SYNTHESIS, CHARECTERIZATION AND ANTIOXIDANT ACTIVITY OF BIS [4-(4'-HYDROXY-3'-METHOXY BENZYLIDENE AMINO PHENYL)] TELLURIDE

*Hiathem J. Kadhum ** Mohammed A. Al-Dewan

*** Shaker A. S. N. AL-Jadaan

[*]Department of Physiology, College of Medicine, University of Basrah, Basrah, Iraq

[**]Department of Physiology, College of Veterinary Medicine, University of Basrah, Basrah, Iraq

[***]Department of Pharmaceutical Chemistry, College of Pharmacy, University of Basrah, Basrah, Iraq

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ABSTRACT

Bis[4-(4'-hydroxy-3'-methoxy benzylidene amino phenyl)] telluride (R_2Te) was prepared from the reaction of two moles of 4-(4'-hydroxy-5'-methoxybenzylidene amino phenyl) mercuric chloride and one mole of $TeBr_4$ in dry dioxane to give diorganyl tellurium dibromide (R_2TeBr_2) which reduced by boiling ethanolic hydrazine hydrate to give the corresponding new telluride (R_2Te).

R_2Te inhibits nitrite induced methemoglobin formation in hemolysate. The time required to convert 50% of the available hemoglobin to methemoglobin was increased from 2.5 to 4, 5.5 and 10.5 minutes by the addition of (1.25, 5 and 20µM) of test compound (R_2Te) respectively, and from 7 to 15, 29 and 54 minutes to convert all of the available hemoglobin to methemoglobin. It seems that R_2Te failed to inhibit nitrite induced hemoglobin oxidation if added after the autocatalytic stage (5-10 minutes after nitrite addition). R_2Te, a novel organotellurium compound, can inhibit nitrite induced methemoglobin formation in hemolysate in a dose dependent manner if added before the autocatalytic stage.

INTRODUCTION

The pharmaceutical proprieties of organotellurium compounds were investigated (1). Several organotellurium compounds show antioxidative, immunomodulating and antitumor activities (2-4). As compared to selenium-containing compounds, a number of water-soluble diorganyl tellurides exhibit significantly higher antioxidant properties (5). Thioredoxin reductase and cancer cell growth inhibited by organotellurium antioxidants analogue of vitamin E (6). Diethyl-2-phenyl-2-tellurophenyl vinylphosphonate is a compound with low toxicity *in vitro* and *in vivo*, as well as also possesses antioxidant activity against iron-induced lipid peroxidation (7).

The apparent potency of organotellurium compounds, together with their relatively simple structure, may represent a new avenue for the development of novel drugs to combat parasitic diseases (8).

Hemoglobin (Hb) is the primary oxygen – transport protein in vertebrate organisms; it can be converted into met hemoglobin by multiple pharmacological and chemical insults, including the nitrites (9). Several free radical species are generated during the course of nitrite induced oxidation of hemoglobin (10).

The formation of methemoglobin occurs in two stages. The first is a slow stage, and the second is a rapid autocatalytic stage (11). The autocatalytic stage results from nitrogen dioxide formed from nitrite through peroxidase activity of methemoglobin. Peroxide and methemoglobin are formed during the initial stage by electron transfer from nitrite (11). Hereditary or acquired methemoglobinemia, contains oxidized ferric iron Fe^{+3} rather than the reduced ferrous form Fe^{+2} found in hemoglobin. Ferric iron has greater affinity for oxygen which shifts the oxygen-Hb dissociation curve to the left resulting in decreased release of oxygen in tissues manifested by hypoxia (12).

The present study was designed to evaluate the protective effect of a novel symmetrical diorganyl telluride (R_2Te) against nitrite induced methemoglobin formation in hemolysate.

EXEPRIMENTAL:

Synthesis

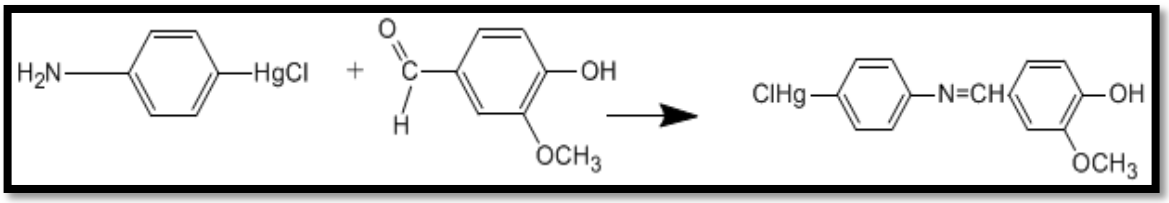
4-Aminophenylmercury chloride was prepared from aniline and mercuric acetate according to a literature method (13); 4-hydroxy-3-methoxybenzaldehyde was purchased

from Jia Xing Zhonghua Chemical Co., Ltd. and used directly. Solvents and reagents were purified by standard methods as necessary.

Melting points were determined by Stuart (SMP 30) melting point apparatus and are uncorrected. FT-IR spectra were recorded for KBr discs with a FT-IR-8400 Schimadzu instrument. NMR spectra were recorded with a Bruker DPX-300 (300 MHz) using TMS as internal standard. Analysis for C, H, and N was done by the Analytical Service Unit, Al al-Bayt University, Jordan. The formation of met- hemoglobin was measured by monitoring absorbance at 631 nm every minute for 1 hour using visible spectrophotometer.

Synthesis of 4-[4'-hydroxy-3'-methoxybenzylidineamino] phenyl mercury chloride

A mixture of 4-aminophenyl mercury chloride (2.63gm, 8mmol) in 50ml absolute ethanol and 4-hydroxy-5-methoxybenzylaldehyde (1.22gm, 8mmol) in 50ml of absolute ethanol containing few drops of glacial acetic acid were refluxed with stirring for 5 hours. After cooling the precipitate was collected by filtration and washed several times with cold ethanol. The solid product was twice re-crystallized from a mixture of ethanol and benzene (3:2) to give orange crystals 3.20gm (86.25%) m.p (197-198) ^{0}C. Elemental analysis found (calculated) C: 36.48(36.37), H: 2.47(2.62) and N: 3.19(3.03).

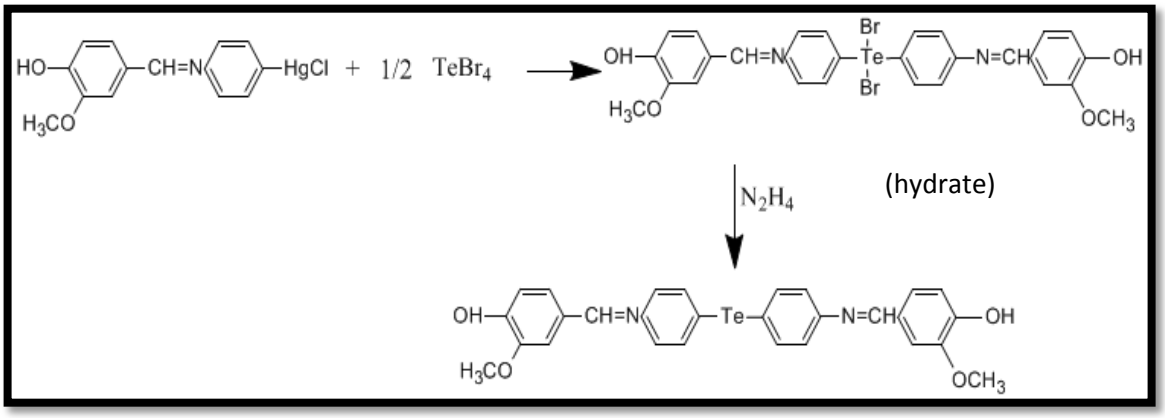


Scheme 1: synthesis of 4-[4'-hydroxy-3'-methoxybenzylidineamino] phenyl mercuric chloride

The FT-IR spectrum of mercurated compound show characteristic bands at 3450 cm^{-1} and 3225 cm^{-1} due to symmetrical and asymmetrical O-H bond, 3090 cm^{-1} due to C-H aromatic, 2870 cm^{-1} due to aliphatic methoxy group and strong band at 1595cm^{-1} due to C=N bond (14).

Synthesis of Bis 4-[4'-hydroxy-3'-methoxybenzylidineaminophenyl] telluride

A mixture of 4-[4'-hydroxy-3'-methoxybenzylidineaminophenyl] mercury chloride (13.91gm, 30mmol) and tellurium tetra bromide $TeBr_4$ (6.71gm, 15mmol) in 150 ml of dry dioxane were refluxed for 6 hours. The resulting solution was filtered hot and cooled to room temperature which deposited 2:1 complexes of dioxane and mercuric bromochloride as white plates which was filtered off. The filtrate was evaporated by rotary evaporator to dryness. The precipitate was dissolved in 50 ml of ethanol and refluxed; a solution of hydrazine hydrate in ethanol was added drop wise to the refluxed solution until nitrogen evolution was ceased. The resulting solution was poured into 500ml of distilled water to afford a yellow solid. The crude product was twice re-crystallized from a mixture of ethanol and dichloromethane to give pale yellow crystals 5.43gm (62.45%) m.p (146-148) ^{0}C. Elemental analysis found (calculated) C: 58.29(57.98), H: 4.22(4.17) and N: 5.08(4.83).

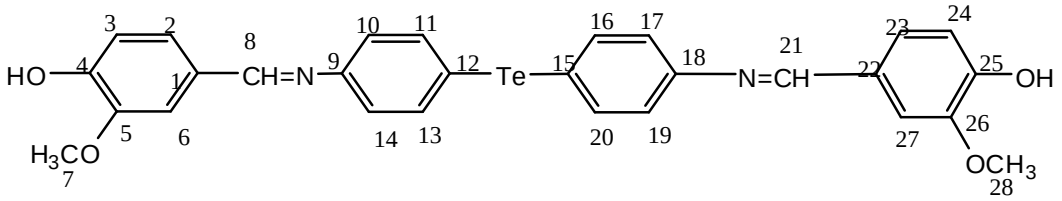


Scheme 2: synthesis of new symmetrical diorganyl telluride.

The FT-IR spectrum of telluride compound show characteristic bands at 3446 cm^{-1} and 3224 cm^{-1} due to symmetrical and asymmetrical O-H bond, 3085 cm^{-1}due to aromatic C-H bond, 2850 cm^{-1}due to aliphatic methoxy group and strong band at 1624 cm^{-1} due to C=N bond.

^{1}H NMR; (300MHz in DMSO-d_6, ppm *vs* TMS); 9.73 (s, 2H, CH=N), 3.87 (s, 6H, OCH_3), 5.11 (s, 2H, O-H), 8.64-6.53 (m, 14H, Ar).

^{13}C NMR;(300MHz in DMSO-d_6); 160.49(C8,C21), 152.99(C9,C18), 149.89(C3,C24), 147.95(C4,C25), 129.77(C1,C22), 125.97(C5,C26) 123.45(C6,C27), 130.12(C11,C13,C16,C20), 55.62(C7,C28), 110.18(C12,C15), 115.49(C2,C23), 122.39(C10,C14,C17,C19) .



Inhibition of nitrite induced methemoglobin formation by new diorganyl telluride (R_2Te)

Blood sample collection and preparation of hemolysate

Blood was obtained by vein puncture from healthy volunteers in ethylene diamine tetra-acetic acid (EDTA) tubes. Blood samples were centrifuged at 2500 rpm for 20 minute to remove the plasma and buffy coat of white cells. The erythrocytes obtained were washed three times with Phosphate Buffer Saline (PBS; pH 7.4). The washed cells were lysed by suspending in 20 volumes of 20mM phosphate buffer (PB; pH 7.4) to yield the required hemolysate concentration of 1:20, then hemolysate was centrifuged at 10000 rpm for 10 minute to remove membrane and supernatant has been utilized for the study (15,16)

Effect of different concentrations of (R_2Te) on nitrite induced met hemoglobin formation in hemolysate

Tow milliliters of each of the following concentrations of R_2Te (1.25, 5, 20µM/L) were added to 0.5ml of freshly prepared hemolysate. The reaction was initiated by the addition of sodium nitrite (final concentration, 0.6mM) to the solution and the formation of methemoglobin was measured by monitoring absorbance at 631 nm every minute for 1 hour using visible spectrophotometer.

Effects of (R_2Te) on nitrite induced methemoglobin formation in hemolysate at different time intervals

Tow milliliters of the highly effective concentration of R_2Te (20 µM), identified in the previous experiment, were added to 0.5ml of freshly prepared hemolysate, either 10 minute before addition of sodium nitrite, and 5 minute and 10 minute after sodium nitrite addition. The formation of methemoglobin was measured by monitoring absorbance at 631 nm every minute for 1 hour using visible spectrophotometer.

RESULTS AND DISSCUSION

In the present study, 4-aminophenyl mercury chloride was reacted with 4-hydroxy-5-methoxybenzaldehyde to give 4-[4'-hydroxy-3'-methoxy benzylidine aminophenyl] mercury chloride as orange crystals in good yield (Scheme 1). Reaction of tellurium tetrabromide with 4-[4'-hydroxy-3'-methoxy benzylidine aminophenyl] mercury chloride in 1:2 molar ratio gave the corresponding diorganyl tellurium dibromide which reduced using ethanolic hydrazine hydrate to give new bis-4 -[4'-hydroxy-3'-methoxy benzylidine amino phenyl] telluride as pale-yellow crystals in good yield (Scheme 2).

In general both compounds prepared are solids with high melting points, the new telluride was soluble in common organic solvents. The carbon, hydrogen, and nitrogen analyses for these compounds agreed well with the calculated values as mentioned above.

The FT-IR spectra of mercurated and tellurated compounds show the disappearance of the stretching bands near 1725cm^{-1} due to the carbonyl bond for aldehyde and disappearance of the characteristic bands for the amine bonds of NH_2 (3460 cm^{-1}-3240 cm^{-1}). This supports the conversion of carbonyl group to azomethine group. The stretching bands could be observed at 1595-1620 cm^{-1} as a strong sharp band due to the -C=N- bond in organomercury and tellurated compounds respectively. In general, the IR spectra of organomercury compound are quite similar to that of new telluride which indicates that telluration has occurred at the position initially occupied by HgCl.

The ^1H NMR spectrum of new symmetrical telluride shows that the resonances of the aromatic rings were observed in the range 6.53-8.64 ppm. The signal characteristic of the

azomethine protons were observed at 9.73 ppm. The signal of hydroxyl protons were observed at 5.11 ppm. The signal of the methoxy groups were observed at 3.87 ppm. The ^{1}H NMR spectra of prepared telluride was agreed well with the structure (17).

The ^{13}C NMR spectra of new symmetrical telluride were agreed well with the structure as mentioned in the synthesis part.

Inhibition of nitrite induced methemoglobin formation

Oxidation of hemoglobin by nitrite to methemoglobin is one of the most employed procedures to oxidize hemoglobin. This process takes place readily after a clear induction time (18). Nitrite oxidizes hemoglobin in two stages, a slow stage followed by a rapid autocatalytic stage (11).

Contrary to widespread opinion, direct oxidation-reduction interaction between hemoglobin and nitrite is absent or negligible under physiological conditions. The driving stage of this process is methemoglobin-catalyzed peroxidase oxidation of nitrite. The product of the oxidation (NO_2) directly oxidizes hemoglobin to methemoglobin peroxide complex without H_2O_2 release into the environment (19).

In the present study nitrite causes a rapid oxidation of hemoglobin to methemoglobin. The oxidation process was delayed by the addition of different concentrations of novel organotellurium compound (1.25, 5, 20µM) in a dose-dependent manner (table 1, figure 1). The time required to convert 50% of the available hemoglobin to methemoglobin was increased from 2.5 to 4.5, 5.5, 10 minute by the addition of (1.25, 5, 20µM) of tested compound (R_2Te) respectively, and the time required to convert all of the available hemoglobin to methemoglobin was increased from 7 to 15, 29, and 54minute by the addition of (1.25, 5, 20µM) of R_2Te respectively (Table 1, figure1)

Table-1- Inhibition of nitrite induced methemoglobin formation by the novel organotellurium compound (R_2Te) in hemolysate

R_2Te (µM/L)	Time to form 50% methemoglobin (min)	Time to form 100% methemoglobin (min)
Control	2.5 ± 0.25	7± 1.5
1.5	4 ± 0.4	15± 0.5
5	5.5 ± 0.66	29± 1.33
20	10.5 ± 0.3	54± 2

Results are means ± standard deviation (SD); n=3

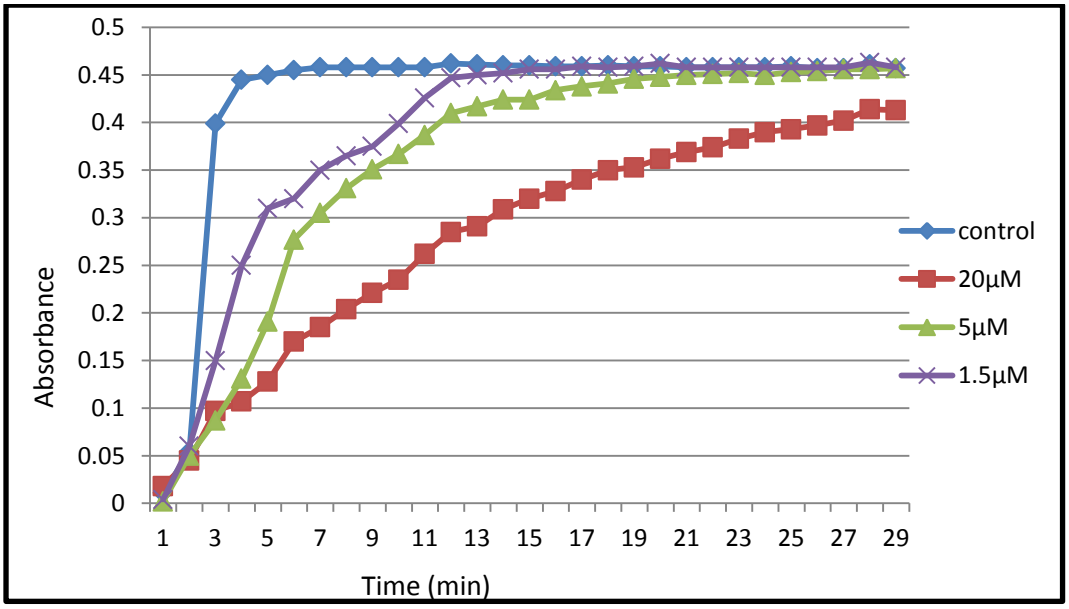


Figure-1-: Time course of methemoglobin formation. Hemolysate was treated with sodium nitrite (0.6mM final concentration). R_2Te (1.25, 5, 20µM) was added before the addition of sodium nitrite. Control without R_2Te.

Since superoxide is implicated in the autocatalytic stage, which carries the reaction to completion (11), R_2Te is able to prevent the onset of autocatalytic stage by scavenging superoxide. Direct interaction between nitrite and R_2Te as a reason for protection against nitrite induced methemoglobin formation is ruled out because the concentration causing protection is very low (1.25, 5, 20µM/L) as compared to nitrite concentration (0.6mM)

The addition of 20µM (the most effective concentration observed in the previous experiment) of R_2Te after the autocatalytic process (5- 10 minute) fails to reverse the effect of oxidation of hemoglobin by sodium nitrite suggesting the protective effect of R_2Te is not due to reduction of methemoglobin to hemoglobin (figure 2).

Many antioxidants like curcumin (15), curcumin analogues (20), plant extracts (21), drugs (16), and vitamins (22) also protect hemoglobin from oxidation by nitrite by inhibiting the autocatalytic stage of methemoglobin formation. Thus, R_2Te may be similar to these materials in inhibiting nitrite induced methemoglobin formation in hemolysate.

Conclusion: Bis(4-[4'-hydroxy-3'-methoxybenzylidineaminophenyl]) telluride , is a novel organotellerium compound can inhibits nitrite induced methemoglobin formation in hemolysate in a dose dependent manner if added before the autocatalytic stage; therefore it has an antioxidant property.

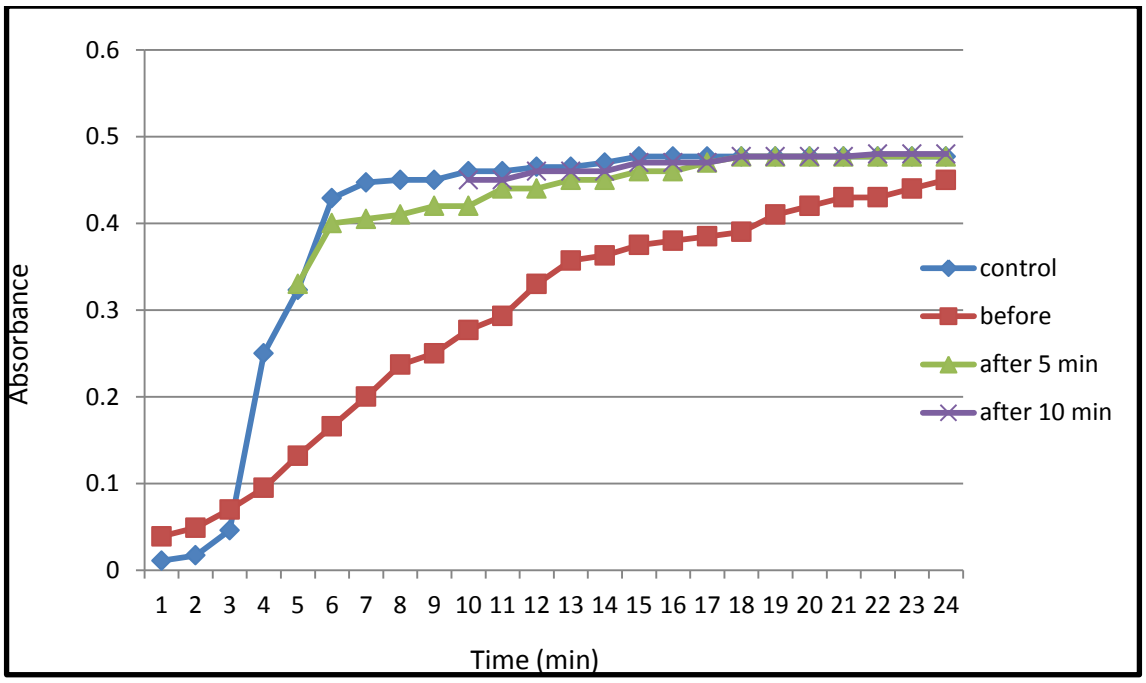


Figure -2- Time course of methemoglobin formation. Hemolysate was treated with sodium nitrite (0.6mM final concentration). R_2Te (20 µM) was added before sodium nitrite, at 5 min, and at 10 min. Control without R_2Te.

تصنيع وتشخيصوالفعالية المضادة للاكسدة لـ بس-4(4-هايدروكسي-3-ميثوكسي بنزلدين امينوفينيل) تلورايد

محمد علي الديوان ، شاكر عبد السالم نعمة الجدعان ، هيثم جواد كاظم

كلية الطب البيطري – جامعة البصرة ،البصرة ، العراق

كلية الصيدله- جامعة البصرة ،البصرة ، العراق

كلية الطب – جامعة البصرة ،البصرة ، العراق

الخلاصة

حضر بس4- (4' هيدروكسي 3'- ميثوكسي بنزيلدين امينو فينيل) تلورايد (R_2Te) من تفاعل مولين من بس 4-(4' هيدروكسي 3'- ميثوكسي بنزيلدين امينو فينيل) كلوريد الزئبق ومول واحد من التليريوم رباعي البروم في الديوكسان الجاف لاعطاء ثنائي الاورجانيل ثنائي التليريوم (R_2TeBr_2) والذي اختزل بواسطة الهيدرازين المائي بعد اذابته وغليانة بالايثانول المطلق لإعطاء التلورايد الجديد. R_2Te منع تكون المتهيموغلوبين في حلالة الدم. لقد زاد الوقت اللازم لتحويل 50% من الهيموغلوبين الى متهيموغلوبين من 2.5 الى 4، 5.5 و10 دقيقة باضافة (1.25، 5 و 20 مايكرومول) من الموكب تحت الاختبار على التوالي ومن 7 الى 15، 29 و54 دقيقة لتحويل كل الهيموغلوبين الى متهيموغلوبين. R_2Te فشل بمنع اكسدة الهيموغلوبين بالنيترايت عند اضافته بعد مرحلة الحفاز التلقائي (5 الى 10 دقيقة من اضافة النايترايت) . R_2Te هو مركب عضوي-تليريوم جديد له القدرة على منع تكون المتهيموغلوبين بواسطة النايترايت في حلالة الدم باسلوب يعتمد على الجرعة عند اضافته قبل مرحلة الحفاز التلقائي.

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