Indazolinones, a New Series of Redox-Active 5-Lipoxygenase Inhibitors with Built-In Selectivity and Oral Activity

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Since the hypothetical mechanisms of hydroperoxydation of arachidonic acid by, respectively, 5-lipoxygenase (5-LPO) and cyclooxygenase (CO) involve a redox cycle, a compound which reduces 5-LPO and CO to their inactive state would give a nonselective inhibitor of both enzymes. Structural modifications of such a compound could be expected to give improved potency and selectivity for 5-LPO and oral activity. Such an approach has led to the discovery of 1,2-dihydroindazol-3-ones which are potent 5-LPO inhibitors with various degrees of selectivity. Structure–activity relationship studies indicated that while N-1,N-2-unsubstituted and N-1-substituted derivatives are orally inactive, *N*-2-alkyl derivatives are orally active and inhibit both 5-LPO and CO. In contrast, *N*-2-benzyl derivatives are selective for 5-LPO but possess only weak oral activity. Further structural modifications have identified ICI 207968 [1,2-dihydro-2-(3-pyridylmethyl)-3*H*-indazol-3-one, **21a**] which combines potent oral activity and high selectivity. Methemoglobin (MHb) induction by **21a** in dog blood precluded its development for clinical use. Attempts at dissociating 5-LPO inhibitory properties and MHb formation showed that MHb formation in vitro seemed to be related to the redox potential of the compounds whereas 5-LPO inhibition was not. This study led to a series of 4-(*N*-*n*-pentylcarbamoyl)indazolinones which maintained in vitro 5-LPO potency but did not induce MHb.

Introduction

Arachidonic acid is metabolized by two major oxidative pathways via the enzymes cyclooxygenase (CO) and 5-lipoxygenase (5-LPO). Inhibition of CO prevents the formation of prostaglandins and is well-established as a clinically effective antiinflammatory therapy.[1-4] Inhibition of the enzyme 5-LPO blocks synthesis of leukotrienes and represents a potentially new treatment for diseases such as rheumatoid arthritis, asthma, and inflammatory bowel disease, in which elevated levels of leukotrienes are believed to play a role.[5-8] Many compounds which inhibit 5-LPO in vitro have been described but most of these agents suffer from a lack of in vivo efficacy and/or selectivity with respect to inhibition of CO. The therapeutic potential of 5-LPO inhibition will only be established when potent, orally active inhibitors free from CO inhibitory activity are evaluated clinically.[9]

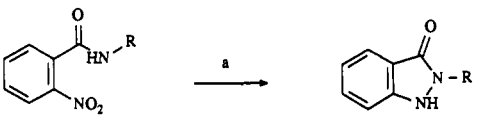
It is well-known that the two arachidonic acid metabolizing enzymes 5-LPO and CO are susceptible to inhibition by compounds with low redox potentials such as phenidone[10] and BW755C.[11] "Redox inhibitors of 5-LPO", as these type of compounds have been loosely termed, generally show poor selectivity for 5-LPO relative to CO. Our aim was to discover selective 5-lipoxygenase inhibitors by incorporating structural features into a "redox-type" inhibitor that would favor inhibition of 5-LPO relative to CO.

In this paper we describe the structure–activity relationships of the indazolinones,[12] a new class of 5-LPO inhibitors. We have discovered that manipulation of the indazolinone N-2-substituent influences both selectivity and oral efficacy. In particular we report that 1,2-dihydro-2-(3-pyridylmethyl)-3*H*-indazol-3-one (**21a**) is a highly selective, potent, orally active inhibitor of 5-LPO and should be a useful agent for elucidating the in vivo roles of leukotrienes.

Biological Testing

Measurement of arachidonic acid metabolites in whole blood was used to monitor the 5-LPO inhibitory potency, selectivity versus CO inhibition and the oral activity of our series. In vitro and ex vivo studies with rat blood and dog blood were carried out as previously described.[13a-e] In the in vitro studies IC_{50} values for both LTB4 [IC_{50}(LT)] and PGE2 [IC_{50}(PG)] inhibition were determined. In the ex vivo studies ED_{50} values for inhibition of LTB4 ex vivo [ED_{50}(LT)] were determined. Methemoglobin induction has also been measured in various rat or dog blood samples (in vitro and ex vivo) or in human blood (in vitro). In vitro

Scheme I. Method A[a]



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[a] (a) Zn/NaOH.

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(1) Samuelsson, B.; Granström, E.; Green, K.; Hamberg, M.; Hammarström, S. *Annu. Rev. Biochem.* **1975**, *44*, 669.
(2) Flower, R. J.; Vane, J. R. *Biochem. Pharmacol.* **1974**, *23*, 1439.
(3) Samuelsson, B.; Goldyne, M.; Granström, E.; Hamberg, M.; Hammarström, S.; Malmsten, C. *Annu. Rev. Biochem.* **1978**, *47*, 997.
(4) Shen, T. Y. *Handbook of Experimental Pharmacology, Vol. 50/II, Anti-inflammatory Drugs*; Vane, J. R., Ferreira, S. H., Eds.; Springer: Berlin, 1979, pp 305-347.
(5) Ferreira, S. H.; Vane, J. R. *Handbook of Experimental Pharmacology, Vol. 50/II, Anti-inflammatory Drugs*; Vane, J. R., Ferreira, S. H., Eds.; Springer: Berlin, 1979, pp 348-398.
(6) Piper, P. J.; Samhoun, M. N. *Br. Med. Bull.* **1987**, *43*, 297.
(7) Salmon, J. A.; Higgs, G. A. *Br. Med. Bull.* **1987**, *43*, 285.
(8) Parker, C. W. *Drug Dev. Res.* **1987**, *10*, 277.
(9) McMillan, R. M.; Foster, S. J. *Agents and Actions* **1988**, *24*, 114.
(10) (a) Blackwell, G. J.; Flower, R. J. *Prostaglandins* **1978**, *16*, 417. (b) Blackwell, G. J.; Flower, R. J. *Br. J. Pharmacol.* **1978**, *63*, 360P.
(11) Higgs, G. A.; Flower, R. J.; Vane, J. R. *Biochem. Pharmacol.* **1979**, *28*, 1959.
(12) Heller, G.; Jacobsohn, P. *Chem. Ber.* **1921**, *54B*, 1107.
(13) (a) Carey, F.; Forder, R. A. *Br. J. Pharmacol.* **1985**, *84*, 34P. (b) Carey, F.; Forder, R. A. *Prostaglandins, Leukotrienes Med.* **1986**, *22*, 57. (c) Forder, R. A.; Carey, F. *Prostaglandins* **1984**, *28*, 666. (d) McMillan, R. M.; Millest, A. J.; Proudman, K. E.; Taylor, K. B. *Br. J. Pharmacol.* **1986**, *87*, 53P. (e) Foster, S. J.; Bruneau, P.; Walker, E. R. H.; McMillan, R. M. *Br. J. Pharmacol.* **1990**, *99*, 113.

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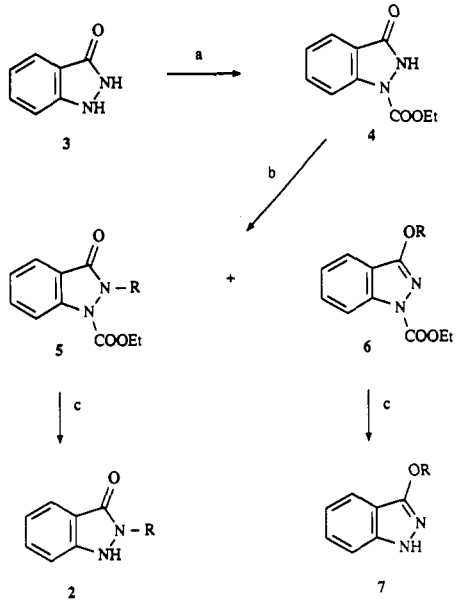
Scheme II. Method B[a]



[a] (a) ClCOOEt, pyridine; (b) RX, KOH, or Et_3N; (c) KOH.

data are quoted as EC_{25} values [EC_{25}(MHb)].

Chemistry

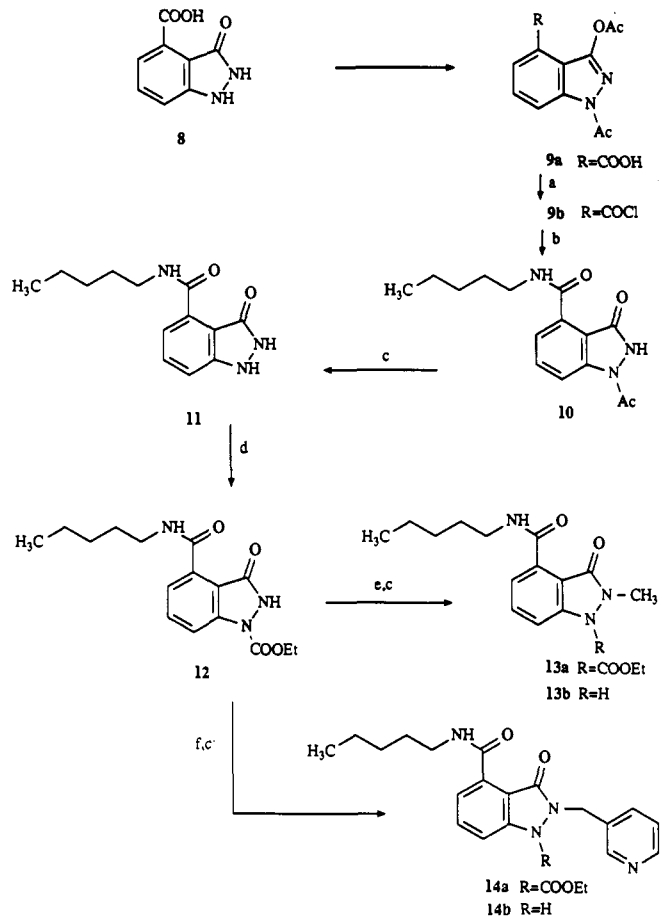
The chemistry of indazoles and indazolinones has been extensively reviewed by Baiocchi et al.[14]

Two different methods were used to obtain N-2-substituted indazolinones. The first one, method A, shown in Scheme I, involved reductive cyclization of an *o*-nitrobenzamide (1) by Zn under basic conditions. This reaction gave indazolinones **2** in variable yields, depending on the susceptibility of the amide bond to hydrolysis. The required *o*-nitrobenzamides were obtained by condensing *o*-nitrobenzoyl chloride with the corresponding amine according to the well-known procedure.[15]

When the reductive cyclization process did not work or when the suitable *o*-nitrobenzamide could not be obtained, the alternative synthesis of N-2-substituted indazolinones **2**, method B, shown in Scheme II, was used. Alkylation of the protected indazolinone **4**, following the conditions described in the literature, gave good to very poor yields.[16] Due to the fact that the conditions used for the alkylation step were similar to those needed for the subsequent deprotection step, it is likely that deprotection occurred during the alkylation and in certain cases this led to mixtures of N-1- and N-2-alkylated compounds along with O-alkylated derivatives (**6**) and in low yields. In these cases alkylation using triethylamine, rather than potassium hydroxide, increased the yield dramatically but a mixture of N-2- (**5**) and O-alkylated compounds (**6**) was still obtained. These two isomers were usually separated by column chromatography and then the carbethoxy groups were removed by rapid treatment with potassium hydroxide at room temperature to give either the N-2-alkylated or the O-alkylated indazolinones **2** or **7**.

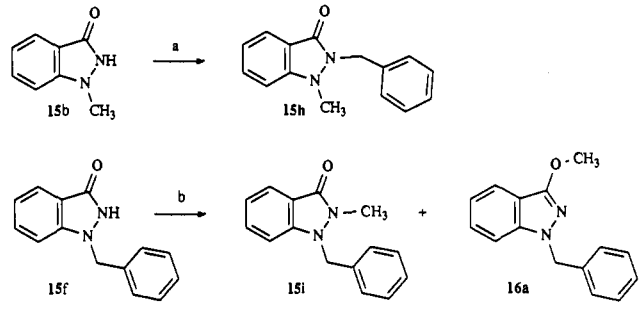
Syntheses of the compounds bearing a 4-carbamoyl substituent (**11**, **13b**, and **14b**) (Scheme III) were performed starting from 4-carboxyindazolinone **8**. Suitable protection of the indazolinone oxygen and nitrogen as acetates gave **9a**,[17] which could be derivatized to 4-carbamoyl derivative **11** via acid chloride **9b** and monoacetate **10** after spontaneous loss of the oxygen protection followed by deprotection of the nitrogen. Reprotection of N-1 of **11** with a carbethoxy residue allowed the alkylation of N-2

Scheme III. 4-Carbamoyl Derivatives[a]



[a] (a) $SOCl_2$, reflux; (b) *n*-pentylamine, Et_3N, CH_2Cl_2; (c) NaOH; (d) ClCOOEt, pyridine; (e) NaH, MeI; (f) 3-pyridylcarbinol, PPh_3, diisopropyl azodicarboxylate.

Scheme IV. Disubstituted Derivatives[a]



[a] (a) $ClCH_2Ph$; Et_3N; (b) CH_2N_2.

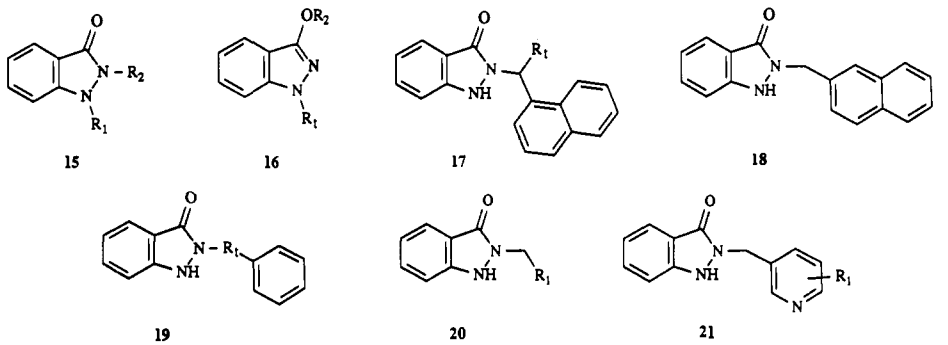
(14) Baiocchi, L.; Corsi, G.; Palazzo, G. *Synthesis* **1978**, 633.
(15) Furniss, B. S.; Hannaford, A. J.; Smith, P. W. G.; Tatchell, A. R. *Vogel's Textbook of Practical Organic Chemistry*, 5th Ed.; Longman Publisher: Birmingham, AL, 1989; p 1261.
(16) Wyrick, S.; Voorstad, P.; Cocolas, G.; Hall, I. *J. Med. Chem.* **1984**, *27*, 768.
(17) Boie, I.; Willibald, P.; Kunits, F. W. Ger. Offen. 2.035.725, July 18, 1970.
(18) Stephenson, F. M. *Organic Synthesis*; Wiley: New York, 1955; Collect. Vol. 3, p 475.
(19) Schmutz, J.; Hunziker, F.; Michaelis, W. *Helv. Chim. Acta* **1964**, *47*, 1986.
(20) (a) Kametani, T.; Sota, K.; Shio, M. *J. Heterocycle Chem.* **1970**, *7*, 815. (b) Ardakani, M.; Smalley, R. *Tetrahedron Lett.* **1979**, *49*, 4765.

Table I. Inhibition of the Production of LTB_4 and PGE_2 in the in Vitro and ex Vivo Whole Blood Tests

compd	R_1	R_2	method	mp, °C (or ref)	formula[c]	in vitro[a] LT	in vitro[a] PG	ex vivo:[b] LT
15a	H	H		(18)		2	30	>100
15b	CH_3	H		(19)		3	20	>100
15c	H	CH_3		(16)		1.3	17	1
15d	C_6H_5	H		(16)		30		
15e	H	C_6H_5		(20a,b)		0.31	2.7	>100
15f	$CH_2C_6H_5$	H		(19)		3.4	31	>100
15g	H	$CH_2C_6H_5$		(16)		0.36	18	20
15h	CH_3	$CH_2C_6H_5$		oil		>30		>100
15i	$CH_2C_6H_5$	CH_3		oil	$C_{15}H_{14}N_2O$[d]	>30		
16a	$CH_2C_6H_5$	CH_3		oil	$C_{15}H_{14}N_2O$	>30		
16b	H	$CH_2C_6H_5$	B	86–87	$C_{14}H_{12}N_2O$	>30		
17a	H		A	198–200	$C_{18}H_{14}N_2O$	0.61	100	>100
17b	CH_3 (*R*)		A	232–233	$C_{19}H_{16}N_2O$	1.4	400	>100
17c	CH_3 (*S*)		A	230–232	$C_{19}H_{16}N_2O{\cdot}0.1CH_2Cl_2$[i]	11	48	
18			B	208–212	$C_{18}H_{14}N_2O$	0.97	18	>100
19a	CH_2CH_2		A	164–165	$C_{15}H_{14}N_2O$	0.55	15	>100
19b	$CH_2CH_2CH_2$		A	140–141	$C_{16}H_{16}N_2O$[g]	0.54	3.8	>100
19c	$CH_2CH_2CH_2CH_2$		A	130–131	$C_{17}H_{18}N_2O$	0.78	26	>100
20a	2-furyl		A	148–149	$C_{12}H_{10}N_2O_2$	0.27	13	15
20b	2-thienyl		A	182–184	$C_{12}H_{10}N_2OS$	0.40	26	8
20c	5-thiazolyl		B	191–192	$C_{11}H_9N_3OS$	1.8	>100	5
20d	3-indolyl		B	194–198	$C_{16}H_{13}N_3O{\cdot}0.1H_2O$[h]	1.1	94	30
20e	4-pyridyl		A	141–142	$C_{13}H_{11}N_3O$	2.4	280	10
20f	2-pyridyl		B	137–138	$C_{13}H_{11}N_3O$	3.1	67	30
21a	H		A	174–175	$C_{13}H_{11}N_3O$	1.5	>300	3
21b	6-CH_3		B	185–186	$C_{14}H_{13}N_3O$	2.5	21	10
21c	5-Br		B	185–187	$C_{13}H_{10}BrN_3O$	1.0	39	10
21d	2-CH_3		B	175–178	$C_{14}H_{13}N_3O$	2.6	58	10
21e	2-Cl		B	161–162	$C_{13}H_{10}ClN_3O$[e]	1.4	12	5
21f	5-CH_3		B	191–192	$C_{14}H_{13}N_3O$	1.8	81	15
21g	6-Cl		B	190–191	$C_{13}H_{10}ClN_3O$[f]	<1	3–30	15
21h	6-CH_3O		B	176–177	$C_{14}H_{13}N_3O_2{\cdot}0.2H_2O$[h]	<1	3–30	10
21i	4-CH_3		B	194–196	$C_{14}H_{13}N_3O{\cdot}0.1H_2O$[h]	3.0	>30	5

[a] IC_{50}'s (μM) in the in vitro test for the inhibition of the production of LTB_4 (LT) and PGE_2 (PG) (see the text). All IC_{50}'s were calculated by nonlinear-regression analysis using two experiments and were significant at the $p < 0.05$ level. [b] EC_{50}'s (mg/kg) in the ex vivo test for the inhibition of the production of LTB_4 (LT) (see the text). All ED_{50}'s have been estimated graphically with at least two different experiments. [c] All compounds gave elemental analyses (C, H, N, Cl, Br) within 0.4% of the theoretical values, except when noted. [d] C: calcd, 75.6; found, 74.4. [e] C: calcd, 60.1; found, 59.6. [f] C: calcd, 60.1; found, 59.2. N: calcd, 16.2; found, 15.7. [g] C: calcd, 76.2; found, 75.4. [h] Water content has been determined by a Karl Fischer assay. [i] Methylene chloride content has been quantified by NMR.

Scheme V. Structures for Tables I and II



and gave ultimately **13b** and **14b** after deprotection and separation of the N-2- and O-alkylated isomers.

Disubstituted derivatives (**15h**, **15i**, **16a**) were synthesized by either alkylation of *N*-1-methylindazolinone **15b** to give **15h** as the sole product or by treatment of *N*-1-benzylindazolinone **15f** with diazomethane to give the *N*-2-methyl derivative **15i** and the *O*-methyl derivative **16a** (Scheme IV).

Results and Discussion

Our approach to the inhibition of 5-LPO was to assess the ability of compounds that possessed weak redox properties to inhibit the enzyme. This approach led to the discovery that indazolinones are inhibitors of 5-LPO. A range of substituted analogues was prepared in order to investigate the influence of structure on potency and selectivity. The biochemical profile of some of the compounds examined is summarized in Table I (see Scheme V for structures).

Unsubstituted indazolinone **15a** inhibited 5-LPO with an IC_{50} of 2 μM but showed poor selectivity and no oral activity. Methyl and benzyl substitution on N-1 (**15b** and **15d**, respectively) did not change the profile of **15a** while the *N*-1-phenyl analogue **15f** was a poorer inhibitor.

Methyl substitution on N-2 (**15c**) gave an interesting compound with a comparable in vitro profile to that of **15a** and this time it shows potent in vivo activity. In contrast, phenyl substitution on N-2 (**15e**) also gave a potent compound in vitro (IC_{50} 0.31 μm), but unfortunately, the compound lacked oral activity.

Notably, however, *N*-2-benzyl derivative **15g** was a more selective lipoxygenase inhibitor which retained the 5-LPO

Table II. Redox Potentials of 5-LPO Inhibitors

compd	redox potential,[a] V	in vitro:[b] LT	compd	redox potential,[a] V	in vitro:[b] LT
15a	0.71	2	**15i**	1.10	>30
15b	0.85	3	**17a**	0.73	0.61
15c	0.66	1.3	**17b**	0.69	1.4
15d	0.88	30	**17c**		11
15e	0.78	0.31	**21a**	0.71	1.5
15f	0.89	3.4	phenidone	0.39	10
15g	0.69	0.36	BW755c	0.31	20
15h	1.08	>30	methyldopa	0.51	

[a] See the Experimental Section. [b] See footnote *a* in Table I. The figures are the same as in Table I; they are reported here for easier comparison with redox potentials.

inhibitory potency of phenyl derivative **15e**. This was the first indazolinone we had prepared in which increased selectivity (IC_{50}(PG)/IC_{50}(LT) ≅50) was observed. N-Disubstituted compounds **15h** and **15i** were inactive as were the compounds substituted on oxygen (**16a** and **16b**).

The encouraging selectivity and oral ex vivo activity shown by **15g** led us to explore this series more thoroughly. A number of substituted benzyl ring analogues of **15g** were prepared.[21] The nature and position of simple substituents on the benzyl ring had no effect on in vitro activity. In contrast, replacement of the *N*-2-benzyl group by a naphthylmethyl moiety produced a dramatic effect. 1-Naphthyl analogue **17a** showed potent inhibition of LTB4 generation in vitro (IC_{50} = 0.61 μM) with a selectivity ratio (IC_{50}(PG)/IC_{50}(LT)) of 160. Its close analogue, 2-naphthyl compound **18**, showed similar potency as an LTB4 inhibitor in vitro but was also more potent against PGE2 generation (IC_{50} = 18 μM), giving it a diminished selectivity ratio of 18.

The pure enantiomers (**17b** and **17c**) of the methylated analogue of **17a** were synthesized. Interestingly, *R* enantiomer **17b** showed a high selectivity ratio of 285, whereas *S* enantiomer **17c**, which was significantly less potent as a 5-LPO inhibitor, showed a much lower selectivity ratio (ca. 4). These results supported our hypothesis that indazolinones might be structurally modified to modulate their specificity toward 5-LPO and CO, without altering their redox properties, as shown for compounds **17a–c** in Table II.

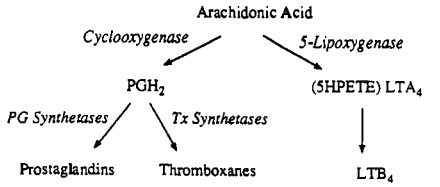
The beneficial effect of inserting a methylene between the indazolinone moiety and a phenyl group on N-2 was studied further. The two, three, and four methylene linked analogues (**19a**, **19b**, and **19c**, respectively) were very active compounds in vitro with IC_{50}(LT)'s in the submicromolar range, but their selectivities were either similar (**19a**, **19c**) or slightly reduced (**19b**) when compared with that of benzyl analogue **15g**.

The structure–activity relationships in the benzyl series were further explored by replacing the phenyl ring with a heterocycle. Simple heterocyclic analogues were as selective as the benzyl compound, with selectivity ratios in excess of 50, as exemplified by the furyl (**20a**), thienyl (**20b**), thiazolyl (**20c**), and indolyl (**20d**) derivatives. Oral activity was a feature in this series and ranged from 30 to less than 5 mg/kg. No explanation has been found to explain these differences.

3-Pyridyl analogue **21a** combined potent inhibition of LTB4 generation in vitro with a high level of selectivity: PGE2 synthesis was only inhibited to 50% by 450 μM **21a**. Furthermore, the exciting in vitro profile of **21a** was reflected in its oral potency and selectivity as an inhibitor of LTB4 synthesis, with an ED_{50} of 3 mg/kg, while not inhibiting PGE2 production at doses as high as 300 mg/kg. 2-Pyridyl isomer **20f** was much less selective and had lower oral potency with an IC_{50}(PG) of 67 μM and an ED_{50}(LT) of 30 mg/kg. Para analogue **20e** had a profile intermediate between those of **21a** and the 2-isomer **20f**.

(21) For the sake of clarity these analogues are not reported.

Scheme VI. Arachidonic Acid Cascade



Following the discovery of **21a**, a range of substituted pyridine analogues was prepared to explore the influence of shape, lipophilicity and pK_a on biological activity (Table I, **21b–i**). Although a number were more potent inhibitors of LTB4 in vitro, none had an overall profile superior to that of the parent compound.

The structure–activity relationships for selectivity among the pyridine-containing indazolinones was reminiscent of those for thromboxane synthetase (TS) inhibition by pyridine derivatives.[22] Thus, a 3 or 4-monosubstituted pyridine was required for optimal inhibition of thromboxane synthetase by pyridine-containing TS inhibitors and for high (>100) LT/PG selectivity in pyridine-containing indazolinones.

In rat whole blood, **21a** was found to be a more potent inhibitor of TxB2 synthesis (IC_{50} = 50 μM) than PGE2 synthesis (IC_{50} = 470 μM).[23] Thus, the impressive LTB4/PGE2 selectivity of **21a** reflects the balance between three effects: (a) reduced LT synthesis as a result of LPO inhibition, (b) elevated PGE2 levels as a consequence of diversion of the pivotal prostaglandin and thromboxane precursor PGH2 to prostaglandin production during TS inhibition, and (c) reduced PGE2 levels due to inhibition of cyclooxygenase (see Scheme VI).[24] Mathematical simulation of the effects of inhibitors of the enzymes of the arachidonic acid cascade confirmed that concurrent weak TS inhibition can enhance the LT/PG selectivity ratios of 5-LPO inhibitors.[25] However, although TS inhibition may contribute to the high LT/PG selectivity in whole blood, **21a** also exhibited 300-fold selectivity for inhibition of 5-LPO in peritoneal macrophages which do not contain TS.[13e]

Redox Potential and Methemoglobinaemia

Since indazolinones have the potential to interact with biological redox systems, we have investigated whether the potency of 5-LPO inhibition by indazolinone analogues was proportional to redox potential alone. In addition, since other redox inhibitors of 5-lipoxygenase have caused induction of methemoglobin (MHb) in animals,[26] the effects

(22) Tanouchi, T.; Kawamura, M.; Ohyama, I.; Kajiwara, I.; Iguchi, Y.; Okada, T.; Miyamoto, T.; Taniguchi, K.; Hayashi, M.; Iizuka, K.; Nakazawa, M. *J. Med. Chem.* **1981**, *24*, 1149.

(23) McMillan, R. M.; Masters, D. J.; Vickers, V. C.; Proudman, K. E.; Foster, S. J.; Pilgrim, W. R.; Bruneau, P. *Br. J. Pharmacol.* **1989**, *96*, 368.

(24) Marcus, A. J. *Inflammation: Basic Principles and Clinical Correlates*; Gallin, J. I., Goldstein, I. M., Snyderman, R., Eds.; Raven Press Ltd.: New York, 1988; p 129.

(25) Several simple simulations can be performed, the following is given as an example. If we consider that four molecules of PGH_2 give three molecules of Tx and one molecule of PG, an inhibitor with an apparent IC_{50}(CO) = 470 μM and an IC_{50}(Tx) = 50 μM would have an actual IC_{50}(CO) = 73 μM.

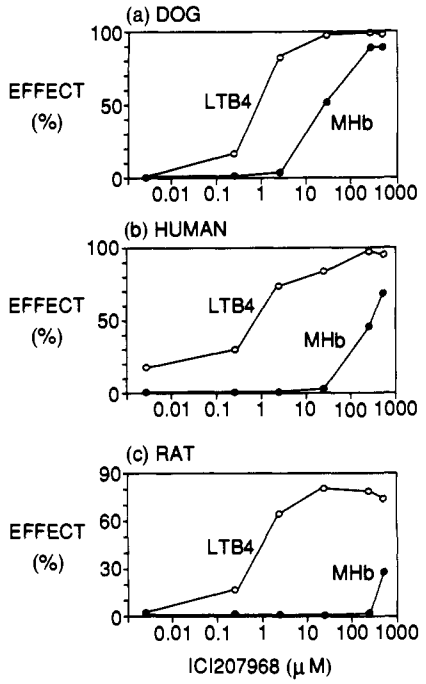


Figure 1. LTB_4 inhibition and MHb induction by ICI 207968 (**21a**) in dog, human, and rat blood (in vitro). LTB_4 synthesis in A23187-stimulated blood was measured after preincubation with ICI 207968 for 15 min at 37 °C. MHb was measured after incubation of blood with ICI 207968 for 2 h at 37 °C. Points represent mean values from duplicate determinations.

of **21a** and related compounds on MHb formation in whole blood have been studied.

As can be seen in Table II, there is clearly no direct relationship between potency and redox potential. The redox potentials of these compounds are higher than those of phenidone[10] (0.39 V) and BW755C[11] (0.31 V), and in the same range as that of methyldopa[27a] (0.51 V).

MHb induction was not observed following oral dosing with **21a** in the rat, even in a subacute study with twice daily dosing at 100 mg/kg po for 10 days. However, therapeutic dosing with the compound in the dog was associated with transient MHb induction. Thus, a single oral dose of 5 mg/kg **21a**, which suppressed ex vivo LTB4 synthesis for 6 h, induced 25% and 20% MHb at 1 and 3 h, although MHb levels had returned to control levels (1–2%) by 6 h (data not shown). This species difference was confirmed in vitro. **21a** (ICI 207968) was a comparable inhibitor of LTB4 synthesis in dog, human, and rat blood (IC_{50} = 0.8, 1.0, and 1.5 μM, respectively) (Figure 1). However, there were marked species differences in the sensitivity to in vitro MHb induction by **21a**, with EC_{25} values in dog, human, and rat blood of 7.5, 80, and 250 μM, respectively (Figure 1).

MHb induction was detected with other indazolinone analogues, for example, the parent unsubstituted indazolinone (**15a**) and the 2-methyl indazolinone (**15c**) (Table III). The problematic MHb induction could be dissociated from inhibition of LTB4 synthesis by incorporation of a 4-(*N*-*n*-pentylcarbamoyl) substituent. In the three examples (**11**, **13b**, **14b**) shown in Table III, the presence of the 4-carbamoyl group prevented MHb induction, but not inhibition of LTB4 synthesis. Interestingly, the 4-carbamoyl analogues exhibited higher redox potentials than their respective parent compound, suggesting that MHb, but not 5-LPO inhibition, may be tightly coupled to redox properties. The problem of MHb induction by **21a** precluded its development for clinical use and, although the 4-carbamoyl analogues maintained in vitro 5-LPO potency and did not induce MHb, none achieved the oral potency of **21a**.

Table III. Inhibition of LTB_4 Synthesis and Formation of Methemoglobin in Vitro in Dog

compd	mp, °C	formula[c]	in vitro LT[a]	in vitro MHb[b]	redox pot.,[g] V
15a	*d*	*d*	0.7	40	0.71
11	167–169	$C_{13}H_{17}N_3O_2$[e]	0.3	>250	0.87
15c	*d*	*d*	0.2	10	0.66
13b	128–129	$C_{14}H_{19}N_3O_2 \cdot 0.6H_2O$[f]	0.3	>250	0.89
21a	*d*	*d*	0.8	7.5	0.71
14b	112–117	$C_{19}H_{22}N_4O_2$	1.5	>250	0.90

[a] IC_{50}'s (μM) in the in vitro test for the inhibition of the production of LTB_4 (LT) in dog blood (see the text). All IC_{50}'s were estimated graphically with at least two different experiments. [b] ED_{25}'s (μM) in the in vitro test for the induction of the production of methemoglobin (MHb) in dog blood (see the text). All EC_{25}'s were estimated graphically with at least two different experiments. [c] All compounds gave elemental analyses (C, H, N) within 0.4% of the theoretical values, except when noted. [d] See Table I. [e] C: calcd, 63.1; found, 62.5. N: calcd, 17.0; found, 16.4. [f] Water content has been determined by a Karl Fischer assay. [g] See the Experimental Section.

Table IV. Activity of 5-LPO Inhibitors in in Vitro and ex Vivo Tests

compd	in vitro[a] LT	in vitro[a] PG	ex vivo:[b] LT
BW755c	20	5	30
phenidone	10	3	5
NDGA	5.0	30	>100
quercetin	10	50	>100
baicalein	10	50	>100
nafazatrom	50	>100	>100
AA861	0.30	15	>100
REV5901	3.0	>300	>100
A-64077	2.2	60	4[c]
BWA4C	0.30	7	20
21a	1.5	>300	3

[a] See footnote *a* in Table I. [b] ED_{50}'s in the ex vivo test (see the text) have been determined graphically from at least three different experiments. [c] Blood withdrawn 3 h after oral dosing.

Conclusions

The results described here with indazolinones support our hypothesis that structural modification of redox-based compounds can modulate 5-LPO inhibitory potency and selectivity independently of changes in redox potential.

(1) Although 5-LPO inhibitory potency of indazolinones was not proportionally related to redox potential, those compounds with highest redox potentials were inactive.

(2) Indazolinones inhibited arachidonic acid metabolism via the CO and 5-LPO pathways, but selectivity within the series could be modulated by specific substitution, most notably with analogues containing naphthyl or pyridine groups.

(3) Increasing the redox potential of indazolinone analogues by incorporation of a 4-carbamoyl substituent reduced MHb induction, but did not reduce 5-LPO inhibitory potency.

A key compound arising from these studies was **21a** (ICI 207968), which combined high selectivity with oral activity as a 5-LPO inhibitor. Table IV compares the profile of

(26) Fort, F. L.; Pratt, M. C.; Carter, G. W.; Lewkowski, J. P.; Heyman, I. A.; Cusick, P. K.; Kesterson, J. W. *Fundam. Appl. Toxicol.* **1984**, *4*, 216.

(27) (a) *The Merck Index*, 11th ed.; Merck: Rahway, NJ, 1978. (b) Chang, J.; Skowronek, M. D.; Cherney, M. L.; Lewis, A. J. *Inflammation* **1984**, *8*, 143. (c) Hope, W. C.; Welton, A.; Fiedler-Nagy, C.; Batula-Bernado, C. B.; Coffey, J. W. *Biochem. Pharmacol.* **1983**, *32*, 367.

this compound with those of previously reported inhibitors. BW 755C and phenidone were orally active, but were more potent against CO than 5-LPO. Earlier redox-based inhibitors nordihydroguaiaretic acid[27b] (NDGA), quercetin,[27b,c] and nafazatrom[28] demonstrated approximately 5-fold selectivity for 5-LPO, but lacked oral activity. More recent compounds demonstrated improved profiles: REV 5901[29] and AA861[30] showed increased selectivity, but were inactive orally, while A-64077[31] and BWA4C[32] were orally active, but possessed only modest selectivity. **21a** is the most selective of the orally active 5-LPO inhibitors and should, therefore, be a useful agent to probe the role(s) of leukotrienes in biological systems.[33]

Experimental Section

Biology. The potency and selectivity of lipoxygenase inhibitors was evaluated by studying eicosanoid generation in whole blood.[13d,e] Male Alderley Park Wistar rats were killed in a carbon dioxide filled chamber. The abdominal wall was opened and blood was withdrawn from the inferior vena cava into heparin (5 units/mL). Human and dog blood was collected from forearm and leg veins, respectively.

For in vitro studies blood samples were pooled and stored on ice prior to use. Heparinized blood samples were incubated with drugs dissolved in DMSO (final concentration 1%) for 15 min at 37 °C, then A23187 (20 μg/mL) was added, and incubations were continued for a further 15 min. Incubations were terminated by addition of 0.1 volume of a "stop cocktail" containing indomethacin (1 mM), EGTA (0.1 M), and sodium azide (0.15 M). The samples were centrifuged (12000g, 2 min) and the supernatant plasma was removed. Plasma samples were stored at −20 °C, prior to assay of leukotriene B_4 and prostaglandin E_2, by radioimmunoassay.

For ex vivo studies in the rat, animals were dosed with compound in HPMC (1 mL/kg) or with vehicle alone. At various times after dosing rats were killed and bled and within 15 min individual blood samples were challenged with A23187 as described above.

For ex vivo studies in dog blood, test compound was administered orally as a lactose mixture in gelatin capsules and blood was collected at various times after dosing.

MHb induction was studied in vitro by incubating heparinized blood with test compound for 2 h or ex vivo in blood removed from animals previously dosed with test compound. MHb was quantified with a IL282 cooximeter.

Electrochemistry. Cyclic voltamperometry[34] was carried out on a Thompson Electrochem instrument (voltage capacity ± 100 V, accuracy ± 0.5 mV). The potentiostat (Ministat 401) was connected to a triangular wave generator (DRG-16) and a Kipp & Zonen X-Y recorder (BD-19). The reference electrode was a saturated calomel electrode. A platinum wire served as working electrode and a spiral platinum wire as the indicating electrode. The solvent used was a solution of DMSO (Prolabo) containing 0.1 M of $(Bu_4N)BF_4$ (Fluka, purum quality). DMSO was used as supplied and the supporting electrolyte was recrystallized twice from hot water and then dried under vacuum at 100 °C overnight. Approximately 3 mg of sample was dissolved in 10 mL of the electrolyte solution, previously introduced into the electrochemical cell. The solution was homogenized and purged with oxygen-free nitrogen for 10 min before recording the cyclic voltamperograms.

Each sample was evaluated at room temperature and at a scan rate of 0.1 V s^{-1} with the Pt wire electrode being cleaned by brief passage through a flame between each different solution. The reported voltage was read on the recorded voltamperograms as the voltage of the half-cathodic peak.

Chemistry. Melting points were determined on a Reichert Jung microscope and are uncorrected. Spectra were recorded for all compounds and were consistent with assigned structures. ^1H NMR spectra were recorded at 90 MHz on a JEOL FX90Q or a Varian EM390 spectrometer. Mass spectra were recorded on a VG1212 or a VG7250 SA. IR spectra were recorded on a Perkin-Elmer 781 infrared spectrophotometer. Elemental analyses were recorded with a Carlo Erba 1106 apparatus and all compounds came within 0.4% of the theoretical value except when noted in Table I or III. All commercially available chemicals were used as supplied by the manufacturer. Phenidone, NDGA, and quercetin were obtained from Sigma Chemical Co. Baicalein was obtained from Appin Chemical Co. Samples of BW755C, nafazatrom, AA861, REV5901, A64077, and BWA4C were synthesized in our laboratories. Column chromatography was performed on silica gel 60 (70–230 mesh) from E. Merck (Darmstadt, Germany).

1,2-Dihydro-1-methyl-2-(phenylmethyl)-3*H*-indazol-3-one (15h). Benzyl bromide (0.342 g, 2 mmol) was added to a solution of 1,2-dihydro-1-methyl-3*H*-indazol-3-one[19] (**15b**; 0.074 g, 0.5 mmol) and triethylamine (0.050 g, 0.5 mmol) in chloroform (5 mL). The mixture was refluxed for 3 days and then evaporated in vacuo to dryness. The residue was purified by column chromatography eluting with methylene chloride to give 0.080 g (67% yield) of an oily product (**15h**) which solidified on standing. ^1H NMR ($CDCl_3$) δ (ppm): 3.16 (s, 3 H, NCH_3), 5.10 (s, 2 H, NCH_2), 7.16 (s, 5 H, Ph), 6.90–7.35 (m, 3 H, arom), 7.90 (d, 1 H, arom).

1,2-Dihydro-1-(phenylmethyl)-2-methyl-3*H*-indazol-3-one (15i). A solution of diazomethane[35] (1 mmol) in ether was added dropwise to a solution of 1,2-dihydro-1-(phenylmethyl)-3*H*-indazol-3-one[19] (**15f**; 0.224 g, 1 mmol) in methylene chloride (10 mL) at room temperature. After addition the mixture was stirred for 1.5 h and then evaporated in vacuo to dryness. The residue was purified by column chromatography using methylene chloride/ether/methanol (70/30/1, v/v) as eluant to give 0.050 g (21% yield) of an oily product (**15i**) as the most polar compound. ^1H NMR ($CDCl_3$) δ (ppm): 3.40 (s, 3 H, NCH_3), 4.84 (s, 2 H, NCH_2), 7.12 (s, 5 H, Ph), 6.80–7.70 (m, 3 H, arom), 7.83 (dd, 1 H, arom).

1-(Phenylmethyl)-3-methoxy-1*H*-indazole (16a). The chromatography performed in the preparation of **15i** also gave 0.150 g (64% yield) of an oily product (**16a**) as the less polar compound. ^1H NMR ($CDCl_3$) δ (ppm): 4.10 (s, 3 H, OCH_3), 5.18 (s, 2 H, NCH_2), 7.12 (s, 5 H, Ph), 6.8–7.5 (m, 3 H, arom), 7.64 (d, 1 H, arom).

1,2-Dihydro-2-(1-naphthylmethyl)-3*H*-indazol-3-one (17a). A solution of sodium hydroxide (4.13 g, 103 mmol) in water (80 mL) was added to a solution of *N*-(1-naphthylmethyl)-2-nitrobenzamide (7.6 g, 25 mmol) in methanol (60 mL). Zinc powder (3.35 g, 76 mmol) was then added to the mixture, which was heated under reflux for 24 h. After cooling, the zinc residue was separated by filtration and the methanol was partially evaporated. The residual solution was then adjusted to pH 7 with aqueous hydrochloric acid. The precipitated solid was collected, dried, and purified by column chromatography, eluting with methylene chloride/ether/methanol (50/50/5, v/v) to give 17a as a white solid after washing with acetone. This solid was recrystallized in acetone/ether to give 3.0 g (44% yield) of product. Mp: 198–200 °C.

The following compounds were made according to the same method, occasionally replacing methanol with ethanol as the

(28) Honn, K. V.; Dunn, J. R. *FEBS Lett.* **1982**, *139*, 65.
(29) Coutts, S. M.; Khandwala, A.; Van Inwegan, R.; Chakraborty, U.; Musser, J.; Bruens, J.; Jariwala, N.; Dally-Meade, V.; Ingram, R.; Pruss, T.; Jones, H.; Neiss, E.; Weinryb, I. *Prostaglandins, Leukotrienes, Lipoxins*; Bailey, J. M., Ed.; Plenum Press: New York, 1985; p 627.
(30) Yoshimoto, T.; Yokoyama, C.; Ochi, K.; Yamamoto, S.; Maki, Y.; Ashida, Y.; Terao, S.; Shiraishi, M. *Biochim. Biophys. Acta* **1982**, *713*, 470.
(31) Carter, G. W.; Young, P. R.; Albert, D. H.; Bouska, J. B.; Dyer, R. D.; Bell, R. L.; Summers, J. B.; Gunn, B. P.; Rubin, P.; Kasterson, J. *New Trends in Lipid Mediator Research*; Zor, V., Naor, Z., Danan, A., Eds.; Karger: Basel, 1989; Vol. 3, p 50.
(32) Jackson, W. P.; Islip, P. J.; Kneen, G.; Pugh, A.; Wates, P. J. *J. Med. Chem.* **1988**, *31*, 499.
(33) Foster, S. J.; Aked, D. M.; McCormick, M. E.; Potts, H. C. *Br. J. Pharmacol.* **1989**, *96*, 38P.
(34) Greef, R.; Peat, R.; Peter, L. M.; Pletcher, D.; Robinson, J. *Instrumental Methods in Electrochemistry*; Ellis Horwood Ltd Publishers: Chichester, U.K., 1985; p. 178.
(35) Prepared and titrated according to the method of Arndt: Arndt, F. *Organic Synthesis*; Wiley: New York, 1943; Collect. Vol. 2, p 165.

reaction cosolvent (reaction time; solvent; yield; mp, °C): 1,2-dihydro-2-(2-thienylmethyl)-3*H*-indazol-3-one (**20b**; 5 h, ethanol, 45%, 182–184), 1,2-dihydro-2-(2-phenylethyl)-3*H*-indazol-3-one (**19a**; 10 h, methanol, 42%, 164–165).

1,2-Dihydro-2-[1(*R*)-(1-naphthyl)ethyl]-3*H*-indazol-3-one (17b). Compound **17b** was made by the same procedure employed in the synthesis of **17a**, except that the cooled reaction mixture was acidified to pH 5 with aqueous hydrochloric acid (2 M) and then extracted with ethyl acetate (three times). The extract was dried ($MgSO_4$) and concentrated and the residue purified by column chromatography, using methylene chloride/ether (90/10, v/v) as eluant to give a white solid (25% yield). Mp: 232–233 °C (recrystallized from ether/pentane). $[\alpha]^{20}_D$ = +166.5° (*c* = 1.0, methanol).

Compound **17c**, the *S* isomer of compound **17b** was made by the same procedure employed in the synthesis of **17b** to give a white solid (30% yield). Mp: 230–232 °C (recrystallized from ether/pentane). $[\alpha]^{20}_D$ = −154.8° (*c* = 1.1, methanol).

1,2-Dihydro-2-(2-furylmethyl)-3*H*-indazol-3-one (20a). Compound **20a** was made by the same procedure employed in the synthesis of **17a** except that the neutralized solution at pH 7 was extracted with methylene chloride (three times). The extract was dried ($MgSO_4$) and concentrated and the residue purified by column chromatography as for **17a** to give a white solid (mp: 148–149 °C) in 48% yield.

1,2-Dihydro-2-(4-pyridylmethyl)-3*H*-indazol-3-one (20e). The same procedure as the one followed to obtain **20a** was used to obtain **20e** with a reaction time of 6 h in 21% yield. Mp: 140–142 °C.

1,2-Dihydro-2-(3-pyridylmethyl)-3*H*-indazol-3-one (21a). A solution of sodium hydroxide (5.3 g, 132 mmol) in water (100 mL) was added to a partial solution of *N*-(3-pyridylmethyl)-2-nitrobenzamide (8.5 g, 33 mmol) in ethanol (110 mL). Zinc powder (4.3 g, 66 mmol) was then added to the mixture, which was heated under reflux for 9 h. After cooling, the zinc residue was separated by filtration and the ethanol was partially evaporated. The residual solution was adjusted to pH 8 with aqueous hydrochloric acid (6 N) and extracted with methylene chloride (2 × 100 mL). The organic phase was washed with brine (1 × 100 mL), dried ($MgSO_4$), and evaporated to dryness. The solid (5.4 g) was purified by column chromatography, eluting with methylene chloride/ether (50/50, v/v) and a progressive gradient of methanol from 5% to 25% (v/v). A white solid was obtained (4.9 g) which was recrystallized from acetone (dissolution in 200 mL, concentration to 160 mL and addition of 40 mL of ether) to give 4.0 g (54% yield) of product. Mp: 174–175 °C.

The following compounds were made according to the same method (reaction time; yield; mp, °C): 1,2-dihydro-2-(3-phenylpropyl)-3*H*-indazol-3-one (**19b**; 10 h, 22%, 140–141), 1,2-dihydro-2-(4-phenylbutyl)-3*H*-indazol-3-one (**19c**; 20 h, 43%, 130–131).

Typical Procedure for Obtaining the Required Nitrobenzamides: 3-[[*N*-(2-Nitrobenzoyl)amino]methyl]pyridine (Starting Material for 21a). A mixture of 2-nitrobenzoic acid (5 g, 30 mmol) and thionyl chloride (5 mL) was heated under reflux for 30 min and then concentrated in vacuo. The residue was dissolved in dry toluene (20 mL) and the volatile material was evaporated. This procedure was repeated twice. The 2-nitrobenzoyl chloride thus obtained was dissolved in methylene chloride (20 mL). This solution was then added to a solution of 3-(aminomethyl)pyridine (3.24 g, 30 mmol) and triethylamine (3.03 g, 30 mmol) in methylene chloride (50 mL) at 0 °C. After the addition was complete, the reaction mixture was washed with water (2 × 20 mL), dried ($MgSO_4$), and evaporated to give 3-[[*N*-(2-nitrobenzoyl)amino]methyl]pyridine as a solid of satisfactory purity and in essentially quantitative yield which was used without further purification.

1,2-Dihydro-2-(2-naphthylmethyl)-3*H*-indazol-3-one (18). A solution of potassium hydroxide (1.4 g, 25 mmol) in ethanol (5 mL) was added to a warm solution of 1,2-dihydro-1-carbethoxy-3*H*-indazol-3-one[12] (4.1 g, 20 mmol) in ethanol (25 mL). 2-(Bromomethyl)naphthalene (11.05 g, 50 mmol) was then added portionwise to the thick suspension of the potassium salt. The mixture was then refluxed for 6 h. The solvent was evaporated and the residue was dissolved in water (50 mL) and extracted with ethyl acetate (3 × 20 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo to dryness. This mixture thus obtained was purified by column chromatography using an increasing gradient of ethyl acetate in methylene chloride (0% to 15%, v/v) as eluent to give from the later fractions 1,2-dihydro-1-carbethoxy-2-(2-naphthylmethyl)-3*H*-indazol-3-one (4.4 g, yield 64%).

A mixture of the latter compound (4 g, 11 mmol) and powdered potassium hydroxide (2.55 g, 46 mmol) in ethanol (25 mL) was refluxed for 0.25 h. After cooling, the mixture was neutralized with acetic acid (2.77 mL, 46 mmol) and the solvent was evaporated in vacuo. The residue was dissolved in methylene chloride (20 mL) and washed with water (2 × 20 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo to dryness to give a solid which was recrystallized from acetone to obtain **18** (2.7 g, 85% yield). Mp: 205–212 °C.

1,2-Dihydro-2-(2-pyridylmethyl)-3*H*-indazol-3-one (20f). A mixture of 1,2-dihydro-1-carbethoxy-3*H*-indazol-3-one[12] (5.2 g, 25 mmol), triethylamine (10.1 g, 100 mmol), and 2-picolyl chloride hydrochloride (7.6 g, 50 mmol) in chloroform (350 mL) was heated under reflux for 72 h. The reaction mixture was then washed with water (2 × 100 mL), dried ($MgSO_4$), and evaporated in vacuo. The residue was dissolved in methylene chloride and purified by column chromatography using an increasing gradient of diethyl ether in methylene chloride as eluant to give from the later fractions 1,2-dihydro-1-carbethoxy-2-(2-pyridylmethyl)-3*H*-indazol-3-one as a solid (1.5 g, 20% yield). Mp: 97 °C.

A mixture of 1,2-dihydro-1-carbethoxy-2-(2-pyridylmethyl)-3*H*-indazol-3-one (1.2 g, 4 mmol), powdered potassium hydroxide (0.24 g, 4 mmol), and ethanol (40 mL) was heated to reflux for 15 h. The mixture was cooled in an ice bath and acidified to pH 5 with acetic acid. The bulk of the ethanol was evaporated and the residue was partitioned between methylene chloride and water. The organic solution was dried ($MgSO_4$) and evaporated to give **20f** as a solid. Mp: 137–138 °C (recrystallized from ethyl acetate) (0.7 g, 78% yield).

Except when a free pyridine was used, the amount of triethylamine was reduced to be equimolar with the alkylating reagent. Thus the following compounds were made according to the same method (alkylating agent; reaction time for alkylation; yield of alkylation; reaction time for hydrolysis; yield of hydrolysis; mp, °C): 1,2-dihydro-2-[3-(6-methylpyridyl)methyl]-3*H*-indazol-3-one (**21b**; chloride,[36] 30 min, 37%, 30 min, 65%, 185–186), 1,2-dihydro-2-[3-(5-bromopyridyl)methyl]-3*H*-indazol-3-one (**21c**; iodide,[37] 45 min, 33%, 30 min, 58%, 185–187), 1,2-dihydro-2-[3-(2-methylpyridyl)methyl]-3*H*-indazol-3-one (**21d**; bromide,[39] 30 min, 25%, 30 min, 33%, 175–178), 1,2-dihydro-2-[3-(2-chloropyridyl)methyl]-3*H*-indazol-3-one (**21e**; chloride,[40] 4 h, 37%, 30 min, 58%, 161–162), 1,2-dihydro-2-[3-(5-methylpyridyl)methyl]-3*H*-indazol-3-one (**21f**; bromide,[42] 1 h, 20%, 10 min, 83%, 191–192), 1,2-dihydro-2-[3-(6-chloropyridyl)methyl]-3*H*-indazol-3-one (**21g**; chloride,[43] 7 h, 34%, 30 min, 36%,

(36) 5-(Chloromethyl)-2-methylpyridine was prepared as a hydrochloride salt of satisfactory purity by the reaction of 5-(hydroxymethyl)-2-methylpyridine and thionyl chloride.

(37) 3-Bromo-5-(iodomethyl)pyridine was prepared as an oil of satisfactory purity by the reaction of a solution of 3-bromo-5-(chloromethyl)pyridine[38] in acetone with sodium iodide.

(38) Kauffmann, T.; Fischer, H. *Chem. Ber.* **1973**, *106*, 220.

(39) Ghera, E.; Ben David, Y.; Rapoport, H. *J. Org. Chem.* **1981**, *46*, 2059.

(40) 2-Chloro-3-(chloromethyl)pyridine was prepared by treatment of 2-chloro-3-(hydroxymethyl)pyridine[41] with thionyl chloride in toluene at 0 °C. The mixture was evaporated to give the chloromethyl product as a hydrochloride salt of satisfactory purity.

(41) Prepared following the same procedure as described for 2-methoxy-5-(hydroxymethyl)pyridine.

(42) 3-(Bromomethyl)-5-methylpyridine was prepared by the reaction of a solution of 3,5-lutidine in carbon tetrachloride with *N*-bromosuccinimide. The solution was cooled and filtered and the filtrate was used directly in the alkylation reaction.

(43) 2-Chloro-5-(chloromethyl)pyridine was prepared as the hydrochloride salt of satisfactory purity by the reaction of 2-chloro-5-(hydroxymethyl)pyridine[44] and thionyl chloride as in footnote 40.

(44) Ziegler, F.; Sweeny, J. G. *J. Org. Chem.* **1969**, *34*, 3545.

190–191), 1,2-dihydro-2-[3-(6-methoxypyridyl)methyl]-3*H*-indazol-3-one (**21h**; chloride,[45] 4 h, 28%, 30 min, 46%, 176–177), 1,2-dihydro-2-(3-indolylmethyl)-3*H*-indazol-3-one (**20d**; bromide,[47] 12 h, 37%, 3 h,[49] 55%, 153–154), 1,2-dihydro-2-[3-(4-methylpyridyl)methyl]-3*H*-indazol-3-one (**21i**; bromide,[50] 2 h, 20%, 30 min, 29%, 194–196), 1,2-dihydro-2-(5-thiazolylmethyl)-3*H*-indazol-3-one (**20c**; chloride,[51] 30 min, 22%, 30 min, 26%, 191–192), 3-(benzyloxy)-1*H*-indazole (**16b**; chloride, 4 h, 22%, 20 min, 65%, 86–87).[52]

1,2-Dihydro-4-(*N*-pentylcarbamoyl)-3*H*-indazol-3-one (11). A solution of 3-acetoxy-1-acetyl-4-(chloroformyl)indazole[17] (prepared from 1.8 g of the corresponding carboxylic acid **9a** according to the procedure followed to obtain the nitrobenzamide precursor of **21a**) in methylene chloride (10 mL) was added dropwise at 0–4 °C to a solution of triethylamine (1.14 mL, 8.2 mmol) and pentylamine (0.88 mL, 7.6 mmol) in methylene chloride (20 mL). The mixture was stirred for 1 h. The volatile material was then removed by evaporation and the residue was dissolved in ethyl acetate (50 mL). The solution obtained was washed with 2 M hydrochloric acid, then with water and was then dried ($MgSO_4$) and evaporated in vacuo to give **10** as an oil (1.5 g, 75% yield) which slowly crystallized and was used in the next step without purification.

A solution of **10** (500 mg, 1.73 mmol) in methanol (5 mL) was mixed with sodium hydroxide solution (1 M, 3.5 mL). After 30 min at 50 °C, the reaction mixture was diluted with water and acidified with hydrochloric acid (2 M). This mixture was then extracted with ethyl acetate (×3). Evaporation of the dried extracts ($MgSO_4$) gave an oil which was purified by column chromatography, eluting with a mixture of methylene chloride and methanol (95/5, v/v) to give **11** (330 mg, 77% yield) as a solid after recrystallization in ether. Mp: 167–169 °C.

1,2-Dihydro-1-carbethoxy-4-(*N*-pentylcarbamoyl)-3*H*-indazol-3-one (12). Ethyl chloroformate (0.76 mL, 3 mmol) was added to a solution of **11** (1 g, 4 mmol) in pyridine (3.3 mL). The mixture was heated under reflux for 30 min and then evaporated to dryness. The residue was triturated in water to give **12** as a white solid (1.05 g, 78% yield).

1,2-Dihydro-2-methyl-4-(*N*-pentylcarbamoyl)-3*H*-indazol-3-one (13g). Compound **12** (3.83 g, 12 mmol) was added portionwise under nitrogen to a suspension of sodium hydride (5.68 g of 50% initially in oil then washed, 14 mmol) in DMF (24 mL). The mixture was stirred for 20 min before adding methyl iodide (0.9 mL, 14 mmol) dropwise to the yellow solution. After 1 h the reaction mixture was poured into hydrochloric acid (0.5 N, 50 mL) and extracted with ethyl acetate (3 × 20 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo to dryness to give **13a** as an orange oil (4 g, quant. yield) which slowly crystallized and was used without purification in the next step.

The obtained compound **13a** was solubilized in a mixture of methanol (40 mL) and aqueous sodium hydroxide (2 N, 12 mL). The solution was heated at 50 °C for 15 min before being concentrated in vacuo. The residue was acidified with hydrochloric acid (1 N) to pH 3 and extracted with ethyl acetate (3 × 20 mL). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The yellow solid obtained (2.8 g) was purified by column chromatography using methylene chloride/methanol (95/5, v/v) as eluent to give **13b** as a yellow solid (2.15 g, 68% yield). Mp: 128–129 °C (after recrystallization in ethanol/water).

1,2-Dihydro-2-(2-pyridylmethyl)-4-(*N*-pentylcarbamoyl)-3*H*-indazol-3-one (14b). 3-Pyridylcarbinol (1.79 mL, 18.4 mmol) and triphenylphosphine (6.6 g, 25 mmol) were added to a solution of **4b** (5.35 g, 16.8 mmol) in THF (60 mL) under nitrogen. A solution of diisopropyl azodicarboxylate (4.96 g, 25 mmol) in THF (40 mL) was then added dropwise for 20 min. After a further 40 min of reaction the mixture was evaporated in vacuo, diluted in ethyl acetate (50 mL), and extracted with hydrochloric acid (2 N, 2 × 30 mL). The aqueous phase was neutralized with sodium bicarbonate and extracted with ethyl acetate (3 × 30 mL). The organic phase was dried ($MgSO_4$) and evaporated in vacuo to dryness to give **14a** as an oil (6.9 g) which was used without purification in the next step.

The compound **14a** obtained was solubilized in a mixture of methanol (70 mL) and aqueous sodium hydroxide (2 N, 16.8 mL). The resulting solution was stirred at room temperature for 1 h before being concentrated in vacuo. The residue was extracted with ethyl acetate (3 × 20 mL). The organic phase was washed with brine, dried ($MgSO_4$), and evaporated in vacuo. The yellow oil obtained was purified by column chromatography using methylene chloride/methanol (95/5, v/v) as eluent to give **14b** (1.15 g, 20% yield), mp: 112–117 °C) alongside a fraction of the O-alkylated isomer.

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Registry No. **9b**, 128484-32-2; **10**, 131589-48-5; **11**, 128484-42-4; **12**, 128484-40-2; **13a**, 128484-39-9; **13b**, 128484-27-5; **14a**, 128484-45-7; **14b**, 128484-29-7; **15a**, 7364-25-2; **15b**, 1006-19-5; **15c**, 1848-40-4; **15d**, 28561-80-0; **15e**, 17049-65-9; **15f**, 2215-63-6; **15g**, 1848-46-0; **15h**, 109632-72-6; **15i**, 4454-32-4; **16a**, 4454-33-5; **16b**, 1848-47-1; **16b** (1-carbethoxy deriv.), 131589-45-2; **17a**, 120273-56-5; **17a** (nitrobenzamide precursor), 120275-47-0; **17b**, 120273-57-6; **17b** (nitrobenzamide precursor), 120275-76-5; **17c**, 120274-33-1; **17c** (nitrobenzamide precursor), 120276-11-1; **18**, 120274-02-4; **18** (1-carbethoxy derivative), 120275-83-4; **19a**, 120273-85-0; **19a** (nitrobenzamide precursor), 19209-03-1; **19b**, 120273-72-5; **19b** (nitrobenzamide precursor), 120275-52-7; **19c**, 120273-79-2; **19c** (nitrobenzamide precursor), 120275-59-4; **20a**, 120273-74-7; **20a** (nitrobenzamide precursor), 120275-54-9; **20b**, 120273-89-4; **20b** (nitrobenzamide precursor), 120275-68-5; **20c**, 120273-62-3; **20c** (1-carbethoxy derivative), 120276-46-2; **20d**, 120275-19-6; **20d** (1-carbethoxy derivative), 131589-46-3; **20e**, 120273-59-8; **20e** (nitrobenzamide precursor), 120275-89-0; **20f**, 131589-44-1; **20f** (1-carbethoxy derivative), 120276-21-3; **21a**, 120273-58-7; **21a** (nitrobenzamide precursor), 120275-62-9; **21a** (1-carbethoxy derivative), 131589-47-4; **21b**, 120274-40-0; **21c**, 120274-44-4; **21c** (1-carbethoxy derivative), 120276-40-6; **21d**, 120274-42-2; **21d** (1-carbethoxy derivative), 120276-39-3; **21e**, 120273-61-2; **21e** (1-carbethoxy derivative), 120276-22-4; **21f**, 120274-41-1; **21f** (1-carbethoxy derivative), 120276-38-2; **21g**, 120274-43-3; **21g** (1-carbethoxy derivative), 120276-35-9; **21h**, 120274-45-5; **21h** (1-carbethoxy derivative), 120276-23-5; **21i**, 120275-21-0; **21i** (1-carbethoxy derivative), 120277-06-7; 3-(aminomethyl)pyridine, 3731-52-0; 1,2-dihydro-1-carbethoxy-3*H*-indazol-3-one, 16105-24-1; 2-(bromomethyl)naphthalene, 939-26-4; 2-picolyl chloride hydrochloride, 6959-47-3; 5-(chloromethyl)-2-methylpyridine, 72093-07-3; 3-bromo-5-(iodomethyl)pyridine, 120276-48-4; 3-(bromomethyl)-2-methylpyridine, 76915-53-2; 2-chloro-3-(chlo-

(45) 5-Chloro-2-methoxypyridine was prepared as the hydrochloride salt of satisfactory purity by the reaction of 2-methoxy-5-(hydroxymethyl)pyridine[46] and thionyl chloride as in footnote 40.

(46) Horn, U.; Mutterer, F.; Weis, C. *Helv. Chim. Acta* **1976**, *59*, 211.

(47) 3-(Bromomethyl)-1-(*tert*-butoxycarbonyl)indole, used as starting material, was obtained, as an oil of satisfactory purity, from indole-3-carboxaldehyde using the process described in ref 48, that is *tert*-butoxycarbonylation with di-*tert*-butyl dicarbonate, reduction with sodium borohydride, and bromination with bromine in the presence of triphenylphosphine.

(48) Schöllkopf, U.; Lonsky, R.; Lehr, P. *Liebigs Ann. Chem.* **1985**, 413.

(49) Hydrolysis was performed at 40 °C.

(50) 3-(Bromomethyl)-4-methylpyridine was prepared from 3,4-lutidine as in footnote 42.

(51) Hatanaka, M.; Ishimaru, T. *J. Med. Chem.* **1973**, *16*, 978.

(52) To obtain the O-alkylated derivative, the less polar compound was selected during chromatography after the alkylating step. The hydrolysis was then conducted as in the case of the N-2-alkylated compounds.

romethyl)pyridine, 89581-84-0; 3-(bromomethyl)-5-methylpyridine, 120276-47-3; 5-(chloromethyl)-2-methoxypyridine, 101990-70-9; 3-(bromomethyl)-1-(*tert*-butoxcycarbonyl)indole, 96551-21-2; 5-chloromethylthiazole, 45438-77-5; *n*-pentylamine, 110-58-7; 3-pyridylcarbinol, 100-55-0; lipoxygenase, 63551-74-6; cyclooxygenase, 39391-18-9; 5-(hydroxymethyl)-2-methylpyridine, 34107-46-5; 3-bromo-5-chloromethyl)pyridine, 120277-69-2; 2-chloro-3-(hydroxymethyl)pyridine, 42330-59-6; 3,5-lutidine, 591-22-0; 3,4-lutidine, 583-58-4; 2-chloro-5-(chloromethyl)pyridine, 70258-18-3; 2-chloro-5-(hydroxymethyl)pyridine, 21543-49-7; 2-methoxy-5-(hydroxymethyl)pyridine, 58584-63-7; indole-3-carboxaldehyde, 487-89-8.

Water-Soluble Cholesteryl-Containing Phosphorothioate Monogalactosides: Synthesis, Properties, and Use in Lowering Blood Cholesterol by Directing Plasma Lipoproteins to the Liver

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The synthesis of several monogalactoside-terminated phosphorothiolated cholesteryl derivatives is described. Monogalactosyl derivatives are coupled by phosphorothiolation to cholesterol by using ethylene glycol units as hydrophilic spacer moieties. The resulting compounds are easily soluble in water. Upon addition of such solutions to human serum (to 2 mM final concentration) the compounds are readily incorporated into lipoproteins. Isolated low-density lipoprotein (LDL) and high-density lipoprotein (HDL), preloaded with the compounds, are rapidly cleared from the circulation by the liver. The hepatic association is blocked by *N*-acetylgalactosamine, which indicates that galactose-specific recognition sites are responsible for the increased liver uptake. The plasma clearance and hepatic uptake of LDL loaded with the compounds is substantially higher (about 2-fold) than clearance and uptake of HDL containing the compounds. The selectivity of the effects of monogalactoside-terminated phosphorothiolated cholesteryl derivatives on the in vivo behavior of LDL as compared to that of HDL indicates that these compounds might be used to lower specifically LDL levels in patients with a high LDL-cholesterol level.

Introduction

High plasma levels of low-density lipoprotein (LDL) cholesterol are correlated with an increased occurrence of atherosclerosis.[1,2] LDL is the major vehicle for the transport of cholesterol in the circulation. A smaller part of the circulating cholesterol is transported by high-density lipoproteins (HDL), the level of which is inversely correlated with atherosclerotic disease.[1] Removal of LDL from plasma occurs mainly via specific LDL (apo B,E) receptors in the liver.[3,4] The importance of these receptors is evident from the congenital disease familial hypercholesterolemia (FH). The homozygous form of the disease is characterized by the lack of functional LDL receptors.[5] As a result, LDL levels in the circulation are 6–8 times above normal and myocardial infarction usually occurs before the age of 20. In heterozygotes, LDL levels are 2–3 times the normal levels. These individuals are still at a much greater risk of myocardial infarction.[5]

Therapies of FH are aimed at lowering the levels of circulating LDL. Current drug therapies of heterozygous FH consist of the administration of inhibitors of HMG-CoA reductase (the rate-limiting enzyme in the synthesis of cholesterol) and/or bile acid sequestrants. These treatments induce an increase in the number of active LDL receptors in liver, which results in lower levels of circulating LDL.[5-8] Homozygotes do not respond appreciably to these therapies.[9]

In our studies, we examined the potential to induce removal of circulating LDL via alternative receptor mechanisms, in particular via hepatic galactose-specific receptors. Two different types of galactose-specific hepatic receptors have been described, specific for parenchymal and Kupffer cells, respectively. The receptor on parenchymal cells is the classical asialoglycoprotein receptor originally described by Ashwell and Morell.[10] The receptor on Kupffer cells was characterized more recently.[11] It specifically recognizes particles larger than 10–15 nm that have exposed galactose residues.[12] In previous papers[13,14]

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(1) Gordon, T.; Castelli, W. P.; Hjortland, M. C.; Kannel, W. B.; Dawber, T. R. *Am. J. Med.* **1977**, *62*, 707–714.
(2) Castelli, W. P.; Doyle, J. T.; Gordon, T.; Hames, C. G.; Hjortland, M. C.; Hully, S. B.; Kagan, A.; Zakel, W. J. *Circulation* **1977**, *55*, 767–772.
(3) Mahley, R. W.; Innerarity, T. L. *Biochim. Biophys. Acta* **1983**, *737*, 197–222.
(4) Dietschy, J. M.; Spady, D. K. *Receptor-Mediated Uptake in the Liver*; Greten, H., Windler, E., and Beisiegel, U., Eds.; Springer Verlag: Berlin, 1986; p 56–65.
(5) Brown, M. S.; Goldstein, J. L. *Science* **1986**, *232*, 34–47.
(6) Endo, A. *J. Med. Chem.* **1985**, *28*, 401–405.
(7) Alberts, A. W.; Chen, J.; Kuron, G.; Hunt, V.; Huff, J.; Hoffman, C.; Rothrock, J.; Lopez, M.; Joshua, H.; Harris, E.; Patchett, A.; Monaghan, R.; Currie, S.; Stapley, E.; Albers-Schonberg, G.; Hensens, O.; Hirshfield, J.; Hoogsteen, K.; Liesch, J.; Springer, J. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 3957–3961.
(8) Bilheimer, D. W.; Grundy, S. M.; Brown, M. S.; Goldstein, J. L. *Proc. Natl. Acad. Sci. U.S.A.* **1983**, *80*, 4124–4128.
(9) Yamamoto, A.; Sudo, H.; Endo, A. *Atherosclerosis* **1980**, *35*, 259–266.
(10) Ashwell, G.; Morell, A. G. *Adv. Enzymol.* **1974**, *41*, 99–128.
(11) Roos, P. H.; Hartman, H.-J.; Schlepper-Schäfer, J.; Kolb, H.; Kolb-Bachofen, V. *Biochim. Biophys. Acta* **1985**, *847*, 115–121.

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