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Naphthol-derived Betti bases as potential SLC6A14 blockers

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Betti bases (aminobenzylnaphthols) have not been studied extensively to explore their possible pharmacological applications. Our group prepared a small and focused library of twenty-three Betti bases from the multicomponent reaction of 2-naphthol with primary or secondary cyclic amines and representative aromatic aldehydes. The compounds were prepared in 52-90% yield using environmentally friendly procedures. The E-factor and the atom economy for our process were 3.92 and 94%, respectively. The study of the anti-proliferative activity against human solid tumor cell lines pointed out that these Betti bases represent privileged scaffolds and could be used for the development of pharmacologically-active compounds in cancer therapeutics. The 50% growth inhibitory (GI_{50}) values of the most potent compounds were in the low micromolar range. Fourteen of these Betti bases were less active in HBL-100 breast cancer cells than towards the breast cancer cell line T-47D. A subset of these Betti bases was further tested against the human breast cancer cell lines MCF-7 and MDA-MB-453. The results indicated a correlation in the sensitivity of T-47D cells to Betti bases. We explored computationally the interaction of the Betti bases with SLC6A14, a Na^+- and Cl^-- dependent influx transporter of both neutral and cationic amino acids that is overexpressed in T-47D cells. SLC6A14 is inhibited by α-methyl-tryptophan, which blocks cell growth via deprivation of amino acid influx. The docking studies indicated that our Betti bases might behave as tryptophan mimetics, blocking this solute carrier transporter and inducing the anti-proliferative effects. Importantly, these Betti bases showed good cytotoxic selectivity towards cancer cells with no activity against normal human fibroblast cells BJ-hTERT.

Keywords

Anti-proliferative activity; Betti bases; breast cancer; multicomponent reactions; solute carrier protein; SLC6A14 ($ATB^{0,+}$)

1. Introduction

Multicomponent reactions (MCRs) represent an extraordinary tool to gain quick access to novel molecular scaffolds. In medicinal chemistry, these small molecules represent ideal candidates in drug discovery [1]. As part of our program directed at the drug discovery of new antitumor agents, we have explored the anti-proliferative activity of small and focused libraries of compounds obtained by diverse types of MCRs (Fig. 1). For example, we have investigated pyrroles (**1**) [2], propargylic enol ethers (**2**) [3], Ugi adducts of grindelic acid (**3**) [4], Hantzsch derived 1,4-dihydropyridines (**4**) and pyridines (**5**) [5], pyrazolodihydropyridines (**6**) [6], Ugi and Passerini tocopherol mimetics (**7** and **8**, respectively) [7], as well as diazepinones (**9**-**10**) [8].

As shown in Fig. 1, all lead compounds representing the aforementioned scaffolds, displayed anti-proliferative activity in human solid tumor cells at the low micromolar range. These results encouraged us to look for other MCRs that could provide diverse molecular templates to explore their biological activity. Thus, we focused our attention at the Betti reaction [9], a variation of the Mannich reaction where the carbonyl compound is replaced by a naphthol.

Betti bases have attracted the interest of organic chemists due to their chelating properties with organometallic reagents that provide highly efficient complex compounds useful in asymmetric synthesis [10]. However, the Betti bases have not been studied extensively to explore their possible pharmacological applications. In this context, some Betti bases have been proposed as antimicrobial agents [11] or as a tool to reverse multidrug resistance against doxorubicin mediated by P-glycoprotein (P-gp/ABCB1) with *N*-tylosil-1-α-amino-(3-bromophenyl)-methyl-2-naphthol (TBN) *in vitro* and *in vivo* [12].

Herein, we report on the anti-proliferative activity of a small library of Betti bases obtained by using environmentally friendly organic synthesis procedures against human solid tumor cells. A small subset of the compounds was further evaluated and investigated computationally in order to predict their ability to block the solute carrier family 6 member 14 transporter (SLC6A14 or $ATB^{0,+}$). The latter is a member of the sodium- and chloride-dependent neurotransmitter transporter family, which functions

as a Na^+- and Cl^-- dependent influx transporter in the cellular uptake of both neutral and dibasic amino acids. In the latter respect, as rapidly proliferating cells, tumor cells display an altered metabolism in order to meet their increasing anabolic requirements [13]. This enhanced cell proliferation results in an increased demand for nutrients including for example glucose and amino acids. Selective amino acid transporters have been reported to undergo up-regulation in cancer in order to meet this increased demand for amino acids [14]. Thus, tumor-selective amino acid transporters such as SLC6A14 are *bona fide* druggable targets for cancer therapeutics.

2. Materials and methods

2.1 Chemicals

All commercially available chemicals were purchased from Aldrich and used without further purification.

2.2 Cell lines and culture

Human fibroblast cells BJ-hTERT and the human cancer cell lines A549 (non-small human lung cancer, NSCLC), HBL-100, MCF-7 and T-47D (breast cancer), as well as HeLa (cervical cancer), were provided by Dr. Raimundo Freire (Hospital Universitario de Canarias, Tenerife, Canary Islands). The NSCLC cell line SW1573 and the colon cancer cell line WiDr were provided by Prof. G. J. Peters (Cancer Center Amsterdam, Vrije Universiteit, Amsterdam, The Netherlands). The breast cancer cell line MDA-MB-453 was provided by CEAMED S. A. (Tenerife, Canary Islands). Cells were grown in RPMI-1640 medium containing 5% fetal bovine serum (FBS), 2 mM L-glutamine, 100 U/mL of penicillin G and 0.1 mg/mL of streptomycin in a 37 ºC, at a 95% humidified atmosphere of 5% CO_2. Cells were maintained in culture in 60 mm cell culture dishes in growth medium (10 mL) and passaged twice weekly.

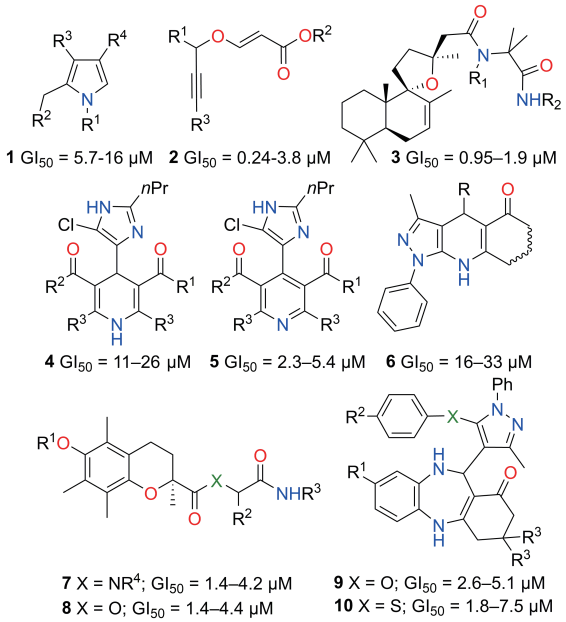

Figure 1. General structure of scaffolds obtained by MCRs, with indication of the anti-proliferative activity (GI_{50}) of the resulting leads.

2.3 Anti-proliferative assays

Cell line suspensions were counted with a Moxi Z automated cell counter (Orflo Technologies, Ketchum, ID 83340, USA) and diluted to reach the appropriate cell densities for inoculation onto 96-well plates. After 24 hours of equilibration in growth medium, the compounds were added at increasing concentrations (0.01-100 μM). The maximal test concentration was set at 100 μM. Cisplatin was used as a positive control of a conventional cytotoxic agent and DMSO (0.25% v/v) was used as negative control. Drug incubation times were 48 hours, following which monolayer cells were fixed onto the wells using 25 μL ice-cold trichloroacetic acid solution (50% w/v) for 60 min at 4 ^{o}C. Then, the plates were rinsed with water, following which 25 μL of a sulforhodamine B (SRB) solution (0.4% w/v in 1% acetic acid) was added for 15 min. Unbound SRB was rinsed with 1% acetic acid. Cell-bound SRB was dissolved with 150 μL of a 10 mM Tris solution at pH 10.5. The optical density of each well was determined at 530 and 620 nm using PowerWave XS microplate reader. This dual wavelength was used to reduce optical interference caused by scratches, fingerprints or other matters that equally absorb light at both wavelengths. The anti-proliferative activity, expressed as 50% growth inhibition (GI_{50}), was calculated according to NCI formulas [15].

2.4 Docking

The 3D structure of the transporter SLC6A14 is not available in Protein Data Bank. Therefore, we used a homology modelling procedure. We selected the *Drosophila's* dopamine transporter in complex with cocaine (PDB ID: 4XP4, resolution of 2.8 Å) as a template. We used HyperChem software (Hypercube Inc., FL, USA) to obtain and optimize the molecular structures and OpenBabel application to convert the files into adequate format before exporting them to docking software Glide v8.2 (Schrödinger, MA, USA). We used "Standard Precision" protocol in Glide with OPLS 2005 force field with post-docking minimization.

3. Results and discussion

3.1 Chemistry

The Betti bases reported in the current study were prepared according to our general synthetic pathway [16], which is outlined in Fig. 2. In this multicomponent organic synthesis process, 2-naphthol (**11**) was reacted with primary or secondary cyclic amines and diverse aromatic aldehydes (see Table 1 for residues). This one-pot multicomponent Betti synthesis was performed under green heterogeneous and neat conditions in the presence of Montmorillonite K30 catalyst, at 60 °C and in relatively short reaction times. Using this method, a small and focused library of Betti bases (**14a-w**) was prepared in good to excellent yield (50-92%). The "greenness" of a chemical reaction can be quantified by calculating both the E-factor and the atom economy, which for our process were 3.92 and 94%, respectively.

3.2 Biological evaluation

The *in vitro* anti-proliferative activity of the twenty-three Betti bases **14a-w** library was studied using our implementation of the NCI protocol [17]. The compounds were screened against a panel of representative human solid tumor cell lines comprised of A549 (NSCLC), HBL-100 and T-47D (breast cancer), HeLa (cervical cancer), SW1573 (alveolar cell carcinoma), and WiDr (colon). The results of the 50% growth inhibition (GI_{50}) values are shown

Table 1. Anti-proliferative activity (GI_{50}) against human solid tumor cell lines of the Betti bases of 2-naphthol[a]

			Cell line					
Compound	Amine	R	A549	HBL-100	HeLa	SW1573	T-47D	WiDr
14a	Pyrrolidine	Ph	> 100	> 100	> 100	> 100	> 100	> 100
14b		*p*−MePh	14 ± 0.9	41 ± 0.6	16 ± 4.7	15 ± 0.8	26 ± 5.2	32 ± 1.5
14c		*p*−NO_2Ph	92 ± 1.0	> 100	15 ± 1.9	63 ± 24	13 ± 3.1	> 100
14d		*p*−CNPh	> 100	> 100	> 100	42 ± 2.0	55 ± 9.1	> 100
14e		*o*-MeOPh	17 ± 0.7	28 ± 5.2	15 ± 3.0	20 ± 3.1	27 ± 3.9	28 ± 2.1
14f		*m*-MeOPh	26 ± 8.1	56 ± 11	22 ± 1.6	29 ± 3.9	29 ± 0.9	36 ± 5.9
14g		*p*−MeOPh	30 ± 6.0	27 ± 5.4	27 ± 2.5	23 ± 4.5	32 ± 4.0	35 ± 5.6
14h		*p*−FPh	21 ± 6.3	48 ± 11	23 ± 5.8	25 ± 4.7	29 ± 1.4	55 ± 6.3
14i		*p*−ClPh	12 ± 2.4	25 ± 2.4	27 ± 2.6	14 ± 0.3	19 ± 2.1	33 ± 2.9
14j		*p*−BrPh	**7.9 ± 0.2**	**9.4 ± 3.2**	**8.3 ± 1.4**	14 ± 2.2	16 ± 5.5	37 ± 12
14k		*p*−3,4-diClPh	12 ± 3.9	22 ± 12	**8.4 ± 2.5**	22 ± 7.4	**9.8 ± 2.3**	36 ± 8.8
14l		4-Pyridyl	40 ± 3.6	62 ± 2.1	37 ± 5.2	18 ± 5.9	64 ± 15	68 ± 16
14m	Piperidine	*p*−MePh	30 ± 8.9	53 ± 4.2	23 ± 3.0	31 ± 7.5	25 ± 3.1	44 ± 4.2
14n		*p*−NO_2Ph	66 ± 26	82 ± 25	**8.1 ± 1.2**	66 ± 10	13 ± 3.3	> 100
14o		*p*−CNPh	79 ± 30	76 ± 27	11 ± 0.7	29 ± 13	**8.5 ± 0.7**	93 ± 0.7
14p	Morpholine	*p*−MePh	28 ± 4.2	24 ± 2.4	18 ± 1.8	18 ± 2.6	28 ± 0.7	31 ± 6.6
14q		*p*−NO_2Ph	22 ± 0.5	31 ± 9.1	13 ± 1.5	34 ± 11	27 ± 3.8	51 ± 7.4
14r		*p*−CNPh	28 ± 3.9	33 ± 1.7	18 ± 1.7	17 ± 7.9	27 ± 4.8	31 ± 6.4
14s	$BnNH_2$	Ph	55 ± 2.5	30 ± 4.8	16 ± 5.4	25 ± 2.5	27 ± 3.5	45 ± 7.0
14t	$BnNH_2$	*p*−CNPh	12 ± 3.3	**5.0 ± 0.1**	**4.1 ± 0.6**	**6.3 ± 1.2**	**8.4 ± 1.2**	30 ± 6.3
14u	*n*-$BuNH_2$	Ph	> 100	> 100	> 100	> 100	> 100	> 100
14v	*i*-$PentNH_2$	Ph	19 ± 3.1	19 ± 2.8	13 ± 1.4	22 ± 3.9	20 ± 2.7	25 ± 4.7
14w	*c*-$PentNH_2$	Ph	18 ± 3.9	24 ± 0.6	18 ± 5.5	15 ± 3.6	25 ± 0.2	33 ± 7.5
CDDP			**4.9 ± 0.2**	**1.9 ± 0.2**	**1.8 ± 0.5**	**2.7 ± 0.4**	17 ± 3.3	23 ± 4.3

[a] GI_{50} values are given in μM. Standard deviation was calculated from two to four independent experiments. Cisplatin (CDDP) was used as a reference cytotoxic drug. Values in bold face represent the best anti-proliferative data against tumor cell lines with GI_{50} values < 10 μM.

in Table 1. Taken collectively, the data show that eighteen out of twenty-three compounds were able to inhibit cell growth in all cell lines with GI_{50} values in the range 4-79 μM. This initial results allow considering Betti bases as privileged structures, with inherent drug-likeness, that can represent an ideal source of a core scaffold for the design and synthesis of combinatorial libraries that could be targeted at various cellular targets including for example receptors, enzymes and other proteins.

From this set of compounds, five compounds displayed GI_{50} values at the low micromolar range (< 10 μM) against at least one cell line. In terms of cytotoxic potency, we could classify them in the following order **14t** > **14j** > **14k** > **14n-o**. From the GI_{50} values we observed that only two compounds were inactive (GI_{50} > 100 μM) in all cell lines studied (**14a** and **14u**), while 14n, 14c and 14d displayed anti-proliferative effects against five, four and two cell lines, respectively.

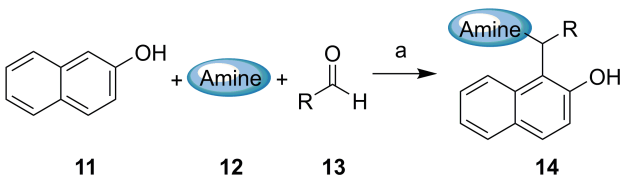

Figure 2. General procedure for the synthesis of Betti bases. Reagents and conditions: (a) Montmorillonite K30, neat, 60 °C or r.t. See Table 1 for amine and R definitions.

When considering the substitution pattern of compounds **14a-w**, we could not infer a clear structure-activity relationship (SAR). Furthermore, the data set for each cell line did not provide a SAR trend. Furthermore, a direct comparison between the GI_{50} data obtained in the breast cancer cells (HBL-100 ad T-47D) drew our attention. As a rule of thumb, HBL-100 cells tended to be more sensitive to drug exposure than T-47D cells. However, fourteen of the Betti bases were less active in HBL-100 cells, whilst seven followed the opposite trend.

In a previous study with tryptophan dipeptides we observed that the compounds were inactive against HBL-100 cells and active towards T-47D [18]. This result was potentially consistent with the fact that T-47D cells overexpress SLC6A14. This protein is a cell membrane transporter that recognizes tryptophan as a substrate with high affinity [19]. In this respect, it was previously shown that the blockade of SLC6A14 induces cell death in SLC6A14-positive tumors and represents the first nutrient transporter to be exploited as a drug target for possible cancer therapeutics [20]. This transporter is markedly up-regulated in some types of carcinomas including breast [21], cervix [22], colon [23]

Table 2. Anti-proliferative activity (GI_{50}) of selected Betti bases against breast cancer cells[a]

		Cell line (subtype)		
Compound	HBL-100 (TNBC)	MDA-MB-453 (HER2+)	MCF-7 (ER+)	T-47D (ER+)
14c	> 100	94 ± 11	63 ± 11	13 ± 3.1
14d	> 100	42 ± 7.0	53 ± 12	55 ± 9.1
14n	82 ± 25	96 ± 9.9	> 100	13 ± 3.3
14o	76 ± 27	59 ± 9.1	56 ± 10	8.5 ± 0.7
α-MT	> 100	> 100	> 100	> 100

[a] GI_{50} values are given in μM. Standard deviation was calculated from two to five independent experiments. α-Methyl-dl-tryptophan (α-MT) was used as a reference drug.

and pancreas [24]. In particular, the blockade of SLC6A14 mainly affects estrogen receptor positive (ER+) breast cancer cells. Therefore, we decided to further explore the activity of a subset of our Betti bases in other breast cancer cell lines.

In HBL-100 cells, the expression of SLC6A14 is undetectable and tryptophan analogues do not affect cell growth [25]. Based on this premise, we selected the pyrrolidine derivatives **14c-d** as representative examples of Betti bases that displayed activity in T-47D cells but inactivity in HBL-100 cells. In addition, we also studied the corresponding piperidine siblings **14n-o** for comparison purposes. As a model to run the study, we selected cell lines that represent the three main types of breast cancer according to the status of estrogen receptor (ER), progesterone receptor (PR) and the human epidermal growth factor receptor 2 (HER2); cells with none of these receptors are knowns as triple negative breast cancer (TNBC) [26]. The results are shown in Table 2. With the exception of **14d**, all compounds were less active against the HER2+ cell line and MCF-7 cells (ER+), when compared to T-47D cells. We speculate that factors other than blockade of SLC6A14 could explain these observed effects. For instance, T-47D cells reportedly harbor a mutant p53, whilst MCF-7 and MDA-MB-453 cells possess wild type p53 [27].

Furthermore, we checked the selectivity towards healthy human cells using as a model the human fibroblast cell line BJ-hTERT. The results indicate that the Betti bases **14c-d** and **14n-o** exhibited good selectivity, since they did not affect cell growth of BJ-hTERT human fibroblast cells ($GI_{50} > 100\ \mu M$).

3.3 Docking studies

Next, we decided to run computational assays to predict the possible mode of interaction of the Betti bases with the SLC6A14 transporter and compared these results to α-MT. We did a bibliographic search of the SLC6A14 gene, the biological transporter of tryptophan. The 3D structure of this transporter is not available in Protein Data Bank, the single worldwide archive of structural data of biological macromolecules. Therefore, we used a homology modelling procedure. We used the amino acid sequence of SLC6A14 to search for an indexed sequence as similar as possible to ours. Thus, we selected the Drosophila's dopamine transporter in complex with cocaine (PDB ID: 4XP4, resolution of 2.8 Å) as a template. This latter transporter also belongs to the solute carrier superfamily and shows 46% amino acid identity and 62% similarity to SLC6A14. Most recently, structural modeling of the human SLC6A14, docking and molecular dynamics studies were undertaken; this study unraveled novel aspects of the human SLC6A14 structure-function relationship, thereby having important implications for cancer treatment through the future design of novel inhibitors of SLC6A14-mediated transport [28].

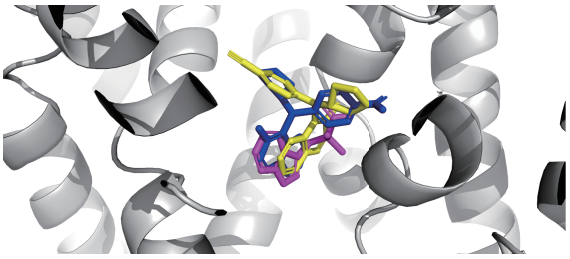

Figure 3. Dock superimposed pose of α-MT (pink) with 14c (blue) and 14d (yellow).

Herein, both α-MT and Betti bases **14** are racemic mixtures. The tragic example of thalidomide marked a turning point in drug development and revealed that the physicochemical and biochemical properties of racemic mixtures and individual stereoisomers can differ significantly. Therefore, we analyzed independently the binding of both enantiomeric series. The docking scores are given in Table 3. In all cases under study, the calculated docking interaction energy is comparable for all compounds and for both enantiomeric series. This apparent lack of selectivity could be attributed to the fact that SLC6A14 not only takes up the 18 of the 20 proteinogenic amino acids, but also d-amino acids such as d-serine, d-alanine, d-methionine, d-leucine and d-tryptophan [29].

Remarkably, the Betti bases **14c-d** displayed comparable docked conformations when superimposed with α-MT, as shown in Fig. 3. These results might support the speculation that Betti bases could behave as tryptophan mimetics, and thus block the transporter SLC6A14 and disrupt cellular uptake of amino acids. From the anti-proliferative data, one cannot rule out the possibility that the compounds inhibit cell growth by mechanisms other than interaction with SLC6A14. However, it is reasonable considering that the Betti bases shown here might serve as a starting point to develop inhibitors of SLC6A14. In support of this notion, the group of Vadivel Ganapathy has recently shown that deletion of the SLC6A14 gene suppresses tumor growth in a mouse model of breast cancer [30].

Table 3. Docking scores of α-MT and selected Betti bases

	Docking interaction energy (kcal mol^{-1})	
Compound	R enantiomer	S enantiomer
14c	-7.1	-6.3
14d	-6.3	-6.3
14n	-5.9	-6.3
14o	-6.3	-6.2
α-MT	-6.8	-6.8

4. Conclusion

In conclusion, we have reported on the anti-proliferative activity of a small and focused library of Betti bases, which were prepared under green conditions. The results prompted us to consider the Betti bases as privileged scaffolds for the design of new chemical entities to be possibly used in the treatment of human diseases such as cancer. Although the exact cellular target remains unknown, we speculate that these compounds might behave as tryptophan mimetics, blocking the solute carrier transporter SLC6A14 and inhibiting cell growth by deprivation of amino acids. In addition, the compounds show excellent selectivity toward healthy cells. Further work is necessary in order to confirm the proposed mechanism of action. However, no assays are available at present to test the inhibition of the transporter SLC6A14 by small molecules. Our current study paves the way for the development of SLC6A14 inhibitors with anti-proliferative activity at the low micromolar range.

Authors' contributions

All authors participated in the design, interpretation of the studies and analysis of data, and review of the manuscript. GB and JMP obtained funding for the study. AP, AG, RA, and KD performed the experiments and the statistical analysis. MXF, GB, and JMP conceived the study and drafted the first manuscript. JMP coordinated the study. All authors participated in the writing process of the manuscript, read, and approved the final manuscript.

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Conflict of interest

The authors confirm that this article content has no conflict of interest.

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