1,5-Disubstituted Indazol-3-ols with Anti-Inflammatory Activity ☆

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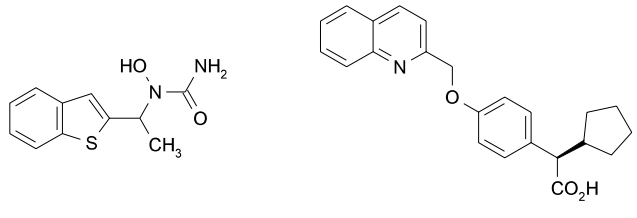
Key Words: *Indazole derivatives; inhibitors of 5-lipoxygenase; antiinflammatory activity; asthma*

Summary

A series of new indazol-3-ol derivatives was synthesized. Some of these compounds exhibit interesting anti-inflammatory activities in various models of inflammation. 5-Methoxy-1-[quinoline-2-yl-methoxy)-benzyl]-1H-indazol-3-ol (**27**) strongly inhibits the oxidation of arachidonic acid to 5-hydroperoxyeicosatetraenoic acid catalyzed by 5-lipoxygenase (IC_{50} = 44 nM). **27** also inhibits the contraction of sensitized guinea pig tracheal segments (IC_{50} = 2.9 μM). In guinea pigs treated with **27** (1 mg/kg i.p.) 2 h before antigen provocation, there was a marked inhibition (47%) of the antigen-induced airway eosinophilia. After topical application of 1 μg/ear **27** inhibits the arachidonic acid induced mouse ear edema (41%).

Introduction

Allergic diseases like asthma are characterized by inflammatory processes. Important mediators involved in these processes are arachidonic acid metabolites, such as leukotrienes, prostaglandins, and thromboxanes. The oxidative formation of leukotrienes is catalyzed by the enzyme 5-lipoxygenase (5-LOX). This enzyme has been found in eosinophils, neutrophils, monocytes, macrophages, mast cells, basophils, and B lymphocytes. Therefore inhibition of this enzyme is a promising approach in the treatment of asthma and other allergic diseases. Recent studies have demonstrated the therapeutic benefit of 5-LOX inhibitors[1]. Zileuton (**1**) is one of the first launched 5-LOX inhibitors for the treatment of asthma (Scheme 1). In comparison to treatment with as needed inhaled salbutamol the additional administration of zileuton in case of mild to moderate bronchial asthma inhibited asthma exacerbations, improved the lung function, and reduced the use of salbutamol [2,3]. BAY × 1005 (**2**) has been reported as leukotriene synthesis inhibitor (Scheme 1). Added to inhaled corticosteroids it has a significant effect on the pulmonary function [4]. Several other 5-LOX inhibitors are under development.



Zileuton (**1**) BAY x 1005 (**2**)

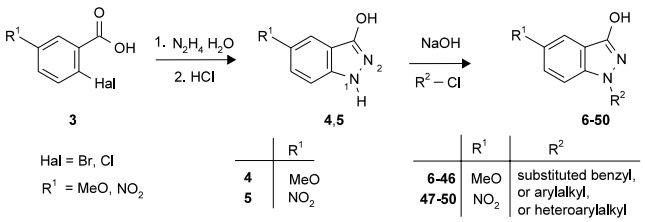
Scheme 1

In the following we report about synthesis and biological activities of indazol-3-ols – a new class of 5-LOX inhibitors.

Results and Discussion

Chemistry

Indazol-3-ols are synthesized by common procedures [5,6]. We used 5-substituted 2-halogenobenzoic acids (**3**) and hydrazine hydrate as starting materials which were heated to give hydrazino benzoic acid derivatives. Acid catalyzed cyclization of these intermediates yielded 5-substituted indazol-3-ols (**4**,**5**). 5-Methoxy-1H-indazol-3-ol (**4**) and 5-nitro-1H-indazol-3-ol (**5**) were used for further derivatisation. We focused on N-1 alkylation with halogenoalkylaryls or -heteroaryls. As potential side products N-2, O- and various bisalkyl derivatives may occur. For the synthesis of 5-substituted 1-alkyl-indazol-3-ols (**6–50**) best selectivity was achieved by using aqueous sodium hydroxide.



Scheme 2

Starting with **4** we synthesized a wide range of substituted 1-benzyl derivatives varying the substitution pattern in the benzyl moiety (Table 1). Many of them proved to be active in the *in vitro* testing of 5-LOX inhibition.

The effect of modification of the substituents in the aromatic ring of the benzyl moiety is summarized as follows:

mono subst. halogen:	4-Cl > 3-Cl > 2-Cl 4-Cl > 4-Br > 4-F > 4-CF_3
disubst. halogen:	2,4-Cl_2 > 3,4-Cl_2 > 2,6-Cl_2> 2-Cl, 6-F
alkyl:	4-tBu > 3-Me, 4-Me > 2-MeO, 4-MeO > 3-MeO, 4-MeO, 5-MeO
(hetero)aryl:	4-O-CH_2-quinolin-2-yl > 4-O-Bzl > Ph

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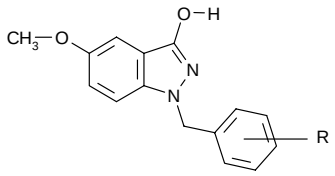
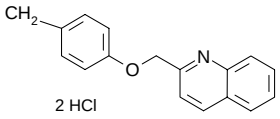
Table 1. 5-LOX inhibition of 1-benzyl-5-methoxy-1H-indazol-3-ols.

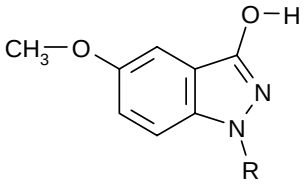


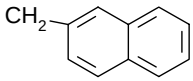
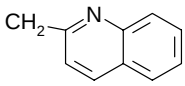
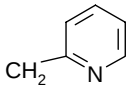
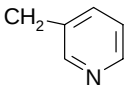
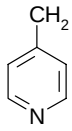
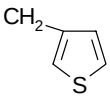
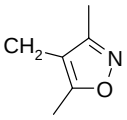
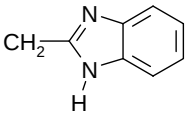
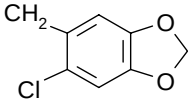
No.	R	Mp (°C)	MW	Analysis C, H, N[a]	IC_{50} [nM][b]
6	4-Br	187–189	333.19	$C_{15}H_{13}BrN_2O_2$	765 ± 132
7	4-Cl	194–196	288.74	$C_{15}H_{13}ClN_2O_2$	400 ± 88
8	3-Cl	167–168	288.74	$C_{15}H_{13}ClN_2O_2$	470 ± 76
9	2-Cl	190–191	288.74	$C_{15}H_{13}ClN_2O_2$	710 ± 120
10	4-F	202–204	272.28	$C_{15}H_{13}FN_2O_2$	1100 ± 146
11	2,4-Cl_2	214–215	323.18	$C_{15}H_{12}Cl_2N_2O_2$	100 ± 32
12	3,4-Cl_2	194–195	323.18	$C_{15}H_{12}Cl_2N_2O_2$	410 ± 33
13	2,6-Cl_2	214–215	323.18	$C_{15}H_{12}Cl_2N_2O_2$	750 ± 156
14	2-Cl, 6-F	189	306.73	$C_{15}H_{12}ClFN_2O_2$	>1000
15	3-Cl, 2-F	194–195	306.73	$C_{15}H_{12}ClFN_2O_2$	829 ± 153
16	4-Cl, 2-NO_2	212–215	333.73	$C_{15}H_{12}ClN_3O_4$	>1000
17	4-CF_3	165–167	322.29	$C_{16}H_{13}F_3N_2O_2$	>1000
18	2-OH, 4-NO_2	224–227	315.29	$C_{15}H_{13}N_3O_5$	>1000
19	2,4-$(OMe)_2$	151–154	314.34	$C_{17}H_{18}N_2O_4$	>1000
20	3,4,5-$(OMe)_3$	180	344.37	$C_{18}H_{20}N_2O_5$	>1000
21	3,4-$(Me)_2$	209	282.35	$C_{17}H_{18}N_2O_2$	977 ± 207
22	4-tBu	202–205	310.40	$C_{19}H_{22}N_2O_2$	696 ± 103
23	4-COOH	220–223	298.30	$C_{16}H_{14}N_2O_4$	>1000
24	4-CH_2-COOH	182–186	312.33	$C_{17}H_{16}N_2O_4$	>1000
25	4-Ph	214–218	330.39	$C_{21}H_{18}N_2O_2$	257 ± 57
26	4-O-Bzl	177.5–179	360.42	$C_{22}H_{20}N_2O_3$	171 ± 31
27 		181	484.39	$C_{25}H_{23}Cl_2N_3O_3$	44 ± 8

Zileuton (**1**)	2160 ± 291
Bay x 1005 (**2**)	18.9 ± 2.9

a: Analysis C, H, N within ± 0.4% unless otherwise indicated. b: Mean of 4 independent experiments.

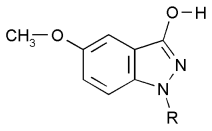
Table 2. 5-LOX inhibition of 1-substituted 5-methoxy-1H-indazol-3-ols (further CH_2 spacer linked derivatives).



No.	R	Mp (°C)	MW	Analysis C, H, N[a]	IC_{50} [nM][b]
28		187	304.35	$C_{19}H_{16}N_2O_2$	265 ± 56
29		180–184	305.34	$C_{18}H_{15}N_3O_2$	> 1000
30		166–167	255.28	$C_{14}H_{13}N_3O_2$	> 1000
31		153	255.28	$C_{14}H_{13}N_3O_2$	> 1000
32		150	255.28	$C_{14}H_{13}N_3O_2$	> 1000
33		186	260.32	$C_{13}H_{12}N_2O_2S$	500 ± 110
34		191–195	273.29	$C_{14}H_{15}N_3O_3$	> 1000
35		210–214	294.32	$C_{16}H_{14}N_4O_2$	> 1000
36		227–229	332.75	$C_{16}H_{13}ClN_2O_4$	874 ± 179

a: Analysis C, H, N within ± 0.4% unless otherwise indicated. b: Mean of 4 independent experiments.

Table 3. 5-LOX inhibition of 1-substituted 5-methoxy-1H-indazol-3-ols (more than one atom between the indazole skeleton and the phenyl ring).



No.	R	Mp (°C)	MW	Analysis C, H, N[a]	IC_{50} [nM][b]
37	$(CH_2)_3Ph$	160	282.34	$C_{17}H_{18}N_2O_2$	150 ± 36
38	$CH_2CH{=}CHPh$	183–187	280.33	$C_{17}H_{16}N_2O_2$	299 ± 47
39	$(CH_2)_2OPh$	165	284.32	$C_{16}H_{16}N_2O_3$	745 ± 130
40	$(CH_2)_3OPh$	138–142	298.34	$C_{17}H_{18}N_2O_3$	655 ± 123
41	$(CH_2)_2COPh$	171–173	296.33	$C_{17}H_{16}N_2O_3$	> 1000
42	$(CH_2)_2\ C_6H_4NO_2(4)$	238–240	313.32	$C_{16}H_{15}N_3O_4$	> 1000
43	$(CH_2)_2OC_6H_4NO_2(4)$	221–224	329.32	$C_{16}H_{15}N_3O_5$	> 1000
44	$(CH_2)_3COC_6H_4F(4)$	163–165	328.35	$C_{18}H_{17}FN_2O_3$	679 ± 118
45	$CH_2CONHC_6H_3(OMe)_2(3,4)$	225	357.37	$C_{18}H_{19}N_3O_5$	> 1000
46	$CH_2CHOHC_6H_3Cl_2(2,4)$	232–235	353.21	$C_{16}H_{14}Cl_2N_2O_3$	> 1000

a: Analysis C, H, N within ± 0.4% unless otherwise indicated. b: Mean of 4 independent experiments.

The most active compounds of this series are **11** and **27**.

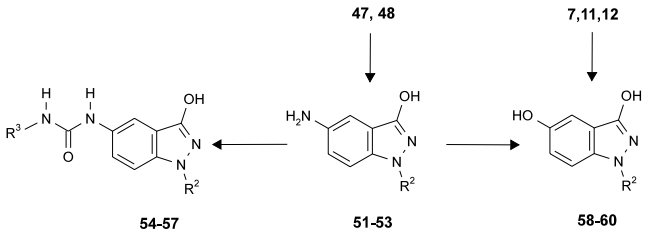
Compounds with a heteroaryl- or naphthyl moiety linked by a methylene group to the indazole skeleton were also prepared (Table 2).

Only the 1-naphthalen-2-ylmethyl derivative **28** and the 1-thiophen-2-ylmethyl substituted indazole **33** inhibited the enzyme 5-LOX in concentrations smaller than 1 μM. The heterocylic substituted compounds show no or only little activity.

Extending the linkage between the indazole skeleton and the aryl ring resulted in some derivatives with moderate *in vitro* activity (Table 3).

The propyl linked (**37**) and the allyl linked (**38**) indazoles have the highest activity in the series of more than one atom spacer linked compounds.

The synthesis of 1-substituted 5-nitro-indazol-3-ols was carried out in analogy to the corresponding 5-methoxy derivatives. In all cases 5-nitro-substitution led to a loss of *in vitro* activity (Table 4).



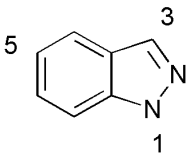
Scheme 3

By reduction of the 5-nitro group we obtained 5-amino-indazol-3-ols (**51–53**) (Scheme 3).

51–53 were precursors of 5-urea derivatives (**54–57**) obtained by reaction with isocyanates. In addition, **51–53** were used to prepare indazol-3,5-diols **58–60** via diazotisation and elimination of nitrogen. Alternatively these compounds are accessible by ether cleavage of **7**, **11**, or **12**. Selected compounds with 5-amino-, 5-urea, and 5-hydroxy-substituent are summarized in Table 4. With the exception of **54**, **59**, **60** none of these compounds showed any activity.

Structure-Activity Discussion

According to the (preliminary) results of the *in-vitro* assay indazole derivatives with the following substitution pattern did not show any 5-LO activity:

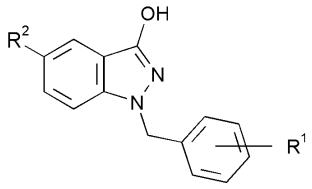


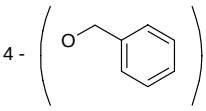
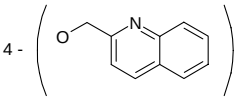
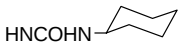
1-benzyl (subst.)	3-OH	5-H
1-aryl (subst.)	3-OH	5-OMe
1-benzoyl (subst.)	3-OH	5-OMe
1-benzyl (subst.)	3-Hal, 3-SH, 3-OR	5-OMe

On the contrary, the combination of the following structural features seemed to be relevant for 5-LO activity:

1-benzyl (subst.)	3-OH	5-OMe, 5-OH, ...

Table 4. 5-LOX inhibition of 5-nitro-, 5-amino-, 5-urea-, and 5-hydroxy-1H-indazol-3-ols.



No.	R^1	R^2	Mp (°C)	MW	Analysis C, H, N[a]	IC_{50} [nM][b]
47	3,4-Cl_2	NO_2	247	338.15	$C_{14}H_9Cl_2N_3O_3$	> 1000
48	2,4-Cl_2	NO_2	236–240	338.15	$C_{14}H_9Cl_2N_3O_3$	> 1000
49	4- 	NO_2	264–266	375.39	$C_{21}H_{17}N_3O_4$	> 1000
50	4- 	NO_2	218–221	426.44	$C_{24}H_{18}N_4O_4$	> 1000
51	4-Cl	NH_2	200	273.72	$C_{14}H_{12}ClN_3O$	> 1000
52	3,4-Cl_2	NH_2	216–220	308.17	$C_{14}H_{11}Cl_2N_3O$	> 1000
53	2,4-Cl_2	NH_2	199–203	308.17	$C_{14}H_{11}Cl_2N_3O$	> 1000
54	4-Cl	$NHCONHC_6H_3Cl_2$(3,4)	282 dec.	461.74	$C_{21}H_{15}Cl_3N_4O_2$	278 ± 60
55	3,4-Cl_2	$NHCONHC_6H_3Cl_2$(3,4)	259–263	496.18	$C_{21}H_{14}Cl_4N_4O_2$	> 1000
56	2,4-Cl_2	$NHCONHC_6H_4OMe$(4)	302–306	457.32	$C_{22}H_{18}Cl_2N_4O_3$	> 1000
57	2,4-Cl_2		308–310	433.34	$C_{21}H_{22}Cl_2N_4O_2$	> 1000
58	4-Cl	OH	n.d. (resin)	274.71	$C_{14}H_{11}ClN_2O_2$	> 1000
59	3,4-Cl_2	OH	239–243	309.15	$C_{14}H_{10}Cl_2N_2O_2$	748 ± 119
60	2,4-Cl_2	OH	216	309.15	$C_{14}H_{10}Cl_2N_2O_2$	258 ± 44

a: Analysis C, H, N within ± 0.4% unless otherwise indicated. b: Mean of 4 independent experiments.

Therefore, we first concentrated our synthetic efforts on the variation of the substituents in the 1-benzyl moiety of the 3-hydroxy-5-methoxy-indazole skeleton.

The resulting structure-activity relationships can be summarized as follows:

– If there is only one substituent in the benzyl moiety, the 4-position seems to be the most active one.

– In the series of halogen derivatives the chloro substituents are superior to bromo or fluoro substituents.

– Among the compounds with dihalosubstituted benzyl groups those with 2,4- or 3,4-substitution prove to be the most active ones.

– Halogen compounds are more active than those with (branched) alkyl, alkyloxy, nitro, OH or CF_3 substituents in the benzyl group.

– Bulky lipophilic substituents in 4-position of the benzyl ring are superior to small substituents.

– Naphthylmethyl or 6-chloropiperonylmethyl moieties in 1-position of the indazole ring reveal similar activity as the benzyl group.

– Heterocyclic analogues of the benzyl group show (almost) no activity.

The distance between the indazole skeleton and the aryl ring system significantly influences 5-LO activity. Linking by

propyl-or allyl-chains results in best activity; compounds with ethyloxy or propyloxy chains were slightly less active. Carbonyl, amido, or hydroxy functions in this part of the molecule lead to loss of activity.

When modifying the 5-position in 3-hydroxy indazoles with selected 1-benzyl substitution we found that 5-nitro and 5-amino derivatives did not show any 5-LO activity. In the series of 5-urea derivatives only for **54** an IC_{50} value < 1 μM was found. However , introduction of the 5-hydroxy group results in compounds almost equipotent to the analogues with 5-methoxy substituent.

Table 5. Effect of the 5-LOX inhibitors on allergen-induced contraction of actively sensitized guinea pig tracheal segments.

Compound	IC_{50} μmol/l (with 95% confidence intervals in parentheses)
12	3 μmol/l = maximum inhibition: 40%
26	1 μmol/l = maximum inhibition: 32%
27	2.9 (1.2–6.9)
2 (BAY × 1005)	2.1 (1.4–2.9)

as well as on the arachidonic acid (AA) induced ear edema in mice.

In vitro experiments for investigation of bronchodilating activity were carried out in the presence of the H_1-receptor antagonist mepyramine and of the cyclooxygenase inhibitor indomethacin to exclude the reactions of histamine and prostaglandins [7]. Compounds were tested in the concentration-range from 0.3 to 10 μM (Table 5). While compounds **12** and **26** exhibited maximum inhibition at 3 μM (40%) and at 1 μM (32%) respectively, compound **27** (IC_{50} = 2.9 μM) showed a strong inhibitory effect. This effect was comparable with **2** (BAY × 1005, IC_{50} = 2.1 μM).

Late phase asthmatic reaction is characterized by a significant increase in the number of eosinophils in the bronchoalveolar fluid resulting from pronounced migration of these cells into the lung [8]. The corresponding model comprises the application of the test compounds to sensitized and ovalbumin (OVA) challenged guinea pigs [9].

In contrast to vehicle only **12**, **26**, and **27** significantly reduced the number of eosinophils in the bronchoalveolar fluid (Table 6). A similar effect could be demonstrated for **2** (BAY × 1005).

Topical application of AA produces an acute transient inflammatory reaction characterized by vasodilation, tissue edema and elevated tissue concentration of prostaglandins and leukotrienes. Neutrophils migrate into the dermis of the ear 30 min after AA-application and reach the maximum concentration 1 h when edema culminates. The quantity of polymorphonuclear leukocytes (PMNL) migrating into the inflammatory sites corresponds to the degree of development of edema [10,11]. 5-LOX inhibitors are known to reduce the development of edema in mice [12].

There were equipotent effects of **27** and **2** (BAY × 1005) after topical application of 0.5 and 1 μg/ear (Table 7).

Table 6. Effect of 5-LOX inhibitors on inflammatory cell influx into BAL fluid in the actively sensitized guinea-pig by prophylactic administration 2 hours prior to antigen challenge.

	n	Eosinophil numbers ($\times 10^6$ cells/animal)	% inhibition of increase
Naive	9	0.83 ± 0.31	
Saline challenged + vehicle	58	0.84 ± 0.45	
OVA challenged + vehicle	56	6.44 ± 2.49$^{###}$	0
OVA challenged + **12** (1.0 mg/kg i.p.)	9	2.92 ± 1.25***	62.9
OVA challenged + **26** (1.0 mg/kg i.p.)	8	3.32 ± 1.55***	55.7
OVA challenged + **27** (free base), (1.0 mg/kg i.p.)	10	3.82 ± 1.87**	46.8
OVA challenged + **27** (free base), (10 mg/kg i.p.)	20	3.82 ± 2.80***	45.5
OVA challenged + **2** (BAY × 1005) (10 mg/kg i.p.)	9	3.23 ± 1.88***	57.3

$^{###}$ $p < 0.001$ versus saline challenged; ** $p < 0.01$, *** $p < 0.001$ versus OVA challenged plus vehicle. Data are presented as mean ± s.d.

Pharmacology

Based on the *in vitro* results the compounds **12**, **26**, and **27** were selected for further pharmacological characterization. The antiinflammatory effect of 5-LOX inhibitors was tested on the *in vitro* model of allergen-induced contraction of tracheal segments and *in vivo* on the antigen-induced bronchopulmonary eosinophilia in actively sensitized guinea pigs

Table 7. Inhibitory effect of 5- LOX inhibitors after topical administration on arachidonic acid (AA)-induced edema in mice.

Compound	Topical Dose µg/ear	Mean % change AA-edema
27 (free base)	0.5	–19 *
	1	–41*
2 (BAY × 1005)	0.5	–17
	1	–48 *
1 (Zileuton)	20	–54 *

* $p < 0.05$ compared to the vehicle-treated group.

Conclusion

The synthesized indazole derivatives enabled us to study the influence of substituents on *in vitro* inhibition of 5-lipoxygenase. According to the *in vivo* results **27** proved to be the most potent compound of the synthesized series. **27** inhibited the contraction of tracheal segments of guinea pigs, reduced the infiltration of eosinophils into the lung (guinea pigs), and exhibited dose dependent antiinflammatory activity in the mouse ear edema model. Therefore **27** is expected to be useful in the treatment of a variety of leukotriene-mediated disorders including asthma.

Experimental Part

Chemistry

Melting points: melting point apparatus by Boetius, uncorrected.– IR spectra: Perkin Elmer FT-IR 1725 X, KBr.– ^{1}H and ^{13}C NMR spectra (TMS as internal standard): Bruker ARX 300 and Bruker AMX 500 spectrometers, 303 K, chemical shifts in δ units. All NH and OH were replaceable by D_2O.– Microanalyses were within ± 0.4% of the theoretical values for all elements listed, unless otherwise stated.- Silica gel chromatography was performed using Merck silica gel 60 (63–200 mesh). Reagents used were purchased from the Aldrich Chemical Co., E. Merck KGaA and Lancaster Synthesis GmbH.

All compounds were characterized by elemental analysis, ^{1}H, ^{13}C-NMR, and IR spectroscopy, melting points and thin layer chromatography. In all cases the IR and NMR spectra indicated the presence of the 3-hydroxy group and not a carbonyl group (no carbonyl band at about $\nu \approx 1700$ cm^{-1} s).

Synthesis of 5-substituted 1H-indazol-3-ols

5-Methoxy-1H-indazol-3-ol (**4**) was prepared by the method of Baiocchi [5].

5-Nitro-1H- indazol-3-ol (**5**) was prepared by the method of Pfannstiel et al. [6].

Synthesis of 1-substituted indazol-3-ols **6–50**

Compounds **6–50** were prepared by similar methods as reported previously [5].

Typical procedures are described in the following:

*1-(4-Benzyloxybenzyl)-5-methoxy-1H-indazol-3-ol (***26***)*

5-Methoxy-1H-indazol-3-ol (**4**) (3.0 g, 18.0 mmol) and 4-benzyloxy-benzyl chloride (4.2 g, 18.0 mmol) were stirred in NaOH (20 ml of 5 N) for 2.5 h at 70°C. After cooling the viscous solid was filtered, washed with water, dissolved in DMF (60 ml), and purified by column chromatography on a silica gel column eluating with dichloromethane-methanol (95:5, *v/v*). The purified fraction was concentrated under reduced pressure and the residue was crystallized with MeCN. The solid was filtered and recrystallized from MeCN in the presence of activated charcoal.

Yield: 1.0 g (15%) **26**, mp 177.5–179 °C.– Anal. ($C_{22}H_{20}N_2O_3$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 3.59 (s, 3H, CH_3), 4.88 (s, 2H, CH_2), 5.07 (s, 2H, CH_2), 6.75–7.31 (12H, aromatic), 10.30 (s, 1H, OH).– ^{13}C-NMR ([D$_6$]DMSO): δ = 50.53 CH_2N, 54.99 CH_3O, 68.75 CH_2O, 99.41–153.73 18 C-aromatic, 157.22 C-OH.

*5-Methoxy-1-[quinoline-2-ylmethoxy)-benzyl]-1H-indazol-3-ol Dihydrochloride (***27***)*

5-Methoxy-1H-indazol-3-ol (**4**) (3.6 g, 21.9 mmol), 2-(4-chloromethyl-phenoxymethyl)-quinoline hydrochloride (6.4 g, 22.6 mmol) and sodium hydroxide (2.4 g, 60.0 mmol) in DMSO (50 ml) were stirred for 6 h at 20–30°C. The mixture was extracted with CH_2Cl_2 (300 ml) and water (400 ml), the CH_2Cl_2 phase was washed successively with water (3 × 400 ml), dried over Na_2SO_4 and concentrated. The crude product was recrystallized twice from EtOAc in the presence of activated charcoal.

Yield: 0.6 g (7%) **27** (free base), mp 165–169 °C. Anal. ($C_{25}H_{21}N_3O_3$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 3.73 (s, 3H, CH_3), 5.22 (s, 2H, CH_2N), 5.34 (s, 2H, CH_2O), 6.96–9.41 (21H, aromatic), 10.46 (s, 1H, OH).– ^{13}C-NMR ([D$_6$]DMSO): δ = 50.83 CH_2N, 55.32 CH_3O, 70.80 CH_2O, 99.75–157.47 22 C-aromatic.

27 (free base) (1.5 g, 3.6 mmol) were dissolved in acetone (550 ml) under reflux. After cooling isopropanolic HCl (1.71 ml of 7 N) were added and the solution was stirred for 3 h at 20–25°C. The product was filtered off and recrystallized from ethanol.

yield: 1.1 g (62%) **27**, mp 181 °C. Anal. ($C_{25}H_{23}Cl_2N_3O_3$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 3.72 (s, 3H, CH_3), 5.26 (s, 2H, CH_2N), 5.69 (s, 2H, CH_2O), 6.95–9.05 (21H, aromatic), 11.5 (s, 2H, OH, NH).– ^{13}C-NMR ([D$_6$]DMSO): δ = 50.74 CH_2N, 55.41 CH_3O, 66.55 CH_2O, 100.16–156.71 22 C-aromatic.

*1-(3,4-Dichlorobenzyl)-5-nitro-1H-indazol (***47***)*

A solution of 5-nitro-1H-indazol-3-ol (**5**) (14.4 g, 80 mmol) and 3,4-dichlorobenzyl chloride (15.6 g, 80 mmol) in NaOH (80 ml of 1 N) was stirred for 4 h at 70°C, additional 3,4-dichlorobenzyl chloride (5.5 g, 28 mmol) and NaOH (20 ml of 1 N) were added and stirred for another 2.5 h at 70°C. After cooling and filtration the solid was recrystallized from n-BuOH with activated charcoal.

Yield 22.1 g (81%) **47**, mp 247 °C. Anal. ($C_{14}H_9Cl_2N_3O_3$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 5.34 (s, 2H, CH_2N), 7.04–8.55 (6H, aromatic).– ^{13}C-NMR ([D$_6$]DMSO): δ = 50.60 CH_2N, 110.27–142.69 12 C-aromatic, 157.62 C-3.

*5-Amino-(2,4-dichlorobenzyl)-1H-indazol-3-ol (***53***)*

1-(2,4-Dichlorobenzyl)-5-nitro-1H-indazol-3-ol (**48**) (16.2 g, 48 mmol) were hydrogenated in dioxane (800 ml) with Ra-Ni (5.0 g, 20 bar, 100°C, 6 h). After filtration and concentration under reduced pressure the crude product was recrystallized from n-BuOH with activated charcoal.

yield: 8.7 g (59%) **53**, mp 199–203 °C. Anal. ($C_{14}H_{11}Cl_2N_3O$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 4.75 (s, 2H, NH_2), 5.24 (s, 2H, CH_2N), 6.67–7.58 (6H, aromatic), 10.25 (s, 1H, OH).– ^{13}C-NMR ([D$_6$]DMSO): δ = 48.52 CH_2N, 100.86–141.63 12 C-aromatic, 154.18 C-3.

*1-[1-(3,4-Dichlorobenzyl)-3-hydroxy-1H-indazole-5-yl]-3-(4-methoxy-phenyl)-urea (***56***)*

A solution of 5-amino-1-(3,4-dichlorobenzyl)-1H-indazol-3-ol (**52**) (1.54 g, 5.0 mmol) and 4-methoxyphenylisocyanate (1.12 g, 7.5 mmol) in THF (75 ml) was stirred for 5 h at room temp. The solution was concentrated under reduced pressure to one third of its volume. After standing overnight the solid was filtered and recrystallized from n-BuOH.

Yield: 1.55 g (68%) **56**, mp 267 °C. Anal. ($C_{22}H_{18}Cl_2N_4O_3$) C, H, N.– ^{1}H-NMR ([D$_6$]DMSO): δ = 3.75 (s, 3H, CH_3), 5.29 (s, 2H, CH_2N), 6.87–8.52 (18H, aromatic), 10.5 (s, 1H, OH).– ^{13}C-NMR ([D$_6$]DMSO): δ = 52.65 CH_2N, 57.76 CH_3O, 111.26–141.71 17 C-aromatic, 155.75, 156.99, 157.31 C-3, C-OCH_3, C=O.

*1-(4-Chlorobenzyl)-1H-indazol-3,5-diol (**58**)*

To a solution of 5-amino-1-(4-chlorobenzyl)-1H-indazol-3-ol (**51**) (2.0 g, 7.3 mmol) in n-BuOH (180 ml) and NaOH (20 ml of 3.3 N) a solution of $NaNO_2$ (1.26 g, 18.2 mmol) in water (5 ml) was dropped at 0 °C. After stirring for 1 h at 0–5 °C the solution was stirred for another hour at 80 °C. After cooling the product was concentrated under reduced pressure to dryness. The residue was extracted with water (100 ml) and tBu-O-Me (200 ml). The ether phase was washed with water (100 ml), dried over Na_2SO_4, and evaporated to dryness.

Yield: 2.01 g (99%) **58**. Anal. ($C_{14}H_{11}ClN_2O_2$) C, H, N.– ^{1}H-NMR ([D_6]DMSO): δ = 5.35 (s, 2H, CH_2N), 7.13–7.78 (7H, aromatic), 10.44 (s, 2H, OH).– ^{13}C-NMR ([D_6]DMSO): δ = 50.96 CH_2N, 110.98–140.46 12 C-aromatic, 154.89 C-3.

*1-(3,4-Dichlorobenzyl)-1H-indazol-3,5-diol (**59**)*

A solution of 1-(3,4-dichlorobenzyl)-5-methoxy-1H-indazol-3-ol (**12**) (4.85 g, 15.0 mmol) in acetic acid (30 ml) and HBr (30 ml of 5.93 N) was refluxed for 4h. After cooling water (250 ml) were added, pH 14 was adjusted by adding conc. NaOH, and the aqueous solution was extracted twice with tBu-O-Me. The aqueous phase was acidified with H_2SO_4, and extracted with tBu-O-Me (3 × 200 ml). The combined ether phases were dried over Na_2SO_4 and concentrated under reduced pressure. The crude solid was crystallized with tBu-O-Me.

yield: 2.40 g (52%) **59**, mp 239–243 °C. Anal. ($C_{14}H_{10}Cl_2N_2O_2$) C, H, N.– ^{1}H-NMR ([D_6]DMSO): δ = 5.33 (s, 2H, CH_2N), 6.98–7.65 (6H, aromatic), 9.13 (s, 2H, OH).– ^{13}C-NMR ([D_6]DMSO): δ = 49.17 CH_2N, 101.45–149.69 12 C-aromatic, 153.33 C-3.

Supplementary Material

NMR data are available from the authors.

Pharmacological Methods

Inhibition of 5-lipoxygenase

Paraffin (2 ml per animal) was administered intraperitoneally to ten male Wistar rats (360–400 g) to induce the production of macrophages. After 4 days the rats were sacrificed. The abdominal cavity was opened, the cavity was washed with sodium-phosphate buffer (20 ml of 3 mM, pH 7, containing 5.5 mM glucose, 150 mM NaCl, 3.8 mM KCl) and the cell suspension was transferred into plastic vials. The cells were centrifuged at 400 g for 10 min. The supernatant was removed and the cells were washed twice with cold sodium-phosphate buffer (50 ml). After addition of aq. $CaCl_2$ (500 µl of 200 mM) and [^{3}H]-arachidonic acid (15 µl, Bio Trend) the vials were closed, shaken gently and centrifuged at 400 g for 10 min. The radioactive supernatant was removed, the cell pellet resuspended and buffer (50 ml) was added. The cells were washed twice.

The incubation mixture (2 ml) contained sodium-phosphate buffer (3 mM, pH 7), indomethacin (0.025 mM), glutathione (0.6 mM), Ca-ionophore (0.01 mg/ml), the prepared cell preparation (500 µl) and the test compound in different concentrations. Nonspecific enzyme activity was determined in presence of E 6080 (20 µM). This mixture was preincubated for 30 min at 37 °C. After addition of aq. $CaCl_2$ (25 µl of 200 mM) incubation was continued for 10 min at 37 °C. The reaction was stopped by addition of formic acid (25 µl).

The reaction products of 5-lipoxygenase were separated by an extraction procedure. Isopropanol/diethyl ether (5 ml of 1/4, *v/v*) was added, the vials were shaken and centrifuged at 3000 g for 5 min at 4 °C. The upper organic phase was transferred into new vials. After addition of aq. NH_3 (25 µl of 25%), n-hexane (2.5 ml) was added, the vials were shaken and centrifuged at 3000 g for 5 min at 4 °C. The vials were placed into a freezer at –70°C. After removing the upper organic phase the lower aqueous phase was thawed. 100 µl of the aqueous phase, which contained the [^{3}H] labeled products (mixture of the polar metabolic products of arachidonic acid), was transferred into scintillation vials. After addition of scintillation fluid (3 ml) the vials were counted for radioactivity in a liquid scintillation counter (Wallac 1409, Berthold) [13–15].

Inhibition of the allergen-induced contraction of tracheal segments in actively sensitized guinea pigs

Actively sensitized guinea pigs were sacrificed and the trachea was removed. The trachea was divided into 3 to 4 small segments and equilibrated for 0.5 hours at 37°C under isotonic conditions (1 g pretension, isometric transducers and amplifier, TSE *in vitro* apparatus, Bad Homburg). The isolated preparations were suspended in 20 ml organ baths containing modified Krebs' solution (4.7 mM KCl, 1.2 mM KH_2PO_4, 1.62 mM $MgSO_4 \times 7\ H_2O$, 118 mM NaCl, 25 mM $NaHCO_3$, 6.1 glucose, 2.52 $CaCl_2 \times 2\ H_2O$), which was gassed with a mixture of 95% O_2 and 5% CO_2. Indomethacin (1 µM) and mepyramine (28 µM) were added to the organ bath to inhibit bronchoactive prostanoid and histamine. After an equilibration period carbachol (0.5 µM) was applied as spasmogen, left in contact with the organs up to the maximum contraction (about 3 – 5 min) and washed out. After 30 minutes 10 µg/ml ovalbumin was added as spasmogen, left in contact with the organs for 1 hour. The contractile reaction of the sensitized tissues was measured after a single challenge of ovalbumin. The test compounds were applied as a suspension in Tween 80 (0.2 ml with a final concentration of 0.0013%) to the organ bath (30 ml), 30 minutes prior to allergen challenge. There was no effect of vehicle (Tween 80) - treated control group at the final concentration of 0.0013% on allergen-induced contraction of tracheal segments. The anaphylactic (contractile) responses were expressed as percentage of the maximum carbachol response obtained at the beginning of the experiment.

Effect on late phase eosinophilia in actively sensitized guinea pigs

Male Dunkin-Hartley guinea pigs (200–250 g) were obtained from Schoenwalde, FRG. Animals were actively sensitized with a suspension of 10 µg ovalbumin (OVA) + 1 mg $Al(OH)_3$ subcutaneously and two weeks later boosted. 7 days after boosting the animals were exposed for 30 s to an aerosol of OVA (5 mg/ml) administered via a nebulizer driven by compressed air at 19.6 kPa in an exposure chamber. To prevent anaphylactic death mepyramine (10 mg/kg i.p.) was given 0.5 minutes before challenge. 24 h later bronchoalveolar lavage (BAL) was performed with 2 × 5 ml saline in animals sacrificed by an overdose of urethane. Lavage fluid was pooled, centrifuged at 400g for 10 minutes and the cell pellet suspended in 1 ml saline. Eosinophils were counted in a Neubauer chamber after staining them using the Becton Dickinson Test kit (No. 5877) for eosinophils. Mean and standard deviation (sd) was calculated. Each group of animals treated with test compounds was compared with the placebo treated challenged group (unpaired *t* test). For drug administration all compounds were administered intraperitoneally suspended in 10% polyethylene glycol 300 and 0.5% hydroxyethyl-cellulose with a volume of 0.1 ml per 100 g body weight. Animals received test compounds prophylactically 2 hours before allergen challenge. Each drug was tested in at least 8–20 animals. For each group mean and standard deviation (sd) was calculated.

Antiinflammatory activity in the AA induced mouse ear edema

Investigations into the effect of test substances on arachidonic acid (AA) induced ear edema were carried out in male mice (25–30 g). The test compounds or vehicle, dissolved in acetone, were administered topically 30 minutes prior to topical application of AA. Arachidonic acid was dissolved in acetone and applied to the inner side of the right ear in a volume of 15 µl (2 mg AA/ear) by an automatic pipette. The left ear received 15 µl acetone alone. One hour after AA application, the thickness of both ears was measured by means of an Oditest dial gauge calipers in units of 0.01 mm. The development of the ear edema was calculated by subtracting the thickness of the left ear (vehicle control) from that of the right ear (treated ear). The percentage inhibition was calculated according to Kotyuk [11].

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