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Cancer Cells Reduction by MTT with new series of cytotoxic 16- (Substituted Benzylidene) Derivatives of Dhea

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Abstract: One of the most important indicators of the effectiveness of potential anticancer therapies is the decrease in the vitality of cancer cells. An innovative series of cytotoxic 16-(substituted benzylidene) derivatives of dehydroepiandrosterone (DHEA) was synthesised and evaluated for their potential anticancer effects in this work. The MTT test was used for the purpose of synthesis. The MCF-7 cell line, which is used to treat breast cancer, the NCI-H460 cell line, which is used to treat lung cancer, and the SF-268 cell line, which is used to treat cancer of the central nervous system, were all evaluated against a constant concentration of each chemical. The MTT assay demonstrated cytotoxic effects that were dose-dependent, with several substances exhibiting significant reduction of growth. Enhanced tumoricidal efficiency was seen in chemical compounds that included hydrophobic substituents, such as isopropyl and alkyl chains, as well as electron-withdrawing groups, such as nitro and cyano. By boosting the number of receptor interactions, hydroxyl functional groups at carbon (3) and carbon (17) also contributed to an increase in cell bioactivity.

Keywords: MTT Assay, 16-Benzylidene DHEA Derivatives, Cytotoxicity, Cancer Cell Reduction, Structure-Activity Relationship, Anticancer Agents

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INTRODUCTION

The area of research into anticancer medications has gone a long way from the early days of exploring nitrogen mustards in 1887. As a result of this research, synthetic steroidal and non-steroidal compounds such as letrozole and anastrozole, exemestane and formestane have been developed. The naturally occurring compounds taxol and vinca alkaloids are two examples of substances that have been the subject of research enquiries.

Cytotoxic Effects and Hormonal Approaches in Cancer Treatment

The cytotoxic effects of anticancer drugs are what truly make them effective, despite the fact that they achieve their effects via a number of different mechanisms. It is possible that these actions will have an impact on any cells that divide rapidly, not only cancer cells. It has been shown beyond a reasonable doubt that hormone medications are beneficial in the treatment of cancer, particularly for breast and prostate cancers. For the purpose of treating these cancers, a number of medications have been produced as a consequence of structural modifications to steroids. These include adrenocorticosteroids, progestins, oestrogens, anti-estrogens, androgens, anti-androgens, and gonadotropin-releasing hormone analogues.

Steroidal Compounds and Novel Anticancer Approaches

There is evidence that some steroidal nitrosoureas compete with oestradiol for binding to cytosolic oestrogen receptors in the uterus of rats. This competition has been shown. The use of chemicals such as 7-hydroxy-6-nitrocoumarin, which have shown particular antiproliferative actions by targeting p38 mitogen-activated protein kinase, is one of the novel approaches that may be utilised to prevent renal cell carcinoma. A further observation that has been made is that Schiff bases that include 5-nitrofuran and modified pyrimidines have antineoplastic properties. It has also been shown that other medicines containing NO2 have a significant tumour suppressive effect.

When linked to progesterone receptors, synthesised amino acids containing steroid linkages, such as (17β)-{N-[N'-(2-chloroethyl)-N'-nitroso] carbamoyl}, have antineoplastic effects. On the other hand, when bound to (17c)-CNC-alanyl-19-nortestosterone, these amino acids exhibit an affinity for androgen receptors. There have been reports of anticancer medicines that include CN-substituted guanidino groups. These medications have been shown to accelerate glycolytic flow, which leads to active cell death, and induce an early rise in extracellular acidification.

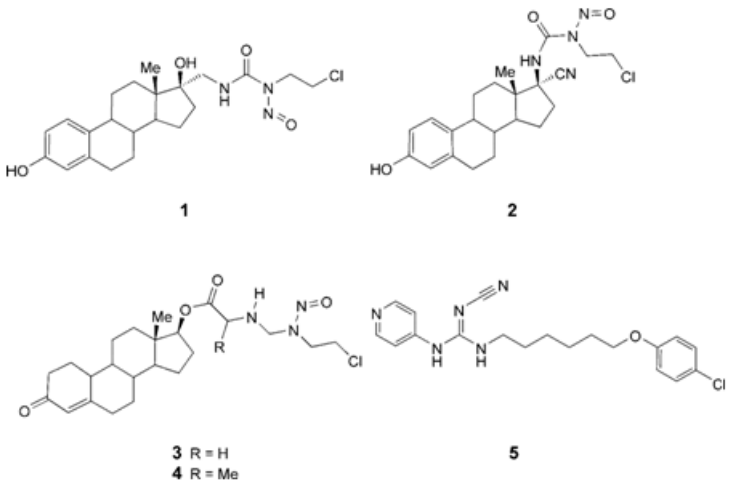


Figure 1. NO_2 and CN groups that are present in these potent anticancer medications into the structure of the steroid

Following this, we proposed adding the NO_2 and CN groups that are present in these potent anticancer medications into the structure of the steroid, building on the information that was already now available. Therefore, we worked at the National Cancer Institute to develop a series of 16-(para-substituted) benzylidene derivatives (androst-5-ene) spanning from 7 to 25. We next investigated the efficiency of these compounds as anticancer agents in vitro using three distinct human cancer cell lines: breast, brain, and lung.

The Scheme demonstrates that 16-benzylidene-substituted androstene derivatives 7–9 were generated by reacting dehydroepiandrosterone (DHA; 6) with 4-nitrobenzaldehyde, 4-formylbenzonitrile, and 4-isopropylbenzaldehyde by the process of aldol condensation. The signal for C6H4—CH= was shown by the 1H-NMR spectra in a range of δ 7.40 to 7.45. The Oppenauer oxidation of these aldol intermediates in

the cyclohexanone/toluene system resulted in the production of three different chemicals, numbered 10–12. The 1H-NMR spectrum revealed the presence of a signal for H—C(4) at a time interval of 5.76 seconds. The presence of a C=O group was indicated by an infrared peak that occurred at 1685 and 1720 cm—1. In methanol, compounds 13–15 were generated by refluxing compounds 10–12 with pyrrolidine. This process was carried out. It was determined that the 1H-NMR signals for H—C(4) and H—C(6) were detected at δ 4.8 (seconds) and 5.1 (meters), respectively. In the subsequent step, the reduction of these enamines with NaBH4 in methanol resulted in the production of 3β-(pyrrolidin-1-yl)-17-ol. Scheme. Synthesis of Compounds 6 ± 25.

In the 1H-NMR spectra, derivatives 16 ± 18 showed a 1-H multiplet at δ 5.37 (H—C(6)). Around 3300 cm —1, an O—H stretching band was seen in the infrared (IR) spectrum. 3,17-diols 19 and 20 were produced by reducing aldol products 7 and 8 with NaBH4. These diols' IR spectra showed O—H stretching at 3350 cm—1, and the reduction of C=O to O—H was validated by the lack of C=O stretching. Compounds 21 and 22 were created when diols 19 and 20 were acetylated, respectively. A wide C=O stretching was seen in the IR spectra at 1730 cm—1. 3-H singlets were detected in the 1H-NMR spectra for compounds 21 and 22 at δ 2.04 (MeOCO—C(3)) and 2.22 (MeOCO—C(17)), respectively, and at δ 2.03 (MeOCO—C(3)) and 2.20 (MeOCO—C(17)). Compounds 23 and 24 were produced by acetylation of products 7 and 8, respectively. The existence of a 3-H singlet at δ 2.05 and 2.04 (MeOCO—C(3)) for 23 and 24 respectively verified their structures. Compound 25 was created by treating compound 18 with Ac2O and dry pyridine; it showed a C=O stretching at 1720 cm—1 in the infrared spectrum. MeOCO was detected as a singlet at δ 2.20 in the 1H-NMR spectrum.

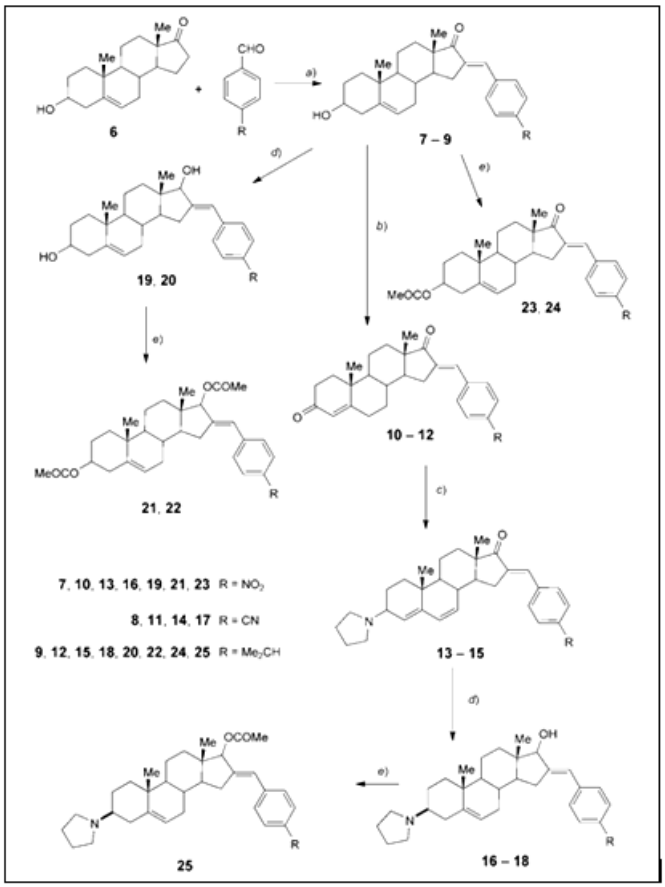


Figure 2. a) NaOH, shaken at r.t. b) Cyclohexanone, (i-PrO)3Al. c) Pyrrolidine, MeOH, reflux. d) NaBH4, MeOH, r.t. e) Ac2O, pyridine, reflux.

OBJECTIVES

1. To get a better understanding of hormonal approaches to cancer therapy and the cytotoxic consequences of these techniques is one of the goals.
2. The reason for doing research on steroidal chemicals and developing novel therapies for cancer
3. The second step is to do molecular docking and ADME/Tox evaluation.

MATERIAL AND METHODS

The whole set of solvents, reagents, and starting ingredients came from Merck AG. The synthesised compounds' purity was determined by using thin layer chromatography (TLC) with a range of solvents with varied polarity. The uncorrected melting points were determined using a Kofler hot stage equipment. Bruker 400 spectrometers were used to record 1H-NMR spectra, and chemical shifts are given as δ (ppm) with tetramethyl silane (TMS) serving as the internal standard. A Shimadzu 470 spectrophotometer was used to record the infrared spectra, which were taken using potassium bromide discs. Finnigan TSQ-70 spectrometers (Finnigan, USA) were used to record the mass spectra at 70 eV.

Substituted benzylidene, also known as (E)-16- substances derived from dehydroepiandrosterone There is a common method to the preparation of 1a-m via aldol condensation.

It was determined that the appropriate aldehyde was used to modify a solution of NaOH (1.75 g) and DHEA (1.0 g, 3.47 mmol) that was dissolved in methanol (20 ml). The reaction mixture was stirred for a period of one hour while it was kept at room temperature. Through the use of analytical thin-layer chromatography, the completeness of the reaction was verified. Following the completion of the reaction, the reactant mixture was then poured into freezing water. After being filtered, washed with cold water, dried under reduced pressure, and refined, the final precipitate was then subjected to crystallisation in methanol. This process of crystallisation was carried out.

Dodecahydro (E)-16-(2-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,15 2-H-cyclopenta-3-hydroxy-10,13-dimethyl[a] One (1a) phenanthren-17(14H)-one

Yield: 23%; mp=213-214°C; IR (KBr, ν_{max}, cm^{-1}): 3475 (OH), 1725(C=O).^{1}HNMR (400 MHz, $CDCl_3$): 1.00(s, 3H, CH_3), 1.07 (s, 3H, CH3), 3.52-3.63(m, 1H, CH-OH), 5.40(s, 1H, H_{vinyl}), 7.28-7.32(m, 2H, H_{phenyl}), 7.42-7.46 (m, 1H, H_{phenyl}), 7.52-7.56(m,1H, H_{phenyl}). MS (EI) m/z (%): 412 (M^++2, 31), 410(M^+, 100).

Dodecahydro (E)-16-(3-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16 2-H-cyclopenta-3-hydroxy-10,13-dimethyl[a] One (1b) phenanthren-17(14H)-one

Yield: 30%; mp= 199-202°C; IR (KBr, ν_{max}, cm^{-1}): 3219 (OH), 1708 (C=O).^{1}HNMR (400 MHz, CDCl3):

0.99(s, 3H, CH3), 1.07(s, 3H, CH_3), 3.48-3.60(m, 1H, CH-OH), 5.41(s, 1H, H_{vinyl}), 7.33-7.43(m, 4H, H_{phenyl}). MS (EI) m/ z (%): 412 (M^++2, 5), 410 (M^+, 15).

Dodecahydro (E)-16-(4-Chlorobenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16 2-H-cyclopenta-3-hydroxy-10,13-dimethyl[a] One (1c) phenanthren-17(14H)-one

Yield: 27%; mp=229-231°C; IR (KBr, ν_{max}, cm^{-1}): 3416 (OH), 1710(C=O). ^{1}HNMR (400 MHz, CDCl3): 0.98(s, 3H, CH_3), 1.07(s, 3H, CH_3), 3.53-3.54(m, 1H, CH-OH), 5.40(s, 1H, H_{vinyl}), 7.39(dd, 1H, H_{phenyl}, J= 8.5Hz),7.46 (dd, 1H, H_{phenyl}, J= 8.5Hz). MS (EI) m/z (%): 412 (M++2, 10), 410 (M^+, 28), 378(18), 351(4), 300(19), 268(10), 214(22), 150(100), 91(87), 79(100).

The dodecahydro of (E)-16-(2,3,4-Trimethoxybenzylidene)-1,3,4,7,8,9,10,11,12,13,15,16 2-H-cyclopenta-3-hydroxy-10,13-dimethyl[a] One (1l) phenanthren-17(14H)

Yield: 20%; mp-199-201°C; IR (KBr, V_{max}, cm^{-1}): 3429 (OH), 1717(C=O). HNMR (400 MHz, CDC_3): 0.98(s, 3H, CH_3), 1.07(s, 3H, CH_3), 3.90(s, 9H, $3OCH_3$), 5.39(s, 1H, Huinki), 6.72(d, 1H, H_{Phenyl}, J=8.4Hz), 7.27(d, 1H, H_{Phenyl}, J=8.4Hz). MS (EI) m/z (%):467(M^++1, 28), 466 (M^+, 100).

(1, 3, 4, 7, 8, 9, 10, 11, 12, 13, 15, 16, and 16-dodecahydro) -(E)-16-(4-(Dimethylamino) benzylidene) 2-H-cyclopenta-3-hydroxy-10,13-dimethyl[a] One (1m) phenanthren-17(14H)-one

Yield: 35%; mp=216-218°C; IR (KBr, ν_{max}, cm^{-1}): 3521 (OH), 1721(C=O). ^{1}HNMR (400 MHz, CDC_3): 0.96(s, 3H, CH3), 1.07(s, 3H, CH_3), 3.03(s, 6H, $2CH_3$), 3.42-3.60 (m, 1H, CH-OH), 5.42(s, 1H, H_{vinyl}), 6.71(d, 1H, H_{phenyl}, J= 8.3Hz), 7.47(d, 1H, H_{phenyl}, J= 8.3Hz). MS (EI) m/z (%): 420(M^++1, 32), 419(M^+, 10).

RESULTS AND DISCUSSION

In order to synthesizes the benzylidene-substituted DHEA derivatives 1a-m, the aldol condensation of DHEA with benzaldehyde derivatives that were a match was used (Figure 2). In vitro cytotoxicity tests were performed on three different cancer cell lines: KB, T47D, and SK-N-M. The chemical 1a-m was evaluated against all three of these cell lines. The MTT reduction assay was used in order to ascertain the percentage of growth inhibition in comparison to the control group, which did not get any treatment from the chemicals under investigation. For your convenience, the following table provides a listing of the substances together with their corresponding 50% growth inhibitory doses (IC_{50}s). Additionally, the data pertaining to etoposide was provided.

According to the cytotoxic results, the majority of the compounds that were obtained by synthesis had a cytotoxic potential that ranged from moderate to high in all three cell lines. Based on the cytotoxic data, it is able to create the structure-activity connection that is as follows:

- By inserting a number of different groups into either the ortho or meta position of the benzylidene

molecule, it was possible to enhance the cytotoxic potential of benzylidene derivatives of DHEA. Groups such as chlorine, trifluoromethyl, methoxy, and methyl are included in this category.

- Each of the three cell lines that were examined had a relatively low level of activity against the compounds that included chlorine, nitro, and fluorine substituents at the para position of the benzylidene pendant (IC50>100 μM). When methoxy, methyl, and trifluoromethyl groups were replaced into the para position of comparable derivatives (compounds 1i-j), the cytotoxic potential of these derivatives was raised. An example of this would be the IC50 values of the para-methyl benzylidene derivative 1j in the KB, T47D, and SK-N-MC cell lines, which were 1.7, 7.6, and 1.0 μM, respectively.
- Compound 1b, which is the most potent synthetic derivative of 3-chlorobenzylidene DHEA, had IC50 values that were comparable to those of etoposide (2.8 and 1.2 μM, respectively). Furthermore, it demonstrated exceptional efficacy against the highly resistant cell lines KB and T47D.

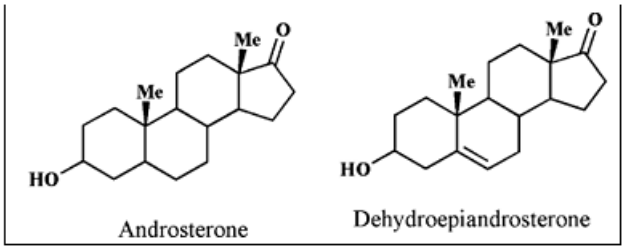


Figure 3. Dehydroepiandrosterone and androsterone chemical structures

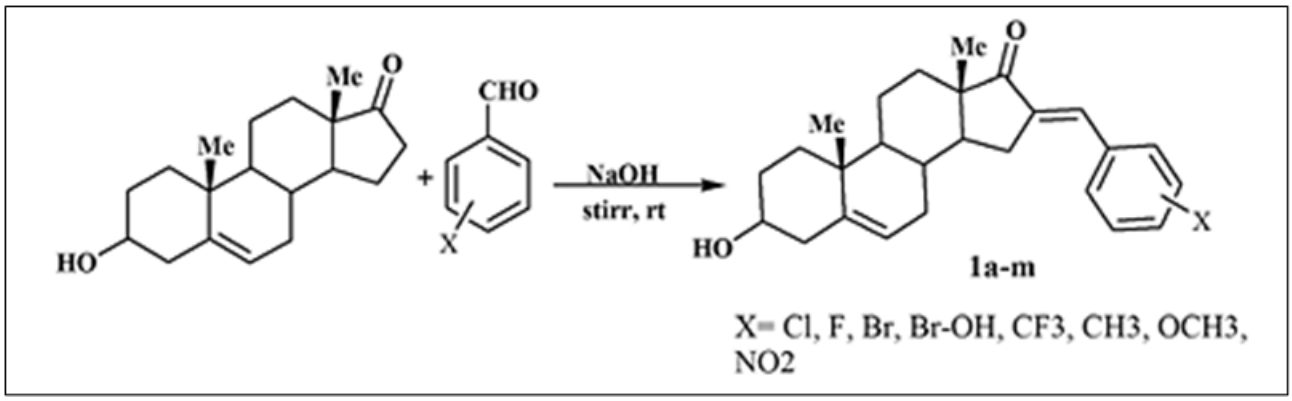
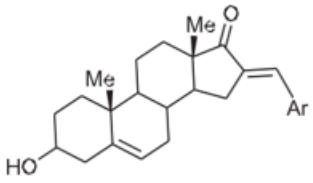
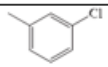
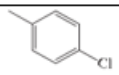
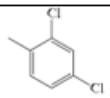
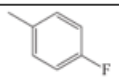
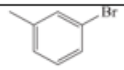
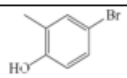
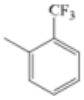
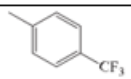
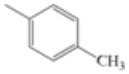
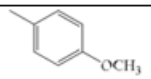
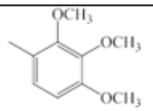
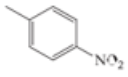


Figure 4. Chemical synthesis procedure for compounds 1a–m.

Table 1.Compounds 1a–m was evaluated for their chemical structures and in vitro cytotoxic activities using the MTT reduction test.



		IC_{50} (µM)[a]		
Compound	**Ar**	**KB**	**T47D**	**SK-N-MC**
1a		2.9(±10.1)	9.6 ±3.1	13.2± 2.2
1b		0.6(±2.0)	1.7±14.8	10.0± 3
1c		>100	>100	>100
1d		>100	>100	3.6±13.26
1e		>100	>100	>100
1f		6.5(±21.1)	>100	3.6±40.7
1g		>100	>100	16.3(±55.9)
1h		1.2(±3.3)	3.6(±7.3)	2.7(±3.6)
1i		2.5(±8.3)	2.4(±9.6)	2.0(±5.3)
1j		1.7(±12.5)	7.6(±17.4)	1.0(±17.4)

1k		1.3(±18)	4.1(±12.3)	5.9(±14.1)
1l		>100	>100	>100
1m		>100	>100	>100
Etoposide	-	2.8(±16.8)	1.2(±8)	3.9(±8.3)

Based on the results in the previous section, it seems that 16-(substituted benzylidene) DHEA derivatives could possess anti-cancer capabilities. The cytotoxic potential of the compounds that are supplied is mostly determined by the position and kind of the substituted group that is located on the benzylidene pendant. In order to generate extremely toxic derivatives of this sort, it is possible to accommodate a number of substituents in either the ortho or meta regions of the benzylidene group.

In Vitro Evaluation of Anticancer Activity of Selected Compounds

The selected compounds were tested in vitro for anticancer activity against three cell lines panel consisting of MCF-7 (breast), NCI-H460 (lung) and SF-268 (CNS) (Table). The compounds were tested at a single concentration (10—4 m) and then incubated for 48 h. The assay, evaluated in terms of percentage of growth inhibition of the cells, showed the compounds 7, 9, 10, 12, 16, and 19 ± 21 to be active. It can be inferred from these results that the steroidal derivatives containing 4- nitro- and 4-isopropylbenzylidene substituents at C(16) are active as anticancer agents, while the compounds with 4-CN substitution did not produce any apoptosis. Compounds 7 and 9 (with 3ß-hydroxy and 17-oxo substitution), and 3,17-diones 10 and 12 have also shown good antiproliferative activity. The diols 19 and 20, obtained after reduction of the 17-oxo function, and the acetylated compounds 21 and 22 have elicited growth inhibition of the cells. The acetates, being susceptible to hydrolysis, might yield their hydroxy analogues, which again show good tumoricidal activity. The results thus highlight the importance of OH functionality in steroidal 16-(para-substituted)benzylidene series of anticancer compounds. The replacement of the OH group at C (3) with 3ß-(pyrrolidin-1-yl) moiety in 16 retained the activity of the compound. The concentrations and the activities are given in the Table.

Table 2. Results of the in vitro Anticancer Assaya)

Compound	**Prefix**	**Growth percentages**			
	NSC	**Lung**	**Breast**	**CNS**	**Activity**
		NCI-H460	MCF 7	SF-268	
7	S716261	35	33	— 35	Active
9	S722255	0	0	3	Active

10	S716263	25	28	— 37	Active
12	S722257	47	27	91	Active
16	S718179	— 91	— 98	— 75	Active
18	S722258	108	101	110	Inactive
19	S716264	— 15	— 90	— 46	Active
20	S722256	5	1	5	Active
21	S718181	65	82	17	Active
23	S718180	106	90	76	Inactive

CONCLUSION

The MTT reduction colorimetric assay was used in order to evaluate the effectiveness of newly synthesised cytotoxic 16-(substituted benzylidene) DHEA derivatives against three unique cancer cell lines. These cell lines were KB, T47D, and SK-N-MC. These newly discovered DHEA benzylidene derivatives have the capacity to induce cell death due to the placement and nature of the substituted group that is located on the benzylidene pendant.

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