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Syntheses and Anti-inflammatory Activities of Substituted Arylamino-[*N'*-benzylidene)acetohydrazides and Derivatives

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The substituted arylamino-(*N'*-benzylidene)acetohydrazides **1–6**, substituted acetic acid arylamino-(*N'*-benzyl)acetohydrazides **7–12** and *N*-(3-chloro-2-oxo-4-phenylazetan-1-yl) (phenylamino)acetamides **13–16**, containing a β-lactam ring were synthesized and studied for their anti-inflammatory activities against carrageenin induced paw oedema in albino rats. Compound **7** was most active (30.6 % at 50 mg/kg p. o.) with minimum acute toxicity. All compounds showed a marked but variable (44–80 %) inhibition of antiproteolytic activity.

Synthese und antiphlogistische Aktivität von substituierten N-Arylaminoessigsäure-(N'-benzyliden)-hydraziden und Derivaten

Die substituierten N-Arylaminoessigsäure-(N'-benzyliden)-hydrazide **1–6**, die substituierten N-Arylaminoessigsäure-(N'-benzyl)-hydrazide **7–12** und die N-(3-Chlor-2-phenyl-4-azetanon-1-yl)-phenylaminoessigsäureamide **13–16**, die einen β-Lactamring enthalten, wurden synthetisiert und auf ihre antiphlogistische Aktivität im Carrageenintest an der Rattenpfote geprüft. Auch die antiproteolytische Wirkung der Verbindungen wurde bestimmt. Sie zeigten eine deutliche aber unterschiedliche (44–80 %) Hemmung der antiproteolytischen Wirkung. Die größte antiphlogistische Aktivität zeigte Verbindung **7** mit 30,6 % bei 50 mg/kg p. o. und minimaler akuter Toxizität.

Various clinically useful anti-inflammatory drugs such as indomethacin or salicylic acid are acidic in nature[1]. It is well documented that the lactam moiety confers acidic properties

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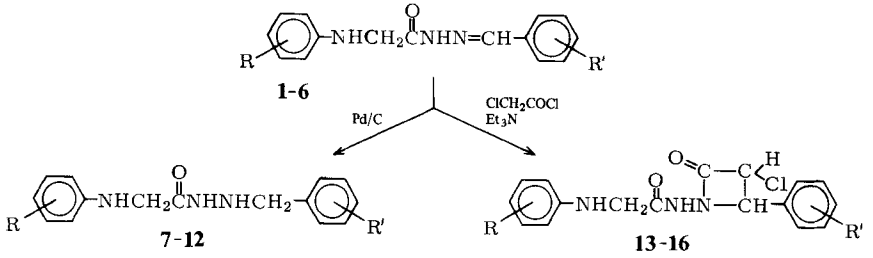
to compounds. This prompted the synthesis of some azetidinoyl acetamides containing a β-lactam ring[2]. The synthesis of substituted N-arylaminoacet-(N'-benzylidene)-hydrazides) **1–6** was done by the condensation of substituted arylamino-acethydrazides with appropriate aldehydes. These benzylidene derivatives were reduced by hydrazine hydrate, using Pd/C as catalyst, to the corresponding benzyl derivatives **7–12**. Benzylidene derivatives were also cyclized to the azetanonyl derivatives **13–16** in the presence of chloroacetyl chloride and triethylamine. The compounds were synthesized by the route given below and screened for antiproteolytic and anti-inflammatory activity as well as acute toxicity.



Experimental Part

M. P.'s: open capillary tubes, uncorr. All compounds were routinely checked by TLC on Silica Gel 'G' for homogenicity.

Substituted N-phenylglycine ethyl esters and 1-(substituted arylamino)acethydrazides were prepared by the reported methods[3].

Substituted N-arylaminoacet-(N'-benzylidene)-hydrazides

A mixture of 0.01 mole substituted arylaminoacethydrazide and 0.01 mole substituted aldehyde was refluxed in 35 ml dry benzene in presence of 2–3 drops of glacial acetic acid for 4–6 h. Benzene was removed and the residue was cooled and filtered. The filtrate, after removal of the solvent, yielded a crude solid which was crystallized from appropriate solvent. The analytical data of the compounds are given in table 1.

Substituted N-arylaminoacet-(N'-benzyl)-hydrazides

0.05 mole substituted N-aryłaminoacet-(N'-benzylidene)-hydrazide in 30 ml N-N'-dimethylformamide, 0.1 g Pd/C catalyst (10 % of Pd) and 0.01 mole hydrazine hydrate was refluxed for 6–8 h. It was filtered, diluted with cold water and then extracted with ether. Evaporation of ether gave the crude product which was crystallized from ether. Analytical data are given in table 2.

N-(3-chloro-2-phenyl-4-azetanon-1-yl)-phenylaminoacetamides

To 0.02 mole substituted N-arylaminoacet-(N'-benzylidene)-hydrazide, 0.02 mole chloroacetyl chloride was added dropwise during 30 min and the reaction mixture was refluxed for 3 h with occasional stirring. The separated solid was washed several times with dioxane. The filtrate along with the washings was concentrated to yield a semisolid residue, which was washed with a mixture of ether/petroleum ether (1:1). The resulting solid mass was crystallized from ethanol/water. Analytical data are given in table 3.

Table 1: *Substituted N-Arylaminoacet-(N'-benzylidene)-hydrazides*

Compd. No.	R	R^1	M.P. °C	Yield %	Recryst. solvent (a)	Molecular formula	Antiproteolytic activity % inhibition 5×10^{-4} M Conc. (b)	Anti-inflammatory activity % inhibition 50 mg/kg p.o.
1**	4-OCH_3	4-OCH_3	196	60	A	$C_{17}H_{19}N_3O_3$	60.0	13.3
2	4-OCH_3	3-NO_2	215	58	A	$C_{16}H_{16}N_4O_4$	54.7	N.S.*
3	4-Cl	4-OCH_3	205	61	B/C	$C_{16}H_{16}N_3O_2Cl$	47.3	4.1
4	4-Cl	3-NO_2	241	55	B/C	$C_{15}H_{13}N_4O_3Cl$	44.0	4.8
5	4-Cl	3-OCH_3, 4-OH	171	57	B/C	$C_{16}H_{16}N_3O_3Cl$	46.7	6.7
6	4-Cl	4-$N(CH_3)_2$	248	63	B/C	$C_{17}H_{19}N_4OCl$	78.8	N.S.*

(a) A: Methanol; B: DMF; C: Water. (b) The values are the mean of two separate experiments. N. S.* – Not studied; ** I. R. (KBr) 1685 (CO), 3300 (NH), 1600 cm^{-1} (CN). All the compounds gave satisfactory elementary analysis (N).

Table 2: *Substituted N-Arylaminoacet-(N'-benzyl)-hydrazides*

Compd. No.	R	R^1	M.P. °C	Yield %	Recryst. solvent (a)	Molecular formula	Antiproteolytic activity % inhibition 5×10^{-4} M conc. (b)	Anti-inflammatory activity % inhibition at 50 mg/kg p.o.
7**	4-OCH_3	4-OCH_3	150	42	A	$C_{17}H_{21}N_3O_3$	52.0	30.6
8	4-OCH_3	3-NO_2	164	45	A	$C_{16}H_{18}N_4O_4$	48.2	N.S.*
9	4-Cl	4-OCH_3	158	44	B/C	$C_{16}H_{18}N_3O_2Cl$	60.0	28.6
10	4-Cl	3-NO_2	204	47	B/C	$C_{15}H_{15}N_4O_3Cl$	44.0	13.3
11	4-Cl	3-OCH_3,4-OH	129	50	A/C	$C_{16}H_{18}N_3O_3Cl$	44.3	N.S.*
12	4-Cl	4-$N(CH_3)_2$	208	52	A/C	$C_{17}H_{21}N_4OCl$	56.3	N.S.*

(a) A: Ether; B: Ethylacetate; C: Petroleum Ether (60–80°). (b) The values are the mean of two replicates. N. S.* – Not studied. ** I. R. – 1690 (CO), 3300 (NH), 2910 cm^{-1} (CH_2). All the compounds gave satisfactory elementary analysis (N).

Table 3: *N-(3-chloro-2-phenyl-4-azetanon-1-yl)-phenylaminoacetamides*

Compd. No.	R	R^1	M.P. °C	Yield %	Recryst. solvent (a)	Molecular formula	Antiproteolytic activity % inhibition 5×10^{-4} M conc. (b)	Anti-inflammatory activity % inhibition 50 mg/kg p.o.
13**	4-OCH_3	4-OCH_3	104	40	C/A	$C_{19}H_{20}N_3O_4Cl$	48.0	3.1
14	4-Cl	4-OCH_3	160	37	B	$C_{18}H_{17}N_3O_3Cl_2$	N.S.*	Nil
15	4-Cl	3-NO_2	124	39	B/A	$C_{17}H_{14}N_4O_4Cl_2$	46.1	17.6
16	4-Cl	3-OCH_3,4-OH	132	40	B/A	$C_{18}H_{17}N_3O_4Cl_2$	N.S.*	8.6

(a) A: Water; B: Methanol; C: Ethanol. (b) Values are the mean of two replicates. N. S.* – Not studied. ** IR – 1710 (CO) (Monocyclic lactam), 3310 (NH), 2900 cm^{-1} (CH_2). All the compounds gave satisfactory elementary analysis (N).

Biological Studies

I.: Determination of Antiproteolytic Activity

Antiproteolytic activity of the compounds was determined by the spectrophotometric method of *Kishor* et al.[4]. The reaction mixture consisted of 0.5 M tris-buffer (pH 8.2), 0.75 mg of crystalline trypsin (Sigma), 3.5×10^{-5} M bovine serum albumin (BSA, substrate), glass distilled water and the test compounds in a total vol. of 1 ml. The compounds were tested at a final conc. of 5×10^{-4} M and were dissolved in DMF. An equiv. amount of DMF was added to control tubes. The test compounds were preincubated with trypsin for 10 min at 37 °C prior to the addition of BSA and the reaction mixture was further incubated for 5 min. The reaction was stopped by addition of 0.5 ml of trichloroacetic acid (15 %, w/v). It was centrifuged and the acid soluble products of protein catabolism were determined by the method of *Lowry* et al.[5].

II: Anti-inflammatory Activity

Anti-inflammatory activity was evaluated against carrageenin induced paw oedema in albino rats. Adult albino rats of either sex weighing 80–120 g were divided into groups of six animals each. A freshly prepared suspension of carrageenin, 0.05 ml (1.0 % in 0.9 % saline) was injected under the planter aponeurosis of the right paw of the rats by the method of *Winter* et al.[6]. One group was kept as control and the animals of the other groups were pretreated with the test drugs suspended in gum acacia given orally 1 h before the carrageenin injection. The vol. of the foot was measured before and 3 h after carrageenin treatment by the micro pipette method as described by *Buttle* et al.[7]. The mean increase in paw volume in each group was measured and the percent antiinflammatory activity was calculated.

III: Acute Toxicity

The compound with highest anti-inflammatory activity against carrageenin induced oedema was investigated for acute toxicity (approximate LD_{50}) in albino mice.

Results and Discussions

Antiproteolytic Activity

14 Compounds were tested for their antiproteolytic activity at 5×10^{-4} M conc. Results are shown in tables 1, 2 and 3.

Maximum activity (78.7 %) was exhibited by compound **6**. The minimum activity (44 %) was found in compound **4** and the corresponding benzyl derivative **10**.

Anti-inflammatory Activity

The compounds showed varying degrees of anti-inflammatory activity at 50 mg/kg oral dose. The results are given in tables 1, 2 and 3.

Structure-activity-relationships revealed that in general the reduction of the benzylidene hydrazides **1, 3** and **4** to the corresponding benzyl hydrazides **7, 9** and **10** markedly enhanced the anti-inflammatory activity, while the cyclization to the corresponding N-(3-chloro-2-phenyl-4-azetanon-1-yl)-phenylaminoacetamides **13–16** (table 3) did not effect the anti-inflammatory activity.

Compound **7** exhibited the highest anti-inflammatory activity (30.6 % at 50 mg/kg p. o.) and was tested for acute toxicity. The ALD_{50} was found to be > 2000 mg/kg.

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Buchbesprechungen

Arch. Pharm. (Weinheim) *317*, 894–896 (1984)

Lexikon der offizinellen Arzneipflanzen von B. Zepernick, L. Langhammer und J.B.P. Lüdcke, VII, 546 S., Preis DM 78,00, Walter de Gruyter Verlag, Berlin – New York 1984.

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