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Preliminary communication

6-(4-Morpholino-phenyl)-4,5-dihydro-2H-pyridazine-3-ones: potent platelet aggregation inhibitors and antithrombotics

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Summary — A series of di- and tri-substituted 6-phenyl-4,5-dihydro-2H-pyridazine-3-ones is described. The compounds were designed to be antithrombotics and were assessed for their inhibitory properties on platelet aggregation and on thrombus formation in an arteriovenous shunt in the rat. The synthesis and physical properties of the compounds are described. The structure–activity relationships reveal that non-aromatic nitrogen-containing heterocycles can confer high activity on the 6-phenyl-pyridazinone system, provided they are combined with an additional electron-withdrawing substituent in the phenyl ring. The most potent compounds (**8i**, **8b**) had an ED_{min} of 1–3 mg/kg after oral administration in the thrombus formation test.

Résumé — 6-(4-Morpholino-phényl)-4,5-dihydro-2H-pyridazine-3-ones, inhibiteurs puissants de l'agrégation plaquettaire et agents antihtrombotiques. *Une série de 6-phényl-4,5-dihydropyridazinones a été préparée. Nos résultats démontrent que les substances ont une activité antiagrégante envers les thrombocytes et une activité antithrombotique chez le rat. Les relations de structure–activité montrent qu'un hétérocycle non-automatique contenant de l'azote confère aux 6-phényl-pyridazinones une bonne activité biologique à condition qu'il y ait sur le phényle un susbstituant électronégatif. Les substituants les plus actifs (**8i**, **8b**) ont une DE_{min} de 1 à 3 mg/kg* po.

dihydropyridazinone (derivatives) / antithrombotic / platelet aggregation inhibitor

Introduction

Platelet adhesion and aggregation play a central role in thrombus formation, and agents which inhibit platelet function represent potential antithrombotic drugs [1].

Many antithrombotic drugs like aspirin (ASA) or sulfinpyrazone interfere merely with one single activation pathway of platelets [2] (pro-aggregatory AA-metabolites *eg* PGH_2 and thromboxanes Tx) and they inhibit at the same time the production of the anti-aggregatory AA-metabolites (PGI_2, PGE_2) [3]. In search of antithrombotics capable of inhibiting platelet aggregation induced by any activator (ADP, AA, Tx, PAF, collagen etc), intereference with platelet function itself looked more promising than inhibition of the formation of one of the several possible platelet activating agents.

Since elevation of platelet cyclic 3',5'-adenosine monophosphate (cAMP) results in inhibition of platelet function [4], inhibitors of platelet phosphodiesterase (the enzyme responsible for breakdown of intracellular cAMP) seemed to be a suitable target for our purpose [5]. Therefore a series of 6-aryl-4,5-dihydro-2H-pyridazine-3-ones, which all contain morpholine in the *p*-position of the aromatic ring and one additional substituent in the *m*-position were synthesised [6–8].

Chemistry

The starting materials were obtained according to the literature by 3 different methods: **1a** and **1b** by succinoylation of 1,2-dihalobenzene (R^1 = X = F or Cl) [6, 9]; **1d** by homologation of 3-trifluoromethyl-4-

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chloro-benzaldehyde to the aroylpropionic acid [10] (*cf* methods K in scheme 3); and **1g** by nitration of *p*-chlorobenzoylpropionic acid [11].

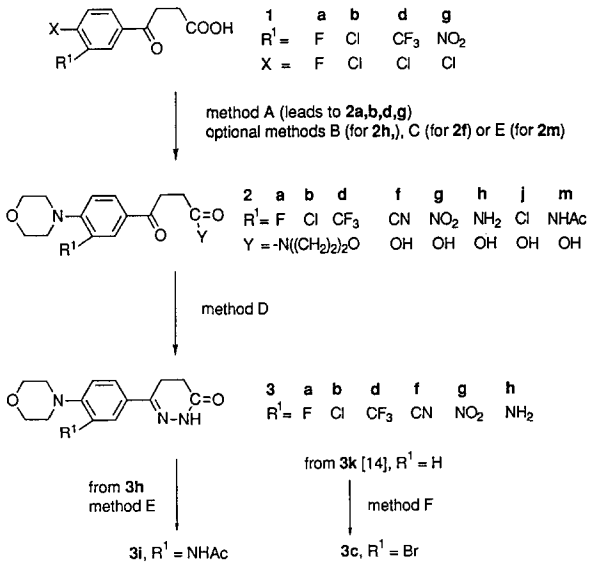
The intermediates **2a,b,d,g** were prepared by nucleophilic displacement of the halogen in 4-chloro/fluoro-3-substituted benzoylpropionic acids (**1a,b,d,g**) with morpholine (method A).

For the conversion of **1** into **2** morpholine was found to be both a good nucleophile and a good solvent. In the case of R^1 = Cl and X = Cl no substitution of X was observed with other amines like thiomorpholine, 3-picolylamine or 3,4-dehydropiperidine, unless the halogen is strongly activated by R^1 = nitro (Goeschke, unpublished results).

The products **2a,b,d** were obtained as morpholine-amides (Y = morpholine), which could either be cyclised directly to the pyridazinones or first hydrolysed with KOH-ethanol to the free acids (Y = OH) *eg* **2j**.

Only with the strongly activating R1 = nitro the free acid **2g** (Y = OH) could be obtained without hydrolysis due to milder reaction conditions (lower temperature, shorter reaction time, DMSO as cosolvent).

The intermediate **2h** was prepared by method B, reducing **2g** with Pd/C, and **2f** as well as the reference compound **2l** [12] by Sandmeyer reaction of **2h** (method C). Compound **2m** was prepared from **2h** by acetylating with acetic anhydride according to method E.

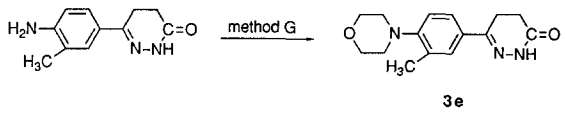


Scheme 1. Synthesis of compounds **3**. A) Morpholine; B) H_2, Pd/C; C) $NaNO_2$, HCl, KCN resp CuCl, resp H_2SO_4; D) H_2NNH_2, EtOH, AcOH; E) Ac_2O; F) Br_2, $CHCl_3$.

The target compounds **3** were obtained by closure of the pyridazinone ring with hydrazine (method D). For free acids (**2f,g,h**) (Y = OH) ethanol was a suitable solvent while for morpholine-amides (**2a,b,d**) (Y = morpholine) 50% acetic acid was the best solvent.

The target compound **3i** was prepared by acetylating **3h** with acetic anhydride (method E) and compound **3c** was synthesised from **3k** [14] by bromination with bromine in chloroform (method F).

Compound **3e** was prepared on a different route from 6-(4-amino-3-methyl-phenyl)-4,5-dihydro-2H-pyridazine-3-one [6] by morpholine ring closure as the last step (method G, scheme 2).



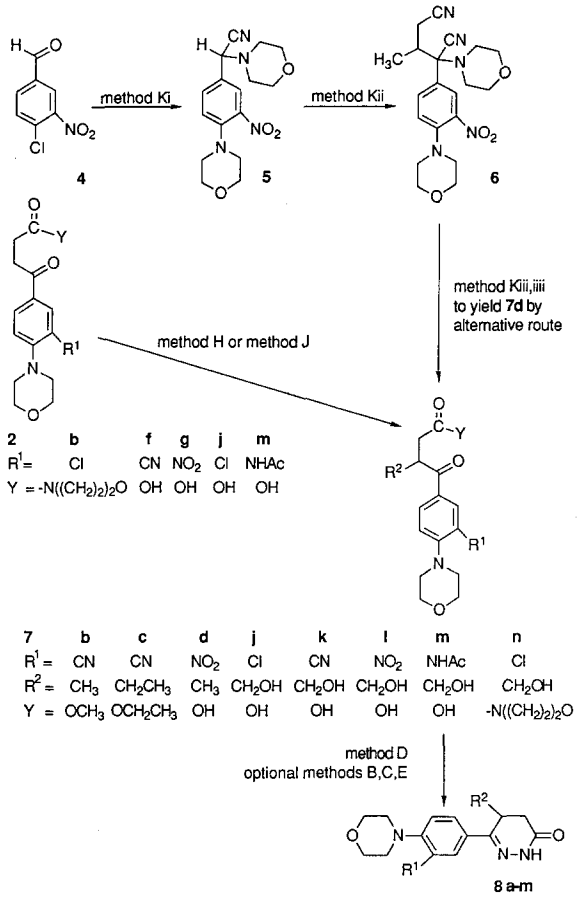
Scheme 2. Synthesis of compound **3e**. G) $(BrCH_2CH_2)_2O$, DMF, N-ethyldiisopropylamine.

Target compounds **8** containing a substituent R^2 in the 5-position of the pyridazinone ring were prepared either *via* method K (modification of the literature [10] with the use of crotononitrile instead of acrylic ester for R^2 = methyl, **7d**) or *via* methods H/J and subsequent cyclisation of the intermediates **7** by method D (scheme 3).

Compound **8d** was synthesized *via* the methods K–D and from **8d** the target compounds **8a,b,e,f,g,h** were prepared by transformation of the nitro-group into the other substituents R^1 *via* standard methods: hydrogenation (**8e** *cf* method B), Sandmeyer reaction (**8a,b** *cf* methods C) or acylation (**8f,g,h** *cf* method E).

Compounds with R^2 = alkyl can also be prepared by the methods J–D. The deprotonation of **2f** (Y = OH, R^1 = CN) with 2 equivalents of base and treatment with alkyl iodides (method J) gives the intermediate esters **7b,c** and after cyclisation with hydrazine (method D) the alkylsubstituted pyridazinones **8b,c**. The alkyl residue can be introduced into position 5 as well as position 4 of the pyridazinone ring by this method. The regioselectivity of the deprotonation of **2f** and subsequent alkylation can be controlled by choice of the appropriate base (Goeschke, unpublished results).

Compounds with R^2 = CH_2OH are prepared by methods H–D. Treatment of **2** (Y = OH: **2f,g,j,m** as well as Y = morpholine: **2b**) with formaldehyde and aqueous base (method H) leads to **7j,k,l,m** resp **7n**, which after cyclisation with hydrazine (method D) give the compounds **8j,k,l,m** with R^2 = CH_2OH. If the



Scheme 3. Synthesis of compounds **8**. H) CH_2O, NaOH to give $R^2 = CH_2OH$; J) NaH, DMF, CH_3J resp CH_3CH_2J to give $R^2 = CH_3$ resp CH_2CH_3; Ki) KCN, morpholine; Kii) Crotononitrile, KOH, MeOH; Kiii) AcOH, H_2O; Kiiii) HCl, H_2O.

free acids **2** *eg* **2j** (Y = OH) is used as the starting material the intermediate **7j** is conveniently isolated in its lactone form [14], obtained by acid treatment of **7j** (Y = OH), and freed of unreacted starting material by washing with base.

Pharmacology/biology

The pyridazinones were assessed for antiplatelet activity by their ability to inhibit human platelet aggregation induced *in vitro* by platelet activating factor (PAF) [15].

Human platelet rich plasma was prepared from citrated whole blood by centrifugation. The aggregatory response of the platelets was assessed in an optical aggregometer (Payton) following the addition of the aggregating agent according to Zucker [16]. A full dose–response curve was obtained to the PAF and subsequently a sub-maximal concentration was chosen for inhibition studies. The extent of inhibition of the control PAF aggregation response was then quantified and the concentration of inhibitor required to inhibit the control response by 50% (IC_{50}, μM) was reported.

In view of the ubiquitous inhibitory role of elevated c-AMP levels in platelets, agents designed to be phosphodiesterase inhibitors will inhibit platelet aggregation irrespective of the aggregating agent used (*eg* ADP, PAF, collagen, thrombin, etc). PAF was selected as aggregating agent for assessing the *in vitro* potency of the potential phosphodiesterase inhibitors described here due to the sensitive and reproducible nature of its aggregatory response.

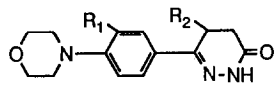
The antithrombotic potential of these compounds was assessed *in vivo* by quantitating inhibition of thrombus formation on a cotton thread inserted into an arterio-venous shunt in the rat [17]. This *in vivo* model was selected because thrombus formation is known to be platelet-dependent and so would be sensitive to potential platelet inhibitors (*eg*, phosphodiesterase inhibitors). Thrombus formation was assessed by weight of thrombus on the cotton thread, 15 min after initiation of blood flow through the arteriovenous shunt. The test was initiated 1 h after oral administration of the compound to the rat. Results are expressed as minimum active dose (ED_{min}) (mg/kg body weight). This is the minimum dose which shows a statistically different effect ($P < 0.05$) from the control experiments.

Results and discussion

The anti-platelet and anti-thrombotic effects of these compounds are expressed in table I as IC_{50} values (μM) and ED_{min} values (mg/kg) respectively. When comparing chemical structure to biological activity in structure activity relationships it should be considered that the *in vitro* biological test system involves platelets in a plasma medium. The correlation of *in vitro* inhibitory potency to the *in vivo* antithrombotic potency after oral administration, however, is complicated as it involves multiple steps. While there is good evidence that inhibition of platelet aggregation correlates with inhibition of thrombus formation, a number of variables come into play when extrapolating *in vitro* anti-platelet effects to *in vitro* antithrombotic effects, the major ones being oral absorption, bio-availability, pharmacokinetics, pharmacodynamics and metabolism.

Therefore the inhibition of platelet aggregation *in vitro* is a good measure of the test compounds'ability to act as a phosphodiesterase inhibitor allowing structure–activity relationships to be drawn up, but the sub-

Table I. 4,5-Dihydropyridazinones, synthetic routes and biological activities.



Compd	*R1*	*R2*	*Synth route*	*mp °C*	*Cryst solvent*	*Formula*[b]	*Antithrombotic activity*[d] ED_{min}	*Antiplatelet activity* [e] IC_{50}
3a	F	H	A,D	167-170	EtOH [a]	$C_{14}H_{16}FN_3O_2$	30	1.4
3b	Cl	H	A,D	184-185	EtOH	$C_{14}H_{16}ClN_3O_2$	10	3.3
3c	Br	H	D,F	175-176	EtOH	$C_{14}H_{16}BrN_3O_2$	10	3.5
3d	CF_3	H	A,D	163-166	EtOH-PE	$C_{15}H_{16}F_3N_3O_2$	10	7.8
3e	CH_3	H	G	208-211	CH_2Cl_2-PE	$C_{15}H_{19}N_3O_2$	10	45
3f	CN	H	A,B,C,D	215-216	CH_2Cl_2-PE	$C_{15}H_{16}N_4O_2$	3	2.4
3g[10]	NO_2	H	A,D	168-171	EtOH [a]	$C_{14}H_{16}N_4O_4$	10	2.6
3h	NH_2	H	A,B,D	250-254	DMF-H_2O	$C_{14}H_{18}N_4O_2$	100	>36
3i	NHAc	H	A,B,D,E	240-242	DMF-EtOH	$C_{16}H_{20}N_4O_3$	10	>16
3k[14] [c]	H	H	D	219-221	EtOH	$C_{14}H_{17}N_3O_2$	>150	60
3l[13] [c]	OH	H	A,B,C,D	216-219	EtOH	$C_{14}H_{17}N_3O_3$	>300	46
8a	Cl	CH_3	K,D,B,C	218-221	DMF-CH_2Cl_2	$C_{15}H_{18}ClN_3O_2$	10	1.6
8b	CN	CH_3	K,D,B,C	223-225	f	$C_{16}H_{18}N_4O_2$	3	0.4
8b [g]	CN	CH_3	A,B,C,J,D	219-221	CH_2Cl_2-MeOH	$C_{16}H_{18}N_4O_2$	3	0.4
8c	CN	CH_2CH_3	A,B,C,J,D	217-219	$CHCl_3$-EtOEt	$C_{17}H_{20}N_4O_2$	n.d.	28
8d	NO_2	CH_3	K,D	227-229	DMF-EtOH	$C_{15}H_{18}N_4O_4$	30	0.54
8e	NH_2	CH_3	K,D,B	207-209	DMF-EtOH	$C_{15}H_{20}N_4O_2$	10	5.3
8f	NHAc	CH_3	K,D,B,E	225-227	EtOH	$C_{17}H_{22}N_4O_3$	3	5.8
8g	NHCOEt	CH_3	K,D,B,E	198-201	EtOH	$C_{18}H_{24}N_4O_3$	3	5.7
8h	NHCHO	CH_3	K,D,B,E	196-198	EtOH	$C_{16}H_{20}N_4O_3$	10	3.0
8j	Cl	CH_2OH	A,H,D	180-181	CH_2Cl_2	$C_{15}H_{18}ClN_3O_3$	1	2.1
8k	CN	CH_2OH	A,B,C,H,D	219-221	DMF-EtOH	$C_{16}H_{18}N_4O_3$	3	2.4
8l	NO_2	CH_2OH	A,H,D	216-217	EtOH [a]	$C_{15}H_{18}N_4O_6$	3	5
8m	NHAc	CH_2OH	A,B,E,H,D	246-248	EtOH [a]	$C_{17}H_{22}N_4O_4$	>30	>140
Acetylsalicylic acid [c]							100	
Anagrelide [c][22]							10	1.2

[a]Reaction solvent. [b]All compounds were analyzed for C, H, N with values ± 0,4% of the theoretical ones. [c]Reference compound listed only for biological comparison. [d]Antithrombotic activity, oral administration, see biological section. [e]*In vitro* anti-aggregatory activity, see biological section. [f]Purified by chromatography. [g]Compound **8b** prepared by two different synthetic routes.

sequent extrapolation to *in vivo* antithrombotic effects must be viewed with caution from the viewpoint of establishing structure–activity relationships. The 6-phenyl-4,5-dihydro-2H-pyridazine-3-ones are known to be phosphodiesterase inhibitors, and many of them bear a nitrogen substituent in the *p*-position of the phenyl ring.

These nitrogens are either in sp^2-configuration, being part of an aromatic ring system (*eg* imazodan [18]), or they are of partial sp^2 character, being acylated (*eg* amipizone [19]). Such a structural feature is in accordance with the "electron delocalisation key group" of a recently postulated PDE inhibitor pharmacophore [20].

Our interest was to explore whether cyclic dialkylated sp^3 nitrogen in this position can also be part of a highly active molecule. We found that non-aromatic nitrogen-heterocycles can confer activity on the phenylpyridazinone system, although to different degrees and only in combination with certain additional substituents.

Morpholine is more active than *eg*, thiomorpholine, piperidine or 4-hydroxypiperidine (Goeschke, unpublished results). An additional substituent (R^1) in the *o*-position to morpholine greatly modifies the activity. Thus electron-donating substituents lead to very weakly active compounds (**3l,h**), while the highest activities are found if R^1 is an electron-withdrawing group (**3f, 8b,d,k**). Without R^1 the activity disappears almost completely (**3k**).

Substitution in the pyridazinone ring with small group (R^2) improves the inhibition of platelet aggregation *in vitro*, whereas it seems to be of little importance for the antithrombotic activity *in vivo*.

Homologation of R^2 to ethyl (**8c**) results in a drastic decrease of the activity in accordance with previously reported findings [21].

Experimental protocols

Melting points were determined on a Büchi or Tottoli capillary melting point apparatus and are not corrected. Structures of all target compounds were confirmed by proton NMR and elemental analysis. Proton NMR spectra were determined on a Bruker WM 250, a Varian HA 100D or on a Varian EM 360 (operating at 60 MHz) spectrometer using Me_4Si as the internal standard. Abbreviations used are: RT = room temperature, PE = petroleum ether. The experimental procedures for the biological tests have been described previously [15–17]. The chemical methods are illustrated by the following representative examples.

*Synthesis of compounds **3***

Introduction of morpholine

*Method A. 3-(3-Chloro-4-morpholino-benzoyl)propionic acid morpholine amide (**2b**).* A mixture of 3-(3,4-dichloro-benzoyl)propionic acid **1b** [9] (200 g, 0.8 mol) and morpholine (360 ml) was stirred for 24 h at reflux temperature. The reaction mixture was then treated at RT with ethyl acetate (2 l) and 2 N HCl (1 l) and the phases were separated. The organic phases were washed with 2 N HCl (3 x 1 l) and extracted with 5 N HCl (4 x 800 ml). The combined 5 N HCl extracts were put to pH 6 with concentrated NaOH. The mixture was extracted with ethyl acetate (2 x 1 l). The organic phases were washed with water, dried and concentrated. The residue (198 g) was recrystallized from ethyl acetate–hexane: yield 172 g (58%) mp = 103–106°C.

*Method A. 3-(4-Morpholino-3-nitro-benzoyl)propionic acid (**2g**).* A mixture of 3-(4-chloro-3-nitro-benzoyl)propionic acid (**1g**) [11] (214 g, 0.84 mol), DMSO (1.7 l) morpholine (80 ml, 0.92 mol) and *N*-ethyldiisopropylamine (143 ml, 0.84 mol) was stirred at 80°C for 3 h. The reaction mixture was concentrated and to the residue was added ethyl acetate (700 ml), water (1 l) and saturated Na_2CO_3 solution (1 l). The aqueous phase was separated and the organic phase washed twice with a 1:1 mixture of water and saturated Na_2CO_3 solution. To the combined aqueous phases was added water (1 l) and concentrated HCl (0.3 l). The suspension was extracted with CH_2Cl_2 (1200 ml and 2 x 200 ml) and the organic phases were washed with water, dried (Na_2SO_4) and concentrated. The residue was treated with hexane (400 ml) cooled to 10°C and filtered: yield 244 g (95%) mp = 115–117°C.

Transformation of the nitro group into other R^1 substituents

*Method B. 3-(3-Amino-4-morpholino-benzoyl)propionic acid (**2h**).* 3-(3-Nitro-4-morpholino-benzoyl)propionic acid (**2g**, 300 g, 0,97 mol), dissolved in dioxane (3 l) was hydrogenated at room temperature in the presence of 15 g of 5% Pd on charcoal. The catalyst was filtered off, resuspended in hot dioxane and filtered again. The combined filtrates were concentrated to a slurry, cooled to 0°C and the product isolated by filtration, washed with MeOH and dried: yield 188 g (70%) mp = 180–182°C.

*Method C. 3-(3-Cyano-4-morpholino-benzoyl)propionic acid (**2f**).* To a solution of 3-(3-amino-4-morpholino-benzoyl)propionic acid (**2h**, 408 g, 1.47 mol) in concentrated HCl (335 ml) and water (1160 ml) 4 N aqueous $NaNO_2$ (460 ml) was added under stirring at 0°C. The reaction mixture was stirred an additional 15 min at 0°C, treated with NH_2SO_3H (30 g), filtered and added at 0°C during 30 min to a stirred solution of cupric cyanide, prepared by mixing a solution of cupric sulfate pentahydrate (395 g, 1.58 mol) in water (1.5 l) with a solution of KCN (440 g, 6.76 mol) in water (800 ml). The reaction mixture was stirred 16 h at RT, treated with water (2 l) and 30% NaOH (400 ml) and washed with ethyl acetate (2 l and 1 l). The organic phases were washed with water (2 x 2 l). The combined aqueous phases were treated with ethyl acetate (2 l) and acidified with concentrated HCl (350 ml) to pH 2–3. The separated copper salts were filtered off, washed with ethyl acetate and the organic phases were washed with 2 N HCl (2 x 1.5 l) and water (3 x 1 l), dried and concentrated. From the cooled suspension the product (105 mg, mp = 149–151°C) was filtered off and from the mother liquor another 99 g of product were obtained by filtration of the ethyl acetate solution through silica gel (50 g) and crystallization: total yield 204 g (48%).

Closing of the pyridazinone ring

*Method D. 6-(3-Cyano-4-morpholino-phenyl)-4,5-dihydro-2H-pyridazine-3-one (**3f**).* To a suspension of 3-(3-cyano-4-morpholino-benzoyl)propionic acid (**2f**, 204 g, 0.7 mol) in ethanol (2.4 l) was added hydrazine hydrate (43 ml, 0.88 mol). The reaction was stirred for 2 h at reflux, cooled and filtered. The product was washed with ethanol, dried and recrystallized from DMF: yield 141 g (70%) mp = 215–216°C.

*Method D. 6-(3-Chloro-4-morpholino-phenyl)-4,5-dihydro-2H-pyridazine-3-one (**3b**).* To a solution of 3-(3-chloro-4-morpholino-benzoyl)propionic acid morpholine amide (**2b**, 88 g, 0.24 mol) in a mixture of AcOH–water = 1:1 (1320 ml) was added hydrazine hydrate (35 ml, 0.74 mol). The reaction was stirred at reflux for 2 h. The suspension was stirred to reach RT and filtered. The product was thoroughly washed with water, dried and recrystallized from ethanol (2 l): yield 60 g (86%) mp = 184–185°C.

*Preparation of additional **3***

*Method E. 6-(3-Acetamido-4-morpholinophenyl)-4,5-dihydro-2H-pyridazine-3-one (**3i**).* To a solution of 6-(3-amino-4-morpholinophenyl)-4,5-dihydro-2H-pyridazine-3-one (**3h**, 10 g, 0.365 mol) and *N*-ethyl-diisopropylamine (6.2 ml, 0.365 mol) in dry DMF (150 ml) was added acetic anhydride (3.5 ml, 0.365 mol) and the mixture was stirred for 48 h at RT. The reaction mixture was concentrated. The residue was taken up in ethyl acetate and this solution was extracted 3 times with 2 N HCl. The combined aqueous phases were treated with concentrated NaOH and 10.6 crude product was filtered off, washed with water and dried. Recrystallization from DMF–ethanol yielded 7.8 g product (68%), mp = 240–242°C.

*Method F. 6-(3-Bromo-4-morpholino-phenyl-4,5-dihydro-2H-pyridazine-3-one (**3c**).* To a solution of 6-(4-morpholino-phenyl)-4,5-dihydro-2H-pyridazine-3-one (**3k**, 1.5 g, 5.8 mmol) in $CHCl_3$ (11 ml), stirred at 5°C was added during 15 min the solution of bromine (0.96 g, 6 mmol) in $CHCl_3$ (4.5 ml), stirring was continued for 30 min at 5°C and 1 h at 25°C. The reaction mixture was treated with 1 N $NaHCO_3$ and a trace of 2 N NaOH to obtain a clear separation of layers. The organic phase was washed with water, dried and evaporated. The residue was dissolved in ethyl acetate–CH_2Cl_2 = 95:5. The solution was freed of the starting material by washing it 3 times with 2 N HCl. The product was then extracted from the organic phase with 5 N HCl. These extracts were put to pH 9 with concentrated NaOH, the product was extracted with ethyl acetate–CH_2Cl_2 = 95:5, the organic phases were washed with water, dried and evaporated. The residue was crystallized from EtOH: yield 1 g (51%) mp = 175–176°C.

*Method G. 6-(4-Morpholino-3-methyl-phenyl)-4,5-dihydro-2H-pyridazine-3-one (**3e**).* A mixture of 6-(4-amino-3-methyl-phenyl)-4,5-dihydro-2H-pyridazine-3-one [6] (2.2 g, 0.011 mol), 2,2'-dibromo-diethylether (3.7 g, 16 mmol), *N*-ethyldiisopropylamine (5.5 ml) and DMF (11 ml) was stirred at 100°C for 6 h. The reaction mixture was cooled off, diluted with ethyl acetate, filtered, the filtrate washed 3 times with water, dried and evaporated. The crude product was purified by flash column chromatography (100 g silica gel, toluene–ethyl acetate = 1:1) and crystallization from CH_2Cl_2–hexane: yield 0.75 g (25%), mp = 208–210°C.

*Synthesis of compounds **8***

Compounds prepared by route K–D

*Compound **8d**. Method K. 3-(4-Morpholino-3-nitrobenzoyl)-butyric acid (**7d**): α-morpholino-α(4-morpholino-3-nitro-phenyl)-acetonitrile (**5**).* A solution of morpholine (76.6 g, 0.88 mol) in THF (100 ml) was added dropwise to a solution of *p*-toluene sulfonic acid (41.8 g, 0.22 mol) in THF (500 ml) at RT with stirring. After 15 min a solution of 4-chloro-3-nitro-benzaldehyde (**4**, 37.1 g, 0.2 mol) in THF (80 ml) was added and the mixture was stirred at reflux for 2 h and then allowed to cool to 60°C. A solution of KCN (16.9 g, 0.26 mol) in water (50 ml) was added and the mixture was heated at reflux for 16 h. The reaction mixture was then concentrated and the residue was dissolved in water (200 ml) and extracted with chloroform (3 x 200 ml). The combined chloroform extract was washed with water (2 x 150 ml) then with saturated sodium metabisulfite (3 x 200 ml), then with saturated brine (3 x 200 ml) and was dried ($MgSO_4$) and concentrated to give a dark red oil. The oil was triturated with ethyl acetate and a little heptane to afford a yellow solid which was collected, washed with heptane and dried at RT *in vacuo* to give crude product (42.5 g). This was recrystallized from ethanol: yield 39.9 g (60%), mp = 133–135°C.

*4-(4-Morpholino-3-nitrophenyl)-4-cyano-4-morpholino-3-methyl-butyronitrile (**6**).* To a stirred solution of (**5**) (16.7 g, 0.05 mol) in THF (150 ml) under nitrogen was added methanolic KOH (5 ml 30%) at 25°C. After stirring for 10 min, a solution of crotononitrile (8.0 g, 0.12 mol) in THF (20 ml) was added during 30 min with water bath cooling. After stirring for another 5 h at RT the reaction mixture was poured into water (500 ml) and extracted with ethyl acetate (2 x 1 l). The combined extract was dried and concentrated to an oily residue which was triturated with ethyl acetate/hexane (1:1) to give a solid, which was collected, washed with ethyl acetate, then hexane and dried *in vacuo* at 80°C: yield 10.4 g (52%), TLC (ethyl acetate) $R_F = 0.58$.

4-(4-Morpholino-3-nitrophenyl)-4-oxo-3-methylbutyronitrile. A mixture of the above intermediate (**6**) (30 g, 0.075 mol), glacial acetic acid (220 ml) and water (20 ml) was heated on a steam bath for 18 h. The mixture was then concentrated and the residue dissolved in dichloromethane (600 ml). The solution was washed with brine (3 x 200 ml), water (3 x 200 ml) and dried, treated with charcoal and concentrated to a red oil: yield 22 g (97%). IR 2250 cm^{-1}.

*3-(4-Morpholino-3-nitrobenzoyl)butyric acid (**7d**).* A mixture of the above intermediate oxonitrile (16.2 g, 0.053 mol), conc HCl (150 ml) and water (150 ml) was heated on an oil bath at 100°C with stirring for 1.5 h. The mixture was then poured into water (1 l) and extracted with ethyl acetate (3 x 750 ml). The combined extract was washed with brine (3 x 150 ml), water (3 x 150 ml), dried, treated with charcoal and concentrated to a red oil (15.8 g). This was dissolved in abs ethanol (120 ml) and allowed to crystallize in the refrigerator. The crystalline product was collected, washed with hexane and dried at 40°C *in vacuo*: yield 9.7 g (57%), mp = 127–129°C.

*Method D. 5-Methyl-6-(4-morpholino-3-nitrophenyl)-4,5-dihydro-2H-pyridazine-3-one (**8d**).* To a suspension of 2-(4-morpholino-3-nitrobenzoyl)butyric acid (**7d**, 27 g, 0.084 mol) in ethanol (100 ml) was added hydrazine hydrate (8.4 g, 0.168 mol). The reaction was stirred for 2 h at reflux and then additional hydrazine hydrate (2.2 g, 0.044 mol) was added. After a further 2 h reflux, the reaction mixture was allowed to cool and crystallize. The product was collected, washed with hexane/ethanol (9:1) and dried *in vacuo* at 80°C: yield 20.5 g (77%), mp = 223–226°C. A sample recrystallized from EtOH/DMF gave 80% return; orange prisms, mp = 227–229°C.

*Transformations of **8d** into **8a,b,e,f,g,h***

*Method B. 6-(3-Amino-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8e**).* 5-Methyl-6-(4-morpholino-3-nitrophenyl)-4,5-dihydro-2H-pyridazine-3-one (**8d**, 15 g, 0.047 mol) dissolved in DMF (500 ml) was hydrogenated at room temperature in the presence of 5% Pd/C (1.5 g) at 3 atm H2. The catalyst was removed by filtration and the filtrate was concentrated to give a grey solid, which was slurried with PE (40–60°C), collected, washed with PE and dried *in vacuo* at 60°C. Recrystallization from EtOH/DMF gave the product: yield 10.1 g (75%), mp = 207–209°C.

*Method E. 6-(3-Acetamido-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8f**).* To a solution of 6-(3-amino-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8e**, 3.0 g, 0.0105 mol) in dry DMF (60 ml) was added di-isopropylamine (1.35 g, 0.0105 mol) followed by addition of acetic anhydride (1.2 g, 0.0108 mol) in dry DMF (15 ml) and the mixture was stirred for 48 h at RT. After concentration the solid residue obtained was slurried with ethyl acetate/PE mixture, collected, washed with PE, dried and recrystallized from ethanol with charcoal clarification treatment: yield 2.3 g (67%), mp = 225–227°C. (The 3-propionamide derivative **8g** was prepared in an analogous manner using propionic anhydride.)

*Method E. 6-(3-Formamido-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8h**).* A mixture of 6-(3-amino-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8e**, 0.5 g, 0.0017 mol) and formic acid (5 ml) was heated at reflux for 30 min, cooled, diluted with water (10 ml) and allowed to crystallize. The solid was collected, washed with water and recrystallized from ethanol: yield 0.25 g (46%), mp = 196–198°C.

*Method C. 6-(3-Cyano-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8b**).* A solution of $CuSO_4 \cdot 5H_2O$ (5.0 g, 0.02 mol) in water (20 ml) was added to a solution of KCN (6.5 g, 0.10 mol) in water (20 ml) at 60°C. The mixture was cooled to RT and was added dropwise to a solution of 6-(3-amino-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8e**, 2.8 g, 0.01 mol) in conc HCl (20 ml) and water (20 ml) diazotised at 0–5°C with a solution of sodium nitrite (0.8 g, 0.011 mol) in water (10 ml) and adjusted to pH 7.0 with dil NaOH. The reaction mixture was stirred for 2 h at RT, then for 2 h at 80°C and cooled. Solid product was collected, dried, dissolved in dichloromethane and chromatographed on silica gel: yield 0.6 g (20%), mp = 223–225°C.

*Method C. 6-(3-Chloro-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8a**).* A solution of 6-(3-amino-4-morpholinophenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8e**, 1.0 g, 0.0034 mol) in conc HCl (9 ml) and water (9 ml) was diazotised at 0–5°C by addition of sodium nitrite (0.29 g, 0.0042 mol) in water (1 ml). After 15 min, unreacted nitrite was decomposed by addition of urea and to the reaction

mixture at 0–5°C was added a solution of cuprous chloride (1.8 g, 0.018 mol) in conc HCl (9 ml) and water (9 ml). The reaction mixture was stirred 1.5 h at RT and was then stirred a further 2 h at 40°C. The mixture was then rendered alkaline to pH 10 by addition of dil NaOH and was extracted with ethyl acetate (2 x 25 ml). The combined organic extract was dried ($MgSO_4$) and concentrated. The solid residue obtained was slurried with ethyl acetate/PE mixture, collected and dried and was then recrystallised from DMF/dichloromethane mixture: yield 0.7 g (67%), mp = 218–221°C.

*Compounds **8b,c** prepared by route J–D*

*Method J–D. 6-(3-Cyano-4-morpholino-phenyl)-5-methyl-4,5-dihydro-2H-pyridazine-3-one (**8b**).* To a suspension of sodium hydride (1.66 g, 50% dispersion in oil, 34.7 mmol) in DMF (60 ml) was added 3-(3-cyano-4-morpholino-benzoyl)propionic acid (**2f**, 5 g, 17.34 mmol). The reaction mixture was stirred at 80°C for 40 min, cooled to –70°C, treated with methyl iodide (3.23 ml, 52 mmol), allowed to warm to RT and treated with water and ethyl acetate. The mixture was adjusted to pH 2 with 2 N HCl and extracted with ethyl acetate. The organic phases were washed with water, dried and evaporated to obtain the crude 3-(3-cyano-4-morpholino-benzoyl)butyric acid methylester **7b**: R_f = 0.66 in ethyl acetate–toluene = 8:2, NMR ($CDCl_3$) δ 1.23 (3H, d), 2.48 (1H, m), 2.94 (2H, m), 3.43 (4H, t), 3.57 (3H, s), 3.92 (4H, t), 7.05 (1H, d), 8.12 (1H, dd), 8.23 (1H, d). The ester was stirred for 16 h at reflux with AcOH (40 ml), water (40 ml) and hydrazine hydrate (2.5 ml, 51.5 mmol). The reaction mixture was concentrated, treated with water, the suspension was washed with ethyl acetate and extracted with CH_2Cl_2. The methylene chloride extracts were dried and evaporated. The residue was dissolved in acetone, filtered, evaporated and the product twice recrystallized from CH_2Cl_2–MeOH–hexane: yield 1.9 g (38%) mp = 221–222°C. NMR (Me_2SO–d6) δ 1.04 (3H, d), 2.24 (1H, d), 2.7 (1H, dd), 3.24 (4H, t), 3.42 (1H, m), 3.78 (3H, t), 7.22 (1H, d), 8.02 (1H, dd), 8.06 (1H, d).

The 5-ethyl derivative **8c** was prepared in an analogous manner using ethyl iodide instead of methyl iodide in the alkylation step to give the intermediate **7c**.

*Compounds **8j,k,l,m** prepared by route H–D*

*Method H, Y = OH resp lactone form: 4-(3-chloro-4-morpholino-benzoyl)-butyrolactone (**7j**).* A mixture of 3-3-chloro-4-morpholino-benzoyl)propionic acid (prepared by hydrolysis of the morpholine amide **2b** in refluxing 5% KOH in EtOH–water = 5:3, mp = 153–154°C) (**2j**, 29.8 g, 0.1 mol), 0.5 N NaOH (220 ml) and 35% aqueous formaldehyde (8.7 ml) was stirred for 16 h at RT, treated with concentrated HCl (11 ml) and stirred another 4 h at RT. The reaction mixture was extracted with ethyl acetate, the organic phases were washed with 2 N aqueous Na_2CO_3, dried and evaporated. The residue was dissolved in EtOH (500 ml), cooled to –70°C and the product filtered off: yield 15 g (49%), mp = 66–68°C.

*Method D, starting from lactone form. 6-(3-Chloro-4-morpholino-phenyl)-5-hydroxymethyl-4,5-dihydro-2H-pyridazine-3-one (**8j**).* A mixture of 4-(3-chloro-4-morpholino-benzoyl)-butyrolactone (**7j**, 3 g, 10 mmol) hydrazine hydrate (1 ml, 21 mmol) and ethanol (40 ml) was refluxed for 5 h and then evaporated. The solution of the residue in ethyl acetate was washed with 2 N Na_2CO_3 and water, dried (Na_2SO_4) and concentrated while cooling. The product was filtered off and recrystallized from CH_2Cl_2: yield 1.75 g (54%), mp = 180–181°C. The compounds **8k,l,m** were prepared in an analogous manner using compounds **2f,g,m** as starting materials leading to **7k,l,m** as intermediates.

*Method H–D. Y = morpholine. 6-(3-Chloro-4-morpholino-phenyl)-5-hydroxymethyl-4,5-dihydro-2H-pyridazine-3-one (**8j**).* A mixture of 3-(3-chloro-4-morpholino-benzoyl)propionic acid morpholine amide (**2b**, 36.7 g, 0.1 mol), 37% formaldehyde (20 ml, 0.2 mol), $NaHCO_3$ (33.6 g, 0.4 mol) and MeOH (200 ml) was stirred for 42 h at 50°C and then evaporated. A suspension of the residue in water was adjusted to pH 3 with 4 N HCl and extracted with ethyl acetate, the organic phases were washed with water, dried and evaporated to yield 37.9 g (95%) of crude 3-(3-chloro-4-morpholino-benzoyl-4-hydroxybutyric acid morpholine amide **7n**, which was without further purification cyclised according to Method D to (**8j**), yield after flash chromatography on silica gel with CH_2Cl_2–MeOH = 97:3 and crystallization from CH_2Cl_2 9.4 g (30%) mp = 180–181°C.

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