Expedited Articles

Nonsteroidal Progesterone Receptor Ligands. 2. High-Affinity Ligands with Selectivity for Bone Cell Progesterone Receptors

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A novel series of nonsteroidal heterocycles was discovered which display cell-type selective, high-affinity (nanomolar) binding to the progesterone receptors from TE85 osteosarcoma cells but >1 μM binding affinity to the progesterone receptors from T47D and ZR75 human breast carcinoma cells. Structure–activity relationships were developed for a set of these compounds, and a representative analog 1-(3,4-dichlorobenzoyl)-3-phenyl-1,4,5,6-tetrahydropyridazine (**1i**, RWJ 25333) was chosen for further evaluation. RWJ 25333 stimulated the *in vitro* proliferation of human osteoblast-like cells but not human breast cells.

Introduction

There are no approved therapies designed to stimulate bone growth and to replace the bone mass lost in osteoporosis. Recent studies have suggested that steroidal progestins may promote bone formation and so might serve as a treatment for established osteoporosis.[1,2] Progesterone receptors have been described in cultured osteoblast-like cells, and steroidal progestins have also been shown to directly affect osteoblast activity.[3-5]

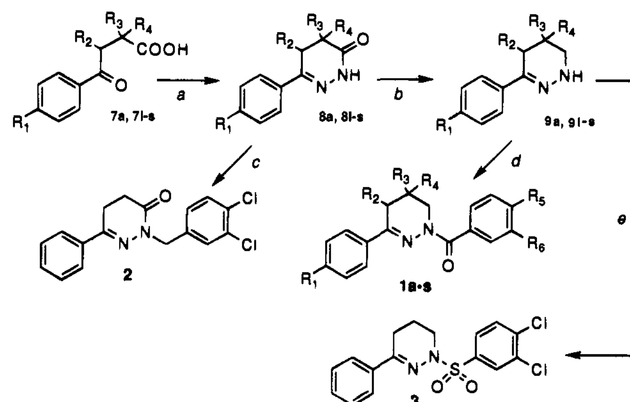
In our studies of nonsteroidal progesterone receptor ligands we have discovered a novel series of compounds that have selective binding affinity for the progesterone receptor from rabbit uterus.[6] We were also interested in exploring the ability of these and similar nonsteroidal ligands to bind the receptors from bone cells. We report here that ligands have been found which not only bind to progesterone receptors from bone cells but are able to stimulate bone cell proliferation *in vitro*.

Interestingly, these compounds also show a high degree of cell-type selectivity. There is precedent for the selectivity of steroid-like compounds for bone over other tissues: Willson et al.[7] have recently described a nonsteroidal estrogen with selectivity for bone over uterus in rats. The structure–activity relationships (SAR) of our nonsteroidal series of tetrahydropyridazines at the bone progesterone receptor were explored and will be discussed. Data showing the selectivity of one of these nonsteroidal progestin-like compounds (RWJ 25333) for human bone cells over human breast cells will also be presented.

Chemistry

1-Aroyl-3-aryl-1,4,5,6-tetrahydropyridazines (**1a–s**) were prepared by condensation of the appropriately substituted benzoyl chlorides and 3-aryl-1,4,5,6-tetrahydropyridazines (**9a,l–s**) as shown in Scheme 1 and as discussed in the previous paper in this series.[6] Compound **2** was synthesized by the addition of 3,4-dichlorobenzoyl bromide to the anion of 3-phenyl-1,4,5,6-tetrahydropyridazin-6-one (**8a**). Sulfonamide **3** was formed by the condensation of **9a** and 3,4-dichlorobenzenesulfonyl chloride in pyridine. Thioamide **4** and 3,4-dichlorobenzyl analog **5** were prepared by treatment of the amide **1i** with phosphorus pentasulfide and by reduction with lithium aluminum hydride, respectively. Dihydropyridazine **6** was formed by base-catalyzed elimination of HBr from 4-bromo-1-(3,4-dichlorobenzoyl)-3-phenyl-1,4,5,6-tetrahydropyridazine which was in turn prepared by reaction of **1i** with bromine in acetic acid (see Scheme 2).

Scheme 1[a]



[a] Reagents: (a) hydrazine; (b) $LiAlH_4$/THF; (c) NaH/DMF/3,4-dichlorobenzoyl bromide; (d) substituted benzoyl chlorides; (e) 3,4-dichlorobenzenesulfonyl chloride/pyridine.

The 5-methyl- and 5,5-dimethyl-1,4,5,6-tetrahydropyridazines **1q** and **1r** were made in four steps starting with the Friedel–Crafts reaction of 2-methyl- or 2,2-dimethylsuccinic anhydride with benzene and aluminum chloride (Scheme 3).[8] The resultant 2-methyl and 2,2-dimethyl keto acids **7q** and **7r** were treated with anhydrous hydrazine in refluxing ethanol to give pyridazinones **8q** and **8r** which were reduced with lithium aluminum hydride in THF to give **9q** and **9r** as shown

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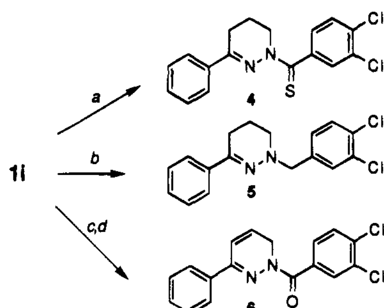
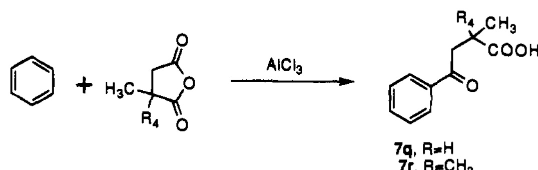
Scheme 2[a]



[a] Reagents: (a) P_2S_5; (b) $LiAlH_4$/THF; (c) Br_2/HOAc; (d) DBU.

Scheme 3



in Scheme 1. Reaction with 3,4-dichlorobenzoyl chloride gave the desired analogs. McEvoy and Allen[9] described the synthesis of 3-methyl-4-oxo-4-phenylbutyric acid (**7s**) which was converted to the 4-methyl analog **1s** by the usual route.

Literature references to the synthesis of intermediate compounds **7a,l–s** to **9a,l–s** are given in tabular form (see Table 1).

Results and Discussion

We have reported that certain 1-aroyl-3-aryltetrahydropyridazines have weak binding affinity for the rabbit uterine progesterone receptor.[6] Because of the growing interest in the effects of steroids on bone, we sought to determine if our compounds would show high-affinity binding to the recently characterized progesterone receptors from cultured TE85 human osteosarcoma cells. Using parent compound **1a** as a template, we explored the effect of selected functional groups on the ability of these compounds to displace the progesterone receptor radioligand (*Z*)-[^{125}I]-17α-(2-iodovinyl)-19-nortestosterone[10] (see Table 2).

While **1a** was inactive (IC_{50} > 1000 nM), addition of electron-withdrawing groups to the benzoyl ring had a profound effect on binding affinity. The 3-chloro analog (**1c**, IC_{50} = 77 nM) had a higher affinity than the 4-chloro analog (**1b**, IC_{50} = 521 nM). Other 4-substituents were uninteresting with 4-methyl (**1d**) having about the same affinity as 4-chloro. The 4-bromo (**1e**), 4-methoxy (**1f**), and 4-*tert*-butyl (**1g**) analogs had IC_{50}'s > 1000 nM. The 3,4-difluoro analog (**1h**, IC_{50} = 515 nM) was about the same as 4-chloro, and 3,4-dichloro (**1i,** IC_{50} = 62 nM) was not significantly different than 3-chloro. Examination of the SAR leads us to the conclusion that a small electron-withdrawing group is needed at the 4-position, while a larger group is required at the 3-position. Accordingly, compound **1j** bearing a 4-fluoro, 3-bromo substitution pattern was synthesized and proved to be a higher affinity ligand (IC_{50} = 38 nM). The 3-trifluoromethyl, 4-chloro analog also had improved affinity (**1k,** IC_{50} = 270 nM) over that of **1b**.

Due to its high affinity, the availability of starting materials, the ease of synthesis, and its identification early in the project, the dichloro analog **1i** was chosen as a scaffold for further exploration of the SAR. Substitution of the phenyl ring at the 3-position of the pyridazine ring with electron-donating groups gave less active or inactive compounds with short alkyl groups (**1l**, R_1 = ethyl) having higher affinity than longer alkyl groups (**1m**, R_1 = butyl). The 4-methoxy analog (**1n**) was inactive. Replacement of hydrogen with a 4-halogen substituent did not greatly affect affinity, with the 4-fluoro analog (**1o**) having slightly more affinity than **1i** or the 4-chloro analog **1p**. A small electronegative group is therefore optimal but not required.

Small alkyl substitutions on the heterocyclic ring also did not greatly affect the binding. 4-Methyl analog **1s** was slightly more potent than the 5-methyl (**1q**) or 5,5-dimethyl (**1r**) variants. It is possible that the alkyl substitution induces subtle disturbances in the conformation of the pyridazine ring affecting the orientation of the carbonyl bond at the 1-position.

Directly altering the carbonyl had dramatic effects. Moving the amide oxygen into the ring to give a pyridazin-6-one (**2**) resulted in only a 2-fold loss of binding affinity, while conversion to the sulfonamide **3** abolished binding completely. Thioamide **4** had the highest affinity with an IC_{50} of 17 nM, while reduction of the amide to the tertiary amine (**5**) resulted in loss of all binding. The introduction of unsaturation into the heterocyclic ring (**6**) also gave an inactive compound. The conclusion drawn from studies with compounds **1i** and **2–6** is that not only must a heteroatom be present for binding to occur but also it must be in the correct orientation to optimally interact with the receptor. Compounds **1i**, **2**, and **4**, among others, are able to comply with this requirement. Several factors may explain the lack of activity of the sulfonamide; the fact that **3** did not bind suggests to us that the vector of the C=X bond is critical and that the tetrahedral sulfur in **3** cannot orient either of the S=O bonds on an appropriate vector. Also, the increased size of the sulfur atom compared with carbon may contribute to this loss of affinity. We attribute the improved affinity of the thioamide **4** to the polarizability and size of the sulfur atom compared to oxygen, perhaps allowing it to interact more efficiently through a hydrogen bond with some element of the receptor pocket. The introduction of a double bond (**6**) may distort the geometry of the pyridazine ring and causes the amide carbonyl to deviate significantly from its optimal vector. Compound **5** has no heteroatom in this position and is inactive.

During this SAR study, the binding affinity of this class of nonsteroidal compounds to the progesterone receptor in bone cells was improved dramatically (from >1000 to 17 nM). The affinities of some of these compounds began to approximate that of steroidal progestins (progesterone, IC_{50} = 17 nM; norethindrone, IC_{50} = 3.2 nM) at the bone receptor. Interestingly, the receptor binding SAR of this subset of tetrahydropyridazines differs somewhat from that of our earlier SAR study[6] in which we measured rabbit uterine progesterone receptor binding. In contrast to the steroidal progestins (progesterone and norethindrone) which showed the expected high-affinity binding to all known progesterone receptors (including those from rabbit uterus, T47D human breast carcinoma cell[11] and the receptors from TE85 bone cells), all of the tetrahydropyridazines described here demonstrated poor binding

Table 1. Intermediates **7–9** and Beilstein References

					formula/Beilstein reference						
compd	R_1	R_2	R_3	R_4	**7**	Beil 10	**8**	Beil 24	**9**[a]	Beil 23	Affords[b]
a	H	H	H	H	$C_{10}H_{10}O_3$	696	$C_{10}H_{10}N_2O$	167	$C_{10}H_{12}N_2$	III 1164	**1a–k,2,3**
l	Et	H	H	H	$C_{12}H_{14}O_3$	II 494	$C_{12}H_{14}N_2O$	V3 342	$C_{12}H_{16}N_2$	*c*	**1l**
m	Bu	H	H	H	$C_{14}H_{18}O_3$	III 3106	$C_{14}H_{18}N_2O$	*c*	$C_{14}H_{20}N_2$	*c*	**1m**
n	OMe	H	H	H	$C_{11}H_{12}O_4$	958	$C_{11}H_{12}N_2O_2$	30[d]	$C_{11}H_{14}N_2O$	e	**1n**
o	F	H	H	H	$C_{10}H_9FO_3$	III 3039	$C_{10}H_9FN_2O$	V3 251	$C_{10}H_{11}FN_2$	*e*	**1o**
p	Cl	H	H	H	$C_{10}H_9ClO_3$	II 483	$C_{10}H_9ClN_2O$	II 79	$C_{10}H_{11}ClN_2$	V6 472	**1p**
q	H	H	Me	H	$C_{11}H_{12}O_3$	711	$C_{11}H_{12}N_2O$	III 503	$C_{11}H_{14}N_2$	V7 04	**1q**
r	H	H	Me	Me	$C_{12}H_{14}O_3$	III 3084	$C_{12}H_{14}N_2O$	III 516	$C_{12}H_{16}N_2$	*c*	**1r**
s	H	Me	H	H	$C_{11}H_{12}O_3$	II 488	$C_{11}H_{12}N_2O$	V3 307	$C_{11}H_{14}N_2$	*c*	**1s**

[a] **9** were oils which were acylated without purification. [b] **9** are the starting materials for **1a–s** and **3**. Compound **2** comes from **8a** and **4–6** from 1i. [c] New compound. [d] Beilstein volume 25. [e] Ref 20 describes an alternate synthesis.

Table 2. Binding Affinity of Tetrahydropyridazines and Analogs for Progesterone Receptors from Human Osteosarcoma Cells

compd	R_1	R_2	R_3	R_4	R_5	R_6	formula	mp (°C)	IC_{50} (nM)[a]
1a	H	H	H	H	H	H	$C_{17}H_{16}N_2O$	110–111	>1000 (1)
1b	H	H	H	H	Cl	H	$C_{17}H_{15}ClN_2O$	132–133	521 (1)
1c	H	H	H	H	H	Cl	$C_{17}H_{15}ClN_2O$	88–89	70.6, 82.8
1d	H	H	H	H	Me	H	$C_{18}H_{18}N_2O$	89–90	416 (1)
1e	H	H	H	H	Br	H	$C_{17}H_{15}BrN2O$	139–140	>1000 (1)
1f	H	H	H	H	OMe	H	$C_{18}H_{18}N_2O_2$	120–121	>1000 (1)
1g	H	H	H	H	*t*-Bu	H	$C_{21}H_{24}N_2O$	92–93	>1000 (1)
1h	H	H	H	H	F	F	$C_{17}H_{14}F_2N_2O$	88–89	515 (1)
1i	H	H	H	H	Cl	Cl	$C_{17}H_{14}Cl_2N_2O$	139–140	62.4 ± 8.2
1j	H	H	H	H	F	Br	$C_{17}H_{14}BrFN_2O$	89–90	38.2 ± 17.9
1k	H	H	H	H	Cl	CF_3	$C_{19}H_{14}ClF_3N_2O$	117–118	270 ± 92
1l	Et	H	H	H	Cl	Cl	$C_{19}H_{18}Cl_2N_2O$	120–121	570 (1)
1m	Bu	H	H	H	Cl	Cl	$C_{21}H_{22}Cl_2N_2O$	93–94	>1000 (2)
1n	OMe	H	H	H	Cl	Cl	$C_{18}H_{16}Cl_2N_2O_2$	121–122	>1000 (1)
1o	F	H	H	H	Cl	Cl	$C_{17}H_{13}Cl_2FN_2O$	112–113	48.2
1p	Cl	H	H	H	Cl	Cl	$C_{17}H_{13}Cl_3N_2O$	95–96	84.0 ± 8.2
1q	H	H	Me	H	Cl	Cl	$C_{18}H_{16}Cl_2N_2O$	124–126	98.0 ± 22.6
1r	H	H	Me	Me	Cl	Cl	$C_{19}H_{18}Cl_2N_2O$	108–111	75.3 ± 9.3
1s	H	Me	H	H	Cl	Cl	$C_{18}H_{16}Cl_2N_2O$	118–120	24.0 ± 3.6
2							$C_{17}H_{14}Cl_2N_2O$	86–87	170 289
3							$C_{16}H_{14}Cl_2N_2O_2S$	140–141	>1000 (1)
4							$C_{17}H_{14}Cl_2N_2S$	151–152	17.5 ± 5.7
5							$C_{17}H_{16}Cl_2N_2$	72–73	>1000 (2)
6							$C_{17}H_{12}Cl_2N_2O$	153–154	>1000 (2)
norethindrone									3.2 ± 0.7
progesterone									17 ± 1.0

[a] Values represent either the mean ± SEM of three to six determinations or, if in parentheses, the result of one or two receptor binding assays.

affinity to receptors from rabbit uterus and from T47D and ZR75 human breast carcinoma cells (IC_{50} >1000 nM). These results suggest that a high degree of cellular selectivity in terms of progestin receptor binding can be achieved with the tetrahydropyridazine ring system. The molecular basis for the selectivity of these nonsteroidal compounds is under investigation.

Early in our evaluation of this series of compounds, we selected what was then our highest affinity ligand (**1i**, RWJ 25333) for testing *in vitro*. We and others have previously reported that norethindrone and other steroidal progestins can stimulate the proliferation of MC3T3 osteoblast-like cells.[12–14] The nonsteroidal ligand RWJ 25333 not only showed high-affinity binding in the MC3T3 bone cells (IC_{50} = 57 nM) but also significantly stimulated the proliferation of this cell line at concentrations of 0.1 and 1 nM (Figure 1). As would be expected from the results of the binding assay, no effect was observed on T47D human breast carcinoma cell proliferation in a similar experiment at concentrations up to 10 μM.

The proliferative response of the MC3T3 cells to RWJ 25333 is biphasic and is similar to that observed with mouse, chicken, and human bone cells after treatment with norethindrone and other steroidal progestins.[14,15] When grown *in vitro*, osteoblasts and osteoblast-like cells require a long time to fully differentiate and express all the characteristics of mature osteoblasts. The proliferation and the differentiation of osteoblasts are interdependent and reciprocial processes.[16] Progestins and progestin-like compounds such as RWJ 25333 may affect both processes, and so the cells are stimulated to differentiate their proliferative response wanes, producing a biphasic growth curve.

Conclusion

We have discovered a novel class of nonsteroidal ligands which can bind with high affinity to the progesterone receptors obtained from osteoblast-like cells. This chemical series displays a well-defined SAR pattern for this receptor and a surprising degree of cellular selectivity, both in terms of receptor binding and biological activity. The progesterone receptor SAR for this series shows a marked cellular selectivity for receptors from human osteosarcoma cells over receptors from breast carcinoma cells. A representative compound, RWJ 25333 (**1i**), was found to mimic the ability of progestational steroids to stimulate the proliferation of osteoblast-like cells without affecting breast cell growth. Additional details on the *in vitro* and *in vivo* biological

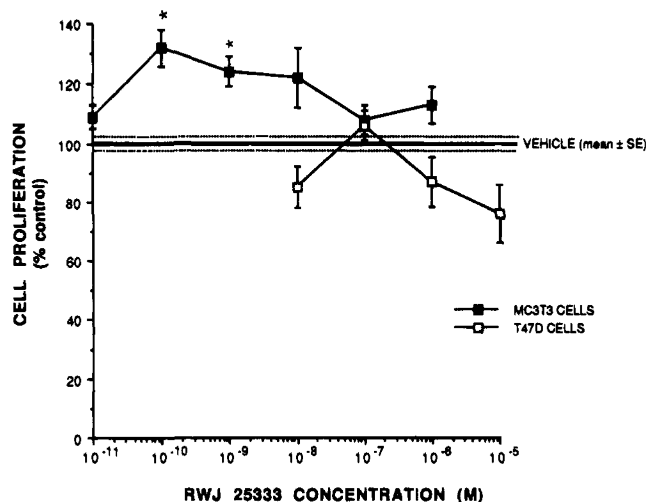


Figure 1. Proliferation of MC3T3 osteoblast-like cells and T47D breast cells after 48 h of RWJ 25333 treatment. Symbols and error bars represent mean ± SE ($N = 6$) of thymidine incorporation mesurements expressed as a percent of vehicle controls. MC3T3 and T47D vehicle control data had SE values of 1% and 3%, respectively. Maximal proliferative responses were observed with these cells when treated with norethindrone at concentrations of 0.01–1.0 nM (148% for MC3T3 cells, 313% for T47D cells). *$p < 0.5$ versus vehicle controls (Dunnett t test).

effects of RWJ 25333 will be presented in a subsequent publication.

Experimental Section

Receptor Binding Assay. Human osteosarcoma cells (TE85) were obtained from Dr. William Lau, J. L. Pettis Veterans Hospital (Loma Linda, CA). A transformed preosteoblastic mouse cell line (MC3T3-E1) was obtained from Dr. Thomas Inhorn, Mt. Sinai Hospital (New York, NY). TE85 and MC3T3 cells were grown in DMEM media (Gibco Laboratories) supplemented with 10% bovine calf serum (BCS, Hyclone Laboratories, Logan, UT). Two human breast carcinoma cell lines were used in these studies: T47D cells obtained from American Type Culture Collection (Rockville, MD) and ZR75 cells obtained from Dr. Rosalyn Blumenthal, Center for Molecular Medicine and Immunology (Newark, NJ). Both breast cell lines were maintained in RPMI media (Gibco Laboratories, Grand Island, NY) supplemented with 10% BCS. Culture media were replaced with RPMI or DMEM containing 10% charcoal-stripped fetal calf serum 24 h before cell harvest. Cells were harvested by trypsinization, counted, and then homogenized on ice in TEG assay buffer (0.01 M TRIS, 1 mM EDTA, 0.01 M molybdic acid, 10% glycerol, pH 7.4). The homogenate was centrifuged for 12 min at 541000g at 4 °C and the supernatant containing the cell cytosol diluted in TEG assay buffer.

Progestin binding was measured in tubes containing the cytosol from 1×10^6 breast (T47D and ZR75) cells, 3×10^6 MC3T3 cells, or 5×10^6 TE85 cells according to the methods of Clark et al.[17] Cell cytosol (200 μL) was incubated overnight at 4 °C with 1 nM of (Z)-[^{125}I]-17α-(2-iodovinyl)-19-nortestosterone[10] and test compounds. Test compounds were solubilized in DMSO and diluted in TEG assay buffer. The final concentration of DMSO in the assay tubes was 5%. Nonspecific binding was measured in the presence of 100-fold excess nonradioactive norethindrone. Unbound tracer was removed by adsorption with dextran-coated charcoal. An aliquot of the bound fraction was counted in a Micromedic Apex gamma counter.

Cell Proliferation Assay. The proliferation of T47D human breast carcinoma cells was estimated by measuring their incorporation of [^{3}H]thymidine into DNA. Cells were plated into 96-well plates and given 24 h to attach. To prepare the cells for the assay, they were maintained for 24 h in media with no FBS. This media was then replaced with media containing 0.03% FBS and RWJ 25333 (10 μM to 0.1 nM). An aliquot of medium containing 0.4 μCi of [^{3}H]thymidine was then added. After 24 h the cells were washed, harvested with trypsin–EDTA, and aspirated onto a glass fiber filter mat. The mats were counted by liquid scintillation spectroscopy and the rate of [^{3}H]thymidine incorporation calculated.

Chemical Synthesis. Melting points were determined on a Hoover capillary melting point apparatus and are uncorrected. Microanalyses were performed on a Perkin-Elmer Model 240c elemental analyzer.

Starting keto acids **7a** and **7d–f** were purchased from Aldrich Chemical Co. Compounds **7l** and **7m** were prepared by the acylation of ethyl- or butylbenzene with succinic anhydride and aluminum chloride. 5-Methyl analogs **7q** and **7r** were prepared by the same procedure using benzene and either 2-methyl- or 2,2-dimethylsuccinic anhydride. 4-Methyl analog **7s** was prepared by a literature route.[9] Treatment of **7a,l–s** with hydrazine in ethanol according to the method of McEvoy and Allen[18] gave 3-aryl-6-oxo-1,4,5,6-tetrahydropyridazines **8a,l–s**. These were reduced by the method of Aubagnac et al[19] to afford 3-aryl-1,4,5,6-tetrahydropyridazines **9a,l–s** which were acylated as described previously.[6] All biologically tested compounds had satisfactory elemental analyses (C, H, N), and spectral data was consistent with the reported structure.

1-(3,4-Dichlorobenzoyl)-3-phenyl-1,4,5,6-tetrahydropyridazine (1i). 3-Phenyl-1,4,5,6-tetrahydropyridazine (**9a**, 2.0 g, 11 mmol) was dissolved in toluene (50 mL) and 3,4-dichlorobenzoyl chloride (2.61 g, 12 mmol) added. The mixture was heated at reflux for 1.5 h and then evaporated at reduced pressure. The residue was dissolved in methylene chloride and filtered with suction through silica gel (100 g). The solvent was removed by evaporation at reduced pressure and the residue recrystallized from ethyl acetate to give 1.80 g (44%) of **1i** as a white solid melting at 139–140 °C. Anal. ($C_{17}H_{14}Cl_2N_2O$) C, H, N.

Likewise **9a** was acylated with various benzoyl chlorides to give **1a–k** while **9l–s** were acylated with 3,4-dichlorobenzoyl chloride to give **1l–s**.

1-(3,4-Dichlorobenzyl)-3-phenyl-1,4,5,6-tetrahydropyridazin-6-one (2). 3-Phenyl-1,4,5,6-tetrahydropyridazin-6-one (**8a**, 3.48 g, 20 mmol) was dissolved in dimethylformamide (50 mL) and cooled to 5 °C, and sodium hydride (0.9 g of 60% in oil) was added. After 30 min, 3,4-dichlorobenzoyl bromide (4.8 g, 20 mmol) was added and stirring continued for 2 days at room temperature. The dark reaction mixture was poured into water (500 mL) and extracted with ethyl ether (2 × 250 mL). The combined extracts were washed with dilute HCl (100 mL of 1 N) and then with water (100 mL), dried over magnesium sulfate, and filtered. The filtrate was evaporated to dryness and the residue purified by column chromatography, eluting with 5% methanol in dichloromethane to give 1.8 g of an off-white solid which was recrystallized from 2-propanol and hexane to give analytically pure **2** as an off-white solid, mp 86–87 °C. Anal. ($C_{17}H_{14}Cl_2N_2O$) C, H, N.

1-((3,4-Dichlorophenyl)sulfonyl)-3-phenyl-1,4,5,6-tetrahydropyridazine (3). 3-Phenyl-1,4,5,6-tetrahydropyridazine (**9a**, 0.77 g, 4.8 mmol) was dissolved in pyridine (20mL) and cooled to 15 °C in an ice bath. 3,4-Dichlorobenzenesulfonyl chloride (1.3 g, 5.3 mmol) was added slowly in portions. When addition was complete, the temperature was allowed to return to 22 °C and the red mixture stirred for 4 h. HCl solution (50 mL of 2 N) was added and the mixture extracted with dichloromethane (3 × 50 mL). The extracts were washed with 2 N HCl and dried over anhydrous potassium carbonate. The filtered solution was concentrated to 50 mL and passed through a short column of silica gel. The eluate was evaporated to dryness and the residue recrystallized from ethyl acetate and dried under vacuum to give 0.22 g of **3**, mp 140–141 °C. Anal. ($C_{16}H_{14}Cl_2N_2O_2S$) C, H, N.

1-(3,4-Dichlorothiobenzoyl)-3-phenyl-1,4,5,6-tetrahydropyridazine (4). Compound **1i** (0.75 g, 2.25 mmol) was dissolved in toluene (50 mL), and phosphorus pentasulfide (1 g, 2.25 mmol) was added under an atmosphere of nitrogen. The mixture was heated to 60 °C for 4 h, allowed to stir at

room temperature overnight, and then heated at 60 °C for an additional 7 h. The reaction mixture was filtered and evaporated to dryness under reduced pressure. The residue was purified by column chromatography on silica gel, eluting with 1:1 dichloromethane:hexane. The fractions containing product were combined, the solvent removed under reduced pressure, and the residue recrystallized twice from ethyl acetate and ether to give 0.19 g of **4** as a bright yellow solid, mp 151–152 °C. Anal. ($C_{17}H_{14}Cl_2N_2S$) C, H, N.

1-(3,4-Dichlorobenzyl)-3-phenyl-1,4,5,6-tetrahydropyridazine (5). Compound **1i** (2.0 g, 6 mmol) was dissolved in THF (150mL) in an atmosphere of nitrogen and the solution cooled to 5 °C in an ice bath. Lithium aluminum hydride (15 mL of 1.0 M solution in THF) was added dropwise over a 15 min period. The mixture was stirred for 45 min at room temperature, and then water (0.6 mL), sodium hydroxide solution (0.6 mL), and more water (1.8 mL) were added. The mixture was dried over magnesium sulfate, filtered, and concentrated under reduced pressure to give a residue which was purified by column chromatography on silica gel eluted with methanol and recrystallized from 2-propanol and hexane to give 1.0 g of **5**, mp 72–73 °C. Anal. ($C_{17}H_{16}Cl_2N_2$) C, H, N.

1-(3,4-Dichlorobenzoyl)-3-phenyl-1,6-dihydropyridazine (6). Compound **1i** (1.0 g, 3 mmol) was dissolved in acetic acid (20 mL) and bromine (0.48 g, 3 mmol in acetic acid) added dropwise until the color was not discharged. The solvent was removed under reduced pressure and the residue recrystallized from ether to give analytically pure 1-(3,4-dichlorobenzoyl)-3-phenyl-4-bromo-1,4,5,6-tetrahydropyridazine. This bromide (0.92 g, 2.2mmol) was dissolved in THF (100 mL) and treated with diazabicycloundecene (1.0 g, 6.6 mmol). The mixture was heated to reflux on a steam bath, affording a yellow precipitate. The reaction was poured into ice and dilute sulfuric acid and extracted with dichloromethane. The organic layer was dried over magnesium sulfate and filtered. The filtrate was evaporated to dryness and the residue purified by column chromatography, eluting with dichloromethane to give **6**, mp 153–154 °C. Anal. ($C_{17}H_{12}Cl_2N_2O$) C, H, N.

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