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Antiproliferative activity in HL60 cells by tetrasubstituted pyrroles: a structure–activity relationship study

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Abstract—A number of tetrasubstituted pyrrole derivatives have been synthesized and evaluated for their in vitro antiproliferative activities using the human promyelocytic leukemia cell line HL60. Tetrasubstituted pyrroles are obtained by irradiation of a silica gel absorbed mixture of a conjugated alkynoate and a primary amine. Active compounds exhibited GI_{50} values in the range 4–45 μM, and only six products showed TGI values within the evaluation range. A structure–activity relationship is also discussed.

Antiproliferative and cytotoxic drugs play a major role in cancer therapy, whether used alone or in concert with other treatment modalities such as surgery, radiation, and biological therapy. In the past 50 years, the mass screening of either synthetic derivatives or natural products has led to the discovery of the currently utilized anticancer drugs.

Despite the original expectation that the synthesis of libraries that contain millions of compounds would produce many drug candidates, it was soon recognized that the quality of a library would determine its success thereby overcoming the problem of efficient hit and lead finding. The quality of a library is determined by its diversity and drug-likeness.

Privileged structures, with their inherent drug-likeness, represent an ideal source of core scaffolds and capping fragments for the design and synthesis of combinatorial libraries targeted at various receptors. Several privileged structure-based combinatorial libraries have been designed and synthesized. These libraries have proved to be an extremely powerful tool to aid the rapid discovery and optimization of potent and selective drugs for a wide variety of cellular targets.[1]

As part of a wide research program aimed at developing new antitumoral agents, we present herein our preliminary results on the antiproliferative activity in HL60 cells of the 20-member library of 1,2,3,4-tetrasubstituted pyrroles **2** shown in Table 1. These heterocycles were selected as starting framework for our drug discovery program because they are recognized pharmacophores present in numerous bioactive natural products[2] and therapeutic compounds.[3] Recently, aryl pyrroles have been reported as potent inhibitors of Ras farnesyltransferase with regression of tumors grown in nude mouse xenograft models.[4]

We have recently reported on a fast, simple, and versatile method for the modular and diversity-oriented synthesis of tetrasubstituted pyrroles.[5] The method comprises two coupled domino processes linked in a one-pot manner: an organocatalyzed domino synthesis of a propargylic scaffold **1** (domino I)[6] and a

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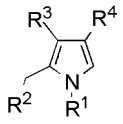
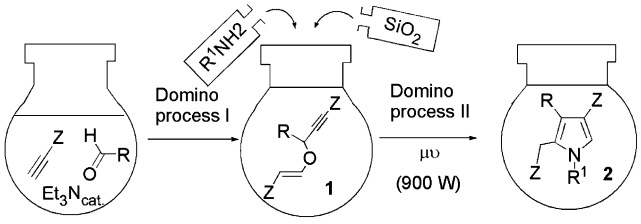
Table 1. Structures of substituted pyrroles and their antiproliferative activity



2

Compds	R^1	R^2	R^3	R^4	GI_{50}, μM[a]	TGI, μM[a]
2a	Bn	H	Me	CO_2Et	35.4 (±13.9)	80.9 (±22.1)
2b	Bn	H	Et	CO_2Me	31.4 (±1.9)	na
2c	Bn	H	*n*Hex	CO_2Me	26.6 (±8.7)	87.2 (±25.6)
2d	Bn	CO_2Et	H	CO_2Me	na	na
2e	Bn	CO_2Et	Me	CO_2Et	35.8 (±12.3)	na
2f	Bn	CO_2Me	Et	CO_2Me	43.1 (±7.6)	na
2g	Bn	CO_2Et	Et	CO_2Et	34.3 (±8.4)	na
2h	Bn	CONHBn	Et	CO_2Me	4.8 (±3.0)	na
2i	Bn	CO_2Me	*i*Pr	CO_2Me	34.7 (±12.9)	na
2j	Bn	CO_2Et	*i*Pr	CO_2Et	17.9 (±7.6)	88.4 (±16.5)
2k	Bn	CO_2Me	*i*Pr	CO_2Et	33.0 (±15.6)	88.3 (±18.8)
2l	Bn	CO_2H	*n*Hex	CO_2Me	40.4 (±8.9)	92.4 (±13.1)
2m	Bn	CO_2Me	*n*Hex	CO_2Me	17.7 (±9.1)	na
2n	Bn	CO_2Et	*n*Hex	CO_2Et	5.1 (±1.6)	94.5 (±11.1)
2o	Bn	CO_2Et	3-Butenyl	CO_2Et	na	na
2p	Bn	CO_2Et	$BnOCH_2$	CO_2Et	na	na
2q	Bn	CO_2Et	*c*Pr	CO_2Et	34.2 (±12.5)	na
2r	Bn	CO_2Et	(*S*)-$(Me)_2CCH(CH_2)_2CH(Me)CH_2$	CO_2Et	na	na
2s	(*S*)-PhCHMe	CO_2Me	Et	CO_2Me	39.1 (±10.6)	na
2t	(*R*,*S*)-$MeCHCH_2CO_2Et$	CO_2Me	Et	CO_2Me	na	na

[a] Values are means of at least three experiments, standard deviation is given in parentheses (na = not active).

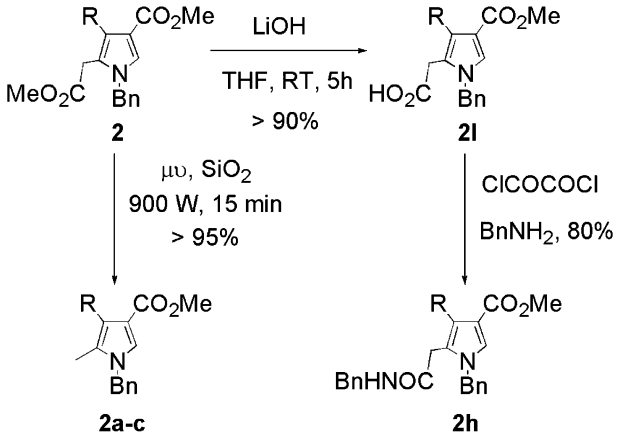


Scheme 1. Synthesis of tetrasubstituted pyrroles starting from conjugated alkynoates and primary amines.

microwave-assisted amine addition–cyclization domino process (domino II) (Scheme 1).

By using this protocol we have prepared the set of tetrasubstituted pyrroles **2a**–**t** shown in Table 1, and we have evaluated their in vitro antiproliferative activities against the human promyelocytic leukemia cell line HL60.

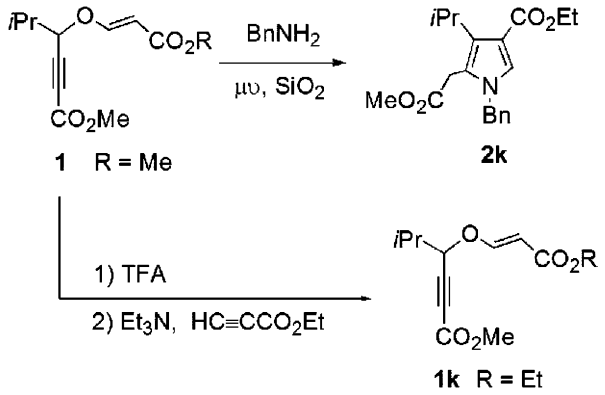
Pyrroles **2d**–**t** were directly obtained from commercially available precursors (methyl or ethyl propiolate, aliphatic aldehydes, and primary amines) in less than 1 h and in 40–55% yield.[5] The method tolerates a wide scope in the primary amine (aromatic, aliphatic, amino acids, etc.) and it is sufficiently mild to allow a range of functionalities on the aldehyde chain. In addition, the aliphatic ester group of pyrroles **2** can be selectively submitted to a microwave-assisted reductive decarboxylation (entries **2a**–**c**) or selectively hydrolyzed to generate a new functional-diversity point on the molecule (entries **2h** and **2l**) (Scheme 2). Finally, pyrrole **2k**, featuring an



Scheme 2. Generation of a new functional-diversity point on the tetrasubstituted pyrrole molecule.

aliphatic methyl ester and an aromatic ethyl ester groups, was synthesized from the appropriate conjugated alkynoate **1k** (Scheme 3).

Cell antiproliferative assays were performed in 96-well plates using the National Cancer Institute protocol with slight modifications.[7] We screened growth inhibition and cytotoxicity of the whole set of compounds **2a**–**t** against HL60 cells after 48 h of stimulation using the sulforhodamine B (SRB) assay.[8] In addition, treated and control cells were analyzed by phase contrast microscopy to assess morphological changes. Three dose response parameters can be calculated, when possible, for each experimental agent from the dose response



Scheme 3. Synthesis of pyrrole **2k** bearing two different ester groups.

curves. Growth inhibition of 50% (GI_{50}), which is the drug concentration resulting in a 50% reduction of cellular net growth when compared with values of untreated control cells, the drug concentration resulting in total growth inhibition (TGI), and the net loss in 50% of cells following treatment (LC_{50}) denoting cell kill.

The GI_{50} and TGI data are listed in Table 1. The results allow us to classify the compounds in two groups according to their antiproliferative profile. The group of active compounds comprises 15 products, which show GI_{50} values in the range 4–45 μM. From this series, compounds **2h** and **2n** were the most active against HL60 cells, with GI_{50} values of 4.8 and 5.1 μM, respectively. The remaining active derivatives showed modest activity with GI_{50} values in the range 15–45 μM. Only five products proved to be inactive at the maximum test concentration, that is, 100 μM. Interestingly, compounds **2a**, **2c**, **2j**–**l**, and **2n** were the only products from the active group series that reached a TGI value. The TGI values for those products were in the range 80–95 μM. None of the evaluated pyrroles was able to show a LC_{50} value. Morphological changes were observed in cells treated with active compounds at 100 μM (results not shown), being shrinkage the sole effect. At lower drug concentrations no clear difference could be observed from control cells.

When considering GI_{50} data the following structure–activity relationship is obtained: (a) the aliphatic substituent on the nitrogen atom of compound **2t** decreases activity when compared to compounds **2f** and **2s**, which posses aromatic side chains; (b) the absence of a substituent in position 3 of the pyrrole ring led to inactive compound **2d**; (c) ethyl ester derivatives **2g**, **2j**, and **2n** are more active than their corresponding methyl esters **2f**, **2i**, and **2m**, respectively; (d) hydrolysis of the aliphatic ester of **2m** led to a less potent derivative **2l**; (e) conversion of the aliphatic methyl ester in **2f** to benzyl amide **2h** led to the most potent compound of the series; (f) substituents other than methyl, ethyl, *i*-propyl, cyclopropyl, and *n*-hexyl at R^3 produces decrease in activity as shown with products **2o**, **2p**, and **2r**; and (g) the absence of an alkoxy carbonyl moiety at R^2 position in **2b** makes the compound more active than **2f**, but such a difference is not observed for compounds **2a** and **2c**, when compared to **2e** and **2m**.

Although the experiments are preliminary, we found that these synthetic derivatives considerably induced growth inhibition in HL60 human leukemia cells in vitro. The observation that the major part of the derivatives evaluated in this study present antiproliferative activity is consistent with considering pyrrole as a privileged structure with the substituents on the pyrrole ring modulating the biological activity.

In summary, we have constructed a 20-member library of tetrasubstituted pyrroles in a simple and direct way. On the basis of GI_{50} and TGI data, a structure–activity relationship was obtained. Some derivatives showed promising antiproliferative activity against HL60 cells. This general methodology allows the quick production of a variety of pyrrole synthons that are useful in combinatorial syntheses for the discovery of novel bioactive compounds.

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8. Pure compounds were initially dissolved in DMSO at 400 times the desired final maximum test concentration, that is, 100 μM. Control cells were exposed to an equivalent concentration of DMSO. Each agent was tested in duplicates at five different 10-fold dilutions. Drug incubation times were 48 h, after which cells were precipitated with 50 μL ice-cold 80% (w/v) trichloroacetic acid and fixed for 60 min at 4 °C. Then the SRB assay was performed. The optical density (OD) of each well was measured at 490 nm using Bio-Tek's Elx800 NB 96-well plate reader. The percentage growth was calculated at each of the drug concentration levels based on the difference in OD at the start and end of drug exposure. Values were corrected for background OD from wells only containing medium.