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17-Oxo-17a-Aza-D-Homo-5-Androsten-3β-Yl Esters: Cytotoxic To Liver and Neuroblastoma Cancer Cell Lines

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ABSTRACT

The activity profile of these compounds is significantly impacted by the introduction and placement of the heteroatom in the parental steroid skeleton. Numerous biologically significant azasteroids have been identified as GABA receptor antagonists, neuromuscular blocking drugs, antifungals, antilipemics, local anesthetics, and antimicrobials. The synthesis and assessment of 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl ester derivatives as possible 5-alpha reductase inhibitors have been reported in recent work from our lab. Using additional human cancer cell lines, the current work was conducted to further explore the cytotoxicity of the newly synthesized derivative. Finasteride, 5-fluorouracil, mitocycin 6, and adriamycin were employed as reference medications for comparison after seven human cancer cell lines were treated to 1X 10-5m (single dose) of compounds 1–8. Following labeling with sulforhodamine B dye (SRB), which binds to basic amino acid residues in the trichloroacetic acid (TCA) fixed cells, the cell proliferation was assessed using an ELISA reader 24 hours later. Every experiment was run in four duplicates. When compared to conventional reference medications, the newly synthesized azasteroids derivatives demonstrated notable cytotoxicity against liver and neuroblastoma cell lines. This suggests that 17a-azasteroids may have the ability to cause in vitro cytotoxicity, together with previously documented antiproliferative activity against prostate cancer cell lines (DU-145) and 5-alpha reductase inhibitory action. The findings of these investigations will serve as a basis for future research into new substances that can be used as chemotherapeutic agents.

Keywords: Human cancer cell lines, cytotoxicity, 17a-aza-D-homosteroids, and sulforhodamine B color

INTRODUCTION

A neoplasm is characterized by an abnormally high cell count, which can be caused by both a decrease in programmed cell death (apoptosis) and an increase in cell growth [1]. Development signals cause cells to die, and a variety of metabolic alterations are hallmarks of this process. Organ size changes and the subsequent emergence of anomalies in a given organ might result from any interaction between the physiological processes of cell proliferation and cell death [2]. Therefore, it makes sense to believe that cytotoxic medicines are effective in treating diseases involving aberrant or unchecked cell proliferation since they either kill cells or stop them from proliferating.
The evolutionary process chose the steroid system to carry out some of the most basic biological tasks, and it has not only served as an inspiration for endocrinologists and biochemists, but it also served as the foundation for the most amazing advancements in medicinal chemistry. In order to create an active molecule with fewer or fewer unwanted side effects, naturally occurring steroids have undergone various

positions of modification. Steroids' chemical characteristics change when one or more of their atoms are swapped out for other groups, which alters their biological function [3]. Many researchers have described the chemistry and pharmacology of 4-aza [4], 6-aza [5], 7-aza-des-A-steroids [6], 19-Nor-10-aza [7], 15,16 di-aza [8,9], 17aza, 17a-aza [10-14], and 4,17-diaza [15] steroids as antagonistic to the gamma amino butyric acid (GABA) receptor, antifungal, antineoplastic, mutagenic, anti-inflammatory [10-14], antilipemic [16], neuromuscular blocking agents [17], local anesthetics, and antimicrobials [6] [18,19].

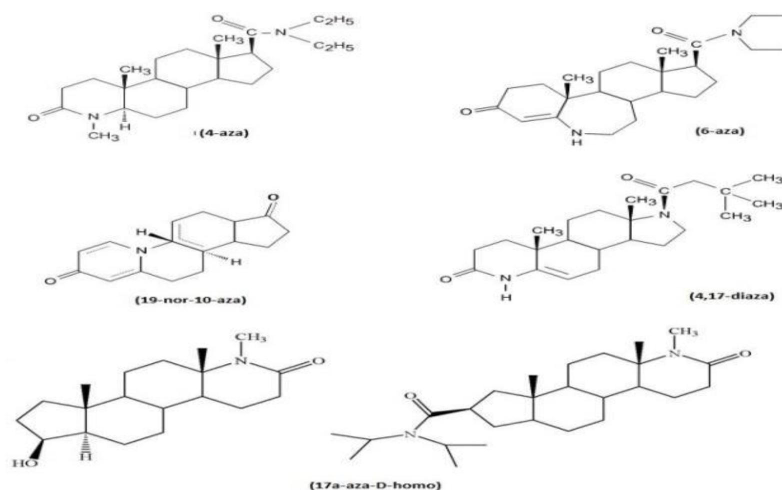

Figure 1: Structure of some reported potent azasteroids

The production of 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl ester steroids (Fig. 2) and their assessment as 5-alpha reductase inhibitors and antiproliferative agents were reported in recent published papers from our lab. When compared to the reference medication finasteride, this class of steroids has demonstrated strong antiproliferative action against the human prostate cancer cell line (DU-145), with IC 50 values ranging from 5.2 to 33.1 µM and When compared to control, there has been a notable rise in the serum androgen level of testosterone [20,21]. We used additional human cancer cell lines to examine the cytotoxicity of the newly synthesized derivative because of the observation that the location of the aza group in the parent steroid skeleton causes significant changes in the pharmacological activity as well as our prior research experience.

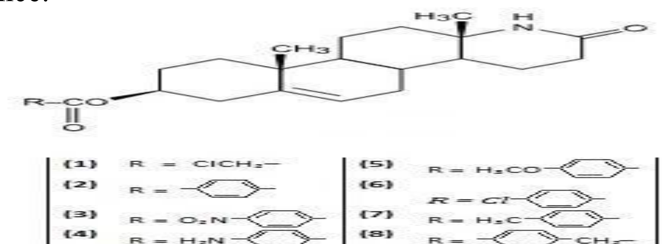

Figure 2 Structure of synthesized and evaluated 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl ester [20,21]

METHODS

A Chemicals and biochemical:

Chemicals of reagent grade were utilized without being purified. Sigma Aldrich was the supplier of complete growth medium (RPMI-1640), fetal bovine serum, tricholoroacetic acid, sodium dihydrogen phosphate, disodium hydrogen phosphate, and dimethyl sulfoxide. Finasteride, of analytical quality (assay 99.9%), was acquired as a gift sample from Cipla in Bombay, India.

B cell culture: The cell line panel included seven human cancer cell lines: Colo-205, HCT-15, 502713 HT-29, and SW-620; Neuroblastoma (IMR-32); and Liver (Hep-2). RPMI-1640 supplemented with 10% heat-inactivated fetal bovine serum, 100 µg/ml streptomycin, and 100 µg/ml penicillin was used to cultivate cells in a CO_2 incubator with 5% CO_2 and 90% relative humidity.

C Test material preparation: A stock solution containing 20 mg/ml of each extract was made. 5% alcoholic extracts were dissolved in DMSO, 50% aqueous extracts in DMSO, and aqueous extracts in distilled water. Working test solutions of 200 g/ml were obtained by serially diluting the stock solution with complete growth medium (RPMI-1640 medium with 2 mM glutamine, 100 g/l streptomycin, pH 7.4, sterilized by filtration and supplemented with 10% fetal bovine serum and 100 units/ml penicillin before use) containing 50 g/ml gentamycin. Gentamycin was added to the entire growing medium used to dilute the stock solution in order to control microbial contamination in the working test solutions, which were neither filtered or sterilized.

D Cytotoxicity assay (Sulforhodamine B SRB assay): 96-well tissue culture plates were used to measure the in vitro cytotoxicity against seven human cancer cell lines [22, 23]. The cells were cultivated in tissue culture flasks with full growth media at 37°C in a carbon dioxide incubator with 5% CO_2 and 90% relative humidity. Depending on the cell's mass doubling times, the necessary cell suspension and density (1-2 lakhs/ml)

lines were created in full growth media in order to assess cytotoxicity. Each well on a 96-well tissue culture plate received an aliquot of 100 µl of the cell suspension. Instead of cell suspension, entire medium was present in the blank wells. Concurrently, a control experiment was conducted using positive controls that contained compounds from proven anticancer medications. For a whole day, the cells were incubated. After 24 hours, the test material (100 l per well) was introduced to the blank and cell suspension wells. The plates were further incubated for 48 hours to allow the cells to proliferate in the presence of the test material. Trichloroacetic acid (50%TCA, 50 l/well) was carefully layered on top of the medium in each well to halt the cell growth at the end of the incubation time. For one hour, the plates were incubated at 4oC. To get rid of TCA, growth medium, low molecular weight metabolites, and serum proteins, the plates were cleaned five times with distilled water before being allowed to air dry. Sulforhodamine B dye staining was used to gauge the cell proliferation. After adding 100 l of sulforhodamine B dye (SRB, 0.4% in 1% acetic acid) to each well, the plates were left to stand at room temperature for half an hour. After four rounds of washing with 1% acetic acid, the plates were dried. To dissolve the dye, 100 l/ml of Tris-HCl buffer (0.01M, pH 10.5) was added to each well. After gently shaking the plates on a shaker for ten minutes, the optical density was measured at 540 nm using an ELISA reader. The mean optical density (OD) value of the experimental set was subtracted from the mean OD value of the corresponding blank to calculate the cell growth. The percentage growth in the presence of test material was computed by taking the growth in the absence of test material as 100%. This was followed by the calculation of the percentage growth inhibition in the presence of test material. Every experiment was run in four duplicates. OD of treated cells divided by OD of control cells × 100 is the cell viability (%).

OUTCOMES AND CONVERSATION

Previous studies on the chemical synthesis and biological assessment of derivatives of 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl ester have demonstrated the significance of lactam for enhanced antiproliferative activity and appropriate structure for the production of novel inhibitors of 5-alpha reductase. Compounds 1–8 were submitted to

the Indian Institute of Integrative Medicine, Jammu (formerly Regional Research laboratory) for an in vitro preclinical antitumor screening program using sulforhodamine B dye (SRB assay) against seven human cancer cell lines derived from liver cancer (Hep-2), neuroblastoma (IMR-32), and colon cancer cell lines (Colo-205, HCT-15, 502713, HT-29, SW-620) in order to investigate the cytotoxic potential of newly synthesized steroidal derivatives [22,23]. A single dose of 1 x 10-5M of the compounds, finasteride, and 5-

As reference medications for comparison, fluorouracil, mitomycin 6, and adriamycin were employed. The percentage growth inhibition of synthetic steroidal derivatives on various cancer cell lines is the result of the results described in Table 1. Active cytotoxic agents were defined as compounds that decreased the proliferation of any one cell line by 40% or more. The findings are based on significance $p<0.05$ and are summarized in Fig. 3 and Table 2 as % growth inhibition ± SEM of four replicate assays against liver and neuroblastoma cell lines.

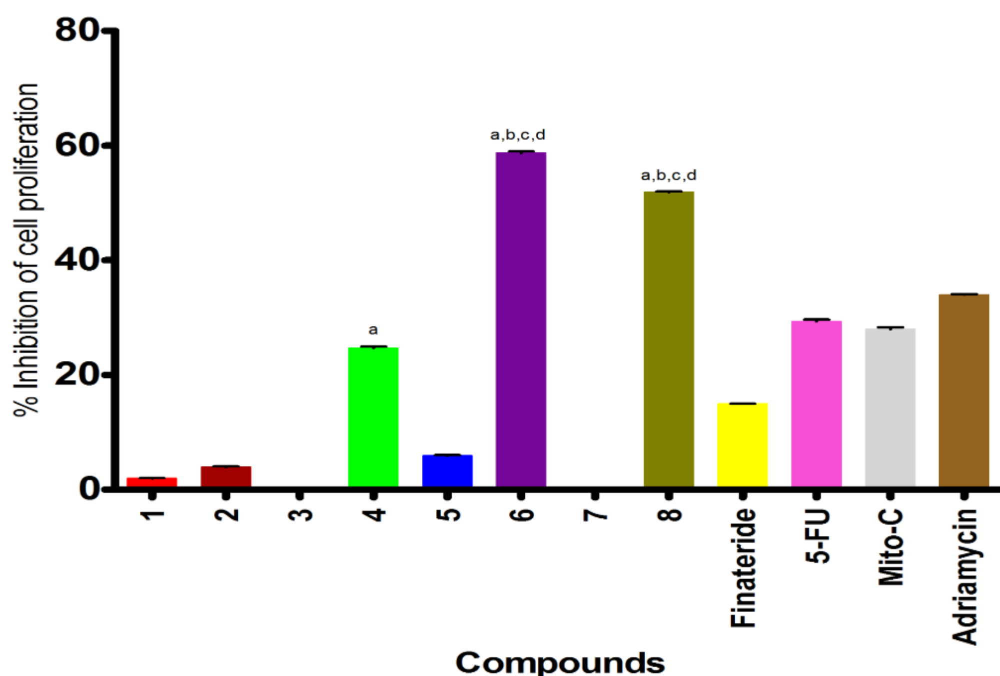

Fig.3 Data expressed as the mean ± SEM against liver cell lines (Hep-2), [a]P<0.05 as compared to Finasteride, [b]P<0.05 as compared to 5-FU, [c]P<0.05 as compared To Mito-C, [d]P<0.05 as compared to Adriamycin.

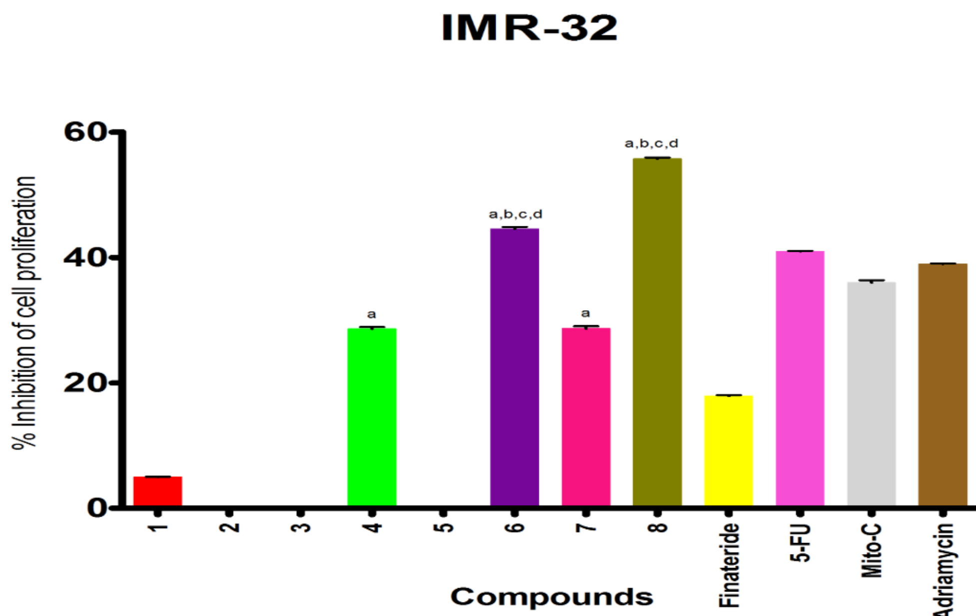

Fig.4 Data expressed as the mean ± SEM against Neuroblastoma cell line (IMR-32), [a]P<0.05 as compared to Finasteride, [b]P<0.05 as compared to 5-FU, [c]P<0.05 as compared to Mito-C, [d]P<0.05 as compared to Adriamycin.

Table 1: Percent Growth inhibition at $1x10^{-5}$ m concentration in the 7 cell lines

Compound	Liver	Neuro blastoma	Colon				
	Hep-2	IMR-32	Colo-205	HCT-15	502713	HT-29	SW-620
1	2	5	32	12	22	2	26
2	4	0	26	8	10	3	16
3	0	0	17	11	14	13	13
4	25	29	22	8	3	0	29
5	6	0	27	17	19	0	27
6	59	45	17	12	25	15	32
7	0	29	2	9	25	11	0
8	52	56	11	4	25	13	17
Finasteride	15	18	23	13	0	0	6
5-FU $1X10^{-5}M$	30	41	38	61	66	51	11
Mito C $1x10^{-5}M$	28	36	0	19	43	Ni	Ni
Adriamycin $1x10^{-5}M$	34	39	13	29	51	Ni	Ni

Not significant inhibition (Ni)

Table 2: % growth inhibition expressed as the mean ± SEM against liver and neuroblastoma cell lines (Hep-2) and (IMR-32)

Compound	% growth inhibition (mean±SEM)^{a,b,cd}	
	Liver (Hep-2)	Neuroblastoma (IMR-32)
1	1.959±0.0325	4.971±0.066
2	3.945±0.0924	0
3	0	0
4	24.713±0.218^{a}	28.588±0.304^{a}
5	5.913±0.096	0
6	58.722±0.249^{a,b,cd}	44.555±0.300^{a,b,cd}
7	0	28.648±0.358^{a}
8	51.884±0.116^{a,b,cd}	55.706±0.246^{a,b,cd}
Finasteride	14.977±0.245	17.918±0.120
5-FU	29.333±0.300	40.954±0.072
Mitomycin	27.984±0.350	35.982±0.382
Adriamycin	33.994±0.100	38.936±0.112

^{a}P<0.05 as compared to Finasteride, ^{b}P<0.05 as compared to 5-FU, ^{c}P<0.05 as compared to Mito-C,^{d}P<0.05 as compared to Adriamycin.

Of the 17a-aza-D-homo-17-one steroids 1–8, 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl 4-chlorobenzoate (6) and 17-Oxo-17a-aza-D-homo-5-androsten-3b-yl phenylacetate (8) showed the highest activity. Compounds 6 and 8 in particular shown notable cell line-selective cytotoxicity against neuroblastoma (IMR-32) and liver (Hep-2) cancer cells, with respective percentages of 59%, 45%, and 52%, 56% (Table 1). The colon cancer cell lines appear to be somewhat resistant to the examined compounds, since no effective growth suppression has been detected when compared to reference medications. Sensitivity to malignant cell lines varied according to cell types. Additionally, compounds 4 and 7 with (NH2-) and 7 (CH3-) are less active with 25 and 0% growth inhibition for Hep-2 cells, respectively, suggesting that the electron withdrawing group at the para position of the aromatic ring system is linked to the inhibition properties of newly synthesized azasteroids toward liver cancer cell lines. However, the results for 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl 4-nitrobenzoate (3) and 17-Oxo-17a-aza-D-homo-5-androsten-3β-yl 4-methoxybenzoate (5), which are completely inactive, contradict this theory. with 0% inhibition, indicating that the electron-withdrawing moiety has little effect on anticellular action. Compound 8 with potent anti-neuroblastoma activity was obtained by introducing a spacer such as -CH2 to separate the carboxylic carbon and phenyl ring (IMR-32). The precise mechanism of action of azasteroids is yet unknown, however based on our previous published investigations [20,21] and current research, they appear to be the most likely candidates to produce an in vitro cytotoxic impact.

FINAL RESULTS

The synthesis, effectiveness (antiproliferative activity and 5-alpha reductase inhibitory), and toxicity profile of the entire group were reported in a recently published research. In the current investigations, cytotoxicity was assessed utilizing seven human cancer cell lines and the sulphoradamine B6 assay (SRB assay). It has been discovered that compounds 6 and 8 are the most effective cytotoxic agents against the liver (Hep-2)

and neuroblastoma (IMR-32) cell lines. These compounds are intriguing due to their 5-alpha reductase inhibitory effect, antiproliferative efficacy against prostate cancer cell lines, and potential as pharmacological instrument and maybe as a precursor to anticancer drugs. Additionally, before being considered as a clinical contender as antitumor drugs, studies must be conducted to thoroughly evaluate the efficacy and potency utilizing various in vitro/in vivo models, including insilico investigations using docking.

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