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N-*i*-Propoxy-*N*-biphenylsulfonylaminobutylhydroxamic acids as potent and selective inhibitors of MMP-2 and MT1-MMP

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Abstract—Structural manipulation of the pharmacophoric model of type **A** selective MMP inhibitors (MMPi), obtained by the insertion of some alkyl substituents R_2 possessing an appropriate geometry, steric bulkiness and lipophilicity, is able to improve potency, in the subnanomolar range on MMP-2, and to give a good MMP inhibition on MMP-14 (MT1-MMP) in the designed MMPi of type **C**, while maintaining a good MMP-1/MMP-2 selectivity profile. The simultaneous inhibition of these two enzymes yields type **C** compounds, which are potent antiangiogenic agents, able to block a chemoinvasion model on HUVEC cells in the micromolar range.
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Often during tumour progression, an overexpression of matrix metallo proteinases (MMPs), produced and secreted as inactive zymogens in the extracellular matrix (ECM) by the tumour cells themselves, or by surrounding stromal cells, is responsible for the deregulation of the extracellular matrix (ECM) functions.[1,2] Normally, the homeostasis of MMPs is maintained by tissue inhibitors, TIMPs, but in cancer progression, control over MMPs activity is lost.[3] Other proteases, such as uPa, MMPs and furin-like serine proteases are responsible for the activation of specific MMPs, such as MMP-2, MMP-9, and MMP-14 (a membrane-associated type MMP which is the principal activator of pro-MMP-2). These three specific MMPs play a significant role in these degenerative processes, and are directly involved in metastatic tumour dispersion and angiogenesis.[4–8]

More recently, MMP-2 has been shown to play other important roles in tumours, for example, in resistance to apoptosis, in the activation of EGF receptors and in cellular prolification.[9–12] All these data, taken together, indicate that MMP-2, MMP-9 and even MMP-14 may represent an important target to develop new potential anticancer drugs.[13,14] In the past few years, some potent 'broad spectrum' MMPi have been proposed and tested against tumours, but at present none of them are on the market.[15,16]

In fact, many of these new molecules have shown a severe musculoskeletal syndrome, with fibroproliferative effects in the joint capsule of the knee.[17–19] These effects are thought to be linked to an impairment of normal tissue remodelling governed by MMP-1 and/or by sheddases such as TNF-α-convertase.[20] For these reasons, a lack of activity with respect to MMP-1 is considered to be an important factor in reducing some of the side effects found for 'non-selective' MMPi.[21] As a matter of fact, the recent development of some synthetic MMPi possessing a good potency and selectivity towards the

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two gelatinases, together with the discovery that some of these molecules active on MMP-2 show important pro-apoptotic effects on tumour cell cultures, confirmed the validity of their use as potential anti-tumour agents.[22–29] Nowadays the development of studies on compounds possessing a selective inhibitory activity on the MMPs that are over-expressed in tumours is very useful, with particular reference to viability control and invasiveness of the cancer cell.[15]

In a recent work, we described some *N*-arylsulfonyl-*N*-alkoxyaminoacetohydroxamic acids of type **A**. Some of the new compounds, when tested in vitro for their inhibitory activity towards some MMPs, showed a remarkable activity and an already good MMP2 selectivity, with IC_{50}s in the nanomolar range. Moreover the potent MMP-2 inhibitor **1**, belonging to the class **A**, proved to possess an appreciable anti-invasive efficacy.[30] In an aim to improve the potency of these molecules towards MMP-2 and MMP-9, and also towards MMP14, where type **A** compounds have proved to be devoid of any activity (see compound **1** in Table 1), we planned a chemical manipulation of this new type of model of pharmacophore in the class of MMPi. Our interest in the study of these inhibitors also on the MMP-14, which is significantly expressed at the membrane level in tumour cells, is linked to the particular role of this MMP in cancer cells. MMP-14, together with TIMP-2, the natural tissue inhibitor of MMP-2, closely controls the activation/inactivation balance of pro-MMP-2, and therefore it may be directly involved in the crucial regulation of the matrix functions around the metastatic cells in invasive tumours, thereby greatly facilitating motility, invasion, metastasis and angiogenesis (see Fig. 1).[31–35] On this basis, we have developed some new *N*-*i*-propoxy-*N*-biphenylsulfonylaminobutylhydroxamic acids of type **C**, which are analogues of the previously studied sulfonamide **1** of type **A**, in order to evaluate their biopharmacological properties. In these new type **C** hydroxamates, we have introduced some alkyl substituents possessing an increasing steric bulkiness and lipophilicity in R_2, on the carbon atom alpha to the hydroxamic group of **A**, thinking that these substituents, if appropriately oriented, might favour lipophilic interactions with the nearby S1 region of the enzyme site, thus further reinforcing the binding of the planned new inhibitors to the active site of MMP-2 and MMP-14. By introducing an asymmetric centre into molecules **C**, this kind of chemical manipulation might allow us to evaluate the enantioselective recognition of the studied MMPs towards this class of compounds and, therefore, the influence of chirality on their biopharmacological properties. According to our hypothesis, in consideration of the differences existing in the S1, S1′ and S2′ pockets between MMP-1, MMP-2 and MMP-14, the combination of appropriate molecular geometries, hindrances and lipophilic characteristics of the groups R, R_1 and R_2, introduced into type **C** compounds might be useful to improve their MMPi potency and selectivity.[36] Furthermore, the new compounds **C** may be able to give an antiangiogenic activity, seeing that they act as potent inhibitors versus MMP-2 and MMP-14, the two MMPs over-expressed in tumours characterised by particular aggressiveness, sparing the inhibitory activity on MMP-1 responsible for undesirable serious side effects.

The synthesis of the new type **C** derivatives is outlined in Schemes 1 and 2. Firstly, in the aim to improve our knowledge on the stereochemical aspects of type **C** compounds action, we planned the synthesis of racemic (*R*/*S*)-**5a** and of its enantiomers (*R*)-**5a** and (*S*)-**5a** then, identified the best stereochemistry on the alpha carbon atom to the hydroxamate binding group, some new type **C** derivatives of *R* configuration were synthesised.

Scheme 1 reports the synthesis of racemic (*R*/*S*)-**5a** and optically active (*S*)-**5a** according to the synthetic method used for type **A** hydroxamates.[30] In the same scheme, there is an attempt to obtain (*R*)-**5a**, using the same synthetic method, by nucleophilic S_N2 replacement reaction of optically active secondary bromides with a cesium salt of the *O*-*i*-propyl-sulfonamide **6**. Unfortunately, only

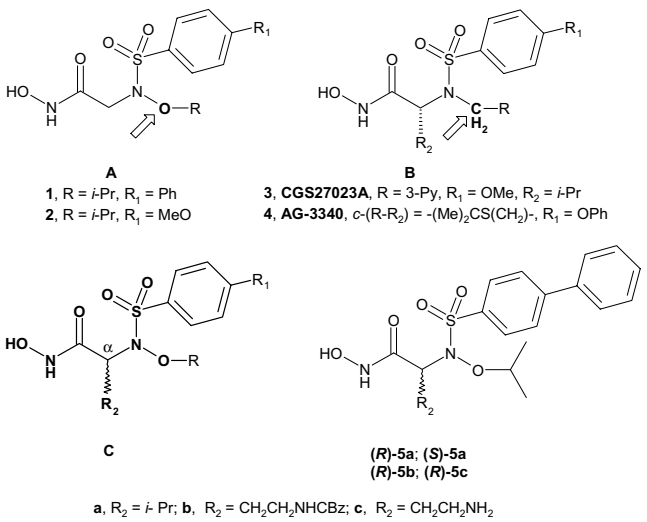
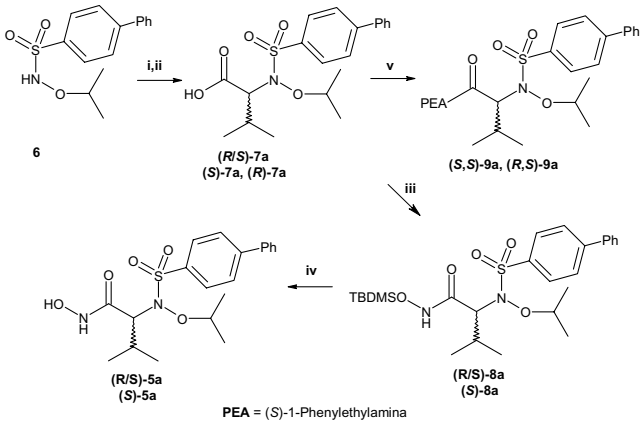
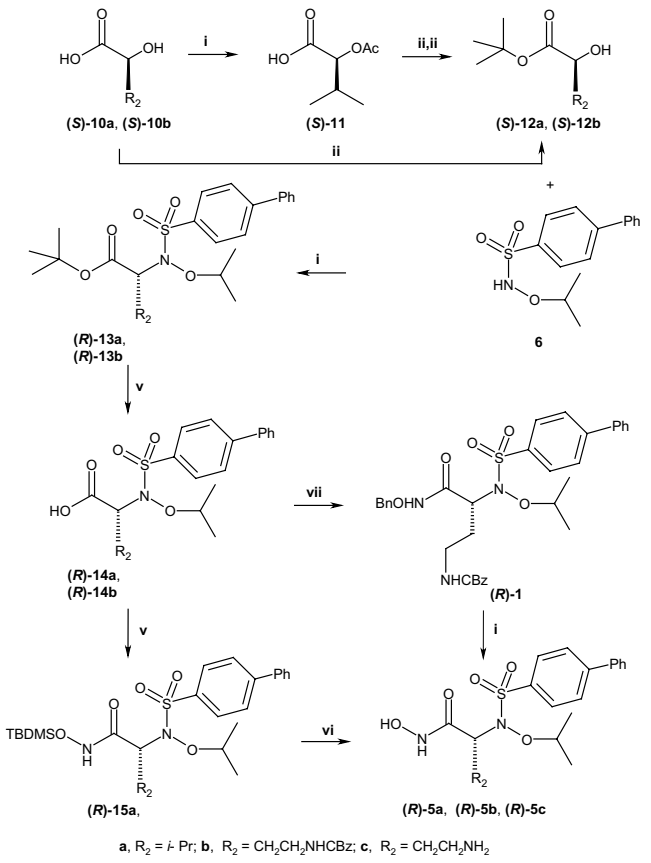


Figure 1.



Scheme 1. Reagents and conditions: (i) Racemic or (*R*)- or (*S*)-$BrCH[CH(CH_3)_2]CO_2$–Bu^t, Cs_2CO_3, Bu_4NHSO_4, anhyd DMF, rt, 3 days; (ii) TFA, anhyd DCM, 0 °C; (iii) $TBDMSONH_2$, EDCI, anhyd DCM, 0–25 °C, 24 h; (iv) TFA, anhyd DCM 0 °C, 5 h; (v) (*S*)-1-PEA, NMM, HOBt, EDCI, anhyd DCM, rt, 12 h.



Scheme 2. Reagents and conditions: (i) AcCl, rt, 18 h; (ii) $(CH_3)_2NCH[O(CH_3)_3]_2$, toluene, 95 °C, 3 h; (iii) LiOH, THF/MeOH/H_2O, rt, 3 h; (iv) TPP, DEAD, anhyd THF, 0 °C, 5 h; (v) TFA, anhyd DCM, 0 °C, 5 h; (vi) $TBDMSiONH_2$, EDCI, anhyd DCM, 0–25 °C, 24 h; (vii) TFA, anhyd DCM 0 °C, 5 h; (viii) $BnONH_2$ HCl, NMM, HOBt, EDCI, anhyd DCM, rt, 24 h; (ix) H_2, Pd/C 10%, MeOH/AcOH.

one of the two desired enantiomeric acids, (*S*)-**7a**, was obtained with an acceptable enantiomeric purity.[37] Only acid (*S*)-**7a** was therefore transformed into its hydroxamate (*S*)-**5a** in accordance with the previously described method. The absolute configuration of (*S*)-**5a** was determined by comparison of its optical characteristics with an authentic sample of its enantiomer (*R*)-**5a** prepared and optically characterised as described below. The enantiopure biphenylsulfonamides of type **C** with the desired *R* configuration, (*R*)-**5a**, (*R*)-**5b**, and (*R*)-**5c**, were synthesised as described in Scheme 2. Optically active alpha-hydroxy-*tert*-butyl-ester, (*S*)-**12a** (R_2 = *i*-Pr) was synthesised by acetylation of the commercial alpha-hydroxy acid (*S*)-**10a** to the stable intermediate (*S*)-**11a** (R_2 = *i*-Pr) and subsequent esterification to (*S*)-**12a** (R_2 = *i*-Pr).[38] On the contrary, the ester (*S*)-**12b** was prepared directly from the alpha-hydroxy acid (*S*)-**10b** ($R_2 = CH_2CH_2NHCBz$) by direct esterification with *N*-(di-*tert*-butoxymethyl)-*N*,*N*-dimethylamine. A Mitsunobu condensation of the *O*-*i*-propyl-sulfonamide **6** with the appropriate alpha-hydroxy-*tert*-butyl-ester, (*S*)-**12a** and (*S*)-**12b**, gave *tert*-butyl esters (*R*)-**13a** and (*R*)-**13b**. Acid cleavage of esters **13** gave acids (*R*)-**14a** and (*R*)-**14b**, which were converted into their *O*-silylates (*R*)-**15a** and (*R*)-**15b**. Acid cleavage of the *tert*-butyl*O*-silylate (*R*)-**15a** and (*R*)-**15b** in the same conditions described above gave the desired hydroxamates (*R*)-**5a** and (*R*)-**5b**. In the case of the compound (*R*)-**5c** ($R_2 = CH_2CH_2NH_2$), the synthetic procedure was modified slightly because it is not possible to obtain (*R*)-**5c** by direct catalytic hydrogenation of its hydroxamate precursor (*R*)-**15b**. In this case, the acid (*R*)-**14b** was converted into its *O*-benzyl-hydroxamate (*R*)-**16**,and then the desired (*R*)-**5c** was obtained by catalytic hydrogenation on 10% Pd/C.[39,40]

Table 1 shows the inhibitory indices (IC_{50}) towards some of the principal MMPs of the new *N*-*i*-propoxy-*N*-biphenylsulfonylaminobutylhydroxamic acids of type **C** (**5a-c**), compared with those of the previously described type **A** MMPi **1** (R = *i*-Pr; R_1 = Ph). In the same table, the IC_{50} values are reported for two related known MMPi of type **B**, CGS27023A (**3**) and AG-3340 (**4**).

For the more interesting compounds and reference drugs, selectivity indices for MMP-2 over the other MMPs studied are also reported, expressed as ratios of their inhibitory indices (Table 1).

On MMP-2, the R_2 substitution on the P1 site with an *i*-Pr group on the carbon atom alpha to the hydroxamate is able to improve the inhibitory potency about 7-fold, passing from **1** (IC_{50} = 12 nM) to racemic (*R*,*S*)-**5a** (IC_{50} = 1.8 nM). The (*R*)-**5a** enantiomer shows an improvement of the potency on MMP-2 of about 20-fold (IC_{50} = 0.09 nM) compared with its racemate (*R*,*S*)-**5a** and about 114 times, compared with its dystomer (*S*)-**5a** (IC_{50} = 10.3 nM). On MMP-9, the same racemate (*R*,*S*)-**5a** (IC_{50} = 19.6 nM) shows an appreciable increase in potency of about 10 times compared with the type **A** compound **1** (IC_{50} = 200 nM). On the same enzyme, the eutomer (*R*)-**5a** shows an IC_{50} = 6.7 nM, about 3 times that of (*R*,*S*)-**5a** and about twice that of its enantiomer (*S*)-**5a** (IC_{50} = 13 nM). (*R*)-**5a** shows good indices of potency also on MMP-3 (IC_{50} = 50 nM), and on MMP-8 where the inhibitory activity of this compound (IC_{50} = 1.6 nM) is particularly significant.

The R_2 substitution on the P1 site with a more hindered group, such as in the benzylethylcarbamate, compound (*R*)-**5b**, reduces the inhibitory activity on MMP-2 about 4.5-fold, even if this remains in the subnanomolar range (IC_{50} = 0.41 nM), compared with that of its R_2 *i*-Pr substituted analogue (*R*)-**5a**. More marked for this compound, (*R*)-**5b**, is the loss of inhibitory activity against MMP-1, where (*R*)-**5b** is 20 times less active (IC_{50} > 3000 nM versus IC_{50} = 147 nM for the parent compound (*R*)-**5a**).

On MMP-9, (*R*)-**5b** shows an IC_{50} = 16 nM, 2.4 times less that of its most potent analogue (*R*)-**5a**.

The best results obtained, with these new type **C** compound, are those found for the inhibition of MMP-14 where (*R*)-**5a** and (*R*)-**5b**, display IC_{50} values in the nanomolar range. Compared with the type **A** compound

Table 1. Inhibitory activity of new type **C** compounds compared with the type **A** inhibitor **1** and the reference drugs **3** and **4** towards some MMPs. MMP-2 selectivity is shown, in parentheses, for the more active compounds[a]

Compd	R_2	IC_{50} (nM)[30]						
		MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-14
1	H	>50,000 (>4000)[a]	12	4500 (375)[a]	>50,000 (>4000)[a]		200 (17)[a]	>10,000 (>833)
(*R*/*S*)-**5a**	*i*-Pr		1.8				19.6	
(*R*)-**5a**	*i*-Pr	147 (1633)[a]	0.09	50 (556)[a]	>1000 (>11,111)	1.6 (18)[a]	6.7 (74)[a]	9.8 (109)[a]
(*S*)-**5a**	*i*-Pr		10.3				13	
(*R*)-**5b**	CH_2CH_2 NHCbz	>3000 (>7300)[a]	0.41	130 (317)[a]	>10,000 (>24,390)		16 (39)[a]	7.7 (19)[a]
(*R*)-**5c**	$CH_2CH_2NH_2$		935				4517	>300
3, CGS27023A		55 (2.8)[a]	20	22 (1.1)[a]	100 (5)[a]		8 (0.4)[a]	8.9 (0.4)[a]
4, AG-3340		8.2 (91)[a]	0.09	0.23 (2.5)[a]	54 (600)[a]		0.26 (2.9)[a]	0.3 (3.3)[a]

[a] Selectivity for MMP-2 over each of the other MMPs, is expressed as the ratio of the IC_{50} value for MMPn over the value for MMP-2.[30]

1, where the $IC_{50} > 10{,}000$ nM, the two MMPi, (*R*)-**5a** and (*R*)-**5b**, show an inhibitory potency that is more than 1000 times higher ($IC_{50} = 9.8$ nM for (*R*)-**5a** and $IC_{50} = 7.7$ nM for (*R*)-**5b**). Finally, the removal of the CBz group on the R_2 substituent of (*R*)-**5b** to obtain the amino derivative (*R*)-**5c** caused a further loss of inhibitory activity of 2280-fold on MMP-2 ($IC_{50} = 935$ nM) and of 282-fold on MMP-9 ($IC_{50} = 4517$ nM). Probably the presence of a protonable nitrogen as in (*R*)-**5c**, is not accepted in this site.

An analysis of the selectivity indices reported in parentheses in Table 1 for the *N*-*i*-propoxy-*N*-biphenylsulfonylaminobutylhydroxamic acids of type **A**.

(**1**), **C** (**5a**–**c**) and the reference drugs **3** and **4** indicates that the newly synthesised type **C** compounds (*R*)-**5a** and (*R*)-**5b** show a high selectivity profile (MMP-1/MMP-2 ratio of 1633 for (*R*)-**5a** and >7300 for (*R*)-**5b**). These two potent MMPi, (*R*)-**5a** and (*R*)-**5b**, show good selectivity indices versus MMP-3, with an MMP-2/MMP-3 ratio of 556 for (*R*)-**5a** and 317 for (*R*)-**5b**, and versus MMP-7, with an MMP-2/MMP-7 ratio >11,111 for (*R*)-**5a** and >24,390 for (*R*)-**5b**. Selectivity indices are lower on MMP-8 (MMP-2/MMP-8 ratio of 18 for (*R*)-**5a**), on MMP-9 (MMP-2/MMP-9 ratio of 74 for (*R*)-**5a** and39 for (*R*)-**5b**) and on MMP-14 (MMP-2/MMP-9 ratio of 109 for (*R*)-**5a** and 19 for (*R*)-**5b**). A comparison of the selectivity indices with the previously studied *N*-4-biphenylsulfonyl-*N*-*i*-propoxyaminoacetohydroxamic acid **1** of type **A** indicates an improvement of the selectivity ratio MMP-1/MMP-2 in the most potent new MMP-2 inhibitor (*R*)-**5b** of almost 2-fold. The new inhibitor (*R*)-**5a** is certainly more potent on MMP-2 than the reference drug **3**, and is as potent, on the same enzyme, as AG-3340 (**4**). On MMP-14, the two new inhibitors show a similar inhibitory potency to those of the known sulfonamides chosen as reference drugs.

The two potent and selective type **C** MMPi synthesised, (*R*)-**5a** and (*R*)-**5b**, were evaluated in the chemoinvasion assay, an in vitro model of angiogenesis, able to quantify the invasive potential of endothelial cells. Figure 2 reports the results of this test, using human umbilical vein endothelial cells (HUVEC), stimulated to invade a reconstituted basement membrane (matrigel) by 3T3 fibroblast-derived conditioned medium (3T3-CM).

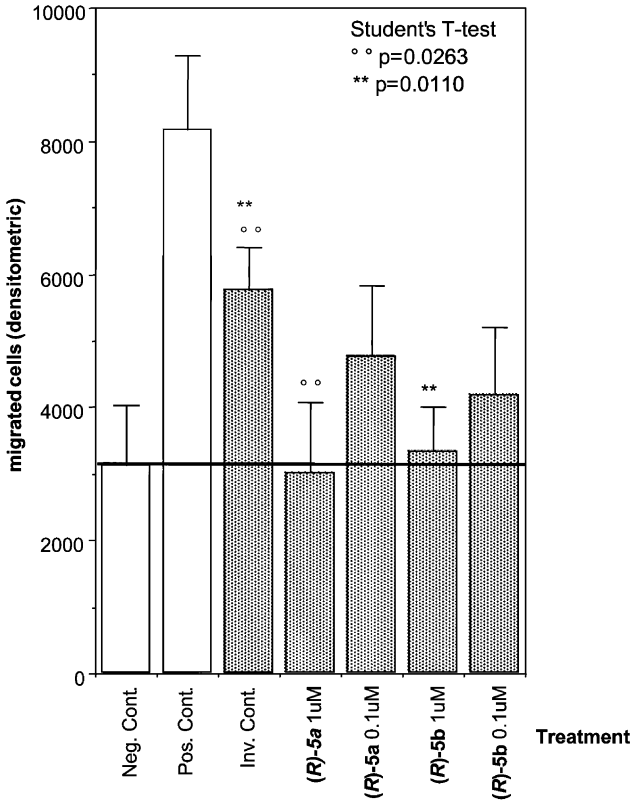


Figure 2. HUVEC–Chemoinvasion Test: Negative control (column 1) represents the background random migration of HUVEC in the presence of serum free, unconditioned medium (SFM). Positive control (column 2) represents the maximal chemotactic response of HUVEC in the presence of 3T3-CM. Invasion control (column 3) represents the maximal ability of HUVEC to cross the matrigel barrier in the presence of 3T3-CM. Columns 4, 5, 6 and 7 represent, respectively, the ability of HUVEC to cross the matrigel barrier in the presence of the (*R*)-**5a** inhibitor 1 and 0.1 μM (columns 4 and 5) and of the (*R*)-**5b** inhibitor at the same concentrations (columns 6 and 7), in the presence of 3T3-CM.

The chemoinvasion assay clearly shows that both (*R*)-**5a** and (*R*)-**5b** are able to reduce considerably the ability of HUVEC cells to cross the matrigel barrier. At a concentration of 1 μM, both inhibitors reduced invasion to basal level, while at 0.1 μM, the effect of (*R*)-**5a** and (*R*)-**5b** was less impressive. On this basis, both (*R*)-**5a** and (*R*)-**5b** inhibitors can be considered to be potent antiangiogenic agents, able to block the chemoinvasion of HUVEC cells in the micromolar range.[41]

In conclusion, in the new *N*-*i*-propoxy-*N*-biphenylsulfonylaminobutylhydroxamic acids of type **C**, designed as analogues of type **A** inhibitors, the R_2 alkyl substituents introduced on the carbon atom alpha to the hydroxamic group of the pharmacophoric portion of **A** seem to confirm our starting hypothesis: this new R_2 substitution site may strongly favour lipophilic interactions with the nearby S1 region of the enzyme site, reinforcing the binding of the planned new inhibitors to the active site of MMP-2. Moreover, in the case of the targeted MMP-14, this chemical manipulation of the type **A** pharmacophore increases the MMPi potency on this crucial MMP more than 1000-fold, shifting their IC_{50} values from the micromolar to the nanomolar range. A similar structural modification on the pharmacophoric portion of MMPi of type **A** does not substantially modify the MMP-1/MMP-2 selectivity profile, but maintains or reinforces the good indices. Finally, the new type **C** MMPi, which prove to be active against these two MMPs, MMP-2 and MMP-14, are potent antiangiogenic agents, able to block the chemoinvasion of HUVEC cells in the micromolar range.

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