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Synthesis of some halogen-containing 1,2,4-triazolo-1,3,4-thiadiazines and their antibacterial and anticancer screening studies — Part I☆

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Abstract

A series of 7-arylidene-6-(2,4-dichloro-5-fluorophenyl)-3-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**3**) were prepared by the condensation of 4-amino-5-mercapto-3-substituted-1,2,4-triazoles (**1**) and 3-aryl-1-(2,4-dichloro-5-fluorophenyl)-2-bromo-2-propen-1-one (**2**). An alternative route for the synthesis of the title compound **3** has been described. The newly synthesised compounds were characterised on the basis of N-analyses, IR, ^{1}H NMR and mass spectral data. Some of the newly synthesised compounds were tested for their antibacterial activities against Gram + ve and Gram − ve bacteria. Among the tested compounds **3n** showed the highest degree of antibacterial activity against *S. aureus* and evaluation of the LD_{50} value of this compound was carried out. Some of the newly synthesised compounds were also screened for their anticancer activities. Among these, compounds **3b**, **3g**, **3n** and **3p** are found to be active against NCI-H460 (lung), MCF7 (breast), SF 268 (CNS) in the preliminary anticancer screening studies. Further, 60-cell-line anticancer studies of these compounds were carried out. The results of such studies are discussed in this paper.

Keywords: 1,2,4-Triazoles; 1,3,4-Thiadiazines; Antibacterial agents; LD_{50} value; Anticancer screening

1. Introduction

1,2,4-Triazoles and *N*-bridged heterocycles derived from them are found to be associated with diverse pharmacological activities [1–4]. Synthesis and reactions of 4-amino-5-mercapto-3-substituted-1,2,4-triazoles have been reviewed by Temple Jr. [5]. The 1,2,4-triazole nucleus has been incorporated into a wide variety of therapeutically important drugs. In recent years fluorinated acetophenone finds an important place in medicinal chemistry as starting material for the preparation of antibacterial agents. Ciprofloxacin, the widely used antibiotic drug, is synthesised starting from 2,4-dichloro-5-fluoroacetophenone. Norfloxacin, Pefloxacin and Endrofloxacin are the other newer fluorine-containing antibiotics and antibacterial agents.

Prompted by the biological properties of 1,2,4-triazole derivatives and 1,3,4-thiadiazines, and in continuation of our studies on *N*-bridged heterocycles [6–8], it was decided to synthesise various halogen-containing 1,2,4-triazolo-[3,4-*b*]-thiadiazines and to screen them for their antibacterial and anticancer properties. The results of such studies are discussed in this paper.

2. Chemistry

2,4-Dichloro-5-fluoroacetophenone and substituted benzaldehydes were allowed to react in the presence of

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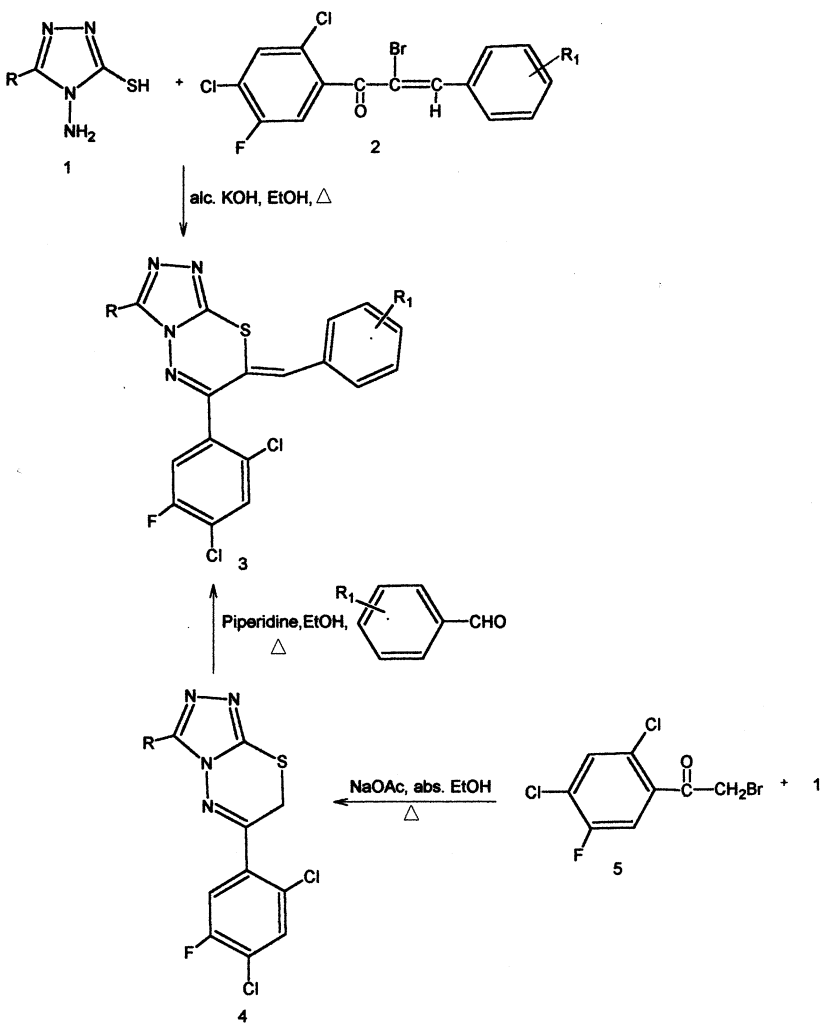
ethanolic potassium hydroxide to yield 3-aryl-1-(2,4-dichloro-5-fluorophenyl)-2-propen-1-ones. Bromination of new chalcones yielded 3-aryl-1-(2,4-dichloro-5-fluorophenyl]-2,3-dibromo-propan-1-ones. Dehydrobromination of these dibromopropanones yielded α-bromo-2-propen-1-ones (**2**). Various 4-amino-5-mercapto-3-substituted-1,2,4-triazoles (**1**) were prepared according to the methods proposed in the literature [9].

The condensation reactions of new α-bromo-2-propen-1-ones (**2**) with aminomercaptotriazoles **1** were carried out as shown in Scheme 1. Such condensation reactions yielded 7-arylidene-3-substituted-6-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**3**). The formation of these products can also be achieved through an alternative method. Aminomercaptotriazoles **1** were condensed with 2,4-dichloro-5-fluorophenacyl bromide to yield 7*H*-3-substituted-6-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**4**) [10]. Condensation of **4** with the suitable aromatic aldehydes in the presence of piperidine also afforded the title compounds **3** (Scheme 1). The analytical data of newly synthesised compounds are listed in Table 1.

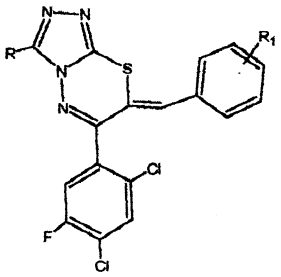
Newly synthesised compounds were characterised on the basis of elemental analysis, IR, ^{1}H NMR and mass spectral data. IR spectra of compounds **3c**, **3g**, **3n** and **3p** were recorded. Compound **3n** showed absorption band at 2983 cm^{-1} due to C–H stretching. The absorption band seen at 1590 cm^{-1} could be attributed to the –C=N– stretching. The absence of characteristic absorption bands due to stretching of the $-NH_2$ and –SH moieties of the triazoles **1** and carbonyl absorption of α-bromo-2-propen-1-ones (**2**) indicates the formation of cyclised products.

The ^{1}H NMR spectra of the compounds **3b**, **3e**, **3g**, **3h**, **3n** and **3q** were recorded. In the ^{1}H NMR spectrum of compound **3b** the signal due to methyl protons was seen at δ 2.49. A singlet appearing at δ 6.74 was attributed to the exocyclic vinylic proton (=CH–) of the compound. The signal due to protons of methylene-



Scheme 1.

Table 1
Analytical data of 7-arylidene-6-(2,4-dichloro-5-fluorophenyl)-3-substituted-1,2,4-triazolo[3,4-*b*]-1,3,4-thiadiazines (**3a**–**3q**)



Compound	R	R_1	Melting range (°C)	Yield (%)	Molecular formula	Anal. %N calculated [found]
3a	H	3,4-methylene-dioxy	150–52	60	$C_{18}H_9Cl_2FN_4O_2S$	12.90 [12.91]
3b [a]	CH_3	3,4-methylene-dioxy	225–30	80	$C_{19}H_{11}Cl_2FN_4O_2S$	12.50 [12.49]
3c [b]	C_2H_5	3,4-methylene-dioxy	184–86	73	$C_{20}H_{13}Cl_2FN_4O_2S$	12.12 [12.13]
3d	C_3H_7	3,4-methylene-dioxy	170–75	60	$C_{21}H_{15}Cl_2FN_4O_2S$	11.76 [11.75]
3e	C_6H_5	3,4-methylene-dioxy	180–82	85	$C_{24}H_{13}Cl_2FN_4O_2S$	10.98 [10.95]
3f	$CH_3O–C_6H_4$	3,4-methylene-dioxy	228–30	70	$C_{25}H_{15}Cl_3FN_4O_3S$	10.33 [10.31]
3g [c]	CH_3	4-chloro	210–12	60	$C_{18}H_{10}Cl_3FN_4S$	12.78 [12.75]
3h [d]	C_2H_5	4-chloro	220–22	65	$C_{19}H_{12}Cl_3FN_4S$	12.38 [12.35]
3i [e]	C_3H_7	4-chloro	160–65	70	$C_{20}H_{14}Cl_3FN_4S$	12.01 [12.00]
3j	C_6H_5	4-chloro	210–12	75	$C_{23}H_{12}Cl_3FN_4S$	10.56 [10.53]
3k	$CH_3O–C_6H_4$	4-chloro	218–220	63	$C_{24}H_{14}Cl_3FN_4OS$	12.44 [12.42]
3l	H	3,4-dimethoxy	170–72	65	$C_{19}H_{13}Cl_2FN_4O_2S$	12.44 [12.41]
3m	CH_3	3,4-dimethoxy	180–82	70	$C_{20}H_{15}Cl_2FN_4O_2S$	12.06 [12.00]
3n [f]	C_2H_5	3,4-dimethoxy	155–57	75	$C_{21}H_{17}Cl_2FN_4O_2S$	11.71 [11.70]
3o [g]	C_3H_7	3,4-dimethoxy	165–67	70	$C_{22}H_{19}Cl_2FN_4O_2S$	11.38 [11.33]
3p	C_6H_5	3,4-dimethoxy	170–73	65	$C_{25}H_{17}Cl_2FN_4O_2S$	10.64 [10.63]
3q [h]	$H_3CO–C_6H_4$	3,4-dimethoxy	210–12	80	$C_{26}H_{19}Cl_2FN_4O_3S$	10.07 [10.01]

[a] ^{1}H NMR (δ ppm): 2.49 (s, 3H, $–CH_3$); 6.74 (s, 1H, =CH–); 6.62 (s, 2H, –O–CH_2–O); 7.3–7.7 (m, 5H, aromatic protons). MS; m/z: 448 (M^+, 65%), 259 ($M^+ - 189$, 100%).

[b] ^{1}H NMR (δ ppm): 3.87 (t, 2H, [$J = 6$ Hz], CH_2); 2.72 (d, 3H, [$J = 6$ Hz], CH_3); 7.03–8.20 (m, aromatic protons).

[c] ^{1}H NMR (δ ppm): 2.5 (s, 3H, CH_3); 7.03 (s, 1H, =CH–); 7.59 (m, 4H, aromatic protons); 7.90–7.91 (d, 1H, [$J = 9$Hz]); 8.05–8.07 (d, 1H [$J = 6.5$ Hz]).

[d] ^{1}H NMR (δ ppm): 1.38 (t, 3H, [$J = 6$ Hz], $–CH_3$); 2.90 (q, 2H, [$J = 6$ Hz], $–CH_2$); 6.66 (1H, =CH); 7.36 (d, 3H, [$J = 9$Hz]); 7.62 (d, 1H [$J = 6$ Hz]).

[e] MS; m/z: 450 (M^+, 45%), 163 [100%, 2,4-dichloro-5-fluorophenyl cation).

[f] ^{1}H NMR (δ ppm): 1.38 (t, 3H, [$J = 6.0$ Hz], $–CH_3$); 2.87 (q, 2H, [$J = 6$ Hz], CH_2); 6.62 (s, 1H, =CH–); 7.03–7.08 (m, 3H, aromatic protons); 7.34 (d, 1H, [$J = 9$Hz], aromatic protons); 7.62 (d, 1H, [$J = 6$ Hz], aromatic protons). MS; m/z: 478 (M^+), 163 (100%, 2,4-dichloro-5-fluorophenyl cation).

[g] ^{1}H NMR (δ ppm): 0.95 (t, 3H, [$J = 6$ Hz], $–CH_3$); 1.6 (sextet, 2H, [$J = 6$ Hz], $–CH_2$); 2.79 (t, 2H, [J = 6 Hz], $–CH_2$); 6.55 (s, 1H, =CH–); 7.00–7.64 (m, 6H, aromatic protons).

[h] ^{1}H NMR (δ ppm): 3.70–3.94 (m, 9H, $–OCH_3$); 6.66 (s, 1H, =CH–); 7.38 (d, 1H, [$J = 9$ Hz], aromatic proton); 7.62 (d, 1H, [$J = 6$ Hz]), aromatic proton); 6.91–7.10 (m, 7H, aromatic protons). MS; m/z: 521 (100%, $M^+ - 35$), 367 (28%, $M^+ -$2,4-dichloro-5-fluorophenyl radical).

dioxy moiety was seen at δ 6.62. The aromatic protons resonated as multiplets in the region δ 7.3–7.7 integrating for five protons. ^{1}H NMR spectrum of the compound **3n** showed a triplet centred at δ 1.38 ($J = 6.0$ Hz) due to $–CH_3$ protons. The signal due to CH_2 protons was seen as a quartet centred at δ 2.87 ($J = 6.0$ Hz). The exocylic vinylic proton resonated as a singlet at δ 6.62. The signals due to three aromatic protons of the 3,4-dimethoxyphenyl ring were seen as closely packed multiplets at δ 7.03–7.08 while two aromatic protons of the 2,4-dichloro-5-fluorophenyl ring appeared as two doublets centred at δ 7.34 ($J = 9$ Hz) and at δ 7.62 ($J = 6$ Hz).

The mass spectrum of compound **3b** showed a molecular ion peak at m/z 448, which is in conformity with the its molecular formula $C_{19}H_{11}Cl_2FN_4OS$. The base peak was seen at m/z 259, which could be attributed to the formation of a cation after elimination of the 2,4-dichloro-5-fluorobenzonitrile radical from the molecular ion. The mass spectra of compounds **3i**, **3n** and **3q** were also recorded and were found to be consistent with the assigned molecular formulae.

Table 2
Antibacterial activities of 7-arylidene-6-[2,4-dichloro-5-fluorophenyl]-3-substituted-1,2,4-triazolo [3,4-*b*]-1,3,4-thiadiazines (**3**) [a]

Compound	*B. subtilis*	*E. coli*	*S. aureus*	*P. aeruginosa*
3b	5.0	5.0	5.0	5.0
3c	1.25	5.0	1.25	5.0
3f	2.5	1.25	1.25	1.25
3g	5	10.0	5.0	1.25
3h	1.25	10.0	10.0	>10.0
3j	1.25	10.0	10.0	>10.0
3j	1.25	1.25	5.0	>10.0
3k	2.5	5.0	5.0	5.0
3m	0.625	0.312	0.625	0.312
3n	0.625	0.312	0.312	0.312
3q	0.625	2.50	2.50	5.0
Furacin	>10	6.0	>10	>10

[a] Minimum inhibitory concentrations in micrograms per millilitre.

3. Biological activity

3.1. Antimicrobial activity

Ten newly synthesised triazolothiadiazines **3** were screened for their antibacterial activities against *S. aureus*, *P. aeruginosa*, *E. coli* and *B. subtilis* by the serial dilution method [11] Furacin was used as standard drug. Among the tested compounds, almost all the newly synthesised compounds showed moderate to good antibacterial activities (Table 2). Compound **3n** showed the highest antibacterial activity against *S. aureus*, *P. aeruginosa* and *E. coli*. Hence, it was decided to carry out the acute toxicity studies of this compound.

3.2. Toxicity studies

For compound **3n**, which showed the highest antibacterial activity, toxicity studies were conducted according to the method suggested by Miller and Tainter [12]. Albino mice of either sex weighing 20–30 g were starved (with free access to water) for 18 h prior to the experiment. The compound was used as a suspension in 2% gum acacia in a fixed volume of 4 ml/kg. The route of administration was intraperitonial (i.p).

The animals were observed continuously for 2 h and once in 30 min for another 24 h for any changes in their behaviour, movements, gait, writhing reflex, etc. At the end of 24 h following drug administration the percentage of mortality was calculated. Up to the dose of 150 mg/kg neither mortality nor behavioural change was observed; at the dose of 300 mg/kg one-fourth of the animals died immediately with convulsions, the remaining died within 24 h. At intermediate doses grooming, rearing and loss of writhing reflex were observed in some of the animals.

The calculated i.p. LD_{50} was 195 ± 50 mg/kg.

Following oral administration LD_{50} was higher than 600 mg/kg.

3.3. Anticancer screening studies

Some of the newly synthesised compounds were screened for their anticancer activities at NIH, Bethesda, MD. The three-cell-line one-dose assay was done for the compounds **3b**, **3e**, **3g**, **3j**, **3m** and **3p**. Compounds **3b**, **3g**, **3m** and **3p** were found to be active in the preliminary screening studies. The three cell lines used in the present investgation are NCI-H 460 (lung), MCF 7 (breast) and SF 268 (CNS). In this current protocol each cell line is preincubated on a microtitre plate, the test agents are then added at a single concentration and the culture incubated for 48 h. End-point determinations are made with Sulphorhodamine B, a protein-binding dye. The results for each test agent are reported as the percent growth of the treated cells when compared with the untreated control cells. The compounds which reduce the growth of any one of the lines to 32% or less (negative numbers indicate the cell kill) are passed on for evaluation in the full panel of 60 cell lines over a five-log dose range. Compounds **3b**, **3g**, **3m** and **3p** are now found to be active against NCI-H 460 (lung), MCF7 (breast) and SF268 (CNS). The results of this study are given in Table 3. Further, 60-cell-line anticancer assay of these compounds have been carried out and their log 10 GI 50, log 10 TGI and log 10 LC_{50} values are given in Table 4.

Table 3
Anticancer activity data of triazolothiadiazine derivatives **3**

Compound	Sample concentration $\times 10^{-4}$ (M)	Growth percentage			
		NCI-H 460 (lung)	MCF-7 (breast)	SF 268 (CNS)	Activity
3b	1.00	45	22	37	active
3e	1.00	68	40	80	inactive
3g	1.00	−71	−72	−30	active
3j	1.00	76	68	77	inactive
3m	1.00	−65	−88	−87	active
3p	1.00	49	32	75	active

Table 4
Anticancer activity data (panel of 60 cell lines) of compounds **3b**, **3g**, **3m** and **3p**

Compound	Panel/cell line	Log 10 GI 50	Log 10 TGI	Log 10 LC_{50}
3b	leukaemia			
	MOLT-4	−4.42	> −4.00	> −4.00
	non-small cell lung cancer			
	EKVX	−4.64	> −4.00	> −4.00
	NCI-H 322 M	−4.62	> −4.00	> −4.00
	NCI-H 522	−4.69	−4.11	> −4.00
	ovarian cancer			
	OVCAR-3	−4.46	> −4.00	> −4.00
	OVCAR-5	−4.49	> −4.00	> −4.00
	breast cancer			
	MCF7	−4.45	> −4.00	> −4.00
	MDA-N	−4.41	> −4.00	> −4.00
3g	non-small cell lung cancer			
	EKVX	−5.03	−4.54	−4.08
	colon cancer			
	HCT-116	−5.03	−4.54	−4.08
	CNS-cancer			
	SF-295	−4.87	−4.45	−4.03
	melonoma			
	LOX IMVI	−5.03	−4.55	−4.08
	SK-MEL-2	−5.09	−4.58	−4.13
	ovarian cancer			
	OVCAR-5	−5.19	−4.55	> −4.00
	renal cancer			
	CAKI-1	−5.00	−4.38	−4.02
	UO-31	−4.95	−4.56	−4.18
	breast cancer			
	MDA-MB-231/ATC-C	−4.80	−4.32	−4.02
	NCI/ADR-RES	−4.93	−4.47	−4.05
3m	leukaemia			
	CCRF-CEM	−5.38	> −4.00	> −4.00
	K-562	−5.51	−4.71	> −4.00
	MOLT-4	−5.47	> −4.00	> −4.00
	RPMI-8226	−5.59	−4.95	> −4.00
	CNS cancer			
	SF-295	−5.52	−4.93	−4.45
	ovarian cancer			
	OVCAR-3	−5.34	−4.80	−4.36
	SK-OV-3	−4.52	−4.11	> −4.00
	renal cancer			
	786-0	−5.53	−5.02	−4.50
	breast cancer			
	MCF7	−5.23	−4.71	−4.23
	MDA-MB-435	4.98	−4.63	−4.29
	MDA-N	−5.27	−4.75	−4.36
3p	leukaemia			
	CCRF-CEM	−5.30	> −4.00	> −4.00
	HL-60(TB)	−6.24	> −4.00	> −4.00
	K-562	−5.01	> −4.00	> −4.00
	MOLT-4	−6.02	−4.16	> −4.00
	RPMI-8226	−5.22	> −4.00	> −4.00
	SR	−5.67	> −4.00	> −4.00
	non-small cell lung cancer			
	NCI-H522	−4.90	−4.42	> −4.00
	colon cancer			
	HCC-2998	−4.84	−4.33	> −4.00
	HCT-116	−5.20	> −4.00	> −4.00
	HCT-15	−4.72	> −4.00	> −4.00
	SW-620	−4.85	−4.04	> −4.00
	CNS cancer			
	SF-268	−4.68	> −4.00	> −4.00
	ovarian cancer			
	OVCAR-4	−4.74	> −4.00	> −4.00
	renal cancer			
	CAKI-1	−4.51	> −4.00	> −4.00
	RXF393	−4.93	−4.26	> −4.00
	breast cancer			
	HS 578T	−4.69	> −4.00	> −4.00
	MDA-MB-435	−4.86	> −4.00	> −4.00
	MCF7	−4.7	> −4.00	> −4.00

4. Experimental

Melting points were determined in open capillaries and are uncorrected. The IR spectra (KBr pellet) were recorded on a Perkin–Elmer model IR spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AC 300F NMR spectrometer. The mass spectra were recorded on a VG 70-S micromass spectrometer operative at 70 eV. Purity of the compounds was checked by TLC using the benzene/methanol (8:2) solvent system. α-Bromo-2-propen-1-ones (**2**), 2,4-dichloro-5-fluorophenacyl bromide (**5**) were prepared according to the methods proposed in the literature [7,13].

*4.1. General procedure for the preparation of 7-arylidene-3-substituted-6-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo[3,4,-b]-1,3,4-thiadizine (**3**)*

Method A: A mixture of 3-substituted-4-amino-5-mercapto-1,2,4-triazoles (**1**) (10 mmol), substituted α-bromo-2-propen-1-one (**2**) (10 mmol) and a solution of ethanolic potassium hydroxide (10%, 2.5 ml) in ethanol (25 ml) was kept under reflux on a water bath for about 5 h. The reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallised from suitable solvents. The analytical data are given in Table 1.

Method B: A mixture of 7*H*-3-substituted-6-(2,4-dichloro-5-fluorophenyl)-1,2,4-triazolo-[3,4-*b*]-1,3,4-thiadiazine (**4**) (10 mmol), substituted aromatic aldehyde (10 mmol) and piperidine (0.1 ml) in ethanol (25 ml) was kept under reflux on a water bath for 5 h. The reaction mixture was cooled and the precipitated solid was filtered, washed with water, dried and recrystallised from ethanol to yield the title compounds.

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