$) , 0.90 (3H, $-CH_3$) , MS (m/z) 338.6: Anal. Calcd for $C_{22}H_{27}NO_2$: C, 78.30%; H, 8.06%; N, 4.15%; O, 9.48%: Found; C, 77.95%; H, 8.23%; N, 4.45%.

Synthesis of Danazol Dimer:

In a flask added 50ml THF and 3gms of copper chloride and 4ml of tetramethyl-ethylenediamine. It was stirred for 20minutes and then to this was added Danazol in lots 5gms of in 20 minutes. The reaction mixture was bubbled with air while addition of Danazol and then bubbling was continued for one hour. After 1 hour reaction was monitored by TLC (Mobile phase :- Hexane: Isopropyl alcohol / 90:10).The reaction mixture is neutralized by dilute HCl. The solid obtained was filtered and washed with water.

Yellow solid ,1.15 g, yield 87%, m.p >275^{0}C, IR (KBr) (ῡmax ,cm-1): 3317 (OH stretching), -C≡C-H stretching absent,, -C≡C- stretching absent due to symmetrical substitution.

1H NMR ($CDCl_3$) δ: 8.06 (2H, -CH=N), 6.11 (2H, -CH=C), 2.68 (2H, -CH), 2.53 (2H, -CH), 2.40(2H, -CH), 2.37 (2H, -CH), 2.26 (2H, -CH), 1.93 (2H, -CH), 1.76 (2H, -CH), 1.72 (2H, -CH), 1.70 (2H, -CH), 1.63 (2H, -CH), 1.58 (2H, -CH), 1.52 (2H, -CH), 1.47 (2H, -CH) , 1.44 (2H, -CH), 1.33 (2H, -CH) , 1.14 (2H, -CH), 1.04 (2H, -CH), 1.03 (6H, $-CH_3$) , 0.93 (6H, $-CH_3$). MS (m/z) 674.3: Anal. Calcd for $C_{44}H_{52}N_2O_4$: C, 78.54%; H, 7.79%; N, 4.16%; O, 9.51%: Found; C, 77.86%; H, 8.19%; N, 4.31%.

Antibacterial Activity Evaluation

Agar Diffusion Method:

The obtained new compound was screened *in vitro* for its antibacterial activities against Gram positive bacteria [*Staphylococcus aureus* and *Bacillus cereus*] and Gram negative bacteria [*Serratia marcesens* and *Proteus mirabilis*], using the agar diffusion technique. The results of the antibacterial activity tests are as below.

Gram positive

Staphylococcus aureus +

Bacillus cereus ++

Gram negative

Serratiamarcesens ++

Proteus mirabilis ++

The width of the zone of inhibition indicates the potency of antibacterial activity :

(-) no antibacterial activity; (+) mild activity with the diameter of the zones equal to 0.5–0.8 cm, (++) moderate activity with the diameter of the zones equal to 1.1–1.2 cm; (+++) marked high activity with the diameter of the zones equal to 1.8–2.0 cm.

CONCLUSION

A novel heterocyclic compound having testosterone moiety has been synthesised. The reaction conditions are very simple and the yields are good for all stages in the synthesis. The newly synthesized compound was tested for antibacterial activity.

ACKNOWLEDGEMENTS

We thank Dy Mach Pharma for providing ethisterone and the gift sample of Danazol and also UGC for the financial aid given for this research work.

REFERENCES

1. Vilhelmsen, M.; Jensen, J.; Tortzen, C.; Nielsen, M. The Glaser-Hay Reaction: Optimization and Scope Based on C-13 NMR Kinetics Experiments. Eur. J. Org. Chem. 2013, 701–711.
2. Glaser, C. Beitrage zur Kenntiniss des Acetyenylbenzols. Ber. Dtsch. Chem. Ges. 1896, 2, 422–424.
3. Hay, A.S. Polymerization by oxidative coupling. II. Oxidation of 2, 6-disubstituted phenols. J. Poly. Sci. 1962, 58, 581.

4. Hay, A.S. Oxidative Coupling of Acetylenes. J. Org. Chem. 1962, 27, 3320.
5. Wong, W. Recent advances in luminescent transition metal polyyne polymers. J. Inorg. Organomet. Polym. Mater. 2005, 15, 197–219.
6. Shi Shun, A.L.K.; Tykwinski, R.R. Synthesis of naturally occurring polyynes. Angew. Chem. Int. Ed. 2006, 45, 1034–1057.
7. Slepkov, A.D.; Hegmann, F.A.; Eisler, S.; Elliott, E.; Tykwinski, R.R. The surprising nonlinear optical properties of conjugated polyyne oligomers. J. Chem. Phys. 2004, 120, 6807–6810.
8. Pan, Y.; Lowary, T.; Tykwinski, R.R. Naturally occurring and synthetic polyyne glycosides. Can. J. Chem. Rev. 2009, 87,1565–1582.
9. Merck Index, Published by Merck Research Laboratories, NJ USA, Fourteenth edition,(2006): 474
10. Merck Index, Published by Merck Research Laboratories, NJ USA, Fourteenth edition,(2006): 642