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# SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF NONACYCLIC AND TRISDECACYCLIC PYRAZINES RELATED TO CEPHALOSTATIN

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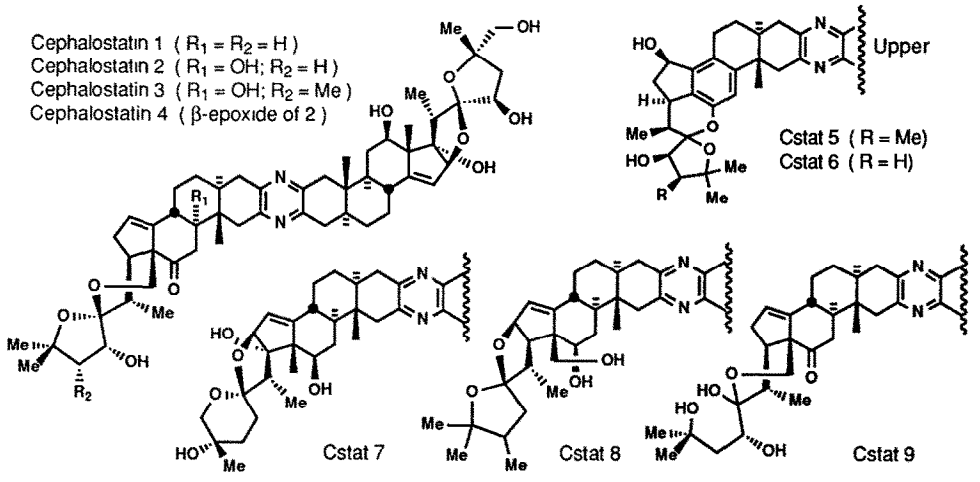
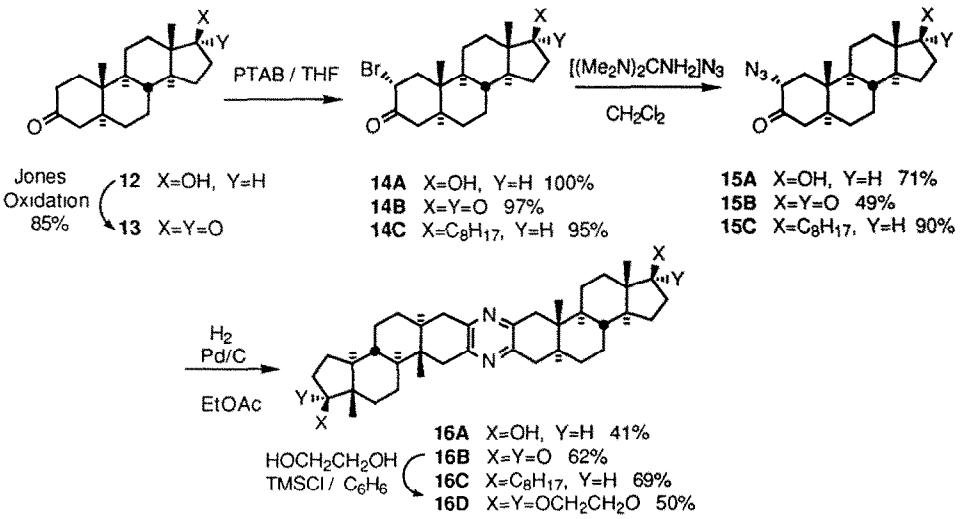
**Abstract:** Steroidal α-azidoketones are converted through catalytic reduction to C2 symmetrical nonacyclic and trisdecacyclic pyrazines. An unusual azide-mediated formation of an unsymmetrical heterobenzyl azide was observed. In vitro testing of the symmetrical pyrazines revealed cytotoxicity of about $10^{-5}$ M; the azidopyrazine was about 100-fold more cytotoxic. Animal testing of one of the derivatives in the xenograft implanted H/1.2-NRK and K/1-NRK cell lines showed inhibition of tumor growth between 50-60%.

The cephalostatins **1-9**[1] are a group of trisdecacyclic pyrazines whose structural elucidation has resulted from the heroic efforts of the Pettit group at Arizona State University. Cephalostatin **1** is the most potent inhibitor of the family with an $ED_{50}$ $10^{-7}$-$10^{-9}$ μg/mL in the P388 cell line. More recently Pettit has also reported that these materials are highly active ($10^{-9}$-$10^{-10}$ M) in a reasonable proportion of the 60 in vitro screens of the National Cancer Institute.[1d]



No synthetic activity has yet been reported in this area. As a prelude to a synthetic program which envisages the total synthesis of Cephalostatin **1**, we deemed it prudent to synthesize and ascertain the anti-cancer activity of a number of simpler, C2-symmetric trisdecacyclic pyrazines. The initial symmetrical nonacyclic pyrazine analogs to be tested were prepared by the standard methods shown in the scheme below.[2]




In the course of scaling up the synthesis to provide 2g of **16A** for animal testing, a minor (5-10%) product, **18A** was observed. A series of control studies showed that excess azide serves as a base to generate α-aminoenone **17A**. Dimerization of this intermediate followed by $S_N2'$ reaction with the hydrazoic acid co-product generates azido-pyrazine **18A**. Independent generation of α-aminoenone **17A** can also be effected by treatment of azide **15A** with DBU (65%). Subsequent reaction of **17A** with hydrazoic acid generated in situ from TMS-azide and methanol in methylene chloride solution in a sealed tube at 70°C for 18h provides azido-pyrazine **18A** in 70% yield. Recent in vitro testing of this interesting derivative reveals substantially increased activity relative to **16A** which lacks the azido group (Entry 5, Table 1).

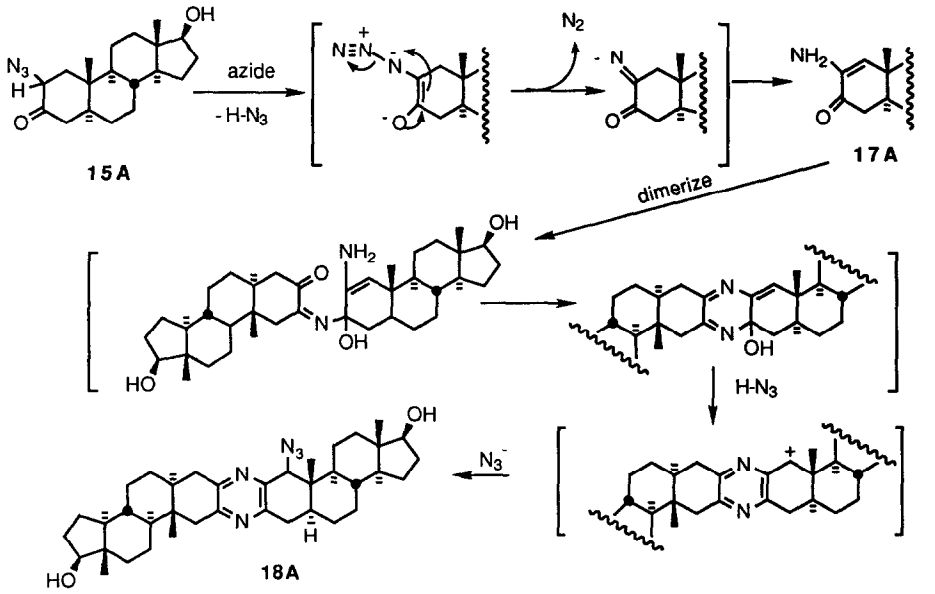
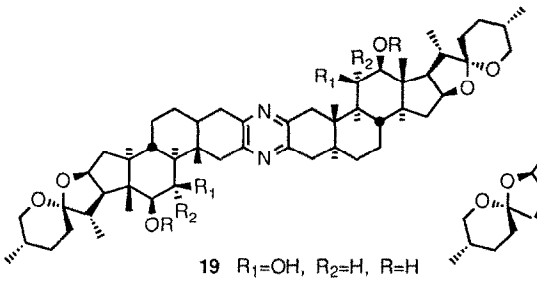



**Table 1. In Vitro Human Tumor Cytotoxicity Assay Results for Polycyclic Pyrazines ($ED_{50}$) [μg /mL (M)]**

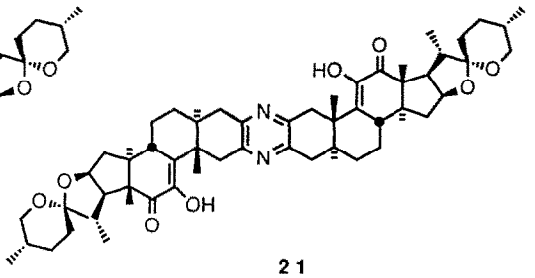
| Compound | M.W. | A-549[a] | MCF-7[b] | HT-29[c] | SK-MEL-5[d] | Malme-3M[e] |
|---|---|---|---|---|---|---|
| 16A | 572 | 35 ($6.1x10^{-5}$) | 28 ($4.9x10^{-5}$) | 7 ($1.2x10^{-5}$) | 36 ($6.3x10^{-5}$) | 39 ($6.8x10^{-5}$) |
| 16B | 568 | 42 ($7.4x10^{-5}$) | 46 ($8.1x10^{-5}$) | 94 ($1.6x10^{-4}$) | >100 | >100 |
| 16C | 764 | >100 | >100 | >100 | >100 | >100 |
| 16D | 656 | 33 ($5.0x10^{-5}$) | 37 ($5.6x10^{-5}$) | 39 ($5.9x10^{-5}$) | 17 ($2.6x10^{-5}$) | 31 ($4.7x10^{-5}$) |
| **18A** | **613** | **0.22 ($3.6x10^{-7}$)** | **0.27 ($4.4x10^{-7}$)** | **0.35 ($5.7x10^{-7}$)** | **0.31 ($5.1x10^{-7}$)** | **0.42 ($6.8x10^{-7}$)** |
| 19 | 884 | 39 ($4.3x10^{-5}$) | 38 ($4.3x10^{-5}$) | 28 ($3.2x10^{-5}$) | 26 ($2.9x10^{-5}$) | 52 ($5.9x10^{-5}$) |
| 20 | 880 | 37 ($4.2x10^{-5}$) | 35 ($4.0x10^{-5}$) | 28 ($3.2x10^{-5}$) | 36 ($4.1x10^{-5}$) | 48 ($5.5x10^{-5}$) |
| 21 | 876 | >100 | >100 | 26 ($3.0x10^{-5}$) | 28 ($3.2x10^{-5}$) | 53 ($5.5x10^{-5}$) |

[a]Lung carcinoma; [b]Breast carcinoma; [c]Colon adenocarcinoma; [d]Melanoma, metastatic to auxillary node; [e]Melanoma, metastatic to lung. MTT assay results determined by the Purdue University Cell Culture Laboratory;[3] these numbers are single determinations and should be regarded as having a S. D. of about ±1/2 log.

In a parallel fashion to the simple nonacyclic derivatives shown in Table 1, commercially available (Aldrich) 3β,12β-diacetoxy-5α-spirostan-11-one was converted to trisdecacyclic pyrazines **19-21** in 13-24% overall yield.[4] Cytotoxicity data for these compounds are also reported in Table 1, structures **19-21**.[4]



**19** $R_1$=OH, $R_2$=H, R=H
**20** $R_1$=O, $R_2$=O, R=H



**21**

Over the past decade, progress has been made in understanding the genetic changes involved in cancers. The ras gene family is important in the etiology of a large variety of human cancers, such as pancreas, colon, and lung.[5,6] In these tumors, the proteins encoded by the ras genes are mutated and are required for the maintenance of the transformed phenotype.[6,7] Because of the importance of mutated ras in cancer, the in vitro cytotoxicity of **16A** and **20** was evaluated against normal and ras transformed epithelial cells.

For these studies, normal rat kidney epithelial (NRKE) cells[8] and the ras transformed cell lines K/1-NRK and H/1.2-NRK were employed.[9] The K/1-NRK line was produced by transformation of NRKE cells with the v-$ras^K$ gene ($gly^{12}$ to $val^{12}$), and the H/1.2-NRK line was produced by transformation with the mutated human c-$ras^H$ gene ($gly^{12}$ to $val^{12}$). NRKE cells are not tumorigenic in several strains of immunologically deficient mice, whereas the H/1.2-NRK and K/1-NRK cells produced carcinomas in these mice. A clonogenicity assay was used to determine the cytotoxicity of **16A** and **20** against the parental NRKE and transformed H/1.2-NRK and K/1-NRK cells. At 3.3 and 33 μM, **20** was more cytotoxic against H/1.2-NRK cells than K/1-NRK or NRKE cells (Table 2). In contrast, **16A** had no differential cytotoxicity at these concentrations.

**Table 2. In vitro cytotoxicity[a] of 16A and 20 against NRKE, H/1.2-NRK and K/1-NRK cells.**

| Compound | μM | Percent Cell Kill | | |
|---|---|---|---|---|
| | | NRKE | H/1/2-NRK | K/1-NRK |
| | | | | |
| **16A** | 3.3 | 100 | 100 | 100 |
| | 33 | 100 | 100 | 100 |
| **20** | 3.3 | 7 | 0 | 0 |
| | 33 | 20 | 100 | 100 |

[a]In the clonogenicity assay all evaluations were done in triplicate, and percent kill values were determined from the colony values for the DMSO-vehicle control groups. The compound was added 24 hr after seeding and was assayed 5 days later The maximum S D. for the percent kill values was ± 20%.

Both **20** and **16A** were evaluated at their maximally tolerated doses (MTD) and 1/2 MTD against H/1.2-NRK and K/1-NRK cells grown as xenograft tumors implanted subcutaneously near the mamary gland of the left axillary region in CD-1 nu/nu mice (8 animals per group). The drug was administered ip daily up to a maximally tolerated dose of 200 mg/kg/day, **20** inhibited the growth of the H/1.2-NRK tumor by 28% and the growth of the K/1-NRK tumor by 26% relative to vehicle control. At a maximally tolerated dose of 150 mg/kg/day, **16A** inhibited the H/1.2-NRK tumor by 28%. However, at 150 and 75 mg/kg/day, **16A** produced a 59 and 51% inhibition, respectively, in the growth of the K/1-NRK tumor. There were no toxic deaths with either compound. Compound **20** was poorly soluble in several pharmaceutical vehicles and this may explain its diminished antitumor activity.

It is interesting to note that the simple polycyclic pyrazines prepared in this study (with the possible exception of heterobenzylic azide **18A** which may behave as an alkylating agent) as well as the cephalostatins themselves are devoid of the functional groups which are commonly associated with anti-cancer agents (enones, quinones, vinyl lactones, alkylating agents, etc.). It seems that the mode of action of these materials likely will be found in terms of their ability to function as a spatially-defined set of hydrogen-bond donors and acceptors which may provide a tightly-bound enzyme inhibitor.

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[9]A full description of the transformed cell lines will be described at a later date by the Lilly group.