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Follicle-Stimulating Hormone (FSH) Activities of Some Synthesized Candidates

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A series of pyridines, pyrimidinone, oxazinones, and their derivatives (1-14) **were synthesized and screened as follicle-stimulating hormone (FSH) agents using abietic acid as the starting material. The pharmacological screening showed that many of these compounds have good follicle-stimulating hormone (FSH) activity comparable to Pergonal® as reference drug. The detailed synthetic pathways of obtained compounds and Follicle-stimulating hormone (FSH) activity were reported.**

Key words: Abietic acid, Oxazinone, Pyrimidinone, Follicle-stimulating hormone (FSH) activity.

Follicle-stimulating hormone (FSH) is a hormone found in humans and other animals. It is synthesized and secreted by gonadotrophs of the anterior pituitary gland[1]. FSH regulates the development, growth, pubertal maturation, and reproductive processes of the body. FSH and luteinizing hormone (LH) act synergistically in reproduction. In addition, there is evidence that gonadotrophin surge-attenuating factor produced by small follicles during the first half of the follicle phase also exerts a negative feedback on pulsatile luteinizing hormone (LH) secretion amplitude, thus allowing a more favorable environment for follicle growth and preventing premature luteinization[2]. In our previous work, we have found that certain substituted pyridines and their derivatives show antimicrobial and pharmacological properties[3-7] and antitumor activities[8,9]. In addition, the biological and analgesic activities of many heterocyclic compounds containing a sulfur atom have been reviewed[10-13]. On the other hand, thienopyrimidine and thioxopyrimidine derivatives have promising biological[14,15] and anticancer activities[16,17]. Also, some new pyridines, pyrimidines, and their derivatives have been synthesized and used as analgesic, anticonvulsant and antiparkinsonian agents[18-23]. Recently, synthetic steroidal and terpinoidal candidates were synthesized and evaluated as 5α-reductase and aromatase inhibitors, anti-inflammatory, anti-Alzheimer, anti-arthritic and immunosuppressive[24-30] agents. In view of these observations and in continuation of our previous work in pharmacological properties of pyridine chemistry, we tested some new fused polycyclic compounds containing pyridine, oxazinone, or pyrimidinone nucleus and tested their follicle-stimulating hormone (FSH) activity.

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EXPERIMENTAL

Chemistry

All synthesized compounds were established by the following apparatus: The IR spectra (KBr) were recorded on a Pye Unicam SP-1000 spectrophotometer. The ^{1}H NMR spectra were recorded at 270 MHz on Varian EM-360 Spectrometer using *TMS* as an internal standard. The Central Services Laboratory, Cairo University, Egypt. The mass spectra were performed using VG 2AB-3F spectrometer (70eV). All reactions were followed by *TLC* (silica gel, aluminum sheets 60 F_{254}, Merck). All physical and chemical data are reported by Abdulla (2008)[31].

Pharmacological screening

In Vitro [^{3}H] thymidine uptake in cultured mouse ovaries

Intact ovaries are obtained from 15 day old mice. They are dissected carefully with the aid of a stereomicroscope and transferred to culture dishes. Each ovary is placed on a strip of lens tissue supported on a stainless steel mesh grid 4 mm above the floor of a plastic Petri dish and incubated in Eagle's medium supplemented with glucose and glutamine. The dishes are gassed with 5% CO^2 in air at 37 °C. Three replicate dishes are used for each concentration of the standard (0.1 and 0.4 IU/ml) and of the test preparation. [^{3}H]Thymidine (0.02 ¼C) is added to each dish the day after the cultures are set up. Three days later the tissue is prepared for counting. Each grid is irrigated with about 5 ml saline solution. The ovary is then transferred to a counting vial and dissolved in Soluene (Packard Instruments Comp, Inc.). Scintillation solution is added for counting in a liquid scintillation counter. Calculate the IC_{90} (¼M) that cause 9o% Up take of [^{3}H]thymidine.

Ovarian weight in HCG-primed rats

Immature female Sprague-Dawley rats weighing 40–45 g receive twice daily for 3 days subcutaneous injections of 3 different doses of the standard or the test preparation both of them together with a total of 25 IU HCG (Primogonyl®, Schering AG, Berlin)dissolved in 2% gelatin solution in saline. Six to 8 animals are used per group. Eighteen hours after the last injection, the animals are sacrificed, the ovaries extirpated, freed from adherent fat and connective tissue, and weighed to the nearest 0.1 mg, finally potency ratios with confidence limits calculated.

Receptor binding assay for FSH

Membrane preparations from bovine testes are used according to the methods of Cheng (1975)[32] and Andersen (1983)[33]. Fresh bovine testes or testes from rats weighing 220-280 g are decapsulated and rinsed with cold 0.025 M Tris-HCl buffer at pH 7.2, containing 0.3M sucrose, and then minced and homogenized with a Polytron homogenizer at maximum speed for 30 s at a concentration of 5 ml buffer per g of tissue. The homogenate is first filtered through 4 layers, and the filtrate is again filtered through 8 layers of cheesecloth. The filtrate is then centrifuged at 12 000 *g* for 30 min at 4 °C. The pellet is discarded and the supernatant is further centrifuged at 100 000 *g* for 1 h at 4 °C. The supernatant is discarded and the pellet re-suspended in cold 0.025 M Tris-HCl buffer at pH 7.2, containing 10 mM $MgCl_2$, at a concentration of 1 ml buffer per g of the original weight of the testis. The isolated membranes are stored at –70 °C in aliquots of 10 ml per vial until use for assays; 12/75 mm glass disposable tubes are used. To each tube, 0.2 ml of 0.025 M Tris-HCl buffer at pH 7.2, containing 10 mM $MgCl_2$ and 0.1% BSA, 0.1 ml of standard FSH or unknown samples in the same buffer, 0.1 ml of 125I-hFSH tracer labeled by the lactoperoxidase method (50 000 cpm, approximately 2 ng), and finally 0.1 ml of plasma membrane receptors of appropriate dilution (approximately 1–2 mg/ml) are added to reach a final volume of 500 ¼l per tube. All the above solutions are kept at 4 °C before use. The tubes are then shaken vigorously and incubated at room temperature for 20 h. Following incubation, the reaction is stopped by adding 3.0 ml of cold 0.025 M Tris-HCl buffer containing 0.1% BSA. After centrifugation at 4 000 rpm for 30 min, the supernatant is drained and the tip of each tube is dried. The pellet remaining at the bottom of the tube is counted in an automatic gamma counter. Specific binding (%) is defined for agents tested and standard is calculated.

Measurement of drug levels in plasma and in different organ samples.

Drug levels in plasma and in different organ samples were measured by liquid chromatography as previously described[34]. Briefly,

samples were prepared by adding 300¼l acetonitrile and 40¼l phosphoric acid 40% to 100¼l plasma or organ homogenate and placing the mixture in a vortex for 5 s. plasma and brain samples were then centrifuged at 14,000 rpm for 5 min and the supernatants (15 and 50 µl, respectively) were injected into the HPLC system. Equipment system with mass spectrometry (API2000, Applied Biosystems, and Foster City, CA, USA with MassLynx Showroom) detector was used. The chromatographic conditions were adapted to each compound to obtain good peak separation and detection sensitivity. Temperature was maintained at 25 °C by a thermo stated cell holder. Measurements with The flow rate 0.22 ml/minA mixture of ammonium formate (20 µM) buffer-acetonitrile-methanol was used as mobile phase. For drugs s in Mass the assay was liner between 400 and 20,000 ng g^{-1} in the organand 100-8500 ng ml^{-1} in plasma.

RESULTS

A series of pyridines, pyrimidinone, oxazinones, and their derivatives **1-14** (Fig. 1) were synthesized before and screened as anti-inflammatory agents using abietic acid as the starting material[31]. Herein, we used these compounds for evaluation as follicle-stimulating hormone (FSH) agents.

Table 1. IC_{90} (µM) of the tested compounds for *In Vitro* [^{3}H] thymidine uptake in cultured mouse ovaries model

Comp. No.	IC_{90} (nM)
1	16.7
2	14.5
3	12.3
4	9.0
5	8.9
6	6.7
7	5.6
8	2.5
9	4.5
10	3.5
11	1.3
12	1.4
13	1.1
14	1.2
Pergonal®	22.8

Pharmacological Screening

In Vitro [^{3}H] thymidine uptake in cultured mouse ovaries

Follicle-stimulating hormone increases dose-dependent the amount of [^{3}H] thymidine uptake by cultured mouse ovaries. This *in vitro* bioassay for FSH uses a tissue specific proliferation response[35, 36]. In this in vitro preliminary screening method the IC_{90} (¼M) or the doses of the tested compounds that cause 90% increases in the amount of [^{3}H] thymidine uptake

Table 2. Relative potency of the tested compounds in ovarian weight in HCG-primed rats model

Compound	Relative potency
1	1.18
2	1.19
3	1.21
4	1.44
5	1.55
6	1.56
7	1.67
8	1.98
9	1.77
10	1.81
11	2.21
12	2.08
13	2.45
14	2.22
Pergonal®	1.00

Table 3. Specific binding of the tested compounds for Receptor binding assay for FSH model

Comp. No.	Specific binding (%)
1	45.77
2	56.67
3	67.56
4	68.45
5	71.34
6	73.23
7	75.32
8	83.87
9	76.53
10	77.94
11	92.64
12	85.76
13	98.46
14	96.55
Pergonal®	58.88

Table 4. *In vivo* pharmacokinetic and pharmacodynamic profiles of the some newly synthesized agents were evaluated in at the end of Experiment in {PM}

Comp. No.	Ovarian Drug Conc in female Sprague-Dawley rats (ovarian weight in HCG-primed rats) PM	Plasma Drug Conc in female Sprague-Dawley rats (ovarian weight in HCG-primed rats) PM
1	2.13	4.34
2	3.22	4.45
3	3.33	4.57
4	3.45	4.78
5	4.32	4.99
6	4.65	5.42
7	4.78	6.65
8	5.33	9.44
9	5.21	7.57
10	5.22	8.37
11	7.54	10.55
12	6.32	9.67
13	9.65	12.76
14	8.67	11.54
Pergonal®	3.33	4.35

by cultured mouse ovaries were calculated and tabulated in Table 1.

Ovarian weight in HCG-primed rats

Follicle-stimulating hormone (FSH) increases the weight of ovaries in immature rats by inducing follicular maturation. This effect is greatly enhanced by simultaneous administration of a constant dose of human chorionic gonadotropin (HCG) for additional luteinization allowing the detection of low amounts of FSH[37]. In this in vivo method the relative potency to standard reference drug Pergonal were calculated and given in Table 2.

Receptor binding assay for FSH

Significant differences between biological activity and receptor binding activity of FSH preparations have been found by Marana *et al*. (1979)[38], Zaid *et al*. (1981)[39], Foulds and Robertson (1983)[40], AND Burgon *et al*. (1993)[41]. This is attributed to the assay principle of measuring binding activity, but not subsequent intracellular signaling. Several receptors binding assay procedures have been described, e.g. Cheng (1975)[39], Andersen (1983)[40] using bovine testes; Reichert (1976 & 1974)[42,43] was using rat testes tubule tissue. So this method calculate the specific binding percentage of each of the tested compounds to its Receptor and given in Table 3.

Pharmacokinetics and pharmacodynamics profiles of the tested agents

Ovarian Drug Conc in female Sprague-Dawley rats (ovarian weight in HCG-primed rats) nM and Plasma Drug Conc in female Sprague-Dawley rats (ovarian weight in HCG-primed rats) nM were measured and given in Table 4 indicating good Pharmacokinetics and pharmacodynamics properties of the tested agents (Table 4).

DISCUSSION

Abietic acid[1] showed good FSH activity and when it converted to its methyl ketone[2] this activity increases, the same happened when converted[2] into the aldol condensation product with thiophen[3]. This may attribute to the increasing of the lipophylic character of the tested compounds similar to those or happened with estrone derivatives where estrone is more active than estradiol and the later more active than estriol. Here we could reah to the first assumption that confirmed from the receptor binding data that lipophillicity increases receptor binding and FSH activity. Building cyan pyridone ring[4] increases the FSH activity and converting the later to its bioisoster thianopyridone[5] increases both receptor binding and FSH activity. The same happened when

converting derivative[5] to derivative[6] this confirms our previous assumption. Building an extra ring system as in derivative[7] increases both receptor binding and FSH activity; this could attributed to the remote modulating effect of the peripheral substituents of the receptor binding and approach. Converting (7) into (8) increases both receptor binding and FSH activity but converting (8) to (9) reducing both receptor binding and FSH activity. Converting (9) into (10) increases both receptor binding and FSH activity, this attributed to the role of the least electronegative nitrogen atom. So we can go to the second assumption that low electro negativity needed to both receptor binding and FSH activity that could be confirmed by the previous observation where derivatives[5-7] with the least electronegative sulfur atom showed higher receptor binding and FSH activity more than those derivatives[5] containing the more electronegative oxygen atom. Building extra pyrimdone to derivative[8] as in derivatives[11-12] increases both receptor binding and FSH activity due the remote effect of substituent and consequently cage deformations that effect receptor binding and approach, but derivative[12] contain more hydrophilic amino group that increases both polarity and electronegativity than compound[11] and consequently leaded to tat compound[12] have lower receptor binding and FSH activity than that of compound[11]. The same happened typically when

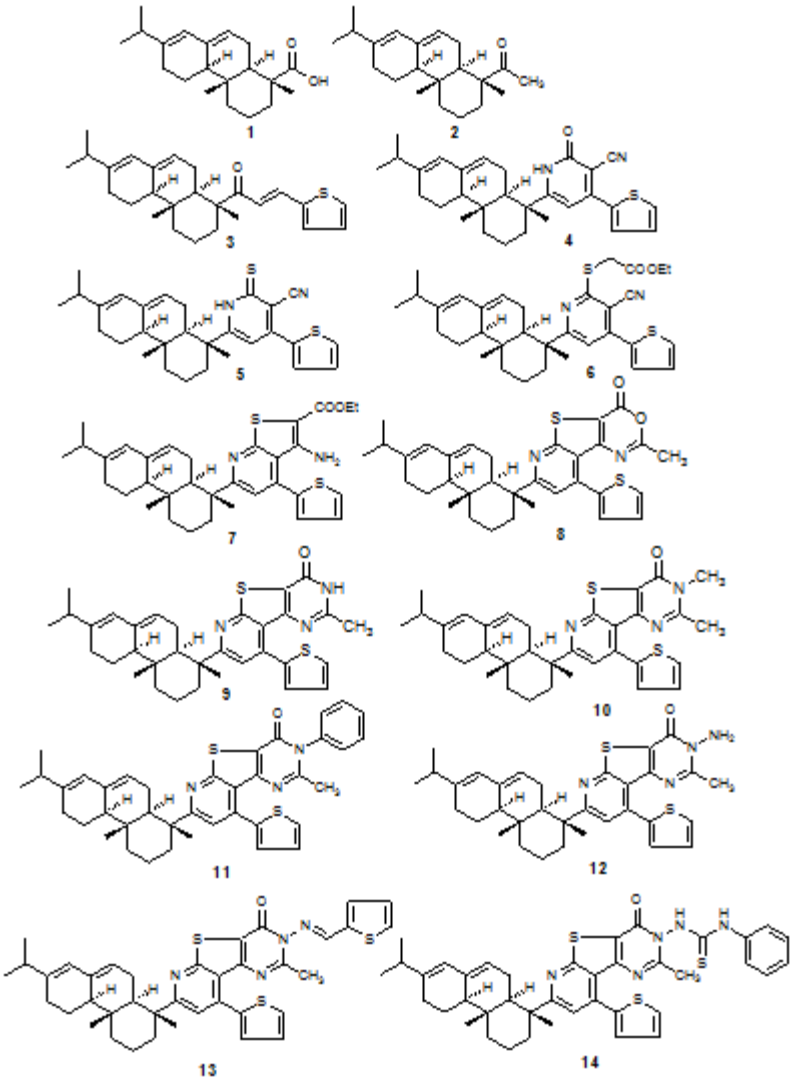

Fig. 1. Chemical structures for testing compounds 1-14

converted derivative[12] to derivatives[13-14], derivative[14] contain higher polar and electronegative atoms that decreases its receptor binding and FSH activity.

Structure activity relationship (SAR)

a) Methyl ketone increases the activity.
b) Building extra ring system increases the activity.
c) Sis membered ring systems of two heteroatoms is more active than those of one hetroatom (increasing the number of nitrogen atoms increases the activity).
d) Sulfur atom increases the activity more than its oxygen bio-isoster.

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