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Anticancer activity of (1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-pyrimidines and -purines against the MCF-7 cell line: Preliminary cDNA microarray studies

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Abstract—Completing a SAR study, a series of (*RS*)-1- or 3-(1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-pyrimidines and (*RS*)-6-substituted-7- or 9-(1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl)-7*H*- or 9*H*-purines have been prepared. Their antiproliferative activities on MCF-7 cells are here presented and discussed. (*RS*)-6-Chloro-9-[1-(9*H*-9-fluorenylmethoxycarbonyl)-1,2,3,5-tetrahydro-4,1-benzoxazepine-3-yl]-9*H*-purine (**28**) is the most active ($IC_{50} = 0.67 \pm 0.18$ μM) of the series so far described. cDNA microarray technology reveals potential drug targets, which are mainly centred on apoptosis regulatory pathway genes.

Advance in the knowledge of molecular biology has thrown light on many aspects of the cell-cycle regulation mechanisms. This has allowed a change in the anticancer therapy trends, from the classic cytotoxic strategies to new non-harmful therapies which target the cell-cycle regulation. One of them is the differentiation therapy, searching for the survival of the cells once the cancerous phenotype is eliminated.[1] Despite these advances, the successful treatment of cancer still remains a significant challenge. We have previously reported the synthesis and biological activity of a series of *O*,*N*-acetals with antitumoural activity, which included seven-membered benzoxaheterocyclic moieties and different nitrogen bases. To complete a structure–activity relationship study, we have prepared the bioisosteric compounds bearing the heteroatoms O, S or N in the cyclic seven-membered moiety and linked them to natural or unnatural pyrimidines, or purines.[2–8,10] Between them, the structures **1**–**12**[6,8] (Fig. 1) can be highlighted due to their notable antitumoural activity ($IC_{50} = 1.25$–6.75 μM on MCF-7 cells), that can be compared to that of drugs currently used in therapy, such as the cytotoxic agent 5-fluorouracil (5-FU, $IC_{50} = 4.32 \pm 0.02$ μM, in vitro studies on MCF-7 human breast cancer cells[8]).

Recently, some molecular aspects of the biological mechanism of these molecules have been reported.[9] These results hint at a completely different mechanism with respect to 5-FU, based on cell-cycle arrest at the phases G_0/G_1 and G_2/M versus the S-phase cell-cycle arrest produced by 5-FU. Moreover, they induce apoptosis. This makes these molecules potential cell-differentiating agents and they become excellent prototypes for optimization studies. For compound **2**, this effect is based on the decrease of the expression of cyclin D1 and the activity of Cdk1, and also produces an increment of the activity of CDKIs such as p21 and p27. The apoptotic activity has been found to be due to a p53 independent apoptotic DNA-fragmentation.

Herein we would like to report the antiproliferative activity found for those bioisosteres containing a

Keywords: Benzoxazepines; MCF-7 cell line; Microarrays; Purines; Pyrimidines.
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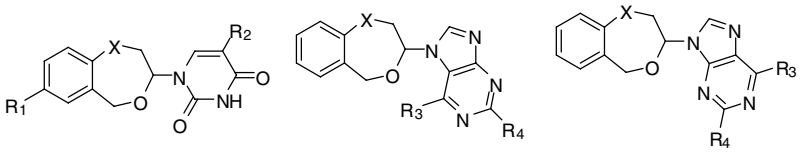


1. X = O, R_1 = OCH_3, R_2 = F
2. X = O, R_1 = R_2 = H

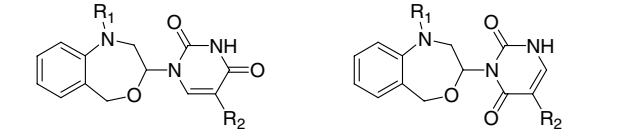
3. X = O, R_3 = Cl, R_4 = H
4. X = O, R_3 = I, R_4 = H
5. X = O, R_3 = R_4 = Cl
6. X = S, R_3 = Cl, R_4 = H

7. X = O, R_3 = Cl, R_4 = H
8. X = O, R_3 = I, R_4 = H
9. X = O, R_3 = R_4 = Cl
10. X = O, R_3 = CF_3, R_4 = H
11. X = S, R_3 = Cl, R_4 = H
12. X = SO_2, R_3 = Cl, R_4 = H

Figure 1. *O*,*N*-Acetals with antitumoural activities against MCF-7 cells.

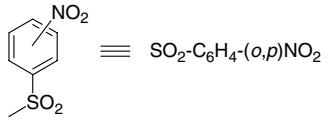
4,1-benzoxazepine *N*-alkylated pyrimidine[7] (**13**–**25**, Fig. 2) or purine[10] (**26**–**38**, Fig. 3).

The structures **28**, **32** and **35** were prepared from the condensation of *O*,*O*-acetal **39** with 6-chloropurine (6-CP), following the reported procedure[10] (Scheme 1). The rate of transformation of the starting *O*,*O*-acetal is very similar to that found for R_1 = nitrobenzenesulfonyl (Ns)[10] with a product yield near to 45%. The alkylation of 6-CP seems to occur to a larger extent on its *N*7 versus its *N*9 position, as the structure **35** could be formed from the 6-chloroderivative **32**. This trend was also found when the nitrobenzenesulfonyl *O*,*O*-acetals were condensed with 6-CP.[10] The more facile chlorine



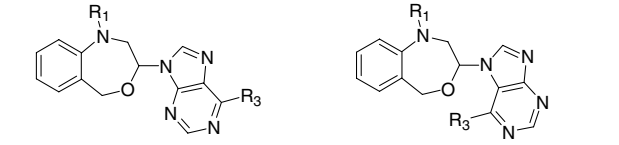
13 R_1 = H, R_2 = F
14 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = F
15 R_1 = SO_2-C_6H_4-*p*NH_2, R_2 = F
16 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = F
17 R_1 = SO_2-C_6H_4-*o*NH_2, R_2 = F
18 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = H
19 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = H

20 R_1 = H, R_2 = F
21 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = F
22 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = F
23 R_1 = SO_2-C_6H_4-*o*NH_2, R_2 = F
24 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = H
25 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = H



≡ SO_2-C_6H_4-(*o*,*p*)NO_2

Figure 2. New benzoxazepine *O*,*N*-acetals containing pyrimidine rings.



26 R_1 = SO_2-C_6H_4-*p*NO_2, R_3 = Cl
27 R_1 = SO_2-C_6H_4-*o*NO_2, R_3 = Cl
28 R_1 = Fmoc, R_3 = Cl

29 R_1 = SO_2-C_6H_4-*p*NO_2, R_3 = Cl
30 R_1 = SO_2-C_6H_4-*o*NO_2, R_3 = Cl
31 R_1 = SO_2-C_6H_4-*o*NH_2, R_3 = Cl
32 R_1 = Fmoc, R_3 = Cl
33 R_1 = SO_2-C_6H_4-*p*NO_2, R_3 = -OH
34 R_1 = SO_2-C_6H_4-*o*NO_2, R_3 = OH
35 R_1 = Fmoc, R_3 = OH
36 R_1 = H, R_3 = SPh
37 R_1 = SO_2-C_6H_4-*p*NO_2, R_3 = SPh
38 R_1 = SO_2-C_6H_4-oNO_2, R_3 = SPh

Figure 3. New benzoxazepine *O*,*N*-acetals containing purine rings.

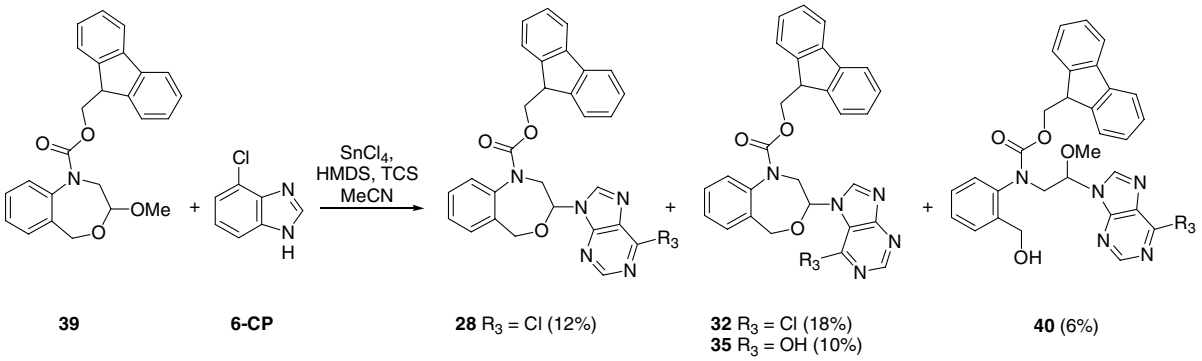
substitution on *N*7-alkylated purines versus the *N*9-alkylated has been previously observed.[10]

The *N*7- or *N*9-binding of the purine ring in **28** and **32** was established from a comparison study of the ^{1}H NMR spectra of *N*9- and *N*7-isomers[11,12]: (i) H3β and H2β appear more unshielded in *N*7-isomers than in *N*9. Both hydrogen atoms are more deshielded in **32**. (ii) H2α appears at slightly lower fields in *N*9- than in *N*7-isomers. In **28**/**32**, the difference in chemical shift for this hydrogen is 0.16 ppm and is more unshielded in **28**. (iii) In every case, H8″ is more deshielded than H2″. Both hydrogen atoms appear at lower fields in *N*7- than in *N*9-isomers. Compound **32** presents more unshielded signals for H8″ and H2″ than **28**. (iv) The most shielded of the two benzylic hydrogen atoms H5 is more shielded in *N*7- than in *N*9-isomers. This hydrogen is also more shielded in **32** than in **28**. To summarise, the ^{1}H NMR spectrum of **28** is more similar to that of the remainder of the *N*9-derivatives, while the ^{1}H NMR spectrum of **32** is more similar to those of the *N*7-isomers. All new compounds gave satisfactory ^{1}H NMR, ^{13}C NMR, HR LSIMS and combustion analyses.

Tables 1 and 2 show the antiproliferative activity (IC_{50} values) on MCF-7 human breast cancer cells found for the pyrimidine and purine derivatives **13**–**38**.[13]

The most potent molecules are the purine derivatives. Compounds **28**, **30**, **32** and **37** present IC_{50} values below 1 μM. We selected **28** and **37** to identify the molecular key targets of its anticancer activity. Completion of the human genome sequence and the advent of DNA microarrays using cDNAs enhanced the detection and identification of hundreds of differentially expressed genes in response to anticancer drugs. In this way we obtained gene-expression patterns of treated human breast cancer cells in comparison with parental MCF-7 cells. For this purpose, we analysed the expression of about 22,000 different human genes using the Agilent 60-mer oligo microarray platform and the Human 1A Oligo Microarray Kit (V2) (Agilent Technologies, CA, USA).

The up-regulated and the down-regulated genes include genes that encode for different metabolic pathways, cellular development process, signal molecules, response to stress, regulation of the cell cycle and apoptosis, etc. Analysis of the mRNAs, which are deregulated (up-regulated or down-regulated) at least 2-fold in treated cells,



Scheme 1. Condensation between the *O*,*O*-acetal **39** and 6-chloropurine (6-CP). Reagents and conditions: **39** (1.0 equiv), 6-CP (1.2 equiv), HMDS (1.2 equiv), TMSCl (1.2 equiv), $SnCl_4$ (1.5 equiv), anhydrous MeCN, 50 °C, 48 h.

Table 1. Antiproliferative activities against the MCF-7 cells for *N*1- (**13**–**19**) and *N*3- (**20**–**25**) pyrimidines

Compound	IC_{50}[a] (μM)	Compound	IC_{50} (μM)
13	>100	**20**	72.40 ± 11.3
14	19.33 ± 1.04	**21**	19.81 ± 0.08
15	14.37 ± 0.69	**22**	22.63 ± 0.11
16	19.70 ± 0.15	**23**	43.70 ± 0.09
17	54.82 ± 1.04	**24**	44.28 ± 4.65
18	39.78 ± 2.60	**25**	50.90 ± 3.87
19	45.17 ± 0.48		

[a] The data are means ± SEM of three independent determinations.

Table 2. Antiproliferative activities against the MCF-7 cells for *N*9- (**26**–**28**) and *N*7-purines (**29**–**38**)

Compound	IC_{50}[a] (μM)	Compound	IC_{50} (μM)
26	2.73 ± 0.17	**33**	>100
27	2.10 ± 0.69	**34**	19.66 ± 5.27
28	0.67 ± 0.18	**35**	53.57 ± 13.1
29	1.22 ± 0.12	**36**	48.92 ± 9.89
30	0.92 ± 0.01	**37**	0.86 ± 0.12
31	9.14 ± 1.24	**38**	2.59 ± 0.57
32	0.84 ± 0.09		

[a] The data are means ± SEM of three independent determinations.

revealed the following results: 26 genes up-regulated and 59 genes down-regulated in **28**-MCF-7 treated cells; and 26 genes up-regulated and 17 genes down-regulated in **37**-treated human breast cancer cells. Each compound revealed a somewhat unique expression pattern together with the up-regulation of significant genes involved in different cellular functions and a significant down-regulation of genes for **28**. One of the more important results in the current study was the ability of **28** to modulate the expression of genes involved in apoptosis or its delay of mitosis. This effect can be explained by the accumulation of cells in the G_2/M checkpoint of cell cycle, particularly GP132, the receptor for an unknown ligand, which activates a G2α protein. This is transcriptionally up-regulated by stress-inducing and cell-damaging agents and is involved in caspase-mediated apoptosis.[14] Similarly, the ERN1 gene that belongs to the Ser/Thr protein kinase family is a potent unfolded-protein response transcriptional activator and acts by triggering growth arrest and apoptosis.[15] However, **37** induced the down-regulation of a gene involved in the metastatic progression of cancer such as RAC1, a Ras-like protein member of the Rho family of the GTPase key downstream target in Ras signalling.[16]

Among the pyrimidine derivatives **13**–**25**, those containing 5-fluorouracil (R_2 = F) show better activities than those derived from uracil (R_2 = H). Bonding of the pyrimidine ring through *N*1 or *N*3 affects the activity though only slightly, when R_1 = H, **13** versus **20**.

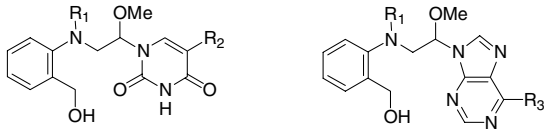
The substitution on *C*6 of the purine ring is essential for the activity. Bulky, lipophilic groups afford the best values of IC_{50} [R_3 = Cl (**26**–**32**), -SPh (**37**–**38**)] while purinone compounds [R_3 = OH (**33**–**35**)] are comparable to the pyrimidine analogues in terms of activity. Bonding of the purine ring through *N*7 or *N*9 affects the activity to a lesser extent, and the positive or negative character of this effect depends on the nature of R_1 (see **26/29** R_1 = *p*Ns, **27/30** R_1 = *o*Ns, **28/32** R_1 = Fmoc).

Both, pyrimidine and purine derivatives, are more potent when R_1 is not hydrogen. The lipophilic character of R_1 increases the activity, and no limit of volume has been observed for the studied groups. The electron-withdrawing character of R_1 could help to increase the activity.[4] Carbonyl derivatives are more potent than the sulfonyl ones.

When R_1 = benzensulfonamido, it can be observed for pyrimidine and purine derivatives, that the *ortho*-substitution on R_1 is preferred to *para*, in terms of potency, and the nitro group renders better results than the amino one. As an exception, compound **37** (R_1 = *p*Ns, R_3 = SPh) is more potent than **38** (R_1 = *o*Ns, R_3 = SPh).

New related acyclic acetals **41**–**51** (Fig. 4) were obtained as minor products in the condensation reaction between the *O*,*O*-acetals and pyrimidine[7] or purine[10] bases. Their antiproliferative activities have also been studied on MCF-7 human breast cancer cells and the IC_{50} values obtained are shown in Table 3.

Acyclic purine *O*,*N*-acetals (**49**–**51**) show higher potency than the pyrimidine acyclic derivatives (**41**–**48**). The *N*7-alkylated purine **50** presented an excellent value of IC_{50}. In contrast to the cyclic analogues, the presence of an *o*NO_2 or *p*NO_2 group does not modify the activity of the *N*9-isomers (**49** and **51**).



41 R_1 = H, R_2 = F
42 R_1 = CO-C_6H_5, R_2 = F
43 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = F
44 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = F
45 R_1 = SO_2-C_6H_4-*o*NH_2, R_2 = F
46 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = H
47 R_1 = SO_2-C_6H_4-*p*NO_2, R_2 = H (*N3*)
48 R_1 = SO_2-C_6H_4-*o*NO_2, R_2 = H

49 R_1 = SO_2-C_6H_4-*p*NO_2, R_3 = Cl
50 R_1 = SO_2-C_6H_4-*o*NO_2, R_3 = Cl (*N7*)
51 R_1 = SO_2-C_6H_4-*o*NO_2, R_3 = Cl

Figure 4. New acyclic *O*,*N*-acetals containing pyrimidine and purine moieties.

Table 3. Antiproliferative activities against the MCF-7 cell line for acyclic *N*1- and *N*3-pyrimidines (**40**–**47**) and *N*9- and *N*7-purines (**48**–**50**)

Compound	IC_{50}[a] (μM)	Compound	IC_{50} (μM)
41	35.97 ± 0.40	**47**	55.22 ± 12.1
42	16.14 ± 0.77	**48**	64.81 ± 0.05
43	55.22 ± 12.1	**49**	18.70 ± 0.08
44	90.99 ± 6.06	**50**	3.25 ± 0.23
45	>100	**51**	11.30 ± 1.27
46	45.76 ± 2.45		

[a] The data are means ± SEM of three independent determinations.

To summarise, the in vitro anticancer activity on MCF-7 cells of new 4,1-benzoxazepines containing pyrimidine or purine rings has been studied. The change to 4,1-benzoxazepine rings has afforded an enhancement of the antiproliferative activity on MCF-7 cells in relation to the previous 4,1-benzoxathiepin[8] and 1,4-benzodioxepin,[2,6,17] with an improvement on the values of IC_{50} from 1.25 μM (for **5**) to 0.67 μM (for **28**). Moreover, the studies by microarray technology showed that the main molecular targets of some of these compounds (**28** and **37**) are pro-apoptotic genes with protein kinase activity such as GP132, ERN1 or RAC1, which prevent the metastatic progression. More detailed results of these key targets will be published in future papers.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2007.12.070.

References and notes

1. Gómez-Vidal, J. A.; Campos, J.; Marchal, J. A.; Boulaiz, H.; Gallo, M. A.; Carrillo, E.; Espinosa, A.; Aránega, A. *Curr. Top. Med. Chem.* **2004**, *4*, 175.
2. Saniger, E.; Campos, J.; Entrena, A.; Marchal, J. A.; Súarez, I.; Aránega, A.; Choquecillo, D.; Niclós, J.; Gallo, M. A.; Espinosa, A. *Tetrahedron* **2003**, *59*, 5457.
3. Saniger, E.; Campos, J.; Entrena, A.; Marchal, J. A.; Boulaiz, H.; Aránega, A.; Gallo, M. A.; Espinosa, A. *Tetrahedron* **2003**, *59*, 8017.
4. Díaz-Gavilán, M.; Rodríguez-Serrano, F.; Gómez-Vidal, J. A.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2004**, *60*, 11547.
5. Núñez, M. C.; Entrena, A.; Rodríguez-Serrano, F.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2005**, *61*, 10363.
6. Núñez, M. C.; Pavani, M. G.; Díaz-Gavilán, M.; Rodríguez-Serrano, F.; Gómez-Vidal, J. A.; Marchal, J. A.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2006**, *62*, 11724.
7. Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Entrena, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *J. Org. Chem.* **2006**, *71*, 1043.
8. Núñez, M. C.; Rodríguez-Serrano, F.; Marchal, J. A.; Caba, O.; Aránega, A.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2007**, *63*, 183.
9. Marchal, J. A.; Núñez, M. C.; Súarez, I.; Díaz-Gavilán, M.; Gómez-Vidal, J. A.; Boulaiz, H.; Rodríguez-Serrano, F.; Gallo, M. A.; Espinosa, A.; Aránega, A.; Campos, J. M. *Breast Cancer Res. Treat.* **2007**, *105*, 237.
10. Díaz-Gavilán, M.; Choquesillo-Lazarte, D.; González-Pérez, J. M.; Gallo, M. A.; Espinosa, A.; Campos, J. M. *Tetrahedron* **2007**, *63*, 5274.
11. For details about the spectroscopic assignment of the *N*9- and *N*7-isomers in alkylated purines, see Ref. 10.
12. There are characteristic differences for each type of isomer in ^{13}C NMR: (i) The most representative features are the chemical shifts (δ) of the quaternary carbons of the purine ring: C4″ is approximately 10 ppm more deshielded in *N*7-isomers than in the *N*9 ones ($\delta C4_{N7}$ = 161 vs $\delta C4_{N9}$ = 151 ppm for our compounds), C5″ appears approximately 9 ppm more deshielded in *N*9-isomers than in the corresponding *N*7 ($\delta C5_{N9}$ = 130 vs $\delta C5_{N7}$ = 121 ppm). The differences in chemical shifts of C4″ and C5″ are 40 ppm in the *N*7-isomers and 20 ppm in the *N*9 ones. C4″ is more deshielded than C2″ in *N*7-isomers, while the opposite occurs to *N*9-isomers. C6″ is approximately 7 ppm more deshielded in *N*9 than in *N*7-isomers ($\delta C6_{N9}$ = 149 ppm between C2″ and C8″) versus $\delta C6_{N7}$ = 142 ppm (higher than C2″ and C8″). (ii) In agreement with previous reports, C2″ and C8″ were found to resonate at lower fields in *N*7 than in *N*9-isomers (0.26 ppm for C2″ and 2.44 ppm for C8″). The value of δC2–δC8 is approximately 4 ppm for *N*7-isomers and approximately 6 ppm for isomers *N*9. To summarise, those carbon atoms of the purine ring that are orientated towards the benzoxazepine fragment appear more shielded than those situated towards the outer part of the molecule. This agrees with previous bibliographical data: Hocková, D.; Budesinsky, M.; Marek, R.; Marek, J.; Holy, A. *Eur. J. Org. Chem.* **1999**, 2675; Garner, P.; Ramakanth, S. *J. Org. Chem.* **1988**, *53*, 1294.
13. For the biological methods see Ref. 4.
14. Lin, P.; Ye, R. D. *J. Biol. Chem.* **2003**, *278*, 14379.
15. Yoneda, T.; Imaizumi, K.; Oono, K.; Yui, D.; Gomi, F.; Katayama, T.; Tohyama, M. *J. Biol. Chem.* **2001**, *276*, 13935.
16. Baugher, P. J.; Krishnamoorthy, L.; Price, J. E.; Dharmawardhane, S. F. *Breast Cancer Res.* **2005**, *7*, R965.
17. Campos, J. M.; Espinosa, A.; Gallo, M. A.; Gómez-Vidal, J. A.; Núñez, M. C.; Aránega, A.; Marchal, J. A.; Rodríguez-Serrano, F. Spanish Patent, P200601538, 2006.