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SHORT COMMUNICATION

SYNTHESIS OF A NOVEL STEROIDAL HETEROCYCLIC AS EFFECTIVE ANTIMICROBIAL AGENT

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The introduction of an isoxazole ring system in a steroidal moiety may leads to the formation of drugs of medicinal importance. In view of therapeutic importance of these steroidal heterocycles, we report the synthesis of cholest-4-eno[2, 3-*d*]isoxazole from cholesterol. The synthesized compound were screened for their antifungal activity by Serial plate dilution method, using Fluconazole as standard drug against dimorphic fungal strain *C. albicans* and other fungal strain *A. niger* in the concentrations of 25, 50 and 100 μg/ml in DMF (solvent). Synthesized compounds showed good antibacterial activity.

Key words: Oppenauer oxidation, Antifungal activity, Serial plate dilution, Annulation.

INTRODUCTION

The steroids form a group of structurally related compounds which are widely distributed in animal and plants. Substituents above the ring system are termed β (heavy line) and one below is termed α (dotted line). The structures of the steroids are based on the 1, 2-cyclo pentane phenenthrene skeleton.

In the majority of steroids, the junction between ring C/D and B/C are Trans (H atom at 8 and 9) and H atom at 14 and CH_3 at 13 are one above the plane and one below the plane of the ring system. The recent pharmaceutical interest in synthesis of heterocyclics (Rodriguez *et al* 1998; Xue *et al* 1998; Park and Kurth, 1999; Laitonjam *et al* 2002; Waldo and Larock, 2005; Xin *et al* 2005; Shang *et al* 2006; Robins *et al* 2007; Kumar, 2011; Mehta and Pathak, 2011; Dahiya and Mourya, 2013) has initiated us to synthesize steroids fused in the 2,3 and 16,17-position to the isoxazole ring system.

METHODS

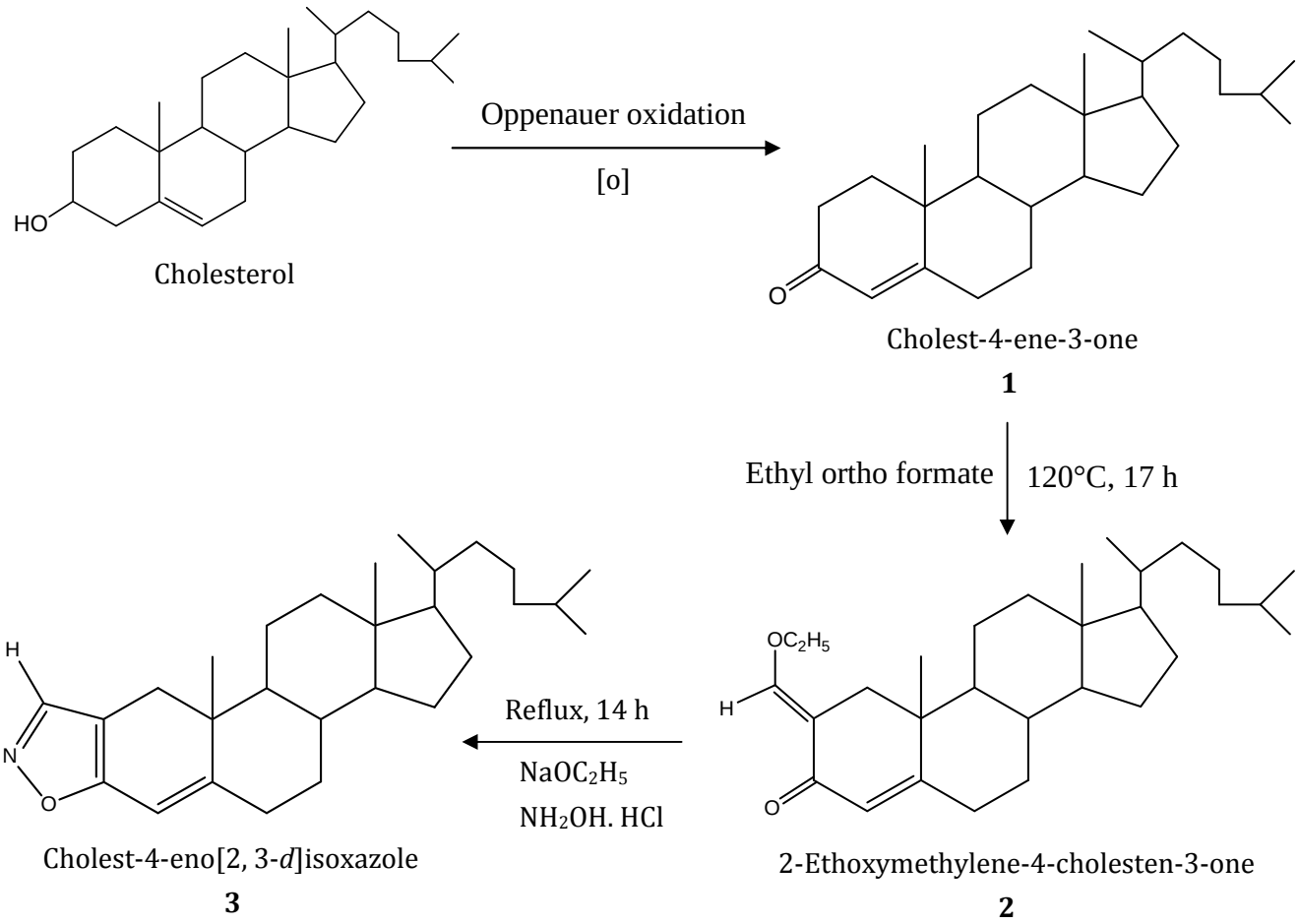
Synthesis of cholest-4-ene-3-one from cholesterol (1)

A mixture of 10 g (0.026 mol) of pure cholesterol, 50 ml cyclohexanone and 200 ml of dry toluene was added in a dry two necked round bottom flask, then the mixture was distilled for 10 min to remove water as azeotrope.

A mixture of 2.8 g aluminium isopropoxide in 40 ml dry toluene was added drop wise to above mixture for 30 min. The combined mixture was cooled and water was added to it, then, the mixture was filtered.

The steam distillation was carried out to remove excess of solvent. Mixture was cooled and extracted with chloroform (3 × 100 ml). The solvent was removed under reduced pressure to yield viscous oily product which was recrystallized with methanol to yield desired compound (**Scheme 1**).

1: Yield 8.1 g, 81.2%; m.p. 80-81°C; R_f 0.36 (Petroleum ether : Ethyl acetate, 9:1, *v/v*); UV λ_{max} (Methanol): 242 nm; IR (KBr) *v*: 2938.7 (C-H str), 1751.7 (-C=O str), 1657.7 (-C=C- str), 868.5 (-C-C- str) cm^{-1}; ^1H NMR ($CDCl_3$): δ 0.71 (s, 3H), 0.82 (s, 3H), 2.54 (t, 2H), 5.72 (s, 1H) ppm; ^{13}C-NMR (DMSO-d_6): δ 196.6, 170.4, 123.6, 58.4, 57.5, 52.3, 45.4, 41.9, 39.7, 39.2, 38.9, 37.8, 36.8, 36.0, 35.5, 34.5, 33.9, 31.2, 29.2, 27.4, 26.6, 24.1(2C), 23.2, 21.8, 19.7, 17.2; Calcd. for



Cholesterol

Cholest-4-ene-3-one

1

2-Ethoxymethylene-4-cholesten-3-one

2

Cholest-4-eno[2, 3-*d*]isoxazole

3

Scheme 1. Synthetic pathway for Cholest-4-eno[2, 3-*d*]isoxazole

$C_{27}H_{44}O$: C, 84.31; H, 11.53; Found: C, 84.33; H, 11.55%.

Synthesis of 2-ethoxymethylene-4-cholesten-3-one (2)

A mixture of 9.6 g (0.02 mol) cholest-4-ene-3-one **1** and 100 ml (0.75 mol) ethyl orthoformate was refluxed at 120°C for 17 h with continuous stirring. On cooling, the crude product obtained was filtered, washed with *n*-hexane and crystallized from benzene-petroleum ether to yield desired compound.

2: Yield: 8.65 g, 81.2%; m.p. 96-98°C; R_f 0.71 (Benzene : Ethyl acetate, 9:1, *v/v*); UV λ_{max} (Methanol): 242 nm; IR (KBr) *v*: 3045.4 (C=C-H str), 2865.4 (C-H str), 1747.7 (C=O str), 1263.5 (C-O str) cm^{-1}; ^1H-NMR ($CDCl_3$): δ 0.69 (s, 3H), 0.82 (s, 3H), 1.25 (t, 3H) 4.32 (q, 2H), 5.81 (s, 1H) 8.26 (s, 1H) ppm; ^{13}C-NMR (DMSO-d_6): δ 189.8, 168.5, 156.3, 128.5, 120.1, 69.5, 59.8, 59.1, 54.6, 46.5, 41.1, 40.9, 40.2, 39.4, 37.6, 37.2, 36.9, 35.3, 32.7, 30.7, 29.6, 26.3, 25.9, 23.9 (2C), 23.2, 20.8, 18.8, 16.7, 15.8; Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98; Found : C, 81.77; H, 10.97%.

Synthesis of cholest-4-eno[2,3-*d*]isoxazole (3)

A suspension of 0.41 g (0.006mol) sodium ethoxide in 30 ml dry ethanol was stirred with 0.2 g (0.003 mol) hydroxylamine hydrochloride. To it, a solution of 1.32 g (0.003 mol) 2-ethoxymethylene-4-cholesten-3-one **2** in 15 ml dry ethanol was added and the reaction mixture was then refluxed for 14 h.

The solvent was removed under reduced pressure and the residue was diluted with ice cold 30 ml water, extracted with chloroform (3 × 50 ml), dried and evaporated to yield desired compound.

3: Yield: 0.65 g, 41%; m.p. 96-98°C; R_f 0.56 (Benzene : Ethyl acetate, 9:1, *v/v*); UV λ_{max} (Methanol): 276 nm; IR (KBr) *v*: 2997.3 (=C-H str), 2873.4 (C-H str), 1646.4 (C=C str), 1461.3 (N-O str), 1225.0 (C-O str), 946.9 (C-C str) cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 0.72 (s, 3H), 0.88 (s, 3H) 2.22-2.36 (m, 6H), 5.43 (s, 1H) 7.41 (s, 1H, isoxazole) ppm; ^{13}C-NMR (DMSO-d_6): 171.2, 157.6, 150.28, 116.7, 113.2, 59.9, 58.7, 54.5, 45.3, 42.8, 41.3, 40.9, 38.7, 38.2, 37.7, 36.9, 35.3, 31.7, 29.8, 28.4, 26.8, 26.0, 24.1 (2C), 22.3, 20.8, 18.8, 14.3; Calcd. for $C_{28}H_{43}NO$: C, 82.09; H, 10.58; N, 3.42; Found : C, 82.10; H, 10.59; N, 3.44.

BIOLOGICAL EVALUATION

Antifungal activity

Antifungal activity study of isoxazole was performed as per the procedure reported by Gupta *et al* 1999. In the present study, serial plate dilution method was used for the evaluation of antifungal activity against fungal diamorphic fungal strain *C. albicans* and other strain *A. niger*. A spore suspension in normal saline was prepared from culture of the test fungi on Mac-Conkey agar media. After transferring growth medium, petri plates were inoculated with spore suspension. After drying, wells were made using an agar punch and test samples, reference drug and negative control (DMF) were placed in labeled wells in each petri plate. Test samples were tested at 25, 50 and 100 μg/ml concentration in DMF. Fluconazole in concentration of 50 μg/ml was used as a standard drug for antifungal activity. The petri plates inoculated with fungal cultures were incubated at 25°C for 48 h. Antifungal activity was determined by measuring the diameter of the inhibition zone for triplicate sets. The diameters obtained for the test sample were compared with that produced by the standard drug fluconazole. The results of antifungal studies are presented in **Table 1** and **Figure 1**.

Table 1. Antifungal activity of newly synthesized compounds

Compound	Diameter of zone of inhibition in mm [mean ± S.D.]*					
	C. albicans			*A. niger*		
	25 μg ml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}	25 μg ml^{-1}	50 μg ml^{-1}	100 μg ml^{-1}
1	5.5±0.132	6.8±0.37	7.6±0.72	7.0±0.87	8.53±0.35	10.8±0.61
3	5.8±1.24	6.9±1.15	9.7±0.47	4.8±0.85	7.4±0.24	12.13±2.51
Fluconazole		13.8±1.62			13.9±1.75	

*S.D. = Standard Deviation; Negative control disc used for solvent had no zone of inhibition.

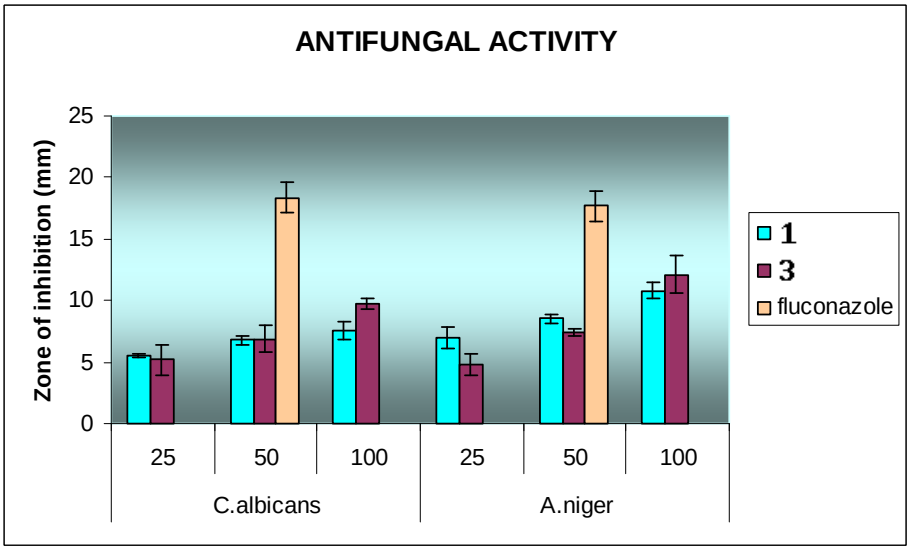


Fig. 1. Antifungal activity of synthesized compounds and fluconazole at the dose 100 μg/ml against *Candida albicans* and *Aspergillus niger*

RESULTS AND DISCUSSION

Cholest-4-ene-3-one **1** (C-H str at 2938.7, C=O str at 1751.7, C=C str at 1657.7 cm^{-1} and singlet of C=C-H at 5.72 ppm) was obtained by oppenauer oxidation of cholesterol with cyclohexanone in presence of aluminium

isopropoxide. 2-Ethoxymethylene-4-cholesten-3-one **2** (=C-H str at 3045.4, C=O str at 1747.7, C-O str at 1263.5, quintet of two hydrogens of OCH_2CH_3 and singlet of one hydrogen of HC=C at 8.26 ppm) was obtained by refluxing of cholest-4-ene-3-one with ethyl orthoformate. Cholest-4-eno[2, 3-*d*]isoxazole (=C-H str at 2997.3, C=C str at 1646.4, N-O str, C-O str at 1225.0 cm^{-1}, singlet of one hydrogen of C=C at 5.43 and singlet of one aromatic hydrogen of isoxazole at 7.41 ppm) was obtained by reaction of 2-ethoxy-methylene-4-cholesten-3-one with hydroxyl-amine hydrochloride in dry pyridine. The synthesis work is fruitless without performing biological activities, so the newly synthesized compounds were screened for antifungal activity against fungi *C. albicans* and *A. niger*.

CONCLUSION

Annulations of heterocyclic ring in steroidal molecules produces pharmacological activities. Out of these compounds, steroidal isoxazoles are with promising antimicrobial activities. Synthesis of cholest-4-eno[2, 3-*d*]isoxazole **3** was accomplished with good yield.

IR and $^1H/^{13}C$ NMR spectral data confirmed the identity of the newly synthesized heterocyclic steroid. The steroidal isoxazole showed promising antifungal activity against fungi *C. albicans* and *A. niger*.

REFERENCES

Dahiya R, Mourya R. Synthetic studies on novel nitroquinazolinone analogs with antimicrobial potential. *Bull. Pharm. Res.* 2013;3(2):51-7.

Gupta R, Pathak D, Jindal DP. Synthesis and study of some 4-aza and 17a-azasteroidal isoxazole. *Eur. J. Med. Chem.* 1999; 34(7-8):659-62. [DOI: http://dx.doi.org/10.1016/S0223-52 34(00)80035-5]

Kumar V. Topological models for the prediction of tyrosine kinase inhibitory activity of 4-anilinoquinazolines. *Bull. Pharm. Res.* 2011;1(2):53-9.

Laitonjam WS, Rajkumar TS, Chingakham BS. Synthesis of some A- and D-ring fused steroidal pyrazoles, isoxazoles and pyrimidines. *Steroids* 2002;67(3-4):203-9. [DOI: http://dx.doi.org/10.1016/S0039-128X(01)00146-5]

Mehta PD, Pathak AK. Synthesis, characterization and *in vitro* antimicrobial activity of novel 4,4′-bis[3-chloro-4-aryl-azetidin-2-one-1-yl]diphenyl sulphones. *Bull. Pharm. Res.* 2011;1(3):38-48.

Park K-H, Kurth MJ. Solid-phase synthesis of novel heterocycles containing thiohydantoin and isoxazole rings. *J. Org. Chem.* 1999;64(25):9297-9300. [DOI: 10.1021/jo991 166p]

Robins LI, Fettinger JC, Tinti DS, Kurth MJ. Selective nucleophilic chemistry in the synthesis of 5-carbamoyl-3-sulfanylmethylisoxazole-4-carboxylic acids. *J. Comb. Chem.* 2007;9(1):139-42. [DOI: 10.1021/cc0601074]

Rodriguez E., Santos J.M., Aparicio D. and Palacios F. An improved and effective method for the preparation of *α, β*-unsaturated oximes and isoxazole derivatives. *Tetrahedron* 1998;54(3-4):599-614. [http://dx.doi.org/10.1016/S0040-4020(97)10320-9]

Shang Y, Fan C, Li M, Zheng C. Synthesis and properties study of novel ferrocenyl isoxazole derivatives. *Appl. Organomet. Chem.* 2006;20(10):626-31. [DOI: 10.1002/aoc.1115]

Waldo JP, Larock RC. Synthesis of isoxazoles via electrophilic cyclization. *Org. Lett.* 2005;7(23):5203-5. [DOI: 10.1021/ol052027z]

Xin Z, Zhao H, Serby MD, Liu B, Schaefer VG, Falls DH, Kaszubska W, Colins CA, Sham HL, Liu G. Synthesis and structure-activity relationships of isoxazole carboxamides as growth hormone secretagogue receptor antagonists. *Bioorg. Med. Chem. Lett.* 2005;15(4):1201-4. [DOI: http://dx.doi.org/10.1016/j.bmcl.2004.11.075]

Xue C-B, Roderick J, Mousa S, Olson RE, Degrado WF. Synthesis and antiplatelet effects of an isoxazole series of glycoprotein IIb/IIIa antagonists. *Bioorg. Med. Chem. Lett.* 1998;8(24):3499-504. [DOI: http://dx.doi.org/10.1016/S0960-894X(98)00637-4]

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