Biochemical Pharmacology, Vol. 32, No. 19, pp. 2863–2871, 1983.
Printed in Great Britain.

0006-2952/83 $3.00 + 0.00

INHIBITION OF PROSTAGLANDIN 15-HYDROXYDEHYDROGENASE BY SULPHASALAZINE AND A NOVEL SERIES OF POTENT ANALOGUES

C. N. BERRY, J. R. S. HOULT, S. H. PEERS and H. AGBACK*

Department of Pharmacology, King's College, Strand, London WC2R 2LS, U.K., and *Department of Chemical Research, Pharmacia AB, S-751 25 Uppsala 1, Sweden

(*Received* 20 *January* 1983; *accepted* 22 *April* 1983)

Abstract—The ability of sulphasalazine, its colonic metabolites and various analogues to inhibit prostaglandin inactivation by two purified preparations of type I NAD^+-dependent prostaglandin 15-hydroxydehydrogenase or in various 100,000 *g* cytosolic supernatants was investigated using $PGF_{2\alpha}$ as substrate and radio-TLC. Bovine lung and human placental PGDH were inhibited in a dose-dependent and apparently non-competitive manner by sulphasalazine and most of the 26 salazine/sulphasalazine analogues tested, but the potencies of the analogues varied considerably. In a survey of structure-activity effects testing 30 drugs at a fixed dose (50 μM) in six test systems, it was established that only two aromatic rings are needed and that optimal PGDH inhibition requires $-CH_2COOH$ and $-OH$ at positions 1 and 2 in the salicyl C ring system. Homosalazine was thus established as the type compound of a novel series of powerful PGDH inhibitors. Electronegative substituents *meta* or *para* in ring B produce compounds with > 150 × inhibitory potency of sulphasalazine, and a significant linear correlation ($r = 0.82$, $P < 0.002$) was found between the inhibitory activity and the Hammett σ substituent constant in this series of ten homosalazine analogues.

Our recent studies using a number of cell-free cytosolic supernatants have shown that sulphasalazine (Fig. 1) is a potent inhibitor (IC_{50} approximately 50 μM) of the inactivation of classical prostaglandins (PG) such as $PGF_{2\alpha}$ [1, 2]. The drug also inhibits the inactivation of prostaglandins in the isolated perfused lung and in the pulmonary circulation of the rat [2]. It was proposed on the basis of this and other evidence that the action against prostaglandin breakdown may explain the prophylactic benefit of sulphasalazine for the prevention of relapse in ulcerative colitis [1–4]. Studies with homosulphasalazine, which contains an additional methylene group at the salicyl carboxyl group, showed that it was an even more potent inhibitor in these test systems [5].

Sulphasalazine has also been shown to be a weak inhibitor of prostaglandin synthesis ([1, 2, 6] and reviewed in [4]), but a recent survey of a number of aspirin-like and sulphasalazine-like drugs revealed that these two groups of agents represent distinct pharmacological types on the grounds of selectivity for inhibition of synthesis and breakdown, respectively, as well as in terms of their clinical properties [7].

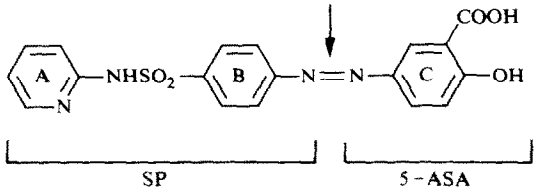


Fig. 1. Structure of sulphasalazine, showing the azo bond which is cleaved by colonic bacteria (arrow) to yield sulphapyridine (SP) and 5-aminosalicylic acid (5-ASA). Full structures of these latter compounds are shown in Table 2. The three rings are designated A, B and C (see text).

The inhibitory effects of sulphasalazine against prostaglandin inactivation were attributed to interactions with prostaglandin 15-hydroxydehydrogenase (type I, NAD^+-dependent PGDH, EC 1.1.1.141), which is the enzyme responsible for the first step in the biological inactivation of classical prostaglandins. The present paper tests this hypothesis directly by using purified PGDH preparations derived from two species, and is also concerned with the identification of the mode of inhibitory action as well as the elucidation of some of the necessary structural features needed for inhibition. A preliminary account of part of this work has appeared previously [8].

MATERIALS AND METHODS

Materials. [^{3}H-9β]-$PGF_{2\alpha}$ (specific activity 16.2 Ci/mmole) was obtained from Amersham International (U.K.), prostaglandin $F_{2\alpha}$ tromethamine salt from Upjohn Company (Kalamazoo, MI), NAD^+ from Sigma (London) Ltd. (Poole, U.K.) and 2-hydroxyphenylacetic acid from Fluka, A.G. (Basel, Switzerland). Sulphapyridine and 5-aminosalicylic acid were kind gifts from May and Baker Ltd. (Dagenham, U.K.). Sulphasalazine, *N*-acetyl-5-aminosalicylic acid and the 26 salazine/sulphasalazine analogues used in this work were prepared in the Department of Chemical Research, Pharmacia AB, Uppsala, Sweden.

Partially purified bovine lung type I NAD^+-dependent prostaglandin 15-hydroxydehydrogenase

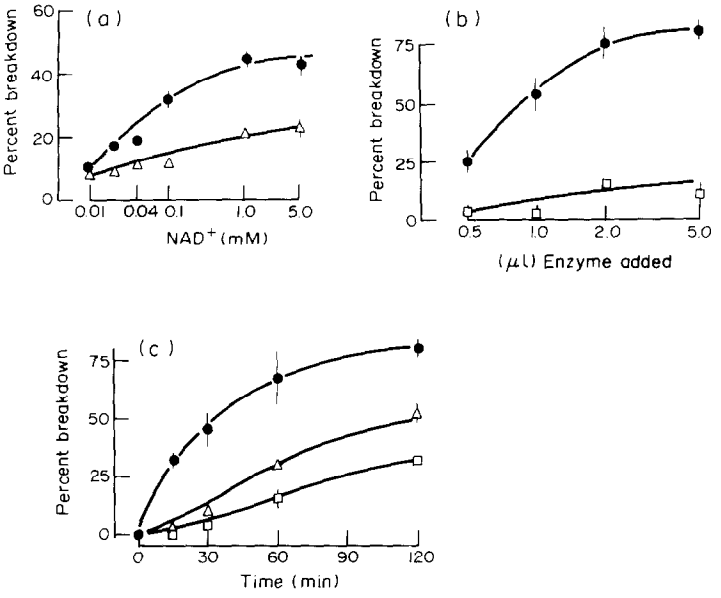


Fig. 2. Purified PGDH: experimental variables and inhibition by sulphasalazine like drugs. Panels a and b show results using human placental PGDH and panel c is of the bovine lung enzyme. Incubations marked △ contained 50 μM sulphasalazine, those marked □ contained 1 μM analogue **i** (Ph CK 47A). In summary, these results show the effects of varying the amount of cofactor NAD^+ added (panel a), the amount of enzyme added (panel b) and the incubation time (panel c) in terms of the extent of breakdown of the 10 μg/ml $PGF_{2\alpha}$ substrate (ordinate scales). Results show mean ± S.E.M., $n = 4$.

(PGDH, 75 mU/ml) was purchased from BDH Ltd. (Poole, U.K.) and purified human placental type I NAD^+-dependent PGDH (200 mU/ml) was a kind gift from Dr. J. Jeffery, Department of Biochemistry, Marischal College, Aberdeen. One mU is defined as that amount of enzyme which oxidizes 1 nmole of prostaglandin E_1 per min at 37°, pH 7.4.

Preparation of 100,000 g *cytosolic supernatants.* Various organs (rat colon, rabbit colon, guinea-pig kidney, human placenta) were homogenized in 4 vols. 50 mM phosphate buffer, pH 7.5 (containing 1 mM EDTA and cysteine) and 100,000 *g* supernatants prepared as described [1, 2].

Inhibition of prostaglandin breakdown in purified PGDH and cytosolic supernatant preparations. This was assayed radiochemically using methods which have been described in detail [1, 2]. In brief, incubation tubes contained 100—200 μU PGDH or 0.18 ml cytosolic supernatant, 0.01 ml NAD^+ (final concentration 5 mM), 0.01 ml vehicle or various concentrations of inhibitor drug and 2 μg (5.7 nmole) $PGF_{2\alpha}$ containing 0.1 μCi radiolabel to a final volume of 0.2 ml, and were incubated at 37° for 15–60 min as appropriate so as to give 60—75% inactivation of $PGF_{2\alpha}$. After extraction, the metabolites were separated by thin-layer radiochromatography in a solvent containing ethyl acetate–acetone–glacial acetic acid (90:10:1, v/v) and the extent of breakdown was calculated by comparing the proportion of counts in the relevant zones of the chromatogram. In some experiments the amounts of $PGF_{2\alpha}$, NAD^+ or enzyme were varied as appropriate, but the experimental procedure was as described above.

Data analysis. Results are expressed as mean values ± S.E.M. In the case of the Michaelis–Menten analysis of the kinetics of PGDH action, values for V_{max} and K_m were calculated by obtaining provisional estimates from application of the Michaelis–Menten equation in its linear form, followed by fine adjustment of the values by fitting the function directly into the hyperbolic form according to the method of Wilkinson [9]. A Commodore PET computer was used for this procedure. Regression lines for other data analyses were fitted by computer using standard procedures of linear regression analysis (method of least squares).

RESULTS

In pilot experiments with the two PGDH preparations purified from bovine lung and human placenta it was found that the rate and extent of $PGF_{2\alpha}$ breakdown increased as a function of the enzyme concentration and was dependent upon the length of incubation. Catalytic activity was also dependent upon the addition of sufficient NAD^+, greatly reduced if carried out in an ice-bath but similar at 22° and 37°. Figure 2 illustrates some of these findings and shows that regardless of the variable under consideration there was a substantial reduction in the extent of inactivation of $PGF_{2\alpha}$ if 50 μM sulphasalazine (compound **xvi**)* or 1 μM CK 47A (compound **i**) was included in the incubation. These results establish that sulphasalazine directly inhibits NAD^+-dependent PGDH.

Figure 3 shows representative dose–inhibition curves for sulphasalazine and some of its analogues against both the bovine lung and human placental PGDH preparations. These curves illustrate the

* For structures and manufacturer's code number of compounds used in this study, see Table 2.

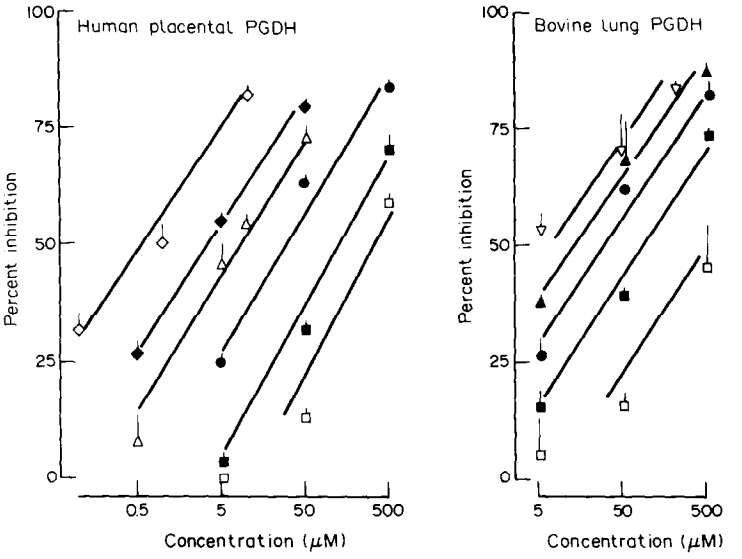


Fig. 3. Dose-dependent inhibition of purified human placental PGDH (left panel) and bovine lung PGDH (right panel) by sulphasalazine and some of its analogues (structures given in Table 2). Three drugs were used in both experiments: ●—sulphasalazine (**xvi**), ■—dihomosulphasalazine (**xviii**, CK 27A) and □—dihomosalazine (**xxvi**, CK 37A). The other compounds are ◇—CL 10A (**xi**), ◆—CL 07A (**xiv**), △—homosalazine (**xv**, CK 35A), ▽—methylsulphasalazine (**xvii**, CH 74A) and ▲—homosulphasalazine (**vii**, CH 44A). Results show mean ± S.E.M., $n = 4$.

large differences in potency between compounds, and show that inhibition of PGDH also occurred in the presence of salazine analogues which lack the pyridine ring system of sulphasalazine (see Table 2 for structures).

In an attempt to discover the mode of action of sulphasalazine and certain of its analogues as inhibitors of PGDH, we incubated various concentrations of substrate ($PGF_{2\alpha}$) with either bovine lung or human placental PGDH in the presence or absence of inhibitor. A typical result is shown in Fig. 4; the double reciprocal plot indicates that 50 μM sulphasalazine considerably (55.2%, $P < 0.001$) reduced the maximal velocity of the reaction but did not affect the Michaelis constant. The results of ten such determinations are collected in Table 1 and show that sulphasalazine or its analogues consistently reduced the V_{max} and, with the exception of experiments 1 and 3, had no significant effect on the Michaelis constant (K_m), i.e. they appeared to act as non-competitive inhibitors. It was not possible to monitor this reaction in the usual manner for determination of inhibitor kinetics (viz. by continuous recording of NADH generation using spectrophotometry) because of the intense colour of these azo-compounds; instead the radiochemical method was used and samples were taken after 30–60 min incubation as appropriate. This may therefore cause the initial velocity of the reaction to be underestimated.

We sought an alternative method of obtaining kinetic data, and Dixon plots of experiments in which

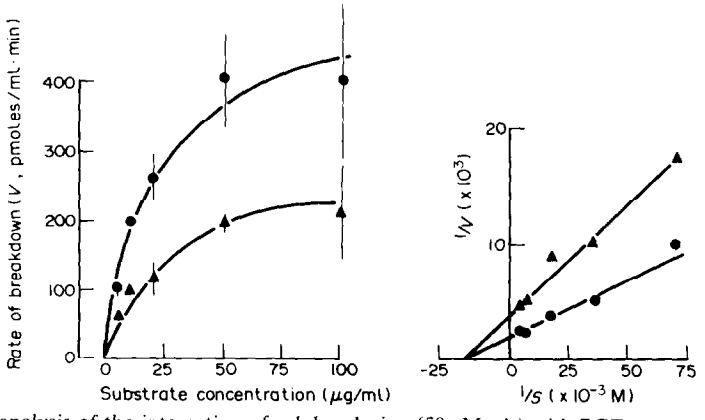


Fig. 4. Kinetic analysis of the interaction of sulphasalazine (50 μM, ▲) with $PGF_{2\alpha}$ as substrate of the human placental enzyme. Samples contained various concentrations of labelled substrate, 5 mM NAD^+ and were incubated for 60 min at 37°. Results show mean ± S.E.M., $n = 4$ (left part of graph). The right hand part shows the double reciprocal plot, with lines fitted by computer analysis (see Materials and Methods).

Table 1. Mode of inhibition of PGDH by sulphasalazine-like drugs

Enzyme preparation/ experiment number	Inhibitor	K_m value (μM)		V_{max} (pmoles/ml/min)		Conclusion
		$PGF_{2\alpha}$	+ inhibitor	$PGF_{2\alpha}$	+ inhibitor	
Bovine lung (1)	SZP, 50 μM	31 ± 5	94 ± 26*	442 ± 46	333 ± 68†	V_{max} down, K_m up
	homoSZP, 50 μM		162 ± 40‡		327 ± 50†	V_{max} down, K_m up
Bovine lung (2)	SZP, 10 μM	79 ± 25	36 ± 11*	1143 ± 300	474 ± 101*	V_{max} down
	SZP, 100 μM		81 ± 35		318 ± 107‡	V_{max} down
Human placenta (3)	SZP, 50 μM	73 ± 16	120 ± 37†	1925 ± 223	1149 ± 198‡	V_{max} down, K_m up
Human placenta (4)	CK 47A, 0.5 μM	135 ± 20	114 ± 42	777 ± 89	92 ± 47‡	V_{max} down
Human placenta (5)	SZP, 25 μM	52 ± 18	44 ± 10	562 ± 140	280 ± 31‡	V_{max} down
	SZP, 50 μM		49 ± 13		252 ± 35‡	V_{max} down
Human placenta (6)	CK 47A, 0.1 μM	118 ± 45	99 ± 26	4171 ± 840	1795 ± 330‡	V_{max} down
	CK 47A, 0.4 μM		151 ± 26		1534 ± 260‡	V_{max} down

Values show mean ± S.D.; line-fitting by computer-determined weighted linear regression analysis of double reciprocal plots as in Fig. 4. Significance of differences compared to control by Student's *t*-test: *$P < 0.01$, †$P < 0.05$ and ‡$P < 0.001$. SZP = sulphasalazine, homoSZP = homosulphasalazine (**vii**, CH 44A) and CK 47A is compound **i**.

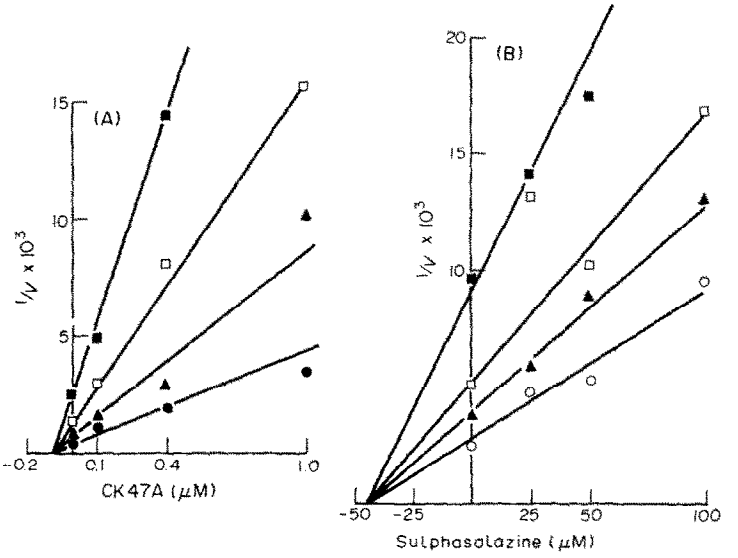


Fig. 5. Dixon plots of PGDH inhibition by CK 47A (analogue **i**, A) and sulphasalazine (**xvi**, B) when tested against $PGF_{2\alpha}$ at four substrate concentrations: ■ = 14.2 μM (5 μg/ml); □ = 28.3 μM; ▲ = 56.6 μM; ○ = 141.5 μM. Results show mean values from four determinations. Lines fitted by eye; K_i values in text computed by linear regression analysis. Initial velocity V expressed as pmoles/ml per min.

sulphasalazine (compound **xvi**) and the analogue CK 47A (**i**) were used as inhibitors are shown in Fig. 5. The results confirm the interpretation above that these drugs are non-competitive inhibitors of human placental PGDH with K_i values of 41.1 ± 2.8 and 0.11 ± 0.01 μM, respectively.

The final series of experiments concerns a survey of the structure–activity requirements for sulphasalazine-like drugs as inhibitors of prostaglandin inactivation by PGDH. For this purpose 30 drugs were used and tested at a fixed concentration (50 μM) in at least four of the following six prostaglandin-metabolizing test systems: semi-purified human placental or bovine lung PGDH (systems A and B) or cell-free 100,000 *g* cytosolic supernatants prepared from rat colon (system C), rabbit colon (system D), guinea-pig kidney (system E) or human placenta (system F).

The 30 drugs are shown in Table 2, which gives their structures and manufacturer's reference code number as well as the combined data for their activities as inhibitors of PGDH. Of the 30 compounds, 6 are analogues of sulphasalazine (with three aromatic rings) and 19 are based on salazine (with two aromatic rings, but lacking the pyridylsulphamoyl moiety), whereas 4 were selected as representative of the colonic metabolites of the parent drug. Sulphasalazine is extensively broken down to sulphapyridine (compound **xxix**) and 5-aminosalicylic acid (compound **xxx**) in the large bowel by bacterial action [10–12] and, based on some preliminary studies, it has been proposed that 5-aminosalicylic acid may be the active therapeutic part [13, 14]. Compound **xxviii** was chosen because this acetyl derivative is the principal urinary metabolite of 5-aminosalicyclic acid [12]. Compound **xxv** (2-hydroxy-

Table 2. Structure–activity relationships of sulphasalazine analogues as PGDH inhibitors

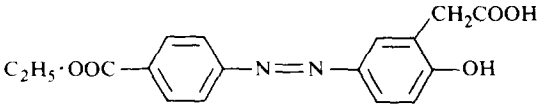
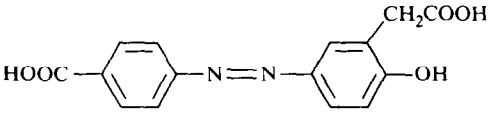
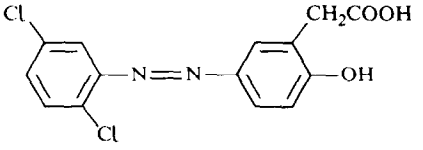
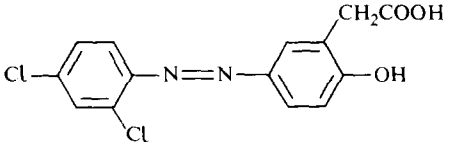
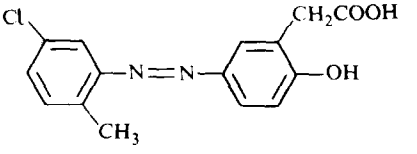
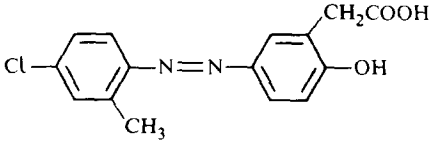
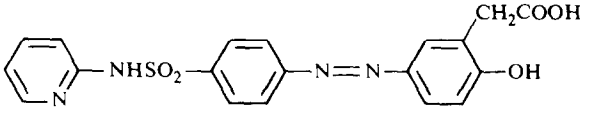
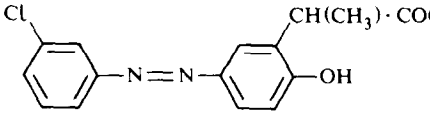
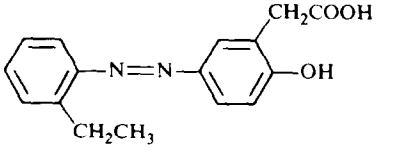
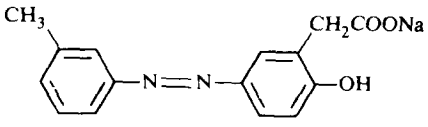
Roman code No. used in this paper		Pharmacia code No.	Overall % inhibition at 50 μM in (n) test systems
	Superactive compounds		
i		CK 47A	84.4 ± 3.7 (9)
ii		CK 61A	80.1 ± 4.0 (6)
iii		CL 17A	78.5 ± 6.9 (5)
iv		CL 15A	77.1 ± 8.2 (5)
	Very active compounds		
v		CL 11A	74.4 ± 8.8 (5)
vi		CL 12A	72.6 ± 11.1 (5)
vii		CH 44A	70.1 ± 5.7 (10)
viii		CL 18A	69.2 ± 11.8 (5)
ix		CL 13A	68.0 ± 11.1 (5)
x		CL 16B	67.2 ± 11.4 (5)

Table 2—*continued*

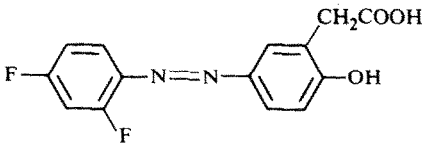
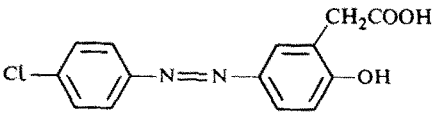
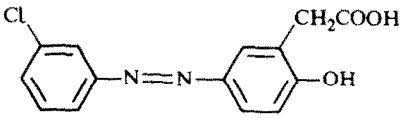
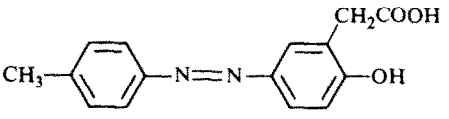
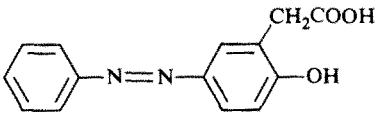
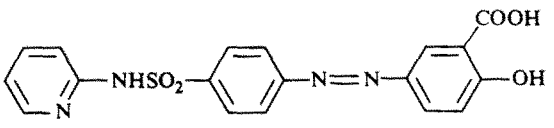
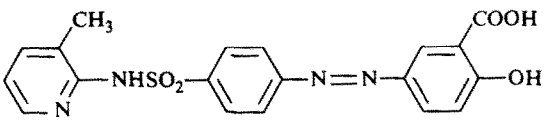
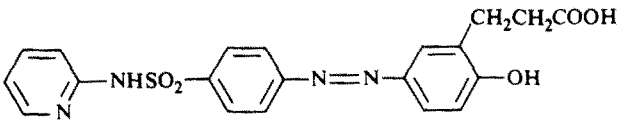
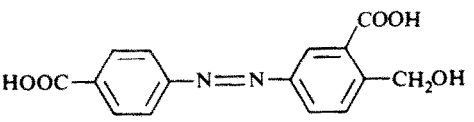
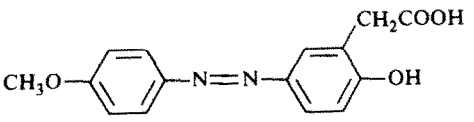
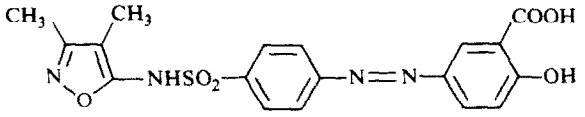
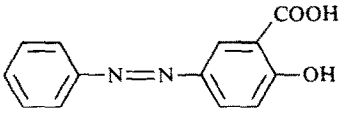
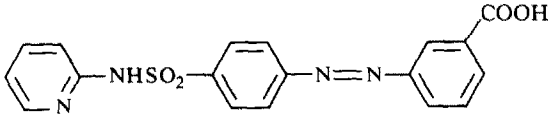
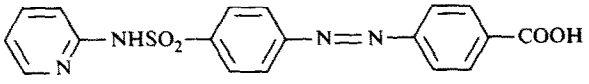
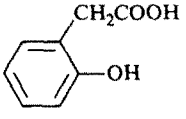
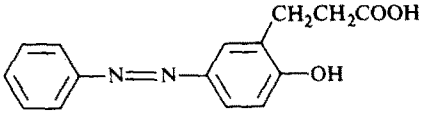
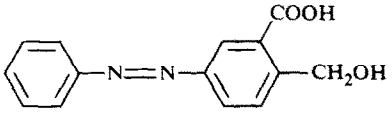
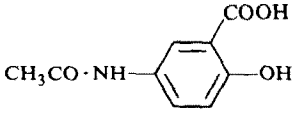
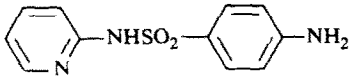
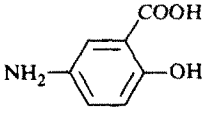
Roman code No. used in this paper		Pharmacia code No.	Overall % inhibition at 50 μM in (*n*) test systems
xi		CL 10A	66.0 ± 8.4 (5)
xii		CK 55A	65.5 ± 8.1 (6)
xiii		CK 56A	63.4 ± 10.7 (6)
xiv		CL 07A	62.9 ± 10.4 (6)
Active compounds			
xv		CK 35A	54.1 ± 7.1 (12)
xvi		SZP	50.4 ± 2.9 (25)
xvii		CH 74A	49.3 ± 7.2 (12)
xviii		CK 27A	45.2 ± 5.2 (7)
xix		CL 08A	44.5 ± 8.5 (6)
xx		CK 58A	43.9 ± 12.9 (6)

Table 2—*continued*

Roman code No. used in this paper		Pharmacia code No.	Overall % inhibition at 50 μM in (*n*) test systems
Mediocre compounds			
xxi		CH 52A	23.6 ± 7.6 (8)
xxii		CK 46A	19.7 ± 4.3 (8)
xxiii		CH 02A	16.2 ± 6.3 (6)
Inactive compounds			
xxiv		CH 19A	13.0 ± 4.5 (7)
xxv		2HPAA	12.7 ± 5.9 (4)
xxvi		CK 37A	9.4 ± 3.0 (7)
xxvii		CL 09A	8.5 ± 5.1 (5)
xxviii		CJ 46A	7.5 ± 2.4 (7)
xxix		SP	3.4 ± 2.0 (7)
xxx		5-ASA	3.4 ± 2.4 (6)

Results show mean ± S.E.M. Basis for arbitrary grouping of compounds is as follows: superactive—inhibition > 75%; very active 55–75%; active 35–55%; mediocre 15–35%; inactive < 15% inhibition. Abbreviations: SZP = sulphasalazine, 2HPAA = 2-hydroxyphenylacetic acid, SP = sulphapyridine, 5-ASA = 5-aminosalicylic acid.

phenylacetic acid, 2-HPAA) was studied because of the high activity of the more complex phenylacetic acid derivatives of the homosalazine and homosulphasalazine type (see below).

The compounds shown in Table 2 are grouped into five classes in descending order of activity. The classification into 'superactive', 'very active', 'active', 'mediocre' and 'inactive' is arbitrary (the quantitative basis for this is indicated in the notes to the table) but is based on results obtained using PGDH derived from five species (human, rat, rabbit, guinea-pig and cow). Results are shown as the mean values ± S.E.M. for percentage inhibition at 50 μM averaged for all test systems studied. These figures indicate that the differences between systems are not large, i.e. that PGDH from the various species shows relatively little variation, at least in terms of inhibition by this class of drugs.

It is also clear that at least two aromatic rings are required for inhibitory activity: the salicyl-based compounds containing just one aromatic ring (**xxv**, **xxviii** and **xxx**) are inactive. Sulphapyridine (**xxix**) is also inactive, indicating that of the two aromatic rings needed, one must be the salicylic acid moiety. That high activity can be achieved with just two aromatic rings (rings B and C, see Fig. 1) joined by an azo-group is indicated by results obtained using a large number of compounds (**i–vi**, **viii–xv**, **xix**, and **xx**), although all of these except **xix** contain an extra methylene group at the salicyl carboxyl function in ring C; as described below this homo-substitution is the key for the production of compounds with greatest inhibitory activity in this series. Nevertheless, salazine (compound **xxii**) has some inhibitory activity (Table 2; see also Fig. 3).

Removal of the hydroxyl group from the ring C salicyl moiety (compound **xxiii**) or substitution of the hydroxyl with carboxyl shifted from the vicinal position (**xxiv**) is highly detrimental to activity (compare these activities with that of the parent sulphasalazine, compound **xvi**). Moreover, interpolation of an additional methylene into the salicyl hydroxyl rather than carboxyl results in considerable loss of activity (compare **xxvii** and **xxii**, or **xix** with **ii**).

In contrast, interpolation of an extra methylene into the salicyl carboxyl group (thus producing phenylacetic acid derivatives) yields compounds with high PGDH inhibitory potency which have been designated as the homo-analogues (compare homosulphasalazine with sulphasalazine **vii**, and **xvi**, Table 2 and [5, 8]; or homosalazine and salazine, **xv** and **xxii**). That the homo-substitution is optimal can be gauged from the fact that dihomo-compounds show reduced activity in relation to their homo-analogues (compare **xviii** with **vii**, or **xxvi** with **xv**, Table 2, and see [8]). Other homosalazine analogues also exhibit high potency, e.g. **i–vi**, **ix–xv** and **xx**, but their activity varies systematically according to the nature of the substituents in ring B as described in the Discussion.

Thus in summary, the critical features for optimal PGDH activity in these test systems are the presence of the two aromatic rings B and C together with the presence of $-CH_2COOH$ at position 1 and $-OH$ at position 2 of ring C. Substituents in ring B cause relatively minor changes in activity, although the extent varies in a systematic way (*q.v.*). Homosalazine thus represents the type compound of a novel series of PGDH inhibitors.

DISCUSSION

This paper provides evidence that sulphasalazine and related drugs interact directly with prostaglandin 15-hydroxydehydrogenase and inhibit it in a dose-dependent and non-competitive manner. This was previously proposed as the likely explanation for the inhibition of prostaglandin inactivation by these drugs in cytosolic supernatants [1, 2, 5] and in the intact lung [2], although other authors who have confirmed the inhibitory effect of sulphasalazine on pulmonary prostaglandin inactivation have suggested the uptake mechanism as the site of action [15, 16]. Our results also confirm that 5-aminosalicylic acid, a colonic metabolite of sulphasalazine and thought to be the active therapeutic part [13, 14], is inactive in this respect, as shown previously in experiments on cytosolic supernatants derived from animals [2] or human colonic mucosa [17].

These experiments also provide comparative data on the PGDH-inhibitory potencies of 26 salazine/sulphasalazine analogues and show that optimal inhibitory activity resides in molecules such as compounds **i** and **ii** (Ph CK 47A and Ph CK 61A) which are based on the homosalazine structure containing only two of the three aromatic rings of the parent compound sulphasalazine. As shown in Fig. 3 and Table 2, homosalazine itself is somewhat more potent than the parent compound and appropriate addition of substituents in the B-ring may increase potency still further. Thus *para*-substitution of carboxyl (compound **ii**) or its ethyl ester (compound **i**) or halogen(s) yields inhibitors (Table 2) which are vastly more potent than sulphasalazine. For example, CK 47A (**i**) has a K_i value some 375 times lower than the parent compound (Fig. 5), and in rat colon 100,000 *g* supernatants inhibits $PGF_{2\alpha}$ inactivation with an IC_{50} value of 0.36 μM, compared to the value of 68 μM for sulphasalazine (C. N. Berry, unpublished experiment). In contrast, substitution with *para*-methoxy (compound **xx**) gives comparatively modest inhibitory capacity.

Further analysis of the data from Table 2 concerning ten compounds (Fig. 6) showed that the overall inhibitory activity is highly correlated ($P < 0.002$) with the Hammett σ substituent constant [18]. Thus inhibition increases as a function of the substituent's electronegativity, i.e. capacity to withdraw electrons from the conjugated ring system. A similar correlation ($r = 0.87$, $P < 0.01$) was found when the same ten analogues were tested at 5 μM as inhibitors of the human placental enzyme: inhibition varied from 23.0 ± 4.0% (CK 58A, compound **xx**) to 97.9 ± 1.8% (CK 61A, **ii**). Thus this correlation between structure and activity is valid when considering the effect of these compounds on a single enzyme preparation or on the overall results obtained using various test systems derived from several species. It was not possible to include all the homosalazine analogues in this analysis because σ constants for substituents at the *ortho* position cannot be obtained unambiguously due to contribution of steric interactions to reactivity. This effectively

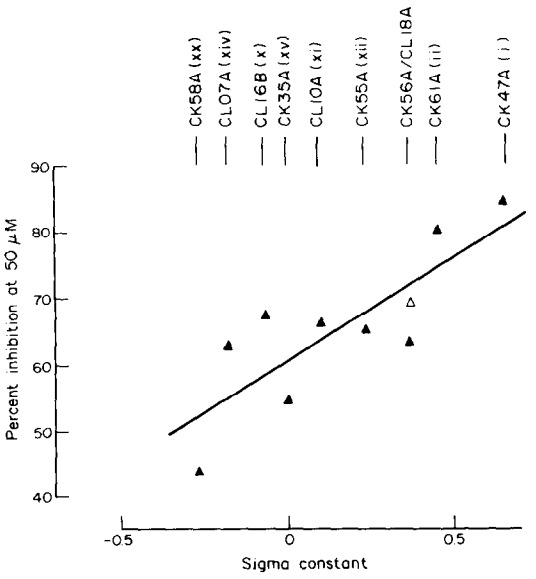


Fig. 6. Relationship between inhibition of PGDH by a series of homosalazine analogues and the Hammett σ constant for the substituent present in the aromatic ring B. The per cent inhibition values are taken from Table 2 and the σ constant values from [18]. The regression line was fitted by computer and shows a significant correlation: $r = 0.82$, $P < 0.002$.

excluded compounds **iii**, **iv**, **v**, **vi** and **ix** from the analysis, although **xi** (CL 10A) was included (*o,p*-fluoro) by assuming the *ortho* contribution to be equivalent to that assigned to the *p*-substituent. Moreover, **viii** (CL 18A), which contains an additional methyl substituent on the carboxyl side chain of ring C, was also included.

These results do not establish the nature of the mechanism whereby the homosalazines as well as sulphasalazine and the other analogues inhibit PGDH. However, a common property of the homosalazine derivatives is the possibility of lactone formation due to the vicinal $-CH_2COOH$ and $-OH$ functions on ring C. These lactones would be potential acylation reagents capable of combining with free amino groups near the active site of the PGDH enzyme. Nevertheless, any theory for the mechanism of action based on this property must take into account the activity of the salicyclic derivatives (e.g. sulphasalazine) which do not lactonize, as well as the fact that dihomo-analogues such as **xviii** and **xxvi** that may also form lactones show reduced activity compared to their homo-analogues. These considerations do not exclude the possibility that an optimum exists for the size or shape of any hypothetical lactone structure and that this also contributes to the overall activity.

The correlation between the Hammett σ constants and inhibitory activity is certainly striking and may permit the design of still more active compounds. For example, we find in preliminary experiments that the p-NO_2 substituent (σ value = 0.78) is as potent an inhibitor of the human placental enzyme as compound **i** (CK 47A). In fact the substituents may influence several variables of possible significance for enzyme inhibition, for example the electronic configuration of ring B and its consequent interaction with a binding site on the enzyme at a region distinct from the active site. Moreover, an electronic effect could be transmitted to ring C and affect the formation or reactivity of the hypothetical lactone.

Thus in conclusion, a critical analysis of these structure–activity results may offer a means of further probing the geometry and function of PGDH. Moreover, in view of the controversy surrounding the question of whether the therapeutic benefits of sulphasalazine are due to inhibition of prostaglandin synthesis or degradation or not to either (see [3, 4, 17] for discussion), some of these novel compounds might be of therapeutic interest.

Acknowledgements—We thank the MRC for a training scholarship (S.H.P.), the British Heart Foundation for partial financial support, Dr. J. Jeffery (University of Aberdeen) and May & Baker Ltd. for gifts of reagents, and Terry McCarthy for devising a computer programme for drawing chemical structures.

REFERENCES

1. J. R. S. Hoult and P. K. Moore, *Br. J. Pharmac.* **64**, 6 (1978).
2. J. R. S. Hoult and P. K. Moore, *Br. J. Pharmac.* **68**, 719 (1980).
3. J. R. S. Hoult, P. K. Moore, A. J. Marcus and J. Watt, *Agents Actions Supp.* **4**, 232 (1979).
4. J. R. S. Hoult and P. K. Moore, in *Inflammatory Bowel Diseases* (Ed. D. Rachmilewitz), p. 174. Martinus Nijhoff, The Hague (1982).
5. P. K. Moore, E. Ramcharan and J. R. S. Hoult, *Eur. J. Pharmac.* **61**, 287 (1980).
6. H. O. J. Collier, A. A. Francis, W. J. McDonald-Gibson and S. A. Saeed, *Prostaglandins* **11**, 219 (1976).
7. P. K. Moore and J. R. S. Hoult, *Biochem. Pharmac.* **31**, 969 (1982).
8. C. N. Berry, J. R. S. Hoult, J. Jeffery, O. T. Mak and S. H. Peers, *Br. J. Pharmac.* **75** (Supp), 7P (1982).
9. G. N. Wilkinson, *Biochem. J.* **80**, 324 (1961).
10. M. A. Peppercorn and P. Goldman, *J. Pharmac. exp. Ther.* **181**, 555 (1972).
11. K. M. Das, M. A. Eastwood, J. P. A. McManus and W. Sircus, *Scand J. Gastroenterol.* **9**, 137 (1974).
12. H. Schröder and D. E. S. Campbell, *Clin. Pharmac. Ther.* **13**, 539 (1972).
13. A. K. Azad Khan, J. Piris and S. C. Truelove, *Lancet* **ii**, 892 (1977).
14. U. Klotz, K. Maier, C. Fischer and K. Heinkel, *New Engl. J. Med.* **303**, 1499 (1980).
15. Y. S. Bakhle, *Eur. J. Pharmac.* **68**, 493 (1980).
16. P. G. Hellewell and J. D. Pearson, *Br. J. Pharmac.* **76**, 319 (1982).
17. K. Hillier, P. J. Mason, S. Pacheco and C. L. Smith, *Br. J. Pharmac.* **76**, 157 (1982).
18. C. D. Ritchie and W. F. Sager, *Prog. phys. org. Chem.* **2**, 323 (1964).