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Synthesis and biological activities of novel 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles

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Abstract

*New series of 3, 6-disubstituted-1, 2, 4-triazolo-[3, 4-b]-1,3,4-thiadiazoles (**5a-j**) have been synthesized by condensation of 3-aryl substituted-4-amino-5-mercapt-(4H) - 1, 2, 4-triazole **(4a)** with various aromatic acids in the presence of phophorus oxychloride. The structure of synthesized compound was supported by elemental analysis, IR, ^{1}HNMR and mass spectral data. The compounds **5a-j** was screened for antifungal activity against Candida albicans and Aspergillus niger and antioxidant activity by DPPH and Nitric oxide methods. Among all the synthsised products, the compounds **5d, 5f** and **5h** bearing hydroxy phenyl ring in 6th position of triazolo-thiadiazole exhibited significant antifungal activity with MIC value at 6.25 µg/ml. All the compounds showed moderate to good antioxidant activity by both the methods.*

Key words: 1, 2, 4-triazole, thiadiazoles, antifungal, DPPH, antioxidant.

INTRODUCTION

The recent literature is enriched with progressive findings about the synthesis and pharmacological activities of fused heterocycles. Heterocycle bearing a triazole or 1,3,4-thiadiazole moiety are reported to possess various biological activities [1-4]. In addition, the N-bridged heterocyclic derived from triazoles have wide applications in medicine[5-7].The 1,3,4-thiadiazole exhibits broad spectrum of biological activities, possibly due to the presence of N-C-S moiety [8].The available therapeutically important medicines Terconazole, Itraconazole, Fluconazole, Cefazoline, Ribavarin, Triazolam, Alprazolam, Etizolam and Furacylin [9] are some examples which contain one of these heterocyclic nucleus. The 1,2,4–triazolo-[3,4-b]-1,3,4-thiadiazole derivatives were obtained by fusing the biolible 1,2,4-triazole and 1,3,4-thiadiazoles ring are reported to possess antifungal [10], antibacterial [11], antiviral [12], anthelmentic [13], anti tumor [14], anti-inflammatory [15] and antituberculer [16] activities.

Prompted by these observation the present study report the synthesis of a series of new 1,2,4-triazolo-[3, 4-b]-1,3,4-thiadiazoles and their antifungal and antioxidant activities.

RESULT AND DISCUSSION

The reaction sequences employed for the synthesis of title compounds are depicted in the Scheme-1. Esterification of 2-[(2', 6'-dichloro phenyl) amino] phenyl acetic acid with ethanol followed by hydrazinolysis with hydrazine hydrate resulted in compound **2**. The acid hydrazide **2** on reaction with carbon disulphide with ethanolic potassium hydroxide affords the corresponding intermediate potassium dithiocarbazinate **3**. The required 3-[2-(2', 6'-dichloro phenyl) amino] benzyl-4-amino-5-mercapto-(4H)-1, 2, 4-triazole **4** was synthesized by refluxing compound **3** with excess of hydrazine hydrate and condensation of **4** with various aromatic acids in the presences of phosphorusoxy chloride yeilded 1, 2, 4-triazolo-[3, 4-b]-1,3,4-thiadiazole **5a-j**. The structure assignments of new compounds were based on their analytical and spectral data. The IR spectra of the compound **4** showed a characteristic weak absorption band at 2608 cm^{-1} attributed to SH group, disappeared in the compounds **5a-j** indicates the formation of triazolo thiadiazole ring system. Further the ^{1}HNMR spectra of the synthesized triazoles **4** showed two characteristic broad singlet at δ 13.6 and 5.9, due to SH proton and NH_2groups respetively. The absence of these absorption due to SH and NH_2 in compound **5a-j** established that the triazoles had converted to triazole-thiadiazole by reacting with the -COOH group of various acid. In summary all the synthesized compounds exhibited satisfactory spectral data consistent with their structures.

Table 1 .The *in Vitro* Antifungal and Antioxidant Activities of compounds 5a-j

Compounds	Ar	Antifungal activity MIC in µg ml^{-1}		Antioxidantactivity% Inhibition at 100 µ M	
		A.niger	*C.albicans*	DPPH method	Nitricoxide method
5a	2-chloro phenyl	25	12.5	70	66
5b	2-nitro phenyl	400	200	55	50
5c	3-methoxy phenyl	100	100	75	80
5d	5-sulpho salicyl	6.25	12.5	82	75
5e	3-bromo phenyl	50	25	50	56
5f	4-hydroxy phenyl	3.12	6.25	80	74
5g	2,4-dichloro phenyl	25	50	65	60
5h	3,4dihydroxyphenyl	3.12	6.25	85	78
5i	3,4-dimethoxy phenyl	12.5	25	64	62
5j	3-pyridyl	50	100	45	54
Ketoconazole	-	6.25	12.5	-	-
Ascorbic acid	-	-	-	85	80

The compounds **5a—j** were screened for antifungal activity against two fungal organisms *viz. Candida albicans* and *Aspergillus niger* in dimethyl sulfoxide (DMSO). Antifungal screening data reports reveal that the compounds **5d, 5f** and **5h** were effective when compared with standard. The compounds **5c** and **5i** showed moderate activity against both the strains. The compounds **5d, 5f** and **5h** in which triazolo thiadiazole moiety bearing hydroxy phenyl ring exhibited good inhibitory activity against both the microorganisms. The compounds **5a-j** was tested for antioxidant property by nitric oxide and DPPH methods. The compounds 5**d, 5f** and **5h** exhibited high antioxidant activity in DPPH methods and the compound **5c** showed equipotent activity in nitric oxide method (Table 1).

MATERIALS AND METHODS

Melting point was measured on Veego digital melting point apparatus and was uncorrected. The IR spectra were recorded using potassium bromide on a Perkin Elmer-FTIR Spectrophotometer.^{1}HNMR spectra were recorded on Bruker Spectrophotometer (400 MHz) in $CDCl_3$ using TMS as an internal standard. Mass spectra were recorded on LC-MSD Trap-SL 2010A-Shimadzu. Micro analysis was performed on a Perkin Elmer-240 CHN elemental analyzer.

Synthesis of 2-[(2', 6'-dichloro phenyl) amino] phenyl acetate (1)

A mixture of 2-[(2', 6'-dichloro phenyl) amino] phenyl acetic acid (0.01M), 100ml of ethanol and 1 ml of sulphuric acid were refluxed for 6h. The completion of the reaction was checked on precoated silica gel G plates using chloroform: methanol (9:1) as an eluent and observed under UV light. After cooling the solution, the product obtained was collected by filtration and recrystallized from ethanol. Colorless powder; Yield: 51%; mp: 122-124^{o}C; FT-IR (cm^{-1}): 1693(C=O), 3404(NH), 1623, 1491, 1462 (C=C), 796(C-Cl). Anal.Calcd.for $C_{16}H_{15}Cl_2NO_2$ (324.2): C, 59.28; H, 4.66; N, 4.32. Found: C, 59.35; H, 4.74; N, 4.43%

Synthesis of 2-[(2', 6'-dichloro phenyl) amino] phenyl acetyl hydrazide (2)

A mixture of compound **1** (0.01 mole) and hydrazine hydrate (0.02 M) in 50ml methanol was heated under reflux for 6h. The completion of the reaction was monitored on silica gel G coated TLC plates using ethyl acetate and petroleum ether (1:1) as an eluent and observed under UV light. The reaction mixture was left over night and solid obtained was collected by filtration and recrystallized from methanol. Colorless powder; Yield: 67 %; mp: 173-174^{o}C; FTIR (cm^{-1}): 3342(NH_2), 3251(NH), 1694(C=O), 791 (C-Cl).Anal.Calcd.for $C_{14}H_{13}Cl_2N_3O$ (310.18): C, 54.21; H, 4.22; N, 13.55. Found: C, 54.13; H, 4.36; N, 13.64 %

Synthesis of 3-[2-(2', 6'-dichloro phenyl) amino] benzyl- 4-amino-5-mercapto-(4H)-1, 2, 4-triazole (4).

A solution of 50ml of alcoholic potassium hydroxide (0.03M) was cooled in an ice bath and compound **2** (0.016M) was added with stirring. Then carbon disulphide (0.025M) was added in small portion with constant stirring. The reaction mixture was agitated continuously for 12h at room temperature. The precipitated potassium thiocarbamate **(3**) was filtered, washed with ethanol, dried and used for the next step.

The above potassium thiocarbamate was mixed with water (8ml) and hydrazine hydrate (0.02 mole) and refluxed until a homogeneous reaction mixture turned green (5h) was obtained. The reaction product was cooled to room temperature and diluted with water. On acidification with acetic acid, the required triazole was precipitated out. The purity was checked by TLC using precoated silica gel G plate with toluene: ethyl acetate: formic acid (5:4:1) as solvent system and observed under UV light. Yellow crystals; Yield 77%; mp188-190^{o}C; FTIR (cm^{-1}): 3400(NH_2), 2618(SH), 1624(NH-NH_2), 801 (C-Cl). ^{1}HNMR δ (ppm): 13.6 (s, 1H, SH), 7.5-8.2 (m, 7H, ArH), 5.9(s, 2H, NH_2) and 2.3 (s, 2H, -CH_2-). Anal.Calcd.for $C_{15}H_{13}Cl_2N_5S$ (366.27): C, 49.19; H, 3.58; N, 19.12. Found: C, 49.14; H, 3.61; N, 19.09%.

General method for the synthesis of 3-[2- (2', 6'-dichloro phenyl) amino] benzyl]-6-substituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles (5a-j).

An equimolar mixture of compound **4** and appropriate aromatic acids in 10ml of phosphorus oxychloride was refluxed for 5h. The reaction mixture was cooled to room temperature and poured onto crushed ice with stirring. Finally powdered potassium carbonate and the required amount of potassium hydroxide were added till the pH of the mixture was raised to 8, to remove the excess of phosphorus oxy chloride. The solid resulted was collected by filtrations, dried and recrystallized from methanol.

*3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(2-chlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole***(5a):**Yield: 60%; mp: 172-173^{o}C; FTIR (cm^{-1}): 3226 (N-H), 1265 (C=N), 1585 (C=C), 756 (C-Cl). ^{1}HNMR (ppm): δ 6.45-7.55 (m, 11H, ArH), δ 2.75(s, 2H, CH_2-), δ 4.1 (s, 1H, NH). EI- MS (m/z %): 485 (M-1). Anal.Calcd.for $C_{22}H_{14}Cl_3N_5S$ (486): C, 54.28; H, 2.90; N, 14.39. Found: C, 54.31; H, 2.89; N, 14.41.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(2-nitrophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5b):**Yield: 72%; mp: 224-225^{o}C; FTIR (cm^{-1}): 3109(NH), 1246 (C=N),1598(C=C), 1549, 1336(C-NO_2), 745(C-Cl). ^{1}HNMR (ppm): δ 6.7-8.1(m, 11H, ArH), δ 2.85(s, 2H, CH_2-), δ 4.3(s, 1H, NH). EI- MS (m/z %): 496(M-1). Anal.Calcd.for $C_{22}H_{14}Cl_2N_6O_2S$ (497): C, 53.16; H, 2.84; N, 16.90. Found: C, 53.24; H, 2.90; N, 16.81.

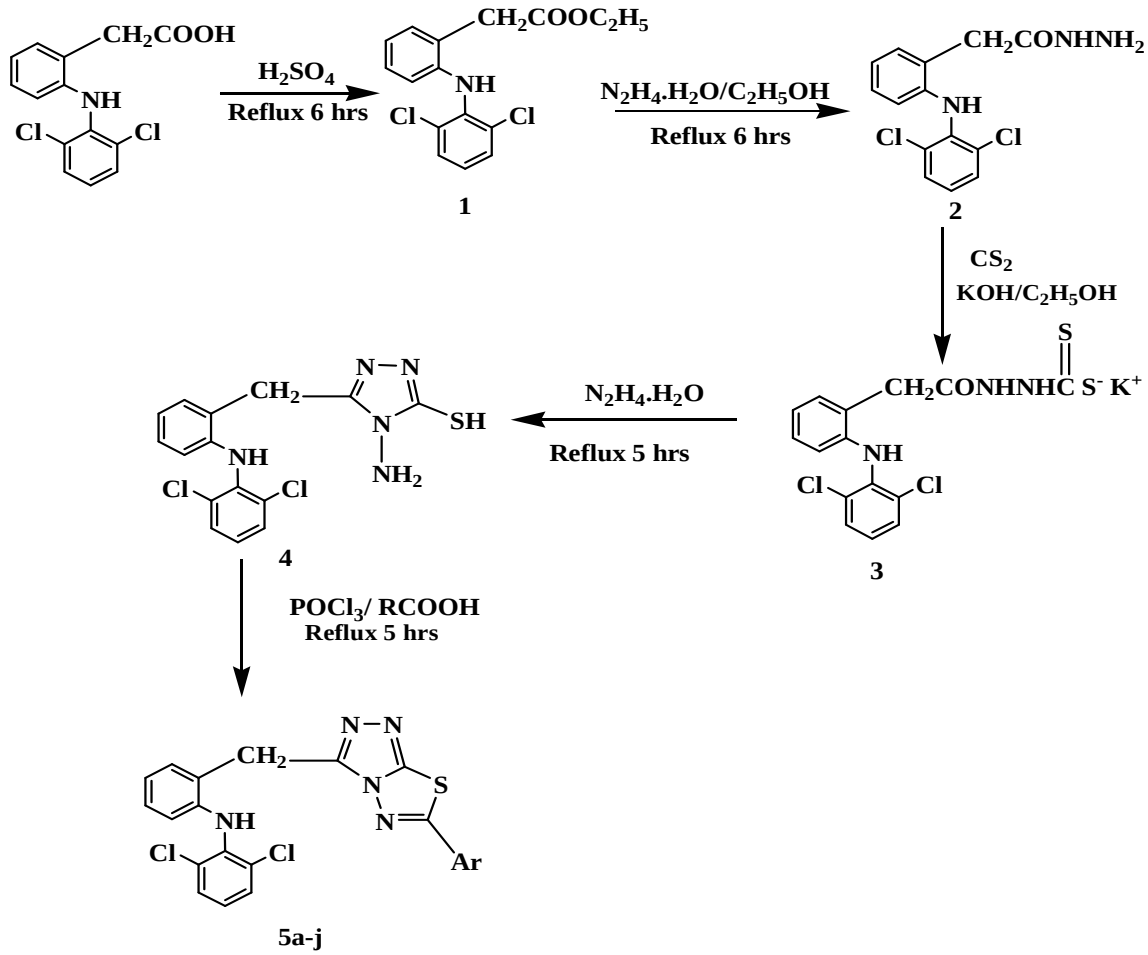
3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(3-methoxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5c):**Yield: 80 %; mp: 176-177^{o}C; FTIR (cm^{-1}): 3285(N-H), 1211 (C=N), 1619(C=C), 1268, 1019(C-O-C), 785(C-Cl). ^{1}HNMR (ppm): δ 6.5-7.75(m, 11H, ArH), δ 3.45(s, 3H, OCH_3), δ 3.15(s, 2H, CH_2-), δ 4.3(s, 1H, NH). EI- MS (m/z %): 481(M-1). Anal.Calcd.for $C_{23}H_{17}Cl_4N_5OS$ (482.3): C, 57.27; H, 3.55; N, 14.52. Found: C, 57.34; H, 3.52; N, 14.59.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(5-sulphonylsalicyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5d):**Yield: 63%; mp: 133-134^{o}C; FTIR (cm^{-1}): 3377 (N-H), 3439(SO_2OH), 1249(C=N), 1617 (C=C), 756 (C-Cl). ^{1}HNMR (ppm): δ 6.45-7.55 (m, 10H, ArH), δ 2.85(s, 2H, CH_2-), δ 4.4 (s, 1H, NH), δ 2.25 (s, 1H, SO_2-OH)). EI- MS (m/z %): 546.99 (M-1). Anal.Calcd.for $C_{22}H_{15}Cl_2N_5O_4S_2$ (548): C, 48.18; H, 2.76; N, 12.77. Found: C, 48.23; H, 2.82; N, 12.80.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(3-bromophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5e):**Yield: 55%; mp: 150-151^{o}C; FTIR (cm^{-1}): 3275 (N-H), 1216(C=N), 1627

(C=C), 759(C-Cl), 655 (C-Br). ^{1}HNMR (ppm): δ 7-8.2 (m, 11H, ArH), δ 2.85(s, 2H, CH_2-), δ 4.3 (s, 1H, NH). EI- MS (m/z %): 530 (M-1). Anal.Calcd.for $C_{22}H_{14}BrCl_2N_5S$ (531): C, 49.74; H, 2.66; N, 13.18. Found: C, 49.80; H, 2.71; N, 13.15.

Synthesis of 3, 6-disubstituted-1, 2, 4-triazolo-[3, 4-b]-1, 3, 4 thiadiazole derivatives (5a-j)



3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(4-hydroxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5f):**Yield: 68%; mp: 148-149^{o}C; FTIR (cm^{-1}): 3276 (N-H), 3440(OH), 1208(C=N), 1638 (C=C), 762 (C-Cl). ^{1}HNMR (ppm): δ 6.5-8.2 (m, 11H, ArH), δ 2.8(s, 2H, CH_2-), δ 4.25 (s, 1H, NH), δ 5.55 (s, 1H, OH). EI- MS (m/z %): 467 (M-1). Anal.Calcd.for $C_{22}H_{15}Cl_2N_5OS$ (468): C, 56.42; H, 3.23; N, 14.95. Found: C, 54.39; H, 3.29; N, 14.90.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(2,4 dichlorophenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5g):** Yield: 75%; mp: 148-149^{o}C; FTIR (cm^{-1}): 3324(N-H), 1222(C=N), 1628 (C=C), 766 (C-Cl). ^{1}HNMR (ppm): δ 7.15-8.35 (m, 10H, ArH), δ 2.65(s, 2H, CH_2-), δ 4.55 (s, 1H, NH). EI- MS (m/z %): 520 (M-1). Anal.Calcd.for $C_{22}H_{13}Cl_4N_5S$ (521): C, 50.69; H, 2.51; N, 13.44. Found: C, 50.74; H, 2.59; N, 13.40.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6(-3,4-dihydroxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5h):** Yield: 62%; mp: 159-160^{o}C; FTIR (cm^{-1}): 3269 (N-H), 3440(OH), 1226(C=N), 1596 (C=C), 758 (C-Cl). ^{1}HNMR (ppm): δ 6.65-7.7 (m, 10H, ArH), δ 2.85(s, 2H, CH_2-), δ 4.5 (s, 1H, NH), δ 5.5 (s, 2H, OH), EI- MS (m/z %): 483 (M-1). Anal.Calcd.for $C_{22}H_{15}Cl_2N_5O_2S$ (484): C, 54.55; H, 3.12; N, 14.46. Found: C, 54.63; H, 3.19; N, 14.50.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(3,4-methoxyphenyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5i):** Yield: 55%; mp: 135-136^{o}C; FTIR (cm^{-1}): 3269 (N-H), 3440(OH), 1226(C=N), 1596 (C=C), 1274, 1015(C-O-C) 758 (C-Cl). ^{1}HNMR (ppm): δ 7.15-8.2 (m, 10H, ArH), δ 2.85(s, 2H, CH_2-), δ 3.35(s, 3H, 3-OCH_3), 3.4(s, 3H, 4-OCH_3), δ 4.5 (s, 1H, NH). EI-MS (m/z %): 511 (M-1). Anal.Calcd.for $C_{24}H_{19}Cl_2N_5O_2S$ (512): C, 56.26; H, 3.74; N, 13.67. Found: C, 56.34; H, 3.79; N, 13.59.

3-[2-[(2', 6'-dichloro phenyl) amino] benzyl]-6-(3-pyridyl)-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole **(5j):** Yield: 64%; mp: 118-120^{o}C; FTIR (cm^{-1}): 3232(N-H), 1259(C=N), 1605 (C=C), 765 (C-Cl). ^{1}HNMR (ppm): δ 6.8-8.5 (m, 11H, ArH), δ 2.9(s, 2H, CH_2-), δ 4.15 (s, 1H, NH). EI- MS (m/z %): 452 (M-1). Anal.Calcd.for $C_{21}H_{14}Cl_2N_6S$ (453): C, 55.64; H, 3.11; N, 18.54. Found: C, 55.72; H, 3.19; N, 18.50.

Antifungal Activity

The *invitro* antifungal activity of compounds **5a-j** were determined using micro dilution susceptibility method against the fungal species *Candida albicans* and *Aspergillus niger* in SDA (Sabouraud Dextrose Agar) medium. Ketoconazole was used as reference standard. The test compounds and ketoconazole were dissolved in DMSO at concentration of 800μg/ml. The two-fold dilution of the solution was prepared (400, 200, 100….3.12 μg/ml).The microorganism suspensions were incubated at 36 °C for 48 h. The MIC (minimum inhibitory concentration) of the compounds were recorded as the lowest concentration of each synthesized compound in the tubes with no turbidity (*i.e.*, no growth) of inoculated fungi.

Antioxidant Activity

The compounds **5a-j** was tested for antioxidant property by nitric oxide and DPPH methods. Ascorbic acid was used as a standard.

Assay for Nitric Oxide (NO) Scavenging Activity

Sodium nitroprusside (5mM) in phosphate buffer pH 7.4 was incubated with 100 µM concentration of test compounds dissolved in methanol and tubes were incubated at 25°C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5ml of incubated solution was taken and diluted with 0.5 ml of griess reagent (1% sulfanilamide, 0.1% *N*-naphthylethylenediamine dihydrochloride and 2% O-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent *N*-naphthylethylenediamine dihydrochloride was read at λ_{max} 546 nm.

Reduction of 1, 1-Diphenyl-2-picrylhydrazyl (DPPH) Free Radical Method

The solutions of test compounds (100mM) were added to DPPH (100mM) in methanol. The tubes were kept at an ambient temperature for 20 min and the absorbance was measured at

λ_{max}517 nm. The difference between the test and the control experiments was taken and expressed as the percent scavenging of the DPPH radical. The nitrogen centered stable free radical DPPH has often been used to characterize antioxidants. A radical scavenging antioxidant reacts with DPPH stable free radical and converts into 1, 1-diphenyl-2-picrylhydrazine. The resulting decolorization is stoichiometric with respect to the number of electrons captured. The change in the absorbance produced in this reaction has been used to measure antioxidant properties.

CONCLUSION

In conclusion, we report that the antifungal and antioxidant studies of newly synthesized 3,6-disubstituted-1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazole derivatives. Compounds bearing electron donating aromatic ring in 6th position of triazolo-thidiazole system showed significantly good antifungal and antioxidant activities.

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