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Anti-Inflammatory, Antiproteolytic and Analgesic Activities of Some New Thiadiazolyl Indoles

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3-[(2-Aminomethyl)-5-mercapto-1,3,4-thiadiazolyl]indole **(1)**, obtained by *Mannich* condensation of 2-amino-5-mercapto-1,3,4-thiadiazole with indole and formaldehyde, was subjected to mercapto-etherification with epichlorohydrin to yield 3-[(2-aminomethyl)-5-(3-epoxypropylmercapto)-1,3,4-thiadiazolyl] indole **(2)**, which, on hydrolysis with NaOH and subsequent treatment with various arylamines, gave the 3-[(2-aminomethyl)-5-(2-hydroxy-3-arylaminopropyl-mercapto)1,3,4-thiadiazolyl]indoles **3a–3l**. All compounds **3a–3l** were studied for their anti-inflammatory activity against carrageenin induced rat paw oedema. Their antiproteolytic properties were also evaluated. An attempt has been made to establish structure-activity relationships.

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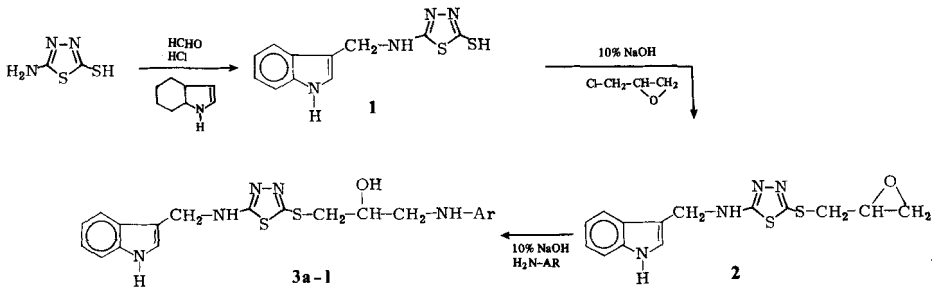
Antiphlogistische, antiproteolytische und analgetische Aktivität einiger neuer Thiadiazolylindole

3-[(2-Aminomethyl)-5-mercapto-1,3,4-thiadiazolyl]indol **(1)**, erhalten durch *Mannich*-Kondensation aus 2-Amino-5-mercapto-1,3,4-thiadiazol, Indol und Formaldehyd, wurde mit Epichlorhydrin zu 3-[(2-Aminomethyl)-5-(3-epoxypropylmercapto)-1,3,4-thiadiazolyl]indol **(2)** umgesetzt, das nach alkalischer Hydrolyse und anschließender Behandlung mit verschiedenen Arylaminen schließlich die 3-[(2-Aminomethyl)-5-(2-hydroxy-3-arylaminopropylmercapto)-1,3,4-thiadiazolyl]indole **3a–3l** ergab. Die Verbindungen **3a–3l** wurden auf ihre antiphlogistische Wirkung am Carragenin-Rattenpfoten-Ödem getestet. Auch ihre antiproteolytischen Eigenschaften wurden untersucht. Es wurde versucht, Strukturwirkungsbeziehungen abzuleiten.

Indomethacin is a drug of choice to treat inflammatory disorders[1]. Further, thiadiazole congeners have recently been evaluated for their anti-inflammatory activity by various groups of authors[2,3,4]. The fact, that propyl derivatives are very strong antiphlogistic agents[5] prompted us to synthesize twelve newer 3-[(2-aminomethyl)-5-(2-hydroxypropyl-3-arylamino-mercapto)-1,3,4-thiadiazolyl]indoles so as to get better therapeutic agents.

The syntheses of the compounds **3a–3l** started from 2-amino-5-mercapto-1,3,4-thiadiazole. It was treated with indole and formaldehyde, in cold, to get the *Mannich* base **1**. The mercaptan **1** was condensed with epichlorohydrin in dry ethanol using 10% NaOH solution, in equimolar amounts, to obtain 3-[(2-aminomethyl)-5-(3-epoxypropylmercapto)-1,3,4-thiadiazolyl]indole **(2)**. The thioether **2**, on hydrolysis with NaOH and subsequent treatment with different aryl amines, produced **3a–3l**. All of these derivatives gave satisfactory elementary analyses. Additional support to their structures was obtained through IR and PMR spectral studies.



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Experimental

MP: in open capillaries (uncorr.). *I.R. spectra*; Perkin Elmer 177 Spectrophotometer in KBr. *NMR spectra*; in C_5D_5N at 90 MHZ.

3-[(2-Aminomethyl)-5-mercapto-1,3,4-thiadiazolyl]indole **(1)**

A mixture of indole, formaldehyde and 2-amino-5-mercapto-1,3,4-thiadiazole[6] (0.1 mol. each) were taken in methanol and 3–4 drops of HCl were added to this solution according to *Vogel*[7]. The

precipitation of the *Mannich* base occurred at once, however, the mixture was refluxed for additional 2 h. The excess of methanol was distilled off and the crude solid base was recrystallized from methanol; m.p. 340 °C, yield 85 %, $C_{11}H_{10}N_4S_2$ (258): Calcd. C 50.4 H 3.8 N 21.4 Found C 50.3 H 3.9 N 21.4. IR: 3340 (Sec. N-H group), 3050, 2910, 2550 (weak S-H group), 1640, 1590, 1510, 1480, 1320 (C-S-linkage) and 750 cm^{-1} PMR: δ (ppm) = 2.1 (d, 2H $C\underline{H}_2$), 4.3 (t, 1H -CH_2-N$\underline{H}$), 4.7 (d, broad, 1H indolyl -NH), 6.7 (5H, 4H phenyl ring and 1H indolyl -C$\underline{H}$) and 8.9 (1H of S-H).

3-[(2-Aminomethyl)-5-(3-epoxypropylmercapto)-1,3,4-thiadiazolyl]indole **(2)**

The formation of this thioether was performed by following the method of *Manverney* and *Busch*[8]. 0.1 mol 3-[(2-aminomethyl)-5-mercapto-1,3,4-thiadiazolyl]indole was dissolved in 100 ml absol. ethanol. A freshly prepared 10 % NaOH solution (40 ml) was added to the above solution by and by. The sodium salt solution was cooled and then treated with 0.15 mol epichlorohydrin. The contents were refluxed for 3 h and the mixture was concentrated by distilling off excess ethanol. The impure thioether was treated with cold water, dried and recrystallized from dioxan/water; m.p. 110–111 °C, yield 54 %, $C_{14}H_{14}N_4OS_2$ (314): Calcd. C 52.8 H 4.4 N 17.6 Found C 52.8 H 4.5 N 17.7. IR: 3350, 1590 (N-H-stretchs and bends), 3030, 1510, (aromatic C-H vibrations), 2900, 1490 (aliphatic C-H stretchs and bends), 1640 (C=N linkage), 1320, 1200, 800 (epoxy linkage, medium), 1050 (-C-S-C-linkage) and 750 cm^{-1}. PMR; δ (ppm) = 1.8 (d, 2H -S-C$\underline{H}_2$), 2.1 (d, 2H -C$\underline{H}_2$-NH-), 2.3 (d, 2H S-CH_2-C$\underline{H}$-C$\underline{H}_2$), 2.45 (dt, 1H -S-CH_2-C$\underline{H}$-C$\underline{H}_2$), 4.3 (t, 1H -CH_2-N$\underline{H}$), 4.65 (d, broad, 1H indolyl-N$\underline{H}$) and 6.7 (s 5H, 4H of phenyl ring and 1H of indolyl-C$\underline{H}$).

3-[(2-Aminomethyl)-5-(2-hydroxy-3-chlorophenylaminopropyl-mercapto)-1,3,4-thiadiazolyl]indole **(3c)**

0.01 mol 3-[(2-aminomethyl)-5-(3-epoxypropyl-mercapto)-1,3,4-thiadiazolyl]indole and 0.01 mol 4-chloroaniline were taken in methanol and 4 ml of 10 % NaOH was added to this solution. the solution was refluxed for 3 h on a steam bath. The contents were cooled and poured on ice for crystallisation of the final product. It was washed several times with cold water to remove excess NaOH, dried and finally recrystallized from methanol/water; m.p. 214 °C, yield 44 %, $C_{20}H_{20}ClN_5OS_2$ (445.5) Calcd. C 53.9 H 4.5 N 15.7 Found C 53.8 H 4.5 N 15.7. IR: 3400, 1250 (O-H stretchs and bends)-3310, 3050, 2900, 2800, 1630, 1590, 1510, 1470, 1310, 1050 and 750 cm^{-1}. PMR: δ (ppm) = 1.8 (d, 2H -S-C$\underline{H}_2$), 2.0 (d, 2H C$\underline{H}_2$-NH-), 2.1 (t, 2H -CH_2-CH(OH)-C$\underline{H}_2$-), 2.82 (m, 1H -C$\underline{H}$-), 4.5 (blunt t, 2H, 1H -CH_2-N$\underline{H}$ and 1H -CH-C$\underline{H}_2$-N$\underline{H}$), 5.0 (d, 1H indolyl-N$\underline{H}$), 6.7–7.0 (m, 8 phenylic protons and 1 indolyl C$\underline{H}$ proton) and 9.5 (d, 1H OH).

Similarly the compounds **3a–l** were synthesized and characterized (Tab. 1).

Anti-inflammatory Activity

The anti-inflammatory activity was assessed by utilizing the method of *Winter*[9]. The animals used were adult albino rats of either sex.

The albino rats, weighing between 100–120 g, were divided in groups of six animals each. 0.05 ml of freshly prepared suspension of carrageenin (1 %) in saline was injected into the planter aponeurosis of the right hind paw of the rats. The test compounds, suspended in 5 % gum accacia, were given orally in dose of 100 mg/kg to each group of animals 1 h prior to the carrageenin injection. In every test, one group served as control which received an equivalent amount of 5 % gum accacia and one group received phenylbutazone as a standard drug. The mean increase in paw vol. due to carrageenin induced oedema, was measured before and 3 h after the carrageenin injection by micropipette method deviced by *Buttle* et al.[10].

Table 1: *Substituted indoles* **3a–3l***

No.	Ar	M.P. °C	Yield %	Molecular formula
3a	$-C_6H_5$	198–200	50	$C_{20}H_{21}N_5OS_2$
3b	$2\text{-}ClC_6H_4$	222	40	$C_{20}H_{26}ClN_5OS_2$
3c	$4\text{-}ClC_6H_4$	214	44	$C_{20}H_{20}ClN_5OS_2$
3d	$4\text{-}BrC_6H_4$	269	42	$C_{20}H_{20}BrN_5OS_2$
3e	$2\text{-}CH_3C_6H_4$	190–192	52	$C_{21}H_{23}N_5OS_2$
3f	$4\text{-}CH_3C_6H_4$	178	50	$C_{21}H_{23}N_5OS_2$
3g	$2\text{-}OCH_3C_6H_4$	196	42	$C_{21}H_{23}N_5O_2S_2$
3h	$4\text{-}OCH_3C_6H_4$	180	54	$C_{21}H_{23}N_5O_2S_2$
3i	$4\text{-}NO_2C_6H_4$	211	80	$C_{20}H_{20}N_6O_3S_2$
3j		219	40	$C_{19}H_{20}N_6OS_2$
3k		234	46	$C_{17}H_{18}N_6OS_3$
3l**		262	48	$C_{18}H_{23}N_5O_2S_2$

* All of the compounds were recrystallized from methanol/water and gave correct elementary analysis for nitrogen.

** Morpholine was used as secondary amine.

Antiproteolytic Activity

The antiproteolytic activity was measured by determining their ability to inhibit trypsin induced hydrolysis of bovine serum albumin. The reaction mixture consisted of 0.05 M tris-buffer (pH 8.2), 0.075 mg of crystalline trypsin (1 g sufficient to hydrolyse 250 g of Casein), 0.03 mM bovine serum albumin (substrate) in a total vol. of 1 ml. The various test compounds were dissolved in dimethyl formamide and were used at a final concentration of $1 \cdot 10^{-3}$M. Equivalent amount of dimethyl formamide added to control tubes, was found to have no effects on the in vitro activity of trypsin during hydrolysis of bovine serum albumin as the substrate. The compounds were incubated with trypsin for 10 min prior to the addition of the substrate (*Chaudhari* et al[11]; *Kishore* et al[12]). The reaction was carried out for 5 min and then stopped by the addition of 0.5 ml of 15 % trichloroacetic acid solution (w/v). The acid soluble products of protein breakdown, obtained after centrifugation in the supernatent fractions, were determined by the method of *Lowry* et al.[13]. Decrease in the formation of the products breakdown in the presence of test compounds was used to determine the antiproteolytic activity of the compounds. Sodium salicylate was used as a reference drug to test the validity of the experiments.

Analgesic Activity

Aconitine induced writhing method of *Bhalla* et al.[14] was used to find out the analgesic action of the test drugs in albino mice. The mice grouped in ten for each dose, were fed with the drug 1/2 h before the i.p. injection of 100 % writhmogenic dose of aconitine (2 μg/mice) and observed for 30 min for any abdominal torsion, stretching of hind legs to the abdominal wall, marked contraction of abdominal area and the periodic arching of the back to rub the abdominal wall on the glazed surface on which the mouse was kept. The standard drug given was acetylsalicylic acid.

Toxicity Study

The method of *Weil*[15] was adopted for the determination of LD_{50} of the synthesized compounds in albino mice. The animals were divided in groups of four each, and were fasted for 18 h prior to the drug administration. The compounds were given in doses of 250, 500, 750 and 1000 mg/kg intraperitoneally and the animals were observed for 24 h mortality.

Results and Discussion

It was observed that three compounds viz., **3c, 3d** and **3j** gave more than 20 % inhibition against inflammation, while other derivatives exhibited a low sensitivity in reducing the size of the oedema (table 2). The dose, at which the compounds were screened, was 100 mg/kg p.o. The standard drug phenyl butazone inhibited the foot oedema by 50.9 % at the same dose.

Compound **3c**, being most active in the above study, was further tested for it's efficacy against aconitine induced writing in albino mice at 100 mg/kg and 150 mg/kg p.o, and showed 40 % and 70 % protection, resp. (table 3), when compared with acetylsalicylic acid (45 mg/kg p.o.) as a reference standard showing 80 % protection.

As far as their antiproteolytic activity is concerned, all of them except **3i**, exhibited good response to this hydrolysis. Compound **3c**, possessed the maximum activity of 87.2 %, whereas the compound **3a** recorded the minimum inhibition of 29.6 %, **3f** and **3j** were other derivatives to show more than 60 % activity while other derivatives exhibited a middle order activity. The compounds were tested for their toxicity and the high LD_{50} values revealed their nontoxic nature and high safety margin.

These observations did not provide any clear correlation between the in vitro and in vivo activities but it is evident that the compound **3c** showed maximum activity in both tests (tab. 2). As far as structure-activity-relationships are concerned, the following points are worth of consideration in this regard.

1. When a substituent with strong-I effect (Cl, Br) is present at the phenyl ring, both the activities increased significantly (compare **3b, 3c** and **3d** with other compounds). The potentiality of the compounds decreased with the decrease in -I effect of the substituents i.e. compound **3h** with a OCH_3 group recorded the minimum inhibition of 4 % against inflammation, the antiproteolysis activity being 50 % (tab. 2).

2. Among the halogen substituents at the phenyl ring, a Cl substituent potentiated the inhibitory activity of the compound to greater extent as compared to a Br substituent (cf **3c** and **3d**).

3. It was also found that substitution at position 4 of the phenyl ring had greater impact on the anti-inflammatory activity as compared to position 2 (compare compounds **3c** with **3d** and **3e** with **3f** resp., tab. 2).

4. It was also found that substitution with CH_3 or OCH_3 at position 2 or 4 of the phenyl ring decreased the anti-inflammatory activity of the compounds markedly (cf compounds **3e–3h**). Contrary to this, the antiproteolytic activity of these compounds was found to be significant (tab. 2).

5. The introduction of a $-NO_2$ group at position 4 of the phenyl ring caused the total loss of inhibitory activity against proteolysis while the anti-inflammatory activity remained measurable (Cf. Compound **3i**).

6. Replacement of the phenyl ring with a pyrrolo ring was found to have a promising effect on both the activities i.e., both the activities increased markedly (cf. Compound **3j**).

Table 2: *Anti-inflammatory and antiproteolytic activities of compounds* **3a–l** *against carrageenin induced oedema in albino rats and trypsin induced hydrolysis of Bovine Serum albumin*

No.	Mean increase in paw volume in mm ± S.E.	% Anti-inflammatory activity at a dose of 100 mg/kg p.o.	% Anti proteolytic activity at concentration of 1×10^{-3} M *	LD_{50} mg/kg i.p.
Phenylbutazone	0.50 ± 0.02	50.9	–	–
Control	1.04 ± 0.01	–	–	–
3a	0.94 ± 0.02	9.6	29.6	750
3b	0.91 ± 0.01	12.5	57.2	> 1000
3c	0.78 ± 0.01	25.0**	87.2	> 1000
3d	0.80 ± 0.04	23.0	57.2	> 1000
3e	0.94 ± 0.03	9.6	50.0	500
Control	1.00 ± 0.01	–	–	–
3f	0.93 ± 0.03	7.0	64.3	> 1000
3g	0.93 ± 0.03	7.00	35.8	750
3h	0.96 ± 0.03	4.0	50.0	–
3i	0.93 ± 0.04	7.0	NiL	–
3j	0.79 ± 0.02	21.0	64.3	> 1000
3k	0.92 ± 0.01	8.0	42.9	500
3l	1.00 ± 0.04	NiL	50.0	–

* Sodium salicylate (reference drug) showed 51 % protection and the values are mean values from two separate experiments.

** p value significant.

Table 3: *Analgesic activity of compound* **3c** *against aconitine induced writhing in albino mice*

No.	Dose in mg/kg p.o.	% Protection
Acetylsalicylic acid	45	80
3c	100	40
	150	70

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Inhaltsstoffe von Boraginaceae, 4. Mitt.[1)]

Anchusosid-3, ein neues Triterpensaponin aus Anchusa officinalis L.

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Aus *Anchusa officinalis* L. wurde das neue pentacyclische Triterpen Anchusosid-3 (**1**) isoliert. Auf der Basis IR, ^{1}H-NMR, partiell relaxierter ^{13}C-NMR und ms Daten sowie chemischer Derivatisierung wird diesem die Struktur der 21-O-β-D-Glukopyranosyl-2α, 3β, 21β, 23-tetrahydroxy-12-oleanen-28-carbonsäure zugeteilt.

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