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Synthesis, Anticancer Activity and Computational SAR Analysis of Acylsulfonylpiperazines Derivatives

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Abstract

A series of 1-acyl-4-sulfonylpiperazine derivatives has been prepared. The antiproliferative effect of these compounds was evaluated in vitro against human prostate cancer cell line C4-2, several among them exhibited interesting growth inhibitory against this particular cell line. Finally, a molecular modeling study was employed to analyze the structure/activity relationships (SAR) of these novel compounds.

Keywords: 1-acyl-4-sulfonylpiperazines; Prostate cancer; Antiproliferative activity; Molecular modelling; SAR

Introduction

Prostate cancer (PC) is the second most commonly diagnosed cancer in men in western world and is the leading cause of cancer death in elderly [1,2]. It is considered a major research and public health priority [3]. Proliferation of cancer cells is at the origin dependent on the action of the hormone responsive androgen receptor, which regulates the expression of genes implicated in the control of metabolism and proliferation of cancer cells. Hormone ablation remains the standard therapy in progressive disease, but unfortunately almost all prostate cancer patients develop hormone refractory prostate cancer (HRPC) and bone metastatic disease. Progression of prostate cancer to androgen independence is the primary barricade in improving patient survival due to complex mechanisms underlying the evolution to androgen independence, and at present, there is no curative treatment for HRPC and bone metastatic disease [4-9]. Development of new drugs with high action against the prostate cancer and with low adverse effects is therefore the most urgent demand to treat cancer [10,11].

In our Laboratory, we have shown that 6-[4-(2-Bromo-5-methoxy-benzoyl)-piperazin-1-yl]-N-phenethyl propionamide (Compound A, Figure 1) induces growth arrest of prostate cancer cell lines in vitro and inhibits LnCap and C4-2 tumour growth in vivo [12].

In continuation of our efforts in search of original molecules with medicinal applications, we decided to design new derivatives of compound A (Figure 1). We have synthesized a novel series of 1-acyl-4-sulfonylpiperazine derivatives in order to obtain new anticancer agents which will be active against C4-2 prostate cancer cells, which are a model of HRPC [13]. Piperazines and substituted piperazines are important pharmacophores that are found in many drugs [14]. Piperazine scaffold acts on different pharmacological targets and is present in a broad range of biological active compounds, including several molecules against cancer [15-21], dual calcium antagonists [22], compounds with effects on dopaminergic neurotransmission [23] and HIV protease inhibitors [24,25].

Preliminary bioassays indicated that compounds 4i, 4k, 4m, 4p displayed an interesting antiproliferative activity on prostate cancer C4-2 cells. Finally, the structure activity relationship (SAR) was studied.

Experimental Methods

Chemistry

Reagents were obtained from Sigma-Aldrich or Acros. Solvents were distilled from the appropriate drying agents immediately prior to use. 1H, 19F , and 13C NMR spectra were recorded at 300.13 MHz, 282.37 MHz and 75.46 MHz respectively with a Brucker Avance 300 spectrometer; the chemical shifts are given in ppm relative to Me4Si for the 1H and 13C, and CCl3F for 19F , as internal standards. Coupling constants were given in Hz. High Resolution Mass Spectrometry (HRMS) were recorded on a Jeol SX 102 spectrometer.

Melting points were recorded at atmospheric pressure unless otherwise stated on a Stuart scientific SMP3 apparatus and were uncorrected. The products were purified by column chromatography. Thin layer chromatography was performed with Merck Silica gel aluminium-backed plate with UV visualization. The following synthetic conditions have not been optimized.

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A series of 1-acyl-4-sulfonylpiperazine derivatives has been prepared. The antiproliferative effect of these compounds was evaluated in vitro against human prostate cancer cell line C4-2, several among them exhibited interesting growth inhibitory against this particular cell line. Finally, a molecular modeling study was employed to analyze the structure/activity relationships (SAR) of these novel compounds.

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In continuation of our efforts in search of original molecules with medicinal applications, we decided to design new derivatives of compound A (Figure 1). We have synthesized a novel series of 1-acyl-4-sulfonylpiperazine derivatives in order to obtain new anticancer agents which will be active against C4-2 prostate cancer cells, which are a model of HRPC [13]. Piperazines and substituted piperazines are important pharmacophores that are found in many drugs [14]. Piperazine scaffold acts on different pharmacological targets and is present in a broad range of biological active compounds, including several molecules against cancer [15-21], dual calcium antagonists [22], compounds with effects on dopaminergic neurotransmission [23] and HIV protease inhibitors [24,25].

Preliminary bioassays indicated that compounds 4i, 4k, 4m, 4p displayed an interesting antiproliferative activity on prostate cancer C4-2 cells. Finally, the structure activity relationship (SAR) was studied.
Synthesis of tert-butyl-1-piperazinecarboxylate (1): In a 1000 mL flask were added 370 mL of CH₂Cl₂ and 12.63 g (147 mmol) of piperazine. To the resulting solution, maintained at 0°C using an ice bath, was added dropwise a di-tert-butyl dicarbonate solution (12.9 g, 47 mmol) and DIPEA (13.6 mL, 78 mmol) in CH₂Cl₂ at 0°C, was stirred for additionnal 1 h, then poured in cold water (50 mL). The solvent was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by column chromatography (Petroleum ether/Ethyl acetate 50:50) to give compound (0.52 g). \( R_f = 0.49 \) (Petroleum ether/Ethyl acetate 50:50).

\(^1\)H NMR (300.13 MHz, CDCl₃): \( \delta 1.35 \) (s, 9H, Boc), 3.10-3.30 (m, 3H, CH₂), 3.45 (m, 3H, CH₂), 3.60 (m, 1H, CH), 3.70 (s, 3H, OCH₃), 3.75 (m, 1H), 5.05-5.35 (m, 2H). 

Synthesis of tert-butyl-4-(2-bromo-5-methoxybenzoyl)piperazine-1-carboxylate (2): 2-bromo-5-methoxybenzoic acid (12 g, 52 mmol) and thionyl chloride were refluxed for 3 h. The excess of thionyl chloride was removed by distillation in vacuo to give dark oil (12.9 g, 99%). To a solution of tert-butyl-1-piperazinecarboxylate (8.7 g, 47 mmol) and DIPEA (13.6 mL, 78 mmol) in CH₂Cl₂ at 0°C, was added a di-tert-butyl dicarbonate solution (12.96 g, 52 mmol). The reaction mixture was stirred at room temperature for 2 h. The progress of reaction was monitored by TLC. Upon completion, the crude product was taken in water and extracted with CH₂Cl₂. The organ layer was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The crude material was purified by column chromatography (Petroleum ether/Ethyl acetate 70:30) to give compound 2 (16 g, 85%). \( R_f = 0.25 \) (Petroleum ether/Ethyl acetate 70:30).

\(^1\)H NMR (300.13 MHz, CDCl₃): \( \delta 1.35 \) (s, 9H, Boc), 3.10-3.30 (m, 3H, CH₂), 3.45 (m, 3H, CH₂), 3.60 (m, 1H, CH), 3.70 (s, 3H, OCH₃), 3.75 (m, 1H), 5.05-5.35 (m, 2H). 

Synthesis of 1N-[(2-bromo-5-methoxybenzoyl)phenylsulfonyl]piperazine (4a-4x): 

- **Synthesis of 1N-[(2-bromo-5-methoxybenzoyl)phenylsulfonyl]piperazine (4a):** In a 1000 mL flask were added 370 mL of CH₂Cl₂ and 12.63 g (147 mmol) of piperazine. To the resulting solution, maintained at 0°C using an ice bath, was added dropwise a di-tert-butyl dicarbonate solution (16 g, 73.5 mmol in 150 mL of CH₂Cl₂). The mixture was stirred for additionnal hour, filtered and the filtrate concentrated to dryness. Water (220 mL), was added to the resulting oil and the mixture filtered. The filtrate was saturated with potassium carbonate and extracted with diethyl ether (3 x 100 mL). The solvent was dried over Na₂SO₄ and concentrated to dryness yielding to 9 g of 1 (oil, 66%). 

- **Synthesis of 1N-[(2-bromo-5-methoxybenzoyl)sulfonyl]piperazine (4i):** In a 1000 mL flask were added 370 mL of CH₂Cl₂ and 12.63 g (147 mmol) of piperazine. To the resulting solution, maintained at 0°C using an ice bath, was added dropwise a di-tert-butyl dicarbonate solution (16 g, 73.5 mmol in 150 mL of CH₂Cl₂). The mixture was stirred for additionnal hour, filtered and the filtrate concentrated to dryness. Water (220 mL), was added to the resulting oil and the mixture filtered. The filtrate was saturated with potassium carbonate and extracted with diethyl ether (3 x 100 mL). The solvent was dried over Na₂SO₄ and concentrated to dryness yielding to 9 g of 1 (oil, 66%).
BrDu staining: C4-2 cells were grown on cover slips and were incubated with the indicated compounds at 25 µM in DMSO. Following 44h of treatment, the cells were then incubated for 4 more hours with 5-bromo-2-deoxyuridine (BrDu) at 100 µM final (Sigma Aldrich, Saint Quentin Fallavier, Saint). Cells were then fixed and permeabilized with cold methanol for 10 min at -20°C. After 3 washes with PBS, DNA was denaturated with 4N HCl for 10 min at RT, and cells were incubated with blocking buffer (PBS; 1% BSA). BrDu was then detected with anti-BrDU monoclonal antibody 1:50 (Dako, Carpinteria, CA) for 1 hour at 37°C. After 3 washes with PBS, cells were incubated with an FITC-conjugated anti-mouse secondary antibody 1:50 (Jackson ImmunoResearch) for 30 min at 37°C, and slides were mounted in mounted with a 4% paraformaldehyde solution. The percentages of proliferating BrdU-positive cells were counted. Experiment were done in duplicate and are expressed as the mean ± SEM percentage decrease of proliferating BrdU-positive cells as compared to control cells treated with DMSO only.

Statistical analysis: Statistical analysis were performed with unpaired Student’s t-test. Differences were considered statistically significant at p<0.05 (*p<0.05; **p<0.01 and ***p<0.001).

Molecular modeling

Molecular modeling calculations were performed on E4 Server Twin 2 x Dual Xeon-5520, equipped with two nodes. Each node: 2 x Intel® Xeon® QuadCore E5520-2.66Ghz, 36 GB RAM. The molecular modeling graphics were carried out on a personal computer equipped with Intel(R) Core(TM) i7-4790 processor and SGI Octane 2 workstations.

The apparent pKa and logD values (pH 7.2) of the newly designed compounds 4a-x were calculated by using the ACD/Percepta software (ACD/Percepta, Advanced Chemistry Development, Inc., Toronto, ON, Canada, 2015, http://www.acdlabs.com/). All compounds were considered neutral in all calculations performed as a consequence of the estimation of percentage of neutral/charged forms computed at pH 7.2 (cytoplasm pH value), using the Henderson−Hasselbalch equation.

Compounds 4a-x were built and, then, subjected to molecular mechanic (MM) energy minimization (ε=80*r) until the maximum RMS derivative was less than 0.001 kcal/A, using Conjugate Gradient [26] as minimization algorithm (Discovery Studio 2017; Dassault System BIOVIA, San Diego, 2016). Atomic potentials and charges were assigned using the CFF forcefield [27]. The conformers obtained for each compound were used as starting structure for the subsequent systematic conformational analysis (Search Small Molecule Conformations; Discovery Studio 2017). The conformational space of the compounds was sampled considering possible piperazine ring conformations, as well as, the two orientation of the amide bond, and by systematically varying all rotatable bonds (r1, r2, r3 and r4) with an increment of 60°C. In the case of compounds 4c, 4n, 4o, 4p, 4r, and 4t the rotatable bonds of the R1 substituent were also systematically varied. The RMSD cutoff for structure selection was set to 0.01 Å. Finally, to ensure a wide variance of the input structures to be successively fully minimized, an energy threshold value of 10^6 kcal/mol was used as selection criteria. The generated structures were then subjected to MM energy minimization (CFF forcefield; ε=80*r) until the maximum RMS derivative was less than 0.001 kcal/A, using Conjugate Gradient as minimization algorithm. Finally, the resulting conformers were ranked by their potential energy values (i.e., ΔE from the global energy minimum) and grouped into conformational families on the basis of dihedral angle values.

The global minimum conformer of each compound and the minimum conformers of the 1n, IIIx, and IVx families of 4a, of the I, and IV families of 4b, of the II, 2, 3, and 4 families of 4c, of IVx family of 4h, of the I, 1a, 1b, 1c, and 1d families of 4k, of IVx family of 4l, of the II, and III families of 4o, of IVx family of 4p, of IVx family of 4s and of IVx family of 4t have been then subjected to DFT calculations. The calculations were carried out using the Gaussian 09 package [28]. All structures were fully optimized in gas-phase at the B3LYP/6-31+G(d,p) level [29,30]. In order to characterize every structure as minimum, a vibrational analysis was carried out at the same level of theory, using the keyword freq. The RMS force criterion was set to 3 × 10^-4 a.u. Molecular charge distribution has been calculated using the natural bond orbital (NBO) method [31]. The atomic charges, derived from the NBO population analysis were used to calculate the dipole moment of the substituent R1.

Results and Discussion

Chemistry

The synthesis of 1-acyl-4-sulfonylpiperazine derivatives 4a-x is shown in Scheme 1. Tert-butyl-1-piperazinecarboxylate (1) was prepared from piperazine and from di-tert-butyldicarbonate. Acylation with 2-bromo-5-methoxybenzoyl chloride of (1) in the presence of DIPEA led to tert-butyl-1-(2-bromo-5-methoxybenzoyl)piperazine-1-carboxylate (2). The tert-butylxycarbonyl (Boc) deprotection with trifluoroacetic acid resulted in 1-N-(2-bromo-5-methoxybenzoyl)piperazine (3). The nucleophilic substitution reactions of 1-N-(2-bromo-5-methoxybenzoyl)piperazine (3) with different aryl and heteroaryl sulfonyl chlorides (RSO2Cl), in the presence of disopropylethylamine (DIPEA) and dichloromethane (CH2Cl2) as solvent at 0°C to room temperature gave the 1-acyl-4-sulfonylpiperazine derivatives (4a-x) in a moderate to good yields ranging from 60 to 81% (Scheme 1).

The presence of NH proton at 2.1 δ value in starting material piperazine (3) and the absence of this proton peak in 1H NMR spectra confirm our products (4a-x). The 1-acyl-4-sulfonylpiperazine derivatives (4a-x) were purified by column chromatography using cyclohexane: ethyl acetate (6:4; or 5:5; 3:7) or petroleum ether : ethyl acetate (7:3; 5:5) as eluent or by recrystallization in acetone. All the newly prepared compounds (4a-x) were given with corrected analytical data. The 1H, 13C NMR spectra data, HRMS data were consistent with the assigned structure. The chemical structure of each compound is reported in Table 1.

Antiproliferative activity

It was previously reported that piperazine derivatives show...
Computational studies and SAR analysis

The conformational space of compounds 4a-x was sampled by means of a systematic conformational search including all rotatable bonds, coupled with molecular mechanic (MM) optimization of the resulting conformations. MM calculations were performed by using the CFF force field [27] for atom parametrization and a distance dependent dielectric constant with a value of 80 to mimic an aqueous environment (Discovery Studio 2017, BIOVIA, San Diego USA; see the Experimental Section for details). The results obtained allowed us to identify all energy minimum structures of compounds 4a-x and, in particular, their global minimum (GM) conformers.

All conformers within 5 kcal/mol from the GM were, then, selected and classified in to families according to the values of their torsion angles (Tables S1-S24). In all the resulting families, the piperazine ring assumes a chair-like conformation. The rotation about the amide bond is accompanied by piperazine chair inversion and generates two parallel sets of conformers, which resulted to be the mirror image of each other and show the same conformational energy, thus behaving as conformational enantiomers (Figure 2).

On the other hand, the rotation about the N-S bond (τ1) gave rise to four families of conformers, herein named I-IV (Figure 3).

The resulting family of conformers can be further classified in to sub-families on the basis of the rotation of the aromatic ring R and the varied substituent R1. In particular, regarding the orientation of R1, when it is an ortho- and meta-substituted phenyl ring, as well as, a benzyl or a heterocycle (i.e., 4b, 4c-g, 4k, 4l, 4u-x; Table 2) two main sets of equilibrium positions were identified within each family, characterized

| Cmp | R          | R1          | Yield(%) |
|-----|------------|-------------|----------|
| 4a  | MeO        | MeO         | 64       |
| 4b  | MeO        |             | 66       |
| 4c  | MeO        |             | 72       |
| 4d  | MeO        |             | 68       |
| 4e  | MeO        |             | 68       |
| 4f  | MeO        |             | 73       |
| 4g  | MeO        |             | 75       |
| 4h  | MeO        |             | 69       |
**Table 1:** Chemical structure, physical data of synthesized compounds (4a-4x).

| Cmp | R           | R<sub>i</sub>          | Yield(%) |
|-----|-------------|------------------------|----------|
| 4i  | -Br         | -Br                   | 65       |
| 4j  | -Cl         | -Cl                   | 63       |
| 4k  |             | -F<sub>3</sub>C       | 60       |
| 4l  | MeO         | MeO                   | 81       |
| 4m  | MeO         | MeO                   | 71       |
| 4n  | MeO         | MeO                   | 69       |
| 4o  | MeO         | MeO                   | 77       |
| 4p  | MeO         | MeO                   | 68       |
| 4q  | MeO         | MeO                   | 67       |
| 4r  |             |                       | 68       |
| 4s  | -NO<sub>2</sub> | -NO<sub>2</sub>     | 77       |
| 4t  |             |                       | 66       |
| 4u  |             |                       | 72       |
| 4v  |             |                       | 67       |
| 4w  |             |                       | 64       |
| 4x  |             |                       | 72       |

*Yields obtained after purification of all compounds by column chromatography or by recrystallization.*

By negative or positive values of the corresponding torsion angle (named t2). The same is valid also for rotation of the di-substituted phenyl ring R (torsion angle named t3). Accordingly, families I-IV were further classified in to sub-families A-D according to the values of t2 and t3 torsion angles (A=+/+, B=+-/-, C=-+/+, D=-/-). It has to be underlined that in the cases in which R<sub>i</sub> is i) an unsubstituted phenyl ring (4a), ii) a *para*-substituted phenyl ring (4c, 4d, 4j, and 4m-1), or iii) a 2,5-disubstituted phenyl ring (4h), due to symmetry reasons, negative or positive values of t2 leads to the same conformation of R<sub>i</sub>, thus, only positive values are reported in the corresponding tables. By consequence, for these compounds, just t3 (positive or negative) values are considered in the classification in to sub-families (i.e., A=+/+, B=+-).

In Table 3 are reported the results obtained for the most active compound of the series, 4k, while the complete set of results for compounds 4a-x is reported in Tables S1-S24.
| Cmp | R<sub>i</sub> | GI<sub>i</sub> (%) at 25 µM |
|-----|-------------|------------------------|
|     |             | Mean | Sem | t-test |
| 4a  | -           | 30.33* | 9.53 | 0.0422 |
| 4b  | -           | 46.27** | 11.01 | 0.0024 |
| 4c  | -           | 60.95** | 0.83 | 0.0005 |
| 4d  | -           | 37.17" | 16.30 | 0.0032 |
| 4e  | -           | 55.59** | 2.95 | 0.0000 |
| 4f  | -           | 35.73** | 5.64 | 0.0006 |
| 4g  | -           | 55.24* | 6.39 | 0.0390 |
| 4h  | -           | 11.11 | 23.06 | 0.3537 |
| 4i  | -           | 67.93** | 4.72 | 0.0027 |
| 4j  | -           | 38.61** | 12.35 | 0.0060 |
| 4k  | -           | 85.29* | 3.68 | 0.0158 |
| 4l  | -           | 32.37 | 4.96 | 0.0893 |
| 4m  | -           | 65.79** | 6.98 | 0.0002 |
| 4n  | -           | 58.01** | 6.35 | 0.0001 |
| 4o  | -           | 60.31 | 6.20 | 0.1675 |
| 4p  | -           | 76.77** | 3.31 | 0.0025 |
| 4q  | -           | 2.81 | 2.81 | 0.4090 |
| 4r  | -           | 43.79** | 4.02 | 0.0004 |
| 4s  | -           | 3.82 | 2.74 | 0.2720 |
| 4t  | -           | 22.43* | 7.24 | 0.0156 |
| 4u  | -           | 57.29* | 3.46 | 0.0135 |
All compounds, with the exception of 4k and 4b, present a conformational preference for the family of conformers named I (τ₁ about 70°), closely followed by family IV (τ₁ about -80°) and, then, at about 2 kcal/mol from the GM, by families II and III (τ₁ about -165° and -68°, respectively). Importantly, if we consider the position of the sulphur bound oxygen atoms with respect to the piperazine nitrogen substituents, family I and IV present the anti and the fully eclipsed conformations, respectively (Figure 3B). Thus, in agreement with previously reported results on benzosulfonamide derivatives [35-37] the two most favoured families of conformers are I and IV, with family I (i.e., anti conformation of the oxygen atoms with respect to the piperazine nitrogen substituents) representing the GM family, as found in the crystal structure of N,N-dimethyltoluene-p-sulfonamide [38].

As mentioned above, two exceptions to the common conformational behaviour showed by compounds 4a-x are represented by compounds 4k and 4b. As expected, a strong influence on the conformational behaviour of 4b is due to the presence of a benzyl ring as R₁, which in all the other derivatives is a substituted phenyl ring (Table 2). Thus, on one hand, due to the steric hindrance of the R₁ substituent, only conformers belonging to family I and IV resulted energetically allowed (Figure 3B), with this latter being the GM family. On the other hand, due to the introduction of an sp³ carbon atom linking the aromatic R₁ moiety to the sulphonamide function, the conformational preference favoured orientation of R (τ₃) depends on the nature of R₁ thus it varies from a compound to another, although just contributing for about 0.4 kcal/mol to the overall conformational energy. Finally, the orientation of the meta-methoxy substituent of R resulted to shift between the two co-planar conformations with respect to the phenyl ring, corresponding to values of about 0° or 180° of the related torsion angle (named τ₄). If we exclude some exception in family IVB and IIIB, the lowest energy conformers of 4a-x presented a τ₄ value of about 0°, although the alternative orientation of the methoxy substituent of R (τ₄=180°) is anyway just 0.2 kcal/mol less favoured.

*GI-growth inhibition; C4-2 Prostate cancer cell line; *p<0.05; **p<0.01; ***p<0.001

**Table 2: In vitro Growth Inhibition (GI) of C4-2 prostate cancer cells by the tested compounds.**

| Fam | ΔEₘₘ⁻ (kcal/mol) | Torsion Angles* (°) |
|-----|-----------------|---------------------|
|     |                 | τ₁                | τ₂                | τ₃                | τ₄                |
| III₂ | 0.00-0.80       | -71               | -49               | -65               | -1                |
| II₂  | 0.02-0.39       | -166              | 71                | 118               | 2                 |
| III₃ | 0.05-0.64       | -166              | 71                | -64               | -1                |
| I₂   | 0.18-0.55       | -71               | -49               | 118               | 2                 |
| l₂   | 0.23-0.45       | 68                | 100               | 114               | 1                 |
| l₃   | 0.27-0.49       | 70                | -100              | 115               | 1                 |
| s₂   | 0.40-1.08       | 68                | 100               | -112              | -6                |
| l₉   | 0.50-0.57       | 71                | -100              | -64               | -2                |
| II₉  | 0.84-1.63       | -154              | -155              | -66               | -1                |
| III₉ | 0.90-1.69       | -75               | 155               | -65               | -1                |
| I₉   | 1.01-1.39       | -155              | -155              | 118               | 2                 |
| IV₉  | 1.05-1.24       | -119              | -160              | -65               | -1                |
| III₉ | 1.06-1.44       | -75               | 155               | 118               | 2                 |
| IV₉  | 1.27-1.49       | -120              | -160              | 119               | 2                 |

*The values reported refer to the lowest and the highest energy conformers of the family. **The values reported refer to the lowest energy conformers of the family. *τ₁ torsion angle is calculated considering e, f, g, and h atoms. *τ₂: f, g, h, and i atoms. *τ₃: a, b, c, and d atoms. *τ₄: j, k, l, and m atoms.

**Table 3: Conformational families of 4k considering MM conformers within 5 kcal/mol from the global minimum.**

| Fam | ΔEₘₘ⁻ (kcal/mol) | Torsion Angles* (°) |
|-----|-----------------|---------------------|
|     |                 | τ₁                | τ₂                |
| III₂ | 28.03**         | 1.41              |
| IV₂  | 48.61*          | 10.40             |
| III₄ | 28.38**         | 9.19              |

*The values reported refer to the lowest and the highest energy conformers of the family. **The values reported refer to the lowest energy conformers of the family. *τ₁ torsion angle is calculated considering e, f, g, and h atoms. *τ₂: f, g, h, and i atoms.
of 4b strongly depends on R rotation, with the lowest energy conformers showing, just in the case of this compound, a τ2 torsion angle value of 180° (Figure 3B and Table S2).

Compound 4k represents the other exception, being also the most active compound of the series, and it is characterized by an ortho-trifluoromethyl-substituted phenyl ring as R1. In the case of 4k, the GM conformer belongs to the IIIc subfamily, closely followed by the conformers of subfamily IIc, IIb, and IIIc, in turn, followed by family I (within 0.5 kcal/mol from the GM), and, at about 1 kcal/mol from the GM, by the conformers of the remaining subfamilies. Thus, the conformational behavior of 4k appears to be significantly different from those of the other compounds, with families II and III becoming energetically preferred over families I and IV, depending on the orientation of the trifluoromethyl substituent. It is noteworthy, that, despite being characterized by different τ2 values, when superimposed by the piperazine ring, the lowest energy conformers of family III and II of 4k present the R1 substituent occupying the same spatial position (Figure 3C and Table 3).

To investigate the peculiar conformational preference of 4b and, especially, 4k with respect to the other derivatives, the lowest energy MM conformer of each family of 4a, 4b, 4c and 4k were subjected to DFT full optimization calculations at the B3LYP/6-31+G(d,p) [29,30] Gaussian 09 package [28]. Moreover, to evaluate the influence of the orientation of the ortho-trifluoromethyl substituent of 4k, we included in DFT calculations the lowest energy conformers of family I-III presenting the opposite orientation of the R1 substituent (τ2 torsion angle); while, to check the energy difference between the two possible R1 orientation (τ3 torsion angle), the lowest energy conformer of the Ic180 conformer family: IA (green), IC (violet), IIA (brown), and IIID (gray; GM conformer). The conformational preference of 4k was confirmed compared to MM results, with the GM belonging to family IIIc (negative τ2 value), followed, within 0.5 kcal/mol from the GM, by subfamily IIc, Ic, and Ic (Figure 4), while the alternative orientation of the trifluoromethyl substituent of 4k (subfamily IIA, positive τ2 value) results in to a conformer with a ΔG_{GM} > 2 kcal/mol. Similar results were obtained for the lowest energy conformer of family Ic (positive τ2 value) with respect to that of family IIc (negative τ2 value), thus, increasing the influence of the orientation of the ortho-substituent on the conformational preference of 4k with respect to MM results.

Remarkably, the DFT full optimization of the MM lowest energy conformer of family IV, belonging, in this case, to IVc subfamily, led to the lowest energy conformer of another family (IIId) (Table 4). Similarly, DFT calculations on 4a and 4e confirm the conformational trend obtained by MM when we consider families I-III (as also resulted extending DFT calculations to 4o), but gave very different results for family IV (Table 4). In particular, the lowest energy conformer of family IV of 4a (subfamily IVc, which closely follows the GM conformer according to MM results; Table S1, SI) became the less favoured of the considered conformers, while the corresponding conformer of 4e (subfamily IVc, Table S5, SI) moves, after DFT optimization, to the conformer belonging to family IIIc (Table 4). To further investigate this issue, DFT calculations were also performed on the MM lowest energy conformers of family I and IV of compounds 4c, 4h, 4l, 4p, 4s, and 4w. Results confirm the family I conformer as the GM, as obtained by MM, but with the family IV conformer showing a ΔG_{GM} of more than 2 kcal/mol, as occurred for 4a (Table 4). Finally, the results obtained for the Ic180 and Ic180 conformers of 4b and the Ic and Ic conformers of 4w, showed an energy difference of less than 0.2 kcal/mol between the two possible orientations of the di-substituted phenyl ring of R (positive/ negative t3 value; Table 4), in agreement with the conformational search results (Table S1-S24, SI).

In all cases, the GM conformers identified by MM were all confirmed by DFT calculations with the only exception of 4b, whose GM still belongs to family IV but presenting a τ2 angle value of 60° (family IVc instead of IVc180). This is particularly important for sulphonamide derivatives which present electron withdrawing substituent on the sulphur atom, since it has been previously demonstrated that, in this case, the different conformers can be considered as rather rigid molecules due to a high barrier of internal rotation around the S-N bond [35], and that this effect is enhanced in the presence of an ortho-substituted phenyl ring with respect to unsubstituted or para-substituted ones [37].

At this stage, in order to properly evaluate the effect of electronic parameters on biological activity, we extended the DFT full optimization...
Table 4: ΔE<sub>GM</sub> values (kcal/mol) and torsion angle values (degrees) of 4a-c, 4e, 4h, 4k, 4l, 4o, 4p, 4s, and 4w conformers obtained after DFT optimization.

As can be evicted from Figure 5, particularly evident is the effect of polarization in affecting the activity of compounds 4h vs 4e and 4g (Table 2); regarding 4k, which also present a similar dipole compared to 4e and 4g, conformational parameters likely play an additional role on its higher biological activity. On the contrary, the meta-substituted analogue of 4k, compound 4l, despite presenting a similar R<sub>1</sub> dipole (data not shown), is endowed by poor biological activity (Table 2). Thus, a proper R<sub>1</sub> polarization is necessary, likely to allow the right orientation in the target binding site, however it is not sufficient to improve biological activity. This suggests the presence of a spatially defined(specific) interaction with the biological target involving the electron-withdrawing ortho-substituent of R<sub>1</sub>. The analysis of the results obtained for compounds 4u-x (Table 2), characterized by the presence of a 2- or 3-thiophenyl substituent (4u and 4v, respectively) and a 2- or 3-furanyl substituent (4w and 4x, respectively), supports this hypothesis (data not shown).
On the other hand, when shifting to the polarization of the para-substituted R1, analogues other interesting considerations can be done (Figure 6).

Also in this case the presence of electron withdrawing substituents, such as in 4m, 4n, and 4r leads to an improvement of the biological activity compared to 4a, while the opposite is true for substituents such as a methoxy (4t) or a methyl (4q, Table 2). However, by increasing the polarization of R1, the biological activity progressively decrease (4r<4m<4n), as it also occurs when the R1 dipole is oriented in the opposite direction as in 4s. In this view, the activity improvement of 4i (para-Br) with respect to 4d (para-F) and 4j (para-Cl) seems not to be connected to R1 polarization but rather to the increased dimension of the bromine atom. A similar SAR can be observed by comparing 4d to 4m (Table 2). In general, the size of the para-substituent seems to be crucial for biological activity (4q vs 4o; Table 2) with bulky hydrophobic substituents leading to increased potency (4p>4c>4o>4r).

In Figure 7 it is reported the superimposition of the DFT GM conformers of the compounds presenting favourable R1 substituents with respect to the unsubstituted phenyl ring of 4a (Figure 7A), the compounds whose R1 do not improve the biological activity with respect to 4a (Figure 7B), and the compounds presenting unfavourable R1 substitution compared to 4a (Figure 7C). Interestingly, in addition to the above discussed favourable steric effects at the para-position of the phenyl ring of R1, another sterically favourable region occupied by both, the DFT GM conformers of 4k and 4b, can be observed.

Conclusion

In summary, we report herein the synthesis of a series of 4a-x derivatives by a multistep reaction starting from piperezine, 2-bromo-5-methoxybenzoic acid and different aryl or heteroarylsulfonyl chlorides.

These compounds were obtained in good yields and were characterized by 19F, 1H, 13C NMR spectroscopies, HRMS and by HPLC. Moreover, their antiproliferative activity against C4-2 prostate cancer cells were evaluated by measuring the percentage of BrdU-positive proliferating cells 48 hours after incubation. Among the series, four compounds (4i, 4k, 4m, 4p) exhibited significant growth inhibitory activities against C4-2 prostate cancer cells.

The 3D-SAR analysis evidenced that the size and the polarizability of the ortho- and para-substituent of R1 seem to be crucial for biological activity suggesting the presence of a spatially defined interaction with the putative biological target involving the electron-withdrawing ortho-substituent and the bulky hydrophobic para-substituent of R1.

Supplementary Material

Computational studies of all compounds are described in SI. The description of all compounds and 1H-NMR and 13C-NMR for target compounds 4i, k, m, p are described in SI.

Conflict of Interest

The authors declare that they have no conflict of interest. We gratefully thank the french Institute of national cancer (INCa) and the French Fondation pour la Recherche Medicale (FRM) for their financial support.

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