

1

3,637,731

**1-(ALKYLSUBSTITUTED PHENYL)IMIDAZOLES
USEFUL IN ACTH RESERVE ASSAY**

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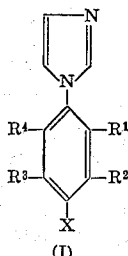
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7 Claims

ABSTRACT OF THE DISCLOSURE

Described and claimed are the 1-(alkylsubstituted-phenyl) imidazoles of the formula



wherein at least one of R^1 through R^4 is a saturated lower aliphatic group of up to six carbon atoms, the remaining R groups being hydrogen, and X is hydrogen with the proviso that X can be lower alkyl when both R^1 and R^4 are lower alkyl.

The compounds of this invention possess selective activity as inhibitors of steroid hydroxylation in the adrenal cortex and are thus useful in the ACTH reserve assay.

BACKGROUND OF THE INVENTION**(1) Field of the invention**

This invention relates to a novel group of 1-(alkyl-substituted phenyl) imidazoles possessing selective activity in warm-blooded animals as inhibitors of adrenocortical steroid hydroxylation.

The anterior pituitary gland secretes important hormones, one of which is the adrenocorticotrophic factor designated ACTH. This polypeptide of 39 amino acid residues, the structure of which was established by Bell and coworkers, acts upon the adrenal cortex stimulating the production of the adrenocortical hormones from the precursor progesterone. In this regard ACTH has been shown to mediate hydroxylation at the 11 β -, 18- and 21-positions of the steroid nucleus.

Diagnostic aids to determine adrenocorticotrophic ability have been sought for some time. It has long been known that the regular sequence of enzymatic hydroxylation reactions in the adrenal cortex is apparently blocked in certain endocrine diseases. The result is that certain factors fail to be elaborated while their precursors accumulate. But it is only more recently that the administration of certain non-steroidal agents has been found to inhibit adrenocortical steroid hydroxylations. Among these compounds are amphenone B [3,3-bis-(p-amino-phenyl)-2-butanone dihydrochloride] and SU-4485 also known as Metopirone [2-methyl-1,2-bis-(3-pyridyl)-1-propanone]. The latter compound appears to inhibit 11 β -hydroxylation specifically so that, when administered, the adrenal glands secrete 11-deoxycortisol instead of cortisol itself. The resumption of cortisol production after administration of SU-4485 is discontinued has suggested the use of this compound in testing pituitary ACTH reserve.

There is, however, a particular need for compounds useful in testing pituitary function that are less toxic than the compounds currently available. Such materials would

2

be useful not only for regulation of corticoid dosage but also possibly for the control of hypertension and electrolyte balance.

Accordingly, it has been found that the novel imidazoles of this invention are good diagnostic aids for the ACTH reserve assay, i.e. for testing pituitary function, in that they inhibit adrenocortical steroid hydroxylation in warm-blooded animals.

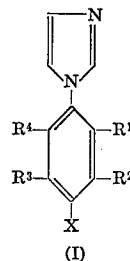
(2) Description of the prior art

Several 1-(substituted aromatic) imidazoles bearing a rather close structural relationship to the compounds of the present invention were synthesized by A. Wohl and W. Marckwald late in the nineteenth century. Preparation of 1-(phenyl) imidazole was reported by these workers at Ber. 22, 568 (1889) and Ber. 22, 1353 (1889). Preparation of 1-(4-methylphenyl) imidazole, 1-(2,4-dimethylphenyl) imidazole and 1-(1-naphthyl) imidazole was disclosed at Ber. 25, 2354 (1892). None of these compounds has been reported to act as an inhibitor of steroid hydroxylation in the adrenal cortex or to be useful in the ACTH reserve assay.

F. W. Kahnt and R. Neher in *Helv. Chim. Acta* 49, 725 (1966) describe the steroid hydroxylation blocking activity at the 11 β -, 17 α -, 18- and 19-positions of the steroid nucleus of 1-, 2- and 4(5)-(benzyl) imidazoles. Unlike the compounds of the present invention in which the aromatic function is bonded directly to imidazole nitrogen, the compounds of Kahnt and Neher all possess a methylene link between imidazole and phenyl (i.e. they are of the benzyl type). Furthermore only in the case of 1-benzyl imidazole is there imidazole substitution on ring nitrogen.

SUMMARY OF DETAILS OF THE INVENTION

The new compounds of this invention are 1-(alkyl-substituted phenyl) imidazoles having the structure

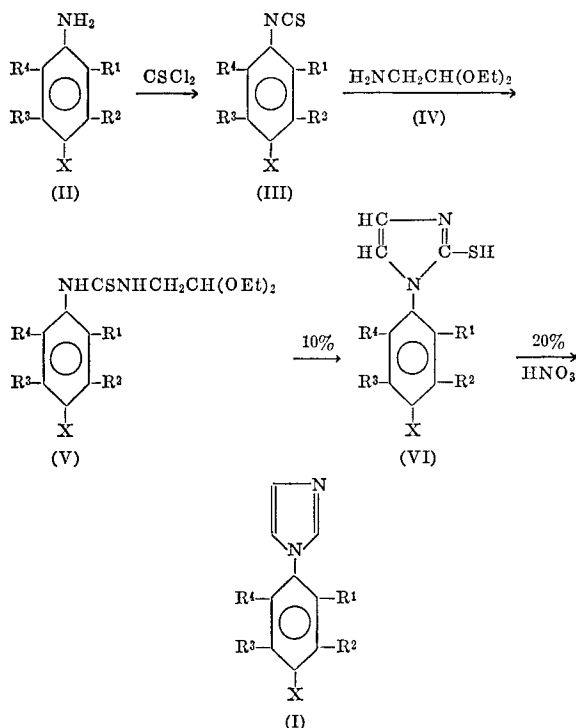


wherein at least one of R^1 through R^4 is a saturated lower aliphatic group of up to six carbon atoms, the remaining R groups being hydrogen, and X is hydrogen with the proviso that X can be lower alkyl. The particularly preferred compounds of this invention which possess a high degree of inhibition of adrenocortical steroid hydroxylation are those in which the groups R^2 , R^3 and X of Formula I are hydrogen and the groups R^1 and R^4 of Formula I are aliphatic or cycloaliphatic groups which taken together have a total of 2 to 8 carbon atoms or which taken individually have up to 6 carbon atoms each. Also quite active and useful are those compounds in which the groups R^1 and R^2 , whatever they may be, are substituted with aliphatic or cycloaliphatic groups as previously described. In the most active compounds of this invention either or both of R^1 and R^4 possess at least 3 carbon atoms.

1-(alkylsubstituted phenyl) imidazoles (I) may be prepared by reacting an alkylphenyl isothiocyanate (III) with an aminoacetal (IV) to give an alkylphenyl acetalthiourea (V) which undergoes acid-catalyzed ring closure to produce the 2-mercaptoimidazole having alkylsubstituted phenyl in the 1-position (VI). The mercapto group is readily removed from the latter compound by treatment with an oxidizing agent to give the 1-(alkylsubstituted

3

phenyl) imidazoles of this invention (I). The process is illustrated by the following scheme:



4

two hours longer. The aqueous mixture was extracted three times with 50 ml. of chloroform, and the isothiocyanate (III) was isolated from the dried extract by distillation.

A solution of 30.0 g. (0.225 mole) of aminoacetal (IV) in 250 ml. of ethanol was treated with 0.225 mole of the isothiocyanate (III). After the addition was complete, the mixture was heated under reflux for 30 minutes and then stripped of ethanol. In a few cases, the intermediate aryl acetalylthiourea (V) was purified, but more usually it was converted directly the mercapto compound (VI) by heating for 30–60 minutes at reflux with 250 ml. of 10% aqueous hydrochloric acid. The mercaptans were isolated by cooling the acid solution to 0° and filtering the crystalline product. Purification was effected by recrystallization.

A slurry of the 2-mercaptoimidazole (VI) and four times its weight of 20% aqueous nitric acid was warmed on a steam bath in a large Erlenmeyer flask. After the vigorous reaction was over, the mixture was cooled, made basic with 15% aqueous ammonia and extracted with three portions of chloroform. The chloroform extracts were dried and evaporated to leave a residue of the crude imidazole which was purified by short-path distillation or by sublimation to give the 1-(alkylsubstituted phenyl) imidazole (I).

Tables I and II which follow list the various alkyl-substituted phenyl isothiocyanates of Formula III and the 1-(alkylsubstituted phenyl)-2-mercaptoimidazoles of Formula VI which were prepared as intermediates in the overall synthesis of the 1-(alkylsubstituted phenyl) imidazoles of Examples 1–11. All synthetic steps were carried out according to the general experimental scheme previously detailed.

TABLE I.—ALKYLSUBSTITUTED PHENYL ISOTHIOCYANATES (III)

No.	R ₁	R ₂	R ₃	R ₄	X	B.P. in ° C. (mm. Hg)	Yield percent
1.....	CH ₃	CH ₃	H	H	H	69 (0.1)	87
2.....	CH ₃	H	H	CH ₃	H	67 (0.4)	80
3.....	(CH ₃) ₂ CH	H	H	H	H	82 (1.8)	68
4.....	C ₂ H ₅	H	H	H	H	53 (0.1)	79
5.....	C ₂ H ₅	H	H	C ₂ H ₅	H	82 (0.35)	88
6.....	H	CH ₃	H	H	H	50 (0.1)	75
7.....	(CH ₃) ₂ C	H	H	H	H	60 (0.1)	82
8.....	CH ₃	H	H	H	H	84 (0.2)	84
9.....	(CH ₃) ₂ CH	H	H	(CH ₃) ₂ CH	H	75 (0.05)	50
10.....	—CH ₂ —CH ₂ CH ₂ —CH ₃	H	H	CH ₃	CH ₃	75 (0.25)	87
11.....	CH ₃	H	H	CH ₃	CH ₃		

TABLE II.—1-(ALKYLSUBSTITUTED PHENYL)-2-MERCAPTOIMIDAZOLES (VI)

No.	Recrystallization solvent	Ml./g.	Percent yield	M.P. (° C.)	Solvent	UV mμ.	Analysis (percent)					
							Calculated			Found		
							C	H	N	C	H	N
1.....	C ₂ H ₅ OH	20	45	263–265	C ₂ H ₅ OH	265	64.69	5.92	13.72	65.37	6.45	13.42
2.....	C ₂ H ₅ OH ¹	-----	72	303–304.5	C ₂ H ₅ OH	264	64.69	5.92	13.72	65.46	6.42	13.58
3.....	C ₂ H ₅ OH	6	68	225–226.5	C ₂ H ₅ OH	265	66.03	6.47	12.84	64.28	5.80	13.64
4.....	C ₂ H ₅ OH	4	87	200–201	C ₂ H ₅ OH	264	64.69	5.92	13.72	64.55	5.99	13.69
5.....	C ₂ H ₅ OH	6	57	193–195	C ₂ H ₅ OH	265	67.22	6.94	12.06	65.57	6.23	12.55
6.....	50% C ₂ H ₅ OH	18	82	147–149	C ₂ H ₅ OH	281	63.15	5.30	14.73	65.35	6.40	12.83
(Used directly for I)												
8.....	33% C ₂ H ₅ OH	15	73	243–245	C ₂ H ₅ OH	263	63.15	5.30	14.73	64.41	5.87	13.81
9.....	C ₂ H ₅ OH	8	82	243–245	C ₂ H ₅ OH	265	69.74	7.02	10.85	64.67	5.81	13.66
10.....	C ₂ H ₅ OH	6	87	213–215	C ₂ H ₅ OH	263	67.81	6.13	12.17	67.57	6.61	12.12
11.....	C ₂ H ₅ OH	20	38	272–274	C ₂ H ₅ OH	264	66.03	6.47	12.84	67.57	6.78	12.25
							66.46	6.17	13.03	63.36	5.29	14.81
							66.60	5.90	13.06	63.22	5.23	14.70

¹ Extraction.

² Decomposition.

The experimental details involved in preparation of the 1-(alkylsubstituted phenyl) imidazoles of this invention according to the foregoing synthetic scheme are as follows:

To a rapidly stirred mixture of 24.9 g. (0.216 mole) of thiophosgene and 350 ml. of water there was added slowly 0.20 mole of the appropriately substituted aniline (II). After the addition was complete, stirring was continued for

EMBODIMENTS OF THE INVENTION

There follows in Table III a set of examples illustrative of the present invention. All compounds comprising the entries in this table were prepared through the intermediates enumerated in Tables I and II according to the general experimental scheme previously described.

TABLE III.—1-(ALKYLSUBSTITUTEDPHENYL) IMIDAZOLES (I)

Example	Compound	B.P. (° C.)	Mm.	M.P. (° C.)	Percent Yield	Solvent	UV, μ		Analysis (percent)					
							λ_{max}	ϵ_{max}	Calculated			Found		
									C	N	H	C	H	N
1	1-(2,3-dimethylphenyl) imidazole	80	0.01	53.5-56.5	60	C ₂ H ₅ OH	272	322	76.71	7.02	16.27	76.67	7.26	16.69
2	1-(2,6-dimethylphenyl) imidazole	80	0.1	82-82.5	90	C ₂ H ₅ OH	264	399	76.80	7.02	16.27	76.80	7.26	16.96
3	1-(2-isopropylphenyl) imidazole	80	0.1	67-68	91	C ₂ H ₅ OH	272	430	76.71	7.02	16.27	76.39	6.75	16.45
4	1-(2-ethylphenyl) imidazole	100	0.25	---	91	C ₂ H ₅ OH	265	452	77.38	7.58	15.04	77.53	7.89	15.51
5	1-(2,6-diethylphenyl) imidazole	95	0.1	72.5-74	91	C ₂ H ₅ OH	261	350	76.71	7.02	16.27	77.44	7.87	15.46
6	1-(3-methylphenyl) imidazole	80	0.1	---	91	C ₂ H ₅ OH	268	303	76.71	7.02	16.27	77.21	7.33	15.57
7	1-(2-ethylphenyl) imidazole	95	0.1	---	91	C ₂ H ₅ OH	262	366	77.96	8.05	13.99	77.16	7.15	15.84
8	1-(2-methylphenyl) imidazole	75	0.05	---	78	C ₂ H ₅ OH	272	477	75.92	6.37	17.71	77.82	8.44	14.02
9	1-(2,6-diisopropylphenyl) imidazole	105	0.1	123-125	62	C ₂ H ₅ OH	264	514	75.92	6.37	17.71	78.23	8.67	14.24
10	1-[8-(1,2,3,4-tetrahydronaphthyl)]-imidazole	90	0.5	68-70	69	C ₂ H ₅ OH	282	7850	77.96	8.05	13.99	75.69	6.56	13.13
11	1-(2,4,6-trimethylphenyl) imidazole	100	0.1	106-109	69	C ₂ H ₅ OH	268	667	77.96	8.05	13.99	77.41	8.18	14.01
					75	C ₂ H ₅ OH	269	343	75.92	6.37	17.71	77.58	8.09	13.99
					80	C ₂ H ₅ OH	271	410	79.60	8.02	12.38	76.24	6.61	13.13
					79	C ₂ H ₅ OH	263	450	78.75	7.12	14.13	75.90	6.48	17.91
					67	C ₂ H ₅ OH	290	141	78.61	8.92	12.12	78.79	8.86	12.20
							273	665	78.22	6.76	14.27	78.54	6.76	14.29
							266	684	76.83	7.70	14.15	76.83	7.70	14.15
							283	864	77.38	7.58	15.04	76.61	7.44	15.04
							271	1,060						

In addition to the compounds specifically described in Table III, several closely related compounds are within the scope of this invention and are useful in the ACTH reserve assay. For example, when the process of the preceding examples is repeated using o-(2-methylbutyl) aniline, 2,4,6-tri-tert-pentylaniline, o-hexylaniline (from aniline and hexene-1 with Al/CiCl₃ at 290° and 240 atm.), 4-indanamine, o-cyclopropylaniline, o-cyclopentylaniline, and o-cyclohexylaniline, the compounds of this invention produced are 1 - (2 - [2 - methylbutyl] - phenyl) imidazole, 1 - (2,4,6 - tri - tert - pentylphenyl) imidazole, 1 - (2 - hexylphenyl) imidazole, 1 - (4 - indanyl) imidazole, 1 - (2 - cyclopropylphenyl) imidazole, 1 - (2 - cyclohexylphenyl) imidazole.

All of the compounds of this invention are in vitro and in vivo inhibitors of steroid hydroxylases, particularly the 11 β -, 18- and 21-hydroxylases. They are useful in diagnosis and evaluation of the functional status of the hypothalamic-anterior pituitary. The following procedure illustrates the method by which the activity of the hydroxylation inhibitors of this invention may be demonstrated.

EXAMPLE A

(1) Enzyme preparation

Bovine adrenal glands were collected as soon as possible after slaughter, freed from connective tissues and adhering fat and placed in Dry Ice. The frozen glands were stored at -20° and processed within 48 hours.

The following operations were carried out in a cold room at 4°. The adrenal cortex of the glands was removed while still frozen and was homogenized with three times its weight of ice-cold 0.25 M sucrose solution, first in a Waring Blendor at ¼ speed for two minutes and then in a Potter-Elvehjem homogenizer. The homogenate was centrifuged at 700×g for 15 minutes to obtain the mitochondrial fraction. This step was followed by centrifugation of the supernatant fluid at 105,000×g for 60 minutes to sediment the microsomes.

The mitochondria and the microsomes were washed by resuspension of these fractions in twice their weight of 0.25 M sucrose solution and centrifugation at 700×g and 105,000×g for 45 minutes respectively.

The mitochondrial and microsomal pellets were resuspended in three times their weight of distilled water and lyophilized. The freeze-dried preparation was stored at -20° under desiccation.

The mitochondrial preparation was used for studying the 11 β -hydroxylation of 11-deoxycorticosterone, the microsomal fraction for C-21 hydroxylation of progesterone and pregnenolone in vitro.

(2) Incubations

All incubations were carried out in 20 ml. beakers at 37° in air with shaking in a Dubnoff Metabolic Incubator. The incubation beakers contained, besides substrate and enzyme, the following components: 3.5 μ moles of TPN (triphosphopyridine nucleotide); 22 μ moles of glucose-6-phosphate; 1 Kornberg unit of glucose-6-phosphate-dehydrogenase; 5 μ moles of MgCl₂; 200 μ moles of Tris buffer [i.e. tris(hydroxymethyl)-aminomethane] at pH 7.4 in a total volume of 4 or 5 ml. The substrate was added to the beakers in 0.05 ml. of propane-1,2-diol before other additions.

After incubation the steroids were extracted with 45 ml. of methylene chloride and centrifuged at 0° for 10 minutes, and the lower layer was quantitatively transferred to another tube and evaporated under nitrogen. The extracted steroids were dissolved in a definite volume of methylene chloride and suitable aliquots were taken for the estimation of products.

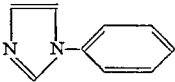
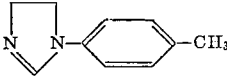
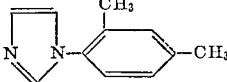
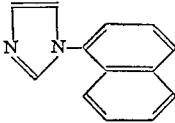
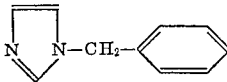
(3) Assay methods

To determine the effect of the compounds tested on steroid 11 β -hydroxylation, 11-deoxycorticosterone was

employed as the substrate. The product, corticosterone, was estimated fluorometrically (Moncloa et al., Endo-

standard "Metopirone" the hydroxylation blocking activity of several prior art compounds.

TABLE V

Compound	Reference	Activity ("Metopirone"=1)
	A. Wohl and W. Marekwald <i>Ber.</i> 22 568 1,353 (1889).....	0.05
	W. Marekwald <i>Ber.</i> 25 2,354 (1892).....	0.05
	W. Marekwald <i>Ber.</i> 25 2,354 (1892).....	2
	W. Marekwald <i>Ber.</i> 25 2,354 (1892).....	2
	F. Kahnt and R. Neher <i>Helv. Chim. Acta</i> 49 725 (1766).....	

crinology, 65, 717 [1959]) as described below:

Aliquots of the sample corresponding to about 0.2, 0.4, 0.6, 1.0 and 2.5 μ g. of corticosterone were taken in thick-wall glass stoppered tubes with a capacity of 10-11 ml. The solvent was blown off under a stream of nitrogen, and the residue was dissolved in 2 ml. of 13% ethanol. Each sample was washed with 5 ml. of petroleum ether by shaking for one minute, centrifuging for five minutes and removing the upper layer by aspiration. The sample was next extracted with 4 ml. of methylene chloride, and the aqueous phase was removed by aspiration. To the methylene chloride extract was added 1 ml. of ice-cold 0.1 N sodium hydroxide solution; the tube was shaken for 15 seconds, centrifuged for 5 minutes, and the sodium hydroxide layer was carefully removed. Three ml. of sulfuric acid reagent was then added; the tube was then shaken vigorously for 1 minute and finally centrifuged for 5 minutes. The sulfuric acid phase was removed and its fluorescence was determined after 50 minutes in an Aminco Bowman spectrophotofluorometer. For corticosterone the monochromator for activation wave length was set at 470 $m\mu$ and that for recording the fluorescence at 515 $m\mu$. With each set of observations, a five point curve for six samples (0.3 to 2.4 μ g.) of corticosterone was established as the standard.

Table IV compares the activity of the commercially available steroid hydroxylation inhibitor SU-4885 ("Metopirone") with that of the 1-alkylsubstitutedphenyl imidazoles of this invention. The "Metopirone" has been given an activity of unity in the procedure detailed above.

TABLE IV

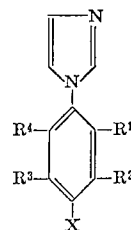
Example	Compound	Activity
1.....	1-(2,3-dimethylphenyl) imidazole.....	1.0
2.....	1-(2,6-dimethylphenyl) imidazole.....	1.0
3.....	1-(2-isopropylphenyl) imidazole.....	2.0-4.0
4.....	1-(2-ethylphenyl) imidazole.....	1.0
5.....	1-(2,6-diethylphenyl) imidazole.....	2.0
6.....	1-(3-methylphenyl) imidazole.....	0.3
7.....	1-(2-t-butylphenyl) imidazole.....	2.0-4.0
8.....	1-(2-methylphenyl) imidazole.....	0.33
9.....	1-(2,6-diisopropylphenyl) imidazole.....	2.0
10.....	1-[8-(1,2,3,4-tetrahydronaphthyl)]-imidazole.....	2.0
11.....	1-(2,4,6-trimethylphenyl) imidazole.....	1.0

Table V presents for comparison with the commercial

Since obvious modifications and equivalents in the invention will be evident to those skilled in the chemical arts, I propose to be bound solely by the appended claims.

The embodiments of the invention in which an exclusive property or privilege is claimed are defined as follows:

1. 1-(alkylsubstitutedphenyl)imidazole of the formula



wherein $X=R^3=H$; and the substituents R^1 , R^2 and R^4 are selected from the groupings: R^1 =isopropyl and $R^2=R^4=H$; R^1 is ethyl and $R^2=R^4=H$; $R^1=R^4$ =ethyl and $R^2=H$; R^1 is tertiary butyl and $R^2=R^4=H$; $R^1=R^4$ =isopropyl and $R^2=H$; and R^1 and R^2 together are $-CH_2-CH_2-CH_2-CH_2-$ and $R^4=H$.

2. A compound of claim 1 wherein R^1 =isopropyl and $X=R^2=R^3=R^4$ =hydrogen, 1 - (2 - isopropylphenyl) imidazole.

3. A compound of claim 1 wherein R^1 =ethyl and $X=R^2=R^3=R^4$ =hydrogen, 1-(2-ethylphenyl)imidazole.

4. A compound of claim 1 wherein $R^1=R^4$ =ethyl and $X=R^2=R^3$ =hydrogen, 1 - (2,6-diethylphenyl)imidazole.

5. A compound of claim 1 wherein R^1 =t-butyl and $X=R^2=R^3=R^4$ =hydrogen, 1 - (2-t-butylphenyl)imidazole.

6. A compound of claim 1 wherein $R^1=R^4$ =isopropyl and $X=R^2=R^3$ =hydrogen, 1-(2,6-diisopropylphenyl) imidazole.

7. A compound of claim 1 wherein R¹ and R² together are —CH₂—CH₂—CH₂—CH₂— and X=R³=R⁴=hydrogen, 1-[8-(1,2,3,4-tetrahydronaphthyl)]imidazole.

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5 NATALIE TROUSOF, Primary Examiner

U.S. Cl. X.R.

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