1, 2, 4 - Triazoles, III[1]:

New 1,5-Diaryl-3-(substituted amino)-1H-1,2,4-triazoles as Anti - inflammatory Agents

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A series of 1,5-diaryl-3-(substituted amino)-1 *H*-1,2,4-triazoles was synthesized and assayed in the rat adjuvant induced arthritis model. Several compounds showed significant anti-inflammatory activity.

1,2,4-Triazole, 3.Mitt.[1]:
Neue 1,5-Diaryl-3-(substituierte amino)-1H-1,2,4-triazole als antiphlogistische Wirkstoffe

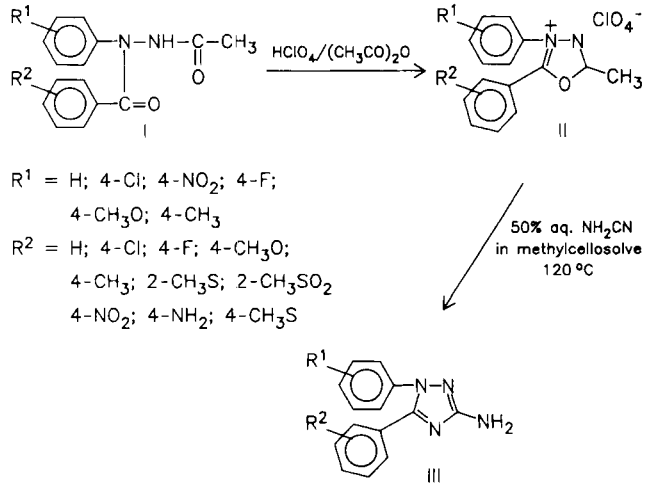
Eine Serie von 1,5-Diaryl-3-(substituierten amino)-1*H*-1,2,4-triazolen wurde hergestellt und auf ihre antiphlogistische Aktivität an Ratten im Adjuvans-Arthritis-Test untersucht. Einige dieser Verbindungen zeigten günstige antiphlogistische Aktivität.

Previously we reported the synthesis of several diaryl-1*H*-1,2,4-triazoles[2], which have useful anti-inflammatory activity. In a continuing search for new and useful agents for the treatment of inflammatory diseases we now report the synthesis of 1,5-diaryl-3-(substituted amino)-1*H*-1,2,4-triazoles[3]. This paper also describes some features of the structure-activity relationships /SAR/ observed in this series.

The known methods[4] described for the synthesis of 3-amino-1*H*-1,2,4-triazoles were not useful for the preparation of the title compounds.

On the basis of *Boyd's* experiences[5] we have developed a general synthetic route for the preparation of 1,5-diaryl-3-(substituted amino)-1*H*-1,2,4-triazoles.

The 1,3,4-oxadiazolium salts **II** were prepared from the N-benzoyl-N'-acetyl-phenylhydrazines **I** and acetic anhydride/perchloric acid below 50 °C, as described[6]. Reaction of **II** with cyanamide at 120 °C in methylcellosolve led to the 3-amino-1,5-diaryl-1*H*-1,2,4-triazoles **III** in high yield (Scheme 1).



Scheme 1

1,5-Diaryl-3-(substituted amino)-1*H*-1,2,4-triazoles [**IV**, **V**, and **VII**] were obtained by alkylation or acylation of **III**, respectively (Scheme 2).

It is interesting that the direct acylation of **III** with sulfonyl chlorides yielded the diacylated compounds **VI a, b**. Reaction of **III** with benzoyl chlorides yielded both the diacylated and monoacylated compounds **VI c** and **VII c**. Reaction of **VI a, b, c** with $NaHCO_3$ led to the monoacylated derivatives **VII a, b, c**.

VI d was obtained with diethyl pyrocarbonate and **VII d** with ethyl chloroformate.

The structures of **1** - **42** were characterized by satisfactory microanalyses and predominantly by IR- and mass spectral data. In the IR-spectra were found the typical μ_{NH_2} bands between 3416 and 3491 cm^{-1} (**1-17**), amide-I bands between 1664 and 1755 cm^{-1} (**18-21**; **28-30**; **36-42**), for sulfonamides (**31-35**) characteristic μ_{NH} bands in the 3202-3231 cm^{-1} region. For methylated compounds (**22-26**) the characteristic methyl signals were found at 2.83-3.1 ppm in their ^{1}H-NMR specra.

Pharmacological methods

Carrageenin-induced oedema (Winter's method[7])

Groups of 5 female CFY rates weighing 120-130 g (LATI Gödöllö) were used. The volume of the right paw was measured by *Lence's* plethysmometer and the compounds were immediately administered at 10, 30 and 50 mg kg/d orally (0.5 ml/100 g with 0.1% carboxymethylcellulose). As a phlogist, carrageenin (0.1 ml 1% in saline) was injected subcutaneously to the right hind paw 1 h later. Thereafter, the volume of the paw was measured 3 h after injection of carrageenin to calculate the swelling rate from the value of pretreatment with phlogist. The experiment was repeated twice to compare the mean value in compounds pretreated group with that in control group.

Cotton pellet-induced granuloma (Winter's method[8])

Groups of 5 male CFY rats weighing 150-180 g were used. Compounds administered orally for 7 days at 6.25, 12.5 and 50 mg/kg, reduced the size of the granuloma formed around a cotton pellet implanted in the axillary space of rats. After 8 days cotton pellets formed were removed, dried over

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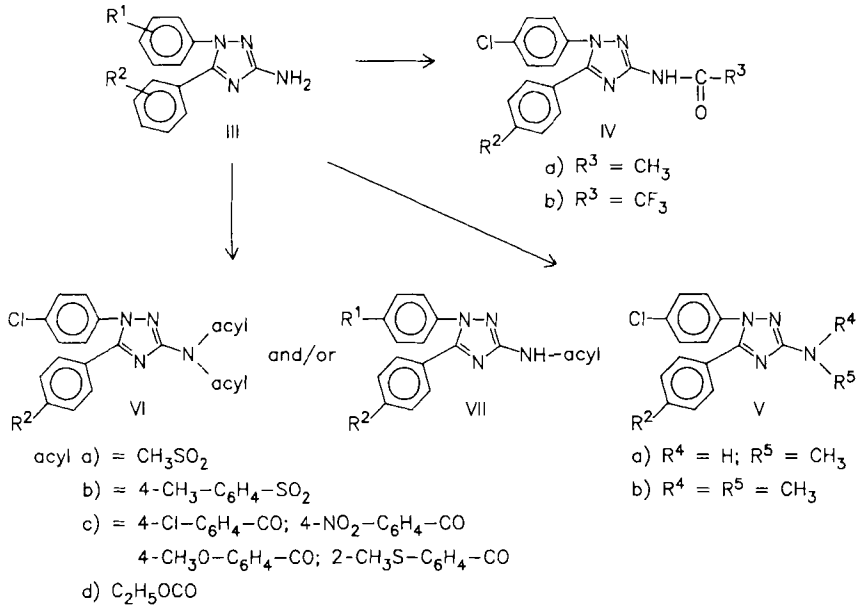

Scheme 2

24 h at room temp. and weighed giving the weight of the connective tissue formed. The experiment was repeated twice.

Adjuvant-induced arthritis (Newbould's-preventive test[9])

Adult Long Evans male rats weighing 200-250 g (Small Animal Breeding Institute Gödöllö) were used. Arthritis was induced in rats by injecting heat-killed Mycobacterium tuberculosis ravenel (*Freund* adjuvant) in 0.1 ml of liquid paraffin suspension into the left hind paw on day 1. Compounds (dissolved in water containing Tween 80) were administered orally at low doses (6.25 and 12.5 mg/kg for 21 days starting on day 1). Control animals were given vehiculum.

Plethysmographic measurements of the primary lesion (injected paw) and the secondary lesion (non-injected paw) were made by mercury displacement on day 0, 3, 6, 10, 13, 17, 20, and 22 after injection of phologist compared with controls.

Phenylquinone-induced writhing in mice[10]

Groups of 5 male CFLP mice weighing 20-25 g were used. Compounds were administered orally at 10, 30 and 100 mg/kg to mice 30 min before intraperitoneal administration of phenylquinone (0.2 ml of a 0.05% solution in 5% ethanol). The analgesic effect was manifested as a reduction in the number of characteristic writhes induced by phenylquinone during 20 min after injection of phlogist. The experiments were repeated twice.

Results

Table 6 contains the biological results of the diphenyl triazoles prepared by us. As a result the general biological features can be summarized, as follows:

1. diphenyltriazoles are less potent or inactive in carrageenin-induced oedema than reference substances
2. they show only a weak analgesic effect in case of single treatment in phenylquinone-induced writhing test
3. their strong inhibitory effects on adjuvant-induced arthritis test refer to some immunological events.

On the basis of the biological data important structure-activity relationships are:

1. *Aryl substitution*

The most active compounds contain halogeno substituents in the para position (e.g. **5**), in some cases methylthio derivatives (e.g. **10**) showed also high activity.

2. *Amino substituent*

The highest activity resided in the unsubstituted amino compounds, although the monomesylated ones proved to be equipotent (e.g. **5**, **9**, **10** or **32**, **34**, respectively). Substitution of the amino group by a benzoyl or ethoxycarbonyl group were found to reduce the anit-inflammatory activity sharply.

The methylated or acetylated compound were less active than the unsubstituted ones.

On the basis of the results obtained compounds **5**, (3-amino-1-(4-chloropheny)-5-(4-fluoropheny)-1*H*-1,2,4-triazole) and **34** (1,5-bis(4-fluorophenyl)-3-methanesulfonamido-1*H*-1,2,4-triazole) were selected for detailed studies (Table 7)

Conclusion

The anti-inflammatory activity of diphenyltriazoles may be due to their immunomodulatory activity and the inhibition of PG-biosynthesis. The most active compound **5** without accumulation in the blood serum and organs shows an anti-inflammatory activity like naproxen in the same activity range.

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Table 1: Anti-inflammatory activity

Comp.	Inhibition of adjuvant induced arthritis in rats* (preventive adm.) % in 6.25 mg/kg po	Comp.	Inhibition of adjuvant induced arthritis in rats* (preventive adm.) % in 6.25 mg/kg po
1	33	**22**	0
2	0	**23**	37
3	46	**24**	73
4	28	**25**	34
5	64	**26**	70
6	0	**31**	30
7	47	**32**	75
8	0	**33**	29
9	63	**34**	64
10	73	**36**	15
11	0	**37**	39
12	17	**38**	4
14	0	**40**	0
18	14	**42**	32
19	30	Naproxen	44

* The %-inhibitory effect shows the decrease of the hind-paw volume after treatment in comparison with the increase of the hind-paw volume of controls.

Table 2

Test	Compound **5**	Compound **34**	Naproxen
inhibition of macrophage PG-biosynthesis % in 10^{-6}M PGI_2/PGF_2	48/27	35/12	42/16
inhibition of carrageenin-induced oedema in rats % in 50 mg/kg po	28	0	51 (ED_{50}:7.8)
inhibition on cotton pellet-induced oedema in rats % in 6x12.5 mg/kg po	30	0	37
inhibition of adjuvant-induced arthritis in rats (preventive admin.), ED_{50}	9.7	7.8	12.0
inhibition of phenylquinone-induced writhing in mice % in 10x6.25 mg/kg po	53	0	ED_{50}:4.7

Experimental Part

Mp: Boetius apparatus, uncorr. Analyses: C, H, N, S and halogen were within ± 0.5% of the theoretical values.

II were prepared by Boyd's method [6] (s. Table 8). - Compounds of types **III** - **VII** were recrystallized from EtOH.

General procedure for the preparation of **III**

A mixture of 27.1 mmole of **II**, 81.3 mmole of triethylamine and 54.2 mmole of cyanamide (50% aqueous solution) in 50 ml of methylcellosolve was heated for 4-6 h at 120 °C, then poured into 500 ml of water. The precipitate formed was filtered, washed with water and dried. Yield: 16-91%.

Table 3: Chemical data of compounds II

Comp. II	R^1	R^2	Mp (°C)	Yield %
1	H	H	209-212	92
2	4-Cl	4-Cl	271-276	98
3	4-F	4-F	250-253	92
4	4-F	4-Cl	247-252	80
5	4-Cl	4-F	243-245	94
6	4-Cl	4-CH_3O	224-225	88
7	4-F	4-CH_3	180-185	64
8	4-Cl	4-CH_3	230-234	88
9	4-CH_3	4-Cl	217-224	85
10	4-Cl	2-CH_3S	190-196	62
11	4-NO_2	4-Cl	250-255	93
12	4-Cl	4-NO_2	272-278	81
13	4-F	H	218-224	77
14	4-Cl	4-NH_2	-	-
15	4-CH_3O	4-CH_3O	160-163	47
16	4-Cl	2-CH_3SO_2	232-235	56
17	4-Cl	4-CH_3S	115-120	98

3-Acetamido-1,5-bis(4-chlorophenyl)-1H-1,2,4-triazole (**18**)

A mixture of 3 mmole of **III**, 15 mmole of acetic anhydride and 10 ml of dioxane was stirred for 1 h at 90 °C, then poured into 50 ml of water. Work-up as in **III**, yield: 90%.

General procedure for the preparation of **IV b**

A mixture of 3 mmole of **III**, 15 mmole of trifluoroacetic anhydride and 10 ml of dioxane was stirred for 1 h at room temp., then poured into 50 ml of water. Work-up as in **III**, yield: 82-99%.

General procedure for the preparation of **V a**

A mixture of 5 mmole of **III** and 7.5 ml of triethyl orthoformate was refluxed for 2 h. After evaporation to dryness the residue was stirred with a solution of 1.1 g of $NaBH_4$ in 20 ml of ethanol for 2 h at room temp. Then the solution was evaporated to dryness in vacuo, the residue was dissolved in 70 ml of ethyl acetate and washed with 70 ml of water, then dried over Na_2SO_4. After removal of the solvent, the residue was recrystallized from ethanol, yield: 71-98%.

General procedure for the preparation of **V b**

A mixture of 5 mmole of 3M H_2SO_4 and 1.05 ml of 35% HCHO was added dropwise during 15 min to a mixture of 2 mmole of **III**, 1.5 mmole of $NaBH_4$ and 14 ml of THF at -10°C, then the mixture was stirred for 2 h at 20°C, the solution was alkalized to pH 8 with 10% NaOH, extracted with CH_2Cl_2, dried over Na_2SO_4, and the solvent was removed in vacuo. Yield: 57-96%.

General procedure for the preparation of **VI a**

15 mmole of methanesulfonyl chloride was added dropwise during 10 min to a solution of 5 mmole of **III** in 15 ml of pyridine at 5°C, then the reaction mixture was stirred for 5 h at room temperature and finally poured into 30 ml of water. - Work-up as in **III**, yield: 75%.

Table 4: Chemical data of the compounds **III**

Comp. III	R^1	R^2	Mp (°C)	Yield (%)	Molecular formula (Mol.w.)
1	H	H	155-156	85	$C_{14}H_{12}N_4$ (236.3)
2	4-Cl	4-Cl	172-173	84	$C_{14}H_{10}Cl_2N_4$ (305.2)
3	4-F	4-F	142-143	75	$C_{14}H_{10}F_2N_4$ (272.3)
4	4-F	4-Cl	136-137	81	$C_{14}H_{10}ClFN_4$ (288.7)
5	4-Cl	4-F	130-132	77	$C_{14}H_{10}ClFN_4$ (288.7)
6	4-Cl	4-CH_3O	174-175	88	$C_{15}H_{13}ClFN_4O$ (300.7)
7	4-F	4-CH_3	115-117	81	$C_{15}H_{13}FN_4$ (268.3)
8	4-Cl	4-CH_3	130-135	87	$C_{15}H_{13}ClN_4$ (284.7)
9	4-CH_3	4-Cl	150-151	91	$C_{15}H_{13}ClN_4$ (284.7)
10	4-Cl	2-CH_3S	126-128	34	$C_{15}H_{13}ClN_4S$ (316.8)
11	4-NO_2	4-Cl	218-221	71	$C_{14}H_{10}ClN_5O_2$ (315.7)
12	4-Cl	4-NO_2	220-222	81	$C_{14}H_{10}ClN_5O_2$ (315.7)
13	4-F	H	145-146	80	$C_{14}H_{11}FN_4$ (254.3)
14	4-Cl	4-NH_2	177-183	97	$C_{14}H_{12}ClN_5$ (285.7)
15	4-CH_3O	4-CH_3O	168-169	84	$C_{16}H_{16}N_4O_2$ (296.3)
16	4-Cl	2-CH_3SO_3	184-186	16	$C_{15}H_{13}ClN_4O_2S$ (348.8)
17	4-Cl	4-CH_3S	183-188	79	$C_{15}H_{13}ClN_4S$ (316.8)

Table 5: Chemical data of the compounds **IV**

Comp. IV	R^2	R^3	Mp (°C)	Yield (%)	Molecular formula (Mol.w.)
18	Cl	CH_3	175-178	90	$C_{16}H_{12}Cl_2N_4O$ (347.2)
19	Cl	CF_3	179-181	93	$C_{16}H_9Cl_2F_3N_4O$ (401.2)
20	F	CF_3	166-169	82	$C_{16}H_9ClF_4N_4O$ (384.7)
21	CH_3	CF_3	134-137	99	$C_{17}H_{12}ClF_3N_4O$ (380.8)

General procedure for the preparation of **VII a**

A mixture of 5 mmole of **VI a** in 40 ml of 6% $NaHCO_3$ was refluxed for 6 h, then acidified with 10% HCl under cooling. Work-up as in III, yield: 68-91%.

1,5-bis(4-Chlorophenyl)-3-(4-toluenesulfonamido)-1H-1,2,4-triazole(**35**)

15 mmole of 4-toluenesulfonyl chloride was added to a solution of 5 mmole of **III** in 15 ml of pyridine at 5°C, then the mixture was stirred for

Table 6: Chemical data of the compounds **V**

Comp. V	R^1	R^2	R^4	R^5	Mp (°C)	Yield (%)	Molecular formula (Mol.w.)
22	Cl	Cl	H	CH_3	188-190	71	$C_{15}H_{12}Cl_2N_4$ (319.2)
23	Cl	F	H	CH_3	186-190	98	$C_{15}H_{12}ClFN_4$ (302.7)
24	Cl	Cl	CH_3	CH_3	136-138	96	$C_{16}H_{14}Cl_2N_4$ (333.2)
25	F	F	CH_3	CH_3	99-100	96	$C_{16}H_{14}F_2N_4$ (300.3)
26	Cl	F	CH_3	CH_3	107-109	83	$C_{16}H_{14}ClFN_4$ (316.8)

Table 7: Chemical data of the compounds **VI**

Comp. VI	R^2	acyl	Mp (°C)	Yield (%)	Molecular formula (Mol.W.)
27	Cl	CH_3SO_2	248-251	75	$C_{16}H_{14}Cl_2N_4O_4S_2$ (461.3)
28	Cl	C_2H_5OCO	114-115	61	$C_{20}H_{18}Cl_2N_4O_4$ (449.3)
29	Cl	2-CH_3S-C_6H_4-CO	186-189	39	$C_{28}H_{22}Cl_2N_4O_2S_2$ (581.5)
30	Cl	4-Cl-C_6H_4-CO	150-155	14	$C_{28}H_{16}Cl_4N_4O_2$ (582.3)

Table 8: Chemical data of the compounds **VII**

Comp. VII	R^1	R^2	acyl	Mp (°C)	Yield (%)	Molecular formula (Mol.w.)
31	Cl	Cl	CH_3SO_2	200-202	84	$C_{15}H_{12}Cl_2N_4O_2S$ (383.3)
32	Cl	F	CH_3SO_2	208-210	68	$C_{15}H_{12}ClFN_4O_2S$ (366.8)
33	F	Cl	CH_3SO_2	205-210	91	$C_{15}H_{12}ClFN_4O_2S$ (366.8)
34	F	F	CH_3SO_2	210-211	78	$C_{15}H_{12}F_2N_4O_2S$ (350.3)
35	Cl	Cl	4-CH_3-C_6H_4-SO_2	203-205	36	$C_{21}H_{16}Cl_2N_4O_2S$ (459.4)
36	Cl	Cl	2-CH_3S-C_6H_4-CO	147-149	49	$C_{22}H_{16}Cl_2N_4OS$ (455.4)
37	Cl	Cl	4-Cl-C_6H_4-CO	209-211	75	$C_{21}H_{13}Cl_3N_4O$ (443.7)
38	Cl	Cl	4-NO_2-C_6H_4-CO	124-126	68	$C_{21}H_{13}Cl_2N_5O_3$ (454.3)
39	Cl	Cl	4-CH_3O-C_6H_4-CO	210-212	83	$C_{22}H_{16}CL_2N_4O_2$ (439.3)
40	Cl	Cl	C_2H_5OCO	151-153	80	$C_{17}H_{14}Cl_2N_4O_2$ (377.2)
41	Cl	NO_2	C_2H_5OCO	160-162	91	$C_{17}H_{14}ClN_5O_4$ (387.8)
42	Cl	F	C_2H_5OCO	118-121	74	$C_{17}H_{14}ClFN_4O_2$ (360.8)

5 h at room temp. and finally poured into 30 ml of water. The precipitate was filtered, washed with water and refluxed with 40 ml of 6% $NaHCO_3$ for 6 h. Then it was acidified with 10% HCl under cooling. Work-up as in **III**, yield: 36%.

General procedure for the preparation of **VI** *and* **VII c**

A solution of 22 mmole of substituted benzoylchloride in 20 ml of benzene was added dropwise during 30 min to a mixture of 10 mmole of **III**, 22 mmole of pyridine and 30 ml of benzene at 5°C. The reaction mixture was stirred for 6-8 h at 80°C and after evaporation of benzene the residue was treated with water. The solid was collected, washed with water and purified over on SiO_2 column using benzene/ acetone 95:5 as eluent. Yield: 14-39% for **VI c**, 49-83% for **VII c**.

1,5-bis(4-Chlorophenyl)-3-[bis(ethoxycarbonyl)-amino]-1H-1,2,4-triazole (**28**)

A mixture of 5 mmole of **III** and 7 ml of diethyl pyrocarbonate was refluxed for 1 h. After evaporation to dryness the residue was recrystallized, yield: 61%.

General procedure for the preparation of **VII d**

8 mmole of ethyl chloroformate was added dropwise during 30 min to a solution of 4 mmole of **III** in 12 ml of pyridine at 5°C, then the mixture was stirred for 2-3 h at room temp. and was finally poured into 120 ml of water. Work-up as in **III**, yield: 74-91%.

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